Structure and function of microorganisms in the methanic sediments of the Helgoland mud area, North Sea, Germany

DISSERTATION

zur

Erlangung des Grades eines

Doktors der Naturwissenschaften

−Dr. rer. nat.−

dem Fachbereich Biologie/Chemie der

Universität Bremen vorgelegt von

Oluwatobi Emmanuel Oni

aus Ogun State, Nigeria

Bremen 2015

1. Gutachter: Prof. Dr. Michael W. Friedrich (Universität Bremen)

2. Gutachter: Prof. Dr. Kai-Uwe Hinrichs (Universität Bremen)

To my parents

To my daughter

In memory of a wonderful colleague and mentor,

Tetsuro Miyatake (R.I.P)

Preface

This doctoral thesis project was mainly supported by the Research Center/Cluster of Excellence 'The Ocean in the Earth System' (MARUM) funded by the Deutsche Forschungsgemeinschaft (DFG) and the University of Bremen. Additional financial support was received from the Max-Planck Society. This work, supervised by Prof. Dr. Michael W. Friedrich, is submitted as a dissertation to obtain a doctoral degree (Dr. rer. nat., Microbial Ecophysiology) from the University of Bremen, Germany.

With this work, my goal is to contribute to the understanding of the geo-microbiological interactions and diagenetic processes occurring in the subsurface sediments of the Helgoland mud area. The laboratory works and analyses, which generated the results presented in this work, were carried out in the University of Bremen (Germany), Alfred Wegener Institute for Polar and Marine Research (Bremerhaven, Germany), MARUM-Center for Marine Environmental Sciences (University of Bremen) and the Johannes Gutenberg University (Mainz, Germany) between 2012 and 2015. Each chapter is presented as "stand-alone" for convenience. Chapter 1 explains the scientific background of the problems addressed in the following parts of the thesis. Chapters 2, 3, and 4 cover three manuscripts that are in review, published, and in preparation for submission respectively to international peer-reviewed journals. Chapter 4, 5, and 7 present additional results which are not yet ready for publication but are relevant for the overall view of the topics addressed in this thesis. Chapter 8 sums up all the work presented in previous chapters and point out important areas for future research.

Table of Contents

Summary

The Helgoland mud area was characterized by high sedimentation rates prior to 1250 AD, most likely accounted for by the disintegration of the Helgoland Island during this period. Presumably, high amounts of terrigenous metals (e.g. Fe and Mn minerals) and organic matter were deposited as a result of the high sedimentation rates. Evident from the deposition of high amounts of organic matter and metals in the past are the shallow sulfate-methane transition zone (due to rapid organic matter-dependent electron acceptor consumption) and elevated concentrations of dissolved metals (Fe and Mn) in the methanic zone of the Helgoland mud area.

Such high concentrations of dissolved Fe and Mn have been observed in other highly-depositional environments (e.g. Argentine Basin, Bothnian Sea, Aarhus Bay etc.). However, whether biotic or abiotic, the source of elevated dissolved iron in the methanic zone is still not known. Amid several hypotheses, the exact mechanisms of iron reduction in methanic zones of marine sediments are also still a matter of debate. Therefore, as a first step to understanding the potential involvement of microorganisms in iron reduction in the methanic zone in marine sediments, this work provides a 16S rRNA gene-based characterization and quantification of bacteria and archaea populations in the surface and subsurface sediments of the Helgoland mud area. In addition, this work links iron reduction in the methanic zone of the Helgoland mud area to biotic activities and suggested microbial populations which may have been involved in iron cycling therein.

High and depth-wisely increasing concentrations of NH_4^+ in pore-water measurements from subsurface sediments of many highly-depositional environments around the world suggest that organic matter degradation is still ongoing in deeper sediments. Nevertheless, molecular information about the composition and diagenetic changes of organic matter from surface to subsurface sediments are few. On this front, this doctoral work shows that the most dominant bacteria and archaea populations in the subsurface sediments of the Helgoland mud area are influenced by concentration of organic matter, thus potentially important for organic matter degradation therein. Molecular characterization of a potentially bio-available portion of sedimentary organic matter (the water-extractable fraction) in the surface and subsurface sediments, using Fourier Ion Cyclotron Resonance Mass Spectrometry, most

importantly reveals that while aliphatic, N-rich compounds, presumably of algal origin are preferentially degraded in the surface sediments, O-rich, aromatic compounds, most likely of terrestrial origin are utilized in deeper sediments. These results are consistent with observations in subsurface soils of peatlands suggesting similar diagenetic alterations of organic matter in marine subsurface sediments.

It is a three decade-old finding that when poorly-crystalline Mn (IV) is added to marine sediments, there is a rapid formation of sulfate which is linked to biological activity. However, knowledge of the diversity of microorganisms involved in this reaction is limited. In experiments investigating the potential for chemolithotrophic Mn (IV) reduction in subsurface sediments of the Helgoland mud area, this work uncovers novel uncultured *Deltaproteobacteria* (tentatively named Marine Sediment Manganese-reducing Enrichment, MSME Cluster) potentially involved Mn (IV)-dependent sulfate formation in marine sediments.

Overall, this work adds to the current body of knowledge on microbe-mineral or geo-microbiological interactions in marine sediments. The finding that hematite enhanced methanogenesis (by 25−48 % faster) in a year-long slurry incubations with sediments from the subsurface sediment of the Helgoland mud area also provide a basis for future studies on how (semi)conductive iron minerals such as hematite, goethite, and pyrite may mediate electron transfer between specific bacterial populations and methanogens in the Helgoland mud area.

Zusammenfassung

Die Helgoländer Schlammzone ("Helgoland Mud Area") ist charakterisiert durch hohe Sedimentationsraten vor dem Jahre 1250, sehr wahrscheinlich ausgelöst durch den Landverlust der Insel während dieser Periode. Vermutlich wurden dabei große Mengen terrigener Metalle (z.B. Eisen- und Manganmineralien) und organischen Materials in diesen Sedimenten abgelagert. Dadurch bedingt ist eine relativ schmale Sulfat-Methan-Übergangszone (aufgrund der hohen Aufzehrungsrate der Elektronenakzeptoren bei hoher Verfügbarkeit organischen Materials) und erhöhte Konzentrationen gelöster Metalle in der Methanzone in diesem Gebiet.

Ähnlich hohe Konzentrationen an gelöstem Eisen und Mangan wurden auch in anderen Umgebungen mit hohem Ablagerungsanteil beobachtet (beispielsweise im Argentinischen Becken, in der Bottensee zwischen Finnland und Schweden, in der Bucht von Aarhus etc.). Jedoch ist bisher nicht bekannt, ob diese hohen Mengen an gelöstem Eisen biotischen oder abiotischen Ursprunges sind, genau wie die exakten Mechanismen der Eisenreduktion in der Methanzone mariner Sedimente noch diskutiert werden. Daher wurde mit dieser Arbeit eine quantitative und qualitative Beschreibung der mikrobiellen Gemeinschaft in dieser Zone auf der Basis von Genabundanzen der kleinen ribosomalen Untereinheit der Bakterien und Archaeen vorgelegt, um in einem ersten Schritt Erkenntnisse über die potentielle Rolle von Mikroorganismen in der Eisenreduktion innerhalb der Methanzone in den schlammreichen Gebieten um Helgoland zu gewinnen. Tatsächlich konnte durch diese Arbeit die Eisenreduktion in Verbindung mit biologischer Aktivität gebracht, und bestimmte mikrobielle Subgemeinschaften als besonders involviert vorgeschlagen werden.

Im Porenwasser vieler dieser ablagerungsreichen Sedimente weltweit werden hohe und proportional mit der Tiefe ansteigende Konzentrationen an NH_4^+ gemessen, was darauf hinweist, dass auch in diesen Tiefen noch organisches Material abgebaut wird. Allerdings existieren nur wenig Informationen über die Zusammensetzung und diagenetischer Veränderungen dieses Materials zwischen den einzelnen Sedimentschichten. Hierbei konnte diese Arbeit zeigen, dass die dominanten Bakterien und Archaeen in diesen Tiefen auf die Konzentrationsänderungen des organischen Materials reagieren, womöglich also auch im Abbau dieses Substrats involviert sind. Die molekulare

Charakterisierung des wasserlöslichen und damit potentiell biologisch verfügbaren Anteils dieses Materials im Oberflächensediment und den Schichten darunter mit Hilfe eines speziellen Verfahrens der Massenspektrometrie („Fourier Ion Cyclotron Resonance Mass Spectrometry“) ergab, dass sauerstoffreiche, aromatische Verbindungen in den tieferen Schichten abgebaut werden, während im Oberflächensediment eher aliphatische, stickstoffreiche Verbindungen bevorzugt werden. Diese Ergebnisse stimmen mit aktuellen Untersuchungen überein, die in den tieferen Horizonten von Torfmoorböden ähnliche Abbaumuster fanden.

Weiterhin beschäftigte sich diese Arbeit mit der mittlerweile drei Jahrzehnte alten Beobachtung, dass der Zusatz von biologisch zugänglichem Mangan(IV) in Meeressedimenten zu einer schnellen, biologisch bedingten Bildung von Sulfat führt. Auch hier ist wenig über die Diversität der involvierten Mikroorganismen bekannt, und auch hier konnte innerhalb der vorliegenden Arbeit der Kenntnisstand erweitert werden, weil neuartige, unkultivierte *Deltaproteobacteria* des MSME Clusters („Marine Sediment Manganese-reducing Enrichment“) gefunden wurden, die sich in Experimenten zur chemolithotrophen Reduktion von Mn(IV) anreicherten.

Insgesamt konnte diese Arbeit den Stand des Wissens über die Interaktionen zwischen Mikroben und Mineralien bzw. ihrer Umwelt in marinen Sedimenten erweitern. Der Befund, dass die Zugabe von Hematit die Methanogenese in den Helgoländer Sedimenten um 25-48% beschleunigen konnte, liefert eine Grundlage für weitere Experimente, die die Rolle von (halb)leitenden Eisenmineralien wie Hematit, Goethit und Pyrit im Elektronentransfer zwischen einzelnen bakteriellen Gemeinschaften und den Methanogenen beleuchten.

List of Manuscripts and Contribution of Authors

Manuscript 1

Microbial communities and organic matter composition in surface and subsurface sediments of the Helgoland mud area, North Sea

Oluwatobi Oni, Frauke Schmidt, Tetsuro Miyatake, Sabine Kasten, Matthias Witt, Kai-Uwe Hinrichs, Michael W. Friedrich

(Under revision for re-submission to *Frontiers in Microbiology, Impact Factor - 4.0*)

Author's contributions

O.O and F.S provided the concept for the study. O.O analyzed pyrosequencing data. F.S analyzed Mass Spectrometry (MS) data with contributions from O.O. M.W. performed MS measurements. S.K supplied fine-resolution Total Organic Carbon data. O.O wrote the manuscript. All authors gave inputs to the manuscript.

Manuscript 2

Distinct microbial populations are tightly linked to the profile of dissolved iron in the methanic sediments of the Helgoland mud area, North Sea

Oluwatobi Oni, Tetsuro Miyatake, Sabine Kasten, Tim Richter-Heitmann, David Fischer, Laura Wagenknecht, Ajinkya Kulkarni, Mathias Blumers, Sergii I. Shylin,Vadim Ksenofontov, Benilde F.O. Costa, Göstar Klingelhöfer, Michael W. Friedrich

(Published in *Frontiers in Microbiology* (2015), *Impact Factor - 4.0*)

Author's contributions

O.O, M.W.F and S.K. developed the concept for the study. O.O wrote the manuscript with inputs from M.W.F, S.K and T.M. O.O performed pyrosequencing analysis. T.R.M and A.K provided the phylogenetic trees. D.F. performed sequential extraction of iron minerals. L.W performed pore-water geochemical analyses. A.K performed clone library. O.O and T.M prepared samples for Mössbauer analysis. M.B, S.I.S, V.K, B.F.O and G.K performed Mössbauer Spectroscopy and analyzed the data, with advice from O.O.

Manuscript 3

Short Communication

Novel uncultured *Deltaproteobacteria* populations (MSME Cluster) are dominant under chemolithotrophic manganese (IV)-reducing conditions in marine sediment slurry incubations

Oluwatobi Oni, Cedric Hahn, Ajinkya Kulkarni, Sabine Kasten, Michael Friedrich

(In preparation for *Microbes and Environments, Impact Factor - 2.2*)

Author's contributions

O.O performed slurry incubation experiments, interpreted all the results and wrote the manuscript. C.H and A.K performed clone library and analyzed clone sequences. S.K provided geochemical data. M.W.F supervised and gave input on the work.

Chapter 1

1. Introduction

1.1. Role of Microorganisms in the formation of geochemical zones in marine sediments

Marine sediments cover 70 % of the Earth's surface. Marine sediments host the largest reservoir of organic carbon (Hedges and Keil, 1995) and microbial biomass (Whitman et al., 1998; Parkes et al., 2000) on earth, thus extremely important in shaping global carbon cycle and climate. The coupling of sedimentary organic matter degradation to the mineralization of terminal electron acceptors (e.g. oxygen, nitrate, manganese, iron and sulfate etc.) by microbes is pertinent to the delineation of distinct geochemical zones in marine sediments (Jørgensen, 2006). Ideally, microorganisms utilize terminal electron acceptors in marine sediments in order of the free energy available from the reactions (Table 1). This results in a redox cascade of electron-accepting processes (EAPs) such as that presented in Fig. 1.

Table 1: Pathways of organic carbon mineralization and their standard Gibbs free energies (ΔG°), per mole of organic carbon (after Jørgensen, 2006).

Pathway	Stoichiometry of reaction	ΔG° (KJ mol $^{-1}$)
Aerobic respiration	$CH_2O + O_2 \rightarrow CO_2 + H_2O$	-479
Denitrification	$5\ CH_2O + 4\ NO_3^- \rightarrow 2\ N_2 + 4\ HCO_3^- + CO_2 + 3\ H_2O$	-453
Mn (IV) reduction	$CH_2O + 3\ CO_2 + H_2O + 2\ MnO_2 \rightarrow 2\ Mn^{2+} + 4\ HCO_3^-$	-349
Fe (III) reduction	$CH_2O + 7\ CO_2 + 4\ Fe(OH)_3 \rightarrow 4\ Fe^{2+} + 8\ HCO_3^- + 3H_2O$	-114
Sulfate reduction	$2\ CH_2O + SO_4^{2-} \rightarrow H_2S + 2\ HCO_3^-$	-77
Methanogenesis	$CH_3\ COO^- + H^+ \rightarrow CH_4 + CO_2$	-28
Fermentation	$CH_3CH_2COO^- + 3\ H_2O \rightarrow CH_3COO^- + HCO_3^- + 3\ H_2O + H^+$	77

However, depending on the abundance of specific electron acceptors or their bioavailability, these cascades may not follow the depicted order. For example, sulfate is the most abundant electron acceptor in marine sediments and although less-energy yielding than most of other electron acceptors,

sulfate reduction is responsible for the oxidation of most of the organic carbon in coastal marine sediments (Jørgensen, 1982).

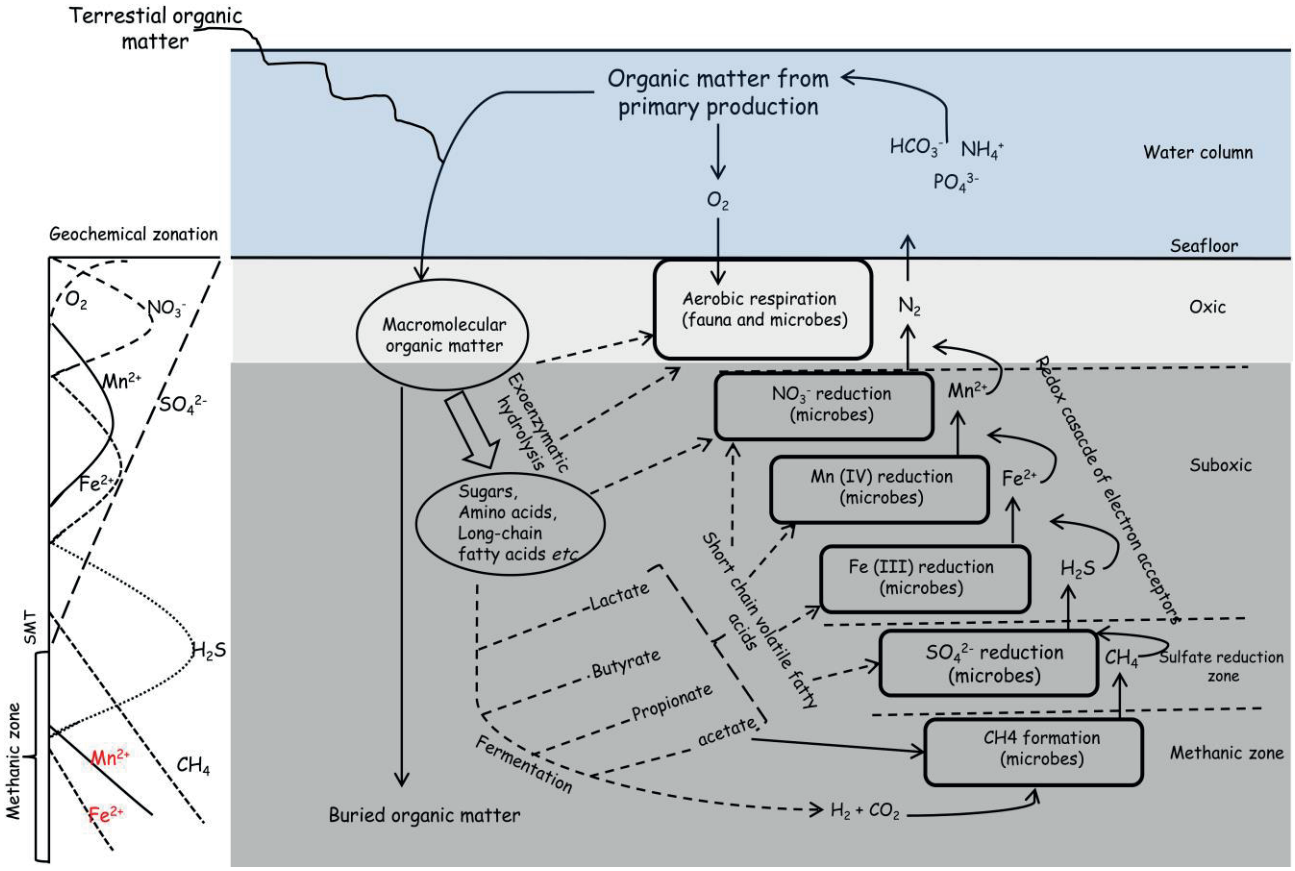

Fig.1. Schematic depiction of organic matter degradation steps, geochemical zonations and redox cascade of electron acceptors mineralization in marine sediments (figure modified from Jørgensen, 2006 and Ahke, 2007).

Due to the involvement of distinct microorganisms in specific EAPs, certain microorganisms may dominate specific redox zones in marine sediments. For example, the intersecting point of opposing fluxes of sulfate and methane in marine sediments (Fig. 1), termed sulfate-methane transition zone (SMT, Iversen and Jørgensen, 1985), is often characterized by a dominance or prominent presence of Anaerobic Methane-oxidizing (ANME) archaea and sulfate-reducing bacteria, which can be found living together in tight consortia (Boetius et al., 2000). Recently, links between Marine Group I archaea and pore-water nitrate concentrations in marine sediments could also be observed in sediments of the Arctic Mid-Ocean Ridge, proffering a possible role for members of this group in the nitrogen cycle (Jorgensen et al., 2012). Therefore, bearing in mind that redox zones in marine sediments are not

always clear-cut and may overlap (Canfield and Thamdrup, 2009) e.g. as result of bioturbation by burrowing organisms, the spatial dominance of certain microorganisms in specific environments can still provide clues for rough predictions of the occurrence of associated geochemical processes in marine sediments.

1.2. Organic matter composition in marine sediments and implication for microorganisms

Organic matter is dispersed in marine sediments in different concentrations depending largely on the size of the organic matter source (terrestrial *vs.* marine), water depth, and sedimentation rates (Hedges and Keil, 1995). Apart from organic matter produced in the marine environment, e.g. algal and bacterial biomass rich in lipids and nitrogenous compounds, marine sediments also receive inputs of terrestrial organic matter, which is mainly derived from plant materials rich in cellulose and lignin (de Leeuw and Largeau, 1993). Regardless of sources, extensive recycling of organic matter occurs in the water column (Hedges and Keil, 1995) and only about 1 % of the organic carbon export reaches the seafloor on a global scale (Hedges and Keil, 1995). This detrital organic matter serves as a main energy source for microorganisms living in marine sediments (Jørgensen and Boetius, 2007).

In surface sediments, easily degradable organic matter is preferentially utilized by microorganisms (Cowie and Hedges, 1994; Wakeham et al., 1997), whereas less reactive organic matter accumulates and is buried in deeper sediments (Zonneveld et al., 2010). Consequently, microorganisms inhabiting deeper sediments have to satisfy their metabolic demands by relying on more recalcitrant organic matter, whose degradation requires longer time scales (Middelburg, 1989; Biddle et al., 2006). There are very few studies (e.g. Xie et al., 2013; Vigneron et al., 2014) on the nature of organic matter mineralized by microorganisms in marine subsurface sediments. However, the consistence of microorganisms dominating subsurface sediments (below 30 cm) across many environments may be due to special adaptations for utilization of less reactive organic matter (Biddle et al., 2006; Inagaki et al., 2006). Dominant Bacteria are usually *Chloroflexi* and candidate division JS1 (Inagaki et al., 2006; Webster et al., 2007; Blazejak and Schippers, 2010; Hamdan et al., 2011; Zhang et al., 2012; Schippers et al., 2012; Vigneron et al., 2014), while dominant Archaea are mostly members of the Miscellaneous Crenarchaeota Group (MCG) and Marine Benthic Group B (MBGB), otherwise

referred to as Deep Sea Archaeal Group (DSAG; Inagaki et al., 2006; Biddle et al., 2006; Teske and Sørensen, 2008; Kubo et al., 2012). How these important groups of microorganisms thrive, and what types of carbon sources they assimilate is still largely unknown.

1.3. The depositional history of the Helgoland mud area, North Sea

The North Sea is characterized by high-energy waves which causes constant redistribution of sediment, especially in the shallow parts (average water depth- < 100 m). Nevertheless, there are a few areas in the North Sea where high deposition of sediments still occur (Lohse et al., 1995). Apart from the Skagerrak, which is the area of highest sediment deposition in the North Sea, the Helgoland mud area in the German Bight represents another area of sustained sediment deposition (Hebbeln et al., 2003). Sustained sediment deposition in the Helgoland mud area is driven by small-scale eddy currents resulting from the interaction between long-shore coastal currents and riverine discharges from the Elbe and Weser (Hebbeln et al., 2003).

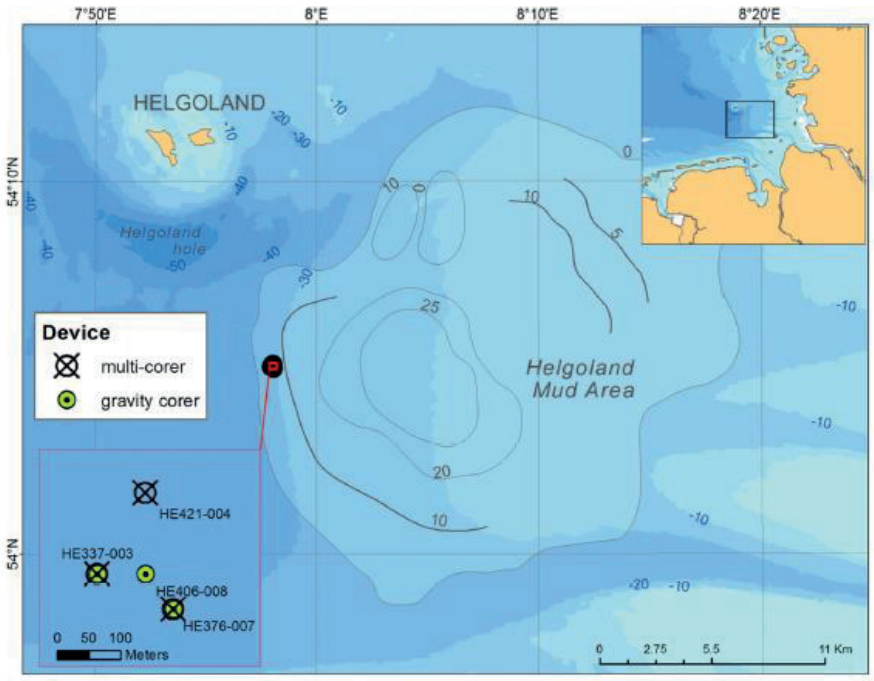

Fig. 2. Map of the Helgoland mud area. Picture taken from Oni et al., 2015

The Helgoland mud area covers an area of 500 km^2 and is overlaid by 20-30 m deep water (Hebbeln et al., 2003). The Helgoland mud area, in contrast to the till-now unfilled Helgoland hole south west, is filled with about 30 m of Holocene sediment (Dellwig et al., 2000; Hebbeln et al., 2003). In periods before 1250 AD (about 750 years before present), average sedimentation rates in the Helgoland mud area (Fig. 3) were estimated to be ~ 13 mm yr^{-1} compared to estimates of average sedimentation rates presently (~ 1.6 mm yr^{-1}). Of all historical events in the North Sea during the middle ages, disintegration of the Helgoland island was the most plausible reason for the higher sedimentation rates before 1250 AD, indicated by the substantial reduction in size of Helgoland after 800 AD (Hebbeln et al., 2003).

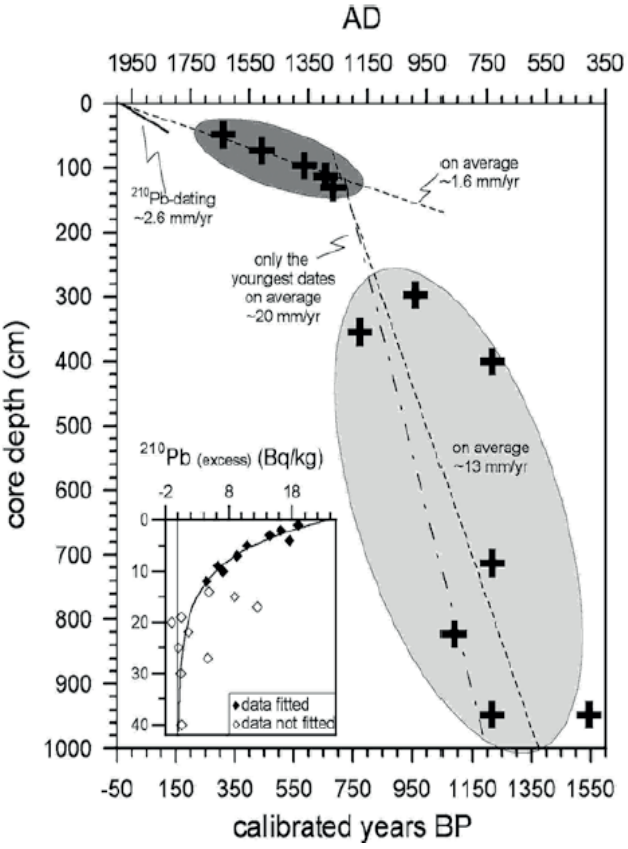

Fig. 3. Stratigraphic display of ^{210}Pb-determined sedimentation rates in sediment core GeoB 4801-1 retrieved from the Helgoland mud area. Light grey shading highlights areas of high sedimentation rates (up to 20 mm yr^{-1}) at the bottom of the core. Dark grey shading depicts areas of lower sedimentation rates (up to ~2.6 mm yr^{-1}) at the top of the core. Picture taken from Hebbeln et al., 2003 with permission from publisher.

1.4. Connections between the depositional history and geochemistry of the Helgoland mud area

Higher sedimentation rates in the past likely had important consequences for the geochemistry of the Helgoland mud area. In other environments such as the western Argentine Basin, mineralogical and bulk solid phase analyses data provided evidences for high deposition of terrigenous material with high metal oxide (e.g. iron and manganese minerals) concentrations (Riedinger et al., 2005). Predictably, such environments are likely to contain high amounts of terrestrially-derived organic matter. Consequently, there is sufficient amount of organic matter to fuel the reduction of oxidants in such environments. As sulfate reduction is mostly responsible for the mineralization of organic matter in coastal marine environments, the rate of sulfate reduction in highly depositional areas is expected to be high. This means that sulfate is quickly depleted (Meister et al., 2013) and conditions of co-existing minimal sulfate and methane concentrations, favoring anaerobic oxidation of methane with sulfate are thus established earlier in comparison to environments where sedimentation is lower and organic matter is buried at a much slower pace. Therefore, highly depositional marine environments are expected to be characterized by shallower SMT (Riedinger et al., 2006; Egger et al., 2014). Rapid upward movement of the SMT may also reduce the contact time between hydrogen sulfide formed from sulfate-dependent AOM and buried metals, allowing the deposition of excess of minerals such as iron (III) and manganese (IV) to depths below the SMT (methanic zone). In these respects, the Helgoland mud area is characterized by a shallow SMT (30-75 cm bsf, Oni et al., 2015) and high concentrations of dissolved iron deep in the methanic zone (Oni et al., 2015). Such elevated concentrations of pore-water dissolved iron deep in the methanic zone have also been found in the subsurface sediments of other highly depositional marine environments such as the Amazon Fan (Flood et al., 1995; Kasten et al., 1998), Peru Margin (D'Hondt et al., 2004), Sea of Okhotsk (Wallmann et al., 2008), Argentine Basin (Hensen et al., 2003; Riedinger et al., 2005, 2014), Zambesi Fan (März et al., 2008), Aarhus Bay (Holmkvist et al., 2011) and Bothnian Sea (Slomp et al., 2013; Egger et al., 2014). The occurrence of such high concentrations of pore-water dissolved iron in the methanic zone, the source of which is so far unknown, is intriguing as it does not conform to textbook knowledge of redox cascades of electron-accepting processes in marine sediments (see Fig. 1, sub-section 1.1). In the Helgoland mud area, excess Fe (III) or Mn (IV) minerals and high flux of organic

matter together thus presents themselves as an important spectacle for studying geo-microbiological interactions.

1.4.1. Proposed hypotheses to explain the source of dissolved iron the subsurface sediments of the Helgoland mud area

In surface marine sediments, iron reduction typically occurs in the top 10 cm below sea floor (Vandieken et al., 2006a, 2006b). Chemical (Canfield, 1989; Canfield et al., 1992; Poulton et al., 2004) and microbial (Roden and Lovley, 1993; Vandieken et al., 2006b; Nickel et al., 2008; Vandieken and Thamdrup, 2013) iron reduction in surface marine sediments have also been well studied. However, the pathways of dissolved iron formation in methane-rich subsurface sediments of some marine environments (see sub-section 1.4) are yet to be fully established and documented. A number of hypotheses, mostly revolving around microbial *vs.* chemical mechanisms, have been put forward to explain the elevated concentrations of pore-water dissolved iron measured at these sites and are briefly discussed below:

The "Cryptic Sulfur Cycle"

One of the central hypotheses that have been used to explain iron reduction in the methanic zone of sediments of the Aarhus Bay, Denmark, is the abiotic reaction between downward-diffusing sulfide from sulfate reduction in the SMT zone, with iron (III) minerals (Fig. 4) deeply buried over geological time scales (Holmkvist et al., 2011).

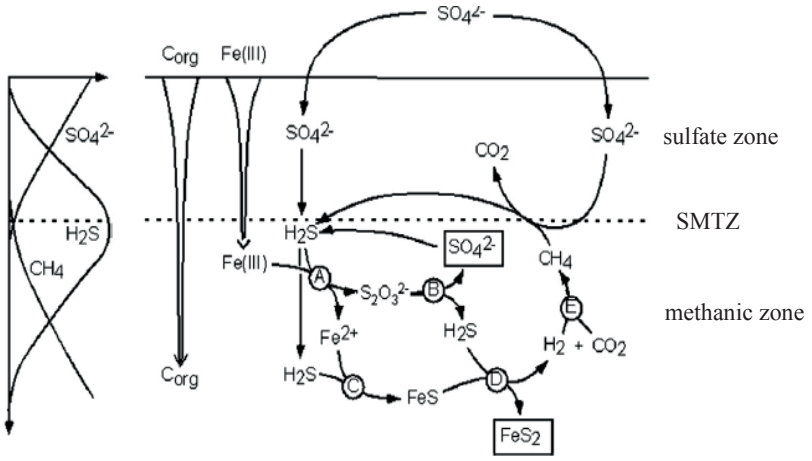

Fig. 4. Conceptual depiction of an iron-driven "cryptic sulfur cycle" in the methanic zone of the Aarhus Bay sediments. Formation of pyrite (FeS_2) results in the presence of excess hydrogen sulfide and excess reducing equivalent from pyritzation (H_2) is proposed to be utilized for methanogenesis by methanogenic archaea. Picture taken from Holmkvist et al., 2011 with permission from publisher.

Disproportionation of intermediate sulfur species e.g. elemental sulfur and thiosulfate, resulting from reaction between sulfide and iron (III) minerals to sulfide and sulfate was put forward as the origin of background sulfate concentrations (< 500 μM) still detected in the methanic zone (Holmkvist et al., 2011). The iron-driven internal cycling of sulfur in steady state, thus re-supplies sulfate for use by sulfate-reducing bacteria, which were detected in unexpectedly high abundance in the methanic zone of the Aarhus Bay and Black Sea sediments (Leloup et al., 2007, 2009). Besides, Fe (III), Mn (IV) may also play a role in the re-supply of sulfate in the methanic zone. Although only shown in marine surface sediments, Mn (IV) is a powerful oxidant for solid phase sulfide minerals (Aller and Rude, 1988; King, 1990) such as mackinawite (FeS) and pyrite (FeS_2) to sulfate, a reaction which although can occur purely chemically (Schippers and Jørgensen, 2001), has also been linked to microbial activity (Aller and Rude, 1988; Thamdrup et al., 1993; Lovley and Phillips, 1994; Tender et al., 2002) but with limited knowledge of diversity of microorganisms involved in nature.

Biological oxidation of organic matter coupled to iron reduction

As in surface marine sediments, organic matter oxidation, e.g. that of glucose or other fermentation end products such as acetate or hydrogen, can be enzymatically coupled to dissimilatory iron reduction (Lovley, 1993) by bacteria such as *Desulfuromonas* (Roden and Lovley, 1993; Coates et al., 1995; Vandieken et al., 2006c) or *Shewanella* (Das and Caccavo, 2000) is a potential pathway for iron reduction in the methanic zones. The oxidation of electron donors such as acetate or hydrogen coupled to iron reduction in the methanic zone may be complicated by the fact that iron reducers and methanogen compete for the same electron donors (Achtnich et al., 1995).

Fermentative processes linked to iron (III) reduction

During the fermentation of complex organic matter, iron (III) mineral may serve as a sink for excess electrons and means of maximizing carbon utilization from organic substrate fermentation (Dobbin et al., 1999; Shah et al., 2014). Dobbin et al. observed that glucose degradation coupled to iron reduction by *Clostridium beijerinckii* did not occur via an electron transport chain *i.e*. was not directly coupled to energy generation by the microorganism. However, the presence of Fe (III) facilitated the regeneration of $NAD(P)^+$ used for generation of additional ATP to that provided by the presence of glucose alone. Since many microorganisms usually dominant in subsurface sediments (see review, Parkes et al., 2014 and refs. therein) have been linked to fermentative metabolism (Dodsworth et al., 2013; Peacock et al., 2013; Hug et al., 2013; Wrighton et al., 2014), organic matter fermentation coupled to iron reduction is conceivable as a hypothesis for the source of iron reduction in the methanic zone of marine sediments.

AOM-driven iron reduction

Traditionally, our idea of methane oxidation in environments was that it was only performed aerobically by methanotrophic bacteria (Hanson and Hanson, 1996) and anaerobically via a consortium of methane-oxidizing archaea (ANME) and a deltaproteobacterial sulfate-reducing partner (Boetius et al., 2000; Orphan et al., 2001). In the last 6-7 years, new environmentally relevant pathways of methane oxidation have been unearthed, with findings that this process can be coupled to terminal electron acceptors other than sulfate, e.g. iron and manganese oxides (Beal et al., 2009;

Wankel et al., 2012; Segarra et al., 2013; Egger et al., 2014) or nitrite and nitrate (Pernthaler et al., 2008; Ettwig et al., 2010; Haroon et al., 2013). Recently, for sulfate-depleted methanic sediments of the Argentine Basin, it was argued that high amounts of pore-water dissolved iron detected must have resulted from methane oxidation (Riedinger et al., 2014). Similarly, Egger and colleagues concluded that high concentration of dissolved iron in the methanic zone of the Bothnian Sea is most likely a result of AOM coupled to iron reduction based on higher rates of methane oxidation observed in their slurry incubation experiments, in the presence of amorphous iron (III) minerals (Egger et al., 2014). However, microorganisms potentially involved in methane oxidation in their incubation studies were not identified.

1.5. Goals of this thesis

The main aims of this thesis have been divided into two major parts which are highlighted as follows:

- As there are still uncertainties surrounding the biotic *vs.* abiotic origin as well as the main pathways of dissolved iron formation in sulfate-depleted methanic sediments, it is important to ascertain whether iron reduction in the methanic zone of the Helgoland mud area was microbially-driven. If microbially-mediated, identify the pathway(s) and microorganisms potentially driving the process .
- High flux of organic matter into the subsurface sediments of the Helgoland mud area made it important to determine the nature of organic matter present therein in relation to the surface sediments which were more recently deposited under comparably lower sedimentation rates. I also aimed to discuss the potential links between the nature/sources of organic matter and microorganisms inhabiting the surface and subsurface sediments of the Helgoland mud area.

Additionally, in a pilot experiment, I aimed to test the potential for chemolithotrophic Mn (IV) reduction in subsurface sediments of the Helgoland mud area.

1.6. Approach to achieving goals

The goals of this thesis were realized through the following approaches:

- Depth-wise molecular characterization of the microbial communities in the surface and subsurface sediments. This helped to get a first insight into the metabolic potentials that may exist in the Helgoland mud area, based on literature knowledge of the environmental roles of microorganisms detected.
- Matching depth-wise distributions of microbial populations with specific geochemical profiles (e.g. dissolved iron concentrations and total organic carbon) to predict roles of distinct microbial populations *in situ*.
- Molecular characterization of water-extractable organic matter as a first step to identifying classes of organic matter being utilized preferentially in the surface and methanic subsurface sediments of the Helgoland mud area.
- Analyze the nature of iron (III) minerals *in situ* to check for the presence of amorphous iron minerals which are more amenable to microbial reduction
- Perform slurry incubation experiments with sediments from the Helgoland mud area to find out which substrates, microbes or processes are most likely or likely not driving iron reduction and manganese reduction *in situ.*

References

Achtnich, C., Bak, F., and Conrad, R. (1995). Competition for electron donors among nitrate reducers , ferric iron reducers , sulfate reducers , and methanogens in anoxic paddy soil. *Biol. Fertil. Soils* 19, 65–72.

Ahke, A. (2007). Composition of molecular organic matter pools, pigments and proteins, in Benguela upwelling and Arctic sediments. Ph. D. thesis, Universität Bremen (Germany). 20. Available at: http://elib.suub.uni-bremen.de/diss/docs/00010817.pdf [Accessed August 27, 2015].

Aller, R. C., and Rude, P. D. (1988). Complete oxidation of solid phase sulfides by manganese and bacteria in anoxic marine sediments. *Geochim. Cosmochim. Acta* 52, 751–765. doi:10.1016/0016-7037(88)90335-3.

Beal, E. J., House, C. H., and Orphan, V. J. (2009). Manganese- and iron-dependent marine methane oxidation. *Science* 325, 184–7. doi:10.1126/science.1169984.

Biddle, J. F., Lipp, J. S., Lever, M. A., Lloyd, K. G., Sørensen, K. B., Anderson, R., Fredricks, H. F., Elvert, M., Kelly, T. J., Schrag, D. P., et al. (2006). Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proc. Natl. Acad. Sci. U. S. A.* 103, 3846–51. doi:10.1073/pnas.0600035103.

Blazejak, A., and Schippers, A. (2010). High abundance of JS-1- and *Chloroflexi*-related Bacteria in deeply buried marine sediments revealed by quantitative, real-time PCR. *FEMS Microbiol. Ecol.* 72, 198–207. doi:10.1111/j.1574-6941.2010.00838.x.

Boetius, A., Ravenschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O. (2000). A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407, 623–6. doi:10.1038/35036572.

Canfield, D. E. (1989). Reactive iron in marine sediments. *Geochim. Cosmochim. Acta* 53, 619–32. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11539783.

Canfield, D. E., Raiswell, R., and Bottrell, S. H. (1992). The reactivity of sedimentary iron minerals toward sulfide. *Am. J. Sci.* 292, 659–683. doi:10.2475/ajs.292.9.659.

Canfield, D. E., and Thamdrup, B. (2009). Towards a consistent classification scheme for geochemical environments, or, why we wish the term “suboxic” would go away. *Geobiology* 7, 385–92. doi:10.1111/j.1472-4669.2009.00214.x.

Coates, J. D., Lonergan, D. J., Philips, E. J., Jenter, H., and Lovley, D. R. (1995). *Desulfuromonas palmitatis sp. nov*., a marine dissimilatory Fe(III) reducer that can oxidize long-chain fatty acids. *Arch. Microbiol.* 164, 406–13. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8588742.

Cowie, G. L., and Hedges, J. I. (1994). Biochemical indicators of diagenetic alteration in natural organic matter mixtures. *Nature* 369, 304–307. doi:10.1038/369304a0.

D’Hondt, S., Jørgensen, B. B., Miller, D. J., Batzke, A., Blake, R., Cragg, B. A., Cypionka, H., Dickens, G. R., Ferdelman, T., Hinrichs, K., et al. (2004). Distributions of microbial activities in deep subseafloor sediments. *Science* 306, 2216–21. doi:10.1126/science.1101155.

Das, A., and Caccavo, F. (2000). Dissimilatory Fe(III) oxide reduction by *Shewanella alga* BrY requires adhesion. *Curr. Microbiol.* 40, 344–7. doi:10.1007/s002849910068.

Dellwig, O., Hinrichs, J., Hild, a, and Brumsack, H.-J. (2000). Changing sedimentation in tidal flat sediments of the southern North Sea from the Holocene to the present: a geochemical approach. *J. Sea Res.* 44, 195–208. doi:10.1016/S1385-1101(00)00051-4.

Dobbin, P. S., Carter, J. P., Garcia-Salamanca San Juan, C., von Hobe, M., Powell, A. K., and Richardson, D. J. (1999). Dissimilatory Fe(III) reduction by *Clostridium beijerinckii* isolated from freshwater sediment using Fe(III) maltol enrichment. *FEMS Microbiol. Lett.* 176, 131–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10418140.

Dodsworth, J. A., Blainey, P. C., Murugapiran, S. K., Swingley, W. D., Ross, C. A., Tringe, S. G., Chain, P. S. G., Scholz, M. B., Lo, C.-C., Raymond, J., et al. (2013). Single-cell and metagenomic analyses indicate a fermentative and saccharolytic lifestyle for members of the OP9 lineage. *Nat. Commun.* 4, 1854. doi:10.1038/ncomms2884.

Egger, M., Rasigraf, O., Sapart, C. J., Jilbert, T., Jetten, M. S. M., Röckmann, T., van der Veen, C., Banda, N., Kartal, B., Ettwig, K. F., et al. (2014). Iron-mediated anaerobic oxidation of methane in brackish coastal sediments. *Environ. Sci. Technol.* 277, 277–283. doi:10.1021/es503663z.

Ettwig, K. F., Butler, M. K., Le Paslier, D., Pelletier, E., Mangenot, S., Kuypers, M. M. M., Schreiber, F., Dutilh, B. E., Zedelius, J., de Beer, D., et al. (2010). Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature* 464, 543–8. doi:10.1038/nature08883.

Flood, R. D., Piper, D., and Klaus, A. (1995). Initial Reports. *Proc. Ocean Drill. Progam.* 155. doi:10.2973/odp.proc.ir.155.1995.

Hamdan, L. J., Gillevet, P. M., Pohlman, J. W., Sikaroodi, M., Greinert, J., and Coffin, R. B. (2011). Diversity and biogeochemical structuring of bacterial communities across the Porangahau ridge accretionary prism, New Zealand. *FEMS Microbiol. Ecol.* 77, 518–32. doi:10.1111/j.1574-6941.2011.01133.x.

Hanson, R. S., and Hanson, T. E. (1996). Methanotrophic Bacteria. *Microbiol. Rev.* 60, 439–471.

Haroon, M. F., Hu, S., Shi, Y., Imelfort, M., Keller, J., Hugenholtz, P., Yuan, Z., and Tyson, G. W. (2013). Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. *Nature* 500, 567–70. doi:10.1038/nature12375.

Hebbeln, D., Scheurle, C., and Lamy, F. (2003). Depositional history of the Helgoland mud area, German Bight, North Sea. *Geo-Marine Lett.* 23, 81–90. doi:10.1007/s00367-003-0127-0.

Hedges, J. I., and Keil, R. G. (1995). Sedimentary organic matter preservation: an assessment and speculative synthesis. *Mar. Chem.* 49, 81–115. doi:10.1016/0304-4203(95)00008-F.

Hensen, C. H. H., Zabel, M., Pfeifer, K., Schwenk, T., Kasten, S., Riedinger, N., Schulz, H. D., and Boetius, A. (2003). Control of sulfate pore-water profiles by sedimentary events and the significance of anaerobic oxidation of methane for the burial of sulfur in marine sediments. *Geochim. Cosmochim. Acta* 67, 2631–2647. doi:10.1016/S0016-7037(00)00199-6.

Holmkvist, L., Ferdelman, T. G., and Jørgensen, B. B. (2011). A cryptic sulfur cycle driven by iron in the methane zone of marine sediment (Aarhus Bay, Denmark). *Geochim. Cosmochim. Acta* 75, 3581–3599. doi:10.1016/j.gca.2011.03.033.

Hug, L. A., Castelle, C. J., Wrighton, K. C., Thomas, B. C., Sharon, I., Frischkorn, K. R., Williams, K. H., Tringe, S. G., and Banfield, J. F. (2013). Community genomic analyses constrain the distribution of metabolic traits across the *Chloroflexi* phylum and indicate roles in sediment carbon cycling. *Microbiome* 1, 22. doi:10.1186/2049-2618-1-22.

Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., Suzuki, M., Takai, K., Delwiche, M., Colwell, F. S., et al. (2006). Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proc. Natl. Acad. Sci. U. S. A.* 103, 2815–20. doi:10.1073/pnas.0511033103.

Iversen, N., and Jørgensen, B. B. (1985). Anaerobic methane oxidation rates at the sulfate-methane transition in marine sediments from Kattegat and Skagerrak (Denmark). *Limnol. Oceanogr.* 30, 944–955.

Jørgensen, B. B. (1982). Mineralization of organic matter in the sea bed—the role of sulphate reduction. *Nature* 296, 643–645. doi:10.1038/296643a0.

Jørgensen, B. B. (2006). "Pathways of organic matter degradation," in *Marine Geochemistry*, eds. H. Schulz and M. Zabel (Bremen: Springer Berlin Heidelberg), 180–193.

Jørgensen, B. B., and Boetius, A. (2007). Feast and famine--microbial life in the deep-sea bed. *Nat. Rev. Microbiol.* 5, 770–81. doi:10.1038/nrmicro1745.

Jorgensen, S. L., Hannisdal, B., Lanzén, A., Baumberger, T., Flesland, K., Fonseca, R., Ovreås, L., Steen, I. H., Thorseth, I. H., Pedersen, R. B., et al. (2012). Correlating microbial community profiles with geochemical data in highly stratified sediments from the Arctic Mid-Ocean Ridge. *Proc. Natl. Acad. Sci.* 109, 2846–2855. doi:10.1594/PANGAEA.786687.

Kasten, S., Freudenthal, T., Gingele, F. X., and Schulz, H. D. (1998). Simultaneous formation of iron-rich layers at different redox boundaries in sediments of the Amazon deep-sea fan. *Geochim. Cosmochim. Acta* 62, 2253–2264.

King, G. M. (1990). Effects of added manganic and ferric oxides on sulfate reduction and sulfide oxidation in intertidal sediments. *FEMS Microbiol. Lett.* 73, 131–138. doi:10.1111/j.1574-6968.1990.tb03933.x.

Kubo, K., Lloyd, K. G., F Biddle, J., Amann, R., Teske, A., and Knittel, K. (2012). Archaea of the Miscellaneous Crenarchaeotal Group are abundant, diverse and widespread in marine sediments. *ISME J.* 6, 1949–65. doi:10.1038/ismej.2012.37.

De Leeuw, J. W., and Largeau, C. (1993). "A review of macromolecular organic compounds that comprise living organisms and their role in kerogen, coal, and petroleum formation," in *Organic Geochemistry, Principles and Applications*, eds. M. Engel and S. A. Macko (New York, NY: Plenum Press), 23–72. Available at: http://link.springer.com/chapter/10.1007/978-1-4615-2890-6_2 [Accessed February 11, 2015].

Leloup, J., Fossing, H., Kohls, K., Holmkvist, L., Borowski, C., and Jørgensen, B. B. (2009). Sulfate-reducing bacteria in marine sediment (Aarhus Bay, Denmark): abundance and diversity related to geochemical zonation. *Environ. Microbiol.* 11, 1278–91. doi:10.1111/j.1462-2920.2008.01855.x.

Leloup, J., Loy, A., Knab, N. J., Borowski, C., Wagner, M., and Jørgensen, B. B. (2007). Diversity and abundance of sulfate-reducing microorganisms in the sulfate and methane zones of a marine sediment , Black Sea. 9, 131–142. doi:10.1111/j.1462-2920.2006.01122.x.

Lohse, L., Malschaert, J. F. P., Slomp, C. P., Helder, W., and van Raaphorst, W. (1995). Sediment-water fluxes of inorganic nitrogen compounds along the transport route of organic matter in the North Sea. *Ophelia* 41, 173–197. doi:10.1080/00785236.1995.10422043.

Lovley, D. R. (1993). Dissimilatory metal reduction. *Annu. Rev. Microbiol.* 47, 263–90. doi:10.1146/annurev.mi.47.100193.001403.

Lovley, D. R., and Phillips, E. J. P. (1994). Novel processes for anaerobic sulfate production from elemental sulfur by sulfate-reducing bacteria. *Appl. Environ. Microbiol.* 60, 2394–2399.

März, C., Hoffmann, J., Bleil, U., de Lange, G. J., and Kasten, S. (2008). Diagenetic changes of magnetic and geochemical signals by anaerobic methane oxidation in sediments of the Zambezi deep-sea fan (SW Indian Ocean). *Mar. Geol.* 255, 118–130. doi:10.1016/j.margeo.2008.05.013.

Meister, P., Liu, B., Ferdelman, T. G., Jørgensen, B. B., and Khalili, A. (2013). Control of sulphate and methane distributions in marine sediments by organic matter reactivity. *Geochim. Cosmochim. Acta* 104, 183–193. doi:10.1016/j.gca.2012.11.011.

Middelburg, J. J. (1989). A simple rate model for organic matter decomposition in marine sediments. *Geochim. Cosmochim. Acta* 53, 1577–1581. doi:10.1016/0016-7037(89)90239-1.

Nickel, M., Vandieken, V., Brüchert, V., and Jørgensen, B. B. (2008). Microbial Mn(IV) and Fe(III) reduction in northern Barents Sea sediments under different conditions of ice cover and organic carbon deposition. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 55, 2390–2398. doi:10.1016/j.dsr2.2008.05.003.

Oni, O., Miyatake, T., Kasten, S., Richter-Heitmann, T., Fischer, D., Wagenknecht, L., Kulkarni, A., Blumers, M., Shylin, S. I., Ksenofontov, V., et al. (2015). Distinct microbial populations are tightly linked to the profile of dissolved iron in the methanic sediments of the Helgoland mud area, North Sea. *Front. Microbiol.* 06, 1–15. doi:10.3389/fmicb.2015.00365.

Orphan, V. J., House, C. H., Hinrichs, K. U., McKeegan, K. D., and DeLong, E. F. (2001). Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science* 293, 484–7. doi:10.1126/science.1061338.

Parkes, R. J., Cragg, B. A., and Wellsbury, P. (2000). Recent studies on bacterial populations and processes in subseafloor sediments : A review. *Hydrogeol. J.*, 11–28.

Parkes, R. J., Cragg, B., Roussel, E., Webster, G., Weightman, A., and Sass, H. (2014). A review of prokaryotic populations and processes in sub-seafloor sediments, including biosphere:geosphere interactions. *Mar. Geol.* 352, 409–425. doi:10.1016/j.margeo.2014.02.009.

Peacock, J. P., Cole, J. K., Murugapiran, S. K., Dodsworth, J. A., Fisher, J. C., Moser, D. P., and Hedlund, B. P. (2013). Pyrosequencing reveals high-temperature cellulolytic microbial consortia in Great Boiling Spring after in situ lignocellulose enrichment. *PLoS One* 8, e59927. doi:10.1371/journal.pone.0059927.

Pernthaler, A., Dekas, A. E., Brown, C. T., Goffredi, S. K., Embaye, T., and Orphan, V. J. (2008). Diverse syntrophic partnerships from deep-sea methane vents revealed by direct cell capture and metagenomics. *Proc. Natl. Acad. Sci. U. S. A.* 105, 7052–7. doi:10.1073/pnas.0711303105.

Poulton, S. W., Krom, M. D., and Raiswell, R. (2004). A revised scheme for the reactivity of iron (oxyhydr)oxide minerals towards dissolved sulfide. *Geochim. Cosmochim. Acta* 68, 3703–3715. doi:10.1016/j.gca.2004.03.012.

Riedinger, N., Formolo, M. J., Lyons, T. W., Henkel, S., Beck, a, and Kasten, S. (2014). An inorganic geochemical argument for coupled anaerobic oxidation of methane and iron reduction in marine sediments. *Geobiology* 12, 172–81. doi:10.1111/gbi.12077.

Riedinger, N., Kasten, S., Gröger, J., Franke, C., and Pfeifer, K. (2006). Active and buried authigenic barite fronts in sediments from the Eastern Cape Basin. *Earth Planet. Sci. Lett.* 241, 876–887. doi:10.1016/j.epsl.2005.10.032.

Riedinger, N., Pfeifer, K., Kasten, S., Garming, J. F. L., Vogt, C., and Hensen, C. (2005). Diagenetic alteration of magnetic signals by anaerobic oxidation of methane related to a change in sedimentation rate. *Geochim. Cosmochim. Acta* 69, 4117–4126. doi:10.1016/j.gca.2005.02.004.

Roden, E. E., and Lovley, D. R. (1993). Dissimilatory Fe(III) reduction by the marine microorganism *Desulfuromonas acetoxidans*. *Appl. Environ. Microbiol.* 59, 734–42. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=202183&tool=pmcentrez&rendertype=abstract.

Schippers, A., and Jørgensen, B. B. (2001). Oxidation of pyrite and iron sulfide by manganese dioxide in marine sediments. *Geochim. Cosmochim. Acta* 65, 915–922. doi:10.1016/S0016-7037(00)00589-5.

Schippers, A., Kock, D., Höft, C., Köweker, G., and Siegert, M. (2012). Quantification of microbial Communities in subsurface marine sediments of the Black Sea and off Namibia. *Front. Microbiol.* 3, 16. doi:10.3389/fmicb.2012.00016.

Segarra, K. E. A., Comerford, C., Slaughter, J., and Joye, S. B. (2013). Impact of electron acceptor availability on the anaerobic oxidation of methane in coastal freshwater and brackish wetland sediments. *Geochim. Cosmochim. Acta* 115, 15–30. doi:10.1016/j.gca.2013.03.029.

Shah, M., Lin, C.-C., Kukkadapu, R., Engelhard, M. H., Zhao, X., Wang, Y., Barkay, T., and Yee, N. (2014). Syntrophic Effects in a Subsurface Clostridial Consortium on Fe(III)-(Oxyhydr)oxide Reduction and Secondary Mineralization. *Geomicrobiol. J.* 31, 101–115. doi:10.1080/01490451.2013.806610.

Slomp, C. P., Mort, H. P., Jilbert, T., Reed, D. C., Gustafsson, B. G., and Wolthers, M. (2013). Coupled dynamics of iron and phosphorus in sediments of an oligotrophic coastal basin and the impact of anaerobic oxidation of methane. *PLoS One* 8, e62386. doi:10.1371/journal.pone.0062386.

Tender, L. M., Reimers, C. E., Stecher, H. A., Holmes, D. E., Bond, D. R., Lowy, D. A., Pilobello, K., Fertig, S. J., and Lovley, D. R. (2002). Harnessing microbially generated power on the seafloor. *Nat. Biotechnol.* 20, 821–5. doi:10.1038/nbt716.

Teske, A., and Sørensen, K. B. (2008). Uncultured archaea in deep marine subsurface sediments: have we caught them all? *ISME J.* 2, 3–18. doi:10.1038/ismej.2007.90.

Thamdrup, B. O., Finster, K. M., Hansen, J. W., and Bak, F. (1993). Bacterial disproportionation of elemental sulfur coupled to chemical reduction of iron or manganese. *Appl. Environ. Microbiol.* 59, 101–108.

Vandieken, V., Finke, N., and Jørgensen, B. B. (2006a). Pathways of carbon oxidation in an Arctic fjord sediment (Svalbard) and isolation of psychrophilic and psychrotolerant Fe (III) -reducing bacteria. *Microb. Ecol. Prog. Ser.* 322, 29–41.

Vandieken, V., Knoblauch, C., and Jørgensen, B. B. (2006b). *Desulfovibrio frigidus sp. nov*. and *Desulfovibrio ferrireducens sp. nov*., psychrotolerant bacteria isolated from Arctic fjord sediments (Svalbard) with the ability to reduce Fe(III). *Int. J. Syst. Evol. Microbiol.* 56, 681–5. doi:10.1099/ijs.0.64057-0.

Vandieken, V., Mussmann, M., Niemann, H., and Jørgensen, B. B. (2006c). *Desulfuromonas svalbardensis sp. nov*. and *Desulfuromusa ferrireducens sp. nov*., psychrophilic, Fe(III)-reducing bacteria isolated from Arctic sediments, Svalbard. *Int. J. Syst. Evol. Microbiol.* 56, 1133–9. doi:10.1099/ijs.0.63639-0.

Vandieken, V., and Thamdrup, B. (2013). Identification of acetate-oxidizing bacteria in a coastal marine surface sediment by RNA-stable isotope probing in anoxic slurries and intact cores. *FEMS Microbiol. Ecol.* 84, 373–86. doi:10.1111/1574-6941.12069.

Vigneron, A., Cruaud, P., Roussel, E. G., Pignet, P., Caprais, J.-C., Callac, N., Ciobanu, M.-C., Godfroy, A., Cragg, B. A., Parkes, J. R., et al. (2014). Phylogenetic and functional diversity of microbial communities associated with subsurface sediments of the Sonora Margin, Guaymas Basin. *PLoS One* 9, e104427. doi:10.1371/journal.pone.0104427.

Wakeham, S. G., Lee, C., Hedges, J. I., Hernes, P. J., and Peterson, M. J. (1997). Molecular indicators of diagenetic status in marine organic matter. *Geochim. Cosmochim. Acta* 61, 5363–5369. doi:10.1016/S0016-7037(97)00312-8.

Wallmann, K., Aloisi, G., Haeckel, M., Tishchenko, P., Pavlova, G., Greinert, J., Kutterolf, S., and Eisenhauer, A. (2008). Silicate weathering in anoxic marine sediments. *Geochim. Cosmochim. Acta* 72, 2895–2918. doi:10.1016/j.gca.2008.03.026.

Wankel, S. D., Adams, M. M., Johnston, D. T., Hansel, C. M., Joye, S. B., and Girguis, P. R. (2012). Anaerobic methane oxidation in metalliferous hydrothermal sediments: influence on carbon flux and decoupling from sulfate reduction. *Environ. Microbiol.* 14, 2726–40. doi:10.1111/j.1462-2920.2012.02825.x.

Webster, G., Yarram, L., Freese, E., Köster, J., Sass, H., Parkes, R. J., and Weightman, A. J. (2007). Distribution of candidate division JS1 and other Bacteria in tidal sediments of the German Wadden Sea using targeted 16S rRNA gene PCR-DGGE. *FEMS Microbiol. Ecol.* 62, 78–89. doi:10.1111/j.1574-6941.2007.00372.x.

Whitman, W. B., Coleman, D. C., and Wiebe, W. J. (1998). Perspective Prokaryotes : The unseen majority. *Proc. Natl. Acad. Sci.* 95, 6578–6583.

Wrighton, K. C., Castelle, C. J., Wilkins, M. J., Hug, L. a, Sharon, I., Thomas, B. C., Handley, K. M., Mullin, S. W., Nicora, C. D., Singh, A., et al. (2014). Metabolic interdependencies between phylogenetically novel fermenters and respiratory organisms in an unconfined aquifer. *ISME J.* 8, 1452–63. doi:10.1038/ismej.2013.249.

Xie, S., Lipp, J. S., Wegener, G., Ferdelman, T. G., and Hinrichs, K. (2013). Turnover of microbial lipids in the deep biosphere and growth of benthic archaeal populations. *Proc. Natl. Acad. Sci. U. S. A.* 110, 6010–4. doi:10.1073/pnas.1218569110.

Zhang, Y., Su, X., Chen, F., Wang, Y., Jiao, L., Dong, H., Huang, Y., and Jiang, H. (2012). Microbial diversity in cold seep sediments from the northern South China Sea. *Geosci. Front.* 3, 301–316. doi:10.1016/j.gsf.2011.11.014.

Zonneveld, K. A. F., Versteegh, G. J. M., Kasten, S., Eglinton, T. I., Emeis, K.-C., Huguet, C., Koch, B. P., de Lange, G. J., de Leeuw, J. W., Middelburg, J. J., et al. (2010). Selective preservation of organic matter in marine environments; processes and impact on the sedimentary record. *Biogeosciences* 7, 483–511. doi:10.5194/bg-7-483-2010.

Chapter 2

Microbial communities and organic matter composition in surface and subsurface sediments of the Helgoland mud area, North Sea

Oluwatobi Oni[1,2,3], Frauke Schmidt[2], Tetsuro Miyatake[1], Sabine Kasten[2,4], Matthias Witt[5], Kai-Uwe Hinrichs[2], Michael W. Friedrich[1,2]

1. Department of Microbial Ecophysiology, University of Bremen, Bremen, Germany.
2. MARUM – Center for Marine Environmental Sciences, University of Bremen, Bremen, Germany
3. Max Planck Institute for Marine Microbiology, Bremen, Germany
4. Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany
5. Bruker Daltonik GmbH, Bremen, Germany

Corresponding author:

Michael W. Friedrich

E-mail: michael.friedrich@uni-bremen.de

Phone: +49-421-218-63060

(In revision for re-submission to *Frontiers in Microbiology*)

Abstract

The role of microorganisms in the cycling of sedimentary organic carbon is a crucial one. To better understand relationships between molecular composition of a potentially bioavailable fraction of organic matter and microbial populations, bacterial and archaeal communities were characterized using pyrosequencing-based 16S rRNA gene analysis in surface (top 30 cm) and subsurface sediments (30-530 cm) of the Helgoland mud area, North Sea. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) was used to characterize a potentially bioavailable organic matter fraction (hot-water extractable organic matter, WE-OM). Algal polymer-associated microbial populations such as members of the *Gammaproteobacteria, Bacteroidetes* and *Verrucomicrobia* were dominant in surface sediments, while members of the *Chloroflexi* (*Dehalococcoidales* and candidate order GIF9) and Miscellaneous Crenarchaeota Groups (MCG), both of which are linked to degradation of more recalcitrant, aromatic compounds and detrital proteins, were dominant in subsurface sediments. Microbial populations dominant in subsurface sediments (*Chloroflexi*, members of MCG, and *Thermoplasmata*) showed strong correlations to total organic carbon content. Changes of WE-OM with sediment depth revealed molecular transformations from oxygen-rich (high Oxygen to Carbon (O/C), low Hydrogen to Carbon (H/C) ratios) aromatic compounds and highly unsaturated compounds towards compounds with lower O/C and higher H/C ratios. The observed molecular changes were most pronounced in organic compounds containing only CHO atoms. Our data thus, highlights classes of sedimentary organic compounds that may serve as microbial energy sources in methanic marine subsurface environments.

Keywords: Helgoland mud area, subsurface sediment, Soxhlet extraction, FT-ICR MS, Total Organic Carbon, water-extractable organic matter, Miscellaneous Crenarchaeota Group (MCG), *Chloroflexi*.

1. Introduction

Marine sediments cover 70 % of the Earth's surface. Organic matter is finely dispersed in these sediments in different concentrations depending largely on the size of the organic matter source, water depth, and sedimentation rates (Hedges and Keil, 1995). Apart from organic matter produced in the marine system, e.g. algal and bacterial biomass rich in lipids and nitrogenous compounds, marine sediments also receive inputs of terrestrial organic matter, which is mainly derived from plant materials rich in cellulose and lignin (de Leeuw and Largeau, 1993). Regardless of sources, extensive recycling of organic matter occurs in the water column (Hedges and Keil, 1995) and only about 1 % of the organic carbon export reaches the seafloor on a global scale (Hedges and Keil, 1995). This detrital organic matter serves as a main energy source for microorganisms living in marine sediments (Jørgensen and Boetius, 2007).

In surface sediments, easily degradable organic matter is preferentially utilized by microorganisms (Cowie and Hedges, 1994; Wakeham et al., 1997), whereas less reactive organic matter accumulates and is buried in deeper sediments (Zonneveld et al., 2010) . Consequently, microorganisms inhabiting deeper sediments have to satisfy their metabolic demands by relying on more recalcitrant organic matter, whose degradation requires longer time scales (Middelburg, 1989; Biddle et al., 2006). There are very few studies (e.g. Xie et al., 2013; Vigneron et al., 2014) on the nature of organic matter mineralized by microorganisms in marine subsurface sediments. However, the consistence of microorganisms dominating subsurface sediments across many environments may be due to special adaptations for utilization of less reactive organic matter (Biddle et al., 2006; Inagaki et al., 2006). Dominant Bacteria phyla are usually *Chloroflexi* and candidate division JS1 (Inagaki et al., 2006; Webster et al., 2007; Hamdan et al., 2011), while dominant Archaea are mostly members of the Miscellaneous Crenarchaeota Group (MCG) and Marine Benthic Group B (MBGB), otherwise referred to as Deep Sea Archaeal Group (DSAG; Inagaki et al., 2006; Biddle et al., 2006; Teske and Sørensen, 2008; Kubo et al., 2012). How these important groups of microorganisms thrive and what carbon sources they assimilate is largely unknown.

Knowledge of the molecular composition of sedimentary organic matter is important to predict the contributions of different organic matter sources to the pool of total organic carbon (TOC) (Meyers and Ishiwatari, 1993), each pool's relevance for shaping the functional diversity of microbial communities (Hunting et al., 2013) and associated energy limitations originating from substrate composition (Lever et al., 2015). However, it is a major challenge to molecularly characterize organic matter in sediments due to analytical limitations (Nebbioso and Piccolo, 2012). In the last decade, Fourier Transform Ion-Cyclotron Resonance Mass Spectrometry (FT-ICR MS) has successfully provided insights into the molecular composition of dissolved organic matter (DOM) in diverse environments (Kim et al., 2004; Koch et al., 2005; Dittmar and Koch, 2006; Hertkorn et al., 2006; Tremblay et al., 2007; Reemtsma et al., 2008; Schmidt et al., 2009; Bhatia et al., 2010; D'Andrilli et al., 2010; Lechtenfeld et al., 2013; Roth et al., 2013; Schmidt et al., 2014) due to its capacity to resolve thousands of individual components of complex organic matter based on precise mass determination. We applied FT-ICR MS to the water-extractable organic matter (WE-OM) fraction, which consists of free and adsorbed pore-water DOM as well as DOM that can be leached from particulate organic matter (Schmidt et al., 2014). Thus WE-OM is representative of both pore-water DOM and its potential particulate precursor pool. This pool of organic matter may also provide utilizable carbon and nitrogen for microorganisms living in sediments and soils (Strosser, 2010; Guigue et al., 2015). However, the ubiquity, distribution, and potential relevance, as a substrate source, of individual groups of DOM molecules for microbes in marine sediments are not known.

Here, we aim at a better understanding of the relationship between the molecular composition of WE-OM and the microbial diversity in marine sediments from the Helgoland mud area in the North Sea. So far, studies on microbial communities in surface (Llobet-Brossa et al., 2002; Mussmann et al., 2005) and subsurface sediments (Köpke et al., 2005; Wilms et al., 2006a, 2006b) in the German Bight of the North Sea have focused on tidal flat sediments. The Helgoland mud area is distinct in that it is one of the few depocenters of fine-grained mud in the open North Sea; in periods before 1250 A.D. this area has experienced higher sedimentation rates (up to 12-fold higher) and deposition of organic matter than nowadays (Hebbeln et al., 2003). Besides, the subsurface sediments (methanic zone) of the Helgoland mud area are characterized by intriguingly high dissolved iron concentrations (Oni et al.,

2015), the source of which is currently not known but predicted to be of microbial origin (Oni et al., 2015). These peculiarities necessitate that extensive research on the existing functional potentials of microorganisms in the Helgoland mud area be done in future. We thus provide an insight into the microbial populations as well as the nature of organic matter present therein. In addition, we discuss potential links between the molecular composition of organic matter and diversity of microbial populations in these surface and sub-seafloor environments.

2. Methods

2.1 Site and Sampling description

Samples from surface sediments (up to 10 cm) and subsurface sediments (up to 530 cm) from the Helgoland mud area (54° 5.00'N 7° 58'E) were collected in 2012, 2013, and 2014 during cruises with the research vessels HEINCKE and UTHÖRN. Sampling sites, coordinates and methods, are described in detail by Oni et al.(2015). Microbial community analysis was performed on samples reported in the aforementioned study. For sediment cores collected in 2012 (core UT2012, surface sediments and core HE376-007, deeper sediments), total organic carbon (TOC), total nitrogen (TN), stable carbon and nitrogen isotope analysis was performed with samples from 0-5 cm, 5-10 cm, and each 25 cm sections of the 500 cm sediment core described in Oni et al. (2015). The same parameters were measured on sediment cores collected in 2013 (core HE406-8-003, deeper sediments). From sediment core HE421-004, only 4-6 cm (surface sediments) was sampled, while sediment core HE406-8 was sampled in 25 cm sections at 100 cm intervals (i.e. 30-55 cm (close to the sulfate-methane transition depth, SMT (75 cm, Oni et al., 2015), termed “SMT area” hereafter), 130-155 cm, 230-55 cm, 330-355 cm, and 430-455 cm (methanic zone)). Samples from cores HE421-003 and HE406-8 were used for studying the molecular composition of organic matter by aqueous soxhlet extraction and subsequent FT-ICR MS analysis of extracts.

2.2 Organic matter analysis

2.2.1. Total organic carbon, total nitrogen, and stable carbon and nitrogen isotopes

To quantify the contents of total organic carbon (TOC), total nitrogen (TN) and their respective stable isotopes, approximately 3 g of wet sediment from each section were decalcified by treatment with 10% HCl. Afterwards samples were washed with ultrapure water and freeze-dried. Samples were ground in a mortar and 10-30 mg of each sample was weighed into tin capsules and analyzed on a Thermo Scientific Flash 2000 elemental analyzer connected to a Thermo Delta V Plus IRMS. All values are mean values of duplicate measurements. Stable isotopic compositions are reported in the $\delta^{13}C$ and $\delta^{15}N$ notation in ‰ relative to the Vienna Pee Dee Belemnite (V-PDB) standard and atmospheric N, respectively. High-resolution TOC contents were determined using a Carbon-Sulphur Determinator (ELTRA CS 2000). About 50 mg of dried and ground sediment were weighted into ceramic crucibles. Two to three drops of ethanol were added to avoid strong bubbling, and subsequently the sediment was decalcified with 12.5 % HCl p.a. and dried on a heating plate at 250 °C. After about two hours the dry sediment was covered by a mixture of steel and tungsten splinters to ensure a homogenous burning of the sample. The analytical precision was better than 1 %.

2.2.2. Soxhlet extraction

A detailed description of extraction procedures and post-extraction steps has been provided in Schmidt et al., 2014. In brief, about 25 g of wet sediment was weighed into pre-combusted glass fiber thimbles (30 x 100 mm, Whatman). Prior to use, thimbles were extracted in ultrapure water for 48 hours to remove potential contaminants. A procedural blank containing thimble and deionized water was run to check for contaminations. The thimbles were placed in the soxhlet extraction unit and WE-OM was extracted from the sediment samples with 200 ml of distilled, de-ionized water for 24 hours. Soxhlet extracts were filtered first with 0.7 µm (GF/F, Whatmann) and then 0.2 µm (cellulose, Sartorius) microbiologically sterile filters before storing extracts at 4°C until further use.

2.2.3. DOM extraction

Soxhlet extracts were acidified to pH 2 with HCl (suprapur, Merck) before concentrating the DOM by solid phase extraction (SPE) using Bond Elut-PPL cartridges (500 mg, 3 ml syringe; Agilent Technologies, Germany) as described by Dittmar et al., 2008. As the extracts were adsorbed to the cartridges, salts were removed by rinsing the cartridges with 6 ml ultrapure water (pH 2). Extracts were eluted with 1 ml of methanol (LiChrosolv, Merck) and stored at -20°C in the dark until FT-ICR MS analyses.

2.2.4. Dissolved organic carbon and total dissolved nitrogen

DOC and total dissolved nitrogen (TDN) concentrations were analyzed in Soxhlet extracts and SPE extracts. First, methanol was removed from aliquots of SPE extracts under a stream of nitrogen and afterwards DOM was re-dissolved in 6 ml ultrapure water. Measurements were performed by high-temperature catalytic oxidation (at 680°C) using a Shimadzu TOC/TN analyzer equipped with infrared and chemiluminescence detector (oxygen flow: 0.6 l min^{-1}). Prior to direct injection onto the catalyst, samples were acidified with 0.12 ml HCl (2 M) in the autosampler and purged with oxygen to remove inorganic carbon. Final DOC concentrations were average values of triplicate measurements.

2.2.5. FT-ICR MS

DOM extracts were analyzed on a Bruker SolariX FT-ICR mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with a 12 T refrigerated actively shielded superconducting magnet (Bruker Biospin, Wissembourg, France) and an electrospray ionization source (ESI, Apollo II electrospray source, Bruker Daltonik GmbH, Bremen, Germany). Prior to measurement, the extracts were diluted with methanol:water (1:1, v/v) mixture to same SPE concentrations for all samples (750 nM DOC/µL). Samples were ionized in negative ionization mode at an infusion flow rate of 5µl min^{-1}. Ion accumulation time was set to 0.05 s and 200 scans were added to one mass spectrum. Acquired mass spectra were calibrated externally with arginine clusters and recalibrated internally with compounds that were repeatedly identified in marine pore-water DOM samples (cf. Schmidt et al., 2014). The root mean square error of the internal calibration was below 0.095 ppm resulting in very reliable molecular formula assignment. Molecular formulas were calculated under consideration of the

following elements $^{1}H_{0-90}$, $^{12}C_{0-60}$, $^{13}C_{0-1}$, $^{16}O_{0-35}$, $^{14}N_{0-4}$, $^{32}S_{0-2}$, $^{34}S_{0-1}$, $^{31}P_{0-2}$ in an m/z range of 180-600. Formulas were restricted to integer double bond equivalent (DBE) values and a molecular element ratio of O/C ≤ 1.2. A mass tolerance of ± 0.5 ppm was considered as a valid formula. Multiple formulas were filtered with the homologous series/building block approach and isotope check (Koch et al., 2007). Molecular formulas containing ^{13}C or ^{34}S were excluded from the final dataset which was limited to peaks with S/N > 7 corresponding to a relative peak intensity of 0.4 %. Relative peak intensities were calculated from the total peak intensity ($\Sigma Int_{allPeaks}$) in the spectra after following equation:

$$\text{Rel. Intensity} = (Int_{Peak}/\Sigma Int_{allPeaks}*1000) \quad (1)$$

In order to reduce the complexity of data characteristically obtained from FT-ICR MS analyses, molecular formulas were first grouped into categories based on their elemental composition: (1) molecular formulas containing C, H and O atoms, (2) molecular formulas consisting of C, H, O and one or two N atoms (CHO-N_{1-2}), (3) molecular formulas consisting of C, H, O and three or four N atoms (CHO-N_{3-4}), (4) molecular formulas containing N and P (CHNOP), (5) molecular formulas containing S (CHOS), (6) molecular formulas containing N and S (CHNOS), (7) molecular formulas containing P and S (CHOPS) as well as (8) those containing P only (CHOP). In addition, molecular formulas in the different categories were divided into five groups based on modified aromaticity index ($AI_{mod,}$ (Koch and Dittmar, 2006), H/C and O/C ratios (e.g.Šantl-Temkiv et al., 2013; Seidel et al., 2014), hereafter referred to as groups 1-5 : (group 1) polycyclic aromates, (PCAs, $AI_{mod} \geq 0.67$), (group 2) highly aromatic compounds, including polyphenols and PCA compounds with aliphatic chains ($0.67 > AI_{mod} > 0.50$), (group 3) highly unsaturated compounds (including humic compounds and carboxyl-rich alicyclic molecules (CRAM; Hertkorn et al., 2006; $AI_{mod} \leq 0.5$ and H/C <1.5), (group 4) unsaturated aliphatic compounds ($2.0 > H/C \geq 1.5$), (group 5) saturated aliphatic compounds (may include carbohydrate-like compounds, saturated fatty and sulfonic acids; $H/C \geq 2.0$). Raw data sheets used for molecular assignments are provided as supplementary material (data sheet S4). ESI negative FT-ICR mass spectra covering all mass ranges and nominal mass 385Da (as an example) in

WE-OM extracted from the sediment cores are also provided in supplementary Figs. S1 and S2. respectively.

2.3 Microbial community analyses

2.3.1. Pyrosequencing and sequence analyses

DNA samples, extracted as described in Oni et al. (2015), from depths 0-5 cm and 5-10 cm (surface sediments), 30-55 cm (SMT area), 180-205 cm, 230-255 cm, 305-330 cm, 355-380 cm, and 480-505 cm (methanic zone), were selected for 454 FLX pyrosequencing at Molecular and Research Testing Laboratory, (Lubbock, Texas, USA). The same primer pairs for bacteria and archaea 16S rRNA gene amplification as reported in Oni et al.(2015) were used. Downstream processing of sequence raw data files (SFF files) were done as reported earlier (Oni et al., 2015). Rarefaction curves (observed species based on 97% OTU cut-off) and microbial diversity and richness indices (Shannon and Chao 1, respectively) were calculated for each sample analyzed using the QIIME version 1.7.0. Species diversity and richness indices along the depth profile for bacteria and archaea were calculated after jackknifing (normalization) the number of sequences to those of the samples with lowest sequence reads. Weighted Paired Group Method of Averaging (WPGMA) cluster diagrams were generated for bacterial and archaeal OTUs.

2.3.4. Statistical analyses

To investigate the strength of relationships between TOC and TN or between TOC and microbial populations with depth, spearman correlations were calculated using PAleotontological STatistics software (PAST, Hammer et al., 2001), version 2.17c.

3 Results

3.1 Organic matter analyses

3.1.1. Total organic carbon, total nitrogen, and stable carbon and nitrogen isotopes

In cores UT-2012 (surface sediments) and HE376-007-5 (deep sediments), mean TOC and TN values showed high variations with depth (Fig. 1). TOC and TN contents ranged between 0.6–2.2 wt % and

0.09–0.2 wt %, respectively, with the highest values of both parameters measured at depths below 300 cm. Depth-wise TOC and TN variations strongly co-varied (Fig. 1; ρ= 0.962, p-value = 1.48E-11, n =22). δ^{13}C-TOC and δ^{15}N values ranged between -23.1 to -23.4 ‰ and 6.9 to 7.1 ‰ in the surface sediment. In deeper sediments, δ^{13}C-TOC and δ^{15}N values showed variations between -24.9 to -25.4 ‰ and 4.6 to 5.5 ‰, respectively (Fig. 1). In cores HE421-003 and HE406-008, TOC, TN, δ^{13}C-TOC and δ^{15}N, distributions were similar to those of cores UT-2012 and HE376-007-5. In core HE421-003, TOC and TN in the surface sediment (4-6 cm) were 0.98 and 0.11 wt %, respectively. In subsurface sediments TOC varied between 0.81 to 1.6 wt % and TN ranged from 0.08 to 0.17 wt %. Both parameters showed the same trend with depth (ρ = 1.000, p value < 0.001, n=6) with the highest values observed in sediments sampled below 230 cm (Fig. 1). Furthermore, both δ^{13}C-TOC and δ^{15}N gradually decreased with depth (Fig. 1).

3.1.2 Water-extractable organic matter analysis

WE-OM fraction in the surface and deep sediments of the Helgoland mud area ranged between 1.2–2.4 % (Table 1) with the highest portion found for the sample from 130–155 cm and the lowest portion for the sample from 230–255 cm. FT-ICR MS analysis resolved thousands of molecular formulas per sample (Table 2). The sample from the surface sediment (4–6 cm) contained a lower number of formulas compared to samples from deeper sediments (30–455 cm). In deeper sediments, numbers of molecular formulas were higher in the samples from the methanic zone (below 130 cm) compared to the sulfate methane transition zone (SMT area; 30 – 55 cm). Intensity weighted averages of molecular masses (m/z_{wa}) were higher in deeper sediments than in surface sediments. Weighted average Double Bond Equivalent (DBE_{wa}) values, which denote the sum of rings and double bonds in the molecular compounds, as well as O/C_{wa} and C/N_{wa} ratios, were generally lower in surface sediments. On the other hand, the H/C_{wa} ratio was higher in the surface sediment compared to the deeper sediments. With respect to relative intensities of peaks, total signal intensities of CHO and N-bearing compounds were highest in all samples. CHO and CHO-N_{1-2} compounds were more enriched in deeper sediments whereas CHO-N_{3-4} and CHNOP compound groups were most abundant in the surface sediments (Fig.

2). Relative signal intensities of CHOS compounds showed no clear trend from surface sediments down to deeper sediments (Fig. 2).

PCAs (group 1) showed the highest relative abundance in sample from the SMT area (4.1 %), followed by sample from the surface sediment (3.8 %). Their abundance decreased at 130 – 355 cm and below, where it ranged around 2.5–2.7 %. In the deepest sample from 430–455 cm, PCA compounds showed a slight increase to 3.4 % (Fig. 3). Highly aromatic compounds (group 2) were comparatively more abundant in all samples and showed similar trends in the subsurface sediments as group 1 (Fig. 3). The decrease in the percentage relative intensities of PCA and highly aromatic compounds below the SMT area appear to be most pronounced in the CHO compounds (Figs. 3 and 4B). Highly unsaturated compounds (group 3) were the most abundant molecular formulas group in all samples (Fig. 3). In the surface sediment they constitute 47 % of all peak intensities while their relative abundance increased in deeper sediments, from 58 % in the SMT area to 61–64% in the methanic zone. Unsaturated aliphatic compounds (group 4) were highest in the surface sediment (40 %) whereas their relative intensities decreased in the deeper sediment to approximately half (19.3–20.3 %) of their total intensities in the surface sediment. The relative abundances of CHO-$N_{3\text{-}4}$, in surface sediments, were highest in in groups 4 and group 1 (Fig. 3). Finally, saturated aliphatic compounds (group 5) were most abundant in the surface sediment (3.4%) in relation to samples from deeper sediments (1.4–2.2 %). CHO-$N_{3\text{-}4}$ formulas made up a small portion (approx. 0.3–1 %) of the compounds in group 5 (Fig. 3).

3.2 Microbial community structure and composition

The bacteria and archaea community structure clearly differed between the surface and deep sediments of the Helgoland mud area as displayed in Figs. 4A and 4B. Specifically, subsurface sediments showed a separation between bacterial populations in the SMT area and the methanic zone (Fig. 4A). However, there was generally no separation of archaea between the SMT area and the methanic zone in the subsurface sediments (Fig. 4B) as also observed by Lazar et al. (2015) in shallow estuarine sediments. Bacterial and archaeal diversities (Shannon index) were generally higher in surface compared to subsurface sediments (Fig. 5). Overall, no clear differences in bacterial species richness

were observed between surface and subsurface sediments (Fig. 5). However, archaea species richness was approximately 4-9 times higher in surface than in subsurface sediments (Fig. 5). Estimates of the number of bacterial and archaeal OTUs detected (based on 97% sequence similarity cut-off) are shown in rarefaction curves (Fig. 6).

In comparison to subsurface sediments, bacterial communities in the surface sediments of the Helgoland mud area were dominated by *Deltaproteobacteria* (33−34 % in surface *vs.* 2.5–7 % in deeper sediments) and *Gammaproteobacteria* (25–29 % in surface *vs.* 1–3 % in deeper sediments). Subsurface sediments are dominated by *Chloroflexi* (27−40 % *vs.* 3 % in surface sediments) as well as candidate division OP9/JS1 (18–27%; not detected in surface sediments). Bacterial populations belonging to *Acidobacteria* (3–4 %), *Verrucomicrobia* (1.5–4.4 %), Cyanobacteria (2–4.5%), and *Bacteroidetes* (3.5–5 %) were more dominant in the surface sediments (Fig. 7A). In contrast, *Spirochaetes* (2.3–5.3 %), salt marsh clone LCP89 (1.22–2.19 %), *Betaproteobacteria* (0.6–1.7 %), *Planctomycetes* (1.2–2.0 %), *Elusimicrobia* (1.7–2.6 %), candidate divisions OP1 (0.71–1.6 %), OP8 (1.7–3.6 %), and WS3 (1.3–4.4 %) were more abundant in all samples from deep sediments compared to surface sediments (Fig. 7A). Other bacterial populations such as *Actinobacteria* and *Firmicutes* were detected both in surface and deep sediments with more or less similar relative abundances (Fig. 7A).

Archaeal populations in surface sediments were largely dominated by *Thaumarchaeota* (31–47 % in surface *vs*. 0.7–1.9 % in deeper sediments) and Parvarchaea (36–40 % in surface vs 1.5–5 % in deeper sediments) while deeper sediments were dominated by Miscellaneous Crenarchaeota Group (MCG; 30-56 % in deeper *vs.* 0.6–4 % in surface sediments) (Fig. 7B). Anaerobic methanotrophic archaea (ANME 1 and ANME-2c) were detected in the deeper sediments with their combined relative abundances highest at 30–55 cm (SMT area). Methanogens belonging to the *Methanosaetaceae*, *Methanosarcinaceae* and *Methanomicrobiales* were more abundant in the deeper sediments, in particular in the methanic zone (305–330 cm and 355–380 cm) (Fig. 7B). Conspicuously, more abundant in samples from deeper sediments were the Marine Benthic Group B (MBGB; 4.7–15 % in

deeper *vs*. 0.5–1.9 % in surface sediments) and *Thermoplasmata* (13–17 % in deeper *vs*. 4–10 % in surface sediments).

Up-to-family-level relative abundance information on bacterial and archaeal populations at each sampled depth are given in supplementary data sheets S1 and S2, respectively.

3.3 Organic matter-linked microbial populations in the subsurface sediments

More sediment samples retrieved from the gravity core (HE 376-007-5) allowed the possibility to match the depth-wise distribution of bacterial and archaeal populations detected in deeper sediments to TOC content at depths from which samples were chosen for microbial molecular analysis. Microbial populations belonging to *Chloroflexi* ($\rho = 0.928$, p-value = 0.01; mainly *Dehalococcoidales*, candidate order GIF9), *Thermoplasmata* ($\rho = 0.812$, p-value = 0.07), and a candidate order of the MCG (pGrfC26; $\rho = 0.899$, p-value = 0.03) showed strong correlations to TOC (Fig. 8, Table S3).

4 Discussion

To investigate how organic matter composition and diagenesis may be associated with specific microbial populations in sediments of the Helgoland mud area, we characterized the molecular composition of the WE-OM pool of bulk organic matter in the surface and subsurface sediments. In addition, general microbial composition of the Helgoland mud area was also studied. Our findings, as discussed below, reveal important differences in the molecular composition of WE-OM and organic matter bioavailability, which likely has consequences on microbial populations dominating in surface and subsurface sediments.

4.1 Sources and bioavailability of organic matter in surface sediments

The relative ^{13}C enrichment values ($\delta^{13}C$ of TOC is -23.1 to -23.4 ‰) in surface sediments is indicative of higher contributions of marine derived organic matter such as algal materials (Dauwe and Middelburg, 1998; Holtvoeth, 2004; Sangiorgi et al., 2005). Algal organic matter consists of a higher portion of aliphatic molecules and it has previously been shown that near-surface pore-water DOM from open marine sites with a predominance of algal material contains more molecular formulas with elevated H/C ratios (Schmidt et al., 2009). In line with this were the high H/C_{wa} ratio and higher

abundances of saturated and unsaturated aliphatic compounds (groups 4 and 5) in WE-OM from the surface sediment of the Helgoland mud area. In the van Krevelen diagram (Figure 9A), this difference in the WE-OM composition between surface and subsurface sediment is illustrated by elevated relative intensities of aliphatic compounds with low O/C ratios in the surficial WE-OM (orange to red color). Not only a change in the main organic matter source could explain the variations between WE-OM in the surface and the subsurface sediment but also differences in the reactivity of different organic matter types. Saturated aliphatic compounds (group 5), which might contain fatty acids and carbohydrates, are considered as easily biodegradable components of marine organic matter and quickly lost during early diagenesis (Freese et al., 2008). Unsaturated aliphatic hydrocarbons (group 4) including N-containing compounds, which are also relatively easy to metabolize, are most likely intermediates of protein degradation (Schmidt et al., 2011, 2014). The higher biodegradability of saturated and unsaturated aliphatic compounds might result in their lower abundances in the deeper sediment compared to samples from the surface sediments (Fig. 3). Similarly, the higher abundance of CHO-$N_{3\text{-}4}$ formulas in the surface sediment relative to deeper sediments suggests that the N-rich compounds are preferentially degraded in the surface sediment. This is consistent with reports of preferential degradation of N-rich organic matter in marine sediments (Cowie and Hedges, 1991; Freudenthal et al., 2001; Sinkko et al., 2013; Schmidt et al., 2014; Barber et al., 2014).

4.2 Sources and bioavailability of organic matter in deeper sediments

The ^{13}C depletion of TOC in sediments from the SMT area and below is consistent with an elevated proportion of terrestrial organic matter in the subsurface sediment (Fig. 1). TOC showed only minor variations in $\delta^{13}C$ in subsurface sediments, which is suggestive on homogeneous sources. High sedimentation rates in the Helgoland mud area in the past (Hebbeln et al., 2003) were presumably associated with a high input of terrestrial, relatively recalcitrant organic matter to deeper sediments, which were partly deposited during periods of heavy storms and disintegration of parts of the Helgoland Island (Hebbeln et al., 2003). Terrestrial organic matter is known to show greater recalcitrance in marine sediments compared to algal-derived organic matter (Andersen and Kristensen, 1992; Meyers and Ishiwatari, 1993; Meyers, 1994; Rontani et al., 2012). One reason for this could be pre-ageing of terrestrial organic matter *en-route* the marine system or its higher susceptibility to

encapsulation by accompanying minerals (Mayer, 1994; Keil, 2011; Lalonde et al., 2012; Riedel et al., 2013; Barber et al., 2014). In general, selective degradation strongly modifies the characteristics of residual organic matter in sediments (Meyers, 1994; Zonneveld et al., 2010). As microbes preferentially degrade the easily-utilizable portion of bulk organic matter, the more recalcitrant fractions selectively accumulate in deeper sediments (Cowie and Hedges, 1994; Wakeham et al., 1997). The generally higher abundances of CHO as well as CHO-N_{1-2} in the deeper sediments (Fig. 2) suggest that in the Helgoland mud area, a larger portion of the compounds represented by these formulas is relatively refractory. In the same vein, PCA compounds (group 1), highly aromatic compounds (group 2) and highly unsaturated compounds (group 3) are likely to harbor a larger proportion of recalcitrant compounds, as they are more abundant in deeper sediments (approx. 80 % *vs*. 60 % in deeper *vs*. surface sediments respectively; Fig. 3). However, we cannot rule out that these differences additionally result from the change in organic matter source from a former more terrestrial influenced system to the modern marine predominated system. Nevertheless, lower H/C_{wa} and higher O/C_{wa} ratios (Table 2) of WE-OM in subsurface sediments relative to surface sediments (see also higher relative intensities of unsaturated formulas with elevated O/C ratios in Fig. 9A) suggest degradation of organic matter over time in the subsurface sediments (Blair et al., 1985; Seidel et al., 2014). Additionally, changes in the abundance of different organic matter groups within the subsurface sediment were most likely related to organic matter degradation (Tfaily et al., 2015). The percentage relative intensities of PCA and highly aromatic formulas (mostly CHO compounds) show a slightly decreasing trend in the methanic zone (Fig. 3). This could be a result of a slow degradation of these formulas groups by microorganisms in the methanic zone.

4.3 Microbial populations and organic matter degradation in surface sediments

The quality and sources of organic matter in surface sediments of the Helgoland mud area is reflected in the microbial community composition. The higher proportion of labile algal-derived aliphatic organic matter was apparently an attractive substrate for the dominant bacterial groups. For example, members of the *Gammaproteobacteria*, *Alphaproteobacteria*, and *Bacteroidetes* have been observed to be involved in the initial degradation of algal-derived organic matter in marine waters and sediments (Gutierrez et al., 2011; Teeling et al., 2012; Landa et al., 2014; Ruff et al., 2014; Miyatake

et al., 2014). In addition, *Flavobacteriaceae*, the dominant members of the *Bacteroidetes* in surface sediments of our study site (S1, Tables 1 and 2), have been consistently enriched in plankton-amended microcosm incubations as well as in natural phytoplankton blooms (Kirchman, 2002; Abell and Bowman, 2005; Bauer et al., 2006; Teeling et al., 2012). A recent study in an Arctic fjord (Smeerenburgfjord, Svalbard) has suggested a role in polysaccharide hydrolysis for members of the *Verrucomicrobia* phylum (Cardman et al., 2014). The occurrences of *Cyanobacteria*, *Acidobacteria* and some members of the *Chloroflexi* (namely candidate class Ellin 6529; S1, Tables 1 and 2) mainly in the surface sediments (Fig. 7A) suggest that they may be better adapted to fresh organic matter. Dominant *Deltaproteobacteria* in surface sediments, namely *Desulfobulbaceae*, *Desulfuromonadaceae*, and *Desulfobacteraceae* (S1, Tables 1 and 2), include various sulfate-, sulfur-, and metal-reducing bacteria that may specialize in the oxidation of low-molecular weight organic compounds fermentatively produced from upstream degradation of the heavier organic molecules (Lovley et al., 1993, 1995; Muyzer and Stams, 2008). Ammonia resulting from organic matter degradation is a potential substrate for the dominant *Thaumarchaeota* (mainly *Cenarchaeaceae*), which include known ammonia-oxidizing archaea such as *Nitrosopumilus maritimus* (Könneke et al., 2005) and *Candidatus* Nitrosopumilus koreensis (Park et al., 2010). Candidate division Parvarchaea also constitute a dominant archaeal group in surface sediment; due to the lack of cultured members, no ecological role can be assigned to this candidate phylum.

4.4 Microbial populations and organic matter degradation in deep sediments

The recalcitrant nature of organic matter in subsurface sediments may have selected for specific microbial populations capable of its utilization, resulting in lower bacterial and archaeal diversity compared to surface sediments (Fig. 5). WE-OM from the subsurface sediments showed higher abundances of highly unsaturated compounds compared to the surface sediment (Fig. 9A) that may include CRAMs (Hertkorn et al., 2006) and some plant-derived materials rich in lignin/lignocellulosic molecules (Sleighter and Hatcher, 2008). Microbial populations dominant in deep sediments of our study site (*Chloroflexi*, candidate division JS1, MCG and *Thermoplasmata*, Figs. 7A and 7B) are consistent with those regularly found in marine subsurface sediments (Parkes et al., 2005; Biddle et al., 2006, 2008; Inagaki et al., 2006; Webster et al., 2007; Durbin and Teske, 2012; Schippers et al.,

2012), most of which have been linked to heterotrophic metabolism (Biddle et al., 2006; Webster et al., 2007; Lloyd et al., 2013). In addition, the strong covariance of *Chloroflexi* (mainly *Dehalococcoidales*, ρ = 0.81 and candidate order GIF 9, ρ = 0.75) and MCG archaea (mainly candidate order pGfrC26, ρ = 0.89) and *Thermoplasmata* (ρ = 0.81) to the depth profile of TOC in sediment core HE376-007-5 (Fig. 8) suggests that these organisms are important for organic matter degradation in subsurface sediments of the Helgoland mud area as well. The observed shift of molecular signatures (mostly among CHO compounds) from high O/C and low to intermediate H/C ratios towards lower O/C and higher H/C ratios with increasing depth in the methanic zone suggests microbial utilization of these O-rich highly unsaturated and aromatic compounds via potential reactions such as reduction or decarboxylation (Fig. 9B). A similar shift has previously been observed in the subsurface sediments of peatlands where organic matter is considerably reactive (Tfaily et al., 2013, 2015). This offers an interesting new perspective to the range of organic matter potentially available for microbes in deep sub-seafloor as complex molecules such as for example, CRAM-like, lignin-like and tannin-like structures, as well as condensed aromatic molecules, have previously not been considered to be an important energy source for subsurface microbes. In line with our finding here, a role in fermentation of plant polymers (such as pyrogallol) has recently been predicted for a member of the candidate order GIF9 (Hug et al., 2013). In addition, members of the *Dehalococcoidia* are also known to be involved in the reductive degradation of substituted aromatic hydrocarbons (Alfreider et al., 2002; Fennell et al., 2004; Wasmund et al., 2014; Pöritz et al., 2015). Candidate order pGrfC26, similar to Rice Cluster IV (Großkopf et al., 1998), and sub-grouped into the MCG-A or class 6 MCG (Meng et al., 2014), have been largely enriched in lignocellulose-amended cultures (Peacock et al., 2013) and may also have a role in the degradation of lignin monomers such as protocatechuate (Meng et al., 2014). Functional potential of organisms such as members of *Chloroflexi* and MCG in the degradation of aromatic compounds may have contributed to the molecular changes in CHO fractions of at least, PCA and aromatic formulas (group 1 and 2) below the SMTZ of our study site. Potential for degradation of aromatic compounds were found in other *Chloroflexi*- and MCG-dominated subsurface sediments- e.g. in the Sonora Margin, Guayamas Basin, where genes

responsible for degradation of aromatic hydrocarbons such as ethylbenzene and ethylphenol increased in proportion with depth (Vigneron et al., 2014).

The presence/higher abundances of candidate lineages such as OP1, OP8, WS3, and LCP-89 and *Planctomycetes* (mostly *Phycisphaerae*), *Elusimicrobia* (formerly Termite Group I), *Spirochaetes* and *Actinobacteria* in the subsurface in relation to the surface sediments of the Helgoland mud area suggests that they are better suited to the conditions or more important therein. *Firmicutes* in our site, mostly belonging to the *Bacillales* and *Clostridiales* (S1, Tables 1-8), appear less selective as they are equally abundant in the surface and subsurface sediments.

4.5 Methanogenesis and AOM

Methanogenesis is the terminal step of organic matter degradation (Schink, 1997). The presence of methanogenic populations belonging to *Methanosarcinaceae* (harbor methylated C1 compounds, hydrogen and acetate utilizers), *Methanosaetaceae* (acetoclastic methanogenesis), *Methanomicrobiales* (hydrogenotrophic methanogenesis) and *Methanocellales* (hydrogenotrophic methanogenesis) suggest the potential for all three major pathways of methanogenesis in our site (Fig. 7B). Methylotrophic methanogenesis has also been reported in members of the *Thermoplasmata* (Paul et al., 2012; Dridi et al., 2012; Poulsen et al., 2013; Iino et al., 2013). *Thermoplasmata* detected in this study all belong to the candidate order E2, a member (*Candidatus* Methanogranum caenicola) of which has recently been reported to reduce methanol to methane using hydrogen as an electron donor (Iino et al., 2013). Although in lower concentrations compared to surface sediments, methanol has been detected in pore waters of subsurface sediments of the Black Sea (Zhuang et al., 2014) and its source has been attributed to degradation of terrestrially-derived macromolecules such as lignin and pectin (Donnelly and Dagley, 1980; Schink and Zeikus, 1980). This may explain the strong covariance of *Thermoplasmata* with TOC ($\rho = 0.812$, p-value = 0.07) in subsurface sediment samples studied here. If the ability to utilize methylated C1 compounds is widespread among members of the candidate order E2, such methanogenic pathway may be very important in subsurface sediments of the Helgoland mud area as *Thermoplasmata* account for up to 17 % of total archaeal populations in deeper

sediments based on our sequencing method (Fig. 8B). However, analysis of *mcrA* genes and incubation studies on these sediment samples will be necessary to verify this hypothesis.

Potential for anaerobic oxidation of methane in the Helgoland mud area is reflected by the abundances of ANME populations (ANME-1 and ANME-2c). The highest combined abundance of ANME populations (approx. 30 % of archaeal populations) and the highest presence of *Deltaproteobacteria* (mostly *Desulfobacteraceae*) found in the SMT area are consistent with the distinctiveness of this zone as the active site for AOM coupled to sulfate reduction (Boetius et al., 2000). Nevertheless, potential for AOM in the Helgoland mud area may extend deeper into the methanic zone as ANME-1 were detected in all samples taken below 30-55 cm depth (4-8 % of archaeal populations), in analogy to previous observations (Lloyd et al., 2011) and ANME-2c were also found in high proportion at 230−255 cm (16 % of archaeal population).

5 CONCLUSIONS

Our study suggests that the amount and composition of organic matter are important determinants of microorganisms dominating surface and subsurface sediments of the Helgoland mud area (e.g. as seen in Fig. 8). While nitrogen-rich, aliphatic organic compounds of presumed algal origin are mostly available for microorganisms in surface sediments, the subsurface sediments are dominated by aromatic and unsaturated phenolic compounds that presumably originate from terrestrial sources. Microorganisms dominating subsurface sediments of our study site are consistent with those commonly found in other marine subsurface sediments. These dominant bacterial and archaeal populations are strongly correlated to the TOC content, suggesting involvement in degradation of organic matter in the Helgoland mud area. Consistently, we observed molecular transformations in the water-extractable (potentially microbially-available) portion of bulk organic matter in subsurface sediments (particularly within the methanic zone) showing a shift from a higher abundance of O-rich molecules in the shallower subsurface (higher O/C ratio) towards a higher abundance of more reduced compounds (with higher H/C and lower O/C ratios). The molecular shifts were most pronounced for CHO compounds. The assemblage of formulas corresponds to PCA, aromatics and highly unsaturated molecules that may include lignins, tannins, CRAM equivalents (groups 1-3), and is consistent with

recent findings that O-rich compounds are also preferentially depleted in highly-reactive peatland subsurface sediments (Tfaily et al., 2015). We therefore conclude that organic matter with such oxygen-rich phenolic and aromatic compounds may be an important energy source for microorganisms inhabiting marine subsurface environments characterized by high depositional rates such as the Helgoland mud area as well. These findings could accelerate ongoing efforts to culture microorganisms or enrich active microbial consortia in the marine subsurface sediments.

Acknowledgement

This study was supported by the Research Center/Cluster of Excellence 'The Ocean in the Earth System' (MARUM) funded by the Deutsche Forschungsgemeinschaft (DFG), by the University of Bremen, and by the European Research Council under the European Union's Seventh Framework Programme–"Ideas" Specific Programme, ERC grant agreement No. 247153 (project DARCLIFE) to KUH. The authors thank the captain, crew, and scientists of R/V HEINCKE expeditions HE376, HE406 and HE421. Dr. Carolina Reyes is thanked for her help with sectioning of sediment core HE376-007-5. We also thank the captain and crew of RV UTHÖRN for their help during UT-2012 sampling. We are grateful to Boris Koch for DOC measurements and Jenny Wendt for help on carbon and nitrogen measurements. We thank Benjamin Löffler and Gerhard Kuhn for carrying out and support with the high-resolution TOC measurements. We acknowledge additional funding by the Max Planck Society and the Helmholtz Association (Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research) in the framework of the research programs PACES I and PACES II.

References

Abell, G. C. J., and Bowman, J. P. (2005). Colonization and community dynamics of class *Flavobacteria* on diatom detritus in experimental mesocosms based on Southern Ocean seawater. *FEMS Microbiol. Ecol.* 53, 379–91. doi:10.1016/j.femsec.2005.01.008.

Alfreider, A., Vogt, C., and Babel, W. (2002). Microbial diversity in an in situ reactor system treating monochlorobenzene contaminated groundwater as revealed by 16S ribosomal DNA analysis. *Syst. Appl. Microbiol.* 240, 232–240. Available at: http://www.sciencedirect.com/science/article/pii/S0723202004701071 [Accessed February 11, 2015].

Andersen, F. Ø., and Kristensen, E. (1992). The importance of benthic macrofauna in decomposition of microalgae in a coastal marine sediment. *Limnol. Oceanogr.* 37, 1392–1403. doi:10.4319/lo.1992.37.7.1392.

Barber, A., Lalonde, K., Mucci, A., and Gélinas, Y. (2014). The role of iron in the diagenesis of organic carbon and nitrogen in sediments: A long-term incubation experiment. *Mar. Chem.* 162, 1–9. doi:10.1016/j.marchem.2014.02.007.

Bauer, M., Kube, M., Teeling, H., Richter, M., Lombardot, T., Allers, E., Würdemann, C. A., Quast, C., Kuhl, H., Knaust, F., et al. (2006). Whole genome analysis of the marine Bacteroidetes'*Gramella forsetii*' reveals adaptations to degradation of polymeric organic matter. *Environ. Microbiol.* 8, 2201–13. doi:10.1111/j.1462-2920.2006.01152.x.

Bhatia, M. P., Das, S. B., Longnecker, K., Charette, M. A., and Kujawinski, E. B. (2010). Molecular characterization of dissolved organic matter associated with the Greenland ice sheet. *Geochim. Cosmochim. Acta* 74, 3768–3784. doi:10.1016/j.gca.2010.03.035.

Biddle, J. F., Fitz-Gibbon, S., Schuster, S. C., Brenchley, J. E., and House, C. H. (2008). Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. *Proc. Natl. Acad. Sci. U. S. A.* 105, 10583–8. doi:10.1073/pnas.0709942105.

Biddle, J. F., Lipp, J. S., Lever, M. A., Lloyd, K. G., Sørensen, K. B., Anderson, R., Fredricks, H. F., Elvert, M., Kelly, T. J., Schrag, D. P., et al. (2006). Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proc. Natl. Acad. Sci. U. S. A.* 103, 3846–51. doi:10.1073/pnas.0600035103.

Blair, N., Leu, A., Muñoz, E., and Olsen, J. (1985). Carbon isotopic fractionation in heterotrophic microbial metabolism. *Appl. Environ. Microbiol.* 50, 996–1001. Available at: http://aem.asm.org/content/50/4/996.short [Accessed January 6, 2015].

Boetius, A., Ravenschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O. (2000). A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407, 623–6. doi:10.1038/35036572.

Cardman, Z., Arnosti, C., Durbin, A., Ziervogel, K., Cox, C., Steen, A. D., and Teske, A. (2014). Verrucomicrobia are candidates for polysaccharide-degrading bacterioplankton in an arctic fjord of Svalbard. *Appl. Environ. Microbiol.* 80, 3749–56. doi:10.1128/AEM.00899-14.

Cowie, G., and Hedges, J. (1991). *Black Sea Oceanography*. , eds. E. İzdar and J. W. Murray Dordrecht: Springer Netherlands doi:10.1007/978-94-011-2608-3.

Cowie, G. L., and Hedges, J. I. (1994). Biochemical indicators of diagenetic alteration in natural organic matter mixtures. *Nature* 369, 304–307. doi:10.1038/369304a0.

D'Andrilli, J., Chanton, J. P., Glaser, P. H., and Cooper, W. T. (2010). Characterization of dissolved organic matter in northern peatland soil porewaters by ultra high resolution mass spectrometry. *Org. Geochem.* 41, 791–799. doi:10.1016/j.orggeochem.2010.05.009.

Dauwe, B., and Middelburg, J. J. (1998). Amino acids and hexosamines as indicators of organic matter degradation state in North Sea sediments. *Limnol. Oceanogr.* 43, 782–798. doi:10.4319/lo.1998.43.5.0782.

Dittmar, T., Koch, B., Hertkorn, N., and Kattner, G. (2008). A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnol. Oceanogr. Methods* 6, 230–235. doi:10.4319/lom.2008.6.230.

Dittmar, T., and Koch, B. P. (2006). Thermogenic organic matter dissolved in the abyssal ocean. *Mar. Chem.* 102, 208–217. doi:10.1016/j.marchem.2006.04.003.

Donnelly, M., and Dagley, S. (1980). Production of methanol from aromatic acids by Pseudomonas putida. *J. Bacteriol.* 142, 916–924. Available at: http://jb.asm.org/content/142/3/916.short [Accessed March 31, 2015].

Dridi, B., Fardeau, M.-L., Ollivier, B., Raoult, D., and Drancourt, M. (2012). Methanomassiliicoccus luminyensis gen. nov., sp. nov., a methanogenic archaeon isolated from human faeces. *Int. J. Syst. Evol. Microbiol.* 62, 1902–7. doi:10.1099/ijs.0.033712-0.

Durbin, A. M., and Teske, A. (2012). Archaea in organic-lean and organic-rich marine subsurface sediments: an environmental gradient reflected in distinct phylogenetic lineages. *Front. Microbiol.* 3, 168. doi:10.3389/fmicb.2012.00168.

Fennell, D. E., Nijenhuis, I., Wilson, S. F., Zinder, S. H., and Häggblom, M. M. (2004). *Dehalococcoides ethenogenes* Strain 195 reductively dechlorinates diverse chlorinated aromatic pollutants. *Environ. Sci. Technol.* 38, 2075–2081. doi:10.1021/es034989b.

Freese, E., Köster, J., and Rullkötter, J. (2008). Origin and composition of organic matter in tidal flat sediments from the German Wadden Sea. *Org. Geochem.* 39, 820–829. doi:10.1016/j.orggeochem.2008.04.023.

Freudenthal, T., Wagner, T., and Wenzhöfer, F. (2001). Early diagenesis of organic matter from sediments of the eastern subtropical Atlantic: evidence from stable nitrogen and carbon isotopes. *Geochim. Cosmochim. Acta* 65, 1795–1808. Available at: http://www.sciencedirect.com/science/article/pii/S0016703701005543 [Accessed January 6, 2015].

Großkopf R, Stubner, S., and Liesack, W. (1998). Novel euryarchaeotal lineages detected on rice roots and in the anoxic bulk soil of flooded rice microcosms. *Appl. Environ. Microbiol.* 64, 4983–9. Available at:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=90952&tool=pmcentrez&rendertype=abstract.

Guigue, J., Lévêque, J., Mathieu, O., Schmitt-Kopplin, P., Lucio, M., Arrouays, D., Jolivet, C., Dequiedt, S., Chemidlin Prévost-Bouré, N., and Ranjard, L. (2015). Water-extractable organic matter linked to soil physico-chemistry and microbiology at the regional scale. *Soil Biol. Biochem.* 84, 158–167. doi:10.1016/j.soilbio.2015.02.016.

Gutierrez, T., Singleton, D. R., Aitken, M. D., and Semple, K. T. (2011). Stable isotope probing of an algal bloom to identify uncultivated members of the *Rhodobacteraceae* associated with low-molecular-weight polycyclic aromatic hydrocarbon degradation. *Appl. Environ. Microbiol.* 77, 7856–60. doi:10.1128/AEM.06200-11.

Hamdan, L. J., Gillevet, P. M., Pohlman, J. W., Sikaroodi, M., Greinert, J., and Coffin, R. B. (2011). Diversity and biogeochemical structuring of bacterial communities across the Porangahau ridge accretionary prism, New Zealand. *FEMS Microbiol. Ecol.* 77, 518–32. doi:10.1111/j.1574-6941.2011.01133.x.

Hammer, Ø., Harper, D. A. T., and Ryan, P. D. (2001). PAST :Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4, 1–9.

Hebbeln, D., Scheurle, C., and Lamy, F. (2003). Depositional history of the Helgoland mud area, German Bight, North Sea. *Geo-Marine Lett.* 23, 81–90. doi:10.1007/s00367-003-0127-0.

Hedges, J. I., and Keil, R. G. (1995). Sedimentary organic matter preservation: an assessment and speculative synthesis. *Mar. Chem.* 49, 81–115. doi:10.1016/0304-4203(95)00008-F.

Hertkorn, N., Benner, R., Frommberger, M., Schmitt-Kopplin, P., Witt, M., Kaiser, K., Kettrup, A., and Hedges, J. I. (2006). Characterization of a major refractory component of marine dissolved organic matter. *Geochim. Cosmochim. Acta* 70, 2990–3010. doi:10.1016/j.gca.2006.03.021.

Holtvoeth, J. (2004). Terrigenous organic matter in sediments of the eastern equatorial Atlantic-distribution, reactivity, and relation to Late Quaternary climate. Ph. D. thesis, Universität Bremen (Germany). 10. Available at: http://elib.suub.uni-bremen.de/publications/dissertations/E-Diss947_Diss.pdf [Accessed January 6, 2015].

Hug, L. A., Castelle, C. J., Wrighton, K. C., Thomas, B. C., Sharon, I., Frischkorn, K. R., Williams, K. H., Tringe, S. G., and Banfield, J. F. (2013). Community genomic analyses constrain the distribution of metabolic traits across the *Chloroflexi* phylum and indicate roles in sediment carbon cycling. *Microbiome* 1, 22. doi:10.1186/2049-2618-1-22.

Hunting, E. R., White, C. M., Gemert, M. Van, Mes, D., Stam, E., Van, H. G., Geest, D., Kraak, M. H. S., and Admiraal, W. (2013). UV radiation and organic matter composition shape bacterial functional diversity in sediments. *Front. Microbiol.* 4, 1–5. doi:10.3389/fmicb.2013.00317.

Iino, T., Tamaki, H., Tamazawa, S., Ueno, Y., Ohkuma, M., Suzuki, K., Igarashi, Y., and Haruta, S. (2013). Candidatus Methanogranum caenicola: a novel Methanogen from the anaerobic digested sludge, and proposal of *Methanomassiliicoccaceae fam. nov*. and *Methanomassiliicoccales ord. nov*., for a Methanogenic lineage of the class *Thermoplasmata*. *Microbes Environ.* 28, 244–250. doi:10.1264/jsme2.ME12189.

Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., Suzuki, M., Takai, K., Delwiche, M., Colwell, F. S., et al. (2006). Biogeographical distribution and diversity of

microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proc. Natl. Acad. Sci. U. S. A.* 103, 2815–20. doi:10.1073/pnas.0511033103.

Jørgensen, B. B., and Boetius, A. (2007). Feast and famine--microbial life in the deep-sea bed. *Nat. Rev. Microbiol.* 5, 770–81. doi:10.1038/nrmicro1745.

Keil, R. G. (2011). Terrestrial influences on carbon burial at sea. *Proc. Natl. Acad. Sci. U. S. A.* 108, 9729–30. doi:10.1073/pnas.1106928108.

Kim, S., Kaplan, L. A., Benner, R., and Hatcher, P. G. (2004). Hydrogen-deficient molecules in natural riverine water samples—evidence for the existence of black carbon in DOM. *Mar. Chem.* 92, 225–234. doi:10.1016/j.marchem.2004.06.042.

Kirchman, D. (2002). The ecology of Cytophaga–Flavobacteria in aquatic environments. *FEMS Microbiol. Ecol.* 39, 91–100. doi:10.1016/S0168-6496(01)00206-9.

Koch, B. P., and Dittmar, T. (2006). From mass to structure: an aromaticity index for high-resolution mass data of natural organic matter. *Rapid Commun. Mass Spectrom.* 20, 926–932. doi:10.1002/rcm.2386.

Koch, B. P., Witt, M., Engbrodt, R., Dittmar, T., and Kattner, G. (2005). Molecular formulae of marine and terrigenous dissolved organic matter detected by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Geochim. Cosmochim. Acta* 69, 3299–3308. doi:10.1016/j.gca.2005.02.027.

Könneke, M., Bernhard, A. E., de la Torre, J. R., Walker, C. B., Waterbury, J. B., and Stahl, D. A. (2005). Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* 437, 543–6. doi:10.1038/nature03911.

Köpke, B., Wilms, R., Engelen, B., Cypionka, H., and Sass, H. (2005). Microbial diversity in coastal subsurface sediments: a cultivation approach using various electron acceptors and substrate gradients. *Appl. Environ. Microbiol.* 71, 7819–30. doi:10.1128/AEM.71.12.7819-7830.2005.

Kubo, K., Lloyd, K. G., F Biddle, J., Amann, R., Teske, A., and Knittel, K. (2012). Archaea of the Miscellaneous Crenarchaeotal Group are abundant, diverse and widespread in marine sediments. *ISME J.* 6, 1949–65. doi:10.1038/ismej.2012.37.

Lalonde, K., Mucci, A., Ouellet, A., and Gélinas, Y. (2012). Preservation of organic matter in sediments promoted by iron. *Nature* 483, 198–200. doi:10.1038/nature10855.

Landa, M., Cottrell, M. T., Kirchman, D. L., Kaiser, K., Medeiros, P. M., Tremblay, L., Batailler, N., Caparros, J., Catala, P., Escoubeyrou, K., et al. (2014). Phylogenetic and structural response of heterotrophic bacteria to dissolved organic matter of different chemical composition in a continuous culture study. *Environ. Microbiol.* 16, 1668–81. doi:10.1111/1462-2920.12242.

Lazar, C. S., Biddle, J. F., Meador, T. B., Blair, N., Hinrichs, K.-U., and Teske, A. P. (2015). Environmental controls on intragroup diversity of the uncultured benthic archaea of the miscellaneous Crenarchaeotal group lineage naturally enriched in anoxic sediments of the White Oak River estuary (North Carolina, USA). *Environ. Microbiol.* 17, 2228–38. doi:10.1111/1462-2920.12659.

Lechtenfeld, O. J., Koch, B. P., Gašparović, B., Frka, S., Witt, M., and Kattner, G. (2013). The influence of salinity on the molecular and optical properties of surface microlayers in a karstic estuary. *Mar. Chem.* 150, 25–38. doi:10.1016/j.marchem.2013.01.006.

De Leeuw, J. W., and Largeau, C. (1993). "A review of macromolecular organic compounds that comprise living organisms and their role in kerogen, coal, and petroleum formation," in *Organic Geochemistry, Principles and Applications*, eds. M. Engel and S. A. Macko (New York, NY: Plenum Press), 23–72. Available at: http://link.springer.com/chapter/10.1007/978-1-4615-2890-6_2 [Accessed February 11, 2015].

Lever, M. A., Rogers, K. L., Lloyd, K. G., Overmann, J., Schink, B., Thauer, R. K., Hoehler, T. M., and Jørgensen, B. B. (2015). Life under extreme energy limitation: a synthesis of laboratory- and field-based investigations. *FEMS Microbiol. Rev.*, 1–41. doi:10.1093/femsre/fuv020.

Llobet-brossa, E., Rabus, R., Böttcher, M. E., Könneke, M., Finke, N., Schramm, A., Meyer, R. L., Grötzschel, S., Rosselló-mora, R., and Amann, R. (2002). Community structure and activity of sulfate-reducing bacteria in an intertidal surface sediment : a multi-method approach. 29, 211–226.

Lloyd, K. G., Alperin, M. J., and Teske, A. (2011). Environmental evidence for net methane production and oxidation in putative ANaerobic MEthanotrophic (ANME) archaea. *Environ. Microbiol.* 13, 2548–64. doi:10.1111/j.1462-2920.2011.02526.x.

Lloyd, K. G., Schreiber, L., Petersen, D. G., Kjeldsen, K. U., Lever, M. A., Steen, A. D., Stepanauskas, R., Richter, M., Kleindienst, S., Lenk, S., et al. (2013). Predominant archaea in marine sediments degrade detrital proteins. *Nature* 496, 215–8. doi:10.1038/nature12033.

Lovley, D. R., Phillips, E. J., Lonergan, D. J., and Widman, P. K. (1995). Fe(III) and S^0 reduction by *Pelobacter carbinolicus*. *Appl. Environ. Microbiol.* 61, 2132–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=167486&tool=pmcentrez&rendertype=abstract.

Lovley, D. R., Roden, E. E., Phillips, E. J. ., and Woodward, J. . (1993). Enzymatic iron and uranium reduction by sulfate-reducing bacteria. *Mar. Geol.* 113, 41–53. doi:10.1016/0025-3227(93)90148-O.

Mayer, L. M. (1994). Surface area control of organic carbon accumulation in continental shelf sediments. *Geochim. Cosmochim. Acta* 58, 1271–1284. doi:10.1016/0016-7037(94)90381-6.

Meng, J., Xu, J., Qin, D., He, Y., Xiao, X., and Wang, F. (2014). Genetic and functional properties of uncultivated MCG archaea assessed by metagenome and gene expression analyses. *ISME J.* 8, 650–9. doi:10.1038/ismej.2013.174.

Meyers, P. A. (1994). Preservation of elemental and isotopic source identification of sedimentary organic matter. *Chem. Geol.* 114, 289–302.

Meyers, P. A., and Ishiwatari, R. (1993). Lacustrine organic geochemistry: an overview of indicators of organic matter sources and diagenesis in lake sediments. *Org. Geochem.* 20, 867–900.

Middelburg, J. J. (1989). A simple rate model for organic matter decomposition in marine sediments. *Geochim. Cosmochim. Acta* 53, 1577–1581. doi:10.1016/0016-7037(89)90239-1.

Miyatake, T., Moerdijk-Poortvliet, T. C. W., Stal, L. J., and Boschker, H. T. S. (2014). Tracing carbon flow from microphytobenthos to major bacterial groups in an intertidal marine sediment by using an in situ ^{13} C pulse-chase method. *Limnol. Oceanogr.* 59, 1275–1287. doi:10.4319/lo.2014.59.4.1275.

Mussmann, M., Ishii, K., Rabus, R., and Amann, R. (2005). Diversity and vertical distribution of cultured and uncultured Deltaproteobacteria in an intertidal mud flat of the Wadden Sea. *Environ. Microbiol.* 7, 405–18. doi:10.1111/j.1462-2920.2005.00708.x.

Muyzer, G., and Stams, A. J. M. (2008). The ecology and biotechnology of sulphate-reducing bacteria. *Nat. Rev. Microbiol.* 6, 441–54. doi:10.1038/nrmicro1892.

Nebbioso, A., and Piccolo, A. (2012). Molecular characterization of dissolved organic matter (DOM): a critical review. *Anal. Bioanal. Chem.* 405, 109–24. doi:10.1007/s00216-012-6363-2.

Oni, O., Miyatake, T., Kasten, S., Richter-Heitmann, T., Fischer, D., Wagenknecht, L., Kulkarni, A., Blumers, M., Shylin, S. I., Ksenofontov, V., et al. (2015). Distinct microbial populations are tightly linked to the profile of dissolved iron in the methanic sediments of the Helgoland mud area, North Sea. *Front. Microbiol.* 06, 1–15. doi:10.3389/fmicb.2015.00365.

Park, B.-J., Park, S.-J., Yoon, D.-N., Schouten, S., Sinninghe Damsté, J. S., and Rhee, S.-K. (2010). Cultivation of autotrophic ammonia-oxidizing archaea from marine sediments in coculture with sulfur-oxidizing bacteria. *Appl. Environ. Microbiol.* 76, 7575–87. doi:10.1128/AEM.01478-10.

Parkes, R. J., Webster, G., Cragg, B. A., Weightman, A. J., Newberry, C. J., Ferdelman, T. G., Kallmeyer, J., Jørgensen, B. B., Aiello, I. W., and Fry, J. C. (2005). Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. *Nature* 436, 390–4. doi:10.1038/nature03796.

Paul, K., Nonoh, J. O., Mikulski, L., and Brune, A. (2012). "*Methanoplasmatales*," Thermoplasmatales-related archaea in termite guts and other environments, are the seventh order of methanogens. *Appl. Environ. Microbiol.* 78, 8245–53. doi:10.1128/AEM.02193-12.

Peacock, J. P., Cole, J. K., Murugapiran, S. K., Dodsworth, J. A., Fisher, J. C., Moser, D. P., and Hedlund, B. P. (2013). Pyrosequencing reveals high-temperature cellulolytic microbial consortia in Great Boiling Spring after in situ lignocellulose enrichment. *PLoS One* 8, e59927. doi:10.1371/journal.pone.0059927.

Pöritz, M., Schiffmann, C. L., Hause, G., Heinemann, U., Seifert, J., Jehmlich, N., von Bergen, M., Nijenhuis, I., and Lechner, U. (2015). *Dehalococcoides mccartyi* strain DCMB5 respires a broad spectrum of chlorinated aromatic compounds. *Appl. Environ. Microbiol.* 81, 587–96. doi:10.1128/AEM.02597-14.

Poulsen, M., Schwab, C., Jensen, B. B., Engberg, R. M., Spang, A., Canibe, N., Højberg, O., Milinovich, G., Fragner, L., Schleper, C., et al. (2013). Methylotrophic methanogenic Thermoplasmata implicated in reduced methane emissions from bovine rumen. *Nat. Commun.* 4, 1428. doi:10.1038/ncomms2432.

Reemtsma, T., These, A., Linscheid, M., Leenheer, J., and Spitzy, A. (2008). Molecular and Structural Characterization of Dissolved Organic Matter from the Deep Ocean by FTICR-MS, Including Hydrophilic Nitrogenous Organic Molecules. *Environ. Sci. Technol.* 42, 1430–1437. doi:10.1021/es7021413.

Riedel, T., Zak, D., Biester, H., and Dittmar, T. (2013). Iron traps terrestrially derived dissolved organic matter at redox interfaces. *Proc. Natl. Acad. Sci. U. S. A.* 110, 10101–5. doi:10.1073/pnas.1221487110.

Rontani, J.-F., Charriere, B., Petit, M., Vaultier, F., Heipieper, H. J., Link, H., Chaillou, G., and Sempéré, R. (2012). Degradation state of organic matter in surface sediments from the Southern Beaufort Sea: a lipid approach. *Biogeosciences* 9, 3513–3530. doi:10.5194/bg-9-3513-2012.

Roth, V.-N., Dittmar, T., Gaupp, R., and Gleixner, G. (2013). Latitude and pH driven trends in the molecular composition of DOM across a north south transect along the Yenisei River. *Geochim. Cosmochim. Acta* 123, 93–105. doi:10.1016/j.gca.2013.09.002.

Ruff, S., Probandt, D., Zinkann, A.-C., Iversen, M. H., Klaas, C., Würzberg, L., Krombholz, N., Wolf-Gladrow, D., Amann, R., and Knittel, K. (2014). Indications for algae-degrading benthic microbial communities in deep-sea sediments along the Antarctic Polar Front. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 108, 6–16. doi:10.1016/j.dsr2.2014.05.011.

Sangiorgi, F., Fabbri, D., Comandini, M., Gabbianelli, G., and Tagliavini, E. (2005). The distribution of sterols and organic-walled dinoflagellate cysts in surface sediments of the North-western Adriatic Sea (Italy). *Estuar. Coast. Shelf Sci.* 64, 395–406. doi:10.1016/j.ecss.2005.03.005.

Šantl-Temkiv, T., Finster, K., Dittmar, T., Hansen, B. M., Thyrhaug, R., Nielsen, N. W., and Karlson, U. G. (2013). Hailstones: a window into the microbial and chemical inventory of a storm cloud. *PLoS One* 8, e53550. doi:10.1371/journal.pone.0053550.

Schink, B. (1997). Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol. Mol. Biol. Rev.* 61, 262–80. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=232610&tool=pmcentrez&rendertype=abstract.

Schink, B., and Zeikus, J. (1980). Microbial methanol formation: a major end product of pectin metabolism. *Curr. Microbiol.* 4, 387–389. Available at: http://link.springer.com/article/10.1007/BF02605383 [Accessed March 31, 2015].

Schippers, A., Kock, D., Höft, C., Köweker, G., and Siegert, M. (2012). Quantification of Microbial Communities in Subsurface Marine Sediments of the Black Sea and off Namibia. *Front. Microbiol.* 3, 16. doi:10.3389/fmicb.2012.00016.

Schmidt, F., Elvert, M., Koch, B. P., Witt, M., and Hinrichs, K.-U. (2009). Molecular characterization of dissolved organic matter in pore water of continental shelf sediments. *Geochim. Cosmochim. Acta* 73, 3337–3358. doi:10.1016/j.gca.2009.03.008.

Schmidt, F., Koch, B. P., Elvert, M., Schmidt, G., Witt, M., and Hinrichs, K.-U. (2011). Diagenetic transformation of dissolved organic nitrogen compounds under contrasting sedimentary redox conditions in the Black Sea. *Environ. Sci. Technol.* 45, 5223–9. doi:10.1021/es2003414.

Schmidt, F., Koch, B. P., Witt, M., and Hinrichs, K.-U. (2014). Extending the analytical window for water-soluble organic matter in sediments by aqueous Soxhlet extraction. *Geochim. Cosmochim. Acta* 141, 83–96. doi:10.1016/j.gca.2014.06.009.

Seidel, M., Beck, M., Riedel, T., Waska, H., Suryaputra, I. G. N. A., Schnetger, B., Niggemann, J., Simon, M., and Dittmar, T. (2014). Biogeochemistry of dissolved organic matter in an anoxic intertidal creek bank. *Geochim. Cosmochim. Acta* 140, 418–434. doi:10.1016/j.gca.2014.05.038.

Sinkko, H., Lukkari, K., Sihvonen, L. M., Sivonen, K., Leivuori, M., Rantanen, M., Paulin, L., and Lyra, C. (2013). Bacteria contribute to sediment nutrient release and reflect progressed eutrophication-driven hypoxia in an organic-rich continental sea. *PLoS One* 8, e67061. doi:10.1371/journal.pone.0067061.

Sleighter, R. L., and Hatcher, P. G. (2008). Molecular characterization of dissolved organic matter (DOM) along a river to ocean transect of the lower Chesapeake Bay by ultrahigh resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Mar. Chem.* 110, 140–152. doi:10.1016/j.marchem.2008.04.008.

Strosser, E. (2010). Methods for determination of labile soil organic matter: An overview. *J. Agrobiol.* 27, 49–60. doi:10.2478/s10146-009-0008-x.

Teeling, H., Fuchs, B., and Becher, D. (2012). Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science (80).* 5567, 608–611. Available at: http://www.sciencemag.org/content/336/6081/608.short [Accessed January 29, 2015].

Teske, A., and Sørensen, K. B. (2008). Uncultured archaea in deep marine subsurface sediments: have we caught them all? *ISME J.* 2, 3–18. doi:10.1038/ismej.2007.90.

Tfaily, M. M., Corbett, J. E., Wilson, R., Chanton, J. P., Glaser, P. H., Cawley, K. M., Jaffé, R., and Cooper, W. T. (2015). Utilization of PARAFAC-Modeled Excitation-Emission Matrix (EEM) fluorescence spectroscopy to identify biogeochemical processing of dissolved organic matter in a northern peatland. *Photochem. Photobiol.* doi:10.1111/php.12448.

Tfaily, M. M., Hamdan, R., Corbett, J. E., Chanton, J. P., Glaser, P. H., and Cooper, W. T. (2013). Investigating dissolved organic matter decomposition in northern peatlands using complimentary analytical techniques. *Geochim. Cosmochim. Acta* 112, 116–129. doi:10.1016/j.gca.2013.03.002.

Tremblay, L. B., Dittmar, T., Marshall, A. G., Cooper, W. J., and Cooper, W. T. (2007). Molecular characterization of dissolved organic matter in a North Brazilian mangrove porewater and mangrove-fringed estuaries by ultrahigh resolution Fourier Transform-Ion Cyclotron Resonance mass spectrometry and excitation/emission spectroscopy. *Mar. Chem.* 105, 15–29. doi:10.1016/j.marchem.2006.12.015.

Vigneron, A., Cruaud, P., Roussel, E. G., Pignet, P., Caprais, J.-C., Callac, N., Ciobanu, M.-C., Godfroy, A., Cragg, B. A., Parkes, J. R., et al. (2014). Phylogenetic and functional diversity of microbial communities associated with subsurface sediments of the Sonora Margin, Guaymas Basin. *PLoS One* 9, e104427. doi:10.1371/journal.pone.0104427.

Wakeham, S. G., Lee, C., Hedges, J. I., Hernes, P. J., and Peterson, M. J. (1997). Molecular indicators of diagenetic status in marine organic matter. *Geochim. Cosmochim. Acta* 61, 5363–5369. doi:10.1016/S0016-7037(97)00312-8.

Wasmund, K., Schreiber, L., Lloyd, K. G., Petersen, D. G., Schramm, A., Stepanauskas, R., Jørgensen, B. B., and Adrian, L. (2014). Genome sequencing of a single cell of the widely distributed marine subsurface *Dehalococcoidia*, phylum *Chloroflexi*. *ISME J.* 8, 383–97. doi:10.1038/ismej.2013.143.

Webster, G., Yarram, L., Freese, E., Köster, J., Sass, H., Parkes, R. J., and Weightman, A. J. (2007). Distribution of candidate division JS1 and other Bacteria in tidal sediments of the German Wadden Sea using targeted 16S rRNA gene PCR-DGGE. *FEMS Microbiol. Ecol.* 62, 78–89. doi:10.1111/j.1574-6941.2007.00372.x.

Wilms, R., Köpke, B., Sass, H., Chang, T. S., Cypionka, H., and Engelen, B. (2006a). Deep biosphere-related bacteria within the subsurface of tidal flat sediments. *Environ. Microbiol.* 8, 709–19. doi:10.1111/j.1462-2920.2005.00949.x.

Wilms, R., Sass, H., Ko, B., Cypionka, H., and Engelen, B. (2006b). Specific bacterial , archaeal , and eukaryotic communities in tidal-flat sediments along a vertical profile of several meters †. 72, 2756–2764. doi:10.1128/AEM.72.4.2756.

Xie, S., Lipp, J. S., Wegener, G., Ferdelman, T. G., and Hinrichs, K. (2013). Turnover of microbial lipids in the deep biosphere and growth of benthic archaeal populations. *Proc. Natl. Acad. Sci. U. S. A.* 110, 6010–4. doi:10.1073/pnas.1218569110.

Zhuang, G.-C., Lin, Y.-S., Elvert, M., Heuer, V. B., and Hinrichs, K.-U. (2014). Gas chromatographic analysis of methanol and ethanol in marine sediment pore waters: Validation and implementation of three pretreatment techniques. *Mar. Chem.* 160, 82–90. doi:10.1016/j.marchem.2014.01.011.

Zonneveld, K. A. F., Versteegh, G. J. M., Kasten, S., Eglinton, T. I., Emeis, K.-C., Huguet, C., Koch, B. P., de Lange, G. J., de Leeuw, J. W., Middelburg, J. J., et al. (2010). Selective preservation of organic matter in marine environments; processes and impact on the sedimentary record. *Biogeosciences* 7, 483–511. doi:10.5194/bg-7-483-2010.

Table 1. Concentrations of dissolved organic carbon (DOC), total dissolved nitrogen (TDN), their ratios in WE-OM and proportion of water extractable organic carbon (WE-OC) in TOC

Sample	DOC (μM)	TDN (μM)	DOC/TDN	WE-OC (mg C/g sed.)	TOC (%)	TOC (mg/g sed.)	WE-OC (%TOC)
HE421-004_4-6 cm	1103.94	134.82	8.19	0.20	0.98	9.79	2.05
HE406-8_30-55 cm	827.34	64.90	12.75	0.14	0.81	8.06	1.72
HE406-8_130-155 cm	913.74	88.62	10.31	0.20	0.86	8.55	2.38
HE406-8_230-255 cm	1078.14	113.82	9.47	0.20	1.60	15.95	1.23
HE406-8_330-355 cm	976.74	103.32	9.45	0.19	1.14	11.36	1.65
HE406-8_430-455 cm	1468.47	154.47	9.51	0.25	1.36	13.55	1.84

Table 2. Number of molecular formulas, weighted averages (wa) of DBE, molar ratios of oxygen, hydrogen, carbon, nitrogen atoms and charge to mass ratios of water-extractable organic matter as obtained from FT-ICR MS analysis.

Sample	Number of formulas	DBE_{wa}	H/C_{wa}	O/C_{wa}	C/N_{wa} ratio	m/z_{wa}
HE421-004_4-6cm	4858	7.22	1.35	0.47	12.83	367.02
HE406-8_30-55 cm	5805	8.88	1.17	0.52	21.92	389.44
HE406-8_130-155 cm	6348	8.42	1.20	0.54	24.46	391.95
HE406-8_230-255 cm	6899	8.53	1.21	0.51	24.55	392.20
HE406-8_330-355 cm	6780	8.43	1.21	0.52	22.47	391.04
HE406-8_430-455 cm	6936	8.70	1.20	0.50	22.01	391.01

Fig. 1

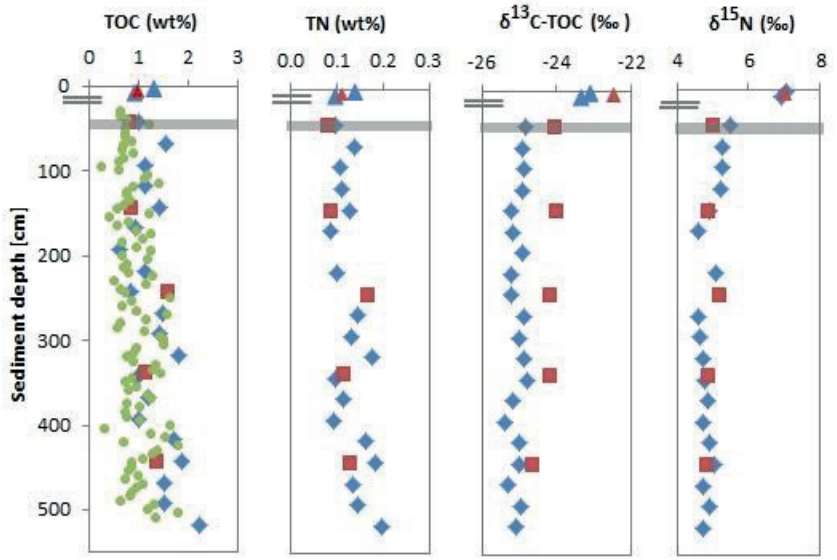

Fig. 1. Depth profiles of TOC, TN, stable carbon and stable nitrogen in surface and subsurface sediments of the Helgoland mud area. Surface sediment; core UT2012 (blue triangle), surface sediment; core HE421-003 (brown triangle), deep sediment; core HE376-007-5 (blue diamond), deep sediment; core HE376-007-2 (high-resolution TOC, green dots), deep sediment; core HE406-008 (brown square). Grey bar represents SMT.

Fig. 2

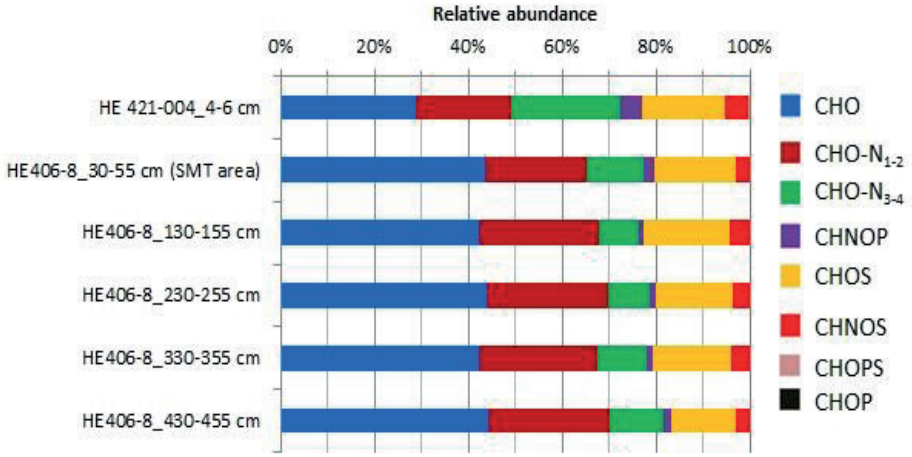

Fig. 2. Depth-wise relative abundance distribution of intensities of molecular formulas groups classified based solely on heteroatoms (N, S, and P) contents. Surface sediment samples (4-6 cm) are obtained from core HE421-003. Deep sediments samples (30-455 cm) are obtained from core HE 406-008.

Fig. 3

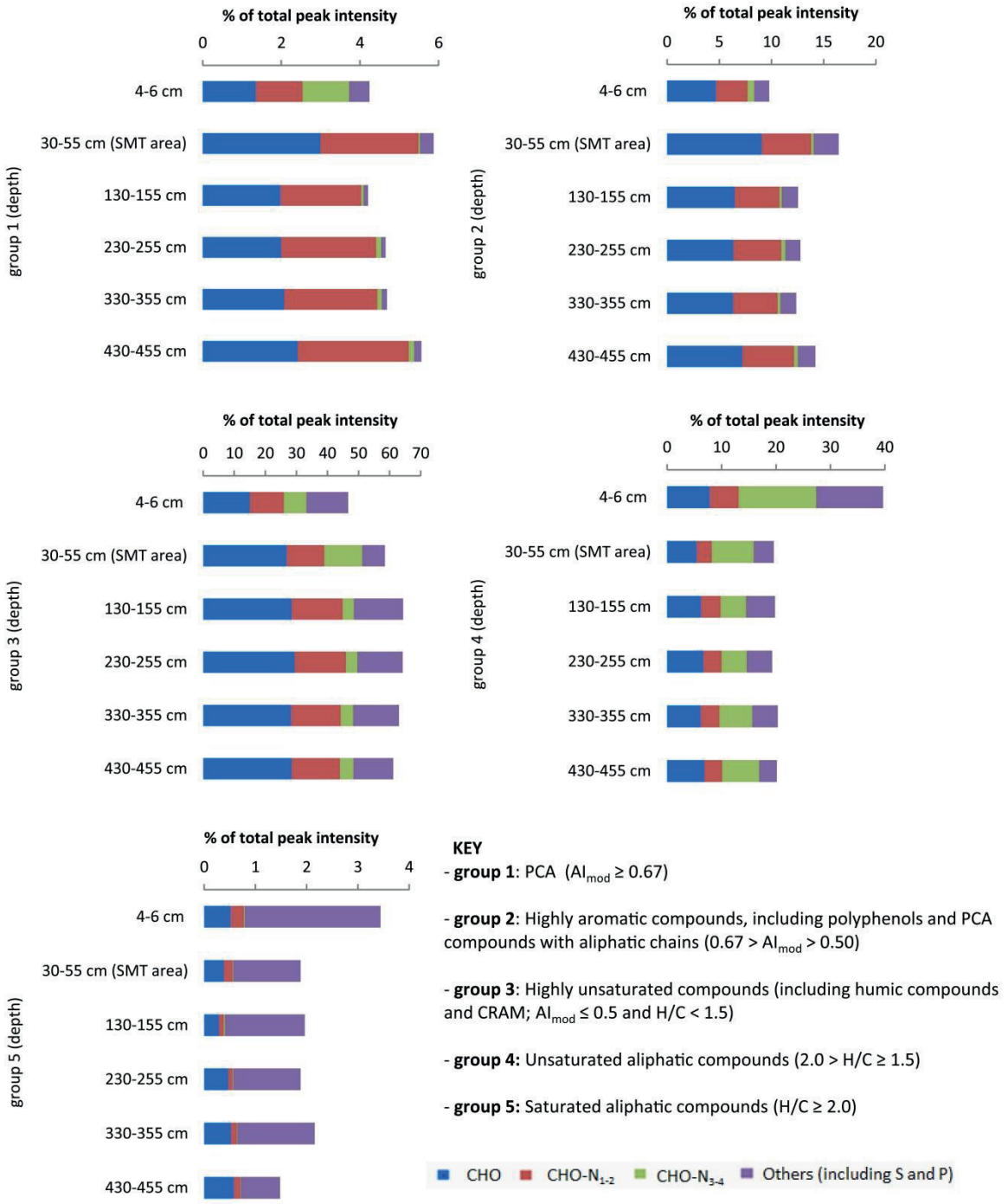

Fig. 3. Depth-wise relative abundance distribution of intensities of compound groups classified based on modified aromaticity index (AI_{mod}), H/C and O/C ratios. At each depth, compound groups are further divided based on heteroatoms (N, S, and P). Surface sediment samples (4-6 cm) are obtained from core HE421-003. Deep sediments samples (30-455 cm) are obtained from core HE 406-008.

Fig. 4

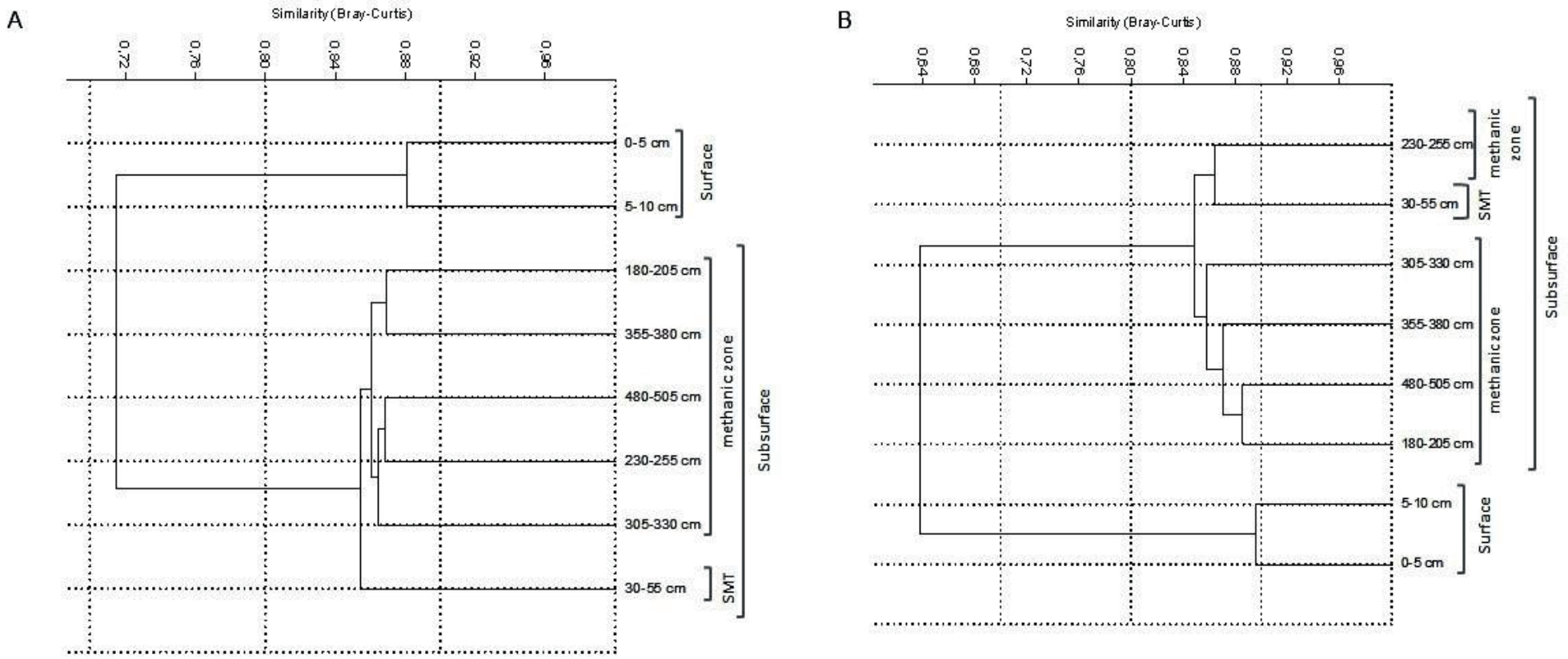

Fig. 4. Weighted Paired Group Method of Averaging (WPGMA) cluster diagrams of bacteria and archaeal OTUs (97% cut off) obtained from pyrosequencing-based 16SrRNA gene sequencing.

Fig. 5

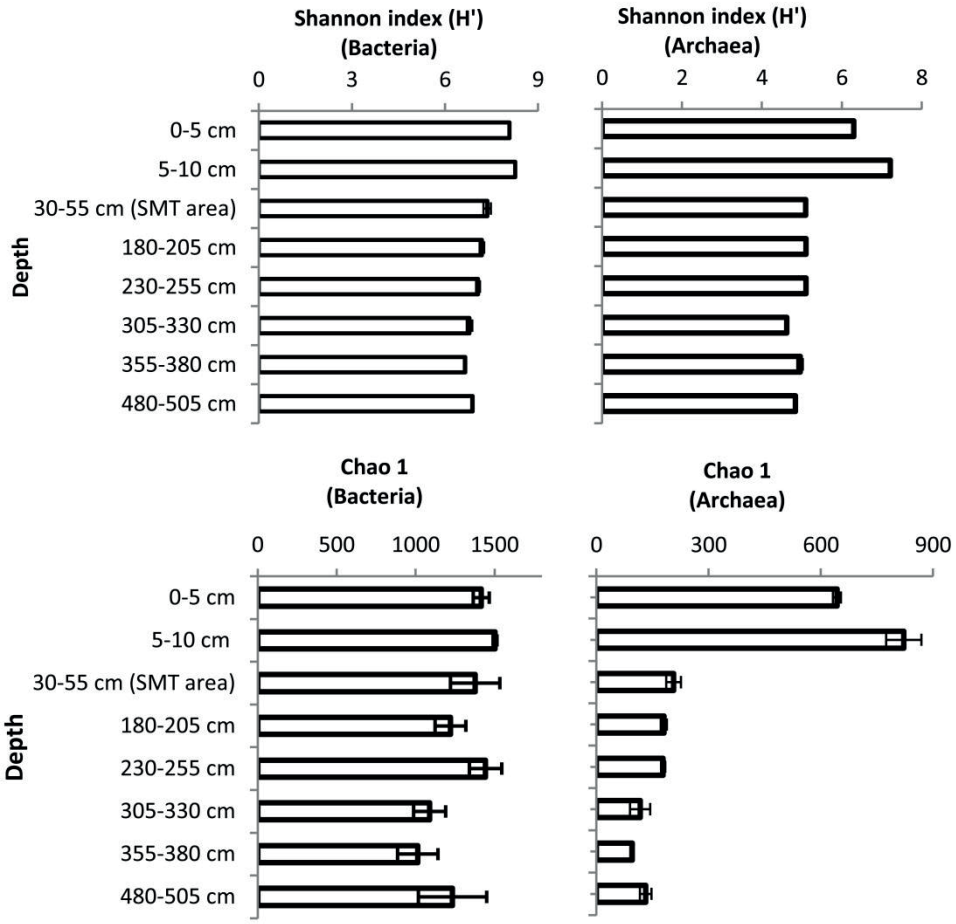

Fig. 5. Shannon and Chao1 diversity indices of Bacteria and Archaea in surface and deep sediments of the Helgoland mud area. Error bar are standard deviations for results of calculations of diversity indices over 3 iterations.

Fig. 6

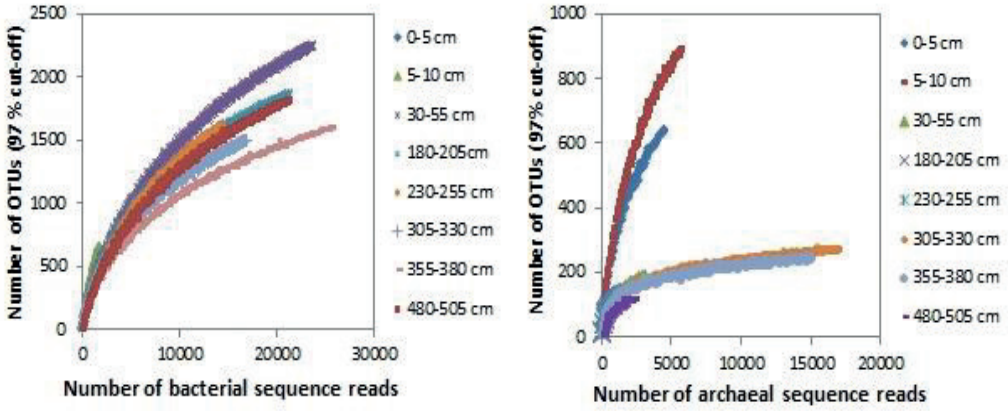

Fig. 6. Rarefaction curves of bacterial and archaeal OTUs (97% cut-off) detected at the depths sampled in the surface and deep sediments.

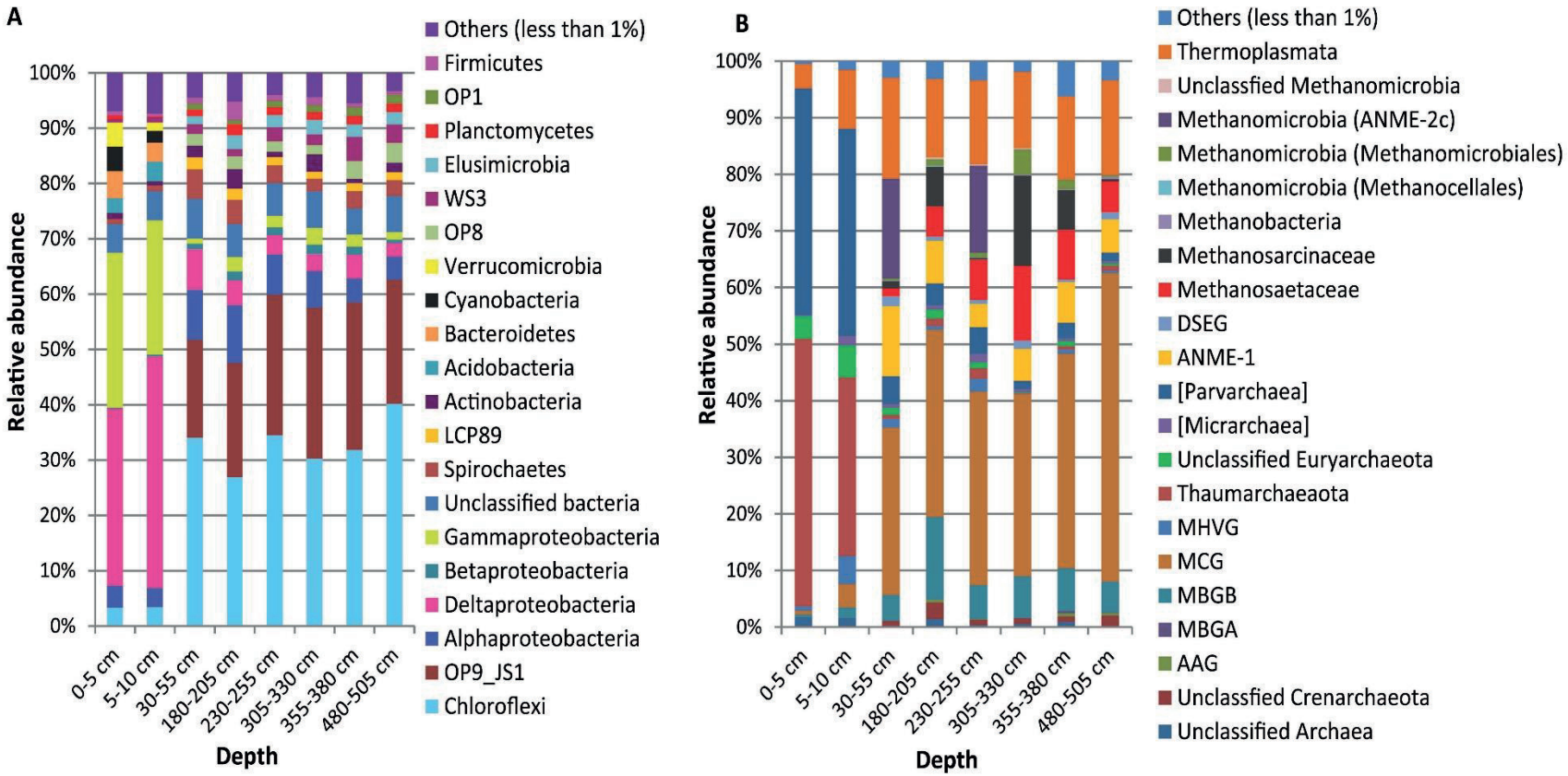

Fig. 7. Composition of Bacteria (A) and Archaea (B) communities in the Helgoland mud area based on pyrosequencing analyses of 16S rRNA gene data.

Fig. 8

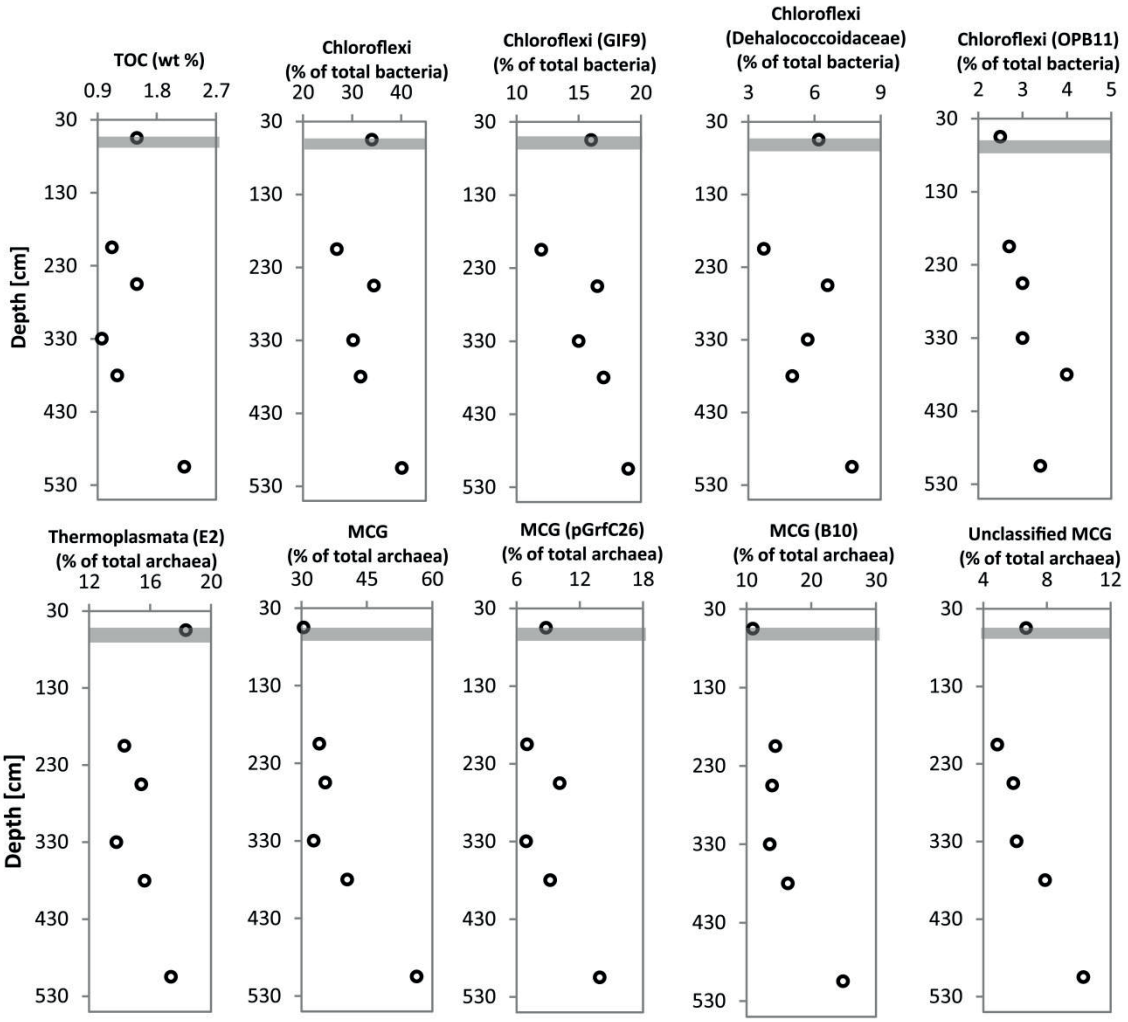

Fig. 8. Depth profiles of dominant Bacteria (*Chloroflexi*) and Archaea (*Thermoplasmata* and MCG) in relation to TOC content at sediment depths from which DNA was extracted for pyrosequencing analysis of 16S rRNA genes. Grey bar represents SMT.

Fig. 9

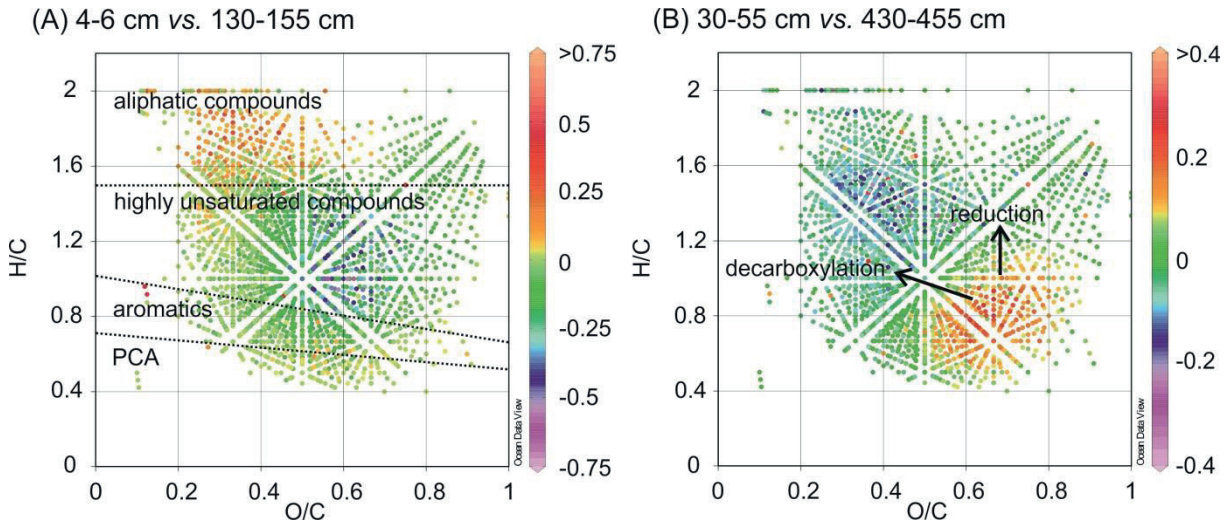

Fig. 9. Differential van-Krevelen diagrams compare the relative formula intensities of CHO compounds between two samples showing molecular variations between surface and subsurface sediments, (A) and between two subsurface sediments, (B). Higher formula intensities in each of the shallower samples relative to the deeper samples is indicated by positive values (orange to red color) whereas negative values (blue to purple color) indicate higher formula intensities in the deeper samples relative to the shallow ones.

Fig. S1

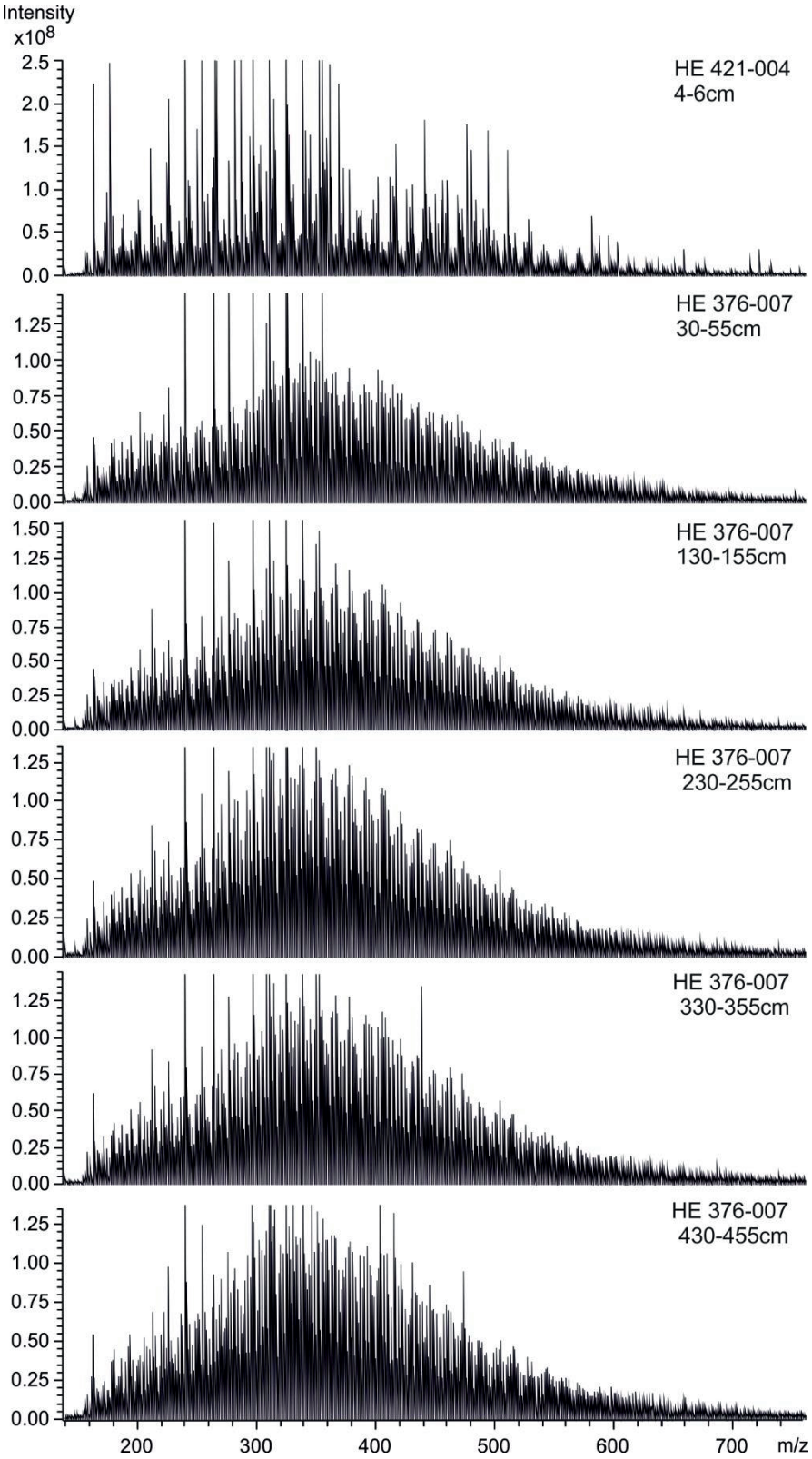

Figure S1. ESI negative FT-ICR mass spectra of WE-OM extracted from the sediment cores of Helgoland mud area. Largest peaks are contaminants (listed in the surfactant database: http://www.terrabase-inc.com//Surfactants.htm) and were removed from the final data set.

Fig. S1

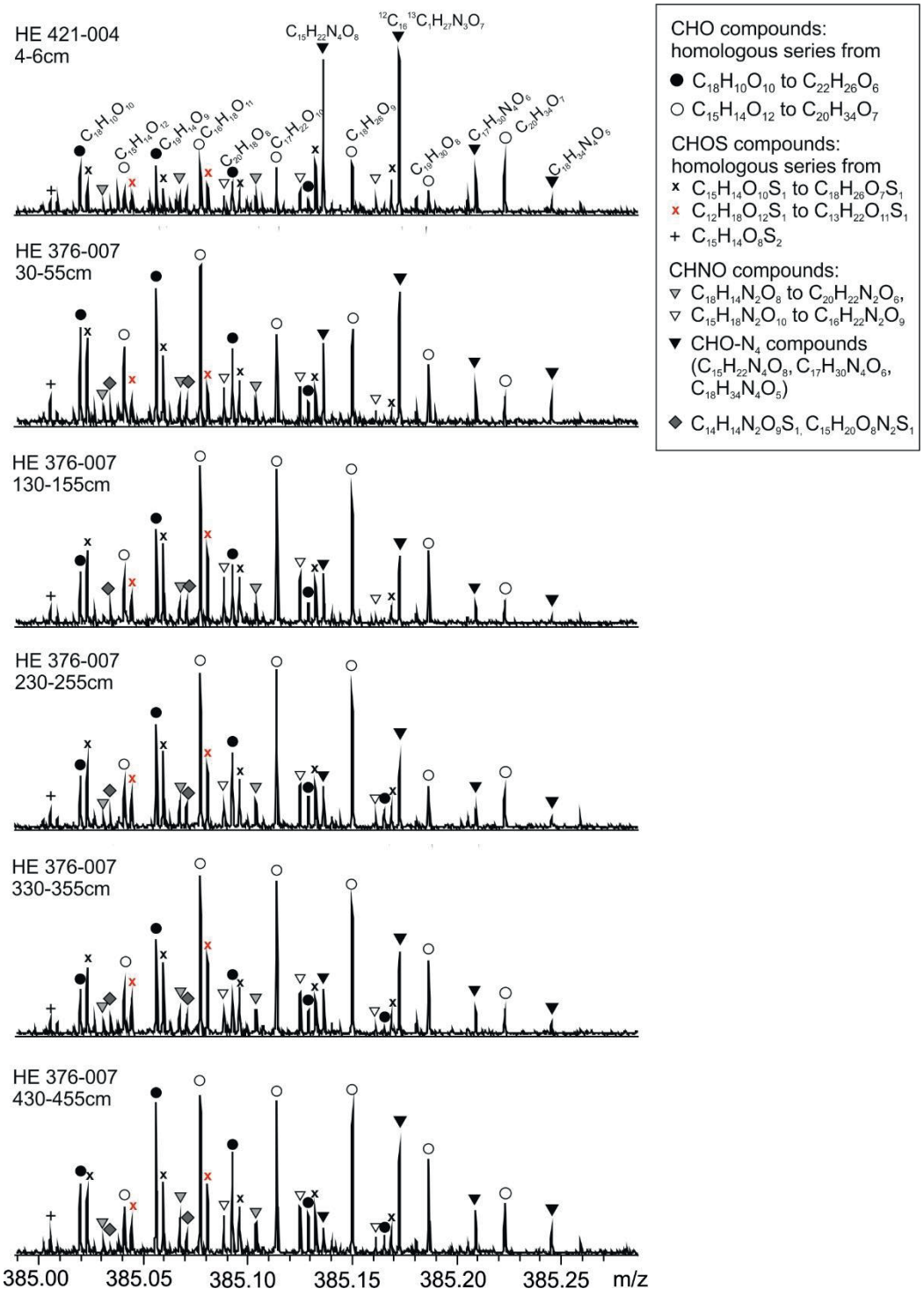

Figure S2. FT-ICR mass spectra on the mass 385 Da for WE-OM with increasing sediment depth from top to bottom. Symbols refer to different compound groups and homologous series. Homologous series are defined as the functional relationship between molecular formulas that differ by a specific mass difference equivalent to a chemical building block (in this case CH_4 replaced by O (0.036 Da)).

Chapter 3

frontiers in Microbiology

ORIGINAL RESEARCH
published: 01 May 2015
doi: 10.3389/fmicb.2015.00365

Distinct microbial populations are tightly linked to the profile of dissolved iron in the methanic sediments of the Helgoland mud area, North Sea

*Oluwatobi Oni[1,2], Tetsuro Miyatake[1], Sabine Kasten[2,3], Tim Richter-Heitmann[1], David Fischer[2,3], Laura Wagenknecht[3], Ajinkya Kulkarni[1], Mathias Blumers[4], Sergii I. Shylin[4,5], Vadim Ksenofontov[4], Benilde F. O. Costa[6], Göstar Klingelhöfer[4] and Michael W. Friedrich[1,2]**

[1] Microbial Ecophysiology Group, Faculty of Biology/Chemistry, University of Bremen, Bremen, Germany, [2] MARUM, Center for Marine Environmental Sciences, University of Bremen, Bremen, Germany, [3] Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany, [4] Institute for Inorganic and Analytical Chemistry, Johannes Gutenberg University, Mainz, Germany, [5] Department of Chemistry, Taras Shevchenko National University of Kyiv, Kyiv, Ukraine, [6] CFisUC, Department of Physics, University of Coimbra, Coimbra, Portugal

Iron reduction in subseafloor sulfate-depleted and methane-rich marine sediments is currently a subject of interest in subsurface geomicrobiology. While iron reduction and microorganisms involved have been well studied in marine surface sediments, little is known about microorganisms responsible for iron reduction in deep methanic sediments. Here, we used quantitative PCR-based 16S rRNA gene copy numbers and pyrosequencing-based relative abundances of bacteria and archaea to investigate covariance between distinct microbial populations and specific geochemical profiles in the top 5 m of sediment cores from the Helgoland mud area, North Sea. We found that gene copy numbers of bacteria and archaea were specifically higher around the peak of dissolved iron in the methanic zone (250–350 cm). The higher copy numbers at these depths were also reflected by the relative sequence abundances of members of the candidate division JS1, methanogenic and *Methanohalobium*/ANME-3 related archaea. The distribution of these populations was strongly correlated to the profile of pore-water Fe^{2+} while that of *Desulfobacteraceae* corresponded to the pore-water sulfate profile. Furthermore, specific JS1 populations also strongly co-varied with the distribution of *Methanosaetaceae* in the methanic zone. Our data suggest that the interplay among JS1 bacteria, methanogenic archaea and *Methanohalobium*/ANME-3-related archaea may be important for iron reduction and methane cycling in deep methanic sediments of the Helgoland mud area and perhaps in other methane-rich depositional environments.

Keywords: candidate division JS1, iron reduction, methanogens, ANME, subsurface sediments, North Sea, SMT, anaerobic oxidation of methane

OPEN ACCESS

Edited by:
David Emerson, Bigelow Laboratory for Ocean Sciences, USA

Reviewed by:
Mustafa Yucel, GEOMAR – Helmholtz Centre for Ocean Research Kiel, Germany
Jeffrey M. Dick, Chiang Mai University, Thailand

****Correspondence:***
Michael W. Friedrich, Microbial Ecophysiology Group, Faculty of Biology/Chemistry, University of Bremen, Leobener Straße, 28359 Bremen, Germany
michael.friedrich@uni-bremen.de

Specialty section:
This article was submitted to Microbiological Chemistry and Geomicrobiology, a section of the journal Frontiers in Microbiology.

Received: *26 January 2015*
Accepted: *10 April 2015*
Published: *01 May 2015*

Citation:
Oni O, Miyatake T, Kasten S, Richter-Heitmann T, Fischer D, Wagenknecht L, Kulkarni A, Blumers M, Shylin SI, Ksenofontov V, Costa BFO, Klingelhöfer G and Friedrich MW (2015) Distinct microbial populations are tightly linked to the profile of dissolved iron in the methanic sediments of the Helgoland mud area, North Sea. Front. Microbiol. 6:365. doi: 10.3389/fmicb.2015.00365

Introduction

The involvement of microorganisms in electron-accepting processes (EAP) in marine sediments results in the formation of redox zones which may overlap with one another (Canfield and Thamdrup, 2009). Regardless, specific EAP may still dominate in certain zones with the result that microorganisms that are involved in these distinct processes are more dominant (Jorgensen et al., 2012). For example, at the sulfate-methane transition (SMT) of marine sediments sulfate reducers belonging to the *Desulfococcus/Desulfosarcina* (DSS) group and methanotrophic archaea (ANME-1, 2, and 3) are often found in relatively higher proportion (Lloyd et al., 2006; Harrison et al., 2009; Knittel and Boetius, 2009) compared to deeper methanic zones. Due to overlapping redox zonation in marine sediments, it may be challenging to accurately correlate down-core microbial community distribution to geochemical profiles. However, Jorgensen et al. (2012) have recently shown that the depth-wise distribution of archaeal populations, Marine Group I, are linked to nitrate profiles of deep sediments from the arctic mid-ocean ridge, thus buttressing their suspected role in the nitrogen cycle (Durbin and Teske, 2011).

Iron reduction is a major electron-accepting process in marine surface sediments and organisms known to be involved in this process have been studied (Roden and Lovley, 1993; Vandieken et al., 2006b; Nickel et al., 2008; Vandieken and Thamdrup, 2013). While members of the *Geobacteraceae* are considered as the major populations mediating metal reduction in freshwater sediments (Coates et al., 1996), other members of the *Desulfuromonadales* (e.g., *Desulfuromusa* and *Desulfuromonas*) are considered to be important in marine sediments (Roden and Lovley, 1993; Vandieken et al., 2006a). Based on most-probable-number (MPN) cell counts, members of the *Desulfuromonadales* were the most abundant iron-reducing bacteria (65% of the total bacteria population) in surface sediments from Aarhus Bay (Vandieken and Thamdrup, 2013).

In contrast to marine surface sediments, microbes involved in iron reduction in subsurface sediments have not been identified so far. At a number of sites elevated amounts of dissolved iron (i.e., Fe^{2+}) in pore-water have been observed: for example, in sediments of the Amazon Fan (Flood et al., 1995; Kasten et al., 1998), Peru Margin (D'Hondt et al., 2004), Sea of Okhotsk (Wallmann et al., 2008), Argentine Basin (Hensen et al., 2003; Riedinger et al., 2005, 2014), Zambesi Fan (März et al., 2008), Aarhus Bay (Holmkvist et al., 2011) and Bothnian Sea (Slomp et al., 2013; Egger et al., 2014). To explain the source of dissolved iron in subsurface sediments, a number of hypotheses may be considered. In Aarhus Bay sediments, a chemical reaction between buried iron(III) minerals and hydrogen sulfide diffusing downward from the SMT has been suggested to explain the formation of Fe^{2+} (Holmkvist et al., 2011). In the sulfate-depleted methanic zone of sediments of the Argentine Basin, it is argued that iron reduction is most likely coupled to the anaerobic oxidation of methane (Fe-AOM; Riedinger et al., 2014). This process has been suggested to be directly or indirectly linked to microbial activity (Beal et al., 2009). In addition, oxidation of products of organic matter fermentation such as acetate and hydrogen coupled to iron reduction by dissimilatory iron-reducing microorganisms (Roden and Lovley, 1993) may also be a possibility. Lastly, the non-dissimilatory reduction could be a potential pathway in which iron(III) oxides serve as an electron sink during fermentation of complex organic matter (Lovley and Phillips, 1986; Dobbin et al., 1999).

Elevated concentrations of dissolved iron measured in the pore-water of sediments below the SMT (methanic zone) in the Helgoland mud area have prompted us to investigate the potential involvement of certain microbial populations in the reduction of iron therein. In this study, we use molecular ecology techniques such as quantitative PCR and 454-pyrosequencing as well as geochemical measurements to estimate cell numbers and determine the proportion of distinct bacteria and archaea populations in the sediments of the Helgoland mud area down to over 530 cm below sea floor in relation to the dissolved iron profile.

Materials and Methods

Site and Sampling Description

The Helgoland mud area (**Figure 1**) in the German Bight of the North Sea extends over ~500 km^2 and has a water depth of less than 30 m (Hebbeln et al., 2003). It represents one of the few depocenters of fine-grained sediments in the North Sea. The average sedimentation rate was estimated to have been high (13 mm yr^{-1}) between 750–1550 before present. Presently, it is at 1.6 mm yr^{-1} (Hebbeln et al., 2003). During RV HEINCKE cruise HE376 in April 2012, subsurface sediment samples were collected from site HE 376-007 (**Table 1**) using a gravity corer (GC; 5 m core length). The upper 20–30 cm of sediment is generally lost when using a GC. Therefore, a multi corer (MUC) was used to collect undisturbed surface sediments (30 cm core length) from the same site during cruises with RV UTHÖRN in September 2012 (UT-2012; **Table 1**) and with RV HEINCKE in April 2014 (HE421-004; **Table 1**). The 5 m-long gravity core HE376-007-5 was cut in 25 cm sections, and subsamples of each section, taken with sterile 10 ml cut-off syringes, were frozen at −80°C for molecular analyses. For the MUC core UT-2012, only the top 10 cm of the 30 cm-long sediment core was processed for molecular analysis as this corresponded to the depth at which high dissolved iron was measured based on previous geochemical investigations at this study site. The sediment samples from the top 10 cm were homogenized and stored in separate 50 ml Falcon tubes at −80°C until further use. For pore-water and solid-phase analyses, a parallel gravity core at site HE376-007 was taken and sampled. Pore-water was retrieved every 25 cm by means of rhizon samplers which have an average pore size of 0.15 μm according to procedures described by Seeberg-Elverfeldt et al. (2005) and Dickens et al. (2007). For the measurement of methane concentrations, 5 ml of wet sediment were taken with cut-off syringes and were inserted into 50 ml headspace vials pre-filled with 20 ml of saturated NaCl solution. The vials were tightly closed with rubber septa, sealed with aluminum crimps and stored inverted at +4°C to minimize methane loss.

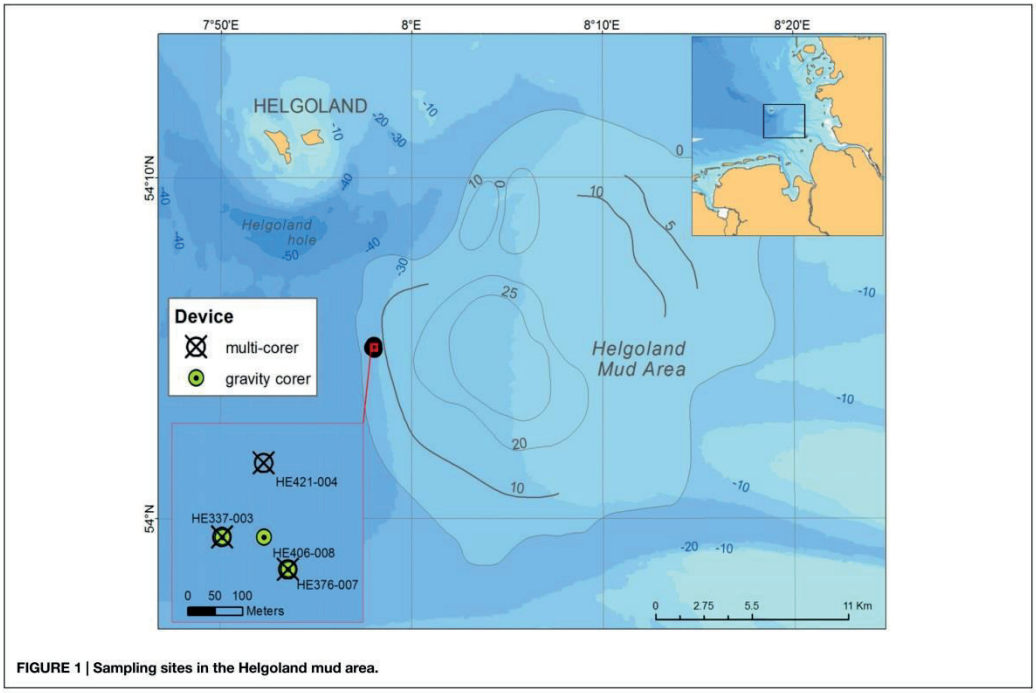

FIGURE 1 | Sampling sites in the Helgoland mud area.

TABLE 1 | Sampling information of sediment samples analyzed in the study.

	Gear	Date	Site	Coordinates
Pore-water profile	Gravity corer	April (2012)	HE376-007	54°5.00′N7°58.05′E
	Multi corer	April (2014)	HE421-004	54°5.10′N7°58.01′E
Molecular analysis	Multi corer	September (2012)	UT-2012	54°5.00′N7°58.05′E
	Gravity corer	April (2012)	HE376-007	54°5.00′N7°58.05′E
Sequential sediment extractions	Gravity corer	April (2012)	HE376-007	54°5.00′N7°58.05′E
Mössbauer spectroscopy	Gravity corer Multi corer	July (2013), April (2014)	HE406-008, HE421-004	54°5.01′N7°58.01′E 54°5.10′N7°58.01′E

For Mössbauer spectroscopy, GC, and MUC cores collected during RV HEINCKE cruises HE406 and HE421 conducted in July 2013 and April 2014, respectively, were used (**Table 1**). In the home lab, the GC retrieved from site HE406-008 was cut into 25 cm sections and the top 10 cm of the MUC core (UT-2012) were sampled as described above. 10 g of wet sediment were transferred into wide-mouth glass vials under a stream of argon gas (99.998% purity, Linde, Germany), frozen at −20°C and then freeze-dried to avoid air oxygen contamination. The freeze-drier chamber was filled with argon, lyophilized samples were removed immediately, sealed with rubber septa, and headspaces were flushed with argon on a manifold.

Geochemical Analyses

Pore-Water Measurements

For the analysis of dissolved iron, 1 ml pore-water aliquots were transferred into cuvettes pre-filled with 50 µl of Ferrospectral solution immediately after pore-water retrieval on board the RV HEINCKE. Dissolved iron concentrations were measured photometrically at a wavelength of 565 nm. 1.5 ml subsamples of pore-water were added to a 2.5% zinc acetate solution on board in order to fix all sulfide present as ZnS. After the cruise, sulfide concentrations were analyzed in the laboratories of the AWI using a spectrophotometer applying the methylene blue method (Cline, 1969). After dilution of pore-water samples, concentrations of ammonium were measured as described by Hall and Aller (1992). Pore-water sulfate concentrations were determined in pore-water aliquots (1:50 dilution) using a Metrohm Compact IC 761 ion chromatograph.

Determination of methane concentrations in pore-water was carried out in the laboratory of the AWI, Bremerhaven. This was done by injecting 20–100 µl of the headspace gas from closed vials containing wet sediments into a Thermo Finnigan TRACE GC equipped with a packed column and an integrated flame ionization detector (FID).

Sequential Extraction of Iron Minerals

Iron adsorbed to particle surfaces as well as bound in carbonates and iron (oxyhydr)oxides was extracted from the sediment using a sequential extraction procedure developed by Poulton and Canfield (2005). In brief, about 80 mg of dry sediment from the gravity core from site HE 376-007 (**Table 1**) were exposed to a sequence of four leaching agents and shaken for a defined period of time at room temperature (**Table 2**). Iron concentrations in the extracts were determined using an ICP-OES (Iris Intrepid, Thermo Elemental) with a relative standard deviation of less than 5%. In order to circumvent matrix effects, we prepared the standards for the ICP-OES with the same relative amount (1:20) of leaching agent as is in the extracts.

Mössbauer Spectroscopy

^{57}Fe-Mössbauer spectra were recorded in transmission geometry with an 8 mCi ^{57}Co source embedded in a rhodium matrix using a conventional constant-acceleration Mössbauer spectrometer (WissEL GmbH, Starnberg, Germany) equipped with a bath helium cryostat. The absorbers were prepared by placing the powdered samples of about 200 mg between acryl platelets of the sealed sample holder. Isomer shifts are given relatively to iron metal at ambient temperature. Simulations of the experimental data were performed with the *Recoil* program (Lagarec and Rancourt, 1997).

Molecular Ecology Analyses

Nucleic Acid Extraction

DNA was extracted from 0.5 to 0.6 g of wet sediment following Lueders et al. (2004) with modifications. Cells were disrupted twice for 45 s by bead-beating. DNA was precipitated with 0.2 volumes of 7.5 M ammonium acetate and one volume of isopropanol for 1 h at room temperature and collected by centrifugation at 17,950 *g*, at 4°C for 20 min. The final DNA pellet was dissolved in 50 μl elution buffer (Qiagen, Hilden, Germany). DNA concentration was measured using NanoDrop 1000 spectrophotometer (Peqlab Biotechnologie, Erlangen, Germany). DNA extracts from all samples were stored at −20°C until further processing.

Quantitative PCR (Q-PCR)

To estimate cell abundances through copy numbers of bacterial and archaeal 16S rRNA genes, Q-PCR was performed using DNA extracts from *Escherichia coli* strain SB1 and *Methanosarcina barkeri* (DSM 800) as standards, respectively. Standard templates were prepared by amplifying the 16S rRNA genes using primer pairs 27F and 907R (Lane, 1991) for bacteria and Ar109F (Grosskopf et al., 1998), and Ar912r (Lueders and Friedrich, 2000) for archaea. The concentrations of purified PCR products were determined using Qubit 2.0 fluorometer (Invitrogen, Darmstadt, Germany). Standard curves were prepared using standard templates. Primer pair 338F (Muyzer et al., 1993) and 518R (Muyzer et al., 1993) were used for quantifying bacterial 16S rRNA gene copies and primer pair Ar806F (Takai and Horikoshi, 2000) and Ar912rt (Lueders and Friedrich, 2002) for archaeal gene copies. Each Q-PCR reaction contained a total volume of 20 μl: 10 μl of master mix (MESA GREEN qPCR master mix, Eurogentec, Cologne, Germany), 0.5 μl of each primer (0.25 μM each; final concentration), 5 μl of RNase-free water, and 4 μl of template DNA. Amplification efficiency of not less than 90% and slope of −3.6 was obtained. Gene copy numbers per gram of wet sediment were calculated using the formula: gene copies = (quantity of DNA [g/μl]/size of amplicon (bp)) × (Avogadro's constant/660) assuming that the average weight of 1 bp is equal to 660 daltons.

Pyrosequencing and Sequence Analysis

DNA samples from depths of 0–5 cm and 5–10 cm (surface sediments), 30–55 cm (SMT), 180–205 cm, 230–255 cm, 305–330 cm, 355–380 cm, and 480–505 cm (methanic zones) were selected for 454 FLX pyrosequencing at Molecular and Research Testing Laboratory, (Lubbock, TX, USA). Primer pairs used for sequencing bacterial 16S rRNA genes were 104F (5′-GGC GVA CGG GTG AGT AA-3′) and 530R (5′-CCG CNG CNG CTG GCA C-3′; Wang and Qian, 2009). Primers 349F (5′-GYG CAS CAG KCG MGA AW-3′) and 806R (5′-GGA CTA CVS GGG TAT CTA AT-3′; Takai and Horikoshi, 2000) were used for archaea. Sequence raw data (SFF files) were subjected to downstream processing using QIIME version 1.6 (Caporaso et al., 2010). Barcodes and low quality sequences (less than 200 bp) were removed using Amplicon Noise (Quince et al., 2011). Taxonomic classification was done based on the Greengenes data base v12_10. The number of sequences per sample obtained can be found in **Table 3**. Raw sequences obtained from pyrosequencing analyses have been uploaded to the MG-RAST metagenomics analysis server for public access (Meyer et al., 2008; MG-RAST ids: 4612914.3, 4612912.3, 4612913.3, 4612915.3, 4612916.3, 4612917.3, 4612911.3, 4612918.3, 4612906.3, 4612904.3, 4612905.3, 4612907.3, 4612908.3, 4612909.3, 4612903.3, 4612910.3).

For phylogenetic analysis, sequences were aligned using ARB version 6.0.2 (Ludwig et al., 2004) and the closest neighbor and type strain sequences were identified and extracted using the SILVA non-redundant reference database [SSU Ref NR 99,

TABLE 2 | Sequential extraction procedure of iron minerals in sediment samples.

Step	Abbreviation	Extraction Agent	Target Fractions
1	FeCarb	1 M Na-acetate (pH 4.5) for 24 h	Adsorbed Fe, Fe carbonates
2	FeOX1	1 M hydroxylamine-HCl in 25 %v/v acetic acid (pH 2) for 24 h	Amorphous or poorly crystalline Fe (oxyhydr)oxides, mainly ferrihydrite and lepidocrocite
3	FeOX2	0.35 M acetic acid/0.2 M Na-citrate/0.28 M Na-dithionite (pH 4.8) for 2 h	Crystalline Fe (oxyhydr)oxides, mainly goethite, hematite
4	FeMag	0.2 M ammonium oxalate/0.17 M (pH 3.2) oxalic acid for 6 h	Crystalline Fe (oxyhydr)oxides, mainly (titano)magnetite, maghemite

TABLE 3 | Number of pyrosequencing-generated bacterial and archaeal 16S rRNA gene sequences analyzed per depth sampled.

Sediment depth	Bacteria	Archaea
0–5 cm	2018	*
5–10 cm	1753	*
30–55 cm	23683	3185
180–205 cm	21403	1579
230–255 cm	14391	16984
305–330 cm	17018	16984
355–380 cm	25792	15079
480–505 cm	21388	2227

*Not applicable.

Version 119 (Quast et al., 2013)]. Tree topologies were calculated with the maximum likelihood and neighbor joining algorithms as implemented in MEGA 6 (Tamura et al., 2013).

Statistical Analysis

In order to test for association/correlation between depth-wise distribution of microbial populations and specific geochemical profiles, spearman correlation (ρ) was used. This calculation was done using R software (http://www.r-project.org).

Results

Geochemical Profiles

Pore-water sulfate concentrations showed a linear decrease with depth from ∼6 mM at the top of the gravity core HE376-007-2 down to below detection limit at a depth of about 70 cm (**Figure 2B**). Concomitantly, methane concentrations increased below this depth reaching a maximum concentration of 3.2 mM around 105 cm (**Figure 2B**). Below 105 cm, methane concentrations ranged between 1 and 2 mM. Sulfide concentrations increased downward up to 350 μM at a depth of about 50 cm and were completely depleted in the methanic zone (**Figure 2B**). Pore-water ammonium gradually increased down-core from 2 mM at the top of the gravity core to more than 8 mM in the methanic zone (**Figure 2B**).

Dissolved iron was detected in the top 15 cm of sediments of the MUC core HE421-004 with a maximum of 210 μM found at 4.5 cm sediment depth (**Figure 2A**). Below the Fe^{2+}-containing

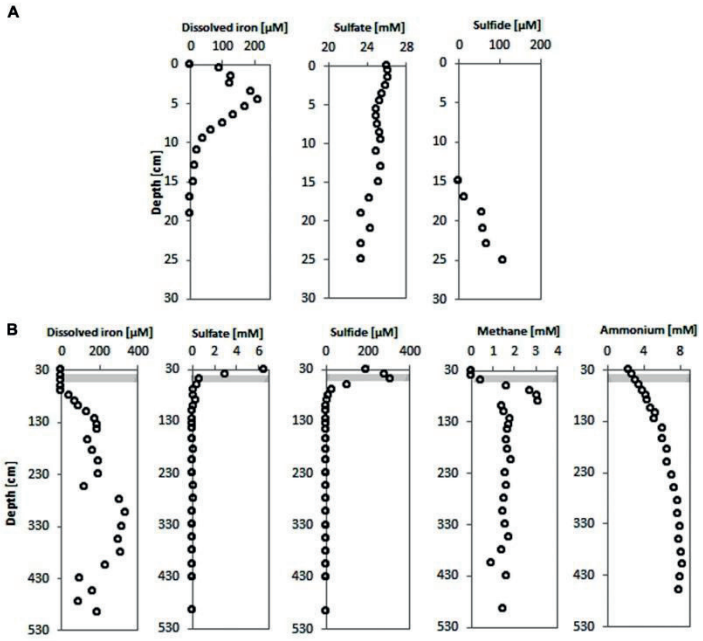

FIGURE 2 | Pore-water concentration profiles of geochemical parameters of surface and deep sediments of the Helgoland mud area. **(A)** Pore-water profiles of dissolved iron, sulfate and sulfide in surface sediments (HE421-004). **(B)** Pore-water profiles of dissolved iron, sulfate, sulfide, methane, and ammonium in deeper sediments (HE376-007-2). Loss of the upper 20–30 cm during gravity corer sampling is taken into consideration by defining the starting depths as 30 cm in geochemical profiles from deep sediment cores. Gray bar represents the SMT.

zone hydrogen sulfide increased downward to 108 μM at 25 cm depth. Sulfate showed only slightly depleted values compared to bottom water concentrations (**Figure 2A**).

In the upper part of the gravity core, dissolved iron could not be detected (**Figure 2B**). Measurements of pore-water samples from the methanic zone showed a gradual increase in dissolved iron as sulfate was depleted (**Figure 2B**). Dissolved iron in the pore-water reached highest concentrations (~330 μM) in the depth interval 280–380 cm (**Figure 2B**).

Sequential Iron Mineral Extraction

Sequential extraction of iron minerals yielded four operationally defined phases as shown in **Table 2** and **Figure 3A**. The most abundant phases are those designated FeCarb, containing adsorbed iron and iron carbonates. These fractions varied between 0.17 and 0.82 weight percent down the depth of the sediment core. FeOX1 and FeOX2 fractions containing amorphous (mainly ferrihydrite and lepidocrocite) and crystalline (mainly goethite and hematite) Fe(oxyhydr)oxides, respectively showed similar concentrations at all depths sampled. These fractions both ranged between 0.14 and 0.41 weight percent. The FeMag fraction assumed to contain mostly magnetite was least abundant and varied between 0.04 and 0.13 weight percent. In general, oxidized iron minerals were found to be most abundant around 305 cm, which corresponded to the depth with the highest dissolved iron concentrations measured.

Mössbauer Spectroscopy

In order to determine the exact nature of the reactive iron mineral(s) present in the Helgoland mud area, Mössbauer spectra of selected sediment samples [0–5 cm (MUC; HE421-004), 30–55 cm, 130–155 cm, 305–330 cm, and 380–405 cm (GC;

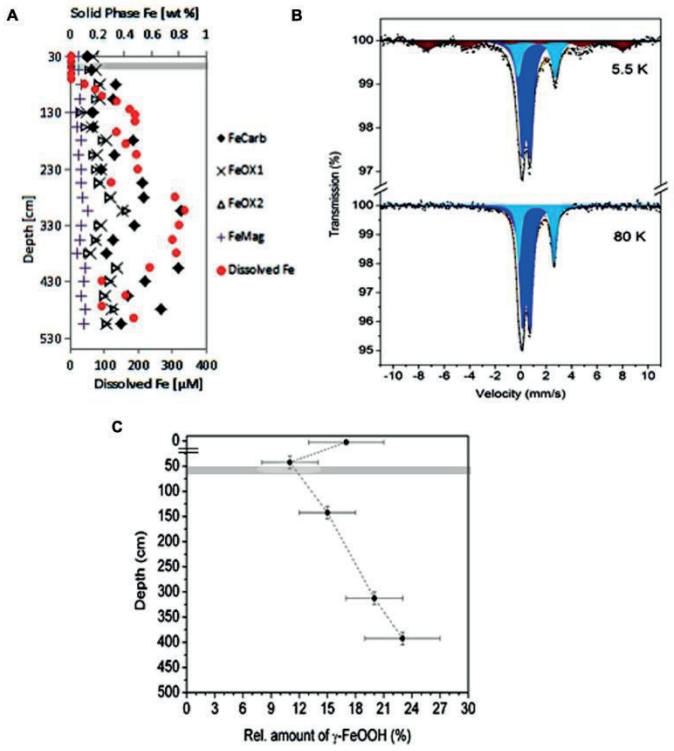

FIGURE 3 | Iron speciation in the Helgoland mud area sediment. **(A)** Sequential extraction of iron minerals in sediment core samples (HE376-007-5). Filled circles in red color, pore-water profile of dissolved iron at the same site for comparison with the distributions of solid phase iron minerals. **(B,C)** Show results of Mössbauer analysis of sediments samples from the surface and deep sediments (HE 421-004 and HE406-008). **(B)** Spectra of iron minerals detected in a sediment sample taken from 380 to 405 cm at 5.5 K (upper panel) and 80 K (lower panel). Illite, light and dark blue; lepidocrocite, brown. **(C)** Depth-wise distribution of the amount of lepidocrocite. The break in the depth axis is the demarcation between samples from surface and deep sediments. Gray bar denotes the SMT.

HE406-008)] were recorded at 5.5 K, 80 K, and 293 K. The spectrum of the sediment from the 380–405 cm depth at 5.5 K is shown in **Figure 3B**. The detailed analysis of the Mössbauer data revealed the presence of illite and lepidocrocite. Thus, two quadrupole doublets with $\delta = 0.43(1)$ mm/s, $\Delta E_Q = 0.62(2)$ mm/s and $\delta = 1.25(1)$ mm/s, $\Delta E_Q = 2.93(2)$ mm/s correspond to high-spin Fe(III) and high-spin Fe(II) of illite respectively, which is in accordance with those reported elsewhere (Murad and Wagner, 1994). The magnetic sextet fitted with $\delta = 0.34(1)$ mm/s, $H_{hf} = 467(6)$ mm/s corresponds to Fe(III) sites of lepidocrocite. The absence of magnetic sites at 80 K (**Figure 3B**) and 293 K (data not shown) spectra excludes aside from lepidocrocite, the presence of other magnetic iron oxides or hydroxides (Greenwood and Gibb, 1971) abundant enough to be detected by our system (detection limit: 3% of total Fe). Although the relation between Fe(III) and Fe(II) doublets of illite does not show significant dependence on depth (data not shown), the relative amount of the lepidocrocite does vary with depth (**Figure 3C**).

16S rRNA Gene Copy Numbers

Gene copy numbers in surface sediments, SMT and methanic zone were estimated by quantifying bacterial and archaeal 16S rRNA genes using qPCR (**Figure 4**). In general, bacterial 16S rRNA gene copies were highest in surface sediments (10^9 copies/grams wet sediment), while gene copies of archaea were highest in the SMT (10^8 copies/gram wet sediment). Archaeal gene copies were one order of magnitude lower than bacterial gene copies both in the SMT (10^7 vs. 10^8 respectively) and at each depth of the methanic zone sampled (10^6 vs. 10^7 respectively). In surface sediments, bacterial gene copies dominated archaeal gene copies by three orders of magnitude (10^9 vs. 10^6 copies/gram wet sediment). Interestingly, in the methanic zone, both archaeal and bacterial gene copies were highest at 275–350 cm corresponding to the depths of maximum pore-water iron concentrations.

Known Potential Iron Reducers in Surface Sediments

Among the sequences retrieved were those from bacteria that are known to possess iron-reducing capabilities such as members of the *Desulfuromonadales*. Relative abundances of *Desulfuromonadales* were 8.2 and 3.5% of the total bacterial community at 0–5 cm and 5–10 cm, respectively (**Figure 5**). *Desulfuromonadales* were not detected in the SMT (30–55 cm). However, they could be detected at all depths of the methanic zone sampled except at 480–505 cm. In the methanic zone, relative abundances of *Desulfuromonadales* at the depths sampled range from 0.01 to 0.1%.

Relationship of Distinct Microbial Populations to the Deep Pore-Water Iron Profile

Since members of the *Desulfobacteraceae* live in syntrophic associations with ANME populations in the reduction of sulfate coupled to the oxidation of methane (Boetius et al., 2000; Knittel and Boetius, 2009), they should be more abundant in the SMT with higher sulfate concentrations compared to the methanic zone where sulfate is almost completely depleted (**Figure 2B**). As predicted, we found that the distribution of the *Desulfobacteraceae* did correspond to the sulfate pore-water profile (**Figure 6**). We then checked for populations that corresponded to the dissolved iron profile and found that the depth-wise distribution of the JS1_SB45 candidate division showed a strong positive covariance ($\rho = 0.943$; p-value $= 0.004$; **Table 4**). Further probing of

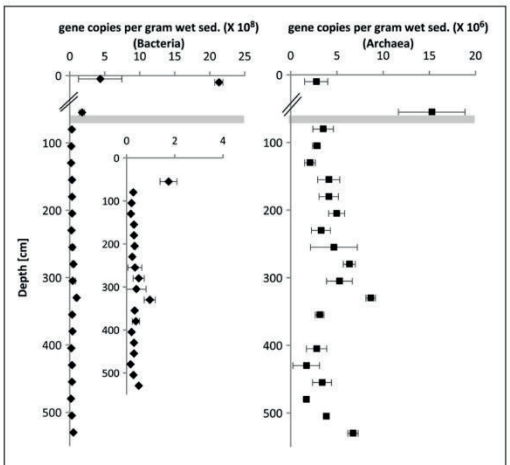

FIGURE 4 | Depth-wise estimation of bacterial and archaeal 16S rRNA gene copies based on Q-PCR. The breaks in the *x*-axes are demarcations between surface (top 10 cm, UT-2012) and deep sediments (≥30 cm, HE376-007-5). Error bars represent the SD of three technical replicates ($n = 3$). Gray bar denotes the SMT.

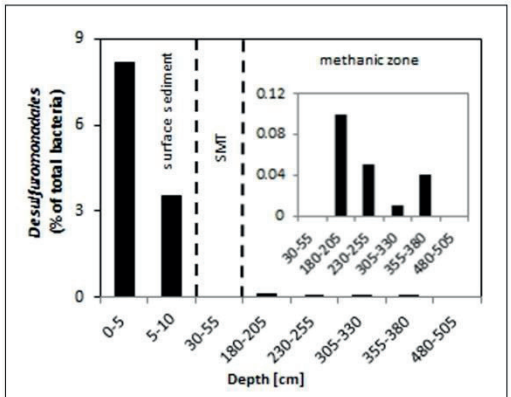

FIGURE 5 | Relative abundance of *Desulfuromonadales* in the surface (top 10 cm, core UT-2012), and deep sediments (≥30 cm, core HE376-007-5). The inset shows a magnified representation of the values from 30 to 505 cm. SMT, 30–55 cm.

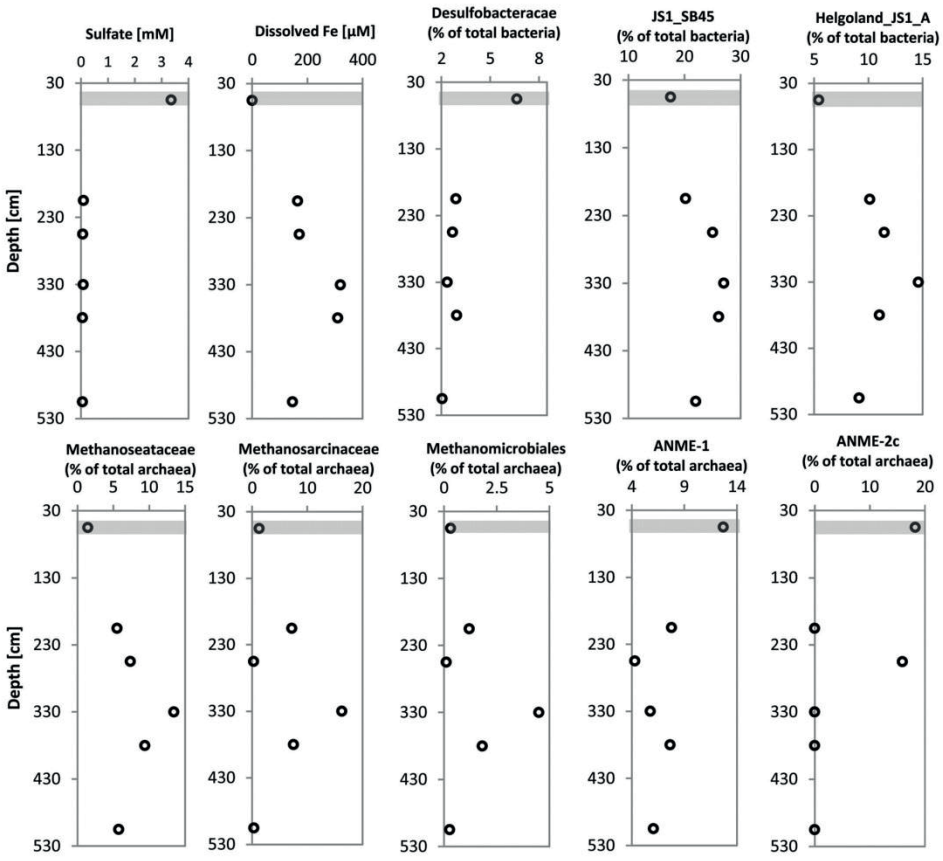

FIGURE 6 | Profiles showing the distribution of specific microbial populations from gravity core HE376-007-5 at selected depths and corresponding concentrations of pore-water sulfate and dissolved iron at the SMT and in the methanic zone (HE376-007-2).

Concentrations plotted for pore-water profiles of sulfate and dissolved iron here are mean concentrations of three depth interval of 30 cm each, for which molecular analyses were performed **(Table 3)**. Gray bar denotes the SMT.

the first five most abundant operational taxonomic units [OTUs; Helgoland_JS1_A to JS1_D (**Figure 7A**)] in the SB45 lineage at each depth sampled revealed that an OTU (Helgoland_JS1_A, **Figure 8**), closely related (100% sequence identity; ∼200 bp sequence length) to clone HB2-8-29 (DQ334649), was the main determinant of the positive relationship between the SB45 lineage and the dissolved iron profile ($\rho = 0.943$; p-value $= 0.004$). Helgoland_JS1_A was also found to mirror the distribution of members of the *Methanosaetaceae* and more specifically a clone 100% identical in sequence to the *Methanosaeta*-related clone MNO686arcE10 (GU996834; $\rho = 1.000$; p-value < 0.005). This *Methanosaeta*-related OTU is designated here as Helgoland_Meth3 (**Figures 7B** and **9**). In addition, other sequences related to methanogenic archaea, *Methanosarcinaceae* and *Methanomicrobiales,* generally showed a similar trend to *Methanosaetaceae* and Helgoland_JS1_A (**Figure 6**). However, at 230–255 cm, they were detected in very low abundances (0.3 and 0.1% respectively). Instead, the relative abundance of ANME-2c archaea sequences increased at the same depth (**Figure 6**) to ∼16% of total archaea. Both ANME-1 and ANME-2c did not show covariance with neither of Helgoland_JS1_A, *Methanosaetaceae* nor the dissolved iron profile (**Figure 6**). In the depths sampled, Helgoland_Meth7 (**Figure 8**), closely related to uncultured archaea in the phylogenetic radiation of obligate methylotrophic methanogens, *Methanohalobium* (Zhilina and Zavarzin, 1987) and methane-oxidizing ANME-3

TABLE 4 | Spearman correlations between depth-wise relative abundance of 16S rRNA genes of specific microbial populations and geochemical parameters.

	Dissolved Fe	Sulfate	JS1	*Desulfobacteraceae*	ANME-2c	ANME-1	*Methanomicrobiales*	*Methanosaetaceae*	*Methanohalobium*/ ANME-3-related archaea
Dissolved Fe	1.0000	0.8717	**0.0048**	0.6228	0.3046	0.2080	0.2080	**0.0048**	0.1108
Sulfate	−0.0857	1.0000	0.7872	0.1108	0.0341	0.7872	0.7872	0.7872	0.8717
JS1	0.9429	−0.1429	1.0000	0.3965	0.3046	0.1108	0.3287	**0.0000**	0.2657
Desulfobacteraceae	−0.2571	0.7143	−0.4286	1.0000	0.3046	0.1108	0.7872	0.3965	0.6228
ANME-2c	−0.5071	0.8452	−0.5071	0.5071	1.0000	0.7489	0.2678	0.3046	0.4679
ANME-1	−0.6000	0.1429	−0.7143	0.7143	0.1690	1.0000	0.7040	0.1108	0.8717
Methanomicrobiales	0.6000	−0.1429	0.4857	0.1429	−0.5409	0.2000	1.0000	0.3287	**0.0048**
Methanosaetaceae	0.9429	−0.1429	1.0000	−0.4286	−0.5071	−0.7143	0.4857	1.0000	0.2657
Methanohalobium/ ANME-3-related archaea	0.7143	0.0857	0.5429	0.2571	−0.3719	0.0857	0.9429	0.5429	1.0000

p-values are the values given on right of the "1.000" (in red) diagonal, while the correlation coefficients are displayed to the left. Significant correlations ($p < 0.05$) are the values printed in bold.

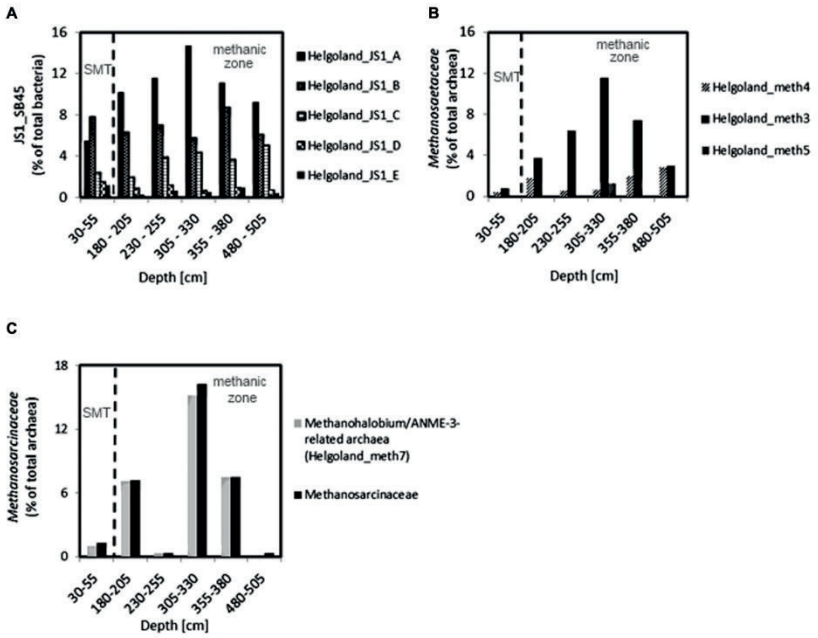

FIGURE 7 | Depth-wise distribution of selected JS1 bacteria, and archaeal OTUs. (A) Relative abundances of most abundant JS1 bacteria OTUs. **(B)** Relative abundance of *Methanosaeta*-related OTUs. **(C)** Distribution of *Methanohalobium*/ANME-3-related archaea (Helgoland_meth7) as the dominant members of *Methanosarcinaceae*. Helgoland_JS1_A, Helgoland_meth3, and Helgoland_meth7 follow the similar depth-wise distribution patterns and show highest abundance at 305–330 cm (dissolved Fe peak).

(**Figure 9**; confirmed using cloned nearly full length 16S rRNA sequences (~1400 bps); see supplementary data for details, Figure S1) almost exclusively dominated sequence reads ascribed to *Methanosarcinaceae* (**Figure 7C**).

Discussion

The observation of elevated concentrations of dissolved iron in the methanic zone of marine sediments has been a matter of

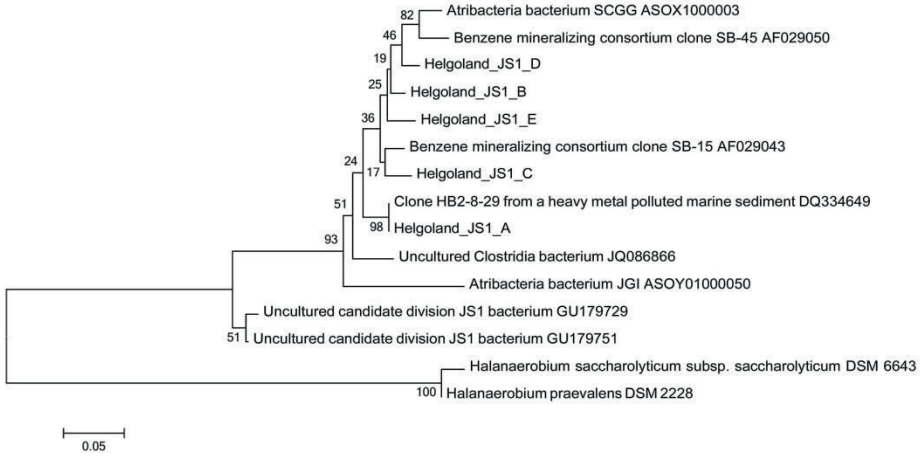

FIGURE 8 | Neighbor-joining tree of 16S rRNA gene sequences showing the phylogenetic affiliation of the most abundant JS1 bacteria sequences (Helgoland_JS1_A-E). Helgoland_JS1_A OTU is closely related to clone from a heavy metal-contaminated site (DQ334649). Scale bar represents 5 base substitutions per 100 nucleotides. *Halanaerobium saccharolyticum* and *H. praevalens* were used as outgroup references.

interest in subsurface geomicrobiology for some time, and it is still not known which microbial population might be involved in the reduction of iron. Here, we find that the distribution of members of the JS1 candidate division, methanogens, and *Methanohalobium*/ANME-3-related archaea co-varies with the profile of pore-water iron, which suggests that these microorganisms might be involved in iron cycling in the methanic zone of the Helgoland mud area.

A number of mechanisms of iron cycling in the methanic zone of marine sediments are discussed till date. One possibility is cryptic sulfur cycling, involving a chemical reaction of downward-diffusing sulfide from the SMT with buried iron (III) minerals, as suggested for Aarhus Bay sediments (Holmkvist et al., 2011). However, in the Helgoland mud sediments this explanation is highly unlikely, because the "sulfidization" front (~75 cm depths bsf) is too distant from the depths of maximum pore-water iron concentrations. Sulfide is completely depleted at 75 cm depths while the maximum of pore-water iron occurs between 275 and 350 cm (**Figure 2B**). This suggests that a different mechanism might be responsible for iron reduction observed below the SMT.

Dissimilatory iron reducers such as members of the *Desulfuromonadales* are present in the methanic zone and are potential contributors to organoclastic or hydrogenotrophic reduction of iron (III) minerals therein. Unlike in the surface sediments, where they may contribute largely to iron reduction (**Figure 2A**), their relative abundance is low in deeper sediments (**Figure 5**). Based on qPCR-derived bacterial gene copies (10^7 gene copies per gram wet sediment; **Figure 4**) and their pyrosequencing-derived relative abundances in the methanic zone (0.01–0.1% of total bacteria; **Figure 5**), they were estimated to account for about 10^3–10^4 gene copies per gram wet sediment at the depths sampled. Assuming those cells would be viable and active *in situ*, their contribution to iron reduction in the methanic zone of our study site cannot be disregarded considering the relatively large amounts of reactive iron minerals such as lepidocrocite as revealed by Mössbauer spectroscopy (**Figures 3B,C**) and sequential iron extraction (**Figure 3A**) that have been deposited during periods of higher sedimentation (750–1000 years ago) and the absence of free sulfide at the depths below 75 cm. These conditions could allow accumulation of copious amounts of dissolved iron even if iron reduction had occurred at low rates. Nevertheless, the distribution of *Desulfuromonadales* did not correspond to the dissolved iron profiles and are thus most likely these bacteria were not the main organisms reducing iron minerals in the methanic zone.

In the deeper subsurface sediment, the distribution of candidate division JS1 (SB45 lineage) bacteria, however, co-varies with the dissolved iron profile and their direct or indirect linkage to iron reduction seems likely. The main driver of this covariance, Helgoland_JS1_A OTU, is closely related to clone HB2-8-29 (DQ334649), which originated from an industrial harbor basin contaminated with heavy metals and where iron reduction was the dominant metal transformation process (Toes et al., 2008; **Figure 8**).

JS1 populations have previously been found in many methane-containing sediments: in methanic subsurface sediments (Webster et al., 2004, 2007; Parkes et al., 2005, 2007), methane seeps and gas hydrate sites (Inagaki et al., 2006; Siegert et al., 2011b; Chevalier et al., 2013; Lee et al., 2013), and mud volcanoes (Pachiadaki et al., 2011). However, there exists only little knowledge regarding the metabolism of these bacteria.

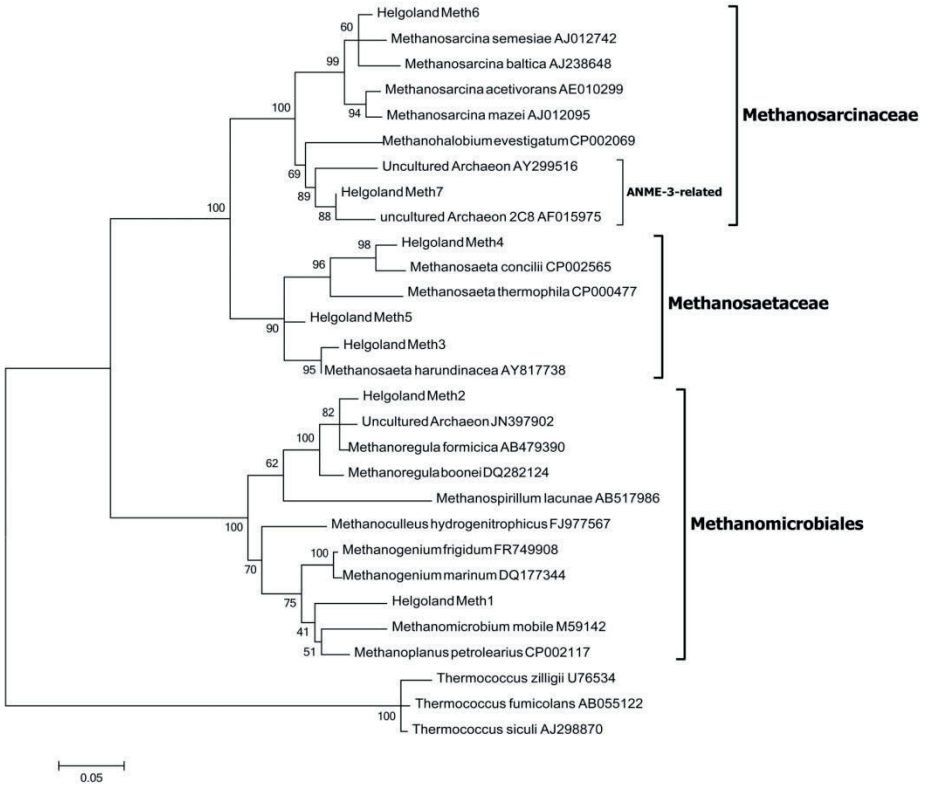

FIGURE 9 | Maximum likelihood tree of 16S rRNA gene sequences showing the phylogenetic affiliations of the methanogen-related sequences detected in the Helgoland mud area. Scale bar represents 5 base substitutions per 100 nucleotides. *Thermococcus zilligii*, *T. fumicolans*, and *T. siculi* served as outgroup references.

JS1 bacteria appear to be heterotrophic based on their ability to utilize acetate and glucose under low sulfate conditions as revealed by stable isotope probing and enrichment studies (Webster et al., 2006, 2011). Emerging lines of evidence from single cell genomics have also predicted a saccharolytic and fermentative lifestyle for some bacteria in the OP9/JS1 lineage (Dodsworth et al., 2013). As evident from the observed depth-wise increase in ammonium concentrations (**Figure 2B**), degradation of organic matter seems to occur in subsurface sediments of the Helgoland mud area. Members of the JS1 (SB45) lineage have been shown to persist in a sulfate-reducing benzene-mineralizing enrichment culture over 3 years (Phelps et al., 1998). Close relatives of the major JS1 (SB45) member detected in the sediments of our study site (Helgoland_JS1_A), have been found in hydrocarbon-contaminated environments (**Figure 8**). For example, North Sea sediments from which clone HB2-8-29 (DQ334649) was retrieved were contaminated with polychlorinated biphenyl and polyaromatic hydrocarbons (Toes et al., 2008). Clone 2_68_H10-1_b (JQ086866; **Figure 8**) was also detected in hydrocarbon contaminated aquifers (Tischer et al., 2013). One could speculate that some members of the JS1 group may generally play a role in the degradation of hydrocarbon compounds. Moreover, specific members of both JS1 and the *Methanosarcinales* appear to co-exist in marine sediments (Mediterranean Sea, Gulf of Mexico) based on a co-occurrence survey (Chaffron et al., 2010). In the same vein, co-occurrence networks between JS1 bacteria and members of the *Methanomicrobiales* were detected (Gies et al., 2014). The covariance of members of the JS1 bacteria (Helgoland_JS1_A) and specific *Methanosaetaceae* populations (Helgoland_meth3) observed in the sediments studied here (**Figures 7A,B**) suggests that certain members of JS1 bacteria and methanogens interact metabolically possibly via the anaerobic microbial food chain (Schink, 1997).

It has been suggested that AOM may be coupled to the reduction of metal oxides in several aquatic environments: for example, in marine sediments (Beal et al., 2009; Wankel et al., 2012; Egger et al., 2014; Riedinger et al., 2014), freshwater/limnic sediments (Sivan et al., 2011; Segarra et al., 2013). Methanotrophic archaea (ANME) populations are known to be involved in the mediation of anaerobic methane oxidation (Hinrichs et al., 1999; Boetius et al., 2000). The detection of ANME-1, ANME-2c, and uncultured *Methanosarcinaceae* (**Figure 6**), which are mostly related to *Methanohalobium*/ANME-3 (**Figure 7C**), suggests a potential for AOM at the SMT and within the methanic zone of the Helgoland mud area. While the distribution of ANME-1 and ANME-2c populations over depth did not match the dissolved iron profile, *Methanohalobium*/ANME-3-related sequences were highly abundant around the peak of dissolved iron (**Figures 6** and **7C**). Thus, anaerobic oxidation of methane or methylated one-carbon compounds may be coupled to iron reduction in the methanic zone of our study site. The involvement of ANME-3-related populations in metal reduction in incubations with sediments from the Eel River Basin has been previously suggested by Beal et al. (2009).

Similar depth-wise distribution profiles of *Methanomicrobiales*, *Methanosaetaceae*, and *Methanohalobium*/ANME-3-related *Methanosarcinaceae* (Helgoland_Meth7; **Figure 9**) hint at a possible co-occurrence of methanogenesis and anaerobic oxidation of methane in subsurface sediments of the Helgoland mud area. Evidence for co-occurrence of both processes has been recently reported in sediments from the Bothnian Sea, a site also characterized by the deposition of high amounts of organic matter, non-sulfidic and high dissolved iron in the methanic zone (Egger et al., 2014). However, microorganisms have not been identified from this site yet. *Methanomicrobiales* and *Methanosaetaceae* harbor obligate hydrogenotrophic and acetotrophic methanogens, respectively. Some methanogenic archaea are capable of coupling hydrogen and acetate oxidation directly to iron reduction in pure culture (Bond and Lovley, 2002; van Bodegom et al., 2004; Liu et al., 2011; Zhang et al., 2012; Yamada et al., 2014). Addition of ferrihydrite as a potential electron acceptor increased *mcrA* gene copy numbers of methanogens in incubations with heavy metal- and hydrocarbon-contaminated mud sediments from Zeebrugge harbor basin (Siegert et al., 2011a). Transient conservation of energy from ferrihydrite reduction by Rice Cluster I methanogens from rice field soils has also been suggested (Lueders and Friedrich, 2002). In the presence of high amounts of reactive iron minerals, methanogenesis can be directly inhibited as electrons are diverted to iron reduction (Bond and Lovley, 2002; van Bodegom et al., 2004). The Helgoland mud area is characterized by high sedimentation rates (Hebbeln et al., 2003) and consequently high burial rates of organic matter and iron minerals. Such conditions may cause some of the electrons for methanogenesis to be shunted to oxidized iron minerals implicating methanogenic archaea to be involved in iron reduction in the subsurface sediments of our study site.

Conclusion

Although the actual mechanisms guiding the interplay of JS1 metabolism, iron reduction, methanogenic, and ANME-3-related archaea activities are not yet clear, our results suggest a close relationship amongst members of the JS1 bacteria, specific methanogens and *Methanohalobium*/ANME-3-related archaea. These associations may also have an influence on iron cycling in subsurface sediments of the Helgoland mud area and other high-accumulation depositional environments such as the Amazon Fan (Flood et al., 1995; Kasten et al., 1998), Argentine Basin (Hensen et al., 2003; Riedinger et al., 2005, 2014), Zambesi Fan (März et al., 2008), and Bothnian Sea (Slomp et al., 2013; Egger et al., 2014), where high sedimentation rates enable the preservation and burial of reactive iron oxides to greater depths. In addition, our results add to the evidences that microorganisms are important in shaping the geochemical environment in sub-seafloor sediments.

Acknowledgments

This study was supported by the Research Center/Cluster of Excellence 'The Ocean in the Earth System' (MARUM) funded by the Deutsche Forschungsgemeinschaft (DFG) and by the University of Bremen. The authors thank the captain, crew, and scientists of R/V HEINCKE expeditions HE376, HE406, and HE421. We also thank the captain and crew of RV UTHÖRN for their help during UT-2012 sampling. In addition, we thank Benjamin Löffler and Roi Martinez for their help with onboard geochemical measurements and creation of the map of the Helgoland mud area, respectively. We acknowledge additional funding by the Helmholtz Association (Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research) in the framework of the research programs PACES I and PACES II. BC gratefully acknowledges the FCT/COMPETE PEst-C/FIS/UI0036/2011 project.

Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmicb.2015.00365/abstract

References

Beal, E. J., House, C. H., and Orphan, V. J. (2009). Manganese- and iron-dependent marine methane oxidation. *Science* 325, 184–187. doi: 10.1126/science.1169984

Boetius, A., Ravenschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., et al. (2000). A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407, 623–626. doi: 10.1038/35036572

Bond, D. R., and Lovley, D. R. (2002). Reduction of Fe(III) oxide by methanogens in the presence and absence of extracellular quinones. *Environ. Microbiol.* 4, 115–124. doi: 10.1046/j.1462-2920.2002.00279.x

Canfield, D. E., and Thamdrup, B. (2009). Towards a consistent classification scheme for geochemical environments, or, why we wish the term "suboxic" would go away. *Geobiology* 7, 385–392. doi: 10.1111/j.1472-4669.2009.00214.x

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7, 335–336. doi: 10.1038/nmeth.f.303

Chaffron, S., Rehrauer, H., Pernthaler, J., and von Mering, C. (2010). A global network of coexisting microbes from environmental and whole-genome sequence data. *Genome Res.* 20, 947–959. doi: 10.1101/gr.104521.109

Chevalier, N., Bouloubassi, I., Birgel, D., Taphanel, M.-H., and López-García, P. (2013). Microbial methane turnover at Marmara Sea cold seeps: a combined 16S rRNA and lipid biomarker investigation. *Geobiology* 11, 55–71. doi: 10.1111/gbi.12014

Cline, J. (1969). Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol. Oceanogr.* 14, 454–458. doi: 10.4319/lo.1969.14.3.0454

Coates, J. D., Phillips, E. J., Lonergan, D. J., Jenter, H., and Lovley, D. R. (1996). Isolation of *Geobacter* species from diverse sedimentary environments. *Appl. Environ. Microbiol.* 62, 1531–1536.

Dickens, G. R., Kölling, M., Smith, D. C., and Schnieders, L. (2007). Rhizon sampling of pore waters on scientific drilling expeditions: an example from the IODP expedition 302, Arctic Coring Expedition (ACEX). *Sci. Drill.* 4, 22–25. doi: 10.5194/sd-4-22-2007

D'Hondt, S., Jørgensen, B. B., Miller, D. J., Batzke, A., Blake, R., Cragg, B. A., et al. (2004). Distributions of microbial activities in deep subseafloor sediments. *Science* 306, 2216–2221. doi: 10.1126/science.1101155

Dobbin, P. S., Carter, J. P., Garcia-Salamanca San Juan, C., von Hobe, M., Powell, A. K., and Richardson, D. J. (1999). Dissimilatory Fe(III) reduction by *Clostridium beijerinckii* isolated from freshwater sediment using Fe(III) maltol enrichment. *FEMS Microbiol. Lett.* 176, 131–138. doi: 10.1111/j.1574-6968.1999.tb13653.x

Dodsworth, J. A., Blainey, P. C., Murugapiran, S. K., Swingley, W. D., Ross, C. A., Tringe, S. G., et al. (2013). Single-cell and metagenomic analyses indicate a fermentative and saccharolytic lifestyle for members of the OP9 lineage. *Nat. Commun.* 4, 1854. doi: 10.1038/ncomms2884

Durbin, A. M., and Teske, A. (2011). Microbial diversity and stratification of South Pacific abyssal marine sediments. *Environ. Microbiol.* 13, 3219–3234. doi: 10.1111/j.1462-2920.2011.02544.x

Egger, M., Rasigraf, O., Sapart, C. J., Jilbert, T., Jetten, M. S. M., Röckmann, T., et al. (2014). Iron-mediated anaerobic oxidation of methane in brackish coastal sediments. *Environ. Sci. Technol.* 277, 277–283. doi: 10.1021/es503663z

Flood, R. D., Piper, D., and Klaus, A. (1995). Initial Reports. *Proc. Ocean Drill. Progam.* 155. doi: 10. 2973/odp.proc.ir.155.1995

Gies, E. A., Konwar, K. M., Beatty, J. T., and Hallam, S. J. (2014). Illuminating microbial dark matter in Meromictic Sakinaw Lake. *Appl. Environ. Microbiol.* 80, 6807–6818. doi: 10.1128/AEM.01774-14

Greenwood, N., and Gibb, T. (1971). *Mössbauer Spectroscopy.* London: Chapman and Hall Ltd, 240. doi: 10.1007/978-94-009-5697-1

Grosskopf, R., Janssen, P. H., and Liesack, W. (1998). Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. *Appl. Environ. Microbiol.* 64, 960–969.

Hall, P., and Aller, R. (1992). Rapid, small-volume, flow injection analysis for ΣCO2 and NH4+ in marine and freshwaters. *Limnol. Oceanogr.* 37, 1113–1119. doi: 10.4319/lo.1992.37.5.1113

Harrison, B. K., Zhang, H., Berelson, W., and Orphan, V. J. (2009). Variations in archaeal and bacterial diversity associated with the sulfate-methane transition zone in continental margin sediments (Santa Barbara Basin, California). *Appl. Environ. Microbiol.* 75, 1487–1499. doi: 10.1128/AEM.01812-08

Hebbeln, D., Scheurle, C., and Lamy, F. (2003). Depositional history of the Helgoland mud area, German Bight, North Sea. *Geo Mar. Lett.* 23, 81–90. doi: 10.1007/s00367-003-0127-0

Hensen, C. H. H., Zabel, M., Pfeifer, K., Schwenk, T., Kasten, S., Riedinger, N., et al. (2003). Control of sulfate pore-water profiles by sedimentary events and the significance of anaerobic oxidation of methane for the burial of sulfur in marine sediments. *Geochim. Cosmochim. Acta* 67, 2631–2647. doi: 10.1016/S0016-7037(00)00199–196

Hinrichs, K. U., Hayes, J. M., Sylva, S. P., Brewer, P. G., and DeLong, E. F. (1999). Methane-consuming archaebacteria in marine sediments. *Nature* 398, 802–805. doi: 10.1038/19751

Holmkvist, L., Ferdelman, T. G., and Jørgensen, B. B. (2011). A cryptic sulfur cycle driven by iron in the methane zone of marine sediment (Aarhus Bay, Denmark). *Geochim. Cosmochim. Acta* 75, 3581–3599. doi: 10.1016/j.gca.2011.03.033

Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., et al. (2006). Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proc. Natl. Acad. Sci. U.S.A.* 103, 2815–2820. doi: 10.1073/pnas.0511033103

Jorgensen, S. L., Hannisdal, B., Lanzén, A., Baumberger, T., Flesland, K., Fonseca, R., et al. (2012). Correlating microbial community profiles with geochemical data in highly stratified sediments from the Arctic Mid-Ocean Ridge. *Proc. Natl. Acad. Sci.* 109, 2846–2855. doi: 10.1594/pnas.12075740109

Kasten, S., Freudenthal, T., Gingele, F. X., and Schulz, H. D. (1998). Simultaneous formation of iron-rich layers at different redox boundaries in sediments of the Amazon deep-sea fan. *Geochim. Cosmochim. Acta* 62, 2253–2264. doi: 10.1016/S0016-7037(98)00093-3

Knittel, K., and Boetius, A. (2009). Anaerobic oxidation of methane: progress with an unknown process. *Annu. Rev. Microbiol.* 63, 311–334. doi: 10.1146/annurev.micro.61.080706.093130

Lagarec, K., and Rancourt, D. G. (1997). Extended Voigt-based analytic lineshape method for determining N-dimensional correlated hyperfine parameter distributions in Mössbauer spectroscopy. *Nucl. Instrum. Methods Phys. Res. B* 129, 266–280. doi: 10.1016/S0168-583X(97)00284-X

Lane, D. J. (1991). "16S/23S rRNA Sequencing," in *Nucleic Acid Techniques in Bacterial Systematics*, eds E. Stackebrandt and M. Goodfellow (New York, NY: John Wiley and Sons), 115–175.

Lee, J.-W., Kwon, K. K., Azizi, A., Oh, H.-M., Kim, W., Bahk, J.-J., et al. (2013). Microbial community structures of methane hydrate-bearing sediments in the Ulleung Basin, East Sea of Korea. *Mar. Petrol. Geol.* 47, 136–146. doi: 10.1016/j.marpetgeo.2013.06.002

Liu, D., Dong, H., Bishop, M. E., Wang, H., Agrawal, A., Tritschler, S., et al. (2011). Reduction of structural Fe(III) in nontronite by methanogen *Methanosarcina barkeri*. *Geochim. Cosmochim. Acta* 75, 1057–1071. doi: 10.1016/j.gca.2010.11.009

Lloyd, K. G., Lapham, L., and Teske, A. (2006). An anaerobic methane-oxidizing community of ANME-1b archaea in hypersaline Gulf of Mexico sediments. *Appl. Environ. Microbiol.* 72, 7218–7230. doi: 10.1128/AEM.00886–886

Lovley, D. R., and Phillips, E. J. (1986). Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Appl. Environ. Microbiol.* 51, 683–689.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, et al. (2004). ARB: a software environment for sequence data. *Nucleic Acids Res.* 32, 1363–1371. doi: 10.1093/nar/gkh293

Lueders, T., and Friedrich, M. (2000). Archaeal population dynamics during sequential reduction processes in rice field soil. *Appl. Environ. Microbiol.* 66, 2732–2742. doi: 10.1128/AEM.66.7.2732-2742.2000

Lueders, T., and Friedrich, M. W. (2002). Effects of amendment with ferrihydrite and gypsum on the structure and activity of methanogenic populations in rice field Soil. *Appl. Environ. Microbiol.* 68, 2484–2494. doi: 10.1128/AEM.68.5.2484

Lueders, T., Manefield, M., and Friedrich, M. W. (2004). Enhanced sensitivity of DNA- and rRNA-based stable isotope probing by fractionation and quantitative analysis of isopycnic centrifugation gradients. *Environ. Microbiol.* 6, 73–78. doi: 10.1046/j.1462-2920.2003.00536.x

März, C., Hoffmann, J., Bleil, U., de Lange, G. J., and Kasten, S. (2008). Diagenetic changes of magnetic and geochemical signals by anaerobic methane oxidation in sediments of the Zambezi deep-sea fan (SW Indian Ocean). *Mar. Geol.* 255, 118–130. doi: 10.1016/j.margeo.2008.05.013

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E. M., Kubal, M., et al. (2008). The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics* 9:386. doi: 10.1186/1471-2105-9–386

Murad, E., and Wagner, U. (1994). The mössbauer spectrum of illite. *Clay Miner.* 29, 1–10. doi: 10.1180/claymin.1994.029.1.01

Muyzer, G., de Waal, E. C., and Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* 59, 695–700.

Nickel, M., Vandieken, V., Brüchert, V., and Jørgensen, B. B. (2008). Microbial Mn(IV) and Fe(III) reduction in northern Barents Sea sediments under different conditions of ice cover and organic carbon deposition. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 55, 2390–2398. doi: 10.1016/j.dsr2.2008.05.003

Pachiadaki, M. G., Kallionaki, A., Dählmann, A., De Lange, G. J., and Kormas, K. A. (2011). Diversity and spatial distribution of prokaryotic communities along a sediment vertical profile of a deep-sea mud volcano. *Microb. Ecol.* 62, 655–668. doi: 10.1007/s00248-011-9855–9852

Parkes, R. J., Cragg, B. A., Banning, N., Brock, F., Webster, G., Fry, J. C., et al. (2007). Biogeochemistry and biodiversity of methane cycling in subsurface marine sediments (Skagerrak, Denmark). *Environ. Microbiol.* 9, 1146–1161. doi: 10.1111/j.1462-2920.2006.01237.x

Parkes, R. J., Webster, G., Cragg, B. A., Weightman, A. J., Newberry, C. J., Ferdelman, T. G., et al. (2005). Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. *Nature* 436, 390–394. doi: 10.1038/nature03796

Phelps, C. D., Kerkhof, L. J., and Young, L. Y. (1998). Molecular characterization of a sulfate-reducing consortium which mineralizes benzene. *FEMS Microbiol. Ecol.* 27, 269–279. doi: 10.1111/j.1574-6941.1998.tb00543.x

Poulton, S., and Canfield, D. (2005). Development of a sequential extraction procedure for iron: implications for iron partitioning in continentally derived particulates. *Chem. Geol.* 214, 209–221. doi: 10.1016/j.chemgeo.2004.09.003

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596. doi: 10.1093/nar/gks1219

Quince, C., Lanzen, A., Davenport, R. J., and Turnbaugh, P. J. (2011). Removing noise from pyrosequenced amplicons. *BMC Bioinformatics* 12:38. doi: 10.1186/1471-2105-12–38

Riedinger, N., Formolo, M. J., Lyons, T. W., Henkel, S., Beck, A., and Kasten, S. (2014). An inorganic geochemical argument for coupled anaerobic oxidation of methane and iron reduction in marine sediments. *Geobiology* 12, 172–181. doi: 10.1111/gbi.12077

Riedinger, N., Pfeifer, K., Kasten, S., Garming, J. F. L., Vogt, C., and Hensen, C. (2005). Diagenetic alteration of magnetic signals by anaerobic oxidation of methane related to a change in sedimentation rate. *Geochim. Cosmochim. Acta* 69, 4117–4126. doi: 10.1016/j.gca.2005.02.004

Roden, E. E., and Lovley, D. R. (1993). Dissimilatory Fe(III) reduction by the marine microorganism *Desulfuromonas acetoxidans*. *Appl. Environ. Microbiol.* 59, 734–742.

Schink, B. (1997). Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol. Mol. Biol. Rev.* 61, 262–280.

Seeberg-Elverfeldt, J., Schlüter, M., Feseker, T., and Kölling, M. (2005). Rhizon sampling of porewaters near the sediment-water interface of aquatic systems. *Limnol. Oceanogr. Methods* 3, 361–371. doi: 10.4319/lom.2005.3.361

Segarra, K. E. A., Comerford, C., Slaughter, J., and Joye, S. B. (2013). Impact of electron acceptor availability on the anaerobic oxidation of methane in coastal freshwater and brackish wetland sediments. *Geochim. Cosmochim. Acta* 115, 15–30. doi: 10.1016/j.gca.2013.03.029

Siegert, M., Cichocka, D., Herrmann, S., Gründger, F., Feisthauer, S., Richnow, H. -H., et al. (2011a). Accelerated methanogenesis from aliphatic and aromatic hydrocarbons under iron- and sulfate-reducing conditions. *FEMS Microbiol. Lett.* 315, 6–16. doi: 10.1111/j.1574-6968.2010.02165.x

Siegert, M., Krüger, M., Teichert, B., Wiedicke, M., and Schippers, A. (2011b). Anaerobic oxidation of methane at a marine methane seep in a forearc sediment basin off sumatra, Indian Ocean. *Front. Microbiol.* 2:249. doi: 10.3389/fmicb.2011.00249

Sivan, O., Adler, M., Pearson, A., Gelman, F., Bar-Or, I., John, S. G., et al. (2011). Geochemical evidence for iron-mediated anaerobic oxidation of methane. *Limnol. Oceanogr.* 56, 1536–1544. doi: 10.4319/lo.2011.56.4.1536

Slomp, C. P., Mort, H. P., Jilbert, T., Reed, D. C., Gustafsson, B. G., and Wolthers, M. (2013). Coupled dynamics of iron and phosphorus in sediments of an oligotrophic coastal basin and the impact of anaerobic oxidation of methane. *PLoS ONE* 8:e62386. doi: 10.1371/journal.pone.0062386

Takai, K., and Horikoshi, K. (2000). Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. *Appl. Environ. Microbiol.* 66, 5066–5072. doi: 10.1128/AEM.66.11.5066-5072.2000

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30, 2725–2729. doi: 10.1093/molbev/mst197

Tischer, K., Kleinsteuber, S., Schleinitz, K. M., Fetzer, I., Spott, O., Stange, F., et al. (2013). Microbial communities along biogeochemical gradients in a hydrocarbon-contaminated aquifer. *Environ. Microbiol.* 15, 2603–2615. doi: 10.1111/1462-2920.12168

Toes, A.-C. M., Finke, N., Kuenen, J. G., and Muyzer, G. (2008). Effects of deposition of heavy-metal-polluted harbor mud on microbial diversity and metal resistance in sandy marine sediments. *Arch. Environ. Contam. Toxicol.* 55, 372–85. doi: 10.1007/s00244-008-9135-4

van Bodegom, P. M., Scholten, J. C. M., and Stams, A. J. M. (2004). Direct inhibition of methanogenesis by ferric iron. *FEMS Microbiol. Ecol.* 49, 261–268. doi: 10.1016/j.femsec.2004.03.017

Vandieken, V., Mussmann, M., Niemann, H., and Jørgensen, B. B. (2006a). *Desulfuromonas svalbardensis* sp. nov. and *Desulfuromusa ferrireducens* sp. nov., psychrophilic, Fe(III)-reducing bacteria isolated from Arctic sediments, Svalbard. Int. *J. Syst. Evol. Microbiol.* 56, 1133–1139. doi: 10.1099/ijs.0.63639-0

Vandieken, V., Nickel, M., and Jørgensen, B. (2006b). Carbon mineralization in Arctic sediments northeast of Svalbard: Mn(IV) and Fe(III) reduction as principal anaerobic respiratory pathways. *Mar. Ecol. Prog. Ser.* 322, 15–27. doi: 10.3354/meps322015

Vandieken, V., and Thamdrup, B. (2013). Identification of acetate-oxidizing bacteria in a coastal marine surface sediment by RNA-stable isotope probing in anoxic slurries and intact cores. *FEMS Microbiol. Ecol.* 84, 373–386. doi: 10.1111/1574-6941.12069

Wallmann, K., Aloisi, G., Haeckel, M., Tishchenko, P., Pavlova, G., Greinert, J., et al. (2008). Silicate weathering in anoxic marine sediments. *Geochim. Cosmochim. Acta* 72, 2895–2918. doi: 10.1016/j.gca.2008.03.026

Wang, Y., and Qian, P.-Y. (2009). Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. *PLoS ONE* 4:e7401. doi: 10.1371/journal.pone.0007401

Wankel, S. D., Adams, M. M., Johnston, D. T., Hansel, C. M., Joye, S. B., and Girguis, P. R. (2012). Anaerobic methane oxidation in metalliferous hydrothermal sediments: influence on carbon flux and decoupling from sulfate reduction. *Environ. Microbiol.* 14, 2726–2740. doi: 10.1111/j.1462-2920.2012.02825.x

Webster, G., Parkes, R. J., Fry, J. C., and Weightman, A. J. (2004). Widespread occurrence of a novel division of bacteria identified by 16S rRNA gene sequences originally found in deep marine sediments. *Appl. Environ. Microbiol.* 70, 5708–5713. doi: 10.1128/AEM.70.9.5708

Webster, G., Sass, H., Cragg, B. A., Gorra, R., Knab, N. J., Green, C. J., et al. (2011). Enrichment and cultivation of prokaryotes associated with the sulphate-methane transition zone of diffusion-controlled sediments of Aarhus Bay, Denmark, under heterotrophic conditions. *FEMS Microbiol. Ecol.* 77, 248–263. doi: 10.1111/j.1574-6941.2011.01109.x

Webster, G., Watt, L. C., Rinna, J., Fry, J. C., Evershed, R. P., Parkes, R. J., et al. (2006). A comparison of stable-isotope probing of DNA and phospholipid fatty acids to study prokaryotic functional diversity in sulfate-reducing marine sediment enrichment slurries. *Environ. Microbiol.* 8, 1575–1589. doi: 10.1111/j.1462-2920.2006.01048.x

Webster, G., Yarram, L., Freese, E., Köster, J., Sass, H., Parkes, R. J., et al. (2007). Distribution of candidate division JS1 and other bacteria in tidal sediments of the German Wadden Sea using targeted 16S rRNA gene PCR-DGGE. *FEMS Microbiol. Ecol.* 62, 78–89. doi: 10.1111/j.1574-6941.2007.00372.x

Yamada, C., Kato, S., Kimura, S., Ishii, M., and Igarashi, Y. (2014). Reduction of Fe(III) oxides by phylogenetically and physiologically diverse thermophilic methanogens. *FEMS Microbiol. Ecol.* 89, 637–645. doi: 10.1111/1574-6941.12365

Zhang, J., Dong, H., Liu, D., Fischer, T. B., Wang, S., and Huang, L. (2012). Microbial reduction of Fe(III) in illite–smectite minerals by methanogen *Methanosarcina mazei*. *Chem. Geol.* 292, 35–44. doi: 10.1016/j.chemgeo.2011.11.003

Zhilina, T. N., and Zavarzin, G. A. (1987). *Methanohalobium evestigatus*, n. gen., n. sp. the extremely halophilic methanogenic Archaebacterium. *Dokl. Akad. Nauk SSSR* 293, 464–468.

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.

Supplementary Material

Distinct microbial populations are tightly linked to the profile of dissolved iron in the methanic sediments of the Helgoland mud area, North Sea

Oluwatobi Oni[1,2], Tetsuro Miyatake[1], Sabine Kasten[2,3], Tim Richter-Heitmann[1], David Fischer[2,3], Laura Wagenknecht[3], Ajinkya Kulkarni[1], Mathias Blumers[4], Sergii I. Shylin[4,6],Vadim Ksenofontov[4], Benilde F.O. Costa[5], Göstar Klingelhöfer[4], Michael W. Friedrich[1,2]

1. SUPPLEMENTARY METHOD

ARCHAEAL 16S rRNA GENE CLONING AND SEQUENCING

Cloning was done to obtain long archaeal 16S rRNA genes (ca. 1.3 to 1.4 kbps) in order to validate the phylogenetic placement of methanogen sequences provided in Fig. 9. DNA extract from two depths of the sediment 55 cm - 80 cm and 330 cm - 355 cm (Fe^{2+} maximum) were used for PCR amplification of archaeal 16S rRNA genes with primer pairs Arch109F

(5' ACKGCTCAGTAACACGT 3') (Großkopf et al., 1998) and Arch1492R (5' GGCTACCTTGTTACGACTT 3') (modified from Miyashita et al., 2009)). Purified PCR products were cloned into pGEM-T vector system (Promega, Mannheim, Germany) and positive clones were selected using the blue-white screening assay. 16S rRNA gene fragments were amplified by colony PCR (Subcloning Notebook Guide, BR152,Promega) using plasmid specific M13F-40 (5' GTTTTCCCAGTCACGAC 3') and M13b (5' CAGGAAACAGCTATGAC 3') primers (Promega) and PCR products were submitted to LGC Genomics (Berlin, Germany) for bi-directional Sanger sequencing using M13 primers. Forward and reverse sequences were merged using SeqMan Pro (DNA Star, version 8.1.2 (33.3), 418) and saved as FASTA files. Clone sequences were aligned using SINA aligner (www.arb-silva.de/aligner) (Pruesse et al., 2007) with the archaeal variability profile. The alignment was imported and curated in ARB version 6.0.2 (Ludwig et al., 2004) using the archaeal SSU filter. Taxonomic identification was done by inserting the aligned sequences into the 16S rRNA gene SILVA non-redundant reference database (SSU Ref NR 99, Version 119 (Quast et al., 2013)) using the ARB Parsimony tool. Sequences belonging to families containing known methanogens were

selected and re-aligned along with archaeal OTUs obtained from 454 sequencing using the SINA aligner. The aligned sequences were used to generate a maximum likelihood tree with 200 bootstraps using MEGA 5.2.2 (Tamura et al., 2011) and the General Time Reversal (GTR) substitution model. Archaeal 16S rRNA sequences from clone libraries have been submitted to NCBI GenBank under the accession numbers KP987241- KP987264.

2. SUPPLEMENTARY RESULT

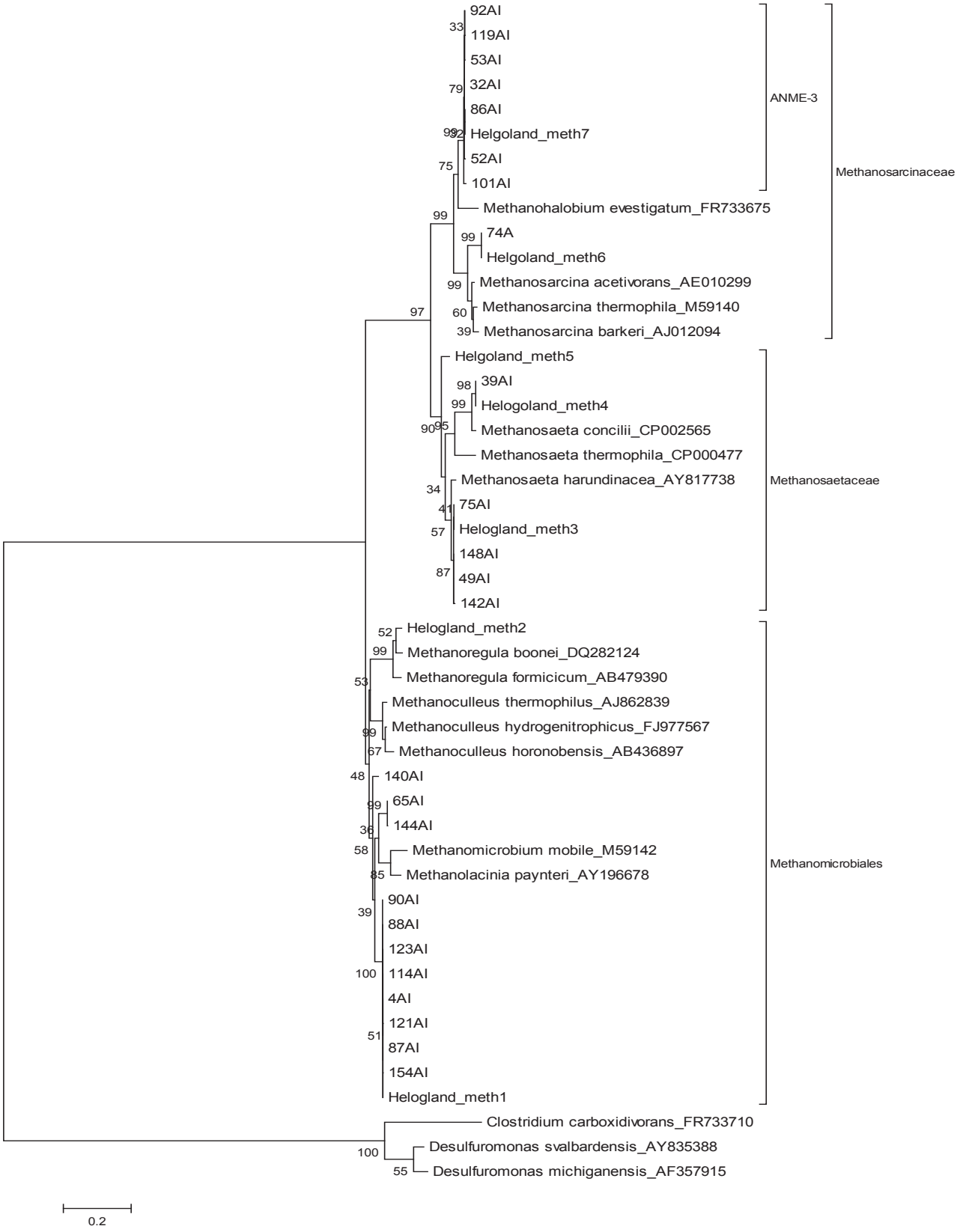

Fig S1.Maximum likelihood tree of archaeal 16S rRNA gene sequences showing the phylogenetic affiliations of methanogen and ANME-3 sequences from 454 sequencing (Helgoland_meth, ca.200bp sequence length) and Sanger sequencing (A/AI; ca. 1.3 to 1.4 kbps). All methanogen sequences

retained their positions as presented in Fig. 9. Helgoland_meth7 is closely related to *Methanohalobium*/ANME-3. *Clostridium carboxidivorans* (FR733710), *Desulfuromonas svalbardensis* (AY835388) and *Desulfuromonas michiganenesis* (AF357915) serve as outgroups. Bar represents 20% sequence divergence.

Reference

Großkopf R., Stubner, S., and Liesack, W. (1998). Novel euryarchaeotal lineages detected on rice roots and in the anoxic bulk soil of flooded rice microcosms. *Appl. Environ. Microbiol.* 64, 4983–9.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., et al. (2004). ARB: a software environment for sequence data. *Nucleic Acids Res.* 32, 1363–71. doi:10.1093/nar/gkh293.

Miyashita, A., Mochimaru, H., Kazama, H., Ohashi, A., Yamaguchi, T., Nunoura, T., Horikoshi, K., Takai, K., and Imachi, H. (2009). Development of 16S rRNA gene-targeted primers for detection of archaeal anaerobic methanotrophs (ANMEs). *FEMS Microbiol. Lett.* 297, 31–7. doi:10.1111/j.1574-6968.2009.01648.x.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., and Glöckner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* 35, 7188–96. doi:10.1093/nar/gkm864.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–6. doi:10.1093/nar/gks1219.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28, 2731–9. doi:10.1093/molbev/msr121.

Chapter 4

Novel uncultured *Deltaproteobacteria* populations (MSME Cluster) are dominant under chemolithotrophic manganese (IV)-reducing conditions in marine sediment slurry incubations

Oluwatobi Oni[1, 2, 3], Cedric Hahn[1], Ajinkya Kulkarni[1], Sabine Kasten[2,4], Michael W. Friedrich[1,2]

1. Department of Microbial Ecophysiology, University of Bremen, Bremen, Germany.
2. MARUM – Center for Marine Environmental Sciences, University of Bremen, Bremen, Germany
3. Max Planck Institute for Marine Microbiology, Bremen, Germany
4. Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Bremerhaven, Germany

(In preparation for submission as a short communication to *Microbes and Environment*)

Abstract

It is a three decade old observation that when poorly-crystalline Mn(IV) is added to marine sediments, there is a rapid formation of sulfate which is linked to biological activity. However, knowledge of the diversity of microorganisms involved in this reaction is limited. The rapid enrichment (55-60 % of total bacteria in 4 days from about 1% on day 0) of novel uncultured *Deltaproteobacteria* (Marine Sediment Manganese-reducing Enrichment, MSME Cluster) in MnO_2 (birnessite)-amended slurry incubations with sediment from the Helgoland mud area further expand the diversity of microbes involved in Mn (IV)-dependent sulfate formation in marine sediments.

Manganese (IV) reduction in marine sediments holds an important role in the cycling of elements such as carbon, sulfur, iron and phosphorus (Burdige, 1993). Regardless, most studies have focused on iron reduction and microorganisms involved (Coates et al., 1995; Zhang et al., 1999; Vandieken et al., 2006; Finke et al., 2007; Vandieken and Thamdrup, 2013) resulting in a dearth of literature addressing the diversity of microbes involved in manganese reduction in marine sediments. Recently, heterotrophic Mn (IV) reduction is receiving more attention (Thamdrup et al., 2000; Vandieken et al., 2012; Berg et al., 2013) despite the critical importance of chemolithotrophic manganese reduction in marine environments already identified almost 30 years ago (Aller and Rude, 1988) . Microorganisms such as species of *Shewanella* (Lovley et al., 1989) are able to gain energy from coupling the oxidation of hydrogen to Mn (IV) reduction. In marine sediments, Mn (IV) can also serve as a powerful oxidant for solid phase and dissolved sulfides (Burdige and Nealson, 1986; Aller and Rude, 1988). Although, Mn (IV) reduction can be exclusively chemical (Schippers and Jørgensen, 2001), it has been shown that some microorganisms are capable of partly mediating the reduction of Mn (IV) with reduced sulfur compounds (Burdige and Nealson, 1986; Lovley and Phillips, 1994). Nevertheless, the diversity of microorganism involved in sulfur-dependent Mn (IV) reduction is not known.

Elevated concentrations of dissolved manganese have been detected in pore water of subsurface sediment of the Helgoland mud area (Fig. S1). The expected depletion of easily-mineralizable organic matter for microbes in subsurface sediments relative to surface sediments, prompted us to test for the potential for chemolithotrophic manganese reduction. In addition, hydrogen is the likely reductant formed from the reaction of FeS with excess sulfide during a “cryptic sulfur cycling” in the methanic zone (Holmkvist et al., 2011) hence, the selection of hydrogen as a candidate electron donor for testing chemolithotropic Mn (IV) reduction in this study.

In slurry incubations with sediments from the Helgoland mud area, we therefore test the potential for chemolithotrophic Mn(IV) reduction. Using molecular techniques such as quantitative PCR (Q-PCR), Terminal Restriction Fragment Length Polymorphisms (TRFLP) and pyrosequencing, we also quantified and characterized shifts in microbial populations in slurry incubations with time in order to

investigate the involvement and possibly expand the diversity of microorganisms linked to chemolithotrophic Mn (IV) reduction in marine sediments.

***Geochemistry*:** Sediment (10 g) collected from subsurface sediments of the Helgoland mud area as described in Oni et al., 2015, was used to inoculate 30 ml of anoxic artificial sea water (ASW) in 120 ml serum vials. ASW medium contained (g/l): NaCl; 26.4, $MgCl_2$; 11.2, $CaCl_2.2H_2O$; 1.5, KCl; 0.7. Any traces of oxygen in slurry incubation was further removed by expelling headspace gases from serum vials under gentle swirling, using a vacuum pump (CVC 3000, Vacuubrand GmbH, Germany). After every 5 min purge, headspaces were filled with N_2 (99.999 % purity) and these procedures were repeated 3 times. In vials, where the addition of an electron donor (H_2/CO_2, 80:20, 0.2 bar) was not required, headspaces were filled N_2 gas. As electron acceptor, birnessite (amorphous MnO_2, 30 mmol L^{-1}) was used. Birnessite was synthesized according to McKenzie (1971). Briefly, 2 moles of concentrated hydrochloric acid was added dropwise to a boiling solution of one mole $KMnO_4$ (Sigma-Aldrich, Taufkirchen, Germany) in 2.5 ml of sterile deionized water, under vigorous stirring. After 10-15 min of further boiling, the mixture was allowed to cool as brownish precipitates settled. The precipitate was washed 5 times with de-ionized water to remove impurities before transfer into centrifuge tubes. Wet precipitates were centrifuged 3 times at 3,834 *g* for 10 min at room temperature using a Sorvall Evolution RC Centrifuge (Thermo Scientific, Germany). After each centrifugation step, the supernatant was decanted and pellet was rinsed with de-ionized water.

Sediment slurries were incubated for 15 days at 10 °C and 1 ml of slurries were anoxically sampled on days 0, 4, 9, and 15 with sterile syringes into 1.5 ml microreaction tubes and centrifuged at 20,000 *g*. 500 µl of the supernatant was fixed in 0.16 M nitric acid (1:10 v/v) and the solution for analysis of geochemical parameters (dissolved Mn, Fe and S). The sediment pellets were frozen at -20°C until use for microbial community analysis. Dissolved Fe, Mn and S in the aqueous phase were determined using inductively-coupled plasma optical emission spectrometry (ICP-OES; 700 Series, Agilent Technologies, Germany). Sulfate concentration was determined using a Metrohm Compact IC 761 ion chromatograph.

Manganese reduction was strongly stimulated in incubations where MnO_2 was added in relation to control incubations without addition MnO_2 (Fig. 1A). However, the formation of 4 − 6.5 times more

dissolved Mn in slurry incubations with H_2/CO_2 and MnO_2 over the course of incubation, compared to sediment slurries treated with MnO_2 only, suggest that hydrogen strongly stimulated manganese reduction directly. In treatments with H_2/CO_2 only, dissolved Mn only accumulated to 50 μM by day 15 from 12 μM on day 0 (Fig. 1A). In these incubations, dissolved Fe increased to an average of ~ 430 μM from ~150 μM on day 0 (*n=2*, Fig. 1B). Systematic tests showed that CO_2 was the component responsible for the unexpectedly high concentration dissolved Fe on day 0 (Fig. S2). This is perhaps caused by a rapid change in pH of slurry by CO_2 headspace addition, resulting in dissolution of bound iron minerals. However, measured pH (7.7, data not shown), was the same across test incubations (Fig.S2) with the different treatments. Equilibrium may have been quickly re-established due to the high pH buffer capacity of marine sediments (Widdicombe et al., 2011), causing pH to remain at 7.7. Overall, the high dissolved Fe is consistent with the presence of excess reactive/easily reducible iron minerals deeply buried in the Helgoland mud area (Oni et al., 2015).

In line with the observations of Aller and Rude (1988), and King (1990), sulfate rapidly developed in slurry incubations containing MnO_2. From day 0 to day 15, average sulfate concentrations (*n=2*) in incubations with MnO_2 and MnO_2 + H_2/CO_2 increased from 28 μM to 730 μM and 35 μM to 1303 μM, respectively (Fig. 1C). The observation of a similar trend but higher concentrations of total dissolved S (Fig. 1D, relative to sulfate concentrations), suggest the presence of soluble intermediate sulfur species. The source of sulfate formed in marine sediments on addition of amorphous Mn (IV) has been predicted to be iron monosulfide (Aller and Rude, 1988; King, 1990) with dissolved sulfur species as intermediates (Aller and Rude, 1988). Mn (IV) in marine sediments can also be reduced by hydrogen sulfide (Burdige and Nealson, 1986). Since free dissolved sulfide is completely depleted in our system (Oni et al., 2015), solid phase sulfide must have accounted for the entire sulfate detected. In slurry incubation treated with H_2/CO_2 only, sulfate was reduced from ~27 μM on day 0 to ~7 μM by day 15 (Fig. 1B). Aller and Rude (1988), King (1990) have reported significant inhibition of sulfate reduction in the presence of MnO_2 reduction. However, $^{35}SO_4$-radiotracer experiments will be necessary to determine whether hydrogen fueled sulfate reduction under MnO_2-dependent metal sulfide-oxidizing conditions in our slurry incubation.

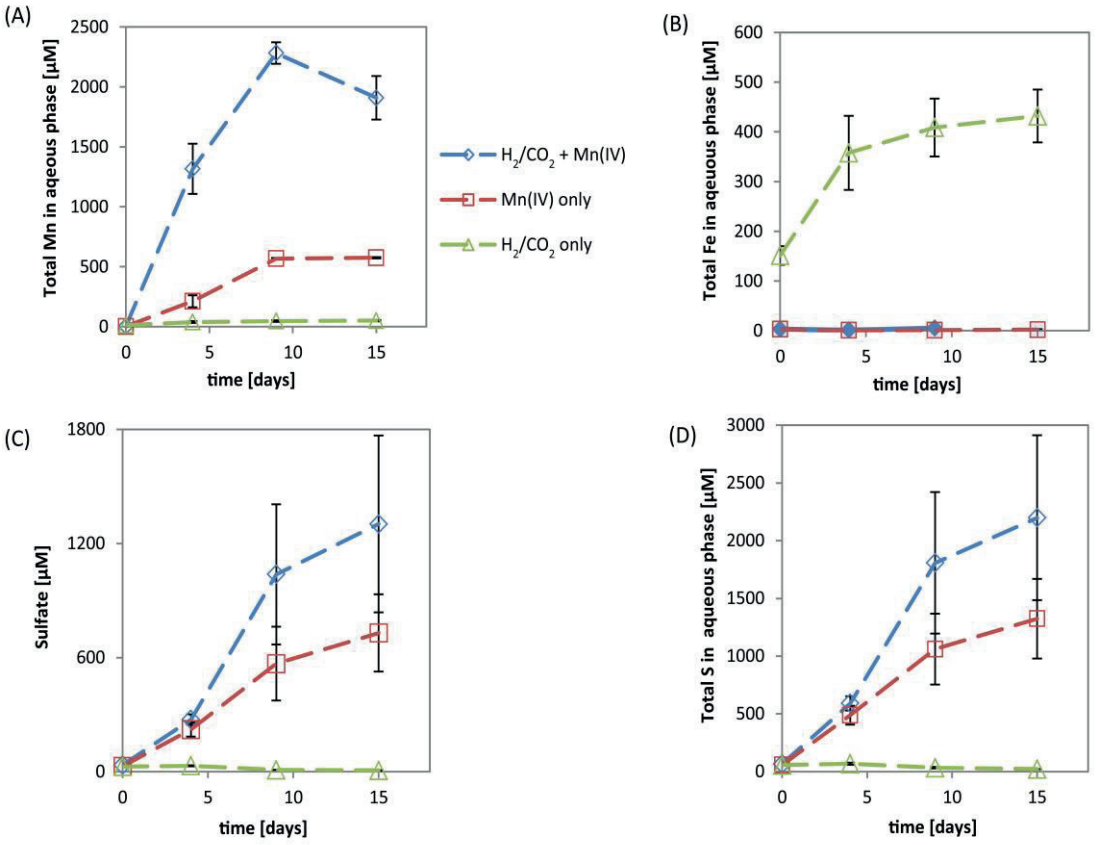

Fig. 1: Time course measurements of total manganese (A), total iron (B), sulfate (C) and total sulfur (D) in aqueous phase of sediment slurry incubations. *n=2*

***Bacterial Q-PCR and Community Analysis*:** Since the difference in dissolved Mn concentrations between slurry incubations treated with H_2/CO_2 + MnO_2 and MnO_2 alone was highest on day 4 (6.5 times compared to 4 times on day 9, Fig. 1A), slurry samples taken at this time point were used to determine changes in the 16S rRNA gene copies and shifts in bacterial community. 16S rRNA gene Q-PCR quantification was performed as described in Oni et al., 2015. Fingerprints of bacterial community shifts were analyzed using Terminal Restriction Fragment Length Polymorphism (T-RFLP, see suppl. info for details). 16S rRNA genes bacterial community composition was mainly determined via pyrosequencing-based sequencing of 16S rRNA gene amplicons (see details of amplicon generation in suppl. info). Amplicon sequencing and analyses of sequences are as described in Oni et al., 2015. Numbers of analyzed reads per sample are given in (Table S2).

TRFLP cluster analyses revealed that by day 4, bacterial communities had shifted similarly in sediment slurry incubations containing MnO_2 irrespective of the presence of H_2/CO_2 (Fig. S3A). The

shift was attributable to the rapid dominance of *Deltaproteobacteria* populations in those incubations in contrast to typical subsurface sediments bacteria, *Chloroflexi* and candidate division OP9 (JS1), dominating incubations on day 0 (Fig. S3B). *Deltaproteobacteria* accounted for 74-76 % of total bacteria population in MnO_2- containing incubations in comparison to incubations treated with H_2/CO_2 alone (18 %), Fig. S3B. Q-PCR (Table 1) shows that gene copies in incubations treated with H_2/CO_2 and MnO_2 were higher than those treated with MnO_2 alone (~ 3 folds) and H_2/CO_2 alone (~10 folds). These results, so far, suggest that: 1. there is a biological component to the oxidation of metal sulfides with MnO_2 in our incubations and *Deltaproteobacteria* area most likely the main players in this process, consistent with earlier reports (Aller and Rude, 1988; King, 1990; Thamdrup et al., 1993; Lovley and Phillips, 1994). 2. In addition to metal sulfides, the presence of hydrogen as an electron donor further enhanced manganese reduction by certain members of the *Deltaproteobacteria*, agreeing with the detection of higher concentrations of dissolved Mn in treatments with both H_2/CO_2 and MnO_2 (Fig. 1A).

Table 1: Q-PCR-based 16S rRNA gene copies in sediment slurry incubations

	gene copies g^{-1} wet sed.$^{-1}$	
Treatments	*day 0*	*day 4*
Mn(IV) + H_2		1.39E+08 ± 2.31E+06
Mn(IV) only	1.09E+07 ± 6.65E+06	4.59E+07 ± 3.12E+06
H_2 only		1.13E+07 ± 2.65E+05

Further analyzing of the distribution of *Deltaproteobacteria* across all treatments showed that the rapid dominance of this group in MnO_2-containing incubations was mainly due to the occurrence of novel unclassified *Deltaproteobacteria* populations (as annotated by the greengenes database v12-10; Fig. 2). To confirm the phylogenetic identities of unclassified *Deltaproteobacteria* populations, full-length 16S rRNA clone sequences (see supplementary information for details) were obtained with DNA extracted from incubations treated with MnO_2 and H_2/CO_2 (representative of MnO_2-containing incubations, Fig. 3). Phylogenetic treeing of pyrosequencing- and clone library-derived *Deltaproteobacteria* sequences (Fig. 3), using ARB and MEGA software (see details in supplementary information), showed that novel unclassified *Deltaproteobacteria* formed a distinct cluster in the phylogenetic radiation of *Desulfobulbus-Desulfocapsa* groups (Fig. 3). This cluster was tentatively

named Marine Sediment Manganese-reducing Enrichment, MSME cluster. Like the MSME cluster, members of the *Desulfobulbaceae* (mostly *Desulfocapsa* groups, Figs. 2 and 3) were more dominant in MnO_2-containing incubations in relation to incubations treated with H_2/CO_2 only (5.0 − 8.4 % *vs.* 0.7 % of total bacteria, respectively). However, while *Desulfocapsa* are known to disproportionate intermediate sulfur species such as elemental sulfur (Janssen et al., 1996; Finster et al., 1998), the roles of MSME cluster are not known yet. With the current results, one could predict that they are involved in the metabolisms of sulfur compounds in our slurry incubations experiments. A close relative of MSME cluster sequences detected in our incubations (Fig. 3) have been found to colonize sulfur bags buried in tidal flat sediments under anoxic conditions (Pjevac et al., 2014). Relatives of *Desulfobulbus-Desulfocapsa* groups were also enriched near active anodes where elevated sulfate concentrations, sulfide depletion and elemental sulfur deposition could be detected (Tender et al., 2002).

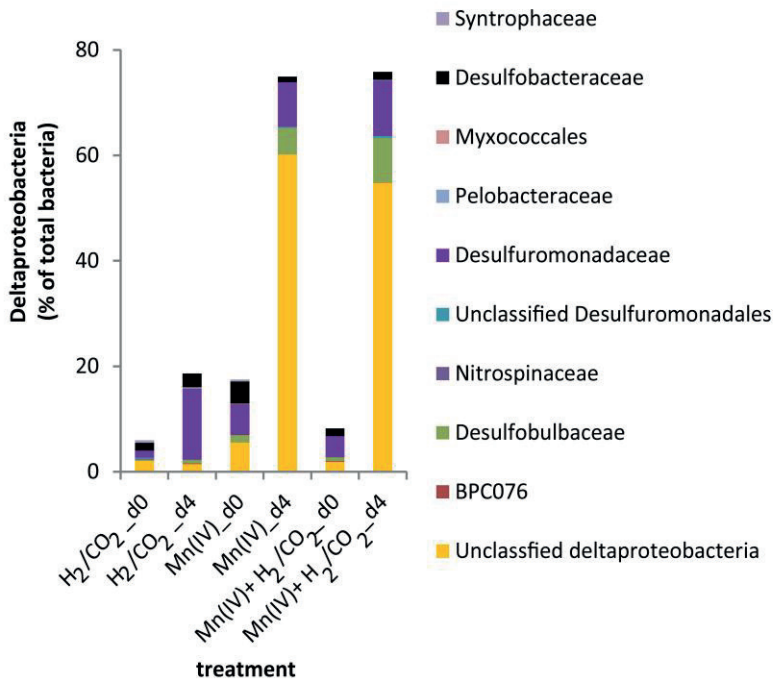

Fig. 2: Relative abundances of pyrosequencing-derived *Deltaproteobacteria* 16S rRNA gene sequence reads in sediment slurry incubations. Unclassified *Deltaproteobacteria* rapidly dominated MnO_2-containing incubations after 4 days.

MnO_2 is able to chemically reduce metal sulfides mainly to elemental sulfur (Schippers and Jørgensen, 2001). It is possible that members of the MSME cluster further metabolized elemental sulfur via disproportionation or directly coupled its oxidation to the reduction of excess MnO_2, forming sulfate (Lovley and Phillips, 1994).

Bacterial groups similar to MSME cluster members, for example, some *Desulfobulbus* species are known to utilize hydrogen as electron donor in addition to being able to oxidize sulfur directly to sulfate by reducing electrodes poised at +512 mV (Holmes et al., 2004), similar to the potential of birnessite (δ-MnO_2/Mn^{2+}; 500-600 mV, Thamdrup, 2000). It is therefore, conceivable that in addition to sulfur compounds, members of the MSME cluster might be able to utilize hydrogen as higher gene copy numbers were detected in slurry incubations treated with MnO_2 + H_2/CO_2 than those treated with MnO_2 only, albeit more or less similar microbial community shifts (Figs. 2, S3A and B). However, MnO_2 reduction with hydrogen could also have been performed by members of the *Desulfuromonadales*, which make up (of total bacterial populations) 10.5 % in MnO_2 + H_2/CO_2 treated incubations compared to 8.4 % in those treated with MnO_2 only (Fig. 2). In incubations treated with H_2/CO_2 only, the dominance of *Desulfuromonadaceae* (Fig. 2) relative to other *Deltaproteobacteria* is likely linked to iron reduction observed in those incubations (Roden and Lovley, 1993; Coates et al., 1995).

***Implications and Conclusions*:** The finding that MSME cluster dominates bacterial populations in MnO_2-containing sediment slurry incubations is interesting. They rapidly responded to the presence of excess manganese (IV) under our incubation conditions although Mn (IV) does not seem to be very abundant *in situ*, compared to Fe (III) minerals. Regardless, with the results presented here, we extended the inventory of microbial populations so far linked the biotic formation of sulfate on addition of Mn (IV). Whether via disproportionation or direct oxidation of elemental sulfur, the exact pathways of Mn (IV) reduction by MSME cluster still needs to be determined.

Fig. 3: Maximum likelihood tree of 16S rRNA gene sequences showing the phylogenetic affiliations of MSME cluster sequences obtained to other *Deltaproteobacteria*. Sequences obtained from cloning area shown in green while representative sequences obtained via pyrosequencing are shown in red. Archaea serve as an out group. Bar represents 20 % sequence divergence.

References

Abdo, Z., Schüette, U. M. E., Bent, S. J., Williams, C. J., Forney, L. J., and Joyce, P. (2006). Statistical methods for characterizing diversity of microbial communities by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. *Environ. Microbiol.* 8, 929–38. doi:10.1111/j.1462-2920.2005.00959.x.

Aller, R. C., and Rude, P. D. (1988). Complete oxidation of solid phase sulfides by manganese and bacteria in anoxic marine sediments. *Geochim. Cosmochim. Acta* 52, 751–765. doi:10.1016/0016-7037(88)90335-3.

Berg, C., Beckmann, S., Jost, G., Labrenz, M., and Jürgens, K. (2013). Acetate-utilizing bacteria at an oxic-anoxic interface in the Baltic Sea. *FEMS Microbiol. Ecol.* 85, 251–61. doi:10.1111/1574-6941.12114.

Burdige, D. J. (1993). The biogeochemistry of manganese and iron reduction in marine sediments. *Earth-Science Rev.* 35, 249–284. doi:10.1016/0012-8252(93)90040-E.

Burdige, D. J., and Nealson, K. H. (1986). Chemical and microbiological studies of sulfide-mediated manganese reduction. *Geomicrobiol. J.* 4, 361–387. doi:10.1080/01490458609385944.

Coates, J. D., Lonergan, D. J., Philips, E. J., Jenter, H., and Lovley, D. R. (1995). *Desulfuromonas palmitatis sp. nov*., a marine dissimilatory Fe(III) reducer that can oxidize long-chain fatty acids. *Arch. Microbiol.* 164, 406–13. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8588742.

Culman, S. W., Bukowski, R., Gauch, H. G., Cadillo-Quiroz, H., and Buckley, D. H. (2009). T-REX: software for the processing and analysis of T-RFLP data. *BMC Bioinformatics* 10, 171. doi:10.1186/1471-2105-10-171.

Finke, N., Vandieken, V., and Jørgensen, B. B. (2007). Acetate, lactate, propionate, and isobutyrate as electron donors for iron and sulfate reduction in Arctic marine sediments, Svalbard. *FEMS Microbiol. Ecol.* 59, 10–22. doi:10.1111/j.1574-6941.2006.00214.x.

Finster, K., Liesack, W., and Thamdrup, B. (1998). Elemental sulfur and thiosulfate disproportionation by *Desulfocapsa sulfoexigens sp. nov*., a new anaerobic bacterium isolated from marine surface sediment. *Appl. Environ. Microbiol.* 64, 119–25. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=124681&tool=pmcentrez&rendertype=abstract.

Hammer, Ø., Harper, D. A. T., and Ryan, P. D. (2001). PAST :Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4, 1–9.

Holmes, D. E., Bond, D. R., and Lovley, D. R. (2004). Electron transfer by *Desulfobulbus propionicus* to Fe(III) and graphite electrodes. *Appl. Environ. Microbiol.* 70, 1234–1237. doi:10.1128/AEM.70.2.1234-1237.2004.

Holmkvist, L., Ferdelman, T. G., and Jørgensen, B. B. (2011). A cryptic sulfur cycle driven by iron in the methane zone of marine sediment (Aarhus Bay, Denmark). *Geochim. Cosmochim. Acta* 75, 3581–3599. doi:10.1016/j.gca.2011.03.033.

Janssen, P. H., Schuhmann, A., Bak, F., and Liesack, W. (1996). Disproportionation of inorganic sulfur compounds by the sulfate-reducing bacterium *Desulfocapsa thiozymogenes gen. nov*., sp. nov. *Arch. Microbiol.* 166, 184–192. doi:10.1007/s002030050374.

King, G. M. (1990). Effects of added manganic and ferric oxides on sulfate reduction and sulfide oxidation in intertidal sediments. *FEMS Microbiol. Lett.* 73, 131–138. doi:10.1111/j.1574-6968.1990.tb03933.x.

Lane, D. J. (1991). "16S/23S rRNA Sequencing," in *Nucleic acid techniques in bacterial systematics*, eds. E. Stackebrandt and M. Goodfellow (New York, NY: John Wiley and Sons), 115–175.

Lovley, D. R., and Phillips, E. J. P. (1994). Novel processes for anaerobic sulfate production from elemental sulfur by sulfate-reducing bacteria. *Appl. Environ. Microbiol.* 60, 2394–2399.

Lovley, D. R., Phillips, E. J. P., and Lonergan, D. J. (1989). Hydrogen and formate oxidation coupled to dissimilatory reduction of iron or manganese by *Alteromonas putrefaciens*. *Appl. Environ. Microbiol.* 55, 700–706.

McKenzie, R. (1971). The synthesis of birnessite , cryptomelane , and some other oxides and hydroxides of manganese. *Mineral. Mag.* 38, 493–502.

Oni, O., Miyatake, T., Kasten, S., Richter-Heitmann, T., Fischer, D., Wagenknecht, L., Kulkarni, A., Blumers, M., Shylin, S. I., Ksenofontov, V., et al. (2015). Distinct microbial populations are tightly linked to the profile of dissolved iron in the methanic sediments of the Helgoland mud area, North Sea. *Front. Microbiol.* 06, 1–15. doi:10.3389/fmicb.2015.00365.

Pjevac, P., Kamyshny, A., Dyksma, S., and Mussmann, M. (2014). Microbial consumption of zero-valence sulfur in marine benthic habitats. *Environ. Microbiol.* 16, 3416–30. doi:10.1111/1462-2920.12410.

Roden, E. E., and Lovley, D. R. (1993). Dissimilatory Fe(III) reduction by the marine microorganism *Desulfuromonas acetoxidans*. *Appl. Environ. Microbiol.* 59, 734–42. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=202183&tool=pmcentrez&rendertype=abstract.

Schippers, A., and Jørgensen, B. B. (2001). Oxidation of pyrite and iron sulfide by manganese dioxide in marine sediments. *Geochim. Cosmochim. Acta* 65, 915–922. doi:10.1016/S0016-7037(00)00589-5.

Tender, L. M., Reimers, C. E., Stecher, H. A., Holmes, D. E., Bond, D. R., Lowy, D. A., Pilobello, K., Fertig, S. J., and Lovley, D. R. (2002). Harnessing microbially generated power on the seafloor. *Nat. Biotechnol.* 20, 821–5. doi:10.1038/nbt716.

Thamdrup, B. (2000). *Advances in Microbial Ecology*. , ed. B. Schink Boston, MA: Springer US doi:10.1007/978-1-4615-4187-5.

Thamdrup, B. O., Finster, K. M., Hansen, J. W., and Bak, F. (1993). Bacterial disproportionation of elemental sulfur coupled to chemical reduction of rron or manganese. *Appl. Environ. Microbiol.* 59, 101–108.

Thamdrup, B., Rosselló-Mora, R., and Amann, R. (2000). Microbial manganese and sulfate reduction in Black Sea shelf sediments. *Appl. Environ. Microbiol.* 66, 2888–97. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=92088&tool=pmcentrez&rendertype=abstract.

Vandieken, V., Mussmann, M., Niemann, H., and Jørgensen, B. B. (2006). *Desulfuromonas svalbardensis sp. nov*. and *Desulfuromusa ferrireducens sp. nov*., psychrophilic, Fe(III)-reducing

bacteria isolated from Arctic sediments, Svalbard. *Int. J. Syst. Evol. Microbiol.* 56, 1133–9. doi:10.1099/ijs.0.63639-0.

Vandieken, V., Pester, M., Finke, N., Hyun, J.-H., Friedrich, M. W., Loy, A., and Thamdrup, B. (2012). Three manganese oxide-rich marine sediments harbor similar communities of acetate-oxidizing manganese-reducing bacteria. *ISME J.* 6, 2078–90. doi:10.1038/ismej.2012.41.

Vandieken, V., and Thamdrup, B. (2013). Identification of acetate-oxidizing bacteria in a coastal marine surface sediment by RNA-stable isotope probing in anoxic slurries and intact cores. *FEMS Microbiol. Ecol.* 84, 373–86. doi:10.1111/1574-6941.12069.

Widdicombe, S., Spicer, J. I., and Kitidis, V. (2011). "Effects of ocean acidification on sediment fauna," in *Ocean Acidification*, eds. P. Gattuso, J and L. Hansson (Oxford: Oxford University Press), 179.

Zhang, C., Stapleton, R., Zhou, J., Palumbo, A., and Phelps, T. (1999). Iron reduction by psychrotrophic enrichment cultures. *FEMS Microbiol. Ecol.* 30, 367–371. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10568845.

Supplementary Methods

Terminal Restriction Fragment Length Polymorphism (T-RFLP)

T-RFLP was carried out on bacterial 16S rRNA genes with forward primer fluorescently labelled with 6-carboxyfluorescine (FAM). 16S rRNA genes were amplified using primer pairs 27f-FAM and 907r (Lane, 1991). PCR reagents and conditions are as follows:

Table S1. Concentration of reagents per PCR reaction

Reagent	Volume per reaction (µl)	Final Concentration
10X AmpliTaq buffer	2.5	1X
2 mM dNTP	2.5	200 µM
20 mg/ml BSA	0.25	200 µg/ml
25 Mm $MgCl_2$	3.0	3 mM
10 µM Forward Primer	0.75	0.3 µM
10 µM Reverse Primer	0.75	0.3 µM
Nuclease-free water	13.125	–
5U/µl AmpliTaq DNA polymerase	0.125	0.025 U
DNA template	2 µl	–
Total Volume	**25 µl**	–

Table S2. PCR Conditions

	Temp (°C)	*Duration (min)*	
Initial denaturation	94	5	
Final denaturation	94	0.5	35 cycles
Annealing	52	1	
Extension	72	1	
Final Elongation	72	7	
Hold	4	∞	

PCR amplicons were purified using MinElute spin columns (Qiagen Hilden, Germany) and ~ 120 ng of purified 16S rRNA amplicons were digested using *Msp I*. Digested amplicons were purified using SigmaSpin post-reaction clean-up columns (Sigma-Aldrich, Germany). Purified digests were analyzed for fragment analysis on an ABI Prism 3130 XL Genetic analyzer (Applied Biosystems CA, USA). Raw data from the fragment analysis run were processed using Genemapper software 3.0 (Applied Biosystems, Germany). A height-based data matrix for cluster analysis was generated using the platform T-RFLP analysis Expedited (T-REX, Culman et al., 2009). Peaks whose height exceeds the standard deviation (assuming zero mean) computed for all peaks heights across all samples were considered as true peaks (Abdo et al., 2006). A T-RF binning window of 0.5 was also selected. Data matrices were imported into the PAST software (Hammer et al., 2001) to generate a Jaccard similarity-based cluster analysis of samples.

Molecular Cloning and Phylogenetic Analysis

Cloning and phylogenetic analysis of full-length16S rRNA genes (ca 1.3 -1,.4 kbps) were done as described in Oni et al., 2015. However, primer pair 27F and 1492R (Lane, 1991) were used for generate full-length bacterial 16S rRNA gene amplicons.

Supplementary data

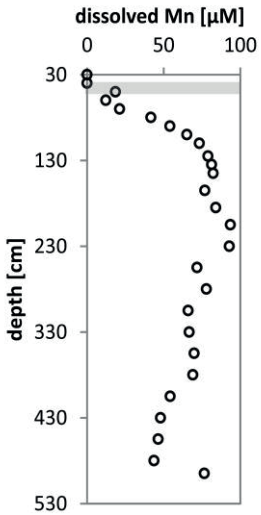

Fig. S1. Geochemical profile (HE376-007-002) of dissolved Mn in the Helgoland mud area. Shaded area represents SMT.

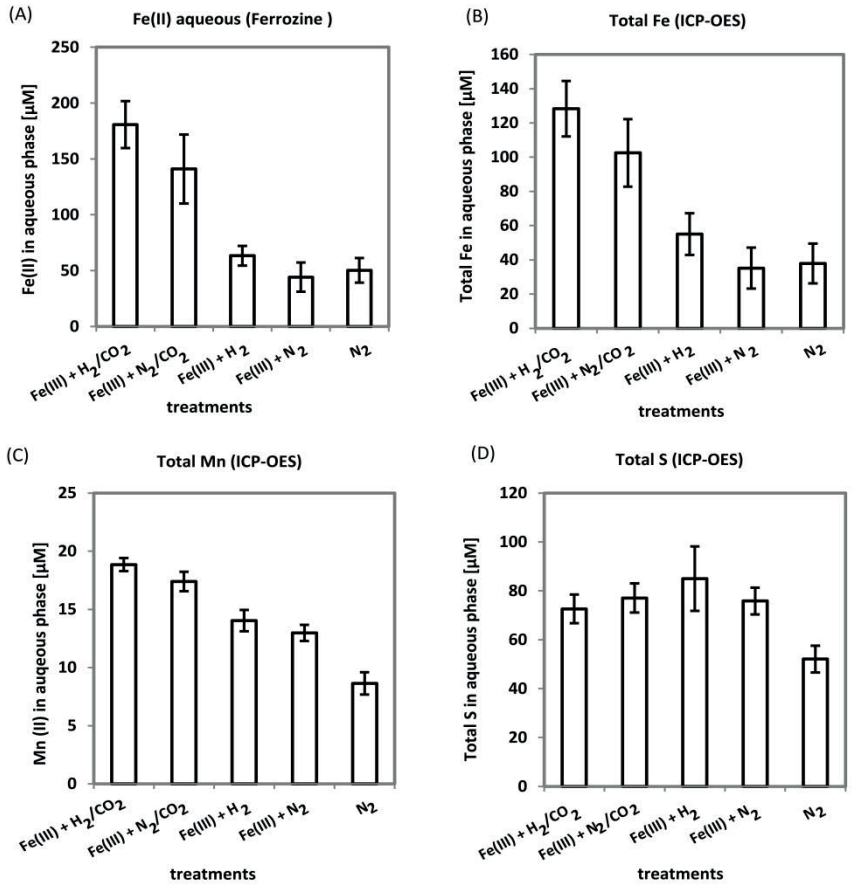

Fig. S2. Effect of CO_2 on the formation of dissolved Fe (Aand B), Mn (C) and S (D) in the aqueous phase of sediment slurry incubations. Dissolved Fe, Mn and S were measured immediately after sediment slurry incubations (with treatments) were prepared. Values from ferrozine and ICP-OES measurements of dissolved iron in aqueous phase are comparable. CO_2 addition is responsible for the rapid formation of dissolved iron.

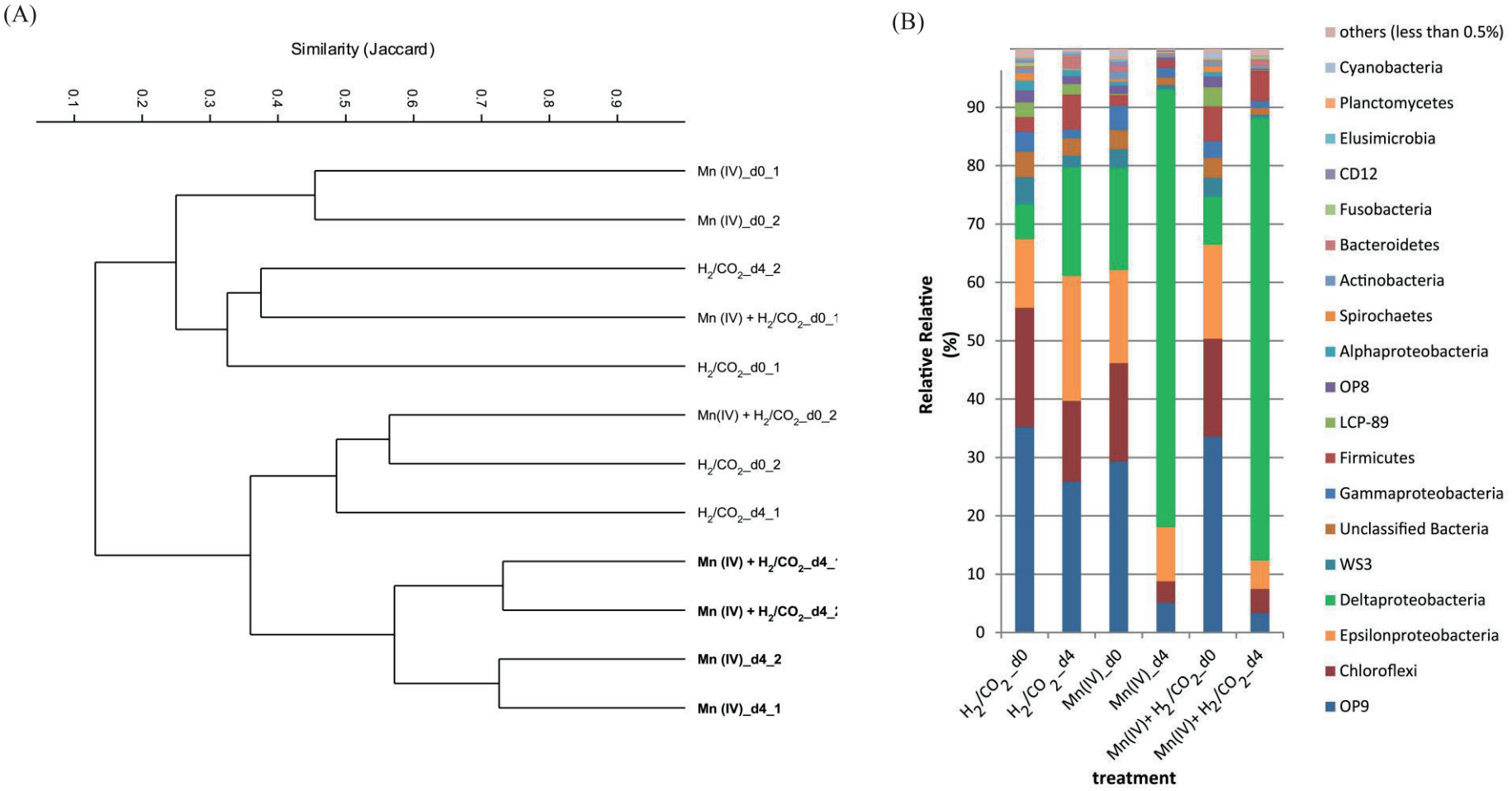

Fig. S3. Microbial community shifts in sediments slurry incubations after 4 days. T-RFLP (A) shows a Mn(IV)-dependent shift in microbial community fingerprint (in **bold e.g Mn (IV) +H_2/CO_2_d4_1**). Pyrosequencing-based relative abundances of bacterial groups (Phylum/Class level) in sediment slurry incubations (B)

Table S2. Number of sequence reads analyzed per sample

	Number of sequences	
Treatments	***day 0***	***day 4***
Mn (IV) + H_2/CO_2	1487	4636
Mn (IV)	2452	4262
H_2/CO_2	3728	2702

References

Abdo, Z., Schüette, U. M. E., Bent, S. J., Williams, C. J., Forney, L. J., and Joyce, P. (2006). Statistical methods for characterizing diversity of microbial communities by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. *Environ. Microbiol.* 8, 929–38. doi:10.1111/j.1462-2920.2005.00959.x.

Aller, R. C., and Rude, P. D. (1988). Complete oxidation of solid phase sulfides by manganese and bacteria in anoxic marine sediments. *Geochim. Cosmochim. Acta* 52, 751–765. doi:10.1016/0016-7037(88)90335-3.

Berg, C., Beckmann, S., Jost, G., Labrenz, M., and Jürgens, K. (2013). Acetate-utilizing bacteria at an oxic-anoxic interface in the Baltic Sea. *FEMS Microbiol. Ecol.* 85, 251–61. doi:10.1111/1574-6941.12114.

Burdige, D. J. (1993). The biogeochemistry of manganese and iron reduction in marine sediments. *Earth-Science Rev.* 35, 249–284. doi:10.1016/0012-8252(93)90040-E.

Burdige, D. J., and Nealson, K. H. (1986). Chemical and microbiological studies of sulfide-mediated manganese reduction. *Geomicrobiol. J.* 4, 361–387. doi:10.1080/01490458609385944.

Coates, J. D., Lonergan, D. J., Philips, E. J., Jenter, H., and Lovley, D. R. (1995). *Desulfuromonas palmitatis sp. nov*., a marine dissimilatory Fe(III) reducer that can oxidize long-chain fatty acids. *Arch. Microbiol.* 164, 406–13. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8588742.

Culman, S. W., Bukowski, R., Gauch, H. G., Cadillo-Quiroz, H., and Buckley, D. H. (2009). T-REX: software for the processing and analysis of T-RFLP data. *BMC Bioinformatics* 10, 171. doi:10.1186/1471-2105-10-171.

Finke, N., Vandieken, V., and Jørgensen, B. B. (2007). Acetate, lactate, propionate, and isobutyrate as electron donors for iron and sulfate reduction in Arctic marine sediments, Svalbard. *FEMS Microbiol. Ecol.* 59, 10–22. doi:10.1111/j.1574-6941.2006.00214.x.

Finster, K., Liesack, W., and Thamdrup, B. (1998). Elemental sulfur and thiosulfate disproportionation by *Desulfocapsa sulfoexigens sp. nov*., a new anaerobic bacterium isolated from marine surface sediment. *Appl. Environ. Microbiol.* 64, 119–25. Available at:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=124681&tool=pmcentrez&rendertype=abstract.

Hammer, Ø., Harper, D. A. T., and Ryan, P. D. (2001). PAST :Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4, 1–9.

Holmes, D. E., Bond, D. R., and Lovley, D. R. (2004). Electron transfer by *Desulfobulbus propionicus* to Fe(III) and graphite electrodes. *Appl. Environ. Microbiol.* 70, 1234–1237. doi:10.1128/AEM.70.2.1234-1237.2004.

Holmkvist, L., Ferdelman, T. G., and Jørgensen, B. B. (2011). A cryptic sulfur cycle driven by iron in the methane zone of marine sediment (Aarhus Bay, Denmark). *Geochim. Cosmochim. Acta* 75, 3581–3599. doi:10.1016/j.gca.2011.03.033.

Janssen, P. H., Schuhmann, A., Bak, F., and Liesack, W. (1996). Disproportionation of inorganic sulfur compounds by the sulfate-reducing bacterium *Desulfocapsa thiozymogenes gen. nov*., sp. nov. *Arch. Microbiol.* 166, 184–192. doi:10.1007/s002030050374.

King, G. M. (1990). Effects of added manganic and ferric oxides on sulfate reduction and sulfide oxidation in intertidal sediments. *FEMS Microbiol. Lett.* 73, 131–138. doi:10.1111/j.1574-6968.1990.tb03933.x.

Lane, D. J. (1991). "16S/23S rRNA Sequencing," in *Nucleic acid techniques in bacterial systematics*, eds. E. Stackebrandt and M. Goodfellow (New York, NY: John Wiley and Sons), 115–175.

Lovley, D. R., and Phillips, E. J. P. (1994). Novel processes for anaerobic sulfate production from elemental sulfur by sulfate-reducing bacteria. *Appl. Environ. Microbiol.* 60, 2394–2399.

Lovley, D. R., Phillips, E. J. P., and Lonergan, D. J. (1989). Hydrogen and formate oxidation coupled to dissimilatory reduction of iron or manganese by *Alteromonas putrefaciens*. *Appl. Environ. Microbiol.* 55, 700–706.

McKenzie, R. (1971). The synthesis of birnessite , cryptomelane , and some other oxides and hydroxides of manganese. *Mineral. Mag.* 38, 493–502.

Oni, O., Miyatake, T., Kasten, S., Richter-Heitmann, T., Fischer, D., Wagenknecht, L., Kulkarni, A., Blumers, M., Shylin, S. I., Ksenofontov, V., et al. (2015). Distinct microbial populations are tightly linked to the profile of dissolved iron in the methanic sediments of the Helgoland mud area, North Sea. *Front. Microbiol.* 06, 1–15. doi:10.3389/fmicb.2015.00365.

Pjevac, P., Kamyshny, A., Dyksma, S., and Mussmann, M. (2014). Microbial consumption of zero-valence sulfur in marine benthic habitats. *Environ. Microbiol.* 16, 3416–30. doi:10.1111/1462-2920.12410.

Roden, E. E., and Lovley, D. R. (1993). Dissimilatory Fe(III) reduction by the marine microorganism *Desulfuromonas acetoxidans*. *Appl. Environ. Microbiol.* 59, 734–42. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=202183&tool=pmcentrez&rendertype=abstract.

Schippers, A., and Jørgensen, B. B. (2001). Oxidation of pyrite and iron sulfide by manganese dioxide in marine sediments. *Geochim. Cosmochim. Acta* 65, 915–922. doi:10.1016/S0016-7037(00)00589-5.

Tender, L. M., Reimers, C. E., Stecher, H. A., Holmes, D. E., Bond, D. R., Lowy, D. A., Pilobello, K., Fertig, S. J., and Lovley, D. R. (2002). Harnessing microbially generated power on the seafloor. *Nat. Biotechnol.* 20, 821–5. doi:10.1038/nbt716.

Thamdrup, B. (2000). *Advances in Microbial Ecology*. , ed. B. Schink Boston, MA: Springer US doi:10.1007/978-1-4615-4187-5.

Thamdrup, B. O., Finster, K. M., Hansen, J. W., and Bak, F. (1993). Bacterial disproportionation of elemental sulfur coupled to chemical reduction of iron or manganese. *Appl. Environ. Microbiol.* 59, 101–108.

Thamdrup, B., Rosselló-Mora, R., and Amann, R. (2000). Microbial manganese and sulfate reduction in Black Sea shelf sediments. *Appl. Environ. Microbiol.* 66, 2888–97. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=92088&tool=pmcentrez&rendertype=abstract.

Vandieken, V., Mussmann, M., Niemann, H., and Jørgensen, B. B. (2006). *Desulfuromonas svalbardensis sp. nov*. and *Desulfuromusa ferrireducens sp. nov*., psychrophilic, Fe(III)-reducing bacteria isolated from Arctic sediments, Svalbard. *Int. J. Syst. Evol. Microbiol.* 56, 1133–9. doi:10.1099/ijs.0.63639-0.

Vandieken, V., Pester, M., Finke, N., Hyun, J.-H., Friedrich, M. W., Loy, A., and Thamdrup, B. (2012). Three manganese oxide-rich marine sediments harbor similar communities of acetate-oxidizing manganese-reducing bacteria. *ISME J.* 6, 2078–90. doi:10.1038/ismej.2012.41.

Vandieken, V., and Thamdrup, B. (2013). Identification of acetate-oxidizing bacteria in a coastal marine surface sediment by RNA-stable isotope probing in anoxic slurries and intact cores. *FEMS Microbiol. Ecol.* 84, 373–86. doi:10.1111/1574-6941.12069.

Widdicombe, S., Spicer, J. I., and Kitidis, V. (2011). “Effects of ocean acidification on sediment fauna,” in *Ocean Acidification*, eds. P. Gattuso, J and L. Hansson (Oxford: Oxford University Press), 179.

Zhang, C., Stapleton, R., Zhou, J., Palumbo, A., and Phelps, T. (1999). Iron reduction by psychrotrophic enrichment cultures. *FEMS Microbiol. Ecol.* 30, 367–371. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10568845.

Chapter 5

Iron reduction in glucose-amended enrichment incubations with sediments from the methanic zone of the Helgoland mud area

Oluwatobi Oni, Sabine Kasten and Michael W. Friedrich

Summary

The very low relative (0.01-0.1%) abundance of iron-reducing *Desulfuromonadales*, in the methanic zone of the Helgoland mud area compared to surface sediments (3.5-8%), raised questions about their viability and iron reduction potential in the methanic zone. The possible biogenic origin of methane formed was also not confirmed. In addition, members of candidate division OP9/JS1 have been linked to iron reduction and fermentative/saccharolytic metabolisms but their possible role in iron reduction has not been tested in our study site. Results from sediment slurry incubations reported here show that *Desulfuromonadales* are viable and are capable of iron reduction in the methanic zone of Helgoland mud area. However, higher rates of iron reduction were observed in the presence of glucose compared to incubations without glucose which suggest that the subsurface sediment of the Helgoland mud area is limited in bio-available organic matter but rich in microbially-utilizable iron (III) mineral. Contrary to crystalline mineral phases such as hematite, easily reducible iron mineral (ferrihydrite and lepidocrocite) almost completely suppressed methanogenesis. JS1 bacteria were outcompeted for organic matter by other fermentative bacteria typically found in surface sediments.

Introduction

The source of elevated dissolved iron concentrations measured in the methanic zone of the Helgoland mud area is currently an important subject of interest (Oni et al., 2015). Among the hypotheses considered is the dissimilatory reduction of iron (III) minerals coupled to the oxidation of organic matter by microbial population such as members of *Desulfuromonadales* (Roden and Lovley, 1993). In the surface sediments of the Helgoland mud area, the relative abundance of *Desulfuromonadales* was higher in the zone of iron reduction (3.5- 8.2 % of total bacteria, Oni et al., 2015). However, in the methanic zone, their relative abundance was only (0.01-0.1%). Nevertheless, it is not known whether *Desulfuromonadales* present in the methanic zone are viable and may have potentially contributed to iron reduction *in situ*.

Recently, members of the candidate division OP9/JS1, mostly found in methane-rich sedimentary environments (Webster et al., 2004; Parkes et al., 2005; Inagaki et al., 2006; Webster et al., 2007; Parkes et al., 2007; Pachiadaki et al., 2011; Siegert et al., 2011; Chevalier et al., 2013; Lee et al., 2013), have also been linked to iron reduction in the methanic zone of the Helgoland mud area, although the mechanisms of iron reduction are not known (Oni et al., 2015). These populations are involved in the degradation of glucose or glucose metabolites in marine sediment enrichment slurries, under low sulfate conditions (Webster et al., 2006, 2011). Their potential for heterotrophic and saccharolytic metabolisms has also been confirmed by single cell genomics (Dodsworth et al., 2013; Nobu et al., 2015).

Glucose is usually one of the dominant monosaccharides in marine surface sediments (Cowie and Hedges, 1984; Tanoue and Handa, 1987; Moers et al., 1990) and has also been detected in considerable amounts in pore-water of subsurface sediments (Seifert et al., 2000). Fatty acids and hydrogen resulting from the fermentation of glucose can be utilized by members of the *Desulfuromonadales* (Coates et al., 1995; Greene et al., 2009). Therefore, using glucose as a potential energy source, we aimed to test the potential for iron reduction by *Desulfuromonadales* in the methanic zone of the Helgoland mud area. In addition, the possibility of biogenic methanogenesis and the competition of methanogens with iron reducers for common electron donors were investigated.

The dynamics of JS1 bacteria during glucose-dependent iron reduction was also studied to obtain preliminary information regarding role of this microbial group in iron reduction.

Materials and Methods

Sampling

A 5 m sediment core (HE376-007-5) was collected from the Helgoland mud area (54° 5.00' N, 7° 58.05' E) using a gravity corer and sectioned as described in Oni et al., 2015. Each 25 cm section of the gravity core was homogenized into 2.6 l jars under N_2 gas (99.999% purity). The headspaces of jars were filled with anoxic artificial sea water (ASW). Jars were tightly sealed and stored at 4°C until use.

Incubation set-up

Incubations were performed in 120 ml serum vials. 10 g of sediment from the methanic zone (305-330 cm; dissolved iron peak, Oni et al., 2015) was used to inoculate sterile anoxic 30 ml of sulfate-free ASW. ASW medium contained (g/l): NaCl, 26.4; $MgCl_2$, 11.2; $CaCl_2.2H_2O$, 1.5; KCl, 0.7. Serum vials were tightly sealed with butyl rubber septa and aluminum crimps. Vials were connected to a manifold and were further made anoxic by repeatedly (3 times for 5 min) expelling headspace gases using a vacuum pump (CVC 3000, Vacuubrand GmbH, Germany). After each step of vacuum application for 5 min, headspaces of vials were filled with N_2 (99.999 % purity). Sediment slurries were finally incubated under N_2 (99.999 % purity) headspaces. Synthetic amorphous FeOOH prepared by titrating $FeCl_2.6H_2O$ dissolved in water against NaOH following the methods of Schwertmann and Cornell (2000). Autoclaved hematite stock solution was prepared using commercially available hematite (α-Fe_2O_3; Bayferrox-110M, Laxness, Germany). To sediment incubations, as electron acceptors, hematite (25 mmol l^{-1}) and amorphous FeOOH (5 mmol l^{-1}) were added, and glucose solution (2mM) was added as electron donor where necessary, based on the experimental set-ups. Each treatment was performed in triplicate and slurry incubations were stored in the dark, without shaking, and at 10°C.

Geochemical Analyses

At specific time points, 1 ml of sediment slurry was drawn from incubations, with a sterile syringe flushed with N_2 gas, into 1.5 ml Eppendorf vials, pre-flushed with N_2 gas. Eppendorf vials were centrifuged at 20,000 *g* for 3 min. 100 µl of supernatant was immediately transferred into ferrozine solution to determine Fe^{2+} concentrations following Viollier et al., 2000. When higher Fe^{2+} concentrations were expected or measurements were not immediately done, supernatants were diluted or fixed in calculated amounts of 0.5 M HCl. 500 µl of supernatant was added to 500 µl zinc acetate solution (5 % v/v), and with this mixture, sulfate concentration in incubations was measured using a Metrohm Compact IC 761 ion chromatograph. The sediment pellets were stored at -20°C for molecular analysis of microbial communities.

Methane and CO_2 concentrations in the headspaces of incubation vials were monitored by injecting 100 µl of headspace gas into a gas chromatograph equipped with a capillary column (Porapak Q, 2 m x 1/8″, inner diameter 2 mm, mesh range:80/100, Agilent, Germany) and a flame ionization detector (FID; Shimadzu GC-2014, Tokyo, Japan). Gas Chromatograph was coupled to a methanizer (nickel reactor, CP 11952, Agilent, Germany) and was operated with the following parameters: H_2 as carrier gas (99.999% purity; 5 bar, 30 ml min^{-1} flow rate), H_2 (40 kPsi) as combustion gas, compressed air (50 kPsi) for combustion and N_2 as make-up gas (5 bar). Detector, injector, column and methanizer were operated at 200°C, 120°C, 70°C, and 350°C, respectively. Chromatographic data was recorded using a Peak Simple data system (SRI, model 202) and concentrations of methane and CO_2 were determined as described in Conrad et al., 1987.

Microbial Community Analysis

DNA Extraction and Pyrosequencing

DNA was extracted from sediment pellets as described in Oni et al., 2015. In preparation for pyrosequencing, PCR amplicons of bacterial and archaeal 16S rRNA genes were generated from DNA templates using primer pairs 27f, 907r for Bacteria and 109f, 912 rtr for Archaea (see Oni et al., 2015 for primer sequences). PCR conditions are as follows: initial denaturation (94°C for 5mins), final denaturation (94°C for 30 secs), annealing temperature (52°C for 1 min, Bacteria; 55°C for 1 min, Archaea), extension (72°C for 1 min), final elongation (72°C for 7 min). Final denaturation, annealing

and final elongation steps were repeated for 35 and 30 cycles for bacterial and archaeal 16S rRNA gene amplification, respectively. Reactions were kept at 4°C until further processing. Amplicons were purified and 16S rRNA genes were sequenced and analyzed as described in Oni et al., 2015.

Results

Geochemical Analysis

Iron reduction occurred most rapidly in glucose-amended incubations (Fig. 1A) with concomitant increase in headspace CO_2 formation (Fig. 1D). Formation of Fe^{2+} in aqueous phase had ceased by day 22 in glucose-amended incubations. At this point, up to 3 times more Fe^{2+} was measured in the overlying water of slurry incubations containing glucose and ferrihydrite compared to incubations containing glucose only or glucose and hematite (Fig.1A). Interestingly, Fe^{2+} concentrations measured in slurry incubations containing glucose or glucose and hematite (250-280 μM) were similar to pore-water iron concentrations measured at the sampled depth *in situ* (~ 300 μM, Oni et al., 2015). By the time iron reduction had ceased in glucose-amended incubations (day 22), concentrations of Fe^{2+} were lowest in control incubations in which glucose was not added (~ 140 μM). Starting concentrations of sulfate in the aqueous phase of slurry incubations were between 122-128 μM across all incubations (Fig.1B). However, in glucose-or glucose and iron (III)-amended incubations, sulfate was reduced to ~70 μM, at a rate of ~2.1-2.3 μM d^{-1}, by day 36 and stayed constant further on. Methane formation was not observed in incubations until after 80 days (Fig. 1C). Afterwards, methanogenesis occurred in treatments containing glucose and hematite (43.9 ± 1.44 nmol g wet $sed.^{-1}$ d^{-1}) and treatments containing glucose only (33.3 ± 8.44 nmol g wet $sed.^{-1}$ d^{-1}). Rates of methanogenesis in treatments with glucose and ferrihydrite (0.3 ± 0.22 nmol g wet $sed.^{-1}$ d^{-1}) were comparable to rates in slurry incubations without additional electron donors (~ 0.1 nmol g wet $sed.^{-1}$ d^{-1}). Overall glucose-dependent methanogenesis was suppressed by ferrihydrite almost completely (74 ±18 %, glucose only treatments; 98.6 ± 1.4 %, glucose and hematite treatments).

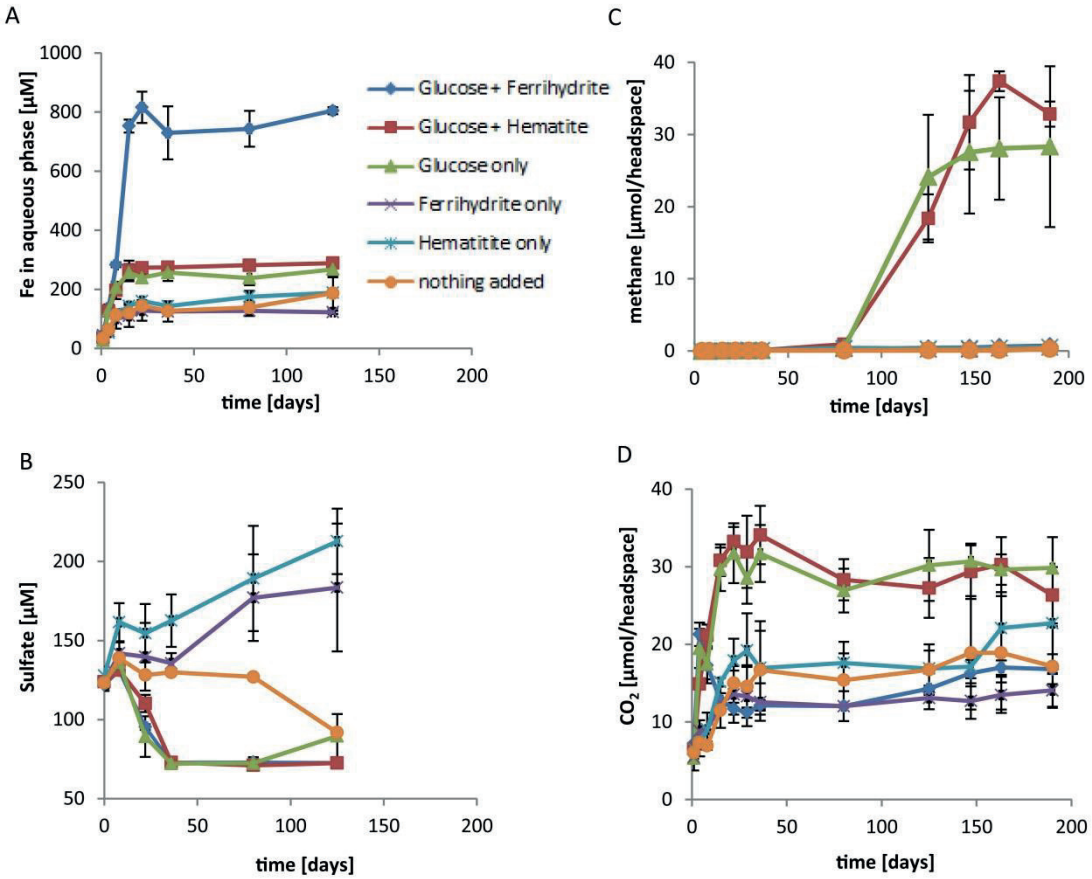

Fig.1. Iron reduction (A), sulfate reduction (B) methane formation (C), CO_2 formation in glucose and iron (III)-amended incubations. Headspace volume is 80 ml. Iron and sulfate concentrations in aqueous phase of slurry incubations were only measured until day 125 while methane formation was measured until day 190. Values are 1 SD of n=3

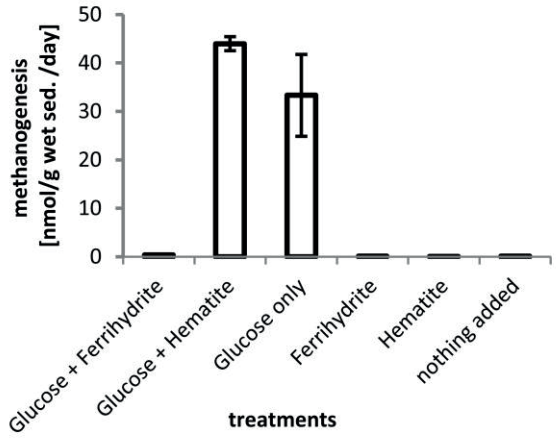

Fig. 2. Methanogenesis rates in sediment slurry incubations. Methane formation from glucose degradation was heavily suppressed by iron reduction.

Microbial Community Composition

Based on geochemical results, microbial community changes were only studied in glucose-containing incubations in order to know which microbial populations were specifically responsive to the presence of reducible iron minerals under glucose amendment as well as archaeal groups involved in methanogenesis. During the course of incubation, typical subsurface sediment bacterial populations such as members of *Chloroflexi* and candidate division OP9/JS1 were replaced by members of the *Firmicutes* (mostly *Clostridiales*, Fig. 3A) and *Gammaproteobacteria* (predominantly *Vibrionaceae* and *Alteromondales*; Fig. 3A, 3C). *Deltaproteobacteria* predominated in incubations with glucose and ferrihydrite, where iron reduction was most pronounced (3.8 %, 13 %, and 18 % of total bacterial populations on day 0, 36, and 125 respectively; Fig. 2B). Specifically, most common iron-reducing

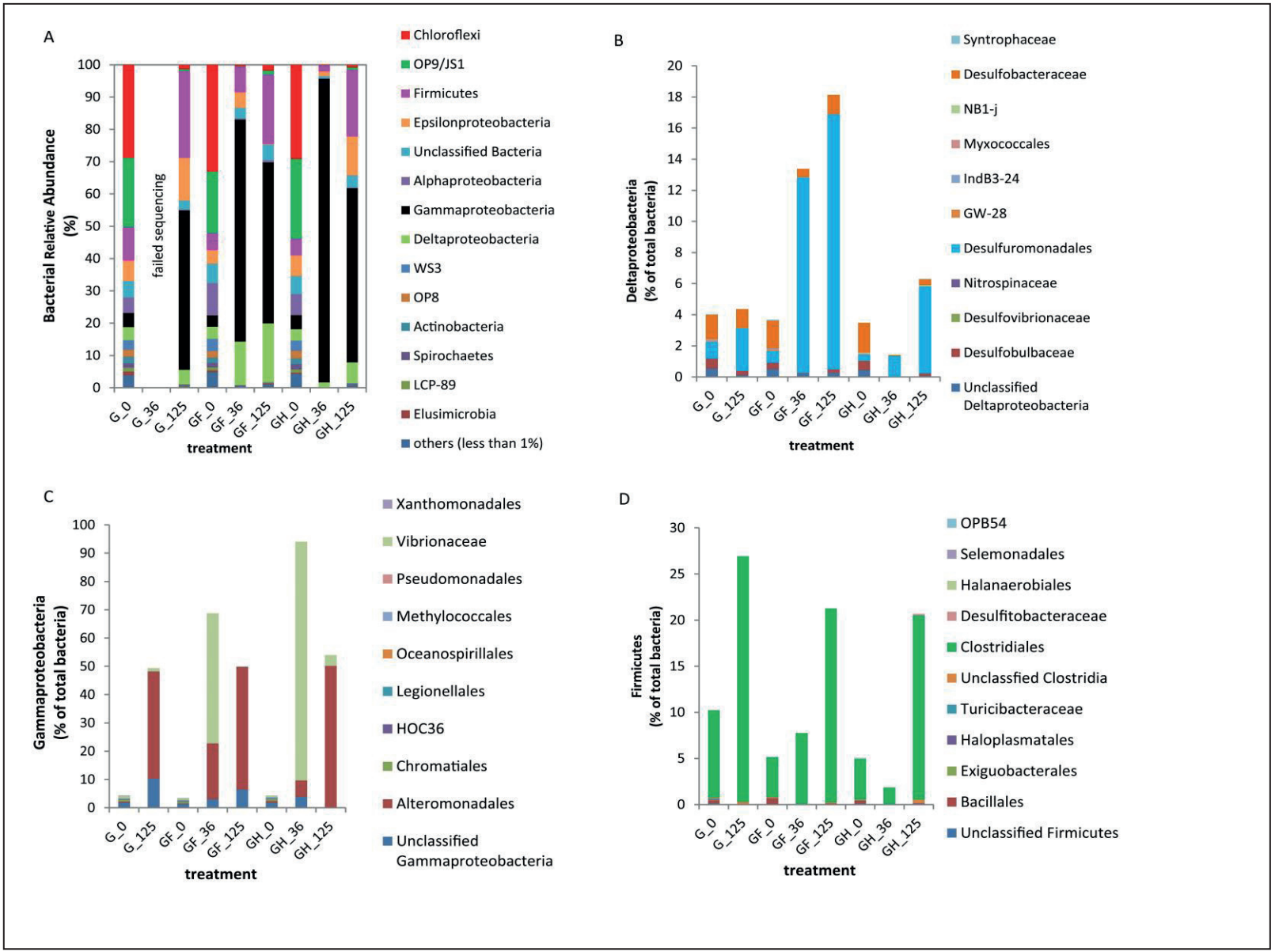
A
Bacterial Relative Abundance (%)
failed sequencing
treatment
G_0
G_36
G_125
GF_0
GF_36
GF_125
GH_0
GH_36
GH_125
Chloroflexi
OP9/JS1
Firmicutes
Epsilonproteobacteria
Unclassified Bacteria
Alphaproteobacteria
Gammaproteobacteria
Deltaproteobacteria
WS3
OP8
Actinobacteria
Spirochaetes
LCP-89
Elusimicrobia
others (less than 1%)
B
Deltaproteobacteria (% of total bacteria)
treatment
Syntrophaceae
Desulfobacteraceae
NB1-j
Myxococcales
IndB3-24
GW-28
Desulfuromonadales
Nitrospinaceae
Desulfovibrionaceae
Desulfobulbaceae
Unclassified Deltaproteobacteria
C
Gammaproteobacteria (% of total bacteria)
treatment
Xanthomonadales
Vibrionaceae
Pseudomonadales
Methylococcales
Oceanospirillales
Legionellales
HOC36
Chromatiales
Alteromonadales
Unclassified Gammaproteobacteria
D
Firmicutes (% of total bacteria)
treatment
OPB54
Selemonadales
Halanaerobiales
Desulfitobacteraceae
Clostridiales
Unclassfied Clostridia
Turicibacteraceae
Haloplasmatales
Exiguobacterales
Bacillales
Unclassified Firmicutes

Fig. 3. Bacterial community composition in glucose/glucose and iron (III) amended incubations. Phylum and class level classification (A), *Deltaproteobacteria* (B), *Gammaproteobacteria* (C), *Firmicutes* (D) relative abundances in slurry incubations. Glucose only (G), Glucose and Ferrihydrite (GF), Glucose and Hematite (GH) represents the different treatments and number in front represent days.

Deltaproteobacteria in marine sediments, *Desulfuromonadales,* dominated Deltaproteobacterial groups with time across the treatments (Fig. 2B). The relative abundances of *Desulfuromonadales* shifted from an average of 0.6 % of total bacteria, on day 0, across sediments slurry incubations, with different treatments, to ~3 % in glucose only, 16 % in glucose and ferrihydrite, and 6 % of total bacteria in glucose and hematite incubations (Fig. 2B)

By day 125, at which point higher methane concentrations were measured in headspaces of incubation vials, relative abundances of methanogens belonging to the *Methanosarcinaceae*, had increased. *Methanosarcinacae*, from ~ 0.9-1.5 % of total archaea on day 0 and 36, across the treatments studied, increased by ~10 fold in glucose- and ~11 fold in glucose and hematite-containing slurry incubations, on day 125 (Fig. 4). Most of the *Methanosarcinaceae* detected in the methanic zone of our study site are related to methane-oxidizing archaea, ANME-3 (Oni et al, 2015). However, the methanogenic populations responsible for methane formation in the incubations, clone A79 (KP987248.1, or Helgoland Meth6, Oni et al., 2015) constituted 1 % of *Methanosarcinceae* (Oni et al., 2015) at the depth from which sediments were sampled for these experiments (305-330 cm). In slurry incubations containing glucose and ferrihydrite, the relative abundances of *Methanosarcinaceae* remained the same on day 125 as day 36.

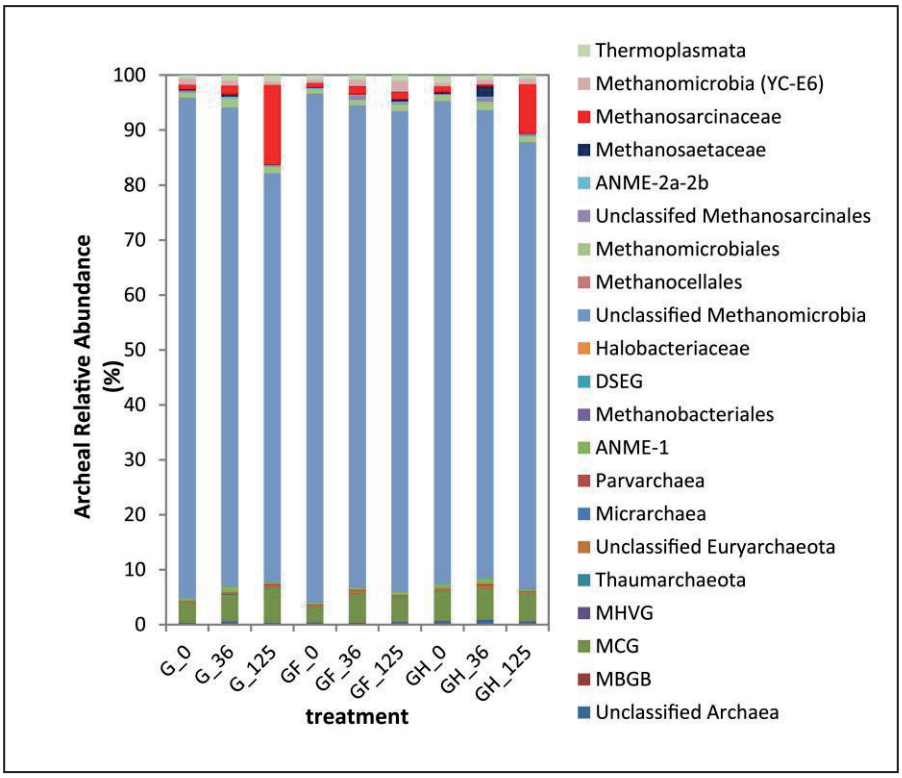

Fig. 4. Archaeal community composition in glucose and glucose plus iron (III) amended incubations. Group affiliations are presented to family level. Glucose only (G), Glucose and Ferrihydrite (GF), Glucose and Hematite (GH) represents the different treatments and number in front represent days. *Methanosarcinaceae* (red), in glucose /glucose and hematite incubations were most abundant on day 125, when higher methane concentrations were detected.

Discussion

To understand the source of iron reduction in methanic sediments of the Helgoland mud area, we have monitored shift in bacteria and archaea communities (especially candidate division JS1 bacteria, *Desulfuromonadales* and methanogens) under glucose-dependent iron-reducing conditions. Our results show that the presence of reactive organic matter (glucose) resulted in immediate iron reduction and stimulation of classical iron reducers in surface marine sediments (*Desulfuromonadales*) that were previously very low in relative abundance. In addition, JS1 bacteria were outcompeted by fast-growing bacterial populations such as *Clostridiales* and *Gammaproteobacteria*. Overall, iron reduction almost completely suppressed methanogenesis.

Fe (III) reduction

The rapid stimulation of iron reduction on addition of glucose (Fig. 1A) compared to glucose-devoid incubations suggests that the methanic sediments of the Helgoland mud area is limited in reactive/bioavailable organic matter. Similar concentrations of Fe^{2+} in slurry incubations containing glucose only or glucose and hematite suggests that microorganisms could not couple degradation of glucose to reduction of crystalline iron mineral phases such as hematite. Nevertheless, the rapid occurrence of high concentrations (up to ~ 270 µM) of Fe^{2+} in the aqueous phase of these incubations shows that the methanic zone of the Helgoland mud area is rich in amorphous iron (III) minerals which are bioavailable (Raiswell et al., 2008). This confirms recent Mössbauer spectroscopy results which showed that amorphous iron (III) minerals (mainly lepidocrocite) constitute up to 24 % of total Fe (III) species in the methanic zone of the Helgoland mud area (Oni et al., 2015). Rapid enhancement of iron reduction in the presence of synthetic lepidocrocite (Fig. 5A) also suggests that iron-reducing microorganisms in the subsurface sediments of the Helgoland mud area seem to be able to utilize lepidocrocite.

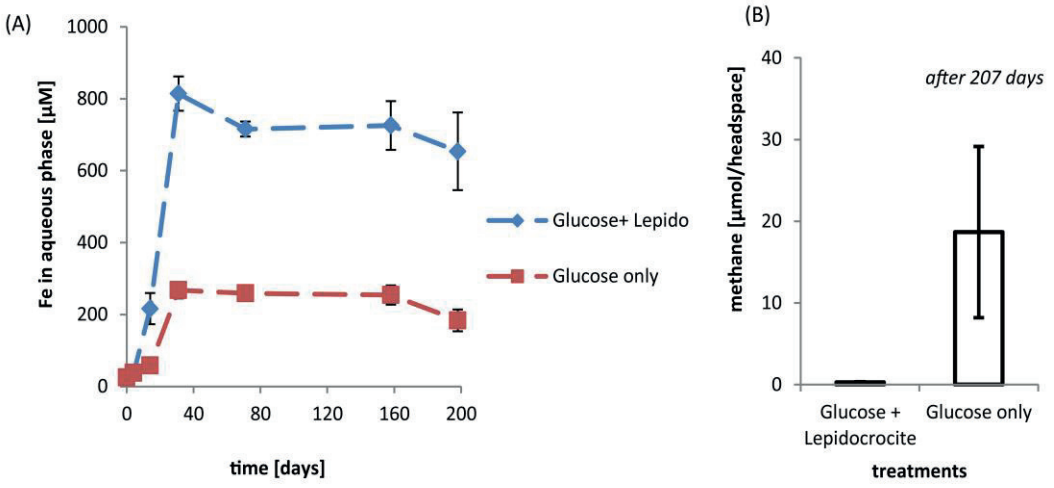

Fig. 5. Effect of glucose-dependent lepidocrocite reduction on methane formation in sediment slurry incubations. Lepidocrocite reduction suppressed methanogensis. Glucose and lepidocrocite were added at concentrations of 2 mM and 5 mmol l^{-1}, respectively.

Members of the *Desulfuromonadales* are usually abundant in surface sediments where fresh organic matter is more abundant and iron reduction is occurring (e.g Aarhus Bay; Vandieken and Thamdrup,

2013, Helgoland mud area; Oni et al., 2015). This explains their increase in relative abundances in iron-reducing slurry incubations when glucose was supplemented (Fig. 3B).

Other dominant bacterial populations such as members of the *Alteromonadales* (specifically, *Psychromonadaceae*; Köpke et al., 2005) and *Clostridiales* (Hammann and Ottow, 1974; Dobbin et al., 1999) have been linked to iron reduction. However, unlike *Desulfuromonadales*, they did not show highest relative abundance in incubations in which most Fe^{2+} concentrations were measured. Thus, iron reduction by these bacterial groups was most likely not coupled to growth but a minor proportion of electrons generated during fermentative degradation of glucose or glucose metabolites may have been transferred to iron (III) minerals (Lovley and Phillips, 1986). Unfortunately, electron balances could not be calculated here because of the difficulty in measuring fermentation intermediates in marine samples via our in-house high pressure liquid chromatography (HPLC) system. Measurements with marine pore-water samples are complicated by the high concentrations of anion chloride that interfere with analytical methods (Glombitza et al., 2014). However, new sample treatment methods and instrument adaptations (e.g. Glombitza et al., 2014) are emerging to alleviate this problem.

Sulfate reduction

The occurrence of sulfate reduction during the phase of iron reduction in all glucose-containing incubations suggests that sulfate reduction was not inhibited despite low starting concentrations of sulfate (~125 μM, Fig. 1B). It also confirms the presence of potentially active sulfate reducers in the methanic sediments of the Helgoland mud area, which are likely limited by reactive organic carbon as sulfate reduction was not glucose-devoid incubations.

Methanogenesis

Methanogenesis by members of the *Methanosarcinaceae* (Fig. 4) suggest that some methanogenic populations in the methanic zone of the Helgoland mud area are viable and methane formed in the Helgoland mud area is likely biogenic. The strong suppression of methanogenesis in slurry incubations containing glucose and ferrihydrite or lepidocrocite (Fig. 5B) may indicate substrate competition between methanogens and iron reducers such as *Desulfuromonadales*. However, this sort of competitive inhibition is not expected in the methanic sediments of the Helgoland, because methanogens were most abundant around the region of highest dissolved iron formation (Oni et al.,

2015). Therefore, classical organoclastic iron reduction by bacterial populations such as members of *Desulfuromonadales* is unlikely to explain iron reduction in the methanic zone of the Helgoland mud area as methanogenesis would have been strongly inhibited.

Proliferation of fast-growing and medium-adapted microbial populations

Together with *Psychromonadaceae* and *Clostridiales,* members of *Vibrionaceae* dominated all glucose-amended incubations (Fig. 3C, 3D). These groups are fast growing microbes often present in marine surface sediment which took advantage of the presence of highly reactive organic matter such as glucose, as their (*Psychromonadaceae* and *Vibrionaceae*) relative abundances were very low at day 0 (Fig. 3C). Their fast growth and high affinity for fresh organic matter may have allowed them to out-compete typical, slow-growing subsurface microbial populations such as JS1 and *Chloroflexi*, which are capable of degrading organic matter but may be better adapted to more recalcitrant organic matter or organic matter at lower concentrations than that used in this study (Thorn and Ventullo, 1988; Hoehler and Jørgensen, 2013). Moreover, the divergence of dominant archaeal populations on day 0 (Fig. 1) from archaeal populations, such as Miscellaneous Crenarchaeota Groups (MCG), ANME populations etc., dominant in original sediment (see Chapter 2, Fig. 7) is likely due to the "Bottle Effect" (Fuchs et al., 2000).This may explain the proliferation of uncultured *Methanomicrobia* which dominated archaeal populations upon storage of sediments at 4°C for 9 months in jars and overlaid with sulfate-free ASW, before this experiment was carried out. Nevertheless, this dominant uncultured *Methanomicrobia* population seemed not to play a role in methanogenesis as their relative abundances stayed almost constant before the onset of methanogenesis after day 80, by *Methanosarcinceae* (clone A79 or Helgoland Meth6, Oni et al., 2015) which were present in the original sediment.

Conclusions

Even though, known iron reducers in marine sediments such as members of *Desulfuromonadales* are present in the methanic zone of the Helgoland mud area and they may have contributed in part to iron reduction therein. However, they are likely not responsible for the iron reduction as they were limited in abundance and are probably limited by the presence of high-quality organic matter contrary to surface sediments (see Chapter 2). In addition, due to substrate competition, iron reduction by these

populations *in situ* will likely inhibit methanogenesis as shown, contrary to observations in the Helgoland mud area, where there seem to be a positive relationship between iron reduction, methanogens and some methane-oxidizing archaea (Oni et al., 2015). In addition, JS1 bacteria are likely not very competitive against physiologically similar microorganisms like *Firmicutes* (Dodsworth et al., 2013; Nobu et al., 2015) for fresh organic matter or organic matter at higher concentrations.

References

Chevalier, N., Bouloubassi, I., Birgel, D., Taphanel, M.-H., and López-García, P. (2013). Microbial methane turnover at Marmara Sea cold seeps: a combined 16S rRNA and lipid biomarker investigation. *Geobiology* 11, 55–71. doi:10.1111/gbi.12014.

Coates, J. D., Lonergan, D. J., Philips, E. J., Jenter, H., and Lovley, D. R. (1995). *Desulfuromonas palmitatis sp. nov*., a marine dissimilatory Fe(III) reducer that can oxidize long-chain fatty acids. *Arch. Microbiol.* 164, 406–13. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8588742.

Conrad, R., Schütz, H., and Babbel, M. (1987). Temperature limitation of hydrogen turnover and methanogenesis in anoxic paddy soil. *FEMS Microbiol. Lett.* 45, 281–289. doi:10.1111/j.1574-6968.1987.tb02378.x.

Cowie, G. L., and Hedges, J. I. (1984). Carbohydrate sources in a coastal marine environment. *Geochim. Cosmochim. Acta* 48, 2075–2087. doi:10.1016/0016-7037(84)90388-0.

Dobbin, P. S., Carter, J. P., Garcia-Salamanca San Juan, C., von Hobe, M., Powell, A. K., and Richardson, D. J. (1999). Dissimilatory Fe(III) reduction by *Clostridium beijerinckii* isolated from freshwater sediment using Fe(III) maltol enrichment. *FEMS Microbiol. Lett.* 176, 131–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10418140.

Dodsworth, J. A., Blainey, P. C., Murugapiran, S. K., Swingley, W. D., Ross, C. A., Tringe, S. G., Chain, P. S. G., Scholz, M. B., Lo, C.-C., Raymond, J., et al. (2013). Single-cell and metagenomic analyses indicate a fermentative and saccharolytic lifestyle for members of the OP9 lineage. *Nat. Commun.* 4, 1854. doi:10.1038/ncomms2884.

Fuchs, B. M., Zubkov, M. V, Sahm, K., Burkill, P. H., and Amann, R. (2000). Changes in community composition during dilution cultures of marine bacterioplankton as assessed by flow cytometric and molecular biological techniques. *Environ. Microbiol.* 2, 191–201. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11220305.

Glombitza, C., Pedersen, J., Røy, H., and Jørgensen, B. B. (2014). Direct analysis of volatile fatty acids in marine sediment porewater by two-dimensional ion chromatography-mass spectrometry. *Limnol. Oceanogr. Methods* 12, 455–468. doi:10.4319/lom.2014.12.455.

Greene, A. C., Patel, B. K. C., and Yacob, S. (2009). Geoalkalibacter subterraneus sp. nov., an anaerobic Fe(III)- and Mn(IV)-reducing bacterium from a petroleum reservoir, and emended

descriptions of the family *Desulfuromonadaceae* and the genus *Geoalkalibacter*. *Int. J. Syst. Evol. Microbiol.* 59, 781–5. doi:10.1099/ijs.0.001537-0.

Hammann, R., and Ottow, J. C. G. (1974). Reductive Dissolution of Fe_2O_3 by Saccharolytic *Clostridia* and *Bacillus polymyxa* under anaerobic Conditions. *Zeitschrift für Pflanzenernährung und Bodenkd.* 137, 108–115. doi:10.1002/jpln.19741370205.

Hoehler, T. M., and Jørgensen, B. B. (2013). Microbial life under extreme energy limitation. *Nat. Rev. Microbiol.* 11, 83–94. doi:10.1038/nrmicro2939.

Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., Suzuki, M., Takai, K., Delwiche, M., Colwell, F. S., et al. (2006). Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proc. Natl. Acad. Sci. U. S. A.* 103, 2815–20. doi:10.1073/pnas.0511033103.

Köpke, B., Wilms, R., Engelen, B., Cypionka, H., and Sass, H. (2005). Microbial diversity in coastal subsurface sediments: a cultivation approach using various electron acceptors and substrate gradients. *Appl. Environ. Microbiol.* 71, 7819–30. doi:10.1128/AEM.71.12.7819-7830.2005.

Lee, J.-W., Kwon, K. K., Azizi, A., Oh, H.-M., Kim, W., Bahk, J.-J., Lee, D.-H., and Lee, J.-H. (2013). Microbial community structures of methane hydrate-bearing sediments in the Ulleung Basin, East Sea of Korea. *Mar. Pet. Geol.* 47, 136–146. doi:10.1016/j.marpetgeo.2013.06.002.

Lovley, D. R., and Phillips, E. J. (1986). Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Appl. Environ. Microbiol.* 51, 683–9. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=238947&tool=pmcentrez&rendertype=abstract.

Moers, M. E. C., Baas, M., de Leeuw, J. W., and Schenck, P. A. (1990). Analysis of neutral monosaccharides in marine sediments from the equatorial eastern Atlantic. *Org. Geochem.* 15, 367–373. doi:10.1016/0146-6380(90)90163-T.

Nobu, M. K., Dodsworth, J. a, Murugapiran, S. K., Rinke, C., Gies, E. A, Webster, G., Schwientek, P., Kille, P., Parkes, R. J., Sass, H., et al. (2015). Phylogeny and physiology of candidate phylum "Atribacteria" (OP9/JS1) inferred from cultivation-independent genomics. *ISME J.*, 1–14. doi:10.1038/ismej.2015.97.

Oni, O., Miyatake, T., Kasten, S., Richter-Heitmann, T., Fischer, D., Wagenknecht, L., Kulkarni, A., Blumers, M., Shylin, S. I., Ksenofontov, V., et al. (2015). Distinct microbial populations are tightly linked to the profile of dissolved iron in the methanic sediments of the Helgoland mud area, North Sea. *Front. Microbiol.* 06, 1–15. doi:10.3389/fmicb.2015.00365.

Pachiadaki, M. G., Kallionaki, A., Dählmann, A., De Lange, G. J., and Kormas, K. A. (2011). Diversity and spatial distribution of prokaryotic communities along a sediment vertical profile of a deep-sea mud volcano. *Microb. Ecol.* 62, 655–68. doi:10.1007/s00248-011-9855-2.

Parkes, R. J., Cragg, B. A., Banning, N., Brock, F., Webster, G., Fry, J. C., Hornibrook, E., Pancost, R. D., Kelly, S., Knab, N., et al. (2007). Biogeochemistry and biodiversity of methane cycling in subsurface marine sediments (Skagerrak, Denmark). *Environ. Microbiol.* 9, 1146–61. doi:10.1111/j.1462-2920.2006.01237.x.

Parkes, R. J., Webster, G., Cragg, B. A., Weightman, A. J., Newberry, C. J., Ferdelman, T. G., Kallmeyer, J., Jørgensen, B. B., Aiello, I. W., and Fry, J. C. (2005). Deep sub-seafloor

prokaryotes stimulated at interfaces over geological time. *Nature* 436, 390–4. doi:10.1038/nature03796.

Raiswell, R., Benning, L. G., Tranter, M., and Tulaczyk, S. (2008). Bioavailable iron in the Southern Ocean: the significance of the iceberg conveyor belt. *Geochem. Trans.* 9, 7. doi:10.1186/1467-4866-9-7.

Roden, E. E., and Lovley, D. R. (1993). Dissimilatory Fe(III) reduction by the marine microorganism *Desulfuromonas acetoxidans*. *Appl. Environ. Microbiol.* 59, 734–42. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=202183&tool=pmcentrez&rendertype=abstract.

Schwertmann, U., and Cornell, R. M. (2000). *Iron oxides in the Laboratory- Preparation and Characterization*. Second. Weinheim: WILEY-VCH Verlag GmbH & Co. KGaA Available at: http://doi.wiley.com/10.1002/jpln.19921550321 [Accessed July 3, 2015].

Seifert, R., Emeis, K., Michaelis, W., and Degens, E. T. (2000). Amino acids and carbohydrates in sediments and interstitial waters from Site 681, Leg 112, Peru Continental Margin. in *Proceedings of the Ocean Drilling Program*, 555–566.

Siegert, M., Krüger, M., Teichert, B., Wiedicke, M., and Schippers, A. (2011). Anaerobic oxidation of methane at a marine methane seep in a forearc sediment basin off Sumatra, Indian Ocean. *Front. Microbiol.* 2, 249. doi:10.3389/fmicb.2011.00249.

Tanoue, E., and Handa, N. (1987). Monosaccharide composition of marine particles and sediments from the Bering Sea and northern North Pacific. *Oceanol. Acta* 10, 91–99.

Thorn, P. M., and Ventullo, R. M. (1988). Measurement of bacterial growth rates in subsurface sediments using the incorporation of tritiated thymidine into DNA. *Microb. Ecol.* 16, 3–16. doi:10.1007/BF02097401.

Vandieken, V., and Thamdrup, B. (2013). Identification of acetate-oxidizing bacteria in a coastal marine surface sediment by RNA-stable isotope probing in anoxic slurries and intact cores. *FEMS Microbiol. Ecol.* 84, 373–86. doi:10.1111/1574-6941.12069.

Viollier, E., Inglett, P. ., Hunter, K., Roychoudhury, A. ., and Van Cappellen, P. (2000). The ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters. *Appl. Geochemistry* 15, 785–790. doi:10.1016/S0883-2927(99)00097-9.

Webster, G., Parkes, R. J., Fry, J. C., and Weightman, A. J. (2004). Widespread occurrence of a novel division of bacteria identified by 16S rRNA gene sequences originally found in deep marine sediments. *Appl. Environ. Microbiol.* 70, 5708–5713. doi:10.1128/AEM.70.9.5708.

Webster, G., Sass, H., Cragg, B. A., Gorra, R., Knab, N. J., Green, C. J., Mathes, F., Fry, J. C., Weightman, A. J., and Parkes, R. J. (2011). Enrichment and cultivation of prokaryotes associated with the sulphate-methane transition zone of diffusion-controlled sediments of Aarhus Bay, Denmark, under heterotrophic conditions. *FEMS Microbiol. Ecol.* 77, 248–63. doi:10.1111/j.1574-6941.2011.01109.x.

Webster, G., Watt, L. C., Rinna, J., Fry, J. C., Evershed, R. P., Parkes, R. J., and Weightman, A. J. (2006). A comparison of stable-isotope probing of DNA and phospholipid fatty acids to study prokaryotic functional diversity in sulfate-reducing marine sediment enrichment slurries. *Environ. Microbiol.* 8, 1575–89. doi:10.1111/j.1462-2920.2006.01048.x.

Webster, G., Yarram, L., Freese, E., Köster, J., Sass, H., Parkes, R. J., and Weightman, A. J. (2007). Distribution of candidate division JS1 and other Bacteria in tidal sediments of the German Wadden Sea using targeted 16S rRNA gene PCR-DGGE. *FEMS Microbiol. Ecol.* 62, 78–89. doi:10.1111/j.1574-6941.2007.00372.x.

Chapter 6

Preliminary results on the effect of methane on iron reduction in slurry incubations with methanic sediments from the Helgoland mud area

Oluwatobi Oni, Sabine Kasten and Michael W. Friedrich

Results in chapter 5 suggested that iron reduction and methanogenesis are unlikely to occur simultaneously in methanic sediments of the Helgoland mud area. Typical organoclastic iron reducers in marine sediments such as *Desulfuromonadales* seemed to out-compete methanogens for similar electron donors. In addition, organic matter in the subsurface sediments of the Helgoland mud area appeared to be not readily-bioavailable, although abundant.

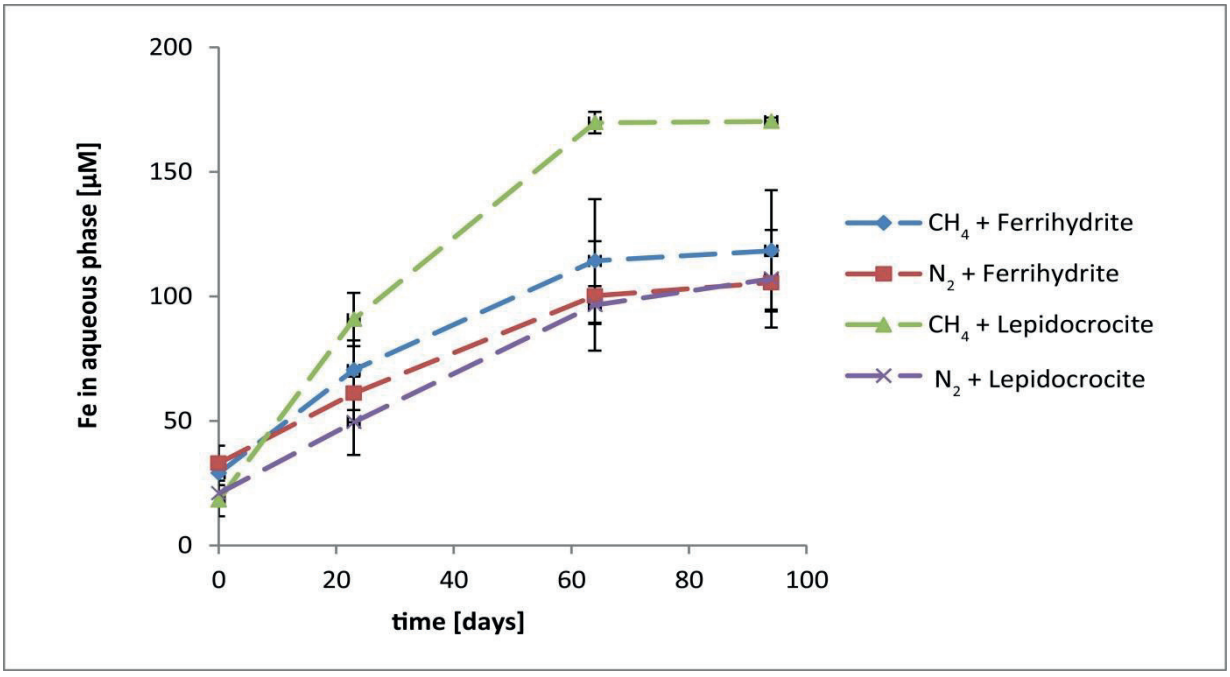

Fig. 1: Effect of methane on iron reduction in sediment slurry incubations. 20 g of sediment (HE 406-008, 275-300 cm) was added to 20 ml ASW. Incubations were set up as described in previous chapters. Slurries were incubated at 25°C with the aim of accelerating methane oxidation; a process which otherwise would be very slow (Nauhaus et al., 2007). Error bars are 1S.D of triplicate measurements.

The concurrent detection of high concentrations of methane and dissolved iron in the methanic zone of our study site hints at the absence of competitive inhibition of methanogenesis and iron reduction.

Therefore, alternative hypotheses had to be tested. In sediment slurry microcosm experiments, we tested the potential for methane to fuel iron reduction in the methanic zone of the Helgoland mud area. Indeed, higher concentrations of dissolved iron could be measured in incubations with methane (0.2 bar) and iron (III) minerals (ferrihydrite, 10 mmol l^{-1} and lepidocrocite, 10 mmol l^{-1}) compared to control incubations with N_2 (0.2 bar) as headspace gas and supplemented iron minerals (Fig. 1). Interestingly, slurries treated with methane and lepidocrocite showed highest dissolved iron concentrations over the incubation period (Fig. 1), consistent with the detection of lepidocrocite as the microbially-relevant iron (III) mineral dominant *in situ* (Oni et al., 2015). This result suggests that lepidocrocite reduction was enhanced by the presence of methane. Nevertheless, the data at hand must be interpreted with caution, as concentrations of dissolved iron so far measured are low (Fig. 1), considering that 10 mmol l^{-1} Fe (III) was added. In Chapter 5 (Figs. 1A and 4), addition of 5 mmol l^{-1} Fe (III) resulted in ~ 800 µM Fe^{2+} in aqueous phase when iron reduction had reached completion whereas here, only ~ 100-170 µM Fe $^{2+}$ was detected, with iron reduction seemingly reaching completion already by day 94. To verify the occurrence of methane oxidation coupled to lepidocrocite reduction more sensitively, $^{13}CH_4$ radiotracer experiments such as in Sivan et al., 2011 and Egger et al., 2014 will be necessary.

References

Egger, M., Rasigraf, O., Sapart, C. J., Jilbert, T., Jetten, M. S. M., Röckmann, T., van der Veen, C., Banda, N., Kartal, B., Ettwig, K. F., et al. (2014). Iron-mediated anaerobic oxidation of methane in brackish coastal sediments. *Environ. Sci. Technol.* 277, 277–283. doi:10.1021/es503663z.

Nauhaus, K., Albrecht, M., Elvert, M., Boetius, A., and Widdel, F. (2007). In vitro cell growth of marine archaeal-bacterial consortia during anaerobic oxidation of methane with sulfate. *Environ. Microbiol.* 9, 187–96. doi:10.1111/j.1462-2920.2006.01127.x.

Oni, O., Miyatake, T., Kasten, S., Richter-Heitmann, T., Fischer, D., Wagenknecht, L., Kulkarni, A., Blumers, M., Shylin, S. I., Ksenofontov, V., et al. (2015). Distinct microbial populations are tightly linked to the profile of dissolved iron in the methanic sediments of the Helgoland mud area, North Sea. *Front. Microbiol.* 06, 1–15. doi:10.3389/fmicb.2015.00365.

Sivan, O., Adler, M., Pearson, A., Gelman, F., Bar-Or, I., John, S. G., and Eckert, W. (2011). Geochemical evidence for iron-mediated anaerobic oxidation of methane. *Limnol. Oceanogr.* 56, 1536–1544. doi:10.4319/lo.2011.56.4.1536.

Chapter 7

Hematite accelerates methanogenic degradation of benzoate in marine sediment slurry incubations

Oluwatobi Oni and Michael W. Friedrich

Via several mechanisms, the methane and iron cycles are connected (Beal et al., 2009; Kato et al., 2012; Jiang et al., 2013; Riedinger et al., 2014; Egger et al., 2014; Rotaru et al., 2014). Most recently discovered of the different mechanisms is the transfer of electrons between fermentative microbes and methanogens via conductive iron mineral as electron conduits, a concept termed "electric syntrophy" (Kato et al., 2012) or direct interspecies electron transfer (DIET, Rotaru et al., 2014). The enhancement of methanogenic degradation of organic matter with (semi)conductive iron minerals such as hematite, goethite, magnetite, pyrite, *etc*. has been demonstrated in rice field soils (Kato et al., 2012; Zhuang et al., 2015a, 2015b) as well as anaerobic sludge and digesters (Rotaru et al., 2014; Viggi et al., 2014). Until now, DIET has not been studied in marine sediments, where it may in fact, play a major role in mediating metabolic interactions between marine microorganisms since conductive minerals are abundant. DIET may be even more important in subsurface sediments due to high energy limitations (see review: Lever, 2011) and reliance of inhabiting microorganisms on fermentative or syntrophic metabolisms. It is noteworthy that varying microbial populations have been reported to mediate DIET in different environments: For example DIET in rice field soils studied by Kato et al. was mainly mediated by *Geobacter* and *Methanosarcinales* whereas in other studies, it could be mediated by other microbes, e.g. *Bacillaceae*, *Peptococcaceae*, *Sedimentibacter* and *Methanobacterium* (Zhuang et al., 2015a, 2015b). This means that marine sediments (especially subsurface sediments), harboring distinct microbial populations, may offer novel microorganisms capable of DIET.

In the subsurface sediments of the Helgoland mud area, a tight correlation has been observed between JS1 bacteria and methanogens, and this association is linked to the iron cycle (Oni et al., 2015). Although not detected by Mössbauer spectroscopy, sequential extraction of iron minerals show that

conductive crystalline iron mineral phases (goethite and hematite) are as abundant as bio-reducible iron mineral phases (lepidocrocite), and both phases follow a similar distribution pattern with depth (Oni et al., 2015). Since JS1 bacteria have been linked to fermentative, saccharolytic and syntrophic metabolism (Dodsworth et al., 2013; Gies et al., 2014; Nobu et al., 2015), it is conceivable their tight correlation with methanogens is due to metabolic dependencies and perhaps facilitated by the iron minerals. This hypothesis is thus, worth testing, and as a first step, it was necessary to test the potential for enhancing methanogenesis with a conductive crystalline iron mineral in sediments of the Helgoland mud area. The choice of benzoate as the electron donor was motivated by the fact that close relatives of JS1 bacteria detected in the Helgoland mud area (Oni et al., 2015) have been found members of the microbial populations in a stable benzene-mineralizing enrichment cultur (Phelps et al., 1998). However, due to the recalcitrant nature and toxicity of benzene, benzoate, being an intermediate in benzene degradation was selected. Sediment slurry incubations were prepared as described in chapters 5 and 6. Benzoate, ferrihydrite and lepidocrocite were added at concentrations of 2 mM (80 µmol), 1 0 $mmol^{-1}$, and 30 $mmol^{-1}$, respectively. Sediment slurries were incubated at 30 °C to allow methanogenesis proceed faster. Methanogenesis started after 168 days in sediment slurries treated with benzoate alone and benzoate with hematite **(Fig. 1A)**. According to the stoichiometry of methanogenic degradation of benzoate, 1 mole of benzoate is expected to yield 3.75 moles of methane (Schink, 1997), which means 300 µmol of methane would be expected if all 80 µmol of benzoate supplemented was turned over, however, only 140 µmol could be recovered experimentally (Fig. 1A), resulting in a 46 % conversion efficiency of benzoate to methane. Addition of ferrihydrite suppressed methanogenic degradation of benzoate but stimulated highest iron reduction (up 400 µM dissolved Fe in aqueous phase, Fig. 1B), suggesting that iron reducers may have outcompeted methanogens for electron equivalents. Relative to incubations without hematite,

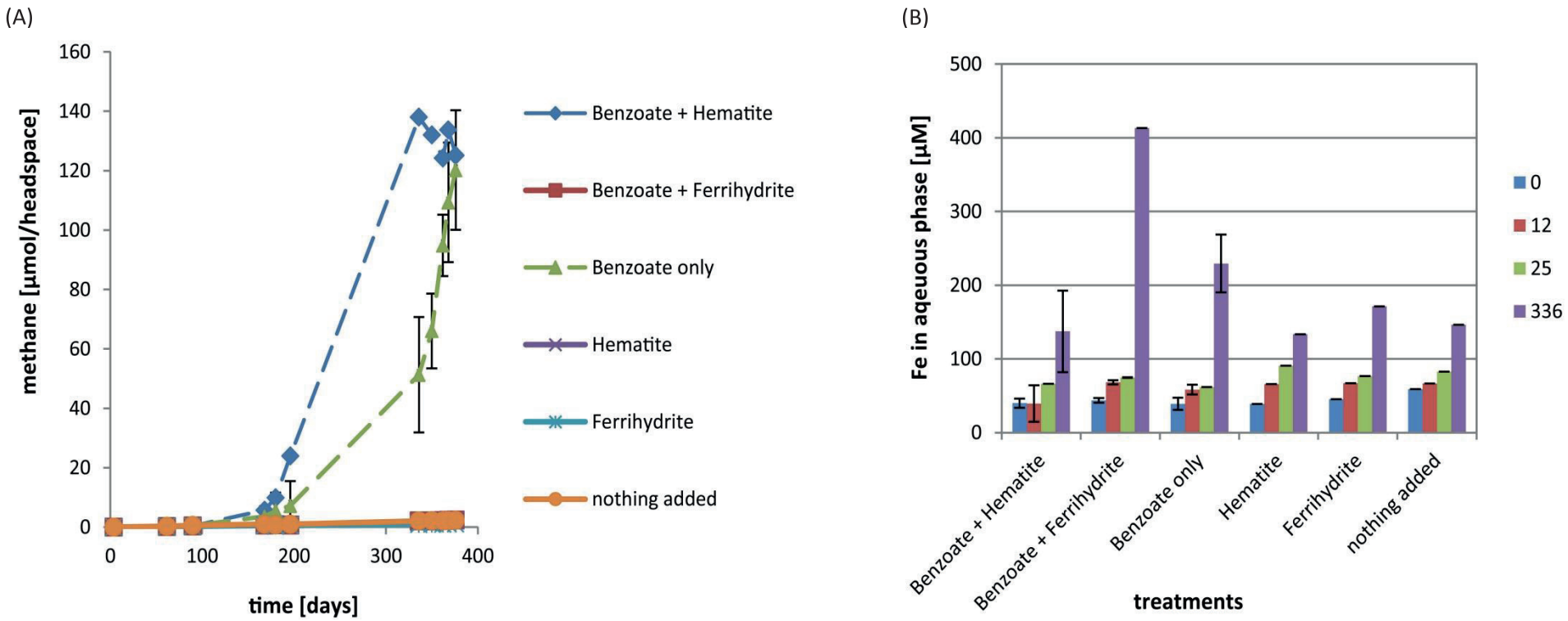

Fig. 1. Methanogenesis (A) and iron reduction (B) in slurry incubations with sediments from the methanic zone of the Helgoland mud area, in the presence of benzoate.

methanogenesis from benzoate was at 25 - 48 % faster in presence of hematite (28.4 ± 9.5 vs 78.7 nmol g wet sed.$^{-1}$ day^{-1}, respectively). The stimulatory effect of hematite is most likely due to DIET since hematite did not stimulate iron reduction to higher concentrations than in control incubations. Therefore, one could speculate that it is only serving a conductive conduit facilitating electron transfer between benzoate degrading microbial populations and methanogens. These results thus provide a strong basis for the identification of microbial populations in the methanic zone of the Helgoland mud area which are capable of methanogenic aromatic compounds degradation via DIET.

References

Beal, E. J., House, C. H., and Orphan, V. J. (2009). Manganese- and iron-dependent marine methane oxidation. *Science* 325, 184–7. doi:10.1126/science.1169984.

Dodsworth, J. A., Blainey, P. C., Murugapiran, S. K., Swingley, W. D., Ross, C. A., Tringe, S. G., Chain, P. S. G., Scholz, M. B., Lo, C.-C., Raymond, J., et al. (2013). Single-cell and metagenomic analyses indicate a fermentative and saccharolytic lifestyle for members of the OP9 lineage. *Nat. Commun.* 4, 1854. doi:10.1038/ncomms2884.

Egger, M., Rasigraf, O., Sapart, C. J., Jilbert, T., Jetten, M. S. M., Röckmann, T., van der Veen, C., Banda, N., Kartal, B., Ettwig, K. F., et al. (2014). Iron-mediated anaerobic oxidation of methane in brackish coastal sediments. *Environ. Sci. Technol.* 277, 277–283. doi:10.1021/es503663z.

Gies, E. A., Konwar, K. M., Beatty, J. T., and Hallam, S. J. (2014). Illuminating microbial dark matter in meromictic Sakinaw Lake. *Appl. Environ. Microbiol.* 80. doi:10.1128/AEM.01774-14.

Jiang, S., Park, S., Yoon, Y., Lee, J.-H., Wu, W.-M., Phuoc Dan, N., Sadowsky, M. J., and Hur, H.-G. (2013). Methanogenesis facilitated by geobiochemical iron cycle in a novel syntrophic methanogenic microbial community. *Environ. Sci. Technol.* 47, 10078–84. doi:10.1021/es402412c.

Kato, S., Hashimoto, K., and Watanabe, K. (2012). Methanogenesis facilitated by electric syntrophy via (semi)conductive iron-oxide minerals. *Environ. Microbiol.* 14, 1646–54. doi:10.1111/j.1462-2920.2011.02611.x.

Lever, M. A. (2011). Acetogenesis in the energy-starved deep biosphere - a paradox? *Front. Microbiol.* 2, 284. doi:10.3389/fmicb.2011.00284.

Nobu, M. K., Dodsworth, J. a, Murugapiran, S. K., Rinke, C., Gies, E. A, Webster, G., Schwientek, P., Kille, P., Parkes, R. J., Sass, H., et al. (2015). Phylogeny and physiology of candidate phylum "Atribacteria" (OP9/JS1) inferred from cultivation-independent genomics. *ISME J.*, 1–14. doi:10.1038/ismej.2015.97.

Oni, O., Miyatake, T., Kasten, S., Richter-Heitmann, T., Fischer, D., Wagenknecht, L., Kulkarni, A., Blumers, M., Shylin, S. I., Ksenofontov, V., et al. (2015). Distinct microbial populations are tightly linked to the profile of dissolved iron in the methanic sediments of the Helgoland mud area, North Sea. *Front. Microbiol.* 06, 1–15. doi:10.3389/fmicb.2015.00365.

Phelps, C. D., Kerkhof, L. J., and Young, L. Y. (1998). Molecular characterization of a sulfate-reducing consortium which mineralizes benzene. *FEMS Microbiol. Ecol.* 27, 269–279. doi:10.1111/j.1574-6941.1998.tb00543.x.

Riedinger, N., Formolo, M. J., Lyons, T. W., Henkel, S., Beck, a, and Kasten, S. (2014). An inorganic geochemical argument for coupled anaerobic oxidation of methane and iron reduction in marine sediments. *Geobiology* 12, 172–81. doi:10.1111/gbi.12077.

Rotaru, A.-E., Shrestha, P. M., Liu, F., Shrestha, M., Shrestha, D., Embree, M., Zengler, K., Wardman, C., Nevin, K. P., and Lovley, D. R. (2014). A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to *Methanosaeta* for the reduction of carbon dioxide to methane. *Energy Environ. Sci.* 7, 408. doi:10.1039/c3ee42189a.

Schink, B. (1997). Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol. Mol. Biol. Rev.* 61, 262–80. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=232610&tool=pmcentrez&rendertype=abstract.

Viggi, C. C., Rossetti, S., Fazi, S., Paiano, P., Majone, M., and Aulenta, F. (2014). Magnetite particles triggering a faster and more robust syntrophic pathway of methanogenic propionate degradation. *Environ. Sci. Technol.* 48, 7536–43. doi:10.1021/es5016789.

Zhuang, L., Tang, J., Wang, Y., Hu, M., and Zhou, S. (2015a). Conductive iron oxide minerals accelerate syntrophic cooperation in methanogenic benzoate degradation. *J. Hazard. Mater.* 293, 37–45. doi:10.1016/j.jhazmat.2015.03.039.

Zhuang, L., Xu, J., Tang, J., and Zhou, S. (2015b). Effect of ferrihydrite biomineralization on methanogenesis in an anaerobic incubation from paddy soil. *J. Geophys. Res. Biogeosciences* 120, 876–886. doi:10.1002/2014JG002893.

Chapter 8

General Discussion and Perspectives

Subsurface microbiology has become an important field in environmental microbiology due to the detection of several uncultured bacterial and archaeal lineages, whose functions are so far poorly understood in subsurface sediments of marine and terrestrial environments. However, microbial populations in subsurface sediments have been linked to a number of elemental cycles (e.g. Fe, Mn, S, C etc), some of which are extremely important for shaping Earth's climate. In this work, bacteria and archaea communities, as they interact with the geochemical environments in the subsurface sediments of the Helgoland mud area, were studied, and results have been discussed in detail in previous chapters. Here, I aimed to synergize the main findings in previous chapters into a clearer picture that presents the structure and potential roles of microorganisms in the subsurface sediments of the

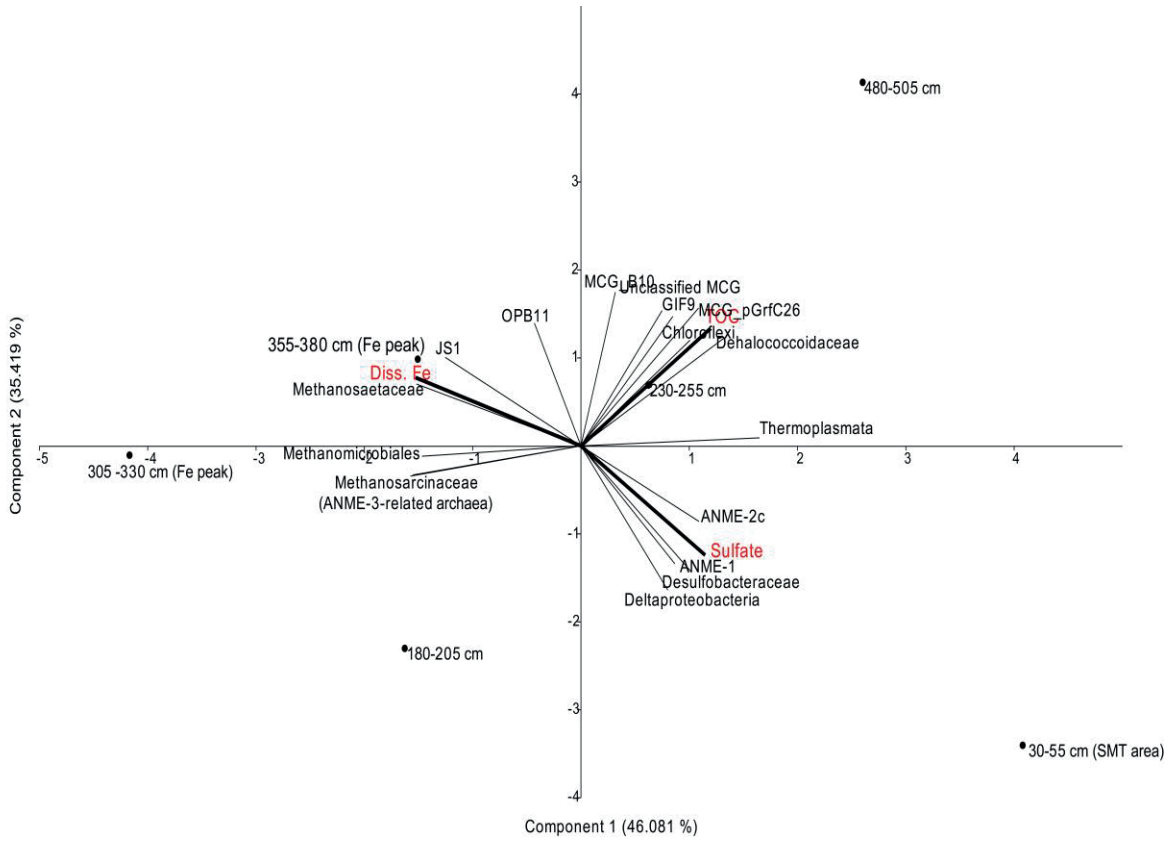

Fig.1. Microbial community structure in subsurface sediments of the Helgoland mud area relative to select environmental parameters as illustrated with the Principal Component Analysis.

Structure of microbial communities in subsurface sediments of Helgoland mud area

Overall, surface and subsurface sediments of the Helgoland mud area are dominated by different microbial populations as seen in Chapter 2 (Figs. 4 and 7). In addition, Fig. 1 shows that TOC, dissolved Fe and sulfate are important factors that influence the distribution of dominant Bacteria and Archaea communities in the subsurface sediments of the Helgoland mud area. The potential for sulfate reduction in the subsurface sediments of the Helgoland mud area seem to be most pronounced around the SMT, where anaerobic oxidation of methane is coupled to sulfate reduction. This process, in our study site, is likely mediated by anaerobic methane-oxidizing archaea, ANME-1 and -2c archaeal populations in partnership with members of the sulfate-reducing *Desulfobacteraceae*, as reported in other studies (Boetius et al., 2000; Orphan et al., 2001; Girguis et al., 2005). The most dominant bacterial and archaeal populations (*Chloroflexi* and MCG, respectively) may be involved in organic matter degradation, as they strongly correlate to TOC content in the subsurface sediments. These results are also consistent with their recently predicted roles in sedimentary organic matter degradation (Kubo et al., 2012; Krzmarzick et al., 2012; Lloyd et al., 2013; Wasmund et al., 2014; Meng et al., 2014; Vigneron et al., 2014b). Interestingly, the depth-wise distribution of the *Thermoplasmata*, seems to be controlled by both sulfate and TOC (Fig. 1 and Chapter 2, Fig. 8), because they were highly abundant around the SMT (30-55 cm) and below. They also increased in relative abundance with increasing TOC content. Genetic evidences from single cells of *Thermoplasmata* recovered from deep anoxic peatlands suggest their ability to reduce sulfite and organosulfate (Lin et al., 2015). Other studies have linked members of the *Thermoplasmata* (*Methanomassiliicoccales*) to methylotrophic methanogenesis (Paul et al., 2012; Dridi et al., 2012; Poulsen et al., 2013; Iino et al., 2013). Terrestrially-derived organic matter, present in subsurface sediments of the Helgoland mud area (see Chapter 2) such as lignin-derived materials, may serve as a source of methoxy compounds for methylotrophic methanogenesis (Schink and Zeikus, 1980; Donnelly and Dagley, 1980). Most importantly, the distribution of JS1 bacteria, methanogens (*Methanosaetaceae* and *Methanomicrobiales*) and ANME-3-related archaea seem to be connected to dissolved Fe concentrations in the methanic zone. Therefore, it can be concluded that metabolic interactions among

JS1, methanogens and ANME-3-related archaea may be important for iron reduction in subsurface sediments of the Helgoland mud area (Oni et al., 2015).

Biotic origin of dissolved iron in the methanic zone of the Helgoland mud area

In surface sediments, where microorganisms are more actively turning over substrates, cell numbers are higher than in subsurface sediments where turnover of substrates are in the order of 100-2000 years (Biddle et al., 2006). However, at distinct zones of high microbial activities in subsurface sediments, such as the SMT (Biddle et al., 2006; Li et al., 2012) and other presumed zones of AOM occurrence (Fernandes et al., 2015), higher cell numbers have been recorded. Higher qPCR-based 16S rRNA gene copies detected around the peak of dissolved iron in the methanic zone (Oni et al., 2015) suggest that iron reduction in our study site is biogenic and that rates of iron reduction are locally high at the depths of elevated iron dissolved Fe concentrations. In other words, not as a result of chemical reaction between downward-diffusing sulfide from the SMT and buried iron (III) minerals or diffusion of dissolved iron from depths above and below the dissolved Fe peak. This is strongly supported by co-occurrence of higher relative abundances of JS1 bacteria, methanogens and ANME-3-related archaeal populations with high concentrations of dissolved iron at 305-330 cm and 355-380 cm (dissolved Fe peaks, Fig. 1; Oni et al., 2015).

Possible pathways of iron reduction in the methanic zone of the Helgoland mud area

Indications of a biotic origin of iron reduction in the methanic zone rule out chemical iron reduction from downward-diffusing sulfide from the SMT. In addition, considering the large distance (over 200 cm) between the SMT (~ 75 cm bsf) and peak of dissolved iron, sulfide diffusing downward would have been quickly exhausted, and the peak of dissolved Fe would be expected at shallower depths. The "cryptic sulfur cycle" as proposed by Holmkvist et al., (2011), also does not completely explain the mechanism of iron reduction the methanic zone of marine sediments because it does not account for the detection of free dissolved Fe in pore water. In the Aarhus Bay sediments, sulfide penetrated up till 350 cm and prior to this depth, free dissolved Fe^{2+} was not detected until sulfide was completely

depleted (Holmkvist et al., 2011). Therefore, iron reduction in deeper methanic sediments could not be well studied in these sediments.

Contrary to surface sediments, oxidation of products (acetate and hydrogen) of organic matter fermentation by bacteria such as members of the *Desulfuromonadales*, is also unlikely to explain iron reduction in the methanic zone. Ideally, iron reducers and methanogens compete for similar electron donors in the environment, thus iron reduction and methanogenesis are not expected to co-occur if both processes are fed by similar electron donors (Achtnich et al., 1995). This was also demonstrated in Chapter 5, where methanogenesis was almost completely inhibited in slurry incubations in which excess iron (III) minerals were being reduced by members of the *Desulfuromonadales*. The very low abundance (0.01− 0.1% of total bacteria) of *Desulfuromonadales* in sediments from the methanic zone compared to the surface sediments adds to the evidence that they do not play a major role in iron reduction in the methanic zone (Oni et al., 2015). Nevertheless, a cooperative interaction may exist between bacterial populations and methanogens in the metabolism of complex organic matter or simpler products of organic matter fermentation such as acetate, which may be linked to iron reduction. In recent findings of Jiang and colleagues (Jiang et al., 2013), isolates of *Clostridia* from rice paddy soils facilitated methanogenesis and growth of methanogens via iron reduction. This occurred via oxidation of acetate coupled to iron (III) mineral (poorly crystalline akagenite) reduction. Methanogens further harnessed electrons that resulted from the transformation of dissolved Fe (see Benner et al., 2002; Hansel et al., 2003; Zegeye et al., 2010) from akagenite reduction to goethite (a conductive iron mineral), which perhaps further facilitated electron transfer (via DIET) between methanogens and *Clostridia*. This sort of mechanism of iron reduction is plausible in the methanic zone of the Helgoland mud area based on the tight linkages between JS1, methanogens and dissolved Fe. Members of the JS1 candidate division have been consistently detected in methane-rich habitats (Webster et al., 2004; Newberry et al., 2004; Parkes et al., 2005; Inagaki et al., 2006; Parkes et al., 2007; Pachiadaki et al., 2011; Chevalier et al., 2013; Lee et al., 2013), and evidences from single cell genomics so far suggest that they are fermentative organisms and capable of syntrophic oxidation of volatile fatty acids (Dodsworth et al., 2013; Gies et al., 2014; Nobu et al., 2015; Carr et al., 2015), physiological potentials also shared by microorganisms such as *Clostridia* (Fontaine et al., 1942;

Schnürer et al., 1997). Considering that sequential extraction of iron mineral phases from methanic sediments of the Helgoland mud area suggested the presence of crystalline iron minerals phases (hematite/goethite), whose depth distribution follow a similar pattern as amorphous iron mineral phases and their concentrations highest around the peak of dissolved Fe (Oni et al., 2015), crystalline iron minerals may have acted as conductive electron conduits facilitating metabolic interactions between JS1 and methanogens. Enhanced benzoate-dependent methanogenesis by hematite in slurry incubation experiments presented in Chapter 7 suggests that there are microbes in the methanic zone of the Helgoland mud area capable of exploiting the presence of conductive iron minerals to facilitate syntrophic interaction with methanogens. A more detailed study of such interactions is required as it affects iron reduction.

Correlation of methane-oxidizing ANME-3-related archaeal populations to dissolved Fe (Fig. 1) suggests that oxidation of methane coupled to iron reduction remains a strong possibility as a source of dissolved Fe^{2+} in the methanic zone of the Helgoland mud area. In slurry incubation experiments of Beal and colleagues, ANME-3 populations were linked to methane oxidation coupled to metal reduction (Beal et al., 2009). In addition, members of the JS1 (SB45 lineages) candidate division were found to be highly enriched in methane and Mn (IV)-containing slurry incubations with sediments from Lake Matano (Glass et al., 2014), suggesting that JS1 bacteria may play a role in methane oxidation coupled to metal reduction. From these points of view, one could hypothesize that *Methanosaetaceae* and *Methanomicrobiales* generated methane, whose oxidation is then coupled to iron reduction by JS1 bacteria and/or ANME-3-related populations via yet unknown mechanisms. In fact, other studies have reported strong correlations between JS1 and other ANME populations (ANME-2, Roalkvam et al., (2011); ANME-1, Vigneron et al., (2014a)). Results presented in Chapter 6 suggest that methane influenced lepidocrocite reduction although $^{13}CH_4$ tracer experiments would be necessary to prove this unambiguously. Meanwhile, characterization of microbial community shifts in those incubations would be important to check if predicted microbial populations potentially involved in anaerobic methane oxidation (JS1 bacteria and ANME-3-related archaea) were enriched. Results so far gathered, clearly indicate that more work still need to be done to decipher the exact mechanisms of iron reduction in the methanic zone of the Helgoland mud area. However, chemical reduction of Fe

(III) minerals with downward-diffusing sulfide from the SMT or organoclastic iron reduction by organisms such as *Desulfuromonadales* are unlikely mechanisms that account for elevated concentrations of dissolved Fe in the methanic zone. Therefore, further research should focus on alternative mechanisms such as syntrophic degradation of acetate (or other complex organic matter) coupled to methanogenesis, via iron mineral or AOM coupled to iron reduction. The latter mechanism seems more likely based on preliminary results in Chapter 6. Regardless of the exact mechanisms, this work provides data suggesting that iron reduction in the methanic sediments of the Helgoland mud area is likely biotic and also suggests microbial populations potentially linked to the process.

Organic matter composition and degradation in the Helgoland mud area

Data presented in Chapter 2 revealed that surface sediments of the Helgoland mud area are dominated by nitrogen-rich, aliphatic compounds which are most likely derived from marine sources (algal materials, Nguyen et al., 2003). Algal-derived organic matter is usually rich in protein, and carbohydrates are more amenable than terrestrially-derived organic matter to microbial degradation (Kristensen, 1994; Sun et al., 1997). Consistently, microorganisms dominating or more abundant in the surface sediments (e.g. members of the *Gammaproteobacteria* or *Bacteroidetes*) are those often associated with algal bloom degradation (Teeling et al., 2012; Klindworth et al., 2014; Ruff et al., 2014; Tan et al., 2015), which eventually sinks to the sea floor to serve as energy sources for surface sediment microorganisms. In deeper sediments, the abundance of organic molecules bearing signatures of terrestrial origin is consistent with higher burial efficiency of terrestrial organic matter in continental margin sediments (Prahl et al., 1994; Burdige, 2005) and aligns with the depositional history of the Helgoland mud area as a highly depositional environment in the past (Hebbeln et al., 2003). Terrestrially-derived organic matter is rich in aromatic hydrocarbons derived mainly from vascular plant materials (Armstroff et al., 2006) and are considered less easily degradable by microorganisms (Andersen and Kristensen, 1992; Meyers and Ishiwatari, 1993; Meyers, 1994; Prahl et al., 1994; Sun et al., 1997). However, FT-ICRMS data (see Chapter 2, Fig. 9) suggests that in the methanic zone, water-extractable aromatic and phenolic organic molecules presumably of terrestrial origin are being degraded. Degradation of such organic compound groups have also been recently

observed in subsurface peatland sediments and was predicted to be as a result of microbial activities (Tfaily et al., 2013, 2015). The possibility of microbial organic matter degradation in the subsurface sediments of the Helgoland mud area was buttressed by the strong correlations between dominant bacteria and archaea populations (*Chloroflexi*, MCG, *Thermoplasmata*) with TOC content (see Chapter 2, Fig. 8 and this Chapter, Fig. 1). Similar microbial populations, dominating marine and terrestrial subsurface sediments (Breuker et al., 2011), coupled with the finding that aromatic and phenolic molecules are also being degraded in peatland subsurface sediments, suggest that similar organic matter diagenetic processes, mediated by similar microbial populations, occur in terrestrial and marine continental margin subsurface sediments. This could mean that as terrestrial organic matter are transported into the ocean, they are selectively enriched and buried deeper in marine sediments due to the preferential removal of more bioavailable marine organic matter, specific microbial communities with special adaptive capabilities to survive in subsurface marine sediments and degrade more recalcitrant organic matter emerge, resulting in more or less similar microbial populations to those dominating terrestrial subsurface environments. The slow (100-2000 years, Biddle et al., 2006) turnover and release of intermediates from recalcitrant organic matter in subsurface sediments could offer slow-growing subsurface microbial populations (Thorn and Ventullo, 1988) a competitive advantage over surface sediment microorganisms which are usually exposed to higher quality and more easily degradable organic matter. This is probably one of the reasons why previous experiments aimed at obtaining enrichment cultures of the dominant subsurface microorganisms using easily degradable substrates resulted in the proliferation of typical surface sediment microorganisms (Köpke et al., 2005; Batzke et al., 2007; Chapters 4 and 5 of this work). A small but positive step in the effort to cultivate subsurface sediment microorganisms is the molecular characterization of organic molecules that are present and being degraded in subsurface sediments. Therefore, the findings presented in Chapter 2, that aromatic and phenolic compounds presumably of terrestrial origin area being degraded in the methanic zone, may accelerate efforts to culture subsurface microorganisms in the Helgoland mud area and perhaps other highly depositional environments. For the future, it would be worthwhile to systematically study microbial community shifts in slurry incubations with marine subsurface sediments, when treated with algal-derived organic matter and model terrestrially derived

organic matter under anoxic conditions, to find out under which conditions more of the typical microbial communities dominating deep subsurface sediments will be recovered.

Chemolithotrophic Mn (IV) reduction in subsurface sediments of the Helgoland mud area

Results in Chapter 4 showed that there is a huge potential for chemolithotrophic Mn (IV) reduction in subsurface sediments of the Helgoland mud area provided excess Mn (IV) is present. The results obtained from Mn (IV)-containing sediment slurry incubations is reminiscent of a 30 year-long observation by Aller and Rude, (1988) that sulfate, resulting from solid phase sulfides, is rapidly formed when amorphous Mn (IV) is added to surficial marine sediments. Aller and Rude suggested that this reaction is likely linked to microbial activity but till now there has been limited information in the literature regarding microorganisms linked to this process and on this front, the finding that novel *Desulfobulbaceae-Desulfocapsa*-related populations, MSME cluster is noteworthy. MSME cluster was not abundant *in situ* (about 1 % of total bacteria) but appear to have responded rapidly to the presence of excess Mn (IV), thus making any conclusions about their real ecological role in the subsurface sediment of the Helgoland mud area premature. Regardless, we extend the inventory of microbial populations so far linked the biotic formation of sulfate on addition of Mn (IV) in marine sediments. Future studies should focus on the mechanisms of sulfate formation by members of the MSME cluster to decipher whether they disproportionate elemental sulfur resulting from chemical reaction between Mn (IV) and FeS (Thamdrup et al., 1993; Schippers and Jørgensen, 2001) or couple its oxidation directly to Mn (IV) reduction (Lovley and Phillips, 1994).

References

Achtnich, C., Bak, F., and Conrad, R. (1995). Competition for electron donors among nitrate reducers , ferric iron reducers , sulfate reducers , and methanogens in anoxic paddy soil. *Biol. Fertil. Soils* 19, 65–72.

Aller, R. C., and Rude, P. D. (1988). Complete oxidation of solid phase sulfides by manganese and bacteria in anoxic marine sediments. *Geochim. Cosmochim. Acta* 52, 751–765. doi:10.1016/0016-7037(88)90335-3.

Andersen, F. Ø., and Kristensen, E. (1992). The importance of benthic macrofauna in decomposition of microalgae in a coastal marine sediment. *Limnol. Oceanogr.* 37, 1392–1403. doi:10.4319/lo.1992.37.7.1392.

Armstroff, A., Wilkes, H., Schwarzbauer, J., Littke, R., and Horsfield, B. (2006). Aromatic hydrocarbon biomarkers in terrestrial organic matter of Devonian to Permian age. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 240, 253–274. doi:10.1016/j.palaeo.2006.03.052.

Batzke, A., Engelen, B., Sass, H., and Cypionka, H. (2007). Phylogenetic and physiological diversity of cultured deep-biosphere Bacteria from Equatorial Pacific Ocean and Peru Margin sediments. *Geomicrobiol. J.* 24, 261–273. doi:10.1080/01490450701456453.

Beal, E. J., House, C. H., and Orphan, V. J. (2009). Manganese- and iron-dependent marine methane oxidation. *Science* 325, 184–7. doi:10.1126/science.1169984.

Benner, S. G., Hansel, C. M., Wielinga, B. W., Barber, T. M., and Fendorf, S. (2002). Reductive dissolution and biomineralization of iron hydroxide under dynamic flow conditions. *Environ. Sci. Technol.* 36, 1705–11. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11993867.

Biddle, J. F., Lipp, J. S., Lever, M. A., Lloyd, K. G., Sørensen, K. B., Anderson, R., Fredricks, H. F., Elvert, M., Kelly, T. J., Schrag, D. P., et al. (2006). Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proc. Natl. Acad. Sci. U. S. A.* 103, 3846–51. doi:10.1073/pnas.0600035103.

Boetius, A., Ravenschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O. (2000). A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407, 623–6. doi:10.1038/35036572.

Breuker, A., Köweker, G., Blazejak, A., and Schippers, A. (2011). The deep biosphere in terrestrial sediments in the chesapeake bay area, virginia, USA. *Front. Microbiol.* 2, 156. doi:10.3389/fmicb.2011.00156.

Burdige, D. J. (2005). Burial of terrestrial organic matter in marine sediments: A re-assessment. *Global Biogeochem. Cycles* 19, 1–7. doi:10.1029/2004GB002368.

Carr, S. a, Orcutt, B. N., Mandernack, K. W., and Spear, J. R. (2015). Abundant Atribacteria in deep marine sediment from the Adélie Basin, Antarctica. *Front. Microbiol.* 6, 872. doi:10.3389/fmicb.2015.00872.

Chevalier, N., Bouloubassi, I., Birgel, D., Taphanel, M.-H., and López-García, P. (2013). Microbial methane turnover at Marmara Sea cold seeps: a combined 16S rRNA and lipid biomarker investigation. *Geobiology* 11, 55–71. doi:10.1111/gbi.12014.

Dodsworth, J. A., Blainey, P. C., Murugapiran, S. K., Swingley, W. D., Ross, C. A., Tringe, S. G., Chain, P. S. G., Scholz, M. B., Lo, C.-C., Raymond, J., et al. (2013). Single-cell and metagenomic analyses indicate a fermentative and saccharolytic lifestyle for members of the OP9 lineage. *Nat. Commun.* 4, 1854. doi:10.1038/ncomms2884.

Donnelly, M., and Dagley, S. (1980). Production of methanol from aromatic acids by *Pseudomonas putida*. *J. Bacteriol.* 142, 916–924. Available at: http://jb.asm.org/content/142/3/916.short [Accessed March 31, 2015].

Dridi, B., Fardeau, M.-L., Ollivier, B., Raoult, D., and Drancourt, M. (2012). *Methanomassiliicoccus luminyensis gen. nov*., sp. nov., a methanogenic archaeon isolated from human faeces. *Int. J. Syst. Evol. Microbiol.* 62, 1902–7. doi:10.1099/ijs.0.033712-0.

Fernandes, C. E. G., Gonsalves, M. J. B. D., Nazareth, D. R., Nagarchi, L., and Kamaleso, S. a. (2015). Microbial iron reduction and methane oxidation in subsurface sediments of the Arabian Sea. *Mar. Pet. Geol.* 67, 327–335. doi:10.1016/j.marpetgeo.2015.05.002.

Fontaine, F., Peterson, W., Elizabeth, M., Johnson, M., and Ritter, G. (1942). A new type of glucose fermentation by *Clostridium thermoaceticum*. *J. Bacteriol.* 43, 701–715. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC373636/ [Accessed August 27, 2015].

Gies, E. A., Konwar, K. M., Beatty, J. T., and Hallam, S. J. (2014). Illuminating microbial dark matter in meromictic Sakinaw Lake. *Appl. Environ. Microbiol.* 80. doi:10.1128/AEM.01774-14.

Girguis, P. R., Cozen, A. E., and DeLong, E. F. (2005). Growth and population dynamics of anaerobic methane-oxidizing archaea and sulfate-reducing bacteria in a continuous-flow bioreactor. *Appl. Environ. Microbiol.* 71, 3725–33. doi:10.1128/AEM.71.7.3725-3733.2005.

Glass, J. B., Bray, M. S., Reed, B. C., Kretz, C. B., Sarode, N. D., Dichris, T. J., Stewart, F. J., Fowle, D. A., and Crowe, S. A. (2014). Anaerobic oxidation of methane coupled to metal reduction in an archean ocean analogue. in *Annual Meeting of The Geological Society of America (Paper No. 191-4)* (Vancouver, Canada). Available at: https://gsa.confex.com/gsa/2014AM/finalprogram/abstract_245503.htm.

Hansel, C. M., Benner, S. G., Neiss, J., Dohnalkova, A., Kukkadapu, R. K., and Fendorf, S. (2003). Secondary mineralization pathways induced by dissimilatory iron reduction of ferrihydrite under advective flow. *Geochim. Cosmochim. Acta* 67, 2977–2992. doi:10.1016/S0016-7037(03)00276-X.

Hebbeln, D., Scheurle, C., and Lamy, F. (2003). Depositional history of the Helgoland mud area, German Bight, North Sea. *Geo-Marine Lett.* 23, 81–90. doi:10.1007/s00367-003-0127-0.

Holmkvist, L., Ferdelman, T. G., and Jørgensen, B. B. (2011). A cryptic sulfur cycle driven by iron in the methane zone of marine sediment (Aarhus Bay, Denmark). *Geochim. Cosmochim. Acta* 75, 3581–3599. doi:10.1016/j.gca.2011.03.033.

Iino, T., Tamaki, H., Tamazawa, S., Ueno, Y., Ohkuma, M., Suzuki, K., Igarashi, Y., and Haruta, S. (2013). Candidatus Methanogranum caenicola: a novel Methanogen from the anaerobic digested sludge, and proposal of *Methanomassiliicoccaceae fam. nov*. and *Methanomassiliicoccales ord. nov.*, for a Methanogenic lineage of the class Thermoplasmata. *Microbes Environ.* 28, 244–250. doi:10.1264/jsme2.ME12189.

Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., Suzuki, M., Takai, K., Delwiche, M., Colwell, F. S., et al. (2006). Biogeographical distribution and diversity of

microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proc. Natl. Acad. Sci. U. S. A.* 103, 2815–20. doi:10.1073/pnas.0511033103.

Jiang, S., Park, S., Yoon, Y., Lee, J.-H., Wu, W.-M., Phuoc Dan, N., Sadowsky, M. J., and Hur, H.-G. (2013). Methanogenesis facilitated by geobiochemical iron cycle in a novel syntrophic methanogenic microbial community. *Environ. Sci. Technol.* 47, 10078–84. doi:10.1021/es402412c.

Klindworth, A., Mann, A. J., Huang, S., Wichels, A., Quast, C., Waldmann, J., Teeling, H., and Glöckner, F. O. (2014). Diversity and activity of marine bacterioplankton during a diatom bloom in the North Sea assessed by total RNA and pyrotag sequencing. *Mar. Genomics* 18 Pt B, 185–92. doi:10.1016/j.margen.2014.08.007.

Köpke, B., Wilms, R., Engelen, B., Cypionka, H., and Sass, H. (2005). Microbial diversity in coastal subsurface sediments: a cultivation approach using various electron acceptors and substrate gradients. *Appl. Environ. Microbiol.* 71, 7819–30. doi:10.1128/AEM.71.12.7819-7830.2005.

Kristensen, E. (1994). Decomposition of macroalgae , vascular plants and sediment detritus in seawater : use of stepwise thermogravimetry. *Biogeochemistry* 26, 1–24.

Krzmarzick, M. J., Crary, B. B., Harding, J. J., Oyerinde, O. O., Leri, A. C., Myneni, S. C. B., and Novak, P. J. (2012). Natural niche for organohalide-respiring Chloroflexi. *Appl. Environ. Microbiol.* 78, 393–401. doi:10.1128/AEM.06510-11.

Kubo, K., Lloyd, K. G., F Biddle, J., Amann, R., Teske, A., and Knittel, K. (2012). Archaea of the Miscellaneous Crenarchaeotal Group are abundant, diverse and widespread in marine sediments. *ISME J.* 6, 1949–65. doi:10.1038/ismej.2012.37.

Lee, J.-W., Kwon, K. K., Azizi, A., Oh, H.-M., Kim, W., Bahk, J.-J., Lee, D.-H., and Lee, J.-H. (2013). Microbial community structures of methane hydrate-bearing sediments in the Ulleung Basin, East Sea of Korea. *Mar. Pet. Geol.* 47, 136–146. doi:10.1016/j.marpetgeo.2013.06.002.

Li, Q., Wang, F., Chen, Z., Yin, X., and Xiao, X. (2012). Stratified active archaeal communities in the sediments of Jiulong River estuary, China. *Front. Microbiol.* 3, 311. doi:10.3389/fmicb.2012.00311.

Lin, X., Handley, K. M., Gilbert, J. A., and Kostka, J. E. (2015). Metabolic potential of fatty acid oxidation and anaerobic respiration by abundant members of Thaumarchaeota and Thermoplasmata in deep anoxic peat. *ISME J.*, 1–5. doi:10.1038/ismej.2015.77.

Lloyd, K. G., Schreiber, L., Petersen, D. G., Kjeldsen, K. U., Lever, M. A., Steen, A. D., Stepanauskas, R., Richter, M., Kleindienst, S., Lenk, S., et al. (2013). Predominant archaea in marine sediments degrade detrital proteins. *Nature* 496, 215–8. doi:10.1038/nature12033.

Lovley, D. R., and Phillips, E. J. P. (1994). Novel processes for anaerobic sulfate production from elemental sulfur by sulfate-reducing bacteria. *Appl. Environ. Microbiol.* 60, 2394–2399.

Meng, J., Xu, J., Qin, D., He, Y., Xiao, X., and Wang, F. (2014). Genetic and functional properties of uncultivated MCG archaea assessed by metagenome and gene expression analyses. *ISME J.* 8, 650–9. doi:10.1038/ismej.2013.174.

Meyers, P. A. (1994). Preservation of elemental and isotopic source identification of sedimentary organic matter. *Chem. Geol.* 114, 289–302.

Meyers, P. A., and Ishiwatari, R. (1993). Lacustrine organic geochemistry: an overview of indicators of organic matter sources and diagenesis in lake sediments. *Org. Geochem.* 20, 867–900.

Newberry, C. J., Webster, G., Cragg, B. A., Parkes, R. J., Weightman, A. J., and Fry, J. C. (2004). Diversity of prokaryotes and methanogenesis in deep subsurface sediments from the Nankai Trough, Ocean Drilling Program Leg 190. *Environ. Microbiol.* 6, 274–287. doi:10.1111/j.1462-2920.2004.00568.x.

Nguyen, R. T., Harvey, H. R., Zang, X., van Heemst, J. D. ., Hetényi, M., and Hatcher, P. G. (2003). Preservation of algaenan and proteinaceous material during the oxic decay of Botryococcus braunii as revealed by pyrolysis-gas chromatography/mass spectrometry and 13C NMR spectroscopy. *Org. Geochem.* 34, 483–497. doi:10.1016/S0146-6380(02)00261-9.

Nobu, M. K., Dodsworth, J. a, Murugapiran, S. K., Rinke, C., Gies, E. a, Webster, G., Schwientek, P., Kille, P., Parkes, R. J., Sass, H., et al. (2015). Phylogeny and physiology of candidate phylum "Atribacteria" (OP9/JS1) inferred from cultivation-independent genomics. *ISME J.*, 1–14. doi:10.1038/ismej.2015.97.

Oni, O., Miyatake, T., Kasten, S., Richter-Heitmann, T., Fischer, D., Wagenknecht, L., Kulkarni, A., Blumers, M., Shylin, S. I., Ksenofontov, V., et al. (2015). Distinct microbial populations are tightly linked to the profile of dissolved iron in the methanic sediments of the Helgoland mud area, North Sea. *Front. Microbiol.* 06, 1–15. doi:10.3389/fmicb.2015.00365.

Orphan, V. J., Hinrichs, K. U., Ussler, W., Paull, C. K., Taylor, L. T., Sylva, S. P., Hayes, J. M., and Delong, E. F. (2001). Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. *Appl. Environ. Microbiol.* 67, 1922–34. doi:10.1128/AEM.67.4.1922-1934.2001.

Pachiadaki, M. G., Kallionaki, A., Dählmann, A., De Lange, G. J., and Kormas, K. A. (2011). Diversity and spatial distribution of prokaryotic communities along a sediment vertical profile of a deep-sea mud volcano. *Microb. Ecol.* 62, 655–68. doi:10.1007/s00248-011-9855-2.

Parkes, R. J., Cragg, B. A., Banning, N., Brock, F., Webster, G., Fry, J. C., Hornibrook, E., Pancost, R. D., Kelly, S., Knab, N., et al. (2007). Biogeochemistry and biodiversity of methane cycling in subsurface marine sediments (Skagerrak, Denmark). *Environ. Microbiol.* 9, 1146–61. doi:10.1111/j.1462-2920.2006.01237.x.

Parkes, R. J., Webster, G., Cragg, B. A., Weightman, A. J., Newberry, C. J., Ferdelman, T. G., Kallmeyer, J., Jørgensen, B. B., Aiello, I. W., and Fry, J. C. (2005). Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. *Nature* 436, 390–4. doi:10.1038/nature03796.

Paul, K., Nonoh, J. O., Mikulski, L., and Brune, A. (2012). "*Methanoplasmatales*," Thermoplasmatales-related archaea in termite guts and other environments, are the seventh order of methanogens. *Appl. Environ. Microbiol.* 78, 8245–53. doi:10.1128/AEM.02193-12.

Poulsen, M., Schwab, C., Jensen, B. B., Engberg, R. M., Spang, A., Canibe, N., Højberg, O., Milinovich, G., Fragner, L., Schleper, C., et al. (2013). Methylotrophic methanogenic Thermoplasmata implicated in reduced methane emissions from bovine rumen. *Nat. Commun.* 4, 1428. doi:10.1038/ncomms2432.

Prahl, F. ., Ertel, J. ., Goni, M. ., Sparrow, M. ., and Eversmeyer, B. (1994). Terrestrial organic carbon contributions to sediments on the Washington margin. *Geochim. Cosmochim. Acta* 58, 3035–3048. doi:10.1016/0016-7037(94)90177-5.

Roalkvam, I., Jørgensen, S. L., Chen, Y., Stokke, R., Dahle, H., Hocking, W. P., Lanzén, A., Haflidason, H., and Steen, I. H. (2011). New insight into stratification of anaerobic methanotrophs in cold seep sediments. *FEMS Microbiol. Ecol.* 78, 233–43. doi:10.1111/j.1574-6941.2011.01153.x.

Ruff, S., Probandt, D., Zinkann, A.-C., Iversen, M. H., Klaas, C., Würzberg, L., Krombholz, N., Wolf-Gladrow, D., Amann, R., and Knittel, K. (2014). Indications for algae-degrading benthic microbial communities in deep-sea sediments along the Antarctic Polar Front. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 108, 6–16. doi:10.1016/j.dsr2.2014.05.011.

Schink, B., and Zeikus, J. (1980). Microbial methanol formation: a major end product of pectin metabolism. *Curr. Microbiol.* 4, 387–389. Available at: http://link.springer.com/article/10.1007/BF02605383 [Accessed March 31, 2015].

Schippers, A., and Jørgensen, B. B. (2001). Oxidation of pyrite and iron sulfide by manganese dioxide in marine sediments. *Geochim. Cosmochim. Acta* 65, 915–922. doi:10.1016/S0016-7037(00)00589-5.

Schnürer, A., Svensson, B. H., and Schink, B. (1997). Enzyme activities in and energetics of acetate metabolism by the mesophilic syntrophically acetate-oxidizing anaerobe *Clostridium ultunense*. *FEMS Microbiol. Lett.* 154, 331–336. doi:10.1016/S0378-1097(97)00350-9.

Sun, L., Perdue, E., Meyer, J., and Weis, J. (1997). Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. *Limnol. ...* 42, 714–721. Available at: http://onlinelibrary.wiley.com/doi/10.4319/lo.1997.42.4.0714/abstract [Accessed August 27, 2015].

Tan, S., Zhou, J., Zhu, X., Yu, S., Zhan, W., Wang, B., and Cai, Z. (2015). An association network analysis among microeukaryotes and bacterioplankton reveals algal bloom dynamics. *J. Phycol.* 51, 120–132. doi:10.1111/jpy.12259.

Teeling, H., Fuchs, B., and Becher, D. (2012). Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science (80).* 5567, 608–611. Available at: http://www.sciencemag.org/content/336/6081/608.short [Accessed January 29, 2015].

Tfaily, M. M., Corbett, J. E., Wilson, R., Chanton, J. P., Glaser, P. H., Cawley, K. M., Jaffé, R., and Cooper, W. T. (2015). Utilization of PARAFAC-Modeled Excitation-Emission Matrix (EEM) fluorescence spectroscopy to identify biogeochemical processing of dissolved organic matter in a northern peatland. *Photochem. Photobiol.* doi:10.1111/php.12448.

Tfaily, M. M., Hamdan, R., Corbett, J. E., Chanton, J. P., Glaser, P. H., and Cooper, W. T. (2013). Investigating dissolved organic matter decomposition in northern peatlands using complimentary analytical techniques. *Geochim. Cosmochim. Acta* 112, 116–129. doi:10.1016/j.gca.2013.03.002.

Thamdrup, B. O., Finster, K. M., Hansen, J. W., and Bak, F. (1993). Bacterial disproportionation of elemental sulfur coupled to chemical reduction of iron or manganese. *Appl. Environ. Microbiol.* 59, 101–108.

Thorn, P. M., and Ventullo, R. M. (1988). Measurement of bacterial growth rates in subsurface sediments using the incorporation of tritiated thymidine into DNA. *Microb. Ecol.* 16, 3–16. doi:10.1007/BF02097401.

Vigneron, A., Cruaud, P., Pignet, P., Caprais, J.-C., Gayet, N., Cambon-Bonavita, M.-A., Godfroy, A., and Toffin, L. (2014a). Bacterial communities and syntrophic associations involved in anaerobic oxidation of methane process of the Sonora Margin cold seeps, Guaymas Basin. *Environ. Microbiol.* 16, 2777–90. doi:10.1111/1462-2920.12324.

Vigneron, A., Cruaud, P., Roussel, E. G., Pignet, P., Caprais, J.-C., Callac, N., Ciobanu, M.-C., Godfroy, A., Cragg, B. A., Parkes, J. R., et al. (2014b). Phylogenetic and functional diversity of microbial communities associated with subsurface sediments of the Sonora Margin, Guaymas Basin. *PLoS One* 9, e104427. doi:10.1371/journal.pone.0104427.

Wasmund, K., Schreiber, L., Lloyd, K. G., Petersen, D. G., Schramm, A., Stepanauskas, R., Jørgensen, B. B., and Adrian, L. (2014). Genome sequencing of a single cell of the widely distributed marine subsurface Dehalococcoidia, phylum Chloroflexi. *ISME J.* 8, 383–97. doi:10.1038/ismej.2013.143.

Webster, G., Parkes, R. J., Fry, J. C., and Weightman, A. J. (2004). Widespread occurrence of a novel division of bacteria identified by 16S rRNA gene sequences originally found in deep marine sediments. *Appl. Environ. Microbiol.* 70, 5708–5713. doi:10.1128/AEM.70.9.5708.

Zegeye, A., Mustin, C., and Jorand, F. (2010). Bacterial and iron oxide aggregates mediate secondary iron mineral formation: green rust versus magnetite. *Geobiology* 8, 209–22. doi:10.1111/j.1472-4669.2010.00238.x.

Acknowledgements

First, I owe my supervisor, Prof. Dr. Michael Friedrich, a lot of gratitude for offering the opportunity to work on this exciting project, setting up the right environment for me to work and for his mentorship.

Thank you, Prof. Kai-Uwe Hinrichs, for agreeing to be the second reviewer of my thesis despite your tightly packed schedule.

I am very grateful to Dr. Sabine Kasten for taking up the tedious work of planning cruises to the Helgoland mud area, so I could have samples to work with. I am also thankful for your great support with the geochemistry aspect of the project.

I am eternally grateful to Dr. Tetsuro Miyatake his immense support right from the start of my doctoral thesis. It is very sad that you are not around to witness this moment and how this project has developed. I really miss you and may your soul rest in perfect peace!

Prof. Dr. Karl-Heinz Blotevogel, I am very grateful for the „daddy“ role you played during the thick and thin times of the MarMic program.

Special thanks to my collaborators from Johannes Gutenberg University, Germany (Dr. Mathias Blumers, Dr. Göster Klingelhöfer, Dr. Vadim Ksenofontov, Sergii Shylin) and University of Coimbra, Portugal (Prof. Benilde F.O. Costa) for all the efforts made to help me with Mössbauer Spectroscopy analysis of iron minerals in my Helgoland mud area samples.

Dr. Frauke Schmidt, I am extremely grateful to you for agreeing to collaborate with me on the molecular characterization of organic matter in my samples and in the process, introducing me to the fascinating world of dissolved organic matter. You are the epitome of niceness!

I am very grateful to my colleagues, Saar, Tim Richter-Heitmann, Dr. Carolina Reyes, Ajinkya Kulkarni, Cedric Hahn, Yin Xiuran for all their assistance and team support along this stressful journey. You guys are the giants on whose shoulders I stood to be able to see far.

Charlotte Wilms, thank you for all the technical assistances you provided here and there to make sure that valuable data for the project are produced. You are a gem!

Thank you very much Tina Stickan! You are the best secretary in the world. I am grateful for all your help in sorting out our administrative issues.

Special thanks to the members of the MarMic Class of 2014. You guys are great!

Joanna-my love, my pillar, I am extremely grateful to you for standing by my side and supporting me during the good and the bad times of my PhD program. Thank you for giving us our bundle of joy, Lily Aderonke Amorkor Oni. For this, I will be grateful to you eternally!

As the Yoruba proverb says „Eegun nla lo n ke yin Igbale“(the most influential masquerade performs last), I cannot thank my parents enough for teaching me to understand the importance of hardwork and patience. I am also very grateful for your support and prayers from my birth up till this moment. May you live long, in good health, to enjoy the fruit of your labor.

Name: ..... Ort, Datum.....

Anschrift:.....

ERKLÄRUNG

Hiermit erkläre ich, dass ich die Doktorarbeit mit dem Titel:

……

……

……

selbstständig verfasst und geschrieben habe und außer den angegebenen Quellen keine weiteren Hilfsmittel verwendet habe.

Ebenfalls erkläre ich hiermit, das es sich bei den von mir abgegebenen Arbeiten um 3 identische Exemplare handelt.

.....
(Unterschrift)