UiT
THE ARCTIC UNIVERSITY OF NORWAY

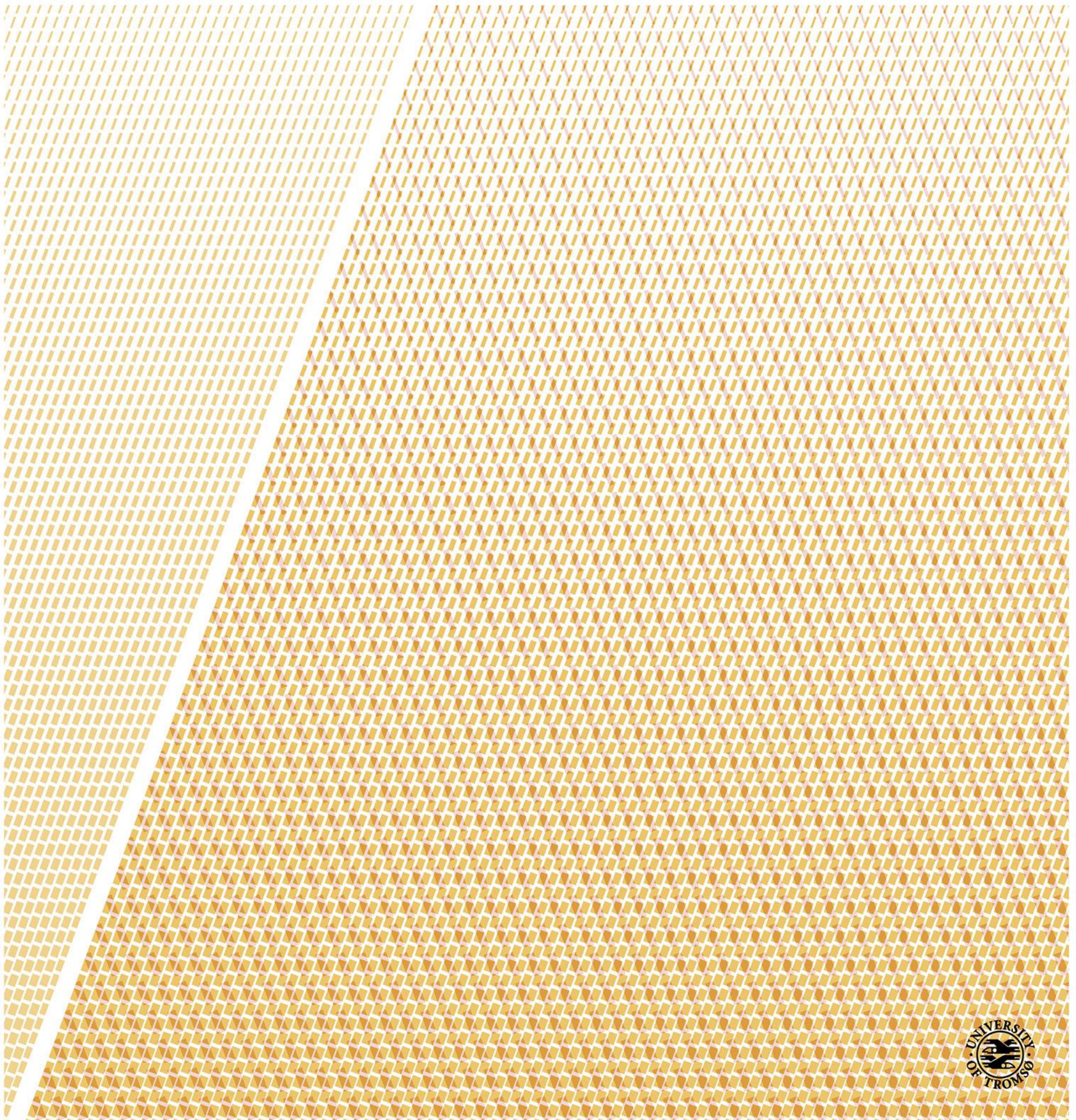
Faculty of Biosciences, Fisheries and Economics
The Norwegian College of Fishery Science

Microbial community variation in an Arctic shelf seafloor
Biogeographic and anthropogenic influences

—

Tan Thi Nguyen

A dissertation for the degree of Philosophiae Doctor – February 2017



Acknowledgements

The PhD work was carried out at the Norwegian College of Fishery Science (NCFS). Financial support was obtained from the UiT-The Arctic University of Norway, the Centre for Marine Bioactives and Drug Discovery (MABCENT-SFI), the program Marine Biotechnology in Northern Norway (MABIT), and Eni Norge through the BARCUT project.

First of all, I would like to express my sincere gratitude to my main supervisor, Prof. Bjarne Landfald, for giving me the opportunity to do the PhD work and for your excellent guidance, discussion and support. Without you, this dissertation would never been completed.

I would also like to thank my co-supervisor Prof. Nils Peder Willassen for your comments in my thesis. Special thanks goes to Assoc Prof. Hans-Matti Blencke for your willingness to read my thesis. Thank you for your very quick response and very useful comments. I also thanks to Peter Arbo, Chun Li, and Ida Kristine Hansen for providing valuable feedback in the introduction part of the thesis.

I express sincere thanks to Bioprospecting research group "Team Klara" for providing a great working environment. We had lots of fun during seminars outside of the campus as well as every second Monday, good discussion and support in each other. I would also like to thank "Qiime Forum" for quick answer my questions and helpful suggestions in bioinformatics.

Thanks to my friends, Ekaterina, Duy, Xuan, Juan, Jaya, Ngan and Thuy for being great friends and great colleagues. Thank you for always having time for conversation and support when things may have seemed too chaotic and unmanageable.

Thank you to my office mates, Birthe Vang and Ida Kristine Hansen for making a great office-team, for discussions about science as well as about personal life, thank you!

A big thanks to my family in Vietnam for your support, belief in me, and the patience to keep the fingers crossed about the years. Many thanks to my dear husband, my best friend, Nam Thanh Le, for your patience, encouraging, and support. You take care of our children and housework without complaining while I was spending long days in the office on both weekdays and weekend days. Without your support, this would not have been possible. To our wonderful son and lovely daughter, Duong and Thao, thank you for waiting until late in the evening and making me happy and laughing. You mean everything to me!

Tromsø, February 2017

Tan Thi Nguyen

Summary

Studying the spatial patterns of microbial diversity is crucial in order to assess the relationship between community structure and ecosystem function. However, due to methodological limitations in the past, relatively little has been known about the biogeography of microbial communities. The introduction of next generation sequencing technologies from 2005 was a game changer, as DNA sequencing suddenly became relatively fast and cost-effective. The application of next generation sequencing in microbial ecology has revealed non-random patterns of microbial diversity in a variety of habitats. The main objective of this thesis was to assess how microbial communities were distributed in an Arctic shelf seafloor across geographic separation and anthropogenic impact by using next generation sequencing approaches.

To assess spatial variations of bacterial, archaeal, and viral communities, and possible coincidences in their biogeographical patterns, surface sediment samples were collected along a 640 km Barents Sea transect. Previous findings were confirmed and extended in the explored marine sediments, as *Deltaproteobacteria* and *Gammaproteobacteria* were dominant bacterial classes, while *Thaumarchaeota* were predominant among the archaeal groups. The viral assemblages appeared dominated by single-stranded DNA (ssDNA) viruses, mostly of the Eukaryotic Circular Rep-encoding ssDNA (CRESS-DNA) group, which include the families *Circo*-, *Nano*-, and *Geminiviridae*. However, the high representation of ssDNA viruses was likely a consequence of amplification bias caused by the use of multiple displacement DNA amplification of the viral preparations.

Although stability in higher taxa composition across geographical distances, were expected due to moderate environmental variations in the sampling area, significant distance-decay of both bacterial and archaeal communities in the Barents Sea seafloor was observed.

Beta-diversity analyses of prokaryotes and viruses showed some degree of community structuring in accordance with the south-north spatial separation. While archaeal communities appeared largely influenced by environmental factors, bacterial communities seemed structured by a relatively equal contribution from environmental and spatial factors. The viral grouping into a southern and a northern region was principally associated with changes in the relative abundance and composition of eukaryotic ssDNA viruses.

To determine whether offshore drilling waste, i.e discharge of rock cuttings and drilling muds, could cause changes in the bacterial communities, sediment corer samples were collected at variable distances from a recently drilled location. The results showed that the bacterial community compositions in the uppermost sediment layer close to the drilling site was significantly different from those of unaffected areas. The bacterial groups most conspicuously associated with the community change were representatives of the orders *Clostridia* and *Desulfuromonadales* and the class *Mollicutes.* These are candidates as microbial bioindicators of the spatial extent and persistence of drilling waste discharge. The effects of drilling waste discharges on bacterial composition were observable in 100 m radius around the drilling location. The drilling waste caused oxygen depletion in the upper sediment layer in close proximity to offshore drilling site.

List of papers

The thesis is based on the following papers, referred to by their roman numerals in the text.

Paper I

Tan T. Nguyen and Bjarne Landfald. 2015. Polar front associated variation in prokaryotic community structure in Arctic shelf seafloor. *Frontiers in Microbiology* ***6****:17*

Paper II

Tan T. Nguyen, Espen M. Robertsen and Bjarne Landfald. 2017. Viral assemblage variation in an Arctic shelf seafloor. *Aquatic Microbial Ecology. In press, doi: 10-3354/ame 01808.*

Paper III

Tan T. Nguyen, Sabine K.J. Cochrane and Bjarne Landfald. Perturbation of seafloor bacterial community structure by drilling waste discharge *(Manuscript)*

The table shows the major contributions

	Paper I	**Paper II**	**Paper III**
Concept and idea	BL	BL	SC, BL
Study design and methods	TTN, BL	TTN, BL	TTN, SC, BL
Data gathering and interpretation	TTN, BL	TTN, EMR, BL	TTN, SC, BL
Manuscript preparation	TTN, BL	TTN, EMR, BL	TTN, SC, BL

TTN = Tan Thi Nguyen; BL = Bjarne Landfald; EMR = Espen Mikal Robertsen; SC = Sabine K. J. Cochrane

Contents

Acknowledgements ... i
Summary ... ii
List of papers ... iv
Contents ... v
List of the figures ... vi
Abbreviations ... vii
1. Introduction ... 1
  1.1 Microbial diversity ... 1
    1.1.1 Microbial alpha-diversity ... 3
    1.1.2 Microbial beta-diversity ... 5
  1.2 Microbial biogeography ... 6
  1.3 Characteristics of marine sediments ... 8
  1.4 Microbial diversity in marine sediments ... 10
    1.4.1 Bacterial diversity in marine sediments ... 10
    1.4.2 Archaeal diversity in marine sediments ... 11
    1.4.3 Viral diversity in marine sediments ... 12
  1.5 Study system: The Barents Sea ... 13
    1.5.1 The Barents Sea and its seafloor ... 13
    1.5.2 Offshore drilling for oil and gas in the Barents Sea ... 15
2. Aims of the thesis ... 18
3. Results and discussion ... 19
  3.1 Methodological consideration ... 19
    3.1.1 Primers for 16S rRNA gene amplification ... 19
    3.1.2 Viral isolation and DNA amplification ... 21
    3.1.3 Bioinformatics and data analysis ... 23
  3.2 Microbial alpha-diversity in Barents Sea sediments ... 25
    3.2.1 The prokaryotic diversity ... 25
    3.2.2 The viral assemblages ... 28
  3.3 Microbial beta-diversity in Barents Sea sediments ... 30
    3.3.1 Spatial variation of prokaryotic and viral communities in the Barents Sea transect ... 30
    3.3.2 Spatial variation of bacterial communities surrounding a freshly drilling location 31
4. Conclusions and further work ... 33
5. References ... 35
Papers ... 46

List of the figures

Figure 1. The contribution by environmental and historical effects on microbial biogeography 8

Figure 2. Diagram of some major seafloor habitats and the profiles of dominant electron acceptors in these habitats 10

Figure 3. Schematic of the circulation patterns in the Norwegian and Barents Seas 14

Figure 4. Distribution of major phylogenetic groups of Bacteria from amplicons of the V1-V2 and V3-V4 regions of 16S rRNA gene and from taxonomic annotation of 16S rRNA genes in metagenomic DNA, all originating from the same sample material.............................20

Figure 5. The percent viral sequence reads exhibiting significant similarity to the Refseq viral genomes deposited in the nr protein database (E threshold value 10^{-3}) 22

Figure 6. Composition of bacterial communities (percent) from various habitats at phylum/class level of taxonomy 26

Figure 7. Composition of archaeal communities (percent) in various habitats at phylum level of taxonomy 27

Figure 8. Percentage composition of viral communities from various habitats. All taxonomic annotations originate from multiple-displacement amplified viral DNA 29

Abbreviations

ACE	Abundance-based coverage estimator
ANOSIM	Analysis of similarities
BLAST	Basic local alignment search tool
CCA	Canonical correspondence analysis
CRESS-DNA	Circular Rep-encoding ssDNA
CsCl	Cesium chloride
dBC	Distance Bray-Curtis
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded DNA
dsRNA	Double-stranded RNA
GLM	Generalized linear model
GS	Grain size
LASL	Linker amplified shotgun libraries
MBGB	Marine benthic group B
MCG	Miscellaneous Crenarchaeotal group
MDA	Multiple displacement amplification
MEGA	Molecular evolutionary genetics analysis
mRNA	Messanger RNA
NGS	Next generation sequencing
NMDS	Nonmetric multidimensional scaling
NPMANOVA	Non-parametric multivariate analysis of variance
OBM	Oil-based drilling mud
OP	Obsidian pool
OTU	Operational taxonomic unit
PCoA	Principal coordinates analysis
PCR	Polymerase chain reaction
QIIME	Quantitative insights into microbial ecology
RDA	Redundancy analysis
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SBM	Synthetic based drilling mud
ssDNA	Single-stranded DNA
ssRNA	Single-stranded RNA
SSU	The small subunit of ribosomal RNA
TOC	Total organic carbon
WBM	Water-based drilling mud

1. Introduction

1.1. Microbial diversity

Microbial diversity is the description of the variety and abundance of microbial organisms at the gene, species, community, and ecosystem levels. It encompasses all species of microorganisms and the ecological processes of which they are part. Thus, microbial diversity comprises aspects of community structure and function.

Traditional cultivation-dependent methods do not provide comprehensive information on microbial community structure, because less than 1% of the microorganisms can be cultured by standard techniques [1]. As a consequence, a few phyla are well characterized, e.g *Proteobacteria, Firmicutes, Actinobacteria* and *Cyanobacteria*, other phyla are underrepresented by cultivation approaches [1].

Development of cultivation-independent techniques has provided new tools for examining the diversity and ecology of microbial communities. Early on, deoxyribonucleic acid (DNA) fingerprinting methods, which separate DNA fragments of whole genomes or individual genes according to their length and/or nucleotide composition, were dominating [2, 3]. These techniques make it possible to compare multiple samples relatively rapidly, but they are limited to predominant taxa. Before the development of next generation sequencing techniques, the most common molecular approaches for estimating microbial diversity were the analysis of 16S rRNA gene PCR amplicons by use of denaturing gradient gel electrophoresis or the construction and Sanger sequencing of gene clone libraries. The 16S rRNA gene has been widely used as a phylogenetic marker because of its high degree of sequence conservation and its presence in all prokaryotes throughout evolution [1, 4]. Horizontal gene transfer of the 16S rRNA gene is thought to be very rare [5]. In addition,

genes encoding 16S rRNA can be obtained in high enough quality and quantity from most environmental samples for successful amplification using polymerase chain reaction (PCR). Establishing 16S rRNA clone libraries, however, is relatively time-consuming, and has limited sequencing depth. It is difficult to draw meaningful conclusions about the true microbial diversity of environmental samples using this technique because of the limited number of clones (typically < 1000).

As an alternative to employing selected phylogenetically informative or functional genes, whole genome sequencing approaches may be used to assess microbial diversity by characterizing the complete genomic material, i.e. the metagenome, of an environmental sample. One of the first major metagenomic sequencing studies to investigate microbial diversity was conducted on Sargasso Sea bacterioplanton by using shotgun sequencing [6]. The study identified at least 1800 different species, including 148 previously unknown bacterial phenotypes and more than 1.2 million unknown genes.

In 2005, the next generation sequencing (NGS) technologies were introduced. The main advantages of NGS are the increased throughput, and the lowered costs per basepair of sequence information. Another advantage is that multiple environmental samples can be combined in a single run, and after sequencing, the reads from each sample can be separated by use of assigned nucleotide barcodes. The application of these methods into the fields of microbiology has changed the conception of microbial diversity to a vast extent [7, 8]. A large proportion of the species richness has been found to consist of “rare species” in almost any environmental sample [8]. However, next generation sequencing has some challenges, i.e. short sequence reads, rather high rates of sequencing errors, as well as handling and processing of huge amounts of data.

1.1.1 Microbial alpha-diversity

There are many different ways to assess microbial diversity, but they largely boil down to two main categories introduced by Whittaker [9]: alpha diversity and beta diversity. The term alpha diversity refers to local diversity, typically within a particular area or, for microorganisms, in one sample. Two aspects taken into account when measuring alpha diversity are richness and evenness.

Species richness is the estimate of the number of different species present in a sample or an ecological community, region or landscape [9, 10]. In community studies based on amplicons of 16S rRNA or some other phylogenetic marker genes, operational taxonomic units (OTUs) are used as proxy for species. OTUs are obtained by clustering sequences into non-overlapping classes based on a similarity threshold [11]. Most commonly, a 97% threshold is used for the 16S rRNA gene to devide prokaryotic microorganisms into separate OTUs [12].

One of the simplest and most commonly used richness estimators in microbiology is the Chao1 index [13]. This non-parametric estimator is based on adjustment of the observed number of taxonomic units in a sample for the prevalence of rare OTUs. The Chao1 richness estimator [14] is expressed as:

$$S_{est} = S_{obs} + f_0,$$

$$\text{where } f_0 = f^2{}_1 / (2f_2)\,,\ f_2 > 0$$

$$\text{or } f_0 = f_1(f_1 - 1)/[2(f_2 + 1)]\,, \text{for } f_2 = 0$$

Here S_{est} is the estimated number of taxonomic units in the assemblage, S_{obs} is the number of species observed in a sample, f_1 is the count of singletons (taxonomic units represented by a single read in the sample, f_2 is the count of doubletons (taxonomic units represented by two reads in the sample).

The Chao1 and other commonly used non-parametric richness estimators, e.g., the abundance-based coverage estimator (ACE) [15], have been shown not to converge by increasing the sequencing depth when applied to communities with high species richness and a large fraction of rare species [16]. This suggests that the estimators undervalue the true microbial diversity [17, 18]. This richness estimates are sensitive to sampling effort, implying that deeper sampling efforts give more true numbers for microbial species present in the sample [18].

Diversity indices are quantitative measures that are influenced by both the species richness and the evenness in species distribution. The most commonly used diversity indices in microbial studies are the Shannon and the Simpson diversity indices. The Shannon index (H') is defined as:

$$H' = -\sum_{i=1}^{s} (p_i log_2 p_i)$$

and the Simpson diversity as:

$$D = \sum_{i=1}^{s} p_i^2$$

where s is the number of taxonomic units and p_i is the proportion of the community represented by taxonomic unit i.

A community with a high number of species that are equally abundant will show high Shannon and low Simpson values, indicating a highly diverse community. However, both indices are also known to vary with sample size [19].

Both Simpson and Shannon indices can serve as a basis for estimating evenness, defined as the quotient between the actual, measured index and its theoretical maximum. A community quantitatively dominated by a minor fraction of all species present is considered

less even than a community in which several different species show similar abundances [10]. The evenness is maximized when all taxonomic types are equally abundant.

1.1.2 Microbial beta-diversity

A main feature of most microbial community studies is analysis of beta-diversity, i.e. comparisons of community composition (presence-absence or relative abundance of species) between two or more samples of equal size [9]. It describes the dynamics of communities across space and time, or along environmental gradients [10]. The beta diversity can be presented by a range of different indices [20]. The Jaccard and Sørensen indices are the two oldest ones and a modified version of the Sørensen index (Bray-Curtis index), based on abundance data instead of presence/absence data, was developed by Bray & Curtis [21]. It is bound between 0 and 1, where 0 means the two samples have the same composition (that is they share all species and at identical proportions), and 1.0 means the two sites do not share any species.

The Bray-Curtis index is given by the formula:

$$dBC = \frac{\sum_{i=1}^{R}|p_i - q_i|}{\sum_{i=1}^{R}(p_i + q_i)}$$

where p_i and q_i are the relative abundances of the ith taxonomic unit in the two samples to be compared and R is the combined richness of the two samples.

There are numerous ways to visualize and analyze beta diversity. Multivariate ordination and hierarchical clustering are commonly used for comparing the (dis)similarities among multiple samples. The hierarchical clustering is generally recommended when distinct discontinuities are expected between samples [22], while the ordination is commonly used for more continuous differences, i.e. as found in gradients [23]. The most common ordination

methods employed in microbial community comparisons are non-parametric, such as nonmetric multidimensional scaling (NMDS), canonical correspondence analysis (CCA), parametric principal coordinates analysis (PCoA), or parametric redundancy analysis (RDA).

To test for significant differences between multivariate groups, several non-parametric analyses have been used [24], among which NPMANOVA (non-parametric multivariate analysis of variance) [25] and ANOSIM (analysis of similarities) [26] are most commonly used in microbial ecology [23]. The NPMANOVA can be used to test for significant differences between the means of two or more groups of multivariate, quantitative data [25]. The test is the fraction of permuted F-statistics that is larger than the observed F-ratio. The ANOSIM test provides a way to do significance tests between two or more groups of sampling units based on permutations of the rank similarity matrix. The ANOSIM test is suitable for testing for spatial and temporal differences in community [26]. ANOSIM gives a p value (i.e. significance level) and an R-value (i.e. measure of compositional differences between groups). An R-value of 1.0 implies that the groups are completely separated while R = zero means no separation has occurred. Generally, R-values > 0.75 are commonly interpreted as well separated multivariate groups, $R > 0.5$ as separated, but overlapping, and $R < 0.25$ as barely distinguishable [26].

1.2 Microbial biogeography

Biogeography describes the distribution of organisms over space, time and along environmental gradients [27]. Biogeographic studies address a variety of questions, e.g., where certain organisms can survive or how historical events or present climate have shaped species distributions [28]. A famous dictum for microorganisms, formulated by Lourens Baas Becking, says that “everything is everywhere, but the environment selects” [29]. The first part of this tenet implies that the small size, high abundance and ease of dispersal of bacteria

should lead to a cosmopolitan distribution of all species (“everything can be everywhere”). The present composition of local communities would then be the result of selection by contemporary environmental conditions (the second part of the Baas Becking statement: “but the environment selects”). The microbial community in marine environments can be shaped by water depth [6, 30], temperature [31, 32], different water masse [33, 34], or chlorophyll-*a* [35, 36].

Recently, multiple studies have demonstrated that historical contingencies, mainly dispersal limitation, are also key factors affecting the spatial distribution of microbial communities [16, 37, 38]. Dispersal is the movement of organisms from one place to another. Low dispersal rates are likely to increase community beta diversity, and high dispersal rates lead to more homogenous communities (decrease in beta-diversity). Dispersal rates may vary widely between different types of environments, e.g., microorganisms in pelagic water masses will disperse faster than in subsurface sediment [36], because oceanic water is subject to more physical mixing. The dispersal of microorganisms in deep ocean upper sediments will likely be more limited than in sediments of shelf seas, where the impacts from e.g. near-bed ocean currents and large benthic organisms are stronger [39].

Four conceptual models have been presented to describe and explain microbial biogeography [37] (Fig.1). If the samples are randomly distributed over space, there is no effect of either current environmental conditions or past historical events (Fig. 1a). Alternatively, the distribution of microorganisms is affected by current environmental variation, and there is very little or no influence of historical events on the microbial distribution (Fig. 1b). In the third model, the historical events are main effect influencing microbial community patterns (Fig. 1c). Finally, both past historical events and contemporary environmental conditions can shape the microbial biogeographic patterns (Fig.1d).

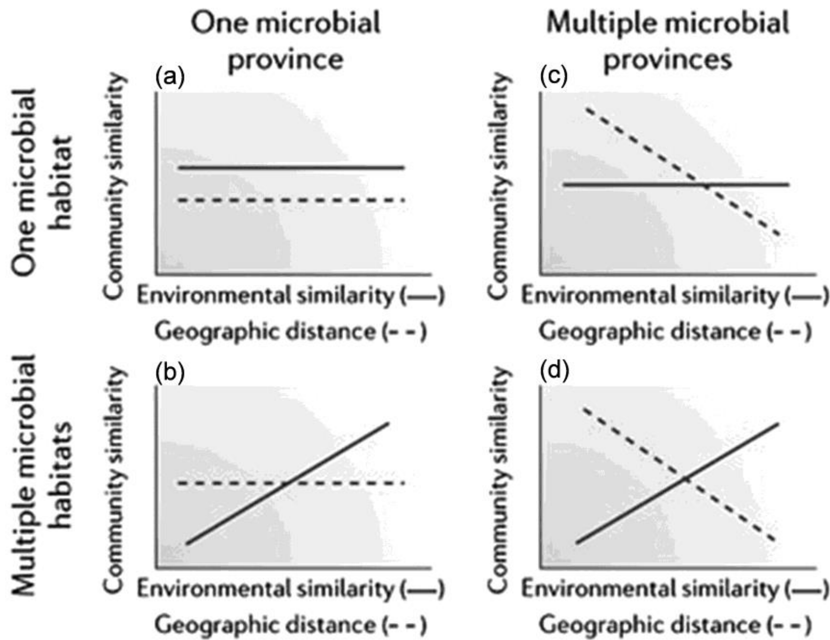


Figure 1. The contribution by environmental and historical effects on microbial biogeography (Figure from Martiny et al. [37])

To test which of the four alternative models fits the experimental data, correlation tests between environmental variation or geographic distance on the one hand, and community variation on the other, can be done. Since these tests are generally based on distance matrices, methods that allow for non-independence of variables, such as complete or partial Mantel tests or bootstrapped regression analyses have to be employed.

1.3 Characteristics of marine sediments

Marine sediments are the result of the accumulation of particles derived from a variety of sources that have been deposited on the ocean floor. They can be grouped and ordered by the grain size or the origin of the deposited material [40]. Grain size classification divides sediments into four main types, ranging from small-scale clay (< 3.9 µm in diameter) via silt particles (< 63 µm) to macroscopic sand (< 2 mm) and gravel (> 2 mm). According to the origin of the deposited material, sediments are divided into five types, including terrigenous,

biogenic, authigenic, volcanogenic and cosmogenous [40]. Terrigenous materials are those derived from the land by rivers, fluxes, winds, glaciers and turbidities. Biogenic sediments come from life in the oceans. The main ingredients of biogenic sediments are calcium carbonate ($CaCO_3$) and silica (SiO_2), which are composed of the hard parts of organisms. Authigenic (or hydrogenous) components are oceanic minerals that precipitate directly from the seawater. They mainly consist of manganese (Mn) and phosphorus (P) containing minerals. Volcanogenic (or lithogenous) sediments are composed of mineral products formed during volcanic eruptions while cosmogenous sediment are extraterrestrial (outer space) particles that have survived the trip through the atmosphere. The terrigenous or volcanogenic fractions are the main constituents of continental shelf and slope sedimentation, while cosmogenous, authigenic and biogenic particles are the major components of the deep-sea sedimentation. The sediments accumulate very slowly on the deep ocean floor, i.e. by less than 1mm per 1000 years, and have low organic carbon content of less than 1%, whilst the shelf sediments accumulate at an average rate of 30 cm/1000 years, with a range from 15-40 cm [41].

In marine sediments, microbial metabolic processes are principally stratified according to the sequential consumption of electron acceptors down the sediment. Oxygen is the main electron acceptor in surface sediment, followed by nitrate (NO_3^-), manganese (Mn^{4+}), ferric iron (Fe^{3+}), sulfate (SO_4^{2-}) and carbon dioxide (CO_2). The penetration of oxygen or nitrate in deep or abyssal marine sediments is on a scale of meters [42], while in organic-rich continental margin or shelf sediments all electron acceptors are consumed in the upper few centimeters (Fig. 2).

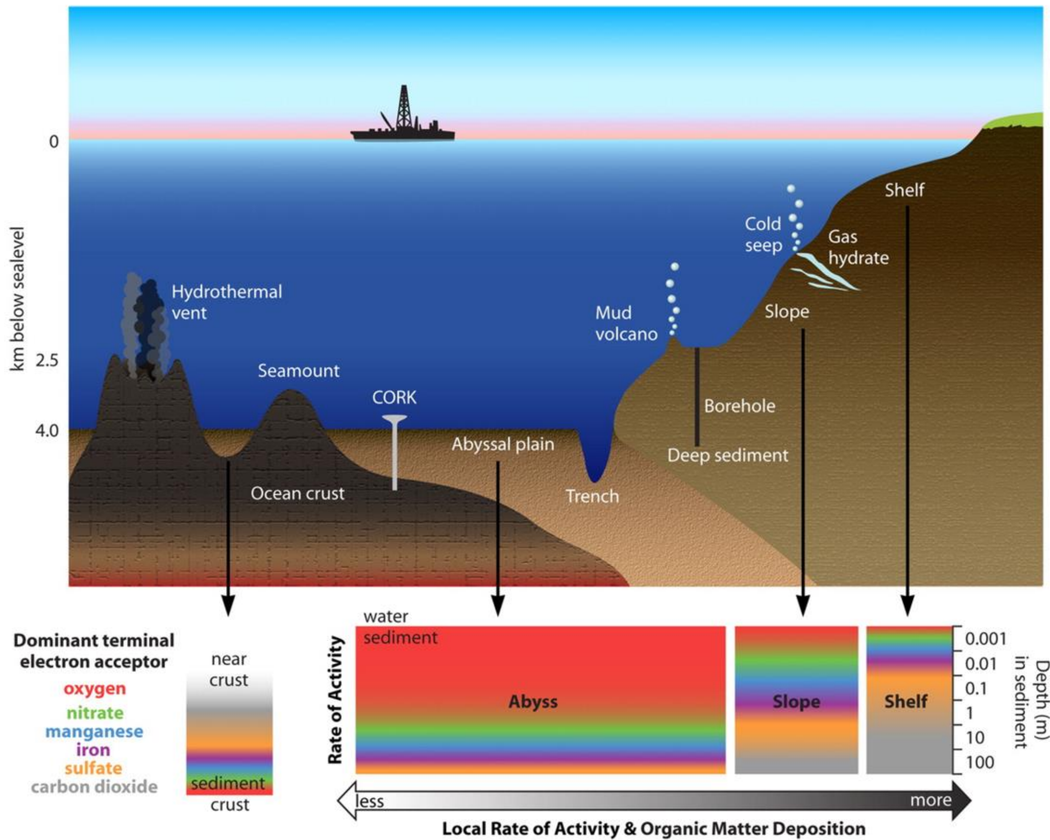


Figure 2. Diagram of some major seafloor habitats and the profiles of dominant electron acceptors in these habitats (Figure from Orcutt et al. [43]).

1.4 Microbial diversity in marine sediments

1.4.1 Bacterial diversity in marine sediments

The global abundance of bacterial and archaeal cells in the marine subsurface sediments is estimated between 2.9 $x10^{29}$ and $3.5x10^{30}$ cells [44, 45], and the densities are higher in shelves/margins than in abyssal sites [42, 46]. In all habitats, however, the abundance of cells decrease logarithmically with sediment depth [45, 46]. Densities of 10^8 to 10^9 cells cm^{-3} in surface sediment decrease to 10^6 to 10^7 cells cm^{-3} at hundreds of meter depths [46]. The numbers of prokaryotic cells are strongly correlated with organic matter burial rates. Factors

that affect the burial rates include the productivity of the overlying ocean, the water depth, the flux of organic matter from land, and the sedimentation rate [45, 47].

Abundant bacterial taxa in marine sediments are *Gammaproteobacteria*, *Deltaproteobacteria*, *Alphaproteobacteria,* *Epsilonproteobacteria*, *Chloroflexi*, *Actinobacteria,* and uncultured candidate phylum *JS1* [36, 43]. In the upper layers, the *Gamma*- and *Deltaproteobacteria* are dominant [48, 49] and the *Deltaproteobacteria* are also abundant in cold seeps with high rates of sulphur reduction and methane cycling [50]. The *Gammaproteobacteria* are consistently found at ocean margin sites, commonly at high concentrations of organic matter. However, they are less common at open-ocean sites, where organic concentrations are low [42]. In the deeper layers of marine sediments, *Epsilonproteobacteria, Chloroflexi* and candidate division *JS1* are dominant [49, 51]. The *Epsilonproteobacteria* are also abundant at hydrothermal vent sites [52]. Uncultured candidate division *JS1* is typically found in organic-rich deep sediments [49], and in subsurface sediments with reduced sulphate [49]. Phylum *Chloroflexi* is a widespread group of bacteria found in a range of microbial communities, not only subsurface sediments [53], but also wastewater, polluted sites [54, 55], and organic-rich subseafloor sediments [53, 56]. The phyla *Actinobacteria* and *Bacteroidetes* are present in most samples from surficial sediments [48, 57, 58] as well as in basalt communities [59, 60], but the groups are rarely observed in hydrate associated and in deep sediments [43].

1.4.2 Archaeal diversity in marine sediments

Archaea were discovered as an independent domain in the 1970s [61]. Two decades later, marine Archaea were first reported in Antarctic coastal surface water [62]. Currently, there are five recognized major groups of Archaea, the *Euryarchaeota*, *Crenarchaeota* and the more recently described *Korarchaeota*, *Nanoarchaeota*, and *Thaumarchaeota*.

The most abundant marine sediment Archaea are the *Crenarchaeota*, *Euryarchaeota*, and *Thaumarchaeota*. The *Thaumarchaeota*, previously classified as a Crenarchaeotal group 1.1a [63], are aerobic, ammonia oxidizing prokaryotes [64], which have been found particularly prevalent in upper layer marine sediments [46, 65, 66]. The *Thaumarchaeota* are also found in the oceans [67], in hydrothermal deposits [68], and in hydrothermal fluids [69], as well as in basalt [60, 69]. Surprisingly, ammonia-oxidizing *Thaumarchaeota* are also dominant in deep layers of sediment [65, 70]. They may have the ability to oxidize ammonia with an alternative elector acceptor, or the *amo* genes present in the organisms could have some other function [71].

The *Crenarchaeota* have been detected in a variety of different marine sediments, including organic-rich shelves/margins [72], cold sediments [56, 73, 74], mud volcanoes [75], and methane hydrate-containing marine sediments [76]. These groups are also found in other environments, wastewater [77], and freshwater sediments [78, 79]. They are anaerobes, facultative anaerobes or aerobes, and reduce sulfur or nitrate [80, 81].

The *Euryarchaeota* are found in marine sediments containing methane hydrates [82-84], also in deep marine subsurface sediments [74, 85]. This phylum includes both methanogenic and anaerobic methane-oxidizing groups.

1.4.3 Viral diversity in marine sediments

Viruses affect all three domains of the tree of life (Bacteria, Archaea and Eukaryota) [86]. Most marine viruses, however, are assumed to be bacteriophages [87]. Phages have been shown to impact processes like horizontal gene transfer, microbial community structuring and biogeochemical cycling [88-90]. Similar to the prokaryotes, viral abundances decrease exponentially with sediment depth [91], and also decrease by moving from shallow shelves to

the abyssal sediments [92]. The viral density increases in areas with high pelagic productivity, where sediments are enriched with suspended particles from the overlaying water [89, 93, 94]. Moreover, marine viral abundance is closely linked with the abundance of their potential hosts, so that any change in the abundance or metabolic state of the prokaryotic host populations will affect viral abundance [89, 95].

Viral genomes may have double-stranded (ds) or single-stranded (ss) DNA or ds or ss RNA. Several studies have indicated the dsDNA group to be the most abundant in marine environments [87, 96-99]. But in recent years, high proportions of ssDNA viruses have been reported in various aquatic environments, including coastal estuaries [100, 101], marine waters [100, 102-104], freshwater [105, 106], and deep seafloor sediments [107]. The study of viral diversity is limited by the sampling method, the isolation, and the lack of viral characterization. It is estimated that less than 1% of the extant viral diversity has been explored so far [108]. Moreover, 60-95% of marine viral metagenomic sequences show no significant similarity to any sequences in databases [102, 109, 110].

1.5 Study system: The Barents Sea

1.5.1 The Barents Sea and its seafloor

The Barents Sea is an Arctic continental shelf sea of approximately 1.4 million km^2, with an average depth of 230 m. The maximum depth is approximately 500 m in the western part of the Bear Island Trough and the shallowest part is around 50 m at the Spitsbergen Bank. The Barents Sea is bordered by the Norwegian and Russian mainlands to the south, the Arctic Ocean and Svalbard to the north, Novaya Zemlya to the east, and the Norwegian Sea to the west [111]. The general oceanographic feature of the Barents Sea is inflow of temperate

Atlantic and coastal water from the west and cold Arctic water from the north (Fig. 3). The Atlantic and Arctic water masses are separated by the Polar Front, which is characterized by marked gradients in both temperature and salinity [112]. The temperature differences are most pronounced in the surface waters, resulting in winter sea ice covering the northern regions and central Barents Sea, while the southern parts of the Barents Sea are ice-free throughout the year. Near the seafloor, the temperature difference is modest, i.e. about 2°C, and the temperature difference has even shown a diminishing trend in recent years [113].

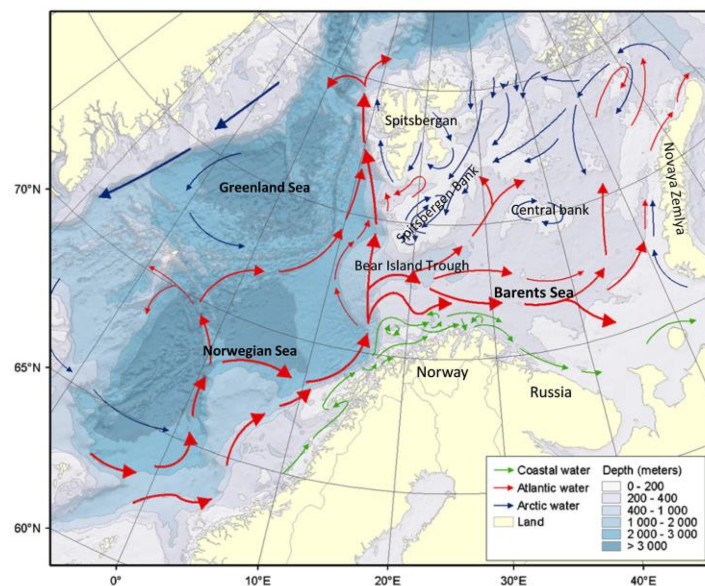


Figure 3. Schematic of the circulation patterns in the Norwegian and Barents Seas. The Figure is modified from Loeng and Drinkwater [111]

The Barents Sea is a spring bloom system. The bloom starts in late March/early April in the south-west areas and spreads north-east, as the icemelt proceeds over the season [114]. During the bloom period, the phytoplankton abundance increases much more rapidly along the polar front than in the Atlantic waters, but after the peak, phytoplankton densities also decrease faster in the front water [115]. The average annual primary production in the Barents Sea is approximately 90 g C m^{-2} year -1 and it varies by 10 -15% from year to year [116]. The primary production in Polar water mainly occurs in the spring, while in the Atlantic water it is stronger in the summer and autumn. The highest primary productivity in the Barents Sea

occurs in the mixed water areas, such as Bear Island, and the Spitzbergen Bank [116]. However, the total annual primary production in the Atlantic water is estimated to be threefold higher than in the Polar water [117].

1.5.2 Offshore drilling for oil and gas in the Barents Sea

The first exploration license for the Norwegian Barents Sea was awarded in 1979 and exploratory drilling began in 1980 by Norsk Hydro [118]. The Snøhvit gas field, operated by StatoilHydro, was the first commercial oil and gas development project in the Barents Sea, while the Goliat field, operated by Eni Norge AS, started production in 2006. By 2013, a total of 97 exploratory wells had been drilled in the Barents Sea. The exploration activities were most intensive in the period 1980-1993 (54 of 97 wells were drilled). From 1994 to 1999, no new wells were drilled in the Barents Sea due to declining interest by the industry. However, optimism returned to the Barents Sea with the discovery of oil in the Goliat field in 2006, and exploration activities are ongoing at present.

During drilling of exploratory and production wells, various types of waste material are generated and discharge of used drilling muds (drilling fluids) and drill cuttings are the ones that affect the seafloor most directly.

The term “drill cuttings” refers to the rock debris produced during the drilling process when the drill bit is driven down through the ground. The rock fragments are carried to the surface by drilling muds that are pumped down through the drill pipe. The largest sources of drilling waste are rock material and small quantities of liquid and solid components of the drilling muds. The volume and level of contaminated drill cuttings waste depend on the type of drilling muds, the depth of the well, and the size of the borehole.

Drilling muds are used to aid the drilling process. Three main types of drilling muds have been used offshore. They include oil-based (OBM), synthetic (SBM), and water-based drilling muds (WBM), as divided by the character of their fluid components. In OBM, the dominating fluid is a mineral oil, while SBM have less toxic organic fluids, like esters, or olefins. Fresh water or salt water are used in WBM. Another component of drilling muds is weight materials that often contain barite, hematite and brines.

During the 1970s and 1980s, drilling wastes were generally discharged from the platforms directly into the ocean. Laboratory and field studies revealed that OBM were toxic and persistent in marine sediment around oil and gas installations [119-121]. Oil-based drilling muds have been found to affect species composition and diversity of the benthic fauna several kilometers away from the drilling locations [119, 121]. Therefore, since 1993 OBM or drill cuttings contaminated with oil-based mud are no longer permitted on the Norwegian Shelf.

Synthetic based drilling muds were developed in the early 1990s to reduce the toxic effects and thereby inflict less harm on the marine environment [122]. The SBM have lower toxicity, faster biodegradability and lower bioaccumulation potential than OBM. However, with effect from 1993, discharges of drilling waste containing more than 1% oil were prohibited in Norwegian water. The use of SBM, therefore, ceased around 1995 in Norway.

Water based muds are the most widely used muds today and are predominantly used in the Barents Sea, due to environmental concerns and compliance with regulations. They are considered less harmful, and environmental impacts have not been recorded more than 200 m from the drilling installations [123-125]. The main effect of WBM is assumed to be the physical impact of the sediment material, e.g. disruption of feeding or respiration, or burial of settled larvae [124, 126]. However, several studies have indicated that the effect of WBM on

the environment seems to be more complex than previously assumed [125, 127], and therefore, this should be investigated further.

Little is known about how the microbial community is affected by water based drilling muds and cutting deposition, because most studies focus on the consequences of using oil-based muds. In a North Sea field study, Sanders and Tibbetts [128] showed that the number of hydrocarbon-oxidizing bacteria and sulfate-reducing bacteria being highest close to the platform. The impact of WBM on microbiota is considered to be least harmful among the different drill cutting types [128], but it still has a significant impact on the microbial compositions in sediment [129].

2. Aims of the thesis

The overall aim of the thesis was to expand the knowledge of the microbial diversity in Arctic marine environments by a comprehensive study of the microbiota of a shelf seafloor. Both the overall taxon composition and its variation due to geographical distance and anthropogenic impacts were explored.

Next-generation sequencing technologies were employed in order to comprehensively catch the diversity of a complex microbial community like this marine seafloor.

The specific research questions addressed in this thesis were:

- What bacterial, archaeal and viral taxa are present in upper Arctic marine sediments and how large are their relative contributions to the overall microbiota pool (**Paper I, II, and III**)?

- Are there common biogeographical patterns among the different main groups of microbes within the same geographical region and what factors are the main drivers of the biogeographical variations **(Paper I, II**)?

- Are anthropogenic insults like deposition of drilling waste onto the seafloor during offshore drilling operations clearly reflected in bacterial community changes, and if so, are there specific taxonomic groups that may serve as bioindicators of such impacts (**Paper III**)?

3. Results and discussion

3.1 Methodological consideration

3.1.1 Primers for 16S rRNA gene amplification

The 16S rRNA gene has become the universal standard in prokaryotic taxonomic classification and a powerful tool for assessing and comparing the diversity of microbial communities. The gene consists of eight highly conserved regions, U1-U8 and nine variable regions, V1-V9. The overall sequence length is about 1550 bp [130]. Primers targeting conserved 16S rRNA gene regions are used to generate amplicons of variable regions that are more informative for taxonomic assignments. However, choosing different primer pairs can result in differences in the inferred community compositions by over- or under-representation of certain phyla [131-134]. In several studies, the V3 or V6 regions individually, or the V4-V6 region, have been targeted in sequencing projects employing high-throughput methods [135, 136]. Amplicons of these regions provide sufficient phylogenetic information, to be close to equivalent to full-length 16S rRNA gene sequences [136]. Other studies suggest that representational characterization of bacterial communities is achieved by use of the V1-V4 region [137], while the V1-V3 and the V4-V7 regions were recommended for analysis of archaeal communities [137]. Recently, Yang et al. [138] compared the individual V1 to V9 regions to the corresponding full-length sequences, and the results indicated that the V4 to V6 regions provided the best choices for representing the full-length 16S rRNA sequences in the phylogenetic analysis, while V2 and V8 were the least reliable regions. However, Baker et al. [130] conclude that no single V region is guaranteed to amplify all taxonomic groups with the same efficiency.

As part of my work, the same source material (station 8 in **Paper I**) was amplified with different primer sets, i.e. V1-V2 (**Paper I**) and V3-V4 (unpublished data). Furthermore, the 16S rRNA gene distribution in a metagenome constructed from the same material was established as part of a master thesis [139]. Both amplicon and metagenomic sequencing confirmed that *Gammaproteobacteria* and *Deltaproteobacteria* had high abundances in the Barents Sea sediments (Fig. 4). The taxonomic distribution was markedly influenced by the choice of 16S rRNA gene primer pairs. The primers amplifying the V1-V2 regions generated much higher fractions of class *Gammaproteobacteria* and *Deltaproteobacteria* than the V3-V4 primers (43% and 23 %, as compared to 28% and 19% of total reads). The V3-V4 amplicons, by contrast, showed higher relative abundances of groups such as *Alphaproteobacteria*, *Chloroflexi*, *Planctomycetes* and *Verrucomicrobia*.

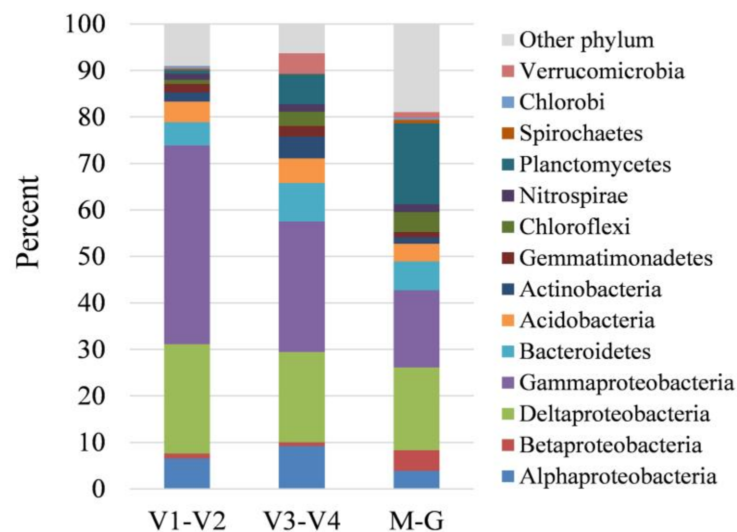


Figure 4. Distribution of major phylogenetic groups of Bacteria from amplicons of the V1-V2 and V3-V4 regions of 16S rRNA gene and from taxonomic annotation of 16S rRNA genes in metagenomic DNA (M-G), all originating from the same sample material.

The relative abundances of taxa were also influenced by the choice of method. Some groups were more strongly represented in the metagenomic data than in the amplicon data, including *Betaproteobacteria*, *Chloroflexi* and *Planctomycetes,* while others, such as *Gammaproteobacteria*, *Alphaproteobacteria* and *Actinobacteria,* showed the opposite trend.

Remarkably, the *Planctomycetes* constituted 17.4 % of the community when based on metagenomic data, but just 0.7 % in V1-V2 and 6.7 % in the V3-V4 amplicons (Fig. 4). Similar results are reported by Poretsky et al. [140], who detected *Planctomycetes* in the metagenomes, but hardly so in 16S rRNA gene amplicons. Overall, our data showed that the inferred taxonomic compositions of the communities were sensitive to primer choice by the 16S rRNA gene amplification and sequencing technology. However, primer choice has more impact on the outcome of microbial community analyses than the choice of sequencing platform [141]. If presupposing that the metagenomic data show the best accordance with the true taxon distribution in the sediment, the V3-V4 amplicons apparently performed better than the V1-V2 amplicons for this type of material.

The choice of metagenomics or 16S rRNA gene sequencing approaches for diversity analyses is dependent on the biological question and objectives of the study. The metagenomic sequencing avoids biases of PCR amplification, but often fails to provide sufficient sequence depth to detect the 16S rRNA genes of rare species in a community [142], especially in complex microbial communities like soils and sediments. The 16S rRNA gene is well suited for analysis of multiple samples providing cost-effective phylogenetic analyses, but PCR amplification biases appears as an inevitable drawback. If using a one-gene PCR amplicon comparison approach, it is possible to include reads that have no significant taxonomic annotation both in various alpha diversity estimates and in comparative beta diversity analyses.

3.1.2 Viral isolation and DNA amplification

To eliminate contamination by cellular material, viral particles can be purified by filtration [143, 144] or purification in a density gradient [145, 146].

In our study, filtration of the samples with 0.2 µm pore size filters was used as purification method. This procedure may not recover large virus particles and thus may bias the viral preparations towards the smaller ssDNA viruses (**Paper II**). However, the purification of viral particles in cesium chloride (CsCl) density gradients also has limitations, as bacteriophages show considerable variability in terms of shape, size and buoyant density [146]. Therefore, if extracting only narrow fractions from CsCl density gradients, certain groups may fall outside the specified density range. Besides, some phage types degrade rapidly in CsCl gradients due to chemical or mechanical stresses [146].

To compare the results from our study with previously published research where CsCl gradient purification has been used for cleaning viral preparations, metagenomes deposited in the Metavir server were re-analyzed in accordance with the procedure for our sequence data. The results showed the proportion of virus-affiliated sequences to be higher in our study than in other studies, i.e. 27-44 % versus 1-32 % (Fig. 5). These figures indicated that the purity of our virus preparations were satisfactory and that the procedure was well suited for purification and isolation of virus from marine sediments.

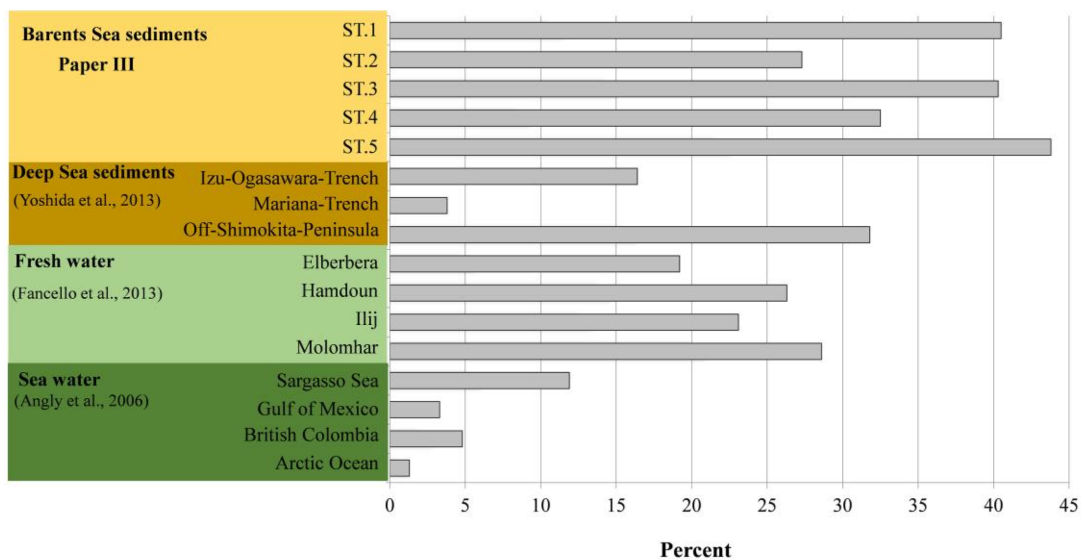


Figure 5. The percent viral sequence reads exhibiting significant similarity to the Refseq viral genomes deposited in the nr protein database (E threshold value 10^{-3})

The quantities of viral DNA from environmental samples are often too small for sequencing due to the small size of viral genomes combined with substantial losses during purification. To increase the amount of DNA template for sequencing, the extracted materials were amplified by multiple displacement amplification (MDA) (**Paper II**). Random hexameric oligonucleotides are used for DNA synthesis with phi29 DNA polymerase [102]. By this method, femtograms of DNA template may be amplified to micrograms of product. However, the polymerase is known to preferentially amplify ssDNA rather than dsDNA [147, 148]. Another common viral DNA amplification technique is to make linker amplified shotgun libraries (LASL), which requires higher initial DNA concentrations than the MDA method. Total viral DNA is randomly sheared into small fragments and end-repaired. The DNA fragments are ligated with oligonucleotide adapters that can be used for PCR amplification [96]. The two different methods can give widely different patterns of diversity even from the same template material, with complete dominance by dsDNA viruses as the characteristic of the LASL method, whereas ssDNA viruses are predominantly represented employing the MDA method [147]. Hence, comparisons between metagenomic studies employing different amplification methods are complicated and of limited value [99]. Several methods have been introduced to obtain less biased viral amplicons, i.e. transposon-based Nextera [149], loop-mediated isothermal amplification of DNA (LAMP) [150], or hydroxyapatite chromatographic separation of viral groups prior to LASL amplification [151], but it still remains a challenge to obtain truly representative of preparations of viral metagenomes.

3.1.3 Bioinformatics and data analysis

Sequences were clustered into Operational Taxonomic Units (OTUs) at a dissimilarity threshold value of 3%, implying that the OTUs served as proxies for species [12]. The total

number of OTUs in an amplicon depends on the sequencing depth. As an example, the clustering of 7761 sequences from the Barents Sea sampling station 6 generated 2116 OTUs, while 164 880 sequences from the same sample, generated in an independent analysis, clustered into 9074 OTUs, i.e. a four-fold increase in the OTU richness (**Paper I**). Therefore, estimation of OTU richness by use of the Chao 1 index had to be based on an even number of sequences from each sample. Singleton OTUs (i.e. OTUs comprising only one sequence among the reads) from both bacterial and archaeal communities were removed before taking any further steps (**Paper I**), as recommended by Huse et al. [152] and Quince et al. [153]. Removal of the singletons will evidently reduce the OTU/species richness in a sample and thereby have an impact on alpha diversity estimates. However, the reads with a minute presence (one individual in tens of thousands) will have insignificant impact on beta diversity [36].

In this thesis, the taxonomic annotations were inferred by similarity-based approaches. The complete or partial gene sequence reads were compared to known sequences present in reference databases. Such comparisons are most commonly done using the Basic Local Alignment Search Tool (BLAST) [154]. This method has higher taxonomic assignment efficiency for short fragments of 1 kb or less than other methods, i.e composition-based methods [155]. However, for next generation sequencing data, BLAST searches demand enormous amounts of time and computing resources for generating alignments of query sequences with reference databases. Moreover, the lack of comprehensive reference genome databases frequently leads to sequences being categorized as “unassigned” or having low-identity matches to the reference sequences. New additions/updates of the same databases, therefore, continuously reduce the fraction of queries that end up as “unassigned”. For example, the novel archaeal class *Thaumarchaeota* was not included in the 2011 version of the Greengenes sequence database, while large numbers of *Thaumarchaeota* were identified

from the same sequence data (**Paper I**) by using Greengenes database of May 2013 when the *Thaumarchaeota* had been introduced into the database. In **Paper II**, the percent open reading frames from our viral assemblages that showed significant similarity to genes of complete viral genomes in the RefSeq database was increased from 23-38% (database release of 2015-01-05) to 27-44% in the updated database of 2016-01-19.

Last but not least, disentangling the effect of environmental factors and historical processes on microbial biogeography were main challenges in our study. There were significant collinearities between several environmental factors, i.e., temperature, phytopigment ratio, water depth and salinity, and the same factors showed marked spatial autocorrelation (**Paper I**). The collinearity caused problems in variation partitioning efforts, such as negative contributions to explained variation in, e.g., partial redundancy analysis. A generalized linear model (GLM) approach proved most suitable for our data (**Paper I**). However, co-variation was still a problem by making the regression coefficients of the linear models sensitive to minor changes in the input data linear models and therefore difficult to interpret [156].

3.2 Microbial alpha-diversity in Barents Sea sediments

3.2.1 The prokaryotic diversity

The taxon composition of the bacterial communities in the Barents sea seafloor was similar to what has been reported from marine sediments elsewhere, that is distinctly higher fractions of *Deltaproteobacteria* (**Paper I**, **III** and references therein), and lower abundances of *Alphaproteobacteria* than commonly found in the pelagic bacterial communities (Fig. 6).

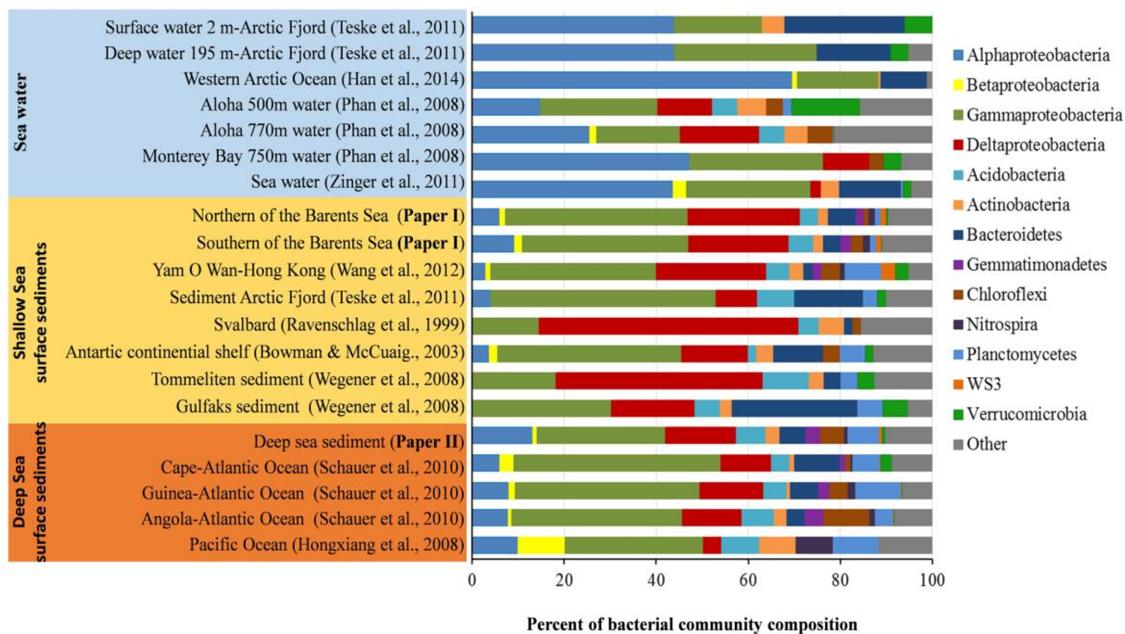


Figure 6. Composition of bacterial communities (percent) from various habitats at phylum/class level of taxonomy

In the uppper layer of sediments, *Gammaproteobacteria* and *Deltaproteobacteria* were dominant members of bacterial communities in both the Barents Sea transect and the undisturbed Bønna continental slope sediments (**Paper I, III**). These groups have been identified as major marine sediment bacterial community [36, 48, 157, 158]. Most *Deltaproteobacteria* sequences were affiliated with the uncultivated deep-sea trench sediment group NB1-j [159] and the sulfate-reducing *Desulfobacteriales*, while the high proportion of *Gammaproteobacteria* reads were affiliated with family *Piscirickettsiaceae* (**Paper I**, **II**). With reference to the discussion of the effect of primer choice above, the *Gammaproteobacteria* and *Deltaproteobacteria* groups in the Barents Sea transect, amplified with V1-V2 primer pairs (**Paper I**), showed higher proportions than at the undisturbed Bønna location, amplified with primer pairs for V3-V4 (**Paper III**) (Fig. 6). However, there were smaller differences in relative abundances of these groups if the Barents Sea transect

communities amplified with V3-V4 primer pairs (unpublished data) were compared with the V1-V2 data of paper I.

The major archaeal taxonomic group observed in the Barents Sea shelf seafloor was the recently established class *Thaumarchaeota* (**Paper I**). This is in agreement with previous studies of marine sediments [74, 160-162]. The *Thaumarchaeota* are associated with an autotrophic ammonia-oxidizing energy metabolism with capacity to utilize low substrate concentrations [64, 163]. Several studies in oceanic waters have shown that the abundance of *Thaumarchaeota* groups varies seasonally, as they appear to be predominant in winter, but all most absent in summer [163-169] (Fig. 7).

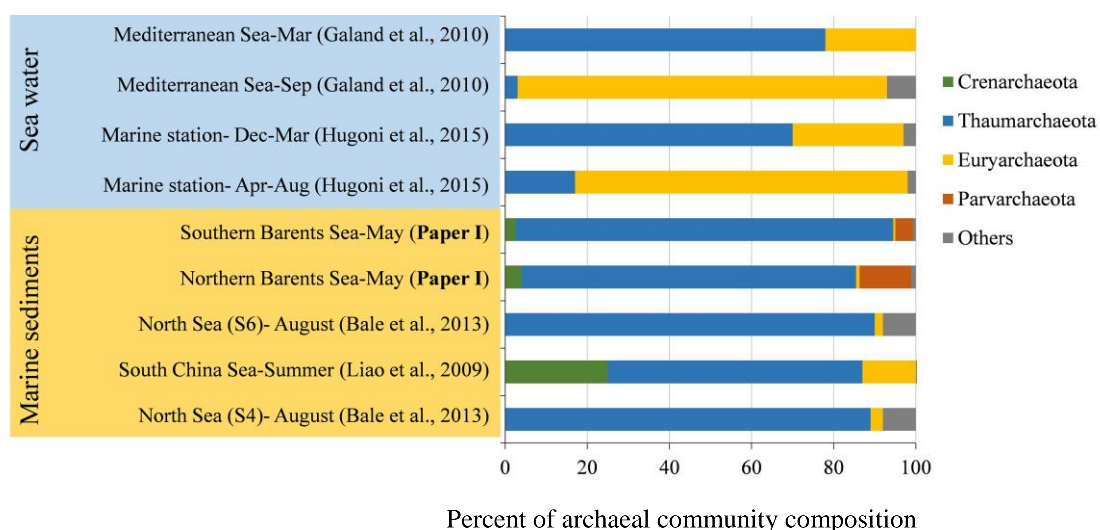


Figure 7. Composition of archaeal communities (percent) in various habitats at phylum level of taxonomy

In contrast to pelagic *Thaumarchaeota***,** benthic *Thaumarchaeota* in the upper layer sediment were on average highest in the summer (August) and lowest in the winter (November) [164]. In our study, the relative abundance of *Thaumarchaeota* was higher in the southern part, where the temperature was higher than in the northern part, when the samples were collected in late May (**Paper I**). This may be expected because deposition of algal bloom derived organic matter onto the Barents Sea seafloor occurs in late spring and summer.

The Bønna bacterial communities close to the fresh drill cuttings affected area gave a different picture of the bacterial composition in the top layer of the sediments. The community structure exhibited a consistent relationship to distance from the drilling location, the most striking features being high abundances of groups *WH1-8* and *Fusibacter,* belonging to the order *Clostridiales* and of *Desulfuromonadaceae* and *Pelobacteraceae*, belonging to the order *Desulfuromonadales*, and the class *Mollicutes* in the two upper layers near the drilling well (**Paper III**). The *Fusibacter* are able to transform glucose to acetate by fermentation [170], and have been speculated to be responsible for the biodegradation of benzene in an iron-reducing enrichment culture [171-173]. The *Desulfuromonadales* are known to reduce sulfur, iron and manganese oxides [174, 175], and are frequently found in clone libraries of marine sediments [48, 176]. The apparent high presence of these members in the top sediment layer near the drilling site make these groups possible candidates as microbial bioindicators of seafloor perturbation by water-based drilling muds (**Paper III**).

3.2.2 The viral assemblages

More than half the metagenomic reads obtained from the Barents Sea sediment viral preparations could not be taxonomically assigned (**Paper II**), indicating that a large fraction of viral diversity remained uncharacterized. The Barents Sea sediment viromes were dominated by ssDNA viruses which belonged to the two major groups *Microviridae* and Eukaryotic Circular Rep-encoding ssDNA (CRESS-DNA), the latter including the families *Circo-, Nano-,* and *Geminiviridae* (**Paper II**). These results were in agreement with previous studies in marine environments [103, 107, 147, 177, 178].

The diversity of viral assemblages, amplified by the MDA method from different environments, i.e., from freshwater, seawater and marine sediments, were compared. The composition of the sediment viromes deviated quite clearly from those of freshwater and

seawater as ssDNA viruses appeared very dominant, while the dsDNA virus types showed high abundance in the freshwater and seawater samples, although the MDA method amplifies ssDNA more efficiently than dsDNA (Fig. 8).

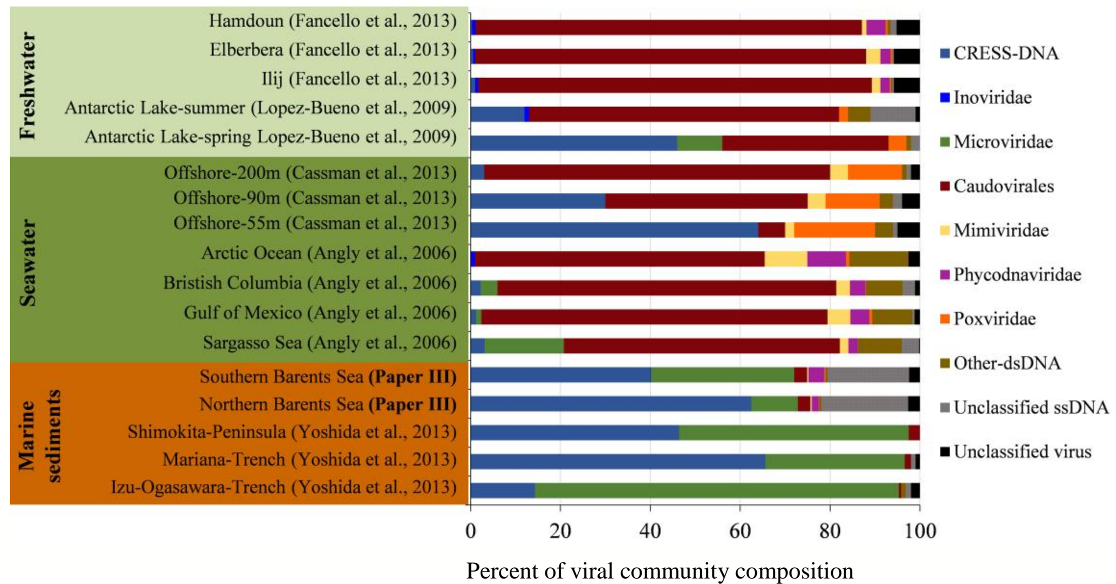


Figure 8. Percentage composition of viral communities from various habitats. All taxonomic annotations originate from multiple-displacement amplified viral DNA

The composition of the Barents Sea sediment viral assemblages showed similarity with three Pacific Ocean marine sediment viromes [107]. Interestingly, the Pacific location with least difference in water depth, showed the highest assemblage similarity (**Paper II**). The similar community features in the seafloor viromes likely reflect corresponding similarities in the prokaryotic and eukaryotic host communities. However, very little is known about the viruses that are associated with marine microbes.

3.3 Microbial beta-diversity in Barents Sea sediments

3.3.1 Spatial variation of prokaryotic and viral communities in the Barents Sea transect

Community distance decays were observed in both the bacterial and archaeal communities in the Barents Sea sediments (**Paper I**). Therefore, it can be concluded that the dispersal rate of the microbes was not high enough to blur the community structuring effects of the extant environmental differences within the sampling area or the possible community differences established by past events. The bacterial biogeographic variation in our study fell into "model four" while archaeal variation patterns fell into "model two" of [37] (Fig. 1d and 1b, respectively in the Introduction). For bacteria, the significant impact of environmental factors on community composition was confirmed when controlling for spatial distance. Although the reciprocal test, i.e. spatial effects when controlling for environmental distance, concluded with marginal non-significance, the bacterial communities seemed influenced by both environmental factors and geographical isolation. For archaea, the community structuring appeared as more exclusively determined by environmental factors. However, the two prokaryotic domains seemed controlled by different environmental factors. The temperature appeared as the strongest driver of bacterial community structuring, while the generalized linear model analysis left this factor uninfluential on archaeal beta-diversity. The organic content and grain size of the sediment also gave some contribution to bacterial variation. Only the level of freshly sedimented phytopigment appeared as a main factor influencing the archaeal distribution pattern (**Paper I**). However, the GLMs were sensitive to minor changes in our input data. Therefore, we could not concluded with high confidence whether this difference had a true ecological basis or rather was a consequence of model lability caused by collinearity.

The viral composition in the Barents Sea sediment also varied according to the south-north biogeographic pattern (**Paper II**). However, the distribution of the dominant group of bacteria-associated virus in the assemblages, i.e the *Microviridae* family of phages, showed no clustering pattern reflecting the one exerted by the bacterial community. The observed Barents Sea virus assemblages clustered into a southern and a northern region primarily by the composition of eukaryotic CRESS-DNA virus assemblages, suggesting that these taxa were inclined to stronger host variations along the south-north axis than the bacteria-infecting viruses. The relative abundance of eukaryotic virus types in the north was higher than in the south (**Paper II**), coinciding with higher levels of chlorophyll *a* and chloroplast 16S rRNA gene frequencies in the northern part, as reported in **Paper I**. Viruses are known to impact blooms of phytoplankton [179, 180]. Hence, the inputs of fresh algal phytodetritus to the seafloor, due to the recent ice margin spring bloom, may have contributed to the observed differences among the eukaryotic virus assemblages.

3.3.2 Spatial variation of bacterial communities surrounding a freshly drilling location

The bacterial community structure within a radius of about 200 m around the drilling location changed both with distance from the drilling site, evidently related to the effect of drilling waste disposal, and by sediment depth (**Paper III**). Most strikingly, the steeper shift towards anaerobicity in the uppermost centimeters in the vicinity of the drilling site as compared with 100 m distance and beyond, coincided with significant changes in bacterial community structure. The community diversity, e.g., richness and evenness values also showed a slight decrease. The observed increase in anaerobicity near the drilling location appeared to contribute directly to the observed re-structuring of the upper sediment bacterial communities. The levels of residual barium indicated residual quantities of drilling waste, but it is unlikely that the changes in the bacterial community were caused by the barium as such,

due to low solubility and very high minimum inhibitory concentrations of barium [181]. The top layer of sediments had less silt and clay fraction than the deeper layers at all the sampling sites, and there were no differences in grain size in corresponding layers related to distance from the drilling location. Therefore, the observed relationship between grain size and bacterial community structure reflected the distinctiveness of the deep communities rather than being a consequence of the drilling waste discharge.

The PCoA ordination partitional all sampling samples into four distinct clusters, among which two were associated with the upper sediment layers visually affected by drilling waste discharge (**Paper III**). Remarkably, high presence of the fermentative *Clostridiales*, anaerobic *Desulfuromonadales*, and parasitic *Mollicutes* was observed in the most affected upper layer, pointing to one or more of these taxa as prospective candidates as microbial bioindicators of seafloor perturbation by water-based drilling muds. Previous studies on oil contamination in marine environments have shown that these groups to increase dramatically after environmental pollution [172, 182-184]. In the Bønna drilling waste, the water-based mud contained no hydrocarbons or other organic ingredients [185], it seems that enhanced anaerobicity, as such, or some other environmental change not revealed by the present study, triggered a similar community response as the oil contamination. To further elucidate the consequences of drilling activity, more information about the interactions of these bacterial groups is needed.

4 Conclusions and further work

The thesis focuses on exploring the biogeographical variation and anthropogenic impacts on prokaryotic and viral communities within an Arctic shelf seafloor like the Barents Sea. The microbial composition and relative abundance of the main phylogenetic groups were similar to what has been reported from comparable marine sediments. The community structure of bacteria along the Barents Sea transect seemed to be influenced by both environmental factors and isolation by distance, while the structure of the archaeal community was mainly influenced by environmental factors. The Barents Sea viromes grouped in accordance with the south−north separation reflect the biogeographical division of the bacterial communities. However, compositional differences in the eukaryotic virus assemblages rather than the bacteriophages appeared to be the primary basis for this spatial separation. Regarding the effects of drilling wastes discharges, the changing of bacterial community by this type of seafloor environmental perturbation at the most affected sites coincided with a steeper decline into anaerobicity down the sediment than at larger distance. The impacts have not been recorded more than 100 m from the freshly drilling site. The thesis also showed the challenges in identifying the actual factors, i.e. environment variations and dispersal limitation that cause compositional shifts, because the various factors interact or show extensive correlation between the spatial and environmental variables.

In order to better evaluate the impacts of dispersal limitation and environmental variables on benthic communities, regular sampling at in permanent stations over time would be needed, and the sampling area should be well designed to eliminate too much co-variations between the variables. Year-round sampling would provide a better understanding of Arctic microbial community diversity during times of changing organic matter deposition.

The interactions between the benthic macrofauna and microbial communities in arctic seafloors are largely unknown. Benthic macrofaunal activities, such as burrowing, irrigation,

foraging and defecation, have large effects on the ecosystem processes, such as organic matter degradation, nutrient cycling, biogeochemical interactions and benthic–pelagic fluxes [186, 187]. The description of co-occurrence patterns, therefore, would be another important step toward a more complete picture of the seafloor biological processes.

Additional approaches, e.g metatranscriptomics and metaproteomics, can be applied to assess functional gene expression and determine microbial functions in the ecosystem processes, especially in offshore drilling activity areas.

5 References

1. Hugenholtz, P. (2002) Exploring prokaryotic diversity in the genomic era. *Genome Biol*. **3**: p. 1-8
2. Liu, W.T., Marsh, T.L., Cheng, H. and Forney, L.J. (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl Environ Microbiol*. **63**: p. 4516-22.
3. Handelsman, J. (2004) Metagenomics: application of genomics to uncultured microorganisms. *Microbiol Mol Biol Rev*. **68**: p. 669-85.
4. Olsen, G.J. and Woese, C.R. (1993) Ribosomal RNA: a key to phylogeny. *FASEB J*. **7**: p. 113-23.
5. Kitahara, K., Yasutake, Y. and Miyazaki, K. (2012) Mutational robustness of 16S ribosomal RNA, shown by experimental horizontal gene transfer in Escherichia coli. *Proc Natl Acad Sci U S A*. **109**: p. 19220-5.
6. Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A., Wu, D., Paulsen, I., Nelson, K.E., Nelson, W., Fouts, D.E., Levy, S., Knap, A.H., Lomas, M.W., Nealson, K., White, O., Peterson, J., Hoffman, J., Parsons, R., Baden-Tillson, H., Pfannkoch, C., Rogers, Y.H. and Smith, H.O. (2004) Environmental genome shotgun sequencing of the Sargasso Sea. *Science*. **304**: p. 66-74.
7. Margulies, M., Egholm, M., Altman, W.E., Attiya, S., Bader, J.S., Bemben, L.A., Berka, J., Braverman, M.S., Chen, Y.J., Chen, Z., Dewell, S.B., Du, L., Fierro, J.M., Gomes, X.V., Godwin, B.C., He, W., Helgesen, S., Ho, C.H., Irzyk, G.P., Jando, S.C., Alenquer, M.L., Jarvie, T.P., Jirage, K.B., Kim, J.B., Knight, J.R., Lanza, J.R., Leamon, J.H., Lefkowitz, S.M., Lei, M., Li, J., Lohman, K.L., Lu, H., Makhijani, V.B., McDade, K.E., McKenna, M.P., Myers, E.W., Nickerson, E., Nobile, J.R., Plant, R., Puc, B.P., Ronan, M.T., Roth, G.T., Sarkis, G.J., Simons, J.F., Simpson, J.W., Srinivasan, M., Tartaro, K.R., Tomasz, A., Vogt, K.A., Volkmer, G.A., Wang, S.H., Wang, Y., Weiner, M.P., Yu, P., Begley, R.F. and Rothberg, J.M. (2005) Genome sequencing in microfabricated high-density picolitre reactors. *Nature*. **437**: p. 376-80.
8. Sogin, M.L., Morrison, H.G., Huber, J.A., Mark Welch, D., Huse, S.M., Neal, P.R., Arrieta, J.M. and Herndl, G.J. (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proc Natl Acad Sci U S A*. **103**(32): p. 12115-20.
9. Whittaker, R.H. (1972) Evolution and Measurement of Species Diversity. *Taxon*. **21**: p. 38.
10. Magurran, A.E. (2004) Measuring Biological Diversity: Vol. Blackwell Publishing, Oxford, UK.
11. Rossello-Mora, R. and Amann, R. (2001) The species concept for prokaryotes. *FEMS Microbiol Rev*. **25**: p. 39-67.
12. Kunin, V., Engelbrektson, A., Ochman, H. and Hugenholtz, P. (2010) Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. *Environ Microbiol*. **12**: p. 118-23.
13. Chao, A. (1984) Non-parametric estimation of the number of classes in a population. *Scand J Stat*. **11**: p. 5.
14. Colwell, R.K., Chao, A., Gotelli, N.J., Lin, S.Y., Mao, C.X., Chazdon, R.L. and Longino, J.T. (2012) Models and estimators linking individual-based and sample-based rarefaction, extrapolation and comparison of assemblages. *J Plant Ecol*. **5**: p. 3-21.
15. Chao, A. and Lee, S.M. (1992) Estimating the number of classes via sample coverage. *J Am Stat Assoc*. **87**: p. 210-217.
16. Gihring, T.M., Green, S.J. and Schadt, C.W. (2012) Massively parallel rRNA gene sequencing exacerbates the potential for biased community diversity comparisons due to variable library sizes. *Environ Microbiol*. **14**: p. 285-90.

17. Hong, S.H., Bunge, J., Jeon, S.O. and Epstein, S.S. (2006) Predicting microbial species richness. *Proc Natl Acad Sci U S A*. **103**: p. 117-22.

18. Quince, C., Curtis, T.P. and Sloan, W.T. (2008) The rational exploration of microbial diversity. *ISME J*. **2**: p. 997-1006.

19. Soetaert, K. and Heip, C. (1990) Sample-size dependence of diversity indexes and the determination of sufficent sample size in a high diversity in deep-sea environment. *Mar Ecol Prog Ser*. **59**: p. 305-307.

20. Anderson, M.J., Crist, T.O., Chase, J.M., Vellend, M., Inouye, B.D., Freestone, A.L., Sanders, N.J., Cornell, H.V., Comita, L.S., Davies, K.F., Harrison, S.P., Kraft, N.J., Stegen, J.C. and Swenson, N.G. (2011) Navigating the multiple meanings of beta diversity: a roadmap for the practicing ecologist. *Ecol Lett*. **14**: p. 19-28.

21. Bray, J.R. and Curtis, J.T. (1957) An ordination of the upland forest communities of southern wisconsin. *Ecol Monogr*. **27**: p. 326-349.

22. Legendre L, L.P. (1998) Numerical Ecology. Elsevier Science: Amsterdam.

23. Ramette, A. (2007) Multivariate analyses in microbial ecology. *FEMS Microbiol Ecol*. **62**: p. 142-60.

24. Schloss, P.D. (2008) Evaluating different approaches that test whether microbial communities have the same structure. *ISME J*. **2**: p. 265-275.

25. Anderson, M.J. (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecol*. **26**: p. 32-46.

26. Clarke, K.R. (1993) Non-parametric multivariate analyses of changes in community structure. *Australian J Ecol*. **18**: p. 117-143.

27. Gaston, K.J. (2000) Global patterns in biodiversity. *Nature*. **405**: p. 220-7.

28. Lomolino, M.V., Riddle, B.R. & Brown, J.H. (2005) Biogeography, 3rd. edn: Sinauer Associates, Sunderland, MA.

29. Baas-Becking, L.G.M. (1934) Geobiologie of Inleiding Tot de Milieukunde. The Hague, The Netherlands.

30. DeLong, E.F., Preston, C.M., Mincer, T., Rich, V., Hallam, S.J., Frigaard, N.U., Martinez, A., Sullivan, M.B., Edwards, R., Brito, B.R., Chisholm, S.W. and Karl, D.M. (2006) Community genomics among stratified microbial assemblages in the ocean's interior. *Science*. **311**: p. 496-503.

31. Pommier, T., Canback, B., Riemann, L., Bostrom, K.H., Simu, K., Lundberg, P., Tunlid, A. and Hagstrom, A. (2007) Global patterns of diversity and community structure in marine bacterioplankton. *Mol Ecol*. **16**: p. 867-80.

32. Fuhrman, J.A., Steele, J.A., Hewson, I., Schwalbach, M.S., Brown, M.V., Green, J.L. and Brown, J.H. (2008) A latitudinal diversity gradient in planktonic marine bacteria. *Proc Natl Acad Sci U S A*. **105**): p. 7774-8.

33. Agogue, H., Lamy, D., Neal, P.R., Sogin, M.L. and Herndl, G.J. (2011) Water mass-specificity of bacterial communities in the North Atlantic revealed by massively parallel sequencing. *Mol Ecol*. **20**: p. 258-74.

34. Galand, P.E., Casamayor, E.O., Kirchman, D.L., Potvin, M. and Lovejoy, C. (2009) Unique archaeal assemblages in the Arctic Ocean unveiled by massively parallel tag sequencing. *ISME J*. **3**: p. 860-9.

35. Horner-Devine, M.C., Leibold, M.A., Smith, V.H. and Bohannan, B.J.M. (2003) Bacterial diversity patterns along a gradient of primary productivity. *Ecol Lett*. **6**: p. 613-622.

36. Zinger, L., Amaral-Zettler, L.A., Fuhrman, J.A., Horner-Devine, M.C., Huse, S.M., Welch, D.B., Martiny, J.B., Sogin, M., Boetius, A. and Ramette, A. (2011) Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. *PLoS One*. **6**: p. e24570.

37. Martiny, J.B., Bohannan, B.J., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., Morin, P.J., Naeem, S., Ovreas,

L., Reysenbach, A.L., Smith, V.H. and Staley, J.T. (2006) Microbial biogeography: putting microorganisms on the map. *Nat Rev Microbiol*. **4**: p. 102-12.

38. Sul, W.J., Oliver, T.A., Ducklow, H.W., Amaral-Zettler, L.A. and Sogin, M.L. (2013) Marine bacteria exhibit a bipolar distribution. *Proc Natl Acad Sci U S A*. **110**: p. 2342-7.
39. Quéric, N.V. and Soltwedel, T. (2007) Impact of small-scale biogenic sediment structures on bacterial distribution and activity in Arctic deep-sea sediments. *Mar Ecol*. **28**: p. 66-74.
40. Paul, P.R. (2009) Invitation to oceanography, edn: Jones and Bartlett Publishers
41. Seiter, K., Hensen, C., Schroter, E. and Zabel, M. (2004) Organic carbon content in surface sediments - defining regional provinces. *Deep-Sea Res PT I-Oceanogr Res Pap*. **51**: p. 2001-2026.
42. D'Hondt, S., Jorgensen, B.B., Miller, D.J., Batzke, A., Blake, R., Cragg, B.A., Cypionka, H., Dickens, G.R., Ferdelman, T., Hinrichs, K.U., Holm, N.G., Mitterer, R., Spivack, A., Wang, G., Bekins, B., Engelen, B., Ford, K., Gettemy, G., Rutherford, S.D., Sass, H., Skilbeck, C.G., Aiello, I.W., Guerin, G., House, C.H., Inagaki, F., Meister, P., Naehr, T., Niitsuma, S., Parkes, R.J., Schippers, A., Smith, D.C., Teske, A., Wiegel, J., Padilla, C.N. and Acosta, J.L. (2004) Distributions of microbial activities in deep subseafloor sediments. *Science*. **306**: p. 2216-21.
43. Orcutt, B.N., Sylvan, J.B., Knab, N.J. and Edwards, K.J. (2011) Microbial ecology of the dark ocean above, at, and below the seafloor. *Microbiol Mol Biol Rev*. **75**: p. 361-422.
44. Whitman, W.B., Coleman, D.C. and Wiebe, W.J. (1998) Prokaryotes: the unseen majority. *Proc Natl Acad Sci U S A*. **95**: p. 6578-83.
45. Kallmeyer, J., Pockalny, R., Adhikari, R.R., Smith, D.C. and D'Hondt, S. (2012) Global distribution of microbial abundance and biomass in subseafloor sediment. *Proc Natl Acad Sci U S A*. **109**: p. 16213-6.
46. Parkes, R.J., Cragg, B., Roussel, E., Webster, G., Weightman, A. and Sass, H. (2014) A review of prokaryotic populations and processes in sub-seafloor sediments, including biosphere:geosphere interactions. *Mar Geol*. **352**: p. 409-425.
47. Berger, W.H. and Wefer, G. (1990) Export production-seasonality and intermittency, and paleoceanographic implications. *Glob Planet Change*. **89**(3): p. 245-254.
48. Bowman, J.P. and McCuaig, R.D. (2003) Biodiversity, community structural shifts, and biogeography of prokaryotes within Antarctic continental shelf sediment. *Appl Environ Microbiol*. **69**: p. 2463-83.
49. Webster, G., Yarram, L., Freese, E., Koster, J., Sass, H., Parkes, R.J. and Weightman, A.J. (2007) Distribution of candidate division JS1 and other Bacteria in tidal sediments of the German Wadden Sea using targeted 16S rRNA gene PCR-DGGE. *FEMS Microbiol Ecol*. **62**: p. 78-89.
50. Muyzer, G. and Stams, A.J. (2008) The ecology and biotechnology of sulphate-reducing bacteria. *Nat Rev Microbiol*. **6**: p. 441-54.
51. Parkes, R.J., Cragg, B.A., Banning, N., Brock, F., Webster, G., Fry, J.C., Hornibrook, E., Pancost, R.D., Kelly, S., Knab, N., Jorgensen, B.B., Rinna, J. and Weightman, A.J. (2007) Biogeochemistry and biodiversity of methane cycling in subsurface marine sediments (Skagerrak, Denmark). *Environ Microbiol*. **9**: p. 1146-61.
52. Nakagawa, S., Inagaki, F., Suzuki, Y., Steinsbu, B.O., Lever, M.A., Takai, K., Engelen, B., Sako, Y., Wheat, C.G. and Horikoshi, K. (2006) Microbial community in black rust exposed to hot ridge flank crustal fluids. *Appl Environ Microbiol*. **72**: p. 6789-99.
53. Webster, G., Parkes, R.J., Cragg, B.A., Newberry, C.J., Weightman, A.J. and Fry, J.C. (2006) Prokaryotic community composition and biogeochemical processes in deep subseafloor sediments from the Peru Margin. *FEMS Microbiol Ecol*. **58**: p. 65-85.
54. Hugenholtz, P., Pitulle, C., Hershberger, K.L. and Pace, N.R. (1998) Novel division level bacterial diversity in a Yellowstone hot spring. *J Bacteriol*. **180**: p. 366-76.
55. Teske, A., Hinrichs, K.U., Edgcomb, V., de Vera Gomez, A., Kysela, D., Sylva, S.P., Sogin, M.L. and Jannasch, H.W. (2002) Microbial diversity of hydrothermal sediments in the

Guaymas Basin: evidence for anaerobic methanotrophic communities. *Appl Environ Microbiol*. **68**: p. 1994-2007.

56. Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., Suzuki, M., Takai, K., Delwiche, M., Colwell, F.S., Nealson, K.H., Horikoshi, K., D'Hondt, S. and Jorgensen, B.B. (2006) Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proc Natl Acad Sci U S A*. **103**: p. 2815-20.
57. Polymenakou, P.N., Lampadariou, N., Mandalakis, M. and Tselepides, A. (2009) Phylogenetic diversity of sediment bacteria from the southern Cretan margin, Eastern Mediterranean Sea. *Syst Appl Microbiol*. **32**: p. 17-26.
58. Nercessian, O., Fouquet, Y., Pierre, C., Prieur, D. and Jeanthon, C. (2005) Diversity of Bacteria and Archaea associated with a carbonate-rich metalliferous sediment sample from the Rainbow vent field on the Mid-Atlantic Ridge. *Environ Microbiol*. **7**: p. 698-714.
59. Santelli, C.M., Orcutt, B.N., Banning, E., Bach, W., Moyer, C.L., Sogin, M.L., Staudigel, H. and Edwards, K.J. (2008) Abundance and diversity of microbial life in ocean crust. *Nature*. **453**: p. 653-6.
60. Mason, O.U., Di Meo-Savoie, C.A., Van Nostrand, J.D., Zhou, J., Fisk, M.R. and Giovannoni, S.J. (2009) Prokaryotic diversity, distribution, and insights into their role in biogeochemical cycling in marine basalts. *ISME J*. **3**: p. 231-42.
61. Woese, C.R. and Fox, G.E. (1977) phylogenetic structure of prokaryotic domain - primary kingdoms. *Proc Natl Acad Sci USA*. **74**: p. 5088-5090.
62. DeLong, E.F., Wu, K.Y., Prezelin, B.B. and Jovine, R.V. (1994) High abundance of Archaea in Antarctic marine picoplankton. *Nature*. **371**: p. 695-7.
63. Schleper, C. and Nicol, G.W. (2010) Ammonia-oxidising archaea--physiology, ecology and evolution. *Adv Microb Physiol*. **57**: p. 1-41.
64. Konneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B. and Stahl, D.A. (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature*. **437**: p. 543-6.
65. Newberry, C.J., Webster, G., Cragg, B.A., Parkes, R.J., Weightman, A.J. and Fry, J.C. (2004) Diversity of prokaryotes and methanogenesis in deep subsurface sediments from the Nankai Trough, Ocean Drilling Program Leg 190. *Environ Microbiol*. **6**: p. 274-87.
66. Durbin, A.M. and Teske, A. (2010) Sediment-associated microdiversity within the Marine Group I Crenarchaeota. *Environ Microbiol Rep*. **2**: p. 693-703.
67. Bano, N., Ruffin, S., Ransom, B. and Hollibaugh, J.T. (2004) Phylogenetic composition of Arctic Ocean archaeal assemblages and comparison with Antarctic assemblages. *Appl Environ Microbiol*. **70**: p. 781-9.
68. Takai, K. and Horikoshi, K. (1999) Genetic diversity of archaea in deep-sea hydrothermal vent environments. *Genetics*. **152**: p. 1285-97.
69. Huber, J.A., Johnson, H.P., Butterfield, D.A. and Baross, J.A. (2006) Microbial life in ridge flank crustal fluids. *Environ Microbiol*. **8**: p. 88-99.
70. Jorgensen, S.L., Hannisdal, B., Lanzen, A., Baumberger, T., Flesland, K., Fonseca, R., Ovreas, L., Steen, I.H., Thorseth, I.H., Pedersen, R.B. and Schleper, C. (2012) Correlating microbial community profiles with geochemical data in highly stratified sediments from the Arctic Mid-Ocean Ridge. *Proc Natl Acad Sci U S A*. **109**: p. e2846-55.
71. Mussmann, M., Brito, I., Pitcher, A., Sinninghe Damste, J.S., Hatzenpichler, R., Richter, A., Nielsen, J.L., Nielsen, P.H., Muller, A., Daims, H., Wagner, M. and Head, I.M. (2011) Thaumarchaeotes abundant in refinery nitrifying sludges express amoA but are not obligate autotrophic ammonia oxidizers. *Proc Natl Acad Sci U S A*. **108**: p. 16771-6.
72. Vetriani, C., Jannasch, H.W., MacGregor, B.J., Stahl, D.A. and Reysenbach, A.L. (1999) Population structure and phylogenetic characterization of marine benthic Archaea in deep-sea sediments. *Appl Environ Microbiol*. **65**: p. 4375-84.

73. Parkes, R.J., Webster, G., Cragg, B.A., Weightman, A.J., Newberry, C.J., Ferdelman, T.G., Kallmeyer, J., Jorgensen, B.B., Aiello, I.W. and Fry, J.C. (2005) Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. *Nature*. **436**: p. 390-4.
74. Sorensen, K.B. and Teske, A. (2006) Stratified communities of active Archaea in deep marine subsurface sediments. *Appl Environ Microbiol*. **72**: p. 4596-603.
75. Heijs, S.K., Haese, R.R., van der Wielen, P.W., Forney, L.J. and van Elsas, J.D. (2007) Use of 16S rRNA gene based clone libraries to assess microbial communities potentially involved in anaerobic methane oxidation in a Mediterranean cold seep. *Microb Ecol*. **53**: p. 384-98.
76. Ingalls, A.E., Shah, S.R., Hansman, R.L., Aluwihare, L.I., Santos, G.M., Druffel, E.R. and Pearson, A. (2006) Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. *Proc Natl Acad Sci U S A*. **103**: p. 6442-7.
77. Collins, G., O'Connor, L., Mahony, T., Gieseke, A., de Beer, D. and O'Flaherty, V. (2005) Distribution, localization, and phylogeny of abundant populations of Crenarchaeota in anaerobic granular sludge. *Appl Environ Microbiol*. **71**: p. 7523-7.
78. MacGregor, B.J., Moser, D.P., Alm, E.W., Nealson, K.H. and Stahl, D.A. (1997) Crenarchaeota in Lake Michigan sediment. *Appl Environ Microbiol*. **63**: p. 1178-81.
79. Schleper, C., Holben, W. and Klenk, H.P. (1997) Recovery of crenarchaeotal ribosomal DNA sequences from freshwater-lake sediments. *Appl Environ Microbiol*. **63**: p. 321-3.
80. Kubo, K., Lloyd, K.G., J, F.B., Amann, R., Teske, A. and Knittel, K. (2012) Archaea of the Miscellaneous Crenarchaeotal Group are abundant, diverse and widespread in marine sediments. *ISME J*. **6**: p. 1949-65.
81. Teske, A. and Sorensen, K.B. (2008) Uncultured archaea in deep marine subsurface sediments: have we caught them all? *ISME J*. **2**: p. 3-18.
82. Goffredi, S.K., Wilpiszeski, R., Lee, R. and Orphan, V.J. (2008) Temporal evolution of methane cycling and phylogenetic diversity of archaea in sediments from a deep-sea whale-fall in Monterey Canyon, California. *ISME J*. **2**: p. 204-20.
83. Heijs, S.K., Aloisi, G., Bouloubassi, I., Pancost, R.D., Pierre, C., Sinninghe Damste, J.S., Gottschal, J.C., van Elsas, J.D. and Forney, L.J. (2006) Microbial community structure in three deep-sea carbonate crusts. *Microb Ecol*. **52**: p. 451-62.
84. Niemann, H., Losekann, T., de Beer, D., Elvert, M., Nadalig, T., Knittel, K., Amann, R., Sauter, E.J., Schluter, M., Klages, M., Foucher, J.P. and Boetius, A. (2006) Novel microbial communities of the Haakon Mosby mud volcano and their role as a methane sink. *Nature*. **443**: p. 854-8.
85. Inagaki, F., Suzuki, M., Takai, K., Oida, H., Sakamoto, T., Aoki, K., Nealson, K.H. and Horikoshi, K. (2003) Microbial communities associated with geological horizons in coastal subseafloor sediments from the sea of okhotsk. *Appl Environ Microbiol*. **69**: p. 7224-35.
86. Rohwer, F. and Thurber, R.V. (2009) Viruses manipulate the marine environment. *Nature*. **459**: p. 207-12.
87. Wommack, K.E. and Colwell, R.R. (2000) Virioplankton: viruses in aquatic ecosystems. *Microbiol Mol Biol Rev*. **64**: p. 69-114.
88. Bouvier, T. and del Giorgio, P.A. (2007) Key role of selective viral-induced mortality in determining marine bacterial community composition. *Environ Microbiol*. **9**: p. 287-97.
89. Weinbauer, M.G. (2004) Ecology of prokaryotic viruses. *FEMS Microbiol Rev*. **28**: p. 127-81.
90. Fuhrman, J.A. (1999) Marine viruses and their biogeochemical and ecological effects. *Nature*. **399**: p. 541-8.
91. Middelboe, M., Glud, R.N. and Filippini, M. (2011) Viral abundance and activity in the deep sub-seafloor biosphere. *Aqua Microb Ecol*. **63**: p. 1-8.
92. Danovaro, R., Dell'Anno, A., Corinaldesi, C., Magagnini, M., Noble, R., Tamburini, C. and Weinbauer, M. (2008) Major viral impact on the functioning of benthic deep-sea ecosystems. *Nature*. **454**: p. 1084-7.

93. Danovaro, R. and Serresi, M. (2000) Viral density and virus-to-bacterium ratio in deep-sea sediments of the Eastern Mediterranean. *Appl Environ Microbiol*. **66**: p. 1857-1861.

94. Hewson, I., O'Neil, J.M., Fuhrman, J.A. and Dennison, W.C. (2001) Virus-like particle distribution and abundance in sediments and overlying waters along eutrophication gradients in two subtropical estuaries. *Limnol Oceanogr*. **46**: p. 1734-1746.

95. Danovaro, R., Corinaldesi, C., Dell'anno, A., Fuhrman, J.A., Middelburg, J.J., Noble, R.T. and Suttle, C.A. (2011) Marine viruses and global climate change. *FEMS Microbiol Rev*. **35**: p. 993-1034.

96. Breitbart, M., Salamon, P., Andresen, B., Mahaffy, J.M., Segall, A.M., Mead, D., Azam, F. and Rohwer, F. (2002) Genomic analysis of uncultured marine viral communities. *Proc Natl Acad Sci U S A*. **99**: p. 14250-5.

97. Breitbart, M., Wegley, L., Leeds, S., Schoenfeld, T. and Rohwer, F. (2004) Phage community dynamics in hot springs. *Appl Environ Microbiol*. **70**: p. 1633-40.

98. Steward, G.F. and Preston, C.M. (2011) Analysis of a viral metagenomic library from 200 m depth in Monterey Bay, California constructed by direct shotgun cloning. *Virol J*. **8**: p. 287.

99. Fancello, L., Trape, S., Robert, C., Boyer, M., Popgeorgiev, N., Raoult, D. and Desnues, C. (2013) Viruses in the desert: a metagenomic survey of viral communities in four perennial ponds of the Mauritanian Sahara. *ISME J*. **7**: p. 359-69.

100. Labonte, J.M. and Suttle, C.A. (2013) Metagenomic and whole-genome analysis reveals new lineages of gokushoviruses and biogeographic separation in the sea. *Front Microbiol*. **4**: p. 404.

101. McDaniel, L.D., Rosario, K., Breitbart, M. and Paul, J.H. (2014) Comparative metagenomics: natural populations of induced prophages demonstrate highly unique, lower diversity viral sequences. *Environ Microbiol*. **16**: p. 570-85.

102. Angly, F.E., Felts, B., Breitbart, M., Salamon, P., Edwards, R.A., Carlson, C., Chan, A.M., Haynes, M., Kelley, S., Liu, H., Mahaffy, J.M., Mueller, J.E., Nulton, J., Olson, R., Parsons, R., Rayhawk, S., Suttle, C.A. and Rohwer, F. (2006) The marine viromes of four oceanic regions. *PLoS Biol*. **4**: p. e368.

103. Tucker, K.P., Parsons, R., Symonds, E.M. and Breitbart, M. (2011) Diversity and distribution of single-stranded DNA phages in the North Atlantic Ocean. *ISME J*. **5**: p. 822-30.

104. Labonte, J.M. and Suttle, C.A. (2013) Previously unknown and highly divergent ssDNA viruses populate the oceans. *ISME J*. **7**: p. 2169-77.

105. Lopez-Bueno, A., Tamames, J., Velazquez, D., Moya, A., Quesada, A. and Alcami, A. (2009) High diversity of the viral community from an Antarctic lake. *Science*. **326**: p. 858-61.

106. Roux, S., Enault, F., Robin, A., Ravet, V., Personnic, S., Theil, S., Colombet, J., Sime-Ngando, T. and Debroas, D. (2012) Assessing the diversity and specificity of two freshwater viral communities through metagenomics. *PLoS One*. **7**: p. e33641.

107. Yoshida, M., Takaki, Y., Eitoku, M., Nunoura, T. and Takai, K. (2013) Metagenomic analysis of viral communities in (hado)pelagic sediments. *PLoS One*. **8**: p. e57271.

108. Mokili, J.L., Rohwer, F. and Dutilh, B.E. (2012) Metagenomics and future perspectives in virus discovery. *Curr Opin Virol*. **2**: p. 63-77.

109. Breitbart, M., Felts, B., Kelley, S., Mahaffy, J.M., Nulton, J., Salamon, P. and Rohwer, F. (2004) Diversity and population structure of a near-shore marine-sediment viral community. *Pro Roy Soc B-Biol Sci*. **271**: p. 565-574.

110. Schoenfeld, T., Patterson, M., Richardson, P.M., Wommack, K.E., Young, M. and Mead, D. (2008) Assembly of viral metagenomes from yellowstone hot springs. *Appl Environ Microbiol*. **74**: p. 4164-74.

111. Loeng, H. and Drinkwater, K. (2007) An overview of the ecosystems of the Barents and Norwegian Seas and their response to climate variability. *Deep Sea Res PT II*. **54**: p. 2478-2500.

112. Loeng, H. (1991) Features of the physical oceanographic conditions of the Barents Sea. *Pol Res*. **10**: p. 5-18.

113. Lind, S. and Ingvaldsen, R.B. (2012) Variability and impacts of Atlantic Water entering the Barents Sea from the north. *Deep-Sea Res PT I-Oceanogr Res Pap*. **62**: p. 70-88.

114. Wassmann, P., Slagstad, D. and Ellingsen, I. (2010) Primary production and climatic variability in the European sector of the Arctic Ocean prior to 2007: preliminary results. *Pol Biol*. **33**: p. 1641-1650.

115. Rey, F. and Loeng, H. (1985) The influence of ice and hydrographic conditions on the development of phytoplankton in the Barents Sea. *Eur Mar Biol Symp*. **18**: p. 49-63.

116. Sakshaug, E., Johnsen, G., Kristiansen, S., von Quillfeldt, C.H., Rey, F., Slagstad, D. and Thingstad, F. (2009) Phytoplankton and primary production . In: Sakshaug E, Johnsen G, Kovacs KM (eds) *Ecosystem Barents Sea*: Tapir Academic Press, Tronheim. 41.

117. Loeng, H. and Drinkwater, K. (2007) An overview of the ecosystems of the Barents and Norwegian Seas and their response to climate variability. *Deep-Sea Res PT II-Top Stud Oceanogr*. **54**: p. 2478-2500.

118. Hasle, J.R., Kjellén, U. and Haugerud, O. (2009) Decision on oil and gas exploration in an Arctic area: Case study from the Norwegian Barents Sea. *Safety Science*. **47**: p. 832-842.

119. Gray, J.S., Clarke, K.R., Warwick, R.M. and Hobbs, G. (1990) Detection of initial effects of pollution on marine benthods- an example from the Ekofisk and Eldfisk oilfields, North-Sea. *Mar Ecol Prog Ser*. **66**: p. 285-299.

120. Daan, R. and Mulder, M. (1996) On the short-term and long-term impact of drilling activities in the Dutch sector of the North Sea. *Ices J Mar Sci*. **53**: p. 1036-1044.

121. Olsgard, F. and Gray, J.S. (1995) A comprehensive analysis of the effects of offshore oil and gas exploration and production on the benthic communities of the Norwegian continental shelf. *Mar Ecol Prog Ser*. **122**: p. 277-306.

122. Neff, J.M., Ostazeski, S., Gardiner, W. and Stejskal, I. (2000) Effects of weathering on the toxicity of three offshore Australian crude oils and a diesel fuel to marine animals. *Environ Toxicol Chem*. **19**: p. 1809-1821.

123. Montagna, P.A. and Harper, D.E. (1996) Benthic infaunal long term response to offshore production platforms in the Gulf of Mexico. *Can J Fish Aquat Sci*. **53**: p. 2567-2588.

124. Currie, D.R. and Isaacs, L.R. (2005) Impact of exploratory offshore drilling on benthic communities in the Minerva gas field, Port Campbell, Australia. *Mar Environ Res*. **59**: p. 217-233.

125. Trannum, H.C., Nilsson, H.C., Schaanning, M.T. and Oxnevad, S. (2010) Effects of sedimentation from water-based drill cuttings and natural sediment on benthic macrofaunal community structure and ecosystem processes. *J Exp Mar Biol Ecol*. **383**: p. 111-121.

126. Hyland, J., Hardin, D., Steinhauer, M., Coats, D., Green, R. and Neff, J. (1994) Environmental-impact of offshore oil development on the outer continental shelf and slope off point Arguello, California. *Mar Environ Res*. **37**: p. 195-229.

127. Schaanning, M.T., Trannum, H.C., Oxnevad, S., Carroll, J. and Bakke, T. (2008) Effects of drill cuttings on biogeochemical fluxes and macrobenthos of marine sediments. *J Exp Mar Biol Ecol*. **361**: p. 49-57.

128. Sanders, P.F. and Tibbetts, P.J.C. (1987) Effects of discarded drill muds on microbial populations. *Philos Trans R Soc Lond B-Biol Sci*. **316**: p. 567-585.

129. Dow, F.K., Davies, J.M. and Raffaelli, D. (1990) The effects of dill cuttings on model marine sediment system. *Mar Environ Res*. **29**: p. 103-134.

130. Baker, G.C., Smith, J.J. and Cowan, D.A. (2003) Review and re-analysis of domain-specific 16S primers. *J Microbiol Methods*. **55**: p. 541-55.

131. Huber, B., Scholz, H.C., Lucero, N. and Busse, H.J. (2009) Development of a PCR assay for typing and subtyping of Brucella species. *Int J Med Microbiol*. **299**: p. 563-73.

132. Kumar, P.S., Brooker, M.R., Dowd, S.E. and Camerlengo, T. (2011) Target Region Selection Is a Critical Determinant of Community Fingerprints Generated by 16S Pyrosequencing. *Plos One*. **6**.
133. He, Y., Zhou, B.J., Deng, G.H., Jiang, X.T., Zhang, H. and Zhou, H.W. (2013) Comparison of microbial diversity determined with the same variable tag sequence extracted from two different PCR amplicons. *BMC Microbiol*. **13**: p. 208.
134. Lee, C.K., Herbold, C.W., Polson, S.W., Wommack, K.E., Williamson, S.J., McDonald, I.R. and Cary, S.C. (2012) Groundtruthing next-gen sequencing for microbial ecology-biases and errors in community structure estimates from PCR amplicon pyrosequencing. *PLoS One*. **7**: p. e44224.
135. Youssef, N., Sheik, C.S., Krumholz, L.R., Najar, F.Z., Roe, B.A. and Elshahed, M.S. (2009) Comparison of species richness estimates obtained using nearly complete fragments and simulated pyrosequencing-generated fragments in 16S rRNA gene-based environmental surveys. *Appl Environ Microbiol*. **75**: p. 5227-36.
136. Huse, S.M., Dethlefsen, L., Huber, J.A., Mark Welch, D., Relman, D.A. and Sogin, M.L. (2008) Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing. *PLoS Genet*. **4**: p. e1000255.
137. Kim, M., Morrison, M. and Yu, Z. (2011) Evaluation of different partial 16S rRNA gene sequence regions for phylogenetic analysis of microbiomes. *J Microbiol Methods*. **84**: p. 81-7.
138. Yang, B., Wang, Y. and Qian, P.Y. (2016) Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. *BMC Bioinformatics*. **17**: p. 135.
139. Erik, K.-S. (2013) Exploring bioinformatic software for taxonomic classification of metagenomes. UiT-The Arctic University of Norway. p. 70.
140. Poretsky, R., Rodriguez, R.L., Luo, C., Tsementzi, D. and Konstantinidis, K.T. (2014) Strengths and limitations of 16S rRNA gene amplicon sequencing in revealing temporal microbial community dynamics. *PLoS One*. **9**: p. e93827.
141. Tremblay, J., Singh, K., Fern, A., Kirton, E.S., He, S., Woyke, T., Lee, J., Chen, F., Dangl, J.L. and Tringe, S.G. (2015) Primer and platform effects on 16S rRNA tag sequencing. *Front Microbiol*. **6**: p. 771.
142. Zengler, K. and Palsson, B.O. (2012) A road map for the development of community systems (CoSy) biology. *Nat Rev Microbiol*. **10**: p. 366-72.
143. Helton, R.R. and Wommack, K.E. (2009) Seasonal dynamics and metagenomic characterization of estuarine viriobenthos assemblages by randomly amplified polymorphic DNA PCR. *Appl Environ Microbiol*. **75**: p. 2259-65.
144. Labonte, J.M., Reid, K.E. and Suttle, C.A. (2009) Phylogenetic analysis indicates evolutionary diversity and environmental segregation of marine podovirus DNA polymerase gene sequences. *Appl Environ Microbiol*. **75**: p. 3634-40.
145. Filippini, M. and Middelboe, M. (2007) Viral abundance and genome size distribution in the sediment and water column of marine and freshwater ecosystems. *FEMS Microbiol Ecol*. **60**: p. 397-410.
146. Thurber, R.V., Haynes, M., Breitbart, M., Wegley, L. and Rohwer, F. (2009) Laboratory procedures to generate viral metagenomes. *Nat Protoc*. **4**: p. 470-83.
147. Kim, K.H. and Bae, J.W. (2011) Amplification methods bias metagenomic libraries of uncultured single-stranded and double-stranded DNA viruses. *Appl Environ Microbiol*. **77**: p. 7663-8.
148. Kim, K.H., Chang, H.W., Nam, Y.D., Roh, S.W., Kim, M.S., Sung, Y., Jeon, C.O., Oh, H.M. and Bae, J.W. (2008) Amplification of uncultured single-stranded DNA viruses from rice paddy soil. *Appl Environ Microbiol*. **74**: p. 5975-85.
149. Marine, R., Polson, S.W., Ravel, J., Hatfull, G., Russell, D., Sullivan, M., Syed, F., Dumas, M. and Wommack, K.E. (2011) Evaluation of a transposase protocol for rapid generation of

shotgun high-throughput sequencing libraries from nanogram quantities of DNA. *Appl Environ Microbiol*. **77**: p. 8071-9.

150. Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. and Hase, T. (2000) Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res*. **28**: e63.

151. Andrews-Pfannkoch, C., Fadrosh, D.W., Thorpe, J. and Williamson, S.J. (2010) Hydroxyapatite-mediated separation of double-stranded DNA, single-stranded DNA, and RNA genomes from natural viral assemblages. *Appl Environ Microbiol*. **76**: p. 5039-45.

152. Huse, S.M., Huber, J.A., Morrison, H.G., Sogin, M.L. and Welch, D.M. (2007) Accuracy and quality of massively parallel DNA pyrosequencing. *Genome Biol*. **8**: R143.

153. Quince, C., Lanzen, A., Curtis, T.P., Davenport, R.J., Hall, N., Head, I.M., Read, L.F. and Sloan, W.T. (2009) Accurate determination of microbial diversity from 454 pyrosequencing data. *Nat Methods*. **6**: p. 639-41.

154. Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res*. **25**: p. 3389-402.

155. Patil, K.R., Haider, P., Pope, P.B., Turnbaugh, P.J., Morrison, M., Scheffer, T. and McHardy, A.C. (2011) Taxonomic metagenome sequence assignment with structured output models. *Nat Methods*. **8**: p. 191-2.

156. Dormann, C.F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J. R. G., Gruber, B., Lafourcade, B., Leitão, P. J., Münkemüller, T., McClean, C., Osborne, P. E., Reineking, B., Schröder, B., Skidmore, A. K., Zurell, D. and Lautenbach, S. (2013) Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography*. **36**.

157. Schauer, R., Bienhold, C., Ramette, A. and Harder, J. (2010) Bacterial diversity and biogeography in deep-sea surface sediments of the South Atlantic Ocean. *ISME J*. **4**: p. 159-70.

158. Baldi, F., Marchetto, D., Pini, F., Fani, R., Michaud, L., Lo Giudice, A., Berto, D. and Giani, M. (2010) Biochemical and microbial features of shallow marine sediments along the Terra Nova Bay (Ross Sea, Antarctica). *Cont Shelf Res*. **30**: p. 1614-1625.

159. Yanagibayashi, M., Nogi, Y., Li, L. and Kato, C. (1999) Changes in the microbial community in Japan Trench sediment from a depth of 6292 m during cultivation without decompression. *FEMS Microbiol Lett*. **170**: p. 271-9.

160. Biddle, J.F., Lipp, J.S., Lever, M.A., Lloyd, K.G., Sorensen, K.B., Anderson, R., Fredricks, H.F., Elvert, M., Kelly, T.J., Schrag, D.P., Sogin, M.L., Brenchley, J.E., Teske, A., House, C.H. and Hinrichs, K.U. (2006) Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proc Natl Acad Sci U S A*. **103**: p. 3846-51.

161. Durbin, A.M. and Teske, A. (2011) Microbial diversity and stratification of South Pacific abyssal marine sediments. *Environ Microbiol*. **13**: p. 3219-34.

162. Liao, L., Xu, X.W., Wang, C.S., Zhang, D.S. and Wu, M. (2009) Bacterial and archaeal communities in the surface sediment from the northern slope of the South China Sea. *J Zhejiang Univ Sci B*. **10**: p. 890-901.

163. Herfort, L., Schouten, S., Abbas, B., Veldhuis, M.J., Coolen, M.J., Wuchter, C., Boon, J.P., Herndl, G.J. and Sinninghe Damste, J.S. (2007) Variations in spatial and temporal distribution of Archaea in the North Sea in relation to environmental variables. *FEMS Microbiol Ecol*. **62**: p. 242-57.

164. Bale, N.J., Villanueva, L., Hopmans, E.C., Schouten, S. and Damste, J.S.S. (2013) Different seasonality of pelagic and benthic Thaumarchaeota in the North Sea. *Biogeosciences*. **10**: p. 7195-7206.

165. Hugoni, M., Agogue, H., Taib, N., Domaizon, I., Mone, A., Galand, P.E., Bronner, G., Debroas, D. and Mary, I. (2015) Temporal Dynamics of Active Prokaryotic Nitrifiers and Archaeal Communities from River to Sea. *Microb Ecol*. **70**: p. 473-483.

166. Wuchter, C., Abbas, B., Coolen, M.J., Herfort, L., van Bleijswijk, J., Timmers, P., Strous, M., Teira, E., Herndl, G.J., Middelburg, J.J., Schouten, S. and Sinninghe Damste, J.S. (2006) Archaeal nitrification in the ocean. *Proc Natl Acad Sci U S A*. **103**: p. 12317-22.

167. Pitcher, A., Wuchter, C., Siedenberg, K., Schouten, S. and Damste, J.S.S. (2011) Crenarchaeol tracks winter blooms of ammonia-oxidizing Thaumarchaeota in the coastal North Sea. *Limnol Oceanogr*. **56**: p. 2308-2318.

168. Galand, P.E., Gutierrez-Provecho, C., Massana, R., Gasol, J.M. and Casamayor, E.O. (2010) Inter-annual recurrence of archaeal assemblages in the coastal NW Mediterranean Sea (Blanes Bay Microbial Observatory). *Limnol Oceanogr*. **55**: p. 2117-2125.

169. Murray, A.E., Wu, K.Y., Moyer, C.L., Karl, D.M. and DeLong, E.F. (1999) Evidence for circumpolar distribution of planktonic Archaea in the Southern Ocean. *Aquat Microb Ecol*. **18**: p. 263-273.

170. Ravot, G., Magot, M., Fardeau, M.L., Patel, B.K., Thomas, P., Garcia, J.L. and Ollivier, B. (1999) Fusibacter paucivorans gen. nov., sp. nov., an anaerobic, thiosulfate-reducing bacterium from an oil-producing well. *Int J Syst Bacteriol*. **49** : p. 1141-7.

171. Santos, F.M., Monfort, L.E., Castro, D.M., Pinto, J.E., Leonardi, M. and Pistelli, L. (2011) Characterization of essential oil and effects on growth of Verbena gratissima plants treated with homeopathic phosphorus. *Nat Prod Commun*. **6**: p. 1499-504.

172. Koo, H., Mojib, N., Thacker, R.W. and Bej, A.K. (2014) Comparative analysis of bacterial community-metagenomics in coastal Gulf of Mexico sediment microcosms following exposure to Macondo oil (MC252). *Antonie Van Leeuwenhoek*. **106**: p. 993-1009.

173. Gieg, L.M., Duncan, K.E. and Suflita, J.M. (2008) Bioenergy production via microbial conversion of residual oil to natural gas. *Appl Environ Microbiol*. **74**: p. 3022-9.

174. Lovley, D.R., Holmes, D.E. and Nevin, K.P. (2004) Dissimilatory Fe(III) and Mn(IV) reduction, in *Advances in Microbial Physiology, Vol. 49*, Poole, R.K., Editor. p. 219-286.

175. Kuyver, J., F.A.Rainey, and F.Widdel. (2005) *Desulfuromonadaceae fam.nov.In: Bergey's Manual of Systematic Bacteriology, 2nd. ED. Vol.2.* . The Proteobacteria, Part C, ed. Brenner, D., J., N.R.Krieg, J.T.Staley, and G.M Garrity eds., Springer New York, p.1006.

176. Mussmann, M., Richter, M., Lombardot, T., Meyerdierks, A., Kuever, J., Kube, M., Glockner, F.O. and Amann, R. (2005) Clustered genes related to sulfate respiration in uncultured prokaryotes support the theory of their concomitant horizontal transfer. *J Bacteriol*. **187**: p. 7126-7137.

177. Desnues, C., Rodriguez-Brito, B., Rayhawk, S., Kelley, S., Tran, T., Haynes, M., Liu, H., Furlan, M., Wegley, L., Chau, B., Ruan, Y., Hall, D., Angly, F.E., Edwards, R.A., Li, L., Thurber, R.V., Reid, R.P., Siefert, J., Souza, V., Valentine, D.L., Swan, B.K., Breitbart, M. and Rohwer, F. (2008) Biodiversity and biogeography of phages in modern stromatolites and thrombolites. *Nature*. **452**: p. 340-3.

178. Rosario, K. and Breitbart, M. (2011) Exploring the viral world through metagenomics. *Curr Opin Virol*. **1**: p. 289-97.

179. Baudoux, A.C., Noordeloos, A.A.M., Veldhuis, M.J.W. and Brussaard, C.P.D. (2006) Virally induced mortality of Phaeocystis globosa during two spring blooms in temperate coastal waters. *Aquat Microb Ecol*. **44**: p. 207-217.

180. Tomaru, Y., Tarutani, K., Yamaguchi, M. and Nagasaki, K. (2004) Quantitative and qualitative impacts of viral infection on a Heterosigma akashiwo (Raphidophyceae) bloom in Hiroshima Bay, Japan. *Aquat Microb Ecol*. **34**: p. 227-238.

181. Sivolodskii, E.P. (2012) Determination of the Sensitivity of Bacteria to Barium Ions, a Taxonomic Marker of the Genus Pseudomonas. *Microbiology*. **81**: p. 112-117.

182. dos Santos, H.F., Cury, J.C., do Carmo, F.L., dos Santos, A.L., Tiedje, J., van Elsas, J.D., Rosado, A.S. and Peixoto, R.S. (2011) Mangrove bacterial diversity and the impact of oil contamination revealed by pyrosequencing: bacterial proxies for oil pollution. *PLoS One*. **6**: p. e16943.

183. Hasegawa, R., Toyama, K., Miyanaga, K. and Tanji, Y. (2014) Identification of crude-oil components and microorganisms that cause souring under anaerobic conditions. *Appl Microbiol Biotechnol*. **98**: p. 1853-61.

184. Suarez-Suarez, A., Lopez-Lopez, A., Tovar-Sanchez, A., Yarza, P., Orfila, A., Terrados, J., Arnds, J., Marques, S., Niemann, H., Schmitt-Kopplin, P., Amann, R. and Rossello-Mora, R. (2011) Response of sulfate-reducing bacteria to an artificial oil-spill in a coastal marine sediment. *Environ Microbiol*. **13**: p. 1488-99.

185. Paulsen, J.E., Cochrane, S. K. J., Leikvin,O., Hansen, J., Torbergsen,H. E and Pierfelici, S. (2014) Assessing exploratory drilling impacts on an Arctic deepwater sea-pen habitat offshore Norway. Report ESP 168343, Society of Petroleum Engineers, 14p

186. Gilbert, F., Aller, R.C. and Hulth, S. (2003) The influence of macrofaunal burrow spacing and diffusive scaling on sedimentary nitrification and denitrification: An experimental simulation and model approach. *J Mar Res*. **61**: p. 101-125.

187. Lohrer, A.M., Thrush, S.F. and Gibbs, M.M. (2004) Bioturbators enhance ecosystem function through complex biogeochemical interactions. *Nature*. **431**: p. 1092-1095.