# Microbial carbon processing in marine sediments:

## *Case studies in North Sea sands and oligotrophic deep-sea sediments*

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# Chapter 1

# INTRODUCTION

# INTRODUCTION

This thesis deals with the bacteria and their contribution to carbon turnover in different marine sediments. Marine sediments play a major role in the oceanic carbon cycle as the main site for mineralisation and oxidation of organic matter (e.g. Jørgensen, 1983, Jahnke, 1996). The investigations presented in this thesis were located on the shelf in the Southern North Sea and the deep Mediterranean. Bacterial communities were investigated through lipid biomarker analysis and fluorescence *in situ* hybridisation.

## Carbon cycle

On a global basis, carbon is cycled through all of Earth's major carbon reservoirs: the atmosphere, the land, the oceans and other aquatic environments, sediments, rocks, and biomass. Global carbon cycling involves the activities of both micro- and macroorganisms and is intimately tied to the oxygen cycle, as oxygenic photosynthesis removes $CO_2$ and produces $O_2$ while respiratory processes produce $CO_2$ and remove $O_2$. Under anoxic conditions the carbon cycle is managed by prokaryotes, with carbon fixation by purple and green bacteria and a variety of chemoautotrophs, and decomposition of complex organic materials to $CH_4$ and $CO_2$ by various groups of anaerobic prokaryotes.

The balance between the oxidative and reductive part of the carbon cycle is critical, because the products of metabolism of some organisms are substrates for others. In terms of decomposition the prokaryotes are much more important than the eukaryotes, and this is especially true for anoxic environments. Jørgensen (2000) gives three reasons for the great importance of bacteria for the biogeochemical cycling:

1) their metabolic versatility with many types of anaerobic metabolisms,

2) their small size which allows them to inhabit nearly all environments and strongly enhances the efficiency of their catalytic activity and

3) the wide range of environmental conditions under which they thrive.

Marine sediments are environments of intense oxidation and mineralisation of organic matter (OM) that has been produced in the photic zone of the world oceans. The flux of OM to the sediment depends on the amount of primary productivity in the photic zone and on the depth of the water column.

The continental shelf is generally characterised by shallow water depths, and due to strong wave action and currents coarse-grained sandy sediments are abundant. Due to the shallowness of this environment 50% of the primary production can settle through the water column. However, the only low accumulation of organic material in shelf sediments may reflect high degradation rates.

The environmental conditions prevailing in the deep sea are totally different from the continental shelf. Due to the high efficiency of recycling in the water column, approximately only 1% of the primary production reaches the deep-sea floor (Suess, 1980). The mechanisms for transport of carbon to the seafloor are either physical transport ("physical carbon pump") driven by water masses that could be subducted to the deep sea or by transport of biologically produced carbon products ("biological carbon pump"). After reaching the seafloor, the OM is subject to physical, chemical and biological reactions collectively referred to as diagenesis (Berner, 1980).

The aerobic mineralisation that starts in the water column continues at the sediment surface with the activity of aerobic bacteria and animals. Due to oxygen depletion, the oxic layer constitutes a more or less thin surface, below which the mineralisation takes place through anaerobic processes. Whereas the aerobic pathways completely oxidise the organic carbon to $CO_2$, the anaerobic degradation includes a variety of different steps, each completing only a partial oxidation of the organic molecules. These molecules are the primary substrates for denitrifying, sulfate reducing and methane producing bacteria, accompanied by the use of

inorganic compounds of nitrogen and sulfur as carriers of chemical energy. Through these processes organic carbon is subsequently oxidised to $CO_2$ whereas reduced molecules, like $N_2$, $H_2S$, and $CH_4$ are produced. The decreasing energy yield of the electron acceptors per mol organic carbon oxidised, together with the subsequent utilisation by bacteria, induce a vertical stratification of electron acceptors in the porewater beginning with oxygen, nitrate, manganese, iron and then sulfate. The gradually decreasing energy yield of the accompanying bacterial metabolisms results in a narrower substrate spectrum with increasing depth. Whereas the denitrifiers are still very versatile with respect to usable substrates, the sulfate reducers are mostly unable to use carbohydrates and amino acids (Jørgensen, 2000). The emerging gap is closed by fermenting bacteria, who take up these compounds and convert them to primarily short chain fatty acids, as well as $H_2$, lactate, alcohols and $CO_2$. The sulfate reducers are commonly divided into complete and incomplete oxidisers. The product of incomplete oxidation is mainly acetate, which can be used by complete oxidisers to produce $CO_2$.

It was due to the investigations of Jørgensen (1982), that the global importance of sulfate reduction became eminent. At the sediment-water interface, dissolved sulfate concentrations are more than 50 times higher than those of other electron acceptors with higher standard free energy (e.g. nitrate, iron, manganese) together (D´Hondt *et al.*, 2002). Below the suboxic zone the redox potential is very low and sulfate reduction becomes the dominating early diagenetic process (Jørgensen, 1983). In near-shore sediments the sulfate reduction is of such great importance that it roughly equals oxic mineralisation (Jørgensen, 1982, Canfield, 1989).

## Methods to investigate the bacterial community

A general problem in marine microbiology is that only a small fraction of bacteria in the ocean is known to science today (Jørgensen, 2000). This is caused by problems of preferential cultivation of only some groups of bacteria that tend to grow on commonly used substrates whereas other groups of organisms are completely lost

during these routine procedures. Nevertheless, new approaches tend to overcome these problems.

## Biomarker studies

If you ask a geologist and an ecologist to define the word biomarker, you will probably receive two different answers. Both would describe a biomarker as a molecule that can only be biosynthesised by one species or a defined group of organisms. For a geologist a reliable biomarker is relatively resistant to diagenesis and therefore preserved in the geological record, whereas ecologists use the term biomarker for molecules that are quickly degraded after death of the organism in order to have a useful specific tracer for living organisms. For microbiological studies, biomarkers should furthermore provide information on microbial identity and biomass (Boschker and Middelburg, 2002). For identification purposes the biomarker is ideally only produced by the organism of interest, but specificity is rarely absolute. The ideal biomarker therefore belongs to a group of compounds in which markers of different organisms can be found. For microbiological studies, investigations of lipids, especially phospholipid-derived fatty acids (PLFA), tend to be a useful tool. For the determination of biomass, it is necessary that the biomarker concentration in the organism of interest is relatively constant, excluding storage products and other accumulating molecules. Microbial ecologists therefore seek biomarkers that are as specific as possible and that have high turnover rates. The use of lipid biomarkers for the identification of microbial communities was promoted during the last 20 years. One early detailed description of fatty acid composition of several bacterial strains was done by Dowling *et al.* (1986), followed by different scientists transferring the knowledge from culture analysis to the investigations of natural prokaryotic communities (e.g. Rajendran *et al.*, 1992, Oude Elferink *et al.*, 1998, Orphan *et al.*, 2001, Wakeham *et al.*, 2003).

In Figure 1 some structures of bacterial fatty acids are given. Branched chain fatty acids often occur in bacterial lipids and are synthesised from malonyl-CoA and primer sources, mainly α–keto acids, whereas straight chain fatty acids are synthesised from acetyl-CoA and malonyl-CoA as a chain extender (Kaneda, 1991). All these fatty acids occur as membrane components. They typically consist of alkyl esters linked to glycerol and a polar head group and are arranged in a bilayer, with the hydrophilic polar head group protruding outside and the hydrophobic fatty acid chains pointing to the inside. Branched chain fatty acids increase the fluidity of membrane lipids due to their space-consuming structure (Kaneda, 1991).

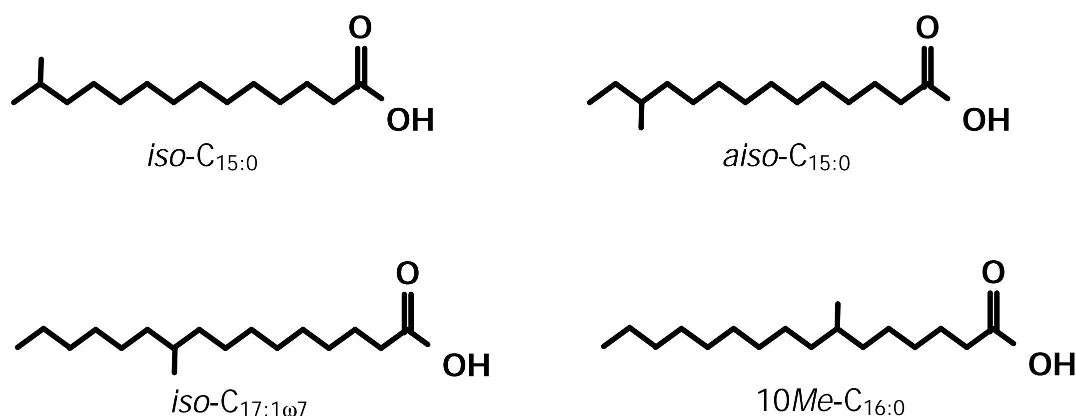


Figure 1: Structures of four typical bacterial fatty acids.

The information gained from studying biomarker can be further increased by simultaneous determination of the stable carbon isotope composition. Natural abundance studies use the small difference in isotopic ratios as found in nature (Peterson, 1999, Hayes, 2001). Carbon stable isotopes are principally useful to distinguish between marine and terrestrial OM sources and to identify different origins in the food web. The carbon source as well as the discrimination between $^{13}C$ and $^{12}C$ are accompanied by enzymatic reactions that affect the stable carbon isotope composition.

Most plants including phytoplankton, incorporate carbon into their biomass using the Calvin-cycle ($C_3$-plants) which discriminates against $^{13}C$ to produce a shift in $\delta^{13}C$ values of about –20‰ from the signature of the carbon source. The $C_4$ plants

use the Hatch-Slack pathway, which leads to an isotopic shift of –7‰. Marine algae use dissolved bicarbonate with a $\delta^{13}C$ value of approximately 0‰, and consequently marine organic matter has values in the range of –20‰ to –22‰. Stable carbon isotope composition of heterotrophic organisms naturally reflect the ratios of their food sources ("you are what you eat") (Pond *et al.*, 1997, Boschker *et al.*, 1999). These differences can be used to investigate organic matter cycling and food web relationships. For interpretation of the resulting data we have to keep in mind that in most enzymatic driven processes fractionation against the heavy carbon isotope $^{13}C$ takes place. Lipids are depleted in the heavy isotope as a result of isotopic fractionation during the oxidation of pyruvate to acetyl coenzyme A (DeNiro and Epstein, 1977), which generally results in 3-6‰ depletion (Hayes, 2001). In contrast to this, Abraham *et al.* (1998) found different fractionation factors for fatty acids from several bacterial strains, which hamper interpretation of results gained from natural communities.

In some environments the differentiation between different carbon sources is more straightforward. Due to the extreme depletion of microbially formed methane, the prokaryotes associated with the process of anaerobic methane oxidation could be traced following the light carbon signal (Elvert and Suess, 1999, Hinrichs *et al.*, 1999). These investigations could be designated as natural $^{12}C$-labelling caused by the unequivocally negative carbon isotope value of methane (-50 to –110‰; Whiticar, 1999), in analogy to the relatively new attempt of $^{13}C$-labelling experiments. Labelling methods are promoted by the increased availability of stable isotope labelled compounds. Furthermore cultivation of organisms on labelled compounds could be a source for labelled OM. The great advantage of stable isotope labelling experiments is that they do not suffer from legal restrictions and health problems associated with radioisotopes and therefore can be used directly in the field (e.g. Middelburg *et al.*, 2000, Moodley *et al.*, 2002, reviewed by Boschker and Middelburg, 2002). Studies in natural environments include the complexity of an ecosystem and minimise the artefacts that occur due to experimental conditions.

The basic idea behind the labelling approach is that a portion of the added stable isotope tracer is incorporated into the biomass of metabolic active organisms. In case of prokaryotes the detection via isotopic measurements of the specific

biomarkers is of common practice. Furthermore, the labelled carbon can be followed through the food web by analysing biomass samples of higher organisms. One early approach was done by Middelburg *et al.* (2000), who investigated the fate of carbon in an intertidal sandflat, by spraying $^{13}$C-bicarbonate on the surface and following its way through the benthic food web. Boschker *et al.* (1998) used labelled $^{13}$C compounds to provide evidence for the identity of bacteria involved in sulfate reduction and methane oxidation.

The role of heterotrophic bacteria in benthic food web studies can be elucidated by the use of $^{13}$C-OM. The first application of this method was done by Blair *et al.* (1996), who used $^{13}$C-labelled algae for *in situ* experiments to trace the fate of phytodetritus in ocean margin sediments. Not only the observation of uptake and incorporation into different benthic compartments is possible, newer investigations also included the respiration of the added material to $^{13}CO_2$ (e.g. Moodley *et al.*, 2000, Witte *et al.*, 2003).

Addition of labelled substrates to an ecosystem followed by biomarker probing enables direct identification of microbes in specific processes. Successful applications of this method are included in this thesis in Chapter 4 and 5.

### FISH studies

Another new and promising approach to study the bacterial community *in situ* is the use of fluorescence *in situ* hybridisation (FISH). Hybridisation of intact cells with fluorescently labelled 16S rRNA targeted oligonucleotides has been introduced to molecular ecology approximately a decade ago as an accurate method of characterising *in situ* microbial populations (Stahl *et al.*, 1988, Amann *et al.*, 1990). The rRNA is part of the ribosomes, which are present in high abundances of up to 10,000 in each bacterial cell. Ribosomes are of homologous origin and show functional constancy and hence the phylogeny of the accompanying organisms can be reconstructed based on the 16S rRNA molecules. Different degrees of conservation allow a differentiation on any taxonomic level, i.e. from species to

domains, which made the 16S rRNA a powerful target for microbial community investigations. During the last decades large databases on rRNA sequences have been established, which are available to the public (e.g. http://www.ncbi.nlm.nih.gov/).

In order to design the specific probes, DNA must be extracted from natural samples. After amplification of rDNA using PCR and specific primers, the cloning and sequencing of the rDNA follows. Afterwards the probe can be used to enumerate whole fixed cells in the original sample by *in situ* hybridisation (Amann *et al.*, 1995). This method does not rely on the culturability of the bacteria inhabiting a certain sediment, which is a major advantage compared to classical methods. Furthermore conclusion on the morphology and the spatial distribution of microbes can be assessed (Amann *et al.*, 1995). Since the first applications of this method, FISH has often been used to quantify microbial communities in marine (e.g. Ravenschlag *et al.*, 2001, Ishii *et al.*, subm.) and freshwater sediments (Altmann *et al.*, 2003).

## Areas of investigations

The investigations presented in this thesis were located on a subtidal sand in the North Sea and in the deep sea of the Eastern Mediterranean. In following the different study sites will be introduced according to their history, sediments and sediment-water exchange processes.

### Continental Shelf Areas and the North Sea

The continental shelf is defined as the part of the continental margin that is located between the shoreline and the continental slope. The shelf gently slopes up to the continental break, which is usually located at depths of about 100-250 m (Brown *et al.* 1989). The continental shelf covers only 7.5% of the oceans surface, but is responsible for 30% of the oceans primary production (Jørgensen, 1996). This high biological productivity is caused by a variety of controlling factors: a) the high

terrestrial run-off through river systems, b) upwelling of nutrient-rich deep waters, c) aeolean input of different trace elements and d) close coupling of benthic and pelagic systems (Wollast, 1991). The shelf areas are very important for the global biogeochemical cycling of organic matter, by representing a close link between the terrestrial environment and the open ocean.

Six main classes of continental sediments can be distinguished after Emery, (1968): I) detrital (sediment presently supplied to the shelf), II) biogenic (e.g. shell debris, fecal pellets, etc.), III) residual (*in situ* weathering of rock outcrops), IV) authigenic (e.g. glauconite, phosphorite, etc.), V) volcanic and VI) relict. Relict sediments were deposited in the geological past. During the Quaternary (last 1.8 Ma) a number of glacial and interglacial periods caused dramatic eustatic fluctuations of the sea level. As the glacial periods predominated during the last million years, the sea level has been lower than it is today, at least for 90% of that time. Therefore, large areas of the shelf have been air-exposed and were subjected to terrestrial conditions, with high deposition rates of terrestrial sediments.

The North Sea covers an area of 575,000 $km^2$, has a mean depth of 70 m and reaches a maximum depth of 725 m. It is a shelf sea area, which is hydrologically coupled to the North-eastern Atlantic, and its present status is caused by great changes over geological time scales.

Fresh water from riverine input and saline water from the Atlantic Ocean influence the biological and chemical regime of the North Sea. The resulting density differences lead to convective mixing. The eminent river loads into this area are furthermore associated by high nutrient influxes, causing a high primary production, especially in southern coastal areas, where values of 370 g C $m^{-2}$ $y^{-1}$ were observed (Wollast, 1991). This high productivity directly leads to one of the most fish-rich areas of the world oceans, causing a high impact of the fishing industry during the last century.

The main Atlantic inflows are through the northern boundary or through the British Channel in the south-west. The main outflow of North Sea water into the Atlantic takes place through the Norwegian Trench, resulting in a mainly counter clockwise circulation.

The German Bight is located in the south-eastern part of the North Sea, with a depth range of 20-40 m. The salinity varies between 27 and 34 PSU, depending on the influx of fresh water from the two main rivers Elbe and Weser. The predominant types of sediments are sand and mud, and mixtures with different shares of both. Part of the globally unique Wadden Sea is located in the German Bight, covering 13% of this area. The Frisian barrier islands separate the Wadden Sea from the remaining German Bight, which is a high-energy environment, with transport processes influenced by tides, waves and high bottom current velocities generated by storm events. Our study area is near the Frisian island Spiekeroog, where mean tidal ranges are approximately 2.5 m, and peak tidal current velocities are 30-60 cm $s^{-1}$ at 1 m above bottom (Antia, 1993).

In areas with severe current velocities coarse-grained sands are the only remaining sediment type. Sands are conventionally classified according to their grain size based on the Udden-Wentworth scale into very fine (63 to 125 µm), fine (125 to 250 µm), medium (250 to 500 µm), coarse (500 to 1000 µm) and very coarse sand (1000 to 2000 µm) (Brown *et al.*, 1989).

Sandy sediments can be permeable, which means that they allow porewater movement and thus advective solute transport through their interstitial pore space. The permeability is the capability of the sediment to permit flow through this space. The porewater movements in permeable sediments provide a fast carrier for the exchange of substances between the water column and the upper sediment layers. $10^{-12}$ $m^2$ is given as the threshold for permeability to allow porewater exchange (Huettel and Gust, 1992). The permeability depends on different factors, like : a) the grain size and sorting degree (Hsü, 1990), b) clogging of sand grains due to bacterial and algal exopolymers (Dade *et al.*, 1990, Yallop *et al.*, 1994), c) bioturbation (Ziebis *et al.*, 1996) as well as d) the density and viscosity of the pore fluid (Klute and Dirksen, 1986).

The driving forces of advective transport processes are pressure gradients induced by surface gravity waves or interactions of bottom currents and sediment topography or biogenic structures (Huettel and Rusch, 2000, Huettel and Webster, 2001). In permeable sediments the water is forced into the sediment where pressure

is increased and porewater emerges just downstream of the highest point of protruding structures, where the pressure is reduced (Figure 2; Huettel and Gust, 1992). The intensity of the porewater flow depends on various factors such as sediment permeability (Huettel and Gust, 1992), flow velocity (Forster *et al.*, 1996), and topography height (Huettel *et al.*, 1996). Permeable sediments can advectively filter particles, like algal cells, from the water column (Pilditch *et al.*, 1998, Huettel and Rusch, 2000).

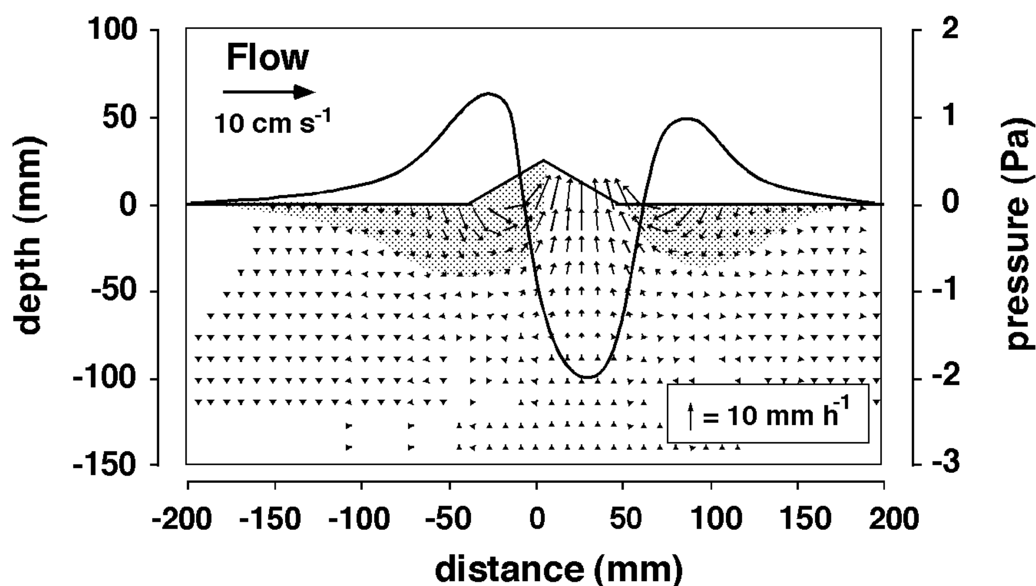


Figure 2: Schematic of the advective porewater flow field under sediment topography exposed to unidirectional flow in a straight open channel. Solid curved line: pressure distribution at the sediment-water interface; shaded areas: water intrusion zones; arrows: direction and magnitude of advective porewater flows. Figure taken from Huettel and Rusch (2000).

Laboratory flume experiments have shown that advective porewater flow in permeable sediments leads to increased oxygen penetration (Ziebis *et al.*, 1996), enhanced nutrient efflux (Huettel *et al.*, 1998) and elevated oxygen consumption (Forster *et al.*, 1996). These insights led to the overturning of the old paradigm that sands act as biogeochemical deserts, which had been caused by the low organic carbon contents of permeable sediments (Boudreau *et al.*, 2001).

In order to further elucidate the processes prevailing in sandy sediments an experimental campaign in the Southern North Sea was initiated at the Max Planck

Institute for Marine Microbiology in Bremen. One main focus lay on the extension of flux investigations to natural sands. For this purpose a new autonomous *in situ* instrument was developed, the benthic chamber lander SANDY (Janssen *et al.*, subm.), which is based on a circular chamber with a rotating stirrer disc similar to the one described by Huettel and Gust (1992). With this instrument it was possible for the first time to measure advection driven processes under controlled *in situ* conditions (Janssen *et al.*, subm.).

The impact of this advective driven transport processes on biogeochemical processes was investigated during the second experimental campaign. Therefore, experiments with labelled POM were conducted in a working group, dedicated to elucidate the transport of algal derived material into the sediment as well as the decomposition, uptake, and incorporation into macrofauna and bacteria. *In situ* nutrient recycling was observed and higher stimulation of nitrification in coarser-grained sediments was examined (Ehrenhauss *et al.*, in press). The uptake of labelled carbon into macrofauna organisms was investigated over a time series on a fine-grained sediment type (Kamp and Witte, in prep.). The transport of algal cells as well as the transport of labelled carbon into sediments of different permeability was furthermore investigated and is included in this thesis (Chapter 3). The special focus of my work lay on the bacteria, which were investigated via specific bacterial biomarkers and fluorescence *in situ* hybridisation (Chapter 2), and their contribution to carbon turnover was investigated (Chapter 4).

## The deep sea and the Mediterranean

The second part of this thesis (Chapter 5) presents a study on carbon turnover in an oligotrophic deep-sea site in the Eastern Mediterranean.

The relative inaccessibility still remains many uncertainties about life in the bathyal, abyssal and hadal zones of the oceans, although more than 75% of the ocean water is part of the deep sea. The deep sea is defined as the region of the

oceans below 1000 m water depth, even though in polar regions characteristic deep-sea fauna organisms can be found in only several hundreds meters depth.

Typical deep-sea sediments consist of muddy sediments of extremely fine-grained red clay with a medium grain size of < 1 µm. The clay particles tend to stick together, caused by electrochemically charged areas, where ions are attracted and hydrogen bondings could emerge (Little, 2000). Furthermore biogenic oozes frequently occur, consisting of shells and skeletal material from pelagic organisms. The composition of this part depends on the faunal distribution of the accompanied organisms in the water column and furthermore on the position of the calcite compensation depth (CCD), defined as the depth of preservation of carbonate above and is normally located between 4 to 5 km below the surface.

Solute-transport in fine-grained, cohesive sediments is mostly driven by molecular diffusion (Huettel *et al.*, 1998). Furthermore, bioturbation and bioirrigation can be of local importance (Huettel *et al.*, 1996, Huettel *et al.*, 1998). Bioturbation refers to the spatial rearrangement of the sediments solid phase by diverse organisms and has great impact on the geochemical processes (Sun *et al.*, 2002). Another important process mediated by organisms living in the sediment is bioirrigation, which is the active pumping of water through burrows in the sediment, which can introduce oxygen-rich water in deeper sediment layers (Levin *et al.*, 1997).

Organisms that inhabit the deep sea face three major environmental extremes: I) high pressure, II) low temperature, and III) low food availability. OM is supplied by the light penetrated epipelagic regions of the oceans. The initial belief for the maintenance of this kind of ecosystem was thought to be OM supply constantly delivered as a fine rain of particles sinking through the water column. Over the last decades great effort was put into the investigations of the deep sea, but only the invention of moored time lapse cameras (Billett *et al.*, 1983, Rice *et al.*, 1986) made documentation of seasonal accumulation of relatively fresh phytodetrital material possible. It derives from phytoplankton blooms occurring in the epipelagic realm in early spring, caused by increasing solar radiation and enhanced nutrient availability due to mixing processes (Purdie, 1996). These blooms often die off rapidly and due to repacking in different matrices (Alldredge and Silver, 1988) they can reach fast

sinking velocities of up to 150 m per day (Lampitt, 1985). The seasonally depositing phytodetritus is essential for life in the deep oceans. A map of the abundances of benthic macrofauna in the deep sea (Belyaev, 1966) shows good correlations with data of the primary production in the worlds oceans (Koblentz-Mishke *et al.*, 1970, reviewed by Hinga *et al.*, 1979). The dependence of benthic and benthopelagic organisms on these seasonal events has been investigated and an increase in activity was observed (reviewed by Gooday, 2002). Furthermore, an influx of detrital flocs with high shares of fecal pellets (Carroll *et al.*, 1998) and large food falls from dead fish and whale carcasses (Rowe *et al.*, 1986, Smith, 1992) contribute to the nutritional supply of these environments during all seasons. The enhanced metabolic activity of the benthic community leads to seasonal fluctuations in sediment community oxygen consumption revealed by investigations e.g. in the North-Atlantic (Pfannkuche, 1993), the Norwegian Margin (Graf, 1989) as well as in the highly oligotrophic Cretan Sea (Duineveld *et al.*, 2000). Even though they are less pronounced, variations in abundances and activity in macro- and megafauna is reported as well, some of them even show seasonal reproduction cycles (reviewed by Gooday, 2002). But due to the logistical constrains concerning the methodical difficulties accessing deep-sea sites as well as the unpredictability of settling phytoplankton blooms, the data on the fate of such a pulse are rare and often contradictory (e.g. Smith Jr. and Baldwin, 1984, Graf, 1989, Sayles *et al.*, 1994, Smith and Kaufmann, 1999). To overcome these difficulties new attempts simulated a settling phytoplankton bloom in chambers of a deep-sea lander system (Moodley *et al.*, 2002, Witte *et al.*, 2003). The study of Witte *et al.* (2003) in the deep North Atlantic revealed, in contrast to previous hypotheses (e.g. Sayles *et al.*, 1994, Witbaard *et al.*, 2000), an immediate doubling of the sediment community oxygen consumption (SCOC), a fast reaction of the macrofauna and a retarded response of bacteria and foraminifera.

The high significance of the predictions gained from experimental simulation of settling phytoplankton, was our motivation for the experimental campaign in the deep Eastern Mediterranean (Chapter 5).

The Mediterranean covers an area of 3,020,000 km$^2$, with a mean and maximum depth of 1450 m and 5092 m, respectively. The Straight of Gibraltar with

only 14.5 km width and 300 m depth separates the Mediterranean from the Atlantic. The Straight of Sicily intersects the westerly and the easterly basins. The younger history of the Mediterranean was accompanied by separations from the Atlantic and Indian Ocean due to a northward drift of the African continent. Simultaneously a climate change caused an almost total withering during the Messinian salinity crises (5 to 6 Ma), resulting in extended salt deposits in the deep sediments. During the Pliocene the basin was again filled with seawater, but during the ice age the connection to the Black Sea was cut off and it was only 10,000 years ago that it was rebuilt, probably in a kind of catastrophic flood intruding the Black Sea lake, often referred to as "Noah's flood" (reviewed by Ryan *et al.*, 2003).

The Cretan Sea is a very unique environment. It is the largest in volume and deepest part of the Aegean Sea. It is connected to the Levantin Basin and the Ionian Sea through the eastern and western straits of the Cretan Arc. The Cretan Basin is an area of recent tectonism, which is dominated by the ongoing subduction of the African Plate below the Eurasian Plate. Investigations of Georgopoulos *et al.* (2000) confirmed that the deep Cretan Sea acts as a reservoir of very dense, high salinity water for the Eastern Mediterranean which causes vertical convection processes, through upwelling of the isopycnals.

An event in the early 1990's dramatically changed the structure of the Cretan Sea and adjacent basins. During this time exceptionally dense water of local origin started to fill up the Cretan Basin and outflow through the Cretan Arc straits into the Levantine and Ionian basins. This process led to an upwelling of the former deep waters and therewith strongly influenced the thermohaline circulation in the Eastern Mediterranean basin. In recent years three distinctive upper and three lower water masses could be identified. The deepest bottom part, the so-called Cretan Deep Water (CDW) is formed locally and in the surrounding shelf area.

The entire Aegean can be classified as highly oligotrophic. Especially the integrated production of the southern part is very low with values of 15.2 g C $m^{-2}$ $y^{-1}$ (Lykousis *et al.*, 2002) and the chlorophyll-*a* concentration in the uppermost sediment layers does not exceed 0.2 µg $g^{-1}$. Most of the export production is being mineralised before it reaches the sediments (97-98% as given by Danovaro *et al.*,

1999). Lykousis *et al.* (2002) declared this region as an "oceanic margin" environment due to the very small fraction of the initial amount of the primary production in the euphotic zone reaching the deep-sea bottom. The general scarcity of food leads to a predominance of the microbial food web in the entire Aegean Sea (Lykousis *et al.*, 2002) and makes it an ideal environment to study benthic response patterns to varying amounts of settling particulate organic matter (POM) which is the main focus of Chapter 5.

## Experimental setup and general methods

North Sea experiments

For the experimental campaign in the North Sea, acrylic cylindrical chambers were used (Figure 3). These were 31 cm high and of 19 cm inner diameter. A horizontal disk (17 cm diameter) installed approximately 10 cm above the sediment surface and set at 20 rpm stirred the water inside the chamber.

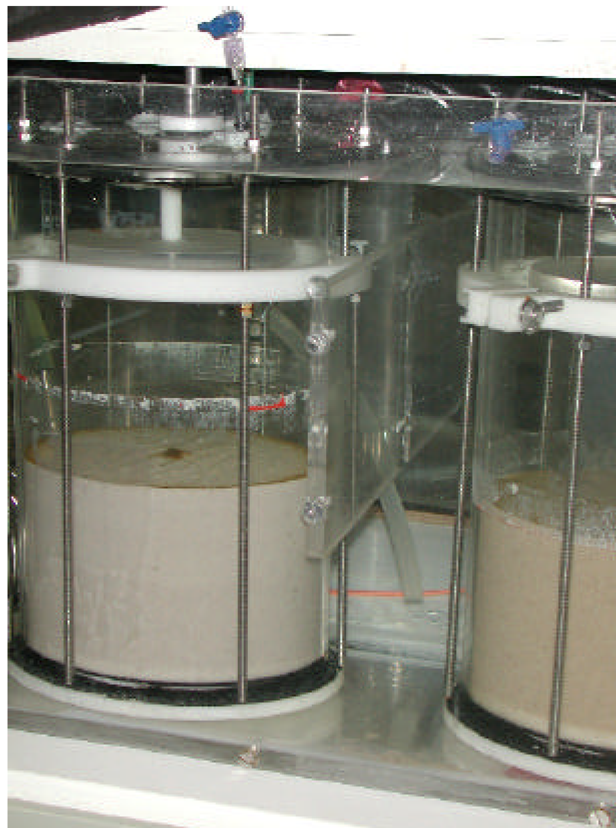

Figure 3: Stirred chambers filled with sediment.

The radial pressure gradient introduced by the rotating water is lowest in the centre of the chamber and highest at the outer rim. The magnitude of this pressure gradient (approximately 0.2 Pa $cm^{-1}$) is comparable to natural condition, when sediment ripples of 2 cm height are exposed to a relatively slow boundary layer flow of 10 cm $s^{-1}$ at 10 cm above the sediment (Huettel and Rusch, 2000, Figure 4).

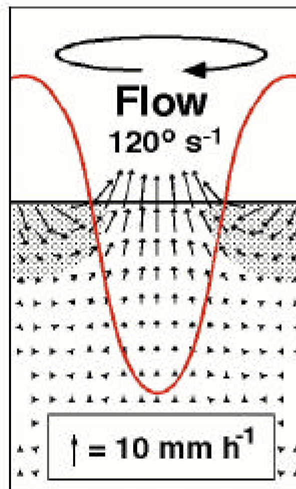


Figure 4: Schematic of the advective porewater flow field in a sediment exposed to a rotating water column in a stirred chamber. Solid curved line: pressure distribution at the sediment-water interface; shaded areas: water intrusion zones; arrows: direction and magnitude of advective porewater flows. Figure taken from Huettel and Rusch (2000).

This pressure gradient induces advective porewater exchange in permeable sediments. The exchange processes occur along the pressure gradient, with flux of water into the sediment at the outer rim of the chambers and out of the sediment at the centre.

The chambers were attached to a frame (Figure 5) for lowering to the sea floor by the ship's winch. For the experiments, the chambers were deployed and recovered by divers.

Figure 5: Framework with the motor device and four brackets for the transporting of the experimental chambers. For the incubations, the motor was connected by wires to the single chamber in order to operate the rotating disk.

## Crete experiments

For the deep-sea experiments in the Cretan Sea, a benthic chamber lander was used, which has first been applied to marine deep-sea science in 1976 by Smith Jr. *et al.* (1976), followed by several other early descriptions (Berelson *et al.*, 1987, Jahnke and Christiansen, 1989). The system used here was described in detail by Witte and Pfannkuche (2000). A lander is a freefall system with ballast that is released acoustically, and glass spheres for buoyancy. The stainless-steel tripod frame carries a platform to which various experimental packages can be attached. The whole system is comparably small (2 m wide, 2.5 m high) and thus easy to handle on board (Figure 6).

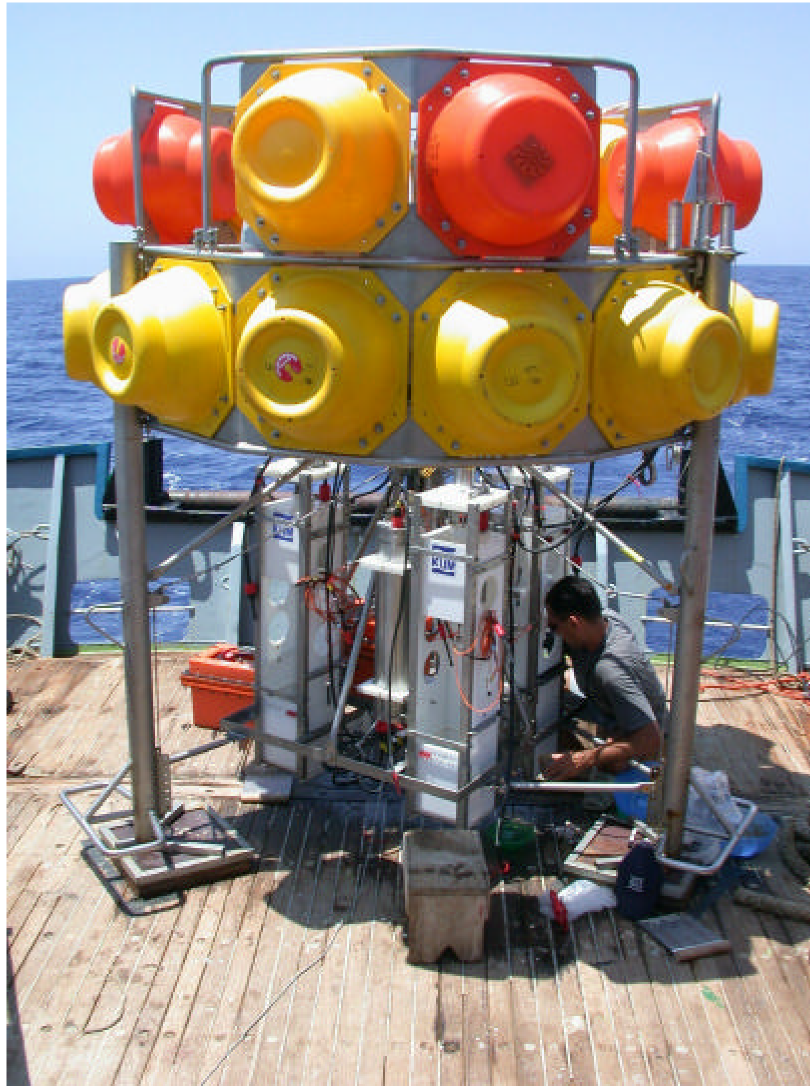

Figure 6: The chamber lander.

For our experiments it was equipped with 3 chambers of 20 × 20 cm, which enclose an area of the seafloor. Each chamber is equipped with a syringe water sampler containing seven glass syringes that take water samples of 50 ml from the overlying chamber water at pre-set time intervals. The chamber lid carries a central stirrer. Approximately 3 h after reaching the deep-sea floor to allow the resuspension plume to vanish, the chambers are driven into the sediment. After implementation of the chamber the lid is closed and the initial water sample is taken. Each chamber lid is additionally equipped with an injection unit for the inoculation with the labelled particulate organic matter (POM) at the beginning of the incubation, approximately 1 h after inserting the chambers into the sediment. In order to retrieve the sediment,

a shutter closes the chambers at the end of the experiments before they are slowly heaved out of the sediment. Afterwards the ballast can be released and the lander ascends to the surface to be recovered by the ship's winch.

In our experiments we injected different amounts of diatom carbon to investigate the influence of the quantity of settling phytodetritus on the benthic response. The syringe samples were analysed to assess oxygen depletion to calculate the sediment community oxygen consumption (SCOC) and to investigate the mineralisation of the added material to $^{13}CO_2$. Samples from the sediment were taken at different depth intervals for lipid analyses and porewater $^{13}CO_2$ to investigate benthic mineralisation and incorporation into different functional groups of bacteria (Chapter 5).

## Algae cultures

For our experiments on the benthic response to settling phytoplankton, an axenic, i.e. contaminant-free, labelled algae clone was precultured in the lab. The special focus on bacteria, which were investigated via their respective biomarkers, required the use of axenic algae cultures. *Ditylum brightwellii* (Bacillariophyceae, Biddulphiales) was used for the experiments in the North Sea, where it is reported to form blooms in spring (Raabe *et al.*, 1997). *Thallassiosira rotula* (Bacillariophyceae, Thallassiosiraceae) was utilised for the Crete experiments, which was isolated from the Mediterranean (http://ccmp.bigelow.org). Axenic clones were cultured in an artificial seawater medium (Grasshoff, 1999) containing 25% $^{13}C$ enriched hydrogencarbonate (99% $NaH^{13}CO_3$, Cambridge Isotope Laboratories). The seawater was enriched with f/2 medium (Guillard and Ryther, 1962).

## Stable carbon isotope analyses

Stable carbon isotope compositions of the fatty acid fractions were determined by gas chromatography - *combustion line* - isotope ratio mass spectrometry (GC-*c*-IRMS, Figure 7).

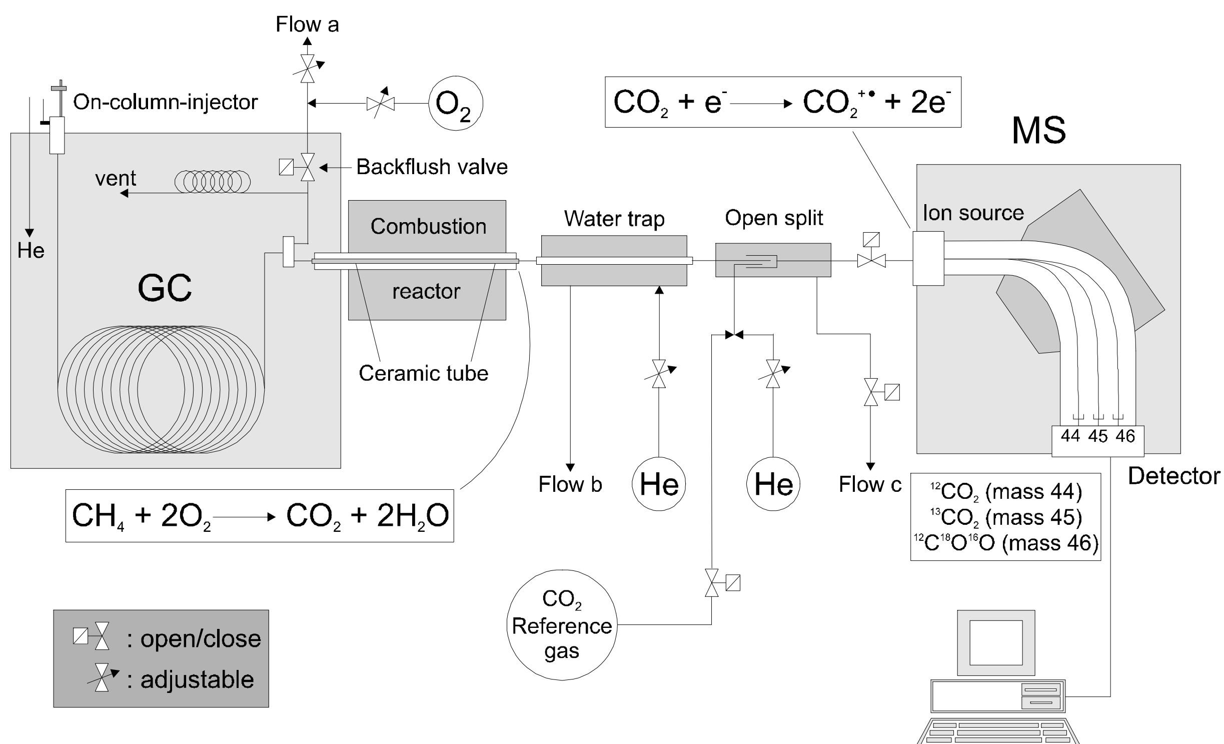


Figure 7: Schematic GC-*c*-IRMS set-up used for the determination of stable carbon isotopes on individual biomarkers.

This technique allows measurements of nanomolar quantities of samples. The GC was equipped with a 50 m HP-5 column (0.32 mm ID, 0.17 µm stationary phase) The carrier gas was He (1.5 ml min$^{-1}$). Initial oven temperature was 60°C held for 1 min, increased to 150°C with 10°C min$^{-1}$, then to 310°C with 4°C min$^{-1}$ and finally kept at 310°C for 15 min. The mass spectrometer and the GC were connected via a combustion interface, which consisted of a ceramic reactor (0.5 mm ID) containing two oxygenated copper wires and one platinum wire (0.1 mm OD each). The oxidation reactor was heated to 940°C to convert the organic compounds

quantitatively to carbon dioxide and water. The oxidising power of the combustion reactor was regularly regenerated by oxygenation of copper at 500°C.

The carbon dioxide produced in the reactor continuously enters the ion source of the mass spectrometer. Ion currents for m/z 44, 45, and 46 resulting from ionisation of carbon dioxide were simultaneously measured using triple Faraday cups connected to high speed amplifiers (Ricci *et al.*, 1994, Merrit *et al.*, 1996). Carbon isotope ratios were determined by integration of each chromatographic peak of all three ion currents (Freeman *et al.*, 1994) and reported in the δ notation as per mil (‰) deviation relative to the Pee Dee Belemnite standard (PDB):

$$\delta\,^{13}C\ (‰) = \left( \frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{standard}} - 1 \right) \times 10^3$$

The integrated ion currents were corrected for background contributions, differences in elution time of m/z 44 and 45 due to separation of the carbon isotope species $^{13}C$ and $^{12}C$ on the chromatographic column (Hayes *et al.*, 1990), and for contributions of $^{17}O$ to m/z 45 (Santrock *et al.*, 1985). The fatty acids were converted to fatty acid methyl esters (FAMEs) using $BF_3$-methanol prior to analyses and therefore the results were corrected for the introduction of the additional carbon atom. *n*-$C_{26}$ was used as injection standard with known isotopic composition (δ=-34.35‰) and $CO_2$-calibration was performed at the start and at the end of each run. Data acquisition and processing were managed using ISODAT software (Version 5.0 and upgrade; Finnigan).

For the calculation of the total incorporation, it is necessary to elucidate the concentrations of the individual biomarkers as well. For this purpose measurements using gas chromatography coupled to a flame ionisation detector (GC-FID, Hewlett Packard 5890 Series II GC) were performed, whereas identification of unknown compounds was carried out using a Thermoquest Trace GC interfaced to a Finnigan Trace MS using the same conditions as given for GC-*c*-IRMS measurements. Double bond positions of fatty acids were determined by the analysis of dimethyl disulphide adducts, prepared after the method of Nichols *et al.* (1986).

The composition of organic carbon was measured by elemental analysis-isotope ratio mass spectrometry (EA-IRMS). The samples were combusted at 1,000°C with copper oxide as oxidant in a CE Instruments CHN-Analyser and the evolved carbon dioxide was passed online via an interface to IRMS (both ThermoFinnigan) after separation by gas chromatography from nitrogen oxides and water. Analyses were carried out by Dr. U. Struck (University of Munich).

$^{13}CO_2$ was measured using a Carlo Erba 1106 Elemental Analyser coupled online with a Finnigan Delta S isotope ratio mass spectrometer by Dr. L. Moodley and J. Nieuwenhuize from the Netherlands Institute of Ecology (NIOO) in Yerseke.

## OBJECTIVES OF THIS THESIS

The aim of this thesis was to investigate the benthic bacterial community of a shallow shelf area and a deep-sea study site and furthermore reveal the benthic response to settling phytoplankton blooms.

Chapter 2, 3 and 4 deal with the North Sea study site. The first study (Chapter 2) gives insights into the bacterial community structure of three North Sea stations with different permeabilities. The investigations were carried out using lipid biomarker analysis and fluorescence *in situ* hybridisation (FISH). The bacterial community structure was investigated for its depth distribution accompanied by the working hypothesis that higher permeability and thereby increased oxygen penetration induces a change in the bacterial distribution in favour of aerobic metabolisers. Furthermore, the integrated approach of two independent techniques should provide information on their combined applicability for *in situ* studies. The results are interpreted with emphasis on the special characteristics of permeable sediments, which tend to mix up bacterial communities that would otherwise be separated.

For the second study (Chapter 3) experiments were conducted at the same sandy sediment stations to ascertain the influence of advection on transport and degradation of algae.

The third study (Chapter 4) was undertaken to investigate the benthic response pattern to a settling phytoplankton bloom. Therefore *in situ* and on-board experiments at the fine sand station were conducted. The major goal of the North Sea experiments was to investigate the reaction of different benthic compartments over depth and time (Figure 8). The working hypotheses were first, that advective porewater flows provide deeper sediment layers with suspended phytoplankton. Second, that bioturbating animals contribute to the downward transport as well and, that animals and bacteria inhabiting these layers take up the material into their biomass and furthermore metabolise it and thereby contribute to the large turnover of carbon in sandy sediments.

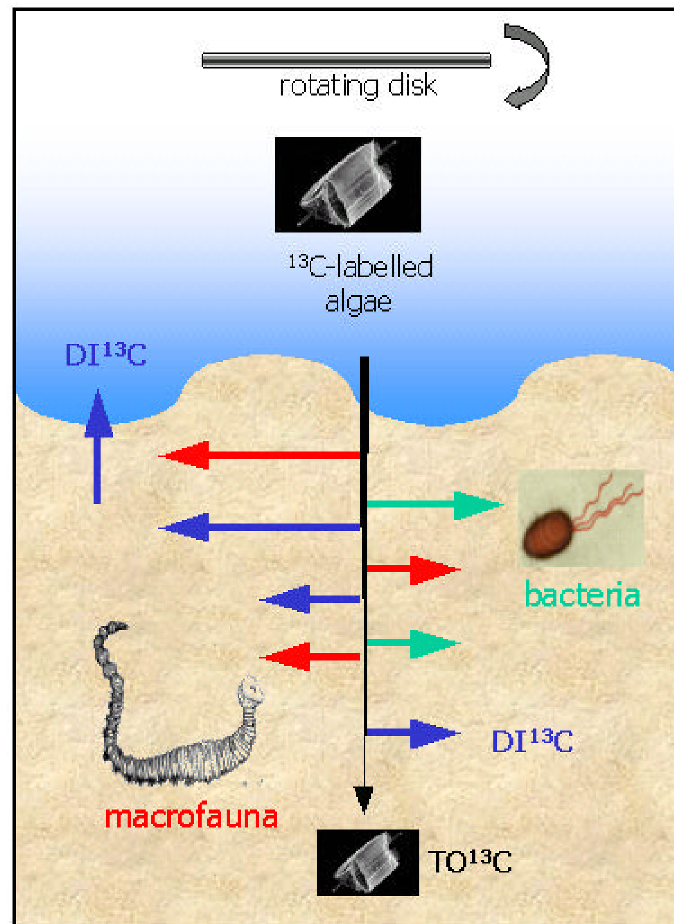


Figure 8: Schematic of the chamber experiments.

Chapter 5 presents a study on the response pattern of a deep-sea benthic community on varying amounts of settling algal carbon. These investigations took place in the oligotrophic deep Cretan Sea. The working hypothesis behind these experiments was that the reaction varies with respect to the amount of settling material. In order to investigate the difference in benthic response, the sediment community oxygen consumption, the mineralisation and the incorporation into bacterial fatty acids were determined after the addition of a small and a large amount of carbon. The large amount was comparable to half of the yearly export production at this study site. These data were used to outline the special adaptation of this oligotrophic deep-sea community.

The results are helpful to improve the understanding of the role of bacteria in marine sediments. The experimental data were interpreted with respect to the carbon cycle in shelf and deep-sea sediments.

## PUBLICATIONS OUTLINE

This thesis includes four articles. One is accepted, two are submitted and one is in preparation. One article deals with distributional patterns of bacteria in three permeable North Sea sands (Chapter 2), two articles are based on $^{13}C$-labelled algae experiments in the North Sea, investigating the transport and degradation of the added material (Chapter 3) and the reaction of the benthic community (Chapter 4). The last article investigates the response of a deep-sea benthic community in the oligotrophic Cretan Sea to varying amounts of settling phytoplankton (Chapter 5).

North Sea studies:

- Chapter 2: S.I. Bühring, Elvert M. & Witte U.

**The microbial community structure of different permeable sandy sediments characterised by the investigation of bacterial fatty acids and fluorescence *in situ* hybridisation.**

This study was initiated by U. Witte. The sampling was carried out by S.I. Bühring and U. Witte. S.I. Bühring performed the analyses, evaluated the data and wrote the manuscript with editorial help and input by M. Elvert and U. Witte. This article has been submitted to *Environmental Microbiology*.

- Chapter 3: S. Ehrenhauss, Witte U., Bühring S.I. & Huettel, M.

**Effect of advective pore water transport on distribution and degradation of diatoms in permeable North Sea sediments.**

U. Witte initiated this study. S.I. Bühring, S. Ehrenhauss and U. Witte carried out the experiments. S. Ehrenhauss evaluated the data and wrote the manuscript with editorial help and input from M. Huettel and U. Witte. This article has been accepted by *Marine Ecology Progress Series*.

- Chapter 4: S.I. Bühring, Ehrenhauss S., Kamp A., Moodley L. & Witte U.

**POC processing in a sublittoral sandy sediment: pulse-chase experiments with $^{13}C$-labelled diatoms.**

U. Witte initiated this study. S.I. Bühring, S. Ehrenhauss, A. Kamp and U. Witte carried out the experiments. S.I. Bühring evaluated the data and wrote the manuscript with editoral help and input by U. Witte. This article has been submitted to *Marine Ecology Progress Series*.

Deep-sea study:

- Chapter 5: S.I. Bühring, Moodley L., Lampadariou N., Tseledipes A. & Witte U.

**Benthic response to varying food input: *in situ* pulse-chase experiments in the deep Cretan Sea (Eastern Mediterranean).**

U. Witte initiated this study. Experiments were carried out by U. Witte, S.I. Bühring, L. Moodley, and N. Lampadariou. S.I. Bühring performed the analyses of the fatty acids and the data evaluation. The manuscript has been written by S.I. Bühring with editorial help and input from U. Witte. This article will be submitted to *Limnology and Oceanography*.

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**Chapter 2**

# THE MICROBIAL COMMUNITY STRUCTURE OF DIFFERENT PERMEABLE SANDY SEDIMENTS CHARACTERISED BY THE INVESTIGATION OF BACTERIAL FATTY ACIDS AND FLUORESCENCE *IN SITU* HYBRIDISATION

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## SUMMARY

This study describes the microbial community structure of three sandy sediment stations in the German Bight of the Southern North Sea that differ with respect to median grain size and permeability. The microbial community was investigated using lipid biomarker analyses and fluorescence *in situ* hybridisation. For further characterisation we determined the stable carbon isotope composition of the biomarkers.

Bacterial biomass was 63 to 170 µg $ml^{-1}$ on the fine sediment, as opposed to 17 and 7 µg $ml^{-1}$ for the medium and coarse sand.

The fatty acid analyses identified biomarkers of different bacterial groups such as members of the *Cytophaga-Flavobacterium* cluster and sulfate reducing bacteria (SRB). The depth profiles of bacterial fatty acid relative abundance revealed pronounced subsurface maxima for the fine sediment. Although oxygen penetrates deeper into the permeable sediments, the SRB biomarkers are similarly abundant, indicating suboxic to anoxic niches in these environments. This finding could be supported by investigations using different fluorescent *in situ* hybridisation probes, targeting *Cytophaga-Flavobacterium*, γ-Proteobacteria, and different members of the SRB. We detected SRB in all sediment types as well as in the surface and at greater depth, which suggests that SRB play a more important role in oxygenated marine sediments than previously thought.

## Key words

North Sea, benthos, sandy sediment, lipid biomarkers, fluorescence *in situ* hybridisation

## INTRODUCTION

Sandy sediments cover large areas of the continental shelf, which is located between the shoreline and the continental slope. During the last decade great scientific effort was paid on the investigations of this environment. Huettel and Gust (1992) were among the first to describe that the interaction of bottom currents with sediment topography results in pressure gradients that induces an advective porewater flow, an exchange process exclusively found on permeable sediments. Sandy sediments with a permeability exceeding $10^{-12}$ $m^2$ are generally thought to allow advective pore water transport (Huettel and Gust, 1992) and thereby supporting aerobic and suboxic metabolism in deeper sediment layers. This process may often homogenise otherwise separated microbial populations by inducing changes in oxygen distribution. Despite the low bacterial abundances of sandy sediments compared to finer compartments (Llobet-Brossa *et al.*, 1998), bacterial assemblages in sandy sediments are characterised by high turnover rates (Rusch *et al.*, 2000; Huettel and Rusch, 2000), which is also reflected by high oxygen consumption rates in permeable sediments (Janssen *et al.*, subm.).

However, little attention has as yet been paid to the composition of the microbial community inhabiting permeable sediments. Rusch *et al.* (2003) studied the spatiotemporal variation and metabolic activity of the microbial community in a coarse-grained sediment and found members of different aerobic and anaerobic bacteria generally low abundant, but with high turnover rates. For the investigation of the composition of the bacterial community fluorescence *in situ* hybridisation (FISH) was applied, using group-specific fluorescently labelled rRNA-targeted oligonucleotides. This new and promising approach of *in situ* detection has the advantage that it does not rely on isolation and cultivation. Since the first descriptions (Amann *et al.*, 1990) and applications of this method (Llobet-Brossa *et al.*, 1998), FISH has often been used to quantify microbial communities in marine (e.g. Ravenschlag *et al.*, 2001; Mußmann *et al.*, subm.) and freshwater sediments (Altmann *et al.*, 2003).

Another approach to investigate bacterial communities *in situ* is the analysis of specific lipid biomarkers (Rajendran *et al.*, 1992; Hinrichs *et al.*, 1999, Rütters *et al.*, 2002a.). The isolation and identification of fatty acids is a good tool, because bacteria synthesise highly specific components, like branched chain fatty acids (Kaneda, 1991). A pioneering work on fatty acid composition of several bacterial strains was done by Dowling *et al.* (1986), followed by different scientists transferring the knowledge from culture analysis to the interpretation of natural prokaryotic communities (e.g. Rajendran *et al.*, 1992; Oude Elferink *et al.*, 1998; Orphan *et al.*, 2001; Wakeham *et al.*, 2003). The information gained from biomarker studies could further be increased by simultaneous determination of the stable carbon isotope composition. Natural abundance studies use the small difference in isotopic ratios as found in nature (Peterson, 1999; Hayes, 2001). Carbon stable isotopes are principally useful to distinguish between marine and terrestrial organic matter sources and to identify different origins in the food web. The carbon source as well as the discrimination between $^{13}C$ and $^{12}C$ accompanying enzymatic reactions affect the stable carbon isotope composition.

So far, only a few studies used the combination of molecular and biomarker approaches (e.g. Orphan *et al.*, 2001; Elvert *et al.*, 2003), which provide an independent verification of the bacterial distributions *in situ*. In this study, we combined lipid biomarker analysis with investigations targeting bacterial taxa using FISH probes to study microbial communities on three different permeable sands in the southern North Sea (Figure 1). The aim of this study was to get a deeper insight into the vertical colonisation patterns of bacteria on sandy sediments and its relationship to sediment permeability.

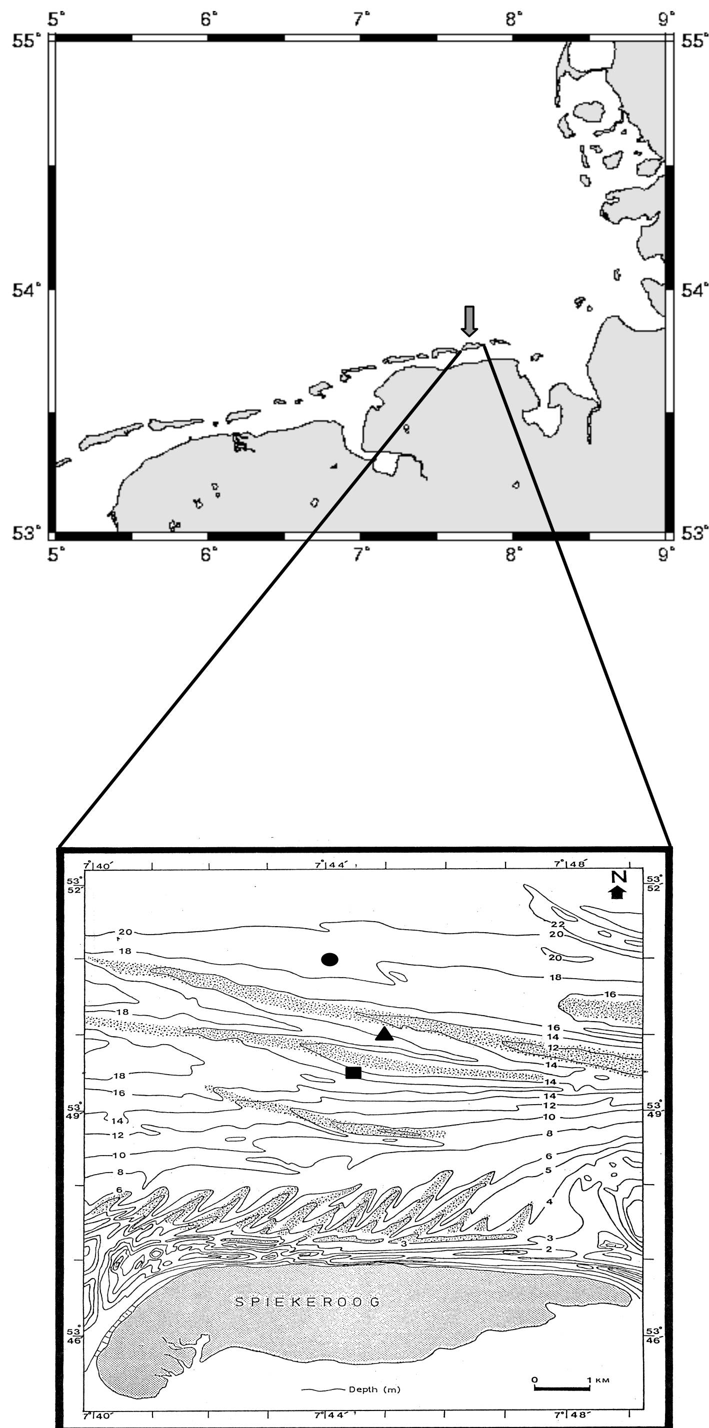


Figure 1: Location and bathymetry of the three stations in the German Bight (southern North Sea) as given by Antia (1993). The circle indicates the fine sand, the triangle the medium and the square the coarse sand.

## RESULTS AND DISCUSSION

### General observations

Bacterial biomass varied distinctly during the investigation of the three sands. 169.6 ± 36 µg $ml^{-1}$ and 63 ± 10 µg $ml^{-1}$ were found on the fine sand, during April and June, respectively. On the medium sand the bacterial biomass accounted for 16.7 ± 16 µg $ml^{-1}$ and on the coarse for 6.5 µg $ml^{-1}$. This increasing biomass on the finer sands can be explained by the larger specific surface area (Dale, 1974). Generally decreasing abundances of bacteria on coarser grained sediments were as well documented by Llobet-Brossa *et al.* (1998). However, bacteria in permeable sediments show high activities despite their low abundances, visible e.g. in high ammonification rates in the porewater (Ehrenhauss *et al.*, in press). Advective porewater exchange occurring in sediments with a permeability exceeding $10^{-12}$ $m^2$ (Huettel and Gust, 1992) is probably causing this high activity in sandy sediments. The accompanying porewater movements provide a fast carrier for the exchange of substances between the water column and the upper sediment layers. Investigations of by Janssen *et al.* (subm.) on the porewater exchange in the same three sediments using the autonomous Lander system SANDY, revealed nearly no advective exchange processes for the fine sand, but tremendously increasing values for the medium and the coarse sands. This circulation of water through the sediment matrix leads to enhanced solute transport processes.

### Fatty acid distribution pattern and stable carbon isotopes

The investigation of the fatty acid composition revealed 23 fatty acids on the three sands. Figure 2 shows the relative abundances of fatty acids from the depth ranges 0-3 cm and 9-10 cm from the three sands. The dark bars indicate fatty acids of presumably bacterial origin. The profiles were dominated by the even chained fatty acids $C_{16:1\omega7}$, $C_{16:0}$, $C_{18:1\omega7}$, and $C_{18:1\omega9}$. $C_{14:0}$, *ai* $C_{15:0}$, *i* $C_{15:0}$ and 10*Me*-$C_{16:0}$ follow in abundance. Other fatty acids could only be found in minor amounts. No cyclic fatty acids were detected.

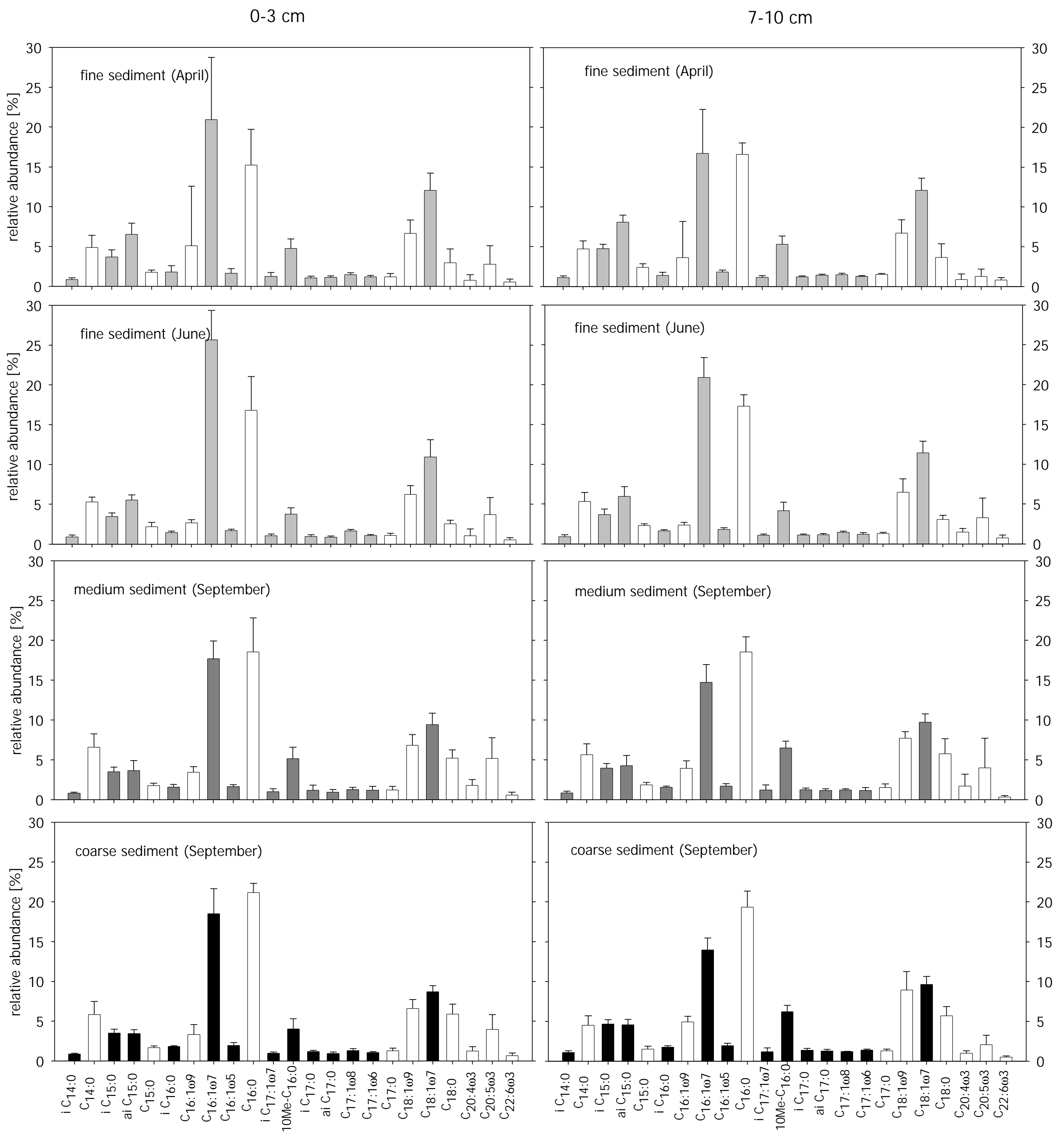


Figure 2: Relative abundance [%] of fatty acids of the three sands. The fine sand was investigated during April and June and the medium and coarse sands during September. The distribution in 0-3 cm sediment depth is plotted on the left and in 7-10 cm sediment depth on the right. The vertical bars indicate S.D.

The profiles determined reflect a prokaryotic dominated community, because mainly fatty acids in the $C_{14}$ to $C_{18}$ range were detected, which are described as characteristic for bacteria (Lechevalier, 1977). These general patterns are comparable to other marine environments (e.g. Rajendran *et al.*, 1992; Boschker *et al.*, 1999; Rütters *et al.*, 2002a.).

The most abundant fatty acids $C_{16:1\omega7}$ and $C_{16:0}$ are generally common in marine sediments (e.g. Rütters *et al.*, 2002a.; Rütters *et al.*, 2002b.; Boschker *et al.*, 2001). $C_{16:0}$ is ubiquitous in marine life forms, is extensively biosynthesised *de novo* and therefore not suitable for biomarker studies (Sargent and Whittle, 1981). Mono-unsaturation at the $\omega$7 position is generally considered as typical for bacteria (Sargent and Whittle, 1981). $C_{16:1\omega7}$ is a constituent of some algae, but could also be found in bacteria; White *et al.* (1996) suggested it as indicative for *Cytophaga*-like bacteria. Members of the *Cytophaga-Flavobacterium* cluster (taxonomically reclassified into the subgroup Bacteroidetes) are described as mostly aerobic, gram-negative bacteria which are specialised in the degradation of complex macromolecules (Reichenbach, 1992) and adapted to low nutrient levels (Höfle, 1983; Stoeck *et al.*, 2002). *Flavobacteria* are also described as obligate anaerobic, fermenting sugars to primarily acetate and succinate. The isotopic signal of –27.1‰ for $C_{16:1\omega7}$ displays a mixed origin, probably terrigenious and marine (Fry and Sherr, 1984) on the fine sand during April with a shift to a marine phytoplankton signal during June with –24.9‰ (Table 1). This is probably caused by the spring phytoplankton bloom generally occurring between March and June in the southern North Sea (Boon *et al.*, 1998). The isotopic composition of $C_{16:1\omega7}$ on the medium sand during September revealed again a shift to higher values (–21.5‰), which is a clear reflection of the marine phytoplankton signal (Fry and Sherr, 1984).

Branched-chain fatty acids such as *i* $C_{15:0}$, *ai* $C_{15:0}$, and *i* $C_{16:0}$ are major constituents of gram-positive bacteria according to White *et al.* (1996) but are also present in other anaerobic bacteria (Findlay and Dobbs, 1993). Our study revealed higher relative abundances for *ai*$C_{15:0}$ compared to *i*$C_{15:0}$ on the fine sand during both seasons and in the upper and lower part of the sediment (Figure 2).

Table 1: Carbon isotope ratios [‰] of different investigated bacterial fatty acids on the fine and medium sediment (medium values from measurements over the whole sediment sampling depth are presented, numbers in brackets indicate S.D.).

| bacterial fatty acid | fine sediment (April) | fine sediment (June) | medium sediment (September) |
|---|---|---|---|
| $iC_{14:0}$ | –23.3 (±1.0) | –20.8 (±2.2) | –20.5 (±1.9) |
| $iC_{15:0}$ | –22.6 (±1.9) | –20.1 (±0.6) | –18.4 (±1.0) |
| $aiC_{15:0}$ | –21.6 (±1.4) | –19.6 (±0.6) | –21.4 (±0.4) |
| $iC_{16:0}$ | –23.4 (±1.8) | –25.6 (±2.7) | –19.0 (±1.8) |
| $C_{16:1\omega7}$ | –27.1 (±1.0) | –24.9 (±0.8) | –21.5 (±1.7) |
| $C_{16:1\omega5}$ | –35.1 (±4.7) | –30.7 (±1.6) | –24.1 (±1.5) |
| $iC_{17:1\omega7}$ | –14.6 (±1.3) | –19.0 (±0.8) | –17.6 (±0.9) |
| 10*Me*-$C_{16:0}$ | –23.7 (±1.0) | –22.2 (±0.8) | –22.5 (±1.1) |
| $iC_{17:0}$ | –21.8 (±1.3) | –21.3 (±2.2) | –21.1 (±1.4) |
| $aiC_{17:0}$ | –18.6 (±2.5) | –16.2 (±1.8) | –17.9 (±1.6) |
| $C_{17:1\omega8}$ | –24.6 (±1.9) | –21.1 (±0.9) | –24.4 (±1.9) |
| $C_{17:1\omega6}$ | –24.8 (±1.4) | –22.1 (±0.9) | –23.0 (±1.2) |
| $C_{18:1\omega7}$ | –30.0 (±1.1) | –25.5 (±0.6) | –24.4 (±1.1) |

Nevertheless, with increasing grain size the relative amount of *ai* $C_{15:0}$ subsequently decreased, displaying equal values for both isomers on the coarse sediment. Analyses of different genera of sulfate reducing bacteria (SRB) revealed that the *anteiso*-form was always more abundant than the *iso*-isomer (Rütters *et al.*, 2001; Rütters *et al.*, 2002 b.). Therefore, the predominance of *ai* $C_{15:0}$ could be an indicator of SRB occurrence on the finer sand. Nevertheless, the stable carbon isotope ratios of *i* $C_{15:0}$ and *ai* $C_{15:0}$ of approximately –20‰ revealed in general a marine signal, indicating that the accompanying bacteria are no chemoautotrophs (Degens, 1969; Ruby *et al.*, 1987).

The mesophilic Gram-negative SRB form coherent groups within the δ-subdivision of the proteobacteria. Findlay and Dobbs (1993) stated that 10*Me*-$C_{16:0}$, *i* $C_{17:0}$, and *ai* $C_{17:0}$ are typical for SRB. Furthermore, 10*Me*-$C_{16:0}$ is considered to be indicative for *Desulfobacter* species by Dowling *et al.* (1986) and for *Desulfobacteriaceae* in general by Rütters *et al.* (2002 b.) and Kuever *et al.* (2001). This fatty acid was present with relative abundances of 4.0 to 5.1% in the upper layer and 4.1 to 6.5% in the deeper sediment layer of all investigated sands (Figure 2).

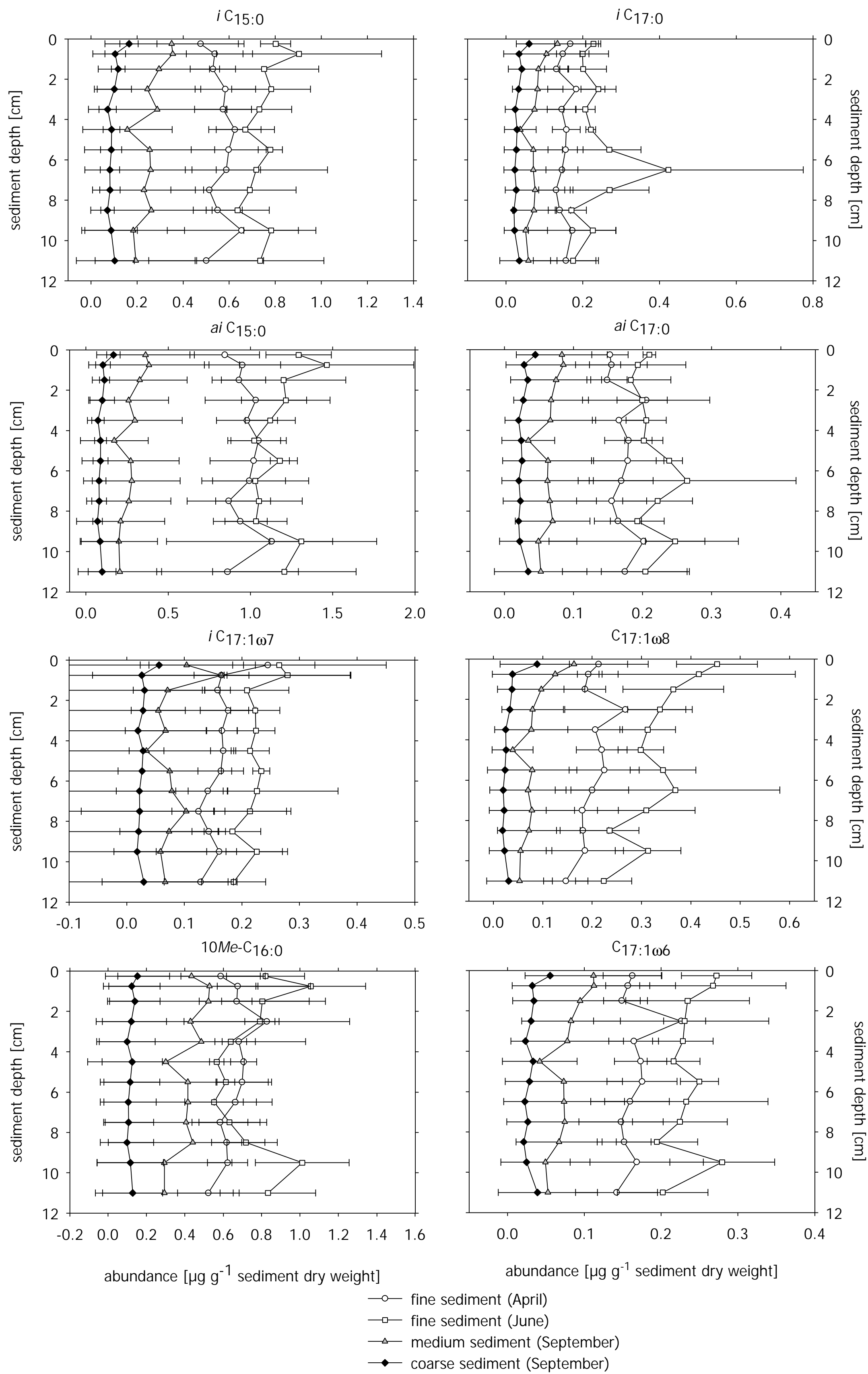


Figure 3: Depth distribution of different bacterial fatty acids on the fine sand in April and June and on the medium and coarse sands during September. The horizontal bars indicate S.D.

Absolute concentrations of $10Me\text{-}C_{16:0}$ (Figure 3) revealed pronounced subsurface peaks on the fine and medium sand. The formation of vertical layers most probably reflects different ecological niches of the respective groups of bacteria, induced by stronger stratification of e.g. nutrients on the finer grained sediment types. The conditions on the coarse sediment seemed to be less varying with depth, probably caused by strong advective flushing. The $\delta^{13}C$ measurements of $10Me\text{-}C_{16:0}$ generally reflect a marine origin with values around –22.8‰, without pronounced differences between the investigated sands or seasons.

The fatty acids $i\,C_{17:1\omega7}$ and $ai\,C_{17:0}$ are relatively common in *Desulfosarcina* (Rütters *et al.*, 2001). These fatty acids accounted for approximately 1% of all fatty acids present in our study, and showed slightly elevated concentrations in the deeper sediment layer (Figure 2). The concentrations versus sediment depth given in Figure 3 revealed comparable behaviour with a subsurface peak at 9.5 cm. Only in the upper sediment layers differences are visible, with higher concentrations for $i\,C_{17:1\omega7}$ for the medium sand in general and the fine sand during June. Another indicator for a similar origin of these two fatty acids is provided by the results obtained from $\delta^{13}C$ measurements (Table 1). Both displayed the most $^{13}C$ enrichment from all investigated bacterial fatty acids with mean values of –17.1 and –17.6% for $i\,C_{17:1\omega7}$ and $ai\,C_{17:0}$, respectively. This is probably due to a different origin and or a discrete way of synthesis, compared to the other investigated bacterial fatty acids.

Members of the family *Desulfosarcina* are nutritionally rather versatile complete oxidisers (Widdel and Bak, 1992). $I\,C_{17:1\omega7}$ is also described as a marker fatty acid for *Desulfovibrio* (Coleman *et al.*, 1993; Llobet-Brossa *et al.*, 2002; Taylor and Parkes, 1983), which could provide an explanation for the slight discrepancy described before with surface and subsurface peaks that could not been observed for $ai\,C_{17:0}$. Members of the genus *Desulfovibrio* have been shown to be able to respire with oxygen (Dilling and Cypionka, 1990), which would provide an explanation for the higher occurrence of $i\,C_{17:1\omega7}$ in upper sediment layers, although they do not seem to grow under oxic conditions (Dannenberg *et al.*, 1992).

One of the most common fatty acids with slightly higher relative shares on the fine sand was $C_{18:1\omega7}$. This fatty acid can be found in several bacteria, e.g. in

*Desulfomicrobium* sp. (Rütters *et al.*, 2002 b.), but is also formed in plants and animals by elongation from $C_{16:1\omega7}$. The same study by Rütters *et al.* (2002 b.) showed that $C_{17:1\omega8}$ is another indicator for *Desulfomicrobium*, where it made up to 7% of the total fatty acids. *Desulfomicrobium* is described as commonly using lactate, pyruvate and ethanol, which are incompletely oxidised to acetate (Widdel and Bak, 1992). $C_{17:1\omega6}$, on the other hand, is a major fatty acid in *Desulfobulbus* spp. (Taylor and Parkes, 1983) and in *Desulforhabdus amnigenus* (Oude Elferink *et al.*, 1998). *Desulfobulbus* spp. are considered to be specialised in the oxidation of propionate (Widdel and Bak, 1992) and till now were only detected well below the oxic surface (Sahm *et al.*, 1999). We found maximum values for $C_{17:1\omega6}$ on the fine sediment in 2.5 cm and 9.5 cm sediment depth in April and June, respectively, and this fatty acid was also detected on the medium and coarse sediments, even though only in low amounts. Knoblauch *et al.* (1999) found $C_{17:1\omega6}$ accounting for over 60% in a *Desulforhopalus* species. *Desulfofrigus* was investigated by Knoblauch *et al.* (1999) as well and its main fatty acids were $C_{16:1\omega7}$ and $C_{18:1\omega7}$. These fatty acids were also two major constituent of our sediments, ranging between 14 and 25% for $C_{16:1\omega7}$ and 9 and 12% for $C_{18:1\omega7}$, indicating *Desulfofrigus* species at our study sites. However, we can not exclude an at least partly diatom origin of $C_{16:1\omega7}$ fatty acid (Kharlamenko *et al.*, 1995), even though the depleted $\delta^{13}C$ values of –30‰ make an exclusive phytoplankton source unlikely.

## Microbial ecology of sandy sediments

Figure 4 displays the percentages of Bacteria detected with probe EUB 338 in comparison to all bacteria detected by DAPI counts. Up to 65% of the DAPI stained cells hybridised with EUB 338. But only in the upper layers of the fine and coarse sediments the share of EUB 338 was higher than 50% whereas in the deep layer of the coarse sediment the fraction of detectable bacteria was very low.

With a set of six probes for mayor phyla within the domain Bacteria, we could affiliate between 19 and 70% of the EUB 338 counts with known bacterial groups. These probes were chosen according to hybridisation results gained on a nearby

sandy site (M. Mussmann, pers. comm.). The relative amounts of the different bacterial groups targeted are given in Figure 5 (see Table 3 for probe specificity). Members of the *Cytophaga-Flavobacterium* cluster were detected on the fine sediment in both sediment layers analysed. On the medium sand they could only be detected in the lower part and on the coarse sediment they were negligible. On the other hand, *Cytophaga* were found to be the largest fraction in sandy sediments of the nearby Jadebusen Bay by Llobet-Brossa *et al.* (1998). Rusch *et al.* (2003) obtained the same result from a coarse-grained Middle Atlantic Bight shelf sediment.

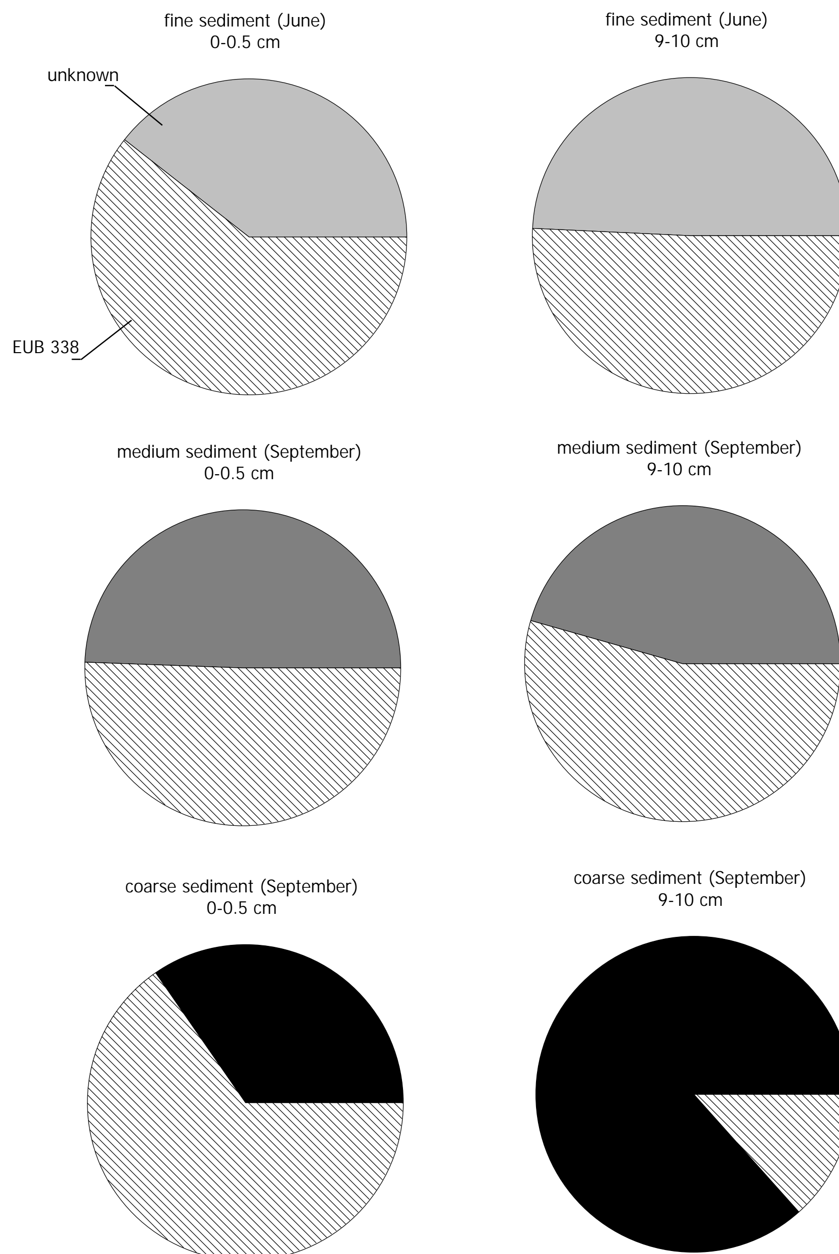


Figure 4: Share of EUB 338 targeted cells from all DAPI counts of the three different sands in 0-0.5 cm depth on the left and in 9-10 cm depth on the right.

In our investigation they seem to play a role only on the fine sand and in the lower part of the medium sand. A typical fatty acid for *Cytophaga-Flavobacterium* is $C_{16:1\omega7}$ (White *et al.*, 1996), which shows similar to the FISH results higher relative amounts on the fine sediment, furthermore stressing their great importance in this sediment type.

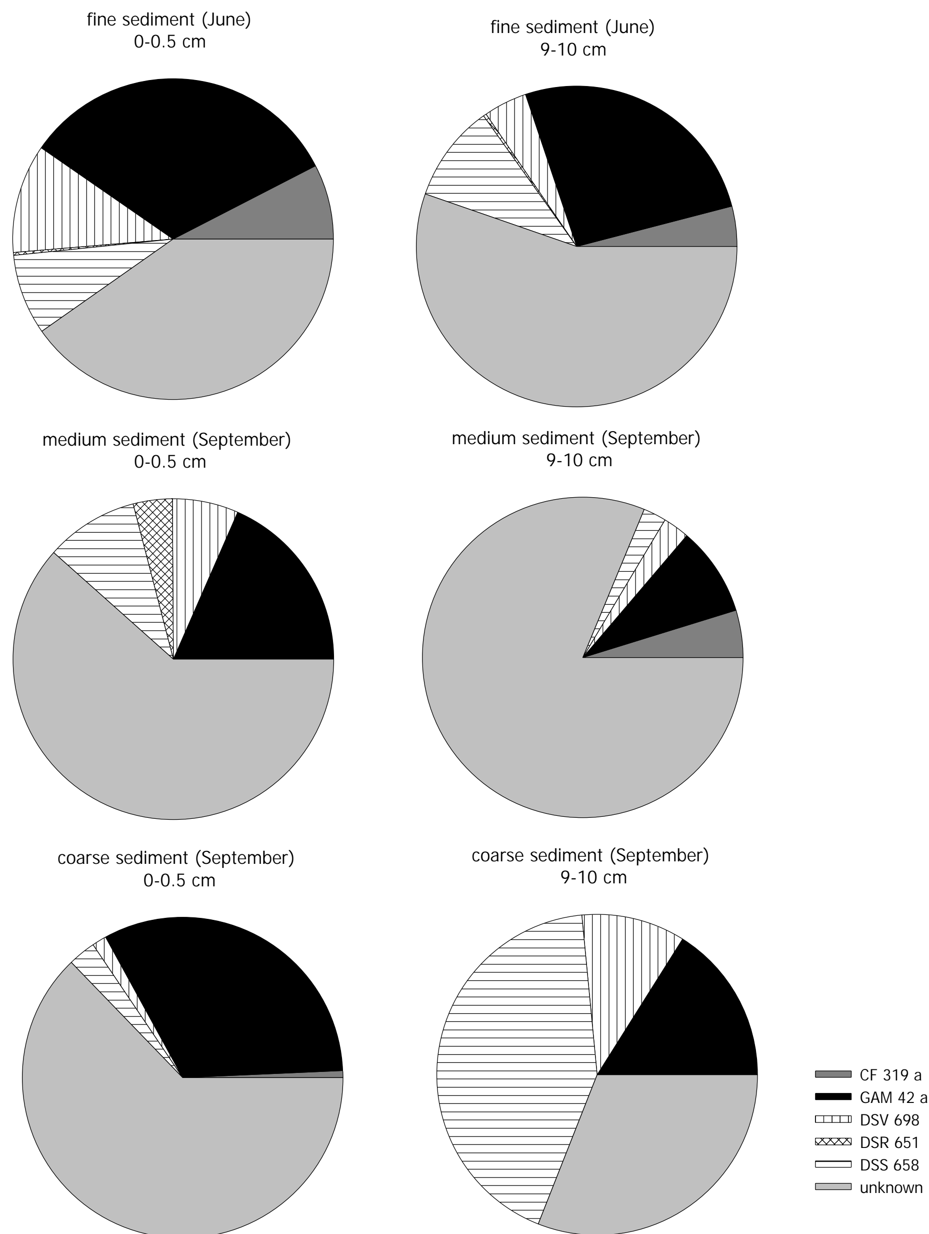


Figure 5: Relative share of the different FISH probes to all EUB 338 counts.

Members of the γ-Proteobacteria detected with the probe GAM 42a were found at all stations and investigated sediments depths ranging from 9-33% of all EUB 338 counts. The fraction of GAM 42a in the upper sediment layer was always greater than in the lower part. Some members of this group are sulfur oxidising bacteria, with members of the genera *Thiomicrospira* (Kuenen *et al.*, 1992; Brinkhoff *et al.*, 1998; Brinkhoff *et al.*, 1999) or *Thiobacillus* (Robertson and Kuenen, 1992) frequently isolated from marine sediments. Considerable amounts of members of this group were as well targeted by Ravenschlag *et al.* (2001) in marine Arctic sediments. SRB of the δ-Proteobacteria were detected using three specific probes (see Table 3). *Desulfovibrio* spp., detected by the probe DSV 698, was present in all samples. Highest shares were found in the upper fine and lower coarse sediment with 11% each and the lowest share was detected in the upper coarse sediment with only 1%. Using probe DSR 651 specific for *Desulforhopalus* species only small amounts were found in the fine sediment and in the upper fraction of the medium sediment, whereas they were absent in all other samples. *Desulfosarcina*, *Desulfofaba*, *Desulfococcus*, and *Desulfofrigus* species were targeted with probe DSS 658. We determined considerable amounts of this group in all samples, with an unexpected result found in the 9-10 cm horizon of the coarse sediment, where they accounted for 43% of all EUB 338 counts.

The FISH data clearly show abundant SRB in all investigated sediments. If we combine the three groups detected by the different probes DSV 698, DSR 651 and DSS 658, we obtain average shares of 6.4 and 4.9% for the upper and lower part of the fine sediment, 6.7 and 1.7% for the medium sediment and 1.4 and 49% for the coarse sand. The high value for the lower coarse sediment is probably an artefact and could be due to problems with the use of the EUB 338 probe caused by the sediment structure. This is also indicated by the high share of DAPI cells, which were not targeted by the EUB 338 probe in this sample (Figure 4). Our results are in good agreement with other studies using FISH. Llobet-Brossa *et al.* (1998), who investigated the microbial community of Wadden Sea sandy sediments from the nearby Jadebusen Bay using FISH, found a maximum of 6.5% of all DAPI counts belonging to SRBs.

The investigation of biomarker fatty acids from SRB supports our FISH findings as well. The highest share was always due to 10*Me*-$C_{16:0}$, which is described as typical for *Desulfobacter* species (Dowling *et al.*, 1986). Relative abundances of fatty acids of presumed SRB origin ($C_{16:1\omega5}$, *i*$C_{17:1\omega7}$, 10*Me*-$C_{16:0}$, *i*$C_{17:0}$, *ai*$C_{17:0}$, $C_{17:1\omega8}$, and $C_{17:1\omega8}$) made up a fraction of approximately 12-14%. Sahm *et al.* (1999) investigated an organic rich sediment (10-12% of the dry weight) of the Aarhus Bay for the abundance and depth distribution of SRB using slot-blot hybridisation of rRNA and found 18 to 25%. They specifically targeted *Desulfovibrio*, which we detected via its diagnostic fatty acid *i*$C_{17:1\omega7}$ and found comparable depth distribution pattern.

### Sulfate reducers under suboxic conditions

The analyses of the bacterial community using specific biomarkers and FISH exposed that SRB were abundant in all three sediment types and depths. Investigations of Ishii *et al.* (subm.) using FISH with catalysed reporter deposition (CARD-FISH) in a sandy core from the backbarrier area of the island Spiekeroog revealed high abundances of *Desulfobulbaceae* in the upper layers and *Desulfosarcinales* up to 40 cm depth. Mußmann *et al.* (subm.) stated that *Desulfosarcinales* are the numerically dominant SRB in sandy sediments of the Wadden Sea (German Bight). Moreover, several different strains of SRB were found in an intertidal sandy sediment in the oxic-anoxic interface by Wieringa *et al.* (2000), as well as they succeeded in isolating a *Desulfomicrobium* species. Together with our own investigations we suggest to reconsider the former believe of restriction of SRB to anoxic sediments.

Our results clearly suggest a stable community of SRB in all three investigated sediments, despite deep oxygen penetration on the medium and coarse sediment (Janssen *et al.*, subm.). This is an astonishing finding, in contrast to the former conviction, that SRB do not sustain under oxic conditions and die off more or less rapidly (Cypionka *et al.*, 1985). Some other authors also observed high abundances of SRB dislocated from black, reduced sediments. For example, Sahm *et al.* (1999) found a peak of rRNA from SRB in 1.5 cm depth even though the maximum of sulfate reduction was detected considerably deeper in the sediment. Investigations of Moeslund *et al.* (1994) leads to the assumption that sulfate reduction may be

underestimated by a factor of 5 in the oxidised zone of the sediment. In hypersaline microbial mats high sulfate reduction rates were detected and SRB were rRNA targeted in the presence of oxygen in (e.g. Canfield and Des Marais, 1991; Minz *et al.*, 1999).

Several SRB are known to grow on alternative electron acceptors, like e.g. Fe (III) (Coleman *et al.*, 1993) nitrate and oxygen (Dilling and Cypionka, 1990; Dannenberg *et al.*, 1992) or use special organic substrates for fermentation (Widdel and Hansen, 1992). Despite the oxygen tolerance, dissimilatory sulfate reduction has not yet been reported to occur in the presence of free oxygen.

Our results confirm the tremendous importance of sulfate reduction in coastal sediments as already stressed by Jørgensen (1982). He stated that the amount of organic matter mineralised by sulfate reduction is comparable to aerobic processes and we propose extension of this statement to oxygenated environments. These results may indicate that there is a so far unknown biochemical pathway for sulfate reduction or microbial interactions, the details of which are not yet known. Thus, further effort has to be put in accurate measurement of sulfate reduction rates in such environments, predicting the activity of SRB populations that were detected using descriptive methods.

## CONCLUSIONS

The three investigated sands revealed colonisation with aerobic bacteria like members of the *Cytophaga-Flavobacterium* cluster, and SRB. The close vicinity of different bacterial communities in permeable sediments is an interesting finding. Such communities would normally be separated between oxygenated and anoxic zones. The observed patterns are probably due to effective transport mechanisms (predominantly advection) which strongly influence benthic life in permeable sediments.

## EXPERIMENTAL PROCEDURES

### Sites and sampling

During three cruises with R.V. "Heincke" in April, June and September 2001, samples were taken from three subtidal stations in the southern North-Sea seawards the island Spiekeroog (Figure 1), which is part of the East Frisian barrier-island system fringing the southern North Sea coast of the Netherlands and Germany. This area is influenced by tides, waves, and storm-generated bottom currents (Antia 1995). Surficial sediments within the area mostly consist of quaternary sandy deposits and our three stations consist of fine, medium and coarse sands with median grain sizes of 164 µm (±1), 299 µm (±3) and 672 µm (±37) (Janssen *et al.*, subm.) and permeabilities of 3.02 (±1.66), 26.27 (±3.26) and 77.24 (±14.36) · $10^{-12}$ $m^2$, respectively. Station locations and sediment characteristics are summarised in Table 2. Salinity of the sampling site was around 33 psu.

Sediment cores were taken with a multiple corer equipped with four tubes with an inner diameter of 36 mm and additional cores of 200 mm diameter were taken by divers. Sediment cores were sliced at intervals of 0.5 cm down to 1 cm depth, and of 1 cm down to 12 cm depth. Each sediment horizon was carefully mixed and subsamples of 10 ml for lipid analyses were taken and directly stored frozen at –20°C.

From two sediment horizons (0-0.5 and 9-10 cm sediment depth) samples of 0.5 ml were taken for FISH analyses. The sediment was fixed for 2-4 h with formaldehyde (3 % final concentration), washed twice with phosphate-buffered saline solution (PBS) (10 mM sodium phosphate [pH 7.2], 130mM NaCl), and then stored in PBS:ethanol (1:1) until analysis.

Table 2: Station characteristics

| Cruise | Date | Position | Permeability k ($10^{-12}$ $m^2$) | Average water depth (m) | Water Temp. (°C) | Sediment POC (% dry mass) | Notes |
|---|---|---|---|---|---|---|---|
| **HE 145** | 08.-<br>18.04.01 | 53°51´N,<br>007°44´E | 3.02<br>(± 1.66) | 19 | 9 | n.a. | 6 cores (Ø200mm)<br>+ 2 cores (Ø 36mm) |
| **HE 148** | 07.-<br>15.06.01 | 53°51´N,<br>007°44´E | 3.02<br>(± 1.66) | 19 | 13 | 0.114<br>(± 0.014) | 2 cores (Ø200mm)<br>+ 2 cores (Ø 36mm) |
| **HE 154** | 24.-<br>30.09.01 | 53°50´N,<br>007°45´E | 26.27<br>(± 3.26) | 16 | 16 | 0.023<br>(± 0.003) | 5 cores (Ø200mm)<br>+ 2 cores (Ø 36mm) |
| | | 53°49.5´N,<br>007°44.5´E | 77.24<br>(± 14.36) | 14 | 16 | 0.032<br>(± 0.003) | 1 cores (Ø200mm)<br>+ 1 core (Ø 36mm) |

## Lipid analysis

Lipids were extracted from the wet sediment using dichloromethane-methanol and esterified following the protocol of Elvert *et al.* (2003).

Concentrations of fatty acid methyl esters (FAMEs) were determined by gas chromatography-flame ionisation detection in a Hewlett Packard 5890 Series II GC, equipped with a split/splitless injector operating in splitless mode. A 50 m apolar HP-5 fused silica capillary column was used (0.32 mm internal diameter, 0.17 µm film thickness; Hewlett Packard). The carrier gas was He at a constant flow rate of 2.0 ml $min^{-1}$. Initial oven temperature was 60°C held for 1 min, increased to 150°C with 10°C $min^{-1}$, then to 310°C with 4°C $min^{-1}$ and finally kept at 310°C for 15 min. The injector temperature was set at 300°C and the detector at 310°C. $C_{19:0}$ fatty acid was added prior to extraction as an internal standard for quantification. Identification of FAMEs was based on the comparison of retention time with known standards. Fatty acid short-hand nomenclature is according to Guckert *et al.* (1985).

Identification of unknown compounds was carried out using a Thermoquest Trace GC interfaced to a Finnigan Trace MS using the same conditions as given for gas chromatography. Mass spectra were collected in full scan (m/z 30-580, 0.9 scans per second), operating in electron impact mode at 70 eV. The samples were injected in splitless mode at an injection temperature of 295°C on a HP-5 MS fused silica capillary column (60 m, internal diameter 0.32 mm; Hewlett Packard) coated with a cross-linked methyl silicone phase (film thickness 0.25 µm). The detector was set at 350 V, the interface was 310°C and the carrier gas was He with a constant flow of 1.4 ml $min^{-1}$.

Double bond positions of fatty acids were determined by the analysis of dimethyl disulphide adducts, prepared after the method of Nichols *et al.* (1986).

Bacterial biomass was calculated using an estimation of Brinch-Iversen and King (1990), where 0.056 g of bacterial carbon fatty acid account for 1 g C-biomass. For calculation we used the bacterial fatty acids *i*$C_{14:0}$, *i*$C_{15:0}$, *ai*$C_{15:0}$, *i*$C_{16:0}$, $C_{16:1\omega7}$, $C_{16:1\omega5}$, *i*$C_{17:1\omega7}$, 10*Me*-$C_{16:0}$, *i*$C_{17:0}$, *ai*$C_{17:0}$, $C_{17:1\omega8}$, $C_{17:1\omega6}$ and $C_{18:1\omega7}$.

$^{13}C/^{12}C$ ratios of biomarkers were determined using GC-*c*-IRMS as previously reported (Elvert *et al.*, 2003). Briefly, the mass spectrometer (Finnigan Delta plus) was connected via a Finnigan Combustion Interface III to a HP 6890 Series GC equipped with a 50 m HP-5 (0.32 mm ID, 0.17 µm stationary phase, Hewlett-Packard). Samples were injected in splitless mode (injector temperature: 290°C) and subjected to the same temperature program as given for GC analyses. The $\delta^{13}C$ values reported have an analytical error of less than ±1.0% and were corrected for the introduction of the additional carbon atoms during derivatisation with $BF_3$-methanol.

## Fluorescence *In Situ* Hybridisation (FISH)

Samples were treated by mild ultrasonication according to Epstein and Rossel (1995) with a Bandelin Sonoplus HD200 (Berlin, Germany). Diluted samples were applied to gelatine-coated microscope slides and immobilised by air drying. *In situ* hybridisation was performed at 46°C for 2.5 h in a hybridisation buffer containing 0.9 M NaCl, 20 mM Tris-HCl (pH 7.4), 0.01% sodium dodecyl sulfate (SDS) and with different amounts of formamide (Table 3). Probe concentrations were 5 ng/µl. Probe Gam 42a was used with a competitor oligonucleotide. All oligonucleotides were purchased from Interactiva (Ulm, Germany) and synthesised with a Cy3 fluorochrome at the 5´-end. After hybridisation the buffer was removed and the slides were washed for 15 min at 48°C in a washing buffer (containing 20mM Tris-HCl [pH 7.4], concentration of NaCl depending on formamide concentration in the hybridisation buffer and 0.01% SDS). Washing buffer was removed with distilled water. Samples were stained with 4´, 6´-diamidino-2-phenylindole (DAPI) to determine the total cell counts. The slides were examined using an Axiophot microscope (Zeiss, Oberkochen, Germany). For each probe and sample 10-20 randomly chosen fields were counted, which corresponds to 700-1000 DAPI-stained cells. Counting results were corrected for subtracting signals observed with probe NON 338.

Table 3: Oligonucleotide probes used in this study

| Probe | Specificity | Formamid concentration in hybridisation buffer |
|---|---|---|
| **EUB 338** | Bacteria | 10% |
| **NON 338** | complementary probe to EUB 338 | 10% |
| **CF 319 a** | *Cytophaga-Flavobacterium* | 35% |
| **GAM 42 a** | γ-Proteobacteria | 35% |
| **DSV 698** | *Desulfovibrio* spp. | 35% |
| **DSR 651** | *Desulforhopalus* spp.<br>*Desulfocapsa* spp.<br>*Desulfobacterium catecholicum* | 35% |
| **DSS 658** | *Desulfosarcina* spp., *Desulfofaba* spp.,<br>*Desulfococcus* spp., *Desulfofrigus* spp. | 60% |

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## Chapter 3

# EFFECT OF ADVECTIVE PORE WATER TRANSPORT ON DISTRIBUTION AND DEGRADATION OF DIATOMS IN PERMEABLE NORTH SEA SEDIMENTS

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**ABSTRACT**

This contribution addresses the incorporation and degradation of diatoms in coastal fine, medium and coarse North Sea sands. During three cruises in 2001 to a highly dynamic, non-depositional area in the southern German Bight, the transport of $^{13}C$-labeled diatoms into these different permeable sand beds was assessed by *in-situ* and on-board chamber experiments. Enhanced advective transport of diatom frustules and $^{13}C$-enriched diatom carbon into sandy sediments with increasing permeability was demonstrated. Highest transport rates were observed in medium and coarse sand where 6% of the added algae were found below 1 cm after 20 h incubation time. In the coarse sand, the high ratio between sand grain and particle size enhanced the delivery of algae to the sediment, but seemed to reduce the filtration efficiency and thus algal retention.

Broken frustules of *Thalassiosira* sp., the diatom which dominated the diatom spring bloom 2001, were found in the medium and coarse sand in autumn. This indicates that advective transport and to some limited extent also bioturbation deposits phytoplankton into these sandy sediments, where strong bottom currents theoretically would prevent the sedimentation of low-density organic material. The trapped cells are rapidly degraded, as observed in our chamber experiments, where 28% of the added diatom carbon was released as DOC per day after the third incubation day.

We conclude that permeable sediments represent expansive coastal filter systems, where high advective flushing rates boost remineralisation of trapped algal cells. These processes promote a fast recycling of organic matter and, thus, may be important for maintaining high primary production rates in shelf environments.

**KEY WORDS**

German Bight – permeable shelf sediments – pore water flow – planktonic and benthic diatoms – $^{13}C$-labeling – remineralization – carbon cycling

## INTRODUCTION

The German Bight is a shallow region of the south-eastern North Sea with depths mainly between 20 and 40 m. This region is characterized by a high primary productivity and large standing stock of phytoplankton except during the winter months (Boon et al. 1998). Offshore of Spiekeroog Island, near-bottom current velocities range from 30 to 60 cm $s^{-1}$ (Antia 1993). In this high-energy environment, tides, waves and storm-generated bottom currents cause frequent sediment erosion, redeposition and lateral transport, resulting in coarse-grained, highly permeable sediments (Antia 1995). Consequently, organic particulate material also goes through many cycles of deposition and resuspension before it is finally completely mineralized or buried (Bacon et al. 1994). For the southern North Sea, it has been postulated that only small amounts of the primary production are incorporated into the sediments, because this material has to be transported to less turbulent zones where it can settle (Creutzberg & Postma 1979). However, Jenness & Duineveld (1985) demonstrated the deposition of considerable amounts of phytoplankton into sandy North Sea sediments without simultaneous mud deposition. In contrast to muddy, cohesive sediments, in which molecular diffusion is the major transport process for solutes through the sediment, advective transport processes gain significance in sediments with permeabilities exceeding $10^{-12}$ $m^2$ (Huettel et al. 1998). The driving forces for these interstitial pore water flows are pressure gradients, which are generated when unidirectional or oscillating bottom currents interact with sediment topography, e.g. sediment ripples and biogenic structures (Huettel & Webster 2001, Precht & Huettel 2003). Advective pore water flows provide an effective transport mechanism for dissolved and particulate matter through the interstitial space (Huettel et al. 1998). Flume experiments have shown that such pore water flows enhance the nutrient release (Huettel et al. 1998), as well as oxygen penetration depth (Ziebis et al. 1996) and consumption (Forster et al. 1996) in permeable sediments. Advective transport of phytoplankton into permeable beds has been demonstrated in flume studies and *in situ* (Pilditch et al. 1998, Huettel & Rusch 2000). Thus, the degradation of organic matter can be shifted from the sediment surface to deeper sediment layers, preventing resuspension of the material by waves and strong bottom currents (Huettel & Rusch 2000).

Nevertheless, the organic carbon content of sandy sediments is generally low (Shum & Sundby 1996), and this has led to the view that the biogeochemical activity in these beds also is low. However, a study of Grant et al. (1991) on the oxygen consumption in shelf sediments revealed that the oxygen uptake in coarse sediment was only two to three times lower than the uptake in a nearby fine-grained sediment despite a 20 times higher carbon content. Consequently, the contribution of sandy sediments to organic matter degradation in the shallow shelf may be larger than inferred from the low organic content (Shum & Sundby 1996).

Spring diatom blooms are often the events of highest yearly new production and carbon sedimentation in the coastal ocean (Goering et al. 1973). Planktonic diatoms do not have any structures facilitating locomotion, but considerable physiological control over buoyancy (Smayda 1970). As some of these controls are energy dependent, sinking rates can increase drastically upon nutrient depletion (Smetacek 1985). Aggregation after intense blooms further can accelerate the sinking rates (Passow 1991). Several authors (Peinert et al. 1982, Brussaard et al. 1995) have shown that sedimentation, and not grazing, is the major loss factor of diatom spring blooms. The sinking dynamics of coastal bloom diatoms are an integral part of their life history and represent the transition from a reproductive pelagic stage to a benthic resting stage, which enables them to survive over long periods in cold, dark environments (Smetacek 1985).

In contrast to planktonic diatoms, benthic diatoms include motile and non-motile species. Epipelic species (growing on mud), for example, usually are motile, while epipsammic species (growing on sand) are usually non-motile. Benthic diatoms are important primary producers in many estuarine, intertidal and shallow-water environments.

The purpose of this study was to assess the vertical distribution of diatoms in coastal sediments with different permeabilities, and the potential role of advective transport processes for this distribution. Therefore, we collected sediment cores in three nearshore subtidal sandy sediments that revealed the distribution and abundance of planktonic and benthic diatoms in the different sands. For the investigation of the entrainment depth and the time scale of the interfacial transport

of planktonic diatoms into the different sands, we conducted three on-board and two *in-situ* chamber experiments. The diatoms were labeled with $^{13}C$, permitting the tracing of the pathway of the algal carbon within the sediment (Levin et al. 1997). In order to assess whether interfacial water flows enhance the degradation of the added diatoms, samples were analyzed for dissolved organic carbon (DOC) content.

## MATERIALS AND METHODS

### Study area

Sediment collection and experiments were carried out on nearshore subtidal sands during 3 cruises of R.V. Heincke (HE 145, HE 148 and HE 154) to an area seawards of Spiekeroog Island (south-eastern German Bight) (Fig. 1). This environment is strongly influenced by tides, waves and storm currents (Antia 1995). The mean tidal range at the study site is 2.5 m. Salinity varied between 31-32 PSU.

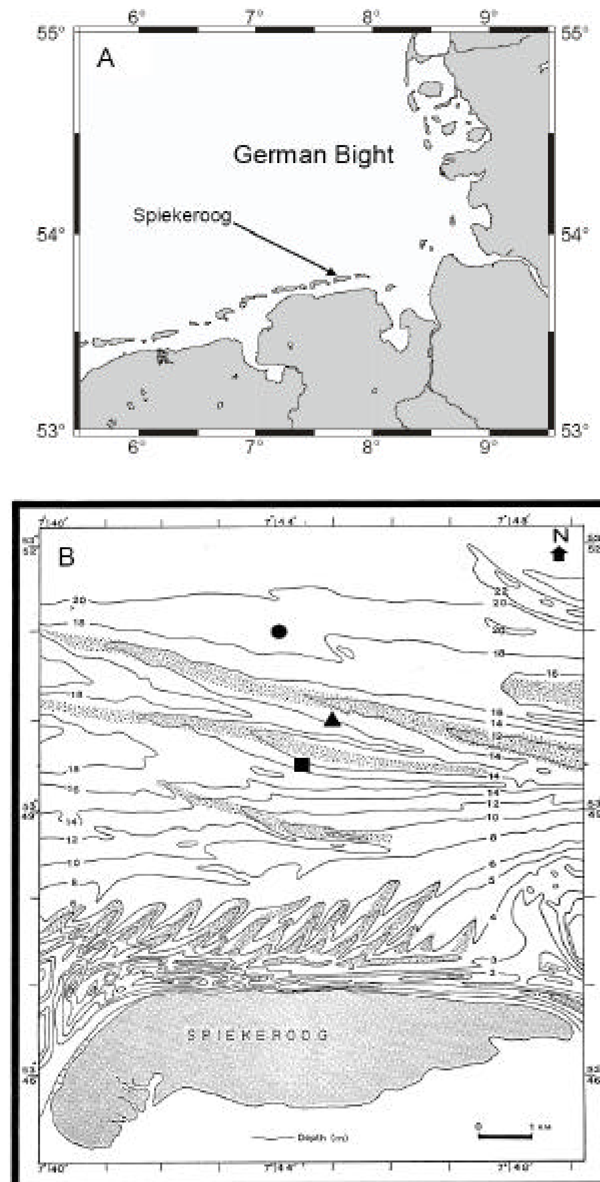


Fig. 1. (A) Location of Spiekeroog Island in the German Bight (south-eastern North Sea). (B) Bathymetry of the Spiekeroog shoreface as given by Antia (1993), and locations of the 3 stations. A circle indicates the station with the fine sand, a triangle the medium and a square the coarse sand.

Table 1. Positions, sediment and water characteristics of the study sites at the south-east corner of the German Bight (Spiekeroog Island). The permeability k, the porosity, the median grain size and the POC concentrations of the sediments are taken from Janssen et al. (submitted). Samples for sediment and water characteristics were taken at the same time as the experimental sediment cores retrieved by divers. Sediment POC values were integrated over the upper 10 sediment cm. For the assessment of background diatom numbers and $PO^{13}C$ in the different sediments, additional sediment cores were taken with a Multiple Corer (fine and medium sand: n = 3; coarse sand: n = 1).

| Cruise | Date | Position | Sand type | k ($10^{-12}$ $m^2$) (±s.d.) | Porosity (vol. %) (±s.d.) | Median Grain size (µm) (±s.d.) | Water depth (m) | Water Temp. (°C) | Bottom water POC (mg $L^{-1}$) (±s.d.) | Sediment POC (% dry mass) | Chamber experiment with diatoms |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **HE 145** | 08.-18.04. 2001 | 53°51´N, 7°44´E | Fine | 3.02 (± 1.66) | 44.9 (± 1.6) | 164 (± 1) | 19 | 9 | 0.96 (± 0.02) | n.a. | On-board (12 h, 30 h, 132 h; n=2) |
| **HE 148** | 07.-15.06. 2001 | 53°51´N, 7°44´E | Fine | 3.02 (± 1.66) | 44.9 (± 1.6) | 164 (± 1) | 19 | 13 | 1.22 (± 0.06) | 0.114 (± 0.014) | *In-situ* (32 h; n=2) |
| **HE 154** | 24.-30.09. 2001 | 53°50´N, 7°45´E | Medium | 26.27 (± 3.26) | 43.2 (± 1.4) | 299 (± 3) | 16 | 16 | 0.61 (± 0.03) | 0.023 (± 0.003) | On-board (12 h, 25 h, 72 h; n=1) + *in-situ* (20 h; n=2) |
| | | 53°49.5´N, 7°44.5´E | Coarse | 77.24 (± 14.36) | 41.1 (± 1.6) | 672 (± 37) | 14 | 16 | 0.61 (± 0.03) | 0.032 (± 0.003) | On-board (20 h; n=1) |

For the measurements, *in-situ* and on-board experiments, three well-studied sites (Antia 1993, Antia 1995) with different sediment characteristics were chosen (Table 1), all located within a radius of 2500 m (Fig. 1).

## Sediment collection

For the characterization and distribution of planktonic and benthic diatom species, 3 cores of fine sand, 3 cores of medium sand and 1 core of coarse sand were taken with a Multiple Corer on the September cruise (HE 154). These cores (10 cm length, 3.6 cm inner diameter) were sliced in intervals of 2 × 0.5 cm and 9 × 1 cm and analyzed in the same manner as described below for the chamber cores.

## Cultivation of $^{13}C$-enriched phytoplankton

For the experiments, an axenic clone of *Ditylum brightwellii* (Bacillariophyceae, Biddulphiales) was cultured in sterile artificial seawater with a salinity of 33 PSU (Grasshoff et al. 1999) enriched with f/2 medium (Guillard & Ryther 1962) at 25°C. *D. brightwellii* is a common species in the German Bight (Drebes 1974), and was also abundant during the spring bloom in 1998 near our station (53°53 ´N, 7°32 ´E) (Lo 1999). The medium contained 25% $^{13}C$-enriched bicarbonate (through addition of 99% $NaH^{13}CO_3$, Cambridge Isotope Laboratories). The algal material was harvested by centrifugation (404 **g**, 4 min), rinsed 3 times with an isotone sodium chloride solution and centrifuged again. From this concentrated material, samples for dry mass, particulate organic carbon (POC), DOC, diatom numbers and labeling efficiency were taken, and then the algae were stored frozen until use. This treatment killed the diatoms and caused breakage of some cells, as observed under the microscope, which led to release of DOC from the cells (19 ± 7% of the added carbon). The axenic state of the culture was verified by microscopic observation of DAPI stained cells. The produced algal carbon contained 15% $\delta^{13}C$ (HE 145), 9% $\delta^{13}C$ (HE 148) and 10% $\delta^{13}C$ (HE 154), and the carbon content of the added algae per chamber corresponded to 0.31 g C $m^{-2}$ (HE 145), 0.36 g C $m^{-2}$ (HE 148) and 0.50 g C $m^{-2}$ (HE 154).

## Experiments

Both *in-situ* and on-board experiments were carried out in acrylic cylindrical chambers (31 cm height, 19 cm inner diameter), which were covered by black foil preventing any light penetration to the incubated water and sediments. The water inside each chamber was stirred by a horizontal disk (17 cm diameter), rotating approximately 10 cm above the sediment surface at 20 rpm. The sediment height in each chamber was approximately 15 cm. The rotating water generates a pressure gradient (ca. 0.2 Pa $cm^{-1}$), comparable to the pressure gradient at a sediment ripple interacting with bottom currents (Huettel & Rusch 2000). This pressure gradient creates advective pore water flows in permeable sediments.

The chambers were deployed and recovered by divers, and for the *in-situ* experiments the algae were directly injected into the chambers by the divers, who sealed the chambers afterwards. Oxygen did not become limiting during the incubation time (always above 75 µM $O_2$), as verified by determination of the final oxygen concentrations in the chamber water using the *Winkler* method (Grasshoff et al. 1999). At the end of the incubation time of 2 × 32 h (fine sand, HE 148) and 2 × 20 h (medium sand, HE 154), the chambers were closed at the bottom with sealing lids and brought back to R.V. Heincke. For the assessment of background values, bottom water was collected 2 m above the seafloor with a rosette equipped with 10 L Niskin bottles at the beginning of the *in-situ* experiments.

The sediment for the on-board incubations was cored and recovered by the divers using the same benthic chambers. On board, the chambers were kept at *in-situ* temperature, and stirring was started immediately. Between the lid of the chambers and the water surfaces, an air-filled space of 4 cm was left to permit gas exchange. The on-board experiments ran for 2 × 12 h, 2 × 30 h and 2 × 132 h (fine sand, HE 145); for 12 h, 25 h and 72 h (medium sand, HE 154) and 20 h (coarse sand, HE 154). During these time periods, water samples for diatom numbers; DOC and bacterial numbers were taken with a syringe, which was connected to a tube, at regular time intervals. The sampled water volume was replaced by 0.2 µm filtered seawater and all results were corrected for this dilution. For the *in-situ* experiments, these samples were only taken at the end of the incubation time.

At the end of all experiments, the entire cores were sliced at intervals of 10 × 1 cm and 2 × 2.5 cm. Every depth interval was carefully mixed and samples for diatom numbers and $^{13}C$ of particulate organic carbon ($PO^{13}C$) were taken. In order to assess the background $PO^{13}C$ values without organic matter addition, 3 (fine and medium sand) or 1 (coarse sand) additional sediment cores were taken with a Multiple Corer for each experiment. These cores were sliced and analyzed in the same manner as described for the chamber cores.

## Analytical techniques

Water samples for diatom numbers were preserved with hexamethylenetetramine buffered formaldehyde (end concentration 2%) and Lugol solution (end concentration 1%) and kept refrigerated in dark glass bottles until analysis. To separate the algae from the sand grains, 1 ml sediment was resuspended two times in 5 ml 0.2 µm filtered seawater, containing formaldehyde and Lugol in the same final concentrations as for the water samples. The supernatant was collected after 30 s of deposition time and filtered on black membrane filters (0.2 µm). All diatom cells of 20 randomly chosen counting grids of three parallel filters per sample were counted under a Zeiss™ Axiophot epifluorescence microscope (excitation wave length 510-560 nm, magnification 1300 ×). Diatom species were identified (Drebes 1974, Pankow 1990) using a Zeiss™ inverted microscope and the method of Utermöhl (1958) at a magnification of 400 ×.

To test the extraction efficiencies, a known concentration of a *Ditylum brightwellii* culture was added to the various sediments and incubated for 1 day in the dark. Diatom cells were extracted with 0.2 µm filtered seawater as described above. Extraction efficiencies were: 76 ± 5% (fine sand), 82 ± 14% (medium sand), 79 ± 9% (coarse sand).

For the dry mass determination of the *D. brightwellii* culture, 1 ml sample was filtered on precombusted (500°C, 6h), pre-weighed GF-F filters, rinsed with distilled water to remove the sodium chloride, dried for 24 h at 60°C and weighed again.

Samples for the carbon content of the culture were filtered on precombusted GF-F filters, pre-treated with 0.1 N HCl for 2 h to remove the bicarbonate and dried at 60°C. Filters were then transferred into tin cups. The particulate organic carbon was measured using a Fisons™ NA1500 elemental analyzer.

For the assessment of the label efficiency of the culture, samples were combusted in a CE Instruments™ CHN-Analyzer and the evolved $CO_2$ was passed online via a ThermoFinnigan™ interface to a ThermoFinnigan™ isotope-ratio mass spectrometer (IRMS) in a continuous flow of helium.

Samples for the $\delta^{13}C$ values and concentration of the sediment POC were stored frozen in precombusted dark glass vials until processing. About 2 g sediment was dried for 48 h at 60°C and pre-treated with approximately 10 ml 2 M HCl overnight to remove the bicarbonate. Sediments were then centrifuged (2800 **g**, 10 min), washed 3 times with distilled water, centrifuged and dried again. Approximately 100 mg of the sediment were exactly weighed into tin cups and samples were measured as described for the label efficiency.

Carbon isotope ratios ($^{13}C/^{12}C$) are expressed in the conventional delta notation ($\delta^{13}C$) relative to Vienna PDB ($^{13}C/^{12}C_{VPDB}$ = 0.0112): $\delta^{13}C$ (‰) = $[(R_{sample}/R_{std}) - 1)] \times 1000$, where $R_{sample}$ and $R_{std}$ are the $^{13}C/^{12}C$ of the sample and standard, respectively (Craig 1957). Incorporation of $^{13}C$ is shown as excess (above background) $^{13}C$ uptake and was calculated according to Moodley et al. (2000) as the product of the POC concentration and excess $^{13}C$ ($E$). $E$ is the difference between the fraction ($F$) of the sample and background: $E = F_{sample} - F_{background}$, where $F = {}^{13}C / ({}^{13}C + {}^{12}C) = R / (R + 1)$ and $R$ = the carbon isotope ratio. $R$ was derived from the measured $\delta^{13}C$ values as $R = (\delta^{13}C / 1000 + 1) \times R_{VPDB}$.

Water samples for DOC were filtered through precombusted GF-F filters into precombusted 4 ml glass vials and stored frozen until analysis. The DOC concentration was measured by high-temperature catalytic oxidation using a Shimadzu™ TOC-5050A analyzer. Three parallels were measured per sample.

For the bacteria counts, water samples were preserved with formaldehyde (end concentration 4%) and kept at 4°C. Bacteria were filtered on black membrane filters (0.2 µm), stained with acridine orange, and 20 randomly chosen counting grids

of 3 parallel filters per sample were counted under a Zeiss™ Axiophot epifluorescence microscope (excitation wave length 450-490 nm, magnification 1300 ×).

## RESULTS

### The distribution of planktonic and benthic diatoms in coastal North Sea sediments of different permeabilities

In general, the medium and coarse sands showed a higher diversity of planktonic and benthic diatom species than the fine sand. Furthermore, the penetration depths of single diatom cells and diatom chains were higher in the coarse-grained sands.
The two major taxonomic divisions, centric (Centrales, Fig. 2) and pennate diatoms (Pennales, Fig. 3), also reflect a major ecological difference, as Pennales are mainly benthic and Centrales are mainly planktonic (Schrader & Schuette 1981). This division was applicable for most species we identified, with the exception of *Plagiogramma brockmanni*, *Nitzschia* spp. (Pennales) and *Actinoptychus senarius* (Centrales), which have been reported as both, benthic and planktonic forms (Drebes 1974). The maximum penetration depth of the dominant planktonic diatom *Coscinodiscus* spp. increased with sediment permeability: 2 cm, 7 cm and 8 cm (total sampling depth) for the fine, medium and coarse sand, respectively (Fig. 2). Broken parts of diatom frustules from mainly centric species were abundant in relatively high numbers in the medium and coarse sand.

Four different pennate diatom species could be found in the medium and coarse sands (Fig. 3), while in the fine sand only 2 different species occurred. The non-motile diatom *P. brockmanni* was the dominant pennate diatom species in all 3 sands, and its abundances were higher compared to the dominant centric diatom species. *P. brockmanni* belongs to the non-motile epipsammic diatom species (Schrader & Schuette 1981), and the single cells are united in long chains (Drebes 1974). The maximum cell number united in a chain did not exceed 5 cells for the fine sand, whereas chains of 9 and 10 cells were found in the medium and coarse sand respectively (Fig. 4). The maximum penetration depth of single cells and chains of *P. brockmanni* both showed a positive correlation with sediment permeability (Fig. 5).

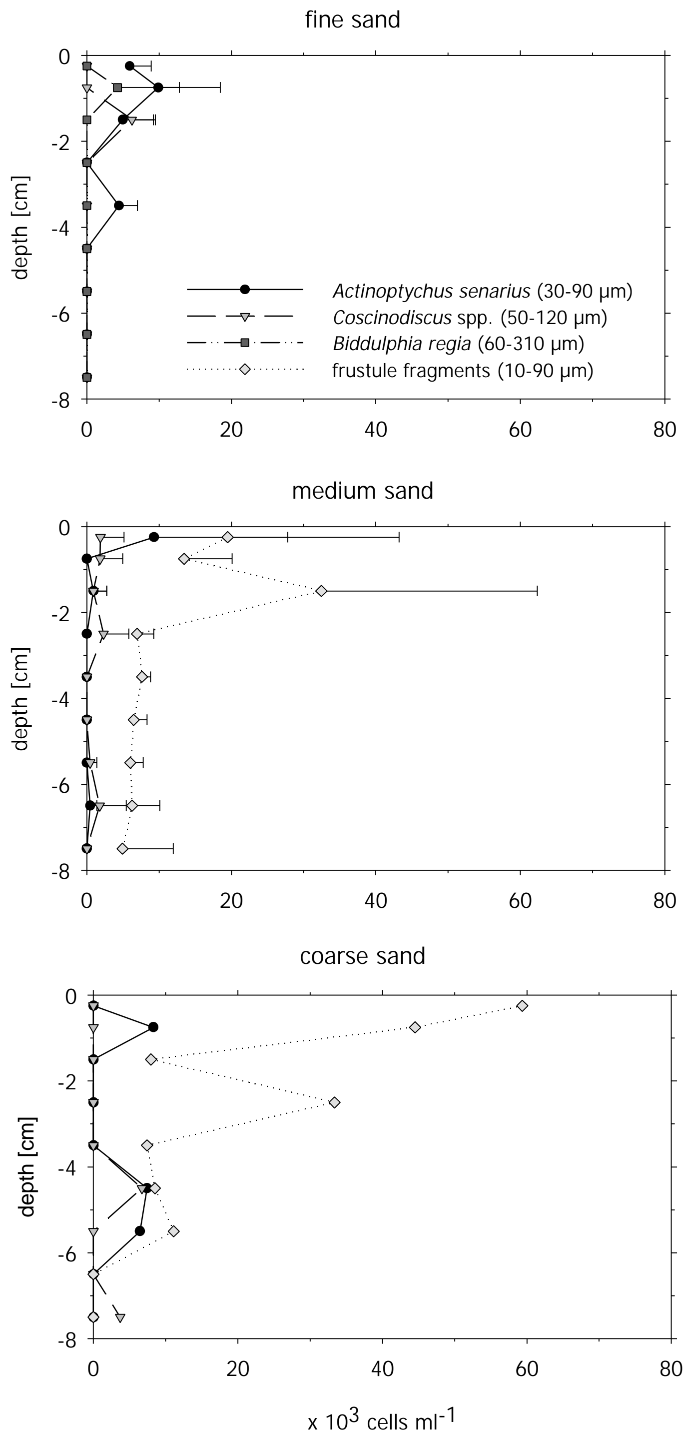


Fig. 2. Vertical distributions and averaged cell numbers (+ s.d.) of centric diatom species in a fine, medium and coarse sand. Sediment cores (fine and medium sand: n = 3; coarse sand: n = 1) were collected in September.

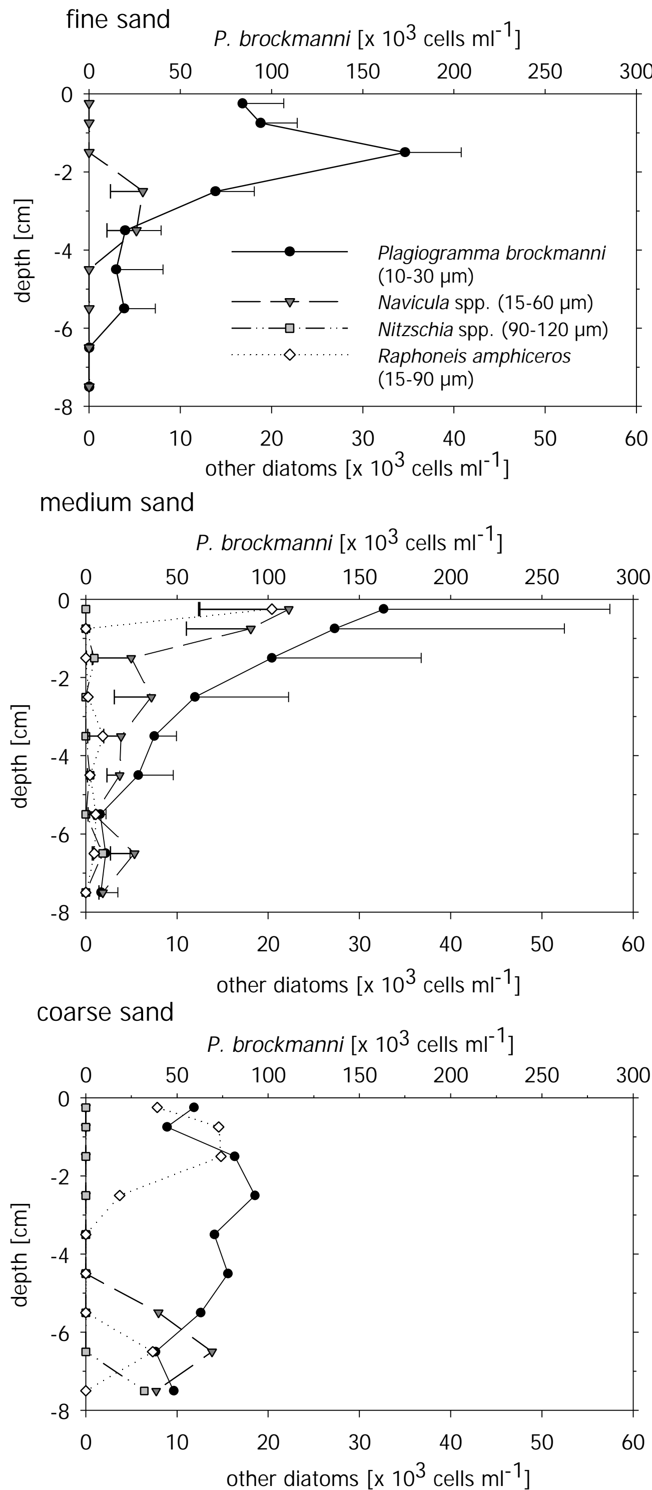


Fig. 3. Vertical distributions and averaged cell numbers of pennate diatom species in a fine, medium and coarse sand. Sediment cores (fine and medium sand: n = 3; coarse sand: n = 1) were collected in September. Upper scale: *Plagiogramma brockmanni* numbers (+ s.d.). Lower scale: Other pennate diatoms (- s.d.).

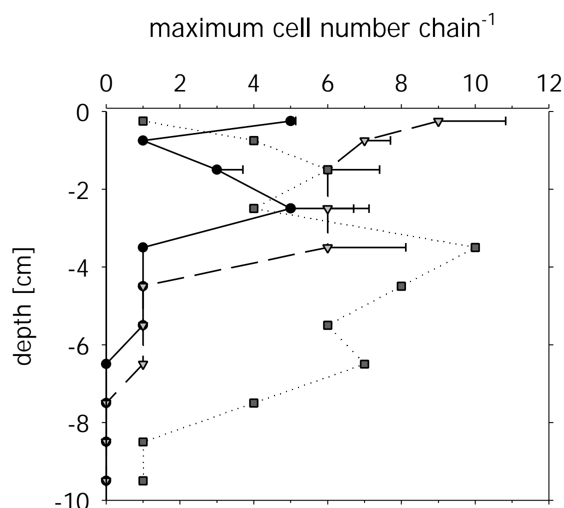


Fig. 4. Maximum cell number united in chains (+ s.d.) of the dominant diatom species *Plagiogramma brockmanni* in a fine (circle), medium (triangle) and coarse sand (square).

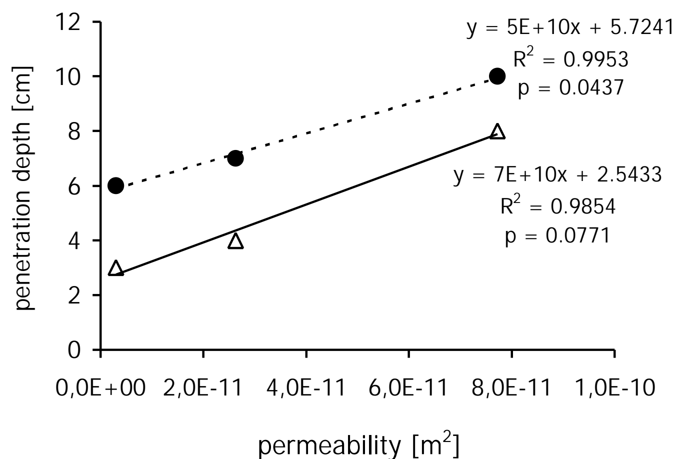


Fig. 5. Maximum penetration depth of *Plagiogramma brockmanni* cells (circles) and chains (triangles) in dependency on the sediment permeability.

## Transport of $^{13}$C-labeled diatoms into sandy sediments of different permeabilities

In all incubations we observed higher penetration depths of *Ditylum brightwellii* cells into the medium and coarse sand and compared to the fine sand. This result was supported by the excess $^{13}$C data, which showed enhanced transport of algal carbon into deeper layers of the coarse-grained sands.

Flux of *D. brightwellii* cells from the water column into or onto the sediment increased with increasing sediment permeability (Table 2). The transport of *D. brightwellii* cells into the fine sand was restricted to the upper 2 cm of the sediment, with most cells accumulated in the upper centimeter (Fig. 6) and less than 3.6% of the added diatoms found below 1 cm (Table 3).

Table 2. Initial *Ditylum brightwellii* cell concentration in the water column and flux of cells into or onto the sediment, increase of bacteria, DOC flux and pH changes measured in the water column of all on-board incubations. The change of cell numbers is given for the first 12 hours of the experiment. No cells were found in the water column after 17 h (fine sand) and 12 h (medium and coarse sand), respectively. PH values are given for the total incubation time. Positive values represent increase; negative values indicate decrease. The pH at the beginning of the experiment was approximately 8.

| **Sand type** | **Initial *D. brightwellii* concentration (± s.d.) (´ $10^3$ cells $m^{-2}$)** | ***D. brightwellii* flux (´ $10^3$ cells $m^{-2}$ $d^{-1}$)** | **Bacteria (´ $10^5$ cells $ml^{-1}$ $h^{-1}$)** | **DOC (µmol $m^{-2}$ $d^{-1}$)** | **pH (pH units $d^{-1}$)** |
|---|---|---|---|---|---|
| **Fine** | 758 (± 126) | -1070 | 1.35 | -1306 (first 72h)<br>+ 7183 (72-132 h) | -0.07 |
| **Medium** | 604 (± 72) | -1208 | 0.55 | -1326 (72 h) | -0.05 |
| **Coarse** | 776 | -1552 | 1.02 | -1728 (20 h) | -0.09 |

Algal penetration depth did not increase with increasing incubation time. After an incubation time of 132 h, the bulk of diatom cells still was found in the uppermost 1 cm of the fine sand and only 1.5% of the added diatoms were found below 1 cm (Table 3). Highest cell numbers were also observed in the upper sediment centimeter in the incubations with medium sand, but with increased incubation time more cells, corresponding to 6.2% (20 h) and 14.3% (72 h) of the added diatom cells, were transported below 1 cm depth of the medium sand (Table 3). Comparable transport rates were recorded in the coarse sand where 5.8% of the added algae were found below 1 cm after 20 h incubation time. Lower total recovery rates of diatoms in the fine sand compared to the coarse-grained sands (Table 3) may be a result of lower extraction efficiencies (see P. 9) and higher uptake by macrofauna, whose biomass were significantly higher on the fine sand (Witte et al. unpublished data).

Table 3. Transport rates of *Ditylum brightwellii* cells (% of total added algal cells) and $PO^{13}C$ (% of total added algal $TO^{13}C$) into the different sediments. The recovery rate of the total algal cells in the sediment was between 10 to 36% (fine sand), 50 to 82 % (medium sand) and 43% for the coarse sand. The recovery rate of the $PO^{13}C$ in the sediment was between 1.2 to 1.5% (fine sand), 2.4 to 5.2% (medium sand) and 4.9% (coarse sand).

| | **Fine sand** | | **Medium sand** | | **Coarse sand** | |
|---|---|---|---|---|---|---|
| **Time (h)** | cells | $PO^{13}C$ | cells | $PO^{13}C$ | cells | $PO^{13}C$ |
| **0-1 cm depth** | (% ± range) | (% ± range) | (% ± range) | (% ± range) | (%) | (%) |
| **12 h** | 32.2 ± 10.5 | 1.38 ± 1.72 | 67.6 | 2.23 | | |
| **20 h** | | | 76.2 ± 5.5 | 1.03 ± 0.16 | 37.6 | 4.48 |
| **25 h** | | | 49.7 | 4.68 | | |
| **30 h** | 31.9 ± 7.3 | 0.50 ± 0.10 | | | | |
| **32 h** | 8.1 ± 2.2 | 0.53 ± 0.02 | | | | |
| **72 h** | | | 67.3 | 3.62 | | |
| **132 h** | 21.2 ± 4.1 | 0.65 ± 0.36 | | | | |
| **1-4 cm** | | | | | | |
| **12 h** | 3.6 ± 0.7 | 0.10 ± 0.17 | 5.3 | 0.81 | | |
| **20 h** | | | 6.2 ± 3.0 | 1.38 ± 1.96 | 5.8 | 0.42 |
| **25 h** | | | 0.0 | 0.50 | | |
| **30 h** | 0.0 ± 0.0 | 0.68 ± 1.04 | | | | |
| **32 h** | 2.4 ± 0.9 | 0.69 ± 0.98 | | | | |
| **72 h** | | | 14.3 | 0.93 | | |
| **132 h** | 1.5 ± 0.7 | 0.73 ± 2.84 | | | | |

Total uptake of excess $^{13}C$ into the fine sand (Fig. 6) was mainly restricted to the upper 2 cm of the sediment after 12 h. With increasing incubation time (30 to 132 h), more excess $^{13}C$ could be detected in deeper sediment layers (2 to 6 cm), but the bulk of diatom carbon still accumulated in the surface layer. In the 12 h incubation with medium sand, highest amounts of excess $^{13}C$ were also detected in the upper 2 cm, but with increasing incubation time (72 h) excess $^{13}C$ was transported deeper into the sediment (12 cm) (Fig. 6). In the incubation with coarse sand (20 h) the labeled algal carbon was found in depths of up to 3 cm (Fig. 6).

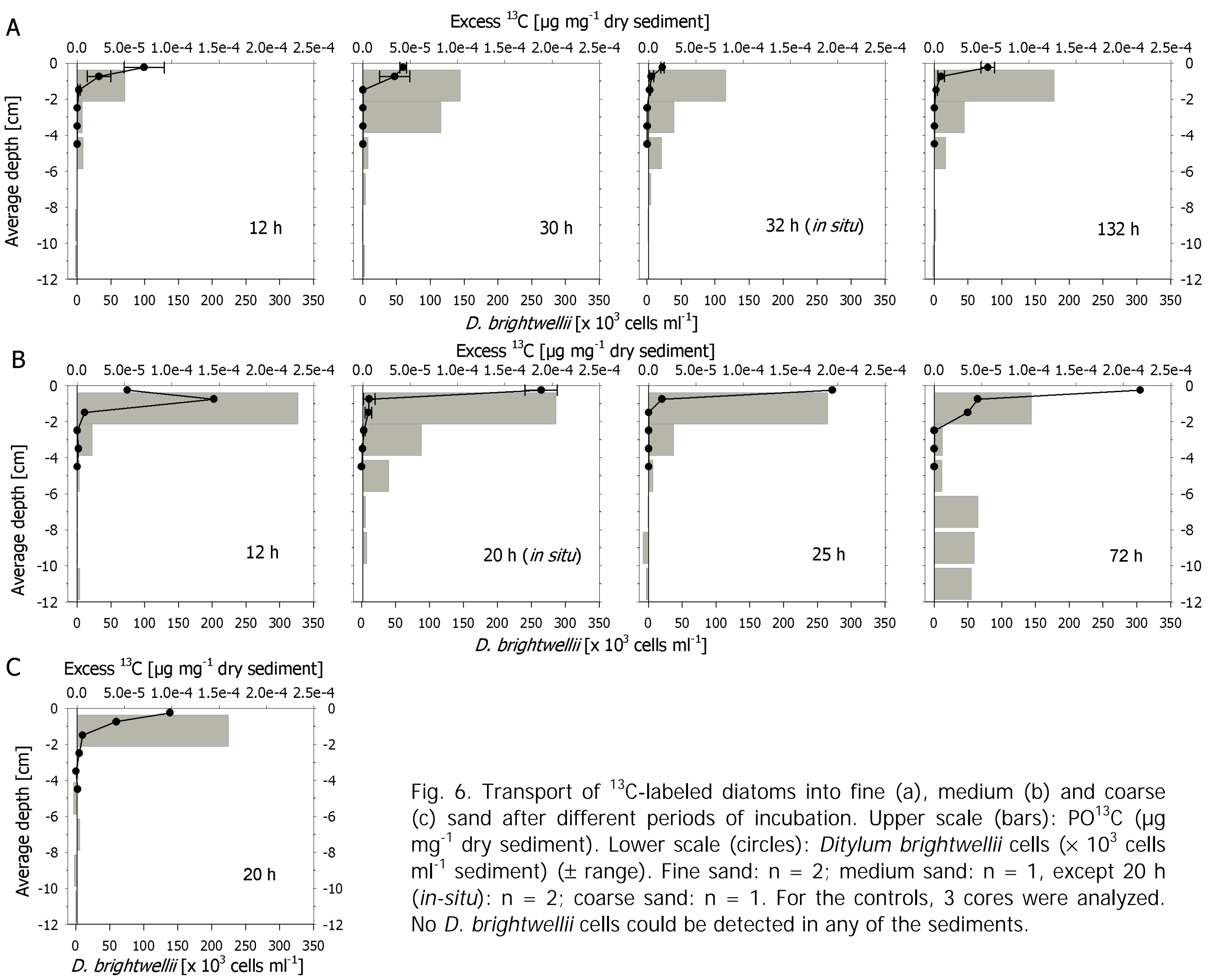


Fig. 6. Transport of $^{13}C$-labeled diatoms into fine (a), medium (b) and coarse (c) sand after different periods of incubation. Upper scale (bars): $PO^{13}C$ (µg mg$^{-1}$ dry sediment). Lower scale (circles): *Ditylum brightwellii* cells (× $10^3$ cells ml$^{-1}$ sediment) (± range). Fine sand: n = 2; medium sand: n = 1, except 20 h (*in-situ*): n = 2; coarse sand: n = 1. For the controls, 3 cores were analyzed. No *D. brightwellii* cells could be detected in any of the sediments.

These results indicate enhanced transport of diatom cells and carbon into deeper sediment layers with increasing permeability (Fig. 7).

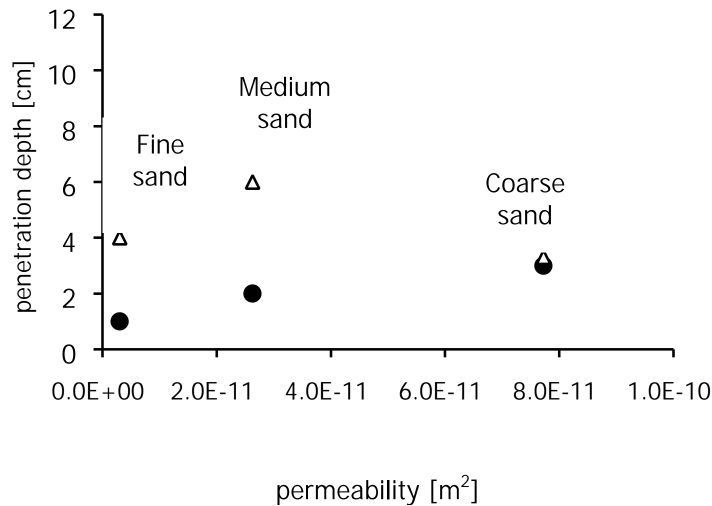


Fig. 7. Penetration depth of *Ditylum brightwellii* cells (circles) and $PO^{13}C$ (triangles) in dependency on the sediment permeability. Please note different incubation times: 30 h (fine sand) and 20 h (medium and coarse sand). This means that the higher penetration depth of algal cells with increasing permeability was obtained in less time.

## DOC, pH and bacterial counts

DOC in the water (Table 2) was consumed in all experiments (approx. -1500 µmol $m^{-2}$ $d^{-1}$), except for of the long-time incubation with fine sand, where DOC concentrations increased after 72 h until the end of the experiment (+7183 µmol $m^{-2}$ $d^{-1}$). Lowest decrease of pH was observed for the medium sand, which also had the lowest bacterial growth in the water during the first day (Table 2). Highest increase in bacterial numbers within the first day occurred in the water of the fine sand incubations.

## DISCUSSION

The incorporation of suspended pelagic diatoms into the sediment usually requires the settling of the algae onto the sediment surface, and then the transfer of the deposited material into deeper layers by biological (Huettel 1990) or hydrodynamical sediment mixing (Jenness & Duineveld 1985). In permeable sediments, flow-induced advective transport can additionally enhance the deposition of suspended algae by direct transfer from the boundary layer into the bed (Huettel et al. 1996).

Planktonic diatom species, dominated by *Coscinodiscus* spp., were present in all investigated sands (Figs. 2 and 3). The maximum penetration depth of *Coscinodiscus* spp. increased with sediment permeability (Fig. 2). This indicates enhanced advective transport of these planktonic diatoms into the highly permeable sands, as could be confirmed by our chamber experiments. Broken parts of diatom frustules from mainly centric species as *Coscinodiscus* spp. and *Thalassiosira* sp., (the latter dominated the diatom spring bloom in 2001 (Ehrenhauss et al. submitted)), were abundant in the medium and coarse sands. As the surficial sediments are frequently mobilized due to tidal flow and waves (Antia 1993), the motion of the sand grains may break-up the diatom frustules, leading to fast decomposition of the organic material in these dynamic sediments. A more detailed interpretation of the data is not possible, as we do not have any information about the sediment history.

The pennate diatom species were dominated by the epipsammic diatom *Plagiogramma brockmanni* in all 3 sands (Fig 3). *P. brockmanni* has been reported as being abundant during the spring bloom in the plankton near the Frisian Islands (Drebes 1974, Lo 1999). Thus, we do not know if the *P. brockmanni* cells we found lived as benthic form in the sediment or originated from the water column. Under epifluorescence microscopy, chlorophyll autofluorescence was present in the bulk of the cells, indicating their living state, however, the non-growing vegetative cells of many diatoms have a long survival time in dark and cold environments (Smayda & Mitchell-Innes 1974). No data are available on the light intensity reaching the sediments, but the sea floor in the southern North Sea is a relatively low light environment (Jerlov 1951). Nevertheless, light may reach the sea floor occasionally in these shallow depths, e.g. during bright summer days. Studies on the continental

shelf of the South Atlantic Bight (14-40 m) revealed that benthic microalgae, which were dominated by diatoms (Nelson et al. 1999), contributed an average of 37 % to the total primary production (Jahnke et al. 2000). Thus further studies on light penetration to the North Sea floor, benthic primary production and sedimentation rates of phytoplankton will be needed to enlighten these processes.

The vertical distribution of *P. brockmanni* in the fine sand with a subsurface maximum at 1.5 cm may be caused by algal growth, or can be explained by the incorporation of the diatoms by moving sediment ripples (Jenness & Duineveld 1985), which causes typical stripes in up to 5 cm depth in the sediment at the base of the ripples. The vertical distribution of *P. brockmanni* in the medium sand did not show stripes, but rather a typical distribution as caused by advection (Huettel & Rusch 2000). According to Huettel & Gust (1992) advective transport processes may have been limited to the upper 2 cm of the fine sand, whereas this pressure-driven pore water flow can be effective to more than 8 cm depth in the medium and coarse sand. This would explain the higher penetration depth of *P. brockmanni* in these sands. Furthermore, the depth in which the longest diatom chains could be found and the maximum chain lengths recorded in the sediment increased with permeability (Fig. 4). These results indicate a positive relationship between the sediment permeability and sand grain size with the maximum penetration depth of single *P. brockmanni* cells or chains (Fig. 5). A negative relationship between the proportion of fine sediments and benthic microalgal biomass in shallow water ecosystems has been shown by Cahoon et al. (1999).

The fine sand had the lowest diversity of pennate diatom species with *P. brockmanni* and *Navicula* spp., whereas all four pennate species were found in the medium and coarse sand. As the phytoplankton composition and abundances in the water column did not differ between the stations (Ehrenhauss et al. submitted), the lower diversity of diatoms in the fine sand can be related to different sediment characteristics. *Nitzschia* spp. and *Raphoneis amphiceros* are relatively big diatom species with a maximum cell size of 120 µm and 90 µm, respectively, which could explain their absence in the fine sand (no advective filtration possible).

The experiments showed fast decrease of the number of added diatom cells in the water (Table 2), resulting from gravitational settling and transport of suspended *Ditylum brightwellii* cells into deeper layers of the permeable sediments (Fig. 6). The penetration depths of diatom frustules and carbon into the sediment increased with sediment permeability and sand grain size (Figs. 6 and 7), except for the coarse sand where excess $^{13}C$ was only found in the upper 3 cm. This may be caused by local sediment inhomogeneities. The coarse sand was not as efficient as trap for diatom cells and carbon as would be expected from its much higher permeability and sand grain size compared to the medium sand (Table 1). The deposition of particles into permeable sediments requires not only the delivery of particles, but also their retention. Fries & Trowbridge (2003) observed that an enhanced fine-particle deposition to permeable sediments depends on the ratio of bed grain size to particle diameter. With increasing permeability of the sand bed, the delivery of particles is enhanced. However, without efficient filtration, a larger particle supply does not necessarily translate to enhanced particle deposition. The importance of interactions between particles and sand grains in the retention of delivered particles can be an explanation for the relatively equal deposition of diatom cells in deeper sediment layers of the medium and coarse sand after 20 h (Table 3).

The distribution of excess $^{13}C$ in the fine and medium sand always exceeded maximum penetration depth of diatom frustules. Excess $^{13}C$ includes not only diatom cells, but also broken parts of the cells, which could not be detected by microscopic observations, and also the incorporation of algal $^{13}C$ into bacteria and meiofauna. High amounts of diatom carbon were found in deeper layers (6-12 cm) of the medium sand after 72 h. As the velocity of the advective flow decrease with depth the penetration of the algae into the sediment is limited (Huettel & Rusch 2000). Algae accumulate in a layer where pore water moves too slowly to overcome the friction between the cells and sand grains, which produces a subsurface maximum. In their labeling study on a sandy and silty intertidal site, Middelburg et al. (2000) showed a faster mixing of algal $^{13}C$ into deeper sediment layers of the sandy sediment compared to the muddy sediment. Higher flux of algal cells (*Dunaliella* sp.) into the sediment with increasing permeability was demonstrated by Huettel & Rusch

(2000). Pilditch et al. (1998) also observed that coarser sediments can be a larger sink for diatoms, when boundary flows interact with biogenic structures.

With increasing grain size of the sand bed, a larger volume of water is forced through the sediment. Due to increasing interfacial flows and the larger interstices between the sand grains, the medium and coarse sands were larger sinks for diatoms than the fine sand. Particles trapped in deeper sediment layers cannot be easily removed again from the sediment by upwelling pore water, as the flow velocity decreases with depth, and relatively high pore water flows are needed to dislocate trapped material again (Huettel & Rusch 2000). Our data furthermore demonstrated that the high sediment permeability and sand grain size of the coarse sand did not lead to higher particle deposition due to reduced retention efficiency.

The lower penetration depth of *D. brightwellii* compared to *Coscinodiscus* spp. may be caused by the relatively short incubation time and lower advective pore water flows in the experiments compared to *in-situ* conditions. The intensity of the advective water flows depends on the sediment permeability (Huettel & Gust 1992), flow velocity (Forster et al. 1996), and topography height (Huettel et al. 1996). The pressure gradient in our stirred chambers (approx. 0.2 Pa $cm^{-1}$) is comparable to the pressure gradient at sediment ripples of 2 cm height exposed to bottom currents of 10 cm $s^{-1}$ at 10 cm above the sediment surface (Huettel & Rusch 2000). Tidal current velocities at our study site are in the range of 30 to 60 cm $s^{-1}$ at 100 cm above the sediment surface (Antia 1993), but consequently lower at 10 cm above the sediment. Roughness elements of approximately 1-4 cm height were always present at the sediment surface of all three stations (Janssen unpublished data). Thus, advective pore water exchange and coherent transport of diatoms inside the chambers would lie in the lower range of rates obtained under natural conditions.

Penetration depth is additionally determined by the size, shape and surface characteristics of the diatom cells (Huettel & Rusch 2000). The cell size of *Coscinodiscus* spp. and *D. brightwellii* lies in the same range (approx. 100 µm), but the shape of these diatoms differs. *Coscinodiscus* spp. has a discoidal shape, whereas *D. brightwellii* has an elongated, prismatic shape with a long spine on both sides, which most likely caused the lower penetration depth compared to

*Coscinodiscus* spp.. As the majority of coastal bloom diatoms are chain-forming and spiny (Smetacek 1985), these characteristics may not only serve as anti-predation device, but also reduce benthic filtration.

Sediment mixing associated with the feeding activities of benthic macrofauna may have also accounted for particle transport into the sediment. Laboratory chamber experiments on the fine sand with *Fabulina fabula* (Bivalvia, Tellinidae), the dominant macrofauna species at our fine station, revealed that advection was responsible for the transport of algal material down to 2 cm depth (Kamp 2002). The presence of *F. fabula* led additionally to the deposition of algae down to 5 to 7 cm depths. Macrofauna biomass was significantly lower in the chambers with medium and coarse sand compared to the fine sand (Witte et al. unpublished data). Therefore, bioturbation seems to be less important for solute and particle transport in the coarse-grained sands. Marinelli et al. (1998) found that advection also was the dominant solute transport process in the upper sediment layers of sandy sediments on the South Atlantic Bight, which had a comparable permeability and sand grain size as our medium and coarse sand.

As our study site represents a highly dynamic environment, the question arises whether moving sediment ripples can burry or remove algae after deposition. Jenness & Duineveld (1985) demonstrated that moving sediment ripples alternately buried algae in sandy North Sea sediments, and release the material again. However, Jenness & Duineveld (1985) also stated that transient deposited algae may provide a food source for benthic organisms. In our chamber experiments, uptake of deposited algal $^{13}C$ into bacteria and macrofauna was already visible in the shortest incubation of 12 h (Kamp 2002, Bühring et al. unpublished data), indicating a fast turnover of algal $^{13}C$ in these sandy sediments. For the fine sand, Kamp (2002) showed that the very abundant (surface) deposit and suspension feeder *F. fabula* was responsible for the highest uptake of algal $^{13}C$ into macrofauna organisms. Another important species in terms of $^{13}C$ uptake was the polychaete *Lanice conchilega*. Because of their ability to feed on suspended particulate matter in the overlying water, *F. fabula* and *L. conchilega* showed higher uptake of algal $^{13}C$ than (surface) deposit feeders as *Echinocardium cordatum*. The dual labeling study of Herman et al. (2000), where pelagic and benthic algae were labeled with $^{15}N$ and $^{13}C$, respectively, demonstrated

also the high importance of pelagic algae as food source for suspension feeders, whereas (surface) deposit feeders depended mostly on microphytobenthos as food source. Investigations on a mobile ripple-forming intertidal sandy sediment in a Dutch estuary (Herman et al. 2000, Herman et al. 2001) showed that the resuspension of benthic microalgae is relatively limited, which shortens the time period of their availability to suspension feeders.

Due to enhanced advective transport of $^{13}C$-enriched diatoms into the medium and coarse sand, the mineralization of the added organic matter was consequently shifted into deeper sediment layers. Total mineralization of the algal carbon to $^{13}CO_2$ was mainly restricted to the upper sediment layer of the fine sand (Bühring et al. unpublished data), whereas $^{13}CO_2$ release from the added algal carbon took place over the total depth of 12 cm in the medium sand (Witte et al. unpublished data).

Remineralization of the algal carbon and release of DOC into the overlying water was not visible during the first 3 days, as DOC was rather consumed (Table 2). Sorption of DOC onto mineral surfaces in the sediments (Keil et al. 1994) or onto the chambers walls can be an explanation for the observed DOC decrease. Nevertheless, DOC decrease (3-5% $d^{-1}$ of the added algal carbon) and $^{13}CO_2$ release (3.4% $d^{-1}$ of the added algal carbon for the fine sand, Bühring et al. unpublished data) were in the same order of magnitude. This indicates that initially the soluble fraction of the added algal carbon (approx. 20% DOC) was decomposed to $CO_2$ by the increasing bacteria population in the water (Table 2). pH decreased slightly in the water column above all tested sediments (Table 2), indicating release of $CO_2$ and further formation of bicarbonate and carbonate ions. The high DOC increase in the chambers with fine sand after the third day (7183 µmol $m^{-2}$ $d^{-1}$, corresponding to 28% of the added algal carbon) reveals that subsequently the particulate fraction of the diatom carbon was rapidly decomposed. This may also explain the low amounts of $PO^{13}C$ (approx. 5%), that could be recovered from the sediment of the chambers (Table 3). Furthermore, the added diatom cells were not all intact due to freezing, that led to release of DOC from the cells (approx. 20%). Additionally, the diatom carbon was incorporated into macrofauna (Kamp 2002) and probably into water column bacteria, which would also contribute to the low amounts of $PO^{13}C$ found in the sediments. Intact and parts of

diatom frustules were still abundant in the sediment, as the dissolution of opal by mainly inorganic dissolution is relatively slow.

## CONCLUSIONS

We have shown that advective interfacial flows carry suspended planktonic diatoms into deeper layers of permeable North Sea sediments. The magnitude and penetration depth of diatoms thereby depends on sand grain size, sediment permeability, diatom cell size, shape and chain length. We propose that colony formation and spines of coastal bloom diatoms may also be an adaptation to reduce benthic filtation, as permeable sediments may advectively filter 100 L $m^{-2}$ $d^{-1}$ (Precht & Huettel 2003). Advective filtration rates within our experimental chambers were most likely a conservative estimate of natural conditions, as near-bottom current velocities can be higher (Antia 1993), as well as the sea bed topography. Our results support the view that permeable sediments have a high filtration capability, trap suspended planktonic diatoms and thus prevent the resuspension by strong bottom currents and waves. Trapped in the sediment, these algae are most likely rapidly degraded, as observed in our experiment, where 28% of the added diatom carbon was released as DOC per day after the third incubation day. Boon et al. (1998) also demonstrated that a substantial part, up to 40%, of the primary production was buried and subsequently degraded in non-depositional areas of the southern and central North Sea. Further studies are needed to resolve these processes, which are a major issue for carbon and nutrient recycling in shelf sediments.

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## Chapter 4

# POC PROCESSING IN A SUBLITTORAL SANDY SEDIMENT: PULSE-CHASE EXPERIMENTS WITH $^{13}$C-LABELLED DIATOMS

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## ABSTRACT

*In situ* and on-board pulse-chase experiments were carried out on a sublittoral fine sand in the German Bight (southern North Sea) to investigate the pathway of settling particulate organic carbon through the benthic food web. Phytoplankton (*Ditylum brightwellii,* Bacillariophyceae), corresponding to 0.31 g C $m^{-2}$ (on-board experiments) and 0.36 g C $m^{-2}$ (*in situ* experiments), was injected into the incubation chambers. The algae were labelled with the stable carbon isotope $^{13}C$ in order to follow the processing of the carbon by bacteria and macrofauna and its mineralisation. On-board incubations lasted 12 h, 30 h, and 132 h, respectively, while the *in situ* experiment were incubated 32 h.

The study revealed a stepwise short-term processing of a phytoplankton bloom settling on a sandy sediment. After 12 h incorporation into a distinct bacterial population had taken place, corresponding mainly to the bacterial fatty acids *i* $C_{15:0}$, *ai* $C_{15:0}$, and *i* $C_{16:0}$. In contrast, some of the biomarker fatty acids of sulfate-reducing bacteria (SRB), like 10*Me*-$C_{16:0}$ and *i* $C_{17:1\omega7}$, showed a late and weak incorporation. After the 12 h incubation the largest fraction of processed carbon was in the bacteria, but after longer incubation times (30 h and 32 h *in situ*) the macrofauna gained more importance, until after 132 h the greatest fraction was mineralised to $CO_2$. Our findings show the fast reaction capacity of the benthic sand community on a settling phytoplankton bloom and a great importance of bacteria in the first steps of algal carbon processing.

## KEYWORDS

Sandy sediment; pulse-chase experiment; $^{13}C$ labelling; bacteria; mineralisation; macrofauna; benthos

## INTRODUCTION

Shelf seas cover one tenth of the ocean area, but contribute 30% to the worlds ocean primary production (Jørgensen, 1996). Sand is the predominant sediment type on the shelf, where in the northern temperate latitudes large phytoplankton blooms occur in spring (Reid *et al.*, 1990). Due to the shallowness of shelf seas, up to 50% of this biomass can settle to the sea floor (Jørgensen *et al.*, 1990), building up the bulk of the food supply for the benthic community (Conley and Johnstone, 1995). Most of the deposited material is directly mineralised in these sediments (Berner, 1982), emphasising the prominent role of the continental shelf in the marine carbon cycle. In these sediments, the organic matter is degraded along aerobic and anaerobic pathways, with oxygen and sulfate as the most important electron acceptors (Jørgensen, 1982). The bacterial communities inhabiting sands are nowadays regarded as highly active (Rusch *et al.*, 2003, Ehrenhauss *et al.*, in press), even though sandy sediments typically show lower bacterial densities than muddy sites (Llobet-Brossa *et al.*, 1998). The high activity is probably caused by advective porewater transport (e.g. Boudreau *et al.*, 2001) or interactions of bottom currents and sediment topography or biogenic structures (Huettel and Rusch, 2000, Huettel and Webster, 2001).

The benthic community processes the arriving organic material by incorporation, excretion and respiration. Benthic organisms and interactions within the benthic community are thus supposed to have a great impact on the fast turnover rates of settling particles in these environments. However, these interactions are poorly studied so far. Information is available on the impact of settling phytoplankton on single benthic compartments like on macrofauna (Boon *et al.*, 1998), on the bacterial response after bloom events (Stoeck and Kroncke, 2001) or on the decomposition of settling particles (Sun *et al.*, 1997). But the *in situ* determination of the benthic community structure is still one of the most urgent problems. Above all, information is missing on microbial communities. Here, investigations of microbial lipid biomarkers have tremendously increased our understanding of the microbial world. They represent a very useful tool to investigate microbial community patterns *in situ* (Rajendran *et al.*, 1992, Hinrichs *et al.*, 1999,

Rütters *et al.*, 2002 a) as shown as well at our study site (Bühring *et al.*, subm.). In addition, the application of isotopically labelled substrates has made tracer studies on carbon cycling by microbial populations possible (Middelburg *et al.*, 2000, Boschker *et al.*, 1998, Moodley *et al.*, 2002, and Witte *et al.*, 2003).

In this study, we report *in situ* and on-board experiments with a simulated phytoplankton bloom on a fine sand station near Spiekeroog Island (German Bight, North Sea). In order to follow the fate of settling phytoplankton, we carried out pulse-chase chamber experiments with $^{13}$C-labelled phytoplankton. The total uptake of algal carbon into macrofauna, the incorporation into bacterial biomarkers and its mineralisation to $^{13}CO_2$ was then followed by isotopic ratio mass spectrometry (IRMS).

## MATERIAL AND METHODS

### Sites and sampling

During two cruises of R.V. Heincke in April (HE 145) and June 2001 (HE 148), experiments were conducted on a fine sand station in the southern North Sea seaward of the island of Spiekeroog (53°51´N, 007°44´E, Figure 1). The German Bight is a shallow part of the southern North Sea. Because of tides, waves and storm-generated bottom currents, the sediment is frequently eroded and redeposited (Antia, 1995). The permeability was $3.02 \pm 1.66 \times 10^{-12}$ m$^2$ and the density was 2.06 $\pm$ 0.02 g ml$^{-1}$ (Janssen *et al.*, subm.). The salinity of the sampling site was 31-32 PSU and the water temperature was 9°C in April and 13°C in June. During HE 145 we conducted on-board incubations of 12 h, 30 h and 132 h duration and during HE 148 an *in situ* experiment of 32 h.

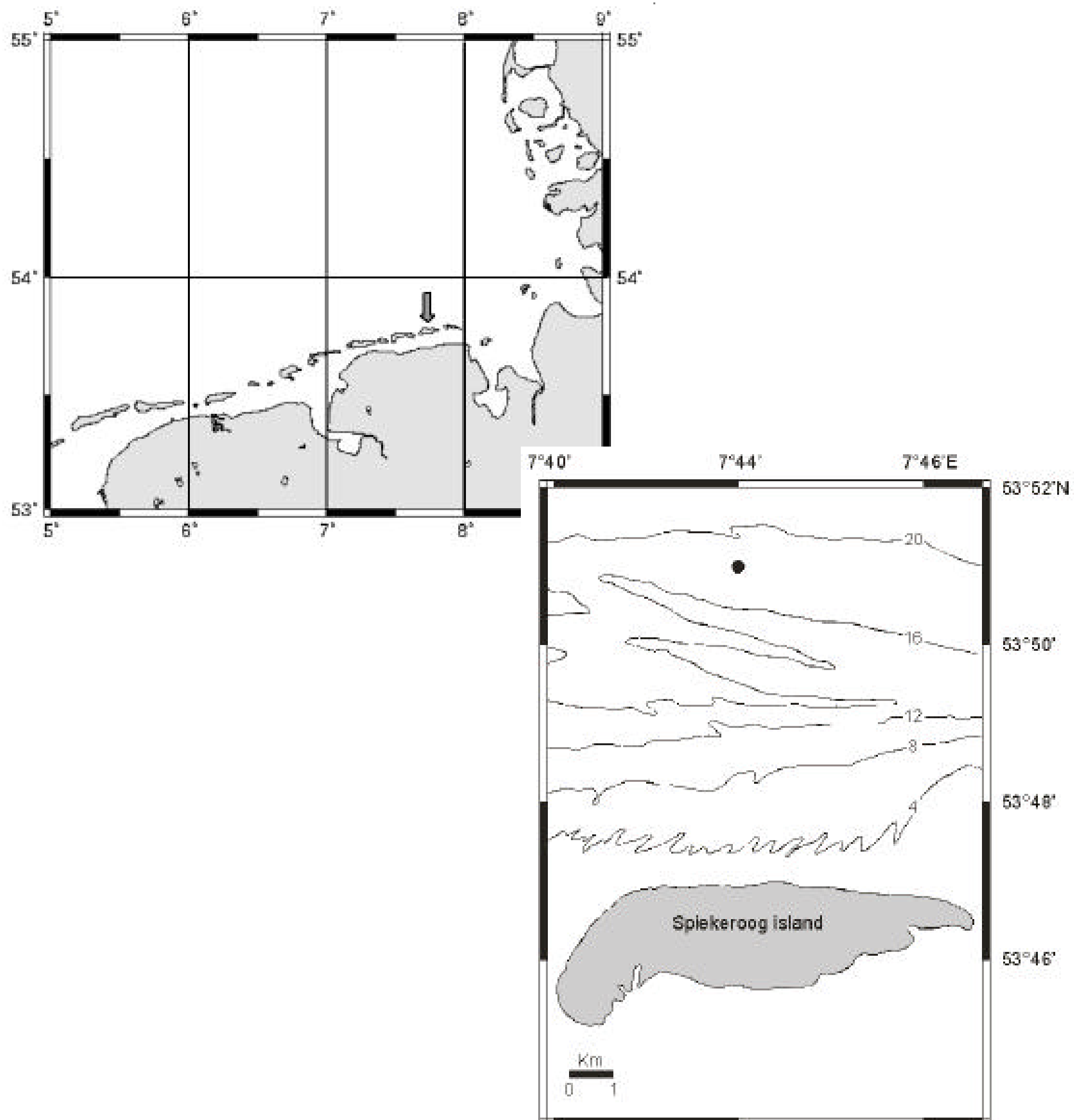


Figure 1: Area of investigation in the German Bight (southern North Sea) and the bathymetry of the area (redrawn after (Antia, 1993)).

Prior to the experiments, we cultured an axenic clone of the diatom *Ditylum brightwellii* (Bacillariophyceae, Biddulphiales) at 25°C in artificial seawater with a salinity of 33 PSU (Grasshoff, 1999) enriched with f/2 medium (Guillard and Ryther, 1962). This medium contained 25% $^{13}$C-bicarbonate (99% $NaH^{13}CO_3$, Cambridge Isotope Laboratories). The algal material was harvested by centrifugation (404 **g**, 4 min), rinsed 3 times with an isotonic sodium chloride solution and centrifuged again. The axenic state of the algae was verified by microscopic observation of DAPI stained cells and by investigation of fatty acid composition. Neither bacteria nor bacterial

fatty acids could be detected. The produced algae consisted of 15 atom% $^{13}C$ (HE 145) and 9 atom% $^{13}C$ (HE 148), the amount of carbon added to each chamber corresponded to 0.31 g C $m^{-2}$ (HE 145) and 0.36 g C $m^{-2}$ (HE 148), respectively.

The experiments were performed in acrylic cylindrical chambers (Huettel and Gust, 1992), 20 cm in diameter and 31 cm in height, which were covered with black foil to prevent light penetration. A horizontal disk with 17 cm in diameter stirred a water column of approximately 10 cm height with 20 rpm. The rotating water generates a pressure gradient (ca. 1.5 Pa $cm^{-2}$), comparable to the pressure gradient at a sediment ripple interacting with bottom currents (Huettel and Rusch, 2000). This gradient induces advective porewater flows in permeable sediments.

Chambers for the on-board experiments were attached to a frame that was lowered to the sea floor by ships winch. They were obtained by divers, who inserted them into the sediment, and directly sealed the bottom before haul back to the ship's lab. They were incubated in duplicates at *in situ* temperature and stirred directly after recovery. For the *in situ* experiments the frame was deployed, divers inserted the chambers into the sediment, and also injected the algae into the chambers. After the incubation time of 32 h, divers closed the two chambers at the seafloor. At the end of the experiments the sediment was sliced at 0.5 cm intervals down to 1 cm, 1 cm intervals down to 10 cm and 2.5 cm intervals below 10 cm sediment depth. The macrofauna was carefully picked from every sediment horizon and the sediment was then homogenised before taking samples for porewater $^{13}CO_2$ and lipid analysis. For the porewater $\delta^{13}CO_2$ analysis 20 ml sediment was centrifuged (2800 **g**, 10 min) through GF-F filters and 2 ml of the porewater was kept at 4°C in a nitrogen flushed 4 ml gas-tight glass scintillation vial containing mercury chloride (end concentration 0.2%). The macrofauna was sorted under the stereo microscope and stored at –20°C. (For further details on subsequent treatment of macrofauna samples see Kamp and Witte (in prep.)). The lipid samples were stored in glass bottles with teflon lids at –20°C.

Sediment cores for background samples were taken with a small multicorer system equipped with four cores with an inner diameter of 36 mm. Sediment cores were sliced at the same depth intervals as for the experiments.

## Lipid analysis

Lipids were extracted ultrasonically four times for 20 min from the wet sediment material with dichloremethane-methanol (2:1 v/v) following an extraction protocol of Elvert *et al.* (2003). The resulting dichloromethane extract was separated from the aqueous methanol phase by adding 0.05 M KCl solution. For saponification an aliquot of the total extract was treated with 6% KOH in methanol for 3 h at 80°C. Neutral lipids were released by addition of 0.05 M KCl for phase separation and extracted with hexane. 25% HCl was added to the aqueous reaction mixture (pH < 1) and free fatty acids were extracted with hexane. The fatty acid fraction was then evaporated to near dryness and derivatized by adding 1 ml $BF_3$-methanol (12-14%, Merck) for 1 h at 60°C to yield fatty acid methyl esters (FAME).

Concentrations of FAMEs were determined by gas chromatography-flame ionization detection (Hewlett Packard 5890, series II), operating in splitless mode. A 50 m apolar HP-5 fused silica capillary column was used (0.32 mm internal diameter, 0.17 µm film thickness; Hewlett Packard). The carrier gas was He (2.0 ml $min^{-1}$). Initial oven temperature was 60°C held for 1 min, increased to 150°C with 10°C $min^{-1}$, then to 310°C with 4°C $min^{-1}$ and finally kept at 310°C for 15 min. The injector temperature was set at 300°C and the detector at 310°C. $C_{19:0}$ fatty acid was added prior to extraction as an internal standard for quantification. Identification of FAMEs was based on the comparison of retention time with known standards. Fatty acid short-hand nomenclature is according to Guckert *et al.* (1985).

Identification of unknown compounds was carried out using a Thermoquest Trace GC interfaced to a Finnigan Trace MS using the same conditions as for gas chromatography. Mass spectra were collected in full scan (m/z 30-580, 0.9 scans per second), operating in electron impact mode at 70 eV. The samples were injected in splitless mode at an injection temperature of 295°C on a HP-5 MS fused silica capillary column (60 m, internal diameter 0.32 mm; Hewlett Packard) coated with a cross-linked methyl silicone phase (film thickness 0.25 µm, Hewlett Packard). The detector was set at 350 V, the interface was 310°C and the carrier gas was He with a constant flow of 1.4 ml $min^{-1}$.

Double bond positions of fatty acids were determined by analysis of dimethyl disulphide adducts, prepared after the method of Nichols *et al.* (1986).

Carbon isotope composition of FAMEs was determined by GC-*c*-IRMS. The mass spectrometer (Finnigan Delta plus) was connected via a Finnigan Combustion Interface III to a HP 6890 Series GC equipped with a 50 m HP-5 (0.32 mm ID, 0.17 µm stationary phase, Hewlett-Packard). The flow rate of the carrier gas He was 1.5 ml $min^{-1}$. Samples were injected in splitless mode (injector temperature: 290°C) and subjected to the same temperature program as given for GC and GC-MS measurements. The oxidation and reduction ovens were operated at 940°C and 640°C, respectively. The results were corrected for the introduction of the additional carbon atoms during derivatization with $BF_3$-methanol.

### $^{13}CO_2$ analysis

The water samples for the $\delta^{13}CO_2$ measurements were filtered (0.2 µm) directly into 12 ml gas-tight glass scintillation vials sealed with a rubber septum and containing mercury chloride in an end concentration of 0.2% to stop all activity. The samples were stored refrigerated until analysis.

Prior to the measurement a headspace was created by injecting 3 ml nitrogen gas and the sample was then acidified with 100 µl and 20 µl sulfuric acid (20%) for the chamber and porewater samples, respectively. The $\delta^{13}CO_2$ and the concentration of $CO_2$ in the headspace was measured using a Carlo Erba 1106 Elemental Analyser coupled online with a Finnigan Delta S isotope ratio mass spectrometer. Reproducibility of the measurements is ~0.10‰.

### Calculations

Carbon isotopic ratios ($^{13}C/^{12}C$) are expressed in the delta notation ($\delta^{13}C$) relative to Vienna Pee Dee Belemnite Standard ($^{13}C/^{12}C_{VPDB}$ = 0.0112): $\delta^{13}C$ (‰) = $[(R_{sample}/R_{std})-1)] \times 1000$, where $R_{sample}$ and $R_{std}$ are the $^{13}C/^{12}C$ of sample and

standard, respectively (Craig, 1957). Total uptake *I* of $^{13}C$ was calculated as the product of excess $^{13}C$ (*E*) and macrofauna biomass (organic carbon content) or carbon content in $CO_2$ concentrations. For bacteria, *I* was calculated after Middelburg *et al.* (2000) from label incorporation into bacterial fatty acids (*i* $C_{15:0}$, *ai* $C_{15:0}$, *i* $C_{16:0}$, *i* $C_{17:1\omega7}$, 10*Me*-$C_{16:0}$, *i* $C_{17:0}$, *ai* $C_{17:0}$, $C_{17:1\omega8}$ and $C_{17:1\omega6}$) as $I_{bact} = \sum I_{bact.fatty\text{-}acids}/(a \times b)$, where *a* is the average phospholipid-derived fatty acid (PLFA) concentration in bacteria of 0.056 g of carbon PLFA $g^{-1}$ biomass (Brinch-Iversen and King, 1990) and *b* is the average fraction specific bacterial PLFA encountered in sediment dominated by bacteria (0.19; calculated after Findlay and Dobbs, 1993, Guezennec and Fiala-Medioni, 1996, Rajendran *et al.*, 1994). *E* is the difference between the fraction *F* of the sample and background: $E = F_{sample} - F_{background}$, where $F = {}^{13}C/({}^{13}C/{}^{12}C) = R / (R + 1)$ and $R = (\delta^{13}C / 1000 + 1) \times R_{VPDB}$ with $R_{VPDB} = 0.0112372$.

The fatty acid composition of the added diatom *Ditylum brightwellii* was dominated by the fatty acid $C_{14:0}$, $C_{15:0}$, $C_{16:3}$, $C_{16:1\omega7}$, and $C_{16:0}$.

In our experiments, the amount of added particulate organic carbon (POC) (0.31 and 0.36 g C $m^{-2}$) corresponds to half of the daily primary production (0.5 g C $m^{-2}$ $d^{-1}$; Wollast, 1991) in the southern North Sea. Thus the experimental conditions were comparable to conditions in the natural environment.

## RESULTS

### Incorporation of $^{13}C$ into bacterial fatty acids

Figure 2 shows the total incorporation of label into selected bacterial fatty acids. $^{13}C$ was detectable in all investigated bacterial fatty acids although in varying amounts. The highest incorporation was found in *i* $C_{16:0}$ and the lowest in 10*Me*-$C_{16:0}$ and *i* $C_{17:0}$. *I* $C_{16:0}$ showed a fast and high incorporation already after 12 h, with decreasing values after 30 h and 132 h. The fatty acids *i* $C_{15:0}$ and *ai* $C_{15:0}$ showed increasing incorporation up to 30 h experimental duration. Only minor incorporation with only slight changes over time was visible for *i* $C_{17:1\omega7}$, 10*Me*-$C_{16:0}$, *i* $C_{17:0}$, *ai* $C_{17:0}$ and $C_{17:1\omega6}$. The fatty acid $C_{17:1\omega8}$ displayed a continuously increasing uptake with

maximum incorporation after 132 h. Bacterial incorporation of $^{13}C$ was generally lower in the 32 h *in situ* experiment in June than the 30 h on-board experiment in April.

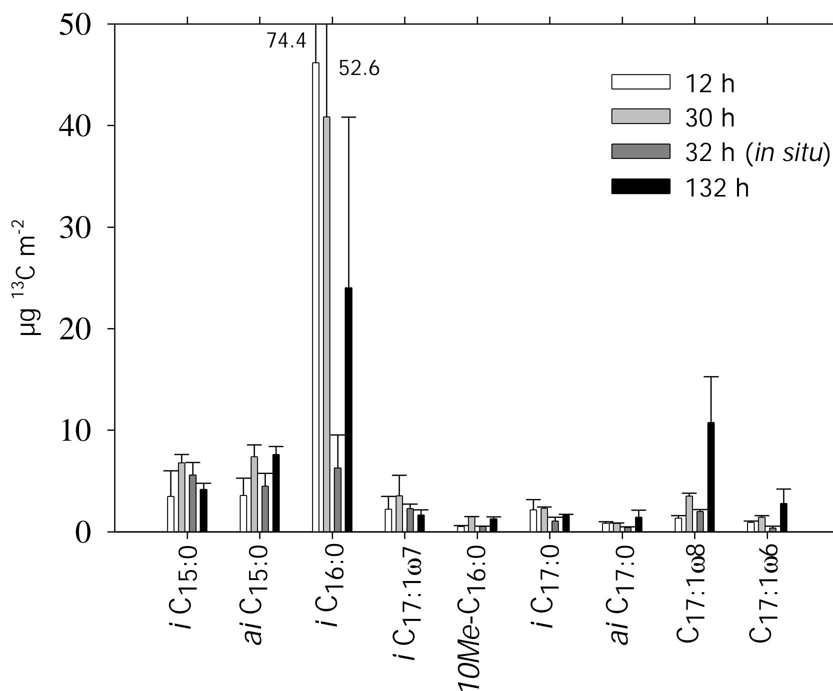


Figure 2: Incorporation of algal $^{13}C$ into bacterial fatty acids [µg $^{13}C$ $m^{-2}$] (sampling depth: 0-12 cm) from the on-board incubations of 12 h, 30 h and 132 h and the 32 h *in situ* experiments. Vertical bars indicate range of two replicates.

The total amount of tracer- $^{13}C$ found in bacterial fatty acids in the April experiments was relatively constant with time with 5765 µg $m^{-2}$ (12.4% of the added $^{13}C$) after 12 h, 6422 µg $m^{-2}$ (13.8%) after 30 h and 5212 µg $m^{-2}$ (11.2%) after 132 h. *In situ*, 2170 µg $m^{-2}$ (6.7%) of the added label were detected in bacterial fatty acids after 32 h of incubation.

The vertical distribution of $^{13}C$ incorporation into bacterial fatty acids within the sediment is shown in Figures 3 A and 3 B. Bulk incorporation took place at the sediment surface. After 12 h, bacterial fatty acids down to 3 cm sediment depth were labelled. After 30 h different fatty acids displayed subsurface incorporation layers between 3 and 5 cm.

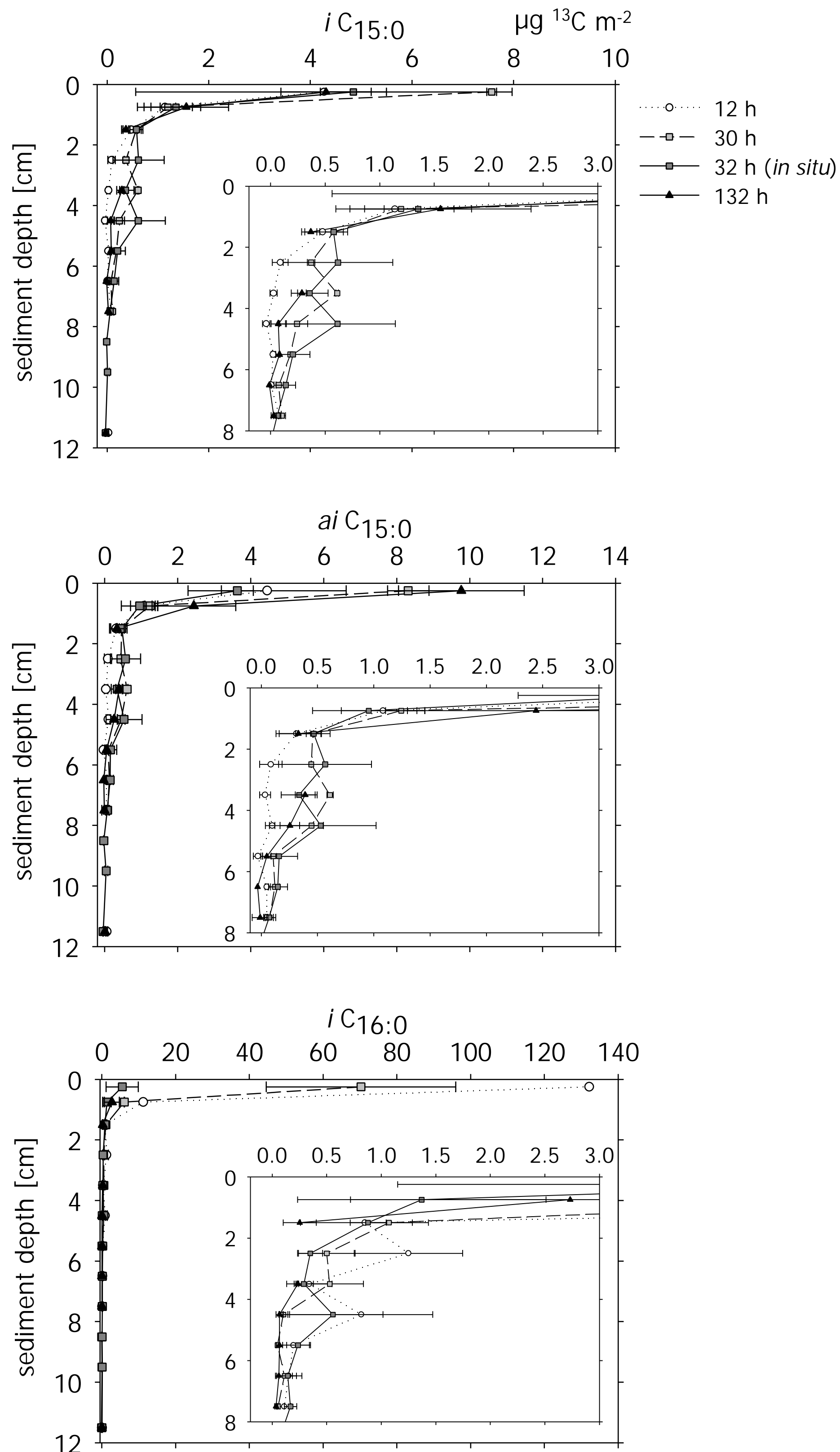


Figure 3 A: Incorporation [µg $^{13}C$ $m^{-2}$] into bacterial fatty acids *i* $C_{15:0}$, *ai* $C_{15:0}$, and *i* $C_{16:0}$ versus sediment depth from the on-board incubations of 12 h, 30 h and 132 h and the 32 h *in situ* experiments. Bars indicate range, shown if two replicates were available. Please note the different scale for total uptake. The inlays display a magnification.

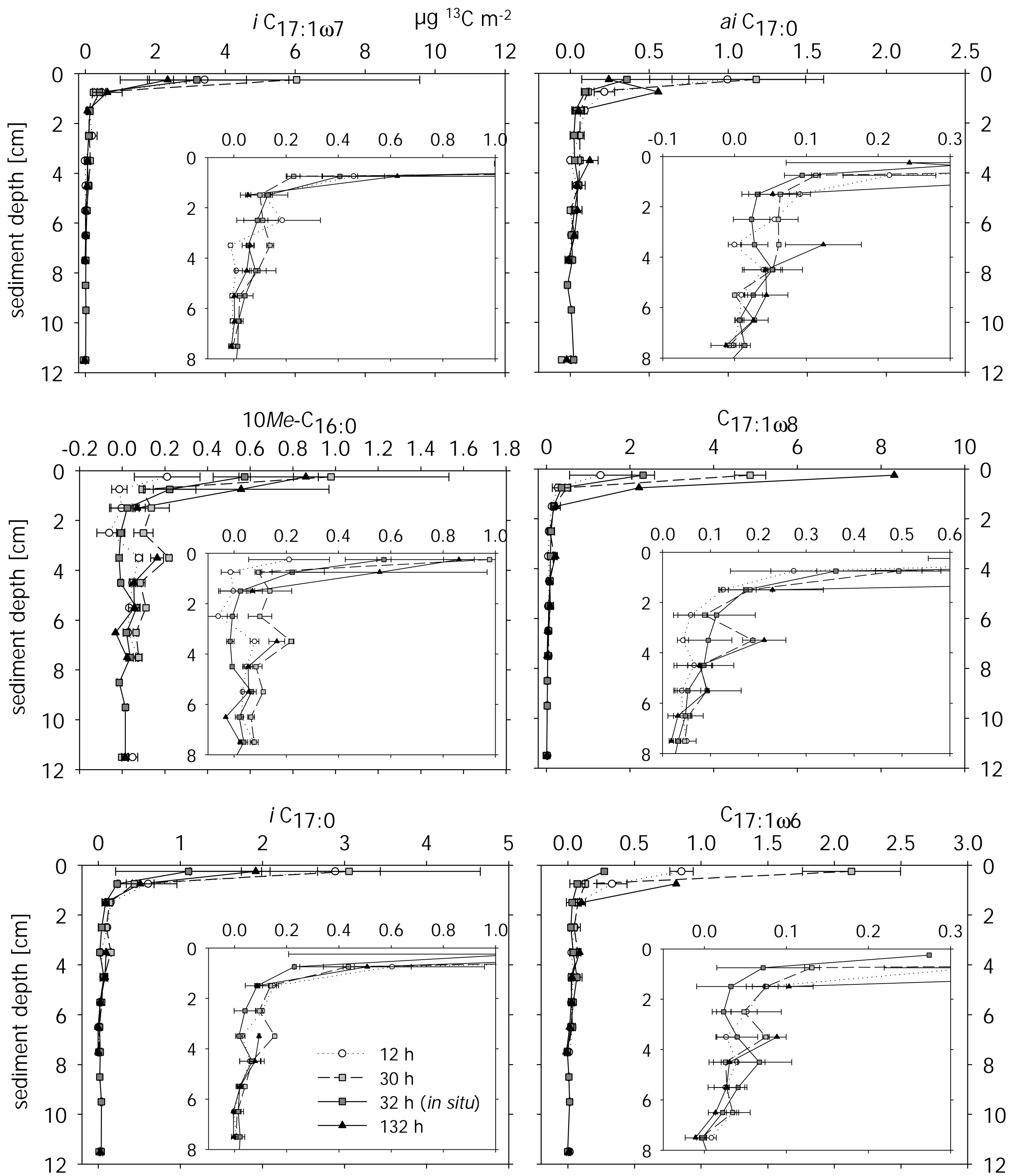


Figure 3 B: Incorporation [μg $^{13}C$ $m^{-2}$] into bacterial fatty acids *i* $C_{17:1\omega7}$, 10*Me*-$C_{16:0}$, *i* $C_{17:0}$, *ai* $C_{17:0}$, $C_{17:1\omega8}$, and $C_{17:1\omega6}$ versus sediment depth from the on-board incubations of 12 h, 30 h and 132 h and the 32 h *in situ* experiments. Bars indicate range, shown if two replicates were available. Please note the different scale for total uptake. The inlays display a magnification.

## Uptake of algal material by macrofauna

The amount of tracer taken up by macrofauna organisms continually increased throughout the experiments (Figure 4). Already after 12 h organisms at all sediment depth had ingested tracer material. Macrofauna labelling increased with time displaying maxima at different depths. After 12 h, 249 µg $m^{-2}$ (0.5%) of the added $^{13}C$-label could be detected in macrofauna organisms, after 30 h and 132 h, macrofauna had ingested 2137 µg $m^{-2}$ (4.6%) and 2900 µg $m^{-2}$ (6.2%), respectively. In the 32 h *in situ* experiment in June we found a very high uptake by macrofauna of 5007 µg $m^{-2}$ (15.5% of the added label).

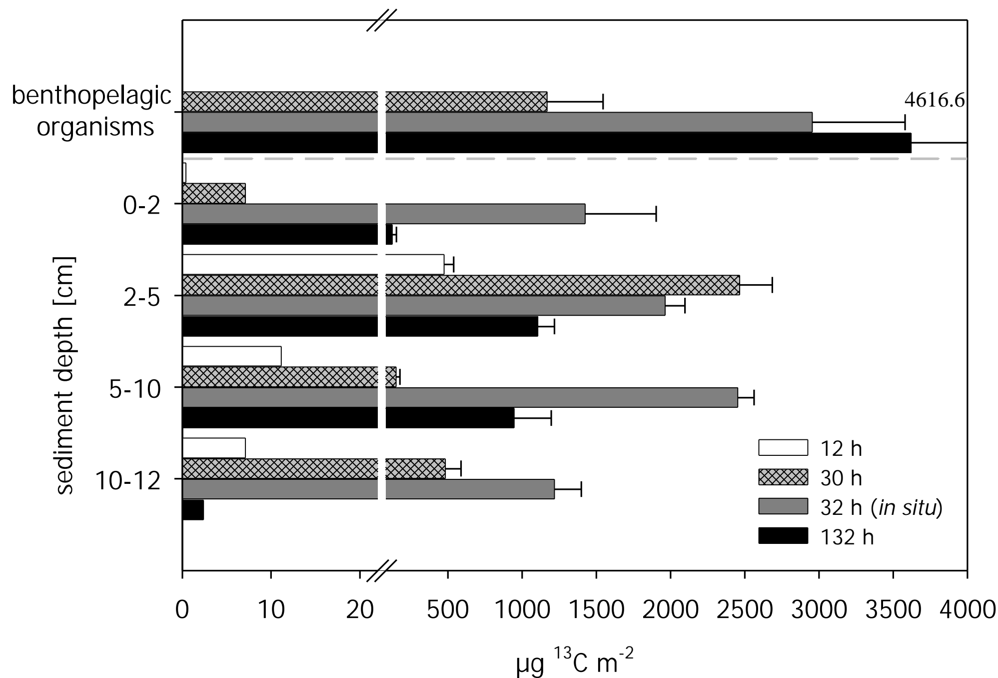


Figure 4: Total uptake [µg $^{13}C$ $m^{-2}$] of label into macrofauna versus sediment depth during the 12 h, 30 h and 132 h on-board incubations and the 32 h *in situ* experiment. Horizontal bars indicate S.D.

## Mineralisation of tracer POC to $^{13}CO_2$

During the on-board experiments, chamber water $S^{13}CO_2$ was determined repeatedly during the experiments. The results, shown as mineralisation to $^{13}CO_2$ [µg $^{13}C$ $m^{-2}$] over time, are given in Figure 5. The labelling of $CO_2$ increased throughout the experimental duration. In the end of our experiments we found the following fluxes of the added label into the $CO_2$ of the overlying water: 1.0% after 12 h, 6.0% after 30 h, 3.1% after 32 h (*in situ*) and 10.7% after 132 h.

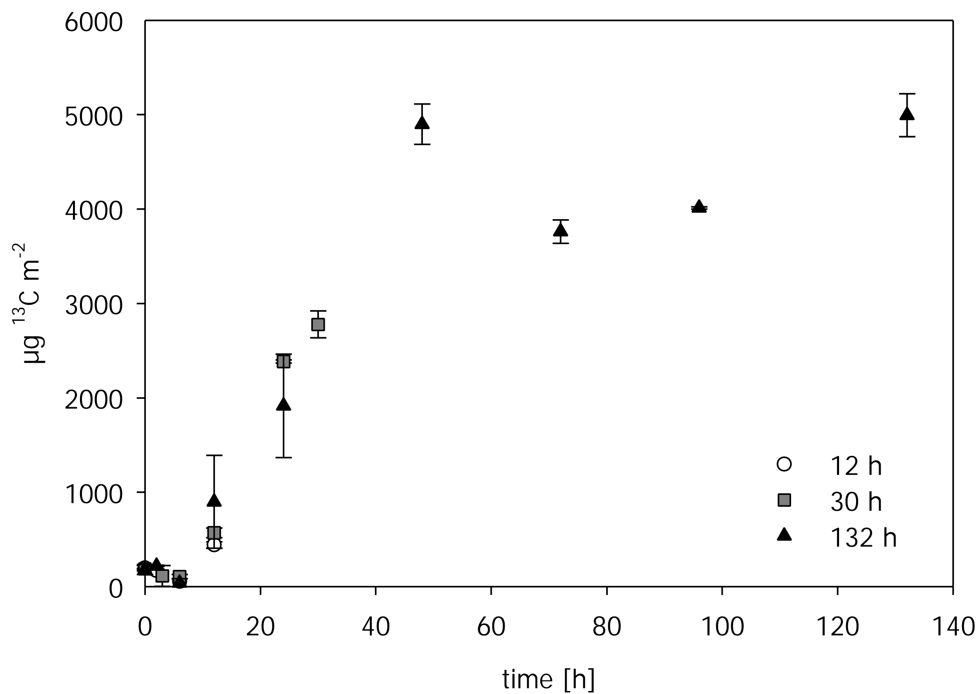


Figure 5: Release of $^{13}CO_2$ to overlying water [µg $^{13}C$ $m^{-2}$] over time for the 12 h, 30 h, 132 h on-board incubations. Vertical bars indicate range of two parallels.

Porewater $^{13}CO_2$ increased throughout the experiment duration over the whole sediment column, indicating that mineralisation of tracer POC occurred down to 12 cm sediment depth (Figure 6). After 132 h, a subsurface peak had developed at 3-4 cm sediment depth. The 32 h *in situ* experiment of the June cruise displayed slightly higher values than the 30 h experiment of HE 145 and in the end 2.9% of the added algae were detectable as $^{13}CO_2$ in the porewater.

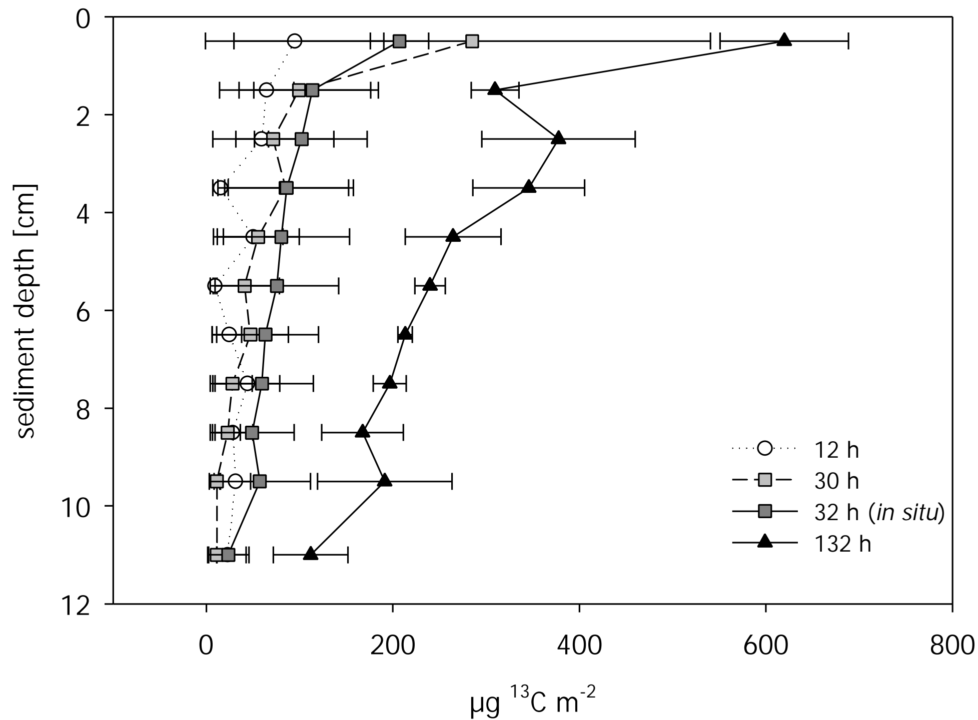


Figure 6: Porewater $^{13}CO_2$ [µg $^{13}C$ m$^{-2}$] versus sediment depth of the 12 h, 30 h and 132 h on-board incubations and the 32 h *in situ* experiment. Horizontal bars indicate range of two parallels.

## DISCUSSION

The decomposition of organic material follows different pathways. At the sediment-water interface aerobic mineralisation takes place through the activity of benthic microorganisms and animals and below the oxygen penetration depth mineralisation occurs via anaerobic processes. The anaerobic food chain consists of different types of bacteria that mediate different metabolic steps, each of which is only partially completing the oxidation of the organic compounds. The participating organisms are usually denitrifying, sulfate reducing, and methane producing bacteria. Our investigations of bacterial biomarkers differentiated bacterial communities with different physiologies.

In our experiments the bacterial community proved the capability for fast processing of organic carbon, but incorporation patterns into the investigated bacterial fatty acids were very different (Figures 2, 3 A and 3 B). This is probably due to different ways of synthesis of the investigated biomarkers and metabolic specificity of the bacteria. The branched-chained fatty acids *i* $C_{15:0}$, *ai* $C_{15:0}$ and especially *i* $C_{16:0}$ showed a fast and high label incorporation. Therefore, these fatty acids must be quickly biosynthesised. They are described as typical for several types of bacteria (Boschker and Middelburg, 2002), but they are particularly abundant in gram-positive prokaryotes (White *et al.*, 1996). The fast incorporation of label indicates that these bacteria have direct access to the diatom material by either using exoenzymes or taking up dissolved organic carbon (DOC), which was already present in the added algal material (Ehrenhauss *et al.*, accepted). Middelburg *et al.* (2000) found fast uptake into *i* $C_{15:0}$, *ai* $C_{15:0}$ and *i* $C_{16:0}$ fatty acids as well, during an experiment on an intertidal sandy site in the Scheldt estuary, where they sprayed $^{13}C$-bicarbonate on the surface and followed its way through the benthic food web. They found evidence for photosynthetically fixed $^{13}C$ entering the microbial food web within hours and maximum labelling of bacterial fatty acids after 1 d. At our study site the maximum of bacterial fatty acid labelling happened between 30 h and 132 h.

Those fatty acids with a weak incorporation after 12 h, like *i* $C_{17:1\omega7}$, 10*Me*-$C_{16:0}$, *i* $C_{17:0}$, *ai* $C_{17:0}$, $C_{17:1\omega8}$, and $C_{17:1\omega6}$, are described as typical for *Desulfobacter*

spp. (Boschker *et al.*, 1998). Low label incorporation into $C_{17}$ fatty acids was also observed by Boschker *et al.*, 1998) during an experiment with $^{13}C$-acetate. They concluded that *Desulfobacter* spp., which is the mainly isolated SRB in coastal sediments, is probably of minor importance. Our experiments showed that this group is active, but the small amount of uptake indicates that they have no direct access on the diatom material. The fatty acid $C_{17:1\omega8}$, which is described as typical for *Desulfomicrobium* spp. (Rütters *et al.*, 2002 b), was the only fatty acid with a clear increase of incorporation until the end of experiment. This pattern could be caused by the incorporation of secondary carbon products (e.g. acetate and other short chain carbon sources) produced by other organisms feeding directly on the diatoms, like fermentative bacteria and meio- or macrofauna.

The total uptake of label into bacterial fatty acids versus sediment depth (Figures 3 A and 3 B) displayed highest turnover rates at the sediment surface. However, especially after 30 h on-board incubation and 32 h *in situ* experiment we found subsurface peaks of incorporation in 3-5 cm depth. Comparing the label incorporation into bacterial fatty acids and the $^{13}CO_2$ in the porewater (Figure 6), we found distinct depths with high mineralisation corresponding with high bacterial incorporation activity, e.g. in the upper sediment layers and at 4.5 cm depth after 12 h. Good correlations of bacterial incorporation and mineralisation could also be found for the 30 h incubation with high values at 3.5 cm sediment depth. These results suggest that "hot spots" of bacterial activity exist in the distinct sediment horizons.

As part of our project, the transport of algal derived $TO^{13}C$ into deeper sediment layers was observed as well by Ehrenhauss *et al.*, accepted), who found uptake down to 6 cm sediment depth after 132 h. One possible explanation for this transport could be advective porewater flow, although at our station with a permeability of $3.02 \pm 1.66 \times 10^{-12}$ $m^2$, advection should only cause transport down to 2 cm into the sediment (Huettel and Gust, 1992). New insights from Janssen *et al.* (subm.) using the *in situ* chamber system SANDY at the same station, make relevance of advection as predominant transport process at our station even more unlikely. They found negligible advective porewater exchange, which emphasise the great importance of macrofauna driven transport at our study site. Deeper penetration of algal derived TOC, together with subsurface maxima in label uptake by bacteria and

mineralisation must be caused by a different transport process, possibly by activity of macrofauna organisms. Right from the beginning of our experiments the uptake into macrofauna was not restricted to distinct depth intervals (Figure 4). The largest uptake was measurable in animals from the sediment water interface, but already after 12 h of incubation animals from all depths were labelled. Deep penetration of labelled particles in our experiments is therefore possibly due to the activity of macrofauna organisms. At our study site *Fabulina fabula* is the most abundant macrofauna organism (Kamp and Witte, in prep.). Lab experiments showed that *F. fabula* is responsible for transport of $TO^{13}C$ up to 7 cm sediment depth after 132 h incubation (Kamp, 2002). In our experiments Kamp and Witte (in prep.) found furthermore high amounts of label ingestion by the polychaete *Lanice conchilega* (Terebellidae). Tube-building polychaetes are known to rapidly subduct sedimenting material to deeper sediment layers (Levin *et al.*, 1997). Transport of POM by the activity of bioturbating animals was also observed by Sun *et al.* (1999) and Blair *et al.* (1996) using labelling experiments. Ehrenhauss *et al.* (accepted) found after 30 h of incubation uptake of $TO^{13}C$ up to 4 cm into the sediment and we found a peak of labelled bacterial fatty acids in the same depth. After 32 h *in situ* experiment the $TO^{13}C$ was found up to 6 cm depth and a peak of bacterial labelling at 4.5 cm. Regarding these results we conclude, that the transport of $TO^{13}C$ by bioturbating animals allows bacteria from deeper layers to access relatively fresh POM and is assumed to be one reason for our subsurface labelling.

Macrofauna organisms also digest the algae and alter them by passage through their gut system. This may also influence the availability of POC for other (micro-) organisms. Some of the detected SRB, like *Desulfosarcina*, are furthermore capable of using autotrophic pathways. Therefore, the uptake of $^{13}C$-bicarbonate released as $^{13}CO_2$ by macro- and meiofauna inhabiting deeper sediment layers could be another explanation for subsurface maxima of incorporation into bacterial fatty acids.

5.0% of the added label was converted to $CO_2$ per day. Release from the sediment could be caused by bioirrigation of macrofauna organisms. We found fluxes of 3.4% of the added label per day into the $CO_2$ in the overlying water. Overall the mineralisation of added organic carbon corresponds to 0.37 and 0.35 mmol $CO_2$ $m^{-2}$

$d^{-1}$, with and without *in situ* experiments, respectively. This is only a small amount of total respiration referring to measurement of Janssen *et al.* (subm.) from the same station, who found a total oxygen uptake (TOU) of 29.2 mmol $O_2$ $m^{-2}$ $d^{-1}$. Assuming an RQ of 1 we found only 1.2 to 1.3 % of total respiration are due to our added algae, revealing that our approach was no enrichment experiment.

In Figure 7 we displayed the relative fractions of the carbon in the different compartments. Clearly visible is the great importance of bacteria on the carbon turnover in this sediment, emphasising their importance for the very first reaction after the arrival of settling phytoplankton blooms. After longer incubation times, the macrofauna gains more importance and the mineralisation is increasing as well.

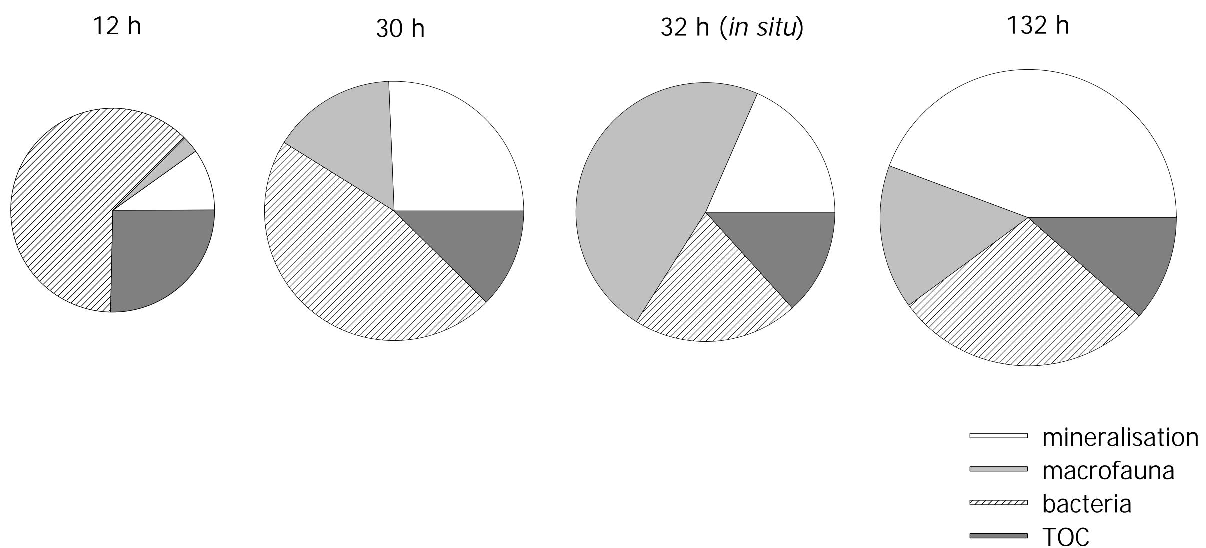


Figure 7: Proportional division of the processed carbon of the different benthic compartments and sediment $TO^{13}C$. The size of the pies corresponds to the total amount of recovered label.

The size of the pies corresponds to the total recovery in % of added label, which was 20%, 30%, 32% and 40% for the 12 h, 30 h, 32 h *in situ* and 132 h experiment, respectively. This results in a total recovered label of 9146, 13823, 10504 and 18375 µg $^{13}C$ $m^{-2}$ for the different incubations.

Differences between the on-board and *in situ* experiments are mainly due to macrofauna uptake. The macrofauna organisms that were found in the chambers of the *in situ* experiment of the June cruise, displayed a much greater share of label uptake compared to the experiments in April. This is probably due to the seasonal variations in abundance and activity, which were higher during June (Kamp and Witte, in prep.). Meio- and macrofauna can furthermore significantly reduce the bacteria due to their feeding activity. That could be an explanation for the lower bacterial incorporation especially during the *in situ* experiment with high macrofauna abundances.

In total 19.1 mg C $m^{-2}$ $d^{-1}$ was processed during the on-board incubations. Taking the *in situ* experiments into account as well, 21.7 mg C $m^{-2}$ $d^{-1}$ was processed. Compared to other investigations using the same labelling approach we found higher values than detected for the abyssal deep sea (4 mg C $m^{-2}$ $d^{-1}$; Witte *et al.*, 2003) and a deep-sea study in 2100 m depth (4.2 mg C $m^{-2}$ $d^{-1}$; Moodley *et al.*, 2002) and comparable values to a study on the continental slope (19 mg C $m^{-2}$ $d^{-1}$; Witte *et al.*, 2003). We have to take into account that we added only approximately half of the daily primary production (0.5 g C $m^{-2}$ $y^{-1}$, Wollast, 1991), implying that overall carbon processing in this sediment is considerably higher. This is an astonishing result, regarding the former belief that sandy sediments are biogeochemical deserts due to their low organic carbon content (Boudreau *et al.*, 2001) and due to low bacterial abundances compared to finer compartments (Llobet-Brossa *et al.*, 1998).

Our experiments revealed the great importance of the benthic bacterial community on the short-term fate of settling phytoplankton blooms. Graf (1992) exposed that bacteria are responsible for the major part of the oxygen consumption. Investigations of the benthic reaction on phytoplankton input by Boon *et al.* (1998) at several stations in the North Sea revealed a temporal uncoupling of macrofaunal and bacterial processes. In the present study, we could observe that bacteria, macrofauna and the mineralisation follow different time pattern in their very short-term reaction.

## CONCLUSIONS

The investigation of turnover on a sandy North Sea station revealed a very rapid reaction of the benthic community to experimental POM addition. Incorporation into bacterial biomarkers shows significant variations between the different bacterial groups in the sediment. Our results suggest that at least two distinct metabolic groups of bacteria were involved in the consumption of the added material, one with direct access on the added material, maybe with subsequent production of fermentative products, and SRB, which take up e.g. acetate or other short chain carbon products. The macrofauna reaction was somewhat retarded compared to the bacteria, as well as the mineralisation of the added material to $CO_2$.

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**Chapter 5**

# BENTHIC RESPONSE TO VARYING FOOD INPUT: *IN SITU* PULSE-CHASE EXPERIMENTS IN THE DEEP CRETAN SEA (EASTERN MEDITERRANEAN)

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## ABSTRACT

The deep Eastern Mediterranean Sea is considered one of the most oligotrophic regions of the world oceans. The scarcity of food and resulting food limitation is very suitable to investigate the response pattern of deep-sea benthic communities to varying food input.

For this purpose, 36 h pulse-chase experiments were performed *in situ* in benthic chambers via addition of $^{13}C$-labeled phytodetritus equivalent to 25 mg C $m^{-2}$ (L experiments) and 250 mg C $m^{-2}$ (H experiments), respectively. The latter amount corresponds to approximately half of the total annual organic carbon flux to the deep sea in the study area. The stable isotope served as a tracer in order to track the microbial processing of the fresh carbon added.

The analysis of the fatty acid composition revealed a bacterially dominated benthic community, with evidence for considerable numbers of sulfate-reducing bacteria (SRB). Eminent differences in incorporation into the various biomarkers were observed, with generally lower incorporation into biomarkers of SRB than into other bacteria. All experiments revealed a fast reaction of the deep-sea benthic community in total, confirming that these deep-sea communities are well adapted to changing conditions in food availability. The overall carbon mineralization increased by 40% (L) and 120% (H), respectively. However the remineralization of fresh labeled carbon was > 20 fold higher in the H experiments. Furthermore a difference between respiration and incorporation was observed, which revealed a preferential assimilation of label (corresponding to the fresh phytoplankton material added) into bacterial fatty acids in contrast to the respiration, where obviously a material of mixed origin was used, visible by comparably low label concentrations in $CO_2$.

## INTRODUCTION

With the discovery of life in the deep ocean and the first profound studies of the deep-sea fauna e. g. with the HMS *Porcupine* in 1868/69 and the HMS *Challenger* 1872-1876, the question arouse how these organisms could survive without obvious food resources. In the early twentieth century the concept that all life in the ocean ultimately depends on the primary production in the euphotic zone was developed, and it was not until the late 1970s that the belief of a constant, fine 'rain' of small particles to the deep-sea floor was challenged by the hypothesis of the aggregation and subsequent rapid sinking of marine snow (Trend *et al.*, 1978). In the early 80s, time-lapse cameras moored in the deep-sea provided photographic evidence for seasonally occurring fluffy layers of fresh phytodetritus covering the deep-sea floor (Billett *et al.*, 1983, Rice *et al.*, 1986). Great effort was put into investigation of both sedimentation events (e.g. Hinga *et al.*, 1979, Rowe and Gardner, 1979, Honjo and Manganini, 1993) as well as the benthic response to such an event. But although data are available for different functional groups (e.g. Boetius and Lochte, 1996, Gooday *et al.*, 1996, Pfannkuche, 1993), the unpredictability of sedimentation events severely hampered an in-depth understanding of the subject. Recent advances were made with studies that attempted to experimentally simulate a settling phytoplankton bloom *in situ* (Moodley *et al.*, 2002, Witte *et al.*, 2003, Witte *et al.*, 2003). With pulse-chase experiments inside the chambers of benthic landers, these investigations combine state of the art lander technology with stabile isotope labeling techniques in order to investigate the reaction of benthic communities to a food pulse.

However, the microbial response varied strikingly between different deep-sea study sites: at the Porcupine Abyssal Plain a delay of 8 days in response of the microbial community was observed by Witte *et al.* (2003), compared to investigations in the deep Sognefjord were the bacteria reacted within 36 h (Witte *et al.*, 2003). The differences between these studies are probably due to the different adaptation of the bacterial community to the varying availability of food at the study sites. The possibility that the magnitude of the microbial reaction depends on the amount of available food was the hypothesis behind our experiments. Therefore, we conducted

pulse-chase experiments with variable quantities of phytoplankton in order to get a closer match to natural conditions.

We present a study on the short-term response of an deep-sea benthic community in the oligotrophic Eastern Mediterranean to differing food inputs. Artificial phytodetritus pulses, consisting of the $^{13}C$-labeled diatom *Thalassiosira rotula*, were created in the chambers of an autonomous benthic lander system (Witte and Pfannkuche, 2000). Two series of experiments were carried out: addition of 250 mg C $m^{-2}$, equivalent to approximately half of the yearly export production in the area of investigation (300-400 mg C $m^{-2}$ $y^{-1}$, calculated after Lykousis *et al.*, 2002 and Danovaro *et al.*, 1999) and 25 mg C $m^{-2}$. In order to follow the microbial utilization of the added POC, changes in sediment community oxygen consumption, $^{13}CO_2$ production and label incorporation into the bacterial fatty acids were monitored.

## MATERIAL AND METHODS

The experiments were carried out in June 2002 with RV PHILIA at 1540 m water depth in the Cretan Sea (35°44.15´N, 025°08.40´E; Eastern Mediterranean, Figure 1). The deep Cretan Sea (NE Mediterranean) is one of the most oligotrophic deep-sea areas of the worlds oceans (Tselepides *et al.*, 2000). It is the largest in volume and deepest of the deep-sea basins of the Aegean Sea. The entire Aegean, and in particular the southern part with an integrated production of 15.2 g C $m^{-2}$ $y^{-1}$ and a chlorophyll-*a* concentration which does not exceed 0.2 µg $g^{-1}$ in the uppermost sediment layers, can be classified as highly oligotrophic (Lykousis *et al.*, 2002). Our experimental site is located at the southern boundary of the Cretan Sea in 1540 m water depth and was intensely studied during the CINCS project (Pelagic-Benthic **C**oupling **IN** the Oligotrophic **C**retan **S**ea, Tselepides and Polychronaki, 2000).

Prior to the experiments, an axenic clone of the diatom *Thalassiosira rotula* (Bacillariophyceae, Thallassiosiraceae) was cultured at 16°C in artificial seawater (Grasshoff, 1999) with a salinity of 35 psu enriched with f/2 medium (Guillard and Ryther, 1962). The medium contained 25% $^{13}C$-bicarbonate (99% $NaH^{13}CO_3$,

Cambridge Isotope Laboratories). The algal material was harvested by centrifugation (404 **g**, 4 min), rinsed three times with an isotonic sodium chloride solution and dried in vacuo. The axenic state of the algae was verified by microscopic observations and by incubation of a few drops of the culture, in the dark, on agar plates containing the culture medium and glucose. The produced algae contained 20 atom% $^{13}$C.

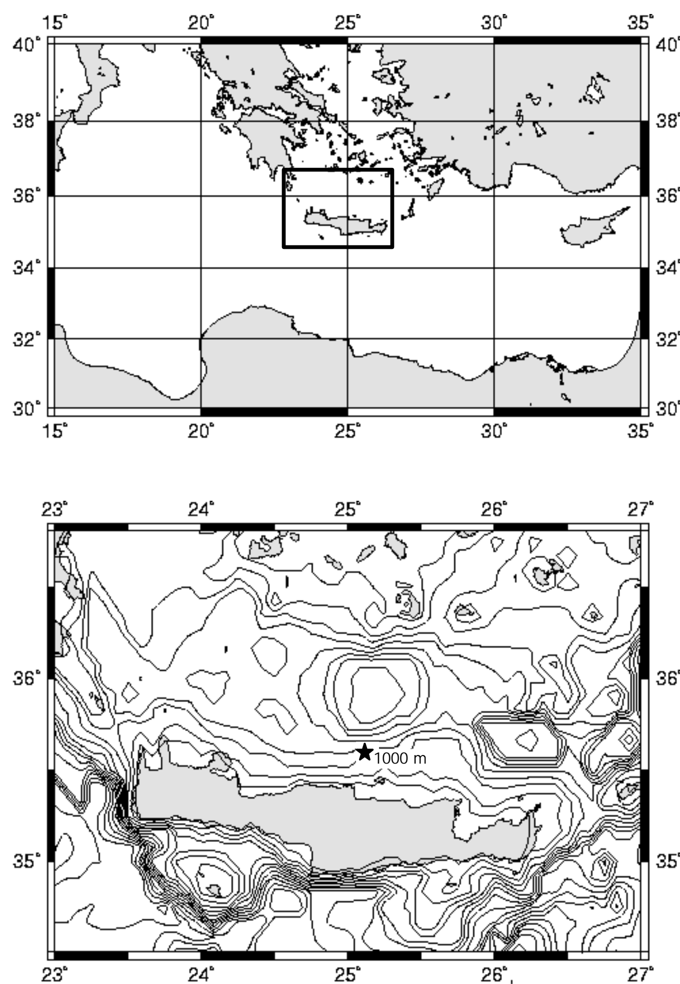


Figure 1: Area of investigation in the NE Mediterranean (http://aquaris.geomar.de)

A deep-sea benthic chamber lander (for further details see Witte and Pfannkuche, 2000) was used for the experiments. It was equipped with three benthic chambers of 0.04 m$^2$ each. The injection of the labeled particulate organic matter (POM) was done at the beginning of each experiment using an injection unit integrated in the lid of each chamber. One hour after insertion of the chambers into the sediment, 1.01 mg C and 10.14 mg C of our algae, respectively (corresponding to 25 mg C m$^{-2}$ and 250 mg C m$^{-2}$) were injected. Experiment duration was 36 h.

The injected algae were suspended in the chamber by a stirrer in order to achieve homogeneous distribution before sedimentation. Each chamber was equipped with a syringe water sampler, which took 7 water samples of 50 ml volume at preprogrammed intervals during the incubation. Sediment community oxygen consumption (SCOC) was determined by Winkler titration of syringe water samples (2 replicates). Water samples for $\delta^{13}CO_2$ were filtered directly into 12 ml gas-tight glass scintillation vials sealed with a rubber septum and containing mercury chloride in an end concentration of 0.2% to stop all bacterial activity. The samples were stored refrigerated until analysis.

Control experiments ran without algal injection, additional samples were taken using a multiple corer at the same location.

5 chambers were sampled in total (4 experimental chambers, which each two parallels for low (L) and high (H) carbon load experiments, and one control chamber) for the analysis of porewater $\delta^{13}CO_2$ and bacterial biomarkers. Sediment was sliced in 1 cm intervals up to 5 cm and 2.5 cm intervals after 5 cm sediment depth. Each sediment horizon was carefully homogenized prior to subsampling for lipid analysis and of porewater $\delta^{13}CO_2$. The samples for lipid analysis were stored frozen at –20°C until analysis and the samples for $\delta^{13}CO_2$ were stored cooled until pressure filtration of the porewater. A 2 ml aliquot of the porewater was kept at 4°C in a nitrogen flushed 4 ml gas-tight glass scintillation vial containing mercury chloride (end concentration 0.2%).

The lipid extraction was performed according to Elvert *et al.* (2003). Concentrations were determined by gas chromatography-flame ionization detection, stable isotope composition by GC-*c*-IRMS and unknown compounds were investigated using GC-MS (for detailed description see Bühring *et al.*, subm.). The carbon isotopic ratios were corrected for the one methyl group inserted during derivatization.

For the $\delta^{13}CO_2$ measurements, a head space was created in the scintillation vials by injecting nitrogen gas and the sample was than acidified with 100 µl and 20 µl sulfuric acid (20%) for the chamber and the porewater samples, respectively. The $\delta^{13}CO_2$ and concentrations of $CO_2$ in the headspace was measured using a Carlo Erba

1106 Elemental Analyser coupled online with a Finnigan Delta S isotope ratio mass spectrometer. Reproducibility of the measurements was ~0.10‰.

The carbon isotope ratios are expressed in the delta notation ($\delta^{13}C$) relative to Vienna PDB: $\delta^{13}C$ (‰) = $[(^{13}C:\ ^{12}C)_{sample}/(^{13}C:\ ^{12}C)_{reference}-1] \times 1000$. Total uptake *I* was calculated as the product of excess (*E*) and the $CO_2$ concentration. For bacteria, *I* was calculated after Middelburg *et al.* (2000) from label incorporation into bacterial fatty acids (*i* $C_{15:0}$, *ai* $C_{15:0}$, *i* $C_{16:0}$, *i* $C_{17:1\omega7}$, 10*Me*-$C_{16:0}$, *i* $C_{17:0}$, *ai* $C_{17:0}$, and $C_{17:1\omega6}$) as $I_{bact}=\sum I_{bact.fatty\text{-}acids}/(a \times b)$, where *a* is the average phospholipid-derived fatty acid (PLFA) concentration in bacteria of 0.056 g of carbon PLFA $g^{-1}$ biomass (Brinch-Iversen and King, 1990) and *b* is the average fraction specific bacterial PLFA encountered in sediment dominated by bacteria (0.19; calculated after Findlay and Dobbs, 1993, Guezennec and Fiala-Medioni, 1996, Rajendran *et al.*, 1994). *E* is the difference between the fraction *F* of the sample and background: $E = F_{sample} - F_{background}$, where $F = {}^{13}C/(^{13}C/^{12}C) = R / (R + 1)$ and $R = (\delta^{13}C / 1000 + 1) \times R_{VPDB}$ with $R_{VPDB} = 0.0112372$.

# RESULTS

## Sediment community oxygen consumption

The bottom water oxygen content obtained from the first syringe samples taken before addition of the algal material was 244.12 ± 1.5 µmol $l^{-1}$ (n=13). The sediment community oxygen consumption (SCOC), as determined from the syringe samples, was 0.633 ± 0.06 mmol $m^{-2}$ $d^{-1}$ for the control chambers. The changes in SCOC due to algae addition are depicted in Figure 2. In L experiments (addition of 25 mg C $m^{-2}$), the SCOC significantly increased (41%), reaching 0.881 ± 0.04 mmol $m^{-2}$ $d^{-1}$. In the H experiments (addition of 250 mg C $m^{-2}$) led to an increase of 118% compared to the control, reaching 1.380 ± 0.12 mmol $m^{-2}$ $d^{-1}$.

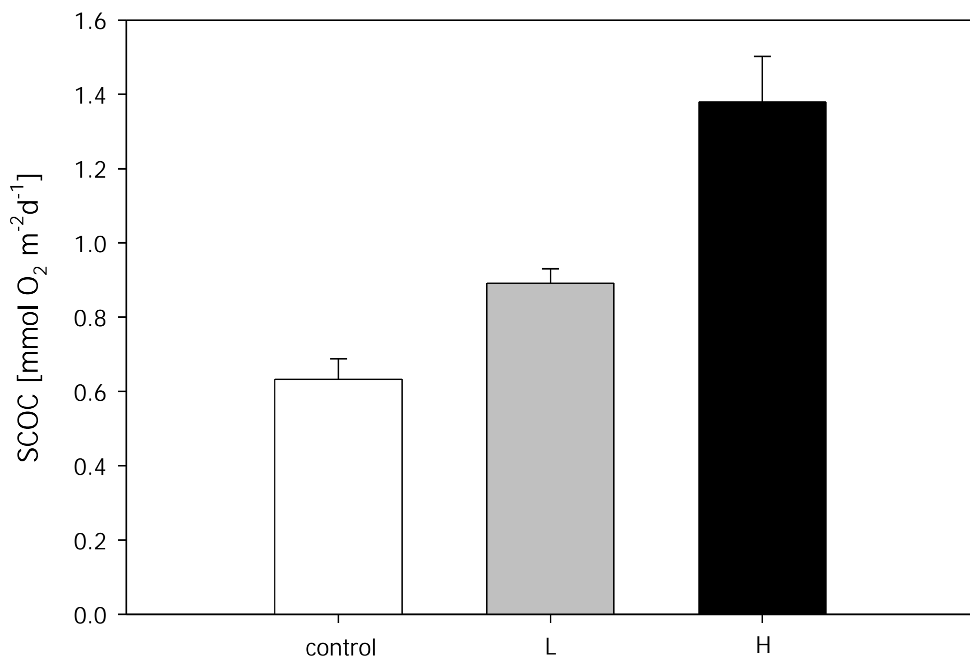


Figure 2: Changes in sediment community oxygen consumption (SCOC) for the control chambers, and the L and H experiments. Error bars represent ± SD.

### Mineralization of tracer POC to $^{13}CO_2$

The $^{13}CO_2$ that accumulated in the overlying water is given in Figure 3. Directly after the algae addition, $^{13}CO_2$ values increased. In the L experiments, approximately 342 µg $^{13}C$ $m^{-2}$ had accumulated in the chamber water after 36 h. In contrast, in type H experiments, already after 6 h values of nearly 7700 µg $^{13}C$ $m^{-2}$ were reached, further increasing to 11504 µg $^{13}C$ $m^{-2}$ after 36 h. The $^{13}CO_2$ in the porewater versus sediment depth is given in Figure 4. After 36 h of the L experiments elevated $^{13}CO_2$ values in the upper sediment layers were detected (116 µg $^{13}C$ $m^{-2}$ in $CO_2$, 0-7.5 cm depth). No subsurface peaks were detected. The H experiments resulted in a totally different profile, reaching values of 832 µg $^{13}C$ $m^{-2}$ (0-7.5 cm). Again a maximum in $^{13}CO_2$ was found in the upper sediment horizon, reaching values of over 2500 µg $^{13}C$ $m^{-2}$. But high remineralization activity was also detectable over the whole sediment sampling depth, reaching values of 756 µg $^{13}C$ $m^{-2}$ at 2-3 cm and 365 µg $^{13}C$ $m^{-2}$ at 4-5 cm.

In total, 459 µg $^{13}C$ $m^{-2}$ (9.1% of added label) and 12336 µg $^{13}C$ $m^{-2}$ (24.5% of added label) were remineralized during the L and H experiments, respectively.

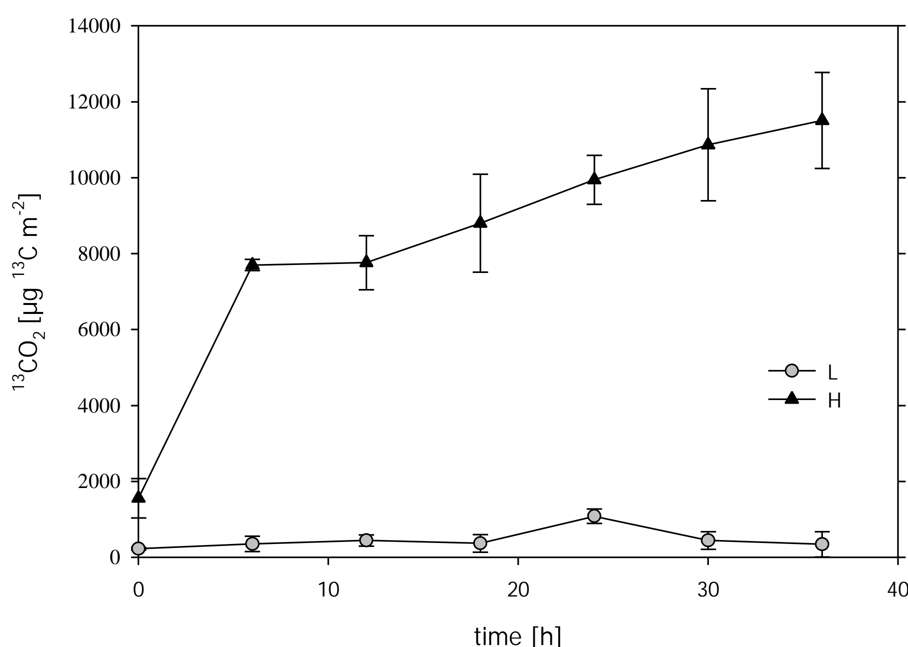


Figure 3: Mineralization of tracer POC to $^{13}CO_2$ measured in the overlying water [µg $^{13}C$ $m^{-2}$] over time for the L and H experiments. Error bars represent range of two replicates.

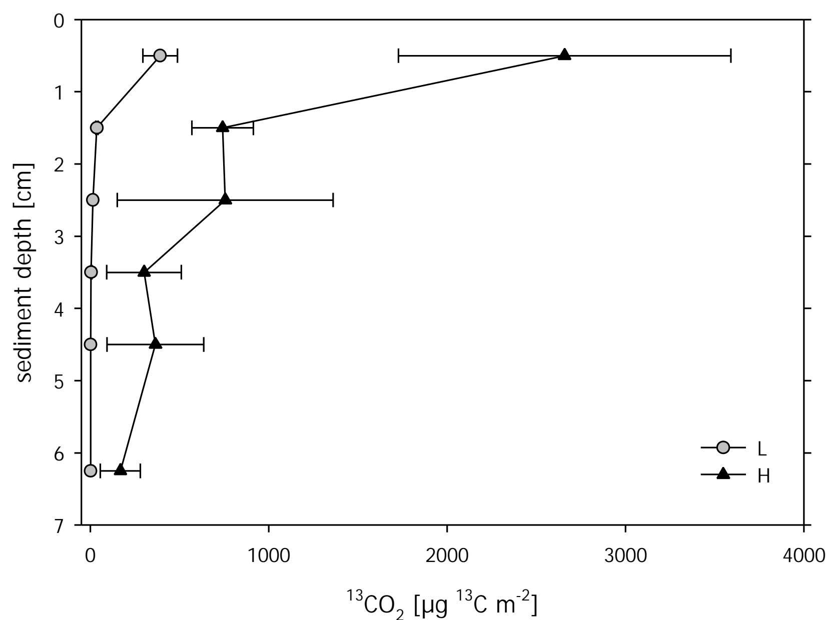


Figure 4: Mineralization of tracer POC to $^{13}CO_2$ in the porewater [µg $^{13}$C $m^{-2}$] versus sediment depth for the L and H experiments. Horizontal bars indicate range of two replicates.

## Relative abundances of bacterial fatty acids

Figure 5 displays the relative abundance of all 23 fatty acids detected at our study site. Highest relative abundances were found for $C_{16:0}$, followed by $C_{18:1\omega9}$, 10*Me*-$C_{16:0}$ and $C_{18:0}$. Only slightly lower values were found for $C_{18:1\omega7}$, $C_{16:1\omega9}$, *i* $C_{15:0}$ and *ai* $C_{15:0}$. The other fatty acids contribute 4% or less. The bacterial biomass was 9.3 ± 1.9 µg $ml^{-1}$ or 357.5 ± 109 µg $m^{-2}$ (0-3 cm).

The depth distribution of selected bacterial fatty acid relative abundances is given in Figure 6. The fatty acids *i* $C_{15:0}$, *ai* $C_{15:0}$, 10*Me*-$C_{16:0}$, *i* $C_{17:0}$, and *ai* $C_{17:0}$ displayed highest relative abundances in the surface layer and subsurface peaks at 3 – 4 cm sediment depth. In contrast, *i* $C_{17:1\omega7}$ exhibited a subsurface peak at 1 – 2 cm and minimum relative abundance in 3.5 cm.

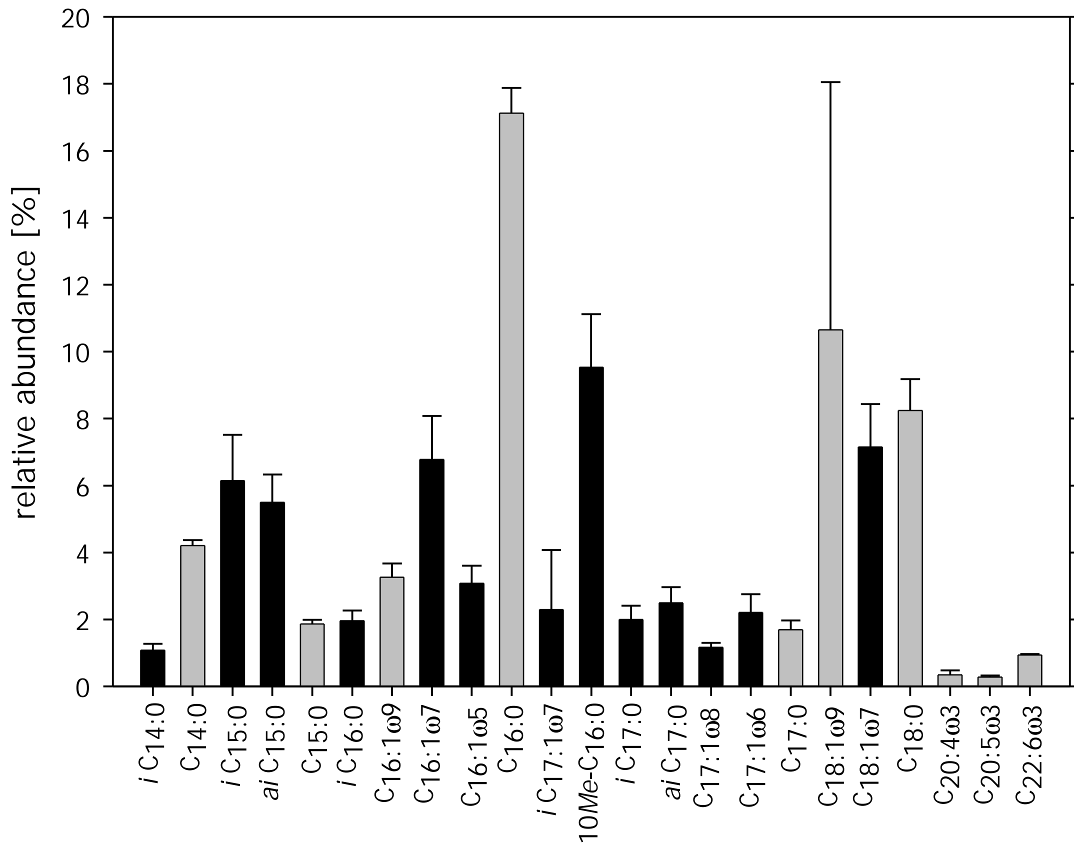


Figure 5: Relative abundances of all fatty acids detected in all experimental and one control chamber. (0-3 cm depth; Error bars indicate ± SD). Black bars indicate fatty acids of bacterial origin.

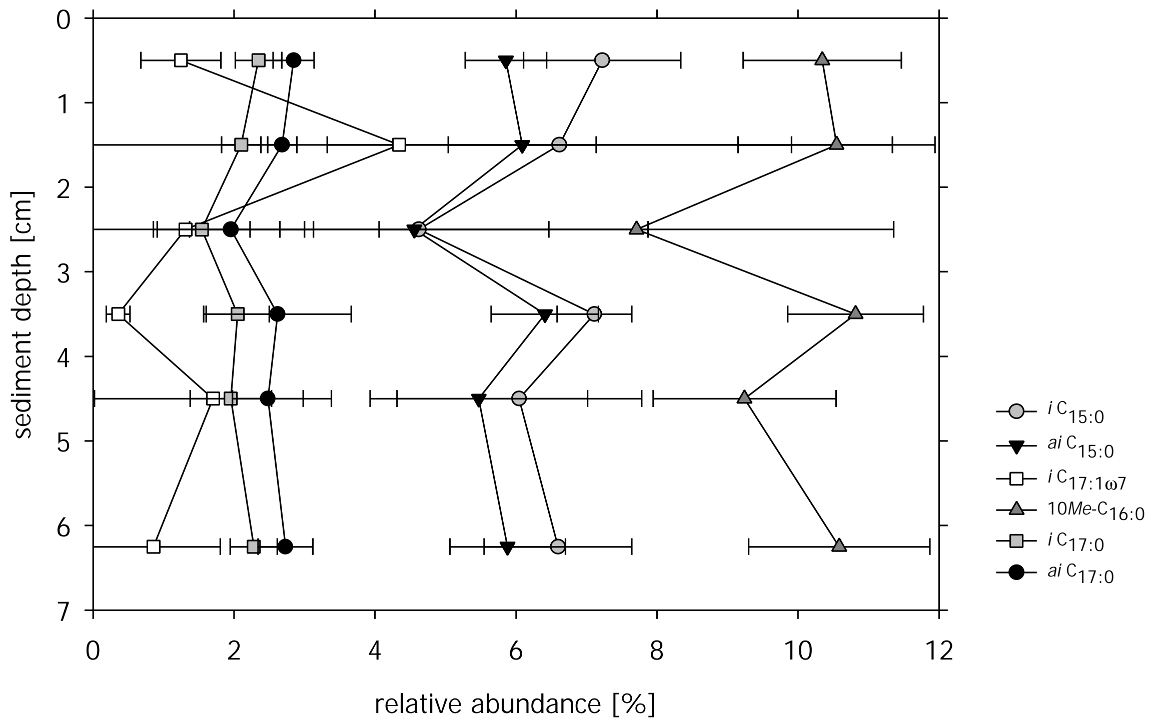


Figure 6: Depth distribution of relative abundances of different bacterial fatty acids. Shown are mean values from all experimental and one control chamber. Error bars indicate ± SD.

## Incorporation of $^{13}$C into bacteria

Incorporation of our deliberate tracer was detectable for all bacterial fatty acids, even though in different amounts. Figure 7 shows the total incorporation of label into selected bacterial fatty acids. In L experiments, most label was incorporated into *i* $C_{16:0}$ and *ai* $C_{15:0}$, with 7.8 and 4.8 µg $^{13}$C m$^{-2}$, respectively, followed by *ai* $C_{17:0}$ with 1.2 µg $^{13}$C m$^{-2}$. In other bacterial fatty acids we found less than 1 µg $^{13}$C m$^{-2}$. In contrast, the H experiments were characterized by elevated incorporation into all bacterial fatty acids. Most label was again incorporated into *i* $C_{16:0}$ and *ai* $C_{15:0}$, followed by *ai* $C_{17:0}$ with 12.3, 6.9 and 2.3 µg $^{13}$C m$^{-2}$, respectively. In total, 107% more label was incorporated into bacteria in H than in L experiments.

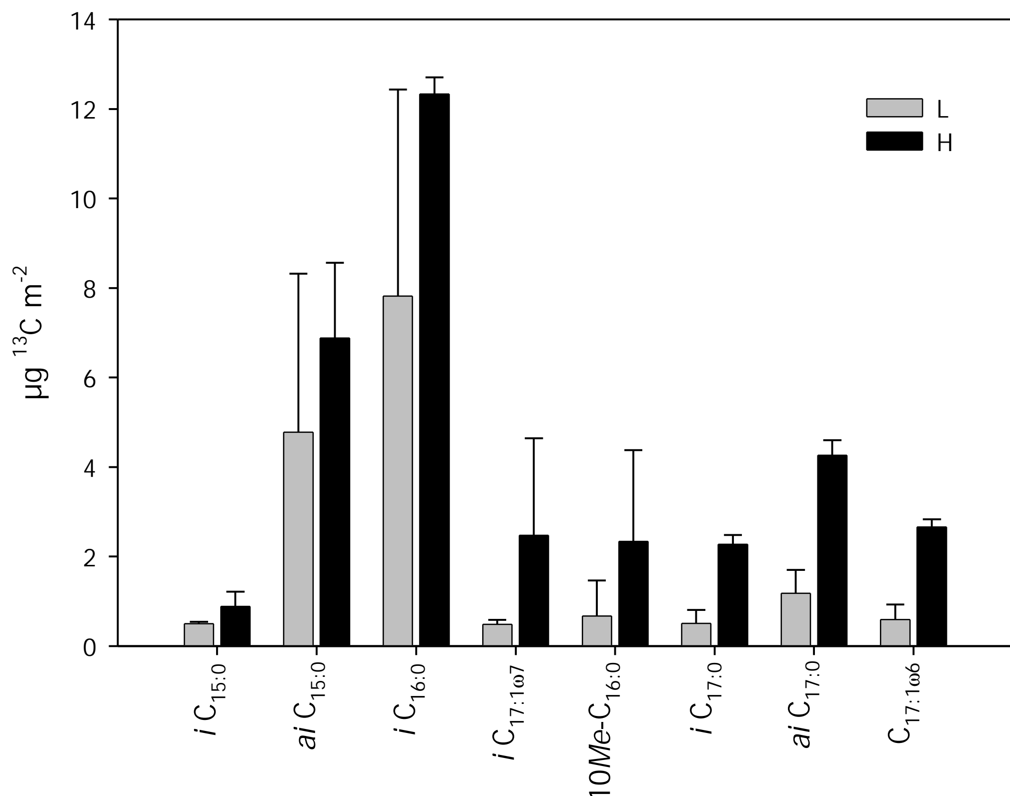


Figure 7: Total incorporation of label into bacterial fatty acids in µg $^{13}$C m$^{-2}$ (sampling depth: 0-7.5 cm). Grey bars represent results from L experiments and black bars from the H experiments. Error bars indicate range of two replicates.

The incorporation into selected bacterial fatty acids versus sediment depth for L and H experiments is displayed in Figure 8. For low carbon addition, most label was incorporated by bacteria in the uppermost sediment layer. But restriction of incorporation to this depth was only visible for *i* $C_{15:0}$ and *ai* $C_{15:0}$. Incorporation of label in deeper sediment layers was obvious for all other fatty acids, normally up to 3.5 cm sediment depth. In contrast, the H experiments displayed a totally different picture with pronounced subsurface peaks for all investigated fatty acids. Comparing *i* $C_{15:0}$ and *ai* $C_{15:0}$, much higher labeling and deeper penetration depth was measured for *ai* $C_{15:0}$. The incorporation into *i* $C_{16:0}$ displayed a very pronounced subsurface labeling maximum between 3.5 and 4.5 cm. Compared to the other investigated bacterial fatty acids, the incorporation into *i* $C_{17:1\omega7}$ during the H experiment was inversely distributed with low values at the surface and highest values in 1.5 cm sediment depth. High incorporation into 10*Me*-$C_{16:0}$ was investigated at the surface and in 2.5 cm depth. The labeling pattern for *i* $C_{17:0}$, *ai* $C_{17:0}$ and $C_{17:1\omega6}$ were very comparable with highest incorporation at the surface.

The total incorporation into bacteria accounted for 1579 µg $^{13}C$ $m^{-2}$ (31.7% of the added label) and 3261 µg $^{13}C$ $m^{-2}$ (6.8% of the added label) for the L and H experiments, respectively.

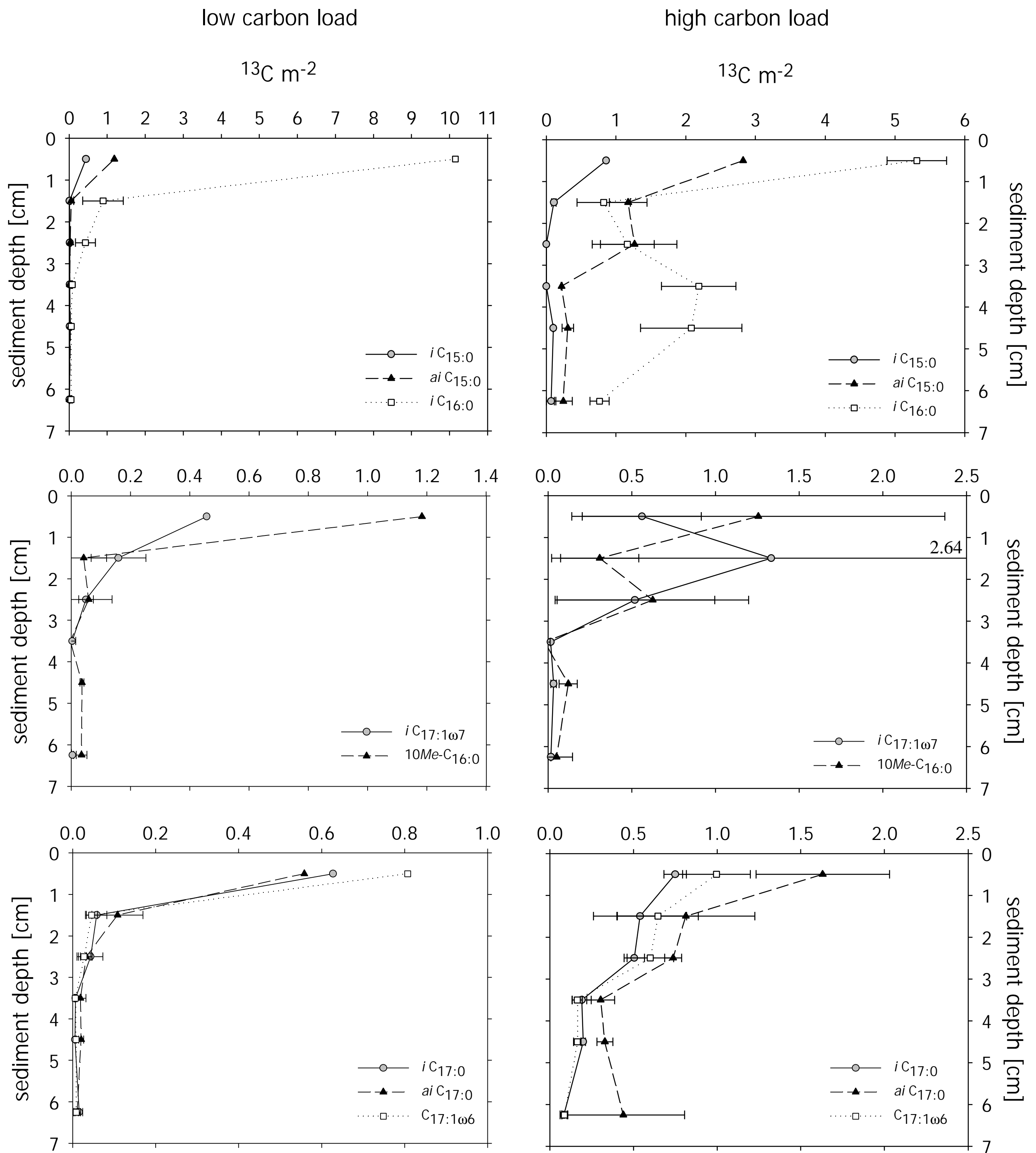


Figure 8: Total incorporation of tracer $^{13}C$ into bacterial biomarkers versus sediment depth from the L and H experiments (horizontal bars indicate range of two replicates).

## DISCUSSION

The special topography of the Cretan Sea, with straits no deeper than 150-1100 m communicating to the adjacent basins, creates an environment with unique geomorphological features (Tselepides *et al.*, 2000). It acts as a reservoir for heat and salt, where deep water formation for the whole Eastern Mediterranean takes place (Miller, 1963). Due to the low terrestrial runoff and its large volume, the Mediterranean is an oligotrophic ocean basin, with increasing nutrient-depletion from west to east (Turley *et al.*, 2000). The integrated production in the Aegean Sea is approximately 15.2 g C $m^{-2}$ $y^{-1}$ (Lykousis *et al.*, 2002), with only 2-3% of the export production reaching the sediment (Danovaro *et al.*, 1999), resulting in a highly food limited deep-sea environment. Lykousis *et al.* (2002) declared this region as an "oceanic margin" environment due to the very small fraction of the initial amount of the primary production of the euphotic zone reaching the deep-sea bottom. The general scarcity of food leads to a predominance of the microbial food web in the entire Aegean Sea (Lykousis *et al.*, 2002). The investigation of the deep-sea benthos in terms of abundance and biomass in this area revealed seasonal variations for the bacteria (Danovaro *et al.*, 2000), as well as for demersal fish and megafauna assemblages (Kallianiotis *et al.*, 2000), while seasonal variations of meiofauna occurred only after a time lag (Danovaro *et al.*, 2000).

The sediment community oxygen consumption (SCOC) in the control chambers was 0.633 ± 0.06 mmol $O_2$ $m^{-2}$ $d^{-1}$, which is slightly higher than in investigations of Lykousis *et al.* (2002), who found 0.432 mmol $O_2$ $m^{-2}$ $d^{-1}$ during Summer 1997 in the Southern Aegean Sea. These values, measured at 1500 m water depth, are much lower than SCOC at abyssal sites of 4000 – 5000 m water depth in the NE Atlantic or the Arabian Sea (0.6-1.4 and 0.9-6.3 mmol $O_2$ $m^{-2}$ $d^{-1}$, respectively (reviewed by Witte and Pfannkuche, 2000)). Several investigations of SCOC on continental margin sediments revealed higher values as well (e.g. 3.6 mmol $O_2$ $m^{-2}$ $d^{-1}$ found in the deep Sognefjord by Witte *et al.*, 2003; oxygen uptake of 3.08 mmol $O_2$ $m^{-2}$ $d^{-1}$ on the Norwegian Continental margin found by Sauter *et al.*, 2001), confirming the extreme oligotrophy of our study site.

The fatty acid composition (Figure 5) revealed a benthic community that is dominated by bacteria (Lechevalier, 1977). The highest relative share of all found bacterial fatty acids owned 10*Me*-$C_{16:0}$. As this fatty acid is described as a biomarker for *Desulfobacter* by Dowling *et al.* (1986) and for *Desulfobacteriaceae* in general (Rütters *et al.*, 2002, Kuever *et al.*, 2001) gives evidence for the great importance of this group at our study site. The *Desulfobacteriacea* belong to the sulfate-reducing bacteria (SRB), which form a coherent group within the δ-subdivision of the proteobacteria. Several other biomarkers confirm the existence of SRB. The fatty acids *i* $C_{17:0}$ and *ai* $C_{17:0}$ are generally considered to be of SRB origin (Findlay and Dobbs, 1993), *i* $C_{17:1\omega7}$ is very common in *Desulfosarcina* (Rütters *et al.*, 2001), and $C_{17:1\omega6}$ is a major fatty acid in *Desulfobulbus* (Taylor and Parkes, 1983). If all biomarkers of described SRB origin are taken together, a relative share of 22.8% from all fatty acids results. This is an astonishing finding, regarding the generally lower sulfate reduction activity in deep-sea sediments compared to shelf areas (Jørgensen, 1982). Only recently Sass *et al.* (2002) isolated a new SRB from Mediterranean deep-sea sediments, which was named *Desulfobulbus mediterraneus* and is able to grow on carbohydrates. Its fatty acids were dominated by $C_{16:1\omega7}$, $C_{16:1\omega5}$, $C_{17:1\omega6}$, and $C_{18:1\omega7}$, which were all found at our study site, providing evidence for occurrence of this or a related species at our station.

No significant increase over time in bacterial fatty acid concentrations from the control chambers and the incubations could be observed. This is probably due to the short incubation time of 36 h, but it can also be assumed that the bacteria predominantly use the new material for energy yielding processes, as shown by Bianchi *et al.* (2003) through glutamate uptake experiments in the southern Aegean Sea. Furthermore one has to keep in mind that we can only monitor the "net biomass" of a microbial community that is constantly grazed upon.

Following the relative abundances of some bacterial fatty acids over the sediment sampling depth (Figure 6) revealed comparable distribution for *i* $C_{15:0}$, *ai* $C_{15:0}$, 10*Me*-$C_{16:0}$, *i* $C_{17:0}$, and *ai* $C_{17:0}$. The comparable oscillation in abundances of these fatty acids could indicate their origin in one bacterial strain or their affiliation to

a strongly associated group of prokaryotes. This group probably favor special conditions, which were similarly vertically stratified across the sediment.

Different depth distribution behavior was only visible in *i* $C_{17:1\omega7}$ (Figure 6), which showed a subsurface maximum at 1.5 cm that was much more pronounced than for the other fatty acids. Furthermore this fatty acid displayed a second peak at 4.5 cm sediment depth, where all the others had distinct minima. *I* $C_{17:1\omega7}$ is a main fatty acid in *Desulfosarcina* (Rütters *et al.*, 2001). This SRB is a complete oxidizer, which is nutritionally very versatile with respect to potential electron donors. This could be advantageous in case of competition for limited carbon sources (Ravenschlag *et al.*, 2000), what makes it not unlikely that these bacteria outcompete other groups at distinct depth intervals, because of locally prevailing conditions.

If we compare the relative abundances of the bacterial fatty acids with the incorporation pattern of our label, we can state that there is a distinct mismatch. The fatty acids *i* $C_{15:0}$ and *i* $C_{16:0}$ showed the highest incorporation from all bacterial fatty acids, even though especially *i* $C_{16:0}$ contribute in average only 2% to all fatty acids. *I* $C_{15:0}$, *ai* $C_{15:0}$ and *i* $C_{16:0}$ are very common in gram-positive prokaryotes (White *et al.*, 1996). The high incorporation of our deliberate tracer into these biomarkers must be caused by a direct access of the corresponding bacteria to the algal material. This could be possible either by uptake of DOC from the algae or with the help of exoenzymes.

Very pronounced is the difference in 10*Me*-$C_{16:0}$, which showed the highest abundance of all bacterial fatty acids, but the label incorporation was comparably low. This fatty acid is typically described as deriving from SRB, mainly for members of the *Desulfobacteriaceae* (Rütters *et al.*, 2002). Other biomarkers of SRB revealed lower incorporation behavior as well, like *i* $C_{17:1\omega7}$, *i* $C_{17:0}$, *ai* $C_{17:0}$, and $C_{17:1\omega6}$. SRB usually take up secondary carbon products, which differ between the species (Widdel and Bak, 1992). Some are even capable of autotrophic growth. The low incorporation of our deliberate tracer suggest that the SRB have no direct access on the added POC, probably relying on the delivery of secondary carbon products like e.g. short chain fatty acids released by fermentative bacteria. It can be assumed that longer

incubation times would probably lead to higher incorporation of label into SRB biomarker. Using a comparable experimental design on shelf sediments Bühring *et al.* (subm.) found increasing incorporation into a biomarker of SRB over a time period of 132 h, supporting this hypothesis.

Taking a closer look on the differences between the L and H experiments, we can state that the incorporation into all investigated bacterial fatty acids was significantly elevated during the H experiments (Figure 7). Taking the differences of all investigated bacterial fatty acids together, an increase of label incorporation of 107% between the two different experimental setups could be found. But the discrepancy in incorporation is not evenly distributed between the different bacterial fatty acids. The increase from L to H experiments in the 17-carbon fatty acids was e.g. 6.4 times higher compared to *ai* $C_{15:0}$ and *i* $C_{16:0}$. Taking into account that the added material was of the same composition while only the quantity varied, it can be speculated that the bacteria corresponding to the 17-carbon fatty acids (mainly SRB) were in a "hibernate"- state and awake in case of a considerable sedimentation event. Another possibility is that other organisms tend to be more active in case of great phytoplankton blooms arriving at the deep-sea bottom. They could produce secondary carbon products and therewith feed the SRB. An increasing activity of bacteria as well as meio- and macrofauna organisms between the two experiments is confirmed by the increase in SCOC (Figure 2), rooting this hypothesis.

Additional to the investigations of the SCOC to determine the benthic response due to algae addition, $^{13}CO_2$ enrichment in $\Sigma CO_2$ is a sensitive tool for detecting respiration of the labeled algal carbon (Blair *et al.*, 1996, Moodley *et al.*, 2002). Our experiments reveal a rapid benthic respiration of the added carbon in the L and H experiments, as reflected in the increase in $^{13}CO_2$ during the investigated time period (Figure 3). It is evident that the addition of the large quantity of organic carbon boosts the remineralization; after only 6 h values as high as 7700 µg $^{13}C\ m^{-2}$ were reached in the overlying water. This very fast reaction could be due to the remineralization of labeled dissolved organic carbon, directly available after injection from the added algae material.

The depth distribution of incorporation of label into bacterial fatty acids (Figure 8) revealed a restriction to the surface sediments for the L experiments. This is in good agreement with the remineralization of POC to $^{13}CO_2$ in the porewater (Figure 4), which also showed a limitation to the upper first cm of the sediment. During the H experiments pronounced subsurface incorporation layers were observed in all investigated bacterial fatty acids with the exception of *i* $C_{15:0}$. In *ai* $C_{15:0}$, 10*Me*-$C_{16:0}$, *i* $C_{17:0}$, *ai* $C_{17:0}$, and $C_{17:1\omega6}$ more or less pronounced peaks at 2.5 cm sediment depth were found. The same could also be observed regarding the $^{13}CO_2$, which leads to the conclusion that bacteria are participating in the formation of the secondary subsurface maxima in label respiration. Completely different incorporation behavior was only observed in *i* $C_{16:0}$, with a broad maximum between 3 and 5 cm and in *i* $C_{17:1\omega7}$, with a minimum value at the surface, a pronounced maximum in 1.5 cm sediment depth and afterwards values that decrease versus zero. These observations furthermore support the idea of a different origin of *i* $C_{17:1\omega7}$, probably corresponding to *Desulfosarcina* or related species (Rütters *et al.*, 2001).

Prior to the remineralization and the incorporation into bacteria, transport of the labeled material to deeper sediment layers must have taken place. Levin *et al.* (1997) observed the rapid subduction of fresh organic matter by bioturbating animals and proposed that this process provides deeper living organisms with relatively fresh material. In oligotrophic sediments infauna often pull settled food material to deeper sediment layers to avoid competition (according to the hypothesis of Jumars *et al.*, 1990). A rapid downward transport of food material down to 10 cm depth in the Cretan Sea was observed by Danovaro *et al.* (1999), as well as high bioturbation activity of infauna at our study site (Tselepides *et al.*, 2000). Nevertheless not only the transport by infauna organisms is of importance for deeper living bacteria, another important component is the gut passage that alters the algae material and may prepares secondary carbon products that could be taken up by SRB. Some of the investigated biomarkers indicate SRBs capable of autotrophic growth (like *Desulfosarcina*), therefore the uptake of labeled inorganic carbon is another possible explanation for subsurface labeling of bacterial biomarkers.

Comparing uptake of label into the different investigated benthic compartments (Figure 9) we can state that only a fraction of the added POC was processed. 31.7% and 6.8% of the added label were incorporated in the bacteria in the L and H experiments, respectively. The low amount of label recovered in the $CO_2$ compared to the incorporation into bacteria during the L experiments (Figure 9) indicate an additional respiration of "old" material already present in the sediment. The comparison with the values calculated from the SCOC (assuming a RQ of 1) reveal that only a share of 1% of carbon mineralization (8.5 mmol $CO_2$) was due to the addition of the low carbon load (L experiments). During the H experiments 11% (228 mmol $CO_2$) were due to the algae addition.

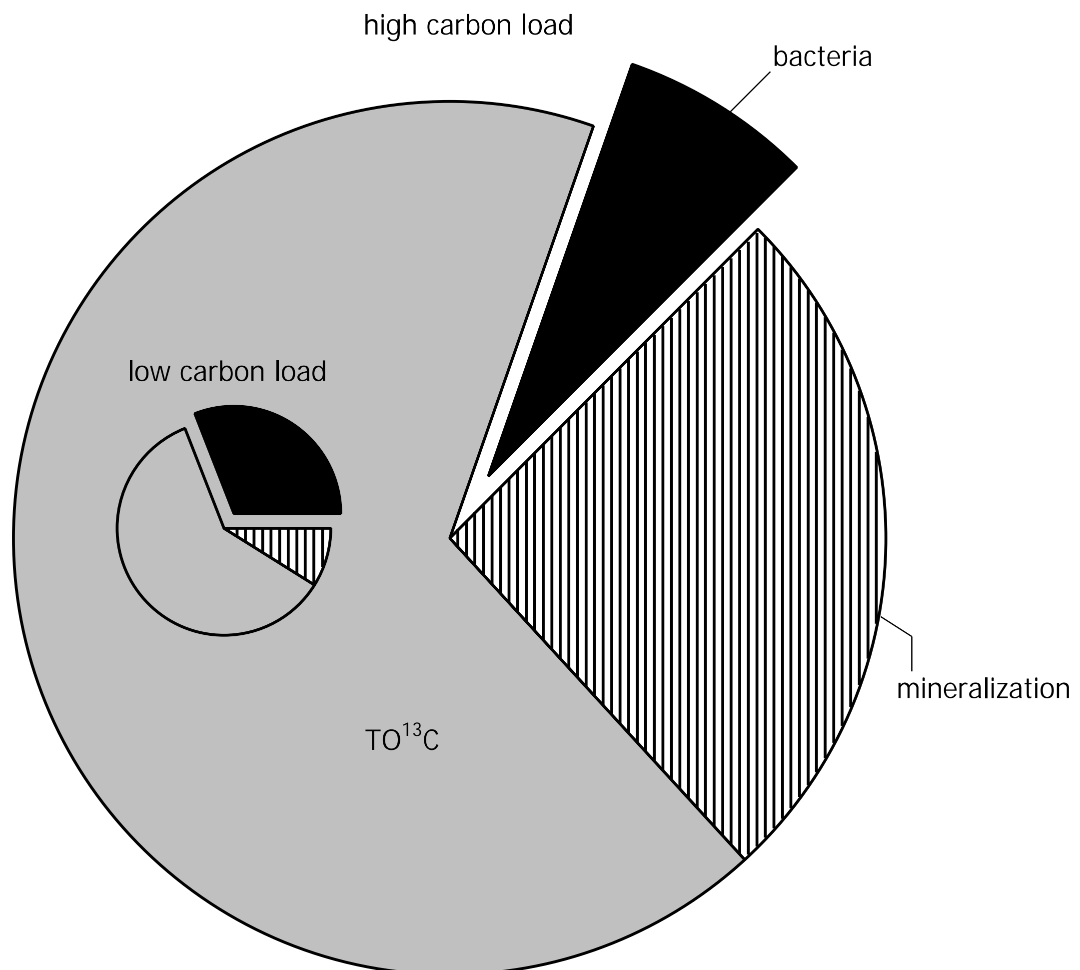


Figure 9: Comparison of total incorporation of label into bacteria, mineralization of tracer POC to $^{13}CO_2$, and remaining TO$^{13}$C and for the L and H experiments. The size of the pies corresponds to the total amount of recovered label.

The amount of processed carbon (incorporation into bacteria plus $^{13}CO_2$ produced) was 7 mg C $m^{-2}$ $d^{-1}$ and 52 mg C $m^{-2}$ $d^{-1}$ for the L and H experiments, respectively. These are astonishingly high values compared to other studies. In similar studies, Witte *et al.* (2003) found 19 mg C $m^{-2}$ $d^{-1}$, investigating the benthic community of the deep Sognefjord and Moodley *et al.* (2002) found 2 mg C $m^{-2}$ $d^{-1}$ at 2150 m water depth in the NE-Atlantic. A direct comparison is hampered by the fact that we did not include macro- and meiofauna into our investigations. But due to the fact that bacteria are described as the primary agents for settling particles arriving at the deep-sea floor (Danovaro *et al.*, 1999), the different observations are comparable and conclude that this oligotrophic benthic community is capable of high carbon processing rates.

Several former investigations revealed an increase in SCOC due to POM addition of approximately 50% (Smith Jr. and Baldwin, 1984, Smith Jr, 1987, Moodley *et al.*, 2002). These investigations led to the speculation that deep-sea benthic communities can only switch between a "low turnover" and a "high turnover" mode. Here we demonstrated the capability to different responses due to the amount of added algae. Assuming that the SCOC is a reliable measure of benthic carbon mineralization (BCR) (Pfannkuche, 1993, Witte *et al.*, 2003), our results clearly show that the benthic community at our deep-sea study site is able to graduate its reaction depending on the amount of settling phytoplankton. Our results therefore strongly suggest to overturn the former belief of the deep-sea benthic community reacting like an on-off switch.

In comparison to other deep-sea studies, we found the strongest potential to increase the initial reaction at our study site in the highly oligotrophic deep Mediterranean (118% increase for the H experiments), followed by the Porcupine Abyssal Plain in the North Atlantic (approximately 75% increase due to POM addition, Witte *et al.*, 2003), an area with strong seasonal influx of phytodetritus (Billett *et al.*, 1983) and the Sognefjord with land-runoff (25% increase due to POM addition, Witte *et al.*, 2003). This comparison suggests a close relation between amplitude of the increase in benthic carbon turnover and food availability, or rather food scarcity in the respected environment.

It could furthermore be presumed that the benthic community is able to increase its reaction when large quantities of food arrive. A high bacterial community activity was as well documented by Bianchi *et al.* (2003). They found hydrolytic activity of benthic bacteria enhanced in the southern compared to the less oligotrophic northern Aegean Sea. They concluded that the low availability of utilizable organic compounds in the southern Aegean Sea leads to bacterial communities well equipped for biopolymer hydrolysis.

## CONCLUSIONS

In the deep Cretan Sea we encountered an astonishingly fast reaction of the benthic compartments, and the possibility of a graded response in this oligotrophic deep-sea environment. The SCOC increased with 41% and 118% in the L and H experiments compared to the control clearly indicate that this deep-sea benthic community does not respond in a kind of on-off reaction. Incorporation into all investigated bacterial fatty acids was detectable in different amounts. The biomarkers of SRB generally showed a lower incorporation, but the increase in incorporation comparing L and H experiments was very pronounced in these biomarkers. Furthermore the labeling reached greater sediment depth in the H experiments. This indicates an enhanced benthic reaction in total if great amounts of settling particles arrive at the deep-sea bottom.

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# Chapter 6

# DISCUSSION

# DISCUSSION

The major aim of this thesis was to get deeper insights into the bacterial community structure and activity as well as their role for the microbial carbon turnover in different marine sediments. The discussion will therefore deal with the comparable specialities of the different investigated environments with respect to the results gained for this thesis.

## Permeable sediments

Sandy sediments have long been regarded as geochemical deserts due to their usually low organic matter content (Boudreau *et al.*, 2001). The North Sea investigations presented in this thesis (Chapter 2, 3, and 4) fortunately contribute to the changing of this misguided view. The investigation of the prokaryotic structure revealed the presence of different groups of bacteria (Chapter 2). Aerobic bacteria as well as sulfate reducing bacteria (SRB) were found. The close coexistence of these bacterial groups is probably propagated by advection, a transport process exclusively found in permeable sandy sediments. This process provides the basis for a fast porewater exchange, which is probably the reason for the mixing of bacterial populations that are otherwise separated between oxic and anoxic zones. A considerable standing stock of SRB was found in all investigated sandy sediments, even though the fast porewater exchange provides oxygenated water to deeper sediment layers and SRBs are described as not being able to sustain under oxic conditions and die off more or less rapidly (Cypionka *et al.*, 1985). These findings suggest anoxic subniches in these sediments. Experiments on sandy sediments with $^{13}C$-labelled algae (Chapter 3) clearly demonstrated the fast transport of diatom frustules and labelled diatom carbon into the sediment, which was enhanced with increasing permeability. This transport, introduced by advection driven porewater exchange, provides organisms deep in the sediment with fresh phytoplankton material. Furthermore, the carbon turnover of a benthic community of a fine sand

station was experimentally investigated (Chapter 4). This study revealed high carbon processing rates of 21.7 mg C $m^{-2}$ $d^{-1}$ on sandy sediments together with a very fast response capacity of the benthic community. We could furthermore demonstrate the importance of bacteria in the very short-term processing of fresh phytoplankton material reaching the sediment, even though bacterial abundances on sandy sediments are low compared to finer compartments (Llobet-Brossa *et al.*, 1998). The reasons for the high turnover rates are probably I) fast transport of algal cells into deeper sediments layers (Chapter 3), II) mixing of bacterial communities with different physiologies (Chapter 2) and III) fast reaction capacity of different benthic compartments (Chapter 4). The studies presented in the Chapters 2, 3 and 4 of this thesis led to the conclusion that sandy sediments, which cover about 70% of the continental shelf, act as expansive coastal filter systems. Despite low standing stocks of reactants our results clearly demonstrate that the benthic community is able to react fast to settling phytoplankton and the high advective flushing rates furthermore boost the remineralisation of trapped algal cells.

## ***In situ*** **investigation of the bacterial community structure using lipid biomarker analyses**

Two study sites were investigated according to their bacterial community composition; Chapter 2 deals with three subtidal sandy sediments in the Southern North Sea (approximately 20 m water depth) and in Chapter 5 biomarker data from a deep-sea study site in the Cretan Sea are discussed (Eastern Mediterranean, 1500 m water depth). The three investigated sandy sediments were dominated by the even chain fatty acids $C_{16:1\omega7}$, $C_{16:0}$, $C_{18:1\omega7}$, and $C_{18:1\omega9}$. The fatty acids $C_{14:0}$, *ai* $C_{15:0}$, *i* $C_{15:0}$ and 10*Me*-$C_{16:0}$ follow in abundance. Other fatty acids could only be found in minor amounts. In general the same fatty acids were found in the deep Cretan Sea, with highest relative abundances for $C_{16:0}$, $C_{18:1\omega9}$, 10*Me*-$C_{16:0}$ and $C_{18:0}$, followed by $C_{18:1\omega7}$, $C_{16:1\omega9}$, *i* $C_{15:0}$ and *ai* $C_{15:0}$. Several of the fatty acids found are described as characteristic for bacteria. They give evidence for the abundance of members of the *Cytophaga-Flavobacterium* cluster ($C_{16:1\omega7}$, White *et al.*, 1996), which are described

as mostly aerobic, gram-negative bacteria. Furthermore, high abundances of branched-chain fatty acids such as *i* $C_{15:0}$, *ai* $C_{15:0}$, and *i* $C_{16:0}$ are indicative for gram-positive bacteria (White *et al.*, 1996) and other anaerobic bacteria (Findlay and Dobbs, 1993). 10*Me*-$C_{16:0}$, *i* $C_{17:0}$, *ai* $C_{17:0}$ are typical for sulfate reducing bacteria (SRB) (Findlay and Dobbs, 1993), a mesophilic gram-negative group of δ-Proteobacteria. Furthermore, 10*Me*-$C_{16:0}$ is considered to be indicative of *Desulfobacteriaceae* (Rütters *et al.*, 2002 b, Kuever *et al.*, 2001). The composition of the bacterial fatty acids of these two study sites is not differing very much, even though these environments have little alike. The dominance of $C_{16:1\omega7}$ is less pronounced in the deep Cretan Sea. This could be due to the higher phytoplankton input into the shelf sediment, because $C_{16:1\omega7}$ is a constituent of some diatom species as well (Kharlamenko *et al.*, 1995). The bacterial fatty acid with the highest abundance in the deep sea was 10*Me*-$C_{16:0}$, indicating a great importance of SRB in this environment.

The high abundances of SRB biomarker at the two study sites is an astonishing finding. At the North Sea study site it is striking, because sulfate reduction is described as a process located in anoxic sediments (Jørgensen, 1983) and SRB normally die off under oxic conditions (Cypionka *et al.*, 1985). For the Crete study it is astonishing, due to the generally lower sulfate reduction activity in deep-sea sediments (Jørgensen, 1982). The findings presented in this thesis therefore suggest a reconsideration of the "SRB-world", which are described as being very important for anaerobic processes on the continental shelf (Jørgensen, 1982), but probably are so far underestimated in other marine habitats.

The general fatty acid distribution patterns found during this study are comparable to other marine environments (e.g. Rajendran *et al.*, 1992, Boschker *et al.*, 1999, Rütters *et al.*, 2002 a). It therefore could be proposed that these pattern generally reflect the fatty acid composition of marine sediments (with the exception of extreme environments, like hot vents or cold seeps). This implies that benthic bacterial communities are generally composed of the same physiological groups. Only the relative abundances vary, reflecting special conditions in the investigated environment. On the other hand could these results as well draw suspicion on the

interpretation of biomarkers on a very specific level. Promising new results to overcome this problem are gained from the examination of intact polar lipids (IPL). These molecules have a greater structural diversity and their analysis has now become possible through coupled high performance liquid chromatography and mass spectrometry (HPLC-MS). So far, only a few research groups have entered this new field and analysed IPLs in marine sediments and environmental sediments in general (Rütters *et al.*, 2002 a, Zink *et al.*, 2003), but the further promotion of this method will strongly improve the interpretation of lipid biomarkers in microbial ecology.

During the different experiments made for this thesis no significant differences were recorded in the absolute abundances of bacterial fatty acids before and after the experiments. Therefore we can conclude that studies of microbial biomarker are not very useful to interpret the activity of bacterial communities. For these questions it is an appropriate attempt to combine $^{13}C$-labelling experiments with subsequent compound-specific isotopic ratio measurements to get an insight into the activity of bacterial communities (Chapter 4 and 5).

## Carbon turnover

The total benthic carbon turnover is generally investigated via Sediment Community Oxygen Consumption (SCOC) (e.g. Smith Jr, 1987). Most previous investigations compared the SCOC with the flux of particulate organic carbon (POC) to the sediments using sediment traps (e.g. Witte and Pfannkuche, 2000, Smith *et al.*, 2001, Giordani *et al.*, 2002, Baldwin and Smith, 2003). They all found that the contribution of carbon flux to SCOC was very low and concluded a lateral input (Giordani *et al.*, 2002, Baldwin and Smith, 2003), which is proposed as an additional food source for deep-sea organisms by other authors as well (Koppelmann, 1994, Danovaro *et al.*, 2000, Bühring *et al.*, 2002). Smith *et al.* (2001) furthermore outlined the importance of dissolved organic carbon (DOC) to the nutrition of benthic communities and proposed that it has a great impact on the mismatch comparing SCOC and POC flux. Our investigation in the deep Cretan Sea revealed that only up to 11% of the carbon mineralisation calculated from the SCOC (assuming an RQ of 1)

was due to our POM addition. Due to the experimental design using a chamber lander we can exclude an additional input during the duration of the experiments, but we can clearly state that the benthic community additionally used material of unknown quality and composition already present in the sediment.

Nevertheless, the interpretation of data resulting from SCOC measurements is hampered by the generality of these results. No further statement on the contribution of the different benthic compartments is possible together with a total non-observance of anaerobic processes. For this thesis two relatively new attempts were combined to overcome these problems. To become uncoupled from natural sedimentation events the first new approach was to experimentally induce a phytoplankton bloom *in situ*. For this purpose the use of chambers was promoted, which were invented and designed to match the natural conditions (for the North Sea experiment presented in Chapter 3 and 4 see Huettel and Rusch, 2000; for the Crete experiments presented in Chapter 5 see Witte and Pfannkuche, 2000). Furthermore the injected algae were labelled with the stable carbon isotope $^{13}C$ in order to follow the uptake and processing of the added material through the benthic food web (reviewed by Boschker and Middelburg, 2002). These new attempts were very promising to match the questions posed in this thesis.

The labelling experiments conducted during this thesis led to calculation of processed carbon. No direct comparison can be conducted due to the incomparable experimental basis with respect to the different environments. In the North Sea the amount of added carbon was comparable to half of the daily export production in this area, which clearly shows that this was not an enrichment experiment. For the deep-sea study in the Cretan Sea, different amounts of POC have been added, with the high amount corresponding to nearly half of the yearly export production in this area. The differences in the amount of processed carbon during these studies are therefore more related to the ability of the respective systems to respond to incoming carbon, than representing total carbon processing rates.

The Cretan Sea study furthermore showed that the deep-sea organisms are capable of graduate their response according to the amount of food arriving clearly reflected by the differences between the L and H experiments. Several former

investigations revealed an increase in SCOC due to POM arrival at the deep-sea floor of approximately 50% (Smith Jr. and Baldwin, 1984, Smith Jr, 1987, Moodley *et al.*, 2002). These investigations led to the speculation that deep-sea benthic communities can only switch between "low turnover" and "high turnover" mode. Our results demonstrated the capability of the benthic community at our deep-sea study site to graduate its reaction depending on the amount of settling phytoplankton. Regarding our results together with other deep-sea studies, it seems obvious that the short-term benthic reaction is increasing with decreasing food availability, with the strongest potential to increase the initial reaction at our study site in the highly oligotrophic deep Mediterranean (118% increase for the H experiments), followed by the Porcupine Abyssal Plain in the North Atlantic (approximately 75% increase due to POM addition, Witte *et al.*, 2003 b) and the Sognefjord (25% increase due to POM addition, Witte *et al.*, 2003 a). This comparison suggests a close relation between amplitude of the increase in benthic carbon turnover and food availability, or rather food scarcity in the respected environment.

Comparing the incorporation of label into the bacteria and the label respiration in the very short term, we can state that more labelled carbon was incorporated than respired. We would expect the respiration to be higher to maintain the energy needs of the cell, but during our experiments 62% and 31% of label was found in the bacteria, compared to 10% and 9% remineralised, for the 12 h North Sea experiments and the 36 h L Crete experiments, respectively. Simultaneously we determined an increase in sediment community oxygen consumption (SCOC) during the Crete experiments, which is a reliable measure for the benthic carbon demand (Pfannkuche, 1993), indicating that the whole benthic community responded with increasing activity. Therefore we can conclude that the benthic community used already present material for respiration as well, when fresh material settles. The higher activity caused by the arrival of fresh phytoplankton material tends to propagate to organisms and places were no fresh material had arrived at that point. This furthermore indicates some unknown communication ways in the benthic community.

In conclusion, it can be stated that marine sediments are sites of high carbon turnover. Even though the inhabiting benthic organisms are normally only seasonally to sporadically be provided with fresh food, they demonstrated a fast reaction capacity in terms of uptake and incorporation as well as respiration. Nevertheless, no general reaction could be recorded and due to the low number of studies using labelling approaches to unravel the benthic reactions in marine sediments, I strongly suggest experiments in different environments in order to get a better understanding of the global carbon turnover. Furthermore, experiments using different DOC substrates could help to elucidate physiologies of bacterial communities *in situ* and variations not only in terms of quantity, but also in terms of quality could be helpful to better understand the processes of carbon turnover in marine sediments.

The inclusion of meiofauna into the budgeting approach could additionally increase the significance of these studies. This could either be done by separation of meiofauna organisms from the sediments or by analyses of biomarkers for groups that are to small for hand-picking. A candidate biomarker for protists is tetrahymanol (Boschker and Middelburg, 2002), however the combination with stable isotope analysis needs to be demonstrated. Furthermore, promising new attempts try to find new and more specific biomarker classes for microbes, like specific amino acids, intact polar lipids (Rütters *et al.*, 2002 a) and rRNA (MacGregor *et al.*, 2002).

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# Chapter 7

## SUMMARY

## ZUSAMENFASSUNG

## SUMMARY

The general aim of this thesis was to elucidate the fate of settling phytoplankton in shelf sands and deep-sea sediments. A special focus was on the participation of the bacterial community on degradation and incorporation of organic matter. Therefore investigations on three subtidal North Sea sandy sediment stations and in the deep Cretan Sea were conducted.

Sandy sediments cover large areas of the continental shelf. These sediments are highly permeable and thus allow advective porewater exchange across the sediment-water interface that is driven by pressure gradients across the sediment surface. In contrast to fine grain sized sediments that are characterised by molecular diffusion, transport and water exchanges in surface layers of permeable sediments are tightly coupled to the water column. In the first part of this thesis, we investigated the importance of permeable sediments for the degradation of diatom blooms with special focus on the bacterial contribution and with respect to the other benthic compartments. The overall working hypothesis was that permeable sediments act like filters for water column derived phytoplankton cells, with the advection driven porewater transport, furthermore enhancing the carbon turnover in sandy sediments. Proving this hypothesis, we demonstrated the importance of permeable sediments for carbon cycling in shelf areas and provide deeper insights into their bacterial colonisation and contribution to turnover of organic matter.

The first study (Chapter 2) addresses the microbial community structure of three stations with fine, medium and coarse sandy sediments in the German Bight (Southern North Sea). The microbial community was investigated using lipid biomarker analysis and fluorescence *in situ* hybridisation (FISH). Here, the bacterial biomass decreased with increasing grain size. Furthermore, fatty acid analyses revealed biomarkers of different bacterial groups such as members of the *Cytophaga-Flavobacterium* cluster and sulfate reducing bacteria (SRB). Despite a high oxygen penetration depth, the SRB biomarkers were abundant in surface horizons indicating suboxic to anoxic micro habitats in these oxygenated environments. This finding is moreover supported by investigations with the taxonomically specific FISH technique.

Staining with probes targeting a *Cytophaga-Flavobacterium*, $\chi$-Proteobacteria, and different members of the SRB displayed positive results in all three sediment types, which suggests that SRB play a more important role in oxygenated shelf sediments than previously thought.

In order to asses the importance of advective porewater transport in permeable sediments, *in situ* and on-board experiments at the same three sandy sediment stations were performed (Chapter 3 and 4). Benthic chamber deployments demonstrated enhanced advective transport of $^{13}C$-labeled diatom carbon and frustules into sandy sediments with increasing permeability. On the coarse sediments highest transport rates were observed, with 21% of the added diatom frustules found below 0.5 cm sediment depth after 20 h. Furthermore, high DOC release rates of 7200 µmol C $m^{-2}$ $d^{-1}$ indicate rapid degradation of trapped diatom cells. These results reveal that advective transport through a sandy matrix filters particulate matter and enhances degradation processes by constantly transporting oxygen-rich water into the particle-rich surface layers of these sediments.

The investigation of the pathway of settling particulate organic carbon (POC) through the benthic food web of the fine sand is given in Chapter 4. This study gives good evidence for a stepwise short-term processing of a settling phytoplankton bloom: $^{13}C$-labelled carbon was primarily incorporated into the bacterial fatty acids *i* $C_{15:0}$, *ai* $C_{15:0}$, and *i* $C_{16:0}$, indicating the participation of a distinct bacterial population after 12 h of incubation. In contrast, some of the biomarker fatty acids of sulfate-reducing bacteria (SRB), such as 10*Me*-$C_{16:0}$ and *i* $C_{17:1\omega7}$, showed a late and weak incorporation. After 12 h incubation the largest fraction of processed carbon was incorporated into bacterial biomass while longer incubation times (32 and 132 h) revealed that the participation of macrofauna gained more importance. After 132 h the greatest fraction of $^{13}C$-labelled organic matter was mineralised to $CO_2$ with a total carbon processing rate of 21.7 mg C $m^{-2}$ $d^{-1}$. Despite the relatively low bacterial biomass in sands, these results give evidence that that sandy sediments have biocatalytic filter properties. Our findings show the fast reaction capacity of the benthic sand community on a settling phytoplankton bloom event with a great importance of bacteria in the first step of algal carbon processing.

The second part of this thesis (Chapter 5) addresses the carbon turnover in the Cretan basin, an oligotrophic deep-sea environment. The main working hypothesis here postulates that the benthic community is able to adjust its reaction capacity gradually according to the amount of settling phytoplankton, rather than just switch between a "low turnover" and "high turnover" mode. The food limitation in this oligotrophic system is very suitable to investigate the response pattern of deep-sea benthic communities to varying food input. For this purpose, *in situ* pulse chase experiments with low and high amounts of diatom carbon were performed using the stable carbon isotope $^{13}C$ as a tracer. The analysis of the fatty acid composition revealed a bacterially dominated benthic community, with evidence for considerable numbers of sulfate-reducing bacteria (SRB). Eminent differences between the incorporation into various biomarkers were observed, with generally lower incorporation into biomarkers of SRB than into other bacteria. The generally lower labelling of SRB is probably caused by incorporation of secondary carbon products (e.g. acetate and other short chain carbon sources) that first have to be produced by other organisms feeding directly on the diatoms, like fermentative bacteria and meio- or macrofauna. Overall carbon mineralization increased by 40% and 120% due to the low and high carbon enrichment, respectively. The amount of processed carbon (incorporation into bacteria plus $^{13}CO_2$ produced) was 6.9 mg C $m^{-2}$ $d^{-1}$ and 52.4 mg C $m^{-2}$ $d^{-1}$ for the low and high carbon addition, respectively. These results demonstrate a graduated reaction capacity, overturning the former belief of an on-off reaction of deep-sea benthic communities. The comparison of label incorporation into bacterial fatty acids and respiration to $^{13}CO_2$ revealed a mixed origin of the material used for respiration, indicating that the accompanied organisms use already present material for respiration as well, when fresh material settles.

This thesis reveals the great importance of permeable sediments for carbon turnover on the continental shelf, due to advective transport processes. The enhanced porewater exchange provides periodic input of fresh phytoplankton material to deeper sediment layers. Permeable sediments, which cover large areas of the continental shelf, represent expansive coastal filter systems, where advective flushing boosts the remineralisation and therefore enhances carbon cycling.

Furthermore, aerobic and anaerobic bacteria were observed to coexist in the same sediment horizons, indicating anaerobic micro environments, and seem to largely contribute to the high turnover rates in these sandy sediments.

The deep-sea study presents first evidence for a graduated reaction capacity of an oligotrophic benthic community to varying food input. Our results alter the former belief that a deep-sea benthic community reacts in a kind of on-off switch to sedimentation events.

The successful applications of labelling approaches presented in this thesis demonstrate the advantage of this approach to unravel the carbon turnover in marine sediments with respect to different benthic compartments.

## ZUSAMMENFASSUNG

Die grundlegende Zielsetzung dieser Doktorarbeit war, dass Schicksal von Phytoplankton nach der Sedimentation in Schelf- und Tiefseesedimente detailliert zu untersuchen. Dabei wurde ein besonderes Augenmerk auf die Zusammensetzung der bakteriellen Gemeinschaft gelegt, die an der Aufnahme und dem Abbau des organischem Materials beteiligt ist. Dazu wurden Untersuchungen in drei subtidalen Sanden der Nordsee und in der Tiefsee des Kretischen Meeres durchgeführt.

Sandige Sedimente bedecken große Flächen des kontinentalen Schelfs. Diese meist hochpermeabel Sedimente sind durch advektiver Porenwasseraustausch entlang der Sediment-Wasser Grenzschicht geprägt. Im Gegensatz zu feinkörnigen Sedimenten, in denen molekulare Diffusion vorherrscht, sind Transportprozesse in permeablen Sedimenten eng an die Wassersäule gekoppelt. Im ersten Teil dieser Arbeit wurde die Bedeutung permeabler Sedimente für den Abbau sedimentierter Diatomeenblüten durch Bakterien und anderer benthischer Organismen untersucht. Die übergreifende Arbeitshypothese war die Bedeutung permeabler Sedimente als Filter für Phytoplankton aus der Wassersäule, wobei advektiv angetriebener Porenwasseraustausch zusätzlich den Kohlenstoffumsatz sandiger Sedimente antreibt. Unsere Ergebnisse belegen die große Bedeutung permeabler Sande für den Kohlenstoffkreislauf in Schelfgebieten. Außerdem wurden tiefere Erkenntnisse über die bakterielle Besiedlung und deren Beitrag zum Umsatz organischen Materials gewonnen.

Kapitel 2 beschäftigt sich mit der Struktur der mikrobiellen Gemeinschaft an drei Standorten in der Deutschen Bucht (südliche Nordsee) mit feinen, mittleren und groben Sandsedimenten. Die bakterielle Gemeinschaft wurde mit den Methoden der Biomarkeranalyse und Fluoreszenz *in situ* Hybridisierung (FISH) untersucht. Wir fanden heraus, dass die bakterielle Biomasse mit zunehmender Korngröße abnimmt. Die Analyse der Fettsäuren enthüllte Biomarker verschiedener bakterieller Gruppen, wie Angehörige der *Cytophaga-Flavobacterium* Gruppe und Sulfat-reduzierende Bakterien (SRB). Trotz großer Sauerstoffeindringtiefen fanden wir Biomarker von SRB in oberflächennahen Sedimenthorizonten, was die Existenz suboxischer bis

anoxischer Mikrohabitate in dieser oxygenierten Umgebung impliziert. Mit zusätzliche Untersuchungen mit taxonomisch spezifischen FISH-Sonden konnten wir diese Befunde bestätigen. Hierbei gelang der Nachweis von *Cytophaga-Flavobacterium*, $\chi$-Proteobacteria und verschiedener SRBs in allen drei Sedimenttypen, was für eine größere Bedeutung der SRBs in oxygenierten Schelfsedimenten als bisher angenommen spricht.

Um mehr über die Bedeutung von advektivem Porenwassertransport in permeablen Sedimenten herauszufinden, wurden *in situ*- und Schiffsexperimente an den gleichen drei sandigen Sedimenten durchgeführt (Kapitel 3 und 4). Messungen mit benthischen Kammern zeigten erhöhten advektiven Transport von $^{13}C$-markiertem Diatomeenkohlenstoff und –zellbruchstücken in Sande mit zunehmender Permeabilität. In grobem Sand wurden die höchsten Transportraten gemessen, wobei 21% der zugegebenen Diatomeen nach 20 h tiefer als 0.5 cm ins Sediment transportiert worden waren. Die hohen Bildungsraten von gelöstem organischen Kohlenstoff (DOC) von 7200 µmol C $m^{-2}$ $d^{-1}$ weisen auf einen schnellen Abbau der Diatomeenzellen im Sediment hin. Diese Ergebnisse zeigen, dass advektiver Porenwasseraustausch durch eine sandige Matrix partikuläres Material aus der Wassersäule filtriert und Abbauprozesse durch permanenten Nachschub von sauerstoffreichem Wasser in die oberflächennahen Schichten beschleunigt werden.

Das 4. Kapitel beschreibt eine Untersuchung im Feinsand über den Fluß des sedimentierten partikulären organischen Kohlenstoffs (POC) durch die benthische Nahrungskette. Über kurze Zeiträume konnte die schrittweise Verarbeitung von sedimentiertem Phytoplankton gezeigt werden: der $^{13}C$-markierte Kohlenstoff war nach 12 h vorwiegend in die Bakterienfettsäuren *i* $C_{15:0}$, *ai* $C_{15:0}$, und *i* $C_{16:0}$ eingebaut. Dies ist ein Hinweis für die Aufnahme in eine spezielle Bakterienpopulation nach 12 h Inkubationsdauer. Die Aufnahme in Biomarker-Fettsäuren von SRBs, wie 10*Me*-$C_{16:0}$ und *i* $C_{17:1\omega7}$, war hingegen weniger ausgeprägt und zeitlich verschoben. Nach 12 h war der größte Anteil des umgesetzen Kohlenstoffs in bakterieller Biomasse gebunden, wohingegen bei längeren Inkubationszeiten (32 und 132 h) eine erhöhte Aufnahme in Makrofaunaorganismen nachweisbar war. Nach 132 h war die größte Fraktion des $^{13}C$-markierten organischen Materials zu $CO_2$ veratmet, mit einer Gesamtumsatzrate von 21.7 mg C $m^{-2}$ $d^{-1}$. Diese Ergebnisse lassen auf

biokatalytische Filtereigenschaften von Sanden schließen, obwohl die bakterielle Biomasse sandiger Sedimente relativ niedrig ist. Wir konnten die schnelle Reaktion der benthischen Sandgemeinschaft auf das Sedimentationsereignis einer Phytoplanktonblüte zeigen, wobei für den ersten Schritt des Kohlenstoffumsatzes die Bakterien eine wichtige Rolle spielten.

Im zweiten Teil dieser Arbeit (Kapitel 5) wurde der Kohlenstoffumsatz im Kretischen Becken, einem oligotrophen Tiefseegebiet, untersucht. Die Hypothese dieser Arbeit war, dass die benthische Gemeinschaft in der Lage ist, ihre Reaktionskapazität der Menge an sedimentiertem Material stufenweise anzupassen. Die Nahrungslimitation in diesem oligotrophen System macht es zu einem sehr geeigneten Gebiet für die Untersuchung der Reaktion einer benthischen Tiefseegemeinschaft auf variable Nahrungszugaben. Dazu wurden *in situ* Experimente mit geringer und hoher Zugabe von Diatomeen, markiert mit dem stabilen Kohlenstoffisotop $^{13}C$, durchgeführt. Die Zusammensetzung der gefundenen Fettsäuren sprach für eine bakteriell dominierte benthische Gemeinschaft, mit beträchtlichen Anteilen von SRBs. Die $^{13}C$-Aufnahme in die einzelnen Biomarker war sehr unterschiedlich, und zeigte generell geringere Aufnahmen in SRB Biomarker. Die schwache Markierung von SRBs liegt wahrscheinlich an der Aufnahme von sekundären Kohlenstoffprodukten (wie z.B. Acetat und andere kurzkettige Kohlenstoffverbindungen), welche erst durch andere Organismen, wie fermentierende Bakterien sowie Meio- und Makrofauna, produziert worden sind. Die benthische Sauerstoffzehrung stieg um 40 bzw. 120% nach der Anreicherung mit der geringen bzw. der hohen Kohlenstoffmenge an. Die Menge umgesetzen Kohlenstoffs (Aufnahme in die Bakterien + produziertes $^{13}CO_2$) stieg von 7 mg C $m^{-2}$ $d^{-1}$ auf 52 mg C $m^{-2}$ $d^{-1}$ zwischen den beiden Versuchsansätzen an. Diese Ergebnisse deuten auf eine abstufbare Reaktion, was der gängigen Vorstellung einer "an-aus Schalter"-Funktionsweise benthischer Tiefseegemeinschaften widerspricht. Der Vergleich der $^{13}C$-Aufnahme in bakterielle Fettsäuren und Respiration zu $^{13}CO_2$ deutete auf einen gemischten Ursprung des veratmeten Materials hin, wobei die beteiligten Organismen im Falle eines Sedimentationsereignisses zusätzlich Material benutzen, das bereits im Sediment vorhanden war.

Mit dieser Doktorarbeit ist es gelungen, die große Bedeutung permeabler Sedimente mit advektiven Porenwassertransport für den Kohlenstoffumsatz des kontinentalen Schelfs zu unterstreichen. Der erhöhte Porenwasseraustausch versorgt tiefere Sedimentschichten periodisch mit frischem Phytoplankton. Permeable Sedimente bedecken große Teile des kontinentalen Schelfs und repräsentieren ausgedehnte Küstenfiltersysteme, in denen advektives Durchspülen des Sedimentes die Remineralisation und damit den Kohlenstoffkreislauf stimuliert. Zusätzlich wurden aerobe und anaerobe Bakterien in den gleichen Sedimentschichten gefunden, was als Hinweis auf anaerobe Mikronischen gedeutet werden kann. Diese Koexistenz hat wahrscheinlich einen maßgeblichen Einfluss auf die hohen Umsatzraten in den untersuchten Sanden.

Die Tiefseeuntersuchung lieferte erste Hinweise für eine abgestufte Reaktionskapazität einer oligotrophen Benthosgemeinschaft auf variable Nahrungszugaben. Diese Ergebnisse widersprechen der gängigen Auffassung, dass benthische Tiefseegemeinschaften als „Standardreaktion" auf Sedimentations-ereignisse reagieren.

Die erfolgreichen Anwendungen des Markierungsansatzes in dieser Arbeit demonstrieren darüber hinaus die Vorteile dieser Methode bei der Entschlüsselung des Kohlenstoffumsatzes durch die verschiedenen benthischen Organismengruppen in marinen Sedimenten.

# LIST OF PUBLICATIONS

## Publications presented in this thesis

1) S.I. Bühring, Elvert M. & Witte U.
**The microbial community structure of three sandy sediments characterised by the investigation of bacterial fatty acids and fluorescence in situ hybridisation.**
This article has been submitted to *Environmental Microbiology*.

2) S. Ehrenhauss, Witte U., Bühring S.I. & Huettel, M.
**Effect of advective pore water transport on distribution and degradation of diatoms in permeable North Sea sediments.**
This article has been accepted by *Marine Ecology Progress Series*.

3) S.I. Bühring, Ehrenhauss S., Kamp A., Moodley L. & Witte U.
**POC processing in a sublittoral sandy sediment: investigations using pulse chase experiments with $^{13}$C-labelled diatoms.**
This article has been submitted to *Marine Ecology Progress Series*.

4) S.I. Bühring, Moodley L., Lampadariou N., Tseledipes A. & Witte U.
**Benthic response to varying food input: in situ pulse-chase experiments in the deep Cretan Sea (Eastern Mediterranean).**
This article will be submitted to *Limnology and Oceanography*.

## Further publications

1) S.I. Bühring & Christiansen, B. (2001)
   **Lipids in selected abyssal benthopelagic animals: links to the epipelagic zone?**
   *Progress in Oceanography*. **50**: 369-382.

2) S.I. Bühring, Koppelmann, R., Christiansen, B. & Weikert, H. (2002)
   **Are Rhodophyceae a dietary component for deep-sea holothurians?**
   *Journal of the Marine Biological Association of the United Kingdom*. **82**: 347-348.

3) B. Christiansen, Bühring, S.I., Pfannkuche, O. & Weikert, H.
   **The benthic mixed layer plankton community at the Porcupine Abyssal Plain, NE-Atlantic: structure and vertical distribution.**
   This article has been submitted to *Marine Biology*.

4) U. Witte, Bühring, S.I., Ehrenhauss, S., Kamp, A. & Moodley, L.
   **Driving forces of carbon turnover in subtidal sandy sediments.**
   in preparation.