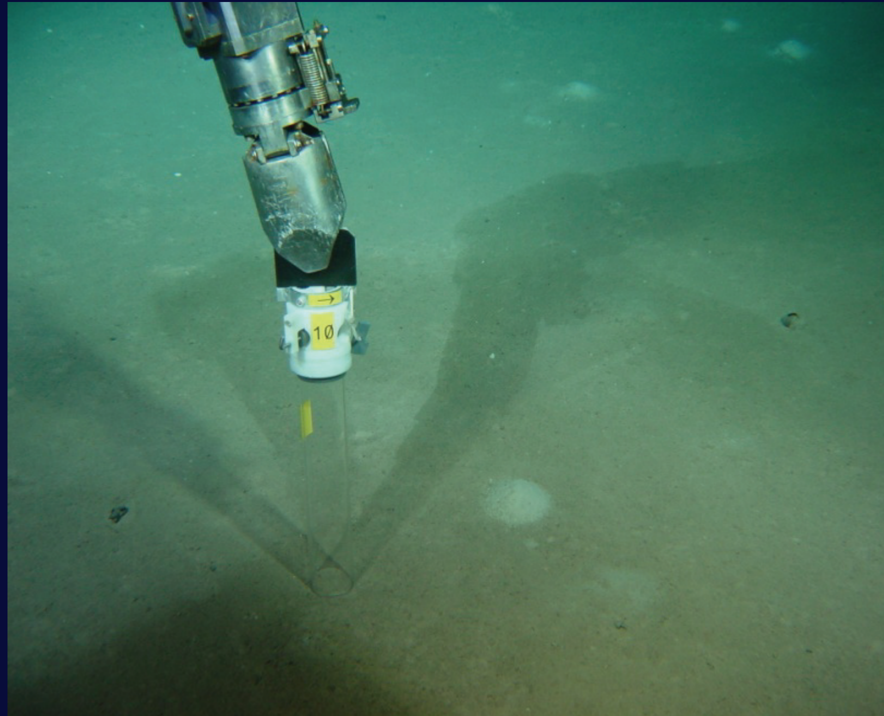
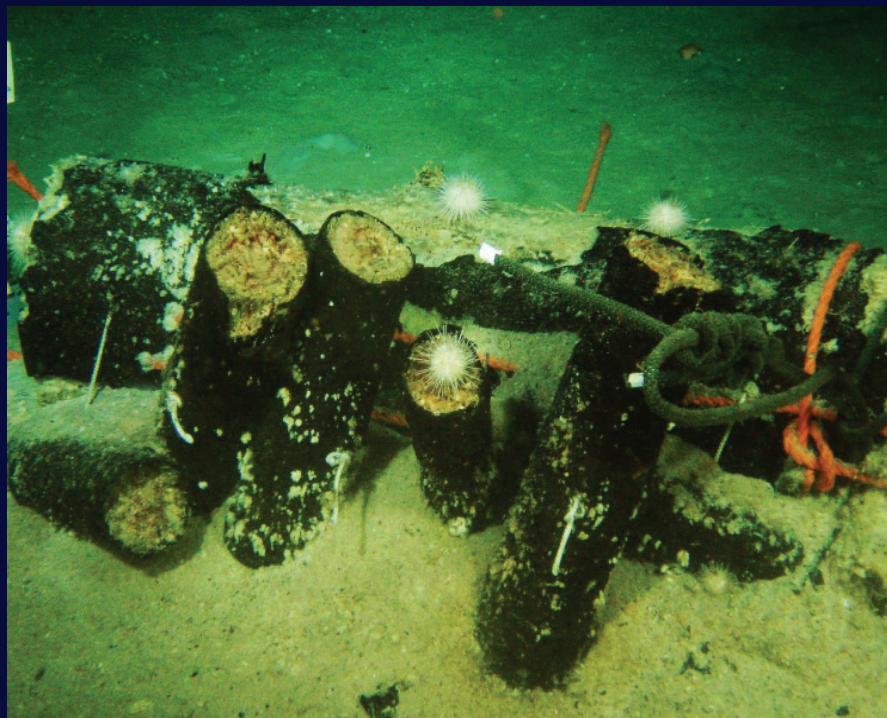
Diversity and ecology of bacterial communities at the deep seafloor





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Diversity and ecology of bacterial communities at the deep seafloor

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Front cover: Upper picture: A push core is taken in the Eastern Mediterranean deep sea with ROV Victor 6000 (Ifremer). Lower picture: Wood colonization experiment in the Eastern Mediterranean deep sea. Copyright Ifremer, France.

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Man cannot discover new oceans

unless he has the courage to loose sight of the shore

(André Gide)

Summary

Understanding biodiversity and its distribution across space, time, and along environmental gradients, is crucial in order to assess the ecological functions of groups of organisms in the environment and gain insights into overall ecosystem functioning. In contrast to the distribution patterns of larger organisms, little is known about the structuring of bacterial communities in the environment. The reason being, that appropriate tools for the study of microbial ecology have only become available during the last decades. Today, molecular tools like fingerprinting or next-generation sequencing enable a time- and cost-effective, high-throughput processing of environmental samples to study bacterial diversity patterns. The application of such tools has revealed non-random patterns of bacterial diversity across space and time, and along environmental gradients in a variety of habitats. However, research on microbial community ecology is just starting to pick up pace, and entire ecosystems, such as the deep seafloor, remain largely uncharted. The deep sea represents the largest ecosystem on Earth and at the same time remains one of the least explored regions on our planet. Bacterial communities play an essential role for carbon and nutrient cycling in deep-sea sediments, and are thus an important component of benthic deep-sea ecosystems. Therefore, the specific investigation of bacterial diversity and its distribution at the deep seafloor in the context of environmental parameters were major objectives of this thesis. The influence of both spatial distance, as an indicator for dispersal limitation, and contemporary environmental factors on bacterial communities were investigated at different spatial scales.

In **Chapter I**, global-scale patterns of bacterial community composition in deep-sea surface sediments were explored. Strong distance-decay relationships (i.e., the decrease in shared bacterial types between samples with increasing distance) and a high degree of endemism suggested a limited dispersal of benthic bacterial populations in the deep sea. In addition, potential members of a core deep-sea surface sediment community were identified.

Results presented in **Chapter II** describe the distribution of bacterial diversity at intermediate (10–3000 km) and large (>3000 km) scales and further test the influence of an underwater mountain range, the Walvis Ridge, on the dispersal of bacterial communities. Both, geographic distance and environmental heterogeneity influenced bacterial diversity at these scales, indicating a complex interplay of local contemporary environmental effects and dispersal limitation. The Walvis Ridge, however, did not appear to present a geographic barrier for the dispersal of bacterial communities.

The relationship between energy availability and bacterial diversity and activity was investigated at the regional scale (7–500 km) in Arctic Ocean deep-sea sediments (**Chapter III**). Phytodetritus input, as a proxy for energy availability at the deep seafloor, was evidenced to be an important driver of changes in bacterial diversity and activity along the investigated depth transects. The results indicate that bacterial communities may exhibit energy-diversity relationships comparable to the ones observed for macrofaunal deep-sea organisms. Furthermore, contrasting responses of individual taxa to changes in phytodetritus input suggested varying ecological strategies among bacterial groups, and may enable the classification of indicator taxa for certain environmental states.

Finally, the influence of a specific source of energy, i.e., wood, on the biogeochemistry and bacterial diversity at the deep seafloor was investigated using experimental wood deployments (**Chapter IV**). The deposition of wood at the deep seafloor presents a localized input of organic matter to an otherwise largely oligotrophic environment. Wood colonization experiments were deployed in the Eastern Mediterranean deep sea for one year, and revealed the development of sulfidic niches and the colonization of specialized communities at these large organic food falls.

Zusammenfassung

Ein besseres Verständnis von Biodiversität und der Verteilung von Biodiversität über Raum und Zeit, sowie entlang von Umweltgradienten ist wichtig, um die ökologische Funktion einzelner Gruppen in der Umwelt zu verstehen, sowie Einblicke in die Funktion gesamter Ökosysteme zu erhalten. Im Gegensatz zu den Verteilungsmustern größerer Organismen, ist wenig über die Strukturierung bakterieller Gemeinschaften in der Umwelt bekannt. Ein Grund hierfür ist, dass geeignete Methoden für die Untersuchung mikrobieller Ökologie erst in den letzten Jahrzehnten verfügbar geworden sind. Heute ermöglichen molekulare Methoden wie das „fingerprinting“ (das Erstellen molekularer Fingerabdrücke bakterieller Gemeinschaften) und neueste Sequenzierungstechniken, einen zeit- und kosten-effizienten, hohen Durchsatz von Umweltproben für die Analyse bakterieller Gemeinschaftsmuster. Die Anwendung derartiger Methoden zeigte, dass bakterielle Gemeinschaften in einer Reihe von Habitaten nicht einfach zufällig in Raum, Zeit, oder entlang von Umweltgradienten verteilt sind. Allerdings steht die Erforschung mikrobieller Gemeinschaftsmuster noch am Anfang, und komplette Ökosysteme, wie z.B. der Tiefseeboden, sind in dieser Hinsicht weitgehend unerforscht. Die Tiefsee stellt das größte Ökosystem der Erde dar, bleibt aber gleichzeitig eines der am wenigsten erforschten Gebiete auf unserem Planeten. Bakterielle Gemeinschaften spielen eine grundlegende Rolle für die Umsetzung von Kohlenstoff und Nährstoffen in Tiefseesedimenten, und sind deshalb ein wichtiger Teil des Tiefseeökosystems. Aus diesen Gründen war die gezielte Untersuchung bakterieller Diversitätsmuster am Tiefseeboden und eine Bewertung im Zusammenhang mit Umweltparametern ein Hauptziel dieser Arbeit. Auf unterschiedlichen räumlichen Skalen wurden der Einfluss geographischer Distanz, als Anzeiger für eine eingeschränkte Verbreitung, sowie der Einfluss von Umweltfaktoren auf bakterielle Gemeinschaften untersucht.

In **Kapitel I** wurden bakterielle Gemeinschaftsmuster in Oberflächensedimenten der Tiefsee auf globaler Ebene untersucht. Die Abnahme gemeinsamer Bakterientypen in Probenvergleichen mit zunehmender Distanz („distance-decay relationship“), sowie ein hoher Anteil endemischer Bakterientypen, deutete auf eine eingeschränkte Verbreitung benthischer bakterieller Populationen in der Tiefsee hin. Des Weiteren konnten potentielle Vertreter einer Kern-Population in Tiefseeoberflächensedimenten identifiziert werden.

Ergebnisse, die in **Kapitel II** präsentiert werden, beschreiben die Verteilung bakterieller Diversität über mittlere (10–3000 km) und große (>3000 km) Distanzen. Außerdem wurde der Einfluss des untermeerischen Bergrückens Walfischrücken auf die Verbreitung bakterieller Gemeinschaften untersucht. Sowohl geographische Distanz als auch Umweltfaktoren

beeinflussten die bakterielle Diversität über diese räumlichen Skalen, was auf ein komplexes Zusammenspiel von lokalen Umweltbedingungen und Verbreitungsgrenzen hindeutet. Der Walfischrücken schien allerdings keine geographische Barriere für die Verbreitung bakterieller Gemeinschaften darzustellen.

Der Zusammenhang zwischen Energieverfügbarkeit und bakterieller Diversität und Aktivität wurde auf regionaler Ebene (7–500 km) in Tiefseesedimenten des Arktischen Ozeans untersucht (**Kapitel III**). Der Eintrag von Phytodetritus (pflanzliche Überreste), als eine der Hauptenergiequellen am Tiefseeboden, konnte als wichtiger Einflussfaktor für Veränderungen in bakterieller Diversität und Aktivität entlang der untersuchten Tiefentransekte nachgewiesen werden. Die Ergebnisse deuten darauf hin, dass Bakteriengemeinschaften ähnliche Zusammenhänge zwischen Energieverfügbarkeit und Diversität aufweisen könnten, wie die, die bei Makroorganismen in der Tiefsee beschrieben worden sind. Des Weiteren wurden unterschiedliche Reaktionen einzelner Gruppen auf Veränderungen in der Phytodetritus-Verfügbarkeit aufgezeigt, die auf unterschiedliche ökologische Strategien dieser Gruppen hinweisen. Zukünftig könnte dies eine Charakterisierung von Indikatorgruppen für bestimmte Umweltbedingungen ermöglichen.

Zuletzt wurde mit Hilfe experimenteller Holzeinträge in die Tiefsee der Einfluss dieser speziellen Energiequelle auf die Biogeochemie und bakterielle Diversität am Tiefseeboden untersucht (**Kapitel IV**). Der Eintrag von Holz in die Tiefsee stellt einen lokalen Eintrag organischen Materials in eine anderenfalls weitgehend nahrungsarme Umgebung dar. Holzbesiedlungsexperimente wurden in der Tiefsee des östlichen Mittelmeeres versenkt und nach einem Jahr konnten die Entwicklung sulfidischer Nischen, sowie die Besiedlung spezialisierter Gemeinschaften an diesen lokalen Nahrungseinträgen beobachtet werden.

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1.

Introduction

1.1 Biodiversity and Biogeography

The term biodiversity originates from a combination of the words "**biological diversity**", and is the sum of all biological variation, from the level of genes to ecosystems (Purvis and Hector, 2000). Diversity can be measured in different ways; for example, **species richness** and **species evenness** are commonly used to describe diversity in given area (BOX 1). Furthermore, it can be differentiated between the use of species incidence (presence-absence) or relative abundance data in comparisons of diversity between samples. The term **alpha-diversity** refers to the diversity within one sample or location and is often measured as species richness (i.e., number of species), while **beta-diversity** compares differences in diversity between two or more samples or locations (BOX 1). Biodiversity is not distributed homogenously across the Earth, but shows patterns of variation between different geographic regions and with time (Gaston, 2000). Accordingly, **biogeography** is the study of the distribution of biodiversity over space, time, and along environmental gradients. Biogeographic studies address a variety of questions, e.g., why are species or broader taxonomic groups confined to their present range, how have historical events such as continental drift or recent climate change shaped species distributions, or, what role do climate, topography and interactions with other organisms play in limiting the distribution of a species (Lomolino et al., 2005). The study of diversity patterns is therefore also important in order to understand the ecological function of a group of organisms in the environment. Historically, studies of biogeography have focused on macroorganisms, and to date little is known about microbial biogeography.

1.1.1 Why study patterns of bacterial biodiversity?

Prokaryotes are highly diverse and represent the majority of diversity on Earth (Dykhuizen, 1998; Pace, 1997; Torsvik and Ovreas, 2002; Venter et al., 2004). Estimates of bacterial diversity reach 2×10^6 species in global oceans (Curtis et al., 2002) and with projections of $4–6 \times 10^{30}$ cells on Earth, prokaryotes represent a significant proportion of the Earth's total biomass (Whitman et al., 1998). Bacteria play an important role in global biogeochemical cycles by contributing significantly to carbon and nutrient recycling (Azam and Worden, 2004; Falkowski et al., 2008), and therefore to ecosystem functioning. In general, biodiversity is assumed to enhance the capacity of ecosystems to recover from perturbations (i.e. ecosystem resilience), both by ensuring the maintenance of specific functions despite species loss, as well as by diversifying organisms' responses to different perturbations (Chapin et al., 2000; Loreau et al., 2001; Naeem and Li, 1997; Naeem, 1998). These ideas are mainly based on studies of macroorganisms, but first

investigations of microbial communities have shown that changes in microbial community composition and structure may also directly affect ecosystem processes (Allison and Martiny, 2008). All of these considerations emphasize the urgent need to better understand patterns of bacterial diversity in the context of space, time as well as according to environmental factors, **if we are to better estimate the effects of future environmental changes on ecosystem functioning**.

BOX 1| Measures of diversity

Alpha-diversity. Alpha diversity, or **species richness**, refers to the total number of species present within a particular area or community. Species richness is sensitive to sampling effort and requires standardized sampling and counting procedures, or the use of estimators that correct for undersampling biases (e.g. Chao1 or ACE estimators). Estimates of **bacterial richness** are described in more detail in BOX 3.

Species evenness. Evenness is a measure of diversity that considers how individuals are distributed among species (Purvis and Hector, 2000), e.g., a sample with 20 species A and 23 species B would be more even than a sample with 20 species A and 100 species B.

Beta-diversity. Beta-diversity compares the variation in community composition (presence-absence) or structure (relative abundance data) between two or more samples (Whittaker, 1972). It describes the dynamics of a community across space, time, or along an environmental gradient (Magurran, 2004). Measures of dissimilarity are obtained in pairwise comparisons between samples. The Bray-Curtis index is commonly used for abundance data (Bray and Curtis, 1957) and the Jaccard index (Jaccard, 1901) for presence-absence data. For more information on the properties of these indices and how they are calculated, see (Anderson et al., 2011; Legendre and Legendre, 1998).

1.1.2 Biogeographic patterns of bacteria

In contrast to classical ecology, where studies have historically focused on plants and animals, the field of microbial ecology is much younger, a major reason being the lack of appropriate methods until a couple of decades ago. With emerging molecular tools the field of microbial ecology is just starting to pick up pace; however, the application of theoretical concepts is largely missing (Prosser et al., 2007). The earliest, and most famous statement in microbial biogeography goes back to Baas-Becking (1934) who wrote: “everything is everywhere, but, the environment selects”. His statement implies that the small size, high abundance, and virtually unlimited dispersal of bacteria should lead to a cosmopolitan distribution, and local communities would then be selected by contemporary environmental conditions. A cosmopolitan distribution of microbial organisms has indeed been proposed by some studies (Fenchel and Finlay, 2004; Finlay and Clarke, 1999; Finlay, 2002). Nonetheless, a number of studies have refined this view, reporting on a **heterogeneous distribution of microbial communities across space, time**

and along environmental gradients in a variety of habitats (see Martiny et al., 2006 for a review). For example: a restricted dispersal of soil bacteria across continents suggested a certain degree of spatial isolation (Fulthorpe et al., 2008), and in another study soil pH was proposed as an important structuring factor for soil bacterial communities (Fierer and Jackson, 2006; Lauber et al., 2009). Bacterial diversity varied with environmental heterogeneity in a salt marsh (Horner-Devine et al., 2004), and also along gradients of primary productivity in aquatic mesocosms (Horner-Devine et al., 2003). In lakes, bacterial communities were shown to vary according to physicochemical characteristics, e.g., pH, temperature, and lake water retention time (Lindstrom et al., 2005; Logue and Lindstrom, 2008). Lastly, seasonal effects have been observed for oceanic bacterioplankton (Fuhrman et al., 2006; Gilbert et al., 2009) and for bacterial communities in coastal sediments (Böer et al., 2009). Studies in the marine realm have mainly focused on pelagic ecosystems. In concordance with results from terrestrial and aquatic ecosystems, they evidenced distinct patterns in bacterioplankton communities that were related to, e.g., water depth (DeLong et al., 2006), temperature and latitude (Fuhrman et al., 2008; Pommier et al., 2007), and different water masses (Agogue et al., 2011; Galand et al., 2009).

The observation of **taxa-area relationships**, i.e., a positive relationship between the number of species in an area and the size of that area, provided further evidence for a microbial biogeography (Bell et al., 2005; Green and Bohannan, 2006; Green et al., 2004; Horner-Devine et al., 2004): relationships were steeper in discontinuous habitats, implying that the distribution of microbial communities may to some degree be shaped by historical contingencies. Indeed, dispersal is likely to be lower in discontinuous habitats, which may favor local adaptation or speciation. This was further affirmed by several studies that found indications of **dispersal limitation** and endemism in bacterial communities. For example, in hot springs, Papke et al. (2003) reported on the genetic isolation of cyanobacteria by geographic barriers, and Whitaker et al. (2003) evidenced a strong relationship between genetic and geographic distance together with endemic populations of hyperthermophilic archaea in geothermal hot springs. Also, studies of less extreme habitats (e.g. soils, marine bacterioplankton) suggested the occurrence of endemism in bacterial communities (Cho and Tiedje, 2000; Fulthorpe et al., 1998; Fulthorpe et al., 2008; Pommier et al., 2007).

Hence, the distribution of microorganisms appears to be non-random and may be driven by contemporary environmental factors as well as by historical effects (e.g., isolation by distance, geographic barriers, past environmental conditions) (Ramette and Tiedje, 2007). An important objective in current microbial ecology is to **disentangle the relative effects of contemporary environmental factors versus the legacies of historical events** on present day distribution

patterns, in order to identify the major underlying processes shaping microbial community composition and diversity (Martiny et al., 2006; Ramette and Tiedje, 2007). The work presented in this thesis was aimed at testing whether and to what extent these concepts may apply to bacterial communities in surface sediments of the largely underexplored deep seafloor.

1.2 The deep-sea environment

The deep seafloor below 200 m water depth covers approximately 65% of the Earth's surface and therefore presents the **largest continuous ecosystem on Earth**, but also one of the least understood (Figure 1). It comprises a variety of habitats (Figure 2a), but most of the deep seafloor is covered with fine-grained sediments (Figure 2b), that are usually well oxygenated across the upper few cm. Currents are generally weak and most of the deep sea is characterized by low temperatures (between -1 and 4°C), high pressures, and the absence of light. Due to the absence of light there is no photosynthetic primary production in the deep sea and fixation of inorganic carbon only takes place at patchily distributed chemosynthetic ecosystems such as hydrothermal vents and cold seeps. Therefore, benthic communities mainly depend on the input of organic matter produced in the euphotic zone. However, most of the organic matter is utilized during its descent through the water column, and only 1–5% of the primary produced organic matter (~ 1 g C m^{-2} yr^{-1}) reaches the seafloor, making the deep sea an oligotrophic and extremely energy-limited environment (Jahnke and Jackson, 1992; Jorgensen and Boetius, 2007; Klages et al., 2003; Lampitt and Antia, 1997; Smith et al., 2008). The deep sea constitutes a **significant long-term sink in the global carbon budget** and may remove carbon from the atmosphere for centuries or millions of years (Jahnke et al., 1990). The recycling of organic matter at the deep seafloor is therefore a key component of the global carbon cycle and links the deep-sea ecosystem to the global biosphere (Seiter et al., 2005; Wenzhöfer and Glud, 2002).

Hence, the **functioning of deep-sea ecosystems is crucial to global biogeochemical cycles**. Additionally, deep-sea ecosystems provide a number of goods and services of importance for society; some examples include the provision of oil and gas, biomass, climate regulation, nutrient regeneration, food (Danovaro et al., 2009), and unexplored genetic diversity (Deming, 1998). Despite its remoteness, the deep sea is exposed to some principal human threats, such as the disposal of wastes (radioactive wastes, munitions and carbon dioxide), contamination during oil and gas extraction, deep-sea fishing, marine mineral extraction, as well as climate change (Glover and Smith, 2003). These factors have yet unknown effects on deep-sea biodiversity and functioning. Even now, the deep sea remains **one of the least explored regions on Earth**, mainly due to the difficulties associated with accessing and sampling this region. However, recent

improvements of underwater technologies, e.g., manned submersibles, remotely operated vehicles, lander systems, and in situ technologies, have enabled *in situ* observations and measurements at the seafloor (Boetius and Wenzhöfer, 2009), as well as targeted sampling and long-term observations, which will help to **develop a better understanding of diversity and ecosystem functioning at the deep seafloor**.

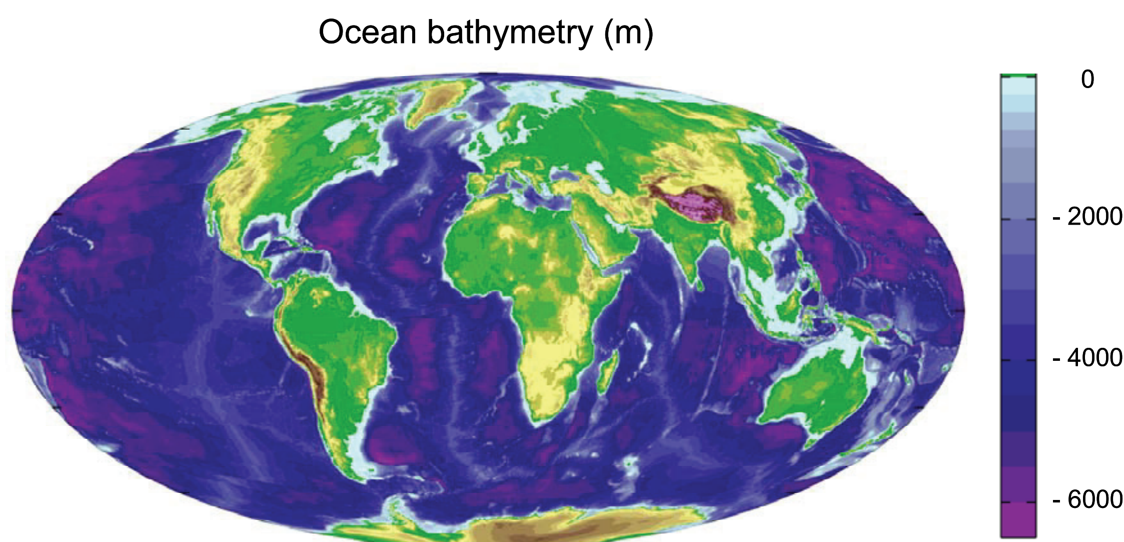

Figure 1 Bathymetry of the ocean floor. The deep seafloor covers almost 95% of the seabed and is represented by all the blue and purple regions, except for the light blue parts primarily in shelf regions around continents (adapted from: http://en.wikipedia.org/wiki/Bathymetry, downloaded: 30.04.2011).

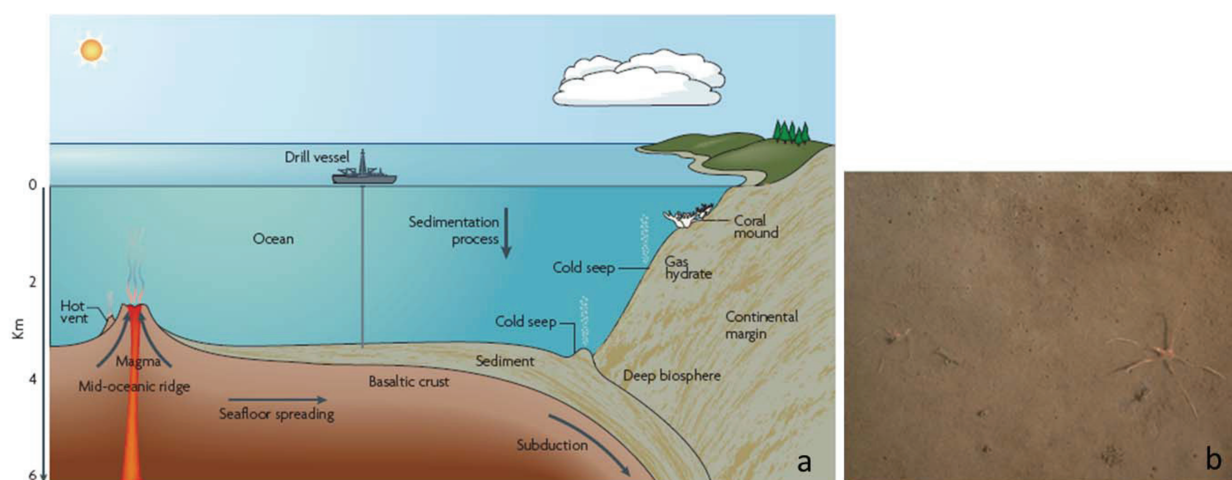

Figure 2 Vertical section of the seafloor (**a**) and bathyal ocean floor at a continental margin (**b**) (adapted from Jorgensen and Boetius, 2007). Image **b** is courtesy of the Monterey Bay Aquarium Research Institute, California, USA.

BOX 2| Early deep-sea research

First deep-sea cruises. Early researchers believed the deep sea to be a desert-like environment, isolated from the rest of the biosphere and devoid of life (Forbes, 1844). This conception was ultimately proven wrong when in 1860 a deep-sea cable was brought up from 1830 m water depth and was covered with organisms. A little over a decade later, the British *Challenger* expedition (1872-1876) provided a first overview of major seafloor features such as the deep ocean basins and discovered more than 4700 new species (Lalli and Parsons, 2004); and more than 50 years after that the second Danish *Galathea* expedition (1950-1952) obtained biological material from over 10,000 m water depth in the Mariana Trench (Bruun et al., 1956).

Microbial deep-sea research. Microbial deep-sea research began in 1882/1883 with the French *Travaillieur* and *Talisman* expeditions, where bacteria were detected in samples from 5000 m depth (Jannasch and Taylor, 1984). Later, living microorganisms were recovered from 10,400 m depth (Zobell, 1952). Due to the low temperatures and high pressure, the cell machinery of deep-sea bacteria must be specifically adapted to work under these conditions (e.g. Bartlett et al., 1989; Chi and Bartlett, 1993; Lauro and Bartlett, 2008; Li et al., 1998; Nakasone et al., 1998; Yayanos, 1995); first isolates were described as psychrophilic ("cold-loving") and piezophilic ("pressure-loving"). Most of the cultivated strains of psychrophilic and piezophilic deep-sea bacteria belong to the *Gammaproteobacteria*, and the genera *Shewanella*, *Photobacterium*, *Colwellia*, *Moritella* (DeLong et al., 1997) and *Psychromonas* (Nogi et al., 2002).

1.2.1 General patterns of faunal diversity in the deep sea

While early researchers believed the deep sea to be a species-poor environment due to the extreme conditions (BOX 2), surprisingly **high species diversity** was discovered at the deep seafloor (Hessler and Sanders, 1967) and extrapolations reached estimates of at least 1 million and up to 10 million species of macrofaunal organisms (Grassle and Maciolek, 1992). Explanations for this tremendous diversity have included effects of predation (Dayton and Hessler, 1972), and the long-term environmental stability in the deep sea that may have allowed diversity to develop through specialized biological interactions (stability-time hypothesis) (Sanders, 1968). But the most widely accepted mechanism proposed to be responsible for high diversity at the deep seafloor, was the concept of patch dynamics, where small-scale disturbances at the seafloor, e.g., temporal and spatial variations in food supply, would permit high local diversity by creating successional sequences that are temporally out of phase (Grassle and Sanders, 1973).

Variations of diversity in the deep sea have historically mainly been studied on mega- and macrofaunal organisms. Patterns have been observed, for example, with changes in sediment grain size (Etter and Grassle, 1992), indicating differences in resource partitioning (Rex and Etter, 2010), and along latitudinal gradients, with some groups exhibiting poleward decreases in species

richness (Rex et al., 1993; Rex et al., 2000; Stuart et al., 2003). The **most prominent patterns of species diversity**, however, have been observed along **bathymetric gradients**, together with an exponential decrease in benthic standing stock, and were mainly explained by the decrease in **food availability** that occurs with increasing water depth (Gooday et al., 1990; Rex and Etter, 2010). For a variety of organisms, unimodal (hump-shaped) relationships between species diversity and water depth were reported, with highest diversity at intermediate water depths, and lower diversity at the shallowest and deepest stations. These were mainly ascribed to changes in food (energy) availability and its potential mediation of biological interactions (Rex, 1973; Rex, 1976; Rex, 1981). Depressed diversity in the abyss is attributed to food limitation constraining the number of species that can survive, resulting in extremely low population densities (Rex, 1973). As food supply increases, diversity may increase because more species can maintain viable populations. The decline of diversity at higher productivity levels may then be due to competitive exclusion (Huston, 1979; Levin et al., 2001; Rex, 1976), higher variability in productivity, oxygen limitation (Levin et al., 2001), and predation (Rex, 1981).

Knowledge on global distribution ranges and large-scale biogeographic patterns of deep-sea fauna is limited because data are scarce. A restriction to specific oceanic regions has been proposed for macrofauna (Vinogradova, 1979; Vinogradova, 1997), but many taxa appear to be broadly distributed across the deep-sea floor (McClain and Hardy, 2010). Additional sampling and more complete taxonomic information will be needed to decipher biogeographic ranges of benthic organisms in the deep sea and disentangle the possible influence of historical events and contemporary environmental factors. A small number of studies have also addressed the distribution and diversity patterns of smaller benthic size classes, suggesting latitudinal patterns for benthic foraminifera (Culver and Buzas, 2000) as well as bathymetric and latitudinal patterns of nematode diversity (Danovaro et al., 2008; Danovaro et al., 2009). The most recent study has indicated that environmental gradients may shape microbial eukaryote community structure at the landscape scale (Scheckenbach et al., 2010). In contrast, biogeographic patterns of bacterial communities at the deep seafloor remain a black box.

1.2.2 Significance of bacteria at the deep seafloor

Seafloor sediments contain 10–10,000-fold more bacterial cells per unit volume than productive ocean-surface waters and bacteria likely comprise the major fraction of the total microbial community of the detritus-fueled oxic seafloor (Jorgensen and Boetius, 2007). Bacterial cell numbers in deep-sea surface sediments reach the order of 10^9 cells per g sediment, which is comparable to coastal sediments (Boetius et al., 1996; Deming and Colwell, 1982; Guezennec and

FialaMedioni, 1996; Harvey et al., 1984; Schauer et al., 2010). Due to a rapid decline of biomass in benthic meio-, macro- and megafaunal organisms and only to a smaller extent in bacterial biomass, the relative contribution of bacteria to benthic biomass increases with water depth, i.e., with decreasing food supply (Rex et al., 2006; Rex and Etter, 2010; Wei et al., 2010) (Figure 3). Thus, in abyssal sediments, bacteria can make up 95% of the total benthic biomass (Pfannkuche, 1992; Rowe et al., 1991). In addition, the more recent discovery of a deep-subsurface biosphere has provided evidence for prokaryotic populations as deep as 800 m below the seafloor (Parkes et al., 2000; Sturt et al., 2004; Teske, 2006; Zink et al., 2003), and may indicate that the largely unexplored deep subsurface ocean floor contains the largest reservoir of microbial life on Earth (Whitman et al., 1998).

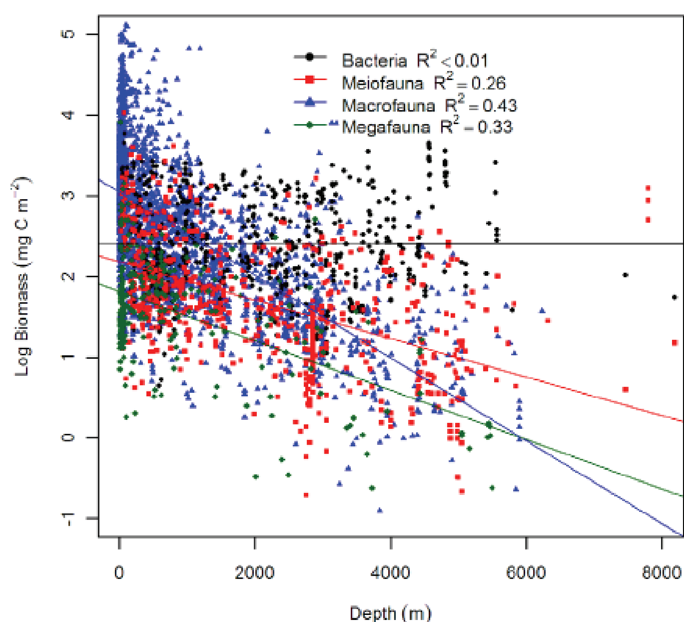

Figure 3 Biomass as a function of depth for four benthic size classes: bacteria, meiofauna, macrofauna and megafauna (Wei et al., 2010). Animal biomass decreases with depth while bacterial biomass stays nearly constant.

Bacteria not only dominate deep-sea sediments in terms of abundance and biomass but they are an integral part of the recycling of organic matter and nutrients, i.e., for ecosystem functioning, at the deep seafloor (Figure 4). They **dominate the turnover of organic matter in deep-sea sediments** (Deming and Baross, 1993; Pfannkuche, 1993) and therefore play an essential role in the **global carbon cycle**. Accordingly, bacteria play an important role in the **deep-sea benthic food web**, by being primary decomposers and constituting an energy (food) source for other groups of organisms such as flagellates and foraminifera (Gooday et al., 1990; Turley, 2000) which present links to higher trophic levels, and emphasize the importance of bacterial communities in deep benthic ecosystems (Deming and Baross, 1993). Understanding the

diversity, distribution, and activity of bacterial communities in deep-sea sediments is therefore an essential step toward a better picture of ecosystem functioning at the deep seafloor and its influence on global biogeochemical cycles.

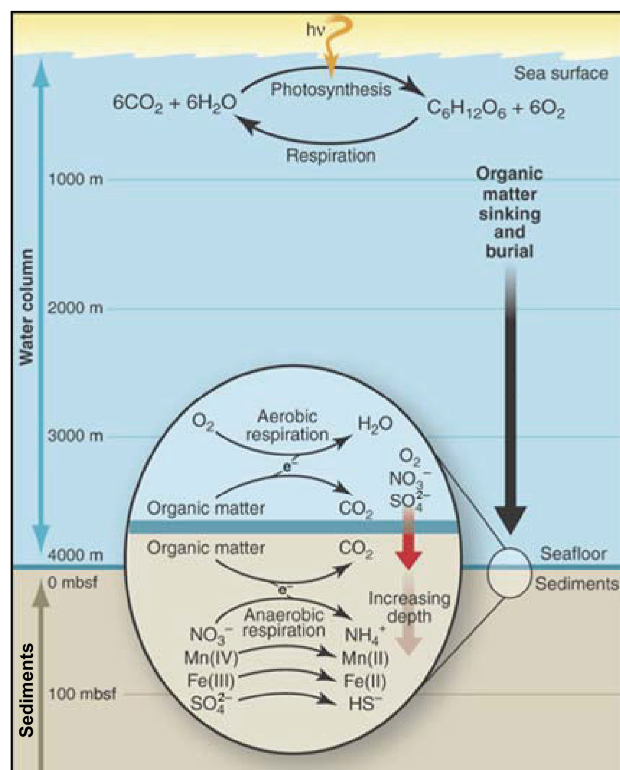

Figure 4 Organic matter respiration in the water column and in deep-sea surface sediments (adapted from DeLong, 2004). Organic matter is produced in the euphotic zone and sinks to the seafloor where bacterial communities play an important role for the turnover of organic matter, by aerobic processes in the upper sediment horizons and anaerobic processes in the deeper sediment layers.

1.2.3 Early studies of bacterial deep-sea communities

A first global-scale assessment of the distribution of bacterial biomass in deep-sea sediments indicated water depth to be a weak predictor of changes in bacterial biomass, while the magnitude of particulate organic carbon (POC) flux proved a strong one (Deming and Yager, 1992). Several other studies also evidenced a **positive relationship of bacterial abundance and biomass at the seafloor with the input of phytodetritus** (Boetius and Damm, 1998; Smith et al., 1997; Smith et al., 2008; Turley and Dixon, 2002). In fact, bacterial communities at the deep seafloor were shown to quickly respond to the deposition of organic matter (Lochte and Turley, 1988; Turley and Lochte, 1990; Witte et al., 2003a). Although sinking particles may already be colonized and partly degraded on their way through the water column (Turley and Mackie, 1994; Turley et al., 1995), bacterial communities at the deep seafloor likely play a significant role for the degradation of organic material at the seafloor. Local communities are much more adapted to the

low temperatures and high pressure than surface-derived bacteria whose metabolic activities may be slowed down under these conditions (Deming and Baross, 1993). Furthermore, the input of phytodetritus and other labile organic materials to deep-sea sediments was shown to induce the production of hydrolytic extracellular enzymes like beta-glucosidase and chitobiase (Boetius and Lochte, 1994; Vetter and Deming, 1994). The availability of energy in the form of organic matter produced in surface waters thus seems to play an important role not only for larger benthic size classes, but also for the distribution of bacterial biomass and activity at the seafloor. However, up to this point in time, the diversity of bacterial communities at the deep seafloor, their major representatives, and distribution patterns remained largely uncharted.

1.2.4 Molecular studies of bacterial diversity at the seafloor

In recent years, several molecular studies have become available that describe bacterial diversity in deep-sea surface sediments. Most of them were based on 16S rRNA gene clone libraries and thus confined to one or a few samples in locally restricted areas. Hence, they do not provide the necessary framework to statistically test links between bacterial diversity patterns and spatial or environmental parameters. However, they have yielded valuable insights into bacterial taxonomic diversity in deep-sea sediments (see also Orcutt et al., 2011). The taxa most commonly reported as dominating deep-sea sediments are *Gammaproteobacteria*, *Deltaproteobacteria*, *Alphaproteobacteria*, *Acidobacteria*, and *Actinobacteria* (Table 1). Some differences can be observed in the taxa and their relative ranking within and between oceanic regions at this broad level of comparison. For example, the Eastern Mediterranean is not dominated by *Gammaproteobacteria*, but rather by *Planctomycetes*, *Acidobacteria* or *Chloroflexi*. Nonetheless, **a systematic and comprehensive description and analysis of bacterial diversity patterns in deep-sea sediments in relation to spatial and environmental parameters is still missing**.

Table 1 Most common bacterial taxa in decreasing order of their dominance as observed in several studies of deep-sea surface sediments. Proteobacterial classes *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, *Gammaproteobacteria* are abbreviated as Alpha-, Beta-, Delta-, Epsilon-, and Gamma-.

Location	Method	Most common taxa	Reference
Arctic	16S rRNA gene clone library	Delta-, Gamma-, Alpha-, Cytophaga, Flavobacteria	(Ravenschlag et al., 1999)
Arctic	16S rRNA gene clone library	Gamma-, Alpha-, Delta-, Beta-	(Tian et al., 2009)
Pacific Arctic Ocean	16S rRNA gene clone library	Gamma-, Beta-, Alpha-, Delta-	(Li et al., 2009)
North Pacific	16S rRNA gene clone library	Gamma-, Delta-, Actinobacteria	(Kouridaki et al., 2010)
Pacific (Sagami Bay, Japan)	16S rRNA gene clone library	Gamma-, Delta-, Epsilon-, Verrucomicrobia	(Urakawa et al., 1999)
Pacific	16S rRNA gene clone library	Gamma-, Cytophaga, Delta-, Alpha-	(Li et al., 1999)
East Pacific Rise	16S-V3 rDNA sequences / DGGE	Chloroflexi, Gamma-, Actinobacteria	(Li et al., 2008)
East Pacific	16S rRNA gene clone library	Gamma-, Alpha-, Delta-, Chloroflexi	(Dang et al., 2009)
Eastern Mediterranean	16S rRNA gene clone library	Chloroflexi, Alpha-, Delta-, Acido-	(Heijs et al., 2008)
Eastern Mediterranean	16S rRNA gene clone library	Acidobacteria, Gamma-, Actinobacteria	(Kouridaki et al., 2010)
Eastern Mediterranean (Sea of Marmara)	Metagenomic study	Planctomycetes, Delta-, Gamma-, Acidobacteria	(Quaiser et al., 2011)
South Atlantic	16S rRNA gene clone library	Gamma-, Delta-, Alpha-, Planctomycetes, Acidobacteria	(Schauer et al., 2010)

1.3 Factors that may influence bacterial diversity and distribution in deep-sea sediments

Although a structuring of bacterial diversity has been demonstrated along spatial and environmental gradients in a variety of habitats, these results cannot be easily translated to the deep-sea environment. The deep seafloor presents a largely under-sampled habitat with many unique characters that may influence the distribution of bacterial diversity in various ways. Here, I consider three major factors that are likely important in structuring bacterial communities in deep-sea surface sediments (in this context defined as the upper 2 cm), and that present the main foci of this thesis.

1.3.1 Dispersal limitation

Theoretically, deep-sea organisms **may show no restriction in their geographical range**. For one, the deep sea is characterized by relatively stable and uniform conditions (e.g., low temperatures, high pressure, absence of light), and may be relatively isolated from atmospheric climatic fluctuations, so that environmental factors may have little structuring effect on deep-sea benthic communities. Physical disturbances such as near-bottom currents (Gage and Tyler, 1991; Gage, 1997; Levin et al., 2001) or periodic benthic storms (Hollister et al., 1984; Richardson et al., 1993), or feeding activity of benthic fauna, may lead to resuspension of deep-sea sediments and facilitate a long-range dispersal of bacteria, comparable to what has been proposed for the larval stages of benthic deep-sea fauna (Rex and Etter, 2010). In fact, broad geographic ranges and little evidence for dispersal limitation have been reported for many faunal deep-sea organisms (McClain and Hardy, 2010), though some macrofauna were shown to be restricted to specific oceanic regions (Vinogradova, 1979; Vinogradova, 1997), or isolated by geographic barriers (Brandt et al., 2005). However, data are still too scarce to allow for well-grounded conclusions.

In contrast to the assumption of large dispersal ranges, **dispersal** of bacterial communities in deep-sea sediments may also be **limited due to a lack of physical mixing**, as currents are usually weak across large parts of the deep-sea floor and bacteria associated with the sediment matrix may be relatively immobile. This would limit the potential for long-range transport in comparison to, e.g., the water column, and should make geographic distance a good predictor for changes in bacterial communities. Furthermore, geological structures such as deep-sea mountain ranges, as shown for peracarid crustaceans (Brandt et al., 2005), or oceanographic properties such as water masses (Galand et al., 2009) may act as biogeographic barriers and limit the dispersal of bacterial communities in the deep sea. As yet, almost no studies testing dispersal ranges of bacterial communities in the deep sea are available. This was investigated in **Chapters I and II**.

1.3.2 Energy availability at the seafloor

The availability of energy at the seafloor is largely dependent on the input of organic matter, primarily as surface-derived phytodetritus, and is a **main factor structuring benthic communities** (Klages et al., 2003; Smith et al., 2008). As mentioned earlier, relationships between energy availability and abundances, biomass, and biodiversity of various size classes of benthic organisms have been described (Gooday, 1988; Soltwedel et al., 2009; Turley et al., 1988; Vanaverbeke et al., 2004; Witte et al., 2003b). The input of phytodetritus to the deep sea was also shown to influence bacterial biomass and activity (Boetius and Lochte, 1994; Deming and Yager,

1992; Lochte, 1992; Vetter and Deming, 1994). However, very little is known on how the composition and structure of benthic bacterial communities could be affected by spatial and temporal variations in phytodetritus availability, which co-varies with water depth, distance from coast, and seasons (Jahnke, 1996; Rowe et al., 1994; Suess, 1980).

Investigations of experimental systems have suggested that the availability of energy, measured as primary productivity or organic carbon concentration, may be an important factor structuring bacterial alpha- and beta-diversity (i.e., changes in richness and community composition and structure, respectively). Primary productivity was shown to influence bacterial community composition in aquatic mesocosms (Horner-Devine et al., 2003), and diversity of *Pseudomonas fluorescens* genotypes in microcosms (Kassen et al., 2000). Furthermore, changes in bacterial community composition in response to organic carbon input were also evidenced in seawater mesocosms (Riemann et al., 2000) and in aquatic batch cultures (Eiler et al., 2003). A recent review on energy/productivity-diversity relationships in aquatic ecosystems suggests that microbial communities may exhibit patterns similar to the ones observed for macroorganisms (Smith, 2007). In classical ecology the study of productivity- and energy-diversity relationships has a long tradition and although there are many possible forms of this relationship, the most commonly observed are positively increasing or hump-shaped curves (Cardinale et al., 2009; Dodson et al., 2000; Mittelbach et al., 2001; Waide et al., 1999).

Studies of bacterial communities in relation to energy availability in natural (marine) ecosystems are sparse. On a global scale, marine bacterioplankton diversity (richness) was weakly positively correlated with chlorophyll a as a proxy for surface productivity (Fuhrman et al., 2008; Pommier et al., 2007). Few studies have reported on specific relationships between organic matter availability and bacterial community composition in benthic marine systems. For example, bacterial community composition was influenced by phytoplankton deposition in North Sea sediments (Franco et al., 2007), and by organic carbon and chlorophyll concentrations in Eastern Mediterranean sediments (Polymenakou et al., 2005). Similarly, chitin enrichments (a main component of particulate organic matter reaching the deep seafloor) resulted in changes in bacterial community structure (Kanzog et al., 2009). Thus, there are strong indications that **energy availability** is an important factor structuring bacterial communities in marine sediments and in other environments, and it **may be of particular importance in the energy-limited deep sea**. It also remains largely unknown which taxa may specifically respond to the input of phytodetritus to deep-sea sediments. Therefore, we investigated how changes in phytodetritus availability affect bacterial diversity and activity in Arctic Ocean sediments in **Chapter III**.

1.3.3 Chemosynthetic ecosystems in the deep sea - Wood falls as a special energy source

Hydrothermal vent (e.g. Fisher et al., 2007) and cold seep ecosystems (e.g. Foucher et al., 2009; Vanreusel et al., 2009) were discovered only about 30 years ago (Figure 5a,b). Here, **chemolithoautotrophic bacteria act as primary producers** in the deep, dark ocean and gain energy by the oxidation of reduced chemical compounds such as sulfide and methane that emerge through hydrothermalism and hydrocarbon venting (Jorgensen and Boetius, 2007). With specific seep and vent microorganisms at the basis of the food chain, such localized habitats are able to sustain rich communities of animals that must, however, be adapted to the extreme conditions, e.g., steep chemical and temperature gradients. In addition to free-living chemolithoautotrophic bacteria, a variety of symbioses with vent and seep fauna have been discovered, where bacterial ecto- and endo-symbionts transform the chemical energy of reduced compounds into food for their hosts (Dubilier et al., 2008). Chemosynthetic ecosystems thus present **biogeochemical and biological hotspots in the deep sea**.

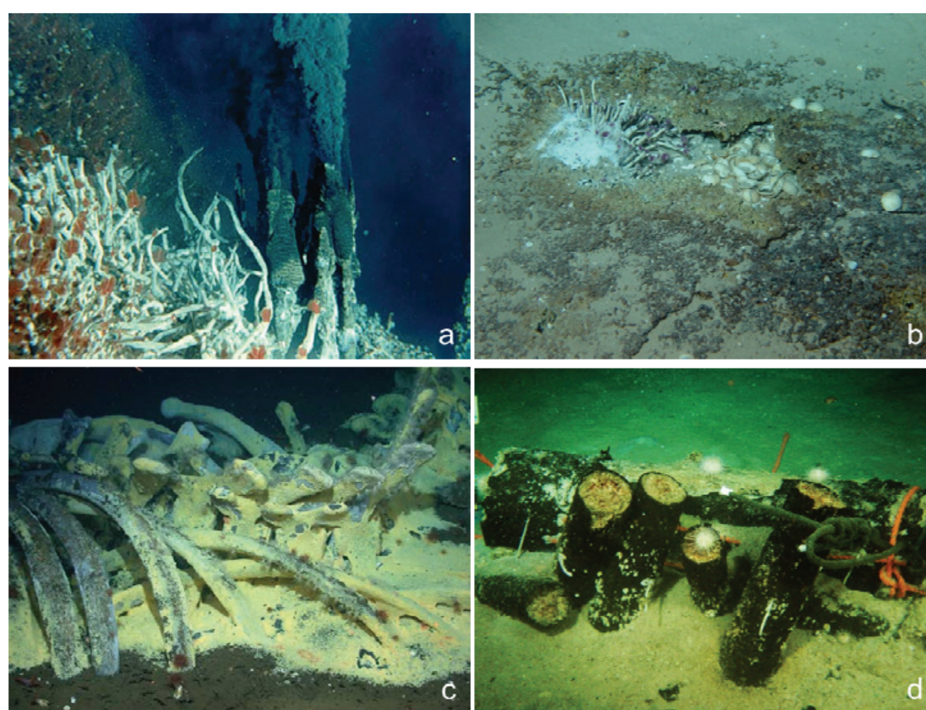

Figure 5 Hydrothermal vent (**a**), cold seep (**b**), whale fall (**c**) and wood fall (**d**) ecosystems provide localized sources of energy to benthic deep-sea communities. Image **a** copyright University of Victoria, Canada; images **b** and **d** are courtesy of Ifremer, France; and image **c** was taken by C. Smith (from Treude et al., 2009).

Due to the patchy distribution of these ecosystems, which are mainly located along mid-ocean ridges or back-arc basins (hydrothermal vents) or along continental margins (seeps), the distribution and evolutionary history of animals thriving in these environments is a major research question. The discovery of diverse deep-sea communities at **whale falls** (whale carcasses deposited at the seafloor) in 1987 marked the exploration of a new, unique kind of deep-sea

ecosystem (Smith and Baco, 2003). Similarly, the input of wood and other plant remains, such as kelp, provide a locally and temporally restricted input of organic matter to the deep sea and serve as a special source of energy in an otherwise largely oligotrophic environment. Local degradation processes at large organic food falls may lead to the establishment of reducing, sulfidic conditions at the seafloor that may facilitate the settlement of chemosynthetic organisms. For instance, the decomposition of the soft tissue and bone lipids of whales leads to the production of sulfide, creating conditions similar to those experienced at vents and seeps (Deming et al., 1997). Observations of shared and closely related organisms at hydrothermal vents, cold seeps, wood falls, and whale carcasses led to the hypothesis that large food falls may present **stepping stones** in the evolution and distribution of chemoautotrophic communities in the deep sea, which are constrained to sulfide- and methane-rich niches for their energy supply (Baco et al., 1999; Distel et al., 2000; Glover et al., 2005; Lorion et al., 2009; Smith et al., 1989). Considerable effort has been spent on the exploration of hydrothermal vent and cold seep ecosystems as well as whale falls, but the role of wood falls as localized boosts of organic material to the deep seafloor remains largely unstudied.

Wood falls are derived from branches and stems of trees, which may be transported by rivers and oceanic currents to the open ocean, where they soak with water and eventually sink to the seafloor (Figure 5d). Their occurrence as specific submarine habitats is traced back in the fossil record to 56 to 34 million years ago (Kiel and Goedert, 2006; Kiel, 2008). Wood falls have been observed in all oceans and at all water depths (Wolff, 1979), though they are likely to be more common off the mouths of rivers, around wooded coastlines, and along shipping routes. The colonization of wood substrates was first examined by Turner (1973) who deployed wood at abyssal depths and showed a significant colonization already after 1–2 months. While some of the taxonomic groups recovered from wood falls have also been reported from other chemosynthetic ecosystems, wood falls may also support highly endemic communities of wood-degrading organisms that are able to utilize the wood under deep-sea conditions. This includes wood-boring bivalves that harbor bacterial symbionts in their gills, and play an important role in the degradation of wood in the deep sea by converting woody plant material to food sources available to other organisms (Turner, 1973). However, free living or other symbiotically associated bacteria in particular, and to a lesser extent fungi, may play a major role in the degradation of wood in aquatic environments (Huisman et al., 2008; Landy et al., 2008), as they are the only organisms known to be able to degrade cellulose and lignin.

Even some decades after the first observations of wood fall environments (Turner, 1973), the succession of colonization and the establishment of biogeochemical gradients at deep-sea

wood falls remain poorly understood. Virtually nothing is known about bacterial communities colonizing deep-sea wood falls or about the effects of wood and its associated degradation processes on surrounding benthic communities. Thus, the impact of large wood falls on bacterial communities and sediment biogeochemistry at the seafloor was investigated using wood colonization experiments (**Chapter IV**).

1.4 Objectives

As outlined above, research on microbial community ecology and the generation and formulation of hypotheses and theories is just starting to pick up pace. New molecular techniques and powerful statistical methods (see 1.5) now provide us with the basic framework to study and evaluate the structuring of complex natural bacterial communities at different spatial scales and evaluate it in an environmental context. We have recently begun to understand that bacterial communities show distinct patterns of diversity across space and time, which may be explained by environmental properties and historical contingencies such as dispersal limitation. It remains an open question, however, whether and to what extent bacterial communities in one of the largest ecosystems on Earth, the deep seafloor, show patterns of distribution and by which factors they are influenced. Given that bacteria dominate deep-sea sediments in terms of biomass and play an important role in carbon cycling at the seafloor, a better understanding of bacterial community dynamics will be crucial in order to better predict the effects of global environmental changes on the structure and functioning of deep-sea ecosystems.

The **overall aim** of this thesis was to develop a better understanding of the factors that shape bacterial communities at the deep seafloor, and to test whether basic concepts of microbial biogeography, e.g., a structuring of diversity across space and with changing environmental parameters that have been observed for terrestrial, aquatic, and marine pelagic communities, can be extended to bacterial communities in deep-sea sediments.

The **specific objectives** were to explore bacterial diversity patterns in pelagic deep-sea surface sediments both at global and at regional spatial scales, as well as in localized wood fall environments, and evaluate them in an environmental context. These objectives were addressed by using high-throughput molecular techniques (see 1.5), and applying multivariate statistics in order to disentangle the effects of different contextual parameters.

The **major questions** addressed in this thesis were:

1) **Do we observe isolation-by-distance effects in bacterial deep-sea communities, possibly resulting from limited dispersal or the presence of geographic barriers?** If a lack of physical mixing leads to a limited dispersal of sedimentary bacterial communities this should be reflected in a strong effect of geographic distance on bacterial community variation. In addition, geographic barriers or oceanographic properties may present barriers for bacterial dispersal. (**Chapters I and II**)

2) **What is the relationship between energy-availability and bacterial diversity and activity in deep-sea sediments?** The supply of organic matter to the deep seafloor has been shown to be a major structuring factor for larger benthic size classes. Also, bacterial biomass and enzymatic activity were shown to respond to the input of organic matter in the form of phytodetritus. But whether and in which way bacterial diversity at the seafloor may be affected by changes in energy availability and how this may be associated with changes in activity remains largely unknown. (**Chapter III**)

3) **How do large wood falls influence the biology and biogeochemistry at the seafloor?** Large organic food falls present a localized source of energy to the otherwise largely oligotrophic deep sea. The role of wood falls for the development of reduced, sulfidic environments at the deep seafloor, and a subsequent attraction of chemosynthetic organisms, is not well understood. It also remains largely unknown which microorganisms colonize the wood and how bacterial communities in surrounding sediments may be affected. Wood colonization experiments help to develop a better understanding of the role of wood falls as biological and biogeochemical hotspots in the deep sea. (**Chapter IV**)

1.5 Methods used to study and interpret patterns of bacterial diversity

Until some decades ago the study of bacteria depended on the cultivation of organisms from environmental samples. While this approach is still important in the description of bacterial strains and their metabolic capabilities, the process is very selective and a large part of the diversity in a sample remains undetected, the so called Great Plate Count Anomaly (Staley and Konopka, 1985). In seawater, for example, the proportion of cultivable bacteria is only 0.0001–0.1% and 0.25% in sediments (Amann et al., 1995; Rappe and Giovannoni, 2003). The identification of the 16S rRNA gene as an important tool for the classification of bacteria and their phylogenetic relationships (Woese, 1987) paved the way to a variety of **cultivation-independent approaches**. Comparative analyses of 16S rRNA gene sequences enabled the description of communities from environmental samples without cultivation (Amann et al., 1995) and whole groups of organisms that are only known from molecular sequences are believed to be quantitatively significant in many environments (Head et al., 1998). The construction of **16S rRNA gene clone libraries** presents an extensive approach for the analysis of 16S rRNA gene sequences from the environment and enables the phylogenetic classification and identification of prokaryotes (Amann et al., 1995; Giovannoni et al., 1990, Chapter II). It is, however, relatively time-consuming and does not allow a rapid assessment of large numbers of samples. Also, the composition of sequences in a clone library does not necessarily reflect the composition of microorganisms in the environment. Quantitative information on members of a bacterial community may be obtained with other methods, such as fluorescence in situ hybridization (FISH) (e.g. Amann and Fuchs, 2008) or quantitative PCR (e.g. Smith and Osborn, 2009), but these methods usually target specific groups and not the entire community.

Only within the last decade other molecular tools have become available, e.g., **fingerprinting** methods and **next-generation high-throughput sequencing** that enable higher throughput of samples and increased sequencing effort. These tools allow for a rapid and cost-effective exploration of bacterial biodiversity in a large number of samples and enable the systematic assessment of bacterial diversity patterns across spatial, temporal and environmental gradients. Here, I will introduce the two main techniques applied during this thesis, automated ribosomal intergenic spacer analysis and 454 massively parallel tag sequencing.

1.5.1 Automated ribosomal intergenic spacer analysis (ARISA)

There is a variety of molecular methods that use chemical or enzymatic reactions to produce specific nucleic acid patterns, i.e., fingerprints, of a community. Some examples are denaturing gradient gel electrophoresis (DGGE), single strand conformation polymorphism (SSCP), amplified ribosomal DNA restriction analysis (ARDRA), and terminal restriction fragment length polymorphism (T-RFLP), of which the latter was used in Chapter II. Among these methods, we applied ARISA, which targets the intergenic transcribed spacer region (ITS) between the 16S and the 23S rRNA genes in the rRNA operon. The length heterogeneity of the ITS region (~ 300–1200 bp) is used to produce a **fingerprint of the microbial community** (Fisher and Triplett, 1999). In brief, community DNA is extracted from an environmental sample and amplified in triplicate with primers targeting the ITS region, one of the primers being fluorescently labeled (Figure 6). The amplification products are cleaned and analyzed via capillary electrophoresis. DNA fragments are separated according to their length, which is determined by comparison to an internal size standard. Each peak of the electropherogram corresponds to one bacterial ARISA operational taxonomic unit (ARISA OTU), not to be confused with OTUs defined at 3% sequence difference in studies of 16S rRNA genes (BOX 3). ARISA profiles are analyzed and standardized by applying a binning procedure (Cardinale et al., 2004; Hewson and Fuhrman, 2006; Ramette, 2009) and by dividing individual peak areas by the total area of peaks in a given sample profile to obtain "relative ARISA OTU abundances".

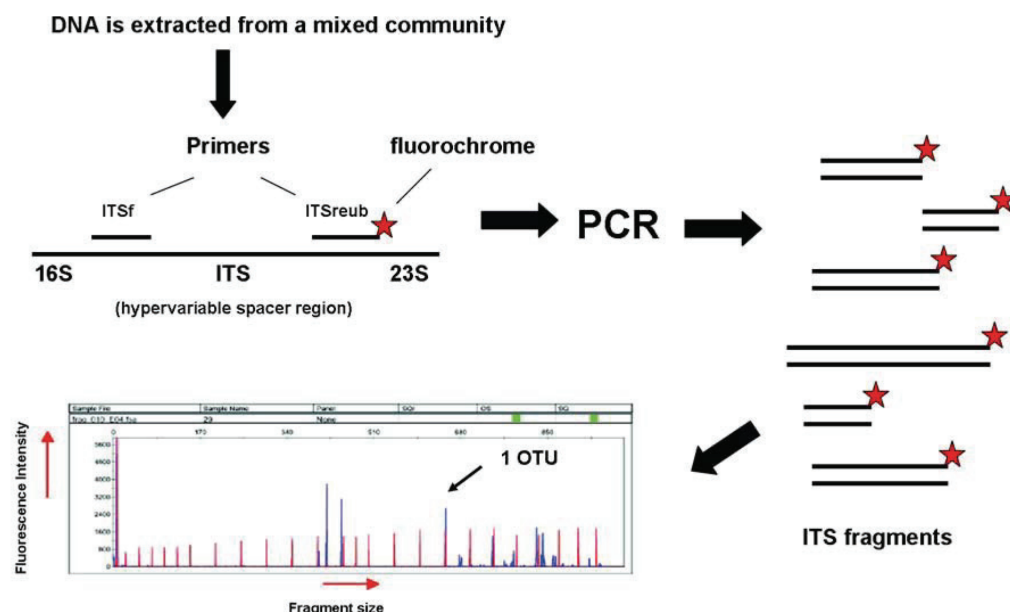

Figure 6 ARISA workflow (Böer, 2008). Amplification of the ITS region produces fragments of different length that are separated by capillary electrophoresis and detected as individual peaks in the electropherogram.

ARISA, per se, does not allow the taxonomic identification of bacteria, but attempts have been made to couple ARISA with 16S-ITS gene clone libraries to infer taxonomy of selected ARISA OTUs (Brown et al., 2005; Schöttner et al., 2011). Remaining limitations of the process are the inability to precisely determine the length of an ARISA OTU, and the fact that several bacterial types may have intergenic spacer genes of the same length, as well as intragenomic diversity between multiple operons (Brown and Fuhrman, 2005), so that unambiguous assignments may not be possible. Some well-known biases have been acknowledged for all PCR-based approaches, such as the preferential annealing (Suzuki and Giovannoni, 1996) and amplification of specific sequences or the formation of chimeric sequences (Head et al., 1998; Wang and Wang, 1997; Wintzingerode et al., 1997). Nevertheless, a number of studies have demonstrated ARISA to be a robust and reproducible method for the assessment of bacterial community profiles (Fisher and Triplett, 1999; Yannarell and Triplett, 2005).

1.4.2 454 massively parallel tag sequencing (454 MPTS)

454 massively parallel tag sequencing is based on high-throughput pyrosequencing that enables a significantly higher throughput than traditional Sanger sequencing based on capillary electrophoresis (Margulies et al., 2005; Sogin et al., 2006). This highly parallel sequencing system is able to sequence 25 million bases in a four-hour run (Margulies et al., 2005). Within the framework of the International Census of Marine Microbes (ICoMM), Sogin and colleagues (2006) introduced a “tag sequencing” strategy that, instead of sequencing nearly full-length sequences of the 16S rRNA gene, is based on sequence tags from a hypervariable region in the 16S rRNA gene, the V6 region (Sogin et al., 2006). In most cases, sequence variation within the V6 region appears sufficient to broadly characterize the phylogenetic lineage of an organism (Kysela et al., 2005).

For the studies in this thesis, 454 massively parallel tag sequencing was performed on a Genome Sequencer FLX System (Roche, Basel, Switzerland) at the Marine Biological Laboratory in Woods Hole, Massachusetts, USA. In the general procedure, extracted DNA is amplified using a cocktail of primers targeting the V6 region of the bacterial 16S rRNA gene and including 454 Life Science’s A and B sequencing adapters. Adapter B enables the immobilization of single-stranded assemblies onto a bead (Figure 7C). Beads are then emulsified in a water-oil mixture containing PCR reagents. A PCR will yield many (about ten million) clonally amplified DNA fragments per bead. The emulsion is broken, DNA strands denatured, and beads with single-stranded DNA templates are deposited in wells of a Pico Titer plate (Figure 7C). Smaller beads with immobilized enzymes are added to the wells (Figure 7D) and sequencing reagents (e.g.,

buffers and nucleotides) are then flowed across the wells of the plate (Figure 7F). Nucleotides are flowed sequentially (TACG) and each of the hundreds of thousands of beads with millions of copies of DNA is sequenced in parallel (massively parallel tag sequencing). The addition of a nucleotide results in a reaction generating a light signal which is recorded by a CCD (charge-coupled device) camera and generates a peak in a flowgram. In follow-on steps, tag sequences are trimmed and low-quality reads removed (Huse et al., 2007; Huse et al., 2008).

Taxonomic assignments are performed with the Global Alignment for Sequence Taxonomy tool (GAST) (Huse et al., 2008; Sogin et al., 2006) and are based on comparisons to a reference database of hypervariable V6 regions within the context of full-length rRNA sequences of known phylotypes. The limited length (50–70 nucleotides after trimming) of the sequences does not allow direct reconstruction of phylogenetic relationships between sequences. But, in contrast to ARISA, 454 MPTS allows the extraction of information about taxonomic identity and previously undocumented microbial diversity. In addition, the enumeration of the number of different rRNA sequences may provide an approximation of the relative abundance of specific microbes in a sample (Sogin et al., 2006), although the occurrence of multiple operons in strains generally limits the quality of quantitative information obtained in studies of the 16S rRNA gene. For further analyses of community patterns, broad (e.g., phylum, class), as well as fine levels of taxonomic resolution (e.g., operational taxonomic units defined at 3% sequence difference, BOX 3) were used.

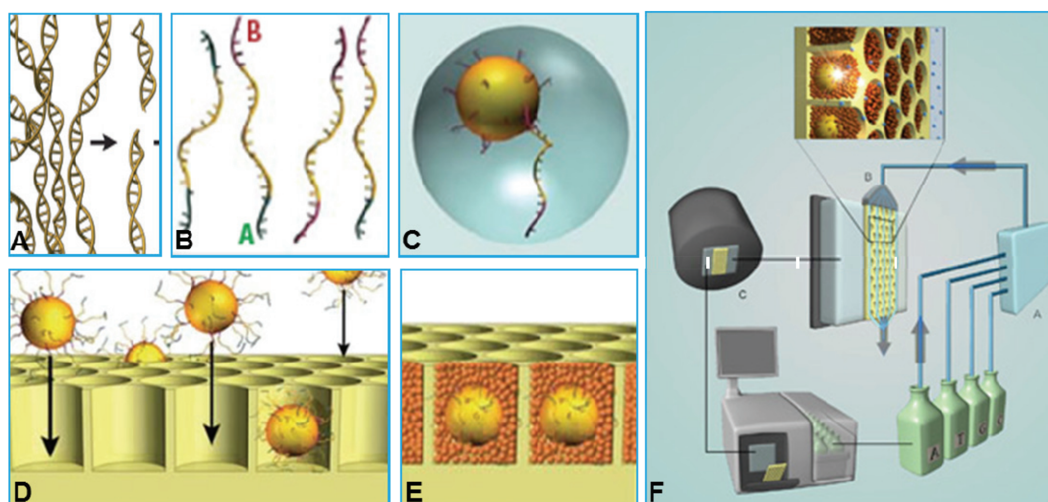

Figure 7 Workflow 454 massively parallel tag sequencing (adapted from Margulies et al. 2005; Roche Genome Sequencer FLX System brochure; www.roche-applied-science.com). For explanations of the individual steps see text.

1.5.3 Extracting ecological information from molecular data

The advent of high-throughput molecular techniques has opened doors to a new era of discovery in microbial ecology. In combination with contextual information on physical, chemical, and biological parameters we are now able to address complex ecological questions, such as about the distribution of bacterial diversity and the main factors driving it. The increasing accumulation of molecular and contextual information, however, also presents computational challenges. This has initiated the incorporation of statistical tools developed by community ecologists into the microbial ecologist's toolbox (Ramette, 2007). Most importantly, **multivariate statistics** enable the analysis of complex datasets, which can be used to test and interpret patterns of bacterial diversity in the context of spatial and environmental parameters, to better understand the factors shaping bacterial communities (e.g. Martiny et al., 2006; Ramette and Tiedje, 2007). For a review of the most commonly used multivariate analysis techniques please refer to Ramette (2007).

BOX| 3 Operational taxonomic units and bacterial richness

Operational taxonomic units (OTU). There is no commonly accepted theoretical species concept for microbes (Rossello-Mora and Amann, 2001; Staley, 2006). The classical definition of a bacterial species is a 70% DNA-DNA hybridization of their genome (Stackebrandt and Goebel, 1994). In addition, microbes whose 16S rRNA gene sequences are <97% (Stackebrandt and Goebel, 1994) or <98.7% (Stackebrandt and Ebers, 2006) identical are likely to be of different species, as these differences correlate with <70% DNA-DNA homology. Though the 3% difference threshold is not flawless, e.g. some microbes have identical rRNA genes but their genome similarities fall below the 70% threshold (Acinas et al., 2004), it serves as a general guideline in molecular studies.
In practice, many researchers define bacterial **OTUs at 3% sequence difference** and this definition was also adopted in Chapter I, and with a slightly more conservative definition at 2% in Chapter II. Furthermore, the studies in this thesis generally focused on relative comparisons of diversity and therefore may to a certain extent overcome problems associated with different OTU definitions (Hughes et al., 2001). Also, highly similar patterns of diversity were observed with ARISA and 454 tag sequencing and on different taxonomic levels of 454 MPTS data (Chapter III, Gobet et al., in preparation), which makes us confident that we are describing ecologically meaningful patterns.

Bacterial richness. Estimates of bacterial richness are often inferred from rarefaction curves, by plotting the number of recovered OTUs versus the number of sequences sampled (Gotelli and Colwell, 2001; Kemp and Aller, 2004, Chapter II). These estimates reveal that the "true" bacterial diversity is usually larger than what was sampled. But even with next-generation sequencing, which allows insights into the rare biosphere (i.e. members of very low abundance), it seems that bacterial diversity is still undersampled (Sogin et al., 2006) and a considerable increase in sequencing effort would be required to more accurately determine the true number of bacterial species present (Quince et al., 2008). Other ways to estimate species richness is the use of coverage-based non-parametric estimators such as the Chao and ACE estimators (Chao, 1984; Chao and Lee, 1992). These estimators are very conservative and may still largely underestimate true microbial diversity (Hong et al., 2006; Quince et al., 2008). The true extent of bacterial richness might never be accurately determined, but the use of relative comparisons of patterns in alpha- and beta-diversity between different samples has been shown to be conserved, regardless of sequencing depth or diversity index used (Shaw et al., 2008).

1.6 Publication Outline

In the following four chapters, I will first present evidence for a spatial isolation of bacterial communities in deep-sea sediments at the global scale as well as the effects of both geographic distance and environmental heterogeneity on benthic bacterial diversity at large (>3000 km) and intermediate (10–3000 km) scales. Following, I will highlight the significance of energy availability in the form of phytodetritus for the diversity and activity of bacterial communities at regional scale (7–500 km) in Arctic Ocean deep-sea sediments. Finally, investigations of experimental wood falls as a special energy source in the deep sea will reveal the colonization of specialized communities and the establishment of sulfidic conditions at these localized organic food inputs in the deep sea.

Chapter I: Biogeography of abyssal seafloor bacteria

Christina Bienhold, Lucie Zinger, Antje Boetius, Alban Ramette

(22.06.2011 – in preparation for The ISME Journal)

This study investigates global biogeographic patterns of bacterial communities in deep-sea surface sediments and reveals isolation-by-distance effects and a high degree of endemism. Samples originate from sample repositories of A. Boetius and additionally include data obtained within the International Census of Marine Microbes (ICoMM) project. The study was designed by C. Bienhold, L. Zinger and A. Ramette. Molecular analyses were performed by C. Bienhold. Statistical analyses were done by C. Bienhold and L. Zinger with input from A. Ramette. The manuscript was written by C. Bienhold with support and input from all co-authors.

Chapter II: Bacterial diversity and biogeography in deep-sea surface sediments of the South Atlantic Ocean

Regina Schauer, Christina Bienhold, Alban Ramette, Jens Harder

(The ISME Journal (2010) 4:159-170)

This study shows that both geographic distance and environmental heterogeneity influence bacterial diversity in deep-sea surface sediments at intermediate (10–3000 km) and large scales (>3000 km). The study was initiated by J. Harder and R. Schauer. Sampling and 16S rRNA gene sequencing were conducted by R. Schauer. Terminal restriction fragment length polymorphism and corresponding data analyses were performed by C. Bienhold. Statistical analyses were done by R. Schauer, C. Bienhold, and A. Ramette. The manuscript was written by R. Schauer with support and input from all co-authors.

Chapter III: The energy-diversity relationship of complex bacterial communities in Arctic deep-sea sediments

Christina Bienhold, Antje Boetius, Alban Ramette

(22.06.2011 – Manuscript has been in review with Proceedings of the National Academy of Sciences, but was not accepted for publication and is currently under review with The ISME Journal)

This study describes energy-diversity relationships for bacterial communities in Arctic deep-sea sediments and highlights potential bioindicator taxa for changes in energy availability. Sampling and initiation of the study were done by A. Boetius, the design of the study was done jointly by all coauthors. Molecular and statistical analyses were done by C. Bienhold with input from A. Ramette. The manuscript was written by C. Bienhold with support and input from all co-authors.

Chapter IV: Biogeochemistry and bacterial diversity of deep-sea wood falls

Christina Bienhold, Petra Pop Ristova, Frank Wenzhöfer, Thorsten Dittmar, Antje Boetius

(22.06.2011 – in preparation for PLoS One)

Wood colonization experiments in the Eastern Mediterranean deep sea were used to study the colonization and development of biogeochemical gradients at large organic food falls at the deep seafloor. A. Boetius initiated the study and F. Wenzhöfer the experiments. Sampling and observations on board were conducted by C. Bienhold. Molecular analyses were performed by C. Bienhold with support by P. Pop Ristova. Microsensor measurements (*ex situ*) of sulfide, oxygen and pH as well as *in situ* benthic chamber incubations were conducted by F. Wenzhöfer. Samples for sulfate reduction rates, anaerobic oxidation of methane and nutrient measurements were prepared by A. Boetius and C. Bienhold. DOC measurements were conducted by T. Dittmar. Bacterial community analyses were conducted by C. Bienhold. The manuscript was written by C. Bienhold with support and input from A. Boetius and comments of the coauthors.

Participation in seminars and courses of the excellence graduate school GLOMAR provided the framework for discussions of the impact of global change on the diversity and function of marine ecosystems and how they may respond to global environmental changes. The study on woods was financed by the CNRS MPG Groupement de Recherche Européene DIWOOD; this project and the ESF Eurocores project CHEMECO provided the framework for discussions of zoology and ecology of large food falls.

2.

Thesis chapters

Chapter I

Biogeography of abyssal seafloor bacteria

Christina Bienhold, Lucie Zinger, Antje Boetius, Alban Ramette

HGF-MPG Group for Deep Sea Ecology and Technology, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany, and Max Planck Institute for Marine Microbiology, Bremen, Germany

In Preparation for The ISME Journal

Abstract

Distinct biogeographical patterns of bacterial communities have been detected in soils, lakes, ocean waters and on the human body in the past decade, indicating strong effects of spatial and temporal variations in the environment on the distribution of bacterial diversity. This study focuses on bacterial community distribution in one of the world's largest ecosystems, the deep-sea ocean floor, which covers almost 70% of Earth's surface. To provide a first comprehensive description of bacterial communities of pelagic surface sediments, and to further determine their specific distribution, we analysed samples from nine oceanic regions across the globe, using 454 massively parallel tag sequencing. Bacterial communities markedly differed between oceanic regions, and the number of shared bacterial types at fine taxonomic resolution decreased with geographic distance, indicating isolation-by-distance effects. Accordingly, these bacterial types displayed a high degree of endemism, suggesting a limited dispersal in deep-sea sediments, as opposed to generally cosmopolitan patterns identified at broad taxonomic resolution. Noticeably, the most sequence-abundant bacterial types were affiliated to *Gammaproteobacteria* and *Actinobacteria* and were widely dispersed. In contrast, the majority of rare members of the bacterial community (~80%) were restricted to a small number of samples, but 20% of them showed large fluctuations in sequence abundance between and within oceanic regions. Our results provide evidence for bacterial endemism in deep-sea sediments that may be the result of long-term isolation processes. In addition we identify possible members of a core deep-sea surface sediment community.

1. Introduction

Increasing evidence suggests that bacterial communities, similar to macroorganisms, exhibit large-scale patterns of biogeography which have been linked to ecosystem properties like productivity, salinity or pH, as well as to historical events such as dispersal limitation (e.g. Fierer and Jackson, 2006; Horner-Devine et al., 2003; Lozupone et al., 2007; Martiny et al., 2006; Ramette and Tiedje, 2007). The structuring of bacterial communities with environmental parameters has also been demonstrated in the marine pelagic realm (e.g. Fuhrman et al., 2006; Galand et al., 2009b), including some investigations at the global scale that evidenced large-scale patterns of bacterial communities along gradients such as latitude and temperature (Fuhrman et al., 2008; Pommier et al., 2007; Rusch et al., 2007). In contrast, little is known if such structuring occurs also in bacterial communities of surface sediments of the deep sea. Pelagic sediments cover approximately 65% of the Earth's surface and are dominated by bacteria in terms of total organism abundance and biomass (Rex and Etter, 2010; Whitman et al., 1998). Our limited understanding of deep-sea sediment microbial communities is largely due to difficulties in accessing and sampling this remote environment. The bacterial communities are responsible for carbon and nutrient recycling at the deep seafloor (Jorgensen and Boetius, 2007), and the characterization of their composition and structure, as well as their patterns of distribution is highly relevant for our understanding of the ecological structure and functioning of this ecosystem. Previous studies describing bacterial communities in deep-sea sediments have been restricted to one or a few samples from locally restricted sites (e.g. Dang et al., 2009; Li et al., 2009; Schauer et al., 2010; Tian et al., 2009), but a global-scale approach comparing bacterial communities of surface deep-sea sediments and their distribution patterns is not available yet.

The deep sea is considered a relatively stable and uniform environment, characterized by a low supply of organic matter, low temperatures, generally oxygenated surface sediments, the absence of light, and high pressure. As a consequence, bacterial communities at the seafloor may show little structuring with environmental parameters. Also, seafloor currents are usually weak and the lack of horizontal mixing may result in a limited dispersal of microbes in deep-sea sediments. An isolation of microbial communities by long distances or geographic barriers may lead to the development of endemic populations through local evolutionary processes (Cho and Tiedje, 2000; Papke et al., 2003; Papke and Ward, 2004; Whitaker et al., 2003). This was supported in a broad comparison of marine pelagic and benthic bacterial communities that evidenced higher provincialism for benthic than for pelagic communities (Zinger et al.,

submitted). However, the specific effects of dispersal limitation on the distribution of bacterial communities at the seafloor, and the mechanisms underlying these patterns remain to be determined.

Here, we provide a first comprehensive description of bacterial communities and their distribution patterns in deep-sea sediments from all major oceans by analysing 454 massively parallel tag sequencing data from 41 deep-sea surface sediments (> 1000 m water depth) collected across the globe. Because the class level presents a common entity in taxonomic studies to which ecological knowledge is often associated (Philippot et al., 2009), we first describe the bacterial classes present in deep-sea sediments. Subsequently, we investigate the dataset at finer taxonomic resolution (i.e. that of operational taxonomic units at 3% sequence difference), to yield insights into community patterns that may be masked by pooling different populations into the same, broad categories. Bacterial community turnover is compared between nine oceanic regions, taking into account their geographic proximity so as to test isolation-by-distance effects. Both, community composition (presence/absence) and structure (relative abundances) are considered in this analysis, as changes may occur at one or both of these levels, which may imply different ecological processes (Anderson et al., 2011; Lozupone et al., 2007). Effects of dispersal limitation were further examined by determining the degree of endemism and cosmopolitanism of bacterial types as well as their ranges of spatial dispersal, in association with their relative abundances to infer potential ecological strategies. In this context, we also investigated the occurrence and distribution of rare types that often account for significant portions of bacterial populations.

2. Material and Methods

2.1 454 massively parallel tag sequencing (454 MPTS)

For samples of the DSS project (Table S1) total community DNA was extracted from 1 g of sediment using Ultra Clean Soil DNA Isolation Kits (MoBio Laboratories Inc., Carlsbad, CA) and stored in a final volume of 100 µl Tris-EDTA buffer. For other projects DNA extraction procedures were done according to publicly available protocols (http://icomm.mbl.edu/microbis). Extracted DNA was amplified using a cocktail of primers targeting the V6 region of the bacterial 16S rRNA gene as published on http://vamps.mbl.edu. Fragments were sequenced by pyrosequencing on a Genome Sequencer FLX system (Roche, Basel, Switzerland) at the Marine Biological Laboratory in Woods Hole, MA, USA. Taxonomic assignments of V6-hypervariable region tags were obtained through comparisons with a reference

database of rRNA sequences using the Global Alignment for Sequence Taxonomy tool (GAST) (Huse et al., 2008; Sogin et al., 2006). Taxonomic assignments were shown to be highly consistent with results based on full-length rRNA sequences (Huse et al., 2008). The total number of sequences in the complete dataset was 761,508, corresponding to 24,398 unique OTUs at 3% sequence difference (hereafter referred to as $OTU_{0.03}$). The percentage of singletons, i.e., sequences occurring only once in the full dataset, was 43%. Based on the fact that public databases do not contain information on the full global bacterial diversity, the taxonomic annotation automatically excluded an increasing amount of sequences with increasing taxonomic resolution, i.e., 93% of all sequences were assigned at phylum level, while only 23% were assigned at the genus level (Class: 82%, Order: 47%, Family: 39%).

2.2 Datasets

Sequence data for 41 deep-sea surface sediment samples (> 1000 m water depth) were obtained through the VAMPS database (Visualization and Analyses of Micbrobial Population Structures, http://vamps.mbl.edu) of the ICoMM project (http://icomm.mbl.edu). Data either originated from own sample repositories or from public ICoMM projects. All samples analyzed here consisted of 0.5-1 g of the top 2-3 cm of pelagic deep-sea sediments composed of clays and biogenic particles. Samples covered water depths between 1025 and 5347 m. A list of all samples used in this study, their corresponding projects and geographic location as well as the number of samples in each region is shown in (Table S1). Sequences are deposited in the Genbank Sequence Read Archives (www.ncbi.nlm.nih.gov) and their accession numbers are provided in Table S1 (submission of DSS sequences is pending). For part of the analyses, samples were classified into nine different oceanic regions: South Pacific (New Zealand, 6 samples), North Pacific (Station M, 16 samples), North Pacific (Japan, 1 sample), Arabian Sea (2 samples), Northeast-Atlantic (3 samples), Eastern Mediterranean (4 samples), Arctic Ocean (5 samples), Antarctic (1 sample), South Atlantic (3 samples) (see also Figure 1).

2.3 Statistical analyses

Overall patterns of bacterial community composition (presence/absence data) and community structure (relative abundance data) were detected with non-metric multidimensional scaling based on Bray-Curtis and Jaccard distances, respectively. For analyses of variations in community structure, data were standardized to relative sequence abundances within samples, where the number of reads for a specific OTU was divided by the total number of reads in the sample. Shared and unique OTUs were calculated with 1000 sequence re-samplings in each

sample based on the smallest dataset (n = 7602 sequences), to account for differences in sequence numbers between samples. To test whether dissimilarity matrices were significantly correlated (Spearman correlation), a Mantel test (Legendre and Legendre, 1998; Mantel, 1967) was applied and significance assessed based on 999 matrix permutations. All statistical analyses were performed in R (v. 2.9.1) (R Development Core Team 2009, http://www.R-project.org) using packages *vegan* (Oksanen et al., 2010), *gplots* (Warnes et al., 2010), *gmt* (Magnusson, 2010), and custom R scripts.

3. Results

3.1 Which bacterial clades comprise the average benthic bacterial deep-sea community?

Based on all samples, an average bacterial deep-sea sediment community was assembled at the class level (Figure 1). Over half of the sequences were affiliated with *Proteobacteria* (55%), where *Gammaproteobacteria* dominated with 28% of relative sequence abundance. In order of decreasing relative abundance, sequences affiliated with *Deltaproteobacteria* (14%), *Actinobacteria* (13%), *Alphaproteobacteria* (12%), *Planctomycetacia* (9%), and *Acidobacteria* (7%) were most commonly detected. Within oceanic regions and between individual samples relative sequence abundances varied considerably (Figure 1). The most abundant classes were consistently present in all oceanic regions. *Gammaproteobacteria* sequences were the most abundant in all deep-sea sediments, except for the Eastern Mediterranean samples that were dominated by *Planctomycetacia*-affiliated sequences.

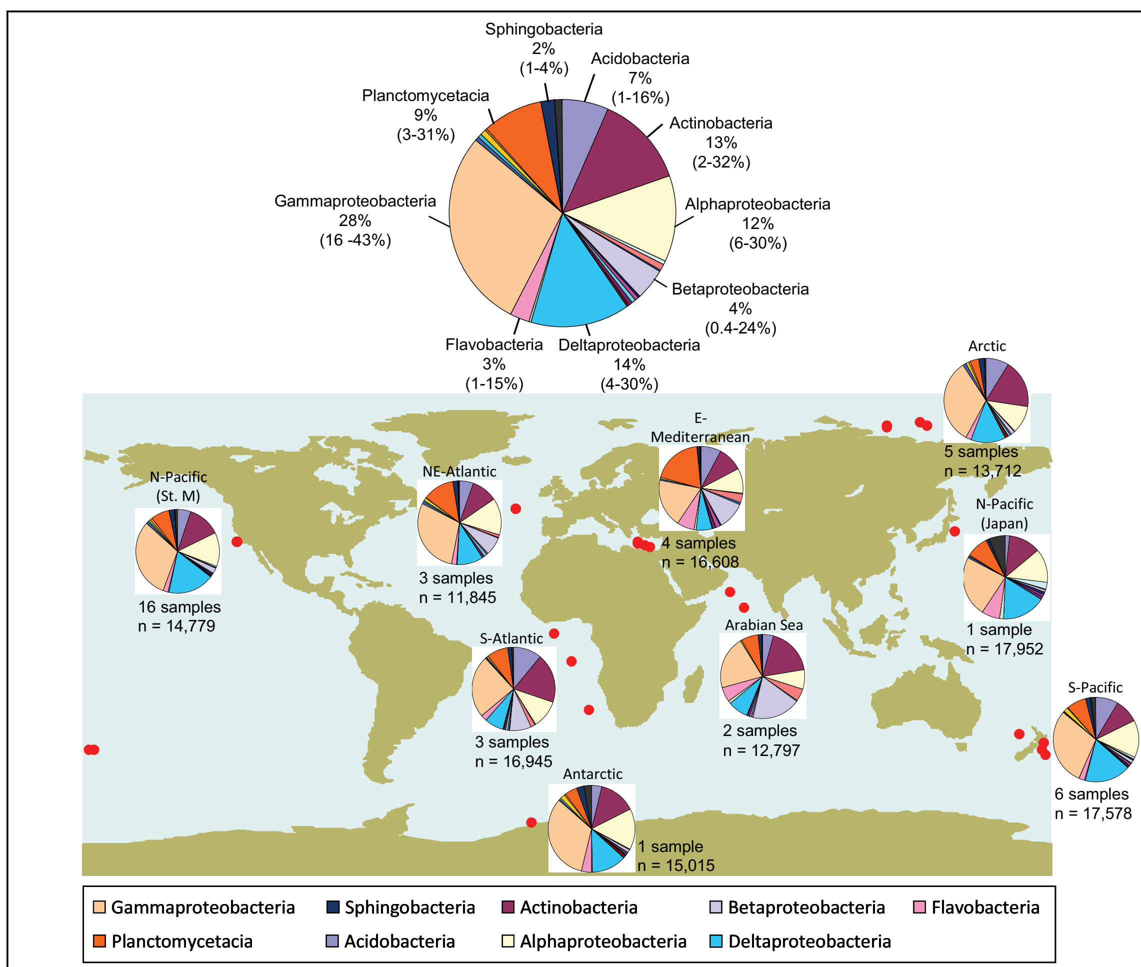

Figure 1 Community composition of deep-sea sediment bacterial communities at the class level. Large pie chart presents the average community based on all global samples and numbers give the relative abundances (≥ 2%) of classes and the range of relative abundances in individual samples. Small pie charts give the average community compositions in nine different oceanic regions. The numbers of samples as well as the number of tag sequences (n) are indicated.

3.2 Do communities differ between oceanic regions? How much?

Although most classes (60%) were shared between all oceanic regions, differences in overall community composition and structure between oceans could be evidenced both at broad and fine taxonomic levels (Figure 2a-b, Figure S1). An effect of oceanic regions on bacterial community composition and structure was confirmed with an analysis of similarity (Class composition: $R = 0.44$, $p = 0.001$; $OTU_{0.03}$ composition: $R = 0.83$, $p = 0.001$; Class structure: $R = 0.31$, $p = 0.002$; $OTU_{0.03}$ structure $R = 0.66$, $p = 0.001$). Consistent with the large amount of shared classes, community composition (presence/absence) at the class level showed large overlaps between oceanic regions, while patterns at the $OTU_{0.03}$ level were consistent when considering presence/absence or relative abundance data (Figure 2a-b). Largest variations within oceanic regions were observed for samples from the Eastern Mediterranean and Arabian Sea.

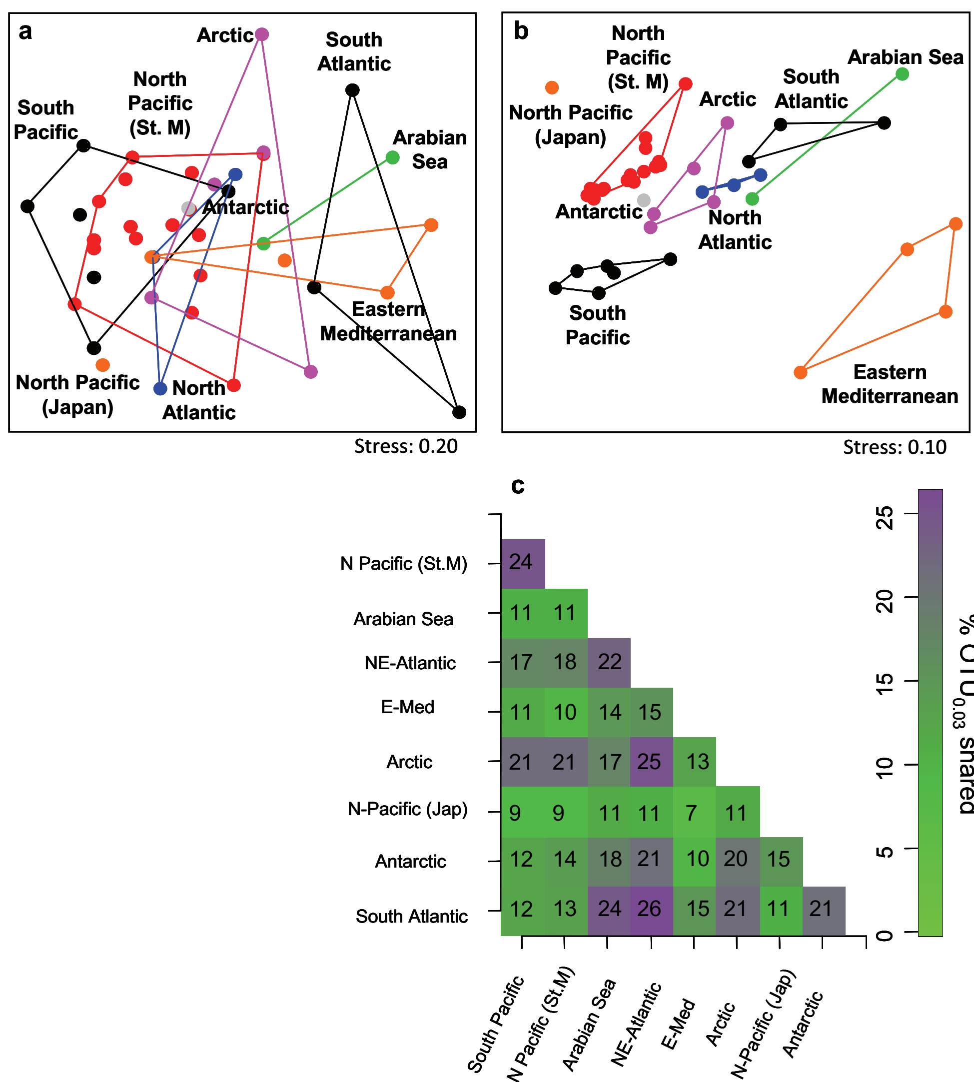

Figure 2 Differences in bacterial communities between oceanic regions. **a** and **b**: Non-metric multidimensional scaling plots for community composition at the class (**a**) and at the 3% OTU level (**b**). Samples originating from different oceanic regions are grouped by colours and lines. **c**: Percent of shared $OTU_{0.03}$ between oceanic regions.

Despite differences in the overlap of oceanic regions in ordination plots, overall patterns in community composition and structure were found to be consistent at the class and $OTU_{0.03}$ level (composition Spearman's r = 0.59, p = 0.001, structure r = 0.74, p = 0.001, as tested by Mantel test with 999 matrix permutations). The proportion of pairwise shared $OTU_{0.03}$ between oceanic regions ranged from 7–26% (Figure 2c). Highest proportions of shared $OTU_{0.03}$ (> 22%) were found for comparisons between Northeast Atlantic vs. South Atlantic, Northeast Atlantic vs. Arctic, South Pacific vs. North Pacific, Arabian vs. South Atlantic. When comparing individual samples, 5–34% of OTUs were shared in pairwise comparisons (Figure S2). The highest proportions of shared $OTU_{0.03}$ were observed for samples from the North Pacific Station M,

which were sampled at one station during September 2007 (http://icomm.mbl.edu/microbis). Also intra-ocean sample comparisons showed relatively high proportions of shared $OTU_{0.03}$ in the Arctic and the Northeast Atlantic oceans. Eastern Mediterranean and southern Arabian Sea samples had least shared $OTU_{0.03}$ with samples from other oceans, consistent with the ordination results.

3.3 What are the proportions of cosmopolitan and endemic OTUs?

At broad taxonomic resolution, i.e., the class level, most taxa were present in all oceans and samples (Figure 3a-b). The patterns found at broad taxonomic resolution, i.e., phylum, class, order, consistently showed a high percentage of cosmopolitan taxa (40 to 25% at the phylum or class, and order level, respectively). This feature shifted at the family level, and a high proportion of endemism was observed at the genus and 3% OTU levels (Figure S3).

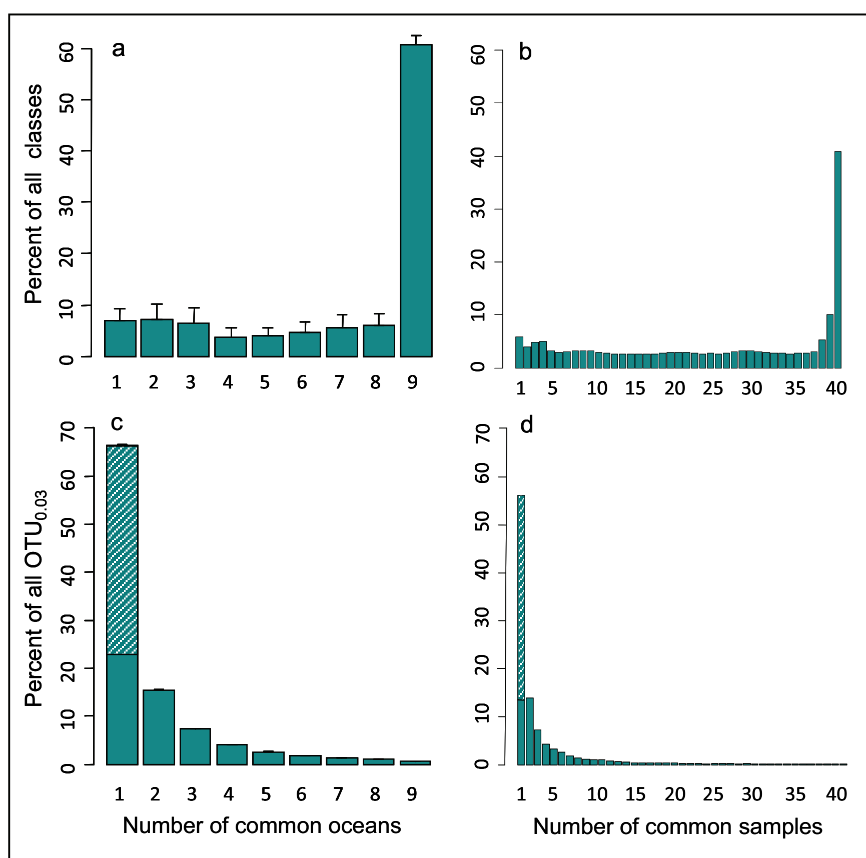

Figure 3 Proportions of unique and cosmopolitan OTUs between oceanic regions and individual samples at the class (a, b) and the $OTU_{0.03}$ level (c, d). 16,164 $OTU_{0.03}$ were unique to one ocean, of which 13,712 were unique to one sample. 149 $OTU_{0.03}$ were common to all oceans and 15 common to all samples. Dashed areas in c and d indicate the fraction accounted for by absolute singletons (i.e. $OTU_{0.03}$ only occurring with one sequence in the whole dataset).

Most $OTU_{0.03}$ (16,164 $OTU_{0.03}$, 66% of all $OTU_{0.03}$) occurred only in one ocean (i.e., were endemic), whereas less than 10% $OTU_{0.03}$ were cosmopolitan, i.e., occurred in five or more oceans, with only 149 (0.6%) $OTU_{0.03}$ detected in all oceanic regions (Figure 3c).
A similar picture emerged when individual samples were considered: most $OTU_{0.03}$ (13,712 $OTU_{0.03}$, 56%) occurred in only one sample and 15 $OTU_{0.03}$ (0.06%) were common to all samples (Figure 3d). It must be noted that absolute singletons, i.e., $OTU_{0.03}$ occurring with only one sequence in the whole dataset, accounted for about 40% of all $OTU_{0.03}$ (Figure 3, hatched area). But, even when removing these singletons, a high amount of $OTU_{0.03}$ remained unique to one ocean or to one sample.

3.4 Do community composition and structure change with geographic distance?

Notably, the proportion of shared $OTU_{0.03}$ between samples decreased significantly with geographic distance (Figure 4). A LOESS curve for the relationship between the number of shared $OTU_{0.03}$ and geographic distance showed a strong decrease in shared $OTU_{0.03}$ until 5,000 km (Figure 4). The curve levelled off between 5,000 and 10,000 km and showed a strong decrease again for larger distances.

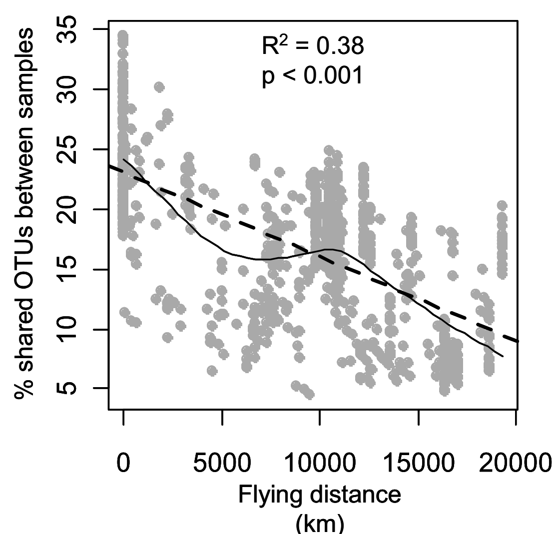

Figure 4 The proportion of shared $OTU_{0.03}$ between samples decreased significantly with geographic distance (defined as straight lines between samples). Solid line is a LOESS curve and dotted line is a linear model fit.

To better resolve the effects of different spatial components, we plotted changes in community composition and structure against geographic distance, latitudinal distance, longitudinal distance and differences in water depth (Figure S4). Bacterial community composition and structure showed significant relationships with all of the investigated

components. The strongest relationship was observed with geographic distance (composition r = 0.56, p = 0.001; structure r = 0.47, p = 0.001), which seemed to be mainly due to changes along longitude (composition: r = 0.53, p = 0.001; structure: r = 0.49, p = 0.001), while latitudinal patterns were less pronounced (composition r = 0.40, p = 0.001; structure: r = 0.24, p = 0.018). A weaker but significant relationship was observed for community changes along water depth (composition: r = 0.41, p = 0.001; structure: r = 0.24, p = 0.005).

3.5 What is the relationship between dispersal and abundance: do more abundant OTUs have larger dispersal ranges?

When considering the relative abundance of unique and cosmopolitan $OTU_{0.03}$, it could be shown that the large proportion of $OTU_{0.03}$ only found in one sample belonged to low abundant (rare) members of the community (Figure 5). At the same time a smaller proportion of $OTU_{0.03}$ was found in all samples, but included all of the more abundant $OTU_{0.03}$. Another way of looking at distributions of $OTU_{0.03}$ is to consider the maximum distance between samples in which a given $OTU_{0.03}$ was detected, regardless of the number of samples it was found in (Figure S5).

Distances ranged between 0 and 19,327 km between samples of the Arabian Sea (Western) and the North Pacific (Station M). Noticeably, all of the most abundant $OTU_{0.03}$ were widely dispersed, up to the maximum distance of 19,327 km (Figure S5). However, the largest fraction of $OTU_{0.03}$ to the total detected $OTU_{0.03}$, occurred at low spatial ranges (0–5,000 km) and had low sequence abundances. The most sequence-abundant, globally dispersed $OTU_{0.03}$ (i.e., found at maximum distances and common to all samples) were affiliated to *Actinobacteria* and *Gammaproteobacteria*. But also some less abundant $OTU_{0.03}$ affiliated to *Gemmatimonadetes*, *Acidobacteria* and *Alphaproteobacteria* were globally distributed.

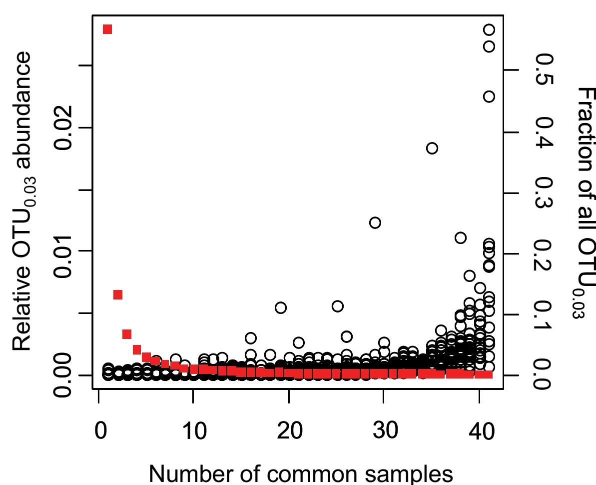

Figure 5 Distribution and abundance of $OTU_{0.03}$. Relative $OTU_{0.03}$ abundance (black circles) and the fraction of all $OTU_{0.03}$ (red squares) as a function of the number of samples an $OTU_{0.03}$ was detected in.

3.6 Do rare types have specific spatial patterns and do they stay always rare?

Single sequence OTU (SSOrel), i.e., $OTU_{0.03}$ occurring as one sequence in at least one sample, which accounted for 39% of all $OTU_{0.03}$, were used to further explore patterns of rare types in the community. Most SSOrel remained at low sequence abundance in the samples they were observed in, e.g., 79% of all SSOrel had a maximum abundance of 10 sequences or lower and only smaller proportions of SSOrel reached maximum abundances of over 100 sequences (maximum abundance of one SSOrel was 4059 sequences) (Figure 6). For a few selected cases higher fluctuations were observed, e.g., maximum abundances of 50 and 4059 sequences, and a random sampling indicated that some SSOrel only occurred at specific sites while others were present in many samples.

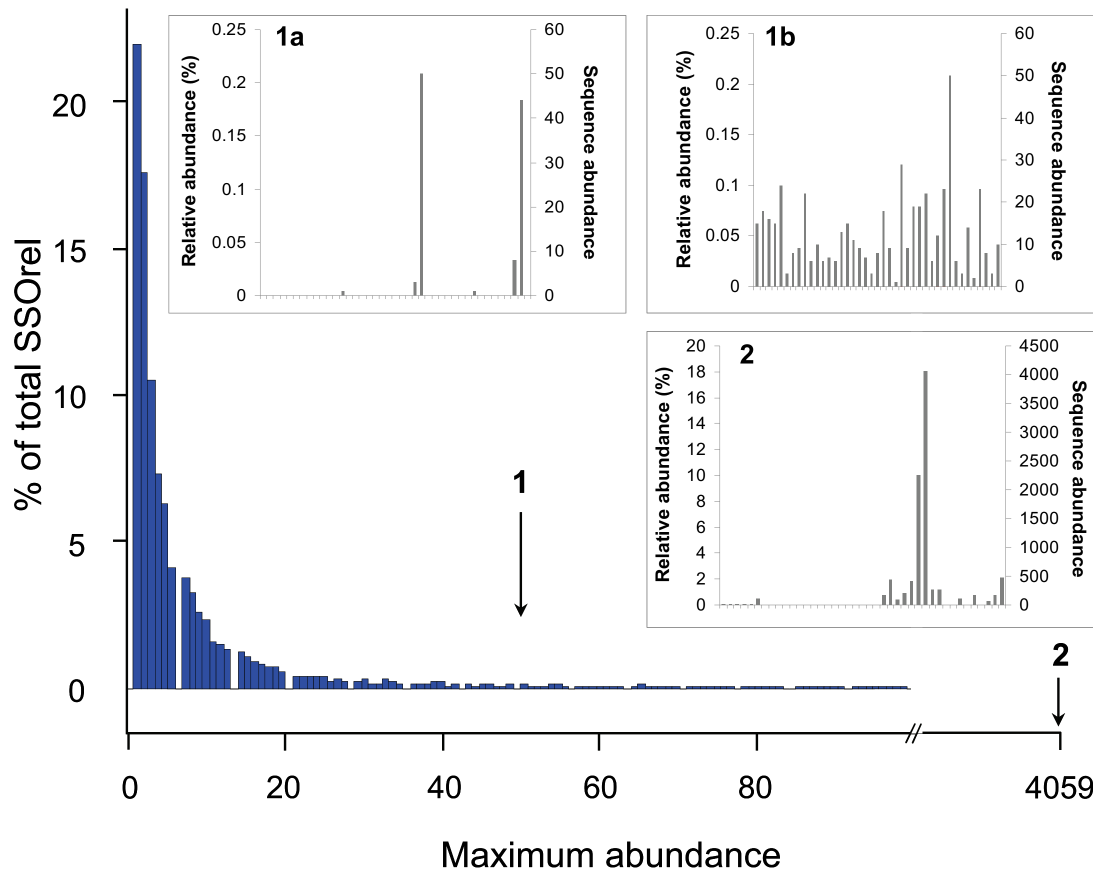

Figure 6 Distribution of the maximum abundance of SSOrel (i.e., $OTU_{0.03}$ occurring as one sequence in at least one sample). Panels 1-2 are exemplary cases of SSOrel showing high fluctuations, with maximum abundances of 50 (1a, b) and 4059 (2) sequences. The patterns demonstrate that some SSOrel may only occur at a few sites (1a, 3), while others are present in many samples (1b). Relative abundances in subpanels are proportions of the total number of sequences.

4. Discussion

4.1 Which bacterial clades comprise the average benthic bacterial deep-sea community?

So far it remains unknown as to which bacterial taxa form typical deep-sea bacterial communities inhabiting pelagic surface sediments, and if these are distinct from deep water or deep subsurface communities. Here, we show that the major taxa observed in globally distributed deep-sea surface sediments were *Gammaproteobacteria*, *Deltaproteobacteria*, *Actinobacteria*, *Alphaproteobacteria*, *Planctomycetacia*, and *Acidobacteria* (Figure 1). This is in agreement with previous studies of deep-sea sites, e.g., in the Eastern Mediterranean, Arctic, East Pacific and South Atlantic Ocean (Dang et al., 2009; Heijs et al., 2008; Li et al., 2009; Schauer et al., 2010; Tian et al., 2009). These studies were mainly based on 16S rRNA gene clone libraries and consistently reported *Gammaproteobacteria*, *Alphaproteobacteria* and *Deltaproteobacteria* among the most dominant representatives of surface deep-sea sediments, which confirms a certain degree of consistency between different molecular methods, when broad taxonomic categories are considered. Benthic bacterial community composition can be differentiated from both surface and deep pelagic communities, where *Alphaproteobacteria* were the dominant group observed (Giovannoni and Stingl, 2005; Zinger et al., submitted). Furthermore, surface waters were shown to be dominated by *Flavobacteria* and *Cyanobacteria*, while *Actinobacteria* and *Deltaproteobacteria* increased in relative sequence abundances in deeper waters. Also the vast subsurface biosphere underlying the surface sediments investigated here can be distinguished in terms of the most commonly reported bacterial types, which belong to the groups *Gammaproteobacteria*, *Chloroflexi* and candidate division JS1 (Fry et al., 2008; Giovannoni and Stingl, 2005; Inagaki et al., 2006; Teske, 2006; Webster et al., 2006; Zinger et al., submitted). This shows that pelagic, benthic surface and deep-subsurface environments exhibit distinct bacterial community signatures already at broad taxonomic resolution, which probably reflect differences in life styles and environmental pressures between these three habitats, as shown for other environments (Philippot et al., 2010).

4.2 Do communities differ between oceanic regions? How much?

A certain degree of provincialism of benthic bacterial communities has been proposed previously (Zinger et al., submitted). To determine specific distribution patterns between oceanic regions, we first investigated differences in bacterial community composition and structure between oceanic regions. The major classes, namely *Gammaproteobacteria*, *Deltaproteobacteria*, *Actinobacteria*, *Alphaproteobacteria*, *Planctomycetacia*, and *Acidobacteria* were widely distributed and appeared in all sediments investigated, similar to what has been reported for marine

bacterioplankton (Pommier et al., 2007). On the one hand, the systematic recruitment of similar taxa at broad taxonomic resolution may support an ecological coherence of high taxonomic ranks (Philippot et al., 2010), with lifestyles adapted to the deep seafloor environment in this case. On the other hand, this observation may also reflect the distribution of a few dominant members of these classes, e.g., as the global dominance of *Alphaproteobacteria* in surface waters is caused by some cosmopolitan *SAR* and *Roseobacter* taxa (Rusch et al., 2007; Venter et al., 2004).

Higher proportions of shared OTUs were observed between oceans that were connected by deep-water ocean currents, e.g., the South and North Pacific, and the Northeast and South Atlantic (Figure 2c), although the southernmost station in the South Atlantic is filled with Antarctic lower circumpolar deep water and would need to be considered separately. Nonetheless, this suggests that dispersal is limited over longer distances and across continents. The lowest proportions of shared OTUs were observed in comparison of all ocean regions with the Eastern Mediterranean. Similar trends have also been evidenced in a metagenome study of deep-water communities (Martin-Cuadrado et al., 2007). The Eastern Mediterranean deep-water current has little exchange with the bottom water of other oceans, and benthic microbes may therefore display limited dispersal, resulting in the development of endemic populations due to local, isolated evolutionary processes. In addition, the Mediterranean deep sea has higher temperatures (13–14°C) compared to other deep-sea regions, and the influence of land, e.g., river input, may be especially pronounced in this enclosed ocean. Low proportions of shared OTUs were also observed when comparing samples with the sample from the North-Pacific (Japan). The sample was taken from a trench system probably also indicating different environmental settings and limited dispersal. However, the nature of dispersal of surface sediment communities remains enigmatic, and needs further investigation. As sedimentation of pelagic bacteria with particles sinking from the surface waters appears not to be a main source for benthic bacterial diversity (Zinger et al., submitted), it is likely that resuspension of sediments by bottom water currents and feeding fauna is a main source of dispersal of benthic surface bacteria.

4.3 What are the proportions of cosmopolitan and endemic OTUs?

At broad taxonomic resolution (i.e., phylum, class), most taxa occurred in all oceans and samples (e.g., 61% and 41% of all classes, respectively) and therefore exhibited cosmopolitanism (Figure 3). But this pattern reversed at the family level (Figure S3) and most genera and $OTU_{0.03}$ were unique to one ocean (66% of all $OTU_{0.03}$) and sample (56% of all $OTU_{0.03}$), indicating a high degree of endemism of bacterial taxa in deep-sea sediments. Absolute singletons, i.e., sequences occurring only once in the dataset, contributed significantly to this trend, but did not change the

overall pattern when removed. For one, the occurrence of large numbers of singletons may indicate that we are still under-sampling bacterial diversity. But, in general, the relevance of rare types in bacterial communities is not well resolved. In agreement with our observations, earlier studies have shown that the removal of singletons or rare types neither affected the overall patterns of bacterial communities nor their ecological interpretation (Gobet et al., 2010; Zinger et al., submitted). The high degree of endemism observed here (including or not singletons) reflects high spatial turnover for bacterial communities in deep-sea sediments and has also been observed for marine bacterioplankton communities (Pommier et al., 2007). In the future, differences in turnover between different ecosystems may be quantified, to determine to what degree sediment or soil associated bacterial communities show stronger dispersal limitation than marine or aquatic pelagic communities, though differences in methodologies, which represent abundant (e.g., 16S rRNA gene clone libraries,), or rare types (e.g., 454 massively parallel tag sequencing) would need to be considered.

4.4 Do community composition and structure change with geographic distance?

The proportion of shared $OTU_{0.03}$ significantly decreased with increasing geographic distance, both on small and large scales, providing strong evidence for isolation-by-distance of bacterial communities in deep-sea surface sediments (Figure 4). This is supported by earlier observations of geographic isolation of bacterial communities in other environments, e.g., for *Pseudomonas* genotypes in soils (Cho and Tiedje, 2000) and for cyanobacteria and archaea in hot springs (Papke et al., 2003; Whitaker et al., 2003). The relationship between the percent of shared $OTU_{0.03}$ with distance showed a strong decrease until 5,000 km which corresponded approximately to the maximum distance of samples within an oceanic region (South Atlantic). Between 5,000 and 10,000 km the curve levelled off and showed a stronger decrease in shared $OTU_{0.03}$ again for larger distances, demonstrating an effect of distance both for within ocean as well as between oceans.

We used different spatial components to better decipher the parameter responsible for community turnover both at the level of community composition (presence/absence) and structure (relative abundances) (Figure S4). The observed patterns were consistent for community composition and structure, indicating that variation was mainly caused by differences in the occurrence of specific $OTU_{0.03}$ (composition) between sites. Changes of overall community composition and structure along latitude were not very pronounced, suggesting that energy gradients associated with latitude might not play an important role in bacterial community composition and structure at the deep seafloor, compared to other ecosystems and other

organisms (Hillebrand, 2004; Soininen et al., 2007). A reason for this may be the isolation of the deep seafloor from climatic, light and temperature fluctuations along latitudes. Strong community variation along longitude suggests that continents and deep-water currents may present an important barrier for dispersal (McClain and Hardy, 2010). However, also spatially structured environmental parameters may account for changes with geographic distance. For example, the role of surface productivity, particle flux, and of other biological factors in the structuring of benthic communities have been suggested previously (Bienhold et al., submitted; Zinger et al., submitted), but need to be further explored for the deep seafloor at the global scale. Furthermore, the geological history of deep-sea basins or past environmental conditions may have played a role in shaping distribution patterns of benthic bacterial communities. Also topographic features such as deep-sea mountain ranges could act as biogeographic barriers which has been demonstrated for some macrofaunal organisms in the South Atlantic that were separated by the Walvis Ridge (Brandt et al., 2005), but could not be confirmed for bacterial communities (Schauer et al., 2010). The weak relationship of bacterial community composition and structure with water depth would indicate that, below 1000 m, bacterial communities may only be slightly affected by subtle changes in biological or physical parameters that are correlated with water depth (e.g., gradients in resource availability, pressure).

4.5 What is the relationship between dispersal and abundance: do more abundant OTUs have larger dispersal ranges?

Most $OTU_{0.03}$ were either unique to one sample or only common to a few samples and were restricted in their dispersal to ranges between 0 and 5,000 km. This spatial range approximately corresponds to the largest distance observed between samples within an oceanic region (South Atlantic), suggesting that most $OTU_{0.03}$ may be limited in their dispersal over larger scales (Figure 5, Figure S5). The majority of $OTU_{0.03}$ were associated with low sequence abundances, thus exhibiting typical features of bacterial populations with a long tail of rare types in rank abundance curves (Pedros-Alio, 2006; Sogin et al., 2006). All of the most abundant $OTU_{0.03}$ were widespread, i.e., occurred in a large number of samples and were found at maximum distances of ~20,000 km. Positive range-abundance relationships for bacterial types have been observed in a variety of other studies (Green and Bohannan, 2006; Nemergut et al., 2011; Pommier et al., 2007), and have also been described as a property of macroorganisms (Brown, 1984). A plausible ecological explanation would be that higher local population size enables a wider dispersal, but we should keep in mind that methods based on sequencing of 16S rRNA genes do not necessarily reflect the true abundance of organisms in the environment (Venter et al., 2004). Sequences belonging

to the globally dispersed $OTU_{0.03}$ were affiliated to taxa commonly observed in deep-sea benthic communities, e.g., *Gammaproteobacteria* and *Actinobacteria* (Dang et al., 2009; Schauer et al., 2010; Tian et al., 2009). These $OTU_{0.03}$ may constitute part of a core bacterial community in deep-sea sediments that may consist of generalists highly adapted to life in the deep sea or that may be able to form dormant stages, which could enable dispersal over large spatial ranges. Our study presents a first step toward the characterization of a core bacterial community in deep-sea sediments that may serve as a bacterial fingerprint of the deep seafloor when contrasting it with other marine habitats, e.g., the pelagic or subsurface biosphere.

4.6 Do rare types have specific spatial patterns and do they stay always rare?

The advent of high-throughput sequencing technology has opened doors to the exploration of a largely underexplored biosphere in bacterial communities (Sogin et al., 2006). But, up to now, there is no general agreement on how a rare member of the community should be defined. Some authors have arbitrarily defined rare types as having a frequency of less than 0.01% within a sample (Galand et al., 2009a), while others have applied systematic cut-offs to explore the effect of removing rare sequences from the dataset on ecological interpretations (Gobet et al., 2010). The occurrence of substantial numbers of rare $OTU_{0.03}$ in our dataset, prompted us to further explore whether rare types are rare in all deep-sea sediment samples or whether patterns in their distribution could be observed. Relative single sequence OTU (SSOrel), i.e., $OTU_{0.03}$ occurring as one sequence in at least one sample, are a possible definition of a rare type (Gobet et al., submitted). Most SSOrel appeared at low abundances in all samples (Figure 6), consistent with other studies investigating variations of rare members in bacterial communities in oceanic waters or coastal sediments (Galand et al., 2009a; Gobet et al., submitted; Kirchman et al., 2010). Nevertheless, members of the rare biosphere may exhibit non-random, ecological patterns (Galand et al., 2009a) and could be relevant to ecosystem functioning by forming a "seed bank" of organisms able to increase in abundance and maintain ecosystem functioning when environmental conditions change (Loreau et al., 2001; Pedros-Alio, 2006).

For a few selected cases we observed that SSOrel may show differing patterns across samples (Figure 6). Disregarding their maximum sequence abundance, some only occurred at specific sites, while others were present in most samples. This illustrates that SSOrel are not distributed randomly across space and time (some stations included several time points), but may also either be limited in their dispersal and/or depend on specific environmental conditions. This was indicated by one example, where an SSOrel, affiliated to *Planctomycetes*, was absent or only present at low abundances in most samples, but showed extremely elevated sequence counts for

two samples, which corresponded to samples from a deep-sea trench in the Eastern Mediterranean, where increased microbial biomass had been evidenced due to the accumulation of phytodetritus (Boetius et al., 1996). This is consistent with earlier studies that have found *Planctomycetes* in association with marine phytodetritus aggregates (Crump et al., 1999; Delong et al., 1993; Fuerst, 1995; Gihring et al., 2009). Further studies would be needed to better understand the community composition and turnover in deep sea surface sediments, including targeted sampling across spatial and temporal gradients of environmental parameters, deep water currents, sinking particles, to follow and resolve the distribution patterns of specific bacterial clades.

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Supplementary information

Table S1 Contextual data for all deep-sea samples: VAMPS (http://vamps.mbl.edu.) sample ID, geographic origin, water depth, oceanic region, and sequence archive accession numbers for Genbank Sequence Read Archives (www.ncbi.nlm.nih.gov) (n.a. = not available, submission of DSS sequences is pending).

Sample ID	Longitude	Latitude	Water depth (m)	Date of sampling	Oceanic region	Sequence Archive accession #
NZS_0003_2007_04_16	-176.714	-42.782	1025	16.04.2007	South Pacific (New Zealand)	SRA009906.1
NZS_0004_2007_04_26	175.930	-42.992	1197	26.04.2007	South Pacific (New Zealand)	SRA009906.1
NZS_0007_2007_04_06	177.141	-44.485	1241	06.04.2007	South Pacific (New Zealand)	SRA009906.1
NZS_0011_2007_04_20	-178.339	-42.531	1400	20.04.2007	South Pacific (New Zealand)	SRA009906.1
NZS_0013_2007_05_30	167.526	-36.920	1217	30.05.2007	South Pacific (New Zealand)	SRA009906.1
DSS_0023_2007_02_11	177.023	-40.022	1181	11.02.2007	South Pacific (New Zealand)	n.a.
SMS_0001_2007_09_19	-123.016	35.164	3953.5	19.09.2007	North Pacific (Station M)	SRA009865.1
SMS_0002_2007_09_19	-123.016	35.164	3953.5	19.09.2007	North Pacific (Station M)	SRA009865.1
SMS_0003_2007_09_19	-123.016	35.164	3953.5	19.09.2007	North Pacific (Station M)	SRA009865.1
SMS_0004_2007_09_23	-123.016	35.164	3953.5	23.09.2007	North Pacific (Station M)	SRA009865.1
SMS_0005_2007_09_23	-123.016	35.164	3953.5	23.09.2007	North Pacific (Station M)	SRA009865.1
SMS_0006_2007_09_23	-123.016	35.164	3953.5	23.09.2007	North Pacific (Station M)	SRA009865.1
SMS_0007_2007_09_19	-123.016	35.164	3953.5	19.09.2007	North Pacific (Station M)	SRA009865.1
SMS_0008_2007_09_19	-123.016	35.164	3953.5	19.09.2007	North Pacific (Station M)	SRA009865.1
SMS_0009_2007_09_19	-123.016	35.164	3953.5	19.09.2007	North Pacific (Station M)	SRA009865.1
SMS_0010_2007_09_23	-123.016	35.164	3953.5	23.09.2007	North Pacific (Station M)	SRA009865.1
SMS_0011_2007_09_23	-123.016	35.164	3953.5	23.09.2007	North Pacific (Station M)	SRA009865.1
SMS_0012_2007_09_23	-123.016	35.164	3953.5	23.09.2007	North Pacific (Station M)	SRA009865.1
SMS_0013_2007_09_19	-123.016	35.164	3953.5	19.09.2007	North Pacific (Station M)	SRA009865.1
SMS_0014_2007_09_19	-123.016	35.146	3953.5	19.09.2007	North Pacific (Station M)	SRA009865.1
SMS_0015_2007_09_23	-123.016	35.164	3953.5	23.09.2007	North Pacific (Station M)	SRA009865.1
SMS_0016_2007_09_23	-123.016	35.164	3953.5	23.09.2007	North Pacific (Station M)	SRA009865.1
DSS_0022_2006_06_07	143.893	39.106	5347	07.06.2006	North Pacific (Japan)	n.a.
DSS_0001_1995_10_03	60.268	16.051	4078	03.10.1995	Arabian Sea	n.a.
DSS_0002_1995_10_21	65.034	10.050	4411	21.10.1995	Arabian Sea	n.a.
DSS_0003_1992_03_27	-19.583	47.167	4560	27.03.1992	North-East Atlantic	n.a.
DSS_0004_1992_04_04	-19.583	47.167	4560	04.04.1992	North-East Atlantic	n.a.
DSS_0005_1992_08_05	-19.583	47.167	4560	05.07.1992	North-East Atlantic	n.a.
DSS_0006_1993_05_26	25.859	34.742	1375	26.05.1993	Eastern Mediterranean	n.a.
DSS_0007_1993_05_27	26.097	34.414	4260	27.05.1993	Eastern Mediterranean	n.a.
DSS_0008_1993_05_29	28.571	33.604	2968	29.05.1993	Eastern Mediterranean	n.a.
DSS_0009_1993_05_31	30.597	32.680	1904	01.06.1993	Eastern Mediterranean	n.a.
DSS_0015_1993_09_04	133.191	78.387	2019	04.09.1993	Arctic Ocean (Laptev Sea)	n.a.
DSS_0016_1993_09_03	130.596	79.652	3427	03.09.1993	Arctic Ocean (Laptev Sea)	n.a.
DSS_0017_1993_09_03	130.596	79.652	3427	03.09.1993	Arctic Ocean (Laptev Sea)	n.a.
DSS_0020_1993_09_15	118.577	77.680	1517	15.09.1993	Arctic Ocean (Laptev Sea)	n.a.
DSS_0021_1993_09_14	118.742	78.667	2620	14.09.1993	Arctic Ocean (Laptev Sea)	n.a.
DSS_0033_2005_02_23	-14.000	-70.000	4300	23.02.2005	Antarctic	n.a.
DSS_0034_2005_03_04	7.347	-28.112	5114	04.03.2005	South-Atlantic (Cape)	n.a.
DSS_0035_2005_03_11	0.897	-9.932	1928	11.04.2005	South-Atlantic (Angola)	n.a.
DSS_0036_2005_03_19	-5.583	0.833	5225	19.04.2005	South-Atlantic (Guinea)	n.a.

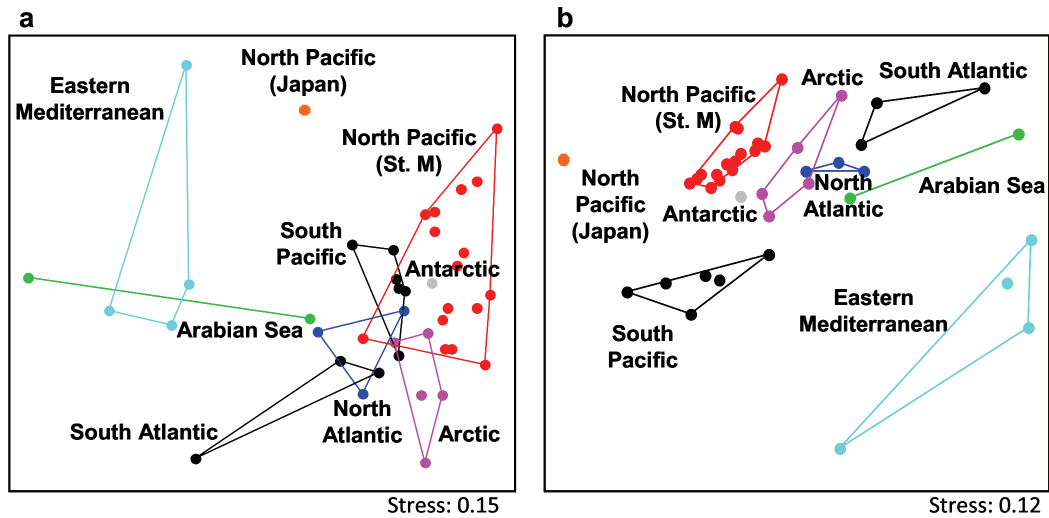

Figure S1 Non-metric multidimensional scaling plots for community structure (considering relative abundances) at the class (a) and at the 3% OTU level (b). Samples originating from different oceanic regions are grouped by colors and lines.

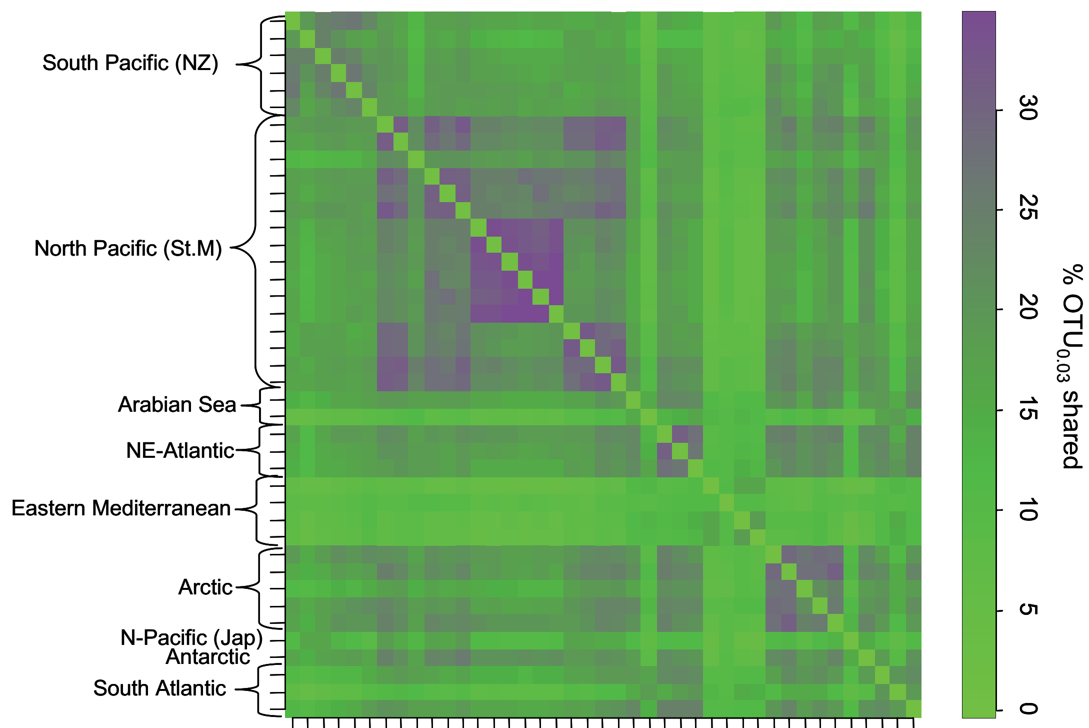

Figure S2 Symmetric heatmap of the percent of shared $OTU_{0.03}$ between samples, indicating the oceanic region of the samples.

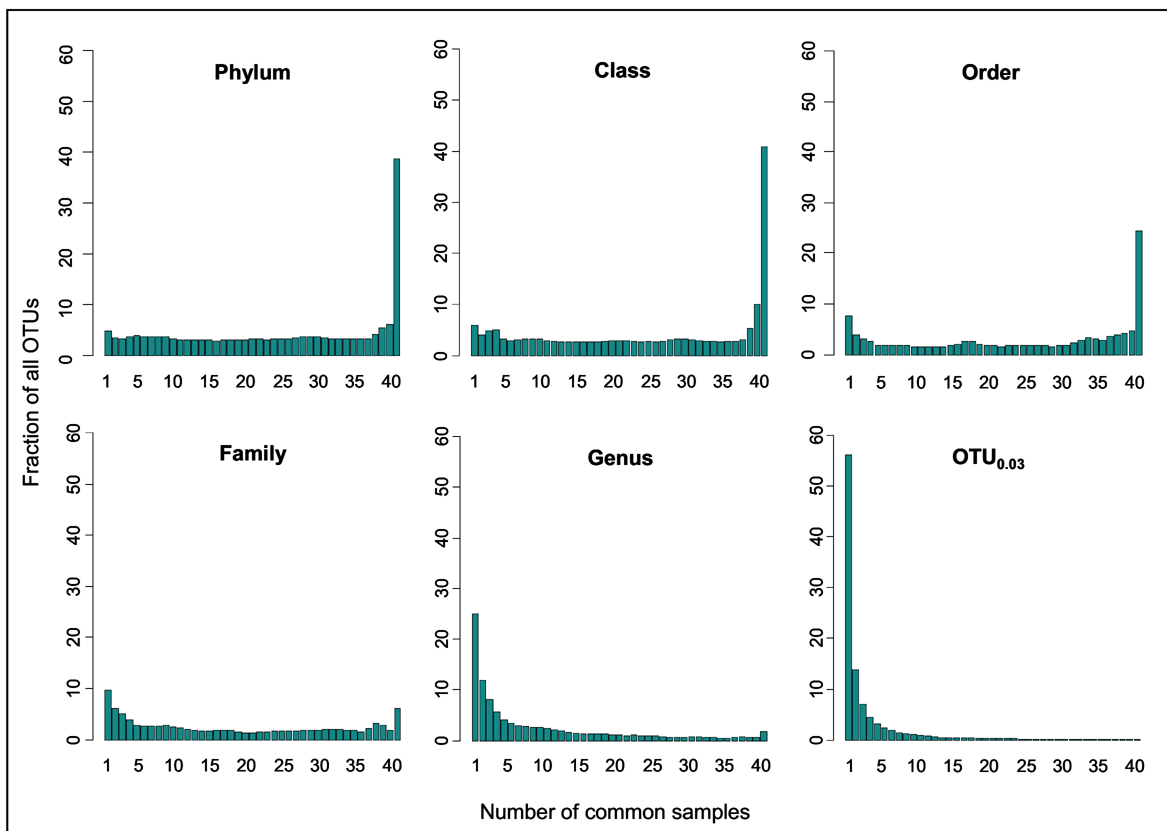

Figure S3 Proportions of unique and cosmopolitan OTU between samples at all taxonomic levels.

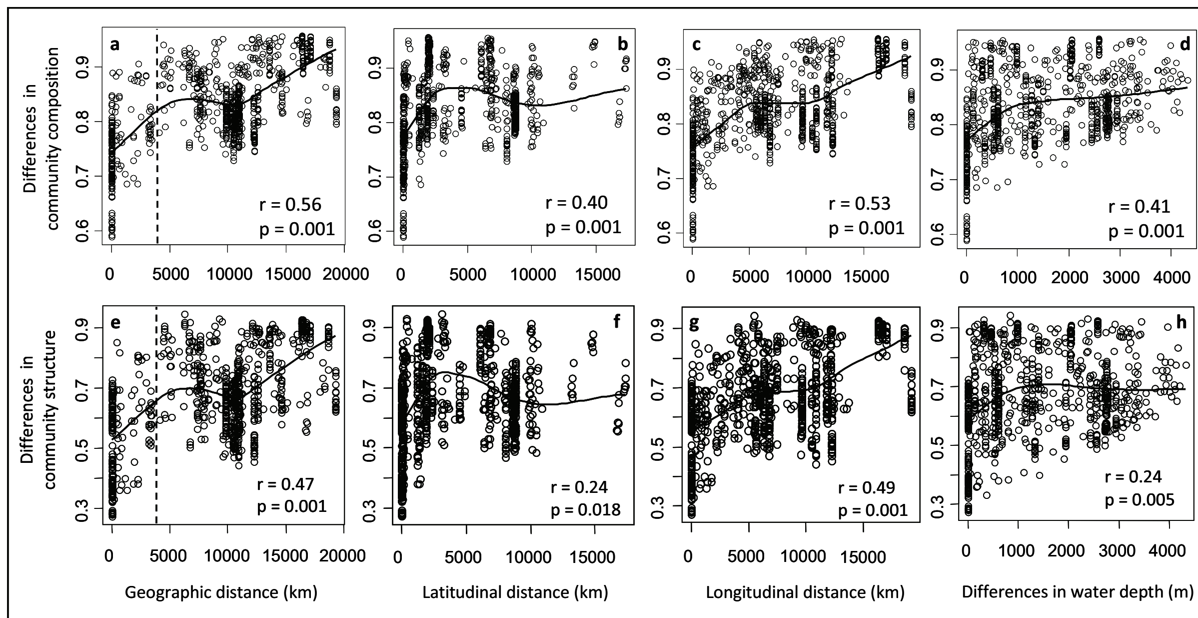

Figure S4 Changes in global bacterial community composition (a-d) and structure (e-h) with a, e) geographic distance, b, f) latitudinal distance, c, g) longitudinal distance, and d, h) water depth. Spearman correlations as tested by Mantel tests with 999 permutations are indicated in the plots. Lines are LOESS curves; the dotted line in a and e marks the maximum distance between samples originating from one ocean (4000 km, South Atlantic).

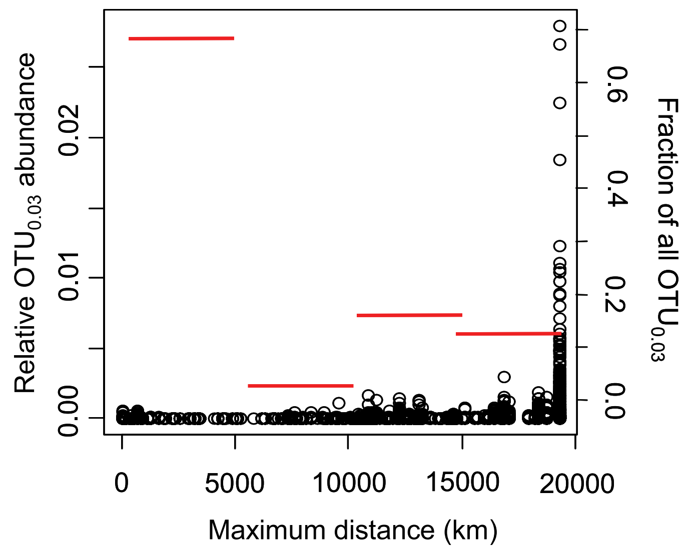

Figure S5 Distribution and abundance of $OTU_{0.03}$. Relative $OTU_{0.03}$ abundance (black circles) and the fraction of all $OTU_{0.03}$ (red bars) as a function of the maximum occupation range of an OTU (maximum distance an OTU was detected at).

Chapter II

Bacterial diversity and biogeography in deep-sea surface sediments of the South Atlantic Ocean

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ORIGINAL ARTICLE

Bacterial diversity and biogeography in deep-sea surface sediments of the South Atlantic Ocean

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Microbial biogeographic patterns in the deep sea depend on the ability of microorganisms to disperse. One possible limitation to microbial dispersal may be the Walvis Ridge that separates the Antarctic Lower Circumpolar Deep Water from the North Atlantic Deep Water. We examined bacterial communities in three basins of the eastern South Atlantic Ocean to determine diversity and biogeography of bacterial communities in deep-sea surface sediments. The analysis of 16S ribosomal RNA (rRNA) gene clone libraries in each basin revealed a high diversity, representing 521 phylotypes with 98% identity in 1051 sequences. Phylotypes affiliated with *Gammaproteobacteria*, *Deltaproteobacteria* and *Acidobacteria* were present in all three basins. The distribution of these shared phylotypes seemed to be influenced neither by the Walvis Ridge nor by different deep water masses, suggesting a high dispersal capability, as also indicated by low distance–decay relationships. However, the total bacterial diversity showed significant differences between the basins, based on 16S rRNA gene sequences as well as on terminal restriction fragment length polymorphism fingerprints. Noticeably, both geographic distance and environmental heterogeneity influenced bacterial diversity at intermediate (10–3000 km) and large scales (>3000 km), indicating a complex interplay of local contemporary environmental effects and dispersal limitation.
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Subject Category: microbial population and community ecology
Keywords: barrier; biogeography; deep sea; *Gammaproteobacteria*; spatial scale

Introduction

Biogeographic patterns in microbial communities are traditionally explained by two factors, the environmental heterogeneity and historical events (Martiny *et al*., 2006; O'Malley, 2008). On the basis of the cosmopolitan hypothesis, 'everything is everywhere, but the environment selects' (Baas-Becking, 1934), environmental conditions have long been considered to have a strong influence on microbial biogeography. The effects of spatial distances (historical events) have been shown to affect microbial diversity in several studies (Papke *et al*., 2003; Whitaker *et al*., 2003; Martiny *et al*., 2006; Ramette and Tiedje, 2007). The relative influences of environmental heterogeneity and historical events on microbial biogeography are still poorly understood. In marine habitats like the deep sea, microorganisms in the surface sediment may be assumed to disperse with oceanic currents. Bioirrigation by the activities of larger benthic organism as well as near-bed currents (Hughes and Gage, 2004; Queric and Soltwedel, 2007) influence the sediment-water interface exchange and consequently lead to the dispersal of particles and therefore of microorganism. Barriers to microbial dispersal could be physical (topography) or physiological conditions (temperature, pH or hydrostatic pressure).

In the eastern South Atlantic Ocean, the Cape Basin is separated from the Angola and Guinea basins by the Walvis Ridge that forms a barrier to the northward and southward flow of water below a depth of about 3000 m (Shannon and Chapman, 1991). Furthermore, the Cape Basin is dominated by Lower Circumpolar Deep Water arriving from Antarctica and the deepest part of the Angola and Guinea Basins are filled with North Atlantic Deep Water originating from the Arctic (Bickert and Wefer, 1996). Noticeably, the Walvis Ridge has been shown to function as a barrier for the dispersal of some crustacean species of *Peracarida* (Brandt *et al*., 2005), but it is not known whether this physical barrier also affects microbial dispersal.

To analyze whether different deep water masses associated with the physical barrier of the

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Walvis Ridge have significant structuring effects on microbial diversity, the bacterial diversity in three deep-sea basin surface sediments was determined by 16S ribosomal RNA (rRNA) gene sequencing and the community fingerprinting method terminal restriction fragment length polymorphism (T-RFLP). The relative contribution of environmental heterogeneity and of historical events on microbial biogeography were assessed for this data set in concert with earlier published data on basaltic lavas in the Pacific Ocean (Santelli *et al*., 2008), shallow permanently cold sediment of the Arctic Ocean (Ravenschlag *et al*., 1999) and Antarctic continental shelf sediment (Bowman and McCuaig, 2003).

Materials and methods

16S ribosomal RNA gene clone libraries construction
Sediment sampling was performed on the DIVA II cruise by a multicorer (Barnett *et al*., 1984) in water depths ranging from 5032 to 5649 m. The sediment cores were sliced on board in layers of 2 cm and the layers were subsampled top-to-bottom by sterile 1- to 2-ml syringes at 4 °C. After storage at −80 °C, DNA was extracted from 0.5 g of the surface sediment sample (0–2 cm) of the Cap, Angola and Guinea I areas (Figure 1, Table 2) after the protocol of the FastDNA SPIN Kit for Soil (Q-BIOgene, Carlsbad, CA, USA). Bacterial 16S rRNA genes were amplified using the primer pair GM3/GM4 (Muyzer *et al*., 1995). The 100-μl reaction contained 30 ng DNA as template, 0.5 μM of each primer, 10 mM of dNTPs, 1 × buffer (Eppendorf, Hamburg, Germany) and 5 U of the Takara-Taq DNA polymerase (TAKARA, Dalian, China). PCRs were performed in 10 replicates with 20 cycles to minimize PCR bias. Final extension was performed 60 min at 60 °C to increase 3′-A-overhang. The amplicons were pooled and purified with a PCR purification kit (Qiagen, Hilden, Germany). Cloning of the amplicons was performed using TOPO TA Cloning Kit for sequencing (pCR4-TOPO, Invitrogen, Karlsruhe, Germany). Clones with a correct insert size of ∼1500 bp were

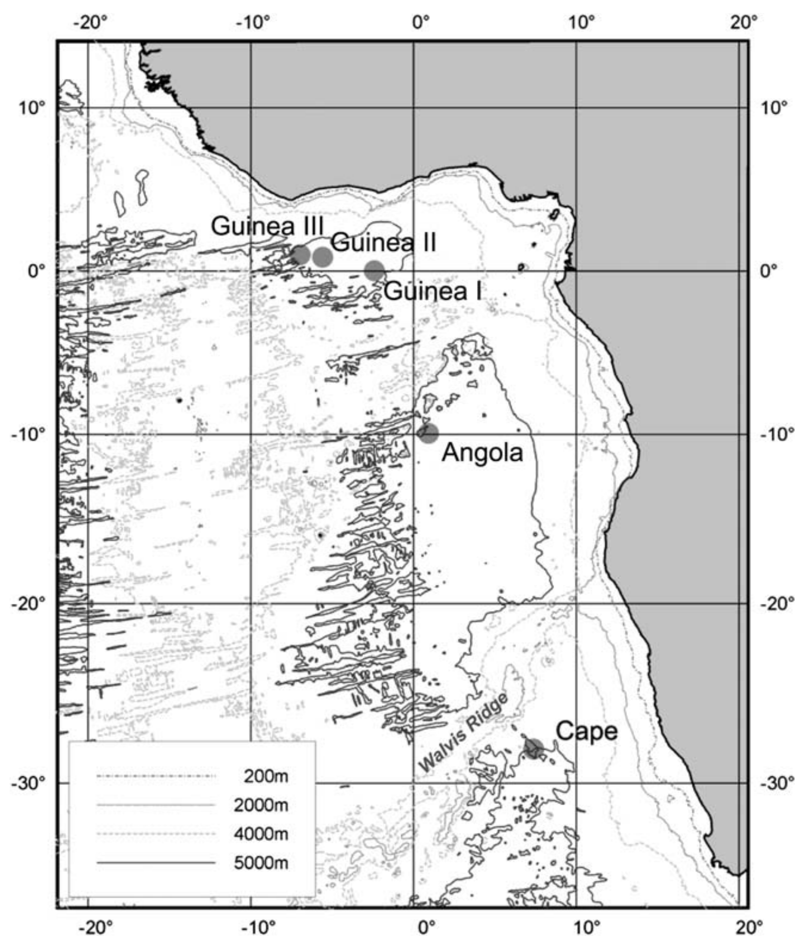

Figure 1 Sampling areas in the South Atlantic Ocean as well as the Walvis Ridge that separates the Cape Basin from the Angola and Guinea basins. For the 16S ribosomal RNA (rRNA) gene approach surface sediment (0–2 cm) of the Cape, Angola and Guinea I areas were used and for the terminal restriction fragment length polymorphism (T-RFLP) analysis 3–5 surface sediments of the Cape, Angola and Guinea I–III areas were analyzed.

sequenced using the vector primers M13 F (5′-GGAA ACAGCTATGACCATG-3′) and M13 R (5′-GTTGTAA AACGACGGCCAGT-3′).

Phylogenetic and sequence analyses
The quality of the obtained sequences was manually checked using Sequence Analysis 5.2 (Applied Biosystems, Weiterstadt, Germany). Full-length sequences were assembled with Sequencher (Gene Code, Ann Arbor, MI, USA). No chimeras were detected with Bellerophon (Huber *et al*., 2004) and CHECK_CHIMERA (Maidak *et al*., 1996). Sequences were imported into the ARB software package (Ludwig *et al*., 2004) and aligned using the ARB FastAligner, then refined manually. The ARB software package was used to generate phylogenetic trees of 810 full-length sequences using the maximum likelihood algorithm with a 50% positional conservation filter and with 100 bootstrap replicates. Sequences reported in this study were deposited at EMBL under the accession numbers AM997284–AM997988 for 705 full-length sequences and under AM997989–998333 and AM997283 for 346 partial sequences.

The software distance-based OTU and richness (DOTUR) was applied to ARB distance matrices generated with the Jukes-Cantor correction to estimate operational taxonomic units (OTU), rarefaction curves of observed OTUs, richness estimators and diversity indices (Schloss and Handelsman, 2005). A sequence identity of 98% was used to define OTUs, as this cut-off roughly corresponds to the species level (Rossello-Mora and Amann, 2001; Stackebrandt and Ebers, 2006). The statistical tool ∫-LIBSHUFF was applied to genetic distance matrices to determine whether differences in library composition were because of chance or to biological effects, and significances were assessed by Monte Carlo permutations and further corrected for multiple comparisons (Schloss *et al*., 2004). The statistical tool SONS (Schloss and Handelsman, 2006) was used on full-length 16S rRNA gene sequences to calculate Chao1 shared richness estimates, the J_{class} index for the ratio of shared to total number of OTUs, and θ_{yc} for the estimated similarity in community structure between any two communities.

Terminal restriction fragment length polymorphism
Terminal restriction fragment length polymorphism analyses included three to five samples of surface sediments (0–2 cm) from several cores of each area, Cape, Angola and Guinea I–III (Figure 1, Table 2). Genomic DNA was extracted from 0.5 g sediment samples using the FastDNA Spin Kit for Soil (Q-Biogene, Irvine, CA, USA). PCR amplification of the 16S rRNA gene was carried out using the fluorescently labelled primers 27F (FAM, 5′-AGAGTTTGA TCCTGGCTCAG-3′) and 907R (HEX, 5′-CCGTCAAT TCCTTTRAGTTT-3′), targeting all bacteria as well as 558F (FAM, 5′-ATTGGGTTTAAAGGGTCCG-3′) (Abell and Bowman, 2005a, b) and 1390R (HEX, 5′-GACGGGCGGTGTGTACAA-3′) (Zheng *et al*., 1996), targeting the class *Flavobacteria*. Undigested and digested amplicons were identified by capillary electrophoresis to verify the absence of false-positive fragments in the undigested control and the completeness of the digestion. PCRs were carried out in a total volume of 25 μl, including 12.5 μl PCR Master Mix (Promega GmbH, Mannheim, Germany), 1 μM forward and reverse primer, and 5–24 ng DNA template. PCR reactions were carried out in triplicates and purified on Sephadex columns (Sephadex G-50 Superfine, Amersham Biosciences AB, Uppsala, Sweden). PCR amplicons (70-120 ng) were digested in a total volume of 10 μl at 37 °C for 3 h using 5 U of the restriction enzyme *AluI* (Fermentas, Burlington, Canada) for bacterial amplicons and 5 U of the enzyme *MspI* (Fermentas) for *Flavobacteria* amplicons. The two restriction enzymes were chosen based on high numbers of unique terminal restriction fragments assessed with *in silico* analyses using enzyme restriction power analysis (http://mica.ibest.uidaho.edu/) as well as on best performance in laboratory experiments (that is, producing maximum numbers of terminal restriction fragments (TRFs)). After heat inactivation (65 °C, 25 min) and purification on Sephadex columns, detection of TRFs was performed on a ABI Prism 3130 XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) equipped with a 80-cm capillary, a POP-7 polymer and the filter set DS-30. The ROX-labelled MapMarker 1000 (Eurogentec, Seraing, Belgium) served as a size standard between 50 and 1000 bp. The fragment profiles were visualized and automatically analyzed with GeneMapper v. 3.7 Software (Applied Biosystems), using standardized settings with a peak detection cut-off set to 30 fluorescence units. The 5′-end labelled TRFs were used as they produced a higher number of fragments in comparison with 3′-end TRFs (Suzuki *et al*., 1998; Osborn *et al*., 2000).

A binning procedure was applied to the GeneMapper output to compensate for slight peak shifts between runs and for TRF size calling imprecision, in order to avoid artificial, technically derived differences between profiles (Hewson and Fuhrman, 2006). The technical variability of peak size calling in different replicates including runs conducted on different days was determined as of ± 0.25 bp (window size of 0.5 bp). The binning function included two different starting points (50 and 50.25 bp) and the binning strategy yielding higher correlation between all samples was selected for further statistical analyses. The binning window was adjusted to 1 bp for samples amplified with *Flavobacteria* primers, because a window frame of 0.5 bp did not yield higher resolution. The computation was carried out with the Interactive Binner function (Ramette, 2009 http://www.ecology-research.com). The output consisted of a table of TRFs with

corresponding relative fluorescence intensities, which are the individual peak area divided by the total area of peaks in a given profile. Master profiles were generated by building a consensus table of the binned TRF profiles for all samples from one basin, averaging the respective relative fluorescence intensities values of all samples. A TRF was considered present if it appeared in one or more PCR parallels, therefore including all natural and technical variability at this level of analysis.

Statistical analyses
Non-metric multidimensional scaling (nMDS) and analysis of similarity (ANOSIM) were carried out with the program PAST (Paleontological Statistics, ver. 1.47, http://folk.uio.no/ohammer/past). Simple and partial Mantel tests were used to determine the significance and correlation coefficients between genetic-, spatial- and environmental distance matrices, using the R package vegan (http://vegan.r-forge.r-project.org/) (Legendre and Legendre, 1998; Mantel, 1967). Spatial dissimilarities based on geographic distances between sites and environmental dissimilarities (temperature, salinity, pH, Eh, TOC, Chl *a* and grain size; Table 1) were used to explain genetic dissimilarity. To determine the strength of the relationship between genetic and geographic distance linear models were fitted and slope coefficients were calculated with their 95% confidence intervals.

Results and discussion

Bacterial biomass and richness in sediments of the South Atlantic Ocean
The cell numbers of the suboxic surface sediments (0–2 cm) in three eastern South Atlantic Ocean basins were $3.4–3.7 \times 10^9$ cells g^{-1} sediment (Table 1). The abundances were in the range found in other deep-sea sediments (9.2×10^8 cells g^{-1} (Deming and Colwell, 1982), 1.5×10^9 cells g^{-1} (Guezennec and Fiala-Medioni, 1996) and 5×10^8 cells g^{-1} (Harvey *et al*., 1984)). The 16S rRNA gene libraries showed a high diversity with up to 20 different phyla in the Cape Basin and 17 phyla in the Angola and Guinea basins (Figure 1). Earlier described deep-sea (Bowman and McCuaig, 2003; Polymenakou *et al*., 2005, 2009; Xu *et al*., 2005) and shallow sediments (Ravenschlag *et al*., 1999) had also found a large diversity, which may be based on a weak and symmetric competition (Grant, 2000). The reciprocal Simpson's indices for all sites were above 50 (Table 2), suggesting evenly distributed diversity profiles as typical dominance profiles show $1/D$ values below approximately 50 (Zhou *et al*., 2002). Total richness estimates (Chao1) (Table 2) and rarefaction curves (Supplementary Figure S1) based on a 98% sequence identity showed that Cape, Angola and Guinea basin surface sediments contained an equal bacterial richness at a significance level of 0.05.

Both analyses predicted a lower richness for the South Atlantic sediments in comparison to the Antarctic sediments and a higher richness in comparison to the Arctic sediment. The library-based equality of richness was supported by the T-RFLP analysis, as basin-specific master profiles showed a comparable OTU richness (167, 190 and 182 TRFs for the Cape, Angola and Guinea Basin, respectively) (Figure 4a).

Bacterial diversity of the 16S ribosomal RNA genes
The clone libraries contained 521 phylotypes with 98% identity in 1051 sequences, containing 705 full-length sequences. Applying a 100% identity threshold revealed 230 sequences, which were present at least twice, with a majority of 176 sequences (18 OTUs) present in all deep-sea sediments. The bacterial communities were dominated by *Proteobacteria*, which accounted for 64, 58 and 63% of all sequences in the Cape, Angola and Guinea Basin, respectively, with the class *Gammaproteobacteria* representing 45, 37 and 40% of all sequences in the respective basins (Figure 2). The class *Gammaproteobacteria* comprised 116 phylotypes (98% identity, 427 sequences), of which 39 phylotypes (138 sequences) were related to known cultivated species. These belonged mainly to families of psychrophilic microorganisms including *Enterobacteriaceae, Alteromonadaceae, Oceanospirillaceae* and *Legionellaceae* (Figure 3a). Among

Table 1 Sediment data (Türkey and Kröncke, in preparation) and cell numbers of microbial communities in the South Atlantic Ocean

Basin	*Depth (mbsl)*[a]	*Temp. (°C)*	*Salinity (‰)*	*pH*	*Eh (mV)*	*TOC (%)*	*Chl a (µg g⁻¹)*	*Grain size (%)*		*Cell counts (cell g⁻¹)*	*MPN (cells ml⁻¹)*
								<63µm	*>63 µm*		
Cape	5032	1.14	34.6	7.74	177	0.83	0.017	92.89	6.87	3.5×10^9	1.22×10^4
Angola	5649	ND	ND	7.72	96	0.9	0.069	83.84	16.4	3.4×10^9	2.67×10^5
Guinea I	5063	2.1	34.9	7.77	183	0.72	0.264	84.23	15.23	3.7×10^9	2.67×10^4
Guinea II	5225	ND	ND	7.76	132	0.77	0.301	84.99	14.46	ND	ND
Guinea III	5525	2.1	34.5	ND	ND	0.76	0.152	86.45	13.34	ND	ND

Abbreviation: ND, not detected.
[a]Meters below sea level.

Table 2 Sampling sites of sediments used for 16S rRNA gene sequencing or for T-RFLP analysis with corresponding richness and diversity indices for bacteria

Sampling area	*Latitude*	*Longitude*	*Depth (mbsl)*[a]	*Stations*[b] *T-RFLP*	*Stations*[b] *16S rRNA gene seq.*	*No. of clones*[c]	*OTU DOTUR 0.02*	*Richness estimator Chao1*[d]	*Simpson 1/D*	*Study*
Cape	28°06′42″ S	7° 20′48″ E	5032	33, 34, 37, 38	33	342 FP	202	466 (369, 620)	77	This study
						228 F	**145**	**508 (351, 785)**	**53**	
Angola	9°56′00″ S	0° 53′48″ E	5649	46, 48, 50	46	354 FP	183	256 (227, 305)	77	This study
						219 F	**126**	**369 (259, 570)**	**59**	
Guinea I	0°00′00″ S	2°25′06″ W	5063	56, 58, 59, 60, 61	60	355 FP	203	369 (308, 465)	125	This study
						258 F	**155**	**489 (348, 735)**	**91**	
Guinea II	0°50′00″ N	5° 35′00″ W	5225	74, 75, 76, 77, 79	—	—				This study
Guinea III	0°37′12″ N	6° 28′06″ W	5525	95, 97, 99	—	—				This study
Antarctic continental shelf	66°31′86″ S	143°38′30″ E	761	—	MERTZ 0–2 cm	590 P	322	899 (713, 1175)	125	Bowman and McCuaig, 2003
Arctic ocean Svalbard	79°42′81″ N	11°05′18″ E	218	—	Station J	123 P	84	125 (104, 167)	167	Ravenschlag *et al*., 1999
East Pacific	9°28′48″ N	104°13′48″ W	2516	—	EPR	**370 F**	**239**	**601 (475, 796)**	**200**	Santelli *et al*., 2008
Rise	−9°50′38″ N	−104°17′86″ W	−2674							
Hawaii	18°52′17″ N	155°14′53″ W	888	—	PV	**472 F**	**276**	**764 (597, 1017)**	**167**	Santelli *et al*., 2008
	−18°58′31″ N	−155°53′42″ W	−1714							

Abbreviations: DOTUR, distance-based OTU and richness; OTU, operational taxonomic units; rRNA, ribosomal RNA; T-RFLP, terminal restriction fragment length polymorphism.
[a]Meters below sea level.
[b]For details see cruise report DIVA II (M63/2).
[c]Number of full-length (F) and partial (P) sequences, full-length sequences and values calculated from them are presented in bold.
[d]Chao1 richness with lower and upper bound of 95% confidence interval.

these phylotypes 11 OTUs (12 sequences) clustered with the NOR5/OM60 clade that includes ‘*Congregibacter litoralis*’ strain KT71, the first marine aerobic anoxygenic phototrophic *Gammaproteobacteria* in culture (Fuchs *et al*., 2007; Yan *et al*., 2009). Three phylotypes (5 sequences) were related to free living (*Thiothrix*) and endosymbiotic sulfur oxi‘dizers and methylotrophic bacteria. A large portion of 77 phylotypes (289 sequences) clustered distinctly from cultured species to JTB255/BD3-6 (38 phylotypes, 192 sequences), BD7-8/MERTZ (10 OTUs, 36 sequences), JTB23/Sva0091 (18 OTUs, 34 sequences) (Figures 3a and b) and to Cret-1F, BD1-1, PWP and South Ionian groups (11 OUT, 27 sequences). These groups included only 16S rRNA gene sequences that originated from other deep-sea or permanent cold marine habitats (Kato *et al*., 1999; Li *et al*., 1999; Ravenschlag *et al*., 1999; Urakawa *et al*., 1999; Bowman and McCuaig, 2003; Polymenakou *et al*., 2005; Xu *et al*., 2005; Zhao and Zeng, 2005).

The *Alpha*-, *Beta*- and *Deltaproteobacteria* accounted together for 18 to 23% of all sequences in the libraries. *Deltaproteobacteria* (11 to 14%) outnumbered *Alphaproteobacteria* (6 to 8%) and *Betaproteobacteria* (1 to 3%) (Figure 2). Other groups with a sequence abundance of over 5%, which occurred in all three basins, were the phyla *Chloroflexi* (1, 10 and 4% for Cape, Angola and Guinea basins, respectively), *Planctomycetes* (6, 4 and 10%), *Acidobacteria* (4, 7 and 5%) and *Bacteroidetes* (10, 4 and 6%).

Bacterial diversity comparison

The proportion of bacteria present in two or three basins was high in the 16S rRNA gene sequences analyses (23%) and in the T-RFLP analyses (58%) (Figure 4a). A third of the fragments (93 TRFs) was detected in the sediments of all basins and represented 82% of the total relative fluorescence intensities. Among the 16S rRNA gene sequences, a shared membership of 19 OTUs (98% identity) was found in all three basins with the statistical tool SONS. The manual assignment in ARB confirmed the small fraction of OTUs detected in all three basins (29 OTUs, 347 sequences), but provided additional information regarding the sequence abundance and identity of each OTU. These were dominated by *Gammaproteobacteria* (76%; Figure 4b). In this class, the common members were related to marine heterotrophic aerobic and facultative anaerobic microorganisms (*Alteromonadaceae* and *Oceanospirillaceae*), photoheterotrophic aerobic bacteria (NOR5/OM60 clade) (Fuchs *et al*., 2007), and to groups consisting of uncultivated bacteria

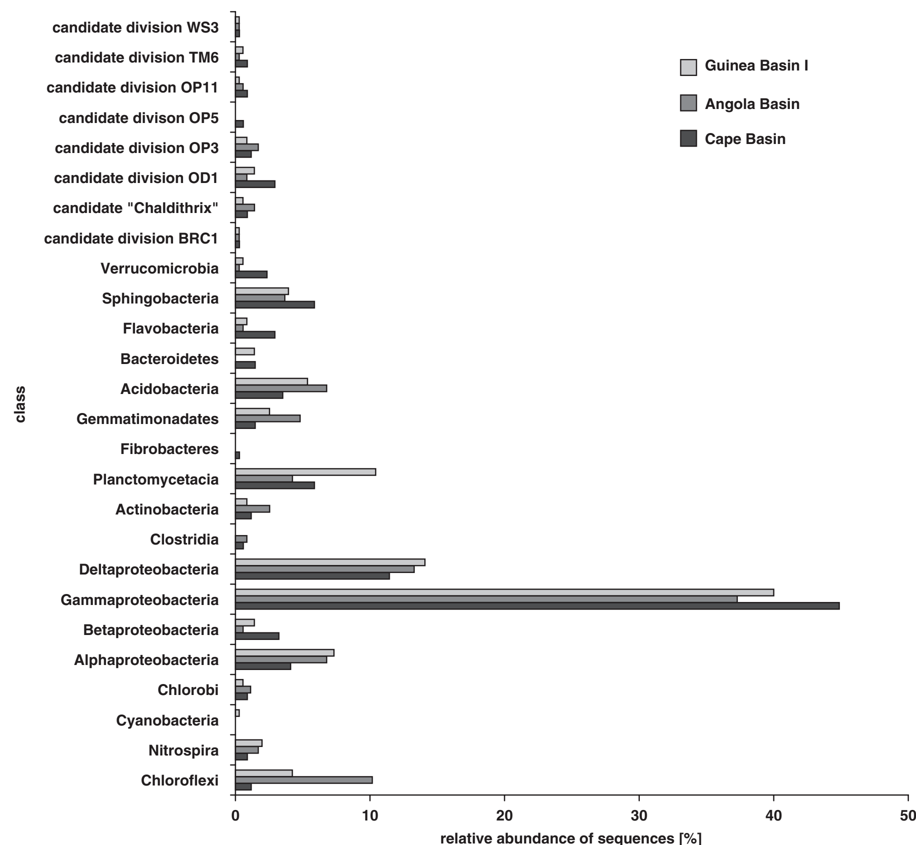

Figure 2 Bacterial diversity in the Cape Basin (342 sequences), Angola Basin (354 sequences) and Guinea Basin (355 sequences). All detected classes in the domain *Bacteria* are shown.

(JTB255/BD3-6, JTB23/Sva0091/BD3-1, BD7-8/MERTZ, Gret-1F and South Ionian).

Phylotypes present in two of three basins belonged to the *Gamma*- and *Deltaproteobacteria* and to the *Chloroflexi*. A major group of *Chloroflexi*-OTUs were restricted to Angola and Guinea basin sediments (7 OTUs, 21 sequences). The $\int$-LIBSHUFF analyses revealed no significant difference between the Angola and Guinea libraries as well as Cape and Guinea libraries (using a minimum *P*-value of 0.0012) (Supplementary Table S1). Thus, common phylotypes dominate the communities of these basins. The largest number of TRFs covered by two basins was found for the Angola and Guinea basins (30 TRFs, 30.5 relative fluorescence intensity) (Figure 4a). High chlorophyll *a* contents were detected in the Angola and Guinea surface sediments indicating a large fraction of fresh, recently arrived organic carbon (Table 1, Türkay and Kröncke, in preparation). This probably originated from a primary productivity in the surface waters that can be linked to the discharge of nutrients from the Congo and the Niger Rivers into the Angola and Guinea basins, respectively (Schefuss *et al*., 2004).

Angola and Cape basins showed significantly different communities ($\int$-LIBSHUFF test, $P = 0.008$) and significantly different *Flavobacteria* T-RFLP profiles (Figure 5b) (analysis of similarity, *R* values 0.869, $P < 0.001$) (Supplementary Table S2). These differences were consistent with a different chlorophyll *a* content as well as a different sediment particle size in the Cape Basin (Table 1) (Etter and Grassle, 1992), indicating that environmental factors seem to influence bacterial communities in deep-sea sediments of the eastern South Atlantic Ocean. It is, however, needed to also take spatial parameters into account in this analysis to strengthen our interpretation concerning environmental or spatial effects on the observed community shifts.

Biogeography: environmental and historical factors

In the eastern South Atlantic Ocean the Walvis Ridge separates the Cape Basin from the Angola and Guinea basins below a depth of about 3000 m and causes different deep water masses in these basins. The dominance of common phylotypes in the 16S rRNA gene libraries and T-RFLP master profiles suggested that microbial dispersal may not be influenced by the Walvis Ridge or by the presence of different water masses. This was

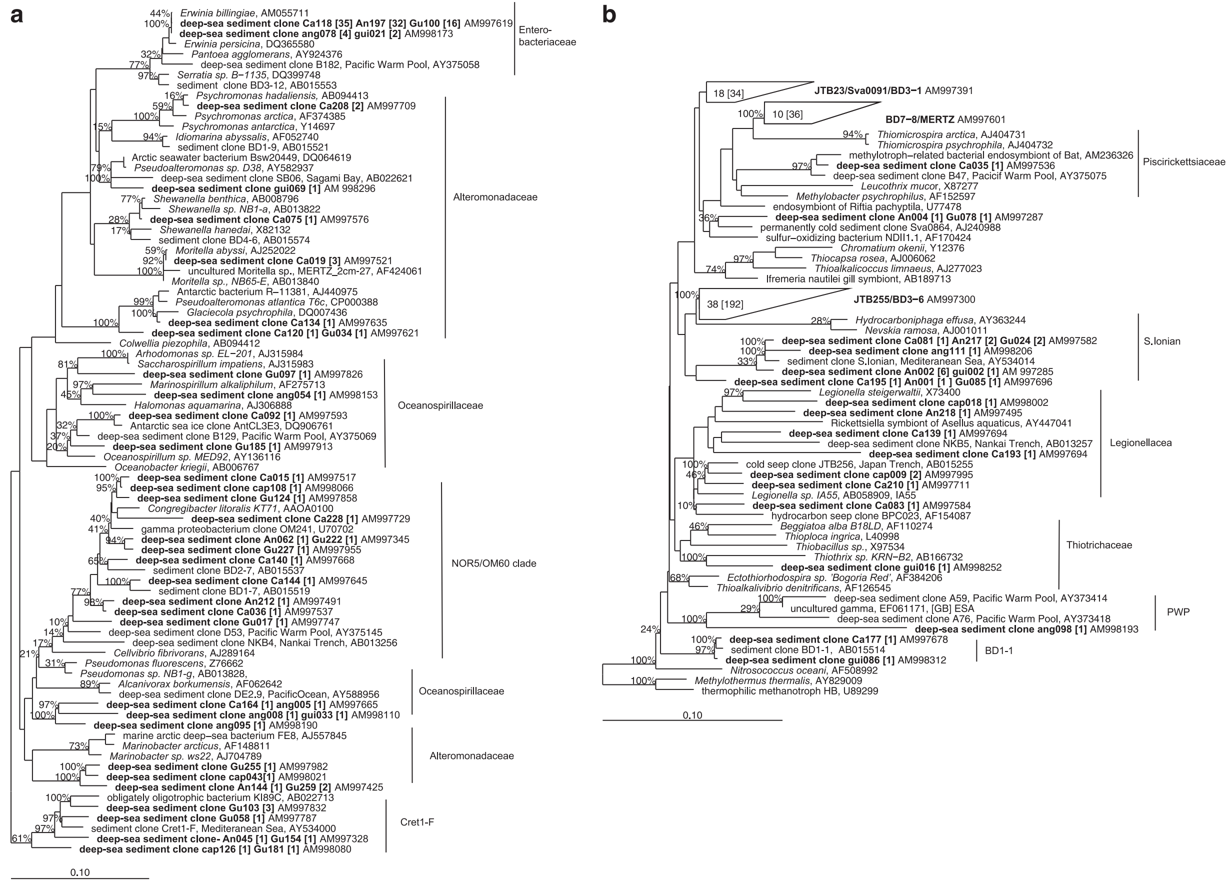

Figure 3 Phylogenetic tree based on 16S ribosomal RNA (rRNA) gene sequences of the class *Gammaproteobacteria* showing position of (**a**) marine heterotrophic aerobic and facultative anaerobic microorganisms and photoheterotrophic aerobic (NOR5/OM60 clade) bacteria and (**b**) potential auto- or mixotrophic sulfur oxidizers and bacteria that inhabit various geographic regions (JTB255/BD3-6). The tree was calculated using the maximum-likelihood algorithm with a 50% positional conservation filter and with 100 bootstrap replicates. The bar represents 10% estimated sequence divergence. Full-length sequences (Ca, An and Gu), partial sequences (cap, ang and gui), the number of OTUs in a cluster and the corresponding number of sequences (squared bracket) are shown.

supported by the significantly similar distance–decay relationships of the TRFs in the pairwise comparison (Cape/Angola, slope coefficient 6.9×10^{-5} and 95% confidence interval (3.4×10^{-5}, 10.3×10^{-5}); Angola/Guinea, slope coefficient 8.7×10^{-5} and 95% confidence interval (1.5×10^{-5}, 15.9×10^{-5})). Phylotypes common in the communities of the South Atlantic Ocean and the Pacific, Antarctic and Arctic Oceans sediments (Supplementary Table S1) indicated that some microorganisms disperse effectively over a huge distance and therefore are cosmopolitan, at least at the resolution of 16S rRNA genes that is insufficient for the classification of microorganisms into species (Konstantinidis and Tiedje, 2005).

To get more information regarding the amount of spatial structure present, we analyzed the relative relationships between genetic diversity and geographic distances. The 16S rRNA gene and TRFs based distance–decay relationships for the South Atlantic Ocean and for all sites were all very low (0.003 to 0.07) (Table 3), as also found in taxa-area relationships for soil and salt marsh communities (0.03 to 0.074) (Green *et al.*, 2004; Horner-Devine *et al.*, 2004; Fierer and Jackson, 2006), suggesting high dispersal rates and low extinction rates because of vast population sizes (Connor and McCoy, 1979).

From the clustering of TRF profiles by basins, as shown by non-metric multidimensional scaling (Figure 5a) associated with large, significant R values for all pairwise comparisons between the deep-sea basins (analysis of similarity, 0.586 to 0.999, $P < 0.001$) (Supplementary Table S2), and from significant differences between the South Atlantic Ocean communities to all other communities ($\int$-LIBSHUFF tests, Supplementary Table S1), it seemed obvious that communities were structured either by the contemporary environment, spatial distances (historical events) or by a combination of both (Martiny *et al.*, 2006; Ramette and Tiedje 2007).

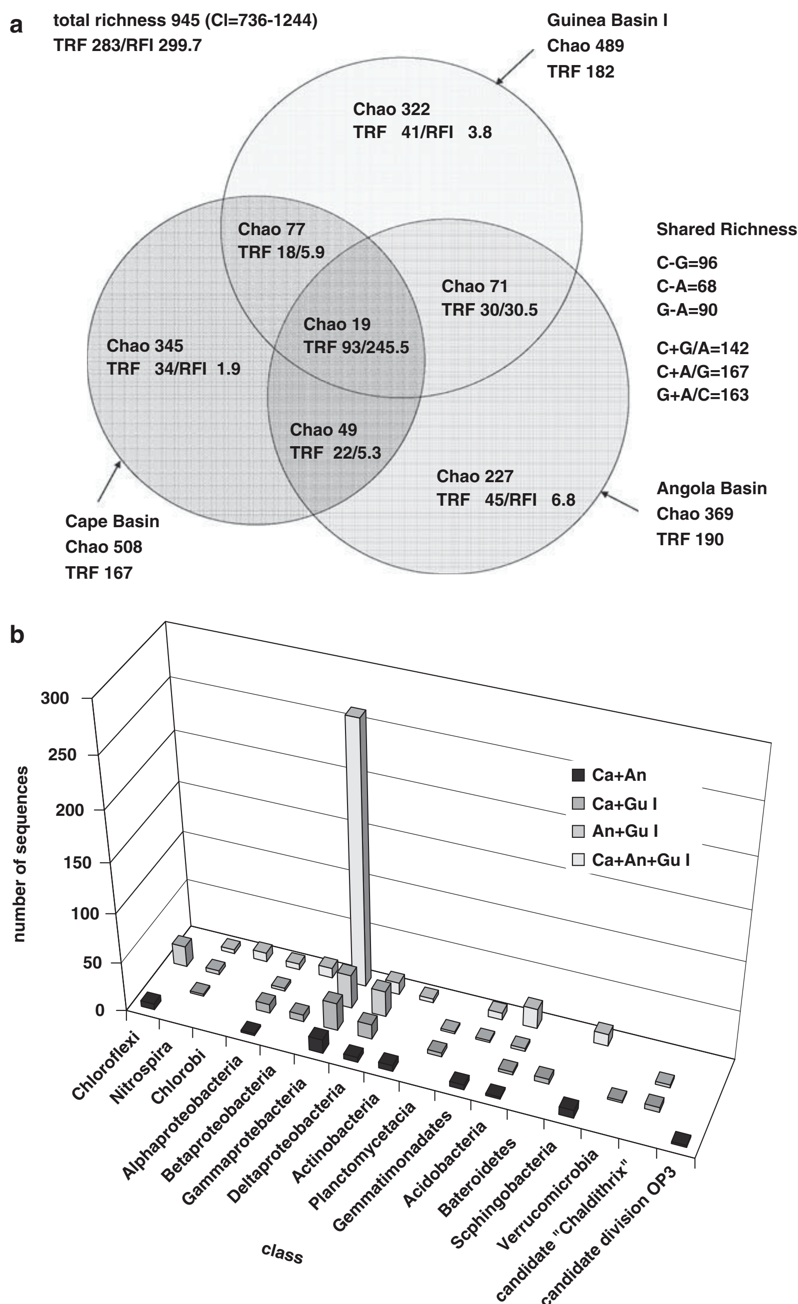

Figure 4 (**a**) Shared and basin-specific SONS generated $OTU_{0.02}$ and terminal restriction fragment (TRF) and corresponding relative fluorescence intensities (RFI) values for the three deep-sea basin of the South Atlantic Ocean. (**b**) Phylogeny of the shared OTUs between three and two deep-sea basins and the corresponding number of sequences.

To disentangle the relative influence of environmental heterogeneity and spatial distance on the distribution of microbial deep-sea sediment communities, we used a combination of simple and partial Mantel tests. For distances of 0–1200 km T-RFLP results showed a comparable influence of both factors (environment $r = 0.636$, $P < 0.001$, geography $r = 0.651$, $P < 0.001$) (Table 2) (Figure 6b). But environment ($r = 0.588$, $P < 0.001$) overwhelmed any effect of geographic factors ($r = 0.278$, $P = 0.009$) for intermediate distances (1200–3500 km), as also supported by significant partial Mantel tests (Table 3). A higher correlation between spatial and genetic distance for small spatial scales (<200 m) was reported for other microbial groups in soil (Cho and Tiedje, 2000), suggesting the existence of

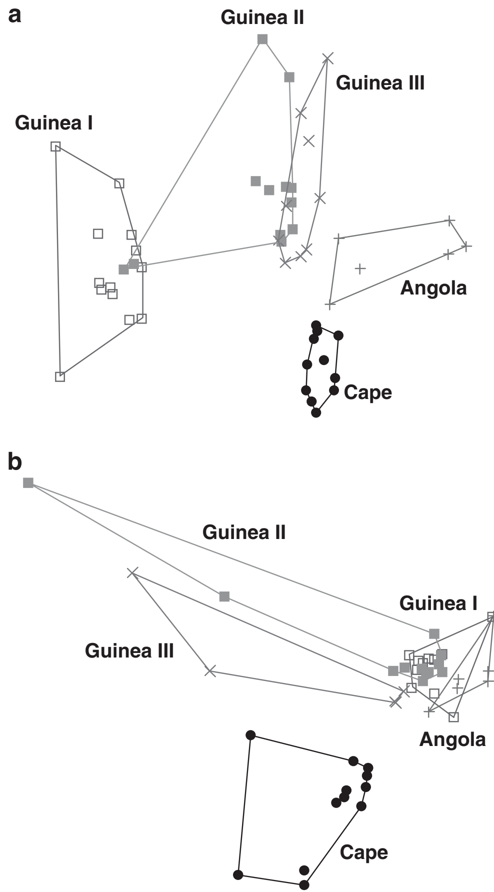

Figure 5 (**a**) Non-metric multidimensional scaling (nMDS) plot using Bray–Curtis distance for the data set derived from amplification with bacterial primers 27F and 907R and subsequent digestion with *AluI*. Stress: 0.15. (**b**) nMDS plot using Bray–Curtis distance for the data set derived from amplification with *Flavobacteria*-specific primers 517F and 1457R and digestion with *MspI*. Stress: 0.07.

endemic taxa, as genetic distance increases with spatial distances.

Mantel tests for 16S rRNA gene sequences revealed that both environment and geography ($r = 0.008$, $P = 0.006$ and $r = 0.024$, $P = 0.001$, respectively) had an influence on the bacterial diversity of the South Atlantic. Significant correlations between genetic and geographic distances (Mantel's coefficient $r = 0.013$, $P = 0.001$) (Table 3, Figure 6a) were as well observed for all deep-sea and coastal sediments.

When compared in more detail, the two methods showed different results for the relative influence of both factors on microbial biogeography. These detected differences reflect different levels of similarity, saturation and resolution of each method and sampling effort, for example, the T-RFLP analyses including Guinea I versus Guinea I+II+III (Table 3). Indeed, the analysis of 16S rRNA gene sequences

Table 3 Slope coefficients and Mantel *r* statistics for genetic distance matrices derived from 16S rRNA gene sequences or T-RFLP

	Slope coefficient (genetic versus geographic distance)		*Simple Mantel tests*			*Partial Mantel tests*		*Number of samples*
	$\times 10^{-5}$	*Log_{10} transformed*	*Geography*[a]		*Environment*	*Geography*	*Environment*	
			Untransformed	*Log_{10} transformed*				
South Atlantic, 0–1200 km distance								
T-RFLP	28.28 [21.34, 35.22][b]	0.074 [0.053, 0.095]	0.651 (<0.001)*	0.593 (<0.001)*	0.657 (<0.001)*	0.054 (0.306)	0.131 (0.134)	14
South Atlantic, 0–3500 km distance								
16S rRNA gene	0.19 [0.17, 0.22]	0.0036 [0.0028, 0.0044]	0.024 (0.001)*	0.012 (0.001)*	0.008 (0.006)*	0.031 (0.002)*	−0.021 (0.981)	3
T-RFLP (Guinea I)	7.5 [5.38, 9.62]	0.076 [0.063, 0.090]	0.698 (0.004)*	0.841 (0.001)*	0.886 (0.001)*	−0.138 (0.86)	0.768 (<0.001)*	11
T-RFLP (Guinea I+II+III)	2.6 [1.24, 3.96]	0.055 [0.042, 0.068]	0.278 (0.009)*	0.532 (0.001)*	0.599 (<0.001)*	−0.227 (0.992)	0.573 (<0.001)*	19
All sites, 0–18 000 km distance								
16S rRNA gene	0.02 [0.019, 0.022]	0.0030 [0.0027, 0.0032]	0.013 (0.001)*	0.013 (0.001)*	NA	NA	NA	7

Abbreviations: NA, no environmental parameters available for all comparisons; rRNA, ribosomal RNA; T-RFLP, terminal restriction fragment length polymorphism.
For simple and partial Mantel tests, *P*-values based on 1000 permutations are indicated in parentheses with * when the *P*-values are still significant after Bonferroni correction for multiple comparisons.
[a]For simple Mantel tests, geographic distances were either left untransformed or log10 transformed to allow for comparisons with previously published studies.
[b]Lower and upper bounds of 95% confidence interval for the slope coefficients are indicated within square brackets.

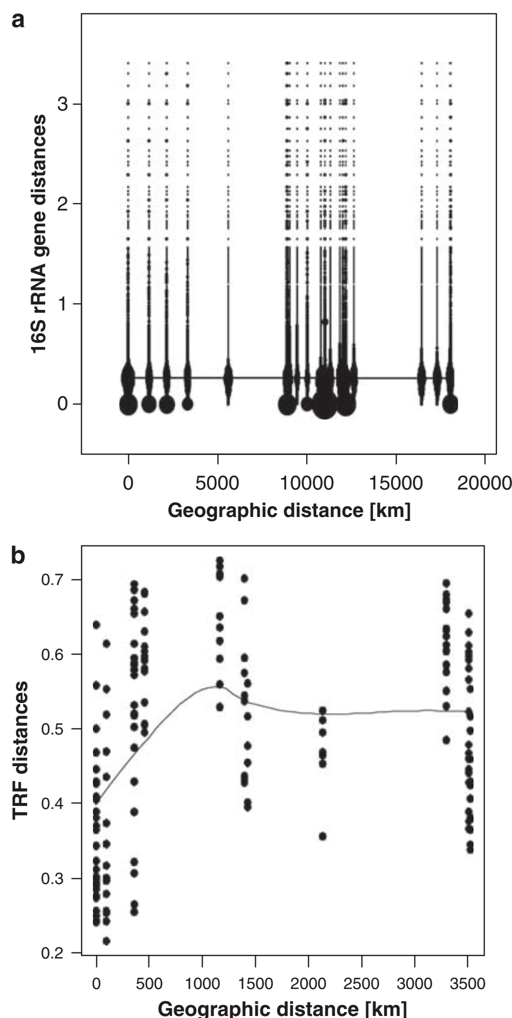

Figure 6 Scatterplots with loess curve presenting (**a**) genetic dissimilarity plotted against geographic distance for genetic dissimilarity of distance matrices derived from 16S ribosomal RNA (rRNA) gene sequences of samples of the South Atlantic and (**b**) for terminal restriction fragment length polymorphism (T-RFLP) profiles of samples from five different stations in the South Atlantic (Cape, Angola, Guinea I, Guinea II and Guinea III).

provides information regarding randomly chosen phylotypes ('sampling communities') where the finding of an OTU is proportional to its abundance in the clone library (Bent and Forney, 2008). In contrast, the fingerprinting method T-RFLP screens for all OTUs present above the detection threshold of the method ('screening' communities; Bent and Forney 2008), typically $>6\times10^2$–10^3 DNA fragment copies per ml samples (Ramette 2009), but does not provide clear taxonomic distinction (Dunbar *et al*., 2001).

Although high dispersal rates were detected for some groups in deep-sea sediments, both T-RFLP and 16S rRNA-based analyses suggest barriers for the dispersal of microorganisms in the deep sea. The influence of both factors at intermediate scales was already shown by other studies (Green *et al*., 2004; Reche *et al*., 2005; Yannarell and Triplett, 2005), but our study suggest an effect of both factors for large scales as well, as shown for soil microbial communities (Fierer and Jackson, 2006). Although the small size, high dispersal rates, large population size and low extinction rates of microorganisms suggest a low effect of geographic barriers on microorganisms (Staley and Gosink, 1999; Beja *et al*., 2002; Finlay, 2002; Ramette and Tiedje, 2007), our study shows that the distribution of microorganisms in deep-sea sediments is limited at intermediate (10–3000 km) and large scales (>3000 km).

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Supplementary Information accompanies the paper on The ISME Journal website (http://www.nature.com/ismej)

Supplementary Information

Microbial biomass

Samples of 0-2 cm depth were sonicated and cell numbers were determined after 4',6-diamidino-2-phenylindole (DAPI)-staining (1 μg/ml). Counts were performed until 1,000 cells were reached for statistical significance (Pernthaler et al, 2003). Most probable number (MPN) determinations were performed in three dilution series with ten-fold dilutions with artificial sea-water medium (Widdel and Bak, 1992), containing casamino acids, glucose, ribose (each 2 g/l), pyruvate, propionate, fumarate (each 1.2 g/l) and yeast extract (Difco, 0.4 g/l). All tubes were incubated for the first 4 weeks at 4°C and afterwards at 12°C. The number of culturable cells/ml was calculated based on visible cell pellets with standard probability tables (American Public Health Association, 1969).

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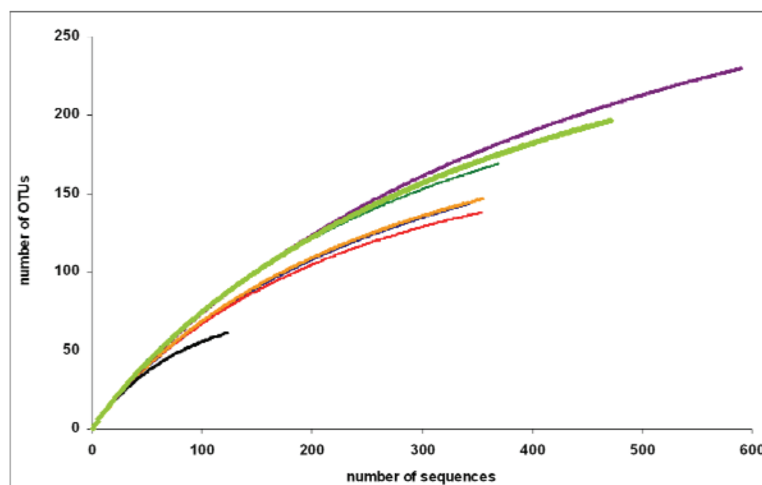

Figure S1: Rarefaction analyses of the relative bacterial richness of different communities compared at a distance of 0.02.

Table S1: Comparison of bacterial community structure by the statistical tools SONS (upper matrix) and ∫-LIBSHUFF (lower matrix).

SONS / ∫-LIBSHUFF	Cape	Angola	Guinea I	Arctic	Antarctic	EPR	Hawaii
Cape		**68**	**96**	**5**	**217**	**42**	**73**
		(0.11/0.57)	(0.14/0.35)	(0.01/0.01)	(0.04/0.02)	(0.04/0.03)	(0.03/0.03)
Angola	0.0008*		**90**	**2**	**46**	**47**	**51**
	0*		(0.16/0.49)	(0.00/0.00)	(0.03/0.02)	(0.03/0.06)	(0.05/0.05)
Guinea I	0.4011	0.0338		**5**	**50**	**53**	**25**
	0.0014	0.0966		(0.01/0.00)	(0.02/0.01)	(0.04/0.04)	(0.04/0.03)
Arctic	0.0602	0.0336	0.0256		**181**	**13**	**5**
	0*	0*	0*		(0.06/0.04)	(0.01/0.01)	(0.01/0.00)
Antarctic	0*	0*	0*	0.4735		**95**	**38**
	0.4116	0*	0*	0.4735		(0.05/0.04)	(0.03/0.02)
EPR	0*	0*	0*	0*	0*		**148**
	0*	0*	0*	0*	0*		(0.1/0.13)
Hawaii	0*	0*	0*	0*	0*	0*	
	0*	0*	0*	0*	0*	0*	

In the upper matrix (SONS analyses) shared $OTU_{0.02}$s are shown in bold numbers and the ratio of shared $OTU_{0.02}$s to the total number of $OTU_{0.02}$s is the first number in parenthesis and the similarity index θ is the second number in
In the lower matrix (∫-LIBSHUFF analyses) significant *P*-level are indicated by an asterisk (*).

Table S2: Analysis of similarity (ANOSIM) of bacterial and Flavobacteria derived T-RFLP profiles.

Parameter R	Angola	Guinea I	Guinea II	Guinea III
Cape	**0.831 (< 0.001)***	**0.999 (<0.001)***	**0.672 (<0.001)***	**0.777 (<0.001)***
	0.869 (<0.001)*	0.841 (<0.001)*	0.595 (<0.001)*	0.534 (0.004)*
Angola		**0.998 (<0.001)***	**0.586 (<0.001)***	**0.716 (<0.001)***
	-	0.415 (0.008)	0.285 (0.027)	0.52 (0.002)*
Guinea I			**0.672 (<0.001)***	**0.976 (<0.001)***
	-	-	0.002 (0.413)	0.569 (0.001)*
Guinea II				**-0.014 (0.49)**
	-	-	-	0.304 (0.05)

P values are indicated in parentheses with * to indicate significant P values following Bonferroni correction for multiple testing. The first (bold) line represents bacterial datasets for each pairwise comparison and the second lines *Flavobacteria* T-RFLP datasets.

Chapter III

The energy-diversity relationship of complex bacterial communities in Arctic deep-sea sediments

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In Review with The ISME Journal

Abstract

The availability of nutrients and energy is a main driver of biodiversity for plant and animal communities in terrestrial and marine ecosystems, but we are only beginning to understand whether and how energy-diversity relationships may be extended to complex natural bacterial communities. Here, we analyzed the link between phytodetritus input, diversity and activity of bacterial communities of the Siberian continental margin (37 to 3427 m water depth). Community structure and functions, such as enzymatic activity, oxygen consumption and carbon remineralization rates, were highly related to each other, and with energy availability. Bacterial richness substantially increased with increasing sediment pigment content, suggesting a positive energy-diversity relationship in such oligotrophic regions. Hump-shaped relationships were indicated when including mesotrophic sites, implying that bacterial communities and other benthic fauna may be structured by similar mechanisms. Dominant bacterial taxa showed strong positive or negative relationships with phytodetritus input and allowed us to identify candidate bioindicator taxa. Contrasting responses of individual taxa to changes in phytodetritus input also suggest varying ecological strategies among bacterial groups along the energy gradient. Our results imply that environmental changes affecting primary productivity and particle export from the surface ocean will also cause shifts in bacterial community structure and function at depth, and that sediment bacterial communities can record shifts in the whole ocean ecosystem functioning.

1. Introduction

The relationship between diversity and bioavailable energy, often measured as photosynthetic productivity, is best described by positive or hump-shaped functions in animal and plant communities (Evans et al., 2005; Mittelbach et al., 2001; Waide et al., 1999). A variety of explanations for this ecological pattern have been proposed, including effects of population size, biomass, competition, evolutionary, environmental or resource heterogeneity (Cardinale et al., 2009; Waide et al., 1999 and references therein). Although microbes dominate most ecosystems in terms of abundance, diversity and biomass (Whitman et al., 1998), we have only recently begun to understand to what degree these relationships may be extended to complex microbial communities (studies reviewed in Smith, 2007). Noticeably, studies have mainly focused on pelagic or simplified ecosystems and came to different conclusions: No relationship of overall richness with productivity could be found in aquatic mesocosms (Horner-Devine et al., 2003), weak positive correlations were shown for global patterns of bacterioplankton diversity (Fuhrman et al., 2008; Pommier et al., 2007), and hump-shaped relationships for genotypes of *Pseudomonas fluorescens* in microcosms (Kassen et al., 2000). Recently, as high-throughput fingerprinting methods became available, the bacterial energy-diversity relationship may now start being addressed in complex aquatic or terrestrial communities (Fuhrman, 2009). Unravelling the relationships between environmental conditions, organism diversity and its links to ecosystem functions remains a priority if we are to better understand effects of global change.

For benthic life, productivity-diversity relationships have been mostly studied along continental slopes, which constitute ideal natural laboratories because of relatively defined variations in energy availability with water depth. Benthic communities in the deep sea depend on the sedimentation of phytodetritus from the productive surface waters, but detritus flux to the seafloor decreases substantially with increasing water depth due to the grazing and remineralization of particles in the water column. As the main source of energy, phytodetritus flux to the deep sea strongly impacts the abundance, biomass and biodiversity of various size classes of benthic organisms (Smith et al., 2008 and references therein). The input of phytodetritus to deep-sea sediments has also been shown to influence bacterial biomass and activity (Boetius and Lochte, 1994; Deming and Yager, 1992; Jorgensen and Boetius, 2007), but studies linking energy availability at the seafloor to bacterial diversity patterns are still rare (Franco et al., 2007; Polymenakou et al., 2005).

Here we tested for the first time bacterial energy-diversity relationships for complex natural bacterial communities in Arctic seafloor on a regional scale, in order to minimize confounding

factors from sampling across different ocean provinces. We have chosen depth transects across the Arctic continental slope, covering a range of phytodetritus fluxes, representing mesotrophic to oligotrophic deep-sea settings. As a common proxy for phytodetritus input to sediments, we used the chlorophyll pigment content of surface sediments (Boetius and Damm, 1998; Dell'Anno et al., 2002; Soltwedel, 2000; Soltwedel et al., 2009). Along this natural energy gradient, we described the shape of the relationships between energy, bacterial activity and bacterial diversity at the community and at different taxonomic levels, and identified bacterial taxa that are most likely affected by changes in energy availability.

2. Materials & Methods

2.1 Study site and contextual parameters

Sediment samples were collected on three transects down the Laptev Sea continental slope during RV Polarstern cruise ARK IX/4 in September 1993 (Boetius and Damm, 1998). The samples analyzed here included 17 stations from the outer Laptev Sea shelf into the deep Eurasian basin (Figure S1). An opening of the ice cover occurred as a temporally and regionally restricted event between June and September for periods of 2-12 weeks at different stations (Fütterer, 1994), leaving the Eastern most transect largely ice free at the time of sampling. Sediment cores were horizontally sliced into 1 cm thick layers and sediment samples from the same stations were used for measuring environmental parameters, potential enzyme activities and DNA extraction. Measurements of chlorophyll pigments, protein concentration and hydrolytic enzyme activities (esterase, lipase, peptidase, beta-glucosidase) have been previously published (Boetius and Damm, 1998). Corresponding phaeopigment concentrations of the same stations were retrieved through the Publishing Network for Geoscientific and Environmental Data" (PANGAEA, doi:10.1594/PANGAEA).

2.2 Community structure analysis

Total community DNA was extracted from 1 g of sediment using UltraClean Soil DNA Isolation Kits (MoBio Laboratories Inc., Carlsbad, CA) and stored in a final volume of 100 µl Tris-EDTA buffer. DNA quantities were spectrophotometrically adjusted with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE) for each step of the molecular protocol.

2.3 Automated ribosomal intergenic spacer analysis (ARISA)

A total of 42 samples composed of three sediment horizons (0-1 cm, 1-2 cm, 4-5 cm) were analyzed by ARISA. DNA quantities were standardized to 10 ng. PCR amplification, separation of fragments by capillary electrophoresis, evaluation of electrophoretic signals, and subsequent binning into OTUs were done as reported elsewhere (Ramette, 2009). An OTU was considered present if it appeared in at least two of the three PCR replicates and fingerprint profiles were standardized by dividing each individual peak area by the total area of peaks in a given profile.

2.4 454 massively parallel tag sequencing

A subset of 10 samples was selected for 454 MPTS. To increase the representativeness of our analyses we combined samples from the upper two sediment layers (0-1 and 1-2 cm), after verifying that no significant differences were found in community structure between these two layers based on ARISA fingerprinting. Pigment concentrations for corresponding samples were averaged accordingly. Extracted DNA was amplified using primers targeting the V6 region of the bacterial 16S rRNA gene and including 454 Life Science's A or B sequencing adapters as published on http://vamps.mbl.edu. Fragments were sequenced by pyrosequencing on a Genome Sequencer FLX system (Roche, Basel, Switzerland) at the Marine Biological Laboratory in Woods Hole, MA, USA. Taxonomic assignments were performed with the Global Alignment for Sequence Taxonomy tool (GAST, Huse et al., 2008; Sogin et al., 2006). Data were normalized to relative sequence abundances within samples, where the number of reads taxonomically assigned was divided by the total number of reads in the sample.

In order to keep analyses over different taxonomic levels consistent, we used a subset of the 454 MPTS dataset for further analysis, in which only sequences with a complete assignment up to genus level were retained. A high Spearman correlation between dissimilarity matrices of the reduced (20% of original) and the original dataset confirmed that ecological patterns were consistent in both datasets (Table S1). To investigate potential differences in the response of rare and common types to changes in phytodetritus input, we first removed all singletons (defined as sequences occurring only once in the dataset), as statistical relationships cannot be tested for these cases. Subsequently we defined subsets of "rare" and "common" types and required that such types appeared in at least four samples so as to obtain enough statistical power to detect individual OTU responses as a function of phytodetritus input.

2.5 Statistical analyses

To identify overall patterns in bacterial beta-diversity, non-metric multidimensional scaling (nMDS) was performed on Bray-Curtis dissimilarity matrices of OTU relative abundance tables. Significant differences of *a posteriori* groupings of samples were assessed with an analysis of similarity (ANOSIM). Mantel tests were used to compare spearman correlations of dissimilarity matrices between different datasets.

To avoid over-determination in modeling the community responses to environmental parameters, forward selection procedures were performed on groups of factors with redundancy analysis (RDA) models. The best fitting models were selected using the Akaike Information Criterion (AIC). Space was modeled by using a polynomial of degree three of the spatial coordinates, from which the terms Y, XY, XY^2, X^3, Y^3 were finally retained after forward selection. Protein, water depth, sediment depth and ice cover were kept as separate categories. The respective effects of various groups of variables on the variation in community composition were investigated by canonical variation partitioning (Legendre and Legendre, 1998; Ramette and Tiedje, 2007). Among different pigment measurements (Boetius and Damm, 1998), phaeopigments explained the highest amount of variation with 4% ($p < 0.001$). Phaeopigments were therefore used to explore the response of the bacterial community to changes in phytodetritus input.

Directed dependencies between the response variables (ARISA diversity and enzymatic activity) and all groups of relevant contextual parameters were displayed in one causal model with path analysis (Legendre and Legendre, 1998). The RV coefficient (Robert and Escoufier, 1976) was used to derive a correlation matrix between groups of variables. Based on our previous statistical analyses an initial model was tested and subsequently improved by comparing the fit of new models to the original matrix using Chi-square tests. Other goodness-of-fit indices (e.g. Bentler Comparative Fit Index, Bayesian Information Criterion) were used to further compare model performance. All statistical analyses were performed in R (v. 2.9.1; R Development Core Team 2009, http://www.R-project.org) using *vegan*, *gmt*, *sem*, and *FactoMine*R packages and custom R scripts.

3. Results

3.1 Relationships between bacterial diversity and function with increasing energy availability

Changes in bacterial alpha-diversity (richness) and beta-diversity (changes in community structure between sites) were strongly related to changes in pigment concentrations (Figure 1a-f): OTU (operational taxonomic unit as defined by ARISA, based on fingerprinting of the intergenic region of the ribosomal genes) richness and pigment concentrations showed a strong positive, linear relationship until pigment concentrations of about 2 $\mu g/cm^3$ sediment were reached, changing into a hump-shaped relationship with a good quadratic model fit for the range of pigment concentrations between 0 and 3 $\mu g/cm^3$ (Figure 1a). Patterns of bacterial community structure also showed high correlations with pigment concentrations ($r = 0.52$, $p = 0.001$, Figure 1d) and lower, yet significant correlations with spatial distance and water depth ($r = 0.24$, $p < 0.001$ and 0.14, $p = 0.011$, respectively) (Figure S2 a, b). When 454 MPTS (pyrosequencing of the variable V6 region of the 16S rRNA gene) was applied to a subset of samples to further explore the response of bacterial taxa to this range of energy availability, a similar linear relationship was found, appearing to level off at higher pigment concentrations (>3 $\mu g/cm^3$) (Figure 1 c). The two molecular techniques, ARISA and 454 MPTS, revealed similar ecological patterns. Additional statistical tests further validated the technical concordance (Table S1; SI text) and the usefulness of combining the two techniques to ecologically interpret the data.

Hydrolytic enzyme activities (esterase, lipase, peptidase, beta-glucosidase) showed a slightly lower, yet significant relationship with pigments ($r = 0.38$, $p = 0.001$, Figure S3) and was also highly correlated with variations in bacterial community structure ($r = 0.63$, $p = 0.001$, Figure 1 g-i). Changes in activity showed a low, but significant correlation with spatial distance ($r = 0.17$, $p = 0.003$), and no correlation with water depth (Figure S2 c, d). Similar results were obtained for oxygen consumption and carbon remineralization rates previously measured at 19 stations in the same area (Boetius and Damm, 1998): Oxygen consumption was significantly correlated with differences in pigment concentrations ($r = 0.59$, $p < 0.001$), spatial distance ($r = 0.26$, $p = 0.01$), and water depth ($r = 0.16$, $p = 0.04$), as were carbon remineralization rates with pigment concentrations ($r = 0.72$, $p < 0.001$), spatial distance ($r = 0.24$, $p = 0.01$), and water depth ($r = 0.31$, $p = 0.004$). Oxygen consumption and carbon remineralization were also correlated to changes in community structure ($r = 0.39$, $p = 0.08$ and $r = 0.66$, $p < 0.001$, respectively).

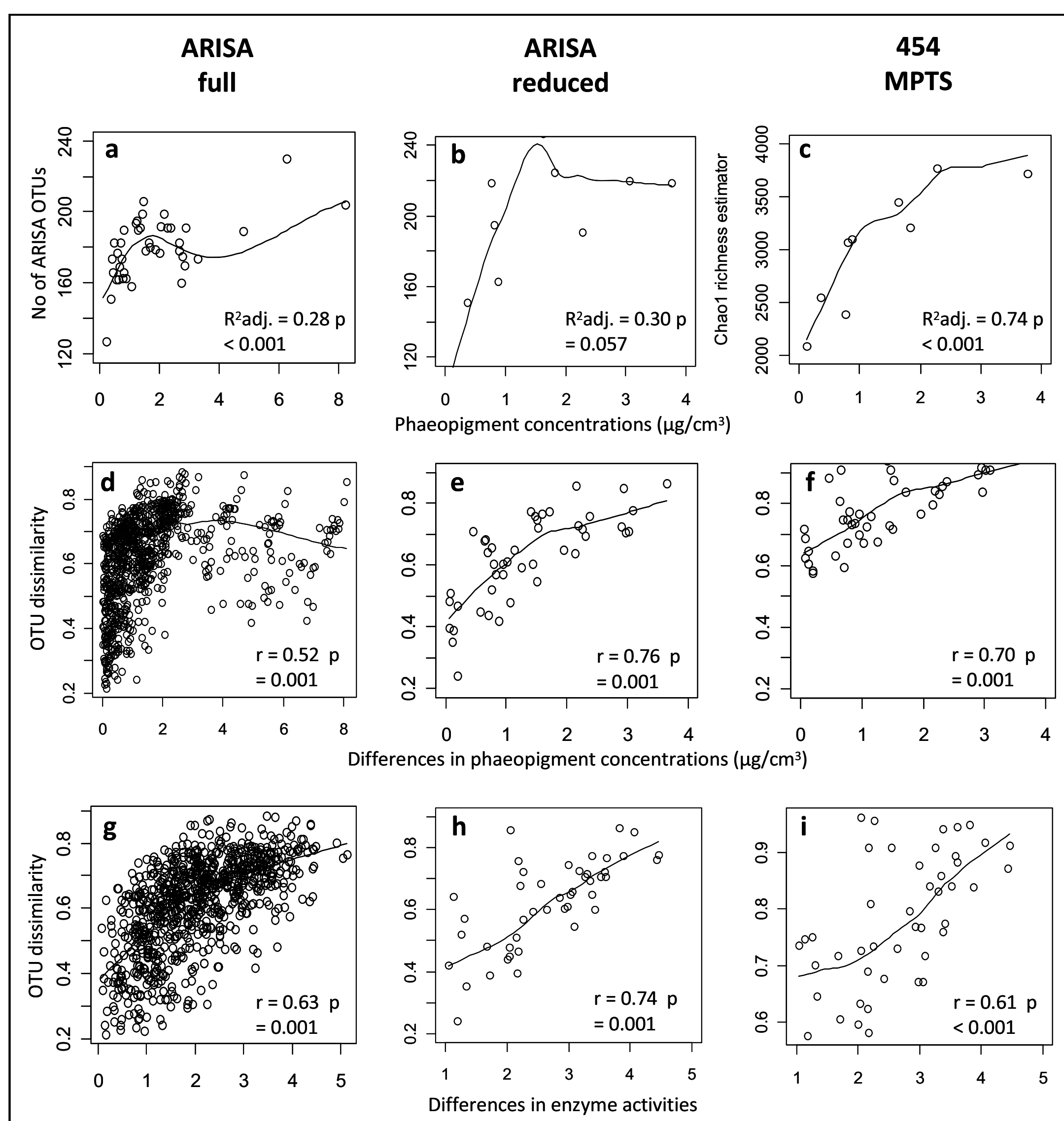

Figure 1 Changes in bacterial OTU richness, community structure and enzyme activity with pigment concentrations and correlation of changes in community structure with changes in enzyme activities for ARISA and 454 MPTS data. The plots in the left column of the figure (a, d, g) are based on the full ARISA dataset, the ones in the middle column (b, e, h) are based on a reduced ARISA dataset containing only samples used for 454 MPTS and plots in the right column (c, f, i) are based on 454 MPTS data. Linear regression R^2 values and Spearman correlations as tested by Mantel tests with 999 permutations are indicated in the plots. The full dataset (a) exhibits a strong linear correlation for very low pigment values (linear model $R^2 = 0.59$, $p < 0.001$), changing into a hump-shaped relationship with a good quadratic model fit when extending the range of pigment concentrations, but removing the three highest pigment values (quadratic model $R^2 = 0.52$, $p < 0.001$). Quadratic model fits for b) and c) are $R^2 = 0.55$, $p = 0.03$ and $R^2 = 0.84$, $p < 0.001$, respectively.

The effects of sedimentary pigments and protein (as proxies for phytodetritus input) on variation in bacterial community structure and function were further investigated by taking into account the confounding effects of spatial distance (geographic distance between samples) and water depth (Figure 2). Variations in bacterial community structure and function were best

explained by changes in pigment and protein concentrations. The full multivariate model explained 47% of the community variation, with pigment and protein concentrations significantly explaining 5% and 6% of the variation, respectively ($p < 0.001$, based on 999 Monte Carlo permutation tests), and water depth and spatial distance explaining 3% and 10%, respectively. Sediment depth and ice cover could only explain a very small amount of variation in the biological data (1%). Co-variation between explanatory variables, i.e. variation that can be explained by the combined effects of several parameters, summed up to 23%. At the functional level, the variation in available energy as represented by pigment concentrations had the largest specific effects on the variation in enzyme activity (16% of the total variation, $p < 0.001$; Figure 2), followed by protein concentration and spatial distance that explained 9% ($p < 0.001$) and 8% ($p < 0.01$) of the variation in enzyme activities, respectively. Water depth alone could not explain any significant part of the variation, while co-variation between variables overall accounted for 33%.

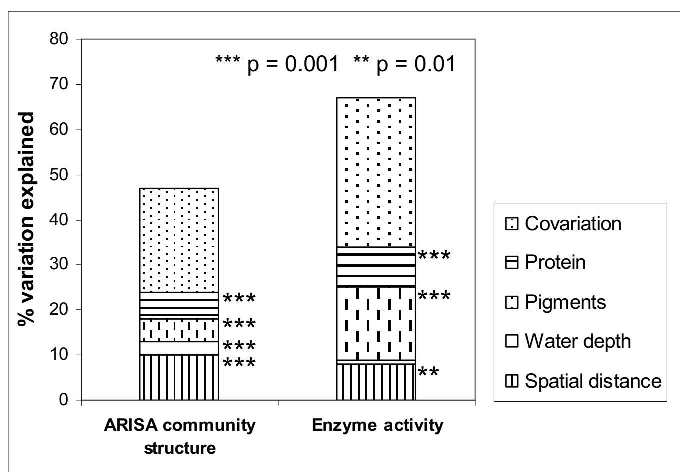

Figure 2 Partitioning of the variation in bacterial community structure (ARISA) and activity (enzyme activity: esterase, lipase, peptidase, beta-glucosidase). Represented are the specific effects of contextual parameters (protein concentration, pigment concentrations, water depth, spatial distance) and total co-variation between these parameters. Statistical significance is indicated as determined by 999 Monte Carlo permutations under the full multivariate model. *** $p < 0.001$, ** $p < 0.01$.

We further validated the causal relationship between bacterial community structure, activity and environmental parameters by path analysis. This method helps to determine the most plausible ecological models among a set of candidate models (Figure 3, Figure S4). The strongest factor directly affecting changes in both bacterial community structure and their activity (enzymatic hydrolysis) was the energy gradient (combination of pigments and protein concentrations as indicators for the presence of labile organic matter, and depth for other flux-related processes; $p < 0.001$). Noticeably, community structure and function were so tightly coupled that modeling a causal relationship in either direction resulted in very similar partial correlation values and overall path models (Figure S4).

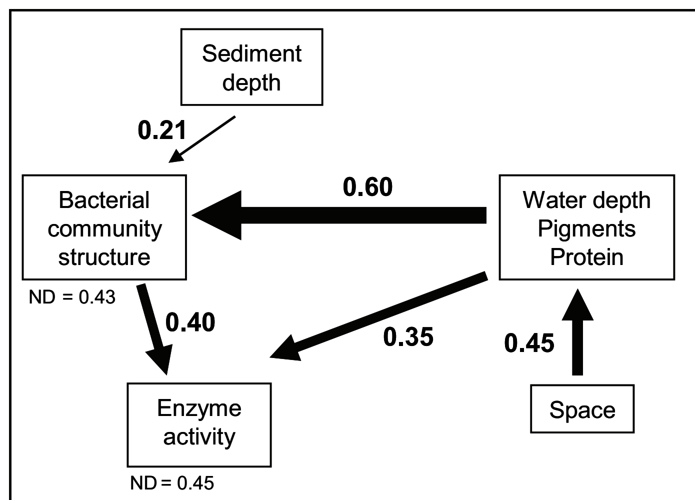

Figure 3 Path analysis of the causal relationships between bacterial community structure, bacterial activity and contextual parameters. The significance of a Chi-square test shows that the model is not significantly different from the correlation matrix of the data based on the RV coefficient ($p = 0.76$). A goodness-of-fit index (0.98) and Bentler Comparative Fit Index (1) indicate an optimal fit of the model. The Bayesian Information Criterion (-16.1) is another measure of the goodness of fit, and was the criterion that was iteratively minimized. The coefficient of non-determination ($ND = 1-R^2$) determines the fraction of the variance in bacterial community structure and enzyme activity that is not explained by the model.

3.2 Response of individual taxa to changes in energy availability

The most abundant sequences in the complete 454 MPTS dataset were affiliated with the phylum *Proteobacteria* (51% of all sequences), followed by *Actinobacteria* (10%) and *Acidobacteria* (9%). On the class level, *Gammaproteobacteria* (26%), *Deltaproteobacteria* (14%), *Actinobacteria* (10%), *Alphaproteobacteria* (7%) and *Acidobacteria* (6%) contained the majority of all sequences (see SI text for sequence taxonomic classification). Taxa showing significant positive or negative relationships with pigment concentrations comprised the dominant fraction of the dataset in terms of relative sequence abundance (> 50%).

Already at coarse taxonomic resolution (i.e. phylum and class level) patterns of community structure with pigments could be detected (Table S2) and variable responses to changes in phytodetritus input were observed (Figure 4 a, b): The major phylum *Proteobacteria* overall strongly responded positively to pigment concentration increase, while its corresponding classes showed positive or no correlations (Figure 4 a, b; Table S3). Examples of classes showing positive linear relationships with pigments included *Gammaproteobacteria* and *Flavobacteria* (phylum *Bacteroidetes*), while no such relationship could be found for other bacterial classes, e.g. *Betaproteobacteria* (Table S3). *Acidobacteria* showed a negative, linear relationship with pigment concentrations. Taxa were also tested for quadratic relationships with pigment concentrations, but only very few significant correlations were found at high taxonomic resolution levels, e.g. for the families *Desulfuromonadaceae* and *Flavobacteriaceae*.

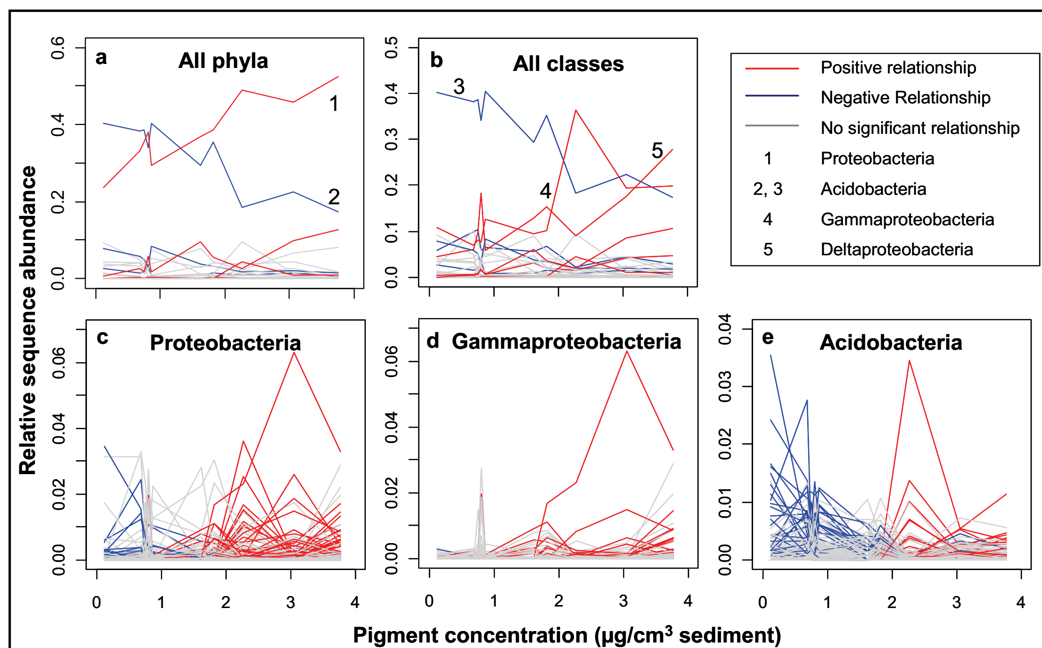

Figure 4 Examples of typical behaviors of taxa (**a, b**) and individual OTUs (**c-e**) with pigment concentration. Significant positive and negative Spearman rank correlation values are displayed in red and blue, respectively, whereas no significant relationships are in gray. The proportions of positively and negatively correlated taxa and OTUs are: For phyla, 21% positive, 16% negative; classes, 40% positive, 20% negative; *Proteobacteria,* 4.6% positive, 0.6% negative; *Gammaproteobacteria*, 3.8% positive, 0.1% negative; and for *Acidobacteria*, 4.8% positive, 1.9% negative.

The significant relationships were, in some cases, consistent at various taxonomic levels, e.g. the class *Gammaproteobacteria*, the family *Psychromonadaceae* and the genus *Psychromonas* were significantly positively related to phytodetritus input (Table S3). Yet, a more complex picture emerged when considering the highest resolution level, i.e. that of individual sequences (Figure 4c-e): *Proteobacteria* and the class *Gammaproteobacteria* showed strong positive relationships with pigment concentrations, but OTUs consisting of individual sequences assigned to these taxa varied in their response from positive to negative, the same being true for OTUs assigned to *Acidobacteria* which overall showed negative relationships with pigments. To investigate potential differences in the response of rare and common types, we created subsets defining "rare" as those that occurred with ≤ 5 sequences in at least four samples and "common" as those occurring > 5 times in at least four samples. Noticeably, of the rare types only 26% showed significant relationships with phytodetritus input, whereas of the more common types 46% showed significant relationships.

4. Discussion

4.1. Change in richness with increasing energy availability

Our results suggest an overall positive response of bacterial OTU richness to energy availability in the form of phytodetritus, which was strongest at oligotrophic conditions defined by low levels of pigment concentrations (<2-3 μg/cm^3 sediment) (Figure 1a-c). Positive relationships between diversity and food availability have also been described for benthic meio- and megafaunal organisms (Soltwedel et al., 2009; Vanaverbeke et al., 1997) and suggest that bacteria and animals of different size classes may be structured by similar ecological mechanisms. Increasing phytodetritus input sustains increasing bacterial abundance and biomass (Wei et al., 2010), potentially enabling more species to coexist. This would be in line with the "*more individuals*" hypothesis (Srivastava and Lawton, 1998) of the species-energy theory (Wright, 1983). A leveling off of richness and the hump shape emerging from the inclusion of sites with higher phytodetritus supply (mesotrophic sites at the upper slope and close to the ice-edge) could be explained by the resource-ratio theory (Tilman, 1980), when other resources, e.g. oxygen, become limiting, favoring the survival of fewer specialists (Levin et al., 2001 and references therein). Other types of community dynamics may also cause this type of relationship, like competition or predation which may put a limit to the number of coexisting species (Levin et al., 2001; Rex, 1976). The patterns we observed at the Arctic continental margin suggest that bacteria may conform to ecological rules that have originally been established for larger organisms. Further studies of natural and experimental systems are needed to test and decipher the mechanisms responsible for the establishment and maintenance of energy-diversity relationships in bacterial communities and if these can be extended to the global scale.

4.2 Changes in community structure and function with increasing energy availability

Not only bacterial richness but also community structure and function were affected by energy availability, suggesting a tight coupling between community structure and functions in organic matter remineralization, such as hydrolytic enzyme activity and oxygen consumption. A close association between community structure and functional patterns has also recently been reported for other Arctic regions (Teske et al., 2011). This may imply that changes in bacterial community structure could directly translate into functional changes that may even affect overall ecosystem functioning, e.g. such as carbon retention and nutrient remineralization. Additional analyses will be needed to investigate not only the role of quantity but also quality of

phytodetrital material for the specific functional response of benthic bacterial communities, which may alter organic matter recycling at the seafloor.

Some of the community variation was explained by pure effects of the categories "spatial distance" and "water depth". Although this suggests some isolation-by-distance processes, we cannot rule out the effects on community variation of other unmeasured biogeochemical parameters that would also be spatially structured (Legendre and Legendre, 1998). Furthermore, the relation between phytodetritus input and bacterial community structure could also be indirectly enforced by top-down effects such as nanoflagellate grazing (Danovaro et al., 1998; Lebaron et al., 1999; Lindstrom, 2000) or viral infection (Danovaro and Serresi, 2000), or food-dependent differences in benthic fauna composition that would affect grazing, defecation, and bioturbation. We identified reproducible ecological patterns of diversity on all taxonomic levels investigated, demonstrating that bacterial diversity is not just randomly distributed along a well-defined energy gradient but compares well with response patterns of other benthic organisms.

4.3 Specific taxa – energy relationships

While bacterial OTU richness generally increased with phytodetritus input, the response varied from positive to negative for individual taxonomic groups of bacteria. Taxa showing significant relationships with phytodetritus input were usually sequence abundant. Common taxa such as the *Gammaproteobacteria* and *Acidobacteria* with strong relationships to energy availability may serve as indicator taxa for certain environmental conditions, e.g. high vs. low phytodetritus availability, and could be helpful for future monitoring studies of benthic ecosystems in the Arctic Ocean. The strong positive correlations with energy availability in the *Gammaproteobacteria*, a globally ubiquitous group in marine sediments, imply that these organisms may include many opportunistic, fast growing bacteria. In contrast, the Acidobacteria may be especially adapted to oligotrophic conditions (Fierer et al., 2007), indicated by their significant negative relationship with pigments. Our results are consistent with other studies that showed differing responses of bacterial taxa to changes in productivity (Horner-Devine et al., 2003; Pommier et al., 2007). This suggests that varying ecological strategies and broad-scale patterns of co-existence or avoidance between bacterial groups may exist. Also at the level of individual OTUs, different relationships are realized, indicating niche differentiation even in closely related bacterial types. Future experimental studies on microbial energy-diversity relationships using quantitative methods such as fluorescence in situ hybridization or quantitative PCR, which generally target specific microbial populations, would need to consider this biological variability in their assays. Interestingly, common types were more likely to show significant relationships with food input than rare types,

possibly supporting the notion that the more abundant types are actively growing and mediating most ecosystem functions (Pedros-Alio, 2006). But, the fact that also 26% of rare types exhibited relationships with food availability, indicates that both common and rare types follow ecological patterns (cf. Galand et al., 2009).

4.4 Further implications

Beyond the insight in energy availability – diversity relationships in complex bacterial communities, this study strongly suggests that any environmental changes affecting primary productivity and particle export will cause shifts in bacterial community structure and function in the Arctic, which in turn could affect key processes such as carbon cycling (Deming and Baross, 1993; Klages et al., 2003). Already now, structural shifts of Arctic ecosystems in response to changing environmental conditions have been observed (Grebmeier et al., 2006) and may further affect benthic-pelagic coupling (Aagaard et al., 1999). Our samples were collected at a time when the Laptev Sea was largely ice-covered throughout the year, explaining why short-term changes in ice cover occurring during our study were mostly not reflected in changes in bacterial community structure and function. Since then, a rapid decline in sea ice cover has occurred, leaving most of this area ice-free during the Arctic summer (Serreze et al., 2007). Such long-term changes in ice cover are predicted to result in changing primary productivity and particle flux (Arrigo et al., 2008; Lalande et al., 2009; Wassmann et al., 2010). Our study thus offers a unique ecological baseline against which ecosystem shifts can be assessed in the future, especially by incorporating bacterial community dynamics in a region increasingly influenced by global change.

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Supplementary Information

Supplementary Material and Methods

Statistical analyses

The Mantel test determines correlation coefficients between dissimilarity matrices. The Procrustes test compares two ordinations using symmetric Procrustes rotation (Peres-Neto and Jackson, 2001), i.e. it uses reduced space instead of complete dissimilarity matrices as in the Mantel test (Legendre and Legendre, 1998; Ramette, 2007).

The ARISA and 454 MPTS tables were Hellinger transformed, in order to standardize the tables and to make them more suitable for multivariate linear analyses (Legendre and Gallagher, 2001). Environmental parameters were log- or square root transformed when necessary, in order to normalize their distribution (Ramette, 2007). Environmental data tables were standardized (z-score transformation; x scaled to zero mean and unit variance) prior to analyses to remove the influence of magnitude differences between scales and units. Chao1 richness estimates (Chao, 1984) were calculated with re-sampling of the OTUs (at 3% difference) based on the smallest dataset (n = 7613 sequences) in order to have comparable estimates between samples.

Taxonomy based on 454 MPTS

454 massively parallel tag sequencing presents a cost-effective, high-throughput technique allowing a much higher sequencing depth (sampling effort) than traditional sequencing of the full length 16S rRNA gene (Margulies et al, 2005; Sogin et al, 2006). Taxonomic assignments of V6-hypervariable region tags were obtained through comparisons with a reference database of rRNA sequences using the Global Alignment for Sequence Taxonomy tool (Sogin et al, 2006; Huse et al, 2008). The retrieved taxonomy was shown to be highly consistent with results based on full-length rRNA sequences (Huse et al, 2008).

Technical considerations

Recent studies using 454 MPTS on Arctic water samples have explored the ecology of rare tags in 454 MPTS datasets (Galand et al, 2009; Kirchman et al, 2010), while other studies have critically discussed the existence of the rare biosphere and suggested that diversity estimates may be inflated by sequencing errors in massively parallel tag sequencing approaches (Kunin et al, 2009; Quince et al, 2009). In our study we neither focused our analysis on rare tags nor put an

emphasis on richness estimates in comparison with other studies, but rather explored relative differences between samples (Reeder and Knight, 2010). To test the impact of rare tag sequences on ecological interpretations of 454 MPTS datasets, Gobet et al. (2010) compared the effects of removing or not the pyrosequencing noise in datasets with the PyroNoise tool (Quince et al., 2009) and demonstrated that the observed variation in profiles were mostly due to non-technical fluctuations in the data, i.e. to real structural and ecological characteristics of the studied data sets. This result and the consistency of our 454 MPTS data with the ARISA data make us confident that the patterns we describe reflect true ecological variations between communities and not sequencing artifacts.

Supplementary Results

ARISA and 454 MPTS datasets

A total of 42 samples composed of three sediment horizons (0-1 cm, 1-2 cm, 4-5 cm) were analyzed by the molecular community fingerprinting technique ARISA, and for each sample 106-230 OTUs were obtained after binning (binning was done to take into account technical imprecision in the OTU definition). A subset of ten samples was selected for 454 MPTS, where the total number of sequences in our dataset was 225,744; the number of sequences from each of the selected samples ranged from 7,613 to 45,891, with 1275 to 4844 unique OTUs at 97% sequence similarity. The proportion of singletons, i.e. sequences that occurred only once in the study, was 65% and 11% respectively, when relating it either to the number of OTUs defined as unique sequences or to the total number of sequences in the dataset.

Comparability between ARISA and 454 MPTS

To demonstrate comparability in the ecological patterns extracted with ARISA and 454 MPTS, a Mantel test with Spearman correlation was used to compare dissimilarity matrices. Correlations were highly significant for comparisons on all taxonomic levels, legitimizing a combination of the two techniques for the interpretation of bacterial ecological patterns in our study (Table S1). In order to keep analyses over different taxonomic levels consistent, we used a subset of the 454 MPTS dataset for further analysis, in which only sequences with a complete assignment up to genus level were retained. A high Spearman correlation between dissimilarity matrices of the reduced (20% of original) and the original dataset confirmed that ecological patterns were consistent in both datasets (Table S1).

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Supplementary Figures

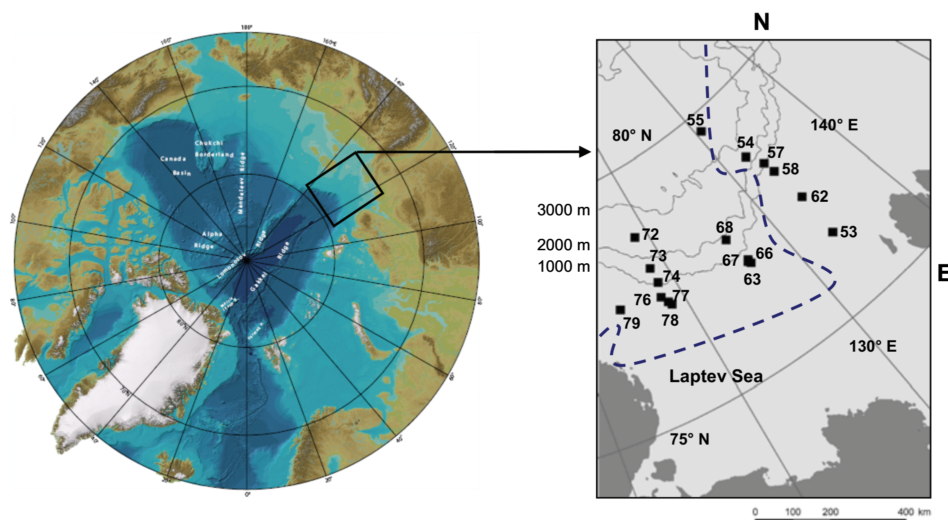


Figure S1 Map of the sampling area in the Arctic Ocean with three water depth transects in the Laptev Sea. The ice cover at the time of sampling is indicated by the dotted line, where the eastern part was ice free and the western part mostly ice covered. (Left: http://www.ngdc.noaa.gov/mgg/bathymetry/arctic, right: the map was generated in ArcMap (ArcGIS Desktop 9.3; ESRI, Kranzberg, Germany). The bathymetry was obtained from Jakobsson et al. (1).

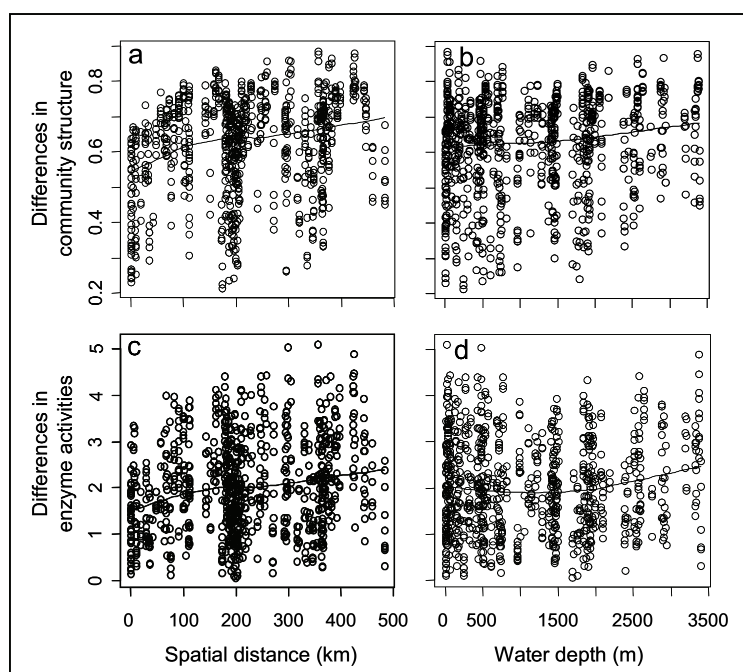


Figure S2 **S**catterplots with LOESS curves displaying the relationships between differences in ARISA community structure and a) geographic distance (Spearman's r = 0.24, p < 0.001 as tested by 999 Monte Carlo permutations), and b) differences in water depth (r = 0.14, p = 0.011), as well as relationships between differences in enzyme activities (esterase, lipase, peptidase, beta-glucosidase) and c) spatial distance (r = 0.17, p = 0.003), and d) differences in water depth (r = 0.05, p = 0.22).

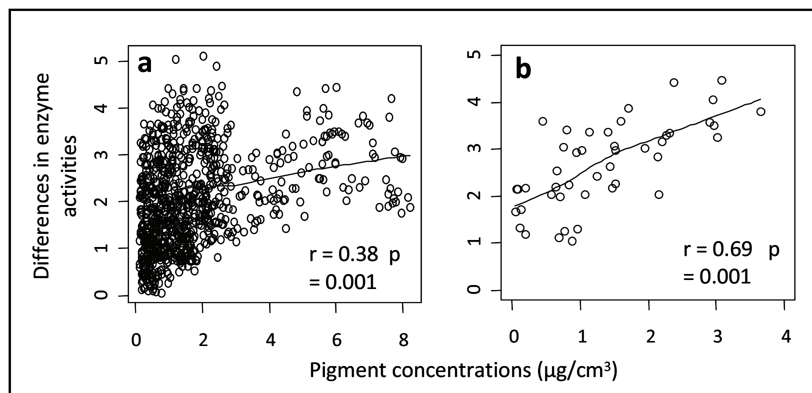
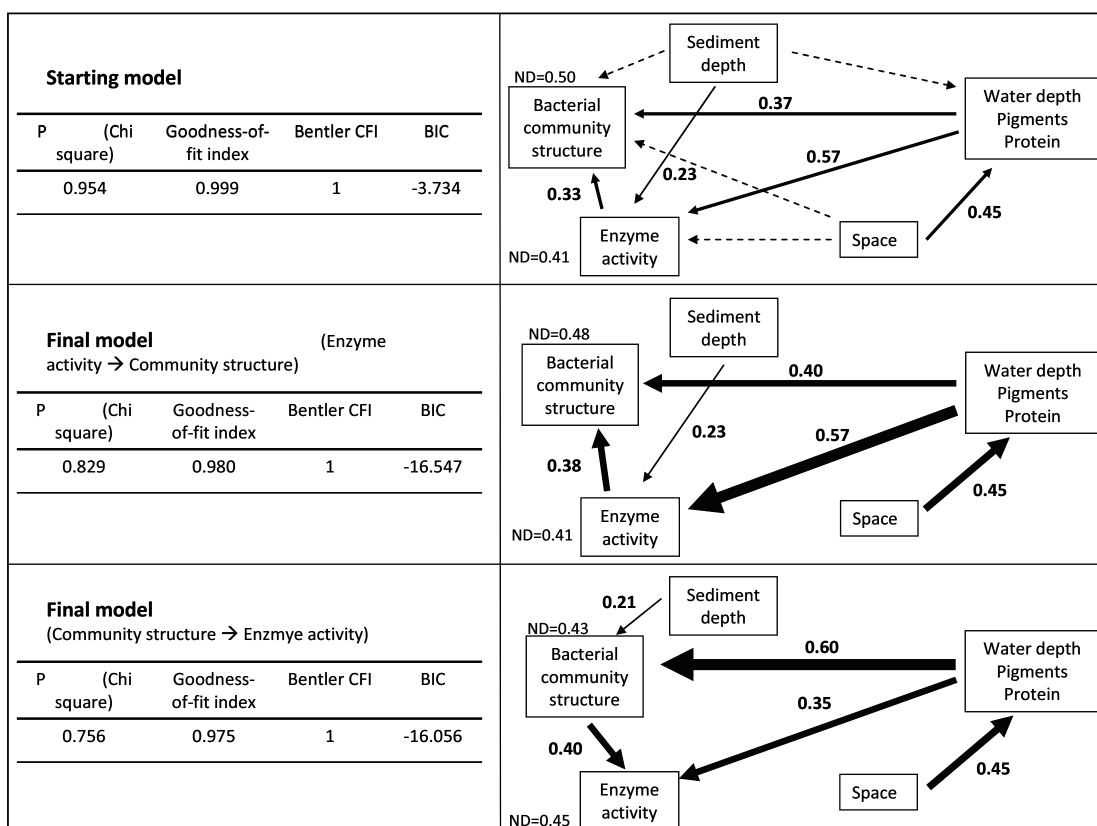


Figure S3 Changes in enzyme activities (esterase, lipase, peptidase, beta-glucosidase) with increasing pigment concentrations a) for the full dataset, and b) for a reduced dataset only including samples used for 454 MPTS. Spearman correlations as tested with the Mantel test and 999 permutations are indicated in the figure.



Starting model

P (Chi square)	Goodness-of-fit index	Bentler CFI	BIC
0.954	0.999	1	-3.734

Final model (Enzyme activity → Community structure)

P (Chi square)	Goodness-of-fit index	Bentler CFI	BIC
0.829	0.980	1	-16.547

Final model (Community structure → Enzmye activity)

P (Chi square)	Goodness-of-fit index	Bentler CFI	BIC
0.756	0.975	1	-16.056

Figure S4 Initial and final path models obtained with structural equation modeling. In the final model (E→A) the path is directed from enzyme activities to ARISA and in final model (A→E) the direction of the path is reversed. The tables show the significance value for the Chi-square test, assessing whether the model is significantly different from a correlation matrix of the data based on the RV coefficient. The goodness-of-fit index and Bentler CFI (Comparative Fit Index) indicate the goodness of fit of the model, the best fit would result in a value of 1. The BIC (Bayesian Information Criterion) is another measure of the goodness of fit and needs to be minimized. The coefficient of non-determination (ND = 1-R^2) determines the fraction of the variance in bacterial community structure and enzyme activity that is not explained by the model.

Supplementary Tables

Table S1 Mantel and Procrustes tests for comparisons between ARISA and 454 MPTS community data, as well as for comparisons between the 454 MPTS taxonomic table with all available taxonomic assignments and a reduced table where only tags are kept that have a full assignment up to the genus level. *p<0.05, **p<0.01, p<0.001

	Taxonomic level	Number of OTU categories	Mantel test	Procrustes test
Compared to ARISA	**Phylum**	19	0.77 ***	0.88 **
	Class	31	0.75 ***	0.79 **
	Order	74	0.73 ***	0.78 **
	Family	163	0.77 ***	0.79 **
	Genus	381	0.73 ***	0.81 **
	Tags (only up to genus)	7,819	0.91 ***	0.93 **
	Tags (all)	38,581	0.91 ***	0.917 **
All assignments / only assignments up to genus	**Phylum**	36 /19	0.70 ***	0.87 **
	Class	36 /31	0.79 ***	0.91 **
	Order	79 / 75	0.94 ***	0.91 **
	Family	176 / 16	0.97 ***	0.91 **
	Genus	381 / 381	0.98 ***	0.99 **
	Tags	38,581 / 7,819	0.99 ***	0.99 **

Table S2 Proportion of variation in different community datasets that is explained by pigment concentrations, as determined by redundancy analysis and 999 Monte Carlo permutations.

	Relative abundance		Presence / Absence	
Taxonomic level	**Proportion explained**	**p**	**Proportion explained**	**p**
Phylum	50	0.005	25	0.01
Class	48	0.005	26	0.01
Order	43	0.005	19	0.01
Family	45	0.005	19	0.01
Genus	39	0.005	19	0.005
OTUs (3%)	37	0.005	21	0.005
Tags (genus)	27	0.005	17	0.005
Tags (all)	30	0.005	17	0.005
ARISA reduced	32	0.005	22	0.005
ARISA full	20	0.005	13	0.005

Table S3 Linear correlations of taxa with phaeopigments. Shaded area for phyla and classes indicate significant relationships at $p<0.05$. For families and genera only significant relationships at $p<0.05$ are shown. Taxa are ordered by decreasing adjusted R^2.

	Taxa	Adj. R^2	p	Sign
Phylum	Proteobacteria	0.81	0.000	+
	Acidobacteria	0.80	0.000	-
	Verrucomicrobia	0.63	0.004	+
	Gemmatimonadetes	0.61	0.004	-
	Deinococcus-Thermus	0.58	0.006	+
	Actinobacteria	0.23	0.092	
	Bacteroidetes	0.21	0.103	
	Planctomycetes	0.19	0.114	
	Nitrospira	0.14	0.154	
	Spirochaetes	0.10	0.200	
	Tenericutes	0.02	0.309	
	Deferribacteres	0.02	0.313	
	Thermotogae	0.00	0.345	
	Chlamydiae	-0.05	0.467	
	Lentisphaerae	-0.09	0.608	
	Chloroflexi	-0.09	0.641	
	Firmicutes	-0.10	0.701	
	Cyanobacteria	-0.11	0.782	
	Fusobacteria	-0.12	0.810	
Class	Verrucomicrobiae	0.80	0.000	+
	Acidobacteria	0.80	0.000	-
	Flavobacteria	0.77	0.001	+
	Gemmatimonadetes	0.61	0.004	-
	Deinococci	0.58	0.006	+
	Gammaproteobacteria	0.56	0.008	+
	Alphaproteobacteria	0.39	0.031	-
	Deltaproteobacteria	0.28	0.066	+
	Actinobacteria	0.23	0.092	
	Erysipelotrichi	0.20	0.108	
	Epsilonproteobacteria	0.19	0.113	
	Planctomycetacia	0.19	0.114	
	Caldilineae	0.15	0.148	
	Nitrospira	0.14	0.154	
	Bacteroidia	0.12	0.174	
	Betaproteobacteria	0.11	0.186	
	Spirochaetes	0.10	0.200	
	Mollicutes	0.02	0.309	
	Deferribacteres	0.02	0.313	
	Anaerolineae	0.01	0.331	
	Thermotogae	0.00	0.345	
	Chlamydiae	-0.05	0.467	
	Sphingobacteria	-0.06	0.503	
	Chloroflexi	-0.06	0.515	
	Dehalococcoidetes	-0.08	0.563	
	Bacilli	-0.08	0.593	
	Lentisphaeria	-0.09	0.608	
	True_Cyanobacteria	-0.11	0.782	
	Fusobacteria	-0.12	0.810	
	Opitutae	-0.12	0.990	

	Taxa	Adj. R^2	p	Sign
	Clostridia	-0.12	0.995	
Family	Flavobacteriaceae	0.85	0.000	+
	Desulfuromonadaceae	0.84	0.000	+
	Rubritaleaceae	0.80	0.000	+
	Acidobacteriaceae	0.80	0.000	-
	Cyclobacteriaceae	0.77	0.001	+
	Alteromonadaceae	0.69	0.002	+
	Rhodobacteraceae	0.67	0.002	+
	Trueperaceae	0.62	0.004	+
	Gemmatimonadaceae	0.61	0.004	-
	Psychromonadaceae	0.58	0.006	+
	Ectothiorhodospiraceae	0.56	0.007	-
	Kordiimonadaceae	0.54	0.010	+
	Coxiellaceae	0.52	0.011	-
	Desulfurellaceae	0.50	0.013	+
	Enterococcaceae	0.50	0.013	+
	Family_II	0.50	0.013	+
	Methylophilaceae	0.50	0.013	+
	Litoricolaceae	0.50	0.014	+
	Nitrospinaceae	0.48	0.016	-
	Francisellaceae	0.48	0.016	+
	Clostridiaceae	0.46	0.019	+
	Desulfohalobiaceae	0.43	0.023	+
	Desulfobulbaceae	0.43	0.024	+
	Campylobacteraceae	0.42	0.025	+
	Halomonadaceae	0.41	0.027	-
	Cytophagaceae	0.40	0.030	+
	Colwelliaceae	0.39	0.031	+
	Phyllobacteriaceae	0.38	0.034	-
	Aerococcaceae	0.36	0.038	+
	Desulfonatronaceae	0.36	0.038	+
	Leptospiraceae	0.36	0.038	+
	Rhodothermaceae	0.34	0.046	-
Genus	Maribacter	0.80	0.000	+
	Rubritalea	0.80	0.000	+
	Desulfuromonas	0.79	0.000	+
	Marinobacterium	0.76	0.001	+
	Desulfosarcina	0.74	0.001	+
	Algoriphagus	0.74	0.001	+
	Desulfobacula	0.71	0.001	+
	Lutibacter	0.71	0.001	+
	Gp10	0.70	0.002	-
	Tenacibaculum	0.65	0.003	+
	Desulfuromusa	0.65	0.003	+
	Truepera	0.62	0.004	+
	Gemmatimonas	0.61	0.004	-
	Ulvibacter	0.61	0.005	+
	Desulfofaba	0.59	0.006	+
	Jannaschia	0.59	0.006	+
	Psychromonas	0.58	0.006	+
	Coxiella	0.55	0.008	-

Taxa	Adj. R^2	p	Sign
Kordiimonas	0.54	0.010	+
Halobacillus	0.53	0.010	+
Octadecabacter	0.53	0.010	+
Gp26	0.52	0.012	-
GpIIa	0.50	0.013	+
Hippea	0.50	0.013	+
Methylophilus	0.50	0.013	+
Litoricola	0.50	0.014	+
Nitrospina	0.48	0.016	-
Francisella	0.48	0.016	+
Loktanella	0.47	0.018	+
Clostridium	0.46	0.019	+
Reinekea	0.45	0.020	+
Desulfocapsa	0.45	0.020	+
Polaribacter	0.45	0.021	+
Glaciecola	0.44	0.022	+
Desulfonauticus	0.43	0.023	+
Leptospira	0.43	0.023	+
Gp4	0.43	0.024	+
Mesorhizobium	0.42	0.025	-
Halomonas	0.41	0.027	-
Gp6	0.40	0.029	-
Desulfobulbus	0.40	0.029	+
Arcobacter	0.40	0.029	+
Colwellia	0.39	0.032	+
Thiobacillus	0.39	0.032	+
Laceyella	0.38	0.033	+
Thalassomonas	0.38	0.034	+
Gp22	0.37	0.037	-
Aerococcus	0.36	0.038	+
Agarivorans	0.36	0.038	+
Arenibacter	0.36	0.038	+
Balneola	0.36	0.038	+
Belliella	0.36	0.038	+
Cellulophaga	0.36	0.038	+
Desulfacinum	0.36	0.038	+
Desulfonatronum	0.36	0.038	+
Dokdonia	0.36	0.038	+
Enterobacter	0.36	0.038	+
Enterococcus	0.36	0.038	+
Hydrogenophaga	0.36	0.038	+
Myroides	0.36	0.038	+
Oceanisphaera	0.36	0.038	+
Pleurocapsa	0.36	0.038	+
Pontibacter	0.36	0.038	+
Pseudonocardia	0.36	0.038	+
Thermosediminibacter	0.36	0.038	+
Nitrospira	0.36	0.039	-
Salinibacter	0.34	0.046	-
Aminobacterium	0.34	0.046	+
Caedibacter	0.33	0.050	+

Chapter IV

Biogeochemistry and bacterial diversity of deep-sea wood falls

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In Preparation for PLoS ONE

Abstract

Large organic food falls to the deep sea such as whale carcasses and wood logs support the development of reduced, sulfidic habitats in an otherwise oxygenated, oligotrophic deep-sea environment. These transient hot spot ecosystems may serve the dispersal of highly adapted chemosynthetic organisms such as thiotrophic bivalves and siboglinid worms. Here we investigated the biogeochemical and microbiological processes leading to the development of sulfidic niches colonized by chemosynthetic organisms, on wood falls deployed at a depth of 1690 m at the Nile deep sea fan (Eastern Mediterranean). Wood-boring bivalves of the genus *Xylophaga* played a key role in the degradation of the wood logs and the provision of organic matter to anaerobic microorganisms such as sulfate-reducing *Deltaproteobacteria*. The macrofaunal organisms and bacterial communities associated with the wood included types reported from other chemosynthetic deep-sea habitats, confirming the potential role of large organic food falls as stepping stones for vent and seep communities. However, also heterotrophic organisms such as polychaetes, crabs and heterotrophic bacteria were attracted to the wood falls, underlining the importance of large food falls as biological hotspots in the deep sea.

1. Introduction

Most of the deep seafloor receives very little supply of energy and nutrients, leading to extremely oligotrophic conditions in large parts of the ocean [1]. Sunken wood, whale carcasses, kelp and other food falls present locally and temporally restricted inputs of organic material to the deep sea that may be quickly localized and exploited by opportunistic fauna [e.g. 2,3,4], and can develop into hotspots of diversity [5]. Locally enhanced degradation processes at these organic falls can lead to reducing conditions and high sulfide concentrations [6], attracting chemoautotrophic bacteria, both free-living and as symbionts of chemosynthetic fauna [e.g. 6,7]. Observations of shared or similar phylogenetic species between wood falls, whale carcasses, hydrothermal vents and cold seeps have led to the hypothesis that organic falls may present stepping stones in the evolution and dispersal of chemoautotrophic communities in the deep sea, which are constrained to sulfide- and methane-rich niches for their energy supply [8,9,10,11]. The chemosynthetic mussel *Idas* sp. which has been collected from active chemosynthetic ecosystems such as pockmarks and mud volcanoes of the Nile Deep Sea Fan [12], has recently been shown to also colonize experimental wood depositions [4]. However, how sulfidic habitats develop at wood falls to attract chemosynthetic organisms is not well understood. It is also largely unknown which bacteria colonize deep-sea wood falls or how the deposition of wood affects surrounding benthic communities [13]. Hence, the aim of this study was to contribute to a better understanding of the microbial ecology and biogeochemistry of wood fall ecosystems and their role as biological and biochemical hotspots in the deep sea.

To reach these objectives, we deployed and revisited three replicate wood parcels at different distances to an active cold seep of the Central Nile deep sea fan (Eastern Mediterranean) [14,15], to observe colonization of the wood and the development of biogeochemical gradients. The main objectives of this study were to observe: I) which organisms colonize the wood and what their potential role may be in the process of wood degradation, II) how sulfidic environments develop, III) which bacterial communities colonize the wood, and IV) how the presence of wood affects bacterial communities in surrounding sediments.

2. Material and Methods

2.1 Description of wood colonization experiments

Each wood colonization experiment consisted of one large Douglas fir log (length: 200 cm, diameter: 30 cm) to which several smaller logs (length: 30-50 cm, diameter: 10-15 cm) were attached, as well as cement stones serving as weights. The large log served as attraction for wood colonizing megafauna, while the smaller logs could easily be collected by an ROV. The wood parcels were deployed at the Central Province Site 2A in the Eastern Mediterranean Sea [14,15] at water depths of about 1690 m during the BIONIL cruise (RV *Meteor M76/2b*) with ROV *Quest 4000* (Marum, Bremen, Germany) in November 2006. Characteristic features of the Central Province are pockmark structures (subcircular depressions a few meter in diameter and about one meter deep) associated with active methane seepage and the occurrence of large flat authigenic carbonate crusts above reduced sediments [14,15,16,17]. Recovery of sub-samples with ROV *Victor 6000* (IFREMER, Toulouse, France) and deposition of an additional wood experiment took place during the MEDECO-2 cruise (RV *Pourquoi Pas?*) in November 2007. Available metadata of both cruises are stored in the PANGAEA database (http://www.pangaea.de/PHP/CruiseReports.php?b=HERMES) and PANGAEA references for the samples are cited accordingly (Table 1).

Table 1 Locations of the four wood colonization experiments and PANGAEA references for deployment and recovery of the experiments.

Wood experiment	Location	Position	Date	PANGAEA event label
Wood#1	Close to carbonate crusts	N 32°32.0496 E 30°21.1248	**Deployment**: 19 Nov 2006 **Recovery**: 11 Nov 2007	M70/2b_841_WOOD-1 MEDECO2-D338-PANIER-1
Wood#2	On carbonate crust	N 32°31.9626 E 30°21.1752	**Deployment**: 19 Nov 2006 **Recovery**: 11 Nov 2007	M70/2b_841_WOOD-2 MEDECO2-D338-Wood2-1
Wood#5	On sediments	N 32°32.0790 E 30°21.3840	**Deployment**: 20 Nov 2006 **Recovery**: 13 Nov 2007	M70/2b_846_WOOD-1 MEDECO2-D339-BOX-4, MEDECO2-D339-BOX-5, MEDECO2-D339-BOX-6
Wood#6	Reference, sampled after 1 d at seafloor	N 32°32.0124 E 30°21.1920	**Deployment**: 10 Nov 2007 **Recovery**: 11 Nov 2007	MEDECO2-Wood5-1 Not available

Wood experiment #2 was deployed on carbonate crust, wood#1 in close proximity to carbonate crusts, and wood#5 was located on fully oxygenated sediments over 350 m away from the other two wood experiments with no indications for past or present gas venting or fluid flow. No methane degassing into the water column was detected at any of the locations. Wood experiment #6 served as a reference sample after less than one day of submersion and was also deployed close to carbonate crusts. Distances between the wood experiments ranged between 31 m between wood#2 and wood#6 and 410 m between wood#1 and wood#5 (Figure 1).

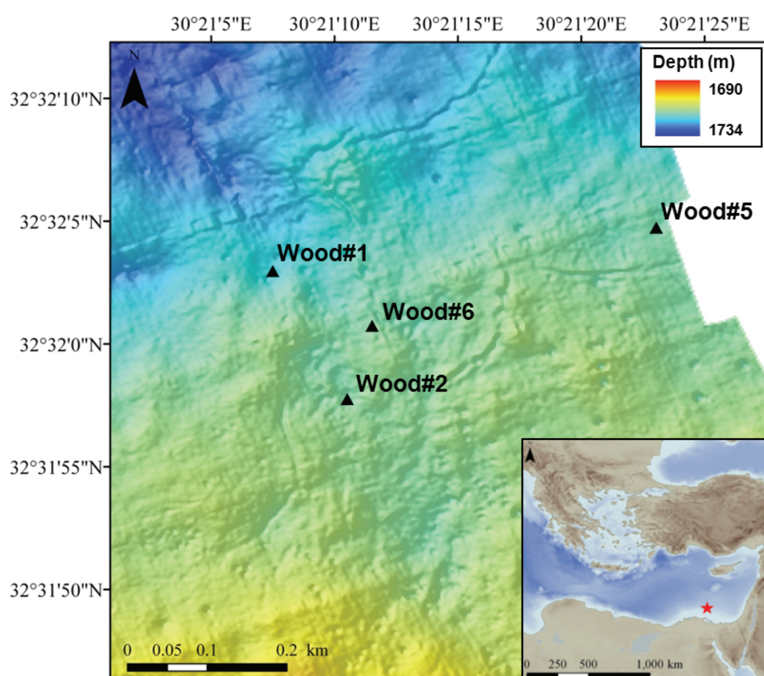

Figure 1 The Pockmark area in the Central Province of the Nile Deep Sea Fan in the Eastern Mediterranean with locations of the four DIWOOD wood colonization experiments: Wood#1 close to carbonate crust, wood#2 on carbonate crust, wood#5 on sediment, wood#6 close to carbonate crust, sampled after less than 1 day of submersion.
The maps were generated in ArcMap (ArcGIS Desktop 9.3) with continental margins provided by ESRI (Kranzberg, Germany) and bathymetry obtained from the 2-minute Gridded Global Relief Data ETOPO2v2 (2006, http://www.ngdc.noaa.gov/mgg/fliers/06mgg01.html). The bathymetry of the Pockmark Area was obtained during Meteor expedition M70/2 (BIONIL) using AUV Aster^x equipped with EM120 multibeam (IFREMER/Geosciences Azur).

2.2 Visual observation and sampling of wood experiments

To monitor the overall condition of the wood experiments after one year at the seafloor we used high quality video and photo surveys by the ROV. Wood logs were sampled for analyses of the bacterial and macrofaunal wood-colonizing communities, and adjacent sediments were sampled for analyses of wood degradation effects on benthic bacterial communities and biogeochemistry. To avoid loss of organisms, each wood log was put by the ROV manipulator into a separate box closed with a lid. Sampling of the wood logs and handling after retrieval took place at *in situ* temperature of 13°C. From each of the wood experiments wood#1, wood#2, and wood#5, three replicate small logs were collected one year after deployment; for the reference wood#6 one small wood log was collected after less than 1 day of submersion. From each small

wood log 2x3 subsamples of the surface (0-2 cm) and the subsurface (2-4 cm) were obtained, cutting 4x4 cm areas on the sides and the middle of the wood log, resulting in 18 subsamples for each wood experiment, and 6 subsamples for wood#6. Any visible organisms (macrofauna) were removed from the wood pieces and samples were preserved for DNA extraction (-20°C) and bacterial cell counts (4% Formalin/Seawater). Macrofauna was collected and preserved for taxonomic analyses.

Sediment cores of up to 28 cm length were taken at distances of 0.5 m and 10 m from the wood experiments. Cores were sub-sampled in 1 cm intervals and fixed for DNA extraction (-20°C) and bacterial cell counts (4% Formalin/Seawater). Porewater was extracted for measurements of sulfate, nutrients and dissolved organic carbon (DOC). Additional cores were used for ex situ measurements of sulfide, oxygen and pH as well as sulfate reduction rates (SRR). Sediment cores could not be obtained for wood experiment #2 as the carbonate crusts covering the sea floor could not be penetrated with push cores.

2.3 Biogeochemical measurements

2.3.1 In situ total oxygen uptake (TOU) with benthic chamber

In situ total oxygen uptake (TOU) was measured at (0.5 m) and away (10 m) wood#1 using a ROV benthic chamber module [6,18]. The centrally stirred chamber encloses 284 cm^2 of sediment with 10–15 cm of overlying bottom water, the latter determined visually with the help of the ROV camera system. At the seafloor, the benthic chamber was operated by the ROV – positioning the chamber at the desired location, gentle insertion of the module into the sediment and starting the measurement.

Mounted oxygen electrodes were used to continuously measure the oxygen concentration in the enclosed water body and the TOU flux (mmol m^{-2} d^{-1}) was calculated from the initial linear decrease in O_2 concentration versus time [18,19].

2.3.2 Ex situ microsensor measurements of sulfide, oxygen and pH

Concentration microprofiles of O_2, H_2S and pH were determined *ex situ* (laboratory) on retrieved push cores, at (0.5 m) and away (10 m) wood#1 and wood#5. Immediately after retrieval, the cores were transferred into an aquarium cooled to *in situ* temperature (13 °C). The sediment cores were fully immersed, and the overlying bottom water was gently stirred by an aquarium pump to create a diffusive boundary layer (DBL) thickness close to *in situ* conditions [20,21]. O_2, H_2S and pH microsensors were mounted on a micromanipulator, which allowed

measuring of concentration profiles with 1 mm resolution. A Clark-type O_2 microelectrode, an amperometric H_2S microelectrode and a pH electrode were used for all *ex situ* measurements [22,23,24]. The sensors were calibrated as described previously [25,26]. Due to a change of pressure and temperature during ascend the cores could to certain extent be disturbed when brought on board, thus microsensor measurements were only started once the overlying water in the core was clear. Three replicate profiles of oxygen, sulfide and pH were measured for every investigated core within approximately 12 h of the core retrieval.

Fluxes were calculated from the steepest porewater gradients in the sediment, according to Fick's first law of diffusion:

$$J_{diff} = \Phi D_{sed} \frac{dc}{dz}$$

where J_{diff} = diffusive flux [mmol m^{-2} d^{-1}], Φ = porosity, D_{sed} = diffusion coefficient in the sediment [m^2 s^{-1}] and dc/dz = concentration gradient. D_{sed} for oxygen and sulfide is 8.9 x 10^{-10} and 6.4 x 10^{-10} m^2s^{-1}, respectively.

2.3.3 Sulfate concentration

Sediment cores were subsampled in 1 cm depth intervals and transferred into plastic centrifuge vials and centrifuged at 3500 x g for 10 min to extract porewater. Subsequently, 500 µl Zincacetate was added to 1 ml porewater and samples were stored at 4°C. Porewater sulfate concentrations were measured in the fixed samples using non-suppressed anion exchange chromatography (Waters IC-Pak anion exchange column, Waters 430 Conductivity detector). As eluent, isophthalic acid (1mmol L21, pH 4.6) containing 10%v/v methanol with a constant flow rate of 1 mL min^{-1} was used. Sulfate concentrations were used for calculations of SR rates.

2.3.4 Porosity

Sediments were sampled in 5 cm depth resolution and stored at 4°C. Porosity was determined as the difference in weight of a defined volume of sediment before and after drying at 60°C until constant weight, and data were used for recalculating solid phase wet weight to units of sediment volume in the SR rate calculations.

2.3.5 Ex situ measurements of sulfate reduction (SR) and anaerobic oxidation of methane (AOM) rates

Push cores were sub-sampled in triplicate with smaller subcores (diameter: 2.8 cm) immediately after recovery. Radiotracer labelled substrates $^{35}SO_4^{2-}$ (SR) and $^{14}CH_4$ (AOM) were injected in 1 cm intervals following the whole core injection method [27]. Experiments were incubated for 10-12 hours at *in situ* temperature and the reactions were stopped by transferring 1 cm slices of the cores into 20 ml 20% Zincacetate for SR or 25 ml NaOH (2.5% w/v) for AOM. Detailed description of the measurements of methane concentration and radioactivity as well as the calculations of the AOM rates can be found in [28]. Sulfate reduction rates were determined using the cold-chromium distillation method [29]. Calculations of the SR turnover rates were done according to [19].

2.3.5. Nutrients

Nutrients (nitrate, phosphate, silicate, ammonium) were measured from the porewater samples with a Skalar Continous-Flow Analyzer according to the method of Grasshoff et al. [30].

2.3.6 Measurements of dissolved organic carbon and total dissolved nitrogen

After extraction of porewater by centrifugation (3500 x g, 10 Min), porewater was filtered through 0.22 µm cellulose-acetate filters. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were analyzed with a Shimadzu TOC-VCPH total organic carbon analyzer equipped with a TNM-1 total nitrogen measuring unit. To remove cells prior to analysis, samples were filtered through GHP Acrodisc 25 mm syringe filters with a 0.2 µm GHP membrane (Pall Life Sciences, USA). Samples were injected manually (100 µL) in order to minimize the amount of required sample volume. Each sample was injected five times and average values of these injections are reported. Outliers were removed. Before analysis, samples were acidified to pH=2 with HCl (10 M, p.a.) and purged with synthetic air for 5 minutes to remove inorganic carbon. Detection limits were 5µM for DOC and TDN (0.06 g C L-1 and 0.07 g N L-1). Analytical errors based on the standard deviations for replicated measurements (at least three measurements per sample) were within 5% for DOC and TDN. Analytical precision and accuracy was tested in each run against deep Atlantic seawater reference material and low carbon water provided by the consensus reference materials program (D.A. Hansell, University of Miami, FL, USA). Procedural blanks, including the filtration step, were obtained with ultrapure water.

2.4 Characterization of bacterial communities

2.4.1 Bacterial cell numbers

Total bacterial cell numbers in sediment samples were determined using acridine orange direct counts (AODC) based on previously described methods [31,32]. Single cell numbers were determined for two replicate filters by randomly counting at least 30 grids per filter. For samples containing wood chips (upper layers next to wood experiments), the duration of sonication (at power: 72/D and a cycle: 30%) was increased from 1 Min 40 sec for sediments to 2x5 Min. Due to the strong fluorescence of wood pieces in acridine orange stained samples, cell numbers for samples containing lots of wood pieces, were verified with cell counts based on the DNA-targeting fluorescent stain 4',6-diamidino-2-phenylindole (DAPI) [33], which gave much less background fluorescence of wood particles. Pure wood samples (0.3 g) were sonicated in 5 ml 4% Formalin/Seawater (FA/SW) for 3x5 Minutes to detach as many cells as possible without breaking them. After each 5 Minute interval, the FA/SW solution was exchanged after wood pieces had settled for about 1 Minute. Supernatants from the different sonication steps were combined and volumes for filtering were adjusted to obtain an even distribution of cells on the filters. Additionally wood pieces remaining after 3x5 minutes of sonication, were again sonicated for 5 minute intervals up to 30 minutes (6x5 Minutes), and the complete volumes filtered, to quantify remaining cells. All samples were kept on ice during sonication. Cell counts for pure wood samples were performed with DAPI staining.

2.4.1 DNA extraction

Total community DNA was extracted from 0.3–0.4 g of wood material that had been cut into very small pieces or from 1 g of sediment using UltraClean Soil DNA Isolation Kits (MoBio Laboratories Inc., Carlsbad, CA) and stored in a final volume of 100 µl Tris-EDTA buffer. DNA quantities were spectrophotometrically determined with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE) and adjusted for each step of the molecular protocol.

2.4.3 Automated ribosomal intergenic spacer analysis (ARISA)

Standardized amounts of 10 ng DNA were amplified in triplicate using bacteria specific ARISA primers ITSF and ITSReub, the latter labeled with the phosphoramidite dye HEX [34]. A standardized amount of PCR product (100 ng DNA) was used for separation of fragments by capillary electrophoresis with the internal size standard MapMarker 1000 ROX (BioVentures Inc., Wahsington DC, USA). Using a standard ARISA protocol, a “fixed window” binning strategy

with a bin size of 2 bp was applied to the ARISA generated data to compensate for slight peak shifts between runs and for TRF size calling imprecision [35] (Interactive Binner function, http://www.ecology-research.com). An OTU was considered present if it appeared in one of the three PCR replicates. Relative fluroscence intensities were calculated by dividing each individual peak area by the total area of peaks in a given profile.

2.4.4 454 massively parallel tag sequencing (454 MPTS)

One sample from each wood experiment and surface sediment samples at (0.5 m) and away (10 m) from wood#1 and #5 were selected for 454 MPTS. For cores covered with wood chips (0.5 m), sediment samples were obtained from the previous sediment surface, below the wood chips, for direct comparison with the surface samples from reference cores 10 m away from the wood experiments. Extracted DNA was amplified using primers targeting the V6 region of the bacterial 16S rRNA gene as published on http://vamps.mbl.edu. Fragments were sequenced by pyrosequencing on a Genome Sequencer FLX system (Roche, Basel, Switzerland) at the Marine Biological Laboratory in Woods Hole, MA, USA. Taxonomic assignments were performed with the Global Alignment for Sequence Taxonomy tool (GAST) [36,37,38]. All data were retrieved from the website VAMPS (Visualization and Analyses of Micbrobial Population Structures, http://vamps.mbl.edu).

2.4.5 Statistical analyses

Overall patterns in bacterial community structure were detected with non-metric multidimensional scaling (nMDS) based on ARISA OTU tables with Bray-Curtis distance as implemented in the R package *vegan*. Analysis of similarity (ANOSIM) was used to assess significant differences between *a posteriori* groupings of samples. All statistical analyses were performed in R (v. 2.9.1) (R Development Core Team 2009, http://www.R-project.org) using *vegan* and custom R scripts.

3. Results

3.1 Visual observations of wood experiments

The *in situ* observations after one year of immersion showed that the wood had been heavily degraded by the activity of wood-boring animals, e.g., indicated by the presence of burrows in the wood (Figures 2 and 3). In an area of about 0.5–1 m around the wood experiments a layer of fine wood chips had accumulated of up to 5 cm thickness. The state of degradation and colonization differed between wood logs but also between different positions on one log. Those sides of the wood logs lying in the sediments appeared to be less colonized and degraded by wood-boring animals than those exposed to the bottom waters.

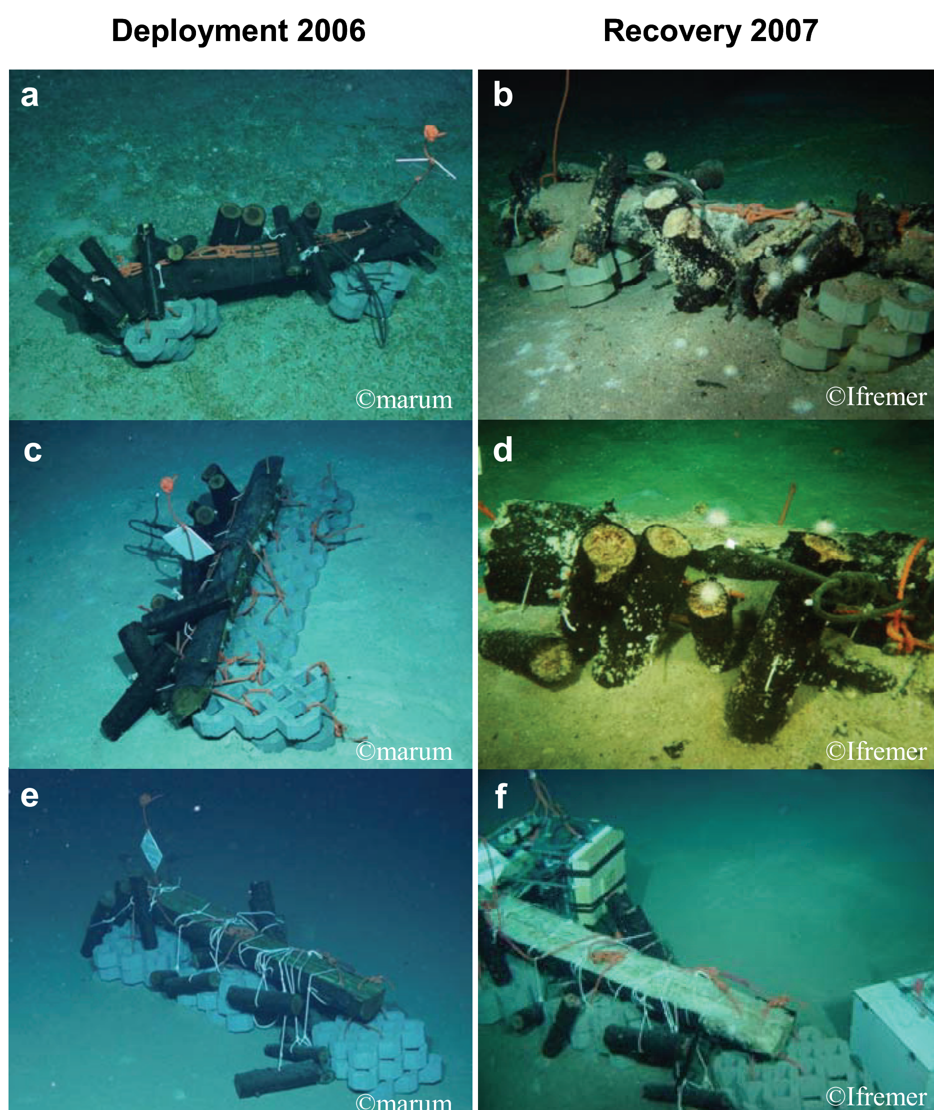

Figure 2 Wood experiments deployed in the Eastern Mediterranean deep sea during the BIONIL cruise in 2006 (RV Meteor) and recovered during the Medeco-2 cruise (RV Pourquoi Pas?) in 2007. a, b) Wood#1 close to carbonate crust, c, d) Wood#2 on carbonate crust, e, f) Wood#5 on sediment

3.2 Faunal diversity at wood falls

3.2.1 Wood-boring bivalves

Observations on board confirmed that a strong degradation of the wood logs had occurred during one year at the seafloor for all wood experiments and large numbers of animals had colonized the wood (Figure 3). The degradation of the wood was mainly due to the activity of wood-boring bivalves, identified as *Xylophaga dorsalis*, Turton, 1819 (T. Haga, National Museum of Nature and Science, Tokyo, pers. information); these organisms made up the main biomass of macrofaunal colonizers (Figure 3a). The size of the specimen ranged between 1–10 mm in shell size. *X. dorsalis* were able to colonize the wood from all sides, especially from the cut ends of the wood logs, but also from the sides and through the bark, indicated by lots of small holes and burrows on the sides of the wood. Even the most inner core (heartwood) showed burrows and individuals of *Xylophaga* in some logs.

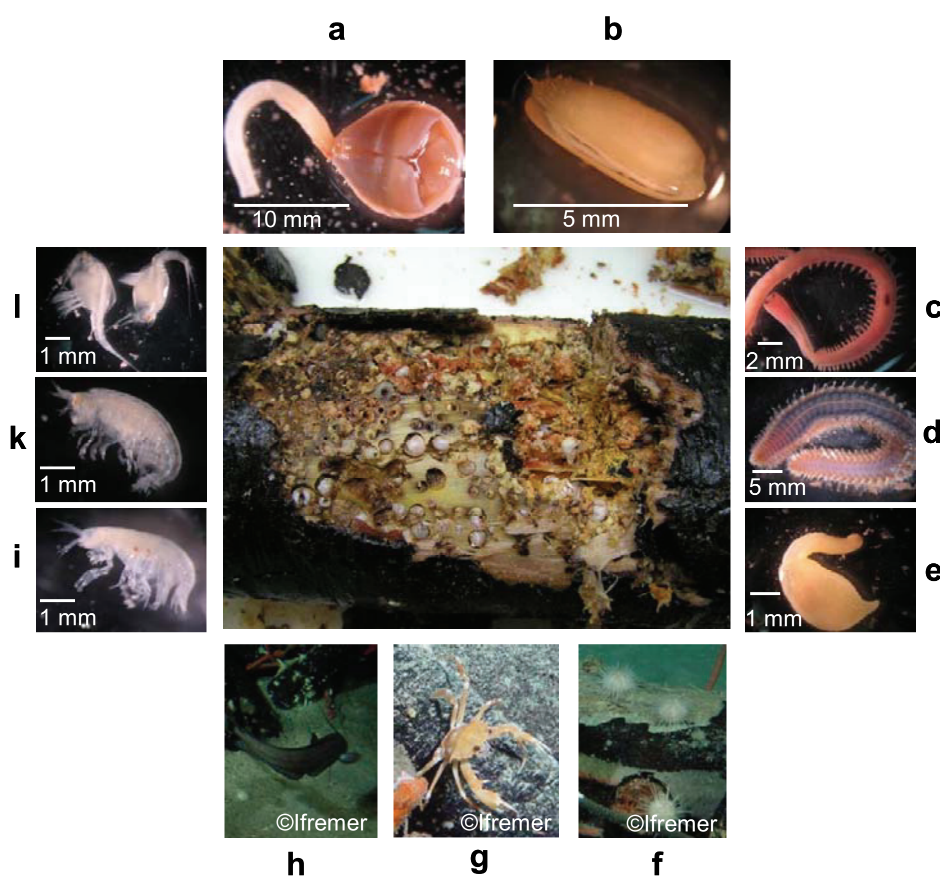

Figure 3 Macrofauna colonizing the wood experiments after one year at the sea floor. a) *Xylophaga dorsalis*, b) *Idas* sp., c) *Glycera noelae* sp. nov. d) *Cryptonome* gen. nov. *conclava*, n. sp., e) *Phascolosoma turnerae*, f) *Asterechinus elegans*, g) *Bathynectes piperitus*, h) unidentified deep-sea fish, i, k) unidentified species of amphipods, l) unidentified species of *Leptostracea*.

3.2.2 Chemosynthetic fauna

Small mussels of the chemosynthetic genus *Idas* were found on all wood experiments submerged for a year, in, on and directly underneath the bark (Figure 3b). Their shell lengths measured between 1 and 6 mm. Based on subsampling of wood log slices, we extrapolated that up to 150 individuals of *Idas* may have occurred on one small wood log of wood#1 and 30-90 individuals on a small wood log of wood#5.

3.2.3 Other fauna

Sea urchins, identified as *Asterechinus elegans* (N. Ameziane, Museum of Natural History, Paris, pers. communication), seemed to be chemically attracted to the wood, as their densities increased with decreasing distance to the wood experiments, e.g., for wood#2 at least 20 sea urchins were counted on and in the close vicinity of the wood (Figure 3f). In addition, crabs (Portunidae; *Bathynectes piperitus* Manning & Holthuis, 1981; B. Richer de Forges, Institut de recherche pour le développement (IRD), New Caledonia, pers. communication) were often observed on and under the wood (Figure 3g), which they probably used as shelter and source of food (e.g., by grazing on polychaetes). Other colonizing macrofauna included *Glycera noelae* sp. nov. [39], sipunculids identified as *Phascolosoma turnerae*, Rice 1985 (G.Y. Kawauchi, Department of Organismic and Evolutionary Biology, Harvard University, pers. communication), amphinomids being described as a new genus and species of Amphinomidae, *Cryptonome* gen. nov. *conclava*, n. sp. [40] and at least three groups of unidentified species of small crustaceans (Figure 3). There were no qualitative differences in the macrofaunal colonization of the three wood experiments that had been submerged for one year related to distance from the seep. No macrofauna was associated with the control wood#6 after less than 1 day of submergence.

3.3 Biogeochemical characterization

Sediment cores retrieved at (0.5 m) the wood experiments revealed a several centimetre (2–4 cm) thick layer of wood chips. The sediment surface below the wood chips was blackened (0–1 cm depth), indicating sulfide production and precipitation with iron. The subsurface sediments showed a brown to grey color (1–5 cm depth) (Figure 4). Control sediment cores (10 m away from wood) differed between experiment#1 and experiment#5: for experiment#1 cores were beige down to max. 4 cm, followed by light gray sediment, changing into dark gray at the bottom 6–8 cm of the cores. For experiment#5 sediments were brown down to 7 cm, then changing in to gray/dark gray sediments. These differences probably reflect biogeochemical differences in the two regions, with experiment#1 being located close to carbonate crusts, indicating an influence

of methane seepage in the past, and experiment#5 being located on pelagic sediments. Several biogeochemical measurements were conducted *in situ* and *ex situ* to describe the influence of the wood on surrounding sediments

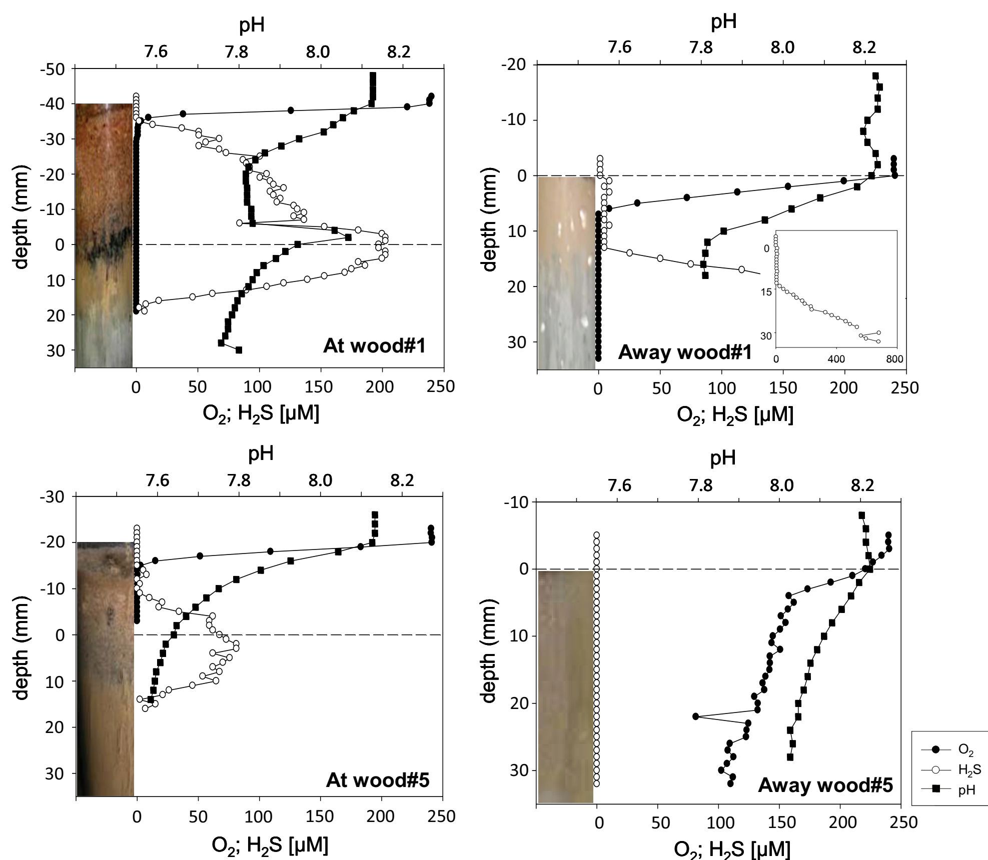

Figure 4 Ex situ microsensor measurements of oxygen, sulfide and pH at (0.5 m) and away (10 m) from wood experiments #1 and #5.

3.3.1 Sulfide, oxygen and pH measurements (ex situ) in the sediments

At both wood experiments (0.5 m) oxygen penetrated not more than 5–10 mm into the wood chip-covered seafloor (Figure 4, Table 2). Elevated concentrations of sulfide were detected at both sites, approximately coinciding with the black layer that was observed visually (3–4 cm depth below surface, former 0–1 sediment surface layer). Measured sulfide concentrations at these depths reached up to 200 µM at wood#1 and up to 80 µM at wood#5. In coherence with their proximity to the active seep, reference cores (10 m away from wood#1 and #5) displayed different profiles. Oxygen penetrated up to 5 mm deep in sediments from the reference of wood#1, whereas sediments away from wood#5 were fully oxygenated down to at least 32 mm. Sulfide concentrations reached a maximum of nearly 800 µM at about 3 cm sediment depth at the

wood#1 reference, and no sulfide was present in control sediments away from wood#5. Diffusive oxygen uptake calculated from oxygen microprofiles was higher at the wood experiments when compared to measurements away from the experiments and was lowest at the reference site away from wood#5 (Table 2).

Table 2 Summary of biogeochemical measurements at the wood experiments and at selected seep and reference sites. TOU: total oxygen uptake, DOU: diffusive oxygen uptake, OPD: oxygen penetration depth, H_2S flux: sulfide flux, SRR: sulfate reduction rate.

	TOU (mmol m^{-2} d^{-1})	DOU (mmol m^{-2} d^{-1})	OPD (mm)	H_2S flux (mmol m^{-2} d^{-1})	SRR (mmol m^{-2} d^{-1})
At wood#1 (0.5 m, ~ 4cm wood chips)	25	4.3	6.7	2.1	1.3
Away wood#1 (10 m)	1	2.3	6.7	2.7	2.5
At wood#5 (0.5 m, ~ 2 cm wood chips)	n.d.	4.4	5.0	1.0	1.9
Away wood#5 (10 m)	n.d.	1.0	> 32	0	0.1 [1]
Arcobacter mat (Pockmark)	71 [2]	27 ± 16 [2]	1.75 [2]	27 ± 16 [2]	9 – 112 [2]
Reference	1 [2]	0.5 ± 0.4 [2]	> 10 [2]	0 [2]	0.14 ± 0.04 [2]

1 Felden, unpubl. data (reference measurement during Bionil cruise 2006, approximately 400 m from wood#5)
2 Grünke et al., in press

3.3.2 Sulfate reduction and anaerobic oxidation of methane

Depth integrated sulfate reduction (SR) rates across 10 cm were 1.3 m^{-2} d^{-1} (at wood#1), 2.5 mmol m^{-2} d^{-1} (away wood#1) and 1.9 mmol m^{-2} d^{-1} (at wood#5), respectively. No samples were available for away wood#5. No anaerobic oxidation of methane (AOM) was detected at any of the sites, hence sulfide production was related to organoclastic sulfate reduction at all sites.

3.3.3 Total oxygen uptake (in situ)

Benthic chamber measurements were conducted at (0.5 m) and away (10 m) from wood#1. Total oxygen uptake (TOU) was higher at the wood (25 mmol m^{-2} d^{-1}), as opposed to 10 m away (1 mmol m^{-2} d^{-1}), evidencing a strongly increased activity of sedimentary communities close to the wood experiment.

3.3.4 Dissolved organic carbon and nutrient concentrations

Non-wood influenced pore waters showed dissolved organic carbon (DOC) concentrations around 300 µM. The wood chip – sediment boundary layer showed elevated DOC

concentrations of 2100 µM at wood#1 and 3000 µM at wood#5. There was no influence of the wood deposition on phosphate, silicate and nitrate concentrations, but ammonium showed elevated concentrations at the wood chip-sediment boundary layer (3.3–10.6 µM). Ammonium concentrations were similar away from wood#1 (1.9–11.3 µM) and there was no ammonium detected away from wood#5.

3.4 Characterization of bacterial communities

3.4.1 Bacterial cell numbers

Bacterial cell numbers of pure wood samples ranged between $2.8*10^8$ cells/g wood for wood#1 and #2 and $7.7*10^8$ cells/g wood for wood#1, and were considerably lower for the freshly submerged wood #6 ($5.6*10^6$ cells/g wood). The wood chips next to the experiments showed even higher cell numbers than the submerged wood with $1.2*10^9$ cells/g at wood#1 and $9.3*10^8$ at wood#5. Close to the seep area, integrated cell numbers across 10 cm sediment depth showed no significant difference between the wood#1 site and its 10 m reference, with $1.1*10^{10}$ cells/cm^2 and $1.2*10^{10}$ cells/cm^2, respectively (Figure 5). Cell numbers were an order of magnitude lower for wood experiment #5, with substantially higher cell numbers at the wood ($5.8*10^9$ cells/cm^2) compared to the reference ($4.7*10^9$ cells/cm^2). Cell numbers decreased with sediment depth in all cores.

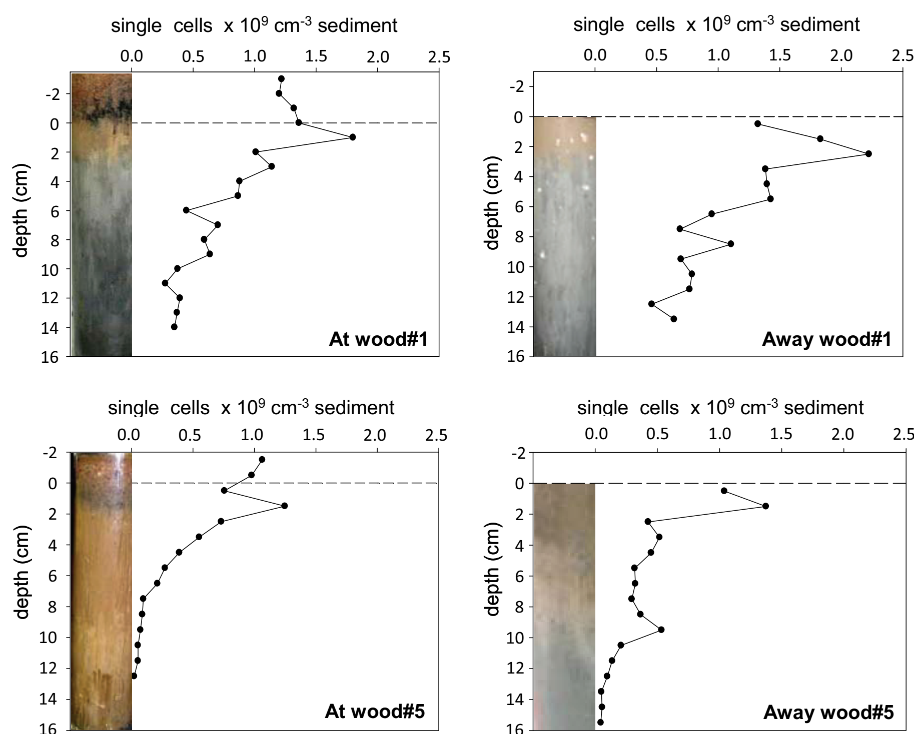

Figure 5 Total bacterial cell numbers of wood and surrounding sediment samples.

3.4.2 Bacterial community structure

Bacterial community structure determined with ARISA fingerprints showed significant differences between individual wood experiments and between wood compared with sediment samples (Figure 6, Table S1), indicating highly specialized assemblages colonizing the wood falls. The large number of replicate samples enabled us to differentiate between heterogeneity within a single wood experiment and differences between wood experiments from different locations. The most prominent differences were observed between wood experiments that had been submerged for one year when compared to the freshly deployed wood#6, indicating the development of autochthonous communities. Statistically significant differences were also observed between the community structures on the three wood experiments deployed in different locations in the Central Nile Deep Sea Fan. No consistent differences could be observed for wood samples from the surface or the inner part of the wood samples.

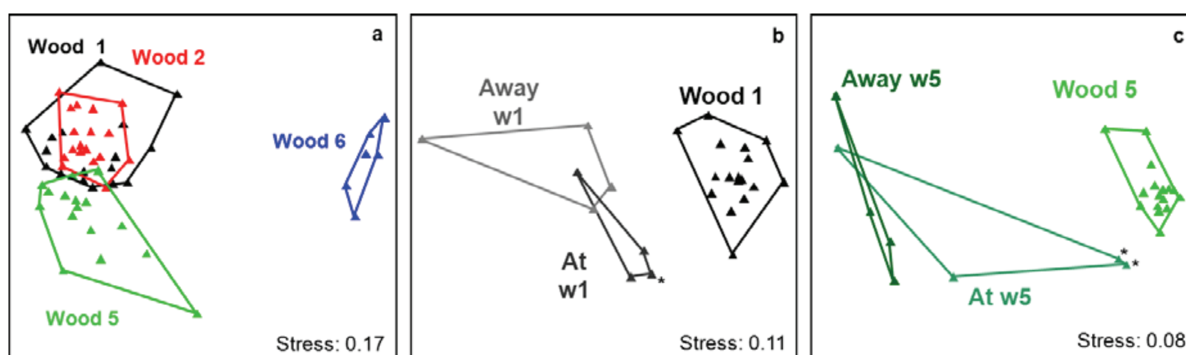

Figure 6 Non-metric multidimensional scaling (nMDS) ordination with Bray-Curtis distance. Each point represents the consensus of 2-3 replicate ARISA profiles. Colors and groupings indicate the origin of the samples. **a** includes a comparison only of the wood experiments, while **b** and **c** show bacterial community structure on the wood experiments in comparison to surrounding sediments at distances of 0.5 m and 10 m from the wood experiments. Asterisks (*) indicate the presence of wood chips in a sample.

3.4.3 Response of specific bacterial taxa to wood input

To identify taxa specifically colonizing and responding to the deposition of wood at the seafloor, we applied 454 massively parallel tag sequencing (MPTS) which enables sequencing of samples at a very high resolution as well as taxonomic classification of sequences [36,37]. Patterns of the relative contributions (relative sequence abundances) of phyla and classes to the overall community clearly differed between the "fresh" control wood and the woods submerged for one year, as well as between woods and sediments (Figure 7, Figure S1). *Proteobacteria*-affiliated sequences dominated in all woods and sediments. The phyla *Actinobacteria*, *Bacteroidetes* and

Firmicutes showed higher relative sequence-abundances at the woods submerged for a year. At the taxonomic resolution of class, the fresh wood (#6) was dominated by *Gammaproteobacteria*, which accounted for most of the proteobacterial sequences (Figure 7, Table 3). In the woods submerged for one year, *Alphaproteobacteria*, *Flavobacteria*, *Gammaproteobacteria*, *Actinobacteria*, and *Clostridia* were present in higher relative abundances. Among the ten most common genera in the freshly submerged wood#6, sequences affiliated to *Pseudoalteromonas*, *Vibrio*, *Burkholderia*, *Pseudomonas*, *Erwinia* and *Ralstonia* were observed. In contrast, woods submerged for one year were dominated by sequences affiliated to the genera *Demequina*, *Conchiformibius*, *Blastopirellula*, *Desulforhopalus*, *Thalassobacter*, and *Iamia*. Wood-chip covered sediments were dominated by *Clostridia*, *Gammaproteobacteria*, *Planctomycetacia* and *Deltaproteobacteria* (Table 3). The most common genera of the active wood-chip-sediment boundary layer included *Coxiella*, *Ralstonia*, *Methylobacterium*, *Reichenbachiella* and *Desulfobacula*.

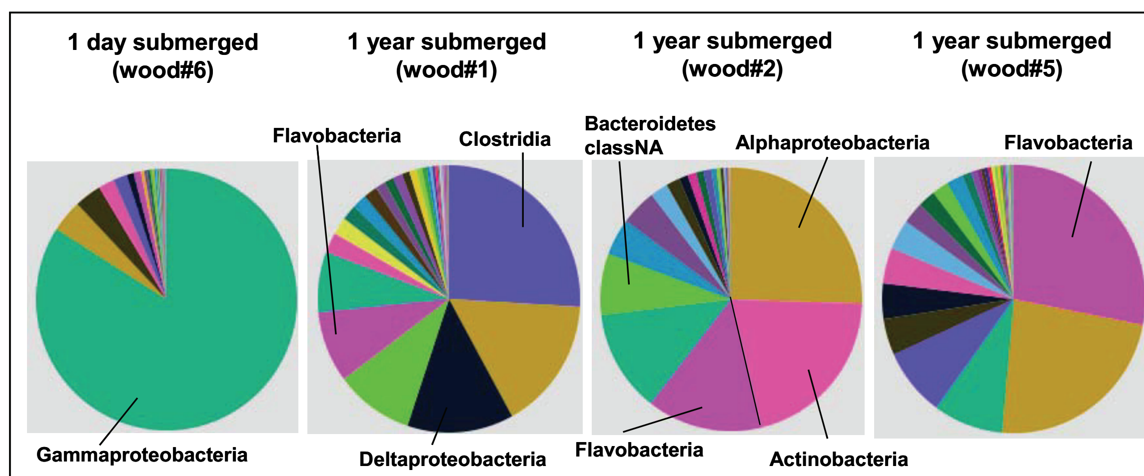

Figure 7 Bacterial community composition of the wood experiments at the class level.

As to the comparison between wood influenced sites and reference sediments, the latter showed a high proportion of *Acidobacteria and Actinobacteria*. *Bacteroidetes* and *Firmicutes* were present with higher relative abundances at and on the woods. The reference sediments showed differences in composition between the oxygen-limited location (*Deltaproteobacteria*, *Holophagae* and *Epsilonproteobacteria)* and the oxic sediments (*Actinobacteria* and *Alphaproteobacteria)* (Table 3, Figure S1). On the class level it was mostly *Clostridia*, *Alphaproteobacteria* and *Planctomycetacia* that showed higher relative abundances at wood#1, and *Flavobacteria*, *Alphaproteobacteria* and *Deltaproteobacteria* that showed higher relative abundances at wood#5.

Table 3 Most common bacterial classes in decreasing order of their relative sequence abundances in wood experiments submerged for 1 day or 1 year, and in wood-influenced or non-wood influenced sediments.

Wood, 1 day submerged (wood#6)	Woods, 1 year submerged (wood#1, #2, #5)	Sediments at wood experiments (0.5 m)	Sediments (oxygen-limited)	Sediments (oxic)
Gammaproteobacteria	Alphaproteobacteria	Clostridia	Gammaproteobacteria	Gammaproteobacteria
Alphaproteobacteria	Flavobacteria	Gammaproteobacteria	Deltaproteobacteria	Acidobacteria
Betaproteobacteria	Gammaproteobacteria	Planctomycetacia	Acidobacteria	Actinobacteria
Actinobacteria	Actinobacteria	Deltaproteobacteria	Holophagae	Alphaproteobacteria
Deltaproteobacteria	Clostridia	Alphaproteobacteria	Epsilonproteobacteria	Holophagae
Flavobacteria	Sphingobacteria	Actinobacteria	Actinobacteria	Deltaproteobacteria
Clostridia	Deltaproteobacteria	Acidobacteria	Clostridia	Planctomycetacia
Sphingobacteria	Bacteroidia	Betaproteobacteria	Betaproteobacteria	Betaproteobacteria
Acidobacteria	Planctomycetacia	Holophagae	Acidobacteria	Gemmatimonadetes
Bacteroidia	Verrucomicrobia	Sphingobacteria	Alphaproteobacteria	Bacilli

4. Discussion

4.1 Faunal diversity at wood falls

4.1.1 Wood-boring bivalves

Wood-boring bivalves (*Xylophaga dorsalis*) had colonized the wood in large numbers after one year and apparently played a key role in the microbial ecology and biogeochemistry of sunken woods (Figure 3a). They were responsible for the rapid degradation and dispersal of wood chips around the wood log, which lead to the emergence of sulfidic zones. In addition, *X. dorsalis* provided colonization surfaces for other organisms that were attracted to the wood, such as polychaetes and sipunculids. Neither one was yet considered to bore into wood or use wood as a nutritional source. Wood-boring bivalves may therefore be considered a keystone species in wood fall habitats, as they transform the energy stored in the wood into nutrients that can be digested by other animals, either in the form of fecal pellets that are used by detritus-feeders or as flesh or carrion used by predators and scavengers [2]. *Xylophaga* depend on wood and they must be able to quickly localize and exploit small specific habitats that are widely scattered on the ocean floor [2]. Tyler and colleagues [41] have described the settlement, growth and population dynamics of *X. depalmai*, but it remains unclear how *Xylophaga* larvae and/or adults detect the presence of wood in the deep sea. A similar wood colonization experiment at the Logatchev vent field (Mid-Atlantic ridge) showed massive population growth of *Xylophaga* and suggested the presence of larvae ~2000 km away from the nearest coast (C. Borowski, pers. communication). From our experiments we can deduce that strong colonization and growth took place within one

year. Based on earlier observations, it is likely that colonization by wood-borers occurs within a few months of wood deposition [2,41,42].

The genus *Xylophaga* (family *Pholadidae*) is known to include opportunistic species that colonize wood at depths greater than 150 m [43]. They are the deep-water counterparts of bivalves of the family *Teredinidae* (also known as "shipworms") that colonize and ingest wood in shallow waters. *Teredinidae* and *Xylophaga* both have endosymbiotic bacteria in specialized cells located in the gills [43,44]. For shallow water *Teredinidae*, symbionts have been characterized as cellulolytic and nitrogen-fixing *Gammaproteobacteria* [45,46] but little is known about the deep-sea *Xylophaga* and their symbionts. Our 454 MPTS dataset revealed sequences affiliated to the 16S rRNA sequence of *Teredinibacter turnerae* that has been isolated from the gills of teredinid species and that has been cultured and described as a cellulolytic nitrogen fixing gammaproteobacterium [46]. These sequences occurred in higher relative abundances in woods submerged for one year while they were virtually absent in wood#6 and in sediment samples away from the wood experiments. *Teredinibacter turnerae* lives as an intracellular endosymbiont in the gills of wood-boring bivalves of the family *Teredinidae* [47]. It probably possesses cellulases and nitrogenase to convert the cellulose in the wood into digestible carbon and supplement the nitrogen-deficient wood-diet of its host. An initial phylogenetic characterization based on 16S rRNA gene clone libraries of bacterial symbionts associated with *Xylophaga* from this study, showed that a majority of clone sequences clustered with those of *Teredinidae* symbionts (C. Borowski, pers. communication), suggesting a close phylogenetic relationship between symbionts of the shallow and deep-sea wood-boring bivalves. It is not clear however whether sequences obtained by 454 MPTS analysis originated from free-living bacteria in the wood or from bacteria associated with *Xylophaga* or other macrofauna that may have been crushed during sampling.

4.1.2 Chemosynthetic organisms

Small chemosynthetic mussels of the genus *Idas* were mainly found on or underneath the bark of wood pieces (Figure 3b). The same genus has been collected from carbonate crusts at active seeps as well as from other wood colonization experiments in the same study area [4,12]. It is known to harbour a variety of bacterial symbionts including sulfide oxidizers [12,48]; these mussels may therefore serve as indicators of sulfidic conditions in their environment. The higher number of *Idas* individuals found on wood#1 (located close to carbonate structures) may indicate that mussels colonize the wood from carbonate crusts in the Pockmark area, but this remains speculative due to the limited number of experiments. Members of the genus *Idas* have also been found on wood falls in other oceanic regions [49,50], as well as in association with whale

carcasses [5,51], and hydrothermal vents [52], and therefore seem to be cosmopolitan among chemosynthetic ecosystems in the deep sea.

4.1.3 Other fauna

Sea urchins (*Asterechinus elegans*) seemed to be attracted to the wood (Figure 3f) and have also been reported from wood falls in the West-Pacific region [53]. Earlier observations of their gut content have indicated that they are a wood-feeding species and that they might have a microbial community associated with their gut content that would be able to support a digestion of wood [53]. In our specimen we also observed wood particles associated with the gut content. Only few amphinomids have yet been recorded from the deep sea [54,55], but they have been found at hydrothermal vents [56,57] and seeps [58]. To our knowledge this is the first record from wood falls (Figure 3d). The sipunculid species *Phascolosoma turnerae* was originally described as a deep-water species found in association with submerged wood, occupying burrows in the wood [59]. Members of this genus have also been reported from seeps [60] and whale carcasses [3,61]. It is not clear, whether the sipunculans enter *Xylophaga* burrows or whether they are able to produce their own burrows [59], but sipunculans have been described to bore into other structures like calcareous rock [62]. In our experiments we could not find indications that sipunculids were able to bore into the wood. It is most likely that *Phascolosoma* used the burrows produced by the wood-boring bivalves as shelter, because neither a mechanism for burrowing into wood nor the ability to utilize wood as a food source is known for sipunculans (Figure 3e). It is likely that also the other macrofaunal organisms, e.g. the polychaete *Glycera noelae* sp. nov. (Figure 3c) and the amphinomid *Cryptonome* gen. nov. *conclava*, n. sp. (Figure 3d) used the burrows of the wood-boring bivalves to enter the wood.

While *Xylophaga* are likely endemic to wood falls in the deeper ocean, several of the macrofaunal organisms recovered from our experiments are shared with whale falls, seeps and/or hydrothermal vents, indicating that these ecosystems may share a close evolutionary history for part of their faunal component [3]. Wood falls present hotspots of diversity in the deep sea, attracting a variety of fauna utilizing the wood for different purposes (nutrition, shelter, grazing). It is yet unclear how organisms localize wood falls in the vast deep-sea environment (e.g., chemical cues like presence of organic matter, degradation products) and what their reproductive and dispersal strategies are.

4.2 Development of sulfidic niches at wood falls

Very few organisms can degrade wood and use it as energy and carbon source. Furthermore, it is known that sunken ships preserve well in cold, salty, anoxic environments [63]. In our experiments, the degradation activity of wood-boring bivalves of the genus *Xylophaga* led to the accumulation of a several centimeter (2–4 cm) thick layer of wood chips in the immediate vicinity of the wood logs, facilitating the microbial production of sulfide. Diffusive and total oxygen uptake rates indicated an increased respiratory activity of sedimentary communities due to the degradation of organic matter derived from the wood falls. The development of anoxic zones subsequently enabled sulfide production by sulfate reducing bacteria, and enhanced sulfide fluxes around the wood experiments. This process could best be observed at wood#5 which was located on pelagic sediments, where oxygen penetrated several centimeters into the sediment and no sulfide was present at the reference location (Figure 4). Wood#1 in contrast was located in an oxygen-limited location, where sulfide was present in control sediments 10 m away from wood#1. Nevertheless, the addition of wood changed the sedimentary environment in that the sulfide maximum shifted from about 4 cm sediment depth to the surface, into the transition zone between wood chips and sediment. Interestingly, the strongly elevated concentrations of dissolved organic carbon at the wood-chip sediment contact zone indicated that cellulose degradation was highest under anoxic conditions and hence enabled by anaerobic benthic bacteria, e.g., fermenters and sulfate reducers. This was confirmed by measurements of sulfate reduction, which also peaked at the wood chip-sediment contact zone. These observations demonstrate that, after one year, the presence of wood at the seafloor has led to the creation of sulfidic niches, comparable to what has been observed at whale falls [6,51].

4.3 Comparison with other chemosynthetic ecosystems

The higher total oxygen uptake at wood#1 (0.5 m) when compared to the reference site 10 m away from the wood, indicated an increased activity of sedimentary communities around the wood falls. In comparison to other sites in the Nile Deep Sea Fan, these values were in the range of those at cold seeps at the rim of the Amon mud volcano [64], or on the border of bacterial mats in the studied Pockmark area [65] (Table 2). Total oxygen uptake rates at the wood experiments were similar to values reported for a whale fall in the Santa Cruz Basin, California, that, in contrast to our wood falls had been submerged for 6–7 years [6].

Sulfide fluxes were elevated at both wood experiments when compared to reference measurements, but were an order of magnitude lower than values measured in a bacterial mat [65]. The measured rates of sulfate reduction (SR) at the wood experiments were higher

compared to reference measurements in the Pockmark area performed during an earlier expedition (Meteor M 70/2 Bionil 2006, 0.1 mmol m^{-2} d^{-1}). Sulfide concentrations at the wood experiments were higher than concentrations measured 1 m away from a whale fall, but an order of magnitude lower than values reported from below a bacterial mat located directly at a whale fall [6]. Sulfide fluxes and sulfate reduction rates reported from other chemosynthetic habitats at cold seeps vary widely, and the rates measured here would fall into the lower range of sulfate reduction rates previously observed [19,65,66,67]. No measurements of oxygen and sulfide were conducted on the wood itself, but also here microoxic/anoxic conditions may develop [7], and the presence of the chemosynthetic mussel *Idas* sp. may be an indicator for this. Hence, our measurements demonstrate that over a period of one year, wood falls in the deep sea can lead to the development of biogeochemical conditions similar to those of other chemosynthetic habitats.

4.4 Bacterial communities

The degradation of submerged woods as a source of energy and carbon requires complex enzymatic transformation of the macromolecular matter by adapted microbial communities [63,68]. Little is yet known about the microbial degradation of cellulose - the second most abundant carbohydrate in the sea and the most abundant on land. In the context of cellulose degradation for biofuels, the potential discovery of novel bacterial types and enzymes adapted to high salinity may even be of interest for industrial applications [69]. In our case, wood degradation appeared to be substantially aided by the activities of wood boring *Xylophaga*, dispersing the wood logs into small chips offering a large surface for further microbial degradation. Accordingly, we observed a substantial increase in cell numbers in the wood within a year, from the freshly submerged woods with low numbers of $5.6*10^6$ cells/g to the wood chip layer with $9.3*10^8$–$1.2*10^9$ cells/g. Next we attempted to identify the most abundant taxa associated with the submerged wood and especially the reactive contact zone between wood chips and the sediments.

The freshly deployed wood (1 day submerged) was dominated by sequences affiliated to *Gammaproteobacteria* (Figure 7). The most common genera indicated both a marine signature, with *Pseudoalteromonas* (strains of which have been shown to produce cellulase) and *Moritella* among the five most common genera, but also a terrestrial signature with typical plant-associated genera like *Burkholderia* (*Betaproteobacteria*), *Erwinia* (*Gammaproteobacteria)* and *Ralstonia* (*Betaproteobacteria*). In woods submerged for one year, *Alphaproteobacteria*, *Flavobacteria*, *Actinobacteria*, and *Clostridia* showed higher relative abundances when compared with the fresh wood. These groups comprise several cellulolytic taxa: currently, the highest proportion of isolated cellulolytic bacteria are

found within the *Firmicutes* (which include *Clostridia*) and *Actinobacteria* [69]. *Clostridia* are obligate anaerobes and may indicate the presence of anoxic conditions in parts of the wood logs and chips that have developed due to wood degradation. The most common genus in woods submerged for one year was *Demequina* (*Actinobacteria*), for which an isolate (*Demequina aestuarii*) was shown to be closely related to *Cellulomonas fermentans* [70], allowing speculations that *Demequina* might play a role in wood degradation in the deep sea. Other observations of distinct genera included the recovery of sequences affiliated with *Teredinibacter* (see Xylophaga section), as well as sequences affiliated with the genera *Cellulophaga* (*Flavobacteria*) [71,72] and *Phycisphaera* (*Planctomycetes*) [73]. Both genera have been previously isolated from marine algae, and were found in high relative abundances in the submerged woods, but barely in the reference wood and sediments, indicating that they may play a role in the degradation of woody material in the deep sea. Furthermore, *Bacteroidetes* and *Firmicutes* had higher relative abundances at and in the wood, whereas relative sequence abundances of *Acidobacteria* and *Actinobacteria* were highest in reference sediments away from wood experiments (Table 3, Figure S1). *Bacteroidetes* may be responsible for the breakdown of a major fraction of complex organic matter [74,75] and both *Bacteroidetes* and *Firmicutes* have been recovered from whale-fall influenced sediments [76]. *Deltaproteobacteria* belonged to the five most common taxa both at the wood experiments as well as at the oxygen-limited site away from wood#1, and included sulfate reducing members of the families *Desulfobacteraceae* and *Desulfobulbacea,* which were also previously shown to be abundant at a whale fall and cold seeps [76,77,78]. *Acidobacteria* and *Actinobacteria* are typical representatives of deep-sea sediments and *Acidobacteria* are known to prefer oligotrophic conditions [79,80,81] which is consistent with their presence in undisturbed reference sediments. The relative importance of *Epsilonproteobacteria* at the oxygen-limited site #1 close to carbonate crusts, is consistent with their dominance at seep and vent ecosystems [82,83,84]. Though *Epsilonproteobacteria* have also been found in association with whale falls [76], we could not confirm their relative importance at wood falls.

Previous studies on natural and experimental wood falls have reported high similarities between bacterial communities on woods at geographically distant sites and have identified the type of wood and duration of immersion as the most important factors structuring sunken wood bacterial communities [13]. Our results showed that even the same type of wood, deployed for the same amount of time selects for differently structured bacterial communities. It remains unknown, whether bacterial colonization takes place from the sediments and/or from the water column. Further studies are required to decipher the factors determining the temporal succession of bacterial communities at wood falls in the deep sea, and to identify the most abundant

functional groups in enzymatic cellulose degradation, fermentation and reduction of sulfate to sulfide, supporting chemosynthetic life.

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Supplementary information

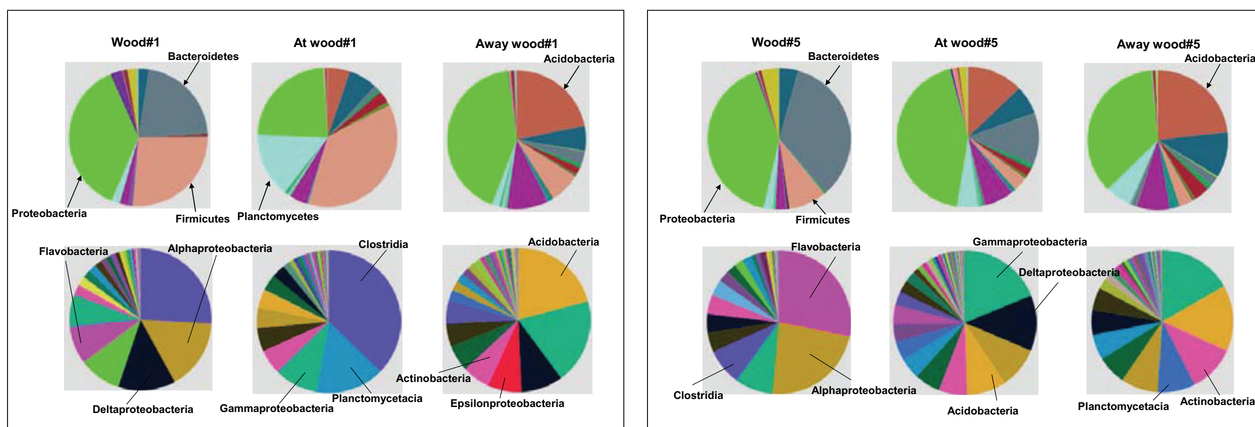

Figure S1 Bacterial community composition of wood experiments and sediments around the wood experiment at the phylum and class level.

Table S1 Analysis of similarity (ANOSIM), testing for significant differences in bacterial community structures between the wood experiments as well as sediments around the wood experiments. *p<0.05, **p<0.01, ***p<0.001 after Bonferroni correction; (*) only significant without Bonferroni correction.

	Wood#1	Wood#2	Wood#5	Wood#6	At wood#1	Away wood#1	At wood#5
Wood#1							
Wood#2	0.48***						
Wood#5	0.2 ***	0.47***					
Wood#6	1***	1***	1***				
At wood#1	0.94**	0.98**	0.90**	0.98(*)			
Away wood#1	0.99**	0.99***	0.94**	1(*)	0.63		
At wood#5	0.96***	0.97**	0.85**	0.81(*)	0.21	0.42	
Away wood#5	1**	1**	1**	1(*)	0.79 (*)	0.52 (*)	0.27

3.

Discussion & Perspectives

The deep sea presents the largest ecosystem on Earth and bacterial communities play a crucial role in the recycling of carbon and nutrients at the seafloor, linking the deep-sea ecosystem to the global biosphere. The remoteness of the deep sea has long hindered its exploration, and still makes research at the deep seafloor technologically challenging and expensive. Owing to these difficulties and to the lack of appropriate molecular techniques until two decades ago, little was known about bacterial diversity and its distribution at the seafloor when I started this thesis. For the thesis it was possible to use an existing deep-sea sample repository covering major oceanic regions, with contextual information available for many of the samples. Furthermore, the latest underwater technology was used to sample and document wood colonization experiments in the deep sea. The application of ARISA fingerprinting and state-of-the-art next-generation sequencing enabled a high throughput and deep sequencing of samples, in order to characterize bacterial community patterns in deep-sea sediments. The implementation of ecological multivariate statistics allowed the subsequent interpretation of bacterial community data in conjunction with contextual parameters. The combination of these techniques could be successfully applied to address ecological questions and may continue to advance our knowledge on microbial community ecology in the future. The results presented in the chapters of this thesis demonstrate that benthic bacterial communities are not randomly distributed across the seafloor. They were rather structured across geographic distance, indicating dispersal limitation, and were strongly influenced by energy availability in the form of phytodetritus or large organic food falls at the seafloor. Furthermore, bacterial functions such as extracellular enzyme activities and carbon remineralization rates varied with energy availability and were correlated to community structure, suggesting a tight coupling between community structure and functions.

3.1 Dispersal limitation

The high endemism of bacterial types (OTUs defined at 3% sequence difference) and the strong distance-decay relationship, i.e., the decrease in shared bacterial types with increasing distance, observed in Chapter I may be due to a lack of physical mixing in the benthic deep-sea environment. However, the observed relationships with geographic distance may to some extent also be caused by spatially structured environmental parameters. In a first approach to incorporate environmental parameters in our global-scale assessment of bacterial biogeography, I tested the effects of surface productivity and total organic carbon concentration at the seafloor on variations in bacterial diversity (Figure 8). As proxies for these parameters, I used productivity indices based on biogeochemical provinces defined by Longhurst et al. (1995) (http://www.vliz.be/vmdcdata/ vlimar/downloads.php), and oceanographic regions based on

total organic carbon measurements (Seiter et al., 2004). A partitioning of the biological variation between geographic distance, water depth, surface productivity and total organic carbon content confirmed a significant effect of geographic distance on bacterial community structure and composition, even when taking the other environmental parameters into account (Figure 8). However, especially differences in total organic carbon concentration (food availability) at the seafloor were evidenced to play an important role in shaping bacterial communities. Surface productivity could not explain any of the variation in bacterial community composition and structure. Discrepancies between surface productivity and total organic carbon availability at the seafloor may be explained by biological processes or hydrographic features that would influence particle flux or by a lateral input of organic material, for example. The result is in agreement with general trends in the deep sea that have identified the availability of organic material as a major determinant for the diversity and biomass of different benthic size classes in the deep sea (Levin et al., 2001; Rex and Etter, 2010; Smith et al., 2008) and is further confirmed at the regional scale (7-500 km) in Chapter III. In general, the influence of both space and environmental conditions on bacterial communities is also suggested for intermediate scales (10–3000 km) in the ocean-wide analysis of bacterial communities in three deep-sea basins of the South Atlantic (Chapter II), and has as well been proposed in other studies of microbial biogeography (Ramette and Tiedje, 2007).

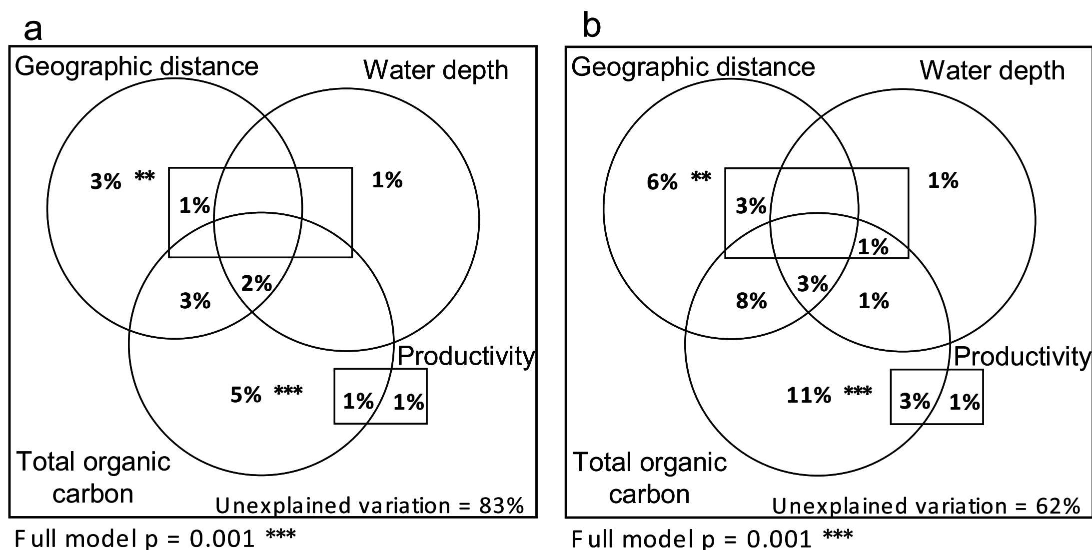

Figure 8 Partitioning of the biological variation in bacterial community composition (a) and structure (b) at the $OTU_{0.03}$ level (OTUs defined at 3% sequence difference) between several contextual parameters: geographic distance between samples, water depth, TOC availability (TOC regions based on Seiter et al. 1994) and surface productivity (Longhurst productivity index based on Longhurst et al. 1995). *** p = 0.001, ** p = 0.01.

The spatial distribution of bacterial and other benthic organisms in the deep sea may be influenced by a number of other factors (e.g., geographic barriers, geological history, deep-water currents) that will require further investigation. Geological structures, for instance, have been shown to limit the dispersal of macrofaunal organisms (Brandt et al., 2005). However, this observation could not be confirmed for bacterial communities where the Walvis Ridge in the South Atlantic was tested as a potential biogeographic barrier (Chapter II). Nevertheless, other, larger topographic features such as the Mid-Atlantic Ridge, or hydrographic features may restrict bacterial distribution. The global-scale study (Chapter I) provided first indications that deep-water currents and water masses may to some extent influence the distribution of bacterial communities in the deep sea. This has also been proposed for larger benthic fauna, many of which have larval stages, which may facilitate dispersal with deep-water currents (Levin et al., 2001; McClain and Hardy, 2010). This assumption would have to be systematically tested by accounting for the strength and direction of deep-water currents that may lead to a directional transport of cells (McClain and Hardy, 2010). Also, the geological history of deep-sea basins may offer clues about possible historical effects on the evolution of different bacterial deep-sea populations (McClain and Hardy, 2010). In the future, a better coverage of deep-sea sediment samples will be needed to improve our ability to statistically test distribution patterns at the global scale.

3.2 Energy availability at the seafloor

Energy availability has been identified as a major structuring factor for benthic organisms, e.g., megafauna and macrofauna, in the deep sea. Here, we show that distinct patterns could also be observed for bacterial alpha- and beta-diversity along gradients of phytodetritus input, as an important source of energy in the deep sea (Chapter III). This emphasizes the general significance of organic matter input for the structuring of benthic deep-sea communities. Our study extends observations of bacterial energy-diversity relationships, primarily obtained from experimental studies (Eiler et al., 2003; Horner-Devine et al., 2003; Riemann et al., 2000), to complex natural bacterial communities in deep-sea sediments. Noticeably, different taxa showed specific responses to changes in phytodetritus input. Some appeared to be opportunistic showing high relative sequence abundances at high levels of phytodetritus availability (e.g., *Gammaproteobacteria*), while others seemed to be adapted to low levels of energy supply (e.g., *Acidobacteria*), which may imply differing ecological strategies of these bacterial groups. Distinct adaptations would have to be confirmed on the genetic and physiological level. Eventually, it

could lead to the definition of indicator taxa for different environmental states, which may be helpful in environmental monitoring studies.

Furthermore, the observed relationship of bacterial alpha-diversity with energy availability, i.e., increasing or hump-shaped curves (Chapter III), indicate that bacteria may exhibit patterns similar to the ones observed for larger benthic size classes in the deep sea (Rex, 1981; Rex and Etter, 2010). Hence, some ecological concepts may universally apply to different domains of life (Martiny et al., 2006; Smith, 2007). However, direct comparisons between bacterial and macrofaunal diversity patterns are restricted by fundamental conceptual differences such as the "species" definitions for animals and bacteria, with that of bacteria being much broader than the one for animals and moreover still a matter of debate (Cohan, 2002; Fraser et al., 2009; Rossello-Mora and Amann, 2001; Staley, 2006). Instead, it may be more important to investigate possible relationships between bacterial and animal communities in the future. Distributions of bacterial and macro- and meiofaunal organisms may be linked through direct or indirect interactions, such as macrofaunal bioturbation, bioirrigation, and defecation. Other effects that may significantly influence bacterial community structure are grazing by nanoflagellates (Danovaro et al., 1998; Lebaron et al., 1999; Lindstrom, 2000), or viral infection (Danovaro and Serresi, 2000; Schwalbach et al., 2004). In the latter cases, simplified and controlled laboratory experiments might be more helpful to yield insights into such kinds of interactions.

Functions such as extracellular enzyme activity and carbon remineralization rates were also highly correlated to changes in phytodetritus availability and bacterial community structure. A close association between community structure and functional patterns has also recently been reported elsewhere (Teske et al., 2011), and implies that community structure may to a certain extent predict functionality. However, further studies of natural and experimental systems will be needed to describe and understand relationships between community composition/structure and associated ecosystem functions (e.g. Gravel et al., 2010). This will be key to a better understanding of how ecosystems will change in response to global environmental changes, which may have profound impacts on the world's marine ecosystems (Hoegh-Guldberg and Bruno, 2010). For example, the projected overall decrease in surface productivity (Behrenfeld et al., 2006; Steinacher et al., 2010) will supposedly lead to changes in particle flux and organic matter availability at the deep seafloor. Based on our results, we would predict that these changes will likely affect bacterial community structure and function which could in turn affect key processes such as carbon cycling (Deming and Baross, 1993; Klages et al., 2003). Most deep-sea sampling campaigns provide snapshots of the investigated locations at a given point in time. Changes in organic matter availability, for example, may also occur on temporal scales, and have

been shown to cause seasonal and interannual variations in benthic bacterial communities (e.g. Boetius et al., 2000; Pfannkuche et al., 1999). Only few long-term observations are available for the deep seafloor, e.g., the HAUSGARTEN in the Arctic (Soltwedel et al., 2005) and Station M in the North Pacific (e.g. Ruhl et al., 2008), but time-series studies will be an essential tool to better assess the potential effects of future climate scenarios (Glover et al., 2010).

3.3 Wood falls as a special energy source in the deep sea

The deposition of wood at the seafloor attracted a variety of organisms, some of which may be endemic to this habitat, e.g., wood-boring bivalves of the genus *Xylophaga* (Chapter IV). The degradation activity of wood-boring bivalves led to the accumulation of wood chips around the wood log, promoting the establishment of anoxic zones and the subsequent production of sulfide by the activity of sulfate-reducing bacteria, eventually leading to the creation of sulfidic niches. Bacteria are also likely responsible for a major part of the decomposition of the wood. Alongside fungi, some bacterial groups are known to be capable of degrading cellulose and lignin (El-Hanafy et al., 2007; Lynd et al., 2002; Nishimura et al., 2006; Pometto and Crawford, 1986), in contrast to animals that can only utilize wood in association with symbiotic partners. Although fungi have been reported from a variety of habitats in the deep sea, e.g., sediments, hydrothermal vents, and the subsurface biosphere (Burgaud et al., 2009; Edgcomb et al., 2011; Nagano et al., 2010; Raghukumar et al., 2010), it is likely that in marine environments bacteria play a larger role for the degradation of wood than fungi (Huisman et al., 2008; Landy et al., 2008). This will have to be further confirmed in future studies of fungal diversity and their metabolic potential on wood falls in the deep sea.

The development of sulfidic niches at the wood falls attracted chemosynthetic fauna, e.g., the chemosynthetic mussel *Idas* sp., and thus supports ideas of the role of food falls as stepping stones for organisms between chemosynthetic habitats in the deep sea. Also, some of the other faunal and bacterial types on the wood have been reported from a variety of chemosynthetic habitats, i.e., whale falls, vents and seeps (Chapter IV). Little is known on what might attract organisms to these specialized habitats, and what their reproductive and dispersal strategies are, as the occurrence of food falls is unpredictable in time and space. The presence of organic matter and degradation products are examples of chemical cues that may enable organisms to detect these habitats.

Furthermore, it remains unclear from where bacteria colonize the wood. Part of the community may be allochthonous and be brought down with the wood itself, but it is unlikely that terrestrial, plant-associated bacteria would be able to survive or stay active in marine water,

under high pressure and cold temperatures. This was also indicated by a low proportion of shared OTUs between the control wood (submerged for 1 day), and the wood experiments (submerged for 1 year), i.e., only 1–2% of OTUs defined at 3% sequence difference were shared between the control and the experiments (data not shown). Colonization may take place from the water column and/or the sediment. In the future detailed analyses of shared bacterial types between wood, sediments and water column may give information about the origin of wood-colonizing bacteria.

As yet, the further succession of colonization and biogeochemical gradients at the wood fall experiments remains unknown, but will be investigated in the future. First observations of our wood colonization experiments after three years at the seafloor indicated a strong decline in the *Xylophaga* population (C. Bienhold, pers. observation). Nevertheless, the slow degradation of the wood may continue to influence the area for several years to come, changing biogeochemical properties and local diversity. For example, depending on the size of the whale, whale-fall ecosystems can persist as a distinct habitat for several decades (Smith and Baco, 2003). The frequency of wood falls to the deep seafloor remains largely unknown. Considering that they have been recovered from all oceans (Wolff, 1979), they may as a whole significantly add to overall diversity in the deep sea by providing specialized niches. In addition, they may serve as "playgrounds" for the discovery of new organisms (see additional contributions to publications: Böggemann et al. 2011, Borda et al. in preparation). Also novel enzymes may be discovered that would be able to perform under high pressure and/or anoxic conditions in these specialized habitats. Here, the application and further development of novel tools such as metaproteomics (Wilmes and Bond, 2006) will aid our ability to detect proteins and pathways that may also be of interest for biotechnological applications (Carere et al., 2008).

3.4 Comparison of bacterial communities in the deep-sea sediments and other environments

The major bacterial taxonomic groups observed in our global comparison of **deep-sea sediments**, were *Gammaproteobacteria*, *Deltaproteobacteria*, *Actinobacteria*, *Alphaproteobacteria*, *Planctomycetacia*, and *Acidobacteria* (Figure 9a). Overall, this is in agreement with previous studies of deep-sea sediments (Table 1, Orcutt et al., 2011). Nonetheless, differences in relative sequence abundances were observed within and between oceanic regions at the broad taxonomic resolution of class, and became even more pronounced at high taxonomic resolution (OTUs defined at 3% sequence difference) where a high amount of endemism was observed (Chapter I). In contrast to these oligotrophic settings, sediments next to wood fall experiments were

dominated by *Clostridia* (Figure 9b), which comprise obligate anaerobes, being consistent with the anoxic conditions that had developed around the wood falls (Chapter IV).

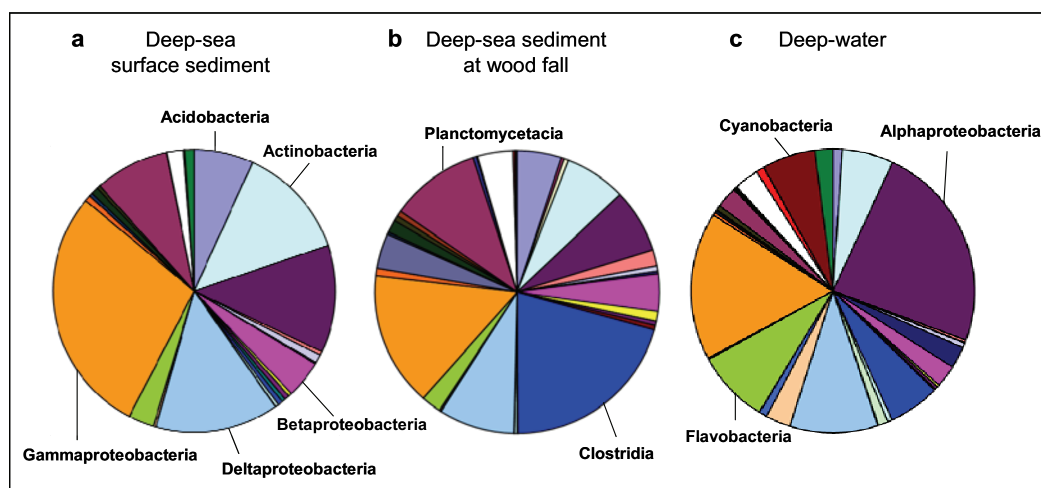

Figure 9 Average bacterial taxonomic composition in **a**) deep-sea surface sediments (≥ 1000 m water depth), n = 41 samples (Bienhold et al. in preparation, Chapter I), **b**) deep-sea sediments at a large wood fall (~ 1700 m water depth), n = 2 samples (Bienhold et al. in preparation, Chapter IV), **c**) deep oceanic waters (≥ 1000 m water depth), n = 24 samples (Zinger et al. submitted).

The average benthic deep-sea community differs from **deep-water** communities in that *Alphaproteobacteria* are the dominant group observed in deep-water masses (Eloe et al., 2011; Galand et al., 2009; Zinger et al., submitted) (Figure 9c). In addition, deep-water communities also show high proportions of sequences affiliated with *Gammaproteobacteria*, *Actinobacteria*, *Deltaproteobacteria*, and *Flavobacteria* (Galand et al., 2009; Zinger et al., submitted). Oceanic surface waters are as well dominated by sequences affiliated with *Alphaproteobacteria*, but show higher proportions of *Cyanobacteria* and *Flavobacteria* (Giovannoni and Stingl, 2005; Zinger et al., submitted). The marine **subsurface biosphere** is dominated by *Gammaproteobacteria* and usually shows high proportions of *Chloroflexi* and the candidate division JS1 (Fry et al., 2008; Inagaki et al., 2006; Teske, 2006; Webster et al., 2006), differentiating it from surface deep-sea sediments. Similar to deep-sea surface sediments, **coastal sediments** are dominated by sequences affiliated with *Gammaproteobacteria*; in addition they may show high proportions of *Planctomycetes* and *Deltaproteobacteria* (Gobet et al., submitted; Llobet-Brossa et al., 1998; Musat et al., 2006). Also *Cyanobacteria*, *Bacteroidetes*, and *Alphaproteobacteria* have been detected in coastal sands in considerable proportions (Gobet et al., submitted). Studies of **terrestrial soils** commonly report *Alphaproteobacteria*, *Acidobacteria*, *Betaproteobacteria*, *Actinobacteria* and *Bacteroidetes* as dominant members (Fierer et al., 2005; Janssen, 2006; Roesch et al., 2007; Torsvik and Ovreas, 2002), but

have also shown *Gammaproteobacteria* to be abundant in some locations (Torsvik and Ovreas, 2002).

This broad comparison demonstrates that at broad taxonomic resolution (i.e., class), bacterial taxa (e.g., *Gammaproteobacteria*) may occur in a large number of different habitats, from the marine pelagic realm over marine sediments and the deep subsurface biosphere to terrestrial soils. However, despite overall similarities in taxonomic composition, some distinct differences can be observed in bacterial community composition and structure (relative abundance) between different environments. This probably reflects the different life styles and environmental pressures in these habitats which may have led to the evolution of distinct bacterial populations. Widely distributed groups like the *Gammaproteobacteria* may be opportunistic and metabolically versatile. Yet, the cosmopolitanism of these bacterial taxa may be due to a few bacterial types (e.g., OTUs at 3% sequence difference) that are highly sequence abundant and wide-spread (see Chapter I). These specific types likely differ between habitats, and comparative analyses between different habitats will need to be refined at higher taxonomic resolution, e.g., with full-length sequencing of the 16S rRNA genes of specific groups.

Concluding remarks

Bacterial communities are not randomly distributed across the deep seafloor. They rather show distinct patterns of community structure and activity in response to changes in energy availability in the form of phytodetritus or large organic food falls and are structured across space both at small (local/regional) and at large (global) spatial scales, indicating limited dispersal ability. In the context of microbial community ecology, we demonstrate that both contemporary environmental conditions as well as isolation by distance effects shape bacterial communities in deep-sea sediments. Some common observations that seem to be emerging for bacterial communities in a variety of habitats include the significance of energy-diversity relationships, effects of dispersal limitation (endemism), and the existence of positive range-abundance relationships (i.e., a positive relationship between the range of occupation and the sequence abundance of a bacterial type, Chapter I). The results of this thesis present a step forward in the endeavor of microbial ecologists to understand and in the long-run predict changes in bacterial diversity and function in response to changing environmental conditions. This ability will be of particular importance for the deep sea that represents the largest ecosystem on Earth. The thesis marks important progress because it presents a comprehensive description of bacterial diversity in pelagic deep-sea sediments and sheds light on the processes shaping bacterial communities at the deep seafloor.

3.5 Perspectives

Functional diversity

The assessment of functional diversity and functional redundancy is important for estimating the resilience and stability of an ecosystem (e.g. Allison and Martiny, 2008; Naeem, 1998). As we have demonstrated, changes in bacterial community structure may be linked to bacterial functions (Chapter III), and it has been suggested previously that microbial community composition may have functional significance (Strickland et al., 2009). However, the specific interrelations between community structure and function will need further investigation. In certain cases, phylogenetic information may be used to predict physiology, but often phylogeny may not be a good predictor of functional traits, e.g., one group may carry out several functions and many functions may be carried out by different taxa (Achenbach and Coates, 2000; Konopka, 2009; Konstantinidis and Tiedje, 2005). Therefore, investigations of functional diversity will be important to be able to better estimate the effects of environmental changes on ecosystem functioning. The metabolic potential and functional diversity of microbial communities may be determined with microarray-based methods (e.g. Wu et al., 2008), in metagenomic studies (e.g. Eloe et al., 2011; Martin-Cuadrado et al., 2007; Quaiser et al., 2011), or by targeting conserved functional genes in fingerprinting assays (Santillano et al., 2010). Metatranscriptomics and metaproteomics offer more extensive approaches for studying functional gene expression of microbial communities and determining microbial functions in the environment (e.g. Gilbert et al., 2008; Wilmes and Bond, 2006). Genes/proteins of interest may be those involved in organic carbon degradation, at wood falls specifically cellulases and ligninases.

Bacteria on sinking particles

For deep-sea sediments, another largely unresolved question is how many of the bacterial types at the seafloor may originate from sinking particles. Although, sedimentation rates across abyssal plains may be as low as a few cm per 1000 years (e.g. Stein and Fahl, 2000), a considerable amount of particle-associated bacteria may be transported to the deep sea with sedimenting particles (Lochte and Turley, 1988; Thiel et al., 1988). These communities have been shown to clearly differ from free-living populations in the water column (Kellogg and Deming, 2009). However, it is largely unknown which proportion of bacteria in deep-sea sediments originate from sinking particles and how many of these bacteria may remain active under deep-sea conditions and contribute to the degradation of organic matter at the seafloor. This would

require comparative studies of microbial communities in the water column, attached to particles and associated with the sediment matrix, and would need to include rRNA-based methods targeting active parts of the population (e.g. Gremion et al., 2003; Logue and Lindstrom, 2008).

Linking bacterial diversity patterns with those of other deep-sea organisms

To better understand co-occurrence and/or avoidance patterns, not only among bacteria, but also in conjunction with meio- and macrofaunal patterns at the deep seafloor, would be another important step toward a more complete picture of the deep-sea ecosystem. First steps toward this goal could be achieved by incorporating existing data on larger benthic fauna, e.g., in collaboration with the Alfred Wegener Institute for the Arctic dataset (Chapter III), or from existing databases such as the ones generated in the Census of Marine Life project CEDAMAR (Census of the Diversity of Abyssal Marine life). Together with the bacterial datasets obtained during this thesis, these data could be used to search for co-occurrence patterns of bacterial and faunal taxa, and would add another important (biological) dimension to the set of contextual parameters tested.

Toward a definition of biogeographic provinces in the deep sea

A long-term perspective in deep-sea ecology would be the classification of biogeographic provinces. This means a delineation of oceanic deep-sea regions based on environmental features such as geology and physico-chemical parameters, but also in respect to biological community characteristics, e.g., community composition. A classification like this could aid ecosystem management strategies in their efforts to regulate human interventions in the deep sea such as the use of deep-sea resources (e.g., oil and gas extraction, deep-sea fishing) and the proposed application of carbon capture and storage (CCS) in the deep sea to mitigate climate change.

First recommendations for a biogeographic classification of benthic deep-sea areas have been proposed in a report of the Intergovernmental Oceanographic Commission (UNESCO, 2009). The areas defined in the report are mainly based on bathymetric and topographic features, but the authors also tried to incorporate information on benthic community composition where possible, although data are limited even for larger benthic organisms. As in most environmental assessments and ecosystem models, bacterial communities were not considered (e.g. Allison and Martiny, 2008). On the long run it would be desirable to incorporate biological information on all benthic size classes to describe ecological biomes in the deep sea. However, corresponding data on benthic community composition are largely missing and it will be impossible to conduct comprehensive biological surveys. Instead, we may have to rely on extrapolations from

relationships between biota and their physical or chemical environment. Information about bacterial communities at the deep seafloor are extremely limited and do not allow for such extrapolations yet. Thus, sampling efforts will need to be further increased in order to delineate effects of environmental parameters on the distribution of bacterial communities at the deep seafloor. For example, results presented in this thesis suggest that the availability of organic matter at the seafloor may be an important predictor of bacterial community composition, and this parameter may be estimated from surface productivity and knowledge on particle flux rates. Furthermore, the proposed classification of biogeographic provinces for the deep seabed (UNESCO, 2009) may serve as a starting point to test the designated areas using community data. Based on the classified provinces and existing information on benthic fauna and bacterial communities, missing data would have to be identified and future sampling efforts directed so as to fill in the gaps.

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Additional contributions to publications

Böggemann M, **Bienhold C**, Gaudron SM (2011) A new species of Glyceridae (Annelida: "Polychaeta") recovered from organic substrate experiments at cold seeps in the eastern Mediterranean Sea. Marine Biodiversity. Published online (DOI: 10.1007/s12526-011-0091-2).

Abstract: A new species of Glyceridae, Glycera noelae sp. nov., was recovered from two distinct colonization experiments based on organic substrates, deployed for 1 year at cold seeps in the eastern Mediterranean Sea (1,694 m depth, Nile Deep-Sea Fan Central Site 2A). The new taxon, which is the first glycerid reported from such an environment, is described by using morphological and molecular methods (18S rDNA, 16S rDNA, COI, ITS1, ITS2).

Borda E, Kudenov JD, **Bienhold C**, Rouse G (2011) A call to revise Amphinomidae (Annelida, Amphinomida): description and affinities of a new genus and species from the Nile deep-sea fan. (22.06.2011, in preparation)

Abstract: The discovery of a new amphinomid from wood falls deployed at cold seeps (1694 m) in the Nile deep-sea fan stresses the need to revise Amphinomidae in order to better characterize amphinomid diversity. *Cryptonome* gen. n. *conclava* sp. n. is the second genus established to include species from chemosynthetic environments and its designation highlights the lingering taxonomic perplexities of amphinomid species presently assigned to *Amphinome* and the erroneous placement of related xylophilic taxa in the genera *Eurythoe*. The phylogenetic affinities of *Cryptonome* to 12 species representing nine amphinomid genera were explored and inferred from two nuclear (18S rDNA and 28S rDNA) and two mitochondrial (COI and 16S rDNA) genes. The phylogenetic hypotheses indicated a close relationship of *Cryptonome* to other opportunistic species of temporary pelagic substrata including *Amphinome* sensu stricto (emended herein) and *Hipponoa*. Hypotheses also supported *Cryptonome* as a distinct lineage, here attributed to genus. *Cryptonome* is distinguished morphologically from all previously described Amphinomidae (rectilinear) genera in: lacking notochaetal hooks, having a reduced caruncle, modified neurochaetae and branchiae on nearly all segments. We emended and restricted six known oceanic flotsam species with stalked heart-shaped caruncles in *Amphinome* sensu stricto. An additional 15 species previously assigned to *Amphinome* may belong to other genera (e.g.

Linopherus) and are here tentatively considered *insertae sedis*, warranting further investigation. Finally, *Eurythoe turcica* and *Eurythoe parvecarunculata* are transferred to *Cryptonome* as new combinations. A revised key to rectilinear (Clade II) amphinomid genera (relevant to this study) is presented.

Boetius A, **Bienhold C,** Schöttner S, Ufkes J, Ramette A (2009) Fingerabdrücke mikrobieller Gemeinschaften im Meer. BIOspektrum 07/2009: 726-729

Die weitgehend unbekannten mikrobiellen Gemeinschaften im Meeresboden können durch den Vergleich von molekularen Fingerabdrücken in ihrer Wechselwirkung mit der Umwelt und dem globalen Wandel untersucht werden.
(Molecular fingerprinting tools allow an assessment of environmental controls of marine microbial community structure, including those related to global change.)

Poster and Oral Presentations

Bienhold C, Wenzhöfer F, Le Bris N, Ramette A and Boetius, A (2008). Wood colonization experiments in the Eastern Mediterranean deep sea. EGU General Meeting, Vienna. Poster presentation.

Bienhold C, Wenzhöfer F, Le Bris N, Boetius A (2008) Wood colonization experiments in the Eastern Mediterranean deep sea. CHEMECO/ DIWOOD Meeting, Paris. Oral presentation.

Bienhold C, Boetius A and Ramette, A (2009). Spatial gradients in microbial biodiversity of Arctic Ocean sediments: from shelf to deep sea. ASLO Conference, Nice. Oral presentation.

Bienhold C, Sogin, M L, Boetius A, Ramette A (2009). Analysis and ecological interpretation of microbial community patterns derived from ARISA fingerprinting and 454 tag sequencing. ICoMM 454 User Meeting, Woods Hole. Poster presentation.

Bienhold C, Boetius A, Sogin M, Ramette A (2009). Contextual interpretation of bacterial diversity in Arctic Ocean sediments. MARUM/GLOMAR PhD student day, Bremen. Oral presentation.

Bienhold C (2009). Deep-sea wood falls – Cities of life in the dark. GLOMAR Retreat, Etelsen. Oral presentation.

Bienhold C, Schulz I, Ufkes J, Wang T, Hubert A-M, Assmy P (2010). Geo-engineering the climate – from science to governance. GLOMAR/MARUM PhD Days, Bremen. Oral group presentation.

Bienhold C, Boetius A, Ramette A (2010) Environmental drivers of benthic bacterial diversity along the Arctic continental slope. ISME-13 Conference, Seattle. Poster presentation.

Bienhold C, Boetius A, Ramette A (2010). A baseline for assessing the impact of global environmental change on bacterial communities in the Arctic. PhD Student Conference "Integrated climate and earth system sciences in Northern Germany", Hamburg. Oral presentation.

Bienhold C, Boetius A, Ramette A (2011). Response of complex bacterial communities to changing energy availability in Arctic deep-sea sediments. Bremen PhD Days in Marine Science 2011, Bremen. Poster presentation.

Cruise Participations

RV Pourquoi Pas?, MEDECO-2, Eastern Mediterranean, Nile deep sea fan (wood experiments, cold seeps). 2 - 29 November 2007.

RV Meteor, M 76/3B, Congo fan (cold seeps, wood experiments). 17 July - 24 August 2008.

RV Maria S. Merian, MSM 13-3, Eastern Mediterranean, Nile deep sea fan (wood experiments, cold seeps). 25 October - 18 November 2009.

Erklärung

Hiermit erkläre ich, Christina Bienhold, dass ich

1. die Arbeit ohne unerlaubte fremde Hilfe angefertigt habe,

2. keine anderen als die von mir angegebenen Quellen
   und Hilfsmittel benutzt habe und

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