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

Tan Thi Nguyen
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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 *Germiniviridae*. 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


Paper II


Paper III

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

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

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

\[ S_{\text{est}} = S_{\text{obs}} + f_0, \]

where
\[ f_0 = f_1^2 / (2f_2), \quad f_2 > 0 \]

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

Here \( S_{\text{est}} \) is the estimated number of taxonomic units in the assemblage, \( S_{\text{obs}} \) 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:

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

where $p_i$ and $q_i$ are the relative abundances of the $i^{th}$ 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 $R = 0$ 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 masses [33, 34], or chlorophyll-<[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).
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 $2.9 \times 10^{29}$ and $3.5 \times 10^{30}$ 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 electron 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 km², 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).
In the upper 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 *Desulfbacteriales*, 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).

![Percent of archaeal community composition](image)

**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


