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"Identification of bacteria degrading sulfated organics in anoxic marine sediments"

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ABSTRACT (ENGLISH)

A large variety of natural organic sulfur compounds exists in the marine environment and intimately couples the major biogeochemical cycles. Amongst these compounds, sulfated polysaccharides are high-molecular-weight (HMW) molecules that are found in large abundances in marine sediments due to their wide range of biogenic sources. The degradation and desulfonation of these HMW compounds in anoxic marine sediments has not been shown so far and the microorganisms performing such a process are virtually unknown. In order to investigate sulfated polysaccharide desulfonation and the microbial players involved therein, anoxic sediment from the Venice Lagoon, Italy, was supplemented with different sulfated polysaccharides including carrageenans, fucoidan, sulfated arabinogalactan and chondroitin sulfate in a microcosm experiment. Bacterial 16S rRNA gene amplicon data from microcosm samples revealed strong shifts in relative abundances of microbial community members induced by substrate additions. The obtained data indicates that phylotypes from the *Clostridiales* and *Spirochaetes* are involved in the degradation of the investigated substrates, and possibly also the desulfonation of these molecules. Measurements of sulfate, hydrogen sulfide and short-chain fatty acids showed that fucoidan and κ-carrageenan were apparently degraded fastest amongst the investigated substrates. Supplementary results from Raman microspectroscopy performed on cells grown in D_2O incubations supplemented with sulfated polysaccharides, showed that bacteria were activated by the model substrates, and future work combining Raman-activated cell-sorting or (2-step) CARD-FISH-based cell-sorting methods may allow for the identification of anaerobic degraders of sulfated polysaccharides.

ZUSAMMENFASSUNG (GERMAN)

Sulfatierte Polysaccharide sind organische Schwefelverbindungen mit hohem Molekulargewicht. Zahlreiche marine Organismen, insbesondere Makroalgen, produzieren sulfatierte Polysaccharide als Zellwandkomponenten. Speziell in marinen Sedimenten finden sich sulfatierte Polysaccharide in Konzentrationen, die für globale Stoffkreisläufe (C, S, O, …) eine wichtige Rolle spielen können. Über den Abbau und die damit verbundene Desulfonierung sulfatierter Polysaccharide, vor allem in anoxischen Sedimenten, ist nur wenig bekannt. Ebenso sind die Mikroorganismen, die an diesen Prozessen beteiligt sind, weitgehend unbekannt. Die vorliegende Arbeit soll helfen, jene Bakteriengruppen aufzuzeigen und zu identifizieren, die im anaeroben Abbau sulfatierter Polysaccharide in marinen Sedimenten involviert sind. Dazu wurden anoxische Mikrokosmen mit Sedimenten aus der Lagune von Venedig, Italien, angesetzt und sulfatierte Polysaccharide (Carrageen, Fucoidan, sulfatiertes Arabinogalactan, Chondroitin Sulfat) einzeln oder als Gemisch beigefügt. Daraus gesammelte 16S rRNA Amplikon Daten zeigten signifikante Anstiege relativer Häufigkeiten mehrerer bakterieller Gruppen in Folge der Substratzugabe. Phylotypen aus den Klassen *Clostridiales* und *Spirochaetes* schienen stark am Abbau und möglicherweise an der Desulfonierung der Substrate beteiligt zu sein. Zusätzliche Messungen von Sulfat, Schwefelwasserstoff und kurzkettigen Fettsäuren zeigten, dass insbesondere Fucoidan und κ-Carrageenan schnell von der Bakteriengesellschaft im Sediment abgebaut werden können. Bakterielle Aktivität am Substrat wurde mittels Raman-Mikrospektroskopie D_2O-markierter Zellen nachgewiesen. Die daraus entstanden Ergebnisse sind vielversprechend für die zukünftige Identifizierung der Mikroorganismen, welche sulfatierte Polysaccharide in anoxischen Sedimenten abbauen können.

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INTRODUCTION

1.1 ORGANIC SULFUR COMPOUNDS AND WHERE TO FIND THEM

The impact of sulfate on biota in the ocean is hardly matched in any other ecosystem, due to its high concentration and availability in seawater. Therefore, many marine organisms have developed strategies to incorporate sulfate for the use in amino acids, vitamins, enzyme co-factors or carbohydrates like glycolipids and polysaccharides. This results in a remarkable range of biogenic organic sulfur molecules of varying size and complexity (Helbert 2017). Organosulfur is especially abundant in marine sediments, where it represents up to 85% of the total reduced sulfur pool, depending on various environmental parameters (Zaback & Pratt 1993; Werne et al. 2004). Biogenic organosulfur includes amino acids as well as various co-enzymes and co-factors. These however, make up just a small part of the total organic sulfur molecules in sediments. The greater part is taken up by various organisms and incorporated into biomolecules as sulfonates ($R\text{-}SO_3^-$) and sulfate esters ($R\text{-}O\text{-}SO_3^-$). Between 20-40% of organosulfur in marine sediments is made up by sulfonated organic material (Vairavamurthy et al. 1994).

Despite their abundance and structural and compositional variety, the impact of organosulfur on the marine sulfur cycle and especially the sediment sulfur cycle is poorly understood. The coupling of the major element cycles of carbon, oxygen and sulfur through formation and dissimilation of organic sulfur compounds is however, apparent (Garrels & Lerman 1981; Anderson & Pratt 1995; Canfield 2000; Werne et al. 2004). Chemical sulfurization or biogenic sulfated or sulfonated material is important for organic matter preservation (de Leeuw & Largeau 1993; Gelin et al. 1996; Werne et al. 2000) and its microbial degradability might significantly influence the burial or remineralization of carbon and sulfur (de Leeuw & Sinninghe Damsté 1990; Werne et al. 2004). As marine sediments cover almost the entire seafloor, the impact of biogeochemical processes including organosulfur generation and dissimilation are of high environmental relevance.

In soils, isotope tracer experiments have shown the considerable turnover of organic sulfur compounds under aerobic conditions within the ecosystem (Ghani et al. 1993). Although no such experiments have been conducted for marine sediments, the high abundance of organosulfur in sediments leads us to hypothesize that a similar turnover could be expected. In regards to anaerobic studies of organosulfur transformations, investigations have often been restricted to low-molecular-weight (LMW) components like taurine (Lie et al. 1999) or isethionate (Lie et al. 1996).

A significant source of complex organosulfur compounds are macroalgae that produce sulfated polysaccharides. Due to their suggested beneficial health effects in humans, these compounds have been of high research interest (e.g. Mulloy et al. 2000; Zhang et al. 2012; Ngo & Kim 2013). Sulfated polysaccharides are common structural components in macroalgae and are seen as an adaption to the osmotic stress of a marine lifestyle (Olsen et al. 2016). Additionally, sulfated polysaccharides have been

discovered in marine angiosperms like the seagrass *Zostera marina* (Olsen et al. 2016) and several marine invertebrates like sea urchins (Vasseur 1948). Besides these predominantly marine sulfated polysaccharides, sulfated glycosaminoglycans, like chondroitin sulfate are common components of animal cartilage (Prestegard et al. 2015) and occur abundantly both in marine and terrestrial ecosystems. The most important macroalgal marine sulfated polysaccharides include fucans from brown algae, galactans like carrageenans and agarans from red algae and sulfated heteropolysaccharides from green algae (Ngo & Kim 2013; Jiao et al. 2011). Sulfated polysaccharides can also be found in the cell walls of diatoms (Anderson et al. 1978; Percival & McDowell 1967; Smestad et al. 1975; Wustman et al. 1998). Therefore, sulfated polysaccharides and consequently sulfatase enzymes needed for their degradation, occur in high amounts during the succession of microbial communities during algal blooms in the oceans (Teeling et al. 2012). As such, sulfated polysaccharides are important compounds in the pelagic environment and will thus also reach benthic environments in significant amounts as detritus within marine snow. Considering the ubiquitous nature of sulfated molecules in marine environments, it can be hypothesized that the degradation of these compounds presents an important component of biogeochemical cycles of marine sediments by influencing the microbial community structures and functioning in these environments.

Organosulfur was recognized as an important microbial substrate after Lie et al. 1999 showed the anaerobic microbial desulfonation of taurine, meaning the enzymatic removal of the sulfur moiety from the sulfonate molecule. In an earlier study it was also shown that relatives of known sulfate-reducing bacteria can use organic sulfur compounds like isethionate as terminal electron acceptor instead of sulfate, whereby they liberated the sulfur moiety from the organic component to subsequently use the sulfite as the electron acceptor (Lie et al. 1996). Although such studies are useful and valuable to understand the mechanisms and processes allowing for organic sulfur compounds to be dissimilated, many of the natural organosulfur molecules are much larger and more complex than the model compounds used in those investigations. Not much is known about microbial removal of sulfur moieties from high-molecular-weight (HMW) organosulfur compounds like sulfated polysaccharides in anoxic marine sediments. Similarly, the identities of microbial degraders of HMW organosulfur are virtually unknown in anoxic sediments. In the last decade, metagenomic analyses have been conducted and suggested several bacterial groups as possible anaerobic organosulfur degraders. Genes encoding for sulfatase enzymes that are essential for cleaving the sulfur moieties off organic molecules were found in various members of the PVC superphylum (Wagner & Horn 2006), suggesting these taxa are important mediators of desulfonation reactions in marine and hypersaline environments (Glöckner et al. 2003; Thrash et al. 2010; Wegner et al. 2013; Spring et al. 2016).

1.2 STATE OF KNOWLEDGE: A GUIDE TO SULFATE ESTER DEGRADATION

A note on terminology within this thesis: Since ester sulfates are often referred to as O-sulfonates, the term *desulfonation* will in the following be used to include the removal of the sulfur moiety both from sulfate esters ($R\text{-}O\text{-}SO_3^-$) and sulfonates ($R\text{-}SO_3^-$).

A prerequisite for the degradation of complex sulfate esters, such as those of sulfated polysaccharides, is both the breakdown of the carbohydrate backbone by glycoside hydrolases and polysaccharide lyases, as well as the action of enzymes that remove any additional moieties like sulfate (Helbert 2017). Desulfonation of organic matter is catalyzed by sulfatase enzymes. The importance of the cooperation of polysaccharide degradation enzymes and sulfatases is highlighted by the existence of "polysaccharide utilization loci". These were first found in *Bacteroidetes*, where genes for the complete degradation of complex polysaccharides, including sulfatases, cluster together in the genome of these polysaccharide degraders (Sonnenburg et al. 2006; Flint et al. 2008; White et al. 2014).

The enzyme family of sulfatases is large and acts very heterogeneously in terms of substrate range (Hanson et al. 2004). Classification of sulfatase enzymes is ongoing and a new system has been suggested recently (Barbeyron et al. 2016; Hagelueken et al. 2006). Since a classification by the substrate range of sulfatases is sometimes ambiguous (Kertesz 1999), several types of sulfatases are distinguished by their mechanisms of action. The most prominent and well-researched group are the formylglycine (FGly)-dependent arylsulfatases, which are grouped together with carbohydrate and sulfated-hormone sulfatases (Boltes et al. 2001; Knaus et al. 2012). All FGly-dependent sulfatases share a conserved amino acid motif and have a post-translational modification of either a serine or cysteine residue to a Cα-formylglycine (Schmidt et al. 1995; Dierks et al. 1999). In sulfate ester desulfonation, FGly-dependent carbohydrate sulfatases cleave off the sulfur moiety by hydrolyzing the O-S bond of the molecule. This pathway yields an alcohol and liberates sulfate from the organic molecule (Boltes et al. 2001).

Apart from hydrolytic desulfonation, another group of sulfatases can cleave sulfate esters oxidatively. In this pathway, α-ketoglutarate is used as electron acceptor for the oxidation of the C-O bond. Inorganic sulfate and an aldehyde are released in this process while the stereochemical configuration of the involved carbon center is inverted (Knaus et al. 2012).

The generation of FGly for FGly-dependent sulfatases has been first described to strictly require oxygen (Dierks et al. 2003). However, a sulfatase in an anaerobic microorganism was found in *Clostridium perfringens* of the phylum *Firmicutes*, which was the first report of a sulfatase within this phylum (Berteau et al. 2006). *C. perfringens* was shown to be able to degrade 4-nitrophenyl sulfate, an LMW organosulfur compound, anaerobically. Its sulfatase shows the posttranslational modification of the cysteine residue to FGly. However, the sulfatase of *C. perfringens* lacks the common sulfatase motif known for all FGly-dependent sulfatases working with oxygen. Apart from presenting a novel type of sulfatase and a sulfatase-maturing enzyme, this study therefore pointed out the gap in knowledge about

sulfatases and a possible underestimation of the abundance and occurrence of sulfatases obtained from metagenomic studies (Berteau et al. 2006).

Which sulfatase mechanism occurs on complex organosulfur compounds in anoxic environments such as marine sediments, and which microorganisms harbor them, is still unknown. Another aspect of desulfonation of organic matter, is the potential to make desulfonated organic molecules available for other groups of degraders that would otherwise not be able to utilize these substrates. As such, organosulfur degradation might impact the biogeochemical element cycles tremendously and be beneficial for co-existing bacterial carbohydrate degraders without desulfonation activity.

1.3 AIM AND INCENTIVES

There is considerable gap in our knowledge regarding the degradation of HMW organosulfur compounds and the identities of the microorganisms responsible. Marine sediments contain large amounts of sulfated polysaccharides from natural sources, which are potentially able to significantly contribute to the major element cycles. However, essentially nothing is known about the microbial degraders of sulfated organics in the anoxic layers of these sediments or if desulfonation of HMW organosulfur compounds occurs therein. Since large areas of marine sediments are devoid of oxygen, anoxic sediments are the focus of this thesis.

The overall aim of this thesis was to examine, if sulfated polysaccharides may be degraded under anoxic conditions in marine sediment and to identify the key bacterial taxa that may be involved in this process. For this purpose, two main experiments were performed. First, controlled microcosm experiments with additions of model sulfated polysaccharides were conducted. From these, 16S rRNA genes were sequenced in order to identify microbial populations that were stimulated by the substrates and were therefore likely involved in the degradation of the compounds. Additionally, the microcosm experiment was coupled with selected biogeochemical measurements to provide information about microbial activity. In a second experiment, sulfated polysaccharides were added to incubations in the presence of D_2O to identify physiologically active cells and confirm activity on the given substrates using Raman microspectroscopy.

MATERIAL AND METHODS

A list of the equipment used within this thesis is provided in **Appendix Table 5**. All used chemicals are listed in **Appendix Table 6**.

2.1 SAMPLE COLLECTION AND CHARACTERIZATION

Sublayer sediment samples (2-15 cm below seafloor) were collected during early May 2016 from a *Zostera marina* associated location inside the Venice Lagoon, Italy, and stored inside an airtight container for transport. The samples were coarse grained and sandy with a dark grey to blackish color and strong sulfidic smell.

2.2 MICROCOSM EXPERIMENT

Artificial seawater was prepared as per Graue et al. 2012 with an addition of 3.9 g $Na_2SO_4^{2-}$ per liter (as suggested by Hirschler et al. 1998) and adjusted to pH 8.1. Resazurin was added to a final concentration of 0.5 mg L^{-1}. The artificial seawater was autoclaved at 121°C for 20 minutes and left inside an anaerobic chamber overnight to remove oxygen from the water, i.e., until resazurin was clear.

Anoxic sediment was mixed at a 2:3 ratio with artificial, anoxic seawater inside an anaerobic chamber. Sediment slurry was stirred thoroughly and homogenized. Large solid components like rocks, shells and patches of seagrass were removed from the slurry.

Microcosms were set up by transferring 30 ml of the sediment slurry to 100 ml glass bottles. All microcosms treatments were done in triplicates. Samples from day 0 were taken from four randomly selected microcosms. Per microcosm, 150 mg of each sulfated polysaccharide from different biological sources were added (**Table 1**). Due to availability, only 70 mg of chondroitin sulfate were added to the respective set of microcosms. Two sets of microcosms were treated with a substrate mix of sulfated arabinogalactan, κ-carrageenan and λ-carrageenan of 30 mg each. Sulfate reduction was inhibited in one set of these mixed substrate treatments by the addition of 28 mM molybdate. Further, one triplicate of microcosms was left untreated to serve as negative control ("no substrate control") (**Table 1)**.

Microcosm bottles were sealed with thick rubber stoppers and aluminum crimps to make airtight and stored at a constant temperature of 20°C, which roughly corresponds to the average sea temperature of the Venice Lagoon in May. From each microcosm, subsamples were taken within the anaerobic chamber after 0, 2, 5, 11 and 18 days of incubation. Subsamples for fluorescence *in situ* hybridization (FISH) were fixed 1:1 with 4% formaldehyde. For H_2S measurements, subsamples were treated with zinc acetate (2% w/v) to preserve sulfide from re-oxidation. Subsamples intended for molecular analysis were immediately frozen on dry ice. All microcosm subsamples were then stored at -80°C until further processing.

Table 1. List of treatments of the microcosm experiment. Due to the heterogeneity of sulfated polysaccharides in nature, substrate molecular weights were estimated from disaccharide molecular weight (MW) and amount of sulfate moieties per disaccharide.

Substrate/ Treatment	Description	Source	Additional Properties	Addition/ Microcosm [mg]	MW [g*mol^{-1}]	Concentration [mM]
κ-Carrageenan [1)]	3-linked β-D-galactose 4-sulfate, 4-linked AnGal	Red algae	1 sulfate group per disaccharide	150	387	12.9
λ-Carrageenan [1)]	3-linked β-D-galactose 4-sulfate, 4-linked AnGal; missing anhydride bridge	Red algae	3 sulfate groups per disaccharide	150	466	10.7
Arabinogalactan sulfated [2)]	3-linked β-D-galactopyranose and β-L-arabinopyranose	Green algae (*Codium fragile)*	appr. 1 sulfate group per disaccharide	150	395	12.6
Fucoidan [3)]	Fucose and galactose (18:1)	Brown algae *(Macrocystis pyrifera)*	appr. 3 sulfate groups per disaccharide	150	424	11.8
Chondroitin sulfate [5)]	Glycosaminoglycan, N-acetylgalactosamine, glucuronic acid	Bovine cartilage	1 sulfate group per disaccharide	70	463	5.0
Substrate mix	κ-/λ-Carrageenan/ Arabinogalactan sulfated	-	-	90 (30/30/30)		
Mo-inhibited mix	κ-/λ-Carrageenan/ Arabinogalactan sulfated; 28 mM Molybdate	-	-	90 (30/30/30)		
No Substrate	-	-	-			

[1)] Jiao, Yu, Zhang, & Ewart, 2011; [2)] Manufacturer's description (ELICITYL, OligoTech®, Grenoble, France); [3)] Li, Lu, Wei, & Zhao, 2008; [5)] Hardingham, 1998

2.2.1 Molecular Biological Analyses of Anoxic Microcosms

DNA Extraction from Anoxic Microcosms

Environmental DNA was extracted from microcosm sediment samples for each sampling time point using a hexadecyltrimethylammonium bromide (CTAB) extraction buffer and phenol-chloroform-isoamyl alcohol (25:24:1) as described by Griffiths et al. 2000, with the following volumes and modifications: to 250 µL sample, 337.5 µl of extraction buffer were added. Samples were vortexed and transferred to Lysing Matrix 'E' tubes (MP Biomedicals) and 37.5 µL sodium dodecyl sulfate (SDS; 20% wt/vol) were added. Samples were left to incubate at 65°C for 1 hour and mixed by inversion every 20 minutes. Consequently, samples were allowed to cool and lysed in a FastPrep-24™ 5G (MP Biomedicals) bead beating system two times for 20 seconds each at maximum speed (setting 6.5). Samples were then centrifuged for 10 minutes at 6,000 rcf, 25°C. Finally, DNA was precipitated by the addition of a 0.6 time volume of 2-propanol and left at 4°C for 1 hour. DNA was pelleted by

centrifugation at 16,000 rcf for 30 minutes at 4°C. DNA was re-suspended in 50 µL of UV-treated MilliQ water. Aliquots of 1:10 and 1:100 dilutions were stored at -20°C until further processing.

Two-Step PCR Barcoding and Bacterial 16S rRNA Gene Amplicon Sequencing

Bacterial 16S rRNA gene sequences from microcosm sediment DNA extracts were amplified by using a two-step PCR barcoding approach as described by Herbold et al. 2015. For primer sequences and master mix preparations see **Appendix Table 3-4**.

Barcoded PCR products were cleaned up using a ZR-96 DNA Clean-up Kit™ (Zymo Research, Irvine, CA, USA), following the manufacturer's protocol. DNA content of PCR products was measured with a Quant-iT™ PicoGreen™ dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA USA). Amplicons were pooled in equimolar amounts and consequently sent to Microsynth (Balgach, Switzerland) to be sequenced on the Miseq System (Illumina, sequencing by synthesis). Sequence data was further processed with the pipeline by Herbold et al. 2015 to cluster reads into operational taxonomical units (OTU), herein referred to as 'phylotypes', using a 99% identity cut-off.

During qualitative gel electrophoresis following the second step PCR reaction, the PCR negative control gave a slight positive band, indicating a possible contamination. However, after sequencing of the 16S rRNA amplicons from the PCR negative control, the phylotype composition appeared highly different from the compositions found in the datasets of the sediment samples. Phylotypes with the highest counts in the PCR negative control sample deviated in relative abundances strongly from all other samples. The analysis pipeline (Herbold et al. 2015) assigned the five most abundant negative control phylotypes to the *Clostridiales*, family JTB215. Consequently, the contamination was considered to be distinguishable from the rest of the dataset. Therefore, analysis was continued with the data and the five phylotypes with the highest read counts in the PCR negative control sample were excluded from all other libraries.

Statistical Analysis

Phylotype count data converted to relative abundances was analyzed using R (R Core Team 2016) with the DESeq2 package version 1.14.1 (Love et al. 2014). In order to guarantee statistical accuracy, samples with low read numbers deviating from the rest of the dataset (total count ≤ 600) were not considered for statistical analysis.

No-substrate-control count data was used as baseline for pairwise differential relative abundance comparison against all other treatments for each given time point. Phylotypes that significantly (FDR-corrected p-value < 0.05) increased in relative abundances compared to the no substrate control were considered 'responders' to substrate additions. Relative abundance of those phylotypes identified as responders was plotted against time. Plots were then filtered manually to determine phylotypes of putative relevance for substrate degradation. Although filtering might lead to the loss of interesting data, especially about low abundance organisms comprising possible substrate specialists, these criteria were

chosen in order to facilitate the analysis and to capture responders with potentially high impact on substrate degradation due to their high relative abundances. Decision making for the filtering was based on the following rules: (1) only the top 100 responding phylotypes with the highest relative abundance were considered (**Appendix Table 9**). Within these 100 phylotypes, those responding to ≥ 3 treatments were considered for further analysis. (2). The phylotypes selected by these criteria were grouped according to their closest matching bacterial relatives on genus level as given by the pipeline by Herbold et al., 2015. From these groups *Spirochaetes* and *Clostridiales* affiliated phylotypes were chosen, since these classes re-occurred most in the set of phylotypes selected by the criteria mentioned above. In order to keep potential information about substrate specific responders, positive responders from classes *Clostridiales* and *Spirochaetes* not fulfilling criterium 2 were subsequently included into the data for analysis.

Generation of Phylogenetic Tree

A phylogenetic tree was calculated by placing query sequences into a guide tree using the Evolutionary Placement Algorithm (EPA) in RAxML (Stamatakis 2014). Briefly, reference sequences were identified by placing short reads into the main reference tree and selecting close relatives in ARB (Ludwig et al. 2004), which were then exported. Additional reference sequences were identified by *blastn* against the NCBI database. These were then aligned and a *de novo* tree was created with FastTree2 using default settings (Price et al. 2010). Query sequences were then placed onto this tree using the EPA. This tree closely resembled previously constructed trees using FastTree2, AxML, PhyML (DNA) in ARB. *Mesoplasma pleciae* was used as an outgroup for rooting the tree.

2.2.2 Determination of Biogeochemical Parameters in Sediment Microcosms

Measurement of Bacterial Metabolites by Capillary Electrophoresis (CE)

Concentrations of short-chain fatty acids (SCFAs) in each microcosm treatment were measured on a P/ACE™ MDQ Molecular Characterization System (Beckman Coulter, Brea, CA, USA). The CE contains a fused silica capillary, to which an electrical field is applied. Anions will travel through the capillary towards the positive pole depending on their charge and size. Different molecules will be detected by a UV-detector measuring molecule specific UV-light absorption at different time points according to molecule retention time. A CEOfix™ Anions 5 Kit (Analis, Suarlée (NAMUR), Belgium) targeting small anions and organic acids was used. At the UV-detector the difference in UV-absorption caused by the substitution of the UV absorbing electrolyte from the kit by the substrate was measured.

Prior to measurement, microcosm sediment samples of each treatment and time point were centrifuged at maximum settings for at least 10 minutes. Clear supernatant pore water was diluted 1:10 in CE buffer solution containing a known volume of caproate (hydroxyprogesterone hexanoate), serving as internal standard to correct for fluctuations in peak retention times. An external standard with equimolar

concentrations of SCFAs was run in parallel to each measurement plate at concentrations of 250 µM, 100 µM and 50 µM.

The peaks were integrated manually by using the 32 karat software (Beckman Coulter, Brea, CA, USA). SCFAs concentrations were calculated by comparison of sample peak areas to the external standard mixture peak areas. An ANOVA with a post-hoc Tukey (Honestly Significant Difference) Test was performed to determine timepoints and substrates with SCFA concentrations that deviated statistically significantly (adjusted p-value ≤ 0.05) from the no substrate control concentrations.

Measurement of Sulfate by Capillary Electrophoresis (CE)

The temporal concentrations of sulfate were analyzed on a P/ACE™ MDQ Molecular Characterization System (Beckman Coulter, Brea, CA, USA). The principles and procedure of anion measurements using a capillary electrophoresis system are described above. Samples were diluted 1:50 in CE buffer solution to decrease interference between sulfate and chloride peaks during the measurements. Measurements of dilutions 1:10 and 1:20 from a previous measurement run on the same samples had to be included for completion of the data set after machine failure. This was done after evaluating, if the sulfate peak was distinguishable from the chloride peak in the spectra. An ANOVA with a post-hoc Tukey (Honestly Significant Difference) Test was performed in R (R Core Team 2016) to determine time points and substrates with sulfate concentrations that deviated statistically significantly (adjusted p-value ≤ 0.05) from the no substrate control concentrations.

Measurement of Sulfide by Cline Assay

Sulfide contents in the pore water of microcosm sediment samples were determined with an assay described by Cline 1969 (as modified by Reese et al. 2011), using N,N-dimethyl-1,4-phenylenediamine sulfate, to form a Methylene Blue complex with hydrogen sulfide. Absorbance of samples was measured with an Infinite® 200 PRO Plate Reader (TECAN, Zurich Switzerland) at a wavelength of 670 nm. Concentrations of hydrogen sulfide were calculated by correlating the measured mean absorbance values of each microcosm sample triplicate to a standard mixture of known sulfide concentration. It was assumed that total headspace sulfide was lost during each time, samples were taken. This loss of sulfide was corrected for by calculating concentration of sulfide in air using a Henry constant of $9.1*10^{-4}$ (Rinker & Sandall 2000) for ambient conditions. The calculated amount of sulfide contained in the headspace was added to the original measurement in pore water. Headspace loss calculations were extrapolated for each time when microcosms had to be unsealed for measurements. An ANOVA with a post-hoc Tukey (Honestly Significant Difference) Test was performed in R (R Core Team 2016) to determine time points and substrates with hydrogen sulfide concentrations that deviated statistically significantly adjusted p-value ≤ 0.05) from the no substrate control concentrations.

2.3 D_2O Incubations: Assessing Substrate Induced Microbial Activity

2.3.1 Anoxic Incubations Using D_2O Labeling

Incubations in deuterium labeled water were performed as previously described for mouse cecum samples by Berry et al. 2015. D_2O was added to a final concentration of 50% in artificial seawater, which was prepared as described above. The artificial, anoxic seawater was added at a 3:2 ratio to anoxic Venetian sediment and homogenized by shaking. Large particles were left to settle and supernatant cell suspension was added to vials containing different substrate treatments. As a negative control for background activity signals, incubations without the addition of any substrate were incubated in 50% D_2O-artifical seawater. To capture background D_2O signals, glucose was added to the sediment incubations using H_2O instead of D_2O. Glucose was used as an easily degradable substrate to serve as positive control. For the assessment of bacterial activity induced by sulfated polysaccharides, a mix of λ-carrageenan and sulfated arabinogalactan was used. Incubations were prepared inside an anaerobic chamber, crimp-sealed and kept at a constant 20°C.

Samples were taken in the course of one week on days 0, 1, 3 and 7. Sample aliquots of 500 µL were mixed with 233 µL PBS:60% glycerol, instantly frozen on dry ice and stored at -80°C until further processing. Additionally, aliquots intended for FISH experiments were fixed with PFA (2% v/v final in PBS) for one hour at room temperature, left overnight at 4°C, washed and re-suspended 1:1 in PBS:ethanol and stored at -20°C until further processing.

2.3.2 D_2O - Raman Analysis for Determining Cell Activity

D_2O incubated samples were spotted onto aluminum slides and prepared as described by Berry et al. 2015. Cell spectra were acquired for single bacterial cells at the range of 400 – 3,200 cm^{-1} on a LabRAM HR Evolution; HORIBA Scientific Raman Spectrometer. Instrument and laser settings were used as previously described (Berry et al. 2015; Eichorst et al. 2015) with a grating of 300 groove/mm.

For each treatment, 30 to 50 single cell spectra were captured and aligned by the phenylalanine peak (1003 cm^{-1}) and baselined via polynomial fitting. The degree of substitution of C-H bonds (2800 – 3100 cm^{-1} peak) by a C-D bond (2040-2300 cm^{-1} peak) was calculated as %CD according to a script by Berry et al. 2015.

Two-Step Catalyzed Reporter Deposition Fluorescence In Situ *Hybridization (CARD-FISH)*

Specific ‘two-step’ FISH probes (Wasmund et al., in-preparation) were designed for *Clostridiales* phylotypes 2 and 95 and *Spirochaetes* phylotypes 8 and 92 (**Appendix Table 7**), which showed significant response to substrate addition as determined by the 16S rRNA gene sequencing. CARD-FISH was performed as described by Pernthaler et al. 2002 on randomly selected microcosm samples and consequently on D_2O incubations described above. The experiment was implemented on epoxy resin coated microscope slides (Marienfeld-Superior, Lauda-Königshofen, Germany) or as preliminary tests on Isopore™ GTTP Membrane Filters (0.2 µm; Merck Millipore, Billerica, MA, USA).

Endogenous peroxidases were inactivated by incubation in 0.1% H_2O_2 for 2 mins in PBS. Since phylotype data indicated Gram-positive target cells, permeabilization was done with lysozyme (10 mg ml^{-1} in TE buffer) and consequent treatment with achromopeptidase (60 U ml^{-1}) in PBS for 30 minutes for each treatment. After the first hybridization with specific probes and subsequent washes to remove unbound probes, an 'anti-adapter-HRP' probe was hybridized to the sample in a second step using 1 µl probe per 300 µL CARD hybridization buffer and kept at 46°C for 2.5 to 3 hours. Afterwards CARD-reaction was performed (Pernthaler et al. 2002), samples were back stained with DAPI and kept at 4°C and in the dark until analysis at a Leica TCS SP8 X Confocal Laser Scanning Microscope (Leica Microsystems, Wetzlar, Germany).

RESULTS

3.1 MOLECULAR BIOLOGICAL ANALYSES OF ANOXIC MICROCOSMS

3.1.1 Bacterial Community Response to Amendment of Sulfated Polysaccharides in Anoxic Microcosms

Various sulfated polysaccharides were amended to anoxic, marine sediment microcosms to serve as model compounds for the investigation of anaerobic degraders of sulfated polysaccharides. Types of treatment and characterization of the substrates are shown in **Table 1**. Chemical structures of substrates can be found in **Appendix Figure 1**. Phylotypes that increased in relative abundances throughout the experiment were considered to be involved in the degradation of the model compounds. In order to identify those phylotypes, bacterial 16S rRNA genes were amplified and sequenced via the Illumina MiSeq platform. Overall, 4,015 phylotypes were detected within the microcosm samples. To monitor the effect of substrate additions on distinct phylotypes, the relative abundances of phylotypes in amended microcosms were compared to relative abundances in no substrate controls at the corresponding time points over eighteen days of incubation. In total, 128 phylotypes deviated significantly (RDF-corrected p-value < 0.05) and positively from the no substrate control during one or more time points after amendment (**Appendix Table 10**). Examining all phylotypes with a significant positive response to substrate addition was beyond the scope of this thesis and therefore only the top 100 phylotypes with the highest relative abundance were considered for further analysis (**Appendix Table 9**). In order to facilitate further analysis, sequencing data was filtered manually as described in the methods section above, determining the phylotypes most likely to correspond to substrate degraders.

At the start of the microcosm experiment (day 0), the sediment community was dominated by members of the *Proteobacteria* (43.55% relative abundance) followed by the *Firmicutes* (13.14% relative abundance) as second most abundant group (**Figure 1**). The *Bacteroidetes* were the third most abundant group on day 0 (9.91% relative abundance). About 6.6% of all phylotypes on day 0 had a relative abundance ≥ 0.1 %. Other phyla showing considerable relative abundances in the incubations were *Fusobacteria* and *Spirochaetes*. By the second day of incubation, the *Firmicutes* presented the most relevant phylum throughout all treatments, including the no substrate control with the exception of κ-carrageenan treatments. Here, *Firmicutes* became the most abundant phylum only at day 11. In general, the relative abundance of *Firmicutes* increased over time, with the exception of the molybdate-blocked treatment, where a steady decrease in *Firmicutes* relative abundance was observed following day 2. In the substrate mix, the relative abundance of *Firmicutes* decreased between day 11 and day 18.

Bacteroidetes and *Spirochaetes* responded differently to different substrate amendments and like that showed a more substrate-specific response than the members of the *Firmicutes* phylum (**Figure 1**). While *Bacteroidetes* decreased in relative abundance in the substrate mix treatment as well as microcosms treated with fucoidan and κ-carrageenan, relative abundances increased between day 11 and day 18 in microcosms treated with sulfated arabinogalactan, chondroitin sulfate, λ-carrageenan and

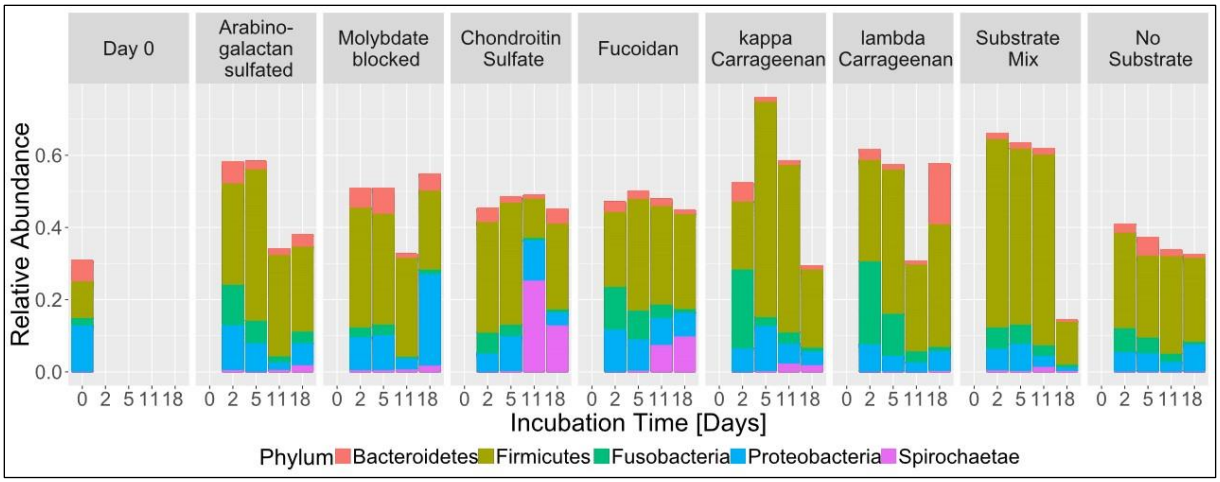

Figure 1. Community response to substrate treatment on phylum level. Day 0 relative abundances apply to all treatments. Plot was generated using R (R Core Team 2016) with the phyloseq package version 1.19.1 (McMurdie & Holmes 2013). Phylotypes with relative abundances < 10^{-5} were not shown.

the molybdate-blocked mix. *Spirochaetes* relative abundances did not show any distinct trend in the no substrate control, while showing a moderate increase for most other treatments. However, *Spirochaetes* relative abundances increased steeply after day 5 for treatments of chondroitin sulfate and fucoidan.

3.1.2 Selected Phylotype Responses to Amendment of Sulfated Polysaccharides in Anoxic Microcosms

Phylotypes 2 and 95 responded significantly and positively to all individual substrates, as well as the substrate mix and the molybdate-blocked mix, during at least one time point (**Figure 2**). Sulfated arabinogalactan, chondroitin sulfate and λ-carrageenan, as well as both the molybdate-blocked mix and the substrate mix led to an early increase of relative abundance in phylotype 2. Phylotype 2 and 95 were both relatively enriched after eighteen days as compared to the no substrate control. Amendment with sulfated arabinogalactan and κ-carrageenan led to a steep increase in relative abundance of both phylotypes during the first eleven days (**Figure 2A**, **C**). However, after eleven days, relative abundances decreased rapidly but remained generally higher than in the respective time points of the no substrate control. Regarding mixed treatments, both mixes (molybdate-blocked and substrate mix) showed similar effects on the relative abundance of phylotype 2 and 95, with the molybdate-blocked mix leading to a more rapid decrease after eleven days of incubation (**Figure 2B, D**).

The maximum relative abundance of phylotype 2 was observed between five and eleven days after amendment of sulfated arabinogalactan (**Figure 2A**). It increased from about 1% at day 0 to about 21% by day 11. Phylotype 95 reached its maximum relative abundance of roughly 21% between five and eleven days after amendment of κ-carrageenan (**Figure 2C**).

Both phylotypes 2 and 95 were affiliated to the phylum *Firmicutes*, order *Clostridiales* by *blastn* (Altschul et al. 1990) against the NCBI database (National Center for Biotechnology Information 1988). On the genus level, phylotype 2 showed a 96% 16S rRNA gene sequence similarity to the closest cultivated relative *Wukongibacter baidiensis strain DY30321* and a 95% 16S rRNA gene sequence

similarity to *Alkaliphilus peptidifermentans strain Z-7036. W. baidiensis strain DY30321* presented also the closest cultivated relative to phylotype 95 (97% 16S rRNA sequence identity). However, phylotypes 2 and 95 showed a 99% 16S rRNA gene sequence identity to one another, thus very likely corresponded to the same genus (Yarza et al. 2014).

Phylotype 15 responded positively and significantly to amendments of chondroitin sulfate, fucoidan and κ-carrageenan, as well as both the substrate mix and the molybdate-blocked mix, during at least one time point (**Figure 3**). Similarly to the observation for phylotypes 2 and 95, addition of κ-carrageenan caused a noticeable increase in relative abundance of phylotype 15 during the first eleven days of incubation, followed by a subsequent decrease. The pattern was similar but more distinct for samples treated with the substrate mix. However, the relative abundance of phylotype 15 remained generally lower in the molybdate-blocked mix than in the substrate mix treatment and reached a plateau after eleven days, indicating a negative effect of inhibition of sulfate reduction activity in this phylotype (**Figure 3B**). Like phylotype 2 and 95, phylotype 15 was assigned to the *Clostridiales* by *blastn*. It showed a 99% 16S rRNA sequence identity to *Clostridium propionicum strain JCM 1430*.

Spirochaetes affiliated phylotype 8 started to increase rapidly five days after the amendment of chondroitin sulfate (**Figure 4A**). After elven days it reached a relative abundance about twenty times higher than for all other treatments. In microcosms amended with sulfated arabinogalactan, fucoidan and κ-carrageenan as well the substrate mix treatment and molybdate-blocked treatment, phylotype 8 responded with a steady increase in relative abundance. When treated with κ-carrageenan and the substrate mix, there was a noticeable decrease after eleven days of incubation (**Figure 4A, B**). *Blastn* revealed a 99% 16S rRNA sequence identity of phylotype 8 to *Sphaerochaeta multiformis strain MO-SPC2*, suggesting it corresponded to the same genus. Phylotype 92, whose closest cultivated relative (96% 16S rRNA gene sequence identity) was also *S. multiformis strain MO-SPC2*, showed increased relative abundances after five days for treatments of κ-carrageenan and fucoidan.

For phylotype 5, which showed a 99% 16S rRNA sequence identity to *Clostridium saccharobutylicum* strain NCP 262 via *blastn*, relative abundances increased very strongly within two to five days when amended either with κ-carrageenan or the substrate mix. The response was transient, with phylotype 5 relative abundances dropping to the base levels found in the no substrate control within eleven days of incubation (**Appendix Figure 2A, B**).

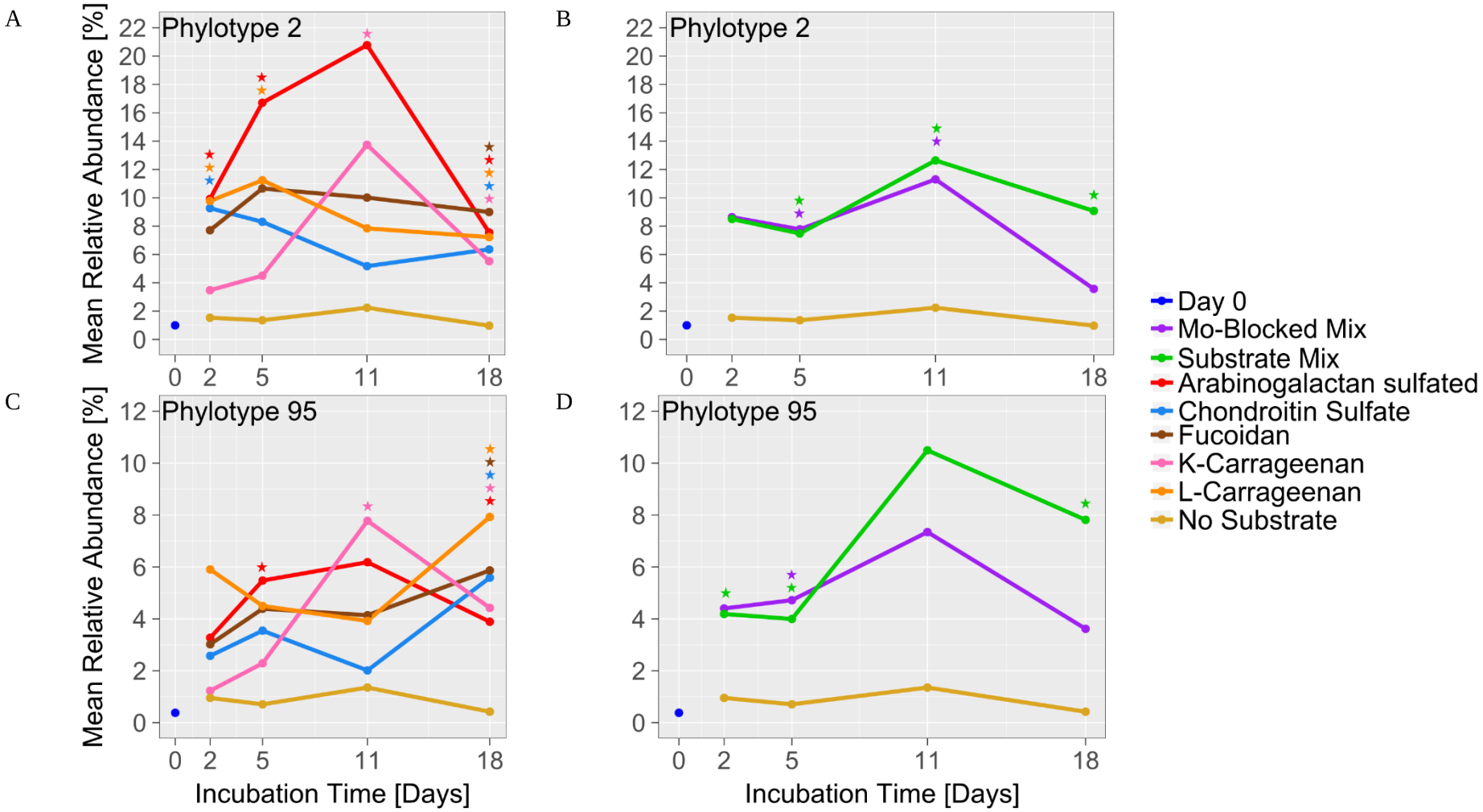

Figure 2. Mean relative abundance of *Firmicutes* (*Wukongibacter*) phylotypes responding positively to all amended substrates. Significant (RDF-corrected *p*-value < 0.05) deviation from no substrate control during a time point is indicated by a (★)-symbol. Top panels show response of phylotype 2 to individual sulfated polysaccharides (A) and substrate mix compared to molybdate-blocked mix (B). Bottom panels show response of phylotype 95 to individual sulfated polysaccharides (C) and substrate mix compared to molybdate-blocked mix (D). Substrate treatments where relative abundances did not deviate significantly from the no substrate control are not shown.

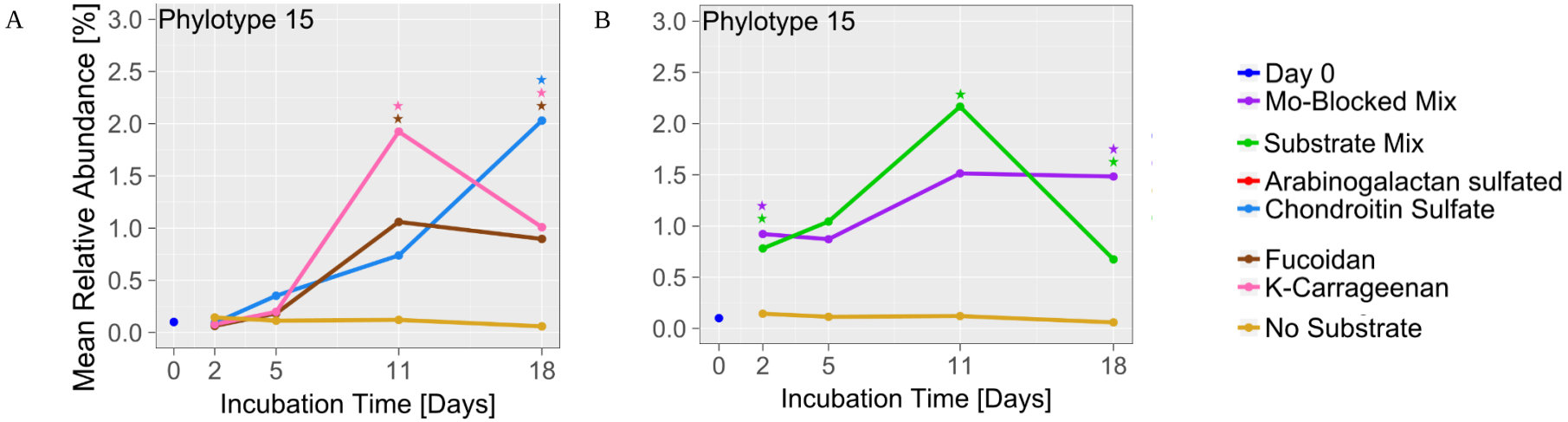

Figure 3. Mean relative abundance of *Firmicutes (Clostridium)* phylotype 15 responding to substrate amendment. Significant (RDF-corrected *p*-value ≤ 0.05) deviation from no substrate control during a time point is indicated by a (★)-symbol. (A) Response to individual sulfated polysaccharides. (B) Response to substrate mix and molybdate-blocked mix. Substrate treatments where relative abundances did not deviate significantly from the control are not shown.

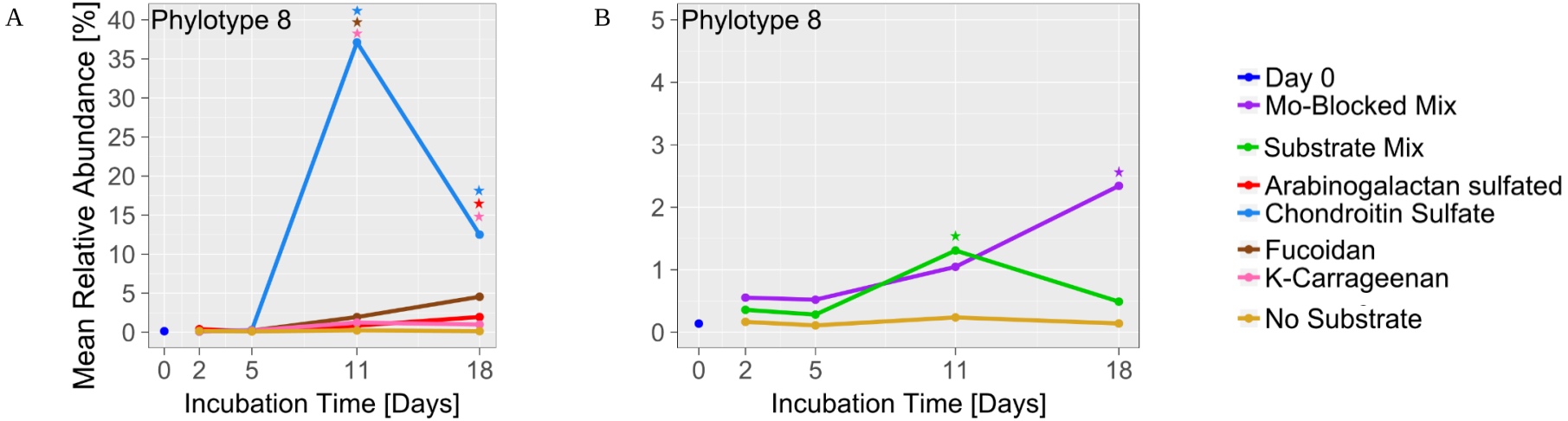

Figure 4. Mean relative abundance of *Spirochaetes* (*Sphaerochaeta*) phylotype 8 responding to substrate amendment. Significant (RDF-corrected p-value < 0.05) deviation from no substrate control during a time point is indicated by a(★)-symbol. (A) Response to individual sulfated polysaccharides. (B) Response to substrate mix compared to molybdate-blocked mix. Substrate treatments where relative abundances did not deviate significantly from the control are not shown.

3.1.3 Phylogenetic Placement of Significantly Responding Phylotypes

In order to examine the phylogenetic identity of phylotypes responding to the sulfated polysaccharide additions described above, phylogenetic analyses were performed. A phylogenetic tree was calculated for relatives of highly responsive phylotypes 2, 95, 8 and 92 as well as for selected *Clostridiales* and *Spirochaetes* phylotypes that showed distinct trends for fewer substrates in the plotted differential comparisons with the no substrate control, and the sequences of the phylotypes obtained in this study were inserted into the tree (**Figure 5**).

Within the resulting phylogenetic tree, two main groups were distinguishable, a *Firmicutes* clade and a clade composed of *Spirochaetes* sequences. Phylotypes 8 and 92 both fell within the *Spirochaetes* group containing an uncultured *Spirochaetes* bacterium from an oil-polluted subtidal sediment and *Sphaerochaeta multiformis*, isolated from subseafloor sediments. Within the *Firmicutes*, phylotype 2 and 95 made up a distinct clade with *Wukongibacter baidiensis* and *Alkaliphilus peptidifermentans* presenting the closest relatives. Phylotype 5 was shown to be closely related to *Clostridium saccharobutylicum* (99% 16S rRNA sequence identity via *blastn*). The phylogenetic tree showed that phylotype 33 fell onto a clade with *Oceanirhabdus sediminicola* as closest cultivated relative. *Blastn* showed only a 94% 16S rRNA gene sequence similarity between phylotype 33 and *O. sediminicola*. Phylotype 15 was positioned on a clade with *Clostridium neopropionicum* and *C. propionicum*.

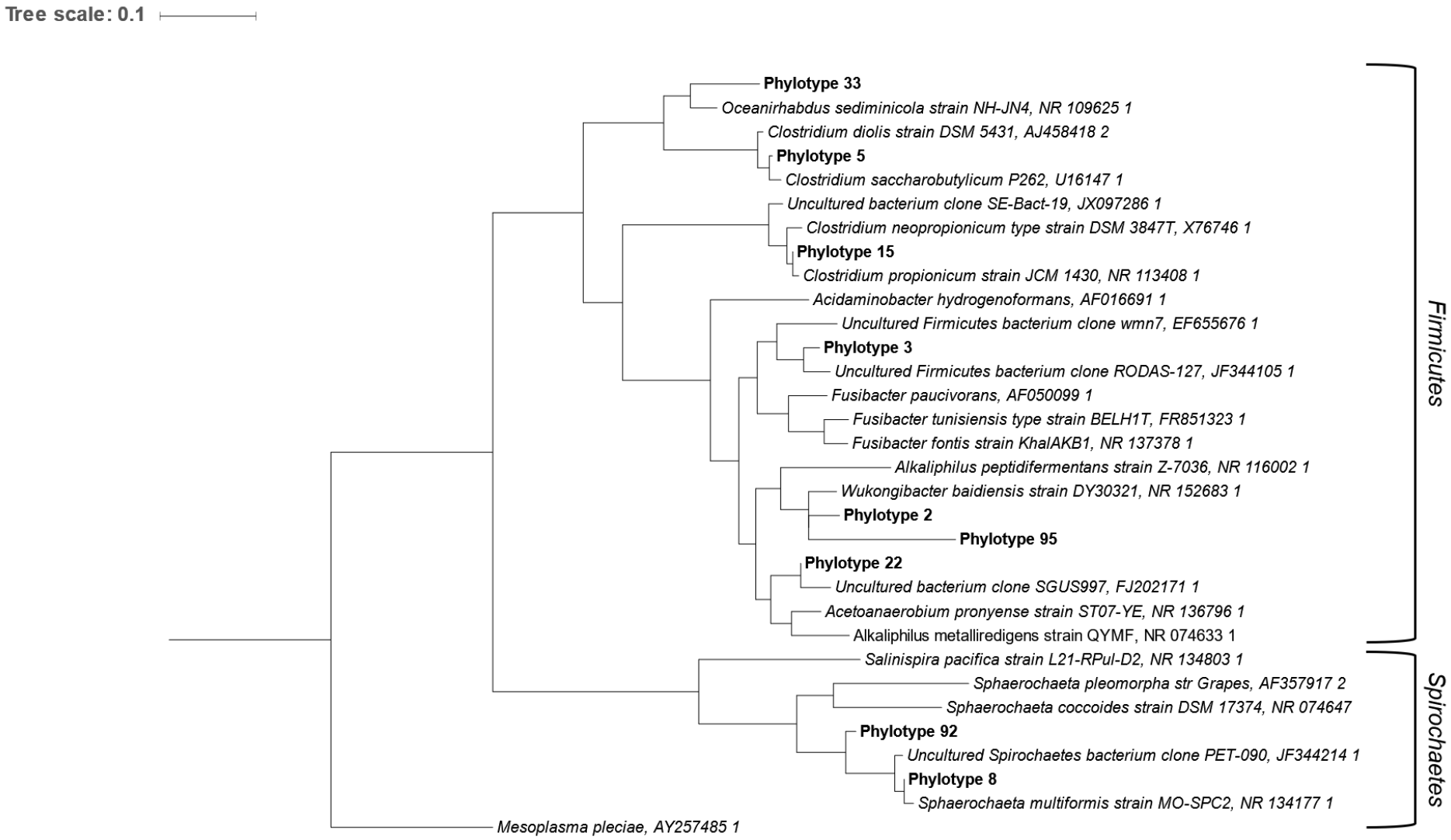

Figure 5. Phylogenetic tree for selected positively responding phylotypes based on 16S rRNA genes. A guide tree was calculated with FastTree2 (Price et al. 2010) and by using the Evolutionary Placement Algorithm (EPA) of RAxML (Stamatakis 2014). *Mesoplasma pleciae* was selected as outgroup for rooting the tree.

3.2 Monitoring of Biogeochemical Parameters in Sediment Microcosms

3.2.1 Temporal Dynamics of Hydrogen Sulfide Induced by Substrate Addition

Over the course of 18 days, the development of hydrogen sulfide (H_2S) concentrations in microcosm sediment pore water was monitored (**Figure 6**). Overall, H_2S concentrations showed different trends depending on the substrate amended during the first two to five days. After that however, H_2S concentrations fell below initial concentrations for all treatments, possibly due to re-oxidation of sulfides or an increasing loss of the effect of zinc acetate treatment for fixation of sulfide towards the end of the experiment. Comparing different sulfated polysaccharide amendments, some variations of the temporal dynamics of H_2S could be observed.

In the no substrate control, H_2S concentration decreased steadily by about 50% from day 0 to the end of the experiment. In contrast, treatment with fucoidan and chondroitin sulfate led to an initial increase of H_2S concentrations. Two days after addition of fucoidan, the concentration of H_2S in sediment pore water rose to about 8.5 mM from an initial concentration of 6.18 mM and was about 3.5 mM higher than in the untreated microcosm on the respective day. Within five days of incubation with fucoidan as well as with chondroitin sulfate, H_2S concentrations fell back to match the day 0 concentration. Consequently, the H_2S concentration decreased further to a value around 2.3 mM in fucoidan treated microcosms and 1.5 mM in chondroitin sulfate treated microcosms until day 11. This corresponded to H_2S concentrations below those in the no substrate control at the respective day. Following day 11, H_2S levels plateaued for fucoidan and chondroitin sulfate amended samples until the end of the experiment.

When treated with sulfated arabinogalactan, H_2S in the pore water of the microcosms decreased already during the first two days of incubation. Until day 5, H_2S concentrations in arabinogalactan treated microcosms followed a trend similar to the no substrate control. After eleven days, H_2S concentrations fell more rapidly in arabinogalactan treatments than in the no substrate control to reach a concentration of about 1.1 mM at the final time point. Similarly, the H_2S concentration in κ-carrageenan-treated samples dropped below the concentration in the control between two and five days of incubation. Compared to all other treatments, κ-carrageenan amendment led to the steepest decrease in H_2S concentration with a minimum concentration of about 0.5 mM after eleven days.

When treated with the substrate mix, there was no detectable change in H_2S concentration for the first two days, suggesting equilibrium conditions between H_2S production and oxidation. Similar to patterns observed for the other treatments, the concentration decreased after the second day. Inhibition of sulfate reduction activity in the substrate mix did not cause a significantly different H_2S trend from the substrate mix without molybdate addition even for the later time points.

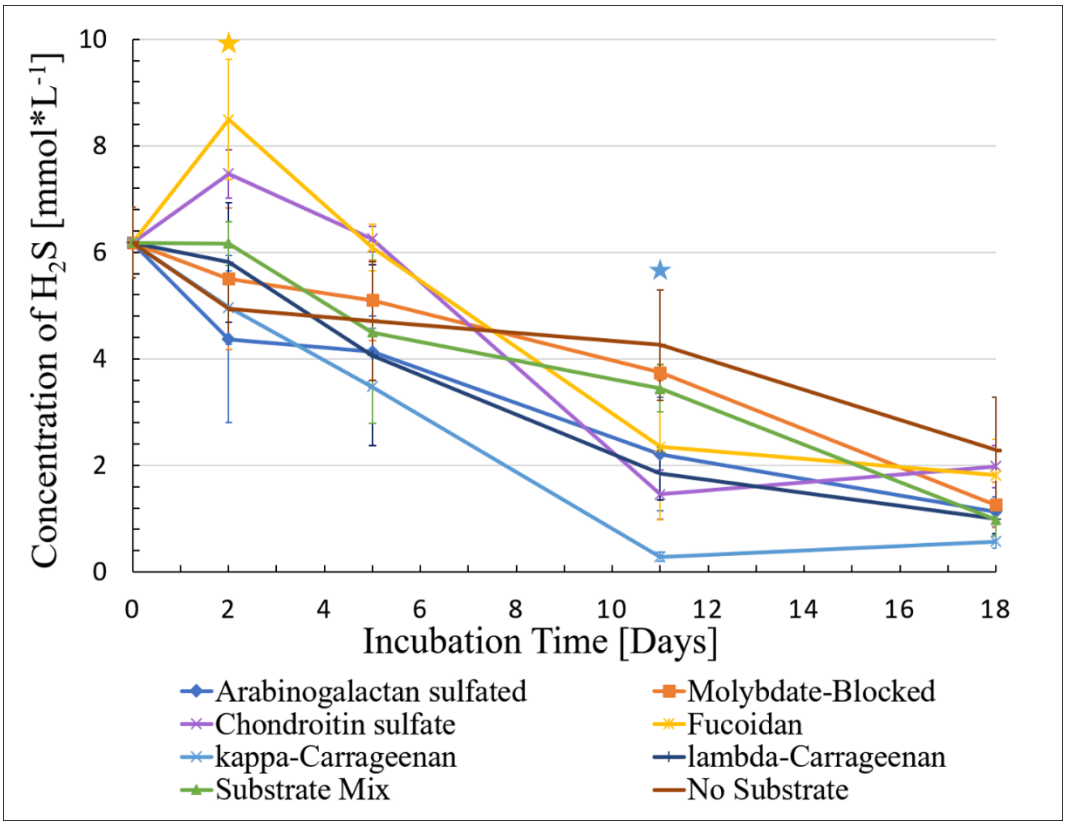

Figure 6. Dynamics of total hydrogen sulfide concentrations induced by substrate addition determined via spectrophotometric measurements. Day 0 concentration corresponds to the background hydrogen sulfide concentration in the artificial seawater medium. Significant (adjusted *p*-value < 0.05) deviation from no substrate control during a time point is indicated by a (★) -symbol.

The development of H_2S concentration in microcosms amended with λ-carrageenan was comparable to that for arabinogalactan and κ-carrageenan treated samples with the concentration falling with time to reach a final concentration of about 1 mM after 18 days. Throughout the duration of the experiment, only treatments of fucoidan, chondroitin sulfate and the substrate mix led to higher H_2S concentrations than in the no substrate control. All positive H_2S trends happened during the first two to five days of the incubations. However, H_2S concentrations only deviated statistically significantly (adjusted *p*-value ≤ 0.05) from the no substrate control for treatments of fucoidan at day 2 and κ-carrageenan at day 11.

3.2.2 Temporal Dynamics of Sulfate Induced by Substrate Addition

Concentration of sulfate in sediment pore water was measured over the course of 18 days in order to determine substrate induced concentration changes over time (**Figure 7**). Of the initial 28 mM sulfate added to the artificial seawater, 23.88 mM were detected in the day 0 pore water samples, suggesting a moderate underestimation of dissolved sulfate concentrations by the capillary electrophoresis. Due to measurement/machine failure, sulfate concentrations could not be determined for chondroitin sulfate and λ-carrageenan treatments.

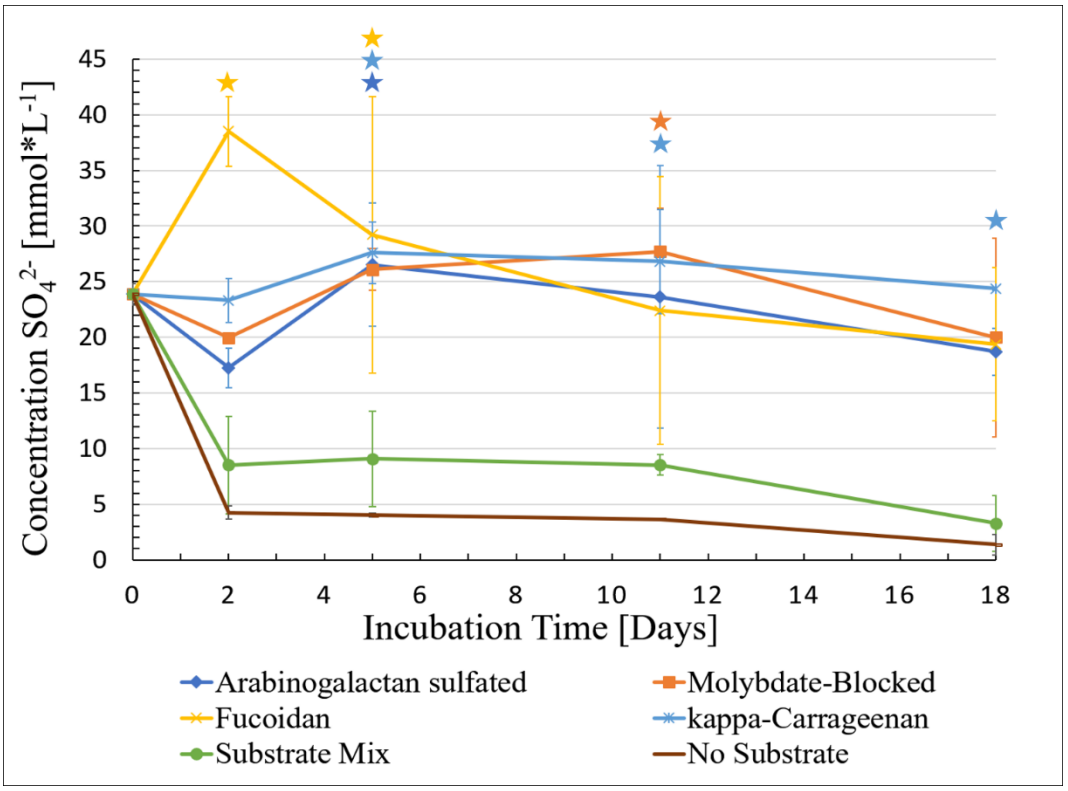

Figure 7. Dynamics of total sulfate concentrations induced by substrate addition measured via capillary electrophoresis. Day 0 concentration corresponds to the background sulfate concentration in the artificial seawater medium. Significant (adjusted *p*-value < 0.05) deviation from no substrate control during a time point is indicated by a (★) -symbol.

Response of sulfate concentration to substrate addition in sediment pore water differed in between individual treatements. However, independent of the amended substrate, sulfate concentrations remained higher than those of the no substrate control during any sampled time point. After five days, sulfate concentrations plateaued for all treatments suggesting that all relevant biogeochemical processes induced by substrate addition happened during the earlier time points. Amendment with fucoidan led to an increase in sulfate concentration of about 15 mM as compared to day 0. The initital increase in sulfate was followed by a decrease below 20 mM sulfate after eighteen days. With the exception of fucoidan, all other treatments showed a decrease of sulfate concentrations during the first two days of incubation. However, for κ-carrageenan-treated samples, concentration decrease appeared negligible. A steep sulfate concentration decrease was observed for samples treated with the substrate mix with a concentration of 8.5 mM at day 2. In the no substrate control, the decrease of sulfate was steeper than in all other treatments, with sulfate levels falling drastically below 5 mM within just two days. After two days, sulfate concentration started to re-approach initial sulfate levels around 20 to 25 mM for microcosms amended with arabinogalactan, κ-carrageenan and the molybdate-blocked mix. In samples treated with the substrate mix, sulfate concentrations stayed well below this value and were more comparable to the no substrate control values. At the final time point sulfate concentration of substrate mix microcosms was at 2.3 mM.

3.3 TEMPORAL DYNAMICS OF SHORT-CHAIN FATTY ACIDS IN ANOXIC MICROCOSMS

Short-chain fatty acids (SCFAs) were measured during several time points of the microcosm experiment, in order to estimate metabolic response of the bacterial community to substrate addition (**Figure 8-9**). With the exception of acetate, initial SCFA concentrations were too low to be distinguishable from background noise.

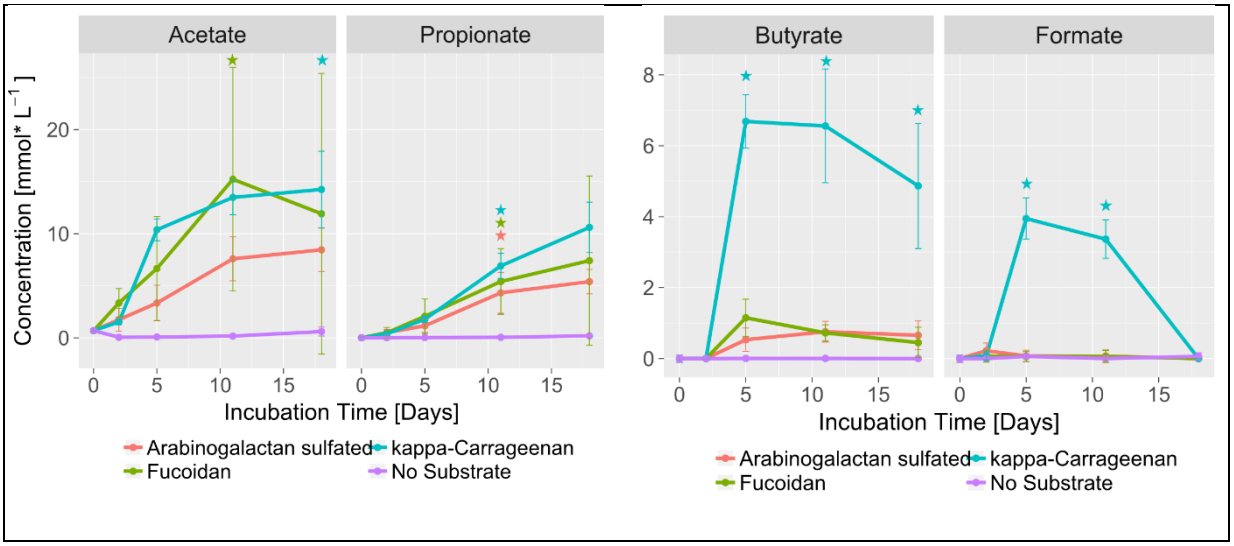

Figure 8. SCFA temporal dynamics as response to individual polysaccharide amendment. Significant (adjusted *p*-value < 0.05) deviation from no substrate control during a time point is indicated by a (★) -symbol.

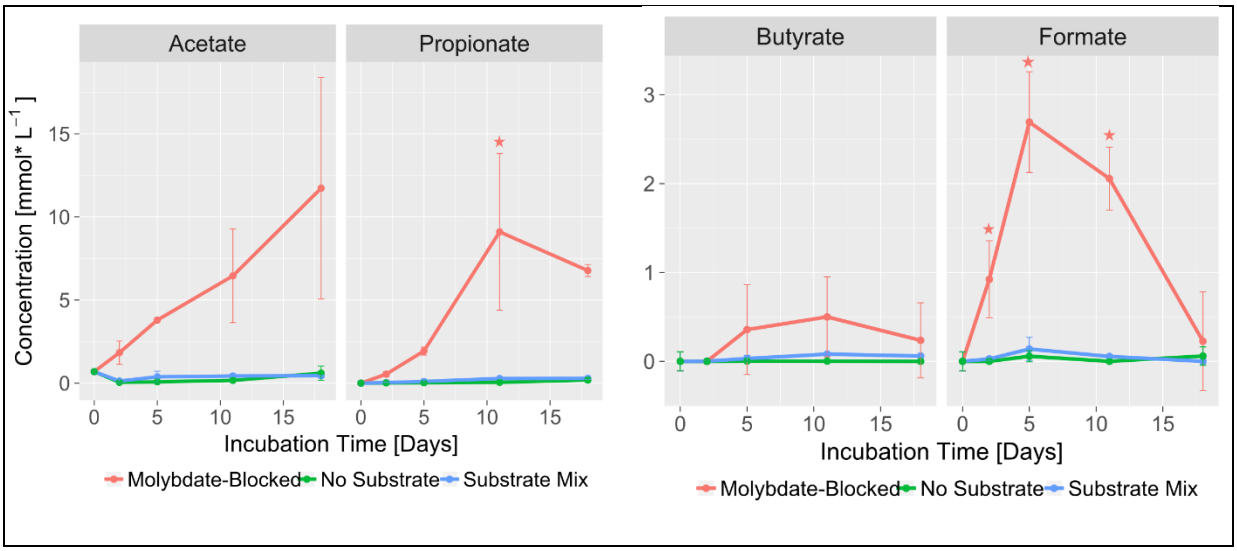

Figure 9. Comparison of SCFA levels in pore water of microcosms treated with substrate mix and molybdate-blocked mix with the no substrate control. Significant (adjusted *p*-value < 0.05) deviation from no substrate control during a time point is indicated by a (★) -symbol.

Independent of the treatment, SCFA concentrations increased during the first five to eleven days, while almost no change in SCFA levels could be observed in the no substrate control.

3.3.1 Acetate

At the start of the microcosm experiment, 0.7 mM acetate were present in sediment porewater (**Figure 8-9**). In the untreated control, the acetate concentration decreased after day 2 and remained between 0 and 0.16 mM. Addition of individual sulfated polysaccharides generally led to the increase and accumulation of acetate throughout the duration of the experiment. The maximum increase of acetate concentrations was observed for fucoidan-treated microcosms, which reached about 14 mM acetate after eleven days of incubation. Similar concentrations were produced through addition of κ-carrageenan after 18 days.

A clear difference could be seen when comparing the acetate concentration of the molybdate-blocked-mix and the substrate mix microcosms (**Figure 9**). While both consisted of the same sulfated polysaccharides, the molybdate-blocked mix caused an increase of acetate in the water, whereas the substrate mix showed acetate levels remaining below 1 mM.

3.3.2 Propionate, Butyrate and Formate

SCFAs propionate, butyrate and formate responded most strongly to the amendment of κ-carrageenan (**Figure 8**). Butyrate increased from 0 to about 7 mM within the first two days. Formate and Propionate increased similiarly when either κ-carrageenan or the molydated-blocked mix were amended to the microcosm. In correspondence to what had been observed for acetate, addition of the substrate mix led to almost no deviation from the no substrate conrol in terms of SCFA production.

3.4 Raman Microspectroscopy Analysis of D_2O Incorporation After Substrate Addition

It was investigated, if the amendment of sulfated polysaccharides induced bacterial activity on the single-cell level. This was done by a Raman-microspectroscopy analysis of cells from incubations in 50% deuterium labeled seawater, amended with a mix of sulfated arabinogalactan, λ- and κ-carrageenan. For this thesis, this approach was modified from soil and gut based methods for the use on marine sediment samples (see Material and Methods). This was therefore the first test of this approach with marine sediments in our laboratory. The amount of substitiution of C-H bonds by C-D bonds was measured as %CD. Values above 10% CD were taken as indicator for strong cell activity as suggested by Eichorst et al. 2015.

A slight increase in activity was seen in the no substrate control, which represented the background activity of the sediment community (**Figure 10**). However, although the %CD increased over time in the majority of measured cells, the activity in the no substrate control did not surpass 10% throughout the experiment.

No significant activity increase was noticable after the first day of incubation in any of the treatments. However, single cell activity of 55.6% of all measured cells surpassed 10% CD in the glucose treated positive control after three days and remained at comparable levels until the final measurement time

point. Samples treated with the substrate mix showed a more moderate increase in %CD until day 3. By day 7 of the experiment 62.3% of all measured cells surpassed the 10% CD threshold as opposed to 61.2% in the postive control.

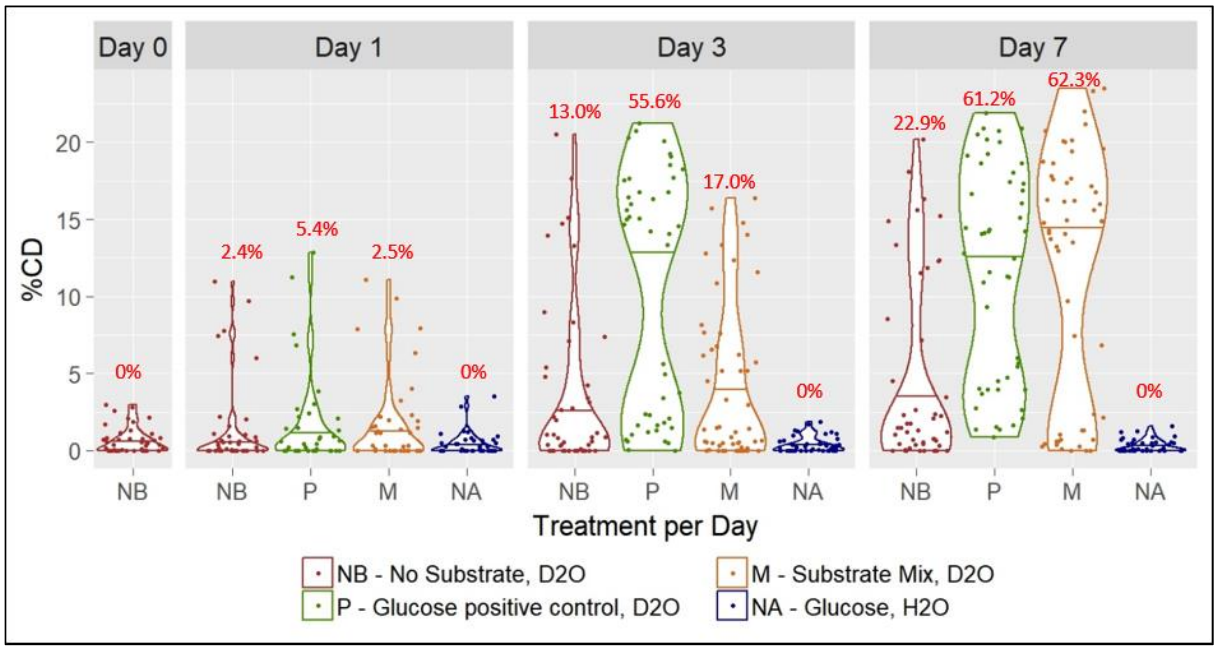

Figure 10. Development of cell activity after substrate amendment as indicated by %CD. Depiction of increasing shift in deuterium labeling intensity and number of labeled cells given by measurement density. Per treatment between 30 and 50 cells were measured. Horizontal lines indicate the median. (NA: Negative Control A, NB: Negative Control B, P: Positive Control, M: Mix) Red numbers indicate the percentage of cells above the 10%CD threshold per time point and treatment.

3.5 Two-Step CARD-FISH

Two-step CARD-FISH was performed with probes specific for *Firmicutes* (*Wukongibacter*) phylotypes 2 and 95, as well as for *Spirochaetes* (*Sphaerochaeta*) phylotypes 8 and 92. The aim of this experiment was to check the applicability of this method and the designed probes, as well as a consequent combination of this approach with D_2O labeling and Raman-microspectroscopy to confirm 'activity' in taxa identified by 16S rRNA gene sequencing. Preliminary tests were done on epoxy resin coated microscope slides with randomly selected sediment samples from the original microcosm setup, described in the method section of this thesis. The microcosm samples were labeled with DAPI and a mix of the specific probes for *Firmicutes*-phylotype 2 and 95. Analysis under a laser-scanning microscope showed rod-shaped cells labeled by the probe-mix (**Figure 11**).

Additionally, two-step CARD-FISH was done for samples from the D_2O incubation with sulfated polysaccharide amendments on microscope slides and membrane filters. Membrane filters should allow for the consequent detachment of cells from the filters and sorting via Raman-microspectroscopy to determine active, labeled cells to be used for genomic analysis. However, only *Spirochaetes* phylotype-specific probes gave a clear signal under the laser-scanning microscope on slides (**Figure 12**).

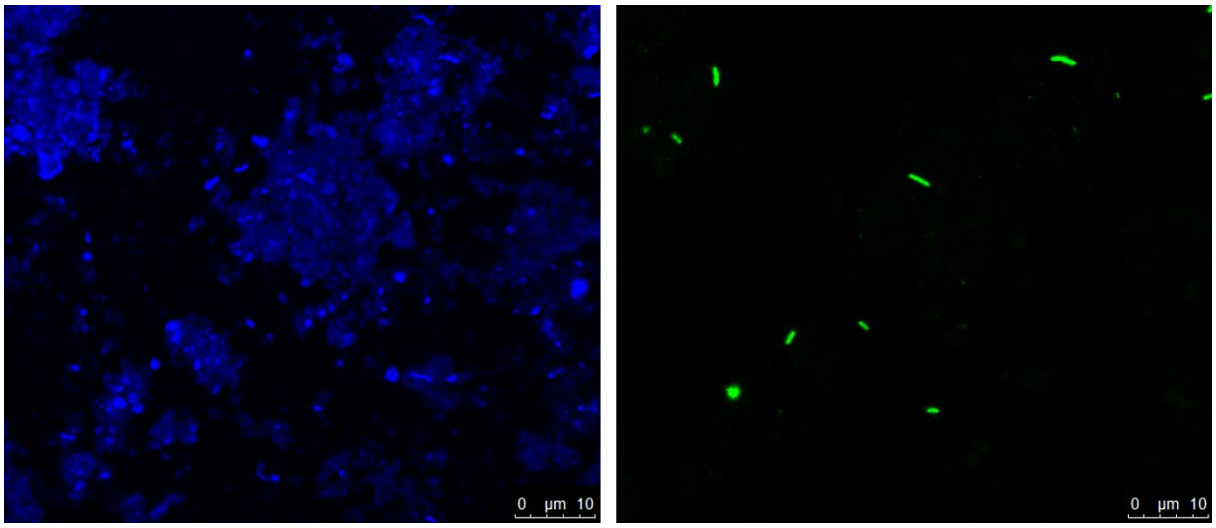

Figure 11. CARD-FISH image of microcosm sediment stained with DAPI (blue) and a mix of phylotype 2 and 95 specific FISH probes (green).

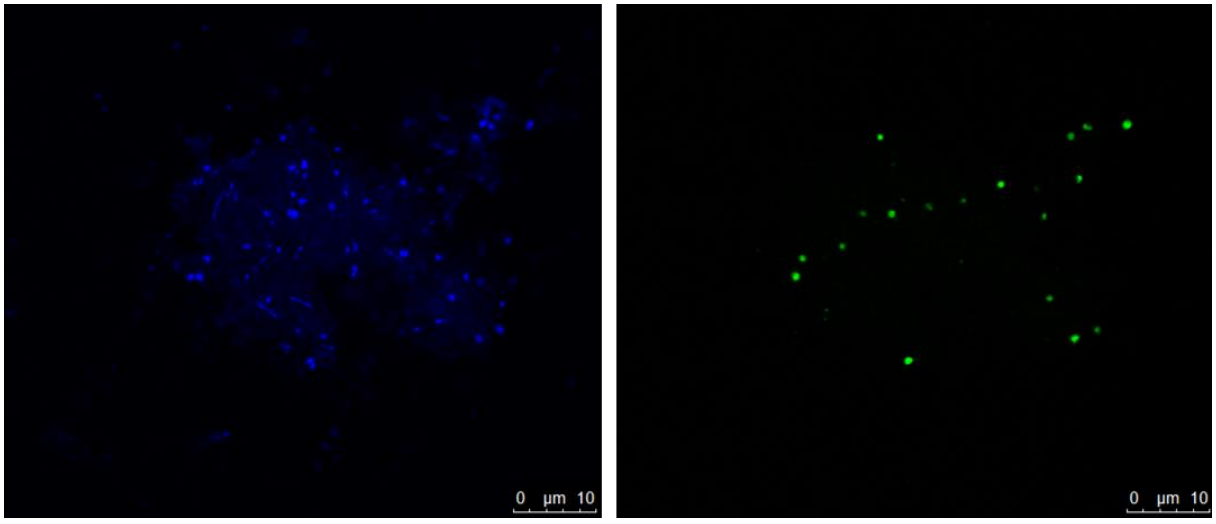

Figure 12. CARD-FISH image of D_2O incubations with substrate mix amendment, stained with DAPI (blue) and a mix of phylotype 8 and 92 specific FISH probes (green).

CARD-FISH on membrane filters only showed usable results for unlabeled microcosm samples. Coccoid cells below a diameter of 2 µm were labeled by the mix of specific probes for phylotypes 8 and 92 on the filter. When the *Wukongibacter*-phylotype specific-probes were used on samples of the D_2O incubations, no specific fluorescence signal was found although high abundance of these phylotypes was evident from both sequencing data and CARD-FISH on microcosm sediment samples. This indicated a deviation of the bacterial community of the D_2O incubation from that of the microcosm sediment. A inhibition of microbial metabolism by 50% D_2O cannot be excluded. Sequencing data from D_2O incubations could give further information on possible community differences or biased growth in one set-up or the other, but was beyond the scope of this thesis.

DISCUSSION

Response of Clostridiales *Phylotypes Suggests Their Involvement in Sulfated Polysaccharide Dissimilation*

In the past, the anaerobic utilization of organosulfur compounds has been investigated mainly for low-molecular-weight compounds like taurine, cysteate (Visscher et al. 1999; Lie et al. 1999) or isethionate (Lie et al. 1996). So far, the desulfonation of high-molecular-weight (HMW) organosulfur compounds has been widely overlooked in anoxic marine sediments. Complex HMW organosulfur compounds from diagenetic (e.g. Berner & Raiswell 1983; Anderson & Pratt 1995; Vairavamurthy & Schoonen 1995) or biological sources (e.g. Pomin & Mourão 2008; Jiao et al. 2011) are abundant in marine sediments and their degradation might therefore be of high environmental impact to biogeochemical cycles and the ecology of microorganisms in marine sediments. This research therefore aimed to investigate the identities and activities of microorganisms in marine sediments that may contribute to the degradation of sulfated polysaccharides.

This thesis shows that various phylotypes within the bacterial community of the investigated marine sediment responded with temporal changes in relative abundances when presented with natural sulfated polysaccharides. This indirectly indicated catabolism of these compounds, whereby substrate additions caused changes in relative abundances of certain populations. The increase in relative abundances of certain bacterial groups in response to substrate amendment gave a first indication for their involvement in the degradation of the sulfated polysaccharides, although it must be noted that some or many of these populations were likely indirectly stimulated by utilizing degraded products, or fermentation products, for instance. Considering this, most phylotypes discussed here were the ones that responded fastest and most drastically, and may therefore be more likely to be involved in the initial degradation of the added substrates. Although specific chemical data regarding the sequence or extent in which the sulfate groups were cleaved off the molecules is not available, one can assume that the complex HMW model organosulfur compounds were indeed desulfonated. For instance, λ-carrageenan is ‘heavily’ sulfated, whereby each monosaccharide is sulfated (see **Appendix Figure 1**). Therefore, any catabolism of this molecule must have required desulfonation prior to catabolism of the individual saccharides. Indeed, λ-carrageenan stimulated a strong response, indicated by changes in the structures of the bacterial communities. Similarly, fucoidan is highly sulfated and led to strong responses in terms of relative abundance increases of several phylotypes and sulfate/sulfide production. Amendment of the lesser sulfated κ-carrageenan (one sulfate moiety per disaccharide) also resulted in significant responses in several members of the bacterial community. In any case, it is stated here that all data presented so far would need to be confirmed or supported with information from other approaches, e.g., isolation of the strains, stable isotope probing or genomics.

The responsiveness of *Firmicutes* (*Wukongibacter*) phylotypes 2 and 95 to all amended substrates made them interesting for further investigation. One must, however, consider that phylotypes with fewer or only one potential substrate may have also been of interest. In fact, those might include true substrate specialists. Some of these will be discussed at a later point of this section. Furthermore, it has to be kept in mind that members of the 'rare biosphere' can be also important for the processing of certain substrates (Sogin et al. 2006; Pester et al. 2010; Jousset et al. 2017). However, priorities were made within this thesis and a full assessment of the 'rare biosphere' and degraders specialized on only one substrate was beyond the scope of this thesis. Further work is needed to understand the impact of also these organisms on HMW organosulfur dissimilation. Still, the significant and immediate increase of phylotypes 2 and 95 was evident and suggested their involvement in the degradation of organosulfur compounds or by-products. An indication that phylotype 2 may be able to profit from the actual sulfated substrates, is that its relative abundance increased significantly for three of the substrates very early already after two days.

Wukongibacter baidiensis was identified as the closest cultivated relative to both phylotypes 2 and 95 based on 16S rRNA gene sequence similarity. With this 16S rRNA gene sequence similarity of 96% and 97% respectively, it is likely that both phylotypes correspond to the genus *Wukongibacter* (Yarza et al. 2014). *Wukongibacter* was recently isolated from mixed hydrothermal sulfides and *W. baidiensis* is the only cultivated relative at the time of the writing of this thesis (Li et al. 2016). No sulfide production has been observed in *Wukongibacter*. However, it has been shown that it is able to utilize several sugars heterotrophically. Similarly, the genus *Alkaliphilus* is described to use proteinaceous substrates and some members can use elemental sulfur and thiosulfate as electron acceptors (Takai et al. 2001). Some *Alkaliphilus* species can ferment carbohydrates like fructose, sucrose and ribose (Wu et al. 2010). For the full degradation of sulfated polysaccharides, the carbohydrate backbone needs to be degraded and sulfate will be released. Therefore, the capacity to ferment the carbon-moiety while using newly freed sulfate might be a favorable characteristic for a degrader of such substrates. Interpretation of the increased relative abundance of phylotype 2 and 95 based on physiological properties of their closest cultivated relatives has to be evaluated cautiously. Its role for organosulfur degradation cannot be confirmed, and even closely related species or even strains of the same species can possess considerably different metabolic and physiological traits (e.g. Gao et al. 2003). However, the results on phylotype 2 and 95 relative abundances can be seen as basis to direct further research foci. The significant increase of phylotype 2 relative abundance for all substrates also during the early time points suggests that it is involved in the dissimilation of the amended compounds.

Several other clostridial phylotypes shown in **Figure 5**, responded positively to different sulfated polysaccharide amendments (**Figure 3, Appendix Figure 2**). In general, these phylotypes responded within in two to five days to the amendments. Among the clostridial responders, clear phylogenetic placement to known classes or families was often not possible. In the following, these clostridial phylotypes that are possibly of importance for organosulfur degradation, will be discussed.

Phylotype 3 responded with an increase from initial relative abundances below 1% to about 13% at day 11 after addition of κ-carrageenan (data not shown). This phylotype had a 95% 16S rRNA sequence identity to *Fusibacter paucivorans* and therefore likely corresponds to the same genus (Yarza et al. 2014). From the already isolated *Fusibacter* species, some were found in petroleum and oil affected environments (Ravot et al. 1999; Smii et al. 2015)**,** where sulfated organic compounds are abundant (reviewed in Orr & Sinninghe Damsté 1990). The capability to dissimilate such compounds would be of benefit for microorganisms in such habitats. However, macroalgal polysaccharides may require different degradation mechanisms than petroleum derived organosulfur. A full genome of a *Fusibacter* strain (*Fusibacter sp. 3D3*) has been sequenced (Serrano et al. 2017). UniProt annotations of this genome do not give any indication about the presence of aryl-sulfatases. However, a different type of sulfatase enzymes active on sulfate esters have been discovered in the *Clostridiales* recently (Berteau et al. 2006) that cannot be identified by the known enzyme motifs previously reported for aryl-sulfatases (Knaust et al. 1998; Dierks et al. 1999). Many sulfatase enzymes have not been biochemically characterized to date and many proteins catalyzing the cleavage of sulfate from macroalgal polysaccharides can still be expected (Helbert 2017).

Close relatives (99% 16S rRNA gene sequence identity) of investigated phylotype 5 such as *C. saccharobutylicum* (Keis et al. 2001) or *C. diolis* (Biebl & Spröer 2002) of the family *Clostridiaceae* (Family I), are known to be degraders of various sugars and starch compounds. There is no indication in the literature that *C. saccharobutylicum* is capable of dissimilating organosulfur compounds, but the ability to catabolize polysaccharides may hint to their involvement in sulfated polysaccharide catabolism. However, an anaerobic sulfatase was found in another *Clostridium* species, namely *C. perfringens* (see Introduction, 1.2), which possesses an anaerobic sulfatase (Berteau et al. 2006) and sulfatase production was reported for a *Clostridium* strain present in rat intestines (Huijghebaert et al. 1982). In this thesis, phylotype 5 responded strongly and only to the addition of κ-carrageenan (**Appendix Figure 2**). Degradation of κ-carrageenan has been shown to require a very distinct set of enzymes including two highly specific sulfatases (neocarrabiose sulfate 4-sulfatase, neocarratetraose 4-O-disulfate 4-sulfatase) (Weigl & Yaphe 1966; McLean & Williamson 1981). Further, the response to κ-carrageenan amendment is transient, with relative abundances of phylotype 5 increasing rapidly within the first days and decreasing after five days until the end of the experiment. Such a pattern might develop, if the organism is capable of degrading the organosulfur compound, giving it a competitive advantage over other microorganisms as long as sulfated substrate is still available and losing such advantage when the substrate is depleted. Filling the gap of knowledge about the physiology and phylogenetic affiliations of these organisms will help finding yet unknown organisms with intriguing catabolic abilities. The class *Clostridiales* seems suitable to contain specialized organosulfur degraders given its high physiological and possibly phylogenetic heterogeneity, and a taxonomic reorganization of this group is expected in the future (De Vos et al. 2009).

Response of Clostridium *phylotype 15 and* Spirochaetes *(*Sphaerochaeta*) phylotypes may be due to desulfonation by other microorganisms*

Phylotype 15 was closely related (99% 16S rRNA sequence identity, *blastn*) to *Clostridium neopropionicum* and *C. propionicum* strains, making it likely to belong to one of these species. These *Clostridium* species have been described to ferment ethanol to small organic compounds like acetate, propionate and ethanol (Tholozan et al. 1992) or lactate and acrylate (Janssen 1991). These compounds are common products of fermentation (Fenchel et al. 2012). For phylotype 15, a lag in response of about five days after substrate addition was observed until relative abundances increased. This indicates that this phylotype might in fact not utilize the initial sulfated polysaccharides, but rather profited from the fermentation products resulting from the degradation by other organisms at a later time point.

Differential comparisons of phylotype relative abundances in substrate addition treatments versus treatments with no substrate additions also showed significant increases in relative abundances for several members of the *Spirochaetes*. *Sphaerochaeta multiformi*s (Miyazaki et al. 2014) presented the closest cultivated relative to phylotype 8 (99% 16S rRNA gene sequence identity) and phylotype 92 (96% 16S rRNA gene sequence identity). *S. multiformis* has been shown to degrade mono-, di- and polysaccharides (Miyazaki et al. 2014). After substrate amendment, both phylotype 8 and 92 showed an initial 'lag phase' of about five days (**Appendix Figure 3**). If the characteristics of the phylotypes' closest relatives gives any indication for the phylotypes' physiological capacities, the delayed response to the amended polysaccharides might hint that these organisms utilize the carbon moieties of the given sulfated polysaccharides after desulfonation by other organisms. An alternative explanation can be that their growth rates may be slower than those of the initial responders and/or may have been present in lower initial relative abundances. To support this, specific *blastp* (National Center for Biotechnology Information 1988) analyses of the above mentioned anaerobic sulfatase against phylum *Spirochaetes* entries in the non-redundant database did suggest various members of this clade contain sulfatases (over 100 hits with an e-value over 10^{-15}), including cultivated organisms such as *Sphaerochaeta globus* (Ritalahti et al. 2011). These organisms may therefore be of particular interest for further studies, since the ability of *Spirochaetes* to degrade sulfated polysaccharides has not been reported so far.

In general the considerable outgrowth of specific phylotypes as the ones discussed above indicates the artificial, closed microcosm environment strongly selected for these phylotypes. Additionally, given also the unusually high (millimolar) SCFA concentrations observed in the microcosms, it seems that certain fermentative bacteria were strongly enriched through substrate addition and microcosms did not present *in situ* conditions. The large relative abundance increase of several phylotypes indicates they are indeed ecological r-strategists and would not dominate the community to such extent in *in situ* conditions. However, even though it seems the microcosm set up did not allow to determine *in situ* conditions, the responding phylotypes are still of high interest for their involvement in sulfated polysaccharide degradations.

Measurements of Sulfate and Sulfide Show Desulfonation of Substrates and Suggest High Desulfonation Efficiency for Fucoidan

In anoxic, marine sediments, sulfate reduction can be expected to occur when organic material and sulfate is available and other electron acceptors are depleted (Jørgensen 1982). Therefore, in a closed system such as the anoxic microcosms used in this study, sulfate was expected to decrease over time with organic matter driving sulfate reduction. Matching this assumption, sulfate decreased rapidly to concentrations below 5 mM in the no substrate control. However, hydrolytic cleavage of sulfated polysaccharides during their degradation, would lead to the liberation of sulfate (Bond et al. 1997; Hanson et al. 2004). The significant increase of sulfate for fucoidan treatments was possibly caused by liberation of sulfate from the substrate. Another indication that sulfate originated from the substrate can be taken from the stoichiometry of the microcosm system. In the case of fucoidan, the significant additional sulfate measured on the second day of incubation corresponds roughly to the total theoretical amount of sulfate that could have been liberated from the amount of fucoidan added (~1 mmol S), assuming a pore water volume of 0.03 L per microcosm (for calculated maximum values of liberated sulfate per substrate see **Appendix Table 1**). It is tempting to interpret the initial increase of sulfate and hydrogen sulfide in fucoidan treatments as the result of a rapid and almost complete sulfate liberation by microbial desulfonation. As such, the sulfate and sulfide dynamics observed for fucoidan treatments would present the pattern to be expected for efficient desulfonation of the substrate. This result was surprising, as hydrolytic degradation of fucoidan has been shown to occur extremely slowly in a previous study on anoxic marine sediments (Arnosti 2000). However, that study was done on permanently cold habitats and warmer temperatures and other environmental conditions might have resulted in different degradation dynamics in this study. Sampling at shorter time intervals especially during the first two days may help to clarify, if sulfate is rapidly released after substrate addition. Further information might be retrieved by measuring additional parameters like iron speciation to investigate the role of pyrite precipitation in the microcosm sediment. In summary, the observed sulfate concentrations over time allowed an inference that most of the substrates were degraded and that desulfonation occurred in the process. However, at this point it is not clear, why addition of the substrate mix did not yield the same results and further research will be necessary to elucidate why it showed different dynamics than the individual treatments.

As hydrogen sulfide is the product of sulfate reduction, it would be expected to increase in the closed, anoxic sediment microcosms used in this study. The decreasing sulfide concentration found in the microcosms, was however, likely due to re-oxidation and loss of sulfide in the gas phase during sampling and opening of the microcosm flasks. Additionally, sulfide fixation via zinc acetate may have been insufficient, especially during the later time points of the experiment, when fresh zinc acetate was not used. For chondroitin sulfate and fucoidan additions, sulfide did increase during the first two days of

incubation. As for fucoidan, increased sulfate levels, as discussed above, indicated that the sulfide concentrations increased due to a higher availability of sulfate for sulfate reduction. Sulfate values are however, not available for chondroitin sulfate, due to measurement/instrument issues. Given the observed increases in sulfide during the early time points after chondroitin sulfate addition, it could be assumed that maximum amounts of sulfate (~ 0.15 mmol) (**Appendix Table 1**) were liberated rapidly from this substrate after amendment.

Accumulation of Short-Chain Fatty Acids Hints on Importance of Fermenters for Substrate Degradation

Fermentation products like short-chain fatty acids (SCFAs) are amongst the most common electron donors for sulfate reduction. The coupling of fermentation and sulfate reduction can serve as an indicator for ongoing fermentation versus sulfate reduction in the sediment. It can be assumed that fermentation products would not accumulate to high concentrations in most anoxic sediments, where an equilibrium between these processes has been stablished (e.g. Rabus et al. 2006; Graue et al. 2012). The low values of all SCFAs measured in the initial (day 0) Venice Lagoon sediments indicate that such an equilibrium between sulfate reduction and fermentation processes was the initial condition, and was obtained over the entire duration the microcosm experiment in the no substrate control. In contrast, SCFAs accumulated in microcosms after treatments with either sulfated arabinogalactan, fucoidan or κ-carrageenan. Such an effect of substrate addition correlates with findings of previous studies (e.g. Fukui et al. 1997; Graue et al. 2012), where amendments of organic matter to anoxic sediments led to the accumulation of acetate, isobutyrate, propionate and ethanol. These results indicate that the substrate additions fueled rather high activities from the hydrolyzing/fermenting populations and that the terminal-oxidizers, such as sulfate reducers, did not initially keep-up with these high substrates loads.

Some differences in SCFA production could be seen from the additions of the different sulfated polysaccharides. Butyrate and formate were produced to a much larger extent in κ-carrageenan treatments as compared to the other investigated treatments. Possibly another organism or group of organisms with different predominant metabolic products was responsible for κ-carrageenan degradation than was for the other two investigated substrates. The high concentrations of acetate in fucoidan treatments along with the possibly fast desulfonation of fucoidan discussed above, indicate that fucoidan was indeed quite efficiently degraded in Venice Lagoon anoxic sediment. Further work is needed in order to find out more about possible substrate specificity and the organisms that could lead to such differences between the SCFAs that are predominantly produced. Environmental conditions as well as substrate priming effects (Arnosti 2004) are possible influencing factors and need further consideration.

The beginning of decreases of butyrate and formate and, in the case of fucoidan, acetate, after eleven days of incubation might be the effect of sulfate reduction gaining in importance at later time points. In general, the individual substrates triggered fermentative processes, as shown by the accumulation of

SCFAs. Although indicators for sulfate reduction could not be seen in the early days of the incubation, it can be assumed that their effect was simply masked by an excess of fermentation products. Coastal sediments usually show very high rates of sulfate reduction (Jørgensen 1982) and also sulfate and hydrogen sulfide dynamics measured in this thesis confirmed ongoing sulfate reduction.

Unexpectedly, the amendment of a mixture of several sulfated polysaccharides yielded opposing results (i.e., sulfate/sulfide concentrations) to when single substrates were added. In fact, it seemed like the SCFA concentrations remained equally as low in the substrate mix amendment as in the untreated sediment. In both, the no substrate control and the substrate mix treatments, the steady-state low SCFA concentrations remained unaltered throughout the duration of the experiment. This could indicate that sulfate reduction and fermentation processes were balanced within those microcosms. However, when sulfate reduction was inhibited within the mix by molybdate additions, SCFAs accumulated as a result of fermentation and a lack of sulfate reduction. Accumulation of acetate after inhibition of sulfate reduction activity by molybdate was also observed by other authors (e.g. Finke et al. 2007). Therefore, the sulfate reduction rates in the substrate mix treatments may have been able to balance the production of fermentation products. Single components of the mix like sulfated arabinogalactan and κ-carrageenan did not show the same trend and SCFAs accumulated even without blocking of sulfate reduction. These observations concur with those made for sulfur species dynamics and their explanation remains unclear. Further research is needed to understand the complexity in the degradation of complex sulfated organics.

Raman Microspectroscopy Combined with D_2O Labeling Shows Single Cell Activity on Substrates

In this study, it was aimed to use Raman microspectroscopy to detect D_2O incorporation into cells made active by the addition of substrates, as previously applied on soil (Eichorst et al. 2015) and gut samples (Berry et al. 2015). This could then be used in combination with specific FISH assays to confirm the activity and identities of populations identified by amplicon sequencing of microcosms. It could also be used in combination with Raman-activated cell sorting (Zhang et al. 2015), to further identify and obtain genomic information from the stimulated/sorted cells. In this study, it was shown that it was feasible to perform Raman-D_2O experiments using cells from anoxic marine sediment samples. Labeling of single cells and acquisition of Raman spectra could be shown within this thesis, whereby stimulation of cells by the substrate additions was confirmed by incorporation of D_2O into single cells. However, labeling signals became visible only after three days or more of incubation. Further, certain populations seemed to have grown/replicated in the 'simplified' conditions used (i.e., most sediment was diluted away in order to reduce stimulation of activity of endogenous organics), thereby outcompeting the other populations. This would not be ideal because subsequent sorting or coupling with FISH would possibly only identify the population/s that grew and then dominated the incubation. This is opposed to experiments with fast growing cells from, e.g., the gut, where D_2O can be detected within hours of incubations, and populations may have little time to increase. These communities do not dramatically shift within those short incubation times, thereby capturing a more natural community (e.g. Berry et al.

2015). In any case, the functionality of the described approach on this type of samples is promising for a possible future combination with other recent methods and may provide different insights into activities and identities of the microorganisms degrading sulfated polysaccharides. For example, Raman-activated cell-sorting (RACS) (Zhang et al. 2015) can be an elegant tool to target single cells of responder phylotypes. This would allow for genome sequencing approaches and the consequent identification of genes indicative of potential degraders of sulfated organics. Such an approach can help to gather more information about the responding organisms and might even lead to their isolation in the future.

Bacterial Community Appears to be Significantly Altered in D_2O Cell Suspensions

Potential degrader phylotypes from the phyla *Firmicutes* and *Spirochaetes* identified in the microcosms from amplicon sequencing data were both successfully targeted via two-step CARD-FISH with cells extracted from the microcosms. In the case of the *Spirochaetes*-phylotypes, the approach was also effective using cells from D_2O incubated cell suspensions amended with sulfated polysaccharides. On the other hand, the fact that despite their high abundance in the microcosm sediments was proven by FISH visualization, the targeted phylotypes 2 and 95 (*Firmicutes*) could not be detected in the D_2O incubations, despite multiple attempts. It was expected that the designed FISH probes would have hybridized to the target cells from the D_2O incubations, since their functionality was assured from their performance on complex microcosm samples. An explanation for the lack of target cells is possibly that phylotypes 2 and 95 abundance were highly decreased or absent from D_2O incubations. Therefore, it appears that the bacterial community was altered over time in the D_2O incubations compared to microcosm sediment. It could also be observed that the morphology of the cells in the D_2O incubations became much more uniform over time, indicating only certain populations survived or grew. Cell suspensions without any sediment matrix were used for the D_2O incubations, which results in a simplification of the system. Other selective changes like loss of mutualistic taxa or lack of extracellular enzymes may be plausible. However, even though the community might be altered, taxa interesting for sulfated polysaccharide remineralization could still be present in the D_2O incubations. Raman microspectroscopy showed general substrate use and bacterial activity in the labeled samples. In fact, the removal of other complex organic compounds available from the natural sediment might result in a more efficient targeting of organosulfur degraders. However, such a system does not mirror natural conditions and therefore DNA was extracted and sequenced directly from microcosm sediment. A trade-off between laboratory efficiency and the conditions closest to the natural ones had to be made and also needs to be considered in future research. Sequencing of D_2O cell suspensions or sorted cells might reveal other potential organosulfur degraders, which were not targeted or of very low abundance in microcosm samples. Another approach could be to label the microcosm sediment slurry directly, which would be challenging due to the high complexity of the sediment matrix and the abundance of natural substrates that may stimulate activity.

CONCLUSION & FUTURE PERSPECTIVE

Changes in relative abundances of various members of the microbial community of anoxic marine sediments from the Venice Lagoon after amendment of sulfated polysaccharides show that sulfated polysaccharides are utilized within anoxic sediments. Strong responses to multiple substrates suggest that members of the *Clostridiales* and *Spirochaetes* are involved in organosulfur degradation. Although non-desulfonating microbes were also likely enriched in the amended microcosms, relative abundance dynamics of potential desulfonating microbes appeared to allow to distinguish them from the former. Isotopic labeling experiments like previously done for soil organosulfur (Ghani et al. 1993) might help to further elucidate the turnover of organosulfur in anoxic sediments and show the true degree of substrate utilization. First results from measurements of sulfate, sulfide and SCFAs in pore water of amended microcosms indicated that heterotrophic bacteria are highly important for desulfonation and degradation of sulfated polysaccharides and that consortia of microorganisms contribute to the overall mineralization of the substrates. Combining Raman-Microspectroscopy with D_2O incubations showed increased activity of bacteria due to the amended substrates. Although communities appeared to be altered between microcosm sediment and D_2O incubations, results are promising to allow for the combination of Raman activated cell sorting. Consequently, genomic data might be obtained. Future work may reveal yet uncharacterized pathways, sulfatase enzymes and unexpected microbial players with fascinating capabilities for the desulfonation and degradation of complex HMW organosulfur in anoxic marine sediments.

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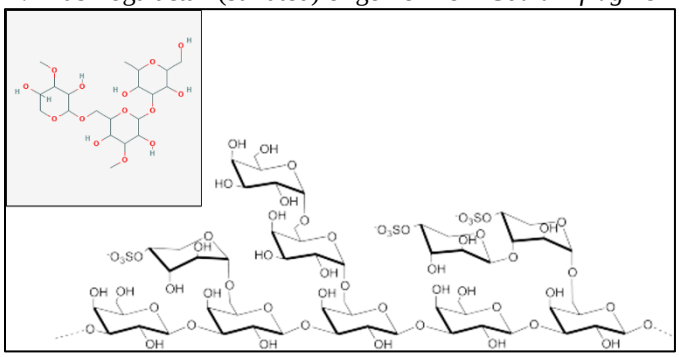
APPENDIX

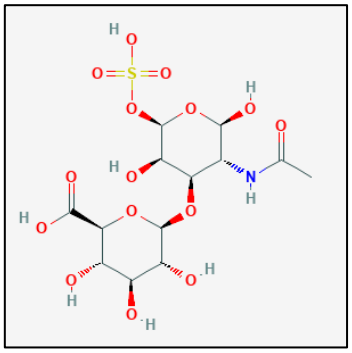
CONTENTS

APPENDIX FIGURES

A: Arabinogalactan (sulfated) oligomer from *Codium fragilis*

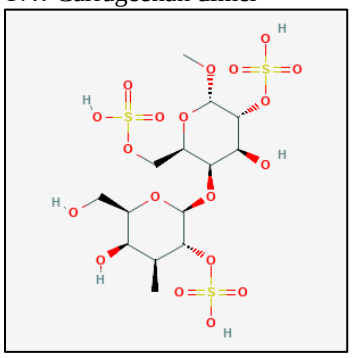


B: Chondroitin sulfate dimer

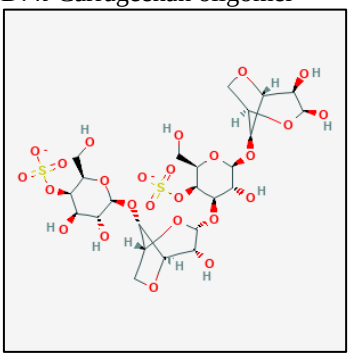


http://www.elicityl-oligotech.com

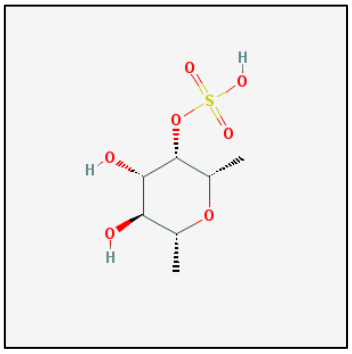
C: λ-Carrageenan dimer



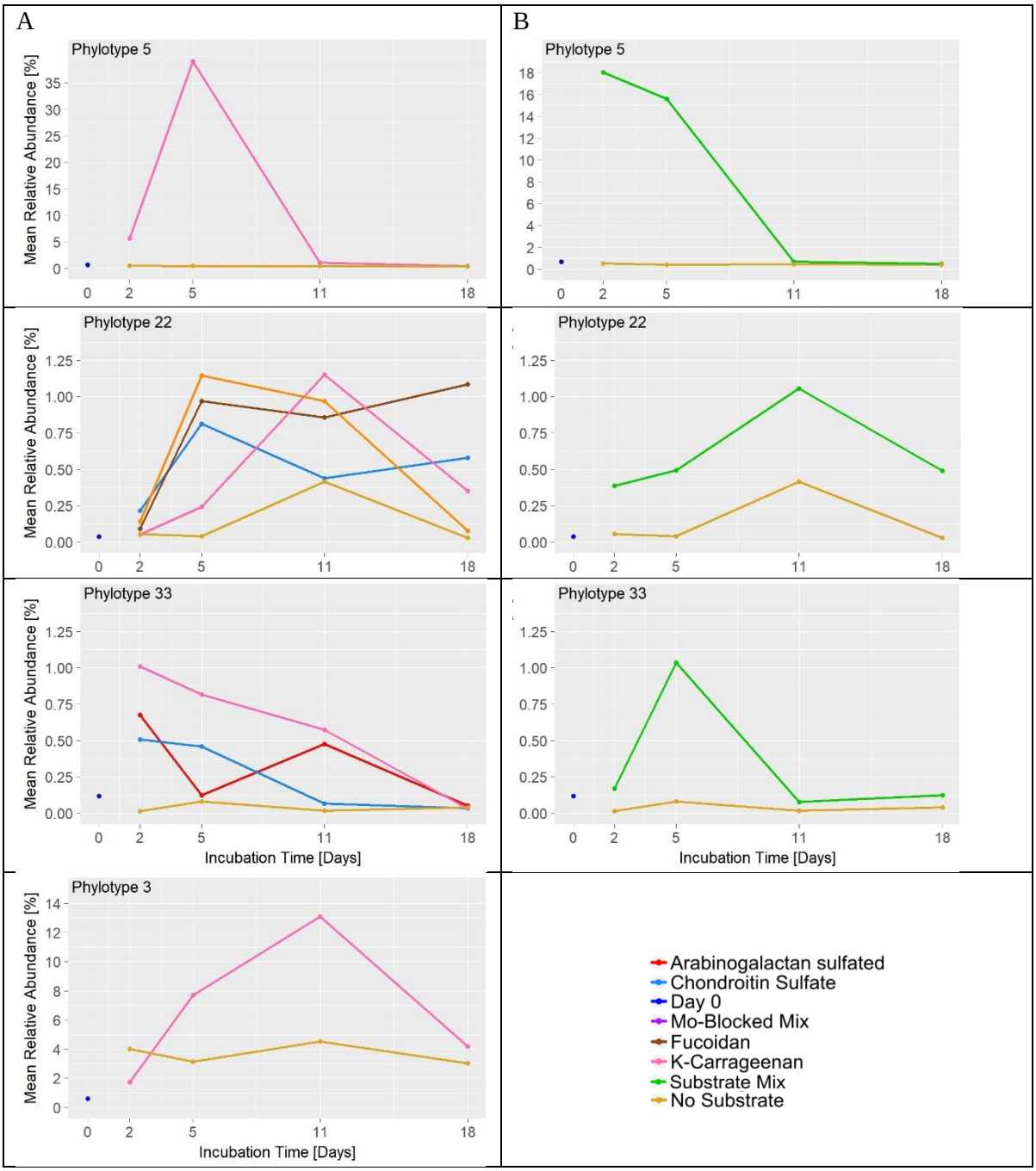
D: κ-Carrageenan oligomer



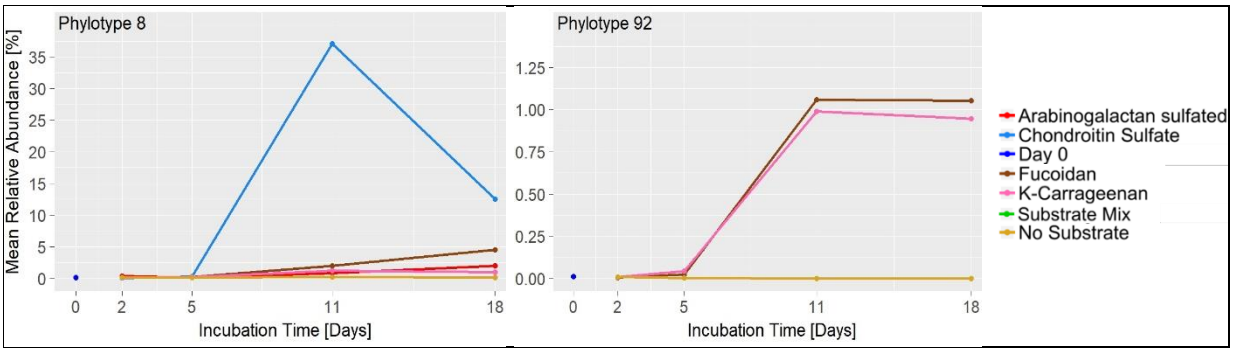
E: Fucoidan monomer



Appendix Figure 1. Potential structures of sulfated polysaccharide units. All 2D images were obtained from Kim et al. 2016. Sulfated polysaccharides are heterogeneous, complex molecules and can differ in sequence and sulfatation depending on their source.



Appendix Figure 2. Mean relative abundance of *Clostridiales* phylotypes 3, 5, 22, 33 responding to substrate amendment. (A) Response of phylotypes to individual sulfated polysaccharides. (B) Response of phylotypes to substrate mix compared to molybdate-blocked mix. Substrates without significant deviation from the control (RDF-corrected p-value < 0.05) at any time point are not shown.



Appendix Figure 3. Comparison of dynamics of mean relative abundances between phylotype 8 and 92 as induced by amendment of sulfated polysaccharides. Both phylotypes show an initial 'lag phase' in their response to substrates. Substrates without significant deviation from the control (RDF-corrected p-value < 0.05) at any time point are not shown. Mixes are excluded from this figure.

APPENDIX TABLES

Appendix Table 1. Calculated maximum releasable sulfate per substrate addition. Due to the heterogeneity of sulfated polysaccharides in nature, substrate molecular weights were estimated from disaccharide molecular weight and amount of sulfate moieties per disaccharide.

Substrate	Mass [mg]	MW Dimer [g/mol]	Molecules sulfate per dimer	Mol sulfate per dimer	Max. liberated sulfate [mmol]
K	150	387	1	0.000387597	0.387596899
L	150	466	2	0.000643777	0.643776824
A	150	395	2	0.000759494	0.759493671
F	150	424	3	0.001061321	1.061320755
C	70	463	1	0.000151188	0.151187905
T	187.5	125	1	0.0015	1.5
K - Mix	30	387	1	7.75194E-05	0.07751938
L - Mix	30	466	2	0.000128755	0.128755365
A - Mix	30	395	2	0.000151899	0.151898734

Appendix Table 2. First step bacterial 16S rRNA gene PCR protocol.

Master Mix	[µL] **x1 (Final Volume: 12.5 µL)**
MilliQ water	8.4
Dream Taq Buffer	1.25
dNTP mix (2.5 mM)	1.25
H_Bact-341F	0.5
H_Bact-785R	0.5
BSA (20µg/µL)	0.05
Dream Taq	0.05

Template – 0.5 µl; **Cycling**: 1x 95°C – 3’; 30x 95°C – 30’’, 52°C – 30’’, 72°C – 45’’; 1x 72°C – 2’

Appendix Table 3. Second step barcode PCR protocol

Master Mix	Volume (final volume: 25 µL)
Deionized water	18.2
Dream Taq Buffer	2.5
dNTP mix (2.5 mM mix)	2.5
Barcode (16.666 µM)	0.6
BSA (20µg/µL)	0.1
Dream Taq	0.1

Template – 1 µl; **Cycling**: 1x 95°C – 3'; 16x 95°C – 30'', 52°C – 30'', 72°C – 30''; 1x 72°C – 2'

Appendix Table 4. Primer sequences for first step bacterial 16S rRNA gene PCR

Primer	Sequence
H_Bact-341F	5'-GCTATGCGCGAGCTGCCCTACGGGNGGCWGCAG-3'
H_Bact-785R	5'-GCTATGCGCGAGCTGCGACTACHVGGGTATCTAATCC-3'

Appendix Table 5. List of equipment.

Equipment	Manufacturer
P/ACETM MDQ Molecular Characterization System (Capillary Electrophoresis)	Beckman Coulter, Brea, CA, USA
Vinyl Anaerobic Chamber	Coy Lab Products, Grass Lake, MI, USA
LabRAM HR Evolution Raman Spectrometer	HORIBA Scientific, Kyoto, Japan
FastPrep-24™ 5G bead beating system	MP Biomedicals, Santa Ana, CA, USA
Lysing Matrix E, 2 mL Tube	MP Biomedicals, Santa Ana, CA, USA
Infinite® 200 PRO Plate Reader	TECAN, Zurich Switzerland
Epoxy resin coated microscope slides	Marienfeld-Superior, Lauda-Königshofen, Germany
Isopore™ GTTP Membrane Filters	Merck Millipore, Billerica, MA, USA).
TCS SP8 X Confocal Laser Scanning Microscope	Leica Microsystems, Wetzlar, Germany

Appendix Table 6. List of chemicals.

Chemical & Kits	Manufacturer
λ-Carrageenan plant mucopolysaccharide	Sigma-Aldrich, St. Louis, MO, USA
Sulfated arabinogalactan polysaccharide (from *Codium f.*)	ELICITYL SA, Crolles, France
κ-Carrageenan plant mucopolysaccharide	Sigma-Aldrich, St. Louis, MO, USA
Chondroitin sulfate sodium salt from shark cartilage	Sigma-Aldrich, St. Louis, MO, USA
Taurine	Sigma-Aldrich, St. Louis, MO, USA
Fucoidan from *Macrocystis pyrifera*	Sigma-Aldrich, St. Louis, MO, USA
Sodium Dodecyl Sulfate (SDS) ≥99 %, for biochemistry	Carl Roth, Karlsruhe, Germany
Resazurin sodium salt	Sigma-Aldrich, St. Louis, MO, USA
Formaldehyde solution 37 %	Carl Roth, Karlsruhe, Germany
Sodium molybdate dihydrate	Carl Roth, Karlsruhe, Germany
Hexadecyltrimethylammonium bromide	Sigma-Aldrich, St. Louis, MO, USA
Roti®-Phenol/Chloroform/Isoamyl alcohol	Carl Roth, Karlsruhe, Germany
Isoamyl alcohol ROTIPURAN® ≥98,5 %	Carl Roth, Karlsruhe, Germany
Zinc acetate dihydrate	Sigma-Aldrich, St. Louis, MO, USA
N,N-dimethyl-1,4-phenylenediamine sulfate	Sigma-Aldrich, St. Louis, MO, USA
ZR-96 DNA Clean-up Kit™	Zymo Research, Irvine, CA, USA
Quant-iT™ PicoGreen™ dsDNA Assay Kit	Thermo Fisher Scientific, Waltham, MA USA
CEOfix™ Anions 5 Kit	Analis, Suarlée (NAMUR), Belgium

Appendix Table 7. Probes designed for phylotypes 2, 95, 8, 92 for two-step CARD-FISH

Identifier	GC-Content [%]	Sequence	Target site	Formamide [%]
2-A	37	5'-ccg aat aca aag cat caa cga cta gaa aaa agc ttc taa ggt acc gtc att tat tc-3'	93-121	25-30
95-A	40	5'-ccg aat aca aag cat caa cga cta gaa aaa agc tcc taa ggt acc gtc att att-3'	93-121	30-35
8-92-A	39	5' -ccg aat aca aag cat caa cga cta gaa aaa acg tyc att gcg gaa gat tct tag-3'	0-20	20-25

Appendix Table 8. List of additional abbreviations used.

Abbreviation	**Stands for**
DNA	Deoxyribonucleic acid
DAPI	4',6-diamidino-2-phenylindole
EDTA	Ethylenediaminetetraacetic acid
HRP	horseradish peroxidase
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PFA	(Para)Formaldehyde
rRNA	Ribosomal ribonucleic acid
TE Buffer	Tris-EDTA-Buffer
ANOVA	Analysis of Variance

Appendix Table 9. Filtered significant results of pairwise differential analysis showing potential responder phylotypes. RDF-corrected *p*-values are given as padj for each individual time point and substrate of a potential positive responder. A = arabinogalactan sulfated, B = molybdate-blocked mix, C = chondroitin sulfate, F = fucoidan from *M. pyrifera*, K = κ-carrageenan, L = λ-carrageenan, M = substrate mix.

Phylotype ID	Substrate	Incub. Time [days]	padj	Phylotype ID	Substrate	Incub. Time [days]	padj	Phylotype ID	Substrate	Incub. Time [days]	padj	Phylotype ID	Substrate	Incub. Time [days]	padj
2	F	18	9.1E-07	15	B	2	5.0E-02	38	K	11	2.3E-05	73	K	11	2.1E-02
2	C	18	5.5E-05	17	K	5	1.7E-08	38	K	18	1.6E-02	73	F	18	4.8E-02
2	K	18	8.4E-05	17	B	5	1.0E-02	38	K	5	3.4E-02	74	K	18	1.4E-02
2	A	18	2.6E-04	17	K	11	1.2E-02	39	K	18	1.6E-02	74	B	18	3.3E-02
2	A	5	2.7E-03	17	B	2	3.1E-02	39	K	5	2.1E-02	75	K	11	1.1E-04
2	C	2	3.5E-03	17	F	5	4.9E-02	39	B	2	2.5E-02	75	K	18	4.1E-03
2	A	2	1.3E-02	18	K	5	2.5E-10	39	K	11	2.9E-02	78	K	11	2.9E-02
2	K	11	2.9E-02	18	K	11	1.8E-04	39	C	11	3.1E-02	80	B	5	3.8E-02
2	B	5	3.8E-02	18	A	2	1.3E-02	39	B	5	4.5E-02	81	K	5	2.1E-02
2	B	2	4.5E-02	18	K	18	4.4E-02	39	F	5	4.9E-02	85	F	18	3.9E-02
4	K	2	2.2E-03	18	B	2	5.0E-02	41	C	11	2.9E-14	86	C	5	1.5E-02
4	A	2	3.5E-02	19	F	18	6.3E-03	45	K	5	3.7E-06	88	K	11	2.9E-02
5	K	5	1.6E-49	22	F	18	3.0E-03	45	B	2	2.8E-02	88	B	5	3.8E-02
5	K	2	8.6E-03	22	C	18	1.1E-02	45	B	5	3.8E-02	91	C	2	2.0E-02
7	A	18	9.4E-03	22	K	18	1.4E-02	53	K	18	8.7E-03	91	C	5	4.9E-02
8	C	11	7.4E-128	22	F	5	4.9E-02	53	C	18	1.5E-02	92	K	11	7.2E-04
8	C	18	1.0E-15	23	C	2	3.5E-03	53	K	11	2.9E-02	92	K	18	1.0E-03
8	A	18	9.4E-03	23	A	2	1.9E-02	53	C	5	4.7E-02	92	F	18	2.6E-03
8	K	11	1.4E-02	27	F	11	3.0E-02	53	F	5	4.9E-02	92	F	11	2.7E-02
8	K	18	2.8E-02	29	C	11	2.1E-03	53	F	11	5.0E-02	93	C	18	1.2E-05
8	F	11	3.5E-02	29	K	11	4.4E-02	55	B	2	3.1E-02	95	F	18	2.2E-06
8	B	18	5.0E-02	29	C	18	4.4E-02	55	B	5	3.8E-02	95	C	18	1.2E-05
10	K	2	5.8E-03	32	A	5	5.0E-05	55	F	11	4.2E-02	95	K	18	4.6E-05
11	B	18	4.7E-02	33	K	11	3.6E-03	55	A	5	4.8E-02	95	A	18	9.4E-03
15	C	18	5.5E-05	33	K	2	6.9E-03	62	F	18	1.9E-03	95	K	11	2.1E-02
15	K	11	4.8E-04	33	A	2	1.3E-02	62	K	18	2.5E-03	95	A	5	3.6E-02
15	F	18	6.3E-03	33	C	2	3.7E-02	62	C	18	3.7E-03	95	B	5	3.8E-02
15	K	18	6.6E-03	33	K	5	4.2E-02	63	K	11	4.8E-02	100	B	2	3.2E-02
15	B	18	3.8E-02	37	K	11	3.6E-03	72	C	2	2.2E-05	100	B	5	3.8E-02
15	F	11	5.0E-02	37	K	18	4.7E-02	72	K	2	4.6E-02				

Appendix Table 10. Phylotypes with significant increase in relative abundance after substrate amendment as compared to the no substrate control. Values indicate total increase or decrease of relative abundances compared to the no substrate control. Significant (RDF-corrected p-value ≥ 0.05) deviations of relative abundances from the no substrate control are framed and indicated in bold type.

			Arabinogalactan (sulfated)				Molybdate-blocked Mix			Chondroitin Sulfate				Fucoidan			κ-Carrageenan				λ-Carrageenan			Substrate Mix		
Phylotype	**Phylogenetic Affiliation (Genus)***	**Day 0**	**Day 2**	**Day 5**	**Day 11**	**Day 18**	**Day 2**	**Day 5**	**Day 18**	**Day 2**	**Day 5**	**Day 11**	**Day 18**	**Day 5**	**Day 11**	**Day 18**	**Day 2**	**Day 5**	**Day 11**	**Day 18**	**Day 2**	**Day 5**	**Day 18**	**Day 2**	**Day 5**	**Day 11**
2	*Fusibacter*	1.0%	**8.9%**	**15.7%**	-1.0%	**6.6%**	**7.6%**	**6.8%**	-1.0%	**8.3%**	-1.0%	-1.0%	**5.4%**	-1.0%	**8.0%**	-1.0%	-1.0%	-1.0%	**12.7%**	**4.5%**	**8.8%**	**10.2%**	**5.0%**	**7.5%**	**6.5%**	**11.6%**
4	*Propionigenium*	1.2%	**7.7%**	-1.2%	-1.2%	-1.2%	-1.2%	-1.2%	-1.2%	-1.2%	-1.2%	-1.2%	-1.2%	-1.2%	-1.2%	-1.2%	**16.5%**	-1.2%	-1.2%	-1.2%	**20.5%**	-1.2%	-1.2%	-1.2%	-1.2%	-1.2%
5	*Clostridium_sensu_stricto_1*	0.7%	-0.7%	-0.7%	-0.7%	-0.7%	-0.7%	-0.7%	-0.7%	-0.7%	-0.7%	-0.7%	-0.7%	-0.7%	-0.7%	-0.7%	**5.0%**	**38.4%**	-0.7%	-0.7%	-0.7%	-0.7%	-0.7%	**17.4%**	**15.0%**	-0.7%
7	*Bacteroidetes_unclassified*	0.2%	-0.2%	-0.2%	-0.2%	**2.3%**	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%
8	*Spirochaeta*	0.1%	-0.1%	-0.1%	-0.1%	**1.8%**	-0.1%	-0.1%	**1.7%**	-0.1%	-0.1%	**37.0%**	**12.4%**	-0.1%	**1.8%**	-0.1%	-0.1%	-0.1%	**1.1%**	**0.9%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**1.2%**
10	*Psychrilyobacter*	0.8%	-0.8%	-0.8%	-0.8%	-0.8%	-0.8%	-0.8%	-0.8%	-0.8%	-0.8%	-0.8%	-0.8%	-0.8%	-0.8%	-0.8%	**3.3%**	-0.8%	-0.8%	-0.8%	-0.8%	**1.7%**	-0.8%	-0.8%	**1.6%**	-0.8%
11	*Sulfurovum*	1.5%	-1.5%	-1.5%	-1.5%	-1.5%	-1.5%	-1.5%	**3.7%**	-1.5%	-1.5%	-1.5%	-1.5%	-1.5%	-1.5%	-1.5%	-1.5%	-1.5%	-1.5%	-1.5%	-1.5%	-1.5%	-1.5%	-1.5%	-1.5%	-1.5%
15	*Incertae_Sedis*	0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**0.8%**	-0.1%	**2.1%**	-0.1%	-0.1%	-0.1%	**1.9%**	-0.1%	**1.0%**	**0.8%**	-0.1%	-0.1%	**1.8%**	**0.9%**	-0.1%	-0.1%	-0.1%	**0.7%**	**0.9%**	**2.1%**
17	*Catenococcus*	0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**1.8%**	**2.0%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**0.8%**	-0.1%	-0.1%	-0.1%	**3.5%**	**0.6%**	-0.1%	-0.1%	-0.1%	-0.1%	**1.9%**	**2.1%**	-0.1%
18	*Vibrio*	0.1%	**1.0%**	-0.1%	-0.1%	-0.1%	**0.9%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**4.8%**	**1.7%**	**0.2%**	-0.1%	-0.1%	-0.1%	**0.4%**	-0.1%	-0.1%
19	*Fusibacter*	0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**1.2%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**1.4%**	-0.1%	**1.0%**	**1.1%**	-0.1%
22	*Fusibacter*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.5%**	**0.9%**	0.0%	**1.0%**	0.0%	0.0%	0.0%	**0.3%**	0.0%	**1.1%**	0.0%	0.0%	**0.5%**	0.0%
23	*Clostridiales_unclassified*	0.1%	**1.6%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**1.5%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%
27	*Desulfobaculum*	0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**4.4%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%
29	*Spirochaeta*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.8%**	**0.5%**	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
32	*Vibrio*	0.1%	-0.1%	**3.6%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%
33	*Clostridium_sensu_stricto_17*	0.1%	**0.6%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**0.4%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**0.9%**	**0.7%**	**0.5%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**0.9%**	-0.1%
37	*Fusibacter*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.8%**	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
38	*Fusobacterium*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.6%**	**2.3%**	**0.6%**	0.0%	0.0%	0.0%	0.0%	0.0%	**0.7%**
39	*Incertae_Sedis*	0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**0.7%**	**0.3%**	-0.1%	-0.1%	-0.1%	**0.4%**	-0.1%	**0.4%**	-0.1%	-0.1%	-0.1%	**0.3%**	**0.4%**	**0.6%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%
41	*Photobacterium*	0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**15.2%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%
45	*Vibrio*	0.0%	0.0%	0.0%	0.0%	0.0%	**0.9%**	**0.7%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**2.1%**	0.0%	0.0%	0.0%	0.0%	0.0%	**1.0%**	**1.2%**	0.0%
48	*Actibacter*	1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	-1.0%	**0.2%**	-1.0%	-1.0%	-1.0%
53	*Anaerovorax*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**1.2%**	0.0%	**0.4%**	**0.9%**	**0.7%**	0.0%	0.0%	0.0%	**0.5%**	**0.3%**	0.0%	0.0%	0.0%	0.0%	**0.8%**	**0.5%**
55	*Anaerotruncus*	0.0%	0.0%	**0.8%**	0.0%	0.0%	**0.5%**	**0.5%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**1.0%**	**0.5%**	**0.3%**
62	*Sedimentibacter*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.7%**	0.0%	0.0%	**0.9%**	0.0%	0.0%	0.0%	**0.9%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
63	*Ralstonia*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
72	*Propionigenium*	0.4%	-0.4%	-0.4%	-0.4%	-0.4%	-0.4%	-0.4%	-0.4%	**1.2%**	-0.4%	-0.4%	-0.4%	-0.4%	-0.4%	-0.4%	**0.1%**	-0.4%	-0.4%	-0.4%	-0.4%	-0.4%	-0.4%	-0.4%	-0.4%	-0.4%
73	*Clostridiales_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	0.0%	0.0%	**0.4%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**1.6%**
74	*Acidiferrobacter*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.6%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
75	*NB1-n_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**1.0%**	**0.7%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
78	*Kazan-2B-17_unclassified*	0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**0.2%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%
80	*Fusibacter*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.5%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
81	*Zobellella*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	**1.1%**	**1.1%**	**0.2%**
85	*Anaerovorax*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
86	*Nitrospiraceae_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**1.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
88	*Incertae_Sedis*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	**0.3%**
91	*Clostridium_sensu_stricto_17*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**1.0%**	**0.8%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
92	*Spirochaeta*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**1.0%**	**1.0%**	0.0%	0.0%	**1.0%**	**0.9%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
93	*Marinifilum*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**2.7%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
95	*Fusibacter*	0.4%	-0.4%	**5.1%**	-0.4%	**3.5%**	-0.4%	**4.3%**	-0.4%	-0.4%	-0.4%	-0.4%	**5.2%**	-0.4%	-0.4%	**5.5%**	-0.4%	-0.4%	**7.4%**	**4.0%**	-0.4%	-0.4%	**5.0%**	**3.8%**	**3.6%**	**10.1%**
100	*Photobacterium*	0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**1.1%**	**1.0%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%
102	*Fusibacter*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
104	*OM1_clade_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
110	*Fusibacter*	0.0%	0.0%	0.0%	0.0%	0.0%	**0.7%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
112	*OM1_clade_unclassified*	0.3%	-0.3%	-0.3%	-0.3%	-0.3%	-0.3%	-0.3%	**0.7%**	-0.3%	-0.3%	-0.3%	-0.3%	-0.3%	-0.3%	-0.3%	-0.3%	-0.3%	-0.3%	-0.3%	-0.3%	-0.3%	-0.3%	-0.3%	-0.3%	-0.3%
113	*NB1-n_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.9%**	0.0%	0.0%	**0.3%**	0.0%	0.0%	**0.2%**	**0.5%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
117	*Photobacterium*	0.0%	0.0%	0.0%	0.0%	0.0%	**0.7%**	**1.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
118	*NB1-n_unclassified*	0.0%	0.0%	0.0%	**1.6%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	**0.5%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
127	*Bacteria_unclassified*	0.0%	0.0%	**0.2%**	**1.5%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
130	*Clostridiales_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
132	*Anaerovorax*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	**0.7%**
144	*Rhodospirillaceae_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**1.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
145	*Intestinimonas*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	**0.4%**	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**
146	*Photobacterium*	0.0%	0.0%	**0.5%**	0.0%	0.0%	**0.5%**	**0.6%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
151	*NB1-n_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.6%**	**1.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
154	*Ilyobacter*	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
158	*Alkalibacter*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**1.8%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
160	*NB1-n_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
162	*OM1_clade_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
169	*Clostridium_sensu_stricto_7*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.7%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
191	*Marinilabiaceae_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**1.1%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
210	*Nitrospiraceae_unclassified*	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
216	*Akkermansia*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
217	*Sva0071_unclassified*	0.5%	-0.2%	-0.5%	-0.5%	-0.5%	-0.5%	-0.5%	-0.5%	-0.2%	-0.5%	-0.5%	-0.5%	-0.5%	-0.5%	-0.5%	-0.5%	-0.5%	-0.5%	-0.5%	-0.5%	-0.5%	-0.5%	-0.5%	-0.5%	-0.5%
218	*Fusibacter*	0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**0.2%**	-0.1%	-0.1%	-0.1%	-0.1%
220	*Vibrio*	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
234	*Desulfococcus*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.5%**	0.0%
240	*NB1-n_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	**0.8%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
242	*Micrococcus*	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
245	*Haldemania*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
247	*Bacteroides*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**1.0%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
252	*Clostridium_sensu_stricto_17*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**
253	*SB-1_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.6%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
254	*Vallitalea*	0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	**1.4%**	**0.1%**	-0.2%	**0.5%**	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%
264	*Staphylococcaceae_unclassified*	0.0%	0.0%	**1.7%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
274	*Enterococcus*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.6%**	0.0%	0.0%	0.0%	0.0%
284	*JTB255_marine_benthic_group_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%
289	*Vibrio*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	**0.4%**	0.0%
296	*SB-1_unclassified*	0.0%	0.0%	0.0%	0.0%	**1.4%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Legend

≥ 20%
15%
10%
5%
1%
0%
-1%
-1.5%
≤ -2%

RDF-corrected p-values ≤ 0.05

Appendix Table 10. Phylotypes with significant increase in relative abundance after substrate amendment as compared to the no substrate control. Values indicate total increase or decrease of relative abundances compared to the no substrate control. Significant (RDF-corrected p-value ≥ 0.05) deviations of relative abundances from the no substrate control are framed and indicated in bold type.

			Arabinogalactan (sulfated)				Molybdate-blocked Mix			Chondroitin Sulfate				Fucoidan			κ-Carrageenan				λ-Carrageenan			Substrate Mix		
Phylotype	**Phylogenetic Affiliation (Genus)***	**Day 0**	**Day 2**	**Day 5**	**Day 11**	**Day 18**	**Day 2**	**Day 5**	**Day 18**	**Day 2**	**Day 5**	**Day 11**	**Day 18**	**Day 5**	**Day 11**	**Day 18**	**Day 2**	**Day 5**	**Day 11**	**Day 18**	**Day 2**	**Day 5**	**Day 18**	**Day 2**	**Day 5**	**Day 11**
301	*Epulopiscium*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
351	*Natranaerovirga*	0.0%	0.0%	0.0%	0.0%	**0.7%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
354	*Clostridium_sensu_stricto_7*	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
363	*KD4-96_unclassified*	0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**0.3%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%
372	*OM1_clade_unclassified*	0.1%	-0.1%	**0.3%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%
385	*Intestinimonas*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**
387	*Flavivirga*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
404	*Profundibacterium*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%
414	*BD1-5_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
425	*Pir4_lineage*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
430	*SB-1_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
438	*Family_XII_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	0.0%	0.0%	0.0%
440	*Acidiferrobacter*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**
446	*Clostridium_sensu_stricto_11*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
448	*Clostridium_sensu_stricto_12*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.5%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**
449	*Fusibacter*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**
489	*Fusibacter*	0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**1.5%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**0.3%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%
501	*NB1-n_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.6%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
523	*Pelagibius*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	0.0%
535	*Peptococcaceae_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.5%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
539	*Vibrio*	0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	**0.0%**	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%	-0.2%
593	*Prolixibacter*	0.0%	0.0%	0.0%	0.0%	**0.4%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
609	*Fusobacteriaceae_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.5%**	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
650	*Fusibacter*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**2.0%**	**2.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
656	*Sphingobacteriales_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
697	*Sva0081_sediment_group*	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
722	*SEEP-SRB1*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
772	*Anaerovorax*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
815	*GR-WP33-30_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
862	*TA18_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
881	*Incertae_Sedis*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**1.0%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
933	*WCHB1-69_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%
952	*Rhodobacteraceae_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%
1033	*Acetobacterium*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
1046	*Spirochaeta*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.4%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
1113	*Gammaproteobacteria_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%
1350	*Family_XII_unclassified*	0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**0.3%**	-0.1%	-0.1%	-0.1%	-0.1%	**0.1%**	**0.3%**
1452	*Family_XII_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**
1606	*Fusibacter*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.2%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
2588	*Fusibacter*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
2625	*Fusibacter*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.6%**	0.0%	0.0%	0.0%	0.0%
2778	*Desulfobaculum*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.8%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
3187	*Photobacterium*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
3664	*Fusibacter*	0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**0.3%**	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	-0.1%	**0.3%**
3700	*Clostridiales_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
3703	*JTB215_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.5%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
3831	*Family_XII_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.3%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
3864	*Family_XII_unclassified*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	**0.1%**	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%