Identity and function of key bacterial groups in Arctic deep-sea surface sediments

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Katy Hoffmann
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1. Gutachter:	Prof. Dr. Antje Boetius
2. Gutachter:	Prof. Dr. Ulrich Fischer
1. Prüfer:	Prof. Dr. Anya Waite
2. Prüfer:	Prof. Dr. Jens Harder

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Dedicated with love to,

Cornelia & Heiko Hoffmann,

Prisca Hoffmann & Jako,

Elisabeth Hoffmann,

Sebastian Wolf

"It has long been an axiom of mine that the little things are infinitely the most important."

- Arthur Conan Doyle -

Katy Hoffmann

ORCID: 0000-0002-7714-4219

Summary

The deep-sea floor covers about 65% of the Earth`s surface and benthic biomass is dominated by highly diverse bacterial communities. Bacterial carbon cycling in deep-sea sediments plays a crucial role in global biogeochemical cycles, and remineralization efficiency of organic carbon can be more than 97%. However, key bacteria relevant for carbon turnover and ecosystem functioning remain unknown. Benthic bacteria mainly depend on organic carbon supply from the surface ocean, and will therefore likely be affected by changing surface ocean conditions. The Arctic Ocean is already impacted by environmental changes more rapidly here than in any other ocean region and will be impacted even more in the future. This turns the Arctic Ocean into an important study site to understand the effects of environmental changes on bacterial communities and ecosystem functioning, such as carbon cycling. At the same time, the Arctic Ocean remains to a large extent understudied, and little is known about the identity of key bacterial groups, which could be useful as indicators to describe the state of the ecosystem and to monitor community response to changing environmental conditions. Consequently, the goals of this thesis include the identification of indigenous key bacteria in deep-sea sediments and their metabolic potential, as well as the development of a better understanding of the specific response of Arctic deep-sea bacterial communities to changes in the supply of organic matter. The Long-Term Ecological Research site HAUSGARTEN (HG) is one out of two open ocean, long-term observatories in a polar region, and therefore provided a unique opportunity to study key bacterial groups from Arctic deep-sea sediments.

Chapters I and II present one of the first characterizations of a globally sequence-abundant sediment bacterial group, the JTB255 marine benthic group (JTB255). Cell counts with newly designed probes evidenced high cell abundances in coastal (Chapter I) and deep-sea sediments (Chapter II). Labeling experiments together with metatranscriptomic data suggested a chemolithoautotrophic lifestyle, with a potential high importance for sulfur-based carbon fixation in coastal sediments (Chapter II). Furthermore, genomic analyses of single cells emerged as a powerful means to provide first insights into the metabolic potential of JTB255 representatives in deep-sea sediments, suggesting a heterotrophic lifestyle with oxygen as terminal electron acceptor (Chapter II). Genomic analysis showed that JTB255 encode enzymes for the oxidative degradation of polymeric cell material such as membranes and cell walls, suggesting recalci-

trant organic carbon sources in marine sediments. Therefore, it is hypothesized for the first time that some representatives of JTB255 might be involved in the cycling of a major class of refractory sediment organic matter, potentially explaining their global ecological success.

In an *ex situ* experimental approach, the response of Arctic benthic bacterial deep-sea communities at HG to different types of detritus was explored (Chapter III). This is the first experimental study investigating the response of bacterial deep-sea communities to the addition of natural food sources by combining measurements of community function with the analysis of high resolution taxonomic community structure. Our results provide evidence that differences in organic matter composition lead to significant changes in bacterial community structure and function at the seafloor, which can affect carbon turnover and retention in the deep sea. In addition, opportunistic groups of bacteria were identified that may serve as indicator taxa for different organic matter sources at this site.

In Chapter IV, a pilot study is presented which addresses an issue often discussed in deep-sea research, i.e. the unknown effects of sample retrieval from high-pressure environments on bacterial communities. Therefore, the influence of de- and recompression on deep-sea sediment bacteria, as inherently imposed during sediment retrieval and subsequent laboratory experiments, was studied in a small-scale experiment. Results indicated few effects of de- and recompression on bacterial community structure within the experimental time frame, but contained evidence for changes in the metabolic activity of specific taxa, after the retrieval of decompressed samples from the seafloor. These observations remain to be verified with further sample replication.

In summary, this thesis contributes to the identification of candidate key bacterial groups. It further provides valuable insights into bacterial diversity and function in Arctic deep-sea sediments and will help to assess impacts of future climate scenarios on pelago-benthic coupling in the Arctic.

Zusammenfassung

Der Meeresboden der Tiefsee umfasst etwa 65% der Erdoberfläche und die enthaltene benthische Biomasse wird von hoch diversen Bakteriengemeinschaften dominiert. Der bakterielle Kohlenstoffumsatz in Tiefseesedimenten, welcher über 97% betragen kann, spielt eine wichtige Rolle in globalen biogeochemischen Stoffkreisläufen. Jedoch sind die für diesen Stoffumsatz und die Funktion des Ökosystems verantwortlichen Schlüsselbakterien noch immer weitgehend unbekannt. Benthische Bakterien sind stark abhängig vom organischen Kohlenstoffffluss aus dem Oberflächenwasser und werden auf diese Weise durch sich ändernde Bedingungen im Oberflächenwasser beeinflusst. Der Arktische Ozean unterliegt starken Umweltveränderungen, welche rascher voranschreiten, als in irgendeiner anderen Meeresregion. Daher wurde der Arktische Ozean zu einem wichtigen Forschungsstandort um die Effekte einer sich verändernden Umwelt auf Bakteriengemeinschaften und die Funktionsweise des Ökosystems, wie etwa dem Kohlenstoffkreislauf, zu verstehen. Gleichzeitig ist der Arktische Ozean großteils unerforscht und wenig ist bekannt über die Identität der vorhandenen Schlüsselbakterien, welche wichtige Indikatoren darstellen könnten, um den Zustand ihres Ökosystems zu beschreiben und die Reaktion der Gemeinschaft auf sich verändernde Umweltbedingungen zu überwachen. Daraus folgend umfassen die Ziele dieser Arbeit die Identifizierung von indigenen Schlüsselbakterien im Tiefseemeeresboden und deren metabolischen Potentials, sowie die Entwicklung eines besseren Verständnisses für spezifische Reaktionen der bakteriellen arktischen Tiefseegemeinschaft zu Nährstoffänderungen. Das ökologische Tiefsee-Observatorium HAUSGARTEN (HG), als bislang eines von zwei polaren Langzeit-Hochseeobservatorien, stellt eine einzigartige Chance dar, Schlüsselbakterien aus arktischen Tiefseesedimenten zu studieren.

Kapitel I und II stellen eine der ersten Charakterisierungen einer globalen, sequenzabundanten Bakteriengruppe, der “JTB255 marine benthic group“ (JTB255), dar. Zellzählungen mit neu entwickelten Sonden bestätigten hohe Zellzahlen in Küsten- (Kapitel I) und Tiefseesedimenten (Kapitel II). Markierungsexperimente zusammen mit metatranskriptomischen Daten suggerierten eine chemolithoautotrophe Lebensweise zusammen mit einer möglichen Wichtigkeit für Schwefel-basierte Kohlenstofffixierung in Küstensedimenten (Kapitel II). Genomische Einzelzellanalysen stellten sich als ein sehr wirkungsvolles Mittel heraus, um erste Einblicke in das metabolische Potential von Vertretern der JTB255 in Tiefseesedimenten zu erhalten, was eine eher heterotrophe

Lebensweise, mit Sauerstoff als terminalem Elektronenakzeptor, nahe legt (Kapitel II). Analysen des Genoms von JTB255 zeigten, dass Enzyme für den oxidativen Abbau von polymerem Zellmaterial, wie etwa Membranen und Zellwänden welche schwer abbaubare organische Kohlenstoffquellen mariner Sedimente darstellen, kodiert sind. Darauf basierend wurde nun erstmals die Hypothese formuliert, dass einige Vertreter von JTB255 an der Umsetzung von schwer abbaubarem organischem Material beteiligt sein könnten, was einen möglichen Grund für ihren ökologischen Erfolg darstellen könnte.

In einem *ex situ* Experiment am HG wurde die Reaktion bakterieller arktischer Tiefseesedimentgemeinschaften, in Bezug auf unterschiedliche Futterquellen hin untersucht (Kapitel III). Dies ist die erste experimentelle Studie die Reaktionen der bakteriellen Tiefseegemeinschaft auf die Zuführung natürlicher Futterquellen hin untersucht, indem funktionale Messungen der Bakteriengemeinschaft mit der hochauflösenden Analyse ihrer taxonomischen Struktur kombiniert wurden. Die erhaltenen Ergebnisse erbrachten den Nachweis, dass Unterschiede in der Zusammensetzung des organischen Materials zu signifikanten Änderungen in der bakteriellen Gemeinschaftsstruktur und –funktion am Meeresboden führen können, was sowohl den Kohlenstoffumsatz als auch seine Speicherung in der Tiefsee beeinflussen kann. Des Weiteren wurden opportunistische Bakteriengruppen identifiziert, welche in dieser Region, als Indikatoren für den Eintrag unterschiedlicher organischer Nährstoffquellen dienen könnten.

Kapitel IV stellt eine Pilotstudie dar, welche ein viel diskutiertes Thema der Tiefseeforschung anspricht und sich dabei mit den noch weitestgehend unbekannten Auswirkungen von Druckänderungen auf Bakteriengemeinschaften, durch Probengewinnung aus der Tiefsee und darauf folgenden Laborversuchen, befasst. Diesbezüglich wurde der Einfluss von De- und Rekompression auf Tiefseesedimentbakterien durch ein Experiment in kleinem Maßstab untersucht. Erste Ergebnisse deuten auf schwache Effekte der De- und Rekompression auf die Gemeinschaftsstruktur der Bakterien, innerhalb des experimentellen Zeitrahmens, hin. Jedoch zeigten sich Hinweise auf eine Änderung der metabolischen Aktivität einiger spezifischer Taxa direkt nach der Gewinnung der dekompressierten Proben. Diese Beobachtungen müssen durch zukünftige Probenreplikationen verifiziert werden. Zusammenfassend trägt diese Arbeit dazu bei, Kandidaten von Schlüsselbakterien zu benennen. Weiterhin ermöglicht sie wertvolle Einblicke in die Diversität und Funktion von Bakterien in arktischen Tiefseesedimenten und wird helfen, Auswirkungen von zukünftigen Klimawandelszenarien auf die arktische pelago-benthische Kopplung besser abschätzen zu können.

Table of Contents

Abbreviations

16S/23S rRNA	Subunits of ribosomal ribonucleic acid
ABYSS	Assessment of bacterial life and matter cycling in deep-sea surface sediments
AODC	Acridine orange direct count
CARD-FISH	Catalyzed reported deposition–fluorescence in situ hybridization
cDNA	Complementary DANN
Chl *a*	Chlorophyll a
CO_2	Carbon dioxide
CTD	Conductivity temperature depth
DIC	Dissolved inorganic carbon
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotidetriphosphate
DOC	Dissolved organic carbon
GC	Guanine-cytosine content
EDTA	Ethylenediaminetetraacetic acid
EEA	Extracellular enzymatic activity
EMP	Earth Microbiome Project
FACS	Fluorescence-activated cell sorting
HG	HAUSGARTEN
ICoMM	International Census of Marine Microbes
JTB255	JTB255 marine benthic group
LTER	Long-term ecological research observatory
MDA	Multiple displacement amplification
MED	Minimum Entropy Decomposition/ oligotyping
$MgCl_2$	Magnesium chloride
MUC	Multiple corer
MUF	4-Methylumbelliferone
NAc	N-acetyl-ß-D-glucosaminide
NGS	Next generation sequencing
*nir*K; NirK	Nitrite reductase gene; nitrite reductase
nMDS	Non-metric multidimensional scaling
O_2	Oxygen
ORFs	Open reading frames
OUT	Operational taxonomic unit
PANGAEA	Publishing network for geoscientific and environmental data
PCR	Polymerase chain reaction
Pg	Petagram
POC/ POM	Particulate organic carbon/ Particulate organic matter

PVA	Polyvinyl alcohol
RAxML	Randomized axelerated maximum likelihood
ROV	Remotely operated vehicle
SCGs	Single cell genomics
SSU	Small subunit
Tris	Tris(hydroxymethyl)-aminomethane
v:v	Volume per volume
W. oceani XK5	*Woeseia oceani* XK5
w:v	Weight per volume

1 Introduction

1.1 A general overview of the deep-sea ecosystem

1.1.1 The deep-sea realm

The ocean can be divided into various zones, depending on depth, and presence or absence of sunlight. The continental shelf, the underwater landmass that extends from a continent, reaches down to about 200 m water depth, and represents the transition zone from shallow-water fauna to deep-sea fauna. Below this, photosynthetic primary production can generally not be sustained anymore due to the absence of sunlight (Hessler, 1974; Merrett, 1989; Sanders et al., 1965). This marks the beginning of the deep sea, where the margin connects the continental shelf and the abyssal plain. The deep sea covers approximately 65% of the Earth's and 93% of the ocean`s surface (Figure 1A), and is thus the largest continuous ecosystem on our planet (Thiel, 2003). At the same time, it remains one of the most remote and least explored ecosystems on Earth. The deep sea is generally characterized by low temperature (−1°C to +4°C), high pressure (up to 1,100 atm; 1 atm = 1 x 10^5 Pa), weak currents, high salinity (~ 35), and the absence of sunlight. The seafloor is morphologically complex, comprising a variety of habitats (Figure 1B), yet, most of the seafloor is comprised of vast flat abyssal plains with an average depth of 3,800 m (Jannasch and Taylor, 1984). They are covered by fine-grained sediments of biogenic, terrigenous, volcanogenic and authigenic particles (Jørgensen and Boetius, 2007).

The main controlling factor of benthic productivity and biomass in the deep sea is the availability of organic matter, sinking from the sunlit productive upper waters. Most of the labile organic matter (> 90%) is recycled in the upper 1,000 m of the water column (Deming and Baross, 1993; Moutin and Raimbault, 2002; Wakeham et al., 1997; Wakeham and Lee, 1993), turning the deep sea into a rather oligotrophic environment with limited carbon input (Jørgensen and Boetius, 2007). Therefore, remineralization (oxygen uptake) is low and oxygen penetrates down into the sediment to several centimeters - at some sites even tens of centimeters (Wenzhöfer and Glud, 2002), making the seafloor surface habitable for a variety of organisms. The organic matter that arrives at the seafloor, which is on average 1 to 5% of the primary produced organic matter from the surface waters, is either remineralized (> 97%) or permanently buried in the sedi-

ment (Berner, 1980; Canfield et al., 1993, Burdige, 2007; Jahnke, 1996; Jahnke and Jackson, 1992; Smith et al., 2008). The remineralization of sedimentary organic matter by microorganisms regenerates inorganic carbon and nutrients for a continued water column production (Wenzhöfer and Glud, 2002). Therefore, deep-sea sediments are a key component of the global carbon cycle, linking the deep-sea ecosystem to the overall biosphere through benthic-pelagic coupling (Seiter et al., 2005; Wenzhöfer and Glud, 2002). Benthic–pelagic coupling describes the deposition of organic matter to benthic habitats (Graf, 1992; Hargrave, 1973; Smetacek, 1985), bioresuspension (Graf and Rosenberg, 1997), and the release of inorganic nutrients from the sediments (Raffaelli et al., 2003); it focusses on the exchange of energy, mass, or nutrients between benthic and pelagic habitats (Griffiths et al., 2017). Besides the input of surface-derived particulate organic carbon (POC), chemosynthetic fixation of inorganic carbon is an important process of (primary) carbon production at deep-sea hydrothermal vents and cold seeps (Jørgensen and Boetius, 2007). In addition, inorganic carbon fixation has been recently suggested to play a role in deep-sea surface sediments, accounting for on average 19% of the total heterotrophic biomass production in the northeastern Atlantic Ocean and in the Mediterranean Sea (Molari et al., 2013), yet we do not know the relevance of it at a global scale. Overall, due to the immense size of the deep-sea ecosystem, covering on average 335 million km^2 (Figure 1A), the processes happening within it, such as the cycling of carbon, are of global importance.

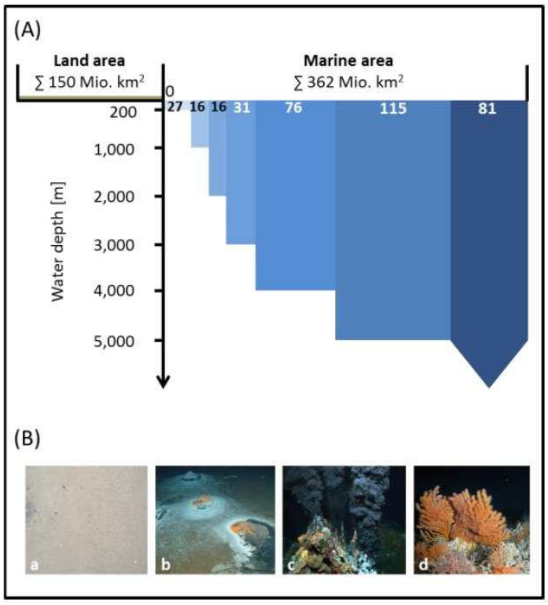

Figure 1. **Deep-sea area and diverse deep-sea environments.** (A) Visualization of the areas [Mio. km^2] covered by land (terrestrial) and the Ocean (marine). The different blue boxes visualize water bodies from 0 to 200 m and afterwards in 1,000 m water depth steps. Numbers in each water column indicate the sediment area covered by the different water depths. The deep sea includes waters from 200 m water depth onwards. Mio.: Million. Modified after Türkay, 2001. (B) Selection of environments on the deep-sea floor. a: soft-bottom sediment at a continental margin; b: cold seep with microbial mats (white); c: black smoker; d: a deep-sea coral reef. Image courtesies a: J. Taylor via OFOS, AWI, Bremerhaven, Germany; b to d: MARUM – Center for Marine Environmental Sciences, University of Bremen.

1.1.2 The role of the deep sea in the global organic carbon cycle

Carbon - next to nitrogen, phosphorus and hydrogen - is the main component of biological compounds, and is therefore crucial for sustaining life on Earth. The carbon cycle describes the flux, recycling, and reuse of carbon between diverse reservoirs (or pools), such as the terrestrial ecosystem, the atmosphere, ocean, and lithosphere (Buesseler et al., 2001), and occurs as the result of various biogeochemical and physical processes. The deep sea plays a key role in the global carbon cycle and climate regulation due to its sheer capacity to store and rework carbon, as indicated in Table 1 (Falkowski et al., 2000). Falkowski and colleagues (2000) highlighted that not atmospheric carbon dioxide (CO_2) concentrations determine the ocean`s CO_2 concentration, but vice versa.

Table 1. **Carbon pools in the major reservoirs on Earth**. Modified after Falkowski et al., 2000.

Pools	Quantity [Pg]
Atmosphere (inorganic)	720
Oceans (total)	38,400
Total inorganic	37,400
Surface water layer	670
Deep sea	36,730
Total organic	1,000
Lithosphere	
Sedimentary carbonates	> 60,000,000
Kerogens	15,000,000
Terrestrial biosphere (total)	2,000
Living biomass	600 – 1,000
Dead biomass	1,200
Aquatic biosphere	1 – 2
Fossil fuels (total)	4,130

Carbon inputs to the sea occur most importantly through the permanent exchange with the atmosphere (as inorganic CO_2). This carbon is sequestered from the sea surface to the deep sea through biological activity, known as the biological pump, and causes atmospheric carbon concentrations to be about 200 to 300 ppm lower than they would be without this mechanism (Heinze et al., 2015; Maier-Reimer et al., 1996; Sarmiento and Toggweiler, 1984). When carbon is introduced to the ocean surface, it is used by a diverse pool of microorganisms that are centrally involved in the fluxes of matter and energy in the marine carbon cycle (Figure 2). Microorganisms constitute approximately half of the natural, carbon-based compounds generated on Earth every year (Arrigo, 2005; Azam and Malfatti, 2007), and thus promote marine life. Phytoplankton (photoautotrophic organisms) capture energy from sunlight at the ocean surface, and uses it to convert inorganic carbon into organic matter (biomass), releasing oxygen (O_2) (Figure 2). This is called primary production because it provides the basis of the food web. When primary producers are being consumed by grazers, a part of their biomass becomes dissolved inorganic carbon (DIC) through respiration. The remainder is released into surface waters as dissolved or particulate organic carbon (DOC and POC, respectively). Furthermore, DOC and POC reach the ocean e.g. through the input of organic matter through river runoff (Raymond and Bauer, 2001) and as chemosynthetically produced organic matter from vents and seeps (Mccarthy et al., 2010; Pohlman et al., 2010). Marine DOC is a large carbon reservoir (~ 1,000 Pg C) cycling in the ocean (Falkowski et al., 2000; Follett et al., 2014). Approximately half of the carbon that is

fixed by marine autotrophs is directly processed and re-introduced into the food web by diverse heterotrophic bacteria (Cole et al., 1988; Ducklow et al., 1993). The remaining organic carbon either becomes transformed into recalcitrant DOC that resists further degradation and is sequestered in the ocean for up to thousands of years (Follett et al., 2014 and references therein), or is exported to the deep-sea floor. This is known as the microbial loop, a complex trophic pathway within the marine microbial food web (Azam and Graf, 1983) (Figure 2).

Deep-sea surface sediments receive on average 0.5% of the carbon fixed in surface waters, accounting for 1 to 10 mmol carbon m^{-2} yr^{-1}, and 1 to 5% of the exported organic material (Jahnke and Jackson, 1992; Jørgensen and Boetius, 2007; Klages et al., 2004; De La Rocha, 2006). In comparison, in coastal and shelf areas up to 50% of the primary production can reach surface sediments (Canfield et al., 1993; Jørgensen, 1982; Wollast, 1991). Exported POC is comprised of phytoplankton aggregates, detritus, living and dead cells, moults, and zooplankton faecal pellets. Zooplankton faecal pellets, rich in chitin, have been found to constitute an important and ubiquitous part of the vertical flux of carbon (Manno et al., 2015; Morata and Seuthe, 2014; Wilson et al., 2013), accounting for up to 100% of total POC (Turner, 2002). Benthic biomass at the deep-sea floor is dominated by heterotrophic bacteria which remineralize organic matter. At the same time the seafloor represents an important long-term sink of the carbon that escapes remineralization for centuries or millions of years (Jahnke, 1996). The importance of heterotrophic bacteria for the function of deep-sea surface sediments represents a main focus of this thesis and is described in more detail in section 1.2.1.

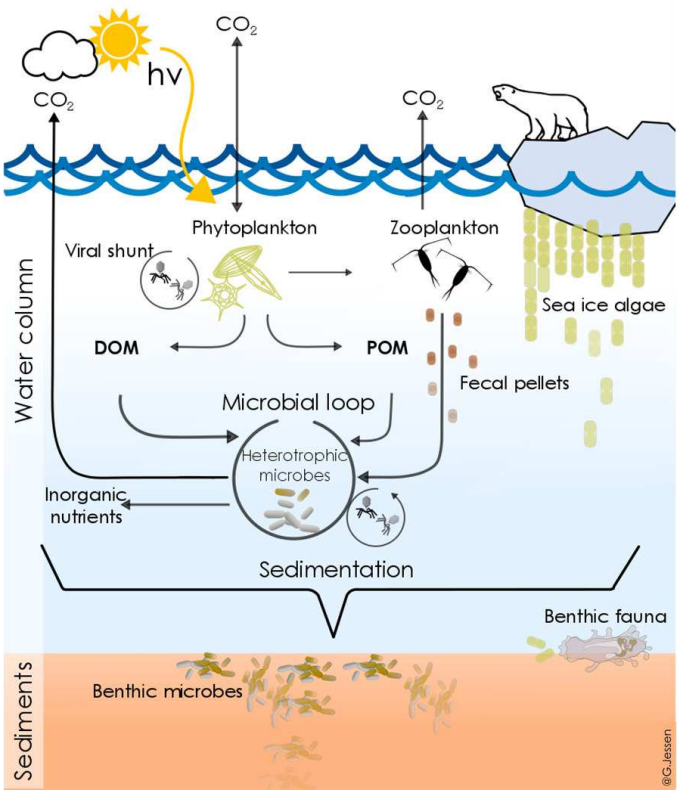

Figure 2. **Simplified illustration of organic matter transformation by bacteria as part of the biological pump in the Arctic marine carbon cycle.** The marine carbon cycle includes diverse processes, several of which are mediated by microorganisms. Key processes include the conversion of inorganic carbon (i.e. CO_2) to organic carbon by photosynthetic phytoplankton (and sea-ice algae in Polar Oceans); the release of dissolved and particulate organic matter (DOM and POM, respectively) from algae; the consumption of algal biomass by grazers (i.e zooplankton) and the mineralization (that is the release of CO_2 via respiration during the catabolism of organic matter) and recycling of organic matter by heterotrophic bacteria, known as the microbial loop. The heterotrophic bacteria are also partially consumed by zooplankton, and the carbon is further transferred up the food web. Heterotrophic bacteria also contribute to the remineralization of organic matter into inorganic nutrients, which are then available for use by algae. Furthermore, viral-mediated cell lysis also contributes to the release of DOM and POM from phytoplankton as well as bacterial pools via the viral shunt. From all of these 'compartments' POM sediments to the benthic seafloor. Modified after Buchan et al., 2014.

1.1.3 The Arctic Ocean ecosystem

Regarding the global carbon cycle, the Arctic Ocean takes up 14% of the global CO_2 from the atmosphere (Bates et al., 2009). Unlike the other oceans, in the Arctic Ocean the carbon export and energy supply to the deep sea is influenced by additional variables. Primary production is constrained by strong seasonality in solar radiation and sea-ice cover (Arrigo, 2014; Janout et al., 2016; Rouse et al., 1997), and can occur, depending on the region (shelves vs. Central Arctic Ocean), between April and October but mainly over a short period of time (Codispoti et al., 2013; Fernández-Méndez et al., 2015; Hill et al., 2013; Matrai et al., 2013; Sakshaug, 2004). Through the short duration

of the algal bloom, which constitutes the largest fraction of the annual surface water production, the overall primary production in the Arctic Ocean is low compared to other oceans (Fernández-Méndez et al., 2014).

Sea ice represents an additional habitat for a variety of microorganisms, including sea-ice algae (Figure 2). In the Arctic Ocean, sea-ice algae and phytoplankton are the two main primary producers (Leu et al., 2011). Sea-ice algae produce large amounts of biomass and can contribute between 20 to 60% of the total primary production (Fernández-Méndez et al., 2015; Leu et al., 2011 and references therein; Wassmann, 2011). At the end of the productive season when the ice melts, and the phytoplankton bloom occurs (Nicolaus et al., 2012; Sakshaug, 2004), sea-ice algae are released to the water column (Fernández-Méndez et al., 2014), and represent an additional important source of organic carbon exported as aggregates to the benthos (Boetius et al., 2013).

The algal bloom represents the largest pulse of biogenic energy to the benthos during the year. Arctic deep-sea sediments receive one main biogenic pulse between July and August (0.07 to 0.1 g C m^{-2} d^{-1}) and experience low food input for most of the year (Fahl and Nöthig, 2007; Schewe and Soltwedel, 2003), turning it into a very oligotrophic environment (Anderson et al., 1990). Furthermore, carbon export flux and the degree of pelagic-benthic coupling depend on oceanographic conditions (i.e. mixing of water masses and depth of the euphotic zone), as well as on the ratio between primary and secondary production which, at the same time, depend on algal standing stocks, types of phytoplankton community present, as well as grazing rates and fecal pellet production (Bourgeois et al., 2017; Reigstad et al., 2008). Therefore, Arctic organisms need to be highly adapted to extreme environmental conditions due to the strong seasonal forcing. Benthic organisms, in particular, need to cope with varying biogenic energy fluxes due to changes in primary productivity that, in turn, fluctuates due to changing ice dynamics (Wassmann, 2011).

Several studies emphasized the strong pelagic-benthic coupling of Arctic environments (Bienhold et al., 2012; Grebmeier et al., 2006; Lalande et al., 2013; Vincent et al., 2009). A strong coupling means a low to moderate pelagic consumption, allowing more carbon of higher quality to reach benthic communities, compared to a low coupling, where the pelagic consumption and recycling of POC is high and the quantity and quality of vertically exported organic material is reduced. Given this strong interconnection of both environments in the Arctic Ocean, changes in one realm are likely to affect the other (Vincent, 2010).

International efforts have been initiated to elucidate the specific role of the Arctic Ocean in the global carbon cycle (see e.g. Klages et al., 2004). Its food web might be highly impacted by environmental changes, which occur more rapidly than in any other ocean region (Arrigo et al., 2008; Michel et al., 2006; Wassmann, 2011). This turns the Arctic Ocean into an important study site to understand the effects of environmental changes on microbial communities and ecosystem functioning, such as carbon cycling.

1.1.4 Recent achievements and challenges of microbial deep-sea research in the Arctic

Microbial life occurs in astonishing abundances in deep-sea environments including the deep water column, surface sediments, and even the deep subsurface sediments. This raises questions about the identities and complex functions of these organisms, in particular regarding the significance of bacterial diversity for redundancy in biogeochemical processes and ecosystem functioning. Bacterial communities are key to understand matter fluxes in the deep sea, and their feedback mechanisms to environmental changes.

Although it is generally accepted that the Arctic Ocean is an important and sensitive region for global climate change, we are only beginning to understand its role in the global carbon cycle and its relationship to global change (Klages et al., 2004). Since the first recovery and analysis of Arctic deep-sea sediments during the Fram expedition of Fridtjof Nansen from 1893 to 1896 (Böggild, 1906; Nansen, 1897), the progress to understand the rapidly changing Arctic ecosystem, particularly its deep-sea benthos, is slow compared to progress in other ocean regions (Stein, 2012). However, due to the relevance of the benthic ecosystem for the entire Arctic Ocean, it is important to monitor how environmental changes, such as sea-ice loss and its subsequent shift in pelagic species composition (e.g. Li et al., 2009b; Nöthig et al., 2015), will affect the resource availability for benthic communities, which in turn will affect rates and pathways of organic matter processing (e.g., flux to the seafloor and benthic organic matter remineralization). Furthermore, changes in the Arctic ecosystem may eventually serve as precursors for other regions in the world where effects are not yet as pronounced. However, despite the recent acceleration of Arctic sea ice decline, there is a large lack of knowledge caused by the Arctic's Ocean difficult accessibility, and an even more difficult accessibility of its deep-sea floor, together with the costly operation of ice-breakers and polar-adapted research technologies required to study this area.

Recent advances in research technologies have allowed quantitative investigations of the Arctic Ocean and its benthos. Varying carbon export rates and qualities of organic matter due to e.g. sea-ice decline and warming waters have been observed (Ardyna et al., 2014; Rossel et al., 2016), e.g. by using annual sediment traps attached to oceanographical moorings that enable to link changes in seasonal export fluxes with changing environmental parameters such as water temperature (Bauerfeind et al., 2009; Lalande et al., 2014). This raised new questions, such as if changing export quantities and qualities could alter sediment bacterial communities, and whether patterns of pelagic-benthic coupling might be altered by climate change in the Arctic. Therefore, many recent studies have investigated sediment processes including the consumption and degradation of deposited organic matter by benthic (bacterial and faunal) communities (Bienhold et al., 2012; Boetius et al., 2013; McMahon et al., 2006; Sun et al., 2009), partially accessed through video-guided sampling gear and remotely operated vehicles (ROV). Furthermore, benthic O_2 fluxes can now be measured through *ex situ* and *in situ* microprofilers, benthic chambers, and eddy covariance (Bourgeois et al., 2017; Cathalot et al., 2015; Donis et al., 2016) in order to calculate carbon utilization and remineralization rates. In addition, cell abundance, biomass, and activity of sediment bacterial populations have been studied in relation to changes in organic carbon quantities or qualities (Jorgensen et al., 2012; Kanzog et al., 2008; Nikrad et al., 2012). These results suggested that benthic bacterial community structures and functions, such as enzymatic activity, O_2 consumption and carbon remineralization rates were highly related to energy availability, and are therefore affected by changing organic matter input conditions due to environmental changes (Bienhold et al., 2012; Han et al., 2016; Jacob et al., 2013).

The development of high-throughput next generation sequencing technologies, such as shotgun metagenomics, proteomics and metatranscriptomics, to study microbial diversity and genetic potential has allowed for the investigation of entire natural communities on very fine scales. However, none of these technologies have been applied so far on Arctic deep-sea sediments. Most of the recent microbial studies have focused on 16S rRNA gene high throughput sequencing (e.g. Danovaro et al., 2016; Han et al., 2016; Jacob et al., 2013; Jorgensen et al., 2012; Zhang et al., 2014; Zinger et al., 2011). In addition, there have been a few and patchily distributed studies concerning the patterns of benthic bacterial communities in the Arctic deep-sea in the last five years (Bienhold et al., 2012; Han et al., 2016; Jacob et al., 2013; Jørgensen et al., 2012). The scarce data make it difficult to estimate long-term benthic community shifts and their

relationship to changing environmental parameters. Furthermore, the ability to sample at high latitudes, at great depths, the retrieval of pressurized deep-sea sediment samples, and the preservation of *in situ* pressure conditions throughout laboratory manipulation experiments remains a challenge (see e.g. Abegg et al., 2008).

1.1.4.1 Effect of hydrostatic pressure change on deep-sea bacterial communities

As high hydrostatic pressure is the most unique physicochemical characteristic of the deep sea, the impact of changing pressure conditions during sample retrieval for *ex situ* studies needs to be addressed when investigating bacterial deep-sea sediment communities and their reaction towards changing conditions. Potential decompression effects on the community are introduced in parallel to 'targeted' manipulations in the laboratory every time the researcher handles decompressed deep-sea samples. Yet, it is often not addressed when samples originate from less than ~ 3,000 m (Follonier et al., 2012). It is known that piezophilic (pressure-dependent) bacterial strains obtained from very deep waters (> 4,000 m) show genetic adaptations to hydrostatic pressure compared to close relatives from surface waters (Lauro and Bartlett, 2008). However, results about hydrostatic pressure effects on bacterial types or overall communities varied between studies and therefore remain unclear. When re-pressurized after decompression during the retrieval, those bacterial communities showed either higher activities than those replicates left decompressed (Nagata et al., 2010), or there were no significant differences in activity (Danovaro et al., 2008). Pressure corers that retain *in situ* conditions have also been developed (Abegg et al., 2008; Jannasch, 1976; Yanagibayashi et al., 1999), but are typically used for samples from greater depths (> 4,000 m). Here, communities that were never decompressed showed a different community composition (Yanagibayashi et al., 1999) and lower activities (Jannasch, 1976) than their decompressed counterparts. Overall, only a few of the earlier studies investigated pressure effects on deep-sea sediment communities from less than 3,000 m water depth (Jannasch, 1976; Jannasch and Wirsen, 1982), and none were obtained from the Arctic deep sea. Overall, it is not well understood if changes of hydrostatic pressure during sample retrieval alter the bacterial community (its abundance, diversity or activity), and thereby our ability to decipher community composition and richness in deep-sea sediments, and its reaction towards environmental changes. This problem will be addressed in Chapter IV of this thesis.

1.2 Bacterial communities of deep-sea sediments

1.2.1 The role of benthic bacteria in the organic carbon cycle at the deep-sea floor

Despite its oligotrophic character, deep-sea surface sediments are an important zone of intense biological activity, dominated by heterotrophic bacteria (Schut et al., 1997). One gram of surface sediment holds on average 10^9 bacterial cells (Parkes et al., 1994), also in the Arctic region (Boetius and Damm, 1998; Schewe and Soltwedel, 2003), which is 10 to 10,000-fold more cells than in productive ocean surface waters, and is comparable to coastal sediments (Boetius et al., 1996; Demingt and Colwell, 1982; Schauer et al., 2010). In seafloor sediment from the very deep (~ 4,000 m), permanently ice-covered central Arctic Ocean also lower cell numbers (down to 6 x 10^7 cells cm^{-3} sediment) were reported, emphasizing the strong oligotrophy of this area (Kröncke et al., 1994). Furthermore, bacteria account for up to 95% of the total benthic biomass (Pfannkuche, 1992; Rowe et al., 1991), followed by meio-, macro-, and megafauna, which decrease in biomass with increasing water depth (Wei et al., 2010) (Figure 3).

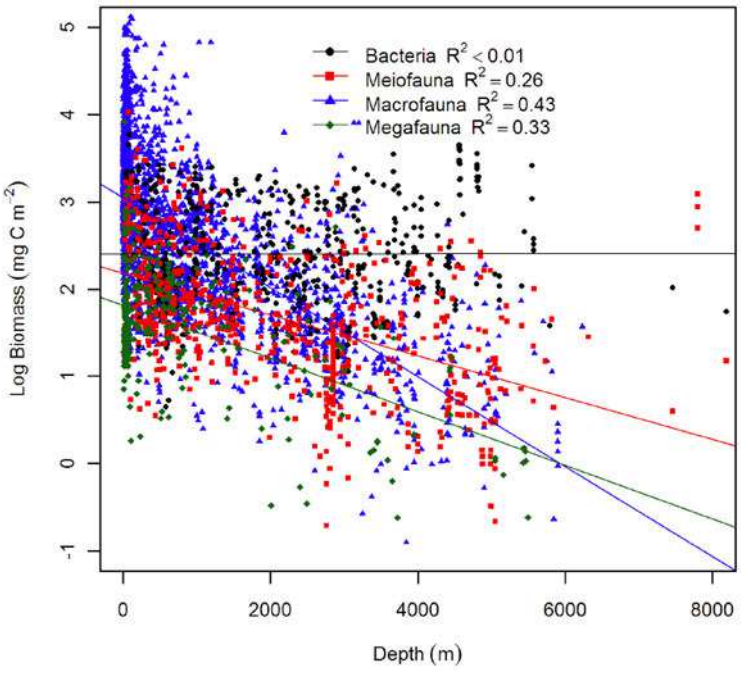

Figure 3. **Benthic biomass as a function of water depth.** Bacterial biomass stays nearly constant while all faunal size classes (meio-, macro-, and mega-fauna) decrease with increasing water depth. Modified after Wei et al., 2010.

The organic matter that arrives at the seafloor is strongly competed for among all faunal size classes (Witte et al., 2003). However, a major fraction of the organic matter consists of less-degradable substances, such as chitin and cellulose, which are not directly available to most marine organisms at higher trophic levels (Boetius and Lochte, 1994). Here, heterotrophic bacteria serve as essential catalysts by recycling the degradation-resistant organic matter into DIC, DOC and inorganic nutrients through extracellular enzymatic breakdown (Deming and Baross, 1993) (Figure 4). Bacterial communities encode a broad range of genetic information to produce specific enzymes for the degradation of various organic materials. This ability provides flexibility and competition advantage for survival under oligotrophic conditions, explaining the dominance of bacterial biomass in deep-sea sediments (Pfannkuche, 1993; Rowe et al., 1991). Therefore, measurements of extracellular enzymatic activity represent an important parameter to assess microbial activity in deep-sea sediments. On a broader scale, O_2 uptake can also be determined as a general measure for remineralization power of the sediment community since most of the heterotrophic activity in sediments is aerobic and consumes oxygen (Pfannkuche, 1993; Smith et al., 2013).

The extent to which bacterial diversity directly affects specific steps in the remineralization of organic matter has not been established yet (Arnosti, 2008). However, heterotrophic bacteria dominate the turnover of organic matter (Deming and Baross, 1993; Pfannkuche, 1993) with turnover times ranging from hours to decades (Boetius and Lochte, 1996; Rullkötter, 2006; Witte et al., 2003). Aerobic bacteria are able to respire a wide range of organic substrates. Oxic respiration is the energetically most favorable terminal electron acceptor process, out-competing all other respiration pathways when O_2 is available. Thus, bacteria in the oxic layers of the sediment play an essential role in the deep-sea food web and enable the transfer of energy between all trophic levels. They are a direct link to higher tropic levels because they represent an energy (food) source for other groups of organisms such as flagellates and foraminifera (Gooday, 1990; Turley, 2000). Another fraction of the remineralized matter (CO_2 and nutrients) is mixed into the water column, fueling life in the water column (Jørgensen and Boetius, 2007). However, ongoing degradation decreases the accessibility of the remaining organic matter to further microbial decomposition (Berner, 1980; Westrich and Berner, 1984).

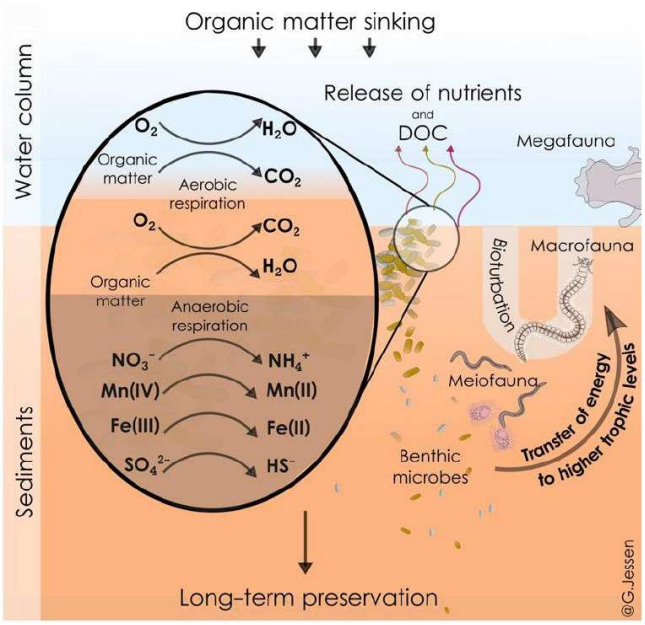

Figure 4. **Recycling of organic matter in deep-sea surface sediments**. Organic matter is produced in the euphotic zone and sinks to the seafloor where bacterial communities play a prominent role for the turnover of organic matter. Aerobic processes occur in the upper sediment layers, where degradation of organic matter is also promoted by bioturbation, and anaerobic processes in the deeper sediment horizons. The main fraction of the organic matter becomes remineralized by bacteria, and nutrients as well as DOC are either used and directly turned into biomass by benthic organisms, or are resuspended back into the overlaying hydrosphere. The smallest fraction of carbon is buried in the sediments for up to millenia. Modified after Delong, 2004.

Only 0.5 to 3% of the organic matter escapes bacterial remineralization (Burdige, 2007; Hedges and Keil, 1995; Jahnke, 1996), and becomes buried in marine sediments and rocks (Figure 4), which comprise the largest pool of stored carbon on Earth (Falkowski et al., 2000). Overall, sediments are the largest (in terms of km^2) interface between the biological and geological carbon cycles (Mayor et al., 2012). Thus, sediments play a key role in the earth system. The buried carbon is responsible for ultimately lowering atmospheric CO_2 with turnover times of millions of years (Rullkötter, 2006). Therefore, on geological time scales, sediments act as a significant sink in the regional and global carbon cycles, and may remove carbon from the atmosphere for centuries to millions of years (Jahnke, 1996). However, deeply buried organic matter, which displays low quality characteristics and is regarded to be highly recalcitrant, still supports microbial life (Parkes et al., 1994; Schippers et al., 2005). Therefore, the deep subsurface comprises a large proportion of the biomass on Earth (e.g., Fry et al., 2008; Orcutt et al., 2013).

Despite the prominent role of deep-sea sediments and their indigenous heterotrophic bacterial community in global organic matter remineralization and burial, only very little knowledge exists addressing key bacteria that may be most relevant in terms of substrate conversion of labile as well as aged, degradation-resistant organic matter in and across deep-sea sediments. In addition, further research is needed to address the impact of variations in organic matter export to the deep sea on sediment bacterial community composition and function (Jacob et al., 2013).

1.2.2 Bacterial community composition in deep-sea surface sediments

Bacterial assemblages in the marine realm contain representatives of all so far described ~50 bacterial phyla, including classified and candidate taxa (Grassle, 1989; Hugenholtz et al., 1998; Rappé and Giovannoni, 2003). They contain a large diversity of bacterial 'species' (estimated to be ~2 x 10^6), usually identified as operational taxonomic units (OTUs) based on genetic similarity (Amaral-Zettler et al., 2010 and references therein; Curtis et al., 2002; Patterson, 1999). These diverse microorganisms are most often genetically and biochemically uncharacterized, and belong to the so-called microbial dark matter (Rinke et al., 2013). Even though the deep sea represents the largest biome of the global biosphere, and may contain millions of bacterial species (Horner-Devine et al., 2004), we are only beginning to understand its diversity and associated functions.

At broad taxonomic resolution (e.g. phylum, class), a rather uniform composition of bacterial communities was described for deep-sea surface sediments across all oceans, as reported in global (Bienhold et al., 2016), regional amplicon-based and clone library-based 16S rRNA gene studies from the Eastern Mediterranean Sea (Pop Ristova et al., 2015), the South Atlantic Ocean (Schauer et al., 2010), the Arctic Ocean (Jacob et al., 2013; Li et al., 2009a; Tian et al., 2009) and Antarctic Ocean (Ruff et al., 2014) as well as the Pacific Ocean (Dang et al., 2009; Santelli et al., 2008; Walsh et al., 2016). *Proteobacteria* (mainly *Gammaproteobacteria*) account for on average 50% of the amplicon-based sequences in deep-sea surface sediments (Bienhold et al., 2016; Zinger et al., 2011). The class of *Gammaproteobacteria* is richer in described genera than all other bacterial phyla except *Firmicutes* (Oren and Garrity, 2015) and exhibits a great variety of morphologies, oxygen concentration and temperature adaptations, as well as metabolic life styles (Schulz et al., 1999; Scott et al., 2006). Further abundant classes are *Alpha*- (~12%), *Beta*- (~8%), and *Deltaproteobacteria* (~10%), as well as *Actinobacteria* (~13%). Other members of the benthic population, such as *Flavobacteria*, *Acidobac-*

teria, *Gemmatimonadetes*, *Clostridia*, and *Bacilli* seem to show a higher degree of endemism with varying abundances in different sampling sites (Bienhold et al., 2016; Pop Ristova et al., 2015; Schauer et al., 2010). The average deep-sea surface sediment bacterial community differs clearly from pelagic, sediment subsurface, hydrothermal vents, seeps, and fresh water systems (see Table 2). Still, at broad taxonomic resolution all major marine ecosystems share a high proportion of phyla and classes. Therefore, differences within and between marine ecosystems rather need to be resolved through analyses at higher taxonomic resolution and by including variations in relative abundances.

1.2.3 The core microbiome of deep-sea surface sediments

The entire microbial collection within one ecosystem, including the microorganisms themselves, and also their overall genetic material, is referred to as a 'microbiome' (Lederberg and McCray, 2001; Whipps et al., 1988). The analysis of the composition and structure of a specific microbiome and its comparison to others may provide important insights into the ecological and biogeochemical functioning of this particular ecosystem (Smith et al., 2009; Zinger et al., 2011). Microorganisms which are both abundant and ubiquitous in an ecosystem, are termed 'core microbiome' (Shade and Handelsman, 2012; Turnbaugh et al., 2007). Representatives of this core microbiome will be referred to as core microorganisms, or, core bacteria. Identifying core microbiomes for ecosystems is a subject of significant interest and importance, representing the first step in defining a community baseline, through which community responses to disturbances can be observed and predicted (Li et al., 2013 and references therein; Shade and Handelsman, 2012). So far, core microbiomes have been proposed for different parts of the human body (Li et al., 2013), plants (Vorholt, 2012), terrestrial systems (Gilbert et al., 2014), and marine ecosystems such as the pelagic realm (Sunagawa et al., 2015), methane seeps (Ruff et al., 2015), and hydrothermal vents (Meier et al., 2016). A detailed overview of abundant microbial groups in different marine systems is given in Table 2. However, the vast majority of microbes are rare types that occur at low abundances within their community and therefore, do not belong to the core microbiome (Jousset et al., 2017; Pedrós-Alió, 2006; Sogin et al., 2006). These rare types may represent a great source of genetic material that could reveal unknown ecological roles in biogeochemical cycles making them crucial components of ecosystems (Campbell et al., 2011; Jousset et al., 2017; Li et al., 2013).

Table 2. **Common and core microbial taxa of diverse ecosystems, focusing on bacteria**. Proteobacterial classes *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, *Gammaproteobacteria* are abbreviated as *Alphap*., *Betap*., *Deltap*., *Epsilonp*., and *Gammap*. JTB255: JTB255 marine benthic group; uncl.: unclassified. *: core microbiome of *Arabidopsis thaliana*.

	Common bacterial taxa at phylum/class level	Reference	Core bacteria	Reference
Atmosphere				
Upper troposphere	*Alphap.*; *Betap.*	DeLeon-Rodriguez et al., 2012	*Afipia*, *Oxalobacteraceae*, *Methylobacterium*	DeLeon-Rodriguez et al., 2012
Continental				
Human body	*Actinobacteria; Bacteroidetes; Firmicutes; Fusobacteria; Proteobacteria*	Grice and Segre, 2012	*Streptococcus; Pasteurellaceae; Propionibacterium; Corynebacterium; Lactobacillus; Bacteroidetes*	Li et al., 2013
Plants	*Alphap.; Betap.; Gammap.; Deltap.; Actinobacteria; Bacteroidetes*	Vorholt, 2012	**Streptomycetaceae; Cornamonadaceae; Bra-dyrhizobiaceae; Cyano-bacteria; Flavobacteriaceae; Micromonosporaceae*	Lundberg et al., 2012; Vandenkoornhuyse et al., 2015
Soil	*Acidobacteria; Alphap.; Betap.; Gammap.; Deltap.; Actinobacteria; Bacteroidetes; Firmicutes; Gemmatimondates*	Lauber et al., 2009		Not available
Freshwater	*Betap.; Actinobacteria; Bacteroidetes; Cyanobacteria; Verrucomicrobia*	Newton et al., 2011		Not available
Marine				
Surface water	*Alphap.; Gammap.; Flavobacteria; Cyanobacteria*	Flombaum et al., 2013; Giovannoni et al., 2005; Sunagawa et al., 2015; Teeling et al., 2016	SAR11*; Prochlorococcus*	Coleman and Chisholm, 2007; Giovannoni et al., 2005; Sunagawa et al., 2015
Deep water	*Alphap.; Gammap.; Deltap.; Actinobacteria*	Zinger et al., 2011		Not available
Coastal sediment	*Alphap.; Gammap.; Deltap.; Flavobacteria; Actinobacteria; Clostridia; Bacilli*	Ruff et al., 2015; Zinger et al., 2011	*Acidiferrobacter;* JTB255*; SSr clades*	Dyksma et al., 2016 *- this thesis -*
Soft bottom deep-sea sediment	*Alphap.; Gammap.; Deltap.; Actinobacteria; Flavobacteria; Acidobacteria; Gemmatimonadetes; Planktomycetes; Clostridia; Bacilli*	Bienhold et al., 2016; Zinger et al., 2011	JTB255; OM1 clade; BD2-11 terrestrial group (uncl.), JTB23 (uncl.)	Bienhold et al., 2016 *- this thesis -*
Cold seep	*Deltap.; Gammap.;* OP9/JS1*; Actinobacteria; Flavobacteria; Epsilonp.*	Pop Ristova et al., 2015; Ruff et al., 2015	*Desulfobacterales, Methylococcales, Thiotrichales*	Ruff et al., 2015
Hydrothermal vent	*Deltap.; Chloroflexi; Epsilonp.;*	Meier et al., 2016; Ruff et al., 2015		Not available
Deep biosphere	*Chloroflexi; Bacilli;* OP9/JS-1	Bienhold et al., 2016; Blazejak and Schippers, 2010		Not available

The few core microbiomes already described have been defined at a high taxonomic resolution, i.e. family to species level (see Table 2 and references therein). This can provide a link to functions, even though functional diversity may still differ for organisms with almost identical 16S rRNA gene sequence (Fox et al., 1992; Hoefman et al., 2014; Jaspers and Overmann, 2004). Key microorganisms of the water column such as *Pelagibacter*, *Prochlorococcus*, *Roseobacter*, and *Crenarchaeota* have been extensively studied (Flombaum et al., 2013; Giovannoni and Stingl, 2005; Morris et al., 2002). Through large-scale surface ocean sampling campaigns, such as TARA Ocean (Karsenti et al., 2011), and the Ocean Sampling Day (Kopf et al., 2015), hundreds of metagenomes have been obtained and made available to the public, which further help to explore the genomic diversity of this realm. Recently, also metagenomics studies of deep-sea waters were carried out (see e.g. Eloe et al., 2011; Martin-Cuadrado et al., 2007; Nagata et al., 2010; Smedile et al., 2012). Compared to the available knowledge on water column bacterial diversity and function, considerably less is known about deep-sea sediments and their microbiomes, and even less about Arctic deep-sea sediments. Furthermore, most deep-sea benthic studies applied tag sequencing (Bienhold et al., 2012; Jacob et al., 2013; Jorgensen et al., 2012; Li et al., 2009a) and, so far, only few published metagenomes of sediments from the deep sea exist (Quaiser et al., 2011), mostly associated to the oil spill in the Gulf of Mexico in 2010 (e.g. (Kimes et al., 2013; Mason et al., 2014). Until now, no Arctic deep-sea sediment metagenome has been published.

In a recent diversity study, Bienhold and colleagues (2016) were able to identify ubiquitous and abundant deep-sea sediment core bacteria at a high taxonomic resolution, and showed that they were distinct from other deep-sea environments, such as vents and seeps. The most abundant ubiquitous bacterial groups were affiliated with the JTB255 marine benthic group (here further referred to as JTB255), class *Gammaproteobacteria*, as well as the actinobacterial OM1 clade. Few other mostly unclassified bacterial groups, possibly associated to the *Gemmatimonadetes* (BD2-11 terrestrial group), the JTB23-group as well as other *Gammaproteobacteria*, were present in more than 90% of the analyzed deep-sea surface samples, and added to the deep-sea sediment core microbiome. In-depth knowledge on these groups of bacteria is rare. The JTB255 group has been observed in high abundance in numerous studies from marine sediments since the late 1990s (see e.g., Bienhold et al. 2016; Ruff et al. 2014; Li et al. 1999; Li et al. 2009; López-García et al. 2003; Schauer et al. 2010; Schauer et al. 2011), but investiga-

tions have never focused directly on the ecophysiology or genetic capabilities of this potential key player microorganism in deep-sea sediments. Most of these identified core bacterial groups consist of an enormous number of uncultured environmental 16S rRNA gene sequences, forming deep-branching lineages at low taxonomic levels (SILVA release 123; Quast et al., 2013). However, no closely related cultivated representative exist, nor any genomic information. Therefore, part of the work presented in this thesis aimed at the specific analysis of the most abundant core bacterium described for deep-sea sediments, the JTB255 (Chapters I and II).

1.2.4 Environmental factors that influence bacterial surface sediment communities in the Arctic deep sea

The flux of organic matter from the euphotic zone to the seafloor is the main source of energy in the deep sea (see section 1.1.2), which decreases in amount and quality with increasing water depth as well as with distance from land (Jørgensen and Boetius, 2007; Klages et al., 2004). Therefore, the POC flux is one of the major driving forces influencing the biomass and diversity of bacterial deep-sea sediment communities (Bienhold et al., 2012; Franco et al., 2007; Polymenakou et al., 2005; Smith et al., 2008b and references therein). The flux of POC to the seafloor in Arctic Oceans varies strongly with season and location, being higher during late summer and at the shelves than in the Central Arctic (Bauerfeind et al., 2009; Lalande et al., 2013, 2014, 2016; Smith et al., 2008b). Deep-sea bacterial communities seem to quickly respond to changes in energy availability as tested in multiple enrichment experiments (Turley and Lochte, 1990; Witte et al., 2003). Arctic POC flux to the seabed is expected to change in its quantity and quality due to ongoing environmental changes, and this will likely affect bacterial diversity and functions (Bauerfeind et al., 2009; Bienhold et al., 2012; Soltwedel et al., 2015). However, observations focusing on microbial responses to energy variations in the deep sea are scarce (e.g. Buhring, 2006; Kanzog et al., 2009; Moodley et al., 2002), especially in polar regions. As a result of increasing warming and sea-ice retreat, the composition, timing and magnitude of Arctic phytoplankton blooms and export fluxes are already changing (Arrigo et al., 2008; Assmy et al., 2017; Bauerfeind et al., 2009; Michel et al., 2006). Increased Atlantic water inflow into the Eurasian Basin of the Arctic Ocean and rising temperatures might favor the growth of different algae blooms and grazers, supporting rather smaller phytoplankton cells (Li et al., 2009b), and enabeling invasive species, such as *Phaeocystis* and *Emiliania huxleyi*, to thrive in the Arctic

Ocean (Assmy et al., 2017; Bauerfeind et al., 2009). This shift in species composition alters the quality and quantity of exported organic matter (Bauerfeind et al., 2009; Hirche and Kosobokova, 2007; Ojaveer et al., 2015; Weslawski and Legezyriska, 1998). Furthemore, a massive export of the aggregate forming sea-ice algae *Melosira arctica* to the deep-sea floor has been recently observed in the Central Arctic Ocean due to rapid ice melt, with a strong impact on the benthic community at 4,000 m depth (Boetius et al., 2013). In addition, Schewe and Soltwedel (2003) have shown that benthic bacteria and meiofauna responded to changes in particle export to the benthos, sampled in summer 1999 at the HG observatory, through changes in their abundances and activities. However, the assessment of the changes in the deep sea due to changes in the export fluxes remain qualitative.

Despite recent advances in Arctic deep-sea research (Klages et al., 2004), studies still lack combined measurements of key community functions, such as the degradation of particulate materials, and whole taxonomic community structure in response to changing environmental conditions (see Appendix Table S1). Only few studies have included analyses of bacterial community structure, either by using fingerprinting methods (Bienhold et al., 2012; Jacob et al., 2013; Kanzog et al., 2009), enrichment and cultivation approaches (Zhang et al., 2011), or cloning and sanger sequencing (Liu et al., 2016; Teske et al., 2011) to investigate changes in whole community structure in laboratory or *in situ* studies (Appendix Table S1). Therefore, it is of prime importance to identify potential key responding bacteria in order to understand matter fluxes and responses in the deep sea, especially in the light of global environmental changes. This research topic is addressed in Chapter III.

1.3 Overall objective and framework of the thesis

Bacterial communities in deep-sea surface sediments play a crucial role in the global carbon cycle. However, the difficult access to samples, the unknown effects of hydrostatic pressure changes during sample retrieval, and the large diversity of benthic microbial communities, pose major challenges to the investigation of life in deep-sea ecosystems. Thus, little is known about the identity of key bacterial groups in deep-sea surface sediments, their global distribution, and ecological function. Ecological baselines need to be established to assess the effects of global change on biological communities in a changing environment, such as the Arctic Ocean. Specifically, changes in the quality and quantity of sinking organic matter in the Arctic Ocean and its effect on deep sea bacterial community structure and functioning need to be addressed to elucidate the potential feedback mechanisms on local and global carbon cycles.

This thesis is written in the framework of the Advanced Investigator Grant ABYSS (294757) to Prof. Dr. Antje Boetius, funded by the European Research Council. The overall scope of the ABYSS project is the 'Assessment of bacterial life and matter cycling in deep-sea surface sediments', with a focus on the diversity, distribution and function of bacteria and their interaction with organic matter.

Within this framework, the overall objective of this thesis was to identify key deep-sea surface sediment bacteria in Arctic deep-sea sediments and to investigate their potential role in the Arctic deep-sea ecosystem.

To address the overall objective, the chapters of this thesis specifically address the following research questions:

- What is the identity of key bacteria in coastal marine sediments and what is their ecosystem function? (Chapter I)
- What is the global abundance and distribution of core sediment bacteria in the deep sea, using as an example the dominant JTB255 group? Which metabolic capacities do these bacteria encode in the Arctic deep sea and what is their putative role in carbon cycling, in contrast to coastal benthic core bacteria? (Chapter II)
- How do benthic bacterial communities respond to changes in organic matter composition in the rapidly changing Arctic? Can we identify key taxa that respond to the input of fresh detritus, and how rapidly do they respire and assimilate carbon? (Chapter III)
- What is the effect of changes in hydrostatic pressure on bacterial communities retrieved from deep-sea sediments? (Chapters III and IV)

1.4 Study site description

The main sampling area was the Arctic long-term ecological research (LTER) observatory HAUSGARTEN located in the eastern Fram Strait (Figure 5). The Arctic Ocean is connected to the Pacific Ocean through Bering Strait and to the Atlantic Ocean by the Fram Strait, whch is the only deep-water connection to the Arctic Ocean. It represents the most important gateway of the Arctic Ocean for the exchange of water masses, sea-ice and heat (Aagaard et al., 1985; Aagaard and Greisman, 1975; Torres-Valdés et al., 2013). Therefore, the Fram Strait is involved in the climate regulation of the entire Arctic region (Hop et al., 2006), and is the first area influenced by climatic and environmental changes, such as rapid ocean warming (Steinacher et al., 2009). It undergoes seasonal fluctuations in sea-ice cover, with an ongoing trend towards strongly reduced ice coverage in summer (Soltwedel et al., 2005; Vinje, 2001). These changes in the physical environment have been reported to affect the pelagic system, leading to changes in the phytoplankton community by migration events, as well as impacting the export of organic matter from the productive layer to the deep sea (e.g. Bauerfeind et al., 2009; Li et al., 2009b).

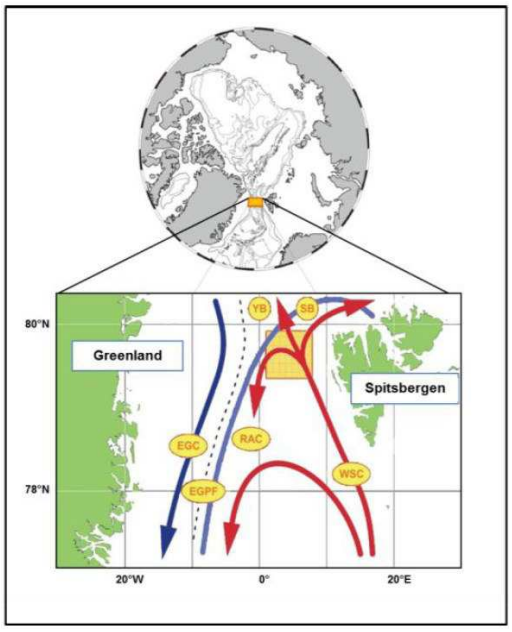

Figure 5. **Main sampling site.** Area of the Fram Strait between Greenland and Spitsbergen. The yellow square marks the position of the HAUSGARTEN observatory. The light blue line indicates the mean recent sea-ice position; EGC (dark blue line): Eastern Greenland Current, which exits the Arctic Ocean through Fram Strait with cold, less saline polar waters; WSC (red line): West Spitsbergen Current, which transports relatively warm and nutrient-rich Atlantic water into the Arctic Ocean; EGPF (dotted line): East Greenland Polar Front; RAC: Return Atlantic Current. YB: Yermak Branch; SB: Svalbard Branch. Modified after Soltwedel et al., 2005.

HG represents the only open ocean, long-term research station in a polar region (Soltwedel et al., 2005). It was established in 1999 to collect both abiotic and biotic data from the transition zone between the North Atlantic and the Arctic Ocean. Biogeochemical measurements of e.g. organic carbon and carbon mineralization rates, physical water properties such as current velocities, temperature, and salinity, as well as organic matter export and its composition from surface waters to the sediment are investigated through repeated sampling at monthly to yearly intervals. These data are partially gained through permanent observatories such as moorings, sediment traps, gliders, and satellites. These, combined with further multidisciplinary approaches, allow the detection of environmental changes and shifts in both pelagic and benthic communities of all faunal size classes.

1.5 Methodological approaches to study key deep-sea sediment bacteria

Deep sea surface sediment samples, including the first two centimeters (0 to 2 cm), were retrieved using a TV-guided multiple corer (MUC), if not stated differently. The main techniques applied in this thesis to study bacteria in deep-sea sediments are: cultivation, 16S rRNA gene amplicon sequencing, bioinformatics analysis of operational taxonomic units, single-cell genome and metagenome analysis, and catalyzed reporter deposition–fluorescence *in situ* hybridization (CARD-FISH).

1.5.1 Cultivation-dependent heterotrophic enrichments of seafloor bacteria

Traditionally, microbiology was based on organism-enrichment from their natural environment, followed by isolation into pure culture. This allowed the study of the microorganism`s physiology. Obtaining pure cultures is, even nowadays, the only way to obtain detailed information on microbial characteristics and to test hypotheses that emerge from genomic data. The power of a pure culture is that every aspect of the cell system can be defined and known exactly. This highlights the need to continue to focus on culturing microorganisms. In this thesis, I aimed to cultivate key bacteria of deep-sea sediments (see Chapter II). Substrate stimulation experiments on slurry sediments were performed to reveal which carbon sources may lead to a successful cultivation of sediment core bacteria when used in broth and solid media. Conditions were selected based on the dominant prevailing physiology of bacteria in deep-sea surface sediments, i.e. aerobic heterotrophy. For cultivation, diluted sediment samples were directly plated on Petri dishes with suitable selective growth media. Growing colonies of diverse microbes can then be analyzed and further purified to obtain pure (axenic) cultures. Furthermore, due to the high pore water content of deep-sea soft bottom surface sediments, cultivation to extinction in liquid broths was tested. A sediment sample was diluted successively multiple times with sterile seawater until mathematically one cell was left in the highly diluted samples. This technique is suited to enrich and finally isolate dominant bacteria based on the laws of statistics. However, cultivation techniques usually enrich for microbes with the desired physiological properties, which are not necessarily the organisms that would prevail under the naturally occurring conditions. Therefore, additional

stimulation experiment incubations under *in situ* pressure conditions at 250 atm (2.5 x 10^7 Pa = 25 MPa) were tested.

It remains highly challenging to recreate the natural requirements of many bacteria during cultivation (Leadbetter, 2003). Also, metabolic behavior in cultures may differ from *in situ* lifestyles (Cottrell and Kirchman, 2000). Since the majority of the vast bacterial diversity cannot be captured by classical cultivation-based approaches (Amann et al., 1995), cultivation-independent methods are used increasingly to characterize bacterial assemblages.

1.5.2 16S rRNA gene amplicon sequencing and OTU generation

Analysis of 16S rRNA gene sequences has greatly advanced our understanding of the phylogenetic diversity of microbes (Hugenholtz et al., 1998). Here, Illumina next generation sequencing (NGS) of partial 16S rRNA genes (also referred to as amplicon or tag sequencing) was applied in Chapters I to IV to capture the total DNA present in a sample (Illumina MiSeq, San Diego, CA, USA). Furthermore, in Chapters II and IV the transcribed 16S rRNA was additionally Illumina-sequenced to characterize the active bacterial community. The general workflow consists of two major steps: The amplicon library preparation and the actual sequencer run. The sequencing library is prepared by barcode and adapter ligation on the DNA target region, which is then PCR-amplified, and gel-purified. Because of the amplification step, amplicon data sets are subject to PCR bias (see e.g. Schirmer et al., 2015). The library is then loaded into a flow cell, the DNA fragments are captured on a lawn of surface-bound oligomers complementary to the adapters of the library and subsequently, each fragment is amplified into distinct, clonal clusters until a high density of sequences per cluster is reached. Sequencing occurs through the incorporation of fluorescently labelled nucleotides into DNA template strands, one base at a time. The nucleotides are identified by fluorophore excitation at the point of incorporation during each cycle (referring here to the incorporation of one base). NGS allows this process to happen across millions of different DNA templates in parallel, highlighting the power of this method. It further allows to sequence from both ends of a DNA template (paired-end sequencing) and merge the resulting fragments, which increases at best the sequencing accuracy when forward and reverse reads fully overlap, but generally improves the length of the amplified fragment when fractions of the forward and reverse reads overlap (see Liu et al., 2012 for details).

The 16S rRNA gene sequences of different bacterial types vary naturally, which is the basis for our current understanding of phylogenetic relatedness of living organisms (Woese, 1987; Yarza et al., 2008). These differences can be used to group amplicon sequences by sequence similarity, and cluster them into operational taxonomic units (OTUs), which are considered the units of highest taxonomic resolution (Blaxter et al., 2005). Subsequently, those OTUs can be used for taxonomic annotation, alpha- and beta-diversity analysis, and other statistical methods to investigate links between community structure and their putative shaping of environmental factors. OTUs from sequence data can be generated through different approaches. Here, I want to shortly explain classical heuristic clustering (Figure 6A) applied in Chapter I, and contrast it with a recently published clustering tool, named SWARM (Mahé et al., 2014) (Figure 6B), which I used in Chapter III and IV, as well as a new approach called oligotyping/ Minimum Entropy Decomposition (MED; Figure 6C) (Eren et al., 2013, 2015) that was used in Chapter II.

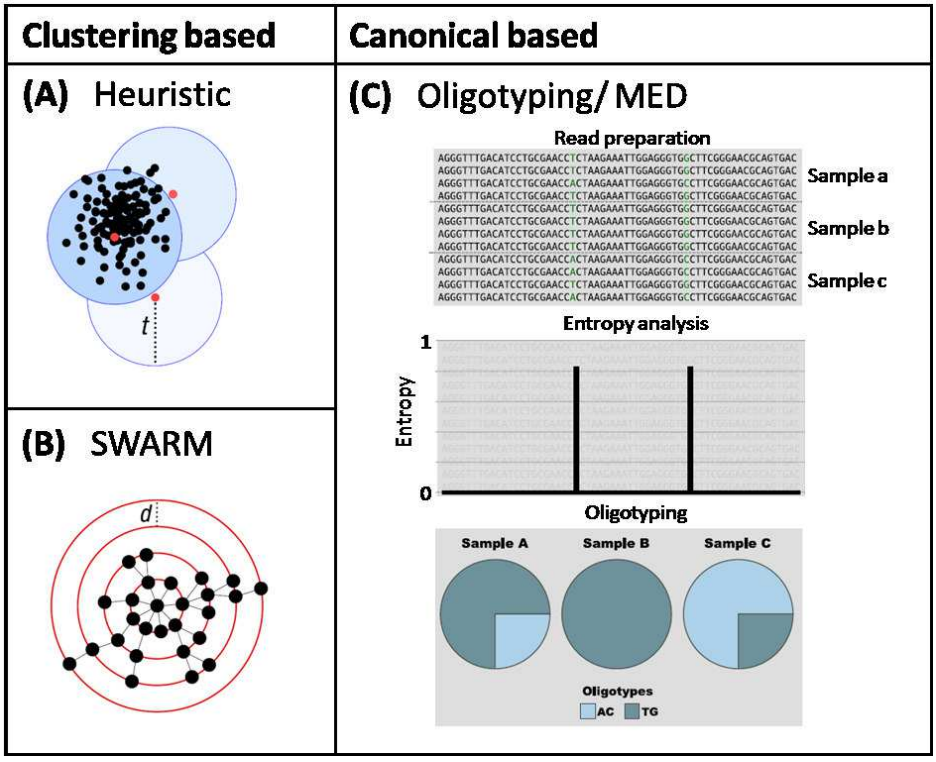

Figure 6. **Approaches to generate operational taxonomic units (OTUs).** (A) Heuristic clustering approach based on the selection of a centroid sequence (red dots) and a global clustering threshold 't'. (B) SWARM-based iterative clustering approach, using varying, local clustering thresholds 'd'. (A) and (B) Modified after Mahé et al., 2014. (C) Major steps of the canonical, cluster-independent Oligotyping analysis. Highly variable nucleotide positions between samples a, b and c are identified by their Shannon entropy, here highlighted in green. Oligotypes are then defined by these high entropy bases (here AC and TG), and oligotype profiles (here displayed as pie charts) are generated to show differences among samples. Modified after Eren et al., 2013.

In the classical heuristic clustering approach one sequence, usually the most abundant or longest sequence of a data set, is chosen as the so-called 'seed' (Edgar, 2013). All sequences with a predefined, *global* distance to the seed sequence are grouped into one OTU around this seed sequence. Mostly this threshold (maximum radius of one OTU) is set at 97 to 99% sequence identity, meeting the overall definition of a species (Stackebrandt and Ebers, 2006; Yarza et al., 2014). The strongest criticism is that this threshold represents an arbitrary similarity cut-off, predefined for the whole dataset (globally), which implies that it is valid for the entire tree of life (Mahé et al., 2014). This does not take into account that lineages evolve at variable rates. It has been shown that in some cases bacterial biodiversity patterns are sensitive to taxon definition (e.g. Cho and Tiedje, 2000) because some lineages evolve slower or faster than implied by this single global cut-off (Koeppel and Wu, 2013; Nebel et al., 2011; Sogin et al., 2006). Furthermore, the affiliation of sequences to a specific OTU is strongly based on the selection of the seed sequence, which may also affect the size of the OTU. This has also been shown to impose a factor of variability for the clustering results (Koeppel and Wu, 2013; Mahé et al., 2014, 2015). It was explicitly shown that one needs to be careful in the definition of a global distance threshold and selection of a seed sequence to not produce erroneous ecological interpretations (Mahé et al., 2014).

In contrast to heuristic clustering, SWARM does not use a fixed global distance threshold for sequence clustering. It rather builds OTUs (for this approach called 'swarms') by using sequence abundance values in an iterative growth process by applying a *local* distance threshold (Mahé et al., 2014). Therefore, the radius and shape of each generated swarm (the implied generation time) can differ from other swarms, and is reported to be more robust (Mahé et al., 2014). It is proposed that swarms may constitute a closer approximation of existing diversity units within a sample, because of the intrinsic 16S rRNA gene sequence differences between bacterial species (Kim et al., 2014). Still, SWARM is a clustering-dependent algorithm (Mahé et al., 2014), shown to partially fail at resolving differences between closely related microbes in complex datasets, potentially missing entities that are ecologically meaningful (Eren et al., 2013).

The JTB255 group is presumed to be the most abundant marine sediment group of bacteria globally, and likewise an important part of the deep-sea sediment core microbiome (Bienhold et al., 2016). In Chapter II, I analyze whether intra-group diversity exists within the JTB255 environmental sequence cluster. For this, I performed Entropy analysis (Oligotyping) (Eren et al. 2013). Oligotyping is based on the identification of

the most discriminating nucleotides (variations) among closely related sequences, up to the level of single nucleotide polymorphisms, through entropy analysis, and generates so-called 'oligotypes' (Eren et al., 2013). Oligotyping aims towards the identification of the minimum number of variations, explaining the maximum of biodiversity (Eren et al., 2013). Minimum Entropy Decomposition is the advancement of oligotyping, applying the underlying principles of oligotyping to the entire community (Eren et al., 2015). This method allows the investigation of 16S rRNA gene diversity among closely related bacteria at the level of 'final operational taxonomic units', due to its focusing on single nucleotide polymorphisms. It represents a non-cluster-based canonical approach; meaning that the algorithm does not compare all nucleotides of sequence reads with each other to evaluate similarity.

1.5.3 Environmental genome analysis techniques

Amplicon sequencing is limited to the amplification of one specific region of interest, here the 16S rRNA gene, to study the diversity and taxonomic composition of a microbial community. Through the sequencing of randomly amplified single cell genomes (single cell genomics; SCGs) (Stepanauskas and Sieracki, 2007) and metagenomic shotgun sequencing (Riesenfeld et al., 2004), as applied in Chapters I and II, genetic information from either one specific organism (SCGs), or, the entire natural community (metagenomics) can be analyzed. Through both, SCGs and metagenomics, the metabolically encoded potential of core bacteria, defined by a high environmental abundance (> 1% of 16S rRNA sequence abundance), can be revealed without the need of cultivation. The analysis of obtained genomic information and the metabolic insights may assist to design and re-adjust cultivation strategies for the respective organism. Both approaches can link phylogenetic (16S rRNA gene) and functional marker genes (Stepanauskas and Sieracki, 2007). However, a major drawback of metagenomics shotgun sequencing is that it cannot unambiguously link the function back to the individual cell, although it provides information about the taxonomic composition and functional potential of the community (Yilmaz and Singh, 2012). This implies the risk of genomic cross-assembly between different cells (Stepanauskas, 2012). Through SCGs, phylogenetic as well as functional genes are sequenced from the same cell, obtained directly from its environment (Swan et al., 2011), overcoming major limitations in metagenomics (Woyke et al., 2010). SCGs consists of a series of consecutive steps (Stepanauskas, 2012; Stepanauskas and Sieracki, 2007), starting with the physical separation of individual

cells via high-speed fluorescence-activated and droplet-based cell sorting (FACS). Cells contained in sediment samples are brought into aquatic suspension in order to obtain FACS-compatible, contamination-free samples. This can be achieved through vortexing of a diluted sediment sample at high speed, followed by a slow centrifugation step to remove large sediment particles. Contaminations through free DNA or two cells being sorted together are considered neglectable for SCGs (Blainey 2013). Following cell lysis, the whole genome of individual cells is randomly amplified through multiple displacement amplification. Subsequently, the DNA is sequenced using Illumina technology, and the obtained sequences are aligned, annotated, and interpreted (Stepanauskas, 2012).

1.5.4 Cell abundance and activity measurements of microbial communities

All genomic data obtained through amplification, such as amplicon, metagenome and single-cell genome sequencing, are limited in their ability to characterize bacterial communities. There is no direct information on metabolic rates, even from RNA sequencing, and also quantitative information about abundances of specific bacterial groups needs to be interpreted with care due to the amplification bias. To gain a more complex, comprehensive understanding of bacterial community composition and functioning, the combination of omics tools with biogeochemical and metabolic data is of great importance. Such data can be derived from cell counts, oxygen respiration rates, and enzymatic activity measurements. This compilation of methods was applied in Chapters I and III.

In order to investigate the biogeochemical recycling of organic matter by the sediment microbial community, parameters, such as the microbial use of oxygen and their extracellular enzymatic activity were measured. Oxygen uptake is an established proxy for total organic carbon remineralization and can be measured directly within the sediment through the use of optodes (Donis et al., 2016; Pfannkuche, 1993; Smith et al., 2013). The nature and characteristics of the extracellular enzymes used by members of heterotrophic communities to hydrolyze complex biopolymers are largely unknown. The enzymatic capabilities of microbial communities can still be probed by measuring the hydrolysis of a suite of substrates of known structure.

I used two artificial substrates, 4-methylumbelliferyl-N-acetyl-ß-D glucosaminide (MUF-NAc) and 4-methylumbelli-feryl ß-D-glucopyranoside (MUF-ß), to assay potential N-acetylglucosaminidase (chito-biase) and beta-glucosidase activities, respectively, *ex situ* in retrieved deep-sea surface sediments.

Enumeration of active bacterial cells belonging to a specific microbial group of interest can further help to overcome limitations of PCR-based techniques, which provide only limited information on the number and spatial distribution of microorganisms. Fluorescence *in situ* hybridization (FISH) helps to visualize and enumerate specific active microbial cells by preserving the microbial communities in their natural habitats (Amann et al., 2001). It uses 16S rRNA-targeted oligonucleotide probes, which only bind to the complementary sequence within the 16S rRNA of their target organism. To greatly enhance the FISH-obtained fluorescence signal for microbial groups with low rRNA content as reported for microorganisms from oligotrophic deep-sea sediments (Amann et al., 2001), FISH was combined with a catalyzed reporter deposition (CARD) based on tyramide signal amplification (Pernthaler et al., 2002). Key targeted groups in this thesis were *Gammaproteobacteria* and the JTB255 (Chapters I and II).

1.6 Publication outline

In the course of this thesis, I identified a core bacterial group in coastal and deep-sea surface sediments, highlighted its global abundance and distribution, and presented a first genomic characterization for this clade (Chapters I and II). Additionally, I evaluated the response of an Arctic bacterial deep-sea community to different organic matter sources in an experimental approach, and identified potential indicator taxa (i.e. key responding groups) (Chapter III). In a pilot study, I assessed the effects of de- and re-compression on the diversity and function of bacterial communities in deep-sea surface sediments (Chapter IV).

Chapter I 'Ubiquitous *Gammaproteobacteria* dominate dark carbon fixation in coastal sediments'

Stefan Dyksma, Kerstin Bischof, Bernhard M. Fuchs, Katy Hoffmann, Dimitri Meier, Anke Meyerdierks, Petra Pjevac, David Probandt, Michael Richter, Ramunas Stepanauskas, and Marc Mußmann
The ISME Journal (published in August 2016; doi: 10.1038/ismej.2015.257)

This study applied a novel combination of molecular and isotopic tracer techniques to identify cosmopolitan chemolithoautotrophic key bacterial groups and their energy sources in coastal sediments. It was shown that three distinct gammaproteobacterial clades, the *Acidiferrobacter*, the JTB255 group, and the SSr clades were ubiquitous and highly abundant in coastal sediments, accounting for approximately half of the dark carbon fixation. Furthermore, the study discussed an unknown metabolic plasticity for sulfur-oxidizing uncultured *Gammaproteobacteria* in marine sediments through the co-localization of key genes of sulfur and hydrogen oxidation pathways, and their expression through meta- and single-cell genomics, as well as metatranscriptomics. Overall, this manuscript highlighted the significance of uncultivated gammaproteobacterial core groups for inorganic carbon turnover in coastal marine sediments

SD, MM, and BMF developed ideas and designed this study. SD and MM collected samples. Experimental work was done by SD, MM, KH*, KB, DP, and RS. Experimental data were contributed by KH*, AM, DM, PP, and MR. SD and MM wrote the manuscript. All co-authors were involved in data interpretation, discussion as well as editing of the manuscript.

This chapter is included in this thesis to provide background information for Chapter II and to validate the newly designed CARD-FISH JTB255 probe sets.

**KH performed comparative counts between her newly designed CARD-FISH probe set and the probe set developed by SD and MM on, both, coastal and deep-sea sediments. KH performed double hybridizations between the two sets of new JTB-probes, the gammaproteobacterial probe (GAM42a), and the Xanthomonadales probe (GAM42a_T1038). KH generated cell images of JTB from coastal and deep-sea sediments. KH discussed CARD-FISH results with SD and MM.*

Contribution of the PhD candidate in % of the total work load (100% for each of the following categories): Experimental concept and design: ca. 0%; Acquisition of (experimental) data: ca. 8%; Data analysis and interpretation: ca. 5%; Preparation of Figures and Tables: ca. 5%; Writing of the manuscript: ca. 0%.

Chapter II 'Diversity and metabolism of the JTB255 clade (Gammaproteobacteria), a global member of deep-sea sediment communities'

Preliminary author list: Katy Hoffmann, Christina Bienhold, Katrin Knittel, Pier L. Buttigieg, Rafael Laso-Pérez, Eduard Fadeev, Josephine Z. Rapp, Antje Boetius, and Pierre Offre.

Planned for submission to The ISME Journal

We combined a diverse set of cultivation-independent and molecular 'omics' techniques, with cultivation-dependent methods to progress on the characterization of the core bacterial group from deep-sea surface sediments: JTB255. This study confirmed the abundance and ubiquitous distribution of JTB255 in deep-sea sediments globally. It refined the phylogenetic position of the JTB255 clade within the *Gammaproteobacteria*, and suggested a grouping into five sequence clusters. Furthermore, we analyzed JTB255`s microdiversity for different marine environments such as sediments, deep-sea water, and polymetallic nodules, indicating a preferential association of sub-groups with certain types of environments. Furthermore, we found strong indications for a heterotrophic lifestyle of JTB255 in deep-sea sediments through the encoding of diverse peptidases and lipases, and compared it to the genetic capacity of published JTB255 genomes from coastal sediments. Given their global distribution, and high abundance we proposed the JTB255 to be a key component of deep-sea benthic ecosystems that may contribute to the remineralization of complex and possibly even aged organic matter at the seafloor.

The study was designed by KH, CB, AB, and PO. KH and JZR collected samples. KH performed lab work. KH and KK designed CARD-FISH probes. KH and CB designed the cultivation experiments. JZR provided the assembled HAUSGARTEN metagenome. KH, RLP, and EF binned JTB255 genomes from the metagenome and anlyzed them. PLB applied oligotyping. KH and PO analyzed the data. KH, PO, and CB discussed the data. KH, PO, and CB wrote the manuscript. The manuscript was edited by all co-authors.

Contribution of the PhD candidate in % of the total work load (100% for each of the following categories): Experimental concept and design: ca. 60%; Acquisition of (experimental) data: ca. 75%; Data analysis and interpretation: ca. 60%; Preparation of Figures and Tables: ca. 80%; Writing of the manuscript: ca. 60%.

Chapter III 'Response of bacterial communities to different detritus compositions in Arctic deep-sea sediments'

Katy Hoffmann, Christiane Hassenrück, Verena Salman-Carvalho, Moritz Holtappels, and Christina Bienhold

Frontiers in Aquatic Microbiology (published in February 2017; doi: 10.3389/fmicb.2017.00266)

In this experimental approach, we combined measurements of community function with high-resolution taxonomic community structure, and statistical methods, in order to evaluate the response of a complex bacterial community from Arctic deep-sea sediments to the input of various organic matter sources. The results were evaluated in the light of a changing Arctic Ocean and provided evidence that differences in organic matter composition led to significant changes in bacterial community structure and function at the seafloor, which may affect carbon turnover and retention in the deep sea. In addition, we identified opportunistic groups of bacteria that may serve as indicator taxa for different organic matter sources in this region.

KH and CB designed experiments. KH performed the experiments. KH, CH, and CB analysed data, and VS-C assisted in data interpretation. MH performed oxygen sensor data analysis and modelling. KH, VS-C, and CB wrote the manuscript with support and input from all co-authors. The manuscript was edited by all co-authors.

Contribution of the PhD candidate in % of the total work load (100% for each of the following categories): Experimental concept and design: ca. 85%; Acquisition of (experimental) data: ca. 100%; Data analysis and interpretation: ca. 75%; Preparation of Figures and Tables: ca. 80%; Writing of the manuscript: ca. 65%.

Chapter IV 'The effect of hydrostatic de- and recompression on bacterial communities sampled from deep-sea sediments'

Katy Hoffmann, Christiane Hassenrück, and Christina Bienhold
In preparation for submission to FEMS Microbiology Letters as a short communication

In this study, we used molecular and statistical methods to assess the influence of de- and recompression on bacterial communities retrieved from high-pressure deep-sea surface sediments at the LTER HAUSGARTEN. Results indicated that hydrostatic pressure changes potentially affected the metabolic activity of bacteria, based on observed altered enzymatic activities and bacterial composition pattern of the actively transcribing bacterial groups (16S rRNA) for certain taxa within the *Proteobacteria* and *Bacteroidetes*. Total community structure (16S rDNA) and total cell numbers remained rather unaffected over short time periods of decompression and over repeated de- and recompression cycles. However, these observations remain to be verified through further sample replication.

KH and CB designed this study. KH did the sampling and laboratory work. KH analyzed the data with input from CH. KH interpreted and discussed the data with input from CH, and CB. KH, CH, and CB wrote the manuscript.

Contribution of the PhD candidate in % of the total work load (100% for each of the following categories): Experimental concept and design: ca. 90%; Acquisition of (experimental) data: ca. 100%; Data analysis and interpretation: ca. 85%; Preparation of Figures and Tables: ca. 80%; Writing of the manuscript: ca. 85%.

2. Thesis Chapters

Chapter I

Ubiquitous *Gammaproteobacteria* dominate dark carbon fixation in coastal sediments

Stefan Dyksma[1], Kerstin Bischof[1], Bernhard M Fuchs [1], Katy Hoffmann[2,3], Dimitri Meier[1], Anke Meyerdierks[1], Petra Pjevac[1,4], David Probandt[1], Michael Richter[5],Ramunas Stepanauskas[6], Marc Mußmann[1]

[1]Department of Molecular Ecology, Max Planck Institute for Marine Microbiology, Bremen, Germany;
[2]HGF-MPG Group for Deep Sea Ecology and Technology, Max Planck Institute for Marine Microbiology, Bremen, Germany;
[3]Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany;
[4]Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, University of Vienna, Vienna, Austria;
[5]Microbial Genomics and Bioinformatics Research Group, Max Planck Institute for Marine Microbiology, Bremen, Germany
[6]Bigelow Laboratory for Ocean Sciences, East Boothbay, USA

The ISME Journal
(10; 1939-1953; August 2016; doi: 10.1038/ismej.2015.257)

Abstract

Marine sediments are the largest carbon sink on earth. Nearly half of dark carbon fixation in the oceans occurs in coastal sediments, but the microorganisms responsible are largely unknown. By integrating the 16S rRNA approach, single-cell genomics, metagenomics and transcriptomics with ^{14}C-carbon assimilation experiments, we show that uncultured *Gammaproteobacteria* account for 70 to 86% of dark carbon fixation in coastal sediments. First, we surveyed the bacterial 16S rRNA gene diversity of 13 tidal and sublittoral sediments across Europe and Australia to identify ubiquitous core groups of *Gammaproteobacteria* mainly affiliating with sulfur-oxidizing bacteria. These also accounted for a substantial fraction of the microbial community in anoxic, 490-cm-deep subsurface sediments. We then quantified dark carbon fixation by scintillography of specific microbial populations extracted and flow-sorted from sediments that were short-term incubated with ^{14}C-bicarbonate. We identified three distinct gammaproteobacterial clades covering diversity ranges on family to order level (the *Acidiferrobacter*, JTB255 and SSr clades) that made up 50% of dark carbon fixation in a tidal sediment. Consistent with these activity measurements, environmental transcripts of sulfur oxidation and carbon fixation genes mainly affiliated with those of sulfur-oxidizing *Gammaproteobacteria*. The co-localization of key genes of sulfur and hydrogen oxidation pathways and their expression in genomes of uncultured *Gammaproteobacteria* illustrates an unknown metabolic plasticity for sulfur oxidizers in marine sediments. Given their global distribution and high abundance, we propose that a stable assemblage of metabolically flexible *Gammaproteobacteria* drives important parts of marine carbon and sulfur cycles.

Keywords

dark carbon fixation; sediment; *Gammaproteobacteria*; sulfur oxidation

Introduction

Marine coastal sediments are global hot spots of carbon remineralization and burial (Hedges and Keil, 1995). In current models of oceanic carbon cycling, the sequestration of microbially altered organic matter is the major mechanism of carbon preservation in sediments (Parkes et al., 1993; Burdige, 2007). Marine sediments are sites not only of carbon remineralization but also of carbon fixation. Recent estimates suggest that marine microbes fix inorganic carbon independent of light (chemolithoautotrophy) in amounts that are in the same order of magnitude as the annual organic carbon burial (Middelburg, 2011). Chemolithoautotrophic microorganisms in marine sediments fix up to 370 Tg C/year, which equals 48% of carbon fixed chemolithoautotrophically in the ocean (Middelburg, 2011). Thereof, 47% are fixed in shallow, near-shore sediments (175 Tg C/year). Near-shore sediments, therefore, contribute more to oceanic carbon fixation than pelagic oxygen minimum zones (OMZs) and hydrothermal vents (Middelburg, 2011). In recent years, chemolithoautotrophy in these marine systems has received much attention. The ecophysiology and genetic composition of key players of carbon (and sulfur) cycling in OMZs and hydrothermal vents, such as the gammaproteobacterial SUP05 clade, have been extensively studied (Lavik et al., 2009; Canfield et al., 2010; Reinthaler et al., 2010; Swan et al., 2011; Grote et al., 2012; Anantharaman et al., 2013; Mattes et al., 2013). This cosmopolitan clade is expected to have an important role in attenuating atmospheric carbon dioxide concentrations, when OMZs expand in a warming climate (Hawley et al., 2014).

In contrast to pelagic OMZs, where the oxic-anoxic/sulfidic interface can be meters thick, in near-shore sediments this interface is only a few millimeters thick, and is characterized by steep biogeochemical gradients and approximately 1000-fold higher cell abundances per sample volume. Biogeochemical evidence indicates that sulfur oxidation is the dominant chemolithoautotrophic process in coastal sediments, while nitrification appears to play only a minor role (Middelburg, 2011; Boschker et al., 2014). Previous studies of benthic autotrophic sulfur oxidizers mostly focused on large, conspicuous sulfur bacteria such as *Beggiatoa*, which are widely distributed but occur in high abundances only in certain habitats (Salman et al., 2013; Ruff et al., 2015). Other *Gammaproteobacteria* affiliating with cultured sulfur oxidizers (*Acidithiobacillus*, *Thiohalophilus* and *Thiomicrospira*) or with uncultured symbiotic sulfur oxidizers among the *Chromaticaeae* and *Ectothiorhodospiracea* have been regularly found in marine and estuarine sediments (Bowman et al., 2005; Orcutt et al., 2011). Consistent with this,

recent molecular and isotopic approaches suggest that some of these are indeed autotrophs (Lenk et al., 2011; Boschker et al., 2014; Vasquez-Cardenas et al., 2015).

Culture-independent molecular studies previously identified predominant carbon fixation pathways such as the Calvin-Benson-Bassham (CBB) cycle and the reductive tricarboxylic acid cycle in marine chemolithoautotrophic bacteria. The key genes encoding ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) form I and form II (*cbb*L, *cbb*M) and the ATP citrate lyase (*acl*AB) in the reductive tricarboxylic acid cycle pathway have been frequently detected in environmental studies (reviewed by Hügler and Sievert, 2011). Likewise, genes encoding subunits of the reverse dissimilatory sulfite reductase (*dsr*AB), of the adenosine-5'-phosphosulfate reductase (*apr*A) and of the thiosulfate-oxidizing Sox-multienzyme complex (*sox*B) have been used to target the diversity of marine benthic sulfur oxidizers (Lenk et al., 2011; Thomas et al., 2014).

To understand how inorganic carbon at sediment surfaces is turned over and possibly buried, detailed knowledge of the microbes driving these processes is essential but currently still lacking. To fill this gap, we surveyed the diversity of candidate bacterial chemolithoautotrophs in 13 coastal surface sediments across Western Europe and Australia. Moreover, we studied whether these chemolithoautotrophic bacteria are also present in anoxic, 490-cm-deep subsurface sediments. We developed a new method to combine ^{14}C-bicarbonate labeling of cells with fluorescence in situ hybridization (FISH), fluorescence-activated cell sorting (FACS) and scintillography to quantify dark carbon fixation by distinct taxonomic groups. Meta- and single-cell genomics along with metatranscriptomics provided evidence for a largely sulfur-based carbon fixation in a selected tidal sediment. Metatranscriptomic reads were mapped against reference databases containing *cbb*L, *cbb*M and *acl*AB sequences to identify active carbon fixation pathways. Metatranscriptomic reads mapped against reference databases containing *dsr*AB, *apr*A and *sox*B sequences indicated sulfur oxidation pathways active in situ. This unique combination of molecular and isotopic approaches provided unprecedented insights into the ecology and ecophysiology of cosmopolitan microorganisms driving a major part of global dark carbon fixation.

Materials and methods

Sediment sampling and characteristics

Between October 2012 and December 2014, we sampled 10 tidal and 3 sublittoral sandy sediments in Western Europe and Australia (Figure 1 and Supplementary Table S1). The 10 tidal sediments were sampled during low tide using polyacryl-cores or cutoff syringes of up to 25 cm length. The three sublittoral, coastal sandy sediments were sampled during cruise He417 with the RV Heincke in March 2014 in the German Bight using multi- and boxcorers. At each site, two to three different sediment layers were selected for molecular analyses (16S rRNA gene amplicon sequencing, catalyzed reporter deposition (CARD-FISH). Sediment for ^{14}C incubations was collected from sites Calais, Courseulles-sur-Mer and from Janssand (Supplementary Table S1). Sediment colors served as a proxy for redox state and active sulfide formation and oxidation (Figure 1). For most of the sites, sediment (i) from the uppermost cm (brownish sediment, sulfide-free), (ii) from the sulfide transition zone (brown to gray, reflecting the presence of iron sulfides) and (iii) from sediment of the sulfidic layer was sampled for molecular analyses (Supplementary Table S1). Sulfide concentrations in pore waters were measured for sites Calais and Courseulles-sur-Mer using the methylene blue method (Cline, 1969). More details on the biogeochemistry of sulfide and oxygen at the study sites Janssand and Königshafen in the German Wadden Sea have been published previously (de Beer et al., 2005; Billerbeck et al., 2006; Jansen et al., 2009). In addition, in April 2005 we sampled a 490-cm-deep subsurface core at site Janssand as described in detail by Gittel et al. (2008). Sulfate and methane concentration profiles and lithological data from this core have been described previously (Gittel et al., 2008; Seidel et al., 2012).

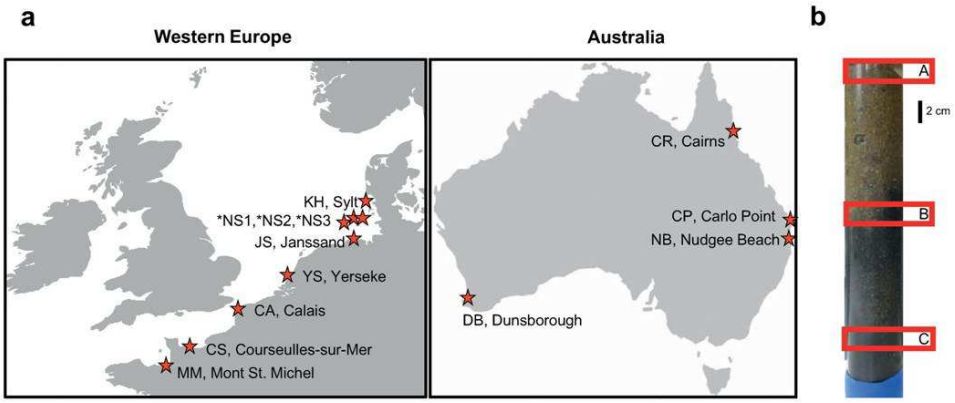

Figure 1. **Sampling sites of the 16S rRNA gene survey** (a). Example for a typical stratification of a sediment core from coastal sandy sediments (b). A = uppermost sediment layer, B = sulfide transition zone and C = sulfidic layer refer to the different sampling depths in this study. During sampling, the sediment colors were used as an indicator for the presence of iron sulfide (dark gray to black). Asterisk indicates samples from sublittoral sediments.

DNA extraction for barcoded 16S rRNA gene amplicon sequencing

For all intertidal and subsurface sediment samples from Europe and Australia, DNA was extracted from 200 to 250 µl sediment recovered from distinct layers using the PowerSoil DNA isolation kit (MoBio Laboratories, Solana Beach, CA, USA). DNA from sites NoahA, NoahB and CCPδ was extracted from 5 g of homogenized surface sediments according to Zhou et al. (1996) including Proteinase K treatment for improved cell lysis.

Barcoded 16S rRNA gene amplicon sequencing

The bacterial diversity in all sediment samples was determined by analyzing the hypervariable V3–V4 region of the 16S rRNA gene using Roche 454 pyro- or Illumina MiSeq-sequencing of barcoded amplicons. Barcoded amplicons from all surface sediments were prepared using primers 341f/785rev (Herlemann et al., 2011; Klindworth et al., 2012). Barcoded amplicons from the 490 cm-deep subsurface sediment from site Janssand were pre-pared by replacing primer 785rev with 907rev (Muyzer et al., 1998; Klindworth et al., 2012). This primer covers a similar bacterial diversity as the reverse primer 785rev used for surface sediments (see above, Figure 2), but it is known to bias against some phyla that are, however, low abundant or absent in marine sediments (Klindworth et al., 2012). In total, 311 196 bacterial 16S rRNA reads were kept for taxonomic classification using the SILVAngs pipeline v115 (Quast et al., 2013) with a clustering at 98% identity. Details on PCR conditions, sequencing and processing are given as Supplementary Information.

Sediment incubations with 14C-bicarbonate

Sediment for ^{14}C-DIC incubations was collected from sites Calais and Courseulles-sur-Mer in July 2013 and from Janssand in April 2014 (Supplementary Table S1). These cores were kept at in situ temperature (15 to 20 °C) and used for ^{14}C-DIC incubations within 48 h after sampling. After slicing, 2 ml of the uppermost sediment layer and from the sulfide transition zone were transferred into 10 ml glass vials. In all, 1 ml of sterile filtered seawater and 1 ml of artificial seawater containing 1.5 mM ^{14}C bicarbonate (specific activity 54.7 mCi mmol^{-1}, Hartmann Analytic, Braunschweig, Germany) were added. Vials were sealed with lab-grade butyl rubber stoppers (GMT Inc., Ochelata, OK, USA) leaving 6 ml of headspace (air). Then, vials were incubated for 20 h with mild agitation (100 r.p.m.) at *in situ* temperature in the dark. In parallel incubations 1 mM thiosulfate was added to slurries from Courseulles-sur-Mer and Calais. Slurry incubations were performed in duplicates (Calais, Courseulles-sur-Mer) or in triplicates (Janssand). Dead controls were included for each site by adding formaldehyde (2%, final concentration) before the incubation.

CARD-FISH and sample preparation for flow cytometry

For CARD-FISH, sediment from sites Calais, Courseulles-sur-Mer and Janssand was fixed immediately after core retrieval as described in Lenk et al. (2011). Cells were detached from 100 to 200 µl sediment by ultrasonic treatment as described previously (Lenk et al., 2011). Permeabilization and CARD-FISH were performed as described by Pernthaler et al. (2002) with the modifications detailed in Supplementary Information. Tyramides labeled with Alexa488 fluorescent dye (Molecular Probes, Eugene, OR, USA) were used for CARD signal amplification. An overview of oligonucleotide probes used in this study is shown in Supplementary Table S2. Novel oligonucleotide probes were designed for the JTB255 group using ARB and the SILVA 16S rRNA reference database release 117 (Pruesse et al., 2007). Please note that the *Xanthomonadales*, which includes the JTB255 clade, are not targeted by probe GAM42a, specific for most *Gammaproteobacteria* (Siyambalapitiya and Blackall, 2005). In line with this, the JTB255 clade could not be detected with probe GAM42a in double hybridizations with the JTB-probe mix (Supplementary Table S2). Therefore, we summed up FISH counts of the JTB255-probe mix and of probe GAM42a to yield the total relative abundance of FISH-detectable *Gammaproteobacteria*.

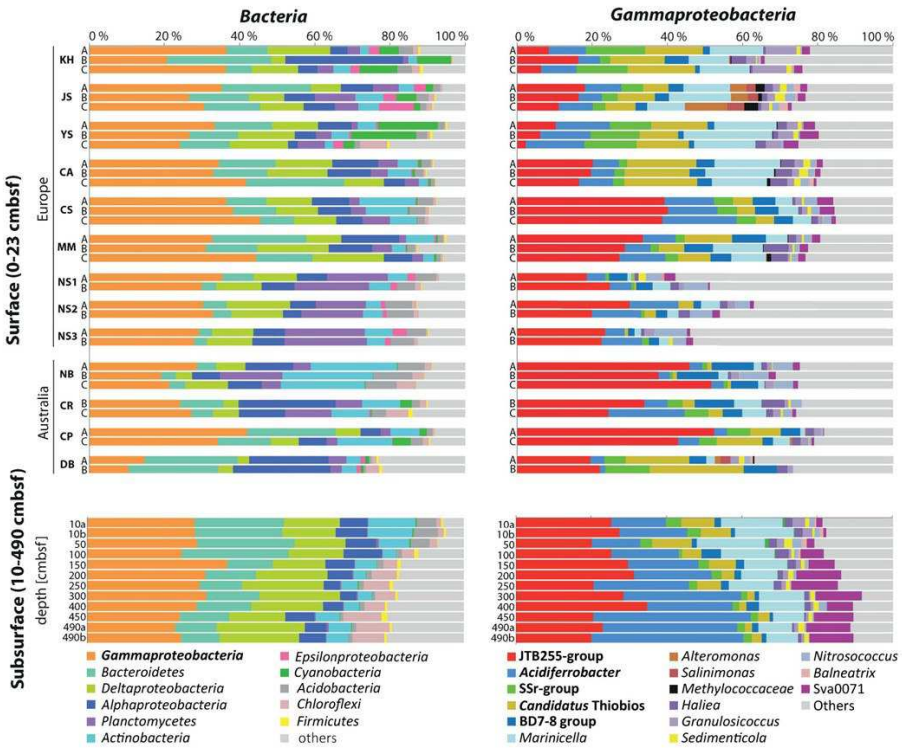

Figure 2. **Relative abundances of the bacterial 16S rRNA gene sequence** (V3-V4 regions) in the 13 coastal sediments. Left panels: relative sequence abundance of most frequent bacterial phyla and classes. Right panels: relative sequence abundance of most frequent taxonomic groups (family to order level) within the class of *Gammaproteobacteria* according to the taxonomy of SILVA release 117 (Pruesse et al., 2007). The five candidate chemolithoautotrophic clades are given in bold. Upper panels: relative sequence abundance in 13 coastal surface sediments from 0 to 23 cm below surface (cmbsf) max. A=uppermost sediment layer (0.5 or 1 cmbsf), B=sulfide transition zone, C=sulfidic layer. For the detailed depths ranges see Supplementary Table 1. Lower panels: relative sequence abundance in subsurface sediments at site Janssand (JS) from 50 to 490 cmbsf. For comparison, samples from 10 cmbsf are included. Sediment samples from 10 cmbsf and 490 cmbsf were run in duplicate (10ab, 490ab). All but one amplicons were pyrosequenced, samples from DB were sequenced via the Illumina MiSeq platform.

FACS and scintillography of sorted cells

We developed a novel protocol to quantify bulk assimilation of radiolabelled substrates in a defined number of cells phylogenetically identified via CARD-FISH before for flow sorting. To minimize cell loss, the filters were handled extremely carefully during the CARD-FISH procedure. Cells were scraped off from membrane filters by using a cell scraper or membrane filters were vortexed in 5 ml of 150 mM NaCl containing 0.05% Tween-80 according to Sekar et al. (2004). Before flow cytometry, large suspended particles were removed by filtration through 8-µm pore-size filter (Sartorius, Göttingen, Germany) to avoid clogging of the flow cytometer.

Flow sorting of cells that were fluorescently labeled by CARD-FISH was performed using a FACS Calibur flow cytometer equipped with cell sorter and a 15-mW argon ion laser exciting at 488 nm (Becton Dickinson, Oxford, UK). Autoclaved Milli-Q water was used as sheath fluid. Cell sorting was done at low flow rate of 12 ± 3 µl min^{-1} or med flow rate of 35 ± 5 µl min^{-1} with single-cell sort mode to obtain the highest purity. The event rate was adjusted with a fluorescence threshold, and sorting was performed at a rate of approximately 25 to 100 particles s^{-1}. Hybridized cells were identified on scatter dot plots of green fluorescence versus 90° light scatter (Supplementary Figure S1). Sediment background such as clay particles was determined by flow-cytometric analysis of sediment hybridized with a nonsense probe (NON338) (Supplementary Figure S1). For subsequent measurements, 50 000 cells were sorted and filtered onto 0.2-µm polycarbonate filters (GTTP, Millipore, Eschborn, Germany). Dead controls of ^{14}C incubated sediments did not show any measurable assimilation of ^{14}C, and were thus not used for cell sorting. Unspecifically adsorbed label in live samples caused only minor radioactive background as determined by spiking experiments with fluorescent beads and *Escherichia coli* cells (Supplementary Figure S2). Fluorescent beads (yellow-green, 1.0 µm, Polyscience, Warrington, PA, USA) or hybridized *E. coli* cells were flow-sorted from of a sample hybridized with the nonsense probe to determine radioactive back-ground. *E. coli* cells were hybridized with EUBI-III probe beforehand. Beads and *E. coli* cells were mixed with the sample in approximately the same quantity as the target populations (10 to 20% of total cells). Two to three repeated sortings were applied to confirm the technical reproducibility from duplicate or triplicate incubations. Collected cell batches on polycarbonate filters were directly transferred into 5 ml scintillation vials and mixed with 5 ml Ultima-Gold XR (Perkin-Elmer, Boston, MA, USA) scintillation cocktail. Radioactivity of sorted cell batches was measured in a liquid scintillation counter (Tri-Carb 2900, Perkin-Elmer).

The purity of flow-cytometric enriched target cells was 93%, and was manually analyzed under an Axioplan epifluorescence microscope (Zeiss, Jena, Germany). For microscopic analysis, filters were counterstained with 1 µg ml^{-1} 4′,6-diamidino-2-phenylindole (DAPI) and at least 1000 DAPI-stained cells were examined for CARD-FISH. Our approach was technically highly reproducible, and the radioactivity linearly increased with the number of sorted cells (Supplementary Figure S2).

For calculation of average cell-specific carbon fixation rates in our slurry experiments, we assumed a background concentration of dissolved inorganic carbon of 2 mM (Billerbeck et al., 2006) as we used local seawater for our experiment (see Supplementary Information for calculations). The relative abundance of assimilating gammaproteobacterial cells in the sulfide transition zone from Calais, Courseulles-sur-Mer and Janssand sediments was determined by microautoradiography (MAR). MAR was performed according to Alonso and Pernthaler (2005) and Lenk et al. (2011) with an exposure time of 2 days. Relative abundance of MAR-positive cells was manually determined under an Axioplan epi-fluorescence microscope (Zeiss).

A single-cell genome of the SSr clade from Janssand sediment

In January 2011, the upper two centimetres of Janssand sediment were sampled for extraction and sorting of single bacterial cells for whole-genome amplification. After extraction, cells were cryopreserved with N,N,N-trimethylglycine ('glycine betaine') (Sigma-Aldrich, St Louis, MO, USA) at a final concentration of 4% according to Cleland et al. (2004), stored at − 80 °C and shipped overseas. Single-cell sorting and whole-genome amplification via multiple displacement amplification were performed at the Bigelow Laboratory Single Cell Genomics Center (https://scgc.bigelow.org) as described by Swan et al. (2011). A single amplified genome (SAG) encoding a single, high quality 16S rRNA gene sequence affiliating with the SSr clade was sent to Max Planck Genome Centre (MP-GC) Cologne for MiSeq (Illumina) sequencing yielding 9 557 547 PE reads. The SAG assemblies were auto-annotated using the Joint Genome Institute IMG-ER pipeline (Markowitz et al., 2012). Details on cell extraction, sequencing and quality control of the assembled genomic data are given as Supplementary information.

cDNA libraries and metatranscriptomic mapping

In April 2013, sediment was sampled from the sulfide transition zone at site Janssand and immediately frozen on dry ice. Total RNA was extracted from sediment in triplicates (one ml each) by the Vertis Biotechnologie AG (Freising, Germany), and bacterial rRNA was depleted with the Ribo-Zero Magnetic Kit (for Bacteria) (Epicentre, Madison, WI, USA). Barcoded RNA TrueSEQ libraries were constructed from RNA extractions and paired-end sequenced using Illumina HiSeq2000 (MP-GC, Cologne, Germany).

After quality trimming at a Phred score 28 using Nesoni clip v.0.115 (http://www.vicbioinformatics.com/software.nesoni.shtml), reads were mapped to reference databases of nucleotide sequences encoding key genes for sulfur oxidation (Sox-multienzyme complex, *sox*B; reverse dissimilatory sulfite reductase, *dsr*AB; adenosine-5′-phosphosulfate reductase, *apr*A; uptake [NiFe]-hydrogenase, *hup*L; ammonia monooxygenase, *amo*A, ribulose-1,5-bisphosphate carboxylase/oxygenase form I, *cbb*l, and form II, *cbb*M; ATP citrate lyase, *acl*AB) and to the SAG using Bowtie2 (Langmead and Salzberg, 2012). Details on program settings and normalization are given as Supplementary information.

Nucleotide accession numbers

All nucleotide sequences obtained in this study have been deposited in GenBank. Sequences of 16S rRNA and hydrogenase gene libraries are available under accession numbers KR824952–KR825244 and KR534775–KR534844, respectively. Amplicon sequences from the 16S rRNA gene surveys were deposited in NCBI BioProjects PRJNA283163 and PRJNA285206. All cDNA reads are available in BioProject PRJNA283210. Fosmid end sequences are available in NCBI's Genome Survey Sequences database (GSS) with the accession numbers KS297884–KS307053. The genome sequence of the SAG WSgam209 is accessible under the IMG Genome ID 2609459745 through the Joint Genome Institute portal IMG/ER (https://img.jgi.doe.gov/ cgi-bin/er/main.cgi), the metagenomic bin Acidi-ferrobacter-a7 is accessible under the IMG Genome ID 2616644801.

Results and Discussion

Identification of candidate chemolithoautotrophs in coastal surface sediments

To identify candidate chemolithoautotrophs in coastal sediments, we studied the bacterial diversity in 10 tidal and 3 sublittoral sandy sediments from Western Europe and Australia (Figure 1 and Supplementary Table S1). We sequenced the V3–V4 region (4300 bp) of tagged 16S rRNA gene amplicons. After quality trimming, 311 196 Illumina-and 454-tag reads were recovered. Taxonomic classification revealed that *Gammaproteobacteria* were consistently among the most abundant clades on class to phylum level, accounting for 12 to 45% of sequences regardless of sampling site, sediment depth or season (Figure 2). These data were supported by CARD-FISH, which showed that *Gammaproteobacteria* make up 19 to 22% of all bacteria at sites Janssand, Calais and Courseulles-sur-Mer (Supplementary Table S5). At all sites, we observed a recurring diversity pattern also at the family to order level within the *Gammaproteobacteria*. We consistently identified candidate chemoautotrophs most closely related to: (1) *Acidiferrobacter thiooxydans* of the family *Ectothiorhodospiraceae*, (2) symbionts of the siboglinid tubeworms such as *Oligobrachia* spp. (henceforth designated as *Siboglinidae* Symbionts related, SSr, see Supplementary Figures S3 and S4), (3) ciliate symbiont Candidatus *Thiobios zoothamnicoli* and (4) BD7-8 clade, including the γ3 symbiont of the marine gutless oligochaete *Olavius algarvensis* (Woyke et al., 2006). Sulfur-dependent chemolithoautotrophy for cultured or symbiotic relatives of these clades has been shown before (Rinke et al., 2006; Lösekann et al., 2008; Hallberg et al., 2011; Kleiner et al., 2012). *Acidiferrobacter thiooxydans* can also grow autotrophically with ferrous iron (Hallberg et al., 2011). Moreover, we previously showed carbon fixation by the SSr- and the *Acidiferrobacter*-related clades, and determined relative cell abundances of up to 8% at site Janssand in the German Wadden Sea (Lenk et al., 2011).

Strikingly, in all tested sediments up to 52% of gammaproteobacterial sequences grouped with the uncultured JTB255 clade. This clade is affiliated with the order *Xanthomonadales* and accounted for the largest fraction of gammaproteobacterial sequences at 10 out of 13 sites (Figure 2). In line with the sequence data, CARD-FISH targeting members of the JTB255 clade revealed rod-shaped cells (Supplementary Figure S5) that made up 3 to 6% of total cell counts in Janssand, Calais and Courseulles-sur-Mer sediments (Supplementary Table S6). So far, the exact environmental function of the JTB255 clade is unknown; however, a sulfur-oxidizing activity has been hypothesized

(Bowman and McCuaig, 2003). In summary, we identified five candidate chemolithoautotrophs that accounted for 28 to 75% of *Gammaproteobacteria* and for 8 to 31% (average = 17%) of all bacterial sequences across all sites. Other potentially autotrophic populations such as sulfur-oxidizing *Epsilonproteobacteria*, anoxygenic phototrophs, the BD1-5/SN-2 clade, nitrifiers and cyanobacteria were found in low abundance or were patchily distributed (Figure 2).

Gammaproteobacteria in subsurface sediments

Because of the high sedimentation rates of 43 mm/year in the German Wadden Sea at site Janssand (Ziehe, 2009), a yet unknown fraction of the surface microbial community including the chemolithoautotrophic *Gammaproteobacteria* is buried into the anoxic subsurface. To study, how these organisms are affected by such a burial, we also analyzed the distribution of chemolithoautotrophic *Gammaproteobacteria* in a 490-cm-deep subsurface core from site Janssand, spanning a sedimentation record of 1000 to 2000 years (Ziehe, 2009). This sediment core displayed a typical sulfate-methane-transition zone in 150 to 200 cm below surface (cmbsf) (Gittel et al., 2008), reflecting the changes in the major metabolic pathways that are active along this depth range. Surprisingly, *Gammaproteobacteria* including the chemolithoautotrophic gammaproteobacterial clades also accounted for a dominant fraction of 16S rRNA gene pyrotags over the entire depth range (21 to 37% of all sequences, Figure 2). This observation was supported by 16S rRNA gene libraries from 200 and 490 cmbsf (Supplementary Figure S4), in which these clades accounted for 95 out of 289 clones (33%). To gain PCR-independent support for the dominance of *Gammaproteobacteria* in subsurface sediments, we fosmid-cloned large metagenomic fragments of ~ 40 kb in size from 490 cmbsf and taxonomically classified the end sequences (Supplementary Table S7), as FISH is commonly too insensitive to comprehensively target subsurface organisms with very low ribosome content (Schippers et al., 2005). In support of our 16S rRNA gene data, 24% of all prokaryote-affiliated fosmid end sequences (n = 4052) showed best hits to *Gammaproteobacteria*, while only approximately 1% affiliated with *Archaea* (Supplementary Table S7). Collectively, these molecular data indicate a relatively stable community structure over the 5-m depth range, despite the measured strong biogeochemical gradients. These data are consistent with similar total cell abundances and a similar composition of major phospholipids over the entire 490 cm depth in the same sediment core (Gittel et al., 2008; Seidel et al., 2012).

Whether these gammaproteobacterial populations are active despite very different biogeochemical settings in the subsurface, or whether they are simply surviving in subsurface sediments with little or no turnover, are currently unclear. Upon burial, marine microbial cells may survive in the subsurface over geological time scales without significant growth (Jørgensen, 2011). Energy for maintenance and survival could be supplied by fermentation of refractory organic matter and by the slow transport and diffusion of dissolved organic compounds to subsurface sediments, which has been demonstrated for the subsurface at site Janssand (Røy et al., 2008; Seidel et al., 2012).

Global occurrence of candidate chemolithoautotrophic Gammaproteobacteria

Our 16S rRNA diversity and FISH data agree well with numerous studies showing a substantial contribution of *Gammaproteobacteria* to microbial communities in diverse marine surface sediments (Hunter et al., 2006; Kim et al., 2008; Schauer et al., 2009; Orcutt et al., 2011; Gobet et al., 2012; Ruff et al., 2015). To examine the geographic distribution of the five candidate chemoautotroph groups in more detail, we did a meta-analysis of 16S rRNA gene sequence data from 65 diversity studies of the sea floor (Figure 3). Although these published data sets hardly covered the extent of microbial diversity at the studied sites, sequences related to the *Acidiferrobacter*, SSr and to a lesser extent *Ca. T. zoothamnicoli* and BD7-8 groups were found in all types of benthic habitats ranging from inter-tidal sediments to deep-sea hydrothermal chimneys (Figure 3). Intriguingly, the JTB255 clade was detected in 92% of all studies (Figure 3) and accounted for the most frequent sequence group among *Bacteria* in Arctic, Antarctic and tropical deep-sea as well as shallow coastal sediments (Wang et al., 2013; Zheng et al., 2014; Liu et al., 2014; Emil Ruff et al., 2014). In temperate Tasmanian and in cold Antarctic coastal sediments, 16S rRNA gene copies of the JTB255 clade accounted for 6 to 9% of total bacterial 16S rRNA gene sequences (Bowman et al., 2005). In summary, our biogeographic survey shows that these five clades are important members of microbial communities in marine surface sediments worldwide, and clearly, the JTB255 clade is one of the most successful bacterial lineages in marine surface sediments.

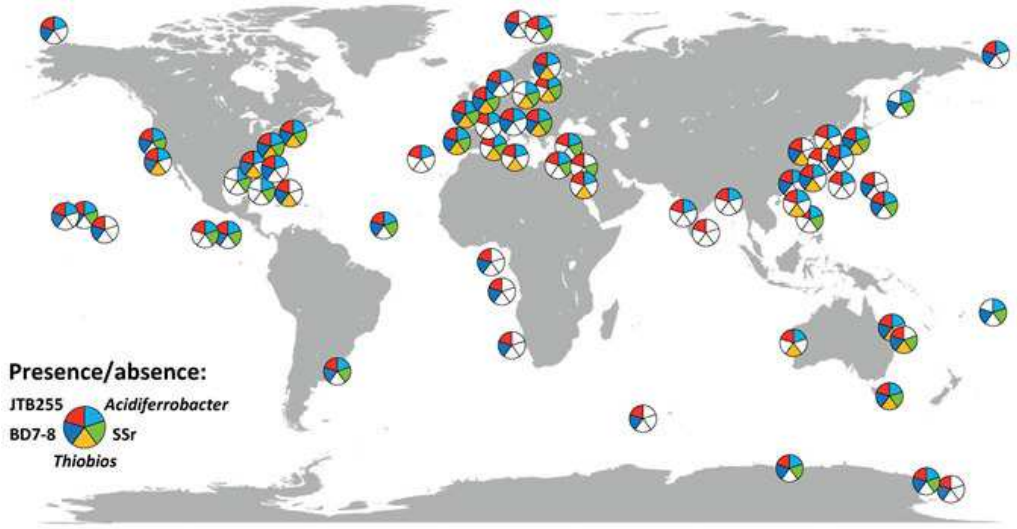

Figure 3. **Biogeographic survey of the major chemolithoautotrophic gammaproteobacterial clades identified in this study.** Only clone sequences (presence/absence) from bacterial diversity studies from 65 marine sea floor surfaces have been considered, deposited in the SILVA database release 117 (Pruesse et al., 2007).

Measuring dark carbon fixation by Gammaproteobacteria in sediments

Our 16S rRNA gene survey suggested that sulfur-oxidizing *Gammaproteobacteria* are potentially the major carbon fixers in dark coastal sediments. However, quantitative data on carbon fixation by distinct bacterial populations in marine sediments are lacking. To determine the contribution of *Gammaproteobacteria* to dark carbon fixation in sediments, we developed a novel approach to quantify assimilation of a radiolabeled compound by specific populations. Previous FACS experiments with autofluorescent, radiolabeled marine bacterioplankton and subsequent scintillography of sorted populations prompted us to use FISH signals instead of autofluorescence or unspecific DNA staining to identify and enrich populations from sediments (Zubkov et al., 2003; Jost et al., 2008). We incubated aerobic sediment slurries prepared from surface and from sulfide transition zone sediments from sites Calais, Courseulles-sur-Mer and Janssand with ^{14}C-labeled bicarbonate and for 20 h in the dark. After detachment of cells from sand grains and CARD-FISH, we sorted fluorescently labeled *Bacteria* (probe EUBI-III) or *Gammaproteobacteria* (probe GAM42a). To account for potentially nitrifying autotrophic *Archaea*, we also sorted cells targeted by the archaeal probe Arch915. Fifty thousand cells were sorted per population, and bulk radioactivity was measured. This workflow allowed us to accurately quantify the bulk assimilation of radiolabeled substrates by a defined population in high throughput. At the analysis level of populations and communities it thereby overcomes limitations in throughput and precision of other

methods such as MAR-FISH, HISH-SIMS and stable isotope probing (Boschker et al., 1998; Lee et al., 1999; Radajewski et al., 2000; Manefield et al., 2002; Musat et al., 2008).

Although the total amount of fixed carbon in sorted populations varied between sites and samples, the ^{14}C-assimilation by sorted *Gammaproteobacteria* ranged from 2.4 to 10.3 Bq and was 2.5- to 5-fold higher than those of sorted *Bacteria* (0.5 to 2.1 Bq) (Figure 4a). At all three sites, the relative abundance of ^{14}C-assimilating *Gammaproteobacteria* was approximately 40 to 50% as determined by MAR (Supplementary Table S8; Lenk et al., 2011) and is similar to the relative sequence frequency of chemoautotrophic subpopulations (Figure 2). The ^{14}C-radioactivity of 50 000 archaeal cells accounted for 1.4 to 3.8 Bq, ranging between the average assimilation by *Bacteria* and *Gammaproteobacteria* (Figure 4b). Addition of nitrate did not stimulate ^{14}C-assimilation in anoxic slurry incubations. The addition of 1 mM thiosulfate doubled the total carbon fixation by *Gammaproteobacteria* in the uppermost surface sediments, but not in the sulfide transition zone at Calais and Courseulles-sur-Mer (Figure 4a). Likewise, thiosulfate did not stimulate carbon fixation in the sulfide transition zone from site Janssand (Zerjatke, 2009). The oxidized surface sediments are possibly limited in electron donors, while the sulfide transition zone contained sufficient reduced sulfur compounds such as free and iron sulfides as energy sources for carbon fixation (Jansen et al., 2009; Supplementary Table S1).

Dark carbon fixation in Gammaproteobacterial clades

To quantify carbon fixed by the candidate chemolithoautotrophic clades, we used the FISH probes available for three of the five clades for cell sorting and subsequent scintillography. Our FISH probes for the SSr and *Acidiferrobacter* clades mostly target sequences retrieved from site Janssand (Supplementary Figure S4; Lenk et al., 2011); therefore, we measured carbon assimilation by specific subpopulations at this site. The *Acidiferrobacter* clade showed the highest ^{14}C-assimilation (6.1 to 10 Bq), while the SSr clade assimilated 2.7 to 6.9 Bq (Figure 4b and Supplementary Figure S6). This is consistent with the chemolithoautotrophic potential encoded in the corresponding genomes (Supplementary Table S5), and is confirmed by the previously detected ^{14}C bicarbonate assimilation by single cells of both clades (Lenk et al., 2011).

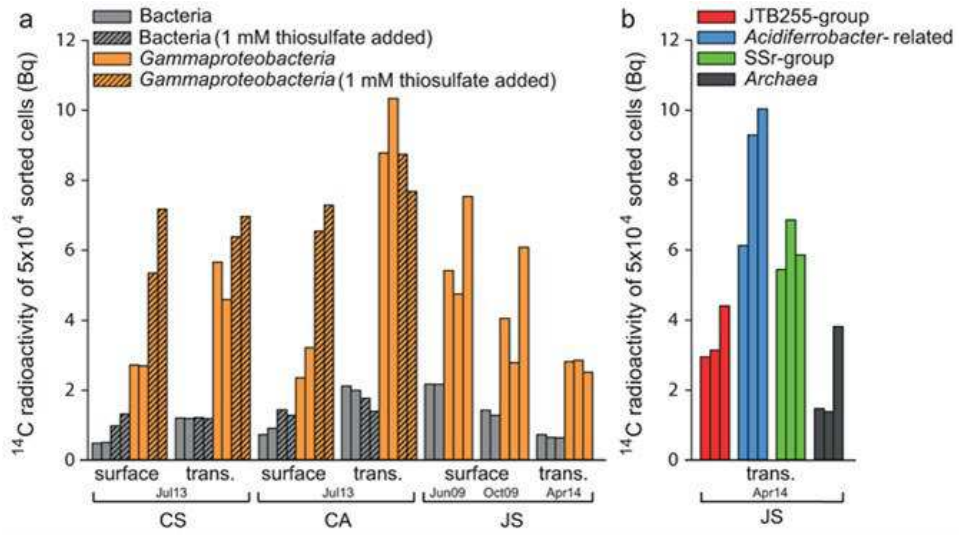

Figure 4 **^{14}C carbon fixed by *Bacteria*, *Gammaproteobacteria* and *Archaea* in three coastal sediments.** Carbon fixation by flow-sorted populations of *Bacteria*, *Gammaproteobacteria* and *Archaea* from the uppermost sediment layer (surface, 0 to 1 cmbsf) and from the sulfide transition zone (trans.) incubated with ^{14}C bicarbonate. ^{14}C-assimilation by *Bacteria* and *Gammaproteobacteria* at three sampling sites (Courseulles-sur-Mer, Calais and Janssand) and in different seasons (a). ^{14}C-assimilation by three gammaproteobacterial clades and Archaea (b). Batches of 50 000 cells were sorted per measurement. The ^{14}C carbon activity is given in Becquerel (Bq).

Our probes for the ubiquitous JTB255 clade display a wider target range; therefore, we used these probes to sort JTB255 cells from Janssand, Calais and Courseulles-sur-Mer. The ^{14}C-assimilation by the JTB255 clade ranged from 3 to 4.4 Bq in Janssand sediment to up to 10.7 Bq in Calais sediment (Figure 4b and Supplementary Figure S6). The ^{14}C-assimilation by the JTB255 clade was slightly less than that of the SSr clade. The addition of thiosulfate did not stimulate the ^{14}C-assimilation by the JTB255 clade in Courseulles-sur-Mer but did slightly stimulate it in Calais sediments, which is consistent with the hypothesized thiotrophy of members of this clade (Bowman and McCuaig, 2003).

The ^{14}C-assimilation by the three gammaproteobacterial clades was up to 10-fold higher than the ^{14}C-assimilation by the entire bacterial community (Figures 4a and b), which largely consisted of heterotrophic bacteria (Figure 2). Using the carbon assimilated by the bulk bacterial community (targeted by probes EUBI-III) as an approximate reference for heterotrophic, anaplerotic carbon fixation (Wood and Werkman, 1936; Li, 1982; Roslev et al., 2004), we conclude that heterotrophic carbon fixation was minor. Moreover, we calculated average carbon fixation rates per sorted gammaproteobacterial cell based on all sorted *Gammaproteobacteria* (1.1 to 3.0 fg C cell $^{-1}$

day $^{-1}$, Supplementary Table S8). Average carbon fixation rates per cell and for each of the three individual subpopulations ranged from 1.1 to 3.5 fg C cell $^{-1}$ day $^{-1}$. These rates are consistent with those of autotrophic freshwater green sulfur bacteria (1.4 to 5.8 fg C cell $^{-1}$ day $^{-1}$) (Musat et al., 2008), and are in the lower range of rates measured for autotrophic marine bacterioplankton (3.5 to 24.7 fg C cell $^{-1}$ day $^{-1}$) (Jost et al., 2008). Collectively, these data strongly support an autotrophic carbon fixation by the *Acidiferrobacter*, SSr and JTB255 clades.

Gammaproteobacteria dominate dark carbon fixation in coastal sediments

The relative contribution of carbon fixed by the *Acidiferrobacter*, SSr and JTB255 clades amounted to 77% of gammaproteobacterial and to 50 to 62% of bacterial dark carbon fixation at Janssand (Figure 5). Although they make up only 19 to 22% of the total microbial community, *Gammaproteobacteria* in total accounted for 70 to 86% of the microbial dark carbon fixation irrespective of sampling site, season and sediment depth (Figure 5 and Supplementary Figure S7).

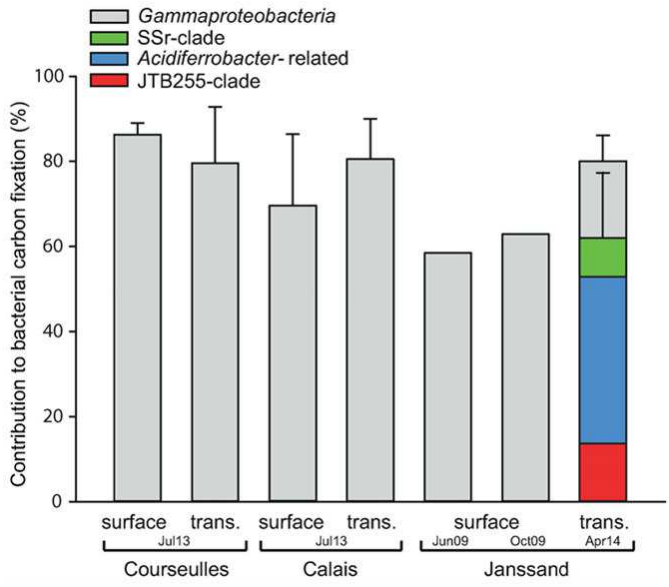

Figure 5 **Relative contribution of *Gammaproteobacteria* to total bacterial dark carbon fixation in three coastal sediments.** The relative contribution of carbon fixed by total *Gammaproteobacteria* and by the *Acidiferrobacter*, SSr and JTB255 clade to total bacterial dark carbon fixation was calculated by integrating the ^{14}C-carbon assimilation per population (dark gray bar) and the relative cell abundances of the respective populations in the sediments. Error bars represent the standard deviation (s.d.) of 3 to 4 replicate incubations. Values from 2009 (site Janssand) are depicted as average of duplicates only. Note that for experiments in 2009 ^{14}C-assimilation by the JTB255 clade was not measured (for details, see Supplementary Information).

Although they can be important autotrophs in organic-poor deep-sea sediments (Molari et al., 2013) nitrifying *Archaea* have a minor role in dark carbon fixation in the sediments we measured. In our study, *Archaea* occurred at low relative abundances and assimilated less ^{14}C than *Gammaproteobacteria* (Figures 4a and b and Supplementary Figure S7).

Genomics suggests thioautotrophy in uncultured Gammaproteobacteria

We previously showed that key genes of sulfur oxidation in Janssand sediments are mainly affiliated with *Gammaproteobacteria* (Lenk et al., 2011). To further investigate the metabolism of the candidate chemolithoautotrophic *Gammaproteobacteria*, we sequenced the amplified genomic DNA of a single cell of the SSr clade from Janssand sediment. In addition, we recovered a metagenomic bin of a member of the *Acidiferrobacter* clade from a deep-sea hydrothermal chimney that displayed 91% sequence identity to 16S rRNA gene sequences from site Janssand (Supplementary Figure S4).

The assembled SAG of the SSr cell ('WSgam209') consists of 1.9 Mbp on 311 scaffolds (Supplementary Table S3). In addition to the cytochrome c oxidase for oxygen respiration, the genome encoded a reverse dissimilatory adenosine-5′-phosphosulfate (APS)-reductase subunit A (AprA), a widely distributed enzyme catalyzing the oxidation of sulfite to sulfate (Meyer and Kuever, 2007). Similar to the 16S rRNA gene also the AprA is affiliated with the sulfur-oxidizing symbionts of *Oligobrachia haakonmosbiensis* (Supplementary Figures S4 and S8), indicating a congruent phylogeny of both phylogenetic markers. Moreover, it encoded the large and small subunits of the RuBisCO form I (CbbL, CbbS) (Supplementary Table S5).

The metagenomic bin of the *Acidiferrobacter*-clade organism ('*Acidiferrobacter*-a7') consists of 2 Mbp on 66 scaffolds (Supplementary Table S3). It contained genes for thiosulfate oxidation (Sox-multienzyme complex, *sox*ABXYZ), sulfite oxidation (*soe*ABC) and carbon fixation (RuBisCO form I subunits *cbb*L and *cbb*S) (Supplementary Table S4). Together with the measured carbon fixation of SSr and the *Acidiferrobacter* clades at site Janssand, the identification of sulfur oxidation and carbon fixation genes in both clades provides the genetic back-ground of their chemolithoautotrophic potential.

Metatranscriptomics underscores the role of Gammaproteobacteria in thioautotrophy

To further test, whether *Gammaproteobacteria* in these sediments are the major active chemolithoautotrophs, we sequenced triplicate metatranscriptomes from the sulfide transition zone of Janssand sediments. Transcript reads were mapped to reference sequences from the GenBank database, functional gene libraries, metagenomic fragments and the SSr-single cell genome recovered from site Janssand (this study; Lenk et al., 2011, 2012).

To identify the expressed carbon fixation pathways, we mapped the metatranscriptomic reads to gene sequences of RuBisCO form I and form II (*cbb*L, *cbb*M) and of ATP citrate lyase (*acl*AB) from cultured representatives and environmental sequences from diverse aquatic environments including sediments.

All transcripts of RuBisCO genes, on average 112 reads, mapped to those of sulfur-oxidizing *Gammaproteobacteria*, confirming active carbon fixation through the CBB-cycle in Janssand sediments. Only very few transcripts (n 6) could be mapped on genes encoding an epsilonproteobacterial ATP citrate lyase (Supplementary Table S4), reflecting the low relative abundance and low activity of sulfur-oxidizing *Epsilonproteobacteria* in these sediments (Figure 2; Lenk et al., 2011).

To identify the expressed sulfur oxidation pathways and the respective organisms in the sulfur transition zone in Janssand sediments, we mapped the metatranscriptomic reads to references databases containing gene sequences encoding SoxB, AprA and subunits of the reverse dissimilatory sulfite reductase, DsrAB. The major fraction (69 to 82%) of transcripts mapped to *dsr*AB, *sox*B and *apr*A sequences that are affiliated with *Gammaproteobacteria* (Figure 6 and Supplementary Figure S8), further supporting their central role in chemolithoautotrophy. Here, *dsr*AB transcripts were fourfold higher than *sox*B transcripts. The identification of the sulfur oxidation pathway prevailing in marine sediments has important implications for modeling of carbon budgets, as the reverse Dsr (rDsr) pathway may allow a higher ATP gain that can be used for carbon fixation than the complete Sox-multienzyme complex (Klatt and Polerecky, 2015). In the rDsr pathway electrons finally enter the electron transport chain at the level of quinone (Holkenbrink et al., 2011), while the Sox-multienzyme complex donates electrons to cytochrome c (Kelly et al., 1997), probably resulting in higher energy yields for rDsr-encoding sulfur oxidizers. However, further in situ studies are essential to confirm this observation.

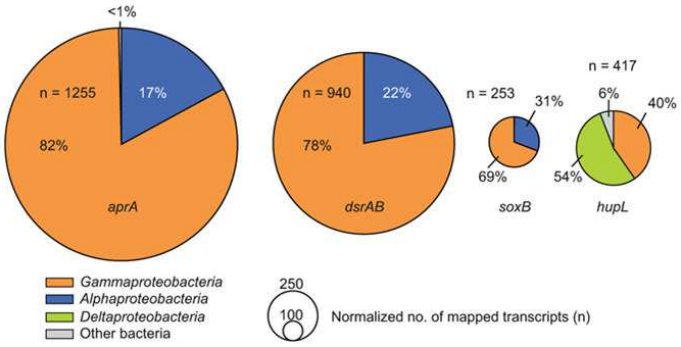

Figure 6 **Taxonomic affiliation of metatranscriptomic reads of key genes from sulfur and hydrogen oxidation pathways.** Metatranscriptomic reads from the sulfide transition zone (Janssand surface sediments 2 to 3 cmbsf) were mapped to key genes of sulfur and hydrogen oxidation genes (*apr*A, adenosine-5′-phosphosulfate reductase; *dsr*AB, reverse dissimilatory sulfite reductase; *sox*B, Sox-multienzyme system; uptake [NiFe]-hydrogenase). Metatranscriptomes were sequenced in triplicates, and the average values are displayed. Transcript abundance is normalized for gene length and number of reads per data set.

Very few reads mapped to bacterial (n 2) and archaeal (n 5) *amo*A genes encoding the ammonia monooxygenase subunit A (AmoA) (Supplementary Table S1). This is consistent with the minor role of *Archaea* in carbon assimilation measured in our study (Supplementary Figure S7), and is also consistent with the very low nitrification rates measured at site Janssand (Marchant et al., 2014). Overall our data confirm previous biogeochemical models suggesting a low impact of nitrification on chemolithoautotrophic production in coastal sediments (Middelburg, 2011; Boschker et al., 2014).

Hydrogen is likely an alternative energy source for sulfur-oxidizing Gammaproteobacteria

Recently, uptake [NiFe]-hydrogenase genes were found in metagenomic bins of *Gammaproteobacteria* from estuarine sediments, indicating alternative energy sources for dark carbon fixation under oxic to suboxic conditions (Baker et al., 2015). As the ability to oxidize hydrogen has been shown to confer metabolic plasticity to symbiotic and pelagic sulfur-oxidizing bacteria (Petersen et al., 2011; Anantharaman et al., 2013; Hansen and Perner, 2015), we tested whether hydrogen could serve as an alternative energy source also for gammaproteobacterial sulfur oxidizers in marine sediments, for example, to respire sulfur under anoxic conditions (Laurinavichene et al., 2007). To overcome the lack of reference sequences from marine sediments, we constructed an uptake [NiFe]-hydrogenase gene library from Janssand sediments. The recovered hydrogenase gene

diversity comprised different physiological groups from phyla such as *Proteobacteria* and *Bacteroidetes* (Supplementary Figure S9). A substantial fraction (40%) of all hydrogenase transcripts were assigned to sequences of diverse *Gammaproteobacteria*, in particular to those grouping with sulfur-oxidizing bacteria (Figure 6 and Supplementary Figure S9). Expression levels of hydrogenase genes were lower than those of sulfur oxidation genes, but were in the same order of magnitude (Figure 6).

To link a potential hydrogen-oxidizing activity with sulfur-oxidizing *Gammaproteobacteria*, we searched for co-localization and co-expression of key genes of both pathways. First, we identified a metagenomic fragment from Janssand, affiliated with *Gammaproteobacteria*, which encoded both the uptake [NiFe]-hydrogenase HupSL and the rDsr operon (Supplementary Figure S10). Notably, the SAG of the SSr-group recovered from site Janssand also encodes an uptake [NiFe]-hydrogenase gene in addition to *apr*A and *cbb*LS. Moreover, these genes were among the top 20 transcribed genes out of 2008 identified genes (Supplementary Figure S11).

Collectively, our single-cell genomic, metagenomic and metatranscriptomic data indicate that *Gammaproteobacteria* in marine surface sediments may use both reduced sulfur species and hydrogen as energy sources for carbon fixation. In fact, the thioautotroph *Sulfurimonas denitrificans* grows more efficiently with hydrogen than with thiosulfate, when the electron acceptor nitrate is limiting (Han and Perner, 2014). Hence, hydrogen oxidation could be a hitherto overlooked energy source for carbon fixation in marine sediments.

Key functions of ubiquitous chemolithoautotrophic Gammaproteobacteria in sediments

Overall, our molecular and ^{14}C-assimilation data suggest that rather than a single group, a stable assemblage of *Gammaproteobacteria* drives dark carbon fixation in coastal surface sediments. In particular, we showed that members of the *Acidiferrobacter*, the JTB255 and the SSr clade occur in sediments worldwide and fix carbon in rates similar to those of uncultured sulfur-oxidizing bacteria from other aquatic habitats. Our genomic and metatranscriptomic evidence supports the previous assumption that chemolithoautotrophy in marine surface sediments is mainly driven by sulfur oxidation (Middelburg, 2011). However, the expression of uptake [NiFe]-hydrogenases in the SSr clade and other *Gammaproteobacteria* suggests that these organisms may also use hydrogen as an energy source for carbon fixation. On the basis of our data, we cannot exclude the possibility that other chemolithoautotrophic pathways such as ferrous iron

oxidation also contributed to dark carbon fixation, but these are probably minor in organic- and sulfide-rich systems. Molecular, isotopic and physiological studies will be critical to determine, how other chemoautotrophic processes such as nitrification, metal oxidation, sulfur disproportionation (Jørgensen, 1990) and possibly also hydrogen-dependent sulfate respiration (Boschker et al., 2014) contribute to dark carbon fixation in marine sediments. Intriguingly, sulfur-oxidizing, autotrophic *Gammaproteobacteria* including members of the SSr clade were recently shown to be associated with heterotrophic, electrogenic 'cable bacteria'. These were hypothesized to use cable bacteria as an electron sink during autotrophic sulfur oxidation (Vasquez-Cardenas et al., 2015). Considering their cosmopolitan distribution, their metabolic lifestyle and their ecological importance, the *Acidiferrobacter*-, the JTB255-, and the three symbiont-related clades may be benthic counterparts to the gammaproteobacterial SUP05 clade, key organisms for sulfur and carbon cycling in hydrothermal plumes and OMZs (Canfield et al., 2010; Wright et al., 2012; Anantharaman et al., 2013; Glaubitz et al., 2013).

Role of chemolithoautotrophic gammaproteobacteria in carbon cycling

Coastal sediments are global hot spots of carbon cycling. The importance of marine vegetation such as sea grass, salt marshes and mangroves for carbon sequestration is already well established (Duarte et al., 2005), but the role of coastal sediments as hot spots of microbial dark carbon fixation was only recently realized (Middelburg, 2011). According to our most conservative estimate, 70% of dark carbon fixation in coastal sediments are driven by chemolithoautotrophic *Gammaproteobacteria* (Figure 5). These could fix 122 Tg C/year in Earth's coastal sediments (assuming a total of 175 Tg/year, from Middelburg 2011), which is similar to the 111 Tg carbon buried yearly by marine vegetated habitats worldwide (Duarte et al., 2005). It is still unclear whether significant amounts of carbon fixed in the dark are buried into subsurface sediments. However, because identical/almost identical chemolithoautotrophic *Gammaproteobacteria* are frequently found in surface and subsurface sediments, they may have the potential to trap inorganic carbon and survive for centuries in subsurface sediments by tapping yet unknown sources of energy. Understanding whether the buried populations are in a state of dynamic equilibrium or whether they merely survive will be essential for assessing their role as a carbon sink.

Even though chemolithoautotrophy in shelf sediments mostly represents a 'secondary production', as it is ultimately based on energy from recycled organic matter (Middelburg, 2011), it may mitigate carbon (and sulfide) emissions from remineralized organic matter already at sediment surfaces. As marine sediments are the main site of global carbon sequestration, it is imperative to understand the processes and microorganisms that govern rates of burial of organic and inorganic carbon in these habitats. Here, our study provides first detailed insights into the microbiology of a largely overlooked aspect of the marine carbon cycle and highlights the environmental importance of widely distributed chemolithoautotrophic, most likely sulfur-oxidizing *Gammaproteobacteria*. As hypoxic events will expand and intensify in a warming ocean, sulfur-dependent carbon cycling will be more prevalent not only in pelagic OMZ, but also in organic-rich coastal sediments. Thus, sulfur-oxidizing and carbon-fixing microorganisms may have an increasingly important role in attenuating the rising emissions of sulfide and inorganic carbon to ocean waters and ultimately to the atmosphere.

Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Information

Methods

Barcoded 16S rRNA gene amplicon sequencing

The bacterial diversity in all sediment samples was determined by analyzing the hypervariable V3-V4 region of the 16S rRNA gene using Roche 454 pyro- or Illumina MiSeq-sequencing of barcoded amplicons. Barcoded amplicons from all surface sediments were prepared using primers 341f/785rev (Herlemann *et al.*, 2011; Klindworth *et al.*, 2012). PCR conditions were as follows: initial denaturation, 95°C for 5 min, followed by 20-28 cycles of 95°C for 30 s, 50°C for 1 min, 72°C for 1 min and final elongation of 72°C for 10 min. In general, the *Taq*-DNA polymerase (Eppendorf, Hamburg, Germany) was used, but for site Dunsborough we applied a high fidelity Phusion polymerase (Thermo Fisher Scientific Inc., USA). Pools of six replicates per sample were sequenced by Max Planck-Genome Centre (MP-GC, Cologne, Germany) using the Roche 454 GS junior sequencer. Barcoded amplicons from Dunsborough sediments were used for preparation of a TruSeq library and sequenced (paired end, 2x 250 bp) at MP-GC using an Illumina MiSeq instrument (Illumina Inc., USA). Barcoded amplicons from the 490 cm-deep subsurface sediment from site Janssand were prepared by replacing primer 785rev with 907rev (Muyzer *et al.*, rev 1998; Klindworth *et al.*, 2012). This primer covers a similar bacterial diversity as the reverse primer 785rev used for surface sediments (see above, Figure 2), but it is known to bias against some phyla that are, however, low abundant or absent in marine sediments (Klindworth *et al.*, 2012). PCR amplification and barcoded pyrosequencing was done by Research and Testing Laboratories, (Lubbock, Texas, USA) using a Roche 454 FLX sequencer. Samples from 10 cmbsf and 490 cmbsf were run in duplicate. Paired end reads were merged using the software package BBmap v4.3 (overlap >15 nucleotides). Finally, all amplicon reads were quality trimmed (>q20) and split by barcodes using MOTHUR (Schloss *et al.*, 2009). Sequences less than 300 bp and those containing ambiguous nucleotides and long homopolymers (>7) were removed. In total 311,196 bacterial 16S rRNA reads were kept for taxonomic classification using the SILVAngs pipeline v115 (Quast *et al.*, 2013) with a clustering at 98% identity.

16S rRNA gene libraries from subsurface sediments and phylogenetic analysis

From the 490 cm-deep subsurface core from Janssand sediment (April 2005) bulk DNA was extracted from 200 and 490 cmbsf according to Zhou *et al*. (1996) with an overnight incubation with proteinase K for improved cell lysis. The crude DNA extract obtained was purified using the Wizard DNA Cleanup system (Promega, Madison, WI, USA). PCRs were performed as described by Lenk *et al.* (2011) with the following thermocycler conditions: initial denaturation, 95°C for 5 min, followed by 25 cycles (490cmbsf) or 19 cycles (200 cmbsf) of 95°C for 1 min, 46°C for 1 min, 72°C for 3 min and final elongation of 72°C for 10 min using Master *Taq* DNA polymerase. The products of 6 (200 cmbsf) and 10 (490 cmbsf) parallel PCR amplifications were pooled and purified (QIAquick PCR purification Kit, Quiagen, Hilden, Germany). Preparative gels were used to get the right size of the PCR amplificates. The gel-excised and extracted (QIAquick Gel Extraction Kit, Quiagen, Hilden, Germany) PCR products were cloned using the TOPO TA Sequencing Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer´s specifications. Clone inserts were sequenced using the BigDye Terminator cycle sequencing kit, version 3.1 (Applied Biosystems, Darmstadt, Germany). Selected nearly full-length sequences were retrieved using the Sequencher 4.9 DNA sequence assembly and analysis software (Gene Codes Corporation, Ann Arbor, MI, USA). All sequences were checked for chimera with the online tool Bellerophon from Greengenes (Huber *et al.*, 2004; DeSantis *et al.*, 2006) and possible chimera sequences were excluded from further analysis.

Phylogenetic trees were calculated by neighbor-joining (Jukes-Cantor correction) and PHYML (Guindon and Gascuel, 2003) maximum-likelihood analyses implemented in the ARB software package (Ludwig *et al.*, 2004) using a 50% base-frequency filter for *Gammaproteobacteria*. RAxML trees (Stamatakis *et al.*, 2004) were calculated using Gamma model of rate heterogeneity at the CIPRES cluster (version 7.2.745, http://www.phylo.org/46). Only sequences with more than 1200 base pairs (bp) were used for tree calculation. Topologies derived by the different approaches were compared to construct a consensus tree. Partial sequences were then inserted into the reconstructed tree by applying parsimony criteria, without allowing changes in the overall topology. Clones library preparation and nearly full-length 16S rRNA gene sequences from Janssand surface sediment have been published elsewhere (Lenk *et al.*, 2011).

CARD-FISH and sample preparation for flow cytometry

For catalyzed reporter deposition-fluorescence *in situ* hybridization (CARD-FISH) sediment from sites Calais, Courseulles-sur-Mer and Janssand was fixed immediately after core retrieval as described in Lenk *et al*. (2011). Cells were detached from 100-200 µl sediment by ultrasonic treatment as described previously (Lenk *et al.*, 2011). The sample was not centrifuged but directly after settlement of the sand grains the supernatant along with the detached cells were filtered onto 25 mm polycarbonate membrane filters with a 0.2 µm pore size (GTTP, Millipore, Eschborn, Germany). Permeabilization and CARD-FISH was performed as described by Pernthaler *et al*. (2002) without embedding in agarose with the following modifications. Endogenous peroxidases were inactivated in 3% H_2O_2 in Milli-Q water for 10 min at room temperature. The temperature for all hybridizations was 46°C and washing was performed at 48°C according to the protocol of Ishii *et al*. (2004). An overview of oligonucleotide probes used in this study is shown in Supplementary Table 2. Novel oligonucleotide probes were designed for the JTB255-group using ARB and the SILVA 16S rRNA reference database release 117 (Pruesse *et al.*, 2007). Please note that the *Xanthomonadales,* which includes the JTB255-clade*,* is not targeted by probe GAM42a, specific for most *Gammaproteobacteria* (Siyambalapitiya and Blackall, 2005). In line with this, the JTB255-clade could not be detected with probe GAM42a in double hybridizations with the JTB-probe mix (Supplementary Table 2). Therefore, we summed up FISH counts of the JTB255 probe-mix and of probe GAM42a to yield the total relative abundance by FISH-detectable *Gammaproteobacteria*.

Calculation of cell-specific carbon fixation rates

The average cell-specific carbon fixation rates (g carbon/cell/h) were calculated from bulk measurements for a sorted population assuming 50,000 cells per sorting event. According to the equation $R = (A * M) / (a * n * t * L)$. A represents the activity of the sorted cell batch (50,000 cells) in Becquerel (Bq), M represents the molar mass of carbon (g/mol), a equals the specific activity of the tracer (Bq/mol), n represents the number of sorted cells, t represents the incubation time in hours and L equals the ratio of total DIC/^{14}C DIC. Note that since the JTB255-group is not targeted by probe GAM42a, we added ^{14}C carbon assimilation of the sorted JTB255-populations to the assimilation of cells sorted by probe GAM42a to yield the total amount of ^{14}C assimilated by all FISH-detectable *Gammaproteobacteria*.

Clone library of uptake [NiFe]-hydrogenase genes from Janssand sediment

A library for the gene encoding the large subunit of an uptake [NiFe]-hydrogenase was established using DNA extracted from sediment of the upper cm, sulfide transition zone and sulfidic layer collected at site Janssand in March 2012. The hydrogenase gene was amplified with the general primers HUPLX1 (forward) and HUPLW2 (reverse) (Csáki *et al.*, 2001). PCR reactions (final volume of 20 µl) contained 10 pmol of each primer, 6.25 nmol of each dNTP, 1x Master *Taq* Buffer and 1U of *Taq* DNA. Thermocycler conditions were as follows: initial denaturation, 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 65°C for 30 s, 72°C for 2 min and final elongation of 72°C for 10 min. Amplified PCR products were cloned using TOPO TA kit for sequencing (pCR4-TOPO, Invitrogen, Germany) and sequenced with the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany) according to manufacturer's instructions. Alternatively, clones were sent for Sanger-sequencing to GATC Biotech AG (Konstanz, Germany). Phylogenetic trees from the deduced amino acid sequences were constructed in ARB (Ludwig *et al.*, 2004) using a maximum-likelihood algorithm (RAxML) (Stamatakis *et al.*, 2004) and the JTT amino acid substitution matrix with 100 bootstrap replicates.

cDNA libraries and metatranscriptomic mapping

In April 2013 sediment was sampled from the sulfide transition zone at site Janssand and immediately frozen on dry ice. Total RNA was extracted from sediment in triplicates (one ml each) by the Vertis Biotechnologie AG (Freising, Germany) and bacterial rRNA was depleted with the Ribo-Zero™ Magnetic Kit (for *Bacteria*) (Epicentre, Madison, *USA*). Barcoded RNA TrueSEQ libraries were constructed from RNA extractions and paired-end sequenced using Illumina HiSeq2000 (MP-GC, Cologne, Germany). After quality trimming at a Phred score 28 using Nesoni clip (v.0.115) reads were mapped to reference databases of nucleotide sequences encoding key genes for sulfur oxidation (Sox multienzyme system, *sox*B; reverse dissimilatory sulfite reductase, *dsr*AB; adenosine-5'-phosphosulfate reductase, *apr*A), hydrogen oxidation (uptake [NiFe]-hydrogenase), ammonia oxidation (ammonia monooxygenase, *amo*A), carbon fixation (ribulose-1,5-bisphosphate carboxylase/oxygenase form I, cbbl; ribulose-1,5-bisphosphate carboxylase/oxygenase form II, cbbm; ATP citrate lyase, *acl*AB) and to the single amplified genome (SAG) using Bowtie2 (Langmead and Salzberg, 2012) with the following settings: match score = 0, mismatch penalty = 5, gap open penalty =

5, gap extension penalty = 5. The minimum alignment score for an alignment considered as valid was defined by -0.25 multiplied by read length for mapping to sulfur and hydrogen oxidation genes as well as for mapping on the SAG and -0.5 multiplied by read length for mapping to carbon fixation genes resulting in a percent identity cut-off of 95% and 90%, respectively. The percent identity cut-off for mapping to ammonia oxidation genes was set at 70%. The numbers of unique mapped reads were normalized by dividing the number of cDNA reads per gene by gene length multiplied by 1000 and adjusting for the total size of the data set. Details on reference databases are given in Supplementary Table 4. Reference sequences of sulfur oxidation genes (*apr*A, *dsr*AB and *sox*B) from site Janssand have been published previously (Lenk *et al.*, 2011; Lenk *et al.*, 2012).

A single cell genome of the SSr-clade from Janssand sediment

In January 2011, sediment from a 10 cm-deep sediment core was sampled at site Janssand. After immediate transfer to the lab the upper two cm including the oxidized and sulfide transition zone were mixed and one ml was transferred to a 15 ml plastic tube. After adding 3 ml of sterile-filtered sea water the slurry was vortexed at maximum speed for 5 min. After settlement of sand grains the supernatant was filtered through a 3 µm pore-size membrane. The filtrate was cryopreserved with N,N,N-trimethylglycine ("glycine betaine") (Sigma-Aldrich, USA) at a final concentration of 4% according to Cleland *et al.* (2004), stored at -80°C and shipped overseas. Single-cell sorting and whole-genome amplification via multiple displacement amplification (MDA) were performed at the Bigelow Laboratory Single Cell Genomics Center (https://scgc.bigelow.org) as described by Swan *et al.* (2011). PCR-screening for 16S rRNA genes of amplified single cell genomes was performed at the MPI Bremen. A single amplified genome (SAG) encoding a single, high quality 16S rRNA gene sequence was sent to Max Planck Genome Centre Cologne for MiSeq (Illumina) sequencing yielding 9,557,547 PE-reads. Reads were assembled with the CLC Genomics software with standard settings. Details on assembled data are given in Supplementary Table 3. The SAG assemblies were auto-annotated using the Joint Genome Institute (JGI) IMG-ER pipeline (Markowitz *et al.*, 2012).

The assembled data set was screened for contamination using k-mer analysis within the IMG-ER pipeline. Finally, two scaffolds showing a k-mer pattern divergent from bulk scaffolds were removed from the dataset. In addition, universal single copy genes

(Ciccarelli *et al.*, 2006; Creevey *et al.*, 2011), ribosomal proteins and tRNA were manually checked for a possible contamination by examining their closest BLAST hits.

Metagenomic sequencing from hydrothermal chimney (Manus Basin) and targeted re-assembly of metagenomic bins

Total DNA from a hydrothermal chimney biofilm obtained from the Manus Basin hydrothermal field (Reeves *et al.*, 2011) was shotgun-sequenced on an Illumina HiSeq sequencer. After quality clipping, sequence reads were normalized to a k-mer depth value of 40 with BBnorm (BBmap package) and assembled using the IDBA-UD iterative assembler (Peng *et al.*, 2012).

Scaffolds from the metagenome assembly larger than 1000 bp were imported into MetaWatt (Strous *et al.*, 2012) and binned by tetranucleotide (N4) frequencies (Teeling *et al.*, 2004) at a high confidence (98%) threshold. Open reading frames (ORFs) were predicted by Prodigal v 2.6.1 (Hyatt *et al.*, 2010) and scaffolds were taxonomically classified by a BLASTP search of the translated ORFs against the NCBI reference Genome database with the BLASTP based module of MetaWatt. N4 based bins were corrected manually based on GC content, coverage, and consistency of taxonomic classification. Selected genome bins were exported from MetaWatt and used as a reference for mapping of raw unassembled reads with BBmap. The initial mapping was performed with a minimum identity threshold of 80%. Mapped reads were subsequently assembled de-novo using the SPAdes assembler V3.1.1 (Bankevich *et al.*, 2012) in single cell mode with k-mer size ranging from 21 to 121 in steps of 10 and 121 as the maximum k-mer size. The new assembly was again binned by N4-frequency, classified with BLASTP and manually refined based on coverage and GC content in MetaWatt. Raw reads were again mapped onto the refined genome bin with more stringent parameters (minimum sequence identity of 95%) and de-novo assembled with SPAdes. Binning, mapping and de-novo assembly with SPAdes were repeated 7 times with increasing read mapping stringency (up to 98%). After the third round of re-assembly binned contigs larger than 10 kb were supplied to SPAdes as "trusted contigs". The completeness and possible redundancy of the final genome bin was evaluated by CheckM lineage-specific workflow and by the HMMER3 (Eddy, 2008) based "Six-frame Pfam" module search against a conserved single-copy gene set (Campbell *et al.*, 2013) in MetaWatt.

Fosmid endsequence analysis

DNA from the 490 cm-deep subsurface sediment core sampled in 2005 (Gittel *et al.*, 2008) was extracted according to Zhou *et al.* (1996). A metagenomic fosmid library was generated comprising 13,680 clones was established as described previously (Mussmann *et al.*, 2005). Five thousand clones were randomly selected for fosmids-end Sanger-sequencing on ABI3730XL capillary sequencers (PE Applied Biosystems, USA) by using fosmid-specific primers. End sequences were translated in all possible six reading frames. All reading frames were searched with BLAST against the NCBI-nr database. Reading frames with significant results (<1e-05 E-value) were considered as protein coding sequences. All protein coding sequences were taxonomically affiliated by BLAST against an in-house database of whole/draft genomes sequences (~10,000) by using a Lowest Common Ancestor (LCA) algorithm to assign an end-sequence to a particular taxonomic rank and group.

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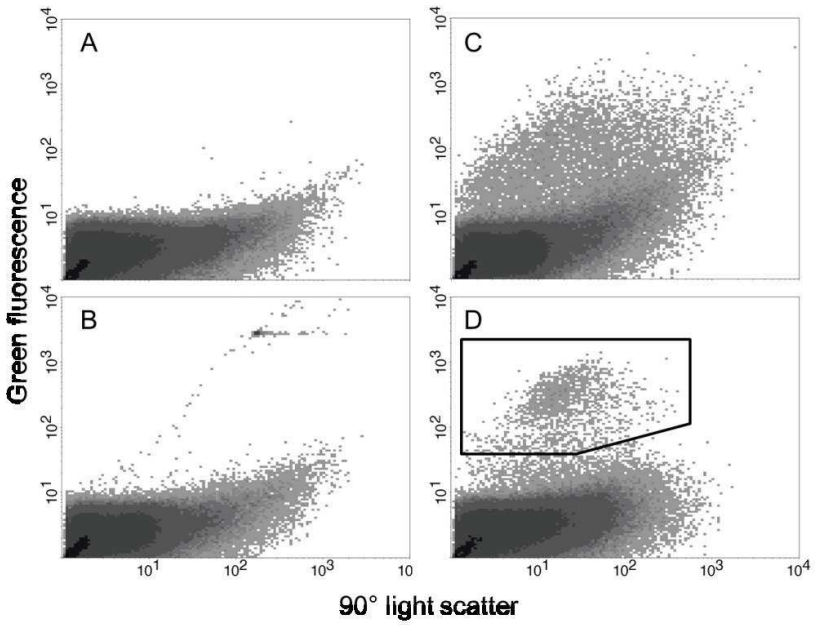
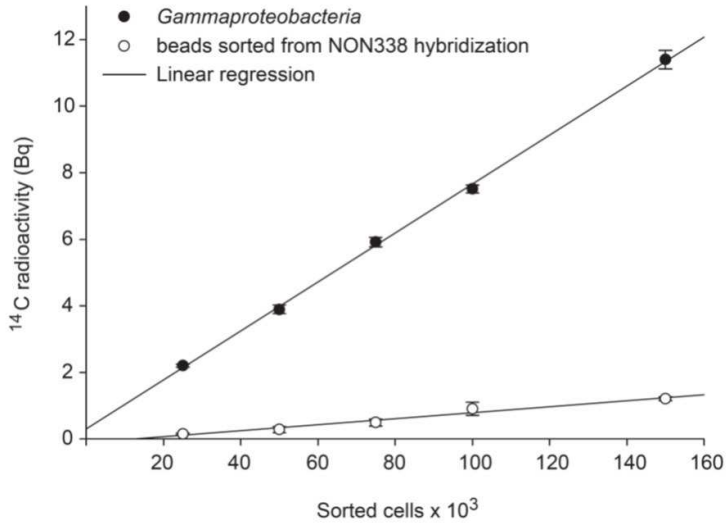
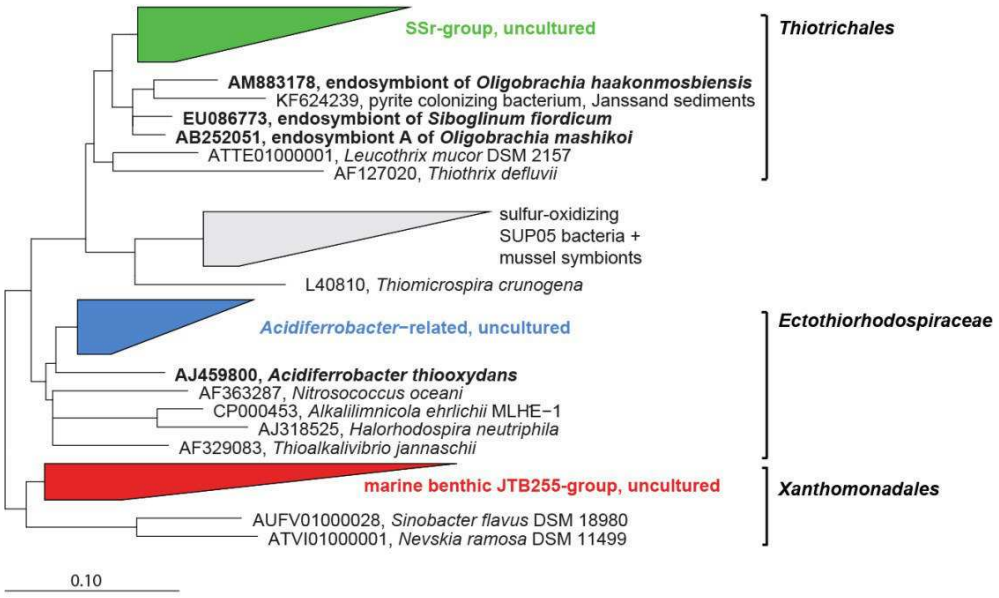
Supplementary Figures and Tables



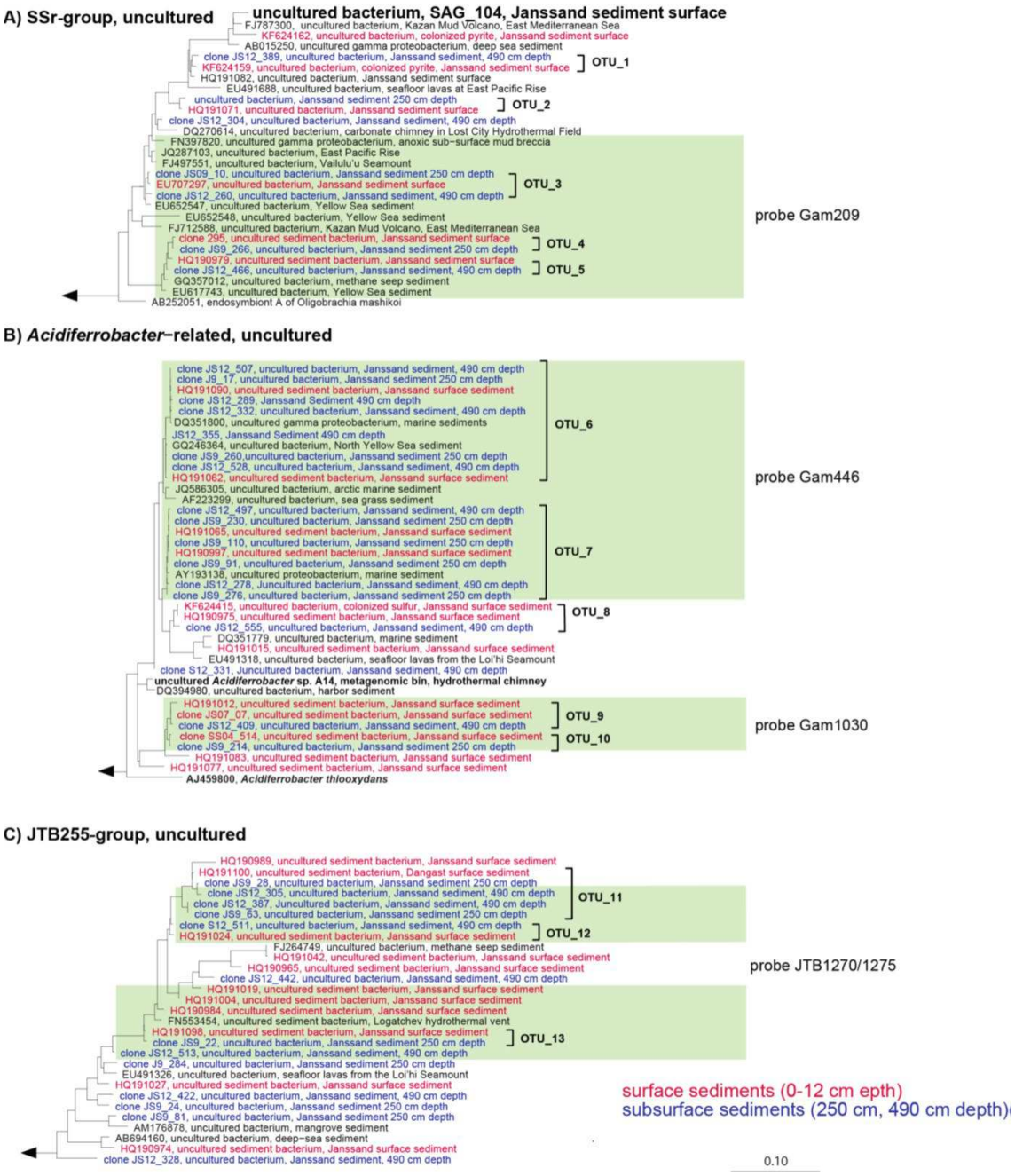
Supplementary Figure 1 Characteristic signatures of sediment samples analyzed by flow cytometry. Density dot plot diagrams of green fluorescence plotted versus 90° light scatter. Each diagram showed 250,000 analyzed events from hybridization with the negative-control probe NON338 (a), NON338 mixed with fluorescent beads (b), EUBI-III (c) and GAM1030/GAM446 (d). The square indicates the sort-gate for cell sorting (d).



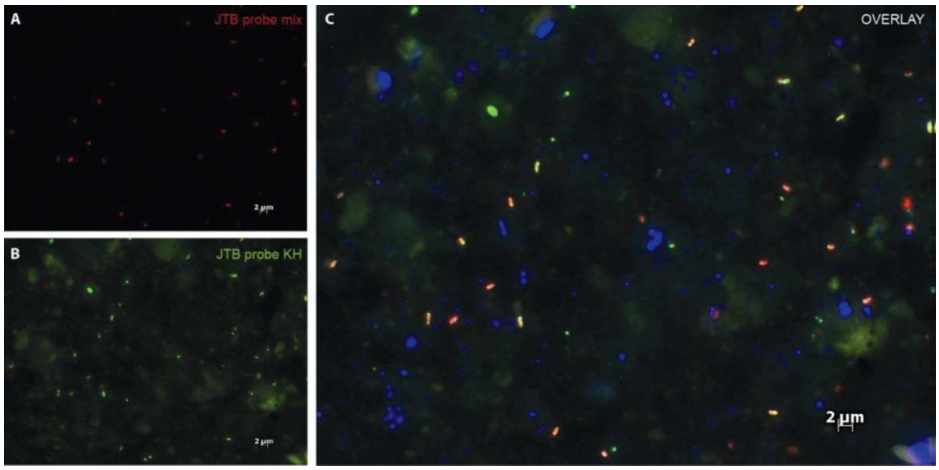
Supplementary Figure 2 Correlation of ^{14}C carbon activity with abundances of sorted *Gammaproteobacteria* cells and fluorescent beads. To determine the unspecific background from ^{14}C bicarbonate incubations, sediments slurries were supplemented with fluorescent beads, hybridized with the negative control probe (NON338) and then sorted for liquid scintillography. ^{14}C carbon activity is given in Becquerel (Bq). Error bars indicate the standard deviation (SD) of triplicate sorting.



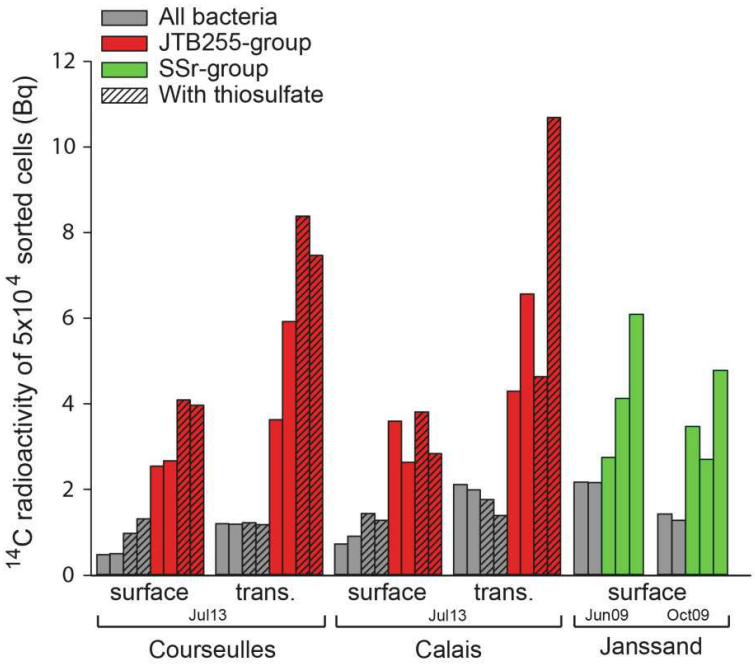
Supplementary Figure 3 16S rRNA phylogeny of the SSr-, the *Acidiferrobacter*-related and the JTB255-clades. Phylogenetic analysis of nearly full-length 16S rRNA gene sequences of three gammaproteobacterial clades abundant and widespread in marine sediments and their closest known relatives. A more detailed view is given in Supplementary Figure 4. The consensus tree was calculated using RAxML, and Phylip maximum likelihood and Neighbor joining algorithms. The taxonomy refers to the SILVA database, release 117 (Pruesse *et al.*, 2007).



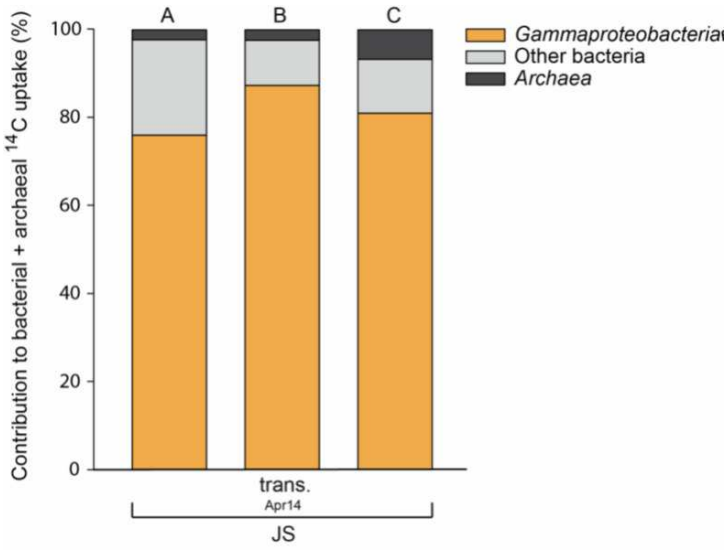
Supplementary Figure 4 Detailed phylogenetic reconstruction of nearly full length and partial 16S rRNA gene sequences of the SSr-, the *Acidiferrobacter*-related and the JTB255-clades. Sequences in blue were recovered from 200 or 490 cmbsf, sequences in red were recovered from the 0-12 cmbsf. OTUs were defined for sequences with 100% sequence identity. Target sequences of probes used for FISH counting and flow sorting are boxed in green. The consensus tree was calculated using RAxML, and Phylip maximum likelihood and Neighbor joining algorithms. Partial sequences were added using the quick-add parsimony tool in ARB. Note that in previous publications the JTB255-clade according to an outdated taxonomy were falsely classified as belonging to the *Sinobacteraceae*. The taxonomy used in this paper is based on to the SILVA database, release 117 (Pruesse *et al.*, 2007).



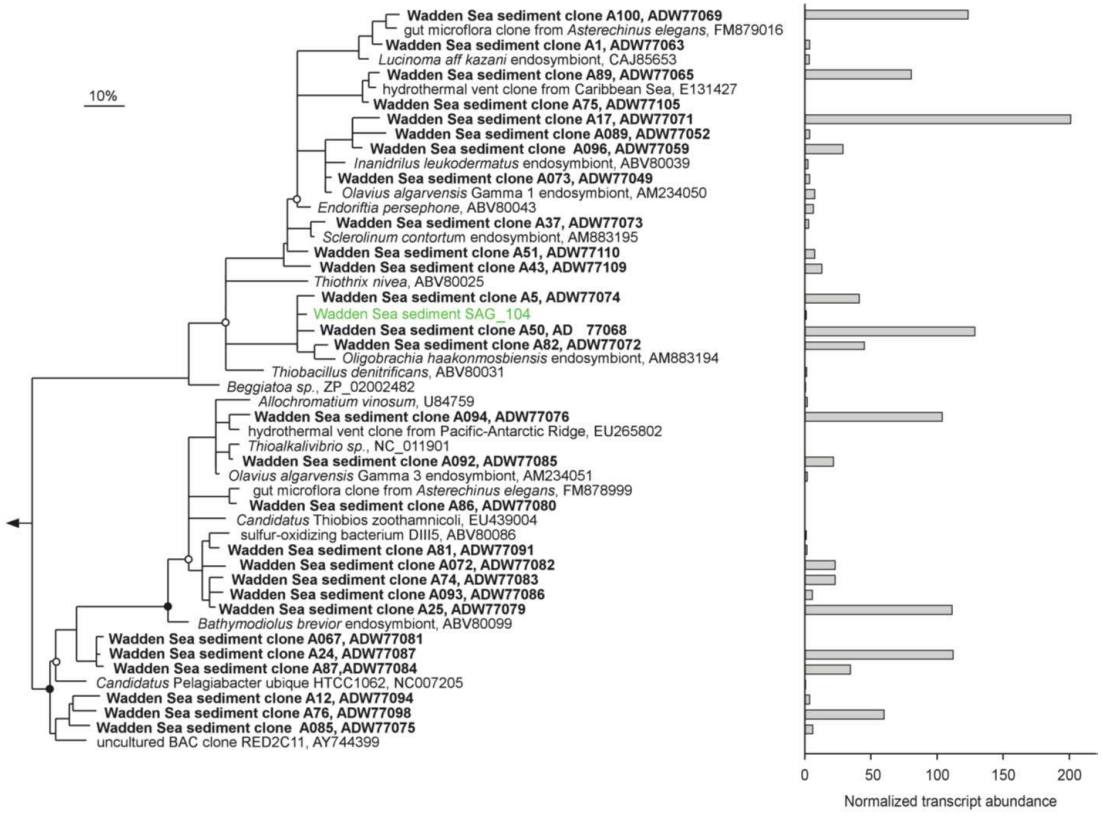
Supplementary Figure 5 Epifluorescence images of cells of the gammaproteobacterial JTB255-clade in Janssand sediment (April 2014). To confirm identity of cells targeted by our "JTB probe mix" (probes JTB843 + JTB1275 + JTB1270), we performed a double hybridization with another JTB255-specific probe ("probe KH", unpublished, Katy Hoffmann). "JTB probe mix", Alexa594, red fluorescence (a), probe KH, Alexa488, green fluorescence (b), overlay of images (c). In blue: DAPI-stain.



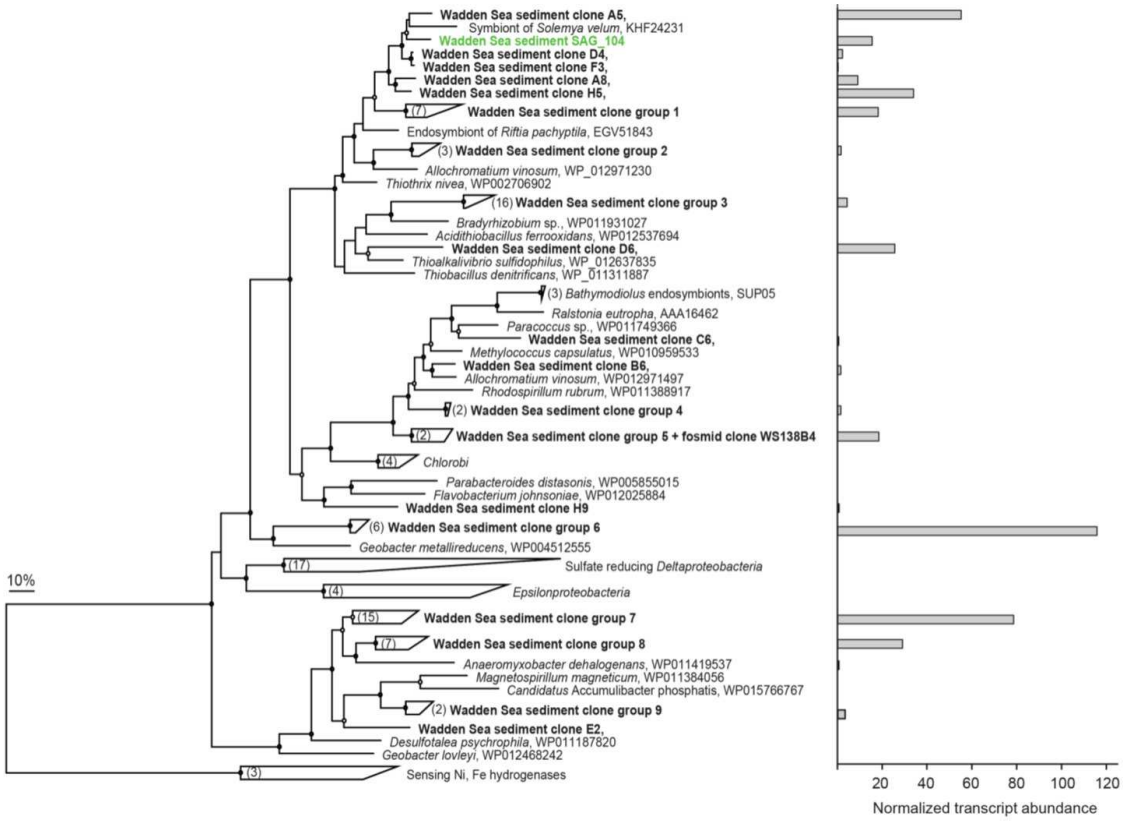
Supplementary Figure 6 ^{14}C carbon fixed by gammaproteobacterial clades identified in this study. ^{14}C-carbon fixation quantified in flow-sorted cells of the JTB255- and SSr-clades from the uppermost sediment layer (surface, 0-1 cmbsf) and from the sulfide transition zone (trans.) incubated with ^{14}C bicarbonate. Batches of 50,000 cells were sorted for quantification. ^{14}C carbon activity is given in Becquerel (Bq).



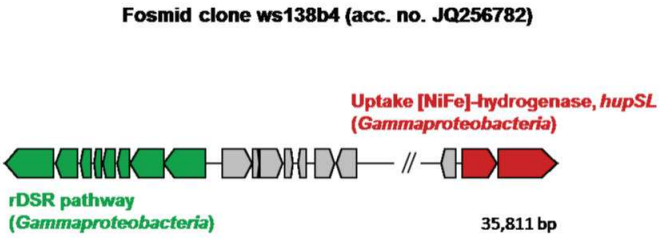
Supplementary Figure 7 Relative contribution of bacterial and archaeal dark carbon fixation at site Janssand from triplicate incubations.



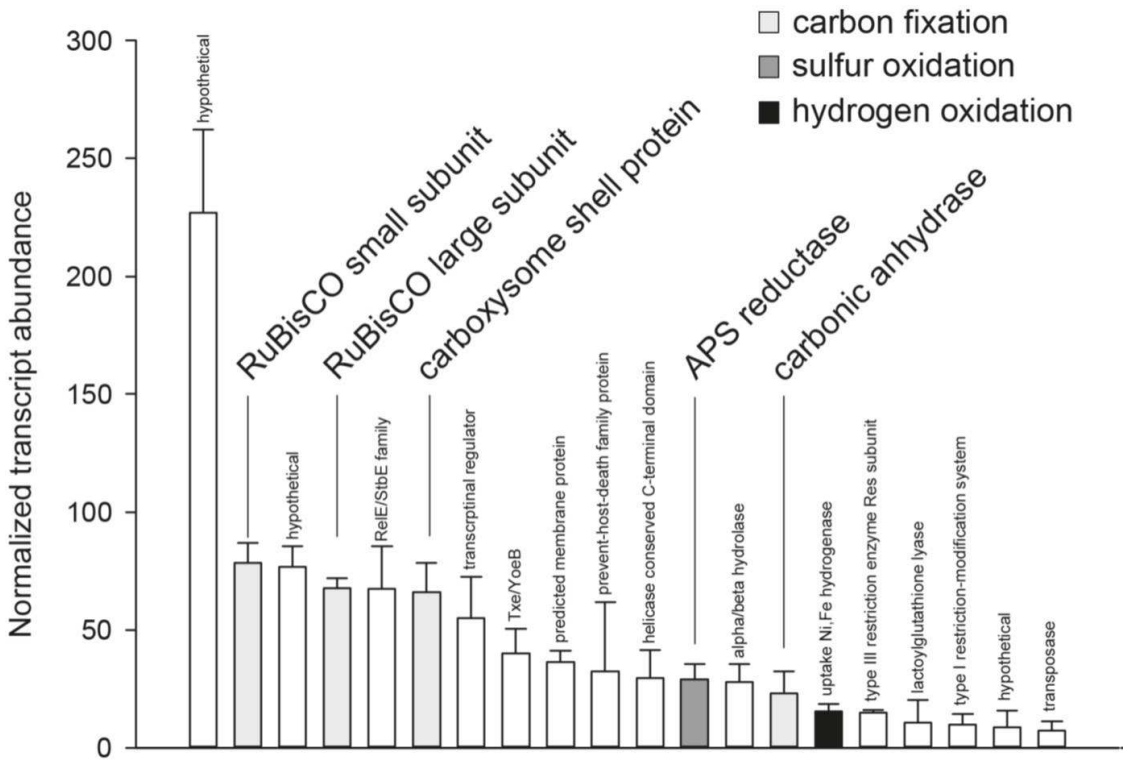
Supplementary Figure 8 Phylogeny of AprA and abundance of metatranscriptomic reads. Phylogenetic tree based on maximum-likelihood (RAxML) of AprA amino acid sequences from site Janssand (in bold) and normalized abundance of their transcripts. Circles indicate lineages with > 70% (closed) and > 50% (open) RAxML bootstrap support. The bar indicates 10% sequence divergence.



Supplementary Figure 9 Phylogenetic tree based on maximum-likelihood (RAxML) of Uptake [NiFe]-hydrogenase amino acid sequences from site Janssand (in bold) and normalized abundance of their transcripts. Circles indicate branches with >70% (closed) and >50% (open) RAxML bootstrap support. The bar indicates 10% sequence divergence.



Supplementary Figure 10 Co-localization of gammaproteobacterial rDSR-operon and *hupSL* genes encoding an uptake [NiFe]-hydrogenase on a metagenomic fosmid clone retrieved from Janssand sediments.



Supplementary Figure 11 Normalized number of metatranscriptomic reads recruited by genes of a single cell genome affiliating with to the SSr-clade (WSgam209). The top 20 transcribed genes are shown including genes for carbon fixation, sulfur and hydrogen oxidation. IMG gene IDs: 2609851537=hypothetical, 2609851423=RuBisCO small subunit, 2609851536=hypothetical, 2609851422=RuBisCO large subunit, 2609852573=RelE/StbE family, 2609851428=carboxysome shell protein, 2609852572=transcriptional regulator, 2609852972=Txe/YoeB, 2609851841=predicted membrane protein, 2609852973=prevent-host-death family protein, 2609853218=helicase conserved C-terminal domain, 2609853000=APS reductase, 2609851840=alpha/beta hydrolase, 2609851425=carbonic anhydrase, 2609852733=type III restriction enzyme Res subunit, 2609852971=lactoylglutathione lyase, 2609851900=type I restriction-modification system, 2609851882=hypothetical, 2609853066=transposase. Note, that the sequence of the uptake [NiFe]-hydrogenase was recovered by PCR derived from MDA product of WSgam209 and is therefore not included in the IMG/ER of WSgam209 submission.

Supplementary Table 1 Cell-specific carbon fixation rates and relative abundance of cells labelled by microautoradiography

Site/Country	Name code	Sampling position (Lat, Lon)	Sampling time	Surface temperature (°C)	Sulfide in sulfide transition zone (µM)	Sampling depth (cm) (A=surface, B= sulfide transition, C= sulfidic)	Performed experiments
Intertidal sands:							
Königshafen/GER	KH	55.03432N, 8.42428E	April 2013	6	0-35*[†]	A: 0-1, B: 3-4, C: 9-10	a
Janssand/GER	JS	53.73668N, 7.69893E	October 2009	-	0-1000[‡]	B: 1-2	a, b[§]
Janssand/GER	JS	53.73668N, 7.69893E	March 2012	8	0-1000[‡]	A: 0-1, B: 7-8, C: 12-13	c
Janssand/GER	JS	53.73668N, 7.69893E	April 2013	13	0-1000[‡]	A: 0-1, B: 2-3, C: 6-7	a, d
Janssand/GER	JS	53.73668N, 7.69893E	April 2014	-	0-1000[‡]	B: 2-3	b, e
Yerseke/NL	YS	51.45950N, 4.08026E	October 2012	15	-	A: 0-1, B: 5-6, C: 7-8	a
Calais/FRA	CA	50.99695N, 1.98212E	October 2012	16	-	A: 0-1, B 18-19, C: 22-23	a
Calais/FRA	CA	50.99695N, 1.98212E	July 2013	21	7-13	A: 0-1, B: 2-3, C: 8-9	b, e
Courseulles-sur-Mer/FRA	CS	49.33955N, 0.46952W	October 2012	18	-	A: 0-1, B: 2-3, C: 6-7	a
Courseulles-sur-Mer/FRA	CS	49.33955N, 0.46952W	July 2013	19	0	A: 0-1, B: 15-16, C: 21-22	b, e
Mont-St-Michel/FRA	MM	48.61387N, 1.74368W	October 2012	18	-	A: 0-1, B: 5-6, C: 7-8	a
Cairns/AUS	CR	16.91796S, 145.77678E	December 2013	25	-	B: 1-1.5, C: 6.5-7	a
Carlo Point/AUS	CP	25.89598S, 153.05691E	December 2013	26	-	A: 0-0.5, C: 6.5-7	a
Nudgee Beach/AUS	NB	27.34243S, 153.10273E	December 2013	26	-	A: 0-0.5, B: 4.5-5, C: 8.5-9	a
Dunsborough/AUS	DB	33.61745S, 115.11572E	December 2014	25	-	A: 0-1, B: 1-3, C: 6-7	a
Sublittoral sands[¶]:							
Noah_A/GER	NS1	53.98767N, 6.23566E	March 2014	-	-	A: 0-2, B: 2-5	a
Noah_B/GER	NS2	53.98983N, 6.88150E	March 2014	7	-	A: 0-2, B: 2-5	a
CCPδ/GER	NS3	54.16883N, 7.99150E	March 2014	6	-	A: 0-2, B: 2-5	a

- no information available; a 16S rRNA gene sequencing; b ^{14}C DIC incubations; c hydrogenase gene library; d metatranscriptomics; e CARD-FISH *(de Beer *et al.*, 2005); [†](Thiermann *et al.*, 1996); [‡](Jansen *et al.*, 2009); [§](Lenk *et al.*, 2011); [¶]water depth: Noah_A, 29 m; Noah_B, 29 m; CCPδ, 20 m

Supplementary Table 2 Oligonucleotide probes used in this study

Probe	Specificity	Sequence (5'-3')	FA* (%)	Reference
EUB I-III†	*Bacteria*	GCWGCCWCCCGTAGGWGT	35	(Amann *et al.*, 1990; Daims *et al.*, 1999)
ARCH915†	*Archaea*	GTGCTCCCCCGCCAATTCCT	35	(Stahl and Amann, 1991)
NON338†	Nonsense probe	ACTCCTACGGGAGGCAGC	35	(Wallner *et al.*, 1993)
ROS537†	*Roseobacter*-clade bacteria	CAACGCTAACCCCCTCC	35	(Eilers *et al.*, 2001)
CF319a†	*Cytophaga*/ *Flavobacteria*	TGGTCCGTRTCTCAGTAC	35	(Manz *et al.*, 1996)
GAM42a†‡	*Gammaproteo-bacteria*	GCCTTCCCACATCGTTT	35	(Manz *et al.*, 1992)
BET42a§	*Betaproteo-bacteria*	GCCTTCCCACTTCGTTT	35	(Manz *et al.*, 1992)
GAM209†‡	SSr-group	CTACTAGTGCCAGGTCCG	25	(Lenk *et al.*, 2011)
GAM209c§		CTAATAGTGCCAGGTCCG	25	(Lenk *et al.*, 2011)
GAM1030†	*Acidiferro-bacter*-related	CCTGTCAACCAGTTCCCG	25	(Lenk *et al.*, 2011)
GAM1030c§		CCTGTCAATCAGTTCCCG	25	(Lenk *et al.*, 2011)
GAM446†	*Acidiferro-bacter*-related	ACCCGCAACTGTTTCCTC	25	(Lenk *et al.*, 2011)
GAM446c1§		ACCCTAAGCTTTTCCTC	25	(Lenk *et al.*, 2011)
GAM446c2§		ACCCTGAGCTTTTCTTC	25	(Lenk *et al.*, 2011)
GAM446c3§		ACCTTAAGCTTTTCTTC	25	(Lenk *et al.*, 2011)
GAM446c4§		ACCAAAAGCTTTTCTTC	25	(Lenk *et al.*, 2011)
GAM446c5§		ACCTTRAGCTTTTCCTC	25	(Lenk *et al.*, 2011)
GAM446h1§		GGTCGCGGGATATTA	25	(Lenk *et al.*, 2011)
GAM446h2§		CCYACTGAAAGTGCTT	25	(Lenk *et al.*, 2011)
JTB1270†	JTB255-group	GAGCTTTAAGGGATTAGCGCACCA	40	This study
JTB1270h¶		TTGCTGGTTGGCAACCCTCTGTAT	40	This study
JTB843†‡	JTB255-group	TGCGACACCGAGGGACAR	10	This study
JTB843c1§		TTCGACACCGAGGGACAR	10	This study
JTB843c2§		TGCGACACCGAGGGGCAR	10	This study
JTB843c3§		TGCGACACCGAAAGACAR	10	This study
JTB843c4§		GGCGACACCGAGGGGCAR	10	This study
JTB843c5§		TGCGACACCGAAGGACTR	10	This study
JTB843us¶		TCCCCCAACATCTAGTTCTCA	10	This study
JTB843ds¶		CGGAGAACTTAACGCGTTAGC	10	This study
JTB1275†‡	JTB255-group	AGCTTTAAGGGATTAGCG	10	This study
JTB1275c1§		AGCTTTAAGGGATTAGCG	10	This study
JTB1275c2§		AGCTTTAAGGGATTAGCA	10	This study
JTB1275c3§		AGCTTTAAGGGATTAGCT	10	This study
JTB1275c4§		CGCTTTAAGGGATTAGCT	10	This study
JTB1275us¶		TTGGCAACCCTCTGTACTCGC	10	This study
JTB1275ds¶		ACTCCATACCGGACTACGACG	10	This study

* Formamide concentration (v/v) in hybridization buffer
† Probe labelled with horseradish peroxidase (HRP)
‡ Probe was applied with competitor
§ Unlabelled competitor oligonucleotide
Unlabelled helper oligonucleotide

Supplementary Table 3 General statistics of assemblies of a single cell genome (recovered from site Janssand) affiliating with the SSr-clade (WSgam209) and a metagenomic bin affiliating with the *Acidiferrobacter*-clade (recovered from a deep-sea hydrothermal chimney)

	WSgam209		Uncultured *Acidiferrobacter*	
	#	% of total	#	% of total
DNA, total number of bases	1,922,127	100	2,041,809	100
DNA coding number of bases	1,660,663	86.4	1,933,109	94.7
G+C content		43.8		68.9
DNA scaffolds	311		66	
Genes total number	2,008	100	2,092	100
Protein coding genes	1,989	99.1	2,059	98.5
Protein coding genes with function prediction	1,488	74	1,748	83.6
Protein coding genes without function prediction	501	25	311	14.9
RNA genes	19	1	33	1.6
tRNA genes	11	0.5	27	1.3
estimated completeness	20%*/22%†		89%‡/71.9%§	

* expressed as % of tRNAs is next cultured relative *Leucothrix mucor* (Grabovich *et al.*, 1999).
† expressed as % of universal single copy genes in SSr_104 (28) out of 40 (Ciccarelli *et al.*, 2006; Creevey *et al.*, 2011).
‡ CheckM (Matsen *et al.*, 2010; Hyatt *et al.*, 2012; Parks *et al.*, 2015)
§ Six Frame PFAM embedded in Metawatt (Strous *et al.*, 2012; Campbell *et al.*, 2013)

Supplementary Table 4 Overview of metatranscriptomic reads mapped on reference databases

Reference database	No. of unique mapped cDNA reads		
	Replicate A (29029446 reads)	Replicate B (29726577 reads)	Replicate C (33590664 reads)
aprA (220 sequences)	603	582	669
dsrAB (133 sequences)	1927	1972	2078
soxB (166 sequences)	226	202	293
Uptake Ni, Fe hydrogenase (203 sequences)	658	532	685
cbbm/cbbl (250 sequences)	196	190	260
aclAB (269 sequences)	0	6	2
amoA (bacteria) (6999 sequences)	2	0	1
amoA (archaea) (8544 sequences)	3	5	3

Supplementary Table 5 Selected IMG gene IDs for WSgam209 and the *Acidiferrobacter* metagenomic bin

IMG/ER Gene ID	Gene product name (IMG/ER)	Type of genome: organism (origin)	Sequence length (aa)	COG ID	COG name	Pfam ID
2609851422	Ribulose 1,5-bisphosphate carboxylase, large subunit, or a RuBisCO-like protein	single cell genome SSr-group: WSgam209 (Janssand sediment)	471	COG1850	Ribulose 1,5-bisphosphate carboxylase, large subunit, or a RuBisCO-like protein	pfam02788
2609851423	Ribulose bisphosphate carboxylase small subunit	single cell genome SSr-group: WSgam209 (Janssand sediment)	113	COG4451	Ribulose bisphosphate carboxylase small subunit	pfam00101
2609851424	Carboxysome shell peptide mid-region	single cell genome SSr-group: WSgam209 (Janssand sediment)	791			pfam12288
2609851425	carboxysome shell carbonic anhydrase	single cell genome SSr-group: WSgam209 (Janssand sediment)	514			pfam08936
2609851426	carboxysome peptide A	single cell genome SSr-group: WSgam209 (Janssand sediment)	84	COG4576	Carboxysome shell and ethanolamine utilization microcompartment protein CcmK/EutM	pfam03319
2609851427	carboxysome peptide B	single cell genome SSr-group: WSgam209 (Janssand sediment)	84	COG4576	Carboxysome shell and ethanolamine utilization microcompartment protein CcmK/EutM	pfam03319
2609851482	cytochrome c oxidase, subunit I	single cell genome SSr-group: WSgam209 (Janssand sediment)	531	COG0843	Heme/copper-type cytochrome/quinol oxidase, subunit 1	pfam00115
2609851483	cytochrome c oxidase, subunit II	single cell genome SSr-group: WSgam209 (Janssand sediment)	377	COG1622	Heme/copper-type cytochrome/quinol oxidase, subunit 2	pfam13442
2609851866	hydrogenase expression/formation protein HypE	single cell genome SSr-group: WSgam209 (Janssand sediment)	333	COG0309	Hydrogenase maturation factor	pfam00586
2609851868	hydrogenase expression/formation protein HypD	single cell genome SSr-group: WSgam209 (Janssand sediment)	364	COG0409	Hydrogenase maturation factor	pfam01924
2609851869	hydrogenase accessory protein HypB	single cell genome SSr-group: WSgam209 (Janssand sediment)	278	COG0378	Ni2+binding GTPase involved in regulation of expression and maturation of urease and hydrogenase	pfam02492
2609851881	hydrogenase (Ni,Fe) small subunit (hydA)	single cell genome SSr-group: WSgam209 (Janssand sediment)	271			pfam01058

Supplementary Table 5 (continued)

IMG/ER Gene ID	Gene product name (IMG/ER)	Type of genome: organism (origin)	Sequence length (aa)	COG ID	COG name	Pfam ID
2609852594	Ni,Fe,NiFeSe hydrogenase small subunit C-terminal	single cell genome SSr-group: WSgam209 (Janssand sediment)	144			pfam14720
2609852596	Ni,Fe-hydrogenase 2 integral membrane subunit HybB	single cell genome SSr-group: WSgam209 (Janssand sediment)	377	COG5557	Ni/Fe-hydrogenase 2 integral membrane subunit HybB	pfam03916
2609852597	Ni,Fe-hydrogenase I large subunit	single cell genome SSr-group: WSgam209 (Janssand sediment)	575	COG0374	Ni,Fe-hydrogenase I large subunit	pfam00374
2609852598	hydrogenase expression/formation protein	single cell genome SSr-group: WSgam209 (Janssand sediment)	199	COG0680	Ni,Fe-hydrogenase maturation factor	pfam01750
2609852649	Carbonic anhydrase	single cell genome SSr-group: WSgam209 (Janssand sediment)	202	COG0288	Carbonic anhydrase	pfam00484
2609852672	hydrogenase maturation protease	single cell genome SSr-group: WSgam209 (Janssand sediment)	173	COG0680	Ni,Fe-hydrogenase maturation factor	
2609853000	dissimilatory adenylylsulfate reductase alpha subunit precursor (EC 1.8.99.2)	single cell genome SSr-group: WSgam209 (Janssand sediment)	524	COG1053	Succinate dehydrogenase/fumarate reductase, flavoprotein subunit	pfam00890
2616652017	Sulfite oxidase subunit A (SoeA)	metagenomic bin Acidiferrobacter_a7 (black smoker chimney, Manus Basin)	943	COG0243	Anaerobic selenocysteine-containing dehydrogenase	pfam04879
2616652018	Sulfite oxidase subunit A (SoeB)	metagenomic bin Acidiferrobacter_a7 (black smoker chimney, Manus Basin)	212	COG0437	Fe-S-cluster-containing dehydrogenase component	pfam13247
2616652019	Sulfite oxidase subunit A (SoeC)	metagenomic bin Acidiferrobacter_a7 (black smoker chimney, Manus Basin)	313	COG3302	DMSO reductase anchor subunit	pfam04976
2616652250	ribulose 1,5-bisphosphate carboxylase large subunit (EC 4.1.1.39)	metagenomic bin Acidiferrobacter_a7 (black smoker chimney, Manus Basin)	472	COG1850	Ribulose 1,5-bisphosphate carboxylase, large subunit, or a RuBisCO-like protein	pfam02788

Supplementary Table 5 (continued)

IMG/ER Gene ID	Gene product name (IMG/ER)	Type of genome: organism (origin)	Sequence length (aa)	COG ID	COG name	Pfam ID
2616652251	ribulose 1,5-bisphosphate carboxylase small subunit (EC 4.1.1.39)	metagenomic bin Acidiferrobacter_a7 (black smoker chimney, Manus Basin)	121	COG4451	Ribulose bisphosphate carboxylase small subunit	pfam00101
2616652516	thiosulfate oxidation protein SoxX	metagenomic bin Acidiferrobacter_a7 (black smoker chimney, Manus Basin)	122			pfam00034
2616652517	thiosulfate oxidation protein SoxY	metagenomic bin Acidiferrobacter_a7 (black smoker chimney, Manus Basin)	155	COG5501	Predicted secreted protein	pfam13501
2616652518	thiosulfate oxidation protein SoxZ	metagenomic bin Acidiferrobacter_a7 (black smoker chimney, Manus Basin)	105			pfam08770
2616652519	thiosulfate oxidation protein SoxA	metagenomic bin Acidiferrobacter_a7 (black smoker chimney, Manus Basin)	278			
2616652520	thiosulfate oxidation protein SoxB	metagenomic bin Acidiferrobacter_a7 (black smoker chimney, Manus Basin)	579	COG0737	2',3'-cyclic-nucleotide 2'-phosphodiesterase/5'- or 3'-nucleotidase, 5'-nucleotidase family	pfam02872

Supplementary Table 6 Relative cell abundance at site Courseulles-sur-Mer (CS), Calais (CA) and Janssand (JS)

		Relative Abundance (%)				
		CS		CA		JS May 2014
Probes	Specificity	Surface	Trans.	Surface	Trans.	Trans.
EUBI-III	*Bacteria*	95.3	92.1	94.7	90.9	94.4
ARCH915	*Archaea*	0.9	0.9	1.3	1.2	1.2
GAM42a	*Gammaproteo-bacteria*	13.5	14.6	15.1	15.3	16.3
JTB1270	JTB255-group	6.2	5.1	5.1	3.4	5.3
GAM209	SSr-group	0.6	1.1	0.9	0.5	1.0
GAM1030/ GAM446	*Acidiferrobacter*-related	2.1	2.1	1.7	0.9	3.1

Note: GAM42a + JTB1270 target most of the *Gammaproteobacteria* (Siyambalapitiya and Blackall, 2005)

Supplementary Table 7 Phylogenetic affiliation of fosmid endsequences recovered from a metagenomic fosmid library from shallow subsurface sediments (490 cm sediment depth), Janssand April 2004

	No. of reads	% of total archaeal and bacterial reads
Archaea	60	1
Bacteria	3992	
Bacterial and archaeal reads	4052	
Gammaproteobacteria	979	24
Planctomycetes	505	12
Alphaproteobacteria	303	7
Deltaproteobacteria	301	7
Actinobacteria	289	7
Firmicutes	173	4
Betaproteobacteria	112	3
Bacteroidetes	95	2
Cyanobacteria	71	2
Chloroflexi	72	2
Acidobacteria	16	0.4
Other *Bacteria*	1076	26
unassigned reads	5118	
all reads	**9170**	

Supplementary Table 8 Cell-specific carbon fixation rates and relative abundance of cells labelled by microautoradiography

Sampling site	Population	Population-specific CO_2 fixation rate (fg C $cell^{-1}$ d^{-1})	MAR positive cells (%)	Cell-specific CO_2 fixation rate (fg C $cell^{-1}$ d^{-1})
Courseulles	All bacteria	0.3	-	-
surface	*Gammaproteobacteria*	1.5	46	3.2
Jul13	JTB255-group	1.1	-	-
Courseulles	All bacteria	0.4	-	-
transition	*Gammaproteobacteria*	2.0	49	4.0
Jul13	JTB255-group	2.1	-	-
Calais	All bacteria	0.4	-	-
surface	*Gammaproteobacteria*	1.6	-	-
Jul13	JTB255-group	1.1	-	-
Calais	All bacteria	0.6	-	-
transition	*Gammaproteobacteria*	3.0	50	5.9
Jul13	JTB255-group	2.2	-	-
Janssand	All bacteria	0.3	-	-
transition	*Gammaproteobacteria*	1.1	-	-
Apr14	JTB255-group	1.5	-	-
	Acidiferrobacter-related	3.5	-	-
	SSr-group	2.5	-	-
	Archaea	0.9	-	-
Janssand	All bacteria	0.9	22 (Lenk *et al.*, 2011)	4.2
surface	*Gammaproteobacteria*	2.4	40 (Lenk *et al.*, 2011)	6.4
Jun09	SSr-group	1.9	25 (Lenk *et al.*, 2011)	7.5
Janssand	All bacteria	0.6	18 (Lenk *et al.*, 2011)	3.3
surface	*Gammaproteobacteria*	1.9	43 (Lenk *et al.*, 2011)	4.4
Oct09	SSr-group	1.6	25 (Lenk *et al.*, 2011)	6.4

- not determined

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Chapter II

Diversity and metabolism of the JTB255 clade (*Gammaproteobacteria*), a global member of deep-sea sediment communities

Katy Hoffmann[1,2]*, Christina Bienhold[1,2], Katrin Knittel[3], Pier L. Buttigieg[1,2], Rafael Laso-Pérez[1], Eduard Fadeev[1,2], Josephine Z. Rapp[1,2], Antje Boetius[1,2], Pierre Offre[1]

[1]HGF-MPG Joint Research Group for Deep Sea Ecology and Technology,
Max Planck Institute for Marine Microbiology, Bremen, Germany
[2]HGF-MPG Joint Research Group for Deep Sea Ecology and Technology,
Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Bremerhaven, Germany
[3] Department of Molecular Ecology,
Max Planck Institute for Marine Microbiology, Bremen, Germany

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Abstract

Life in the deep-sea floor is dominated by bacteria with regard to biomass, phylogenetic and metabolic diversity. Because deep-sea sediments cover > 65% of Earth's surface, its core bacterial groups may represent some of the most abundant microorganisms on a global scale and could have key functions in marine ecosystems. The JTB255 marine benthic group (JTB255) is ubiquitous and potentially abundant in marine sediments, as indicated by next-generation sequencing surveys. The present study aimed at characterizing the phylogenetic structure, abundance, microdiversity patterns and metabolic potential of this enigmatic group of bacteria with a major focus on deep-sea representatives. JTB255 forms a monophyletic clade sub-divided in at least five major lineages, each of which has been detected in various deep-sea habitats. Analysis of JTB255 oligotypes in distinct habitat categories tentatively suggests that a majority of those bacteria could be preferentially associated with coastal, margin and deep-sea realms. We also show that JTB255 is an abundant member of deep-sea bacterial communities accounting for 5 ± 2% of indigenous microbial cells. Genomic analysis suggests that deep-sea representatives of JTB255 encode various extracellular hydrolases, essentially peptidases and lipases, suggesting the ability to degrade both cell wall and cellular membranes, which were previously shown to form an essential part of the aged, refractory necromass. Those potential metabolic capabilities could be a determinant of the ecological success of JTB255 in deep-sea sediments.

Keywords:

Woeseiaceae; organo-heterotrophy; core bacteria; Arctic; single cell genomics; metagenomics.

Introduction

Marine sediments cover approximately 70% of the Earth`s surface and host bacterial communities distinct from those of the overlaying water masses (Bienhold et al., 2016; Zinger et al., 2011). Most of the seafloor (approx. 90%) lies outside of the shelf regions in water depths much below 200 m referred to as deep sea, which thus constitutes one of the largest ecosystems on Earth (Costello et al., 2010; de Lavergne et al., 2016). Deep-sea surface sediments are dominated by bacterial cells in abundance and biomass (Pfannkuche, 1992; Rowe et al., 1991), which play major roles in the biogeochemical cycling of carbon and other essential elements (Jørgensen and Boetius, 2007). The sheer size of the seafloor habitat suggests that core groups of bacteria, i.e. groups that are abundant and widely distributed (Bienhold et al., 2016; Shade and Handelsman, 2012), may represent some of the most abundant microorganisms on Earth, and presumably perform major ecosystem functions. However, in contrast to marine surface waters, where core bacterial groups such as SAR11 and *Prochlorococcus* have been the object of extensive research for more than a decade (Coleman and Chisholm, 2007; Giovannoni et al., 2005), the identity and ecological function of the core taxa of the seafloor microbiome remain poorly investigated.

The 16S rRNA gene sequence cluster JTB255 marine benthic group – hereafter referred to as JTB255 – is globally distributed in both coastal and deep-sea marine sediments as indicated by 16S rRNA gene sequencing surveys (Bienhold et al., 2016; Bowman et al., 2005; Dyksma et al., 2016; Lenk et al., 2011; Li et al., 1999; Mußmann et al., 2017). Those studies suggest high relative sequence abundances of JTB255 in marine sediments, ranging from 1 to 22%. Also JTB255 cell densities appear to be high; in coastal sediments they reach on average 6% of total cells as determined by CARD-FISH (Dyksma et al., 2016; Mußmann et al., 2017; Probandt et al., 2017). However, such quantitative global estimates of JTB255 cell numbers in deep-sea sediments by microscopy are still missing (Dyksma et al., 2016; Probandt et al., 2017). Furthermore, recent studies have suggested that JTB255 might be sub-divided into several clades of coastal and deep-sea organisms and be related to *Chromatiales* (Du et al., 2016; Dyksma et al., 2016; Mußmann et al., 2017). Currently, their phylogenetic position within the *Gammaproteobacteria* remains uncertain, as well as the phylogenetic structure of the group itself. There are only few hints available from metagenomics studies about the metabolic potential and physiology of JTB255 bacteria. Current evidence for JTB255 in coastal sediments points towards an aerobic or facultatively anaerobic,

chemoorganoheterotrophic lifestyle (Du et al., 2016), but recent data also indicate a potential for facultative chemoautotrophy as suggested by the presence of genes encoding a ribulose-1,5-bisphosphate carboxylase/oxygenase (form II RubisCO), a key enzyme of the Calvin-Benson-Bassham cycle (Dyksma et al., 2016; Mußmann et al., 2017). Thiosulfate and hydrogen were suggested as putative electron donors for the autotrophic lifestyle of coastal JTB255, based on the presence of genes encoding a Sox-pathway for thiosulfate oxidation, a dissimilatory adenosine-5'-phosphosulfate reductase and an oxygen-tolerant [NiFe] uptake hydrogenase (Hup) and its accessory proteins (Dyksma et al., 2016; Mußmann et al., 2017). However, so far, our knowledge of the genetic and metabolic diversity of JTB255 bacteria is restricted to some partial genome sequences obtained from single cell sequencing and metagenomics studies of coastal JTB255 populations (Mußmann et al., 2017), and a recently published genome of coastal *Woeseia oceani* XK5 (Du et al., 2016). This study contributes the first genomic data for JTB255 bacteria inhabiting deep-sea sediments, which differ substantially in environmental characteristics and microbial community compositions from coastal areas (Canfield et al., 1993; Zinger et al., 2011).

Based on current knowledge and new results, mostly from our study of Arctic deep-sea sediments, the following hypotheses are tested: I) deep-sea and coastal JTB255 belong to different sub-clades, II) JTB255 define a lineage that is an integral part of *Chromatiales*, III) JTB255 cells are abundant and globally distributed in deep-sea surface sediments and are thus core members of the deep-sea sediment microbiome, IV) deep-sea representatives of JTB255 have habitat-specific traits supporting an aerobic, chemoorganoheterotrophic lifestyle.

Materials and methods

Study sites

Sediment samples were obtained from various oceanic regions, including the Arctic Ocean, South and North Pacific, South and North Atlantic, Indian, and Antarctic Ocean, and covered water depths between 75 to 5,500 m (Table S1). Several of the samples were kindly provided by other research groups (Table S1). For the purpose of this study, we defined sediment horizons ranging from 0.00 to 0.1 m as surface sediments, horizons from 0.1 to 1 m as subsurface sediments and horizons below 1 m as deep subsurface. Upon sample retrieval, all subsamples were treated as described in the following sections according to methodological requirements.

Epifluorescence microscopy and Catalyzed Reporter Deposition Fluorescence In Situ Hybridization (CARD-FISH)

Sediment samples (0.5 g) were fixed, sonicated, and stained with the nucleic acid stain 4`-6-diamidino-2-phenylindole (DAPI) as described by Schauer and colleagues (2011). Total cells were counted to a minimum number of 1,000 cells per sample in 20 independent grids, using a Zeiss Axio Imager M1 epifluorescence microscope equipped with a 100x/1.25 oil plan-apochromat objective. A list of all processed samples can be found in the Supplementary Information (Table S2). CARD-FISH was performed according to a previously published protocol (Ishii et al., 2004). The oligonucleotide probe GAM42a together with its competitor BET42a was used to assess the abundance of *Gammaproteobacteria* (Manz et al., 1992). Probes JTB819a and JTB897 were designed to target JTB255, and applied together with a competitor cJTB897, which targets organisms with one mismatch to the probes (see Supplementary Information and Table S3). Total (DAPI) and specific (*Gammaproteobacteria*; JTB255) cell counts were obtained from the same filters and cells cm^{-3} were calculated from the cell counts applying a microscope factor of 13,104. Replicate filters (2 to 18) were counted for each sampling site.

Cell abundance and biomass estimation

The global abundance and biomass of JTB255 and *Gammaproteobacteria* in marine surface sediments was extrapolated from CARD-FISH cell counts obtained from 23 deep-sea and 2 coastal sites (Table S1 and S2). Cell sizes were measured with a stage micrometer (100 lines with 100 µm intervals) and a graticule of 1 µm intervals (Zeiss,

Germany). Cell volumes were calculated based on cell shapes and sizes, assuming 40% spherical, 40% rod-shaped and 20% comma shaped JTB255 cells. The observed cell measures are given in the results section of the Supplementary Information. Biomass was estimated using a volume to carbon conversion factor of 3 x 10^{-13} g C μm^{-3} (Børsheim et al., 1990).

Estimates of global cell abundances in deep-sea sediments were calculated from average cell numbers across the 23 globally distributed sampling sites. Average cell numbers of *Gammaproteobacteria* and JTB255 were then multiplied with the area of global ocean deep-sea floor, here defined as sediments below 200 m water depth, which cover approximately 333 x 10^6 km^2 (Costello et al., 2010; de Lavergne et al., 2016). In addition, we made a projection of cell abundances for the total area of marine surface seafloor, i.e. 359 x 10^6 km^2 (Costello et al., 2010; de Lavergne et al., 2016; Sverdrup et al., 1942) (see Supplementary Information for calculations). An estimate of JTB255 and gammaproteobacterial cell abundances within the upper 10 cm of the sediment was achieved by integrating the average cell numbers per sediment layer (0 to 1 cm, 1 to 2 cm, 2 to 3 cm, 3 to 5 cm, 5 to 10 cm) to an average per 10 cm. Furthermore, we added up cell numbers obtained with the probes Gam42a (Manz et al., 1992) and the probes JTB819a and JTB897, to allow a more accurate global estimate of gammaproteobacterial cells in deep-sea surface sediments, as the probe Gam42a misses several gammaproteobacterial groups, including JTB255 (Dyksma et al., 2016) and most *Xanthomonas* (Siyambalapitiya and Blackall, 2005). Furthermore, here we report cell counts based on CARD FISH filters, a procedure known to result in lower cell counts compared to the AODC method (Schippers et al., 2005; Webster et al., 2009). Additional information is given as Supplementary Information within the method section.

Relationships of JTB255 cell counts with water depth, latitude, total organic carbon (TOC), and primary productivity were investigated using linear regression analysis, supported by Pearson and Spearman correlation statistics. For the relationship of JTB255 cell counts with sediment depth and oxygen concentration, general linear mixed models were used within the R package lmerTest using sediment depth as a random factor.

Phylogenetic analyses

Phylogenetic placement of JTB255 within the *Gammaproteobacteria* was addressed using the 16S rRNA gene as marker gene. A total of 423 sequences (Table S4), representing validly described families within 14 gammaproteobacterial orders (Williams et al., 2010), were selected to cover the basal segment of *Gammaproteobacteria*. Among those sequences, 49 were assigned to JTB255. Sequences were aligned using the iterative refinement method L-INS-i (Katoh and Toh, 2008) implemented in Mafft v7 (Katoh and Standley, 2013). Parameters for the alignment were set as follows: 200PAM/k=2 scoring matrix, a gap opening penalty of 1.53, and an offset value of 0.123. Scoring and trimming of unreliable sections of the alignment were performed using BMGE (Criscuolo and Gribaldo, 2010) in default settings. Phylogeny was inferred using a maximum likelihood approach as implemented in IQ Tree (Nguyen et al., 2014). Selection of the nucleotide substitution model was performed with the procedure implemented in IQ Tree. Based on the Bayesian Information Criterion (BIC), a GTR model including eight categories of distribution-free rates was the best-fit to the sequence alignment, which was in good agreement with best-fit models returned by the Akaike (AIC) and corrected Akaike (AICc) information criteria. Branch support was assessed using 1 000 replicates of ultrafast bootstrap (Minh et al., 2013) and the Shimodaira–Hasegawa approximate likelihood-ratio test (Guindon et al., 2010).

Nucleic acid extraction, amplicon sequencing, and minimum entropy decomposition

Based on 16S rRNA gene sequences, we aimed at assessing patterns of microdiversity within the JTB255 group across a range of marine environments (Table S1), using the clustering-independent oligotyping approach based on minimum entropy decomposition (MED) (Eren et al. 2013). DNA extraction, amplicon sequencing of the V3-V4 region of the 16S rRNA gene and sequence processing were performed as described in Hoffmann et al. (2017).

To support a reliable identification of JTB255 sequences in the amplicon datasets, a reference dataset of JTB255 sequences from various environments was constructed based on the SILVA SSU Ref database release 123 (Quast et al., 2013) (Table S4). Additional sequences were retrieved from single-cell genomes (Mußmann et al. 2017, and M06) (see Table S4), and assembled from metagenomes from deep-sea HAUSGARTEN sediments (AS6 to AS10, Table S4, Methods section “Metagenomics and binning”). The curated reference sequence dataset was used to build a nucleotide

BLAST database, using BLAST v2.2.31+. Sequences from the amplicon datasets were then screened for potential JTB255 sequences. JTB255 sequences recovered with BLASTN from 35 environmental samples (Table S5) were analyzed for single nucleotide polymorphism using MED v2.1 (Eren et al., 2013) (Table S6). Further details on the assembly of the JTB255 reference set and MED procedure are provided as Supplementary Information.

Single-cell genomics

Single cells were sorted from Arctic deep-sea surface sediment collected at the LTER HAUSGARTEN in Fram Strait in June 2014 (Table S1). Sediments were diluted after sediment retrieval (1:1 [v:v]) using 0.22 µm-filtered seawater. Aliquots of 20 mL were shipped at 5°C to the Bigelow Laboratory Single Cell Genomics Center (SCGC; https://scgc.bigelow.org). At the SCGC, cells were sorted in a total of eight 384-well plates and lysed using cold (four plates) and room temperature (four plates) KOH (Raghunathan et al., 2005). Detailed procedures for single-cell sorting, cell lysis, multiple displacement amplification (MDA), cell screening, genome sequencing and assembly are provided in Swan *et al*. (2011) and on the centers` webpage (https://scgc.bigelow.org).

Metagenomics and binning

Shotgun metagenomic sequencing was conducted on DNA extracts from LTER HAUSGARTEN (Table S1). DNA was extracted from 0.5 g of the uppermost centimeter of the sediment with the MoBio PowerSoil Kit (MoBio Laboratories Inc., Carlsbad, CA, USA), following the manufacturer's instructions, and multiple extractions were pooled to generate a total of 1 to 2 µg DNA for the preparation of a standard Illumina TruSeq sequencing library. A total of 4,108,971 paired-end reads of 300 base pair (bp) length and an average insert size of 400 bp were generated and sequenced on an Illumina MiSeq machine at CeBiTec, University of Bielefeld. Reads were trimmed, quality filtered for common contaminants and merged with tools from the bbtools suite (v. 35.50 BBMap - Bushnell B. - sourceforge.net/projects/bbmap/). The processed reads were assembled using SPAdes v3.6 (Bankevich et al., 2012).

Binning of assembled genome fragments was performed in Metawatt (Strous et al., 2012) based on GC-content, tetranucleotide frequencies and read coverage. Taxonomic assignment of the bins was performed using the BLASTP module of Metawatt and the associated reference genome database. Publicly available genomes of JTB255 organisms were added to the reference genome database to enable taxonomic assignments to JTB255. Added genomes included a partial single cell genome (ID 2651869504), two metagenomic bins (ID 2651869885; ID Ga0136857) (Mußmann et al., 2017), and the closed genome of the recently isolated strain *W. oceani* XK5 (Du et al., 2016). This procedure led to the extraction of two metagenomic bins assigned to JTB255. Targeted reassembly of the JTB255 bins was performed with SPAdes v3.6 and repeated four times with increasing read mapping stringency (up to 98%). Scaffolds with a sequence length < 500 bp were excluded. Purity, completeness and redundancy were checked using CheckM (Parks et al., 2015).

Annotation of partial genomes

The syntactic and functional annotation of the single cell genomes was done via the MicroScope platform (Vallenet et al., 2009, 2013; Vallenet and Labarre, 2006). Open reading frames (ORFs) of the metagenomic bins were predicted with Prodigal v 2.6.1 (Hyatt et al., 2010). Predicted ORFs were then annotated against the PFAM database 30.0 using HMMER. Genes of interest were searched in both metagenomic bins using gene databases obtained from NCBI genes. Automatic annotations were manually curated by examining their closest BLAST hits using NCBI BLASTn and BLASTp, and analyzing protein sequences for their domains using Pfam v30.0 (Finn et al., 2016) as well as InterProScan sequence searches (Jones et al., 2014). Carbohydrate-active enzymes (CAZymes) were automatically annotated based on HMMER searches using the dbCAN online tool (Yin et al., 2012).

Data deposition

Data are accessible via the Data Publisher for Earth & Environmental Science PANGAEA (DOI: 10.1594/PANGAEA.874860). All genomic data including single cell genomes M06 and B02, metagenomic bin1_HGIV and bin2_HGIV, and raw paired-end, primer-trimmed reads are available under INSDC accession: PRJEB20570. The data were archived using the brokerage service of GFBio (Diepenbroek et al., 2014).

Results and Discussion

Phylogeny of JTB255

Analyses of currently available full-length 16S rRNA-gene sequences of JTB255 suggested a grouping into five well-supported sequence clusters, which are referred to as cluster 1 to 5 (Figure 1). The intra-group similarity within those clusters ranged from 88 to 98%, indicating a clustering at family level (Yarza et al., 2014). The five clusters may therefore represent five distinct families within the JTB255. Except for cluster 5, all clusters contained sequences from both deep-sea and coastal environments, and cluster 5 also hosted sequences from deep-sea sediments (Figure 1). Previous reports had suggested JTB255 sequence clusters to be associated with either deep-sea or coastal sediments (Mußmann et al., 2017). Association of JTB255 sequence clusters with either deep-sea or coastal sediments was, however, based on very few sequences (< 10) and require further validation once more global data become available. We similarly observed that all sequences in cluster 5 were derived from hydrothermal vent sediments (at three distinct sampling sites), but this association is at best tentative because of the small number of sequences (6) currently available. Clusters 1, 3, 4 and 5 were retrieved following three independent re-iterations of the maximum likelihood-based inference of the phylogeny further suggesting that those groups of sequences represent genuine lineages (see Supplementary Information). Sequences within cluster 3 were characterized by a 16 nucleotide insertion between nucleotide position 77 and 78 of the 16S rRNA gene sequence of *E. coli* K12 (substr. MG1655), which further support their classification in a distinct sequence cluster. Sequences within cluster 3 originate from several sampling sites and environments, including deep-sea sediments and the water column, indicating that some members of the JTB255 could have a pelagic life-style. Cluster 1 included the 16S rRNA gene sequence of the bacterium *W. oceani* XK5 (Du et al., 2016), which was recently isolated from marine coastal sediments and currently constitutes the sole cultivated representative of JTB255.

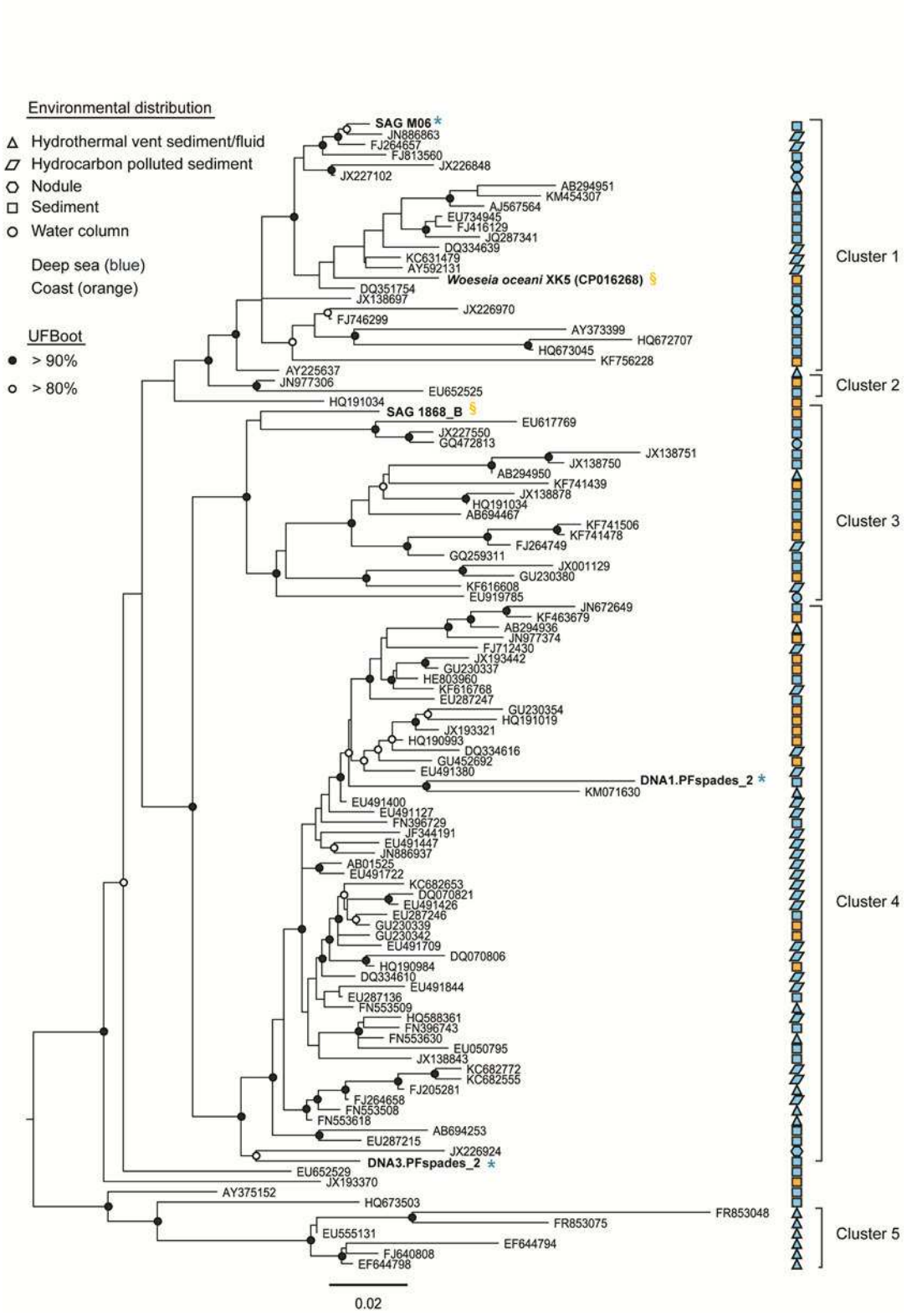

Figure 1. **Maximum likelihood tree (after automatic model selection with IQ-Tree, version 1.4.2) of the 16S rRNA gene of the JTB255 group within the *Gammaproteobacteria* (n = 110 sequences).** More details and strain abbreviations are listed in Table S1 and S4. § highlights 16S rRNA-gene sequences where a coastal genome is available; * highlights 16S rRNA-gene sequences where a deep-sea genome is available. Newly designed JTB255 probes JTB819a and JTB897 cover all sequences displayed within this tree.

At a broader phylogenetic scale, analyses of gammaproteobacterial 16S rRNA gene sequences indicate that JTB255 define a well-supported monophyletic clade that comprises sequences exclusively retrieved from marine habitats (Figure 2) supporting previous results (Dyksma et al., 2016; Mußmann et al., 2017). An intra-group similarity of JTB255 sequences of 85% suggested a phylogenetic placement of this clade between family and order level (Yarza et al., 2014). Our phylogenetic analyses of a larger sequence set including more deep-sea representatives, further supports this JTB255 sublineage within a larger sequence cluster, which, besides JTB255, is comprised of sequences exclusively retrieved from continental habitats (Figure 2), and included the 16S rRNA gene sequences of *Steroidobacter denitrificans* (Fahrbach et al., 2008), *Povalibacter uvarum* (Nogi et al., 2014) and the wrongly named '*Pseudomonas*' VM15C strain (Sakazawa et al., 1981). The taxonomic classification of the genera *Steroidobacter* and *Povalibacter* is, so far, only tentative and both were suggested to be members of the *Nevskiales* (Naushad and Adeolu, 2015), a recently proposed gammaproteobacterial order including organisms initially misclassified within the *Xanthomonadales*. We believe that the phylogenetic affinity between the JTB255 clade and the putative group of microorganisms including both *Steroidobacter* sp. and *Povalibacter* sp. remained thus far unnoticed because available phylogenies were based on a very partial taxon sampling that excluded at least one of the two groups. Our phylogenetic analysis placed the JTB255-*Steroidobacter*-*Povalibacter* sequence cluster among the basal taxa of the *Gammaproteobacteria* (Williams et al., 2010), but its position relative to other basal taxa remains so far unresolved (Figure *2*). The placement of *W. oceani* XK5 (*Woeseiaceae*) and therefore JTB255 within the *Chromatiales* (Du et al., 2016; Mußmann et al., 2017) should be considered only tentative, considering that phylogenomic analyses question the unity of *Chromatiales* (Williams et al., 2010), which also appeared as a polyphyletic group in our 16S rRNA phylogeny (Figure 2).

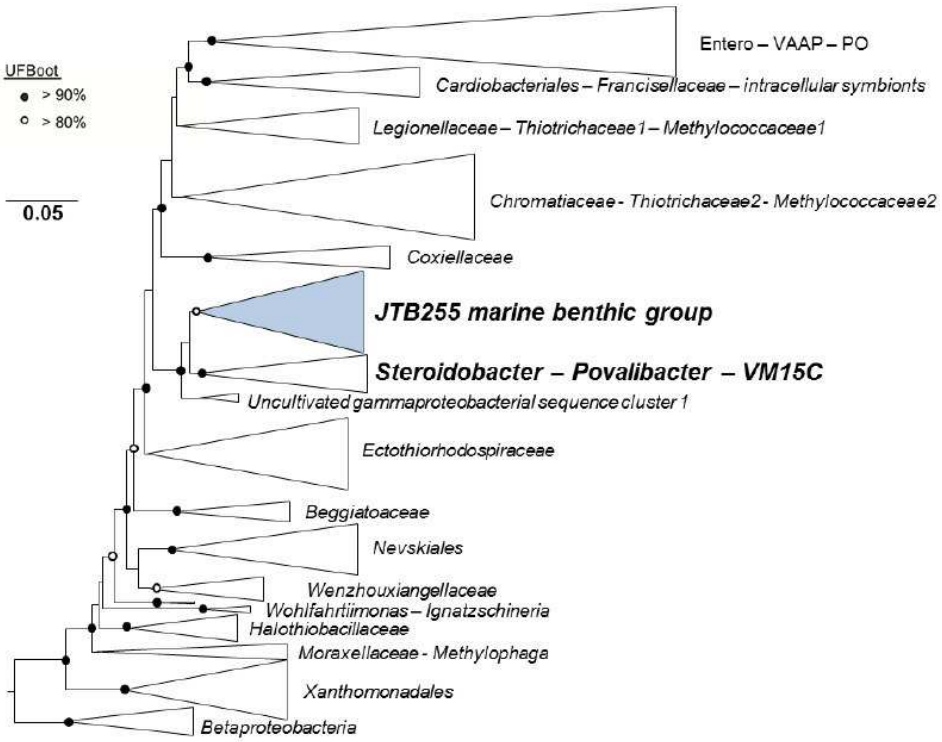

Figure 2. **Maximum likelihood tree (after automatic model selection with IQ-Tree, version 1.4.2) of the 16S rRNA gene of *Gammaproteobacteria*, focusing on its basal part (n = 423 sequences).** Entero – VAAP – PO: *Enterobacteriales*; *Vibrionales*; *Aeroanaerodales*; *Alteromonadales*; *Pasteurellales*; *Pseudomonadales*; *Oceanospirillales*.

Global abundance and distribution of JTB255 in deep-sea sediments

Based on their cosmopolitan distribution and abundance in sequencing surveys, *Gammaproteobacteria* and more specifically the JTB255 clade were proposed to be part of a core microbiome of marine sediments (Bienhold et al., 2016; Mußmann et al., 2017). However, only few quantitative JTB255 cell counts are available so far (Dyksma et al., 2016; Mußmann et al., 2017; Probandt et al., 2017), and the inference of population densities and cell abundances from sequence counts is potentially misleading (Weber and Pawlowski, 2013). Here we provide the first global cell count survey for deep-sea *Gammaproteobacteria* (Supplementary Information) and JTB255, including 23 deep-sea samples and two additional coastal samples (Figure 3). The two JTB255 probes designed in this study (Table S3) targeted 93% of the currently available JTB255 16S rRNA-gene sequences *in silico* (SILVA release 123) (Quast et al., 2013), including all of the five newly proposed JTB255-clusters (Figure 1, Table S4). Previous probes (Dyksma et al., 2016) covered approximately 25% of the groups` diversity and targeted only parts of sequence cluster 1 and 4 proposed in this study (Figure 1). It is also im-

portant to note that the probe Gam42a that binds to the 23S ribosomal part of *Gammaproteobacteria* did not cover JTB255, in line with previous reports (Dyksma et al. 2016). JTB255 cells were relatively abundant in the top 2 cm of deep-sea sediments at latitudes from 55°S to 89°N, with cell numbers from 1.7 x 10^8 cells cm^{-3} sediment in continental margin sediments (Fram Strait; AO.4a) to 4.1 x 10^6 cells cm^{-3} at the Logatchev hydrothermal vent field (Mid-Atlantic Ridge; NA.1) (Table S2), with no apparent latitudinal pattern (Table S7). Independent of sample origin, JTB255 cell morphology varied between spherical, rod, and comma shaped, with an average diameter of 0.4 µm and an average volume of 0.13 $µm^3$ (Figure 4B and Supplementary Information). Consistent with other molecular surveys (e.g., Li et al. 1999b; Bowman & McCuaig 2003; Bienhold et al. 2016; Learman et al. 2016), JTB255 cells were detected in all but one (NA.2) of the 23 surveyed deep-sea surface sediment samples (Figure 4A; Table S2). This station was located at a hydrothermal vent field where sediment were covered by white mats containing filamentous bacteria and elemental sulfur (Schauer et al., 2011). Cell numbers of JTB255 in sediments across all other deep-sea sites corresponded to 1 to 9% of total cells obtained by DAPI staining of CARD-FISH filters. This is in the same range as counts reported from tidal (3 to 6%; Dyksma et al. 2016) and sublittoral coastal sandy sediments (1 to 6%; Probandt et al. 2017), and highlights their global contribution to bacterial communities of marine sediments. In the water column, sequence counts of JTB255 are low (0.2 to 0.7%) (Table S5), suggesting lesser relevance of JTB255 in the planktonic fraction. However, so far no cell counts are available for JTB255 inhabiting the water column, to test this hypothesis.

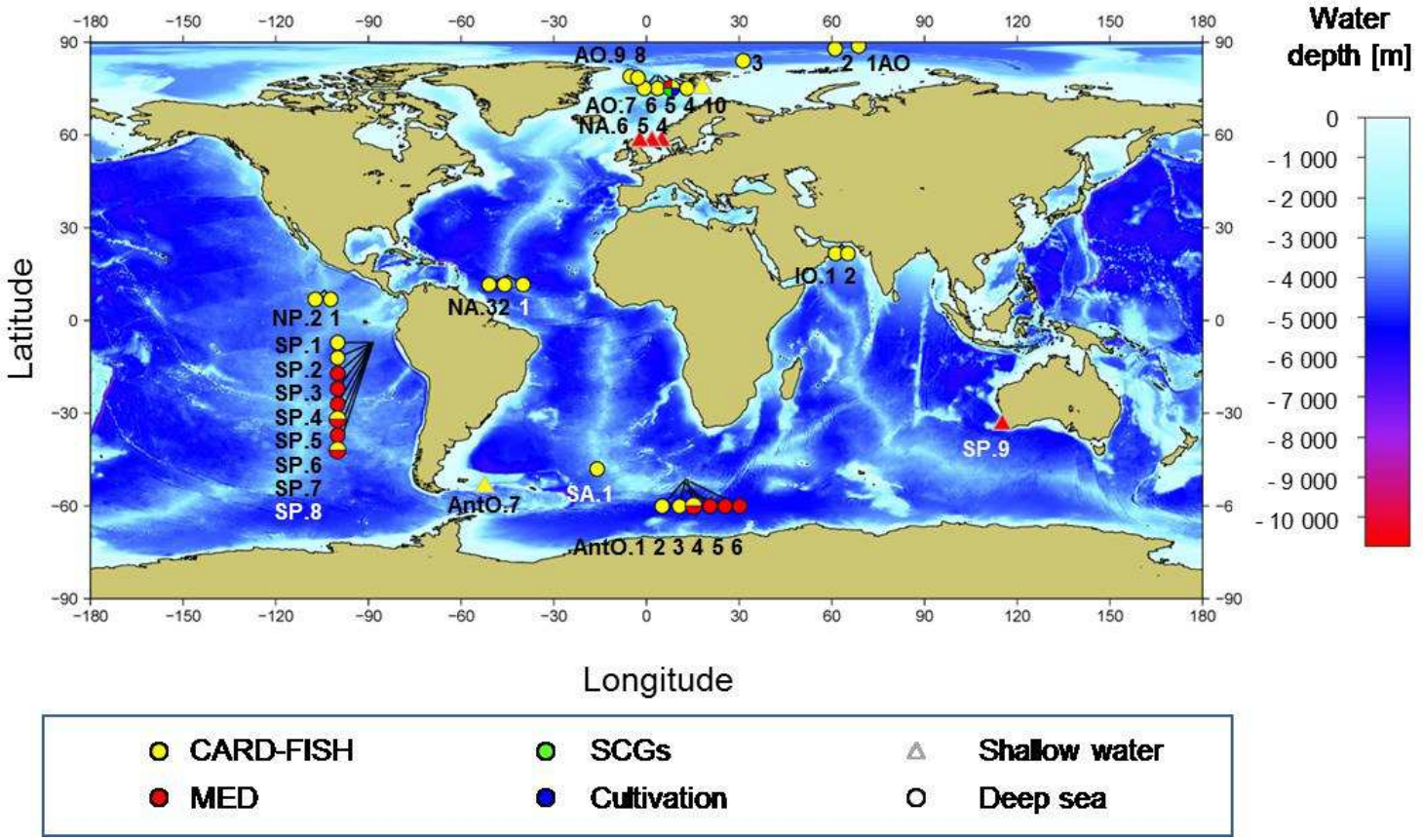

Figure 3. **Geographic location of the sampling sites used in this study.** Different colors of the sampling sites indicate different methods applied at each site: CARD-FISH counts (yellow); minimum entropy decomposition (MED; red), single cell and meta-genomics (SCGs; green), and cultivation approaches (blue). The line color and shapes of the symbols per site highlight the location of the sampling site in shallow waters (grey, triangle) or the deep sea (black, circle). The short labels of the sites are listed in detail in Table S1. This map was created with R version 3.3.2, using the core distribution (R Development Core Team, 2016), together with the marmap package (Pante and Simon-Bouhe, 2013).

Besides their abundant and ubiquitous global distribution in deep-sea surface sediments, we observed variations in cell numbers that were related to environmental parameters. JTB255 cells decreased significantly with increasing water depths from 75 to 5 503 m ($p < 0.001$, Table S7, Figure S1), with some outliers towards the upper and lower part of the gradient. More samples are needed to verify this observation. Furthermore, there was a trend of decreasing cell numbers with increasing sediment depth down to 0.16 m (Table S8, Figure S2). This could be related to a decrease in labile organic matter as potential energy and carbon source with increasing water and sediment depth (Jacob et al., 2013; Klages et al., 2004; Lalande et al., 2016; Learman et al., 2016; Schewe and Soltwedel, 2003). This is affirmed by positive, though weak, correlations of JTB255 cell densities with sediment TOC content and surface ocean primary productivity estimates, as further proxies for organic matter availability. These observed correlations could support the hypothesis of a chemoorganoheterotrophic, opportunistic lifestyle of JTB255 (Mußmann et al., 2017; Du et al. 2016). However, JTB255 showed no growth in response to various stimulation experiments with labile particulate (Hoffmann et al., 2017) and dissolved organic matter sources in short-term enrichment experiments of 4 to 19 weeks (Supplementary Information). Hence, the link between organic matter availability, cell abundance and growth rate remains unclear. A range of other energy-related parameters may co-vary with water depth, e.g. quality of organic matter, total bacterial and faunal community abundance and composition, and may generate the patterns observed here. Decreases in JTB255 cell numbers with increasing sediment depth may reflect variations in chemical gradients, most importantly oxygen (Table S1, Figure S2, Table S8) JTB255 cells were either absent or only present at low abundances in anoxic samples, indicating an aerobic or facultatively anaerobic lifestyle of this group.

Overall, *Gammaproteobacteria* and JTB255 appear to be globally ubiquitous and numerically abundant bacterial groups in marine surface sediments. Based on average cell counts from 23 widely distributed deep-sea sites, we estimate their global abundances to be $1.1 \pm 0.4 \times 10^{27}$ gammaproteobacterial cells and $5.8 \pm 2.1 \times 10^{26}$ JTB255 cells across deep-sea surface sediments (top 10 cm) (see Supplementary Information for further details). For JTB255 abundances from deep-sea sediments combined with cell abundances from coastal sediments (Dyksma et al., 2016), this would add up to $6.3 \pm 2.3 \times 10^{26}$ cells and an estimated biomass of 0.01 Pg carbon (1×10^{13} g carbon, based on calculations results reported as Supplementary Information) in marine surface sediments. As a comparison, the most abundant photosynthetic marine microorganism, *Pro-*

chloroccocus, has a mean global abundance of 3 x 10^{27} cells (Flombaum et al., 2013). And the most abundant bacterial clade in the water column, SAR11, was estimated to amount to 2.4 x 10^{28} cells globally (Morris et al., 2002). JTB255 cells appear to have a higher biovolume (on average 0.13 µm^3) compared to the two water column clades *Prochlorococcus* (0.10 µm^3; Morel et al. 1993) and SAR11 (0.01 µm^3; Rappé et al. 2002), and thus may account for a considerable fraction of microbial biomass in the oceans. Their physiology and ecological function remains largely uncharted, but may be highly relevant for ecosystem functioning in marine (deep-sea) sediments.

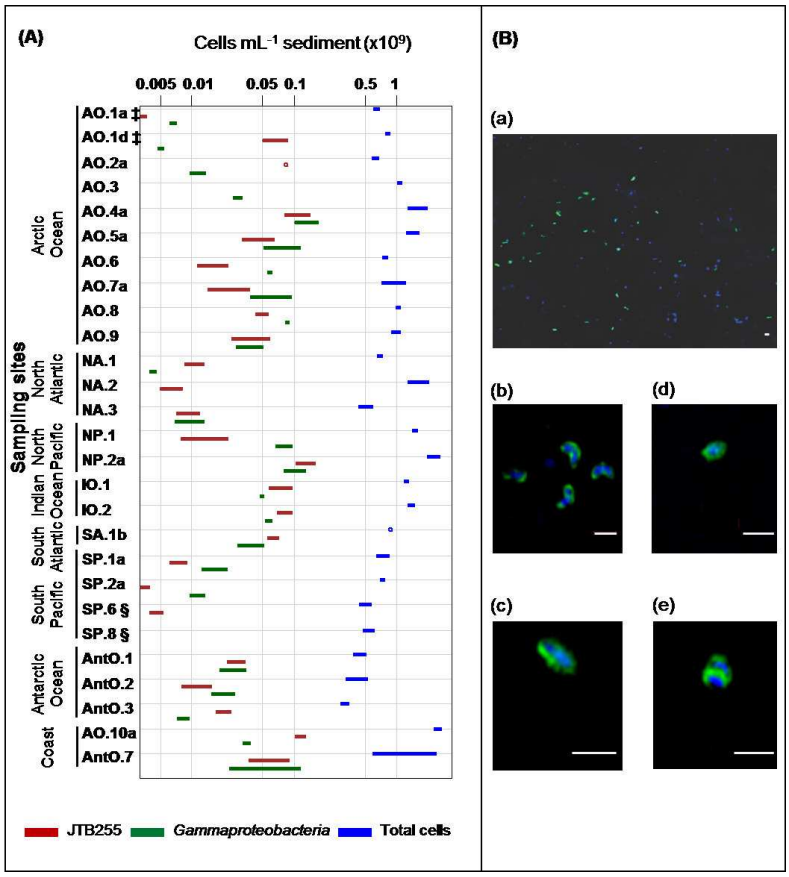

Figure 4. **Counts and cell shapes of JTB255 in marine deep-sea sediments.** (A) Cell counts of marine sediments (0 to 2 cm). Total cell counts (DAPI stain) displayed in blue; CARD-FISH counts of gammaproteobacterial cell counts displayed in green and JTB255 cells displayed in red. Shown is the cell count range from minimum to maximum cell numbers based on technical replication counts. For details see Table S2. Please note that JTB255 (here in red) is not targeted by probe Gam42a, specific for most *Gammaproteobacteria* (here in green). ‡ Sample with different food conditions; § polymetalic nodules. Explanation of the sampling site short labels can be found in Table S1. (B) CARD-FISH based epifluorescence (a) and SR-SIM (b to e) images of cell shapes and size of the gammaproteobacterial JTB255 group in deep-sea surface sediments of the Artic Ocean (site AO.5). In blue: DNA-staining of total cells using DAPI; in green: rRNA-staining of the JTB255 marine benthic group using ALEXA488. Images b to e show the different cell shapes observed for the JTB255-clade: comma (b), rod (c) and spherical (d + e) shape. White bars indicate a length of 1 µm. High-resolution images were captured using superresolution structured illumination microscopy (ELYRA PS.1, Zeiss). A 63x/oil plan-apochromat objective was used with a grating of two for DAPI and three for the CARD-FISH stain Alexa488.

Microdiversity within the JTB255 using minimum entropy decomposition

We explored diversification within the JTB255 phylogenetic cluster across various marine habitats (Figure 3) using Minimum Entropy Decomposition (MED) (Eren et al., 2013), to identify oligotypes, here defined as closely related short 16S rRNA gene sequences differentiated by single nucleotide polymorphisms (Eren et al., 2013). The number of oligotypes ranged from 0 to 10 in deep subsurface sediments (0 to 38 sequences) to 142 to 202 (3 252 to 27 045 sequences) in deep-sea surface sediments (Figure S2, Table S5). Overall, there were clear differences in the composition of JTB255 oligotypes according to their habitat origin, as indicated by cluster analysis based on Bray-Curtis dissimilarity (Figure 5A). The proportion of shared oligotypes was highest (58%) within samples from a deep-sea nodule field at the Clarion Clipperton Fracture Zone and lowest for samples from deep subsurface sediments at the South West Indian Ridge (0.3%) and coastal surface sediments from the Australian coast and German Bight (0.3%). These numbers should be considered preliminary due to the limited number of samples. Most oligotypes, i.e. 29%, were shared between deep-sea subsurface sediments and polymetallic nodules in deep-sea sediments. Sediment and water column JTB255-assemblages shared on average only 1% of their oligotypes. The largest differences in the presence of oligotypes were observed in a comparison of either coastal or deep-sea deep subsurface sediments with all other habitats, with on average 0.3% shared oligotypes. However, only 12% of all oligotypes were unique to one habitat, indicating a broad distribution of JTB255 oligotypes in the marine environment at this level of taxonomic resolution and limited sampling effort. Accordingly, the differences between habitats were mainly caused by variations in the relative abundances of oligotypes, which suggests that different habitats may select for specific oligotypes, as a result of environmental filtering. Further research will need to resolve the presence of potential JTB255 ecotypes in the marine realm, e.g. by analyzing oligotype patterns across spatial and temporal environmental gradients and including other genetic marker genes (see e.g., Davies et al., 2014; Buttigieg and Ramette, 2015).

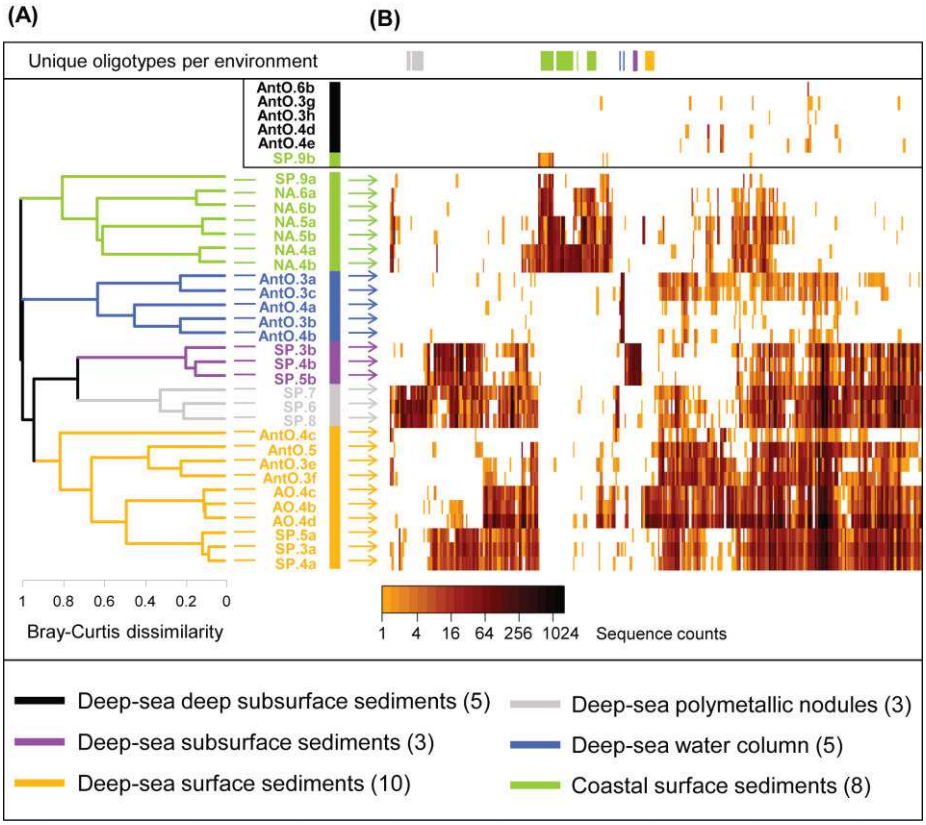

Figure 5. **JTB255-oligotypes.** (A) Cluster diagram of oligotypes per sampling site (listed by their short labels; see Table S1) based on Bray-Curtis dissimilarity on relative sequence proportions using complete linkage hierarchical clustering. Samples with a JTB255 sequence number below 100 were not included in the cluster analysis. (B) Heatmap illustrating MED oligotype counts (log2 transformed). Darker shades indicate higher sequence counts; white represents absence of an oligotype. Samples not included in the dendrogram are visualized in the heatmap in an extra panel. Unique oligotypes per environment are depicted above the heatmap, with colors corresponding to the sampled environment. Numbers in parentheses indicate the number of samples per environment.

Comparative genomics of JTB255 bacteria in deep-sea surface sediments

Single-cell genomics and metagenomics were used to gain further insights into the genomic diversity and metabolic potential of JTB255 bacteria inhabiting deep-sea surface sediments. Samples originated from LTER HAUSGARTEN, station IV (2,403 m water depth), where JTB255 bacteria were especially abundant based on cell counts (Table S2; Figure 4A). Only two out of 498 sorted single-cells were identified as JTB255 bacteria, which was lower than the counted proportion of cells (5 ± 1% of total cell counts) in the original sample. This observation suggests that JTB255 bacteria were either excluded during the sorting procedure or that the utilized lysis protocol was not suited to recover nucleic acids from those cells (Supplementary Information).

Draft genome assemblies obtained from sorted JTB255 single-cells, hereafter referred to as B02 and M06, spanned 0.2 and 1.1 Mb, respectively (Table S9). Two meta-

genomic bins, assigned to JTB255 and referred to as bin1_HGIV and bin2_HGIV were also recovered totalizing 1.6 and 1.8 Mb (Table S9), respectively. Completeness estimates of M06, bin1_HGIV and bin2_HGIV ranged between 16.3 and 52.7% suggesting a tentative genome size range of 3.4 to 6.8 Mb. For comparison, the closed genome sequence of *W. oceani* XK5 spans a total of 4.1 Mb (Du et al., 2016). Phylogenetic analyses of JTB255 16S rRNA gene sequences indicate that M06 and *W. oceani* XK5 belong to JTB255 cluster 1 (Figure 1) and both share 96% 16S rRNA gene identity, suggesting that the respective organisms represent closely related taxa (see Supplementary Information for more details). In contrast, a SAG (1868_B) and metagenome bin (20_j1) previously recovered from coastal sediments (Mußmann et al., 2017) were assigned to cluster 3 and a metagenome bin (WOR_SG8_31) obtained from estuarine sediment (Baker et al., 2015; Mußmann et al., 2017) appeared to be only distantly related to the remaining sequences included in our phylogenetic analysis (Figure 1 and Supplementary Information). Annotation of the metagenome bins and SAGs recovered from coastal and estuarine sediments (Baker et al., 2015; Mußmann et al., 2017) suggested a potential for a facultative chemolithoautotrophic lifestyle, which was supported by isotopic tracer experiments using ^{14}C-CO_2 (Dyksma et al., 2016). Genomic features indicative of a similar chemolithoautotrophic metabolism (i.e. Sox gene cluster, Hup-like [NiFe] uptake hydrogenase, RubisCO and phosphoribulokinase), are absent in the genome of *W. oceani* XK5 and were not found in any of the metagenome bins and SAGs obtained from deep-sea sediments in this study (Table S10). Additional data are necessary to clarify whether members of JTB255 cluster 1 generally lack a potential for chemolithoautotrophy and whether such potential is present in JTB255 bacteria inhabiting deep-sea sediments.

Comparative enzyme annotation of SAGs and metagenomic bins provided a first glimpse into the metabolic potential of the deep-sea surface sediment JTB members (Table S10*)*. Genes encoding an oxygen reductase (a heme-copper oxidase) and membrane-bound F_1F_0 ATP synthase are encoded in bin2_HGIV indicating that energy is conserved via oxidative processes using molecular oxygen as terminal electron acceptor. This prediction is consistent with long term observations of seafloor oxygen concentrations at the HGIV station, which indicate that the first 8 cm of the sediment are fully oxygenated, i.e. from 300 µmol L^{-1} at the sediment-water interface down to 50 µmol L^{-1} at 8 cm, with an oxygen flux of -1.02 ± 0.3 mmol O_2 m^{-2} d^{-1} (Cathalot et al., 2015; Donis et al., 2016). Although homologs of dissimilatory nitrite and nitric oxide

reductases are encoded in *W. oceani* XK5 and our metagenome bins and SAGs, the physiological role of those proteins remain unclear (see Supplementary Information).

A total of 36 different peptidase families (Table S10 and S11) and nine glycosyl hydrolase (GH) families (Table S9), were encoded in the obtained single cells and metagenomics bins from deep-sea surface sediments of which approximately 25% may be involved in the breakdown of organic matter (Fernández-Gómez et al., 2013). Genes encoding peptidases occur in nature at relatively high frequencies within all living cells, ranging from on average 1% to 6% of the total gene count (Potempa and Pike, 2005). We found a broad set of different peptidases encoded within all available JTB255 genomes (Table S11), with the highest numbers in members of JTB255 cluster I (*W. oceani* XK5 and M06). *W. oceani* XK5 and M06 had average encoded peptidases of approximately 3.7% and 3.5%, respectively, of the total gene counts per genome (Table S11). Furthermore, the relatively high number of peptidases in JTB255 was remarkable, especially combined with its low number of GHs, suggesting a preference for proteins over polysaccharides and, hypothetically, even a niche specialization of JTB255 on the degradation of proteins. Moreover, they encoded as many peptidases as other protein specialists, such as *Bacteroidetes* (see Fernández-Gómez et al., 2013 for further information). Based on single cell genomics, it was previously suggested that detrital protein degradation could be a key function of uncultivated subsurface seabed archaea, inhabiting organic rich shelf sediments (Lloyd et al., 2013). The peptidases associated with the archaeal SAGs were partly also encoded within the JTB255 genomes. Intriguingly, all JTB255 genomes from deep-sea and coastal sediments contained a predicted D-amino acid targeting peptidase (Merops family M19), which was hypothesized to be used by archaea for the degradation of aged, refractory compounds from cell walls (Lloyd et al., 2013; Lomstein et al., 2012). In total, 51 different peptidase families were found to be encoded within the different JTB255 genomes: 71% were encoded, both, in deep-sea and coastal JTB255 genomes, the remaining 29% might be specific for the coastal habitat (Table S10 and S11), pointing towards flexibility in the enzymatic repertoire of JTB255. However, as the here obtained genomic sequences of deep-sea JTB255 were incomplete, it is possible that a larger diversity of deep-sea specific peptidases remains to be discovered. Approximately 27% of the 51 identified peptidases tentatively have a nutritional role within JTB255 (see Table S11). Both classes of peptidases, exo- as well as endopeptidases (based on their action at or away from the termini, respectively), were present in each of the investigated JTB255 genomes. Especially exopeptidases play a

major role in nutrition due to their depolymerizing activity (Rao et al., 1998). Furthermore, peptidases of all six catalytic types, which are grouped depending on the nature of the functional group at their active site, were found encoded within deep-sea and coastal JTB255. Yet, JTB255 obtained from coastal sediments appeared to have encoded nearly three times more families belonging to the metallopeptidases then was observed within JTB255 genomes derived from the deep sea. The incompleteness of the newly obtained metagenome bins and SAGs does, however, not allow a complete reconstruction of the hypothetical amino acid and carbohydrate metabolism of deep-sea representatives of the JTB255 clade and their potential to use proteins and polysaccharides as primary source of carbon and energy needs to be further confirmed. No stimulation of growth of JTB255 could be achieved in short-term food enrichment experiments, using different polymeric organic matter sources such as chitin, polyvinyl alcohol, and diverse algal detritus (Table S12). Future tests should take into account the potentially slower growth rate of deep-sea bacteria, and should include substrates representative of the more refractory proteinceous material as a potential food source for JTB255.

Conclusion

JTB255 is a clade of bacteria globally abundant in marine surface sediments as indicated by cell and sequence counts. It represents a core group of marine sediment communities. Phylogenetic analyses indicate that JTB255 can be divided in at least five major sub-clusters. Members of these clusters have been detected in a broad range of habitats including continental shelf, margin, deep-sea and ridge sediments, as well as the pelagic realm. Composition of JTB255 oligotype assemblages appeared to be non-random and reflect their environmental origin, suggesting that similar sediments recruit similar sets of oligotypes. Some oligotypes might be preferentially associated with either coastal or deep-sea sediments, suggesting that adaptation to distinct sediment categories could operate at high taxonomic resolution. The genome of *W. oceani* XK5 encodes an extensive set of extracellular hydrolases, mainly peptidases and lipases, as well as all necessary pathways to conserve energy and assimilate carbon from various amino and fatty acids. Various components of this machinery were also detected in partial genomes recovered from deep-sea sediments suggesting the ability to grow on cell wall and membranes, as well as refractory proteins, which form a substantial part of the microbial necromass, and potentially explain the global ecological success of JTB255. Further cultivation and characterization of JTB255 isolates and their genomes will be needed to understand their ecological niche and function.

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Supplementary Information

Methods

Oligonucleotide probe design, CARD-FISH protocol adaptation, and probe validation

Probe design was based on SILVA SSU Ref database release 123 (Quast et al., 2013), by searching for strictly conserved target regions which cover the diversity of the JTB255-clade. Specificity of new oligonucleotide probes was tested *in silico* using the ARB probe match tool (Ludwig et al., 2004). A competitor oligonucleotide cJTB897 (one base altered to probe JTB897), matching *Bacteroidetes* sequences, was designed and applied together with the newly designed JTB255 probes in the CARD-FISH experiments, to minimize non-specific hybridization of non-targeted genotypes. HRP-labelled probes and competitor oligonucleotides were purchased from biomers.net GmbH (Ulm, Germany).

Oligonucleotide probe and competitor optimization was performed using surface sediments from the LTER HAUSGARTEN sampling site (Table S1). Different formamide concentrations were tested. The newly designed probes, in combination with their oligonucleotide competitor, performed best in a mix with a formamide concentration of 20%. Furthermore, the permeabilization step of the CARD-FISH protocol (Ishii et al., 2004) was modified for JTB255 enumerations: Cells were permeabilized by incubating polycarbonate membrane filters successively in a lysozyme solution (1,000 kU mL^{-1}) for 60 min and in an achromopeptidase (both purchased from Sigma-Aldrich, Germany) solution (90 U mL^{-1}) for 30 min, both at 37°C.

The obtained signals were cross-validated via double hybridizations of the newly designed probes and the competitor used in a mix with 1:1:1 (v:v:v) concentration ratio against: i) the gammaproteobacterial probe Gam42a and its competitor cBET42a to validate observed cells belonging to the *Gammaproteobacteria*; ii) probe GAM42a_T1038 targeting *Xanthomonas,* to validate that JTB255 do not belong to *Xanthomonas*; iii) probe CF319a targeting *Bacteroidetes,* to validate the performance of the oligonucleotidic competitor cJTB897; as well as iv) another set of CARD-FISH probes and competitors designed for JTB255 from coastal sediments (Dyksma et al., 2016) (Table S3). We observed that the specific CARD-FISH probe Gam42a, binding to the 23S ribosomal part of *Gammaproteobacteria*, did not cover JTB255, which confirmed results by Dyksma et al. (2016). We were further able to validate this finding *in silico* based on few 23S rRNA gene sequences of the JTB255 obtained from the two single cell ge-

nomes (M06, B02) and metagenomics bins using the ARB probe match tool (Ludwig et al., 2004). Furthermore, in multiple double-hybridizations of our newly designed JTB255 probe-mix and the JTB255 probes designed by Dyksma et al. (2016), the signal overlap between both probe sets varied between 90 to 95% for coastal and deep-sea surface sediments, although the theoretical coverage of our new probe-mix exceeded that of other JTB255 targeting probes by 3.7-fold (Table S3). This discrepancy might be due to a lower cell abundance of those JTB255 cells that our probe set binds additionally.

Cell abundance and biomass estimation

Samples covered a latitudinal range from 89°N to 55°S down to 5,525 m water depth and 16 cm sediment depth; 73% of the 23 samples of deep-sea sediments were collected in the Northern Hemisphere. Counts were performed for two more shallow water (75 m and 140 m), and two subsurface sediment samples (0.1 – 1 m), and two samples from polymetallic nodules (Table S2). From the total and deep-sea seafloor area, we subtracted the global average area of oceanic ridges (length of ~80 000 km; width of ~20 km) of 1.6×10^6 km^2 (The Editors of Encyclopædia Britannica; 2015[1]).

Assembling a reference set of JTB255 16S rRNA gene sequences

We included in the reference set of JTB255 189 16S rRNA gene sequences, of which 188 were between 1,049 and 1,879 bp length. One sequence (AS2) was short (537 bp) but was kept as part of the reference data set because it originates from a single cell genome (Table S4). The assignment of the reference sequence set to the monophyletic JTB255 clade within the *Gammaproteobacteria* was confirmed using phylogenetic inference methods in an IQ Tree implemented maximum-likelihood phylogenetic analysis (described in detail in methods section "Phylogenetic analysis").

Extracting JTB255 16S rRNA gene sequences from amplicon data

Potential JTB255 sequences were identified in our environmental 16S tag sequence datasets (described in methods section "Nucleic acid extraction, amplicon sequencing, and minimum entropy decomposition") using the sequences in our curated reference set to build a nucleotide BLAST database, generated using BLAST version 2.2.31+. With this database, we ran BLASTN against the 16S rRNA gene amplicon sequences (V3V4 re-

[1]https://www.britannica.com/science/oceanic-ridge, 21.04.2017, 17 o`clock.

gion) from diverse marine environments (Table S5) with an E-value cutoff of < $1e^{-10}$ and retained only the top two hits. Hits were successively filtered by retaining only sequences with a minimum pairwise sequence identity of > 95% towards the reference set, an alignment length > 400 bp, a query starting position > 15 and a query end < 413. All sequences that passed the identity, length and query start/end thresholds were used as input for MED (Eren et al., 2013).

Screening for microdiversity in the JTB255

The potential JTB255 sequences (165,436 sequences), recovered with BLASTN from our 35 environmental samples (Table S5), were analyzed for subtle variable nucleotide positions using MED v2.1 (Eren et al., 2013). MED requires a block of sequences with even lengths as input. Therefore, prior to MED, all sequences were normalized in their length by gap padding. On average, 43 to 45 gaps were added per sequence. Subsequently, MED was run using the following non-default values (Eren et al., 2015): a minimum substantive abundance of an oligotype (block size: -M) of 50, and a maximum number of discriminants to use for decomposition (-d). We also performed entropy normalization heuristics and outlier replacement (-R). A tabular report on the MED results can be found in Table S6.

Analysis of microdiversity

The relative diversity of the oligotypes originating from the analyzed JTB255-sequences from the different environments was quantified through inter- and extrapolation of Hill numbers using iNEXT (Chao et al., 2014). The difference of the JTB255 community between samples (beta-diversity) was assessed by calculating Bray-Curtis dissimilarities from the relative sequence abundance of oligotypes. Samples with less than 100 sequences were excluded from the Bray-Curtis-based dissimilarity analysis. JTB255 community dissimilarities between the different samples were visualized as a dendrogram based on complete linkage hierarchical clustering. To also visualize the differences in sequence counts associated per oligotype across samples, a heatmap was produced. As input data, 16S oligotype counts were $\log_2$ transformed, to better resolve the large number of low sequence counts in the MED matrix. All analyses were conducted in R using the core distribution of the program (version 3.3.2; R Development Core Team 2016) and the following packages: vegan version 2.4-1 (Oksanen et al., 2015), iNEXT version 2.0.12 (Hsieh et al., 2016), and gplots version 3.0.1 (Warnes et al., 2016).

Microcosm stimulation experiments

Growth stimulation experiments were conducted with sediment samples from the LTER HAUSGARTEN from 0 to 2 cm sediment depth (Table S1), using information on the predicted genes and JTB255`s potential physiological abilities. Immediately after recovery, sediments were 1:1 diluted (v:v) with sterile filtered sea-water and kept at *in situ* temperature and in darkness. In order to stimulate growth, either chitin flakes (0.5 mg carbon mL^{-1} slurry) or polyvinyl alcohol (PVA, 5 mg mL^{-1} slurry) and the cofactor pyrroloquinoline quinone (PQQ; 10^{-5} mg mL^{-1} slurry) were added to the slurry as carbon and energy sources (Sigma Aldrich, Taufkirchen, Germany). Chitin flakes as well as PVA were autoclaved before addition to the slurry. PQQ was dissolved in 1 M NaOH solution, filter sterilized and stored at 4°C. Controls consisted of chitin or PVA and PQQ in filter sterilized sea water, as well as sediment slurry with no addition. All samples were incubated in duplicates at 4°C and 12°C each for 62 days at 180 rpm and in darkness. JTB255 cell numbers were monitored every four weeks via CARD-FISH.

Isolation approaches

We approached cultivation of the JTB255 by inoculating deep-sea surface sediment from LTER HAUSGARTEN (Table S1) into two media: The 2216E medium (BD Difco, Detroit, USA) and a modified HaHa-medium (after Hahnke & Harder 2013). The 2216E medium was previously successfully used for the isolation of a closely cultivated representative of the JTB255, named *Woeseia oceani* XK5 (Du et al., 2016). The HaHa-medium was adjusted based on genomic information we gained from single cell genomics. The following modifications to the original HaHa-medium were applied: PVA was added (5 g L^{-1}) as main energy and carbon source and trace amounts of yeast extract and casamino acids (0.1 g L^{-1}) were also supplied to the medium. PQQ was given as additional trace compound (0.1 mg L^{-1}). The concentrations of the following salts NaCl, $CaCl_2 \cdot 6\ H_2O$, KCl, H_3BO_3 and $SrCl_2$ were doubled whereas NaF was excluded, in comparison to Hahnke and Harder (2013).

For each medium, we used a broth and a solid variant, by adding 12.6 g L^{-1} agarose to the broth. In the case of liquid cultivations, 1 mL of sediment was serially diluted in 9 mL of broth six times, followed by a ten times serial dilution of 1 mL in 4 mL broth down to cell extinction (e.g. 10^9 times). From a cell dilution by a factor of 10^6 or more, 0.1 mL aliquots of dilutions were spread onto either 2216E or HaHa-PVA-PQQ plates. Non-complemented slurries, as well as HaHa-PVA-PQQ and 2216E liquid media and

plates, without inoculum, served as controls. Broth and solid cultivations, and the respective controls were incubated as described for the stimulation experiments. Growth in broth cultures was analyzed through the generation of clone libraries. DNA was extracted and Sanger sequenced as described in the following section.

PCR and 16S rRNA gene clone library construction

Clone libraries from liquid cultures were constructed, in order to identify JTB255-cell enrichments. Therefore, one drop of the culture was pipetted on a glass slide and microbial presence and vitality checked via bright field microscopy (Zeiss Axio Imager M1 epifluorescence microscope, Germany). For DNA extraction, 1 mL of liquid culture was taken from the highest dilution which still showed cells and from the first tube that appeared sterile. The liquid was centrifuged at 13 000 x g for 10 min at room temperature, and the supernatant discarded. The cell pellet was resuspended in 20 µL of PCR water and cells were disrupted by freeze-thawing three times. Partial 16S rRNA genes were amplified using the primer pair 27F/907R (Hongoh and Kudo, 2003; Muyzer et al., 1995). The 25 µL reaction mix contained 10 to 50 ng DNA as template, 0.5 µmol L^{-1} of each primer, 0.2 mmol L^{-1} of each dNTP nucleotide, 10 x taq buffer, and 1.25 U Taq DNA polymerase (Peqlab, Erlangen, Germany). PCRs were performed with 30 cycles. After 4 min of initial denaturation at 96°C, each cycle consisted of 1 min at 96°C short denaturation, 1 min at 55°C annealing, and 3 min at 72°C elongation. The amplicons were purified using a PCR purification kit (QIAGEN, Hilden, Germany), ligated into the pGEM-T easy vector (Promega, Mannheim, Germany) and cloned using the One Shot ® TOP10 Chemically Competent *E. coli* cells according to the manufacturer's instructions (Invitrogen, Karlsruhe, Germany). Through blue-white selection, successfully transformed cells were picked and grown in liquid medium, followed by a PCR-based insert-screening of the grown clones using the vector primer pair M13F/M13R. The liquid cultures having clones with the correct insert size were subsequently sequenced. The sequence reaction was performed in a 5 µL reaction, using the BigDye® Terminator v3.1 Cycle Sequencing Kit (ThermoFisher, Life Technologies GmbH, Darmstadt, Germany) together with the 907R primer as the only modification of the kits` manual. After the initial denaturation at 96°C for 20 sec, 59 cycles were run on the DNA template including 10 sec at 96°C of short denaturation, 5 sec at 55°C for annealing and 4 min at 60°C for the elongation. Sanger sequencing was conducted on Sephadex-purified sequencing reaction products. In total, 304 clones were sequenced.

Results and Discussion

Phylogeny of JTB255

Placement of five sequences possessing a similar 16 nucleotide insertion in the cluster 1 remain to be validated once a more comprehensive dataset of JTB255 16S rRNA gene sequences is available. The well-supported co-clustering of the clusters 3 and 4 was not always retrieved and therefore requires further evaluation.

Global abundance and distribution of Gammaproteobacteria in deep-sea sediments

Cell numbers of *Gammaproteobacteria* ranged between 1.4 x 10^6 cells cm^{-3} sediment in Central Arctic Ocean sediments (AO.3) to 1.7 x 10^8 cells cm^{-3} in the South Atlantic (SA.1a) (Figure 3, Figure 4A, Table S2), corresponding to < 1% to 19% of total cell counts. This is a broad range, but comparable to what has been reported for coastal sediments (Dyksma et al., 2016). On a global scale *Gammaproteobacteria* were estimated to constitute 1.2 ± 0.6 x 10^{27} cells in deep-sea surface sediments (top 10 cm). This estimate is likely still an underestimate and may need to be revised in the future using improved CARD-FISH probes. Details on applied calculations are given within the methods section of the manuscript and the Supplementary Information.

Cell sizes of deep-sea surface sediment JTB255

JTB255 cell morphology varied between spherical, rod, and comma shaped (Figure 4B). Spherical JTB255 cells constituted for approximately 40% of all counted JTB255 cells and had a relative biovolume of 0.07 μm^3 per cell (diameter [r] = 0.25µm). Rod-shaped JTB255 cells constituted for approximately 40% of all counted JTB255 cells and had a relative biovolume of 0.18 μm^3 per cell (r = 0.2 µm, height = 1.2 µm), and comma-shaped JTB255-cells of approximately 20% abundance, had a biovolume of 0.13 μm^3 (r = 0.2 µm, height = 0.8 µm).

Microdiversity within the JTB255 using minimum entropy decomposition

We ran MED on a total of 165,436 sequences extracted from initially 2,684,975, found to be highly similar to a manually curated set of JTB255 sequences (see Method section within the Supplementary Information) from 35 samples of five surveyed sites (Table S1 and S5). After quality filtering done by MED (see Method section within the Supplementary Information), 149,952 JTB255 sequences with an average read length of 427 bp were obtained. A total of 288 oligotypes were identified (Table S5; Figure S4),

covering the effective number (Chao et al., 2014) of the typical (based on the exponential of Shannon entropy) as well as the most abundant (based on inverse Simpson concentration) JTB255 oligotypes at most surveyed sites based on rarefaction (Figure S3).

We observed JTB255 oligotypes in samples of deep-sea water and deep subsurface sediment, which supports that the absolute abundance of JTB255 in the marine realm is higher than described in the results and discussion section of the main manuscript, as JTB255 are not only present in marine surface sediments but also in the deep subsurface and the water column. Especially the finding that diverse subgroups of the JTB255 exist in the water column was not reported before. However, samples deriving from the deep subsurface and from the deep-sea water column had a very low relative sequence and oligotype abundance of the JTB255 (number of sequences (nSeqs) = 0 to 38, number of oligotypes (nOligotypes) = 0 to 10 for deep subsurface sediments and nSeqs = 128 to 653, nOligotypes = 18 to 104 for water column samples). Both environments appeared to be undersampled, based on species richness, Shannon entropy and inverse Simpson (Figure S3). Also, no cell numbers could be obtained and we probably reached the detection limit for CARD-FISH counting which is at about 10^3 cells mL^{-1} (Kirschner et al., 2012; Wagner et al., 2003).

JTB255 communities in the analyzed coastal sediments were less complex, represented by fewer oligotypes and lower sequence abundances (nOligotypes 12 to 92 derived from 59 to 3,146 sequences) compared to deep-sea surface sediments (Figure 5B). Whether this observation points towards a different JTB255 community in coastal sediments, possibly encoding different functions, remains unknown especially as we are unaware whether the 16S rRNA gene offers enough resolution power (Fox et al., 1992; Hoefman et al., 2014; Jaspers and Overmann, 2004), and microdiversity should rather be assessed with other marker genes (Lerat et al., 2003; Mende et al., 2013; Yilmaz et al., 2011).

Comparative genomics of JTB255 bacteria in deep-sea surface sediments

We hypothesized that firm attachment of JTB255 bacteria to sediment particles/grains might explain their exclusion during the cell-sorting procedure, however JTB255 bacteria, efficiently/successfully labeled using CARD-FISH in non-sonicated sediment samples, were microscopically not observed attached to surfaces. Furthermore, JTB255 cell counts in sonicated and not-sonicated sediments resulted in comparable numbers (Figure S5). Based on the observation that an additional lysis step was necessary to hybrid-

ize CARD-FISH probes to JTB255 cells, a more stringent lysis protocol including a higher lysis temperature was applied in a second attempt at recovering DNA from sorted cells, however without success.

B02, bin1_HGIV and bin2_HGIV were not assigned to a 16S rRNA gene sequence cluster since none of those partial genomes includes a 16S rRNA gene. A previously published single cell genome (1868_B) obtained from coastal sediment (Mußmann et al., 2017) was in contrast placed in JTB255 cluster 3 (Figure 1) and shares only 92% 16S rRNA gene identity with both *W. oceani* XK5 and M06. Two additional metagenome bins (referred to as 20_j1 and WOR_SG8_31) were previously assigned to JTB255 (Mußmann et al., 2017) and those were obtained from coastal and estuarine sediments, respectively. Although 20_j1 encodes only short 16S rRNA gene fragments (≤ 207 bp), it shares 99.7% whole-genome average nucleotide identity (ANI) with 1868_B (Mußmann et al., 2017) indicating that 20_j1 is also a representative of JTB255 cluster 3. The metagenome bin WOR_SG8_31 encodes a longer 16S rRNA gene fragment (537 bp), which shares no more than 84% identity with the 16S rRNA gene of *W. oceani* XK5 and M06 and 92% with that of 1868_B.

A homolog of a dissimilatory copper-dependent nitrite reductase (NirK), an enzyme of the denitrification pathway, was detected in the partial genome of M06. Genomes obtained from JTB255 bacteria in coastal sediments (Du et al., 2016; Mußmann et al., 2017) encoded a truncated denitrification pathway to nitrous oxide and the ability of *W. oceani* XK5 to grow as a facultative anaerobe using both oxygen and nitrate as terminal electron acceptors was confirmed. Furthermore, the sister sequence-cluster of JTB255, identified through phylogenetic analysis also comprises mainly facultative anaerobe nitrate reducers (e.g. Nogi et al. 2014). Considering the oxic seafloor conditions at the HGIV site and the absence of genes encoding nitrate and nitric oxide reductases, the denitrifying potential of deep-sea sediment JTB255 remains to be clarified.

SAG M06 encoded a polyvinyl alcohol dehydrogenase (PVA-DH), associated to polyvinyl alcohol-like compound (PVA) degradation (Nogi et al., 2014; Shimao et al., 1986). PVA-DH from the deep-sea SAG M06 might not be used to degrade PVA but structurally related, polymeric organic molecules, but the natural analogues of PVA are not known.

Growth stimulation experiments

Experiments aimed at stimulating the growth of JTB255 bacteria in deep-sea sediments in order to gain further insights into their physiology. Both defined (i.e. chitin, PVA) and complex (i.e. four different Arctic algae) sources of biological polymers were used to stimulate growth of JTB255 bacteria in sediment slurries (Table S12), based on their predicted ability to catabolize proteins, few polysaccharides, and specifically polyvinyl alcohol or respective structural analogues. Sources of biological polymers were used at concentrations providing 57.2 mg of carbon L^{-1} of sediment slurry. In addition, liquid and solid media were prepared with on average 200 mg carbon L^{-1} medium, and inoculated with 1 mL of 1:1 (v:v) sediment:seawater slurry. Arctic deep-sea sediments were used for the incubations, which were conducted for 23 and up to 133 days at three different temperatures (0, 4 and 12°C) and hydrostatic pressures (1, 250 and 550 atm) (Table S12). None of the incubations led to an increase in JTB255 cell abundances after the different incubation periods, as determined by CARD-FISH counts and Sanger sequencing of the 16S rRNA gene (Table S12), suggesting that JTB255 bacteria did not respond to the substrate inputs under the given conditions. However, total microbial cell counts and thereof *Gammaproteobacteria* increased in cell abundance, through food input from on average $1.3 \pm 0.2 \times 10^9$ and $3.9 \pm 0.3 \times 10^7$ to up to $2.0 \pm 0.2 \times 10^9$ and $3.2 \pm 0.1 \times 10^8$ cells mL^{-1} sediment, respectively (see Table S12 for more details).

The apparent lack of stimulation of the JTB255 bacteria might have resulted from a combination of several factors: (i) Substrates might have been used at inadequate concentrations. The deep-sea sediment samples originate from an Arctic site at 2,500 m water depth (Table S1), which receives low inputs of nutrients from the water column (Bauerfeind et al., 2009). The average input of particulate organic matter (POM) is 1 mg C mL^{-1} yr^{-1} at 200 to 300 m water depth, which is up to 100 times lower than POM fluxes in coastal and shelf areas (Canfield, 1993; Jørgensen, 1982; Wollast, 1991). The POM flux to the sediment is dominated by fecal pellets, calcium carbonate, refractory particulate organic carbon, and biogenic particulate silica (Bauerfeind et al., 2009; Lalande et al., 2013). This observation suggests that members of the JTB255 clade in those sediments are adapted to low nutrient concentrations. *W. oceani* XK5, a potential cultivated relative of JTB255, does grow on an agar medium containing 5 g L^{-1} peptone and 1 g L^{-1} yeast extract, indicating that at least some members of the JTB255 clade can grow in rich media. There is, however, no evidence indicating that growth conditions of *W. oceani* XK5 apply to the entire JTB255-clade and physiological variability among

members of this lineage remains so far unclear. (ii) Sources of biological polymers used for the incubations are inadequate to stimulate JTB255 bacteria from this site, which might require different polymers or prefer entirely different substrates. The abundance of JTB255 bacteria appears to remain relatively stable over long (i.e. 113 days) incubation periods in sediment slurries (Table S12; based on CARD-FISH counts) but also *in situ* (same Arctic site) when artificially deprived of nutrient input from the water column for an extended (365 days) period of time (Jacob et al., pers. communication), based on relative sequence abundances, suggesting that JTB255 bacteria might use nutrient sources endogenously produced in HGIV sediments. Based on the obtained genomic results, which led to the hypothezise that JTB255 are able to degrade aged detrital organic matter, proteinaceous refractory matter, and cells walls would be substrates that should be tested in future cultivation studies. (iii) The type and concentration of substrates were adequate to stimulate JTB255 bacteria in/from the sediments used in this study, but other factors (e.g. other macro or micronutrients, predators) limit the growth of JTB255 bacteria in the sediment slurries, and in the liquid and solid media.

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Supplementary Figures

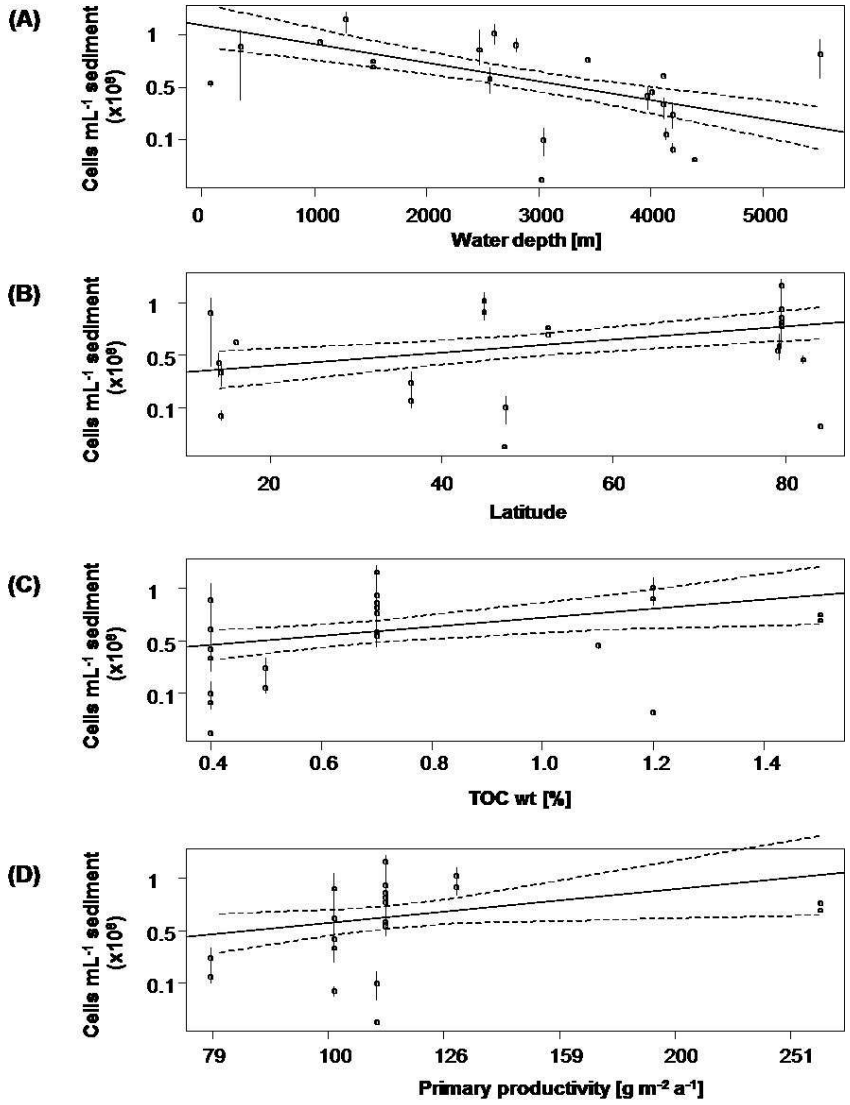

Figure S1. **Relationship between cell numbers and (A) sediment depth, (B) latitide, (C) total organic carbon (TOC), (D) primary productivity (log_{10}-transformed).** Points represent mean counts. Error bars represent range between minimum and maximum JTB255 counts.Trendline based on linear regression and 95% confidence interval; see Table S7.

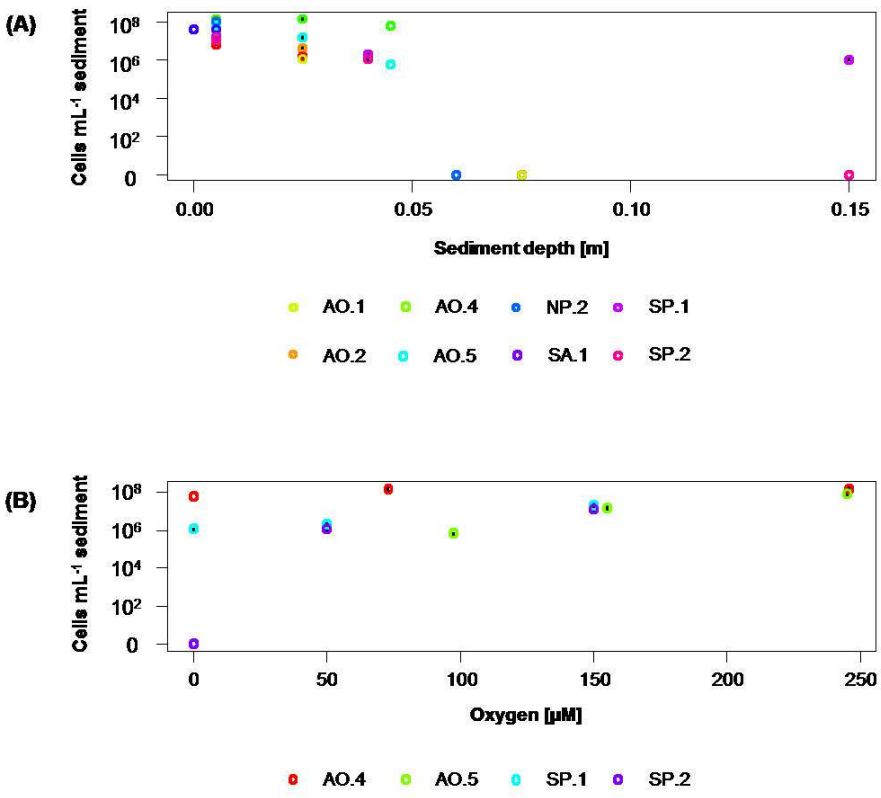

Figure S2. **Relationship between JTB255 cell numbers and (A) sediment depth or (B) oxygen concentration within the sediment.** Points represent mean counts and error bars represent the range between minimum and maximum JTB255 cell counts. Colors correspond to sampling sites.

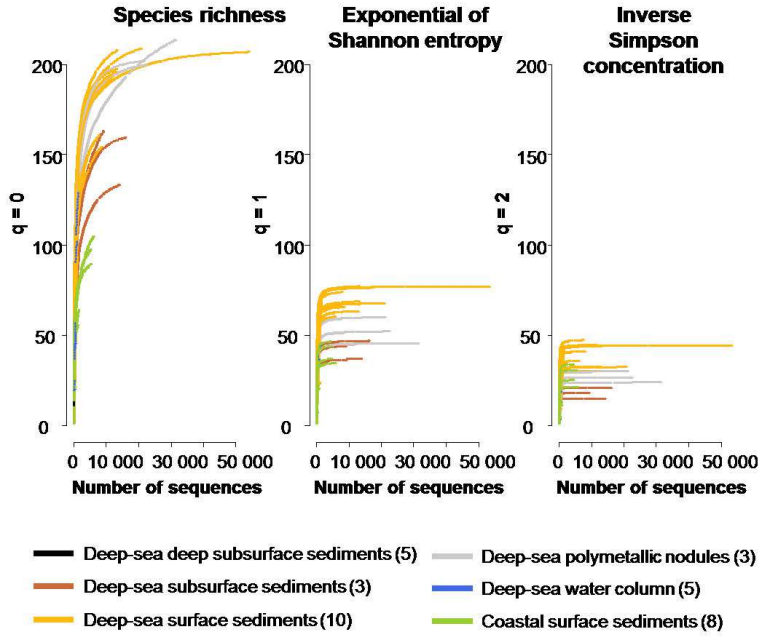

Figure S3. **Hill numbers of 16S rRNA-gene tag sequences based on the iNEXT package used for MED analysis.** Numbers in parentheses indicate the number of samples per environment.

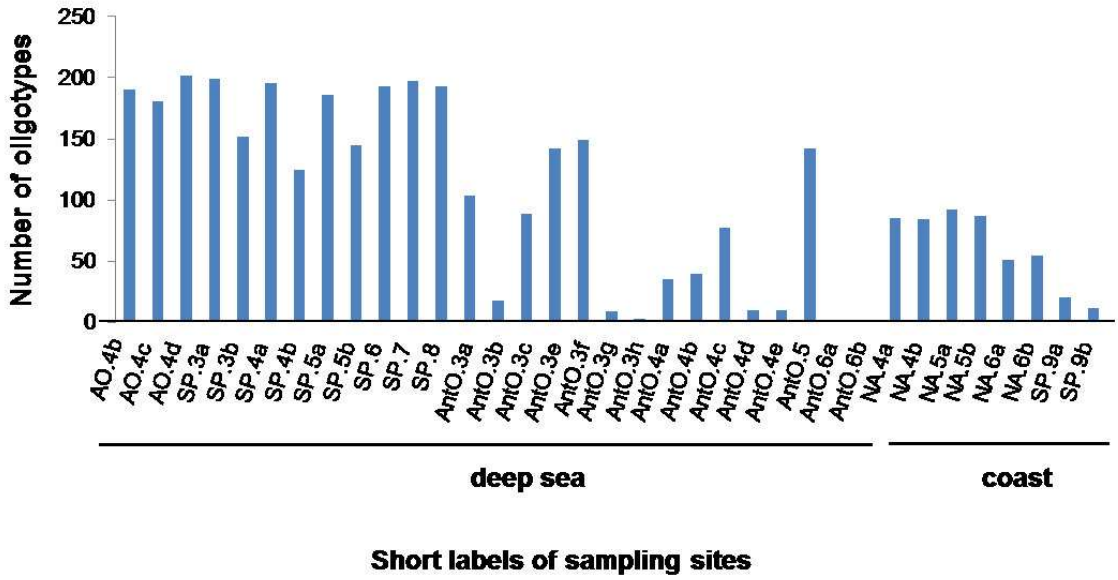

Figure S4. **Histogramm displaying the number of oligotypes identified at each sampling site.** More details and sampling site abbreviations are listed in Table S1 and S5.

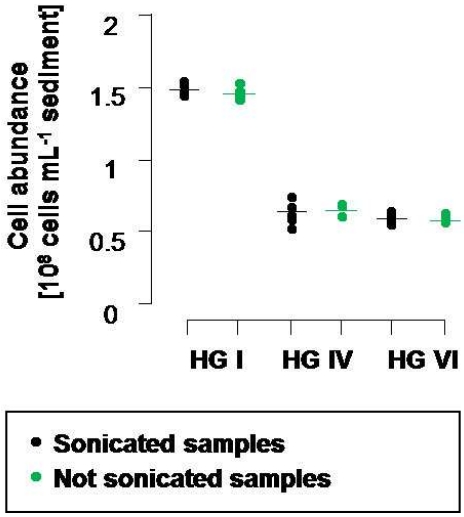

Figure S5. **Differences in cell abundance of JTB255 bacteria based on CARD-FISH counts of sonicated (black dots) or not sonicated (green dots) sediments at three different sampling sites (HGI, IV and VI).** The horizontal black lines indicate the mean value per treatment and station (n = 5).

Supplementary Tables

Table S1. **Overview of sampling sites, site characteristics, and perfomred experiments within this study.** The short label was chosen based on sample origin. Small letters within the short label indicate samples obtained from the same sampling site with distinct features. MUC: Multiple Corer; PC: Push Corer; GC: Gravity Corer; Explanation of short labels: AO: Arctic Ocean; NA: North Atlantic (Logatchev hydrothermal vent field); NP: North Pacific; IO: Indian Ocean; SA: South Atlantic; SP: South Pacific; AntO: Antarctic Ocean.

Short label	Year of sampling	PANGAEA ID	Sampling gear	Longitude	Latitude	Water depth [m]	Sediment depth [m]	Environment	Method used	Reference	chl.*a* [µg mL^{-1}]	TOC [µg C mg^{-1}]	oxygen concentration [µM]
Deep sea													
AO.1a§‡	2012	PS80.3/350-1	MUC	61°10.2'E	87°56.0'N	4380	0.00 - 0.01	surface sediment	CARD-FISH	this study	0.08	2.79	N/A
AO.1b§	2012	PS80.3/350-1	MUC	61°10.2'E	87°56.0'N	4380	0.01 - 0.05	surface sediment	CARD-FISH	this study	0.02	5.32	N/A
AO.1c§	2012	PS80.3/350-1	MUC	61°10.2'E	87°56.0'N	4380	0.05 - 0.10	surface sediment	CARD-FISH	this study	0.01	N/A	N/A
AO.1d‡	2012	PS80.3/350-1	MUC	61°10.2'E	87°56.0'N	4380	0.00 - 0.01	surface sediment	CARD-FISH	this study	0.08	N/A	N/A
AO.2a§	2012	PS80.3/361-1	MUC	68°37.7'E	88°49.6'N	4373	0.00 - 0.01	surface sediment	CARD-FISH	this study	0.12	5.55	N/A
AO.2b§	2012	PS80.3/361-1	MUC	68°37.7'E	88°49.6'N	4373	0.01 - 0.05	surface sediment	CARD-FISH	this study	0.02	6.61	N/A
AO2c§	2012	PS80.3/361-1	MUC	68°37.7'E	88°49.6'N	4373	0.05 - 0.10	surface sediment	CARD-FISH	this study	N/A	N/A	N/A
AO.3	2012	PS80.3/225-1	MUC	31°14.7'E	84° 02.0'N	4012	0.00 - 0.01	surface sediment	CARD-FISH	this study	N/A	N/A	N/A
AO.4a§	2014	PS85.2/470-3	MUC	06°05.6'E	79°08.0'N	1282	0.00 - 0.01	surface sediment	CARD-FISH	this study	N/A	N/A	197 - 295
AO.4b§	2014	PS85.2/470-3	MUC	06°05.6'E	79°08.0'N	1282	0.02 - 0.03	surface sediment	CARD-FISH	this study	N/A	N/A	20 - 126
AO.4c§	2014	PS85.2/470-3	MUC	06°05.6'E	79°08.0'N	1282	0.04 - 0.05	surface sediment	CARD-FISH	this study	N/A	N/A	0
AO.5a§¶	2014	PS85.2/460-4	MUC	04°10.9'E	79°03.9'N	2403	0.00 - 0.01	surface sediment	CARD-FISH; SCG; Metagenomics	this study	1.33	7.45	210 - 280
AO.5b§¶	2014	PS85.2/460-4	MUC	04°10.9'E	79°03.9'N	2403	0.02 - 0.03	surface sediment	CARD-FISH	this study	0.84	7.09	140 - 170
AO.5c§¶	2014	PS85.2/460-4	MUC	04°10.9'E	79°03.9'N	2403	0.04 - 0.05	surface sediment	CARD-FISH	this study	0.9	7.06	85 - 110
AO.5d¶▪	2015	PS93.2/050-5	MUC	04°10.8'E	79°03.9'N	2465	0.00 - 0.02	surface sediment	MED	this study	1.91	6.64	N/A

Table S1. (continued)

Short label	Year of sampling	PANGAEA ID	Sampling gear	Longitude	Latitude	Water depth [m]	Sediment depth [m]	Environment	Method used	Reference	chl.*a* [µg mL^{-1}]	TOC [µg C mg^{-1}]	oxygen concent-ration [µM]
AO.5e¶▪	2015	PS93.2/050-6	MUC	04°10.8'E	79°03.9'N	2465	0.00 - 0.02	surface sediment	MED	this study	1.91	6.61	N/A
AO.5f¶▪	2015	PS93.2/050-6	MUC	04°10.8'E	79°03.9'N	2465	0.00 - 0.02	surface sediment	MED	this study	1.91	6.64	N/A
AO.5g¶	2016	PS99/060-3	MUC	04°05.4'E	79°04.8'N	2467	0.00 - 0.02	surface sediment	Cultivation	this study	N/A	N/A	N/A
AO.6	2014	PS85.2/464-1	MUC	03°35.9'E	79°02.8'N	3442	0.00 - 0.01	surface sediment	CARD-FISH	this study	N/A	N/A	N/A
AO.7a§	2014	PS85.2/465-4	MUC	02°45.6'E	79°08.6'N	5525	0.00 - 0.01	surface sediment	CARD-FISH	this study	2.40	13.49	N/A
AO.7b§	2014	PS85.2/465-4	MUC	02°45.6'E	79°08.'N	5525	0.02 - 0.03	surface sediment	CARD-FISH	this study	2.51	13.67	N/A
AO.7c§	2014	PS85.2/465-4	MUC	02°45.6'E	79°08.6'N	5525	0.04 - 0.05	surface sediment	CARD-FISH	this study	0.43	11.35	N/A
AO.8	2014	PS85.2/436-1	MUC	05°17.4'W	78°58.4'N	1056	0.00 - 0.01	surface sediment	CARD-FISH	this study	N/A	N/A	214 - 270
AO.9	2014	PS85.2/454-3	MUC	02°48.9'W	78°30.4'N	2558	0.00 - 0.01	surface sediment	CARD-FISH	this study	N/A	N/A	176 - 281
NA.1	2007	MSM04.3/251	PC	45°00.0'W	14°40.0'N	3021	0.00 - 0.01	surface sediment	CARD-FISH	Schauer et al., 2011	N/A	N/A	N/A
NA.2	2007	MSM04.3/259	PC	44°58.8'W	14°45.1'N	3032	0.00 - 0.01	surface sediment	CARD-FISH	Schauer et al., 2011	N/A	N/A	anoxic; SRR
NA.3	2007	MSM04.3/271	PC	44°58.7'W	14°45.2'N	3043	0.00 - 0.01	surface sediment	CARD-FISH	Schauer et al., 2011	N/A	N/A	N/A
NP.1	2014	AT26.23/12	PC	104°14'W	9°53'N	2800	0.00 - 0.01	surface sediment	CARD-FISH	Dyksma, unpublished data	N/A	N/A	N/A
NP.2a§	2014	AT26.23/05	PC	104°16'W	9°54'N	2600	0.00 - 0.01	surface sediment	CARD-FISH	Dyksma, unpublished data	N/A	N/A	N/A
NP.2b§	2014	AT26.23/05	PC	104°16'W	9°54'N	2600	0.1 - 0.12	subsurface sediment	CARD-FISH	Dyksma, unpublished data	N/A	N/A	N/A
IO.1	2007	ME74.2/962-1	MUC	63°10.0'E	24°42.0'N	1526	0.00 - 0.02	surface sediment	CARD-FISH	Sokoll et al., 2012	N/A	N/A	N/A
IO.2	2007	ME74.2/962-2	MUC	63°10.0'E	24°42.0'N	1527	0.00 - 0.02	surface sediment	CARD-FISH	Sokoll et al., 2012	N/A	N/A	N/A
SA.1a§	2009	PS73/127-7	MUC	16°00.2'W	47°59.5'S	4113	0	fluffy layer on top of sediment	CARD-FISH	Thiele et al., 2014	N/A	N/A	N/A
SA.1b§	2009	PS73/127-7	MUC	16°00.2'W	47°59.5'S	4113	0.00 - 0.01	surface sediment	CARD-FISH	Thiele et al., 2014	N/A	N/A	N/A

Table S1. (continued)

Short label	Year of sampling	PANGAEA ID	Sampling gear	Longitude	Latitude	Water depth [m]	Sediment depth [m]	Environment	Method used	Reference	chl.*a* [µg mL^{-1}]	TOC [µg C mg^{-1}]	oxygen concent-ration [µM]
SP.1a§	2015	SO242.2/147/148 /151	MUC	88°24.8'W	7°06.0'S	4198	0.00 - 0.01	surface sediment	CARD-FISH	this study	0.12	9.43	~150
SP.1b§	2015	SO242.2/147/148 /151	MUC	88°24.8'W	7°06.0'S	4198	0.03 - 0.05	surface sediment	CARD-FISH	this study	0.04	7.27	~50
SP.1c§	2015	SO242.2/147/148 /151	MUC	88°24.8'W	7°06.0'S	4198	0.14 - 0.16	subsurface sediment	CARD-FISH	this study	0.05	6.93	0
SP.2a§	2015	SO242.2/194	MUC	88°31.6'W	7°04.6'S	4130	0.00 - 0.01	surface sediment	CARD-FISH	this study	0.08	8.91	~150
SP.2b§	2015	SO242.2/194	MUC	88°31.6'W	7°04.6'S	4130	0.03 - 0.05	surface sediment	CARD-FISH	this study	0.03	7.47	~50
SP.2c§	2015	SO242.2/194	MUC	88°31.6'W	7°04.6'S	4130	0.14 - 0.16	subsurface sediment	CARD-FISH	this study	0.03	7.1	0
SP.3b§	2015	SO242.2/146	MUC	88°27.8'W	7°04.4'S	4130	0.14 - 0.16	subsurface sediment	MED	this study	0.03	7.51	~5
SP.4a§	2015	SO242.2/146	MUC	88°27.8'W	7°04.4'S	4130	0.00 - 0.01	surface sediment	MED	this study	0.09	9.31	~150
SP.4b§	2015	SO242.2/146	MUC	88°27.8'W	7°04.4'S	4130	0.14 - 0.16	subsurface sediment	MED	this study	0.03	7.53	~5
SP.5a§	2015	SO242.2/146	MUC	88°27.8'W	7°04.4'S	4130	0.00 - 0.01	surface sediment	MED	this study	0.09	9.32	~150
SP.5b§	2015	SO242.2/146	MUC	88°27.8'W	7°04.4'S	4130	0.14 - 0.16	subsurface sediment	MED	this study	0.03	7.54	~5
SP.6a¶	2015	SO242.2/198	MUC	88°27.0'W	7°04.6'S	4197	0.00 - 0.01	polymetallic nodule	CARD-FISH	this study	N/A	N/A	N/A
SP.6b¶	2015	SO242.2/198	MUC	88°27.0'W	7°04.6'S	4197	0.00 - 0.01	polymetallic nodule	MED	this study	N/A	N/A	N/A
SP.7	2015	SO242.2/194	MUC	88°31.6'W	7°04.6'S	4130	0.00 - 0.01	polymetallic nodule	MED	this study	N/A	N/A	N/A
SP.8a¶	2015	SO242.2/198	MUC	88°27.0'W	7°04.6'S	4197	0.00 - 0.01	polymetallic nodule	CARD-FISH	this study	N/A	N/A	N/A
SP.8b¶	2015	SO242.2/198	MUC	88°27.0'W	7°04.6'S	4197	0.00 - 0.01	polymetallic nodule	MED	this study	N/A	N/A	N/A

Table S1. (continued)

Short label	Year of sampling	PANGAEA ID	Sampling gear	Longitude	Latitude	Water depth [m]	Sediment depth [m]	Environment	Method used	Reference	chl.*a* [µg mL^{-1}]	TOC [µg C mg^{-1}]	oxygen concent-ration [µM]
AntO.1	2012	PS79.3/086-28	MUC	12°02.2'E	51°59.2'S	3969	0.00 - 0.01	surface sediment	CARD-FISH	Ruff et al., 2014	N/A	N/A	N/A
AntO.2	2012	PS79.3/141-9	MUC	12°37.1'E	51°16.1'S	4114	0.00 - 0.01	surface sediment	CARD-FISH	Ruff et al., 2014	N/A	N/A	N/A
AntO.3a§	2013	PS81.8/631	CTD	13°22.2'E	52°25.4'S	2500	0.00	water column	MED	this study	N/A	N/A	N/A
AntO.3b§	2013	PS81.8/631	CTD	13°22.2'E	52°25.4'S	4130	0.00	water column	MED	this study	N/A	N/A	N/A
AntO.3c§	2013	PS81.8/631	CTD	13°22.2'E	52°25.4'S	4319	0.00	water column	MED	this study	N/A	N/A	N/A
AntO.3d¶	2013	PS81.8/606-1	MUC	13°13.6'E	51°26.6'S	4196	0.00 - 0.01	surface sediment	CARD-FISH	this study	0.34	5.09	N/A
AntO.3e§▪	2013	PS81.8/639	MUC	13°18.3'E	52°26.1'S	4375	0.00 - 0.05	surface sediment	MED	this study	0.26	3.38	N/A
AntO.3f§▪	2013	PS81.8/659	MUC	13°19.2'E	52°22.1'S	3941	0.00 - 0.05	surface sediment	MED	this study	0.33	4.14	N/A
AntO.3g§	2013	PS81.8/656	GC	13°19.0'E	52°21.9'S	3968	1.10	deep subsurface sediments	MED	this study	0.42	3.78	N/A
AntO.3h§	2013	PS81.8/656	GC	13°19.0'E	52°21.9'S	3968	4.10	deep subsurface sediments	MED	this study	0.23	4.25	N/A
AntO.4a§	2013	PS81.8/663	CTD	12°31.6'E	52°38.7'S	2501	0.00	water column	MED	this study	N/A	N/A	N/A
AntO.4b§	2013	PS81.8/663	CTD	12°31.6'E	52°38.7'S	3651	0.00	water column	MED	this study	N/A	N/A	N/A
AntO.4c§	2013	PS81.8/661	MUC	13°8.20'E	52°26.46'S	4415	0.00 - 0.05	surface sediment	MED	this study	0.55	3.6	N/A
AntO.4d§	2013	PS81.8/657	GC	13°8.11'E	52°26.45'S	4448	1.10	deep subsurface sediments	MED	this study	0.7	4.86	N/A
AntO.4e§	2013	PS81.8/657	GC	13°8.11'E	52°26.45'S	4449	4.10	deep subsurface sediments	MED	this study	0.73	4.06	N/A
AntO.5	2013	PS81.8/626	MUC	12°27.28'E	54°55.98'S	4864	0.00 - 0.05	surface sediment	MED	this study	0.03	3.99	N/A
AntO.6a§	2013	PS81.8/653	GC	14°10.83'E	52°10.22'S	3709	1.10	deep subsurface sediments	MED	this study	0.35#	2.98	N/A
AntO.6b§	2013	PS81.8/653	GC	14°10.83'E	52°10.22'S	3709	4.10	deep subsurface sediments	MED	this study	0.23	3.33	N/A

Table S1. (continued)

Short label	Year of sampling	PANGAEA ID	Sampling gear	Longitude	Latitude	Water depth [m]	Sediment depth [m]	Environment	Method used	Reference	chl.*a* [µg mL^{-1}]	TOC [µg C mg^{-1}]	oxygen concent-ration [µM]
Coast													
AO.10a§	2015	PS93.2/067-2	MUC	09°17.17'E	78°19.98'N	75	0.00 - 0.01	surface sediment	CARD-FISH	this study	N/A	N/A	N/A
AO.10b§	2015	PS93.2/067-2	MUC	09°17.17'E	78°19.98'N	75	0.02 - 0.03	surface sediment	CARD-FISH	this study	N/A	N/A	N/A
AntO.7	2012	PS79.3/177-3	MUC	52°21.48'W	53°48.90'S	140	0.00 - 0.01	surface sediment	CARD-FISH	Ruff et al., 2014	N/A	N/A	N/A
NA.4a§	2014	HE432/0011-3	MUC	07°57.46'E	54°10.43'N	19	0.00 - 0.01	surface sediment	MED	Proband et al., 2017	N/A	N/A	fully oxic
NA.4b§	2014	HE432/0011-3	MUC	07°57.46'E	54°10.43'N	19	0.01 - 0.02	surface sediment	MED	Proband et al., 2017	N/A	N/A	fully oxic
NA.5a§	2014	HE432/0005-18	MUC	04°44.97'E	55°15.51'N	42	0.00 - 0.01	surface sediment	MED	Proband et al., 2017	N/A	N/A	suboxic to anoxic
NA.5b§	2014	HE432/0005-18	MUC	04°44.97'E	55°15.51'N	42	0.01 - 0.02	surface sediment	MED	Proband et al., 2017	N/A	N/A	suboxic to anoxic
NA.6a§	2014	HE432/0001-6	MUC	04°10.11'E	55°30.05'N	21	0.00 - 0.01	surface sediment	MED	Proband et al., 2017	N/A	N/A	rather oxic
NA.6b§	2014	HE432/0001-6	MUC	04°10.11'E	55°30.05'N	21	0.01 - 0.02	surface sediment	MED	Proband et al., 2017	N/A	N/A	rather oxic
SP.9a§	2014	N/A	MUC	115°06.94'E	33°37.05'S	0	0.00 - 0.01	surface sediment	MED	Dyksma et al., 2016	N/A	N/A	N/A
SP.9b§	2014	N/A	MUC	115°06.94'E	33°37.05'S	0	0.01 - 0.02	surface sediment	MED	Dyksma et al., 2016	N/A	N/A	N/A

‡ Sample with different food conditions
§ Different depth horizons of same sampling site
¶ Different methods applied on sediments of the same sampling site
▪ Natural replicates taken at the same sampling site

Table S2. **Abundance overview.** (A) Sample information. Sampling site short labels can be found in Table S1 (B) Cell counts (minimum and maximum counts as well as % abundance based on DAPI counts) of total cells using the DNA stain DAPI, of the Gammaproteobacteria using the Gam42a CARD-FISH probe, and JTB255 group using probe mix described in Material and Methods section as well as SI methods. (C) Overview of vailable 16S rRNA amplicon sequences from the same sites of which cell counts were obtained. Displeyed is the relative 16S rRNA gene abundance. Grey samples indicate samples not obtained from surface sediments. N/A: no data available.

(A)			(B)						(C)		
			CARD-FISH based cell numbers/ abundances						Amplicon sequence abundances		
Short label	**Water depth [m]**	**Sediment depth [m]**	**Total cells [cells mL^{-1}]**	***Gammaproteobacteria* [cells mL^{-1}]**	***Gammaproteobacteria* [%][a]**	**JTB255 [cells mL^{-1}]**	**JTB255 [%][a]**	**No. of replicates**	***Gammaproteobacteria* [%]**	**JTB255 [%]**	**No. replicates**
Deep sea											
AO.1a§‡	4380	0.00 - 0.01	6.18E+08 - 6.58E+08	3.26E+06 - 3.47E+06	<1 - <1	6.42E+06 - 6.94E+06	1 - 1	2	50.16	0.98	1
AO.1b§	4380	0.01 - 0.05	9.63E+07 - 9.98E+07	0.00E+00	0	1.31E+06 - 1.73E+06	1 - 2	2	N/A	N/A	1
AO.1c§	4380	0.05 - 0.10	2.07E+07 - 2.51E+07	0.00E+00	0	0.00E+00	0	2	N/A	N/A	1
AO.1d‡	4383	0.00 - 0.01	8.07E+08 - 8.48E+08	5.03E+07 - 8.43E+07	6 - 10	4.84E+06 - 5.24E+06	< 1 - < 1	2	N/A	N/A	N/A
AO.2a§	4373	0.00 - 0.01	6.01E+08 - 6.43E+08	1.32E+07 - 1.71E+07	3 - 3	9.98E+06 - 1.32E+07	2 - 2	2	36.00	8.00	1
AO.2b§	4373	0.01 - 0.05	8.39E+07 - 8.82E+07	4.72E+05 - 5.20E+05	<1 - <1	9.83E+05 - 1.29E+06	1 - 1	2	N/A	N/A	1
AO2c§	4373	0.05 - 0.10	8.04E+07 - 8.45+E07	4.67E+05 - 5.12E+06	<1 - <1	0.00E+00	0	2	N/A	N/A	1
AO.3	4012	0.00 - 0.01	1.05E+09 - 1.09E+09	1.38E+06 - 1.91E+06	<1 - <1	2.66E+07 - 3.06E+07	3 - 3	2	23.13	10.63	1
AO.4a§	1282	0.00 - 0.01	1.34E+09 - 1.94E+09	8.39E+07 - 1.40E+08	6 - 7	1.05E+08 - 1.66E+08	8 - 9	18	39.26	14.20	1
AO.4b§	1282	0.02 - 0.03	2.25E+09 - 2.47E+09	6.78E+07 - 7.79E+07	3 - 3	1.22E+08 - 1.64E+08	5 - 7	2	N/A	N/A	N/A
AO.4c§	1282	0.04 - 0.05	2.05E+09 - 2.07E+09	3.73E+07 - 4.85E+07	2 - 2	5.83E+07 - 5.98E+07	3 - 3	2	N/A	N/A	N/A
AO.5a§	2403	0.00 - 0.01	1.29E+09 - 1.61E+09	3.24E+07 - 6.25E+07	3 - 4	5.24E+07 - 1.12E+08	4 - 7	12	34.13	15.00	1
AO.5b§	2403	0.02 - 0.03	1.21E+09 - 1.43E+09	1.03E+07 - 1.47E+07	2 - 2	9.80E+06 - 1.32E+07	< 1 - < 1	2	N/A	N/A	N/A
AO.5c§	2403	0.04 - 0.05	1.01E+09 - 1.32E+09	5.17E+06 - 5.73E+06	<1 - <1	7.01E+06 - 9.32E+06	< 1 - < 1	2	N/A	N/A	N/A
AO.6	3442	0.00 - 0.01	7.65E+08 - 7.96E+08	1.18E+07 - 2.19E+07	2 - 3	5.68E+07 - 5.98E+07	7 - 8	2	35.50	13.10	1
AO.7a§	5525	0.00 - 0.01	7.53E+08 - 1.19E+09	1.51E+07 - 3.60E+07	2 - 3	3.90E+07 - 9.17E+07	5 - 8	4	35.44	12.90	1
AO.7b§	5525	0.02 - 0.03	9.02E+08 - 9.43E+08	1.01E+07 - 1.34E+07	2 - 2	1.27E+07 - 1.62E+07	1 - 2	2	N/A	N/A	N/A
AO.7c§	5525	0.04 - 0.05	9.87E+08 - 1.21E+09	1.83E+06 - 2.33E+06	<1 - <1	2.21E+06 - 3.84E+06	< 1 - < 1	2	N/A	N/A	N/A
AO.8	1056	0.00 - 0.01	1.03E+09 - 1.06E+09	4.42E+07 - 5.46E+07	4 - 5	8.52E+07 - 8.82E+07	8 - 8	2	36.80	10.80	1
AO.9	2558	0.00 - 0.01	9.37E+08 - 1.05E+09	2.53E+07 - 5.57E+07	3 - 5	2.83E+07 - 4.89E+07	3 - 5	4	48.08	15.60	1

Table S2. (continued)

(A)			(B)						(C)		
			CARD-FISH based cell numbers/ abundances						Amplicon sequence abundances		
Short label	**Water depth [m]**	**Sediment depth [m]**	**Total cells [cells mL^{-1}]**	***Gammaproteobacteria* [cells mL^{-1}]**	***Gammaprote-obacteria* [%][a]**	**JTB255 [cells mL^{-1}]**	**JTB255 [%][a]**	**No. of replica-tes**	***Gammaprote-obacteria* [%]**	**JTB255 [%]**	**No. repli-cates**
NA.1	3021	0.00 - 0.01	6.75E+08 - 6.98E+08	8.89E+06 - 1.29E+07	1 - 2	4.07E+06 - 4.37E+06	< 1 - < 1	2	N/A	N/A	N/A
NA.2	3032	0.00 - 0.01	1.35E+09 - 2.01E+09	5.15E+06 - 7.82E+06	<1 - <1	0.00E+00	0	4	N/A	N/A	N/A
NA.3	3043	0.00 - 0.01	4.43E+08 - 5.79E+08	7.45E+06 - 1.16E+07	2 - 2	7.09E+06 1.29E+07	2 - 2	4	N/A	N/A	N/A
NP.1	2800	0.00 - 0.01	1.47E+09 - 1.53E+09	8.18E+06 - 2.19E+07	<1 - 1	6.90E+07 - 9.30E+07	5 - 6	2	N/A	N/A	N/A
NP.2a§	2600	0.00 - 0.01	2.04E+09 - 2.55E+09	1.10E+08 - 1.56E+08	5 - 6	8.24E+07 - 1.26E+08	4 - 5	8	N/A	N/A	N/A
NP.2b§	2600	0.01 - 0.12	1.03E+09 - 1.11E+09	0.00E+00	0	0.00E+00	0	2	N/A	N/A	N/A
IO.1	1526	0.00 - 0.02	1.23E+09 - 1.27E+09	6.01E+07 - 9.31E+07	5 - 7	4.91E+07 - 5.01E+07	4 - 4	2	N/A	N/A	N/A
IO.2	1527	0.00 - 0.02	1.33E+09 - 1.44E+09	7.12E+07 - 9.32E+07	5 - 6	5.47E+07 - 5.97E+07	4 - 4	2	N/A	N/A	N/A
SA.1a§	4113	fluffy layer	8.85E+08 - 8.95E+08	1.32E+08 - 1.72E+08	15 - 19	4.06E+07 - 4.16E+07	5 - 5	2	N/A	N/A	N/A
SA.1b§	4113	0.00 - 0.01	8.78E+08 - 8.81E+08	5.69E+07 - 6.79E+07	6 - 8	2.91E+07 - 4.93E+07	3 - 6	2	N/A	N/A	N/A
SP.1a§	4198	0.00 - 0.01	6.63E+08 - 8.26E+08	6.46E+06 - 8.74E+06	1 - 1	1.31E+07 - 2.18E+07	2 - 3	4	21.96 - 22.55	9.14 - 9.85	3
SP.1b§	4198	0.03 - 0.05	4.78E+08 - 5.31E+08	4.72E+06 - 5.22E+06	1 - 1	1.84E+06 - 2.22E+06	< 1 - < 1	2	N/A	N/A	1
SP.1c§	4198	0.14 - 0.16	7.01E+07 - 7.53E+07	0.00E+00	0	9.82E+05 - 1.11E+06	1 - 1	2	N/A	N/A	1
SP.2a§	4130	0.00 - 0.01	7.11E+08 - 7.43E+08	2.21E+06 - 3.36E+06	<1 - <1	1.01E+07 - 1.31E+07	1 - 2	4	17.39 - 25.63	7.27 - 10.23	3
SP.2b§	4130	0.03 - 0.05	3.75E+08 - 4.37E+08	1.37E+06 - 1.62E+06	<1 - <1	9.86E+05 - 1.22E+06	< 1 - < 1	2	N/A	N/A	1
SP.2c§	4130	0.14 - 0.16	9.88E+07 - 1.34E+08	0.00E+00	0	0.00E+00	0	2	N/A	N/A	1
SP.6	4197	nodule	4.53E+08 - 5.55E+08	4.04E+06 - 5.11E+06	<1 - 1	0.00E+00	0	2	20.44 - 22.83	9.12 - 10.00	3
SP.8	4197	nodule	4.84E+08 - 5.85E+08	0.00E+00	0	0.00E+00	0	2	N/A	N/A	N/A
AntO.1	3969	0.00 - 0.01	3.94E+08 - 4.92E+08	2.30E+07 - 3.22E+07	6 - 7	1.97E+07 - 3.28E+07	5 - 7	8	31.94 - 46.33*	5.48 - 7.52*	2
AntO.2	4114	0.00 - 0.01	3.32E+08 - 5.04E+08	8.31E+06 - 1.54E+07	3 - 3	1.62E+07 - 2.57E+07	5 - 5	8	26.67 - 26.93*	2.24 - 4.97*	2
AntO.3	4196	0.00 - 0.01	2.97E+08 - 3.32E+08	1.80E+07 - 2.37E+07	6 - 7	7.64E+06 - 9.28E+06	3 - 3	6	19.51 - 28.76	2.82 - 3.81	2

Table S2. (continued)

(A)			(B)						(C)		
			CARD-FISH based cell numbers/ abundances						Amplicon sequence abundances		
Short label	**Water depth [m]**	**Sediment depth [m]**	**Total cells [cells mL^{-1}]**	***Gammaproteobacteria* [cells mL^{-1}]**	***Gammaproteobacteria* [%][a]**	**JTB255 [cells mL^{-1}]**	**JTB255 [%][a]**	**No. of replicates**	***Gammaproteobacteria* [%]**	**JTB255 [%]**	**No. replicates**
Coast											
AO.10a§	75	0.00 - 0.01	2.43E+09 - 2.68E+09	1.07E+08 - 1.27E+08	4 - 5	3.29E+07 - 3.69E+07	1 - 1	2	N/A	N/A	N/A
AO.10b§	75	0.02 - 0.03	2.32E+09 - 2.58E+09	7.54E+07 - 8.70E+07	3 - 3	4.01E+06 - 4.57E+06	< 1 - < 1	2	N/A	N/A	N/A
AntO.7	140	0.00 - 0.01	6.05E+08 - 2.36E+09	3.73E+07 - 8.74E+07	4 - 6	2.45E+07 - 1.11E+08	4 - 5	6	23.78 - 24.49*	2.96 - 3.13*	2

‡ Sample with different food conditions; § Different depth horizons of same sampling site

[a] relative cell abundance in relation to total cells; based on CARD-FISH counts

* 16S rRNA-gene abundance based on total RNA extraction and cDNA synthesis (Ruff et al., 2014)

Table S3. **16S rRNA oligonucleotide probes and hybridization conditions used for CARD-FISH analysis in this study.** Probe position is indicated in the probe naming, i.e. JTB819a shows the probe at position 819 according to the *E. coli* gene numbering. FA: formamide concentration in the hydridization buffer; c: competitor without horseradish-peroxidase (HRP-) labeled oligonucleotide probes; small letters in the probe names (a and b) indicate when one probe is a variance of the other with one base pair difference. *Gammap*.: *Gammaproteobacteria*.

Probe	Taxonomy Target taxon	Coverage/ Hits target group [%] I-	Hits non-target groups (false-positives)	FA [%]*	Position Length (bp)	Probe sequence [5'-3']	Target Molecule	Reference
JTB819a†	*Proteobacteria* -> *Gammap.* -> most JTB255	57	uncultivated and Incertae Sedis *Gammap.*	20	819 – 836 18	5'-CCG ACA TCT AGT TCT CAT-3'	16S rRNA	this study
# JTB819b†	*Proteobacteria* -> *Gammap.*-> most JTB255	38	subsets of *Thiotrichaceae; Aquicella*, *Coxiella, Salini-spheraceae* group ZD0417	20	819 – 836 18	5'-CCA ACA TCT AGT TCT CAT-3'	16S rRNA	this study
JTB897†‡	*Proteobacteria* -> *Gammap.* -> most JTB255	86	subset of *Granulosicoccus*	20	897 – 914 18	5'-TTG AGT TTT ACC GTT GCC-3'	16S rRNA	this study
cJTB897§	competitor against subgroups of *Bacteroidetes, Spirochaetae, Firmic-utes, Cyanobacteria*	-	-	20	897 – 914 18	5'-TTG AGT TTC ACC GTT GCC-3'	16S rRNA	this study
GAM42a†‡	*Proteobacteria* -> *Gammap.,* no match on JTB255	27	-	35	1027 – 1043 17	5'- GCC TTC CCA CAT CGT TT-3'	23S rRNA	Manz et al., 1992
cBET42a§	competitor against *Betaproteobacte-ria* used in combination with the Gam42a probe	-	-	35	1027 – 1043 17	5'- GCC TTC CCA CTT CGT TT-3'	23S rRNA	Manz et al., 1992
GAM42a_T1038†‡	*Xanthomona*	27	-	35	1027 – 1043 17	5'- GCC TTT CCA CAT CGT TT-3'	23S rRNA	Siyambalapitiya and Blackall, 2004
CF319a§	most *Flavobacteria,* some *Bac-teroidetes,* some *Sphingobacteria*	38	-	35	319 – 336 18	5'- TGG TCC GTG TCT CAG TAC-3'	16S rRNA	Manz et al., 1996
JTB1270†	*Proteobacteria* -> *Gammap.*-> most JTB255	22	167/179	40	1270 – 1293 24	5'-GAG CTT TAA GGG ATT AGC GCA CCA-3'	16S rRNA	Dyksma et al., 2016
JTB1270h	helper oligonucleotide	-	-	40	1270 – 1293 24	5'-TTG CTG GTT GGC AAC CCT CTG TAT-3'	16S rRNA	Dyksma et al., 2016
JTB843†‡	*Proteobacteria* -> *Gammap.*-> most JTB255	5	41/47	10	843 – 860 18	5'-TGC GAC ACC GAG GGA CAR-3'	16S rRNA	Dyksma et al., 2016

Table S3. (continued)

Probe	Taxonomy Target taxon	Coverage/ Hits target group [%] ⊢	Hits non-target groups (false-positives)	FA [%]*	Position Length (bp)	Probe sequence [5'-3']	Target Molecule	Reference
JTB843c1§	competitor	-	-	10	843 – 860 18	5'-TTC GAC ACC GAG GGA CAR-3'	16S rRNA	Dyksma et al., 2016
JTB843c2§	competitor	-	-	10	843 – 860 18	5'-TGC GAC ACC GAG GGG CAR-3'	16S rRNA	Dyksma et al., 2016
JTB843c3§	competitor	-	-	10	843 – 860 18	5'-TGC GAC ACC GAA AGA CAR-3'	16S rRNA	Dyksma et al., 2016
JTB843c4§	competitor	-	-	10	843 – 860 18	5'-GGC GAC ACC GAG GGG CAR-3'	16S rRNA	Dyksma et al., 2016
JTB843c5§	competitor	-	-	10	843 – 860 18	5'-TGC GAC ACC GAA GGA CTR-3'	16S rRNA	Dyksma et al., 2016
JTB843us	helper oligonucleotide	-	-	10	843 – 863 21	5'-TCC CCC AAC ATC TAG TTC TCA-3'	16S rRNA	Dyksma et al., 2016
JTB843ds	helper oligonucleotide	-	-	10	843 – 863 21	5'-CGG AGA ACT TAA CGC GTT AGC-3'	16S rRNA	Dyksma et al., 2016
JTB1275†‡	*Proteobacteria* -> *Gammap.*-> most JTB255	25	187/202	10	1275 – 1292 18	5'-AGC TTT AAG GGA TTA GCG-3'	16S rRNA	Dyksma et al., 2016
JTB1275c1§	competitor	-	-	10	1275 – 1292 18	5'-AGC TTT AAG GGA TTA GCG-3'	16S rRNA	Dyksma et al., 2016
JTB1275c2§	competitor	-	-	10	1275 – 1292 18	5'-AGC TTT AAG GGA TTA GCA-3'	16S rRNA	Dyksma et al., 2016
JTB1275c3§	competitor	-	-	10	1275 – 1292 18	5'-AGC TTT AAG GGA TTA GCT-3'	16S rRNA	Dyksma et al., 2016
JTB1275c4§	competitor	-	-	10	1275 – 1292 18	5'-CGC TTT AAG GGA TTA GCT-3'	16S rRNA	Dyksma et al., 2016
JTB1275us	helper oligonucleotide	-	-	10	1275 – 1295 21	5'-TTG GCA ACC CTC TGT ACT CGC-3'	16S rRNA	Dyksma et al., 2016
JTB1275ds	helper oligonucleotide	-	-	10	1275 – 1295 21	5'-ACT CCA TAC CGG ACT ACG ACG-3'	16S rRNA	Dyksma et al., 2016

* Formamide concentration (v:v) in hybridization buffe

† Probe labelled with horseradish peroxidase (HRP)

‡ Probe was applied with competitor

CARD-FISH probe excluded from the analysis due to non-target group hits.

§ Unlabelled competitor oligonucleotide

⊢ coverage based on 755 16S rRNA-gene sequences from SILVA release 123 (Quast et al., 2013)

Table S4. **Accesion numbers of high quality sequences used for phylogenetic analysis of the structure of JTB255 and which provided the sequences for the reference database used for oligotyping.** Sequence length varied from 1,400 to 1,600 bp, with AS2 as the only exeption (sequence length of 523 bp). Environment categories used for the labelling of the sequences are: coastal sediments (CS), hydrocarbon-polluted sediments (HPS), hydrothermal vent (HV), nodules (Nod), deep-sea sediments (DSS), polar sediments (PS) and water column (WC).

Due to its size this table was not included within this thesis.

Table S5. **Minimum entropy decomposition (MED) analysis.** Total number of prokaryotic 16S rRNA gene-based tag sequences, JTB255-associated sequences and MED nodes (oligotypes) that the JTB255 sequences formed, are displayed. For details on amplicon sequencing, JTB255 sequence filtering and the generation of MED nodes, see Material and Methods section as well as supplementary method information. For a description of used short labels, see Table S1.

Short label	Sampling site	Environment	Sample description	Library size (right from the sequencer)	Library size (total sequences) quality trimmed, raw reads	Pobable JTB255 sequences after BLAST (e-10) and BLAST hit filtering	relative sequence abundance of JTB255 (%)	MED output: JTB255 sequence number	JTB255 MED nodes
AO.4b▪	Fram Strait; HAUSGARTEN	deep sea	surface sediment	63 205	39 494	7 200	18.2	6 840	190
AO.4c▪	Fram Strait; HAUSGARTEN	deep sea	surface sediment	347 613	35 727	6 417	18.0	6 088	181
AO.4d▪	Fram Strait; HAUSGARTEN	deep sea	surface sediment	191 218	150 516	28 145	18.7	27 045	202
SP.3a§	South Pacific nodule field	deep sea	surface sediment	268 953	120 540	11 471	9.5	10 694	199
SP.3b§	South Pacific nodule field	deep sea	subsurface sediment	280 176	129 174	8 585	6.6	8 305	152
SP.4a§	South Pacific nodule field	deep sea	surface sediment	188 962	80 075	7 308	9.1	6 837	196
SP.4b§	South Pacific nodule field	deep sea	subsurface sediment	285 650	129 761	7 398	5.7	7 224	125
SP.5a§	South Pacific nodule field	deep sea	surface sediment	129 720	81 057	7 002	8.6	6 658	186
SP.5b§	South Pacific nodule field	deep sea	subsurface sediment	271 925	95 284	4 940	5.2	4 750	145
SP.6	South Pacific nodule field	deep sea	polymetallic nodule	373 521	164 402	16 781	10.2	15 991	193
SP.7	South Pacific nodule field	deep sea	polymetallic nodule	249 480	114 273	11 370	9.9	10 888	197
SP.8	South Pacific nodule field	deep sea	polymetallic nodule	259 329	121 865	11 900	9.8	11 459	193
AntO.3a§	Sout West Indian Ridge	deep sea	water	128 078	115 674	787	0.7	653	104
AntO.3b§	Sout West Indian Ridge	deep sea	water	44 248	40 301	154	0.4	128	18
AntO.3c§	Sout West Indian Ridge	deep sea	water	103 980	94 542	573	0.6	500	89
AntO.3e§	Sout West Indian Ridge	deep sea	surface sediment	55 752	35 477	3 599	10.1	3 252	142
AntO.3f§	Sout West Indian Ridge	deep sea	surface sediment	59 037	37 381	4 746	12.7	4 055	149

Table S5. (continued)

Short label	Sampling site	Environment	Sample description	Library size (right from the sequenc-er)	Library size (total sequences) quality trimmed, raw reads	Pobable JTB255 sequences after BLAST (e-10) and BLAST hit filtering	relative sequence abun-dance of JTB255 (%)	MED out-put: JTB255 sequence number	JTB255 MED nodes
AntO.3g§	Sout West Indian Ridge	deep sea	deep subsurface	159 425	99 407	16	0.0	12	9
AntO.3h§	Sout West Indian Ridge	deep sea	deep subsurface	115 178	64 969	3	0.0	3	3
AntO.4a§	Sout West Indian Ridge	deep sea	water	139 092	125 032	309	0.2	256	35
AntO.4b§	Sout West Indian Ridge	deep sea	water	138 198	122 886	327	0.3	288	40
AntO.4c§	Sout West Indian Ridge	deep sea	surface sediment; seep influenced	39 162	25 807	664	2.6	613	77
AntO.4d§	Sout West Indian Ridge	deep sea	deep subsurface	92 251	59 052	54	0.1	38	10
AntO.4e§	Sout West Indian Ridge	deep sea	deep subsurface	146 723	89 397	34	0.0	31	10
AntO.5	Sout West Indian Ridge	deep sea	surface sediment; Reference site	87 522	40 667	4 651	11.4	4 413	142
AntO.6a§	Sout West Indian Ridge	deep sea	deep subsurface	169 752	105 279	0	0.0	0	0
AntO.6b§	Sout West Indian Ridge	deep sea	deep subsurface	48 417	26 209	19	0.1	19	1
NA.4a§	German Bight	coast	surface sediment	38 958	30 477	3 287	10.8	2 452	85
NA.4b§	German Bight	coast	surface sediment	48 688	37 423	3 727	10.0	2 754	84
NA.5a§	German Bight	coast	surface sediment	136 399	98 527	5 141	5.2	3 146	92
NA.5b§	German Bight	coast	surface sediment	136 745	93 261	4 399	4.7	2 633	87
NA.6a§	German Bight	coast	surface sediment	28 572	21 045	1 228	5.8	688	51
NA.6b§	German Bight	coast	surface sediment	32 334	23 528	1 574	6.7	845	55
SP.9a§	Australia	coast	surface sediment	53 601	27 819	1 356	4.9	335	20
SP.9b§	Australia	coast	surface sediment	16 684	8 647	271	3.1	59	12

§ Different depth horizons of same sampling site

▪ Natural replicates taken at the same sampling site; data published with ENA Accession Number: PRJEB17614 (Hoffmann et al., 2017) from LTER HAUSGARTEN

Table S6**. Minimum entropy decomposition (MED) output results.**

Parameter	Value
Library version	2.1
Multi-threaded	True
Mapping file	None
Quick (and dirty) analysis requested	False
Merge homopolymer splits	False
Skip removing outliers	False
Try to relocate outliers	True
Min entropy for a component to be picked for decomposition (-m)	0.0965
Perform entropy normalization heuristics	True
Max number of discriminants to use for decomposition (-d)	4
Min total abundance of oligotype in all samples	0
Min substantive abundance of an oligotype (-M)	50
Maximum variation allowed in each node (-V)	4
Number of sequences analyzed	165,436
Average read length (without gaps)	427
Number of characters in each alignment	471
Number of raw nodes (before the refinement)	288
Outliers removed due to –M	25,663
Outliers removed due to –V	6,705
Total number of outliers removed during the refinement	32,368
Relocated outliers originally removed due to -M	15,234
Relocated outliers originally removed due to –V	1,650
Total number of relocated outliers	16,884
Number of samples found	34
Number of final nodes (after the refinement)	288
Number of sequences represented after quality filtering	149,952
Final number of outliers due to –M	10,429
Final number of outliers due to -V	5,055
Final total number of outliers	15,484

Table S7. **Regression and correlation statistics for log10-transfomred JTB255 cell counts with environmental parameters.** For more details see Materials and Methods.

Parameter	R^2	F-value	[a]DF	p-value	R (Pearson)	Rho (Spearman)	Correlation
Water depth [m]	0.31	29.25	1,61	< 0.001	-0.57	-0.66	negative
Latitude	0.08	6.44	1,61	0.014	0.31	0.35	positive
[b]TOC wt [%]	0.09	7.15	1,61	0.01	0.32	0.46	positive
[c]PP [g m^{-2} yr^{-1}]	0.06	4.73	1,57	0.034	0.28	0.62	positive

[a] Values given as numerator, denominator degrees of freedom

[b] Total organic carbon (TOC); Seiter et al., 2004

[c] Calculated based on log10-transformed primary productivity (PP) values; Seiter et al., 2004

Table S8. **Relationship between JTB255 cell counts (log10-transformed) and sediment depth and oxygen concentration, using the implementation of general linear mixed models in the R-package lmerTest.**

Parameter	R^2	F-value	[a]DF	p-value	Correlation
Sediment depth [m]	0.27	20.18	1,31.59	<0.001	negative
[b]Oxygen [µM]	0.49	53.67	1,31.85	<0.001	positive

[a] Values given as numerator, denominator degrees of freedom

[b] Calculated based on log10-transformed oxygen concentrations

Table S9. **Characteristics of genome bins and single cell genomes investigated in this study**

Data types		Single cell genomes			Genome bin				Isolate	Isolate: outgroup
(Meta)genome name		**SAG M06**	**SAG B02**	**SAG 1868_B[a]**	**bin1_HGIV**	**bin2_HGIV**	**bin 20_j1[a]**	**bin JSS_woes1[a]**	***Woeseia oceani* XK5[b]**	**Steroidobacter denitrificans DSM 18526**
Data set ID				ID 2651869504 (IMG Genome)			ID 2651869885 (IMG Genome)	ID Ga0136857 (IMG Gold AP)	NZ_CP016268 (Genebank)	NZ_CP011971.1 (Genebank)
Source		This study	This study	Mußmann *et al*., 2016	This study	This study	Mußmann *et al*., 2016	Mußmann *et al*., 2016	Du *et al*., 2016	Fahrbach *et al*., 2008
Sample origin		deep-sea surface sediment, 2 500 mwd, Fram Strait, Arctic Ocean[c]	deep-sea surface sediment, 2 500 mwd, Fram Strait, Arctic Ocean[c]	coastal, marine tidal sediment Janssand, Germany[c]	deep-sea surface sediment, 2 500 mwd, Fram Strait, Arctic Ocean[c]	deep-sea surface sediment, 2 500 mwd, Fram Strait, Arctic Ocean[c]	coastal, marine tidal sediment Janssand, Germany[c]	coastal, marine tidal sediment Janssand, Germany[c]	coastal, marine sediment Xiqoshi island, China[c]	anoxic digested sludge
Overall genome information	Assembly size [bp]	1,067,518	161,171	2,277,554[a]	1,571,316	1,773,239	2,404,210[a]	8,121,065[a]	4,059,891	3,467,246
	N50 [bp]	24,372	16,243	22,940[a]	1,955	1,615	20,713[a]	17,507[a]	-	-
	No. Scaffolds	87	13	358[a]	1,048	1,310	298[a]	607[a]	1	1
	GC [%]	53.9	51.1	59.9[a]	56.0	55.9	56.5[a]	57.8[a]	57.8	61.7
	Predicted genes	1067	190	2 333[a]	2,236	2,712	2,424[a,f]	9,718[a,e,f]	3,646	3,103
	Completeness [%][d]	16.3	N/A[h]	48.3[a]	41.9	52.7	48.4[a]	(92.5)[a,g]	100	100
	tRNAs	12	0	26[a]	13	3	25[a]	54[a]	45	47
	rRNAs	5S; 16S; 23S (1x)	5S; partial 16S (1x)	5S; 16S; 23S (1x)[a]	-	-	5S; 16S; 23S[a]	5S; 16S; 23S[a]	5S; 16S; 23S (1x)	5S; 16S; 23S (1x)
	Contamination [%][d]	0.2	0.0	0.4	10.7	9.2	2.6	100	0	0
	Strain heterogeneity[d]	0.0	0.0	0	2.9	7.5	0	15.5	0	0

[a] for details see Mußmann et al., 2017

[b] for details see Du et al., 2016

[c] Geographical location and additional information of sampling sites are presented in Table S1

[d] based on CheckM analysis using y-Proteobacteria for the gene marker set

[e] IMG gene calling

[f] RAST gene calling

[g] includes a 2.2x duplication of single copy genes

[h] too small obtained partial genome to provide any type of meaningful estimate

Table S10. List of manually annotated genes encoded by HAUSGARTEN partial singel cell genomes (B02 and M06) and metagenomic bins (bin1_HGIV and bin2_HGIV) focussed on in this study. XK5: *Woeseia oceani* XK5; bin1: bin1_HGIV; bin2: bin2_HGIV; bin20: bin20_j1; binJSS: binJSS_woes1

Due to its size this table was not included within this thesis.

Table S11. **Overview of the encoding of different peptidases within JTB255 genomes.**

Clan	Family	Hits in XK5	Hits in M06	Hits in B02	Hits in bin1	Hits in bin2	Hits in SAG 1866B	Hits in bin20	Hits in binJSS	Protease types	Biological function
Aspartic Peptidases	A8	1	1				1	1	3	Endopeptidases	Signal peptidases.
	A24	1			1		1	1	3	Endopeptidases	Secretion of proteins, enzymes and toxins but also gene transfer and biofilm formation.
Cysteine Peptidases	C1	1								Endo- and exopeptidases	Component of digestive vacuoles and lysosomal system of eukaryotes. Homologs in bacteria.
	C26	2	1		2		2	1	3	Omega peptidases	Lysosomal enzyme possibly involved in folyl poly-gamma-glutamates. Homologs in bacteria.
	C39	1	1		1				2	Endopeptidases	Processing of bacteriocin.
	C82	4	1		2	2	3	5	14	Transpeptidases and peptide hydrolases	Cell wall biosynthesis (cross-linking of peptide chains and adjacent glycans).
Metallo Peptidases	M2	1					1	1	5	Exopeptidases	Processing of angiotensin. Homologs in bacteria.
	M3	2	1			1	2	2	6	Various types	Predominantly intracellular peptidases, possibly involved in degradation of oligopeptides including signal peptides.
	M4	1								Endopeptidases	Mostly secreted enzymes, role in bacterial nutrition.
	M14	2	1	1	1		2	1	1	Carboxypeptidases	Nutritional protein degradation, processing of bioactive peptides and metabolism of bacterial cell wall.
	M15	2			1			1		Carboxy- and dipeptidases	Bacterial cell wall biosynthesis and metabolism. Also includes bacteriophage endolysins.
	M16	3	3		1		2	2	14	Endopeptidases	N-terminal mitochondrial signal peptide-processing peptidases. Homologs known in bacteria.
	M17	3							6	Aminopeptidases	Breakdown of peptide products from the proteasome. Intracellular aminopeptidases in bacteria.

Table S11. (continued)

Clan	Family	Hits in XK5	Hits in M06	Hits in B02	Hits in bin1	Hits in bin2	Hits in SAG 1866B	Hits in bin20	Hits in binJSS	Protease types	Biological function
Metallo Peptidases	M19	1	2		1		2	1	3	Dipeptidases	Membrane lipoprotein in humans. Homologs known in bacteria, described i.e. for recalcitrant organic matter degradation.
	M20	10			2		3	1	24	Exopeptidases	Hydrolyze the late products of protein degradation to complete protein conversion to free amino acids. Periplasmic enzyme in *Pseudomonas* sp.
	M22	1 (+1?)			1	1	1	1	4	?	?
	M23	8	3		2	3	6	10	21	Endopeptidases	Used by some bacteria to lyse cell walls of other bacteria (defensive or feeding mechanism). *Lysobacter enzymogenes* is capable of lysing soil nematodes.
	M24	7	1		1	2	2	2	12	Exopeptidases	Removal of translation-initiating methionine.
	M28	5	1		1	2	1		9	Exopeptidases	Protein-processing and non-peptidase homologs.
	M41	1 (+1?)				1	2	1	1	Endopeptidases	Cleavage of specific membrane proteins (protein processing function).
	M42	1					1		3	Aminopeptidases	In *Lactococcus lactis*, glutamyl aminopeptidase contribute to growth in milk.
	M48	7	1			2	5	4	12	Endopeptidases	In bacteria, a function in abnormal protein degradation is known (heat-shock response).
	M49	1			1		1	1	2	Dipeptidylpeptidases	Housekeeping peptidases involved in the catabolism of intracellular peptides.
	M50	1			1		1	2	2	Endopeptidases	Function in intramembrane proteolysis. Regulatory processes.
	M55	2	1							Aminopeptidases	In *Bacillus subtilis*, a member of this family is thought to have a role in mitigating nutrient deficiency.
	M56	1								Endopeptidases	Function in antibiotic resistance (Beta-lactams).
	M61	2				2	1		4	Aminopeptidases	A glycyl aminopeptidase in *Sphingomonas capsulata* is a secreted enzyme thought to play a role in nutrition.
	M64	1							4	Endopeptidases	In *Clostridium ramosum*, the peptidase is involved in immunoglobulin degradation and thought to enable a commensal lifestyle in the human intestine.
	M75	2								Endopeptidases	?
	M78	(1?)							4	Endopeptidases	In *Bacillus subtilis*, the protein is involved in the processing of a transposon immunity protein (repressor).

Table S11. (continued)

Clan	Family	Hits in XK5	Hits in M06	Hits in B02	Hits in bin1	Hits in bin2	Hits in SAG 1866B	Hits in bin20	Hits in binJSS	Protease types	Biological function
Metallo Peptidases	M79	1	1				2	2	6	Endopeptidases	Processing of prenylated proteins
	M81	1								Peptidases	In a *Sphingomonas* sp. a member of this family cleaves microcystin toxins, which are used as carbon and nitrogen sources.
Mixed Catalytic Types	(S58)	2								Aminopeptidases	Aminopeptidase DmpA is thought to be part of the general peptidase pool of *Ochrobactrum anthropi*.
Serine Peptidases	S1	3	1	2		1	2	3	5	Endopeptidases	DegP proteins in *Escherichia coli* are perisplasmic peptidases that have a dual function as chaperone and heat-shock response proteins (degrade denatured periplasmic proteins).
	S8	3	3		1		2	1	10	Endopeptidases	In bacteria, archaea and fungi, those peptidases are secreted or membrane-bound and are generally thought to be involved in nutrition.
	S9	13	5				3	3	15	Various types	Thought to be involved in the degradation of biologically active peptides. Various cellular locations. Homologs present in bacteria.
	S10	1								Carboxypeptidases	Possible structural role in eukaryotes. Homologs present in bacteria.
	S11	1	1			1			4	Carboxypeptidases	Involved in the synthesis of bacterial cell walls.
	S13	1			1				3	Carboxypeptidases and atypical endopeptidases	Involved in the biosynthesis and turnover of the bacterial cell wall crosslinks.
	S14	1			1				1	Endopeptidases	Protein quality control and regulatory degradation
	S15	2				1			1	Dipeptidyl peptidase	In lactobacilli, it is believed to be involved in the degradation of caseins.
	S16	1			1				1	Endopeptidases	Thought to be the degradation of unfolded proteins
	S24	1			1	1	1	1	5	Autolysed protein	Regulatory functions. Some members are involved in the SOS response.
	S26	2								Endopeptidases	Signal peptidases
	S33	8	5	1	3	5	9	12	25	Exopeptidases	Generally secreted or periplasmic enzymes thought to have a role in nutrition including proline-rich substrates.
	S41	4			1	1	3	3	9	Endopeptidases	In archaea and bacteria, possible role in the turnover of cytoplasmic proteins / incorrectly folded proteins.

Table S11. (continued)

Clan	Family	Hits in XK5	Hits in M06	Hits in B02	Hits in bin1	Hits in bin2	Hits in SAG 1866B	Hits in bin20	Hits in binJSS	Protease types	Biological function
Serine Peptidases	S46						1	1	1	Dipeptidases	Probably has a nutrional role, perhaps required for the further digestion of peptides generated by proteinases.
	S49	3	1		1				5	Endopeptidases	In *E. coli*, a member is involved in the degradation of cleaved signal peptides present in the periplasm.
	S51	1				1			2	Exopeptidases	Function in nutrition (*Salmonella spp.*; cyanobacteria)
	S66	1							2	Carboxypeptidases	Bacterial cell wall metabolism and processing of bacteriocins
Threonine Peptidases	T3	4	1		3	2	4	3	11	Aminopeptidases	In *Escherichia coli*, a member is soluble in the periplasm.
Unclassified Types	-	1				1	5		3	Putative aminopeptidases	?
Number of total families		50	21	3	24	17	29	27	41		
Number of peptidases		136	37	4	32	30	74	68	276		
% of encoded genes		3.7	3.5	2.1	1.4	1.1	3.2	2.8	2.8		

Table S12. **Cultivation approaches.** (A) Isolation experiments with commercial (2216E) or self-designed broth used as liquid and solid medium. (B to D) Growth stimulation experiments of JTB255 bacteria in 3.5-fold with sea-water diluted sediment slurries. For additional information on medium composition and experimental set up, see Material and Methods section and supplementary methods information. *Gammap*.: *Gammaproteobacteria*; JTB: JTB255 marine benthic group; EHUX: *Emiliania huxleyi*; TWEI: *Thalassiosira weissflogii*; BCLA: *Bacillaria* sp.; MARC: *Melosira arctica*.

(A)

Isolation experiments		Day 30	Day 62
Solid media; 4°C	HaHa + PVA + PQQ	no growth	
	2216E medium	no growth	
Solid media; 12°C	HaHa + PVA + PQQ	no growth	
	2216E medium	no growth	
Liquid broth media; 4°C	HaHa + PVA + PQQ	no JTB growth	no JTB growth
	2216E medium	no JTB growth	no JTB JTB 255 growth
Liquid broth media; 12°C	HaHa + PVA + PQQ	no JTB growth	no JTB growth
	2216E medium	no JTB growth	no JTB growth

Table S12. (continued)

(B)

Stimulation experiments in slurry at 0°C		Day 0					Day 28					Day 133					Replicates
Pressure	Food source	Total [cells mL^{-1}]	*Gammap.* [cells mL^{-1}]	*Gammap.* [%]	JTB [cell mL^{-1}]	JTB [%]	Total [cells mL^{-1}]	*Gammap.* [cells mL^{-1}]	*Gammap.* [%]	JTB [cell mL^{-1}]	JTB [%]	Total [cells mL^{-1}]	*Gammap.* [cells mL^{-1}]	*Gammap.* [%]	JTB [cell mL^{-1}]	JTB [%]	
1 atm	unfed	1.11E+09	2.37E+07	2.10	5.39E+07	5.65	1.51E+09	7.08E+07	5.45	7.27E+07	5.66	1.58E+09	7.03E+07	4.35	4.37E+07	2.77	1
	chitin						1.62E+09	1.31E+08	13.32	9.48E+07	5.84	1.75E+09	1.32E+08	7.43	1.18E+08	6.74	1
	EHUX						1.83E+09	1.39E+08	13.99	9.58E+07	5.24	1.82E+09	1.64E+08	9.26	1.56E+08	8.53	1
	TWEI						2.32E+09	2.67E+08	27.24	6.05E+07	2.6	1.86E+09	2.78E+08	25.27	6.12E+07	3.28	1
250 atm	unfed						1.43E+09	5.83E+07	2.27	8.40E+07	5.86	1.82E+09	3.94E+07	2.46	7.22E+07	3.98	1
	chitin						2.06E+09	1.51E+08	10.32	9.74E+07	4.74	1.70E+09	4.53E+07	2.93	7.56E+07	4.45	1
	EHUX						1.82E+09	1.52E+08	11.16	1.01E+08	5.62	1.75E+09	1.42E+08	8.12	8.23E+07	4.70	1
	TWEI						2.15E+09	1.72E+08	17.32	8.90E+07	4.14	1.59E+09	1.63E+08	9.76	4.03E+07	2.53	1
550 atm	unfed						1.66E+09	2.72E+07	1.81	8.40E+07	5.07	1.20E+09	5.78E+07	4.55	2.69E+07	2.23	1
	chitin						1.55E+09	9.54E+07	6.53	9.41E+07	6.07	1.34E+09	5.01E+07	3.56	1.51E+07	1.13	1
	EHUX						1.38E+09	1.58E+07	8.47	6.05E+07	4.37	1.48E+09	8.40E+07	6.21	4.70E+07	3.17	1
	TWEI						1.59E+09	1.92E+07	22.76	2.69E+07	1.69	9.92E+08	6.52E+06	0.70	1.31E+07	1.32	1

Table S12. (continued)

(C)

Stimulation experiments in slurry at 0°C		Day 0					Day 28					Replicates
Pres-sure	Food source	Total [cells mL^{-1}]	*Gammap.* [cells mL^{-1}]	*Gam-map.* [%]	JTB [cell mL^{-1}]	JTB [%]	Total [cells mL^{-1}]	*Gammap.* [cells mL^{-1}]	*Gammap.* [%]	JTB [cell mL^{-1}]	JTB [%]	
1 atm	unfed	1.19E+09 - 1.27E+09	2.13E+07 - 2.52E+07	1.90 - 2.31	4.37E+07 - 4.51E+07	3.45 - 3.80	1.20E+09 - 1.37E+09	2.20E+07 - 2.48E+07	2.11 - 2.71	4.80E+07 - 4.80E+07	4.00 - 4.01	3
	chitin						1.08E+09 - 1.54E+09	4.71E+07 - 4.83E+07	3.63 - 3.84	1.97E+07 - 2.62E+07	1.70 - 1.88	3
	EHUX						1.34E+09 - 1.44E+09	1.53E+08 - 1.55E+08	10.39 - 10.41	2.84E+07 - 4.59E+07	1.97 - 3.43	3
	TWEI						1.05E+09 - 1.07E+09	1.24E+08 - 1.37E+08	7.10 - 7.87	2.18E+07 - 2.20E+07	2.08 - 2.11	3
	BCLA						1.17E+09 - 1.19E+09	1.49E+08 - 1.49E+08	9.54 - 9.56	1.97E+07 - 1.98E+07	1.67 - 1.78	3
	MARC						1.29E+09 - 1.31E+09	1.37E+08 - 1.39E+08	7.87 - 7.92	2.84E+07 - 2.86E+07	2.20 - 2.43	3
250 atm	unfed						1.19E+09 - 1.23E+09	4.58E+07 - 4.61E+07	4.26 - 4.38	3.93E+07 - 3.98E+07	3.31 - 3.72	3
	chitin						9.81E+08 - 9.81E+08	6.32E+07 - 6.62E+07	5.95 - 5.97	2.84E+07 - 2.84E+07	2.90 - 2.92	3
	EHUX						9.94E+08 - 1.16E+09	2.35E+08 - 2.38E+08	16.72 - 16.98	1.31E+07 - 1.86E+07	1.13 - 1.87	3
	TWEI						9.15E+08 - 9.22E+08	1.26E+08 - 1.27E+08	8.61 - 8.74	6.55E+06 - 6.55E+06	0.71 - 0.72	3
	BCLA						8.95E+08 - 8.98E+08	1.37E+08 - 1.41E+08	9.11 - 9.51	1.09E+07 - 1.11E+07	1.22 - 1.34	3
	MARC						1.00E+09 - 1.07E+09	1.31E+08 - 1.48E+08	9.00 - 9.73	1.75E+07 - 1.76E+07	1.74 - 1.81	3

Table S12. (continued)

(D)

Stimulation experiments in slurry		Day 0					Day 30					Day 62					Replicates
Temperature	Food source	Total [cells mL^{-1}]	*Gammap.* [cells mL^{-1}]	*Gammap.* [%]	JTB [cell mL^{-1}]	JTB [%]	Total [cells mL^{-1}]	*Gammap.* [cells mL^{-1}]	*Gammap.* [%]	JTB [cell mL^{-1}]	JTB [%]	Total [cells mL^{-1}]	*Gammap.* [cells mL^{-1}]	*Gammap.* [%]	JTB [cell mL^{-1}]	JTB [%]	
4°C	unfed	1.62E+09	8.60E+07	5.822	7.37E+07	4.55	1.38E+09	1.90E+08	11.86	6.28E+07	4.54	1.51E+09	3.56E+08	19.35	5.73E+07	3.79	1
	chitin flakes						1.76E+09	4.95E+08	28.05	5.87E+07	3.33	1.75E+09	2.13E+08	11.24	5.19E+07	2.97	1
	PVA + PQQ						8.65E+08	8.05E+07	8.55	5.32E+07	6.15	1.07E+09	2.14E+08	15.91	5.87E+07	5.51	1
12°C	unfed	1.62E+09	8.60E+07	5.822	7.37E+07	4.55	1.12E+09	6.55E+07	6.21	4.37E+07	3.89	1.03E+09	2.73E+07	2.77	3.55E+07	3.45	1
	chitin flakes						1.08E+09	1.47E+08	14.63	5.87E+07	5.43	1.37E+09	2.32E+07	1.68	4.78E+07	3.48	1
	PVA + PQQ						9.94E+08	7.37E+07	6.88	5.32E+07	5.36	9.17E+08	2.87E+07	2.58	2.46E+07	2.68	1

Chapter III

Response of bacterial communities to different detritus compositions in Arctic deep-sea sediments

Katy Hoffmann[1,2]*, Christiane Hassenrück[1], Verena Salman-Carvalho[1,2,]
Moritz Holtappels[3] and Christina Bienhold[1,2]

[1]HGF-MPG Joint Research Group for Deep Sea Ecology and Technology,
Max Planck Institute for Marine Microbiology, Bremen, Germany
[2]Biosciences, HGF-MPG Joint Research Group for Deep Sea Ecology and Technology,
Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Bremerhaven, Germany
[3]Biosciences, Bentho-Pelagic Processes, Alfred-Wegener-Institut Helmholtz-Zentrum
für Polar- und Meeresforschung, Bremerhaven, Germany

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Abstract

Benthic deep-sea communities are largely dependent on particle flux from surface waters. In the Arctic Ocean, environmental changes occur more rapidly than in other ocean regions, and have major effects on the export of organic matter to the deep sea. Because bacteria constitute the majority of deep-sea benthic biomass and influence global element cycles, it is important to better understand how changes in organic matter input will affect bacterial communities at the Arctic seafloor. In a multidisciplinary *ex situ* experiment, benthic bacterial deep-sea communities from the Long-Term Ecological Research Observatory HAUSGARTEN were supplemented with different types of habitat-related detritus (chitin, Arctic algae) and incubated for 23 days under *in situ* conditions. Chitin addition caused strong changes in community activity, while community structure remained similar to unfed control incubations. In contrast, the addition of phytodetritus resulted in strong changes in community composition, accompanied by increased community activity, indicating the need for adaptation in these treatments. High-throughput sequencing of the 16S rRNA gene and 16S rRNA revealed distinct taxonomic groups of potentially fast-growing, opportunistic bacteria in the different detritus treatments. Compared to the unfed control, *Colwelliaceae, Psychromonadaceae*, and *Oceanospirillaceae* increased in relative abundance in the chitin treatment, whereas *Flavobacteriaceae, Marinilabiaceae*, and *Pseudoalteromonadaceae* increased in the phytodetritus treatments. Hence, these groups may constitute indicator taxa for the different organic matter sources at this study site. In summary, differences in community structure and in the uptake and remineralization of carbon in the different treatments suggest an effect of organic matter quality on bacterial diversity as well as on carbon turnover at the seafloor, an important feedback mechanism to be considered in future climate change scenarios.

Keywords

climate change, surface sediment, food pulse experiment, opportunistic bacteria, 16S Illumina sequencing, Arctic algae.

Introduction

Deep-sea sediments below 200 m water depth cover approximately 65% of the Earth's surface. In this environment, particle flux from surface waters is the main source of energy and carbon to benthic communities, with only 1 to 5% of the exported organic material arriving at the seafloor (Klages et al., 2003; Jørgensen and Boetius, 2007). A small fraction (1 to 2%) of the arriving carbon is remineralized in surface sediments within a few days, while much of the remainder gets buried, turning the seabed into the globally most important long-term sink for carbon (Jørgensen and Boetius, 2007). Bacterial communities may constitute up to 90% of total benthic biomass in the deep sea (Rowe, 1991; Pfannkuche, 1992), serving as essential catalysts in carbon and nutrient cycling (Jørgensen and Boetius, 2007). The availability of organic matter has been identified as a major driver structuring deep-sea communities, including bacterial communities (e.g., Zinger et al., 2011; Bienhold et al., 2012, 2016). Assessing the response of bacterial communities to changing carbon input is therefore important to understand matter fluxes in the deep sea, especially in the context of global climate change.

In this regard, the Arctic Ocean plays a prominent role because of the rapid environmental changes occurring in this region. As a consequence of increasing surface water temperatures and sea-ice retreat, the composition and amount of Arctic phytoplankton and sinking organic matter is currently undergoing major changes (Michel et al., 2006; Arrigo et al., 2008; Bauerfeind et al., 2009). In extreme cases, the sudden, massive export of fresh sea-ice algae to the deep-sea floor in the Central Arctic resulted in local hypoxic conditions beneath such algal patches (Boetius et al., 2013). Despite these changes in the quality and quantity of organic matter exported to the deep-sea benthos, little is known about the effects on benthic community structure and functioning. Therefore, the major objective of this study was to evaluate the response of Arctic benthic bacterial deep-sea communities to different organic matter sources.

Methodologically, the retrieval of deep-sea sediment samples, and the large diversity of benthic bacterial communities are two major challenges in this area of research. In contrast to logistically challenging *in situ* experiments, *ex situ* studies (Boetius and Lochte, 1994; Boetius and Damm, 1998; Mayor et al., 2012) allow for comparisons between different treatments under controlled conditions, including sample replication. Previous studies have provided simple nutrient sources to investigate the response of bacterial communities to organic matter input (Jannasch et al., 1973; Jannasch and Wirsen, 1982; Deming and Colwell, 1985). Few have also used more complex food

sources, such as chitin and whole algae (Deming, 1985; Moodley et al., 2002; Kanzog et al., 2009), which represent more environmentally relevant sources of organic matter. Results indicated that bacterial communities respond to the deposition of organic matter within a few days, as recorded by increases in the activity of extracellular enzymes, bacterial biomass, and community oxygen consumption (Turley and Lochte, 1990; Witte et al., 2003b). However, only more recent studies included analyses of whole bacterial community structure, or single taxonomic groups by applying fingerprinting methods (Yanagibayashi et al., 1999; Kanzog et al., 2009), cloning, and sanger sequencing (Toffin et al., 2004; Parkes et al., 2009; Gärtner et al., 2011). Other studies that sampled along natural environmental gradients of organic matter availability have identified potential opportunistic groups, e.g., *Alteromonadaceae, Psychromonadaceae*, and *Flavobacteriaceae* which were positively related with organic matter availability (Bienhold et al., 2012; Jacob et al., 2013). But, experimental studies still lacked combined measurements of community function and high-resolution taxonomic community structure in response to the addition of natural complex food sources.

Here, we used deep-sea surface sediments from the Arctic Ocean Long-Term Ecological Research Observatory (LTER) HAUSGARTEN located in the Fram Strait (Soltwedel et al., 2015) to perform experimental carbon additions, and monitor the response of benthic bacterial communities. The particulate organic carbon flux at this well-studied site is generally less than 30 mg C m^{-2} d^{-1} (~1 mg C mL^{-1} yr^{-1}) at 200 to 300 m water depth, and is mainly composed of fecal pellets, calcium carbonate, refractory particulate organic carbon, and biogenic particulate silica (Bauerfeind et al., 2009; Lalande et al., 2013). Since 2005, strong declines in diatom numbers, such as *Thalassiosira weissflogii*, have led to shifts in phytoplankton community composition. At the same time, a dominance of coccolithophores, especially *Emiliania huxleyi*, as well as the prymnesiophyte *Phaeocystis* was observed in the sinking material (Bauerfeind et al., 2009; Soltwedel et al., 2015), but effects on benthic communities remain unknown.

In this study, deep-sea sediment slurries were amended with different types of organic matter, including four different naturally occurring Arctic algae species, and chitin as the most abundant biopolymer in the oceans, e.g., as a main component of fecal pellets. Incubations were performed at *in situ* temperature and atmospheric pressure, with parallel *in situ* pressure incubations as controls. After 23 days, changes in key community functions were assessed along with changes in bacterial taxonomic compo-

sition. In contrast to natural environmental gradients that may have persisted over extended periods of time, the present study has a short experimental duration and specifically targets fast-growing bacterial groups. We hypothesized (i) that the input of different particulate organic carbon sources to deep-sea sediments differentially affects microbial degradation rates and growth, (ii) that community structure varies with organic matter types, and (iii) that we can identify rapidly responding, i.e., fast-growing, potentially polymer-degrading bacterial groups, representing an opportunistic part of the sediment microbiome.

Materials and methods

Sediment Sampling

Surface sediments were sampled at the central station of the LTER HAUSGARTEN, located in the Fram Strait west of Spitsbergen (79° 03.86′ N, 4° 10.85′ E; 2,470 m water depth). A TV-guided multiple corer (MUC; Barnett et al., 1984) was used for retrieving undisturbed sediments during RV Polarstern cruise PS93.2 (PS93/050-5 and -6; Soltwedel, 2015). Surface sediments were fully oxic (300 µmol L^{-1} O_2) and consisted of fine clays (Kanzog et al., 2008). Immediately after sediment retrieval, a control sample 'day 0', representing the *in situ* bacterial community structure, was taken and frozen at –80°C for RNA and at –20°C for DNA extractions.

For the feeding experiment, the first two centimeters of ten cores were combined into one sterile glass bottle and transferred into a cold room (0°C, *in situ* temperature). The sediment was 3.5-fold diluted with sterile-filtered and air saturated bottom water (35 PSU; 0°C), in order to supply enough oxygen for at least one community duplication. The well-mixed slurry was divided into six sterile 1 L glass bottles. One bottle with slurry remained as unfed control. The other five bottles were amended with substrates to final concentrations of 0.2 mg organic carbon per mL undiluted sediment, which corresponds to 16.6 mmol L^{-1}organic carbon, and is comparable to an annual input of particulate organic carbon at this site (Bauerfeind et al., 2009; Kanzog and Ramette, 2009; Lalande et al., 2013). All treatments were divided into ten replicates of 50 mL each, transferred and sealed into sterile, gas-permeable polyethylene (PE) bags with no headspace or air bubbles.

Incubation

Slurry incubations were started within 24 h after retrieval of the samples, using pressure vessels described in Boetius and Lochte (1994). Ten pressure vessels were filled with fully oxygenated sterile-filtered bottom water, and six PE bags were added to each of them (one replicate bag of each of the five treatments, and one control bag). Five of these pressure vessels were incubated at atmospheric pressure (1 atm), the other five at *in situ* pressure (250 atm). Pressure was applied as hydrostatic (water) pressure, using a mechanical pump. The PE bag material was oxygen permeable, so that oxygen diffusion into the bags prevented the slurries from becoming anoxic (Supplementary Table S1; Supplementary Data). All incubators were stored at 0°C in the dark for 23 days.

Food Sources Used in this Study

We selected five different, naturally occurring, complex substrates to test functional and structural responses of Arctic deep-sea bacterial communities. These were: chitin (CHI), *Thalassiosira weissflogii* (TWEI), *Emiliania huxleyi* (EHUX), *Bacillaria sp*. (BCLA), and *Melosira arctica* (MARC).

Chitin is one of the main components of, e.g., fecal pellets, exoskeletons of worms, arthropods, and mollusks, and thus constitutes a key source of carbon and nitrogen to deep-sea life (Wakeham and Lee, 1993). Here, we used a commercially available product (flakes from shrimp shells, Sigma–Aldrich, Germany). As more complex detritus we added different algae: the centric diatom TWEI, representing a major fraction of sinking particulate organic matter at HAUSGARTEN observatory, as well as the coccolithopore EHUX that has been reported to migrate from the Atlantic towards the Arctic (Bauerfeind et al., 2009; Winter et al., 2014). Furthermore, we provided detritus of the sea-ice algae BCLA (Stecher et al., 2015), a pennate diatom, and MARC, a centric diatom. As a consequence of sea-ice melting, these sea-ice algae can cause massive sedimentation events that strongly impact local sediment turnover rates (McMahon et al., 2006; Boetius et al., 2013). In reference to the incubations, the term phytodetritus includes all algae used (EHUX, TWEI, BCLA and MARC), of which EHUX and TWEI belong to phytoplankton, and BCLA and MARC to the sea-ice algae.

Algal Cultivation, Sterilization, and Tests Prior to Usage

TWEI, EHUX, and BCLA were collected during several Arctic cruises, and were cultivated in F/2-medium (Guillard and Ryther, 1962; Guillard, 1975). Algal mats dominated by MARC (but also containing few other species of pennate diatoms and *Chaetoceros* sp.) were collected directly from the ice during Polarstern cruise PS86 in July 2014 (Boetius, 2015) and frozen at –20°C.

The phytoplankton species (TWEI and EHUX) were incubated in 2.5 L F/2 medium at 18°C and 15 µE using 5 L beakers, and were resuspended twice a week by gentle shaking. TWEI and EHUX were transferred into new media every 10 days. 100 mL of the 2.5 L culture were used to inoculate fresh medium, while the remainder was centrifuged at 1811 × *g*, and the pellet was frozen at –20°C. BCLA was grown at 0°C and 10 µE in 400 mL medium in a 1 L beaker. Due to slower growth, it was transferred and harvested once a month as described above.

For inactivating algal-attached bacteria, we sterilized the algae with microwave radiation at 600 W for three minutes before adding them to the slurry. Sterilization may alter organic matter lability, as has been shown for the process of autoclaving (Turley and Lochte, 1990; Wolf and Skipper, 1994). This may, to some extent, cause changes similar to the natural aging of organic matter, but was not quantified in more detail here. This treatment also led to a disintegration and homogenization of the algae cultures. The effective sterilization was tested by measurements of enzyme activity, oxygen uptake, bacterial cell numbers, and bacterial genetic diversity, which were all negative or without significant increase or change. The carbon and nitrogen content of the different food sources were determined according to Grasshoff et al. (1983), using a Fisons element analyzer type NA 1500 (Fisons plc, UK). C:N ratios were 8:1 for CHI, 10:1 for TWEI, 11:1 for EHUX, 12:1 for BCLA and 23:1 for MARC.

Acridine Orange Direct Cell Counts (AODC)

To determine prokaryotic cell numbers, 1 mL sediment slurry was fixed with sterile filtered formalin/seawater at a final concentration of 2% and stored at 4°C. Each sample was filtered on a 0.2 µm polycarbonate filter, stained with acridine orange as DNA dye (0.01% final concentration), and counted using an epifluorescence microscope (Axiophot II Imaging, Zeiss, Jena, Germany). For each sample, 30 random grids from two replicate filters (technical replicates) of three biological replicates, representing three out of five replicate PE-bags per treatment, were counted (Meyer-Reil, 1983). Bacterial biomass was estimated using a conversion factor of 3×10^{-13} g C μm^{-3} biovolume (Børsheim et al., 1990), and assuming an average cell volume of 0.07 μm^3 (Boetius and Lochte, 1996).

Potential Extracellular Enzymatic Activity (EEA)

By measuring the potential hydrolytic activity of two abundant enzymes, N-acetylglucosaminidase (chitobiase) and beta-glucosidase, in a modification of the method by Hoppe (1983) we tested, which of the supplied macromolecular organic substrates induced a production of extracellular enzymes in the community. Two 11 mL subsamples were taken from each of the five replicate PE-bags, and mixed with the methylumbelliferon (MUF)-labeled artificial substrates 4-methylumbelliferyl ß-D-glucopyranoside (MUF-beta-glucosidase) and 4-methylumbelliferyl-*N*-acetyl-ß-D glucosaminide (MUF-chitobiase), at saturation levels (100 µmol L^{-1}) according to the method described in Boetius and Lochte (1994; Supplementary Table S2). The assay

was run at 0°C for three hours at atmospheric pressure. We measured the release of the fluorochrome (MUF) after one and three hours, using a RF-5300 spectrofluorophotometer (Shimadzu Scientific, USA; emission: 455 nm, excitation: 365 nm). Fluorescent activity was calibrated with MUF standards ranging between 0 and 6 nmol mL^{-1}. Extracellular enzymatic activity (EEA) was then calculated per volume of sediment and time (μmol mL^{-1} d^{-1}). Since substrate concentrations are at saturation level within the incubation time, enzyme activities represent maximum velocities (Vmax). Enzymatic activity measurements may be confounded by competitive inhibition between the fluorogenic substrate and the added carbon (Arnosti, 2011). However, previous studies have reported an induction of enzyme production for beta-glucosidase and chitobiase by their respective substrates (Boetius and Lochte, 1994; Boetius and Lochte, 1996).

Oxygen Uptake

Oxygen concentrations were measured at the end of the incubation in each of the five replicate PE-bags per treatment as an indicator of community activity over the duration of the experiment. Optodes were connected to the FireStingO_2 fiber-optic oxygen meter (both obtained from Pyro Science GmbH, Germany), and calibrated at *in situ* temperatures by a two-point calibration using air-purged seawater and oxygen-free seawater by adding sodiumhydrogensulfite (Sigma Aldrich, Germany). The measuring accuracy was between 0.2% at 20% oxygen and 0.01% at 1% oxygen (FireSting O_2, Fiber-optic Oxygen Meter manual, Pyro Science GmbH, Germany). The PE-bags were gently shaken to mix the slurries and punctured immediately after retrieval from the incubator with the needle-protected optode fiber. Oxygen concentrations were measured in the center of the slurries. Because the PE-bags were oxygen-permeable, the measured oxygen concentration at the end of the 23 days of incubation was in steady state, i.e., the microbial respiration in the bag was balanced by the diffusive transport of oxygen across the PE-foil. From previous tests, an oxygen diffusion coefficient of 2.65×10^{-12} m^{-2} s^{-1} was determined and in combination with the oxygen difference across the PE-foil, the bulk flux and thus the respiration rate in each of the bags were determined (Supplementary Data; Supplementary Table S1; Supplementary Figure S1).

DNA/RNA Extraction and cDNA Generation

The analysis of 16S rRNA-genes (16S rDNA) reveals all taxa present in a given sample, while the analysis of the 16S rRNA identifies those taxa actively transcribing RNA under a given condition. From the latter, the metabolically active bacterial community can be inferred, and community shifts may be more easily detected (Moeseneder et al., 2005; Gaidos et al., 2011).

For DNA extraction, 7 mL slurry of three replicate PE-bags per treatment were sampled, centrifuged (3 min at 3000 rpm to remove the overlaying water) and stored at –20°C. Total DNA was extracted from 0.5 g sediment with the UltraClean Soil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA), and quantified using a microplate spectrometer (Infinite®200 PRO NanoQuant, TECAN Ltd, Switzerland). For assessing the active fraction of the bacterial community, 20 mL slurry of one replicate per treatment were sampled, immediately frozen in liquid nitrogen and stored at –80°C. Total RNA was extracted from 5 g sediment with the MoBio PowerSoil RNA extraction Kit (MoBio Laboratories Inc., Carlsbad, CA, USA). After DNAse digestion and purification using the RNeasy MinElute Cleanup Kit (QIAGEN, Germany), the purity and quantity were determined by electrophoresis using the 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). The extracts were translated into cDNA using the qScriptTM cDNA SuperMix Kit (Quanta Biosciences, Gaithersburg, MD, USA).

Amplicon Sequencing and Sequence Processing

For 16S cDNA amplicon library preparation, the standard instructions of the 16S Metagenomic Sequencing Library Preparation protocol (Illumina, Inc., San Diego, CA, USA) were followed. The hypervariable V3–V4 region of the bacterial 16S cDNA and rDNA was sequenced using the bacterial primers S-D-Bact-0341-b-S-17 (5′-CCTACGGGNGGCWGCAG-3′) and S-D-Bact-0785-a-A-21 (5′-GACTACHVGGGTATCTAATCC-3′; Klindworth et al., 2013). Sequences were obtained on the Illumina MiSeq platform in a 2 × 300 bp paired-end run as well as in a 2 × 250 bp paired-end run on the Illumina HiSeq platform (CeBiTec Bielefeld, Germany). Raw paired-end reads were primer-trimmed using cutadapt (Martin, 2011). For quality trimming, a sliding window of four bases and a minimum average quality of 15 was applied in trimmomatic v0.32 (Bolger et al., 2014), and the reads were merged using PEAR v0.9.5 (Zhang et al., 2014). Clustering into OTUs was done with the swarm algorithm using default parameters (v2.0; Mahé et al., 2014). One representative sequence

per OTU was taxonomically classified with SINA (SILVA Incremental Aligner; v1.2.11; Silva reference database release 123) at a minimum alignment similarity of 0.9, and a last common ancestor consensus of 0.7 (Pruesse et al., 2012). OTUs that were classified as chloroplasts, mitochondria, *Archaea*, and those not classified at the domain level were excluded from further analyses, as well as OTUs that occurred with only a single sequence in the whole dataset.

Statistical Analysis

Enzyme activity, cell abundances, and respiration rates based on oxygen measurements were calculated per volume of undiluted sediment. Differences between treatments were calculated based on ANOVA (analysis of variance) with Tukey HSD *post hoc* tests at a significance threshold of 0.05 (Hothorn et al., 2008). For cell counts, where technical replicates were available, mixed model ANOVA and the implementation of Tukey HSD in the R package multcomp were used. EEA and AODC data were square-root transformed to meet the assumptions of ANOVA.

Observed richness (number of OTUs) and evenness (inverse Simpson index) of the communities, were calculated via repeated random subsampling to the minimum library size of the amplicon data set (i.e., 26,712 sequences). The change in community structure (beta-diversity) between samples was assessed by calculating Bray–Curtis dissimilarities from relative OTU abundances [%], to produce non-metric multidimensional scaling (NMDS) plots, and to visualize community similarity between the 16S rRNA and rDNA datasets as well as between the different treatments.

The R-package ALDEx2 (ANOVA Like Differential Expression) was used to identify differentially abundant OTUs and families between treatments in the rDNA data set. Only OTUs that were present in two out of three replicates were used for the ALDEx2 analysis. Prior to analysis, proportional 16S rDNA based OTU abundances were centered log ratio (clr-) transformed using the aldex.clr function in R with 128 Dirichlet instances (Fernandes et al., 2014). Taxa were classified as differentially abundant at an adjusted parametric significant threshold of 0.05 (Benjamini and Hochberg, 1995), and at an unadjusted, non-parametric significance threshold of 0.05. Furthermore, the pairwise average difference and the effect size between the treatments at day 23 compared to the start of the incubation were calculated in the rDNA data set (Fernandes et al., 2014). Taxa were identified as responding to the treatments at an effect size of more than 4 and an average difference between treatments of more than $\log_2 2$ or less than

$\log_2 0.5$, i.e., one duplication or decrease by half in relative abundance. Since only one sample per treatment was available for the rRNA data set, no statistical analyses could be performed. Here, only abundant taxa with a relative sequence abundance of at least 1% (on OTU level) in at least one sample were screened for responses to the carbon treatments, and only taxa exhibiting at least one duplication or a decrease by half after 23 days are reported. On family level, only differentially abundant taxa with relative sequence abundances above 2% in at least one sample will be discussed.

All statistical analyses were conducted in R using the core distribution (version 3.3.0; R Development Core Team, 2014) and the following packages: vegan (Oksanen et al., 2015), ALDEx2 (Fernandes et al., 2014), nlme (Pinheiro et al., 2016), and multcomp (Hothorn et al., 2008).

Data Accession Numbers

Data are accessible via the Data Publisher for Earth & Environmental Science PANGAEA[1] (Hoffmann et al., 2016). Raw paired-end sequence, primer-trimmed reads are available on ENA Accession Number: PRJEB17614. The data were archived using the brokerage service of GFBio (Diepenbroek et al., 2014).

Results

To assess the response of an Arctic benthic bacterial community to different detritus additions, we determined bacterial cell numbers, estimated biomass, and measured enzymatic activities of two extracellular enzymes, as well as oxygen uptake. Variations in total (16S rDNA) and metabolically active (16S rRNA) bacterial community structure were assessed with 16S tag sequencing of the V3-V4 region. The observed effects represent net community changes, as sediments were unsieved, allowing for bacterial grazing by, e.g., protists, and viral pressure. All data presented below are based on incubations conducted at atmospheric pressure (1 atm). General patterns were comparable between incubations at atmospheric and *in situ* pressure conditions at 250 atm. Corresponding results are shown in the Supplementary Material (Supplementary Figures S2–S6 and Supplementary Tables S3 and S4). We present here comparisons between the initial sediment community at day 0 and the community incubated for 23 days with or without (unfed control) carbon amendments, if not stated otherwise.

Cell Biomass

Prokaryotic cell numbers in sediments retrieved from the central HAUSGARTEN station were on average $1.4 \pm 0.2 \times 10^9$ cells (mL sediment)$^{-1}$ ($n = 3$). After 23 days of incubation, cell numbers increased in all treatments, and in the unfed control, with highest cell numbers of about 4×10^9 cells mL^{-1} in the CHI treatment (ANOVA, $F_{1,9} = 152.7$, $p < 0.05$) (Figure 1A; Table 1). Accordingly, bacteria in CHI treatments showed the highest estimated assimilation of carbon into biomass, i.e., 17% of the supplied carbon, while bacteria assimilated around 8% of the supplied carbon in MARC treatments, and ≤5% in the remaining algae treatments (Table 1). Based on the cell numbers, estimated duplication times ranged between 33 days for the control incubation and 12 days for the CHI-treated samples. The unfed control may serve as an approximation of community doubling times in Arctic deep-sea surface sediments, which can thus be estimated to about four weeks. The corresponding biomass calculations represent rough estimates of carbon conversion into bacterial biomass, as we did not quantitatively determine changes in cell volumes.

Enzymatic Activity

Chitobiase and beta-glucosidase enzymatic activities increased significantly in all carbon-amended samples, while enzymatic activities in the unfed control remained low (approximately 0.01 µmol mL^{-1} d^{-1}), and were close to starting conditions (chitobiase: ANOVA, $F_{11,48}$ = 5,811, $p < 0.05$; beta-glucosidase: ANOVA, $F_{11,48}$= 5,297, $p < 0.05$; Figures 1B,C). Highest chitobiase activity was measured in the BCLA-fed sediments with 0.25 µmol mL^{-1} d^{-1}, which was 16-fold higher than in the unfed control, followed by CHI amended samples with 0.16 µmol mL^{-1} d^{-1}. The remaining algae-fed communities showed a moderate increase in chitobiase activity (0.10 to 0.13 µmol mL^{-1} d^{-1}). All carbon treatments were significantly different from the unfed control and from each other (Tukey HSD, $p < 0.05$; Figure 1B). Highest beta-glucosidase activity was induced in the EHUX treatments with 0.15 µmol mL^{-1} d^{-1}, which represents a 30-fold higher activity compared to the unfed control. Lowest beta-glucosidase activity was induced in the CHI and MARC treatments, turning over only 0.04 µmol mL^{-1} d^{-1}. TWEI and BCLA addition resulted in moderate beta-glucosidase activities. All carbon treatments were significantly different from the unfed control and from each other (Tukey HSD, $p < 0.05$; Figure 1C), except for beta-glucosidase activity in the CHI and MARC treatments. Calculation of the total hydrolysis potential of both measured enzymes over the duration of the experiment showed that communities in BCLA and EHUX treatments had the highest potentials (52 and 38 µmol C mL^{-1} d^{-1}, respectively), whereas MARC treatments had the lowest (17 µmol C mL^{-1} d^{-1}).

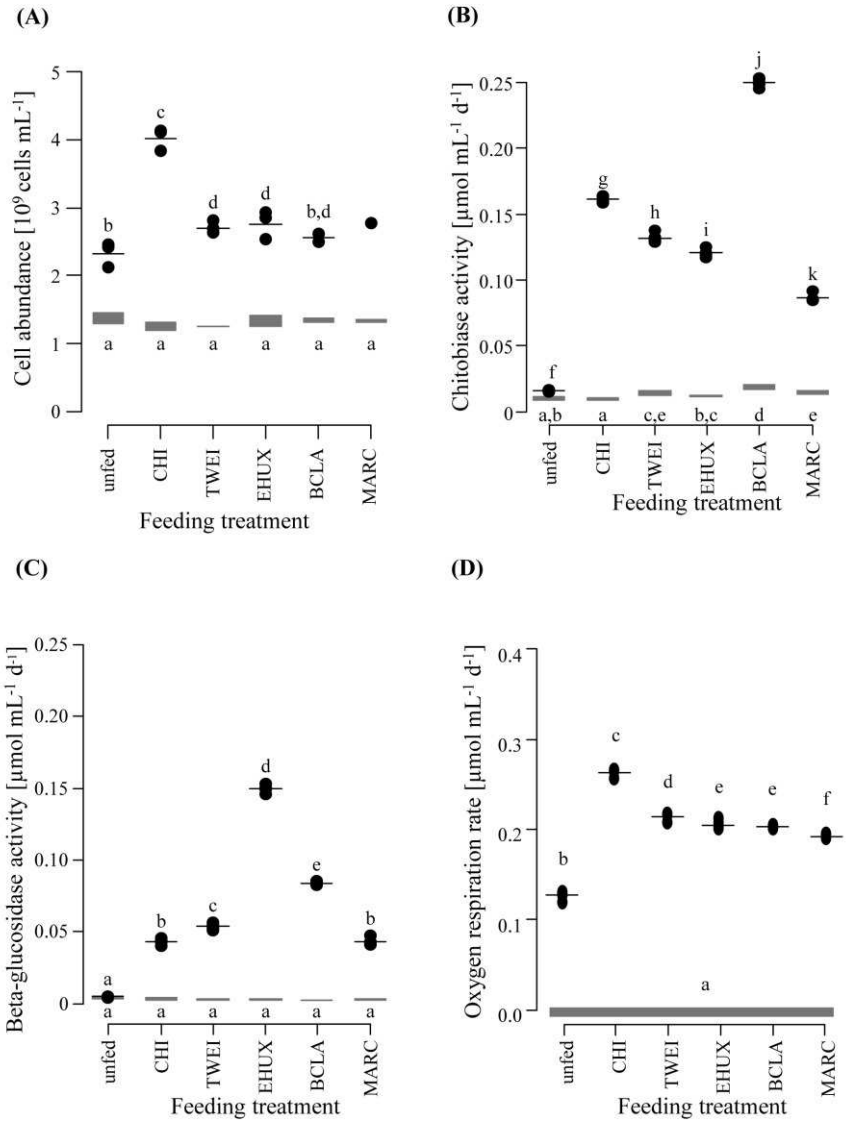

Figure 1**. Changes in cell abundance (A; *n* = 3), extracellular enzyme activity (B: chitobiase, C: beta-glucosidase; *n* = 5), and oxygen respiration rates (D; *n* = 5) of the sediment community in the different treatments under atmospheric pressure conditions.** Gray bars show the range at the beginning of the incubation. Black dots show measurements after 23 days of incubation, with the horizontal black line indicating the mean value per treatment. Lower case letters indicate groups of treatments that are significantly different from each other based on Tukey HSD at a significance threshold of $p < 0.05$. Day 0: starting conditions, unfed: control sediments after 23 days of incubation, carbon amendments: chitin (CHI), *Thalassiosira weissflogii* (TWEI), *Emiliania huxleyi* (EHUX), *Bacillaria* sp. (BCLA), *Melosira arctica* (MARC). Only one measurement of cell abundance was available for MARC-treated sediment.

Oxygen Uptake

Over the 23 days of incubation, oxygen concentrations declined significantly in all treatments (ANOVA, $F_{6,28} = 3{,}381$, $p < 0.05$; Figure 1D; Table 1). Inferred oxygen uptake among the treated sediment slurries ranged between 6.1 µmol O_2 mL^{-1} and 4.4

μmol O_2 mL^{-1} sediment over 23 days for CHI and MARC treatments, respectively (Table 1). The unfed control showed lowest oxygen uptake of 2.9 μmol O_2 mL^{-1}sediment in 23 days. Based on the calculated oxygen uptake, 19 and 9% of the added carbon have been respired at the end of the incubation period for CHI and MARC treatments, respectively (Figure 1D; Table 1). Oxygen uptake calculated for the addition of the planktonic algae and BCLA were equally moderate, with approximately 10% of the added carbon being respired (Table 1).

Bacterial Diversity and Community Structure (16S rDNA and rRNA)

General Trends

In total, 32,502 to 277,806 rDNA and 26,712 to 125,131 rRNA reads were generated per sample, corresponding to 1,731 to 12,549, and 3,012 to 16,384 swarmed, non-singleton OTUs, respectively. Changes in community structure were assessed separately for the 16S rDNA and rRNA sequence datasets, and samples from the two different approaches had an average intra-sample Bray–Curtis dissimilarity of 30% on family level, and 50% on OTU level. However, overall variations in community structure between treatments were significantly correlated between 16S rDNA and rRNA data on OTU and family level (Figure 2; Mantel test based on Bray-Curtis dissimilarity, $r = 0.7$, $p < 0.05$ for both taxonomic levels). Also, variations in community structure were highly correlated between experiments at atmospheric and at *in situ* pressure of 250 atm (Mantel test based on Bray–Curtis dissimilarity, $r = 0.9$, $p < 0.05$ on OTU and family level for rRNA and rDNA). Because data from biological replicates were available, we mainly focus on rDNA results under atmospheric pressure conditions here. Differences in the trends between rDNA and rRNA datasets are explicitly mentioned.

Bacterial communities were most diverse at the start of the incubation, with an observed bacterial richness of 5,777 OTUs and an inverse Simpson diversity index of 359 (Table 2). Diversity decreased significantly after 23 days in all treatments (OTU number: ANOVA, $F_{6,13} = 136.8$, $p < 0.05$; inverse Simpson index: ANOVA, $F_{6,13} = 345.3$, $p < 0.05$; Table 2), but not in control sediments. After 23 days of incubation, effective species richness was highest in the unfed samples with an inverse Simpson index of 193, followed by the CHI treatments with 56. Phytodetritus treatments resulted in the lowest alpha-diversity, with inverse Simpson indices ranging between 25 and 38.

Table 1. **Carbon utilization in the different treatments at atmospheric pressure.** All values are calculated as carbon content or oxygen uptake per mL wet sediment. The total carbon added for the different detritus treatments was equivalent to 16.6 µmol carbon mL^{-1} sediment. Day 0: starting conditions (*in situ* community), unfed: control sediments after 23 days of incubation, carbon amendments: chitin (CHI), *Thalassiosira weissflogii* (TWEI), *Emiliania huxleyi* (EHUX), *Bacillaria* sp. (BCLA), *Melosira arctica* (MARC); C: carbon; n/a: not applicable.

	Treatment	Cell abundance [x10^9 cells mL^{-1}]	Cell biomass [µmol C mL^{-1}] [a]	Stimulated net bacterial biomass yield [µmol C ml^{-1}] [b]	C assimilation into biomass [% of added C] [c]	O_2 uptake in 23 days [µmol O_2 mL^{-1}]	Net C respiration in 23 days [µmol C mL^{-1}] [d]	Net C respired in 23 days [% of added C] [e]	Total C used in 23 days (respired + assimilated) [µmol mL^{-1}] [f]	Total C used [% of added carbon] [g]	Bacterial growth efficiency [%] [h]
day0	unfed	1.4 ± 0.1	2.3 ± 0.1								
	CHI	1.3 ± 0.1	2.1 ± 0.1								
	TWEI	1.3 ± 0.0	2.1 ± 0.0								
	EHUX	1.4 ± 0.1	2.3 ± 0.1								
	BCLA	1.4 ± 0.0	2.3 ± 0.1								
	MARC	1.4 ± 0.0	2.3 ± 0.0								
day23	unfed	2.3 ± 0.2	3.9 ± 0.3	n/a	n/a	2.9 ± 0.1	n/a	n/a	n/a	n/a	n/a
	CHI	4.0 ± 0.1	6.7 ± 0.2	2.8 ± 0.4	17.0 ± 2.3	6.1 ± 0.1	3.2 ± 0.1	18.7 ± 0.5	6.0 ± 0.4	36.0 ± 2.3	47.2 ± 3.3
	TWEI	2.7 ± 0.1	4.5 ± 0.1	0.7 ± 0.4	3.9 ± 1.0	4.9 ± 0.1	2.0 ± 0.1	11.9 ± 0.5	2.7 ± 0.1	16.0 ± 0.8	23.8 ± 4.6
	EHUX	2.8 ± 0.2	4.6 ± 0.3	0.6 ± 0.2	4.4 ± 2.5	4.7 ± 0.1	1.8 ± 0.1	10.6 ± 0.6	2.5 ± 0.3	15.3 ± 2.0	27.0 ± 13.7
	BCLA	2.6 ± 0.1	4.3 ± 0.1	0.4 ± 0.2	2.4 ± 0.9	4.7 ± 0.0	1.8 ± 0.0	10.3 ± 0.1	2.1 ± 0.2	12.7 ± 0.9	18.6 ± 5.5
	MARC	2.8 ± n/a	4.6 ± n/a	1.1 ± n/a	6.5 ± n/a	4.4 ± 0.0	1.5 ± 0.0	8.7 ± 0.2	2.5 ± n/a	15.1 ± n/a	42.8 ± n/a

([a]) using a conversion factor of 1.67 fmol C per cell (Boetius and Lochte, 1996; Børsheim et al., 1990), representing an estimate of bacterial biomass; ([b]) bacterial biomass yield in carbon-amended treatments minus bacterial biomass yield in unfed control; ([c]) biomass yield divided by carbon added; ([d]) respiration in carbon-amended treatments minus respiration in unfed control as derived from oxygen uptake, and assuming a conversion factor of 1; ([e]) respired carbon divided by carbon added; ([f]) sum of respired and assimilated carbon; ([g]) total carbon used divided by total carbon added; ([h]) biomass yield divided by total carbon used (assimilated + respired).

The sediment community at the start of the incubation was dominated by *Proteobacteria*, comprising more than 60% of 16S rDNA sequences, and by members of the *Bacteroidetes, Planctomycetes, Chloroflexi*, and *Acidobacteria*, all of which showed relative sequence abundances between 5 and 10% (Figure 3). An NMDS plot based on Bray–Curtis dissimilarity revealed three distinct groups of samples at a 70% dissimilarity threshold (Figure 2). Bacterial community structure in the unfed control on day 23 was still very similar to the initial bacterial community. CHI treatments grouped separately, but were still similar to the unfed treatments. Phytodetritus treatments formed a third group, which was clearly separated from the unfed control and CHI treatments (Figure 2).

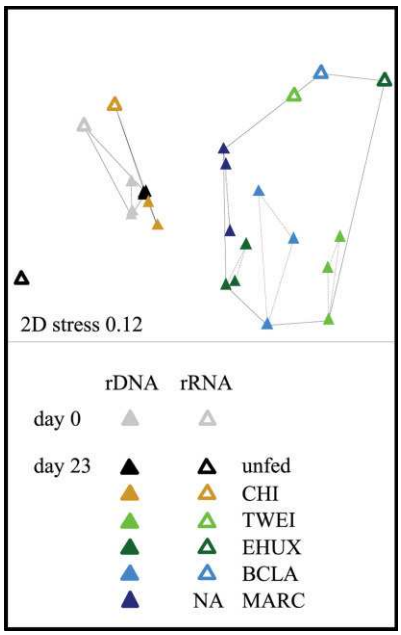

Figure 2. **Non-metric multidimensional scaling (NMDS) plot based on Bray–Curtis dissimilarity of the total (16S rDNA; *n* = 3) and active (16S rRNA; *n* = 1) bacterial community in the different sediment treatments under atmospheric pressure conditions.** Hulls displayed by solid lines are based on a dissimilarity threshold of 70%. Hulls displayed by dashed lines are based on a dissimilarity threshold of 32%, and shows clusters of biological replicates. Day 0: starting conditions, unfed: control sediments after 23 days of incubation, carbon amendments: chitin (CHI), *Thalassiosira weissflogii* (TWEI), *Emiliania huxleyi* (EHUX), *Bacillaria* sp. (BCLA), *Melosira arctica* (MARC). No rRNA data is available for MARC-treated sediment.

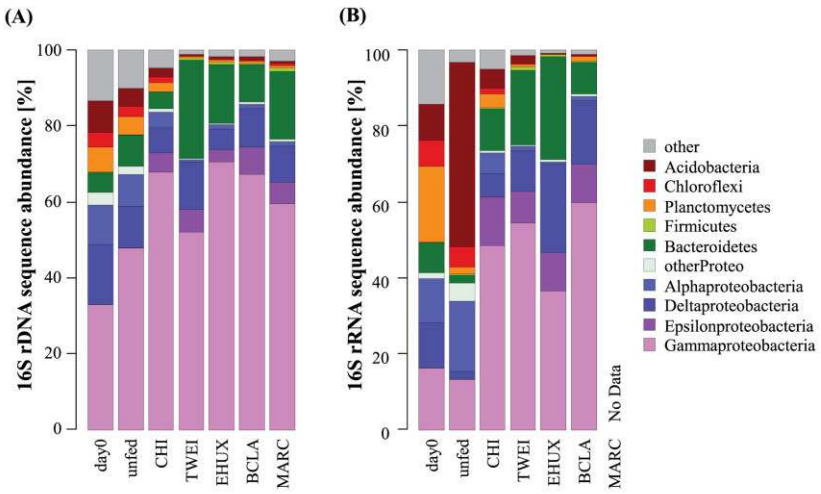

Figure 3. **Dominant phyla of the total (A: 16S rDNA; *n* = 3) and active (B: 16S rRNA; *n* = 1) bacterial community in the different sediment treatments under atmospheric pressure conditions.** For the total bacterial community, sequences from replicate samples were pooled for the calculation of relative sequence abundances. For *Proteobacteria*, class-level resolution is shown. Day 0: starting conditions, unfed: control sediments after 23 days of incubation, carbon amendments: chitin (CHI), *Thalassiosira weissflogii* (TWEI), *Emiliania huxleyi* (EHUX), *Bacillaria* sp. (BCLA), *Melosira arctica* (MARC).

Overall, 30 bacterial families of eight different phyla had a relative sequence abundance, i.e., sequence proportion >2% in at least one sample, and were identified as differentially abundant on rDNA level (Figure 4). About one third of these differentially abundant families (12 families), which were mainly affiliated with *Gamma*- and *Deltaproteobacteria*, and *Bacteroidetes*, increased significantly during incubations, whereas the other two thirds (18 families) decreased. The 12 families, which increased most strongly in relative sequence abundance in the different treatments, were identified as key responding, opportunistic bacterial families. These included four families responding to all treatments, including the unfed control: *Colwelliaceae, Moritellaceae, Psychromonadaceae*, and *Shewanellaceae*. In addition, four families responded to all carbon amendments: *Desulfuromonadaceae*, unclassified *Desulfuromonadales* and *Alteromonadales* families, as well as *Campylobacteraceae*. Another three families showed a strong positive response in relative sequence abundance especially to the supply of phytodetritus: *Flavobacteriaceae, Geobacteraceae*, and *Marinilabiaceae*. The *Oceanospirillaceae* increased significantly in the unfed control treatment, on average sixfold stronger than in any other treatment except for the CHI sample. Furthermore, minor changes in the unfed control treatment included an increase in relative sequence abundance of four gammaproteobacterial families, and a slight decrease of two families belonging to the *Chloroflexi* and *Acidobacteria*. Otherwise, no changes in the majority of

the most abundant families were detected (Figure 4). In total, the 12 opportunistic families increased from 3% relative sequence abundance at the beginning of the incubation to on average 89% in carbon treatments after 23 days of incubation (Supplementary Tables S4A, B). The only case of pronounced differences between rDNA and rRNA results was the unfed control after 23 days. On the level of active transcription (rRNA), diverse families belonging to the phylum *Acidobacteria*, i.e., subgroups 6, 10, 26 (unclassified), NS72, and SVA0725, as well as the alphaproteobacterial family *Sphingomonadaceae* increased strongly in relative sequence abundance. This was not the case on rDNA level, which still closely resembled the starting community composition.

Chitin Treatments

Overall, a different set of families responded to the CHI treatment compared to the phytodetritus treatments. CHI treatment led to a significant increase in nine out of the 30 differentially abundant families, whereas three decreased compared to the initial sediment community. In contrast to phytodetritus treatments, *Colwelliaceae, Psychromonadaceae*, and *Oceanospirillaceae* were most strongly affected by CHI treatments, increasing from < 1% of 16S rDNA relative sequence abundance to 25, 12, and 8%, respectively (Figure 4; Supplementary Table S4A). Overall, most bacterial families with higher relative sequence abundances in the unfed controls, e.g., *Oceanospirillaceae*, or in phytodetritus treatments, e.g., *Campylobacteraceae* and *Desulfuromonadaceae*, also increased in relative abundance in CHI treatments (Figure 4).

Phytodetritus Treatments

All phytodetritus treatments led to stronger changes in community structure than observed for unfed controls and CHI treatments after 23 days of incubation (Figure 2). On average, eleven families increased and 15 decreased in relative sequence abundance in phytodetritus treatments, and they were very similar in their phylogenetic affiliation across all treatments (Figure 4; Supplementary Table S4A). The families most strongly increasing in relative sequence abundance for the different algal treatments were *Flavobacteriaceae, Moritellaceae*, and *Desulfobacteraceae* for TWEI, *Marinilabiaceae*, unclassified *Desulfuromonadales* families, and *Geobacteraceae* for TWEI as well as EHUX, and an unclassified group of *Alteromonadales* for EHUX. *Desulfuromonadaceae, Shewanellaceae*, and *Campylobacteraceae* responded strongest to BCLA. All of the abundant families classified as opportunistic in algal treatments (EHUX, TWEI, BCLA) also increased significantly in MARC treatments.

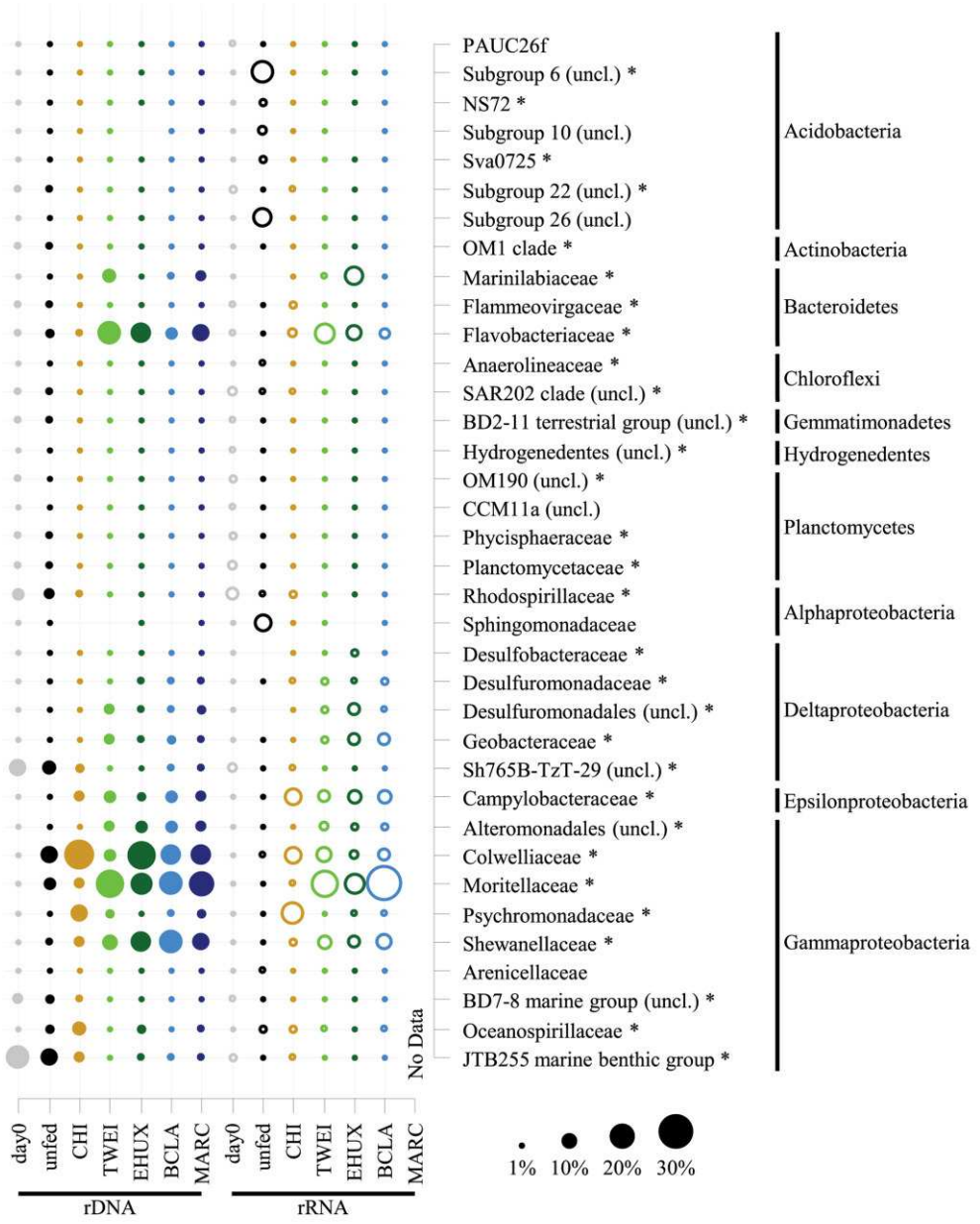

Figure 4. **Dot plot showing relative sequence abundances of dominant bacterial families, and their phylum affiliation, of the total (16S rDNA; *n* = 3) and active (16S rRNA; *n* = 1) bacterial community in the different sediment treatments under atmospheric pressure conditions.** For the total bacterial community, sequences from replicate samples were pooled for the calculation of relative sequence abundances. For families of the *Proteobacteria*, class-level resolution is shown. For taxa that were unclassified at the respective level of resolution, the next higher taxonomic rank is shown. Asterisks mark differentially abundant taxa between treatments based on 16S rDNA samples (ALDEx2 analysis). All groups that are not marked by an asterisk were only abundant in 16S rRNA data, for which no replicates are available and therefore no analysis of differential abundance could be performed. Day 0: starting conditions, unfed: control sediments after 23 days of incubation, carbon amendments: chitin (CHI), *Thalassiosira weissflogii* (TWEI), *Emiliania huxleyi* (EHUX), *Bacillaria* sp. (BCLA), *Melosira arctica* (MARC). No rRNA data is available for MARC-treated sediment.

Response of Bacterial Groups at High Taxonomic Resolution

The analysis of differentially abundant bacterial groups at a higher taxonomic resolution, i.e., at OTU level, revealed intra-genus differences in their response to the different treatments in four out of the 30 differentially abundant families. Within the genera *Arcobacter* (*Campylobacteraceae*), *Colwellia* (*Colwelliaceae*), *Moritella* (*Moritellaceae*) and *Psychromonas* (*Psychromonadaceae*), several OTUs displayed opposing trends among the treatments, in addition to the overall pattern observed at lower taxonomic resolution (SI text).

Discussion

Environmental changes in the Arctic Ocean lead to changes in the quality and quantity of organic matter exported from surface waters to the deep sea (McMahon et al., 2006; Bauerfeind et al., 2009; Boetius et al., 2013), but little is known about the effects on benthic community structure and function. In this study, we aimed at evaluating the response of benthic bacterial communities of the Arctic deep-sea floor to different organic matter sources in short-term, *ex situ* experiments. Using an experimental setup allowing for approximately one community duplication (Table 1), we were able to observe changes in community functions and, for the first time, identified bacterial groups that responded within days to the addition of different naturally occurring organic matter sources, i.e., chitin and different species of algae.

Responses in growth, extracellular enzymatic activities, and oxygen uptake were observed for all organic matter treatments compared to unfed control incubations after 23 days. This showed that bacterial communities actively responded to the input of organic matter by initiating degradation of the organic matter and producing biomass. This observation is supported by previous studies from various deep-sea environments (Deming, 1985; Turley and Lochte, 1990; Boetius and Lochte, 1994, 1996; Witte et al., 2003a). Although carbon uptake appeared to be slightly lower for incubations under *in situ* pressure (see also: Jannasch, 1979; Jannasch and Wirsen, 1982), oxygen consumption was higher, resulting in hypoxic conditions after 23 days. Despite this discrepancy, similar results were obtained for incubations at atmospheric and *in situ* pressure (Figures 1 to 4; Supplementary Figures S2 to S6), of which the latter need to be interpreted with caution, as we cannot clearly distinguish between the effects of pressure and oxygen depletion in the high-pressure treatments.

Table 2 **Alpha diversity indices of the bacterial community in the incubation experiment.** Richness and evenness were estimated based on OTU number (nOTU) and the inverse Simpson index (invS) of rDNA (n = 3) and rRNA (n = 1) datasets, and are given as mean ± standard deviation where applicable. Differences between treatments were assessed with ANOVA at a significance threshold of 0.05. Letters indicate significantly different groups based on pairwise TukeyHSD post-hoc test. Day 0: starting conditions, unfed: control sediments after 23 days of incubation, carbon amendments: chitin (CHI), *Thalassiosira weissflogii* (TWEI), *Emiliania huxleyi* (EHUX), *Bacillaria* sp. (BCLA), *Melosira arctica* (MARC).

		nOTUs		invS	
	treatment	value	TukeyHSD	value	TukeyHSD
rDNA					
	day0	5,777 ± 56	a	359 ± 28	a
	unfed	5,113 ± 54	a	193 ± 22	b
	CHI	3,479 ± 359	b	56 ± 8	c
	TWEI	1,323 ± 95	c	26 ± 0	d
	EHUX	1,786 ± 44	d	25 ± 10	d
	BCLA	1,686± 286	d	35 ± 1	e
	MARC	1,922 ± 131	d	38 ± 2	e
rRNA					
	day0	7,816	NA	1,230	NA
	unfed	2,767	NA	45	NA
	CHI	4,602	NA	67	NA
	TWEI	2,362	NA	32	NA
	EHUX	2,358	NA	34	NA
	BCLA	2,498	NA	34	NA
	MARC	NA	NA	NA	NA

In addition to the response in bulk growth and activity measurements, we observed clear shifts in diversity (Table 2) and community structure (Figure 2) as a result of organic matter additions. The responding taxa were generally consistent between rDNA and rRNA, indicating that shifts in bacterial community structure quickly translated from a higher activity (rRNA) to cell replication (rDNA). Furthermore, changes in community structure were largely consistent between incubations at 1 and 250 atm, indicating that most of the opportunistic bacterial groups appear to cope well with de-/re-compression for the investigated water depth (2,500 m). Accordingly, pressure effects have repeatedly been hypothesized to be negligible below approximately 200 to 300 atm (Yayanos, 1986; Follonier et al., 2012; Picard and Daniel, 2013). Obligate piezophilic bacteria mainly derive from below 6,000 m water depth (Yayanos, 1986), and most bacterial groups from shallower water depths are described to be rather piezotolerant (Picard and Daniel, 2013).

Changes in the Untreated Control Community

For both, atmospheric and *in situ* pressure incubations, an increase in bacterial biomass in control treatments indicated that the presence of sedimentary organic carbon provided sufficient energy for an increase of the bacterial standing stock. This may be partly due to the onset of a settling phytoplankton bloom in the area during the time of sampling (Soltwedel, 2015), but also to the presence of refractory organic material. The initial (*in situ*) sediment bacterial community (Figure 3) was very similar to previous reports from this site (Jacob et al., 2013), and from other deep-sea surface sediments globally (Zinger et al., 2011; Bienhold et al., 2016; Learman et al., 2016). OTUs affiliating with the gammaproteobacterial families *Oceanospirillaceae* and *Colwelliaceae* showed a strong increase in relative sequence abundance in the unfed control treatments after 23 days of incubation, but also in CHI treatments. These groups are known to include polymer degraders (Methé et al., 2005; Weiner et al., 2008). Additionally, the genera *Colwellia* and *Marinomonas* sp. (*Oceanospirillaceae*) have been reported to produce polyhydroxyalkanoate compounds, which serve as intracellular carbon and energy reserves (Methé et al., 2005; Cai et al., 2011). This may be a beneficial strategy for organisms living in organic-poor sediments of the Arctic deep sea.

We also observed a conspicuous increase in relative rRNA sequence abundance of deep-branching acidobacterial groups and taxa belonging to the *Sphingomonadaceae* in the unfed controls after 23 days of incubation. We speculate that the increased relative rRNA sequence abundance may represent a strategy, where in lack of nutrients, energy is invested in maintaining the metabolism rather than increasing biomass (Morita, 1982; Watson et al., 1998 and references therein). However, this pattern was not reproducible in incubations at 250 atm, and it remains unknown what caused this discrepancy. Due to the lack of fresh organic material in control incubations, bacteria adapted to more oligotrophic conditions and the breakdown of refractory, aged organic material may become more important. Indeed, *Sphingomonadaceae* are described to play important roles in oligotrophic environments, and in the degradation of recalcitrant polyaromatic compounds (Ohta et al., 2004; García-Romero et al., 2016). In spite of their frequently observed oligotrophic character, members of this family are widespread in nature, occurring in soils, freshwater, and marine habitats (Cavicchioli et al., 1999; Yabuuchi and Kosako, 2005; Vaz-Moreira et al., 2011).

Acidobacteria have also been reported to cope well with low organic matter availability (Fierer et al., 2007; Bienhold et al., 2012). These groups may thus be representatives of a background community, surviving in the oligotrophic conditions that prevail at the deep Arctic seafloor for most of the year.

Effect of Detritus-Type on Bacterial Community Structure and Function

Chitin addition caused strong functional changes in the sediment microbial community, i.e., the largest increase in net biomass (highest bacterial growth efficiency), highest oxygen consumption rates, and a strong increase in chitobiase activity (Figure 1; Table 1). Despite these effects, and even though overall diversity decreased significantly (Table 2), community composition remained very similar to its initial structure and to control incubations (Figure 2; Supplementary Figure S4). The sediment community thus seemed to be well adapted to the degradation of this type of polymeric organic matter. Since chitin is the most abundant biopolymer in the ocean (Gooday, 1990), and bacteria are the main decomposers of this material in the deep-sea benthos (Christiansen and Boetius, 2000; Kanzog et al., 2009), benthic communities may constantly be exposed to relatively high levels of chitin, and our results may therefore reflect an inherent adaptation to the degradation of this carbon source. The decrease in community diversity probably reflects the loss of taxa that were overgrown by chitin degraders.

In contrast to CHI treatments, the addition of algae caused a much more moderate response in bulk community function, including lower estimated bacterial growth efficiencies. This is in line with reports by Mayor et al. (2012), who showed that benthic bacteria displayed lower growth efficiencies when respiring diatoms compared to fecal pellet material, and confirms that resource quality may influence carbon uptake and retention potential in natural settings.

There are several potential explanations for the lower bacterial growth efficiencies in algae treatments. Some of the tested algae may contain compounds that suppress growth of certain bacterial groups (Guedes et al., 2011; Qin et al., 2013). As one example, the production of dimethylsulphopropionate (DMSP)-related substances by EHUX has been suggested to act as protection against grazers (Hansen et al., 1996; Fileman et al., 2002), and may also inhibit bacterial attachment (Saha et al., 2012). Viral lysis might act as another structuring factor (Proctor and Fuhrman, 1990; Fuhrman and Noble, 1995), as viruses are also considered to be favored by high energy input and biological productivity (Cochlan et al., 1993; Bratbak et al., 1994). Furthermore, meiofauna,

e.g., nematodes, may be direct or indirect beneficiaries of the fresh phytodetritus, by either feeding on the algae or on the bacteria (Guilini et al., 2010; Ingels et al., 2010). Numerous studies have shown that fauna may be rapid consumers of fresh phytodetritus at the seafloor (e.g., Blair et al., 1996; Moodley et al., 2002, 2005, Witte et al., 2003a), in particular in the Arctic, following the deposition of ice algae or phytoplankton (McMahon et al., 2006; Morata et al., 2011, 2015). To further resolve the substrate degradation cascade, labeled substrates could be used in future experiments (Witte et al., 2003a,b; Guilini et al., 2010).

Compared to CHI treatments, we observed a much stronger decrease in bacterial diversity as well as shifts in community structure in all phytodetritus treatments, indicating the response of a few adapted groups outcompeting others. This suggests that changes in community composition needed to precede changes in community function (Schindler, 1987; Niederlehner and Cairns, 1994). This may be due to the fact that the input of relatively fresh algae used in this study is less common than the ubiquitously available chitin. Also, while chitin is solely composed of *N*-acetyl-glucosamine subunits, and only a small set of enzymes is necessary to degrade it, the degradation of algae is more complex, involving a cascade of multiple enzymes and bacterial groups (Xing et al., 2015; Teeling et al., 2016).

Changes in community structure and function were very similar between the phytoplankton (TWEI, EHUX) and sea-ice algae (BCLA, MARC) treatments, despite presumable differences in their nutritional value (McMahon et al., 2006; Sun et al., 2007). However, the input dynamics of these organic matter sources in the natural system would differ profoundly. Specifically, a slow rain of phytoplankton cells that are already being degraded on their way through the water column represent a stark contrast to a highly concentrated and localized input of large ice algae aggregates sinking rapidly to the deep seafloor. These scenarios would likely result in differential impacts on community structure, functioning, and carbon turnover.

Taxon-Specific Responses to Carbon Amendments

Previous studies have already identified links between organic matter quantity and bacterial community structure in marine sediments (Franco et al., 2007; Bienhold et al., 2012; Learman et al., 2016). Here, we specifically addressed the response of bacterial communities to different types of organic matter when provided in a single, large pulse. Overall, we identified 12 bacterial families on both rRNA and rDNA level that in-

creased in relative sequence abundance in the organic matter treatments, and that were consistent between incubations under atmospheric and *in situ* pressure conditions. These opportunistic bacterial groups may represent the specific community fraction that contributed to the increase in enzyme activity, biomass, and oxygen respiration, and that might thus be of key importance for initial polymer degradation at the deep-sea floor. The gammaproteobacterial families *Colwelliaceae, Psychromonadaceae*, and *Oceanospirillaceae* were found in highest abundances in CHI treated sediments. *Gammaproteobacteria* are a globally ubiquitous group in marine environments that have been described as versatile opportunists and copiotrophs (Glöckner et al., 1999; Bienhold et al., 2012). Indeed, representatives of the family *Oceanospirillaceae*, e.g., the genera *Marinomonas* and *Neptunomonas*, have been described to degrade chitin (Nogi et al., 2004; González and Whitman, 2006; Jung, 2006). Also representatives of the families *Colwelliaceae* and *Psychromonadaceae* appear to have a broad substrate spectrum including complex organic compounds (Methé et al., 2005; Auman et al., 2006). Several members of the *Colwelliaceae* have been reported to hydrolyze chitin (Huston et al., 2000, 2004; Ivanova et al., 2004). We therefore identify these bacterial groups as potential opportunists, especially in scenarios of high chitin input, such as mass sedimentation events of crustaceans (Sokolova, 1994; Christiansen and Boetius, 2000).

For *Psychromonadaceae*, only some species are described as being able to degrade chitin (e.g., strain *Psychromonas ingrahamii* 37; Riley et al., 2008), whereas other species, e.g., *Psychromonas arctica*, appear to lack this ability (Groudieva et al., 2003). This supports our findings of a strong increase in relative sequence abundance for diverse OTUs affiliated with *Psychromonas* in CHI amended slurries on the one hand, and a decrease of other *Psychromonas*-associated OTUs on the other hand, which instead showed a stronger response in algae treatments (SI text). This deviation is consistent with previous reports for this genus in a similar environment (Bienhold et al., 2012), and demonstrates the usefulness of community information at a high taxonomic resolution. In the future, genomic and physiological studies may reveal in more detail the different metabolic niches that these taxa occupy.

Several other families were identified with most pronounced shifts in the algae treatments. *Flavobacteria* from Arctic sediments were recently described to exhibit a strong positive correlation with energy availability (Bienhold et al., 2012), consistent with the association of copiotrophy to the phylum *Bacteroidetes* (Fierer et al., 2007). Also, *Flavobacteria* and *Alteromonadales*, in particular *Pseudoalteromonadales*, have

shown strong responses to phytoplankton blooms (Teeling et al., 2016; Xing et al., 2015). Representatives of the order *Pseudoalteromonadales*, e.g., *Pseudoalteromonas haloplanktis*, which shares closest sequence identity to the responsive clade in our study, can produce anti-biofilm molecules (Parrilli et al., 2016). It may be able to degrade extracellular polymeric substances produced by phytoplankton and ice algae like MARC by using its diverse set of enzymes, including peptidases, amylase, and alpha-glucosidase (Médigue et al., 2005). In the future, extended monitoring periods after the addition of organic matter, combined with metagenomic and metatranscriptomic approaches, may yield further insights into succession patterns, species competition, and niche adaptation, e.g., as observed for bacterial communities associated with phytoplankton blooms in the water column (Teeling et al., 2012).

Most of the opportunistic bacterial groups identified in this study contain psychrophilic and psychrotolerant cultured representatives (Médigue et al., 2005; Methé et al., 2005; Auman et al., 2006; Riley et al., 2008; Lee et al., 2012) with mostly heterotrophic lifestyles (e.g., Médigue et al., 2005; Methé et al., 2005), the potential for motility (e.g., Médigue et al., 2005; Auman et al., 2006; Lee et al., 2012), and biofilm formation (e.g., Médigue et al., 2005; Methé et al., 2005; Riley et al., 2008; Baek et al., 2015). The latter two features may aid the association with, and directed degradation of particulate organic matter in the deep sea (O'Toole et al., 2000; Golyshin et al., 2002), but the relevance of such mechanisms remains speculative and will require further investigations. Most of the groups are not specific or exclusive for the sediment environment, but cover a wide range of natural habitats, and appear to have a rather cosmopolitan character (e.g., Médigue et al., 2005; Methé et al., 2005; Zhao et al., 2005; Auman et al., 2006; Xing et al., 2015). Nevertheless, many of the closely related cultured representatives were isolated from polar marine environments, and are described to produce cold-adapted enzymes, capable of degrading high-molecular-weight organic compounds, e.g., *Alteromonadales* (Médigue et al., 2005), *Colwelliaceae* (Methé et al., 2005), *Shewanellaceae* (Zhao et al., 2010), *Psychromonadaceae* (Riley et al., 2008), *Moritellaceae* (Lee et al., 2012; Malecki et al., 2013), *Flavobacteriaceae* (Pati et al., 2011; Baek et al., 2015; Xing et al., 2015), and *Oceanospirillaceae* (Weiner et al., 2008). Future research may therefore identify explicit Arctic and/or sediment ecotypes within these groups, which need to be further metabolically characterized using genomic and physiological methods, in order to assess their role in the turnover of organic matter in Arctic marine sediments.

Conclusion

The observed differences in community structure, carbon uptake, and remineralization between the treatments suggest that changes in the type of organic matter exported to the seafloor will have consequences for bacterial diversity and carbon turnover. The families *Colwelliaceae, Psychromonadaceae*, and *Oceanospirillaceae* may represent indicator groups for CHI input, and *Flavobacteriaceae, Pseudoalteromonadaceae*, and *Marinilabiaceae* for phytodetritus input in this Arctic region. However, quantitative methods targeting specific groups of interest, such as Catalyzed Reporter Deposition-Fluorescence *In Situ* Hybridization (CARD-FISH) and quantitative PCR, are needed to confirm the changes observed in the compositional sequencing datasets, and to further link taxonomic groups (at high taxonomic resolution) to specific community functions. Furthermore, the metabolic potential of these key responding groups remains to be investigated to better understand their role in ecosystem functioning. Future studies should combine *ex situ* and *in situ* observations at extended time periods. Experimental approaches will be helpful in order to monitor changes and succession patterns in community structure and function after disturbances. Additionally, long-term environmental observations are needed to better assess the effects of shifts in organic matter quality and quantity on ecosystem functioning in the context of seasonal variations and spanning several years.

Author Contributions

KH and CB designed experiments. KH performed the experiments. KH, CH, and CB analyzed data, and VS-C assisted in data interpretation. MH performed oxygen sensor data analysis and modeling. KH, VS-C, and CB wrote the manuscript with support and input from all co-authors. All authors critically revised the article and gave their approval of the submitted version.

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Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Abbreviations

16S rDNA, 16S rRNA gene; BCLA, *Bacillaria* sp.; CHI, chitin; EEA, extracellular enzymatic activity; EHUX, *Emiliania huxleyi*; LTER, Long-Term Ecological Research Observatory; MARC, *Melosira arctica*; TWEI, *Thalassiosira weissflogii*.

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Supplementary Data

O_2 respiration experiments in permeable plastic bags

Plastic bags were filled with sediment slurries to a final volume (V) of 50 mL. The initial O_2 concentration (C_0) in the bags was 300 µmol L^{-1}. The bags were stored in a container filled with sterile filtered, air saturated seawater (C_0 = 300 µmol L^{-1}). The plastic material was O_2 permeable with a thickness (x) of 0.12 mm. From previous tests it was known that the O_2 diffusion coefficient (D) is 2.65 x 10^{-12} m² s^{-1}. The change of O_2 concentration over time in the bags was a function of the microbial respiration rate (R), which we assumed to be constant (zero order kinetics), and the O_2 flux (F) through the plastic foil of an area (A) of 81 cm². The flux, in turn, was a function of the different O_2 concentrations inside ($C_{(t)}$) and outside (C_0) the bags. This can be expressed by the equation:

Equation (1) can be solved for C $\quad \frac{dC}{dt} = R + \frac{(C_0 - C(t))}{x} D \frac{A}{V}$

Equation (1) can be solved for C $\quad C(t) = \frac{R}{K}(1 - e^{-Kt}) + C_0$

with the time constant K given by $\quad K = \frac{D\,A}{x\,V}$

In the bags, the O_2 concentrations decrease exponentially and approach a steady state (Supplementary Figure S1). After a long incubation time (i.e. $t >> K$) the O_2 respiration rate will be balanced by the inward flux of O_2 and the steady state solution is

$$C(t) = \frac{R}{K} + C_0$$

For the given volume (50 mL), area (81 cm²), diffusion coefficient (2.65 x 10^{-12} m² s^{-1}), and foil thickness (0.12 mm) the time constant K is 0.3 d^{-1}. This means that 63% of the total decrease of O_2 takes place within $1/K$ = 3.2 days. Consequently, the O_2 concentration is in steady state after 23 days of incubation and the respiration rate can be calculated from the O_2 concentration at day 23 by rearranging equation (4)

$$R = (C_{23d} - C_0)K$$

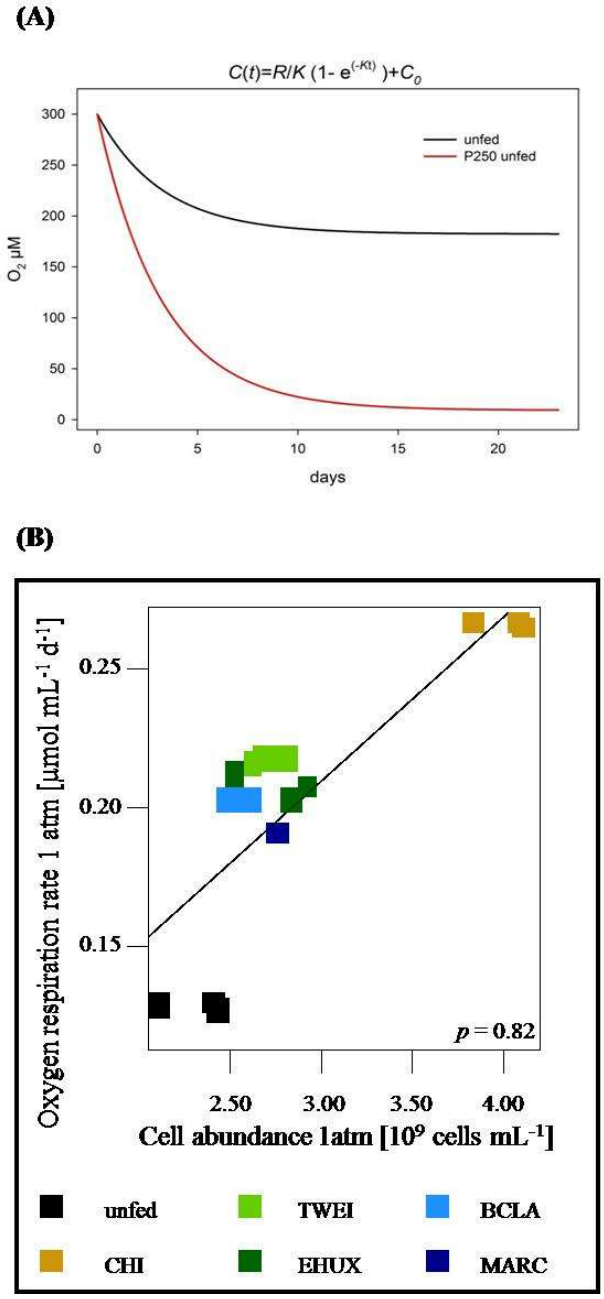
Response of bacterial groups at high taxonomic resolution

When analyzing differentially abundant bacterial groups at a higher taxonomic resolution, i.e. at OTU level, we identified intra-genus differences in their response to different treatments in four out of the 30 differentially abundant families. The family *Campylobacteraceae* consisted of one genus, *Arcobacter*, which strongly increased in all

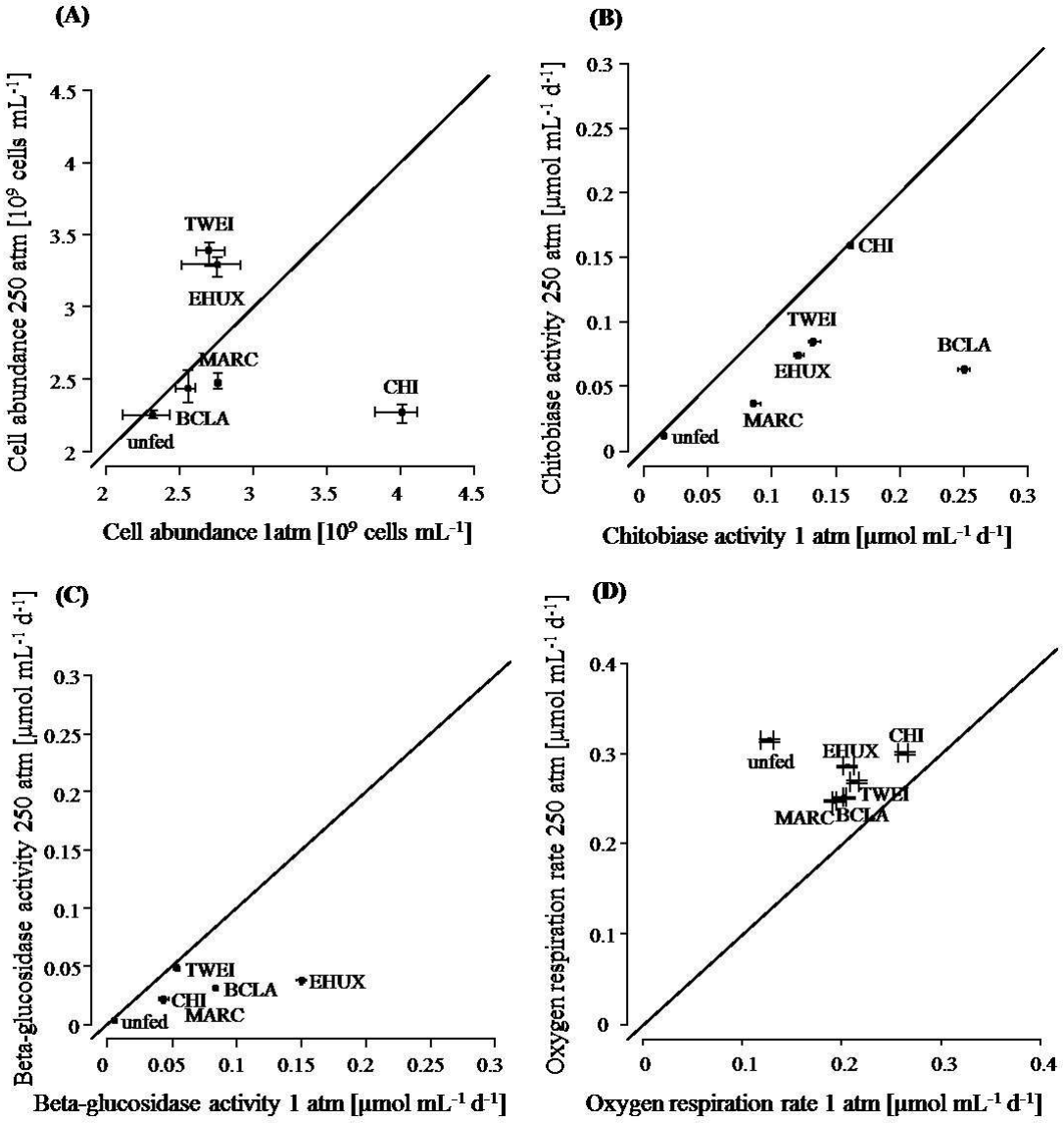
carbon-amended treatments. At a higher resolution, three different OTUs were assigned within this genus, of which two (OTU4 and 130) responded strongest to phytodetritus treatments, but OTU18 showed a 38% stronger relative sequence increase in CHI treatments compared to phytodetritus treatments (Supplementary Table S4). Similar observations were made for the gammaproteobacterial genera *Colwellia*, *Moritella,* and *Psychromonas*. Eight out of ten OTUs affiliating with the genus *Colwellia* responded to all treatments including the control. The *Colwellia* OTU22, however, showed a much stronger relative sequence response in the unfed control and EHUX treatment, i.e. a 57% stronger increase compared to all other treatments. *Colwellia* OTU12 increased by 71% in relative sequence abundance in the CHI and EHUX treatments, compared to other treatments. The genus *Moritella* included nine OTUs, of which seven showed a similar increase in relative sequence abundance in all treatments including the control, while OTUs 13 and 30 showed a 6 to 25% stronger increase in relative sequence abundance in TWEI and MARC treatments compared to all other treatments. The family *Psychromonadaceae* responded strongly to CHI treatments and was mainly represented by the genus *Psychromonas*, compared to the initial sediment community. While four out of five OTUs within this genus responded most strongly to CHI treatments in relative abundance, OTU55 responded much stronger to TWEI and MARC treatments, i.e. with a 99.9% higher increase in relative sequence abundance. A full list of differentially abundant OTUs is included in Supplementary Table S4.

Supplementary Figures and Tables

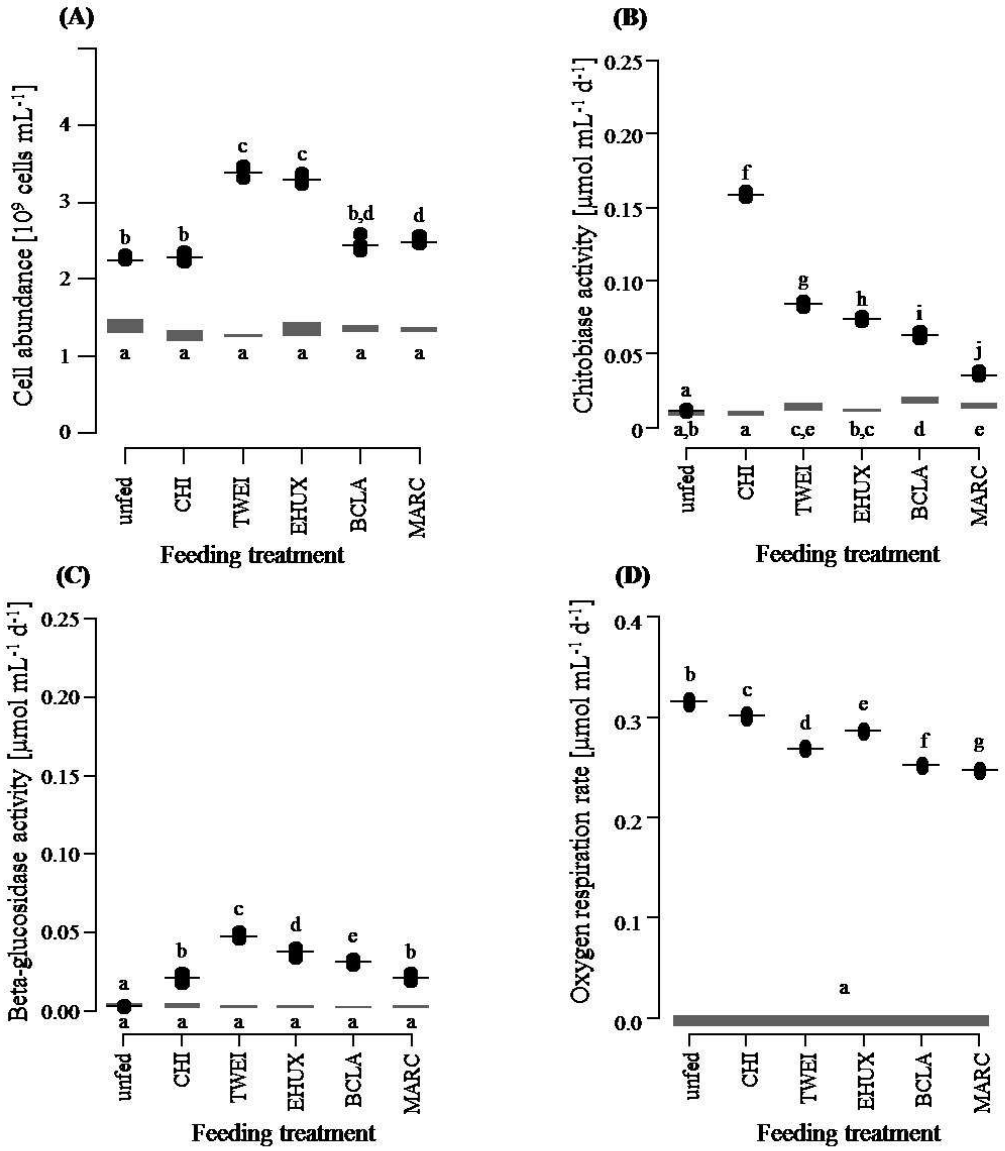
Supplementary Figures



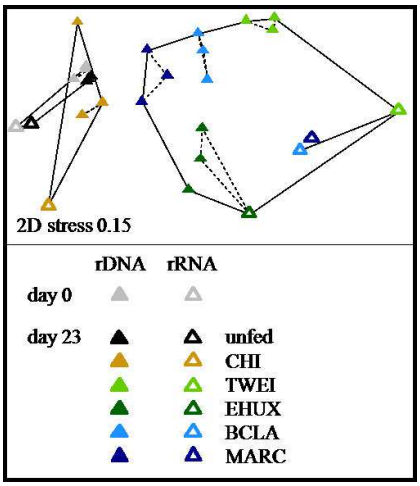
Supplementary Figure S1. **Oxygen.** (A) Modeled O_2 concentration [µM] in the incubated bags as a function of time (in days). Unfed control sediment after 23 days of incubation under 1 atm (unfed) and 250 atm (P250 unfed). (B) Correlation between cell abundance and oxygen respiration rate of the sediment community after 23 days of incubation for the different incubation treatments under atmospheric pressure conditions supporting the oxygen model. Absolute values (solid squares) are shown per replicate of each treatment (n = 3). The black line indicates a 1:1 ratio between each treatment for oxygen and cell abundance measurements; *p* = correlation of Pearson product-moment correlation coefficient. Sediment treatments: unfed control sediment after 23 days of incubation (unfed), sediments amended with chitin (CHI), *Thalassiosira weissflogii* (TWEI), *Emiliania huxleyi* (EHUX), *Bacillaria* sp. (BCLA) and *Melosira arctica* (MARC). Only one measurement of cell abundance was available for MARC-treated sediment.



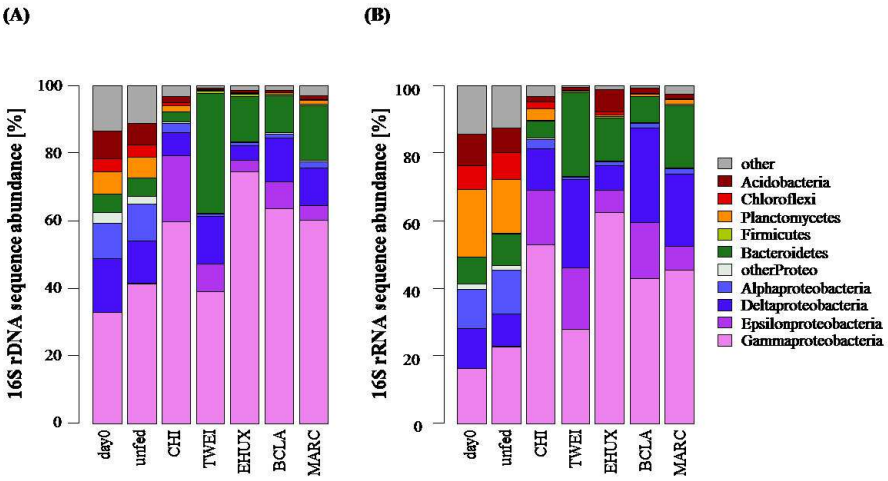
Supplementary Figure S2. **Correlation of cell abundance (A; n = 3), extracellular enzymatic activity (B: chitobiase, C: beta-glucosidase; n = 5), and oxygen respiration rates (D; n = 5) of the sediment community after 23 days of incubation under atmospheric and 250 atm *in situ* pressure conditions**. Mean values (solid circles) are shown with whiskers indicating the range per treatment. The black line indicates identical values under both pressure conditions. Sediment treatments: unfed control sediment after 23 days of incubation (unfed), sediments amended with chitin (CHI), *Thalassiosira weissflogii* (TWEI), *Emiliania huxleyi* (EHUX), *Bacillaria* sp. (BCLA) and *Melosira arctica* (MARC). Only one measurement of cell abundance was available for MARC-treated sediment. Only one measurement of cell abundance was available for MARC-treated sediment.



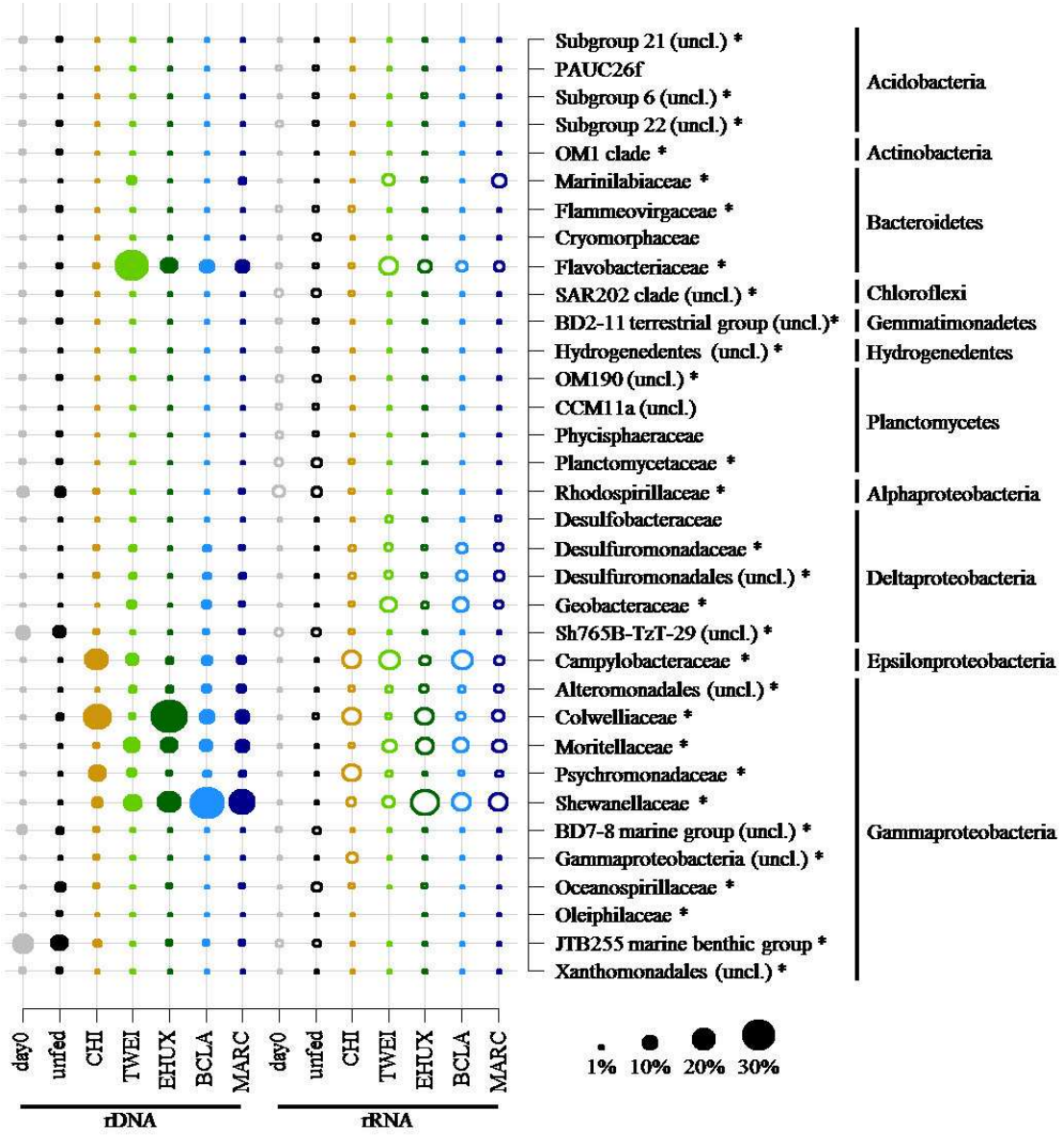
Supplementary Figure S3. **Changes in cell abundance (A; n = 3), extracellular enzyme activity (B: chitobiase, C: beta-glucosidase; n = 5), and oxygen respiration rates (D; n = 5) of the sediment community in the different treatments under 250 atm *in situ* pressure conditions.** Gray bars show the range at the beginning of the incubation. Black dots show measurements after 23 days of incubation, with the horizontal black line indicating the mean value per treatment. Letters indicate groups of treatments that are significantly different from each other based on TukeyHSD at a significance threshold of $p < 0.05$. Sediment treatments: unfed control sediment after 23 days of incubation (unfed), sediments amended with chitin (CHI), *Thalassiosira weissflogii* (TWEI), *Emiliania huxleyi* (EHUX), *Bacillaria* sp. (BCLA) and *Melosira arctica* (MARC). Only one measurement of cell abundance was available for MARC-treated sediment.



Supplementary Figure S4. **Non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis dissimilarity of the total (16S rDNA; n = 3) and active (16S rRNA; n = 1) bacterial community in the different sediment treatments under 250 atm in situ pressure conditions.** Hulls displayed by solid lines are based on a dissimilarity threshold of 70%. Hulls displayed by dashed lines are based on a dissimilarity threshold of 32%, and shows clusters of biological replicates. Sediment treatments: unfed control sediment after 23 days of incubation (unfed), sediments amended with chitin (CHI), *Thalassiosira weissflogii* (TWEI), *Emiliania huxleyi* (EHUX), *Bacillaria* sp. (BCLA) and *Melosira arctica* (MARC). Only one measurement of cell abundance was available for MARC-treated sediment. No rRNA data is available for MARC-treated.



Supplementary Figure S5. **Dominant phyla of the total (A: 16S rDNA; n = 3) and active (B: 16S rRNA; n = 1) bacterial community in the different sediment treatments under 250 atm *in situ* pressure conditions.** For the total bacterial community, sequences from replicate samples were pooled for the calculation of relative sequence abundances. For *Proteobacteria*, class-level resolution is shown. Sediment treatments: unfed control sediment after 23 days of incubation (unfed), sediments amended with chitin (CHI), *Thalassiosira weissflogii* (TWEI), *Emiliania huxleyi* (EHUX), *Bacillaria* sp. (BCLA) and *Melosira arctica* (MARC). No rRNA data is available for MARC-treated sediment.



Supplementary Figure S6. **Dot plot showing relative sequence abundances of dominant bacterial families, and their phylum affiliation, of the total (16S rDNA; n = 3) and active (16S rRNA; n = 1) bacterial community in the different sediment treatments under 250 atm *in situ* pressure conditions.** For the total bacterial community, sequences from replicate samples were pooled for the calculation of relative sequence abundances. For families of the *Proteobacteria*, class-level resolution is shown. For taxa that were unclassified at the respective level of resolution, the next higher taxonomic rank is shown. Asterisks mark differentially abundant taxa between treatments based on 16S rDNA samples (ALDEx2 analysis). All groups that are not marked by an asterisk were only abundant in 16S rRNA data, for which no replicates are available and therefore no analysis of differential abundance could be performed. Sediment treatments: unfed control sediment after 23 days of incubation (unfed), sediments amended with chitin (CHI), *Thalassiosira weissflogii* (TWEI), *Emiliania huxleyi* (EHUX), *Bacillaria* sp. (BCLA) and *Melosira arctica* (MARC). No rRNA data is available for MARC-treated sediment.

Supplementary Tables

Supplementary Table S1. **Test of the oxygen permeability of the bag material (PE) in sea water.** PE-bags were filled with a highly oxygen-reduced (N_2-purged) sterile slurry, and oxygen diffusion into the bags from the surrounding water in the incubators was monitored using optodes. Subsequently, equation (1) was used to back calculate the diffusion coefficient.

Measuring points [h]	**c(O_2) in bags [µmol L^{-1}]**			**Average [µmol L^{-1}]**	**O_2 difference [µmol L^{-1}]**
	Replicate 1	Replicate 2	Replicate 3		
0 (start)	21	23	23	22.3	-
1	32	37	33	34.0	11.7
2	43	44	43	43.2	9.2
3	47	51	45	47.7	4.4
5	61	60	63	61.3	6.8
7	71	73	74	72.4	5.5
10	85	90	87	87.6	5.1
12	95	99	100	98.0	5.2

Supplementary Table S2. **Extracellular enzymes and their target substrates used in this study.** Enzyme classifications and names correspond to the IUBMB enzyme nomenclature. The natural substrates represent examples of a variety of compounds hydrolysed by these enzymes.

Enzyme	**Natural substrate (selection)**	**Artificial assay substrate**	**Final concentration of artificial substrate [µmol L^{-1}]**
Beta-glucosidase (E.C. 3.2.1.21)	cellulose	4-methylumbelliferyl ß-D-glucopyranoside (MUF-ß)	100
N-acetyl-ß-glucosaminidase (E.C. 3.2.1.30)	chitin	4-methylumbelliferyl-N-acetyl-ß-D glucosaminide (MUF-NAc)	100

Supplementary Table S3. **Alpha diversity indices of the bacterial community in the incubation experiment at 250 atm *in situ* pressure conditions.** Sediment treatments: untreated sediment at the beginning of the incubation (day0), unfed control sediment after 23 days of incubation (unfed), sediments amended with chitin (CHI), *Thalassiosira weissflogii* (TWEI), *Emiliania huxleyi* (EHUX), *Bacillaria* sp. (BCLA), and *Melosira arctica* (MARC). Richness and evenness were estimated based on OTU number (nOTU) and the inverse Simpson index (invS) of rDNA (n= 3) and rRNA (n = 1) datasets, and are given as mean ± standard deviation where applicable. Differences between treatments were assessed with ANOVA at a significance threshold of 0.05. Letters indicate significantly different groups based on pairwise TukeyHSD post-hoc test.

		nOTUs		invS	
	treatment	value	TukeyHSD	value	TukeyHSD
rDNA					
	day0	5,777 ± 56	a	359 ± 28	a
	unfed	5,438 ± 139	a	259 ± 73	a
	CHI	2,842 ± 176	b	19 ± 8	b
	TWEI	1,092 ± 76	c	13 ± 0	b
	EHUX	1,693 ± 56	d	27 ± 10	b
	BCLA	1,494± 22	d	18 ± 1	b
	MARC	2,210 ± 157	e	27 ± 2	b
rRNA					
	day0	7,816	NA	1,230	NA
	unfed	7,352	NA	626	NA
	CHI	4,536	NA	30	NA
	TWEI	1,254	NA	16	NA
	EHUX	1,831	NA	25	NA
	BCLA	1,823	NA	18	NA
	MARC	2,962	NA	33	NA

Supplementary Table S4. **Bacterial taxa potentially affected by treatments based on relative OTU abundances at (A) 1 atm and (B) 250 atm.** For the rDNA dataset (n = 3) OTUs are shown, which were detected as differentially abundant between treatments at an adjusted parametric, and an unadjusted non-parametric significance threshold of 0.05, and further exhibited at least a doubling or a decrease by half, as well as an effect size larger than 4 between the start of the incubation and any of the sediment treatments at day 23. Additionally, OTUs from the rRNA (n = 1) dataset are shown, which constituted more than 1% sequence abundance in at least one sample, and exhibited at least a doubling or a decrease by half between the start of the incubation and any of the sediment treatments at day 23. Sediment treatments: untreated sediment at the beginning of the incubation (day0), unfed control sediment after 23 days of incubation (unfed), sediments amended with chitin (CHI), *Thalassiosira weissflogii* (TWEI), *Emiliania huxleyi* (EHUX), *Bacillaria* sp. (BCLA) and *Melosira arctica* (MARC).

Due to its size this table was not included within this thesis. Please refer to the online supporting information to the published manuscript at Frontiers in Microbiology, Aquatic Microbiology (http://journal.frontiersin.org/article/10.3389/fmicb.2017.00266/full#supplementary-material).

Chapter IV

The effect of hydrostatic de- and recompression on bacterial communities sampled from deep-sea sediments

Katy Hoffmann[1,2]*, Christiane Hassenrück[3], and Christina Bienhold[1,2]

[1]HGF-MPG Joint Research Group for Deep Sea Ecology and Technology,
Max Planck Institute for Marine Microbiology, Bremen, Germany
[2]HGF-MPG Joint Research Group for Deep Sea Ecology and Technology,
Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Bremerhaven, Germany
[3] Tropical Marine Microbiology, Leibniz Centre for Tropical Marine Research,
Biogeochemistry/ Geology, Bremen, Germany

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Abstract

Approximately 65% of the Earth`s surface is covered by deep-sea sediments, which are characterized by high hydrostatic pressure. Bacteria dominate the deep-sea benthos in terms of biomass and abundance, and play an important role in carbon remineralization. To study bacterial processes at the seafloor, sediments are typically sampled and returned to the sea surface, and are thereby subjected to pressure changes. Whether or to which extent pressure changes affect deep-sea bacterial viability, diversity, and activity remains largely unexplored, but may strongly affect our judgment of community structure and activity in deep-sea environments. Here we compare bacterial communities from deep-sea sediments collected with a pressure-retaining sediment sampler and push cores that involve decompression of samples, together with an *ex situ* experiment testing the effects of repeated de- and recompression on the bacterial community. We assessed bacterial abundance, extracellular enzymatic activity, community composition and diversity. Enzymatic activities were highest in sediments retrieved with the pressure-retaining sediment sampler, suggesting that enzymatic activities determined from decompressed samples may underestimate *in situ* activities. In contrast, bacterial community composition remained similar across most samples, indicating no or only small effects of de- and recompression. This was challenged by strong changes in community composition at rRNA level in the push core sampled immediately after retrieval, indicating potential changes in the metabolic activity of specific taxa. Considering the need for further sample replication, our results should initiate further discussions on the effects of sample retrieval from high-pressure environments on bacterial communities.

Keywords

hydrostatic pressure, pressure corer, pressure incubator, decompression effects, 16S Illumina sequencing, surface sediments.

Introduction

Deep-sea environments below 200 m water depth make up approximately 65% of the Earth's surface, and are characterized by high hydrostatic pressure, darkness, and cold temperatures. Hydrostatic pressure increases by approximately 1 atmosphere (1 atm = 1 x 10^5 Pa) for every 10 m of water depth. With an average water depth of 3,800 m, the majority of oceanic environments are thus subject to hydrostatic pressures of several 100 atm (Jannasch and Taylor, 1984). Yet, the role that hydrostatic pressure plays for the activity and distribution of life in the deep sea is only vaguely understood (Chastain, 1991; Nagata et al., 2010; Tamburini et al., 2013).

Bacteria are highly abundant and diverse down to the deepest parts of the oceans, and even occur at hundreds of meters below the seafloor in deep subsurface sediments (Fry et al., 2008; Inagaki et al., 2015; Orcutt et al., 2013). In deep-sea surface sediments, bacteria account for up to 90% of the total benthic biomass (Pfannkuche, 1992; Rowe, 1991) and serve as essential catalysts for carbon and nutrient recycling (Deming and Baross, 1993). Therefore, bacterial communities are key to understanding matter fluxes in the deep sea and potential feedback mechanisms as a consequence of environmental changes. Many isolated strains from the deep sea are piezophilic (formerly 'barophilic') (Zobell & Johnson 1949; Bartlett 2002), i.e. they show optimal growth at pressures higher than atmospheric pressure (Jebbar, 2015; Katoh and Toh, 2008; Lauro et al., 2013). But many deep-sea isolates are also 'piezotolerant', i.e. they tolerate high pressure, but have their growth optimum at 1 atm (Lauro et al., 2013; Nogi et al., 1998; Xu, 2003). The distribution of these traits across the diverse taxonomic groups in deep-sea sediments, and the respective relevance for ecosystem functioning remains unknown.

Little is known about the effects of changes in hydrostatic pressure on the activity of indigenous bacterial populations, such as the expression and function of enzymes, respiration rates, cell division, growth, and DNA replication (Bartlett, 2002; Hoffmann et al., 2017; Picard and Ferdelman, 2011). Additionally, it is not well understood if and how decompression during sampling leads to loss of DNA and RNA and how this affects our ability to decipher community composition and richness in deep-sea sediments. Methodologically, the retrieval of pressurized deep-sea sediment samples, and the preservation of *in situ* pressure conditions throughout laboratory manipulation experiments are major challenges in deep-sea research (see e.g., Kallmeyer et al. 2003; Abegg et al. 2008), and most studies involve slow (i.e. over few hours during retrieval

of sampling platforms) decompression during the process of sample recovery and experimentation (Boetius and Damm, 1998; Boetius and Lochte, 1994; Eardly et al., 2001; Hoffmann et al., 2017).

Experimental results on the effects of pressure on microbial activity vary widely, and also depend on the duration of experiments. Early publications suggested a suppression of activity under high pressure conditions for incubation periods of several days to 51 weeks (Jannasch et al., 1973; Jannasch and Wirsen, 1982). However, recent data indicate either no effect (Danovaro et al., 2008) or higher activities of deep-sea microbes under *in situ* pressure conditions when compared to atmospheric pressure for the same time scales (Nagata et al., 2010; Tamburini et al., 2013b). It is thus difficult to draw conclusions about the inhibitory or stimulatory effects of hydrostatic pressure on deep-sea microbial community activity, i.e. whether we under- or overestimate activity in *ex situ* measurements. Also the effects of successive pressure shocks on the metabolic rates of natural microbial populations are underexplored (Tamburini et al., 2013). To our knowledge, no studies have yet explored the effects of changes in hydrostatic pressure during sample retrieval on the diversity of the total and active fraction of benthic bacterial communities in the deep sea at a high taxonomic resolution. Changes in bacterial community structure may originate from losing or fragmenting DNA during the sampling process, e.g. because of the disintegration of cells. Further research is thus needed in order to validate *in situ* turnover rates in deep-sea sediments, and to identify taxonomic groups of bacteria that are most strongly affected by changes in pressure.

In order to investigate the effects of pressure changes on a complex natural bacterial community, we retrieved deep-sea surface sediments from 2,500 m water depth in the Arctic Ocean, using a pressure-retaining sediment sampler (hereafter referred to as pressure corer), and a standard remotely operated vehicle (ROV)-operated sediment corer (push corer). We assessed i) the effects of sudden (seconds) versus slow changes in pressure (~ 3 hours), as well as ii) immediate and delayed (7 days) effects of repeated de- and recompression events on bacterial community activity and diversity. This included measurements of extracellular enzyme activity, cell abundance, and the composition of the total and active fraction of the bacterial community via sequencing of the 16S rRNA gene (hereafter referred to as rDNA) and the 16S rRNA (hereafter referred to as rRNA). The overall aim was to evaluate potential biases in the interpretation of bacterial community measurements that may be associated with changes in hydrostatic pressure during sample retrieval and subsequent experimentation.

Methods

Sediment samples were obtained from soft-bottom deep-sea sediments at the central station of the Long-Term Ecological Research Observatory (LTER) HAUSGARTEN located in the Fram Strait (79° 03.86' N, 4° 10.85' E; 2,470 m water depth). Undisturbed sediment samples were either retrieved using a pressure corer (courtesy of Fumio Inagaki and Yuki Morono, JAMSTEC, Japan) or push cores (dimensions: 0.07 x 0.2 m). Sampling was conducted with the ROV QUEST 4000m (MARUM, Bremen, Germany) during RV Polarstern cruise PS93.2 in 2015 (pressure corer samples: PS93/081-01; push core samples: PS93/081-01 and PS93/052-1; Soltwedel, 2015).

The pressure corer consisted of a stainless steel chamber, into which a single sediment core was placed, and sealed by a valve, maintaining in situ hydrostatic pressure conditions during sample retrieval (Figure 1). Comparative sediment sampling was conducted with twelve push cores during the same and a subsequent ROV dive. These sediment samples were decompressed during retrieval from the seafloor over a time frame of 2 to 3 hours. One pressurized sediment core could be retrieved with the pressure corer from a water depth of 2,500 m maintaining at least 100 atm. Immediately after sediment retrieval, hydrostatic pressure was released from the pressure corer within a time frame of approximately 2 seconds, and the first 2 cm of sediment from the pressure corer as well as of one push core were sampled using cut-off plastic syringes (day 0). From each core, 2 mL of surface sediment were frozen at -20°C for subsequent DNA extraction and 5 mL were frozen at -80°C for RNA extraction. Two subsamples of 1 mL each from the pressure corer and five subsamples from the push core were taken and prepared for microbial cell counting. Another five subsamples from each core were taken for onboard measurements of the extracellular enzymatic activity of chitobiase and beta-glucosidase, which are needed for the degradation of e.g. chitin and cellulose, respectively. Both substrates represent abundant compounds in the marine environment (Boetius and Lochte, 1994; Lee, 1980).

For the *ex situ* recompression experiment, the first 2 cm of the remaining eleven push cores were combined into one sterile glass bottle and transferred into a cold room (0°C, *in situ* temperature). The sediment was 3.5-fold diluted with sterile-filtered bottom water (35‰, 0°C). The well-mixed slurry was equally divided into 40 replicates of 35 mL each, and sealed into sterile, gas-permeable polyethylene (PE) bags with no headspace or air bubbles (experimental work-flow see Figure S1). Subsequently, recompression experiments were started within 12 hours after sample retrieval, using a

pressure incubator described in Boetius and Lochte (1994). Ten of the 40 PE-bags served as controls without any recompression. The remaining 30 PE bags were added to the pressure incubator filled with fully oxygenated sterile-filtered bottom water. Pressure was applied as hydrostatic pressure to *in situ* pressure (250 atm), using a mechanical pump. Hydrostatic pressure was maintained in the incubator for five minutes, followed by a sudden release back to atmospheric pressure conditions. Ten PE-bags each were removed after 1, 5 and 10 cycles of re- and decompression. Five out of the ten PE-bags from the control and the recompression treatments were sampled immediately (day 0.5) for microbial cell counting, extracellular enzymatic activity and molecular analysis as described above. The other five PE-bags were incubated in darkness at 0°C and atmospheric pressure in fully oxygenated sterile-filtered bottom water for seven days (day 7) to test for longer term effects of pressure changes on the bacterial community (Figure S1).

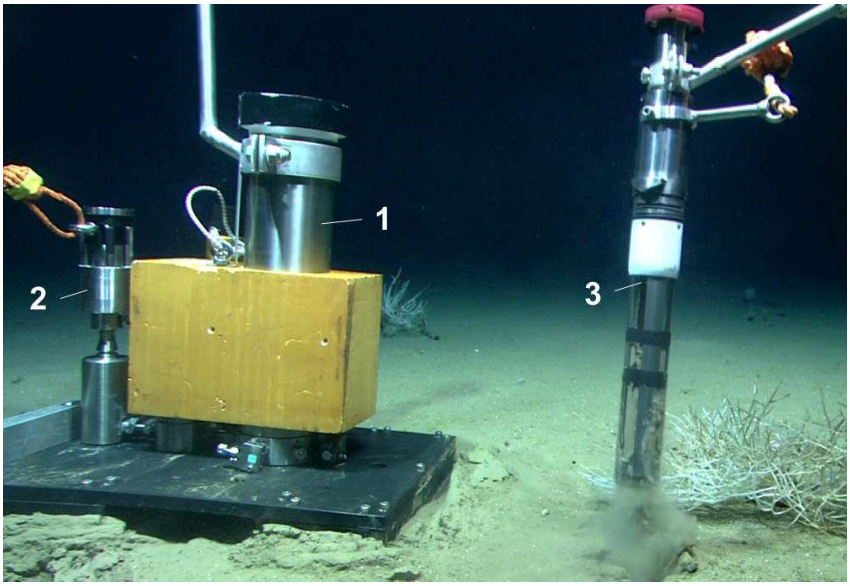

Figure 1. **Sediment sampling procedure at the deep seafloor using a pressure corer provided by of Fumio Inagaki and Yuki Morono, JAMSTEC, Japan.** 1) chamber, 2) pressure valve, 3) pressure corer. Image courtesy of ROV QUEST 4000 MARUM – Center for Marine Environmental Sciences, University of Bremen.

Microbial cell counts using Acridine Orange staining, as well as extracellular enzymatic activity measurements were performed as described by Hoffmann and colleagues (2017). Values were calculated per volume of undiluted sediment. Differences between the pressure corer and the push core at day 0 were assessed with t-tests, whereas the effects of the recompression treatments and resting time were assessed using ANOVA (analysis of variance) with TukeyHSD post-hoc tests (Hothorn et al., 2008). The composition of the total and metabolically active fraction of the bacterial community was investigated as described previously by sequencing the V3-V4 region of 16S rDNA and the 16S rRNA (Hoffmann et al., 2017). No replication of rDNA and rRNA samples was possible due to the limited amount of sample material; therefore only one sample per treatment was sequenced. Sequence quality control, clustering into operational taxonomic units (OTUs) and taxonomic classification also followed the workflow described by Hoffmann and colleagues (2017). All statistical analyses were conducted in R using the R core distribution (version 3.3.0; R Development Core Team, 2014) and the following additional packages: vegan (Oksanen et al., 2015), gplots (Warnes et al., 2016), and ALDEx2 (Fernandes et al., 2014).

Data are accessible via the Data Publisher for Earth & Environmental Science PANGAEA (Hoffmann et al., 2017; https://doi.pangaea.de/10.1594/ PANGAEA.874784). Raw paired-end, primer-trimmed reads are available under ENA Accession Number: ERP022564. The data were archived using the brokerage service of GFBio (Diepenbroek et al., 2014).

Results and Discussion

Microbial cell abundances in sediments retrieved from the central HAUSGARTEN station either with the pressure corer or with push cores were on average 1.9 ± 0.2 x 10^9 cells (mL sediment)$^{-1}$ (Figure 2A). Cell numbers remained stable over the different cycles of re- and decompression and were comparable to cell numbers from this site reported in other studies (Hoffmann et al., 2017; Schewe and Soltwedel, 2003). After seven days of resting, cell numbers increased in all recompression treatments (1x, 5x, 10x), and in the control evenly to 2.4 ± 0.2 x 10^9 cells (mL sediment)$^{-1}$ (Table 1). The increase in microbial cell abundance by approximately 25% over one week was within the range of the estimated microbial generation times of four weeks at HAUSGARTEN in unfed surface sediment samples (Hoffmann et al., 2017). Our results indicated that cell integrity was preserved and that the sediment microbial community remained viable after a maximum of ten cycles of re- and decompression (Table 1). This is in accordance with studies showing that bacterial death upon decompression was mainly restricted to gas-vacuolated bacteria from surface waters (Hemmingsen and Hemmingsen, 1980). In contrast, bacteria without gas vacuoles were reported to survive rapid decompression from up to 1,048 atm (Chastain, 1991; Hemmingsen and Hemmingsen, 1980).

Table 1. **Analysis of variance (ANOVA) testing for the effects of pressure (repeated re- and decompression) and time (0.5 and 7 days) on microbial cell counts and extracellular enzymatic activities; n = 5.**

	Tested effect	F-ratio	df[a]	*p*-value
Cell counts	Pressure	1.7	3,32	0.189
	Time	1,444.1	1,32	< 0.001
	Pressure x Time	0.5	3,32	0.684
Chitobiase	Pressure	43.2	3,32	< 0.001
	Time	26.4	1,32	< 0.001
	Pressure x Time	17.5	3,32	< 0.001
Beta-glucosidase	Pressure	7.2	3,32	0.001
	Time	404.2	1,32	< 0.001
	Pressure x Time	3.5	3,32	0.026

[a] degrees of freedom

Chitobiase and beta-glucosidase enzymatic activities were highest in sediments retrieved with the pressure corer (0.028 ± 0.0008 and 0.007 ± 0.0009 µmol mL^{-1} d^{-1}, respectively) compared to push core samples that were slowly decompressed within 2 to 3 hours (0.023 ± 0.0002 and 0.005 ± 0.0003 µmol mL^{-1} d^{-1}, respectively; Figure 2B and

C). This difference was significant for chitobiase activity (Welch's t-test, $t = 13.0$, $df = 4.5$, $p < 0.001$), but not for beta-glucosidase activity. Both enzymatic activities decreased from the first sampling (day 0) to the start of the recompression experiment approximately 12 hours later (0.009 ± 0.0002 and 0.003 ± 0.0004 µmol mL^{-1} d^{-1}, respectively). This provides further evidence that enzymatic activities determined from decompressed samples may underestimate *in situ* activities (Nagata et al., 2010). During repeated re- and decompression, time as well as hydrostatic pressure changes had significant effects on the activity of both analyzed enzymes (Table 1). At day 0.5, highest activities for both enzymes were recorded after five cycles of re- and decompression (Figure 2B and C). Chitobiase activity varied between 0.010 and 0.015 µmol mL^{-1} d^{-1} among recompression treatments and sampling times, although no consistent patterns were observed (Figure 2B). In contrast to chitobiase, beta-glucosidase activities increased significantly by 31% at day 7, and there was no difference between the different recompression treatments anymore (Table 1; Figure 2C). Overall, the differences in enzymatic activities between the recompression treatments were ambiguous, without showing a clear trend related to effects of hydrostatic pressure variations. This is consistent with previous studies, which have reported variable results, depending on the substrates and enzymatic activities (or other measures of activities) investigated. Several earlier studies have described that bulk hydrolytic enzyme activities, including chitobiase and beta-glucosidase, may be associated with piezotolerant communities (Meyer-Reil and Köster, 1992; Riemann and Helmke, 2002), with similar activities at atmospheric and *in situ* pressure (Lochte, 1993; Poremba, 1995)**.** Other studies have reported conflicting results, e.g. the adaptation of extracellular enzymes, including chitobiase, to certain pressure regimes (Herndl and Reinthaler, 2013; Hood, 1973; Somero, 1992). One explanation for the ambiguous results obtained in this study may be the measurement of bulk enzymatic activities, since different bacterial groups may express homologous enzymes that are more or less sensitive to pressure (Herndl and Reinthaler, 2013; Somero, 1992). It is thus difficult to generalize results, although we see clear indications for a pressure effect on bulk chitobiase activity in deep-sea sediments. Future research will need to resolve in more detail the specific pressure adaptations of various bacterial groups and corresponding metabolic pathways.

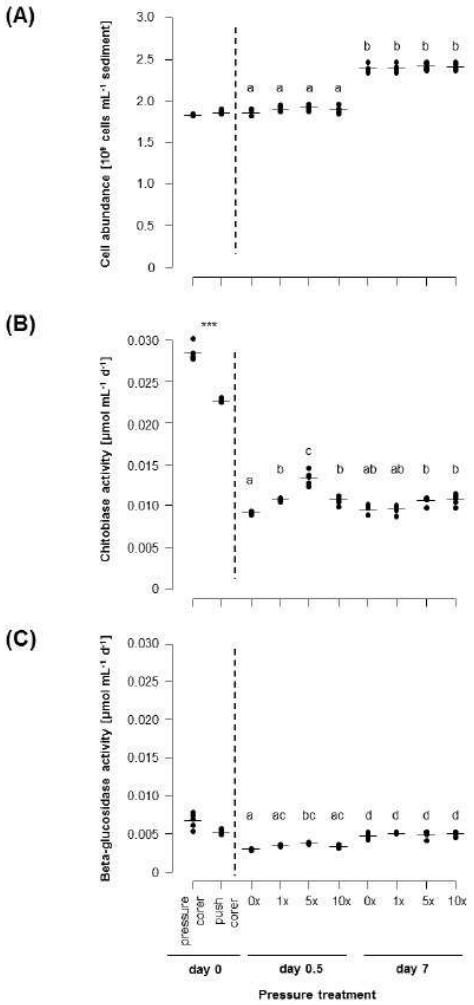

Figure 2. **Changes in cell abundance (A) and extracellular enzyme activity (B: chitobiase, C: beta-glucosidase), of the sediment community in the different pressure treatments.** Black dots indicate individual measurements with the horizontal black line representing the mean value per treatment. The dotted line visually separates samples analyzed directly after sampling (day 0) from those analyzed after an incubation time of 0.5 to 7 days. Lower case letters indicate groups of treatments that are significantly different from each other based on TukeyHSD at a significance threshold of $p < 0.01$. Differences between treatments tested via Welch's t-test are marked with asterisk following the significance code $p < 0.001$ '***'. Sediment treatments: sediment samples retrieved under pressure using a pressure corer and subsampled right after depressurization (pressure corer), sediment samples from a push corer decompressed during retrieval (push corer), sediment samples 0x, 1x, 5x or 10x re-and depressurized and analyzed immediately afterwards (day 0.5) or after 7 days. Only two measurements of cell abundance were available for sediments retrieved via pressure corer, allowing no statistical testing, otherwise n = 5.

High-throughput sequencing allowed us to analyze the effect of changes in hydrostatic pressure on bacterial community composition and diversity at a high taxonomic resolution. With this approach we were able to identify bacterial groups potentially affected by short term decompression effects, such as the loss of rDNA and rRNA within 2 to 3 hours as well as bacterial groups that appeared rather unaffected. In total, 34,781 to 173,432 sequences were generated per sample, corresponding to 31,339 swarmed, non-

singleton OTUs (Table 2). Within most sediment samples, the total (rDNA) and active fraction (rRNA) of the bacterial community had an intra-sample average Bray-Curtis (BC) dissimilarity of 60% on OTU level and an average OTU turnover of 70%, which is in agreement with other publications from the same sampling site (Hoffmann et al., 2017) (Table 2). Bacterial communities in sediment retrieved with a push core and sampled directly after retrieval showed the highest BC and OTU turnover between rRNA and rDNA, i.e. 95% and 92%, respectively. Within the total fraction of the bacterial community (rDNA), all samples were very similar to each other with a BC dissimilarity of 20 to 40%, and they displayed an OTU turnover of 55 to 65% (Figure S2). Similar patterns were observed for the active fraction (rRNA), e.g. BC dissimilarity of 20 to 40% and OTU turnover of 50 to 60% between the different samples, excluding the push core sample at day 0 (Figure S2). These values are in the same range as previous estimates of the heterogeneity of bacterial communities between deep-sea sediment samples from the same site (Hoffmann et al., 2017). Repeated de- and recompression of deep-sea sediment samples thus did not result in significant differences in community structure at the level of rRNA or rDNA. An exception to this pattern was observed in the active bacterial community (rRNA) in the push core sample at day 0. The high dissimilarity to other samples was caused by a highly reduced number of observed OTUs and a concurrent low inverse Simpson diversity of 24 compared to an average of 900 across all other samples (Table 2).

Table 2. **Sequence numbers as well as alpha and beta diversity indices of the bacterial community.** Number of sequences and observed OTUs per treatment and the inverse Simpson index of rDNA and rRNA (n = 1) data sets are displayed, as well as intra-sample Bray-Curtis dissimilarities (BC) and OTU turnover (Jaccard dissimilarity; JC) between rDNA and rRNA. Sediment treatments: sediment samples retrieved under pressure using a pressure corer and subsampled immediately after decompression (pressure corer), sediment samples from a push corer completely decompressed during retrieval (push corer), sediment samples 0x, 1x, 5x or 10x re-and decompressed and analyzed immediately afterwards (day 0.5) or seven days later (day 7).

Treatment	Sequence number		OTU number		Inverse Simpson		BC	JC
	rDNA	rRNA	rDNA	rRNA	rDNA	rRNA		
Day 0								
Pressure corer	65,846	137,846	5,606	9,039	319	968	0.59	0.72
Push corer	57,639	101,442	5,861	1,184	322	24	0.95	0.92
Day 0.5								
0x	52,617	34,781	6,320	8,005	292	924	0.58	0.68
1x	56,110	82,651	6,241	8,183	281	907	0.58	0.67
5x	84,050	98,100	6,153	8,701	262	928	0.56	0.65
10x	173,432	127,716	6,549	8,647	303	1,007	0.55	0.60
Day 7								
0x	62,766	132,557	6,466	8,165	358	872	0.53	0.66
1x	43,446	67,262	5,861	8,464	290	1,000	0.57	0.69
5x	83,887	83,804	4,743	8,344	299	771	0.59	0.72
10x	47,514	36,280	5,224	8,279	309	929	0.57	0.70

The sediment community in all samples was dominated by *Gamma*-, *Delta*- and *Alphaproteobacteria*, together comprising more than 50% of 16S rDNA and rRNA sequences per sample, as well as by members of the *Acidobacteria*, *Phycisphaerae*, *Flavobacteria*, *Acidimicrobiia*, *Epsilonproteobacteria* and the SAR202 clade of the *Chloroflexi,* all of which showed relative sequence abundances between 3 and 5%. Thus, the bacterial community structure was very similar to previous reports from this site (Jacob et al., 2013). Overall, the bacterial community in all samples and on both genetic levels (rDNA and rRNA) was dominated by 32 OTUs, which had relative sequence abundances of at least 1% in at least one sample. Excluding the 16S rRNA community of the push core sample at day 0, these taxa comprised on average 13% of the community on rDNA and 5% on rRNA level. Repeated re- and decompression did not substantially change community structure, neither immediately after the compression cycles, nor after a time lag of 7 days (Figure 3). Therefore, we conclude that although (repeated) fast de- and recompression to up to 250 atm affected extracellular enzymatic activities, especially chitobiase, it does not appear to significantly affect bacterial viability and thereby diversity. This is in agreement with studies by Follonier and colleagues (2012), who

reported that changes in hydrostatic pressure start to show effects on molecular systems only from 300 atm onwards. This observation could also be explained by the slow community turnover in deep-sea sediments, with generation times of ~30 days at HAUSGARTEN in unfed surface sediment samples (Hoffmann et al. 2017). Accordingly, in this experiment, bacterial cell numbers only increased by 25% over 7 days, and this did not lead to a significant shift in community structure

Prominent differences in bacterial community structure compared to all other samples were, however, observed at rRNA level for sediments sampled right after push core retrieval (day 0; Figure 3; Figure S2). Here, the community was dominated by 20 of the 32 OTUs, which were, with one exception, only found in this sample with relative sequence abundances of at least 1% (Figure 3). In total they accounted for approximately 67% of all rRNA sequences in the push core sample at day 0. This uneven OTU distribution, reduced number of total OTU, and high dominance of only a few OTUs were also reflected in the low inverse Simpson index calculated for this sample (Table 2). The drastic change in community alpha diversity was not observed at rDNA level. Compared to the other samples in the data set, rRNA sequences from the push core sample (day 0) showed a higher proportion of OTUs affiliated with the gammaproteobacterial genera *Moritella*, *Colwellia*, and unclassified *Shewanellaceae*, and *Alteromonadales* as well as unclassified *Flavobacteriaceae*, the epsilonproteobacterial *Arcobacter*, and deltaproteobacterial *Geopsychrobacter*, *Desulfuromonas*, and unclassified *Desulfuromonadales* (Figure 3).

The observed discrepancy between the composition of the total (rDNA) and active (rRNA) fraction of the community from sediments sampled right after push core retrieval on day 0 could be caused by a methodological bias, and we currently lack replication to verify this pattern. However, no technical problems occurred during sample retrieval, preservation, RNA extraction, library preparation, and sequencing, and the obtained RNA extract was of high quality, comparable to all other samples (Figure S3). A technical bias thus appears highly unlikely. Yet, sample replication for rDNA and rRNA samples is needed to substantiate these results. From a biological point of view, the increased relative sequence abundance at rRNA level may be caused i) by an increased activity of the aforementioned taxa or ii) by a decreased activity of all other bacterial groups.

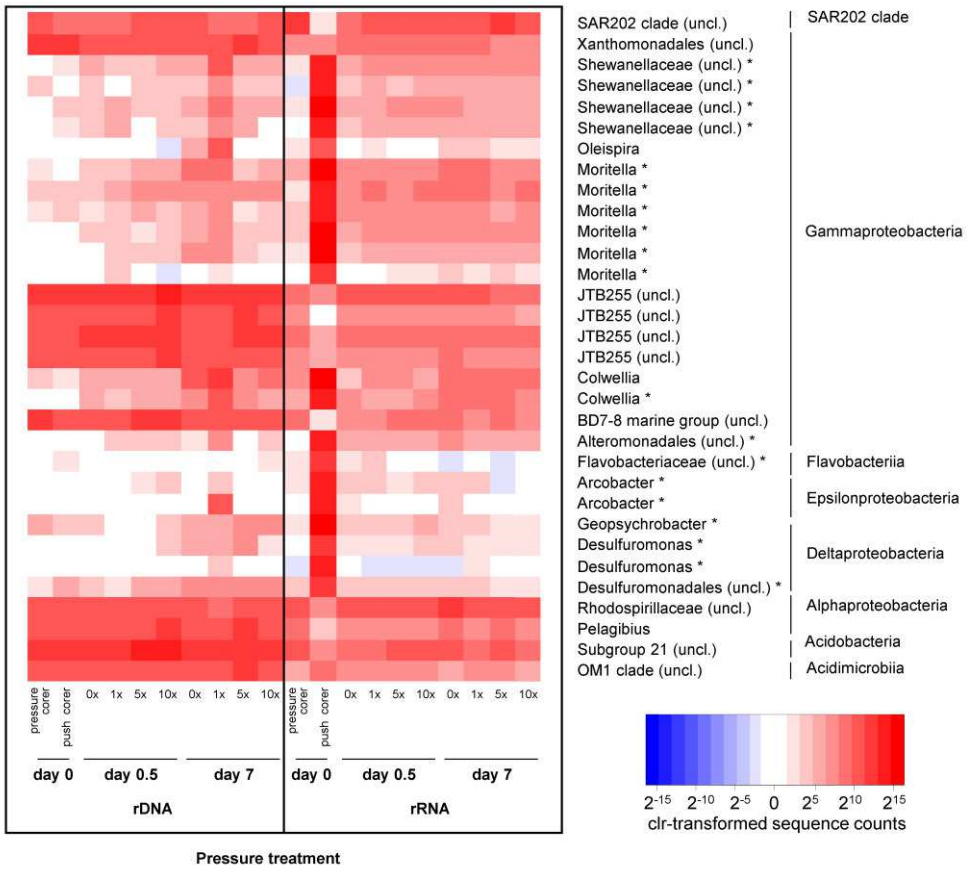

Figure 3. **Heatmap of the sequence proportion of dominant OTUs of the total (16S rDNA; n = 1) and active (16S rRNA; n = 1) bacterial community in the different pressure treatments.** Taxonomic affiliation given on genus and class level. Sequence counts were centered log ratio (clr-) transformed using the aldex.clr function in R with 128 Dirichlet instances (Fernandes et al., 2014). Only abundant taxa with a relative sequence abundance of at least 1% (on OTU level) in at least one sample are shown**.** For taxa that were unclassified (uncl.) at the respective level of resolution, the next higher taxonomic rank is shown. Asterisks highlight the taxa that only showed relative sequence abundance of at least 1% in the rRNA community of the push core sample at day 0. JTB255: JTB255 marine benthic group. Sediment treatments: sediment samples retrieved under pressure using a pressure corer and subsampled right after depressurization (pressure corer), sediment samples from a push corer decompressed during retrieval (push corer), sediment samples 0x, 1x, 5x or 10x re-and decompressed and analyzed immediately afterwards or after 7 days.

Addressing explanation i), most of the piezophilic and piezotolerant bacteria characterized so far, are affiliated with the class *Gammaproteobacteria* (Kato and Qureshi, 1999), specifically the genera *Colwellia*, *Shewanella*, *Photobacterium*, *Oceanospirillaceae* and *Moritella* (see e.g. Kato et al., 1998; Methe et al., 2005; Nogi et al., 2004; Wang et al., 2008; Peoples and Bartlett, 2017). Pressure-regulated genes have been described for several of these groups and studies have indicated an extensive restructuring of piezophilic cell machineries, when exposed to atmospheric pressure (Kato et al., 1995, 1997; Kato and Qureshi, 1999). The observation that several of the dominant taxa in the rRNA sample from the push core (day 0) include potential piezophilic members thus suggests that an increased activity of these groups may present a stress response to decompression. Conversely, addressing explanation ii), this would suggest that the majority of the deep-sea sediment bacterial community would be impaired by decompression. This appears unlikely, as most piezophilic bacteria were retrieved from water depths greater than 2,500 m. Still, whether i) few taxa strongly increased in activity through decompression or ii) the majority decreased in activity, cannot be fully resolved within this study due to the compositional character of our data (Tsilimigras and Fodor, 2016). The obvious difference between the push core sample (day 0) with the distinct rRNA profile and every other sample, was the duration of the decompression process. The sediment retrieved with the pressure corer was decompressed within two seconds, which was also the case for the decompression events during the *ex situ* recompression experiments (day 0.5 and day 7), while the push core sample was decompressed over 2 to 3 hours during retrieval of the ROV. Notably, after approximately 12 hours of storage at 1 atm (day 0.5), the sediment community in the push core sample showed a high similarity between rRNA and rDNA community profiles again (Figures 3 and S2). Ribosomal RNA is hypothesized to have a half-life of 30 minutes up to four hours (Basturea et al., 2011; Molin et al., 1977). Therefore, the fast decompression with the pressure corer and pressure incubators followed by an immediate sampling did probably not leave enough time to observe changes in rRNA expression patterns. At the same time, the resting of samples for 7 days then provided enough time for a restructuring of the cells and the return to a previous state (Peoples and Bartlett, 2017). This would explain why we only observed the conspicuous change in rRNA community structure for the sample analyzed directly after sediment retrieval with a decompression time of approximately 3 hours during sample recovery. Our results hence suggest that there may be a lag phase before changes at rRNA level can be observed, and that there may be a recovery back to

the initial community structure after a maximum of 12 hours (Figure 3). Community profiles then remained similar even after 7 days of resting/storage at atmospheric pressure, and samples clustered together with BC dissimilarities of 43% and an OTU turnover of 65%, (Figure S2). These values are close to the range observed among PCR replicates of the same sample (Hassenrück, pers. comm.), and support our hypothesis that decompression effects might be reversible after a resting period.

The implications of our results still need to be further explored and validated by sample replication. However, our observations may then be highly relevant for sampling and analyses of rRNA from bacterial deep-sea communities, where potential inferences about *in situ* physiological states and activities are of interest, especially considering that sample retrieval for benthic deep-sea research usually involves slow sample decompression and variable time intervals before sample preservation. Similar conclusions were drawn from other studies, e.g. Edgcomb and colleagues (2014) observed altered community gene expression for water column samples obtained from the deep sea (2,222 m), by applying metatranscriptomics to samples preserved *in situ* (no decompression) and collected with niskin bottles (decompression during sample recovery). Their results indicate, e.g. an enhanced transcription of genes associated with cell membrane repair in decompressed samples (Edgcomb et al., 2014). An *in situ* preservation of samples from the deep sea should thus be the preferred way of sampling, and further technological developments are required to make these sampling strategies more feasible. In addition, our results may initiate discussions on how short resting periods after sample retrieval may help to recover an initial community status for decompressed samples, but this clearly needs to be further explored and replicated. It also remains to be investigated whether our observations pertain to sample retrieval from shallower and deeper water depths. Future experiments should thus target a range of water depths between 1,000 and 10,000 m and include replication for rDNA and rRNA samples to verify observed changes in the composition of the active and total bacterial community. Furthermore, they should aim for a preservation of sediment samples *in situ*, directly after retrieval onboard after slow decompression, and several hours after retrieval. Expanding the molecular analysis of the sediment community to metatranscriptomics would further allow the identification of the genes involved in a potential stress response to pressure changes.

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Supplementary Figures

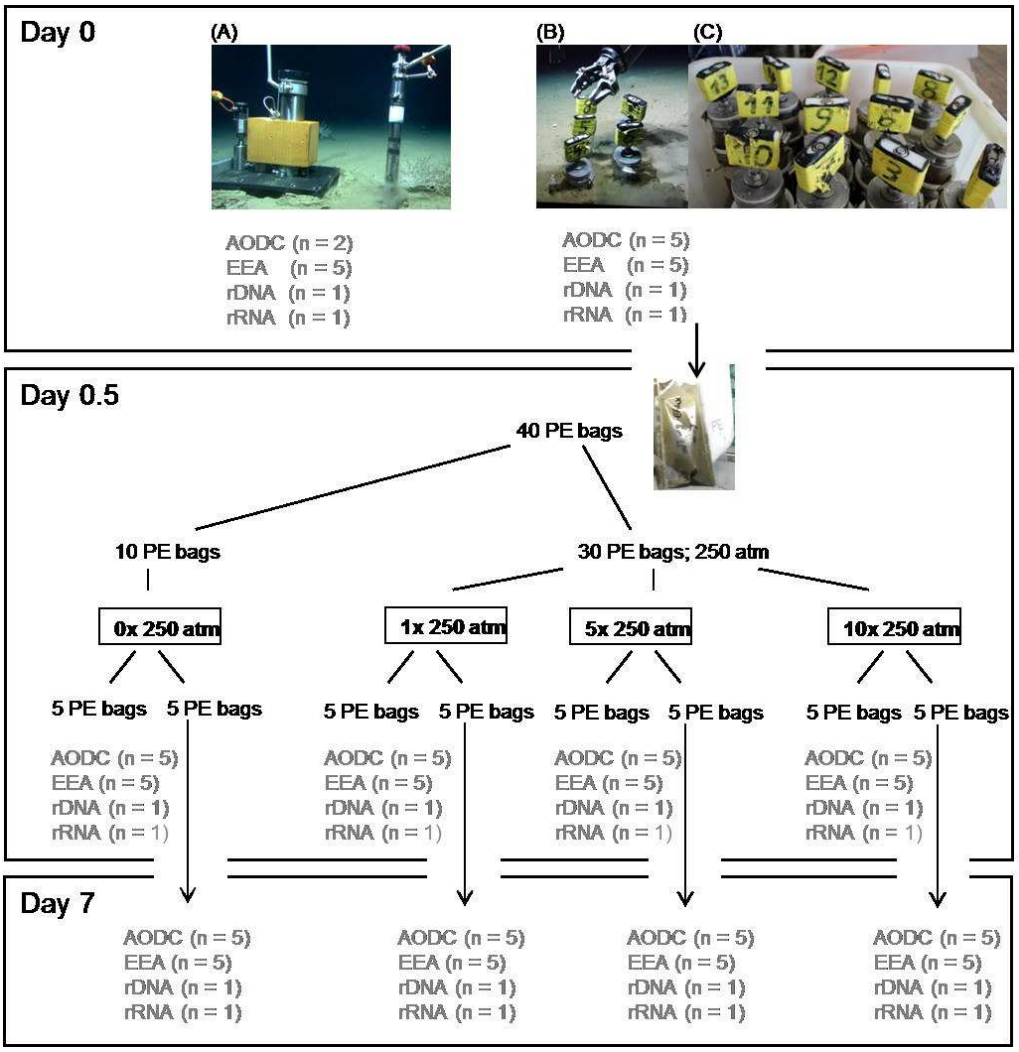

Figure S1. **Work flow of the hydrostatic pressure experiment.** (A) Pressure corer, (B and C) Push cores; For A and B: image courtesy of ROV QUEST 4000 MARUM – Center for Marine Environmental Sciences, University of Bremen; C: image courtesy Josephine Rapp, AWI. Sediment treatments: sediment samples retrieved under pressure using a pressure corer and subsampled right after depressurization (pressure corer), sediment samples from a push corer decompressed during retrieval (push corer), sediment samples 0x, 1x, 5x or 10x re-and depressurized and analyzed immediately afterwards (day 0.5) or after 7 days. PE bags: Polyethylen bags; AODC: Acridine Orange Cell Counts; EEA: extracellular enzymatic activity.

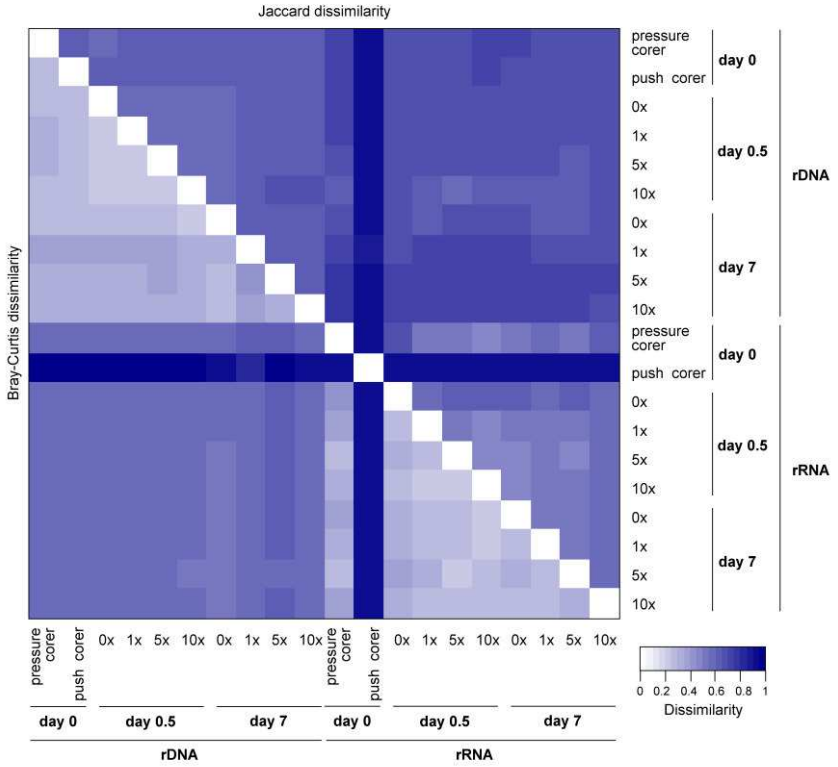

Figure S2. **Dissimilarity heatmap.** Pairwise Bray-Curtis (lower triangle) and Jaccard dissimilarity (OTU turnover; upper triangle) of the total (rDNA; n = 1) and active (rRNA; n = 1) bacterial community in the different pressure treatments. Sediment treatments: sediment samples retrieved under pressure using a pressure corer and subsampled right after depressurization (pressure corer), sediment samples from a push corer decompressed during retrieval (push corer), sediment samples 0x, 1x, 5x or 10x re-and decompressed and analyzed immediately afterwards or after 7 days.

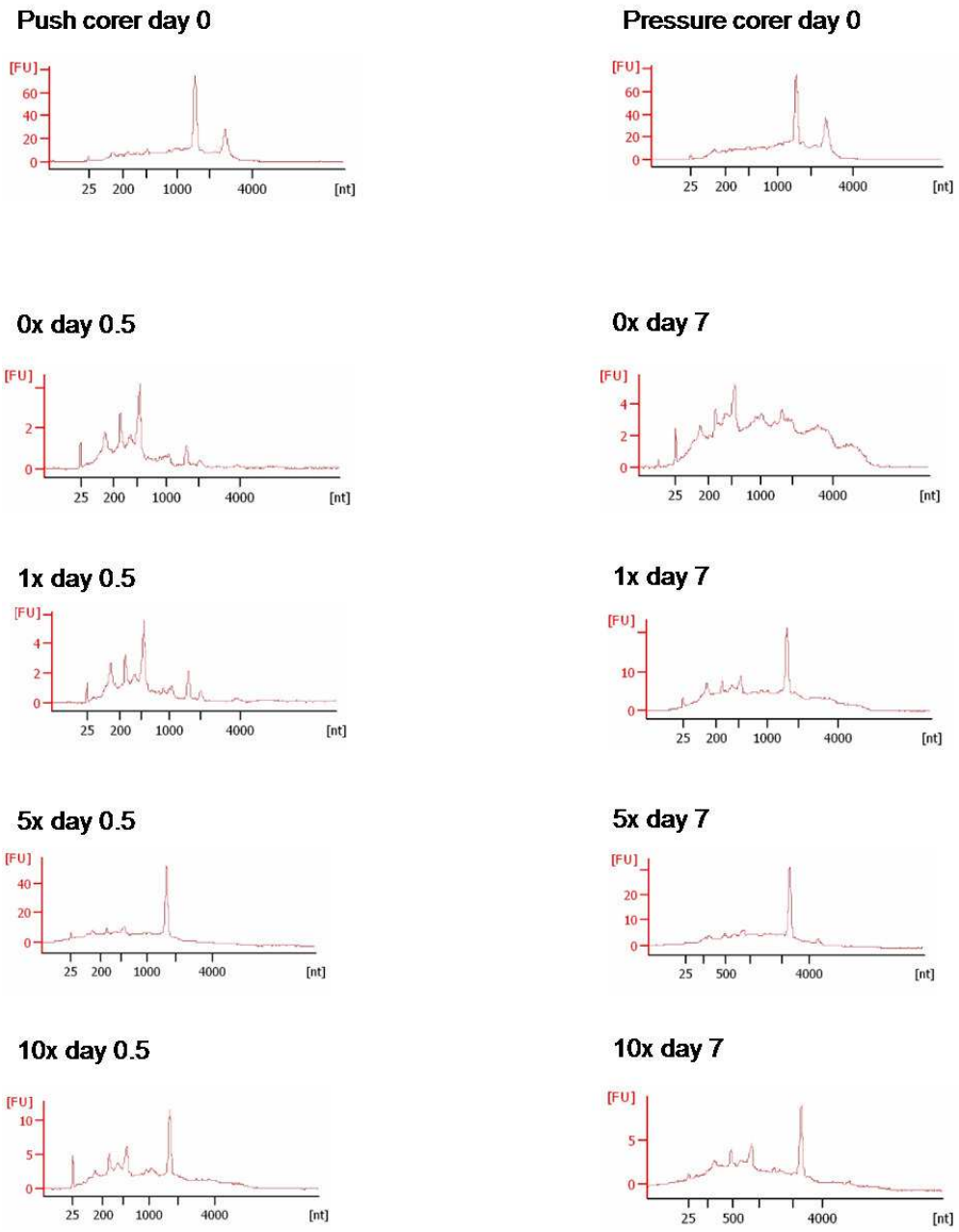

Figure S3. **Prokaryotic total RNA analysis(16S rRNA; n = 1)**. 2100 bioanlyzer. Expected peak for 16S rRNA signal at round 1,800 nt. FU: height threshold. Sediment treatments: sediment samples retrieved under pressure using a pressure corer and subsampled right after depressurization (pressure corer), sediment samples from a push corer decompressed during retrieval (push corer), sediment samples 0x, 1x, 5x or 10x re-and depressurized and analyzed immediately afterwards or after 7 days.

3. Discussion

Marine deep-sea sediments represent one of the largest biomes on Earth, which, at the same time, remain one of the most remote and least explored and understood ecosystems. Deep-sea sediments are dominated by bacteria in terms of biomass and abundance, and their potential in driving global carbon and nutrient cycles has sparked a great scientific interest over the last decades, especially in the light of environmental change. Bacterial communities are key to understanding matter fluxes in the deep sea and feedback mechanisms to environmental changes and impacts, but the involved key bacterial groups for biogeochemical processes and ecosystem functioning in deep-sea sediments are still largely unexplored.

The Arctic Ocean is strongly affected by environmental change, such as increasing air and water temperatures. Due to the proposed strong coupling between surface ocean productivity and organic matter deposition in the Arctic Ocean (Bienhold et al., 2012; Grebmeier et al., 2006; Lalande et al., 2013; Vincent et al., 2009), changes in the upper water column, e.g., sea-ice retreat, or changes in phytoplankton composition are likely to also affect the deep-sea benthos through changes in the exported organic matter quality and quantity (Bauerfeind et al., 2009; Vincent et al., 2009). Thus, the Arctic Ocean became an important study site to identify key bacterial groups relevant for carbon cycling, and potential environmental change effects on bacterial deep-sea sediment communities (Arrigo et al., 2008; Michel et al., 2006; Wassmann, 2011). Therefore, an increasing effort is devoted to investigating the size, structure, and metabolic activity of bacterial populations in sediments of a changing Arctic Ocean (see e.g. Bienhold et al., 2012; Jacob et al., 2013; Ravenschlag et al., 1999, 2001), as the close relationship between diversity, environmental stability, and functioning is a fundamental ecological tenet.

Due to the remoteness of the Arctic deep sea benthos, potential key bacterial groups, their functional role within the deep-sea sediment, and potential indicator taxa for different environmental states were not well studied when I started my PhD thesis. Some bacterial groups are especially abundant within the Arctic sediment ecosystem, or show distinct variations along gradients of energy supply (Bienhold et al., 2012, 2016; Jacob et al., 2013; Jorgensen et al., 2012).These bacterial groups may be useful indicators to describe the state of the ecosystem, whether it is close to its baseline and presumably stable, or whether the system is undergoing changes. The overarching goal of

this thesis was thus to identify key bacteria in Arctic deep-sea sediments and to investigate their potential role in the Arctic ecosystem.

Owing to the location of the long-term ecological research site HG, I was able to obtain deep-sea sediments from the deep gateway between the North Atlantic Ocean and the Arctic Ocean – a geographic region where changes in organic matter export were already observed (Bauerfeind et al., 2009; Lalande et al., 2014; Schewe and Soltwedel, 2003). In the course of this thesis, I aimed to reach the before-stated overarching goal to identify key bacteria and investigate their ecosystem role through two different approaches: I) I analyzed the abundance and metabolic potential of distinct groups at this site, focusing on one of the most sequence-abundant bacteria within HG sediments, the JTB255. To do so, I applied culture-independent techniques such as amplicon sequencing, single-cell genomics, and metagenomics analysis, fluorescence *in situ* hybridization, and oligotyping, in combination with cultivation approaches (Chapter I and II). II) Additionally, I analyzed the ecology and dynamics at the scale of the whole community. I investigated the response of a bacterial community to different habitat-relevant organic matter sources, thereby identifying key responsive bacterial taxa, and potential indicator taxa for different organic matter sources (Chapter III). Furthermore, I performed a pilot study to reveal the need of critical method verification regarding sample retrieval from the deep-sea floor, testing whether decompression has effects on the data we obtain from the microbial sediment community (Chapter IV).

Overall, the combination of a range of molecular methods and experimental analyses techniques to investigate both, an entire community responding to organic matter addition, and the JTB255 in detail as one representative of highly abundant sediment bacterial groups, revealed bacterial groups with different lifestyles within the HG community, i.e. abundant but slowly responsive, and rare but fast responsive bacteria. This finding led to the advanced identification and description of specific bacterial groups that may be of general relevance for deep-sea sediment ecosystem functioning, and represents the most important outcome of my dissertation. In the long-term my work, together with ongoing and future investigations of bacterial communities in Arctic deep-sea sediments, will form a foundation to better understand this ecosystem and to identify functionally important key bacterial groups, aiming to describe an Arctic deep-sea sediment core microbiome.

3.1 The potential role of abundant but functionally enigmatic bacterial members of Arctic deep-sea surface sediments

The sequence-abundant members of the HG sediment community were *Proteobacteria*, *Acidobacteria*, and *Planctomycetes* (for more details see Chapters III and IV), as similarly reported previously from this site (Jacob et al., 2013), and from other Arctic (Bienhold et al., 2012; Han et al., 2016; Jorgensen et al., 2012), and global deep-sea surface sediments (Bienhold et al., 2016; Learman et al., 2016; Zinger et al., 2011). At higher taxonomic resolution (i.e. family level), abundant bacterial groups represented mostly environmental sequence clusters that are unclassified at or below family level, such as the gammaproteobacterial group JTB255, the deltaproteobacterial group Sh765B-TzT-29 (12% sequence abundance), the SAR202 clade (*Chloroflexi*; 2%), the BD2-11 terrestrial group (*Gemmatimonadetes*; 2%), the actinobacterial OM1 clade (2%), the proteobacterial group JTB23 (1%), members of the *Flavobacteriaceae* (2%), and the *Planctomycetaceae* (2%) (Appendix Figure S1). The overlap and occurance of sequence-abundant bacterial groups in several independent studies indicates that these groups represent important community members in deep-sea sediments of the Arctic (Bienhold et al., 2012; Han et al., 2016; Jacob, 2014; Jacob et al., 2013; Li et al., 2009a), and on a global scale (e.g. Bienhold et al., 2016; Li et al., 1999; Ruff et al., 2015; Zinger et al., 2011).

Most of these sequence abundant bacterial types did not show strong changes towards stimuli such as variations in pressure (Chapter IV) or organic matter input (Chapter III), and their role in the sediment community remained unknown. To tackle their enigmatic metabolic potential and ecosystem function, we focused on the JTB255 as a representative of the most abundant community members in the Arctic surface sediment community at HG (Chapters II and III) with so far functionally elusive features (Chapter I and II). Since its initial description by Li and colleagues in 1999, many molecular surveys (e.g. Bienhold et al., 2016; Bowman et al., 2005; Schauer et al., 2010) have indicated a global distribution and high relative sequence abundance of JTB255 in marine benthic environments (up to 22%), suggesting a competitive success and significance in ecosystem functioning. Prior to the completion of this thesis, only one study focused on the potential function and ecological niche of this highly abundant group, studying JTB255 in coastal sediments (Mußmann et al., 2017). Taken together, the findings of this study and experimental results obtained within Chapter I suggest a capability of

hydrogen and thiosulphate-based chemolithoautotrophy of this group in coastal sediments. A chemolithoautotrophic lifestyle of JTB255 in coastal sediments, as one of the most abundant coastal sediment bacteria, is in agreement with the suggested importance of coastal sediments for oceanic dark carbon fixation (Middelburg, 2011). Recently, biogeochemical budgeting revealed coastal sediments as hot spots of dark carbon fixation (175 Tg C yr^{-1}). Coastal sediments may be responsible for 78% of sedimentary dark carbon fixation, which exceeds numbers for total carbon fixed in the dark ocean, pelagic oxygen minimum zones, and hydrothermal vents (Dunne et al., 2007; Middelburg, 2011). However, the only cultivated representative of the JTB255 isolated from coastal surface sediments, *Woeseia oceani* XK5 (Du et al., 2016), showed strong indications for a chemoheterotrophic lifestyle (Chapter II). Moreover, we could not find any evidence of a chemolithoautotrophic lifestyle for deep-sea sediment JTB255 members in this study (Chapter II). However, due to the partial character of obtained deep-sea JTB255 genomes, further tests are needed to determine whether heterotrophicity might be a specific characteristic of JTB255 representatives from a certain sub-cluster, or if it represents a genuine characteristic of JTB255 bacteria inhabiting deep-sea sediments (Chapter II).

Deep-sea sediments are characterized by their high efficiency (> 97%) to remineralize organic matter (Burdige, 2007; Hedges and Keil, 1995; Jahnke, 1996), supporting mainly a heterotrophic bacterial lifestyle (Schut et al., 1997). A major component of marine organic matter are detrital proteins (Wakeham et al., 1997). Bacterial cell walls, e.g. derived from dead bacterial biomass, so-called 'necromass', are considered to form the most persistent sedimentary detrital matter (Lomstein et al., 2012; Pedersen et al., 2001; Steen et al., 2013), and potentially contribute to organic matter preservation (Parkes et al., 1993). Genes encoding various extracellular hydrolases, primarily peptidases and lipases were found in deep-sea representatives of JTB255, suggesting their ability to degrade both cell wall and cellular membranes as sources of carbon and energy (Chapter II). This adaptation would fill a widespread niche and could explain their ubiquitous distribution and generally high abundance in global ocean sediments. Furthermore, this niche adaptation would represent a highly important role of JTB255 in sustaining the sediment community during starvation conditions, as JTB255 may serve as essential catalyst for recycling the degradation-resistant, aged organic matter not available to most other sediment organisms (Deming and Baross, 1993; Westrich and Berner, 1984). However, through deamination of amino acids in the course of peptide

degradation, ammonia is released (Canfield et al., 1993; Schulz and Zabel, 2006). Aerobic ammonia-oxidizing bacteria and archaea, such as *Planctomycetes* and *Thaumarcheota*, respectively, convert ammonia into toxic nitrite during nitrification within deep-sea surface sediments (Junier et al., 2010; Parkes et al., 2014). The encoded nitrite reductase (NirK) within the JTB255 might thus have a role in nitrite detoxification, as a response to increased nitrite concentration, rather than respiration (Chapter II) (Beaumont et al., 2002, 2004, 2005). This reaction was described already within oxic sediments (Beaumont et al., 2002, 2004, 2005) and would explain the presence of a *nirK*-gene within representatives of the JTB255 in fully oxic deep-sea surface sediments at HG. If nitrite is efficiently removed, no accumulation would be traceable in surface sediments. Whether JTB255 are able to detoxify nitrite using NirK remains highly speculative due to the unavailability of isolates of JTB255 representatives from deep-sea sediments to test this hypothesis.

In addition to the putative heterotrophic physiology, I also hypothesize an adaptation of the JTB255 to oligotrophic conditions. This assumption is based on characteristics that tentatively represent oligotrophic organisms (Horner-Devine et al., 2004; Lauro et al., 2009; Pianka, 1970), e.g. a potentially low 16S rRNA gene copy number (Chapter II), its hypothetical ability for detoxification (Chapter II), its low (or slow) responsiveness to environmental stimuli, such as changes in food quantity and quality (Chapter II, III and Appendix Table SI), or hydrostatic pressure (Chapter IV), its high cell abundance and wide dispersal in global sediments (Chapter I and II). Furthermore, a hypothetic use of old proteinaceous matter in sediments would represent a constant energy and carbon source independent of organic matter export from surface waters on long time scales. This would promote a constant, possibly slow, growth rate of JTB255, resulting in a relatively constant abundance and large population size over time. These features are further considered as characteristics of oligotrophic bacteria (Lauro et al., 2009; Pianka, 1970), leading me to the hypothesis that oligotrophy and the use of aged, recalcitrant organic matter may be coupled in the case of JTB255. An oligotrophic lifestyle of JTB255 in Arctic deep-sea surface sediments, and potentially even globally, remains to be verified and a collection of ideas for this are given within section 4: Proposed future work. A potential coupling between oligotrophy and the degradation of recalcitrant organic matter could further be analyzed through the investigation of the lifestyle of other highly abundant bacterial groups within deep-sea sediments and their genomically encoded degradation potential.

The broad 16S rRNA gene diversity within JTB255 (Chapter II) indicates that their impacts on marine biogeochemical cycles probably extend beyond their involvement in detrital protein degradation, as already hypothesized for (certain) JTB255 bacterial types inhabiting coastal sediments. So far the environmental function of the JTB255 group remains largely unknown, and it is therefore of crucial importance to obtain more complete genomes of JTB255 representatives belonging to the proposed different phylogenetic clusters. This may help to study whether physiologically different sub-groups of JTB255 exist, what their respective life strategies might be, and whether a distribution according to environmental parameters exists, e.g. coastal versus deep-sea sediment clades.

3.2 Bacterial groups with a fast response to organic matter input in Arctic deep-sea surface sediments

This thesis provides a better understanding of the bacterial HG community not only in a single snapshot, but more dynamically (see also Kanzog et al., 2009). Furthermore, it represents one of the first studies that analyzed bacterial response to different organic matter sources at a high taxonomic resolution (family to OTU level) through a stimulation experiment, to allow the identification of potentially important indigenous sediment bacteria for the maintenance of the ecosystem function to cycle carbon (Chapter III). Intriguingly, it was mainly initially the rare types (< 1% of relative sequence abundance; Campbell et al., 2011) of the bacterial sediment community that responded to the addition of organic matter, and to changes in its composition. They became the most abundant bacterial taxa within this community in the course of a simulated changing environment (Chapter III and IV). In most marine environments, relatively few groups of exceptionally abundant bacteria co-exist alongside a high number of rare types (Bienhold et al., 2016; Jacob et al., 2013; Pedrós-Alió, 2006; Sogin et al., 2006). However, among these rare types many may be crucial for the functional performance of the ecosystem (Jousset et al., 2017 and references therein) (Chapter III). They become highly abundant under changing conditions, and provide insurance effects by promoting the maintenance of ecosystem function (Lauro et al., 2009; Yachi and Loreau, 1999). One example are bacterial groups, such as *Colwellia* and *Oceanospirillales,* which became abundant, and functionally important, after an oil spill in the Gulf of Mexico (Bælum et al., 2012; for more examples see Appendix Table S1). Therefore, in the age of next generation sequencing, being able to identify the rare bacterial biosphere may become increasingly important with understanding their role in events of environmental change (Jousset et al., 2017). These rare bacterial types may represent good indicator taxa for changing conditions on short time scales. In the frame of this thesis, we were able to propose potential indicator taxa for changing conditions in organic matter quality (Chapter III) Yet, in order to identify truly abundant sediment bacteria (as described in section 3.1), and to distinguish them from rare bacterial types, a high, year-round sampling density is of great importance.

One limitation of the *ex situ* approach presented in Chapter III was that the supplied phytodetritus was relatively fresh, and rather similar to the sudden, fast organic matter export observed by Boetius and colleagues (2013) in the Central Arctic Ocean. During the process of slowly sinking through the water column, the composition of organic

matter would have been altered (Lee, 2002), more or differently than our sterilization step did. The time of sediment sampling could represent another factor that influences the natural community and its response to organic matter addition. The natural bacterial sediment community might differ in the relative abundance of its bacterial groups and their activity when sampled before, during, or after the organic matter from the surface water reaches the seafloor. So far, very limited knowledge exists on how the bacterial community within sediments changes seasonally. However, none of the responsive groups in the *ex situ* stimulation experiment were already abundant in the natural sediment sampled, based on their low relative sequence abundance in the control sample (Chapter III), even though a natural phytoplankton bloom began to settle at the sampling site during the time of sampling (Soltwedel, 2015; Soltwedel et al., 2015). This leads to the assumption that a simple change in quantity of the exported material (e.g., comparing unfed to chitin-fed treatments) rather affects the activity of the community than the bacterial community composition, while a change in freshness (quality) of the organic material rather affects the bacterial community structure. We did observe this tendency in Chapter III, however, the experiments presented there represent a case study and further tests are required to elucidate how the bacterial deep-sea community responds towards different states of organic matter degradation exported to the seafloor. Furthermore, it will be interesting to investigate whether the altered bacterial community returns to the original composition (resilience), or whether the community composition remains altered. In both cases, it might be worthwhile testing whether the time to reach the old or new baseline is correlated with the amount of the organic matter pulse. Going one step further, it would be interesting to see whether an altered community composition will still perform the same functions due to functional redundancy (Louca et al., 2016; Morton et al., 2017), or whether also its function will be altered (Figure 7).

In studies on other systems than the marine deep sea, i.e. soil, freshwater, and coastal sediments, bacterial communities demonstrated a measurable level of resilience by returning in structure and composition to states that resembled their original states after short-term changes (Allison and Martiny, 2008; Jiang et al., 2016; Mohit et al., 2015; Shade et al., 2012). This return to the initial ecosystem state might be coupled to a bacterial succession as observed e.g. in freshwater ecosystems or marine surface waters over the course of an algal bloom (Shade et al., 2007; Teeling et al., 2012, 2016). A successive occurrence of different bacterial groups seems likely also in deep-sea surface sediments, i.e. over the course of the degradation of an organic matter pulse. The fast-

responding bacterial types might be important for the initial break down of more fresh organic matter. Ongoing degradation decreases the susceptibility of the remaining organic matter to further microbial decomposition (Berner, 1980; Westrich and Berner, 1984). A successive occurrence of different bacterial groups that are slightly different in their enzymatic potential may take place, including also the rather oligotrophic bacterial types, such as the JTB255 based on first genomic results discussed within Section 3.1, which may degrade the accumulating recalcitrant organic matter (Chapter II). However, the temporal resolution of our experimental setup (Chapter III) did not allow for resolving a successive occurrence of different lineages. Until today, neither the successive change in community composition nor the change in enzymatic expression cascades due to changes in organic matter quality has been addressed in detail for deep-sea sediments. To achieve this, it would be necessary to add additional observation time points to the experimental set up, and to increase the study time to up to several months in order to analyze whether the system returns to its initial diversity.

Few studies systematically examined how frequencies, intensities or size of environmental change affected the bacterial community structure, its resilience and function (Horner-Devine et al., 2004). Through our case study (Chapter III) we observed a tendency – a scenario of how the benthic bacterial community might react towards changes in (natural) organic matter quality. However, especially environmental and *in situ* studies are needed to further progress on the understanding of how and which bacterial groups are responsive towards changing environmental conditions.

3.3 Different bacterial life strategies within Arctic deep-sea sediments

Through the analyses I performed in the course of this thesis, I identified two distinct groups of bacteria in Arctic deep-sea sediments: (1) abundant but slow-responsive bacteria, such as the JTB255, and (2) rare but fast-responsive bacterial groups. The finding of two major, distinct trophic strategies within one community does not represent a new concept, but dates back to the late 1960`s (MacArthur and Wilson, 1967; Pianka, 1970). Theories describe on the one hand the so-called 'r-strategists' with a potential for fast growth rates, which are presumably adapted to high substrate levels and unstable environments due to their ability to reproduce quickly (Table 3). Therefore, they are also referred to as 'copiotrophs'. Lauro and colleagues (2009) could show that copiotrophs have a higher genetic potential to sense environmental stimuli and respond to changes on relatively short time scales, thereby providing a so-called 'insurance' effect by promoting the maintenance of ecosystem function (Jousset et al., 2017). On the other hand, under conditions of low substrate concentrations, r-selected organisms are outcompeted by K (carrying capacity)-selected organisms (Jannasch and Egli, 1993; Schut et al., 1997; Veldkamp et al., 1884). The so-called 'K-strategists' or oligotrophs are well adapted to nutrient depletion and oligotrophic environments, and typically arise at later successional stages than r-strategists. They are usually strong competitors under low nutrient conditions, and therefore rather stable in their abundance (MacArthur and Wilson, 1967; Pianka, 1970). K-strategists may serve as indicator taxa on much longer time scales, changing only slowly with time. They may also be important for the seafloor ecosystem on a longer time scale as they might be able to access and catabolize the recalcitrant organic matter pool buried within the sediments (Lauro et al., 2009) (Table 3), not usable for most other sediment organisms (Niggemann, 2005). Concluding, I propose that those bacterial types identified in my thesis as rare but potentially fast-responsive (Chapter III), represent r-strategists, while the abundant but slow-responsive types, such as JTB255 (Chapters I and II), represent K-strategists. A multitude of in-between bacterial types very likely form a continuum that bridge the oligotrophic and copiotrophic extremes.

Table 3. **Physiological and genomic characteristics of copiotrophic and oligotrophic lifestyles.** Characteristics marked with an asterisk (*) are from Lauro et al. 2009. Modified after MacArthur & Wilson 1967; Pianka 1970; and Lauro and colleagues, 2009**.**

Physiological/genomic characteristics	Copiotroph	Oligotroph
Type of selection	r-like	K-like
Selection favors…	Fast growth rate; High r_{max}; Early reproduction; Attached lifestyle	Slow, constant growth rate; Lower resource threshold; Delayed reproduction; Free-living lifestyle
Population size	Variable in time; Usually well below carrying capacity of environment	Fairly constant in time; At or near carrying capacity of the environment
Growth strategy	Feast and famine	Equilibrium
Consistent cell yield during nutrient limited growth	No	Yes
Growth rate dependence on media richness	Yes	No
Substrate specificity	High	Low
Cultivability	High	Low
Response to environmental stimuli/ stress	High	Low
Lag phase after starvation	Yes	No
*Genome size	Large (4.8 Mb)	Small (3.9 Mb)
*rRNA operon number	Many (9)	Few (1)
Electron acceptors	Wide variety	Mainly oxygenic
*Chitinase	High (0.06%)	Low (0%)
*Degradation of xenobiotics, recalcitrant, aromatic compounds	Low (0.06%)	High (0.12%)
*Lipid transport and metabolism	Low (2.96%)	High (4.41%)
*Genes related to detoxification	Low (0%)	High (0.08%)
*Dehydrogenases related to short chain alcohol dehydrogenases	Low (0.48%)	High (1.14%)

The Arctic deep-sea surface sediments are generally described as oligotrophic environments, however, feast and famine situations on the seafloor alternate due to strong seasonality in surface water phytoplankton blooms and particle export to the seafloor (Anderson et al., 1990; Bauerfeind et al., 2009; Bienhold et al., 2012; Klages et al., 2004). In such an environment, an interplay of organisms from both life-strategical groups could maintain community functioning, i.e. overcome both, starvation conditions (K-strategists), and intense food availability conditions (r-strategists). It is evident that the presence of both strategic groups is functionally important for the community, and for the functioning of the overall ecosystem. On a short time scale, rather the rare types may become abundant under changing conditions, and provide their insurance effect for the ecosystem functioning (Jousset et al., 2017; Yachi and Loreau, 1999). Overall, I therefore propose that representatives of both life strategies, r- and K-selected bacterial groups, should contribute to the description of a deep-sea sediment core microbiome.

3.4 Long-term perspectives to describe the Arctic deep-sea sediment core microbiome

Core microorganisms are currently identified based on two parameters: numerical abundance and ubiquitous distribution across samples in their respective habitat or ecosystem (Shade and Handelsman, 2012) (Appendix Table S2). Furthermore, based on their abundance and wide distribution, it is hypothesized that core bacteria represent those members of a community that contain fundamentally important genetic functions to sustain the functionality of that microbial community (Shade and Handelsman, 2012 and references therein). Thus, identifying core microbiomes represents a first step in defining a community baseline (Appendix Table S2), through which community responses to changing conditions can be observed and possibly even predicted (Li et al., 2013 and references therein; Shade and Handelsman, 2012). Gaining this knowledge represents a subject of significant interest and importance especially for sediments in a changing Arctic Ocean.

Based on their high abundance and wide distribution, JTB255 have been proposed as one core bacterial group of the deep-sea sediment microbiome (Bienhold et al., 2016). Furthermore, this thesis showed that the JTB255 constituted a core group for overall marine sediments, i.e. deep sea and coasts (Chapters II and I, respectively). However, based on the case study presented in Chapter III, other potentially important K-strategists of high relative sequence abundance (> 1%), but slow responsiveness, might likewise be considered core bacteria of HG deep-sea sediments. These are the Sh765B-TzT-29 clade, the SAR202 clade, the BD2-11 terrestrial group, the OM1 clade, the *Planctomycetaceae*, and the JTB23 clade (see also section 3.1; Appendix Figure S1). Unfortunately, no published isolates exist for any of these bacterial groups from deep-sea sediments. As an exception, the *Flavobacteriaceae* occur in high relative sequence abundances, and also responded fast towards energy addition (Chapter III). They might combine characteristics of both proposed life strategies in the sediments tested here. Nevertheless, all of the above-named bacterial groups fulfill both criteria of the core microbiome concept, and therefore represent candidate core bacterial groups for Arctic and potentially even overall deep-sea surface sediments (see also Bienhold et al., 2016).

In section 3.2 and 3.3, I highlighted that ecosystem-relevant bacterial groups may not solely be represented by bacterial groups of putatively constant high ecosystem abundance. The pool of rare bacterial groups may include the potential for a fast response to changing environmental conditions, by increasing in abundance and potentially activity (see also Chapter III). Examples of these rare but potentially functionally important bacterial groups include *Colwelliaceae*, *Moritellaceae*, *Shewanellaceae*, and *Campylobacteraceae*, which represent tentative r-strategists in the HG sediment community (Chapter III; see also section 3.2 and Appendix Figure S1). These types need to be considered as likewise relevant to the ecosystem as they may serve and maintain respective ecosystem functions (Jousset et al., 2017). In conclusion, besides the numerically abundant groups such as JTB255, OM1 clade, and Sh765B-TzT-29 (Bienhold et al., 2016), also these rare types should be included as candidates of a core microbiome - in this case of the Arctic deep sea surface sediment - to gain a more complete picture of the community, understand its activity and reactivity, and to possibly better predict community responses to environmental changes on short (r-strategists) and long (K-strategists) time scales (Figure 7). A short overview of currently available knowledge on each of the Arctic core K- and r-strategists proposed here and on their potential relevance in the ecosystem is given as Supplementary Information in the Appendix.

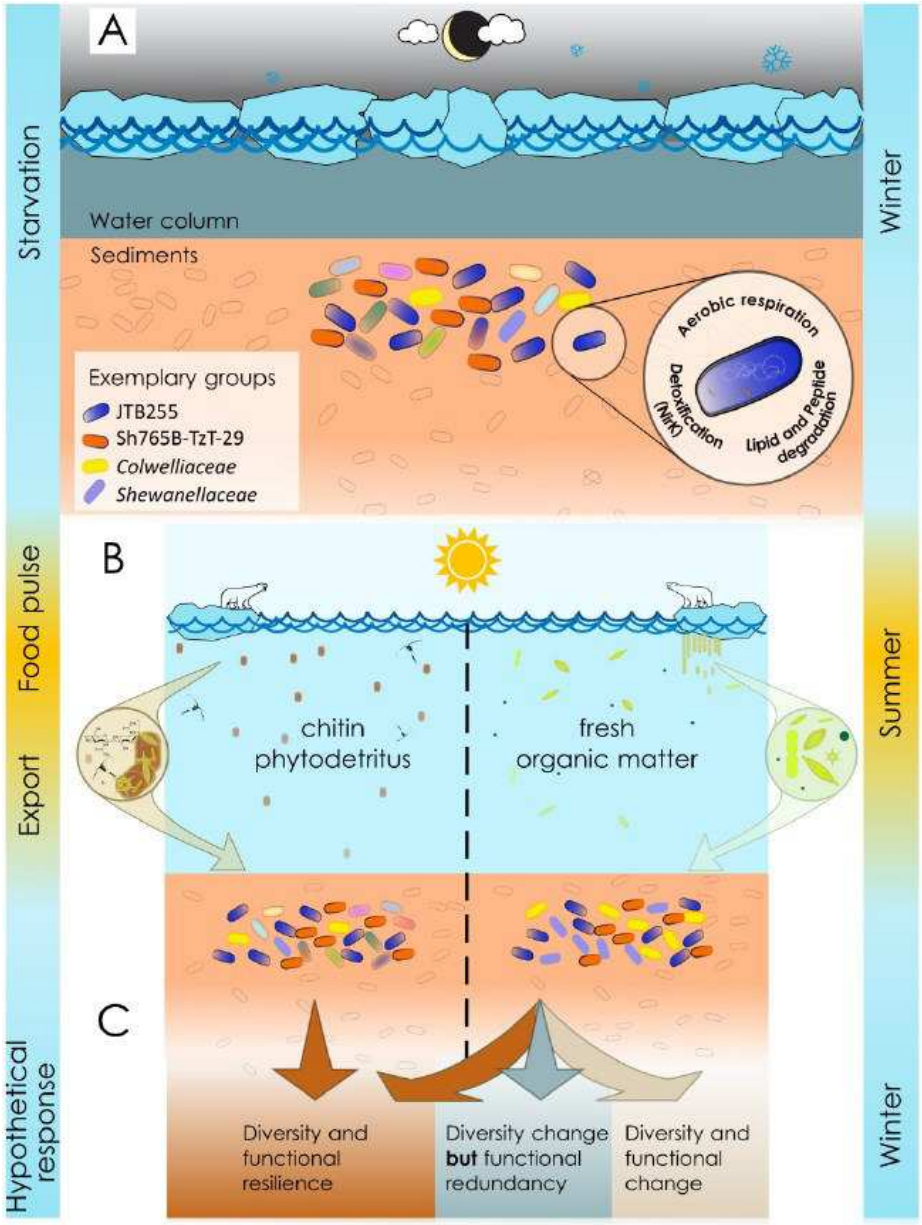

Figure 7. **Schematic model.** This schematic figure represents a projection and a hypothetical extrapolation of the results obtained within this thesis back into the natural context. (A) Graphical visualization of the condition of the bacterial community in oligotrophic Arctic deep-sea surface sediments before the input of a food pulse during summer to autumn. The JTB255 and Sh765B-TzT-29 are shown as representatives of the most abundant bacterial taxa in HAUSGARTEN sediments (exemplary groups; relative sequence abundance >1%). Also, representatives of less abundant bacterial groups (<1%, or "rare groups") such as *Colwelliaceae* and *Shewanellaceae* are displayed. The JTB255 are the most dominant community members and our findings of their main genomic capabilities are summarized in the inlet. Figure part (B) shows schematically how the bacterial community changes in its composition depending on the exported organic matter from the water column into the sediment. The left panel of (B) shows the scenario of an algal bloom typical for the region during summer. Based on our data, we expect the benthic bacterial community to change only moderately in its composition due to the fact that the input of organic carbon resembles a familiar food source (food quality and quantity), i.e. already partially degraded matter consisting of mainly chitin (e.g. fecal pellets). The right panel of (B) depicts the scenario of fresh organic material, e.g. algae such as *Melosira arctica,* getting rapidly exported to the seafloor. This event is rather untypical for the studied region, and in this case based on our data we expect a major change in the benthic bacterial community. Those bacterial taxa that showed initially a low abundance in the sediment

[(A); <1%] strongly increase here, and reach numbers comparable to those bacteria initially categorized as highly abundant groups. In contrast to this change, initially abundant bacteria do not, or only very slowly, respond to the food input in terms of changing their relative abundance in the community, such as JTB255. These bacterial groups are considered to remain abundant throughout the fluctuations of a year, and therefore contrast the strategy of those bacterial groups that quickly respond to an untypical food input, such as *Colwellia* and *Shewanella*. Especially these "rare but reactive" community members may have highly relevant functions for the ecosystem, both, in maintaining and shaping it. In interplay, the rare but responsive bacteria may respond explicitly to the fresh organic matter, degrading labile compounds quickly, while the highly abundant but slow responsive bacterial groups, such as JTB255, may rather degrade the recalcitrant, aged organic matter. Together, those two strategical groups of microorganisms may ensure the high remineralization efficiency of up to 97% in deep-sea sediments. This schematic continues to present the possible stages a benthic community can reach (C) after the organic matter pulse got exported to the sediment and the bacterial community responded. These assumptions are not based on data acquired in this thesis and therefore remain speculative. The left panel of (C) hypothesizes that the community may return to its initial (baseline) diversity and function, which appears likely when it received a food pulse of familiar quality, e.g. dominated by chitin. On the other hand, after receiving a rather unfamiliar food pulse (middle panel of C), it may remain changed in its structure, but due to the insurance effect of the prior rare groups, the function of the overall community remains similar to baseline functionality. The right panel of (C) shows the most extreme scenario where a change in food export (quality or quantity) alters both, the community structure and function, permanently. The details of which organic matter quantity or quality causes which of the presented scenarios of sediment community reactivity, especially on longer time scales, remains to be further studied.

The bacterial groups presented here as candidate core groups (Table 4) may tentatively serve as major drivers of the organic carbon cycle within HG deep-sea surface sediments. The overlap of my findings with other published results (see references within Table 4) are first indications that the identified core candidates at HG sediments might be expandable to the regional Arctic or even global deep-sea sediment community. All here-identified potential K-strategists, including the *Flavobacteriaceae*, were present in at least 90% of deep-sea sediments surveyed in a large, global study (Bienhold et al., 2016), pointing towards potentially important ecosystem functions. Also, all here-proposed r-strategists keep re-occurring in studies addressing the bacterial diversity of diverse deep-sea surface sediments, only at lower relative sequence abundances (see reference selection within Table 4 and Supplementary Information in the Appendix for more details). However, based on the currently available data it remains unclear whether the individual proposed candidate core groups represent the most important functional traits within the specific ecosystem, as no data currently exist for the proposed candidate core bacterial groups that clearly links a specific bacterial group with its local function.

Table 4. Proposal of core bacteria of deep-sea surface sediments.

Core candidate	References
JTB255 (*Proteobacteria*)	Chapter II; Li et al., 1999; Schauer et al., 2010; Bienhold et al., 2016; Mußmann et al., 2017 and references therein
Sh765B-TzT-29 (*Proteobacteria*)	Chapter III; Cerqueira et al., 2017; Probandt et al., 2017; Siegert et al., 2011; Zhou et al., 2015
SAR202 clade (*Chloroflexi*)	Chapter III; Ruff et al., 2015; Morris et al., 2004; Landry, 2016; Jorgensen et al., 2012
BD2-11 terrestrial group (*Gemmatimonadetes*)	Chapter III; Ruff et al., 2015; Bienhold et al., 2016; Bowman & McCuaig, 2003
OM1 clade (*Actinobacteria*)	Chapter III; Bienhold et al., 2016; Chen et al., 2016; Nigro et al., 2012
Flavobacteriaceae (*Bacteroidetes*)	Chapter III; Bienhold et al., 2012; Bienhold et al., 2016; Ravenschlag et al., 2001 and references therein; Jacob et al., 2013; Llobet-Brossa et al., 1998; Gray & Herwig, 1996
Planctomycetaceae (*Planctomycetes*)	Chapter III; Ravenschlag et al., 2001; Ruff et al., 2015; Kirkpatrick et al., 2006; Wagner & Horn, 2006
JTB23 (*Proteobacteria*)	Chapter III; Kouridaki et al., 2010; Schauer et al., 2010; Ruff et al., 2015; Bienhold et al., 2016
Colwelliaceae (*Proteobacteria*)	Chapter III; Jung, 2006; Nogi et al., 2004; Huston et al., 2004
Moritellaceae (*Proteobacteria*)	Chapter III; Lee et al., 2012 and references therein
Shewanellaceae (*Proteobacteria*)	Chapter III; Zhao et al., 2005; Zhao et al., 2010 and references therein
Campylobacteraceae (*Proteobacteria*)	Chapter III; Li et al., 1999; Llobet-Brossa et al., 1998; Bidle et al., 1999

The challenge in addressing this gap is twofold: firstly, the most functionally important candidate core bacterial groups need to be identified from the deep-sea sediment microbiome. Secondly, it needs to be clarified whether these candidate core bacterial taxa cover all ecosystem-relevant functions, or rather represent functionally redundant groups. A promising approach to address both challenges at once was recently presented by Louca and colleagues (2016). It is based on the observation that high taxonomic variability does not necessarily translate into functional diversity. Based on this observation, different taxa can be grouped together based on their function (Morton et al., 2017). These functions can, for a start, be on a rather broad level, such as the here-proposed differentiation into K- and r-strategists. Based on this, potential candidate core bacterial types from both functional traits could be suggested at a local scale, e.g. for HG deep-sea surface sediments. With increasing knowledge of the different members of the bacterial community, these groupings can be refined, differentiated e.g. by their specific oxygen requirements (aerob, facultative anaerob, anaerob), use of (alternative) electron acceptors, or use of different quantities and qualities of organic matter (high/low affinities, fresh/recalcitrant, complex/simple). The mutual integration of both, function and taxonomy, therefore reduces the overall ecosystem complexity, and rather

functionally relevant core candidates can be identified. As a future perspective for the Arctic deep-sea sediment community, such an approach can only be realized when sufficient numbers of closely related cultured representatives or genomes will become available (Louca et al., 2016). Without isolates and experimental knowledge about the distinct physiology of representatives of the candidate core groups at high taxonomic levels, the translation to environmental metabolic potentials can hardly be realized.

Given the high sequence abundances of the potential K-strategic bacterial groups in deep-sea surface sediments at HG (Appendix Figure S1), and the strong responsiveness towards organic matter addition of the potential r-strategic bacteria, I followed several different strategies in the course of this thesis attempting to cultivate representatives of any of the proposed bacterial core groups. I tested complex and specific media, different complex substrates including chitin, algae, and a synthetic polymer (PVA, see Chapter II), which I offered at different temperature and pressure conditions over varying time periods (up to 133 days) (see Supplementary Information of Chapter II and Appendix Figure S2). I successfully obtained isolates for the four proposed r-strategic bacterial groups and the *Flavobactericeae* (Appendix Figure S2). In turn, none of the proposed K-strategist could be obtained in culture. This indicates that all tested media, broad-range and defined, were not suited to match the specific requirements of these types of bacteria (media composition is described in detail in the Supplementary Material of Chapter II). A fundamental redesign of culturing conditions may be needed as these types of bacteria are to an unknown extent considered oligotrophic and very slow-growing. This innovative process should include the exploration of genomic information and can be initiated by analyzing the partial genomes obtained during this thesis in more detail (Chapter II, Appendix Table S3).

Even though the overall function of a benthic bacterial community as the driver of remineralization processes seems evident, the specific functions to maintain ecosystem functioning and efficiency are still largely unknown. Therefore, an analysis and clustering of the bacterial community based on functional redundancy as proposed by Louca and colleagues (2016) represents a desirable, but still a far-future aim in microbial ecology. My suggestions on how to progress on the analysis and functional description of proposed candidate key bacteria for the functioning of the HG sediment ecosystem, together with few ideas on how to widen the study of their potential functional relevance to overall Arctic or even global sediments, are given within section 4 as proposed future work.

4 Proposed future work

This thesis provides first advances in the identification and analysis of potentially highly ecosystem-relevant bacterial groups, and their response towards environmental changes. Studies in the near future should now focus on further analyzing the proposed candidate core bacteria and their response to changes at high temporal resolution.

For the analysis of JTB255, specific CARD-FISH probes were designed (Chapter I and II). With these probes, the quantitative abundance of JTB255 should be monitored over one year at different sites within the same oceanic region, such as HG, to better understand their dynamics also in the context of seasonal variations. Such seasonal changes are often not as pronounced in the deep sea as in shallow water systems, which makes it more difficult to detect effects with local, short-term measurements, and clearly needs extensive time series measurements. At HG, the sampling time points could be synchronized with the automated sampling events executed by the sediment trap moorings. To allow such a high sampling density of the seafloor, automated sampling systems are needed for on-site (*in situ*) collection and sample fixation. Furthermore, automated cell counting using image processing, already applied for water column samples, should be improved for the counting of sediment samples to speed up sample analysis, and to ensure the comparability of counts. This approach will help to verify the hypothesis that JTB255 represent K-strategists that remain relatively stable in their abundance throughout the year, independently of settling phytoplankton blooms from surface waters. However, fluctuations might be hidden within the JTB255 group itself, i.e. at level of the five proposed phylogenetic clades, or at an even finer microdiversity level (Chapter II). Therefore, new sets of CARD-FISH probes should be designed, each set covering one phylogenetic JTB255 clade. The quantitative abundance of those sub-groups can be counted in parallel to the counting of the overall group. For a faster quantification, specific PCR and qPCR primers should be designed based on the CARD-FISH probes. To analyze changes on microdiversity level, e.g. closely related short 16S rRNA gene sequences differentiated by single nucleotide polymorphisms, oligotyping (Eren et al., 2013) should be performed on the same sediments that are also used for cell counts. Through this, we would gain information on fluctuation and responses of the JTB255 group at different resolutions. To test for correlations, e.g. of JTB-types and their abundances with season, sea-ice condition, and POM content of the sediment, metadata need to be collected at each station in parallel to the sediment sampling event. In addition, a

periodic sampling of sediment, e.g. monthly, for metagenome and metatranscriptome analysis should be done. Meta-omics approaches can also assist in revising the observed tentative pressure effects on rRNA gene level of distinct members of the sediment community discussed in Chapter IV.

For most of the proposed candidate core bacteria (r- and K-strategists), CARD-FISH probes exist as well. All of these groups should be counted in parallel to the JTB255, to prove whether bacterial groups categorized as r-strategists indeed fluctuate while the K-strategists remain stable. This would also allow a comparison of absolute numbers of the different strategic members of the community, and not merely relative numbers as is done with whole community tag sequencing. In the course of this thesis, additional single cell genomes and cultured strains of proposed candidate core bacteria were produced (Appendix Table S3, Appendix Figure S2), and should be analyzed in the future to explore their encoded metabolic potential and putative functional relevance in HG deep-sea sediments. Furthermore, obtaining single cell genomes of target bacterial groups should be improved. Therefore, a technique presented by Tamminen and Virta (2015) should be tested. With this technique, individual microbial cells from highly complex environmental samples can be trapped into polyacrylamid droplets. The cells are lysed within the doplets and the single cell genomes amplified using MDA. A PCR-based approach is subsequently used to fluorescently label the droplets containing genomic DNA of the target genomic sequence, i.e. from the JTB255. Labeled and unlabeled doplets can than be differentiated using fluorescence-activated cell sorting to screen and capture genetic material of interest (Tamminen and Virta, 2015).

The CARD-FISH counts of specific bacterial groups at a high temporal and widespread spatial resolution, the increased generation of metagenomes and metatranscriptomes from the HG sediments, and the integration of single-cell genome data and culture experiments on distinct physiological characteristics, will in the future help to understand (i) which bacteria respond first to changing conditions, (ii) if a succession of different bacterial types can be observed, and (iii) if the community will eventually return to its initial diversity or balance on a different diversity profile. In order to monitor functional fluctuation, the total observed bacterial diversity should be grouped into K- and r-strategists, and with increasing functional information can be categorized into finer functional types as proposed within section 3.4. Eventually, community changes at the level of overall abundances of these functional groups can be used for monitoring and prediction.

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Appendix

Additional information of proposed key bacterial candidates of HAUSGARTEN deep-sea surface sediments

JTB255

see Chapters I and II

Sh765B-TzT-29

Deltaproteobacterial group Sh765B-TzT-29 is widely distributed throughout coastal (Probandt et al., 2017) and deep-sea sediments (Cerqueira et al., 2017; Siegert et al., 2011). Only few studies exist where the Sh765B-TzT-29 group is mentioned. Therein, sequence-based abundances ranged from 2% in coastal sediments (Probandt et al., 2017) to 12% in the Arctic deep sea (Chapter III; Appendix Figure S1). This uncultivated cluster was initially believed to belong to the family *Geobacteraceae* and was first identified in uranium mill tailings (Geißler, 2003), associated with specific redox zones of dissolved and particulate metals such as Fe, Cu and Ba (Cerqueira et al., 2017; Steen et al., 2016). Members of this clade may be closely related to metal-reducing *Deltaproteobacteria* (Cerqueira et al., 2017). Furthermore, this group has recently been linked tentatively to methane oxidation in culturing experiments (Siegert et al., 2011), and to complex metal-contaminated organic matter degradation (Baldwin et al., 2015).

SAR202 clade

The SAR202 clade represents an environmental, monophyletic 16S rRNA gene clone cluster within the phylum *Chloroflexi* (Morris et al., 2004) and shows extensive internal diversity (Yilmaz et al., 2016). Sequences of members of the bacterial SAR202 clade are frequently found in 16S rRNA gene clone libraries obtained from marine sediments, but also from marine waters, pelagic freshwater, soil, and deep subsurface terrestrial and marine environments (Jorgensen et al., 2012; Morris et al., 2004 and references therein). Cultivation-independent rRNA gene cloning and sequencing results suggest that members of this diverse group are ubiquitous and potentially abundant in the marine environment. Their increasing abundance with water depth to around 10% of the microbial community below the euphotic zone (Morris et al., 2004) suggests they may be major mediators of recalcitrant organic carbon sequestration and turnover in the deep ocean (Landry, 2016). However, few single cell genomes were retrieved from marine deep

waters (Landry, 2016; GenBank no. AQTZ00000000.1) but currently no functional information exists on the SAR202 in deep-sea surface sediments. In the course of my studies, 20 single cell genomes from the SAR202 clade could be retrieved at HG deep-sea surface sediments, where they make up 2% of the bacterial 16S rRNA sequence counts. Those single cell genomes will be further analyzed to obtain insights into their phylogenetic diversity and metabolic potentials in deep-sea surface sediments.

BD2-11 terrestrial group

Even less knowledge is available for the BD2-11 terrestrial group, which is part of the *Gemmatimonadetes*. BD2-11, also named PAUC43f/BD2-11 (Bowman and McCuaig, 2003), represents an environmental cluster without cultivated representatives and is found associated with sponges and deep-sea sediments in the marine realm (Bowman and McCuaig, 2003; Chen et al., 2016; Fan et al., 2012, 2013). However, in a global survey of deep-sea surface sediments based on NGS, representatives of the BD2-11 terrestrial group were present in at least 90% of all surveyed sites (Bienhold et al., 2016). Furthermore, its relative sequence abundance in surface sediments ranges from < 1 to 2% (Appendix Figure S1), which makes it an interesting candidate as a core bacterium of deep-sea sediments.

OM1 clade

Representatives of the actinobacterial OM1 group show a global distribution in deep-sea surface sediments, comparable to those of the JTB255 and where proposed already by Bienhold and colleagues (2016) to be part of a deep-sea sediment core microbiome. Its sequence-based abundance ranges from < 1 to 2% in deep-sea sediments (Bienhold et al., 2016) (Appendix Figure S1). So far, the OM1 clade was reported to be widely distributed in the photic zone of the ocean (Ghai et al., 2013), mostly near the deep chlorophyll maximum (Yilmaz et al., 2016). Within the water column, OM1 representatives are described as small, oligotrophic, and free-living bacteria with streamlined genomes (Milici et al., 2016), and a proposed photoheterotrophic lifestyle (Ghai et al., 2013). If OM1 clade representatives in deep-sea sediments turn out to have comparable small cell sizes, which is comparable to the size of large viruses, their cell counting using staining methods such as CARD-FISH will be highly challenging. Furthermore, investigations are needed to reveal their lifestyle and functional relevance in deep-sea sediments.

Flavobacteriacea

Representatives of the *Flavobacteriacea* appear in all types of marine environments, especially in the water column associated to phytoplankton blooms. Here, they participate in the initial degradation of complex organic matter and profit first from a decaying phytoplankton bloom (Teeling et al., 2012). However, in diverse studies, *Flavobacteriaceae* are described as an integral part of surface sediments (e.g. Bienhold et al., 2012; Jacob et al., 2013; Llobet-Brossa et al., 1998; Ravenschlag et al., 2001), with a wide distribution (Bienhold et al., 2016). Their sequence and CARD-FISH count based cell abundances range from < 1 to 3%, and 3 to 11%, respectively (Bienhold et al., 2016; Ravenschlag et al., 2001). *Flavobacteria* from Arctic sediments were recently described as potential opportunistic groups, exhibiting a strong positive correlation with organic matter availability (Bienhold et al., 2012; Jacob et al., 2013) (Chapter III), consistent with the association of copiotrophy to the phylum *Bacteroidetes* (Fierer et al., 2007). Within sediments, flavobacterial cells were partially found attached to sediment particles and other organic matrices (Ravenschlag et al., 2001), and are capable of degrading high-molecular-weight organic compounds (Baek et al., 2015; Pati et al., 2011; Xing et al., 2015). *Flavobacteria* were observed in aerobic (Chapter III) as well as in anaerobic sediments and a potential ecological relevance of these bacteria as hydrolytic fermentative organisms was proposed (Rosselló-Mora et al., 1999). Hence, *Flavobacteria* are responsible for a major fraction of organic matter remineralization in the oceans (Kirchman, 2002), and represent a potential functionally important bacterial group for the deep-sea ecosystem.

Planctomycetes

The order *Planctomycetales* is part of the P*lanctomycetes*, *Verrucomicrobia*, *Chlamydiae* (PVC)-superphylum and contains mainly aerobic organisms, but one particular group is capable of anaerobically oxidizing ammonium (Kirkpatrick et al., 2006; Ravenschlag et al., 2001; Wagner and Horn, 2006). *Planctomycetes* are reported from aquatic and terrestrial ecosystems, due to their diverse metabolism, with relatively few cultured chemoorganotrophic isolates (Glöckner et al., 2003; Wagner and Horn, 2006). They colonize macroalgae, which secrete various sulfated glycopolymers, degraded by *Planctomycetes* (Lage et al., 2014). In a sediment study *Planctomycetes* made up between 1 and 4% of DAPI-stained cells (Ravenschlag et al., 2001), and made up around 2% relative 16S rRNA gene sequence abundance at HG deep-sea surface sediments (Appendix

Figure S1). Of special interest appears the uncultured class OM190, recently proposed as indicator taxa for deep-sea surface sediments (Ruff et al., 2015).

JTB23

JTB23 is a group of only environmental 16S rRNA gene sequences which originate from diverse marine habitats, mainly deep-sea surface sediments (Kouridaki et al., 2010; Ruff et al., 2015; Schauer et al., 2010). Their phylogenetic rank is not yet validated and JTB23 represent either a class within the *Proteobacteria* (SILVA database) or a family, within the *Gammaproteobacteria* (Li et al., 1999b). JTB23 representatives were present in 90% of all analyzed sites in a global survey on deep-sea surface sediments conducted by Bienhold and colleagues (2016) and therefore displays a highly distributed bacterial group. However, its sequence abundance ranged from < 1 to 1% based on the few studies available and based on its abundance in HG sediments (Chapter III; Appendix Figure S1). No information are available concerning its potential function.

Colwelliaceae

Colwelliaceae are distributed throughout the marine realm and are strictly psychrophilic (Methé et al., 2005). They are known to include polymer degraders, and have a broad substrate spectrum including complex organic compounds such as chitin (Auman et al., 2006; Huston et al., 2000, 2004; Ivanova et al., 2004; Methe et al., 2005). Additionally, the genus *Colwellia* has been reported to produce polyhydroxyalkanoate compounds, which serve as intracellular carbon and energy reserves (Cai et al., 2011; Methe et al., 2005). Genomic information and cultivated representatives are available (Methé et al., 2005) (Appendix Figure S2 and Appendix Table S2).

Moritellaceae

The family *Moritellaceae* is composed of aerobic, motile, psychrophilic bacteria, found in seawater, fish farms, and marine sediments, including the deep sea (Lee et al., 2012 and references therein). Cultivated representatives are capable to degrade high-molecular-weight organic compounds, such as chitin (Lee et al., 2012; Malecki et al., 2013).

Shewanellaceae

Shewanellaceae are ubiquitous in marine environments such as surface, coastal, and deep sea water as well as in sediments, and some strains were also found in freshwater systems (Zhao et al., 2010 and references therein). *Shewanella* can grow anaerobically and is well known for its ability to oxidize organic matter, and high-molecular-weight organic compounds (Zhao et al., 2005, 2010). Genomic information and cultivated representatives are available (Zhao et al., 2005, 2010) (Appendix Figure S2 and Appendix Table S2).

Campylobacteraceae

Mainly sequences related to *Arcobacter* spp. (*Epsilonproteobacteria*) were repeatedly retrieved from marine sediments (Bidle et al., 1999; Li et al., 1999a, 1999b). Total cell counts exist from Wadden Sea sediments, where the genus *Arcobacter* accounted for up to 2% (Llobet-Brossa et al., 1998). An ability to carry out nitrate reduction and sulfide oxidation has been reported (Teske et al., 1996), and it strongly reacted on organic matter input in an aerobic food pulse experiment (Chapter III).

Colwelliaceae, Moritellaceae, Shewanellaceae, and *Campylobacteraceae* made up a minor fraction of Arctic deep-sea sediment sequences (0.2%, 0.1%, 0.1%, and 0.01%, respectively) but steeply increased upon organic matter input to 5 to 10% sequence abundance (Chapter III). Therefore, we identified these bacterial groups as potential opportunists, and I propose them as core bacterial candidates based on their functional importance they might have for the deep-sea ecosystem.

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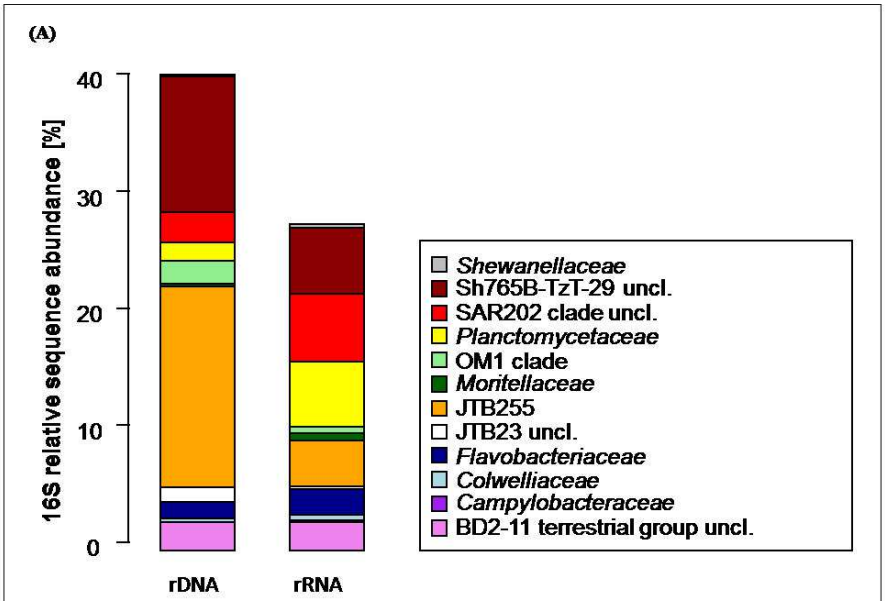
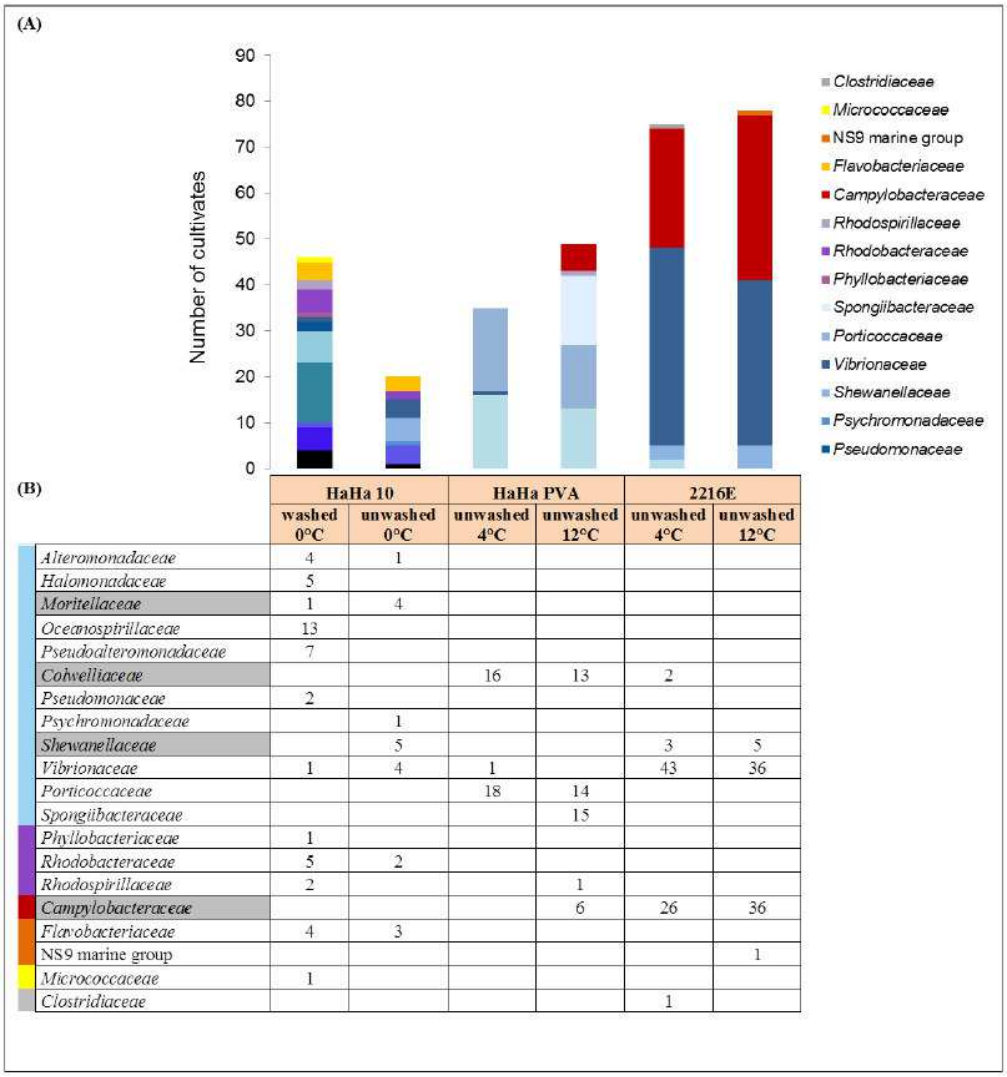
Supplementary Figures



(B)	16S rDNA [%]	16S rRNA [%]
JTB255 (*Proteobacteria*)	17.5	3.8
Sh765B-TzT-29 uncl. (*Proteobacteria*)	11.7	5.7
SAR202 clade uncl. (*Chloroflexi*)	2.4	5.7
BD2-11 terrestrial group uncl. (*Gemmatimonadetes*)	2.4	2.5
OM1 clade (*Actinobacteria*)	2.2	0.5
Flavobacteriaceae (*Bacteroidetes*)	1.6	2.2
Planctomycetaceae (*Planctomycetes*)	1.6	5.6
JTB23 uncl. (*Proteobacteria*)	1.4	0.3
Colwelliaceae (*Proteobacteria*)	0.2	0.5
Moritellaceae (*Proteobacteria*)	0.1	0.7
Shewanellaceae (*Proteobacteria*)	0.1	0.3
Campylobacteraceae (*Proteobacteria*)	< 0.1	< 0.1

Figure S1. **Proposed key bacteria of HG deep-sea surface sediments (0 to 2 cm; station IV) on family level based on relative sequence abundances of the total (16S rDNA; n = 3) and active (16S rRNA; n = 1) fraction.** For the 16S rDNA community, sequences from replicate samples were pooled for the calculation of relative sequence abundances. JTB255: JTB255 marine benthic group; uncl.: unclassified. (A) Visual overview of relative sequence abundances. (B) Proposed core bacterial families sorted by relative 16S rDNA sequence abundances. Phylum level given in parentheses.



(B)

	HaHa 10		HaHa PVA		2216E	
	washed 0°C	unwashed 0°C	unwashed 4°C	unwashed 12°C	unwashed 4°C	unwashed 12°C
Alteromonadaceae	4	1				
Halomonadaceae	5					
Moritellaceae	1	4				
Oceanospirillaceae	13					
Pseudoalteromonadaceae	7					
Colwelliaceae			16	13	2	
Pseudomonaceae	2					
Psychromonadaceae		1				
Shewanellaceae		5			3	5
Vibrionaceae	1	4	1		43	36
Porticoccaceae			18	14		
Spongiibacteraceae				15		
Phyllobacteriaceae	1					
Rhodobacteraceae	5	2				
Rhodospirillaceae	2			1		
Campylobacteraceae				6	26	36
Flavobacteriaceae	4	3				
NS9 marine group						1
Micrococcaceae	1					
Clostridiaceae					1	

Figure S2. **Isolation approaches of bacteria from Arctic deep-sea surface sediments.** Bacterial cultivates obtained from incubated sediment from HG station IV (0 to 2 cm) on marine agar (HaHa) which was either ten times nutrient-enriched or supplemented with polyvinyl alcohol (PVA), or an 2216E agar. Sediment used for cultivation was either used unwashed or was pre-washed with sterile-filtered seawater to maintain mainly sediment-attached bacterial groups for cultivation. Different incubation temperatures were applied and ranged from *in situ* 0°C to 12°C in order to increase bacterial replication rates.

Supplementary Tables

Table S1. Selection of scientific studies which analyzed deep-sea sediment bacterial responses towards changes in organic matter quantity and quality through, e.g., mass sedimentations or organic matter enrichments. The table shows the most responsive bacterial taxa identified within studies where applicable. T-RFLP: Terminal-restriction fragment length polymorphism; clone library: 16S rRNA gene clone library; POM: particulate organic matter; DOM: dissolved organic matter.

Author	**Loction**	**Amendments/other comments**	**Taxa (method)**
		Organic enrichments	
Jannasch and Wirsen, 1982	Atlantic	14C-labelled sodium glutamate, casamino acids, glucose, sodium acetate; ***in situ* pressure incubation**	N/A
Tabor et al., 1982	Atlantic	14C-glutamate, acetate	N/A
Deming, 1985	Atlantic	Yeast extract, chitin	N/A
Deming and Colwell, 1985	Atlantic	14C-amino(glutamic) acid, yeast extract	N/A
Turley and Lochte, 1990	Atlantic	Phyto-, zoodetritus	N/A
Boetius and Lochte, 1994	Atlantic	POM, DOM from net plankton	N/A
Boetius and Lochte, 1996	Arctic	Glycine, glucose, albumin, chitin, cellulose, starch, Tween	N/A
Yanagibayashi et al., 1999	Japan Trench	Marine broth; ***in situ* pressure incubation**	*Shewanella, Moritella* (**clone library, T-RFLP**)
Moodley et al., 2002	Atlantic	13C-enriched *Thalassiosira*	N/A
Witte et al., 2003	Atlantic	13C-labeled, *Thalassiosira*	N/A
Toffin et al., 2004	Pacific	Yeast extract; mix of glucose, fructose, galactose, acetate, alctic acid, glycolate, succinate, mannitol, ethanol, glycerol, benzoate, salicylic acid, casamino acids, peptone	*Firmicutes, Proteobacteria, Spirochaeta, Acetobacterium, Marinilactibacillus* (**DGGE, clone library**)
Biddle et al., 2005	Chile margin	Marine broth	*Photobacterium profundum, Halomonas boliviensis, Shewanella benthica, Halomonas* sp., *Vibrio* sp. (**Sanger sequencing**)
Moodley et al., 2005	North Sea, Mediterranean, Atlantic	13C-enriched Skeletonema costatum	N/A
Bühring et al., 2006	Mediterranean	13C-enriched *T.rotula*; ***in situ* Lander experiment**	N/A

Table S1. (continued)

Author	Loction	Amendments/other comments	Taxa (method)
Parkes et al., 2009	**Gulf of Mexico, Pacific, Indian Ocean**	**Broth with nitrate or metals;** *in situ* pressure incubation	***Carnobacterium, Clostridium, Pseudomonas, Marinilactibacillus; Firmicutes; Carnobacterium), Clostridium, Marinilactibacillus, Marinobacter*** (DGGE, Sanger sequencing)
Kanzog et al., 2009	Arctic	Chitin; ***in situ* Lander experiment; *in situ* pressure incubation**	N/A (**T-RFLP**)
Gärtner et al., 2011	Mediterranean	N-acetyl-D-glucosamine	*Bacillus, Halobacillus, Arthrobacter, Micrococcus, Sreptomyces, Blastococcus, Pseudonocardia, Micromonospora, Aurantimonas, Paracoccus, Citreicella, Sphingobium, Erythrobacter, Pontibacter, Leeuwenhoekiella, Stenotrophomonas, Microbulbifer, Marinobacter, Pseudomonas, Alteromonas, Pseudoalteromonas* (**Sanger sequencing**)
Mayor et al., 2012	Atlantic	13C-labelled diatoms and faecal pellets	*(PCR-DGGE)*
		Large food falls	
Smith et al., 1989	Pacific	Whale carcasses	White microbial mats suggesting *Beggiatoa gigantea* (**observation by eye**)
Deming et al., 1997	Pacific	Whale carcasses	N/A
Christiansen and Boetius, 2000	Arabian Sea	Crab carcasses	N/A
Palacios et al., 2006	Pacific	Wood falls	N/A
Palacios et al., 2009	Pacific; Mediterranean	Wood falls	N/A (**CE-SSCP fingerprinting**)
Treude et al., 2009	Pacific	Whale carcasses	N/A
Goffredi and Orphan, 2010	Pacific	Whale carcasses	*Bacteroidetes, Delta-, Epsiolnproteobacteria, Firmutes* (**clone library**)
Fagervold et al., 2012	Pacific; Mediterranean	Wood falls	*Bacteroidia, Marinilabiaceae, Firmicutes, Planctomycetes, Spirochetes* (**clone library**)
Boetius et al., 2013	Arctic	Sea-ice algae *Melosira arctica*	N/A
Bienhold et al., 2013; Pop Ristova et al., 2017	Mediterranean; Atlantic	Wood falls	*Delta-*, *Epsilonproteobacteria, Demequina, Sulfurovum, Sulfurimonas, Leisingera, Desulfobulbus, Desulfobacula*, SEEP-SRB4 (**ARISA, 454 pyrosequencing**)

Table S2. **Terms relevant to core microbiomes, defined based on the results obtained within this study**. Modified after Shade and Handelsman, 2012.

Term	Definition	Examples	Reference
Biome	The world`s major divisions, defined by temperature gradients in latitude and altitude, precipitation, seasonality, and light; each of which represents a collection of ecosystems and habitats.	Subtropical; Mediterranean; polar	Walter and Box, 1976
Ecosystem	The interactions and dynamics of physical, chemical, and biological components in a locality.	Hydrothermal vent, seep, or coral reef community	Odum, 1953
Habitat	The physical and chemical parameters of an environmental area that determine niche spaces.	Termite hindgut	Whittaker et al., 1972
Microbiome	The entire microbial collection within a reasonably well defined habitat or ecosystem, which has distinct physiochemical properties. Thus, the term does not only include the involved microorganisms, but also spans their overall genetic material. Optimally, each microbiome is characteristic for its specific environment and distinct from others.	Deep-sea surface sediment (soft bottom); seep; human gut	Bienhold et al., 2016; Lederberg and McCray, 2001; Whipps et al., 1988
Environment	The sum of all external conditions affecting the life, development, and survival of an organism.	Hydrothermal vent; deep sea; seep	Johnson et al., 1997
Core microbiome	Organisms common across microbiomes, generally abundant, and hypothesized to play a key role in ecosystem functioning with the defined system boundary, here that of the ecosystem of the deep-sea soft-bottom.	Seep; human gut; soil	Bienhold et al., 2016; Shade and Handelsman, 2012; Turnbaugh et al., 2007
Indicator taxa	Define a trait or characteristic of the ecosystem they are part of, or a deviation of the ecosystem from its baseline.	Upper water column: SAR11; Prochlorococcus; SAR406	Aylagas et al., 2017; Fortunato et al., 2013
Baseline	Also named the foundation or benchmark of a system (mainly an ecosystem) which is defined by the biotic and abiotic conditions, and the organisms present at a defined time point.	*To be determined*	Dayton et al., 1998
Insurance Hypothesis	While a high biodiversity of an ecosystem may allow the encoding of slightly different metabolic potentials and survival strategies, functionally similar species ensure community functioning during the resistance period under environmental stress.	Rare bacterial taxa increase in sediments due to the oil spill in the Gulf of Mexico	Bælum et al., 2012; Jousset et al., 2017; Yachi and Loreau, 1999

Table S3. **Single cell genomes retrieved from Arctic deep-sea surface sediments**. SAGs: Single amplified genomes; JTB255: JTB255-marine benthic group; uncl.: unclassified.

Phylum	Class	Family	Assembly size [Mb]	Completeness [%]	No. of SAGs
Thaumarchaeota	*Nitrosopumilales*	*Nitrosopumilaceae*	0.7	46	1
Thaumarchaeota	*Nitrosospaerales*	*Nitrosospaeraceae*	0.3	16	1
Chloroflexi	SAR202 clade	SAR202 uncl.	0.1 – 1.9	0 – 46	20
Acidobacteria	*Acidobacteria*	Subgroup-21 uncl.	0.5 – 1.3	7 – 38	4
Acidobacteria	*Acidobacteria*	Subgroup-5 uncl.	0.1 – 1.4	5 – 29	6
Bacteroidetes	*Cytophagia*	*Rhodothermaceae*	1.3	28	1
Gemmatimonadetes	*Gemmatimonadetes*	BD2-11 uncl.	0.4 – 0.9	14 – 40	2
Deferribacteres	*Deferribacteres*	PAUC34f	0.5 – 3.3	5 – 51	7
Lentispherae	*Oligosphaeria*	Oligosphaeria uncl.	0.4 – 2.1	0 – 46	4
Proteobacteria	*Alphaproteobacteria*	*Rhodobacteraceae*	0.1 – 1.5	4 – 33	10
Proteobacteria	*Deltaproteobacteria*	*Desulfobacteraceae*	0.1	22	1
Proteobacteria	*Deltaproteobacteria*	*Nitrospinaceae*	0.6	14	1
Proteobacteria	*Deltaproteobacteria*	GR-WP33-30 uncl.	0.8	14	1
Proteobacteria	*Deltaproteobacteria*	*Cystobacteraceae*	0.3	4	1
Proteobacteria	*Deltaproteobacteria*	SAR324 uncl.	0.3 – 1.5	7 – 44	7
Proteobacteria	*Epsilonproteobacteria*	*Sulfurimonas*	0.5	11	1
Proteobacteria	JTB23	JTB23 uncl.	1.4	8	1
Proteobacteria	*Gammaproteobacteria*	JTB255	0.2 – 1.1	11 – 38	2
Proteobacteria	*Gammaproteobacteria*	*Zhongshania*	0.3	36	1
Proteobacteria	*Gammaproteobacteria*	*Piscirickettsiaceae*	0.2 – 1.1	5 – 33	4
Proteobacteria	*Gammaproteobacteria*	OM182 clade	1.1 – 3.5	4 – 41	9
Proteobacteria	*Gammaproteobacteria*	*Halioglobus*	0.4	12	1
Proteobacteria	*Gammaproteobacteria*	*Thiohalophilus*	0.1	14	1
Proteobacteria	*Gammaproteobacteria*	*Porticoccus*	0.3	14	1
Proteobacteria	*Gammaproteobacteria*	BD7-8 uncl.	0.3	10	1
Proteobacteria	*Gammaproteobacteria*	*Colwelliaceae*	0.7 – 2.2	0 – 28	4
Proteobacteria	*Gammaproteobacteria*	*Alteromonadaceae*	0.4 – 1.0	21 – 36	3

Miscellaneous

Additional co-author contributions:

Deep-sea bacterial community structure at the Arctic long-term observatory HAUSGARTEN (Fram Strait)

Pala C.[1], Carvalho V.[1,2], Bienhold C.[1,2], Rapp J.[1,2], Hoffmann K.[1,2], Jacob M.[1,2], Boetius A.[1,2]

[1] Max Planck Institute for Marine Microbiology, Bremen, Germany;

[2]Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Bremerhaven, Germany

In preparation.

Abstract

The overall aim of this project is to design specific probes for Fluorescence *In Situ* Hybridization (FISH) to enumerate the dominant bacterial taxa in sediment samples from different depths at the Arctic LTER HAUSGARTEN, representing the varying conditions of organic matter input to the seabed. In the FISH approach we use specific probes for selected groups of interest, allowing a verification and expansion of the data acquired through sequencing. Further, it works towards the understanding of taxon-specific responses to changes in organic matter supply. We focused on six bacterial groups dominant in the HAUSGARTEN seabed, for only four of which probes have been publicly available before. For the remaining two groups, we designed probes using alignments of partial 16S rRNA gene tag sequences as well as reconstructed full-length 16S gene sequences from genomic and metagenomic data retrieved from HAUSGARTEN sediments and from the public databases. Suitable target sites for nucleic acid probing were identified using the Probe Design tool of the ARB software package, and probe specificity was tested *in silico* using the SILVA and RDP databases.

The role of SAR202 *Chloroflexi* in sediments of the LTER HAUSGARTEN (Fram Strait) and other environments

Carvalho V.[1,2], Offre P.[1], Pala C.[1], Rapp J.Z.[1,2], Hoffmann K.[1,2], Bach M.[1], Boetius A.[1,2]

[1]Max Planck Institute for Marine Microbiology, Bremen, Germany

[2]Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Bremerhaven, Germany

In preparation.

Abstract

Bacteria of the SAR202 clade reveal a high relative abundance (~10%) in the meso- and bathypelagic waters between the twilight zone and the deep dark ocean. Recently, they were also identified among the dominant community members in deep-sea sediments, for example, around hydrothermal vents, and in sponge tissue. Their functional role is almost entirely unknown, and only one recent study revealed the potential connection of water-borne SAR202 and the degradation of recalcitrant organic matter based on single-cell genomics. We study the oxic oligotrophic deep-sea sediments (>2000 m water depth) of the Fram Strait – the deep-water gateway between the Arctic and North Atlantic. Also here, SAR202 is a relevant member of the benthic bacterial community (~1.5%) as revealed by 16S rDNA tag sequencing and specific CARD-FISH cell counting. 16S rRNA tag sequencing and metatranscriptomics indicate that they are also relevant members of the active community, as their relative abundance rises to ~4.9%. We integrated our data with those of the public databases and could reveal a high phylogenetic diversity within the SAR202 clade without a yet clear separation of lineages representing distinctive habitat adaptations. Data mining also revealed an additional five partial single-cell genomes of water-borne SAR202. To complement these, we produced 20 single-cell genomes from benthic Fram Strait SAR202, spanning almost the entire SAR202 diversity. Our aim is the identification of phylogenetic clusters and subtypes, possibly pelago- vs. bathy-types, to investigate the ecophysiological role of the different SAR202 in their respective habitats and niches by applying comparative 'omics analyses.

Oral presentations:

Hoffmann K, Offre P, Knittel K, Buttigieg PL, Rapp JZ, Fadeev E, Bienhold C, Salman-Carvalho V, Stepanauskas R, and Boetius A (2016). Ecophysiology of the JTB255 marine benthic group: a dominant bacterial clade in deep-sea sediments. ISME16 Conference, Montreal, Canada, August 2016.

Hoffmann K**,** Bienhold C, and Boetius A (2015). Response of Arctic Deep-Sea Bacteria to Changing Nutrient Inputs. PhD days Sylt, Germany, April 2015.

Hoffmann K, Bienhold C, and Boetius A (2015). Fed Up: Impact of Different Food Pulses on Bacterial Diversity and Activity in Arctic Deep-Sea Sediments. ASLO Conference Granada, Spain, February 2015.

Hoffmann K, Bienhold C, and Boetius A (2014). Effects of Hydrostatic Pressure on the Activity and Diversity of Arctic deep-sea Bacteria. PhD days Helgoland, Germany, May 2014.

Hoffmann K, Kjeldsen KU, Lever MA, Risgaard-Petersen N, and Jørgensen BB (2013). Sulfate-Reducing Microbial Community Composition in the Marine Deep Biosphere. YouMaRes 4.0 Conference Oldenburg, September 2013.

Cruise participations:

2015 R/V Polarstern, PS93.2, HAUSGARTEN, Fram Strait, Arctic Ocean, Alfred Wegener Institute, Germany

2014 R/V Polarstern, PS85.2, HAUSGARTEN, Fram Strait, Arctic Ocean, Alfred Wegener Institute, Germany

Universität Bremen

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ERKLÄRUNG

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(Unterschrift)