Distribution of *Dehalococcoidia* in marine sediments and strategies for their enrichment

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"Life on earth is such a good story you cannot afford to miss the beginning.

Beneath our superficial differences we are all of us walking communities of bacteria.

The world shimmers a pointillist landscape made of tiny living beings"

Lynn Margulis

Dedicado a mis tíos,

Dr. José Luis Gallardo y Dra. María del Carmen López,

por plantar la semilla del interés por la Ciencia en mi adolescencia,

por su ejemplo y por todo su apoyo

DECLARATION OF INDEPENDENT WORK

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ABSTRACT

The marine subsurface is one of the largest microbial habitats on Earth. Microbial community studies based on the 16S rRNA gene have revealed that bacteria of the class *Dehalococcoidia,* phylum *Chloroflexi*, are one of the most widespread and abundant bacteria inhabiting the marine subsurface. However, their physiology and ecological role are unknown. In this study, marine sediments were cultivated with various potential electron acceptors to explore respiration modes catalysed by *Dehalococcoidia*. The cultivation revealed that *Dehalococcoidia* can be cultivated in an anoxic minimal medium amended with hydrogen and acetate as potential electron donor and carbon source, respectively, at a temperature of 30ºC and at atmospheric pressure, and thus *Dehalococcoidia* are not strict piezophilic bacteria. An increase in *Dehalococcoidia* 16S rRNA gene copy numbers of one order of magnitude, as evidenced by quantitative PCR with newly designed primers, could be observed in a time span of months for several sediment cultures, indicating that their replication times are not in geological times, i.e., thousands to millions of years. *Dehalococcoidia* 16S rRNA gene copy numbers (in the range of 10^2–10^5 ml^{-1} culture) among the various sediment cultures inoculated with diverse sediments and amended with different potential electron acceptors suggested no specific respiratory mode to be preferred by subseafloor *Dehalococcoidia*. Additionally, subseafloor *Dehalococcoidia* were not reliant on organohalide respiration under the conditions tested, in contrast to cultivated *Dehalococcoidia* species such as *Dehalococcoides mccartyi*. Interestingly, among the tested organohalide compounds, 1,2,3-trichlorobenzene was transformed to 1,3-dichlorobenzene in sediment cultures. Inhibition studies using antibiotics against Gram-positives and microbial community analyses by 454-pyrosequencing of 16S rRNA genes, indicated members affiliated with the phylum *Firmicutes* were involved in the transformation of 1,2,3-trichlorobenzene.

The presence, abundance, and distribution of *Dehalococcoidia* were investigated in sediments of the Arctic Baffin Bay. Geochemical studies showed the shelf as an area rich in organic matter, with indications for the presence of sulphate reduction (highest *dsrA* gene copies and a decrease in sulphate concentration in its pore-waters). On the other hand, the basin area was comparably poor in organic matter with high concentrations of iron(II) and manganese(II) in its pore-waters. *Dehalococcoidia* were present at all investigated sites and depths in the range of 1.1 x 10^3–8.4 x 10^5 16S rRNA gene copy numbers g^{-1} sediment. *Dehalococcoidia* copy numbers were highest and generally stable with depth in shelf sites of the Baffin Bay compared to any other area. In contrast, *Dehalococcoidia* copy numbers pronouncedly decreased with depth at basin sites. *Dehalococcoidia* accounted for the highest proportion of the total bacterial 16S rRNA genes when low bacterial copy numbers were found, mostly in samples from deeper sediment layers, indicating that subseafloor *Dehalococcoidia* are

resilient to burial. Illumina sequencing of 16S rRNA genes gave further evidence that *Dehalococcoidia* were mostly associated with shelf sediments. The relative abundance of *Dehalococcoidia* and, most specifically, the clade GIF-9 within the class *Dehalococcoidia*, positively correlated with organic matter content, and negatively correlated with sulphate and manganese(II) pore-water concentrations. Thus, a potential fermenting metabolism is likely for GIF-9 members. Apart from *Dehalococcoidia*, the Baffin Bay bacterial community was dominated by members of the class *Betaproteobacteria* (with relative abundance of 38 to 64%), and specifically the order *Burkholderiales*, which strongly correlated to manganese(II) pore-water concentration, suggesting a metal respiratory metabolism. In contrast to *Dehalococcoidia*, the class *Betaproteobacteria* is not commonly found as widely distributed and abundant bacterial group in the marine subseafloor, and its presence and high abundance in sediments of the Baffin Bay may be the result of the glacial conditions in the area.

ZUSAMMENFASSUNG

Marine Sedimente sind einer der größten mikrobiellen Lebensräume der Erde. 16S rRNA-basierte Studien der mikrobiellen Gemeinschaften haben gezeigt, dass Bakterien der Klasse *Dehalococcoidia* des Phylums *Chloroflexi* ubiquitär und abundant in marinen Sedimenten vorkommen. Die Physiologie und ökologische Funktion dieser Bakterien ist weitestgehend unbekannt. In der vorliegenden Arbeit wurden marine Sedimente mit verschiedenen potenziellen Elektronenakzeptoren versetzt, um die Stoffwechselcharakteristika der Klasse *Dehalococcoidia* zu identifizieren. Die Kultivierungsexperimente zeigten, dass *Dehalococcoidia*-Spezies in anoxischem Minimalmedium versetzt mit Wasserstoff und Acetat als Elektronendonor und Kohlenstoffstoffquelle bei 30°C und atmosphärischem Druck erfolgreich kultiviert werden können und dementsprechend nicht strikt barophil sind. Zur Quantifizierung von *Dehalococcoidia*-Spezies wurde ein qPCR-Verfahren entwickelt. Vereinzelt wurde in den Labormikrokosmenansätzen ein Anstieg der *Dehalococcoidia*-spezifischen 16S rRNA-Genkopienzahl innerhalb der mehrmonatigen Inkubation verzeichnet. Signifikante Änderungen der *Dehalococcoidia*-spezifischen 16S rRNA-Genkopienzahl in Abhängigkeit von den verschiedenen Elektronenakzeptoren wurden nicht beobachtet und ließen damit keine Rückschlüsse auf spezifische Stoffwechselprozesse zu. Weiterhin konnte für marine *Dehalococcoidia*-Spezies im Gegensatz zu kultivierten Süßwasserspezies wie z.B. *Dehalococcoides mccartyi* keine Abhängigkeit von Organohalid-Respiration nachgewiesen werden. Interessanterweise wurde als einzige der getesten chlorierten Kohlenwasserstoffe 1,2,3-Trichlorbenzol zu 1,3-Dichlorbenzol umgesetzt. Studien zur Inhibierung von gram-positiven Bakterien mit Antibiotika sowie 16S rRNA-basierte Analysen der mikrobiellen Gemeinschaften mittels Pyrosequenzierung deuteten auf die Relevanz von *Firmicutes*-verwandten Spezies zur Transformation von 1,2,3-Trichlorbenzol.

Das Vorkommen, die Abundanz und räumliche Verteilung von *Dehalococcoidia* wurde in Sedimenten der nördlichen Baffin Bay untersucht. Geochemische Studien zeigen, dass große Mengen an organischer Substanz im Schelf festgelegt sind. Hohe Kopienzahlen der dissimilatorischen Sulfitreduktase (*dsrA*) verbunden mit einer Sulfatzehrung im Porenwasser deuten auf die Relevanz von Sulfatreduktion hin. Das Baffinbecken ist dagegen vergleichsweise arm an organischer Substanz mit hohen Eisen(II)- und Mn(II)-Konzentrationen im Porenwasser. Das Markergen für *Dehalococcoidia* wurde in Sedimentproben verschiedener Transekte und vertikaler Zonierungen mit einer Abundanz von 10^3 bis 10^5/g Sediment nachgewiesen. In den Schelfsedimenten trat das *Dehalococcoidia*-Gen mit einer Abundanz von 10^5/g Sediment auf und zeigte keine signifikante Änderung entlang der vertikalen Zonierung. Im Gegensatz dazu nahmen die Kopienzahlen des Markergens in den Sedimentproben des Baffinbeckens mit zunehmender Tiefe rasch ab. Lagen bakterielle

Gene in geringen Kopienzahlen vor, was zumeist in den tieferen Sedimentschichten beobachtet wurde, machten *Dehalococcoidia*-Gene den Großteil der bakteriellen 16S rRNA-Gene aus. Illuminasequenzierung der 16S rRNA-Gene zeigte darüber hinaus, dass *Dehalococcoidia* vorrangig in Schelfsedimenten vorkamen. Die relative Abundanz von *Dehalococcoidia*, insbesondere der Gruppe GIF-9 innerhalb der *Dehalococcoidia*, korrelierte positiv mit dem Gehalt an organischer Substanz und negativ mit der Sulfat- und Mangan(II)-Konzentrationen des Porenwassers. Dementsprechend ist ein fermentativer Stoffwechsel für Spezies der Gruppe GIF-9 in Betracht zu ziehen. Diese Hypothese wird durch die Ergebnisse der Kultivierungsexperimente gestützt. Neben *Dehalococcoidia* wurde die mikrobielle Baffin-Bay-Gemeinschaft von Spezies der Klasse *Betaproteobacteria* (mit einer relativen Abundanz von 38 bis 64%), insbesondere der Ordnung *Burkholderiales,* dominiert. Das Vorkommen von *Burkholderiales*-Spezies zeigte eine starke Korrelation mit den Eisen(II)- und Mangan(II)-Konzentrationen und deutet auf eine mikrobielle Eisen- und Manganreduktion hin. Im Gegensatz zur Klasse *Dehalococcoidia* kommen *Betaproteobacteria* nur vereinzelt in marinen Sedimenten vor. Deren Vorkommen und hohe Abundanz in den Baffin Bay-Sedimenten resultieren möglicherweise aus den eiszeitlichen Bedingungen.

LIST OF PUBLICATIONS

Results from this thesis led to three peer-reviewed publications in scientific journals and to a contribution to one scientific cruise report. This thesis is based on those original articles and on unpublished data.

- **PUBLICATION I**

  Algora C., Gründger F., Adrian L., Damm V., Richnow H.-H., Krüger M. (2013). Geochemistry and microbial populations in sediments of the Northern Baffin Bay, Arctic. *Geomicrobiology Journal* 30 (8), 690 – 705;

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  Author contributions

  Martin Krüger developed the concept and coordinated this study. The manuscript concept was developed by Camelia Algora, Lorenz Adrian and Martin Krüger. Volkmar Damm made possible and coordinated the ARK XXV/3 expedition and supplied geological data. Hans-Hermann Richnow supplied ideas for the study concept, field sampling and analysis of isotope data. Friederike Gründger and Camelia Algora designed and performed the field work on board of the Polarstern and produced the data in the laboratory after the expedition. Additionally, Friederike Gründger and Martin Krüger supplied extra data on microcosms and coordinated the geological analysis of sediment samples at BGR. Camelia Algora interpreted the results and drafted the manuscript. Lorenz Adrian and Martin Krüger revised the manuscript.

- **PUBLICATION II**

  Wasmund K., **Algora C**., Müller J., Krüger M., Lloyd K. G., Reinhardt R., Adrian L. (2015). Development and application of primers for the class *Dehalococcoidia* (phylum *Chloroflexi*) enables deep insights into diversity and stratification of subgroup in the marine subsurface. *Environmental Microbiology*.17:3540-3556; DOI:10.1111/1462-2920.12510

  Author contributions

  Kenneth Wasmund and Lorenz Adrian developed the concept of this study. Kenneth Wasmund designed the primers and performed alignments with several bioinformatics software. The practical work in the laboratory was conducted by Kenneth Wasmund, Camelia Algora and Josefine Müller. Karen G. Lloyd, Martin Krüger and Camelia Algora designed, coordinated, and performed field sampling of sediments, as well as

the biogeochemical analysis of sediments. Richard Reinhardt performed the pyrosequencing of the samples. Kenneth Wasmund performed the sequence bioinformatics analysis, Unifrac analysis and produced the phylogenetic trees. Kenneth Wasmund interpreted the results and drafted the manuscript. Lorenz Adrian and Camelia Algora revised the manuscript.

- **PUBLICATION III**

Algora C., Vasileiadis S, Wasmund K, Trevisan M, Krüger M, Puglisi E, Adrian L. (2015). Manganese and iron as structuring parameters of microbial communities in subsurface sediments of the Arctic Baffin Bay. FEMS Microbiology Ecology. 91:fiv056; DOI: 10.1093/femsec/fiv056

Author contributions

Camelia Algora, Lorenz Adrian, and Kenneth Wasmund developed the concept of this study, designed the laboratory experiments and coordinated the study. Sotirios Vasileiadis, Marco Trevisan, and Edoardo Puglisi performed the Illumina sequencing. Sotirios Vasileiadis performed the sequence bioinformatics analysis and the statistical analysis. Camelia Algora and Martin Krüger supplied samples and designed the field sampling. Camelia Algora performed the field sampling. The laboratory work was conducted by Camelia Algora and Kenneth Wasmund. Camelia Algora was the main responsible of the data interpretation. Camelia Algora drafted the manuscript. Lorenz Adrian and Kenneth Wasmund revised the manuscript and supplied ideas.

- **CRUISE REPORT**

Gründger F. and **Algora C**. (2010), Chapter 13, Biogeochemistry and Geomicrobiology, p. 136-139. *In* Damm V. (Editor), 2010. Cruise Report: The expedition of the research vessel "Polarstern" to the Arctic in 2010 (ARK-XXV/3). *Reports on Polar- and Marine Research*

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Camelia Algora and Friederike Gründger designed and performed the field and laboratory practical work on board of the Polarstern. The whole concept of the study was designed by Martin Krüger together with Hans Hermann Richnow and Lorenz Adrian. Camelia Algora wrote the manuscript, Friederike Gründger revised it.

ABBREVIATIONS

bp	base pairs
BGR	Bundesanstalt für Geowissenschaften und Rohstoffe
BSA	bovine serum albumin
cmbsf	centimetres below surface
DCB	dichlorobenzene
DNA	deoxyribonucleic acid
GC-FID	gas chromatography associated to a flame ionization detector
ICP-MS	inductively coupled plasma mass spectrometry
Ma	*Megaannum*, one million years
NW	North-West
mbsf	meters below surface
OTU	operational taxonomic unit
PCR	polymerase chain reaction
PCE	tetrachloroethene
PVC	polyvinyl chloride, synthetic plastic
qPCR	quantitative PCR
RDase	reductive dehalogenase
*rdh*A	reductive dehalogenase-homologous gene
RNA	ribonucleic acid
rpm	rounds per minute
rRNA	ribosomal ribonucleic acid
spp	species
SW	South-West
SD	Standard Deviation
TCB	trichlorobenzene
TCE	trichloroethene
TOC	total organic carbon
TC	total carbon
UFZ	Helmholtz-Zentrum für Umweltforschung
v/v	volume per volume
VPDB	Vienna PeeDee Belemnite standard

LIST OF CONTENTS

1 INTRODUCTION

Microorganisms are almost ubiquitously distributed throughout our planet, independently of how high or low the temperature or the pressure or the osmotic pressure or the pH is. Deep marine sediments are a vast underexplored environment on Earth, where we are currently starting to get an idea how and to which extend life is thriving there. In the last decades, scientific expeditions have drilled into the marine subsurface and tried to give some answers to those questions. We now know that microorganisms are present and active down to depths of 2,458 meters below seafloor (mbsf) (Inagaki et al 2015). Furthermore, microorganisms are present in marine sediments sometimes in massive amounts, which range from 10^6 cells per cm^3 at depths of 1,000 mbsf to 10^9 cells per cm^3 at surface sediments (Parkes et al 1994, Parkes et al 2000, Parkes et al 2014). We also know that these marine sediment microorganisms belong to diverse microbial groups. Nevertheless, many more scientific efforts are needed to unravel microbial life underneath the seafloor, such as: i) which metabolisms do the various microbes have, i.e., what are their carbon and energy sources?; How do they conserve energy?; Which compounds do they use as terminal electron acceptors in their respiratory chain?; Are all of them actively thriving or in stationary phase?, and/or: ii) what geochemical parameters from the sediments affect microbial diversity?; Does microbial diversity change with depth and within geochemical gradients? Studying life in the subseafloor enlarges our understanding of the concept of life, especially in energy terms, since very little nutrients are present in deep marine sediments, where light and phototrophic production is absent. The present study is a contribution to provide more knowledge on microbial life in deep marine sediments, with focus on a microbial group that is one of the most abundant and widespread in marine sediments: the class *Dehalococcoidia* within the phylum *Chloroflexi*.

1.1 MARINE SEDIMENTS AND THE BIOGEOCHEMICAL PROCESSES WITHIN

Marine sediments are deposits of eroded rocks and soil particles transported by rivers, currents and the wind together with material produced in oceanic processes, i.e., submarine volcano products, seawater precipitates, products from or/and remains of marine organisms that deposit at the seafloor and accumulate over years (Schulz and Zabel 2006). Marine sediments occupy the surface underneath the oceans, which is close to 70% of the Earth's surface and with depths that vary depending on the location and that can reach 10 km (Parkes et al 2000, Parkes et al 2014). This is a vast volume of our planet, so any process happening in the marine sediments may have critical consequences globally.

Marine sediments are naturally heterogeneous in their conditions, including: variable temperatures that range from 4°C at the seafloor to 100–150°C in areas affected by thermogenic processes, variable pressures that can reach up to 1,100 bar depending on the

depth, variable mineral composition and organic matter content (Jørgensen and Boetius 2007). Many of these conditions influence others, for example i) sediment porosity is influenced by pressure at variable sediment depths, and ii) the quantity and presence of oxidants or electron acceptors are influenced by the mineral composition of the marine sediments. In this line, organic matter content depends on the primary productivity within the overlying water column (which in turn depends on the proximity to the coast for input of fertilizing minerals and sea-ice free period in Polar Regions), water depth, sediment depth, distance to land, and latitude (Biddle et al 2006, Franco et al 2007, Hamdan et al 2013, Jørgensen et al 2012, Lipp et al 2008). Thus, there are oligotrophic areas such as the South Pacific Gyre, where the sediment cover is thin (Jørgensen and Boetius 2007, Parkes et al 2014), and organic-rich areas such as coastal sediments at continental margins (Parkes et al 2014).

It has been estimated that 5 to 10 billion tons of organic matter is present in oceanic waters, sinking to the sediments continuously (Jørgensen 1982). The bulk of this organic matter is oxidized during sedimentation in the water column and within the first cm of the marine sediments. The rest of it (about 15,000 x 10^{18} g C; (Hedges and Keil 1995)) accumulates in the sediment, representing the major reservoir of organic carbon on the planet. Organic matter is oxidized with oxygen via aerobic respiration of marine biota, which are chiefly microorganisms. In marine sediments, oxygen is consumed usually within the first near-surface mm, sometimes within the first metre depending on the sediment type, i.e., productivity in overlying water column (Cai and Sayles 1996, Jørgensen and Boetius 2007, Wenzhöfer and Glud 2002). Once oxygen is depleted, organic matter is oxidised by other oxidants via anaerobic respiration. These anaerobic oxidants are subsequently used by microorganisms in a higher to lower standard free energy-yields order as follows: nitrate, manganese(VI), iron(III) minerals, sulphate, and bicarbonate (Froelich et al 1979). All these reactions establish a zonation in sediments (D'Hondt et al 2004, Froelich et al 1979, Nealson 1997) and are enzymatically mediated by microorganisms.

Evidence of microbial processes in deep sediments of hundreds of mbsf was provided by pore-water analysis of oxidant (i.e., oxygen, nitrate, sulphate, etc.) concentrations and of released dissolved products such as sulphide and methane in depth profiles (D'Hondt et al 2002, D'Hondt et al 2004). Sulphate reduction and methanogenesis have been intensively studied in marine sediments worldwide (D'Hondt et al 2002). Sulphate reduction is one of the main processes of organic matter degradation in marine sediments due to the high content of sulphate ions in seawater (about 27 mM in average), which diffuses to the marine sediments (D'Hondt et al 2002, Froelich et al 1979). Once sulphate is depleted in the sediment, the main known microbial process in marine sediments is methanogenesis (D'Hondt et al 2002). Although sulphate reduction is usually the dominant pathway for the anaerobic oxidation of

organic carbon in marine sediments worldwide, other anaerobic pathways may play significant roles in the oxidation of organic carbon in specific marine sediments. For example, in Arctic near-surface sediments with high amounts of iron and manganese oxides, iron and manganese reduction are the dominant processes of organic carbon oxidation accounting between 50% and 90% of anaerobic oxidation of organic carbon (Nickel et al 2008, Vandieken et al 2006, Vandieken et al 2014). Iron reduction accounted for 25% of the total annual organic carbon oxidation in Arctic sediments of Northeast Greenland, where nitrate reduction accounted for 4%, manganese reduction below 1%, sulphate reduction for 33%, and oxygen respiration for 38% (Rysgaard et al 1998). In this way, the microbial metabolic activity within the marine sediments contributes substantially to Earth's biogeochemical cycles, especially over geological timescales (D'Hondt et al 2002, D'Hondt et al 2004, Wellsbury et al 2002).

1.2 MICROBIAL LIFE IN MARINE SEDIMENTS

Although deep marine sediments were for a long time considered without life due to the absence of energy supply, the high hydrostatic pressure and low temperatures, the Danish Galathaea deep-sea expedition already in 1951 showed that deep marine sediments harboured microbial life. Abundance of 10^4 to 10^6 cells per ml of viable bacteria in marine sediments of the Philippine Trench exceeding 10,000 m water depth was observed by microscopy and cultivation (Morita and Zobell 1955, Zobell and Morita 1959). Later on, detailed microbial investigations under the Ocean Drilling Program identified active metabolic processes and the presence of microorganisms in every single drilled location (Jørgensen and Boetius 2007, Parkes et al 2000, Parkes and Sass 2009). This gave further evidence for the presence of microorganisms and microbially mediated processes in deep marine sediments. Since then, various studies have demonstrated the existence of prokaryotes in all investigated deep marine sediments. Evidence for the presence of living bacterial cells was shown in terms of the presence of intact cells, visualized with fluorescent microscopy (Parkes et al 1994), or intact cell molecular constituents, such as membrane lipids, DNA and RNA, isolated with molecular biology techniques (Biddle et al 2006, Krüger et al 2002, Lipp et al 2008, Schippers et al 2005). Microscopic cell-counting after staining with the fluorescent DNA stain acridine orange demonstrated that deep marine sediments (even deeper than 500 mbsf) harbour significant numbers of microbial cells, with maximum cell numbers in surface sediments of around 10^9 cells cm^{-3}, which decreased logarithmically to 10^7 at 518 mbsf in Pacific Ocean sediments (Parkes et al 1994). Quantitative-PCR (qPCR) studies corroborated the microscopic cell counting results, showing highest 16S rRNA gene copy numbers of 10^8 cells of prokaryotes cm^{-3} in near-surface sediments on the Peru continental margin and numbers decreasing to 10^6 gene copies cm^{-3} by 40 mbsf (Schippers and Neretin 2006). More importantly, catalysed reporter deposition-fluorescence *in situ* hybridization (CARD-FISH) targeting ribosomal RNA

(rRNA) within the cells revealed that high numbers (up to 10^7 cells of prokaryotes cm^{-3}) of deeply buried (>400 mbsf) bacterial cells are alive as they do have ribosomes (Schippers et al 2005).

DNA-targeted molecular biological techniques allowed phylogenetic studies by partial sequencing of the 16S rRNA genes after generation of clone libraries that identified the most abundant microbial groups (Durbin and Teske 2011, Inagaki et al 2003, Inagaki et al 2006, Kormas et al 2003, Reed et al 2002, Webster et al 2006). Microorganisms inhabiting marine sediments predominantly belong to the phyla *Proteobacteria*, *Chloroflexi*, and candidate phylum JS1 within the *Bacteria*, and the crenarchaeotal groups of *Miscellaneous Crenarchaeotal Group* (MCG) and *Marine Bentic Group B* (MBG-B) within the *Archaea* (Fry et al 2008, Parkes et al 2014). Many of these microbial phyla, such as the candidate phylum JS1 (Webster et al 2004), have no isolated members, and others, such as the phylum *Chloroflexi*, have isolated members, however very distantly related to the sequences retrieved from marine sediments.

Cultivation efforts of marine sediment bacteria have yielded isolates belonging to the phyla *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* (D'Hondt et al 2004). *Firmicutes* and *Actinobacteria* bacteria are fast growing microorganisms that form spores, which may be reasons for their successful cultivation in laboratory media. A likely explanation for the low cultivation efficiencies (lower than 0.1% generally (Parkes and Sass 2009)) of the dominant microbial phyla, i.e., *Chloroflexi* and JS1, may be partly due to the energy limitation conditions in marine sediments, which when starving microorganisms are exposed to the rich in nutrients laboratory media, may experience a substrate-shock, possibly as a result of uncoupling of metabolic reactions (Parkes and Sass 2009). No isolate from the widely distributed marine sediment *Chloroflexi* or candidate phylum JS1 has been obtained so far. Members from the phylum *Chloroflexi* were maintained in anoxic medium (Köpke et al 2005, Lysnes et al 2004, Webster et al 2011), however never isolated. The isolation of a *Chloroflexi* from marine sediments may lead us to a better understanding of their physiology, metabolism and ecological role in marine sediments.

Due to the widespread presence and high abundance of *Chloroflexi* in marine sediments worldwide, *Chloroflexi* may play a critical role in the subseafloor ecosystem. Up to date, scientific surveys in marine sediments have focused firstly on biogeochemical processes, and secondly, on the microorganisms associated with these processes. However, microorganisms associated with biogeochemical processes identified until now did not belong to the dominant bacterial groups identified with DNA-based techniques, e.g., *Chloroflexi*, candidate phylum JS1. Thus, very little is known regarding the dominant bacterial groups such as *Chloroflexi* and

an in depth study on them may contribute to better understanding of the marine sediment ecosystem.

1.3 ABUNDANCE AND DISTRIBUTION OF *CHLOROFLEXI* IN MARINE SEDIMENTS

Bacterial members affiliated with the phylum *Chloroflexi* (formerly described as the 'Green non-sulphur' Bacteria) are almost ubiquitously found in marine sediments (Biddle et al 2006, Coolen et al 2002, Fry et al 2008, Inagaki et al 2006, Nunoura et al 2009, Parkes et al 2005, Parkes et al 2014). Indeed, sequences affiliated to *Chloroflexi* are reported to be present in 66% of the bacterial 16S rRNA gene clone libraries from marine sediments deeper than 2 mbsf (205 prokaryotic clone libraries analysed by (Parkes et al 2014)). Moreover, those 16S rRNA clone libraries pointed out *Chloroflexi* as one of the most abundant, if not the most abundant, bacterial phylum in marine sediments, with an average abundance of 25.5% (Parkes et al 2014). Sometimes, sequences affiliated to *Chloroflexi* add up to 80% of the total bacterial 16S rRNA genes recovered in some marine sediment sites (Parkes et al 2005). In many occasions, members of the *Chloroflexi* are associated with high organic matter contents (Parkes et al 2014), and are therefore thought to be heterotrophic and have a role in sedimentary organic matter mineralization (Parkes et al 2014).

The phylum *Chloroflexi* is deeply branching within the domain *Bacteria* and some of its first isolated members such as *Chloroflexus*, *Herpetosiphon* and *Thermomicrobium* are thermophiles, chemoorganotrophs or/and conserve energy from light (Garrity and Holt 2001, Oyaizu et al 1987). Members of the *Chloroflexi* have in common a unique, single-layer membrane and are therefore monoderms, whereby the great majority stain Gram-negative (Gupta et al 2013, Sutcliffe 2010, Sutcliffe 2011). The phylum currently encompasses six named classes as well as candidate classes consisting of sequences derived from uncultured organisms (Figure 1), which are metabolically diverse bacteria, ranging from aerobic thermophiles, anoxygenic phototrophs to organohalide-respiring bacteria (Gupta et al 2013, Hugenholtz and Stackebrandt 2004, Löffler et al 2013, Sekiguchi et al 2003, Yabe et al 2010, Yamada et al 2006). In the last years, the isolation of a novel species of a nitrite-oxidizer and a nitrate- and iron-reducers evidenced the expanded metabolic diversity still to be discovered within the phylum *Chloroflexi* (Kawaichi et al 2013, Sorokin et al 2012).

Chloroflexi sequences retrieved from marine sediments are usually affiliated to the classes *Anaerolineae*, *Caldilineae* (Blazejak and Schippers 2010) and *Dehalococcoidia* (Löffler et al 2013), as well as to other groups not classified as classes and comprising exclusively environmental sequences such as the formerly known subphylum IV (Parkes et al 2014). Classes *Anaerolineae* and *Caldilineae* were formerly known as subphylum I (Hugenholtz and

Stackebrandt 2004, Yamada and Sekiguchi 2009) and their cultured members are organoheterotrophs (Sekiguchi et al 2003, Yamada et al 2006). Class *Dehalococcoidia* was the formerly known subphylum II (Hugenholtz and Stackebrandt 2004) with several cultured members known for their ability to respire with organohalides (Bowman et al 2013, Löffler et al 2013, Moe et al 2009). Among these three *Chloroflexi* classes, the most frequently detected phylotypes retrieved from marine sediments affiliate to the *Dehalococcoidia* (Durbin and Teske 2011, Inagaki et al 2003, Inagaki et al 2006, Jørgensen et al 2012, Webster et al 2006, Wilms et al 2006). Moreover, PCR-independent metagenomic studies identified both *Dehalococcoidia* affiliated 16S rRNA gene sequences and other DNA sequences most similar to known *Dehalococcoidia* (Biddle et al 2008, Biddle et al 2011). Each of these three classes may have different niches in marine sediments, as suggested by microbial community surveys of South Pacific abyssal marine sediments, which evidenced a change in *Chloroflexi* abundance and in *Chloroflexi* diversity and composition with depth (Durbin and Teske 2011). In this study, sequences retrieved from oxic upper layer sediments (60-70 cmbsf) contained 13% of *Chloroflexi* of the so-termed "*Chloroflexi* VIb" (Durbin and Teske 2011) and the class *Anaerolineae* (Yamada et al 2006). However, sequences retrieved from the anoxic deeper sediment part increased proportionally with depth and belonged mainly to the class *Dehalococcoidia* (Durbin and Teske 2011).

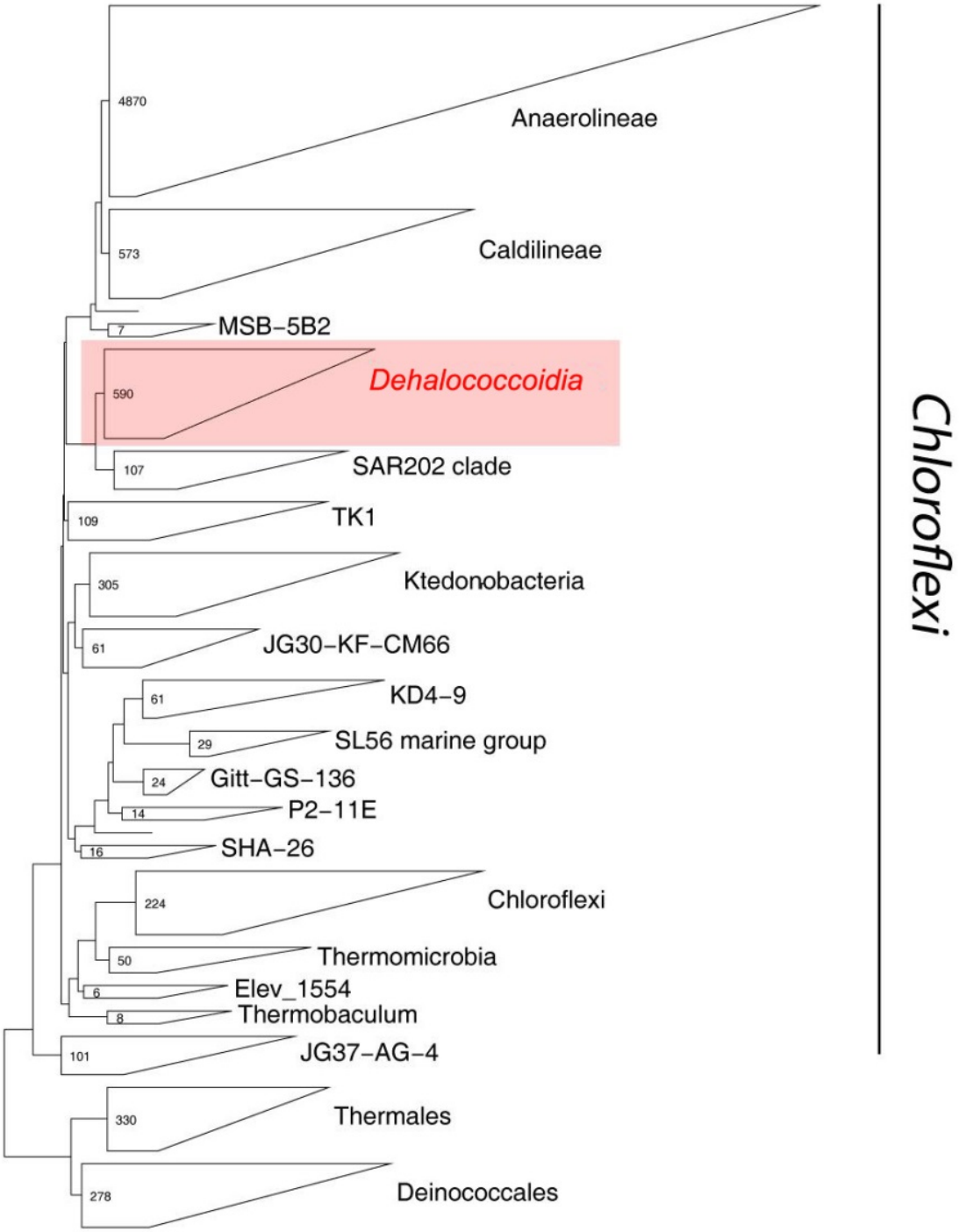


Figure 1. Phylogenetic tree of the phylum *Chloroflexi* as classified by the SILVA 100 16S rRNA gene database. The phylum *Chloroflexi* includes six named classes: *Anaerolineae, Caldilineae, Dehalococcoidia, Ktedonobacteria, Chloroflexi*, and *Thermomicrobia*. Among the many groups, the class *Dehaloccocoidia* includes the organohalide-respiring bacteria: *Dehalococcoides mccartyi* (Löffler et al 2013), *Dehalobium chlorocoercia* DF-1 (May et al 2008) and *Dehalogenimonas lykanthroporepellens* strains BL-DC-8 & BL-DC-9 (Moe et al 2009), and sequences retrieved from marine sediments.

But what do we know about bacteria of the class *Dehalococcoidia*? The class *Dehalococcoidia* includes to date only a few isolated species: *Dehalococcoides mccartyi* (various strains isolated (Löffler et al 2013)), *Dehalogenimonas lykanthroporepellens* (Moe et al 2009), *Dehalogenimonas alkenigignens* (Bowman et al 2013), and *Dehalobium chlorocoercia* strain DF-1 (May et al 2008). All of them are phylogenetically relatively closely related and all of them are known for exclusively growing by respiring organohalides, a process called "organohalide respiration" (Hug et al 2013b, Taş et al 2010b). In addition to the isolates, the mixed cultures KB-1 (Duhamel et al 2004) and ANAS (Richardson et al 2002, West et al 2008) are known to contain organohalide-respiring *Dehalococcoidia* from the genera *Dehalococcoides*. Moreover, several studies of mixed cultures from marine sediments enriched with organohalides have detected *Dehalococcoidia* 16S rRNA gene sequences (Bedard et al 2007, Fagervold et al 2005, Fagervold et al 2007, Kittelmann and Friedrich 2008a, Kittelmann and Friedrich 2008b, Watts et al 2005). All these organohalide-respiring *Dehalococcoidia* are phylogenetically close to each other, forming a cluster within the class when 16S rRNA genes are analysed (Figure 2). Indeed, *Dehalococcoidia* 16S rRNA gene sequences retrieved from marine sediments are in most cases, relatively distantly related to cultivated organohalide-respiring *Dehalococcoidia*, forming a so-called "sister lineage", as well as several other deeper branching clades (Durbin and Teske 2011, Inagaki et al 2006). Therefore, organohalide respiration may be a possible metabolism for marine *Dehalococcoidia*, and evidence for organohalide respiration in the marine subsurface was found when amplifying and sequencing the gene of the key enzyme for organohalide respiration, i.e., reductive dehalogenase (Futagami et al 2009). However, it cannot be concluded that all marine sediments containing *Dehalococcoidia* have a metabolism based on organohalide respiration, since marine sediment *Dehalococcoidia* and organohalide-respiring *Dehalococcoidia* form separate phylogenetic clusters. In fact, attempts to enrich and isolate marine sediment *Dehalococcoidia* have been unsuccessful so far (Köpke et al 2005, Süβ et al 2004). Getting to know marine sediment *Dehalococcoidia* is an important task because they form a huge proportion of the microbial sub-seafloor biosphere and may play key roles for the functioning of the planet.

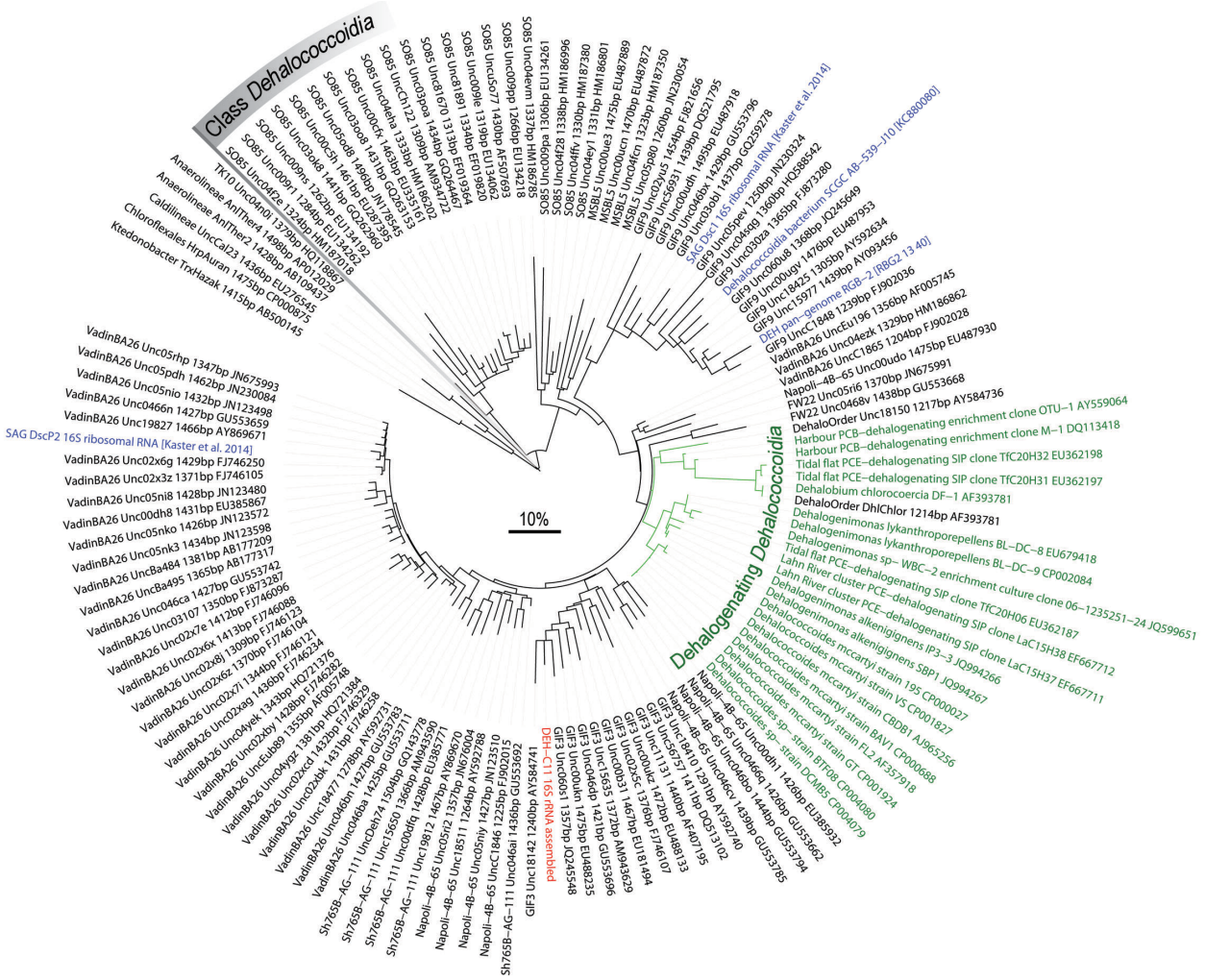


Figure 2. Phylogenetic tree of the class *Dehalococcoidia* based on 16S rRNA genes from the SILVA 100 database representing the different clusters of *Dehalococcoidia* sequences retrieved from uncultured organisms, mostly from the subseafloor. Organohalide-respiring *Dehalococcoidia* members (isolated spp. or from enrichments or stable isotope probing studies) are shown in green. 16S rRNA gene sequences from published single cell genomes of the *Dehalococcoidia* are shown in blue. A 16S rRNA gene from an unpublished single cell genome is shown in red. The tree was calculated using the maximum likelihood algorithm, courtesy of Dr. Kenneth Wasmund, UFZ–Leipzig.

1.4 ORGANOHALIDE RESPIRATION IN MARINE SEDIMENTS

Cultivated members of the *Dehalococcoidia* are highly specialized on organohalide respiration for growth, i.e., they exclusively use organic compounds carrying halogen substituents as terminal electron acceptors in their respiratory chain, coupled to hydrogen as the sole electron donor (Hug et al 2013b, Richardson 2013, Taş et al 2010b). In this way, the organohalide compound is being reduced and a halogen is removed from the organohalide compound. This process has been extensively studied as some halogenated compounds, such as tetrachloroethene (PCE) and trichloroethene (TCE), for example, are major groundwater pollutants worldwide. PCE and TCE are transformed via organohalide respiration into the dichloroethenes 1,2 *cis*-DCE, 1,2 *trans*-DCE or 1,1 DCE by a wide spectrum of microorganisms, such as the proteobacterial *Sulfurospirillum multivorans*, *Desulfuromonas* spp*., Geobacter lovleyi,* and the Firmicutes *Desulfitobacterium* spp. and *Dehalobacter restrictus* (Christiansen and Ahring 1996, Gerritse et al 1996, Holliger et al 1998, Utkin et al 1994). However, only members of *Dehalococcoides mccartyi* within the *Dehalococcoidia* are known to completely metabolically respire PCE to the non-toxic ethene (Maymó-Gatell et al 1997). Various *Dehalococcoides mccartyi* strains have been observed to grow not only with chlorinated aliphatics but also with many other aromatic organohalides. For instance, *Dehalococcoides mccartyi* strain CBDB1 is able to respire a broad spectrum of chlorinated and brominated aromatic compounds including chlorobenzenes, bromobenzenes, chlorophenols, dioxins and polychlorinated biphenyls (PCBs) (Adrian et al 2007, Bunge et al 2003, Cooper et al 2015, Wagner et al 2012, Yang et al 2015).

Organohalide-respiring microorganisms represent a bioremediation option for contaminated sites because the majority of organohalides that have been extensively used in agriculture and industry are highly toxic and persistent in the environment (Braeckevelt et al 2011, Cichocka et al 2010, Imfeld et al 2011, Löffler and Edwards 2006, Maphosa et al 2012, Mészáros et al 2013, Narihiro et al 2010, Pérez-de-Mora et al 2014, Taş et al 2010a, Taş et al 2011, Verce et al 2015). Despite being mainly known for their anthropogenic origin, organohalides are also naturally produced by a wide array of biological and chemical processes in the environment, and oceans are the largest source of biologically produced halogenated organic compounds on Earth (Gribble 2003, Häggblom and Bossert 2003). Regarding the biological processes, an immense number of marine organisms including algae, sponges, corals, invertebrates and bacteria, among others, produce halogenated compounds, the polybrominated ones being the most common ones (Ashworth and Cormier 1967, Baker and Duke 1973, Herrera-Rodriguez et al 2011, Lira et al 2011, Pauletti et al 2010, Pedersén et al 1974, Utkina et al 2001, White and Hager 1977). Chemical processes include i) geothermal processes at high temperature and

pressure, e.g., volcanic eruptions, and ii) halogenation during the degradation and diagenesis of organic matter (Häggblom and Bossert 2003, Keppler et al 2000). Chemically halogenated organic matter buried in marine sediments can therefore be used for respiration by organohalide-respiring bacteria. Thus, organohalide-respiring bacteria have long evolved to thrive on natural organohalogens during Earth's history. In this respect, halogenation and dehalogenation are part of the halogen cycle on Earth. Evidence of dehalogenation processes mediated by microorganisms in marine and estuarine environments already exists (Ahn et al 2003, Futagami et al 2013, Häggblom et al 2003, King 1988, Monserrate and Häggblom 1997). In fact, reductive dehalogenase-homologous (*rdh*A) genes have been amplified from various marine sediment locations within the Nankai Trough plate-subduction area in Japan (Futagami et al 2009), suggesting the occurrence of organohalide respiration in marine sediments. Sediment slurries incubated over 200 days showed activity with halophenols (bromophenols, chlorophenols, iodophenols) in shallow sediments and, after RNA studies, *Desulfuromonadales* bacteria were identified as predominant (Futagami et al 2013). In terrestrial uncontaminated soils, *Dehalococcoidia* correlated positively with organochlorine concentration and organic carbon content. In addition, *Dehalococcoidia* numbers increased when cultured with enzymatically produced organochlorines concomitant to chlorine accumulation (Krzmarzick et al 2012), indicating that some *Dehalococcoidia* identified in pristine sites may have an organohalide-respiring metabolism. The metabolism of marine subsurface *Dehalococcoidia* from pristine sediments that fall outside of the clade containing cultured species (i.e., the predominant groups of *Dehalococcoidia* found in the marine subsurface (Kittelmann and Friedrich 2008a)), is completely unknown, i.e., whether they respire either organohalides or other known anaerobic compounds, e.g., sulphate, nitrate, iron, manganese, possibly even fermentation. For this reason, enrichments and isolates of *Dehalococcoidia* from the marine subsurface may substantially aid in understanding the widely distributed, abundant and enigmatic marine subsurface *Dehalococcoidia*. Additionally, more information on the specific ecological distributions and their relationships with the geochemistry of the marine subsurface will also greatly aid in our understanding of these microorganisms.

1.5 STUDY AREA: BAFFIN BAY

During this thesis work, focused sampling of marine sediment cores from the Baffin Bay was undertaken and studied for their geochemistry and microbial communities, and therefore this section aims to provide a background into the characteristics of the Baffin Bay and its subsurface. The Baffin Bay is a relatively isolated sea located between the Eastern coast of Northern Canada and Greenland (Figure 3). From North to South, the Baffin Bay connects the Arctic Ocean to the Labrador Sea and the North Atlantic Ocean. It is thought that in the late

Paleogene, the Baffin Bay formed the main link for surface waters exchange between the Arctic and the Atlantic Ocean (Srivastava et al 1989). Bathymetrically, the Baffin Bay is an ocean basin formed of a large and deep plain in the centre of the bay with depths that exceed 2,300 m and two continental shelves, one shelf on the side of Greenland, and the other shelf on the side of the Baffin Island, in Canada. The continental shelf on the side of Greenland is much wider than the continental shelf at the Baffin Island. In both shelves, and running across them, there are submarine canyons that cut the shelves (Tang et al 2004). The current circulation is cyclonic (counter-clockwise) with the formation of an eddy at the northern part of the Baffin Bay. Two main currents are present in the Baffin Bay: the subsurface, salty and warm West Greenland Current going northward along the coast of Greenland and the near-surface Baffin Island Current with fresh and cold waters coming from the Arctic Ocean, which enters the Baffin Bay through the Kane Basin and the Smith Sound and flows southwards along the Western coast of Canada to the Labrador Sea (Münchow et al 2015, Tang et al 2004). Three water masses are present in the Baffin Bay although not in all areas. The most surficial water mass, present in the upper 100–300 m is an Arctic water mass, however not present in the southeast of the Baffin Bay. An intermediate water mass at 300–800 m, named West Greenland Intermediate water, is present in the central area of the Baffin Bay. Below 1,200 m, there is a deep Baffin Bay water mass, which covers all areas of high depths (Tang et al 2004).

The Baffin Bay, as any other Arctic region, experiences contrasted sun irradiance periods that turn into contrasted seasonal periods of daylight and temperature along the year. Extreme low air temperatures in winter result in sea-ice formation on the surface of the ocean waters, which restricts the primary production performed by photosynthetic planktonic organisms and algae (Hulth et al 1996). Although some algae are photosynthetically active under the sea-ice cover, this algal primary production of biomass is also restricted to the summer months, as it depends on the sea-ice thickness, snow cover and the water column stratification (Boetius et al 2013). Consequently, to the restriction of the primary production in the Arctic, the organic matter supply to Arctic sediments is reduced (Hulth et al 1996). The Baffin Bay is sea-ice covered most of the year. It is completely free of sea-ice only in the months of August and September (Tang et al 2004). Sea-ice strongly restricts access to this area for navigation and of course, for sampling. This partially explains why very few microbiological studies have been performed in the Baffin Bay.

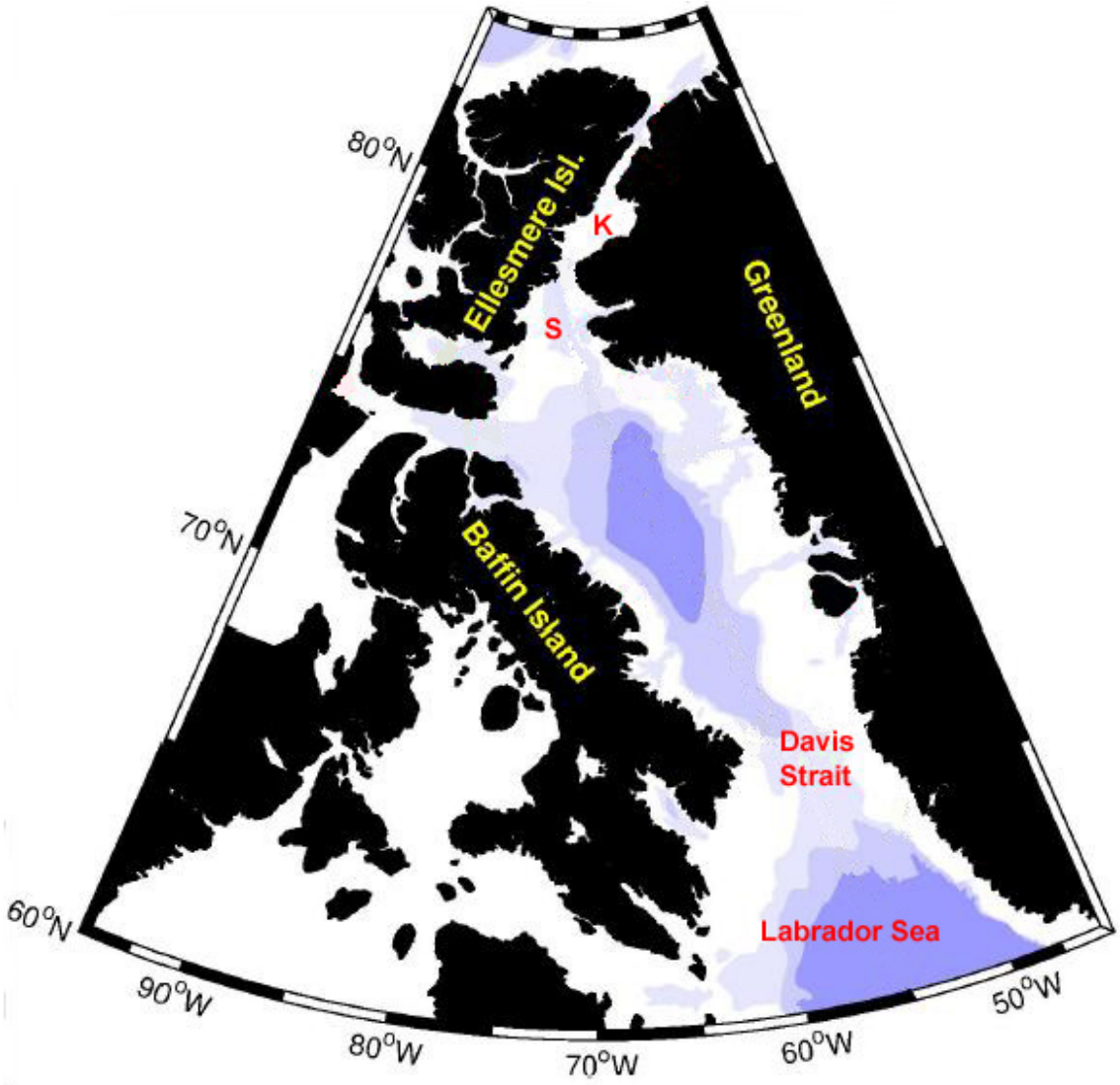


Figure 3. Baffin Bay map modified from (Tang et al 2004). K: Kane Basin, S: Smith Sound. Baffin Island and Ellesmere Island belong to Canada. Darker blue colour corresponds to deeper bathymetry.

In 1985, the Ocean Drilling Program (ODP) Leg 105 sampled the southern Baffin Bay at the site 645, which is located on the slope off Baffin Island, for geological and geochemical purposes. This study also included two further sites in the Labrador Sea (Srivastava et al 1989). During ODP Leg 105, cores down to 1,147 mbsf of depth were sampled at site 645, with a water depth of 2,018 m in the only study ever done in Baffin Bay sediments so far. Site 645 had high sedimentation rates, averaging to 60 m Ma^{-1}, strongly influenced by terrigenous input of clay, silt, sand, and abundant dropstones coming from nearby continental lands and minor biogenic components (Srivastava et al 1989, Stein et al 1989). Geochemical characterization of core pore-waters at site 645 indicated a decrease of sulphate concentrations in the upper 25 mbsf from 22 mM to less than 1 mM, and complete sulphate depletion at 35 mbsf (Zachos and Cederberg 1989). This sulphate depletion corresponds to Pliocene-Pleistocene hemipelagic sediment layers with high sedimentation rates above 120 m Ma^{-1} (Zachos and Cederberg 1989). The decrease in pore-water sulphate concentrations is

most likely the result of microbial sulphate reduction. Sulphate reduction products are sulphide and bicarbonate. Sulphide may lead to the formation of pyrite, which is a common mineral in the upper sediment layers of site 645 where sulphate is depleted. In addition, bicarbonate may cause a decrease of pore-water calcium and magnesium ions in pore-water depth profiles, which was the case in upper sediment layers, and may be linked to carbonate precipitation of calcite and dolomite minerals (Zachos and Cederberg 1989). Organic carbon content at site 645 was relatively high and increased with depth with values that ranged between 0.5% to almost 3% (Stein et al 1989). The composition of this organic matter is mostly terrigenous, and thus originating by nearby terrestrial lands (Stein et al 1989).

In 2007, as part of a study for the international Polar Year, Galand and co-workers took one sample of the Baffin Bay intermediate water mass at the southern Baffin Bay at a depth of 1,000 m in the water column for microbiological studies. Galand and co-workers investigated if there were patterns of microbial biogeographical distribution in various Arctic water bodies, and found that it was explained by the water circulation and hydrography of the Arctic Ocean (Galand et al 2010).

The Baffin Bay is an excellent location to conduct studies of microbial communities in sediments for several reasons: i) the nature of its sediments, particularly the quantity and composition of its mostly terrestrially derived organic matter ii) topographically, it is a relatively isolated ocean basin with lower influence from global ocean circulation. This relative isolation may therefore have reduced ubiquitous bacterial dispersal, and selected for bacteria highly adapted to the processes within this area, e.g., glacial processes, and iii) it combines an ocean basin with continental shelves and deep abyssal plain so that the influence of gradients of environmental parameters (e.g., water column depth, sediment depth, distance to land) on the distribution of microorganisms can be studied.

For these reasons, the Baffin Bay was selected as the study site. Emphasis was laid on investigating the presence, distribution, and diversity of bacteria of the class *Dehalococcoidia* in Baffin Bay sediments.

1.6 AIM OF THE STUDY

The overall aim of this study was to understand the ecological role of *Dehalococcoidia* in marine sediments. As a step towards this overall aim, it was tried to identify physiological characteristics of marine *Dehalococcoidia.* To accomplish the aim, two main experimental approaches were chosen:

Approach 1. Use of cultivation techniques to describe the physiology of marine *Dehalococcoidia*, and in particular, their respiration mode. The cultivation relied on batch cultures containing marine sediment bacteria as inoculum. The medium was based on the growth medium described for *Dehalococcoides mccartyi*, which are phylogenetically the closest and best known cultivated members to marine *Dehalococcoidia*. As a tool to evaluate growth of *Dehalococcoidia*, qPCR and chemical analysis of metabolic products was chosen. Several tasks within this approach were defined:

a) Study if *Dehalococcoidia* could be anaerobically cultivated in the laboratory at atmospheric pressure, within a reasonable growing timespan, i.e., months.

b) Study if *Dehalococcoidia* physiology may be similar to *Dehalococcoides mccartyi* in that they use organohalides as a terminal electron acceptors in their respiratory chain.

c) Study of other types of respiratory modes, e.g., sulphate, iron, manganese or humic acids.

Approach 2. Description of the natural occurrence of *Dehalococcoidia*, their distribution and abundance in marine sediments associated to biotic (i.e., other bacterial groups), and abiotic (i.e., sediment geochemistry, geographical location, depth) parameters in marine sediments, to identify natural conditions promoting their growth. Such an investigation was performed with an in situ approach in sediments of the Baffin Bay in the Arctic. To accomplish this second approach, three specific tasks were defined:

a) Description of the presence, abundance and distribution of *Dehalococcoidia* in marine sediments at different sites and depths with a quantitative approach using qPCR.

b) Description of the microbial diversity in a geochemical gradient of organic matter contents and biogeochemical parameters within a transect from the shelf and into the basin of the Baffin Bay, including also other bacterial groups associated with *Dehalococcoidia*.

c) Identification of correlations of *Dehalococcoidia* abundance with biogeochemical parameters to obtain indications of preferable growth conditions to extrapolate to *Dehalococcoidia* physiology.

2 MATERIAL & METHODS

2.1 CHEMICALS

All chemicals were purchased from Sigma-Aldrich (MO, USA), Merck KGaA (Darmstadt, Germany) or AppliChem (Darmstadt, Germany) at highest purity available. The gases "Biogon" (80% nitrogen, 20% carbon dioxide; v/v), hydrogen, and nitrogen were purchased from Air Liquide (Paris, France). Humic acids were bought from Carl Roth (Karlsruhe, Germany).

2.2 SEDIMENT SAMPLES

Sediments from various sources were used. These sediment sources include the Greenlandic side of the Baffin Bay, the continental shelf at the central off Chile, Århus Bay in Denmark, and the continental shelf at the South-West of Ireland (Table 1). Sediments from the Baffin Bay were collected during the ARK XXV/3 expedition on board of the research vessel Polarstern in August–October 2010. Other sediments were collected during other expeditions and kindly supplied by Dr. Timothy G. Ferdelman from the Max Planck Institute for Marine Microbiology in Bremen, Germany.

Baffin Bay sediments were cored at 34 sites of the Baffin Bay, which extended from the coast of Greenland to the central area of the Baffin Bay (Damm 2010). Due to the geology of the area, with abundant dropstones brought by glaciers, some of the sites yielded unsuccessful recovery of sediments. From those cores with successful recoveries, ten cores were selected based on recovery length (longest cores), intact state of recovered sediments, and location of the site. Selected sediments were used for two biogeochemical and microbiological studies: i) involving three geographical distinct areas "Northern Greenlandic shelf" (sites 363 and 371 in Figure 4), "central deep basin" (sites 389, 391, and 453 in Figure 4), and "Southern slope" (sites 486 and 488 in Figure 4), (Algora et al 2013), and ii) involving seven sites along a North-to-South, shelf-to-basin transect (Figure 5) (Algora et al 2015).

Table 1. Oceanographic details from the sites where the sediment samples used in this study were cored.

Sample location	Cruise	Collection date	Site	Coordinates (latitude, longitude)	Water depth (m)	Sediment depth (mbsf)	References
Århus Bay, Denmark	–	2008	Århus M1 GC A/2	not known	not known	4.44[a]	from Dr. T.G. Ferdelman, MPI Bremen. Metrol project: http://metrol.mpi-bremen.de
Eastern slope, Porcupine Seabight, SW continental margin of Ireland	IODP Leg 307	2005	U1318	51°26.16'N, 11°33.0'W	423	23.15[a]	(Webster et al 2009)
NW-shoulder, Challenger Mound, Porcupine Seabight, SW continental margin of Ireland	IODP Leg 307	2005	U1317	51°22.8'N, 11°43.1'W	781–815	227[a]	(Webster et al 2009)
Central off Chile continental margin	SO-156/3	2001	GeoB 7155-4	34°35.00'S, 72°53.11'W	2,744	4.37–4.42[a]	(Treude et al 2005)
Central off Chile continental margin	SO-156/3	2001	GeoB 7165-2	36°32.32' S, 73°40.02' W	799	6.35–6.40[a]	(Treude et al 2005)
Baffin Bay, Greenland	ARK-XXV/3	2010	363	76° 52.92' N, 71° 34.01' W	938	4.69[b]	(Algora et al 2013)
Baffin Bay, Greenland	ARK-XXV/3	2010	365	76° 39.04' N, 71° 18.79' W	658	3.67[b]	(Algora et al 2015)
Baffin Bay, Greenland	ARK-XXV/3	2010	371	75º 58.24' N, 70° 34.86' W	598	4.05[b]	(Algora et al 2013)
Baffin Bay, Greenland	ARK-XXV/3	2010	383	75º 17.69' N, 69º 53.75' W	674	2.32 [b]	(Algora et al 2015)
Baffin Bay, Greenland	ARK-XXV/3	2010	387	74° 50.42' N, 69º 27.14' W	1,300	3.32[b]	(Algora et al 2015)
Baffin Bay, Greenland	ARK-XXV/3	2010	389	74° 37.05' N, 69º 13.75' W	1,716	4.24[b]	(Algora et al 2013)
Baffin Bay, Greenland	ARK-XXV/3	2010	391	74° 23.36' N, 69º 01.22' W	1,864	4.27[b]	(Algora et al 2013)
Baffin Bay, Greenland	ARK-XXV/3	2010	453	73° 19.37' N, 64° 58.11' W	2,300	4.69[b]	(Algora et al 2013)
Baffin Bay, Greenland	ARK-XXV/3	2010	486	72° 24.51' N, 60° 48.85' W	645	4.69[b]	(Algora et al 2013)
Baffin Bay, Greenland	ARK-XXV/3	2010	488	72° 08.80' N, 60° 58.86' W	1,493	4.69[b]	(Algora et al 2013)

Sediment depth: [a] refers to the depth that was used for inoculum for sediment cultures, [b] refers to the deepest part of the core that was retrieved, from seafloor until the indicated depth. For this later case, the entire core, from 0 mbsf to the indicated depths, was used for various analyses. SW─South-West, NW─North-West.

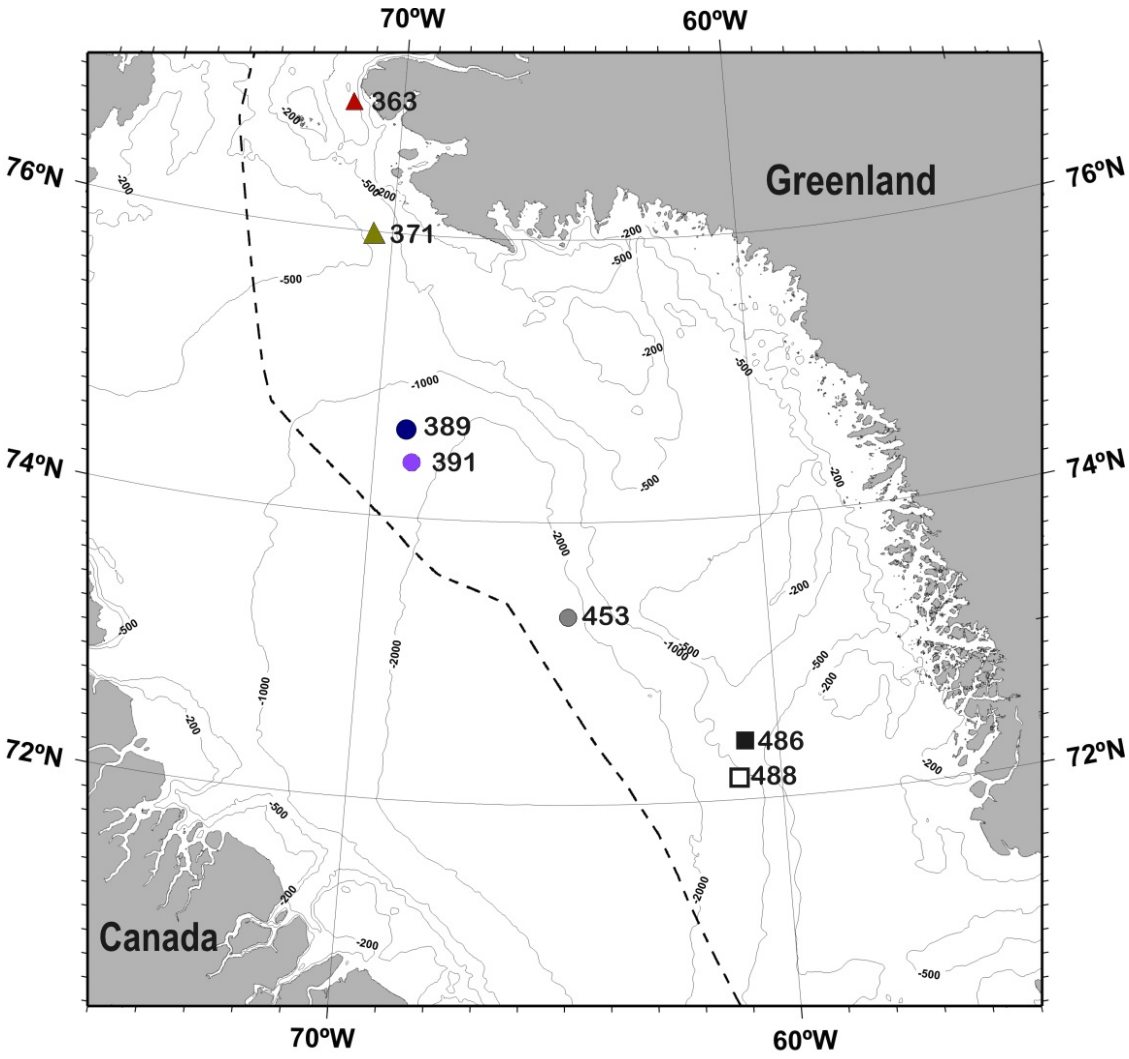


Figure 4. Map of the Baffin Bay indicating investigated sites from three distinct geographical areas. These areas were: "Northern Greenlandic shelf" (triangle symbol; sites 363 and 371), "central deep basin" (circle symbol; sites 389, 391, and 453), and "Southern slope" (square symbol; sites 486 and 488). These sites were studied for differences in geochemical parameters and for the presence, distribution, and abundance of specific microbial groups. The symbol and colour code are consistently used for each site in all figures of this thesis. Modified from (Algora et al 2013).

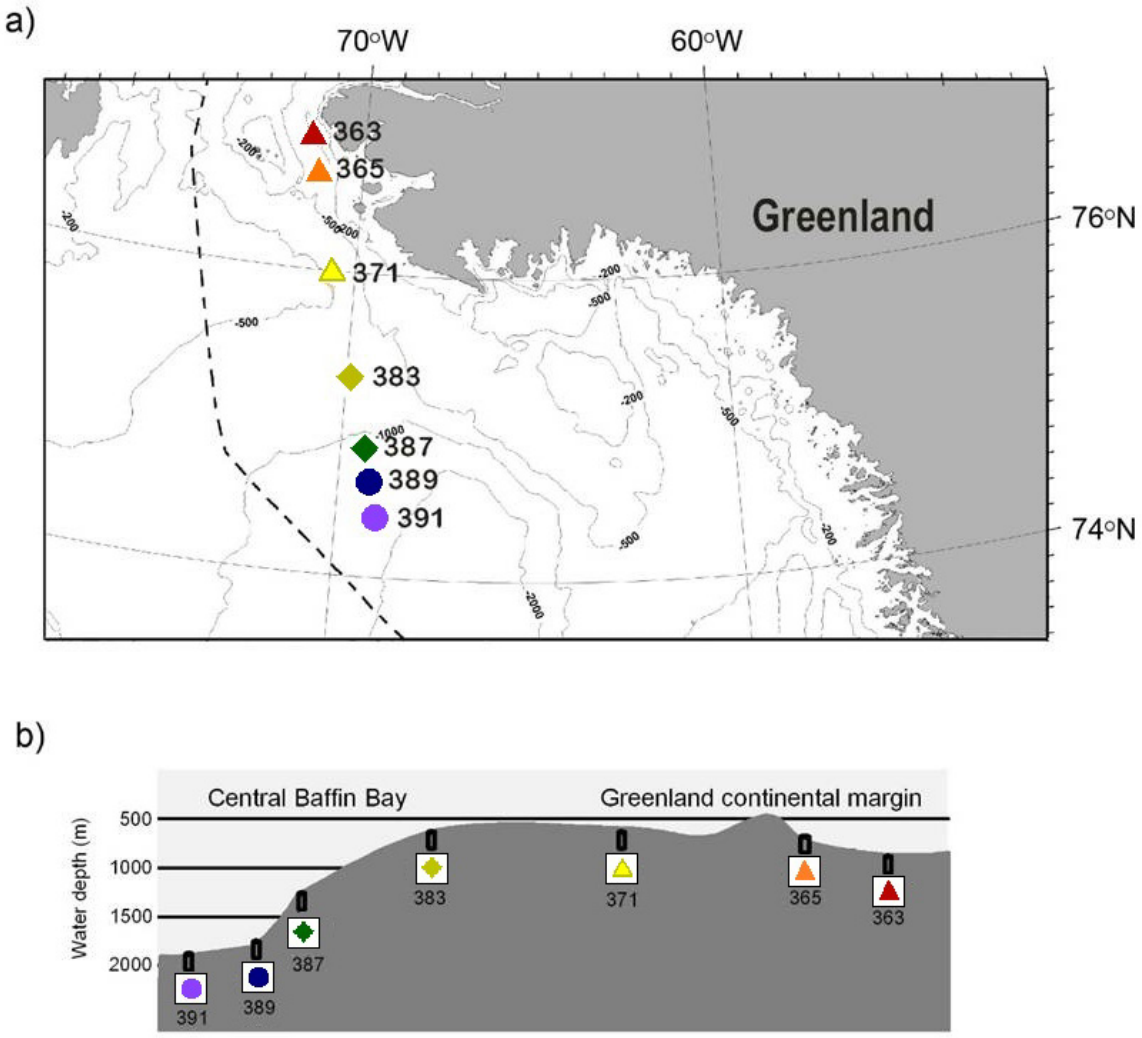


Figure 5. Map of the Baffin Bay showing the location of the sediment sampling sites investigated for a study focused on the microbial diversity associated to geochemical parameters along a North-to-South, shelf-to-basin transect; a) locations from the seven sites within the Baffin Bay that comprise the transect; and b) sediment cut-section scheme of the seven sites investigated. Modified from (Algora et al 2015).

2.2.1 Core sampling

Core sampling and sediment processing for those sediments that were donated by other groups are described elsewhere (see Table 1 for references). Sediments from the Baffin Bay were sampled within this work with a gravity corer equipped with a 4.70 m core barrel (Rehau AG & Co.) and a 90 mm (outside diameter) PVC liner as described (Algora et al 2013, Damm 2010). Recovered cores were divided into one metre sections on board using a PVC tube cutter and a clean masonry spatula, capped and sealed with electrical tape. All cores were kept at 4°C until further subsampling (Figure 6).

Figure 6. Core sampling at the Baffin Bay by means of a gravity corer during the expedition ARK XXV/3.

2.2.2 Core subsampling

Core subsampling consisted of retrieving five different types of samples from each core: i) sediment for free gases (not adsorbed to sediments, i.e., methane and carbon dioxide) analyses, ii) pore-water for dissolved ion concentration analyses, iii) sediment for anaerobic cultivation of indigenous sediment microorganisms iv) sediment for DNA-based analyses v) sediment for organic carbon content, elemental composition, and mineralogy.

Samples for gases, pore-water, and DNA-based analysis were collected at specific depth-sampling points, every 50 cm along the core and starting at 25 cmbsf for all cores (see scheme in Figure 7).

For gases, 5 ml sediment was sampled with sterile needle-adapter-cut syringes immediately after drilling a hole on the PVC liner at each depth-sampling point (Figure 7). Once sampled, liner holes were immediately sealed with electrical tape and the sampled sediment was placed in 50 ml glass serum vials which were previously filled with 20 ml of a 1 M NaOH solution in order to stop any microbial activity (Figure 7). Filled vials were instantly sealed with gas-tight rubber stoppers and aluminium crimp caps and mixed until the sediment was completely suspended in the solution. Vial storage was at 4°C until vial headspace analysis was performed by Gas Chromatography (GC).

Figure 7. Scheme of depth-sampling points for every core (left), and sediment sampling for gas analyses (right).

Pore-water samples were taken immediately after gas sediment sampling (at the same core positions). Between 8 to 10 ml pore-water was recovered from all cores at every depth-sampling point using a rhizon sampler (CSS-F, 5 cm or 10 cm porous length, 2.5 mm tip diameter, Rhizosphere Research Products, Wageningen, NL). Rhizon samplers were fine plastic tubes with a porous membrane on one end, which was introduced into the sediment, and a syringe adaptor on the other end of the tube (Figure 8). The pore-water sampling was done at 4°C inside a cool room.

Rhizons used for pore-water sampling were previously dipped in double distilled water for 1–2 h before use, checked for proper functioning (Figure 8), and introduced into each depth-sampling point inside each core section using the holes already made from the gas sampling in a 4°C room (Figure 8). Rhizons where connected to a sterile syringe which had the plunger partly out to create vacuum and facilitate pore-water extraction from sediment (Figure 8). Through the rhizon porous membrane, the pore-water penetrated and flowed to the syringe barrel where accumulated. The rhizon was left inside the sediment until the syringe was filled and, in any case, for at least 4 hours. The pore-water samples were transferred to polypropylene vials, preserved with 1% (v/v) of a 65% (v/v) concentrated nitric acid solution, and stored at 4°C until analysed (Figure 8). The polypropylene vials were previously washed with 1% (v/v) nitric acid solution and rinsed with double-distilled water. Rhizons were cleaned and rinsed with double-distilled water and further re-used.

Figure 8. Pore-water sampling procedure, which was performed using rhizon samplers. See text for further details.

For retrieval of the remaining sample types, each core section was sliced in two halves. For that, the PVC liner was laterally cut with two sledge-mounted vibrating saws (Figure 9). The two sediment halves were separated with a clean stainless steel thread and a clean masonry spatula. One half was used for stratigraphic studies and stored at the core archive of the Bundesanstalt für Geowissenschaften und Rohstoffe (BGR) in Hannover, Germany, after the expedition. The other half was used for sediment sampling on board, which was done immediately after core slicing. Before sediment sampling, the sediment sliced area was removed to avoid contamination with a sterilized masonry spatula and sterile scalpels. All sediment samples taken for microbiological purposes were strictly sampled from the untouched and uncontaminated interior of every core.

Figure 9. Slicing of one-metre core sections into two halves, which was executed for further sampling of sediment for microbiological purposes.

Sediment samples for anaerobic cultivation of microorganisms were taken with sterile needle-adapter-cut 5 ml syringes immediately after the core was sliced. Sediment samples of 3–20 ml volume were inoculated into glass serum bottles that contained various volumes (27, 45, 75, or 90 ml) of sterile anoxic minimal media. The minimal media components, which are detailed in Appendix 1 (section 6.1), contained in some cases amendments of yeast extract (0.02%), selenium (6 µg l^{-1}) and tungsten (8 µg l^{-1}), and acetate (5 mM). The medium was buffered and reduced with various reducing agents (either titanium (III) citrate, or iron sulphide, or sodium sulphide together with L-cysteine; see Appendix 1) at least a day prior to sediment inoculation and had a headspace atmosphere of "Biogon" (consisting of 80% nitrogen and 20% carbon dioxide; v/v) which occupied ~50% of the glass serum bottle volume. Glass serum bottles were sealed with rubber or Teflon-lined butyl rubber stoppers and aluminium crimps. Prior to inoculation, glass serum bottles containing the reduced and sterile medium were de-crimped, de-capped, and inoculated with sediment contained in the needle-adapter-cut syringe and with help of the plunger. Once inoculated, the glass serum bottle was partly capped and purged with a Biogon stream which was supplied with a sterile needle connected to a 0.2 µm filter to the Biogon rubber pipe source for the headspace exchange (Figure 10, right). Afterwards, the glass serum bottle was capped, crimped and gently mixed.

Figure 10. Sediment sampling for inoculation in glass serum bottles (left). Headspace gas was purged with the mixture gas Biogon (80% N_2, 20% CO_2; v/v) to eliminate the air which may have entered during the inoculation procedure (right).

Sediment samples for DNA-based analyses were retrieved with sterile needle-adapter-cut 5 ml syringes at every depth-sampling point, or as close as possible, in an effort to avoid possible contaminated sediment after the gases or pore-water sampling at each sampling point. Sediment samples were placed in sterile 15 ml Falcon tubes, labelled and immediately frozen at -80°C (Figure 11). Sediment samples were stored at -80°C until analysed.

Figure 11. Sediment sampling for further DNA-based analyses.

Sediment samples for analysis of organic carbon content, elemental composition, and mineralogy were collected at various intervals of 50–100 cm along the core. Sediment samples were placed in sterile Schott glass bottles of 100 or 250 ml (Schott Duran), which were closed with butyl septa and plastic screw caps under a nitrogen atmosphere and stored at 4°C until measured.

2.3 SEDIMENT GEOCHEMICAL ANALYSIS

The determination of methane concentrations from the headspace of the sediment sample vials was performed using a GC-FID equipped with a 6' Hayesep D column (SRI 8610C, SRI Instruments) at 60°C. This was done by collaborators at the BGR as described elsewhere (Algora et al 2013).

Pore-water samples were analysed to obtain ion compositions using an inductively coupled plasma mass spectrometry (ICP-MS) instrument (Perkin Elmer Sciex Elan 5000). This was performed by collaborators in the BGR as previously described (Algora et al 2013).

Sediment carbon content was analysed as total organic carbon (TOC) and total carbon (TC) from the sediment samples frozen at -80ºC, after the sediment sample for DNA was already analysed, from cores 363, 365, 371, 383, 387, 389, and 391 (Figure 5). For that, a sample from the frozen sediment from each core and depth was thawed, dried, homogenized with a mortar, and grinded. One gram of the dry sediment was analysed in a LECO RC-412 carbon determinator (LECO Corporation) with a temperature program of 80°C min^{-1} from 100°C to 530°C for the measurement of TOC, followed by 100°C min^{-1} from 530°C to 1000°C for the measurement of total inorganic carbon. All measurements were done in duplicate and values are reported in weight percentages as mean of duplicate measurements.

Sediment samples from cores 363, 389, and 486 (Figure 4) were examined for sediment mineralogy by X-Ray Powder Diffraction (XRD) patterns, for sediment elemental composition by wavelength dispersive x-ray fluorescence spectrometry (WD-XRFS) in their mineral material, and for TOC and TC. These analyses were performed by collaborators at the BGR as described elsewhere (Algora et al 2013).

In addition, stable isotope composition of the sediment organic content (organic carbon ^{13}C) for cores 363, 389, and 486 was determined in order to obtain an indication of the sediment organic carbon origin, either terrestrial or marine. For this, 5 mg of sediment, which was previously freeze-dried and de-carbonated with hydrochloric acid, was wrapped in tin caps (3.5 x 5 mm, HEKAtech) and analysed in an Elemental Analyzer (EuroEA3000, Euro Vector) coupled via a ConFlow III (Thermo Fisher Scientific) to a MAT 253 isotope ratio mass spectrometer (Thermo Fisher Scientific) as previously described (Algora et al 2013). Triplicate ^{13}C values are expressed in delta notation ($\delta^{13}C$) relative to the Vienna PeeDee Belemnite (VPDB) standard and reported in per mil (‰) (Coplen 2011).

$$\delta^{13}C = (({}^{13}C/{}^{12}C)_{sample} / ({}^{13}C/{}^{12}C)_{VPDB\text{-}standard})\text{-}1$$

2.4 CULTIVATION OF MICROORGANISMS IN SEDIMENT CULTURES

For the cultivation of microorganisms indigenous to the sediments, a defined anoxic minimal liquid medium was used. The medium composition is given in Appendix 1, section 6.1, and was based on the medium used for the cultivation of *Dehalococcoides*

mccartyi strain CBDB1 as described by L. Adrian (Adrian 1999). The medium was prepared by adding a mineral solution (Widdel 1980), a trace metal solution "SL-9" (Tschech and Pfennig 1984), sodium acetate (5 mM) as carbon source, and resazurin as redox indicator to Milli-Q water (see Appendix 1, section 6.1, for specific components and concentrations in the solutions). In order to eliminate oxygen from the medium, dissolved air was removed by applying vacuum to a one- or two-litre glass Schott bottle filled with the medium for at least one hour. Subsequently, the medium was purged with pure nitrogen gas for 30 min and further transferred to an anaerobic glovebox where it was dispensed in aliquots to glass serum bottles. Aliquot volumes were 15 ml, 30 ml, 60 ml or 90 ml, leaving a headspace of 40–55% volume in the glass serum bottle. Filled glass serum bottles were left inside the anaerobic glovebox for fifteen minutes to one hour, to exchange the gas phase to the glovebox atmosphere composed of nitrogen and 2–3% hydrogen. Glass serum bottles were then sealed with Teflon-lined butyl rubber stoppers and aluminium crimp caps. The bottles were autoclaved, and once they reached ambient temperature, buffered with bicarbonate to pH 7.0–7.3 (see Appendix 1, section 6.1.3, for bicarbonate solution). Afterwards, reducing agents (Table 2, see Appendix 1, sections 6.1.5 and 6.1.6 for preparation of the solutions) were added. Reducing agents were allowed to equilibrate for at least 12 h, to overnight. Vitamins were added from a filtered stock solution (Vitamin 7; (Adrian 1999)).

Table 2. Reducing agents combinations used in this study.

Mixture	**Composition**	**Concentration**
I	Titanium (III) citrate 15% (0.1 M Ti(III), 0.2 M citrate)	1.3%
II	Iron sulphide solution	1.6%
III	Sodium sulphide L-cysteine	0.3 mM 0.2 mM

For inoculation, 9–10% (v/v; wet sediment) of sediment (various origins, Table 1) was transferred to the glass serum bottles containing the sterile medium with sterile scalpels inside an anaerobic glove box, except sediments from the Baffin Bay which were inoculated on board as previously described. The sediment inocula were always taken from inner undisturbed core parts, avoiding contamination with the liner or any other recipient holding core intervals.

The headspace gas phase from the glass serum bottles was interchanged to the gas mixture Biogon by five cycles of vacuum and gas mixture addition, and further pressurized to 0.2 bar overpressure. In addition, hydrogen was added up to a pressure of 0.3 bar, which was supplied as a potential electron donor for microorganisms. Various compounds (Table 3) were individually supplied as potential electron acceptors through the septum with a syringe from sterile stock solutions. Some compounds were added as solids, which were autoclaved inside the glass serum bottle with the medium, except for 4-bromo-3,5-dimethoxy benzoic acid and 2,4,6-tribromoresorcinol. Sediment cultures were set up at least in duplicates. Controls without sediment, without electron acceptors, and with autoclaved sediments were always prepared, to evaluate possible abiotic processes within the sediment or medium and microbial growth without electron acceptors. Antibiotics against Gram-positives (vancomycin and ampicillin) and the inhibitor against methanogens (2-bromoethanesulfonate) were used to detect a possible effect on compound transformation and obtain an indication of the type of microorganism performing the transformation. Sediment cultures were statically incubated at 30ºC in the dark.

Table 3. Compounds added as potential electron acceptors to the sediment cultures. Concentration refers to final concentration in sediment cultures.

Electron acceptor			
Type	**Name**	**Stock solution details**	**Concentration**
Halogenated	1,2,3-Trichlorobenzene (1,2,3-TCB)	Crystals solved in 1,2,4-TCB in a 1:1 solution	15 µM
	1,2,4-Trichlorobenzene (1,2,4-TCB)	Liquid. In a 1:1 solution with 1,2,3-TCB	15 µM
	2-Monochlorophenol (2-MCP)	Solved in methanol	50 µM
	2,3-Dichlorophenol (2,3-DCP)	Solved in methanol	50 µM
	Tetrachloroethene (PCE)	Solved in acetone	90 µM
	4-Bromo-3,5-dimethoxy benzoic acid	Added as crystals	~ 0.1 g l^{-1}
	2,4,6-Tribromoresorcinol	Added as crystals	~ 0.1 g l^{-1}
	Hexachlorobenzene	Added as crystals	~ 2 g l^{-1}
Organic	Humic acids	Added as solid	~ 0.3 g l^{-1}
Inorganic	Ferric iron, $Fe(OH)_3$	Added from an anaerobic sterile solution	5.3 mM
	Manganese oxides, MnO_2	Added from an anaerobic sterile solution	10 mM
	Sulphate, Na_2SO_4	Added from an anaerobic sterile solution	20 mM

Sediment cultures were sampled at different time points of incubation to monitor the 16S rRNA gene copy numbers of *Dehalococcoidia* with molecular biology methods (see section 2.7). These time points were within 8 and 17 months of incubation for Århus sediment cultures, and 4, 10, and 12 months of incubation for Chile sediment cultures. The sampling consisted of withdrawing 1 ml (or eventually 1.5 ml) of culture after gentle homogenization of the deposited sediment particles. The sample withdrawal was performed through the septum with sterile needles and syringes. Samples were stored in 2 ml Eppendorf tubes at -80ºC.

2.5 ISOLATION OF PURE STRAINS BY CULTIVATION IN DEEP-AGAROSE DILUTION TUBES

2.5.1 Media, solutions and preparation procedure

Growth of bacteria in semisolid media was obtained by preparing deep-agarose dilution tubes. For this, glass tubes of 16 ml volume were filled with 2 ml of 2% (final concentration in the tube of 0.3%) low-melting agarose (SeaPlaque®, Biozym Scientific), autoclaved and allowed to cool down at ambient temperature. Sterile agarose in the tubes was melted in a 80 ºC preheated heatblock, and cooled down at 60ºC before further addition of 10 ml of sterile anoxic medium, which was buffered with sodium bicarbonate and warmed to 35ºC in a water bath. Further, the tubes were closed with rubber caps and maintained inside a 32–35 ºC water bath. Addition of 0.2 ml titanium (III) citrate and electron acceptors in identical concentrations to liquid sediment cultures was performed. Tubes were sealed with butyl rubber stoppers and plastic screw caps. The headspace atmosphere was exchanged to the Biogon gas mixture through a series of seven vacuum and Biogon gas exchange steps. Tubes were mixed by turning them up and down and maintained at 35ºC for at least one hour to allow equilibration of the reducing conditions. Inoculation was done through the rubber stopper using the previously set up sediment cultures when the sediment cultures were one week old.

A series of dilutions of four deep-agarose dilution tubes per sediment culture was established aiming to obtain single colonies (Figure 12). For first dilution of the deep-agarose tubes, 0.5 ml liquid sediment culture was added as inoculum. Second and third dilution deep-agarose tubes were inoculated with 0.2 ml from the previous dilution tube and the forth dilution tube was inoculated with 0.5 ml from the third dilution tube. Deep-agarose dilution tubes without inoculum were set up as medium blanks. Overpressure of 0.1 bar hydrogen gas was applied to each tube headspace as an

available potential electron donor. Tubes were mixed by two times turned up and down for mixing and solidified in an ice water bath. Incubation was performed in the dark at 30ºC. Tubes were visually observed periodically to monitor colony formation.

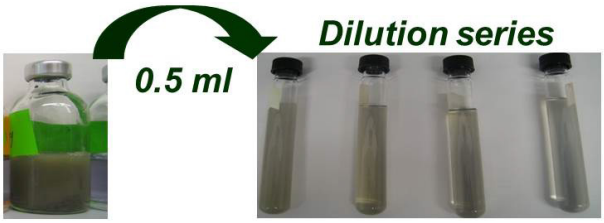


Figure 12. Deep-agarose dilution tubes were inoculated with 0.5 ml from a liquid sediment culture and a dilution series was established aiming to obtain single colonies.

2.5.2 Picking and transferring of colonies

Selected single colonies formed in the tubes were picked inside an anaerobic glovebox and transferred into 10 ml sterile anoxic medium. The 10 ml medium was pH buffered and reduced with titanium (III) citrate prior to colony transfer. The picking process involved using a sterile Pasteur pipette or a sterile 0.60 mm diameter x 80 mm length needle connected to a syringe, which already contained a small volume (1–2 ml) of sterile medium reduced with and titanium (III) citrate. Once the colony was inoculated into the 10 ml liquid medium, the electron acceptor that the colony was exposed to in the deep-agarose tube was added in the same concentration. The glass serum bottle headspace was flushed with Biogon to an overpressure of 0.2 bar. Hydrogen was added to an overpressure of 0.3 bar. Incubation was done statically in the dark at 30 ºC.

New deep-agarose dilution tubes were prepared in duplicate from the cultures inoculated from single colonies to observe the morphology of colonies and to compare it to the former original colony. The agarose tube preparation was done after two weeks of incubation of the cultures inoculated from colonies.

A selection of colonies, either directly picked from the deep-agarose tubes, or 1.5 ml from liquid sub-cultured colonies was sampled for later DNA isolation and 16S rRNA gene amplification, and stored at -20 ºC until analysis. The picking and transferring procedure was done inside the glovebox and with the help of some anoxic medium (same as used for the liquid cultivation of the sediment cultures) reduced with titanium (III) citrate.

2.6 ANALYTICAL METHODS

2.6.1 Analyses of trichlorobenzenes by gas chromatography

The concentrations of tri- and di-chlorobenzenes in sediment cultures were periodically monitored. For that, 0.5 ml culture aliquots were taken with sterile plastic syringes and needles through the culture septum after homogenization of deposited sediment particles. Aliquots were transferred into a 20 ml headspace vial, mixed with 0.5 ml aqueous 1 M NaCl solution to increase volatilization of organic compounds. Subsequently, vials were sealed with Teflon-coated stoppers and aluminium crimps. Vials were preconditioned for 30 min shaking at 70°C in a HP 7694 auto-sampler (Agilent Technologies) prior to the automatic injection into the Gas Chromatograph (GC) for determination of the concentration of chlorinated benzenes. The GC was a HP 6890 GC system (Agilent Technologies) equipped with a flame ionization detector (FID). The capillary column was a HP 5 (30 m length, 0.32 mm diameter, 0.25 μm film thickness). Helium was the carrier gas. The GC temperature program ('DCB_TCB method') was as follows: initial temperature of 55°C for 1 min; increase at a rate of 10°C per min until 90°C was reached; increase at a rate of 6°C per min until 130°C was reached; increase of 30°C per min until 220°C were reached. The injector and detector temperatures were 250°C and nitrogen was the makeup gas, set at a flow of 20 ml min^{-1}.

Standards of pure 1,2,3-TCB, 1,2-DCB and 1,3-DCB were prepared for peak identification (based on the respective compound retention time) and quantitation. Five-point standard curves were prepared by spiking different volumes of 5–100 μM 1,2,3-TCB, and 1,3-DCB and 1,2-DCB in cultivation serum bottles filled with 50 and 45 ml sterile anoxic medium, and were measured in triplicate. Standard curves were associated to linear regression lines for calibration. Detection limits were 1 μM for trichlorobenzenes and 5 μM for dichlorobenzenes.

2.6.2 Analyses of chlorophenols by gas chromatography

The concentration of 2-chlorophenol and 2,3-dichlorophenol in sediment cultures were periodically monitored. For that, a derivatization process (i.e., acetylation) was applied involving the addition of $NaHCO_3$ and acetic acid prior to analysis. Thus, the 2-chlorophenol and 2,3-dichlorophenol were derivatized to the esters 2-chlorophenyl acetate and 2,3-dichlorophenyl acetate, respectively. A 0.5 ml sample from sediment cultures was taken with sterile plastic syringes through the sediment culture septum. The 0.5 ml sample was loaded in a 20 ml headspace vial, which already contained 5–10 mg of $NaHCO_3$. Immediately after, 5 μl of pure acetic acid was added, and the vial

was closed with Teflon-coated stoppers and aluminium crimps. Samples were mixed by shaking vials with the hand and were analysed with the same GC, and using the same GC column and method as described for trichlorobenzenes. Vials were preconditioned in an auto-sampler in the same way as for the trichlorobenzenes analyses. The GC temperature program started at 50°C for 1 min followed by an increase of 30°C per min until 150°C was reached; increase at a rate of 5°C per min until 180°C was reached; increase of 30°C per min until 250°C were reached. The FID temperature was 250°C and nitrogen was the makeup gas, set at a flow of 20 ml min^{-1}.

2.6.3 Cell visualization by epifluorescence microscopy

Cells were visualized by epifluorescence microscopy after staining with SYBR Green I (SYBR® Green I Nucleic Acid Gel Stain - 10,000 x concentrate in DMSO, Invitrogen). SYBR Green I was diluted 1:100 using sterile TE-buffer (consisting of 10 mM Tris and 1 mM EDTA, at pH 7.2), aliquoted, and stored at -20ºC. The staining was performed for 10 min in the dark in a ratio of 20:1 (v/v), where 20 µl of a sample from a sediment culture was mixed with 1 µl of diluted SYBR I Green solution (final concentration in the sample of SYBR Green I was 1:2000). Then, a sample of 18 µl from the stained sediment culture was loaded on an agarose-coated slide for cell immobilization, and subsequently covered with a cover glass. The agarose-coated slides were prepared by homogeneously covering a glass slide with ~2 ml of liquefied agarose solution and dried overnight inside a clean bench (Adrian et al 2007). The agarose solution was prepared with two grams of low-melting agarose (SeaPlaque®, Biozym Scientific) solved in 120 ml water, stirred, and heated until liquefied. The agarose-coated slides allowed cell immobilization in a defined focus level between the slide and the cover glass (Adrian et al 2007). Fluorescence microscopy was performed by using a Nikon Eclipse TE300 microscope associated to a Nikon DXM 1200F digital Camera.

2.7 MOLECULAR BIOLOGY METHODS

2.7.1 DNA isolation from sediments, cultures and colonies

DNA isolation from sediments and sediment cultures was done using the FastDNA Spin Kit for Soil (MP Biochemicals) with a FastPrep instrument (FastPrep FP120; Savant Instruments) following the recommendations of the manufacturer with the following general modifications: the silica matrix settled for 30 min prior to removal of supernatant, and samples were incubated for 15 min at 42°C prior to final DNA elution to increase yields. DNA was aliquoted and stored at -20ºC.

Further specific modifications, in particular groups of samples, were applied: i) for sediments from the Baffin Bay, DNA was isolated from 0.86 g of sediment. For the bead beating step, beads and 780 µl of sodium phosphate buffer were added. In the final step, DNA was eluted in 100 µl DNase/pyrogen-free ultra-pure water supplied with the kit. DNA was isolated in triplicate for each sediment sample. Triplicates were pooled and stored at -20ºC; ii) for sediments from Chile and Århus, DNA were isolated from 0.5 g of sediment, and eluted in 30 µl DNase/pyrogen-free ultra-pure water; and iii) for sediment cultures, 1 ml (eventually 1.5 ml for subcultures with lower biomass) was sampled with sterile syringes after homogenization of the sediment culture by mixing by hand. The extracted 1 ml sample was placed in a sterile Eppendorf tube and centrifuged for 20 min at 13,200 rpm. The supernatant from the tube was discarded. The pellet was re-suspended in sodium phosphate buffer supplied by the kit. DNA was eluted in 30, 40, or 50 µl DNase/ pyrogen-free ultra-pure water supplied with the kit.

DNA isolation from colonies was performed using the Nucleospin Tissue Kit (Macherey & Nagel). For that, a colony was picked from a deep-agarose dilution tube and placed into an Eppendorf tube with some sterile medium. A total of 16 colonies were selected for DNA isolation. Additionally, DNA was isolated from samples (1 ml) taken from sub-cultured colonies, which were incubated over 3–6 months. A total of 16 sub-cultured colonies were selected for DNA isolation. For both cases, samples were centrifuged for 15–20 min at 10,400 rpm, and the supernatant was removed. In the case that no visible pellet could be observed, a volume of 0.2 ml was left in the Eppendorf tube. Pellets were re-suspended with Buffer T1 (180 µl) and Proteinase K (25 µl), previously solved in 3.35 ml of Buffer PB, and mixed; all supplied by the manufacturer. The re-suspended pellets were then incubated at 56ºC for 2 h in a heat-block at 1,100 rpm, and the following steps were done as recommended by the manufacturer. DNA was eluted in 30 µl of elution buffer BE, supplied by the manufacturer. For colonies transferred in a medium with humic acids, a second wash step with Buffer B5 was repeated to further remove humic acids, and the eluted DNA was further diluted, to avoid interference of humic acids in downstream processes, i.e., PCR.

2.7.2 Determination of DNA concentration

The DNA concentration was determined by measuring the absorbance at 260 nm using a Nanodrop spectrophotometer (NanoDrop ND 1000, NanoDrop Technologies). The ratio of absorbance 260/280 was used to evaluate the purity of the DNA in the samples, considering values of ~1.8 as pure DNA.

The concentration of purified amplicons amplified for further high-throughput sequencing (either 454-pyrosequencing or Illumina) were determined for each sample using a Quant-iT™ *PicoGreen*® dsDNA Assay Kit (Invitrogen), and from these concentrations, the samples were pooled in equimolar amounts for all samples derived from each of the primer sets, i.e., bacterial and universal primer sets. These two pools were then mixed, so 71% of the total amplified sequences were derived from bacterial primers, and 29% of the total amplified sequences were derived from universal primers, in a final tube containing all amplicons prior to the 454-pyrosequencing. For the Illumina sequencing, bacterial amplicons were mixed with archaeal amplicons (archaeal data and information is not shown in this study; for further information see (Algora et al 2015)). The final ratio of amplified sequences was 62.5% bacterial and 37.5% archaeal in a final tube.

2.7.3 Quantification of the 16S rRNA gene by qPCR

For qPCR analysis, 1 µl of DNA template was used. For the case of sediment samples, the DNA template was ten-fold diluted to avoid PCR inhibition by co-extracted organic substances, such as humic acids, coming from the sediment sample.

All the qPCR assays were performed on triplicate samples using a StepOne detection system (StepOne/ StepOnePlus version 2.0, Applied Biosystems) and analysed with the StepOne v2.1 software. Resulting amplicons from the qPCR assay were checked by agarose gel electrophoresis when necessary, e.g., when melt-curve irregularities were detected.

Amplification by qPCR of various microbial groups

For amplification of the 16S rRNA gene from the various targeted microbial groups, i.e., total *Bacteria*, class *Dehalococcoidia*, order *Desulfuromonadales*, genera *Desulfitobacterium* spp. and *Dehalobacter* spp., and total *Archaea*, various primer combinations were used as specified in Table 4.

For the quantification of *Bacteria* and *Dehalococcoidia* 16S rRNA gene copy numbers, the qPCR reaction mix had a final reaction volume of 20 µl. The qPCR reaction mix components were 10 µl of SensiMix SYBR (Bioline) (including DNA polymerase, dNTPs, SYBR Green I dye, stabilizers and the ROX reference dye), 7 µl of PCR-grade water, and each primer to a final concentration of 1 µM.

Table 4. Combination of primers used to amplify and quantify 16S rRNA genes of the various microbial groups by qPCR.

Targeted microbial group	**Set of primers***	**Chemistry**
Total *Bacteria* (variable region v3)	341f and 534r	SYBR Green I
Total *Archaea*	Arch349F and Arch806R, with the TaqMan probe Arch516F	TaqMan
Class *Dehalococcoidia*	DEH-Fa and DEH-R[1]	SYBR Green I
Class *Dehalococcoidia*	DehalF5 and DehalR[2]	SYBR Green I
Order *Desulfuromonadales*	GEO494F and GEO825R	SYBR Green I
Genus *Dehalobacter*	Dre441F and Dre645R_Ch	SYBR Green I
Genus *Desulfitobacterium*	DSB406F and DSB619R	SYBR Green I

*Primer sequences and references are specified in Table 7.

[1]Used for samples of sediments and sediment cultures from Baffin Bay and Chile.

[2]Used for samples of sediment cultures from Chile, Ireland, and Århus sediments (Table 1). Primers were designed based on Dehalococcoidia 16S rRNA gene sequences retrieved from sediments of Chile, site 7155, after a clone library, and using the 'probe design' function of the ARB software package (http://www.arb-home.de/) (Ludwig et al 2004), together with the SILVA database (Pruesse et al 2007) release 'SILVA 100'. Primers were designed by a collaborator, Dr. Kenneth Wasmund, UFZ–Leipzig.

The PCR program for both *Bacteria* and *Dehalococcoidia* (DEH-Fa and DEH-R) sets of primers included a touchdown program as described in Table 5. Once amplification was completed, a melt-curve was run to check the specificity of products. The melt-curve had the following parameters: initial denaturation for 15 min at 95°C, renaturation for 1 min at 60°C, which was followed by a gradient (collecting fluorescent signal data every 0.3°C) until 95°C was reached, and finally followed by 15 min at 95°C, as described (Algora et al 2013, Wasmund et al 2015).

Table 5. PCR touchdown program for qPCR amplification of *Bacteria* and *Dehalococcoidia* (primer set DEH-Fa and DEH-R).

Step	Temperature	time
Initial denaturation	95ºC	15 min

Number of cycles

	1		1		1		1		1		33	
Step	T	time	T	time	T	time	T	time	T	time	T	time
Denaturation	95ºC	30 s	95ºC	30 s	95ºC	30 s	95ºC	30 s	95ºC	30 s	95ºC	30 s
Annealing	65ºC	30 s	64.6ºC	30 s	64.2ºC	30 s	63.8ºC	30 s	63.4ºC	30 s	63ºC	30 s
Elongation*	72ºC	45 s	72ºC	45 s	72ºC	45 s	72ºC	45 s	72ºC	45 s	72ºC	45 s

* Fluorescence was acquired during each elongation step

The PCR program for *Dehalococcoidia* using the primers DehalF5 and DehalR is described in Table 6. Melt-curve parameters were the same as for the bacterial and DEH-Fa and DEH-R set of primers.

Table 6. PCR touchdown program for qPCR amplification of *Dehalococcoidia* (primer set DehalF5 and DehalR).

Step	Temperature	time
Initial denaturation	95ºC	15 min

Number of cycles

	1		1		1		1		1		1	
Step	T	time	T	Time	T	time	T	time	T	time	T	time
Denaturation	95ºC	30s	95ºC	30s	95ºC	30s	95ºC	30 s	95ºC	30s	95ºC	30s
Annealing	66ºC	30s	64.8ºC	1 min	63.6ºC	30s	62.4ºC	30 s	62.2ºC	30s	61.2ºC	30s
Elongation*	72ºC	40s	72ºC	40s	72ºC	40s	72ºC	40 s	72ºC	40s	72ºC	40s

Followed by 35 cycles at

Step	Temperature	time
Denaturation	95ºC	30 s
Annealing	60ºC	30 s
Elongation*	72ºC	40 s

* Fluorescence was acquired during each elongation step

The qPCR assay for quantification of total *Archaea* and order *Desulfuromonadales* 16S rRNA gene copy numbers in sediment samples from the Baffin Bay was performed with the primers described in Table 7, and as previously reported (Algora et al 2013, Holmes et al 2002, Schippers and Neretin 2006, Takai and Horikoshi 2000). The quantification of *Dehalobacter restrictus* and *Desulfitobacterium* spp. in sediment cultures from Chile, site 7155, was performed as previously described (Smits et al 2004) (Table 7). The reverse primer, Dre645R, targeting the *Dehalobacter* 16S rRNA gene was modified (see Table 7) accordingly to *Dehalobacter* sequences found in Chile sediments after a clone library of the 16S rRNA gene was set up, amplified with the primers 27f and 1492r (Table 7). The clone library and the Dre645R primer modification were performed by a collaborator, Dr. Kenneth Wasmund, UFZ–Leipzig.

Conversion of qPCR data to gene copy numbers ml^{-1}culture or g^{-1} sediment

For the determination of the number of 16S rRNA gene copies for total *Bacteria* and *Dehalococcoidia* in the qPCR assays, a DNA standard was prepared after cloning 16S rRNA genes into a pGEM-T vector. The 16S rRNA gene used came from a *Dehalococcoidia* member amplified using the primers 27f and 1492r (Table 7), from sediment samples of Chile as described (Wasmund et al 2015).

For the determination of DNA concentration (ng µl^{-1}) in the DNA standard, a NanoDrop ND 1000 (NanoDrop Technologies) was used as previously described (section 2.7.2). The DNA concentration was measured in triplicate, and converted to 16S rRNA gene copies µl^{-1} with the formula in Equation 1.

$$16S\ rRNA\ gene\ copies\ \mu l^{-1} = \frac{\{[DNA](ng\ \mu l^{-1})\}\ \mathrm{x}\ \{6.022\ x\ 10^{23}(molecules\ mole^{-1})\}}{length\ (bp) x\ 10^{9}(ng\ g^{-1})\ x\ 650\ (g\ mole\ of\ bp^{-1})}$$

Equation 1. Formula used for the conversion of DNA concentration (ng µl^{-1}) of a specific DNA strand of a given length (bp) to the number of DNA copies µl^{-1}.The formula assumes an average weight of one bp to be 650 Dalton, and thus, one bp mole to weight 650 g. The molecular weight of a DNA strand is estimated by multiplying 650 times the bp length. 6.022 x 10^{23} is Avogadro's number (http://cels.uri.edu/gsc/cndna.html, and (Ritalahti et al 2006)).

Subsequently, the calculated number of 16S rRNA gene copies in the DNA standard was brought to 10^{11} copies µl^{-1} by dilution with molecular-grade water, which was used as a quantified stock solution, aliquoted, ten-fold diluted, and stored at -80ºC (Wasmund et al 2015). A standard curve was either freshly made after thawing an aliquot of the quantified stock solution, or by thawing ten-fold diluted standard aliquots (10^7–10^2) from a -80ºC stock. In each qPCR assay, a standard curve ranging from 10^7 to 10^2 16S rRNA gene copies µl^{-1} was always included in triplicate. The standard curve was used for i) the calculation of qPCR amplification efficiency after association to a linear regression line, and ii) conversion from the qPCR threshold cycle values (Ct; defined as the qPCR cycle number where the intensity from the fluorescence reaches a set threshold (Ritalahti et al 2006)) to gene copies µl^{-1} in the qPCR reaction. The amplification efficiency was calculated from the calibration curve slope according to the formula $10^{(-1/slope)}$. Each qPCR run was checked for amplification efficiencies, and only those qPCR runs which ranged between 90 and 110% were further processed. The conversion of Ct values to 16S rRNA gene copy numbers µl^{-1} in each qPCR reaction for each sample was performed using the standard curve, and calculated by the StepOne v2.1 software (Applied Biosystems). Further conversion of 16S rRNA gene copy

numbers μl^{-1} per reaction to 16S rRNA gene copies ml^{-1} or g^{-1} of sample was calculated as previously described (Ritalahti et al 2006), and according to Equation 2.

$$gene\ copies\ ml^{-1} or\ g^{-1}\ of\ sample = \frac{(gene\ copies\ per\ reaction)\ x\ \left(eluted\ total\ volume\ of\ DNA\ in\ the\ DNA\ isolation\ (\mu l)\right)}{\left(volume\ of\ DNA\ added\ per\ reaction\ (\mu l)\right)\ x\ \left(volume\ or\ weight\ of\ sample\ (ml\ or\ g)\right)}$$

Equation 2. Formula for the conversion of gene copies per reaction to gene copies per ml or g of sample. The number of gene copies per reaction is given by the qPCR assay after using the standard curve. The eluted volume of DNA within each DNA isolation from each sample, was either 30, 40, or 50 µl for sediment cultures, or 100 µl for sediments. The volume of DNA added per reaction was 1 µl. The volume of sediment culture sample from which the DNA was isolated was either 1 ml or 1.5 ml, and the weight of sediment sample was 0.5 or 0.86 g.

Standard curves for the determination of *Archaea* and the order *Desulfuromonadales* were done as described (Schippers and Neretin 2006).

2.7.4 Quantification of the functional genes *mcrA* and *dsrA* by qPCR

The quantitative amplification of the functional genes of the *dsrA*, which encodes the dissimilatory sulphite reductase of sulphite/sulphate reducers and the *mcrA* encoding for the methyl coenzyme M reductase subunit α gene of methanogens/anaerobic methanotrophs was carried out as described (Algora et al 2013).

All primers used in this study for qPCR assays are presented in Table 7.

Table 7. Primers used in this study.

Primer name	Sequence (5'-3')	Target gene	Target group	Reference
27F	AGAGTTTGATCMTGGCTCAG	16S rRNA	*Bacteria*	(Weisburg et al 1991)
1492R	GGTTACCTTGTTACGACTT	16S rRNA	*Bacteria*	(Lane 1991)
519R	TATTACCGCGGCKGCTG	16S rRNA	*Bacteria*	(Lane et al 1985)
341F	CCTACGGGAGGCAGCAG	16S rRNA	*Bacteria*	(Muyzer et al 1993)
534R	ATTACCGCGGCTGCTGGCA	16S rRNA	*Bacteria*	(Muyzer et al 1993)
343F	TACGGRAGGCAGCAG	16S rRNA	*Bacteria*	(Liu et al 2007)
534R	ATTACCGCGGCTGCTGGC	16S rRNA	*Bacteria*	(Liu et al 2007)
DEH-Fa	TACGGGAGGCAGCAGCDA	16S rRNA	*Dehalococcoidia*	(Wasmund et al 2015)
DEH-R	GRRAGGGTCGATACYCC	16S rRNA	*Dehalococcoidia*	(Wasmund et al 2015)
Dehal-F5	ATCTCYCRGCTYAACYGGGA	16S rRNA	*Dehalococcoidia* Chile sediments	This study. Designed by Wasmund
Dehal-R	ARRAGGGTCGATACYCC	16S rRNA	*Dehalococcoidia* Chile sediments	This study. Designed by Wasmund
GEO494F	AGGAAGCACCGGCTAACTCC	16S rRNA	*Desulfuromonadales*	(Holmes et al 2002)
GEO825R	TACCCGCRACACCTAGT	16S rRNA	*Desulfuromonadales*	(Anderson et al 1998)
Arch349F	GYGCASCAGKCGMGAAW	16S rRNA	*Archaea*	(Takai and Horikoshi 2000)
Arch806R	GGACTACVSGGGTATCTAAT	16S rRNA	*Archaea*	(Takai and Horikoshi 2000)
Arch516F	TGYCAGCCGCCGCGGTAAHA CCVGC	16S rRNA	*Archaea*	(Takai and Horikoshi 2000)
U789F	TAGATACCCSSGTAGTCC	16S rRNA	Prokaryotes	(Baker et al 2003, Barns et al 1994)
U1068R	CTGACGRCRGCCATGC	16S rRNA	Prokaryotes	(Lee et al 2011)

Primer name	Sequence (5'-3')	Target gene	Target group	Reference
ME1F	GCMATGCARATHGGWATGTC	*mcrA*	methanogens	(Hales et al 1996)
ME3R	TGTGTGAASCCKACDCCACC	*mcrA*	methanogens	(Wilms et al 2007)
DSR1F+	ACSCACTGGAAGCACGGCGG	*dsrA*	sulphate reducers	(Kondo et al 2004)
DSR-R	GTGGMRCCGTGCAKRTTGG	*dsrA*	sulphate reducers	(Kondo et al 2004)
Dre441F	GTTAGGGAAGAACGGCATCTGT	16S rRNA	*Dehalobacter restrictus*	(Smits et al 2004)
Dre645R_Ch	CCTCTCCTGTCCTCAAGCCAHM	16S rRNA	*Dehalobacter restrictus*	This study. (Smits et al 2004), modified by Wasmund
DSB406F	GTACGACGAAGGCCTTCGGGT	16S rRNA	*Desulfitobacterium*	(Smits et al 2004)
DSB619R	CCCAGGGTTGAGCCCTAGGT	16S rRNA	*Desulfitobacterium*	(Smits et al 2004)
RRF2	SHMGBMGWGATTTYATGAARR	*rdh*-genes	*Dehalococcoides mccartyi*	(Krajmalnik-Brown et al 2004)
B1R	CHADHAGCCAYTCRTACCA	*rdh*-genes	*Dehalococcoides mccartyi*	(Krajmalnik-Brown et al 2004)
RDH F1C	TTYMVIGAYITIGAYGA	*rdh*-genes	*Dehalococcoides mccartyi*	(Chow et al 2010)
RDH R1C	CCIRMRTYIRYIGG	*rdh*-genes	*Dehalococcoides mccartyi*	(Chow et al 2010)
dehaloF3	ATCGWTSMRGGTAT	*rdh*-genes	*Dehalobacter restrictus* and *Desulfitobacterium* spp.	(von Wintzingerode et al 2001)
dehaloR2	TYTGTACCATAGCC	*rdh*-genes	*Dehalobacter restrictus* and *Desulfitobacterium* spp.	(von Wintzingerode et al 2001)
dehaloF5	GGTTGCATTGCYGTCAT	*rdh*-genes	*Dehalobacter restrictus* and *Desulfitobacterium* spp.	(von Wintzingerode et al 2001)
dehaloR4	TGCTTYATGGAACCAGG	*rdh*-genes	*Dehalobacter restrictus* and *Desulfitobacterium* spp.	(von Wintzingerode et al 2001)
M13F	GTAAAACGACGGCCAGT	-	pGEM –T vector	
M13R	GCGGATAACAATTTCACACAGG	-	pGEM –T vector	

IUPAC-Code: A – adenine; B – cytosine/ guanine/ thymine; C – cytosine; D – adenine/guanine/ thymine; G – guanine; H – adenine/cytosine/ thymine; I – inosine; K – guanine/ thymine; M – adenine/cytosine; R – adenine/ guanine; S – cytosine/ guanine; T – thymine; V – adenine/ cytosine/ guanine; W – adenine/ thymine; Y – cytosine/ thymine

2.7.5 Amplification of reductive dehalogenase genes

Several sets of primers were used for amplification of the functional gene of the key enzyme for organohalide respiration, i.e., the reductive dehalogenase (RDase). Amplification of reductive dehalogenase homologous genes (*rdh*-genes) in sediment cultures was performed with four sets of primers. Two sets were designed for targeting the *rdh*-genes from *Dehalococcoides mccartyi* (Chow et al 2010, Krajmalnik-Brown et al 2004) and the other two set was designed for targeting the *rdh*-genes from *Dehalobacter restrictus* and *Desulfitobacterium* spp. (von Wintzingerode et al 2001).

The first set of primers, RRF2 and B1R (Table 7), were specific for *Dehalococcoides mccartyi* and were used as described (Krajmalnik-Brown et al 2004). In addition, the primers RDH F1C and RDH R1C (Table 7) were used with a S-Tbr DNA polymerase (DyNAmo II, Finnzymes) that is specific for short primers (Isenbarger et al 2008). The reaction mix contained 2.5 µl of buffer and 0.7 DNA polymerase (DyNaMo Taq), 2.5 µl of dNTPs (0.2 mM; final concentration in PCR reaction mix), 1 µl of each primer (0.8 µM; final concentration in PCR reaction mix), 0.75 µl of $MgCl_2$ (1.5 mM; final concentration in PCR reaction mix), 0.1 µl BSA (0.2 g l^{-1} final concentration PCR reaction mix; New England Biolabs), 1 µl of DNA template, and molecular grade water

up to 25 µl. The PCR program was the following: initial denaturation at 95ºC for 2 min, 44 cycles of denaturation at 95 ºC for 30 s, annealing at 47 ºC for 30 s, elongation at 72ºC for 1 min 20 s, and a final elongation step of 72ºC for 5 min.

For the amplification of *rdh*-genes described for *Dehalobacter restrictus* and *Desulfitobacterium* spp., and dechlorinating mixed cultures, the pair of degenerate primers dehaloF3 together with dehaloR2, and a second pair of primers dehaloF5 together with dehaloR4 were used (Table 7). The PCR program for both primer sets involved the following parameters: initial denaturation at 95ºC for 5 min, 35 cycles of denaturation at 95ºC for 30 s, annealing at 50ºC for 30 s, and elongation at 72ºC for 60 s, and a final elongation step at 72ºC for 7 min. Individual PCR reactions had a total volume of 25 µl, and contained 12.5 µl of master mix (Qiagen), which already contained DNA polymerase, buffer and dNTPs (https://www.qiagen.com/de/products/catalog/assay-technologies/end-point-pcr-and-rt-pcr-reagents/taq-pcr-master-mix-kit), 1.5 µl of each primer (0.6 µM; final concentration in PCR reaction mix), 1.5 µl of $MgCl_2$ (1.5 mM; final concentration in PCR reaction mix), 0.1 µl BSA (0.2 g l^{-1} final concentration PCR reaction mix; New England Biolabs), 6.9 µl of molecular grade water, and 1 µl of DNA template.

2.7.6 Amplification of 16S rRNA gene from colonies

For PCR amplification of nearly entire-length bacterial 16S rRNA genes from the various colonies, the primers 27F and 1492R (Table 7) were used. Single PCR reactions were performed using a Taq PCR Master Mix Kit (Qiagen), and consisted of 12.5 µl of master mix (Qiagen), which already contained DNA polymerase, buffer and dNTPs, both primers (0.2 µM final concentration in PCR reaction mix), BSA (0.08 g l^{-1} final concentration in PCR reaction mix; Promega), 1 µl of DNA template, and molecular grade water up to 25 µl.

The PCR program consisted of an initial denaturation of 95°C for 4 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 2 min, elongation at 72°C for 3 min, and a final elongation step of 72°C for 10 min.

2.7.7 Amplification of 16S rRNA gene for 454-pyrosequencing

Bacterial 16S rRNA genes amplification was done using the primers: 27F and 519R (Table 7), targeting the V1–V3 hypervariable regions of the 16S rRNA gene, and the universal primers U789F and U1068R for both *Bacteria* and *Archaea* targeting the hypervariable V6 region of the 16S rRNA gene (Baker et al 2003, Lee et al 2011, Wang and Qian 2009).

Primers were extended with barcoded multiplex identifiers (MID), which were sequences of 10 bp specific to each sample (added to both forward and reverse primer sequences, except for the universal reverse primer which had no MID sequence). The MIDs allowed the later determination of the origin of each sequence from pooled sequencing libraries. In addition, all primers were extended with the library "key" TCAG sequence next to the barcoded MID sequence, or to the primer in case of the universal reverse primer, and followed by the adapter A (CGTATCGCCTCCCTCGCGCCA) in the forward primers, and adapter B (CTATGCGCCTTGCCAGCCCGC) in the reverse primers. The design of these fusion primers was done as requested by 454/Roche (http://454.com/products-solutions/experimental-design-options/amplicon-sequencing. asp). Total amplicon lengths were 599 bp for bacterial primers and 373 bp for universal primers.

PCR single reactions contained 10 µl of 2x Phusion Flash High-Fidelity Buffer (Finnzymes), which already contained the Phusion® High-Fidelity DNA Polymerase (Finnzymes) and dNTPs, each primer in a final concentration of 5 µM, and 1.0 µl of DNA template, and deionized molecular-grade water up to 20 µl.

Bacterial PCR thermocycling conditions comprised an initial denaturation of 98°C for 10 s, followed by 30 cycles of 98°C for 5 s, 52°C for 10 s and 72°C for 10 s. PCR thermocycling conditions for PCRs using universal primers were the same as for PCR reactions using bacterial primers, except the number of cycles was 35. PCR reactions were done in five replicates for each sample. Amplicons from the same sample were pooled

2.7.8 Amplification of the 16S rRNA gene for Illumina sequencing

For Illumina sequencing, 16S rRNA genes were amplified with the bacterial primer set 343F and 534R (Table 7) targeting the V3 hypervariable region of the 16S rRNA gene. To enable multiplexed sequencing in a single Illumina run, forward primers were extended at the 5´end with a 'barcode' sequence of 7 bp. The 'barcode' sequence was specific for each sample and differed to the rest of the barcodes by at least two bases. In between the primer and the barcode, the 2 bp "TA" linker was inserted in order to reduce possible bias effects of the barcodes (Degnan and Ochman 2012, Vasileiadis et al 2012, Wu et al 2010). Amplicon lengths were 190 bp.

Single PCR reactions contained 4 µl Phusion® High-Fidelity Buffer (Finnzymes), 200 µM of each dNTP, 1 µM of each primer, 0.5 U of Phusion® High Fidelity

Polymerase (Finnzymes), 1 µl of DNA template, and molecular biology grade water up to 20 µl.

Bacterial PCR conditions were: initial denaturation of 98°C for 30 s, followed by 35 cycles of a two-step cycling program of 98°C for 10 s and 72°C for 30 s, and a final elongation of 72°C for 1 min. Triplicate PCR reactions per sample were performed, and amplicons from each triplicate PCR reaction from each sample were pooled.

2.7.9 Agarose gel electrophoresis and amplicon purification

After PCR, amplicons were loaded on agarose gels, and gel electrophoresis was run to check if successful amplification of the correct band size occurred. For that, agarose gels were prepared by solving 1–1.5 g of agarose in 100 ml of 1 x TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.3). Agarose gels with loaded amplicons and a DNA ladder (GeneRuler^{TM} 100 bp Plus DNA Ladder, Fermentas) were run for 30 min at 110 V, as a standard basis, and for 70 min at 90 V, when a better band size separation was needed (i.e., when bands were excised from agarose gels). The running buffer in the electrophoresis chamber was 1 x TAE. Then, staining of the agarose gel with ethidium bromide was performed and amplicons were visualized under UV-light with a UV-detector (BioRad Gel Doc^{TM} XR+Imager).

When needed, and prior to loading into agarose gels, amplicon purification from the PCR reaction was done using a Wizard® SV Gel and PCR Clean-Up Kit (Promega) following the recommendations from the manufacturer.

2.7.10 Elution of amplicons from agarose gels

The elution of amplicons from agarose gels were done prior to 454-pyrosequencing and Illumina sequencing. After gel electrophoresis, bands of the correct size were excised using sterile scalpels, placed in sterile Eppendorf tubes, weighed and purified using a Wizard® SV Gel and PCR Clean-Up Kit (Promega) following the manufacturer's recommendations.

2.7.11 Cloning of 16S rRNA gene amplicons from *Dehalococcoidia* qPCR amplifications

Selected amplicons amplified in a qPCR assay with primers targeting the 16S rRNA gene of the class *Dehalococcoidia* were cloned in order to determine their phylogenetic affiliation within this class.

Purified amplicons were cloned into *Escherichia coli* using the pGEM-T vector system (Promega). The ligation reaction was performed according to the manufacturer's instructions, and incubated for 1h at ambient temperature. The pGEM-T vector containing the corresponding ligated amplicons was introduced in *Escherichia coli* strain JM109 high-efficiency competent cells (Promega). The transformation protocol provided by the manufacturer was followed to transform the JM109 strain of *E.coli.* Transformed cells were plated in LB agar plates, supplemented with ampicillin (100 μg ml^{-1}), X-gal (80 μg ml^{-1}) and IPTG (0.1 mM). Colonies were allowed to grow on the plates overnight in an incubator at 30ºC. White colonies (containing the cloned fragment) were picked using sterile toothpicks and transferred to a new LB agar plate supplemented with ampicillin, IPTG and X-gal. Plates were incubated overnight at 30ºC to allow colonies to grow. Part of each isolated white colony was transferred with a sterile toothpick to a sterile Eppendorf tube containing 100 μl of molecular-grade water. One Eppendorf tube was used for each colony. Colony cells inside the Eppendorf tube were intensively vortexed to homogeneously dissolve them in the water. Eppendorf tubes containing colony cells were incubated on the bench at ambient temperature for 2h. After this time, the colony dissolved in water was used as template for a colony PCR reaction to confirm the presence of cloning fragments with the correct size.

PCR single reactions contained 3.5 μl of buffer (Fermentas), 3.5 μl dNTPs, 1 μl M13F primer (Table 7), 1 μl M13R primer (Table 7), 2.8 μl $MgCl_2$, 0.175 μl BSA (0.2 g l^{-1} final concentration PCR reaction mix; New England Biolabs), 0.175 μl Taq DNA Polymerase (Fermentas), 1 μl of template, and sterile molecular-grade water up to 35 μl. The PCR program consisted of an initial denaturation of 95ºC for 5 min, 40 cycles of 95ºC for 30 s, 55ºC for 30 s, and 72ºC for 40 s and a final elongation of 72ºC for 5 min.

2.7.12 454-pyrosequencing, Illumina sequencing and subsequent data analysis

The emulsion PCR and sequencing was done by means of the GS FLX Titanium chemistry, following protocols from the manufacturer and using a 454 GS FLX pyrosequencer (Roche), as recommended by the developer, and performed by a collaborator, Dr. Richard Reinhardt and his group at the Max Planck Genome Centre in Cologne.

Initial quality filtering and extraction of raw pyrosequencing reads was performed using the 'amplicon' settings of the GS Run Processor (Roche). Additional quality control processing included screening sequences using the Greengenes (DeSantis et al 2006) 'Trim' tool (http://greengenes.lbl.gov/cgi-bin/nph-trim_fasta_by_qual.cgi) with the

following settings: good quality score=25, window size=25 bp, and window threshold=90%. Following this, sequences with >1 mismatch to the barcode, >2 mismatches to the forward primer, <200 bp in length and containing homopolymers of >10 bp were removed using *mothur* (v1.35.0) (Schloss et al 2009). Further, bases after 300 bp were removed from the 3' ends of all reads. Chimeric sequences were then detected using the 'chimera.uchime' command within *mothur* (Edgar et al 2011), using the silva.gold.alignment file (supplied by the *mothur* website) as a reference alignment. Potential chimeric sequences were then removed. Taxonomic classification of sequence reads was performed using 'classify.seqs' command within *mothur* with a bootstrap cut-off of 50% and using the SILVA taxonomy files (v119) supplied by the *mothur* website (http://www.mothur.org/wiki/Silva_reference_files). The classify.seqs command is the *mothur* implementation of the RDP naïve Bayesian rRNA Classifier (Wang and Qian 2009). The analysis of the 454-pyrosequencing data was performed in collaboration with Dr. Kenneth Wasmund, UFZ–Leipzig.

The Illumina sequencing was performed by using an Illumina Genome Analyzer IIx with the TruSeq SBS Kit v5-GA sequencing reagents (Illumina Inc) by our collaborators Dr. Edoardo Puglisi and Dr. Sotirios Vasileiadis from the Universita Cattolica del Sacro Cuore, at Piacenza, Italy as described (Algora et al 2015). The subsequent bioinformatics sequence data analysis was performed by a collaborator Dr. Sotirios Vasileiadis, using *mothur* v1.28 (Schloss et al 2009) together with the statistical analyses (Spearman's rank correlation and Hierarchical clustering with the UPGMA algorithm), which were performed in R (R Development Core Team 2011) as described (Algora et al 2015).

2.7.13 Sanger sequencing and subsequent data analysis

Sanger sequencing of 16S rRNA gene amplicons was performed with the 27F primer using an ABI Prism® 3139xl genetic analyzer instrument (Applied Biosystems) at the Environmental Microbiology Department of the UFZ–Leipzig.

Prior to sequencing, a PCR reaction with the BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems) was performed. Single reactions contained BigDye (1 µl), primer 27F (1 µl; Table 7), BigDye Terminator 5 x sequencing Buffer (1 µl), molecular-grade sequencing water (4–6 µl) and amplicons (1–3 µl). The PCR program was 25 cycles of 96ºC for 30 s, 55ºC for 15 s, 60ºC for 4 min. Afterwards, amplicons were precipitated with ethanol. For that, 10 µl of amplicons were mixed with 10 µl sodium acetate, (3 M, pH 4.8), 150 µl ethanol (100%) and 80 µl molecular-grade water, and

centrifuged for 10 min at 12,000 rpm. The supernatant was discarded, and 300 µl ethanol (70%) was added, and subsequently centrifuged. The supernatant was discarded and the pellet was vacuum-dried before injected to the sequencer.

The sequences obtained were analysed for their quality with the software Chromas Lite (Technelysium Pty Ltd). Sequences were compared to public databases using the Blast tool (Basic Local Alignment Search Tool; (Altschul et al 1990)) and the NCBI database (http://www.ncbi.nlm.nih.gov/blast/).

3 RESULTS

3.1 APPROACHES TO CULTIVATE *DEHALOCOCCOIDIA* FROM VARIOUS MARINE SEDIMENTS

Quantification of *Dehalococcoidia* was performed by qPCR using the primers DehalF5 and DehalR, which are specific for the class *Dehalococcoidia*, and target the 16S rRNA gene (Table 7). 16S rRNA gene copy numbers of *Dehalococcoidia* were used as a proxy to monitor *Dehalococcoidia* growth with time, and thus investigate if *Dehalococcoidia* could be cultivated in the laboratory at atmospheric pressures and to which time scale, i.e., within a timespan of days, weeks, months, or years, they may grow. Additionally, 16S rRNA gene copy numbers of *Dehalococcoidia* were compared among the various sediment cultures supplied with different potential electron acceptors to investigate which potential electron acceptor may promote *Dehalococcoidia* growth. Total bacterial numbers were additionally monitored with a 16S rRNA gene-targeted qPCR assay.

3.1.1 *Dehalococcoidia* abundance under various terminal electron acceptors in sediment cultures

Dehalococcoidia were detected with the qPCR assay in all sediment cultures. The sediment cultures with highest *Dehalococcoidia* numbers were those inoculated with sediments of the Århus core at depths of 430–440 cmbsf, with values between 10^4–10^5 16S rRNA gene copies ml^{-1} culture. Cultures set up with sediments from site 1317 of Ireland also showed values of 10^4 16S rRNA gene copies ml^{-1}. The rest of cultures showed values of ~10^3 16S rRNA gene copies ml^{-1}, as it was the case for cultures inoculated with sediments from the site 1318 of Ireland and Chile, sites 7155 and 7165. Values lower than 10^3 16S rRNA gene copies ml^{-1} could not be precisely quantified as they were out of range of the calibration curve.

In order to study if *Dehalococcoidia* numbers increased in the sediment cultures with time, a culture was specifically monitored from start of the incubation and after 68 days. This culture was inoculated (13%, v/v) with a sediment culture that had been previously set up with sediments from Chile site 7155, and that dehalogenated 1,2,3-trichlorobenzene (as further detailed in section 3.2). 16S rRNA gene copy numbers of *Dehalococcoidia* increased one order of magnitude, from a number of 687 16S rRNA gene copies ml^{-1} at the start point to a number of 2.1×10^4 after 68 days. Moreover, nearly no change was detected in total bacterial 16S rRNA gene copy numbers with time, from a 16S rRNA gene copy number of 3.8×10^7 at the start, to a number of 2.7×10^7 after 68 days of incubation (Figure 13). The relative proportion of

Dehalococcoidia with respect to the total bacterial community, were of 0.002% at the start of the incubation time, which increased to 0.08% after 68 days of incubation

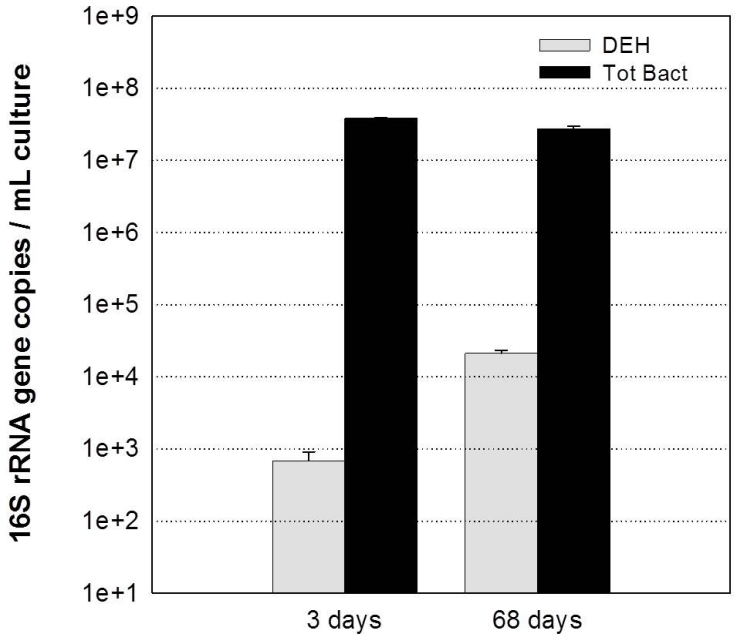


Figure 13. 16S rRNA gene copy numbers of *Dehalococcoidia* ("DEH") and total *Bacteria* ("Tot Bact") after 3 and 68 days of incubation, in a culture inoculated with a previously established sediment culture set up with sediments from Chile, site 7155. Shown are standard deviations of triplicate measurements in the qPCR assay.

Cloning and sequencing of the *Dehalococcoidia* qPCR amplicons revealed a phylogenetical affiliation of the twelve cloned sequences to the class *Dehalococcoidia* (Figure 14). These cloned *Dehalococcoidia* sequences did not fall into the clade Ord-DEH, which includes the organohalide-respiring characterized *Dehalococcoidia*, but were affiliated with the *Dehalococcoidia*-sister clades, particularly DSC-D (the name of the clades of *Dehalococcoidia* are indicated as described by (Wasmund et al 2015)).

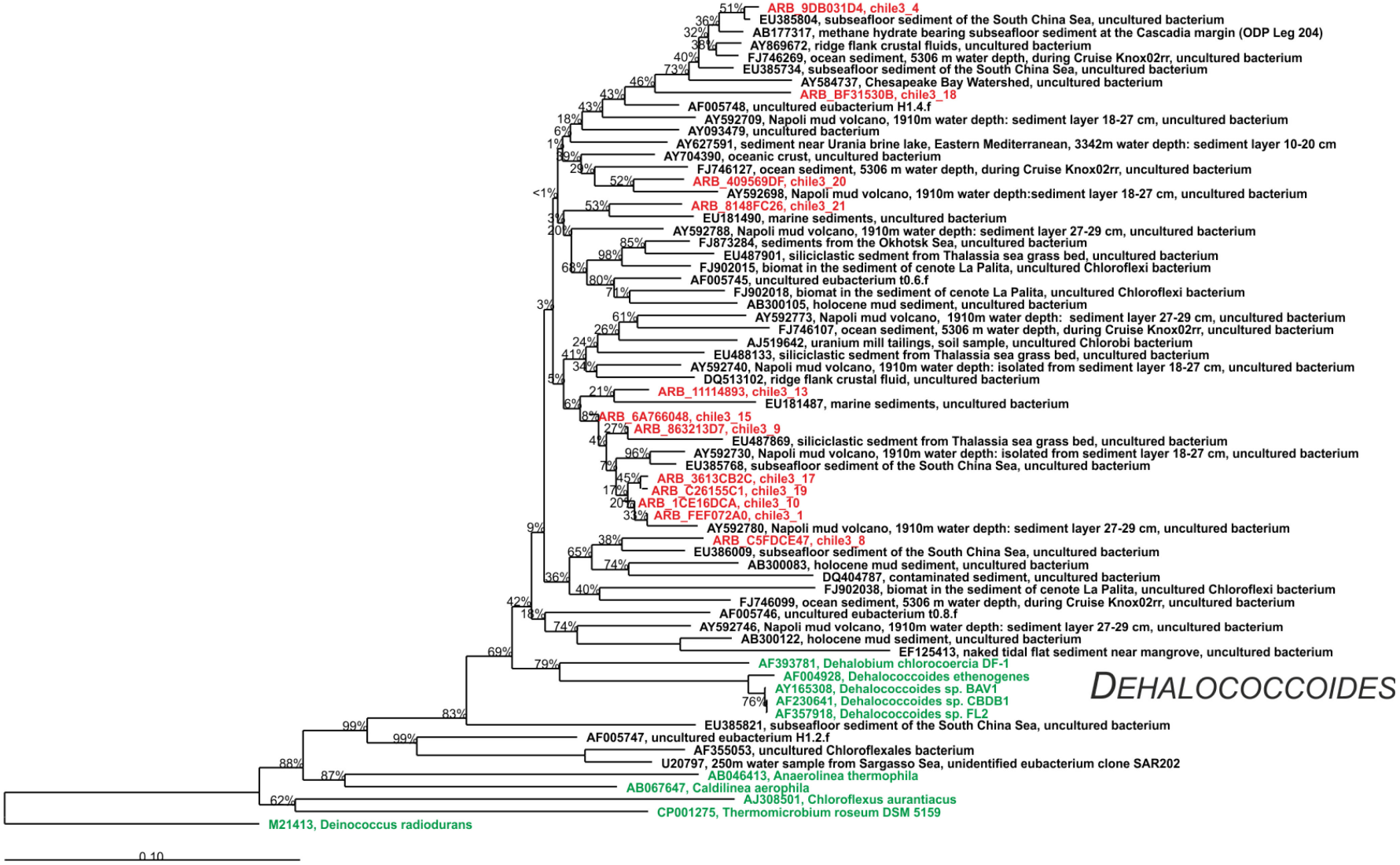


Figure 14. Neighbour joining tree based on partial 16S rRNA sequences done with the SILVA 100 database and the ARB program. Shown in red are the cloned sequences from the amplicons amplified with qPCR from a sediment culture inoculated with sediments of Chile site 7155. Shown in green are cultured strains.

Dehalococcoidia abundance among the various tested electron acceptors showed altogether no clear preferential growth under any of the tested electron acceptors, either halogenated or non-halogenated, i.e., 16S rRNA gene copies ml^{-1} varied no more than one order of magnitude at a given time point. However, differences in *Dehalococcoidia* abundance were observed in the various tested sediments.

For cultures inoculated with Århus sediments from depths of 410-420 cmbsf, *Dehalococcoidia* copy numbers were highest in cultures containing sulphate and 2,4,6-tribromoresorcinol (upper panel, Figure 15). In addition, *Dehalococcoidia* copy numbers were stable after nine months with a value of 10^4 16S rRNA gene copies ml^{-1} in cultures containing sulphate. *Dehalococcoidia* copy numbers could not be measured in the time point of nine months of incubation in cultures amended with 2,4,6-tribromoresorcinol.

In sediment cultures inoculated with sediments from Århus at a depth of 430-440 cmbsf, *Dehalococcoidia* copy numbers increased with time exclusively in those cultures supplied with sulphate and 2,4,6-tribromoresorcinol (lower panel, Figure 15). *Dehalococcoidia* copy numbers of 1.4×10^4 16S rRNA gene copies ml^{-1} culture were observed in sediment cultures amended with sulphate, which increased an order of magnitude to 2.6×10^5 copies ml^{-1} after nine months of cultivation. Similarly, *Dehalococcoidia* numbers increased one order of magnitude, from 1.3×10^4 to 1.4×10^5 16S rRNA gene copies ml^{-1} culture, in sediment cultures amended with 2,4,6-tribromoresorcinol after an incubation time of nine months.

No substantial differences were detected in *Dehalococcoidia* numbers in the presence of various reducing agents: either the inorganic reducing agent sodium sulphide (sediment cultures supplied with 2-chlorophenol and TCB were reduced with sodium sulphide), or the organic compound (titanium III citrate), which may be fermented.

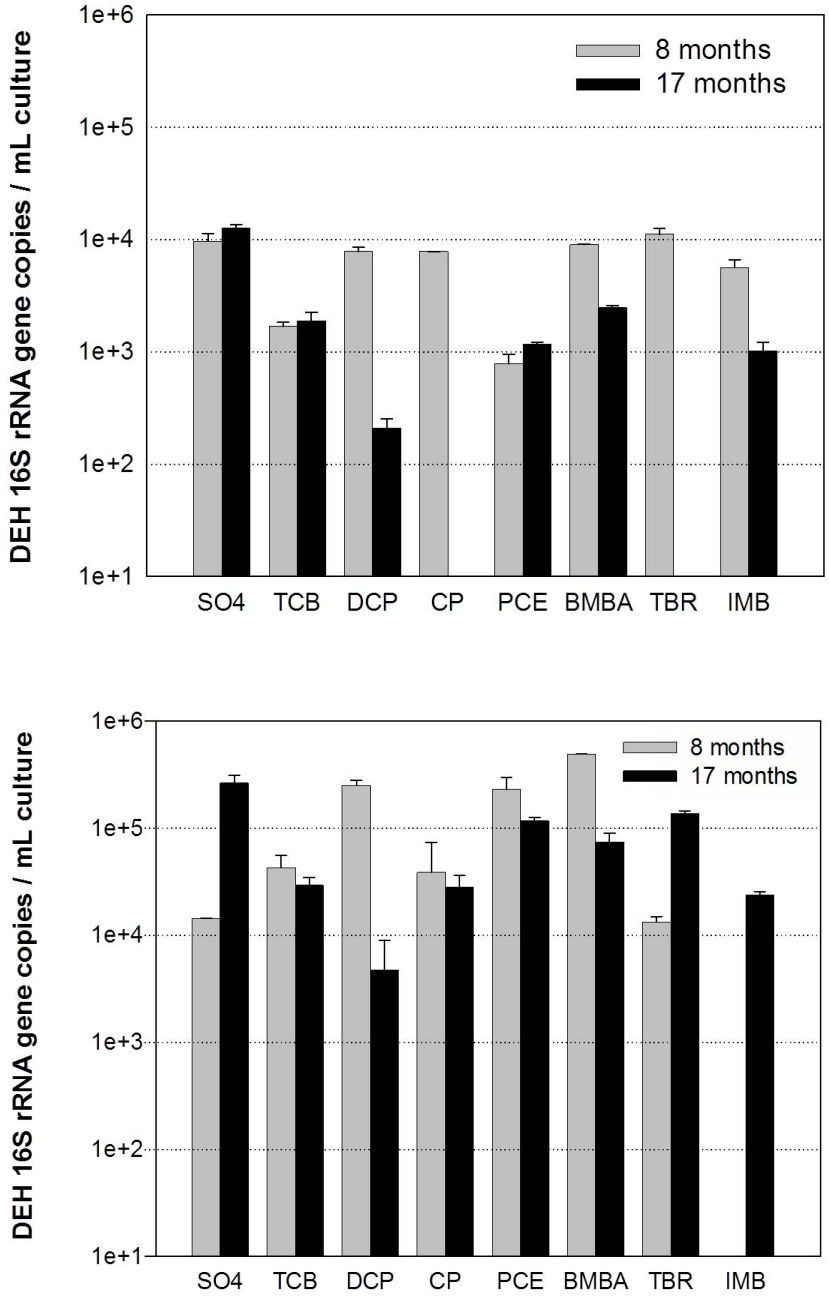


Figure 15. *Dehalococcoidia* quantification of sediment cultures set up with sediments from Århus Bay, measured as 16S rRNA gene copy numbers by qPCR. In the upper panel, cultures inoculated with the top sediment layer from Århus Bay (410-420 cmbsf) are shown. The lower panel shows cultures from the deeper sediment layer from Århus Bay (430-440 cmbsf). Standard deviations refer to triplicate measurements of the qPCR assay. Abbreviations: SO4 - sulphate; TCB - 1,2,3- and 1,2,4-trichlorobenzene mixed in a 1:1 proportion; DCP - 2,3-dichlorophenol; CP - 2-chlorophenol; PCE - tetrachloroethene; BMBA - 4-bromo-3,5-dimethoxybenzoic acid; TBR - 2,4,6-tribromoresorcinol; IMB - 1-iodo-2,6-dimethoxybenzene. TCB and CP sediment cultures were reduced with Na_2S, all others with titanium (III) citrate.

Differences of two orders of magnitude in *Dehalococcoidia* copy numbers were found between the cultures set up with sediments from the two sites of the Porcupine Seabight (Ireland; Table 1). Site 1318 had *Dehalococcoidia* numbers of 10^3 16S rRNA gene copies ml^{-1} culture, compared to site 1317, which had *Dehalococcoidia* numbers of 10^5 16S rRNA gene copies ml^{-1} (Figure 16). In site 1318, only those sediment cultures incubated with 2,4,6-tribromoresorcinol, 2,3-dichlorophenol, iron(III), and tetrachloroethene had *Dehalococcoidia* numbers above the detection threshold of 10^3 16S rRNA gene copies ml^{-1} (Figure 16). Sediment cultures inoculated with site 1317 sediments showed highest *Dehalococcoidia* numbers of 9.2 x 10^4 16S rRNA gene copies ml^{-1} within a culture that contained no supplied electron acceptor, which was prepared as a control. In addition, for site 1317, increased *Dehalococcoidia* numbers of 4.8 x 10^4 and 8.2 x 10^4 16S rRNA gene copies ml^{-1} were observed in cultures amended with sulphate and 2,4,6–tribromoresorcinol, respectively.

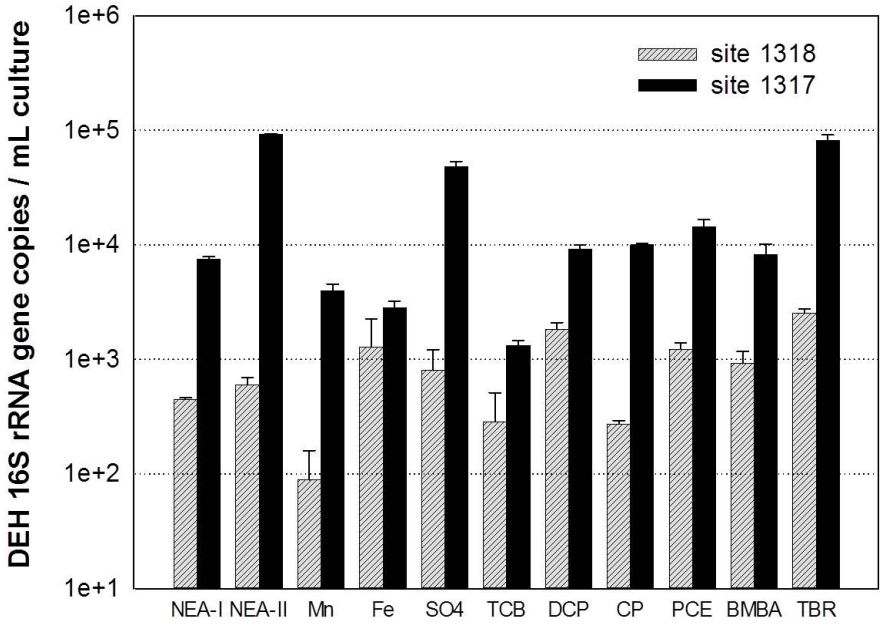


Figure 16. *Dehalococcoidia* quantification of sediment cultures set up with sediments from site 1318 at a depth of 23.15 mbsf, and site 1317 at a depth of 227 mbsf. Both sites are located in the Porcupine Seabight at the continental margin of Ireland (see Table 1 for details). Standard deviations of triplicate measurements from the qPCR assay are shown. Abbreviations: NEA - no electron acceptor as a control, NEA-I and NEA-II are two parallel sediment cultures without electron acceptor; Mn – manganese(IV); Fe – iron(III); SO4 - sulphate; TCB - 1,2,3- and 1,2,4-trichlorobenzene mixed in a 1:1 proportion; DCP - 2,3-dichlorophenol; CP - 2-chlorophenol; PCE - tetrachloroethene; BMBA - 4-bromo-3,5-dimethoxybenzoic acid; TBR - 2,4,6-tribromoresorcinol.

In line with the sediment cultures inoculated with sediments from Århus Bay, sediment cultures from Ireland indicated that sulphate and 2,4,6–tribromoresorcinol may promote *Dehalococcoidia* growth. However, the high numbers of *Dehalococcoidia* in a culture supplied without any electron acceptor reveals that the inoculated sediment may be a source of nutrients and/or electron acceptors which may support *Dehalococcoidia* growth in sediment cultures.

Sediment cultures inoculated with sediment from the coast of central off Chile, site 7165 showed similar *Dehalococcoidia* numbers of 4 x 10^3 16S rRNA gene copies ml^{-1} culture (mean of duplicates) for all electron acceptors (upper panel, Figure 17). Therefore, none of the electron acceptors supplied specifically enriched *Dehalococcoidia.*

For the case of sediment cultures set up with sediment from site 7155, similar *Dehalococcoidia* numbers of 10^3 16S rRNA gene copies ml^{-1} culture (mean of duplicates) were observed after 4 months of incubation for all electron acceptors tested (lower panel, Figure 17). However, after an incubation time of 10 months, highest *Dehalococcoidia* abundance (and the only ones above the threshold) was observed in sediment cultures supplied with sulphate and 2–chlorophenol as potential electron acceptors and in those amended with no electron acceptor. Highest *Dehalococcoidia* increments of an order of magnitude compared to the previous time point of 4 months were observed in those sediment cultures supplied with 2–chlorophenol and without any potential electron acceptor (lower panel, Figure 17). For a third time point corresponding to a year of incubation, sediment cultures supplied with iron(III) and 2,3–dichlorophenol had highest *Dehalococcoidia* numbers and increases in respect to the previous measured time point. Altogether, sediment cultures from site 7155 did not indicate a sustained growth of *Dehalococcoidia* under any of the potential electron acceptors tested here.

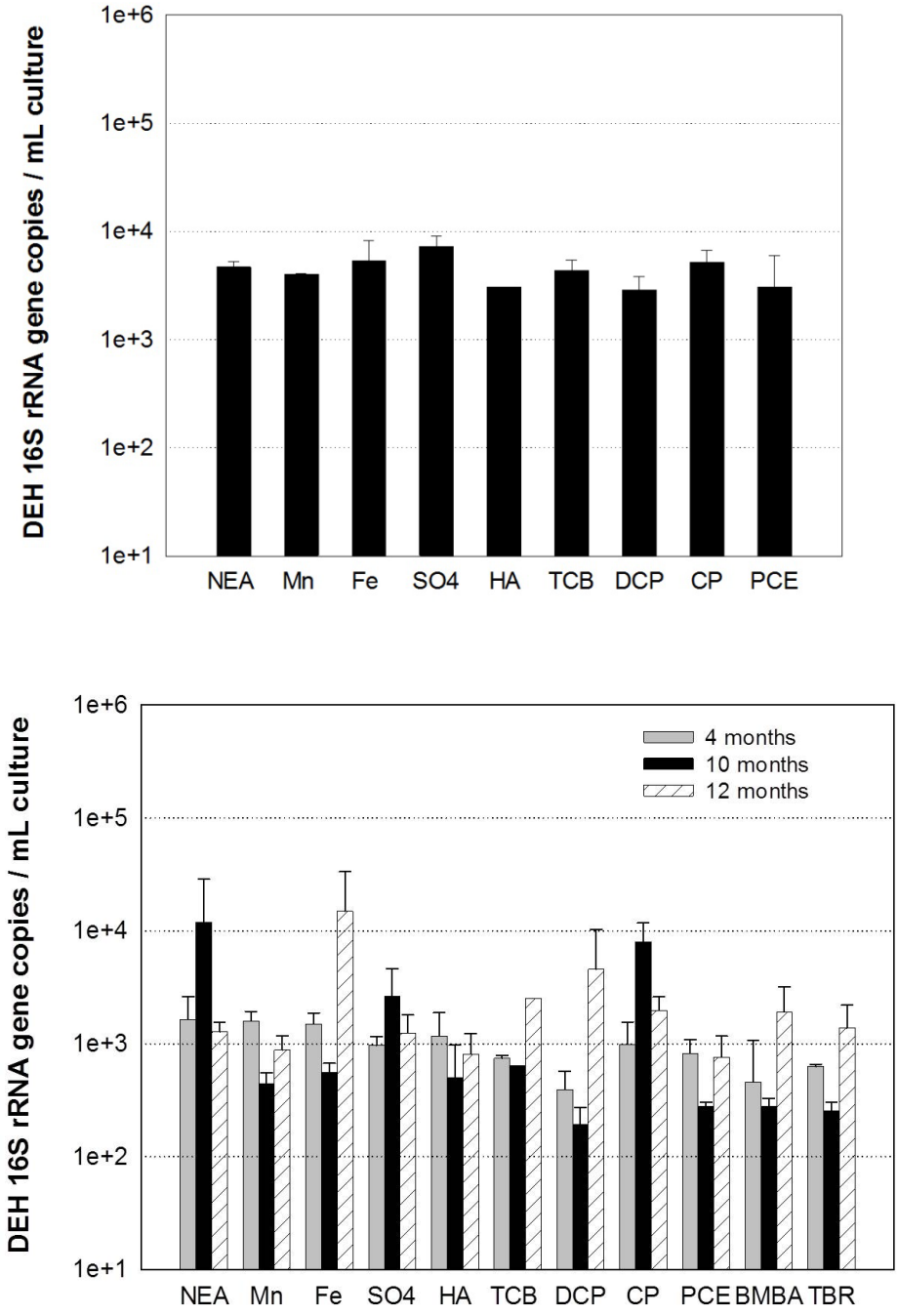


Figure 17. *Dehalococcoidia* quantification of sediment cultures set up with sediments from Chile, site 7165 at a depth of 635– 640 cmbsf (upper panel), and site 7155 at a depth of 437– 442 cmbsf (lower panel). Shown are means of duplicate cultures ± SD. Abbreviations: NEA – no electron acceptor, as a control; Mn – manganese(IV); Fe – iron(III); SO4 – sulphate; HA – humic acids; TCB – 1,2,3- and 1,2,4-trichlorobenzene mixed in a 1:1 proportion; DCP – 2,3-dichlorophenol; CP – 2-chlorophenol; PCE – tetrachloroethene; BMBA – 4-bromo-3,5-dimethoxybenzoic acid; TBR – 2,4,6-tribromoresorcinol.

Due to the variability of the results in *Dehalococcoidia* numbers from the different time points of sediments from Chile, an assay to prove if the methods that were used (i.e., sampling of sediment culture and subsequent DNA isolation and qPCR) were reliable for quantification of bacteria was conducted. For this, three samples of 1, 2, and 4 ml were taken from a sediment culture of Chile site 7165, followed by DNA isolation and the performance of triplicate qPCR measurements with primers targeting for total *Bacteria* (primer pair 341F and 534R, Table 7). Results showed a cycle of difference between the 1 and 2 ml sampled sediment cultures in the qPCR assay and 2 cycles of difference between the 2 and 4 ml sampled sediment cultures. Thus, this approach of sample withdrawal from the sediment culture, DNA isolation, and qPCR assay was reliable for quantifying 16S rRNA gene copy numbers.

In general, these experimental results show that *Dehalococcoidia* were growing in the sediment cultures, demonstrating that they withstood the changes in temperature and pressure during the sampling campaign and that the supplied media based on the medium used for *Dehalococcoides mccartyi* strain CBDB1 was appropriate for the cultivation of marine *Dehalococcoidia*.

3.1.2 Total *Bacteria* abundance under various terminal electron acceptors in sediment cultures

The total bacterial community abundance within Chile site 7155 sediment cultures was measured with primers targeting the 16S rRNA gene of all bacteria (primer pair 341F and 534R, Table 7). The results showed similar total bacterial abundance for all electron acceptors of 10^7 16S rRNA gene copies ml^{-1} culture after four months of incubation (Figure 18). Therefore, *Dehalococcoidia* represent around 0.01% of the total bacterial community in the Chile 7155 sediment cultures. A second time point, after ten months of incubation, showed a decrease in total bacterial numbers for all electron acceptors except for sulphate.

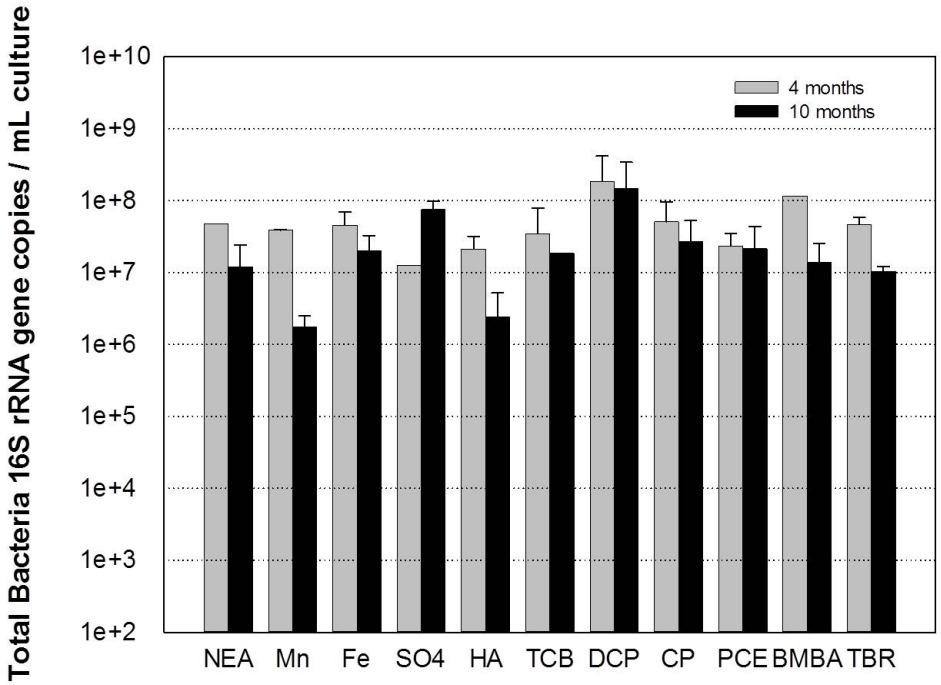


Figure 18. Total bacterial 16S rRNA gene copy numbers in sediment cultures set up with sediments from the continental margin of Chile, site 7155 (see Table 1 for details). Shown are means of duplicate cultures ± SD. Abbreviations: NEA – no electron acceptor, as a control; Mn – manganese(IV); Fe – iron(III); SO4 – sulphate; HA – humic acids; TCB – 1,2,3- and 1,2,4-trichlorobenzene mixed in a 1:1 proportion; DCP – 2,3-dichlorophenol; CP – 2-chlorophenol; PCE – tetrachloroethene; BMBA – 4-bromo-3,5-dimethoxybenzoic acid; TBR – 2,4,6-tribromoresorcinol.

3.1.3 Colony formation in deep-agarose dilution tubes under various terminal electron acceptors

None of the deep-agarose dilution tubes prepared with the various electron acceptors for both Århus and Ireland sediments showed any colony formation, except two tubes, which may also have been due to contamination. One of these deep-agarose tubes was a third sequential dilution from a sediment culture inoculated with sediments from site 1317 from Ireland, amended with humic acids, which had white colonies. The second deep-agarose tube was inoculated with an Århus sediment culture inoculum, amended with 4-bromo-3,5-dimethoxybenzoic acid, and showed growth of small white colonies all over the tube, but particularly under the surface, and although the redox dye resazurin within the agarose tube did not change in colour (i.e., to pink; when at positive redox potentials), maybe traces of oxygen may have induced these colonies growth.

In contrast, deep-agarose dilution tubes prepared from cultures inoculated with sediments from Chile, site 7155, showed many colonies after an incubation time of two weeks to one month. Colonies in the first dilution were small and very numerous, however with increasing dilution, colonies began to be less numerous and bigger.

Deep-agarose dilution tubes prepared with 2,3-dichlorophenol, humic acids, iron(III), tetrachloroethene, and with no potential electron acceptor had the highest colony density from all. Colony morphologies varied from small black and/or white in tubes amended with 2,3-dichlorophenol or with no electron acceptor, to large ones, in the case of humic acids and iron(III) (Figure 19). These large colonies were brown for humic acids and dark red for iron, which were especially large. In agarose tubes with PCE, colonies were black and with a black surrounding halo (Figure 19). This black colour from the colonies may be iron sulphide, which is a black precipitate. Iron sulphide results from the conversion of sulphate to sulphide by sulphate-reducing bacteria and the further combination of sulphide with iron(II), which is present in the medium. Sulphate may come from the inoculum (i.e., the sediment), as no sulphate is provided in the medium. PCE conversion to lower chlorinated compounds was not observed. Thus, the black colour from these colonies may indicate that the colonies may be formed by sulphate reducers. Deep-agarose dilution tubes amended with humic acids developed gas bubbles throughout the tube which may be due to methanogens forming methane and/or fermenters forming carbon dioxide. Growth in agarose tubes amended without any electron acceptor indicated that bacteria may be using the citrate from titanium (III) citrate or residual compounds from the sediment inoculum, which even though diluted, may still be present. Deep-agarose dilution tubes containing sulphate were observed to develop a black precipitate in parts of the tube after longer periods of incubation indicating the presence of sulphate reducers.

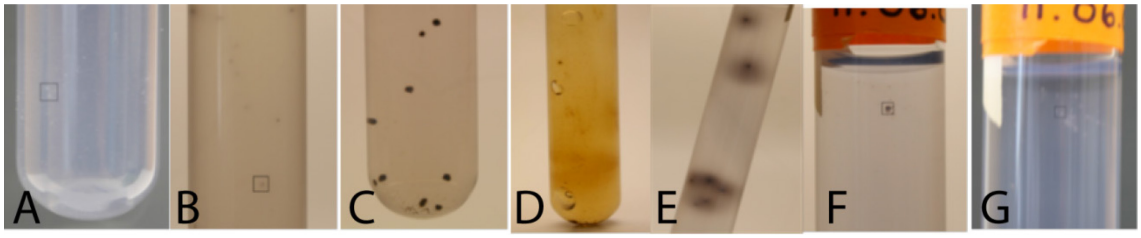


Figure 19. Colony colours, shapes and visual appearance varied from white to black, brown or red in the semisolid deep-agarose dilution tubes. Colonies from deep-agarose dilution tubes amended with no electron acceptor (A, B), with iron(III) (C), humic acids (D), PCE (E) and with 2,3-dichlorophenol (F, G) are shown.

Some colonies were selected for sub-cultivation (Figure 20). For that, colonies were picked with sterile Pasteur pipettes or with sterile syringes associated to thick sterile needles. Picked colonies were immediately transferred into liquid. Deep-agarose dilution tubes were prepared again from these sub-cultivated in-liquid-medium colonies after two weeks of incubation. These newly prepared deep-agarose dilution tubes showed in general consistent morphology with the original picked colony (A, B and C in Figure 20). However, in some other cases, the newly prepared agarose tubes showed

differing colonies to the original colony (see E in Figure 20), indicating that more than one colony was picked or that contamination occurred in the newly prepared agarose tubes. The newly prepared agarose tubes had high colony concentration with smaller colony sizes, due to competition for space and nutrients. Those deep-agarose dilution tubes amended with humic acids showed gas bubbles, as observed previously.

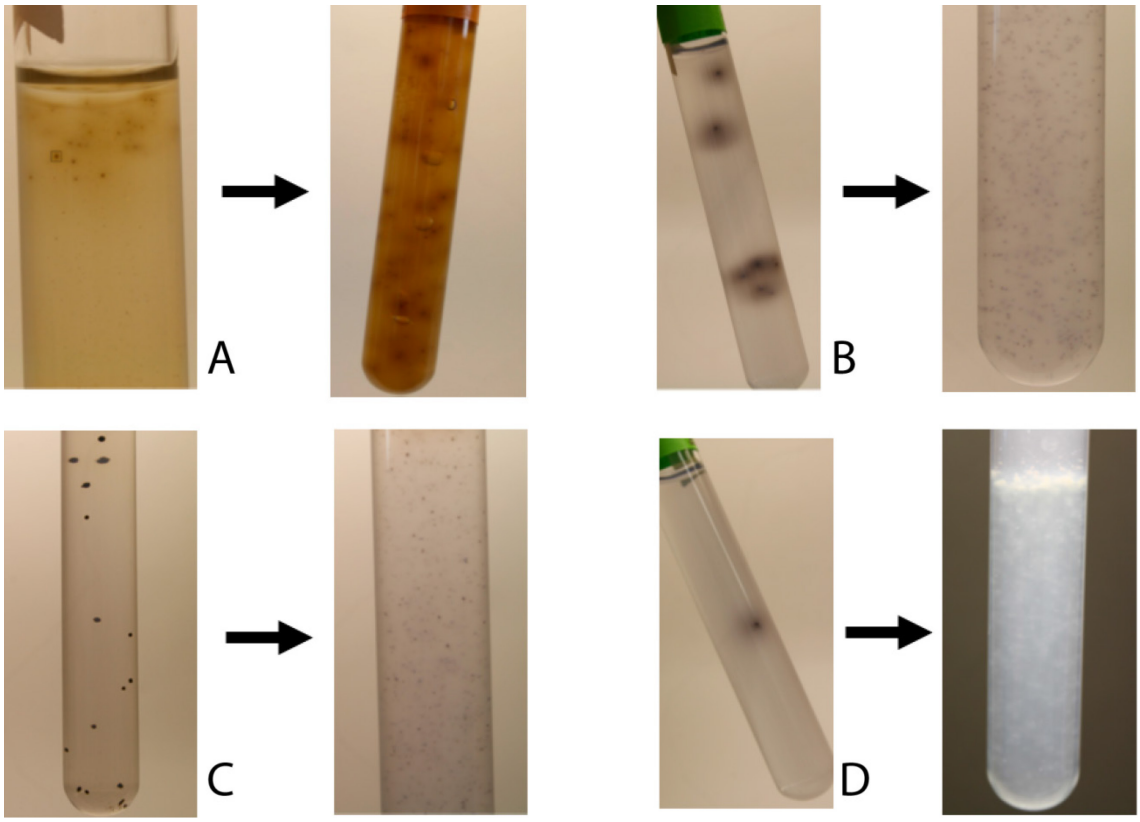


Figure 20. Colonies from sub-cultivated deep-agarose dilution tubes compared to the original colony. Colonies formed in deep-agarose dilution tubes amended with humic acids (A), with PCE (B and D), and with iron(III) (C).

3.1.4 Colony identification after 16S rRNA gene sequencing

Thirty of the observed colonies were picked, DNA was isolated from them, the 16S rRNA gene was amplified and amplicons were sequenced. All sequenced colonies belonged to the phylum *Firmicutes*, class *Clostridia*, although three colonies belonged to the class *Bacillales* (Table 8). Many of them showed high similarity to the genera *Pelotomaculum* (8/30 sequences), *Gracilibacter* (4/30) and *Pelosinus* (4/30), *Vulcanibacillus* (3/30), *Clostridium* (3/30), *Morella* (2/30) and *Desulfotomaculum* (2/30).

Table 8. Colony similarities to closest cultured bacterial member based on the 16S rRNA gene partial sequence. Colonies 1-16 belong to colonies which were picked exclusively to know their phylogenetic affiliation. Colonies 17-32 come from sub-cultivated colonies, from which 1 ml of liquid culture was sampled for DNA to find out their phylogeny. All of them were cultivated with titanium (III)-citrate as reducing agent. The assessment of the 16S rRNA gene sequence similarity was done using Blast (Basic Local Alignment Search Tool; (Altschul et al 1990)) and the NCBI database. Abbreviations: TCB – 1,2,3- and 1,2,4-trichlorobenzene mixed in a 1:1 proportion; BMBA – 4-bromo-3,5-dimethoxybenzoic acid.

No.	Electron acceptor	Closest cultivated bacterial sequence	Literature
1	2,4,6-tribromo-resorcinol	*Gracilibacter thermotolerans* strain JW/YJL-S1 Firmicutes; Clostridia; Clostridiales; Graciibacteraceae; Gracilibacter. 95% coverage; 88% identity	(Lee et al 2006)
2	SO_4	*Gracilibacter thermotolerans* strain JW/YJL-S1 Firmicutes; Clostridia; Clostridiales; Graciibacteraceae; Gracilibacter. 93% coverage; 96% identity	(Lee et al 2006)
3	TCB	Candidatus *Heliobacterium aridinosum* Firmicutes; Clostridia; Clostridiales; Heliobacteriaceae; Heliobacterium 95% coverage; 88% identity	Girija *et al*, unpublished
4	BMBA	*Thermincola carboxydiphila* strain 2204 Firmicutes; Clostridia; Clostridiales; Peptococcaceae; Thermincola. 100% coverage; 89% identity	(Sokolova et al 2005)
5	humic acids	*Sporotalea propionica* strain TmPM3 Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Sporotalea 100% coverage; 85% identity	(Boga et al 2007)
6	humic acids	*Pelotomaculum isophthalicicum* Firmicutes; Clostridia; Clostridiales; Peptococcaceae; Pelotomaculum. 99% coverage; 85% identity	(Qiu et al 2006)
7	humic acids	*Desulfotomaculum sp.* 16S rRNA gene, DSM 7440 Firmicutes; Clostridia; Clostridiales; Peptococcaceae;Desulfotomaculum. 92% coverage; 85% identity	(Stackebrandt et al 1997)
8	Fe(III)	*Pelotomaculum isophthalicicum* Firmicutes; Clostridia; Clostridiales; Peptococcaceae; Pelotomaculum 99% coverage; 87% identity	(Qiu et al 2006)
9	PCE	*Pelotomaculum isophthalicicum* Firmicutes; Clostridia; Clostridiales; Peptococcaceae;Pelotomaculum 90% coverage; 90% identity	(Qiu et al 2006)
10	Methanol	*Pelotomaculum isophthalicicum* Firmicutes; Clostridia; Clostridiales; Peptococcaceae; Pelotomaculum. 100% coverage; 86% identity	(Qiu et al 2006)
11	2-Chloro-phenol	*Clostridium sp. JC3* Firmicutes; Clostridia; Clostridiales; Clostridiaceae;Clostridium. 94% coverage; 83% identity	Syutsubo, *et al*, unpublished
12	Fe(III)	*Lutispora thermophila* Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Lutispora. 99% coverage; 87% identity	(Shiratori et al 2006)
13	Fe(III)	*Pelotomaculum isophthalicicum* Firmicutes; Clostridia; Clostridiales; Peptococcaceae;Pelotomaculum. 100% coverage; 87% identity	(Qiu et al 2006)

No.	Electron acceptor	Closest cultivated bacterial sequence	Literature
14	Fe(III)	*Pelotomaculum isophthalicicum* Firmicutes; Clostridia; Clostridiales; Peptococcaceae;Pelotomaculum. 100% coverage; 86% identity	(Qiu et al 2006)
16	Fe(III)	*Pelosinus sp. UFO1* Firmicutes; Clostridia; Clostridiales; Veillonellaceae;Pelosinus. 100% coverage; 99% identity	Ray *et al*, unpublished
18	PCE	*Pelosinus sp. UFO1* Firmicutes; Clostridia; Clostridiales; Veillonellaceae;Pelosinus. 100% coverage; 99% identity	Ray *et al*, unpublished
19	BMBA	*Moorella glycerini strain JW/AS-Y6* Firmicutes; Clostridia; Thermoanaerobacterales;Thermoanaerobacteraceae; Moorella group; Moorella. 94% coverage; 84% identity	(Slobodkin et al 1997)
20	BMBA	*Pelotomaculum isophthalicicum* Firmicutes; Clostridia; Clostridiales; Peptococcaceae;Pelotomaculum. 99% coverage; 84% identity	(Qiu et al 2006)
21	BMBA	*Pelotomaculum isophthalicicum* Firmicutes; Clostridia; Clostridiales; Peptococcaceae;Pelotomaculum. 100% coverage; 84% identity	(Qiu et al 2006)
22	TCB	*Vulcanibacillus modesticaldus* Firmicutes; Bacillales; Bacillaceae; Vulcanibacillus. 99% coverage; 95% identity	Swiderski, unpublished
23	TCB	*Vulcanibacillus modesticaldus* Firmicutes; Bacillales; Bacillaceae; Vulcanibacillus. 100% coverage; 93% identity	Swiderski, unpublished
24	TCB	*Vulcanibacillus modesticaldus* Firmicutes; Bacillales; Bacillaceae; Vulcanibacillus. 99% coverage; 93% identity	Swiderski, unpublished
26	2,3-dichloro-phenol	*Clostridium sp. AAN11* Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium. 91% coverage; 84% identity	Ueno & Yamazawa, unpublished
27	2,3-dichloro-phenol	*Clostridium acetireducens strain DSM 10703* Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium. 95% coverage; 91% identity	(Orlygsson et al 1996)
28	none	*Desulfotomaculum carboxydivorans strain CO-1-SRB* Firmicutes; Clostridia; Clostridiales; Peptococcaceae;Desulfotomaculum. 100% coverage; 84% identity	(Parshina et al 2005)
29	none	*Moorella glycerini strain JW/AS-Y6* Firmicutes; Clostridia;Thermoanaerobacterales; Thermoanaerobacteraceae; Moorella group; Moorella. 95% coverage; 82% identity	(Slobodkin et al 1997)
30	Fe(III)	*Pelosinus sp. UFO1* Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Pelosinus. 100% coverage; 99% identity	Ray *et al*, unpublished
32	Fe(III)	*Pelosinus sp. UFO1* Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Pelosinus. 100% coverage; 99% identity	Ray *et al*, unpublished

3.2 ORGANOHALIDE TRANSFORMATION IN MARINE SEDIMENT CULTURES

Sediment cultures were amended with various halogenated compounds (1,2,3-TCB and 1,2,4-TCB, 2,3-dichlorophenol, 2-monochlorophenol, 2,4,6-tribromoresorcinol, 4-bromo-3,5-dimethoxybenzoic acid, hexachlorobenzene, hexabromobenzene, or PCE) as potential electron acceptors for all tested sediments (Århus, Ireland, Chile). From the halogenated compounds, the concentrations of 1,2,3-TCB, 1,2,4-TCB, 2,3-dichlorophenol, 2-monochlorophenol, PCE, and hexachlorobenzene transformation products in the cultures were monitored with GC-FID. Exclusively 1,2,3-TCB was transformed to 1,3-DCB in two sediment cultures, one inoculated with sediments from Århus (after 346 days of incubation), and the other with sediments from site 7155 of Chile as detailed in section 3.2.1. No further transformation of 1,3-DCB to monochlorobenzene was observed.

3.2.1 Transformation of 1,2,3-TCB to 1,3-DCB in marine sediment cultures

Complete transformation of 1,2,3-TCB (80 μM, which was solved in 1,2,4-TCB in a 1:1 proportion) to 1,3-DCB was observed in one sediment culture inoculated with sediments from Chile, site 7155 (at a depth of 437–442 cmbsf, Table 1), after six months of incubation. This sediment culture did however not transform 1,2,4-TCB.

After complete 1,2,3-TCB transformation, the sediment culture was transferred in three parallels with a 13% (v/v) inoculum to fresh medium amended with 80 μM of 1,2,3–TCB (solved in 1,2,4-TCB in a 1:1 proportion). One of the three daughter cultures showed complete conversion of 1,2,3-TCB to 1,3-DCB after two months of incubation. In the other two replicate daughter cultures, no transformation of 1,2,3-TCB to DCBs was observed.

Further transferring consisted of six subcultures (using inocula of 4% (v/v) in fresh medium, and amended with 80 μM 1,2,3-TCB solved in acetone for three subcultures, and 40 μM for the other three), and aimed for an enrichment in a larger number of subcultures and thus higher total biomass, and for an enrichment of specifically 1,2,3-TCB transforming microorganisms when increasing the 1,2,3-TCB concentrations. However, none of the six subcultures transformed 1,2,3-TCB.

After this, and to reproduce the organohalide transformation, a new batch of sediment cultures was set up. Sediment cultures were prepared in four replicates with 9 or 6% inoculum (v/v) of sediment from Chile site 7155 and amended with 45 μM of 1,2,3-TCB, solved in acetone. Sediment cultures were prepared in four parallels and reduced

either with titanium (III) citrate, iron sulphide, or sodium sulphide together with L-cysteine (Table 2). All sediment cultures reduced with iron sulphide showed a change of colour of the redox dye resazurin after the inoculation of the sediment, indicating a positive redox potential in the cultures; and these cultures were discarded. Cultures reduced with titanium (III) citrate or sodium sulphide together with L-cysteine remained anoxic (at negative redox potentials as indicated by the transparent colour of the redox indicator resazurin), and they were monitored and further maintained.

The formation of 1,3-DCB was observed in two of the four parallel sediment cultures (labelled as G0, see section 3.2.2, Figure 23) reduced with titanium (III) citrate after 58 days (Figure 21). On day 65, all the cultures were amended with 40 μM of 1,2,3-TCB, before G0–a had depleted all 1,2,3-TCB. The parallels G0–b and G0–d transformed 1,2,3-TCB to 1,3-DCB more slowly. The formation of 1,3-DCB was observed after an incubation time of 270 and 325 days for G0–b and G0–d, respectively (results not shown). All cultures were amended with more 1,2,3-TCB at day 65, and subsequently whenever 1,2,3-TCB was depleted. In all cultures and also in negative controls without bacteria, a slow decrease of 1,2,3-TCB was observed and was attributed to the sampling procedure and the piercing of the Teflon liner of the rubber stoppers.

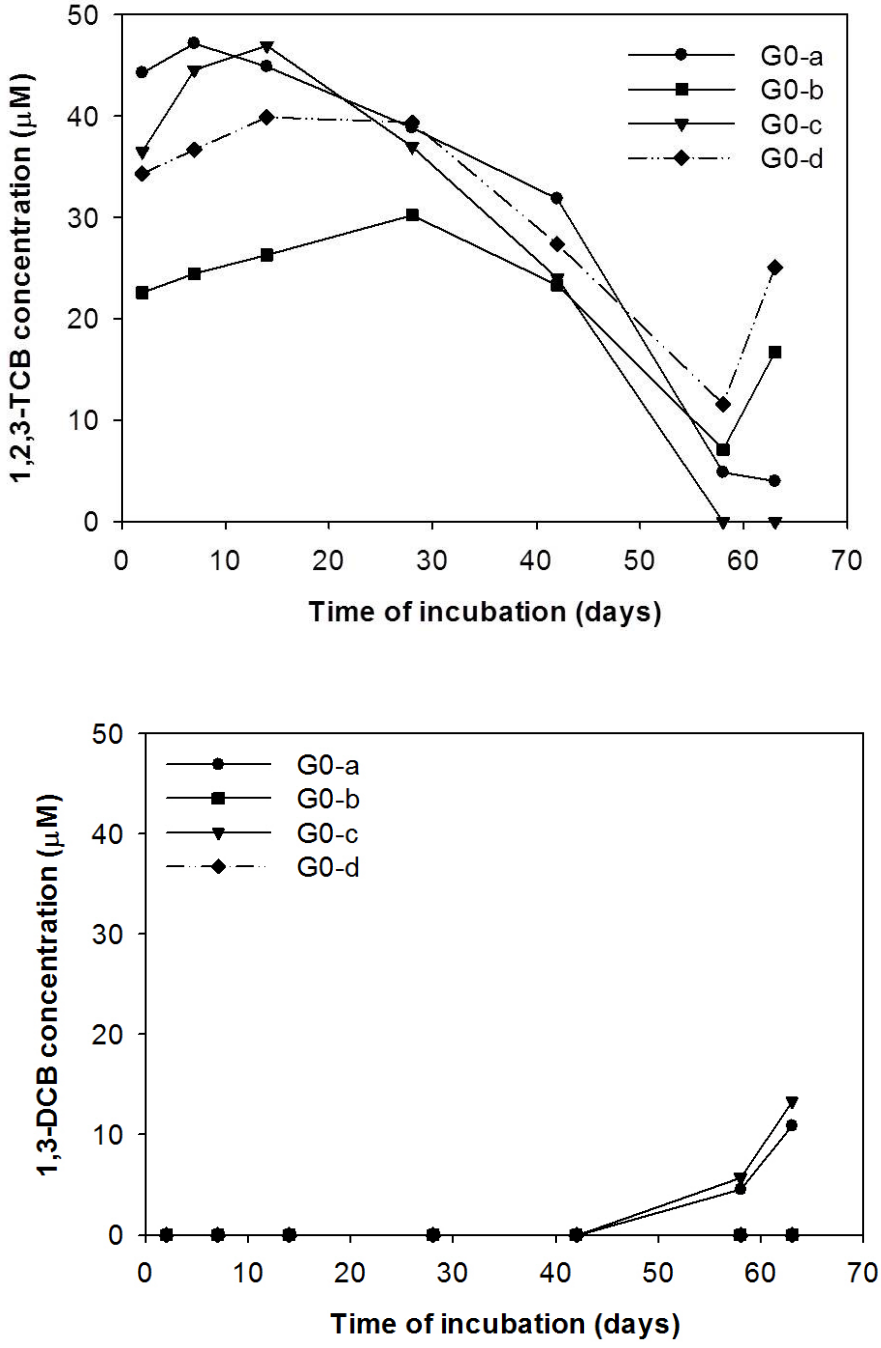


Figure 21. Time-course of 1,2,3-TCB transformation (upper panel) and 1,3-DCB formation (lower panel) in four parallel G0 cultures reduced with titanium (III) citrate. G0 cultures were inoculated with 9% (v/v) sediment from Chile site 7155 (from a depth of 437–442 cmbsf). No production of dichlorobenzene was observed in medium blanks or in autoclaved sediment cultures reduced with titanium (III) citrate.

Three of the four parallel sediment cultures reduced with sodium sulphide plus L-cysteine transformed 1,2,3-TCB to 1,3-DCB (Figure 22). The sediment culture G0–e was not observed to form 1,3-DCB within 277 days. 1,2,3-TCB (40 µM) was supplied to the cultures after 65 and 221 days. The formation of 1,3-DCB was observed within 42 days for two sediment cultures (G0–g and G0–h, lower panel of Figure 22).

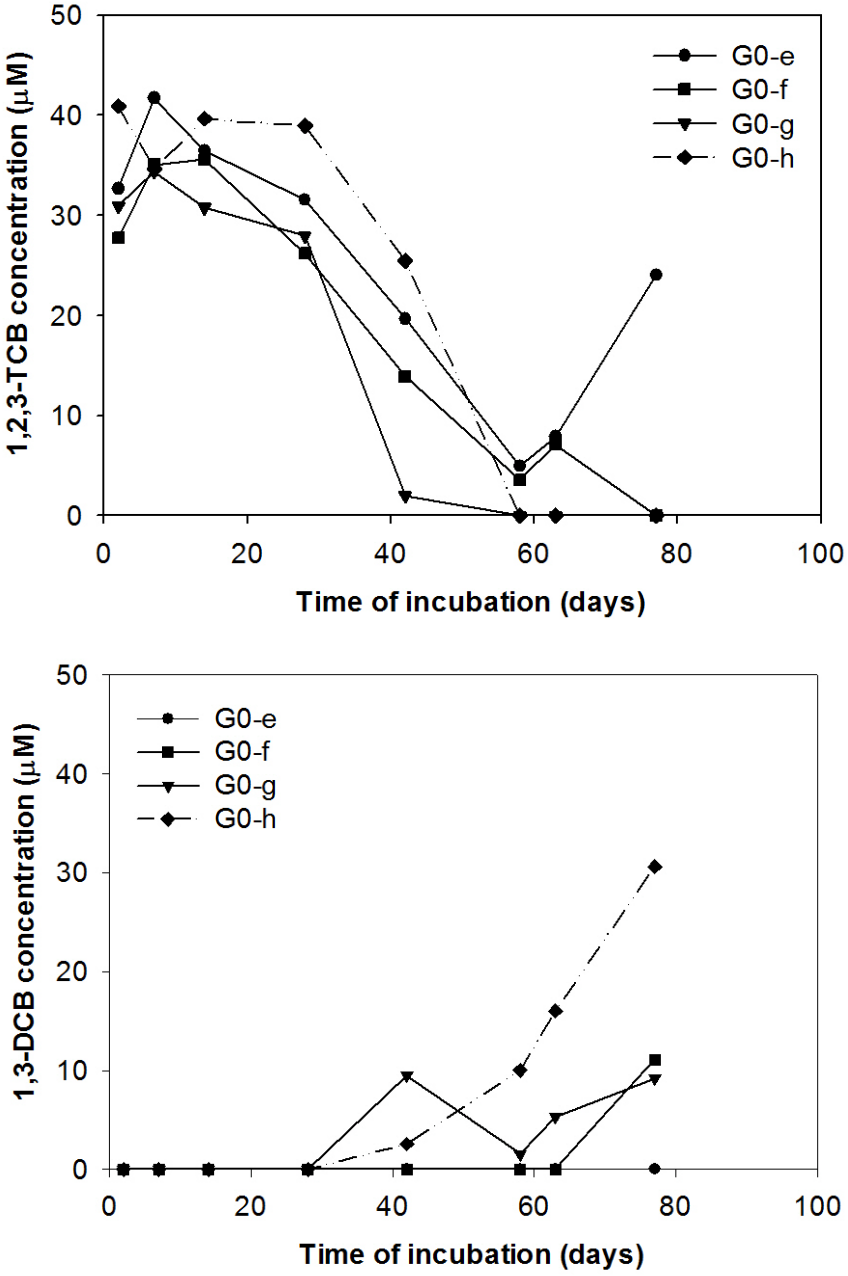


Figure 22. Time-course of 1,2,3-TCB transformation (upper panel) and 1,3-DCB formation (lower panel) in four parallel G0 cultures reduced with sodium sulphide and L-cysteine. G0–e, –f, –g cultures were inoculated with 6% (v/v), and G0–h culture with 9% (v/v) of sediment from Chile site 7155 (at a depth of 437–442 cmbsf). No production of dichlorobenzene was observed in medium blanks or in autoclaved sediment cultures reduced with sodium sulphide and L-cysteine.

Additionally, triplicate sediment cultures reduced with either titanium (III) citrate or sodium sulphide with L-cysteine were supplied with vancomycin or with ampicillin plus the methanogen-inhibitor 2-bromoethanesulfonate (BES). None of the cultures supplied with antibiotics and/or BES did transform 1,2,3-TCB to dichlorobenzenes. Thus, the specific enrichment of dehalogenating bacteria by the addition of vancomycin or ampicillin/BES was not possible, suggesting that Gram-positives may have a direct or indirect role in 1,2,3-TCB transformation.

3.2.2 Enrichment of trichlorobenzene dechlorinating bacteria from sediment cultures

The enrichment procedure consisted of transferring 9% of inoculum into fresh media once all the 1,2,3-TCB was completely transformed or about to be transformed in the culture. The hypothesis of this experiment was that the microorganisms performing 1,2,3-TCB transformation obtain a growth advantage over other microorganisms by repeated subcultivation in the presence of organohalides. Four subsequent transfers (subcultures G1–G4; Figure 23) maintained organohalide transformation in cultures reduced with titanium (III) citrate (Table 9). In cultures reduced with sodium sulphide and L-cysteine, two subsequent transfers (subcultures G1–G2) maintained organohalide transformation (Table 9).

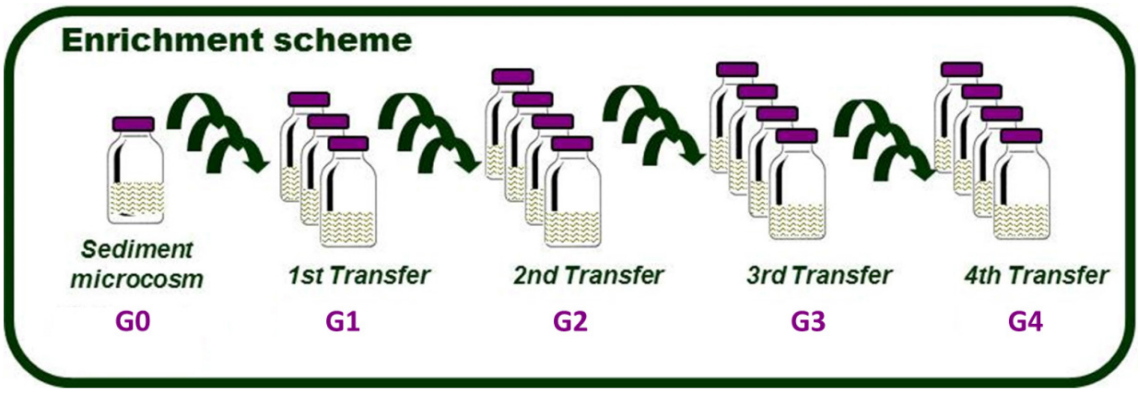


Figure 23. Enrichment scheme of dehalogenating cultures. The nomenclature G0–G4 will be consistently used in this thesis to describe the different cultures.

Table 9. Summary of cultures set up within this study reduced either with titanium (III) citrate or sodium sulphide together with L-cysteine. The total number of cultures per generation (G0–G4; "Total") and the number of cultures that transformed 1,2,3-TCB to 1,3-DCB ("Active") are shown.

Reduced with:	G0		G1		G2		G3		G4	
	Total[1]	Active[2]	Total[1]	Active[2]	Total[1]	Active[2]	Total[1]	Active[2]	Total[1]	Active[2]
Titanium (III) citrate	4	4	34	29	46	38	16	7	8	2
Sodium sulphide and L-cysteine	4	3	20	10	15	6	3[a]	0	6[a]	0

[1]Total number of cultures; [2]Number of active cultures, where formation of 1,3-DCB was observed associated to 1,2,3-TCB transformation. [a]Inoculum was a G2 o G3 culture which was originally reduced with titanium (III) citrate.

A first set of G1 subcultures containing 2% (v/v) inoculum from G0–a, –c, –g, and –h was prepared in triplicate. This first set of subcultures was prepared when G0 cultures had been incubated for 73 days and the formation of 1,3-DCB had been observed (Figure 21 and Figure 22). The three triplicate G1–c subcultures transformed 1,2,3-TCB to 1,3-DCB. Two from the three G1–c subcultures had to be discarded due to the change in colour to pink of the resazurin, which indicated that the subcultures were no longer reduced. The third G1–c subculture was maintained and subsequently transferred. G2 (three subcultures), G3 (16 subcultures) and G4 (six subcultures) were obtained and dehalogenated 1,2,3-TCB to 1,3-DCB. All G3 and G4 subcultures of this study were subcultures of this G1–c subculture. G1–a, and –g did not transform 1,2,3-TCB to 1,3-DCB. One of the triplicates from G1–h showed transformation of 1,2,3-TCB to 1,3-DCB and was transferred into fresh medium, however, no active subculture was obtained from it.

A second set of G1 subcultures were produced with a higher proportion of G0 inoculum of 9% (v/v), when the G0 cultures were 245 days old for G1–a and G1–c, and 277 days for G0–f, –g, and –h. The subcultures G1–a and –c, as well as G1–f, –g and –h were prepared in parallel, respectively. G1–b and –d subcultures were prepared later on, after dechlorination in the G0–b and –d cultures was observed. All G1 subcultures transformed 1,2,3-TCB to 1,3-DCB either if reduced with titanium (III) citrate (Figure 24) or with sodium sulphide together with L-cysteine (Figure 25). All G1 subcultures transformed 1,2,3-TCB to 1,3-DCB in a shorter time spam that the G0 culture from which they were inoculated.

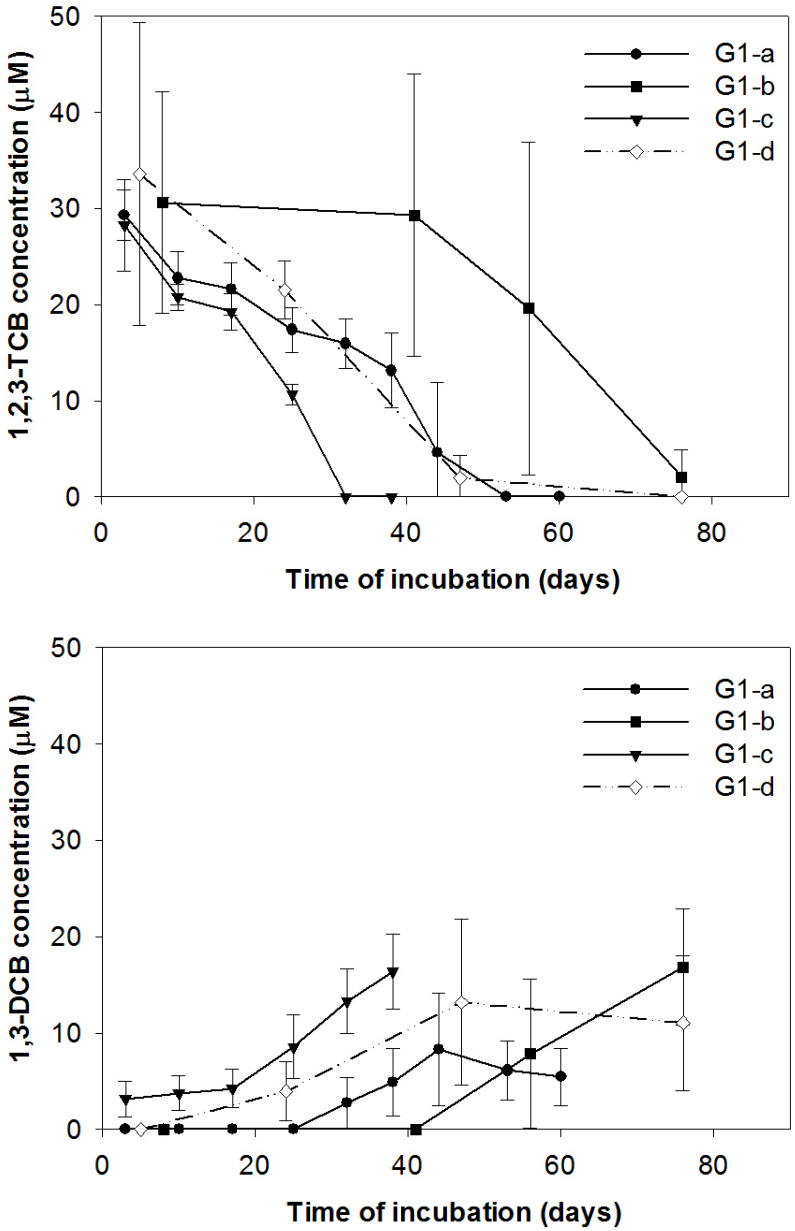


Figure 24. Time-course of 1,2,3-TCB transformation (upper panel) and 1,3-DCB formation (lower panel) in twelve G1 subcultures reduced with titanium (III) citrate. The mean of 1,2,3-TCB and 1,3-DCB concentration in triplicate subcultures are plotted ± SD. The "–a, –b, –c, –d" in the labels refer to the specific G0 culture from which each G1 subculture was inoculated from. For example, G1–a are triplicate subcultures inoculated with 9% (v/v) of culture G0–a. Subcultures G1–a and –c are parallels, inoculated and measured at same time. The subcultures G1–b and –c were set up later at different times.

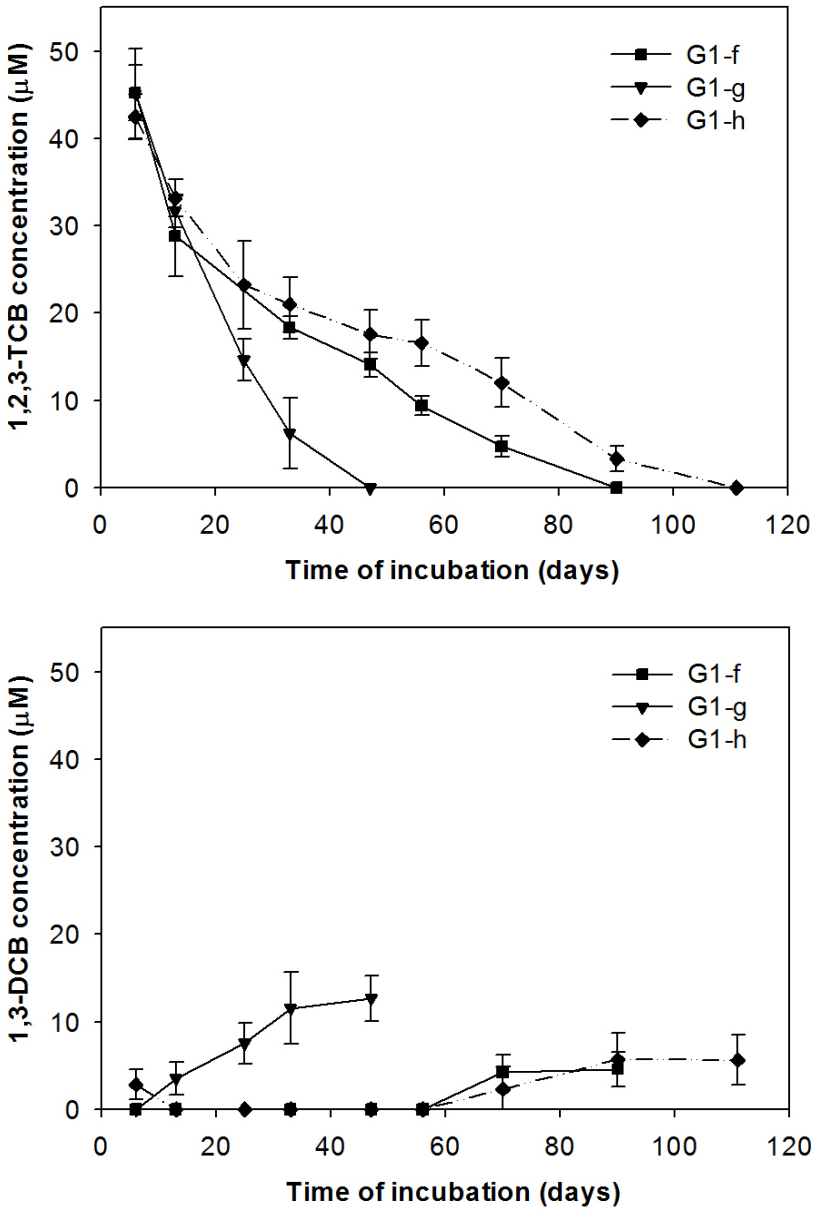


Figure 25. Time-course of 1,2,3-TCB transformation (upper panel) and 1,3-DCB formation (lower panel) in six parallel G1 subcultures reduced with sodium sulphide and L-cysteine. The mean of 1,2,3-TCB and 1,3-DCB concentration in duplicate subcultures are plotted ± SD. The "–f, –g, –h" in the labels refer to the specific G0 culture from which each G1 subculture was inoculated from. For example, G1–f refers to duplicate subcultures inoculated with 9% (v/v) of culture G0–f. No G1–e subcultures were set up as G0–e did not transform 1,2,3-TCB to 1,3-DCB. All subcultures were set up and measured at same time point-s until 1,2,3-TCB was depleted and subsequently transferred to fresh medium.

The subcultures G1–a, –c, –d, and –g were selected for further subculturing. 1,2,3-TCB transformation to 1,3-DCB was observed in the great majority of G2 subcultures (Table 9). Activity (defined here as formation of 1,3-DCB from 1,2,3-TCB) was observed in a greater proportion of G2 subcultures reduced with titanium (III) citrate compared to those reduced with sodium sulphide and L-cysteine (Table 9).

G2 subcultures transformed 1,2,3-TCB in a longer time spam than G1 and sometimes also than G0 cultures, either when reduced with titanium (III) citrate or sodium sulphide together with L-cysteine (Figure 26).

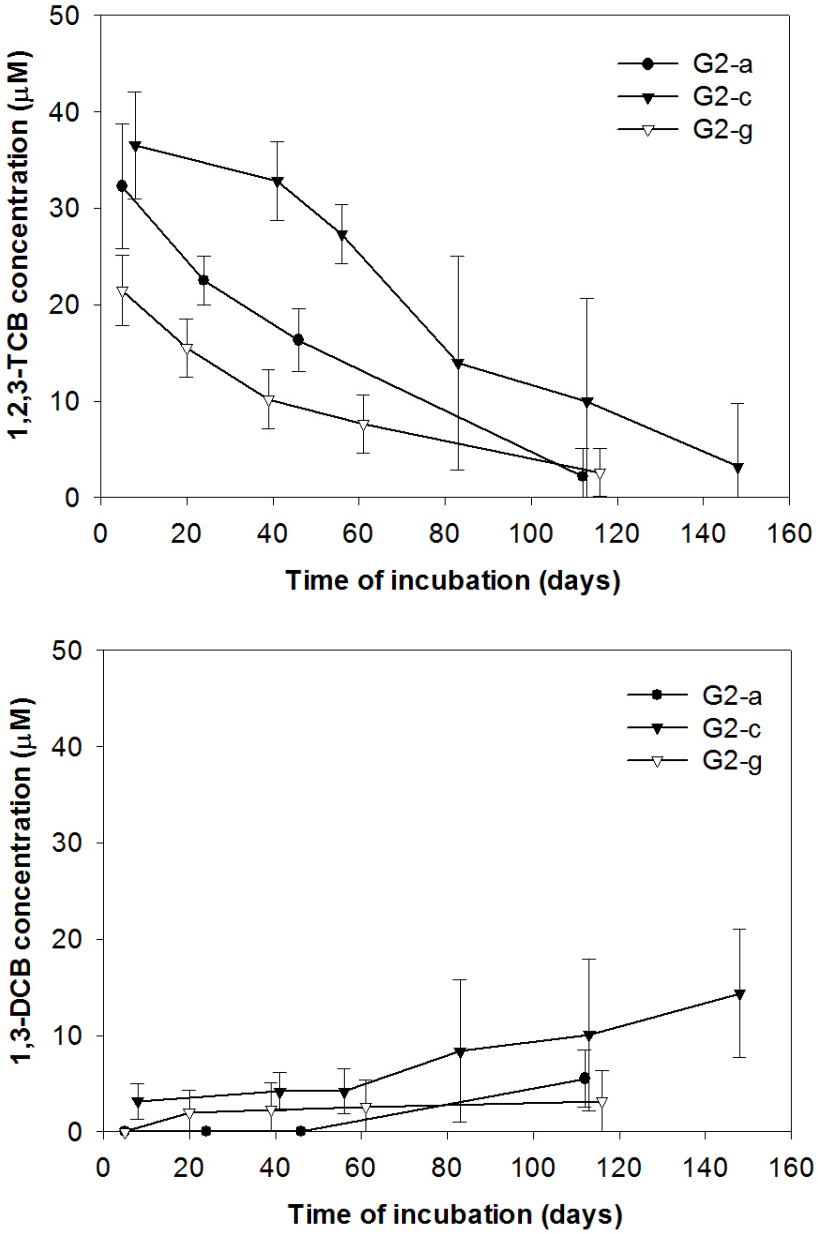


Figure 26. Time-course of 1,2,3-TCB transformation (upper panel) and 1,3-DCB formation (lower panel) in 24 G2 subcultures. G2–a and –c are each the mean of concentration values coming from nine parallel subcultures reduced with titanium (III) citrate ± SD. G2–g are the mean of concentration values of six parallel subcultures reduced with sodium sulphide and L-cysteine ± SD.

G3 subcultures were reduced with titanium (III) citrate or sodium sulphide plus L-cysteine. The G3 subcultures reduced with sodium sulphide plus L-cysteine were inoculated with 9% (v/v) of a G2 subculture which was previously reduced with titanium (III) citrate to investigate if a change of reducing agent may affect dechlorination. No active G3 subculture reduced with sodium sulphide and L-cysteine was observed in any of the triplicates prepared. Sixteen G3 subcultures were prepared and reduced with titanium (III) citrate and seven of them were active. Out of those seven subcultures, five and two, respectively, were parallel subcultures. The five G3 parallel subcultures formed 1,3-DCB after 21 (three subcultures) and 42 (two subcultures) days of incubation. The two G3 parallel subcultures formed 1,3-DCB within 85 and 134 days of incubation. One of each parallels (a total of two G3 subcultures) were further transferred to fresh medium. A total of eight G4 subcultures were prepared and two of them were active after 149 and 274 days of incubation. A further transfer, G5 triplicate subcultures reduced with titanium (III) citrate, was additionally prepared. During the monitoring time of 167 days, no formation of 1,3-DCB was observed. Due to time limitations, the G5 subcultures could not be further monitored.

G2 and G3 subcultures (inoculated with 12% v/v from G1–b and –f, and G2–c and –g) either reduced with titanium (III) citrate or with sodium sulphide and L-cysteine were tested with hexachlorobenzene (HCB) instead of 1,2,3-TCB to investigate if the subcultures may further transform higher chlorinated compounds. However after 337 days of incubation, no lower halogenated products (1,2,3-TCB or 1,3-DCB) were observed in any of the six subcultures tested.

In addition, it was investigated if hydrogen (1 bar overpressure) and acetate (5 mM) affected organohalide transformation. For that, 32 subcultures (either G2 or G3) were prepared amended with 60 µM of 1,2,3-TCB solved in acetone. From those, eight were amended with hydrogen and acetate, eight with acetate alone, eight with hydrogen alone, and eight without hydrogen and acetate. After 253 days of incubation, 1,3-DCB was observed in five of the subcultures. One of the eight parallel subcultures amended only with hydrogen, one of the subcultures with neither hydrogen nor acetate, and three of the subcultures amended only with acetate showed 1,3-DCB formation (Table 10).

Table 10. Transformation of 1,2,3-TCB to 1,3-DCB by G2 and G3 subcultures after 253 days of incubation in the presence or absence of hydrogen and/or acetate. "+" refers to culture amended with either acetate (Ac) or hydrogen (H_2), and "–" to non-amended.

Culture generation	Number of cultures per treatment	Number of cultures which transformed 1,2,3-TCB			
		Ac+/H_2+	Ac+/H_2–	Ac–/H_2+	Ac–/H_2-
G2	6	0	3	1	1
G3	2	0	0	0	0

3.2.3 Quantification of total *Bacteria* and known organohalide-respiring bacteria by qPCR

Three parallel G0 cultures were selected for the quantification of total *Bacteria* and other known organohalide-respiring bacteria, i.e., *Dehalococcoidia*, *Dehalobacter*, and *Desulfitobacterium*. These G0 cultures were G0–a, –b, and –c, which were reduced with titanium (III) citrate. Bacterial quantification was performed at various time points of incubation (i.e., 3, 36, 74, and 238 days) while dehalogenation of 1,2,3-TCB occurred, except for G0–b (Figure 21). The formation of 1,3-DCB was observed after 270 days of incubation for the culture G0–b.

After three days of incubation, the average number (± SD) of total bacterial 16S rRNA gene copies was $1.2 \times 10^7 \pm 2.9 \times 10^6$ ml^{-1} for the triplicate G0 cultures. Bacterial 16S rRNA copies decreased with incubation time to $2.6 \times 10^6 \pm 2.2 \times 10^6$, $6.8 \times 10^6 \pm 2.9 \times 10^6$, and $1.9 \times 10^6 \pm 4.6\ 10^5$ ml^{-1}, for the time points of 36, 74, and 238 days, respectively (Figure 27).

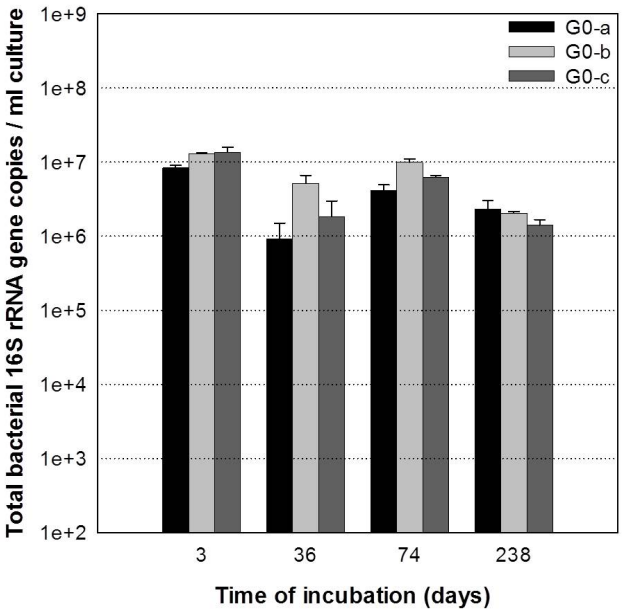


Figure 27. Total bacterial 16S rRNA gene copy numbers in G0 cultures reduced with titanium (III) citrate. Three parallel G0 cultures are shown. G0–a and G0–c completely dehalogenated 1,2,3-TCB within 238 days. Shown are means of qPCR triplicate measurements ± SD.

In the G0 cultures, *Dehalococcoidia* 16S rRNA genes were amplified with both the primer pairs DEH-Fa and DEH-R (matching to most *Dehalococcoidia*), and Dehal-F5 and Dehal-R (matching to Chile-specific *Dehalococcoidia*) (Table 7), although at low copy numbers for both primer pairs. The average number of *Dehalococcoidia* 16S rRNA gene copies of $2.8 \times 10^3 \pm 1.3 \times 10^3$ ml^{-1} (amplified with DEH-Fa and DEH-R) was observed in triplicate G0 cultures after three incubation days. *Dehalococcoidia* 16S rRNA gene copies decreased with time to average values of $5.3 \times 10^2 \pm 9.1 \times 10$, $6.5 \times 10^2 \pm 1.8 \times 10^2$, and $3.5 \times 10^2 \pm 1.6 \times 10^2$, for the time points of 36, 74, and 238 days of incubation, respectively (Figure 28).

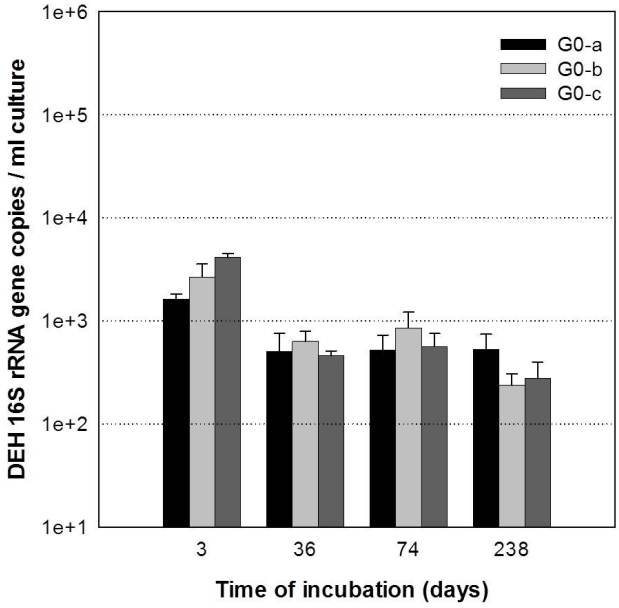


Figure 28. *Dehalococcoidia* 16S rRNA gene copy numbers in G0 cultures reduced with titanium (III) citrate amplified the primer pair DEH-Fa and DEH-R targeting most *Dehalococcoidia*. Three parallel G0 cultures are shown. G0–a and G0–c completely dehalogenated 1,2,3-TCB within 238 days. Shown are means of qPCR triplicate measurements ± SD.

The proportion of *Dehalococcoidia* 16S rRNA gene copies relative to total bacterial 16S rRNA gene copies in G0 parallel cultures was ~0.02%, which did not increase with time meanwhile dehalogenation was taking place (Table 11).

Table 11. Proportion of *Dehalococcoidia* 16S rRNA gene copies relative to total bacterial 16S rRNA gene copies in triplicate G0 cultures at different time points is shown.

	Time of incubation (days)			
Culture	3	36	74	238
G0-a	0.02%	0.05%	0.01%	0.02%
G0-b	0.02%	0.01%	0.01%	0.01%
G0-c	0.03%	0.02%	0.01%	0.02%

G1 and G2 subcultures were also monitored for *Dehalococcoidia* copy numbers. In the qPCR assay, amplification was obtained for G1 and G2 with both the primer pairs (DEH-Fa and DEH-R; Dehal-F5 and Dehal-R). However, the melt-curves from the qPCR assay revealed no clear peaks indicative of specific amplification, and further visualization of these amplicons by agarose gel electrophoresis revealed no clear bands. Together, these results indicated that the primers amplified non-specifically for G1 and G2.

In addition, quantification of 16S rRNA gene copies from *Dehalobacter* and *Desulfitobacterium* was performed using the primers Dre441F and Dre645R_Ch, and DSB406F and DSB619R (Table 7), respectively, in the triplicate G0 cultures. For the quantification of 16S rRNA gene copy numbers, no standard for both *Dehalobacter* and *Desulfitobacterium* was run in the qPCR assay due to lack of cloned 16S rRNA genes of *Dehalobacter* and *Desulfitobacterium.* In Table 12, the values for the threshold cycle (Ct) for the triplicate G0 cultures is shown as a proxy to indicate *Dehalobacter* and *Desulfitobacterium* increase or decrease with time. As both *Dehalobacter* and *Desulfitobacterium* qPCR assays were performed in the same qPCR run, Ct values can be compared among the samples. The qPCR assay revealed that *Dehalobacter* 16S rRNA gene copies decreased with time (Ct values increased with time, Table 12) in G0–a and –c cultures, which dehalogenated 1,2,3-TCB during the time span of 3–238 days of incubation (Figure 21). The G0–b culture showed an increase in *Dehalobacter* 16S rRNA gene copies after 36 and 74 days compared to the Ct value of 3 days (Table 12). However, G0–b did not dehalogenated 1,2,3-TCB during the first 74 days (Figure 21). Thus, *Dehalobacter* increase cannot be associated to dehalogenation in G0–b. *Desulfitobacterium* 16S rRNA gene copies in G–a increased with time with respect of the first time point of 3 days. However, for G–b and –c, *Desulfitobacterium* copies remained stable with time (Table 12).

Table 12. qPCR threshold cycles (Ct) for each sample amplified with primers targeting the 16S rRNA gene of *Dehalobacter* and *Desulfitobacterium.* Shown are mean of duplicate values for Ct ±SD run in the same qPCR assay. Triplicate G0 cultures at different time points of incubation were analysed together with a sediment sample from Chile, site 7155 (at a depth of 437–442 cmbsf), and a negative control (1 µl water as template). Samples with Ct values ≤29 indicate abundant 16S rRNA gene copies, meanwhile Ct values of 38–40 indicate few or no16S rRNA gene copies

Sample		*Dehalobacter*	*Desulfitobacterium*
		Ct mean ± SD	**Ct mean ± SD**
Sediment[1]		24.7 ± 0.1	27.5 ± 0.1
Negative Control		37.0 ± 0.1	35.5 ± 0.1
G0-a	3 days	30.0 ± 0.2	26.2 ± 0
	36 days	31.7 ± 0	23.0 ± 0.1
	74 days	32.8 ± 0	24.0 ± 0.1
	238 days	35.0 ± 0.2	25.3 ± 0
G0-b	3 days	32.4 ± 0.3	18.5 ± 0
	36 days	28.1 ± 0.2	19.9 ± 0
	74 days	29.4 ± 0.2	20.8 ± 0.1
	238 days	31.4 ± 0.7	21.1 ± 0.3
G0-c	3 days	30.7 ± 0.1	19.4 ± 0.2
	36 days	31.7 ± 0.2	21.2 ± 0.1
	74 days	32.8 ± 0.3	21.7 ± 0.1
	238 days	33.0 ± 0.2	21.7 ± 0

[1] 1 µl DNA template was used from DNA isolated from Chile site 7155.

No amplification of known reductive dehalogenase genes was possible with the various primers either for *Dehalococcoides mccartyi* reductive dehalogenase genes *rdhA* (primers RRF2 and B1R; RDH F1C and RDH R1C, Table 7) or for *Dehalobacter-Desulfitobacterium rdhA* genes (primers dehaloF3 and dehaloR2; dehaloF5 and dehaloR4, Table 7).

3.2.4 Bacterial community changes in enrichment cultures studied by 454-pyrosequencing

Several cultures from different enrichment stages (G0, G2, and G4) were selected together with a sediment sample from Chile site 7155 (depth of 437–442 cmbsf) to study the development of the microbial community by using 454-pyrosequencing. Various samples were taken at different time points as specified in Table 13. For the case of titanium (III) citrate-reduced cultures: G0–c was prepared with 9% of sediment inoculum and completely transformed 1,2,3-TCB to 1,3-DCB within 58 days of

incubation (Figure 21); G2 and G4 subcultures transformed 1,2,3-TCB within 53 and 149 days, respectively (Table 13). For the case of sodium sulphide and L-cysteine-reduced cultures: G0–g was prepared with 6% of sediment inoculum and formed 1,3-DCB within 42 days, and depleted 1,2,3-TCB after 58 days (Figure 22). The G2 subculture formed 1,3-DCB within 20 days. G2 had 5 μM of 1,2,3-TCB after 61 days when sampled.

Table 13. Samples used for the study of the microbial community by 454-pyrosequencing.

Reducing agent in culture	none	titanium (III) citrate			Na_2S and L-cysteine	
Sample origin	sediment	G0–c	G2	G4	G0–g	G2
Time 1,2,3-TCB depletion (days of incubation)	n.a.	58	53	149	58	>61[k]
Time points of sampling for 454-pyrosequencing (days of incubation when sampled)	n.a.	3[a] 36[a] 74[a] 238[a]	78[a]	1[b] 31[b] 149[b]	3[a] 36[a] 74[a] 238[a]	4[a] 61[a]

n.a. – not applicable; [a] A sample of 1 ml was withdrawn from the culture [b] A sample of 1.5 ml was withdrawn from the culture. [k]Complete depletion of 1,2,3-TCB in the culture could not be monitored as it was transferred after 65 days.

The microbial community was studied by amplifying bacterial and archaeal 16S rRNA genes with two sets of primers (Table 14). Five replicates from parallel PCRs were pooled for each sample. The total number of bacterial and archaeal sequences obtained after the 454-pyrosequencing are detailed in Table 14.

Table 14. Sequences obtained from the 454-pyrosequencing of sediment and sediment cultures.

Primer pair[1]	**Target group**	**Size of amplified PCR product (bp)**	**Average size of sequence after trimming[2] (bp)**	**Total number of sequences**	**Total number of sequences per Domain**
27F and 519R	*Bacteria*	599	300	44019	44019
U789F and U1068R	*Bacteria* and *Archaea*	373	286	28202	*Archaea*: 26
					Bacteria: 28176

[1]Sequences are detailed in Table 7; [2] The trimming was done for quality control purposes of the sequences with the program *mothur* as described in the Material and Methods section.

The results from samples amplified with the primers 27F and 519R are described in this section. Those amplified with the universal primers, U789F and U1068R, are described in the next section (3.2.5).The number of bacterial sequences obtained in total and for the phyla *Firmicutes*, *Proteobacteria*, and *Chloroflexi* for each sample is shown in

Table 15.

Table 15. Number of sequences obtained for total *Bacteria*, phyla *Firmicutes*, *Proteobacteria*, and *Chloroflexi* after 454-pyrosequencing as classified by *mothur* in samples of sediment and sediment cultures.

Sample		**Number of sequences, total *Bacteria***	**Number of sequences, phylum *Firmicutes***	**Number of sequences, phylum *Proteobacteria***	**Number of sequences, phylum *Chloroflexi***
sediment		2895	7	2884	0
TiCi[a]	G0 – 3 days	2430	1767	595	7
	G0 – 36 days	636	596	31	0
	G0 – 74 days	1006	902	28	0
	G0 – 238 days	1485	1334	71	1
	G2 – 78 days	3999	3999	0	0
	G4 – 1 day	8610	8601	7	0
	G4 – 31 days	7059	7059	0	0
	G4 – 149 days	2657	2657	0	0
Na_2S[b]	G0 – 3 days	2759	684	2029	6
	G0 – 36 days	1199	1084	91	0
	G0 – 74 days	708	659	32	2
	G0 – 238 days	293	279	8	0
	G2 – 4 days	360	144	158	0
	G2 – 61 days	2924	2233	280	0

[a] Titanium (III) citrate-reduced cultures; [b] Na_2S and L-cysteine-reduced cultures.

The bacterial community structure experienced a clear shift from a dominance of the phylum *Proteobacteria*, class *Gammaproteobacteria,* in the sediment, to a dominance of the phylum *Firmicutes* in the cultures, either reduced with titanium (III) citrate or with sodium sulphide and L-cysteine.

A shift to 75% relative abundance to the *Firmicutes* occurred within three days of incubation in the G0 culture reduced with titanium (III) citrate (Figure 29). The dominance of *Firmicutes* was maintained in further subcultures (G2 and G4), where the 100% of the bacterial community was affiliated with the *Firmicutes*. Other phyla such as *Deinococcus-Thermus*, *Actinobacteria*, *Aquificae*, and *Bacteroidetes* were also present in the G0 culture, although at considerably lower relative abundance (≤ 5%) than *Proteobacteria* and *Firmicutes*. The phylum *Deinococcus-Thermus* was the third most abundant phyla, with a maximum relative abundance of 5% at the time point of 74 days of incubation (Figure 29).

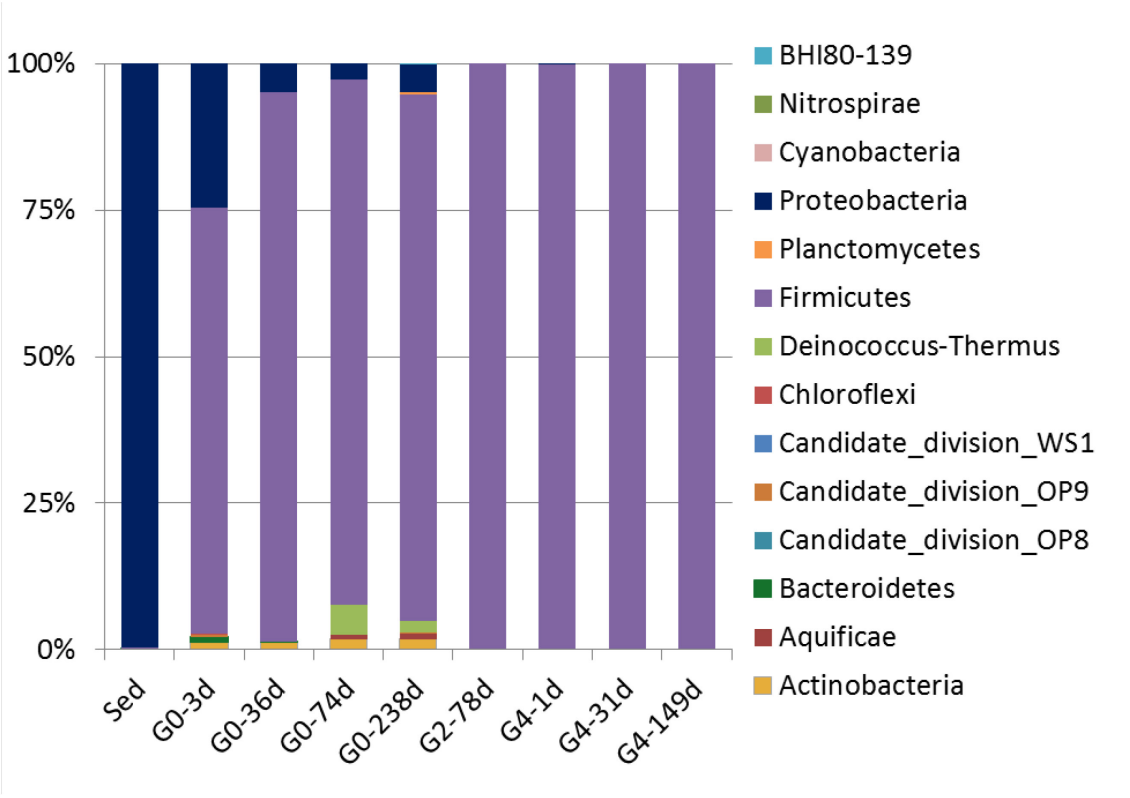


Figure 29. Bacterial community analysis of titanium (III) citrate-reduced cultures. Bacterial community structure is shown as relative proportions of phyla in sediments from Chile, site 7155 ("Sed"), and in sediment culture G0 and subcultures G2 and G4 from the Chile sediment. The labels indicate the subculture (see Figure 23) and the incubation time in days.

Higher diversity of *Firmicutes* was observed in G0 culture than in G2 or G4 subcultures, with members affiliated to the genera *Gracilibacter, Heliorestis, Desulfotomaculum, Thermincola, Caldalkalibacillus,* and *Geosporobacter* being present (Figure 30). In G0, a strong shift was observed for the first 36 days of incubation. After 36 days of incubation, a stable bacterial community was established with variations only in the relative abundance of the different genera (Figure 30). In subcultures G2 and G4, *Anaerobacter* sequences were dominant, forming 90% of the bacterial community (Figure 30). The remaining 10% of the sequences were affiliated with the *Clostridium, Desulfosporosinos, Tepidanaerobacter* and *Gracilibacter*.

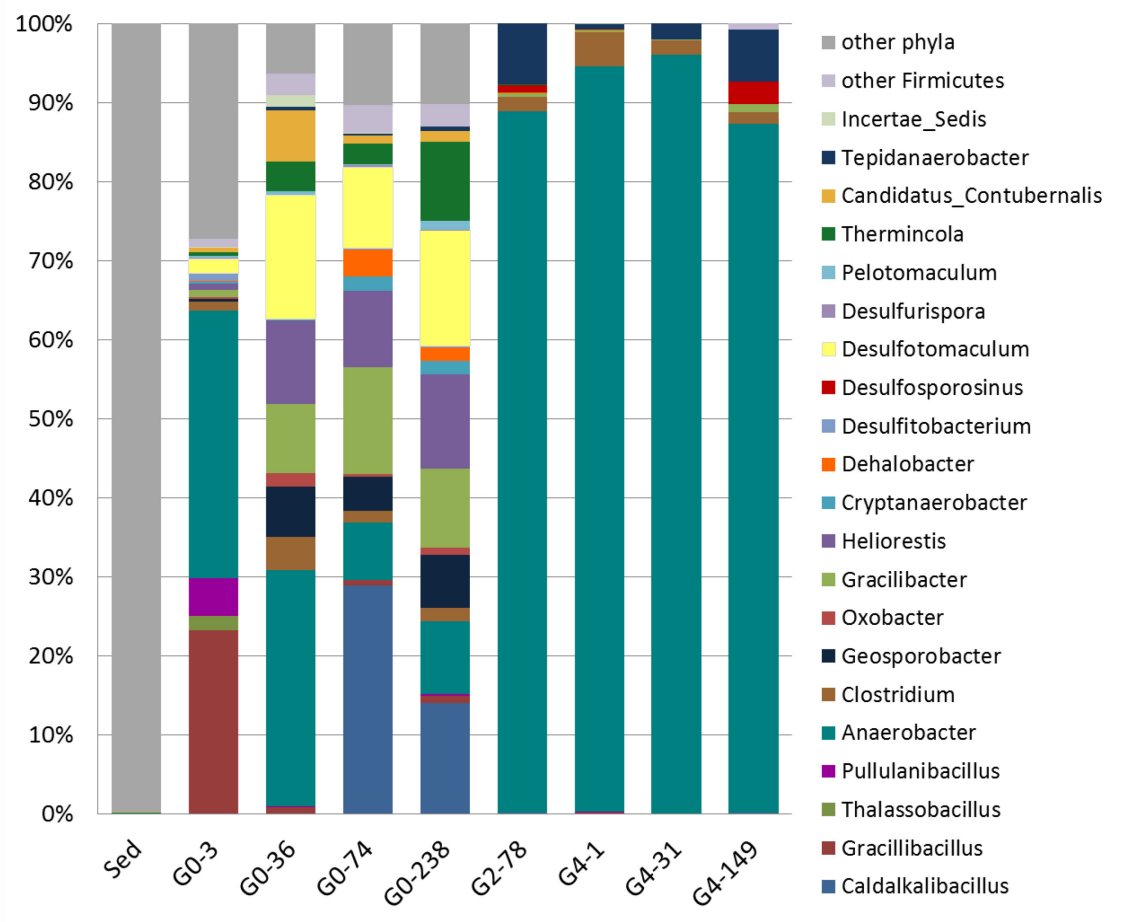


Figure 30. Relative abundance of *Firmicutes* in cultures reduced with titanium (III) citrate and sediments from Chile, site 7155. Shown proportions are at the genus level for the phylum *Firmicutes*. Phyla other than the *Firmicutes* are indicated by the "other phyla" proportion in the stacked-bar section in grey. The proportion for "other Firmicutes" refers to genus within the *Firmicutes* at presence lower than 1%. For details on the labels and the cultures see the legend of Figure 29.

More in depth analysis of sequences from within the phylum *Firmicutes* showed several sequences closely associated phylogenetically to known organohalide-respiring microorganisms such as *Dehalobacter* and *Desulfitobacterium* to be present in the sediment cultures. The relative abundance of *Dehalobacter* sequences in G0 increased with time from 0.1% and 0% on days 3 and 36, respectively, to 3% on day 74, when complete 1,2,3-TCB transformation had occurred and the culture had further been amended with 40 µM 1,2,3-TCB on day 65. However, *Dehalobacter* sequences decreased to 2% on day 238 (Table 16). The number of *Dehalobacter* sequences was very low or even below the detection limit in further G2 and G4 subcultures (Table 16 and Figure 30). *Desulfitobacterium* sequences were detected in G0, however at very low relative abundance which did not increase with time. In subcultures G2 and G4, no *Desulfitobacterium* sequences were detected (Table 16).

Table 16. Relative abundance of various genera within the family *Peptococcaceae* in sediment and cultures reduced with titanium (III) citrate from Chile site 7155. The relative abundance of each genus with respect to whole bacterial community is shown. Sequences were obtained after 454-pyrosequencing and classified by *mothur* in each sample using the primer pair 27F and 519R. Cultures G0 and G2 are shown at different time points as stated in Table 13.

Sample ➔	**Sediment**	**G0**				**G2**	**G4**		
Incubation days ➔	**-**	**3**	**36**	**74**	**238**	**78**	**1**	**31**	**149**
Dehalobacter	0%	0.1%	0%	3%	2%	0.1%	0%	0%	0%
Desulfitobacterium	0%	0.9%	0%	0.2%	0.1%	0%	0%	0%	0%
Desulfosporosinus	0%	0.1%	0%	0%	0.1%	1%	0%	0%	3%
Desulfotomaculum	0%	2%	16%	10%	15%	0%	0%	0%	0%
Desulfurispora	0%	0.2%	0%	0.3%	0.1%	0%	0%	0%	0%
Pelotomaculum	0%	0.2%	0.5%	0.1%	1%	0%	0%	0%	0%
Thermincola	0%	0.4%	4%	3%	10%	0%	0%	0%	0%

For cultures reduced with sodium sulphide and L-cysteine, *Firmicutes* also took over as the dominant bacterial phylum (Figure 31). This shift to *Firmicutes* dominance happened at a slower rate than for those cultures reduced with titanium (III) citrate, with 25% of the bacterial community belonging to *Firmicutes* after three days of incubation (Figure 31). After 36 days of incubation, 90% of the bacterial total community belonged to members of the phylum *Firmicutes* (Figure 31).

In the G2 subcultures, the bacterial community evolved differently to the community in cultures reduced with titanium (III) citrate, with other bacterial phyla apart from *Firmicutes* also being detected. G2 subcultures showed proportions of the phylum *Proteobacteria* of 44% and 10% at time points of 4 and 61 days of incubation, respectively. In these G2 subcultures, the phylum *Bacteroidetes* increased from 2% to 10% for the period of time between 4 to 61 days.

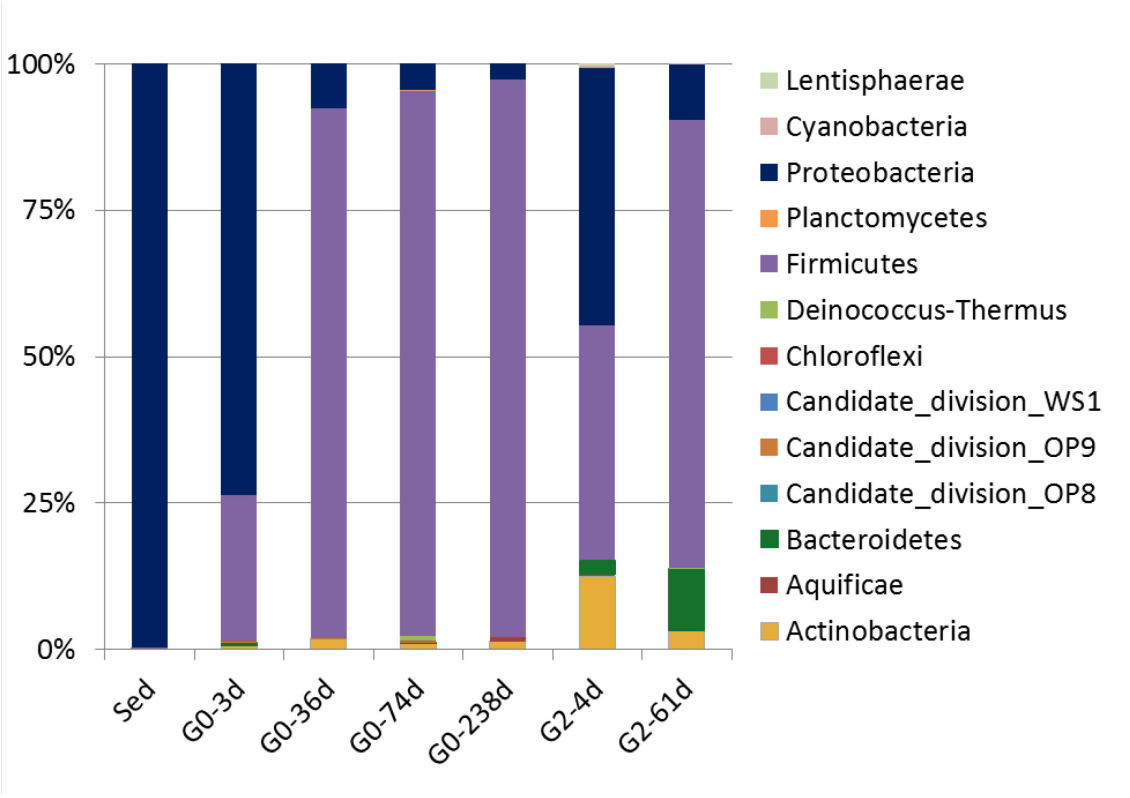


Figure 31. Bacterial community analysis of sodium sulphide plus L-cysteine-reduced cultures. Bacterial community structure is shown as relative proportions of phyla in sediments from Chile, site 7155 ("Sed"), and in culture G0 and subculture G2 from the Chile sediment. The labels indicate the subculture (see Figure 23) and the incubation time in days.

The diversity within the phylum *Firmicutes* in the sulphide-reduced sediment cultures was different to the titanium (III) citrate-reduced sediment cultures. Most importantly, very few *Anaerobacter* members were observed in either G0 or G2 cultures. High proportions of *Desulfosporosinus* were present in G0 (36–238 days of incubation) and G2 cultures, together with *Gracillibacter, Gracillibacillus,* and *Cryptaenobacter* as most dominant genera (Figure 32).

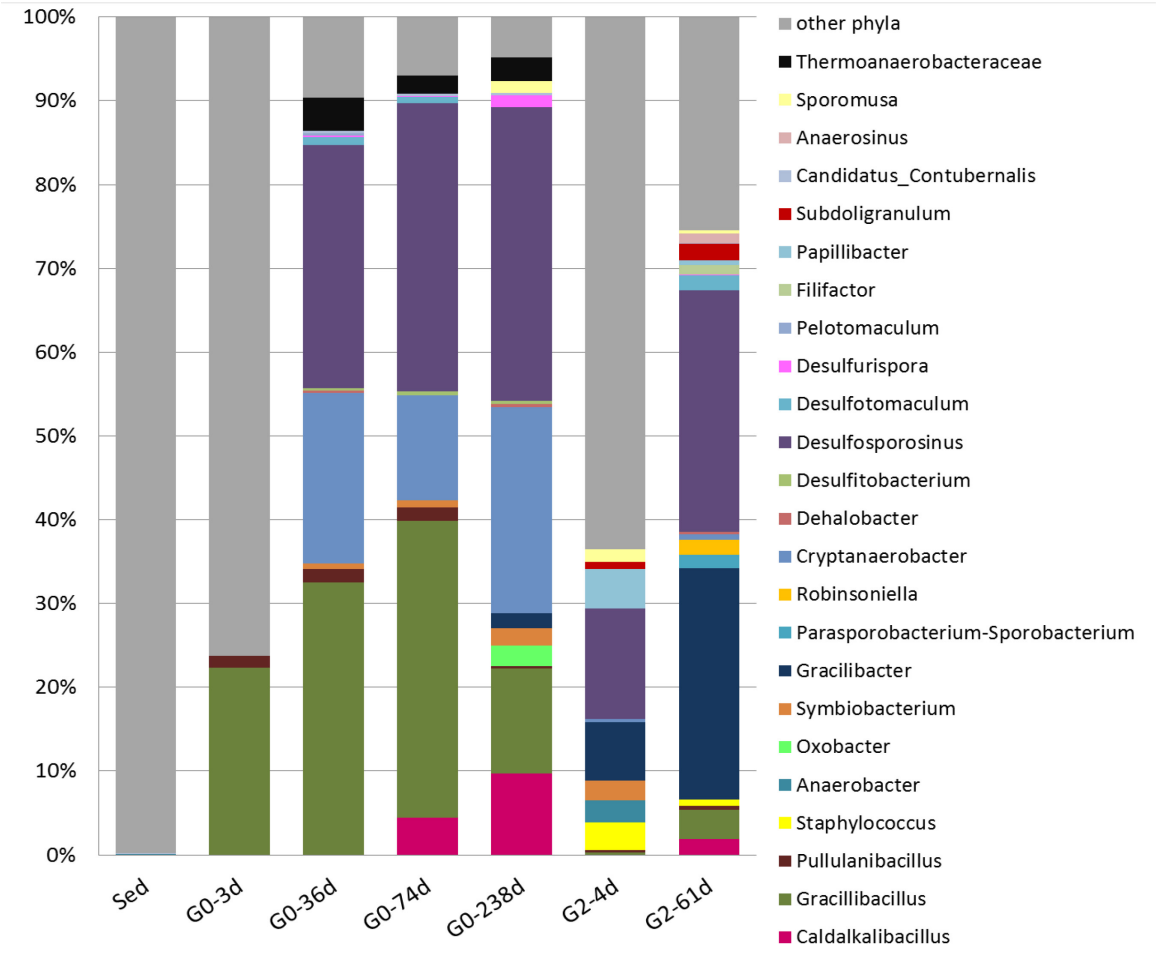


Figure 32. Relative abundance of *Firmicutes* in cultures reduced with sodium sulphide and L-cysteine and sediments from Chile, site 7155. Shown proportions are at the genus level for the phylum *Firmicutes.* Phyla other than the *Firmicutes* are indicated by the "other phyla" proportion in the stacked-bar section in grey. The proportion for "other Firmicutes" refers to genus within the *Firmicutes* at presence lower than 1%. For details on the labels and the cultures see the legend of Figure 31.

More in depth analysis of sequences from the family *Peptococcaceae* (phylum *Firmicutes*), indicated the presence of *Dehalobacter* and *Desulfitobacterium* sequences at very low relative abundance (Table 17). A stable enrichment in *Desulfosporosinus* was achieved in the G0 culture with time from 0% to 34% relative abundance after 3 and 74–238 incubation days, respectively, and further on, in subculture G2 (Table 17).

Table 17. Relative abundance of various genera within the family *Peptococcaceae* in sediment and cultures reduced with sodium sulphide and L-cysteine from Chile site 7155. The relative abundance of each genus with respect to whole bacterial community is shown. Sequences were obtained after 454-pyrosequencing and classified by *mothur* in each sample using the primer pair 27F and 519R. Cultures G0 and G2 are shown at different time points as stated in Table 13.

Sample ➔	**Sediment**	**G0**				**G2**	
Incubation days ➔	**-**	**3**	**36**	**74**	**238**	**4**	**61**
Dehalobacter	0%	0%	0.3%	0%	0.3%	0%	0.3%
Desulfitobacterium	0%	0%	0.2%	0.4%	0.3%	0%	0%
Desulfosporosinus	0%	0%	29%	34%	34%	12%	27%
Desulfotomaculum	0%	0%	1%	0.7%	0%	0%	2%
Desulfurispora	0%	0%	0.2%	0.1%	1%	0%	0.1%
Pelotomaculum	0%	0%	0.2%	0%	0%	0%	0%
Thermincola	0%	0%	0%	0%	0.3%	0%	0%

3.2.5 Microbial community study with universal primers in the enrichment cultures using 454-pyrosequencing

Samples from the same G0, G2, and G4 cultures as for the bacterial-specific community study were used together with a sediment sample from Chile, site 7155 (depth of 437–442 cmbsf) (Table 13) for the amplification of 16S rRNA genes with the universal primers U789F and U106R, which targets both *Archaea* and *Bacteria*.

Less time points compared to the bacterial-specific community study (amplified with primers 27F and 519R) were selected, and related to an incubation time when 1,2,3-TCB was about to be depleted. These time points were 36 and 238 incubation days for G0, 149 days for G4 in cultures reduced with titanium (III) citrate, and 238 days for G0 and 173 days for G2 in cultures reduced with sodium sulphide and L-cysteine.

The sequences amplified with the universal primers belonged mostly to *Bacteria* (Table 14). Archaeal sequences were only found in G0 cultures reduced with titanium (III) citrate making up 0.31% of the total microbial sequences. No archaeal sequences were amplified in sediment cultures reduced with sodium sulphide and L-cysteine, as well as in the sediment sample. *Archaea* were therefore most likely not associated to the transformation of 1,2,3-TCB in sediment cultures.

The bacterial sequences amplified with the universal primers also confirmed the shift from phylum *Proteobacteria* in sediment to phylum *Firmicutes* in the cultures as previously observed (Figure 33). However, there were minor differences, especially that there was a larger contribution of *Nitrospirae* sequences in G0 after 238 incubation days than previously observed, and the *Proteobacteria* sequence contribution was also lower for cultures reduced with sodium sulphide and L-cysteine than previously observed.

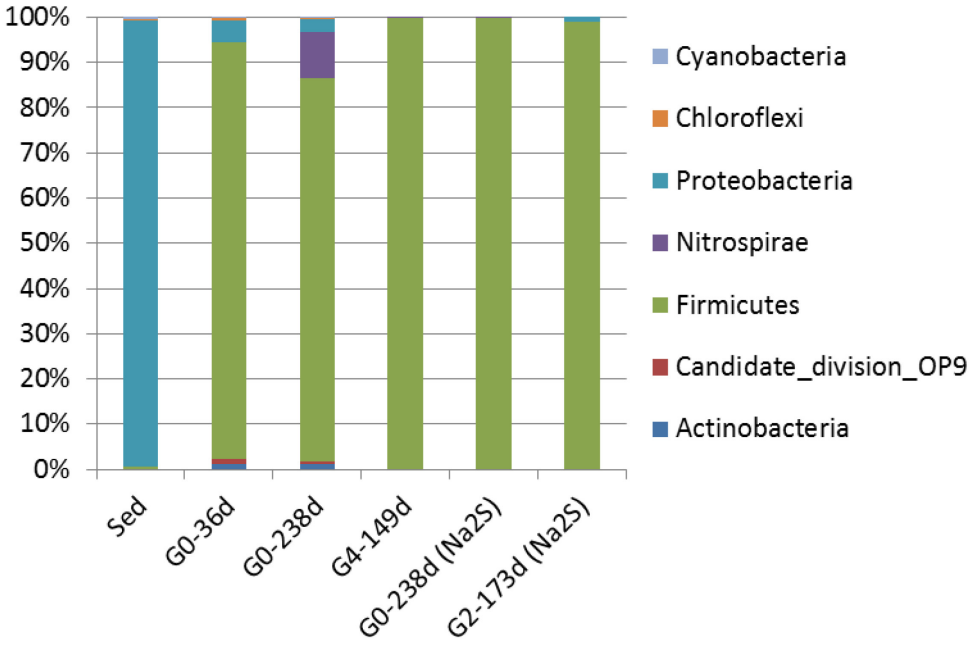


Figure 33. Bacterial relative phyla abundance of samples amplified with universal primers in sediment and cultures from Chile, site 7155. G0 and G4 are cultures reduced with titanium (III) citrate and G0 and G2 are cultures reduced with sodium sulphide plus L-cysteine. The labels indicate the subculture (see Figure 23) and the incubation time in days (see Table 13). Those cultures reduced with sodium sulphide and L-cysteine are indicated with "(Na_2S)".

Within the *Firmicutes*, large relative shares belonged to *Gracilibacter* (in G0), *Tepidanaenobacter* (in G4), and *Sporomusa* (in G0 and G2 cultures reduced with sodium sulphide and L-cysteine) in contrast to the bacterial-specific community analyses (Figure 34).

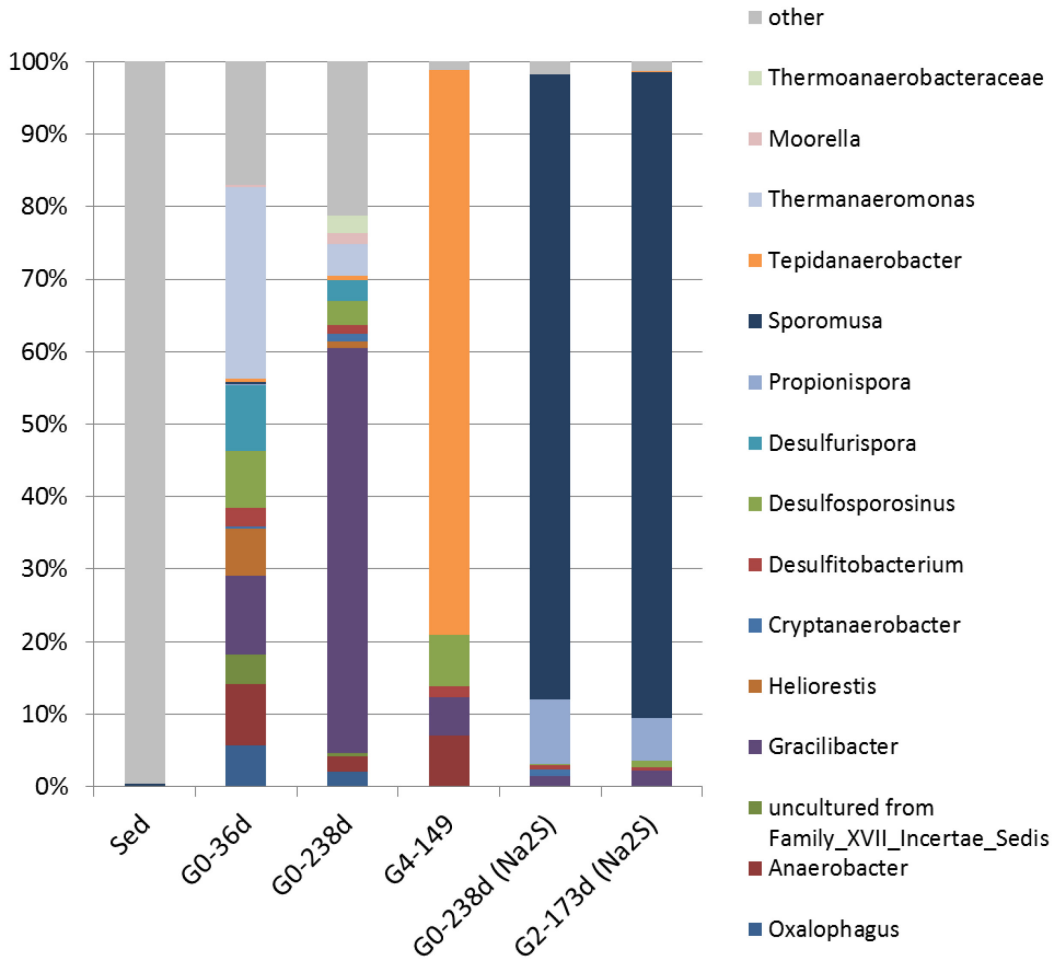


Figure 34. Relative abundance of *Firmicutes* in samples amplified with universal primers. Shown proportions are at the genus level for the phylum *Firmicutes* from cultures reduced with sodium sulphide plus L-cysteine set up with sediments from Chile site 7155. Phyla other than the *Firmicutes* are indicated by the "other phyla" proportion in the stacked-bar section in grey. The labels indicate the subculture (see Figure 23) and the incubation time in days (see Table 13). Those cultures reduced with sodium sulphide and L-cysteine are indicated with "(Na_2S)".

Among phylotypes that were present in all samples are *Desulfosporosinus* and *Desulfitobacterium* (Figure 34 and Table 18). The relative abundance of *Desulfosporosinus* was however, less than in the bacterial-specific community analyses. In addition, no *Dehalobacter* sequences were observed, in contrast to the bacterial-specific community analyses (Figure 34 and Table 18).

Table 18. Relative abundance of sequences amplified with universal primers and affiliated to specific genera within the family *Peptococcaceae* in sediment and cultures from Chile site 7155. The relative abundance of each genus with respect to whole bacterial community is shown.

Reducing agent	none	Titanium (III) citrate			Sodium sulphide and L-cysteine	
Sample ➔	**Sediment**	**G0**		**G4**	**G0**	**G2**
Incubation days ➔	**-**	**36**	**238**	**149**	**238**	**173**
Dehalobacter	0%	0%	0%	0%	0%	0%
Desulfitobacterium	0%	2%	1%	1%	0.5%	0.4%
Desulfosporosinus	0%	8%	3%	7%	0.2%	1%
Desulfotomaculum	0%	0.1%	0.1%	0%	0%	0%
Desulfurispora	0%	9%	3%	0%	0%	0%
Pelotomaculum	0%	1%	0.1%	0%	0.1%	0%
Sporomusa	0.3%	0.2%	0%	0%	84%	88%

3.3 BAFFIN BAY GEOCHEMISTRY AND MICROBIAL COMMUNITIES

This chapter describes the *in situ* study performed in sediments of the Baffin Bay, in the Arctic. The goal of this study was to gain insights into the biogeochemistry and microbial communities which may drive the major element cycles (i.e., carbon, sulphur, iron, manganese) in sediments of the Baffin Bay. For that purpose, sediments were cored at 34 sites, as previously described (section 2.2). Ten sites were selected based on the site location and the recovery of the core after sampling (highest recovery length of 4.69 m), and further sampled for the site- and depth-dependent geochemistry and microbiology study (described in section 2.2.2). These ten sites were from various locations of the Greenlandic part of the Baffin Bay, which included the continental shelf, slope and basin areas. Sites 363, 365, 371 belonged to the Northern continental shelf (Figure 4 and Figure 5). Site 383 was located in between the shelf and the beginning of the continental slope (Figure 5). Site 387 belonged to the continental slope (Figure 5). Sites 389, 391, and 453 were located at the central deep basin, and sites 486 and 488 were at a Southern continental slope (Figure 4 and Figure 5). In this chapter, the geochemistry (including the geology, and the solid- and interstitial-phase geochemistry of sediments; section 3.3.1), and microbiology (microbial ecology deciphered with molecular biology techniques; section 3.3.2) of the cores from the selected ten sites of the Baffin Bay are described.

3.3.1 Geochemistry of Baffin Bay cores

An overview of the oceanographic data of the ten investigated sites and the corresponding sediment recoveries of each core is presented in Table 19.

Table 19. Oceanographic data of the selected sites in the Baffin Bay investigated in this study. The corresponding core recovery after the sediment sampling at each site is presented as "core depth". Apart from the geochemistry analysis, each core was further analysed for either one or two microbiology studies, named study A (including three geographically distinct areas: shelf, basin, and Southern slope), and study T (including sites located along a transect from shelf to basin).

Site	Area	Study	Latitude	Longitude	Water Depth (m)	Core depth (m)
363	Shelf	A & T	76° 52.92' N	71° 34.01' W	938	4.69
365	Shelf	T	76° 39.04' N	71° 18.79' W	658	3.67
371	Shelf	A & T	75º 58.24' N	70° 34.86' W	598	4.05
383	Shelf-slope	T	75º 17.69' N	69º 53.75' W	674	2.32
387	Slope	T	74° 50.42' N	69º 27.14' W	1,300	3.32
389	Basin	A & T	74° 37.05' N	69º 13.75' W	1,716	4.24
391	Basin	A & T	74° 23.36' N	69º 01.22' W	1,864	4.27
453	Basin	A	73° 19.37' N	64° 58.11' W	2,300	4.69
486	S Slope[a]	A	72° 24.51' N	60° 48.85' W	645	4.69
488	S Slope[a]	A	72° 08.80' N	60° 58.86' W	1,493	4.69

[a]Southern Slope

The geology of the cores varied among the different sites (see core photos in the Appendix 2, section 6.2) indicating changes in the local conditions. In general, many dropstones were found in the cores as a result of glacial transport.

Cores from sites 363 and 371 had similar sediment composition of olive-grey silty muds along the entire core length, except in deeper parts where a change in colour to greyish-brown occurred. In deeper sections of the core from site 371 (346–395 cmbsf), sediments became coarser (sandy and silty mud with dropstone granules) and changed to a dark-brown colour.

Shelf site 365 had olive-grey silty muds in the core section from the surface down to ~200 cmbsf. At increased depths, site 365 sediment became gradationally coarser to silty muds with sand granules (200–274 cmbsf) and to muddy sands (274–322 cmbsf). A gradational change of colour from the olive-grey to brownish-grey was observed at 150 cmbsf for site 365, which turned to greyish-red (185–192 cmbsf), and to a distinct red colour lamination (247–260 cmbsf), and finally, to brown in deeper sediments (300 to circa 322 cmbsf).

The core from site 383, a transition site from shelf to slope areas, was composed of olive–grey silty muds in surface sediments, which turned to brown silty muds (core sections of 46–57 cmbsf, and 112–154 cmbsf) and to brownish-black sandy and silty muds (57–100 cmbsf, and 154 to 232 cmbsf).

Shallow sediment layers from basin sites 389, 391, 453 and slope site 387 had diatom-bearing silty muds, which extended from the surface to 64–63 cmbsf for sites 389 and 391, to 100 cmbsf for site 453, and to 21 cmbsf for slope site 387. Sites 387, 389 and 391 had increased composition of sand in their sediments, with alternating layers of silty muds and sandy muds throughout the three cores. Changes in sediment colour were observed along the core lengths in sites 387, 389 and 391. Site 387 sediment colour changed from yellowish-brown (21–52 cmbsf) to a layer where a transition from reddish- to brownish-colours were observed (52–71 cmbsf), and to brown (71–332 cmbsf). Site 389 changes in sediment colour were mainly in the shallower 200 cmbsf, where a gradational change from yellowish-brown to olive-grey was observed at 64–100 cmbsf, followed by a layer of olive-grey sediments (100–145 cmbsf), which changed to brown (145–166 cmbsf) and to greyish brown (166–424 cmbsf). Site 391 sediments changed from yellowish-brown to olive-grey (63–97 cmbsf) to dark greenish-grey (97–100 cmbsf), to a layer of dark yellowish-brown to light olive-grey (100–120 cmbsf), to pale brown (120–123 cmbsf), moderate brown (123–142cmbsf), brownish-grey (145–200 cmbsf), and to brownish-black (200–420 cmbsf). Site 453 sediments were composed of silty muds (100–300 cmbsf) with intercalations of clayey silt and sand at section 200–273 cmbsf. From 300 to 430 cmbsf, sediments contained increased concentrations of carbonate, with a layer of carbonate-rich brownish-grey silty marl at 300–317 cmbsf. Silty marls were observed from 300 to 430 cmbsf at site 453. At deeper sediment layers (430–470 cmbsf) of site 453, dark-olive silty muds were observed.

Southern slope sites 486 and 488 were composed of olive-grey silty muds throughout the entire cores, which eventually changed colour to more brownish in some sections of both cores.

The main minerals composing the mineral fraction of the sediments for three analysed sites (363, 389 and 486) were quartz and feldspar. In deeper layers of site 363, the main mineral fraction changed to be composed of only quartz (454 cmbsf) and quartz with dolomite (460 cmbsf). The mineral composition of site 389 changed from quartz and feldspar (51–192 cmbsf; except layers at 129 and 157 cmbsf that were composed of only quartz) to quartz and feldspar together with dolomite and calcite at 216 cmbsf, followed by layers of quartz and calcite (240–304, and 358 cmbsf; except a intercalated

layer of quartz and feldspar at 320 cmbsf) and only quartz in deepest layers (387–409 cmbsf).

The chemical composition of the sediment was of various oxides (50% silica, 13% alumina, 4% magnesia, 3–7% calcium oxide, 2–4% sodium oxide, 3% potassium oxide). Among them, iron(III) oxide values of 5% and 7% were observed for sites 363 and 486, respectively. Site 389 had an iron(III) oxide value of 6–7%, except at sediments layers of 187–192 cmbsf where the value decreased to 3–4% (see Appendix 3, section 6.3). A manganese(II) oxide value of ~ 0.1% was observed in all sediments analysed expect at a depth of 51 cmbsf from site 389, where a value of 0.7% was observed (see Appendix 3, section 6.3).

Values for the total organic carbon (TOC) in samples from shelf sites 363, 371 and the uppermost 125 cmbsf of 365 were higher than from other sites (Figure 35). The highest TOC value was 3% at surface sediments of site 371. Sites from the continental slope and central deep basin had similar TOC values around 0.5–1%. Lowest TOC values were found in cores 453, 486, and deepest sections of the shelf site 365. TOC decreased gradually with depth generally for all cores except for site 391, which at 175 cmbsf increased to values higher than 1.5%, which were maintained until the deepest part of the core.

In addition, total carbon (TC) was measured and the values indicated a contribution of carbonates at some sediment layers of sites 383, 389, 391, and 453. The highest TC value corresponded to site 453 at 277 cmbsf (Figure 36), which had a contribution of 3% for carbonates. Sediment layers from depths of 122–415 cmbsf at the site 389 had carbonate contributions of ~1.5%. Sediment layers at 122–214 and 221–410 cmbsf for sites 383 and 389, respectively, had carbonate contributions of ~ 1%.

The stable isotopic composition of organic carbon, measured as $\delta^{13}C_{org}$ calibrated vs. the VPDB standard, was measured from the sites 363, 389 and 486. Shelf site 363 had average $\delta^{13}C_{org}$ values of -25‰, which became lighter (-26 – -27‰) in deepest core sections (Figure 37). Sites 389 and 486 had lighter overall $\delta^{13}C_{org}$ values than site 363. Site 389 had average δ^{13} C_{org} values of -28‰, with lighter values of -31‰ at core sections of 130 cmbsf, and heavier values of -25‰ at deepest sections of the core (~ 400 cmbsf). Site 486 had average $\delta^{13}C$ values of -28 ‰, which became lighter with depth.

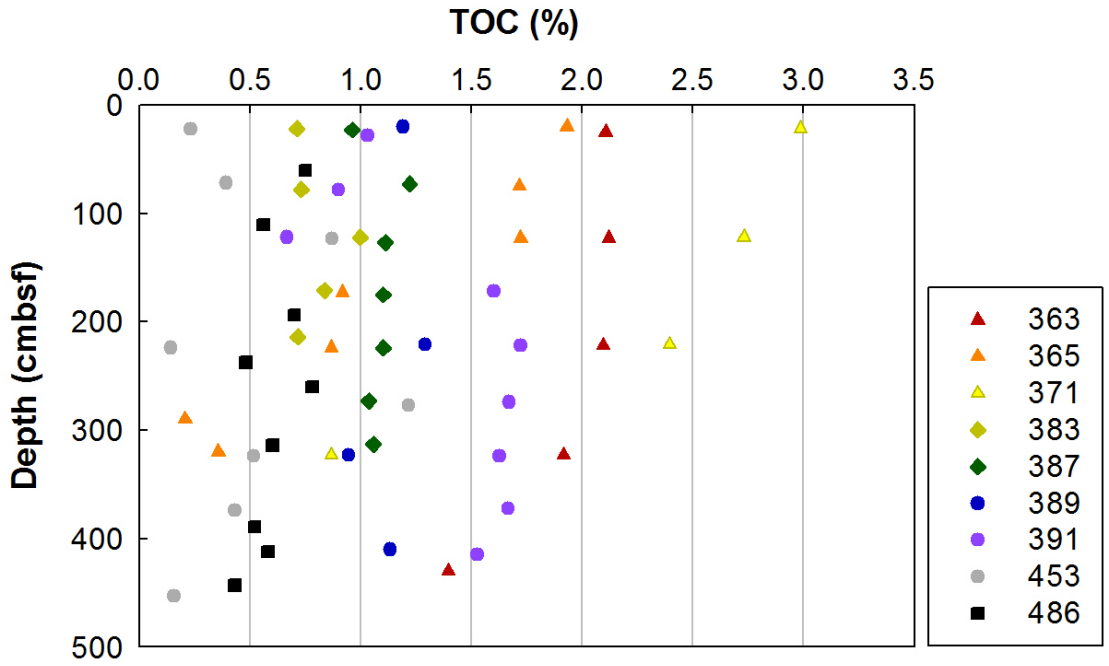


Figure 35. Total organic Carbon (TOC) values for sediments from the Baffin Bay. Shown are depth profiles of all the sites analysed within this study. The symbol and colour code for each site are consistently used in all graphs of this thesis.

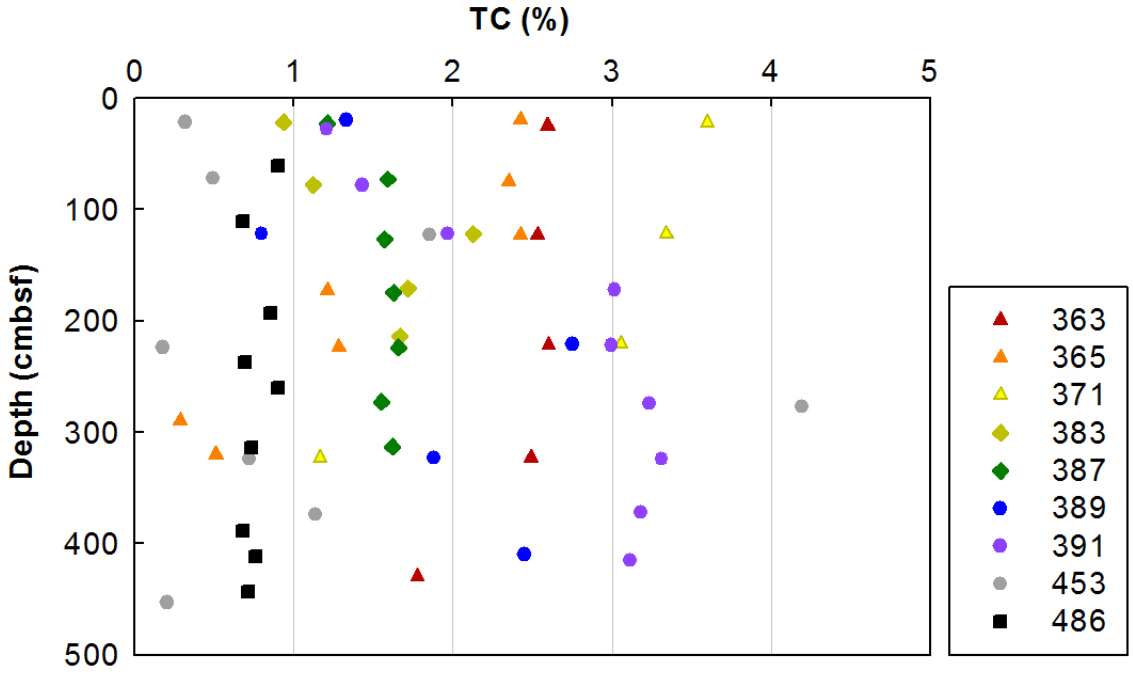


Figure 36. Total Carbon (TC) values of sediment sites from the Baffin Bay. Shown are depth profiles of all sites analysed within this study.

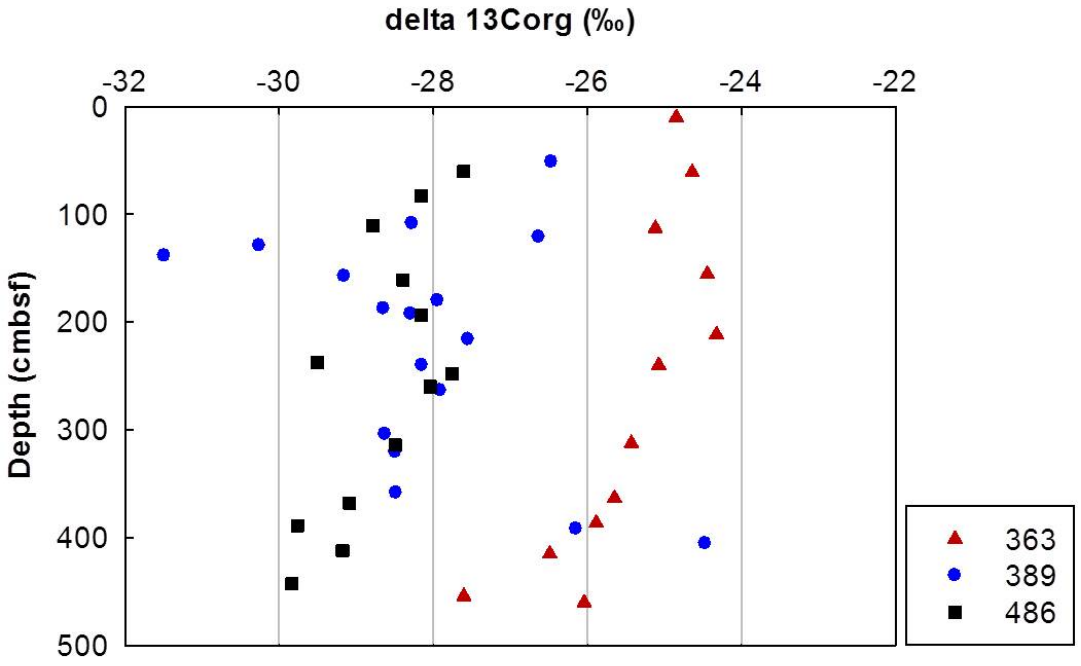


Figure 37. Organic carbon isotopic composition of sediment from the Baffin Bay. Shown are depth profiles of three sites that were selected, one site per area (shelf, basin, slope).

Pore-water constituents of sediments from the Baffin Bay included the ions sodium, chloride, potassium, magnesium, sulphate, iron(II), and manganese(II).

Sodium and chloride depth-dependent concentrations were stable with mean values of 461 mM ± 10 mM and 534 mM ± 13 mM, respectively.

Potassium concentrations of 10 mM were observed in near-surface sediments, which decreased to 8−9 mM with increasing depth.

Magnesium depth-dependant concentrations were stable at 45−50 mM in sediments from all sites, except for sites 363 and 453, where concentrations decreased with increasing depth (results not shown; for details see (Algora et al 2013)).

Sulphate concentrations were 25−27 mM in near-surface sediments and decreased gradually with increasing depths except for sites 365, 383, 389, and 391, which remained stable (Figure 38). Pore-water at sites 363 and 453 had highest decreases in sulphate concentrations with increasing depths, reaching 18 mM and 4 mM, respectively, at 450 cmbsf (Figure 38).

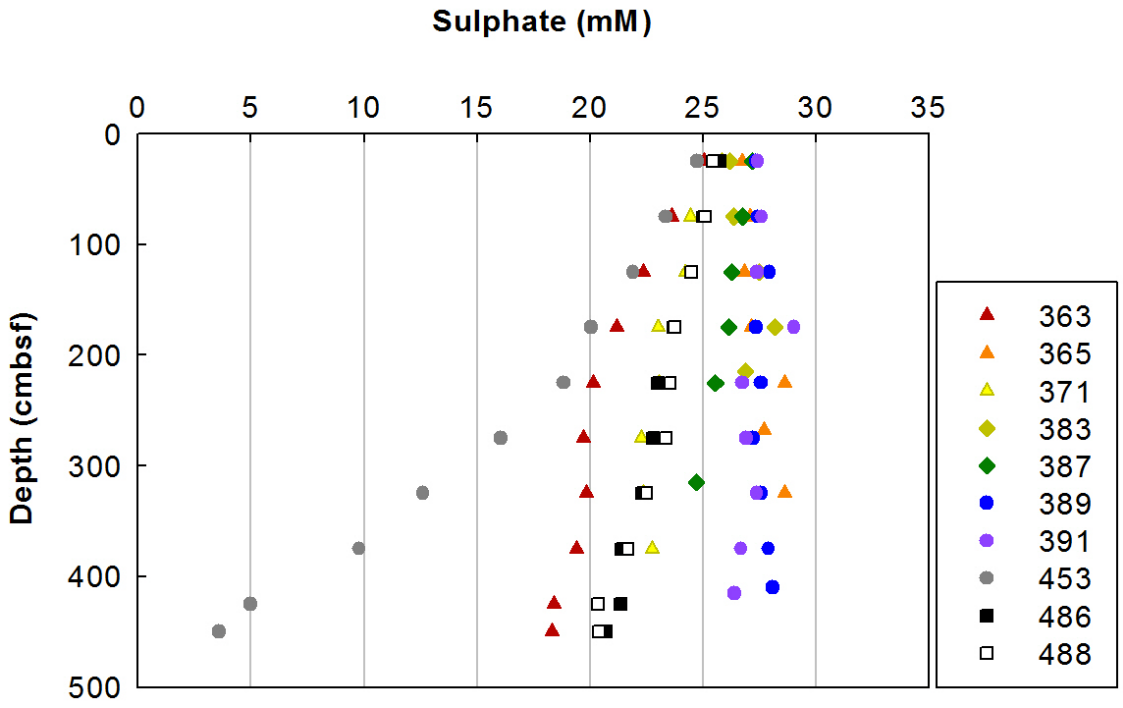


Figure 38. Sulphate concentration in pore-water of sediments from the Baffin Bay. Shown are depth profiles of all the sites investigated within this study.

Iron(II) was present in the pore-water from all sites as shown by the depth profiles (Figure 39). However, the concentration of Fe(II) differed substantially among the various sites and depths. At shelf sites 363, 365, and 371, Fe(II) concentration increased with increasing depth up to 32 µM for site 363, and up to 57 µM for sites 365 and 371, at the deepest parts of the cores. However, Fe(II) concentration increased at higher rates at site 365 (29 µM were measured at 175 cmbsf), compared to sites 371 and 363 (Figure 39). Fe(II) concentration decreased with increasing depth at site 383, except at 175 cmbsf. However, site 387 showed increasing concentration of Fe(II) with increasing depth reaching 58 µM. At the basin sites 389 and 391, Fe(II) concentration increased with increasing depth. The maximum Fe(II) concentration was 27 µM for site 389, observed at the depths of 75 and 225 cmbsf. For site 391, a substantially high value (maximum of all measured values) of 138 µM at 175 cmbsf was detected (Figure 39). Fe(II) concentration at site 453 increased with increasing depth to 15 µM. At Southern slope sites 486 and 488, near-surface Fe(II) concentration of 28 µM and 47 µM were detected, respectively. At both sites, the concentration of Fe(II) decreased with increasing depth to subsequently increase at deeper core sections (Figure 39).

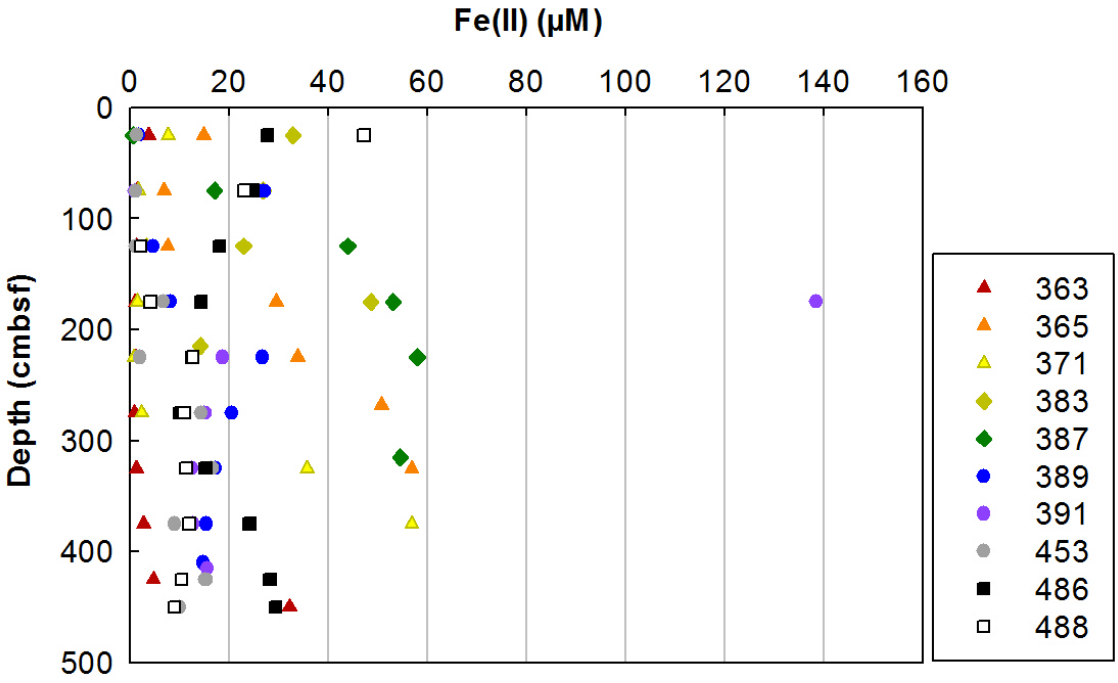


Figure 39. Iron(II) concentration in pore-water of sediments from the Baffin Bay. Shown are depth profiles of all the sites investigated within this study.

Manganese(II) was detected in the pore-water of all sites, however, with high variabilities in its concentration among the various sites and depths, which resulted in distinct trends in depth profiles (Figure 40). The concentration of Mn(II) in depth profiles from shelf sites 363 and 371 slightly increased with increasing depth (Figure 40). Similarly, the Mn(II) concentration in shelf site 365 increased with depth, however at higher rates, reaching a maximum concentration of 23 µM at 225 cmbsf. The Mn(II) concentration in the depth profiles of sites 383 and 387 were stable at a value of 15–20 µM, except at surficial sediments where 39 µM and 3 µM was observed, respectively.

The sediment profiles of sites 389 and 391 indicated a pronounced increase in Mn(II) concentration at 75–175 cmbsf, especially for site 389, where Mn(II) concentrations reached a maximum of 115 µM. At both sites, the Mn(II) concentration decreased to ~20 µM at 225/275 cmbsf and remained stable with increasing depth (Figure 40). The concentration of Mn(II) at site 453 increased with increasing depth to 16 µM at 375 cmbsf, to subsequently slightly decrease at deepest core sections. The Mn(II) concentration at site 486 was stable at ~20 µM with depth, and at site 488 decreased with increasing depth from 50 µM (at 25 cmbsf) to 24 µM (at 450 cmbsf).

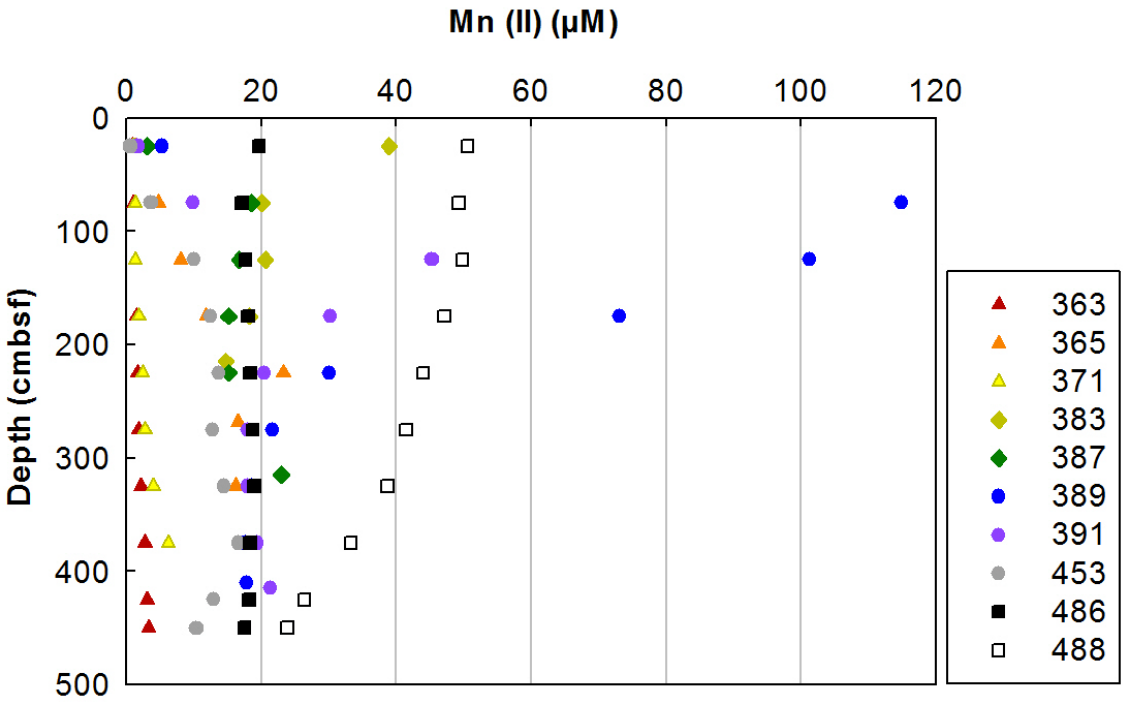


Figure 40. Mn(II) concentration in pore-water of sediments from the Baffin Bay. Shown are depth profiles of all the sites investigated within this study.

Methane was detected at all sites, however with no specific trend in the depth profiles. Methane concentration values were in general low with a maximum concentration of 17 µM at site 486 (Figure 41). Deepest core sections (225/275–425 cmbsf) of Southern slope sites 486 and 484 showed highest methane concentrations of ~15 µM, followed by shelf sites 363 and 365, with methane concentrations of ~11 µM (Figure 41).

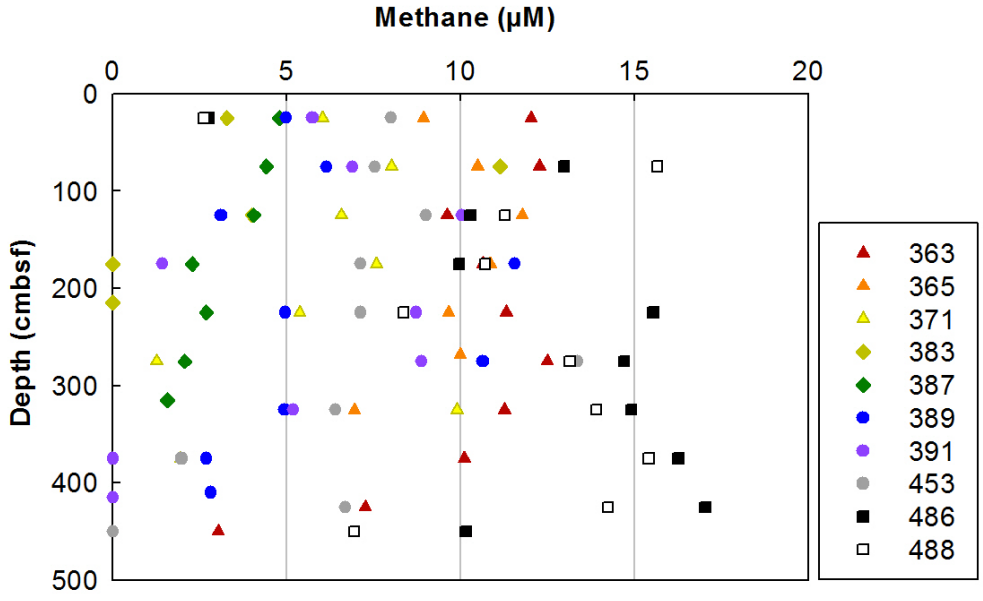


Figure 41. Methane concentration in sediment depth profiles from cores of the Baffin Bay.

3.3.2 Microbial ecology of Baffin Bay cores

The microbial ecology study investigated i) the microbial composition present in sediments of the Baffin Bay, ii) if differences in the microbial composition existed among the various sediment sites, and iii) if these differences in the microbial composition may be associated to changes in environmental parameters, i.e., sediment geochemistry, depth, and geographical location within the Baffin Bay.

The microbial composition was studied in terms of the abundance and spatial distribution of specific microbial groups, the microbial community structure and diversity. For that, two approaches were designated, the first one named "study A" focused on the abundance and spatial distribution of *Bacteria*, *Archaea* and specific microbial groups, e.g., *Dehalococcoidia*, in three geographically distinct areas: Northern Greenlandic shelf, central deep basin, and a Southern slope (Figure 4). The second approach was named "study T" and focused on the microbial community structure and diversity in sediments along a North-to-South, shelf-to-basin transect. This transect was 267 km long, from the Northern Greenlandic shelf to the central deep basin of the Baffin Bay (Figure 5).

Study A: Microbial composition in three distinct areas of the Baffin Bay

The abundance and site- and depth-dependant distribution of microbial populations were investigated in a total of seven sediment sites from the areas: Northern Greenlandic shelf (sites 363 and 371, Figure 4), central deep basin (sites 389, 391, and 453, Figure 4), and Southern slope (sites 486 and 488, Figure 4). This study involved the quantification of total *Bacteria* and *Archaea*, as well as specific microbial phylogenetical groups, i.e., the class *Dehalococcoidia* and the order *Desulfuromonadales*, and specific microbial physiological groups, i.e., sulphate-reducers, and methanogens. Quantification of *Bacteria*, *Archaea*, *Dehalococcoidia* and *Desulfuromonadales* was performed by qPCR using primers targeting the 16S rRNA gene (Table 7). Quantification of the physiological groups of sulphate-reducers and methanogens was done by qPCR with primers targeting the functional genes *dsrA* and *mcrA* (Table 7).

- **Quantification of total *Bacteria* and *Archaea* in sediment cores**

In general, highest 16S rRNA gene copy numbers for both *Bacteria* and *Archaea* were observed in uppermost sediments for all sites, except for site 486, where the highest number of bacterial copies (7.9×10^7 g^{-1}) was detected at 120 cmbsf. Similar patterns in

the depth profiles were observed for both archaeal and bacterial 16S rRNA gene copy numbers.

In the Northern Greenlandic shelf sites 363 and 371, 16S rRNA gene copy numbers for *Bacteria* were stable with increasing depth, averaging $1.4 \times 10^7 \pm 4 \times 10^6$ for site 363 at 25–430 cmbsf, and $2.3 \times 10^7 \pm 1.8 \times 10^7$ for site 371 at 22–271 cmbsf. Similar depth profile patterns were observed for archaeal 16S rRNA gene copies at both shelf sites. Archaeal gene copy numbers were in average $2.1 \times 10^7 \pm 8.4 \times 10^6$ for site 363 at 25–430 cmbsf, and $1.5 \times 10^7 \pm 4.11 \times 10^6$ for site 371 at 22–271 cmbsf. In core sections deeper than 300 cmbsf for site 371 and deeper than 425 cmbsf for site 363, both bacterial and archaeal copy numbers decreased one or two orders of magnitude (Figure 42).

In the central deep basin sites, bacterial copy numbers of 4.1×10^7 and 6.6×10^7 g^{-1} were detected at ~25 cmbsf in sites 391 and 389, respectively (Figure 43). Bacterial copy numbers rapidly decreased down to three orders of magnitude with increasing depth, and became in average 2.2×10^4 g^{-1} at 221–410 and 172–415 cmbsf for sites 389 and 391, respectively (Figure 43). Similarly to *Bacteria*, archaeal copy numbers declined rapidly and steadily with increasing depth at sites 389 and 391 (Figure 43). The highest archaeal copy number of all sites was 1.8×10^8 g^{-1} and was detected at 20 cmbsf at site 389. Archaeal copy numbers at site 389 decreased to 8.4×10^6 g^{-1} at 75 cmbsf and to 8×10^5 g^{-1} at 122 cmbsf. A similar pattern was found at site 391, with highest archaeal copy numbers of 5.4×10^6 g^{-1} at 28 cmbsf that declined to 2.9×10^4 at 122 cmbsf. In general, no amplification was obtained in the qPCR assay for *Archaea* in both sites 389 and 391 at sediments deeper than 175 cmbsf, indicating that the presence of *Archaea* at increasing depths was very rare.

At site 453, the bacterial and archaeal depth profiles differed substantially from the other central deep basin sites. Bacterial copy numbers were in average $2.3 \times 10^6 \pm 1.6 \times 10^6$ g^{-1} at site 453. An order of magnitude decrease in bacterial copy numbers (1.9×10^5 g^{-1}) was observed at 277 cmbsf (Figure 43), where a high percentage of carbonates in the sediment had been detected. Archaeal copy numbers at site 453 were one order of magnitude lower than the bacterial copy numbers, and could be detected throughout the core, except at 22 cmbsf (Figure 43).

In the site 486 from the Southern slope, bacterial copy numbers of 1×10^7 g^{-1} were observed at 20 cmbsf and increased to a maximum value of 7.9×10^7 g^{-1} at 120 cmbsf (Figure 44). At 173–321 cmbsf, bacterial copy numbers remained stable with increasing depth, averaging $1.2 \times 10^7 \pm 1.2 \times 10^6$ g^{-1}. Bacterial copy numbers decreased at layers

deeper than 321 cmbsf down to a minimum number of 1.6 x 10^6 g^{-1} by 448 cmbsf (Figure 44).

Site 488 from the Southern slope showed a maximum bacterial copy number of 5.6 x 10^6 g^{-1} at 70 cmbsf, and a minimum of 2.8 x 10^5 g^{-1} at 171 cmbsf. Surface sediments (20–70 cmbsf) had considerable higher bacterial copy numbers, nearly one order of magnitude, than the deeper core sections (Figure 44).

Archaeal copy numbers were generally one order of magnitude lower than bacterial at Southern slope sites 486 and 488, in contrast to the shelf and basin areas, where similar bacterial and archaeal copy numbers were observed. The only exception was surface sediments of site 488, and deep sediments (421 cmbsf) of site 486, where similar archaeal and bacterial copy numbers were found (Figure 44).

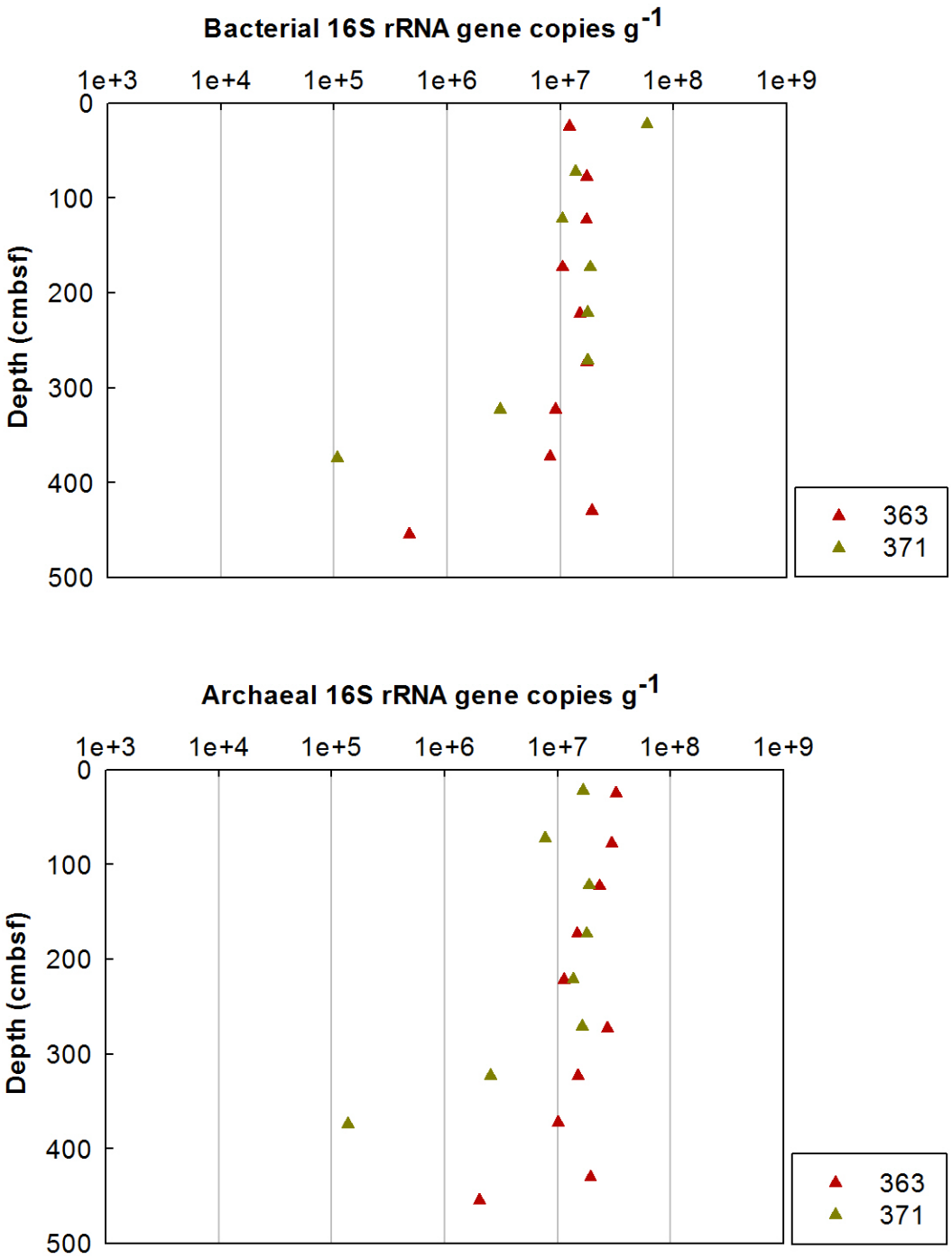


Figure 42. Depth profiles of total *Bacteria* (upper panel) and *Archaea* (lower panel) quantified as 16S rRNA gene copy numbers per gram of sediment (wet weight) for the sites 363 and 371 within the Northern Greenlandic shelf area of the Baffin Bay.

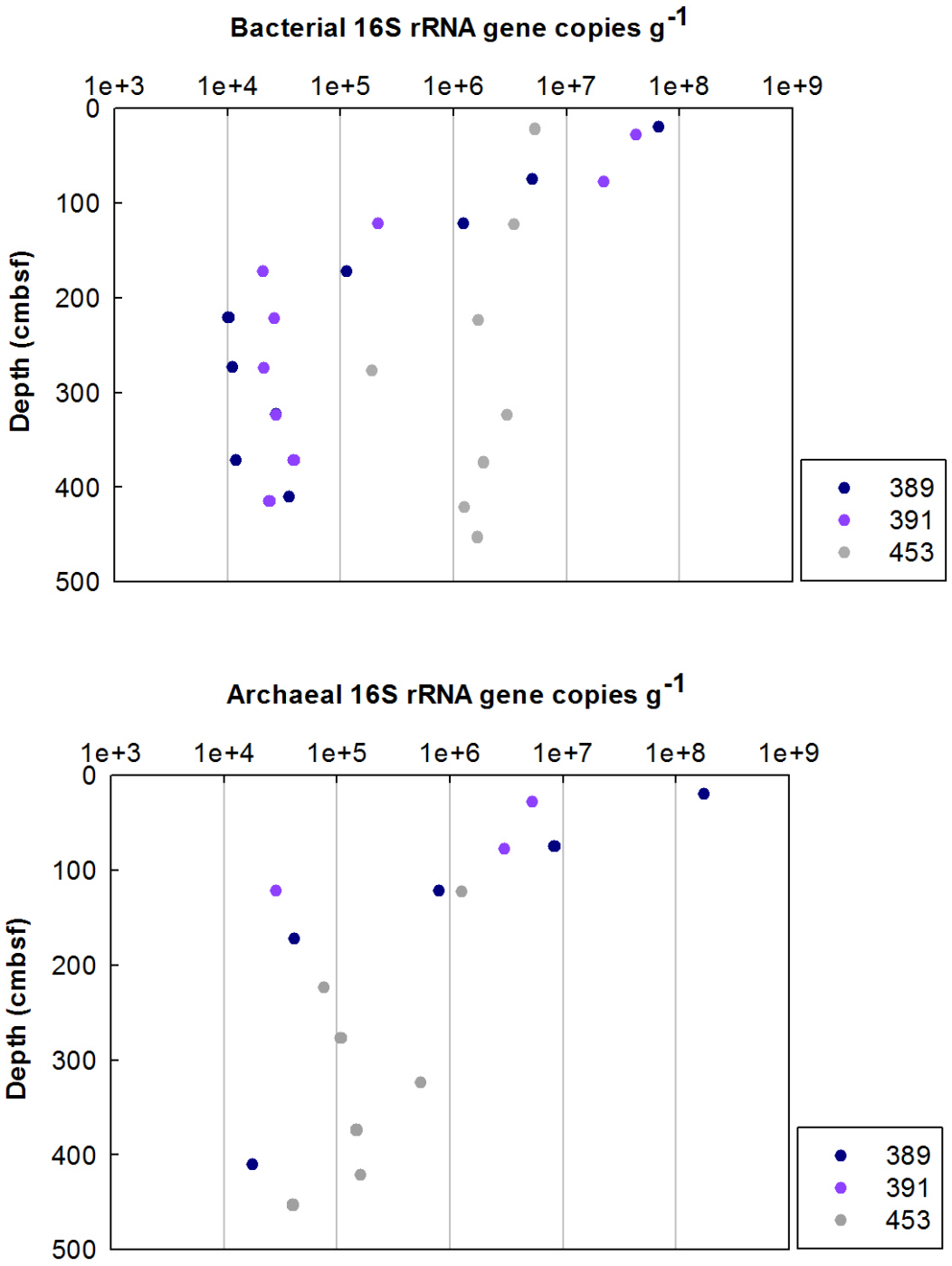


Figure 43. Depth profiles of total *Bacteria* (upper panel) and *Archaea* (lower panel) quantified as 16S rRNA gene copy numbers per gram of sediment (wet weight) for the sites 389, 391, and 453 within the central deep basin area of the Baffin Bay.

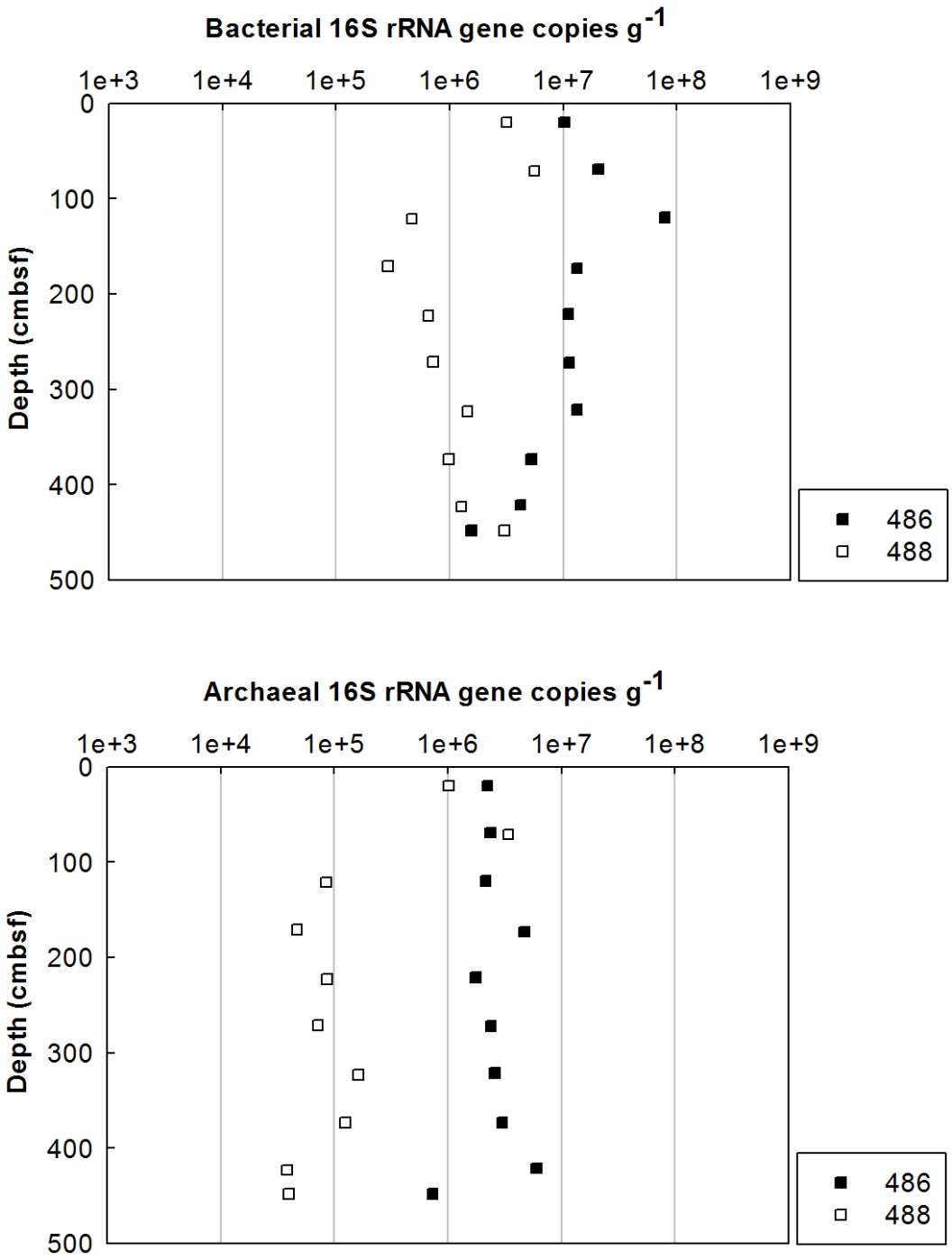


Figure 44. Depth profiles of total *Bacteria* (upper panel) and *Archaea* (lower panel) quantified as 16S rRNA gene copy numbers per gram of sediment (wet weight) for the sites 486 and 488 within the Southern slope area of the Baffin Bay.

- **Quantification of class *Dehalococcoidia* and order *Desulfuromonadales* in sediment cores**

Members from the class *Dehalococcoidia* were quantified with the primers DEH-Fa and DEH-R targeting the 16S rRNA gene (Table 7). From the three areas under investigation, highest *Dehalococcoidia* 16S rRNA gene copy numbers, of 3.7 x 10^5 ± 2.8 x 10^5 g^{-1} in average, were detected in sediment sites 363 and 371, from the Northern Greenlandic shelf (Figure 45). Lowest *Dehalococcoidia* copy numbers, of 5 x 10^4 g^{-1} ± 3.7 x 10^4 in average, were found at Southern slope site 486 (Figure 47).

In the central deep basin, highest *Dehalococcoidia* copy numbers were observed in shallow sediments, with 4 x 10^5 g^{-1} at depths of 22 and 78 cmbsf at sites 453 and 391, respectively (Figure 46). At site 453, similar *Dehalococcoidia* copy numbers with increasing depth (1.7 x 10^5 ± 1.3 x 10^5 in average) were found. However, sites 389 and 391 had pronounced decreases in the number of *Dehalococcoidia* copies with increasing depth (Figure 46). At site 389, the minimum value (1.1 x 10^3 g^{-1}) of *Dehalococcoidia* copy numbers from all sites and depths was detected at 221 cmbsf.

Even though shelf sites 363 and 371 showed highest *Dehalococcoidia* copy numbers, with a maximum of 8.4 x 10^5 g^{-1} at site 363, and depth of 78 cmbsf, the relative proportion of *Dehalococcoidia* with respect of the total bacterial 16S rRNA gene copy numbers (calculated as 16S rRNA gene copy numbers of *Dehalococcoidia* divided by total bacterial 16S rRNA gene copies), was fewer than 10%, with the only exception of site 371, at 374 cmbsf, where *Dehalococcoidia* accounted for 29% of the total bacterial 16S rRNA gene copy numbers (Figure 48). In contrast, sites 389 and 391, in the central deep basin, had low *Dehalococcoidia* copy numbers at 150–400 cmbsf, nevertheless, *Dehalococcoidia* accounted for more than 50% for site 391 and between 11–51% for site 389 (Figure 48).

Southern slope site 488 had a distinct depth profile of *Dehalococcoidia* copy numbers, which differed from the rest of the sites, as numbers increased more than one order of magnitude in deepest core sections (423–448 cmbsf) compared to shallower sediments (20–171 cmbsf). Highest *Dehalococcoidia* copy numbers of 6.4 x 10^5 g^{-1} were detected at 448 cmbsf, where *Dehalococcoidia* accounted for 21% of the total bacterial copy numbers (Figure 47 and Figure 48).

The order *Desulfuromonadales* includes known members of *Bacteria* able to reduce insoluble Fe(III) and Mn(IV) oxides such as *Geobacter* spp. and *Desulfuromonas* spp. The primers GEO494F and GEO825R (Table 7) targeted the order *Desulfuromonadales*

and were used here as an approximation to provide information on potential metal reducing bacteria in the sediments of the Baffin Bay.

Desulfuromonadales 16S rRNA gene copy numbers were in general one order of magnitude less abundant than bacterial and archaeal copy numbers (Figure 45, Figure 46, and Figure 47). The depth profile for *Desulfuromonadales* exhibited similar patterns to the total bacterial and archaeal copy numbers, and did not relate to the concentration of iron(II) and manganese(II) in the pore-water. For instance, at deep core sections of 454 and 323–374 cmbsf at shelf sites 363 and 371, respectively, the concentration of iron(II) notably increased (Figure 39); however, the copy numbers of *Desulfuromonadales* decreased two orders of magnitude (Figure 45). Similarly, substantial high concentrations of iron(II) at 175 cmbsf of site 391, and of manganese(II) at 75–175 cmbsf of site 389 were observed (Figure 39 and Figure 40); however, at those depths and sites, no *Desulfuromonadales* could be detected (Figure 46). *Desulfuromonadales* accounted for more than 20% of the total 16S rRNA bacterial sequences in near-surface shelf sediments, where a low concentration of iron(II) or manganese(II) was measured (Figure 39, Figure 40, and Figure 48).

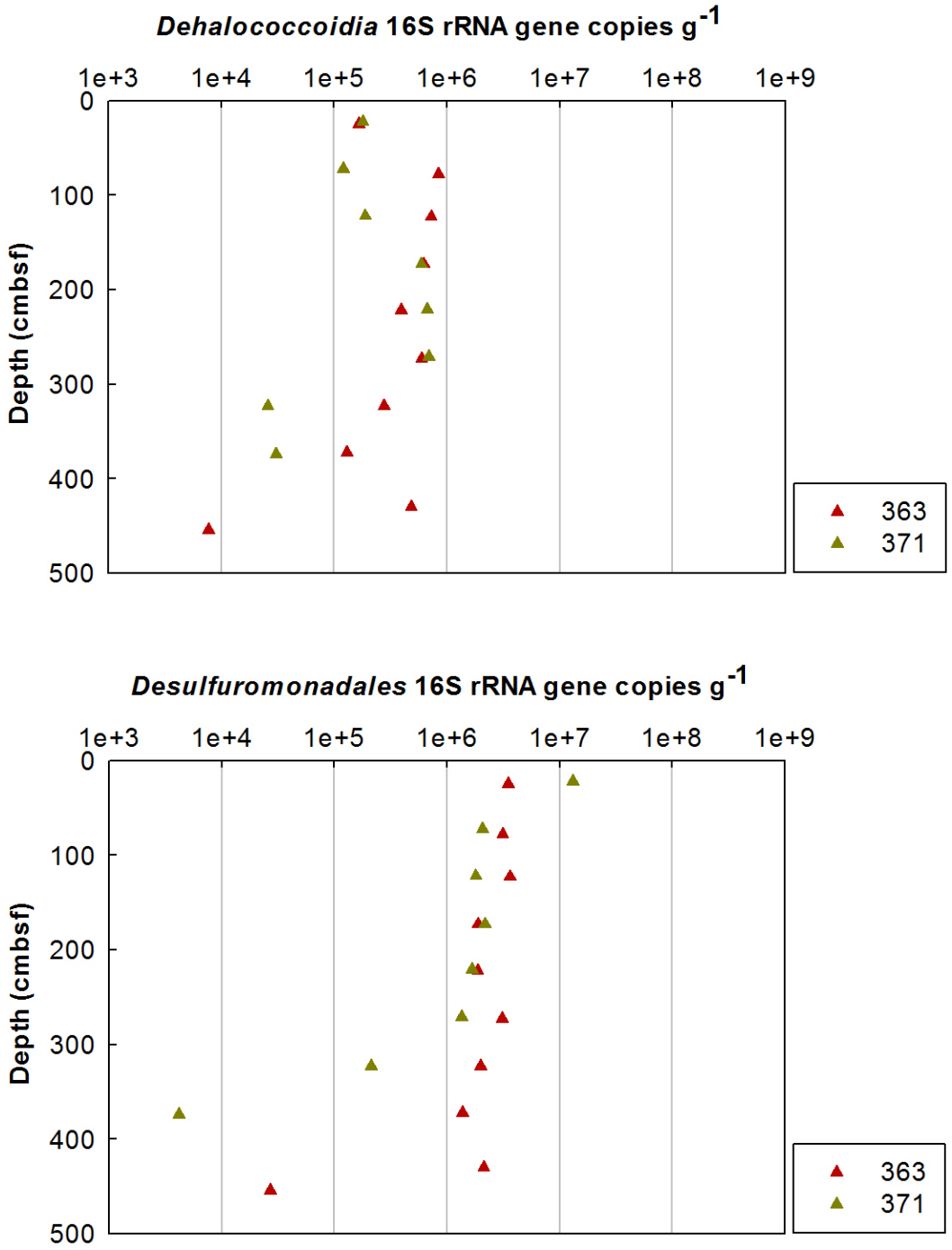


Figure 45. Depth profiles of the class *Dehalococcoidia* (upper panel) and order *Desulfuromonadales* (lower panel) quantified as 16S rRNA gene copy numbers per gram of sediment (wet weight) for the sites 363 and 371 within the Northern Greenlandic shelf area of the Baffin Bay.

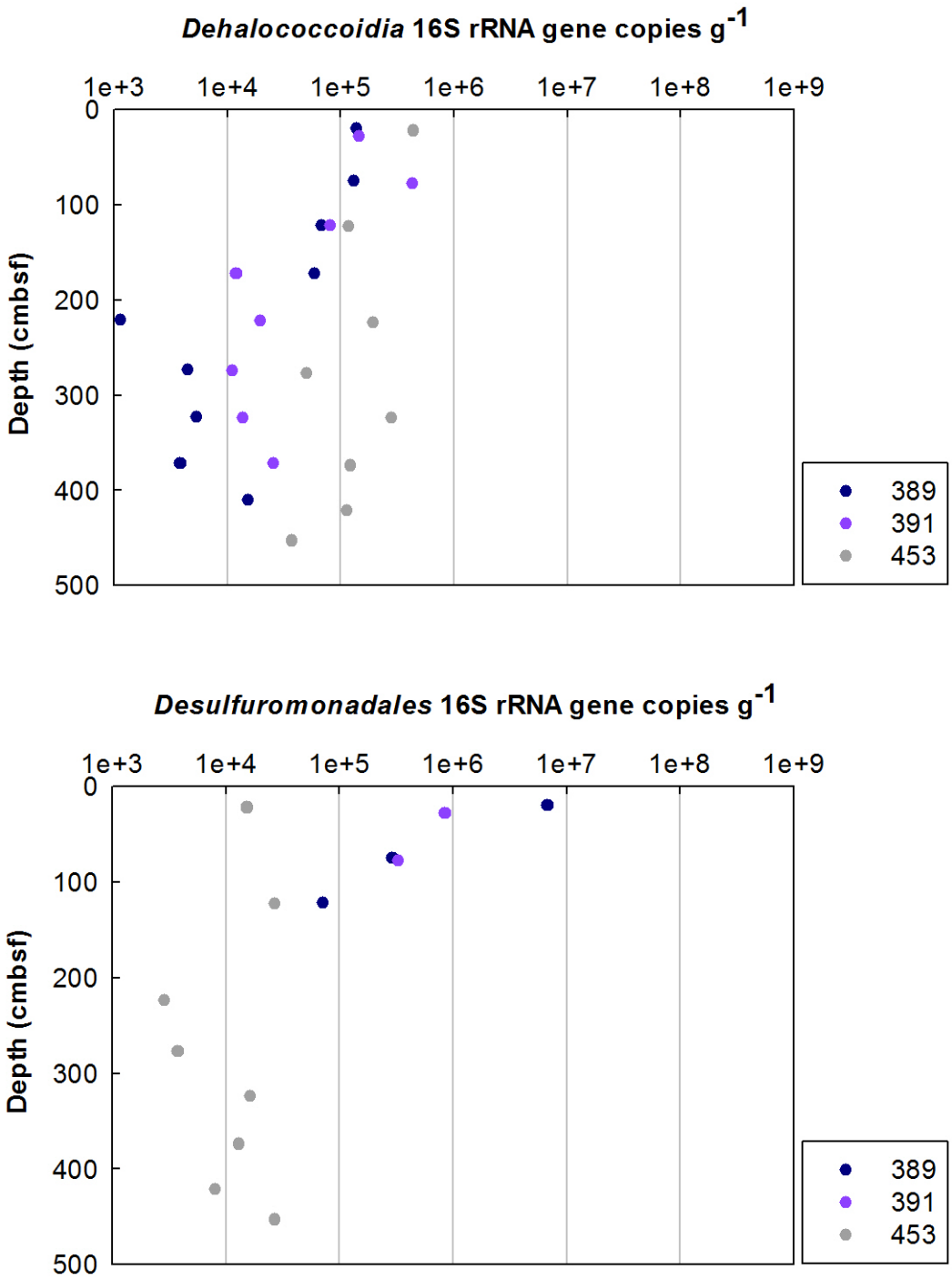


Figure 46. Depth profiles of the class *Dehalococcoidia* (upper panel) and order *Desulfuromonadales* (lower panel) quantified as 16S rRNA gene copy numbers per gram of sediment (wet weight) for the sites 389, 391, and 453 within the central deep basin area of the Baffin Bay.

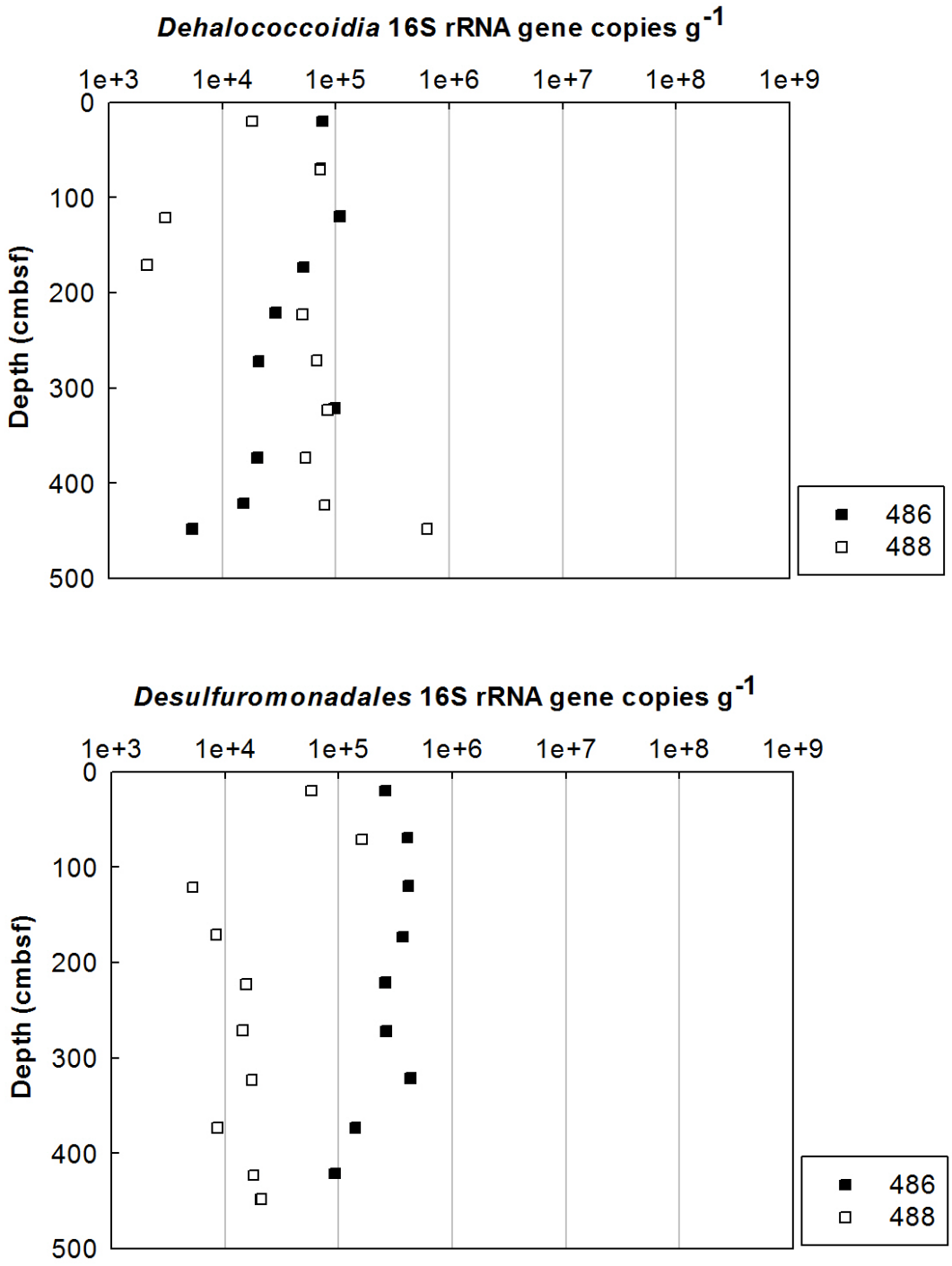


Figure 47. Depth profiles of the class *Dehalococcoidia* (upper panel) and order *Desulfuromonadales* (lower panel) quantified as 16S rRNA gene copy numbers per gram of sediment (wet weight) for the sites 486 and 488 within the Southern slope area of the Baffin Bay.

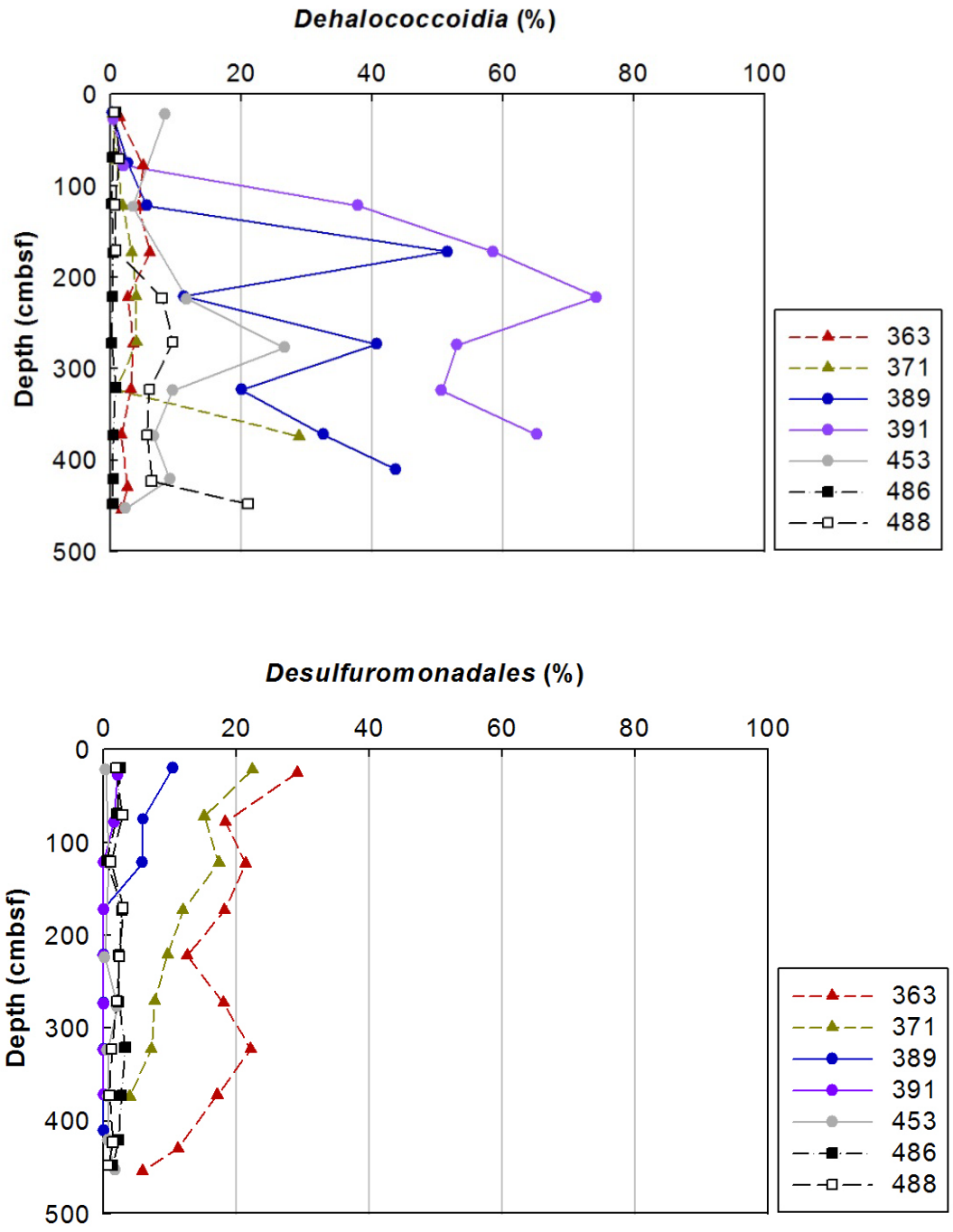


Figure 48. Relative proportion of the 16S rRNA gene copy numbers of class *Dehalococcoidia* (upper panel) and order *Desulfuromonadales* (lower panel) to the total 16S rRNA gene copy numbers of *Bacteria* for all sites of the Baffin Bay investigated in this study.

- **Quantification of functional genes (*dsrA*, *mcrA*) in sediment cores**

The functional genes *dsrA* and *mcrA* encode the enzymes dissimilatory sulphite (bi)-reductase subunit A (*dsrA*) and the α-subunit of the methyl coenzyme M reductase (*mcrA*). The qPCR analysis of *dsrA* and *mcrA* genes was used here as a proxy to quantify the physiological microbial groups of sulphate reducers and methanogens/ anaerobic methanotrophs, respectively.

dsrA genes were detected in sediment sites from the Northern Greenlandic shelf and the Southern slope at all depths except at 374 cmbsf of site 371 (Figure 49 and Figure 51). However, at central deep basin sites 389 and 391, *dsrA* genes were only detected in shallow sediments (20–122 and 28–78 cmbsf at sites 389 and 391, respectively; Figure 50). *dsrA* genes at site 453 were detected at 123–374 cmbsf (Figure 50).

The number of *dsrA* copies was highest in the Northern Greenlandic shelf than in any other area, with maximum *dsrA* copies of 1.4×10^{9} g^{-1} at site 371 and a depth of 22 cmbsf, which decreased with increasing depths down to a minimum of 1.2×10^{7} g^{-1} at 323 cmbsf (Figure 49). In central deep basin sites 389 and 391, *dsrA* gene copies were highest in shallowest sediments (Figure 50). *dsrA* gene copies at site 453 were the lowest of all sites.

In the Southern slope site 486, *dsrA* copy numbers remained generally stable with depth, at a value of $8.6 \times 10^{6} \pm 3.3 \times 10^{6}$ g^{-1} in average (Figure 51). However, at site 488, *dsrA* copy numbers varied with increasing depth, from an average value of $3.4 \times 10^{6} \pm 1.6 \times 10^{6}$ g^{-1} in the uppermost 75 cmbsf, to a minimum value of 7.8×10^{4} g^{-1} at 121, and to an average value of $5.3 \times 10^{5} \pm 2.2 \times 10^{5}$ at deep core sections of 171–448 cmbsf (Figure 51).

In general, few *mcrA* gene copy numbers were observed in any of the sites from all areas. The maximum number of *mcrA* gene copies was 2.9×10^{6} g^{-1}, observed at a depth of 20 cmbsf at site 389 (Figure 50.). Lowest *mcrA* gene copies were detected at sites 453 and 488 (Figure 50 and Figure 51). Generally, depth profiles for *mcrA* gene copies were similar to those of *dsrA*. However, *mcrA* gene copies were one or two order of magnitude less abundant than *dsrA* (Figure 49, Figure 50, and Figure 51).

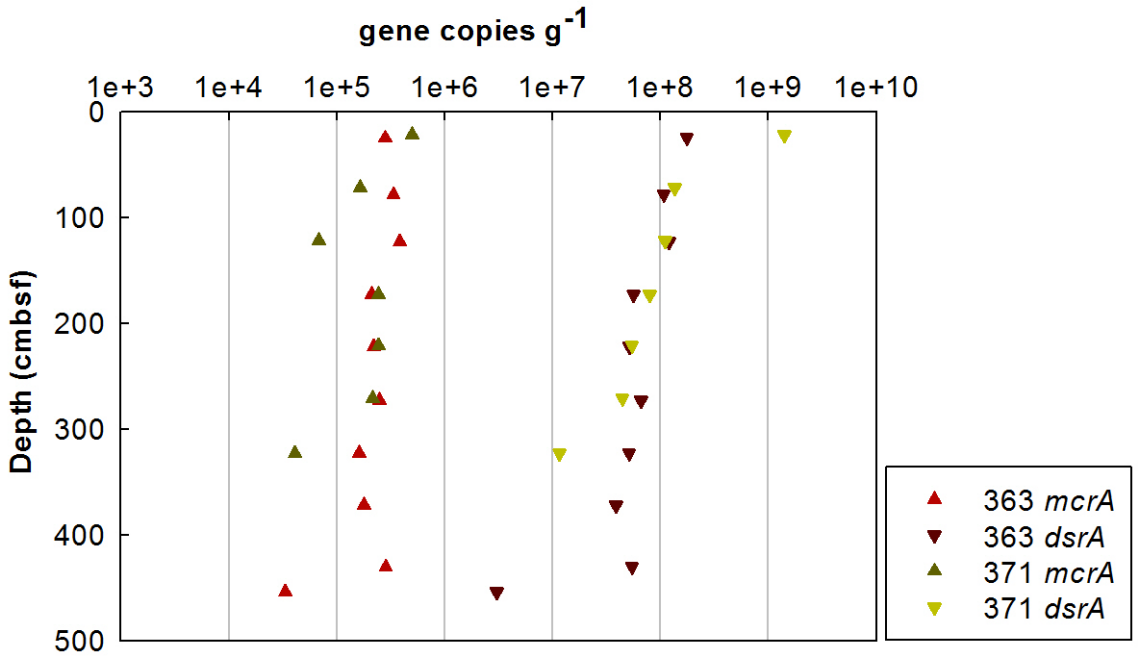


Figure 49. Depth profiles of functional genes *mcrA* and *dsrA* of methanogens/anaerobic methanotrophs and sulphate reducing prokaryotes, respectively, per gram of sediment (wet weight) for two selected sites within the Northern Greenlandic shelf area within the Baffin Bay.

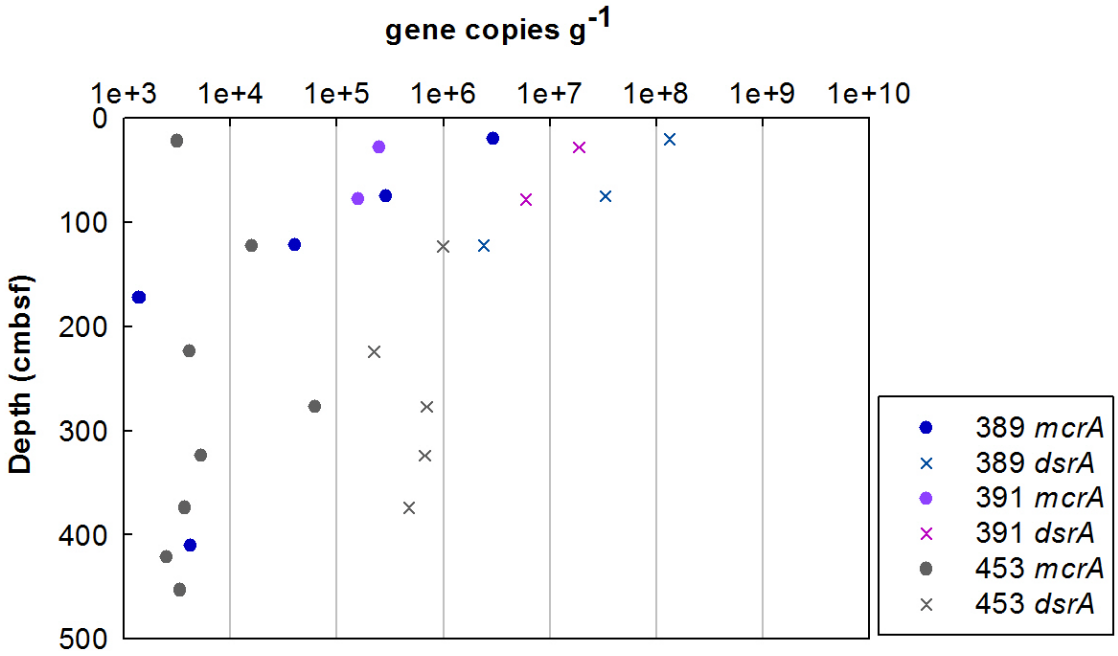


Figure 50. Depth profiles of functional genes *mcrA* and *dsrA* of methanogens/anaerobic methanotrophs and sulphate reducing prokaryotes, respectively, per gram of sediment (wet weight) for two selected sites within the central deep basin area within the Baffin Bay.

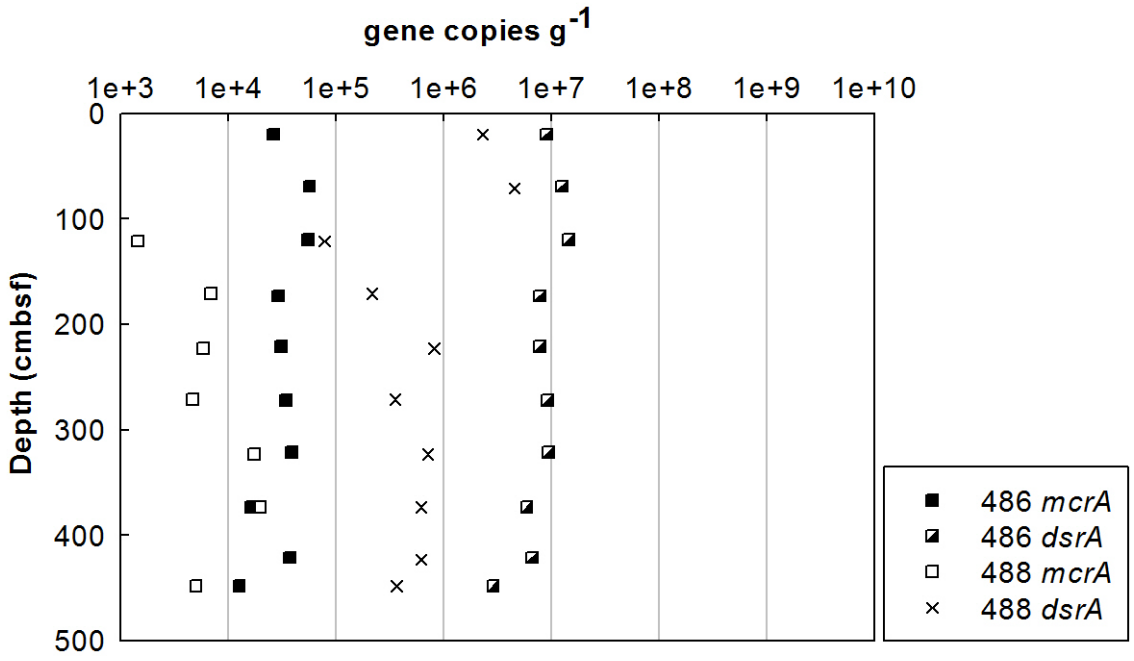


Figure 51. Depth profiles of functional genes *mcrA* and *dsrA* of methanogens/anaerobic methanotrophs and sulphate reducing prokaryotes, respectively, per gram of sediment (wet weight) for two selected sites within the Southern slope area within the Baffin Bay.

Study T: Microbial composition in sediments along a shelf to basin transect

The microbial community structure and diversity was investigated using high-throughput Illumina sequencing in a total of seven sites (363, 365, 371, 383, 387, 389, and 391; Figure 5) along a North-to-South, shelf-to-basin transect. Several samples from various depths were selected for each site (five from site 363, seven from site 365, four from site 371, three from site 383, seven from site 387, five from site 389, and nine from site 391), forming a total of 40 samples (see right-hand legend in Figure 52, where site_depth in cmbsf is shown). The bacterial community structure was determined as relative abundance of bacterial taxonomical groups at the phylum and/or class and/or order level present. The diversity was determined as numbers of operational taxonomic units (OTUs). In addition, the correlation of specific bacterial groups to geochemical parameters, i.e., TOC content, and concentration of sulphate, iron(II), and manganese(II), was also investigated.

- **Bacterial community structure analysed by Illumina sequencing**

The Illumina sequence analysis of the 40 samples yielded a total of 113,120 sequences (2,828 sequences per sample) after quality filtering and normalising the number of sequences in any sample to the lowest number of sequences found in a sample. The sequences had a length of ≥110 bp and were taxonomically classified using the SILVA and Greengenes databases via the naïve Bayesian classification method (Wang et al 2007).

The phylum *Proteobacteria* was dominant within the bacterial community at all sites and depths. More in-depth classification of the proteobacterial sequences showed that 38–64% of the total bacterial community belonged to the class *Betaproteobacteria*, and 6–13% to the class *Alphaproteobacteria* (Figure 52). Other classified groups within the phylum *Proteobacteria*, although in minor relative abundance, were the classes *Deltaproteobacteria* and *Gammaproteobacteria.* The betaproteobacterial sequences affiliated mostly (average of 99.7%) to the order *Burkholderiales* (Figure 52).

The second most abundant phylum was *Chloroflexi* accounting for 8–22% of the total bacterial community (Figure 52). More in-depth classification indicated that 75% of these *Chloroflexi* belonged to the class *Dehalococcoidia.* Relative abundance for *Dehalococcoidia* was 6%–17% of the total bacterial community. The third most abundant phylum was the *Actinobacteria* (Figure 52).

Hierarchical clustering of the analysed bacterial community structure in all the samples resulted in two main clusters of samples showing highest similarities (dendrogram at the right part of Figure 52, shaded in grey). One cluster (upper cluster in Figure 52) included near-surface sediments of 25 cmbsf from all sites, and deeper layers from shelf sites 363, 371, and 365, as well as two samples from basin sites 391 and 389 at 125 and 410 cmbsf, respectively. In the lower cluster (in Figure 52), there were mostly deeper layer samples from basin and slope sites and the shelf sites 365, 371, and 383. One of the main differences between the two clusters was the higher relative abundance of the phylum *Chloroflexi* in the samples from the upper cluster. Indeed, highest relative abundance of the class *Dehalococcoidia* was present in samples of the shelf sites 363 and 371 at 425 and 225 cmbsf, respectively. Samples in the lower cluster showed higher relative abundance of the class *Betaproteobacteria* and specifically from the order *Burkholderiales* than the first cluster (Figure 52).

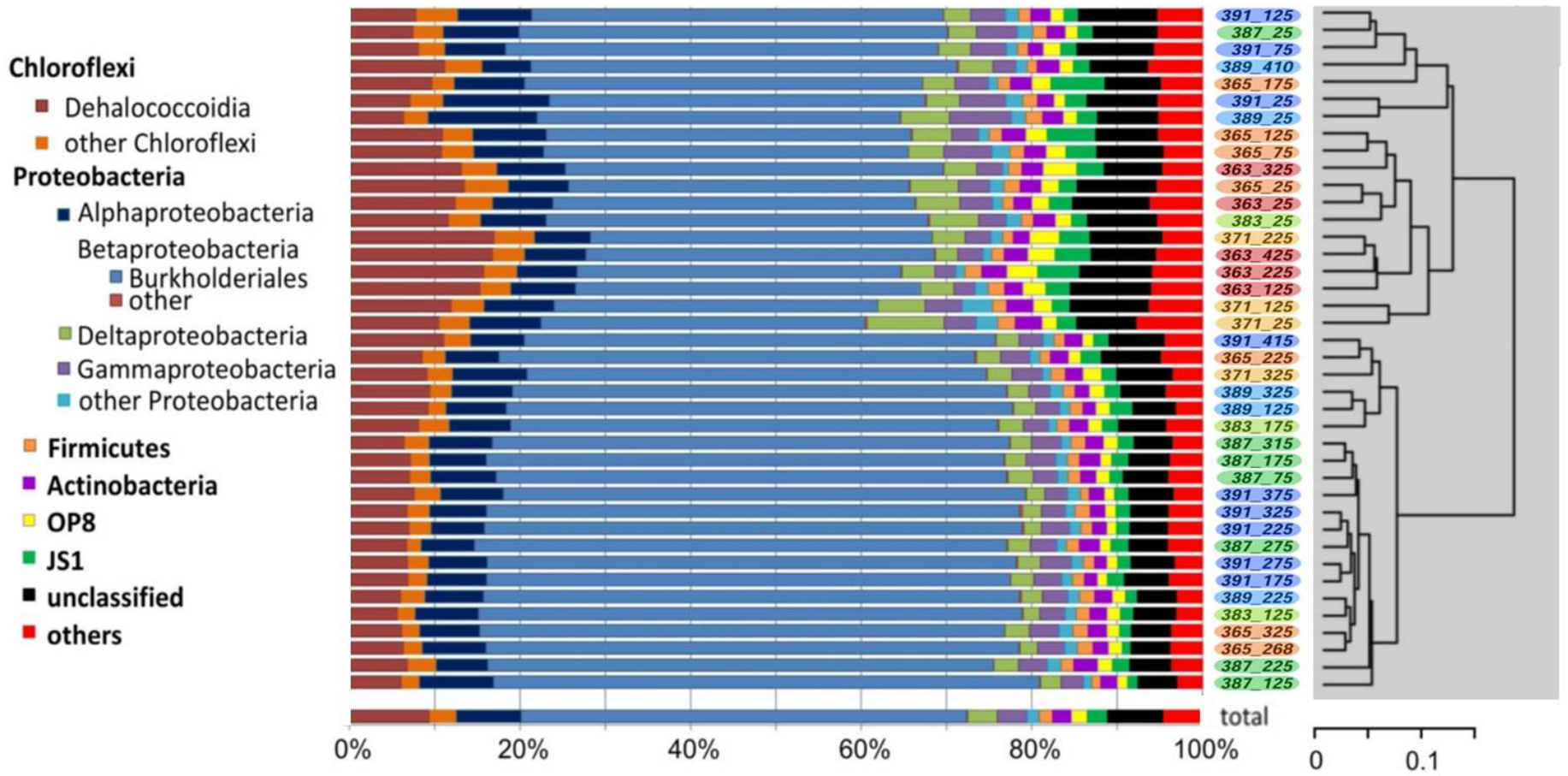


Figure 52. Relative abundance of classified bacterial sequences in samples (bar plot, with colour-code legend on the left) and the associated hierarchical clustering (UPGMA, right and shaded in grey) calculated using the Bray-Curtis dissimilarities. The scale below the bar plot indicates relative abundance in samples and in total. Sediment sample names indicate site and depth in cmbsf where the sample is coming from (e.g., sample "391_125" comes from site 391 at depth 125 cmbsf). The site labels are shaded in colours that correspond to the colour code in Figure 5. Modified after (Algora et al 2015).

➢ Bacterial diversity analysed as number of OTUs

The bacterial diversity in samples from the various sites and depths was measured as number of OTUs, defined here as groups of sequences with an identity of ≥ 97%. Highest OTU numbers were detected at 25 cmbsf, with values ranging around 550 OTUs for all sites (Figure 53). In general, OTU numbers decreased with increasing depth in all sites. The depth profiles of OTUs indicated highest diversity in shelf sites 363, 365, and 371, and in shallow sediments down to 125 cmbsf of basin site 389.

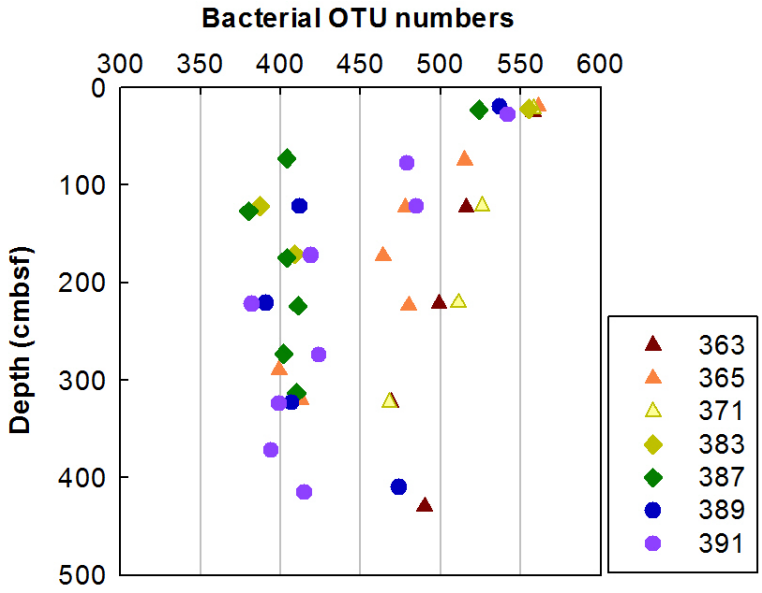


Figure 53. Bacterial diversity, measured as OTU numbers, in depth profiles for each site of the Baffin Bay. Colour code according to each site as given in Figure 5.

➢ Association of bacterial groups to geochemical parameters

The association of OTUs (≥ 97% sequence identity) to the geochemical parameters was evaluated with Spearman's rank correlation tests. An OTU correlated significantly to a geochemical parameter when the p-value was <0.05. OTUs having significant Spearman correlation with an environmental parameter were taxonomically classified using *mothur* and the Greengenes database via the naïve Bayesian classification method. The relative amount of OTUs affiliated to a taxonomical group and significantly correlating with a specific geochemical parameter in relation to the total number of OTUs from that taxonomical group was calculated to evaluate the proportion of a taxonomical group correlating to a specific geochemical parameter (Figure 54). The highest 25% identified correlations were defined as strong (either positive or negative).

OTUs that were strongly positive correlating with Mn(II) concentrations affiliated to the families *Alcaligenaceae*, *Burkholderiaceae* and *Comamonadaceae* within the class

Betaproteobacteria (Figure 54). Indeed, ≥ 90% of the OTUs belonging to these three betaproteobacterial families indicated a strong positive correlation with the concentration of Mn(II), and a negative correlation with TOC content. The opposite was observed for the GIF-9 cluster within the class *Dehalococcoidia* and members of candidate phylum "JS1", both with ≥ 90% of the OTUs having strong positive correlation with TOC and negative correlation with Mn(II) (Figure 54). Positive correlations with TOC were also observed for nearly half of the OTUs of the order *Dehalococcoidales* within the class *Dehalococcoidia*. Moreover, *Dehalococcoidia* (order *Dehalococcoidales* and GIF-9) showed a negative correlation with sulphate concentration. Similarly, ≥ 90% of the OTUs falling into the candidate phylum "OP8" correlated negatively with sulphate and positively with TOC. Taxa that strongly positive correlated with the concentration of Fe(II) belonged to the class *Alphaproteobacteria*. Around 40% of OTUs falling into *Alphaproteobacteria* strongly correlated to Fe(II), and another 40% correlated to sulphate (Figure 54).

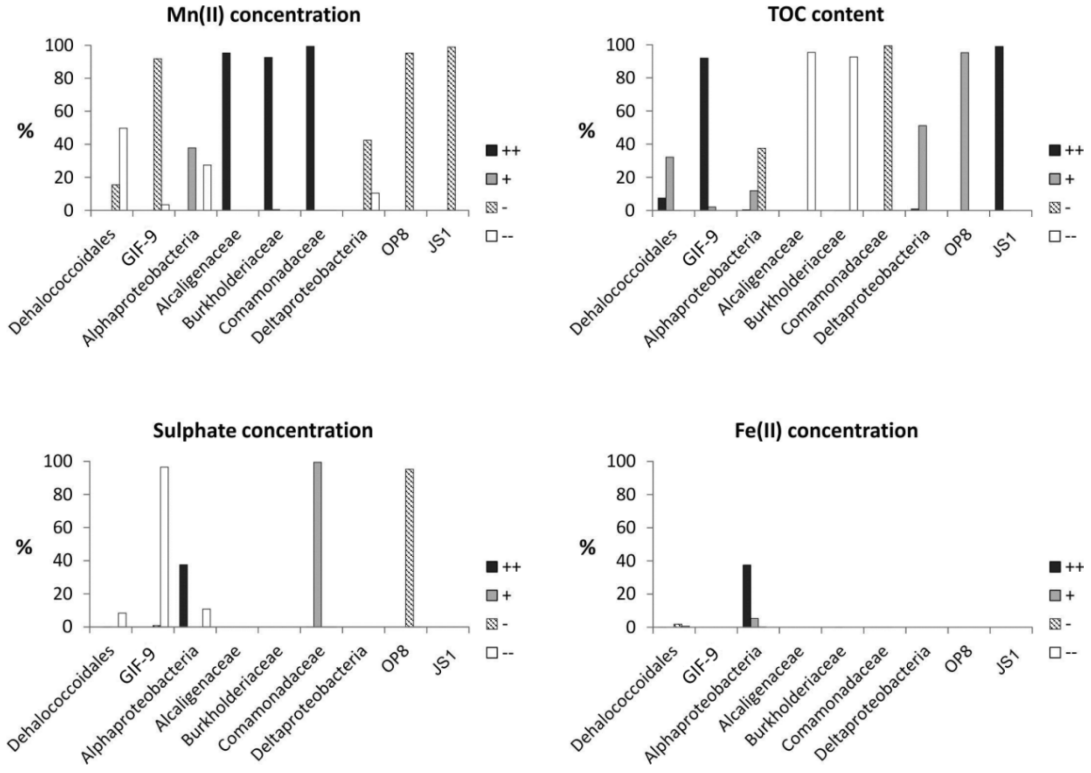


Figure 54. The relative amount of OTUs within a selected taxonomical group which had a significant (*P<0.05*) correlation with a geochemical parameter is shown. The correlation was individually calculated for each OTU by Spearman rank correlation tests. The top 25% positive and/or negative correlations are defined as "strong positive" (++) or "strong negative" (--). The remaining significant correlations are indicated as positive (+) or negative (-). The order *Dehalococcoidales* and GIF-9 belong to the class *Dehalococcoidia*. The families *Alcaligenaceae*, *Burkholderiaceae* and *Commamonadaceae* belong to the class *Betaproteobacteria.* Modified after (Algora et al 2015).

4 DISCUSSION

Marine sediments comprise a vast area of our planet, and are a major habitat for huge amounts of highly diverse and novel microorganisms (Parkes and Sass 2009). Members belonging to the class *Dehalococcoidia* are among the most abundant and widespread microorganisms in marine sediments (Durbin and Teske 2011, Parkes et al 2014). However, very little is known apart from their presence in marine sediments deciphered after 16S rRNA gene clone library studies. The ecological role of marine *Dehalococcoidia* is completely unknown. This study is a contribution for gaining some insights into the ecological role of marine *Dehalococcoidia*, and some understanding of the reasons why marine *Dehalococcoidia* are so abundant and widespread in marine sediments. For that, an *in situ* study in sediments of the Baffin Bay was carried out, investigating the distribution of marine *Dehalococcoidia* in various depths and sites, and the environmental parameters, i.e., depth, geographical location, geochemistry, which may influence such a distribution and abundance. The geochemical parameter of organic carbon was observed to strongly and positively correlate to the abundance of a specific group within the *Dehalococcoidia*, named GIF-9. Other parameters, such as sulphate-, and manganese(II)- concentration negatively correlated with GIF-9 abundance. Interestingly, the clade GIF-9 was present in shelf sediments (site 371), but not in basin sediments (site 453) of the Baffin Bay (Wasmund et al 2015). High differences among the presence and abundance of the members encompassing the class *Dehalococcoidia* were observed depending on the depth and site of the Baffin Bay, suggesting variability in the diversity of *Dehalococcoidia* based on sediment local conditions. In addition, marine *Dehalococcoidia* were observed to persist with depth and account for high percentages (up to 70%) of the total bacterial community at depths where low abundance of total *Bacteria* was detected.

Additionally, an *ex situ* study that relied on the cultivation of marine sediments from various locations and using an anoxic defined minimal medium supplied with various potential electron acceptors was performed to investigate possible respiration modes for *Dehalococcoidia*. Sulphate, iron(III), manganese(IV), and various halogenated compounds were selected as potential electron acceptors that were individually supplied to sediment cultures. Halogenated compounds were chosen because the phylogenetically closest bacteria to marine *Dehalococcoidia* are strict organohalide-respiring bacteria, such as *Dehalococcoides mccartyi* (Löffler et al 2013, May et al 2008, Moe et al 2009). Although dehalogenation of 1,2,3-TCB was observed in this study, it could not be associated to *Dehalococcoidia* growth, suggesting that marine

Dehalococcoidia did not respire the halogenated compounds used here. However, no other electron acceptors tested here promoted a substantial growth of *Dehalococcoidia*, suggesting alternative respiratory modes or fermentation as a mode of living for marine *Dehalococcoidia*.

Altogether, the present study suggests marine *Dehalococcoidia* to be highly diverse displaying different respiratory modes. Some of the marine *Dehalococcoidia* are most likely heterotrophic bacteria, e.g., clade GIF-9 (Hug et al 2013a, Wasmund et al 2014). *Dehalococcoidia* are widespread in marine sediments most likely due to their resilience, i.e., their ability to survive burial, high pressures, long periods of starvation and/or low concentration of nutrients, which is an advantage in an environment depleted in nutrients such as deep marine sediments, and/or may use recalcitrant organic substrates that most other bacteria cannot metabolize.

4.1 *IN SITU* ABUNDANCE OF *DEHALOCOCCOIDIA*

The determination of the abundance of the class *Dehalococcoidia* (phylum *Chloroflexi*) in different marine sediment samples aimed to identify natural conditions, e.g., sediment geochemistry, depth, and geographical location that support *Dehalococcoidia* natural occurrence in an attempt to better understand the ecological role of *Dehalococcoidia* in marine sediments. For this, an *in situ* approach determining the abundance of *Dehalococcoidia* 16S rRNA genes in sediments of the Baffin Bay was performed. The abundance was measured here as "relative abundance" of *Dehalococcoidia* 16S rRNA genes within the total bacterial 16S rRNA genes, and as "absolute abundance", which refers to the number of 16S rRNA gene copies amplified and quantified with a qPCR assay targeting specifically *Dehalococcoidia*. The relative abundance was determined from the amplicon sequencing of 16S rRNA genes performed with Illumina sequencing.

In Baffin Bay samples, members of the class *Dehalococcoidia* were present at all sediment sites and depths, suggesting a widespread distribution, which agrees with the ubiquitous presence of *Dehalococcoidia* found in marine sediments worldwide in previous studies (Fry et al 2008, Parkes et al 2014).

Relative abundance of bacterial classes in Baffin Bay sediments indicated *Dehalococcoidia* as the second most abundant class, which accounted for 6 to 17% of the total bacterial community, after bacteria of the class *Betaproteobacteria* (between 38 to 64%). On the phylum level, the relative abundance for *Chloroflexi* was of 8 to 22%, which was slightly less than the average relative abundance of 25.5% for *Chloroflexi*, as

determined in a review of bacterial communities in various subsurface marine sediments (Parkes et al 2014).

Absolute abundance of *Dehalococcoidia* was around 10^5 16S rRNA gene copy numbers per gram of sediment for most Baffin Bay sediments. Shelf areas showed, however, one order of magnitude higher copy numbers than other areas (Figure 45, Figure 46, and Figure 47, and (Wasmund et al 2015)). Total bacterial numbers were maintained around 10^7 16S rRNA gene copies g^{-1} along the studied sediment profile in shelf sites of the Baffin Bay. However, basin sites of the Baffin Bay showed significant decreasing numbers of total *Bacteria* from nearly 10^8 to 10^4 16S rRNA gene copies g^{-1} with increasing depths (this study Figure 43, and (Algora et al 2013, Wasmund et al 2015)). At similar depth ranges (from seafloor down to 6 mbsf), sediments from other locations showed similar copy numbers of *Dehalococcoidia* and an order of magnitude difference in copy numbers depending on basin/shelf sample origin. For instance, the number of *Dehalococcoidia* was of 10^5 16S rRNA gene copies g^{-1} in basin sediments from the forearc basin in Sumatra, meanwhile shelf sediments from Århus Bay showed one order of magnitude higher copy numbers of 10^6–10^7 g^{-1} (Wasmund et al 2015). The percentage of *Dehalococcoidia* 16S rRNA gene copy numbers in relation to the total bacterial 16S rRNA gene copy numbers showed *Dehalococcoidia* to account for ≤ 10% of the bacteria in shelf sediments of the Baffin Bay (Figure 48 and (Wasmund et al 2015)). Interestingly, *Dehalococcoidia* appears to be a resilient bacterial group that persists with burial and make up to 74% (at a depth of 222 cmbsf at site 391), and more than 10% when low bacterial copy numbers are found at deeper sediment layers, i.e., depths of 374, and 290–320 cmbsf from the shelf sites 371, and 365, respectively; and depths of 150–400 cmbsf at basin sites 389, 391 and 224–277 cmbsf at site 453 (Figure 48 and (Wasmund et al 2015)). Thus, *Dehalococcoidia* may have metabolisms requiring low energy for cell maintenance and/or use recalcitrant substrates, which are not degraded in shallow sediments.

Both absolute and relative abundance for *Dehalococcoidia* were highest at the shelf compared to any other area. Geochemically, the shelf has higher contents of organic carbon than the slope or basin, and highest indications of sulphate reduction, i.e., highest abundance of *dsrA* genes (functional markers for sulphate-reducing microorganisms), and decreasing sulphate concentrations from ~25 to ~20 mM at depths below 4 mbsf. Iron and manganese reduction also occur in the shelf sediments, although at depths of ≥200 cmbsf. In sediments of the Baffin Bay, values for the content of organic carbon strongly correlated with the relative abundance of *Dehalococcoidia,*

and especially, the clade GIF-9 (formerly known as NT-B4, (Reed et al 2002)). Accordingly, a literature review has described an average relative abundance of 41.3% of bacterial 16S rRNA gene sequences belong to the phylum *Chloroflexi* in various shelf sediment sites with high organic carbon contents (Parkes et al 2014). Some examples of sediments containing high organic carbon contents are Mediterranean sapropels and the Peru margin. In sediments from Mediterranean sapropels, almost the whole bacterial community belonged to *Dehalococcoidia* (Coolen et al 2002). In the Peru Margin, an average of 41% of the *Chloroflexi* correlated to 16S rRNA gene libraries from subsurface organic rich shelf sediments (Parkes et al 2014). A recent study specifically found the occurrence of the clade GIF-9 correlated with values of organic carbon content through different depths of a single core from mud flats of Helgoland, North Sea (Oni et al 2015). Previous studies have also suggested that the GIF-9 clade is associated to organic-rich, as well as methane-bearing marine sediments (Harrison et al 2009, Takeuchi et al 2009, Teske et al 2011). Together, previous studies and this study from Baffin Bay sediments indicated that members of the GIF-9 are associated with organic rich sedimentary environments. The GIF-9 clade of the *Dehalococcoidia* may therefore have an ecological role in the carbon cycle for the degradation of organic matter; however their exact role, e.g., whether they are involved in a primary or secondary stage of the degradation of organic matter, or their metabolisms, remain currently unknown.

Further indications about the ecological role and metabolism of the GIF-9 clade can be inferred from the specific profiling of *Dehalococcoidia* 16S rRNA genes in sediments of Baffin Bay sites 371 and 453 (Wasmund et al 2015). In general, *Dehalococcoidia* were more diverse at shelf site 371 than at basin site 453. Site 371 contains higher organic carbon content than site 453. Interestingly, the clade GIF-9 accounted for a high percentage of the *Dehalococcoidia* present in sediments of site 371, and especially at a depth of 173 cmbsf. However, no *Dehalococcoidia* belonging to the GIF-9 clade was present at site 453. Clade GIF-9 therefore appears to be a *Dehalococcoidia* clade inhabiting shelf sediments rich in organic matter, as they were also found in high percentages in Århus Bay sediments and at the Peru margin site 1227, which at depths of 60 mbsf accounted for the 95% of the total *Dehalococcoidia* present in the sample (Inagaki et al 2006, Wasmund et al 2015). Interestingly, GIF-9 was absent at site 453 of the Baffin Bay basin and also at sites from the Arctic Mid-Ocean Ridge cores GC6 and GC12 (Wasmund et al 2015), where low organic matter content were found (Jørgensen et al 2012). Therefore, microbial ecology studies in the Baffin Bay and other areas give

indications that GIF-9 may be heterotrophic or fermenting bacteria thriving in organic-matter rich sediments from coastal margins containing complex organic matter.

Recently, genomic content from members of GIF-9 clade were obtained. These include a single cell named DEH-J10 retrieved from Århus Bay sediments (Wasmund et al 2014), a draft genome curated from metagenomic data named RBG-2 from sediments of an aquifer next to the Colorado River (Hug et al 2013a), and a partial (~35%) single cell named Dsc1 retrieved from site 1230 of the Peru Margin (Kaster et al 2014). Insights into the metabolism of DEH-J10 indicated genes encoding for enzymes involved in β-oxidation of organics, aromatic compounds, and other organics completely to CO_2 via the Wood-Ljungdahl pathway (this pathway is reversible and can function for autotrophic CO_2 fixation), in addition to fermentation (Wasmund et al 2014). Thus, a heterotrophic, fermentative metabolism is most likely for DEH-J10, which may explain the strong association of GIF-9 clade to organic matter in sediments of the Baffin Bay. Furthermore, RBG-2 annotations indicated homoacetogenic metabolisms, producing acetate as final product of the fermentation of glucose or plant polymers, i.e., pyrogallol. Therefore, RBG-2 may have a saprophytic role in aquifer sediments. Additionally, the RBG-2 genome encodes for the degradation of fatty acids via β-oxidation and also the Wood-Ljungdahl pathway (Hug et al 2013a). Thus, both DEH-J10 and RBG-2 genomic insights agree with the hypotheses put forward based on ecological observations, providing further indications that the GIF-9 clade is most likely directly involved in the remineralisation of organic matter and performs fermentation. These features may be the reason why GIF-9 is commonly found in organic-rich areas.

Furthermore, more than 90% of the OTUs affiliated to GIF-9 strongly negatively correlated to sulphate concentration (Figure 54 and (Algora et al 2015)). In sediment depth profiles from a site in Århus Bay and site 1227 of the Peru margin, clade GIF-9 was detected in sediment depths with and without sulphate (Inagaki et al 2006, Wasmund et al 2015). In Århus Bay, GIF-9 accounted for the highest relative proportion of total *Dehalococcoidia* in sediments with low sulphate concentration. In the Peru margin, highest relative proportion of GIF-9 from the total *Dehalococcoidia* was found underneath the sulphate-methane transition zone. The presence of GIF-9 in sediments with low or no sulphate in Århus Bay and Peru margin, together with the strong negative correlation shown in Baffin Bay sediments, indicate that GIF-9 members most likely are not sulphate-reducing bacteria, or that they are linked to high-affinity sulfate reduction, e.g., through syntrophic associations with microbes specialised for the reduction of low concentrations of sulphate (Tarpgaard et al 2011).

Further, no genes encoding for sulphate respiration and for a respiratory metabolism in general, were found in both DEH-J10 and RBG-2.

Further research to get more insights into the metabolisms and ecological roles of bacteria of the class *Dehalococcoidia*, and especially the clade GIF-9, may include cultivation with different organic substances to document possible growth, as well as cultivation-independent studies such as stable isotope probing and activity measurements in sediments. In this line, it will be very helpful to investigate the organic matter composition associated to GIF-9 members to suggest potential organic substrates for GIF-9 bacteria cultivation and also as correlation studies in the field.

4.2 ELECTRON ACCEPTORS IN THE BAFFIN BAY

The Baffin Bay is located in the Arctic, between Canada and Greenland. It is a remote area, covered by ice during the great majority of the year except for the summer months, thus, affecting primary production, sedimentation rates, and organic content in its sediments. Few studies have been carried out so far in sediments of the Baffin Bay. Here, I studied the geochemistry and the indigenous microorganisms inhabiting the Baffin Bay sediments at various sites and depths for gaining understanding of the element cycling in its sediments (Algora et al 2013, Tang et al 2004). Various locations were investigated including shelf, slope, and basin sites. In general, shelf sediments had highest content of organic matter and sulphate decreases in concentration, indicating that sulphate reduction was an important mineralization pathway for organic matter. Additionally, highest bacterial and archaeal 16S rRNA gene copy numbers, and highest bacterial diversity over nearly entire core lengths was observed in shelf sediments. Highest *dsrA* gene copy numbers in shelf sediments revealed that most likely sulphate was the main terminal electron acceptor for microorganisms. On the contrary, sediments from the basin and slope had comparably much lower content of organic matter, which was mainly mineralized by iron(III)- and manganese(IV)-reduction. Depth affected more dramatically bacterial and archaeal abundance and diversity in basin sites, with highest 16S rRNA gene copy numbers and OTUs in uppermost sediments, which decreased substantially with increasing depths. 16S rRNA gene copy numbers from members of the order *Desulfuromonadales,* which encompasses known metal reducers such as the genera *Geobacter* and *Desulfuromonas*, revealed no association with iron or manganese concentrations. Thus, other metal reducers may be present in Baffin Bay sediments which may mediate the reduction of iron(III) and manganese(IV) oxides. Altogether, the analysis of the geochemistry and the microbial communities revealed

strong differences based on the local sedimentary conditions. These differences were especially evident between shelf and basin sites.

4.2.1 Sulphate reduction and methanogenesis in shelf sediments

Due to the high concentration of sulphate in seawater, sulphate is the quantitatively major electron acceptor for the microbial mineralization of organic matter in many marine sedimentary environments (D'Hondt et al 2002, Jørgensen 1982). In the samples measured here from sediments of the Baffin Bay, sulphate was available down to a depth of 5 mbsf. At shelf and slope sites, as well as at basin site 453, sulphate concentrations decreased with increasing depths, indicating that sulphate reduction in sediments of the Baffin Bay occurs. Sulphate reduction was also measured in slurries prepared with and without various substrates including hydrocarbons, monomeric and polymeric carbohydrates (i.e., chitin, cellulose, peptone) from Baffin Bay sediments (Algora et al 2013). Potential rates of sulphate reduction in the slurries indicated the shelf as the most active area in sulphate reduction as a result of highest organic carbon content compared to other sampled areas, which agrees with previous reports from Arctic sediments. In those reports, *in situ* sulphate reduction was limited by organic carbon content independently of the temperature (Arnosti and Jørgensen 2006, Vandieken et al 2006). Methane concentrations in Baffin Bay sediments were very low indicating that the sulphate-methane transition zone was below the studied sediment layers. Previous pore-water measurements of the ODP site 645 showed depleted concentrations of sulphate at 35 mbsf (Srivastava et al 1989). A plausible explanation for such a deep sulphate-methane transition zone compared to other locations in the world may be the low organic content. Low TOC values were especially found at basin sites where long water columns and distance to land result in decreased sedimentation of organic matter. Distance to land is critical for organic matter content in Baffin Bay sediments due to low primary productivity in the overlying water column, which is the result of seasonal ice cover. Terrestrial origin of the organic matter is indicated by the isotope value of the organic carbon (Algora et al 2013, Sackett 1964, Srivastava et al 1989). Thus, shelf areas have increased organic carbon coming from Greenland.

Sulphate reducing bacteria use sulphate as terminal electron acceptor in their respiratory chains. The enzyme dissimilatory sulphite reductase (Dsr), encoded by the genes *dsrA and B*, catalyses the final reaction in sulphite reduction, which is the reduction of sulphite to sulphide, and is present in all known sulphite/sulphate reducers using the canonical sulphite/sulphate reduction pathway (Wagner et al 1998). Therefore, *dsrA* genes are used as a proxy for presence and quantification of sulphate reducing

microorganisms in marine sediments (Kondo et al 2004). *dsrA* genes were abundant in Baffin Bay sediments, indicating the potential for sulphate reduction at all depths and sites. The abundance of *dsrA* genes in Baffin Bay sediments was higher than at other locations such as the Peru margin ODP site 1227 or the Wadden Sea in Germany (Blazejak and Schippers 2011, Schippers and Neretin 2006, Wilms et al 2007). Within Baffin Bay sites, *dsrA* gene abundance was highest in shelf sediments and Southern slope site 486, indicating increased numbers of sulphate reducing microorganisms at sites with higher organic carbon content.

The gene encoding for the alpha subunit from the methyl coenzyme M reductase (*mcrA*) is present in methanogenic and anaerobic methanotrophic archaea (Friedrich 2005, Hallam et al 2003, Nunoura et al 2008). *mcrA* gene abundance was used here for the detection and quantification of methanogens and methanotrophs. Both low methane concentrations and low abundance of *mcrA* indicated a minor role of methanogenesis and/or methanotrophy in sediments of the Baffin Bay. Only in the shelf area, increased *mcrA* gene copy numbers evidenced the presence of methanogens, although at low abundance. Interestingly, slurries prepared with shelf sediments and the aliphatic hydrocarbons hexadecane and hexadecanoic acid showed methane formation (Algora et al 2013). However, methane was not formed in shelf sediment slurries amended with aromatic hydrocarbons (Algora et al 2013). As a conclusion, methanogenesis and methane oxidation do not play a pronounce role in the Baffin Bay in the sediment depths analysed. Most likely, the low organic carbon content in Baffin Bay sediments is mineralized using electron acceptors other than CO_2, i.e., sulphate, iron, or manganese, which yield higher energy. Therefore, organic matter quantity and availability are driving the element cycles in marine sediments of the Baffin Bay as in many other marine sediments worldwide inhabited by heterotrophic microorganisms.

4.2.2 Importance of metal biogeochemistry in the central Baffin Bay

In the Baffin Bay, accumulation of Mn(II) and Fe(II) in pore-water profiles indicated the reduction of manganese and iron oxides (i.e., MnO_2 and Fe_2O_3, respectively) from depths of 0.25 mbsf down to 4.7 mbsf. Thermodynamically, Mn(IV) provides highest energy yields followed by Fe(III) and then by sulphate, in the anoxic mineralization of organic matter mediated by microorganisms (Froelich et al 1979). Mn(IV) and Fe(III) are reduced to Mn(II) and Fe(II) in this process. The reduced forms were observed in high concentrations in pore-water profiles from the central basin sites indicating that metal element cycling is important in basin sediments of the Baffin Bay. Basin sediments were characterized by low organic carbon contents, which may be mainly

mineralized via Mn(IV) and Fe(III) reduction as organic carbon content decreases at those depths where Fe(II) and Mn(II) concentration in pore-water increased. Generally, low concentrations of manganese oxides are found in marine sediments (≤ 20 µmol cm^{-3}) and at shallow depths of ≤ 2 cmbsf (Thamdrup 2000). Thus, manganese is rapidly depleted in surface sediments, and therefore collectively plays a minimal role for the mineralization of the bulk of organic matter, especially in marine sediments rich in organic matter (Nealson and Saffarini 1994, Sørensen and Jørgensen 1987, Vandieken et al 2006). However, some sediment sites have high manganese oxide concentrations of 25 to 185 µmol cm^{-3}, such as sediments from the Panama Basin, the Black Sea, the Barents Sea and some parts of the Skagerrak (Canfield et al 1993, Nickel et al 2008, Thamdrup 2000, Vandieken et al 2006), where manganese oxides are found reaching depths down to 410 cmbsf. Manganese reduction contributes from 25% to nearly 100% to the anaerobic carbon oxidation at those sites. In particular, in Arctic sediments from the Barents Sea, Mn(IV) and Fe(III) are reported to contribute between 69% to more than 90% to the mineralization of organic matter within near-surface sediments of 10 cm (Vandieken et al 2006). Mn(IV) and Fe(III) reduction processes are therefore of importance as the main respiratory pathways for the carbon mineralization in Arctic sediments (Vandieken et al 2006). A reason for metal cycling predominance in polar areas may be that polar ice sheets are enriched in iron, manganese and organic matter (Lannuzel et al 2014). During the ice melt, iron, manganese and organic matter fuel phytoplankton and microbial growth in the seawaters (Lannuzel et al 2013, Lannuzel et al 2014, Martin and Fitzwater 1988). Some of the iron and manganese is deposited on the seafloor, usually associated with organic particles and/or sediment grains (Lannuzel et al 2014, Raiswell et al 2006). Apart from sea-ice, glacial melt-water and glacial sediments transported at the base of icebergs additionally contribute to the release of bioavailable iron into the ocean waters (Bhatia et al 2013, Raiswell et al 2006). The Baffin Bay is ice-covered most of the year except the summer months (Tang et al 2004). Therefore, Baffin Bay sediments may be supplied with manganese, iron, and organic matter from the melting of the sea-ice and the surrounding glaciers.

Well-known bacterial groups associated to iron and manganese reduction are members of the order *Desulfuromonadales*, class *Deltaproteobacteria* (Childers et al 2002, Lovley et al 2004). In sediments of the Baffin Bay, members of the *Desulfuromonadales* were detected. *Desulfuromonadales* 16S rRNA gene abundance followed a similar pattern to total bacterial abundance with depth, independently of Fe(II) and Mn(II) concentrations in the pore-water. Furthermore, no positive correlation was found between the relative abundance of the class *Deltaproteobacteria* and Fe(II)

or Mn(II) pore-water concentrations (Figure 54). On the contrary, 40% of the OTUs affiliated to *Deltaproteobacteria* showed a negative correlation to Mn(II) pore-water concentration. This suggests that *Deltaproteobacteria* are not predominantly involved in the reduction of Fe(III) and Mn(IV) in Baffin Bay sediment pore-water. In the microbial community analysis of Baffin Bay sediments, the class *Betaproteobacteria* was dominant, especially in basin sediments. Pore-water concentration of Mn(II) negatively correlated with microbial diversity (Algora et al 2015), indicating the enrichment for a few specialized microbial groups, i.e., members of the class *Betaproteobacteria*, which may be the bacteria performing the largest share of the Mn(IV) reduction, and therefore oxidation of organic matter in the Baffin Bay.

4.3 *BURKHOLDERIALES* DOMINATE IN BAFFIN BAY SEDIMENTS

The class *Betaproteobacteria*, with a relative abundance of 38 to 64%, as determined from 16S rRNA gene amplicon sequencing, was dominant in all analysed sediments from the Baffin Bay. The average presence of *Betaproteobacteria* in marine sediments is of 5% according to various studies from subseafloor sediments bordering the Pacific Ocean, reviewed by (Fry et al 2008), and of 2% according to (Parkes et al 2014), which reviewed bacterial communities from various subseafloor sediment studies worldwide combining 205 prokaryotic 16S rRNA gene libraries. Thus, sediments from the Baffin Bay are characterized by a considerably high abundance of *Betaproteobacteria* in their bacterial community. Other marine sediments with high abundance of *Betaproteobacteria* that substantially exceed the 2–5% average were found in the Cascadia Margin site 889/890 (relative abundance of betaproteobacterial clones of 20% at 9 mbsf, 55% at 198 mbsf, 47% at 222 mbsf, and 5% at 234 mbsf (Marchesi et al 2001)). Betaproteobacterial clones from the Cascadia Margin site 889/890 were phylogenetically most similar to *Ralstonia pickettii*, which belongs to the order *Burkholderiales*. *R. pickettii* is a heterotrophic bacterium inhabiting oligotrophic habitats (Ryan et al 2007) that include metal-enriched environments such as in acid mine drainage (Kimura et al 2011), and in deep-sea basalts and sediments from the Mid-Atlantic Ridge (Rathsack et al 2009).

Within the class *Betaproteobacteria*, 99.7% on average of the clones found in sediments of the Baffin Bay belonged to the order *Burkholderiales*. The order *Burkholderiales* encompasses a metabolically diverse group of bacteria that includes aerobic, facultative anaerobic, diazotrophic, chemoorganotrophic, and chemolitotrophic microorganisms (Garrity et al 2005). Interestingly, *Burkholderiales* genomes encode many oxygenases able to degrade a wide range of aromatic compounds (Pérez-Pantoja et al 2012).

In the Baffin Bay, ≥ 90% of OTUs affiliated to *Burkholderiales* positively correlated to Mn(II) concentrations and negatively to organic matter content. In basin and slope sites, as well as deep sediment layers from shelf site 365, higher relative abundance of *Burkholderiales* in accordance to lower organic matter content and higher Mn(II) concentration were detected. These results, together with the negative correlation of pore-water Mn(II) concentration with the microbial diversity (measured as number of OTUs) already mentioned in the previous chapter, suggest that *Burkholderiales* may be involved in manganese cycling. To my knowledge, no studies in the marine subsurface link *Burkholderiales* abundance to the concentration of manganese. However, in the terrestrial subsurface, an association of the order *Burkholderiales* with metal cycling was observed after the isolation of *Rhodoferax ferrireducens* from aquifer subsurface sediments of Oyster Bay, Virginia, USA (Finneran et al 2003). *R. ferrireducens* is a psychrotolerant bacterium able to use Fe(III)- and Mn(IV)- oxides, nitrate, fumarate and oxygen as electron acceptors, with acetate or lactate as electron donors (Finneran et al 2003). Indeed, many iron reducers are also able to reduce manganese oxides (Lovley et al 2004, Nealson and Saffarini 1994). A couple more studies in the terrestrial subsurface found *Burkholderiales* associated to Fe(III) reduction in either contaminated groundwater or sediment, both after stimulation with acetate (Handley et al 2014, Livermore et al 2013). Further terrestrial environments include ferromanganese nodules either in natural freshwater sediments (Stein et al 2001) or in anthropogenic sugarcane and rice paddy fields (Hu et al 2015). Identified *Burkholderiales* in the ferromanganese nodules belonged to the genus *Leptothrix*, which are known to oxidize Mn(II) and Fe(II) coupled to oxygen or nitrate reduction (Carlson et al 2013, De Vrind-De Jong et al 1990, Stein et al 2001). However, in the Baffin Bay subsurface, the *Burkholderiales* are more probable associated to the Mn(IV) and Fe(III) reduction than Mn(II) and Fe(II) oxidation, as deduced from the accumulation of the soluble metal forms Mn(II) and Fe(II) in the pore-waters. In addition, Fe(II) accumulation at the bottom of cores 365 and 371 are associated with higher relative abundance of *Burkholderiales*. Interestingly, the bacterial community in deep sediment layers from sites 365 and 371, where increased Fe(II) concentrations were observed, cluster together with samples from the basin sites where the metal cycling of both manganese and iron prevails. Thus, I hypothesize that *Burkholderiales* are possibly reducing both Mn(IV) and Fe(III) in the Baffin Bay. Future studies on cultivation of Baffin Bay sediments in microcosms amended with Fe(III) and Mn(IV) may lead to clear relationships of betaproteobacterial growth associated to reduction of metal oxides.

Burkholderiales are prevalent in glaciers or glacially-associated environments. Examples for this are an ice-core from Greenland frozen for over one-thousand-years (Sheridan et al 2003), permanent lake ice from Antarctica (Gordon et al 2000), moraine chronosequences in the primary succession of a recently deglaciated and unvegetated soil from a Peruvian receding glacier (Nemergut et al 2007), Arctic moraines (Mapelli et al 2011), and subglacial sediments (Carr et al 2013, Foght et al 2004, Lanoil et al 2009, Skidmore et al 2005, Stibal et al 2012, Yde et al 2010). The Baffin Bay is ice-covered for the whole year except the summer and receives sediments and water from glacial melt (Bhatia et al 2006, Stibal et al 2012, Wadham et al 2008). I therefore hypothesize that the sedimentary *Burkholderiales* detected in the Baffin Bay could come from the sediments underneath glaciers in Greenland and Canada. The *Burkholderiales* may be transported by icebergs and released to the Baffin Bay waters attached to sediment particles or iron aggregates after the ice-melt. Thus, *Burkholderiales* may colonize sediments from the Baffin Bay, similarly to the way that iron and manganese particles or organic matter are delivered to the oceans and later on to the sediments (Lannuzel et al 2014, Raiswell et al 2006). *Burkholderiales* can be aerobic and facultative anaerobic bacteria using nitrate or iron for respiration (Willems et al 1991). For example, facultative anaerobic bacteria from the genus *Rhodoferax* (family *Comamonadacea*; order *Burkholderiales*) may reduce iron (Stibal et al 2012, Willems et al 1991). *Rhodoferax*–related bacteria have been commonly detected in basal ice from glaciers (Lanoil et al 2009, Skidmore et al 2005, Yde et al 2010). *Rhodoferax* can deal with oxic conditions, which is an advantage compared to other iron reducers such as members of the *Geobacteraceae* family (order *Desulfuromonadales*; phylum *Deltaproteobacteria)* in periods with oxic-anoxic conditions such as glacial sediments or sea-ice where melting occurs annually (Yde et al 2010), and may be the reason of *Burkholderiales* predominance in sediments of the Baffin Bay over *Desulfuromonadales*. Therefore, *Burkholderiales* may change from aerobic to nitrate to Mn(IV) and Fe(III) respiration in sediments for adaptation in glacial sediments in Greenland, as already suggested (Stibal et al 2012), and may be the case for marine sediments of the Baffin Bay, which may be the key to their dominance in Baffin Bay sediments.

4.4 *DEHALOCOCCOIDIA* CULTIVATION

The close phylogenetical affiliation of *Dehalococcoides mccartyi*, *Dehalogenimonas lykanthroporepellens, Dehalogenimonas alkenigignens,* and *Dehalobium chlorocoercia strain DF-1*, which are all strict organohalide-respiring bacteria (Löffler et al 2013, May

et al 2008, Moe et al 2009), to marine *Dehalococcoidia* members may suggest an organohalide-respiring metabolism of marine *Dehalococcoidia* (Adrian 2009). However, the presence of organohalide compounds in the sediment cultures did not enhance growth of marine *Dehalococcoidia*, suggesting that either they use other organohalides not tested here or they do not use organohalides. Interestingly, I observed the conversion of 1,2,3-TCB to 1,3-DCB in one sediment culture, which was further transferred. In the subculture, the transformation of 1,2,3-TCB to 1,3-DCB was observed together with marine *Dehalococcoidia* growth of one order of magnitude within two months of incubation time. However, organohalide respiration could not be linked to marine *Dehalococcoidia* growth as further transfers failed to transform 1,2,3-TCB, maybe due to the transfer of low biomass within the inoculum, or exposure to inhibitory concentrations of 1,2,3-TCB. Cloned sequences of the *Dehalococcoidia* present in that subculture revealed an affiliation to *Dehalococcoidia* sister-clade DSC-D, falling far from the Ord-DEH cluster of the known dehalogenating *Dehalococcoidia* (name of clades as indicated by (Wasmund et al 2015)). Further sediment cultivation amended with 1,2,3-TCB revealed members of the phylum *Firmicutes* as the bacteria transforming 1,2,3-TCB to 1,3-DCB. Regarding the wide diversity and high abundance of the *Dehalococcoidia* in marine sediments, alternative metabolisms other than organohalide respiration are plausible. In fact, recent genomic data from four marine *Dehalococcoidia* did not contain genes related to organohalide respiration, i.e., RDases (Hug et al 2013a, Kaster et al 2014, Wasmund et al 2014).

In this study, no electron acceptor among those tested was observed to preferably and substantially support growth of *Dehalococcoidia* in the various sediments. All tested electron acceptors resulted in similar *Dehalococcoidia* 16S rRNA gene copy numbers, indicating a lack of a respiratory metabolism with these electron acceptors, or that the preferred electron donors/vitamins/minerals were not supplied, or that growth rates were not detectable within the available incubation time. Thus, marine *Dehalococcoidia* may be fermenters, as already suggested by the *in situ* approach and genomic studies (Hug et al 2013a, Wasmund et al 2014). An alternative explanation may be that the *Dehalococcoidia* have diverse metabolisms, including both respiration and fermentation. Metabolic diversity within the class *Dehalococcoidia* may explain the patchy *Dehalococcoidia* abundance observed for some sediments amended with electron acceptors and monitored at different incubation times. For example, cultures prepared with sediments originating from Århus and a site in Ireland gave some indications of sulphate as potential electron acceptor favouring the growth of marine *Dehalococcoidia*. In addition, a single cell retrieved from Århus sediments named

DEH-C11, which belongs to the class *Dehalococcoidia* (specifically to the *Dehalococcoidia* sister-clade DSC-GIF3-B), contains a *dsr* gene operon as well as genes for arsenate reduction, suggesting that *Dehalococcoidia* may, in fact, respire certain compounds (Dr. Kenneth Wasmund, personal communication). On the other hand, *Dehalococcoidia* abundance in sediment cultures from Chile varied randomly, and clearly showed that none of the tested electron acceptors favoured growth. Although variability in the abundance of *Dehalococcoidia* could be due to heterogeneities or/and errors in the sampling and analysing method (sediment sub-sample extraction together with DNA isolation and qPCR assay), a test trial indicated this method as reliable for total bacterial identification. Altogether, *Dehalococcoidia* variable abundance indicates diverse metabolisms, as already suggested by *in situ* studies (Wasmund et al 2015) and as previously mentioned. In this case, an approach whereby each separate clade is targeted by quantitative methods such as qPCR may be most appropriate than per whole class, since the quantification of some clades and therefore their growth may be masked by changes in quantities of others. For instance, an approach to investigate if the clade GIF-9 encompasses fermenting microorganisms, or if the clade DSC-GIF3 includes potential sulphite/sulphate reducers, as recently suggested by DEH-C11 single-cell study (Dr. Kenneth Wasmund, personal communication), might be more suitable than investigating the entire class *Dehalococcoidia*. Moreover, addition of a sediment inoculum less than 9–10% used here may be beneficial, as the inoculated sediment supplies with nutrients and electron acceptors, e.g. sulphate, which may additionally support microbial growth independently of the compounds added by the medium.

Although no clear respiratory mode could be deciphered, *Dehalococcoidia* were detected in the sediment cultures and 16S rRNA gene copy numbers were maintained with time indicating that it is possible to cultivate them using a minimal mineral medium under atmospheric pressures and at temperatures of 30ºC. Therefore, the marine *Dehalococcoidia* are not obligate piezophilic or psychrophilic bacteria. For instance, *Dehalococcoidia* numbers increased one order of magnitude after an incubation time of two months in a subculture amended with 1,2,3-TCB. Thus, growth rates for marine *Dehalococcoidia* members are not of hundreds of years as may be thought for subseafloor microorganisms. Previous authors suggested that subsurface bacteria may be damaged when exposed to high substrate concentrations due to uncoupling of reactions (Parkes et al 2014). However, it seems not to be the case either for the used medium here, or for the marine *Dehalococcoidia* bacteria.

Nevertheless, a transferable enrichment of marine *Dehalococcoidia* was not achieved and a plausible reason may be that *Dehalococcoidia* were out-competed by other faster growing organisms such as those from the phylum *Firmicutes*, which were observed to be present in some of the sediment cultures by clone libraries and 454-pyrosequencing, in particular, in enrichments from Chile site 7155 (discussed below). In addition, all investigated colonies that were formed in deep-agarose dilution tubes from Chile site 7155 (the rest of sites and sediments showed very few to no colonies) belonged to members of the *Firmicutes*. Possible ways to eliminate fast growing Gram-positives are using antibiotics specific for Gram-positive cell types, i.e., ampicillin, vancomycin, penicillin, in order to avoid out-competition. Additionally, using a medium with very low nutrient concentrations to avoid spore germination or even cultivating at low temperatures may be also favourable for cultivation of indigenous microorganisms in marine sediments.

Isolation of marine *Dehalococcoidia* in deep-agarose dilution tubes was not possible. A possible reason is that the *Dehalococcoidia* may form too small or non-visible colonies as indeed other cultivated *Dehalococcoidia* such as *Dehalococcoides mccartyi* strain CBDB1 colonies are hardly visible. In addition, some microorganisms may not be isolated as pure strain, but may live in symbiosis or syntrophy with other strains or may need other microorganisms for, e.g., quorum sensing, so isolation may also be difficult or even impossible if the metabolite exchange between microbial species is not known.

Altogether, marine *Dehalococcoidia* are able to be maintained in culture under varying conditions, e.g., Mn(IV)-, Fe(III)-, sulphate-, and bicarbonate-reducing conditions. Nevertheless, these organisms are still hard to isolate. To my knowledge, no previous study has enriched or maintained in sediment cultures members of the marine *Dehalococcoidia*, so it is not possible to compare with such other reports for obtaining further overall conclusions. From my perspective, it may be reasonable to hypothesize that enrichments of *Dehalococcoidia*, which may lead to cell densities high enough to give improved chances of isolation, were not possible due to lack of suitable substrates, e.g., electron donor/acceptor combinations. Better knowledge on the chemical nature of the complex organic matter present in marine sediments may substantially aid for this endeavour of suppling suitable substrates for cultivation of marine *Dehalococcoidia*.

4.5 DEHALOGENATION OF ORGANOHALIDES IN SEDIMENT CULTURES

In sediment cultures from Chile, 1,2,3-TCB was transformed to 1,3-TCB. Previous studies in the marine subsurface also observed the transformation of organohalides, i.e.,

2,4,6-tribromophenol, 2,4,6-triiodophenol, and TCE in deep marine sediments from the Nankai Trough, Japan (Futagami et al 2009, Futagami et al 2013). Thus, organohalide respiration may occur in deep marine sediments. Organohalides are naturally produced in the marine environment (Gribble 2003) and buried to marine sediments. Thus, organohalides may be potential electron acceptors in the marine subsurface. Interestingly, the marine subsurface is most likely one of the very few environments on Earth that may be truly uncontaminated, i.e., deep sediments deposited prior to the industrial revolution are completely pristine. Therefore, the marine subsurface is a well-suited environment to study the natural cycle of halogens. Reductive dehalogenase homologous gene (*rdhA*) sequences have been amplified from various subsurface marine sediments (Futagami et al 2009, Futagami et al 2013), however not always associated to dehalogenation activities (Futagami et al 2013). The organohalide 1,2,3-TCB was never observed to be transformed by autochthonous microbiota in deep marine sediments. Interestingly, chlorinated compounds are usually more resistant to microbial attack than brominated compounds, although all can be transformed by specialist bacteria such as *Dehalococcoides mccartyi* strain CBDB1 (Cooper et al 2015, Wagner et al 2012, Yang et al 2015). To study the dehalogenating capacity of the sediment cultures transforming 1,2,3-TCB, my colleague Myriel Cooper (UFZ–Leipzig) performed activity tests using a methyl viologen-based resting cell activity assay (Cooper 2015). Various brominated compounds were selected for the activity test because brominated compounds are more common than chlorinated compounds in the marine environment (Ballschmiter 2003). Activity was found for 2,4,6-tribromophenol, 2,4- and 2,6-tribromophenol, 1,2,4-tribromobenzene, and 4-bromo-3,5-dimethoxybenzoic acid (Cooper 2015). Hexachlorobenzene, hexabromobenzene, PCE and TCE were not transformed. Most likely, the microorganisms responsible for the organohalogen transformations may have a smaller set of reductive dehalogenases (RDases), than for instance, *Dehalococcoides mccartyi* strains, resulting in a narrower catalytic spectrum. Moreover, I was not successful in amplifying *rdhA* genes with the primers available in the literature that target *rdhA* genes from *Dehalococcoides mccartyi*, *Dehalobacter restrictus* and *Desulfitobacterium* spp. (Krajmalnik-Brown et al 2004, von Wintzingerode et al 2001). However, biochemical analysis using 2-iodopropane as a specific inhibitor for B_{12} enzymes indicated a potential RDase was performing the 1,2,3-TCB transformation reaction with a cobalamin as a reactive centre of the enzyme (Cooper 2015). Therefore, the *rdhA* sequence of the sediment cultures may differ from the *rdhA* of *Dehalococcoides mccartyi*, *Dehalobacter restrictus* and *Desulfitobacterium* spp., suggesting the need to improve *rdhA* primers to further

evaluate the potential of organohalide transformation by molecular biology studies in marine deep sediments. On the contrary, many deep marine sediments that amplified *rdhA* genes with the available primers showed no activity when exposed to organohalides in cultures (Futagami et al 2013). Thus, there is a need for better primers for functional *rdhA* genes in the marine subsurface. Other PCR-independent approaches such as metagenomics could also be an avenue to explore marine subsurface *rdhA* genes in the future.

The microorganisms transforming 1,2,3-TCB to 1,3-DCB in sediment cultures were Gram-positives, as indicated by the lack of organohalide transformation when amended with either ampicillin or vancomycin. The microbial community in the cultures was dominated by members of the phylum *Firmicutes*. Interestingly, members of the *Firmicutes* have been suggested as bacteria transforming naturally occurring organohalides in uncontaminated environments (Krzmarzick et al 2014). Additionally, several pure strains belonging to *Firmicutes*, such as *Dehalobacter* spp. and *Desulfitobacterium* spp., are described as dehalogenating organohalogens (Gerritse et al 1996, Holliger et al 1998, Miller et al 1997, Nelson et al 2014, Utkin et al 1994).

The type of the reducing agent used, i.e., titanium (III) citrate or sodium sulphide plus L-cysteine, had an effect on the composition of the microbial communities as deciphered by 454-pyrosequencing of 16S rRNA genes. Sediment cultures with either reducing agents transformed 1,2,3-TCB to 1,3-TCB, and for both cases, the microorganisms transforming 1,2,3-TCB belonged to the phylum *Firmicutes*, as indicated by inhibitor studies. Interestingly, in sediment cultures reduced with titanium (III) citrate, the genus *Anaerobacter* took over nearly the entire bacterial community with a relative abundance of 90% in the most enriched subcultures, which were transferred four times. *Anaerobacter* does not appear in the sediment culture line reduced with sodium sulphide plus L-cysteine suggesting that it may ferment citrate, and thus titanium (III) citrate enriched for *Anaerobacter.* Therefore, the use of a specific reducing agent, as any medium component, strongly affects the microbial community, especially if it is an organic reducing agent.

For sediment cultures (generation 0) reduced with titanium (III) citrate, members affiliated to *Dehalobacter* and *Desulfitobacterium* were observed, indicating a potential role for organohalide transformation. However, *Desulfitobacterium* relative abundance was very low, and did not increase with dehalogenation and enrichment in any sediment culture or subculture reduced with any of the reducing agents used here. However, *Desulfitobacterium* 16S rRNA gene copy numbers increased with dehalogenation and

with respect to the start point of incubation for one of the two sediment cultures investigated, which dehalogenated 1,2,3-TCB. Nevertheless, *Desulfitobacterium* are metabolically versatile bacteria that can use other electron acceptors than organohalides (Villemur et al 2006), and thus an increase in numbers in one sediment culture may not indicate a role in the organohalide transformation. Most likely, the genus *Desulfitobacterium* does not play a substantial role in the transformation of 1,2,3-TCB in the sediment cultures and enrichments.

On the other hand, *Dehalobacter* relative abundance increased with time (forming 3% of the total bacterial community) while dehalogenation occurred in sediment cultures reduced with titanium (III) citrate. Therefore, the genus *Dehalobacter* may play a role for the dehalogenation of 1,2,3-TCB as they are known obligate organohalide-respiring microorganisms, which cannot derive energy via fermentation or respiration of other substrates (Holliger et al 1998, Yoshida et al 2009). Additionally, *Dehalobacter* are known for respiring both aliphatic, e.g., PCE (Holliger et al 1998), and aromatic, e.g., DCBs (Nelson et al 2014). However, no increase in 16S rRNA gene copy numbers was observed in any of the investigated sediment cultures reduced with titanium (III) citrate. This may be due to the use of primers specific for *Dehalobacter restrictus,* which may not match the *Dehalobacter* sequences in the sediment cultures. Interestingly, no increase in *Dehalobacter* relative abundance was observed in sediment cultures reduced with sodium sulphide plus L-cysteine. Thus, most likely, *Dehalobacter* had no role in dehalogenation in those cultures.

Desulfotomaculum has also been documented to transform organohalides (Duan 2014). In particular, *Desulfotomaculum guttoideum* strain VN1 is reported to dechlorinate 1,2,4- and 1,2,3-TCB, and 1,2-DCB, and debrominate hexabromobenzene, 1,2,4-tribromobenzene and monobromobenzene to benzene (Duan 2014). Interestingly, strain VN1 used citrate as an electron donor for dechlorination (Duan 2014). Members affiliated to *Desulfotomaculum* are observed in sediment cultures reduced with titanium (III) citrate, with an increase in their relative abundance from 2% at the start point to 15% during dehalogenation. Thus, *Desulfotomaculum* may play a role in the 1,2,3-TCB dehalogenation in sediment cultures reduced with titanium (III) citrate, and as observed for strain VN1, which was isolated from freshwater sediments, may use citrate as an electron donor in dechlorination. The use of citrate as an electron donor is likely as the relative abundance of *Desulfotomaculum* in sediment cultures reduced with sodium sulphide plus L-cysteine is low (max of 1%). However, no *Desulfotomaculum* could be detected in further enrichments reduced with titanium (III) citrate (subcultures G2 and

G4), and in few proportions (up to 2%) in subcultures reduced with sodium sulphide plus L-cysteine.

Although *Dehalobacter* and *Desulfotomaculum* may have a role in the dehalogenation of 1,2,3-TCB in sediment cultures reduced with titanium (III) citrate, both of them were not present in subcultures reduced with titanium (III) citrate and in cultures reduced with sodium sulphide plus L-cysteine. Thus, there must have been another *Firmicutes* member that performed the transformation of 1,2,3-TCB in subcultures and sediment cultures reduced with sodium sulphide plus L-cysteine. However, no other known organohalide-respiring microorganism was detected in the cultures. It is possible that members affiliated to the *Desulfosporosinus* may be the bacteria dehalogenating 1,2,3-TCB because *Desulfosporosinus* were present in the microbial community increasing in their relative abundance throughout dehalogenation in the sediment cultures reduced with sodium sulphide plus L-cysteine. Furthermore, *Desulfosporosinus* phylotypes were present in further transfers (G2) and were increasing in their relative abundance after complete dehalogenation of 1,2,3-TCB after two months of incubation (Figure 32). In addition, *Desulfosporosinus* phylotypes increased in relative abundance in the most enriched subculture reduced with titanium (III) citrate after complete dehalogenation (G4, 149 incubation days; Figure 30.). *Desulfosporosinus* spp. are known to be sulphate-reducing bacteria (Ramamoorthy et al 2006, Vatsurina et al 2008), however the medium used in the present study contains no sulphate, and thus they may respire alternative electron acceptors other than sulphate in the sediment and transferred cultures. Interestingly, a novel group within the *Firmicutes* named the 'Gopher group' has been enriched in cultures using halogenated aromatic compounds, i.e., chlorinated xanthones (Krzmarzick et al 2014). Members of the 'Gopher group' are related to the genera *Dehalobacter, Desulfitobacterium* and *Desulfosporosinus* (Krzmarzick et al 2014). The members of the *Firmicutes* transforming 1,2,3-TCB in sediment cultures in this study may be also related to the 'Gopher group', adding up evidence to the phylum *Firmicutes* to be dehalogenating-bacteria in pristine environments such as deep marine sediments.

4.6 *FIRMICUTES* ARE COMMON CULTURED BACTERIA FROM MARINE SEDIMENTS

The bacterial community in sediments from Chile was dominated by members of the phylum *Proteobacteria*, with minimal presence of the phylum *Firmicutes*. However, as soon as the sediments were exposed to the mineral medium and a temperature of 30ºC, *Firmicutes* took over the entire microbial community. Many bacteria from marine

sediments, e.g., phyla *Proteobacteria*, *Chloroflexi* are most likely non-spore forming microorganisms that may live in a dormant state in sediments, meanwhile other microorganisms may be in non-vegetative state as spores. Spores may be as abundant as vegetative cells in deep marine sediments (Lomstein et al 2012). Furthermore, the ratio of spores to vegetative cells was found to increase with depth (Fichtel et al 2007). Interestingly, deeper sediments have been observed to have greater microbial 'culturability' (Parkes et al 2014), which might be due to higher numbers of spores in deeper sediment layers. Isolated bacterial members from the marine subsurface belong to the phyla *Actinobacteria*, *Firmicutes, Proteobacteria* and *Bacteroidetes.* Spore-forming microorganisms like those from phyla *Actinobacteria* and *Firmicutes* may out-compete marine *Dehalococcoidia* or any other group when grown in a rich or even any medium due to its fast growth and the quick response of spore to the favourable conditions of a nutrient-rich medium.

All isolated colonies from deep-agarose dilution tubes of sediments originating from Chile site 7155, belonged to the phylum *Firmicutes*. Similarly, the whole microbial community evolved into *Firmicutes* along time in sediment cultures. Together these results suggest that sediments from Chile site 7155 contained spores which quickly germinated upon incubation in favourable medium, however, not for the other Chilean sediments, i.e., site 7165, and the other sediments from other locations, i.e., Århus or Ireland. Isolation and cultivation of *Firmicutes* was also observed by other studies and the great majority of the cultivated bacteria from marine sediments so far belong to the phylum *Firmicutes* (D'Hondt et al 2004, Köpke et al 2005, Parkes et al 2009, Süβ et al 2004).

5 CONCLUSION

This PhD work aimed to gain more insights into microbial life in marine sediments, with the focus to understand the bacterial class of the *Dehalococcoidia*, phylum *Chloroflexi*, which is widely distributed and highly abundant in marine sediments. Specifically, the ecological role of marine *Dehalococcoidia* was investigated within this study. For that, experiments based on the cultivation of marine sediments (*ex situ* study) and the distribution of marine *Dehalococcoidia* in marine sediments associated to environmental parameters, i.e., depth, geographical location, geochemistry (*in situ* study) were carried out.

Within the *ex situ* study, the cultivation of various marine sediments with different potential electron acceptors was carried out aiming for an identification of a specific respiration mode performed by marine *Dehalococcoidia*. The different electron acceptors tested here included halogenated (chlorinated and brominated organohalides) and non-halogenated compounds (sulphate, manganese(IV), and iron(III)). Halogenated compounds were selected as known members of the class *Dehalococcoidia*, such as *Dehalococcoides mccartyi*, strictly depend on organohalide respiration. Cultivation of marine sediments may be challenging when the indigenous microorganisms inhabiting them may be piezophilic, or have extremely slow metabolic rates. However, the cultivation experiments carried out within this study showed that marine *Dehalococcoidia* can be cultivated in the laboratory at atmospheric pressures and at temperatures of 30ºC. A medium containing hydrogen as an electron donor and acetate as carbon source allowed *Dehalococcoidia* growth and maintenance in reduced anoxic minimal medium. It has been suggested that microorganisms inhabiting the marine subsurface may divide on geological time scales (i.e., thousands to millions of years), however, *Dehalococcoidia* growth was observed after months of incubation, similarly to other anaerobic microorganisms, when cultivated with a reduced and anoxic minimum medium. Results from the cultivation of marine *Dehalococcoidia* indicated that they may use terminal electron acceptors other than the organohalides used here, which included chlorinated and brominated aliphatics and/or aromatics, suggesting that a respiratory mode other than organohalide respiration is likely. From all the tested organohalides, only 1,2,3-TCB was dehalogenated to 1,3-DCB in sediment cultures. However, in-depth investigations of the enrichment cultures over four generations indicated no role for members of the *Dehalococcoidia* in the dehalogenation. On the contrary, enrichment of members of the family *Peptococcaceae,* phylum *Firmicutes*, was attained.

Additionally, the cultivation of marine *Dehalococcoidia* with other potential electron acceptors such as sulphate, iron(III), manganese(IV), and humic acids indicated that for some sediments, i.e., from Århus and Ireland, sulphate promoted an increase in marine *Dehalococcoidia* 16S rRNA gene copy numbers, however, not for sediments from Chile, where none of the tested electron acceptor promoted a sustained *Dehalococcoidia* growth with time. Thus, marine *Dehalococcoidia* may have diverse respiratory modes. Therefore, an approach per clade, instead of per class, may be more useful in order to decipher the respiratory mode of specific groups within the class *Dehalococcoidia*. In addition, high *Dehalococcoidia* 16S rRNA gene copy numbers in sediment cultures amended with no electron acceptor indicated that a respiratory mode other than the tested in the current study may also be likely.

Within the *in situ* study, the distribution of marine *Dehalococcoidia* in sediments of the Baffin Bay, in the Arctic, was investigated. Several sites at distinct geographical locations within the Baffin Bay were analysed for its geochemistry and microbial composition. The focus was placed on the identification of natural occurring conditions promoting *Dehalococcoidia* abundance. These natural conditions may include biotic factors, i.e., *Dehalococcoidia* may be associated to the presence of other bacterial groups, or abiotic factors, i.e., *Dehalococcoidia* may be associated to specific environmental parameters such as depth or sediment geochemistry. *Dehalococcoidia* members were present in all sediment sites and depths from different geographical locations and different sediment geochemical conditions within the Baffin Bay, indicating a wide distribution. The highest abundance of *Dehalococcoidia* was at shelf sites, which were richer in organic matter than basin sites. However, *Dehalococcoidia* accounted for the greatest proportion of the total bacterial 16S rRNA gene copy numbers (sometimes higher than 50%) in deeper core sections where low *Bacteria* were found, particularly at the central deep basin of the Baffin Bay, indicating that *Dehalococcoidia* members are bacteria that are resilient to burial.

When associations of microbial distributions in relation with geochemical parameters in the Baffin Bay were studied, *Dehalococcoidia* and particularly the clade GIF-9, correlated positively with organic matter and negatively with sulphate and manganese(II) concentrations. Other bacteria that showed similar correlations to geochemical parameters were members of the candidate phyla “JS1” and “OP8”. In fact, higher relative abundance of JS1 and OP8 were observed at sites and depths where *Dehalococcoidia* relative abundance was also higher. These depths and sites were grouped (by hierarchical clustering) in a cluster containing near-surface sediments from

all sites, and deeper layers from shelf sites. A bacterial phylum that correlated differently to the geochemical parameters in the Baffin Bay was the *Proteobacteria.* More specifically, the class *Betaproteobacteria* positively correlated to manganese(II) and negatively to organic matter. Additionally, about 40% of the members belonging to the class *Alphaproteobacteria* correlated positively to sulphate and another 40% to iron(II) concentration.

Altogether, the identification of natural conditions in the Baffin Bay promoting higher *Dehalococcoidia* relative abundance (positive correlation to organic matter, and negative to sulphate and manganese), and quantified abundance (higher *Dehalococcoidia* 16S rRNA gene copy numbers in the shelf as measured with qPCR), and intrinsic characteristics observed (high resilience at sites and depths where low overall bacteria were found), together with the cultivation with various electron acceptors described in the current PhD thesis, provide a better understanding of the ecological role of marine *Dehalococcoidia* in marine sediments.

6 APPENDIX

6.1 APPENDIX 1

The medium was prepared from sterile concentrated solutions (20 ml mineral *Widdel solution*; 10 ml trace metal *SL9 solution*; 5 ml sodium acetate 1 M and 50 µl of the redox indicator resazurin) and brought up to 1 litre with Milli Q water. The content of the stock solutions used in the medium and the respective components and concentrations is the following:

6.1.1 Mineral solution "*Widdel solution*"

Stock solution 50x (Widdel 1980):

KH_2PO_4	10 g l^{-1}
NH_4Cl	13.5 g l^{-1}
NaCl	50 g l^{-1}
$MgCl_2 \times 6H_2O$	20.5 g l^{-1}
KCl	26.0 g l^{-1}
$CaCl_2 \times 2H_2O$	7.5 g l^{-1}

Sterilization by autoclaving at 121ºC for 40 min.

6.1.2 Trace metal solution "*SL-9 solution*"

Stock solution 100x (Tschech and Pfennig 1984):

H_2O	500 ml
$(CH_2CO_2H)_3$, (NTA)	12.8 g
$FeCl_2 \times 4H_2O$	2 g
$ZnCl_2$	70 mg
$MnCl_2 \times 2H_2O$	80 mg
H_3BO_3	6 mg
$CoCl_2 \times 6H_2O$	190 mg
$CuCl_2 \times 2H_2O$	2 mg
$NiCl_2 \times 6H_2O$	24 mg
$Na_2MoO_4 \times 2H_2O$	36 mg
NaOH	added up to pH 6.0
H_2O	added up to 1000 ml

Sterilization by autoclaving at 121ºC for 40 min.

6.1.3 Bicarbonate solution

For the bicarbonate solution, 7.06 g of $NaHCO_3$ were dissolved in 84 ml of anaerobic and sterile Milli Q water, which was previously saturated with CO_2, sealed under a headspace atmosphere of CO_2. It was sterilized by autoclaving.

6.1.4 Vitamin 7 solution

Stock solution of 2000x (Adrian 1999, Widdel 1980):

4-Aminobenzoic acid	40 mg l^{-1}
D (+)-Biotin	10 mg l^{-1}
Niacin	100 mg l^{-1}
Ca-D (+) pantothenate	50 mg l^{-1}
Pyridoxine hydrochloride	150 mg l^{-1}
Thiamine chloride-di-hydrochloride	100 mg l^{-1}
Cyanocobalamin	100 mg l^{-1}

Sterilization by filtering using a Minisart filter with a 0.2 µm pore size (Sartorius), and preserved in the dark at 4ºC

Addition of 0.5 ml of vitamin solution to 1 litre of medium

6.1.5 Titanium (III) citrate solution as reducing agent

The titanium (III) citrate solution was prepared according to (Zehnder and Wuhrmann 1976). For that, an anoxic sodium citrate solution (1 M; 10 ml) was mixed with a titanium (III) chloride solution (15%; 5.14 ml) under a stream of nitrogen. The final solution was adjusted to a pH of 7.0 with sodium carbonate (added as solid) and diluted with anoxic water to obtain a final concentration of 0.1 M of titanium (III) and 0.2 M citrate. The final titanium (III) citrate solution was filter-sterilized, and stored in a sterile brown-glass vial with biogon (80% N_2, 20% CO_2 v/v) in the vial headspace, which was kept in the dark.

6.1.6 Iron sulphide solution as reducing agent

For the preparation of the iron sulphide (FeS) solution, two solutions were prepared and then mixed. The first solution was Na_2S in a concentration of 400 mM. The second solution was $FeCl_2$ in a concentration of 10 mM. Both solutions were filter-sterilized by using a Minisart filter with a 0.2 µm pore size (Sartorius). Then, 12 ml of the $FeCl_2$ solution was supplied to a sterile anoxic glass vial, and 0.3 ml of the Na_2S solution was added. The FeS solution was always mixed before used. All reducing agent solutions were prepared shortly before used.

6.1.7 Medium composition and concentration

Substance		Concentration
Salts		**mM**
	KH_2PO_4	1.5
	KCl	7.0
	NH_4Cl	5.0
	NaCl	17.1
	$MgCl_2$ x $6H_2O$	2.0
	$CaCl_2$ x $2H_2O$	1.0
Buffer		**mM**
	$NaHCO_3$	30.0
Trace elements		**µM**
	Iron, $FeCl_2$ x $4H_2O$	10.060
	Boron, H_3BO_3	0.098
	Manganese, $MnCl_2$ x $2H_2O$	0.494
	Cobalt, CoCl x $6H_2O$	0.799
	Nickel, $NiCl_2$ x $6H_2O$	0.088
	Copper, $CuCl_2$ x $2H_2O$	0.012
	Zinc, $ZnCl_2$	0.514
	Molybdenum, Na_2MoO_4 x $2H_2O$	0.149
	+ NTA as a complexing agent	
Vitamins		**µM**
	4-Aminobenzoic acid (PABA), $C_7H_7NO_2$	0.15
	D (+) – Biotin (B7), $C_{10}H_{16}N_2O_3S$	0.02
	Niacin (B3), $C_6H_5NO_2$	0.41
	Ca-D (+)-pantothenate (B5), $C_{18}H_{32}CaN_2O_{10}$	0.10
	Pyridoxine hydrochloride (B6), $C_8H_{12}ClNO_3$	0.36
	Thiamine (B1), $C_{12}H_{17}N_4OS$	0.15
	Cyanocobalamin (B12), $C_{63}H_{88}CoN_{14}O_{14}P$	0.04
Reducing agent		
	Titanium (III) citrate	~1.4 mM in regard to Ti(III)
	Sulphide / L-cysteine	0.3 mM / 0.2 mM
Redox indicator		
	Resazurin	0.5 mg l^{-1}

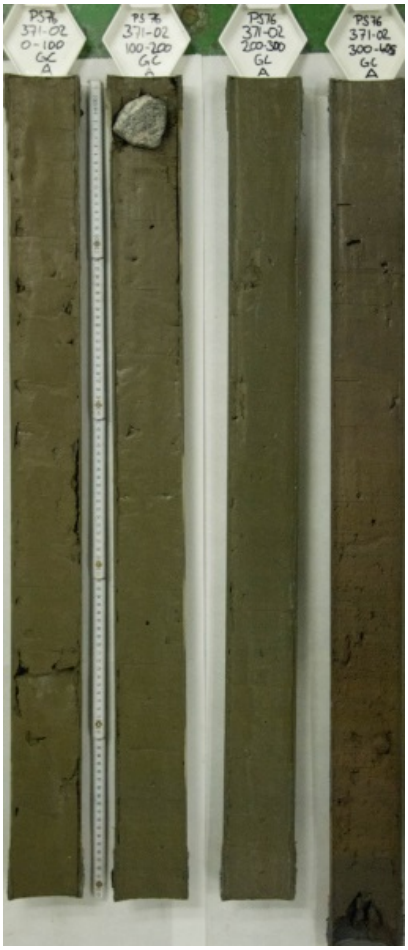
6.2 APPENDIX 2

Cores (divided into one-metre sections) investigated in this study from the sampled sediment sites during the ARK XXV/3 expedition at the Baffin Bay. Photos are courtesy of Dr Thomas Pletsch from BGR-Hannover, Germany.

Site 363

Site 365

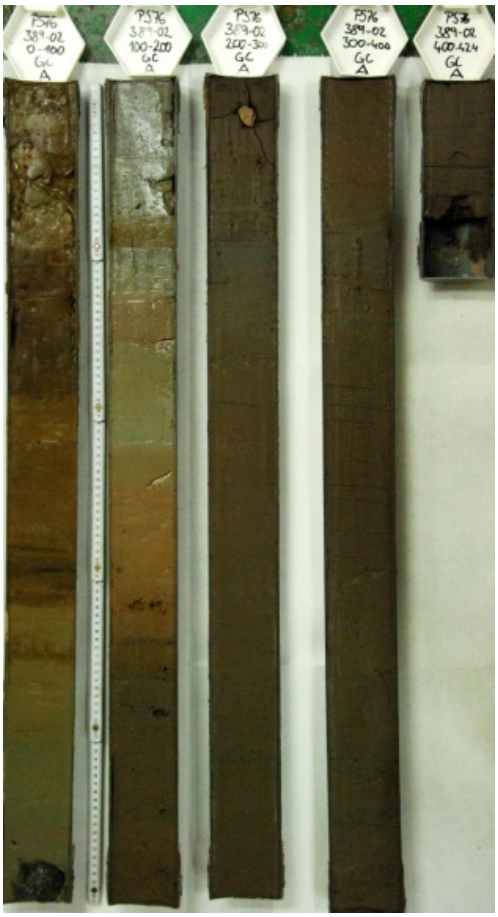
Site 371



Site 383

Site 387

Site 389



Site 391

Site 453

Site 486

Site 488

6.3 APPENDIX 3

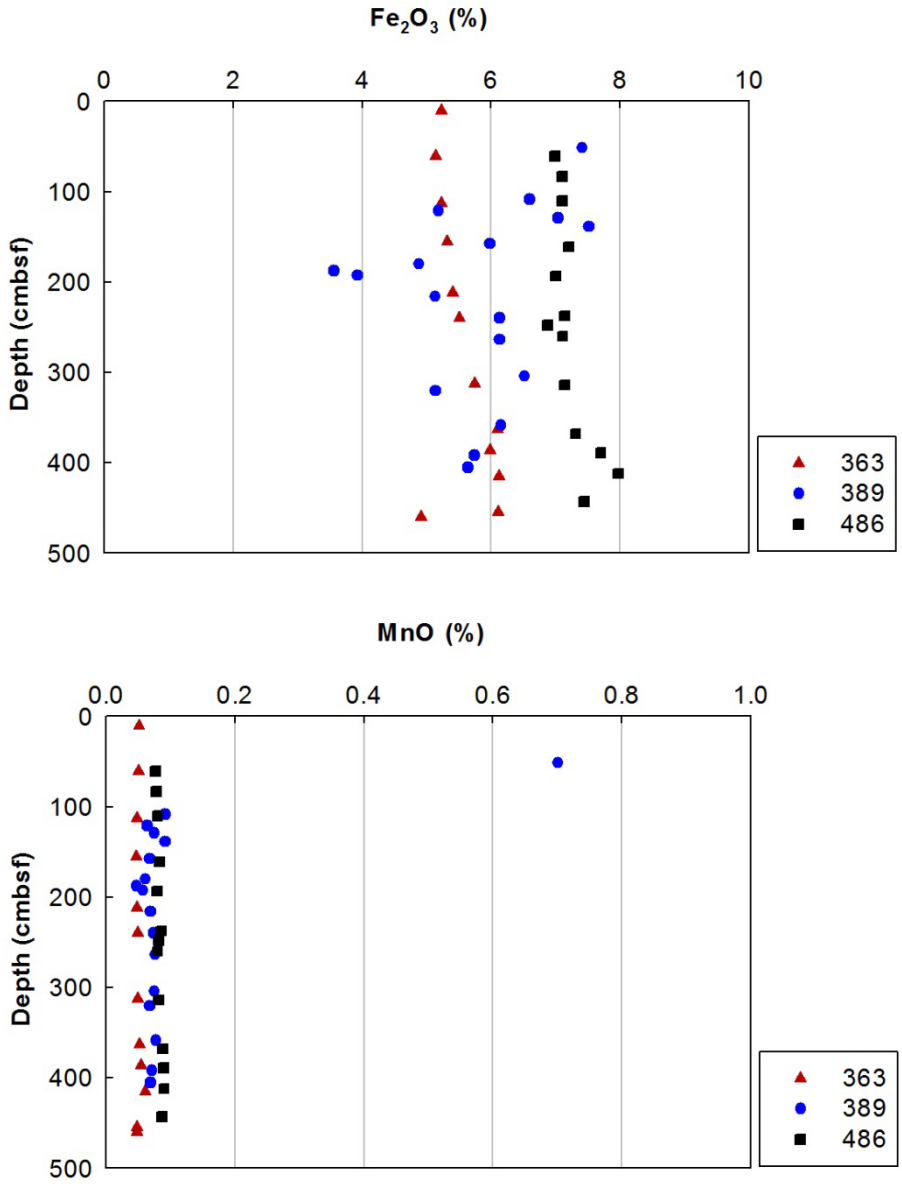


Figure 55. Iron oxide (Fe_2O_3; upper panel) and manganese oxide (MnO; lower panel) percentages in the mineral solid phase of sediments from the Baffin Bay. Shown are depth profiles of three sites that were selected, one site per area (Northern Greenlandic shelf, central deep basin, Southern slope), for elemental composition of the mineral fraction in sediment samples analysed at the BGR-Hannover.

7 REFERENCES

Adrian L (1999). Reduktive Dechlorierung von Trichlorbenzolen durch anaerobe Mikroorganismen. Doktor der Naturwissenschaften thesis, Technischen Universität Berlin.

Adrian L, Hansen SK, Fung JM, Görisch H, Zinder SH (2007). Growth of *Dehalococcoides* strains with chlorophenols as electron acceptors. *Environ Sci Technol* **41:** 2318-2323.

Adrian L (2009). ERC-group microflex: microbiology of *Dehalococcoides*-like *Chloroflexi*. *Rev Environ Sci Biotechnol* **8:** 225–229.

Ahn YB, Rhee SK, Fennell DE, Kerkhof LJ, Hentschel U, Häggblom MM (2003). Reductive dehalogenation of brominated phenolic compounds by microorganisms associated with the marine sponge *Aplysina aerophoba*. *Appl Environ Microbiol* **69:** 4159-4166.

Algora C, Gründger F, Adrian L, Damm V, Richnow HH, Krüger M (2013). Geochemistry and microbial populations in sediments of the Northern Baffin Bay, Arctic. *Geomicrobiol J* **30:** 690-705.

Algora C, Vasileiadis S, Wasmund K, Trevisan M, Krüger M, Puglisi E *et al* (2015). Manganese and iron as structuring parameters of microbial communities in Arctic marine sediments from the Baffin Bay. *FEMS Microbiol Ecol* **91:** fiv056.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). Basic Local Alignment Search Tool. *J Mol Biol* **215:** 403-410.

Anderson RT, Rooney-Varga JN, Gaw CV, Lovley DR (1998). Anaerobic benzene oxidation in the Fe(III) reduction zone of petroleum-contaminated aquifers. *Environ Sci Technol* **32:** 1222-1229.

Arnosti C, Jørgensen BB (2006). Organic carbon degradation in Arctic marine sediments, Svalbard: a comparison of initial and terminal steps. *Geomicrobiol J* **23:** 551-563.

Ashworth RB, Cormier MJ (1967). Isolation of 2,6-dibromophenol from marine hemichordate *Balanoglossus biminiensis*. *Science* **155:** 1558-1559

Baker GC, Smith JJ, Cowan DA (2003). Review and re-analysis of domain-specific 16S primers. *J Microbiol Methods* **55:** 541-555.

Baker JT, Duke CC (1973). Isolation from the hypobranchial glands of marine molluscs of 6-bromo-2,2-dimethylthioindolin-3-one and 6-bromo-2-methylthioindoleninone as alternative precursors to tyrian purple *Tetrahedron Lett* **14:** 2481-2482.

Ballschmiter K (2003). Pattern and sources of naturally produced organohalogens in the marine environment: biogenic formation of organohalogens. *Chemosphere* **52:** 313-324.

Barns SM, Fundyga RE, Jeffries MW, Pace NR (1994). Remarkable archaeal diversity detected in a Yellowstone National Park hot-spring environment. *Proc Natl Acad Sci USA* **91:** 1609-1613.

Bedard DL, Ritalahti KA, Löffler FE (2007). The *Dehalococcoides* population in sediment-free mixed cultures metabolically dechlorinates the commercial polychlorinated biphenyl mixture aroclor 1260. *Appl Environ Microbiol* **73:** 2513-2521.

Bhatia M, Sharp M, Foght J (2006). Distinct bacterial communities exist beneath a high Arctic polythermal glacier. *Appl Environ Microbiol* **72:** 5838-5845.

Bhatia MP, Kujawinski EB, Das SB, Breier CF, Henderson PB, Charette MA (2013). Greenland meltwater as a significant and potentially bioavailable source of iron to the ocean. *Nat Geosci* **6:** 274-278.

Biddle JF, Lipp JS, Lever MA, Lloyd KG, Sorensen KB, Anderson R *et al* (2006). Heterotrophic *Archaea* dominate sedimentary subsurface ecosystems off Peru. *Proc Natl Acad Sci USA* **103:** 3846-3851.

Biddle JF, Fitz-Gibbon S, Schuster SC, Brenchley JE, House CH (2008). Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. *Proc Natl Acad Sci USA* **105:** 10583-10588.

Biddle JF, White JR, Teske AP, House CH (2011). Metagenomics of the subsurface Brazos-Trinity Basin (IODP site 1320): comparison with other sediment and pyrosequenced metagenomes. *ISME J* **5:** 1038-1047.

Blazejak A, 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.

Blazejak A, Schippers A (2011). Real-time PCR quantification and diversity analysis of the functional genes *aprA* and *dsrA* of sulfate-reducing prokaryotes in marine sediments of the Peru continental margin and the Black Sea. *Front Microbiol* **2:** 253.

Boetius A, Albrecht S, Bakker K, Bienhold C, Felden J, Fernández-Méndez M *et al* (2013). Export of algal biomass from the melting Arctic sea ice. *Science* **339:** 1430-1432.

Boga HI, Ji R, Ludwig W, Brune A (2007). *Sporotalea propionica* gen. nov sp nov., a hydrogen-oxidizing, oxygen-reducing, propionigenic firmicute from the intestinal tract of a soil-feeding termite. *Arch Microbiol* **187:** 15-27.

Bowman KS, Nobre MF, da Costa MS, Rainey FA, Moe WM (2013). *Dehalogenimonas alkenigignens* sp nov., a chlorinated-alkane-dehalogenating bacterium isolated from groundwater. *Int J Sys Evol Microbiol* **63:** 1492-1498.

Braeckevelt M, Seeger EM, Paschke H, Kuschk P, Kaestner M (2011). Adaptation of a constructed wetland to simultaneous treatment of monochlorobenzene and perchloroethene. *Int J Phytorem* **13:** 998-1013.

Bunge M, Adrian L, Kraus A, Opel M, Lorenz WG, Andreesen JR *et al* (2003). Reductive dehalogenation of chlorinated dioxins by an anaerobic bacterium. *Nature* **421:** 357-360.

Cai WJ, Sayles FL (1996). Oxygen penetration depths and fluxes in marine sediments. *Mar Chem* **52:** 123-131.

Canfield DE, Jorgensen BB, Fossing H, Glud R, Gundersen J, Ramsing NB *et al* (1993). Pathways of organic carbon oxidation in three continental margin sediments. *Mar Geol* **113:** 27-40.

Carlson HK, Clark IC, Blazewicz SJ, Iavarone AT, Coates JD (2013). Fe(II) oxidation is an innate capability of nitrate-reducing bacteria that involves abiotic and biotic reactions. *J Bacteriol* **195:** 3260-3268.

Carr SA, Vogel SW, Dunbar RB, Brandes J, Spear JR, Levy R *et al* (2013). Bacterial abundance and composition in marine sediments beneath the Ross Ice Shelf, Antarctica. *Geobiology* **11:** 377-395.

Childers SE, Ciufo S, Lovley DR (2002). *Geobacter metallireducens* accesses insoluble Fe(III) oxide by chemotaxis. *Nature* **416:** 767-769.

Chow WL, Cheng D, Wang S, He J (2010). Identification and transcriptional analysis of trans-DCE-producing reductive dehalogenases in *Dehalococcoides* species. *ISME J* **4:** 1020-1030.

Christiansen N, Ahring BK (1996). *Desulfitobacterium hafniense sp* nov, an anaerobic, reductively dechlorinating bacterium. *Int J Syst Bacteriol* **46:** 442-448.

Cichocka D, Nikolausz M, Haest PJ, Nijenhuis I (2010). Tetrachloroethene conversion to ethene by a *Dehalococcoides*-containing enrichment culture from Bitterfeld. *FEMS Microbiol Ecol* **72:** 297-310.

Coolen MJL, Cypionka H, Sass AM, Sass H, Overmann J (2002). Ongoing modification of Mediterranean Pleistocene sapropels mediated by prokaryotes. *Science* **296:** 2407-2410.

Cooper M (2015). Modes of anaerobic respiration catalysed by members of the class *Dehalococcoidia*. Doktor der Naturwissenschaften thesis, Technischen Universität Berlin.

Cooper M, Wagner A, Wondrousch D, Sonntag F, Sonnabend A, Brehm M *et al* (2015). Anaerobic microbial transformation of halogenated aromatics and fate prediction using electron density modeling. *Environ Sci Technol* **49:** 6018-6028.

Coplen TB (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Commun Mass Spectrom* **25:** 2538-2560.

D'Hondt S, Rutherford S, Spivack AJ (2002). Metabolic activity of subsurface life in deep-sea sediments. *Science* **295:** 2067-2070.

D'Hondt S, Jørgensen BB, Miller DJ, Batzke A, Blake R, Cragg BA *et al* (2004). Distributions of microbial activities in deep subseafloor sediments. *Science* **306:** 2216-2221.

Damm V (2010). Cruise Report: The expedition of the research vessel "Polarstern" to the arctic in 2010 (ARK-XXV/3). *Ber Polarforsch Meeresforsch*

De Vrind-De Jong EW, Corstjens P, Kempers ES, Westbroek P, De Vrind JPM (1990). Oxidation of manganese and iron by *Leptothrix discophora* - use of N,N,N',N'-tetramethyl-*para*-phenylenediamine as an indicator of metal oxidation. *Appl Environ Microbiol* **56:** 3458-3462.

Degnan PH, Ochman H (2012). Illumina-based analysis of microbial community diversity. *ISME J* **6:** 183-194.

DeSantis TZ, Hugenholtz P, Keller K, Brodie EL, Larsen N, Piceno YM *et al* (2006). NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. *Nucleic Acids Res* **34:** W394-W399.

Duan TH (2014). Bio-transformation of chlorobenzenes by anaerobic mixed cultures and a pure bacterial strain. Doktor der Naturwissenschaften thesis, Technischen Universität Berlin.

Duhamel M, Mo K, Edwards EA (2004). Characterization of a highly enriched *Dehalococcoides*-containing culture that grows on vinyl chloride and trichloroethene. *Appl Environ Microbiol* **70:** 5538-5545.

Durbin AM, Teske A (2011). Microbial diversity and stratification of South Pacific abyssal marine sediments. *Environ Microbiol* **13:** 3219-3234.

References

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27:** 2194-2200.

Fagervold SK, Watts JEM, May HD, Sowers KR (2005). Sequential reductive dechlorination of meta-chlorinated polychlorinated biphenyl congeners in sediment microcosms by two different *Chloroflexi* phylotypes. *Appl Environ Microbiol* **71:** 8085-8090.

Fagervold SK, May HD, Sowers KR (2007). Microbial reductive dechlorination of aroclor 1260 in Baltimore Harbor sediment microcosms is catalyzed by three phylotypes within the phylum *Chloroflexi*. *Appl Environ Microbiol* **73:** 3009-3018.

Fichtel J, Köster J, Rullkötter J, Sass H (2007). Spore dipicolinic acid contents used for estimating the number of endospores in sediments. *FEMS Microbiol Ecol* **61:** 522-532.

Finneran KT, Johnsen CV, Lovley DR (2003). *Rhodoferax ferrireducens* sp nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe(III). *Int J Sys Evol Microbiol* **53:** 669-673.

Foght J, Aislabie J, Turner S, Brown CE, Ryburn J, Saul DJ *et al* (2004). Culturable bacteria in subglacial sediments and ice from two Southern Hemisphere glaciers. *Microbiol Ecol* **47:** 329-340.

Franco MA, De Mesel I, Diallo MD, Van der Gucht K, Van Gansbeke D, Van Rijswijk P *et al* (2007). Effect of phytoplankton bloom deposition on benthic bacterial communities in two contrasting sediments in the southern North Sea. *Aquat Microb Ecol* **48:** 241-254.

Friedrich MW (2005). Methyl-coenzyme M reductase genes: unique functional markers for methanogenic and anaerobic methane-oxidizing *Archaea*. In: Leadbetter JR (ed). *Environmental Microbiology*. pp 428-442.

Froelich PN, Klinkhammer GP, Bender ML, Luedtke NA, Heath GR, Cullen D *et al* (1979). Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. *Geochim Cosmochim Acta* **43:** 1075-1090.

Fry JC, Parkes RJ, Cragg BA, Weightman AJ, Webster G (2008). Prokaryotic biodiversity and activity in the deep subseafloor biosphere. *FEMS Microbiol Ecol* **66:** 181-196.

Futagami T, Morono Y, Terada T, Kaksonen AH, Inagaki F (2009). Dehalogenation activities and distribution of reductive dehalogenase homologous genes in marine subsurface sediments. *Appl Environ Microbiol* **75:** 6905-6909.

Futagami T, Morono Y, Terada T, Kaksonen AH, Inagaki F (2013). Distribution of dehalogenation activity in subseafloor sediments of the Nankai Trough subduction zone. *Philos Trans R Soc London Ser B* **368:** 20120249.

Galand PE, Potvin M, Casamayor EO, Lovejoy C (2010). Hydrography shapes bacterial biogeography of the deep Arctic Ocean. *ISME J* **4:** 564-576.

Garrity GM, Holt JG (2001). Phylum BVI, Chloroflexi phy. nov. . In: Boone DR, Castenholz RW (eds). *Bergey's Manual of Systematic Bacteriology: The Archaea and the deeply branching and phototrophic bacteria*, 2nd edn. Springer: New York. pp 427–446.

Garrity GM, Bell JA, Lilburn T (2005). Class II. *Betaproteobacteria* class. nov. In: Brenner DJ, Krieg NR, Staley JT (eds). *Bergey's Manual of Systematic Bacteriology*. Springer US. pp 575-922.

Gerritse J, Renard V, Gomes TMP, Lawson PA, Collins MD, Gottschal JC (1996). *Desulfitobacterium* sp strain PCE1, an anaerobic bacterium that can grow by reductive dechlorination of tetrachloroethene or ortho-chlorinated phenols. *Arch Microbiol* **165:** 132-140.

Gordon DA, Priscu J, Giovannoni S (2000). Origin and phylogeny of microbes living in permanent Antarctic lake ice. *Microbiol Ecol* **39:** 197-202.

Gribble GW (2003). The diversity of naturally produced organohalogens. *Chemosphere* **52:** 289-297.

Gupta RS, Chander P, George S (2013). Phylogenetic framework and molecular signatures for the class *Chloroflexi* and its different clades; proposal for division of the class *Chloroflexi* class. nov into the suborder *Chloroflexineae* subord. nov., consisting of the emended family *Oscillochloridaceae* and the family *Chloroflexaceae* fam. nov., and the suborder *Roseiflexineae* subord. nov., containing the family *Roseiflexaceae* fam. nov. *Anton Leeuw Int J G* **103:** 99-119.

Häggblom MM, Bossert ID (2003). Halogenated organic compounds: a global perspective. In: Häggblom MM, Bossert ID (eds). *Dehalogenation: microbial processes and environmental applications*. Kluwer Academic: New York. pp 3–29.

Häggblom MM, Ahn YB, Fennell DE, Kerkhof LJ, Rhee SK (2003). Anaerobic dehalogenation of organohalide contaminants in the marine environment. *Adv Appl Microbiol* **53:** 61-84.

Hales BA, Edwards C, Ritchie DA, Hall G, Pickup RW, Saunders JR (1996). Isolation and identification of methanogen-specific DNA from blanket bog feat by PCR amplification and sequence analysis. *Appl Environ Microbiol* **62:** 668-675.

Hallam SJ, Girguis PR, Preston CM, Richardson PM, DeLong EF (2003). Identification of methyl coenzyme M reductase A (*mcrA*) genes associated with methane-oxidizing archaea. *Appl Environ Microbiol* **69:** 5483-5491.

Hamdan LJ, Coffin RB, Sikaroodi M, Greinert J, Treude T, Gillevet PM (2013). Ocean currents shape the microbiome of Arctic marine sediments. *ISME J* **7:** 685-696.

Handley KM, Wrighton KC, Miller CS, Wilkins MJ, Kantor RS, Thomas BC *et al* (2014). Disturbed subsurface microbial communities follow equivalent trajectories despite different structural starting points. *Environ Microbiol* **17:** 622-636

Harrison BK, Zhang H, Berelson W, Orphan VJ (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.

Hedges JI, Keil RG (1995). Sedimentary organic matter preservation: an assessment and speculative synthesis. *Mar Chem* **49:** 81-115.

Herrera-Rodriguez LN, Meyer H-P, Robins KT, Khan F (2011). Perspectives on biotechnological halogenation Part II: Prospecting for future biohalogenases. *Chim Oggi* **29:** 47-49.

Holliger C, Hahn D, Harmsen H, Ludwig W, Schumacher W, Tindall B *et al* (1998). *Dehalobacter restrictus* gen. nov. and sp. nov., a strictly anaerobic bacterium that reductively dechlorinates tetra- and trichloroethene in an anaerobic respiration. *Arch Microbiol* **169:** 313-321.

Holmes DE, Finneran KT, O'Neil RA, Lovley DR (2002). Enrichment of members of the family *Geobacteraceae* associated with stimulation of dissimilatory metal reduction in uranium-contaminated aquifer sediments. *Appl Environ Microbiol* **68:** 2300-2306.

Hu M, Li F, Lei J, Fang Y, Tong H, Wu W *et al* (2015). Pyrosequencing revealed highly microbial phylogenetic diversity in ferromanganese nodules from farmland. *Environ Sci Proc Imp* **17:** 213-224.

Hug L, Castelle C, Wrighton K, Thomas B, Sharon I, Frischkorn K *et al* (2013a). Community genomic analyses constrain the distribution of metabolic traits across the *Chloroflexi* phylum and indicate roles in sediment carbon cycling. *Microbiome* **1:** 22.

Hug LA, Maphosa F, Leys D, Loeffler FE, Smidt H, Edwards EA *et al* (2013b). Overview of organohalide-respiring bacteria and a proposal for a classification system for reductive dehalogenases. *Philos Trans R Soc London Ser B* **368:** 20120322.

Hugenholtz P, Stackebrandt E (2004). Reclassification of *Sphaerobacter thermophilus* from the subclass *Sphaerobacteridae* in the phylum *Actinobacteria* to the class *Thermomicrobia* (emended description) in the phylum *Chloroflexi* (emended description). *Int J Sys Evol Microbiol* **54:** 2049-2051.

Hulth S, Hall POJ, Blackburn TH, Landen A (1996). Arctic sediments (Svalbard): pore water and solid phase distributions of C, N, P and Si. *Polar Biol* **16:** 447-462.

Imfeld G, Pieper H, Shani N, Rossi P, Nikolausz M, Nijenhuis I *et al* (2011). Characterization of groundwater microbial communities, dechlorinating bacteria, and in situ biodegradation of chloroethenes along a vertical gradient. *Water Air Soil Pollut* **221:** 107-122.

Inagaki F, Suzuki M, Takai K, Oida H, Sakamoto T, Aoki K *et al* (2003). Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. *Appl Environ Microbiol* **69:** 7224-7235.

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 USA* **103:** 2815-2820.

Inagaki F, Hinrichs KU, Kubo Y, Bowles MW, Heuer VB, Hong WL *et al* (2015). Exploring deep microbial life in coal-bearing sediment down to ~2.5 km below the ocean floor. *Science* **349:** 420-424.

Isenbarger TA, Finney M, Ríos-Velázquez C, Handelsman J, Ruvkun G (2008). Miniprimer PCR, a new lens for viewing the microbial world. *Appl Environ Microbiol* **74:** 840-849.

Jørgensen BB (1982). Mineralization of organic matter in the sea bed - The role of sulfate reduction. *Nature* **296:** 643-645.

Jørgensen BB, Boetius A (2007). Feast and famine - microbial life in the deep-sea bed. *Nat Rev Microbiol* **5:** 770-781.

Jørgensen SL, Hannisdal B, Lanzen 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 USA* **109:** E2846-E2855.

Kaster A-K, Mayer-Blackwell K, Pasarelli B, Spormann AM (2014). Single cell genomic study of *Dehalococcoidetes* species from deep-sea sediments of the Peruvian Margin. *ISME J*.

Kawaichi S, Ito N, Kamikawa R, Sugawara T, Yoshida T, Sako Y (2013). *Ardenticatena maritima* gen. nov., sp nov., a ferric iron- and nitrate-reducing bacterium of the phylum *Chloroflexi* isolated from an iron-rich coastal hydrothermal field, and description of *Ardenticatenia* classis nov. *Int J Sys Evol Microbiol* **63:** 2992-3002.

Keppler F, Eiden R, Niedan V, Pracht J, Scholer HF (2000). Halocarbons produced by natural oxidation processes during degradation of organic matter. *Nature* **403:** 298-301.

Kimura S, Bryan CG, Hallberg KB, Johnson DB (2011). Biodiversity and geochemistry of an extremely acidic, low-temperature subterranean environment sustained by chemolithotrophy. *Environ Microbiol* **13:** 2092-2104.

King GM (1988). Dehalogenation in marine sediments containing natural sources of halophenols. *Appl Environ Microbiol* **54:** 3079-3085.

Kittelmann S, Friedrich MW (2008a). Novel uncultured *Chloroflexi* dechlorinate perchloroethene to trans-dichloroethene in tidal flat sediments. *Environ Microbiol* **10:** 1557-1570.

Kittelmann S, Friedrich MW (2008b). Identification of novel perchloroethene-respiring microorganisms in anoxic river sediment by RNA-based stable isotope probing. *Environ Microbiol* **10:** 31-46.

Kondo R, Nedwell DB, Purdy KJ, Silva SD (2004). Detection and enumeration of sulphate-reducing bacteria in estuarine sediments by competitive PCR. *Geomicrobiol J* **21:** 145-157.

Köpke B, Wilms R, Engelen B, Cypionka H, Sass H (2005). Microbial diversity in coastal subsurface sediments: a cultivation approach using various electron acceptors and substrate gradients. *Appl Environ Microbiol* **71:** 7819-7830.

Kormas KA, Smith DC, Edgcomb V, Teske A (2003). Molecular analysis of deep subsurface microbial communities in Nankai Trough sediments (ODP Leg 190, Site 1176). *FEMS Microbiol Ecol* **45:** 115-125.

Krajmalnik-Brown R, Holscher T, Thomson IN, Saunders FM, Ritalahti KM, Loffler FE (2004). Genetic identification of a putative vinyl chloride reductase in *Dehalococcoides* sp strain BAV1. *Appl Environ Microbiol* **70:** 6347-6351.

Krüger M, Eller G, Conrad R, Frenzel P (2002). Seasonal variation in pathways of CH_4 production and in CH_4 oxidation in rice fields determined by stable carbon isotopes and specific inhibitors. *Global Change Biol* **8:** 265-280.

Krzmarzick MJ, Crary BB, Harding JJ, Oyerinde OO, Leri AC, Myneni SCB *et al* (2012). Natural Niche for Organohalide-Respiring Chloroflexi. *Appl Environ Microbiol* **78:** 393-401.

Krzmarzick MJ, Miller HR, Yan T, Novak PJ (2014). Novel *Firmicutes* group implicated in the dechlorination of two chlorinated xanthones, analogues of natural organochlorines. *Appl Environ Microbiol* **80:** 1210-1218.

Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML, Pace NR (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc Natl Acad Sci USA* **82:** 6955-6959.

Lane DJ (1991). 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds). *Nucleic acid techniques in bacterial systematics*. Wiley: New York.

Lannuzel D, Schoemann V, Dumont I, Content M, de Jong J, Tison JL *et al* (2013). Effect of melting Antarctic sea ice on the fate of microbial communities studied in microcosms. *Polar Biol* **36:** 1483-1497.

Lannuzel D, van der Merwe PC, Townsend AT, Bowie AR (2014). Size fractionation of iron, manganese and aluminium in Antarctic fast ice reveals a lithogenic origin and low iron solubility. *Mar Chem* **161:** 47-56.

Lanoil B, Skidmore M, Priscu JC, Han S, Foo W, Vogel SW *et al* (2009). Bacteria beneath the West Antarctic Ice Sheet. *Environ Microbiol* **11:** 609-615.

Lee OO, Wang Y, Yang J, Lafi FF, Al-Suwailem A, Qian P-Y (2011). Pyrosequencing reveals highly diverse and species-specific microbial communities in sponges from the Red Sea. *ISME J* **5:** 650-664.

Lee Y-J, Romanek CS, Mills GL, Davis RC, Whitman WB, Wiegel J (2006). *Gracilibacter thermotolerans* gen. nov., sp nov., an anaerobic, thermotolerant bacterium from a constructed wetland receiving acid sulfate water. *Int J Sys Evol Microbiol* **56:** 2089-2093.

Lipp JS, Morono Y, Inagaki F, Hinrichs KU (2008). Significant contribution of Archaea to extant biomass in marine subsurface sediments. *Nature* **454:** 991-994.

Lira NS, Montes RC, Tavares JF, da Silva MS, da Cunha EVL, de Athayde-Filho PF *et al* (2011). Brominated Compounds from Marine Sponges of the Genus *Aplysina* and a Compilation of Their C-13 NMR Spectral Data. *Mar Drugs* **9:** 2316-2368.

Liu Z, Lozupone C, Hamady M, Bushman FD, Knight R (2007). Short pyrosequencing reads suffice for accurate microbial community analysis. *Nucleic Acids Res* **35:** e120.

Livermore JA, Jin YO, Arnseth RW, Lepuil M, Mattes TE (2013). Microbial community dynamics during acetate biostimulation of RDX-contaminated groundwater. *Environ Sci Technol* **47:** 7672-7678.

Löffler FE, Edwards EA (2006). Harnessing microbial activities for environmental cleanup. *Curr Opin Biotechnol* **17:** 274-284.

Löffler FE, Yan J, Ritalahti KM, Adrian L, Edwards EA, Konstantinidis KT *et al* (2013). *Dehalococcoides mccartyi* gen. nov., sp. nov., obligate organohalide-respiring anaerobic bacteria relevant to halogen cycling and bioremediation, belong to a novel bacterial class, *Dehalococcoidia* classis nov., order *Dehalococcoidales* ord. nov. and family *Dehalococcoidaceae* fam. nov., within the phylum *Chloroflexi*. *Int J Sys Evol Microbiol* **63:** 625-635.

Lomstein BA, Langerhuus AT, D'Hondt S, Jørgensen BB, Spivack AJ (2012). Endospore abundance, microbial growth and necromass turnover in deep sub-seafloor sediment. *Nature* **484:** 101-104.

Lovley DR, Holmes DE, Nevin KP (2004). Dissimilatory Fe(III) and Mn(IV) reduction. *Adv Microb Physiol* **49:** 219-286.

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.

Lysnes K, Thorseth IH, Steinsbu BO, Ovreas L, Torsvik T, Pedersen RB (2004). Microbial community diversity in seafloor basalt from the Arctic spreading ridges. *FEMS Microbiol Ecol* **50:** 213-230.

Mapelli F, Marasco R, Rizzi A, Baldi F, Ventura S, Daffonchio D *et al* (2011). Bacterial communities involved in soil formation and plant establishment triggered by pyrite bioweathering on Arctic moraines. *Microbiol Ecol* **61:** 438-447.

Maphosa F, Lieten SH, Dinkla I, Stams AJ, Smidt H, Fennell DE (2012). Ecogenornics of microbial communities in bioremediation of chlorinated contaminated sites. *Front Microbiol* **3:** 351.

Marchesi JR, Weightman AJ, Cragg BA, Parkes RJ, Fry JC (2001). Methanogen and bacterial diversity and distribution in deep gas hydrate sediments from the Cascadia Margin as revealed by 16S rRNA molecular analysis. *FEMS Microbiol Ecol* **34:** 221-228.

Martin JH, Fitzwater SE (1988). Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. *Nature* **331:** 341-343.

May HD, Miller GS, Kjellerup BV, Sowers KR (2008). Dehalorespiration with polychlorinated biphenyls by an anaerobic ultramicrobacterium. *Appl Environ Microbiol* **74:** 2089-2094.

Maymó-Gatell X, Chien YT, Gossett JM, Zinder SH (1997). Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. *Science* **276:** 1568-1571.

Mészáros É, Imfeld G, Nikolausz M, Nijenhuis I (2013). Occurrence of *Dehalococcoides* and reductive dehalogenase genes in microcosms, a constructed wetland and groundwater from a chlorinated ethene contaminated field site as indicators for in situ reductive dehalogenation. *Water Air Soil Pollut* **224:** 1768.

Miller E, Wohlfarth G, Diekert G (1997). Comparative studies on tetrachloroethene reductive dechlorination mediated by *Desulfitobacterium* sp. strain PCE-S. *Arch Microbiol* **168:** 513-519.

Moe WM, Yan J, Nobre MF, da Costa MS, Rainey FA (2009). *Dehalogenimonas lykanthroporepellens* gen. nov., sp nov., a reductively dehalogenating bacterium isolated from chlorinated solvent-contaminated groundwater. *Int J Sys Evol Microbiol* **59:** 2692-2697.

Monserrate E, Häggblom MM (1997). Dehalogenation and biodegradation of brominated phenols and benzoic acids under iron-reducing, sulfidogenic, and methanogenic conditions. *Appl Environ Microbiol* **63:** 3911-3915.

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

Münchow A, Falkner KK, Melling H (2015). Baffin Island and West Greenland Current systems in northern Baffin Bay. *Prog Oceanogr* **132:** 305-317.

Muyzer G, De Waal EC, Uitterlinden AG (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.

Narihiro T, Kaiya S, Futamata H, Hiraishi A (2010). Removal of polychlorinated dioxins by semi-aerobic fed-batch composting with biostimulation of "*Dehalococcoides*". *J Biosci Bioeng* **109:** 249-256.

Nealson KH, Saffarini D (1994). Iron and manganese in anaerobic respiration: environmental significance, physiology, and regulation. *Annu Rev Microbiol* **48:** 311-343.

Nealson KH (1997). Sediment bacteria: who's there, what are they doing, and what's new? *Annu Rev Earth Planet Sci* **25:** 403-434.

Nelson JL, Jiang J, Zinder SH (2014). Dehalogenation of chlorobenzenes, dichlorotoluenes, and tetrachloroethene by three *Dehalobacter* spp. *Environ Sci Technol* **48:** 3776-3782.

Nemergut DR, Anderson SP, Cleveland CC, Martin AP, Miller AE, Seimon A *et al* (2007). Microbial community succession in an unvegetated, recently deglaciated soil. *Microbiol Ecol* **53:** 110-122.

Nickel M, Vandieken V, Bruchert V, Jørgensen BB (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 2 Top Stud Oceanogr* **55:** 2390-2398.

Nunoura T, Oida H, Miyazaki J, Miyashita A, Imachi H, Takai K (2008). Quantification of *mcrA* by fluorescent PCR in methanogenic and methanotrophic microbial communities. *FEMS Microbiol Ecol* **64:** 240-247.

Nunoura T, Soffientino B, Blazejak A, Kakuta J, Oida H, Schippers A *et al* (2009). Subseafloor microbial communities associated with rapid turbidite deposition in the Gulf of Mexico continental slope (IODP Expedition 308). *FEMS Microbiol Ecol* **69:** 410-424.

Oni OE, Schmidt F, Miyatake T, Kasten S, Witt M, Hinrichs K-U *et al* (2015). Microbial communities and organic matter composition in surface and subsurface sediments of the Helgoland mud area, North Sea. *Front Microbiol* **6:** 1290.

Orlygsson J, Krooneman J, Collins MD, Pascual C, Gottschall JC (1996). *Clostridium acetireducens* sp nov, a novel amino acid-oxidizing, acetate-reducing anaerobic bacterium. *Int J Syst Bacteriol* **46:** 454-459.

Oyaizu H, Debrunnervossbrinck B, Mandelco L, Studier JA, Woese CR (1987). The green non-sulfur bacteria: a deep branching in the eubacterial line of descent. *Syst Appl Microbiol* **9:** 47-53.

Parkes RJ, Cragg BA, Bale SJ, Getliff JM, Goodman K, Rochelle PA *et al* (1994). Deep bacterial biosphere in Pacific Ocean sediments. *Nature* **371:** 410-413.

Parkes RJ, Cragg BA, Wellsbury P (2000). Recent studies on bacterial populations and processes in subseafloor sediments: a review. *Hydrogeol J* **8:** 11-28.

Parkes RJ, Webster G, Cragg BA, Weightman AJ, Newberry CJ, Ferdelman TG *et al* (2005). Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. *Nature* **436:** 390-394.

Parkes RJ, Sass H (2009). Deep sub-surface. In: Schaechter M (ed). *Encyclopedia of Microbiology*, 3rd edn. Academic Press. pp 64-79.

Parkes RJ, Sellek G, Webster G, Martin D, Anders E, Weightman AJ *et al* (2009). Culturable prokaryotic diversity of deep, gas hydrate sediments: first use of a continuous high-pressure, anaerobic, enrichment and isolation system for subseafloor sediments (DeepIsoBUG). *Environ Microbiol* **11:** 3140-3153.

Parkes RJ, Cragg B, Roussel E, Webster G, Weightman A, Sass H (2014). A review of prokaryotic populations and processes in sub-seafloor sediments, including biosphere:geosphere interactions. *Mar Geol* **352:** 409-425.

Parshina SN, Sipma J, Nakashimada Y, Henstra AM, Smidt H, Lysenko AM *et al* (2005). *Desulfotomaculum carboxydivorans* sp. nov., a novel sulfate-reducing bacterium capable of growth at 100 % CO. *Int J Sys Evol Microbiol* **55:** 2159-2165.

Pauletti PM, Cintra LS, Braguine CG, da Silva Filho AA, Andrade e Silva ML, Cunha WR *et al* (2010). Halogenated indole alkaloids from marine invertebrates. *Mar Drugs* **8:** 1526-1549.

Pedersén M, Saenger P, Fries L (1974). Simple brominated phenols in red algae. *Phytochem* **13:** 2273-2279.

Pérez-de-Mora A, Zila A, McMaster ML, Edwards EA (2014). Bioremediation of Chlorinated Ethenes in Fractured Bedrock and Associated Changes in Dechlorinating and Nondechlorinating Microbial Populations. *Environ Sci Technol* **48:** 5770-5779.

Pérez-Pantoja D, Donoso R, Agulló L, Córdova M, Seeger M, Pieper DH *et al* (2012). Genomic analysis of the potential for aromatic compounds biodegradation in *Burkholderiales*. *Environ Microbiol* **14:** 1091-1117.

Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J *et al* (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* **35:** 7188-7196.

Qiu YL, Sekiguchi Y, Hanada S, Imachi H, Tseng IC, Cheng SS *et al* (2006). *Pelotomaculum terephthalicum* sp. nov. and *Pelotomaculum isophthalicum* sp. nov.: two anaerobic bacteria that degrade phthalate isomers in syntrophic association with hydrogenotrophic methanogens. *Arch Microbiol* **185:** 172-182.

R Development Core Team (2011). R: A language and environment for statistical computing, reference index version 2.14.1. R Foundation for Statistical Computing.

Raiswell R, Tranter M, Benning LG, Siegert M, De'ath R, Huybrechts P *et al* (2006). Contributions from glacially derived sediment to the global iron (oxyhydr)oxide cycle: Implications for iron delivery to the oceans. *Geochim Cosmochim Acta* **70:** 2765-2780.

Ramamoorthy S, Sass H, Langner H, Schumann P, Kroppenstedt RM, Spring S *et al* (2006). *Desulfosporosinus lacus* sp nov., a sulfate-reducing bacterium isolated from pristine freshwater lake sediments. *Int J Sys Evol Microbiol* **56:** 2729-2736.

Rathsack K, Stackebrandt E, Reitner J, Schumann G (2009). Microorganisms Isolated from Deep Sea Low-temperature Influenced Oceanic Crust Basalts and Sediment Samples Collected along the Mid-Atlantic Ridge. *Geomicrobiol J* **26:** 264-274.

Reed DW, Fujita Y, Delwiche ME, Blackwelder DB, Sheridan PP, Uchida T *et al* (2002). Microbial communities from methane hydrate-bearing deep marine sediments in a forearc basin. *Appl Environ Microbiol* **68:** 3759-3770.

Richardson RE, Bhupathiraju VK, Song DL, Goulet TA, Alvarez-Cohen L (2002). Phylogenetic characterization of microbial communities that reductively dechlorinate TCE based upon a combination of molecular techniques. *Environ Sci Technol* **36:** 2652-2662.

Richardson RE (2013). Genomic insights into organohalide respiration. *Curr Opin Biotechnol* **24:** 498-505.

Ritalahti KM, Amos BK, Sung Y, Wu QZ, Koenigsberg SS, Löffler FE (2006). Quantitative PCR targeting 16S rRNA and reductive dehalogenase genes simultaneously monitors multiple *Dehalococcoides* strains. *Appl Environ Microbiol* **72:** 2765-2774.

Ryan MP, Pembroke JT, Adley CC (2007). *Ralstonia pickettii* in environmental biotechnology: potential and applications. *J Appl Microbiol* **103:** 754-764.

Rysgaard S, Thamdrup B, Risgaard-Petersen N, Fossing H, Berg P, Christensen PB *et al* (1998). Seasonal carbon and nutrient mineralization in a high-Arctic coastal marine sediment, Young Sound, Northeast Greenland. *Mar Ecol Prog Ser* **175:** 261-276.

Sackett WM (1964). The depositional history and isotopic organic carbon composition of marine sediments *Mar Geol* **2:** 173-185.

Schippers A, Neretin LN, Kallmeyer J, Ferdelman TG, Cragg BA, Parkes RJ *et al* (2005). Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria. *Nature* **433:** 861-864.

Schippers A, Neretin LN (2006). Quantification of microbial communities in near-surface and deeply buried marine sediments on the Peru continental margin using real-time PCR. *Environ Microbiol* **8:** 1251-1260.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB *et al* (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75:** 7537-7541.

Schulz HD, Zabel M (eds) (2006) *Marine Geochemistry*. Springer: Berlin, Heidelberg, New York.

Sekiguchi Y, Yamada T, Hanada S, Ohashi A, Harada H, Kamagata Y (2003). *Anaerolinea thermophila* gen. nov., sp nov and *Caldilinea aerophila* gen. nov., sp nov., novel filamentous thermophiles that represent a previously uncultured lineage of the domain Bacteria at the subphylum level. *Int J Sys Evol Microbiol* **53:** 1843-1851.

Sheridan PP, Miteva VI, Brenchley JE (2003). Phylogenetic analysis of anaerobic psychrophilic enrichment cultures obtained from a Greenland glacier ice core. *Appl Environ Microbiol* **69:** 2153-2160.

Shiratori H, Reno H, Ayame S, Kataoka N, Miya A, Hosono K *et al* (2006). Isolation and characterization of a new *Clostridium* sp. that performs effective cellulosic waste digestion in a thermophilic methanogenic bioreactor. *Appl Environ Microbiol* **72:** 3702-3709.

Skidmore M, Anderson SP, Sharp M, Foght J, Lanoil BD (2005). Comparison of microbial community compositions of two subglacial environments reveals a possible role for microbes in chemical weathering processes. *Appl Environ Microbiol* **71:** 6986-6997.

Slobodkin A, Reysenbach AL, Mayer F, Wiegel J (1997). Isolation and characterization of the homoacetogenic thermophilic bacterium *Moorella glycerini* sp. nov. *Int J Syst Bacteriol* **47:** 969-974.

Smits THM, Devenoges C, Szynalski K, Maillard J, Holliger C (2004). Development of a real-time PCR method for quantification of the three genera *Dehalobacter*, *Dehalococcoides*, and *Desulfitobacterium* in microbial communities. *J Microbiol Methods* **57:** 369-378.

Sokolova TG, Kostrikina NA, Chernyh NA, Kolganova TV, Tourova TP, Bonch-Osmolovskaya EA (2005). *Thermincola carboxydiphila* gen. nov., sp nov., a novel anaerobic, carboxydotrophic, hydrogenogenic bacterium from a hot spring of the Lake Baikal area. *Int J Sys Evol Microbiol* **55:** 2069-2073.

References

Sørensen J, Jørgensen BB (1987). Early diagenesis in sediments from Danish coastal waters: microbial activity and Mn-Fe-S geochemistry. *Geochim Cosmochim Acta* **51:** 1583-1590.

Sorokin DY, Luecker S, Vejmelkova D, Kostrikina NA, Kleerebezem R, Rijpstra WIC *et al* (2012). Nitrification expanded: discovery, physiology and genomics of a nitrite-oxidizing bacterium from the phylum *Chloroflexi*. *ISME J* **6:** 2245-2256.

Srivastava SP, Arthur M, Clement B, al. e (1989). Leg 105: College Station, TX (Ocean Drilling Program). *Proc Ocean Drill Prog Initial Reports*. http://www-odp.tamu.edu/publications/105_IR/VOLUME/CHAPTERS/ir105_01.pdf.

Stackebrandt E, Sproer C, Rainey FA, Burghardt J, Päuker O, Hippe H (1997). Phylogenetic analysis of the genus *Desulfotomaculum*: Evidence for the misclassification of *Desulfotomaculum guttoideum* and description of *Desulfotomaculum orientis* as *Desulfosporosinus orientis* gen. nov., comb. nov. *Int J Syst Bacteriol* **47:** 1134-1139.

Stein LY, La Duc MT, Grundl TJ, Nealson KH (2001). Bacterial and archaeal populations associated with freshwater ferromanganous micronodules and sediments. *Environ Microbiol* **3:** 10-18.

Stein R, Littke R, Stax R, Welte DH (1989). Quantity, provenance, and maturity of organic matter at ODP sites 645, 646, and 647: implications for reconstruction of paleoenvironments in Baffin Bay and Labrador Sea during Terciary and Quaternary time. In: Srivastava SP, Arthur M, Clement B, al. e (eds). *Leg 105: College Station, TX (Ocean Drilling Program)*. Proceedings of the Ocean Drilling Program, Scientific Results: http://www-odp.tamu.edu/publications/105_SR/105TOC.HTM.

Stibal M, Hasan F, Wadham JL, Sharp MJ, Anesio AM (2012). Prokaryotic diversity in sediments beneath two polar glaciers with contrasting organic carbon substrates. *Extremophiles* **16:** 255-265.

Sutcliffe IC (2010). A phylum level perspective on bacterial cell envelope architecture. *Trends Microbiol* **18:** 464-470.

Sutcliffe IC (2011). Cell envelope architecture in the *Chloroflexi*: a shifting frontline in a phylogenetic turf war. *Environ Microbiol* **13:** 279-282.

Süβ J, Engelen B, Cypionka H, Sass H (2004). Quantitative analysis of bacterial communities from Mediterranean sapropels based on cultivation-dependent methods. *FEMS Microbiol Ecol* **51:** 109-121.

Takai K, 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.

Takeuchi M, Komai T, Hanada S, Tamaki H, Tanabe S, Miyachi Y *et al* (2009). Bacterial and Archaeal 16S rRNA Genes in Late Pleistocene to Holocene Muddy Sediments from the Kanto Plain of Japan. *Geomicrobiol J* **26:** 104-118.

Tang CCL, Ross CK, Yao T, Petrie B, DeTracey BM, Dunlap E (2004). The circulation, water masses and sea-ice of Baffin Bay. *Prog Oceanogr* **63:** 183-228.

Tarpgaard IH, Røy H, Jørgensen BB (2011). Concurrent low- and high-affinity sulfate reduction kinetics in marine sediment. *Geochim Cosmochim Acta* **75:** 2997-3010.

Taş N, Heilig HGHJ, van Eekert MHA, Schraa G, de Vos WM, Smidt H (2010a). Concurrent hexachlorobenzene and chloroethene transformation by endogenous dechlorinating microorganisms in the Ebro River sediment. *FEMS Microbiol Ecol* **74:** 682-692.

Taş N, van Eekert MHA, de Vos WM, Smidt H (2010b). The little bacteria that can - diversity, genomics and ecophysiology of '*Dehalococcoides*' spp. in contaminated environments. *Microbiol Biotechnol* **3:** 389-402.

Taş N, van Eekert MHA, Wagner A, Schraa G, de Vos WM, Smidt H (2011). Role of "Dehalococcoides" spp. in the anaerobic transformation of hexachlorobenzene in European rivers. *Appl Environ Microbiol* **77:** 4437-4445.

Teske A, Durbin A, Ziervogel K, Cox C, Arnosti C (2011). Microbial community composition and function in permanently cold seawater and sediments from an Arctic fjord of Svalbard. *Appl Environ Microbiol* **77:** 2008-2018.

Thamdrup B (2000). Bacterial manganese and iron reduction in aquatic sediments. *Adv Microb Ecol* **16:** 41-84.

Treude T, Niggemann J, Kallmeyer J, Wintersteller P, Schubert CJ, Boetius A *et al* (2005). Anaerobic oxidation of methane and sulfate reduction along the Chilean continental margin. *Geochim Cosmochim Acta* **69:** 2767-2779.

Tschech A, Pfennig N (1984). Growth yield increase linked to caffeate reduction in *Acetobacterium woodii Arch Microbiol* **137:** 163-167.

Utkin I, Woese C, Wiegel J (1994). Isolation and characterization of *Desulfitobacterium dehalogenans* gen.-nov., sp.-nov., an anaerobic bacterium which reductively dechlorinates chlorophenolic compounds. *Int J Syst Bacteriol* **44:** 612-619.

Utkina NK, Denisenko VA, Scholokova OV, Virovaya MV, Gerasimenko AV, Popov DY *et al* (2001). Spongiadioxins A and B, two new polybrominated dibenzo-p-dioxins from an Australian marine sponge *Dysidea dendyi*. *Journal of Natural Products* **64:** 151-153.

Vandieken V, Nickel M, Jørgensen BB (2006). 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.

Vandieken V, Finke N, Thamdrup B (2014). Hydrogen, acetate, and lactate as electron donors for microbial manganese reduction in a manganese-rich coastal marine sediment. *FEMS Microbiol Ecol* **87:** 733-745.

Vasileiadis S, Puglisi E, Arena M, Cappa F, Cocconcelli PS, Trevisan M (2012). Soil bacterial diversity screening using single 16S rRNA gene V regions coupled with multi-million read generating sequencing technologies. *PLoS One* **7:** e42671.

Vatsurina A, Badrutdinova D, Schumann P, Spring S, Vainshtein M (2008). *Desulfosporosinus hippei* sp nov., a mesophilic sulfate-reducing bacterium isolated from permafrost. *Int J Sys Evol Microbiol* **58:** 1228-1232.

Verce MF, Madrid VM, Gregory SD, Demir Z, Singleton MJ, Salazar EP *et al* (2015). A Long-Term Field Study of In Situ Bioremediation in a Fractured Conglomerate Trichloroethene Source Zone. *Bioremediation J* **19:** 18-31.

Villemur R, Lanthier M, Beaudet R, Lépine F (2006). The *Desulfitobacterium* genus. *FEMS Microbiol Rev* **30:** 706-733.

von Wintzingerode F, Schlötelburg C, Hauck R, Hegemann W, Göbel UB (2001). Development of primers for amplifying genes encoding CprA- and PceA-like reductive dehalogenases in anaerobic microbial consortia, dechlorinating trichlorobenzene and 1,2-dichloropropane. *FEMS Microbiol Ecol* **35:** 189-196.

Wadham JL, Tranter M, Tulaczyk S, Sharp M (2008). Subglacial methanogenesis: a potential climatic amplifier? *Global Biogeochem Cycles* **22:** GB2021.

Wagner A, Cooper M, Ferdi S, Seifert J, Adrian L (2012). Growth of *Dehalococcoides mccartyi* strain CBDB1 by reductive dehalogenation of brominated benzenes to benzene. *Environ Sci Technol* **46:** 8960-8968.

Wagner M, Roger AJ, Flax JL, Brusseau GA, Stahl DA (1998). Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. *J Bacteriol* **180:** 2975-2982.

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* **73:** 5261-5267.

Wang Y, 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.

Wasmund K, Schreiber L, Lloyd KG, Petersen DG, Schramm A, Stepanauskas R *et al* (2014). Genome sequencing of a single cell of the widely distributed marine subsurface *Dehalococcoidia*, phylum *Chloroflexi*. *ISME J* **8:** 383-397.

Wasmund K, Algora C, Müller J, Krüger M, Lloyd KG, Reinhardt R *et al* (2015). Development and application of primers for the class *Dehalococcoidia* (phylum *Chloroflexi*) enables deep insights into diversity and stratification of subgroups in the marine subsurface. *Environ Microbiol* **17:** 3540-3556.

Watts JEM, Fagervold SK, May HD, Sowers KR (2005). A PCR-based specific assay reveals a population of bacteria within the *Chloroflexi* associated with the reductive dehalogenation of polychlorinated biphenyls. *Microbiology-Sgm* **151:** 2039-2046.

Webster G, Parkes RJ, Fry JC, Weightman AJ (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.

Webster G, Parkes RJ, Cragg BA, Newberry CJ, Weightman AJ, Fry JC (2006). Prokaryotic community composition and biogeochemical processes in deep subseafloor sediments from the Peru Margin. *FEMS Microbiol Ecol* **58:** 65-85.

Webster G, Blazejak A, Cragg BA, Schippers A, Sass H, Rinna J *et al* (2009). Subsurface microbiology and biogeochemistry of a deep, cold-water carbonate mound from the Porcupine Seabight (IODP Expedition 307). *Environ Microbiol* **11:** 239-257.

Webster G, Sass H, Cragg BA, Gorra R, Knab NJ, Green CJ *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.

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173:** 697-703.

Wellsbury P, Mather I, Parkes RJ (2002). Geomicrobiology of deep, low organic carbon sediments in the Woodlark Basin, Pacific Ocean. *FEMS Microbiol Ecol* **42:** 59-70.

Wenzhöfer F, Glud RN (2002). Benthic carbon mineralization in the Atlantic: a synthesis based on in situ data from the last decade. *Deep Sea Res Part 1 Oceanogr Res Pap* **49:** 1255-1279.

West KA, Johnson DR, Hu P, DeSantis TZ, Brodie EL, Lee PKH *et al* (2008). Comparative genomics of "*Dehalococcoides ethenogenes*" 195 and an enrichment culture containing unsequenced "*Dehalococcoides*" strains. *Appl Environ Microbiol* **74:** 3533-3540.

White RH, Hager LP (1977). Occurrence of fatty acid chlorohydrins in jellyfish lipids. *Biochemistry* **16:** 4944-4948.

Widdel F (1980). Anaerober Abbau von Fettsäuren und Benzoesäure durch neu isolierte Arten Sulfat-reduzierender Bakterien, Universität Göttingen.

Willems A, Deley J, Gillis M, Kersters K (1991). *Comamonadaceae*, a new family encompassing the *Acidovorans* ribosomal RNA complex, including *Variovorax-Paradoxus* gen-nov, comb-nov, for *Alcaligenes paradoxus*. *Int J Syst Bacteriol* **41:** 445-450.

Wilms R, Köpke B, Sass H, Chang TS, Cypionka H, Engelen B (2006). Deep biosphere-related bacteria within the subsurface of tidal flat sediments. *Environ Microbiol* **8:** 709-719.

Wilms R, Sass H, Köpke B, Cypionka H, Engelen B (2007). Methane and sulfate profiles within the subsurface of a tidal flat are reflected by the distribution of sulfate-reducing bacteria and methanogenic archaea. *FEMS Microbiol Ecol* **59:** 611-621.

Wu JY, Jiang XT, Jiang YX, Lu SY, Zou F, Zhou HW (2010). Effects of polymerase, template dilution and cycle number on PCR based 16 S rRNA diversity analysis using the deep sequencing method. *BMC Microbiol* **10:** 255.

Yabe S, Aiba Y, Sakai Y, Hazaka M, Yokota A (2010). *Thermosporothrix hazakensis* gen. nov., sp nov., isolated from compost, description of *Thermosporotrichaceae* fam. nov within the class *Ktedonobacteria* Cavaletti et al. 2007 and emended description of the class *Ktedonobacteria*. *Int J Sys Evol Microbiol* **60:** 1794-1801.

Yamada T, Sekiguchi Y, Hanada S, Imachi H, Ohashi A, Harada H *et al* (2006). *Anaerolinea thermolimosa* sp nov., *Levilinea saccharolytica* gen. nov., sp nov and *Leptolinea tardivitalis* gen. nov., so. nov., novel filamentous anaerobes, and description of the new classes anaerolineae classis nov and *Caldilineae classis* nov in the bacterial phylum *Chloroflexi*. *Int J Sys Evol Microbiol* **56:** 1331-1340.

Yamada T, Sekiguchi Y (2009). Cultivation of uncultured *Chloroflexi s*ubphyla: significance and ecophysiology of formerly uncultured *Chloroflexi* 'subphylum I' with natural and biotechnological relevance. *Microbes Environ* **24:** 205-216.

Yang C, Kublik A, Weidauer C, Seiwert B, Adrian L (2015). Reductive dehalogenation of oligocyclic phenolic bromoaromatics by *Dehalococcoides mccartyi* strain CBDB1. *Environ Sci Technol* **49:** 8497–8505.

Yde JC, Finster KW, Raiswell R, Steffensen JP, Heinemeier J, Olsen J *et al* (2010). Basal ice microbiology at the margin of the Greenland ice sheet. *Ann Glaciol* **51:** 71-79.

Yoshida N, Ye L, Baba D, Katayama A (2009). A novel *Dehalobacter* species is involved in extensive 4,5,6,7-tetrachlorophthalide dechlorination. *Appl Environ Microbiol* **75:** 2400-2405.

Zachos JC, Cederberg T (1989). Interstitial water chemistry, Leg 105 sites 645, 646, and 647, Baffin Bay and Labrador Sea. In: Srivastava SP, Arthur M, Clement B, al. e (eds). *Leg 105: College Station, TX (Ocean Drilling Program)*. Proceedings of the Ocean Drilling Program, Scientific Results: http://www-odp.tamu.edu/publications/105_SR/105TOC.HTM.

Zehnder AJB, Wuhrmann K (1976). Titanium (III) citrate as a nontoxic oxidation-reduction buffering system for culture of obligate anaerobes. *Science* **194:** 1165-1166.

Zobell CE, Morita RY (1959). Deep-sea bacteria. *Galathea Report* **1:** 139-154.