Microbial Community Structure and Dynamics of Wadden Sea Sediments (Dangast)

Dissertation
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
- Dr. rer. nat. -

dem Fachbereich Biologie/Chemie der
Universität Bremen
vorgelegt von

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Bremen
September 2000

Die vorliegende Arbeit wurde in der Zeit von Oktober 1997 bis September 2000 am Max Planck Institut für Marine Mikrobiologie angefertigt.

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Tag des Promotionskolloquiums: 30.10.2000

Per a na Corinna

This thesis would not have been possible without the help of many people, which I would like to thank all. I am indebted to Rudi Amann and Ramon Rosselló-Mora for their invaluable support over these years. In particular, I am most thankful to Ramon, who guided me and encouraged me to spend "some" time in Germany.

També vull donar les gracies a tots els que m'heu ajudat, tan professional com personalment. Especialment als amics i familiars, per escoltar-me i ajudar-me sempre que ho he necessitat.

Contents

Summary

Intertidal sediments are complex systems that play a prominent role in the global marine carbon cycle. The oxidation of organic carbon is mainly performed by microorganisms. There is a need to study marine sediment microbial diversity and structure to better understand the factors regulating these systems.

In this study, the microbial community together with the biogeochemical processes of the intertidal sediments from Dangast were analyzed. A FISH protocol was optimized to study the microbial diversity present in marine sediment samples. A maximum of 80 % of the microbial community present in Dangast sediments (up to 4.4 $x10^9$ cells cm^{-3}) hybridized with the universal probe EUB338, reflecting high bacterial activity in the sediments (chapter 2). The combination of different techniques showed that sulfate was the predominant electron acceptor for organic matter oxidation in those sediments and that *Desulfococcus-Desulfosarcina* group and *Desulfovibrio* spp. were the key populations for the terminal mineralization step with up to 2.9×10^8 and 2.8×10^8 cells per cm^{-3}, respectively (chapter 3 and 4).

This approach was followed for a period of two years. Seasonal changes of the microbial activity were reflected on periodic fluctuations of the stratification of different microbial populations as well as changes of geochemical parameters. Total bacterial counts (DAPI) as well as the percentage of detectable cells by FISH increased with temperature. The increase of the temperature resulted in an increase of bacterial activity. Sulfate reduction was enhanced with temperature most probably due to the dependence of the sulfate reducing bacteria (SRB) on the metabolites of the fermenting bacteria (chapter 5). Undisturbed sediment mesocosms were used to test this hypothesis. The results showed that the anaerobic mineralization of organic matter in marine sediments takes place stepwise with the SRB community limited by the availability of metabolites from the temperature-dependent fermentative microbial processes.

Zusammenfassung

Sedimente der Gezeitenzone spielen eine bedeutende Rolle des marinen Kohlenstoffkreislaufs, in denen das organische Material überwiegend von Mikroorganismen oxidiert wird. Um die System-regulierenden Faktoren besser zu verstehen, ist es notwendig, die mikrobielle Diversität und Gemeinschaftsstruktur zu untersuchen.

In dieser Arbeit wurde die mikrobielle Lebensgemeinschaft in Sedimenten der Gezeitenzone in Dangast unter gleichzeitiger Betrachtung geochemischer Prozesse mit molekularbiologischen Methoden analysiert. Dazu wurde ein Protokoll für eine Fluoreszenz in-situ Hybridisierung (FISH) optimiert, um so die mikrobielle Diversität in marinen Sedimentproben zu erfassen. Bis zu 80% (ca. 4.4 x 10^9 Zellen/cm^3) der mikrobiellen Lebensgemeinschaft im Dangaster Sediment hybridisierten mit der Sonde EUB338, spezifisch für einen Großteil der Eubakterien, was eine hohe mikrobielle Aktivität in diesem Sediment indiziert (Kapitel 2). Die Kombination verschiedener methodischer Ansätze zeigte eine große Bedeutung des Sulfats als Elektronen-Akzeptor für die terminale Mineralisation des organischen Kohlenstoffs. Dabei stellten sich Vertreter der *Desulfococcus-Desulfosarcina*-Gruppe und der *Desulfovibrio* spp. als Schlüsselarten mit bis zu 2.9 x 10^8 und 2.8 x 10^8 Zellen pro cm^3 heraus (Kapitel 3 und 4).

Über einen Zeitraum von zwei Jahren wurde die Dynamik dieser Parameter im Sediment verfolgt. Fluktuationen in der vertikalen Stratifizierung verschiedener Bakterien-Populationen sowie geochemischer Parameter korrelierten mit der gemessenen mikrobiellen Aktivität. Gesamtzellzahl-Zählungen anhand von DAPI und der Anteil mit FISH detektierbarer Zellen erhöhten sich mit der Temperatur. Eine Erhöhung der Temperatur führte zu einer verstärkten Aktivität der fermentierenden Fraktion der Gemeinschaft somit zu vermutlich erhöhten Metabolit-Konzentrationen. Den Sulfat-reduzierenden Bakterien verfügten damit über zusätzliches Substrat und verstärkten ihre Aktivität, was sich in einer höheren Sulfat-Reduktionsrate ausdrückte (Kapitel 5). Um diese Hypothese zu testen, wurden ungestörte Sedimentkerne in Mikrokosmos-Experimenten untersucht. Die Ergebnisse zeigten, daß die anaerobe Mineralisation des organischen Materials schrittweise über gärende und Sulfat-reduzierende Bakterien erfolgt, wobei letztere durch die Verfügbarkeit der Metabolite der Fermentierer limitiert sind.

1

General introduction
(results and discussion)

Introduction

„...Microbial diversity has become an important issue due to the importance of microorganisms in energy and matter transformation. Knowledge about bacterial community structure and diversity is essential to understand the relationships between environmental factors and ecosystem functions..."

Total bacterial diversity in soil and sediment communities.

V. Torsvik. 1996. J. Industr. Microbiol.

1. Marine sediments

1.1 Redox processes in marine sediments.

Marine sediments occupy 70% of the Earth's surface. Since most of the deposited organic matter in marine sediments is remineralized, they play an important role in the marine carbon cycle (13). The pore water composition in marine sediments mirrors the biogeochemical processes taking place throughout their vertical profile. According to the typical zonation (14), marine organic matter is oxidized by the oxidant yielding the greatest free energy change per mol of organic carbon oxidized. When this oxidant is depleted, oxidation will proceed utilizing the next most efficient (i.e. most energy producing) oxidant, and so on until either all oxidants are consumed or oxidizible organic matter is depleted (14). Therefore, organic matter oxidation in marine sediments is related to the consumption of oxygen followed by depletion of nitrate, the build up of Mn(II) and Fe(II) due to reduction of Mn(IV) and Fe(III) oxyhydroxides, respectively. Below this „suboxic" zone, sulfate reduction dominates

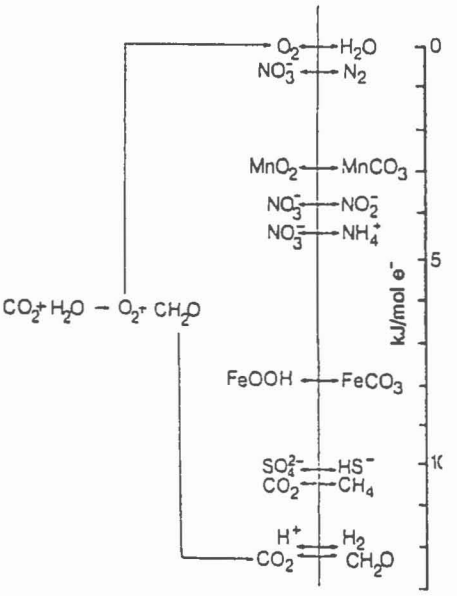

Figure 1. Free energy changes of proces involving two redox couples. From Fenc and Finlay (1995).

(the „sulfidic zone"), and beneath this, when sulfate is depleted, methanogenesis predominates (Fig.1).

This successional sequence is entirely based on equilibrium considerations, and is in many respects a realistic description of nature. However, the location of the different boundary layers is dependent on other factors. The depth at which the sediment becomes anaerobic depends on input of organic carbon, on light, which drives photosynthesis, and on bioturbation. In shelf seas, up to 50% of the production ends on the sediment surface, and the aerobic/anaerobic boundary layer is typically situated 1-6 mm beneath the surface (24). In the oceans, productivity is much lower and only 1% of the primary production of the surface waters reaches the bottom and the oxic zone of deep-sea sediments is much thicker (up to 50 cm; (22)). It is characteristic for sediments with a low input of organic matter that the suboxic zone situated between the oxic and the sulfidic zones is also relatively thick. Contrarily, in areas of high productivity, the anaerobic and sulfidic zone may extend almost to the sediment surface. The degradation and oxidation of the organic matter below the oxic zone is mostly carried out by microorganisms.

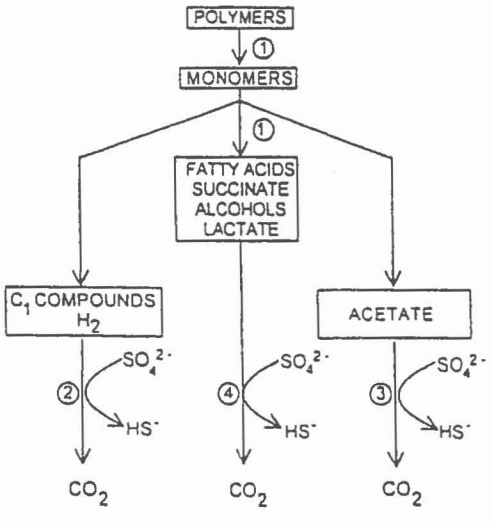

Figure 2. Carbon and electron flow through the various trophic groups of microorganisms involved in sulfate-dependent degradation of complex organic matter in a marine sediment. Groups pf bacteria involved: 1, primary fermenting bacteria; 2 to 4 SRB. From Widdel (1988).

Some of these organisms hydrolyze and ferment the macromolecules to smaller molecules, while others use oxidants other than O_2 to respire the compounds to CO_2 (20). Anaerobic mineralization takes place stepwise, involving a sort of food chain composed of several functional types of bacteria (13). Anaerobically respiring bacteria are dependent on the metabolites of fermenting bacteria and at the same time, the fermenters depend on methanogens and/or sulfate reducers for the removal of H_2. Due to the high concentration of sulfate in seawater, sulfate reduction is the dominating process in marine anoxic habitats, accounting in most cases for 90-99% of the anaerobic mineralization (59). In the presence of active sulfate reduction, there is very little or no methanogenesis. Therefore, only in high productive areas will

sulfate be totally depleted at a depth where there is still enough degradable organic material allowing the process of methanogenesis. The reason is competition for the two substrates common to methanogens and sulfate reducers; acetate and H_2.

In marine sediment communities, a variety of fermenting bacteria is responsible for the hydrolysis of organic polymers, yielding different fatty acids, alcohols and H_2 as metabolites (Fig.2). In sulfate containing sediments, these are further degraded by sulfate reducers, which are responsible for the terminal anaerobic mineralization. In the absence of sulfate, other types of fermenters (e.g. obligate acetogens) are necessary for the conversion of all substrates (butyrate, propionate, etc.) to acetate and H_2, which can be used by methanogens for terminal mineralization.

1.2. Intertidal mudflat sediments

Intertidal mudflats of the North Sea are highly influenced by river input. The dissolved and suspended matter in river waters pass through the coastal areas on their way to the sea. About 90% of the global flux of river particles and organic carbon are trapped in delta and shelf regions (15). Up to 30% of the marine primary production takes place in the area which encompasses shelf and coastal systems. Some 25-50% of this plankton primary production sinks to the sediment (62).

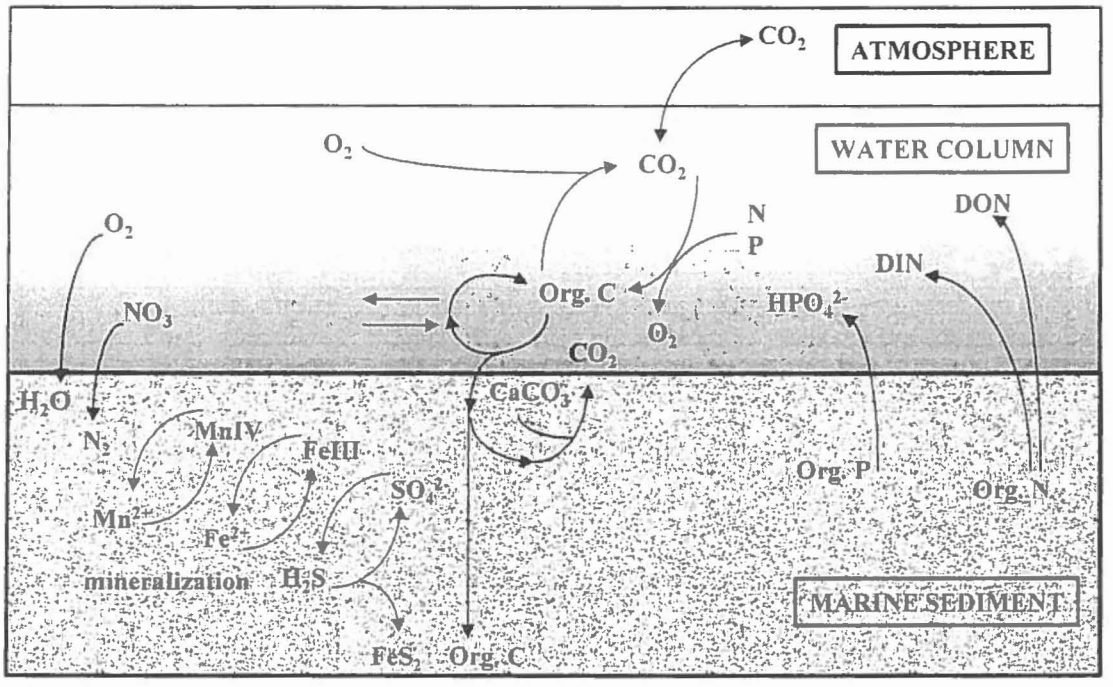

Figure 3. Summary of the typical processes in coastal marine sediments. Modified from Jørgensen (1996).

Most of the deposited organic matter is again mineralized. The budgeting of intertidal reactions is complicated by the drastic and seasonal variability of this environment. Variations of temperature, nutrient supply and substrate composition are typically high in intertidal mudflats. On top of these changes daylight- and tidal-cycles are additionally superimposed (47). Many biogeochemical reactions are mainly controlled by temperature (28). The sediment temperature varies seasonally, but depends also on the day and night cycle. Especially important for intertidal sediments are the tides inundating the sediment with waters of different temperatures. These temperature gradients in near surface sediments are smoothed by the burrowing activities of macrobentic animals, resulting in enhanced thermal diffusivities (25).

The organic matter degradation consuming oxygen and nitrate takes place in a very thin layer near the sediment surface. The oxygen penetration depth changes over the year, depending on the deposition rate of organic carbon, oxygen concentration in the overlying water, light, and bioturbation (24, 47). Manganese oxides become reduced and Mn^{2+} is lost to the water column. Iron oxyhydroxides are reduced and the Fe(II) is bound by sulfide. High concentrations of organic matter preferentially stimulate sulfate reduction, which, therefore, becomes equivalent in importance to oxygen consumption (21). A sulfate concentration of 28 mM is typical for marine systems; for this reason sulfate depletion is often not seen until deep in vertical sediment profiles (Fig.3).

2. The microbial communities of marine sediments

2.1. Identification and quantification of microorganisms (molecular techniques)

Despite their environmental importance, the microbial community structure of marine sediments is poorly studied. Several attempts to describe marine sediment microbial communities have been based on cultivation (8, 23, 33, 38) and were therefore biased by a distorted representation of the true community composition (57). In a study carried out by Jørgensen and Bak (23), the populations of sulfate reducing bacteria (SRB) of Baltic Sea sediments were analyzed. The population sizes of SRB were studied by the MPN technique. The densities of SRB were 1 to 3 orders of magnitude higher than those previously reported from marine sediments. However,

there was evidence of an underestimation of the cell numbers in the deeper sediment layers. Delille and co-workers (1995) carried out a seasonal study to analyze the heterotrophic bacterial populations present in subantartic marine sediments. Up to 1742 isolated strains were identified, and about 75% of the isolates were identified as non-fermentative Gram-negative rods. They were included in two distinct clusters: group A (*Pseudomonas* spp.) and group B (*Xanthomonas* spp., *Flavobacterium* spp., and *Cytophaga* spp.) (8). Together with a low bacterial diversity, a thousandfold discrepancy in cell numbers between direct bacterial enumeration using DAPI stain method and agar plate counts was found.

At least three explanations were suggested by Rehnstam et al. (43) to explain the low numbers and the low bacterial diversity found by this approach: (i) many bacteria in marine sediments could be viable but not culturable; (ii) many bacteria observed under the microscope during direct counts could be inactive; (iii) many bacteria could be viable and culturable, but have a low plating efficiency.

In the late 1980s, Carl Woese, Norman Pace and co-workers established an approach to investigate phylogenetic relationships between microorganisms based on the comparative analysis of rRNA sequences (37, 61). This rRNA approach (Fig. 4) has since then been applied to many microbial communities. Total DNA is extracted from the environmental sample and the 16S rDNA genes are amplified by PCR. These rRNA genes can be cloned and sequenced. The sequences obtained can be compared with databases to yield information about their identity. Oligonucleotide probes (i.e. short labelled fragments of DNA) can be designed to specifically target one group of retrieved sequences. Finally, the populations behind these sequences are identified by oligonucleotide probing of intact fixed samples. Thereby identification, quantification and analysis of the spatial distribution of populations is possible (2).

By application of the rRNA approach, molecular techniques have greatly increased our knowledge of the marine sediment microbial communities and allow us to study diversity, distribution and abundance of microorganisms in their natural environment without the need of isolation. The 16S rDNA libraries yield information on the similarity of the sequences in comparison with the available database and give an estimation of the genetic diversity of the sample. The use of this technique in marine sediments suggested the presence of hitherto uncultured organisms (5, 10, 30, 41, 42, 45, 58). RFLP and DGGE are different fingerprinting techniques available to study the diversity of microbial communities. These techniques rely on the nucleic

acid (DNA and/or RNA) extraction of environmental samples. The extracted nucleic acids are PCR-amplified and separated on an agarose or polyacrylamide gel by electrophoresis. The separation is based on the different sizes of the PCR products

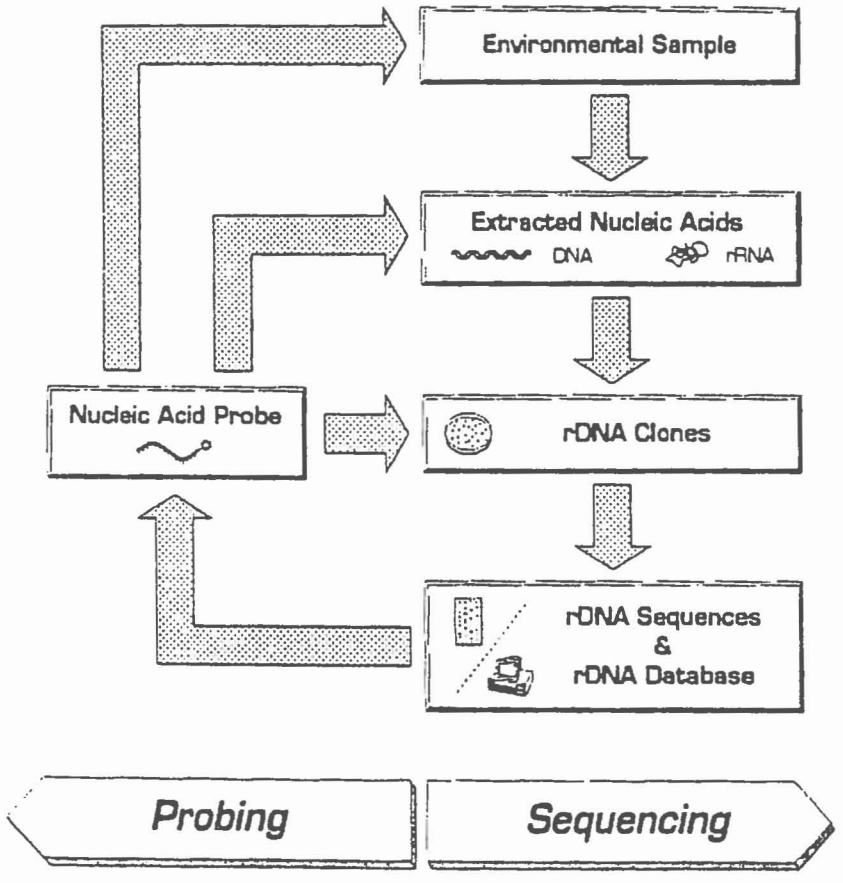

Figure 4. Principal phases of the rRNA approach, sequencing and probing. From Amann et al. 1997.

(RFLP) or on differences in sequence composition (DGGE). The resulting band patterns reflect a part of the microbial diversity present in the original sample. More details about the identity of single bands can be retrieved by blotting and hybridization or by sequencing of gel bands (36). Several studies investigating the microbial diversity in marine sediments were based on RFLP (17) and DGGE (52, 55).

However, these techniques can not prove that the retrieved sequences were from cells thriving in this habitat. PCR-independent hybridization techniques based on rRNA or rDNA are necessary to accurately quantify the abundance of the different phylogenetic groups present in natural microbial communities (2). As described above, these techniques rely on the design of probes to target one group of specific sequences. PCR independent techniques such as dot-blot and slot-blot hybridization rely on the nucleic acid (DNA and/or RNA) extraction of environmental samples. The

extracted nucleic acids are fixed on a membrane and hybridized with the specific probes selected to identify and quantify the relative abundance of specific rRNA sequences in the original sample. Microbial diversity and structure of SRB in marine sediments has been studied using the quantitative slot blot hybridization technique (9, 35, 41, 44, 48-50). However, these results can not be directly translated into cell numbers because of the differences in absolute rRNA content per cell among the different members of the microbial community (2). To quantify the δ–proteobacterial SRB cells in marine sediments, Lillebaek and co-workers used specific antibodies (31). Though single cell identification and quantification was possible with this technique, the need of pure cultures to obtain the antibodies restricted and biased the technique, thus leading to a distorted representation of the true community composition.

Fluorescence in situ hybridization (FISH) with rRNA oligonucleotide probes has single cell resolution, thus, quantification as well as the analysis of the spatial distribution of populations in marine sediments is possible(2). Unfortunately, this technique was not applicable to marine sediments at the beginning of this work. Therefore, no FISH based study on microbial community structure of marine sediments was available.

2.2. Microbial community composition of marine sediments

Molecular approaches allow the characterization of the environmental populations independently of the idiosyncratic biases of culture-dependent techniques (2). The main fraction of retrieved 16S rDNA sequences from marine sediments have been affiliated to members of the α-, β-, γ- and δ-subclass of proteobacteria, the *Cytophaga-Flavobacterium* group, the Planctomycetales, the Verrucomicrobiales, and the *Actinobacteria* group (40).

The comparison of these sequences with the rRNA database of cultured and uncultured organisms give useful hints on potential metabolic groups present in marine sediments; for instance, 50% of the γ-proteobacterial sequences are most closely related to those of endosymbiotic sulfur-oxidizing bacteria (6, 17, 58). The major part of the δ-proteobacterial sequences are related to SRB (5, 6, 10, 29, 58).

The ε-proteobacterial sequences found in marine sediments have 98% sequence similarity to *Arcobacter* spp. (6, 29, 58). The first members of this group were isolated from clinical samples and were described as potentially pathogenic. Nevertheless, *Arcobacter* spp., which represent the most aerotolerant of the former campylobacters, have now also been isolated from various environmental samples and the capacity for denitrification has been reported (54).

Cytophaga-Flavobacterium sequences have been repetitively retrieved from typically anaerobic marine sediments (5, 17, 27, 29, 45, 58). Although culturable members of the *Cytophaga-Flavobacterium* group are known to be mainly aerobic and microerophilic, their hydrolytic fermentative capacity under anaerobic conditionas been shown (46).

Sequences belonging to other bacterial groups as β- and γ- subclass of proteobacteria (5, 6, 17, 29), Actinobacteria (5, 17, 29), and Gram positive bacteria with low GC content of the DNA (26) have been often retrieved from marine sediments, however, it is difficult to speculate about their potential metabolic importance in this ecosystem.

Outline of the experimental work

The object of this thesis was to study the microbial community present in the temperate marine mudflat sediments of the German North Sea coast in terms of diversity, abundance and distribution of microorganisms together with the biogeochemical characterization of their natural environment. The combination of different techniques enabled us to study the activity as well as structure of the microbial community in this system.

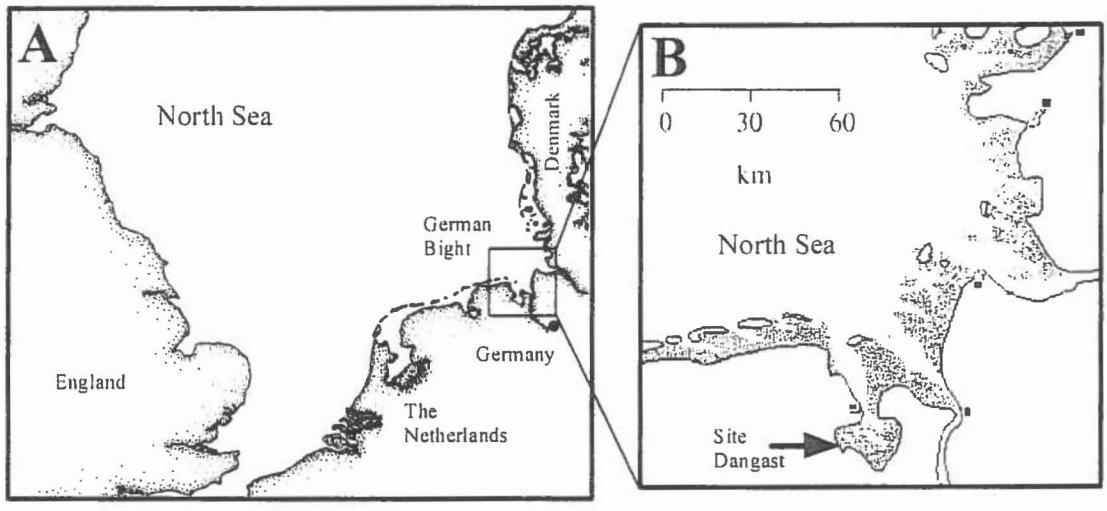

Figure 5. The sampling area is located within the Jade Bay wich is part of the German Wadden Sea.

The sampling station, "Site Dangast", is situated in the Jade Bay which is in the coastal area to the west of the Weser estuary in the German Bight (Fig. 5). The Jade Bay is under influence of the fluvial input of the river Weser, and the mean tidal range in the southern part reaches 3.75 m. The first 10 cm of the sediment are build almost completely of mud (clay and silt grain size fraction < 63 μm). Correspondingly, the pore water contents decreases continuously with depth. The sediment experienced tides which exposed it to the air for about 5 h and left it inundated for about 7 h. The environment provides a situation similar to an estuarine system rich in organic matter. The sediments display 3 distinct colors: the oxic upper part is brown which turns within the first 1-2 cm into a darker olivegreen color with some greyish diffuse streaks. At about 9-12 cm depth, the coupling of increasing accumulation of iron monosulfide (acid-volatile sulfide, AVS) with decreasing porosity is indicated by a diffuse blackening of the sediment, remaining black until the end of the investigated

sediment section. The sediment is characterized by bioturbation, and living polychetes were found down to about 16 cm below surface.

To study the microbial community in marine sediments, a new protocol for the fluorescence in situ hybridization technique (FISH) was developed (Chapter 2). With that work, the direct identification and quantification of the microbial communities present in a marine sediment was possible for the first time and the first detailed descriptions of the microbial community structure of such systems became possible. Several questions were in the focus of interest: Which are the abundant populations in terms of numbers? Are there links between specific stratification and functional zones? Which are the mechanisms that play an important role in the development of the microbial communities in marine sediments? Are these microbial communities stable over time? There was a clear need for a combined study of the microbial diversity and the analysis of biogeochemical processes to improve our understanding of the phenomena that rule the marine sediment ecosystems. The first integrated study showed that sulfate was the predominant electron acceptor for organic matter oxidation in Dangast sediments (Chapter 3). The identification and quantification of the different SRB populations and their activity in the biogeochemical processes in Dangast were therefore studied (Chapter 4).

A seasonal study was designed to follow the dynamics of both biological and geochemical parameters. The microbial community was monitored for two years period (Chapter 5). Stratification changes of different microbial populations as well as changes in geochemical parameters were related to seasonal changes in microbial activity. Strong evidences for the control of the microbial community dynamics by fermentative bacteria and by the temperature as the determining factor of geochemical and microbial fluctuations were found. Therefore microcosms consisting of the undisturbed upper 8 centimeters of the intertidal marine mudflat sediment of Dangast were used to follow the changes of the microbial community structure and its metabolic functioning as a reaction to temperature changes (Chapter 5).

Synopsis of the results and general discussion

Microbial community composition of Wadden Sea sediments. The Wadden Sea sediments of the German North Sea coast are highly influenced by the discharges of eutrophic fresh waters of the rivers Ems, Weser and Elbe. They are among the most active areas for decomposition of organic material in the German Bight. These environmental conditions result in high abundance and activity of microorganisms. This is reflected in high total cell counts determined by DAPI from 5.8 $x10^9$ (surface) to 1.0 $x10^9$ cells cm^{-3} (at 9 cm depth). The decrease in cell number throughout the vertical profile is a normal situation for near-surface estuarine sediments (24, 32, 51, 60). Bacteria dominated the microbial community detected by FISH in these sediments. Up to 4.4 $x10^9$ cells cm^{-3} at the sediment surface hybridized with the probe EUB338 originally assumed to be universal for the Bacteria phyla. Recently, however the probe EUB338 was shown not to detect all Bacteria (7). Some bacterial phyla, most notably the Plantomycetales and Verrucomicrobia, are missed by this probe. Preliminary results showed an increase between 2% and 10% of the detectable population (according to DAPI staining) when the newly designed complementary probes EUB338-II and EUB338-III were used. Only small fraction of the microorganisms could be affiliated to *Archaea* by FISH. However, it is not possible to exclude that *Archaea* not detected by our FISH protocol make up a large part of those DAPI stained cells.

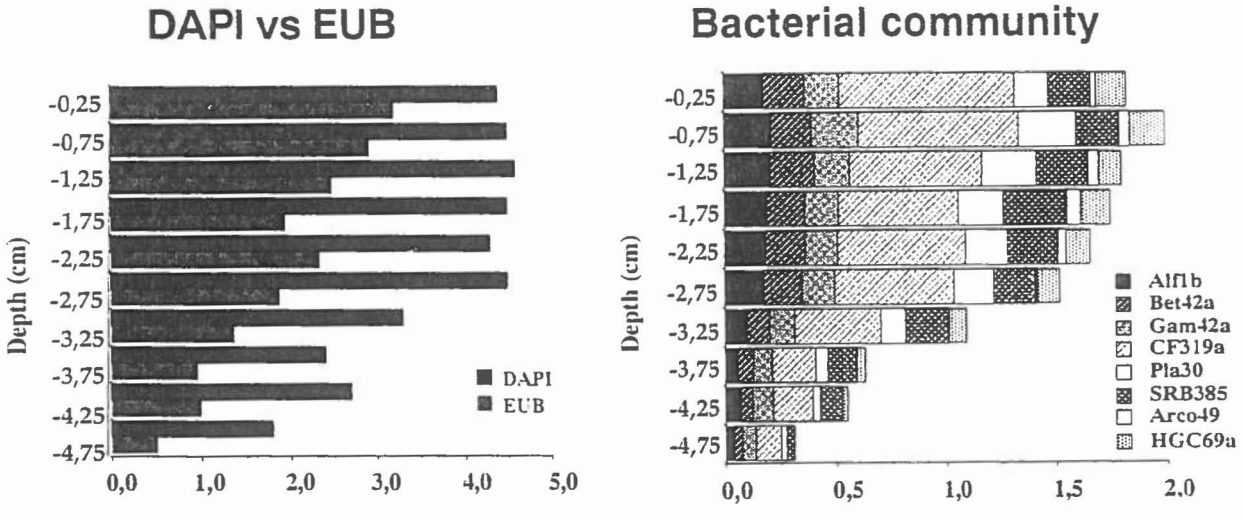

Figure 6. Vertical profiles of Dangast sediment. Total DAPI cells and cells detected with the probe EUB338 (left). Microbial community composition detected with a set of probes (right). From Llobet-Brossa et al. 1998.

Abundance of major phylogenetic groups. The microbial community structure was studied with a set of probes for major phyla within the domain *Bacteria*. Between 17% to 44% of the total DAPI cell counts in the upper 5 cm of the sediment were identified (Fig.1). This means that the majority of the detected bacteria could be affiliated to known groups and that only between 8.5% to 35.8% of the EUB338 counts remained unidentified. The largest group detected were members of the *Cytophaga-Flavobacterium* cluster of the CFB phylum (C/F). Between 15% to 25% of the total detectable bacteria could be affiliated to this group. It is remarkable that later on members of this cluster have been also found to be important in many other marine environments (11, 16, 42, 46). Recently, it has been suggested that C/F might play an important role as fermenters in the anaerobic decomposition of complex organic matter in marine sediments (46). The δ-proteobacterial SRB were the second most abundant group. Sulfate reduction is a key process in this coastal sediment and is thought to be mainly catalyzed by δ-proteobacterial SRB. Therefore, a multiple probe approach was applied to identify SRB. The general probe SRB385 (1) was combined with a set of 15 more specific δ-proteobacterial SRB probes. Altogether, this set of 15 probes detected approximately twice as many cells than probe SRB385. SRB could be detected through the whole sediment even in the most upper layer of the sediment where sulfate reduction is not expected to be the predominant process (14). However, this situation does not seem to be unusual for marine systems and likely reflects anaerobic microniches in the most upper layer of the sediments (18, 21). Planctomycetes, α-, β-, γ- and ε-proteobacterial members, and Gram-positives with high GC DNA content were present in the sediment throughout the vertical profile at relative low abundance (1% to 6.1%, (Fig.6)). Surprisingly, *Arcobacter* spp. was present with more than 10^7 cells cm^3 and limited to the upper 3 cm of sediment. The first members of this group were isolated from clinical samples and were described as potentially pathogenic. Nevertheless, *Arcobacter* spp., which represent the most aerotolerant of the former campylobacters, have now also been isolated from various environmental samples and a denitrification ability has been reported (54). These results showed the applicability of FISH to identify and quantify the different microbial populations present in marine sediments, and therefore the first description of the microbial community structure in such system.

The community of sulfate-reducing bacteria. Since sulfate concentration in sea water is about 28 mM, it is still abundant in deeper layers of the sediment, and consequently the dominant electron acceptor (12). The sulfur isotopic discrimination between sulfide and the AVS-fractions is a clear indication that microbial dissimilatory sulfate reduction took place in this sediments. This is due to a preferential intracellular, enzymatic reduction of $^{32}SO_4^{2-}$ compared to $^{34}SO_4^{2-}$. The diversity and abundance of SRB and their activity in the biogeochemical processes was studied combining cultivation dependent and cultivation independent microbiological approaches with a thorough biogeochemical analysis of the sediment. Combined application of molecular and microbiological tools demonstrated the presence of diverse SRB across the studied vertical sediment profile.

Substrates added to MPN cultures yielded similar types of srb as previously isolated in enrichment cultures. For example, cultures with either lactate or H_2/CO_2/acetate known as substrates typical for *Desulfovibrio* spp., were indeed dominated by members of this species. In addition, H_2/CO_2, the utilization of which requires the capacity for chemolithotrophic growth as known from *Desulfobacterium autotrophicum*, yielded predominantly members of the genus *Desulfobacterium*. MPN-cultures with acetate, the utilization of which requires the capacity of complete oxidation, were dominated by members of the genera *Desulfobacter* and *Desulfosarcina*, both known to possess this capacity. With respect to the presumably active part of the SRB population, a good correlation was observed between SRR and results from 16S rRNA based approaches, i.e. FISH and DGGE of reversley transcribed 16S rRNA. Thus presence of SRB in a given sediment horizon did not always correlate with measureable activity. With respect to the activity of the different SRB populations, members of the *Desulfococcus/Desulfosarcina* group and *Desulfovibrio* spp. were presumably the key populations for the terminal mineralization step in the anaerobic zone in Dangast sediments with 2.9×10^8 and 2.8×10^8 cells per cm^{-3} of sediment, respectively. Counts of other SRB populations, e.g., *Desulfonema* spp., *Desulforhopalus* spp., *Desulfobacter* spp., and *Desulfobacterium* spp. were always below 0.3×10^8 cells cm^{-3} of sediment.

The apparent activation energy calculated from an Arrhenius equation using the depth-integrated SRR for the 10 and 20°C incubations gives a value of 66 kJ mol^{-1} which is similar to observations on a seasonal base in tidal mudflats (Böttcher et al., in

prep.) and general findings on microbial sulfate reduction. The maximum in bacterial sulfate-reduction within the sub-oxic zone, together with enhanced dissolved Fe(II) and Mn(II) concentrations and limited accumulation of free hydrogen sulfide has been observed earlier (4, 34, 56) and is likely related to sulfate reduction taking place in anoxic microniches within the oxidized sediments (18). Hydrogen sulfide which is produced during bacterial dissimilatory sulfate reduction can react with iron compounds to form iron sulfides and may be re-oxidized by the reaction with Fe(III) or Mn(IV) compounds to intermediate sulfur species or sulfate (4, 56). Therefore, the concentrations of dissolved Fe(II) and Mn(II) observed in the pore-waters are not necessarily the result of microbial reduction of the respective oxyhydroxides but may also result from the chemical reduction of Fe(III) and Mn(IV) by H2S. The decrease in SRR near the sediment-water interface is most likely also caused by reoxidation reactions with reactive metal (oxyhydr)oxides in the surface sediments (56).

Seasonal microbial community dynamics and activities. The biogeochemical characterization and the description of the microbial community of Dangast sediments was studied for a period of two years. The seasonal changes were highly similar in the two years studied. Stratification changes of the different microbial populations monitored by FISH as well as changes in geochemical parameters were related to the seasonal changes in microbial activity.

Total cell numbers varied seasonally from 4.3 $x10^9$ (at the sediment surface) and 0.9 $x10^9$ cells cm^{-3} (at 9 cm below the sediment surface) to 5.8 $x10^9$ (at the sediment surface) and 1.1 $x10^8$ cells cm^{-3} (at 9 cm depth below the sediment surface) with higher numbers during summer and lower during winter. The seasonal changes of cell numbers correlated with seasonal changes of temperature and biogeochemical activity. The microbial community detected by FISH was dominated by Bacteria. Between 2.8 $x10^9$ and 4.4 $x10^9$ cells cm^{-3} at the sediment surface hybridized with the probe EUB338. Seasonal changes of EUB338 detectable cells were found to be comparable to those of total cell numbers. An increase in the number of detectable cells correlated with higher microbial activities as shown by DIC, SRR, Mn, Fe, and several other measurements. The number of detected cells decreased exponentially with depth, and this could be related to the availability of the organic matter in the sediments (20, 32). Although the organic matter content was fairly constant for the upper 10 cm of the

sediment. its quality as related to the $\delta^{13}C$ values decreased with depth resulting likely in lower availability (4).

C/F was the largest group of bacteria detected by FISH. C/F counts were lower in winter (maximum of 6.1 $x10^8$ cells/cm^3) than in the rest of the year, and reached the highest values in summer (10.1 $x10^8$ cells/cm^3). A positive linear regression between C/F and total bacterial cells was found (Fig.7). This suggested that the size of the C/F population ruled the microbial community dynamics. This observation supports the idea that the size of the microbial community in marine sediments is limited by the size of the hydrolytic and fermentative population present (12). SRB was the second most abundant functional group detected by FISH. Members of the *Desulfosarcina-Desulfococcus* cluster and of *Desulfovibrio* spp. together accounted for approximately 95% of the total SRB. The minimum detection of SRB was during winter with $4.0x10^8$ cells/cm^3, and the maximum during summer with 7.4 $x10^8$ cells/cm^3. A peak at around 2 to 3 cm depth was found in winter while in summer this peak was shallower at 1 to 2 cm depth. These changes coincided with a strong increase of SRR in summer. These SRR per cell were in the lower range of the specific SRRs of mesophilic SRB in pure cultures at exponential growth (19). A positive correlation between *Desulfovibrio* spp. and C/F suggested that the increased presence of C/F in warmer seasons might strongly increase fermentative processes (46), and the release of compounds that favor SRB like *Desulfovibrio* spp. (Fig.7).

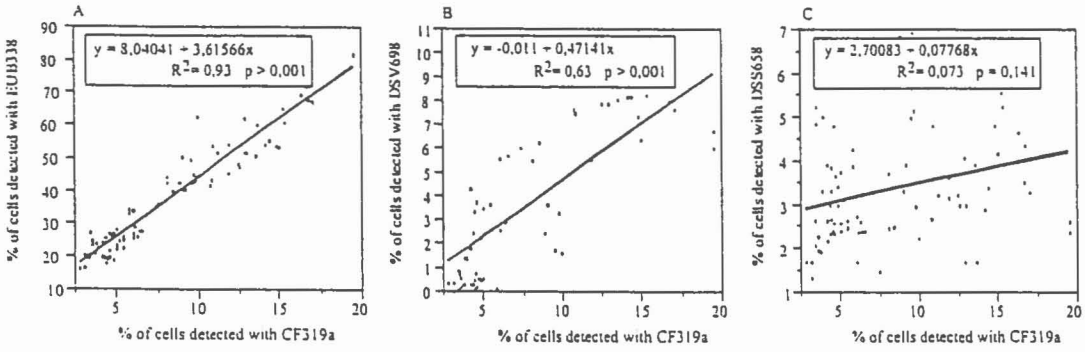

Figure 7. Linear regression between percentage of cells detected in Dangast sediment with probe CF319a specific for C/F and (A) cells detected with the bacterial probe EUB338, (B) cells detected with the probe DSV698 specific for *Desulfovibrio* spp. and (C) cells detected with the probe DSS658 specific for members of the *Desulfosarcina- Desulfococcus* cluster.

Arcobacter spp. duplicated in summer with respect to winter abundance. Detection of *Arcobacter* spp. was limited to the upper 2 cm of the sediment and coincided with the change of the sediment color, likely reflecting redox potential changes (3). Coinciding with higher SRR in summer, the location of Arcobacter spp.

was limited to a very thin zone in the upper part of the sediment, while in winter Arcobacter spp. was detected in deeper layers.

This study showed that the abundance and activity of bacteria decreased during cold periods. Relationships between bacterial distribution, temperature, and the zonation of reactions coupled to the microbial oxidation of organic matter suggested that temperature was the major factor limiting the fermentation processes and therefore the microbial population.

An experimental approach. Undisturbed sediment microcosms were used to study microbial processes, such as community structure changes and metabolic functioning as a response to temperature perturbation.

Total cell numbers detected by DAPI staining and the number of cells detected with bacterial probe EUB338 followed the same temperature dependent dynamics. Higher detection correlated with higher temperature. A positive correlation was found between C/F and temperature, and also between C/F and the total bacterial community detected by the probe EUB338. An increase of SRB numbers with temperature was found. However, the response of members of the *Desulfosarcina-Desulfococcus* cluster and *Desulfovibrio* spp towards temperature was different. A positive correlation between *Desulfovibrio* spp. and temperature was found while abundance of *Desulfosarcina-Desulfococcus* remained stable throughout the incubation period at all temperatures. *Desulfovibrio* spp. blooms correlated with the increase of temperature. In general, incomplete oxidizers like *Desulfovibrio* spp. are less versatile in substrate spectrum than complete oxidizers (39). The increased presence and activity of C/F in warmer seasons might strongly increase fermentative processes (46), thus increasing the release of fementative products that might favor SRB like *Desulfovibrio* spp..

Interestingly, the location of the *Arcobacter* spp. population was limited to the upper part of the sediment. This population was located in higher layers within the vertical sediment profile when the sulfate reduction was enhanced due to temperature increase.

These results suggest that the anaerobic mineralization of organic matter in marine sediments takes place stepwise with the SRB community limited by the availability of metabolites from the fermentative microbial processes. The increase of temperature results in the enhancement of the fermentative processes, therefore,

temperature is the main factor ruling the microbial community structure and activity in this system. Changes in both microbial structure and activity are reflected on the biogeochemical characterization of the sediment. Future studies are now required to test this hypothesis.

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Microbial community composition of Wadden Sea sediment as revealed by fluorescence in situ hybridization

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1998, p. 2691–2696 Vol. 64, No. 7
0099-2240/98/$04.00+0

Microbial Community Composition of Wadden Sea Sediments as Revealed by Fluorescence In Situ Hybridization

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Received 25 February 1998/Accepted 14 April 1998

The microbial community composition of Wadden Sea sediments of the German North Sea coast was investigated by in situ hybridization with group-specific fluorescently labeled, rRNA-targeted oligonucleotides. A large fraction (up to 73%) of the DAPI (4′,6-diamidino-2-phenylindole)-stained cells hybridized with the bacterial probes. Nearly 45% of the total cells could be further identified as belonging to known phyla. Members of the *Cytophaga-Flavobacterium* cluster were most abundant in all layers, followed by the sulfate-reducing bacteria.

Marine sediments cover 70% of the total earth; consequently, they play an important role in the global cycling of carbon and nutrients (36). Early diagenetic processes are catalyzed mainly by the microorganisms that colonize the marine sediments (41). However, despite their environmental importance, the bacterial community structures of marine sediments remain poorly studied (23). Several attempts to describe marine sediment microbial communities have already been made. Most of these have been based on cultivation (see, e.g., references 6, 20, and 30) and were therefore subject to restrictions and biases leading to a distorted representation of the true community composition (2). Molecular techniques have greatly increased our knowledge of marine microbial diversity. For example, 16S rDNA libraries of marine plankton (see, e.g., references 7, 8, 15, and 33) and sediment (see, e.g., references 16 and 25) suggested the presence of hitherto-uncultured organisms. Techniques such as reassociation analysis of DNA (45), denaturing gradient gel electrophoresis (44), and restriction fragment length polymorphism (25) have yielded insight into bacterial diversity and community composition. However, phylogenetically based oligonucleotide hybridization techniques permit not only the monitoring of individual phylogenetic groups but also a quantification of their abundance in the natural habitats (2). Marine sediment microbial diversity has been studied by using the quantitative slot blot hybridization technique (9, 24, 35). However, these results cannot be directly translated into cell numbers because of the differences in absolute rRNA content per cell among the different members of the community (2). In situ hybridization with rRNA-targeted fluorescent oligonucleotide probes, in contrast, permits the identification and quantification of individual cells (2) and has demonstrated great power in the analysis of bacterial community composition in several environments (14, 32, 40, 42, 51). To date this method has not been tested with marine sediments.

In the present work we describe, to our knowledge for the first time, the community composition and vertical distribution of a marine sediment determined by using the in situ hybridization technique. The sampling area is located within the Jadebusen Bay, which is a part of the German Wadden Sea that forms the southern boundary of the North Sea, extending from The Netherlands to Denmark. The area of study is under the influence of the fluvial input of the river Weser, although it is not exposed to the extreme seasonal changes of salinity observed in the Weser estuary (37). The sediment is silty and experiences tides which expose it to air for about 5 h and leave it inundated for about 7 h, with some variability due to the wind velocity and direction (37).

Two cores were obtained from the near shore intertidal mud and sand flats of Dangast on 9 November 1997. One core was completely composed of mud (mud core). The second core originated from an artificial beach which contained a superficial layer (1 to 2 cm) of a thick-grained sand (beach core). Samples were transported at 4°C and processed immediately upon return to the laboratory, within 1 h of sampling. Sediment cores were sliced in 0.5-cm sections and fixed directly in ethanol (96%) or in 4% formaldehyde–phosphate-buffered saline (PBS) (composed of 0.13 M NaCl, 7 mM Na_2HPO_4, and 3 mM NaH_2PO_4 [pH 7.2 in water]) for 2 to 4 h on ice. The formaldehyde-fixed samples were then washed in PBS and stored in ethanol-PBS (1:1) at −20°C. Samples were diluted and treated by mild sonication with an MS73 probe at a setting of 20 for 30 s (Sonopuls HD70; Bandelin, Berlin, Germany). Samples were then mixed with 0.05% agarose, and 10 μl was dropped onto glass slides and dried at room temperature. Glass slides were immersed in 50, 80, and 96% ethanol for 3 min each. The inclusion in agarose did not result in artifacts such as autofluorescence and did not impede the access of the probes to the sample, in contrast to what was observed in similar studies using antibodies (3).

Oligonucleotide probes were synthesized with Cy3 fluorochrome at the 5′ end (Interactiva Biotechnologie GmbH, Ulm, Germany). Hybridizations and microscopy counts of hybridized and DAPI (4′,6-diamidino-2-phenylindole)-stained cells were performed as previously described (40). The probes and formamide concentrations used are given in Table 1. The slides were examined with an Axiophot II microscope (Zeiss, Jena, Germany). For each probe and sample, between 700 and 1,000 DAPI-stained cells and the respective hybridized cells in 10 to 20 independent fields were counted. Standard deviations of counts ranged between 2 and 8%. They are relatively high for those probes that gave low cell counts, and this is mainly due to the heterogeneity of the sample. Counting results were always corrected by subtracting signals observed with the probe NON338 (40).

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TABLE 1. Total DAPI cell counts and relative percentages of hybridized cells with specific probes

Samples and depth (cm)	Absolute DAPI counts (cells/cm^3 [10^8]) (mean ± SD)	% of cells hybridized with probe[a]: EUB338 (mean ± SD)	ALF1b	BET42a	GAM42a	CF319a	PLA30	SRB385	HGC69a	ARC94	DNMA657	% Affiliated bacteria[b]
Mud core												
0.5	44 ± 8	73.0 ± 6	3.5	4.1	3.6	18.1	3.5	4.4	3.2	1.1	0.3	41.5
1.0	45 ± 4	65.0 ± 3	4.7	4.0	4.7	16.3	5.7	4.3	3.6	1.0	0.2	44.3
1.5	46 ± 4	54.0 ± 5	4.5	4.3	3.5	13.0	5.4	5.1	2.3	1.1	0.4	39.2
2.0	45 ± 3	43.5 ± 11	4.3	3.7	3.4	12.1	4.5	6.5	3.1	1.3	0.6	38.9
2.5	43 ± 2	54.5 ± 3	4.4	4.0	3.4	13.6	4.4	5.3	2.6	0.9	0.5	38.6
3.0	45 ± 6	41.8 ± 7	3.9	3.8	3.3	12.0	4.0	4.3	2.4	0.1	0.5	33.8
3.5	33 ± 5	41.3 ± 4	3.1	3.0	3.4	11.8	3.3	6.1	2.5	0.01	0.3	33.2
4.0	24 ± 5	40.0 ± 8	2.5	2.8	3.5	8.1	2.2	5.6	1.7	0.02	0.2	26.4
4.5	27 ± 5	37.0 ± 9	2.7	2.0	3.4	6.4	1.3	3.5	1.0	0.01	0.4	20.3
5.0	18 ± 4	28.6 ± 7	2.3	2.0	3.3	6.2	1.3	2.0	0.1	0.01	0.3	17.2
Beach core												
0.5	6.1 ± 9	40.0 ± 6	1.5	1.0	0.8	7.5	2.8	2.9	0.9	0.9	0.2	18.3
1.0	7.8 ± 7	45.0 ± 3	2.0	1.2	1.3	7.7	3.0	3.5	1.4	0.9	0.3	21.1
1.5	25 ± 3	54.0 ± 2	3.5	3.8	3.0	8.5	3.7	3.8	1.6	1.5	0.4	29.4
2.0	32 ± 4	50.0 ± 7	3.3	3.5	3.2	8.7	4.1	4.2	1.3	1.6	0.3	29.9
2.5	22 ± 5	43.5 ± 5	3.4	3.6	3.1	8.3	3.2	5.2	1.2	1.3	0.2	29.3
3.0	23 ± 6	41.2 ± 2	2.9	3.3	2.8	6.2	3.4	4.9	1.0	1.0	0.4	25.5
3.5	20 ± 7	40.0 ± 11	2.5	3.0	2.7	5.7	3.1	3.6	0.4	0.8	0.2	21.8
4.0	12 ± 4	35.0 ± 9	2.3	2.8	2.6	5.0	2.8	2.3	0.8	0.3	0.3	18.9

[a] Oligonucleotide probes (formamide concentration used for experiments): domain *Eukarya*, EUK516 (20%); domain *Archaea*, ARCH915 (20%): domain *Bacteria*, EUB338 (35%); α subclass of *Proteobacteria*, ALF1b (20%); β subclass of *Proteobacteria*, BET42a (35%): γ subclass of *Proteobacteria*, GAM42a (35%); sulfate reducers of the δ subclass of *Proteobacteria*, SRB385 (20%); *Cytophaga-Flavobacterium* cluster, CF319a (35%); *Planctomycetes*, PLA30 (20%); gram-positive bacteria with high G+C content, HGC69a (20%); *Arcobacter* spp., ARC94 (20%) (40, 51); *Desulfonema magnum*, *D. limicola*, and *D. ishimotoei*, DNMA657 (35%) (12). Probes BET42a, GAM42a, and PLA30 were used with competitor (22, 27).

[b] Results from the addition of counts with the following specific probes: ALF1b, BET42a, GAM42a, SRB385, CF319a, PLA30, HGC69a, and ARC94.

Regarding the specificity of DAPI staining and fluorescent probes, a few cells (<1%) showed very weak staining with DAPI after hybridization with EUB338-Cy3. However, by taking micrographs of those cells, it became clear that they were stained with DAPI, and we regarded this phenomenon to be a result of effective absorption of DAPI emission by Cy3. Furthermore, we observed an effect similar to that reported by Zarda et al. (51). After 3 months of storage of fixed sediments in PBS-ethanol at −20°C, an increased detection of members of the α subclass of *Proteobacteria* with probe ALF1b was observed. Values increased from about 1% to a maximum of 4.1% of the DAPI counts. For the identification of members of the δ subclass of *Proteobacteria*, we used the probe SRB385, which is targeted to most of the known sulfate-reducing bacteria of this subclass (1). We are aware that this probe does not target all members of the δ subclass and that it is complementary to some organisms which are not affiliated with the δ subclass, such as numerous gram-positive bacteria (32, 50). Fluorescence in situ hybridization (FISH) was done on formaldehyde-fixed cells, which renders most gram-positive bacteria unreactive with fluorescent oligonucleotide probes, and consequently it is likely that most of the organisms detected by the SRB385 probe belong to the δ subclass.

Total cell counts and domain-specific probing. Total cell counts determined by DAPI staining in the surface sediments were in accordance with what has been reported previously for similar environments (21, 38, 48). As shown in Table 1 and Fig. 1, DAPI-stained-cell counts were relatively constant in the top 3 cm of the mud core, i.e., 4.3×10^9 to 4.5×10^9 cells/cm^3, and decreased with depth to 1.8×10^9 cells/cm^3. In contrast, we detected many fewer microorganisms (6×10^8 to 8×10^8 cells/cm^3) in the top 1 cm of the beach core, which corresponded to the sand layer. This observation is in accordance with a lower microbial load in sand flats than in mud flats (19, 23). The total cell counts increased below the sand-mud boundary at the 1.5-cm depth to 2.5×10^9 cells/cm^3, although they remained lower than in the mud core.

FISH resulted in the detection of a large fraction of the microbial community living in the top 5 cm of the Wadden Sea sediment. Up to 73% of the DAPI-stained cells hybridized with our set of probes (Table 1). Our detection yields are comparable to those obtained for activated sludge (40) and freshwater (31) but are higher than those obtained for soil (51) or seawater (32). In addition, hybridized cells were visualized with strong fluorescent signals (Fig. 2), which directly demonstrates a high cellular rRNA content (2). The Wadden Sea sediments of the German North Sea coast are highly influenced by the discharges of eutrophic freshwater from the Ems, Weser, and Elbe rivers. They are among the most active areas for decomposition of organic material in the German Bight (17). These environmental conditions would explain the high abundance and activity of microorganisms observed.

The microbial communities analyzed were dominated by *Bacteria* (probe EUB338), whereas *Archaea* (probe ARCH915) and *Eucarya* (probe EUK516) were detected only in numbers that remained below the detection limit, set at 1% of the DAPI-stained cells. Although similar low counts of members of the domain *Archaea* were observed in bulk soil (51), results of other molecular studies on marine and nonmarine environments indicated higher abundance (8, 11, 29). With the still relatively large amount of cells that are not detected by FISH, we currently cannot exclude the possibility that *Archaea* make up a large part of those DAPI-stained cells which did not hybridize with ARCH915.

The fraction of detectable bacteria in the mud core strongly decreased over the vertical profile (Table 1; Fig. 1), from 73% in the uppermost layer to 28.6% at the 5-cm depth. Similar results were obtained for the beach core. However, we found

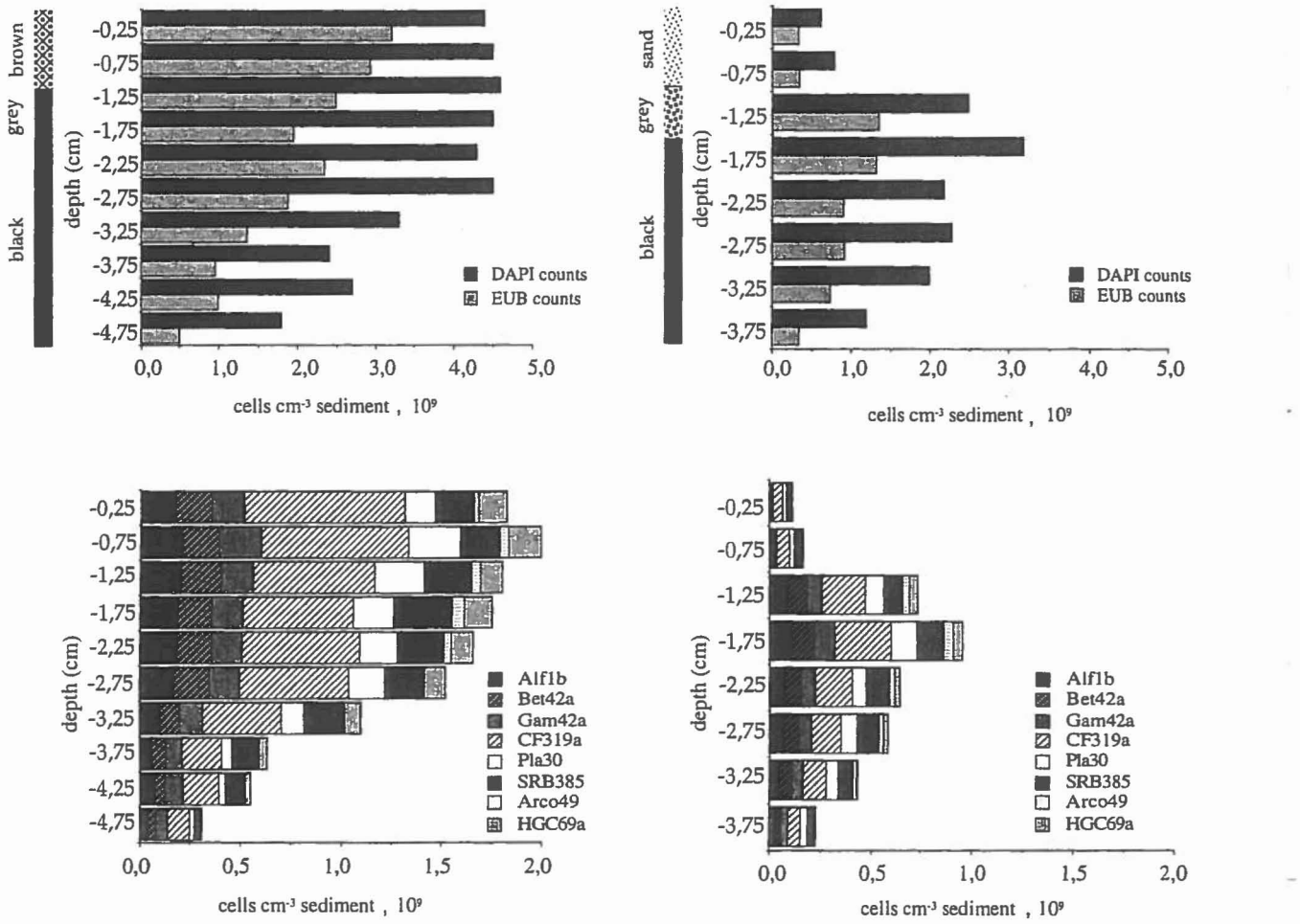

FIG. 1. Vertical profiles of the mud core (left) and beach core (right). In both cases the absolute numbers of bacteria detected are given. The drafted diagram of the cores is given on the left of each DAPI-EUB profile, indicating the transition zone to the iron-sulfide precipitation layer (black).

lower detection rates in the sand layer of the core (40 to 45%) than in the mud layers. These yield differences may be related to the organic-matter content of the sediment (19, 23). Below the sand-mud interface, detection yields were similar to those for the mud core (Table 1; Fig. 1). Thus, not only the absolute cell number but also the fraction of cells detectable by FISH decreased thorough the profiles. Our results are in accordance with the correlation between total cell number and total bacterial production and activity, with the uppermost layers of mud flats showing the highest values (5, 48).

Abundances of major bacterial groups. With a set of eight probes for major phyla within the domain *Bacteria*, we could affiliate between 17 and 44% of the total DAPI cell counts with known bacterial groups (Table 1; Fig. 1). This means that the majority of the detectable bacteria could be affiliated to a known group, and only between 8.5 to 35.8% of the EUB338 counts remained unaffiliated.

The most abundant phylogenetic group in Wadden Sea sediments was the *Cytophaga-Flavobacterium* cluster. This is remarkable, since high numbers of *Cytophaga-Flavobacterium* had so far not been found in marine sediments by either molecular methods (16, 36) or culture-based analysis (6, 10). Most of the cells identified within this cluster showed a homogeneous morphology of thin long rods. Their relative abundance ranged from 5 to 6.2% of the DAPI counts in the deepest layers to 18.1% in the uppermost layer of the mud core. This result means that between 15 and 25% of the total detectable bacteria could be affiliated to this group. Significant numbers of *Cytophaga-Flavobacterium* members in marine environments have so far been found only in the water column associated with macroscopic marine aggregates (7) or with alga blooms in sea ice (4). The members of the *Cytophaga-Flavobacterium* cluster are mainly aerobic, gram-negative bacteria which are specialized for the degradation of complex macromolecules (18, 34). Since the bacterial use of electron acceptors in sediments is stratified according to decreasing redox potentials (41) and since the oxygen depletion in the Wadden sediments occurs within the first 5 mm (37), we can only speculate on the energy metabolism of the *Cytophaga-Flavobacterium* cells found below the oxic zone. However, considering the brightness of the hybridization (2), the cells detected by probe CF319a seem to be intact and metabolically highly active.

The sulfate-reducing members of the δ subclass of *Proteobacteria* detected with the probe SRB385 (SRBs) made up the second-largest group, with a maximum of 6.5% of DAPI counts. We observed positive signals through the whole vertical profile, with a maximum at the 2-cm depth. The relative abundance of SRB counts through the sediment profile, together

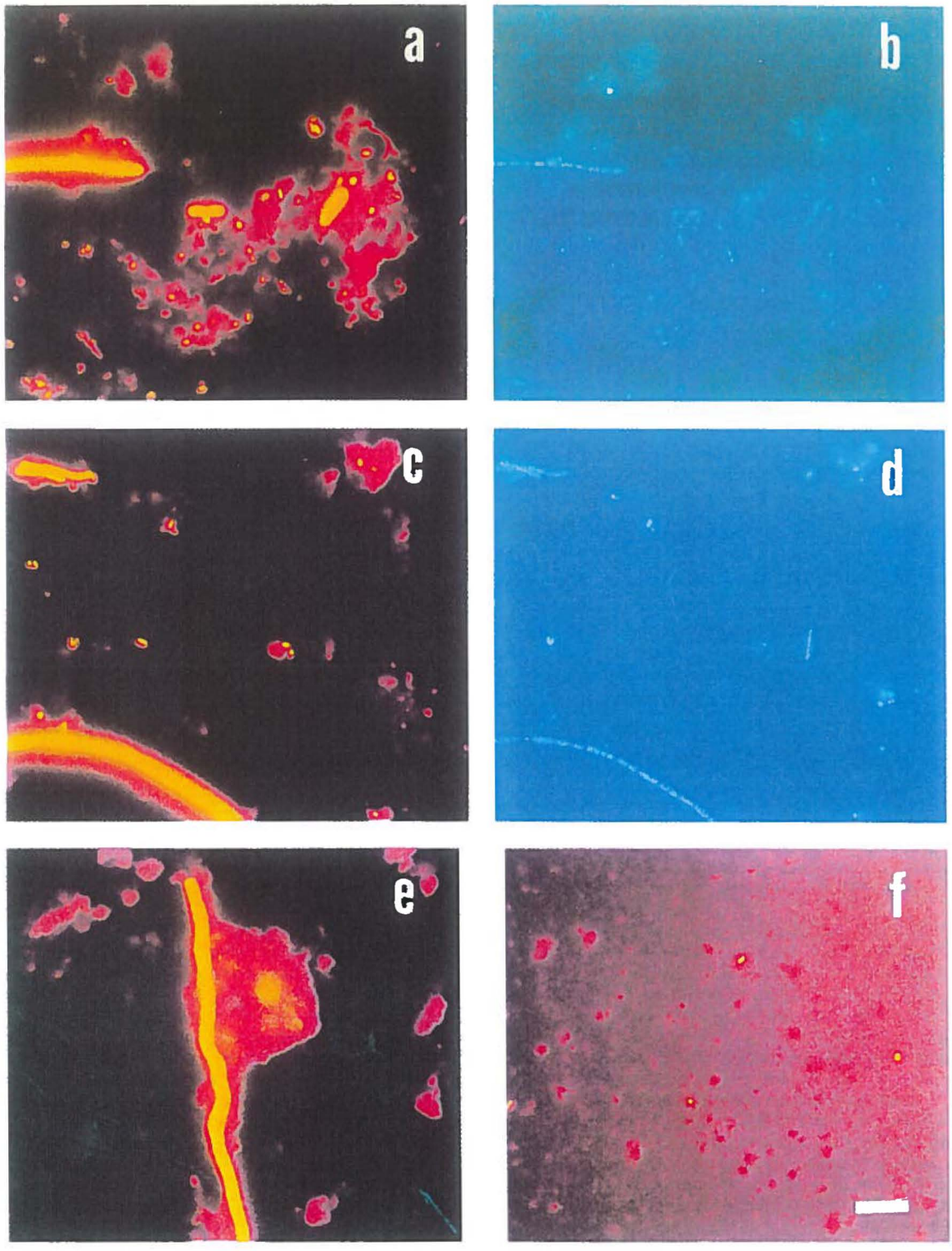

FIG. 2. Epifluorescence micrographs of bacteria in sediment samples from the Jadebusen Bay of the German Wadden Sea. (a) Hybridization with probe EUB338, specific for *Bacteria*. (b) Same microscopic field as in panel a with UV excitation (DAPI staining). (c and d) Identical microscopic fields with probe SRB385 (c) and DAPI staining (d). (e) Hybridization with probe DNMA657, specific for *Desulfonema*. (f) Specific hybridization for *Arcobacter* with probe ARC94. Bar, 10 μm (applies to all panels).

with their relatively high amounts in the upper layers of the sediment where sulfate reduction should not be the predominant process (37), was in accordance with results obtained for comparable environments (9, 20, 39). The morphology of SRBs was quite variable (Fig. 2). Among them were large filamentous bacteria whose affiliation with the genus *Desulfonema* (49) was confirmed with the probe DNMA657 (Fig. 2). A maximum of 2.7×10^7 cells cm^{-3} was found at a depth of 2

to 2.5 cm in the mud core (Table 1). Although *Desulfonema* organisms made up only 9% of all SRBs, these bacteria contribute significantly to the total bacterial biomass in the Wadden sediments due to their large size (Fig. 2).

In contrast to the high abundance of members of the *Cytophaga-Flavobacterium* cluster, we found relative low numbers of *Proteobacteria* (α, β, and γ subclasses), *Planctomycetes*, and gram-positive bacteria with a high G+C content (each accounted for 1 to 5.7%). The relatively low numbers of members of α-subclass *Proteobacteria* in sediments was unexpected, since they have been described as a predominant group in marine plankton (13, 15, 26).

One of our most surprising results was the presence of members of the genus *Arcobacter* at $>10^7$ cells cm^{-3} (1.3% of DAPI counts) (Table 1) in the upper layers of the sediments. All cells detected with the probe ARC94 showed the small, bow-shaped rod morphology (Fig. 2) characteristic of the members of this genus in the ε subclass of *Proteobacteria* (46). There was a clear stratification, with higher counts in the upper 3 cm (Fig. 1). Almost no *Arcobacter* organisms were detected at below 3.5 cm. Although members of this genus have recently been detected in different natural ecosystems (40, 43, 46, 47), they have not previously been reported to be significant in marine sediments. Arcobacters represent the most aerotolerant of the former campylobacters (46). An ability for denitrification has been reported (43). It is therefore not surprising that *Arcobacter* spp. could be found only in the upper layers. Even though the relative abundance is low, the total cell counts for a single genus (exceeding 10^7 cm^{-3}) are high, and the number is nearly identical to that for *Arcobacter* organisms observed in activated sludge (40).

Overall, these results indicate that the community structure in the sediment differs significantly from that in the overlying water column. However, there should be a direct interaction between the water phase and the sediment, which has implications for the community development. In this respect, we think that one of the most interesting findings in our work is the unexpected high abundance of members of the *Cytophaga-Flavobacterium* cluster. This group has also been found to be a major constituent of the macroaggregate-attached bacterial communities in marine environments (7). Indirect observations (28) indicate that the sedimentation of microbial aggregates plays an essential role in the formation of the microbial communities in marine sediments, not only by the input of organic material but also by the input of established microbial communities. Our results support this hypothesis, but there is a question as to whether a high abundance of *Cytophaga* and *Flavobacterium* is common in marine sediments.

Future investigations on marine sediments will combine a larger set of specific probes with the analysis of biogeochemical processes to more fully understand the structure and function of marine sediments.

This study was supported by funds of the Max Planck Society.

Bo Barker Jørgensen and Jakob Pernthaler are acknowledged for critically reading the manuscript and for helpful comments on earlier versions.

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3

The biogeochemistry, stable isotope geochemistry and microbial community structure

PERGAMON

Continental Shelf Research 20 (2000) 1749–1769

CONTINENTAL SHELF RESEARCH

The biogeochemistry, stable isotope geochemistry, and microbial community structure of a temperate intertidal mud at: an integrated study

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Received 10 May 1999; received in revised form 13 October 1999; accepted 23 October 1999

Abstract

An integrated study, combining biogeochemical, stable isotope, micro-sensor, sedimentological, phase-analytical, and molecular ecological methods, was carried out in April 1998 in a temperate intertidal mud at (Site Dangast; German Wadden Sea of the southern North Sea). The biogeochemical zonation was investigated in relation to the vertical abundance of total and sulfate-reducing bacteria, crustaceans, nematodes, agellates, and ciliates. Total organic carbon (TOC) contents of the sediments ranged between 1.0 and 3.3% dry weight and were related to the abundance of clay minerals, indicating sorption processes on mineral surfaces to control organic matter burial. The sediments above 9 cm below sea oor contained an excess of TOC compared to the relationship between TOC and pyrite sulfur proposed for normal marine sediments. The downcore variation of the carbon isotopic composition of organic matter re ected the preferential microbial degradation of labile (marine) organic matter relative to a more resistent (terrestrial) organic matter fraction. The oxygen penetration depth was 4.6 mm in the light and 1.2 mm in the dark, and coincided with the maximum abundance of ciliates, crustaceans and heterotrophic agellates. Although sub-oxic conditions were indicated by the presence of dissolved Fe(II) and Mn(II) to about 15 cm depth, bacterial sulfate reduction rates between 14 and 225 nmol cm^{-3} d^{-1} were measured using radio-tracers with a rst maximum at around 2 cm depth. Up to 80% of the total cells as detected by DAPI-staining hybridized with a *r*RNA-targeted oligonucleotide probe speci c for the domain bacteria (EUB338).

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Sulfate-reducing bacteria as detected by probe SRB385 showed high abundance (up to 7% of total cells) in the upper 5 cm of the sediment. Total and cell numbers of sulfate reducers were highest at about 2 cm and decreased with depth. Cellular sulfate reduction rates were estimated from the SRB counts by uorescence in situ hybridization and the measured sulfate reduction rates and ranged between 0.06 and 0.55 fmol SO_4^{2-} $cell^{-1}$ day^{-1} which is at the lower end determined for pure cultures. From a comparison of cellular SRR and stable sulfur isotope ($^{34}S/^{32}S$) fractionation between coexisting dissolved pore water sulfate and sedimentary reduced sulfur species with laboratory studies a signi cant contribution of bacterial disproportionation reactions within the oxidative part of the sedimentary sulfur cycle is indicated.

Keywords: Mud at; Sulfate reduction rates; Metal oxides; Biogeochemistry; Stable isotopes; Bacterial abundance; Wadden sea; North Sea

1. Introduction

In marine sediments, the degradation of organic matter (OM) by bacteria is coupled to the consumption of oxygen, nitrate, manganese and iron oxyhydroxide, and sulfate as the nal electron acceptors. In coastal marine sediments OM oxidation proceeds to a large part via anaerobic microbial activity using dissolved sulfate as the nal electron acceptor (Jørgensen, 1982) and is associated with the formation of hydrogen sul de according to the overall reaction (for typical marine organic matter)

$$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) \quad 53\,SO_4^{2-} \quad 14\,H^+$$
$$\rightarrow 106\,HCO_3^- \quad 16\,NH_4^+ \quad HPO_4^{2-} \quad 53\,H_2S. \qquad (1)$$

The rates of dissimilatory sulfate reduction in intertidal sediments may vary considerably between about 0.2 and 104 mmol m^{-2} day^{-1} (Trudinger, 1992) and are generally controlled by temperature, the availability of dissolved organic compounds (Vosjan, 1974), and the abundance of sulfate-reducing bacteria. The reduced sulfur species produced can be lost from the pore waters mainly by precipitation of iron sul des, by re-oxidation, and by release from the intertidal sediments to surface waters or the atmosphere. Although a number of studies investigated the biogeochemical zonation related to the oxidation of organic matter in coastal marine sediments (e.g., Sørensen and Jørgensen, 1987; Thamdrup et al., 1994; Moeslund et al., 1994), the corresponding bacterial distribution was typically not characterized. Especially, the observation of signi cant bacterial sulfate reduction in the suboxic zone of the sediments (e.g., Moeslund et al., 1994) requires the presence of a high number of sulfate-reducing bacteria in the non-sul dic part of the sediments and e ective mechanisms for the reoxidation of hydrogen sul de. Analytical techniques for the in situ enumeration and characterization of bacterial cells have been sucessfully applied to coastal marine sediments (Llobet-Brossa et al., 1998) but a combination of these molecular ecological methods with biogeochemical techniques to the sulfur–carbon–manganese–iron cycles in near-surface sediments has not been carried out, yet.

In the present study we have for the rst time combined methods to measure the geochemical and stable isotopical composition of pore waters and sedimentary solid phases with uorescence in situ hybridization ("FISH"; Amann et al., 1995) with *r*RNA-targeted oligonucleotide probes to determine the vertical abundance of total and sulfate-reducing bacteria. The biogeochemical zonation in the porewater–sediment system related to the degradation of organic matter is characterized, and sulfur isotopes are used to evaluate the dominant reactions in the sedimentary sulfur cycle. In previous studies a strong in uence of mineral surfaces on the availability of organic matter and redox-sensitive metals in tidal sediments has been demonstrated (e.g., DeFlaun and Mayer, 1983). Therefore, the abundance of phyllosilicate minerals in the sediments was analyzed. Finally, the consequence of the biogeochemical zonation on the depth-dependent abundance of crustaceans, nematodes, heterotrophic agellates, and ciliates was considered.

2. Study area

The river Weser is one of the four major rivers draining into the German Bight in the southern North Sea. The Jade Bay, a meso- to macrotidal embayment, is situated in the coastal area to the west of the Weser estuary in the northern part of Lower Saxony (Germany). The Jade Bay is in uenced by the uvial input of the river Weser, and the mean tidal range in the southern part reaches 3.75 m (Irion, 1994). The sampling site is located 2 km west of the small village of Dangast (Fig. 1), about 30 m east of a tidal creek, the "Dangast Tief" and about 15 m north of the shore line. The top 10 cm of the sediment completely consisted of mud (grain size fraction < 63 μm). During the tidal cycles, the sediment falls dry for about 5 h and is inundated for about 7 h, with some variation due to the wind velocity and direction (Llobet-Brossa et al., 1998). At every low tide, freshwater is owing via a sluice into a small habor situated at the inlet of the Dangast Tief. Therefore, the environment is similar to an estuarine system. During monthly sampling, salinity in the pore waters of the mud at, however, was found for a year to vary only between 22 and 30, and averaging about 26 (Böttcher, unpublished data).

3. Material and methods

Several parallel sediment cores (PVC tubes; 2.6–5.9 cm wide; 20–40 cm long) were taken in a mud at area of about 2 m^2 on 15th April, 1998, during low tide (12 MET). In situ pH values were measured with a Ross electrode which was inserted into the sediment through the holes in a PVC tube of one core in the eld (precision: $\pm$ 0.02 pH units). The pore water temperature was measured with a digital sensor (GTH 1150 digital thermometer) in situ (precision: $\pm$ 0.5°C). The other sediment cores were closed with air-tight rubber stoppers on both ends and transported cool and dark to the laboratory until further processing within a few hours.

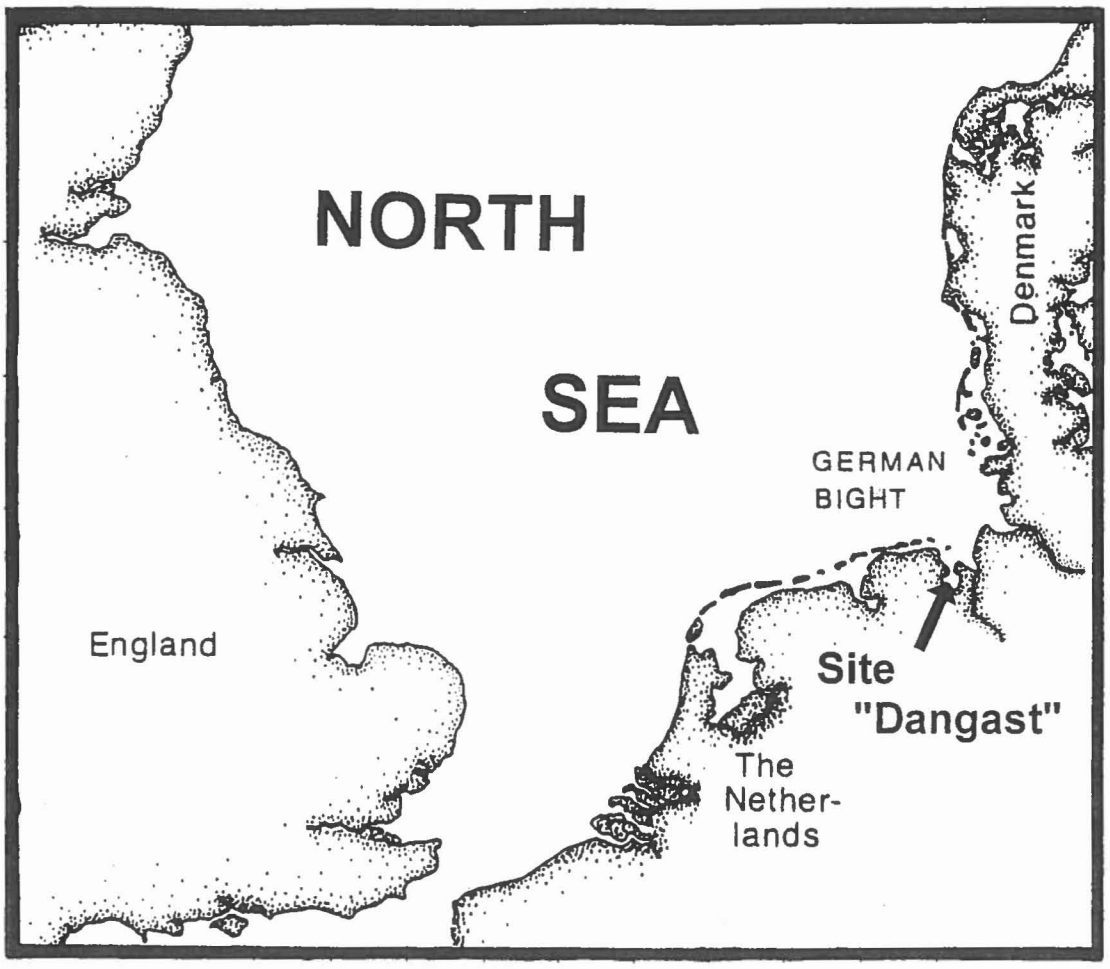

Fig. 1. The study area (modi ed after Sündermann, 1994). Arrow indicates sampling site.

Two parallel sediment cores for FISH were prepared as described by Llobet-Brossa et al. (1998). Hybridization, DAPI-staining, and microscopy counts of hybridized and DAPI-stained bacterial cells were performed as described previously (Snaidr et al., 1997). Oligonucleotide probes for the domain bacteria and speci c for sulfate-reducing bacteria of the δ-subclass of Proteobacteria were EUB338 and SRB385, respectively (Amann et al., 1992). For enumeration of heterotrophic agellates, ciliates, nematodes, and crustaceans, a sediment core (2.6 cm i.d.) was cut in the eld into 2 mm slices down to 2 cm depth and xed with glutaraldehyde (f.c. 1.6%). The following laboratory methods were modi ed after Epstein (1995) and references therein. In two parallel subsamples, the organisms were extracted by isopycnic centrifugation in a Percoll-seawater density gradient. After double staining with the uorescent dyes DAPI and FITC (uorescin-5-isothiocyanate), the organisms were concentrated on black polycarbonate membran lters (1.2 μm pore size) and counted with an epi uorescence microscope (UV and blue light excitation). Abundances are given as numbers of organisms per volume wet sediment.

Pore waters were removed from the sediment by a pore water press (0.45 μm pore size; polyacetate lters) under inert gas in a N_2- lled glove bag. Concentrations of dissolved Fe, Mn and sulfate were measured on diluted acidi ed (2% HNO_3) solutions by ICP-OES (Perkin-Elmer Optima 3000 XL) using Sc as an internal standard (precision 2σ: 5%). Hydrogen sul de was measured in samples preserved with 2% $ZnCl_2$ solution according to Cline (1969) (precision 2σ: 10%). Dissolved inorganic carbon species (ΣCO_2) and NH_4^+ were measured by ow-injection analysis (Hall and Aller, 1992). Samples for ΣCO_2 were preserved with Na_2MoO_4 (precision 2σ: 5%). Salinity of ltered samples was measured with a refractometer (precision: $\pm$ 0.3).

Microsensors for oxygen and hydrogen sul de with tip diameters of 5 μm and a stirring sensitivity <2% were constructed, calibrated, and applied as previously described (Revsbech, 1989; Kühl et al., 1998). Pro les were measured in sediment cores immediately after sampling *on site*, and in a ow chamber (Lorenzen et al., 1995) in the laboratory. Cores were incubated for several hours in the dark or with 430 μmol photons $m^{-2}\,s^{-1}$ before recording dark or light pro les, respectively. The spatial resolution of all measurements was 0.1 mm as controlled by a motor-driven micromanipulator (Märtzhäuser Wetzlar, Germany). Data acquisition was done automatically with a personal computer and the software package LabView® (National Instruments, USA). For each incubation, 20 pro les were measured at di erent sites in the sediment. Total oxygen uptake of the sediment in the dark was calculated as oxygen ux through the di usive boundary layer following Fick's rst law of di usion and using the di usion coefficient for oxygen under the measuring conditions ($1.537 \times 10^{-5}\,cm^2\,s^{-1}$) from Li and Gregory (1974). Bacterial sulfate reduction rates were measured using the whole-core incubation technique and the injection of a $^{35}SO_4^{2-}$ tracer (Fossing and Jørgensen, 1989). Three parallel cores (2.6 cm diameter) were equilibrated at 10°C and incubated with the radio-tracer for 5 h in the dark.

Amounts of iron (hydr)oxides and extractable manganese were determined on freeze-dried sediments by extraction for 1 h at room temperature with a dithionite–citrate–acetic acid solution (Can eld, 1989). Fe and Mn concentrations were measured by ame atomic absorption spectroscopy (Perkin Elmer AAS) (precision 2σ: 4%). Reproducibility from selected doublicate extractions was within 10%. Total reduced sulfur (TRS; sum of iron monosul des, pyrite and elemental sulfur) was determined on freeze-dried samples according to the one-step Cr(II) digestion method (Fossing and Jørgensen, 1989) where H_2S was trapped quantitatively as Ag_2S in a 1 M $AgNO_3$ solution and quanti ed gravimetrically (precision 2σ: 10%). The sedimentary sulfur fractions containing acid volatile sul de (AVS) plus pyrite, and elemental sulfur were distilled separately from Zn-acetate xed samples using a cold or hot acidic Cr(II) chloride solution, respectively (Allen and Parkes, 1995). Total sulfur (TS; sum of pyrite, iron monosul des, S°, elemental and organic sulfur, pore water sulfate) was measured on selected freeze-dried samples using a LECO (precision 2σ: 2.3%; Dellwig et al., 1999), and corrected for pore water sulfate contribution (Table 1). The grain size distribution of a sediment core taken in May 1998 was analyzed on freeze-dried samples after removal of organic matter by H_2O_2 using a Laser particle analysis system (Fritsch Analysette 22).

Table 1
Geochemical composition of sediments

Depth (cmbsf)	SiO_2 (%)	TiO_2 (%)	Al_2O_3 (%)	CaO (%)	MgO (%)	Na_2O (%)	K_2O (%)	Fe (%)	Fe (%)	Mn (ppm)	Mn (ppm)	Zn (ppm)
0–1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.66	n.d.	796	n.d.
1–2	59.0	0.69	12.33	7.75	2.19	2.33	2.54	4.11	1.63	843	618	175
2–3	59.5	0.68	11.89	7.51	2.14	2.37	2.48	3.97	1.58	719	610	164
3–4	63.5	0.65	10.92	6.53	1.86	2.00	2.36	3.55	0.88	618	339	147
4–5	76.8	0.48	7.13	3.82	1.10	1.36	1.79	2.12	0.61	340	198	91
5–6	80.7	0.43	6.21	3.10	0.88	1.10	1.64	1.75	0.63	271	198	76
6–8	80.0	0.45	6.27	3.15	0.89	1.04	1.64	1.77	0.74	278	238	80
8–10	80.6	0.45	6.00	2.97	0.85	1.05	1.65	1.68	0.64	247	196	74
10–12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.59	n.d.	160	n.d.
12–14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.63	n.d.	161	n.d.
14–16	83.0	0.41	5.60	2.58	0.77	0.90	1.56	1.56	0.47	247	199	70
16–18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.48	n.d.	202	n.d.
18–20	79.3	0.45	6.69	3.09	0.98	1.03	1.66	1.91	0.47	255	301	92
20–22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.33	n.d.	257	n.d.

Extractable Fe and Mn in dithionite-citrate-acetic acid solution.
Corrected for pore water sulfate.
Total reduced sulfur (TRS).
[d]Pyrite Acid volatile sul de (AVS). ppm: mg/kg. cmbs: cm below surface.

The carbon isotopic composition of TOC was measured on freeze-dried subsamples at the Department of Geosciences of the University of Bremen. Sediments were weighed into Ag capsules and the carbonate fraction was removed by reaction with diluted HCl. The dried sample was combusted in a Carlo Erba EA 1500 elemental analyzer coupled to a Finnigan Delta E mass spectrometer. The $^{13}C/^{12}C$ isotope ratios are given in the usual δ-notation versus the V-PDB standard and were calibrated with an in-house (Wadden Sea) sediment standard. Reproducibility was $\pm$ 0.2‰. The CO_2 signal on mass 44 recorded with the mass spectrometer was also used to determine the TOC content. Total carbon (TC) was measured on freeze-dried samples using a LECO SC-444 instrument (precision 2σ: 4.0%; Dellwig et al., 1999) and total inorganic carbon (TIC) on a CM 5012 CO_2 coulomat with a CM 5130 acidi cation device (UIC) (precision 2σ: 1.1%; Dellwig et al., 1999). Accuracy was tested by measuring in-house standards. TOC contents were obtained from the di erence of TC and TIC. These TOC results were used to test accuracy of the TOC data obtained by elemental analysis with the isotope mass spectrometer. For stable sulfur isotope analysis, pore water sulfate was precipitated from ltered Zn-acetate preserved samples as $BaSO_4$, carefully washed and dried. Sulfur isotope ratios ($^{34}S/^{32}S$) of the barium sulfate and the silver sul de samples from the TRS and AVS pyrite destillations were measured by C-irmMS at the Institute of Chemistry and Biology of the Marine Environment (ICBM), Oldenburg. Measurements were carried out using a Finnigan MAT 252 mass spectrometer coupled to a Carlo Erba

Pb (ppm)	Zr (ppm)	TC (%)	TIC (%)	TOC (%)	$\delta^{13}C$ (‰)	TS (%)	TRS (%)	S^0 (ppm)	$\delta^{34}S$ (‰)	$\delta^{34}S^d$ (‰)	H_2O (%)
n.d.	n.d.	4.81	1.48	3.33	21.7	0.31	0.37	670	15.2	15.4	75
63	205	4.54	1.43	3.11	21.8	0.34	0.36	n.d.	14.6	n.d.	70
62	206	4.12	1.29	2.83	22.0	0.33	0.32	n.d.	15.3	n.d.	69
55	233	n.d.	n.d.	2.20	21.9	n.d.	0.26	280	16.1	15.9	62
34	259	2.50	0.76	1.74	22.2	0.28	0.20	n.d.	15.9	n.d.	55
30	253	n.d.	n.d.	1.53	22.1	n.d.	0.26	n.d.	n.d.	n.d.	48
31	270	n.d.	n.d.	1.08	22.6	n.d.	0.22	n.d.	15.5	n.d.	39
30	318	n.d.	n.d.	1.11	22.5	n.d.	0.29	180	16.2	16.7	36
n.d.	n.d.	1.65	0.56	1.09	22.5	0.27	0.28	n.d.	16.4	n.d.	36
n.d.	n.d.	n.d.	n.d.	1.05	22.7	n.d.	0.30	190	16.1	16.6	28
29	248	n.d.	n.d.	1.01	22.9	n.d.	0.34	380	17.1	16.6	27
n.d.	n.d.	1.65	0.57	1.08	22.9	0.51	0.42	n.d.	17.0	n.d.	30
34	262	n.d.	n.d.	1.90	23.2	n.d.	0.83	650	18.2	n.d.	38
n.d.	n.d.	2.61	0.96	1.65	23.2	0.88	0.77	n.d.	21.1	n.d.	35

EA1108 elemental analyzer via a Finnigan Con o II split interface as described by Böttcher et al. (1998a). Isotope ratios are given in the δ-notation versus the Vienna-Canyon Diablo Troilite (V-CDT) standard. Reproducibility was better than $\pm$ 0.2‰. International standards IAEA-S-1 and IAEA-S-2 were used to calibrate the mass spectrometer. $\delta^{34}S$ values of 32.2, 20.6, 16.3, and 17.3 were obtained for the international standards IAEA-S-3 (Ag_2S), NBS-127 ($BaSO_4$), and IAEA-S-4 (S°), and NBS-123 (ZnS), respectively (Böttcher et al., 1997).

Concentrations of major, minor and several trace elements were measured on fused borate glass beads of selected freeze-dried samples by X-ray uorescence spectroscopy using a Philips PW 2400 XRF spectrometer at the ICBM. Analytical precision and accuracy of XRF measurements was tested by replicate analysis of international and in-house standards. Precisions (SD (2σ)) were 0.8% (Si, Ti, Al, Fe), 1.2% (Ca), 8.4% (Pb), 2.5% (Zn), and 1.4% (Zr) (Dellwig et al., 1999). Selected bulk sediment samples were analyzed for the mineralogical phase composition by FTIR spectroscopy (Mattson 3000 type FTIR spectrophotometer) and powder X-ray di raction (Siemens X-ray di ractometer; Ni- ltered Cu k_α radiation) according to Flehmig and Kurze (1973).

4. Results and discussion

4.1. Sediments

During sampling at low tide, the pore water temperature ranged between 10°C at the sediment surface and 7°C below about 6 cm depth, and the pore water salinity was

Table 2
Mineralogical composition of selected sediment sections

Depth (cm)	Water (%)	Quartz (%)	Phyllosilicates (%)	Feldspars (%)	Carbonates (%)	LOI (%)
0–1	75	28	39	5	11	17
2–3	69	26	39	5	10	15
3–4	62	31	40	3	10	9
4–5	55	52	25	5	6	8
10–12	33	52	19	4	5	6
20–22	35	47	31	5	8	n.d.

Water contents are given with respect to the original mass of wet sediment, all other data are given on a dry weight base.

Pyrite contents where generally about 1%. The phyllosilicate fraction consists of illite, smectite, kaolinite and chlorite.

The feldspar fraction consists of microcline and albite. The carbonate fraction consists of calcite with about 1% dolomite.

n.d.: not determined. LOI: loss on ignition.

constant at 26 in the top 20 cm. The sediments displayed two distinct color changes: The oxic surface layer was brown. Within the top 1–2 cm the color turned to dark olive green color while at about 9–11 cm depth, the increased accumulation of iron monosul des was indicated by a di use blackening of the sediment which remained black to the bottom of the investigated sediment section. This vertical zonation was also found in biological and geochemical parameters (see below). The sediment was characterized by bioturbation throughout the whole cores, and living polychaetes were found down to about 16 cm below sea oor (cmbsf).

Analysis of the grain size distribution of surface sediments from an adjacent mud at was carried out on sediments sampled in May 1998 and showed that the top 10 cm entirely consisted of mud (grain sizes <63 μm). Of this mud 26% account for the clay size fraction (<2 μm). According to phase analysis carrried out on bulk sediments and texture preparates using FTIR spectroscopy and powder X-ray di raction, the clay mineral fraction consists of illite, kaolinite, chlorite, and smectite. This is in agreement with ndings from measurements on the grain size fraction <2 μm by Engelhardt and Brockamp (1995). These authors found a narrow variation of clay mineral composition in tidal sediments from the North Sea, the Jade Bay and suspended matter in the East-Frisian Wadden Sea, with an approximate composition of 55% illite, 20% smectite, 15% kaolinite and 10% chlorite. Besides the clay mineral components, di erent amounts of quartz, carbonates (essentially calcite), feldspars and minor authigenic pyrite were found in the sediments in the present study (Table 2). The phase analytical results are in agreement with the main and minor element composition of bulk sediments (Table 1). The signi cant downcore decrease in water content of the sediments (Table 1; Fig. 2) is mainly due to changes in the relative proportions of mineral phases, and minor to a collapse of the "card

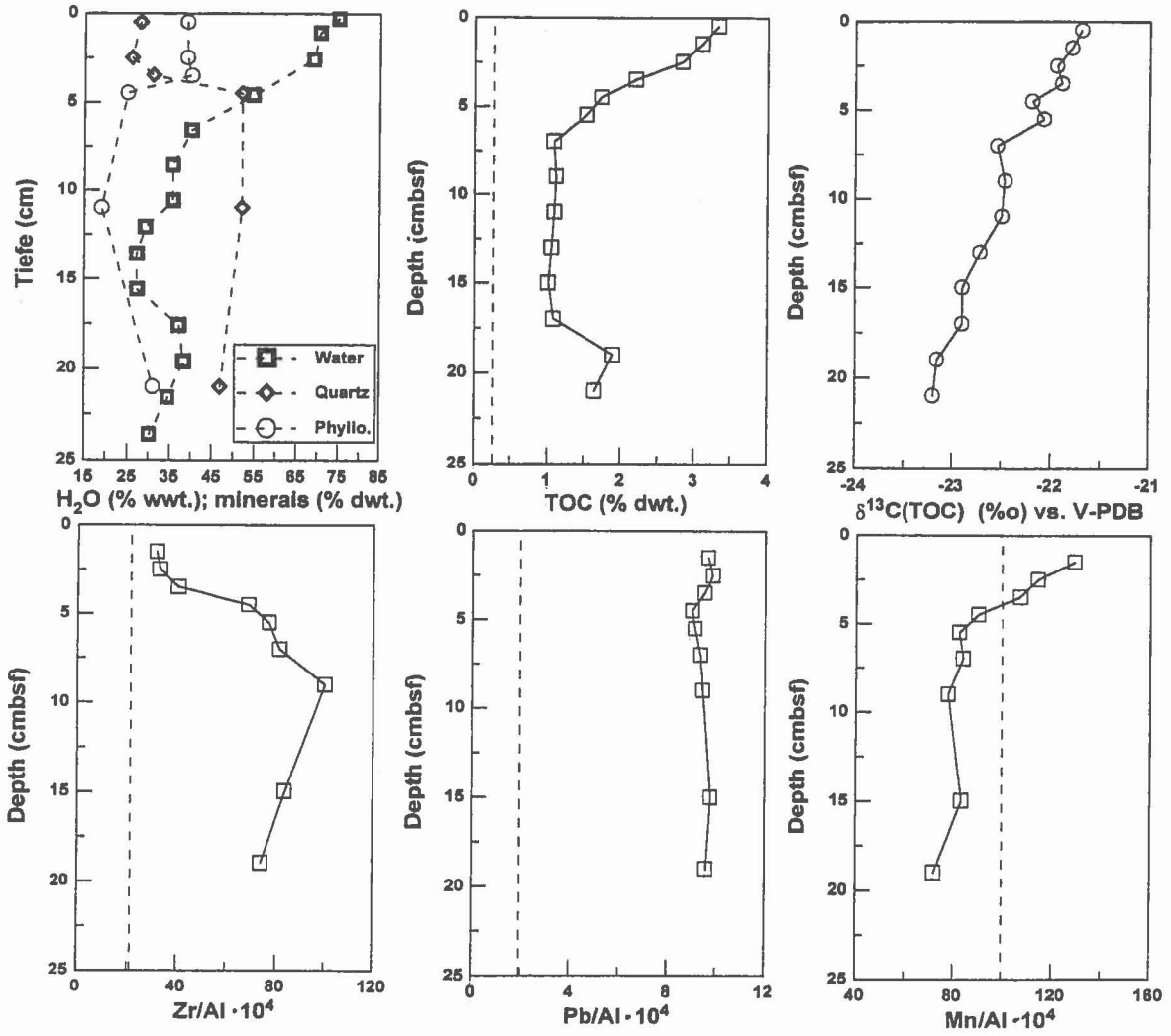

Fig. 2. Downcore variation of selected geochemical parameters. Zr, Pb and Mn data were normalized to Al. Dashed lines indicate average shale.

house"-structure of the clay minerals in the deeper part of the investigated mud at.

Clay minerals are capable of adsorbing large amounts of organic matter (e.g., Keil et al., 1994; Mayer, 1994), leading to a positive correlation between mineral surface areas/mud contents of sediments and organic matter contents as found in various marine sediments, including those from intertidal settings (e.g., DeFlaun and Mayer, 1983; Delafontaine et al., 1996; Böttcher et al., 1998b). A positive correlation was also observed in the present study between phyllosilicates, as determined by phase analysis (Table 2), and the TOC contents (Fig. 3). Relationships between TOC and pore water contents were observed earlier for tidal sediments from the German Wadden Sea (Delafontaine et al., 1996; Böttcher et al., 1998b). These ndings are supported by the results of the present study (Fig. 3). The slightly higher variability observed in the higher water content of the mud at is due to sedimentological changes of the deeper sediment in the investigated mud at, as noted above.

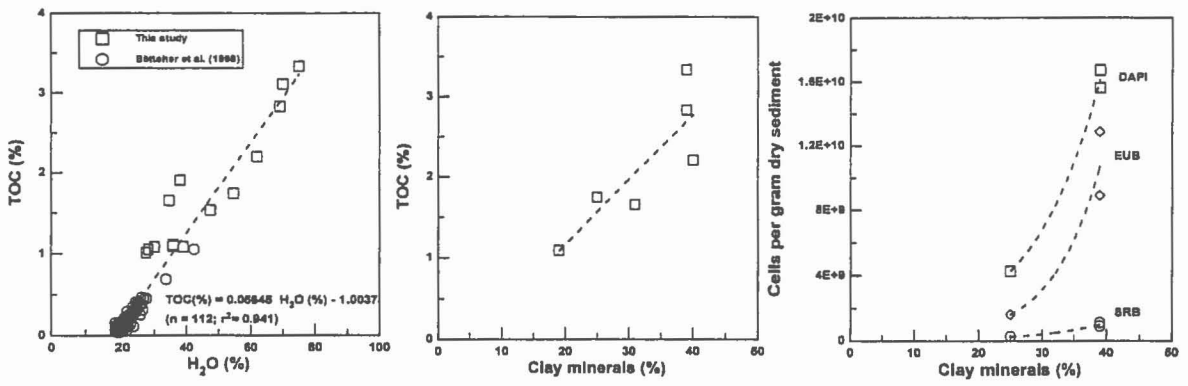

Fig. 3. Scatter plots of TOC versus water and phyllosilicate mineral contents, and bacterial cell numbers versus phyllosilicate contents.

Besides organic matter, trace metals also enter the coastal sediments primarily sorbed to the surface of clay minerals. Depending on the sensitivity of the respective trace metals to changes in the physico-chemical environment (pH, pE) the trace element signature may be altered by diagenetic processes. In order to take the downcore variation in clay mineral contents into consideration, the measured total sedimentary metal contents were, therefore, normalized to the Al contents (Wedepohl, 1971). A normalization of the metal contents to Ti (not shown) gave downcore patterns similar to those of the Me/Al ratios (Fig. 2), indicating that a signi cant in uence of heavy minerals can be ruled out. The entire investigated sediment section was signi cantly enriched in Pb when compared to the geogenic background level ($Pb/Al \approx 2.5 \times 10^{-4}$) indicating anthropogenic contamination. The downcore variation of the Zn/Al ratios (not shown) resembled those of Pb/Al ratios. The small variability in Pb/Al (Fig. 2) indicates an intense mixing of the sediments, either by bioturbation or by resuspension. Similar results were reported by Irion (1994) for the clay mineral fraction in mud ats of the Jade Bay.

When compared to average shale (Mn/Al 96×10^{-4}), the surface sediments are enriched in manganese, but are depleted in this element below about 3 cm (Fig. 2). This indicates that Mn(II) was mobilezed after reduction of Mn(IV)oxyhydroxides. Mn(II) di using to the sediment surface was re-oxidized and the Mn(IV)oxyhydroxides were re-precipitated (Burdige, 1993). Most of the solid-phase manganese near the surface and almost all of it in the deeper sediment is extractable with Na-dithionite (Fig. 4). Besides residual oxides, not easily available for chemical and microbial reduction, authigenic Mn(II)-bearing carbonates can be expected to form in the zone of increased alkalinity (Böttcher, 1998). Similar to manganese, iron showed an enrichment of Fe(III) oxihydroxides near the sediment surface and the (extractable) iron fraction available for chemical and microbial reduction decreased downcore (Table 1). In the deeper part of the sediments (below about 9 cm) iron monosul des may also have contributed to the extractable iron fraction.

Zirconium is present in the heavy mineral fraction and, therefore, the Zr/Al ratios correspond directly to the quartz contents (Fig. 2), indicating changes in the hydrodynamic conditions during the deposition of the investigated sediment section. It

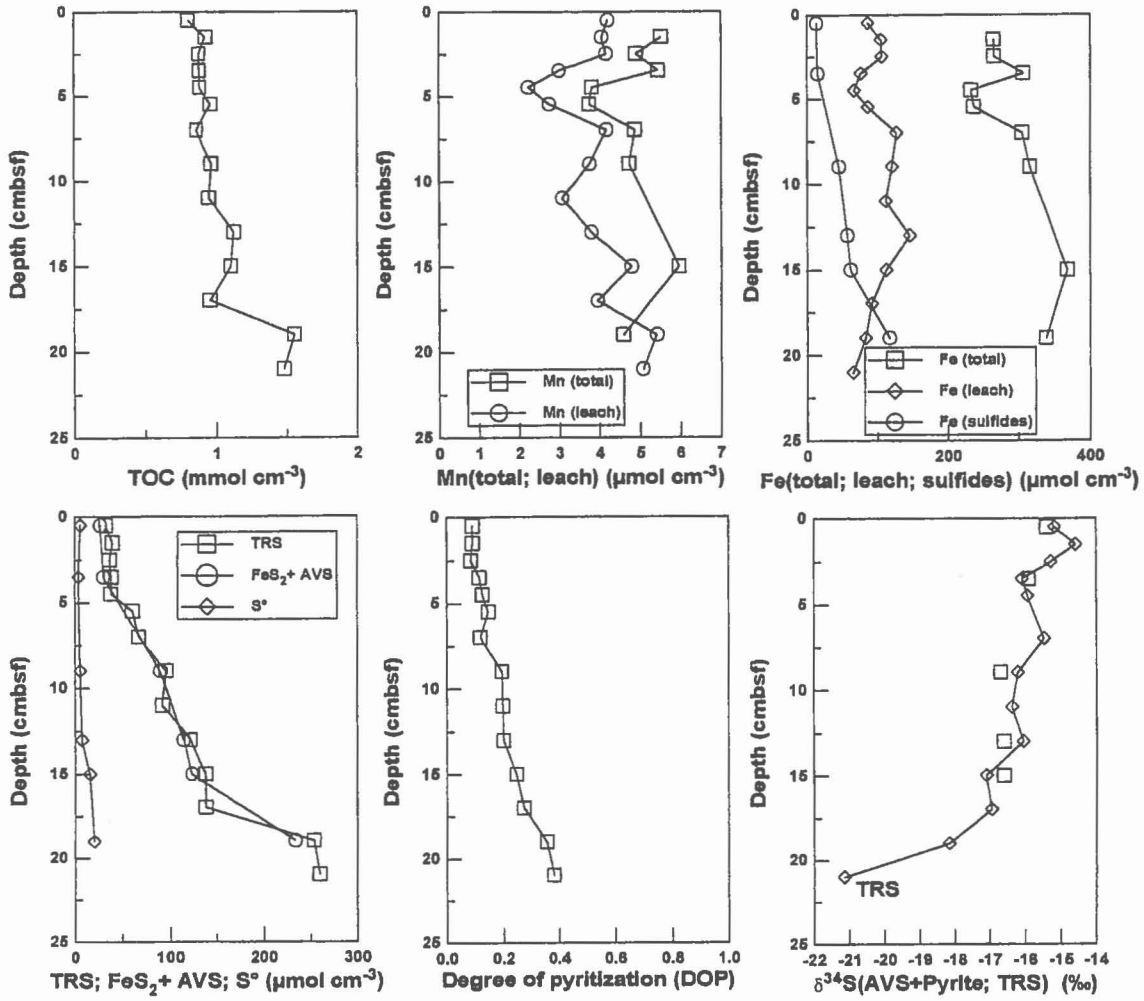

Fig. 4. Downcore variation of selected geochemical parameters based on volume wet sediment, degree of pyritization (DOP) and stable isotopic composition of metal sul des.

should be noted that the downcore variations of the element contents (Table 1) change signi cantly when related to the volume of fresh sediment (Fig. 4). This is caused by the strong e ect of "de-watering" with increasing sediment depth on the normalization to unit volume. Since the elements under consideration are mainly related to mineral surfaces the downcore variation on a dry-weight basis is considered to be most relevant for the present discussion.

The stable carbon isotope ratio of TOC at the sediment surface was 21.7‰ (Fig. 2), similar to previous results from tidal ats of the East Frisian Wadden Sea (Salomons and Mook, 1981; Böttcher et al., 1997, 1998b). A steady downcore decrease in $\delta^{13}C$ values was found to 23.2‰ at 21 cm depth (Fig. 2). This depth-dependent isotope variation can be explained by the preferential microbial degradation of labile (marine) organic matter relative to a more resistent (terrestrial) organic matter fraction in these muddy sediments (Böttcher et al., 1997). For terrestrial particulate organic matter, supplied by rivers to the North Sea or derived from the erosion of Holocene peat in the coastal area of Lower Saxony a $\delta^{13}C$ value of about 27‰ can

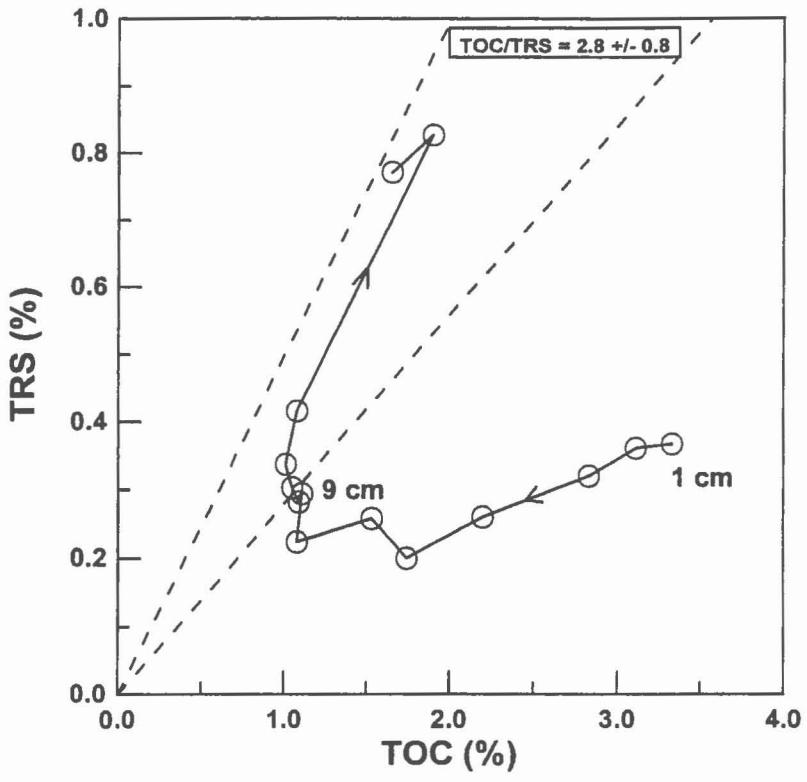

Fig. 5. Scatter plot of TOC versus TRS contents. Dashed lines mark the boundaries proposed for normal marine sediments (Berner, 1984).

be expected (Salomons and Mook, 1981; Böttcher and Scholz-Böttcher, unpublished data). Di erent sources of marine organic matter in the German Wadden Sea show highly variable $\delta^{13}C$ values between about 10– 20‰ (Böttcher et al., 1997, 1998b) but are generally enriched in ^{13}C compared to the terrestrial fraction. Anthropogenic sources, like beach tar, are isotopically similar to the terrestrial fraction (Böttcher et al., 1998b). Application of a binary mixing model between TOC derived from marine ($\delta^{13}C$ 19‰) and terrestrial ($\delta^{13}C$ 27‰) sources yields a contribution of 66% marine organic carbon to the surface sediments. The marine fraction then decreases to 48% at 21 cm depth.

For a number of di erent sediments, the biogeochemical reactions related to speci c depositional environments are re ected by the relationship between total organic carbon and pyritic sulfur (e.g., Berner, 1984). For "normal" marine sediments deposited under an oxic water column, a TOC/TRS ratio of 2.8 ± 0.8 was observed. From Fig. 5 it can be seen that only below a burial depth of about 10 cm a composition assumed to represent "normal" marine sediments was approached. Above this burial depth there is an excess of TOC. Schimmelmann and Kastner (1993) found that it required approximately 500 years of primarily bacterially mediated diagenesis for varved sediments in the Santa Barbara basin to approach the TOC/TRS ratio of normal marine sediments. This indicates that coastal marine sediments, although in uenced by complex processes near the sediment–water interface, may nally reach the geochemical signatures of "normal" marine environments. Critical factors are the

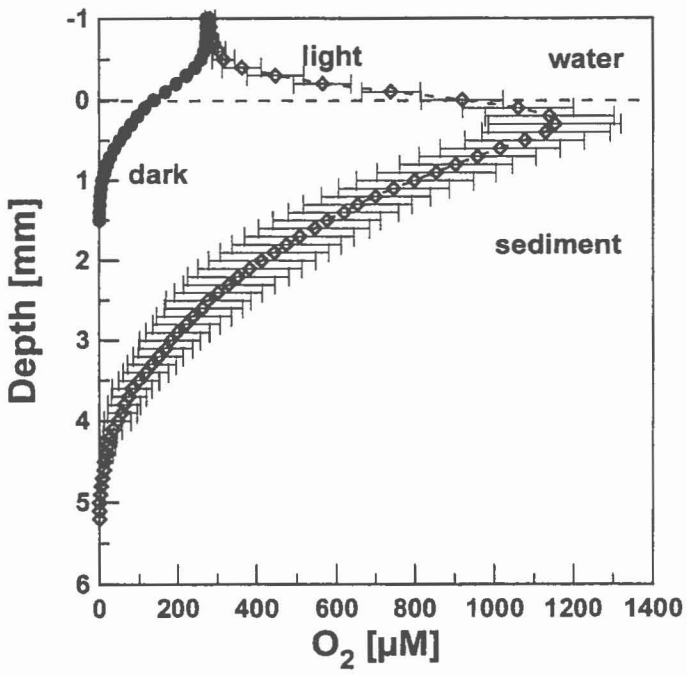

Fig. 6. Dissolved oxygen concentrations during dark (closed symols) and light (open symbols) incubation in the laboratory. Temperature and salinity were adjusted to be close to in situ conditions (11°C; salinity: 31).

amount and quality of buried organic matter and iron compounds in their relationship to microbial and benthic activity, and the hydrodynamic conditions. The generally low degree of pyritization is in agreement with a relatively high amount of non-marine organic matter. The sulfur fraction in the sediment mainly consists of pyritic sulfur, with minor acid volatile sul des, and elemental sulfur (Table 1, Fig. 4). Sulfur in organic matter was additionally present as a minor component (Böttcher, unpublished results). The lower concentrations of TRS at the sediment surface are probably due to bacterial or chemical re-oxidation processes.

4.2. Pore waters

The pore water composition sensitively mirrors the biogeochemical processes in the sediment. According to the typical zonation (e.g., Burdige, 1993), organic matter oxidation should be related to the consumption of oxygen followed by depletation of nitrate, the build-up of Mn(II) and Fe(II) due to reduction of Mn(IV) and Fe(III)oxyhydroxides, and later to the depletion in sulfate. In accordance with this classical scheme, oxygen was only found within a very thin layer at the sediment surface (Fig. 6). Light intensities during sampling ranged from 390–540 µmol photons $m^{-2}\,s^{-1}$ with occasional peaks up to 1300 µmol photons $m^{-2}\,s^{-1}$, which is similar to the intensity applied in the laboratory (430 µmol photons $m^{-2}\,s^{-1}$). Oxygen pro les measured in the eld and under light incubation in the laboratory showed an oxygen peak at 0.2–0.4 mm depth due to photosynthetic oxygen production by benthic diatoms and cyanobacteria. Maximum values were in the range of 899–1402 µM,

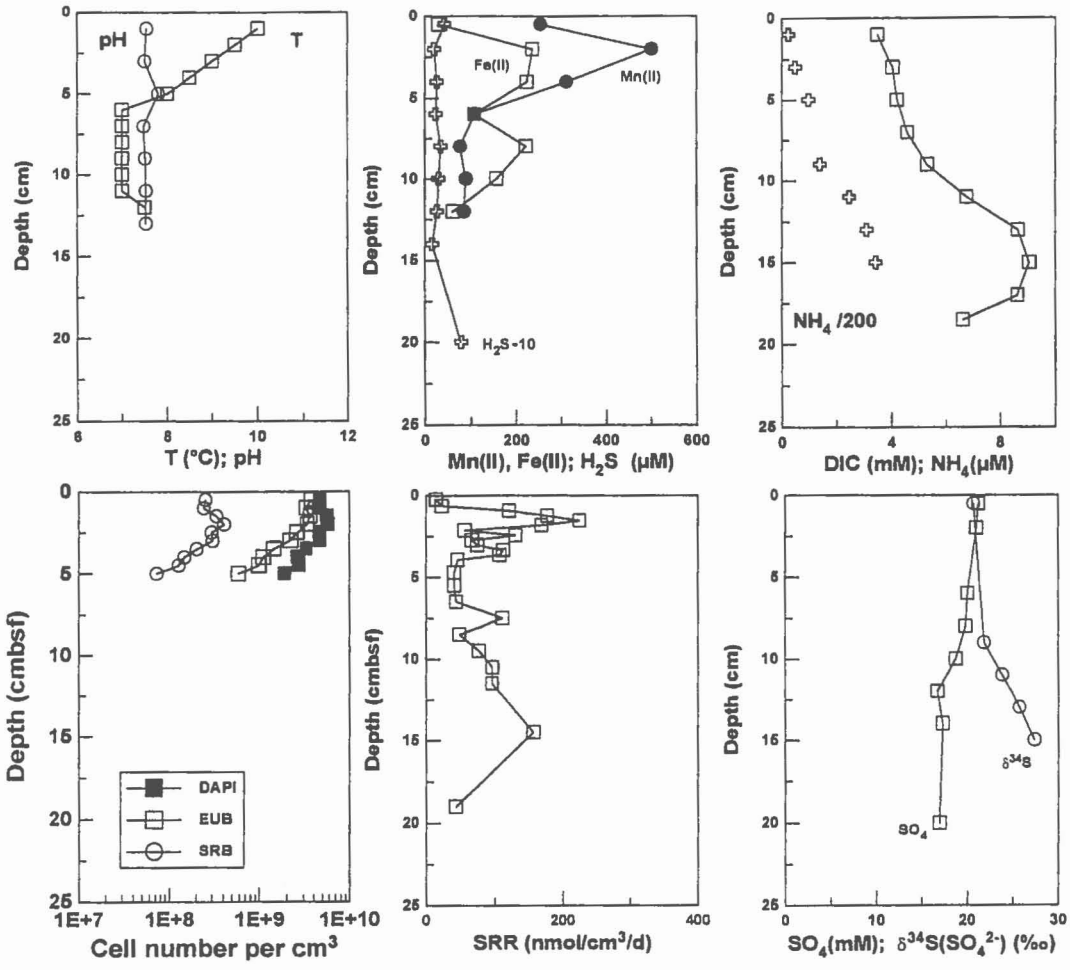

Fig. 7. Downcore variation of pore water parameters and bacterial cell numbers.

corresponding to a 3–5-fold oxygen oversaturation. The maximum oxygen penetration depth in the light was 4.4–4.9 mm. Under eld conditions a slightly lower oxygen penetration depth of 3 mm was observed (not shown). When incubated in the dark, oxygen disappeared within 1.1–1.3 mm depth (Fig. 6). Total oxygen uptake in the dark was calculated from 20 pro les and ranged from 29.9–52.7 mmol m^{-2} d^{-1} with a mean of 39.1 mmol m^{-2} d^{-1}. This is higher than the oxygen ux reported for estuarine Weser sediments at similar temperatures (Sageman et al., 1996).

No hydrogen sul de was detected in the sediment cores under any condition within the top 20 mm, the maximum depth reached by our microsensor measurements. Sub-oxic, non-sul dic conditions were indicated by the presence of dissolved Fe(II) and Mn(II) between about 5 mm and at least 15 cm depth (Fig. 7). In-situ pH values remained more or less constant with values between 7.5 and 7.8 (Fig. 7), but the build-up of signi cant amounts of DIC and NH_4^+ re ects the oxidation of organic matter. The highest concentrations of DIC were found below about 10 cm depth where net sulfate reduction took place as indicated by the decrease in pore water sulfate concentration (Fig. 7). The microbial sulfate reduction rates (SRR) varied

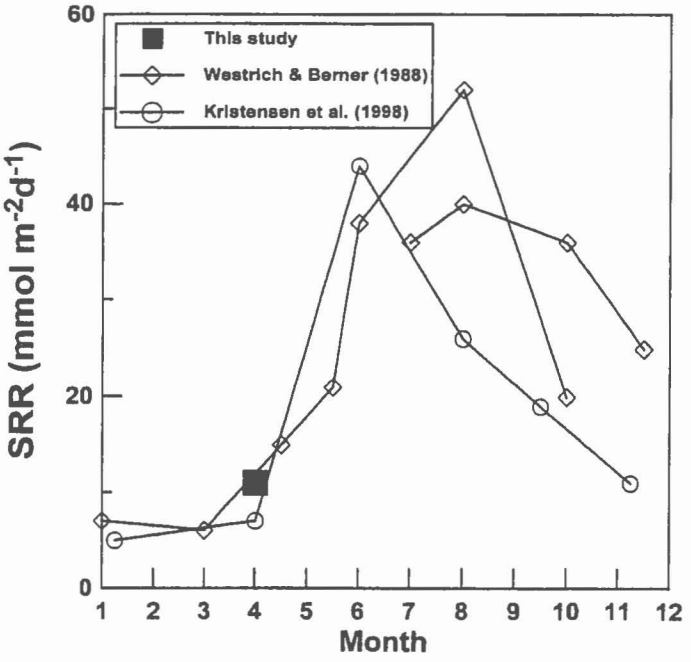

Fig. 8. Depth-intergrated sulfate reduction rates compared to seasonal variations found by previous studies in temperate muddy sediments. Data of Westrich and Berner (1988) are given in mmol/L/yr.

between 14 and 225 nmol cm^{-3} d^{-1} and showed two maxima in the sub-oxic zone, the rst at around 2 cmbsf and the second at around 12 cmbsf (Fig. 7). The depth-integrated sulfate reduction rate (0–15 cm) was 11 ± 2 mmol m^{-2} d^{-1}, which is well within the range observed in ne-grained tidal and sub-tidal sediments of the North Sea (Kristensen et al., 1998; Jørgensen, 1989). A good agreement was also found between the Dangast site and previous investigations on seasonal changes of SRR in temperate muddy sediments (Kristensen et al., 1998; Westrich and Berner, 1988 (Fig. 8)), indicating that, besides the amount and qualitity of organic matter, temperature is important for the activity of sulfate reducing bacteria (SRB) and the related biogeochemical processes.

It is somewhat surprising, that the maximum of sulfate-reduction was found within the sub-oxic zone, with high concentrations of dissolved Fe(II) and Mn(II) and no accumulation of free hydrogen sul de (Fig. 7). Hydrogen sul de concentration exceeded 5 μM only below about 17 cm depth. Similar results were reported by Moeslund et al. (1994) and Thamdrup et al. (1994) and are likely related to sulfate reduction taking place in anoxic microniches within the sub-oxic sediments. Hydrogen sul de produced during bacterial dissimilatory sulfate reduction can react with iron compounds to form iron sul des or may be re-oxidized by the reaction with Fe(III) or Mn(IV) compounds to sulfur species of intermediate oxidation state or sulfate (Thamdrup et al., 1994; Burdige, 1993). Therefore, the concentrations of dissolved Fe(II) and Mn(II) observed in the pore-waters are not necessarily the result of microbial reduction of the respective oxyhydroxides but may also result from the re-oxidation of hydrogen sul de produced during dissimilatory sulfate reduction.

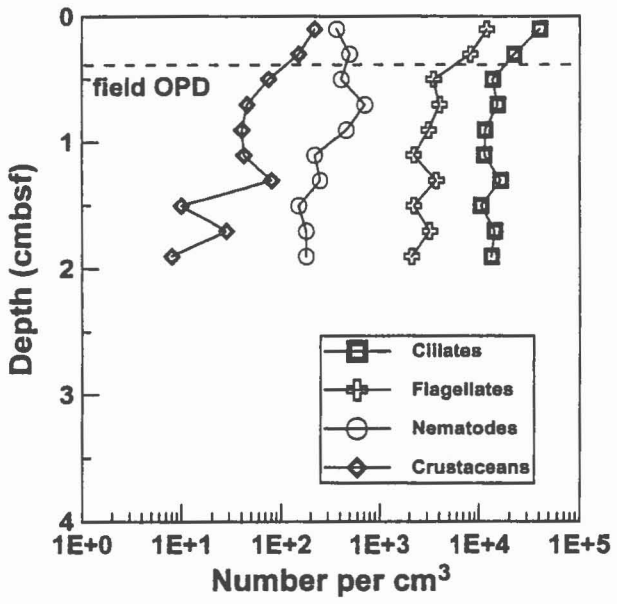

Fig. 9. Vertical abundance of crustaceans, nematodes, heterotrophic agellates, and ciliates. Data are the average of two subsamples from one core. Dashed line indicates oxygen penetration depth.

The coincidence of the rst maximum (2 cm) of Fe(II) and Mn(II) concentrations, sulfate reduction rate and abundance of sulfate reducing bacteria (see below) strongly supports this possibility.

4.3. Microbial community structure

The downcore variation of total (DAPI-stained) bacterial cells and hybridized Gram-negative SRB as detected by FISH was similar to that of the sulfate-reducing activity (Fig. 7) with a maximum at 2 cm depth. Up to 80% of the total, DAPI-stained cells hybridized with probe EUB338, and up to 7% of the detected cells showed positive signals with a probe designed for sulfate reducers (SRB385). The number of SRB counted within the rst 5 cm of the sediment varied between 0.7×10^8 and 4.2×10^8 cells per cm^{-3} wet sediment and showed a maximum at about 2 cmbsf (Fig. 7). When combined with the measurements of the bulk sulfate reduction rates, the cellular sulfate reduction rates can be estimated to range between 0.06 and 0.55 fmol SO_4^{2-} $cell^{-1}$ day^{-1} with the lowest value at 0.25 cmbsf but more or less constant numbers further down to 5 cm. This is within the lower range observed in pure culture experiments (e.g., Kaplan and Rittenberg, 1964) but higher than recent estimates based on *r*RNA-slot blot hybridization (Sahm et al., 1999). It should be noted, however, that the oligonucleotide probe SRB385 does not detect all Gram-negative sulfate-reducing bacteria. A currently on-going survey with a set of probes for de ned genera of sulfate reducing bacteria indicates that the numbers of SRBs might have been underestimated by a factor of about 2 (M. Mussmann, personal communication 1999), but that does not change the conclusions given above.

It is also worth to note, that a positive relationship seems to exist between the abundance of bacteria (recalculated to mass of dry sediment) and the amount of

phyllosilicates (Fig. 2). In agreement with earlier ndings in intertidal sediments (DeFlaun and Mayer, 1983), this relationship indicates that solid interfaces may play a role in determining the abundance of bacteria in the sediments, probably via the availability of organic compounds and their fermentation products, and of iron and manganese compounds (e.g., Ban eld and Nealson, 1997).

The depth-dependent variation of the abundances of ciliates, crustaceans, and heterotrophic agellates all followed the same pattern, with the highest numbers of organisms occurring at the sediment–water interface and decreasing signi cantly below 4 mm depth (Fig. 9). This boundary directly coincides with the oxygen penetration depth observed in the laboratory and in the eld. It indicates that the presence of dissolved oxygen is a critical parameter for the relation between abundances and sediment depth. It should be noted that this general behavior was found for three di erent size classes of ciliates (>100 μm, 30–100 μm and <30 μm in length; Beardsley, 1999). The in uence of dissolved oxygen on the depth distribution of ciliates in intertidal North Sea sediments has been shown previously in laboratory experiments (Berninger and Epstein, 1995). In contrast, the numbers of nematodes showed a maximum at 6 mm depth, suggesting that they can survive periods without oxygen (Ott et al., 1991). This allows them to exploit resources, such as sediment-attached bacteria, over a broader scale than the other investigated organisms.

4.4. *Sulfur isotope fractionation*

The microbial dissimilatory sulfate reduction leads to a discrimination of ^{34}S and ^{32}S between the sum of (minor) AVS and pyrite sulfur, and dissolved porewater sulfate (Figs. 3 and 7). It has been found experimentally that the isotopic composition of metal sul des should nearly re ect the isotope ratios of the reduced sulfur species produced by bacterial processes (Böttcher et al., 1998c). The application of a Rayleigh equation to the pore water sulfate concentrations and sulfur isotope ratios, assuming that the supply rate of dissolved sulfate from the sediment–water interface was much slower than the bacterial sulfate reduction rate ("closed system", Hartman and Nielsen, 1969) yields an isotope fractionation due to microbial sulfate reduction of 26‰ (n 5; r^2 0.88). On the other hand, the observed sulfur isotope fractionation between sulfate and coexisting sul des ranged between 36 and 54‰. Although, most of these data are within the range found in experiments with pure cultures of sulfate-reducing bacteria (ε up to 46‰; Kaplan and Rittenberg, 1964), isotope discrimination in the deeper part of the sediment section is signi cantly higher. This is also shown, when the combined eld data on the speci c sulfate reduction rates and the degree of isotope fractionation based on the sulfate-TRS pair are compared to the relationship found experimentally at near optimum temperatures for members of genus *Desulfovibrio* (Kaplan and Rittenberg, 1964). This indicates that besides microbial sulfate reduction, reactions in the oxidative part of the sulfur cycle including the bacterial disproportionation of sulfur species with intermediate oxidation states (e.g., S°, $S_2O_3^{2-}$; Can eld and Thamdrup, 1994; Cypionka et al., 1998) contribute to the overall fractionation between pore water sulfate and reduced sulfur

species. The complexity of the reactions contributing to the overall isotope partitioning is also obvious from the downcore decrease of the isotopic composition of the TRS (essentially pyrite) fraction of the sediment section (Fig. 5) which is the opposite of the expected trend based on the pore water sulfate data (Fig. 7). Probably, enhanced re-oxidation of pyrite near the sediment–water interface led to the slight enrichment in ^{34}S as it has been found in experimental studies (Nakai and Jensen, 1964).

5. Conclusions

By the combination of biogeochemical, stable isotope, phase-analytical, and molecular–ecological methods it was possible to obtain new quantitative information on the relationships between bacterial cell distribution, clay mineral abundance, quality and availability of organic matter, and the zonation of primary and secondary biogeochemical reactions coupled to the microbial oxidation of organic matter in an intertidal mud at. The abundances of heterotrophic agellates, cilitaes, and crustaceans appeared to be partially controlled by the oxygen penetration depth which reached down to a maximum depth of 4.6 mm (light). Activity and abundance of sulfate reducing bacteria were highest in the sub-oxic zone near the sediment surface (about 2 cmbsf) and decreased further donwcore. Coexising high concentrations of dissolved Fe(II) and Mn(II) indicate that part of the H_2S produced during dissimilatory sulfate reduction was reoxidized by the chemical reduction of Fe(III) and Mn(IV)(oyxhydr)oxides. The calculated cell-speci c sulfate reduction rates are at the lower end of results observed in experiments with pure cultures. Cellular rates have been found to determine the magnitude of stable sulfur isotope fractionation in pure cultures of sulfate-reducing bacteria. The comparison of the quantitative eld results with experimental studies indicates that the sedimentary sulfur isotopic signal was not only determined by sulfate reduction alone, but additionally in uenced by reactions in the oxidative part of the sulfur cycle, including bacterial disproportionation of sulfur intermediates.

Acknowledgements

We wish to thank J. Hinrichs, B. Schnetger (ICBM Oldenburg), S. Fleischer and K. Neumann (MPI Bremen) for technical support. H.-J. Brumsack, G. Gerdes, J. Rullkötter (ICBM Oldenburg and Wilhelmshaven), and M. Segl (University of Bremen) kindly allowed access to analytical facilities. We thank G. Eickert, A. Eggers, and V. Hübner (MPI Bremen) for constructing microsensors. FTIR spectroscopy was carried out by P.-L. Gehlken. Stimulating discussions with J. Rullkötter, J.K. Volkman, H.-J. Brumsack, R. Rosselló-Mora, and M. Delafontaine are gratefully acknowledged. R. Rosselló-Mora critically read the manuscript. The study was supported by Max Planck Society, Munich. MEB whishes to thank B. Thomson and O. Coleman for musical inspiration. The comments of two journal reviewers helped to improve the manuscript.

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4

Vertical abundance and activity of sulfate-reducing bacteria in a temperate intertidal sediment

Vertical community structure and activity of sulfate-reducing bacteria in a temperate intertidal sediment: A multi-methods approach

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The community structure of sulfate-reducing bacteria (SRB) in an intertidal mud flat of the German Wadden Sea (Site Dangast) was studied and related to sedimentary biogeochemical gradients and processes. Below the penetration depths of oxygen (~3 mm) and nitrate (~ 4 mm), the presence of dissolved Fe and Mn and the absence of dissolved sulfide indicate suboxic conditions within the first 10 cm of the sediment. Moderate to high bacterial sulfate reduction rates were measured with radiotracers throughout the investigated sediment and dissimilatory sulfate reduction was also proven by the presence of acid-volatile sulfides (AVS, essentially iron monosulfide). Stable sulfur isotope ($^{34}S/^{32}S$) discrimination between dissolved sulfate and AVS was dominated by sulfate reduction, but a contribution from anaerobic metabolism of sulfur intermediates is likely. The diversity of SRB was studied using denaturant gradient gel electrophoresis (DGGE) of 16S rDNA and counting viable cells with the most probable number (MPN) technique. Phylogenetic groups of SRB identified with these two techniques were evenly distributed across the vertical profile (0-20 cm) of the studied sediment. However, application of fluorescence in situ hybridization (FISH) demonstrated a maximum of the *Desulfovibrio* and *Desulfosarcina-Desulfococcus-Desulfofrigus* groups between 2 and 3 cm depth. These two groups encompass acetate and lactate utilizing SRB. The coincidence of this SRB maximum with a local maximum of sulfate reduction rates and the depletion of acetate and lactate explains well the biogeochemical processes related to sulfate reduction in the vertical profile of the studied sediment.

Continental margin sediments include shelf sediments and intertidal mud flats of the coastal ranges. These sediments are generally characterized by an exceptionally high input of organic matter. Up to 30% of the oceanic primary production takes place in the shelf areas. From this 25-50% sinks to the sediment (Wollast 1991). In addition, the deltaic areas of the shelf regions are receiving up to 90% of the global flux of

particles and organic matter transported by rivers to the oceans (Gibbs 1981). Since most of the deposited matter is remineralized (Berner 1982), continental margin sediments play a prominent role in the marine carbon cycle, even though they encompass only 10% of the total ocean area. The oxidation of organic carbon is performed by microorganisms that employ an array of electron acceptors, each allowing a different yield of free energy. In accordance with the decreasing yields, a zonation of the oxidants is usually observed in the vertical sediment profiles: O_2, NO_3^-, Fe(III) and Mn(IV), and finally SO_4^{2-} (Froelich et al. 1979). Using the $^{35}SO_4^{2-}$ radiotracer technique it was shown that up to about 50% of the organic matter in marine sediments is mineralized via microbial sulfate reduction (Jørgensen 1982b). Since sulfate concentration in ocean water is about 28 mM, it may still be abundant in deeper layers of the sediment, and consequently the dominant electron acceptor (Fenchel et al. 1998). The areal rates of dissimilatory sulfate reduction in intertidal sediments may vary considerably between about 0.2 and 104 mmol $\cdot$ m^{-2} $\cdot$ d^{-1} (Trudinger 1992), and even higher sulfate reduction rates (SRR) have been reported (Skyring 1987). SRR are generally controlled by temperature (Vosjan 1974), the availability of reactive organic compounds (Schubert et al. 2000), and correlated to the abundance of sulfate-reducing bacteria (Sahm et al. 1999; Böttcher et al. 2000).

The importance of sulfate reduction for the remineralization of organic carbon in marine sediments is reflected also by the variety of metabolic capacities of sulfate-reducing bacteria (SRB) isolated from this environment. Pure cultures of SRB have been shown to utilize organic substrates such as short chain fatty acids, alcohols and aromatic compounds, and to completely oxidize acetate to CO_2 (Widdel 1988; Rabus et al. 2000); all important substrates in the anoxic zones of marine sediments. Phylogenetic analysis of isolates (Devereux et al. 1990) and the introduction of 16S rRNA based molecular tools (Amann et al. 1995) allowed to identify and quantify SRBs in their natural habitat. Only recently this type of investigation was applied to marine sediments (Devereux et al. 1996; Llobet-Brossa et al. 1998; Ravenschlag et al. 1999; Sahm et al. 1999; Bowman et al. 2000; Ravenschlag et al. 2000; Wieringa et al. 2000).

The present study focuses on the diversity, abundance and activity of SRB and their relation to the biogeochemical processes in a tidal sediment of the southern North Sea, (Wadden Sea, Site Dangast). This site is located in the intertidal zone and is close to a river outlet. Special interest in this site arose from high bacterial abundance and

activity (Llobet-Brossa et al. 1998; Böttcher et al. 2000). Cultivation dependent and independent microbiological approaches are combined with a thorough biogeochemical analysis of the site to advance our understanding of the relationship between the vertical distribution and activity of sulfate-reducing bacteria in temperate marine surface sediments.

Materials and Methods

Study site□The river Weser is one of four major rivers draining into the German Bight of the southern North Sea. The Jade Bay, a meso- to macrotidal embayment, is situated in the coastal area to the west of the Weser estuary in the northern part of Lower Saxony (Germany). The Jade Bay is under influence of the fluvial input of the river Weser, and the mean tidal range in the southern part reaches 3.75 m (Irion 1994). The sampling station, "Site Dangast", is located 2 km west to the small village Dangast, about 25 m west of a tidal creek ("Dangast Tief") and about 15 m north from the shore-line (Fig. 1).

The sediment experienced tides which exposed it to the air for about 5 h and left it inundated for about 7 h, with some variability due to the wind velocity and direction (Llobet-Brossa et al. 1998).

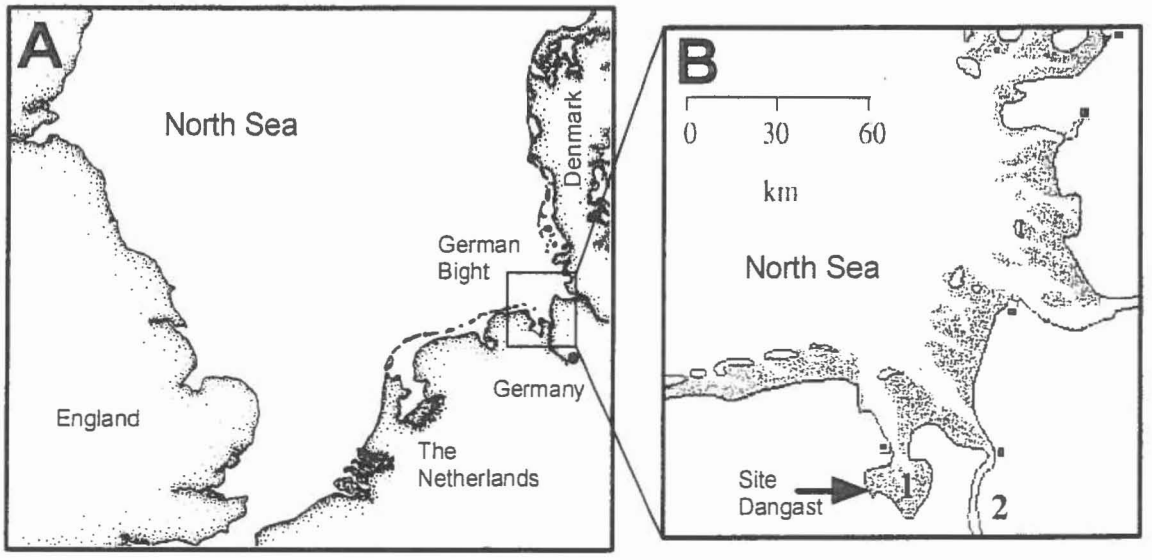


Figure 1. Map of the sampling site, Site Dangast. A, Position of the German Bight in the North Sea. The black dot (□) marks the location of the city Bremen. B, Position of the sampling site (Site Dangast) in the Jade Bay (**1**) is indicated by the arrow. The river Weser is indicated by (**2**).

Processing of sediment samples□Sediment cores were obtained on June 28th, 1999 at low tide between 7 and 9 am with polycarbonate tubes (diameter 8-10 cm; length 50 cm). The sediment cores were closed with air-tight rubber stoppers on both ends

and transported cool (approx. 4°C) and dark to the laboratory for further processing within about 3 hours.

Sediment cores were sliced by extruding them from the polycarbonate tube and cutting with a thin aluminum plate into the following layers: 0-0.5, 0.5-1, 1-2, 2-3, 3-4, 4-5, 5-10, 10-15 and 15-20 cm (top-down). Sediment samples for enumeration of viable cells (MPN) and molecular analysis (DGGE and FISH) were taken from the same sediment layers of a single core. For geochemical measurements of pore water and sediments, parallel cores were used and sliced in 1 cm layers under inert gas (N_2) in a temperature controlled room (4 °C). All cores were taken from an area of 1 by 2 m.

In September 1999 additional samples of Wadden Sea surface sediments (2-5 cm depth) were taken from the same site in Dangast and from Horumersiel, located approx. 20 km north of Dangast.

Sediment characterization□A variety of methods were applied to study physicochemical properties and biological activities related to sulfate-reducing bacteria of the sediment under investigation.

(i) Characterization of organic material and sulfur speciation□Pore water contents in the sediments were determined gravimetrically by drying sediment sections at 70°C until weight constance. Total carbon was measured using a LECO® induction furnace and total inorganic carbon on a CM 5012 coulomat with a CM 5130 acidification modul (UIC). Total organic carbon (TOC) contents were obtained from the difference of total carbon and total inorganic carbon. Phospholipid fatty acids (PLFA) were extracted essentially as described by Bligh and Dyer (1959). The procedure involves a one-phase extraction, fractionation on silicic acid, derivatization to methyl-ester, and analysis by capillary gas chromatography. The polar lipids were fractionated by the use of silicic acid columns (Isolute SPE columns, IST Mid Glamorgan, UK) with chloroform, acetone and methanol as eluents. The mild alkaline methanolysis procedure was used to transmethylate the ester linked fatty acids of phospholipids to methyl ester (Palojärvi and Albers 1998). Nonadecanoic acid methyl ester was utilized as an internal standard. 2.0 µl sample volume was injected splittless onto a fused silica capillary column (Optima-5-MS, Macherey und Nagel, Düren, Germany). The fatty acid methyl esters were identified by the use of a mass spectrometer (GC-Q, Finnigan, Bremen, Germany) and quantified by the use of a flame-ionization-detector (GC-Autosystem, Perkin Elmer, Überlingen, Germany).

The GC-temperature-program is described elsewhere (Palojärvi and Albers 1998). The mass spectra and retention times of 68 fatty acid methyl esters were determined by injection of authentic standards (Supelco, Deisenhofen, Germany and BioTrend, Köln, Germany). Unsaturated *trans* -isomers were not determined. Biomass was determined by phosphate analysis of PLFA. After the Bligh and Dyer (1959) extraction, aliquots of all samples were used for calorimetric phosphate analysis to determine the viable biomass (Findlay et al. 1985; Findlay et al. 1989).

Total sulfur (TS; sum of pyrite, iron monosulfides (AVS), S°, organic sulfur, pore water sulfate) was measured using a LECO® induction furnace, and corrected for pore water sulfate contribution. The AVS fraction was separated from the wet, Zn-acetate preserved sediment by the reaction with cold 6 M HCl containing $SnCl_2$ (Chanton et al. 1987) in a stream of nitrogen. The addition of $SnCl_2$ increased the recovery of the AVS fraction within the first 5 cm, but no further influence was observed at greater depths (Böttcher, unpublished data). The sum of pyrite and elemental sulfur was obtained by the distillation with hot acidic Cr(II)chloride solution (Fossing and Jørgensen 1989). H_2S was trapped as Ag_2S in a $AgNO_3$ solution and quantified gravimetrically.

For stable sulfur isotope analysis ($^{34}S/^{32}S$), pore water sulfate was precipitated from filtered Zn-acetate preserved samples as $BaSO_4$, carefully washed and dried. Sulfur isotope ratios of the AVS and pore water sulfate fractions were measured by C-irmMS. Samples were converted to SO_2 using a Eurovector elemental analyser which was connected to a Finnigan MAT DELTA$^+$ gas mass spectrometer via a Finnigan Conflo II split interface. Isotope ratios are given in the δ-notation versus the SO_2-based Vienna-Canyon Diablo Troilite (V-CDT) standard. International standards IAEA-S-1, IAEA-S-2, IAEA-S-3, and NBS 127 were used to calibrate the mass spectrometer.

(ii) Pore waters□Air and pore water temperatures were measured with a digital sensor (GTH 1150 digital thermometer) at the beginning and the end of the sampling session. After transport to the laboratory, subcores (3.6 cm diameter) for pore water analyses were taken in a temperature-controlled room. Pore waters were separated from the sediment by centrifugation in closed centrifugation vessels under inert gas. Prior to analyses, pore waters were filtered through membrane filters (0.45 µm; Sartorius) and acidified with nitric acid (reagent grade quality) into pre-cleaned PE

bottles. Concentrations of dissolved iron, manganese and sulfate were analyzed after appropriate dilution by means of ICP-OES (Perkin Elmer Optima 3000 XL). It is assumed that dissolved iron essentially consisted of Fe(II), although a contribution from complexed Fe(III) can not be completely ruled out (Luther III et al. 1996). Dissolved sulfate was additionally quantified gravimetrically as $BaSO_4$ from the pore water of a parallel sediment core which was immediately cut into sections and preserved in 20% zinc acetate solutions. The results of the two methods agreed very well. H_2S was measured in samples preserved with 2% $ZnCl_2$ solution according to Cline (Cline 1969). Salinity of filtered samples was measured with a hand refractometer.

Pore waters for the analysis of volatile fatty acids (vfa) analysis was extracted by centrifugation of the sediment in precombusted glas centrifuge tubes. 2 ml of the supernatant were sampled with a glas syringe into precombusted borosilicate vials (4 ml) with teflone lined caps and frozen (-20°C) until analysis. The vfa were measured by HPLC as 2-nitrophenyl hydrazin derivats as described by Albert and Martens (1997). A Sykam S1211 HPLC pump combined with a linear UV/VIS detector and a Gilson 232XL autosampler were used for separation and measurement of the acids. The detected signal was recorded and integrated by a Knauer Eurochrom 2000 integration software. For the separation of the acids a 25 cm LiChrosphere RP8 column with a 1.5 cm LiChrosphere RP8 guard column from Knauer was used. 2.5 cm polymeric reversed phase (PRP-1) cartridge (Hamilton) installed in the sample loop was used as a sample concentrator. Differing from the original method, the flow rate was reduced to 1 ml/min, the concentration of tetrabutylammonium hydroxide of 'solvent A' was reduced to 1 mM and the concentration of tetradecyltrimethylammonium bromids in 'solvent B' to 25 mM. Additionally, the pH of the solvent was adjusted with HCl instead of phosphoric acid. The detection limit for glycolate and lactate was 0.2 µM, for acetate and propionate 0.3 µM and formate 0.6 µM.

(iii) Microsensor measurements- Profiles of oxygen, hydrogen sulfide, and nitrate concentrations at the sediment-water interface were measured in the laboratory with microelectrodes. The determination of oxygen profiles was done with Clark-type O_2 sensors with guard cathodes (Revsbech 1989). The O_2 sensors had tip diameters between 10 µm and 20 µm, a stirring sensitivity <2 %, and a 90 % response time t_{90}< 1 s. To calibrate the sensors, readings in the overlying air saturated water and in the

anoxic zone of the sediment were taken. A two point calibration curve was calculated with oxygen solubility values for different temperatures and salinities based on equations from Garcia and Gordon (1992). Amperometric H_2S microsensors were constructed, calibrated and applied as described by Kühl et al (1998). Nitrate was measured with a microbiosensor in which nitrate and nitrite are reduced to N_2O by bacteria and N_2O is detected electrochemically (Larsen et al. 1997). The nitrate sensor showed no detectable stirring sensitivity and a 90 % response time $t_{90} < 45$ s with a tip diameter of 70 µm. Calibration was done by measuring sensor readings in nitrate solutions with salinity and temperature identically to the sample.

From steady-state oxygen profiles areal fluxes of oxygen were calculated based on Fick´s first law of one dimensional diffusion $J = D_e * dC(z)/dz$ (Kühl et al. 1996) with D_e as effective diffusion coefficient and dC/dz as concentration gradient for oxygen. Volumetric production and consumption rates were calculated based on Fick´s second law of diffusion using the second derivative of the measured concentration profile. Nitrate values were calculated correspondingly. Furthermore, it was also determined if consumed nitrate originated from the water phase (Dw) or was produced by nitrification (Dn) by calculating back a concentration profile from an activity profile with no nitrate production (Meyer et al. 2000).

Sulfate reduction rates (SRR)□Bacterial sulfate reduction rates (SRR) were measured using the whole-core incubation technique with the injection of a carrier-free $^{35}SO_4^{2-}$ tracer (Jørgensen 1978; Fossing and Jørgensen 1989). Although the sediment temperature during sampling varied only slightly and was close to 17°C, much higher changes in pore water temperatures were found on a daily base between 26th and 30th of June, 1999 (Böttcher, unpublished data). Therefore, sediment cores were equilibrated after sampling in the laboratory at 10 and 20°C for several hours and subsequently incubated with the radiotracer (~ 200 kBq per injection along 1 cm intervals) for 4.5 h in the dark. Activities counted with a Packard liquid scintillation counter were corrected for blank contributions derived from the counting and the distillation procedures. The apparent activation energy calculated from an Arrhenius equation using the depth-integrated SRR for the 10 and 20°C incubations gives a value of 66 kJ · mol^{-1} which is similar to observations on a seasonal base in tidal mudflats (Kristensen et al. 2000) and general findings on microbial sulfate reduction (Westrich and Berner 1988).

Nucleic acid extraction and DGGE amplification□In addition to the sediment core used for MPN counts and vfa analysis, a second core was processed for molecular analysis. Replicate sediment cores were analysed to avoid changes in the community composition caused by handling and containment as described before (Rochelle et al. 1994). DNA and RNA of each sediment horizon were extracted from 1.5 ml wet sediment by bead-beating, phenol extraction and isopropanol precipitation as described previously (Sahm and Behringer 1998). PCR amplification specific for SRB of the δ-subclass of *Proteobacteria* was carried out with the forward primer SRB385 carrying a GC-clamp and the reverse primer 907 (Sass et al. 1998). DGGE, excision of bands, reamplification, and sequencing were performed as previously described (Muyzer et al. 1996).

DGGE partial sequences were added to an alignment of about 15,000 homologous bacterial 16S rRNA genes (Maidak et al. 2000) by using the aligning tool of the ARB program package (Strunk et al. 1998-2000). Aligned sequences were inserted within a stable tree by using the ARB parsimony tool (Ludwig et al. 1998). Accession numbers of the partial sequences are XXX and YYY, and deposited at ZZZ.

In situ hybridization and cell counts□Hybridizations, microscopic examination and counting of hybridized cells and total cell counts were performed as previously described (Snaidr et al. 1997; Llobet-Brossa et al. 1998). For each sample between 700 and 1000 DAPI stained cells were analyzed and counted; duplicates were carried out for each sample. The oligonucleotide probes used in this study were purchased from Interactiva (Ulm, Germany) with Cy3 fluorochrome at the 5' end. Probes used are listed in Table 1. For fluorescence in situ hybridization (FISH) of sediment samples two replicate sediment cores were cut and processed as described before (Llobet-Brossa et al. 1998). From MPN-tubes that showed growth, aliquots of 1-2 ml were withdrawn with N_2-flushed syringes. Cells were sedimentated by centrifugation and washed once with 1x PBS. Subsequent fixation and hybridization were performed as previously described (Snaidr et al. 1997).

TABLE 1. Oligonucleotide probes used in this study.

Probe	Target	Sequence (5'-3') of probe	[%] FA[a] in situ	Reference
EUB338	Bacteria 16S rRNA, position[b] 338-355	GCT GCC TCC CGT AGG AGT	0-35	27
SRB385	δ-Subdivision of Proteobacteria 16S rRNA, position 385-402	CGG CGT CGC TGC GTC AGG	35	27
DNMA657	*Desulfonema sp.* 16S rRNA, position 657-676	TTC CGY TTC CCT CTC CCA TA	35	27
DSV698	*Desulfovibrio* (16 species) 16S rRNA, position 698-717	GTT CCT CCA GAT ATC TAC GG	35	29
DSV1292	*Desulfovibrio* (13 species) 16S rRNA, position 1292-1310	CAA TCC GGA CTG GGA CGC	35	29
DSV407	*Desulfovibrio* (3 species) 16S rRNA, position 407-424	CCG AAG GCC TTC TTC CCT	50	29
DSD131	*Desulfovibrio* (1 species) 16S rRNA, position 131-148	CCC GAT CGT CTG GGC AGG	20	29
DSV214	*Desulfovibrio/D'microbium* 16S rRNA, position 214-230	CAT CCT CGG ACG AA TGC	10	29
DSS658	*Desulfosarcina/D'coccus/D'frigus* 16S rRNA, position 658-675	TCC ACT TCC CTC TCC CAT	60	29
DSB985	*Desulfobacter/D'bacula* 16S rRNA, position 985-1003	CAC AGG ATG TCA AAC CCA G	20	29
221	*Desulfobacterium* 16S rRNA, position 221-238	TGC GCG GAC TCA TTC AAA	35	29
660	*Desulfobulbus* 16S rRNA, position 660-679	GAA TTC CAC TTT CCC CTC TG	60	29
DSBO224	*Desulfobotulus* 16S rRNA, position 224-242	GGG ACG CGG ACT CAT CCT C	60	29
DSMA488	*Desulfovibrio/D'monile* 16S rRNA, position 488-507	GCC GGT GCT TCC TTT GGC GG	60	29
DSR651	*Desulforhopalus* 16S rRNA, position 651-668	CCC CCT CCA GTA CTC AAG	35	29
DTM229	*Desulfotomaculum* 16S rRNA, position 229-246	AAT GGG ACG CGG AXC CAT	15	29
SVAL428	*Desulfotalea/D'fustis* 16S rRNA, position 428-446	CCA TCT GAC AGG ATT TTA C	25	36
NON338	none (negative control)	ACT CCT ACG GGA GGC AGC	0-35	27

[a] Percentage of formamide (FA) in hybridization buffer.
[b] *Escherichia coli* numbering.

Media and enumeration of viable cells□A defined, bicarbonate-buffered, sulfide-reduced (1 mM) mineral medium, essentially having the same salt composition as natural seawater was used for cultivation experiments (Widdel and Bak 1992). Na-dithionate at a final concentration of 10 μg/ml was applied as an additional reductant. Organic substrates were added from concentrated stock solutions. Gaseous substrates (H_2/CO_2) were supplied by applying an overpressure of 1 atm to the headspace of the culture tubes.

Viable sulfate-reducing bacteria were enumerated using an MPN technique with liquid media and with agar shakes. Samples from each layer of the sediment core were transferred via a funnel to glas bottles (250 ml volume) and mixed 1:1 with substrate-

free, anoxic media under a steady stream of N_2/CO_2 (90:10 [v/v]), yielding approximately 100 ml of homogenate. The bottles were then anoxically sealed with butyl-rubber stoppers and screw caps. These sediment slurries were diluted in steps of 1:10 by transferring aliquots to substrate-free, anoxic media under N_2/CO_2 (90:10 [v/v]) in butyl-rubber sealed glass-bottles (250 ml volume of bottle, 100 ml total volume of dilution). The transfer of sediment suspensions between the glass-bottles was carried out with N_2-flushed syringes. Prior to transfer, the sediment suspensions were shaken vigorously to achieve optimal mixing. These "master"-dilutions were used to inoculate culture tubes for MPN-counts in liquid media. Similarily, "master"-dilutions were prepared for the inoculation of MPN-counts in agar-shakes. However, in this case not every single sediment layer was diluted but rather a homogenous mix of the top 5 cm of the sediment core.

(i) MPN-counts with liquid media□These MPN-counts were carried out in glass tubes (160 × 16 mm) sealed with butyl-rubber stoppers and screw caps that contained anoxic media with or without substrate under an N_2/CO_2-atmosphere (90:10 [v/v]). Substrates used were as follows: H_2/CO_2 (90:10, [v/v], applied with 1 atm to the gas head space); H_2/CO_2 + 2 mM acetate; 15 mM formate; 7.5 mM acetate; 5 mM propionate; 5 mM lactate. Each tube contained 9 ml medium and was inoculated with 1 ml from the corresponding "master" dilution. Three replicates were prepared for each substrate condition. To sustain reduced conditions, 0.5 mM Na_2S were added after about four weeks of incubation. The tubes were incubated at 23 °C for 10 months. Growth of sulfate-reducing bacteria was determined by measurement of the optical density (660 nm) and sulfide using the semiquantitative method described by Cord-Ruwisch (1985) and by macroscopic and microscopic examination.

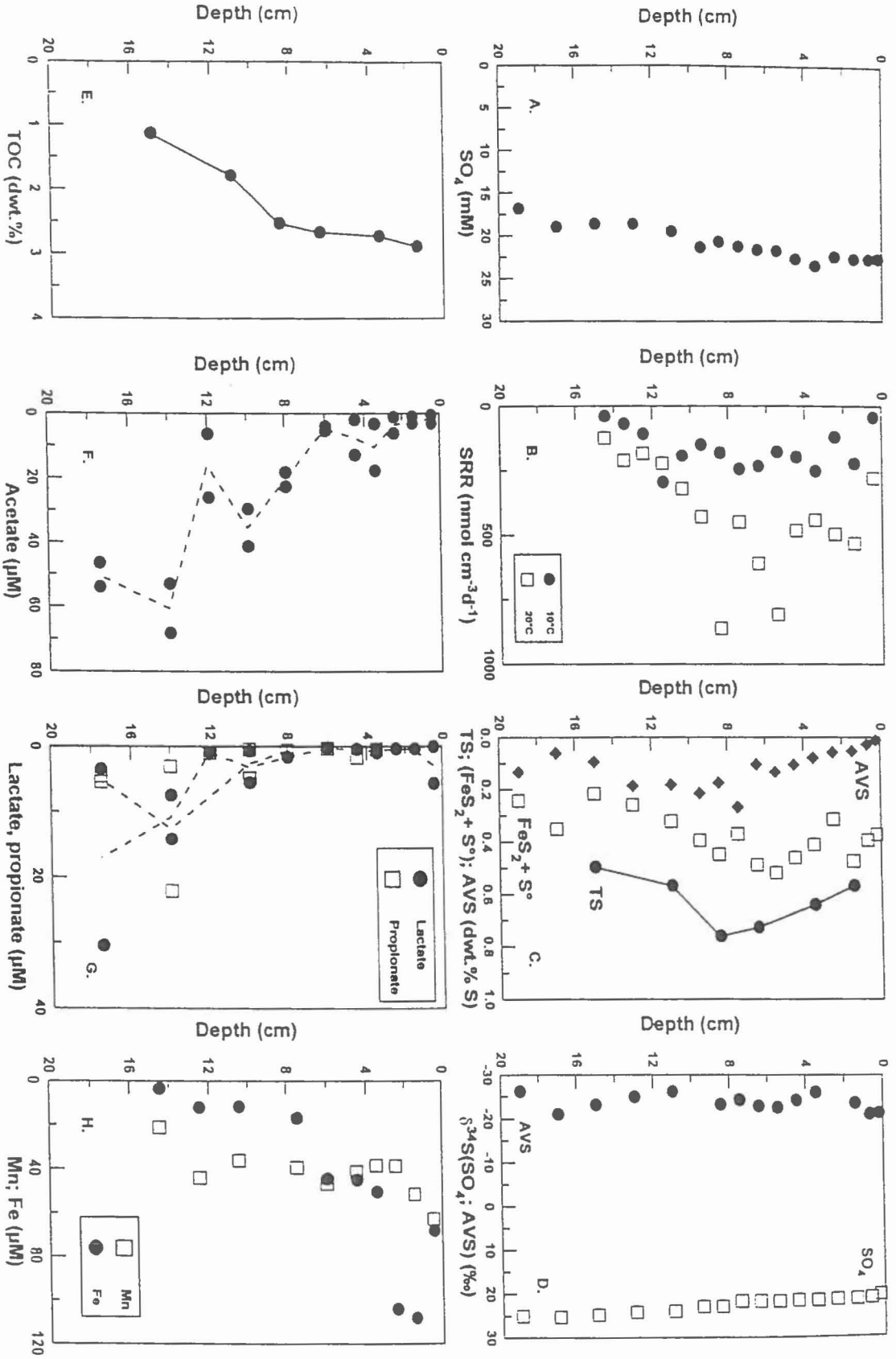
(ii) MPN-counts in agar-shakes and isolations□MPN-counts in agar shakes were conducted to obtain single colonies. Butyl-rubber stopper sealed glass tubes containing approximately 9 ml of anoxic media with molten agar and substrates as described above were prepared as described previously for the isolation of sulfate-reducing bacteria (Widdel and Bak 1992). The tubes were inoculated with 1 ml from the corresponding master dilution. Replicates and incubation conditions were as described above for MPN-counts in liquid media. Agar shakes were incubated at 23 °C. Growth was determined by microscopically observing formation of brownish colonies which are typical for sulfate-reducing bacteria. Such colonies were picked by

means of finely drawn Pasteur pipettes (Widdel and Bak 1992) and transferred to liquid medium containing the corresponding substrate.

Results

Sediment□Downcore temperatures in the sediments varied between about 16.0 and 17.5 °C during sampling with corresponding air temperatures between 16.0 °C (in the wind) and 20.2 °C (without wind). The sediment cores displayed 2 distinct color changes: the oxic upper part was brown which turned within the first 1-2 cm into a darker olive green color with some greyish diffuse streaks. At about 9-12 cmbsf (cm below surface), the coupling of increasing accumulation of iron monosulfide (acid-volatile sulfide, AVS) with decreasing porosity was indicated by a diffuse blackening of the sediment, remaining black until the end of the investigated sediment section. These vertical changes were also reflected by biological and geochemical parameters. The sediment was characterized by bioturbation, and living polychetes were found down to about 16 cmbsf. An analysis of the grain size distribution of surface sediments was carried out on sediments recovered in May 1998 (Böttcher & Hespenheide, unpublished data) and showed that the first 10 cm almost completely consisted of mud (clay and silt grain size fraction < 63μm; essentially phyllosilicates (Böttcher et al. 2000). An increase of the sand grain fraction was generally observed below that depth (Böttcher et al. 2000). Correspondingly, the pore water contents decreased continuously with depth. During sampling in June 1999, for instance, maximum water contents of 67% were observed near the surface (0.5 cm depth) which decreased to 52% and 29% at 10 and 29 cm depth, respectively. The TOC (Fig. 2E) decreased with depth in parallel with the water content.

Acid volatile sulfides (AVS; essentially iron monosulfide) were found in all sections and showed a maximum at around 8 cm depth (Fig. 2C). The stable sulfur isotopic signature of AVS should essentially mirror that of H_2S which is derived from the overall metabolic processes in the sulfur cycle (Böttcher et al. 1998). The AVS fraction ($\delta^{34}S$ values between -21.1 and -26.2‰) was significantly enriched in the lighter sulfur isotope compared to coexisting pore water sulfate ($\delta^{34}S$ values between +20.4 and +25.4‰; Fig. 2D). Apparent sulfur isotope enrichment factors between -41 and -52‰ are calculated from the data given in Fig. 2D which are at the upper end or even exceed the results obtained in experiments with pure cultures of sulfate-reducing bacteria (Kaplan and Rittenberg 1964; Chambers et al. 1975).


A.
Depth (cm)
SO4 (mM)
B.
SRR (nmol cm-3d-1)
10°C
20°C
C.
TS; (FeS2+ S°); AVS (dwt.% S)
AVS
FeS2+ S°
TS
D.
δ34S(SO4; AVS) (‰)
SO4
E.
TOC (dwt.%)
F.
Acetate (µM)
G.
Lactate, propionate (µM)
Lactate
Propionate
H.
Mn; Fe (µM)
Mn
Fe

Pore waters□The salinities of the pore waters during sampling in June 1999 were rather constant with 28‰. This corresponds well to earlier data obtained during sampling on a seasonal base over a two year period, where it has been found that salinities in the pore waters of the mud flat at Site Dangast varied between 22 and 30‰, averaging about 26‰ (Böttcher, unpublished data).

During sampling in June 1999 the microsensor measurements were impeded by high bioturbation activity in the upper 1.5 mm of the sediment and, therefore, no steady state O_2 profiles could be gained. During this measurement only the oxygen penetration depths (3.1 mm ± 0.17 mm) could be determined. Under similar environmental conditions but slightly less bioturbation a sediment core from the same site was investigated in June 1998 and the results are presented in Fig. 3A. In the upper 0.7 mm the sediment was significantly supersaturated with respect to oxygen as it has been found previously for a situation in April 1998 (Böttcher et al. 2000). The maximum concentration occurred at 0.3 mm depth with 0.43 mol/m^3. Below 0.7 mm the oxygen concentration declined constantly and the oxygen penetration depth was 2.7 mm. The volumetric rate calculations also showed a distinct area of oxygen production in the upper 0.7 mm of the sediment sample (Fig. 3A) and a smaller production zone at 1.2 mm - 1.4 mm. Between those layers and below 1.4 mm oxygen was consumed.

Nitrate concentrations from measurements in June 1999 are plotted in Fig. 3B . Down from the water/sediment interface the nitrate concentration increased to a maximum value of nearly 0.01 mol/m^3 at 1.2 mm depth. At 2.8 mm the concentration was still higher than in the water phase but declined in deeper layers. Nitrate could be measured down to 4 mm. The calculated activity rates showed a clear separation between nitrate production in the upper 2.3 mm of the sediment and nitrate consumption below that layer (Fig. 3B). Highest production rates occurred 0.2 mm above the maximum nitrate concentration.

No hydrogen sulfide could be detected in the sediment cores under any conditions within the first 20 mm, the maximum depth reached by our microsensor measurements. Sub-oxic, non-sulfidic conditions were indicated by the presence of dissolved Fe(II) and Mn(II) (Fig. 2H) and absence of sulfide at concentrations exceeding 5 μM between about 5 mm down to at least 12 cm depth (data not shown). Sulfate remained essentially constant within the first 4-5 cm and decreased further

downcore (Fig. 2A), associated with an enrichment in ^{34}S. Despite of minor changes in dissolved sulfate, moderate to high microbial sulfate reduction rates were measured with radio tracers through the whole sediment core and showed a maximum with up to 485 nmol · cm^{-3} · d^{-1} in the sub-oxic zone at around 7 cmbsf (Fig. 2B). The apparent activation energy calculated from an Arrhenius equation using the depth-integrated SRR for the 10 and 20°C incubations gives a value of 66 kJ mol^{-1}. This value was used to relate the measured SRRs to the mean temperature of 17°C measured during field sampling.

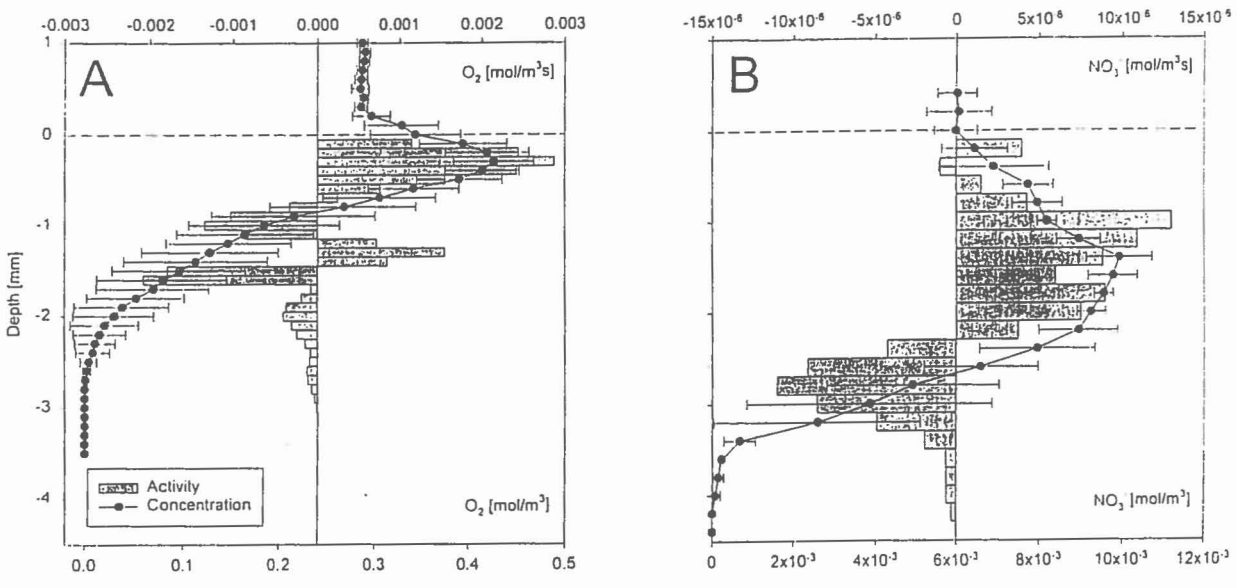


Figure 3. Microsensor measurements of the vertical sediment profile. A, Oxygen concentrations determined with an O_2 microsensor and calculated oxygen production/consumption rates (activity) in a sediment core from Dangast (6/98). B, Nitrate concentrations determined with a NO_3^- microsensor and calculated nitrate production/consumption rates (activity) in a sediment core from Dangast (7/99).

The concentrations of volatile fatty acids (vfa; acetate, propionate, and lactate; Fig. 2F and 2G) were determined in the pore waters of two sediment cores. In both cases concentrations of vfa except for acetate were below 5 µM in the first 5 cm of the sediment. Acetate concentration increased with depth to concentrations of about 50-60

μM. Concentrations of glycolate and formate on the other hand did not exceed 5 μM throughout the entire core. Elevated concentrations of lactate and propionate ranging between 10 and 30 μM were detected in the deepest horizon of 15-20 cm. This accumulation of organic acids occurred only below the maximum of the sulfate reduction rates (Fig. 2B, F and G).

PLFA analysis□Three different depth-depending PLFA patterns were observed in the studied sediment cores (Table 3). The first 0.5 cm are dominated by high amounts of polyunsaturated PLFA. In the depth between 0.5 and 10 cm, the PLFA patterns did not show any pronounced variations. The PLFA patterns included many branched fatty acids, cyclopropane fatty acids and a high amount of c11 18:1. The deepest layer is distinguished from the upper layers by the low amount of c9 16:1 and a higher amount of 18:0. The content of phospholipid phosphate decreased with depth (967 nmol · g^{-1} dry sediment at 1 cm depth to 228 nmol · g^{-1} dry sediment at 15-20 cm depth).

DGGE profiles of sediment samples□Changes in the diversity of the SRB population with depth were analyzed by DGGE of PCR-amplified 16S rDNA fragments and of the reversely transcribed 16S rRNA fragments. In the same sediment sample, the electrophoretic profiles of the 16S rDNA fragments were more complex than the ones observed after reverse transcription of the 16S rRNA (Fig. 4).

DGGE of 16S rDNA showed between 6 and 9 bands of different intensities. There were no major changes between different sediment layers, except for the last layer at 15-20 cm depth.

DGGE of 16S rRNA showed only two to four bands in all the samples. In contrast to the 16S rDNA-based DGGE, these were not evenly distributed but appeared as distinct patterns. It was not possible to get any PCR product from the last layer. As rRNA-based DGGE is influenced by the "activity-regulated" ribosome contents, this pattern might better indicate the identity and distribution of the active SRBs in the sample.

Table 2.: Vertical PLFA compositions of site Dangast. PLFA were analysed as fatty acid methyl esters and are shown in relative amounts [mol %]. PLFA methyl esters present in all horizonts less than 0,5 % are not listed. Not detectable PLFA were abbreviated with nb.

	Sediment horizonts [cm]								
PLFA methyl ester[a]	**0.0-0.5**	**0.5-1.0**	**1.0-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**	**5-10**	**10-15**	**15-20**
i14:0	1.4	0.9	1.0	1.1	1.0	0.8	1.0	0.4	0.3
c7 14:1	0.7	nd	0.1	0.3	nd	nd	0.3	0.2	0.6
14:0	6.3	3.6	3.5	0.4	3.1	0.9	3.3	1.6	1.6
i15:0	2.0	3.2	3.8	4.1	3.8	3.9	3.8	3.1	3.2
a15:0	2.8	5.5	6.6	7.3	6.9	7.3	7.6	7.3	8.0
15:0	3.6	2.8	2.1	2.2	2.0	2.0	2.1	1.8	1.4
16:3[b]	8.4	nd	nd	nd	nd	nd	nd	nd	nd
i16:0	nd	1.6	1.7	1.8	1.7	1.8	1.6	1.7	1.6
c7 16:1	1.2	1.8	1.6	1.8	1.7	1.8	1.5	1.5	1.3
c9 16:1	16.5	17.2	17.8	19.2	18.3	19.5	17.9	14.0	9.4
c11 16:1	4.1	1.6	2.1	2.4	2.3	2.7	2.6	2.2	1.4
c13 16:1	1.3	2.4	2.2	2.5	2.4	2.9	2.5	2.8	2.7
16:0	10.5	19.2	17.6	18.2	17.1	17.4	16.7	15.7	17.4
i17:1	0.3	1.2	1.2	1.1	1.4	1.1	0.8	1.2	2.9
10me16:0	0.4	1.3	1.7	1.9	1.9	2.3	2.4	2.3	2.1
a17:1	0.5	0.2	0.7	0.7	0.7	0.5	0.5	0.6	nd
i17:0	0.5	0.8	0.9	1.0	1.0	1.0	0.9	1.1	1.2
a17:0	0.4	1.1	1.2	1.3	1.3	1.4	1.4	1.8	2.0
c9 17:1	3.2	1.5	1.3	1.4	1.5	1.4	1.4	1.2	0.5
cyc 17:0	0.7	1.3	1.5	1.6	1.6	1.6	1.6	0.3	0.2
17:0	1.0	1.6	1.6	1.6	1.6	1.5	1.5	1.5	1.2
c6 18:2	1.9	0.8	0.7	0.6	0.5	0.5	0.5	0.4	0.1
c6 18:3	nd	0.6	0.6	0.5	0.5	0.5	0.6	0.5	nd
c9 18:2	1.4	0.4	0.7	0.7	0.8	0.7	0.4	0.6	0.7
c9 18:1	3.4	4.4	3.6	4.1	3.8	3.5	4.0	4.3	4.1
c11 18:1	3.9	10.7	11.6	13.3	13.3	13.3	12.7	15.6	14.6
c13 18:1	0.5	4.2	3.0	0.7	2.2	2.4	3.3	4.1	8.0
18:0	2.4	3.2	2.8	3.3	2.6	2.4	2.7	3.8	8.2
cyc19:0	nd	0.4	0.6	0.3	0.5	0.1	nd	0.5	0.7
c5 20:4	1.6	0.9	0.8	1.0	0.9	0.8	0.7	1.4	0.5
c5 20:5	13.7	2.1	1.4	1.2	1.0	0.8	0.8	2.3	0.4
c9 20:2	nd	0.8	0.2	nd	0.8	0.2	0.2	1.6	nd
c4 22:6	5.7	0.7	0.7	0.5	0.2	0.2	0.2	0.2	nd
24:0	nd	0.2	0.2	nd	0.1	1.6	0.3	0.2	0.1
branched[c]	8.8	16.7	20.2	22.1	20.6	20.7	21.2	20.3	22.9
saturated[d]	32.5	47.1	47.7	46.6	47.0	46.6	47.8	43.9	49.9
unsaturated[e]	67.5	53.0	52.3	53.4	53.0	53.5	52.2	56.1	50.1

[a]used short-hand nomenclature: i=iso-branched; a=anteiso-branched; c=*cis*; cyc=cyclopropane; me=methylgroup; x:y x= number of carbon atoms; y=number of double bonds
[b]position of double bond not determined
[c]include all iso, anteiso and 10Me branched PLFA-methyl esters
[d]all PLFA methyl esters without a double bond in the carbon chain
[e]all PUFA methyl esters with one or more double bonds in the carbon chain

Identification of the most prominent DGGE bands by sequencing All the bands analyzed by sequencing were shown to originate from members of the δ-subclass of *Proteobacteria*, the taxonomic group that encompasses most gram-negative SRB (Fig. 5). Bands 1 and 2 were affiliated with a sequence similarity of 99% to *Desulfobulbus* sp. (accession numbers L40786 and L40785, respectively). DGGE bands 3 and 4 were found to be closely related to *Desulfovibrio* species. Band 3 was related to *Desulfovibrio caledoniensis* (accession number U53465) sharing similarities of 97%, and band 4 was related to *Desulfovibrio* sp. Ac5 (accession number AF228117) with 99% similarity. Band number 5 was identify as *Desulfobacter* sp. with 96% similarity (accession number L40787). Bands 6 and 7 were related with 99% similarity to *Desulfonema limicola* (accession number U45990), and to *Desulfonema ishimotoei* (accession number U45991), respectively. The band number 8 had 98% sequence similarity with *Desulfuromonas palmitatis* (accession number U28172).

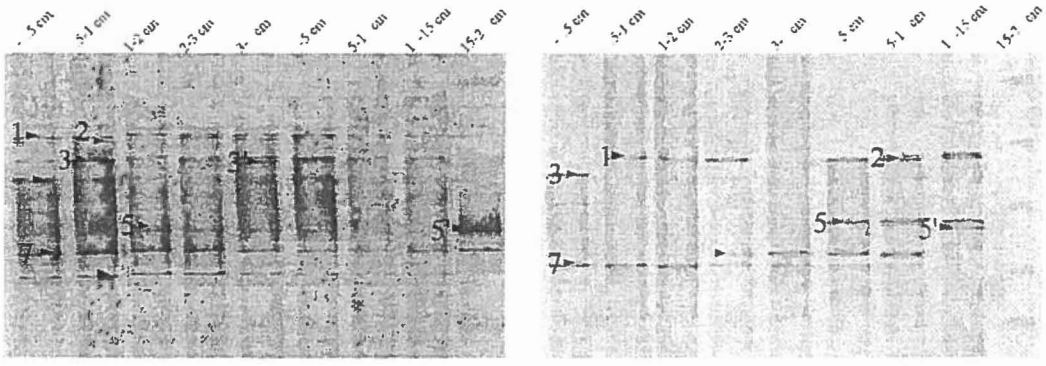


Figure 4. DGGE profiles of 16S rDNA (A) and reverse transcribed 16S rRNA (B) by using the SRB specific forward primer SRB385 which carries a GC-clamp and the reverse primer 907 (Sass et al. 1998). DGGE bands 6 and 7 affiliate with the *Desulfonema* group. It has to be noted, that probe DSS658 does not target this group.

MPN counts of SRB A variety of defined substrates was used to meet specifc substrate preferences of different SRBs possibly occurring in the studied sediment. For example, chemolithoautotrophic SRBs, such as *Desulfobacterium autotrophicum,* can grow with H_2 and CO_2 as the sole sources of energy and carbon, respectively. The bacterial numbers from MPN counts with liquid media are shown in Table 4.

During incubation of MPN tubes first a fast growing population and then after prolonged incubation a slow growing population developed. After about six weeks of

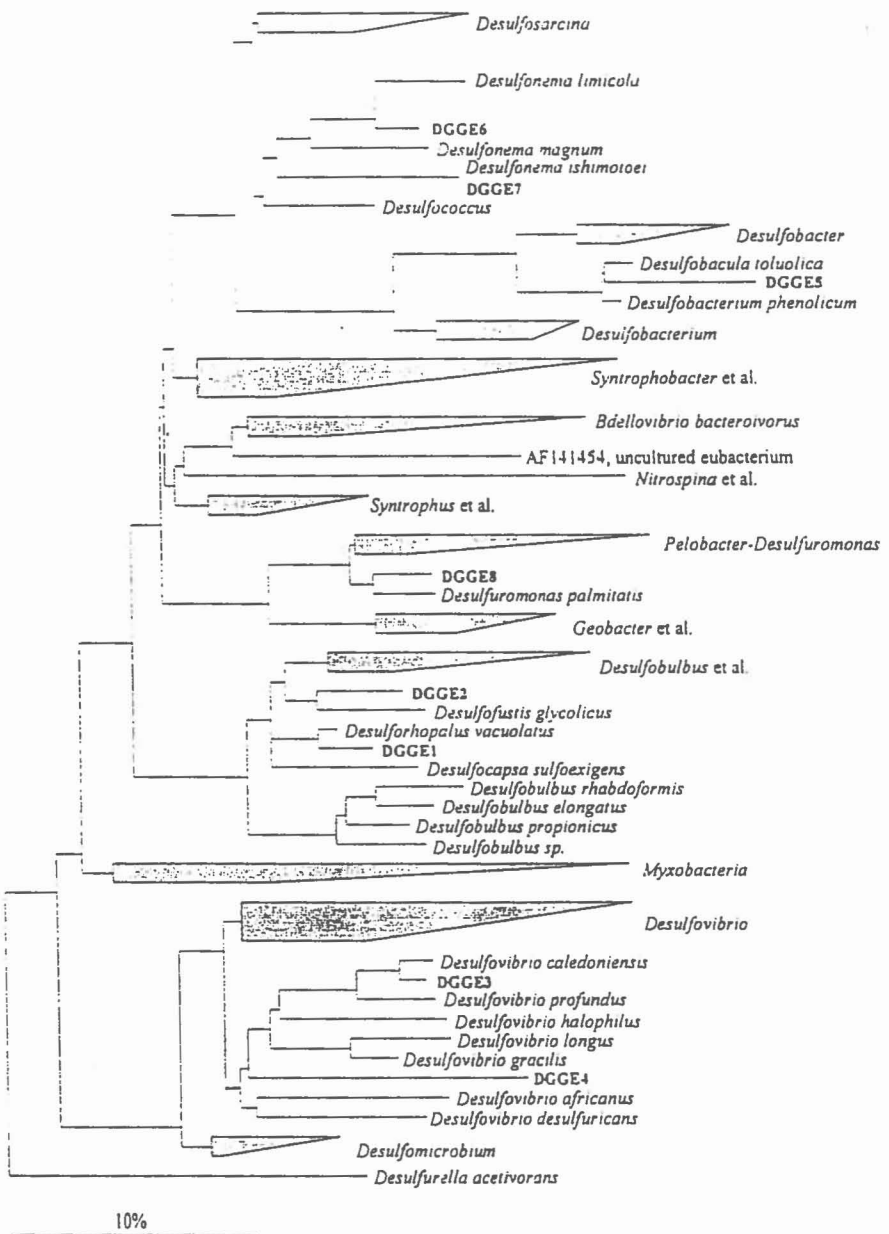


Fig. 5. Phylogenetic affiliation of the sequenced DGGE fragments. The tree is based on the result of the parsimony analysis including only complete or almost complete 16S rRNA sequences of representative bacteria (Maidak et al. 2000). The topology of the tree resulted from the insertion of the partial DGGE sequences into the tree without modifying its topology during sequence positioning (Strunk et al. 1998-2000).

incubation only low cell numbers ranging between 1.1×10^3 and 4.6×10^4 cells per ml could be observed. The only exception were MPN cultures with $H_2 + CO_2 +$ acetate as substrates yielding counts of up to 4.6×10^5 cells per ml. In general, cells grew homogeneously in the medium. With all substrates tested, rather similar cell numbers were obtained for all horizons analyzed. Thus this first population of fast growing SRBs appears to be evenly distributed across a depth profile of 0 to 15 cm. Cell numbers decreased by about one order of magnitude in horizon 15 to 20 cm. Prolonged incubation (10 months) of the MPN cultures allowed the detection of slowly growing but more abundant SRBs. Counts increased up to 1.1×10^7 cells per ml. In contrast to the faster growing SRBs, here cells did not grow homogeneously distributed in the medium, but rather formed flocs (Fig. 6) and aggregates, sometimes similar of the cell packages known from *Desulfosarcina* spp. As with the fast growing SRBs, MPN counts indicated that cells were evenly distributed throughout the horizons, with a decrease in cell numbers only in the last horizon.

From the highest dilution steps that still showed growth, samples were recovered for FISH analysis, the results of which are summarized in Table 5.

MPN in agar shakes yielded numbers of colony forming units essentially in the same range as those observed with MPNs in liquid media. Colonies from the highest dilution were transferred to fresh liquid media containing the same substrates as used in the corresponding agar shakes. Subsequent analysis of growth cultures by FISH revealed no other phylogenetic groups as already identified in MPNs with liquid media.

FISH of sediment samples□Total cell counts of the upper 20 cm of the sediment were determined microscopically (Table 6). From top to bottom DAPI counts strongly decreased from 53.9×10^8 in the first cm to 1.0×10^8 cells per cm^{-3} of sediment in the zone below 15 cm.

The microbial community dwelling in Dangast sediments was dominated by Bacteria. In the first 0.5 cm of the sediment, up to 82.3% of the total microorganisms hybridized with the probe EUB338 (Table 6). Archaeal and eucaryal counts remained below the detection limit of 0.1% of the DAPI stained cells.

Recently, the bacterial probe EUB338 was shown not to detect all members of the domain *Bacteria* (Daims et al. 1999). Some bacterial phyla, most notably the

Table 3. Total cell counts of SRB from different sediment horizons from Site Dangast after 1.5 and 10 months of incubation at 23 °C[a].

Sampling depth	$H_2 + CO_2$		$H_2 + CO_2 + Ac$		Formate		Acetate		Lactate		Propionat	
	1.5 months	10 months	1.5 months	10 months	1.5 months	10 months	1.5 months	10 months	1.5 months	10 months	1.5 months	10 months
0 – 0.5	$> 1.1 \times 10^3$	4.6×10^6	4.6×10^4	$> 1.1 \times 10^7$	nd	$> 1.1 \times 10^6$	4.6×10^3	$> 1.1 \times 10^5$	1.1×10^4	nd changes	$> 1.1 \times 10^4$	$> 1.1 \times 10^5$
0.5 – 1	4.6×10^3	1.1×10^7	1.1×10^5	$> 1.1 \times 10^7$	nd	nd changes	1.1×10^4	4.6×10^5	$> 1.1 \times 10^4$	3.9×10^5	$> 1.1 \times 10^4$	$> 1.1 \times 10^5$
1 – 2	4.6×10^3	$> 1.1 \times 10^6$	1.1×10^5	1.1×10^7	$> 1.1 \times 10^4$	4.6×10^5	$> 1.1 \times 10^4$	nd changes	4.6×10^5	nd changes	$> 1.1 \times 10^4$	4.6×10^5
2 – 3	$> 1.1 \times 10^3$	4.6×10^6	$> 1.1 \times 10^4$	$> 1.1 \times 10^6$	$> 1.1 \times 10^4$	$> 1.1 \times 10^6$	1.1×10^4	$> 1.1 \times 10^6$	4.6×10^3	4.6×10^6	$> 1.1 \times 10^4$	$> 1.1 \times 10^6$
3 – 4	$> 1.1 \times 10^3$	$> 1.1 \times 10^6$	1.1×10^5	$> 1.1 \times 10^6$	4.6×10^4	4.6×10^6	1.1×10^4	nd changes	4.6×10^4	4.6×10^6	$> 1.1 \times 10^4$	$> 1.1 \times 10^6$
4 – 5	$> 1.1 \times 10^3$	4.6×10^6	4.6×10^5	1.1×10^6	4.6×10^3	4.6×10^6	$> 1.1 \times 10^4$	4.6×10^5	4.6×10^4	4.6×10^6	$> 1.1 \times 10^4$	4.6×10^6
5 – 10	nd	4.6×10^6	4.6×10^5	4.6×10^6	$> 1.1 \times 10^4$	1.1×10^7	4.6×10^4	1.1×10^7	$> 1.1 \times 10^4$	nd changes	$> 1.1 \times 10^4$	4.6×10^5
10 – 15	1.1×10^3	4.6×10^6	$> 1.1 \times 10^5$	4.6×10^6	$> 1.1 \times 10^4$	1.1×10^6	$> 1.1 \times 10^4$	3.9×10^6	4.6×10^4	4.6×10^6	1.1×10^4	1.1×10^6
15 - 20	nd	$> 1.1 \times 10^6$	1.1×10^3	4.6×10^5	4.6×10^3	4.6×10^5	$> 1.1 \times 10^3$	$> 1.1 \times 10^4$	4.6×10^3	4.6×10^5	4.6×10^3	nd changes

[a] To confirm the low numbers of bacterial cells observed after the short incubation period a control experiment was performed. A comparative MPN study was carried out with sediment (2-5 cm depth) from the same location at Dangast and from sediment at Horumersiel, north of Jadebusen in September 1999. The latter site is characterized by a locally high organic input and a high sulfidogenic activity. The MPN tubes were incubated for 2.5 months at 23 °C. MPN counts with all substrates tested ($H_2 + CO_2$, $H_2 + CO_2$ + 2mM acetate, 7.5 mM acetate, 5 mM lactate and 5 mM propionate) were between 1.1×10^4 an 1.1×10^5 per ml for the Dangast sediment. In contrast, MPN counts for the Horumersiel site were between 1.1×10^6 and 1.1×10^8 cells per ml.

nd, not detected

Planctomycetales and *Verrucomicrobia*, are missed by this probe. Consequently, total bacterial numbers monitored in this study were most probably underestimated. Initial experiments with the newly designed additional probes EUB338-II and -III (Daims et al. 1999) indicated that this underestimation may amount to 4-10% of the total microbial community.

Detection rates with the probe EUB338 decreased exponentially over the vertical profile. Thus, not only the absolute cell numbers decreased with depth, but also the percentage of detectable cells with our FISH protocol.

Group specific probing□A set of 15 different probes specific for SRB of the δ–subclass of *Proteobacteria* were tested (Table 1). Only five of these probes (i.e. 221, DSB985, DSR6512, DSS658, and DSV698) gave counts above the detection limit set at 0.1% of total DAPI counts (Table 7). The target groups of the other probes were either not present in high abundance (>0.1% of the total cell counts) or not detectable by FISH, e.g. due to a low ribosomal content per cell.

The most abundant SRB present in the sediments were members of the *Desulfosarcina-Desulfococcus-Desulfofrigus* group (probe DSS658) and *Desulfovibrio* spp. (probe DSV698) with maximum values of 2.9×10^8 and 2.8×10^8 cells per cm^{-3} of sediment, respectively. The counts of these two groups decreased strongly with depth to values of 1.0×10^6 and 0.6×10^6 cells per cm^{-3} of sediment at 20 cm, respectively.

SRB detected with the probe DSR651 specific for *Desulforhopalus* were also counted with decreasing numbers from top to bottom from 1.1×10^8 cells per cm^{-3} of sediment in the first 0.5 cm to 2.7×10^7 cells per cm^{-3} sediment between 10 and 15 cm depth. Cell numbers between 15 and 20 cm depth were below the detection limit.

Desulfobacter spp. (probe DSB985) were found over the whole sediment profile in relatively low abundance. The cell numbers were decreasing from 1.1×10^8 to 0.1×10^6 cells per cm^{-3} throughout the sediment. *Desulfobacterium* detected with the probe 221 were below the detection limit in the sediment except for the layers between 1 and 2 cm depth were between 0.5×10^7 and 1.0×10^7 cells per cm^{-3} were found.

The total SRB community was found to be maximum within the upper 3 cm of the sediment with numbers up to 6.6×10^8 cells per cm^{-3}, accounting for 8.6 to 12.3% of

Table 4. Identification of SRB by FISH[a] after 1.5 and 10 months of incubation.

Sampling depth	$H_2 + CO_2$		$H_2 + CO_2 + Ac$		Formate		Acetate		Lactate		Propionat	
	1.5 months	10 months	1.5 months	10 months	1.5 months	10 months	1.5 months	10 months	1.5 months	10 months	1.5 months	10 months
0 – 0.5	DSB985	DSB985[b] /221	DSV698	DSV698[c]	nd	n.d.	DSB985	DSB985 /DSS658	DSV698	n.d.	660	n.d.
0.5 – 1	n.d.	DSB985	DSV698	DSV698[c]	nd	n.d.	DSB985	DSB985 /DSS658	DSV698	DSV698[c]	660	n.d.
1 – 2	221	221	DSV698	DSV698[c]	n.d.	DSV698	DSB985	DSB985	DSV698	n.d.	660	n.d.
2 – 3	221	221	DSV698	DSV698[c]	n.d.	DSV698	DSB985	DSB985	DSV698	DSV698[c]	660	660
3 – 4	221	221	DSV698	DSV698[c]	DSV698 /DSV214	DSV698	n.d.	DSB985	DSV698	DSV698[c]	n.d.	n.d.
4 – 5	221	nd	DSV698	DSV698[c]	DSV698 /DSV214	DSV698	DSB985	DSB985	DSV698	DSV698[c]	n.d.	n.d.
5 – 10	nd	221	DSV698	n.d.	DSV698	DSV698	DSB985	DSB985	DSV698	DSV698[c]	660	660
10 – 15	221	221	DSV698	n.d.	n.d.	DSV698	DSB985	DSB985	DSV698	DSV698[c]	660	660
15 - 20	nd	221	DSV698	n.d.	DSV698 /DSV214	n.d.	DSB985	DSB985	DSV698	n.d.	660	660

[a] Probes are described in Table 1.
[b] Positive hybridization with more than one probe.
[c] Lower brightness of the signal after 8.5 months incubation.

total cells. The number of SRB cells detected by FISH was decreasing over the vertical profile to 1.7×10^6 cells per cm^{-3} (or as low as 1.7% of total cells) at 20 cm depth.

TABLE 5. Quantification of SRB in Dangast sediments by FISH.

	% of hybridized cells[a] (mean ± SD)							
horizon	DAPI ($x10^8$)[b]	Probe EUB338	Probe 221	Probe DSB985	Probe DSR651	Probe DSS658	Probe DSV698	Total SRB
0-0.5	53.9±2.2	82.3±14	n.d.[c]	0.21±0.07	2.0±0.7	2.5±1	4.3±0.9	8.6
0.5-1	53.7±0.9	69.7±13	0.22±0.04	0.18±0.1	1.9±0.7	4.7±1.3	5.3±1.1	12.3
1-2	56.5±1.3	69.8±5	n.d.	n.d.	2.1±0.7	5.3±0.9	3.9±0.8	11.3
2-3	53.5±2.3	53.1±3	0.15±0.03	n.d.	1.7±0.6	3.8±0.8	4.4±0.4	10.0
3-4	33.1±1.5	45.6±4	n.d.	0.1±0.08	2.4±0.9	2.4±0.7	4.2±0.7	9.1
4-5	25.5±2.3	37.4±6	n.d.	0.1±0.09	1.7±0.8	3.5±0.9	6.2±1.2	11.5
5-10	17.0±2.1	25.0±3	n.d.	n.d.	0.7±0.3	2.2±0.4	3.3±0.3	6.3
10-15	9.0±3.3	17.0±2	n.d.	n.d.	0.3±0.4	1.7±0.5	1.0±0.7	3
15-20	1.0±4.6	13.0±6	n.d.	0.1±0.04	n.d.	1.0±0.6	0.6±0.4	1.7

[a] Percent detection compared to DAPI. Numbers have been corrected by subtracting NON338 counts. Means and standard deviations were calculated from the counts of two parallel cores.
[b] Number of cells per cm^{-3}.
[c] Not detected.

Discussion

Biogeochemistry of the sediment□The observed decrease in pore water content with sediment depth is due to changes in the sedimentological and mineralogical properties of the sediment caused by a mixing of minerals of the mud fraction with increasing amounts of the sand grain fraction (essentially quartz). In contrast to quartz, phyllosilicate minerals, and especially smectite, are able to adsorb significant amounts of organic matter (Keil et al. 1994; Mayer 1994). A parallel decrease of pore water and TOC contents with depth has been described earlier for tidal sediments of the German Wadden Sea (Delafontaine et al. 1996; Böttcher et al. 1998; Böttcher et al. 2000; Volkman et al. 2000). Stable carbon isotope measurements on TOC at Site Dangast have additionally shown that the labile fraction, which is of marine origin, decreases with depth due to the preferential biodegradation of marine organic matter (Böttcher et al. 1998; Böttcher et al. 2000). The accumulation of AVS throughout the investigated sediment layers is a clear indication for the dissimilative activity of sulfate-reducing bacteria under anaerobic conditions (e.g., Morse et al. (1987). Solid phase sulfur with higher oxidation states (essentially pyrite with minor elemental

sulfur) were mostly found in higher contents through the sediment core with depth variations essentially decoupled from the AVS trend (Fig. 2C).

The sulfur isotopic discrimination between sulfate and the AVS-fractions is a clear indication that microbial dissimilatory sulfate reduction took place. This is due to a preferential intracellular, enzymatic reduction of $^{32}SO_4^{2-}$ compared to $^{34}SO_4^{2-}$ (Kaplan and Rittenberg 1964; Rees 1973). The isotope enrichment factors observed in the sediment partly exceeds the maximum found in pure cultures. The enhanced sulfur isotope discrimination may be due to the activity of sulfate reducers in the Dangast sediments that have not been available in previous experiments on isotope discrimination and/or a contribution from the oxidative part of the sulfur cycle. H_2S is to a significant portion reoxidized and sulfur species with intermediate oxidation states may be formed (Jørgensen 1982a). It has been shown experimentally that bacterial disproportionation of the latter leads to the formation of ^{32}S-enriched H_2S (Canfield et al. 1998; Cypionka et al. 1998) which may contribute to the observed overall isotope effect. Bacteria which are able to disproportionate elemental sulfur, for instance, have been isolated from the Dangast sediments and their ability to discriminate sulfur isotopes has experimentally been confirmed (Canfield et al. 1998).

Pore water composition The compositions of interstitial waters sensitively mirror the biogeochemical processes in the sediment. According to the typical zonation scheme as proposed by Froelich et al. (1979), organic matter oxidation should be related to the consumption of oxygen followed by nitrate, the build up of Mn(II) and Fe(II) due to the reduction of Mn(IV) and Fe(III) (oxyhydr)oxides, and later to the depletion in sulfate.

Highest oxygen concentration in the photic zone are not directly linked to maximum nitrate production (Fig. 3). For nitrate production the actual oxygen production rates seem to be more important than the oxygen concentration. In the top 0.7 mm, where oxygen is produced via photosynthesis and oxygen concentrations are high, nitrate production rates are relatively low. The nitrate concentration in the upper part of the sediment is clearly influenced by diffusion out of the sediment. That is the reason why at the depth of high nitrate production at 1 mm the concentration is still moderate. Net nitrate consumption appears only below 2 mm depth, where nearly no oxygen is available. A distinct layer of denitrification between 2.2 mm and 3.4 mm could be identified.

Sulfate concentrations at the sediment-water interface correspond well to the measured salinities, indicating that dilution of seawater with sulfate-poor freshwaters took place in the surface waters of the coastal region. The decrease in sulfate was associated with an enrichment in ^{34}S. This is expected for a system, where the consumption of sulfate by bacteria exceeds the supply of sulfate by transport from the sediment water interface or by reoxidation of sulfide (Hartman and Nielsen 1969).

The measured sulfate reduction rates are within the range observed previously at the same site (Böttcher et al. 2000) and in other fine-grained tidal sediments of the North Sea (Oenema 1990; Kristensen et al. 2000). The downcore variation of SRR found in the present study is essentially identical to measurements carried out during summer time on the same mudflat 1 year before (Böttcher and Hespenheide unpublished data). The same is also found for the community size and composition of SRB (Böttcher et al. 2000). This indicates an apparent reproducibility in seasonal variations of boundary conditions (e.g., bioturbation) through the years. The maximum in bacterial sulfate-reduction within the sub-oxic zone, together with enhanced dissolved Fe(II) and Mn(II) concentrations and limited accumulation of dissolved sulfide has been observed earlier and is most likely related to reoxidation of sulfide (Moeslund et al. 1994; Thamdrup et al. 1994; Böttcher et al. 2000). Hydrogen sulfide which is produced during bacterial dissimilatory sulfate reduction can react with iron compounds to form iron sulfides and may be re-oxidized by the reaction with Fe(III) or Mn(IV) compounds to intermediate sulfur species or sulfate (Burdige 1993; Thamdrup et al. 1994; Böttcher and Thamdrup 2001). Therefore, the concentrations of dissolved Fe(II) and Mn(II) observed in the pore-waters are not necessarily the result of microbial reduction of the respective oxyhydroxides but may also result from the re-oxidation of sulfide produced during dissimilatory sulfate reduction. The observed decrease in SRR near the sediment-water interface is most likely superimposed by reoxidation reactions with reactive metal (oxyhydr)oxides in the surface sediments (Moeslund et al. 1994; Thamdrup et al. 1994). During short-time incubations of coastal sediments, Moeslund et al. (Moeslund et al. 1994) observed 5-fold higher SRR at short compared to enhanced incubation times. They related this phenomenon mainly to the reoxidation and/or liberation of H_2S during incubation with time.

Microbial sulfate reduction depends on the activity of fermentative bacteria which are able to degrade polymeric substances to smaller molecules like vfa, which are the

actuai substrates of sulfate-reducers (e.g., Sørensen et al. 1981; Christensen 1984). The SRR is, therefore, also linked to the production rate of vfa. The measured concentrations of volatile fatty acids in interstitial waters, therefore, depend on both, the production and the consumption rates. The reactions responsible for the quantitative balance in different environments, however, are still not completely understood. In the present study high concentrations of acetate and lactate coincide with low sulfate reduction rates (Fig. 2). This is in agreement with observations by Novelli et al. (1988) and Holmer and Kristensen (1996). One may speculate that this correlation may be due to changes in sedimentological (e.g., decreased water content), the TOC composition and an overall decrease in the availability of nutrients.

The sulfate reduction rates per cell observed in these sediment samples were around 1 fmol $SO_4^{2-} \cdot cell^{-1} \cdot day^{-1}$. This result is in agreement with previous reports (Sahm et al. 1999; Böttcher et al. 2000; Ravenschlag, 2000). They are at the lower end of cellular sulfate reduction rates determined for pure cultures of different psychrophilic, mesophilic, and thermophilic sulfate reducing bacteria (Canfield et al. 2000).

Sulfate reducing bacteria The distribution of SRB in the vertical profile of the sediment was studied with four independent techniques.

First, the phospholipid fatty acid (PLFA) analysis allows to study the totality of PLFA present in a sediment horizon without any discrimination between phylogenetic groups. An identified individual PLFA can be used as a biomarker for a specific group of organisms. The top 0.5 cm of the sediment were dominated by polyunsaturated PLFA, which are typical for eucaryotic organisms (Vestal and White 1989; Findlay and Dobbs 1993), including protozoa, algae and higher plants. The sediment layers between 0.5 and 15 cm typically contained the PLFA C11 18:1, characteristic of anaerobic bacteria (Findlay et al. 1990; Findlay and Dobbs 1993; Vestal and White 1989). Thus the shift in PLFA pattern around 0.5 cm depth correlates well with the oxygen penetration as observed by the microsensor study (Fig. 3A). In the deepest studied horizon (15-20 cm) a pronounced decrease in the diversity of PLFA indicated also a general decrease in microbial diversity. This is in good agreement with the changes of DGGE patterns (Fig. 4) and decrease in viable cell and FISH numbers (Table 3 and 5). Throughout the studied sediment horizons PLFA markers characteristic of SRB genera were identified pointing to an even distribution of SRB

in the sediment profile. We found the marker fatty acids 10Me16:0 for *Desulfobacter* (Taylor and Parkes 1983) and i17:1 for *Desulfovibrio/Desulfomicrobium* (Vainshtein et al. 1992) in significant quantities in all horizonts. We also found a17:1, a fatty acid which can be detected in *Desulfococcus* and *Desulfosarcina* (Kohring et al. 1994). The highest relative percentage of i17:1 were discovered in 15 to 20 cm depth. Whereas the most relative amount of 10Me16:0 was found between 4.0 and 15 cm depth

Second, DGGE analysis was carried out to profile the diversity of SRB (Fig. 4A). A similar electrophoretic pattern was observed in the different horizons within the upper 15 cm of the sediment, showing an even distribution of SRB. Phylogentic identification of the most prominent bands showed diverse phylogenetic affiliation within the δ-subclass of *Proteobacteria*. Interestingly most sequences were closely related to SRBs originating from marine environments, e.g. *Desulfonema ishimotoei* (isolated from Dangast, the present study area, ref. Fukui et al. 1999) and *Desulfobacula toluolica* (isolated from Eel Pond, Woods Hole, USA, ref. Rabus et al. 1993).

Third, the combined MPN/FISH approach was used to identify and quantify viable cells in the presence of a variety of substrates typical for SRBs. Regardless of the substrates used or the length of the incubation period, an even distribution of cells throughout the vertical profile of the sediment was observed (Table 4). Identification of SRB in highest dilution showing growth was performed with FISH (Table 5). With respect to the individual substrates added to the MPN cultures, results were in good agreement with the nutritional properties of known SRBs. For example, cultures with either lactate or H_2/CO_2/acetate known as substrates typical for *Desulfovibrio*, were indeed dominated by members of this species. In addition, H_2/CO_2, the utilization of which requires the capacity for chemolithoautotrophic growth as known from *Desulfobacterium autotrophicum* (Brysch et al. 1987), yielded predominantly members of the genus *Desulfobacterium*. MPN-cultures with acetate, the utilization of which requires the capacity of complete oxidation, were dominated by members of the genera *Desulfobacter* and/or *Desulfobacula*, both known to possess this capacity. During incubation generally two types of populations could be observed under all conditions (i.e. with every substrate used and with every sampling depth tested). After a short incubation time of about six weeks a more rapidly growing population of less

abundant cells developed. The more abundant population required a prolonged incubation time of about 10 months. It should be noted that this population type did not grow homogenously in the culture medium but formed flocs. The observation that these SRB predominantly grow in flocs pose some difficulties to an absolute quantitative interpretation of the MPN-results and may explain the lower cells numbers determined with MPN (Table 4) as compared to FISH (Table 5). Interestingly, analysis of the flocs in the MPN tubes with FISH revealed the presence of very small bacteria in the flocs that could not be detected by mere phase contrast microscopy.

Fourth, the analysis of the active SRB or those with a high ribosome content was performed using FISH and DGGE analysis of reversly transcribed 16S rRNA fragments. With respect to the sensitivity of the method, DGGE profiles from reversly transcribed 16S rRNA are assumed to reflect the ribosome content of cells, rendering this method a possible tool to indirectly identify cell activity. Except for two sequences (DGGE 4 and 6; Fig. 4) all of those identified by DGGE profiling of 16S rDNA (Fig. 4A, Fig. 5) were also found in the DGGE profiles of 16S rRNA. However, in the latter case a discrimination of detectable bands with depth was found. For example, DGGE band 5 is evenly distributed throughout the studied sediment horizons if monitored with the 16S rDNA-based DGGE profiling. In contrast, this band could only be detected in the lower part (below 3 cm) of the sediment, if monitored by 16S rRNA-based DGGE profiling. This could indicate that the *Desulfobacter/Desulfobacula* type SRB represented by DGGE band 5 are more active only in the lower part of the sediment, even though they are also present in the upper part. The opposite was observed for DGGE band 3, representing *Desulfovibrio* type SRB.

The examination of the microbial community in Dangast by FISH showed that SRB account for a major part of the detectable Bacteria. Up to 6.5×10^8 cells per cm^{-3} were identified as SRB. This number of cells was always higher than the number of viable SRB observed using MPN. Similar discrepancy between MPN counts and for instance DAPI based cell counts have been described (Sievert et al. 1999). The absolute numbers of the different physiological groups might be underestimated since typically less than 1% of the total bacterial population in natural habitats may be accessible by current cultivation dependent methods (Amann et al. 1995). In addition,

the association of cells in flocs or with particles can not be ruled out. Clumping may also lead to an underestimation, since the MPN evaluation assumes that only a single cell is required to initiate growth at the highest dilution.

The *Desulfosarcina-Desulfococcus-Desulfofrigus* group (targeted by probe DSS658) was identified as the most abundant SRB when sediment samples were directly analysed by FISH. This group was also identified by PLFA analysis of sediment samples. However, this group was not identified when MPN and DGGE methods were applied. Possible explanations could be on the one hand that the cultivation conditions used for MPNs select for SRBs not affiliating with the *Desulfosarcina-Desulfococcus-Desulfofrigus* group, and on the other hand that the primers used for DGGE are not targeting this group (Ravenschlag et al. 2000). The second most abundant group of SRB identified in the sediment samples by FISH belong to the *Desulfovibrio* group. In this case, identification was possible with all three other techniques.

Conclusions

A combined microbiological, molecular, biogeochemical and isotope geochemical approach was applied to gain new information on the relationship between abundance, community structure and activity of the sulfate-reducing bacteria (SRB) of a surface marine sediment. Dissolved oxygen and nitrate were only found within the first few mm of the top sediment with different microscaled zones of formation and consumption, as obtained from profile modeling. Bacterial dissimilatory sulfate reduction was measured through the whole investigated sediment section. However, further reaction and reoxidation of hydrogen sulfide led to the development of suboxic pore water conditions. Microbial and chemical reactions led to the accumulation of dissolved iron and manganese in the suboxic zone. Maximum sulfate reduction rates (SRR) were found in the first 2 to 10 cm coinciding with a maximum of active SRBs as detected by FISH. Cellular SRR calculated from combined FISH and volumetric SRR data are within the range observed in experiments with pure cultures.

The combined application of molecular (DGGE of PCR-amplified 16S rDNA) and microbiological (PLFA and MPN analyses) tools demonstraed that diverse SRB are evenly found across the studied vertical sediment profile, regardless of the biogeochemical zonation. Thus presence of SRBs in a given horizon cannot always be related to either abundance or cellular activity. However, with respect to the presumably active part of the SRB population, a good correlation was observed

between the different techniques. The SRB community in the upper sediment was dominated by SRBs targeted by probes DSS685 and DSV698 and thus most likely by members of the *Desulfosarcina-Desulfococcus-Desulfofrigus* and *Desulfovibrio* groups, as revealed by FISH and in the case of the *Desulfovibrio* group, by DGGE of reversly transcribed 16S rRNA. It is known that these SRB possess the capacity to utilize acetate and lactate. This is in agreement with low concentrations of lactate and acetate in the upper layers of the sediment. Based on the present results we suggest that the *Desulfosarcina-Desulfococcus-Desulfofrigus* group and *Desulfovibrio* spp. - at the sampling time point in June 1999 - are the key populations for the terminal mineralization step in the anaerobic zone of the temperate intertidal mud flat.

Acknowledgments

We wish to thank B. Schnetger and H. Lüschen (ICBM Oldenburg) for technical support, and S. Fleischer, K. Neumann, J. Botzenhardt, C. Probian and D. Lange (MPI Bremen) for help in the laboratory. The authors are indebted to T. Kjaer for providing the biosensors for the nitrate measurements, and to G. Eickert, A. Eggers, and V. Hübner for constructing O_2 and H_2S microsensors. H.-J. Brumsack (ICBM Oldenburg) kindly allowed access to analytical facilities. We wish to thank the Nationalparkverwaltung Niedersächsisches Wattenmeer for the allowance to work in the tidal flats. The study was supported by Max Planck Society, Munich.

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Microbial Community Structure and Dynamics of Wadden Sea Sediments (Dangast)

Dissertation
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
- Dr. rer. nat. -

dem Fachbereich Biologie/Chemie der
Universität Bremen
vorgelegt von

Enric Llobet Brossa

PART 2

Bremen
September 2000

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Seaonal microbial community dynamics and activities of the temperate tidal sediment of Dangast

Microbial Community Dynamics and Activities of the Temperate Tidal Mudflat Sediment of Dangast

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Changes in microbial communities and microbial activities in a temperate intertidal mudflat sediments were followed throughout two years. Abundances and vertical distribution of bacterial populations present in those marine sediments were monitored by fluorescence in situ hybridization (FISH). Biogeochemical and microsensor measurements were used to follow the seasonal changes in chemical composition of sediments and activities.

A vertical zonation was found in both biological and geochemical parameters. Between 82.5% and 15.6% of the total microbial community hybridized with the bacterial probe EUB338. Three different populations of bacteria, members of the *Cytophaga-Flavobacterium* cluster, sulfate reducing bacteria from the δ-subclass of Proteobacteria, and *Arcobacter* spp., were monitored. Stratification changes of the different microbial populations as well as changes in geochemical parameters were related to seasonal changes in the microbial activity. Temperature was found to be the major factor determining the dynamics of the system.

Microcosms consisting of the undisturbed upper 8 centimeters of these intertidal marine sediments were used to follow the changes of the microbial community structure and its metabolic functioning as a reaction to temperature perturbations. The sediment was incubated at different temperature regimes, each consisting of a transient and a subsequent stable period. The biogeochemistry of the sediments was followed by microsensors, sulfate reduction rate measurements, and the microbial community was analyzed by fluorescence in situ hybridization (FISH) at various times during the experiment. Results were in agreement with the field data. Bacterial distribution and the zonation of primary and secondary reactions coupled to the microbial oxidation of organic matter were related to the temperature and to the microbial activity. Total bacterial counts (DAPI) as well as the percentage of detectable cells by FISH increased with temperature. The increase of the temperature resulted in an

increase of the *Cytophaga-Flavobacterium* fermentative population, causing an increase of the hydrolytic and fermentative processes. Sulfate reduction was enhanced with temperature due to the dependence of the sulfate reducing bacteria (SRB) on the metabolites of the fermenting bacteria. However, different SRB populations responded differently to increased temperature and, thus, to enhanced availability of fermentation products.

Continental margin sediments include shelf sediments and intertidal mud flats of the coastal ranges. These sediments are generally characterized by an exceptionally high input of organic matter. Up to 30% of the oceanic primary production takes place in the shelf areas. From this approximately 25-50% sinks to the sediment (42). In addition, the deltaic areas of the shelf regions are receiving up to 90% of the global flux of particles and organic matter transported by rivers to the oceans (14). Since most of the deposited matter is remineralized, continental margin sediments play a prominent role in the marine carbon cycle, even though they encompass only 10% of the total ocean area. The oxidation of organic carbon is performed by microorganisms that employ an array of electron acceptors, each allowing a different yield of free energy. In accordance with the decreasing yields, a zonation of the oxidants is usually observed in the vertical sediment profiles (13). Therefore, there is a distinct vertical zonation of the dominant energy metabolisms in such systems, with a) aerobic respiration in the uppermost few millimeters, b) nitrate respiration (denitrification) in the following few millimeters-centimeters, c) manganese (Mn(IV)) and d) iron (Fe(III)) reduction in the main part of the oxidized anoxic zone, which may be a few to ten centimeters deep, e) sulfate reduction, which mostly extends as the dominant process down to one or several meters depth, and f) methanogenesis, which is the terminal metabolism for organic matter after depletion of the mentioned oxidants (10, 21).

Although this early diagenetic processes are mainly biotic and catalyzed by microorganisms, little is know about the microbial community dwelling in such systems. The introduction of 16S rRNA based molecular tools such as fluorescent in situ hybridization (FISH) allowed to identify and quantify microorganisms in their natural habitat (1). Recently this type of investigation was applied to marine sediments (25, 29, 30). However, only few studies combined this molecular techniques with biogeochemical measurements to relate bacterial distribution to the

zonation of primary and secondary reactions coupled to the microbial oxidation of organic matter in marine sediments (3, 30).

The present study focuses on the diversity, abundance and dynamics of the microbial community and their relation to the biogeochemical processes in a tidal sediment of the southern North Sea, (Wadden Sea, Site Dangast). For a two years period, three different microbial populations were specifically followed (members of the *Cytophaga/Flavobacterium* cluster, sulfate-reducing bacteria and *Arcobacter* spp.) and related to the biogeochemical cycles in the sediment. Microcosms of the undisturbed upper 8 centimeters of these intertidal marine sediments were used to follow changes of the microbial community structure and its metabolic functioning under controlled conditions.

Materials and methods

Sampling site. The site is located within the Jadebusen Bay, which is part of the German Wadden Sea (Fig.1). The sediment experienced tides which exposed it to the air for about 5 h and left it inundated for about 7 h, with some variability due to the wind velocity and direction (25). The environment provides a situation similar to an estuarine system. Undisturbed sediment samples were obtained on seasonal basis between February 1998 and January 2000 (Feb98, Jul98, Oct98, Jan99, Jun99, and Jan00). Parallel sediment cores were closed with air-tight rubber stopers on both ends and transported cool and dark to the laboratory. They were further processed within a few hours.

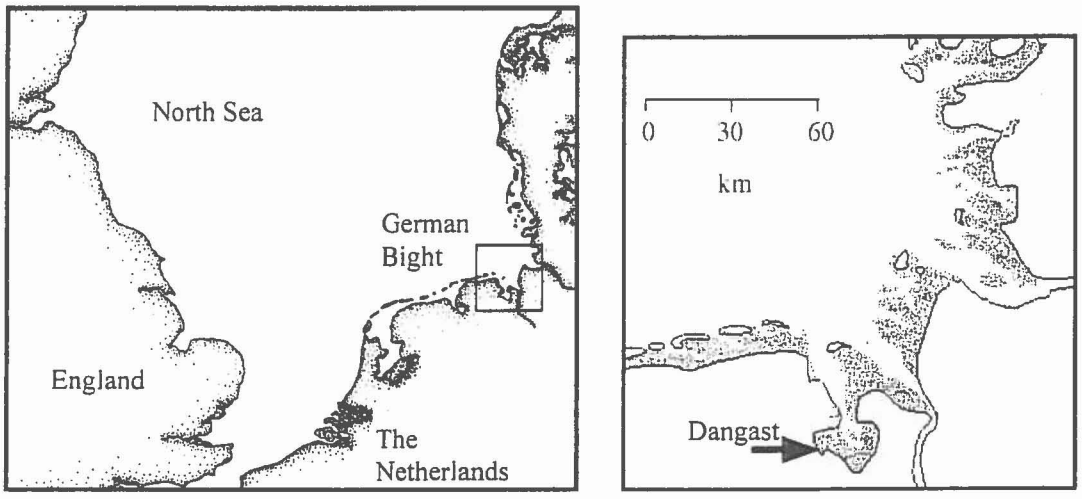


Fig. 1. Map of the sampling site, Site Dangast.

Mesocosm setup. On January 2000, the undisturbed upper 8 cm of the sediment were recovered and incubated in 6 flow chambers (12,5 x 12,5 x 8 cm) with sieve plates at both ends allowing laminar water flow above the sediment surface (Fig.2). Pairs of the flow chambers were kept in aquaria with 15 L of seawater each, which served simultaneously as reservoirs and for temperature control of the sediment cores. Temperature of each reservoir was separately adjusted with a thermostat (Julabo F32-HC, Julabo Seelbach, Germany). The water was aerated, and the salinity was adjusted to 28 ppt every 12 hours. Three liters of seawater were replaced every day. To reduce evaporation and heat loss, and to avoid photosynthesis in the reservoir, the water was covered with hollow plastic spheres (Allplas, Capricorn Chemicals Corp., Secaucus, NJ, USA). A water pump (MI Mouse, Sicce, Padova, Italy) connected to the flow chamber generated a constant laminar water current above the sediment surface with a velocity of 0.85 cm s^{-1}. The sediment surface was exposed to light at 35 µE m^{-2} s^{-1} with a day-night cycle of 10 and 14 hours, respectively.

For an initial period of three days, temperature was maintained constant at 4°C to stabilize the system. In the subsequent experiment, the temperature was raised in steps of 2°C and 4°C per day over a period of 5 days in setup B and C, respectively. Final temperatures of 14°C and 24°C for the setup B and C reached after one week were maintained constant for days 7 to 15 of the experiment. Setup A was kept at 4°C during the whole experiment (Fig.2).

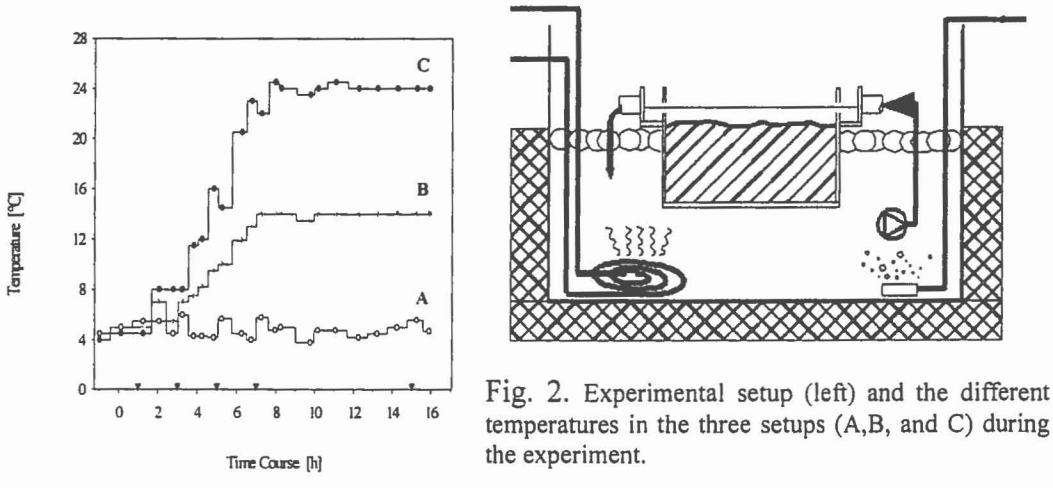


Fig. 2. Experimental setup (left) and the different temperatures in the three setups (A,B, and C) during the experiment.

Replicate samples from each setup were taken at day 1, 3, 5 and 7, and at the end of the experiment at day 15 for analysis of various microbial populations. For sediment and pore water analysis and sulfate reduction rate determination, samples were taken at day 1 and 15.

Sediment and pore water analysis. Biogeochemical analyses of the sediment with respect to pore water contents, dithionite-extractable iron and manganese, total reduced inorganic sulfur (TRIS), total organic carbon (TOC), and total sulfur were performed in Jul98, Oct98, Jan99, and Jan00 as described before (3). Pore waters were analyzed for salinity, temperature, dissolved iron, manganese, sulfate, H_2S, bacterial sulfate reduction rates (SRR), CO_2, and NH_4^+.

From the mesocosm, two parallel sediment cores (PVC tubes; 2.6 cm wide) for each setup were recovered at the end of the experiments after 19 days of incubation for the measurement of bacterial sulfate reduction rates (SRR) (12, 18). Two additional cores (3.6 cm wide) were sampled for the determination of sediment and pore water properties. Water contents and total organic carbon (TOC) were determined as previously described (3). Dissolved sulfate concentrations were measured gravimetrically as $BaSO_4$ from Zn-acetate fixed samples.

Microsensors measurements. 0_2 and H_2S microsensors were constructed, calibrated, and applied as previously described (7, 24, 32). Profiles were measured in sediments cores immediately after sampling.

During the course of the mesocosm experiment, O_2 and H_2S were also measured. Oxygen was measured at day 1, 3, 5, 7, and 15, and 7, and H_2S at day 1, 3, 7, and at the end of the incubation (day 15).

Oxygen uptake rate. The oxygen uptake rate was calculated based on volumetric rates according to Ficks second law including a consumption term

$$\frac{\partial c(z,t)}{\partial t} = D_S \cdot \frac{\partial^2 c(z,t)}{\partial z^2} - C \qquad (1)$$

where

$$D_S = \phi^2 D_0 \qquad (2)$$

represents the sediment diffusion coefficient (40), D_0 the molecular diffusion coefficient of oxygen, ϕ the porosity, which for the sediment surface (0 to 0.5 cm) was determined as $\phi = 0.85$, and C the volumetric oxygen consumption rate. Under steady-state conditions, the initial term of equation (1) equals zero. Local volumetric oxygen

consumption rates were calculated for every measurement step starting with the sediment surface, by calculating the gradient between the respective upward and downward fluxes for every point. The areal net oxygen uptake rate J_v was subsequently calculated by integration of the local rates over the oxygen penetration depth.

Temperature characteristics. The effect of temperature increase on the rate of oxygen-dependent and sulfate reduction processes is described by calculating the apparent temperature characteristics μ, which is based on the Arrhenius equation (17),

$$k = A \cdot \mu^{-RT} \tag{3}$$

where k is the process rate, A is the Arrhenius constant, μ the temperature characteristics in [J mol^{-1}], R the gas constant (R = 8.3144 J mol^{-1} K^{-1}), and T the absolute temperature [K]. Using the integrated form of equation (3), the temperature characteristics is represented by the slope of an Arrhenius plot, i.e. ln k versus $(RT)^{-1}$. Q_{10}, which is the factor describing the increase of the process rate with a temperature increase of 10K was calculated by

$$Q_{10} = 10^{E_a \cdot 10K \cdot [R \cdot T \cdot (T+10K)]^{-1}} \tag{6}$$

for the temperature range applied in the experiment (4 to 24°C).

As temperature response in the case of this study may be in the form of increasing metabolic activity of the cells as well as an increase in (active) cell numbers, we calculated the temperature characteristics for both the areal net oxygen uptake rates as well as for the cell-specific uptake rates. Cell-specific temperature characteristics were calculated by dividing the oxygen uptake and sulfate reduction rates of the respective layer in the three setups at day 15 by the corresponding cell numbers as counted with DAPI, and with probes EUB338 and the SRB probes, respectively.

In situ hybridization and cell counts. Field and mesocosm samples were processed for fluorescence in situ hybridization (FISH). Two replicate cores of each sampling date were cut in 0.5 cm slices in the upper 5 cm, and in 1.0 cm slices the following 5 cm. Samples were then immediately processed to avoid changes in the community composition caused by handling and containment (33) as described before (25).

Hybridization, microscopic counts of hybridized cells and total cell counts were performed as previously described (1, 25, 39). The probes used in this study are listed in table 1. For each probe and sample between 700 and 1000 DAPI stained cells and its respective hybridized cells as well as duplicates of each sample were counted.

Statistical analysis of the data. Cell counts from field and mesocosm samples were checked for normal distribution. To relate the dependent variables, two-way analysis of variance was conducted, i.e. bacterial counts, SRR, temperature, etc. for a significance level of 0.05. Where significance was found, the Tukey-Kramer HSD and the Hsu-MCB tests with $\alpha \square 0.05$ were applied. All tests were done with the software package JMP 3.2 (JMP® Statistics and Graphics Guide, version 3. SAS Institute Inc., Cary, NC, USA).

TABLE 1. Probes used in this study

Probe	Target	Sequence (5'-3') of probe	[%] FA[a] in situ	Reference
EUB338	Bacteria 16S rRNA, position[b] 338-355	GCT GCC TCC CGT AGG AGT	0-35	27
Arch915	*Archaea* 16S rRNA, position 915-935	AG GAA TTG GCG GGG GAG CAC	35	27
CF319a	*Cytophaga-Flavobacterium* cluster of the CFB phylum 16S rRNA, position 319-336	TGG TCC GTG TCT CAG TAC	35	27
ARC94	*Arcobacter sp.* 16S rRNA, position 94-111	TGC GCC ACT TAG CTG ACA	20	27
DNMA657	*Desulfonema sp.* 16S rRNA, position 657-676	TTC CGY TTC CCT CTC CCA TA	35	27
DSV698	*Desulfovibrio* (16 species) 16S rRNA, position 698-717	GTT CCT CCA GAT ATC TAC GG	35	29
DSV1292	*Desulfovibrio* (13 species) 16S rRNA, position 1292-1310	CAA TCC GGA CTG GGA CGC	35	29
DSV407	*Desulfovibrio* (3 species) 16S rRNA, position 407-424	CCG AAG GCC TTC TTC CCT	50	29
DSD131	*Desulfovibrio* (1 species) 16S rRNA, position 131-148	CCC GAT CGT CTG GGC AGG	20	29
DSV214	*Desulfovibrio/D'microbium* 16S rRNA, position 214-230	CAT CCT CGG ACG AA TGC	10	29
DSS658	*Desulfosarcina/D'coccus/D'frigus* 16S rRNA, position 658-675	TCC ACT TCC CTC TCC CAT	60	29
DSB985	*Desulfobacter/D'bacula* 16S rRNA, position 985-1003	CAC AGG ATG TCA AAC CCA G	20	29
221	*Desulfobacterium* 16S rRNA, position 221-238	TGC GCG GAC TCA TTC AAA	35	29
660	*Desulfobulbus* 16S rRNA, position 660-679	GAA TTC CAC TTT CCC CTC TG	60	29
DSBO224	*Desulfobotulus* 16S rRNA, position 224-242	GGG ACG CGG ACT CAT CCT C	60	29
DSMA488	*Desulfovibrio/D'monile* 16S rRNA, position 488-507	GCC GGT GCT TCC TTT GGC GG	60	29
DSR651	*Desulforhopalus* 16S rRNA, position 651-668	CCC CCT CCA GTA CTC AAG	35	29
DTM229	*Desulfotomaculum* 16S rRNA, position 229-246	AAT GGG ACG CGG AXC CAT	15	29
SVAL428	*Desulfotalea/D'fustis* 16S rRNA, position 428-446	CCA TCT GAC AGG ATT TTA C	25	36
NON338	none (negative control)	ACT CCT ACG GGA GGC AGC	0-35	27

[a] Percentage of formamide (FA) in hybridization buffer.
[b] *Escherichia coli* numbering.

Differences in mean penetration depth and rates of the mesocosm sampleswere analyzed between setups and between days of measurement for each setup by multiple pairwise comparison using the Tukey-Kramer-test statistics and the nonparametric Kruskal-Wallis test. The significance level chosen was 0.05.

Results

Biogeochemistry of the sediment samples. The sediment displayed two distinct color changes; the oxic upper part was brown which turned into a darker olive-green color within the first 2-3 cm depending on the season; at about 9-11 cm depth, a diffuse blackening indicated the beginning of the iron monosulfide zone. Similar vertical stratification was also found in biological and geochemical parameters (see below).

The upper 10 cm of sediment consisted to 100% of mud with a grain size < 63 mm. Only the Jan99 sample had a higher concentration of larger quartz grains in the upper 3 cm. In situ sediment temperatures varied between 4 and 24°C and were constant over the vertical profile in the different seasons.

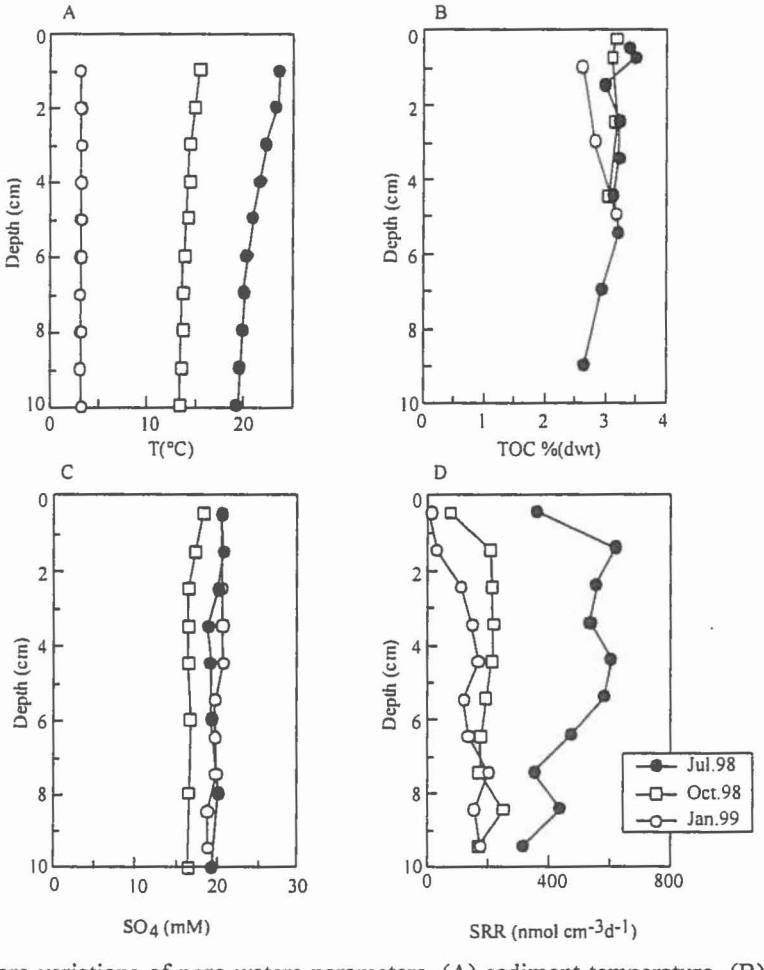


Fig. 3. Downcore variations of pore waters parameters, (A) sediment temperature, (B) total organic carbon, (C) sulfate concentration, and (D) microbial sulfate reduction rates.

TOC contents of the sediments (Fig.3) were fairly constant for all the depths in the different samples (3.2% dwt ± 0.15) except for the upper 3 cm of the winter sample for which the TOC was lower (2.7% dwt ± 0.14). Stable carbon isotope ratio (δ^{13} C) was measured for the Jul98 sample. Different sources of marine organic matter in the German Wadden Sea show values for δ^{13} C between -10 and -20 ‰ but are generally enriched in ^{13}C compared to terrestrial material. Application of a mixing model between marine (δ^{13} C = -19 ‰) and terrestrial (δ^{13} C = -27 ‰) TOC sources to the values observed in July (-22.5 ‰ at the sediment surface and -23.3 ‰ at 21 cm depth) revealed a contribution of 63 % (surface) and 57 % (bottom) of marine organic carbon to the sediments.

Pore waters. The pore water composition indicates a sub-oxic zone between 5 mm to 13-15 cm depth. Dissolved Mn(II) and Fe(II) was present throughout the period of this study with significantly higher concentrations during summer than winter (data not shown). Accumulation of free H_2S occurred within this sub-oxic zone only in the July sample. In the rest of the analyzed samples, free H_2S was detected only in deeper layers.

Sulfate profiles were highly similar for the different sampling dates with maximum values of up to 20 mM near the sediment surface , followed by a zone of constant [SO_4^{2-}] between 15-20 mM and decreasing only below 15-17 cm depth. Lower concentrations were found in Oct98 (Fig.3).

Sulfate reduction rates (SRR) were measured in Jul98, Oct98, Jun99 and Jan00 (Fig.3). They varied between 11 nmol cm^{-3} d^{-1} and 643 nmol cm^{-3} d^{-1} with the highest values during summer and the lowest during winter. The highest SRR were always found within the suboxic zone. The depth integrated SRR of the upper 15 cm were in a good agreement with the range observed in similar sediments of the North Sea and with the seasonal changes of SRR in temperate muddy sediments (26).

DIC values and [NH_4^+] obtained in summer and autumn samples showed a positive linear regression and indicated less organic matter mineralization in autumn than in summer for the upper 10 cm of the sediment (data not shown).

Microsensor measurements. The oxygen penetration depth into the sediment varied seasonally. Lateral heterogeneity was negligible, except for the Jul98 sample when significant bioturbation and irrigation was observed. Field measurements showed the deepest oxygen penetrations to 2.5-3.3 mm depth in Jul98. In Oct98 and

Jan99 oxygen penetration reached depths of 1 and 1.6 mm respectively (data not shown). No H_2S could be detected in any sample under any conditions within the first 20 mm, the maximum depth reached by our microsensor measurements.

Biogeochemistry of the microcosm sediments. The upper 8 cm of the sediment in January 2000 used in the experiments had a more or less constant TOC content of 2.66 % ± 0.13 dwt. (n = 4).

The oxygen penetration depth into the sediment varied with temperature. Profiles were measured at different positions at least 10 mm away from each other, avoiding points of high photosynthetic or bioturbational activity. In the setup A no significant change in O_2 penetration depth was found for the first week. However, a slight increase in the penetration depth was found at the end of the experiment. In setups B and C, O_2 penetration depth was decreasing from 3.725 ± 0.20 mm to 2.125 ± 0.12 mm, and from 3.550 ± 0.20 mm to 3.050 ± 0.27 mm, respectively (Fig.4). The apparent temperature characteristic (μ) for the oxygen-dependent processes of the sediment at the end of the experiment was 42.5 KJ mol^{-1}. This temperature characteristic corresponds to a Q_{10} value of 1.9. (Table 2).

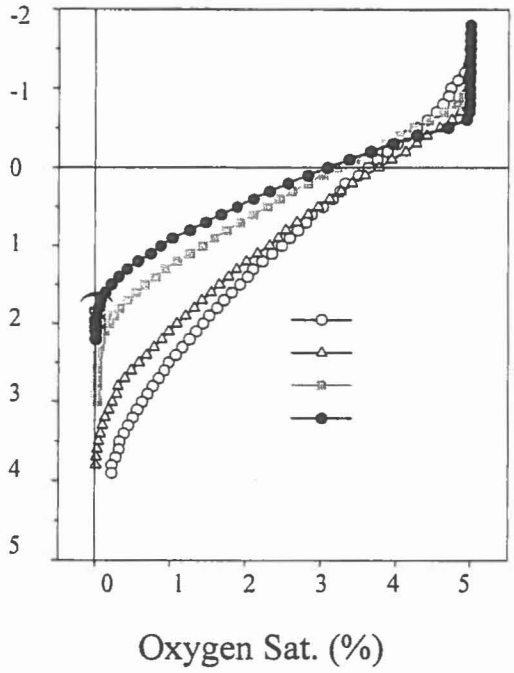


Fig.4. O_2 penetration depth in the different setups at different temperatures.

No free H_2S could be detected in any sample under any conditions within the first 4 cm of the sediment with microsensor measurements. No significant change of the SRR between the beginning and the end of the experiment ($p>0.01$) was observed at 4°C. Higher SRR however, were found in the sediments after two weeks at 14°C and

24°C (Fig.5). The apparent temperature characteristic (μ) for the depth-integrated sulfate reduction rates of the upper 6.5 cm of the sediment at the end of the experiment was of 68.3 kJ mol^{-1}. This corresponds to a Q_{10} value of 2.7. (Table 2).

Sediment microbial community.

i)**Total cell counts.** Over 2,6 millions of cells were counted. Total cell counts decreased exponentially with depth (Table 3, Fig.6). Two distinct zones were found throughout the year. Maximum values were always found in the upper 3 cm.

The total cell counts integration of the upper 9 cm of the sediment showed seasonal changes that were recurrent through the two year period analyzed. There were minimum values during winter (4.6 - 0.9 x10^9 cells/cm^3) followed by an increase of cell numbers in summer (5.8 - 1.1 x10^9 cells/cm^3).

TABLE 2. Temperature characteristic of oxygen-dependent processes and sulfate-reduction processes.

Process	Activation [1]	μ (J_v)	Q_{10} [2]
Oxygen Consumption Rates	**Net activation**	42.5[3]	1.86
	Cell-specific temperature characteristic		
	DAPI	37.6	1.73
	Eub	27.7	1.50
Sulfate Reduction Rates	**Net activation**	68.3[4]	2.71
	Cell-specific temperature characteristic		
	SRB	36.6	1.71

[1] values given in [kJ mol^{-1}].
[2] average value for a 10K increase in the range of 277K to 297K.
[3] value refers to the depth-integrated volumetric rates with depth = 2 to 4 mm.
[4] value refers to the depth-integrated volumetric rates from 1 to 6 cm depth.

ii) Domain specific hybridisation. The microbial community dwelling in the Wadden Sea sediments of the German North Sea detected by FISH was dominated by

Bacteria. Between 82% and 61% of the total microorganisms hybridized with the probe EUB338 (Table 3) in the upper most layers of the sediment.

Recently, the bacterial probe EUB338 was shown not to detect all Bacteria (5). Some bacterial phyla, most notably the Plantomycetales and Verrucomicrobia, are missed by this probe. Therefore, six different layers of the Jul98 sample were hybridized with the two supplementary probes EUB338-II and EUB338-III. An increase between 2% and 10% of the detection rates was found. Consequently, total bacterial numbers monitored in this study were most probably underestimated.

Archaeal and eucaryal counts usually remained below the detection limit of 0.1% of the DAPI stained cells, except for the summer samples in which eucarya were detectable in the most upper layers of the sediment.

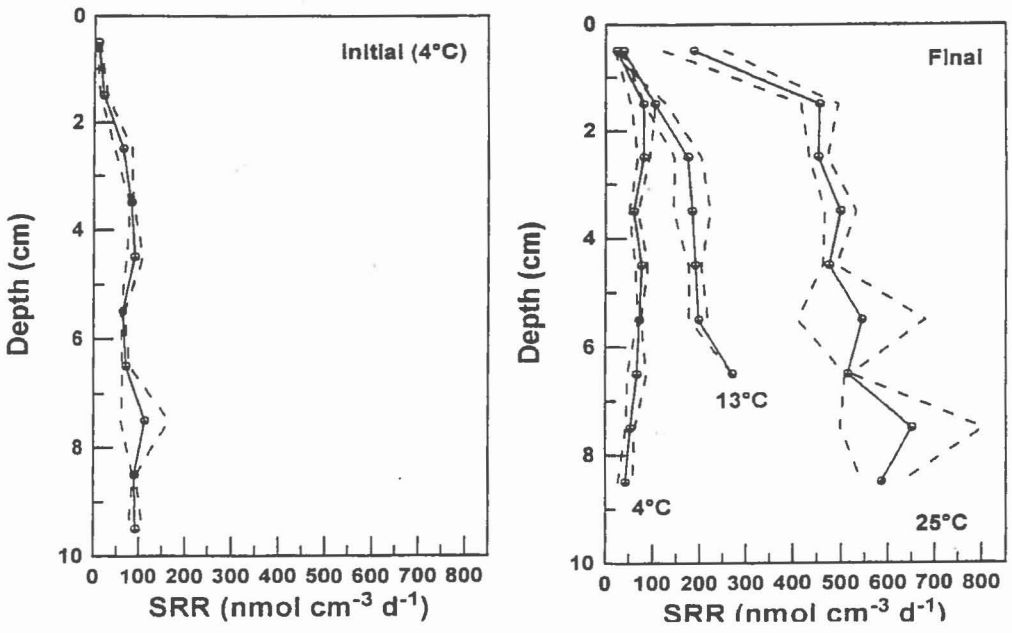


Fig.5. Sulfate reduction rates of (A) field sample during January 2000 and (B) at the end of the incubation at different temperatures.

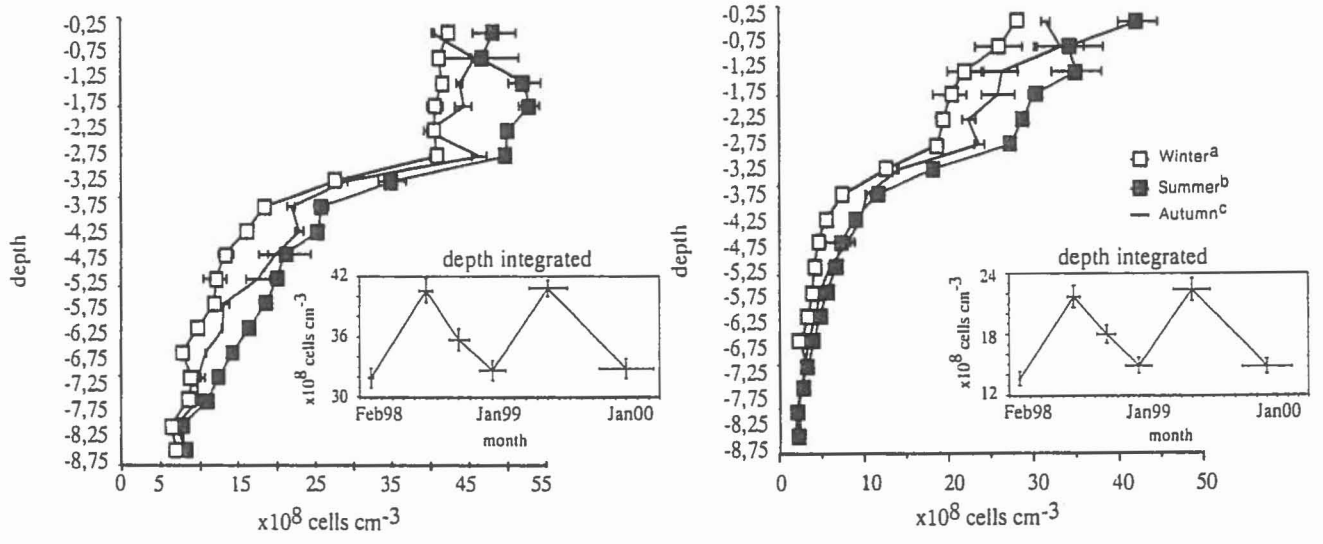
Detection rates with the probe EUB338 decreased exponentially over the vertical profile in all samples. Thus, not only the absolute cell numbers decreased with depth in each sample, but also the percentage of detectable cells with our FISH protocol.

Seasonal changes of EUB338 detectable cells were found to be comparable to those of total cell numbers (Fig.6). Depth integrated numbers for the 9 cm studied were minimum during winter with 9.9 $x10^8$ cells, 10.8 $x10^8$ cells and 10.3 $x10^8$ cells in Feb98, Jan99 and Jan00, respectively, and maximum in summer with 15.8 $x10^8$ cells

and 16.2 x10^8 cells in Jul98 and Jun99, respectively). The depth integrated number of cells detected in Oct98 with the probe EUB338 was 13.0 x10^8.

iii)Abundance of members of the *Cytophaga-Flavobacterium* cluster. CF319a detectable cells turned out to be the largest group of bacteria detected by FISH. Abundances ranged from 2.8% to 19.5% of total DAPI counts (Table 3). This means that between 16.5% and 29.1% of the bacteria detected by FISH could be affiliated to this group.

The depth integrated number of cells detected with the probe CF319a increased from winter to summer. During winter 2.1 x10^8 cells, 2.1 x10^8 cells and 2.0 x10^8 cells were detected in the upper 9 cm of the sediment in Feb98, Jan99 and Jan00, respectively. During summer the depth integrated detection was 3.7 x10^8 cells, and 3.8 x10^8 cells in Jul98 and Jun99, respectively. In Oct 98 3.2 x10^8 cells were detected using this probe. Detectability with the probe CF319a decreased exponentially over the vertical profile in all the samples.



[a] mean of winter samples (Feb98, Jan99, Jan00)
[b] mean of summer samples (Jun98, Jul99)
[c] mean of autumn samples (Oct98)

Figure 6. Vertical distribution and depth integrated cell numbers. Total DAPI counts (left) and cells detected with the probe EUB338.

iv)The genus *Arcobacter*. Detectability with the probe ARC94 was generally very low (Table 3). Almost no cells of *Arcobacter* spp. were detected deeper than 5 cm. The depth integrated values for the upper 5 cm of the sediment were lower during winter with, 3.7 $x10^7$ cells, 3.9 $x10^7$ cells and 3.8 $x10^7$ cells in Feb98, Jan99, and Jan00, respectively. In Jul98 and Jun99, 5.8 $x10^7$ cells and 7.4 $x10^7$ cells were found, respectively, in the upper 5 cm of the sediment.

Detectability with this probe decreased exponentially over the vertical profile during winter but not in summer. During summer, double amount of cells in the upper 2 cm than in the following 3 cm of sediment was detected.

v)Identification of sulfate reducing bacteria. To identify sulfate reducing bacteria (SRB) a multiple probe approach was applied. The general probe SRB385 that targets many SRB of the δ-subclass of Proteobacteria, and a set of 15 more specific probes for δ-proteobacterial SRB (Table 1).

Seasonal dynamics of SRB385 probe counts were found to be similar to the one observed with the total bacterial counts. The depth integrated number of cells detected in the upper 9 cm of the sediment in winter was, 0.9 $x10^8$ cells , 0.6 $x10^8$ cells, and 1.0 $x10^8$ cells in Feb98, Jan99, and Jan00, respectively. During summer, the integrated number of cells was higher with 1.7 $x10^8$ cells in both, Jul98 and Jun99. SRB385 positive cells were detected throughout the whole vertical profile (Table 3).

Six of the 15 probes gave detection rates above the limit of detection of 0.1%. Those were: DNMA657 for members of the genus *Desulfonema*, DSV698 for members of the genus *Desulfovibrio*, DSS658 specific for the *Desulfosarcina-Desulfococcus* group, DSR651 specific for *Desulforhopalus* spp., DSB985 specific for *Desulfobacter* spp., and probe 221 detecting *Desulfobacterium* spp. With this set of probes were detected approximately twice as many cells than with the probe SRB385.

The depth integrated number of cells detected in the upper 9 cm of the sediment in winter was, 1.4 $x10^8$ cells , 1.2 $x10^8$ cells, and 1.6 $x10^8$ cells in Feb98, Jan99, and Jan00, respectively. During summer, the integrated number of cells was higher with 3.5 $x10^8$ cells and 3.8 $x10^8$ cells in Jul98 and Jun99, respectively. A peak of detected cells at around 2 to 3 cm depth was found in winter while in summer this peak was shallower at 1 to 2 cm depth.

Table 3. DAPI and FISH counts of field samples

	Absolute DAPI counts (cells/cm^3 [10^8]) (mean ± SD)						
depth (cm)	Nov. 97	Feb. 98	Jul. 98	Oct. 98	Jan. 99	Jun. 99	Jan. 00
0,25	44,0	46,0±0,1	50,0±1,4	44,0±1,4	45,7±1,0	53,9±2,2	46,1±0,5
0,75	45,0	44,5±0,7	46,5±2,1	49,5±2,1	44,9±1,2	53,7±0,9	45,3±0,4
1,25	46,0	45,5±0,7	54,5±2,1	47,5±2,1	44,9±1,5	57,4±1,8	45,3±0,5
1,75	45,0	43,0±0,0	57,5±2,1	48,0±1,4	44,6±1,4	55,5±1,1	45,2±0,4
2,25	43,0	43,5±0,7	54,0±1,4	43,5±0,7	44,5±0,7	53,2±1,5	44,7±0,4
2,75	45,0	43,5±0,7	53,0±1,4	50,0±1,4	44,8±1,4	53,7±1,6	45,1±0,5
3,25	33,0	31,5±0,7	40,0±1,4	32,5±0,7	31,2±0,7	37,5±1,7	30,8±0,8
3,75	24,0	21,5±0,7	30,0±0,1	25,5±2,1	22,3±2,1	28,7±1,0	22,4±1,1
4,25	27,0	20,0±1,4	29,5±2,1	26,5±0,7	20,0±0,7	28,7±1,1	19,0±1,6
4,75	18,0	15,9±0,2	27,0±2,8	23,5±2,1	17,5±2,1	22,3±1,8	17,6±1,1
5,25	N[b]	13,9±0,2	24,0±1,4	21,2±3,0	16,1±3,0	23,3±1,3	16,8±1,3
5,75	N	14,5±0,7	22,0±1,4	16,8±1,1	15,5±1,1	22,4±1,6	15,8±1,3
6,25	N	13,3±0,4	20,5±0,7	16,5±0,7	13,2±0,7	19,5±0,7	N
6,75	N	11,5±0,7	18,5±0,7	14,3±0,4	11,3±0,4	17,3±1,2	N
7,25	N	12,0±1,4	15,5±0,7	13,3±0,4	13,3±0,4	16,3±0,7	N
7,75	N	12,0±1,5	14,5±0,7	13,2±0,2	12,3±0,2	14,5±0,3	N
8,25	N	9,5±0,3	11,8±1,1	10,5±0,7	10,0±0,7	11,0±0,6	N
8,75	N	10,5±0,7	12,0±0,1	11,9±0,2	10,9±0,2	11,6±1,1	N
	% of cells hybridizes[a] with probe EUB338 (mean ± SD)						
depth (cm)	Nov. 97	Feb. 98	Jul. 98	Oct. 98	Jan. 99	Jun. 99	Jan. 00
0,25	73,0	60,5±4	82,0±4	72,3±6	62,5±4	82,3±14	62,3±4
0,75	65,0	51,8±9	68,0±12	67,6±16	62,9±1	69,7±13	60,8±4
1,25	54,0	43,5±5	61,2±16	55,7±10	50,7±3	65,5±7	52,1±8
1,75	43,5	42,6±2	53,8±4	54,3±4	49,8±2	54,0±13	45,9±6
2,25	54,5	42,8±5	54,4±8	52,0±6	45,1±5	54,1±10	44,3±5
2,75	41,8	42,0±5	51,2±4	47,5±1	43,0±1	52,1±14	42,7±3
3,25	41,3	40,5±3	45,6±18	43,4±16	40,6±6	48,4±11	40,6±7
3,75	40,0	34,6±2	44,5±5	40,3±4	33,5±4	42,7±9	33,8±8
4,25	37,0	27,4±1	39,8±1	36,2±4	28,2±8	40,0±12	27,6±9
4,75	28,6	26,7±1	37,4±9	34,3±3	26,9±5	34,7±9	27,3±6
5,25	N	27,0±3	30,9±3	27,9±6	25,8±4	30,0±11	24,8±8
5,75	N	25,7±3	30,6±2	28,1±3	24,6±3	29,7±8	24,4±7
6,25	N	24,6±2	29,3±2	25,7±4	25,1±9	26,3±8	N
6,75	N	20,8±2	26,2±2	23,0±4	21,2±2	27,0±7	N
7,25	N	23,0±2	24,9±1	22,6±5	23,5±4	25,2±5	N
7,75	N	20,0±2	23,9±1	18,9±1	24,1±5	22,2±4	N
8,25	N	20,2±1	20,0±1	18,4±1	20,6±4	19,8±8	N
8,75	N	16,5±1	20,4±1	19,5±2	16,8±3	20,1±6	N

[a] Percent detection compared to DAPI. Numbers have been corrected by subtracting NON338 counts. Mean and standard deviation were calculated from the counts of two parallel cores.
[b] not done.

% of cells hybridizes with probe CF319a (mean ± SD)

depth (cm)	Nov. 97	Feb. 98	Jul. 98	Oct. 98	Jan. 99	Jun. 99	Jan. 00
0,25	17,5	13,7±1,0	19,5±0,1	16,7±0,5	12,9±1,0	19,5±1,4	12,5±0,5
0,75	16,0	11,2±1,6	16,8±0,8	17,1±0,2	9,9±0,5	16,4±0,1	9,3±0,6
1,25	13,0	9,8±0,7	15,4±0,3	14,4±1,9	9,0±1,4	15,3±0,5	8,7±0,7
1,75	12,1	8,8±4,2	15,0±0,2	14,2±1,0	9,5±1,5	14,9±0,7	7,0±0,7
2,25	13,6	9,6±1,2	11,9±2,0	13,0±0,2	9,7±1,2	10,9±0,4	9,7±0,9
2,75	12,0	10,7±1,5	13,6±1,3	12,6±1,5	9,7±1,4	12,9±0,4	10,6±0,9
3,25	11,8	9,1±3,1	11,9±0,2	10,8±0,9	9,2±1,9	12,5±0,4	9,3±0,5
3,75	8,1	5,8±2,0	8,1±1,7	8,4±0,1	5,8±1,1	8,1±0,1	5,9±0,4
4,25	6,4	3,5±1,7	7,1±0,4	7,5±1,5	5,0±1,1	7,0±0,1	5,5±0,6
4,75	6,2	4,7±1,4	7,0±2,2	6,0±2,3	4,8±2,0	7,1±0,8	4,8±0,1
5,25	N	4,6±0,3	6,4±0,4	6,5±0,4	4,4±1,4	6,4±0,4	4,2±0,3
5,75	N	4,3±0,3	6,3±0,4	6,4±0,4	4,4±0,5	6,6±1,4	4,3±0,5
6,25	N	4,2±0,3	6,1±0,4	6,0±0,4	3,6±0,5	6,3±0,7	N
6,75	N	3,5±0,2	5,4±0,4	5,4±0,4	3,7±0,6	6,0±0,7	N
7,25	N	3,9±0,3	5,2±0,3	5,0±0,3	3,6±0,4	5,4±0,7	N
7,75	N	3,4±0,2	5,0±0,3	4,8±0,3	3,8±0,8	4,6±0,7	N
8,25	N	3,4±0,2	4,2±0,3	4,4±0,3	3,1±0,8	4,6±1,1	N
8,75	N	2,8±0,2	4,2±0,3	4,5±0,3	3,1±0,1	4,3±0,1	N

% of cells hybridizes with probe Arc94 (mean ± SD)

depth (cm)	Nov. 97	Feb. 98	Jul. 98	Oct. 98	Jan. 99	Jun. 99	Jan. 00
0,25	1,10	0,70±0,01	1,05±0,07	1,10±0,14	0,70±0,01	1,10±0,11	0,85±0,10
0,75	1,00	0,80±0,14	1,25±0,07	1,05±0,07	0,90±0,14	1,75±0,07	0,65±0,11
1,25	1,10	0,85±0,07	1,25±0,07	1,15±0,07	0,75±0,07	1,50±0,10	0,80±0,12
1,75	1,30	0,75±0,07	1,00±0,14	0,95±0,07	0,80±0,08	1,10±0,11	0,80±0,07
2,25	0,90	0,50±0,57	0,64±0,51	0,85±0,07	0,65±0,10	0,75±0,09	0,60±0,50
2,75	0,10	0,47±0,61	0,25±0,06	0,18±0,08	0,55±0,60	0,50±0,12	0,50±0,65
3,25	0,01	0,12±0,12	0,12±0,10	0,03±0,00	0,09±0,11	0,10±0,10	ND
3,75	0,02	0,04±0,04	0,06±0,05	0,02±0,01	0,03±0,05	0,07±0,03	0,05±0,04
4,25	0,01	ND[c]	0,02±0,01	0,01±0,01	ND	ND	ND
4,75	0,01	ND	ND	0,02±0,01	ND	ND	ND
5,25	N	ND	ND	ND	ND	ND	ND
5,75	N	ND	ND	ND	ND	ND	ND
6,25	N	ND	ND	ND	ND	ND	N
6,75	N	ND	ND	ND	ND	ND	N
7,25	N	ND	ND	ND	ND	ND	N
7,75	N	ND	ND	ND	ND	ND	N
8,25	N	ND	ND	ND	ND	ND	N
8,75	N	ND	ND	ND	ND	ND	N

[a] Percent detection compared to DAPI. Numbers have been corrected by subtracting NON338 counts. Mean and standard deviation were calculated from the counts of two parallel cores.
[b] not done.
[c] not detected.

% of cells hybridizes[a] with probe SRB385 (mean ± SD)

depth (cm)	Nov. 97	Feb. 98	Jul. 98	Oct. 98	Jan. 99	Jun. 99	Jan. 00
0,25	4,4	3,6±0,1	4,7±0,2	4,5±0,3	3,6±0,6	4,7±0,2	3,6±0,6
0,75	4,3	3,5±0,3	4,5±0,2	4,8±0,2	1,8±0,4	4,5±0,2	1,8±0,4
1,25	5,1	4,3±0,3	6,2±0,3	5,2±0,1	4,1±0,3	6,2±0,3	4,1±0,3
1,75	6,5	5,1±0,1	6,8±0,4	5,1±0,1	4,4±0,5	6,8±0,4	4,4±0,5
2,25	5,3	4,2±0,2	6,0±0,3	5,1±0,1	1,7±0,2	6,0±0,3	1,7±0,2
2,75	4,3	4,4±0,2	6,3±0,1	4,9±0,1	2,7±0,1	6,3±0,1	2,7±0,1
3,25	6,1	4,6±0,1	6,4±0,1	5,3±0,2	2,2±0,2	6,4±0,1	2,2±0,2
3,75	5,6	4,6±0,5	6,1±0,4	5,5±0,3	1,9±0,1	6,1±0,4	1,9±0,1
4,25	3,5	2,5±0,1	5,1±0,1	3,5±0,0	1,8±0,3	5,1±0,1	1,8±0,3
4,75	2,0	1,9±0,1	4,6±0,4	3,2±0,4	1,6±0,2	4,6±0,4	1,6±0,2
5,25	N[b]	1,5±0,1	4,2±0,4	2,5±0,1	1,4±0,5	4,2±0,4	1,4±0,5
5,75	N	1,3±0,1	5,3±0,8	2,6±0,1	0,5±0,3	5,3±0,8	0,5±0,3
6,25	N	1,8±0,1	4,5±0,2	2,2±0,1	0,9±0,4	4,5±0,2	N
6,75	N	2,0±0,1	4,4±0,1	3,0±0,2	0,8±0,3	4,4±0,1	N
7,25	N	1,9±0,1	4,9±0,3	2,4±0,1	0,8±0,4	4,9±0,3	N
7,75	N	0,8±0,3	3,1±0,2	2,6±0,1	0,5±0,3	3,1±0,2	N
8,25	N	1,1±0,3	3,4±0,1	2,5±0,7	0,1±0,5	3,4±0,1	N
8,75	N	0,7±0,1	2,4±0,6	0,9±0,1	0,5±0,2	2,4±0,6	N

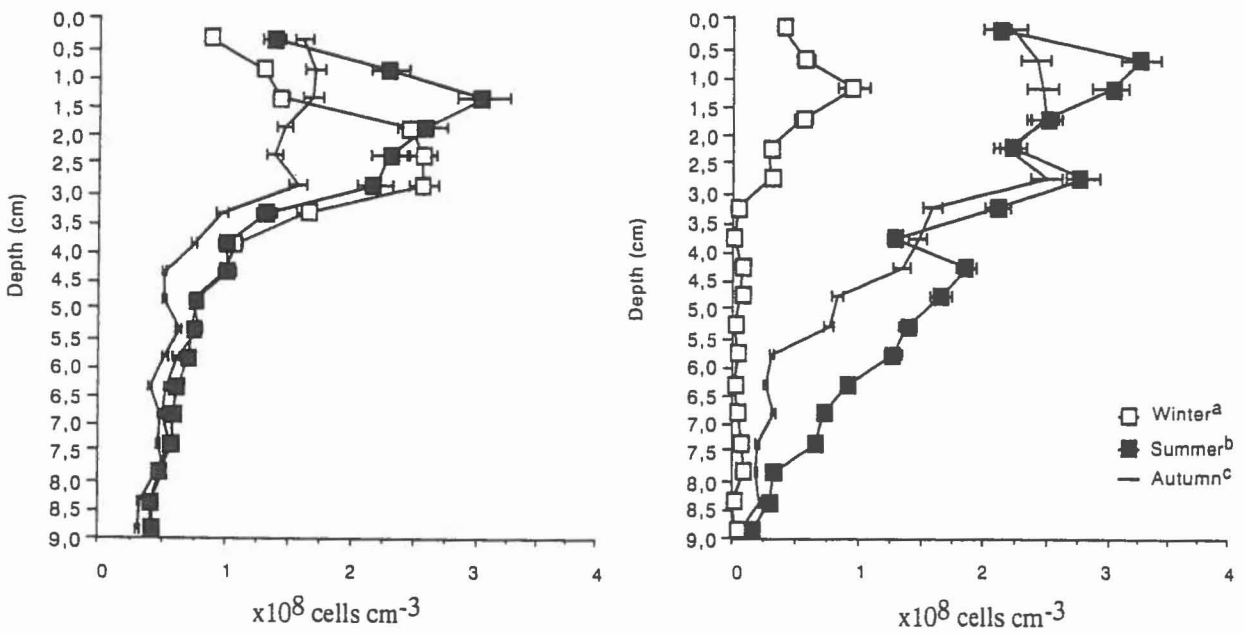
% sum[d] of detected SRB (mean ± SD)

depth (cm)	Nov. 97	Feb. 98	Jul. 98	Oct. 98	Jan. 99	Jun. 99	Jan. 00
0,25	14,0	3,1±2,0	9,4±1,4	11,5±0,2	3,1±1,0	9,4±1,4	3,6±0,6
0,75	13,4	5,6±2,5	14,3±1,5	11,0±1,5	3,9±0,8	14,3±1,5	1,8±0,4
1,25	10,9	6,3±3,0	13,5±1,5	11,6±1,2	7,0±0,4	13,5±1,5	4,1±0,3
1,75	8,7	9,3±2,5	10,6±1,9	11,1±2,1	6,8±0,4	10,6±1,9	4,4±0,5
2,25	10,4	8,2±1,4	9,2±1,8	10,9±4,1	6,1±0,4	9,2±1,8	1,7±0,2
2,75	6,8	7,7±1,8	12,0±1,7	10,9±2,3	6,9±0,5	12,0±1,7	2,7±0,1
3,25	7,6	6,6±1,5	11,7±1,5	10,4±1,0	4,1±1,1	11,7±1,5	2,2±0,2
3,75	6,4	4,4±1,7	8,0±2,6	11,6±2,5	4,0±0,5	8,0±2,6	1,9±0,1
4,25	6,1	6,0±1,7	14,3±2,1	9,5±1,1	4,3±0,4	14,3±2,1	1,8±0,3
4,75	6,0	4,2±1,8	13,2±2,4	7,3±0,9	3,9±0,6	13,2±2,4	1,6±0,2
5,25	N	5,0±2,0	11,8±1,7	8,1±0,7	4,0±0,7	11,8±1,7	1,4±0,5
5,75	N	2,7±2,1	11,7±1,5	5,3±1,3	3,8±0,6	11,7±1,5	0,5±0,3
6,25	N	3,6±1,5	9,2±1,1	4,3±1,0	2,1±0,5	9,2±1,1	N
6,75	N	4,0±1,0	8,5±2,0	6,2±1,0	1,6±0,4	8,5±2,0	N
7,25	N	3,6±1,1	10,3±2,1	5,0±0,9	1,4±0,3	10,3±2,1	N
7,75	N	1,2±0,9	5,9±1,2	5,3±0,7	1,9±0,5	5,9±1,2	N
8,25	N	2,6±1,1	7,0±1,2	6,1±0,8	1,3±0,2	7,0±1,2	N
8,75	N	1,3±1,2	4,0±1,2	1,6±0,9	1,3±0,5	4,0±1,2	N

[a] Percent detection compared to DAPI. Numbers have been corrected by subtracting NON338 counts. Mean and standard deviation were calculated from the counts of two parallel cores.
[b] not done.
[d] Sum of the percentages detected with all the SRB specific probes used in this study.

vi)Relative abundance of different SRB populations. Members of the genus *Desulfovibrio* and the *Desulfosarcina-Desulfococcus* group were identified as the most abundant groups of SRB in the sediments. Up to 9.1 % and 7.1 % of total DAPI counts could be affiliated to *Desulfovibrio* spp. and *Desulfosarcina-Desulfococcus* group, respectively. Counts of *Desulfonema* spp., *Desulforhopalus* spp., *Desulfobacter* spp., and *Desulfobacterium* spp. were always below 2.5%.

Distinct seasonal variations were found with the different probes used. In February, a maximum of *Desulfovibrio* spp. was detected with 0.9 $x10^8$ cells/cm^3, while during summer and autumn maximum values of 3.9 $x10^8$ cells/cm^3 and 3.1 $x10^8$ cells/cm^3 , respectively were found (Fig.7).



[a] mean of winter samples (Feb98, Jan99, Jan00)
[b] mean of summer samples (Jun98, Jul99)
[c] mean of autumn samples (Oct98)

Figure 7. Vertical distribution of *Arcobacter* spp. cells (left) and sulfate-reducing bacteria (right)

Probe DSS658 specific for the *Desulfosarcina-Desulfococcus* group showed no statistically significant differences (p□0.01) on the maximum cell number between winter and summer (between 3.0 $x10^8$ cells/cm^3 and 3.1 $x10^8$ cells/cm^3, respectively). However, the location of their maximum abundance differed in 1 cm over the vertical profile with a deeper location during winter (at 2.5 cm depth). Similar profiles of

Desulfobacter spp. were found, although at much lower abundances of 0.3 $x10^8$ cells/cm^3 and 0.1 $x10^8$ cells/cm^3 during winter and summer, respectively.

Desulforhopalus spp. were found throughout the year with a maximum in summer of 1.3 $x10^8$ cells/cm^3 and minimum in winter of 0.9 $x10^8$ cells/cm^3. No significant change in the depth distribution was found. Similar dynamics and zonation through the year was found for members of the genus *Desulfonema* with low numbers of 0.1 $x10^8$ cells/cm^3 and 0.3 $x10^8$ cells/cm^3 during winter and summer, respectively.

Microcosms microbial community.

i)Total cell counts. Total cell counts of 0.5 cm sections of the upper 6 cm of the undisturbed sediments at setup A, B, and C were determined microscopically (Table 4). Total cell counts strongly decreased with depth in all setups. Total cell counts did not significantly change over the 13 days of incubation in setup A. In the setups B and C, the total cell numbers increased with temperature during the first week. The maximal cell number increased from 45 $x10^8$ cells cm^{-3} to 54.3 $x10^8$ and 57.4 $x10^8$ cells cm^{-3} in setup B and C, respectively. Cell counts were found to be stable in both setups during the last week of the experiment when temperature was maintained constant.

ii)Domain specific hybridisation. The vast majority of positive signals were assigned to members of the domain *Bacteria*. *Archaea* detection was below the detection limit of our FISH protocol.

Detection rates with the probe EUB338 decreased exponentially over the vertical profile in all the samples. Cell detection in the control setup A with EUB338 remained stable over time with a mean value of 30.5 $x10^8$ (surface) and 3.6 $x10^8$ cells cm^{-3} (bottom), throughout the vertical profile (Table 4). There was an increase of detection with EUB338 in setups B and C with the increase of temperature to 40.5 $x10^8$ cells cm^{-3} (surface) and 4.8 $x10^8$ cells cm^{-3} at 6 cm depth in setup B, and to 44.5 $x10^8$ (surface) to 5.4 $x10^8$ cells cm^{-3} at 6 cm depth in setup C. EUB338 values remained constant the second week of the experiment in both setups.

iii)Abundance of members of the *Cytophaga-Flavobacterium* cluster. In all the samples, members of the *Cytophaga-Flavobacterium* cluster of the CFB phylum were the most abundant group with maximum values of 5.7 $x10^8$, 5.7 $x10^8$, and 6.2 $x10^8$ cells cm^{-3} at the beginning of the experiment in setups A, B, and C, respectively. A significant positive correlation ($p>0.01$) of the number of cells detected with the probe

TABLE 4. DAPI and FISH counts from mesocosm samples.

	Absolute DAPI counts (cells/cm^3 [10^8]) (mean ± SD)									% of cells hybridized with probe EUB338[a] (mean ± SD)								
depth	A	B4	B8	B12	B14	C4	C12	C20	C24	A	B4	B8	B12	B14	C4	C12	C20	C24
-0.25	46.1±2	43.6±1	48.9±1	51.4±1	51.6±5	45.5±3	51.3±3	54.1±6	53.9±1	63.4±2	65.7±2	72.1±3	78.5±4	79.1±3	69.9±3	73.2±2	79.8±3	82.3±2
-0.75	45.3±1	43.3±3	45.0±3	52.3±2	51.1±2	44.9±3	50.7±1	54.9±5	53.8±1	67.4±3	64.4±2	65.3±2	66.2±2	67.4±2	59.8±2	63.4±4	68.5±5	69.7±4
-1.25	45.3±2	43.6±3	50.7±2	54.5±4	54.3±2	42.8±2	53.5±1	57.2±5	57.4±2	53.6±5	55.5±3	58.9±3	62.3±1	64.0±1	54.0±1	62.3±1	65.3±3	65.5±2
-1.75	45.2±3	45.1±2	49.8±1	55.2±5	53.7±3	43.8±2	50.9±1	55.4±3	55.5±5	52.6±3	57.3±2	56.8±3	56.2±5	57.5±4	50.4±1	54.3±5	57.2±2	54.0±3
-2.25	44.8±1	45.2±3	49.5±3	49.0±3	50.9±3	45.1±7	50.3±2	54.1±4	53.3±3	46.0±4	45.8±2	49.9±3	54.0±5	54.9±4	44.1±2	51.2±2	53.7±7	54.1±3
-2.75	45.1±2	46.1±4	47.9±5	50.1±3	49.5±3	44.0±2	52.8±2	54.6±3	53.8±3	43.4±1	48.5±1	46.9±1	45.4±3	46.1±3	49.2±3	48.6±4	45.3±5	52.1±3
-3.25	30.8±2	32.0±4	32.2±4	31.5±3	32.3±5	31.5±2	32.0±3	34.0±1	35.5±3	40.7±3	41.6±5	40.5±4	39.8±3	40.5±2	41.2±4	42.3±2	45±2	48.4±5
-3.75	22.4±5	23.5±5	22.0±7	23.0±5	24.8±6	22.5±1	23.0±5	26.0±1	26.8±1	33.0±2	30.5±7	35.5±2	39.9±2	40.6±5	34±4	37.2±3	39.8±1	42.7±2
-4.25	19.0±4	22.0±2	23.5±1	25.3±1	26.5±2	23.0±4	23.0±5	24.0±6	26.8±2	28.5±5	28.5±1	31.2±3	35.6±1	36.7±1	28±3	35.5±1	39.1±1	40.0±2
-4.75	17.6±1	18.5±7	18.3±2	19.0±2	21.0±1	19.0±5	20.0±4	22.0±5	22.3±3	26.9±1	28.3±5	30.5±5	33.3±1	34.9±3	27.5±2	30.3±1	33.2±3	34.7±1
-5.25	16.8±2	17.0±6	17.0±2	17.5±2	17.5±2	16.5±5	15.0±3	18.0±2	19.0±4	25.4±1	26.5±4	28.7±1	28.8±1	29.4±2	26±1	28.2±1	28.6±5	30.0±4
-5.75	15.8±1	16.0±5	16.3±3	15.5±4	16.5±2	15.5±3	16.0±4	16.0±2	17.8±3	24.2±1	25.5±3	27.1±1	28.5±1	29.3±2	25±1	27.6±2	28.3±1	29.7±1

	% of cells hybridized with probe CF319a[a] (mean ± SD)									% sum of detected SRB[b] (mean ± SD)								
depth	A	B4	B8	B12	B14	C4	C12	C20	C24	A	B4	B8	B12	B14	C4	C12	C20	C24
-0.25	12.5±0.1	13.2±0.4	13.8±0.2	14.8±0.2	15.9±0.2	13.7±0.5	18.5±0.4	18.7±0.4	19.5±0.3	3.1±0.5	4.2±0.2	7.3±0.5	7.9±0.5	7.7±0.4	4.0±0.2	7.3±0.1	8.5±0.5	7.8±0.2
-0.75	9.3±0.1	10.2±0.4	10.4±1	12.0±0.1	12.8±0.5	11.2±0.1	16.6±0.7	16.8±0.5	16.4±0.3	3.9±0.4	5.1±0.5	9.1±0.4	9.5±0.4	9.3±0.2	4.7±0.1	9.6±0.5	10.3±0.3	9.4±0.3
-1.25	8.7±0.3	9.8±0.2	10.3±0.1	11.1±0.1	12.2±0.3	9.±0.18	16.0±0.5	16.1±0.6	15.3±0.4	7.0±0.4	6.2±0.1	9.6±0.3	10.1±0.2	8.7±0.7	5.6±0.2	10.5±0.4	10.9±0.3	9.3±0.1
-1.75	7.0±0.2	9.0±0.1	9.7±0.3	14.0±0.2	13.4±0.5	8.8±0.3	15.6±0.4	15.7±0.4	14.9±0.3	6.8±0.3	7.2±0.1	9.1±0.4	9.2±0.4	7.5±0.7	6.9±0.2	9.2±0.3	9.7±0.2	9.0±0.4
-2.25	9.7±0.3	8.6±0.3	9.4±0.2	10.6±0.1	12.3±0.4	9.6±0.3	13.5±0.4	13.6±0.7	10.9±0.4	6.1±0.4	6.4±0.4	9.7±0.1	10.4±0.3	10.1±0.6	6.9±0.3	11.0±0.2	9.2±0.4	7.7±0.5
-2.75	10.6±0.1	8.1±0.2	8.9±0.1	10.4±0.3	11.8±0.5	10.7±0.4	13.5±0.6	13.6±0.5	12.9±0.5	6.9±0.2	6.3±0.3	9.4±0.2	9.9±0.1	9.8±0.5	6.1±00.4	9.8±0.1	9.0±0.6	9.1±0.2
-3.25	9.3±0.1	8.9±0.3	8.3±0.1	9.5±0.1	10.4±0.6	9.3±0.7	10.±0.21	11.2±0.2	12.±0.15	4.1±0.4	4.2±0.6	6.4±0.3	8.2±0.4	10.4±0.5	5.2±0.5	8.5±0.4	10.±0.30	9.4±0.3
-3.75	5.9±0.1	5.8±0.1	6.5±0.2	7.8±0.3	8.3±0.5	5.5±0.5	6.5±0.4	7.3±0.2	8.1±0.7	4.0±0.1	3.5±0.4	7.9±0.3	7.7±0.1	8.4±0.2	4.3±0.1	7.6±0.2	7.5±0.4	6.6±0.3
-4.25	5.5±0.2	4.3±0.1	5.9±0.2	6.3±0.2	7.3±0.7	5.2±0.5	5.9±0.5	6.9±0.1	7.0±0.2	4.3±0.1	3.3±0.2	8.2±0.3	7.5±0.1	9.4±0.2	5.5±0.2	10.1±0.4	11.4±0.2	12.1±0.1
-4.75	4.8±0.3	4.3±0.3	3.5±0.1	4.6±0.1	5.3±0.8	4.7±0.6	5.3±0.5	6.3±0.4	7.1±0.1	3.9±0.3	3.4±0.5	6.6±0.4	9.0±0.2	8.7±0.4	4.1±0.5	9.6±0.4	10.8±0.2	12.1±0.2
-5.25	4.2±0.1	4.5±0.2	4.3±0.3	4.5±0.1	5.0±0.5	3.5±0.4	4.7±0.4	5.3±0.6	6.4±0.3	4.0±0.5	2.6±0.1	7.5±0.1	7.5±0.3	6.4±0.5	3.9±0.1	9.4±0.6	10.6±0.3	11.8±0.2
-5.75	4.3±0.1	4.5±0.2	4.7±0.1	4.9±0.1	6.4±0.5	4.2±0.4	4.3±0.6	5.9±0.4	6.6±0.3	3.8±0.6	3.7±0.2	5.4±0.1	6.6±0.2	6.1±0.7	2.9±0.3	7.6±0.1	8.9±0.4	10.4±0.2

CF319a and temperature was found (Table 5). The maximum number of *Cytophaga-Flavobacterium* cells at the end of the experiment was 5.8 $x10^8$, 8.4 $x10^8$, and 10.2 $x10^8$ cells cm^{-3} in A, B, and C, respectively.

iv) Identification and quantification of *Arcobacter* sp. Detection with probe ARC94 was generally low between 0.4 $x10^8$ and 0.9 $x10^8$ cells cm^{-3} (Table 6). The detection was limited to the upper 4 cm of the sediment. Detection of *Arcobacter* sp. was positively correlated with temperature for the upper 2 cm of the sediment and negatively correlated for the samples located between 2 and 4 cm depth (Fig.8).

TABLE 5

Variable	temperature	DAPI	EUB338	CF319a	Sum of SRB	DSV698	DSS658
temperature	1,0000	0,9029	0,8195	0,8539	0,7797	0,7774	0,4124
CF319a	0,8539	0,8746	0,7911	1,0000	0,7235	0,7834	0,3162

v)Identification and quantification of SRB. The number of cells identified as SRB was increasing with temperature from 3.2 $x10^8$ to 4.9 $x10^8$, and from 3.1 $x10^8$ to 5.1 $x10^8$ cells cm^{-3} in setup B and C, respectively. When temperature was kept constant at 4°C in setup A, the maximum number of SRB was always at the range of 3.1 $x10^8$ cells cm^{-3} of sediment.

Different populations of SRB were identified with the multiple probe approach used. With six of the probes used, the detection rates were above the detection limit of the technique. These probes were: DNMA657, for members of the genus *Desulfonema*; DSV698, for members of the genus *Desulfovibrio;* DSS658, specific for the *Desulfosarcina-Desulfococcus* group; DSR651, specific for *Desulforhopalus* spp.; DSB985, specific for *Desulfobacter* spp.; and probe 221, detecting *Desulfobacterium* spp..

The *Desulfosarcina-Desulfococcus* group was the most abundant group of SRB in the sediment at 4°C accounting for 52 to 93% of the total SRB. The cell counts for this group were almost constant over the two weeks experiment in all setups at about 2.5 $x10^8$ cells cm^{-3} of sediment.

FISH detection of *Desulfovibrio* spp. was increasing with temperature from 0.9 x10^8 to 2.9 x10^8 cells cm^{-3} in both setup B and C (Fig.9). The number of *Desulfovibrio* spp. detected in setup A remained constant around 0.9 x10^8 cells cm^{-3} throughout the experiment.

Counts of *Desulfonema* spp., *Desulforhopalus* spp., *Desulfobacter* spp., and *Desulfobacterium* spp. were always below 0.3 x10^8 cells cm^{-3} of sediment.

A positive correlation was found between temperature and the fraction of incomplete oxidizers among the SRB . Contrarily, no correlation between temperature and the complete oxidizer fraction of SRB was found (Table 5).

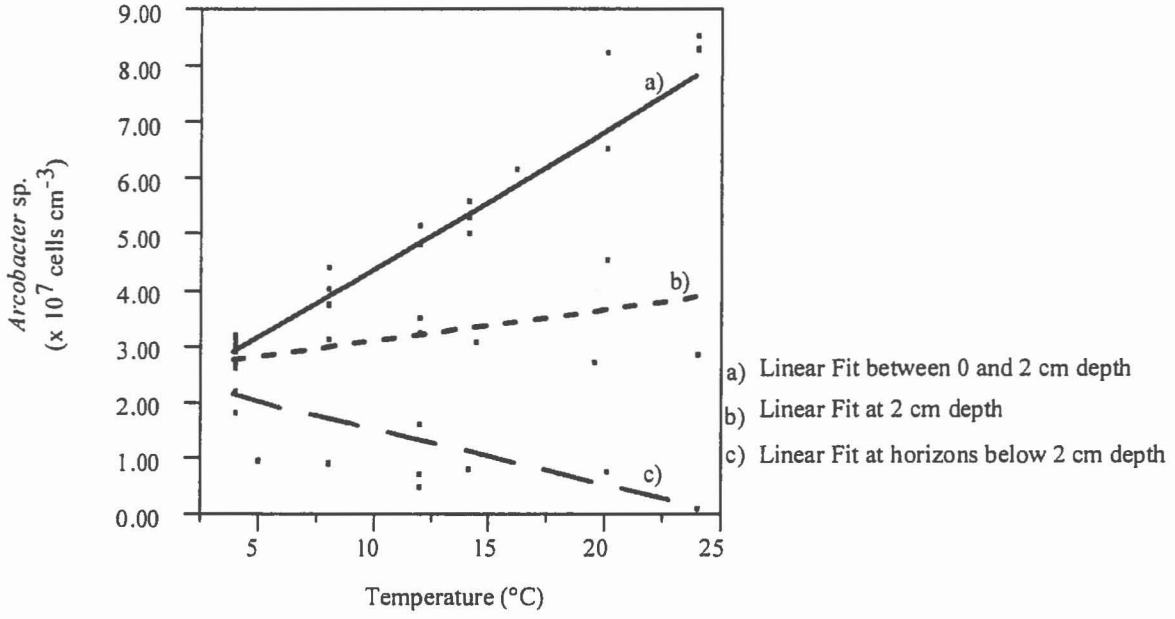


Fig.8. Depth dependent correlation between *Arcobacter* spp. and temperature.

Discussion

The trends in functional parameters, i.e. SRR, and patterns of the microbial community were in agreement between the undisturbed mesocosms and the field data collected for two years.

The control setup at 4°C showed no significant changes in the microbial community structure and activity throughout the two weeks of incubation. After a shift in temperature the microbial community changes but, subsequently stayed constant. This likely reflects that temperature was the major factor driving the changes.

Biogeochemistry. The pore water composition mirrored the biogeochemical processes in the sediment. In accordance with the zonation scheme proposed by Froelich (13). Only in a very thin layer at the sediment surface oxygen and nitrate are

available as terminal electron acceptors. The aerobic/anaerobic boundary layer is typically situated 1-6 mm beneath the surface (21). Oxygen penetration depth changes over the day depending on the light conditions, and over the year depending on the deposition rate of organic carbon, oxygen concentration in the overlying water, light, and bioturbation (21, 35). The O_2 penetration into the sediment varied seasonally and this penetration was maximal during Jul98 when the in situ temperature of the sediment was 20° C higher than in winter. Similar positive correlation between O_2 penetration depth and temperature has been reported due to an increased photosynthesis (6).

However, a decrease in O_2 penetration depth in the mesocosm was found with the increase of temperature. The depth at which the sediment became anaerobic was dependent on the net respiratory oxygen demand. As described before (21, 22), the respiration activity was clearly enhanced under elevated temperatures, leading to a higher carbon turnover and, thus, to a stronger substrate flux of organic matter from deeper sediments, ending up in oxygen limitation and lower penetration of oxygen, respectively. An unexpected slight increase of the mean O_2 penetration depth in the setup with constant temperature was observed. This increase might have been caused by different processes. Due to a lack of allochthonous input from the bulk water during the 15 days of incubation, a net loss of organic matter from the sediment, especially of the labile fraction in the surface layer, is likely to occur. Subsequently, substrate at the surface might become limiting, causing a deeper penetration of oxygen. On the other hand, a clear change in the surface structure could be observed during the experiment. While in the medium and high temperature setups, the surface became smooth, it remained rough in the low temperature setup. Whether this was due to enhanced EPS formation or reduced meiofaunal bioturbation activity under elevated temperatures, is not clear. Whereas no indication of advective transport was found in the profiles measured in setup A, it cannot be excluded that additionally hydrodynamic effects due to differences in surface roughness, and subsequent differences in the effective thickness of the diffusive boundary layer, influenced the depth of oxygen penetration under the three conditions.

The oxygen profiles were used to calculate the temperature characteristics of oxygen-dependent processes in the sediment. The net Q_{10} value was higher than the cell-specific values showing that the increase in activity is due to both, an increase of the size of the active community and enhanced cell metabolism.

Below the oxic zone, suboxic nonsulfidic conditions were indicated by the presence of dissolved Fe(II) and Mn(II) down to 15 cm depth. Here the highest SRR were found but no accumulation of free H_2S was observed. This compound might react with iron compounds to form iron sulfides or be re-oxidized by Fe(III) or Mn(IV) to intermediate sulfur species or sulfate (3). The coincidence of maximal SRR and high concentrations of Fe(II) and Mn(III) strongly supports this hypothesis. Significant amounts of DIC in this zone reflected oxidation of organic matter, and both microbial SRR as well as DIC content varied seasonally. Maximum SRR and DIC contents were found in summer indicating that temperature might promote the activity of SRB. Similar results have been found in sediments from the North Sea (21, 23).

Similarly, the net SRR measured in the mesocosms were increasing with temperature. The SRR per cell were also increasing with temperature from 0.2 to 2.8 fmol SO_4^{-2} $cell^{-1}$ day^{-1}. However, the Q_{10} values showed that the increase in SRR was more influenced by the increase of the SRB active community size than by the increase of the SRR per cell. These SRR per cell were at the low end of the specific SRRs of mesophilic SRB pure cultures at logarithmic growth (18). Nevertheless, the rates observed were in a reasonable range compared to natural environments.

The respiration rates of SRB in such marine systems are generally limited by the availability of electron donors. Although TOC content was fairly constant throughout the year, the increase of fermentative processes with higher temperatures might result in the releasing of compounds that favor SRB growth. The stable carbon isotope ratio ($\delta^{13}C$) measured was in accordance to previous observations in tidal flats of the Wadden Sea (2, 37). The difference in the $\delta^{13}C$ values between surface and deeper layers are best explained by the preferential degradation of the more labile marine organic matter and a higher persistence of a more resistant terrestrial fraction (2).

Microbial community, structure and dynamics. Total cell counts determined in the sediment cores were in good accordance with previous observations (25), in which microbial abundances also decreased exponentially with depth. The upper 2-3 cm of the sediment had cell densities twice as high as in the deeper sediment. The boundary between these two zones coincided with the first color change, which has been related to the location of the oxidized-reduced boundary layer (3). Statistically significant minimum (p□0,01) at a depth of 1 cm detected during summer might be related either to grazing activities by eukaryotes or viral lysis (16).

As previously observed (25), a significant fraction of DAPI stained cells hybridized with the bacterial probe EUB338, and only a very small fraction could be affiliated to *Archaea*. Similar observations have been reported in bulk soil (43) and in marine sediments (36). In contrast, higher abundance of Archaea have been reported in marine bacterioplankton (8, 15). Therefore, we can not exclude that Archaea not detected by our FISH protocol make up a large part of those DAPI stained cells that did not hybridized with one of the three domain-specific probes, EUB338, ARCH915 and EUK516. The possibility that EUB338 likely underestimated counts of Bacteria has been mentioned before.

Detection rates with bacterial probe EUB338 followed the same seasonal dynamics as those of total cell counts. Higher detection rates in summer correlated with higher microbial activity as shown by DIC, and SRR.. Similar correlations between seasonal activity and bacterial biomass have been shown (4). The percentage of detected cells decreased exponentially with depth, and this could be related to the organic matter quality of the sediments (20, 26). Although the organic matter content was fairly constant for the upper 10 cm of the sediment, its quality as judged from related to the $\delta^{13}C$ values decreased with depth resulting likely in lower availability (2).

Total cell numbers in the mesocosms determined in all sediment cores as well as their decrease with depth were in close accordance with the field observations. There was a positive correlation between total cell numbers and temperature. This is in agreement with the seasonal relation between bacterial biomass and temperature pointed before. As for the field samples, the major fraction of the total DAPI stained cells hybridized with the bacterial probe EUB338, and only a very small fraction could be affiliated to *Archaea*.

Detection rates with bacterial probe EUB338 followed the same temperature dependent dynamics as the total cell numbers. Higher detection rates correlated with higher temperature (Fig.4). This is in agreement with the decrease of the available organic matter throughout the vertical profile in this site.

Based on a previous report, the *Cytophaga-Flavobacterium* cluster was the most abundant group found in this sediment (25). Since then, members of this cluster have been found to be important in many other marine environments (9, 15, 31, 34). It has been recently suggested that organisms affiliated with *Cytophaga-Flavobacterium*

might play an important role in the anaerobic decomposition of complex organic matter in marine sediments by catalyzing the rate-limiting first step fermentation (34).

CF319a counts were lower in winter than in the rest of the year, and reached the highest values in summer. A positive linear regression (R^2= 0,959, Fig. 6) between the numbers of *Cytophaga-Flavobacterium* detectable cells and total bacterial cells was found. This suggested that the size of the *Cytophaga-Flavobacterium* population ruled the microbial community dynamics. This observation supports the idea that the size of the microbial community dwelling in marine sediments is limited by the size of the hydrolytic and fermentative population present (10). Hydrolytic and fermentative processes are influenced by organic matter content and temperature (34). Therefore, it is reasonable to expect a correlation between the number of *Cytophaga-Flavobacterium* cells, organic matter content and sediment temperature (38). Such a correlation was found to occur only with temperature. Thus, temperature seems to be the primary factor governing microbial community dynamics in the intertidal sediment of Dangast.

The *Desulfosarcina-Desulfococcus* cluster was found to dominate the SRB community, in accordance with other marine sediments examined by FISH (30). This group is not detected by SRB385 probe (30), which explains the significant underestimation of SRB by this probe. Besides the *Desulfosarcina-Desulfococcus* cluster, *Desulfovibrio* spp. cells were the second dominant group of SRB detected. Together these two groups accounted for 95% of the total SRB, and are assumed to be responsible for the sulfate reduction rates observed.

While population size of *Desulfosarcina-Desulfococcus* cluster remained stable throughout the year, *Desulfovibrio* spp. cells showed a distinct seasonal dynamics. From nearly undetectable in winter they increased to densities that were comparable to those of the *Desulfosarcina-Desulfococcus* cluster in summer, thus doubling the total number of SRB. These changes coincided with a strong increase of SRR in summer. Calculated SRR per cell were minimal in winter with about 0.2 fmol $SO_4^=$ $cell^{-1}$ day^{-1} and maximal in the warmer seasons with 0.8 - 1.0 fmol $SO_4^=$ $cell^{-1}$ day^{-1}. Although these results could be compared to rates observed with pure cultures (19), seasonal differences might not be only related to temperature changes, but to changes in the community composition and its size.

Arcobacter spp. duplicated in summer with respect to winter abundances (Fig.5). As previously observed (25), almost no *Arcobacter* spp. were detected below 4 cm depth.

Interestingly, the disappearance of this organisms coincides with the first color change of the sediment which might reflect redox potential changes (3), which determine the availability of electron acceptors for dissimilatory respiration (10).

These three bacterial populations were followed in the mesocosm setups. As previously reported, *Cytophaga-Flavobacterium* group was the most abundant group found in this sediment. A more pronounced presence of *Cytophaga-Flavobacterium* in the sediment might strongly increase fermentative processes (34), leading to higher concentrations of compounds feeding downstream members of the bacterial community. Indeed, there was a close correlation between *Cytophaga-Flavobacterium* cell and the total bacterial community detected by the EUB338 probe (Table 4).

SRB was the second bacterial group followed during the experiment. Members of the *Desulfosarcina-Desulfococcus* cluster and of *Desulfovibrio* spp. together accounted for approximately 95% of the total SRB. An increase of SRB numbers with temperature was found. However, the response of these two populations to the increase of temperature was different. Upon sampling *Desulfosarcina-Desulfococcus* cluster was found to numerically dominate the SRB community in the winter sediment. The abundance of this group remained stable throughout the incubation period at all temperatures. Contrarily, a positive correlation between *Desulfovibrio* spp. and temperature was found. *Desulfovibrio* spp., that nearly triplicates their population size in summer, are fast growing, incomplete oxidizers with higher SRR than those of complete oxidizers like the *Desulfosarcina-Desulfococcus* cluster (41). These changes coincided with a strong increase of SRR. It is noteworthy that *Desulfovibrio* spp. blooms are related to the increase of temperature of the sediment. In general, incomplete oxidizers like *Desulfovibrio* spp. are less versatile in substrate spectrum than complete oxidizers (27, 28). Therefore, the *Desulfovibrio* spp. are likely more dependent on the fermenting metabolites than *Desulfosarcina-Desulfococcus* cluster. Indeed, a close correlation between the seasonal abundances of *Cytophaga-Flavobacterium* cells and *Desulfovibrio* spp. populations have been reported from field data, whereas there is no correlation between *Cytophaga-Flavobacterium* members and the *Desulfosarcina-Desulfococcus* cluster (Table 4). This suggests that the increased presence of *Cytophaga-Flavobacterium* members with temperature might strongly increase fermentative processes (34), thus releasing higher concentrations of compounds that favor SRB like *Desulfovibrio* spp.. Thus, the

higher abundance of *Desulfovibrio* spp. in summer might contribute to the increase of cellular SRR.

Also *Arcobacter* spp. showed a distinct response to temperature changes. There was a positive correlation between the detection rates of this population and the increase of temperature in the upper 2 cm of the sediment. In contrast, a negative correlation was revealed between 2 and 4 cm depth (Fig.5). *Arcobacter* spp. were rare beneath 4 cm depth. Interestingly, the location of the *Arcobacter* spp. population was limited to the most upper part of the sediment. There is evidence that its location is reflecting changes in the redox potential of the local environment. Changes in the redox potential have been found in intertidal sediments where the increase of sulfate reduction was related to changes in the location and thickness of the sub-oxic zone (2). The positive correlation between detection of *Arcobacter* spp. and temperature in the upper 2 cm of the sediments and the subsequently negative correlation in deeper layers suggest that the increase of the population with temperature occurs simultaneously to its location change.

Conclusions

This study shows that members of the *Desulfosarcina/Desulfococcus* group and *Desulfovibrio* spp. are the key populations within the sulfate-reducing bacterial community for the anaerobic mineralization of organic matter in this coastal marine sediments. Seasonal differences on the abundance and activity of sulfate-reducers seems to be due to the dependence of the *Desulfovibrio* spp. on the metabolites of the *Cytophaga-Flavobacterium* fermentative bacterial population (11). The hydrolytic and fermentative processes are influenced by organic matter content and temperature (34).

Although the microcosms can not exactly reflect fluctuations in the field, e.g., the constant supply with freshly sedimented materials is absent, the trends observed in microbial community composition and metabolic functioning were in agreement with the field data. The response of the microbial community to temperature perturbation was very fast. This good adaptation might reflect the capability of the microbial community to react to the rapid environmental changes characteristic of intertidal marine systems. Thus, it is likely that temperature is the limiting factor for the fermentative bacterial population in these organic matter rich sediments, which subsequently might rule changes of the SRB population.

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