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

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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 mudflat (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, flagellates, 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 floor 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 reflected the preferential microbial degradation of labile (marine) organic matter relative to a more resistant (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 flagellates. 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 first maximum at around 2 cm depth. Up to 80% of the total cells as detected by DAPI-staining hybridized with a *rRNA*-targeted oligonucleotide probe specific for the domain bacteria (EUB338).

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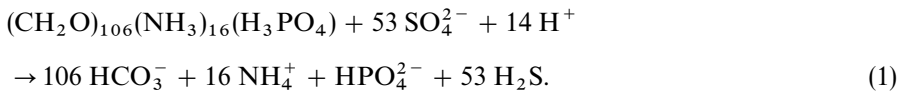
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Sulfate-reducing bacteria as detected by probe SRB385 showed high abundance (up to 7% of total cells) in the upper 5 cm of the sediment. Total and cell numbers of sulfate reducers were highest at about 2 cm and decreased with depth. Cellular sulfate reduction rates were estimated from the SRB counts by fluorescence 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 ($^{34}\text{S}/^{32}\text{S}$) fractionation between coexisting dissolved pore water sulfate and sedimentary reduced sulfur species with laboratory studies a significant contribution of bacterial disproportionation reactions within the oxidative part of the sedimentary sulfur cycle is indicated. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Mud flat; 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 final electron acceptors. In coastal marine sediments OM oxidation proceeds to a large part via anaerobic microbial activity using dissolved sulfate as the final electron acceptor (Jørgensen, 1982) and is associated with the formation of hydrogen sulfide according to the overall reaction (for typical marine organic matter)



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 sulfides, 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 significant 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-sulfidic part of the sediments and effective mechanisms for the reoxidation of hydrogen sulfide. Analytical techniques for the in situ enumeration and characterization of bacterial cells have been successfully 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 first time combined methods to measure the geochemical and stable isotopical composition of pore waters and sedimentary solid phases with fluorescence 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 influence 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 flagellates, 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 influenced by the fluvial 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 μ m). 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 flowing via a sluice into a small harbor 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 flat, 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 flat area of about 2 m² 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 field (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.

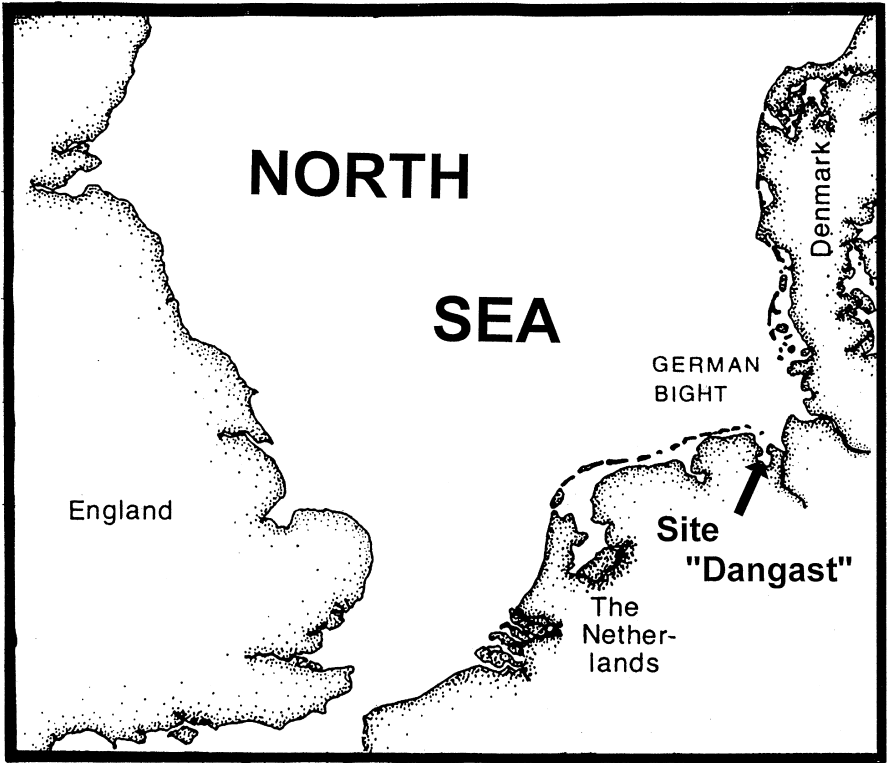


Fig. 1. The study area (modified after Sündermann, 1994). Arrow indicates sampling site.

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 specific for sulfate-reducing bacteria of the δ -subclass of Proteobacteria were EUB338 and SRB385, respectively (Amann et al., 1992). For enumeration of heterotrophic flagellates, ciliates, nematodes, and crustaceans, a sediment core (2.6 cm i.d.) was cut in the field into 2 mm slices down to 2 cm depth and fixed with glutaraldehyde (f.c. 1.6%). The following laboratory methods were modified 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 fluorescent dyes DAPI and FITC (fluorescein-5-isothiocyanate), the organisms were concentrated on black polycarbonate membran filters (1.2 μm pore size) and counted with an epifluorescence 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 filters) under inert gas in a N_2 -filled glove bag. Concentrations of dissolved Fe, Mn and sulfate were measured on diluted acidified (2% HNO_3) solutions by ICP-OES (Perkin-Elmer Optima 3000 XL) using Sc as an internal standard (precision 2σ : 5%). Hydrogen sulfide 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 flow-injection analysis (Hall and Aller, 1992). Samples for ΣCO_2 were preserved with Na_2MoO_4 (precision 2σ : 5%). Salinity of filtered samples was measured with a refractometer (precision: ± 0.3).

Microsensors for oxygen and hydrogen sulfide 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). Profiles were measured in sediment cores immediately after sampling *on site*, and in a flow chamber (Lorenzen et al., 1995) in the laboratory. Cores were incubated for several hours in the dark or with 430 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ before recording dark or light profiles, respectively. The spatial resolution of all measurements was 0.1 mm as controlled by a motor-driven micromanipulator (Märzthäuser Wetzlar, Germany). Data acquisition was done automatically with a personal computer and the software package LabView® (National Instruments, USA). For each incubation, 20 profiles were measured at different sites in the sediment. Total oxygen uptake of the sediment in the dark was calculated as oxygen flux through the diffusive boundary layer following Fick's first law of diffusion and using the diffusion coefficient for oxygen under the measuring conditions ($1.537 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$) from Li and Gregory (1974). Bacterial sulfate reduction rates were measured using the whole-core incubation technique and the injection of a $^{35}\text{SO}_4^{2-}$ 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 (Canfield, 1989). Fe and Mn concentrations were measured by flame atomic absorption spectroscopy (Perkin Elmer AAS) (precision 2σ : 4%). Reproducibility from selected duplicate extractions was within 10%. Total reduced sulfur (TRS; sum of iron monosulfides, 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 quantified gravimetrically (precision 2σ : 10%). The sedimentary sulfur fractions containing acid volatile sulfide (AVS) plus pyrite, and elemental sulfur were distilled separately from Zn-acetate fixed samples using a cold or hot acidic Cr(II) chloride solution, respectively (Allen and Parkes, 1995). Total sulfur (TS; sum of pyrite, iron monosulfides, S^0 , 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 ₂ O ₃ (%)	CaO (%)	MgO (%)	Na ₂ O (%)	K ₂ O (%)	Fe (%)	Fe ^a (%)	Mn (ppm)	Mn ^a (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.

^aExtractable Fe and Mn in dithionite–citrate–acetic acid solution.

^bCorrected for pore water sulfate.

^cTotal reduced sulfur (TRS).

^dPyrite + Acid volatile sulfide (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 ¹³C/¹²C 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 $\pm 0.2\text{‰}$. The CO₂ 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 CO₂ coulomat with a CM 5130 acidification device (UIC) (precision 2σ : 1.1%; Dellwig et al., 1999). Accuracy was tested by measuring in-house standards. TOC contents were obtained from the difference 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 filtered Zn-acetate preserved samples as BaSO₄, carefully washed and dried. Sulfur isotope ratios (³⁴S/³²S) of the barium sulfate and the silver sulfide samples from the TRS and AVS + pyrite distillations 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 (%)	$\delta^{13}\text{C}$ (‰)	TS ^b (%)	TRS (%)	S ^o (ppm)	$\delta^{34}\text{S}^c$ (‰)	$\delta^{34}\text{S}^d$ (‰)	H ₂ O (%)
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 ConFlo 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 $\pm 0.2\text{‰}$. International standards IAEA-S-1 and IAEA-S-2 were used to calibrate the mass spectrometer. $\delta^{34}\text{S}$ 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^o), 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 fluorescence 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 (Matsun 3000 type FTIR spectrophotometer) and powder X-ray diffraction (Siemens X-ray diffractometer; Ni-filtered $\text{Cu } k_\alpha$ 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^a

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.

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

Pyrite contents were 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 monosulfides was indicated by a diffuse 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 floor (cmbsf).

Analysis of the grain size distribution of surface sediments from an adjacent mud flat 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 carried out on bulk sediments and texture prepares using FTIR spectroscopy and powder X-ray diffraction, the clay mineral fraction consists of illite, kaolinite, chlorite, and smectite. This is in agreement with findings 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, different 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 significant 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

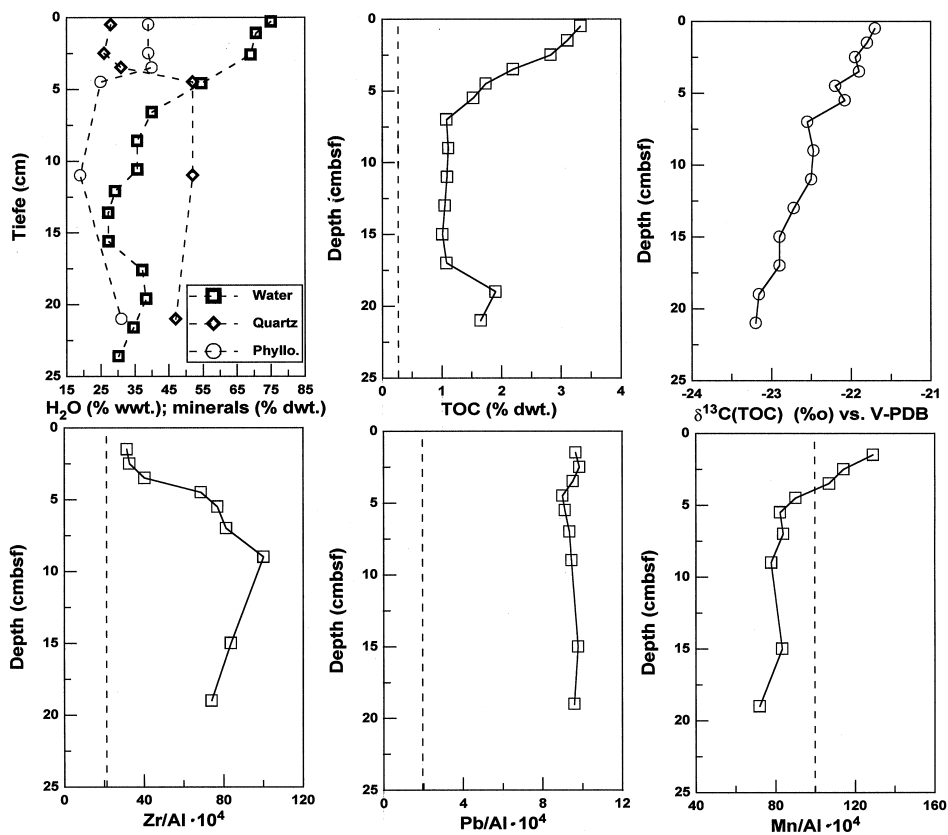


Fig. 2. Downcore variation of selected geochemical parameters. Zr, Pb and Mn data were normalized to Al. Dashed lines indicate average shale.

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

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 findings are supported by the results of the present study (Fig. 3). The slightly higher variability observed in the higher water content of the mud flat is due to sedimentological changes of the deeper sediment in the investigated mud flat, as noted above.

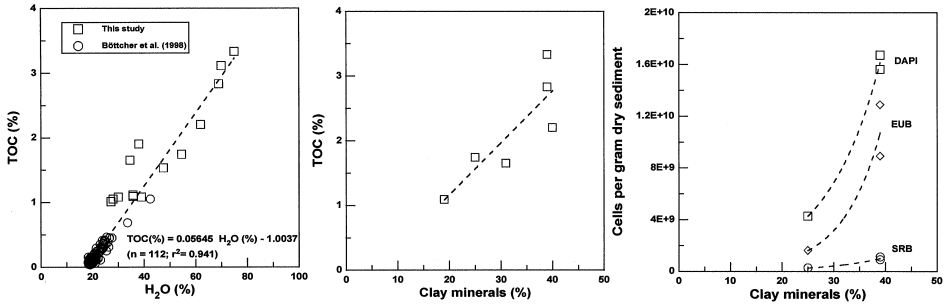


Fig. 3. Scatter plots of TOC versus water and phyllosilicate mineral contents, and bacterial cell numbers versus phyllosilicate contents.

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 significant influence of heavy minerals can be ruled out. The entire investigated sediment section was significantly enriched in Pb when compared to the geogenic background level ($\text{Pb}/\text{Al} \approx 2.5 \times 10^{-4}$) 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 flats of the Jade Bay.

When compared to average shale ($\text{Mn}/\text{Al} = 96 \times 10^{-4}$), 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 mobilized after reduction of Mn(IV)oxyhydroxides. Mn(II) diffusing 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) oxyhydroxides 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 monosulfides 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

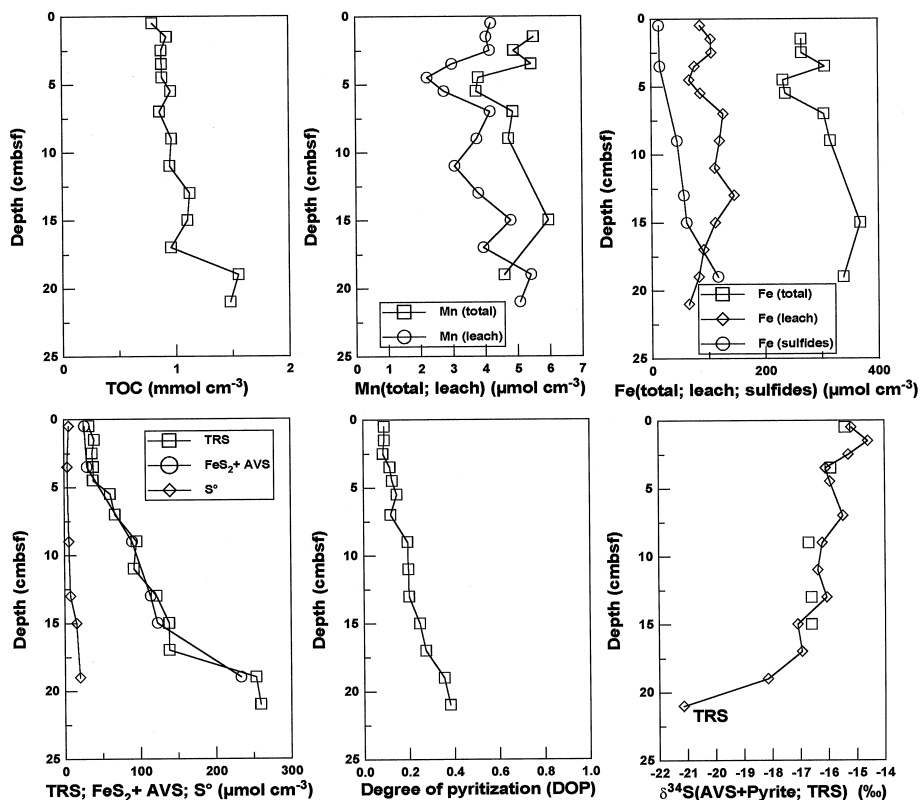


Fig. 4. Downcore variation of selected geochemical parameters based on volume wet sediment, degree of pyritization (DOP) and stable isotopic composition of metal sulfides.

should be noted that the downcore variations of the element contents (Table 1) change significantly when related to the volume of fresh sediment (Fig. 4). This is caused by the strong effect 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 flats of the East Frisian Wadden Sea (Salomons and Mook, 1981; Böttcher et al., 1997, 1998b). A steady downcore decrease in $\delta^{13}\text{C}$ 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 resistant (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 $\delta^{13}\text{C}$ value of about -27‰ can

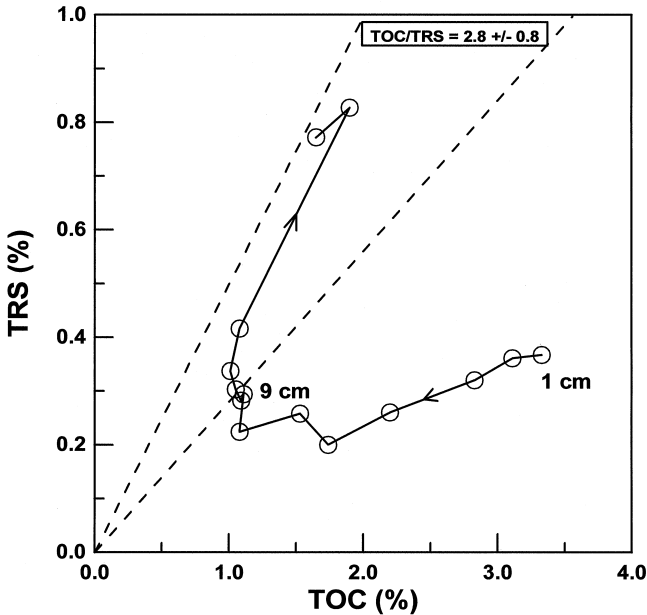


Fig. 5. Scatter plot of TOC versus TRS contents. Dashed lines mark the boundaries proposed for normal marine sediments (Berner, 1984).

be expected (Salomons and Mook, 1981; Böttcher and Scholz-Böttcher, unpublished data). Different sources of marine organic matter in the German Wadden Sea show highly variable $\delta^{13}\text{C}$ 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 ($\delta^{13}\text{C} = -19$ ‰) and terrestrial ($\delta^{13}\text{C} = -27$ ‰) 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 different sediments, the biogeochemical reactions related to specific depositional environments are reflected 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 influenced by complex processes near the sediment–water interface, may finally reach the geochemical signatures of “normal” marine environments. Critical factors are the

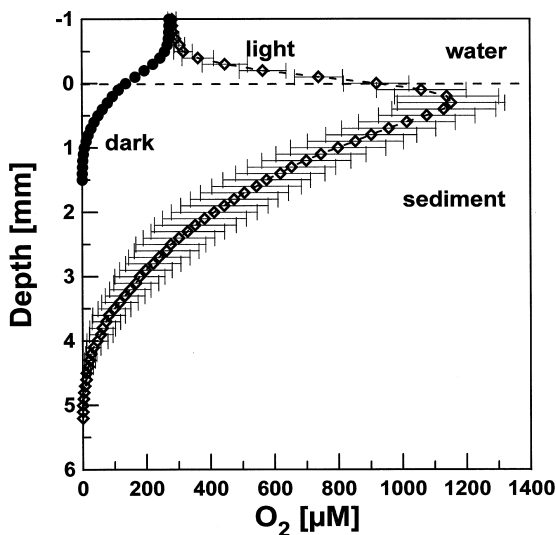


Fig. 6. Dissolved oxygen concentrations during dark (closed symbols) and light (open symbols) incubation in the laboratory. Temperature and salinity were adjusted to be close to in situ conditions (11°C; salinity: 31).

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 sulfides, 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 depletion 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 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ with occasional peaks up to 1300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, which is similar to the intensity applied in the laboratory (430 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Oxygen profiles measured in the field 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 ,

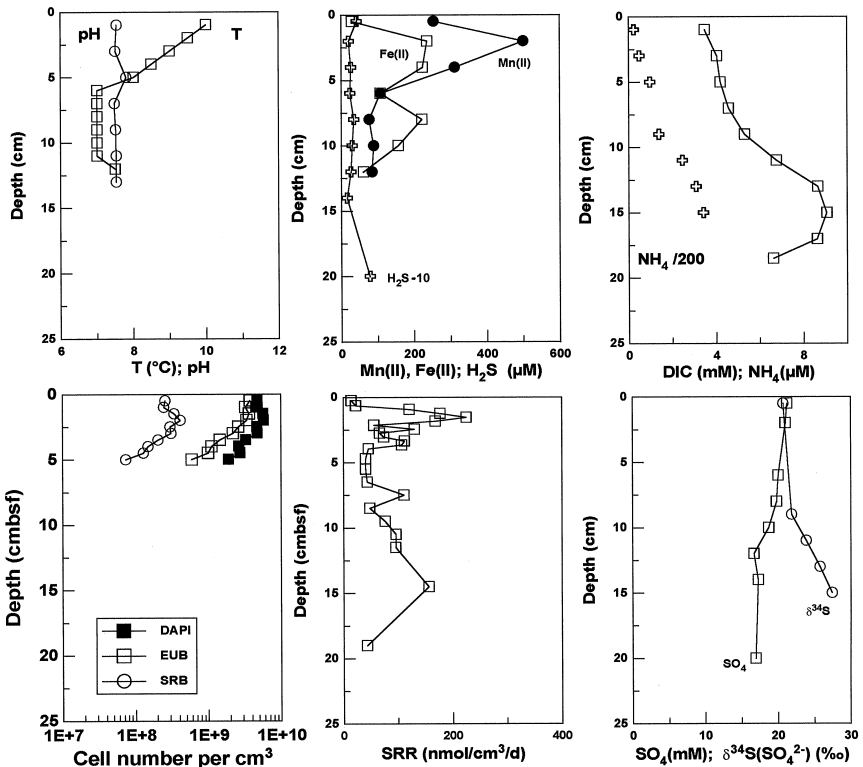


Fig. 7. Downcore variation of pore water parameters and bacterial cell numbers.

corresponding to a 3–5-fold oxygen oversaturation. The maximum oxygen penetration depth in the light was 4.4–4.9 mm. Under field 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 profiles 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 flux reported for estuarine Weser sediments at similar temperatures (Sageman et al., 1996).

No hydrogen sulfide was detected in the sediment cores under any condition within the top 20 mm, the maximum depth reached by our microsensors. Sub-oxic, non-sulfidic 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 significant amounts of DIC and NH₄⁺ reflects 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

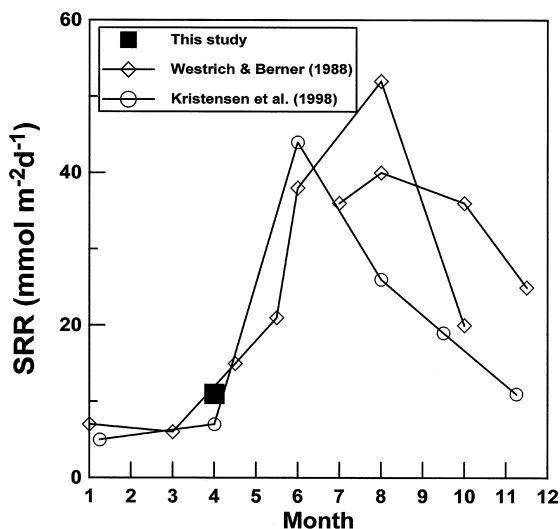


Fig. 8. Depth-integrated sulfate reduction rates compared to seasonal variations found by previous studies in temperate muddy sediments. Data of Westrich and Berner (1988) are given in mmol/L/yr.

between 14 and 225 nmol cm⁻³ d⁻¹ and showed two maxima in the sub-oxic zone, the first 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 fine-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 quality 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 sulfide (Fig. 7). Hydrogen sulfide 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 sulfide produced during bacterial dissimilatory sulfate reduction can react with iron compounds to form iron sulfides 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 sulfide produced during dissimilatory sulfate reduction.

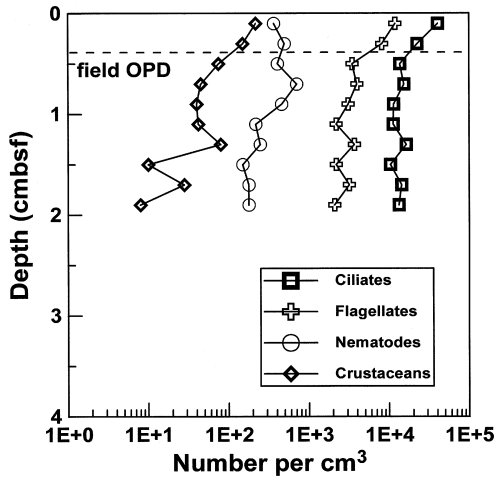


Fig. 9. Vertical abundance of crustaceans, nematodes, heterotrophic flagellates, and ciliates. Data are the average of two subsamples from one core. Dashed line indicates oxygen penetration depth.

The coincidence of the first 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 first 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 $0.55 \text{ fmol SO}_4^{2-} \text{ cell}^{-1} \text{ day}^{-1}$ 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 defined 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 findings 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., Banfield and Nealson, 1997).

The depth-dependent variation of the abundances of ciliates, crustaceans, and heterotrophic flagellates all followed the same pattern, with the highest numbers of organisms occurring at the sediment–water interface and decreasing significantly below 4 mm depth (Fig. 9). This boundary directly coincides with the oxygen penetration depth observed in the laboratory and in the field. 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 different size classes of ciliates ($> 100 \mu\text{m}$, $30\text{--}100 \mu\text{m}$ and $< 30 \mu\text{m}$ in length; Beardsley, 1999). The influence 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 sulfides should nearly reflect 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 sulfides 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 significantly higher. This is also shown, when the combined field data on the specific 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° , $\text{S}_2\text{O}_3^{2-}$; Canfield 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 mudflat. The abundances of heterotrophic flagellates, ciliates, 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 downcore. Coexisting 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)(oxyhydr)oxides. The calculated cell-specific 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 field results with experimental studies indicates that the sedimentary sulfur isotopic signal was not only determined by sulfate reduction alone, but additionally influenced by reactions in the oxidative part of the sulfur cycle, including bacterial disproportionation of sulfur intermediates.

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References

- Allen, R.E., Parkes, R.J., 1995. Digestion procedures for determining reduced sulfur species in bacterial cultures and in ancient and recent sediments. *American Chemical Society Symposium Series* 612, 243–257.
- Amann, R.I., Ludwig, W., Schleifer, K.H., 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews* 59, 143–169.
- Amann, R.I., Stromley, J., Devereux, R., Key, R., Stahl, D.A., 1992. Molecular and microscopic identification of sulfate-reducing bacteria in multispecies biofilms. *Applied and Environmental Microbiology* 58, 614–623.
- Banfield, J.F., Nealson, K.H., 1997. Geomicrobiology: interactions between microbes and minerals. *Reviews in Mineralogy* 35, 448.
- Beardsley, C., 1999. Verteilung benthischer Mikro- und Meiofauna in einem Schlickwatt der Nordsee. Diplom Thesis, University of Hannover, 88 pp.
- Berner, R.A., 1984. Sedimentary pyrite formation — an update. *Geochimica et Cosmochimica Acta* 48, 605–615.
- Berninger, U., Epstein, S.S., 1995. Vertical distribution of benthic ciliates in response to the oxygen concentration in an intertidal North Sea sediment. *Aquatic Microbiology and Ecology* 9, 229–236.
- Böttcher, M.E., 1998. Manganese(II) partitioning during experimental precipitation of rhodochrosite-calcite solid-solutions from aqueous solutions. *Marine Chemistry* 62, 287–297.
- Böttcher, M.E., Brumsack, H.-J., de Lange, G.J., 1998a. Sulfate reduction and related stable isotope (^{34}S , ^{18}O) variations in interstitial waters of the eastern Mediterranean. *Proceedings of the Ocean Drilling Program, Scientific Results*, Vol. 160, pp. 365–373.
- Böttcher, M.E., Oelschläger, B., Höpner, T., Brumsack, H.-J., Rullkötter, J., 1998b. Sulfate reduction related to the early diagenetic degradation of organic matter and “black spot” formation in tidal sandflats of the German Wadden Sea: stable isotope (^{13}C , ^{34}S , ^{18}O) and other geochemical results. *Organic Geochemistry* 29, 1517–1530.
- Böttcher, M.E., Rusch, A., Höpner, T., Brumsack, H.-J., 1997. Stable sulfur isotope effects related to local intense sulfate reduction in a tidal sandflat (southern North Sea): results from loading experiments. *Isotopes in Environmental and Health Studies* 33, 109–129.
- Böttcher, M.E., Smock, A.M., Cypionka, H., 1998c. Sulfur isotope fractionation during experimental precipitation of iron(II) and manganese(II) sulfide at room temperature. *Chemical Geology* 146, 127–134.
- Burdige, D.J., 1993. Biogeochemistry of manganese and iron reduction in marine sediments. *Earth Science Reviews* 35, 249–284.
- Canfield, D.E., 1989. Reactive iron in marine sediments. *Geochimica et Cosmochimica Acta* 53, 619–632.
- Canfield, D.E., Thamdrup, B., 1994. The production of ^{34}S -depleted sulfide during bacterial disproportionation of elemental sulfur. *Science* 266, 1973–1975.
- Cline, J.D., 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnology and Oceanography* 14, 454–458.
- Cypionka, H., Smock, A., Böttcher, M.E., 1998. A combined pathway of sulfur compound disproportionation in *Desulfovibrio desulfuricans*. *FEMS Microbiology Letters* 166, 181–186.
- DeFlaun, M.F., Mayer, M., 1983. Relationships between bacteria and grain surfaces in intertidal sediments. *Limnology and Oceanography* 28, 873–881.
- Delafontaine, M.T., Bartholomä, A., Flemming, B.W., Kurmis, R., 1996. Volume-specific dry POC mass in surficial intertidal sediments: a comparison between biogenic muds and adjacent sand flats. *Senckenbergiana Maritima* 26, 167–178.
- Dellwig, O., Watermann, F., Brumsack, H.-J., Gerdes, G., 1999. High-resolution reconstruction of a Holocene coastal sequence (NW Germany) using inorganic geochemical data and diatom inventories. *Estuarine, Coastal and Shelf Science* 48, 617–633.
- Engelhardt, H.-J., Brockamp, O., 1995. Biodegradation of clay minerals: laboratory experiments and results from Wadden Sea tidal sediments. *Sedimentology* 42, 947–955.
- Epstein, S.S., 1995. Simultaneous enumeration of protozoa and micrometazoa from marine sandy sediments. *Aquatic Microbial Ecology* 9, 219–227.

- Flehmig, W., Kurze, R., 1973. Die quantitative infrarotspektroskopische Phasenanalyse von Mineralgemengen. *Neues Jahrbuch Mineralogische Abhandlungen* 119, 101–112.
- Fossing, H., Jørgensen, B.B., 1989. Measurement of bacterial sulfate reduction in sediments: evaluation of a single-step chromium reduction method. *Biogeochemistry* 8, 205–222.
- Hall, P.O.J., Aller, R.C., 1992. Rapid, small-volume, flow-injection analysis for ΣCO_2 and NH_4^+ in marine and freshwaters. *Limnology and Oceanography* 37, 1113–1119.
- Hartman, M., Nielsen, H., 1969. $\delta^{34}\text{S}$ -Werte in rezenten Meeressedimenten und ihre Deutung am Beispiel einiger Sedimentprofile aus der westlichen Ostsee. *Geologische Rundschau* 58, 621–655.
- Irion, G., 1994. Morphological, sedimentological and historical evolution of Jade Bay, southern North Sea. *Senckenbergiana Maritima* 24, 171–186.
- Jørgensen, B.B., 1982. Mineralization of organic matter in the sea bed — the role of sulphate reduction. *Nature* 296, 643–645.
- Jørgensen, B.B., 1989. Sulfate reduction in marine sediments from the Baltic Sea–North Sea transition. *Ophelia* 31, 1–15.
- Kaplan, I.R., Rittenberg, S.C., 1964. Microbiological fractionation of sulphur isotopes. *Journal of General Microbiology* 34, 195–212.
- Keil, R.G., Martlucan, D.B., Prahl, F.G., Hedges, J.I., 1994. Sorptive preservation of labile organic matter in marine sediments. *Nature* 370, 549–552.
- Kristensen, E., Jensen, M.H., Jensen, K.M., 1998. Sulfur dynamics in sediments of Königshafen. In: Gätje, C., Reise, K. (Eds.), *Ökosystem Wattenmeer, Austausch-, Transport- und Stoffumwandlungsprozesse*. Springer, Berlin, pp. 233–256.
- Kühl, M., Steuckart, C., Eickert, G., Jeroschewski, P., 1998. A H_2S microsensor for profiling biofilms and sediments: application in an acidic lake sediment. *Aquatic Microbial Ecology* 15, 201–209.
- Li, Y.H., Gregory, S., 1974. Diffusion of ions in sea water and in deep-sea sediments. *Geochimica et Cosmochimica Acta* 38, 703–714.
- Llobet-Brossa, E., Rosselló-Mora, R., Amann, R., 1998. Microbial community composition of Wadden sea sediments as revealed by fluorescence in situ hybridization. *Applied Environmental Microbiology* 64, 2691–2696.
- Lorenzen, J., Glud, R.N., Revsbech, N.P., 1995. Impact of microsensor-caused changes in diffusive boundary layer thickness on O_2 profiles and photosynthetic rates in benthic communities of microorganisms. *Marine Ecology Progress Series* 119, 237–241.
- Mayer, L.M., 1994. Relationships between mineral surfaces and organic carbon concentrations in soils and sediments. *Chemical Geology* 114, 347–363.
- Moelsund, L., Thamdrup, B., Jørgensen, B.B., 1994. Sulfur and iron cycling in a coastal sediment: radiotracer studies and seasonal dynamics. *Biogeochemistry* 27, 129–152.
- Nakai, N., Jensen, M.I., 1964. The kinetic isotope effect in the bacterial metabolism and oxidation of sulphur. *Geochimica et Cosmochimica Acta* 28, 1893–1911.
- Ott, J.A., Novak, R., Schiemer, F., Hentschel, U., Nebelsick, M., Polz, M., 1991. Tackling the sulfide gradient: a novel strategy involving marine nematodes and chemoautotrophic ectosymbionts. *Marine Ecology* 12, 261–279.
- Revsbech, N.P., 1989. An oxygen microelectrode with a guard cathode. *Limnology and Oceanography* 34, 474–478.
- Sageman, J., Skowronek, F., Dahmke, A., Schulz, H.D., 1996. Pore-water response on seasonal environmental changes in intertidal sediments of the Weser Estuary, Germany. *Environmental Geology* 27, 362–369.
- Sahm, K., MacGregor, B.J., Jørgensen, B.B., Stahl, D.A., 1999. Sulphate reduction and vertical distribution of sulphate-reducing bacteria quantified by rRNA slot-blot hybridization in a coastal marine sediment. *Environmental Microbiology* 1, 65–74.
- Salomons, W., Mook, W.G., 1981. Field observations of the isotopic composition of particulate organic carbon in the southern North Sea and adjacent estuaries. *Marine Geology* 41, M11–M20.
- Schimmelmann, A., Kastner, M., 1993. Evolutionary changes over the last 1000 years of reduced sulfur phases and organic carbon in varved sediments of the Santa Barbara Basin, California. *Geochimica et Cosmochimica Acta* 57, 67–74.

- Snaidr, J., Amann, R., Huber, I., Ludwig, W., Schleifer, K.-H., 1997. Phylogenetic analysis and in situ identification of bacteria in activated sludge. *Applied Environmental Microbiology* 63, 2884–2896.
- Sørensen, J., Jørgensen, B.B., 1987. Early diagenesis in sediments from Danish coastal waters: microbial activity and Mn–Fe–S geochemistry. *Geochimica et Cosmochimica Acta* 51, 1583–1590.
- Sündermann, J., 1994. *Circulation and Contaminant Fluxes in the North Sea*. Springer, Berlin.
- Thamdrup, B., Fossing, H., Jørgensen, B.B., 1994. Manganese, iron, and sulfur cycling in a coastal marine sediment Aarhus Bay, Denmark. *Geochimica et Cosmochimica Acta* 58, 5115–5129.
- Trudinger, P.A., 1992. Bacterial sulfate reduction: current status and possible origin. In: Schidlowski, M., Golubic, S., Kimberley, M.M., McKirdy, D.M., Trudinger, P.A. (Eds.), *Early Organic Evolution*. Springer, Berlin, pp. 367–377.
- Vosjan, J.H., 1974. Sulphate in water and sediment of the Dutch Wadden Sea. *Netherlands Journal of Sea Research* 8, 208–213.
- Wedepohl, K.-H., 1971. Environmental influences on the chemical composition of shales and clays. *Physics and Chemistry of the Earth* 8, 305–333.
- Westrich, J.T., Berner, R.A., 1988. The effect of temperature on rates of sulphate reduction in marine sediments. *Geomicrobiological Journal* 6, 99–117.