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Activity and distribution of bacterial populations in Middle Atlantic Bight shelf sands

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Abstract

Spatiotemporal variation and metabolic activity of the microbial community were studied in coarse-grained Middle Atlantic Bight shelf sediments in relation to pools of dissolved and particulate carbon. Algal cells were present 8–11 cm into the sediment, probably due to hydrodynamically induced pore water flushing and sediment mixing. The coarse-grained (>70 μm) fraction of the sediment held the major share (61–98%) of benthic bacteria. Bacterial and algal cell abundances, exoenzymatic activity, and [DOC] generally showed higher values in May/July 2001 than in August/December 2000. Carbohydrates and proteins were hydrolyzed at potential rates of 1–12 nmol cm^{-3} h^{-1} (β-glucosidase) and 3–70 nmol cm^{-3} h^{-1} (aminopeptidase), respectively. Fluorescence in situ hybridization analyses of the benthic microbes assigned 45–56% of DAPI-stained cells to Eubacteria and less than 2% to Eukarya. The prokaryotic community was dominated by planctomycetes and members of the Cytophaga/Flavobacterium cluster. Near the sediment surface, iodonitrotetrazolium violet reducing cells, that are considered actively respiring, amounted to 15–29% of total bacteria. Despite a low organic content (particulate organic carbon <0.03%) and relatively low bacterial abundances ($<10^9$ cm^{-3}), the Middle Atlantic Bight shelf sediments showed organic matter turnover rates that are comparable to those found in organic-rich finer-grained deposits. Our findings suggest a high biocatalytic filtration activity in these coarse permeable sediments.

Keywords: Carbon mineralization; Fluorescence in situ hybridization; Marine sediment; Variability

1. Introduction

Amid the physical, biological and chemical parameters that define microbial niches in marine sediments, hydrodynamic forces play a dominant role in shallow nearshore environments [1–3]. On many shelves, strong bottom currents, driven by wind, waves and tides cause sediment transport [4,5], and benthic organisms living in such environments have to withstand the ensuing mechanical stress. Frequent resuspension and deposition cycles [6] winnow fine or less dense material from the sediment and force small organisms (including bacteria) to adhere firmly to the heavy mineral grains or to move deeper into the sediment. The sand beds resulting from the frequent winnowing are characterized by open pore space that may host an abundant interstitial fauna that can significantly affect the sedimentary bacterial population through grazing [7,8].

In contrast to fine-grained coastal sediments, where molecular diffusion limits aerobic and suboxic bacterial metabolism to a thin surface layer, the high permeability of sands allows for pore water flows that are driven by convection or advection [9–18]. These flows can reach several decimeters below the sediment–water interface [17], carry dissolved oxidants [15] and particulate organic matter (POM), and thereby may support aerobic and suboxic metabolism in deeper layers. Thus, living conditions in sandy sediments are not only shaped by the purely physical impact of hydrodynamic forces, but also by biogeochemical processes that are consequences of pore water and particle movement.

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Heterogeneous fields of advective or convective pore water flow in the porous sediment entail a complex spatial and temporal structure of the biogeochemical zonation in permeable sand beds. Changes in the flow field or infaunal activity make microhabitats near the dynamic sediment surface highly variable [19], and many bacteria must temporarily endure adverse conditions or rapidly follow their optimal niche. The partitioning of microbial cells between solid surfaces and the pore fluid in permeable sediments is considered to be controlled by the physical and chemical properties of these compartments [20,21], by morphology and physiology of the bacterial cells [20–22], by hydrogeological properties of the sediment [22,23], and by the flow field. Fluid motion makes bacteria collide not only with grain surfaces, but also with POM or with protozoan predators, thus influencing the microbial food web [24]. Advection can also increase uptake of dissolved organic matter by bacteria attached to particles and, thus, stimulate their metabolic activity [25,26].

The lower specific surface area, lower organic content, or higher predation pressure in coarse-grained sediments may be major reasons for bacterial cells being less abundant and phylogenetically different from those in fine-grained sediments. However, one previous fluorescence in situ hybridization (FISH) study found the microbial community composition in permeable nearshore sand to be similar to that in a nearby cohesive muddy sediment, with representatives of the Cytophaga/Flavobacterium cluster being most abundant in both sediment types [27]; this phylogenetic group comprises mostly aerobic, Gram-negative bacteria that can degrade complex macromolecules. Despite oxic conditions, sulfate reducing Proteobacteria were present in the uppermost layer of these two sediments [27] as well as in the oxic zone of several other marine sediments [28]. These results indicate that the effective transport mechanisms in coastal environments (advection, convection, bioturbation, physical mixing) may often homogenize microbial populations that otherwise would separate between oxygenated and anoxic zones.

In the context of hydrodynamical and biogeochemical peculiarities of permeable marine sediments, this ecological study focused on the microbial populations of a subtidal shelf sand, sampled during four hydrodynamically different time periods and seasons. Applying both biogeochemical and molecular methods, we set out to investigate links between organic carbon, bacterial abundance, distribution, metabolic activity, and community structure.

2. Materials and methods

Sandy sediment cores taken by SCUBA divers were sliced to provide information on seasonal and depth-related variation. Subsamples were separated into pore water, and fine-grained and coarse-grained fractions, and the distribution of cells and organic material between these compartments was determined. We measured the total abundance of benthic microbes and the proportion of active cells, as inferred from the presence of ribosomes and electron transport systems. To investigate interactions between bacteriobenthos and their biogeochemical environment, we quantified organic substrates (algal cells, particulate organic carbon (POC)), decomposition intermediates (dissolved organic carbon (DOC)) and end products (dissolved inorganic carbon (DIC)), and the potential rates of exoenzymatic biopolymer hydrolyses. In select samples, the microbial community structure was analyzed by FISH. Our set of hybridization probes was assembled with the objective to cover the major groups of fermenting and respiring bacteria that had been previously found in mesophilic marine sediments. Based on the currently known rRNA sequences, all existing probes lack satisfactory specificity for the δ subgroup of Proteobacteria or the sulfate reducing species therein. We therefore targeted this potentially important metabolic group by applying a highly specific probe for one of its largest genera, *Desulfovibrio*.

2.1. Study site

The sampling site (39°27′N, 074°14′W) was on the wide, sandy shelf of the Middle Atlantic Bight offshore of Tuckerton, NJ, USA. It was located within a region that contains the Long-term Ecosystem Observatory at ca. 15 m water depth (LEO-15; map e.g. in [29]) and was sampled in August 00, December 00, May 01, and July 01. Tidal amplitudes along the Middle Atlantic Bight coast are about 0.5 m, and mean currents on the inner shelf are generally directed SW at speeds of 0.1–0.2 m s^{-1} [30]. Our study site can be affected by Mullica River and occasionally Hudson River outflow [31]. Bottom shear stresses are influenced by both tidal currents and surface gravity waves, as well as occasional hurricane force storms; during non-storm periods, peak sediment entrainment and transport are linked to the tidal cycle [30]. In the LEO-15 region, the water column is generally isothermal in winter, but can be highly stratified in summer [31]. Episodic upwelling of offshore bottom waters into surface layers can last for days or weeks and lead to phytoplankton blooms [32].

During the study period, bottom water temperatures ranged between 1°C and 23°C (IMCS; http://marine.rutgers.edu/rodan/). As deduced from satellite maps of sea surface temperature and ocean color (IMCS; http://marine.rutgers.edu/cool/newevery.leo.html) as well as casts of CTD-fluorometer and rosette sampling, there was an upwelling event in mid-July 2001, and the water was rich in phytoplankton during the May 01 and July 01 cruises.

2.2. Current and wave measurements

On all cruises, we deployed a tripod for additional sea-

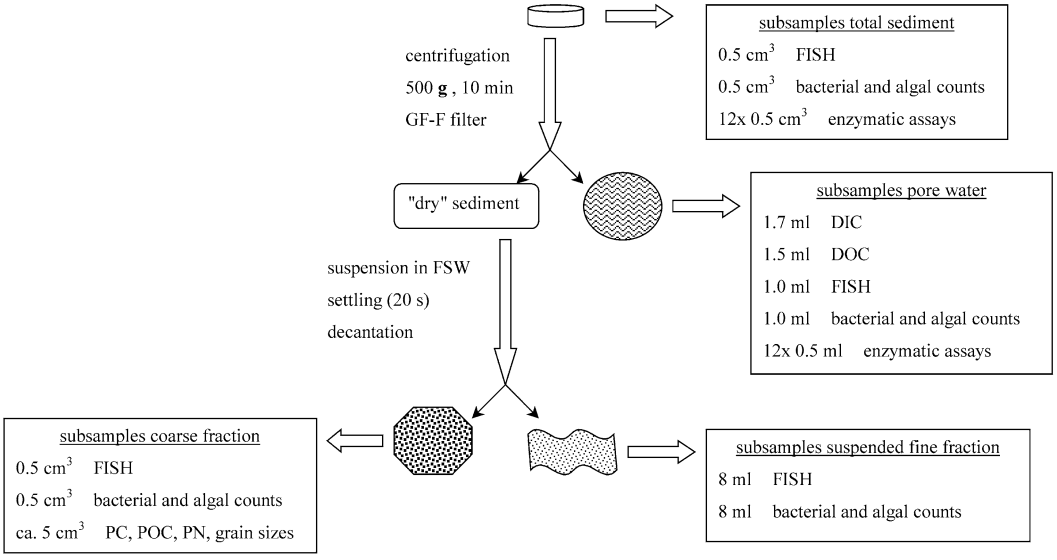

Fig. 1. Schematic outline of the subsampling and separation of sediment fractions.

floor experiments [33] that carried an acoustic Doppler 3D current meter with a temperature and a pressure sensor (Nortek Aquadopp) at a fixed height of 1 m above the bottom. The transducers faced outwards from the tripod frame, and a blanking distance of 35 cm was used to minimize flow interference from the frame. Data were collected at a frequency of 1 Hz. The resultant of the downstream and cross-stream velocities was calculated and is reported as horizontal current speed. Wave troughs and crests were determined from the pressure data (sensor resolution: 1 mm), and individual wave heights were calculated as the average of the differences between preceding and following troughs and the crest.

2.3. Sediment sampling

On 12 August 00, 08 December 00, 30 May 01, and 24 July 01, eight sediment cores (3.6 cm i.d., 15 cm long) each were retrieved by SCUBA divers. After collecting the overlying water, the cores were sectioned onboard ship at depth intervals of 0.5 cm (down to 3 cm), 1.0 cm (down to 6 cm), and 2.0 cm (down to 12 cm). In each depth, the slices of all parallel cores were combined in order to obtain a sufficient sample volume.

2.4. Separation of sediment fractions

To examine total sediment, its pore water, coarse fraction (effective diameter >70 μm), and fine fraction (effective diameter <70 μm) separately, we combined equivalent (by depth) slices of eight parallel cores and treated them as outlined in Fig. 1. Briefly, after putting aside 'total sediment' subsamples, we obtained pore water from the remaining sediment by centrifugation ($500\times g$, 10 min) through GF-F filters. The centrifuge tubes were fabricated according to [34], with minor changes to prevent leaking. 5 cm^3 of sediment was carefully suspended in filtered (pore size 0.2 μm) local sea water (FSW, 3 ml per cm^3 of sediment), and the coarse particles were allowed to settle for 20 s. After decanting the supernatant, the settled sediment was resuspended and separated from its supernatant twice more. The decanted suspensions contained the fine fraction, and the settled remainder was termed 'coarse fraction'. Subsampling of the suspensions and the coarse fraction is described in Fig. 1.

2.5. Pore water DIC and DOC

1.7 ml of pore water (DIC subsample) was stored in gas-tight glass vials containing 100 μl of saturated $HgCl_2$ solution, and 1.5 ml of pore water (DOC subsample) was stored in Eppendorf tubes at −20°C until analysis. DIC was measured according to [35] using a flow-injection system. We used freshly prepared $NaHCO_3$ solutions for calibration. The detection limit was 0.05 mM, and the analytical precision was 0.01 mM.

The DOC subsample was analyzed for total and inorganic carbon (DC, DIC) in a Shimadzu TOC-5050A autoanalyzer by combustion (680°C) and acidification (H_3PO_4), respectively, followed by infrared detection of the produced gases. DOC concentrations were calculated as [DOC] = [DC]−[DIC]. As a consequence of the preservation method, these DIC concentrations were not the original ones, i.e. invalid as a parallel set of [DIC] data. We used freshly prepared solutions of glycolate, phthalate, and $NaHCO_3/Na_2CO_3$ for calibration. The detection limit was 0.042 mM, and results were reproducible within ±2%.

2.6. Particulate carbon and nitrogen (PC and PN)

A freeze-dried subsample of the coarse fraction was homogenized. PC and PN content were measured using a

Fisons NA1500N elemental analyzer with sulfanilamide as the calibration standard. For POC determination, samples were pre-treated with 2.5 N HCl, washed twice with distilled water, dried and re-weighed. The POC contents given refer to original dry weight. The detection limit was 0.01% (dry weight), and results were reproducible within ± 2%.

2.7. Bacterial and algal counts

2.7.1. Total/coarse

In a sterile glass tube, 0.5 cm^3 of sediment was mixed with 1.5 ml of FSW and 20 μl of iodonitrotetrazolium violet (INT) solution (final concentration: 0.02%). Incubation for 30 min in the dark was followed by addition of formalin (final concentration: 3%) and storage in the dark at 4°C until analysis [36]. To dislodge cells from the sand grains, the samples were subjected to ultrasonic treatment (30% pulsed, Bandelin M72 probe) for 150 s and washed with 7×1.5 ml of distilled water. This protocol combined suggestions of [37] and results of tests run in our laboratory to evaluate procedural effects on cell yield.

2.7.2. Pore water/suspended fine fraction

In a sterile glass tube, the pore water (or suspension) subsample was incubated with INT (final concentration: 0.02%) for 30 min in the dark, followed by addition of formalin (final concentration: 2.7% and 3%, respectively). All samples were stored dark at 4°C until analysis.

2.7.3. Bacterial cell counts

0.5–2.0 ml of cell suspension (resulting from grain size fractionation or ultrasonic treatment) was stained with DAPI and concentrated on a polycarbonate membrane filter (0.2 μm pore size). Epifluorescent and bright field illumination (Zeiss Axioskop) were used to count cells stained with DAPI and holding formazan deposits, respectively [38,39]. With a magnification of 1300×, cell numbers were determined from two parallel filters per sample, in 12 randomly chosen counting grids each.

2.7.4. Algal cell counts

4–15 ml of cell suspension was concentrated on a polycarbonate membrane filter (0.8 μm pore size) to view chloroplasts under epifluorescent illumination (Zeiss Axioskop). Using a magnification of 400×, cell numbers were determined in 24 randomly chosen counting grids.

2.8. Enzymatic assays: β-glucosidase and aminopeptidase

The potential activities of β-glucosidase and aminopeptidase were determined according to [40,41]. Briefly, the subsamples were incubated with 4-methylumbelliferyl-β-D-glucoside and L-leucine-4-methyl-7-coumarinylamide, respectively, at approximate in situ temperature. Each assay was run for three different incubation times, that ranged between 1 h and 5 h, and the enzymatic reactions were stopped by addition of NaOH (β-glucosidase) and HCl (peptidase), respectively. Subsequent centrifugation (28 000×*g*, 5 min) enforced particle settling, and the supernatant was stored in the dark at −20°C until analysis. The concentration of the fluorochromic reaction products was quantified using a Hitachi F-2000 spectrofluorometer.

2.9. FISH

2.9.1. Sample fixation

In a 2-ml Eppendorf tube, 0.5 cm^3 of sediment was fixed with formalin (final concentration: 3.7%) for 1–24 h. After centrifugation (15 000×*g*, 5 min), the supernatant was removed, and the pellet was washed with 1.5 ml phosphate-buffered saline (PBS) buffer (pH 7.3) twice. Then the pellet was preserved by addition of 0.75 ml of PBS buffer and 0.75 ml of ethanol, and stored at −20°C until analysis. Cell suspensions were prepared as described for bacterial counts and concentrated on a polycarbonate membrane filter (0.2 μm pore size). The filter was washed with distilled water, allowed to air-dry, and stored in a petri dish at −20°C until hybridization.

2.9.2. Whole cell hybridization

Filter sections were incubated with Cy3-labeled oligonucleotide probes in an equilibrated humidity chamber at 46°C for 2 h [42,43]. Information on the probes, their targets, and the stringency conditions we applied is given

Table 1
Probe and competitor oligonucleotides used for FISH, stringency conditions applied, target organisms and sites

Probe	Target organisms	Target rRNA and site	Formamide (%)	Competitor	References
EUB I–III	domain Bacteria	16S (338–355)	35	–	[44]
EUK 516	domain Eukarya	18S (502–516)	20	–	[27]
NON 338	negative control	16S (338–355)	35	–	[45]
CF 319a	Cytophaga/Flavobacterium cluster	16S (319–336)	35	–	[27,45]
PLA 886	Planctomycetes, some Eukarya	16S (886–904)	35	cPLA 886	[45,46]
ALF 968	α subclass of Proteobacteria	16S (968–986)	35	–	[47]
BET 42a	β subclass of Proteobacteria	23S (1027–1043)	35	cBET 42a	[27,42]
GAM 42a	γ subclass of Proteobacteria	23S (1027–1043)	35	cGAM 42a	[27,42,45]
DSV 698	*Desulfovibrio* spp.	16S (698–717)	35	–	[48]

Table 2
Overview of bottom water temperatures (T), current speeds (v), and wave heights (h) spatially and temporally close to sampling

Sampling date		12 August 00	08 December 00	30 May 01	24 July 01
T (tripod)	°C	17.7	6.0	14.0	20.7
T (node)	°C	18.3 ●	5.9 ●	no data	21.6 ↑
v (tripod)	m s^{-1}	0.24 ± 0.13	0.10 ± 0.05	0.12 ± 0.04	0.22 ± 0.05
v (node)	m s^{-1}	1.14 ↑	0.76 ↓	no data	no data
h (tripod)	m	0.47 ± 0.34	0.16 ± 0.09	0.15 ± 0.10	0.13 ± 0.09

Data from the tripod instruments represent averages over the measurement period (August 00: 39 min, December 00: 119 min, May 01: 125 min, July 01: 20 min). Node data (averaged over 1 h) were obtained from continuous acquisition by LEO-15 instruments at Node B (http://marine.rutgers.edu/rodan/). Note that Node B current speeds were measured between 5.6 m and 7.6 m above the seafloor, whereas tripod measurements were at 1 m above the seafloor. Trends within the last days to weeks before sampling are indicated as ● (no major changes), ↑ (increase), and ↓ (decrease).

in Table 1 (and references therein). Where indicated, equimolar amounts of unlabeled competitor oligonucleotides were used to reduce single-mismatch binding [42]. To avoid unspecific binding of probes to detritus particles and pollutants, 2% blocking reagent was added to the hybridization buffer [49]. The hybridized filter sectors were counter-stained with DAPI, rinsed briefly with distilled water, air-dried in the dark, and mounted on glass slides in Citifluor™/Vectashield® (3:1).

2.9.3. Microscopic evaluation

The slides were examined by epifluorescence microscopy (Zeiss Axioskop) using a magnification of 1300×. Cells showing probe-conferred fluorescence and DAPI-stained cells in the same field of vision were enumerated in 12 or 24 randomly chosen counting grids per filter, corresponding to 1300–6200 DAPI-stained cells. The number of NON 338 positive cells was subtracted from the signal obtained with the other probes. The detection limit was 1–2% of DAPI-stained cells.

2.10. Statistical tests

Correlations among variables were tested for significance according to [50]. To compare the number of probe-detected cells (Fig. 6) to the number of false positives, we applied a t-test or a Fisher/Behrens test, depending on the result of an F-pretest for possible differences in variance [50].

3. Results

3.1. Sampling situations

Temperature, bottom current speed, and wave conditions at the study site before and during sampling are given in Table 2 according to the available information from the LEO-15 node and our tripod measurements. The situation on 12 August 00 can be characterized as the onset of the late summer and autumn storm period, whereas 08 December 00 was during the early winter cooling. On 30 May 01, we sampled during spring bloom conditions, and 24 July 01 was during a mid-summer phytoplankton bloom that developed in response to an upwelling event.

3.2. Abundance and depth distribution

The depth profiles of bacterial cell abundance are shown in Fig. 2. Except for an outlier at 3–4 cm depth, bacterial abundances in December 00 hardly varied below 2 cm depth and were slightly elevated closer to the surface. In May 01 and July 01, higher cell numbers were recorded throughout the sediment column and decreased towards greater depths. Maximum abundance of bacteria was not observed at the sediment surface, but at 2–3 cm (May) or 0.5–1.5 cm (July) depth. The profile in August 00 exhibited intermediate abundances.

3.3. Metabolic activity

To evaluate the activity of benthic bacteria, we used three indicators:

1. the pool sizes of organic substrates (algae, TOC, TN), decomposition intermediates (DOC) and end products (DIC)
2. the potential rates of extracellular hydrolysis of biopolymers, like polypeptides (aminopeptidase assay) and certain polysaccharides (β-glucosidase assay)
3. the physiological state of the cells as reflected by their ribosome content (FISH detectability) and activity of electron transport systems (INT reduction).

3.3.1. Pool sizes along decomposition pathway

The sandy sediment at our study site contained only small amounts of organic material. Its total nitrogen content was below 0.010% in all samples, and POC ranged between 0.015% and 0.030%, tending to higher contents near the sediment surface in summer and to lower contents deeper in the sediment and in winter (data not shown). We roughly approximated bacterial and algal cells in our samples as spheres of 1 μm and 10 μm diameter, respectively, and converted their abundances (Figs. 2 and 3), to cellular organic carbon using empiric relationships between biovolumes and cellular carbon given in the literature [51–

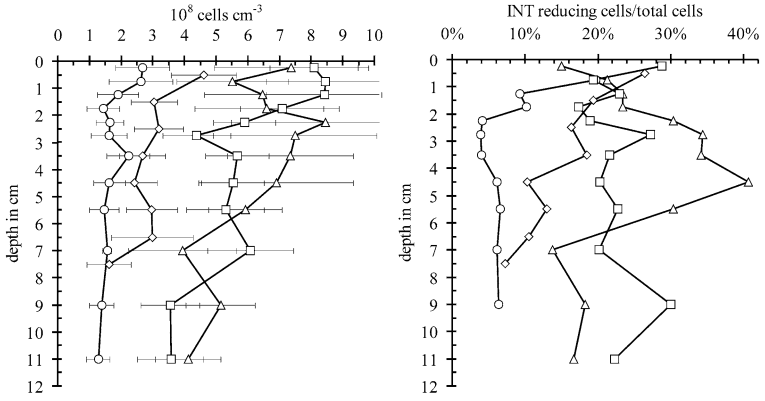

Fig. 2. Depth distribution of bacterial cells in the 'total sediment' samples of August 00 (◇), December 00 (○), May 01 (△), and July 01 (□). Left panel: total abundance; error bars indicate ±1 S.D. within 24–36 replicate enumerations. Right panel: percentage of INT reducing cells.

54]. The calculations resulted in estimates of 4–106 μg cm^{-3} algal carbon and 7–89 μg cm^{-3} bacterial carbon, corresponding to less than 0.01% of the POC in the sediment being cellular carbon.

The depth profiles of algal cells, DOC and DIC are shown in Fig. 3. The December 00 situation was characterized by relatively uniform pore water DIC concentrations that slightly exceeded bottom water values. [DOC] was low, except for slightly elevated concentrations in 0–1 cm and 6–8 cm depth (Fig. 3). There were no significant correlations between [DIC], [DOC], POC content, and cell numbers of algae, bacteria and INT reducing bacteria in these winter samples.

The profiles recorded in May 01 and July 01 were very different: algal cells were enriched in the upper 3 cm, and the pore water was rich in DOC (Fig. 3). Maximum concentrations of both DOC and DIC were found in 1.5 cm depth in May, but at the sediment surface in July. There were numerous significant correlations between [DIC], [DOC], POC content and algal cell numbers in May 01 and July 01; correlations to the abundances of total and INT reducing bacteria, however, were only detected in July (Fig. 4).

3.3.2. *Exoenzymatic hydrolysis of biopolymers*

At each sampling date, hydrolysis catalyzed by aminopeptidase occurred at rates three to nine times as high as rates of β-glucosidic hydrolysis (Fig. 5). Exoenzymatic turnover rates were low but slightly increasing towards the sediment surface in August 00, low and nearly depth-invariant in December 00, and high in May and July 01, especially in the upper sediment layers. This variability of turnover rates suggests the activity of extracellular hydrolases depends on the presence of organic matter, as demonstrated by the parallel time course of algal cell numbers in the sediment (Fig. 3) and by correlation to the profiles of algae and organic carbon in the summer months (Fig. 4). Except for December 00, benthic bacterial abundances were correlated with the turnover rates of β-glucosidase, but not of peptidase, and the microbial reduction of INT appeared related to neither of the exoenzymes assayed (Fig. 4).

Assuming that the POC pool exclusively comprised substrates of β-glucosidase, its depolymerization time would be in the order of weeks to months. If all POC were polyleucin, it would be converted to DOC within days to weeks. These estimations over simplify the variety of degradation processes and pools involved [55], but do roughly indicate the time scales of POC turnover.

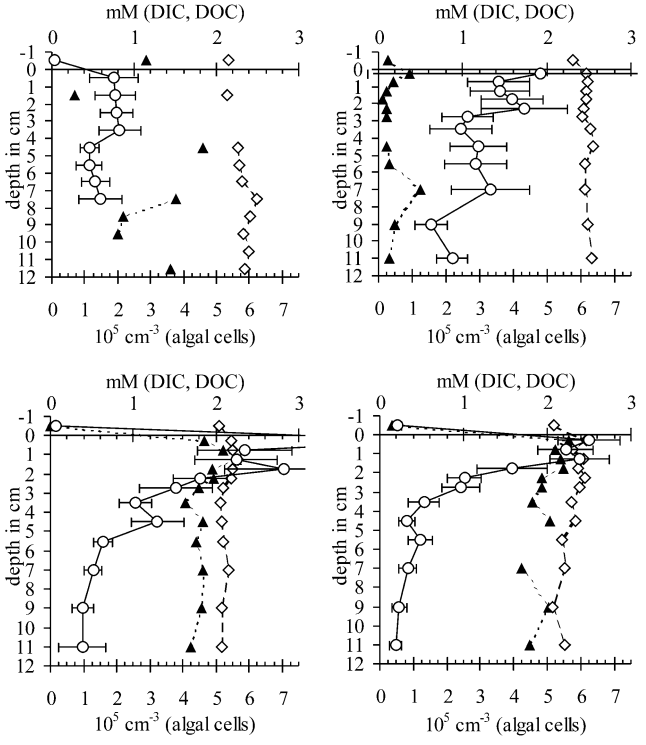

Fig. 3. Algal cell numbers (○) and concentrations of DOC (▲) and DIC (◇) in the sediment and overlying bottom water. Error bars indicate ±1 S.D. within 24 replicate enumerations of algal cells. Upper left: August 00, upper right: December 00, lower left: May 01, lower right: July 01.

3.3.3. Physiological state

Being aware of limitations of the method (see Section 4), we used INT reduction as an indicator for cells with active electron transport systems. The relative abundance of bacteria capable of reducing INT (Fig. 2) temporally paralleled the concentrations of POC (profiles not shown) and DOC (Fig. 3) and tended to decrease with sediment depth. In August 00, the depth profile of INT reducing cells was shaped similar to the profiles recorded for algae (Fig. 3) and exoenzymatic activity (Fig. 5). In December 00, participation in INT reduction decreased considerably within the uppermost 2 cm. In May 01, the relative abundance of INT reducing cells showed a wide peak centered around 4–5 cm depth (Fig. 2), where there were also enrichments of algal cells and [DOC] (Fig. 3). Later in summer (July 01), we observed equally large shares of INT reducing bacteria down to 11 cm depth.

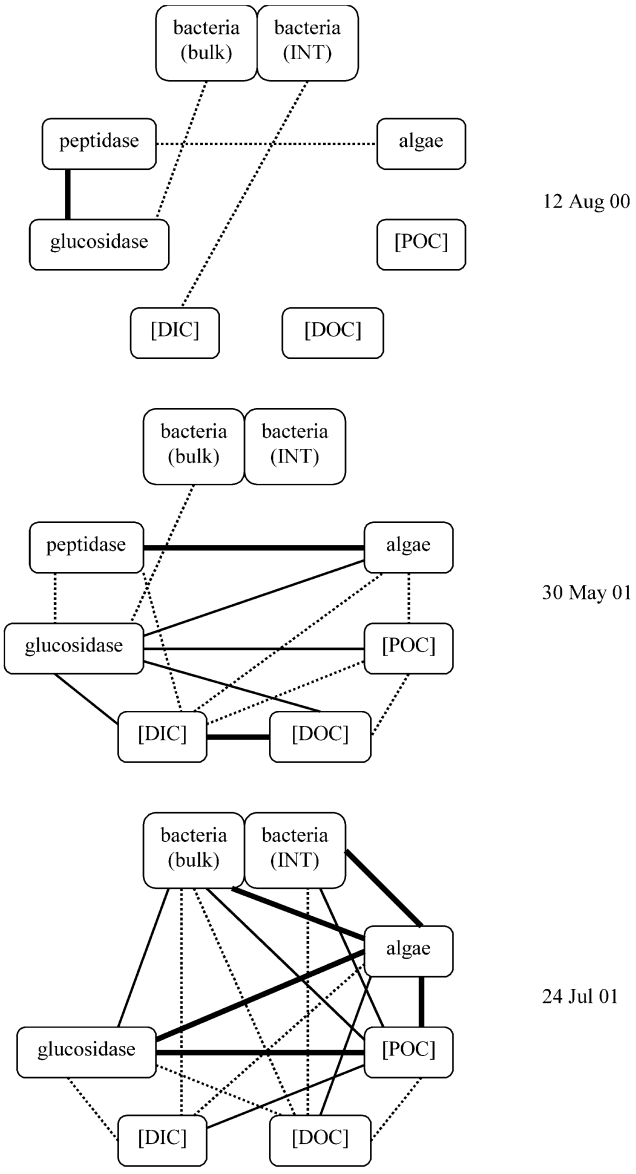

Fig. 4. Correlations between the abundances of bacteria, INT reducing bacteria and algal cells, sedimentary concentrations of POC, DOC, and DIC, and sedimentary rates of β-glucosidase and aminopeptidase activity. The latter was not measured in July 01. Levels of significance: 0.1% bold line, 1% fine line, 5% broken line.

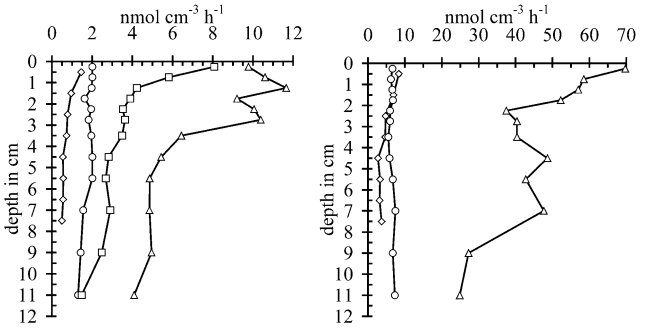

Fig. 5. Exoenzymatic activities in the 'total sediment' samples of August 00 (◇), December 00 (○), May 01 (△), and July 01 (□). Left panel: β-glucosidase, right panel: aminopeptidase.

In the sediment, 45–56% of DAPI-stained cells could be hybridized with the eubacterial probes EUB I–III (Fig. 6), with FISH detection revealing high cellular contents of 16S rRNA, related to metabolic activity in terms of ribosomal polypeptide synthesis. Within the limited set of samples hybridized, the relative abundance of active eubacterial cells showed no significant temporal changes or variation with sediment depth. The share of EUB I–III detected cells was lower in the planktonic microbial population of the bottom water than in the benthic population (Fig. 6).

3.4. Association with sediment fractions

The solid fraction of our permeable shelf sand consisted mainly of particles >70 μm (the 'coarse fraction'), whereas finer particles contributed less than 0.3% (dry weight) or 1 mg cm^{-3} (wet volume). The sediment was much richer in POC than in DOC, which constituted 2–3% of sedimentary organic carbon, without variation in depth nor between the sampling dates.

The pore water generally contained less than 0.2% of total bacteria in the same depth interval. The share of bacterial, INT reducing and algal cells associated with the fine fraction of the sediment is illustrated in Fig. 7. Fine/total ratios obtained in December 00 or at great depth are based on low absolute numbers (Figs. 2 and 3) and should, therefore, be interpreted cautiously. Association with the fine fraction was stronger in INT reducing than in bulk bacteria, indicating that cells attached to finer particles were metabolically more active than those attached to coarser grains. The shares of both bulk and INT reducing bacteria found in the fine fraction were higher in August 00 and December 00 than in May 01 and July 01 (Fig. 7) due to distinct temporal differences in 'total sediment' abundances (Fig. 2), but only minor ones in the fine fraction. Thus, temporal variation in bacterial cell numbers occurred mainly in the coarse fraction.

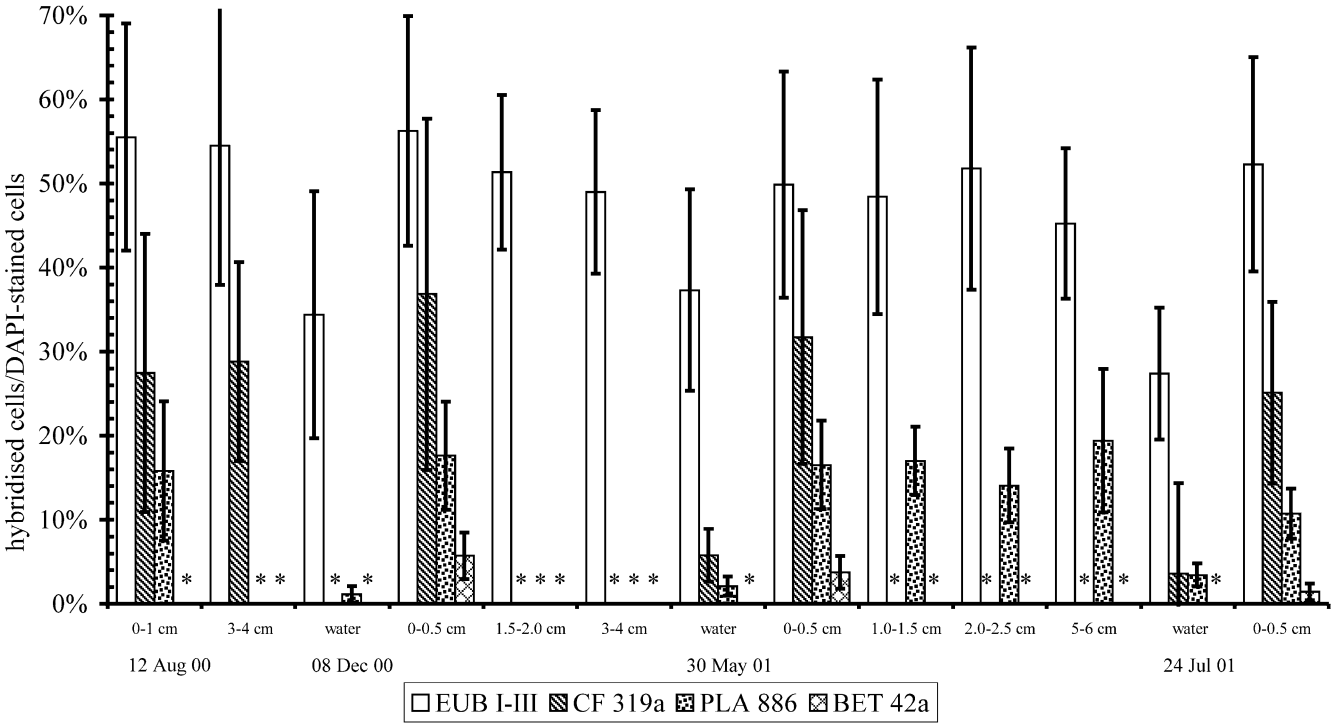

Fig. 6. Number of cells detected by the rRNA targeted oligonucleotide probes, given in % of DAPI-stained cells, false positives (NON 338) subtracted. Open bar: EUB I–III, hatched: CF 319a, stippled: PLA 886, diamond: BET 42a. Error bars indicate ±1 S.D. within 12–64 replicate enumerations. *: not assayed. Not shown: EUK 516: 1.9 ± 1.5% in August 00 (0–1 cm), below detection in December 00, May 01, July 01 (all 0–0.5 cm); ALF 968: below detection in August 00 (0–1 cm) and all May 01 and July 01 sediment samples; GAM 42a: 1.5 ± 1.1% in July 01 (0–0.5 cm), below detection in August 00 (0–1 cm), December 00 (0–0.5 cm) and all May 01 sediment samples; DSV 698: below detection in all May 01 sediment samples.

Except for the upper 3 cm in December 00, algae belonged to the fine fraction to a larger extent than the bulk of bacterial cells. In May 01, algal fine/total ratios were slightly elevated between 2.5 and 6 cm depth (Fig. 7), where also high levels of 'total sediment' INT reduction were observed (see above). In July 01, the fine fraction contained locally greater shares of algal, INT reducing and bulk bacterial cells at 1.5–4 cm depth (Fig. 7).

3.5. Community structure

The results of our FISH analyses are summarized in Fig. 6. Roughly half of the DAPI-stained cells could be assigned to the domain Bacteria and less than 2% to the domain Eukarya. Planctomycetes and species of the Cytophaga/Flavobacterium cluster dominated the prokaryotic microbial community in the sediment, but not in the overlying bottom water. The benthic community also comprised some β-Proteobacteria. With a single exception, we could not detect any Proteobacteria of the α- and γ-subclass (Fig. 6). Down to 6 cm depth, no *Desulfovibrio* sp. were found even in May 01, when DOC concentrations were highest. Within the limited set of samples studied and the limited phylogenetic resolution of the FISH probes we used, the composition of the benthic microbial community did not change significantly with seasons or sediment depth.

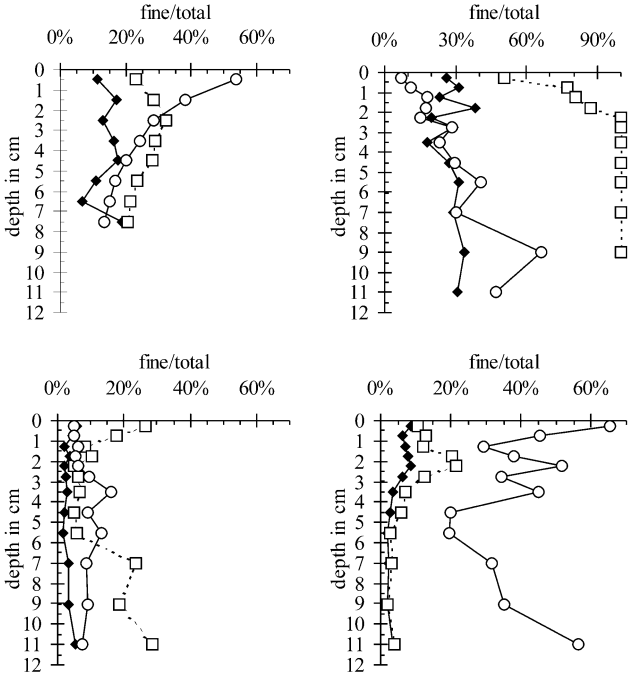

Fig. 7. Cells associated with the fine fraction compared to total sediment: bacteria (♦), INT reducing bacteria (□), algae (○). Upper left: August 00, upper right: December 00, lower left: May 01, lower right: July 01.

4. Discussion

4.1. Shelf sand – a physically dynamic habitat

The data we collected indicate possible adaptations of a benthic bacterial population to the hydrodynamic forces acting on a sandy shelf sediment in the Middle Atlantic Bight. Unlike most depth distributions of bacterial cells in sediments less affected by waves and bottom currents, two of our four profiles showed maximum abundances below the sediment surface (Fig. 2). We hypothesize a loss of cells from the most exposed upper layer due to hydrodynamical stress. This view, though corroborated by observations in an intertidal sandflat [56], lacks direct support by our restricted set of hydrodynamic data (Table 2) and the snapshot nature of our sampling.

Interstitial and interfacial water flows are common in permeable shallow water sediments [12–18]. Bacteria attached to sand grains should be less susceptible to removal and are therefore expected to dominate such sediments. Our finding that free-living cells comprised less than 0.2% of total bacteria in our shelf sand supports this supposition. Likewise, bacteria attached to particles of the fine fraction were of minor abundance, as the 'coarse fraction' was mass dominant, held the major share of the sedimentary bacteria (Fig. 7), and was responsible for the spatiotemporal variability of cell numbers. In spite of possible nutritional disadvantages, the less mobile microhabitat of coarser grains apparently can sustain a population of sand bacteria.

4.2. Sand bacteria and their biogeochemically dynamic habitat

In permeable sediments, physical and chemical interfaces move frequently, and benthic microbes experience highly variable biogeochemical conditions. The activity of extracellular hydrolases, considered rate-limiting in the microbial degradation of organic matter in waters and sediments [57–59], may be crucial in making optimal use of the intermittently supplied POM. The rates of biopolymer hydrolysis measured in LEO-15 sand were in the same range as those reported from sediments with relatively low organic content or comparable hydrodynamic regime [59–61]. In a coarse-grained Antarctic sediment exposed to strong bottom currents, the activity of aminopeptidase exceeded that of β-glucosidase only by a factor of about 10 [59], similar to measurements in fresh diatom aggregates [62] as well as in our shelf sand. Benthic microbes in nutrient-poor habitats may rely on high peptidase activity to obtain sufficient amounts of organic nitrogen, whereas ample supply of fresh POM implies less of a requirement for protein hydrolysis. The relatively weak preponderance of peptidase compared to β-glucosidase activity in our study suggests that the benthic community at LEO-15 is not severely limited by nitrogen. The seasonality of exoenzyme activity (Fig. 5) and correlations with algal material, POC and DOC (Fig. 4) favor the scenario of limitation by organic matter quantity rather than its nutritional quality. The overall low activity in August 00 may indicate that episodes of very strong bottom currents and wave action (Table 2) periodically remove extracellular enzymes and/or compounds that are involved in regulating their activity.

Evaluating the metabolic status of bacterial cells or populations is largely based on criteria like membrane integrity, cellular reducing potential, specific enzyme activity, or rRNA content [63]. Methodical biases include differences and interactions between natural and tracer substrates with respect to concentrations, uptake and transport rates, or substrate specificity. In LEO-15 sand, the relative size of the metabolically active subpopulation of benthic microbes, as inferred from INT reduction, was closely related to organic matter (and we suspect oxygen) supply to the sediment. Both the total abundance of bacterial cells and the proportion of INT reducing cells changed with time and sediment depth (Fig. 2), mainly in the coarse fraction. With the fine fraction holding relatively large shares of algal cells (Fig. 7), it can be assumed to represent the episodic input of suspended POM to the sediment community throughout the year. By contrast, the coarse fraction and associated microbes represent a matrix that receives these POM inputs and responds to intermittent supply. This view is supported by depth profiles of INT reduction (Fig. 2) that often paralleled those of algal cells, [DOC] or exoenzymatic activity. Reversible adsorption of organic material to mineral surfaces [64,65] may buffer its episodic influx towards microbial utilization. Additionally, oxygen supply plays a key role in sediments like the LEO-15 sand, because the stimulation of benthic mineralization by oxygen has been found strongest in coarse-grained, organic-poor sediments [66]. At all times sampled, current and wave impact (Table 2) on the permeable bottom facilitated advective flows that could enhance the influx of oxygen and organic compounds, and thus, intensify POM degradation (Reimers et al., in preparation).

Thus, pool sizes in the course of POM degradation, exoenzymatic turnover rates and the physiological state of cells paint a coherent picture of the microbial population of a permeable shelf sediment as being versatile with respect to the mechanical and biogeochemical dynamics of their habitat.

4.3. Bacterial community composition of a permeable subtidal sediment

The molecular analyses of cells from LEO-15 sand indicate a microbial community almost exclusively composed of planctomycetes and members of the Cytophaga/Flavobacterium cluster (Fig. 6).

Many cultured species of the Cytophaga/Flavobacterium group produce exoenzymes and aerobically degrade

a variety of high molecular mass organic compounds [67–70]. FISH studies proved them a dominant group in marine bacterioplankton [69,71,72] and highly abundant also in marine sediments [27,45]. Throughout our study at LEO-15, the Cytophaga/Flavobacterium cluster was dominating the benthic bacterial community, but occurred in much lower concentrations in bacterioplankton samples from the overlying water (Fig. 6). With the water column differing drastically from the sediment physically and chemically in many respects, differences in microbial community structure are not surprising. However, it is also reasonable to propose that the pore space of permeable sediments may hold a microbial subpopulation similar to that of the water column, since it is tightly coupled to the bottom water by interfacial and interstitial water flows. Investigations to test this hypothesis were beyond the scope of our present study.

Planctomycetes are a very widespread group of chemoheterotrophic bacteria typically degrading polymeric substances aerobically, but they have also been detected in anoxic zones of marine sediments [45]. Planctomycetes were also a major group in the benthic microbial community of the permeable sand we studied (Fig. 6). Together with the Cytophaga/Flavobacterium cluster, they formed a benthic community of bacteria likely to degrade biopolymers. Indeed, we measured considerable potential rates of carbohydrate and peptide hydrolysis in the LEO-15 sediment (Fig. 5). Although both POM supply (Fig. 3) and exoenzymatic activities (Fig. 5) varied markedly with depth and seasons, our set of FISH probes failed to detect corresponding changes in microbial community composition (Fig. 6). Adaptation to spatiotemporal dynamics of our sandy sediment could have been achieved by changes in cell number or activity that affected all bacterial groups equally. An alternate explanation is that the limited number of samples, relatively wide statistical variation, and the broad specificity of the probes we used were not sensitive to possible differences.

Phytoplankton blooms have been shown to be accompanied by pronounced changes in bacterioplankton abundance, productivity, exoenzymes, and community composition [32,73,74]. The sediment surface sample taken during the July 01 bloom situation was unique with respect to detectable numbers of γ-Proteobacteria (Fig. 6), a group with many copiotrophic members [75]. Moreover, the ratio between CF 319a and PLA 886 positive cells was 2.4 in July 01 compared to 1.7–2.1 in the other months (Fig. 6). According to ongoing studies by Llobet-Brossa et al. (personal communication), high CF:PLA ratios are frequently found in sediments rich in labile organic carbon. In our case, they might reflect a community response to the ample supply of fresh algal material.

The general dominance of planctomycetes and Cytophaga/Flavobacterium relatives, both preferably attaching to particles [68,70,71,76,77], agrees well with our finding that less than 0.2% of the sand bacteria were discovered free-living in the pore water. Proteobacteria, that are typically free-living [71], occurred in low abundances. Some isolated strains of planctomycetes are capable of oxidizing organic substrates via nitrate reduction [76], and members of the Cytophaga/Flavobacterium cluster have been shown to degrade monomeric organic compounds in coastal waters near our study site [69]. Hence, these groups could comprise both the hydrolyzing and the mineralizing subpopulation of microbes inhabiting LEO-15 sand, and some species might even be able to run both kinds of heterotrophic metabolism. Species that both ferment and mineralize, however, may fill the niche less successfully than a consortium of mineralization specialists, e.g. Proteobacteria, and fermentation specialists.

Considering the general oxic appearance of our cores and the penetration of oxygen deep into the permeable sand (Reimers et al., in preparation), sulfate reducers are not necessarily expected to be active, but have repeatedly been observed in high abundances, mostly *Desulfovibrio* sp., in oxic marine sediments [28]. We failed to detect them at LEO-15; due to the narrow specificity of the probe, however, we cannot exclude the presence of other sulfate reducing bacteria in this sediment. With the molecular methods applied to our sand still requiring thorough revision and improvement, we wish to have our FISH data viewed conservatively and interpreted with caution.

In conclusion, we have identified and quantified the major phylogenetic groups represented in the active microbial community of a permeable shelf sediment. This ecological study also gives evidence for the bacterial population of marine sands responding to the biogeochemical variability of their habitat. Hydrodynamical impact on the benthic community appeared probable, but remains to be proven by further investigations.

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References

[1] Shimeta, J., Starczak, V.R., Ashiru, O.M. and Zimmer, C.A. (2001) Influences of benthic boundary-layer flow on feeding rates of ciliates

and flagellates at the sediment–water interface. Limnol. Oceanogr. 46, 1709–1719.
[2] Eckman, J.E. (1985) Flow disruption by an animal tube mimic affects sediment bacterial colonization. J. Mar. Res. 43, 419–436.
[3] Berninger, U. and Huettel, M. (1996) The impact of oxygen flow dynamics in photosynthetically active sediments. Aquat. Microb. Ecol. 12, 291–302.
[4] Jenness, M.I. and Duineveld, G.C.A. (1985) Effects of tidal currents on chlorophyll a content of sandy sediments in the southern North Sea. Mar. Ecol. Progr. Ser. 21, 283–287.
[5] Dyer, K.R. (1986) Coastal and Estuarine Sediment Dynamics. John Wiley and Sons, Chichester.
[6] van Raaphorst, W., Malschaert, H. and van Haren, H. (1998) Tidal resuspension and deposition of particulate matter in the Oyster Grounds, North Sea. J. Mar. Res. 56, 257–291.
[7] Young, I.M., Griffiths, B.S., Robertson, W.M. and McNicol, J.W. (1998) Nematode (*Caenorhabditis elegans*) movement in sand as affected by particle size, moisture and the presence of bacteria (*Escherichia coli*). Eur. J. Soil Sci. 49, 237–241.
[8] Epstein, S.S. (1997) Microbial food webs in marine sediments. I. Trophic interactions and grazing rates in two tidal flat communities. Microb. Ecol. 34, 188–198.
[9] Rocha, C. (1998) Rhythmic ammonium regeneration and flushing in intertidal sediments of the Sado estuary. Limnol. Oceanogr. 43, 823–831.
[10] Golosov, S.D. and Ignatieva, N.V. (1999) Hydrothermodynamic features of mass exchange across the sediment–water interface in shallow lakes. Hydrobiology 409, 153–157.
[11] Rocha, C. (2000) Density-driven convection during flooding of warm, permeable intertidal sediments: the ecological importance of the convective turnover pump. J. Sea Res. 43, 1–14.
[12] Webb, J.E. and Theodor, J. (1968) Irrigation of submerged marine sands through wave action. Nature 220, 682–683.
[13] Thibodeaux, L.J. and Boyle, J.D. (1987) Bedform-generated convective transport in bottom sediment. Nature 325, 341–343.
[14] Forster, S., Huettel, M. and Ziebis, W. (1996) Impact of boundary layer flow velocity on oxygen utilization in coastal sediments. Mar. Ecol. Progr. Ser. 143, 173–185.
[15] Huettel, M., Ziebis, W., Forster, S. and Luther III, G.W. (1998) Advective transport affecting metal and nutrient distribution and interfacial fluxes in permeable sediments. Geochim. Cosmochim. Acta 62, 613–631.
[16] Pilditch, C.A., Emerson, C.W. and Grant, J. (1998) Effect of scallop shells and sediment grain size on phytoplankton flux to the bed. Cont. Shelf Res. 17, 1869–1885.
[17] Falter, J.L. and Sansone, F.J. (2000) Hydraulic control of pore water geochemistry within the oxic-suboxic zone of a permeable sediment. Limnol. Oceanogr. 45, 550–557.
[18] Huettel, M. and Rusch, A. (2000) Transport and degradation of phytoplankton in permeable sediment. Limnol. Oceanogr. 45, 534–549.
[19] Goni-Urriza, M., de Montaudouin, X., Guyoneaud, R., Bachelet, G. and de Wit, R. (1999) Effect of macrofaunal bioturbation on bacterial distribution in marine sandy sediments, with special reference to sulphur-oxidising bacteria. J. Sea Res. 41, 269–279.
[20] Rijnaarts, H.H.M., Norde, W., Bouwer, E.J., Lyklema, J. and Zehnder, A.J.B. (1996) Bacterial deposition in porous media: Effects of cell-coating, substratum hydrophobicity, and electrolyte concentration. Environ. Sci. Technol. 30, 2877–2883.
[21] Simoni, S.F., Bosma, T.N.P., Harms, H. and Zehnder, A.J.B. (2000) Bivalent cations increase both the subpopulation of adhering bacteria and their adhesion efficiency in sand columns. Environ. Sci. Technol. 34, 1011–1017.
[22] Heise, S. and Gust, G. (1999) Influence of the physiological status of bacteria on their transport into permeable sediments. Mar. Ecol. Progr. Ser. 190, 141–153.
[23] Newby, D.T., Pepper, I.L. and Maier, R.M. (2000) Microbial transport. In: Environmental Microbiology (Maier, R.M., Pepper, I.L. and Gerba, C.P., Eds.), pp. 147–175. Academic Press, London.
[24] Shimeta, J., Jumars, P.A. and Lessard, E.J. (1995) Influences of turbulence on suspension feeding by planktonic protozoa: experiments in laminar shear fields. Limnol. Oceanogr. 40, 845–859.
[25] Logan, B.E. and Kirchman, D.L. (1991) Uptake of dissolved organics by marine bacteria as a function of fluid motion. Mar. Biol. 111, 175–181.
[26] Kiørboe, T., Ploug, H. and Thygesen, U.H. (2001) Fluid motion and solute distribution around sinking aggregates. I. Small-scale fluxes and heterogeneity of nutrients in the pelagic environment. Mar. Ecol. Progr. Ser. 211, 1–13.
[27] Llobet-Brossa, E., Rosselló-Mora, R. and Amann, R. (1998) Microbial community composition of Wadden Sea sediments as revealed by fluorescence in situ hybridization. Appl. Environ. Microbiol. 64, 2691–2696.
[28] Wieringa, E.B.A., Overmann, J. and Cypionka, H. (2000) Detection of abundant sulphate-reducing bacteria in marine oxic sediment layers by a combined cultivation and molecular approach. Environ. Microbiol. 2, 417–427.
[29] Scala, D.J. and Kerkhof, L.J. (2000) Horizontal heterogeneity of denitrifying bacterial communities in marine sediments by terminal restriction fragment length polymorphism analysis. Appl. Environ. Microbiol. 66, 1980–1986.
[30] Keen, T.R. and Glenn, S.M. (1995) A coupled hydrodynamic-bottom boundary layer model of storm and tidal flow in the Middle Atlantic Bight of North America. J. Phys. Oceanogr. 25, 391–406.
[31] Boehme, S.E., Sabine, C.L. and Reimers, C.E. (1998) CO_2 fluxes from a coastal transect: a time series approach. Mar. Chem. 63, 49–67.
[32] Kerkhof, L.J., Voytek, M.A., Sherrell, R.M., Millie, D. and Schofield, O. (1999) Variability in bacterial community structure during upwelling in the coastal ocean. Hydrobiology 401, 139–148.
[33] Reimers, C.E., Stecher, H.A., Taghon, G.L. and Fuller, C.M. (2002) In situ measurements of solute transport velocities in permeable shelf sands. AGU Ocean Sciences Meeting, Honolulu, HI.
[34] Saager, P.M., Sweerts, J.-P. and Ellermeijer, H.J. (1990) A simple pore-water sampler for coarse, sandy sediments of low porosity. Limnol. Oceanogr. 35, 747–751.
[35] Hall, P.O.J. and Aller, R.C. (1992) Rapid, small-volume, flow injection analysis for ΣCO_2 and NH_4^+ in marine and freshwaters. Limnol. Oceanogr. 37, 1113–1119.
[36] Zimmermann, R., Iturriaga, R. and Becker-Birck, J. (1978) Simultaneous determination of the total number of aquatic bacteria and the number thereof involved in respiration. Appl. Environ. Microbiol. 36, 926–935.
[37] Epstein, S.S. and Rossel, J. (1995) Enumeration of sandy sediment bacteria: search for optimal protocol. Mar. Ecol. Progr. Ser. 117, 289–298.
[38] King, L.K. and Parker, B.C. (1988) A simple, rapid method for enumerating total viable and metabolically active bacteria in groundwater. Appl. Environ. Microbiol. 54, 1630–1631.
[39] Preuß, G. and Hupfer, M. (1998) Ermittlung von Bakterienzahlen in aquatischen Sedimenten. In: Mikrobiologische Charakterisierung aquatischer Sedimente (VAAM, Eds.), pp. 2–34. Oldenbourg, Munich.
[40] Boetius, A. and Lochte, K. (1994) Regulation of microbial enzymatic degradation of organic matter in deep-sea sediments. Mar. Ecol. Progr. Ser. 104, 299–307.
[41] Bélanger, C., Desrosiers, B. and Lee, K. (1997) Microbial extracellular enzyme activity in marine sediments: extreme pH to terminate reaction and sample storage. Aquat. Microb. Ecol. 13, 187–196.
[42] Manz, W., Amann, R., Ludwig, W., Wagner, M. and Schleifer, K.-H. (1992) Phylogenetic oligodeoxynucleotide probes for major subclasses of Proteobacteria: problems and solutions. Syst. Appl. Microbiol. 15, 593–600.
[43] Snaidr, J., Amann, R., Huber, I., Ludwig, W. and Schleifer, K.-H.

(1997) Phylogenetic analysis and in situ identification of bacteria in activated sludge. Appl. Environ. Microbiol. 63, 2884–2896.
[44] Daims, H., Brühl, A., Amann, R., Schleifer, K.-H. and Wagner, M. (1999) The domain-specific probe EUB338 is insufficient for the detection of all Bacteria: development and evaluation of a more comprehensive probe set. Syst. Appl. Microbiol. 22, 434–444.
[45] Ravenschlag, K., Sahm, K. and Amann, R. (2001) Quantitative molecular analysis of the microbial community in marine arctic sediments (Svalbard). Appl. Environ. Microbiol. 67, 387–395.
[46] Neef, A., Amann, R., Schlesner, H. and Schleifer, K.-H. (1998) Monitoring a widespread bacterial group: in situ detection of planctomycetes with 16S rRNA-targeted probes. Microbiology 144, 3257–3266.
[47] Bouchez, T., Patureau, D., Dabert, P., Juretschko, S., Doré, J., Delgenès, P., Moletta, R. and Wagner, M. (2000) Ecological study of a bioaugmentation failure. Environ. Microbiol. 2, 179–190.
[48] Manz, W., Eisenbrecher, M., Neu, T.R. and Szewzyk, U. (1998) Abundance and spatial organization of Gram-negative sulfate-reducing bacteria in activated sludge investigated by in situ probing with specific 16S rRNA targeted oligonucleotides. FEMS Microbiol. Ecol. 25, 43–61.
[49] Nogales, B., Moore, E.R.B., Llobet-Brossa, E., Rosselló-Mora, R., Amann, R. and Timmis, K.N. (2001) Combined use of 16S ribosomal DNA and 16S rRNA to study the bacterial community of polychlorinated biphenyl-polluted soil. Appl. Environ. Microbiol. 67, 1874–1884.
[50] Sachs, L. (1997) Angewandte Statistik. Springer-Verlag, Berlin.
[51] Ritzrau, W. and Graf, G. (1992) Increase of microbial biomass in the benthic turbidity zone of Kiel Bight after resuspension by a storm event. Limnol. Oceanogr. 37, 1081–1086.
[52] Harvey, H.R., Tuttle, J.H. and Bell, J.T. (1995) Kinetics of phytoplankton decay during simulated sedimentation: Changes in biochemical composition and microbial activity under oxic and anoxic conditions. Geochim. Cosmochim. Acta 59, 3367–3377.
[53] Sinsabaugh, R.L. and Findlay, S. (1995) Microbial production, enzyme activity, and carbon turnover in surface sediments of the Hudson River estuary. Microb. Ecol. 30, 127–141.
[54] Smayda, T.J. (1978) From phytoplankters to biomass. In: Phytoplankton Manual (Sournia, A., Ed.), pp. 273–279. UNESCO, Paris.
[55] Westrich, J.T. and Berner, R.A. (1984) The role of sedimentary organic matter in bacterial sulfate reduction: The G model tested. Limnol. Oceanogr. 29, 236–249.
[56] Rusch, A., Forster, S. and Huettel, M. (2001) Bacteria, diatoms and detritus in an intertidal sandflat subject to advective transport across the water-sediment interface. Biogeochemistry 55, 1–27.
[57] Meyer-Reil, L.-A. (1987) Seasonal and spatial distribution of extracellular enzymatic activities and microbial incorporation of dissolved organic substrates in marine sediments. Appl. Environ. Microbiol. 53, 1748–1755.
[58] Vetter, Y.-A. and Deming, J.W. (1994) Extracellular enzyme activity in the Arctic Northeast Water polynya. Mar. Ecol. Progr. Ser. 114, 23–34.
[59] Fabiano, M. and Danovaro, R. (1998) Enzymatic activity, bacterial distribution, and organic matter composition in sediments of the Ross Sea (Antarctica). Appl. Environ. Microbiol. 64, 3838–3845.
[60] Poremba, K. and Hoppe, H.-G. (1995) Spatial variation of benthic microbial production and hydrolytic enzymatic activity down the continental slope of the Celtic Sea. Mar. Ecol. Progr. Ser. 118, 237–245.
[61] Goto, N., Mitamura, O. and Terai, H. (2001) Biodegradation of photosynthetically produced extracellular organic carbon from intertidal benthic algae. J. Exp. Mar. Biol. Ecol. 257, 73–86.
[62] Grossart, H.-P. and Ploug, H. (2001) Microbial degradation of organic carbon and nitrogen on diatom aggregates. Limnol. Oceanogr. 46, 267–277.
[63] Howard-Jones, M.H., Frischer, M.E. and Verity, P.G. (2001) Determining the physiological status of individual bacterial cells. Methods Microbiol. 30, 175–206.
[64] Thimsen, C.A. and Keil, R.G. (1998) Potential interactions between sedimentary dissolved organic matter and mineral surfaces. Mar. Chem. 62, 65–76.
[65] Ding, X. and Henrichs, S.M. (2002) Adsorption and desorption of proteins and polyamino acids by clay minerals and marine sediments. Mar. Chem. 77, 225–237.
[66] Dauwe, B., Middelburg, J.J. and Herman, P.M.J. (2001) The effect of oxygen on the degradability of organic matter in subtidal and intertidal sediments of the North Sea area. Mar. Ecol. Progr. Ser. 215, 13–22.
[67] Shewan, J.M. and McMeekin, T.A. (1983) Taxonomy (and ecology) of *Flavobacterium* and related genera. Annu. Rev. Microbiol. 37, 233–252.
[68] DeLong, E.F., Franks, D.G. and Alldredge, A.L. (1993) Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol. Oceanogr. 38, 924–934.
[69] Cottrell, M.T. and Kirchman, D.L. (2000) Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. Appl. Environ. Microbiol. 66, 1692–1697.
[70] Weller, R., Glöckner, F.O. and Amann, R. (2000) 16S rRNA-targeted oligonucleotide probes for the in situ detection of members of the phylum Cytophaga-Flavobacterium-Bacteroides. Syst. Appl. Microbiol. 23, 107–114.
[71] Nold, S.C. and Zwart, G. (1998) Patterns and governing forces in aquatic microbial communities. Aquat. Ecol. 32, 17–35.
[72] Cottrell, M.T. and Kirchman, D.L. (2000) Community composition of marine bacterioplankton determined by 16S rRNA clone libraries and fluorescence in situ hybridisation. Appl. Environ. Microbiol. 66, 5116–5122.
[73] Riemann, L., Steward, G.F. and Azam, F. (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. Appl. Environ. Microbiol. 66, 578–587.
[74] Fandino, L.B., Riemann, L., Steward, G.F., Long, R.A. and Azam, F. (2001) Variations in bacterial community structure during a dinoflagellate bloom analyzed by DGGE and 16S rDNA sequencing. Aquat. Microb. Ecol. 23, 119–130.
[75] Glöckner, F.O., Fuchs, B.M. and Amann, R. (1999) Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridisation. Appl. Environ. Microbiol. 65, 3721–3726.
[76] Fuerst, J.A. (1995) The planctomycetes: emerging models for microbial ecology, evolution and cell biology. Microbiology 141, 1493–1506.
[77] Gray, J.P. and Herwig, R.P. (1996) Phylogenetic analysis of the bacterial communities in marine sediments. Appl. Environ. Microbiol. 62, 4049–4059.