Bacterial community patterns along small- and large-scale environmental gradients in Arctic deep-sea sediments

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Bakterielle Gemeinschaftsmuster entlang klein- und großskaliger Gradienten
in arktischen Tiefsee-Sedimenten

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SUMMARY

The deep-sea floor is characterized by highly diverse seascapes such as canyons, seamounts, ridges, fractures, trenches and wide deep basins. Any bathymetric incline is linked to a change in hydrostatic pressure, in the availability of organic material as well as in the susceptibility to physical disturbances, splitting the deep-sea environment into highly heterogeneous habitats of different dynamics. Focussing on vast, apparently desert-like plains of deep-sea sediments, major biogeochemical cycling is concentrated on top sediment layers. There, biogeochemical processes are mainly governed by the quantity and quality of organic matter input originating from surface water production, by the composition and activity of the benthic fauna dealing with it, and ultimately by large- and small-scale disturbances affecting these interactions. In Arctic waters, these processes are reinforced by the seasonal occurrence of mass sedimentation of organic material during ice melting processes, accompanied by manifold current-driven transport processes. Key players in biochemical cycling are bacteria, standing at the basis of organic material conversion and ultimately of the energy flow through the ecosystem, and forming up to 90% of the deep-sea benthic biomass. Due to their high potential for adaptation and catalysis, any minute change in nutrient supply due to physical or biological disturbances is immediately mirrored by the composition, metabolic activity and distribution of microbial communities in deep-sea sediments.

One focus of this study was to detect local coherences between bacterial community patterns and an ice-edge related input of organic material (Fram Strait, Arctic Ocean) as well as physical disturbances (Ardencaple Canyon, Greenland Sea). Such large-scale patterns along a depth-dependent gradient were compared to small scales patterns within the sediment column. A further aim was to assess the local impact of small biogenic structures on benthic bacterial communities by increasing microhabitat heterogeneity through the modification of near-bottom flows and hence deposition patterns in deep-sea surface sediments. As the retrieval and incubation procedures of all deep-sea sediments sampled for this study were performed in absence of pressure-retaining gears, bacterial activity has been assessed by different approaches to estimate bulk metabolic pathways. A direct viable count method has been adapted to deep-sea sediment samples in minimizing detrimental decompression effects during sample-processing.

General large-scale, depth-dependent patterns of prokaryotic distribution, activity and community composition have been investigated along a depth transect - following a bathymetric decline from the continental slope down to the abyssal plain in Fram Strait (Arctic Ocean, 1250 m to 5600 m). Aside from any depth-dependent zonation, the
Summary

A prokaryotic community mainly depended on the ice-edge induced flux of phytodetrital and terrigenous particles. A decreasing bacterial viability with increasing water and sediment depth (0 to 5 cm) was significantly correlated with esterase activity (FDA-turnover rates) as well as with bacterial incorporation rates based on thymidine and leucine incorporation (as a measure for DNA and protein synthesis, respectively). The effect of biological disturbances by macro/megafauna seemed to be compensated over this large-scale depth range, allowing a so-called ‘bacterial viability boundary’ to establish generally at approximately 3-4 cm sediment depth. However, there were indications for a locally enhanced bacterial response to ice-edge-induced particle flux, which was reinforced by an increased bacterial ribotype richness (assessed by terminal restriction fragment length polymorphism [T-RFLP] analysis). In this context, the Molloy Hole (the deepest Arctic depression) has been shown to act as a huge sedimentation trap of both, organic material and probably particle-attached bacteria. Hence, at large scales, general depth-dependent patterns seem to be governed by a complex interaction of nutrient availability and current-driven distribution processes, mainly dependent on the ice-edge induced flux of phytodetrital and terrigenous particles.

As an example for a modification of large-scale gradients, the Ardencaple Canyon was characterized by a spatial coherence of benthic bacterial community patterns along its course from the Eastern Greenland continental rise towards the Greenland Basin. Comparing benthic bacterial assemblages from the northern to the southern canyon levees and central areas, lowest community complexity was found in the central parts of the channel. High similarities between benthic bacterial assemblages of different regions along the Canyon as well as increasing prokaryotic incorporation rates and total organic matter concentrations were observed with increasing distance from the continental shelf. These findings pointed to a combination of an ice-edge induced particle flux, episodic down-slope and canyon-guided transport mechanisms, while past events of mass sediment transport seem to have a long-term effect on the distribution of benthic prokaryotic communities along the sediment column.

As an example for a modification of small-scale gradients, different morphotypes of biogenic structures at the deep-sea sediment surface revealed that depressions such as burrows and elevations like sessile epibenthic sponges allowed laterally transported organic particles and prokaryotic cells to accumulate in their immediate sedimentary microenvironment. Bacteria immediately responded to the increased availability of organic material left as faeces or re-suspended by crawling and feeding macroorganisms by increased colonisation and cellular biomasses. Through the same actions, a significant proportion of bacteria had been removed by predation or sediment relocation. A specific
benthic bacterial and archaeal community in the immediate surroundings of *Tentorium semisuberites* suggested a metabolic inhibition and/or an active particle sorting by the sponge. Creating small-scale patches of high prokaryotic activity due to an increased quality of organic substrate and specific prokaryotic community, biogenic sediment structures obviously bear the potential to favour habitat heterogeneity in deep-sea sediments.

Physical and biological factors along environmental gradients in deep-sea sediments have been shown to affect the distribution, composition and metabolic activity of bacterial communities and *vice versa*. At large scales, bacterial community patterns have been shown to be mainly governed by physicochemical factors, whereas modifications at small scales proved to be controlled by biological factors such as bioturbation. Ultimately, highly dynamic regions such as the Ardencaple Canyon as well as minute obstacles at the sediment surface create hot spots of biogeochemical cycling. Hence, there is a huge potential to increasing deep-sea habitat heterogeneity and thus affecting benthic bacterial community patterns, while the question of the driving forces (biological/ physical disturbances) remains a question of scale.
ZUSAMMENFASSUNG


Ein Beispiel für die **Modifikation großskaliger Gradienten** ist der Ardencaple Canyon, in dessen Verlauf vom ost-grönländischen Kontinentalabhang in Richtung Grönländbecken sich eine große räumliche Übereinstimmung der Gemeinschaftsmuster benthischer Bakterien zeigte. Beim Vergleich der benthischen Bakteriengemeinschaft der nördlichen und südlichen Hänge und des zentralen Canyonbereichs wurde die Bakteriengemeinschaft mit der geringsten Komplexität in den zentralen Abschnitten gefunden. Es zeigten sich starke Ähnlichkeiten zwischen den benthischen Bakteriengemeinschaften verschiedener Bereiche im Canyonverlauf sowie zunehmend erhöhte prokaryotische Inkorporationsraten und Konzentrationen organischer Materials mit zunehmender Entfernung vom Kontinentalrand. Diese Ergebnisse deuten auf eine
Kombination zwischen Eisrand-induziertem Partikeleintrag, episodischen Hangrutschungen und Transportprozessen im Canyon hin, während ehemalige, massenhafte Sedimenttransporte einen anhaltenden Effekt auf die Verteilung benthischer, prokaryotischer Gemeinschaften innerhalb der Sedimentsäule zu haben scheinen.


Die vorliegende Arbeit hat gezeigt, dass physikalische und biologische Faktoren entlang von Umweltgradienten die Verteilung, Zusammensetzung und metabolische Aktivität von Bakteriengemeinschaften in Tiefsee-Sedimenten beeinflussen und vice versa. Im großskaligen Bereich richteten sich die bakteriellen Gemeinschaftsmuster hauptsächlich nach physikalisch-chemische Faktoren, während Veränderungen im kleinskaligen Bereich durch biologische Faktoren wie Bioturbation kontrolliert wurden. Dabei dienten hochdynamische Regionen wie der Ardencaple Canyon gleichermaßen als Erzeuger von ’hot spots’ biogeochemischer Umsatzprozesse wie winzigste Erhebungen an der Sedimentoberfläche. Insgesamt ergibt sich ein immenses Potential zur Vergrößerung der Lebensraumheterogenität und somit zur Beeinflussung benthischer Bakteriengemeinschaftsmuster, wobei die Frage nach den treibenden Kräften (biologische / physikalische Störungen) eine Frage der Skala bleibt.
I GENERAL INTRODUCTION

...if we go too far in pooling organisms together, we will lose all the natural history that ultimately drives evolution of both organisms and communities. If we stop short and retain many groups, we will be confronted with a complexity that will escape analysis. Yet, it is often advantageous to ignore the detail in order to look for general patterns...


These considerations of Carlos Pedrós-Alió seemed to me most adequate when I started to approach the question on bacterial community dynamics along small- and large-scale gradients in deep-sea sediments. As rapidly reacting key players in global cycling processes, bacteria are the most suitable indicators for changes in environmental conditions. The first challenge was to select the most important environmental gradients amongst the multitude of possibilities at a given scale. A similar challenge was the environment in question itself, the deep sea. Despite recent advances in technology, it is still a hardly accessible sampling area, and deep-sea organisms suffer from decompression during retrieval.

To grasp the driving coherences between bacterial community dynamics and deep-sea environmental gradients, it appeared to be most promising to establish general patterns first. Bearing Pedrós-Alió’s words in mind, however, the details must not be completely ignored, as the effect of a single changing detail might be sufficient to alter a general pattern - which is, in the end, a question of time, scale and definition.

Of course, we are still a long way from knowing about all the details of global importance. Still, we must learn that many patterns, which were assumed to be general, are in fact not general. Every now and then, we celebrate those studies which succeed in determining linear relationships between more than three different environmental parameters. Hence, we should do our best to extend our knowledge on ecosystem functioning step by step.
I.1. Environmental gradients in deep-sea habitats

According to the American Heritage College Dictionary (3rd edition, 2000), a gradient is defined as:

1. A rate of inclination; a slope.
2. An ascending or descending part; an incline.
3. **Phys.** The rate at which a physical quantity, such as pressure, changes with respect to a given variable, especially distance.
4. **Math.** A vector having coordinate components that are the partial derivatives of a function with respect to its variables.
5. **Biol.** A series of progressively changing differences in the growth rate, metabolism, or physiological activity of a cell, an organ, or an organism.

Hence, gradients are – per definition – ubiquitous. Exactly this aspect, however, is responsible for both, benefits and difficulties, in studying community dynamics along environmental gradients. Whether of physical, (geo-)chemical or biological nature, gradients can be oriented in horizontal, vertical and diagonal directions, either at a macro (m–km), meso (cm–m), micro (mm–cm) or nano (μm–mm) scale. The scale range of organisms colonizing habitats from $10^{-6}$ m (e.g. bacteria) to $10^8$ m (e.g. an entire ocean basin) entails that the assessment of the development and relevance of environmental gradients on the whole is a great task.

The deep sea is one of the most interesting habitats with respect to the variability of environmental gradients and their effect on biological processes in space and time. Comprising approximately 65% of the Earth’s surface, deep-sea environments are characterized by hyperbaric, aphotic and low-temperature conditions, and high topographic variability (Thistle 2003). The highly diverse seascapes include canyons, seamounts, ridges, fractures and trenches, but also biogeochemical oases such as cold seeps, mud volcanoes, carbonate mounds, brine pools, gas hydrates, hot vent systems and deep-water coral reefs (Tyler 2003). Much effort was put into studying microbial diversity and metabolism in those hot-spot, chemosynthetic ecosystems (for review see Jørgensen & Boetius 2007), totally outshining the apparently vast desert-like plains of deep-sea sediments. As a stage of extensive biogeochemical cycling, the top sediment layer of this wide seafloor realm is defined as the deep seafloor for the purpose of this study. Independent of the scientific progress made in unravelling the deep-sea ecosystem, the basic physico–chemical characteristics form at least three types of gradients of high importance to benthic organisms:
The depth gradient

At first sight, the most striking inclination in a marine benthic system is the graduation from the shore, through the intertidal zone, over the gently sloping continental shelf to the shelf break and down to the deep ocean floor with its submerged valleys, troughs and mountains. Figure 1 illustrates these topographic features for the Barents Sea and the Fram Strait, which will be addressed in more detail below (chapter II.1.1). As any bathymetric incline or decline is linked with a change in hydrostatic pressure, the predominant environmental gradient is – according to the above definition – of physical nature.

Fig. 1: Bathymetry of the Barents Sea, displaying the continental shelf and slope at the transition to the Arctic Sea, its abyssal plain with ridge offshoots and fracture zone, where the Fram Strait leads into the Greenland Sea (source: Stein & MacDonald 2004).

The pressure gradient

Starting from a standard atmosphere at Earth mean sea level of approximately 1 bar, a 10 m increase in water depth equals a pressure increase of approximately 1 bar. A depth-related pressure gradient cannot be modified, neither by physical nor by biological factors. Being one of the largest environmental gradients on this planet (in terms of spatial extension), its stability stands out against nutritional gradients.
The biochemical (nutritional) gradient

The export of particulate organic matter (POM) from the ocean’s surface waters constitutes the bulk of nutrient supply for benthic life in the deep sea, although only a small fraction of this material reaches the deep seafloor (De La Rocha & Passow 2007, and references therein). Mainly consisting of phytoplankton, marine snow, faecal pellets, (dead) zooplankton and molts (Iken et al. 2005), this material undergoes different steps of degradation during its passage from the epi- to the meso- and bathypelagic (photic to aphotic) zone. In Arctic waters, the export of ice-trapped POM (e.g. ice algae) takes place as periodic mass sedimentation during melting processes in summer (Nürnberg et al. 1994), which will be addressed in more detail below. The quantity and quality of organic matter settling to the deep seafloor is thus affected by its original composition, residence time in the water column and manifold transport processes (Suess 1980). At the bottom, nutritional gradients are formed through the differences in POM concentration between different locations at the sediment-water interface and within the sediment column. Beside a biochemical modification through colonizing benthic organisms, a physical modification of such gradients may ensue from water mixing by convection, currents, and epi-/infraunal activities (processes are addressed in more detail below).

This short overview points out that the investigation of community responses to a single environmental gradient is not sensible enough, as each habitat may feature a number of associated ecological gradients of considerable variability. Representing transitions of gradually changing conditions, environmental gradients can be treated as links between habitats of specific physicochemical properties. One of the key endeavours in ecology is thus to gain an understanding of the physical and biogeochemical properties of habitats by concentrating on the most important gradients and their interactions.

As demonstrated by the example of three basic gradients occurring in the Arctic deep sea, environmental gradients interact with and determine each other:

1) Any bathymetric incline or decline is bound to a change in hydrostatic pressure.
2) The depth-related photic gradient determines primary production in surface waters and consequently quantity, sinking velocity and thus quality and quantity of phytodetrital matter reaching the deep seafloor.
3) The depth-related pressure gradient alters the composition and ultimately the metabolic activity potential of particle-associated microbial communities, thus affecting the nutritional gradient formation at the deep seafloor.
4) Depth gradients within the sediment column are linked to a concentration gradient of organic and inorganic substances, similarly affecting the activity and composition of sediment-inhabiting organisms.
Definition of gradient scales and directions in deep-sea sediments

Based on the considerations above, we can distinguish between a ‘**vertical large-scale gradient**’, when focussing on the pressure aspect (Fig. 2 a), and a ‘**vertical small-scale gradient**’ (Fig. 2 b), referring to the biochemical variation in sediments. Like in other benthic ecosystems, nutrients in deep-sea sediments can form a distinct small-scale concentration gradient either along the sediment-water interface or within the sediment column.

![Fig. 2: Schematic overview of vertical depth gradients in deep-sea sediments: at large scales down the continental slope (a) or at small scales down the sediment column (b). They can be driven by substances used as nutrients (POM), or by physical or chemical factors, which may in turn differ in relation to scale and direction.](image)

For ecological purposes, the term ‘horizontal’ is generally used for lateral interactions, e.g. for erosion at large scales. While wave action is the most important energy source in shallow marine ecosystems, comparable transport and deposition/erosion mechanisms in deep environments are attributed to bottom currents (Aller 1997, Gage 1996, Ledbetter & Klaus 1987, Richardson *et al.* 1993). Interacting with the (micro-) topographic features at the deep seafloor, lateral advection contributes to the horizontal distribution patterns of organic matter and associated microorganisms (Boetius & Damm 1998, Ritzrau & Thomsen 1997, Witte & Pfannkuche 2000).

Horizontal gradients are most pronounced at the transition between habitats (or patches) of different biogeochemical conditions, affecting concentration profiles and zonation patterns within or between habitats. Ultimately, gradients between habitats can be considered as links between different physical, chemical or biological states on the metabolism and the distribution of benthic organisms, which again mingle with different spatial scales. In this respect, patches of different biogeochemical properties at the sediment-water interface are connected by ‘**horizontal small-scale gradients**’, whereas links between habitats as topographic alterations of the deep seafloor (e.g. continental slope, rise, basins, channel systems, trenches, troughs, ridges) should be defined as ‘**horizontal large-scale gradients**’. In this study, however, the large-scale gradient from the continental slope down to the abyssal plain is treated as ‘**vertical large-scale gradient**’, emphasizing the impact of pressure as an abiotic factor.
1.2. Habitat dynamics in deep-sea sediments

Any modification of vertical and horizontal gradients by locally variable biological and physical factors affects habitat heterogeneity in space and time. Following the ‘Habitat Heterogeneity Hypothesis’ (Grassle & Sanders 1973), the lower physical energy and biological activity in the deep sea leads to the persistence of spatial heterogeneity as a source of faunal diversification through niche partitioning. However, the ‘Intermediate Disturbance Hypothesis’ (Connell 1978) challenges the attempt to use the habitat ‘equilibrium’ as an explanation for diversity patterns. Originally developed for tropical rain forests and coral reefs, the importance of physical disturbance as a dramatic modification of any habitat equilibrium in affecting species diversity was discussed for a variety of habitats at different scales. Physical disturbances in marine systems (e.g. iceberg scouring, turbidites or benthic storms) result in a decrease in the abundance of organisms and in subsequent succession patterns during re-colonisation of limited habitat resources (Gage 1996, Rosenberg et al. 2003, Woodin et al. 1998). In this respect, species diversity is not only a function of scale of disturbance, but also of time, frequency and of competition (Connell 1978). Depending on the tolerance to physical stress and on the response to biological processes, each species will have a different optimal performance zone along the respective gradient. As demonstrated by the impact of iceberg scouring on macro-benthic recolonization patterns (Gutt & Starmans 2001), the numbers of species distributed along a gradient decreased towards the extremes. The co-existence of species may be facilitated by refuges which reduce the risk of mortality by predation or competitive interaction. The period of coexistence in deep-sea environments may also be prolonged by slow growth rates (‘Dynamic Equilibrium Theory’; Huston 1979).

In this study, large-scale habitat heterogeneity refers to topographic features (e.g. canyons, trenches, troughs, ridges, sea mounts) and long-range, physicochemical disturbances. Canyons are very important areas of large-scale disturbance as they act as conduits for drainage flows from the shelf to the deep sea (Carter 1988, Puig et al. 2000, Puig et al. 2003, Schmidt et al. 2001). Along canyons, a funnelling of local currents may facilitate the transport of organic material. Hence, they can be considered as areas of organic matter deposition and erosion (Griggs et al. 1969, Rowe et al. 1982, Gage et al. 1995, Vetter & Dayton, 1998, Gerino et al. 1999, Duineveld et al. 2001). In such systems, benthic communities are exposed to continuously changing physical and biogeochemical conditions leading to episodic or periodic habitat modifications. Large-scale disturbances may occur as massive down-slope transport of material by slides, turbidity currents from the continental shelf down the slope to the basin or so-called benthic storms (Biscay &
Small-scale habitat heterogeneity refers mainly to micro-topographic features at the sediment-water interface which are generated by the activities of benthic organisms (Fig. 3). Biologically produced sediment structures (e.g. crawling and resting traces, pits, feeding structures, dwellings), sessile and projecting epibenthic species (e.g. some foraminifers, sponges, crinoids) and non-living obstacles at the sediment-water interface (e.g. carcasses, whale bones, drop stones) can create dynamic microhabitats (Gage 1996). In this study, such biogenic structures are defined as areas of small-scale disturbances, affecting horizontal gradients at the sediment-water interface as well as vertical gradients within the sediment column. Macrofaunal feeding activities lead, in most cases, to the re-structuring, re-suspension and re-distribution of sediment (and bound organic compounds). These processes involve a physical modification of existing gradients, e.g. in the distribution of nutrients (Davis 1993) or particle size (Thomsen & van Weering 1998, Turnewitsch 2001, Woodin et al. 1998).

Fig. 3: Seafloor photograph showing small-scale habitat heterogeneity created by epi- and infaunal re-structuring of the deep-sea sediment surface (area covered ca. 0.6 m², at approximately 1500 m water depth). Morphotypes of ‘Lebensspuren’ are defined according to Young (1985) as circular grooves (a), burrows (b), pennatulid with its crenulated plough (c), branched ridges (d), ophiuroid with its resting traces (e), simple grooves (f), depressions (g).
Depending on their shape and height, emergent structures passively modify near-bottom flow patterns. Consequently, they alter the deposition rates of particles and cause small-scale gradients by continuously altering nutrient concentrations and particle composition (Carey 1983, Eckman 1985, Witte et al. 1997). The generation of biogenic structures may be regarded as episodic disturbance events. It alters the deep seafloor at small scales and finally provides a surge of spatially and temporally variable habitats to small biota such as bacteria and meiofauna (Jumars 1975, Jumars & Eckman 1983). The concept of a non-competitive co-existence of species by increased habitat heterogeneity led to the ‘Spatial Temporal Mosaic Theory’ (Grassle 1989). It describes the spatial and temporal dynamics of patches characterized by different habitat conditions which lead to locally increased species abundance and evenness.

1.3. Why dealing with microbial communities?

Similar to terrestrial systems, the deep-sea biological response to uni-directional, vertical gradients (either due to physical or nutritional conditions) is zonation. This is reflected in variations in the metabolism of organisms distributed along these gradients and in changes in the community structure (Baker 1909). The effect of environmental parameters on the distribution, functioning and growth of populations (autecology) is regulated by nutritional constraints according to Liebig’s (1840) *Law of the Minimum* and Shelford’s (1913) *Law of Tolerances*. Consequently, the biomass of an organism is determined by the nutrients present at lowest concentrations in relation to the requirements of that organism. By contrast, the occurrence, abundance and growth of an organism pre-suppose a complex, specific set of physicochemical conditions in addition to nutrients.

Since microbes are r-strategists, they not only have short generation times but have also large surface to volume ratios, permitting a more intimate contact with the environment, a greater uptake potential for nutrients and a more rapid turnover of nutrients and organic matter (Black 1996). A high potential for adaptation and catalysis enables them to respond rapidly in numerical and metabolic terms to changes in the supply and quality of organic material at the ocean floor (Gooday & Turley 1990 and references therein). Hence, microorganisms are ideal indicators for minute disturbance events (e.g. physical disturbances, episodic nutrient pulses) at short time scales (from days to weeks). Microbial communities undergo successional processes which may be disrupted by disturbance, but homeostatic forces act to restore the community balance. Microorganisms act as integrated assemblages of populations, which occur and interact within a habitat (synecology), according to the mechanisms of competition for available
niches. As a result, they have a functional role in maintaining community stability. Owing to a highly hierarchical organisation, microbial communities have often been used as model organisms for understanding general population interactions (Atlas & Bartha 1993).

According to Marshall (1993), microbial ecology has three main goals with respect to understanding the role of microorganisms in their natural habitats:

1. to define population dynamics in communities
2. to define physicochemical characteristics of microenvironments
3. to understand the metabolic processes of microorganisms from specific habitats.

Because of their ubiquity, diverse metabolic capabilities and enzymatic efficiency, microorganisms play a major role in biochemical cycling (Pomeroy 1974, Jørgensen 1989), in terms of global transfer and conversion of materials and ultimately of energy flow through ecosystems. In addition to their vital importance in global cycling processes, microorganisms account for up to 90% of the deep-sea benthic biomass (Pfannkuche 1992). Environmental gradients or habitat heterogeneity in deep-sea sediments are therefore expected to affect the distribution, composition and functional metabolic activity of microbial communities and vice versa.

**Bacterial role in deep-sea carbon cycling**

It was the concept of Pomeroy (1974) which invited scientists to reconsider the view of microorganisms as a black box, largely ignoring their role in the cycling of carbon and other elements. His proposition of a diffuse network in which a variety of microorganisms have quantitatively significant roles in both production and consumption finally led to the development of the ‘microbial loop’ concept (Azam *et al.* 1983). From that moment on, the importance of prokaryotes as key players in global carbon cycling became evident: as the only organisms capable of utilizing dissolved organic carbon (DOC), they transform it to biomass, particulate organic carbon (POC). Thus, they reintroduce this carbon to higher trophic levels. The amount of transferred carbon depends on the bacterial assimilation efficiency (generally assumed to be 50%) which, in turn, depends on the amount of refractory compounds (Pomeroy 2001).

High molecular weight molecules which are too complex to be transported across the bacterial cell's membrane are decomposed through a multiple extracellular enzyme hydrolysis system. The closer bacteria are to a given substrate, the higher is the hydrolytic
efficiency which has been proven for particle-attached consortia (Hoppe et al. 1993, Turley 1994). It turned out that heterotrophic bacteria on the aggregates utilize a substantially higher portion of the aggregate-associated POC than previously assumed, in particular amino-acid carbon (Grossart & Ploug 2001). These findings support the statement of Cho & Azam (1988) that bacteria rather than the particle-feeding zooplankton are the principal mediators of particle decomposition. Owing to these particle-associated microorganisms, any surface-derived organic material is continuously degraded during its passage through the water column (Cho & Azam 1988, Tseytlin 1987). Hence, the quantity and quality of organic carbon reaching the deep seafloor is mirrored by the bacterial biomass in surficial sediments (Deming & Yager 1992).

**Importance of POM-characteristics for deep-sea carbon cycling**

Initially, it was suggested that mineralization mainly takes place in the water column and that the nutrient exchange across the sediment–water interface in the deep sea could thus only be of little significance. It turned out that the extent of deep-sea benthic mineralization depends on (1) the origin and composition of POM and (2) its residence time in the water column:

*ad 1)* In polar regions, the amount, distribution and quality of POM at the deep seafloor may be intensified by a combination and a variability of physical factors, i.e. the strong seasonality in irradiance and ice coverage (Hargrave et al. 1994) in conjunction with the release of ice-trapped POM during melting processes in summer (Nürnberg 1994). In terms of periodic pulses, marginal ice zones are of key importance. Here, upwelling processes favour primary production and subsequently an enhanced sedimentation of POM. Ice-associated diatom mats contribute up to 57% of the total Arctic primary production (Gosselin et al. 1997), and account for the bulk of the ice-edge related particle flux. Forming large aggregations, diatoms are subject to events of rapid mass sedimentation to the seafloor, where they form distinct patches (e.g. McMahon et al. 2006, Tamelander et al. 2006). Being a highly bioavailable substrate, they subsidize deep-sea benthic organisms, leading to a locally enhanced metabolic activity (Legendre et al. 1992).

*ad 2)* The extent of microbial degradation is determined by the particle’s residence time in the water column (Davey et al. 2001, Tholosan et al. 1999, Turley & Stutt 2000), which in itself is a function of particle size. In addition to phytoplankton aggregations (e.g. diatoms), secondary aggregation of smaller particles and macromolecules (‘marine snow’) produces large particles (50–500 μm in diameter) which only need a few days or
weeks to settle at the deep seafloor. These large particles comprise only 10% of the particulate organic matter in the ocean but contribute 90% of the organic deposition on the seafloor (e.g. Chesselet 1980). Aggregate sinking velocity can be accelerated by trapped sediments (e.g. melting of ice-rafting sediments from continental glaciers) or decelerated by internal gas bubble formation (e.g. phytoplankton activity within marine snow aggregates). Hence, the particle’s consistence has important implications for its residence time in the water column which determines the exposure to microbial degradation and the bioavailability of surface-derived organic material at abyssal depths.

I.4. Outline of the thesis

At first sight, the different manuscripts merged in this thesis may appear nearly as heterogeneous as the deep-sea environment itself. In fact, however, they all focus on the interaction between bacterial communities and the trophic state of (micro-) environments in testing the following superordinate hypotheses:

(1) A depth-dependent gradient of bacterial distribution, activity and community composition in Arctic deep-sea sediments is locally interfered by enhanced ice-edge related input of organic material and large-scale disturbance.

(2) Small biogenic structures at the sediment surface increase spatial and temporal heterogeneity, leading to a local enhancement of microbial activity and to a shift in the benthic microbial deep-sea community.

To give an overview of the different scales and locations at which these hypothesis were tested, the study areas are presented first in the ‘Material & Methods’ section, followed by a summary of the scale-dependent sampling strategy. Restrictions in sample size and scale (which are typical in deep-sea research) necessitated the adaptation of methods commonly used in microbial ecology. Considerations on the choice of the methods used introduce the presentation of the adapted protocols. The remaining methods are described in more detail in Publications I-IV. The outcomes of these studies are briefly presented in conjunction with some unpublished data in the ‘Results’ section, and ultimately discussed in a broader context in the ‘Discussion’ section, according to the following topics:
**Vertical large- and small-scale gradients (bathymetric transect)**

The investigation of depth-dependent patterns of bacterial distribution, activity and diversity comprises a comparison of vertical environmental gradients at large scales (along the continental slope, down to the abyssal plain) and at small scales (down the sediment column). To gain rapid and reliable information on the activity status of natural deep-sea bacterial communities, a direct viable count method has been adapted to deep-sea sediments in minimizing detrimental decompression effects during sample-processing (Publication I).

**Modifications of large-scale gradients (channel dynamics)**

A channel system situated at the Greenland continental margin was investigated with respect to the potential of contributing to habitat heterogeneity and of experiencing periodic large-scale disturbance such as down-slope sediment slides and funnelled organic matter transport. The assessment of microbial diversity patterns is endorsed by activity measurements and analyses of nutrient concentrations along the course of the canyon (vertical gradient) and between the channel base and the levees (horizontal gradient), to explore the ecological impact of channel dynamics (Publication II).

**Modifications of small-scale gradients (biogenic structures)**

This chapter focuses on the response of bacteria and archaea inhabiting deep-sea sediments characterized by low hydrodynamic energy to biogenic disturbance at horizontal and vertical small (centimetres) scales. As a model for the active and passive modification of near-bottom flow and deposition patterns, the surroundings of small epibenthic sponges were investigated with respect to microbial community dynamics (distribution, composition, activity) (Publication III). The effect of bio-engineering in modifying horizontal small-scale gradients at the sediment-water interface and vertical small-scale gradients in sediments beneath was tested by comparing the metabolic responses of benthic bacterial communities around different sediment structures of biological origin (Publication IV).
II MATERIAL AND METHODS

II.1. Study areas, objects and sampling design

Marine Arctic waters play an important role in the global climate system. Low-salinity water (combined fluxes of the Bering Strait inflow and riverine freshwater input) is transported with the surface outflows of the East Greenland Current (EGC) along the western coast of Greenland to the south. As these outflows exceed the input of freshwater, an inflow of Atlantic water to the Arctic is induced. The only connection between the central Arctic Ocean and the Nordic Seas, where intermediate and deep waters are exchanged, is the Fram Strait (Fahrbach et al. 2001). The Fram Strait is bordered in the east by the Svalbard archipelago (with the continental shelf extending to the Yermak Plateau), and in the west by Greenland (with a steep incline of the continental slope towards the Lena Trough). Reaching from 78°N to 80°N and from 0°E to 7.5°E, the area features a complex sequence of transform faults and spreading centres (from south to north: Hovgaard Ridge System, the Molloy Fracture Zone and the Spitsbergen Fracture Zone).

Atlantic waters of comparably high temperature and nutrient content are transported by the West Spitsbergen Current (WSC) in the eastern parts of the Fram Strait towards the north (Manley 1995). The WSC splits at approximately 79°N into the re-circulating Return Atlantic Current (RAC), the Spitsbergen Branch (SB) at the Yermak Plateau (heading to the Arctic Ocean) and the Yermak Branch (YB), heading to the Nansen Basin. The two currents determine the dynamics and extent of the Marginal Ice Zone (MIZ) which is characterized by high seasonal variations of ice coverage (and phytodetrital input) in the central and northeastern parts of the Fram Strait. Areas sampled for the investigation on the impact of vertical and horizontal gradients on bacterial community dynamics in deep-sea sediments are subject to this circulation system in the Fram Strait and in the western Greenland Sea (Fig. 4). The interaction between southward outflows and induced inflows of Atlantic waters forms a horizontal (‘estuarine’) circulation which is most sensitive to climate change. Cooling and freezing of surface sea water lead to an increased density, sinking and exchange with waters at greater depths, initiating a vertical circulation: the thermohaline circulation (THC) which is of global importance to the oceans’ ‘Great Conveyor Belt’ (Broecker et al. 1985). In this respect, the potential impact of a changing THC on flow and conditions within the marine Arctic is of highest importance.

The most sensitive areas are those that currently receive most of the heat input from inflowing warm Atlantic water, such as the Fram Strait (Polyakov et al. 2005).
Material & Methods

Fig. 4: Study areas in the Fram Strait, with sampling stations along the depth transect of HAUSGARTEN (A) and in the western Greenland Sea at the eastern continental rise, along the Ardencaple Canyon (B). Northwards warm water inflow through the West Spitsbergen Current (WSC) splits into the Return Atlantic Current (RAC), the Spitsbergen Branch (SB) the Yermak Branch (YB); the East Greenland Current (EGC) transports cold water flows southwards out of the Arctic Sea (Map source: National Geographic Society, Washington, D. C.).

The following sections present details on the study areas sampled to assess habitat heterogeneity at large scales and small scales. The samples were taken in summer 2000 and 2001 during two expeditions (ARK XVI/PS57 and ARK XVII/PS59) onboard the German research icebreaker ‘RV Polarstern’ by a multiple corer (MUC). An additional sampling campaign was conducted onboard the French RV ‘L’Atalante’ in late summer 2001, where targeted pushcore samples were taken by the manipulator arms of the remotely operated vehicle (ROV) “Victor 6000”. The sampling designs are presented subsequent to the respective study areas: MUC sampling is described in more detail following Section II.1.2 (also referring to Section II.1.1); details on ROV pushcore sampling and small-scale sub-sampling are presented subsequent to subsections A and B in Section II.1.3. Bathymetric details of all stations and sampling foci according to the definition of small- and large-scale gradients are given in Table 1 at the end of this chapter.
II.1.1 The HAUSGARTEN depth transect (off Spitsbergen)

The ‘hot spot’ for climate change monitoring at 79°N (WSC splitting area, see above) led the Alfred Wegener Institute for Polar and Marine Research (AWI) to establish the unique deep-sea long-term observatory ‘HAUSGARTEN’ (Soltwedel \textit{et al.} 2005). This thesis deals with samples taken during the initial phase after the observatory’s establishment in 1999. The bathymetric transect of HAUSGARTEN comprises nine stations along a depth transect from the Vestnesa Ridge to the Molloy Hole crossing the central HAUSGARTEN area at 79°04’N and 04°10’E (Fig. 5). The stations sampled ranged from 1250 to 5600 m water depth at nominal 1000 m depth intervals (details in Table 1). Most of these stations are actually part of the standard annual sampling program of the long-term observatory. At the time of sampling (in summers 2000 and 2001), this area was subjected to drifting ice flows of variable concentrations. This depth transect comprises the most significant morphological features in the Fram Strait: over a horizontal distance of only 10 km, the seafloor plunges from 2500 m down to 5400 m, with local slopes reaching values of nearly 42° decline. Initially characterised as a nodal deep (Thiede \textit{et al.} 1990), the ‘Molloy Hole’ reaches a maximum depth of 5669 m and has an almost circular shape of approximately 30 km in diameter (Klenke & Schenke 2002). It is the deepest known depression of the Arctic Ocean, the Norwegian-Greenland Sea and the Atlantic Ocean north of 60° latitude.

![Fig. 5: Location of sampling stations in the Fram Strait along the depth transect from the Vestnesa Ridge to the Molloy Hole. Stations sampled in 2000 and 2001 onboard RV ‘Polarstern’ are indicated by multiple corers. During an additional sampling campaign to the central HAUSGARTEN area (summer 2001), the ROV ‘Victor 6000’ was deployed from RV ‘L’Atalante’ (see II.1.3 for details). Points of equal depth are connected by isobaths (blue lines). High line density thus indicates steep slopes; depth marks are approximate values.](image)
II.1.2. The Ardencaple Canyon (off eastern Greenland)

The Ardencaple Canyon – a channel system in the deep western Greenland Sea - was investigated with respect to bacterial community dynamics in relation to large-scale physical disturbances (episodic suspension-loaded shelf-drainage flows) along a distance gradient from the continental shelf (Fig. 6A).

The Ardencaple Canyon connects the Greenland continental margin with the deep Greenland Basin (Frahm 2003). The canyon is up to 4 km in width and follows a meandering course over 200 km in north-easterly direction. Setting off at about 74°05'N / 12°50'W and vanishing in a deep-sea fan at around 74°50'N / 6°00'W, the canyon declines (average 0.2°) from water depths of approximately 2800 m to 3550 m. Thereby it heads against the flow of southerly directed deep-water currents. Sampling sites along the canyon down to the Greenland Basin were regionally grouped in relation to the distance from a bowl-shaped depression, defined as channel setting off (I = 0 km; II = 2 km; III = 22 km; IV = 47 km; V = 82 km; VI = 137 km). The Ardencaple Canyon was sampled at a total of 16 stations inside the central canyon and on the northern and southern levees (Fig. 6A). The maximum height of levees from the channel floor is approximately 125 m, with the southern levee generally towering above the northern levee (Fig. 6B).

Fig. 6: Location of sampling stations along the Ardencaple Canyon sampled in 2000 and 2001 from RV 'Polarstern' indicated by multiple corers. (A) multiple corer sampling sites at regions I to VI with increasing distance from the channel head in the central area (C), at northern (N) and southern levees (S). Points of equal depth are connected by isobaths (blue lines). (B) Canyon profile indicating different levels of elevation of southern and northern levees and the average width of the central area.
Sometimes completely ice-covered during winter, the Ardencaple Canyon area undergoes strong fluctuations in ice coverage during the summer months, generally reaching a minimum extent at 50-100 km offshore in August/September (Ramseier et al. 2001). During both sampling periods, the channel area was characterized by drifting ice flows, adjacent to the MIZ above the continental rise (v. Juterzenka, personal observations).

**Sampling and sub-sampling strategy (for Section II.1.1 and II.1.2)**

Sediments along the depth transect crossing the central HAUSGARTEN area and along the Ardencaple Canyon were sampled by a multiple corer (MUC). This gear enables to obtain cores with a virtually undisturbed sediment surface (Barnett et al. 1984). Sub-sampling of cores (Fig. 7) was performed using pre-washed PE-syringes (1.2 cm in diameter) with cut-off ends. The upper five centimetres of the sediments were sliced horizontally in 1-cm layers using a sterile plastic spatula. These sample sets intrinsically combine vertical large-scale (metres to kilometres) with small-scale aspects (centimetres down the sediment column).

Samples were either immediately processed for assessing bacterial activity and viability (performed at 2°C *in situ* temperature), or preserved in 4% formalin for bacterial cell counts. For T-RFLP analysis, undiluted sediment portions were shock-frozen in liquid nitrogen and stored at -80°C until sample processing (for protocols see Section II.2 as well as Publications I and II).
II.1.3. Biogenic structures

A) Epibenthic sessile organisms

The hadromerid demosponge *Tentorium semisuberites* (Schmidt 1870) was chosen to study the impact of small-scale habitat heterogeneity to microbial communities in their sedimentary microenvironment. Belonging to the class of Demospongia (O. Hadromerida, Fam. Polymastiidae, Gen. Tentorium; Vosmaer 1885), *T. semisuberites* occurs at depths from 26 to 2800 m in the Arctic Ocean, the White Sea and the northern Atlantic Ocean (Hansson 1999). It is one of the most common sponge species of Arctic deep-sea soft-bottom sediments (Barthel & Tendal 1993). It lives partially buried in the sediment, either anchoring with long root-like spicules in the sediment or using shell rudiments or small stones as substrate (HAUSGARTEN area; this study). Observed *in situ*, the Arctic deep-sea morphotype is typically mushroom-shaped, with a smooth surface and usually one to six short, tubular papillae on top. Its erect shape increases three-dimensional habitat complexity. Owing to decompression during retrieval of samples, specimens shrunk by approximately 40% of the size observed *in situ*. Under atmospheric conditions, they take on a cone shape (Fig. 8). While *T. semisuberites* living in shallower and warmer waters can grow up to 3.5 cm in height and 3 cm in width (Hansson 1999), the specimens sampled for the present study generally measured 1-2 cm in diameter.

![Fig. 8: Tentorium semisuberites (Demospongiae), sampled by push-coring using the ROV ‘Victor 6000’. Close-up images of a core surface taken under atmospheric pressure conditions illustrate that decompression during retrieval causes the demosponge to shrink, as shown by supervision (A) and lateral view (B).](image)

**Sampling and sub-sampling strategy**

Samples were taken from the central HAUSGARTEN area (Section II.1.1) during an expedition with the French RV ‘L’Atalante’ in summer 2001. Targeted sampling was performed with pushcores (60 mm diameter, 40 cm length), manipulated by the ROV “Victor 6000” (Fig. 9 a). The sedimentary microenvironment of *T. semisuberites* was compared to nearby reference sediments lacking apparent biogenic structures (Fig. 9 d, [image])
e). The study comprised four paired sample sets defined as couple 1 to 4 (Fig. 10), each comprising the sedimentary vicinity of a *T. semisuberites* and a reference sampled in approximately 0.5 to 1 m distance from the sponge. Each sponge was carefully removed from the sediment surface, and immediately preserved in 4% formalin for identification and further analysis (Pape *et al.* 2006). Subsequently, the upper 2 cm of sediment of each push-corer were sliced, leaving out a 0.5 cm border to avoid any smear-effects due to pushcoring. After homogenization, the surface sediment of each core was portioned for the assessment of bacterial abundance, prokaryotic production measurements and fingerprinting analyses (T-RFLP; see Publication III); remains were frozen for dry and ash-free dry weight determinations.
B) Biogenic sediment structures

The deep-sea sediment surface is pockmarked by a high variety of microto-topographic features, originating from the activities of macrobenthic organisms (‘Lebensspuren’, Gage & Tyler 1991). Such ‘disturbing’ features comprise approximately 3–4% of all deep basins on Earth. Sediment mounds and tracks from crawling, feeding and resting, and dwellings such as burrows and robust sediment structures, are among the most common morphotypes. Such small, biologically generated sediment structures were taken along the HAUSGARTEN depth transect (Section II.1.1) and the Ardencaple Canyon (Section II.1.2), in order to investigate the impact of small-scale disturbances on bacterial community dynamics.

The origin of most biogenic structures could not always be explicitly assigned to specific organisms. All burrow entrances sampled for this study which were most likely generated by crustaceans (e.g. the isopod *Mesidothea megalura*), were not inhabited at the time of sampling. Some faeces and trace samples could be assigned to the deep-sea holothurian *Elpidia heckeri* Baranova, 1989. Other traces might originate from feeding or crawling activities of organisms living at the sediment surface (e.g. polychaetes, crustaceans, echinoderms). Directions and age of the traces could not be defined. For details on size scales and ‘architecture’ of the biogenic sediment structures investigated see Figure 11; details of the stations sampled are given in Table 1.

Fig. 11: Morphotypes of small biologically generated sediment structures sampled from sediment cores taken at 2500-5500 m water depth. A) crawling track (probably from a mollusc), B) crawling tracks of *Elpidia heckeri*, Holothuroidea, C) faeces of *E. heckeri*, D) faeces (probably from a polychaete), E) burrow entrance (probably of an isopod), F) plough trace of a tubiculous polychaete; scale bars comply with 1 cm.
**Sampling and sub-sampling strategy**

Biologically produced sediment structures were randomly gathered by MUC sampling along the HAUSGARTEN depth transect and the Ardencaple Canyon (Fig. 7). Frequently sampled biogenic structures such as faeces, burrows, polychaete plough traces and crawling tracks were sampled to assess the impact of macrofaunal bioengineering at small scales on benthic bacterial distribution and activity. By means of pre-washed PE-syringes with cut-off ends (0.5 cm in diameter), these biogenic sediments were sampled in comparison to nearby reference sediments (3–5 cm distance, same core). The uppermost five centimetres of the sediments were sliced into 1-cm layers and either immediately processed to assess the bacterial carbon metabolism or preserved in 4% formalin for bacterial cell counts (see Section II.2 for modified protocols). Figure 12 shows exemplarily the sampling design along small-scale horizontal gradients of the most frequently gathered biogenic structures.

![Sampling and sub-sampling strategy](image)

Fig. 12: Small-scale sub-sampling of (a) faeces and tracks produced by *E. heckeri* (Holothuroidea) compared to nearby reference sediments by PE-syringes (0.5 cm in diameter). The horizontal sampling direction is given by syringe I to V, following a uniform strategy of serial samples around burrows (b) and along plough traces of tubiculous polychaetes (c) and crawling tracks (d). Minimum distances between sampling points were 0.5 cm.
Table 1: Overview of coordinates and bottom depths of stations along the depth transect from the Vestnesa Ridge to the Molloy Hole (DT) within the central HAUSGARTEN area (HG) and along the Ardencaple Canyon (AC) sampled during RV Polarstern (PS57, PS59) and RV L’Atalante (At) expeditions in summer 2000 and 2001. Each station is related to the topic of bacterial community dynamics either along large-scale physical and biogeochemical gradients (LSG) or at small-scale gradients in sediments surrounding *T. semisuberites* (SSG-ts) and biogenic sediment structures (SSG-bs).

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<th>Latitude</th>
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</table>
II.2 Experimental procedures and development of methodology

The existence of a rapidly growing, metabolically active deep-sea microbial community has been known for decades (Jannasch et al. 1976, Marquis & Matsamura 1978). It was only in the late 1970s, when the introduction of new laboratory techniques allowed the science of microbial ecology to advance significantly. First direct cell counts by fluorescence microscopy clearly outshone the <1% efficiency of the traditional plate-counting method (Jannasch 1959). The ensuing development of radio-tracer incorporation techniques (Fuhrmann & Azam 1982, Kirchman et al. 1985) proved the true importance of prokaryotes not only in being present, but also in being highly active. Being the only organisms capable of transferring dissolved organic carbon to higher trophic levels, they even form their own food web, the ‘microbial loop’ (Azam et al. 1983). Only two years later, first molecular techniques allowed research in microbial diversity (Olsen et al. 1986) without the need of cultivation, which can only be applied to approx. 10% of the members of many communities (Amann et al. 1995, Hugenholtz et al. 1998). To name a few among these techniques: cloning and sequencing of taxonomically useful genes (Pace 1997), fluorescent in-situ hybridization (FISH) to identify individual cells (Amann et al. 1995), and community fingerprinting approaches such as denaturing gradient gel electrophoresis (DGGE) (Muyzer et al. 1995) and terminal restriction fragment length polymorphism (T-RFLP) (Liu et al. 1997).

Playing a major role in biogeochemical cycling, bacteria are considered to be the main heterotrophic link between particulate and dissolved organic matter in marine ecosystems (Azam et al. 1983, Cho and Azam 1990, Fuhrman et al. 1989). Hence, the question of whether or not bacterial cells are growing, dividing, or simply viable is crucial for the assessment of their potential role in deep-sea benthic ecosystems, as the relationships between bacterial dynamics and environmental parameters are important to understand microbial communities and fluctuations in geochemical cycles. Garland and Mills (1991) suggested that the application of the community-level approach to assays of microbial function provides more sensitive and ecologically meaningful measures of heterotrophic microbial community structure.

Any change of physical or biogeochemical conditions influences the size and composition of microbial communities (Rajendran 1995), but also the activity and viability of these bacteria (Helbling 1995). In addition, pressure gradients shape the metabolism and composition of a given community (Gage & Tyler 1991).

Hence, the quantification of bacterial numbers, their physiological state and community composition are most important for our understanding of the ecological
scope of physical and biological gradients at large scales (i.e. along a transect of several thousands of metres) and at small scales (i.e. biogenic structures of a few centimetres). Following these considerations, a combination of bacterial cell and viability counts, measurements of carbon metabolism in terms of protein and DNA synthesis and community fingerprinting by T-RFLP were chosen for this thesis (Table 2).

Deep-sea sampling at different scales, however, showed the need for a compromise between sample size and an efficient methodology, which necessitated the adaptation of methods commonly used in microbial ecology. Details on methods applied for the assessment of bacterial and archaeal community dynamics along large- and small-scale gradients are given in Publications I-IV, while schematic overviews on modified protocols are given in the following sub-sections II.2.1 and II.2.2.

Table 2: Compilation of parameters and methods used to assess prokaryotic community patterns along the depth transect from the Vestnesa Ridge to the Molloy Hole (DT), within the central HAUSGARTEN area (HG) and along the Ardencaple Canyon (AC), related to large-scale physical and biogeochemical gradients (LSG) or at small-scale gradients in sediments around Tentorium semisuberites (SSG-ts) and of biogenic sediment structures (SSG-bs).

<table>
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<tr>
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<th>method</th>
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<td>T-RFLP</td>
<td>LSG (DT, HG, AC), SSG-ts</td>
<td>chapter III, II, III</td>
</tr>
</tbody>
</table>
II.2.1 Adaptation of bacterial viability measurements

As epifluorescent direct count techniques fit best for the enumeration of total bacteria in environmental samples (Kepner & Pratt 1994, and references therein), a combination with viability staining appeared very promising for characterizing the activity status of natural bacterial communities. In this respect, the dual staining Live/Dead® (L/D) Bacterial Viability Kit (BacLight™) technique was adapted to marine sediment samples to provide rapid and reliable information on the activity status of natural deep-sea bacterial communities minimizing detrimental decompression effects. Figure 13 shows a schematic overview of the processing steps. The profound background, development and evaluation of this method are discussed in detail in Publication I; protocols for bacterial cell counts using common DAPI staining (Porter & Feig 1980) are described in the Materials and Methods section of Publications I-IV.

Fig. 13: Schematic overview of processing steps for the methodological evaluation of Live/Dead (L/D) Bacterial Viability Kit (BacLight™) technique compared to DAPI staining (Publication I).


II.2.2 Adaptation of prokaryotic incorporation measurements

The high sensitivity of prokaryotic incorporation rates to changes in temperature and pressure bears the major difficulty in handling deep-sea sediment samples without pressure-retaining gears. Depending on retrieval time, community composition and barotolerance of the respective members, the detrimental effect of decompression is thought to be different for microorganisms sampled from different water depths (Pagan & Mackey, 2000; Park & Clark, 2002).

Additional difficulties in measuring natural rates of microbial metabolic activities lie in the fact that most methods require a manipulation of the system in ways that can alter such rates: for example, simply the removal of sediment from an environmental sample can change various parameters in concentration and zonation (e.g. oxygen); enclosing a sediment sample produces wall effects that can change rates of microbial activity; any addition of nutrients, especially to microbial assemblages adapted to oligotrophic conditions, bears a risk of misinterpretation (Fry 1990).

One approach is to measure bacterial uptake rates for tracer levels of radio-labelled substrates to determine the heterotrophic potential for the utilization of organic solutes (Wright & Hobbie 1965). This approach assumes that microorganisms take up solutes according to saturation kinetics and that the rates of uptake increase with increasing substrate concentration to a maximum uptake rate ($V_{\text{max}}$). This value is a function of the community under study and is very sensitive to environmental changes (e.g. seasonal and spatial differences), even when they do not affect population size.

Information on the bacterial metabolism is expected to reflect the impact of vertical and horizontal, environmental gradients at large and small scales, depending on the bioavailability of nutrients and community adaptations at a given water depth. Particle-associated, heterotrophic bacteria particularly utilize amino-acid carbon (Grossart & Ploug 2001). Hence, to assess the response of benthic bacteria to deposited POM, it appeared most promising to assess bacterial carbon metabolism by determining the incorporation of radio-labelled L-[\text{14C}] leucine, combined with the incorporation of radio-labelled (Methyl-[\text{3H}] thymidine, giving complementary results (Riemann & Bell 1990). As DNA is synthesized in growing cells at a rate proportional to biomass, the uptake rate of radio-labelled thymidine reflects the growth rate (Van Es & Meyer-Reil 1982) specific for bacteria, as [\text{3H}] thymidine labels bacterial DNA only (Moriarty 1986).

The protocol for dual-labelling incorporation used for small-scale sediment samples has been slightly modified according to Tibbles et al. (1992) and Kirschner &
Velimirov (1999). Details on isotopic specificities, amounts applied to respective samples and on the conversion of bacterial uptake rates into secondary production are found in the material and methods section of Publications II-IV. Figure 14 shows the extraction procedure of prokaryotic protein and DNA adapted to small sample sizes.
III. RESULTS

III.1. Bacterial dynamics along vertical environmental gradients

III.1.1 Large-scale patterns with increasing water depth

Bacterial abundance and viability

Focussing on the uppermost centimetre of the sediment, total bacterial cell numbers showed a significant, negative linear relationship ($r^2 = 0.79; p \leq 0.001$) with increasing water depth along the bathymetric HAUSGARTEN transect from the Vestnesa Ridge to the Molloy Hole (Fig. 15 A). Maximum bacterial abundance ($4.9 - 5.4 \times 10^8$ cells cm$^{-3}$) was found in sediments of the shallowest sampling stations (1246 m – 1284 m) and at the central HAUSGARTEN station at about ~2500 m water depth. Total bacterial cell numbers decreased linearly towards the Molloy Hole (~5600 m), reaching $1.8 - 2.3 \times 10^8$ cells cm$^{-3}$ in surface sediments. Similarly, the fraction of viable bacteria decreased significantly with increasing water depth ($r^2 = 0.92; p \leq 0.001$) (Fig. 15 B). Highest values were found in surface sediments of the shallowest sampling stations ($3.5 - 4.0 \times 10^8$ viable cells cm$^{-3}$), corresponding to viability percentages of 71-74% (active versus total cells counted by BacLight staining). Lowest numbers of viable bacteria were obtained at the deepest stations ($0.2 - 0.9 \times 10^8$ viable cells cm$^{-3}$), representing a percentage of 9 – 40% viability at given water depths.

As shown in Publication I, the mean bacterial abundance showed no significant difference between the two cruises ARK XVI and ARK XVII in relation to water depth (1200 – 5600 m), allowing to group data sets from both cruises for regression analysis.

Fig. 15: Total bacterial cell numbers (A) and the fraction of viable bacteria (B) based on BacLight counts in the uppermost centimetre of sediments along the bathymetric transect of HAUSGARTEN (see Section II.1.1) sampled in 2000 and 2001 (data grouped).
Bacterial activity

Thymidine incorporation rates as a measure for DNA synthesis ranged between 8.1 and 1.6 fmol g$^{-1}$ h$^{-1}$ in surface sediments (Fig. 16 A), showing a significant, negative linear relationship with water depth ($r^2 = 0.83; p \leq 0.001$). Leucine incorporation rates ranged between 393 and 59 fmol g$^{-1}$ h$^{-1}$, decreasing significantly with increasing water depth ($r^2 = 0.74; p \leq 0.002$) along the HAUSGARTEN transect (Fig. 16 B). The molar ratio of leucine : thymidine incorporation showed no depth-dependent pattern, ranging between 32.4 and 52.2.

![Fig. 16: Results on prokaryotic incorporation rates based on radio-labelled thymidine (A) and leucine (B) in surface sediments (0 – 1 cm) along the bathymetric transect of HAUSGARTEN sampled in 2001.]

Bacterial viability in the uppermost centimetre of sediments along the transect was significantly correlated with respective thymidine incorporation ($r^2 = 0.96; p \leq 0.001$) as well as with esterase activity ($r^2 = 0.80; p \leq 0.01$), an extracellular enzymatic cleavage which indicates the bioavailability of organic compounds (Fig. 17).

![Fig. 17: Correlation between bacterial viability and esterase activity (green circles) and between bacterial viability and bacterial incorporation rates based on radio-labelled thymidine (orange circles) in the uppermost centimetre of sediments sampled along the bathymetric transect of HAUSGARTEN in 2001.]


**Bacterial community composition**

T-RFLP analyses of bacterial communities along the HAUSGARTEN depth transect showed high dependencies with water depth and particle flux. As a measure for ribotype richness, the number of operational taxonomic units (OTUs) was found to increase down to 4000m (Fig. 18 A), and was significantly correlated with CPE concentrations ($r^2 = 0.71; p < 0.01$; Fig. 18 B).

![Fig. 18: Distribution of bacterial operational taxonomic units (OTUs) in surficial sediments along the HAUSGARTEN depth transect from 1000 – 5500m water depth (A) and in relation to CPE concentrations (B).](image)

Applying the Dice similarity index to the presence (1) and absence data (0) of aligned bacterial OTUs ($\pm 1$ bp), the unweighted pair group method (UPGMA) resulted in a clustering of similar bacterial communities in the uppermost centimetre of sediments (0 – 1 cm) as a function of depth (Fig. 19): Communities in sediments of the shallowest and deepest stations clustered apart, high similarities were found in sediments at 1524 – 3348 m water depth.

![Fig. 19: UPGMA dendrogram of bacterial communities in surficial sediments along the HAUSGARTEN depth transect from 1000 – 5500m water depth (Dice = 0.9809)](image)
III.1.2 Small-scale patterns down the sediment column

Bacterial abundance

Total bacterial cell numbers based on BacLight counts (viable + dead cells) generally showed a significant, gradual decline with increasing sediment depth within the top 5 cm at different stations along the transect across the continental margin. Whereas highest bacterial cell numbers were found in the uppermost sediment layer ($5.4 - 4.9 \times 10^8$ cells cm$^{-3}$), lowest values ($1.2 - 0.8 \times 10^8$ cells cm$^{-3}$) were observed at the deepest stations (about 5600 m) within the fifth centimetre of sediment. As an exception to a generally decreasing trend with increasing water and sediment depth, relatively high values were obtained at 2500 m water depth ($5.3 - 3.5 \times 10^8$ cells cm$^{-3}$ within the first and $3.1 - 2.1 \times 10^8$ cells cm$^{-3}$ within the fifth centimetre of sediment). Figure 20 A exemplarily shows the mean bacterial abundance in the top five centimetres of sediment, displaying a general decrease with increasing water depth.

Fig. 20: Total bacterial abundance (A) and respective fraction of viable cells (B) based on BacLight viability stain counts down the sediment column, within each sediment layer (0-1 cm, 1-2 cm, 2-3 cm, 3-4 cm, 4-5 cm) at different water depths along the transect across the continental margin, sampled in summer 2000. Standard deviations are given for the average per centimetre within the sediment column defined as mean (5 cm)$^{-1}$. 

---

Results
Bacterial viability

The fraction of viable bacteria in relation to total cell counts (BacLight staining) decreased significantly with increasing sediment depth (for details see Table 3 in Publication I), with highest percentages of viable benthic bacteria in the uppermost sediment layer at shallow water depth (71-74%) and lowest values within deeper sediment layers at great water depths (9-11%). Figure 20 B exemplarily shows the bacterial viability in relation to the mean cell abundances in the top 5 centimetres of sediment, displaying a general decrease with increasing water depth.

A two way analysis of variance revealed significant differences in bacterial viability from different water depths (p < 0.001) and sediment sections (p < 0.001). Considering vertical distribution patterns within the sediment columns at different water depths, abundances and viability decreased by about 47% and 60% of their surface values on an average. The upper three centimetres of sediment mainly supported an activity 2-3 times higher than the subjacent sediment layers, indicating some kind of ‘viability boundary’ (Fig. 21), which was confirmed by pairwise multiple comparison (Student-Newman-Keuls Method) by grouping stations to water depth categories (Publication I).

![Bacterial viability boundary](image)

Fig. 21: Bacterial viability boundary at different sediment sections (0-5 cm) and depth categories a (upper slope, 1000-1500 m), b (lower slope, 2300-2500 m), c (rise, 2800-4000 m) and d (basin, 5000-5600 m). Arrows indicate sediment layers with no significant difference. Viability is given as percentage of active versus total cells counted by BacLight staining.
**Results**

**Bacterial incorporation**

Thymidine incorporation rates within sediment columns at different water depths ranged between 22.5 and 0.05 fmol g\(^{-1}\) h\(^{-1}\) as a measure for DNA synthesis, in general significantly decreasing with increasing sediment depth (Table 3, A\(_x\)-y1). Leucine incorporation rates ranged between 1.6 and 729.4 fmol g\(^{-1}\) h\(^{-1}\), showing significant negative linear relationships with sediment depth at different stations along the transect (Table 3, A\(_x\)-y2). In contrast to thymidine, leucine incorporation into bacterial protein was significantly correlated with bacterial viability of respective sediment layers along the transect (Table 3, B\(_x\) y2). The molar ratio of leucine:thymidine incorporation showed no depth-dependent pattern, ranking between 26.1 and 98.2.

Table 3: Results of linear correlation (y = ax + m) either between water depth (A\(_x\); n=14) or percent bacterial viability (B\(_x\); n=7) and thymidine (y\(_1\)) and leucine incorporation (y\(_2\)) given as fmol g\(^{-1}\) h\(^{-1}\) within each sediment section. P from critical values of the correlation coefficient r\(^2\) (n=14)

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<th>r(^2)</th>
<th>P</th>
<th>B(_x)</th>
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</table>

*hs* highly significant (P<0.002); *s* significant (p < 0.005 – 0.05); *ns* not significant.

### III.2. Bacterial dynamics along the Ardencaple Canyon

**Bacterial abundance and viability**

Total bacterial cell numbers based on total L/D counts (living + dead cells) ranged between 1.2 ± 0.4 and 3.9 ± 1.2 x 10\(^8\) cells g\(^{-1}\). The abundance of viable bacteria ranged between 0.3 (9.3%) ± 0.3 and 3.0 (77.5%) ± 1.0 x 10\(^8\) cells g\(^{-1}\). In contrast to total bacterial abundance, bacterial viability showed significant differences between the respective sediment layers when comparing all stations along the Ardencaple Canyon (p < 0.0001), displaying increasing numbers of viable bacteria in sediments from the northern and southern levees with increasing distance from the continental shelf. For both
Count methods, significant variations (p < 0.01 to p < 0.0001) were found along a small-scale, vertical gradient down the sediment column, as well as in sediments at the northern and southern levees, and in the central channel region (Publication II).

**Bacterial incorporation**

Incorporation rates for thymidine as a measure for DNA synthesis ranged between 0.6 ± 0.2 and 15.3 ± 4.8 fmol g\(^{-1}\) h\(^{-1}\) and for leucine as a measure for protein synthesis between 0.2 ± 0.03 and 5.3 ± 1.1 pmol g\(^{-1}\) h\(^{-1}\). Values of both methods gave significant differences between stations (p < 0.0001) as well as down the sediment column (p<0.0001). A high interaction (p < 0.0001) between specific sediment layers at different stations with increasing distance from the continental shelf was only found for leucine incorporation rates as well as for cell-specific leucine incorporation (for details see Table 2 of Publication II).

**Bacterial community patterns**

The bacterial ribotype richness within the upper sediment layer (0 - 1 cm) proved to increase at the northern levees with increasing distance from the shelf, whereas the deeper sediment layers (4 - 5 cm) of respective positions relative to the channel were similar along the canyon, characterising the canyon profile with a pronounced decline at channel centre stations (Fig. 3 of Publication II). Displaying distinct clusters within top and respective deeper sediment layers (Fig. 4 of Publication II) bacterial communities of both levees were closely related to each other and to assemblages in the centre, generally shifting down-slope (Fig. 22).

---

Fig. 22: ‘Trans-canyonal’ displacement of bacterial communities, based on UPGMA analyses of bacterial OTUs in 0-1 cm (A) and 4-5 cm (B) sediment layers. Stations of high similarity in bacterial community composition along the northern (N) and southern (S) levees as well as along the central channel (C) are indicated by arrows in down-slope direction and along the canyon course.
III.3 Biogenic structures (small-scale gradients)

III.3.1 Impact of epibenthic structures (Tentorium semisuberites)

Bacterial abundance
Sediments in the immediate vicinity of all four sponges (Tentorium semisuberites) supported significantly higher (p < 0.001) mean bacterial cell abundances ($5.3 \pm 0.5 \times 10^8$ cells g$^{-1}$) (Fig. 23 A) and higher organic carbon concentrations ($461.5 \pm 72.6$ μg POC) compared to sediments lacking any protruding structures (mean = $2.1 \pm 0.7 \times 10^8$ cells g$^{-1}$ and $381.8 \pm 81.7$ μg POC, respectively). A two-way ANOVA showed that 78.7% of the variance in cell abundances was linked to the presence of sponges (p < 0.0001), whereas a small (9.5%) but also significant (p < 0.002) amount of variance was dependent on the location of the sample pairs. In contrast, 93.4% of the variance in POC content was due to the different locations of the samples, whereas a small (5.4%), but similarly significant (p < 0.0001) amount of variance was dependent on the presence of sponges.

Bacterial incorporation rates
Incorporation rates based on thymidine (mean = $4.81 \pm 1.7$ fmol g$^{-1}$ h$^{-1}$) and leucine (mean = $377.8 \pm 105.7$ fmol g$^{-1}$ h$^{-1}$) were significantly higher (p < 0.001) in the sponge surroundings than in control sediments (mean = $2.5 \pm 1.2$ fmol thymidine g$^{-1}$ h$^{-1}$ and $137.0 \pm 68.6$ fmol leucine g$^{-1}$ h$^{-1}$) (Fig. 23 B, C). Sample pairs were significantly different (p < 0.0001). Cell specific thymidine and leucine incorporation rates of sponge and control sediments generally differed significantly (p < 0.003 and p < 0.013), but not between pairs (Table 1 in Publication III).

Molar ratios between leucine and thymidine uptake have shown to be significantly higher (p<0.008) in the sponge microenvironment (mean = $82.7 \pm 18.9$) compared to unshaped sediments (mean = $52.3 \pm 6.9$) (Fig. 23 D), with significant differences between sample pairs (p < 0.008).
Results

Fig. 23: Bacterial abundance (A), \(^{3}\text{H}\)-thymidine (B), \(^{14}\text{C}\)-leucine incorporation (C) and respective leucine/thymidine ratio (D) displayed as mean values (n = 3; 0-2 cm upper sediment layer) in the close sediment vicinity of 4 sponges (*Tentorium semisuberites*, plain bars) in relation to a nearby plain control sediment (empty bars). Each sponge and nearby control sediment sample is defined as sample pair 1 to 4.

**Prokaryotic community patterns**

UPGMA analysis, based on peaks ≥ 1% of total peak area, indicated some kind of clustering of the bacterial (756 binary characters) and archaeal (273 binary characters) community influenced by *Tentorium semisuberites*, supported by some unique OTUs compared to unshaped sediments (Fig. 5 in Publication III). The prokaryotic community structure was related to the respective bacterial cell distribution. However, the differences in community composition could neither be directly linked to the enrichment, nor to quality of particulate organic matter (POC) in sediments next to the sponge.

**III.3.2 Impact of biogenic sediment structures**

**Bacterial abundance and biomass**

There was no general depth-dependent trend of decreasing bacterial cells down the sediment column beneath tracks, burrows and plough traces. Merely track sediments
Results

contained generally more bacteria \((42.7 \pm 8.5 \times 10^8 \text{ cells cm}^{-3})\) than sediments of reference samples \((34.7 \pm 4.7 \times 10^8 \text{ cells cm}^{-3})\), whereas bacteria in sediments near biogenic structures generally displayed legibly higher mean cellular biomasses compared to controls (Fig. 2 in Publication IV). Along tracks, bacteria displayed high variations in their horizontal distribution and mean cellular biomasses (Fig. 24 A). Numbers and biomasses of bacterial cells showed a polynomial trend along the course of a semicircular plough trace (Fig. 24 B), whereas bacterial abundance significantly increased \((p < 0.0001)\) with increasing distance from the burrow centre (Fig. 24 C), ranging from 5.0 to \(8.1 \times 10^8 \text{ cells cm}^{-3}\).

**Bacterial incorporation rates**

Displaying a clear decreasing trend down the sediment column \((p < 0.0001)\), both bacterial protein- and DNA synthesis were mostly higher in biologically structured sediments than in references, especially beneath faeces (Fig. 3 a–c in Publication IV).
IV DISCUSSION

IV.1. Bacterial dynamics along vertical gradients in benthic deep-sea environments

*General depth-dependent patterns at large scales*

The synchronous effect of temperature and hydrostatic pressure on growth is a common property of deep-sea microorganisms (Kato 1995, Morita 1980, Yayanos 1986). Consequently, microbial activity patterns are suggested to mirror physiological adaptations and community shifts along a vertical, large-scale, depth-dependent gradient as a function of changing pressure conditions. In fact, bacterial abundance, activity and viability in the uppermost centimetre of sediments displayed a significant, depth-dependent decline along a vertical, large-scale gradient of increasing water depth from about 1250 to 5600 m. These observations fit well with findings from the Arabian Sea (Bak & Nieuwland 1997) where bacteria significantly decreased with increasing water depth from 70 m to 5100 m in density ($4 - 0.5 \times 10^9$ cells $cm^{-3}$) and cell size which is a function of activity (Gasol *et al.* 1995). Our study shows that a general depth-dependent decrease in bacterial numbers was associated with lower bacterial viability, which stands in contrast to the findings of Decamp (1998).

In this context, detrimental effects on bacterial cells due to decompression are coming to the fore: as the degree of cell disruption depends on the decompression rate (Park and Clark, 2002), it has been suggested that a comparison of bacterial activities in sediments sampled from different water depths bears a high risk of misinterpretation. Additionally, rather long incubation times of methods commonly applied to estimate bacterial activity may enhance this effect. Decompression time series revealed a reduction of bacterial viability of up to 25% within the first 20 hours after sample retrieval (Publication I). A highly significant correlation between a rapidly assessed bacterial viability and incorporation rates (implying a long-time incubation), however, indicated that only parts of the bacterial community were affected by detrimental decompression effects (Hemmingsen and Hemmingsen 1980, Yayanos & Dietz 1982).

Actually, only few studies focus on extensive bacterial community analyses along a gradient of increasing water depth. It has been only within the last 20 years that we have begun to recognize the phylogenetic diversity of the microbial world, thus the majority of deep-sea bacteria is still unknown. The recent development of rapid molecular techniques to screen communities (Avaniss-Aghajani *et al.* 1994, Bruce *et al.* 1992, Lee *et al.* 1996, Liu *et al.* 1997, Massol-Deya *et al.* 1995, Muyzer *et al.* 1993) now allows comparing multiple sample sets in shortest time.
Exemplarily, the study of Li et al. (1999) offers an extensive overview of bacterial community composition from different depths of about 1200 m to 6500 m along the continental slope towards the Japan Trench (Table 4). The sequences analyzed clustered into the Proteobacteria, Gram-positive Bacteria, Cytophaga, Planctomycetales, and Actinomycetales, whereas many sequences showed no phylogenetic affiliation with known bacteria.

Table 4: Compilation of benthic bacterial cluster analysis with high similarity to known sequences; data are based on 75 DNA sequences (149 clones) from different depths of the deep-sea in the range of 1159 m to 6482 m (Li et al. 1999).

<table>
<thead>
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<th>β-Proteobacteria</th>
<th>γ-Proteobacteria</th>
<th>δ-Proteobacteria</th>
<th>ε-Proteobacteria</th>
<th>Cytophaga</th>
<th>Gram-pos. Bacteria</th>
<th>Plancto- &amp; Actino-mycetales</th>
<th>No. sequ (clones)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1159</td>
<td>4(5) Pedomicrobium, Rhodospirillum, Richettsia, Rhizobium</td>
<td>1 Alcaligenes, Burkholderia</td>
<td>4(9) Stenotrophomonas, Pseudomonas, Solomys, Vibrio</td>
<td>2 Alvinella, Arcobacter</td>
<td>3 Rhodoferax, Capnocytophaga</td>
<td>1 Planctomyces</td>
<td>17(23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1521</td>
<td>1 Richettsia</td>
<td>2 Solomys, Pseudomonas, Vibrio</td>
<td>2 Alvinella</td>
<td>3 Cytophaga, Gallibacter, Rhodothermus</td>
<td>1 Clostridium, Bacillus</td>
<td>1 Saccharomycetales, Micromonospora</td>
<td>16(16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2339</td>
<td>5(8) Pseudomonas, Solomys, Vibrio, Xanthobacter</td>
<td>1 Nitrospira</td>
<td>1 Clostridium, Bacillus</td>
<td>16(16)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>3671</td>
<td>1 Desulfitoccus</td>
<td>3(19) Pseudomonas, Vibrio</td>
<td>1 Alvinella</td>
<td>1 Clostridium, Bacillus</td>
<td>1 Microbacterium</td>
<td>7(24)</td>
<td></td>
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<tr>
<td>4031</td>
<td>1 Methylobacterium, Sphingomonas</td>
<td>3(15) Solomys, Pseudomonas, Vibrio</td>
<td>1 Alvinella</td>
<td>1 Clostridium, Bacillus</td>
<td>8(18)</td>
<td></td>
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<tr>
<td>6482</td>
<td>5(36) Solomys, Pseudomonas, Vibrio, Shewanella</td>
<td>3(36) Pseudomonas, Vibrio, Xanthobacter</td>
<td>1 Cloroflexus, Bacillus</td>
<td>5(38)</td>
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<tr>
<td>6379</td>
<td>8(9) Richettsia</td>
<td>3(2) Desulfitoccus</td>
<td>2 Solomys, Pseudomonas</td>
<td>2(3) Alvinella, Campylobacter</td>
<td>2(4) Rhodoferax, Micrococcus</td>
<td>12(17)</td>
<td></td>
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</table>

Variations in bacterial activity may not only be related to syntrophic and competitive interactions occurring between different physiological types of microorganisms (Fenchel & Finlay 1995), but also to a variable bioavailability of substrates. The trophic state of the deep sea is basically dependent on the flux of organic material being continuously degraded during sinking (Cho & Azam, 1988). As a key factor for benthic bacterial distribution, activity and community composition, the amount of usable organic carbon at the deep-sea floor is determined by its residence time in the water column and finally by water depth (Davey et al. 2001, Tholosan et al. 1999, Turley & Stutt 2000).

Without breaking up the general depth-dependent trend of decreasing bacterial cell numbers and activity, there were two regions along the HAUSGARTEN transect breaking ranks: we found a slight increase in bacterial abundance and viability in sediments of the central HAUSGARTEN area (2500 m) as well as in sediments of the
Molloy Hole (5600 m). The latter, characterised by similar silt and clay components and an almost absent sandy fraction, has already been shown to act as a huge sedimentation trap, as indicated for example by high amounts of sediment-bound pigments (Soltwedel et al. 2003).

We found a significant, negative correlation between CPE concentrations and the number of bacterial OTUs as a measure for ribotype richness. As chloroplastic pigments are only stable for a few days, one might speculate about a chemotactic attraction of bacteria from surrounding habitats. Accordingly, the bacterial community structure showed highest similarities in uppermost sediment layers between 79°04’N to 79°07’N and 03°36’E to 04°53’E, additionally influenced by current-driven and down-slope transports.

Along the HAUSGARTEN transect ranging from 1250 to 5600 m, bacterial distribution, viability and activity showed a generally decreasing trend with increasing water depth. Similarities between bacterial communities along the transect were governed by a complex interaction of ice-edge-related nutrient availability and current-induced distribution processes.

General depth-dependent patterns at small scales

A depth-dependent decline of bacterial abundance and activity patterns was also observed down the sediment column, similar to comparable studies from other deep-sea areas (Bak & Nieuwland 1997, Duineveld et al. 2000, Eardly et al. 2001). Implications for activity and biomass variations within the sediment column have been related to factors like quality and availability of organic material as well as of electron acceptors (Boetius et al. 2000). Due to the lack of such data, we can only assume that changing physicochemical conditions strongly determine the decline in bacterial abundance and viability with increasing sediment depth.

The data on bacterial abundance, viability and activity demonstrated that sufficient organic carbon seems to reach even the deeper sediment layers, being able to support a relatively large number of bacteria, comparable to shallower stations (Tselepides et al. 2000).

However, the sediment column displayed a significant drop by at least half of the initial bacterial viability as a function of sediment depth. Except for sediments of rises, the ‘viability boundary’ position was mostly found between 3-4 cm sediment depth. By grouping all data from different stations into depth-range categories (upper and lower
slope, rise and basin), the influence of bioturbation on the small-scale gradient down the sediment column seemed to be compensated over this large-scale depth range.

Similarly, bacterial activity in eastern North Atlantic sediments obviously dropped only in the 4-5 cm section, without significant variation in the sediment sections above (Eardly et al. 2001). The top layer of sediments along the Ardencaple Canyon was generally characterised by highest bacterial abundance, activity and viability, decreasing significantly with increasing sediment depth. In sediments of the central channel as well as of the northern and southern levees, bacterial community composition proved to show clusters within the top and the deeper sediment layers, respectively (Publication II). Similarly, Ravenschlag et al. (2001) observed a changing prokaryotic community between 3 and 5 cm depth in sediments near Svalbard (Fig. 25). Applying fluorescence in situ hybridization (FISH), the efficiency of which is directly linked to the cellular metabolic state (Karner & Fuhrman 1997), they found a decreasing contribution of ecologically relevant clusters reacting with a strong increase in numbers to an input of complex organic substrates (Rosselló-Mora et al. 1999).

Consequently, the appearance of a ‘viability boundary’ seems to be closely related to the distribution and availability of organic matter and oxygen within the sediment column, and a spatial, small-scale community shift.

Any depth-related gradient of physical and chemical properties provides niches for a variety of metabolically diverse microorganisms in marine sediments, reflecting a stratification of the major phylogenetic groups down the sediment column (Davey et al. 2001, Llobet-Brossa et al. 1998, Tholosan et al. 1999, Urakawa et al. 2000). In this respect, the agreement of lacking, significant differences in leucine incorporation rates at
stations of highest similarity in bacterial community composition along the canyon may, in fact, indicate the occurrence of similar physiological types of microorganisms.

| General depth-dependent patterns of prokaryotic distribution, activity and community composition at small scales are characterized by the presence of a ‘viability boundary’ at 3-4 cm sediment depth. |

**IV.2 Modifications of large-scale gradients (channel dynamics)**

In contrast to data from adjacent regions in the Fram Strait, where bacterial viability proved to decrease as a function of water depth, the fraction of viable cells in sediments along the *Ardencaple Canyon* showed rather stable to slightly increasing values with increasing depth and distance from the continental shelf (Publication II). Similar results were obtained for bacterial uptake rates based on thymidine and leucine incorporation, fitting well to increasing esterase activity estimations along the canyon course (Soltwedel *et al.* 2005.). These patterns lead to the assumption that quality rather than quantity of organic compounds might influence the bacterial viability ratio (Boetius & Damm 1998, Stephens *et al.* 1997).

A local accumulation of highly active benthic bacteria can be related to a mass sedimentation of highly labile organic compounds from high primary production at the ice-edge, as it has been shown to happen during melting processes in ice-covered areas of the Arctic (Hebbeln 2000, Hebbeln & Wefer 1991, Ramseier *et al.* 1999, Schewe & Soltwedel 2003). Increased values of n-alkenes, n-alkenoles and fatty acids in surficial sediments along the Ardencaple canyon gave additional indications for the input of land-derived lipid biomarkers by ice-melting induced sedimentation (Kierdorf & Stein 2004). Hence, the observed increase of benthic bacterial activity and viability along the channel with increasing distance from the continental rise seems to be related to an ice-edge-related particle flux of mainly terrigenous matter.

A locally enhanced benthic response in the Arctic Ocean may also be related to lateral transport mechanisms (Boetius & Damm 1998, Soltwedel *et al.* 2000). A combination of both factors seemed to prevent clear depth-dependent bacterial distribution patterns along a transect from the deep Arctic Eurasian Basin to the Lomonosov Ridge, covering a depth range from 258 to 4427 m water depth (Kröncke *et al.* 1994).

Several indications, however, did not support the assumption of any ongoing, lateral advection nor sediment mass transports along the Ardencaple Canyon: Pirrung *et al.* (2002) found a stable gradient in magnetic susceptibility down the continental shelf, with
Discussion

a more or less uniform distribution within deep-basin sediments. Based on this information, a continuous decrease of sediment-bound pigments (phytodetritus) and particulate proteins along the Ardencaple Canyon (Soltwedel et al. 2005), brought the authors to draw similar conclusions. Hence, they suggested that increasing bacterial activities in the sediments with increasing distance from the continental margin may be explained by a change in bacterial assemblages.

In fact, subsequent community fingerprinting analyses of bacteria in sediments of equal stations as defined by Soltwedel et al. (2005) revealed an increase of bacterial ribotype richness in top sediment layers along the Ardencaple Canyon (Publication II).

In this context, sinking particles have shown to carry large numbers of cyanobacteria and bacteria down to great depths of up to 4000 m (Danovaro et al. 2000, Lochte & Turley 1988, Turley & Mackie 1995, Vanucci et al. 2001). Consequently, any increasing, ice-edge induced flux of particulate organic material with increasing distance from the continental shelf may account for a species inoculation.

A comparison of the bacterial community structures in upper (0-1 cm) and lower (4-5 cm) sediment layers of both levees and the central area of the canyon, however, led us to suggest a past sediment transport event: bacterial communities in sediments from northern levees were found to be closely related to those from the central canyon and the southern levees, thereby displaying a ‘trans-canyonal’ down-slope shift. Furthermore, a decline in bacterial ribotype richness particularly pronounced in deeper sediment layers of the central channel region may be an indication for previous canyon-guided transport mechanisms.

Physical disturbances at large scales seem to have a long-term effect on the distribution of prokaryotic communities. Similar diversities in sediments of different channel sections combined with varying cell-specific incorporation rates seem to result from a highly complex interaction between past down-slope sediment transports, canyon-guided transport mechanisms and ultimately ice-edge induced particle flux.

IV.3 Modifications of small-scale gradients (biogenic structures)

The generally low hydrographic energy (e.g. currents, internal waves) in the deep sea allows sedimentary structures to persist over long periods of time (Gage 1996 and references therein). The stability of such heterogeneous microhabitats may reduce benthic succession dynamics and consequently support a prolonged colonisation period of low-
competitive species (Etter & Mullineaux 2001). In this context, a deceleration of current velocities by protruding objects at the sediment–water boundary layer may lead to a sediment stabilisation, conditioning the substratum for further benthic succession.

Experiments with artificial tube lawns revealed an important deceleration of the current velocity, increasing with higher polychaete population densities (Friedrichs et al. 2000). Passive effects of different protruding objects on the micro-scale flow field have been extensively discussed, but revealed no definite conclusions on a sediment stabilisation or destabilisation effect of the surrounding sediments (Graf & Rosenberg 1997 and references therein).

Near-bottom currents around small epibenthic sponges have shown to cause a differential deposition of particles as a function of particle size (Carey 1983, Eckman 1985). Such a passive interaction may explain our results of an enhanced bacterial abundance and activity in the sedimentary microenvironment of the demosponge Tentorium semisuberites in relation to sediments without any protruding obstacles (Publication III). This result fits well with the findings of Soltwedel and Vopel (2001), reporting on higher total bacterial biomasses around Thenea abyssorum compared to control sediments, which resulted from either higher total cell abundance (mean: 80.4 ± 17.6 x 10⁸ cells 5 cm⁻³) or a higher mean biomass per cell (28.9 fg C cell⁻¹). In order to examine this effect on cellular biomasses, we conducted bacterial activity estimations based on simultaneous incorporation of H³-thymidine and C¹⁴-leucine, which allows to precise the bacterial responses to the substrate supply. Protein synthesis rate was generally conspicuously higher than the DNA synthesis rate (ratio 82.65 ± 18.9), displaying uptake ratios 4 (sponge sediments) to 2 times (control sediments) higher than expected (Kirchman 1992). As previously reported (Chin-Leo 1990), the shifting between protein and DNA synthesis (unbalanced growth) seemed to be mainly caused by substrate supply, as the Leu/TdR ratios correlated with an increased concentration of particulate organic matter (POC) in the sponge surroundings (Publication IV).

Additionally, the micro-scale flow patterns around sponges may be modified by their filtration activity, coming along with an active particle sorting as a function of pore size (Witte et al. 1997, Kowalke 1999). Transient bacterial cells may be trapped within the vascular system, attached to the sponge surface or deposited through downward vortexing at the lee-side of the sponge (Witte et al. 1997). However, Tentorium semisuberites, in fact, seems to govern a specific microenvironment with some unique prokaryotic community compounds (Publication IV). Regarding endosymbiotic sponge associations, associated bacterial or archaeal cells may be actively released, and mix with the benthic prokaryotic community in the sponge’s surroundings.
In case of *Tentorium semisuberites*, such inoculation mechanisms could only explain the benthic specific archaeal community, as this sponge proved to exclusively harbour a methanotrophic archaeal consortium (Fig. 26). Hence, the sedimentary microenvironment of *Tentorium semisuberites* displays a small, but ‘hot’ spot of microbial productivity along a small-scale horizontal gradient governed by the variation of small-scale flow patterns.

**Fig. 26:** Epifluorescence micrographs of identical tissue sections of *Tentorium semisuberites*. Numerous sponge-associated Archaea (small signals, indicated by arrows) are visualized by fluorescence in situ hybridisation (FISH) with the Cy3-labelled oligonucleotide probe ARCH915 (a). With DAPI staining (b), nuclei of sponge cells (ce) are visible in addition to the microbial DAPI signals in the tissue surrounding the canals (ca). Bar=10 µm (from: Pape et al., 2006).

Several studies have demonstrated the potential of bioturbation to support an accumulation and activity enhancement of microbial communities in different ecosystems (e.g. Aller 1984, Deming 1985, Grossmann 1991, Herndl & Peduzzi 1989, Hoppe 1988, Reichardt 1991, Wassmann 1996). Indications of a contribution of small biologically produced sediment structures to any habitat heterogeneity in deep-sea sediments were supported by highly variable bacterial incorporation rates in sediments along traces, of burrows and faeces.

Soltwedel and Vopel (2001) found significantly higher bacterial numbers in sediments beneath double-ridge crawling trails (103.2 ± 11.0 x 10^8 cells 5 cm^-3) compared to control sediments (41.6 ± 12.9 x 10^8 cells 5 cm^-3) in general. The results reported here showed large differences between sampled trails of different depths, as only half of the trail samples supported a slightly higher bacterial activity in relation to control sediments.