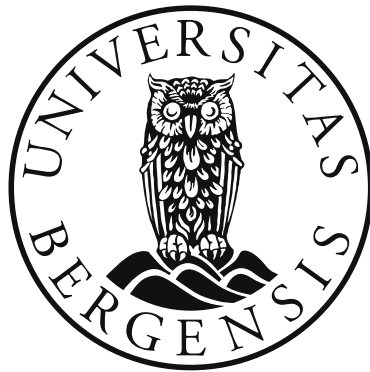# Impact of environmental parameters and dispersal on microbial communities in hydrothermal areas of the Nordic Seas

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Thesis for the degree of philosophiae doctor (PhD)
at the University of Bergen

2017

Date of defence: September 29th

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SCIENTIFIC ENVIRONMENT

UNIVERSITETET I BERGEN

The Research Council of Norway

ACKNOWLEDGEMENTS

I think that I usually show when I am grateful to somebody, and that you already know if you should be in this section. Therefore, I will let your imagination decide what I would have written for you, and skip this political exercise.

Thank you everybody for these four great years. Love you all!

ABSTRACT

The oceans are an extremely diverse environment due to the numerous physicochemical gradients occurring throughout the water column. Microorganisms have adapted to live in virtually all available niches. Therefore, an important focus in marine microbiology is the investigation of changes in marine microbial community structures in response to changes in physicochemical characteristics. In the past decades, with the advent of new technologies, major advances have been made in our understanding of microbial communities, but we have only scratched the surface of the extent and the complexity of the microbial realm.

The work presented here investigates microbial communities in an area that has so far been little studied, the Nordic Seas. These waters, situated between Norway and Greenland, are a complex entanglement of water masses and host hydrothermal activities along the Arctic Mid-Ocean Ridge. They are therefore a suitable area for studying the influences of hydrothermal activities and dispersal processes on microbial communities. The work uses a holistic approach to investigate these influences on individual prokaryotic species and on microbial communities in hydrothermal deposits, hydrothermal plumes and the water column. In this prospect, interdisciplinary methods were used such as culture in- and dependent microbiology techniques, multivariate analysis, chemical modeling, and oceanographic tools.

The influence of the hydrothermal environment was visible on a newly isolated *Bacteroidetes* species by, for example, the ability to grow under various oxygen concentrations and build biofilms. Also, the influence of hydrothermal fluid chemistry was visible within hydrothermal chimneys, where different functional groups inhabited different sections of the chimney in response to change in chemical energy landscapes. However, the specific chemistry of the hydrothermal plume did not have a visible influence on microbial community structure. Instead, the pelagic communities seemed to be subject to the dispersal influence of water masses.

For the first time, this study describes the major factors influencing microbial physiology and microbial community structures in a hydrothermal area of the Nordic Seas. By showing the stronger influence of dispersal through water masses rather than environmental selection through hydrothermal chemistry on the distribution of pelagic microorganisms, it lays the basis for further investigations of the geography of microbial communities of the Nordic Seas.

LIST OF PUBLICATIONS

Paper 1

Le Moine Bauer, S., Roalkvam, I., Steen, I. H., Dahle, H. (2016): *Lutibacter profundi* sp. nov., isolated from a deep-sea hydrothermal system on the Arctic Mid-Ocean Ridge and emended description of the genus *Lutibacter*. *International Journal of Systematic and Evolutionary Microbiology* **66** 2671-2677

Paper 2

Dahle, H., Le Moine Bauer, S., Baumberger, T., Stokke, R., Thorseth, I. H., Steen, I. H.: Taxonomic and functional microbial profiling in cross-sections of two hydrothermal chimneys with contrasting energy-landscapes. Submitted to *Environmental Microbiology*

Paper 3

Le Moine Bauer, S., Stensland, A., Daae, F. L., Sandaa, R., Thorseth, I. H., Steen, I. H., Dahle, H.: Microbial and viral community structures in water masses surrounding hydrothermal systems in the Nordic Seas. *Unpublished manuscript*

INTRODUCTION

1   The ocean hosts highly diverse microbial communities

The ocean is a very disparate environment. For example, the temperature of the seas varies between slightly negative values in the deep sea to higher than 300 °C in hydrothermal fluids. Salinity can reach more than 4 % in the Red Sea or be as low as 1 % in the Baltic Sea. Oxygen, an element vital for some organisms and poisonous for others, can saturate the surface waters and be completely absent in oxygen minimum zones. Other changing parameters across the seas are UV and light exposure, nutrient concentrations, land or ice sheet influence, human activity impact, hydrostatic pressure, and many others. Taken together, the worlds oceans comprise an immense variety of environments available for microbes to flourish. An important focus in marine microbiology aims at understanding how these changes in the environment shape microbial community structures, i.e. why are some microorganisms found in some places and others not?

The different factors influencing community structures can be gathered into four categories (Vellend, 2010). (i) Diversification, speciation or mutation represent the process of evolution, leading to the appearance and disappearance of species. (ii) Dispersal is the movement of species. On a human scale of distances, it is mainly considered as a passive process for microorganisms (movement as stowaways, or through air and water flows). (iii) Selection represents the influence of environmental physicochemical characteristics on community structures. Finally, (iv) drift represents the stochastic variations happening in a community over time. Different scenarios have been proposed where each of these categories are given more or less strength (Leibold *et al.*, 2004; Nemergut *et al.*, 2013).

During my research, I focused on three important factors influencing microbial community structures in the aphotic pelagic ocean and hydrothermal systems. First, I studied the influence of energy landscapes, component of the category (iii) "selection" (**Paper 1**, **Paper 2** and **Paper 3**), and then the influence of depth and water masses, respectively components of the categories (iii) "selection" and (ii) "dispersal" (**Paper 3)**

1.1   Energy landscapes influence the distribution of prokaryotes

Energy availability is one of the most fundamental requirements for the growth of prokaryotes. This energy can be divided in two types: photic energy and chemical energy. Photic energy fuels only the growth of photoautotrophs in the upper 200 m of the ocean. It does not directly influence the deep

aphotic ocean, even if exported organic matter from the photic layer is used as an electron donor in the deep sea. Therefore, light as an energy source will not be further described here.

Until the discovery of the first vent field near the Galapagos Islands in 1977 (Corliss *et al.*, 1979), light was considered to be the primary source of energy for any biological community, either directly or indirectly. Research has now shown that light-independent primary production also occurs in aphotic environments like the deep sea, sediments and hydrothermal vent fields (reviewed in Orcutt *et al.*, 2011). In the absence of light, lithoautotrophs harvest energy by accelerating naturally occurring chemical reactions between reduced compounds (electron donors) and oxidized compounds (electron acceptors). In the oceans, a broad range of both reduced compounds (hydrogen, methane, reduced sulfur and metal compounds, ammonium and organic matter) and oxidized compounds (oxygen, oxidized nitrogen and sulfur compounds, metal oxides and carbon dioxide) can be found and coupled in different "redox" reactions (Table 1). Each reaction releases a different amount of energy. Nevertheless, the necessary compounds for all reactions are not present everywhere in the oceans, and the most efficient reactions cannot always take place. Therefore, there exists an infinite amount of niches along the various chemical gradients that different microbial functional groups are exploiting. Correlations between chemical energy landscapes and the activity and structure of prokaryotic communities have been found in hydrothermal vents (e.g. **Paper 2**; Schrenk *et al.*, 2003; Takai and Nakamura, 2010; Flores *et al.*, 2011; Dahle *et al.*, 2015), marine sediments (e.g. Urakawa *et al.*, 2000; Durbin and Teske, 2011; Bienhold *et al.*, 2012; Jorgensen *et al.*, 2012; Frindte *et al.*, 2015) and oxygen minimum zones (e.g. Ulloa *et al.*, 2012). Below, I describe the differences in chemistry and microbial communities in the aphotic water column and hydrothermal vent systems.

|	Reaction name	Equation
Aerobic	Methane oxidation	$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$
	Oxic respiration/remineralization	$CH_2O + O_2 \rightarrow CO_2 + H_2O$
	Sulfide oxidation with oxygen	$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+$
	Fe(II) oxidation with oxygen	$Fe^{2+} + 1/4O_2 + H^+ \rightarrow Fe^{3+} + 1/2H_2O$
	Hydrogen oxidation	$H_2 + 1/2O_2 \rightarrow H_2O$
	Nitrification	$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$
	Mn(II) oxidation with oxygen	$Mn^{2+} + O_2 \rightarrow MnO_2$
Ana.	Sulfate-dependent anaerobic oxidation of methane	$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$
	Anammox	$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$
	Sulfate reduction	$CH_2O + 1/2SO_4^{2-} \rightarrow HCO_3^- + 1/2H_2S$
	Methanogenesis from H_2/CO_2	$H_2 + 1/4HCO_3^- + 1/4H^+ \rightarrow 1/4CH_4 + 3/4H_2O$

Table 1: Common redox reactions fueling microbial communities in the aphotic ocean and in hydrothermal systems. Ana., Anaerobic. Modified from Orcutt *et al*. (2011).

1.1.1 The aphotic water column

Energy sources

Among the electron acceptors listed in Table 1, oxygen (O_2) has the highest redox potential, i.e. it has the highest tendency of acquiring electrons and thereby being reduced. The upper part of the water column is naturally rich in oxygen due to photosynthesis and atmospheric exchange. Convection linked to the great oceanic conveyor belt (Broeker, 1991) provides a mean of transportation of oxygen to the deep sea. Apart from oxygen minimum zones, oxygen is the most widely used electron acceptor for redox reactions in the water column (Orcutt *et al.*, 2011). Nitrate can also be present as a result of remineralization of organic matter. However, its lower redox potential restricts its use as an electron acceptor primarily to low-oxygen zones (Ward *et al.*, 2009; Kraft *et al.*, 2011).

The main electron donor in the water column is organic matter, present as dissolved organic carbon (Arístegui *et al.*, 2002) or as marine snow, i.e. primary production from the photic layer sinking down in the form of aggregates (Figure 1; Alldredge and Silver, 1988). Thus, a major redox reaction is the aerobic oxidation of organic matter (Cho and Azam, 1988). This reaction fuels the growth of heterotrophs, i.e. microorganisms that cannot directly fix carbon dioxide. Ammonium is another electron donor present in the water column (Figure 1), coming mainly from the degradation of organic matter. In turn, it can be oxidized by oxygen in most of the water column (nitrification). It can also react with nitrites in a process called anammox in anoxic environments (Strous *et al.*,
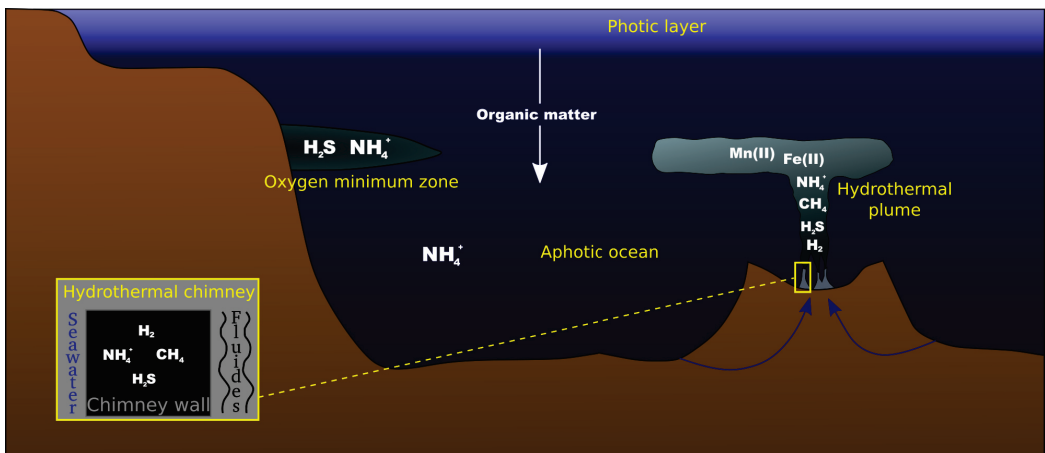
Figure 1: Common electron donors mentioned in the text (in white). In the aphotic ocean and hydrothermal plumes, they are usually oxidized by oxygen. In oxygen minimum zones, often found along the continental margin in upwelling areas, the reduced compounds can be oxidized for example by nitrate, nitrite or sulfate. The caption represents the cross section of a hydrothermal chimney wall. In the caption, the electron donors were positioned randomly, as the same reduced compound can be involved in different redox reactions at different places within the chimney wall. Similar situations can be found in other structures, like hydrothermal deposits.

1999). In contrast with organic matter oxidation, these reactions fuel the growth of autotrophs, i.e. microorganisms that can fix carbon dioxide directly.

Distribution of prokaryotes and viruses

The aphotic water column is oligotrophic, i.e. energy sources are scarce. Nevertheless, several ubiquitous deep-sea prokaryotic species have the potential for autotrophic growth (Swan *et al.*, 2011). Studies have shown that autotrophic ammonia-oxidizing *Thaumarchaeota* (Brochier-Armanet *et al.*, 2008) are dominating the aphotic water column (**Paper 3**; Karner *et al.*, 2001). To date, all marine *Thaumarchaeota* isolates are closely related to the *Nitrosopumilus maritimus* SCM1 strain (Könneke *et al.*, 2005; Qin *et al.*, 2014; Elling *et al.*, 2015; Bayer *et al.*, 2016). This low phylogenetic diversity has also been found in culture-independent environmental studies (**Paper 3**; Zaballos *et al.*, 2006). However, genomic and culture-dependent studies have shown strong phenotypic variations in closely-related *Nitrosopumilus spp.*, such as the ability to use urea as a substrate or to be motile (Park *et al.*, 2014; Qin *et al.*, 2014; Bayer *et al.*, 2016). This physiological diversity, associated to a high affinity for ammonia (Martens-Habbena *et al.*, 2009) and a very efficient CO_2 fixation mechanism (Könneke *et al.*, 2014), allows *Thaumarchaeota* to thrive in the oligotrophic water column. Autotrophic sulfur oxidation can also take place in the water column, for example by the mixotrophic SAR324 *Deltaproteobacteria* (Sheik *et al.*, 2014). Its abundance is correlated with low oxygen concentrations (Wright *et al.*, 2012). Similarly, the members of the sulfur-oxidizing SUP05 *Gammaproteobacteria* clade are more abundant in low-oxygen zones (Glaubitz *et al.*, 2013).

In contrast to *Thaumarchaeota*, members of Marine Group II *Archaea* (MGII) are less abundant, heterotrophic, and contribute more to surface water communities (reviewed in Zhang *et al.*, 2015). Nonetheless, MGII members have been found in the deep sea (Deschamps *et al.*, 2014), and in **Paper 3** we report MGII abundances as high as 34% of the total prokaryotic community in samples from 400 to 600 m depth and 2400 to 2800 m depth. However, there are no cultivated representatives to date and very little is known about their ecological role in the aphotic ocean. Heterotrophic *Bacteria* are also present with, for example, the ubiquitous SAR202 cluster (*Chloroflexi*), which shows increasing abundance with depth (e.g. Morris *et al.*, 2002; Varela *et al.*, 2008; Schattenhofer *et al.*, 2009; Lekunberri *et al.*, 2013; Guerrero-Feijóo *et al.*, 2016). Other heterotrophic *Bacteria* such as *Gammaproteobacteria*, *Alphaproteobacteria*, *Bacteroidetes*, *Actinobacteria* or *Planctomycetia* are also present in the aphotic ocean.

Viral communities are known to respond to changing prokaryotic community structures (Riemann *et al.*, 2000; Sandaa *et al.*, 2009). Therefore, they are indirectly influenced by chemistry, and different viral communities are expected in different environments. Compared to prokaryotes, little is known about viruses in the aphotic water column. Nevertheless, they are known to be very abundant (typically ca. 15-fold the amount of prokaryotes; Suttle, 2007), extremely diverse (Angly *et al.*, 2006; Gustavsen *et al.*, 2014), and represent a strong top-down control on prokaryotes (Proctor and Fuhrman, 1990; Bratbak *et al.*, 1994; Weitz and Wilhelm, 2012). Viruses of *Archaea* have been shown to have a strong impact on their host communities in deep marine sediments (Danovaro *et al.*, 2016). Therefore, similar effects can be expected in the *Archaea*-dominated aphotic ocean. Recently, culture independent methods have lead to the sequencing of the first *Thaumarchaeota* and MGII viruses (Labonté *et al.*, 2015; Philosof *et al.*, 2017, respectively).

1.1.2   Hydrothermal systems

Energy sources

Underwater hydrothermal systems are situated in zones with volcanic and/or tectonic activity (Tivey, 2007). Here, seawater that penetrates into the crust is heated by the magma chamber, and eventually rises towards the seafloor (Alt, 1995). During its journey through the crust, the seawater experiences multiple interactions with the minerals. As a result, the fluids that are ultimately emitted are enriched in metals, H_2S, CO_2, CH_4, and H_2, but depleted in Mg, Ca, and Na (Alt, 1995). However, the chemistry of the fluids varies according to the geology of the area. For example, mantle-hosted hydrothermal fields, like the Rainbow Field, situated on ultramafic peridotites usually have elevated concentrations of H_2 and CH_4, i.e. in the millimolar range (Charlou *et al.*, 2002). In contrast, crust-hosted fields like the ones at the Arctic Mid-Ocean Ridge situated on volcanic basalt usually have higher CO_2 concentrations (Pedersen, Thorseth, *et al.*, 2010). Also, the presence of sediments such as at Loki's Castle Vent Field and in the Guaymas Basin results in increased concentrations of NH_4^+, and possibly CH_4 and H_2 in the fluids (Von Damm *et al.*, 1985; Baumberger *et al.*, 2016). However, the geology of tectonic and volcanic areas is complex and varies in time and space. Therefore, fluid chemistry is often the result of multiple influences.

When reaching the surface of the crust, the reduced fluids mix with oxidized seawater in hydrothermal deposits and chimneys, creating sharp redox and temperature gradients. The potential energy available for microbial growth throughout these mixing gradients has been modeled several times (e.g. McCollom and Shock, 1997; Amend *et al.*, 2011; LaRowe *et al.*, 2014; Dahle *et al.*, 2015). At high hydrothermal fluid:seawater ratios, results show that anaerobic processes like the

reduction of sulfur compounds and methanogenesis are the most exergonic reactions (Figure 1). At low hydrothermal fluid:seawater ratios, available energy from aerobic processes like aerobic oxidation of sulfide, ammonia (if present) or methane is higher. Aerobic oxidation of hydrogen can potentially also fuel autotrophic growth, despite the high rates of abiotic hydrogen oxidation (Foustoukos *et al.*, 2011). The chemistry of the fluids is of primary importance: For example, sulfide oxidation represents most of the energy available at the Jan Mayen Vent Fields, while methane oxidation is more important at Loki's Castle Vent Field (Dahle *et al.*, 2015). In general, sulfide oxidation is a major source of energy in basalt-hosted vent fields, while sediment inputs will provide energy from methane, hydrogen, and ammonium oxidation.

When exiting the crust, the fluids dilute in seawater and become the rising hydrothermal plume. After sufficient mixing, the plume reaches the same buoyancy as the surrounding water and stops rising. It then starts spreading horizontally. The plume is dynamic: its position is not stable as it varies with the currents, the intensity of hydrothermal venting and possible eruptions (Von Damm, 1995). Within hydrothermal plumes, the fluids are diluted ca. 10 000 times (Lupton *et al.*, 1985), allowing oxic reactions to take place (McCollom, 2000). Due to different affinities with oxygen, the electron donors are oxidized successively (Figure 1): H_2 first, followed by H_2S, NH_4, CH_4, Fe and Mn (Kadko *et al.*, 1990). The H_2 signal disappears within hours (Lilley *et al.*, 1995), while iron and manganese inputs can be traced several thousands of kilometers from the origin (Fitzsimmons *et al.*, 2017).

Distribution of prokaryotes and viruses

The chemical and thermal gradients present in hydrothermal systems allow different microbial functional groups to thrive. Various studies report the presence of a broad range of microorganisms in hydrothermal deposits, plumes, chimneys and sediments, diffuse venting fluids, microbial mats and in and on the macrofauna as symbionts (reviewed in Flores and Reysenbach, 2011; Orcutt *et al.*, 2011; Sievert and Vetriani, 2012). The communities change with the variations in the hydrothermal fluid:seawater ratio (**Paper 2**; Schrenk *et al.*, 2003; Flores *et al.*, 2011). Furthermore, communities have been shown to change over short and long time scales (McCliment *et al.*, 2006; Brazelton *et al.*, 2010). Nonetheless, some microbial phylogenetic groups are found in most hydrothermal systems.

Under anoxic conditions, the presence of methanogenic *Methanococcales* has been reported (**Paper 2**; Jones *et al.*, 1983; Higashi *et al.*, 2004), as well as various sulfate reducers from the

Deltaproteobacteria class (**Paper 2**; Huber *et al.*, 2003; Frank *et al.*, 2013; Cao *et al.*, 2014) and anaerobic methane-oxidizing *Archaea* (Brazelton *et al.*, 2006). Towards the most oxidized end of the gradient, several studies report the abundance of sulfur- and hydrogen-oxidizing *Epsilonproteobacteria* (**Paper 2**; Reysenbach *et al.*, 2000; Lanzén *et al.*, 2011; Urich *et al.*, 2014). These *Bacteria* can be found in sediments, mineral structures, and are also known to dominate in microbial mats (Campbell *et al.*, 2006; Stokke *et al.*, 2015). Methane-oxidizing *Methylococcales* from the *Gammaproteobacteria* class can be found in similar oxic conditions (Urich *et al.*, 2014; Dahle *et al.*, 2015; Steen *et al.*, 2016). High concentrations of Fe(II) can foster the growth of iron-oxidizing *Zetaproteobacteria* in microbial mats and hydrothermal deposits (Vander Roost *et al.*, submitted; Emerson and Moyer, 2002; Emerson *et al.*, 2007; Fleming *et al.*, 2013). Ammonia-oxidizing *Thaumarchaeota* were found within chimney walls and sediments (**Paper 3**; Jaeschke *et al.*, 2014). However, no known ammonia oxidizers could be detected in some situations favorable for ammonia oxidation (e.g. Dahle *et al.*, 2015; Steen *et al.*, 2016). Various heterotrophs are also present in hydrothermal systems, for example *Archaeoglobales, Thermococcales* and *Thermotogales* (**Paper 2**; Wery *et al.*, 2002; Takai and Nakamura, 2010; Dahle *et al.*, 2015).

In contrast to hydrothermal chimneys and deposits, microbial communities in hydrothermal plumes have been less investigated. The plume community is a mixture of pelagic and benthic microorganisms, but the factors influencing the relative input of each source are unclear, (**Paper 3**; Dick *et al.*, 2013). Sulfur-oxidizing SUP05 *Gammaproteobacteria* and SUP01 *Epsilonproteobacteria* clades have been found to be very abundant in some plumes (Sunamura *et al.*, 2004; Dick and Tebo, 2010). Meta -genomic and -transcriptomic analysis of SUP05 populations have shown their ability to oxidize hydrogen too (Anantharaman *et al.*, 2013). *Nitrosomonas*-like *Bacteria* (Lam *et al.*, 2004) and *Thaumarchaeota* (Baker *et al.*, 2012) oxidize ammonia, and a broad range of microorganisms seems to oxidize methane (Li *et al.*, 2014). High manganese oxidation rates are found in plumes (Dick *et al.*, 2006). However, little is known about the microorganisms involved in this process, with only a few *Bacillus* species identified with the ability to oxidize manganese in plumes (Dick *et al.*, 2006).

Little is known about viral communities in hydrothermal systems. The abundance of virus-like particles in the diffuse flow may be higher (Ortmann and Suttle, 2005) or similar (Wommack *et al.*, 2004) to the surrounding seawater. The virus-to-prokaryote ratio seems to be lower within chimneys than in the surroundings waters, possibly due to a prevalence of the lysogeny approach in more extreme conditions (Yoshida-Takashima *et al.*, 2012). Such mutualistic strategy between viruses and

prokaryotes is supported by the discovery of several genes related to microbial metabolic pathways on hydrothermal viral genomes (Anantharaman *et al.*, 2014; Anderson *et al.*, 2014).

1.2 Depth and water masses influence the distribution of viruses and prokaryotes

1.2.1 Depth

Several variables are changing with increasing seawater depth. First, the sunlight penetrates on average only the upper 200 m of the water column, leaving most of the sea in darkness. As a result, temperature decreases with depth, even if the water temperature below the thermocline is relatively stable. In surface waters, the phytoplankton makes use of the solar energy to grow, leading to the depletion of nutrients. Below, the sinking biomass is remineralized, and therefore deep waters often have higher nutrient concentrations. Surface waters also have high concentrations of oxygen, due to photosynthesis and sea-air exchanges. An oxygen minimum is usually found in the thermocline, where it is consumed by remineralization. Finally, pressure increases by 1 bar per 10 m of water depth.

These changing variables influence pelagic microbial communities. Surface waters show higher prokaryotic abundance (typically 10^5 to 10^6 cells ml^{-1}) than deep waters (typically 10^3 to 10^5 cells ml^{-1}; **Paper 3**; Reinthaler *et al.*, 2006; De Corte *et al.*, 2012). Contrarily, prokaryotic richness is often higher in deep waters (**Paper 3**; Sunagawa *et al.*, 2015). Changes in community structures have been observed along depth gradients (**Paper 3**; DeLong *et al.*, 2006; Zaballos *et al.*, 2006; Ghiglione *et al.*, 2008). General patterns show that the photic layer often exhibit higher abundances of the ubiquitous SAR11 cluster (Morris *et al.*, 2002), MGII *Archaea* (Massana *et al.*, 1997), and phototrophic *Eukarya* and *Bacteria* such as the very abundant *Prochloroccus* (Partensky *et al.*, 1999). With depth, the relative proportion of *Archaea* (mainly *Thaumarchaeota*) in the prokaryotic community increases (**Paper 3**; Karner *et al.*, 2001; Winter *et al.*, 2009). The distribution of heterotrophs in the aphotic water column is unclear, but links to the concentration of particulate organic matter have been proposed (Nagata *et al.*, 2000). Some studies also suggest that water masses and currents have a role in deep-sea microbial dispersion(**Paper 3**; Lauro *et al.*, 2007; Agogué *et al.*, 2011). In general, the causes for changes in microbial community structures with depth in the aphotic ocean are not clearly understood, and the study of piezophily is still in its infancy.

Virus community structure has been shown to change with depth (**Paper 3**; Hurwitz *et al.*, 2015). The virus-to-prokaryote ratio increases with depth, even though viral abundance decreases (**Paper 3**; Parada *et al.*, 2007). The effects of depth on viral communities seem however to vary between different oceanic provinces (De Corte *et al.*, 2012, 2016).

1.2.2 Water masses

The concept of water mass was defined by Helland-Hansen in 1916, along with the introduction of the θ-S plot (potential temperature-salinity plot; Helland-Hansen, 1916). The concept describes a volume of water with similar physicochemical characteristics. The boundaries of water masses are often defined by temperature and salinity thresholds (Emery and Meincke, 1986; Fogelqvist *et al.*,
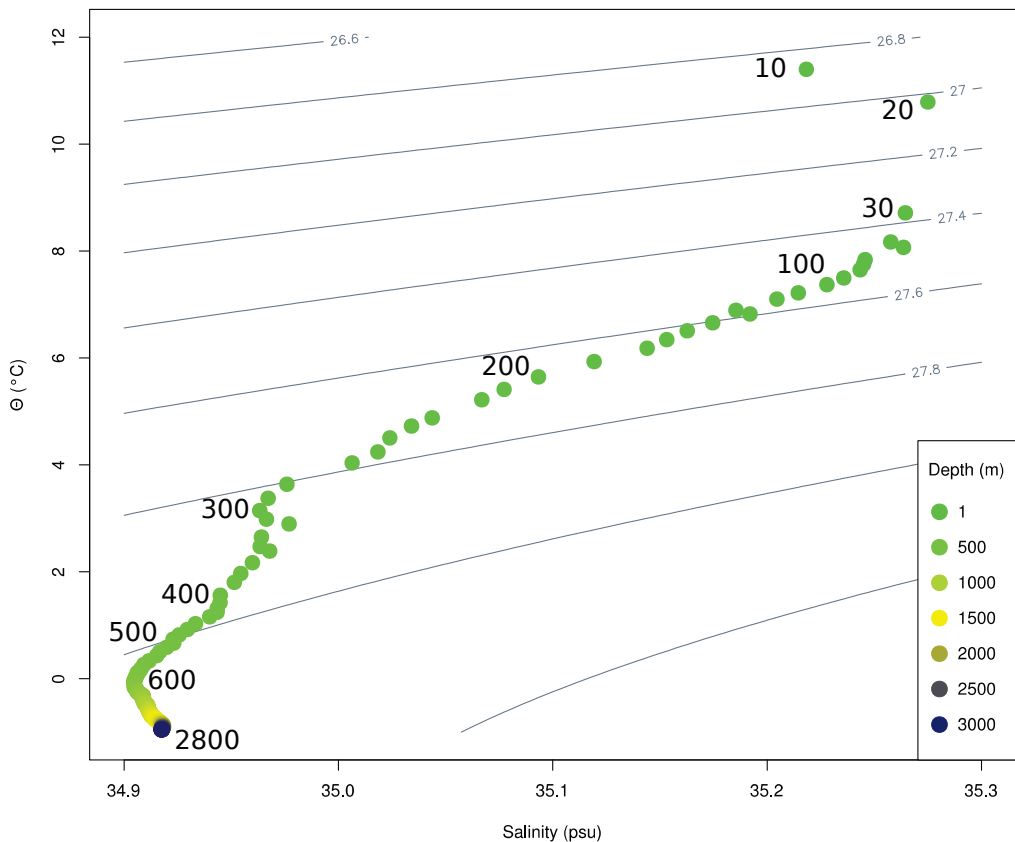
Figure 2: Example of a θ-S plot. Data is from the Norwegian Sea (CTD 1, G.O. SARS 2013 cruise; Figure 3). The profile is typical of the area, with the warm and saline Atlantic Surface Water. The Arctic Intermediate Water layer can be observed at ca. 600 m depth by a weak salinity minimum. Below, the Norwegian Deep Water shows little variability in salinity and potential temperature. As density is a function of temperature and salinity, isopycnals can also be represented on a θ-S plot. Here, the lines represent isopycnals of the potential density with a reference pressure of 0 (σ_{θ}).

2003; Rudels *et al.*, 2005). Therefore, the use of *θ-S* plots has become a major tool for categorizing water samples (Figure 2).

Water masses are usually in movement due to currents and are often connected directly or indirectly to the Great Ocean Conveyor Belt (Broeker, 1991). They are separated from each other by density gradients, bathymetry changes and oceanic fronts. Therefore, in the absence of mixing, these barriers force microbial dispersion to follow the water masses (e.g. Wilkins *et al.*, 2013; Winter *et al.*, 2013). As a result, several studies have shown that different water masses often host separate microbial communities (e.g. **Paper 3**; Pinhassi *et al.*, 2003; Teira *et al.*, 2006; Hamilton *et al.*, 2008; Han *et al.*, 2015; Techtmann *et al.*, 2015; Hernando-Morales *et al.*, 2016; Djurhuus *et al.*, 2017). Conversely, microbial communities geographically distant from each other but from the same water mass can be very similar (**Paper 3**; Agogué *et al.*, 2011). Therefore, the use of geographic distance *per se* as a factor for microbial dispersal in the ocean seems inappropriate.

To my knowledge, **Paper 3** was the first study to investigate the differences of viral community structures between water masses. The results showed that changes in prokaryotic community structures between two water masses were not always triggering a change in the T4-like myovirus community structure.

2   Different studying approaches for different questions

The understanding of the great complexity of the microbial world requires to answer many interrogations. Most of these can be categorized into the very elementary questions "Who? Where? What? Why? How?". Each of these questions are nonetheless very different, and there is no ultimate method that answers them all. Instead, a multitude of techniques exist, each of them addressing a specific aspect of the microbial world. It is therefore important to combine various approaches to obtain a complete picture of the ecosystems. In my thesis, I have used two different approaches to help me understand which type of microorganisms lives where, and why. Below, I introduce these methods: The isolation and characterization of new prokaryotic species and the deep-sequencing of marker genes.

2.1   Isolation and characterization of new prokaryotic species

The cultivation of isolates was one of the first methods used in microbiology, far before the advent of genomic technologies. When genomic science started to develop, isolates were the only source for high-quality genome constructions (Giovannoni and Stingl, 2007). Nowadays, genome-centric

metagenomics allow the sequencing of genomes directly from environmental samples (reviewed in Garza and Dutilh, 2015) but the cultivation of *Bacteria* and *Archaea* remains a major tool in modern microbiology. From a biotechnological point of view, the isolation of a microorganism is the best method to understand its putative applications (Joint *et al.*, 2010). For example, new methods in the cultivation of soil microorganisms have recently led to the discovery of a new antibiotic (Ling *et al.*, 2015). From an ecological point of view, isolate cultures are the only way to study and characterize in-depth a microorganism as an entity, and to understand its physiology and requirements. Thorough culture and genome studies help understanding the putative role and the distribution of the isolate in the environment (see Description of the study for **Paper 1**). Furthermore, conflicts between genome analysis and culture observations remind us how little we understand about microbial physiology. For example, genome analysis proposed that *Lutibacter profundi* LP1^T is able to use gliding motility (Wissuwa *et al.*, 2017) while the cells were observed as non-motile under any tested condition (**Paper 1**). Finally, from a phylogenetic point of view, cultured species can give clues about the characteristics of other clones belonging to the same clade. Several clades remain with no cultivated representative (Hug *et al.*, 2016; Solden *et al.*, 2016), and therefore little is known about their role in the ecosystem. For all these reasons, thorough characterization of isolates in the laboratory remains the only valid approach to identify and define novel bacterial and archaeal species.

Nevertheless, the traditional way of culturing has several limitations. The most well-known is "The great plate count anomaly" (Staley and Konopka, 1985), representing the ratio between the diversity of prokaryotes cultivable in the laboratory and the diversity of prokaryotes in the environment, typically less than 1%. One of the reasons for this low culturability is arguably linked to the difference in chemical and physical characteristics between laboratory and *in situ* growth conditions. For example, some substrates in growth media may be inadequate, whereas other chemicals, like oxygen, can inhibit the growth of some microorganisms at certain concentrations. Another example is the very much used ZoBell's Marine Broth 2216 medium, which contains dissolved organic carbon concentrations 170 times higher than the concentrations in the pelagic ocean (Giovannoni and Stingl, 2007). Consequently, many of the successful isolates are opportunistic fast-growing r-strategists that normally occur in low abundances and exert minor influences in the oligotrophic seas. Ultimately, a serious hurdle in the pursuit of microorganisms isolation is the loss of interactions among these organisms. Some interactions may be necessary as one organism may be releasing growth factors required for other ones (D'Onofrio *et al.*, 2010).

To face these difficulties, scientists have developed new methods to solve the problems of opportunistic r-strategists, lack of *in situ* condition knowledge and growth factors requirements:

Diffusion chambers in the environment, dilutions to extinction, co-cultures, cultures in microdroplets of agarose, among others (reviewed in Stewart, 2012). Using these new methods, two strains from major marine prokaryotic groups have been isolated: the member of the SAR11 clade *Candidatus* Pelagibacter ubique HTCC1062 (Rappé *et al.*, 2002) and the *Thaumarchaeota Nitrosopumilus maritimus* SCM1 (Könneke *et al.*, 2005). The SAR11 clade is ubiquitous and dominates the prokaryotic community in surface oceanic layers (Morris *et al.*, 2002). The isolation and sequencing of *P. ubique*, together with various metagenomic studies, have shed light on the metabolism, distribution and environmental role of the SAR11 clade (reviewed in Tripp, 2013; Giovannoni, 2017). Contrarily to SAR11, *Thaumarchaeota* species are dominating the deeper waters (**Paper 3**; Karner *et al.*, 2001) where they play an important role in ammonia oxidation. The comparison of various cultured *Nitrosopumilus sp.* has shown their ability to use different nitrogen substrates (Qin *et al.*, 2014; Bayer *et al.*, 2016). This allows them to occupy several different niches, possibly explaining their high abundance in the deep sea.

These two examples show how growth experiments of isolated microbial strains is an important tool to understand the distribution and potential role of microorganisms in their environment.

2.2   Deep sequencing of marker genes

The culturing of isolates helps us to understand the function of one element in the ecosystem. However, "the great plate anomaly" leads to a poor understanding of the prokaryotic diversity from culture-dependent studies. Therefore, other approaches are needed to answer the question "Who is out there?". A major advance was made with the development of next-generation sequencing technologies, for example 454-pyrosequencing, Illumina, Ion Torrent, and Oxford Nanopore sequencing. These technologies decreased considerably the sequencing price per DNA base pair, and allowed to sequence directly millions of sequences from the environment, bypassing the culture-dependence of traditional microbiology methods (reviewed in Goodwin *et al.*, 2016). In order to study microbial diversity, next-generation sequencing technologies are associated with a method called fingerprinting, which uses DNA marker sequences to group all organisms in an environment according to taxonomy and evolutionary relatedness.

In order to provide valuable information, a good marker sequence should be present in a broad range of species, have a sufficient variability to identify accurately different species, have conserved sites for the design of universal primers, and be of a length suitable for analysis (Kress and Erickson, 2012). The *g23* gene used in **Paper 3** is not optimal to study total viral diversity as it

targets only T4-like myoviruses (Filée *et al.*, 2005). However, there is no universal gene available that capture the whole viral diversity, and the *g23* gene remains one of the best marker genes for capturing a broad group of marine viruses that have shown to be important players in the ocean (Frank and Moebus, 1987; Angly *et al.*, 2006; Breitbart, 2012).

The gene encoding 16S rRNA, proposed in 1986 (Pace *et al.*, 1986), became quickly the most used barcode for the identification of prokaryotes. The main reason is that it is found in all known *Bacteria* and *Archaea* (Woese, 1987), allowing the identification and classification of virtually any prokaryote on Earth. Within the gene sequence, several conserved sites allow the design of universal primers, while highly variable regions allow high identification accuracy (Janda and Abbott, 2007). Finally, due to intensive worldwide sequencing, the 16S rRNA databases contain an enormous amount of sequences: The GenBank database is the largest one, with more than 18 500 sequences from published microorganisms and several million environmental clone sequences (spring 2017).

Nevertheless, some problems are inherent to the 16S rRNA gene: The similarity between two sequences does not always reflect the divergence level of two organisms (Fox *et al.*, 1992), some cases of mosaicism within the 16S rRNA gene have been reported (Schouls *et al.*, 2003), and some hypervariable regions of the 16S rRNA gene were estimated to be unreliable (Yang *et al.*, 2016). Furthermore, since the primers are designed from existing databases, they may restrict the discovery of completely new taxa. Finally, the different reference databases available for assigning taxonomy to the sequences do not agree on all taxon names (Youssef *et al.*, 2015). For all these reasons, the results obtained from ecological studies solely based on the 16S rRNA gene need to be interpreted with caution.

Nonetheless, the deep-sequencing of the 16S rRNA gene has allowed high resolution studies of prokaryotic phylogeny and community structure. The 16S rRNA gene is now extensively used in oceanic ecological studies (e.g. Huber *et al.*, 2007; Rusch *et al.*, 2007; Ghiglione *et al.*, 2012; Sunagawa *et al.*, 2015), leading to a better understanding of the distribution of prokaryotic communities in the ocean. The most important contribution of the 16S rRNA gene is however phylogenetic, with the separation of prokaryotes in two domains: *Bacteria* and *Archaea* (Woese, 1987). Ever since, novel phylogenetic groups are discovered regularly (e.g. Brown *et al.*, 2015), continuously increasing the extent of the total prokaryotic diversity.
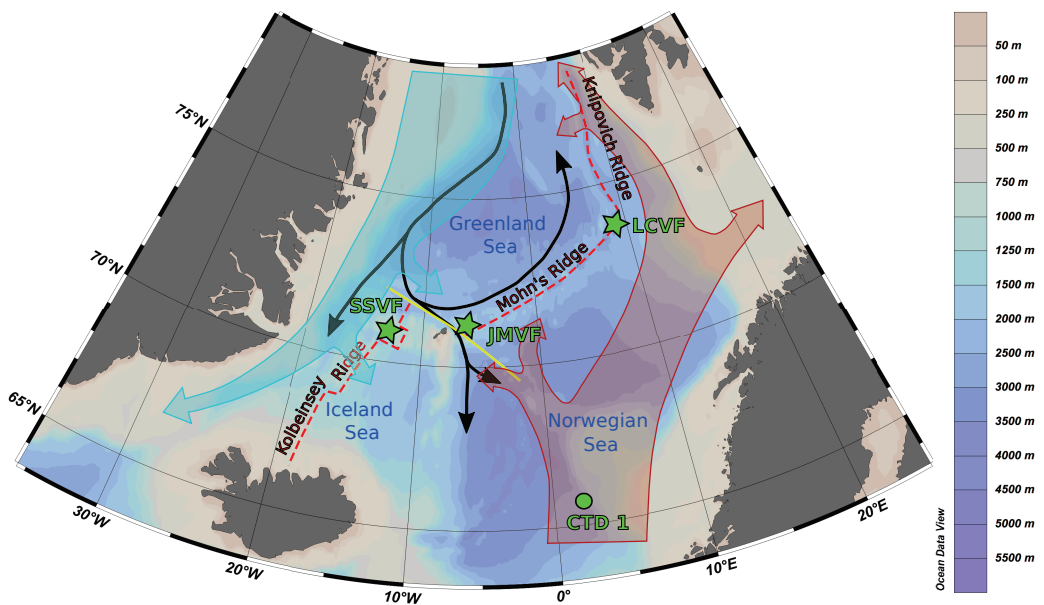
3 The Nordic Seas

Since the creation of the Center for Geobiology at the University of Bergen, annual cruises with the G.O. SARS research vessel have been arranged for collecting samples from the Nordic Seas, with a particular focus on the Arctic Mid-Ocean Ridge (AMOR). The Nordic Seas possess unique features, with a complex oceanographic situation and an ultra-slow mid-ocean spreading ridge. There is therefore a lot to learn from integrated studies of the Nordic Seas. The work presented in this thesis is supported by samples mainly taken at the AMOR, at the middle of the Nordic Seas.

3.1 Oceanography

The Nordic Seas are situated between the Greenland-Scotland Ridge to the south and the Fram Strait-Spitsbergen-Northern Norway transect to the north. They are composed of the Norwegian, Greenland and Island Seas (Figure 3). The diverse topography is made of continental shelfs, mid-oceanic ridges and deep basins, with the deepest point in the Norwegian Sea, at more than 3800 m depth. The area is relatively small, around $2.5 \cdot 10^6$ km^2 (ca. 0.75 % of the world's ocean) but has a major role in several processes: The Atlantic Surface Waters on the eastern side contribute significantly to the abnormally high temperature of Northern Europe (Rhines and Häkkinen, 2003), the sea surface temperature and ice cover of the Nordic Seas influence the atmospheric circulation of the northern hemisphere (Deser *et al.*, 2004; Magnusdottir *et al.*, 2004), the uptake of atmospheric CO_2 in the Nordic Seas is one of the highest in the world's oceans (Takahashi *et al.*, 2002), and it is the most active place for deep water formation in the northern hemisphere (Aagaard *et al.*, 1985). Finally, it is the main connection between the Arctic Ocean and other oceans. Therefore, the Nordic Seas and Arctic Ocean together have been referred to as the Arctic Mediterranean Sea among oceanographers (Krümmel, 1879). Scientists started very early to explore the Nordic Seas, and Helland-Hansend and Nansen had already accurately mapped the water circulation at the beginning of the last century (Helland-Hansen and Nansen, 1909). There are two main inputs of surface waters: The warm and saline Atlantic Surface Water enters the Nordic Seas from the south between Iceland and the Shetlands, while the colder and fresher Polar Surface Water enters from the north through the Fram strait (Figure 3; Blindheim and Østerhus, 2005). The former divides in several branches, with a portion heading west towards Jan Mayen, another one following the Norwegian coast and entering the Barents Sea, and the remaining reaching the western coast of Svalbard. There, a part recirculates back into the Greenland Sea, and the rest enters the Arctic Ocean through the Fram Strait. Oppositely, the fresher Polar Surface Water heads south towards the Atlantic Ocean along the Greenland shelf (Haine *et al.*, 2015), with some parts deflected towards

Jan Mayen and the Iceland Sea. The Nordic Sea Deep Water is made of locally formed deep waters in the Greenland Sea and deep water input from the Arctic Ocean. Finally, the Arctic Intermediate Water layer is situated between the surface and deep waters. This layer is created in the Greenland Sea, and is detectable between 500 and 800 m deep as a weak salinity minimum and an oxygen maximum (Figure 2; Blindheim, 1990).

Besides their spatial heterogeneity, the characteristics of the Nordic Seas also change over time, with periods of warming, cooling, or changing salinity (reviewed in Dickson and Østerhus, 2007). Since the 1980's, the salinity and temperature in the Nordic Sea Deep Water have increased. This is due to the cessation of deep water formation in the Greenland Sea, resulting in a higher inflow of saline and warm Arctic Ocean Deep Water to the Nordic Seas (Østerhus and Gammelsrød, 1999; Dickson and Østerhus, 2007).

Figure 3: Bathymetric map of the Nordic Seas. The dashed line represents the Arctic Mid-Ocean Ridge and the green stars the location of the three hydrothermal systems described in this thesis. The red arrows represent the warm and saline Atlantic Surface Water and the blue arrows the cold and fresher Polar Surface Water within the East Greenland Current. The black arrows represent the flow of the Deep Nordic Waters. The water mass circulation has been simplified and the Arctic Intermediate layer is not represented. SSVF, Seven Sisters Vent Field; JMVF, Jan Mayen Vent Fields; LCVF, Loki's Castle Vent Field; CTD 1, location of the CTD profile shown in figure 2.

3.2   Hydrothermal activity

The AMOR stretches from Iceland to Siberia, and splits the Nordic Seas in two from south to north (Figure 3). In the Nordic Seas, the ridge can be divided in three main fragments: The Knipovich ridge to the north, the Mohn's ridge up to Jan Mayen, and the Kolbeinsey ridge down to Iceland. The two later ones are separated by the Jan Mayen Fracture Zone. The AMOR is the slowest spreading ridge in the world along with the Southwestern Indian Ridge. The slowest spreading rates are however measured near Siberia, whereas spreading rates between Svalbard and Iceland are around 15 to 20 mm per year (Vogt, 1986). The ridge hosts several hydrothermal vent fields (Pedersen, Thorseth, *et al.*, 2010). Three of them are connected to findings reported in this thesis: Loki's Castle Vent Field (**Paper 1**), Jan Mayen Vent Fields (**Paper 2** and **3**), and the Seven Sisters Vent Field (**Paper 3**).

Loki's Castle Vent Field (LCVF) was discovered in 2008 at the transition between the Mohn's Ridge and the Knipovich Ridge (Figure 3, Pedersen, Rapp, *et al.*, 2010). It is a basalt-hosted deep-sea vent field, situated at ca. 2400 m depth. Two sites found on top of two mounds of hydrothermal deposits vent black smoker fluids from up to 11-meter tall chimneys (Figure 4a). The fluids are hot (up to 317 °C) and slightly acidic (pH 5.5). On the northeastern side, a dense field of small barite chimneys emits ca. 20 °C fluids (Eickmann *et al.*, 2014). The high CO_2 values (23.8 mmol kg^{-1}) reflect the presence of basaltic crust, while high CH_4 and H_2 values (13.5 and 4.9 mmol kg^{-1}, respectively) suggest the influence of the ultramafic mantle (Pedersen, Thorseth, *et al.*, 2010). The influence of the sedimentary Bear Island fan results in high NH_4^+ concentrations in the fluids (1.6 mmol kg^{-1}) and possibly also explains the high CH_4 and H_2 concentrations (Baumberger *et al.,* 2016). Due to its high depth, no gas bubbles are observed in the water column. The vent macrofauna is dominated by *Sclerolinum contortum* tube worms, amphipods, *Pseudosetia griegi* and *Skenea spp.* gastropods and tube-dwelling *Polychaeta* (Pedersen, Rapp, *et al.*, 2010). *Lutibacter profundi* (**Paper 1**) has been isolated from an *Epsilonproteobacteria*-dominated mat found on the high-temperature chimneys (Figure 5).

The Jan Mayen Vent Fields (JMVF) were discovered in 2005 at the southern end of the Mohn's Ridge (Figure 3, Pedersen *et al.*, 2005). It is composed of three different venting areas: The Troll wall at ca. 500 m depth, the Soria Moria Vent Field at ca. 700 m depth and the newly discovered Perle and Bruse Vent Field at ca. 580 m depth (Figure 4b). Each field has several chimneys and venting areas. Up to 10-meter tall chimneys produce hot (ca 270 °C) white smoker fluids surrounded by diffuse venting area. High CO_2 concentrations (up to 91.7 mmol kg^{-1}) result in more acidic fluids (pH 4.10-5.20) than at LCVF. CH_4 and H_2 concentrations (0.01-0.09 mmol kg^{-1} and

0.01-0.1 mmol kg^{-1}, respectively) were first described as lower than at LCVF (Pedersen, Thorseth, *et al.*, 2010). However, in **Paper 2**, we measured CH$_4$ concentrations of 5.4 mM in fluids at the Bruse Vent Field. Reasons for these high concentrations are yet to be clarified (Tamara Baumberger, pers. comm.). There is no sedimentary input at JMVF, resulting in low NH$_4^+$ concentrations (0.03-0.14 mmol kg^{-1}). The macrofauna lacks vent specialists, and is dominated by *Heliometra glacialis* and *Gorgonocephalus eucnemis* echinoderms, as well as crustacean clouds (Schander *et al.*, 2010). In **Paper 2**, we investigated sections of two chimneys: one from the Soria Moria Vent Field and one from the Perle and Bruse Vent Field (Figure 6). In **Paper 3**, we sampled the plumes above the Soria Moria Vent Field, measuring CH$_4$ concentrations up to 795.6 nmol kg^{-1}.

The Seven Sisters Vent Field (SSVF) was discovered in 2013 at the northern end of the Kolbeinsey Ridge (Figure 3, Marques *et al.*, in prep.). It is the first shallow (130 m depth) basalt-hosted hydrothermal vent field discovered at a mid-oceanic ridge. Diffuse venting takes place on the top of several flat-topped volcanoes exhibiting pinnacle-like structures and volcaniclastic breccias without clear chimney-like orifice (Figure 4c). Due to its lower depth, the clear fluids from SSVF are less warm (up to 200 °C) than the two other fields described here and do not contain metals. The pH is around 5 and the high CO$_2$ concentrations (2.94-5.58 mmol kg^{-1}) reflect the volcanic influence. CO$_2$-dominated bubbles are also present. Similarly to the JMVF, low NH$_4^+$ (0.05 to 0.06 mmol kg^{-1}), H$_2$ (<0.01 mmol kg^{-1}) and CH$_4$ (0.01 to 0.03 mmol kg^{-1}) concentrations show the absence of sedimentary influence. Biological samples from SSVF are scarce. Nonetheless, video footage suggests that colonial ascidians and *Urticina eques* and *Hormatia sp.* anemones dominate the field. In **Paper 3**, we took different water samples within and near the plumes of the SSVF.

3.3 Microbiology

Only few studies have investigated microbial communities in the Nordic Seas. Furthermore, most of these studies have sampled on the periphery of the seas: Virus-plankton interactions in Norwegian fjords (Pagarete *et al.*, 2013; Storesund *et al.*, 2015), bioprospecting in Norwegian coastal waters (Dang *et al.*, 2016; De Santi, Altermark, *et al.*, 2016; De Santi, Willassen, *et al.*, 2016), or prokaryotic community structures near Svalbard (Wilson *et al.*, 2017). As well, the Alfred Wegner institute performs multidisciplinary studies at the Arctic Long-Term Ecological Research Hausgarten site in the Fram Strait (Soltwedel *et al.*, 2005). Other studies in the Nordic Seas have focused on benthic and coral-associated microbiomes (Storesund and Øvreås, 2013; Jensen *et al.*, 2015). Noteworthy is the genome sequencing of *Lokiarchaeota* from sediments near LCVF (Spang *et al.*, 2015). Also, the Center for Geobiology has been studying various hydrothermal microbial

mats and ecosystems along the AMOR (Dahle *et al.*, 2013; Stokke *et al.*, 2015; Steen *et al.*, 2016). The open waters of the Nordic Seas have only been little sampled for prokaryotic studies before **Paper 3**. In two samples taken north of Jan Mayen at 50 m and 2000 m depth, Zaballos and his colleagues reported the influence of depth on community structure, and the presence of ubiquitous prokaryotes, such as *Thaumarchaeota*, SAR11, and SAR324 (2006).
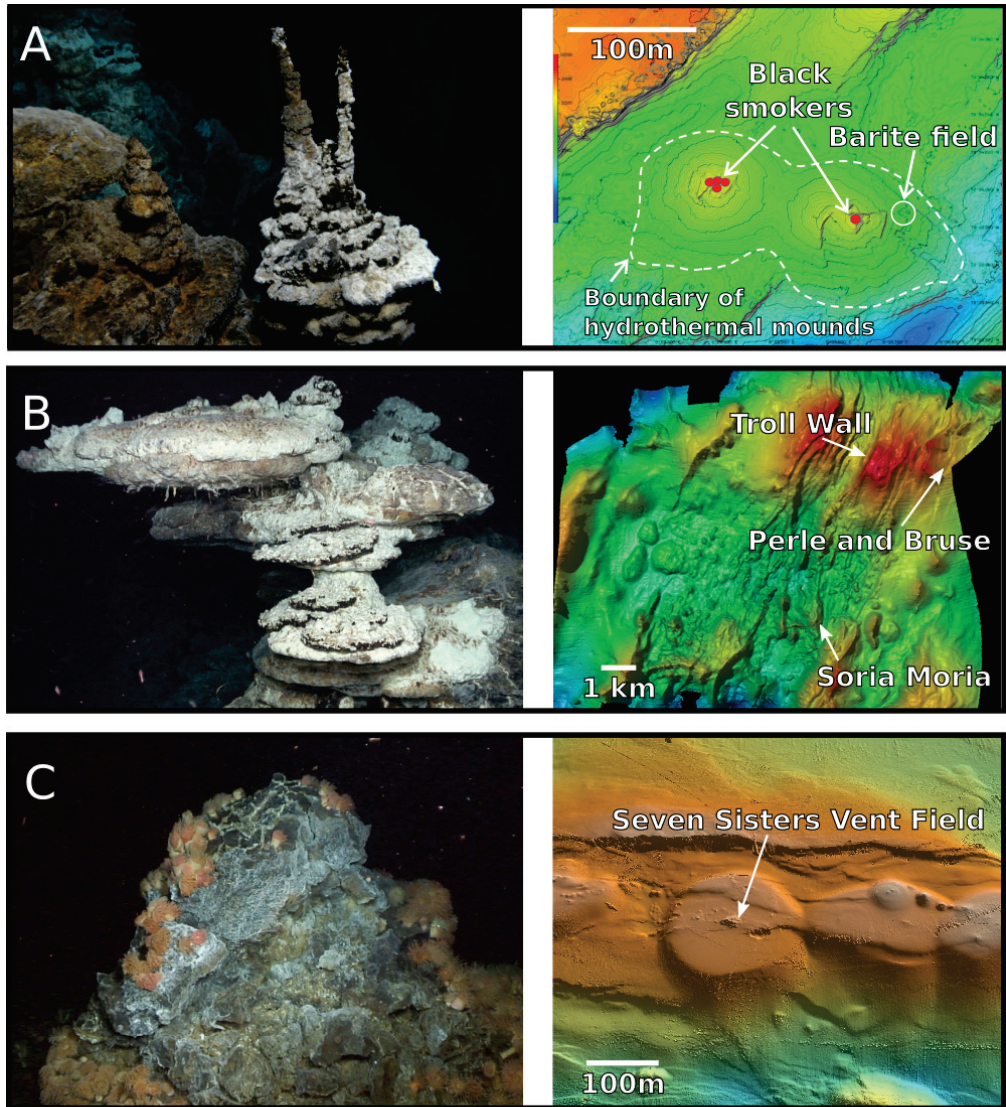
Figure 4: Photographies and topography of the hydrothermal vent fields described in this thesis. (A) The Loki's Castle Vent Field (Steen *et al.*, 2016). (B) The Jan Mayen Vent Fields (Pedersen *et al*, 2010). (C) The Seven Sisters Vent Field. Diffuse venting happens throughout a big part of the flat-toped volcano (Marques *et al.*, in prep.).

AIMS OF THE STUDY

Although hydrothermal systems along the AMOR have been studied extensively over the last years, a number of fundamental questions remain: What factors determine variations in microbial communities between and within hydrothermal systems? How important is the impact of hydrothermal plumes on microbial communities in the water column compared to other factors? The following sub-goals were set:

(i) Investigate the role of *Bacteroidetes* as organotrophic consumers in hydrothermal systems and identify possible adaptations of prokaryotes for life in this extreme environment.

(ii) Explore the connections between geological setting, chemical energy landscapes and microbial communities in hydrothermal systems on the AMOR, including both seafloor deposits and hydrothermal plumes.

(iii) Identify the most important environmental factors explaining beta-diversity patterns in the Nordic Seas, regarding both prokaryotic cells and viruses.

DESCRIPTION OF THE STUDY

The aims described for this study entangle the investigation of various aspects of the microbial communities in the Nordic Seas: hydrothermal environment adaptation, community structuring influence of chemical landscapes in hydrothermal chimneys, and community structuring factors in the dark pelagic ocean. Therefore, the publications presented here are very different, by the problem they address, the methods used, and the spatial scale of the survey. In this section, I introduce each of the publications in relation to ongoing projects from the Center for Geobiology.

Paper 1

**Paper 1** is part of a series of publications aiming for a deep ecological understanding of a microbial mat. The mat was first sampled in 2009 on the 'João' black smoker chimney at LCVF (Figure 5A). Transcriptomic analysis of the mat showed the dominance of *Epsilonproteobacteria* belonging to the *Sulfurovum* genus (Dahle *et al.*, 2013). The same paper gave evidences that hydrogen and sulfur species oxidation, aerobic respiration and denitrification took place in the mat. Imaging techniques revealed that *Sulfurovum* cells grew as long sheathed filaments on which *Bacteroidetes* cells grew as ectobionts (Figure 5B, Stokke *et al.*, 2015). The paper also described the sheath as a complex polysaccharide and proposed a first representation of the interactions between *Sulfurovum* and *Bacteroidetes*. Attempts to isolate microorganisms from the mat led to the isolation of *Lutibacter profundi* LP1[T]. Its characterization (**Paper 1**) and further genome analysis (Wissuwa *et al.*, 2017) aimed at unraveling the physiology adaptation of the species to hydrothermal unstable conditions, as well as increasing our knowledge on the role of *Bacteroidetes* within the microbial mat community.
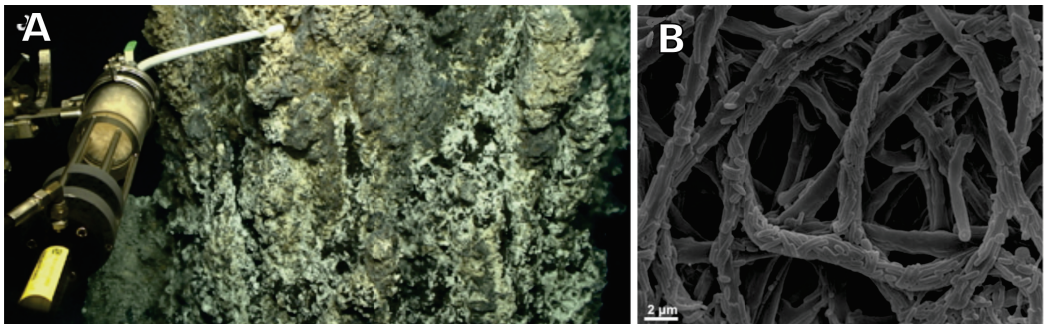
Figure 5: The biofilm from the "João" chimney. (A) Sampling in 2009 (Dahle *et al.*, 2015). (B) Electron microscopy of the *Epsilonproteobacteria* filaments with *Bacteroidetes* cells on top (Stokke *et al*. 2015).

Paper 2

Chemical energy availability is a major factor in shaping autotrophic prokaryotic communities in deep-sea hydrothermal systems. From one chimney to another, differences in fluid chemistry lead to variations in the energy available for autotrophic growth (McCollom, 2000; Amend *et al.*, 2011; LaRowe *et al.*, 2014). As a result, prokaryotic communities are expected to differ between vent fields. However, direct comparisons between modeled and observed communities have been performed only recently (Dahle *et al.*, 2015; Lin *et al.*, 2016). For the first time, **Paper 2** compared energy availability models with prokaryotic community changes throughout the cross section of two hydrothermal chimney walls taken at the Perle and Bruse Vent Field and the Soria Moria Vent Field (Figure 6). The paper aimed at describing the different prokaryotic functional groups present throughout the chimney walls, as well as testing the efficiency of the models to predict the structure of prokaryotic communities.
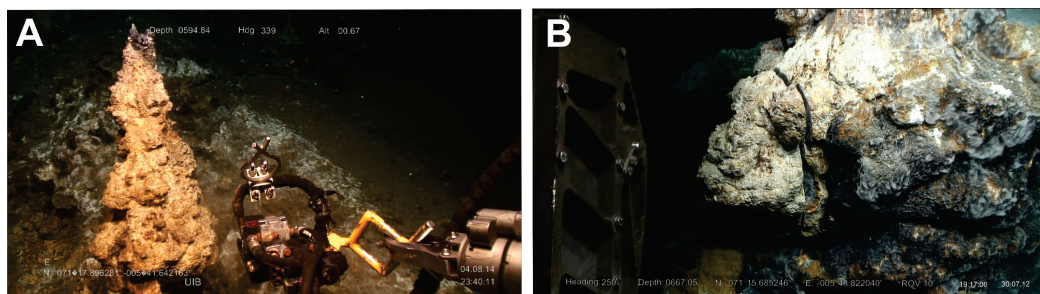
Figure 6: The hydrothermal chimneys sampled for **Paper 2**. (A) At the Bruse Vent Field. (B) At the Soria Moria Vent Field. Photographies from the Center for Geobiology, University of Bergen.

Paper 3

The Nordic Seas are oceanographically complex with many interacting water masses and a slow-spreading mid-oceanic ridge harboring hydrothermal activity (Figure 3). The importance of water masses for microbial dispersion has been shown (e.g. Hernando-Morales *et al.*, 2016; Djurhuus *et al.*, 2017), while the influence of hydrothermal plumes on microbial community structures remain unclear (reviewed in Dick *et al.*, 2013). In the Nordic Seas open-waters, little is known about how these factors influence the prokaryotic and viral communities, as only two samples have ever been taken for prokaryotic analysis in these waters (Zaballos *et al.*, 2006). **Paper 3** aimed at describing the prokaryotic and viral communities present in the Nordic Seas, as well as understanding the underlying factors structuring these communities.

DISCUSSION

Results and discussions of my work are already presented in the respective publications and manuscripts. Nonetheless, I wanted here to briefly discuss two aspects of my research that were only partially discussed or absent from the publications.

1   Why different conclusions on plume microbial communities?

Studies of hydrothermal plumes around the world have reached different conclusions regarding the presence of a specific plume community in contrast to background waters (See discussion and references in **Paper 3**). Surprisingly, reasons for these contrasting observations have rarely been discussed. Here, I continue the discussion of **Paper 3**, with a focus on the reasons for the different observations.

One can hypothesize about the presence of endemic communities inhabiting the plumes, i.e. unique to the plume. On one hand, the idea is supported by the ecological niche that is different from the surrounding ones: More and different electron donors than in the background water column and no mineral or biological supporting structures (for example hydrothermal chimneys or macrofauna) compared to hydrothermal sea floor. On the other hand, the 10 000 fold dilution of the fluids in seawater (Lupton *et al.*, 1985), the continuous movement of the plume and the change of energy landscape on short time/distance scale (Kadko *et al.*, 1990; Lilley *et al.*, 1995) stand against the development of a stable endemic community. Microbial cells are however known to fall from the non-buoyant plume (Sylvan *et al.*, 2012), and the community could therefore be recirculated from old to young plume through convection movements (Dick *et al.*, 2013; Sheik *et al.*, 2015). Nevertheless, clones endemic to plumes are yet to be detected.

Consequently, differences between plume and background pelagic microbial communities are expected to result from the inflow into the plume of microorganisms from hydrothermal chimneys, hydrothermal deposits, or macrofauna. Dick and his colleagues proposed five factors likely to influence the pelagic *vs.* benthic balance of microorganisms within the plume (Dick *et al.*, 2013): the fluid flux of the plume, the likeliness of the near vent environment to be entrained, the bathymetry of the surroundings, the energy potential of the plume for microbial growth, and the ability of the pelagic microbial community to use the electron donors present in the plume. I would suggest that the influence of water movements, with strong currents likely to homogenize the plume and background water, should be added to the list. Different plumes can exhibit radically different characteristics, explaining different observations. As shown in **Paper 3**, most of these factors are very difficult to quantify, and the situation in plumes can result from several opposing factors.

The plume community is also changing on a spatial scale, with the influence of the benthic community being stronger in the very young plume (Sheik *et al.*, 2015). However, recent plume research on the Gakkel Ridge in the Arctic Ocean showed that prokaryotic communities in the early buoyant plume were similar to background waters whereas the late non-buoyant plume hosted specific prokaryotic communities (Gunter Wegener, pers. com.). The problem here is that most of the studies use a binary categorization of the samples ("plume" *vs.* "background"), omitting to estimate the age of the plume (e.g. **Paper 3**; Lesniewski *et al.*, 2012; Anderson *et al.*, 2013; Anantharaman *et al.*, 2016; Li *et al.*, 2016). Temporal variability is also likely, as a stronger inflow of benthic microorganisms can be expected with eruptions or periodic high hydrothermal activity. These spatial and temporal variations may lead to different results between studies on the same plume.

Another possibly important reason for contradictory results is the non-consistency of the approaches used by different research groups. First, the markers used to localize the plume are not always the same (e.g. CH_4 concentration, turbidity, redox potential, temperature or pH anomalies). Recent research shows that the plume identified by gases may be different from the plume identified by metals (Stensland *et al.*, submitted). Therefore, the use of different plume markers between studies can lead to inconsistencies in the sampling. Furthermore, both sampling methods (e.g. Niskin bottles, remotely operated vehicle sampling, sediment traps) and analysis strategy (e.g. clone libraries or deep sequencing of various marker genes) vary between studies. These methodological differences, added to the difficulty of sampling the buoyant plume and the low amount of samples in some studies are likely to add to the discrepancy of the results published.

In **Paper 3** we also demonstrate how different water masses, even adjacent to each other, can host different microbial communities. Therefore, another potential source for different results could be the choice of the "background" referential. Some studies take background samples far away from the plume (e.g. Lesniewski *et al.*, 2012), or above the plume (e.g. Sheik *et al.*, 2015). These samples can belong to other water masses and therefore host different microbial communities. This stresses the importance of choosing background samples carefully, as close as possible to the plume, and at the same depth as the samples taken within the plume.

In conclusion, observed differences in plume microbial communities can be real or linked to methodology issues. Very rigorous sampling of all sections of the plume, as well as background waters from several depths near the plume and further away are needed for an accurate comparison of the microbial community structures between plume and background water.

2   Multivariate analysis and oceanographic knowledge in marine microbial ecology.

In **Paper 3**, we have shown that microbial community structures can vary between water masses. Oppositely, water masses may host similar microbial communities over great distances (Agogué *et al.*, 2011). A logical question is therefore: why are different water masses hosting different communities? A possible explanation is the variability in physicochemical characteristics of seawater. Multivariate analysis is a powerful tool in order to test for correlations between changes in such variables. In **Paper 3**, forward constrained analyses showed the importance of depth in structuring microbial communities, while refuting the importance of hydrothermal plume chemistry. However, conclusions based on the sole multivariate analyses would have omitted the main structuring factor. Only by associating these analyzes with oceanographic knowledge did we realize the importance of considering water masses in order to understand changes in microbial community structures. In the literature, simple regression or multivariate analyzes are often used as sole analysis to find correlations between OTU distributions and physicochemical variables. Sometimes, the ecological meaning of these correlations is difficult to understand. Here, I defend my opinion that simple regression methods and multivariate analyzes should be more carefully used and that implementing knowledge about the studied environment, in this case oceanographic knowledge, to microbial community studies would lead to a better interpretation of the results.

A first problem is that there exists a multitude of statistical approaches that are suited to different types of datasets and analyzes. For microbiologists, it is easy to get confused, and only a thorough review of the literature or the help of a statistician is needed in order to properly understand the analysis and avoid mistakes.

A second problem concerns the choice of the independent variables tested to explain OTU distributions. Specifically, it is important to question if it has an ecological meaning to test a variable. For example, in **Paper 3**, salinity and temperature variations were estimated to be too low to influence the community (less than 0.1 psu, and ca. 1 °C, respectively), and removed from the constrained analysis. Similar evaluations are likely not always taken into account in the literature. For example, in a recent study of a meridional Atlantic transect, Milici and his colleagues investigated the factors influencing bacterial diversity and evenness, bacterial cell abundances, and primary production in the surface waters (Milici *et al.*, 2016). Among others, they investigated correlations with salinity, which varies only of ca. 3 psu over most of the several thousand kilometer long transect. Most of the marine species isolated and characterized in laboratories are known to grow very well with salinity variations at least one order of magnitude higher (e.g. **Paper 1**; Shivaji

and Reddy, 2014; Dar Jean *et al.*, 2016; Kouzui *et al.*, 2016), and therefore the small variations observed are likely to have little ecological influence on microbial communities.

This leads to a third problem, the interpretation of observed correlations. The results of such analyzes are often given *per se*, without questioning "Beyond the correlation, why is it like that?". Complementing the analysis with oceanographic knowledge helps seeing a broader picture, detect confounding variables not analyzed in the regression analysis, and therefore better interpret the results. Milici and his colleagues did not take into account the distributions of water masses in their study (Milici *et al.*, 2016). Using the temperature and salinity data they provided, it is possible to calculate water density and visualize the different water masses present along their transect. A low-density surface water mass, possibly the Tropical Surface Water (Stramma and England, 1999; Talley *et al.*, 2011), can be identified near the equator, and its localization overlaps with an area of lower bacterial cell abundance described in their study (personal analysis, data not shown). Further analyzes of their dataset including water mass distributions, bathymetry, and geographical position of the samples could have possibly explained why certain correlations were found between their variables.

Finally, the use of oceanographic knowledge allows to examine each water mass, and understand what are the historical reasons that led to the physicochemical characteristics and the microbial community in a specific place. For example, in **Paper 3**, the circulation of the water masses between the Arctic, Atlantic and Pacific Oceans allowed us to understand why we did not find *g23* sequences closely related to the ones retrieved by Filée *et al.* in the Pacific Ocean (Filée *et al.*, 2005). Also, knowledge about water mass distributions and microbial dispersal allows us to hypothesize which communities are to be expected at different places of the Nordic Seas, and better organize future sampling strategies to test these hypotheses. This is valuable information that is not given by multivariate analyzes and simple regression methods.

CONCLUSION AND FUTURE PERSPECTIVES

The work presented in this study gathers analyzes of the microbial communities of the Nordic seas at different focus levels. By its holistic approach, the study provides a better understanding of the strong influence of hydrothermal systems on microbial communities, but also shows the limit of this influence, as water masses seem to have a more powerful structuring effect on pelagic microbial communities.

The role of Bacteroidetes as organotrophic consumers in hydrothermal systems and possible adaptations of prokaryotes for life in this extreme environment

The characterization of *Lutibacter profundi* LP1^T in **Paper 1** has shown that the strain possesses several features resulting from the adaptations to life on hydrothermal chimneys. For example, the ability to produce exopolymers and aggregate to build biofilms are beneficial in the fluctuating physical and chemical conditions within hydrothermal systems. The ability to grow under various oxygen concentrations also allows *L. profundi* to adapt to changing fluid flows through the biofilm. The metabolic properties of *L. profundi* showed that the strain can degrade complex polymers, possibly confirming the hypothesis of an opportunistic growth on the *Epsilonproteobacteria* filaments (Stokke *et al.*, 2015). The genome analysis of *L. profundi* by our group confirmed these adaptations and hypothesized others, like the use of *s*ulfide-oxidation genes in prevention of sulfide poisoning from the fluids (Wissuwa *et al.*, 2017). However, analyzes of the genome of *L. profundi* coupled to growth experiments have also shown some contradictions (e.g. about gliding ability), emphasizing the importance of isolate characterization. Future work could aim at the isolation of the dominant *Epsilonproteobacteria* species in the microbial mat, in order to gain a better understanding of the ecology of the mat. The isolation of other prokaryotes from LCVF would also allow a comparative analysis of the adaptations to the environment at LCVF. An *Alphaproteobacteria* isolated from a microbial mat at the barite field shared some characteristics with *L. profundi* (e.g. tolerance to different oxygen concentrations and ability for denitrification; Le Moine Bauer *et al.*, in prep.), suggesting that similar adaptations to hydrothermal conditions could be found throughout the prokaryotic community at LCVF.

Connections between geological setting, chemical energy landscapes and microbial communities in hydrothermal systems on the AMOR, including both seafloor deposits and hydrothermal plumes

Paper 2 showed that the chemistry of the fluids is of major influence on prokaryotic community structures. Chimneys with different chemistry were shown to host different prokaryotic species and functional groups. As well, the modeled distribution of functional groups based on energy landscapes showed high similarities with field observations. Nevertheless, some discrepancies were also observed, suggesting the influence of other factors on the prokaryotic community structures. For example, some microorganisms possibly grow on substrates produced by other microorganisms, chemical compounds like H_2S can be poisonous to some species, and temperature may be inappropriate for the growth of some functional groups. In addition, factors controlling the distribution of organotrophs and heterotrophs in hydrothermal structures are largely unknown and need to be investigated. Finally, several OTUs identified in the samples could not be classified in any functional group, as they are only distantly related to any organism with known metabolic capabilities. Further research will aim at filling these knowledge gaps to improve the modeling of prokaryotic community structures. To do so, shotgun metagenomic sequencing of the different parts of the chimneys would help understanding the putative functions of the dominant microorganisms. This knowledge could in turn also be used to ease the isolation of these microorganisms in laboratories. Species characterization, will be of major importance for the understanding of the prokaryotic communities within chimney walls. Specifically, growth experiments with different energy sources, carbon substrates, temperatures, and concentrations of potential inhibiting compounds (heavy metals, sulfide) will be very valuable.

Chemical landscapes had no visible influence on plume microbial communities, fueling the debate regarding the existence of specific plume communities. Future work on plume microbial communities in the Nordic Seas should propose a more systematic sampling of the plumes and background samples than presented in **Paper 3**. This includes considering the influence of depth on microbial communities and the distribution of the different water masses in the area. Furthermore, methods for estimating the age of the plume should be implemented in the study.

Important environmental factors explaining beta-diversity patterns in the Nordic Seas, regarding both prokaryotic cells and viruses

Paper 3 highlighted that the strong influence of chemistry on prokaryotic communities found in chimney walls in **Paper 2** could not be detected in the water column. Specifically, plume chemistry had little influence on the abundance, biodiversity and distribution of OTUs of prokaryotes and T4-like myoviruses. Instead, we found that in the Nordic Seas the circulation of the different water masses plays an important role in the distribution of prokaryote and T4-like myovirus OTUs. However, this analysis only gave primary insights into the microbial communities of the Nordic Seas. With a proper understanding of water mass distribution, future studies using similar methods could choose to sample different locations to (i) understand the reasons for different microbial communities in different water masses, (ii) analyze other water masses like the Atlantic Surface Water and the Polar Surface Water, (iii) analyze the uniformity of microbial communities in a specific water mass, and (iv) investigate the change of communities at water mass boundaries. Specifically to (iv), the Arctic Mid-Ocean Ridge is a complex area where Arctic and Atlantic Surface Waters mix. The study of communities over the ridge could potentially help understanding the mixing processes.

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