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# Response of deep-sea benthic microbial communities to particulate organic matter supply:

# *In situ* experiments in the Fram Strait (Arctic Ocean)

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# Summary

Particulate organic matter (POM) settling to the deep seafloor is the nutritional basis for benthic life. This material loses much of its labile organic compound during descent in the water column so that the remains reaching the deep seafloor are less degradable substances such as cellulose, chitin and various proteinaceous structural compounds. Benthic microbial communities are able to cleave structural polymeric compounds by extracellular enzymes into smaller organic compounds that can be taken up and used to support microbial metabolism. In temperate and polar regions, seasonal phytoplankton blooms may lead to huge pulses of detrital matter to the deep oceans floor. Microbial organisms respond quickly to such inputs of organic material to the sea bed by increased extracellular enzyme production, DNA and protein synthesis, respiration and microbial biomass.

Up to now, only few studies have focused on the effects of changes in environmental parameters such as POM supply on deep-sea benthic microbial populations. Particularly from high northern latitudes only little is known about the function and structure of microbial communities in deep-sea sediments. The fact that Arctic regions figure most immediately and dramatically in scenarios of global warming, including changes in productivity and food input to the deep seafloor, makes the need for research all the more compelling.

The aim of this study was to investigate *in situ* the effects of a sudden large POM input on the function and structure of heterotrophic microbial assemblages in high northern latitude deep-sea sediments. Three *in situ* studies, each divided into a short- (seven days and three month) and long-term (one year) experiment, were carried out by using a Sediment Tray Free Vehicle (STFV) which was deployed in the Arctic Ocean at the experimental site of the deep-sea long-term observatory HAUSGARTEN (Fram Strait, 2500 m water depth) during expeditions with *RV Polarstern* in summer seasons 2003, 2004 and 2005. The present study describes for the first time the deployment of a STFV as experimental approach for microbial long-term investigations at the deep seafloor.

Special emphasis was placed on the enrichment of deep-sea sediments with chitin as one of the most important biopolymer in aquatic ecosystems. Additionally, microbial response to POM supply was studied in association with different sediment types to assess how variations in sediment characteristics (e.g. particle size, organic carbon content) affects the efficiency of microbial utilisation of particulate organic material. For this purpose, natural (deep-sea sediments) and artificial (glass beads, sand) sediments were enriched with organic substrates and incubated at the deep seafloor.
Different functional parameters (microbial cell number, biomass, enzyme activities) were measured and bacterial community composition was determined by using the fingerprint method of terminal-restriction fragment length polymorphism (T-RFLP).

Briefly the following can be concluded from the experiments presented as independent manuscripts in this thesis:

- A functional *in situ* reaction of microbial assemblages in Arctic deep-sea sediments to large chitin supply required a certain period of time, longer than one week, and was triggered by a preceding shift in the bacterial community structure.

- *In situ* incubation for one year led to a substantial increase of microbial cell numbers, biomass and chitobiase activity associated with increasing chitin concentrations, indicating that chitin may serve as important substrate for microbial organisms in extreme environments such as the Arctic deep sea, providing a potential carbon and nitrogen source.

- Bacterial community structure in chitin-enriched sediments subjected to the long-term incubation was found to be different from that of non-enriched sediments and of sediments subjected to a shorter incubation time, suggesting that chitin had a time-dependent effect on the resident bacterial communities. Although chitin input contributes to variability in community structure, variations in its concentration seemed to play a minor role in determining shifts in the bacterial community composition.

- Deep-sea microbial communities also responded to variations in sediment characteristics (i.e. particle size, particle shape, organic carbon content) by changing their composition, abundance and activity. Results presented here support the general assumption that microbial communities in natural environments prefer to colonise sediments with smaller grain sizes and higher

organic carbon contents. This relationship between sediment type and microbial functioning was also evident when chitin was added, indicating that chitin particles were more available for microbial attack in fine sediments than in those composed of larger grain size. In terms of community structure, bacterial assemblages in the Arctic deep sea seemed to be more affected by variation in physical characteristics of sediment than in the amount and/or availability of organic substrates.

# Zusammenfassung

Benthische Gemeinschaften in der Tiefsee werden insbesondere durch den geringen Eintrag von partikulärem organischem Material aus der euphotischen Zone geprägt. Aufgrund vielfältiger biologischer Abbau- und Umwandlungsprozesse während der Sedimentation in der Wassersäule besteht das organische Material, welches den Tiefseeboden erreicht, hauptsächlich aus schwer abbaubaren Substanzen (z.B. Cellulose, Chitin). Benthische mikrobielle Lebensgemeinschaften sind in der Lage, das sedimentierte partikuläre, organische Material mit Hilfe von extrazellulären Enzymen in niedermolekulare organische Substanzen zu spalten, und für die Biomasseproduktion und Zellvermehrung zu nutzen.

Saisonale Veränderungen in der Sedimentationsrate bewirken, dass in gemäßigten und polaren Gebieten als Folge der Phytoplanktonblüte pulsartige Einträge von organischem Material stattfinden. Mikroorganismen reagieren sehr rasch auf diesen Nahrungseintrag durch erhöhte Produktion von extrazellulären Enzymen, DNA- und Proteinbiosynthese, Respiration und Biomasseproduktion.

Bislang gibt es nur wenige Studien, die sich mit den Auswirkungen solcher pulsartigen Nahrungseinträge auf die funktionellen und strukturellen Eigenschaften von mikrobiellen Gemeinschaften in Tiefseesedimenten beschäftigen, dies gilt besonders für Tiefsee-Ökosysteme polarer Regionen. Im Zuge der zunehmenden globalen Klimaänderung, deren Auswirkungen sich in der Arktis auffallend schnell und dramatisch abzeichnen, ist zu erwarten, dass Qualität und Quantität der im arktischen, marinen Ökosystem zur Verfügung stehenden Nahrung verändert werden.

Ziel der Arbeit war es, die Auswirkungen eines pulsartigen Eintrags von partikulärem, organischen Material nach einer kurzen (sieben Tage bzw. drei Monate) und einer längeren (ein Jahr) Inkubations-Periode auf die Funktion und Struktur heterotropher, mikrobieller Gemeinschaften in arktischen Tiefseesedimenten zu untersuchen. Es wurden insgesamt drei Inkubations-Experimente mit Hilfe eines Sedimentkammer-Freifallsystems im arktischen Ozean an der Tiefsee-Langzeitstation HAUSGARTEN (Fram Strasse, 2500 m Wassertiefe) während Expeditionen mit *FS Polarstern* im

Sommer 2003, 2004 und 2005 durchgeführt und ausgewertet. Die vorliegende Arbeit beschreibt erstmals den Einsatz eines Sedimentkammer-Freifallsystems als experimentelle Plattform für mikrobielle Langzeit-Untersuchungen in der Tiefsee.

Im Mittelpunkt der Untersuchungen stand dabei die Anreicherung von Tiefseesedimenten mit Chitin, als eines der am häufigsten vorkommenden Biopolymere in aquatischen Ökosystemen. Zusätzlich galt es zu ermitteln, welchen Einfluss verschiedene Sedimenttypen (von fein- bis grobkörnig) auf die Effizienz der mikrobiellen Verwertung von partikulärem organischem Material (z.B. Chitin) haben. Dazu wurden natürliche (Tiefseesediment) und künstliche Sedimente (Glasperlen, Sand) mit organischem Substrat angereichert und am Tiefseeboden inkubiert. Verschiedene funktionelle Parameter (mikrobielle Zellzahl, Biomasse, Enzymaktiviäten) wurden gemessen und die strukturelle Zusammensetzung der Bakteriengemeinschaft mit Hilfe der molekularbiologischen Terminale-Restriktions-Fragment-Längen-Polymorphismus (T-RFLP) Methode ermittelt.

- Die Untersuchungen haben ergeben, dass eine funktionale Reaktion der mikrobiellen Gemeinschaften in arktischen Tiefseesedimenten auf einen plötzlichen Chitin-Eintrag erst nach einer Zeitverzögerung von mehr als einer Woche erfolgte, welcher eine Änderung in der Zusammensetzung der bakteriellen Gemeinschaft voranging.

- Nach einer Langzeit-Inkubation von einem Jahr wurde ein signifikanter Anstieg in der mikrobiellen Zellzahl, Biomasse und Enzymaktivität als Funktion zunehmender Chitinkonzentrationen ermittelt. Diese Ergebnisse weisen darauf hin, dass Chitin eine potentiell wichtige Kohlenstoff- und Stickstoffquelle für mikrobielle Gemeinschaften in arktischen Tiefseesedimenten darstellen kann.

- Eine erneute Veränderung in der Zusammensetzung der Bakteriengemeinschaft in den mit Chitin angereicherten Sedimenten nach der Inkubation von einem Jahr deutete auf einen zeitabhängigen Effekt von Chitin auf die angesiedelten Bakteriengemeinschaften hin. Unterschiede in der Chitinkonzentration spielten jedoch eine eher unwesentliche Rolle in der Strukturierung der Bakteriengemeinschaft.

- Die Analysen zeigten, dass die Funktion und die strukturelle Zusammensetzung der mikrobiellen Gemeinschaften ebenfalls durch die Beschaffenheit der

Sedimente (Größe und Form der Sedimentpartikel, organischer Kohlenstoffgehalt) beeinflusst wurden. Tiefsee-Mikroorganismen besiedelten bevorzugt natürliche Sedimente mit kleinen Partikeln und hohem organischen Gehalt. Sowohl Zellzahl als auch Enzymaktivität nahmen mit zunehmender Partikelgröße und abnehmendem organischen Gehalt ab. Diese Beziehung zwischen Sedimenttyp und Funktionalität von mikrobiellen Gemeinschaften wurde auch nach der Zugabe von Chitin beobachtet, was auf eine bessere Verfügbarkeit der Chitinpartikel in natürlichen Sedimenten hinwies. Die Struktur der bakteriellen Gemeinschaften in der arktischen Tiefsee schien stärker durch die physikalischen Eigenschaften des Sediments beeinflusst als durch den Gehalt bzw. die Verfügbarkeit von organischem Substrat.

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# Abbreviations

| | |
|---|---|
| ANOVA | Analysis of Variance |
| db-RDA | distance-based Redundancy Analysis |
| DOC | Dissolved Organic Carbon |
| DOM | Dissolved Organic Matter |
| FISH | Fluorescence *In Situ* Hybridisation |
| HERMES | Hotspot Ecosystems on the Margins of European Seas |
| MIZ | Marginal Ice Zone |
| MUC | Multiple Corer |
| NE | North-East |
| NMDS | Non-metric Multidimensional Scaling |
| OTU | Operational Taxonomic Unit |
| PCR | Polymerase Chain Reaction |
| POC | Particulate Organic Carbon |
| POM | Particulate Organic Matter |
| RDA | Redundancy Analysis |
| rRNA | ribosomal Ribonucleic Acid |
| ROV | Remotely Operated Vehicle |
| STFV | Sediment Tray Free Vehicle |
| T-RFLP | Terminal Restriction Fragment Length Polymorphism |
| UPGMA | Unweighted Pair-Group Average |

# Introduction

## History of deep-sea microbiology

The *Challenger* Expedition (Fig. 1) across the oceans of the globe is commonly credited as the historical beginning of deep-sea biology. The result of nearly 70000 nautical miles surveying and exploring was the 'Report of the scientific results of the exploring voyage of *H.M.S. Challenger* during the years 1872-76', which catalogued about 4000 previously unknown species of animals. With these findings the previous theory of an 'azoic deep sea', suggested in the 1840s by EDWARD FORBES, was obliterated. In addition, during the *Challenger* Expedition the discovery of *Bathybius haeckeli* by THOMAS HENRY HUXLEY (1868) as foremost and universal microorganism was rectified. It became clear that HUXLEY'S observation of whitish, jellied material encasing coccolithophores in sediment samples was in actuality an alcohol precipitate of organic debris and did not represent, as asserted by HUXLEY, the 'Urschleim' (primodal slime) defined by ERNST HAECKEL.

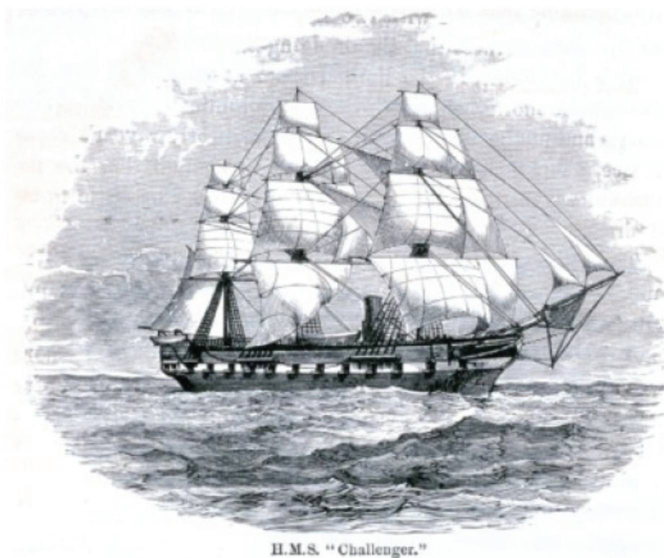



**Fig. 1.** The corvette *H.M.S. Challenger*. During the expedition (1872-1876) 492 deep-sea soundings, 133 bottom dredges, 151 open water trawls, 263 serial water temperature observations, and about 4000 species of marine life were discovered (MURRAY, 1895).

The fact that the conceptual and methodological basis for studying microbial life in the sea, other than by use of the microscope, was not available to the scientists of the *Challenger* Expedition and the general inaccessibility of the study area, have contributed to the relatively late and sporadic start of deep-sea microbiology. First microbiological studies in

the deep oceans were aimed at the distribution and characteristics of bacteria in seawater. CERTES (1884), for example, found bacteria in almost every water and sediment samples he collected from depths to 5000 m during the *Travallier* and *Talisman* expeditions (1882 and 1883). FISCHER (1886) observed a decrease in bacterial numbers with increasing water depth using the pour-plate technique introduced by ROBERT KOCH (1881). The period of preliminary exploration terminated in 1914, probably as a result of World War I that restricted generally research activities at sea.

The initial stage of a definable deep-sea microbiology was initiated in the middle of the 20th century by studies of ZOBELL & JOHNSON (1949) on the effect of hydrostatic pressure on microbial activities. ZOBELLS'S participation of the round-the-world deep-sea expedition on the Danish research vessel *Galathea* (1950-1952) and his subsequent work pioneered research in microbial aspects of the deep sea (MORITA & ZOBELL, 1955; ZOBELL & MORITA, 1957). Sediment samples that were taken from depth of more than 10000 m were shown to contain millions of viable bacteria per gram sediment (ZOBELL, 1952). Thus, the first evidence was produced that life may exist at a pressure of up to 1000 bar. During this time the term 'barophilic' was first used to define the requirement of pressure higher than 1 bar for optimal growth. Further treatises on pressure as well as temperature as important ecological, physiological, and molecular parameter in microbial metabolism were later discussed comprehensively in a number of reviews (e.g. ZOBELL; 1968, 1970; MORITA, 1976; MARQUISE & MATSUMURA, 1978). Deep-sea microbiology in the 1970's became entirely focused on the development of field and laboratory instruments to achieve sampling and sample handling without decompression. The development of pressure retaining samplers was undertaken to find barophilic bacteria regardless of the fact that ZOBELL'S report (1952) on bacteria in one of the deepest parts of the ocean was accomplished without any special high pressure sampling equipment (JANNASCH et al., 1976; JANNASCH & WIRSEN, 1977; TABOR et al., 1981).

## Advance in deep-sea microbial research

Deep-sea microbiology and microbial ecology in general attempt principally to answer three basic questions: (1) what is there (i.e. a description of the physiological and morphological types of microorganisms present; (2) how many are there (i.e. a quantification of the different types and their distribution); and (3) what are they doing (i.e. the *in situ* metabolic activities of the different types and their interaction with higher forms of life as well as biochemical processes). A classical approach for studying microbial activities in terrestrial and aquatic habitats is to measure the metabolic transformation of certain substrates during incubation samples directly in their environment. Applied to the deep sea, the advantages of *in situ* studies were the complete avoidance of pressure and temperature changes that might affect the populations of microorganisms. Although the inaccessibility of deep-sea sites represent a major disadvantage for taking series of samples, a number of time-course incubation experiments were carried out and led to calculations of growth and transformation rates (e.g. JANNASCH et al., 1971; MURAOKA, 1971).

### Progress in deep-sea technology

Especially the use of the first deep-ocean submersible *Alvin* (Fig. 2) offered new possibilities for deep-sea incubation studies. So called “bottle racks” (glass bottles housed in pressure tight aluminium cylinders) deposited by *Alvin* have been used for many years in studying the decomposition of solid material such as various kinds of seaweeds, wood, paper, chitin, agar and radiolabeled dissolved organic substrates (JANNASCH & WIRSEN, 1973). Because experimentation with *Alvin* on the deep ocean floor was restricted by weather conditions, expense, and a depth limitation of 4000 m at present, the possibility of using free-falling vehicles for microbiological experiments have been explored and found to be extremely useful. Since ISAACS`s (1969) original work, free vehicles of various configuration and degree of technical sophistication have been used, with the more expensive ones controlled by acoustic means and the others by simple preset timers. Tripod devices equipped with flotation and releasable weights could be launched from a ship to descend to principally any depth. Several *in situ* studies conducted with tripods revealed that this experimental approach offered the possibility to assure a statistically significant data amount due to nearly unlimited and

relative inexpensive deployments (WADA et al., 1975; WIRSEN & JANNASCH, 1976; JANNASCH & WIRSEN, 1980; WIRSEN & JANNASCH, 1986).

**Fig. 2.** Deep-ocean submersible *Alvin.* It is owned and operated by Woods Hole Oceanographic Institution (WHOI) and has been in operation since 1964. The global first deep-ocean submersible carries two scientists and a pilot up to 4000 m water depth and is equipped with video cameras and robotic arms.

Especially in the last few years, some advanced sampling systems and new technologies were developed which led to further progresses in the understanding of the deep-sea ecosystem, including microbiological processes. The classical design of tripod device was modified and clearly improved by the incorporation of different experimental devices such as micro-electrode profilers, respiration chambers, sonar systems and other incubation modules. Thus, these lander systems represent an ideal experimental platform to study, for example, the uptake or incorporation of organic carbon by microbial organisms (e.g. Benthic Chamber Lander (BCL); Fig. 3a) and the kinetics of prominent biogeochemical reactions (e.g. Biogeochemical Observatory (BIGO), both developed at IFM-GEOMAR, Kiel, Germany). Benthic lander systems used as free-falling vehicles allow incubation experiments directly at the deep seafloor. In combination with sediment trays they can be used to obtain information on, for example, sediment colonisation by benthic organisms or the significance of patchy organic inputs against a background of low productivity (e.g. GRASSLE, 1977; LEVIN & SMITH, 1984; SMITH, 1985, 1986). Such Sediment Tray Free Vehicles (STFVs) were also used for the present study and will be discussed thus later in more detail.

A useful tool for collecting undisturbed replicate sediment samples from the seafloor is the multiple corer (MUC; BARNETT, 1984; Fig. 3b). Up to 12 cores can be taken simultaneously, using plastic tubes with a length of approximately 60 cm and a

diameter of about 5-10 cm. The additional equipment with a video camera allows the observation of the multiple corer positioning at the seafloor and provides thus selective sampling. The combination of the conventional multiple corer with specific pressure-retaining chambers (Multi Autoclave Corer (MAC), MARUM, Germany) provides a useful approach to retrieve samples under *in situ* conditions.

Remotely Operated Vehicles (ROVs) are among the most important tools for precise sampling and experimentation in the deep sea. With these unmanned vehicles it is at present possible to dive to 6500 m water depth. In general, ROVs can be equipped with many different tools (e.g. video equipment, manipulators and a variety of sampling and *in situ* measurement devices). The precise underwater positioning and the stable and well defined height above seafloor allows selective sampling by push-coring (Fig. 3c) of different habitats (e.g. bacterial mats, black smokers at hydrothermal ridges, cold seeps at continental margins or cold water coral reefs).

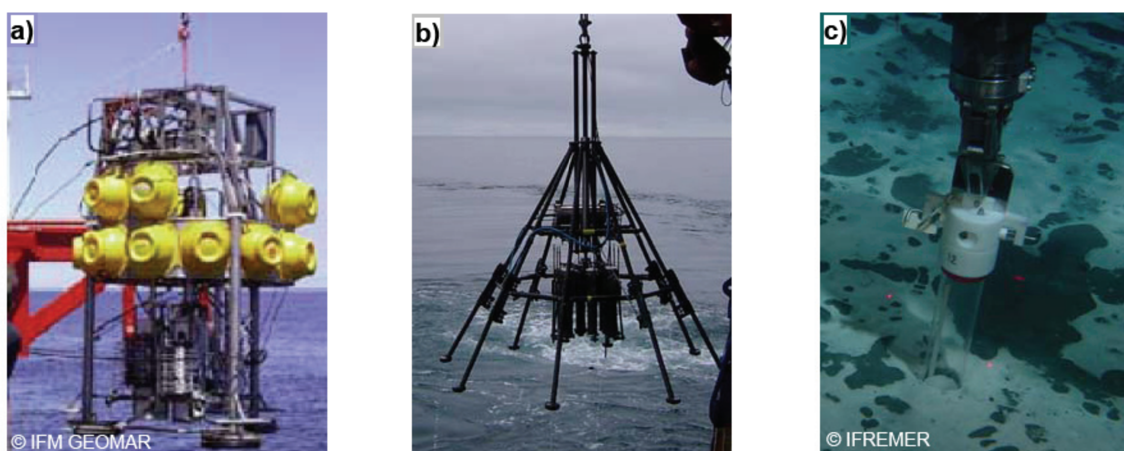



**Fig. 3.** (**a**) Benthic lander system, (**b**) multiple corer (MUC) for taking sediment samples, (**c**) sampling of bacterial mats at the deep seafloor by the ROV *VICTOR 6000* (IFREMER).

## Discoveries in deep-sea microbial ecology

With the development of advanced instruments for observing, mapping and sampling the seabed, the perception of the deep seafloor has changed fundamentally from the one of a species-poor habitat to one that is rich in species (GAGE & TYLER, 1991). In respect to the microbial life at the deep seafloor the assumption was that the presence of microbial organisms was restricted to the thin surface layer of the planet, where the

organic matter derived from photosynthesis is present and available as an energy and nutrient source. With the Deep-Sea Drilling Project started in 1968, highly diverse core material from subsurface sediments and the ocean crust was collected over the past decades, precipitating a new perception of the ocean floor as a dynamic environment. The biological implication of this new viewpoint became strikingly clear with the discovery of unusually dense assemblages of benthic communities such as giant clams, tube worms and microbial mats associated with hydrothermal vents (BALLARD, 1977; LONDSDALE, 1977). A few years later, other chemosynthetic oasis such as mud volcanoes, gas chimneys and pockmarks or oil and asphalt seeps, summarised as cold seep ecosystems, were discovered in different regions (KENNICUTT, 1985; SUESS et al., 1985; KULM et al., 1986) which are characterised by the venting hydrocarbon that can be oxidised by a range of microorganisms using oxygen or sulphate as the terminal electron acceptor (WIDDEL et al., 2000).

Due to their slow *in situ* growth rate, most deep-sea microorganisms are extremely resistant to cultivation so that only about few hundred pure cultures are available. Or, in other words, only 0.01 to 0.1% of the identified marine bacteria are in culture today. The introduction of rRNA sequencing to identify marine bacteria revolutionised the understanding of these organisms and provided significant information on the phylogeny of marine taxa (PACE et al., 1985). Microbial ecogenomics (also called environmental genomics or metagenomics), direct environmental shotgun sequencing, genomics of cultured microbes and functional genomics, together with different microbial ecological methods such as microarrays, real-time PCR (Polymerase Chain Reaction), FISH (Fluorescence *in situ* Hybridisation), T-RFLP (Terminal Restriction Fragment Length Polymorphism) and microautoradiography contributed significantly to the understanding of the hitherto uncultivated microbes of the deep sea.

Despite progress in technology and methods, only a tiny fraction of the pattern and environmental factors that drive or influence microbial biodiversity at the seafloor is known. However, large projects such as the Census of Marine Life and the EU project HERMES (Hotspot Ecosystems on the Margins of European Seas) have been created to study the diversity of ocean life in general. The International Census of Marine Microbes was initiated to develop a strategy to catalogue all known diversity of single-cell organism (incl. bacteria, archaea, protista, and associated viruses). This programme concentrates on the exploration and discovery of unknown microbial diversity and the placement of that knowledge into appropriate and evolutionary context. Up to now, most of the studies aimed at the investigation of environmental

microbial biodiversity have been restricted to ocean waters. Hence, patterns in microbial biodiversity and environmental factor that drive microbial community structure and functioning at the ocean floor remain widely unexplained, although the necessary methods and technologies are available.

## Sources for organic matter to the deep seafloor and their effects on benthic microbial organisms

### The deep seafloor

The deep sea is the most extensive ecosystem on the planet; roughly 60% of the Earth's solid surface (more than 300 x $10^6$ $km^2$) lies beneath water depth exceeding 1000 m (GAGE & TYLER, 1991). By definition, the deep seafloor ranges between the shelf break (~200 m depth) and the bottom of the Challenger Deep (~11000 m; Fig. 4). The most important characteristic of the deep seafloor is low biological productivity and, except for hydrothermal vent communities, the energy for the deep-sea biota is ultimately derived from an attenuated 'rain' of organic matter from the upper water column (typically 1-10 g organic carbon $m^{-2}$ $y^{-1}$). The very low organic-energy flux, combined with low temperatures (-1 to +4°C) in the deep sea, yields communities characterised by relatively low rates of growth, respiration, reproduction, and generally low biomass (SMITH & DEMOPOULOS, 2003).

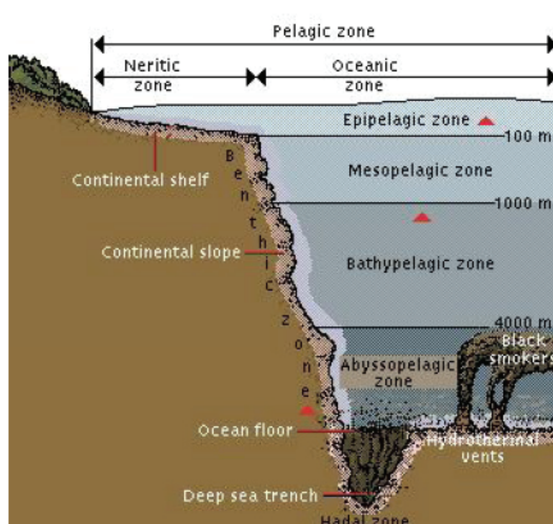



**Fig. 4.** Classification of marine depth zones (according to TARDENT (1993), and others).

The major part of organic matter input to the deep seafloor is derived from carbon production by photosynthesis in the upper water column, or lateral transport from continental shelves (ROWE & STARESINIC, 1979, Fig. 5). In high northern latitudes, ice coverage is the most relevant limiting factor for primary production during the Arctic summer. Ice melting during Arctic

spring and summer gives rise to a strongly stratified and nutrient-rich euphotic zone, with a distinct phytoplankton bloom. This bloom is typically short and constitutes a large fraction of the annual production, which ranges in the Arctic seas from 10 to 100 g C $m^{-2}$ (SUBBA RAO & PLATT, 1984). While sinking through the water column, organic material is altered through grazing by zooplankton, and degradation by microbial and chemical processes. Thus, concentration of the more labile, easily degradable organic compounds such as starch, proteins and lipids gradually decreases with increasing depth (HOPPE et al. 1993), and less than 10% of the primarily produced material reaches the deep-sea bottom in 4000 to 5000 m depth (WEFER, 1989) – compared to up to 50% of the primary production in coastal/shelf areas (WALSH et al., 1981).

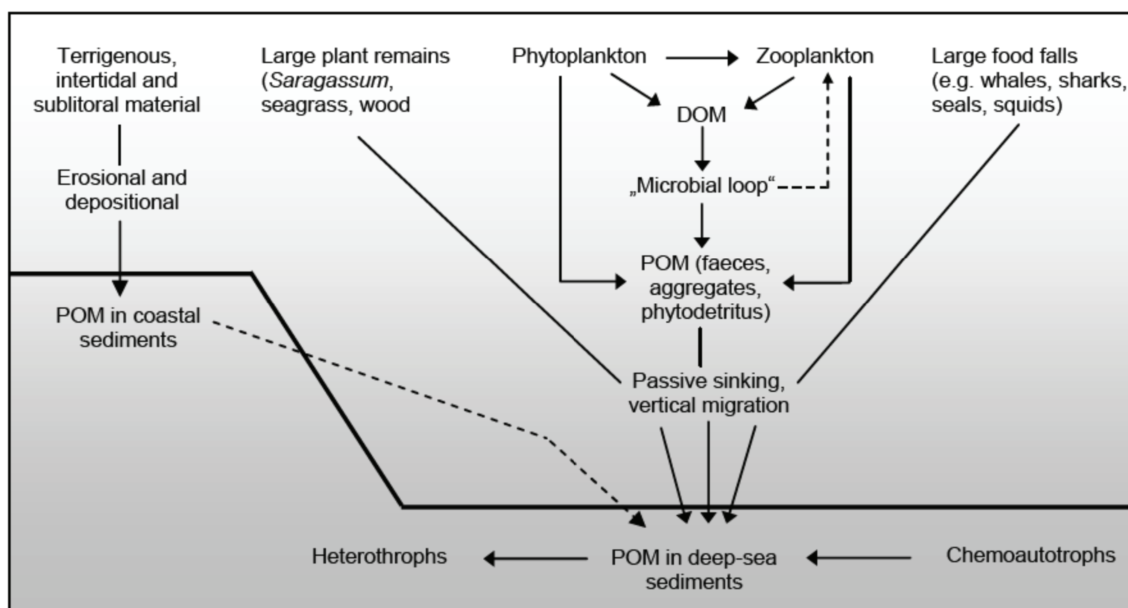



**Fig. 5.** Conceptual model of the potential sources, transport pathways, sinks and utilisation for organic matter in the deep sea (modified from ROWE & STARESINIC, 1979).

Total microbial biomass was found to correlate inversely, though poorly, with ocean depth, somewhat positively with the total organic carbon in sediments, but most strongly with the flux of particulate organic carbon (POC) to the seafloor (DEMING & BAROSS, 1992; DEMING & YAGER, 1992; KRÖNCKE et al., 1994). GOODAY & TURLEY (1990) described the deep sea as a food-limited environment in which the abundance, biomass and distribution of benthic organisms (meiofauna, macrofauna, and deposite-

feeding megafauna) is directly related to the amount and quality of food reaching the sediment surface. Benthic bacteria, which may contribute as much as 90% of the total benthic biomass (ROWE et al., 1991; TIETJEN, 1992; PFANNKUCHE, 1993), make no exception to this rule (DANOVARO et al., 2000), and are expected to live in a 'feast or famine' fashion (WIRSEN & JANNASCH, 1986). Seasonal events of massive sedimentation of phytoplankton detritus and other sources of pulse nutrient input necessitate fast reaction to this sudden nutrient supply, since there is a fierce competition for these available food resources. Therefore, deep-sea microbial organisms have to be able to increase their set of enzymatic 'tools' in order to utilise this resource. The efficiently and economic utilisation of sedimented POM is thus the key to survival of deep-sea bacteria (VETTER & DEMING, 1994).

### Input of phytodetrital material

In many areas of the world's oceans, food input to the deep sea occurs as large seasonal pulses of phytodetrital material (e.g. GRAF, 1989; PFANNKUCHE, 1993; SMITH et al., 1994). The first reports of mass sedimentation of organic matter came from the NE Atlantic (1370-4500 m depth), where phytodetritus (including intact diatoms and cyanobacteria) has been found to occur as green, cm-thick carpets (BILLETT et al., 1983; RICE et al., 1986) or as patches of green fluff concentrated in pits and animal tracks covering the bottom with 70 to 390 mg C $m^{-2}$ (THIEL et al., 1988), which is rather high compared to a total POC deposition of 1 g C $m^{-2}$ $yr^{-1}$ in the North Atlantic Ocean (HONJO & MAGANINI, 1993). Such large pulses can completely cover the seafloor (North Pacific; LAUERMAN & KAUFMANN, 1998 and equatorial Pacific; SMITH et al., 1996). Phytodetritus-like material has also been found to cover the continental slope, and fill burrows on the abyssal rise (NW Atlantic; ALLER & ALLER, 1986; HECKER, 1990). At most sites, primary production in overlaying waters is characterised by strong seasonality, and the seafloor accumulation of phytodetritus has been causally related to major flux events resulting from spring and/or summer phytoplankton blooms. The presence of relatively undegraded algal cells and viable cyanobacteria reflect its rapid sedimentation, of approximately four to six weeks, until reaching the benthos at 4000 m depth (LOCHTE, 1992), contrary to earlier assumptions of a slow and sparse rain reaching the deep-sea bottom throughout the year (RILEY, 1964).

Investigations on pelago-benthic coupling in the open ocean have shown many kinds of responses on phytodetritus supply: increasing biochemical activity (GRAF, 1989),

enhanced oxygen consumption of the sediment community (PFANNKUCHE, 1993; DRAZEN et al., 1998), stimulated feeding activity (BILLETT et al., 1983; GOODAY, 1988; LOCHTE & TURLEY, 1988; GOODAY & TURLEY, 1990), and increased microbial biomass (LOCHTE, 1992; PFANNKUCHE, 1993). A number of short-term studies based on incubation experiments with detrital matter had been performed to assess the response of deep-sea microorganisms on such organic matter supply. Samples were enriched with original phytodetritus (LOCHTE & TURLEY, 1988), artificial detritus (TURLEY & LOCHTE, 1990), and $^{14}C$-labelled microalgae (LOCHTE, 1992) to simulate inputs of phytodetritus. Overall, these studies indicated that benthic deep-sea microbial communities can react quickly within few days to such inputs of organic carbon to the sea bed by rapid colonisation, growth, and decomposition rates. It could be shown that bacterial growth on naturally occurring deep-sea phytodetritus is due to an adapted deep-sea population originating from the sea bed (TURLEY & LOCHTE, 1990). The use of labelled phytodetritus has proven to be a powerful tool in evaluating the potential of the deep-sea benthos to process labile carbon and to follow the fate of a phytodetritus pulse at the abyssal seafloor (BLAIR et al., 1996; LEVIN et al., 1997, 1999). Incubation experiments of POREMBA (1994) with deep-sea sediments and $^{14}C$-labelled microalgae as food source showed a 2-step kinetic of respiration of algae detritus probably caused by the initial attack on easily degradable compounds of the complex biological material (e.g. monomeric and oligomeric substances), and a later degradation of less labile components (e.g. macromolecules). During the initial rapid phase the conversion of organic carbon is highly efficient (60-80%), resulting in high biomass production and low microbial respiration. After this initial period the less effective utilisation of organic matter (10-20%) leads to lower biomass production and higher rates of respiration (TURLEY & LOCHTE, 1990). Hence, whereas approximately 1-2% of sedimented phytodetritus carbon is oxidised by benthic microorganisms within few days, remineralisation rates and efficiency of organic carbon conversion to bacterial biomass are dependent on the state of degradation of the organic matter reaching the deep seafloor. With several enrichment studies the assumption have been supported that microbial growth response is related rather to the quality or 'freshness' than quantity of organic matter (KÖSTER et al., 1991; BOETIUS & LOCHTE, 1994).

Temporal and quantitative links between 1) upper mixed-layer processes, 2) deposition of organic material, and 3) benthic response of natural communities, are very difficult to determine, because the observations, generally based on short-term experiments, are usually too limited and infrequent to observe the whole cycle of reactions. This led to

somehow contradictory results on bentho-pelagic coupling obtained from different studies (LOCHTE et al., 1993; SAYLES et al., 1994; SMITH & DRUFFEL, 1998; PFANNKUCHE et al., 1999; LOCHTE & PFANNKUCHE, 2002). Experimental enrichments with phytodetritus conducted directly at the seafloor gave new insights on the speed and reaction amplitudes of benthic organisms (Fig. 6).

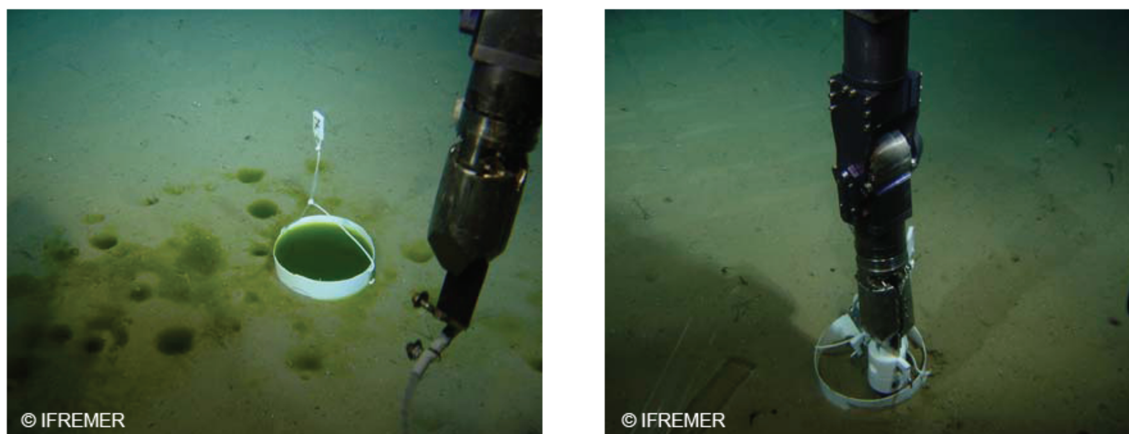



**Fig. 6.** Simulation of large phytodetritus input at the deep seafloor. Artificial detritus has been transferred by a tube into a plastic ring placed on the seafloor. After two weeks, sediments within and outside the ring were sampled by push cores with the ROV *VICTOR 6000* (IFREMER) for subsequently analysis of biotic and abiotic parameters in the sediments (SOLTWEDEL & SCHEWE, unpublished data).

Different studies were carried out to investigate the short-term response of benthic communities from different regions to the enrichment with labelled phytodetritus. (MOODLEY et al., 2002; WITTE et al., 2003a, b; BÜHRING et al., 2006). Results of MOODLEY et al. (2002) have shown that in deep-sea sediments from the NE Atlantic (2170 m water depth) the majority of the tracers was incorporated by bacteria as well as foraminifera, indicating the central role of these organisms in the rapid initial processing of fresh organic carbon in deep-sea sediments. Results of WITTE and colleagues (2003a) for the continental slope (1265 m water depth) showed that microorganisms in deeper sediment layers (below 5 cm) were also capable of very fast responses to POM, and emphasised the importance of vertical transport of 'fresh' POM mediated by larger organisms for biogeochemical cycling in deep-sea sediments. In addition, results of WITTE et al. (2003a) suggested that a large fraction of the material is

passed through the gut system of larger animals, where its quantity and quality are altered, before it becomes available for other (micro) organisms.
From previous studies there is growing evidence that indigenous deep-sea bacteria can react quickly to inputs of organic material other than ‘common’ phytodetritus (e.g GARDENER et al., 1983; DESBRUYÈRES et al., 1985). Important contributions to the energy supply into the deep sea are food falls. These can be of plant origin (e.g. kelp, seagrass) or include the bodies of large vertebrates, and large energy rich invertebrates such as cephalopods and crustaceans.

## Food falls of plant origin

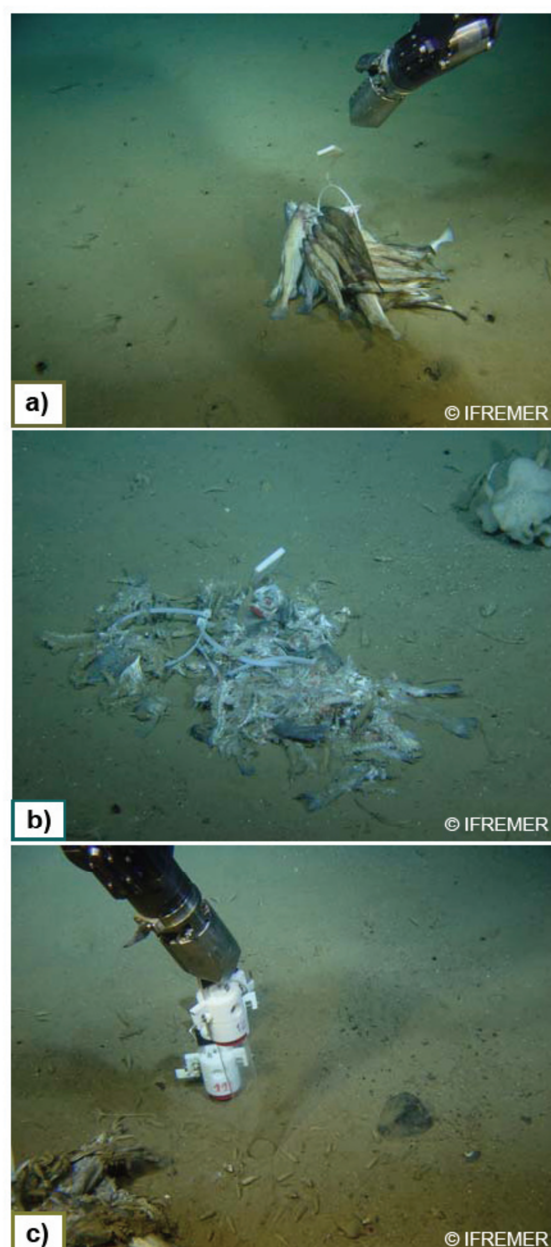
Macrophyte detritus removed from habitats such as seagrass meadows or kelp forest and transported offshore to the seafloor via bottom currents also play an important role as islands of concentrated organic nutrients on the deep seafloor (SUCHANECK et al., 1985; HARROLD et al., 1998). Forest of giant kelp, *Macrocystis pyrifera*, that line the rocky coasts of temperate seas throughout the world’s oceans are among the most productive aquatic communities on earth (HARROLD et al., 1998). Much of the production is exported as large parcels of macroalgal drift. Debris from seagrass such as *Thalassia* were observed in samples and bottom photographs within the Lisbon and Setubal canyon systems that probably originated from coastal lagoons and estuaries near the head of the canyons (GAGE & TAYLOR, 1991). Pelagic macrophyte (*Sargassum*) was found in considerable quantities on the ocean floor in the Central Pacific, the Sargasso and Caribbean Sea, the Gulf of Mexico, and NW Atlantic (Grassle & MORSE-PORTEOUS, 1987) transported into the deep sea by wind-induced circulations (JOHNSON & RICHARDSON, 1977).
Wood falls seem to be most common in deep-sea basins and trenches associated with island arcs, and areas with massive fluvial export (ALONGI, 1990). Its appearance in the deep sea may be followed by the rainy season in the tropics or the spring runoff in temperate latitudes where food is brought down to the sea by rivers, becomes waterlogged, and sinks to the seafloor (TURNER, 1973). The Arctic Ocean receives about 10% of the global continental fluvial water outflow and tree trunks or pieces of wood were observed several times during expeditions in the Fram Strait (KLAGES, pers. communication).
In recent studies, experimental kelp and wood emplacements in the California Borderland Basins region were used to investigate the extent of such massive organic

enrichments in deep-sea sediments (HANNIDES et al., 2005). Results have shown that in surface sediments around kelp and wood falls (up to 1 m distance) pore water sulphide and organic carbon were higher than in background sediments. The enrichment in kelp falls, however, peaked out in months, while wood falls only generated significant signals on the order of years (HANNIDES et al., 2005). In general, only few studies have focussed on microbial assemblages associated with large kelp and wood falls and the ecological importance of these large organic matter inputs to deep-sea benthic microorganisms is only beginning to be understood. Recently, long-term experiments with naturally and experimentally immersed sunken wood indicated a correlation between microbial cellulytic activity and cell numbers (PALACIOS et al., 2006).

### Food falls introduced by animal carcasses

Particular emphasis has been placed on the importance of organic-rich microenvironments, such as those provided by decaying bodies of marine mammals, fish or invertebrates reaching the deep seafloor. Nekton carcasses from fishes and birds (mesocarrion ~1 kg) to seals and dolphins (macrocarrion ~100 kg) and the largest cetaceans (megacarrion >100000 kg) comprise a fraction of total organic fallout (BRITTON & MORTON, 1994). These constitute point sources of organic input to the deep-sea benthos, and are of particular importance as a food source to large motile scavenging fauna (STOCKTON & DELACA, 1982 and references therein). Natural food falls have been simulated in the deep ocean worldwide using autonomous free-falling vehicles incorporating time-lapse camera systems and baited traps (WITTE, 1999; PRIEDE & BAGLEY, 2000) and elicit a very rapid response (e.g. within weeks) from this scavenging fauna (ISAACS & SCHWARZLOSE, 1975; THURSTON, 1979; SMITH et al., 1989; PREMKE et al., 2006). Parcels of fish (~50 kg cod) placed at the Arctic deep seafloor at 2500 m water depth showed, for example, that the fish bait was consumed almost completely within two weeks by invertebrate scavengers (Fig. 7; SOLTWEDEL & SCHEWE, unpublished data). The impact of those large food falls on surrounding sediment assemblages remains largely unevaluated. The enormous mass of soft tissue on a whale carcass, for example, is likely to yield intense organic enrichment of the ambient sediments in a generally organic-poor environment.

**Fig. 7.** (**a**) Deployment of a fish parcel (~50 kg cod) at 2500 m water depth by the ROV *VICTOR 6000*. (**b**) Two weeks after fish emplacements baits were almost completely decomposed. Remaining bones were colonised mainly by isopods (*Mesidothea megatura*) and amphipods (*Eurythenes gryllus*). (**c**) Sediment sampling by a push corer around the carcasses.




Previous studies have shown that sharp gradients in microbial activity exist within few meters of these falls, which are characterised by intense sulphate reduction and sulphide generation (NAGANUMA et al., 1996; HANNIDES et al., 2005). Sulphides are the typical products of anaerobic protein degradation (ALLISON, 1988) and were also produced by sulphate-reducing bacteria, utilising hydrogen produced through the anaerobic oxidation of lipids which is abundant in whale bones (SMITH, 1998). Overall, the phylogenetic resemblance of metazoans living in habitats around whale falls (and wood falls as well) with those of highly reduced deep-sea environments like hydrothermal vents and cold seeps (BACO et al., 1999), and the overlap in species among these habitats (SMITH & BACO, 2003; GLOVER et al., 2005) has led to the hypothesis that deep-sea organic rich matter deposits could play a major role in the adaptation and evolution of chemoautotrophic communities at the ocean basin (e.g. SMITH et al., 1998).

## Mass sedimentation of zooplankton and their role for chitin transport to the deep seafloor

Several authors reported abundant dead bodies of salps (Thaliacea: Tunicata) being transported into deep waters by currents that were probably associated with infrequent salp blooms (e.g. CACCHIONE et al., 1978). WIEBE et al. (1979) observed a vertical migration of massive numbers of salps in the NW Atlantic Ocean. They estimated that this organic matter reaching the deep seafloor might represent over 100% of the daily deep-sea benthic infauna energy requirement. Large numbers of the swimming crab *Charbdis smithii* (Crustacea: Decapoda) were detected by CHRISTIANSEN & BOETIUS (2000) in the deep northern Arabian Sea (~4000 m depth). According to the authors calculations, such large food falls may represent a significant carbon input of at least 10 – 30% of the annual POC flux as measured in sediment traps in this region. Mass sedimentation of large numbers of zooplankton was also observed in higher latitudes. SOKOLOVA (1994), for example, found high concentrations of Antarctic euphasiids at the deep-sea bed in the South Atlantic Ocean. German observation revealed numerous plankters on the top of sediment in grab samples in the Central Arctic (KROENKE, unpublished data). WĘSŁAWSKI & LEGEŻYŃSKA (1998) reported on a high number of dead zooplankton, mainly composed of crustaceans, in grab samples collected in the Kongsfjorden (79°N, Svalbard archipelago). Reasons for zooplankton non-consumptive mortality can be starvation, pollution, environmental stresses, harmful algal blooms and pathogens bacteria (e.g. HALL et al., 1995; GOMEZ-GUTIERREZ et al., 2003).

A large fraction of the carbon input by zooplankton to the deep seafloor is provided by chitin. Apart from the shells of crustaceans, sources of chitin also include exoskeletons of worms and molluscs, the egg shells of nematodes, cuttlefish bone and squid pen (GOODAY, 1990). Shedding of zooplankton carapaces and casing of their fecal pellets may also be an important source of chitin (YOSHIKOSHI & KÔ 1988). In addition, several common genera of diatoms, such as *Thalassiosira* and *Skeletonema*, produce chitin as a significant portion (up to 33%) of their biomass (SMUCKER & DAWSON 1986). Several million tons of chitinous material settle every year to the seafloor (CAUCHIE, 2002), mainly originating from the mass occurrence of krill in the Antarctic (SIEGEL, 2005) and copepods in the Arctic Ocean (HIRCHE, 1997). The highest production recorded in marine environments is associated with the hydrothermal vent vestimentiferan *Riftia pachyptila* (GAILL et al., 1997). Previous studies by SHILLITO et al. (1999) showed that the quantity of chitin generated by *Riftia* for building its tube could reach 100 times that

produced by other marine species. The benthic macrofauna of deep seas is dominated by peracarid crustaceans (CARTES & MAYNOU, 1998) whose chitin content can be estimated to about 7% of the whole body dry weight. On this basis, CAUCHIE (2002) used a mean value of 40 mg chitin $m^{-2}$ $yr^{-1}$ as an estimation of the combined chitin production by copepods, decapods, isopods and leptostracans in the oceanic benthos.

The oceans would be completely depleted of carbon and nitrogen in a matter of decades if this highly insoluble polysaccharide is not returned to the ecosystem in biologically useful form (KEYHANI & ROSEMAN, 1999). Studies have shown that recycling of chitin occurs both in the water column and sediments (GOODAY, 1990). Although other heterotrophic organisms may be capable to decompose chitin (LEE, 1980), the breakdown of such degradation-resistant organic matter is mainly accomplished by bacteria which are able to extracellularly cleave structural polymeric compounds. The rate of chitin degradation will be enhanced by phenomena of adherence of chitinolytic microflora and passage through animal guts. The importance of these processes is highlighted by the repeated finding of chitinolytic bacteria, principally of the genera *Vibrio* and *Photobacterium*, associated with zooplankton and particulate matter (e.g. HOOD & MEYERS, 1977). Estimation of population density of chitinolytic bacteria, both as total counts and as percentages of total heterotroph, have shown considerable variation, but consistently higher counts have been reported from marine sediments than from the overlying seawater (GOODAY, 1990 an references therein). DEMING (1985) suggested that a considerable portion of chitinous material is recycled while still in suspension and rapidly degrades in sediments, since oceans sediments contain only traces of chitin. POULICEK et al. (1986) presented estimates of chitin contents of deep-sea sediments showing no marked accumulations, values ranging from 60-160 µg (g decalcified sediment)$^{-1}$ being similar to those of shallow-sea sediments.

In spite of being recognised as most important biopolymer in marine environments (GOODAY, 1990), surprisingly few ecological studies have examined the effect of chitin on microbial communities in deep-sea sediments (e.g. Deming, 1985). The available studies based on laboratory enrichment experiments with deep-sea sediments have shown that natural microbial assemblages are able to produce high amounts of chitinolytic enzymes when chitin becomes available as a substrate, and they utilise this carbon and nitrogen source with relative high growth efficiency (BOETIUS & LOCHTE, 1996). Measurements of chitinolytic enzyme activities in sediments of the northern Arabian Sea after mass sedimentation of dead crabs at the seafloor showed values that were substantial higher than those detected at other times in this region as well as

those in oceans (CHRISTIANSEN & BOETIUS, 2000), suggesting that even huge chitin amounts as introduced by large food falls will be quickly remineralised.

In summary, all of such massive organic food falls have in common that they are unpredictable both in time and space. Therefore, they cannot be measured by conventional estimates of vertical flux but may be important in the supply and structuring of the deep-sea benthic communities. Studies on microbial community responses to changes of their environmental conditions such as food supply are most important, since they play a major role in the decomposition of organic material on the deep-sea bed (ROWE et al., 1991) being able to consume at least 13-30% of the total biological consumption of organic carbon (ROWE & DEMING, 1985). They can respond rapidly to the arrival of organic matter, producing enzymes that break it down to smaller fractions, which they can incorporate to fuel their metabolism. This process dominates the biogeochemistry of the sediment-water interface and, therefore, the rate and nature of what gets laid down in the sediments and what is provided for higher trophic levels as nutritional source.

Ecological studies of microbial communities that follow changes in composition and functioning resulting from organic substrate enrichments are of outstanding interest. Up to now, however, most of such studies were mainly based on investigations in marine shallow water or freshwater environments (e.g. RIEMANN & MIDDELBOE, 2002; KIRCHMANN et al., 2004) and thus, it is generally unclear how the structure and function of microbial communities in deep-sea sediments, adapted to steady-state conditions, is affected by environmental changes such as variations in POM supply, particularly in polar regions. The fact that Arctic regions figure most immediately and dramatically in scenarios of global warming makes the need for research all the more compelling.

Against this background, the present study focused on the microbial response to POM supply at high northern latitude deep-sea sediments with special emphasis on the enrichment with chitin. In a broader context the results are assumed to contribute to a better understanding of microbial community functioning and structural dynamics under deep-sea conditions. Hence, *in situ* experiments were carried out for different periods directly at the seafloor.

This thesis addressed the following scientific questions:

- How does Arctic deep-sea microbial community structure and functioning respond to the addition of chitin as one of the most important biopolymer in marine environments (manuscript I)?

- How do variations in sediment characteristics (grain size, grain shape, organic carbon content) influence the colonisation by Arctic deep-sea microbial assemblages under *in situ* conditions (manuscript II)?

- Are there any differences in microbial *in situ* response to POM inputs under different sediment conditions (manuscript II and III)?

# Material and Methods

## Study area

*In situ* studies were conducted at the polar deep-sea long-term observatory HAUSGARTEN in the eastern Fram Strait, west off Svalbard (Arctic Ocean). The HAUSGARTEN observatory was established in summer 1999 by the Alfred Wegener Institute for Polar and Marine Research (AWI) and includes 15 permanent sampling sites along a depth transect (1200 to 5500 m water depth) and along a latitudinal transect following the 2500 m water depth crossing the central HAUSGARTEN station (Fig. 8). This central station (79°5' N / 4°2' E) served as experimental area for the present investigations at the deep seafloor which were carried out during *RV Polarstern* cruises in Nordic summer seasons from 2003 to 2005.

Hydrographic conditions in the HAUSGARTEN area are characterised by relatively warm and nutrient-rich Atlantic Water transported by the West Spitsbergen current (WSC) into the central Arctic Ocean (MANLEY, 1995). In the western part of the Fram Strait, approximately 75% of the total Arctic Ocean water outflow is transported in a southerly direction within the East Greenland Current (EGC; MURRAY, 1998). This circulation patterns in the Fram Strait result in highly variable sea-ice cover, with permanent ice-covered areas in the west, permanent ice-free areas in the south-east,

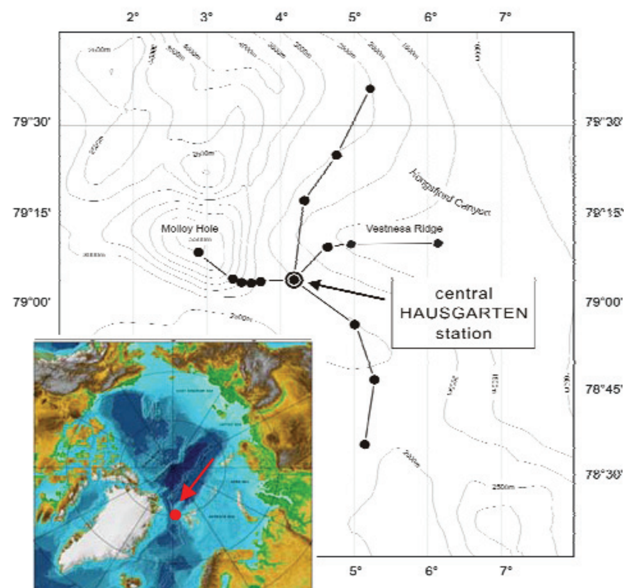



**Fig. 8.** Map of the deep-sea long-term observatory HAUSGARTEN in the Eastern Fram Strait, west off Svalbard (Arctic Ocean).

and seasonally varying conditions in the central and north-eastern parts, where the HAUSGARTEN area is located (SOLTWEDEL et al., 2005). Bioproduction and consequently the flux of POM to the seafloor at the HAUSGARTEN area are dependent on the position of the ice-edge and can vary considerably from year to year (SCHEWE & SOLTWEDEL, 2003). High primary production in the marginal ice zone (MIZ) of the Fram Strait supplies the benthos during spring and summer with high amounts of relative undegraded organic matter (mean primary productivity in summer is ~500 mg C $m^{-2}$ $d^{-1}$ [SMITH et al., 1987]), which is partly transported in a northerly direction under the ice within the WSC. Ice-edge-related POM in the western Fram Strait is transported by the EGC in a southerly direction into ice-free regions of the NW Greenland Sea.
At HAUSGARTEN, the bottom water temperature during the expeditions was about -0.8°C and bottom water oxygen concentration was 280 to 320 µmol $L^{-1}$. Mean bottom currents are relatively weak, ranging from 5.3 cm $s^{-1}$ to 6.6 cm $s^{-1}$ at 0.1 and 0.9 m above the bottom, respectively (SAUTER, unpublished data).

## Experimental design and methods

All results presented in this thesis are based on *in situ* incubation experiments using a Sediment Tray Free Vehicle (STFV) designed by the Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER, France). The STFV consists of an aluminium-framed tripod with four identical round trays (80 cm in diameter and 13 cm high; Fig. 9). Each tray was equipped with four separate, round PVC chambers (227 $cm^2$ per chamber). In order to prevent disturbance of the sediments inside the chambers, the trays were closed during deployment and recovery by means of a remotely controlled mechanism: Before the deployment, the trays were closed by a drilled disc (Fig. 9, I). After about fifteen hours at the seafloor, a magnesium pin was electrolysed, allowing the disc to revolve under the action of a coil spring. A bolt resting on the expendable weight was blocked and sediment trays were in the 'open' position (Fig. 9, II). For recovering the STFV, the expendable weight was released by an automatic acoustic command from the ship and due to the positive buoyancy the STFV was transported to the surface. At the same time, the bolt was resolved, and the drilled disc already closed the chambers inside the trays (Fig. 9, III).

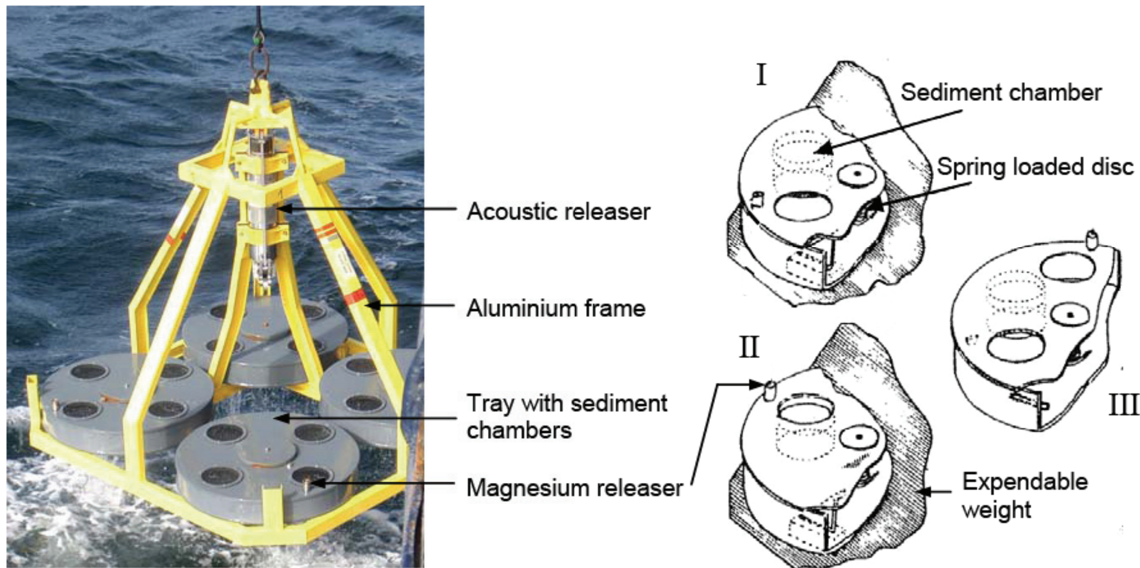



**Fig. 9.** Sediment Tray Free Vehicle (STFV) composed of an aluminium frame and four trays, each of which is equipped with four chambers. The sequence I-III shows the mechanism to open and close the trays by a rotating disc (modified from DESBRUYÈRES et al., 1980).

For the different incubation experiments, chambers in the sediment trays were prepared as described below:

### Experiment I

Deep-sea sediment from the central HAUSGARTEN station was enriched with a 'low', 'medium' and 'high' chitin concentration corresponding to 1.3, 3.0 and 7.0 g $m^{-2}$ chitin (purified chitin flakes from crab shells, Sigma-Aldrich, Germany), respectively. Because of logistic constrains, the experiment was carried out for a short (seven days) and a longer (one year) incubation period.

### Experiment II

Natural and artificial sediments were chosen to provide different sediment types ranging from fine (deep-sea sediments; particle size < 63 µm), medium (uniform glass beads; diameter 100 to 200 µm), and coarse (sand; large, rounded and sub-rounded particle with a mean grain size of 1 to 2 mm) sediments (Fig. 10). In addition to the deployment of one tray with 'pure' sediment types, we used a second tray containing the different sediments enriched with chitin (3 g $m^{-2}$). Similar to the first experiment, incubation took place for a short (seven days) and a longer time period (one year).

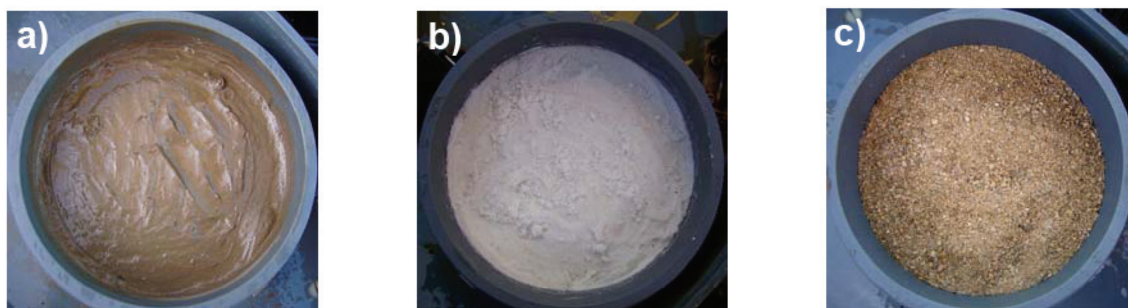



**Fig. 10.** Natural and artificial sediments filled in the chambers of the trays. (**a**) Deep-sea sediments from the HAUSGARTEN central station (<63 µm grain size). (**b**) Glass microbeads (diameter 200 µm). (**c**) Coarse sandy sediments (mean grain size 1 to 2 mm).

### Experiment III

Deep-sea sediments, glass beads, and sand were enriched with cultured microalgae, yeast, and fish carcass, respectively (see Fig. 1 in manuscript III). In contrast to the other *in situ* studies, first sampling took place three months after the tray deployment. Second sampling of sediment trays was carried out also after a longer incubation period for one year.

Detailed description of sediment preparation (e.g. defaunation, organic substrate concentration), sediment sampling and sample processing is given in the respective manuscripts.

The following parameters were determined from all three experiments:

- Total microbial cell number and biomass was analysed by epifluorescence microscopy after staining with acridine orange according to the methods of MEYER-REIL (1983) and GROSSMANN & REICHARDT (1991).
- Terminal restriction fragment length polymorphism analysis (T-RFLP, LIU et al. 1997) was used to investigate microbial community composition at the different stages of the experiments.

In addition to that:

- Extracellular enzymatic activity of chitobiase was measured in experiment I and II (manuscript I, II) according to the method of BOETIUS & LOCHTE (1994).

- The organic carbon content of sediments at different incubation stages was determined by using an Elemental Analyser (EuroVector, Milan, Italy) in experiment II (manuscript II).
- Hydrolytic activity potential of microbial organisms particularly involved in the primary decomposition of organic matter was analysed in samples from the third experiment (manuscript III) by using the fluorogenic model substrate fluoresceinediacetate (MEYER-REIL & KÖSTER, 1992).

# Manuscripts

Three manuscripts developed in the context of this thesis and written by me as first author are based on the performance of *in situ* experiments at the deep seafloor during three expeditions with *RV Polarstern*.

## Manuscript I (experiment I)

**Kanzog C**, Ramette A, Quéric N-V, Klages M (submitted). Response of benthic microbial communities to chitin enrichment: an *in situ* study in the deep Arctic Ocean.

Division of work: I developed the idea and carried out all practical work and statistical analysis (together with 2nd author). Laboratory work (i.e. molecular methods) was supported by the 3rd author. I wrote the manuscript in discussion with the 2nd author and later with the 4th author.

Context: The first manuscript describes the role of chitin as potential food source for microbial communities in high northern latitude deep-sea sediments. Microbial reaction to large chitin supply has been studies after a short (seven days) and longer (one year) incubation period at the seafloor. We asked whether higher amounts of chitin introduced into natural sediments lead to an increased microbial growth and activity, and possibly to significant changes in bacterial community composition.

## Manuscript II (experiment II)

**Kanzog C**, Ramette A (submitted). Microbial colonisation of artificial and deep-sea sediments in the Arctic Ocean.

Division of work: I conducted the sampling and all the laboratory work. The2nd author did the major part of statistical analysis. I had the lead in drafting and writing the manuscript which was discussed with the 2nd author.

Context: The second manuscript deals in general with the effect of natural (fine-grained deep-sea sediment) and artificial sediments (glass beads, sand) on the colonisation process by Arctic deep-sea microbial organisms during a short (seven days) and a longer (one year) period at the seafloor. Chitin was concomitantly added to these sediments to assess microbial response to high carbon input. In general, the study presented in this manuscript disentangled the effects of multiple factors (sediment type,

chitin addition, and incubation time) and of their interactions on abundance, biomass, activity and community structure of microbial communities in the deep Arctic Ocean.

Manuscript III (experiment III)

**Kanzog C**, Schewe I (manuscript). Microbial responses to large POM supply in natural and artificial sediments at the Arctic deep seafloor.

Division of work: The experimental set-up was designed by the 2nd author. Sampling was carried out by both authors. I did the data analysis and wrote the manuscript in discussion with the 2nd author.

Context: The third manuscript describes microbial response to various large food deposits after two different incubation periods (three months and one year) at the seafloor. Furthermore, the relevancy of differences in sediment quality (i.e. sediment particle size, particle shape, organic carbon content) on microbial responses to these organic enrichments was investigated. The third manuscript is linked to the other manuscripts both methodically and content-based and some further important data (e.g. meio- and macrofauna parameter) will be included into this preliminary version before it is ready to submission.

# Manuscript I

## Response of benthic microbial communities to chitin enrichment: an *in situ* study in the deep Arctic Ocean

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Keywords: chitin; deep-sea sediments; microbial communities

## Abstract

*In situ* enrichment experiments were carried out in the Arctic deep sea to test the response of sediment-inhabiting microbial communities to chitin supply. Chambers of a Sediment Tray Free Vehicle were filled with natural deep-sea surface sediment enriched with 1.3 to 7.0 g $m^{-2}$ of chitin. The effects of chitin enrichment were assessed on the microbial hydrolytic activity potential, cell number and community structure at two sampling times (seven days and one year). The input of chitin had no effect on microbial abundance and chitobiase activity after seven days of incubation, whereas community structure in enriched sediments, determined by terminal restriction fragment length polymorphism analysis of 16S rRNA genes, was different from the controls. After one year, microbial numbers and activity significantly increased in sediments enriched with high chitin concentrations and bacterial community structure was different from that of non-enriched sediments or of sediments subjected to shorter incubation time. The present study suggests that microbial communities in Arctic deep-sea sediments are significantly affected by chitin input into the sediments and initial changes in community structure appear to be precondition for subsequent enhanced growth and activity following large chitin input.

## Introduction

Sinking particulate organic matter (POM) that reaches the deep seafloor mainly consists of less degradable material including cellulose, chitin and various proteinaceous structural compounds. As a major component of cuticles and exoskeletons of worms, molluscs and arthropods, chitin is probably the most important biopolymer in marine environments (GOODAY, 1990). Although other organisms may be able to decompose chitin, the breakdown of such degradation-resistant organic matter deposited in deep-sea sediment surfaces is mainly accomplished by bacteria which are able to extracellularly cleave structural polymeric compounds (DEMING & BAROSS, 1993).

A variety of time course experiments to test the functional responses of benthic deep-sea bacteria to chitin input have been performed [e.g. North Atlantic (DEMING, 1985); Arctic continental slope (BOETIUS & LOCHTE, 1996); Arabian Sea (CHRISTIANSEN & BOETIUS, 2000)]. These studies indicated that chitin supplies induced the synthesis of specific enzymes and that indigenous deep-sea bacteria were able to decompose even high amounts of it. Previous experiments, however, were conducted under laboratory

conditions and did not provide detailed information on the variation in microbial community over periods longer than weeks. *In situ* experiments focussing on microbial response to organic enrichments in the deep-sea are scarce (e.g. MOODLEY et al., 2002; BÜHRING et al., 2006), especially in polar regions.

Studies have reported mass sedimentation events of crustaceans in different ocean regions (SOKOLOVA, 1994; CHRISTIANSEN & BOETIUS, 2000), which represent a high input of chitinous material. The fact that high concentrations of the Arctic copepod *Calanus hyperboreus* were repeatedly observed over the ocean floor of the Greenland Sea (HIRCHE et al. 2006) suggests that high amounts of chitinous material would be naturally available when this large aggregation of organisms dies or moults. In addition, shifts in the distribution and abundance of key copepod species from the Arctic Ocean, possible caused by environmental changes associated with climate changes, may also contribute to enhanced chitin input to the deep seafloor (HIRCHE & KOSOBOKOVA, 2007). Therefore, investigations are necessary to better understand the effects of chitin input on deep-sea microbial assemblages. Here, for the first time, short-term (seven days) and long-term (one year) incubation experiments were carried out *in situ* to assess the effects of varying concentrations of chitin on benthic deep-sea microbial communities. We tested the hypothesis that a higher amount of chitin introduced into the sediments leads to increased microbial growth, activity and possibly to significant changes in bacterial community structure.

## Material and methods

*Experimental design*

A Sediment Tray Free Vehicle (STFV), containing deep-sea sediments enriched with different chitin concentrations, was deployed for seven days and one year at 2500 m water depth in the long-term observatory HAUSGARTEN (79°5' N / 4°2' E), in the eastern Fram Strait, west off Svalbard (SOLTWEDEL et al., 2005). The STFV was designed by the Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) and consisted of four identical trays (Fig. 1), each of which was equipped with four separate chambers (0.02 $m^2$). Only one tray was used for our enrichment experiments.

Natural deep-sea sediments from the central HAUSGARTEN station were collected with a multiple corer (MUC; BARNETT et al., 1984) that was composed of a mixture of 37% sand (particle size 63-2000 µm), 43% silt (4-63 µm) and 20% clay (<4 µm). Surface sediments (0-3 cm) of several sediment cores were pooled and transferred into

all four lander chambers. Defaunation was obtained by freezing sediments at -30°C for 48 h. Following sediment freezing and subsequent thawing, samples were taken for initial analysis of microbial parameters (0 day).

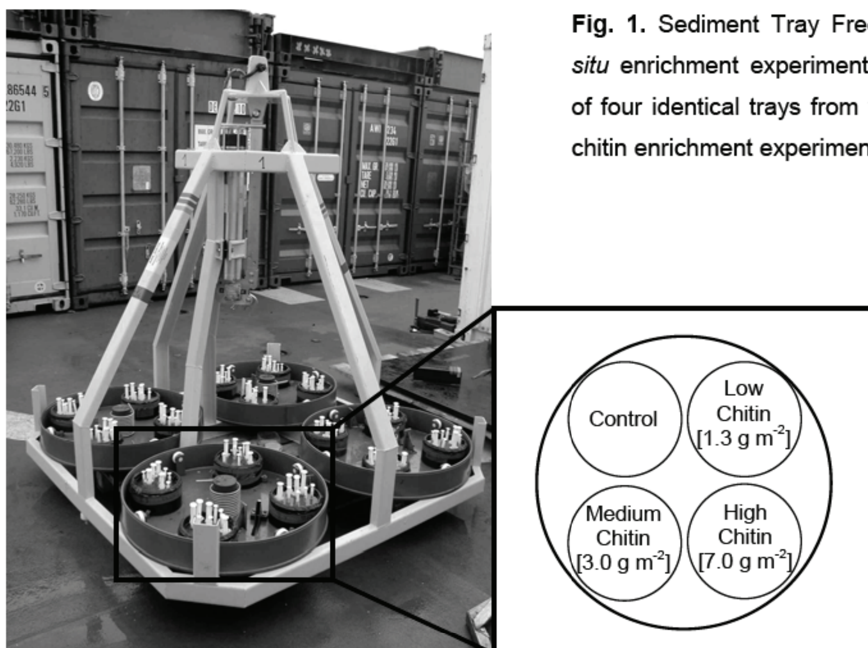


**Fig. 1.** Sediment Tray Free Vehicle (STFV) for *in situ* enrichment experiments. The lander consisted of four identical trays from which one was used for chitin enrichment experiments.

Afterwards, a 'low', 'medium' and 'high' chitin concentration corresponding to 1.3, 3.0 and 7.0 g $m^{-2}$ chitin (purified chitin flakes from crab shells, Sigma-Aldrich, Germany), respectively, were mixed with the upper layers of the sediments placed in the chambers. The additions were equivalent to a substrate input of 0.5 to 3.0 g C $m^{-2}$, which are well above the background value of chitin in deep-sea sediments of 0.1 mg $m^{-2}$ (POULICEK & JEUNIAUX, 1989). A control treatment consisted of one chamber filled with deep-sea sediments, without any addition of organic substrate.

*Sub-sampling and sample processing*

During *RV Polarstern* cruise ARK XX/1 in July 2004, the STFV was deployed for seven days. After recovery and subsequently sampling, trays were renewed and deployed at the same station for one year. Sampling of the one-year experiment took place during RV *Polarstern* expedition ARK XXI/1b in July 2005. After each recovery of the trays, eight samples from the first centimetre of sediments in each chamber were taken by using plastic syringes with cut-off ends (2.5 cm in diameter; Fig. 1). Sub-sampling was carried out in a room at 4°C. Sediments from each chamber were pooled and

homogenised before determining the abundance, enzymatic activity and changes in bacterial community structure.

*Microbial biomass*

For total microbial cell numbers and biomass determination, 2 ml of the sediment sub-sample (2.3 $cm^3$) were taken by means of plastic syringes with cut-off ends (1.2 cm in diameter) and stored refrigerated in 9 ml 2% formaldehyde solution. After staining with acridine orange, total cell counts were determined by epifluorescence microscopy according to the method of MEYER-REIL (1983). Volumetric measurements of the microbial cells were conducted with the New Porton grid, measuring randomly 50 cells per filter as described by GROSSMANN & REICHARDT (1991). Microbial biomass was estimated using a conversion factor of $3 \times 10^{-13}$ g C $\mu m^{-3}$ (BØRSHEIM et al., 1990). For each sample 40 counted grids from two replicate filters were analysed.

*Enzyme activity*

To assess the chitinolytic capability of microbial organisms, we used the enzyme assay for chitobiase activity, providing a simple and sensitive determination of extracellular enzymatic hydrolysis rates after relatively short incubation time and adaptable at low temperature (HOPPE, 1983). The extracellular enzymatic activity of chitobiase was measured fluorometrically using the methylumbelliferone (MUF) labelled substrate *N*-acetyl-$\beta$-glucosaminide (Sigma-Aldrich) according to BOETIUS & LOCHTE (1994). Previous trials to determine the substrate saturation level gave a final concentration of 100 µmol MUF-substrate. Relative units of fluorescence were calibrated with seven MUF standard concentrations between 10 and 300 nmol. The enzyme activity was calculated per volume of sediment and time (µmol $cm^{-3}$ $h^{-1}$) by linear regression. For each sample three measurements per incubation were performed.

*Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis*

Sediment samples for microbial community analysis were stored at -30°C until analysis. Total DNA was extracted and purified using a FastDNA®SPIN Kit for soil (Qbiogene, Heidelberg, Germany) following manufacturer's instructions.

Analysis of community structure focussed exclusively on bacterial groups since they are one of the most important agents in the mineralisation of chitin (GOODAY, 1990). PCR amplification (0.25 µmol 27F-FAM/1492R, 1.5 mmol KCl, 10 mmol Tris-HCl pH 9, 1.5 mmol $MgCl_2$, 250 µmol dNTP, 1.25 U *Taq*-Polymerase) was performed by using the following cycling conditions: a 3-min hot start at 95°C, followed by 29 cycles consisting

of denaturation (1 min at 95°C), annealing (1 min at 55°C), and extension (1 min at 72°C). A final extension at 72°C was then done for 7 min. Fluorescently labelled PCR products were run onto 1% agarose gel and purified using QIAquick Gel Extraction kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Restriction digest (50-µl) were performed with *Hha*I and *Msp*I (10 U; Amersham Pharmacia Biotech) at 37°C for 5 h, desalted (isopropanol 100%) and dried prior to storage at -20°C. Fragment separation was performed by Gene Analysis Service GmbH (Berlin, Germany) using an ABI Prism 310 capillary analyser (PE Applied Biosystems, CA, USA). Peak size determination of end-labelled fragments, considered here as different Operational Taxonomic Units (OTUs), was done by comparison with an internal size standard (GS2500 TAMRA, PE Applied Biosystems). Peaks between 50 and 1000 base pairs long were analysed using Genescan analytic software 2.02 (ABI). An additional check for artefacts was manually performed and peaks whose areas were smaller than 1% of the total peak area were excluded from data analysis. Due to the limited amount of available material, T-RFLP analyses were done once for each treatment.

*Data analysis*

Microbial cell number and chitobiase activity were $\log_{10}$-transformed prior to performing statistical analyses in order to normalise their distribution and homogenise treatments variances (Crochan test of homogeneity, SOKAL & RHOLF, 1995). Analysis of variance (ANOVA) was applied to each data set to test the effects of chitin input and incubation time, and of their interactions. *Post-hoc* comparisons between treatment means were done using Tukey's Honestly Significant Difference (HSD) test at $P < 0.05$.

T-RFLP fingerprints obtained from the two different restriction digests were combined to generate a binary matrix according to the presence or absence of bacterial OTUs. The unweighted pair group with arithmetic mean (UPGMA) method was applied with the Dice similarity index (DICE, 1945). Nodal support in the resulting dendrogram was determined by performing 100 bootstrap replicates (Hammer *et al.*, 2001). In addition, non-metric multidimensional scaling (NMDS; SCHIFFMAN & REYNOLDS, 1981) was used to obtain an ordination of the samples based on a matrix of Dice dissimilarities of T-RFLP profiles. A canonical redundancy analysis (RDA) was used to determine how well different factors could explain the variation in community structure, and in order to assess the respective importance of the different factors and of their covariation on shaping community structure, a variation partitioning procedure (RAMETTE, 2007) was subsequently applied to the community data. Statistical analyses were performed with

the statistical software JMP IN version 5.1 (SAS Institute, Cary, NC) and with the R package (R version 2.4.0; The R foundation for Statistical Computing).

## Results and discussion

*Effects of chitin addition on microbial abundance and activity*

Changes in microbial biomass were mainly explained by variation in microbial cell numbers and less by changes in cell volume (data not shown), thus we mainly focussed our analysis on variations in microbial cell numbers. Each factor taken alone (chitin input and incubation time) as well as the interaction between factors had highly significant effects on microbial abundance and chitobiase activity (Table 1). In sediments incubated for one week, regardless of the chitin amounts added, no significant changes in microbial abundance were observed and initial chitobiase activity also remained constantly low in all treatments (Fig. 2a, b). These results indicate that within one week, a pulse input of chitin to the seafloor has no effect on microbial cell numbers and chitobiase activity. These observations are corroborated by other enrichment studies that showed time lags of the microbial response to nutrient pulses of up to two weeks (e.g. TURLEY & LOCHTE, 1990; BOETIUS & LOCHTE, 1994). More specifically, BOETIUS & LOCHTE (1996) detected an increase in chitobiase activity ten days after chitin addition. Investigations of DEMING (1985) with deep-sea sediments amended with chitin showed that bacterial doubling time were in the order of weeks or months.

**Table 1.** Analysis of variance of microbial abundance and activity as a function of chitin input and incubation time.

| Source | Sum of squares | df[b] | Mean square | *F* ratio[c] |
|---|---|---|---|---|
| *Microbial cell number*[a] | | | | |
| Chitin input | 0.51 | 3 | 0.17 | 153.404*** |
| Incubation time | 1.313 | 2 | 0.656 | 592.487*** |
| Chitin input × Incubation time | 0.409 | 6 | 0.068 | 61.537*** |
| | | | | |
| *Chitobiase activity* | | | | |
| Chitin input | 21.252 | 3 | 7.084 | 201.375*** |
| Incubation time | 21.617 | 2 | 10.808 | 307.248*** |
| Chitin input × Incubation time | 42.598 | 6 | 7.1 | 201.822*** |

[a]The total cell number was $\log_{10}$ transformed prior to performing statistical analyses to normalize its distribution. [b]Degrees of freedom. [c]The *F* ratios were all highly significant with $P < 0.001$ (***).

Thus, bacterial growth is sufficiently slow to preclude detection of significant increases in cell number after seven days of incubation. The one-year incubation led to a substantial increase of microbial cell numbers associated with increasing chitin concentrations (Fig. 2a). Total cell counts in sediments incubated with the highest (7.0 g $m^{-2}$) and medium (3.0 g $m^{-2}$) chitin amount were, respectively, about nine and five times higher than prior to incubation, whereas in low chitin enrichments (1.3 g $m^{-2}$) only a marginal increase was found. Similar trends were recorded for microbial chitobiase activity (Fig. 2b). Whereas the activity levels in control samples and sediments enriched with low chitin amounts were not significantly different from the short-term incubation, four-fold and 30-fold activity increases were found in medium and high treatments, respectively (Fig. 2b).

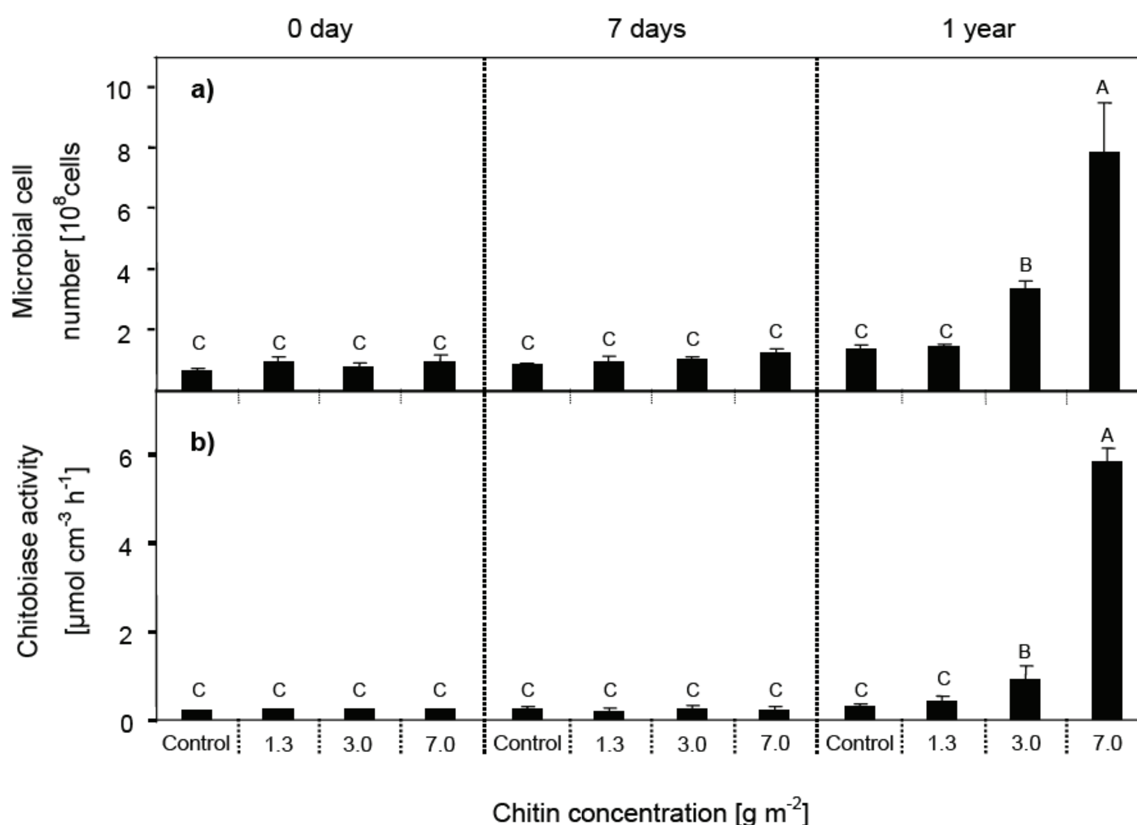


**Fig. 2.** Microbial cell number (**a**) and chitobiase activity (**b**) in control and chitin enriched sediments after seven-day and one-year incubations. Bars represent mean values ± SD calculated from replicate samples. Different letters (A, B, C) above the bars indicate significant differences between mean treatments as determined by *post hoc* Tukey's HSD tests at $P < 0.05$.

To estimate changes in activity potential that were not related to variation in microbial biomass, chitobiase activity was normalised by the total cell number. The highest chitobiase activity per cell was measured in sediments with highest chitin input after one year (7.5 $10^{-18}$ mol $cell^{-1}$ $h^{-1}$), which was approximately three times higher than in the other incubations at the same time. Previous investigations on microbial activity in continental slope sediments have shown positive correlations between chitobiase and organic matter supply to the benthos (VETTER & DEMING, 1994; BOETIUS & LOCHTE, 1996). Our results showed that the highest chitin amount of 7.0 g $m^{-2}$ can induce increasing specific chitobiase activity to the same extent as what was measured only a couple of weeks after sedimentation of a large numbers of crab carcasses by CHRISTIANSEN & BOETIUS (2000). This chitin amount of 7.0 g $m^{-2}$ is larger than the chitin input that may result from the sedimentation of one crab in a square metre of sediment (about 1.0 g; CHRISTIANSEN & BOETIUS, 2000), and much more than the background chitin value in deep-sea sediments of 0.1 mg $m^{-2}$ (POULICEK & JEUNIAUX, 1989). Hence, we suggest that, if a large amount of chitinous material naturally becomes available in deep-sea areas, as would be potentially produced by mass sedimentation of Arctic copepods for example (HIRCHE et al., 2006), benthic microbial communities may react by a significant increase in their cell numbers and chitinolytic enzyme production.
To assess the substrate conversion by microbial organisms, we divided the biomass yield after one year by the organic carbon amount introduced by the chitin concentrations. Results indicated that with increasing chitin-carbon supply about 6%, 26% and 31% were converted into microbial biomass after one year. It is important to note that microbial biomass could have also been actually higher than what we found, since meiofaunal and microeucaryotic grazers were not completely avoided by the 5 mm mesh size covering the trays. It is also likely that additional organic materials may have been introduced from the water column to the sediment trays during one year. This effect, however, contributed only marginally to changes in microbial biomass, as suggested by the only small variations in microbial abundance detected in control sediments (Fig. 2a).

*Shifts in bacterial community structure as a response to chitin inputs*

Cluster analysis of T-RFLP data indicated the existence of three well-defined clusters (Fig. 3), which were consistent with differences in incubation times. Within each of these clusters, communities in medium and high chitin treatments were more similar to each other than the communities from the low chitin treatment. In addition, bacterial

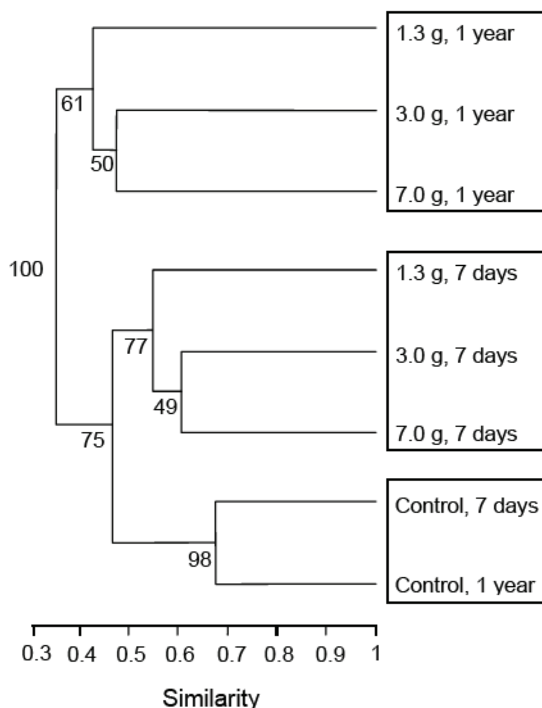


**Fig. 3.** Cluster analysis of T-RFLP data. The UPGMA dendrogram was constructed based on OTU presence/absence data obtained by T-RFLP analysis of the different sediment samples. Nodal support was assessed by 100 bootstrap replicates.

community structure was found to be gradually more dissimilar over the course of the experiment, suggesting that chitin had a time-dependent effect on the resident bacterial communities. Indeed, sample points were found to be more spread in the two-dimensional NMDS plot with increasing incubation time, indicating more variability in community structure (Fig. 4). This observation was confirmed by redundancy analysis that revealed that chitin input and incubation time together significantly contributed to the variation in community patterns ($P$ = 0.031 based on 1000 Monte Carlo permutations). Further variation partitioning of the biological variation into the respective effects of the two factors indicated that chitin input was the most important factor and explained about twice as much biological variation (17%) as duration of incubation (9%; data not shown). The total amount of unexplained variation was about 80%, which yet suggests that unmeasured factors would also need to be added in the future to the ecological model to better understand community dynamics in deep-sea sediments. Several studies have already shown that microbial communities are able to respond within short time to variations in the quality and quantity of organic matter supply by changes in their composition (RIEMANN et al., 2000; EILER et al. 2003). These short-term shifts, however, were generally accompanied by a functional response of bacterial communities. Interestingly here, changes in community structure that were detected after one week occurred without concomitant changes in microbial growth and activity. Few studies have evidenced that metabolic change following alterations of environmental conditions (e.g. substrate amendments) may occur without simultaneous changes in bacterial community

structure (RIEMANN & MIDDELBOE, 2002; FINDLAY & SINSABAUGH, 2003). In contrast, mesocosm experiments conducted with manipulated dissolved organic matter (DOM) concentrations in freshwater environments (JUDD et al., 2006) demonstrated that effects of changing DOM supply on bacterial productivity were mediated by initial shifts in community structure. Similarly, our results suggest that for deep-sea sediments, a functional response of microbial communities following a large chitin input may be triggered by an initial change in community structure before efficient utilisation of chitin compounds can be made. It may be also envisaged that the selection of specific populations (e.g. efficient chitin degraders) may accelerate over time as a function of the amount of carbon entering into the system.

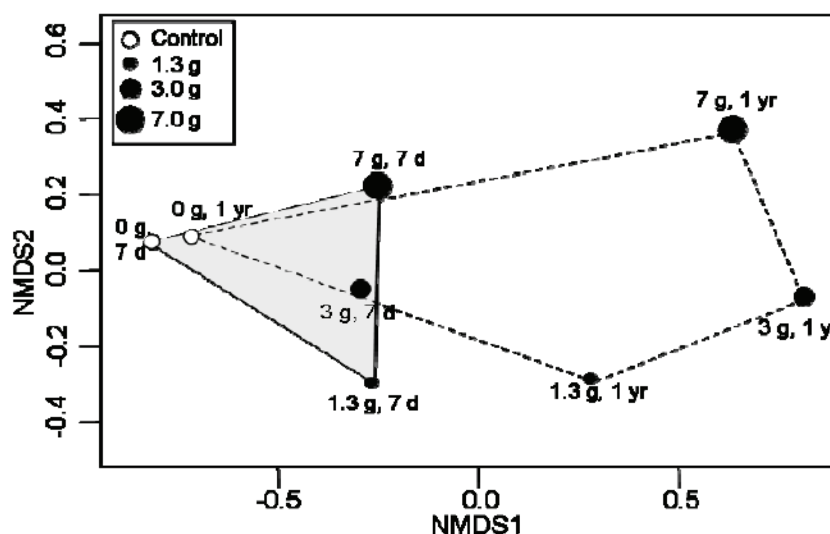


**Fig. 5.** Non-metric multidimensional scaling plot of T-RFLP profiles. The overall stress of the ordination (i.e. how well the Dice dissimilarity matrix fits the two-dimensional space) was 3.9%, indicating that the representation of the original matrix in the ordination space was optimal. Samples corresponding to one-week and one-year incubation times are connected by a continuous line and a dashed line, respectively.

Our results evidenced clear chitin-dependent responses of benthic microbial communities in the deep Arctic Ocean and underline their important role in recycling this highly insoluble organic substrate, although further experiments in other deep-sea regions and greater replication are needed to establish the generality of these findings. To our knowledge, this is the first *in situ* study that describes such phenomenon and that also considers microbial abundance, activity potential, and changes in community structure over a long-term period. Future investigations will be dedicated to the

assessment of the fate and rate of chitin degradation by using radiolabeled substrates. Furthermore, molecular analyses of the community composition of those different samples are needed to identify the microbes that are conjointly enriched under high chitin inputs and thus particularly involved in recycling this highly insoluble organic substrate and maintenance of ecosystem balance.

## Acknowledgements

We gratefully like to thank the ship officers and crew of the German research-icebreaker *Polarstern* for their help during the expeditions to HAUSGARTEN in summers 2004 and 2005. Experiments with our free-falling benthic lander would not have been possible without the support of J. Wegner and I. Schewe. We are also indebted to I. Kolar for assistance with the sample preparation, biochemical analyses. We wish to thank A. Boetius, Gustavo Fonseca and Fabiane Gallucci for useful comments and discussions.

## References

Barnett PRO, Watson J & Conelly D (1984) A multiple corer for taking virtually undisturbed samples from shelf, bathyal and abyssal sediments. *Oceanol Acta* **7**: 399-408.

Boetius A & Lochte K (1994) Regulation of microbial enzymatic degradation of organic matter in deep-sea sediments. *Mar Ecol Prog Ser* **104**: 299-307.

Boetius A & Lochte K (1996) Effect of organic enrichments on hydrolytic potentials and growth of bacteria in deep-sea sediments. *Mar Ecol Prog Ser* **140**: 239-250.

Børsheim KY, Bratbak G & Heldal M (1990) Enumeration and biomass estimation of planktonic bacteria and viruses by transmission electron microscopy. *Appl Environ Microbiol* **56**: 352-356.

Bühring SI, Lampadariou N, Moodley L, Tselepides A & Witte U (2006) Benthic microbial and whole-community response to different amounts of $^{13}$C-enriched algae: *In situ* experiments in the deep Cretan Sea (Eastern Mediterranean). *Limnol Oceanogr* **51**: 157-165.

Christiansen B & Boetius A (2000) Mass sedimentation of the swimming crab *Charybdis smithii* (Crustacea: Decapoda) in the deep Arabian Sea. *Deep Sea Res II* **47**: 2673-2685.

Deming JW (1985) Bacterial growth in deep-sea sediment trap and boxcore samples. *Mar Ecol Prog Ser.* **25**:305-312.

Deming JW & Baross JA (1993) The early diagenesis of organic matter: bacterial activity. Organic geochemistry: principles and application (Engel MH, Macko SA, eds.), pp. 119-144. Plenum Press, New York.

Dice LR (1945) Measures of the amount of ecological association between species. *Ecol* **26**: 297-302.

Eiler A, Langenheder S, Bertilsson S & Tranvik LJ (2003) Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. *Appl Environ Microbiol* **69**: 3701-3709.

Findlay SEG & Sinsabaugh RL (2003) Response of hyporheic biofilm metabolism and community structure to nitrogen amendments. *Aquat Microb Ecol* **33**: 127-136.

Gooday GW (1990) The ecology of chitin degradation. *Adv Microb Ecol* **11**: 387-430.

Grossmann S & Reichardt W (1991) Impact of *Arenicola marina* on bacteria in intertidal sediments. *Mar Ecol Prog Ser* **77**: 85-93.

Hammer O, Harper DAT & Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4**: 1-9.

Hirche H-J & Kosobokova K (2007) Distribution of *Calanus finmarchicus* in the northern North-Atlantic and Arctic Ocean: Expatriation and potential colonisation. *Deep Sea Res II* **54**: 2729-2747.

Hirche H-J, Muyakshin S, Klages M & Auel H (2006) Aggregation of the Arctic copepod *Calanus hyperboreus* over the ocean floor of the Greenland Sea. *Deep Sea Res I* **53**: 310-320.

Hoppe H-G (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. *Mar Ecol Prog Ser* **11**: 299-308.

Judd KE, Crump BC & Kling GW (2006) Variation in dissolved organic matter controls bacterial production and community composition. *Ecol* **87**: 2068-2079.

Meyer-Reil LA (1983) Benthic response to sedimentation events during autumn to spring at a shallow water station in the Western Kiel Bight. *Mar Biol* **77**: 247-256.

Moodley L, Middelburg JJ, Boschker HTS, Duineveld GCA & Pel R *et al*. (2002) Bacteria and Foraminifera: key players in a short-term deep-sea benthic response to phytodetritus. *Mar Ecol Prog Ser* **236**:23-29.

Poulicek M & Jeuniaux C (1989) Chitin biomass in marine sediments. *Chitin and Chitosan* (Skjak-Brek G, Anthonsen T, Sandford P, eds.), pp. 151-160. Elsevier, London.

Ramette A (2007) Multivariate analyses in microbial ecology. FEMS *Microbiol Ecol* **62**: 142-160.

Riemann L & Middelboe M (2002) Stability of bacterial and viral community compositions in Danish coastal waters as depicted by DNA fingerprinting techniques. *Aquat Microb Ecol* **27**: 219-232.

Riemann L, Steward GF & Azam F (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. *Appl Environ Microbiol* **66**: 578-587.

Sokal RR & Rohlf FJ (1995) *Biometry*, 3rd edition. Freeman WH and Company, San Francisco.

Sokolova MN (1994) Euphausiid 'dead body rain' as a source of food for abyssal benthos. *Deep Sea Res I* **41**: 741-746.

Soltwedel T, Bauerfeind E & Bergmann M *et al*. (2005) HAUSGARTEN - multidisciplinary investigations at a deep-sea long-term observatory in the Arctic Ocean. *Oceanograph* **18**: 46-61.

Turley CM & Lochte K (1990) Microbial response to the input of fresh detritus to the deep-sea bed. *Palaeogeogr Palaeoclimatol Palaeoecol (Global and Planetary Change Section)* **9**: 3-23.

Vetter Y-A & Deming JW (1994) Extracellular enzyme activity in the Arctic Northeast Water polynya. *Mar Ecol Prog Ser* **114**: 23-34.

Węsławski JM & Legeżyńska J (1998) Glaciers caused zooplankton mortality? *J Plankton Res* **20**: 1233-1240.

# Manuscript II

## Microbial colonisation of artificial and deep-sea sediments in the Arctic Ocean

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Keywords: deep sea; chitin; microbial colonisation; sediments

## Abstract

Deep-sea mud and artificial sediments (glass beads, sands) were incubated in the Arctic deep sea at 2500 m water depth to investigate how different sediment types affect colonisation by microbial communities under *in situ* conditions. Chitin was concomitantly added to those sediments to assess microbial response to high carbon input. Microbial abundance, biomass, chitobiase activity and changes in community structure were monitored after seven days and one year. In control sediments without chitin addition, no significant changes in microbial abundance, biomass and activity were observed after one year. In presence of chitin, however, considerable increases in these parameters were recorded in all three sediment types. Regardless of chitin addition, deep-sea sediments were always associated with higher values of microbial abundance, biomass and activity compared with artificial sediments. Sediment type was always found to be the most significant factor explaining variation in enzymatic activity and bacterial community structure as compared to the effects of chitin amount, incubation time, and changes in cell number or biomass. Overall, this is the first *in situ* study that disentangles the effects of multiple factors and of their interactions on abundance, biomass, activity and community structure of microbial communities in the deep Arctic Ocean.

## Introduction

The surface conditions of deep-sea sediments may frequently change due to disturbances such as near-bottom currents that can induce regular sediment transport or that can erode the sediment surface (GAGE & TYLER, 1991). The impact of benthic organisms and their activities can further affect the physical and chemical nature of the sediment surface including the abrasion, translocation and mixing of sediments (FINDLAY et al., 1990). The intensity of such processes and their impacts in deeper waters, however, may be enhanced if human disturbances such as commercial trawling (KAISER, 1998), mining (RADZIEJEWSKA & STOYANOVA, 2000), and oil exploration (JONES et al., 2006) are superimposed. Azoic sediment surfaces will temporarily occur as a result of such disturbances, until benthic organisms are able to recolonise the sediments. Consequently, surface sediments at the deep seafloor typically consist of a mosaic of old and newly colonised surfaces (KITAZATO, 1995).
Several field experiments in the deep sea have been conducted to study the patterns of recolonisation and succession of macro- and meiobenthic organisms (e.g. LEVIN &

SMITH, 1984; GRASSLE & MORSE-PORTEUS, 1987; KITAZATO, 1995). The process of microbial colonisation in deep-sea sediments is, however, still largely unexplored. A number of *in situ* studies in the Central Indian Ocean Basin using benthic hydraulic disturber (Deep-Sea Sediment Resuspension System; DSSRS) have directly examined the impact of deep-sea mining on microbial communities by comparing e.g. bacterial abundances and activities in pre- and post-disturbed sediments (NAIR et al., 2000; RAGHUKUMAR et al., 2001). These experiments, however, did not examine the effects of variations of sediment characteristics (e.g. particle size, particle shape) on changes in structure or composition of microbial communities. Noticeably, most studies dealing with microbial colonisation processes in sediments have been performed by time-course experiments in the laboratory with artificial (YAMAMOTO & LOPEZ, 1985) or abiotic natural sediments (FINDLAY et al., 1992), and mainly focussed on estuarine and intertidal microbial communities. The available studies overall indicated that sediment characteristics such as grain size, grain shape and organic carbon of sediments are important factors influencing the microbial colonisation of sediments (e.g. NICKELS et al., 1981). For example, the microbial abundance in marine sediments is directly controlled by sediment surface area and inversely correlated with sediment grade (LLOBET-BROSSA et al., 1998). A positive relationship has also been found between bacterial abundance and organic carbon content in sediments (KÖSTER et al., 2005).

Sediment particles may protect solid substrates from degradation when the pores are too small for microorganisms to penetrate, and such a protection has been reported previously in the case of chitin (OU & ALEXANDER, 1974). The latter compound is probably the most important biopolymer in marine environments (GOODAY, 1990). In the Arctic Ocean that is characterised by large stocks of copepods (HIRCHE, 1997), high input of chitinous material at the deep seafloor may originate from mass aggregation of planktonic copepods over the seafloor (HIRCHE et al., 2006) in addition to the sedimentation of their exoskeletons, exuviae and casing of faecal pellets.

The present study is the first to document microbial colonisation patterns of natural and artificial sediments for a short (seven days) and long (one year) period directly at the Arctic deep seafloor. Natural and artificial sediments were used to investigate the effect of fine, medium, and coarse sediments on growth, activity and structure of sediment-colonising microbial communities. In addition, this study determines concomitant, functional changes in microbial communities by measuring the microbial response to particulate organic material such as chitin associated with those different sediment types. Our main hypothesis was that there may be variations in microbial growth,

activity and community structure as a response to chitin input, and that this response would be modulated by the nature of available sediments.

## Material and methods

### *Experimental design*

*In situ* experiments were carried out in the eastern Fram Strait, west off Svalbard, (79°5’ N / 4°2’ E), more precisely at a central station that belongs to a number of stations along a depth transect from 1200 to 5500 m water depth established in the deep-sea long-term observatory HAUSGARTEN (Fig. 1; SOLTWEDEL et al., 2005). The central HAUSGARTEN station at 2500 m water depth served as an experimental area for our study that used a Sediment Tray Free Vehicle (STFV) designed by the Institut Français de Recherche pour l’Exploitation de la Mer (IFREMER) to deploy treated samples *in situ*. The STFV consists of an aluminium framework that supports four identical round trays, each of which was equipped with four separate round chambers (0.02 $m^2$ per chamber).

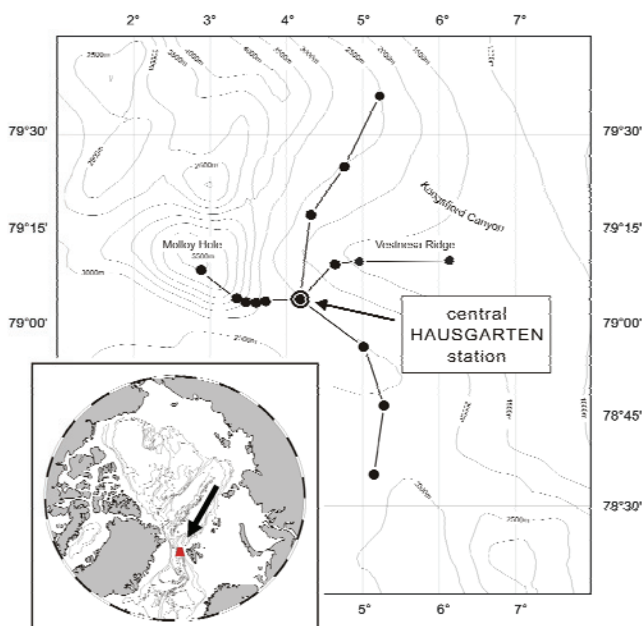


**Fig. 1.** Map of the deep-sea long-term observatory HAUSGARTEN in the Eastern Fram Strait. Fifteen permanent sampling sites along a depth and a latitudinal transect crossing the central HAUSGARTEN station at 2500 m water depth, serves as experimental area for long-term studies on the deep seafloor.

Only two trays with three chambers each were used for the experiments (Fig. 2), whereas the remaining trays were deployed for different purposes. The STFV was equipped with a remotely controlled mechanism consisting of spring-loaded discs to open and close the chambers during the passage to and from the deep seafloor to the surface. Each chamber was covered by a grid (5 mm mesh size) to prevent interferences from larger animals.

Prior to STFV deployment, deep-sea sediments from the central HAUSGARTEN station were collected with a multiple corer (MUC; BARNETT et al., 1984) at the same depth and close to the same position where the benthic lander ultimately was deployed. One chamber of each tray was filled to one centimetre from its top with surface sediments (0-3 cm) pooled from several sediment cores. The second chamber was filled with glass microbeads (diameter 100 to 200 µm; MHG Strahlanlagen GmbH, Düsseldorf, Germany) consisting of simple smooth and spherical particles. The third chamber contained commercially available axenic coarse sand that was selected on the basis of sub-rounded to sub-angular particles with larger mean grain sizes (1-2 mm). In deep-sea mud, silt (4 to 63 µm) was the dominant grain-size fraction (43%), followed by sand (≥ 63 µm, 37%), and clay (< 4 µm, 20%), as determined by using a Coulter LS 100 particle size analyser.

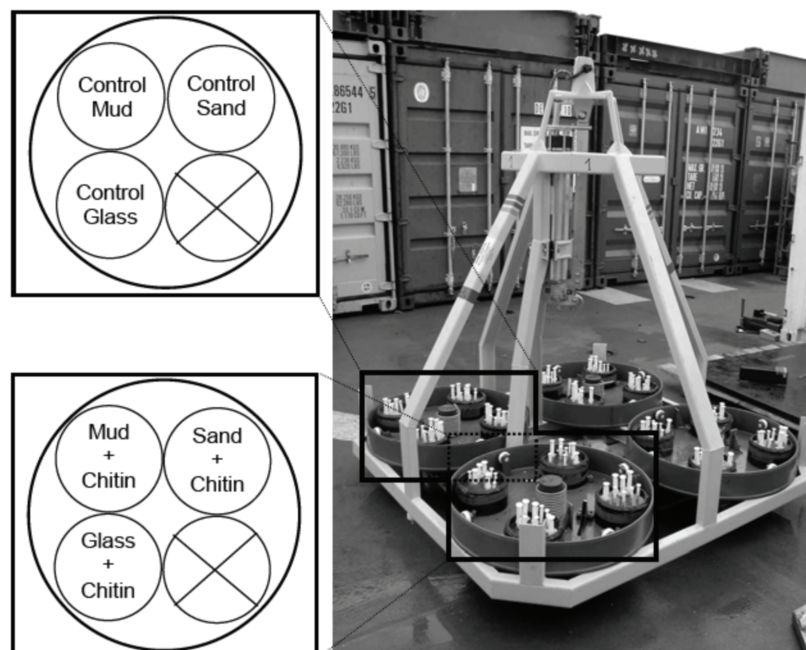


**Fig. 2.** Sediment Tray Free Vehicle (STFV) for *in situ* enrichment experiments. The STFV consisted of four identical trays from which only one was used for chitin enrichment experiments. Samples were taken using plastic syringes following tray recovery.

The pore size of artificial sediment composed of glass spheres was calculated as described by SHARMA & MCINERNEY (1994). The organic carbon content of the different sediment type was determined by using an Elemental Analyser (EuroVector, Milan, Italy).
Following defaunation by freezing sediment samples at -30°C for at least 48 h and subsequent thawing, samples were taken for initial analyses of microbial parameters (0 day). A chitin concentration of 3 g $m^{-2}$ chitin (purified chitin flakes from crab shells; Sigma-Aldrich, Hamburg, Germany) was then mixed thoroughly with the upper sediment layers and placed in the lander chambers of the first tray. The addition of chitin was equivalent to a substrate input of 1.3 g organic C $m^{-2}$. Chambers of the second tray were filled only with natural and artificial sediments without any addition of organic substrate and served as a control. During the *RV Polarstern* cruise ARK XX/1 in July 2004, the STFV was deployed for seven days. After recovery and subsequent sampling, trays were renewed and prepared again in the same way as described above. Afterwards, the STFV was deployed at the same station for one year. Sampling of the one-year experiment was carried out during the expedition ARK XXI/1b of *RV Polarstern* in July 2005. The bottom water temperature during the expeditions was about -0.8°C and bottom water oxygen concentration was 280 to 320 µmol $l^{-1}$. Mean bottom currents are relatively weak, ranging from 5.3 cm $s^{-1}$ to 6.6 cm $s^{-1}$ at 0.1 and 0.9 m above the bottom respectively (SAUTER, unpublished data).

*Sub-sampling and sample processing*

After recovery of the trays, eight samples from the uppermost sediment layers of each chamber were taken by means of plastic syringes with cut-off ends (2.5 cm in diameter). Sub-sampling was carried out in a cold room at 4°C. The first sediment centimetre from each chamber was pooled and homogenised before determining total microbial cell number, enzymatic activity, and community structure.

*Microbial biomass*

For the determination of microbial cell numbers and biomass, 2 ml of the sediment sample were taken by means of plastic syringes with cut-off ends and stored refrigerated in 9 ml 2% formaldehyde solution. The total cell number was enumerated by epifluorescence microscopy after staining with acridine orange according to the method of MEYER-REIL (1983). Volumetric measurements of the microbial cells were conducted with the New Porton grid, measuring randomly 50 cells per filter as described by GROSSMANN & REICHARDT (1991). Microbial biomass was estimated using

a conversion factor of $3 \times 10^{-13}$ g C $\mu m^{-3}$ (Børsheim et al., 1990). For each sample 40 counted grids from two replicate filters were analysed.

*Enzyme activity*

The extracellular enzymatic activity of chitobiase was measured fluorometrically (Hitachi, F-2000 spectrofluorometer) using the methylumbelliferone (MUF) labelled substrate *N*-acetyl-$\beta$-glucosaminide (Sigma-Aldrich) according to Boetius & Lochte (1994). Previous trials to determine the substrate saturation level gave a final concentration of 100 µmol MUF-substrate. Relative fluorescence units were calibrated with seven MUF standard concentrations between 10 and 300 nmol. Enzyme activity was calculated per volume of sediment and time (nmol $h^{-1}$) by linear regression. For each sample three measurements per incubation were performed.

*Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis*

Sediment samples for microbial community analysis were stored at -30°C until analysis. Total DNA was extracted and purified using a FastDNA®SPIN Kit for soil (Qbiogene, Heidelberg, Germany) following manufacturer's instructions. Bacterial 16S rRNA genes were amplified using universal primers 27F and 1492R (both synthesised by Interactiva; Ulm, Germany). The bacterial specific forward primer 27F was end-labelled with phosphoramidite fluorochrome 5-carboxyfluorescein (5´6-FAM). The PCR cocktail contained 1.5 mmol KCl, 10 mmol Tris-HCl pH 9, 1.5 mmol $MgCl_2$, 250 µmol dNTP, and 1.25 U *Taq*-Polymerase (Amersham Pharmacia Biotech, Freiburg, Germany). PCR amplification was performed by using the following cycling conditions: a 3-min hot start at 95°C, followed by 29 cycles consisting of denaturation (1 min at 95°C), annealing (1 min at 55°C), and extension (1 min at 72°C). A final extension at 72°C was then done for 7 min. Fluorescently labelled PCR products were run onto 1% agarose gel and purified using QIAquick Gel Extraction kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

Purified PCR products were digested in a 50-µl reaction volume with 10 U of tetrameric restriction endonucleases *Hha*I and *Msp*I, separately (Amersham Pharmacia Biotech) at 37°C for 5 h. Following desalting by isopropanol precipitation, fragment separation was performed by Gene Analysis Service GmbH (Berlin, Germany) using an ABI Prism 310 capillary analyser (PE Applied Biosystems, CA, USA).

Peak size determination of end-labelled fragments, considered here as different Operational Taxonomic Units (OTUs), was done by comparison with an internal size standard (GS2500 TAMRA, PE Applied Biosystems). Peaks between 50 and 1000

base pairs long were analysed using Genescan analytic software 2.02 (ABI). An additional check for artefacts was manually performed and peaks whose areas were smaller than 1% of the total peak area were excluded from data analysis. T-RFLP analyses were done once for each treatment due to the limited amount of available material.

*Data analysis*

The three variables, i.e. total microbial cell number, biomass, and chitobiase activity, were $log_{10}$-transformed prior to performing statistical analyses in order to normalise their distribution and to insure that treatments variances were homogeneous. Three-way Analyses of variance (ANOVA) were applied to test the effects of each factor (sediment type, chitin addition, incubation time) and of their interactions on each dataset. Differences between treatment means of total microbial cell numbers, biomass, chitobiase activity and organic carbon contents were evaluated by using pairwise Tukey's Honestly Significant Difference (HSD) test at $P < 0.05$ following significant ANOVA tests.

For each sample, T-RFLP fingerprints obtained from the two different restriction digests were combined to generate a binary matrix according to the presence or absence of OTUs. Non-metric multidimensional scaling (NMDS; SCHIFFMAN & REYNOLDS, 1981) was used to obtain an ordination of samples based on a matrix of Jaccard dissimilarities of T-RFLP profiles. The NMDS algorithm ranks distances between samples, and uses those ranks to map the objects non-linearly onto a simplified, two-dimensional ordination space so as to preserve their ranked differences, and not the original distances (LEGENDRE & LEGENDRE, 1998; RAMETTE, 2007). A stress function (values between 0 and 1) indicates how different the ranks on the ordination configuration are from the ranks in the original distance matrix. Twenty iterations of the NMDS procedure based on different random initial positions of the samples were performed so as to obtain an ordination with the lowest stress value (i.e. the best goodness-of-fit). Various groupings among samples were depicted on the ordination. Those groupings were tested for significance using Analysis of similarities (ANOSIM; CLARKE, 1993) tests based on 1000 permutations. This non-parametric, multivariate procedure compares ranks of distances between groups with ranks of distances within groups. The resulting $R$ test statistic measures whether separation of community structure is found ($R$ = 1), or if no separation occurs ($R$ = 0). $R$ values >0.75 are commonly interpreted as well separated, $R > 0.5$ as separated, but overlapping, and $R < 0.25$ as barely separable (CLARKE & GORLEY, 2001).

A distance-based redundancy analysis (db-RDA; LEGENDRE AND ANDERSON, 1999) was used to determine how well different factors explain the variation in bacterial community structure. The overall procedure consists of the following steps: a dissimilarity matrix among samples was calculated by using the Jaccard coefficient (LEGENDRE & LEGENDRE, 1998). A principal coordinate analysis was then applied to the dissimilarity matrix to obtain new, uncorrelated coordinates which were then analysed as independent variables in redundancy analysis (RAMETTE, 2007). In order to assess the respective importance of the different factors and of their covariation on community structure, a variation partitioning procedure (BORCARD et al., 1992; RAMETTE & TIEDJE, 2007) was subsequently applied to the data. This technique assesses the respective effects of each factor after controlling for the effects of other factors using a combination of simple and partial redundancy analyses. Significances of the respective effects of each factor were tested by 1000 Monte Carlo permutation tests. Variation partitioning analysis was also used to assess the respective contribution of each factor and of factor covariation on changes in chitobiase activity. Statistical analyses were performed with the statistical software JMP IN version 5.1 (SAS Institute, Cary, NC) and with the *vegan* package (R version 2.4.0; The R foundation for Statistical Computing).

## Results and discussion

*Effects of sediment types, chitin enrichment and incubation time on microbial abundance and activity*

Three-way ANOVA revealed that not only each factor taken alone (sediment type, chitin input, and incubation time) had highly significant effects on microbial cell number, biomass and chitobiase activity, but that factor interactions were also generally significant to explain variation of the measured variables (Table 1; Fig. 3). Microbial cell number was, however, the only exception since a non-significant $F$ ratio was obtained when the interactions among the three factors were evaluated. The interactions between chitin and incubation time explained the largest variation of all pair wise interaction terms, although sediment type and incubation time displayed the largest variation when each factor was considered separately (Table 1).

More detailed analyses of the variation in microbial cell number (Figs. 3a, 3b), microbial biomass (Figs. 3c, 3d) and chitobiase activity (Figs. 3e, 3f) indicated overall very similar patterns, i.e. generally higher values for deep-sea mud than for glass or sand substrates and a dramatic increase in chitin-enrichments only after one-year.

**Table 1.** Effects of sediment type, chitin input and incubation time on microbial cell numbers, microbial biomass and chitobiase activity.

| | Microbial cell number | | Microbial biomass | | Chitobiase activity | |
|---|---|---|---|---|---|---|
| Factors[1] | df[2] | *F* ratio[3] | df | *F* ratio | df | *F* ratio |
| Sediment | 1 | 288.3*** | 1 | 246.8*** | 1 | 402.3*** |
| Chitin | 2 | 102.56*** | 2 | 652.1*** | 2 | 91.3*** |
| Time | 2 | 262.2*** | 2 | 775.3*** | 2 | 170.0*** |
| SedimentxChitin | 2 | 5.5* | 2 | 8.5** | 2 | 7.4** |
| Sediment×Time | 4 | 6.4** | 4 | 428.7*** | 4 | 54.7*** |
| ChitinxTime | 2 | 43.0*** | 2 | 13.2*** | 2 | 35.5*** |
| Sediment×ChitinxTime | 4 | 1.2$^{NS}$ | 4 | 8.4*** | 4 | 5.5** |

[1]Three-way ANOVAs were performed on $log_{10}$-transformed variables. Factors consisted of two levels for chitin input (presence or absence), and three levels for sediment types (mud, beads, sand) and for incubation time (0 day, 7 days, 1 year). Statistical differences between treatment means are indicated in Figure 3.

[2]Degrees of freedom (df) for each factor or interactions thereof.

[3]*F* ratios are ratios of the mean-square value for a given source of variation to the residual mean-square value. Associated probabilities are indicated as not significant (NS) when $P > 0.05$, (*) when $P < 0.05$, (**) when $P < 0.01$, and (***) when $P < 0.001$.

In the no-chitin, control treatments, initial microbial cell numbers were significantly higher in natural sediments from the study site (0.6 x $10^8$ cells $cm^{-3}$), than in glass beads (0.1 x $10^8$ cells $cm^{-3}$) and sands (0.2 x $10^8$ cells $cm^{-3}$; Figs. 3a, 3b). This initial colonisation on glass and sand surfaces may be explained from the fact that it was not feasible to bring the different sediment types at 2500 m depth under sterile conditions and thus, a low level of colonisation from other sources had to be tolerated. This background colonisation did not, however, change during the course of the experiment (Fig. 3). When chitin was added to the experimental system, the only significant changes in cell number occurred after one year, whereas no noticeable differences were found for shorter incubation times regardless of sediment type (Fig. 3b). Natural sediments were then clearly associated with the highest cell number followed by sand and glass beads.

Changes in microbial biomass in the different treatments also confirmed the trends observed for microbial cell number (Figs. 3c, 3d). Indeed, in treatments without chitin, no changes were observed over time whereas chitin addition was associated with higher microbial biomass but only after one-year incubation.

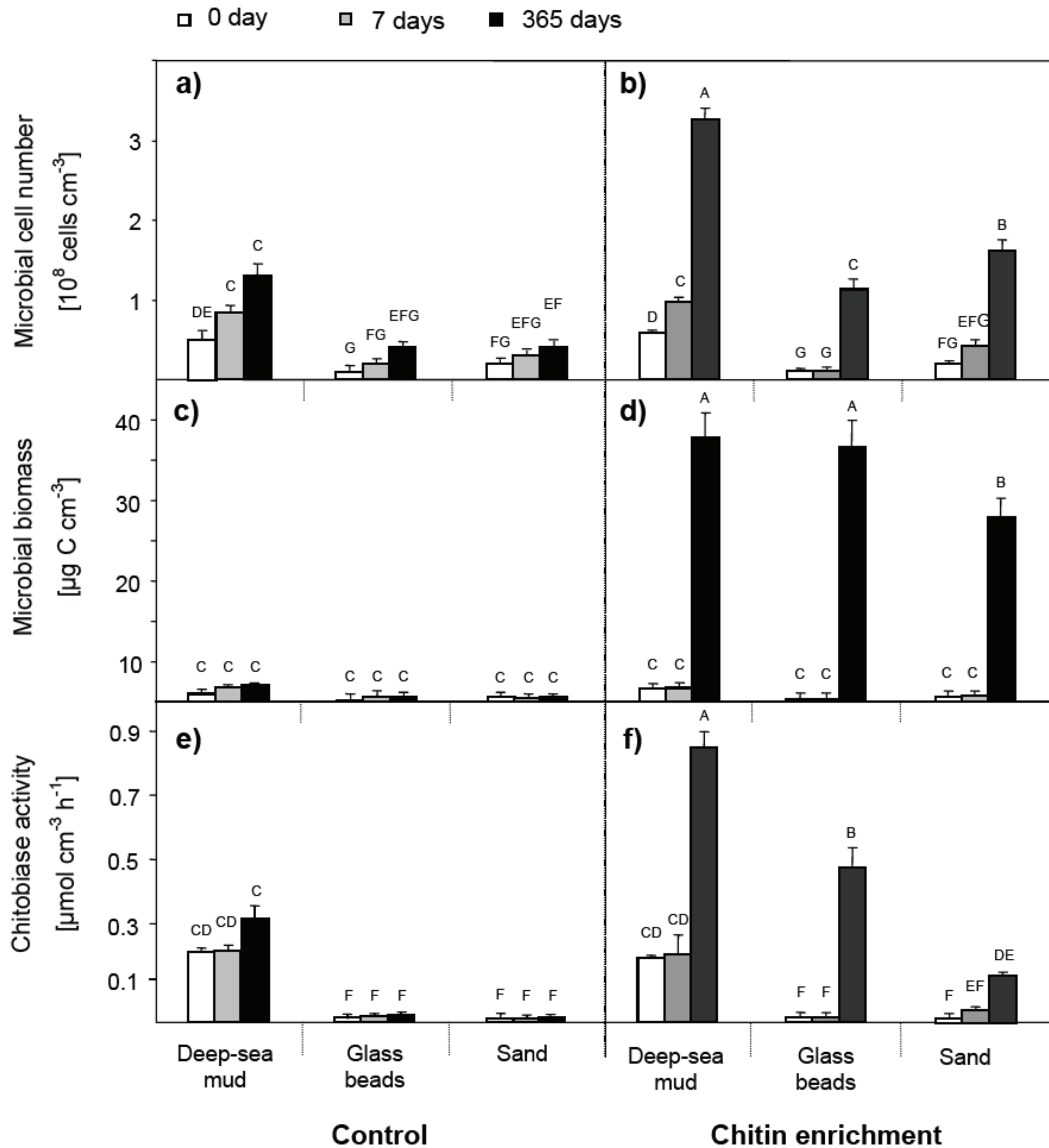


**Fig. 3.** Mean microbial cell number, biomass and chitobiase activity in control (a, c, e) and chitin-enriched (b, d, f) sediments at different incubation times. The concentration of 3.0 g chitin $m^{-2}$ added to the sediments corresponds to 1.3 g C $m^{-2}$. Different capital letters above the bars indicate significant differences between mean treatments as determined by *post hoc* Tukey's HSD tests at $P < 0.05$ which can be directly compared within panels (a, b), (c, d), and (e, f), but not between them.

In contrary to cell number though, no significant difference between deep-sea mud and glass beads were observed in microbial biomass after one year. Interestingly, while in chitin enriched deep-sea mud changes in microbial biomass were mainly accompanied by increased cell numbers, the increased microbial biomass for artificial sediments was mainly associated with a substantial increase in the mean cell volume and less to the increase in cell number (Figs. 3b, 3d). With the glass beads used in our experiment, the calculated pore size of 17 µm was much larger than an average bacterial cell and thus adequate for the colonisation by larger bacterial rods, the latter being not detected by microscopic observations in natural sediments as well as control sediments without chitin (data not shown).

Measurements of extracellular enzymatic chitobiase activity clearly showed differences between treatments (Table 1), consistent with interactions of sediment type, chitin input and incubation time, with sediment type having the largest effect on chitobiase activity (Figs. 3e, 3f). Initial chitobiase activity from deep-sea mud of about 0.2 µmol $h^{-1}$ corresponded to that of the natural environment and to values estimated for other deep-sea sediments (BOETIUS, 1995; BOETIUS & LOCHTE, 1994, 1996). A large increase in enzymatic activity was detected only after one-year incubation (Fig. 3f), which clearly suggests that the addition of chitin induced the production of chitobiase. Furthermore, there was also a significant relationship between decreasing particle size (from sand, glass, to mud, respectively) and increasing chitobiase activity. Those observations support the idea that small particles offer more areas for bacterial attachment, colonisation (e.g. DEFLAUN & MAYER, 1983; YAMAMOTO & LOPEZ, 1985) and activity than coarser particles, regardless of chitin addition.

The fact that seven-day incubation was apparently too short to record significant microbial response to the chitin input is not surprising since microbial colonisation of new sediments may proceed over a course of several days to weeks (SMITH et al., 1982; YAMAMOTO & LOPEZ, 1985) and time lags of more than one week may be needed to observe enhanced enzyme production as a response to chitin pulses (e.g. BOETIUS & LOCHTE, 1996; KANZOG et al., submitted). Due to logistical limitations it was, however, not possible to obtain samples on a shorter timeframe. Our results at least identified some lower and upper time bounds within which significant changes in natural phenomena took place at the bottom of the Arctic Ocean.

Because the effects of incubation time, sediment type and chitin addition were tightly intertwined, further analyses were needed to disentangle the respective effects of each factor and to quantify their covariation (Figs. 4a, 4b). Sediment type was found as the

most important factor (18%, $P < 0.001$) to explain variation in microbial activity when the effects of incubation time, chitin addition and concomitant changes in cell number were controlled for (Fig. 4a). The relative effects of chitin input and incubation time, although significant, only accounted for 6% of the variation in enzymatic activity (Fig. 4a). Interestingly, there was also a high covariation between cell number and both sediment type and incubation time, which accounted for 16 and 18%, respectively, of the variation in chitobiase activity. When microbial biomass was used instead of microbial cell number as a factor (Fig. 4b), a larger proportion of the overall variation in microbial activity could be explained with 70% (Fig. 4b) instead of 60% (Fig. 4a) of the explained variation. The largest amount of the variation in activity could then be explained by the presence of different sediment types and by fluctuations in microbial biomass, as well as the covariation between those factors. Noticeably, neither pure effects of chitin nor incubation time were found as significant when sediment type and microbial biomass where considered (Fig. 4b).

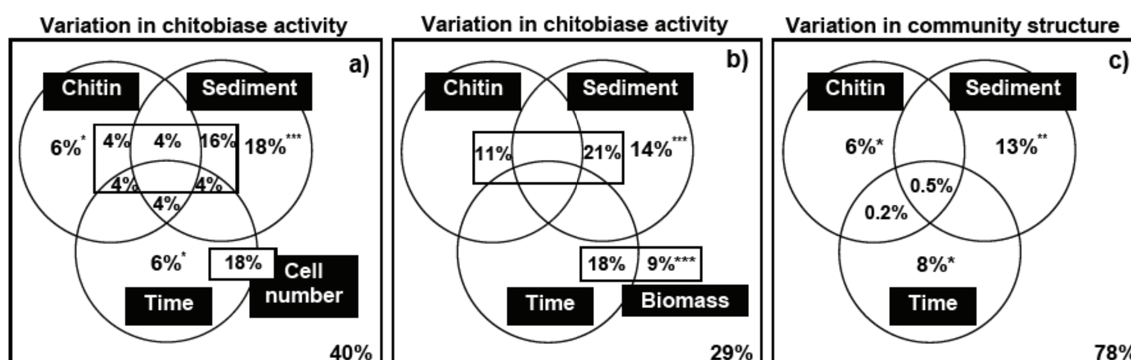


**Fig. 4.** Variation partitioning analysis as a function of sediment type, chitin input, incubation time, microbial abundance and biomass. The respective contribution (as percentages of the total biological variation) of each factor and of their covariation was disentangled by using variation partitioning and distance-based redundancy analyses. **a)** Variation in chitobiase activity was partitioned into the respective effects of chitin input, sediment type, incubation time and total cell number. **b)** Variation in chitobiase activity was partitioned as for the a) panel but with total microbial biomass replacing microbial cell number. Percentages within the square inserted into the circles represent covariation between microbial growth parameter (cell number, biomass) and the respective effects of chitin input, sediment type and incubation time. **c)** Variation in community structure as determined by T-RFLP was partitioned in the effects of the four factors. The respective effects of total cell number and microbial biomass as well as their covariation with other factors did not contribute significantly to the community variation (all below 5%) and are not displayed. The significance of each fraction was tested by using 1000 permutations of the data ($P \leq 0.05$, *; $P < 0.01$, **; $P < 0.001$, ***). Negative variations were not shown, and the amount of unexplained variation is indicated at the bottom right hand corner.

The fact that sediment type was found as the most important factor influencing chitobiase activity might be explained as followed: since a substantial fraction of the extracellular enzymes are bound to the cell membranes or other surfaces (MEYER-REIL, 1990; MARTINEY & AZAM, 1993), the enzyme production may be induced only in microorganisms in the vicinity of nutrient particles (KARNER & HERNDL, 1992). Thus, the degradation of chitin would be initiated only when cells come into contact with the food source. In deep-sea mud composed of finer particles it would thus be more likely that cells came into contact with the chitinous substrate. In addition, the diffusion of extracellular enzymes and monomers liberated by the enzymes away from the cells may probably be more pronounced in coarse sediments than in sediments composed of smaller particles.

Another important factor promoting microbial sediment colonisation is the organic carbon content of the sediments (CAMMEN, 1982; DEFLAUN & MAYER, 1983; YAMAMOTO & LOPEZ, 1985). The organic carbon content of deep-sea mud we used was about 0.9% by weight at the start of the experiment and did not significantly change after short- and long-term incubation (Table 2). Glass beads and sand contained even less organic carbon (460 and 180 times less, respectively) than deep-sea mud prior to incubation. After one year, in both artificial sediments (without and with chitin) the organic carbon content was significant higher than at the start of the experiment. With the assumption that only a minor proportion of sediment organic carbon originates from bacterial carbon (0.6 to 8.5%; DALE, 1974), we presume that beside the gradual accumulation of sedimentary organic material the enhanced bacterial production of extracellular chitinolytic enzymes in chitin treatments contributed to the formation of a complex organic matrix within sediments. This process of sediment stabilisation by microbial exudates could have also contributed to the further increase in organic carbon content. Despite this increase in organic carbon content in artificial sediments, values were always lower than those from natural sediments, supporting the finding that in fine-grained sediments the organic carbon content is generally higher than in sediments composed of larger particles (DEFLAUN & MAYER, 1983). It may thus also be hypothesised that the low carbon content limited microbial colonisation in the artificial sediments.

**Table 2.** Organic carbon content of natural and artificial sediment in control and chitin-enriched treatments after different incubation times at the seafloor.

| | Control (days) | | | Chitin-enrichment (days) | |
|---|---|---|---|---|---|
| | 0 | 7 | 365 | 7 | 365 |
| Deep-sea sediment | 0.935 ± 0.029 (A) | 0.989 ± 0.009 (A) | 1.670 ± 0.050 (A) | 1.889 ± 0.101 (A) | 2.143 ± 0.171 (A) |
| Glass beads | 0.002 ± 0.002 (E) | 0.005 ± 0.003 (D) | 0.043 ± 0.006 (C) | 0.008 ± 0.002 (D) | 0.174 ± 0.041 (B) |
| Sand | 0.005 ± 0.001 (D) | 0.007 ± 0.001 (D) | 0.058 ± 0.001 (C) | 0.009 ± 0.004 (D) | 0.065 ± 0.011 (C) |

All data are given as means of three repetitions ± standard deviation. Different letters (in parentheses) indicate significant differences between mean treatments as determined by *post hoc* Tukey's HSD tests at $P < 0.05$.

*Effects of sediment types, chitin enrichment and incubation time on bacterial community structure*

The variation in the bacterial community structure was analysed by distance-based redundancy analysis (db-RDA) so as to determine whether the factors sediment type, chitin input, incubation time and microbial growth had overall significant effects on the variation of microbial assemblages. The respective effects of microbial cell number and biomass did not contribute significantly to the variation in T-RFLP data ($P > 0.05$; data not shown). The other three factors investigated (chitin input, incubation time and sediment type), however, had each significant effects on the variation in bacterial community patterns ($P < 0.05$; Fig. 4c). Similar to variation in chitobiase activity (Figs. 4a, 4b), sediment type was the most important factor and explained much more biological variation (13%) as duration of incubation (8%) or chitin input (6%). Interestingly, the low amount of covariation among factors could indicate that the factors mostly behave independently from each other (LEGENDRE & LEGENDRE, 1998). Although the three factors significantly explained the variation in the T-RFLP data, there was still a large amount of biological variation that could not be explained (i.e. 78%). Hence, additional environmental parameters would need to be further incorporated in the model to fully explain the changes in diversity patterns.

In order to obtain a finer understanding of the effects of the three factors on bacterial assemblages, non-metric multidimensional scaling (NMDS) ordination (Fig. 5) in association with non-parametric ANOSIM tests were also used to assess the statistical significance between groups of samples based on their T-RFLP profiles. Control samples without additional chitin and samples amended with chitin fell into two overlapping (Fig. 5b), but significantly different groups (ANOSIM $R = 0.326$, $P = 0.005$). The shift in community structure apparently caused by the amendment of sediments with chitin can be explained by the fact that increased food availability in a food-limited environment like the deep sea often leads to a shift to more opportunistic species (in this case probably chitin-specific bacteria), increased dominance, and decreased diversity (SNELGROVE et al., 1996). Short- as well as long-term incubation times, regardless of chitin addition or sediment type seemed to induce community shifts with more pronounced differences after one-year incubation ($R = 0.33$, $P = 0.005$; Fig. 5c). This may probably be explained by a gradual increase of the bacterial colonisation of the defaunated and artificial sediments over the whole duration of the experiment. When samples were grouped by sediment type (natural sediments, glass beads and sand; Fig. 5d), the ANOSIM test also showed significant differences in the bacterial assemblages ($R = 0.247$, $P = 0.017$). Previous studies based on phospholipid fatty acids analyses of microbial communities have already shown that surface morphology of sediment grains has an effect on the community composition (NICKELS et al., 1981; FINDLAY et al., 1992). Similarly, our results suggest that different sediment types used for this experiment may affect not only microbial growth and activity, but also the structure of bacterial communities. Because the ANOSIM $R$ values were generally below 0.5, those groups were most likely separated but overlapping to certain extent (CLARKE & GORLEY, 2001), as may occur if the community structures changed over time but kept some common structure with their initial states (i.e. that at the beginning of the experiments).

Although our study was carried out *in situ*, under natural conditions at the seafloor, it is important to note that the artificial nature of tray experiments might still have modified natural conditions, e.g. by creating hydrodynamic biases (SMITH, 1985; SNELGROVE et al., 1995). These tray-induced variations may also influence benthic biological processes, such as sediment deposition, erosion and nutrient flow. Our sediment trays were approximately 20 cm above the seafloor, and therefore, sediments within the chambers inside the trays were disconnected from the surrounding seafloor.

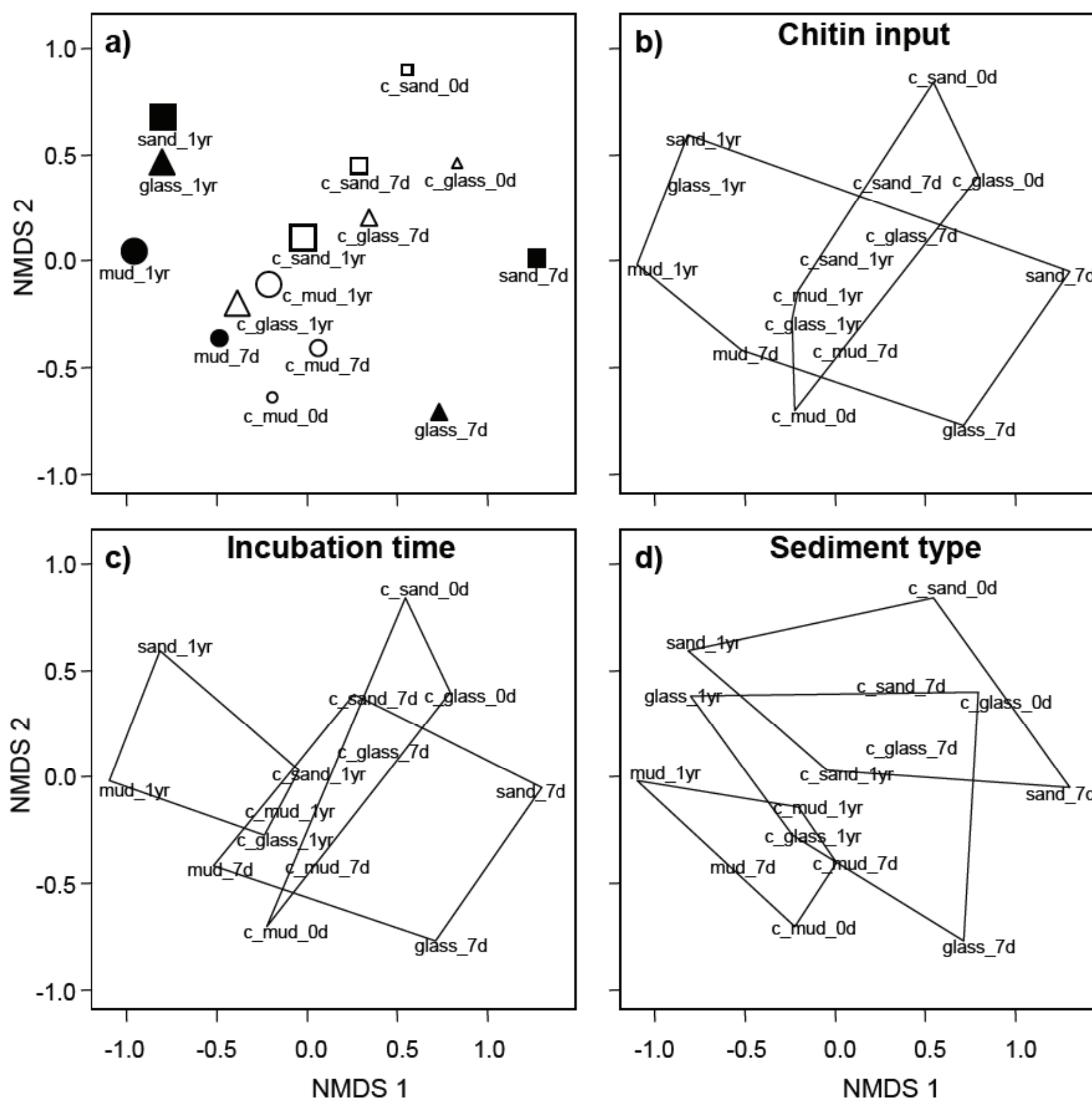


**Fig. 5.** Non-metric multidimensional scaling (NMDS) ordination of T-RFLP data based on Jaccard dissimilarities among samples. **a)** The overall stress of the ordination was 15.2% based on 20 iterations to find a solution with minimal stress. In the first panel, symbols were assigned to each treatment as follows: squares, triangle and circle represent sand, glass, and mud treatment, respectively, whereas full and empty symbols correspond to chitin-amended and not-amended controls (also with a 'c' prefix). The size of the symbols increases with incubation time of the respective samples. To further facilitate the interpretation of the NMDS result, geometric shapes were superimposed on the ordination. They depict **b)** chitin-amended samples vs. no-chitin control samples, **c)** different incubation times (0, 7, 365 days), and **d)** the three sediment types (deep-sea mud, sand, glass).

Hence, colonisation mainly occurred by the already existing communities present in the samples and (re)suspended microorganisms transported by near-bottom currents. Another factor that we could not control for is grazing that may have prevented microbial communities to proliferate to their full potential size after one-year incubation. Indeed, although grids (5 mm mesh size) were applied to avoid interferences of larger-size animals, we could not exclude meiofauna (e.g. nematodes) or microeukaryotic organisms as potential grazers. Hence, microbial abundance and biomass could actually be higher than what is reported here. In addition, grazing may have affected also community structure, since with increasing sediment particle smoothness the susceptibility of bacteria to predation increases (NICKELS et al., 1981). Although it is difficult to control for all experimental biases when working under *in situ* conditions, we assume that all treatments inside the chambers were similarly affected by those environmental conditions and that sediment trays allowed at least valid inter-treatment comparisons to be made. This approach may thus contribute to a better understanding microbial colonisation processes under deep-sea conditions.

In conclusion, deep-sea microbial communities respond to variation in sediment types and chitin input by changing their composition, abundance and activity. In general, our observations support the idea that microbial communities in natural environments prefer to colonise sediments with smaller grain sizes and higher organic carbon contents. The effect of chitin was only seen after one year of incubation, which gives an upper time bound to observe significant changes in microbial response due to chitin input. Future studies will incorporate this knowledge and will address the identification of the microbes associated with high chitin degradation rates and of environmental variables that may contribute to the variation of microbial community structure in the Arctic Ocean.

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## References

Barnett PRO, Watson J& Conelly D (1984) A multiple corer for taking virtually undisturbed samples from shelf, bathyal and abyssal sediments. *Oceanol Acta* **7**: 399-408.

Boetius A (1995) Microbial hydrolytic enzyme activities in deep-sea sediments. *Helgoländer Meeresunters* **49**: 177-187.

Boetius A & Lochte K (1994) Regulation of microbial enzymatic degradation of organic matter in deep-sea sediments. *Mar Ecol Prog Ser* **104**: 299-307.

Boetius A & Lochte K (1996) Effect of organic enrichments on hydrolytic potentials and growth of bacteria in deep-sea sediments. *Mar Ecol Prog Ser* **140**: 239-250.

Borcard D, Legendre P & Drapeau P (1992) Partialling out the spatial component of ecological variation. *Ecol* **73**: 1045-1055.

Børsheim KY, Bratbak G & Heldal M (1990) Enumeration and biomass estimation of planctonic bacteria and viruses by Transmission Electron Microscopy. *Appl Environ Microbiol* **56**: 352-356.

Cammen LM (1982) Effect of particle size on organic content and microbial abundance within four marine sediments. *Mar Ecol Prog Ser* **9**: 273-280.

Clarke KR (1993) Non-parametric multivariate analysis of changes in community structure. *Aust J Ecol* **18**: 117-143.

Clarke KR & Gorley RN (2001) PRIMER v5: User manual/tutorial, PRIMER-E. Plymouth UK.

Dale NG (1974) Bacteria in intertidal sediments: Factors related to their distribution. *Limnol Oceanogr* **19**: 509-518.

DeFlaun MF & Mayer LM (1983) Relationships between bacteria and grain surfaces in intertidal sediments. *Limnol Oceanogr* **28**: 873-881.

Findlay RH, Trexler B, Guckert JB & White DC (1990) Laboratory study of disturbance in marine sediments: response of a microbial community. *Mar Ecol Prog Ser* **62**: 121-133.

Findlay RH, Kim SK & Butman CA (1992) Colonisation of freshly deposited barite and silica sediments by marine microorganisms in a laboratory flume flow. *Mar Ecol Prog Ser* **90**: 73-88.

Gage JD &Tyler PA (1991) *Deep-sea Biology: A natural history of organisms at the deep-seafloor*. Cambridge Univ Press, Cambridge.

Gooday GW (1990) The ecology of chitin degradation. *Adv Microb Ecol* **11**: 387-430.

Grassle JF & Morse-Porteous LS (1987) Macrofaunal colonisation of disturbed deep-sea environments and the structure of deep-sea benthic communities. *Deep Sea Res* **34**: 1911-1987.

Grossmann S & Reichardt W (1991) Impact of *Arenicola marina* on bacteria in intertidal sediments. *Mar Ecol Prog Ser* **77**: 85-93.

Hirche HJ (1997) Life cycle of the copepode *Calanus hyperborius* in the Greenland Sea. *Mar Biol* **128**: 607-608.

Hirche HJ, Muyakshin S, Klages M & Auel H (2006) Aggregation of the Arctic copepod *Calanus hyperboreus* over the ocean floor of the Greenland Sea. *Deep Sea Res I* **53**: 310-320.

Jones DOB, Hudson IR & Bett BJ (2006) Effects of physical disturbance on the cold-water megafaunal communities of the Faroe-Shetland Channel. *Mar Ecol Prog Ser* **319**: 43-54.

Kaiser MJ (1998) Significance of bottom-fishing disturbance. *Conserv Biol* **12**: 1230-1235.

Karner M & Herndle GH (1992) Extracellular enzymatic activity and secondary production in free-living and marine-snow-associated bacteria. *Mar Biol* **113**: 341-347.

Kitazato H (1995) Recolonisation by deep-sea benthic foraminifera: possible substrate preferences. *Mar Micropaleontol* **26**: 65-74.

Köster M, Dahlke S & Meyer-Reil L-A (2005) Microbial colonization and activity in relation to organic carbon in sediments of hypertrophic coastal waters (Nordrügensche Bodden, Southern Baltic Sea). *Aquat Microb Ecol* **39**: 69-83.

Legendre P & Anderson MJ (1999) Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecol Monogr* **69**: 1-24.

Legendre P & Legendre L (1998) *Numerical Ecology*. Elsevier Science BV, Amsterdam.

Levin LA & Smith CR (1984) Response of background fauna to disturbance and enrichment in the deep sea: a sediment tray experiment. *Deep Sea Res I* **3**: 1277-1285.

Llobet-Brossa E, Rossello-Mora R & Amann R (1998) Microbial community composition of Wadden Sea sediments as revealed by fluorescence in situ hybridization. *Appl Environ Microbiol* **64**: 2691-2696.

Meyer-Reil LA (1983) Benthic response to sedimentation events during autumn to spring at a shallow water station in the Western Kiel Bight. *Mar Biol* **77**: 247-256.

Meyer-Reil LA (1990) Microorganisms in marine sediments: consideration concerning activity measurements. *Arch Hydrobiol Beih Ergebn Limnol* **34**: 1-6.

Nair S, Mohandass C, Loka Bharathi PA, Sheelu G & Chandralata R (2000) Microscale response of sediment variable to benthic disturbance in the Central Indian Ocean Basin. *Mar Geores Geotechnol* **18**: 273-283.

Nickels JS, Bobbie RJ, Martz RF, Smith GA, White DC & Richards NL (1981) Effect of silicate grain shape, structure, and location on the biomass and community structure of colonising marine microbiota. *Appl Environ Microbiol* **41**: 1262-1268.

Ou LT & Alexander M (1974) Influence of glass microbeads on growth, activity and morphological changes of *Bacillus megaterium*. *Arch Microbiol* **101**: 35-44.

Radziejewska T & Stoyanova V (2000) Abyssal epibenthic megafauna of the Clarion-Clipperton area (NE-Pacific): Changes in time and space versus anthropogenic environmental disturbance. *Ocanological Studies* **29**: 83-101.

Raghukumar C, Loka Bharathi PA, Ansari ZA, Nair S, Ingole B, Sheelu G, Mohandass C, Nagender Nath B & Rodrigues N (2001) Bacterial standing stock, meiofauna and sediment-nutrient characteristics: indicators of benthic disturbance in the Central Indian Basin. *Deep Sea Res II* **48**: 3381-3399.

Ramette A (2007) Multivariate analyses in microbial ecology. *FEMS Microb Ecol* **62**: 142-160.

Ramette A & Tiedje JM (2007) Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. *Proc Nat Acad Sci USA* **104**: 2761-2766.

Rublee P & Dornseif BE (1978) Direct counts of bacteria in the sediments of a North Carolina salt marsh. *Estuaries* **1**: 188-191.

Schiffman SS & Reynolds ML (1981) Introduction to multidimensional scaling. *Theory, methods and applications*. Academic Press, London, UK.

Sharma PK & McInerney MJ (1994) Effect of grain size in bacterial penetration, reproduction, and metabolic activity in porous glass beads chambers. *Appl Environ Microbiol* **60**: 1481-1486.

Smith GA, Nickels J, Bobbie RJ, Richards NL & White DC (1982) Effects of oil and gas well-drilling fluids on the biomass and community structure of microbiota that colonise sands in running seawater. *Arch Environ Contam Toxicol* **11**: 17-23.

Smith CR (1985) Food for the deep sea: utilisation, dispersal, and flux of nekton falls at the Santa Catalina Basin floor. *Deep Sea Res* **32**: 417-442.

Snelgrove PVR, Butman CA & Grassle JF (1995) Potential flow artefacts associated with benthic experimental gear: Deep-sea mudbox examples. *J Mar Res* **53**: 821-845.

Snelgrove PVR, Grassle JF & Petrecca RF (1996) Experimental evidence for aging food patches as a factor contributing to high deep-sea macrofaunal diversity. *Limnol Oceanogr* **41**: 605-614.

Soltwedel T, Bauerfeind E & Bergmann M *et al*. (2005) HAUSGARTEN-multidisciplinary investigations at a deep-sea long-term observatory in the Arctic Ocean. *Oceanogr* **18**: 46-61.

Yamamoto N & Lopez G (1985) Bacterial abundance in relation to surface area and organic content of marine sediments. *J Exp Mar Biol Ecol* **90**: 209-220.

Weise W & Rheinheimer G (1978) Scanning electron microscopy and epifluorescence investigation of bacterial colonisation of marine sediments. *Microb Ecol* **4**: 175-188.

# Manuscript III

## Microbial responses to large POM supply in natural and artificial sediments at the Arctic deep seafloor

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Keywords: microbial community, deep-sea sediments, organic matter supply, *in situ* experiment

## Abstract

This study aimed for the first time at the investigation of heterotrophic microbial responses to different organic matter inputs under natural conditions in the Arctic deep sea, and how different sediment types influence this response. Artificial sediments and deep-sea mud were enriched with microalgae, yeast and fish and incubated at 2500 m water depth. The effect of organic enrichment on microbial abundance, biomass, potential hydrolytic activity, and community structure was recorded after three months and one year of incubation. Already three months of incubation under rich microalgae and yeast concentrations caused a significant microbial reaction by increased abundance and hydrolytic activity in all sediment types. This effect was mostly distinctive in treatments enriched with microalgae. Microbial growth after three months in treatments with fish was observed exclusively in the artificial sediments. After one year, there was a reduction in microbial abundance and activity, particularly in the artificial sediments enriched with organic substrates. Determination of bacterial community structure by terminal restriction fragment length polymorphism (T-RFLP) analysis indicated a shift in the community composition in treatments with organic substrates after the long-term incubation. Changes in community structure might be explained by decreasing bacterial Operational Taxonomic Units (OTU) richness in natural sediments, whereas a trend toward increasing OTU richness was observed in artificial sediments. The present results suggest that the magnitude of deep-sea microbial population response to environmental changes such as large inputs of organic material is determined by the type of organic food stuff as well as sediment properties such as particle size and particle shape. In addition, removal of organic materials by invertebrate feeding appears to exceed the rate of degradation by microorganisms.

## Introduction

The seasonal deposition of relatively ‘fresh’ detrital material within a short period of time after the spring phytoplankton bloom represents a major particulate organic carbon (POC) input to the deep-sea community (RICE et al., 1986). While 10 to 40% of the primary production may leave the upper water layers, most gets remineralised during its descent (TURLEY et al., 1995) so that only a low amount of the total surface primary production arrives on the deep-sea bed (SUESS, 1980). The abundance and biomass of benthic organisms, inclusively deep-sea microbial communities, is directly

related to the amount of food reaching the sediment surface (GOODAY & TURLEY, 1990; DANOVARO et al., 2000). Rapid responses to fluctuations in nutrient supply in the form of enhanced growth and activity have also been reported (TURLEY et al., 1988; TURLEY & LOCHTE, 1990).

There is growing evidence that indigenous deep-sea bacteria can also react quickly to organic material other than phytodetritus. KOHLMEYER (1980) artificially deposited wood peaces on the deep seafloor for periods of several months and showed a dense colonisation by bacterial populations. Results of long-term incubation experiments with wood parcels exposed on the deep seafloor indicated a correlation between cellulolytic activity and bacterial cell numbers (PALACIOS et al., 2006). At least, bacteria (and their predators) have a key function for decomposition processes of larger detritus remains like zooplankton carcasses, crustacean moults and carcasses of larger organisms (GOODAY & TURLEY, 1990; CHRISTIANSEN & BOETIUS, 2000; SMITH & BACO, 2003).

In recent years, progress in deep-sea technology facilitated new *in situ* studies on the fate of organic matter in deep waters and sediments. Experiments using free falling devices (WIRSEN & JANNASCH, 1986) or benthic chamber landers (WITTE et al., 2003a, b) have been performed to investigate the effects of pulsed food supply on microbial communities in the deep-sea sediments. Most of these studies have been limited in duration to hours (MOODLEY et al., 2002; BÜHRING et al., 2006) or several days (WITTE et al., 2003a, b). Until now, virtually nothing is known about changes in microbial community structure in deep-sea sediments caused by large organic matter inputs, particularly in high northern latitudes. Climate driven changes in primary production, most dramatically recorded in Arctic environments (HASSOL, 2004), have effects on the food supply even to the deep seafloor and may influence community structure and functioning of the total sediment inhabiting microbiota.

The present study proves the hypothesis of microbial response to various food deposits after different time periods ranging from three months up to one year of incubation at the seafloor (2500 m water depth). The study aims at describing possible changes in abundance, biomass, activity, and structure of microbial communities as a reaction to a sudden bulk of food supply. Furthermore, we investigated the relevancy of differences in sediment quality on microbial responses to these organic enrichments.

*Material and methods*

During the *RV Polarstern* Arctic winter cruise ARK XIX/1b in March 2003, a Sediment Tray Free Vehicle (STFV) was deployed for three months at the experimental site (2500 m water depth) of the deep-sea long-term observatory HAUSGARTEN (79°5' N / 4°2' E), in the eastern Fram Strait, west off Svalbard (SOLTWEDEL et al., 2005). After recovery and sampling during the cruise ARK XIX/3c in June 2003, trays of the STFV were renewed and deployed at the same station for a long-term experiment of one year. Sampling of this long-term experiment was carried out in July 2004, during the expedition ARK XX/1 of *RV Polarstern* as well.

*Experimental design*

The STFV was composed of four identical trays, each of which was equipped with four separate round chambers (0.02 $m^2$ per chamber). Each chamber was covered by a grid (5 mm mesh size) to prevent disturbances of the experiments by larger animals. A detailed description of the STFV is given by KANZOG & RAMETTE (submitted).

Prior to STFV deployment, deep-sea sediments from the experimental site of the HAUSGARTEN were collected with a multiple corer (MUC; BARNETT et al., 1984) at the same depth and close to the same position where the STFV was ultimately deployed. One chamber of each tray was filled to one centimetre from its top with deep-sea sediments, which have been pooled from several sediment cores. The second chamber was filled with glass microbeads (diameter 250 to 500 µm; MHG Strahlanlagen GmbH, Düsseldorf, Germany) consisting of simple smooth and spherical particles. The third chamber contained commercially available axenic coarse sand that was selected on the basis of sub-rounded to sub-angular particles with larger mean grain sizes (1-2 mm). In deep-sea sediment, silt (4 to 63 µm) was the dominant grain-size fraction (43%), followed by sand (≥ 63 µm, 37%), and clay (< 4 µm, 20%), as determined by using a Coulter LS 100 particle size analyser. For defaunation, natural and artificial sediments filled in the chambers were subsequently deep-frozen at -30°C for 48 h at least.

To simulate the availability of various types of organic food, natural and artificial sediments within chambers were prepared as follows (Fig. 1):

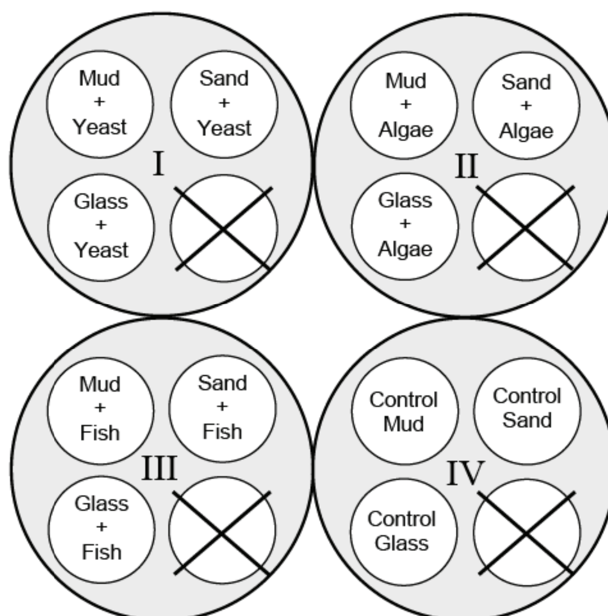
(I) Commercial available bakers's yeast was mixed with the upper layers of sediments filled in the chambers from the first tray (26 mg C $cm^{-3}$).

(II) In the second tray, sediments were enriched with a concentrated solution of microalgae *Nannochloropsis* sp (2.4 mg C $cm^{-3}$; Z+L, Langen, Germany).

(III) Cod fish carcass (80 g, equivalent to 33 mg C $cm^{-3}$) was placed on sediments filled in chambers of the third tray.
(IV) For reference investigations each type of sediment (deep-sea sediment, glass beads, sand) was deployed without any addition of organic material.

*Sub-sampling and sample processing*
Immediately after the recovery of the trays, samples from the first sediment centimetre of each chamber were taken by means of plastic syringes with cut-off anterior ends (2 cm in diameter). Sub-sampling of the syringes was carried out in a cold room at 4°C.

**Fig. 1.** Schematic representation of experimental treatments in the sediment trays. It should be noted that only three chambers of each tray were used for the experiment, whereas the remaining chambers were deployed for purposes not directly linked to this study.




*Microbial biomass*
Microbial cell number and biomass was determined by epifluorescence microscopy after staining with acridine orange according to the method of MEYER-REIL (1983) and GROSSMANN & REICHARDT (1991). Microbial biomass was estimated using a conversion factor of 3 x $10^{-13}$ g C $\mu m^{-3}$ (BØRSHEIM et al., 1990). For each sample 40 counted grids from 2 replicate filters were analysed.

*Enzyme activity*
The activity of extracellular and intracellular microbial ester-cleaving enzymes was measured by using the fluorogenic model substrate fluorescein-di-acetate (FDA; Sigma-Aldrich). The method, developed by MEYER-REIL & KÖSTER (1992), determines

the potential hydrolytic activity of microbial organisms particularly involved in the primary decomposition of organic matter. Relative units of fluorescence were calibrated with five FDA standard concentrations between 10 and 300 nmol. Enzyme activity was calculated per volume of sediment and time (nmol $h^{-1}$ $cm^{-3}$) by linear regression. For each sample three measurements per incubation were performed.

*Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis*

Sediment samples for microbial community analysis were stored at -30°C until analysis. Total DNA was extracted and purified using a FastDNA®SPIN Kit for soil (Qbiogene, Heidelberg, Germany) following manufacturer's instructions.

PCR amplification of bacterial 16S rRNA genes, restriction digest with the tetrameric restriction enzymes (*Hha*I or *Msp*I; Amersham Pharmacia Biotech) and determination of end-labelled fragments have been done as described previously (KANZOG & RAMETTE, submitted). T-RFLP analyses were done once for initial samples and treatments subjected to the one-year incubation.

*Data analysis*

Organic substrates added to the sediments were different in their organic carbon content, ranging from 2.4 to 33 mg C $cm^{-3}$. Therefore, in order to compare the different treatments, microbial cell number, biomass, and esterase activity were standardised by a substrate input of 2.4 mg C $cm^{-3}$. All parameters were $log_{10}$-transformed prior to performing statistical analyses in order to normalise their distribution and to ensure that treatments variances were homogeneous (SOKAL & RHOLF, 1995). Three-way Analysis of variance (ANOVA) was applied to each data set to test the effects of each factor (sediment type, organic matter input, incubation time) and of their interactions. *Post-hoc* comparisons between means of microbial cell numbers, biomass, and activities were done using Tukey's Honestly Significant Difference (HSD) test at $P < 0.05$.

For each sample, T-RFLP fingerprints obtained from the two different restriction digests were combined to generate a binary matrix according to the presence or absence of bacterial Operational Taxonomic Units (OTUs; corresponding to terminal restriction fragments). Non-metric multidimensional scaling (NMDS; SCHIFFMAN & REYNOLDS 1981) was used to obtain an ordination of samples based on a matrix of Jaccard dissimilarities of T-RFLP profiles. Various groupings among samples were tested for significance using Analysis of similarities (ANOSIM; CLARKE, 1993) tests.

## Results and discussion

The visual analysis of different treatments after three months revealed that the soft parts of fish carcasses were completely scavenged and only bones remained. No visible difference existed between treatments with yeast and control sediments. The greenish colour of the sediment surface in microalgae-treatments indicated algae remains. After one year, only few bones of the fish carcasses were still present in the respective sediment treatments. The other organic-treatments did not differ visually to the control treatments.

*Changes in microbial abundance and biomass followed by organic substrate addition*

Three-way analyses of variance revealed that each factor taken alone (sediment type, organic matter input, and incubation time) had highly significant effects on microbial cell number, biomass and esterase activity. Additionally, the general significance of factor interactions might explain variations of the measured variables (Table 1; Fig. 2). The interaction between sediment type and incubation time described the largest variation of all pair wise interactions. Both factors displayed also the largest variation when each factor was considered alone (Table 1).

**Table 1**. Effects of organic matter (OM) input, sediment type and incubation time on microbial cell number, biomass and esterase activity

| | Microbial cell number | | Microbial biomass | | Esterase activity (FDA-turnover) | |
|---|---|---|---|---|---|---|
| Factors[1] | df[2] | *F* ratio[3] | df | *F* ratio | df | *F* ratio |
| OM input | 1 | 282.4*** | 1 | 215.7*** | 1 | 18.9*** |
| Sediment | 2 | 690.1*** | 2 | 428.9*** | 2 | 83.8*** |
| Time | 2 | 513.5*** | 2 | 482.2*** | 2 | 58.3*** |
| OM input×Sediment | 2 | 32.1*** | 2 | 33.7*** | 2 | 3.8** |
| Sediment ×Time | 2 | 84.6*** | 2 | 67.5*** | 2 | 11.2*** |
| Time× OM input | 4 | 40.6*** | 4 | 24.3*** | 4 | 3.5*** |
| OM input×Sediment×Time | 4 | 16.5*** | 4 | 17.0*** | 4 | $1.4^{NS}$ |

[1]Three-way ANOVAs were performed on $log_{10}$-transformed variables. Factors consisted of two levels for organic matter (OM) input (presence or absence), and three levels for sediment types (mud, beads, sand) and for incubation time (0 day, 3 month, 1 year).

[2]Degrees of freedom (df) for each factor or interactions thereof.

[3]*F* ratios are ratios of the mean-square value for a given source of variation to the residual mean-square value. Associated probabilities are indicated as (NS) when not significant $P > 0.05$, (*) when $P < 0.05$, (**) when $P < 0.01$, and (***) when $P < 0.001$.

More detailed analyses of changes in microbial cell number, biomass and esterase activity indicated overall higher values for deep-sea sediments than for the artificial sediments and generally higher values for treatments with microalgae than for those enriched with yeast and fish (Fig. 2). Highest values for microbial cell number and biomass in algae-treatments were found in samples of deep-sea sediments and glass beads after three months. Interestingly, there was no significant difference between both sediment types (Tukey HSD, $P > 0.05$), suggesting that algae cells were apparently just as well available to colonise in glass beads than in deep-sea sediments. The one-year incubation led to unchanged (deep-sea sediment) and reduced (glass beads and sand) microbial abundances in these treatments as compared to the three-month incubation. Microbial biomass, however, increased as a function of decreasing sediment particle size (Fig. 2). This increase was mainly based on a substantial growth of microbial cell size as it was detected in deep-sea mud and glass beads. Mean volume of microbial cells in these treatments increased substantially from 0.09 $\mu m^3$ at the beginning of the experiment to 0.5 and 0.2 $\mu m^3$, respectively, after one year. An increase in microbial cell volume is typical of detritus-decomposing bacteria as seen in shallow-water environments (LINLEY & NEWELL, 1984) as well as the deep sea (TURLEY & LOCHTE, 1990).

Microbial abundance in treatments enriched with yeast increased significantly after three month only in deep-sea sediments and glass beads. In sediments enriched with fish, an increase was significant only for artificial sediments. Microbial biomass in yeast- and fish-treatments did not change in any of the different sediment types after three months. Changes in microbial abundance in treatments with yeast and fish investigated after one-year deployment validated the trend towards decreasing values observed for the microalgae-treatments. Indeed, microbial cell number was unchanged in deep-sea sediment or reduced in the artificial sediments compared to the three-month experiment. This may indicate that the microbial populations of those treatments were in a stationary growth phase already after three months of treatment colonisation.

*Changes in microbial hydrolytic enzyme activity following organic substrate addition*

Measurements of esterase activity showed distinct differences between treatments (Fig. 2). Here the type of the colonised sediment appeared to have the largest effect on potential activity of microbial organisms (Table 1). Similar to the observations for microbial abundance and biomass, the most explicit response was found in treatments with microalgae (Fig. 2).

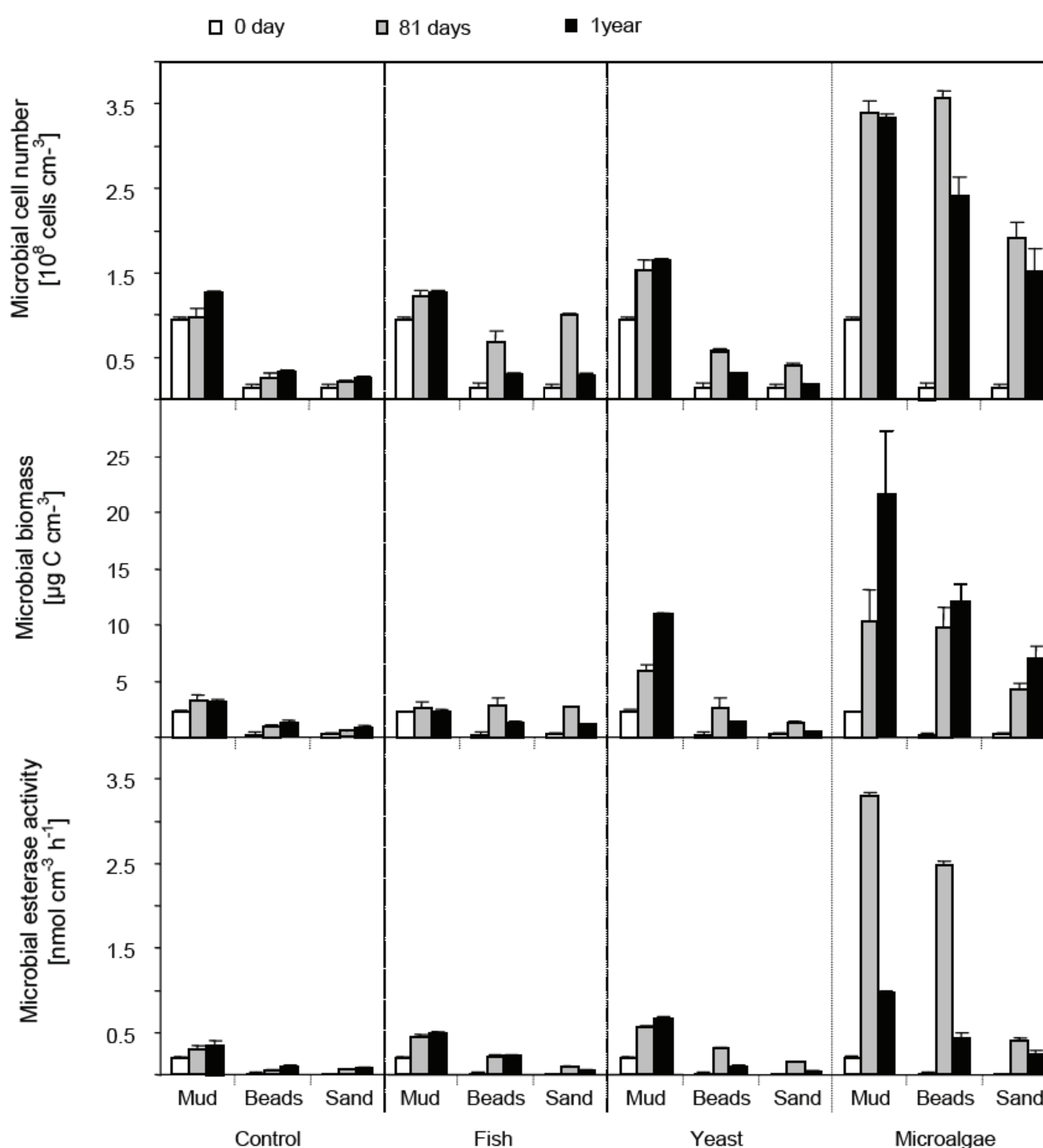



**Fig. 2.** Mean microbial cell number, biomass, and esterase activity in control and organic substrate-treatments at different incubation times. Significance of differences between mean treatments was determined by *post hoc* Tukey HSD tests at $P < 0.05$ were.

Activity level in these treatments was significant higher in all sediment types than in the respective controls, regardless of incubation time. Furthermore, there was also a clear relationship between increasing particle size (from mud, glass beads to sand) and decreasing esterase activity. Such results further support the idea that small particles offer more areas for bacterial attachment, and therefore for colonisation (e.g. DEFLAUN & MAYER, 1983) and activity than coarser particles, independent of the availability of potential food for the organisms (Fig. 2). This decreasing trend in enzymatic activity with increasing sediment particles size was also observed after one year, but activity values were generally lower as compared to the three-month incubation (Fig. 2), leading to the assumption that most of the algae material was already utilised. Increased esterase activity in treatments with fish and yeast were detected exclusively in deep-sea mud and glass beads after the three-month experiment and values were substantially lower than those from the microalgae-treatments.

Overall, results suggest that the utilisation of organic matter introduced by microalgae provided sufficient energy for an increase of the microbial standing stock already after an incubation period of three months. This observation was not surprising since previous studies have shown a rapid response (within days to weeks) by enhanced growth and activity of deep-sea microbial communities after the addition of 'fresh' organic matter such as $^{13}C$-enriched diatoms (MOODLEY et al., 2002), dissolved and particulate organic matter derived from phytoplankton (BOETIUS & LOCHTE, 1994) and natural deep-sea phytodetritus (LOCHTE & TURLEY, 1988). The trend towards decreasing microbial biomass and activity as a function of increasing sediment particle size may indicate that detrital matter was more available for microbial communities in natural than in the artificial sediments. A similar relationship between microbial activity and different sediment types enriched with chitin was reported previously by KANZOG & RAMETTE (submitted). Microbial response to yeast input was less pronounced than in treatments with microalgae, presumably caused by the higher content of refractory compounds which were less accessible for bacterial degradation (e.g. rigid chitinous cell wall). Results from treatments with fish indicated that microbial organisms in deep-sea sediments might benefit just marginally from the availability of large food falls. Experiments conducted in the Santa Catalina Basin by SMITH (1985, 1986) showed that the organic content of food falls such as fish carcass was almost totally utilised by motile scavengers such as crustaceans and bacteria were excluded to a large extent from the decomposition process. Although chambers of trays were covered by a grid (5 mm mesh size), meio- and macrofauna organisms could not be excluded as potential

predators for this large food sources. Previous tray-experiments conducted in the deep sea have shown that the overall colonisation rate by macrofauna organisms was clearly higher in screened (2 mm mesh size) than in unscreened sediment trays after an incubation of two months, possible caused by the protection from larger predatory animals (GRASSLE & MORSE-PORTEOUS, 1987). In the same study, juvenile isopods (*Gnathia* sp. A) and lyssianassid amphipods (*Tryphosella* sp. B) were present after two months in screened trays that were filled with sediments and fish. Lyssianassid amphipods are known as deep-sea scavengers attracted to dead animal tissue and also juvenile specimen smaller than 15 mm were found during decomposition of large fish baits in the area of investigation (PREMKE et al., 2006). Hence, it is likely that small macrofauna individuals contributed to a large extent to the decomposition of the fish carcass. The implication of the rapid consumption of the fish carcass by scavengers is that the energy and nutrients would be dissipated before microorganisms are able to benefit from them. Interpretation of the short-term microbial response in artificial sediments amended with fish should be carefully done. Due to larger interstices between particles as a result of packing, nutrients released from fish decomposition might have been more available for microbial organisms in medium (glass beads) and coarse (sandy) sediments than in fine-grained natural sediments (SHARMA & MCINERNEY, 1994). In summary, removal of organic materials by invertebrate feeding appeared to exceed the rate of degradation by microorganisms. Furthermore, it is most likely that microbial biomass was modified by potential grazers that were able to drop through the grid. The fact that microbial organisms, especially bacteria, are more susceptible to predation in sediments with smooth particles than in those composed of particles where shelter in form of surface irregularities are present (NICKELS et al., 1981; FINDLAY et al., 1992) may explain the decrease in microbial biomass, particular pronounced in the artificial sediments after one year.

*Changes in bacterial community structure followed by organic substrate addition*

The NMDS ordination of T-RFLP data suggest that the patterns underlying bacterial community structure in different treatments are rather complex (Fig. 3). When samples were grouped by sediment types (deep-sea mud, glass beads and sand) and incubation time (0 days and one year), the ANOSIM test did not show significant differences in the bacterial assemblages. Control samples without any additional organic substrate and samples enriched with the organic substrates fell, however, into

two slightly overlapping (Fig. 3), but significantly different groups (ANOSIM $R$ = 0.21, $P$ = 0.04).

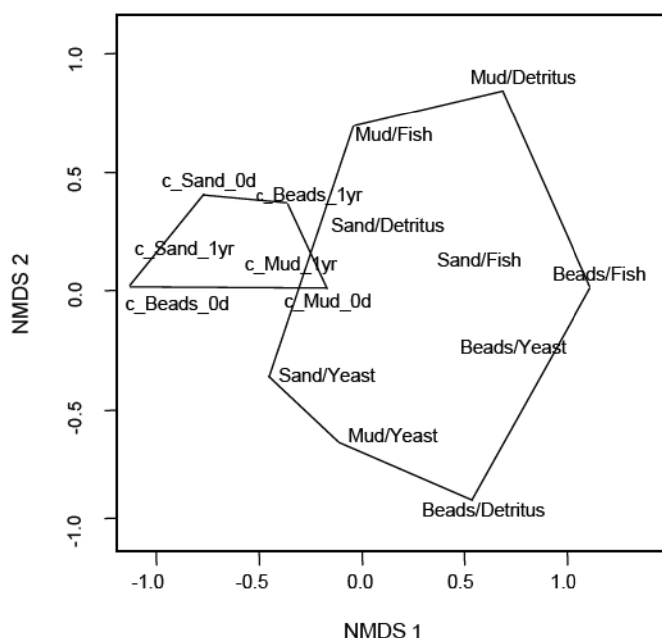



**Fig. 3.** Non-metric multidimensional scaling (NMDS) ordination of T-RFLP data based on Jaccard dissimilarities among samples. Geometric shapes were superimposed on the ordination to depict organic substrate-amended samples vs. control samples (also with a 'c' prefix).

In contrast to observations reported for shallow waters (BISSETT et al., 2007; EILER et al., 2003), increased food availability in the deep sea often leads to a decreased diversity (SNELGROVE et al., 1996). To evaluate whether this hypothesis was supported by our data, bacterial Operational Taxonomic Units (OTU) richness for the different samples was analysed in more detail (Fig. 4). The total number of different restriction fragment (corresponding to different OTUs) was higher in deep-sea sediment than in sand and glass beads. Interestingly, a trend towards a reduction of OTU richness in the long-term incubation experiment was evident in natural sediments, regardless of organic substrate inputs. More unique OTUs (unique here being defined as OTU exclusively occurring in a given treatment) were found in organic enriched deep-sea sediments than in the respective control after one year. This suggests that the overall community shift may correspond to a trend toward more specific subpopulations which were able to use the respective substrate, i.e. a decrease in diversity. Noticeably, there was a trend towards increasing OTU richness in the artificial sediments after one year (Fig. 4) which might be explained by the gradual colonisation through different bacterial populations during the long-term experiment. Despite organic substrate addition, this

increase was associated with an increasing number of unique OTUs in samples with glass beads.

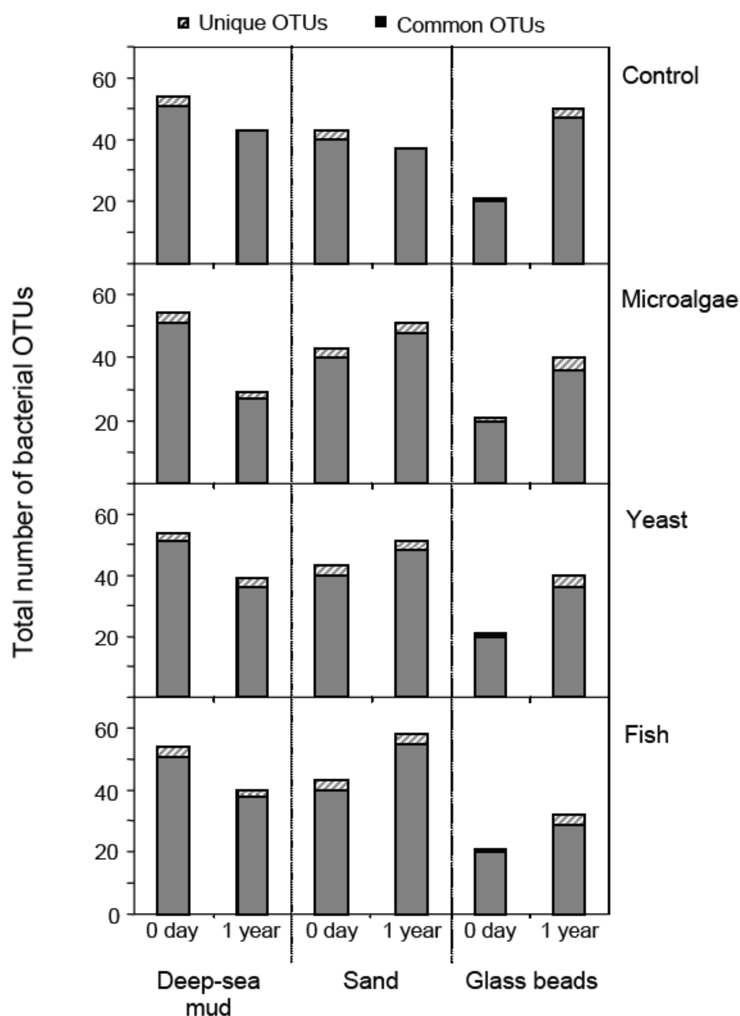



**Fig. 4.** OTU richness in the different treatments. Number of total and unique bacterial OTUs (i.e. exclusively occurring in a given treatment) as obtained by T-RFLP at 0 day and after one-year incubation.

Although the present data are insufficient to demonstrate a causal link between the shifts in community structure and the different factors investigated (OM input, sediment type and incubation time), it raises the assumption that among the effects of organic enrichments, the structure of sediments itself might also drive the successions of deep-sea microbial population (KANZOG & RAMETTE, submitted).

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## References

Barnett PRO, Watson J & Conelly D (1984) A multiple corer for taking virtually undisturbed samples from shelf, bathyal and abyssal sediments. *Oceanol Acta* **7**: 399-408.

Bissett A, Burke C, Cook PLM & Bowman JP (2007) Bacterial community shifts in organically perturbed sediments. *Environ Microbiol* **9**: 46-60.

Boetius A & Lochte K (1994) Regulation of microbial enzymatic degradation of organic matter in deep-sea sediments. *Mar Ecol Prog Ser* **104**: 299-307.

Børsheim KY, Bratbak G & Heldal M (1990) Enumeration and biomass estimation of planktonic bacteria and viruses by Transmission Electron Microscopy (TEM). *Appl Environ Microbiol* **56**: 352-356.

Bühring SI, Lampadariou N, Moodley L, Tselepides A & Witte U (2006) Benthic microbial and whole-community response to different amounts of $^{13}$C-enriched algae: *In situ* experiments in the deep Cretan Sea (Eastern Mediterranean). *Limnol Oceanogr* **51**: 157-165.

Christiansen B & Boetius A (2000) Mass sedimentation of the swimming crab *Charybdis smithii* (Crustacea: Decapoda) in the deep Arabian Sea. *Deep Sea Res II* **47**: 2673-2685.

Clarke KR (1993) Non-parametric multivariate analysis of changes in community structure. *Aust J Ecol* **18**: 117-143.

Clarke KR & Gorley RN (2001) PRIMER v5: User manual/tutorial, PRIMER-E. Plymouth UK.

Danovaro R, Marrale D, Dell`Anno A, Della Croce N, Tselepides A & Fabiano M (2000) Bacterial response to seasonal changes in labile organic matter composition on the continental shelf and bathyal sediments of Cretan Sea. *Prog Oceanogr* **46**: 345-366.

DeFlaun MF & Mayer LM (1983) Relationships between bacteria and grain surfaces in intertidal sediments. Limnology and Oceanography, *Limnol Oceanogr* **28**: 873-881.

Eiler A, Langenheder S, Bertilsson S & Tranvik LJ (2003) Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. *Appl Environ Microbiol* **69**: 3701-3709.

Findlay RH, Kim SK & Butman CA (1992) Colonisation of freshly deposited barite and silica sediments by marine microorganisms in a laboratory flume flow. *Mar Ecol Prog Ser* **90**: 73-88.

Gooday AJ & Turley CM (1990) Responses of benthic organisms to inputs of organic material to the ocean floor. *Phil Trans R Soc Lond A Math Phys Sci* **331**: 119-138.

Grassle JF & Morse-Porteous LS (1987) Macrofaunal colonisation of disturbed deep-sea environments and the structure of deep-sea benthic communities. *Deep Sea Res* **34**: 1911-1987.

Grossmann S & Reichardt W (1991) Impact of *Arenicola marina* on bacteria in intertidal sediments. *Mar Ecol Prog Ser* **77**: 85-93.

Hassol SJ (2004) Impacts of a warming Arctic: Arctic climate impact and assessment. Cambridge University Press, pp. 144.

Kanzog C & Ramette A Microbial colonisation of artificial and deep-sea sediments in the Arctic Ocean. submitted Dec. 2007 in *FEMS Microbiology Ecology*

Kohlmeyer H (1980) Bacterial attack on wood and cellophane in the deep-sea. In: Biodeterioration: proc. 4th Int. Symposium. Oxley T.A., Allsopp D., Becker G. (eds.), London: Pitman Publishing Ltd, pp. 187-192.

Linley EAS & Newell RC (1984) Estimates of bacterial growth yields based on plant detritus. *Bull Mar Sci* **35**: 409-425.

Lochte K & Turley CM (1988) Bacteria and cyanobacteria associated with phytodetritus in the deep sea. *Nature* **333**: 67-69.

Meyer-Reil LA (1983) Benthic response to sedimentation events during autumn to spring at a shallow water station in the Western Kiel Bight. *Mar Biol* **77**: 247-256.

Meyer-Reil LA & Köster M (1992) Microbial life in pelagic sediments: the impact of environmental parameters on enzymatic degradation of organic material. *Mar Ecol Prog Ser* **81**: 65-72.

Moodley L, Middelburg JJ, Boschker HTS, Duineveld GCA, Pel R, Herman PMJ & Heip CHR (2002) Bacteria and Foraminifera: key players in a short-term deep-sea benthic response to phytodetritus. *Mar Ecol Prog Ser* **236**: 23-29.

Nickels JS, Bobbie RJ, Martz RF, Smith GA, White DC & Richards NL (1981) Effect of silicate grain shape, structure, and location on the biomass and community structure of colonising marine microbiota. *Appl Environ Microbiol* **41**: 1262-1268.

Palacios C, Zbinden M, Baco AR, Treude T, Smith CR, Gaill F, Lebaron P & Boetius A (2006) Microbial ecology of deep-sea sunken wood: quantitative measurements of bacterial biomass and cellulolytic activities. *Cah Biol Mar* **47**: 415-420.

Premke K, Klages M & Arntz WE (2006) Aggregations of Arctic deep-sea scavengers at large food falls: temporal distribution, consumption rates and population structure. *Mar Ecol Prog Ser* **325**: 121-135.

Rice AL, Billett DSM, Fry J, John AWG, Lampitt RS, Mantoura RFC & Morris RJ (1986) Seasonal deposition of phytodetritus to the deep seafloor. *Proc R Soc Edinburgh* **88B**: 265-279.

Schiffman SS & Reynolds ML (1981) Introduction to multidimensional scaling. Theory, methods and applications London, UK: Academic Press.

Sharma PK & McInerney MJ (1994) Effect of grain size in bacterial penetration, reproduction, and metabolic activity in porous glass beads chambers. *Appl Environ Microbiol* **60**: 1481-1486.

Smith CR (1985) Food for the deep sea: utilisation, dispersal, and flux of nekton falls at the Santa Catalina Basin floor. *Deep Sea Res I* **32**: 417-442.

Smith CR (1986) Nekton falls, low-intensity disturbance and community structure of infaunal benthos in the deep-sea. *J Mar Res* **44**: 567-700.

Smith CR & Baco AR (2003) Ecology of whale falls at the deep-seafloor. *Oceanogr Mar Biol Ann Rev* **41**: 311-354.

Sokal RR & Rohlf FJ (1995) Biometry, 3rd edition. Freeman WH and company, San Francisco.

Soltwedel T, Bauerfeind E & Bergmann M *et al*. (2005) HAUSGARTEN - multidisciplinary investigations at a deep-sea long-term observatory in the Arctic Ocean. *Oceanogr* **18**: 46-61.

Suess E (1980) Particulate organic carbon flux in the oceans - surface productivity and oxygen utilisation. *Nature*, **288**, 260-263.

Turley CM & Lochte K (1990) Microbial response to the input of fresh detritus to the deep-sea bed. *Palaeogeogr Palaeoclimatol Palaeoecol* **9**: 3-23.

Turley CM, Lochte K & Patterson DJ (1988) A barophilic flagellate isolated from 4500 m in the mid-North Atlantic. *Deep Sea Res* **35**: 1079-1092.

Turley CM, Lochte K & Lampitt RS (1995) Transformations of biogenic particles during sedimentation in the northeastern Atlantic. *Phil Trans R Soc Lond B* **348**: 179-189.

Wirsen CO & Jannasch HW (1986) Microbial transformations in deep-sea sediments: free-vehicle studies. *Mar Biol* **91**: 277-284.

Witte U, Aberle N, Sand M & Wenzhöfer F (2003a) Rapid response of a deep-sea benthic community to POM enrichment: an *in situ* experimental study. *Mar Ecol Prog Ser* **251**: 27-36.

Witte U, Wenzhöfer F, Sommer S, Boetius A, Heinz P, Aberle N, Sand M, Abraham W-R, Jörgensen BB & Pfannkuche O (2003b) *In situ* experimental evidence of the fate of a phytodetritus pulse at the abyssal seafloor. *Nature* **424**: 763-766.

# General Discussion

In the first part of this section, different sediment tray designs associated with free-falling devices will be discussed with special emphasis on the suitability for experimentation at the deep seafloor. The second part focuses on *in situ* observations of benthic microbial communities in the Arctic deep sea, particularly on functional and structural changes as a response to changes in environmental conditions such as POM supply and sediment structure. Finally, some future perspectives will be pointed out.

## Progress in deep-water studies using benthic experimental gear: Sediment Tray Free Vehicles (STFVs)

Sediment Tray Free Vehicles (STFVs) can be excellent experimental tools to improve our understanding of the complex ecological processes that occur in the deep sea. Especially colonisation experiments have yielded important generalisations on life in the deep-sea environment by using STFVs (e.g. DESBRUYÈRES et al., 1985; GRASSLE & MORSE-PORTEOUS, 1987). Generally, one important aspect in designing and deploying experimental instruments at the seafloor is to minimise alteration in the bottom flow regime. Flow artefacts affect sample collection depending on the kind and magnitude of the flow disturbances, and on the sensitivity of the organisms to such hydrodynamic effects. Today several different designs of STFVs are available and the problem of hydrodynamic bias has been circumvented by improving framework and tray designs. STFV equipped with square sediment trays (Fig. 11a) was designed at the Woods Hole Oceanographic Institution and results from several deployments were described in a number of publications (e.g. LEVIN & SMITH, 1984; MACIOLEK et al., 1987). Flume simulations indicated that a second boundary layer developed above the elevated experimental sediment surface and led to complex, constantly changing flow conditions over the tray sediments (SNELGROVE et al., 1995). Tray-induced flow disturbances may influence benthic biological processes in various directions, including transport of organic matter, sediment deposition, and regulation of nutrient and oxygen flux in the sediment-water interface. In order to reduce flow disturbances to the natural boundary

layer, tray design was modified by using round shaped sediment trays with a sloping edge to avoid eddy shedding downstream of the leading edge (Fig. 11b; SNELGROVE et al., 1995). Both types of STFV (Fig. 11a, b) have in common that lids cover the trays to retain sediments during transport to and from the bottom. When the STFV rests on the seafloor, lids are raised above the top of the trays. Acoustic command actuates release of the ballast (steel plates) so that tray floats up to meet the lid. The fact that these lids are permanently present in some distance above the experimental sediment surface may additionally affect vertical inflow of particulate matter settling through the water column over time and supply of larvae and other organisms. This is certainly a significant disadvantage when the experimental approach addresses the investigation of colonisation processes under natural conditions.

A recent modification of STFV developed by the Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER, Fance) was used for the studies presented in this thesis. As described previously in the 'Material and Methods' section, this STFV supports rotating discs to open and close the chambers inside the trays without additional lids. Furthermore, in contrast to other STFV types, the buoyancy spheres are mounted on a cable, floating a couple of meters above the frame. Thus, the vertical flow towards the experimental sediment surfaces in the trays is largely unaffected due to the lack of a screening effect by a lid and the general simple, 'open' design of this STFV (Fig. 11c).

Experimental sediments from STFVs described in Figure 11 are physically isolated above the natural bottom, precluding colonisation by lateral movement from the surrounding sediment, which can bias colonisation and successional patterns as reported by SMITH & BRUMSICKLE (1989) for macrofaunal colonisation in a low-energy intertidal site. When within-sediment migration is less likely to be important, then experiments with sediment trays raised above the seafloor can be a valuable approach. STFVs provide the opportunity to obtain information on temporal variability in rates of colonisation and effects of treatments in comparison to controls. With regard to the current available types of STFVs, the IFREMER design, which was used for the studies presented here, seems to be most suitable for conducting such experiments under virtually natural conditions at the seafloor.

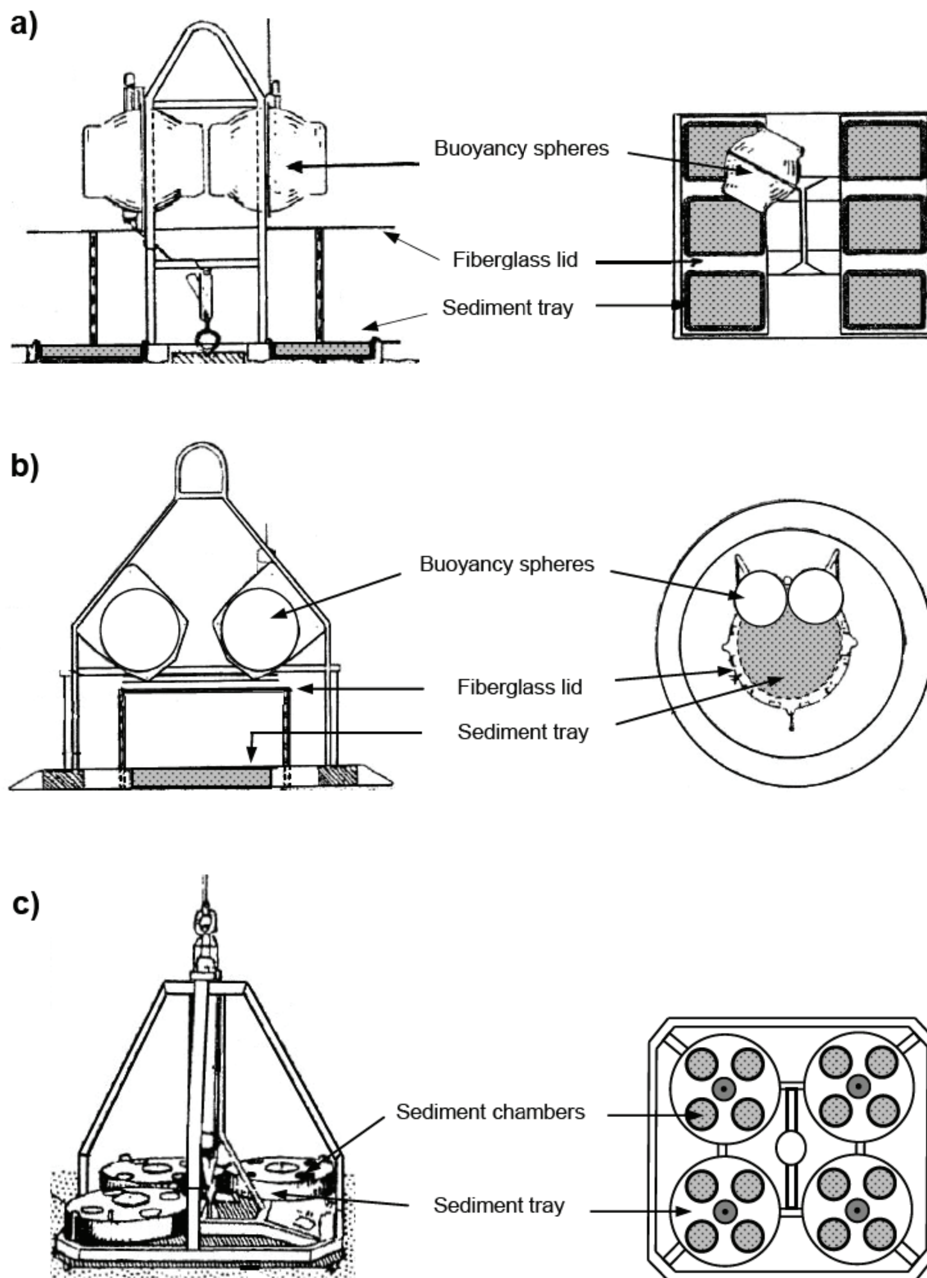


**Fig. 11.** Sediment Tray Free Vehicles (STFVs) designed at the Woods Hole Oceanographic Institution carrying six square trays (**a**). Improved design composed of a circular tray located at the centre of a large disc (**b**; modified from MACIOLEK et al., 1987). Recent design of STFV with rotating discs designed by the IFREMER (**c**; see **Fig. 9**, 'Material and Methods' section for detailed description). Left hand is side view; right hand is top view.

Until now, STFVs were mainly applied to investigate the role of non-equilibrium processes in controlling diversity and community structure of macrofauna organisms (e.g. LEVIN & SMITH, 1984; GRASSLE & MORSE-PORTEOUS, 1987).
The present study is the first that used this kind of experimental approach to study deep-sea microbial populations. Although difficult to control for all experimental biases when working under *in situ* conditions, the assumption is most likely that all treatments were similarly affected by environmental conditions. The experimental approach with STFVs allows thus comparing individual treatments with each other. Therefore, the use of STFVs may contribute to a better understanding of the effects of changes in environmental factors on microbial community structure and functioning under deep-sea conditions.

## Functional and structural response of microbial communities to chitin supply to the Arctic deep seafloor

In my thesis, I focused on POM availability at the deep seafloor, because food energy is thought to be one of the most important factor regulating the standing stock, structure, and function of deep-sea microbial communities. Chitin was chosen for the experiments to simulate a sudden massive input of refractory organic material as would be introduced by large food falls. Chitin degradation by microbial organisms in the marine environment follows a highly complex series of coordinated processes, comprising at least four major steps: sensing of chitin either by random collision or by chemotaxis; attachment to the chitin to stay in close proximity to the nutrients; expression of enzymes and other proteins required for catabolism of the polymer; uptake and catabolism of the hydrolysis products of the glycosidases (KEYHANI & ROSEMAN, 1999). Laboratory based studies have shown that chitin availability induces the synthesis of specific enzymes and that bacteria may than utilise this carbon and nitrogen source with relatively high growth efficiencies (e.g. DEMING, 1985; BOETIUS & LOCHTE, 1996). The calculations of VETTER & DEMING (1994) indicated that secretion of enzymes can only result in an energy gain via uptake of produced hydrolysate when external concentrations of polymeric substrates are very high. The amounts of chitin used for these *in situ* experiments (1 to 7 g $m^{-2}$) were well above the background value of chitin in deep-sea sediments of 0.1 mg $m^{-2}$ (POULICEK & JEUNIAUX, 1989). Consistent with these previous findings, high chitinolytic enzyme activities and microbial biomasses were detected in sediment enriched with chitin, substantially exceeding

those in unenriched control sediments. These significant functional responses occurred only after one year, whereas no noticeable differences were found after seven days (manuscript I). The assumption that within one week, pulses of chitin to the seafloor has no effect on microbial activity and biomass is corroborated by findings reported in manuscript II. Additionally enrichments experiments conducted at *in situ* temperature (-1°C) and atmospheric pressure suggested that the activation of the specific extracellular enzyme precedes any microbial growth, which was only detectable approximately 12 days after the chitin supply to sediments from the central HAUSGARTEN station (KANZOG, unpublished data). This indicated that the production of extracellular enzymes as one of the first steps in the benthic microbial response to chitin input, which is a precondition for microbial cells to gain access to organic macromolecules. Microbial growth in deep-sea sediments is characterised by long lag phases of about 10 days (DEMING, 1985; BOETIUS & LOCHTE, 1996), and thus seven days was apparently too short to record significant increases in microbial abundance, even after the enrichment with chitin.

*In situ* experiments described in manuscript III were carried out to assess microbial response to food falls composed of a large amount of microalgae, yeast and fish carcass. The most significant changes in growth and activity were found as a reaction of microbial organisms in sediments enriched with microalgae. Results from treatments with yeast and fish are not further considered for the following discussion. The addition of microalgae showed clear microbial responses after three months of incubation, suggesting that *in situ* reaction of deep-sea microbial organisms to inputs of organic matter by enhanced biomass production is detectable after weeks or months, thus in accordance with earlier findings based on *in situ* measurements of substrate utilisation rates in similar sediments (JANNASCH & WIRSEN, 1980). The increased microbial growth and activity three months after the addition of microalgae (*Nannochloropsis* sp.) may represent a ‘maximum’ response, as the utilised algae material provided an easily degradable carbon source which was not representative for an *in situ* input of pre-processed, particulate carbon reaching the Arctic deep seafloor. Microbial biomass in algae-treatments was nevertheless substantially lower than those detected in treatments with chitin. This was probably attributed to competition for this food source composed of algae material with other opportunistic species of deep-sea organisms. Rapid responses to phytodetritus input by a number of deep-sea benthic taxonomic groups other than microbes, ranging from protozoa (GOODAY, 1988; DRAZEN et al., 1998), megafauna (Smith et al., 1994; Lauerman et al., 1997; BETT et al., 2001), to

macrofauna (SWEETMAN & WITTE, 2008), have been identified. Microbial organisms are perhaps the greatest opportunists reflecting their rapid reaction by increased enzyme production, DNA and protein synthesis, and respiration after the addition of POM (LOCHTE, 1992; TURLEY et al., 1995). It is most likely, however, that larger organisms were also able to benefit from this potential food input due to the experimental design.
With the addition of chitin, an important source of both carbon and nitrogen was supplied to those microbial organisms with chitinolytic activities, which were able to efficiently utilise this food source for their maintenance and growth. This clearly showed the metabolic versatility of deep-sea adapted microbes, enabling the breakdown of this less labile refractory substrate that was unavailable to other organisms. My results suggested that in response to a large chitin supply, bacterial communities undergone a shift in their structure before they were able to react by functional changes (manuscript I). These shifts in the community structure seemed to occur very rapid (within seven days) when chitin became available (manuscript I). Virtually nothing is known about possible shifts in benthic deep-sea microbial community structure that could be associated with organic matter supply, although such shifts are evident in the community structure of megafauna and macrofauna organisms (e.g. SNELGROVE et al., 1996; BILLETT et al., 2001). For example, *in situ* experiments using colonisation trays showed a fauna with high density but low diversity composed of more opportunistic species in organic enriched sediments, while control unenriched trays were found to host a low density but higher diverse fauna (SNELGROVE et al., 1996). It is therefore reasonable to expect that chitin addition led to a decreased diversity and to a community which was dominated by few chitin-specific bacteria. Although this cannot be verified by the present data, a trend towards a reduction of OTU richness only in sediments enriched with chitin after one year seems to corroborate this assumption. These observations may support the general hypothesis that increased food availability in an oligotrophic environment such as the deep sea may lead to a shift to more opportunistic species, increased dominance, and decreased diversity (SNELGROVE et al., 1996).

## Effects of different sediment types on structure and functioning of colonising Arctic deep-sea microbial communities

From the early literature, general relationships between the nature of sediments and benthic microbiota are well known. Fine-grained sediments carry high organic matter contents and support a higher microbial biomass as compared to coarse sediments (e.g. HARGRAVE, 1972; DALE, 1974; DEFLAUN & MAYER, 1983; MEYER-REIL, 1993; MAYER, 1994). This means that grain size is inversely correlated to available surface area, porosity, organic matter content, and also microbial biomass. The effects of sediment properties are obviously more strongly reflected in microbial biomass than in the activity of microbial communities, which seems to be primarily controlled by the availability and nutritional quality of degradable organic matter (BOTT & KAPLAN, 1985; FABIANO & DANOVARO, 1998). These findings were mainly based on laboratory studies (e.g. YAMAMOTO & LOPEZ, 1985; FINDLAY et al., 1992; SHARMA & MCINERNEY, 1994; KÖSTER et al., 2005), but colonisation patterns in laboratory microcosms may differ from field situation due to experimental artefacts (TARGATZ et al., 1983). For logistic reasons, *in situ* studies aiming at microbial colonisation in deep-ocean environments are scarce.

The aim of experiments described in manuscript II and III was to investigate microbial colonisation (measured in growth and activity) due to different sediment types, ranging from fine to coarse sediments, after the incubation at the deep seafloor. In addition to fine-grained deep-sea sediments, artificial sediments were selected to depict simple model sediment particles within the size range representative for coarse sediments. Coarse sediments of terriginous origin can be transported to the deep ocean by turbidity currents and sediment slumps from the continental slopes (PROSPERO, 1981). Drilling activities during offshore exploration result in the deposition of drill cuttings in size of coarse gravel which change the bottom grain structure (NEFF et al., 2000; BREUER et al., 2004). In polar regions, ice-rafted sediments also contribute to the coarse sediment fraction at the deep-sea bed (GAGE & TYLER, 1991).

Results indicated that the structure and function of microbial deep-sea populations was clearly influenced by the nature of sediments (i.e. sediment particle size, particle shape, organic carbon content). Generally, my observations are consistent with previous findings that sediments composed of small particles with surface irregularities and high organic carbon content promote higher microbial attachment, and therefore higher colonisation and activity than sediments with coarser particles, having smooth

surfaces and low organic carbon content (e.g. NICKELS et al., 1981). Regardless of substrate addition and incubation time, deep-sea sediments always exhibited higher values for microbial abundance, biomass and activity compared to the artificial sediments glass beads and sand. Since many of the enzymes produced by microorganisms are attached to their membrane, microbial organisms must come in contact with POM to hydrolyse it. The observations reported in manuscript II suggested that chitin particles were apparently more available for the colonisation by chitinolytic microorganism, and thus for degradation in deep-sea sediments than in the artificial sediments.

In summary, present *in situ* studies give evidence of clear chitin-dependent responses of microbial communities, indicating that microbial growth in sediments of the deep Arctic Ocean is limited by carbon and nitrogen sources. My observations demonstrated the significance of nutrient availability in controlling the microbial response to changes in their environment, and the importance of efficient and economic utilisation of the sedimenting POM on the survival of microbial organisms in this extreme environment. Experiments with different sediment types emphasised the role of sediment texture as significant environmental parameter in structuring deep-sea microbial communities. In addition to that, sediment texture was found to be also most important in determining the rate or organic matter decomposition due to influencing the accessibility of nutrient particles in sediments, and thus availability and colonisation of these particles by microbial organisms.

## Future perspectives

This experimental approach by using a STFV allowed for the first time to identify some lower and upper time bounds within which significant changes in microbial community functioning and structure took place at the Arctic deep seafloor. Because the artificial nature of STFV-experiments may influence biological processes by creating hydrodynamic biases, *in situ* studies placing sediments flush with the seafloor may provide a useful alternative to overcome this problem. Flume simulations have shown that the flow across so called ‘Flush Sediment Trays’ closely approximates that predicted for the natural environment (SNELGROVE et al., 1995). In addition, this tray design avoids physically isolation of experimental sediments from the surrounding bottom and, therefore, exhibits a further improvement over sediment trays that sit

elevated above the seafloor. Until now, experiments using these sediment trays in the deep sea were carried out by means of submersibles (SNELGROVE et al., 1992; 1994). The experimental approach of 'Flush Sediment Trays' in association with a benthic lander provide the opportunity for *in situ* experiments without the use of submersibles (Fig. 12) and were used first at the deep-sea long-term observatory HAUSGARTEN during *RV Polarstern* cruise in summer 2007.

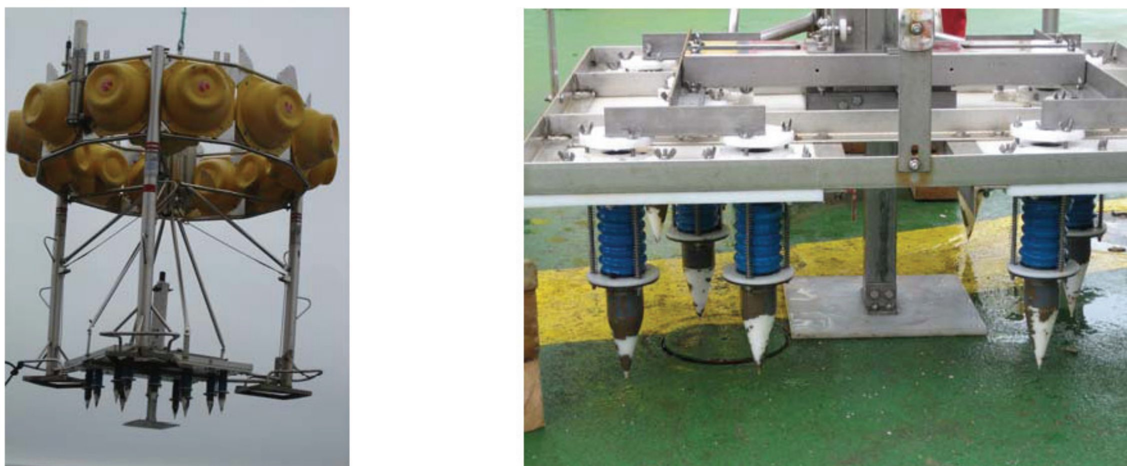

**Fig. 12.** Flush Sediment Trays mounted on a benthic lander. This tray design allows colonisation of defaunated sediments filled in the trays through windows covered by a mesh (mesh size 250 µm) at the periphery of the tray. Rubber gaiter covers the tray during transport to and from the bottom (AWI, Germany).

Concerning future research in deep-sea microbial ecology, the proceeding global warming gives reason to study more in detail microbial response to changes in their environmental conditions, particularly to variations in POM supply in high northern latitudes. Under the force of a changing physical environment due to dramatic northwards retreat of the MIZ and concomitant de-stratification of ice-free waters, increased vertical mixing and primary production (MCGOWAN et al., 1998), as well as shifts in species composition and population dynamics are expected. Indeed, recent studies on the distribution of zooplankton across the central Greenland Sea suggest replacement of key copepod species from the Arctic Ocean (*Calanus glacialis* and *C. hyperboreus*) by those from the North Atlantic (*C. finmarchicus*, HIRCHE & KOSOBOKOVA, 2007). The export of *C. finmarchicu* characterised by a high biomass and productivity would represent a large source of carbon that will sink in the area of

expatriation. WĘSŁAWSKI & LEGEŻYŃSKA (1998) assume that shifts to low-salinity waters due to massive discharge of freshwater following an increase in melting of Arctic glaciers may lead to enhanced mortality of zooplankton organisms such as calanoid copepods. Climate-induced variations in the distribution and abundance of zooplankton organisms may lead to significant changes in organic matter inputs, including chitin production and transport in the aquatic biosphere of high northern latitudes, which will have effects on food supply even to the deep seafloor. It is therefore most important to understand more fully the microbial ecology of deep-sea bacteria due to their important role in nutrient recycling and maintenance of deep-sea ecosystem balance.

There are difficulties associated with assessment of rates of chitin degradation in natural environments and therefore, we do not know much about the rate of chitin degradation and its ecological role in carbon and nitrogen cycling, especially in deep-sea ecosystems. As particulate substrate, typical procedures designed for soluble organic substrates are not directly applicable. Also, the experimental substrate may be supplied to the environment in various forms ranging from the crude natural state to purified forms, or even radiolabeled forms. Most previous investigators working in temperate habitats have used gravimetric procedures for analysing chitin degradation. In this procedure, natural carapaces of larger invertebrates such as shrimp or crab have been placed in the environment, recovered and weighted at subsequent time intervals (HOOD & MEYERS, 1977). In contrast, uniformly $^{14}C$-labeled chitin, prepared by injecting molting blue crabs with the chitin precursors N-acetyl-D-[1-$^{14}C$]glucosamine, was used as a substrate by BOYER & KATOR (1985). In this regard, experimental approaches using $^{14}C$-labelled chitin may enhance our knowledge about the fate and rate of chitin and should considerably increase the understanding about the relative contribution of chitin carbon to supporting bacterial growth in deep-sea environments.

Additionally, there are still so many open questions about environmental factors that drive microbial functioning and diversity at the ocean floor and the spatial and temporal scales on which they operate. In this respect, the role of environmental conditions (e.g. deep-sea storms, resuspension events) needs to be incorporated in our understanding and conceptual and mathematical models of the microbial role in the biogeochemistry of the deep sea and hence the cycling of carbon on a global scale. Future studies should focus on the phylogenetic determination of deep-sea microbial communities as it is essential for the identification of microbes that are involved in the degradation and/mineralisation of organic matter (e.g. chitin) and/or favoured by a certain sediment

texture. The application of molecule techniques will generally contribute to more information about microbial biodiversity in the deep sea, which is proposed to be high in deep-sea sediments due to wide ranges in temperature, pressure and food resources (DEMING & BAROSS, 2000).

# References


Allison PA (1988) The role of anoxia in the decay and mineralisation of proteinaceous macro-fossils. *Palaeobiology*, **14**, 139-154.

Aller JY & Aller RC (1986) Evidence for localised enhancement of biological activity associated with tube and burrows structures in deep-sea sediments at the HEBBLE site, western North Atlantic. *Deep Sea Research*, **33**, 755-790.

Alongi DM (1990) Bacterial growth rates, production and estimates of detrital carbon utilisation in deep-sea sediments of the Solomon and Coral Seas. *Deep Sea Research*, **37A**, 731-746.

Baco AR, Smith CR, Peek AS, Roderick GK & Vrijenhoek RC (1999) The phylogenetic relationships of whale-fall vesicomyid clams based on mitochondrial COI DNA sequences. *Marine Ecology Progress Series*, **182**, 37-147.

Ballard RD (1977) Notes on a major oceanographic find. *Oceanus*, **20**, 35-44.

Barnett PRO, Watson J & Conelly D (1984) A multiple corer for taking virtually undisturbed samples from shelf, bathyal and abyssal sediments. *Oceanology Acta*, **7**, 399-408.

Bett BJ, Gabriella Malzone M, Narayanaswamy BE & Wigham BD (2001) Temporal variability in phytodetritus and megabenthic activity at the seabed in the deep Northeast Atlantic. *Progress in Oceanography*, **50**, 349-368.

Billett DSM, Lampitt RS, Rice AL & Mantoura RFC (1983) Seasonal sedimentation of phytoplankton bloom to the deep-sea benthos. *Nature*, **302**, 520-522.

Blair NE, Levin LA, DeMaster DJ & Plaia G (1996) The short-term fate of fresh algal carbon in continental slope sediments. *Limnology and Oceanography*, **41**, 1208-1219.

Boetius A & Lochte K (1994) Regulation of microbial enzymatic degradation of organic matter in deep-sea sediments. *Marine Ecology Progress Series*, **104**, 299-307.

Boetius A & Lochte K (1996) Effect of organic enrichments on hydrolytic potentials and growth of bacteria in deep-sea sediments. *Marine Ecology Progress Series*, **140**, 239-250.

Bott TL & Kaplan KA (1985) Bacterial biomass, metabolic state, and activity in stream sediments: relation to environmental variables and multiple assay comparison. *Applied and Environmental Microbiology*, **50**, 508-522.

Boyer JN & Kator HI (1985) Method for measuring microbial degradation and mineralisation of $^{14}$C-labeled chitin obtained from the blue crab, *Callinectes sapidus*. *Microbial Ecology*, **11**, 185-192.

Breuer E, Stevenson AG, Howe JA, Carroll J & Shimmield GB (2004) Drill cutting accumulations in the Northern and Central North Sea: a review of environmental interactions and chemical fate. *Marine Pollution Bulletin*, **48**, 12-25.

Britton JC & Morton B (1994) Marine carrion and scavengers. *Oceanography and Marine Biology: An Annual Review*, **32**, 369-434.

Bühring SI, Lampadariou N, Moodley L, Tselepides A & Witte U (2006) Benthic microbial and whole-community response to different amounts of $^{13}$C-enriched algae: *In situ* experiments in the deep Cretan Sea (Eastern Mediterranean). *Limnology and Oceanography*, **51**, 157-165.

Cacchione DA, Rowe GT & Malahoff A (1978) Submersible investigation of Outer Hudson Submarine Canyon. In: Stanley DJ, Kelling G (eds.), *Sedimentation in submarine Canyons, Fans, and Trenches*. Dowden, Hutchinson & Ross, Stroudsburg, Pennsylvania, pp. 42-50.

Cartes JE & Maynou F (1998) Food consumption by bathyal decapod crustacean assemblages in the western Mediterranean: predatory impact of megafauna and food the consumption food supply balance in a deep-water food web. *Marine Ecology Progress Series*, **171**, 233-246.

Cauchie H-M (2002) Chitin production by arthropods in the hydrosphere. *Hydrobiologia*, **470**, 63-96.

Certes A (1884) Note relative à l´action des hautes pressions sur la vitalité des microorganismes d´eau douce et déau de mer. *Comptes rendus des séances et memoires de la société de biologie*, **36**, 220-222.

Christiansen B, Boetius A (2000) Mass sedimentation of the swimming crab *Charybdis smithii* (Crustacea: Decapoda) in the deep Arabian Sea. *Deep Sea Research II*, **47**, 2673-2685.

Dale NG (1974) Bacteria in intertidal sediments: Factors related to their distribution. *Limnology and Oceanography*, **19**, 509-518.

Danovaro R, Marrale D, Dell`Anno A, Della Croce N, Tselepides A & Fabiano M (2000) Bacterial response to seasonal changes in labile organic matter composition on the continental shelf and bathyal sediments of Cretan Sea. *Progress in Oceanography*, **46**, 345-366.

DeFlaun MF & Mayer LM (1983) Relationships between bacteria and grain surfaces in intertidal sediments. *Limnology and Oceanography*, **28**, 873-881.

Deming JW (1985) Bacterial growth in deep-sea sediment trap and boxcore samples. *Marine Ecology Progress Series*, **25**, 305-312.

Deming JW & Baross JA (1992) The early diagenesis of organic matter: bacterial activity. In: Engel MH, Macko SA (eds.), *Organic Geochemistry: Principles and Application*. Plenum Press, New York, pp. 119-144.

Deming JW & Yager PL (1992) Natural bacterial assemblages in deep-sea sediments: towards a global view. In: Rowe GT and Pariente V (eds.), *Deep-sea Food Chains and the global Carbon Cycle*. Kluwer Academic Publishers, Dodrecht, pp. 11-28.

Deming JW & Baross JA (2000) Survival, dormancy and non-culturable cells in extreme deep-sea environments. In: Colwell, RR, Grimes, DJ (eds.), *Non-culturable Microorganisms in the Environment*. ASM Press, Washington DC, pp. 147-197.

Desbruyères D, Bervas JY & Khripounoff A (1980) Un cas de colonisation rapide d'un sédiment profond. *Oceanologica Acta*, **3**, 285-291.

Desbruyères D, Deming JW, Dinet A & Khripounoff A (1985) Réactions de l`écosystème benthique profond aux peturbations: nouveaux résultats expèrimentaux. In: Laubier L, Monniot C (ed.), *Peuplements profonds du Golfe de Gascogne*. Brest, IFREMER, pp. 193-208.

Dover C van (2000) *The Ecology of Deep-Sea hydrothermal Vents*. Princeton University Press, Princeton, pp. 352.

Drazen JC, Baldwin RJ & Smith KL (1998) Sediment community response to a temporally varying food supply at an abyssal station in the NE pacific. *Deep Sea Research II*, **45**, 893-913.

Fabiano M & Danovaro R (1998) Enzymatic activity, bacterial distribution, and organic organic matter composition in sediments of the Ross Sea (Antarctica). *Applied and Environmental Microbiology*, **64**, 3838-3845.

Findlay RH, Kim SK & Butman CA (1992) Colonisation of freshly deposited barite and silica sediments by marine microorganisms in a laboratory flume flow. *Marine Ecology Progress Series*, **90**, 73-88.

Fischer B (1886) Bakteriologische Untersuchungen auf einer Reise nach Westindien. *Zeitschrift für Hygiene*, **1**, 424-464.

Gage JD & Tyler PA (1991) *Deep-Sea Biology. A natural History of Organisms at the Deep Seafloor*. Cambridge University Press, pp. 504.

Gaill F, Shillito B, Ménard F, Goffinet G & Childress JJ (1997) Rate and process of tube production by the deep-sea hydrothermal vent tubeworm *Riftia pachyptila*. *Marine Ecology Progress Series*, **148,** 135-143.

Gardener WD, Hinga KR & Marra J (1983) Observations on the degradation of biogenic material in the deep ocean with implications on accuracy of sediment trap fluxes. *Journal of Marine Research*, **41**, 195-214.

Glover AG, Goetze E, G DT & Smith CR (2005) Morphology, reproductive biology and genetic structure of the whale-fall and hydrothermal vent specialist, *Bathykurila guaymasensis* Pettibone, 1989 (Annelida: Polynoidae). *Marine Ecology*, **26**, 223-234.

Gomez-Gutierrez J, Peterson WT, De Robertis A & Brodeut RD (2003) Mass mortality of krill caused by parasitoid ciliates. *Science*, **301**, 339.

Gooday AJ (1988) A benthic foraminiferal response to the deposition of phytodetritus in the deep sea. *Nature*, **332**, 70-73.

Gooday AJ, Turley CM (1990) Responses of benthic organisms to inputs of organic material to the ocean floor. *Philosophical Transactions of the Royal Society London A*, **331**, 119-138.

Gooday GW (1990) The ecology of chitin degradation. *Advances in Microbial Ecology*, **11**, 387-430.

Graf G (1989) Benthic-pelagic coupling in a deep-sea benthic community. *Nature*, **341**, 437-439.

Grassle JF (1977) Slow recolonisation of deep-sea sediment. *Nature*, **265**, 618-619.

Grassle JF & Morse-Porteous LS (1987) Macrofaunal colonisation of disturbed deep-sea environments and the structure of deep-sea benthic communities. *Deep Sea Research*, **34**, 1911-1987.

Grossmann S & Reichardt W (1991) Impact of *Arenicola marina* on bacteria in intertidal sediments. *Marine Ecology Progress Series*, **77**, 85-93.

Hall LWJ, Ziegenfuss MC, Anderson RD & Killen WDJ (1995) Use of estuarine water column tests for detecting toxic conditions in ambient areas of the Chesapeake Bay water-shed. *Environmental Toxicology and Chemistry*, **14**, 267-278.

Hannides AK, Smith CR & Baco-Taylor AR (2005) Massive organic matter fall processing and organic enrichment in the deep-sea sediments: the role of fall type. *Eos Transaction, American Geophysical Union*, **86** (52), Fall Meeting Suppl.

Hargrave BT (1972) Aerobic composition of sediment and detritus as a function of particle surface area and organic content. *Limnology and Oceanography*, **17**, 583-596.

Harrold C, Light K & Lisin S (1998) Organic enrichment of submarine-canyon and continental-shelf benthic communities by macroalgal drift imported from nearshore kelp forest. *Limnology and Oceanography*, **43**, 669-678.

Hassol SJ (2004) Impacts of a warming Arctic: Arctic climate impact and assessment. Cambridge University Press, pp. 144.

Hecker B (1990) Photographic evidence for the rapid flux of particles to the sea floor and their transport down the continental slope. *Deep Sea Research*, **37**, 1773-1782.

Hirche H-J (1997) Life cycle of the copepode *Calanus hyperborius* in the Greenland Sea. *Marine Biology*, **128**, 607-608.

Hirche H-J & Kosobokova K (2007) Distribution of *Calanus finmarchicus* in the northern North-Atlantic and Arctic Ocean - Expatriation and potential colonisation. *Deep Sea Research II*, **54**, 2729-2747.

Honjo S & Maganini SJ (1993) Annual biogenic particle fluxes to the interior of the North Atlantic Ocean; studied at 34° N, 21° W and 48° N, 21° W. *Deep Sea Research*, **40**, 587-607.

Hood MA & Meyers SP (1977) Rates of chitin degradation in an estuarine environment. *Journal of Oceanographical Society Japan*, **33**, 328-334.

Hoppe H-G, Ducklow H & Karrasch B (1993) Evidence for the dependency of bacterial growth on enzymatic hydrolysis of particulate organic matter in the mesopelagic ocean. *Marine Ecology Progress Series*, **93**, 277-283.

Huxley TH (1868) On some organisms living at great depths in the North Atlantic Ocean. *Quarterly Journal of Microscopy Science*, **8**, 203-212.

Isaacs JD (1969) The nature of oceanic life. *Scientific American*, **221**, 146-162.

Isaacs JD & Schwartzlose RA (1975) Active animals of the deep seafloor. *Scientific American*, **233**, 85-91.

Jannasch HW & Wirsen CO (1973) Deep-sea microorganisms: *In situ* response to nutrient enrichment. *Science*, **180**, 641-643.

Jannasch HW & Wirsen CO (1977) Retrieval of concentrated and undecompressed microbial populations from the deep sea. *Applied and Environmental Microbiology*, **33**, 642-646.

Jannasch HJ & Wirsen CO (1980) Studies on the microbial turnover of organic substrates in deep-sea sediments. In: Roumas, R (ed.), *Biogeochimie de la Matiere Organique a l`Interface Eau-Sediment Marine*. Reprinted in Actes des Colloques du CNRS, **293**, 285-598.

Jannasch HW, Eimhjellen K, Wirsen CO & Farmanfarmaian A (1971) Microbial degradation of organic matter in the deep sea. *Science*, **171**, 672-675.

Jannasch HW, Wirsen CO & Taylor CD (1976) Undecompressed microbial populations from the deep-sea. *Applied and Environmental Microbiology*, **32**, 360-367.

Johannessen OM et al. (2004) Arctic climate change: observed and modelled temperature and sea-ice variability. *Tellus Series A - Dynamic Meteorology and Oceanography*, **54**, 328-341.

Johnson DL & Richardson PL (1977) On the wind-induced sinking of *Sargassum. Journal of Experimental Marine Biology and Ecology*, **28**, 255-267.

Kennicutt M (1985) Vent-type taxa in a hydrocarbon seep region on the Lousiana slope. *Nature*, **317**, 351-353.

Keyhani NO & Roseman S (1999) Physiological aspects of chitin catabolism in marine bacteria. *Biochimica et Biophysica Acta*, **1473**, 108-122.

Kirchman DL & White J (1999) Hydrolysis and mineralisation of chitin in the Delaware Estuary. *Aquatic Microbial Ecology*, **18**, 187-196.

Kirchman DL, Dittel AI, Findlay SEG & Fischer D (2004) Changes in bacterial activity and community structure in response to dissolved organic matter in the Hudson River, New York. *Aquatic Microbial Ecology*, **35**, 243-257.

Koch R (1881) Zur Untersuchung von pathogenen Organismen. *Mitteilungen aus dem Kaiserlichen Gesundheitsamte*, **1**, 1-48.

Köster M, Charfreitag O & Meyer-Reil L-A (1991) Availability of nutrients to a deep-sea benthic microbial community: results from a ship-board experiment. *Kieler Meeresforschungen, Sonderheft*, **8**, 127-133.

Kröncke I, Tan TL & Stein R (1994) High benthic bacteria standing stock in deep Arctic basins. *Polar Biology*, **14**, 423-428.

Kulm LD et al. (1986) Oregon subduction zone: venting, fauna, and carbonates. *Science*, **231**, 561-566.

Lauerman LML & Kaufmann RS (1998) Deep-sea epibenthic echinoderms and a temporally varying food supply: results from a one-year time series in the NE Pacific. *Deep Sea Research II*, **45**, 817-842.

Lee JJ (1980) A conceptual model of marine detrital decomposition and the organisms associated with the process. *Advanced Aquatic Microbiology*, **2**, 257-291.

Levin LA & Smith CR (1984) Response of background fauna to disturbance and enrichment in the deep sea: a sediment tray experiment. *Deep Sea Research I*, **3**, 1277-1285.

Levin LA, Blair NE, DeMaster DJ, Plaia G, Fornes W, Martin C & Thomas C (1997) Rapid subduction of organic matter by maldanid polychaetes in the North Carolina Slope. *Journal of Marine Research*, **55**, 595-611.

Levin LA, Blair NE, Martin CM, DeMaster DJ, Plaia G & Thomas CJ (1999) Macrofaunal processing of phytodetritus at two contrasting sites on the Carolina margin: *in situ* experiments using $^{13}C$-labeled diatoms. *Marine Ecology Progress Series*, **182**, 37-54.

Lochte K (1992) Bacterial standing stock and consumption of organic carbon in the benthic boundary layer of the abyssal North Atlantic. In: Rowe GT, Pariente V (eds.), *Deep Sea Food Chains and the global Carbon Cycle*. Kluwer Academic Press, Dodrecht, pp. 1-10.

Lochte K & Turley CM (1988) Bacteria and cyanobacteria associated with phytodetritus in the deep sea. *Nature*, **333**, 67-69.

Lochte K & Pfankuche O (2002) Processess driven by the small size organisms at the water-sediment interface. In: Wefer G, Billett D, Hebbeln D, Jörgensen BB, Schlüter M, van Weering T (eds.), *Oceans Margin Systems*. Springer Verlag, Berlin Heidelberg, pp. 405-418.

Lochte K, Ducklow HW, Fasham MJR & Stienen M (1993) Plankton succession and carbon cycling at 47°N, 20°W during the JGOFS North Atlantic Bloom Experiment. *Deep Sea Res*earch, **40**, 91-114.

Londsdale P (1977) Clustering of suspension-feeding macrobenthos near abyssal hydrothermal vents at the oceanic spreading centres. *Deep Sea Res*earch, **24**, 857-863.

Maciolek N, Grassle JF& Hecker B *et al*. (1987) Study of biological processes on the U.S. north Atlantic slope and rise. *Final Report Prepared for U.S. Department of the Interior, Minerals Managment Service*, Washington, D.C. 20240, pp. 358.

Manley O (1995) Branching of Atlantic water within the Greenland-Spitsbergen Passage: an estimate of recirculation. *Journal of Geophysical Research*, **100** (C10), 20627-20634.

Marquise R & Matsumura P (1978) Microbial life under pressure. In: Kushner DJ (ed.), *Microbial Life in extreme Environments*. Academic Press, New York.

McGowan JA, Cayan DR & Dorman LM (1998) Climate-ocean variability and ecosystem response in the Northeast Pacific. *Science*, **281**, 210-217.

Mayer LM (1994) Relationships between mineral surfaces and organic carbon concentrations in soil and sediments. *Chemistry and Geology*, **114**, 347-363.

Meyer-Reil LA (1983) Benthic response to sedimentation events during autumn to spring at a shallow water station in the Western Kiel Bight. *Marine Biology*, **77**, 247-256.

Meyer-Reil LA (1993) Mikrobielle Besiedlung und Produktion. In: Meyer-Reil LA, Köster, M (eds.) *Mikrobiologie des Meeresbodens*. Gustav Fischer Verlag, Jena, pp. 38-81.

Meyer-Reil LA & Köster M (1992) Microbial life in pelagic sediments: the impact of environmental parameters on enzymatic degradation of organic material. *Marine Ecology Progress Series*, **81**, 65-72.

Moodley L, Middelburg JJ, Boschker HTS, Duineveld GCA, Pel R, Herman PMJ & Heip CHR (2002) Bacteria and Foraminifera: key players in a short-term deep-sea benthic response to phytodetritus. *Marine Ecology Progress Series*, **236**, 23-29.

Morita RY (1976) Survival of bacteria in cold and moderate hydrostatic pressure environments with special reference to phsycrophilic and barophilic bacteria. 26th Symp Soc Gen Microbiol In: Gray T, Postgate JR (eds.), *The Survival of vegetative Microbes*. Cambridge University Press, New York, pp. 279-298.

Morita RY & ZoBell CE (1955) Occurrence of bacteria in pelagic sediments collected during the Mid-Pacific Expedition. *Deep Sea Research*, **3**, 66-73.

Muraoka JS (1971) Deep ocean biodeterioration of materials. *Ocean Industry* (February 1971), pp. 21-23.

Murray J (1895) A summary of the scientific results. In: *Report of the Scientific Results of the Voyage of HMS Challenger*, *Summary of Scientific Research*, **1**, 1-1608.

Murray JL (1998) Physical/geographical characteristics of the Arctic. In: Wilson SJ, Murray JL, Huntington HP (eds.), *AMAP assessment report: Arctic pollution issue*. Arctic Monitoring and Assessment Programme (AMAP), Oslo, pp. 9-24.

Naganuma T, Wada H & Fujioka K (1996) Biological community and sediment fatty acids associated with the deep-sea whale skeleton at the Torishima Seamount. *Journal of Oceanography*, **52**, 1-15.

Neff JM, McKelvie S & Ayers RC (2000) Environmental impacts of synthetic based drilling fluids. *Report prepared for MMS by Ayers R and Associates*, Inc., August 2000, US Department of interior, minerals management service, Gulf of Mexico, OCS Region, New Orleans, OCS study MMS 2000-064.

Nickels JS, Bobbie RJ, Martz RF, Smith GA, White DC & Richards NL (1981) Effect of silicate grain shape, structure, and location on the biomass and community structure of colonising marine microbiota. *Applied and Environmental Microbiology*, **41**, 1262-1268.

Pace N, Stall D, Lane D & Olsen G (1985) Analysing natural microbial populations by rRNA sequence. *American Society for Microbiology News*, **51**, 4-11.

Palacios C, Zbinden M, Baco AR, Treude T, Smith CR, Gaill F, Lebaron P & Boetius A (2006) Microbial ecology of deep-sea sunken wood: quantitative measurements of bacterial biomass and cellulolytic activities. *Cahiers de Biologie Marine*, **47**, 415-420.

Pfannkuche O (1993) Benthic response to the sedimentation of particulate organic matter at the BIOTRANS station, 47°N, 20°W. *Deep Sea Research*, **40**, 135-149.

Pfannkuche O, Boetius A, Lochte K, Lundgreen U & Thiel H (1999) Responses of deep-sea benthos to sedimentation patterns in the North-East Atlantic in 1992. *Deep Sea Research I*, **46**, 573-596.

Poulicek M & Jeuniaux C (1989) Chitin Biomass in marine sediments. In: Skjak-Braek G., Anthonsen T., Sandford P. (eds), *Chitin and Chitosan*. Elsvier, London, pp. 151-160.

Poulicek M, Goffinet G, Voss-Foucart MF, Bussers JC, Jasper-Versali MF & Toussaint C (1986) Chitin degradation in natural environment (mollusk shells and crab carapaces). In: Muzzarelli C, Jeuniaux C, Gooday G W (eds.), *Chitin in Nature and Technology*. Plenum Press, New York, pp. 547-550.

Premke K, Klages M & Arntz WE (2006) Aggregations of Arctic deep-sea scavengers at large food falls: temporal distribution, consumption rates and population structure. *Marine Ecology Progress Series*, **325**, 121-135.

Priede IG & Bagley PM (2000) *In situ* studies on deep-sea demersal fishes using autonomous unmanned lander platforms. *Oceanography and Marine Biology: An Annual Review*, **38**, 357-392.

Prospero JM (1981) Eolian transport to the World Ocean. In: Emiliani, C (ed.), *The oceanic Lithosphere. The Sea*. Whiley, New York, **7**, 801-874.

Rice AL, Billett DSM, Fry J, John AWG, Lampitt RS, Mantoura RFC & Morris RJ (1986) Seasonal deposition of phytodetritus to the deep-sea floor. *Proceedings of the Royal Society of Edinburgh*, **88B**, 265-279.

Riemann L, Steward GF & Azam F (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. *Applied and Environmental Microbiology*, **66**, 578-587.

Riley GA (1964) Organic aggregates in seawater and the dynamics of their formation and utilisation. *Limnology and Oceanography*, 8, 372-381.

Rowe GT & Staresinic N (1979) Source of organic matter to the deep-sea benthos. *Ambio Special Report*, **6**, 19-23.

Rowe GT & Deming JW (1985) The role of bacteria in the turnover of organic carbon in deep-sea sediments. *Journal of Marine Research*, **43**, 925-950.

Rowe G, Sibuet M, Deming JW, Khripounoff A, Tietjen J, Macko SA & Theroux R (1991) 'Total' sediment biomass and preliminary estimates of organic carbon residence time in deep-sea benthos. *Marine Ecology Progress Series*, **79**, 99-114.

Sayles FL, Martin WR & Deuser WG (1994) Response of benthic oxygen demand to particulate organic carbon supply in the deep sea near Bermuda. *Nature*, **371**, 686-689.

Schewe I & Soltwedel T (2003) Benthic response to ice-edge-induced particle flux in the Arctic Ocean. *Polar Biology*, **26**, 610-620.

Sharma PK & McInerney MJ (1994) Effect of grain size in bacterial penetration, reproduction, and metabolic activity in porous glass beads chambers. *Applied and Environmental Microbiology*, 60, 1481-1486.

Shillito B, Ravaux J, Gaill F, Delachambre J, Thiébaut E & Childress JJ (1999) Preliminary data on carbon production of deep-sea vent tubeworms. *Marine Ecology Progress Series*, **183**, 275-279.

Siegel V (2005) Distribution and population dynamics of *Euphausia superba*: summary or recent findings. *Polar Biology*, **29**, 1-22.

Smith CR (1985) Food for the deep sea: utilisation, dispersal, and flux of nekton falls at the Santa Catalina Basin floor. *Deep Sea Research I*, **32**, 417-442.

Smith CR (1986) Nekton falls, low-intensity disturbance and community structure of infaunal benthos in the deep sea. *Journal of Marine Research*, **44**, 567-700.

Smith CR & Baco AR (2003) Ecology of whale falls at the deep seafloor. *Oceanography and Marine Biology: An Annual Review*, **41**, 311-354.

Smith CR & Demopoulus A (2003) Ecology of the deep Pacific Ocean floor. In: Tyler PA, (ed.), *Ecosystems of the World, Vol. 28: Ecosystems of the Deep Ocean*. Amsterdam: Elsvier, pp. 181-220.

Smith CR & Brumsickle SJ (1989) The effects of patch size and substrate isolation on colonisation modes and rates in an intertidal sediment. *Limnology and Oceanography*, **34**, 1263-1277.

Smith CR, Hoover DJ, Doan SE, Pope RH, Demaster DJ, Dobbs FC & Altabets MA (1996) Phytodetritus at the abyssal seafloor across 10° of latitude in the central equatorial Pacific. *Deep Sea Research II*, **43**, 1309-1338.

Smith CR, Maybaum HL, Baco AR, Pope RH, Carpenter SD, Yager PL, Macko SA & Deming JW (1998) Sediment community structure around a whale skeleton in the deep northeast Pacific: macrofaunal, microbial, and bioturbation effects. *Deep Sea Research II*, **45**, 335-364.

Smith KL & Druffel ERM (1998) Long time-series monitoring of an abyssal station in the NE Pacific. *Deep Sea Research II*, **45**, 843-880.

Smith KL, Kaufmann RS & Baldwin RJ (1994) Coupling of near-bottom pelagic and benthic processes at abyssal depth in the eastern North Pacific Ocean. *Limnology and Oceanography*, **39**, 1101-1118.

Smith WO, Cota GF & Kelly HP (1987) Phytoplankton biomass and productivity in the marginal ice zone of the Fram Strait during summer 1984. *Journal of Geophysical Research*, **92**, 6777-6786.

Smucker RA & Dawson R (1986) Products of photosynthesis by marine phytoplankton: chitin in TCA 'protein' precipitates. *Journal of Experimental Marine Biology and Ecology*, **104**, 143-152.

Snelgrove PVR, Grassle JF & Petrecca RF (1992) The role of food patches in maintaining high deep-sea diversity: Field experiments with hydrodynamically unbiased colonisation trays. *Limnology and Oceanography*, **37**, 1543-1550.

Snelgrove PVR, Grassle JF & Petrecca RF (1994) Macrofaunal response to artificial enrichments and depressions in a deep-sea habitat. *Journal of Marine Research*, **52**, 345-369.

Snelgrove PVR, Butman CA & Grassle JF (1995) Potential flow artefacts associated with benthic experimental gear: Deep-sea mudbox examples. *Journal of Marine Research*, **53**, 821-845.

Snelgrove PVR, Grassle JF & Petrecca RF (1996) Experimental evidence for aging food patches as a factor contributing to high deep-sea macrofaunal diversity. *Limnology and Oceanography*, **41**, 605-614.

Soltwedel T et al. (2005) HAUSGARTEN - multidisciplinary investigations at a deep-sea long-term observatory in the Arctic Ocean. *Oceanography*, **18**, 46-61.

Sokolova MN (1994) Euohasiid 'dead body rain' as a source of food for abyssal benthos. *Deep Sea Research I*, **41**, 741-746.

Stockton WL & DeLaca TE (1982) Food falls in the deep sea: occurrence, quality, and significance. *Deep Sea Research*, **29**, 157-169.

Subba Rao DV & Platt T (1984) Primary production of the Arctic waters. *Polar Biology*, **3**, 191-201.

Suchaneck TH, Williams SL, Ogden JC, Hubbard DK & Gill IP (1985) Utilisation of shallow-water seagrass detritus by Caribbean deep-sea macrofauna: $^{13}$C evidence. *Deep Sea Research*, **32**, 201-214.

Suess E et al. (1985) Biological communities at vents sites along the subduction zone off Oregon. *Bulletin of the Biological Society of Washington*, **6**, 475-484.

Sweetman AK & Witte U (2008) Response of an abyssal macrofaunal community to a phytodetrital pulse. *Marine Ecology Progress Series*, **355**, 73-84.

Tabor PS, Deming JW, Ohwada K, Davis H, Waxman M & Colwell RR (1981) A pressure-retaining deep ocean sampler and transfer system for measurement of microbial activity in the deep sea. *Microbial Ecology*, **7**, 51-65.

Targatz ME, Deans CH, Moore JC & Plaia GR (1983) Alteration in composition of field- and laboratory-developed estuarine benthic communities expose to di-*n*-butyl phtalate. *Aquatic Toxicology*, **3**, 239-248.

Thiel H, Lochte K, Gooday AJ, Hemleben C, Mantoura RFC, Patching JW, Pfannkuche O, Riemann F, Schriefer G & Turley CM (1988) Phytodetritus on the deep-sea floor in a central oceanic region of the Northeast Atlantic. *Biological Oceanography*, **6**, 206-239.

Thurston MH (1979) Scavenging abyssal amphipods from the north-east Atlantic Ocean. *Marine Biology*, **51**, 55-58.

Tietjen JH (1992) Abundance and biomass of metazoan meiobenthos in the deep sea. In: Rowe GT, Pariente V (eds.), *Deep-sea Food Chains and the global Carbon Cycle*. Proccedings. ATO ARW, Kluwer Academic Publisher, Dodrecht, pp. 45-62.

Turley CM & Lochte K (1990) Microbial response to the input of fresh detritus to the deep-sea bed. *Palaeogeography Palaeoclimatology Palaeoecology* **S9**, 3-23

Turley CM, Lochte K & Lampitt RS (1995) Transformations of biogenic particles during sedimentation in the northeastern Atlantic. *Philosophical Transactions of the Royal Society London B*, **348**, 179-189.

Turner RD (1973) Wood-boring bivalves, opportunistic species in the deep sea. *Science*, **180**, 1377-1379.

Vetter Y-A & Deming JW (1994) Extracellular enzyme activity in the Arctic Northeast Water polynya. *Marine Ecology Progress Series*, **114**, 23-34.

Wada E, Koike I & Hattori A (1975) Nitrate metabolism in abyssal waters. *Marine Biology*, **29**, 119-124.

Walsh JE (2004) Cryosphere and Hydrology. In: Symon C, Arris L, Heal B (eds.), *Arctic Climate. Impact and Assessment*. Cambridge University Press, Cambridge, pp. 242.

Wefer G (1989) Particle flux in the ocean: effects of episodic production. In: Berger W H, Smetacek V, Wefer G (eds.), *Productivity of the Oceans: Present and Past*. John Wiles & Sons, New York, pp. 139-154.

Węsławski JM & Legeżyńska J (1998) Glaciers caused zooplankton mortality? *Journal of Plankton Research*, **20**, 1233-1240.

Wiebe PH, Madin LP, Haury LR, Harbison GR & Philbin LM (1979) Diel vertical migration by *Salpa aspera* and its potential for large-scale particulate organic matter transport to the deep-sea. *Marine Biology*, **53**, 249-255.

Widdel F, Musat F, Knittel K & Galushko A (2000) Anaerobic degradation of hydrocarbons with sulphate as electron acceptor. In: Barton LL, Hamilton WA (eds.), *Sulphate-reducing Bacteria: Environmental and Engineered Systems*. Cambridge University Press, Cambridge, pp. 265-304.

Wirsen CO & Jannasch JH (1976) Decomposition of solid organic material in the deep sea. *Environmental Scientific Technology*, **10**, 8801-8886.

Wirsen CO & Jannasch HW (1986) Microbial transformations in deep-sea sediments: free-vehicle studies. *Marine Biology*, **91**, 277-284.

Witte U (1999) Consumption of large carcasses by scavenger assemblages in the deep Arabian Sea: observation by baited camera. *Marine Ecology Progress Series*, **183**, 139-174.

Witte U, Aberle N, Sand M & Wenzhöfer F (2003a) Rapid response of a deep-sea benthic community to POM enrichment: an *in situ* experimental study. *Marine Ecology Progress Series*, **251**, 27-36.

Witte U, Wenzhöfer F, Sommer S, Boetius A, Heinz P, Aberle N, Sand M, Abraham W-R, Jörgensen BB & Pfannkuche O (2003b) *In situ* experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor. *Nature*, **424**, 763-766.

Yamamoto N & Lopez G (1985) Bacterial abundance in relation to surface area and organic content of marine sediments. *Journal of Experimental Marine Biology and Ecology*, **90**, 209-220.

Yoshikoshi K & Ko Y (1988) Structure and function of the peritrophic membranes of copepods. *Bulletin of the Japanese Society Scientific Fisheries*, **54**, 1077-1082.

ZoBell CE (1952) Bacterial life at the bottom of the Philippine Trench. *Science*, **115**, 507-507.

ZoBell CE (1968) Bacterial life in the deep sea. *Bulletin of the Misaki Marine Biology Institute*, Kyoto University, **12**, 77-96.

ZoBell CE (1970) Pressure effects on morphology and life processes of bacteria. In: Zimmerman HM (ed.), *High pressure effects on cellular processes*. New York, pp. 85-130.

Zobell CE & Johnson FH (1949) The influence of hydrostatic pressure on the growth and viability of terrestrial and marine bacteria. *Journal of Bacteriology*, **57**, 179-189.

ZoBell CE & Morita RA (1957) Barophilic bacteria in some deep-sea sediments. *Journal of Bacteriology*, **73**, 563-568.

# Annex

**Table S1.** Patterns of bacterial OTUs from control and chitin-enriched sediments after seven days and one year of incubation at the seafloor (manuscript I).

| bp | 7 days | | | | 1 year | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 1.3 g | 3.0 g | 7.0 g | Control | 1.3 g | 3.0 g | 7.0 g |
| 54 | - | - | - | - | - | - | + | - |
| 55 | - | - | - | - | - | - | + | - |
| 56 | - | + | - | + | + | - | - | - |
| 57 | - | - | + | - | + | - | - | - |
| 58 | + | - | - | - | - | + | - | + |
| 59 | - | - | + | - | - | - | - | - |
| 61 | + | - | - | - | - | - | - | - |
| 64 | + | - | - | - | - | - | - | - |
| 71 | - | - | - | - | - | - | - | + |
| 73 | + | - | - | - | - | - | - | - |
| 80 | - | - | - | - | - | + | - | - |
| 82 | - | - | - | + | - | - | - | + |
| 84 | - | - | + | - | - | - | - | - |
| 86 | - | - | + | + | - | + | - | - |
| 87 | + | - | - | - | + | - | - | + |
| 88 | - | - | + | + | - | + | + | + |
| 89 | + | + | + | + | - | - | + | - |
| 91 | + | + | + | + | + | + | - | + |
| 93 | + | + | + | + | + | + | + | + |
| 94 | + | + | - | + | - | + | + | + |
| 95 | - | - | + | - | - | - | - | - |
| 97 | - | - | + | - | - | + | - | + |
| 99 | + | + | + | + | + | + | - | - |
| 100 | + | + | + | + | + | + | - | - |
| 103 | - | + | - | - | - | - | - | - |
| 104 | - | + | + | + | - | - | - | - |
| 120 | + | - | - | - | - | - | - | - |
| 122 | + | - | - | - | - | - | - | - |
| 126 | - | - | - | - | - | + | + | - |
| 128 | - | - | - | - | - | - | + | + |
| 133 | - | - | - | - | - | + | - | - |
| 137 | + | - | - | - | + | - | - | - |
| 138 | + | - | - | - | + | - | - | - |
| 140 | - | + | + | - | - | - | - | - |
| 143 | - | - | - | - | - | - | - | + |
| 144 | + | - | + | + | - | - | - | - |

Continuation Table S1

| bp | 7 days | | | | 1 year | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 1.3 g | 3.0 g | 7.0 g | Control | 1.3 g | 3.0 g | 7.0 g |
| 148 | + | - | - | - | + | - | - | - |
| 154 | - | - | - | + | - | - | - | - |
| 157 | + | - | - | - | + | - | - | - |
| 158 | - | - | - | - | - | - | + | + |
| 159 | - | - | - | - | - | + | + | + |
| 160 | - | + | + | - | - | + | - | - |
| 167 | - | + | - | - | - | - | - | - |
| 168 | - | + | + | - | - | - | - | - |
| 200 | - | - | - | + | - | - | + | + |
| 203 | + | - | - | + | + | - | - | + |
| 204 | - | + | + | - | - | - | - | + |
| 205 | - | + | - | - | - | - | - | - |
| 207 | + | - | - | - | + | - | - | - |
| 208 | + | - | - | - | + | - | - | - |
| 210 | - | + | + | + | + | - | + | + |
| 218 | - | - | - | + | - | - | - | - |
| 219 | - | + | + | + | - | - | - | - |
| 223 | + | - | + | - | + | - | - | - |
| 224 | + | - | - | + | - | - | - | - |
| 228 | - | - | - | - | - | + | + | + |
| 230 | - | - | - | - | - | + | + | + |
| 241 | + | - | - | - | - | - | - | - |
| 244 | - | - | - | - | - | - | - | + |
| 294 | + | - | - | - | - | - | - | - |
| 296 | + | - | - | - | + | - | - | - |
| 299 | - | - | - | - | - | - | + | - |
| 348 | + | - | + | + | + | - | - | - |
| 349 | + | - | + | - | - | - | - | - |
| 367 | - | - | - | - | - | + | - | - |
| 371 | - | + | + | + | + | - | + | + |
| 372 | - | + | - | - | - | - | - | + |
| 377 | + | + | - | - | + | - | - | - |
| 380 | + | + | + | - | + | + | - | - |
| 382 | + | - | - | - | + | - | - | - |
| 384 | + | + | + | - | - | + | + | + |
| 385 | - | + | - | - | - | + | - | - |
| 393 | + | + | + | + | + | + | - | + |
| 400 | - | - | - | + | - | - | - | - |
| 430 | - | - | - | - | - | + | - | - |
| 440 | - | - | - | - | - | + | - | - |
| 443 | + | - | + | + | + | + | + | - |
| 453 | - | + | - | - | - | - | - | - |
| 455 | + | + | + | + | + | + | + | - |
| 470 | + | + | + | - | - | - | - | - |
| 477 | + | - | - | - | - | + | - | - |
| 480 | + | + | + | + | + | + | - | + |

Continuation Table S1

| bp | 7 days | | | | 1 year | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 1.3 g | 3.0 g | 7.0 g | Control | 1.3 g | 3.0 g | 7.0 g |
| 492 | - | - | + | + | + | - | + | - |
| 496 | + | + | - | + | + | + | - | - |
| 500 | - | - | - | - | - | + | + | + |
| 501 | - | - | - | - | - | - | - | + |
| 503 | + | + | + | + | + | + | + | + |
| 512 | + | + | + | + | + | + | + | - |
| 515 | - | - | - | - | - | + | - | - |
| 528 | - | + | - | - | + | - | - | - |
| 540 | - | - | - | - | - | + | - | - |
| 543 | + | + | - | - | - | - | - | - |
| 547 | - | - | - | - | + | - | - | + |
| 560 | + | - | - | - | + | - | - | - |
| 656 | + | - | - | + | + | - | - | - |
| 566 | - | + | - | - | - | + | + | - |
| 570 | - | + | - | + | - | - | - | + |
| 575 | + | - | - | - | - | - | - | - |
| 580 | - | + | - | - | + | - | - | - |
| 581 | + | - | - | - | + | - | + | - |
| 646 | - | + | - | - | - | - | + | - |
| 764 | - | - | - | - | - | + | - | - |
| 794 | - | - | - | - | - | - | + | - |
| 835 | - | - | - | - | - | - | + | - |
| 854 | + | + | - | - | - | - | - | - |
| 858 | - | - | - | - | - | - | + | - |
| 994 | - | - | - | - | - | + | - | - |

Table S1 is based on bacterial OTUs presence (+) or absence (-) data obtained by T-RFLP analysis of the different treatments.

**Table S2.** Patterns of bacterial OTUs from natural and artificial sediments in control and chitin-enriched treatments after different incubation times at the seafloor (manuscript II).

| bp | 0 day | | | 7 days | | | | | | 1 year | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | | Control | | | Chitin-treatment | | | Control | | | Chitin-treatment | | |
| | glass | sand | mud | glass | sand | mud | glass | sand | mud | glass | sand | mud | glass | sand | mud |
| 54 | - | - | - | - | - | - | - | - | - | - | - | - | + | - | + |
| 55 | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + |
| 56 | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - |
| 57 | + | - | - | + | - | - | + | - | + | + | + | + | - | - | - |
| 58 | + | - | - | + | + | + | - | + | - | - | - | - | - | - | - |
| 60 | - | + | + | - | + | - | - | - | + | - | - | - | - | - | - |
| 61 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 64 | - | - | + | - | - | + | - | - | - | + | - | - | - | - | - |
| 73 | - | - | + | - | - | + | - | - | - | - | - | - | - | - | - |
| 80 | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - |
| 81 | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - |
| 83 | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| 84 | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - |
| 85 | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| 87 | - | - | + | - | - | + | - | - | + | - | - | + | + | + | - |
| 88 | - | - | - | - | - | - | - | - | + | + | - | - | + | + | - |
| 89 | - | - | - | - | - | + | + | + | + | - | + | - | - | - | + |
| 90 | - | + | + | - | - | + | - | - | + | - | + | + | - | + | - |
| 91 | - | - | + | - | - | + | - | - | + | - | + | - | - | - | - |
| 92 | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - |
| 93 | - | - | + | - | - | + | - | - | + | - | - | - | - | - | - |
| 94 | - | - | - | - | - | - | - | - | + | + | - | + | + | + | + |
| 95 | - | - | + | - | - | + | - | - | + | + | - | - | - | - | + |
| 96 | - | - | - | - | - | - | - | - | + | - | - | - | + | - | - |
| 98 | + | - | + | + | - | + | - | - | + | + | - | + | + | + | - |
| 99 | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| 100 | - | + | + | - | + | + | - | - | + | - | - | + | + | - | - |
| 120 | - | - | + | - | - | + | - | - | - | - | - | - | - | - | - |
| 122 | - | - | + | - | - | + | - | - | - | - | - | - | - | - | - |
| 126 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| 127 | - | + | - | - | + | - | - | - | - | - | - | - | - | - | - |
| 129 | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + |
| 135 | - | + | - | - | + | - | - | - | - | - | + | - | - | - | - |
| 136 | - | + | + | - | + | + | - | - | - | - | - | + | - | - | - |
| 137 | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - |
| 138 | - | - | + | - | - | + | - | + | + | + | + | + | - | - | - |
| 140 | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - |
| 141 | - | - | - | - | - | - | - | + | - | - | + | - | - | - | - |
| 143 | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - |
| 144 | + | + | + | + | + | + | - | + | + | + | + | - | - | - | - |
| 148 | - | - | + | - | - | + | + | + | - | - | - | + | - | - | - |
| 149 | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - |
| 150 | - | - | - | - | - | - | - | + | - | - | - | - | + | - | - |
| 151 | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - |
| 152 | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - |
| 154 | - | - | - | - | + | + | + | - | - | + | - | + | - | + | - |
| 156 | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 158 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | + |
| 159 | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + |
| 160 | - | - | + | - | - | + | - | - | + | - | - | - | - | - | - |
| 164 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - |
| 168 | - | - | + | - | - | - | - | - | + | - | - | - | - | - | - |
| 178 | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - |

Continuation Table S2

| bp | 0 day | | | 7 days | | | | | | 1 year | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | | Control | | | Chitin-treatment | | | Control | | | Chitin-treatment | | |
| | glass | sand | mud | glass | sand | mud | glass | sand | mud | glass | sand | mud | glass | sand | mud |
| 180 | - | + | - | - | + | - | - | - | - | - | - | - | - | - | - |
| 194 | - | + | - | - | + | - | - | + | - | - | - | - | + | + | - |
| 198 | + | + | - | + | + | - | - | - | - | + | + | - | - | + | + |
| 200 | - | + | - | - | - | - | - | - | - | - | + | - | - | - | - |
| 201 | - | - | - | + | + | + | - | + | - | + | + | + | + | + | - |
| 203 | + | + | + | - | - | - | - | - | - | - | + | - | - | - | - |
| 205 | - | - | - | - | - | + | - | - | + | + | + | + | - | - | - |
| 207 | - | + | + | + | + | + | - | + | - | - | - | + | - | - | - |
| 208 | + | - | + | + | + | - | - | + | + | + | - | + | + | + | + |
| 210 | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - |
| 211 | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 212 | - | + | - | - | + | - | - | - | - | - | - | - | - | - | - |
| 215 | - | + | - | - | - | - | - | - | + | + | - | - | - | + | - |
| 218 | - | - | + | - | - | + | - | - | + | - | - | - | - | - | - |
| 224 | - | - | + | - | - | + | - | - | + | + | - | + | - | - | - |
| 225 | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - |
| 227 | - | - | - | - | - | - | - | - | - | - | - | - | + | - | + |
| 230 | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + |
| 231 | - | - | - | - | - | - | + | + | - | - | - | - | - | - | - |
| 240 | - | - | + | - | - | + | - | - | - | - | - | - | - | - | - |
| 241 | - | - | + | - | - | + | - | - | - | - | - | - | - | - | - |
| 294 | - | - | + | - | - | + | - | - | - | - | - | + | - | - | - |
| 296 | - | - | + | - | - | + | - | - | - | - | - | - | - | - | + |
| 300 | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - |
| 317 | - | + | - | - | - | - | - | + | - | - | - | - | - | - | - |
| 345 | - | - | - | - | + | + | + | - | + | + | - | + | - | - | - |
| 347 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 348 | - | - | + | - | - | + | - | - | + | - | - | - | - | - | - |
| 349 | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| 370 | - | - | - | - | - | - | - | - | + | - | + | + | - | - | + |
| 372 | - | + | - | - | + | - | - | - | - | - | - | - | - | - | - |
| 374 | - | - | - | - | + | + | + | + | - | - | - | + | - | - | - |
| 376 | - | + | - | - | - | - | - | - | - | - | + | - | - | + | - |
| 377 | - | - | + | + | - | + | + | - | + | + | - | + | - | - | - |
| 379 | - | - | + | - | - | + | - | - | + | + | + | + | - | - | - |
| 380 | - | - | - | - | - | + | + | - | - | + | - | - | - | + | + |
| 382 | - | - | + | - | + | - | - | - | - | - | + | - | - | + | - |
| 383 | - | - | + | - | - | + | - | - | - | - | - | - | - | - | - |
| 386 | - | - | - | - | - | + | - | - | + | + | + | + | + | + | - |
| 394 | - | - | + | - | - | - | - | - | - | - | - | - | + | - | - |
| 416 | - | - | - | - | - | - | + | - | - | + | - | - | - | - | - |
| 427 | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - |
| 431 | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - |
| 435 | + | + | - | + | + | - | - | - | - | - | - | - | - | - | - |
| 438 | - | + | - | - | + | + | - | - | - | - | + | + | + | - | + |
| 442 | - | - | + | + | + | - | + | - | + | + | + | - | + | - | - |
| 443 | + | + | - | - | - | - | - | + | - | - | - | - | - | - | - |
| 447 | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - |
| 451 | - | - | - | + | + | + | + | - | + | + | + | + | + | + | + |
| 454 | - | + | + | - | - | - | - | - | - | + | - | - | - | - | - |
| 455 | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - |
| 460 | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - |
| 465 | - | - | + | - | - | + | - | - | + | - | - | - | + | - | - |
| 468 | - | - | + | - | - | + | - | - | - | - | - | - | - | - | - |
| 475 | - | - | + | - | - | + | - | - | + | + | + | + | + | + | + |
| 477 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 480 | - | - | + | - | - | - | - | - | - | - | - | - | + | - | - |

Continuation Table S2

| bp | 0 day | | | 7 days | | | | | | 1 year | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | | Control | | | Chitin-treatment | | | Control | | | Chitin-treatment | | |
| | glass | sand | mud | glass | sand | mud | glass | sand | mud | glass | sand | mud | glass | sand | mud |
| 482 | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - |
| 484 | - | - | - | + | + | - | - | - | + | - | + | + | + | + | + |
| 491 | + | + | - | + | + | + | + | - | - | - | - | + | + | - | - |
| 496 | + | + | + | + | - | + | + | + | - | + | + | - | - | - | - |
| 498 | + | - | + | - | - | - | - | - | - | - | - | - | - | - | + |
| 500 | + | + | + | + | + | + | - | - | + | + | + | + | + | + | + |
| 503 | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| 506 | - | - | - | - | - | - | - | - | - | + | + | - | + | - | - |
| 509 | - | - | + | - | - | + | - | - | + | + | + | + | - | - | + |
| 513 | - | - | - | - | - | - | + | + | - | - | - | - | + | + | - |
| 516 | + | - | - | + | - | - | - | - | - | + | + | + | - | - | - |
| 526 | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - |
| 528 | - | - | - | - | - | - | - | - | - | + | - | + | + | + | - |
| 540 | + | - | - | + | - | + | + | - | - | - | - | - | - | - | - |
| 543 | - | - | + | - | - | - | - | - | - | - | - | + | - | - | - |
| 548 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - |
| 556 | - | - | - | - | + | + | + | - | - | - | + | + | - | - | - |
| 560 | + | + | + | + | + | + | + | - | - | + | + | + | - | - | - |
| 565 | - | - | + | - | - | - | + | - | - | - | - | - | - | - | + |
| 566 | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - |
| 570 | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - |
| 575 | - | - | + | - | - | - | + | - | - | - | - | + | - | - | - |
| 579 | + | - | - | + | - | + | - | - | - | + | - | - | - | - | + |
| 581 | - | - | + | - | + | - | + | - | - | + | + | - | - | - | - |
| 597 | - | + | - | - | - | - | - | - | - | - | - | - | - | + | - |
| 617 | + | - | - | + | - | - | - | - | - | - | - | - | - | - | - |
| 624 | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + |
| 648 | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - |
| 672 | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| 694 | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - |
| 711 | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - |
| 764 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| 794 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| 834 | + | - | - | + | - | - | - | - | - | - | - | - | - | - | - |
| 848 | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| 853 | - | - | + | - | - | + | - | - | - | + | + | + | - | - | + |
| 858 | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |

Table S2 is based on bacterial OTU presence (+) or absence (-) data as obtained by T-RFLP analysis of the different treatments. Unique OTUs (i.e. exclusively occurring in a given treatment) are labelled in dark.

**Table S3.** Patterns of bacterial OTUs from natural and artificial sediments in control and organic substrate-treatments prior to and after one year of incubation (manuscript III).

| bp | 0 days | | | 1 year | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | | Control | | | Treatment | | | | | | | | |
| | | | | | | | Detritus | | | Yeast | | | Fish | | |
| | glass | sand | mud | glass | sand | mud | glass | sand | mud | glass | sand | mud | glass | sand | mud |
| 54 | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - |
| 56 | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - |
| 57 | + | - | - | - | - | + | - | - | - | - | - | + | - | - | - |
| 58 | + | - | - | + | + | + | + | - | + | + | + | + | - | + | - |
| 59 | - | + | + | - | - | - | - | - | - | - | - | - | + | + | + |
| 60 | - | + | - | - | - | - | - | - | - | - | - | - | + | - | + |
| 61 | - | - | + | - | - | + | - | - | - | - | - | + | - | - | - |
| 63 | - | - | + | + | - | - | - | - | - | + | - | - | - | - | + |
| 72 | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| 75 | - | - | + | - | - | + | - | - | - | + | - | - | + | + | - |
| 77 | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| 80 | - | - | - | + | - | - | - | - | + | + | - | - | + | + | + |
| 81 | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| 83 | - | - | - | - | - | - | - | - | - | + | - | + | - | - | - |
| 84 | - | - | - | + | - | - | - | - | - | + | - | - | + | + | - |
| 87 | - | - | + | - | - | + | + | + | - | + | - | - | - | - | - |
| 88 | - | - | - | + | + | - | - | + | - | + | - | - | + | - | + |
| 89 | - | - | - | + | - | - | - | + | + | + | - | - | + | + | + |
| 90 | - | + | + | - | + | + | + | + | - | + | + | + | + | + | - |
| 91 | - | - | + | + | + | - | + | - | - | + | + | - | - | - | - |
| 93 | - | - | + | - | + | + | - | - | + | + | + | + | - | + | + |
| 95 | - | - | + | + | - | - | - | + | - | + | - | - | - | + | - |
| 97 | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - |
| 98 | + | - | + | + | + | + | + | + | - | + | + | + | - | + | - |
| 100 | - | + | + | - | - | + | + | - | - | - | - | + | - | + | - |
| 106 | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - |
| 112 | - | - | - | - | - | - | - | - | - | + | - | + | + | - | - |
| 114 | - | - | - | - | - | - | - | + | - | - | - | - | + | - | - |
| 120 | - | - | + | + | - | + | - | - | - | - | - | - | - | - | - |
| 122 | - | - | + | - | - | + | - | - | - | + | - | - | - | + | - |
| 123 | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - |
| 125 | - | - | - | - | - | - | - | - | - | + | - | + | - | + | - |
| 127 | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 130 | - | - | - | - | - | - | + | - | + | - | - | - | - | - | - |
| 134 | - | + | - | - | + | - | + | - | - | - | - | - | - | + | - |
| 135 | - | - | - | - | - | - | + | - | - | + | - | - | + | + | - |
| 137 | - | + | + | + | + | + | - | - | - | - | - | - | - | - | - |
| 138 | - | - | + | + | + | + | - | + | - | + | - | - | + | + | - |
| 140 | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - |
| 142 | - | + | - | - | + | - | - | - | - | - | - | - | - | - | + |
| 144 | + | + | + | + | + | + | - | + | - | + | - | - | + | + | - |
| 146 | - | - | - | - | - | - | + | - | - | + | - | - | - | + | + |
| 148 | - | - | + | + | - | + | - | - | - | + | - | - | + | + | - |
| 153 | - | - | - | - | - | - | + | + | - | - | - | + | - | - | - |
| 154 | - | - | - | - | - | - | - | - | - | + | - | - | + | + | - |
| 156 | - | + | + | + | + | + | - | + | - | + | - | - | - | - | - |
| 158 | - | - | + | - | - | - | + | - | - | + | - | - | - | - | - |
| 160 | - | - | + | - | - | + | - | + | + | - | - | + | - | + | - |
| 164 | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - |
| 167 | - | - | + | - | - | - | - | - | - | + | - | - | - | + | - |
| 168 | - | - | - | - | - | - | + | - | - | - | - | - | - | + | - |
| 174 | - | - | - | - | - | - | + | - | - | + | - | - | + | + | + |

Continuation Table S3

| bp | 0 days | | | 1 year | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | | Control | | | Treatment | | | | | | | | | |
| | | | | | | | Detritus | | | Yeast | | | Fish | | | |
| | glass | sand | mud | glass | sand | mud | glass | sand | mud | glass | sand | mud | glass | sand | mud |
| 176 | - | - | - | - | - | - | + | + | - | - | - | - | - | - | - |
| 180 | - | + | - | - | - | - | - | - | + | - | - | - | - | + | + |
| 181 | - | - | - | - | - | - | - | - | - | + | - | - | - | + | - |
| 182 | - | - | - | - | - | - | - | - | + | + | - | - | - | + | - |
| 184 | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| 188 | - | - | - | - | - | - | + | + | - | - | - | - | - | + | - |
| 194 | - | + | - | - | - | - | + | - | - | - | - | - | - | - | - |
| 195 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | + |
| 196 | - | - | - | - | - | - | + | - | - | + | - | - | - | - | + |
| 198 | + | + | - | + | + | + | - | + | - | - | - | - | - | - | + |
| 200 | - | + | - | - | + | - | - | + | - | + | + | + | + | + | + |
| 203 | + | + | + | + | + | + | - | + | - | + | + | + | - | + | + |
| 205 | - | - | - | - | + | - | - | - | - | + | + | + | + | - | - |
| 207 | - | + | + | + | + | + | - | + | - | - | + | + | - | + | + |
| 208 | + | - | + | - | - | + | - | - | - | + | + | + | - | + | + |
| 210 | - | - | - | - | - | - | + | - | - | - | - | + | - | - | + |
| 211 | + | + | - | + | - | + | + | + | - | - | - | - | - | - | - |
| 212 | - | + | + | - | - | - | + | - | - | + | - | + | - | - | - |
| 215 | - | + | - | - | - | - | + | - | + | - | - | + | + | + | - |
| 216 | - | - | - | - | - | - | + | - | - | - | - | + | - | - | - |
| 218 | - | - | + | + | - | - | + | - | - | - | - | + | - | + | + |
| 220 | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - |
| 223 | - | - | + | + | - | + | - | + | - | + | - | - | - | + | + |
| 225 | - | - | - | - | - | - | - | - | - | + | - | + | - | + | - |
| 227 | - | - | - | - | - | - | + | - | - | - | - | - | - | + | - |
| 235 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| 236 | - | - | - | - | - | - | - | - | + | - | - | - | - | + | + |
| 240 | - | - | + | - | - | - | - | - | - | - | - | + | - | - | - |
| 241 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 243 | - | - | - | - | - | - | + | - | - | - | - | - | - | + | - |
| 246 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - |
| 251 | - | - | - | - | - | - | - | - | - | + | - | - | - | + | - |
| 264 | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 275 | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| 281 | - | - | - | - | - | - | - | - | - | + | - | - | - | + | - |
| 293 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 295 | - | - | + | + | - | + | - | - | - | + | - | - | - | - | - |
| 300 | - | - | - | - | - | - | - | - | - | + | - | - | + | + | - |
| 304 | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 305 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - |
| 317 | - | + | - | - | - | - | - | - | - | - | - | - | - | + | - |
| 323 | - | - | - | - | - | - | - | - | - | + | - | - | - | - | + |
| 347 | - | - | + | + | - | - | + | + | - | - | - | + | - | - | - |
| 348 | - | + | + | - | - | + | - | + | + | + | - | - | - | + | - |
| 357 | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - |
| 366 | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + |
| 368 | - | + | - | - | - | - | - | - | - | - | - | - | - | - | + |
| 370 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - |
| 371 | - | + | - | - | + | + | + | + | - | - | - | - | - | - | - |
| 373 | - | + | - | + | - | - | - | - | - | - | + | + | - | + | + |
| 376 | - | - | + | + | + | + | + | + | - | + | - | - | - | - | + |
| 380 | - | - | + | + | + | + | + | - | - | + | + | + | - | - | - |
| 381 | - | - | + | - | - | + | - | + | + | + | + | + | - | + | - |
| 383 | - | + | + | + | + | - | - | + | + | - | - | + | - | - | + |
| 386 | - | - | - | - | - | - | - | - | + | + | + | - | - | - | - |
| 390 | - | - | - | + | - | - | - | - | - | - | - | - | - | + | + |
| 393 | - | - | + | + | + | + | + | + | + | + | - | - | - | + | + |

Continuation Table S3

| bp | 0 days | | | 1 year | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | | Control | | | Treatment | | | | | | | | |
| | | | | | | | Detritus | | | Yeast | | | Fish | | |
| | glass | sand | mud | glass | sand | mud | glass | sand | mud | glass | sand | mud | glass | sand | mud |
| 395 | - | - | - | - | - | - | - | - | - | + | + | + | - | + | - |
| 397 | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - |
| 400 | - | - | - | - | - | - | + | + | - | - | - | - | - | - | - |
| 403 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - |
| 406 | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - |
| 413 | - | - | - | - | - | - | - | - | + | - | - | - | + | + | - |
| 417 | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| 422 | - | - | - | - | - | - | + | + | + | - | - | - | - | - | - |
| 427 | - | - | - | + | - | - | - | + | - | - | + | - | - | - | - |
| 429 | - | - | - | - | - | - | + | + | - | - | - | - | + | - | - |
| 432 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - |
| 435 | + | + | - | + | + | - | - | + | - | - | - | - | - | - | - |
| 438 | - | + | - | - | - | - | - | + | - | - | - | - | + | + | - |
| 440 | - | - | - | - | - | - | - | - | - | - | - | - | + | - | + |
| 443 | + | + | - | + | + | - | - | + | + | - | - | - | - | - | - |
| 447 | - | - | - | - | - | - | - | - | - | - | + | - | + | - | - |
| 453 | - | + | + | + | + | + | - | - | + | - | - | - | - | - | - |
| 456 | + | - | - | + | - | - | - | + | - | - | - | - | + | - | - |
| 457 | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - |
| 460 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| 465 | - | - | + | + | - | - | - | + | - | - | - | - | - | - | + |
| 468 | - | - | + | - | - | - | - | - | - | - | - | - | + | - | - |
| 475 | - | - | + | - | - | - | - | + | + | - | - | - | - | - | - |
| 477 | - | - | + | - | - | - | - | - | - | - | + | - | + | - | - |
| 480 | - | - | + | + | + | + | - | + | - | - | - | - | - | - | - |
| 485 | - | - | - | - | - | - | - | + | - | - | + | - | - | - | - |
| 492 | + | + | - | - | + | + | - | - | - | - | - | - | - | - | - |
| 496 | + | + | + | - | + | + | - | - | - | - | + | - | + | - | + |
| 498 | + | - | + | + | + | - | - | - | - | - | + | - | - | - | + |
| 502 | + | + | + | + | + | + | - | - | - | - | + | - | - | - | + |
| 509 | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - |
| 510 | - | - | + | + | + | + | - | - | - | - | + | - | - | - | + |
| 514 | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + |
| 516 | - | - | - | - | - | - | - | - | + | - | + | - | - | - | - |
| 524 | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - |
| 526 | + | - | - | - | + | + | - | + | + | + | + | - | - | + | - |
| 528 | - | - | - | - | - | - | - | - | + | - | + | + | - | - | - |
| 540 | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - |
| 543 | + | - | + | - | - | - | - | + | - | - | - | - | - | - | - |
| 546 | - | - | - | + | - | + | - | + | - | - | - | - | - | - | - |
| 553 | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - |
| 561 | - | + | + | - | + | + | - | + | - | - | - | - | - | - | - |
| 563 | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - |
| 564 | + | + | + | + | + | + | - | - | - | - | + | + | - | - | - |
| 567 | - | - | - | - | - | - | - | + | - | + | + | + | - | - | - |
| 574 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 577 | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - |
| 580 | + | - | - | - | - | + | - | - | - | - | + | + | - | - | - |
| 581 | - | - | + | + | - | + | - | - | + | - | - | - | - | - | - |
| 587 | - | - | - | - | - | - | - | - | + | + | + | + | - | - | - |
| 588 | - | - | + | + | + | + | - | + | - | - | - | - | - | - | - |
| 593 | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - |
| 598 | - | + | - | + | + | - | - | - | - | - | + | - | - | - | - |
| 624 | + | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| 848 | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 881 | - | - | - | - | - | - | - | - | - | + | + | + | - | - | - |

Table S3 is based on bacterial OTU presence (+) or absence (-) data as obtained by T-RFLP analysis of the different treatments. Unique OTUs (i.e. exclusively occurring in a given treatment) are labelled in dark.

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