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Full Length Research Paper

Vertical shifts in the microbial community structure of organic-rich Namibian shelf sediments

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This study investigates the diversity and abundance of bacteria in organic-rich Namibian shelf sediments from two sampling stations, using the 16S rRNA library approach and Catalyzed Reporter Deposition Fluorescent *in situ* Hybridization (CARD-FISH). Six clone libraries were constructed. Clone libraries were dominated by Delta-proteobacteria (up to 48%) and Gamma-proteobacteria (up to 98%). Bacteroidetes were dominant in the clone library of the top 6 cm (up to 17%), while actinobacteria dominated at a depth of 10 to 12 cm (up to 34%). Sequences that were related to bacteria with hydrolytic and fermenting abilities include members from the Gamma-proteobacteria, Bacteroidetes, Actinobacteria, and Acidobacteria. Cloned sequences within the Delta-proteobacteria affiliates to sulfate reducing bacteria, including Desulfarculaceae, Desulfobacteraceae, Desulfobulbaceae, and Desulfuromonadales and were detected throughout the sediment. The two sampling stations differed in microbial diversity with a higher diversity prevailing at the station with higher metabolic rates for organic matter decomposition. At both sampling stations a shift in microbial community composition with depth was observed and is explained by gradients in organic substrate availability within the sediment, which affects the life strategies adopted by bacteria, resulting in niche diversification and ultimately affects bacterial community composition and structure throughout the sediment depth.

Key words: Namibia, upwelling, microbial, diversity, abundance.

INTRODUCTION

Knowledge of the phylogeny and distribution of microorganisms within marine sediments is essential for understanding the biogeochemical function and ecology of sediment bacteria globally (Lin et al., 2006; Gray and Head, 2001). Previous studies performed in sediments from the highly productive continental shelf off Namibia in the Benguela upwelling region at two closely spaced sampling stations with contrasting biogeochemical rates, revealed a steep downward decrease in biogeochemical rates that compared well with gradients in the abundance of active bacteria (Julies et al., 2010). The distribution of the metabolically active members within a fraction of the microbial community was determined in the previous study, using Catalyzed Reporter Deposition Fluorescent *In Situ* Hybridization (CARD-FISH) (Julies et al., 2010). However, this technique fails to provide a detailed characterization of the overall microbial diversity (Mills et al., 2005). Therefore, the aim of our study is to characterize the microbial community involved in the different steps of organic carbon mineralization along a vertical depth profile within sediments from the highly productive continental shelf off Namibia in the Benguela upwelling area. To our knowledge only one other study

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by Schäfer et al. (2007) tried to characterize the microbial community in surface and sub-surface sediments on the Namibian coast. However, they analyzed samples from the continental slope, while our study focused on sediment from the shelf.

Our study provides new insights into the microbial ecology in these upwelling sediments by linking identity and activity of microorganisms involved in the transformation of organic carbon. We hypothesize that the organic-rich sediments on the Namibian shelf support a high microbial diversity, especially in the top 6 cm of the sediment where higher biogeochemical rates were measured, and that the gradients in microbial activity with depth is reflected in the microbial diversity and abundance, because of different selective pressures existing throughout the depth of the sediment. Therefore, we expect a change in the composition of the microbial community with depth.

We studied microbial diversity in Namibian shelf sediments by polymerase chain reaction (PCR) based 16S rRNA cloning and sequence analysis. Clone libraries are useful for obtaining a snap-shot of microbial diversity within the sediment. This study provides an initial insight into the microbial diversity in the sediment and understanding of the community structure illuminates the key players in organic carbon transformation within the Namibian upwelling system, which improves understanding of productive marine ecosystems.

MATERIALS AND METHODS

Study site

Sediment samples were collected during March, 2004 on an expedition to the Namibian shelf with the RV Alexander von Humboldt from two stations that were in close proximity to each other. Station 3 is located at S22° 38.03; E14° 18.27 and Station 5 at S23° 45.08; E14° 18.27. The water depth is 70 m at Station 3 and 112 m at Station 5. The sediment at Station 3 had a dark, olive green mud and the first 5 cm was a soft fluff. Below 5 cm depth the sediment consisted of mica and fine sand. No bacterial mat was visible and only a low abundance of *Thiomargarita* was detected. The top 15 cm of the sediment at Station 5 was extremely soft and fluffy, had an olive green colour. At a depth of 15 to 20 cm depth light-coloured precipitates were observed. Sediment at Station 5 was covered by a thick layer of *Beggiatoa.*

At the time of sampling, the bottom water temperature was 13.3°C at Station 3 and 11.8°C at Station 5. Concentrations of dissolved oxygen in the bottom water measured about 2 m above the sediment surface were less than 4.5 µmol L^{-1} at both stations. The rate of biogeochemical reactions involved in organic carbon mineralization, measured as sulfate reduction rates and potential hydrolysis rates differed significantly at the two sampling stations (Julies et al., 2010). Dissolved organic carbon (DOC) concentrations were 1.5 and 1.3 mmol L^{-1} in the top 2 cm of the sediment at Stations 3 and 5, respectively. At a depth of 10 to 12 cm DOC concentrations were 1.6 and 2.0 mmol L^{-1} at Stations 3 and 5, respectively (Julies et al., 2010). Sulfate reduction rates at Station 3 were 162 nmol cm^{-3} d^{-1} in the top 2 cm, while it were only 4 nmol cm^{-3} d^{-1} at a depth of 10 to 12 cm (Julies et al., 2010). At Station 5 sulfate reduction rates were 41 nmol cm^{-3} d^{-1} in the top 2 cm of the sediment and 28 nmol cm^{-3} d^{-1} at 10 to 12 cm depth (Julies et al., 2010). Hydrolysis rates of polysaccharides were measured in a previous study and were between 11 and 23 nmol cm^{-3} d^{-1} in the top 2 cm of the sediment at Station 3, while it was between 8 and 21 nmol cm^{-3} d^{-1} at Station 5 (Julies et al., 2010). At a depth of 10 to 12 cm hydrolysis rates were between 3.5 and 7 nmol cm^{-3} d^{-1} at Station 3 and 6.5 to 8 nmol cm^{-3} d^{-1} at Station 5 (Julies et al., 2010).

Sampling

Sediment samples were obtained with a multiple sediment corer (MUC), from which 26 mm diameter sediment sub-cores were taken with a length of 14 and 16 cm at Station 3 and 5, respectively. Sub-cores were collected from the same cast and stored at 12°C until they were sectioned within 6 h after collection. Two parallel cores were sectioned per station. Cores were sectioned in two centimeter intervals, which correspond to a sediment volume of about 10 cm^3. Half of the sediment from each section was stored immediately at -20°C for construction of 16S rRNA clone libraries. The other half was divided into 0.5 cm^3 aliquots that were fixed for 24 h in 1.5 ml formaldehyde (4% v:v). The sediment was then washed twice with 1× phosphate-buffered saline (PBS; 4 mmol L^{-1} sodium phosphate [pH 7.2], 130 mmol L^{-1} NaCl) and it was finally stored in a mixture of 1x PBS and ethanol (2:3 v:v) at -20°C.

DNA extraction, PCR amplification and construction of 16S rRNA clone library

Total community DNA was extracted directly from duplicate sub-cores obtained at each sampling station. From each core 0.5 g (wet weight) sediment from three different depths was used to extract DNA using the BIO 101 Fast DNA spin protocol. 16S rRNA clone libraries were constructed for the following depth intervals: the surface of the core (0 to 2 cm), the middle section (4 to 6 cm) and the bottom of the core (10 to 12 cm). These depths were chosen because biogeochemical rate measurements indicated the highest rates of hydrolysis and sulfate reduction occur in the topmost 5 cm of the sediment and there is a steep decrease in rates below 5 cm depth (Julies et al., 2010). Purified DNA from the sediment was used as a template for the polymerase chain reaction (PCR) amplification. *Bacteria*-specific 16S rRNA primer pairs were used for amplification and included the forward primer GM3F (5'-AGAGTTTGATCMTGGCTCAG-3') and the reverse primer GM4R (5'-TACCTTGTTACGACTT-3') (Muyzer et al., 1995). Primers were synthesized by Pharmacia Biotech. The PCR mastermix consisted of 5 µL of 10× Taq-polymerase buffer, 4 µL dNTPs (2.5 mmol L^{-1} each), 5 µL bovine serum albumin (3 mg ml^{-1}), 0.15 µL of Taq polymerase (5 U $µl^{-1}$), 1 µL of each of the primers (50 µmol L^{-1}), 2 µL of DNA extract and 31.8 µL ultrapure, sterile water. The PCR was run on a Master-cycler (Eppendorf) at the following cycling conditions: 1 cycle at 96°C for 3 min followed by 30 cycles consisting of three steps, the first step at 96°C for 1 min followed by an annealing step at 50°C for 1 min and a third step at 72°C for 3 min. The final cycle was run at 72°C for 10 min PCR products were inspected by gel electrophoresis on 1% (w:v) agarose gels. PCR products were purified with the QIAquick PCR purification kit (Qiagen). Purified PCR products were ligated and cloned into high efficiency competent cells of *Escherichia coli* (JM109) using the TOPO TA cloning kit (Invitrogen). The transformed cells were plated on Luria-Berani (LB) selective agar plates which contained ampicilin (100 µg ml^{-1}), isopropyl-ß-D-thiogalactopyranoside (IPTG, 0.5 mmol L^{-1}), and 5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside (X-Gal, 80 µg ml^{-1}) and incubated overnight at 37°C. X-Gal and IPTG were added for the purpose of blue/white colony screening to distinguish between recombinant (white) and non-recombinant (blue) colonies. After incubation white colonies were transferred into LB culture

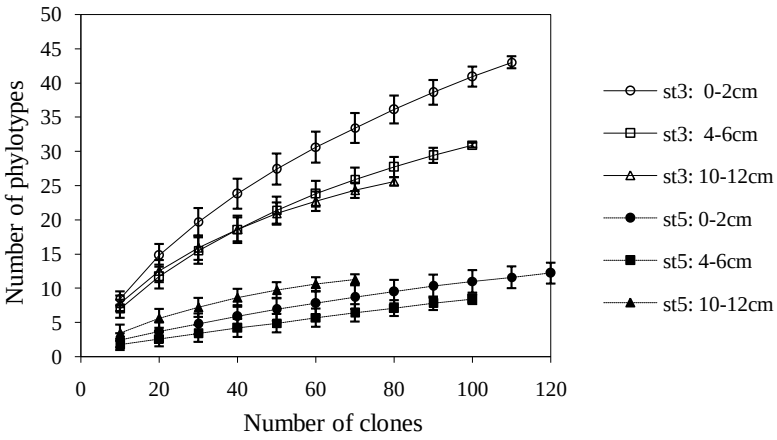


Figure 1. Rarefaction analysis of the clone libraries obtained from Station 3 and Station 5 was performed with the free online analytic rarefaction 1.3 software (Holland 2003, Analytic Rarefaction 1.3. Accessed June, 2006 www.uga.edu/~strata/software/). The error bars are 99% confidence levels.

medium (10 g L^{-1} Bacto-tryptone, 5 g L^{-1} NaCl, 5 g L^{-1} Bacto-yeast extract, 15 g L^{-1} agar, 100 µg mL^{-1} ampicilin, 80 µg mL^{-1} x-Gal, and 0.5 mmol L^{-1} IPTG) for 18 h to allow amplification. Plasmid was extracted from clones with correctly sized inserts of 1.5 kb using the Montage Plasmid $Miniprep_{96}$ kit (Millipore, Bedford, Mass). Plasmid quality was checked with gel electrophoresis.

For sequencing of plasmid inserts, 1 µL of plasmid DNA (100 ng $µL^{-1}$) was used and sequencing was performed with ABI BigDye on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Partial sequencing of 300 to 500 base pairs (bp) was performed using the forward and reverse16S rRNA primers GM1F (5'-CCAGCAGCCCGGTAAT-3') and GM1R (Muyzer et al., 1993). In order to obtain full sequences, clones were amplified with vector primers M13F (5'-GTAAAACGACGGCCAG-3') and M13R (5'-CAGGAAACAGCTATGAC-3') (Messing, 1983). Sequences were aligned and compared to 16S rRNA sequences that were present in the dataset of the Ribosomal Database Project (RDP) in January, 2004 (~95 000 rRNA sequences, http://rdp.cme.msu.edu) by using the automatic aligning tool of the ARB software (Ludwig et al., 2004) and the results were corrected manually where necessary. Subsets of sequences were selected for nearly full-length sequencing (> 1400 bp). Nearly full length sequences were deposited in GENBANK (http://www.ncbi.nlm.nih.gov/Genbank/index.html) and the accession numbers are EU290675-EU290740.

Phylogenetic analysis

Phylogenetic distances were calculated using the Jukes-Cantor model. Tree topologies were evaluated by the neighbor joining, maximum parsimony and maximum likelihood method. For this study we defined that > 97% similarity in sequences represented the same rDNA clone type. Sequences were analyzed for chimeras using the Bellerophon software program (Huber et al., 2004). Sequences that showed potential chimeric origin were excluded from further analysis (n = 1).

Analysis of coverage by clone libraries

A total of six clone libraries have been constructed. The proportion of operational taxonomic units (OTUs) that were represented in the different clone libraries were estimated using rarefaction analysis (Holland, 2003, Analytic Rarefaction 1.3. Accessed June, 2006 www.uga.edu/<strata/software/).

Catalyzed reporter deposition fluorescent *In Situ* hybridization (CARD-FISH)

Relative abundance of bacteria was determined at both Station 3 and 5. Duplicate sub-cores were sectioned in two centimeter intervals. The fixation, preservation, sonication, hybridization and staining of bacterial cells in the sediment and subsequent microscopic evaluation are described in our previous study at these two sampling stations (Julies et al., 2010). Information on the probes and conditions used during hybridization to quantify active members of the Gamma-proteobacteria, SRB, and Bacteroidetes phylum are also summarized in our previous study (Julies et al., 2010). In this study, we determined the abundance of active members of the Alpha-proteobacteria with probe Alf968 (Neef, 1997), Planctomycetes with probe Pla46 (Neef et al., 1998), and Actinobacteria with probe HGC69a (Roller et al., 1994) at Station 5. The probe HGC69a targets part of the 23S rDNA, while all the other probes used target part of the 16S rRNA. All nucleotide probes used were purchased from Interactiva (Ulm, Germany).

RESULTS

Coverage by clone libraries

At Station 3, a total of 314 bacterial clones (135 clones from the top, 87 clones from the middle and 92 clones from the bottom) were sequenced. The clone library consisted of 282 partial and 32 full length sequences. At Station 5, a total of 423 bacterial clones (182 clones from the top, 105 clones from the middle and 136 clones from the bottom) were sequenced. The clone library consisted of 405 partial and 18 full length sequences (a). Rarefaction analysis indicated that the size of the clone libraries were sufficiently large to cover most of the diversity in the sediment at Station 3 (Figure 1).

Diversity and vertical zonation of bacteria

Station 3

The bacterial 16S rRNA clone libraries were diverse at Station 3 and included sequences affiliated with most phyla previously detected in marine sediments. Members of the proteobacteria dominated the clone libraries, in particular members from the Delta-proteobacteria (34% at 0 to 2 cm; 48% at 4 to 6 cm; 20% at 10 to 12 cm) and Gamma-proteobacteria (14% at 0 to 2 cm; 25% at 4 to 6 cm; 8% at 10 to 12 cm) (Figures 3a-c). Members of the Bacteroidetes were also dominant, especially in the top 2 cm of the sediment where they made up 17% of the total clones (Figure 3a). Members from the alpha-proteobacteria, ε-proteobacteria, Candidate divisions BRC1 and OP11 and Bacteria/Hyd24-12 were only observed in the clone library of the top sediment, while members of the Nitrospirae were only observed in the clone library from the bottom sediment (Figures 3a-c). All other OTUs were detected throughout the sediment at Station 3.

Station 5

The bacterial 16S rRNA clone libraries at Station 5 represented fewer OTUs (Figures 3d-f), compared to Station 3. The clone libraries were dominated by Gamma-proteobacteria (51 to 98%) (Figures 3d-f) throughout the sediment and by Actinobacteria in the bottom sediment (37% of total clones obtained in the bottom sediment) (Figure 3f). Other OTUs represented less than 5% of the sequences in the clone libraries at Station 5. Cloned sequences affiliated to the Bacteroidetes bacteria were only observed in the clone library of the top sediment, while sequences representing Actinobacteria were only observed in the clone library from the bottom sediment (Figures 3d and f).

The sequences affiliated to the Alpha-proteobacteria were closely related to uncultured bacteria and *Ahrensia.* The majority of the sequenced clones of the Bacteroidetes phylum in the clone libraries were closely related to Sphingobacteria and Flavobacteria. At both stations the clone libraries were dominated by Gamma-proteobacteria affiliated to uncultured sulfide oxidizing symbionts. Most of the Delta-proteobacteria in the clone libraries was sulfate reducing bacteria (SRB), belonging to the Desulfarculaceae, Desulfobacteraceae, Desulfobulbaceae, Desulfuromonadales and a few other cultured representatives (Figure 4). Almost full length sequences affiliated to a variety of genera, including *Desulfocapsa, Desulfatibacillum, Desulfosarcina, Desulfobacula* and *Desulfobacterium.*

Blast results from cloned full length sequences that are affiliated to the Planctomycetes revealed that they are related to uncultured members and the genera *Pirellula* and "*Candidatus Scalindua brodae"* (AY254883.1).

Distribution and abundance of bacteria

More than 80% of the total DAPI cell counts hybridized to the bacterial probe, EUB I-III, in the top 2 cm of the sediment (Figures 2a and b), indicating high amounts of 16S rRNA in bacterial cells and revealing high metabolic activity of microorganisms. The absolute prokaryote abundance in the top 2 cm of the sediment was similar at both sampling stations (Figures 2a and b). At a depth of 2 to 4 cm absolute abundance of bacteria is two-fold higher at Station 3, compared to Station 5. However, below 4 cm depth Station 5 had a 3 to 4 fold higher abundance of prokaryote cells than Station 3.

The relative abundance of the metabolically active SRB, Bacteroidetes bacteria, and Gamma-proteobacteria was determined with CARD-FISH in our previous study at these two sampling stations (Julies et al., 2010) and the results are briefly summarized here in Figure 2c for the purpose of comparison to our clone library results. Due to the detection of Planctomycetes, Actinobacteria and Alpha-proteobacteria in the clone libraries, specific probes targeting these bacteria were used to establish their relative abundance. The relative abundance of active Planctomycetes, Actinobacteria, and Alpha-proteobacteria at Station 5 decreases with depth from ~24, 3, and 23%, respectively of the total DAPI stained cells in the top 2 to 4 cm of the sediment to ~7, 1, and 4%, respectively of the total DAPI stained cells at a depth of 12 to 14 cm (Figure 2d).

DISCUSSION

Shift in microbial community structure with depth

Dissolved organic carbon (DOC) is labile to degradation in the surface sediment and become increasingly resistant to degradation below a depth of 10 cm (Julies et al., 2010). Thus, there is an increasing substrate limitation for the active bacteria with depth, because DOC becomes increasingly unavailable to the microorganisms with increasing depth (Julies et al., 2010). This results in vertical depth gradients of organic compounds that serve as energy sources for microorganisms. We examined the effect of these gradients on the composition of the microbial community by constructing clone libraries at the two sampling stations from three vertical depths within which the biogeochemical environment and availability of DOC differ. This study revealed a significant shift in the microbial community structure with depth.

Microbial response to the available DOC in the sediment depends on the physiological capabilities of the microorganisms (Madrid et al., 2001), which ultimately determines which microorganisms can co-exist successfully within the environment. Microorganisms adapt to exploit the various pools of organic carbon throughout the sediment depth indicating a vertical niche separation. The partitioning of resources within the

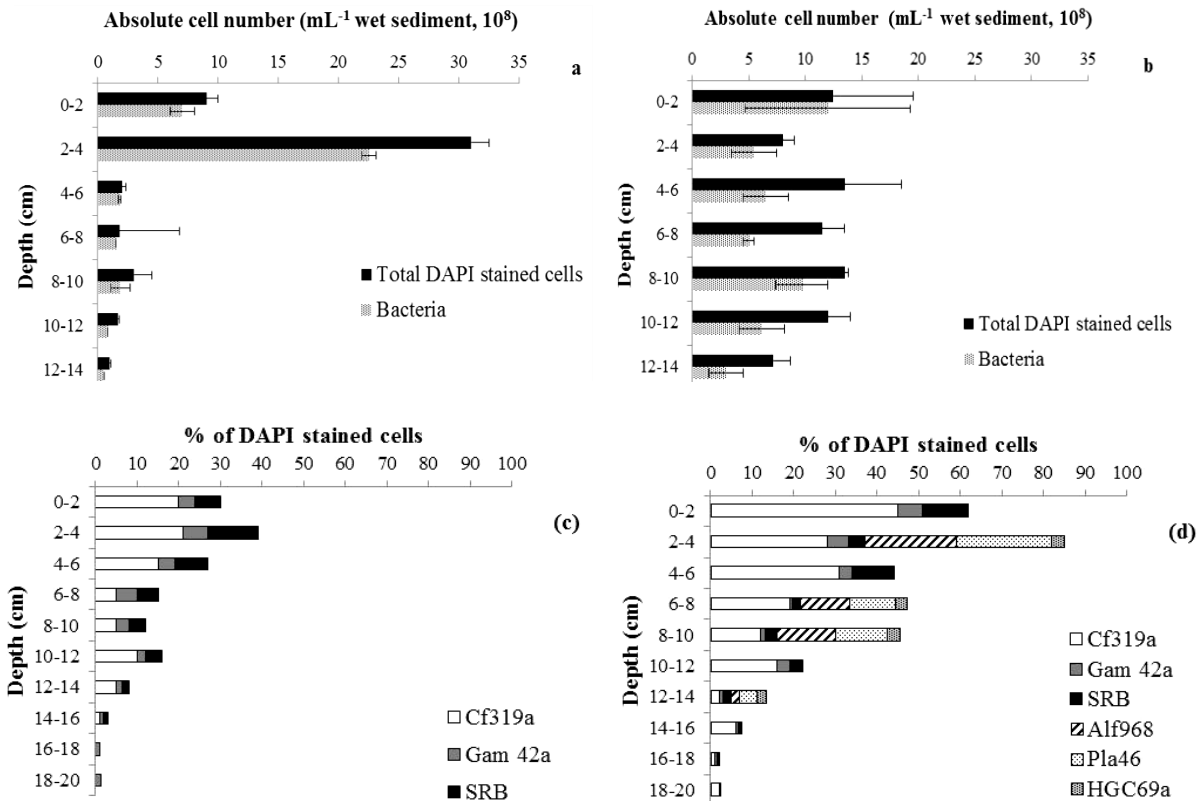


Figure 2. (a and b) Abundance of bacteria at (a) Station 3, and (b) Station 5. The data is from duplicate cores. (c) and d) Relative abundance of the different groups of bacteria at (c) Station 3, and (d) Station 5. The relative abundance of SRB is the total abundance established with the probes DSR 651, DSS 658, and DSB 985. The relative abundance of the different phylogenetic groups is the averages from duplicate cores.

sediment creates specific niches, enhancing microbial specialization and division into distinct ecological guilds (Torsvik et al., 2002). OTU niche specificity was also observed in gas hydrates from the Gulf of Mexico (Mills et al., 2005). We illustrate that such a niche partitioning also exists in Namibian sediments by analyzing the various OTUs distribution and abundance throughout the sediment depth at both sampling stations.

Alpha-proteobacteria and *ε-proteobacteria*

Alpha-proteobacteria were detected in the water column of other oxygen minimum zone areas, where their relative abundance may reach up to 20%, but their abundance decrease with depth (Fuchs et al., 2005). This explains their low contribution to clone libraries in Namibian shelf sediments (less than 3%) and other marine sediments (Llobet-Brossa et al., 1998). The sequences were closely related to uncultured bacteria and *Ahrensia.* Members of *Ahrensia* have diverse metabolisms (Uchino et al., 1998), among which the ability of thiosulfate oxidation exists (Teske et al., 2000). ε-Proteobacteria may live in deep anoxic waters as obligate or facultative chemoorganotrophs by fermenting organic matter (Lin et al., 2006). They can act as sulfur reducers and/or oxidizers and have been detercted in the 16S rRNA gene libraries from redoxclines of anoxic waters of the Cariaco Basin and the Black Sea (Lin et al., 2006). The restriction of *α*- and ε-proteobacteria to the top 2 cm of the sediment may be explained by the high sulfate reduction rates (100 to 220 nmol.cm^{-3}.d^{-1}) in the top 2 cm of the sediment at Station 3 (Julies et al., 2010), providing ample substrate for sulfur oxidizers and reducers. Further evidence for this is provided by the 5 times higher abundance of active Alpha-proteobacteria in the top 2 to 4 cm (Figure 2d) where higher sulfate reduction rates occur compared to the sediment at a depth of 12 to 14 cm where rates of sulfate reduction are lower, at Station 5.

Bacteriodetes

The Bacteroidetes typically contains bacteria with hydrolytic and fermenting abilities (Weller et al., 2000) and active members were previously detected in anoxic sediments (Llobet-Brossa et al., 1998). The presence of Bacteroidetes bacteria in clone libraries up to a depth of

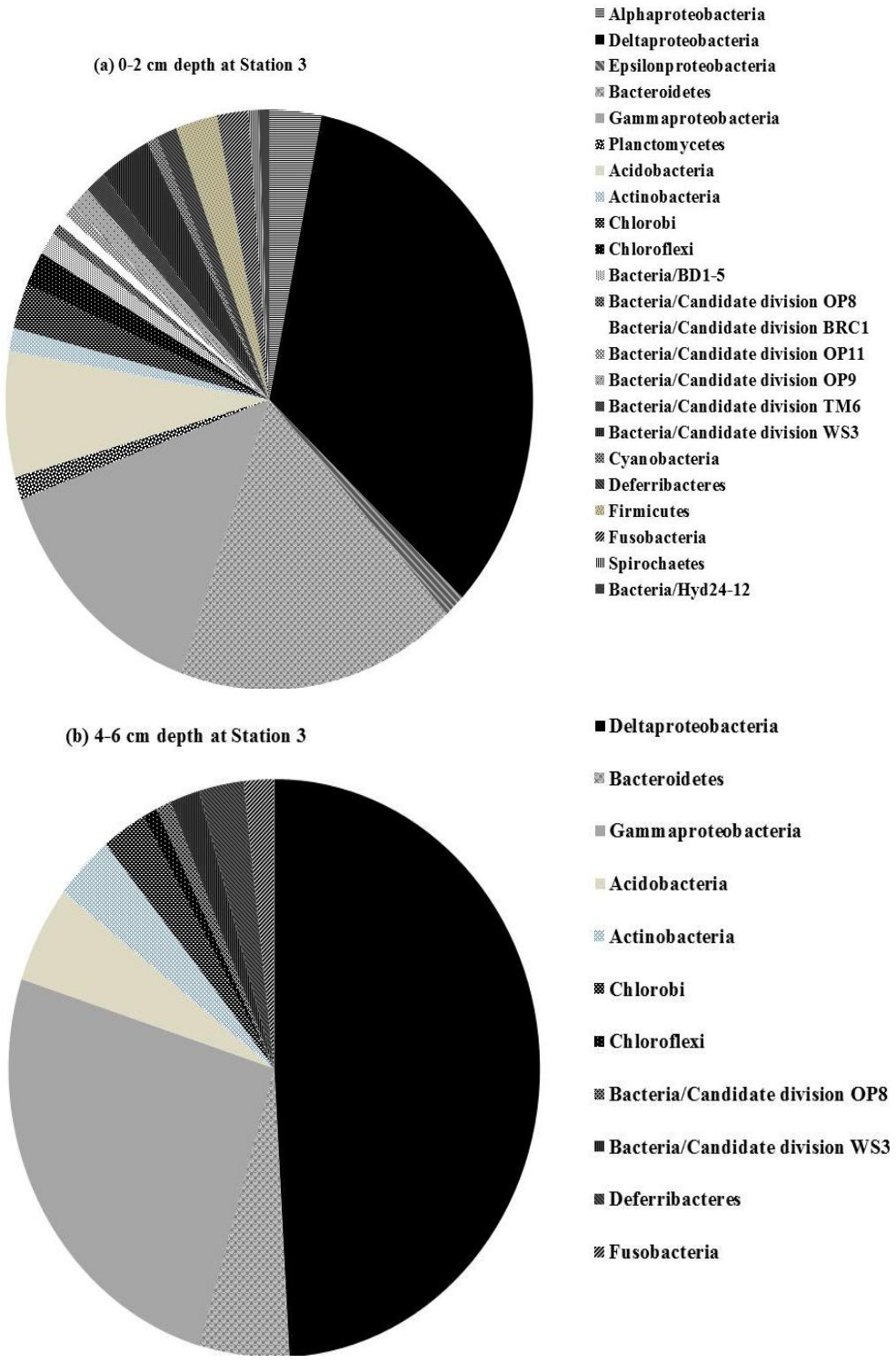


Figure 3. Microbial diversity at Station 3 (a-c) and Station 5 (d-f) at different depth intervals of 0 to 2 cm, 4 to 6 cm and 10 to 12 cm. The figure indicates the relative contribution of various operational taxonomic units (OTUs) to the clone library from duplicate cores at each depth interval.

12 cm, suggest that anaerobic members from the Bacteroidetes play an important role in hydrolytic and fermenting processes in anoxic, organic-rich sediments. They can be classified as copiotrophs and are dominant in environments with high carbon availability and re-mineralization rates (Fierer et al., 2007). Such an

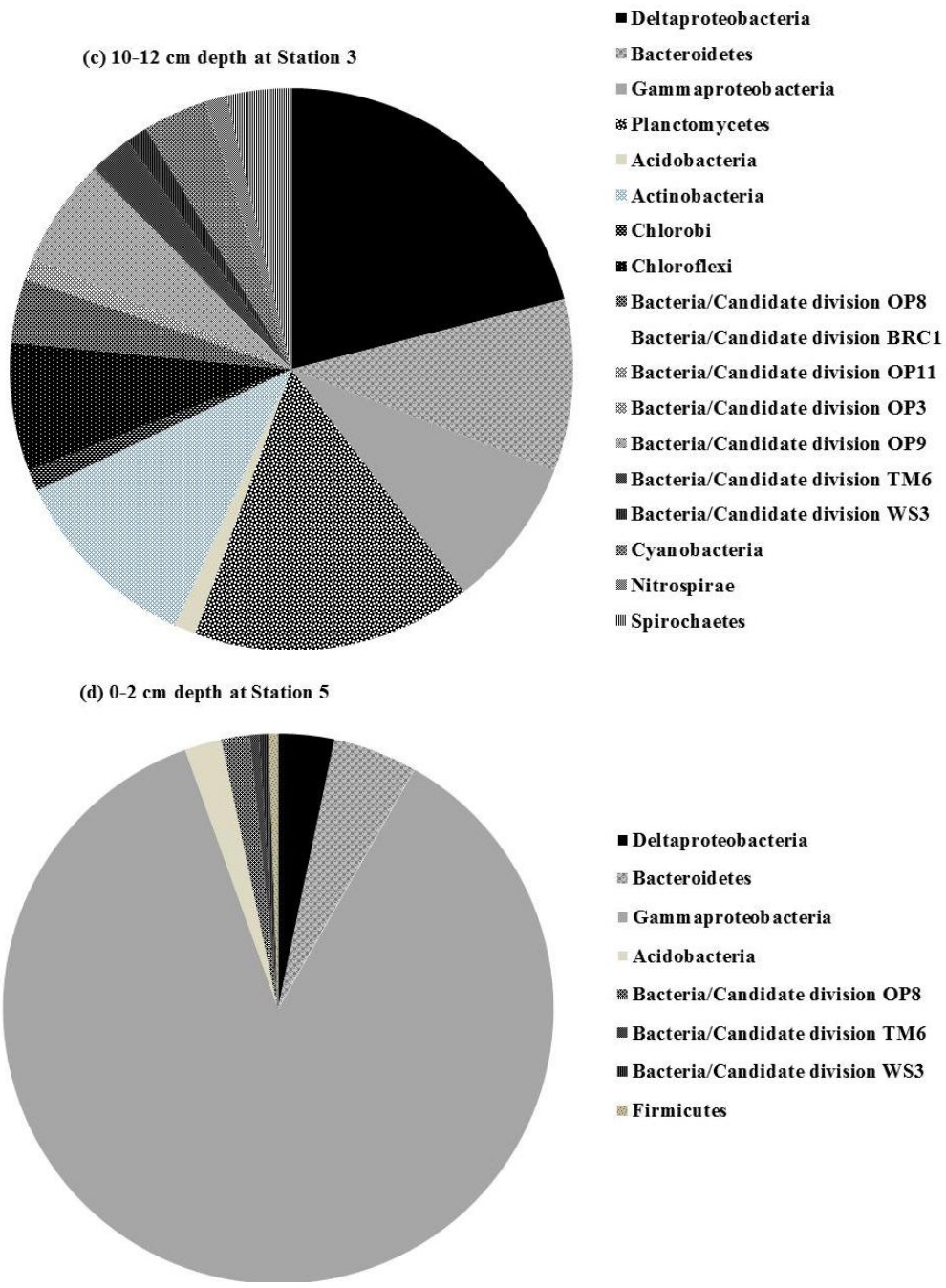
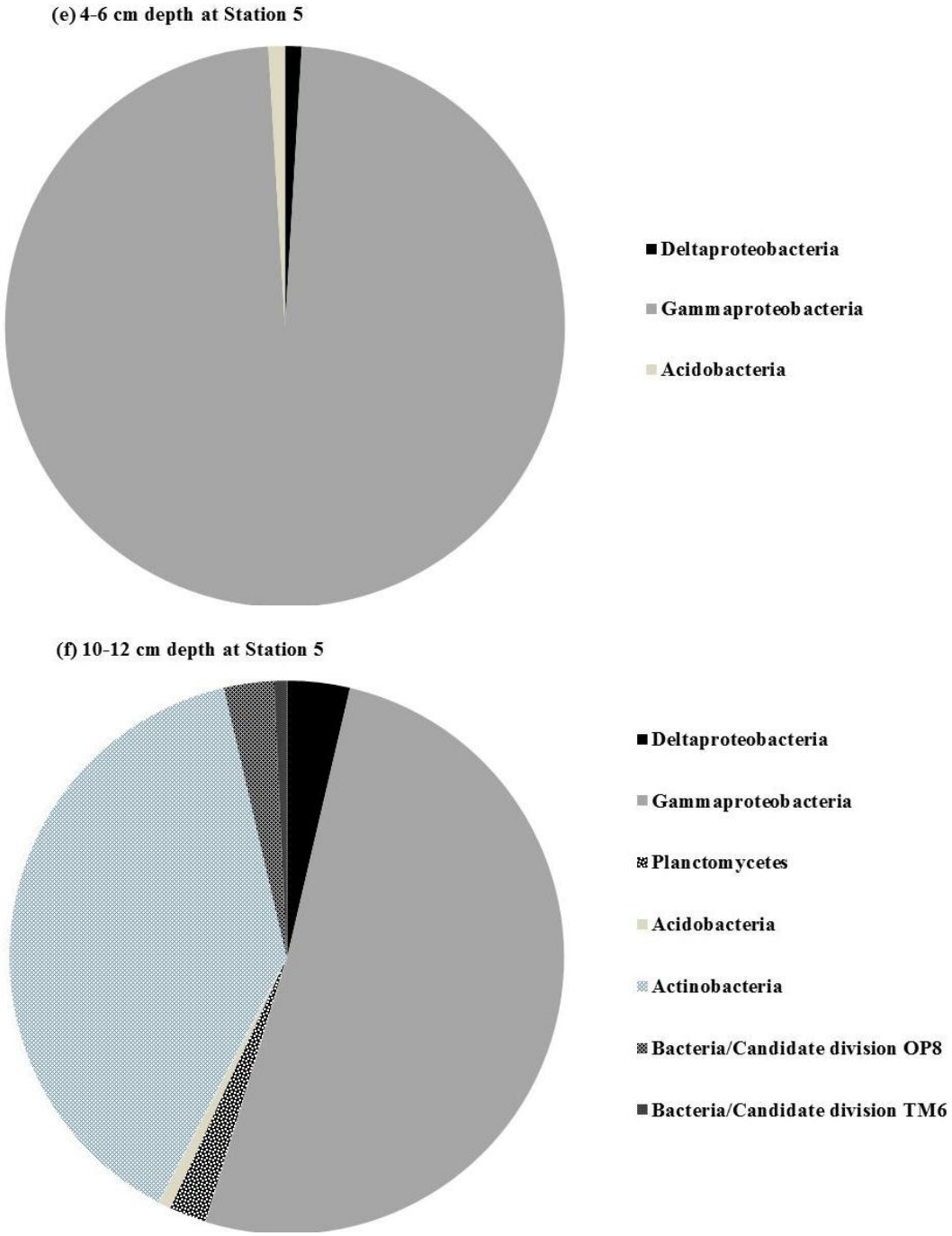


Figure 3. Cont.

environment exists in the top 10 cm of Namibian shelf sediment.

Gammaproteobacteria

Gamma-proteobacteria occurred throughout the sediment and dominated the clone libraries, especially at Station 5. Most of the Gamma-proteobacteria affiliated to uncultured sulfide oxidizing symbionts. No bacterial mat was observed at Station 3 and a low abundance of large sulfur bacteria, capable of sulfide oxidation were recorded. However, previous recordings were made of *Beggiatoa* and *Thiomargarita* mats at these two stations (Brüchert et al., 2006). Thus, bacterial sulfide oxidation in the surface sediment is performed by both the large-sulfur bacteria and sulfide oxidizing Gamma-proteobacteria.



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Delta-proteobacteria

In our clone libraries the majority of Delta-proteobacteria belonged to the Desulfarculaceae, Desulfobacteraceae and Desulfobulbaceae and active members of these groups were also detected in clone libraries of other marine sediment (Dhillon et al., 2003). CARD-FISH with specific probes, targeting the genera *Desulfocapsa, Desulfatibacillum, Desulfosarcina, Desulfobacula* and *Desulfobacterium,* to which almost full length sequences in the clone libraries affiliated, indicated a high activity of these bacteria. The high species diversity of SRB in the clone libraries can be explained by their diverse metabolic capabilities (Devereux et al., 1992). They can use a variety of carbon sources, such as different volatile or long-chain fatty acids, alcohols or aromatic compounds

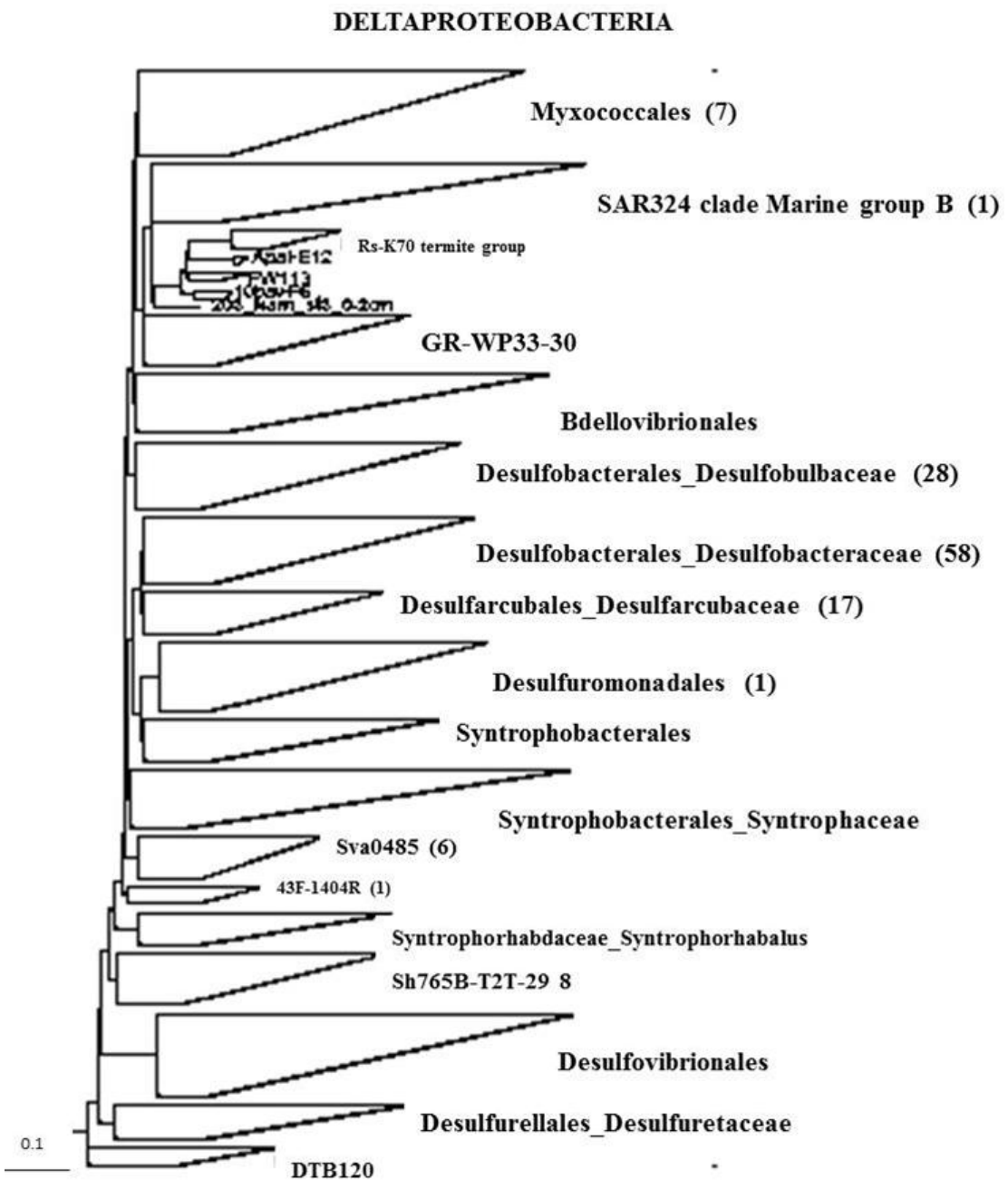

Figure 4. Phylogenetic affiliation of sequences in the clone libraries to various families within the delta-proteobacteria. The number of sequences in the different groups of delta-proteobacteria is shown in brackets. Scale bar = 10% estimated sequence divergence.

(Widdel and Hansen, 1992). The high diversity of SRB throughout the sediment depth is a result of niche partitioning, which is the way in which the different species distribute the available resources in the environment. The diverse physiological abilities of SRB allow them to occur throughout the sediment, while their absolute abundance is limited by the quantity and quality of substrate available.

Planctomycetes

Planctomycetes can oxidize organic substrates through nitrate reduction (Fuerst, 1995) and some are also capable of heterolactic acid fermentation (Glöckner et al., 2003). "*Candidatus* Scalindua brodae" is capable of anaerobic ammonium oxidation (ANAMMOX) (Kirkpatrick et al., 2006). Cloned sequences of this genus in the water

column of the Namibian shelf were previously reported (Woebken et al., 2007). The high relative abundance of active Planctomycetes in the top 2 to 4 cm of the sediment (24% of the total DAPI stained cells) suggests that these bacteria play a significant role in the oxidation of organic substrates, and are probably also involved in ANAMMOX within the top sediment.

Actinobacteria

Actinobacteria contribute to only a small fraction of the bacterial communities in marine sediments (Goodfellow and Williams, 1983). Although these bacteria dominated the clone library in the bottom sediment of Station 5 (38%), CARD-FISH data revealed that the number of active Actinobacteria within the sediment at Station 5 is low (1 to 3% of the total DAPI stained cells). A few studies reported a significant contribution of these bacteria to clone libraries from marine sediments (Ward and Bora, 2006; Stach and Bull, 2005). Actinobacteria are known for their ability to degrade complex polymers such as alginates, laminarin and hydrocarbons (Goodfellow and Williams, 1983). The physiological abilities of Actinobacteria allow active members of these bacteria to thrive in organic-rich Namibian shelf sediments where an increase in the complexity of dissolved organic carbon with depth occurs.

Differences between the two sampling stations

There exist a de-coupling of initial and terminal processes involved in organic carbon mineralization and a greater accumulation of increasingly refractory DOC with depth at Station 3 compared to Station 5 (Julies et al., 2010). The higher amount of refractory organic carbon in the deeper sediment (10 to 12 cm) at Station 3 makes it an environment with limited carbon resources. Competition for the limited, accessible DOC within the deeper sediment at Station 3 may lead to niche specialization and diversification, resulting in the higher OTU diversity reflected by the clone libraries. Therefore, the differences in microbial community structure and diversity between the two sampling stations reflect ecophysiological requirements of the bacteria, in particular with regard to the availability of dissolved organic carbon substrates.

This study revealed that microbial communities in organic rich sediments are structured by environmental factors, such as the quantity and quality of DOC as reflected by differences in microbial diversity with depth and between the sampling stations as well as biological factors, such as microbial physiological abilities, resulting in niche partitioning. We recognize that the physiology of most of the microorganisms are poorly understood and that other factors such as predation may also be selective factors structuring microbial communities, however, this initial survey of microbial community diversity in Namibian shelf sediments is an attempt to improve our understanding of the microbial ecology of such organic-rich sediments.

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