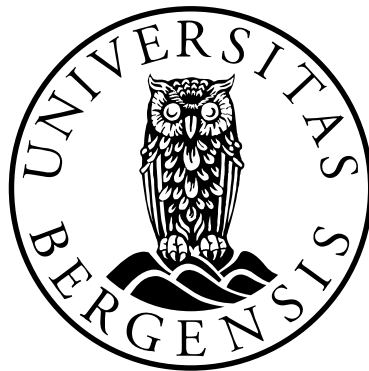# Bacterial community structures in the Arctic Ocean: the effect of increased carbon load on nutrient competition and bacterial diversity

Birte Töpper

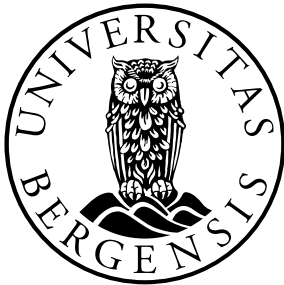Dissertation for the degree philosophiae doctor (PhD)

at the University of Bergen

2012

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Scientific environment

Acknowledgments

First of all, I would like to thank my three supervisors Ruth-Anne Sandaa, Frede Thingstad and Aud Larsen. It was nice to know that I always could come to you with questions and I appreciate that you read the first draft of my first paper…☺

Ruth-Anne, you helped me a lot with problems in the lab and with writing during the last years. You were always positive and god-humoured so it was easy for me to come to you in case I needed help or advice. Especially in the end of my PhD, your support was of particular importance for me. Thank you!

I want to thank the Marine Microbiology group at UiB, which I enjoyed to be a part of. I met the major part of the group for the first time at Bergen airport on the way to Svalbard, where I had my first working day as PhD student. I felt very welcome in the group during this very intensive time in Ny Ålesund. Thanks for the opportunity to let me experience this wonderful place. But of course, I also want to thank all past and present members / visitors of the group who I met later on in Bergen. In particular, I want to thank Evy and Hilde for help in the (very well organised) lab and with paper work, Jorun for taking care of me when I arrived in Bergen, and all the students, post docs, visitors etc. with whom I spend so many nice lunch breaks and free time together.

Of course, I also would like to thank all the other nice people I met at work, for helping me to solve problems, discussions or for just spending time together at the Jule- or Påskefrokost.

Thanks to my "active" colleagues Antonio C., Jessica, Paolo and Selina for all the nice running trips along Fjellveien, Stolzen runs and hiking trips on Bergen's mountains. I really enjoyed this a lot! Paolo, thanks for being a great office mate for almost the whole time of my PhD; Jessica, thanks for helping me **always** with **everything**…you were a great support to me during the last years! Selina, thank you

Abstract

The Arctic Ocean is a unique marine environment with respect to irradiation periodicity and intensity, temperature, ice formation and strong stratification. Changes due to global warming might be more pronounced in this system compared to other oceans, as the melting of both sea ice and permafrost will accompany high nutrient input to the Arctic Ocean simultaneous with increasing light penetration of the water column. Microbial communities play an important role in carbon cycling in the ocean, as they constitute both the main primary producers (phytoplankton) and the main consumers of dissolved organic carbon (heterotrophic bacteria) which make dissolved organic carbon available for higher trophic levels. Increasing atmospheric carbon dioxide (CO_2) might also have a positive effect on the production of dissolved organic carbon in the oceans. This may in particular impact heterotrophic bacteria and, thereby, the trophic state of the ocean. In this thesis, *in situ* investigations of the microbial community and the trophic state of a representative Arctic marine system are presented (**Paper I**) as well as nutrient manipulation experiments performed in mesocosms in the Arctic (**Papers II, III** and **IV**) and in the laboratory (**Paper V**). In the *in situ* study performed in Fram Strait (**Paper I**), the microbial community demonstrated net-autotrophy although the microbial biomass was dominated by heterotrophs. In three nutrient manipulation experiments we studied the effects of (i) increased organic carbon load on mineral nutrient competition between bacteria and phytoplankton (**Paper II**), (ii) increased organic carbon load on the bacterial community structure (**Paper III**), (iii) increased partial pressure of CO_2 ($p$$CO_2$) on

bacterial community shifts in response to increasing organic carbon load (**Paper IV**) and (iv) carbon complexity and viral lysis on the bacterial community structure and diversity (**Paper V**). All experiments showed that the bacterial community was affected by increasing nutrient loads. During the mesocosm experiment in Kongsfjorden, Svalbard, a shift from net-autotrophy to net-heterotrophy was detected with increasing glucose addition, which was interpreted as a consequence of stimulated bacterial competition for mineral nutrients (**Paper II**). In the same experiment, pronounced changes in the bacterial community composition due to glucose addition were seen (**Paper III**). Furthermore, investigations of bacterial community responses to glucose addition under conditions intended to simulate ocean acidification showed a significant interaction between glucose and seawater acidification in the Fram Strait (**Paper IV**). Finally, natural bacterial communities incubated in the laboratory in the presence of a single carbon compound (glucose) demonstrated lower diversity and richness compared to those incubated with complex algal-derived carbon compounds (provided by *Thalassiosira sp.* and *Phaeocystis pouchetii* respectively) (**Paper V**). These observations are likely the consequence of co-acting bottom-up (carbon composition) and top-down (host-virus-interactions) regulatory mechanisms acting upon the bacterial communities under investigation.

List of publications

Paper I

Seuthe L., **Töpper B.**, Reigstad M., Thyrhaug R., Vaquer-Sunyer R. (2011) *Microbial communities and processes in ice-covered Arctic waters of the northwestern Fram Strait (75 to 80° N) during the vernal pre-bloom phase.* Aquatic Microbial Ecology 64: 253-266, doi: 10. 3354/ame01525

Paper II

Thingstad T.F., Bellerby R.G.J., Bratbak G., Borsheim K.Y., Egge J.K., Heldal M., Larsen A., Neill C., Nejstgaard J., Norland S., Sandaa R.A., Skjoldal E.F., Tanaka T., Thyrhaug R., **Töpper B.** (2008) *Counterintuitive carbon-to-nutrient coupling in an Arctic pelagic ecosystem.* Nature 455: 387-391, doi: 10.1038/nature07235

Paper III

Töpper B., Larsen A., Thingstad T.F., Thyrhaug R., Sandaa R.-A. (2010) *Bacterial community composition in an Arctic phytoplankton mesocosm bloom: the impact of silicate and glucose.* Polar Biology 33: 1557-1565, doi: 10.1007/s00300-010-0846-4

Paper IV

Ray J.L., **Töpper B.**, Shu A., Silyakova A., Spindelböck J., Thyrhaug R., Dubow M.S., Thingstad T.F., Sandaa R.-A. *Pyrosequencing reveals effect of increased pCO2 on bacterial community shifts in response to glucose addition in Fram Strait seawater mesocosms.* Manuscript submitted to FEMS Microbiology Ecology

Paper V

Töpper B., Thingstad T.F., Sandaa R.A. *Effects of differences in organic supply on bacterial diversity subject to viral lysis.* Manuscript submitted to FEMS Microbiology Ecology

List of abbreviations

DGGE   Denaturing gradient gel electrophoresis

DNA   Deoxyribonucleic acid

DOC   Dissolved organic carbon

OTU   Operational taxonomic unit

PCR   Polymerase chain reaction

POC   Particulate organic carbon

RNA   Ribonucleic acid

rDNA   Ribosomal DNA

rRNA   Ribosomal RNA

TOC   Total organic carbon

1. Introduction

Due to increasing fossil fuel consumption, atmospheric CO_2 levels have increased dramatically over the last few decades and are estimated to continue to rise by about a factor of two relative to the present value of 380 µatm by the year 2100 (IPCC's "business as usual scenario" IS92a). Atmospheric CO_2 is in equilibrium with the CO_2 in surface seawater (Hedges 2002), thus the increase in atmospheric CO_2 might lead to higher carbon incorporation by photosynthesis and subsequently increasing levels of dissolved organic carbon (DOC) in the oceans (Riebesell et al. 2007). Global warming will accelerate melting of sea ice and permafrost, and may by this cause a general increase in nutrient input to the upper layer of the Arctic Ocean, due to increase of DOC input from rivers, reduced vertical transport of inorganic nutrients (because of increased vertical stability) and changes in light availability caused by reduced ice cover (Vadstein 2011).

Depending on the dominance of either organic matter production by photosynthesis or consumption by heterotrophic bacteria, the ocean is reported to be either net-autotrophic (**Paper I**) or net-heterotrophic (delGiorgio et al. 1997). In a future world with presumably greater carbon availability, there is a need to identify and understand the factors that might affect this balance. More knowledge is also needed about processes that regulate DOC accumulation in the upper layer of the ocean and its function in the global carbon cycle. Heterotrophic bacteria are among the most important players in the microbial food web, as they utilise DOC and make it

available for higher trophic levels (fig. 1). Changes in bacterial community structures due to an enhanced organic carbon load (Øvreås et al. 2003, **Papers III**, **IV** and **V**) might affect the base of the marine food web and are thus important to study with regard to carbon fluxes in marine systems. Climate change may have its fastest impact in the Arctic (Manabe & Stouffer 1994, Stroeve et al. 2007), making investigations of the Arctic Ocean habitat even more meaningful with respect to enhancing our knowledge of the microbial system and its response to a changing carbon load.
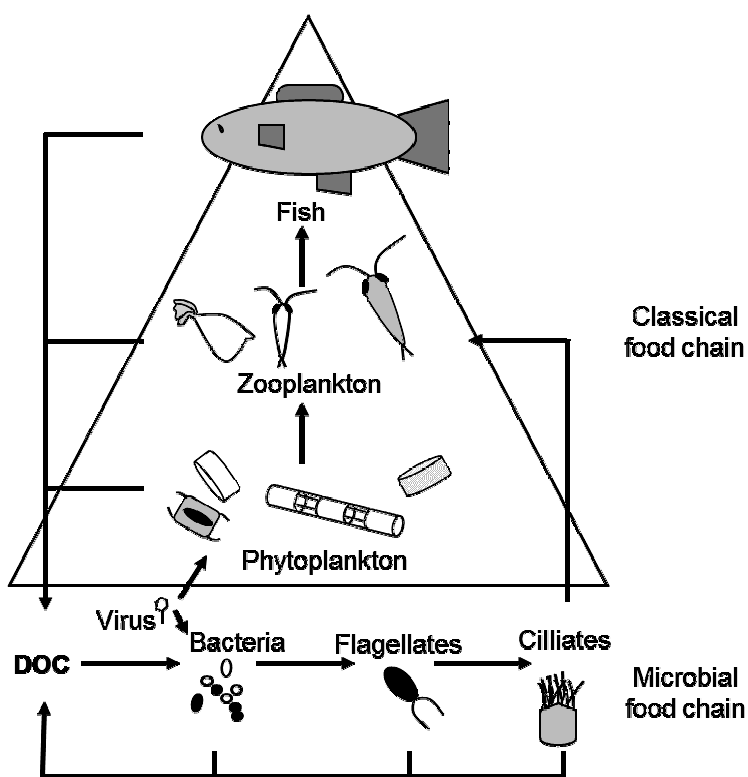
Fig. 1 Schematic diagram of the marine food web modified from Larsson et al. (2000).

Scope of this study

The aim of this study was to investigate the effects of higher nutrient load, as predicted for the near future, on the structure of the Arctic bacterial community. Mesocosm experiments in the fjords of Svalbard (Kongsfjord) and in Fram Strait (**Papers II**, **III** and **IV**), as well as a laboratory experiment (**Paper V**), were performed in order to study nutrient competition and diversification within the bacterial community in response to increased nutrient load. Bacterial community dynamics were investigated using denaturing gradient gel electrophoresis (DGGE) of 16S ribosomal RNA gene fragments (rDNA) (**Papers III** and **V**), while phylogeny/taxonomy and diversity of the bacterial communities were investigated by cloning and sequencing (**Paper V**) as well as pyrosequencing of 16S rDNA fragments (**Paper IV**).

The Arctic Ocean habitat was investigated under *in situ* conditions to describe the microbial community and present-day nutrient conditions (**Paper I**). A broad spectrum of abiotic and biotic factors were analysed during a research cruise on the Norwegian Coastguard ship "KV Svalbard" to the East Greenland shelf located in Fram Strait. Nutrient conditions as well as the abundance of heterotrophic and autotrophic microorganisms and their processes were analysed in order to determine the trophic state of the system.

In addition to organic carbon compounds, mineral nutrients such as nitrate and phosphorus are also essential for bacterial growth. Limited availability of mineral nutrients to bacteria might therefore lead to carbon accumulation. Competition for

mineral nutrients between bacteria and phytoplankton is an important process that determines the availability of mineral nutrients for bacteria in the oceans and is thus of essential importance to the carbon cycle. **Paper II** presents a nutrient competition mesocosm experiment in Kongsfjorden, Svalbard. The results show how microbial communities in the Arctic cope with carbon- and mineral nutrient- limited situations, and the implied consequences for the trophic state of the system. Furthermore, the results from this experiment clearly demonstrate shifts in the bacterial community composition in response to variation in nutrient conditions (**Paper III**).

Increasing organic carbon concentrations in the oceans are expected to affect bacterial community structures and diversity. An elevated oceanic CO_2 level, and resultant ocean acidification, predicted for the near future might influence the response of heterotrophic bacteria to increased organic carbon load. Some bacteria may exhibit greater sensitivity to acidification and changing carbon load than others, ultimately leading to shifts in bacterial diversity. The response of bacterial communities to an increasing supply of organic carbon under ambient and artificially acidified conditions was investigated during an on-board mesocosm experiment using water from Fram Strait (**Paper IV**).

In addition to substrate composition, lysis by viruses is also assumed to be a major factor determining bacterial community size and compositions. While substrate composition is thought to mainly regulate the community composition of the bacteria, viral lysis is believed to control richness and evenness (diversity) of the bacterial community. In a predator free laboratory experiment where the bacterial community

was virus controlled, two complex algal carbon pools and one simple carbon compound (glucose) were provided as carbon sources in order to investigate changes in bacterial diversity in response to the complexity of the carbon pool. The combined effect of carbon complexity and viral lysis on bacterial diversity is discussed in **Paper V**.

2. Microbial ecology in the Arctic

Microbial ecology describes the relationship of microorganisms to each other and to their environment. Both, abiotic (e.g. temperature, nutrients, light) and biotic factors (e.g. the structure of the microbial food web and interactions within this food web) play important roles for determining the bacterial community, as the interaction of these factors create different niches that are filled by different bacterial species.

2.1 Description of the Arctic Ocean habitat for microbial communities

The Arctic Ocean differs in many aspects from other marine environments, particularly with regard to low temperatures and extreme fluctuations in irradiance due to ice coverage and strong seasonality. The winter season in the Arctic is characterised by little or no sun light, of which only a fraction is able to penetrate the thick sea ice and snow layers to the water column below. This mainly affects photosynthetic organisms like phytoplankton, which require sunlight for carbon fixation. Most pronounced changes for the marine environment occur during the spring, when melting sea ice and rapidly increasing day length allow greater penetration of light to the water column, which together with strong stratification, are followed by increased photosynthetic activity (Sakshaug 2004, **Paper I)**. During this time, the pelagic microbial community starts to adapt to both increasing nutrient concentrations (Yager et al. 2001) and prey numbers (Seuthe et al. 2007).

This PhD work was performed in an area west of Svalbard, including coastal areas (Kongsfjorden, **Papers II** and **III**) as well as Fram Strait (**Papers I** and **IV**), which is situated between Greenland and Svalbard and connects the Arctic Ocean to the North Atlantic (fig.2).
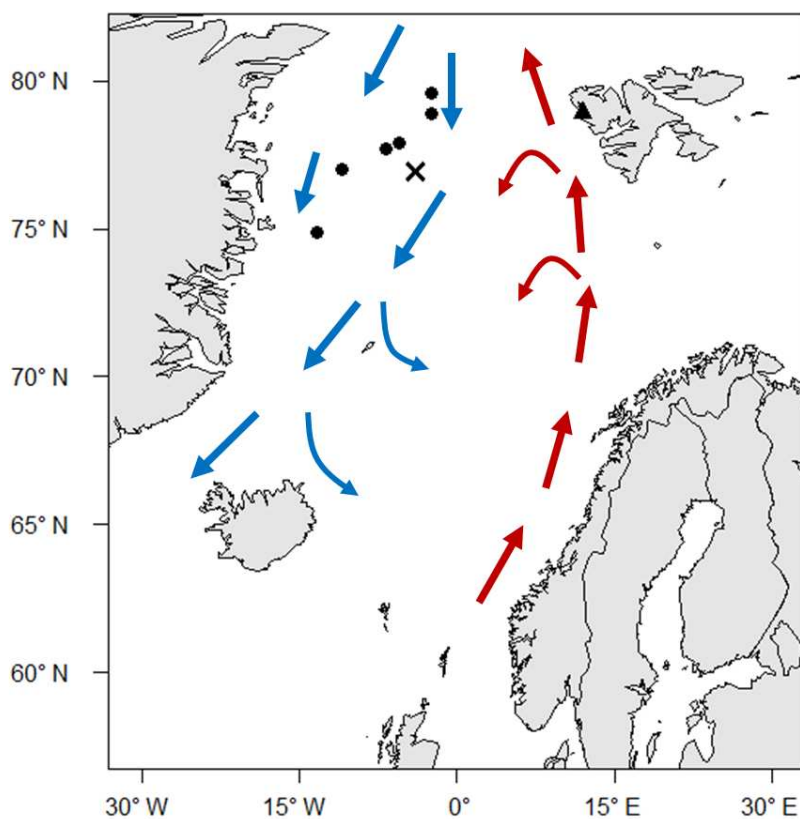
Fig. 2 Current system in Fram Strait with north-flowing warm Atlantic current (Atlantic water) and south-flowing cold East Greenlandic current (Arctic water). The different positions where the experiments were performed are marked on the map: ▲Kongsfjorden (**Papers II** and **III**), • Fram Strait (**Paper I**), ✖ Fram Strait (**Paper IV**).

The Fram Strait is characterised by two different water masses: warm (> 0°C) saline (> 34 ppm) Atlantic water in the eastern part of Fram Strait streaming northward along the Svalbard archipelago, and cold (< 0°C) fresher arctic water (< 34.7 ppm) streaming southward along the East coast of Greenland (Schlichtholz & Houssais 2002). The surface water layer was of arctic nature in the western Fram Strait (**Papers I** and **IV**) while water collected in Kongsfjorden (**Papers II** and **Paper III**) originated from Atlantic water.

Low temperatures in the Arctic might affect some members of the microbial food web more than others, which might also have an effect on the competition for nutrients. Bacteria have been suggested to be more sensitive to low temperatures than members of the phytoplankton community (Pomeroy & Deibel 1986), thus, the low water temperatures in the Arctic Ocean might disadvantage bacteria more than phytoplankton with respect to nutrient competition. However, heterotrophic bacteria living in permanently cold water were reported to achieve activity rates during the summer period that were comparable to rates observed for bacteria in temperate waters. Bacteria present in Arctic water should therefore not exhibit exceptional sensitivity to temperature when compared to bacteria present in warmer regions (Pomeroy & Wiebe 2001, Kirchman et al. 2005). In addition, increasing requirement for organic nutrients by heterotrophic bacteria at temperatures around 0ºC on one hand (Wiebe et al. 1992), and reduced utilisation of organic nutrients on the other hand (Pomeroy & Wiebe 2001), represent the contradictory results concerning temperature effect. An easy stimulation of bacterial growth by additional nutrients in

the experiments in the Fram Strait (**Paper IV**) and in Kongsfjorden (**Papers II** and **III**) suggests temperature to be of minor importance in our experiments. In addition, the bacterial community in Kongsfjorden managed to outcompete the phytoplankton for mineral nutrients (**Paper II**) which indicates that bacteria are not more affected by temperature than phytoplankton. Based on these data, we conclude that availability of nutrients and the structure of the microbial food web, are more important for bacterial growth than temperature in Arctic environments.

2.2 The trophic state of the marine microbial system

During photosynthesis, CO_2 is converted to organic carbon using energy obtained from sunlight. About 50 % of global primary production occurs in the oceans (Field et al. 1998), and half of the primary production in the oceans passes into the pool of dissolved organic carbon (DOC) (Williams 2000). In the ice covered waters of the Barents Sea, however, between 18 and 55 % of primary produced carbon was reported to pass into the DOC pool (Vernet et al. 1998). Also other processes like viral lysis, sloppy feeding and excretion of waste products by aquatic organisms are producing DOC and supply heterotrophic bacteria with fresh DOC (Nagata et al. 2000) as a source of carbon and energy (Ducklow 2000). Some studies have shown that a large proportion of DOC in marine systems is of bacterial origin (Ogawa et al. 2001, Lonborg et al. 2009) and it has even been reported that heterotrophic bacteria contribute more to the DOC pool than phytoplankton (Vadstein et al. 2012).

While heterotrophic bacteria and phytoplankton utilise different carbon sources for energy acquisition and growth, both use mineral nutrients (phosphorus and nitrogen) from the same pool (fig. 3). Phosphorus and nitrogen sources occur in subnanomolar to micromolar concentrations (**Paper I**) in the Arctic Ocean, and limitation of these nutrients seems to be a regular phenomenon in planktonic systems (Vadstein 2011). Thus, bacteria and phytoplankton need to compete for these resources (Thingstad et al. 1998, Vadstein 2000) which seem to play an important role in structuring the microbial community (Havskum et al. 2003, **Paper II**).
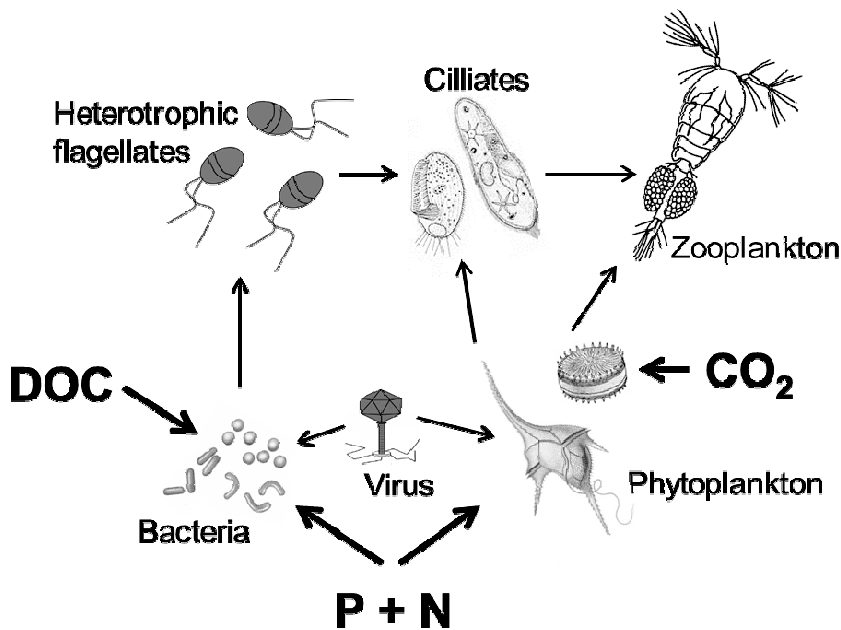
Fig. 3   Schematic diagram of the microbial part of the marine food web with emphasis on competition for essential nutrients. Both, bacteria and phytoplankton compete for the same mineral nutrient pool (phosphorus [P] and nitrogen [N]), while they used different carbon sources (dissolved organic carbon [DOC] and CO_2) for growth. Virus are capable of lysing bacteria and phytoplankton cells, and through lysis release nutrients back to the dissolved nutrient pools.

The predicted expansion of the DOC pool due to elevated atmospheric CO_2 plays an important role for the nutrient competition between heterotrophic bacteria and autotrophic phytoplankton, and as such may be one of the major factors regulating the net trophic state of the ocean. The trophic state of the ocean is determined by whether heterotrophic bacteria or autotrophic phytoplankton are better competitors for mineral nutrients. Bacteria with higher surface to volume ratios are expected to be better competitors in nutrient uptake than larger phytoplankton cells, however, the ability of bacteria to be successful competitors depends highly on the availability of DOC. In case of DOC limitation, which theoretically might be caused by the dominance of phagotrophs (Thingstad et al. 1997, **Paper II**), mineral nutrient uptake by heterotrophic bacteria is limited and thus, more mineral nutrients are available for the phytoplankton community. With increasing DOC addition to such a system, more mineral nutrients can be immobilised in bacterial biomass leading to a decrease in the phytoplankton community (**Paper II**). Due to increased uptake of mineral nutrients by bacteria, the system reaches mineral nutrient limitation and a further increase of DOC does not increase bacterial growth and organic carbon accumulates (**Paper II**). Thus, mineral nutrient availability and nutrient competition abilities of bacteria and phytoplankton are important factors for regulation of bacterial communities and accumulation of organic carbon in the system.

In the Arctic Ocean, nitrogen is considered to be the limiting factor for autotrophic growth, while phosphorus and silicate are assumed to be less limiting (Spies et al. 1988, Lara et al. 1994). Concentrations of nitrogen and phosphorus in Fram Strait

during early spring were lower (**Paper I**) than during the winter period (Sakshaug 2004). This, together with the dominance of phototrophic protists (**Paper I**), indicate that the system has started to change from nutrient rich (winter) to nutrient limited during the spring bloom. The N:P ratio of Arctic waters has been reported to range from 11-16 (Sakshaug 2004) and, as the species-dependent N:P ratio is of average 16, as described by the Redfield ratio of C:N:P = 106:16:1 (Redfield 1934), ratios smaller than 16, as in **Paper I**, are presumably nitrogen limited. Nitrogen limitation might be followed by DOC accumulation in summer, as it was shown for the East Greenland current (Daly et al. 1999). In marine systems, phosphorus limitation seems to be more common for heterotrophic bacteria than nitrogen limitation. This might be due to a stronger competition for phosphorus sources compared to nitrogen sources, as phosphorus is normally less biologically available than nitrogen (Vadstein et al. 2012). However, knowledge about factors that limit bacterial growth in the Arctic is still scarce (Thingstad et al. 2002).

In Fram Strait, the system in early spring was metabolically (net community production) driven by small phototrophs, and was thus net-autotrophic (**Paper I**). But regarding biomass and abundance, the system was net-heterotrophic when considering the bacteria as part of the heterotrophic community. This combination of high phototrophic turnover rates and dominance of heterotrophic biomass has also been reported for other open-ocean studies (Gasol et al. 1997), and seems to be caused by high abundance of fast-growing, small phytoplankton which dominated in samples from our study conducted in Fram Strait (**Paper I**). Increasing organic carbon load in

the form of glucose seems to promote the heterotrophic component of the microbial community, converting the system from net-autotrophy to net-heterotrophy in Kongsfjorden (**Paper II**) and in Fram Strait (pers. comm. Anna Silyakova, Bjerknes Centre for Climate Research, Norway), indicating an advantage for heterotrophic bacteria in mineral nutrient competition when DOC is in excess. Based on these observations, a future world, with possible higher organic carbon load, might lead to a dominance of heterotrophic processes in the ocean.

2.3 The role of silicate for nutrient competition

Diatoms frustules are composed of silicate, thus diatom growth and proliferation is dependent upon availability of dissolved silicate in seawater. By promoting diatoms by the addition of silicate (**Papers II** and **III**), more mineral nutrients would become immobilised in diatom biomass, which could result in reduced bacterial DOC consumption and subsequent DOC accumulation (Havskum et al. 2003). Havskum and colleagues (2003) reported that heterotrophic bacteria from Isefjorden (Denmark) managed to outcompete phytoplankton for mineral nutrients only when phytoplankton (diatoms) were not supported by addition of silicate. The presence of silicate led to immobilisation of mineral nutrients in phytoplankton biomass resulting in mineral nutrient limitation for bacteria. Contrary to that study, the promoting effect of silicate did not advantage the diatoms in Arctic waters (**Paper II**). Regardless of silicate addition, increased organic carbon addition to carbon-limited bacteria caused a decrease in gross primary production and chlorophyll a, most likely due to increased

mineral nutrient uptake by bacteria. Different environmental conditions in the Arctic Ocean might have affected the microbial community and their competitive ability for nutrients differently than in other oceans. Although the bacterial community managed to outcompete the phytoplankton regardless silicate addition, the presence of silicate still seemed to affect the bacterial community. This was demonstrated by a more stable bacterial community with fewer changes in the bacterial community pattern in all the silicate treatments compared to the treatments without silicate that showed more pronounced changes (**Paper III**). As diatoms did not dominate in all silicate treatments other explanations than a dominance of diatoms due to silicate addition are probable. Differences in the viral community have been observed in the different silicate treatments (pers. comm. Ruth-Anne Sandaa, UiB, Norway) with a higher abundance of virus that had large genome sizes (>350 kb) in the silicate treatments compared to the treatments without silicate. Viruses with genome sizes in this range have been shown to lyse phytoplankton cells (Sandaa 2008) which might have provided the bacterial community with additional nutrients and, by this, might have created more stable nutrient conditions for the bacterial community in the silicate treatments.

3. Carbon sources in the ocean

The organic carbon compounds of the ocean are classified in different groups, by size and biodegradability by bacteria. Total organic carbon (TOC) is the amount of carbon bound in organic compounds and consists of particulate organic carbon (POC) and dissolved organic carbon (DOC). DOC is the most abundant form of carbon in the oceans and accounts for 700 Gt which is similar to the amount of atmospheric CO_2 (750 Gt) (Nagata 2008). Labile DOC, like sugars and amino-acids, originate mainly from primary production (Skoog et al. 2005) and account for less than 1 % of the DOC pool. However, the high turnover makes its consumption still to the major flux of energy in pelagic ecosystems (Nagata 2008). DOC concentrations in the Arctic Ocean were reported to be 105 µM on average and, thus, approximately 25 to 45 µM higher than in other systems (Daly et al. 1999). However, a major part of the DOC in the Arctic Ocean is of terrestrial origin (Anderson 2002, Rachold et al. 2004) which is characterised as refractory carbon compounds and not easily degradable by marine heterotrophic bacteria.

3.1 Glucose –a source of dissolved organic carbon

The DOC pool in the ocean consists of different carbon sources, e.g. sugars, amino acids, organic acids and lipids (Fogg 1983). Glucose, used as primary source of energy in cells, is typically the most abundant free neutral sugar in seawater (Rich et

al. 1996, Skoog et al. 1999). However, glucose is highly diluted in the ocean and accounts for less than 1 % of bulk DOC (Nagata 2008). Other studies also suggest that glucose accounts for just a small portion of the bulk DOC in the ocean (Tanaka et al. 2008), however, the glucose concentrations are highly variable depending on location. In the Atlantic, concentrations between 10 and 1000 nM glucose have been measured, with highest concentrations in coastal waters (Vaccaro et al. 1968). Further, low concentrations of glucose were measured in the Ross Sea (0-14 nM), while in the Arctic Ocean, glucose concentrations between 42 and 90 nM were detected (Rich et al. 1997).

In this thesis, glucose was chosen as the major carbon source to create conditions with high organic carbon load. Monosaccharides are simple molecules that are thought to be easily degraded by the major part of the bacterial community. The use of glucose as carbon source in previous nutrient manipulation experiments (Øvreås et al. 2003, Alonso & Pernthaler 2006, Alonso-Saez et al. 2009) allows the comparison of our results with these studies. In our experiments, between 5.3 and 159 µM glucose were added daily to the mesocosms (**Papers II**, **III** and **IV**) which corresponds to 0.5 – 3-fold Redfield ratio values (Redfield 1934) for glucose relative to nitrogen and phosphorus additions. Differences between ≤ 1-fold and 3-fold Redfield ratios for glucose-C addition were observed for both the bacterial community composition (**Paper IV**) and the state of the bacterial community, whether they were mineral nutrient or carbon limited (**Paper II**). Some bacteria have been shown to utilise

glucose at concentrations over the Redfield ratio by forming carbon-rich inclusion bodies (Thingstad et al. 2005).

Although the bacterial community responded to glucose additions with increasing abundance in the different experiments (**Papers II**, **III**, **IV** and **V**), no correlation between glucose turnover time and bacterial abundance was observed in the mesocosm experiment in Kongsfjorden (Cuevas 2010, **Papers II** and **III**). This suggests that other factors than glucose might have influenced the growth of the bacterial community. The addition of mineral nutrients in excess of the Redfield ratio stimulated bacterial growth in the different experiments. This was followed by enhanced immobilisation of mineral nutrients into bacterial biomass, which might have resulted in increased production of DOC by the nutrient stressed phytoplankton community compared to non-stressed phytoplankton (Borsheim et al. 2005). The phytoplankton community might thereby provide the bacterial community with more DOC sources which might be favoured by the bacteria over glucose.

In the laboratory experiment (**Paper V**), higher bacterial abundance in the glucose treatment compared to the two phytoplankton treatments suggests that some members of the bacterial community might prefer glucose as a main carbon source, as no other carbon sources were available in sufficient amounts. Different bacteria species have shown to prefer different carbon sources (Allers et al. 2007) and, thus, the addition of glucose as main carbon source might have led to bacterial community shifts dominated by glucose utilizers. Thus, the supply of glucose alone might not be sufficient for investigating the effect of high organic carbon load on microbial

communities. A more diverse organic carbon pool would have imitated the natural conditions in a more representative way and might have resulted in a different response in the bacterial community with presumably higher bacterial diversity.

3.2 Algal carbon sources and carbon complexity

In **Paper I** we showed that the bacterial abundance was positively correlated with phytoplankton abundance (chlorophyll a). Different phytoplankton species exude different DOC compounds (Myklestad 1995, Aluwihare & Repeta 1999), and different bacterial species prefer different carbon sources (Allers et al. 2007). The structure of the bacterial community is therefore partially regulated by the composition of the co-existing phytoplankton community (Sapp et al. 2007, **Papers III** and **V**). Differences in phytoplankton composition in mesocosms with and without silicate amendment were suggested as a possible reason for the differences found in the bacterial community structure (**Paper III**). Furthermore, bacterial communities exposed to carbon compounds released by either *Thalassiosira sp.* or *Phaeocystis pouchetii* differed in community composition and richness (**Paper V**). *Thalassiosira* and *Pheocystis* are reported to release carbon compounds that differ both in chemical composition and relative lability. For example, *Thalassiosira weissflogii* has been reported to produce more recalcitrant DOC compounds than *Phaeocystis sp.* (Aluwihare & Repeta 1999). Although the applicability of these observations to the investigation conducted with *Thalassiosira sp.* and *Phaeocystis pouchetii* is uncertain, it provides a plausible explanation for the lower species richness found in the

Thalassiosira sp. treatment compared to the *Phaeocystis p.* treatment (**Paper V**). Furthermore, *Phaeocystis sp.* has been shown to produce acrylic acid, a potential inhibitor of bacterial growth (Sieburth 1959, Guillard & Hellebust 1971, Davidson & Marchant 1987). Other studies have reported no negative effect of acrylic acid on bacterial growth (Noordkamp et al. 2000) and bacterial abundance and bacterial activity were reported to increase during *Phaeocystis* blooms (Putt et al. 1994, Rousseau et al 2000). However, differences in carbon compounds released by *Phaeocystis* and by *Thalassiosira* influenced bacterial community structure, as shown by differences in community composition and species richness (**Paper V**).

As indicated above, DOC release seems to be even more pronounced in situations where the phytoplankton community is mineral nutrient-stressed (Borsheim et al. 2005). In situations with high organic carbon load, carbon utilisation by bacteria will require more mineral nutrients leading to mineral nutrient limitation, such as was observed during the mesocosm study in Kongsfjorden (**Paper II**). If high organic carbon load in the ocean is followed by mineral nutrient-stressed phytoplankton, the phytoplankton community in a future (i.e. acidified) ocean might produce even more DOC. This would further promote heterotrophic processes within the microbial food web, leading clearly to net-heterotrophy in the oceans.

The strong effect of additional organic carbon sources in the form of glucose on the bacterial community in Kongsfjorden (**Paper II**), however, suggests carbon limited bacterial growth, and thus low accessibility of a possible increased pool of organic carbon compounds produced by mineral nutrient-stressed phytoplankton. Other

studies have also reported lower algal DOC production with high nutrient load than with low nutrient load (Vadstein et al. 2012), which might explain the carbon limitation after nutrient addition in our experiment (**Paper II**).

3.3 The effect of acidification on bacteria exposed to high organic carbon load

In addition to an increase in DOC load, a decrease in pH is also reported for the ocean as a consequence of an elevated CO_2 level (Caldeira & Wickett 2003). This might affect different heterotrophic bacteria species differently as shifts in the bacterial community composition due to ocean acidification have been reported in earlier studies (Witt et al. 2011).

In **Paper IV**, mesocosms held at the ambient $p$$CO_2$ level (250 µatm) resulted in more pronounced changes of the bacterial community composition in response to high organic carbon load than the mesocosms manipulated to a $p$$CO_2$ level of 400 µatm. This finding suggests that bacterial responses to increasing DOC levels under predicted conditions of increasing atmospheric CO_2 and subsequent ocean acidification may indeed be altered relative to responses occurring at present-day $p$$CO_2$ levels in the oceans.

There might not necessarily be a direct link between changes in the bacterial community structure and changes in the CO_2 concentration. Earlier reports have shown that neither bacterial abundance nor bacterial activity changed due to increased CO_2 concentrations (Allgaier et al. 2008). Ocean acidification is expected to change

the chemistry of the oceans which might result in increased production of particulate organic matter, such as transparent exopolymer particulates (TEP) (Engel et al. 2004). TEP provide large surfaces for bacterial attachment and are thus positively correlated with the abundance of attached bacteria (Allgaier et al. 2008). However, TEP-attached bacteria were reported to respond less to increased CO_2 compared to free-living bacterial species (Allgaier et al. 2008). Thus, a possible explanation for the minor changes within the bacterial community structure in the high glucose treatment with increased pCO_2 (**Paper IV**) might be the dominance of attached bacteria that relied more on algal carbon sources than on added glucose.

4. Bacterial diversity in Arctic water

The analysis and comparison of bacterial diversities are challenging, as most indices of diversity are based on assessment of the total number and range of bacterial species present in a sample. The bacterial "species" concept is highly questionable for several reasons (Claridge et al. 1997, Cohan 2002). Firstly, high frequencies of gene exchange within and between the clusters of related bacteria that are recognised as a single species (Cohan 2002) make it difficult to discriminate between closely related species. Secondly, the frequent occurrence of mutations might increase the discrepancy within clusters recognised as a single species. Thus, the term OTU (operational taxonomic unit) is preferentially used as the unit for diversity analyses, and is in most studies defined as any cluster of 16S rDNA gene fragments with 97% or higher nucleotide sequence identity (Kunin et al. 2010, Pommier et al. 2010, **Paper IV**).

Factors determining patterns of bacterial diversity are very complex in the environment. Viral lysis and the structure of the phytoplankton community seem to be important biotic impact factors for determining bacterial diversity (**Paper V**), but also abiotic factors like substrate quantity and quality (**Paper V**), water depth and closeness to the coast (Galand et al. 2010, Pommier et al. 2010) play important roles for bacterial diversity determination. The abundant and active taxa, which are thought to contribute most to nutrient and energy flow and that are most subject to predation and viral lysis, define the diversity of an ecosystem (Pedros-Alio 2006). Rare

organisms apparently contribute little to most biogeochemical processes, and apparently evade main mortality processes such as viral lysis and predation (Fuhrman 2009). On the other hand, higher 16S rRNA : 16S rDNA ratios found for rare bacteria compared to abundant ones suggest that rare bacteria combine fast growth with strong top-down viral control of abundance (Campbell et al. 2011, Thingstad 2000). Top-down control by viruses might thereby prevent the rare bacteria from becoming abundant, although they still might have important functions within the microbial food web, e.g. as nitrogen fixation or acting as a seed bank for niche exploitation (Montoya et al. 2004, Pedros-Alio 2006, Fuhrman 2009).

4.1 Diversity shifts due to nutrient manipulation

Rapid growth of opportunist bacteria in a high nutrient load ocean may reduce bacterial diversity, as suggested by the low Shannon indices in the nutrient manipulation experiments with water from Fram Strait (**Paper IV**). In this study, the estimated Shannon indices ranged from 1.4 to 2.3 which is lower than the reported Shannon indices from other Arctic studies (Malmstrom et al. 2007). Even higher Shannon indices were measured in the Mediterranean Sea with values between 4.0 and 5.5 for the upper layer (Pommier et al. 2010) which suggests that bacterial diversity might increase with decreasing latitudes, as has also been discussed for species richness (see below). The low diversity measured in **Paper IV** might not only be a consequence of high nutrient load in general, but also an effect of the use of a single carbon compound (glucose), which has been previously shown to select for

lower diversity than complex carbon sources (**Paper V**). In natural systems, a decrease in bacterial diversity due to higher nutrient load, as seen in our Fram Strait mesocosms (**Paper IV**), seems unexpected. For example, coastal regions are characterised by generally higher nutrient concentrations than open oceans (Vaccaro et al. 1968), but bacterial diversity was reported to decrease with the distance from the coastline (Pommier et al. 2010) which was likely attended by a decrease in nutrients. Further, greater resource diversity could generate more niches that can be filled by different bacteria (Fuhrman 2009) and ultimately lead to increased diversity.

Several reports document a gradient of decreasing bacterial richness from low to high latitudes (Pommier et al. 2007, Fuhrman et al. 2008). Thus, bacterial richness in the Arctic Ocean is expected to be lower than in lower latitude oceans, which was also corroborated in this thesis. The estimated OTU richness in the Fram Strait ranged between 70 and 126 (**Paper IV**) and was similar to other studies performed in the Arctic (Malmstrom et al. 2007, Pommier et al. 2007), although much lower than richness values observed for the Mediterranian Sea (980 - 3193 OTUs in Pommier et al. 2010). The estimated richness values in **Paper IV** are in accordance with other Arctic studies, although diversity estimations were lower than previously reported (Malmstrom et al. 2007). As diversity is a measure of both species richness and evenness (Shannon & Weaver 1949), the observed low evenness in the Fram Strait experiment (between 0.16 and 0.29, **Paper IV**) might explain the lower diversity but comparable richness estimates in **Paper IV** as compared to other studies (Pommier et al. 2010, **Paper V**).

4.2 Dominant bacterial groups under different carbon loads

Under an ambient CO_2 atmosphere, high glucose concentrations favoured bacteria belonging to the *Gammaproteobacteria* group (**Papers IV** and **V**), as has been reported in previous studies (Pinhassi & Berman 2003, Øvreås et al. 2003, Harvey et al. 2006). The high abundance of *Gammaproteobacteria* in carbon rich treatments might be caused by their rapid cell growth in response to nutrient addition (Nyström et al. 1990) as also discussed in **Paper V**. *Alpha-* and *Gammaproteobacteria* as well as *Bacteroidetes* are the dominant bacteria groups in the world oceans (Pommier et al. 2007, Galand et al. 2009), including the Arctic Ocean (Bano & Hollibaugh 2002, Pommier et al. 2007, Kirchman et al. 2010). Although a dominance of *Gammaproteobacteria* due to high nutrient load under ambient CO_2 conditions (**Papers IV** and **V**) might suggest a shift in bacterial communities towards *Gammaproteobacterial* dominance in the future, the experimental combination of high nutrient load and increased CO_2 resulted rather in a shift towards *Flavobacteria* dominance (**Paper IV**).

The release of DOC by phytoplankton is extremely important to meet the carbon demand of heterotrophic bacteria, of which especially the *Alphaproteobacteria* group have been shown to grow in the presence of algal blooms (Riemann et al. 2000, Pinhassi et al. 2004, Bruckner et al. 2008). In **Paper V**, a shift in dominance from *Gamma-* to *Alphaproteobacteria* occurred in response to carbon compounds released by *Thalassiosira sp.* but not by *Phaeocystis p.*. This underlines the important role of the structure of co-existing phytoplankton communities in determining bacterial

community composition, and indirectly emphasises the importance of sea ice melting in the Arctic for promoting phytoplankton growth and bacterial community dynamics. Increased light penetration into the water column as a consequence of thinning ice layers might lead to increased primary production by phytoplankton (Sakshaug 2004). It has been reported that bacterial diversity can vary with primary production (Horner-Devine et al. 2003). For example, diversity within the *Cytophaga-Flavobacteria-Bacteroidetes* group was characterised by a hump-shaped relationship with primary productivity, while *Alphaproteobacterial* diversity was characterised by a U-shaped relationship. However, other studies have not reported a clear relationship between bacterial diversity and primary production (Pommier et al. 2010). Other factors, including increasing mixing depth of Arctic water due to disappearing ice layers might thus have a more severe effect on the bacterial diversity, as the depth of mixing is suggested to have a strong impact on the phytoplankton community structure (Sakshaug 2004), and might thus also affect the bacterial diversity (**Paper V**).

4.3 Factors regulating bacterial diversity

Several factors have been reported to have an impact on the composition of bacterial communities (fig. 4). Substrate composition and concentration, phytoplankton composition as well as viral lysis seem to play important roles in forming bacterial communities (**Papers III**, **IV** and **V**). Assuming that bacteria growth is substrate-specific and that different species are limited by different substrates, bacterial diversity would therefore reflect substrate diversity. This assumption seems to be

confirmed by the results of this thesis, as the bacterial community exposed to complex algal carbon sources was more diverse and species-rich than bacterial communities exposed to just one carbon source (**Paper V**). In this study, however, the diversity in the simple carbon treatment (glucose) was still high, therefore results were interpreted as an interplay of both bottom-up (carbon composition) and top-down (viral lysis) factors (**Paper V**). Lysis of host bacteria by viruses has been suggested to be the major top-down factor influencing the diversity of the bacterial community (Fuhrman 1999, Thingstad 2000). Viral infection is host-specific and depends on host abundance, which determines collision rates between bacterial hosts and their viruses. By reducing the abundance of fast-growing bacteria, viruses provide slow-growing non-host bacteria an opportunity to co-exist with fast-growing bacteria and thereby increase bacterial diversity. It is generally considered that viral lysis is the main factor affecting species richness and evenness of bacterial host groups (**Paper V**), while substrate composition determines which bacteria may successfully compete for available niches (Thingstad 2000).

Grazing by heterotrophic protists is an important factor that influences bacterial communities, although it is thought to occur in non-selective manner which regulates general bacterial abundance rather than bacterial diversity (Gonzalez et al. 1990, Sandaa et al. 2009). It has been shown that grazing by flagellates mainly depends on the size (Simek & Chrzanowski 1992) and motility of prey bacteria (Simek & Chrzanowski 1992, Gonzalez et al. 1993, Matz & Jurgens 2003). As cell size was reported to be strain specific (Boenigk et al. 2004), grazing might also have an effect

on the structure of the bacterial community. In addition, it has been shown that

heterotrophic flagellates may have specific prey preferences (Caron 1987), and could

thus impact the taxonomic composition of the bacterial community (Juergens et al.

1994, Simek et al. 1997, Riemann et al. 2000). Virus recognition of bacterial hosts,

however, is mediated by physical contact of viral particles with surface receptor

molecules on the bacterial cell, a process which is likely to confer greater precision

upon viral selection of bacterial hosts than upon size-selective grazing by flagellates.

Grazing is therefore assumed to be of minor importance for the bacterial community

composition relative to regulation by viral lysis.
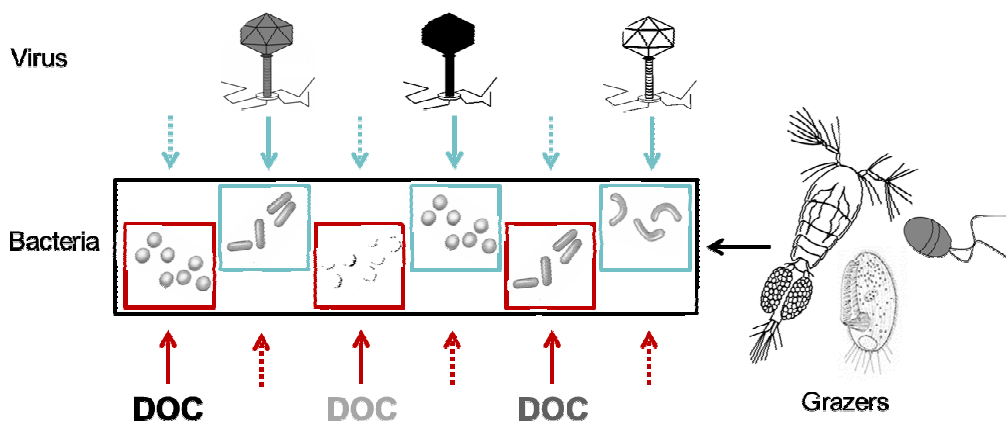
Fig.4 Schematic diagram of processes determining bacterial diversity in the ocean. The black box illustrates the whole bacterial community with different bacterial groups formed by viral control (blue) and / or DOC sources (red). Different viruses and DOC compounds are illustrated by different shades of grey. Grazing is thought to occur non-host-selectively, thus reducing the general abundance of the whole bacterial community rather than of specific bacterial groups. Viral lysis of host groups, which can be both bacteria and phytoplankton, replenishes the dissolved nutrient pool and stimulates continued host growth. In addition to DOC, mineral nutrients (N and P) are essential for bacterial growth.

Viral lysis as top-down factor and substrate composition as bottom-up factor are major factors affecting bacterial community structures, as discussed in **Paper V**. Abiotic factors such as light, temperature and water depth also create different niches which are filled by different bacteria. Many factors therefore play a role in regulating bacterial community structures, although they might act on different taxonomic levels (class, family, species) of bacterial diversity. The degree to which each of the factors impacts upon the bacterial community structure is difficult to measure, as many factors often act in concert and are difficult to separate.

5. Methods and experimental approaches

The study of microbial ecology in the ocean is marked by several challenges. Both the small size of microorganisms and their frequent recalcitrance to cultivation (Staley & Konopka 1985) hinder direct experimental observations. Development of non-cultivation-based techniques has provided new and informative insight into naturally occurring marine microbial communities. Currently, the most common approach to study bacterial community structures utilises comparisons of 16S rRNA gene sequences, which have previously been used as phylogenetic markers for taxonomic and evolutionary studies (Sogin et al. 1972). Application of 16S rDNA sequence analysis to complex microbial communities has uncovered many hitherto undescribed bacterial groups, and led to the discovery that the majority of the marine bacterial taxa are novel and not represented in culture collections (Giovannoni et al. 1990, Pedros-Alio 2006, Ward et al. 1990).

5.1 Approaches to analyse bacterial community structures

Different methods exist to extract information provided in 16S rRNA gene (rDNA) sequences and that can be used for bacterial community studies (Malmstrom et al. 2007, Pommier et al. 2007, Pommier et al. 2010). Fingerprint methods are used to assess gene diversity of microbial communities without direct organism identification or enumeration of microorganisms. Fingerprinting typically involves amplification of genes of interest by PCR followed by analysis of nucleotide sequences of the

amplicon "library" generated. Such analytical methods exploit sequence-dependent denaturation chemistry of amplicons (Denaturing gradient gel electrophoresis, DGGE), variable length of rRNA subunit intergenic spacer regions (Automated ribosomal intergenic spacer analysis, ARISA) or occurrence of specific endonuclease restriction sites within amplicon sequences (Terminal Restriction Fragment Length Polymorphism, T-RFLP) (Øvreås et al. 2003, Schwalbach et al. 2004, Fuhrman et al. 2008). Diversity estimations are generally calculated from variations in banding patterns generated when sample products are separated by gel electrophoresis.

In this thesis, DGGE was used to study trends in bacterial community structure in response to high nutrient load (**Papers III** and **V**). DGGE is especially suited for community comparisons and description of community dynamics within bacterial communities subjected to different experimental manipulations and over time (Øvreås et al. 2003, Pinhassi et al. 2006, Allers et al. 2007). However, there are several limitations to the method that should be considered during data analysis. Firstly, the low resolution of this method only allows detection of the most abundant bacteria (i.e. those abundant enough to have visual representation by electrophoresis). Secondly, sequence heterogeneity at the 16S rDNA region precludes determination of total bacterial richness (Fogel et al. 1999). Furthermore, similar DGGE patterns do not necessarily indicate identical composition of bacterial communities (**Paper V**) thus, this method may not be sufficient to fully characterise changes in bacterial community composition and diversity. Additionally, obtaining good-quality sequence information

from excised DGGE bands is possible but not straightforward, making taxa-level comparisons difficult.

Clone libraries are used for sequence analysis of complex microbial communities on a larger scale (Giovannoni et al. 1990, Rappe & Giovannoni 2003). Compared to DGGE, this method allows us to describe bacterial diversity in addition to community structure (**Paper V**), as a larger number of sequences is obtained, making species richness and evenness estimations possible. Sequencing of clone libraries is a straightforward method that in general gives high quality sequence information. However, insufficiently high numbers of sequences due to throughput limitations might make proper richness estimations difficult. As estimates from metagenomic shotgun sequencing (Venter et al. 2004) suggest very high microbial richness in marine systems, even 1000 clones per sample were stated as clearly insufficient for diversity studies (Pommier et al. 2010). Normally, not more than a few hundred sequences are obtained by this method. Further, clone libraries are subject to PCR bias and only detect the most abundant taxa, or those which are readily amplified by primers targeting 16S rDNA (Pedros-Alio 2006). It has been reported that taxa accounting for less than 0.1 % of the total bacterial diversity are difficult to retrieve by amplification with primers targeting 16S rDNA (Muyzer et al. 1993). Biases can also occur as a result of differences in amplification efficiency of templates and formation of chimeric or heteroduplex molecules (Fogel et al. 1999, Acinas et al. 2005). As it is not likely that all rDNA sequences will be amplified with the same

efficiency, PCR-based diversity estimation methods are an inappropriate choice when absolute quantification of bacterial taxa in environmental samples is desired.

Today's high throughput sequencing technologies such as pyrosequencing (Ronaghi et al. 1998), provide deeper insight into the richness of environmental samples, as this method allows deeper sequencing coverage of bacterial metacommunities or amplification products (Sogin et al. 2006, **Paper IV**). In this thesis, pyrosequencing of specific 16S rRNA gene fragments was applied to investigate the diversity of seawater mesocosm bacterial communities after experimental manipulation. Although this study is also subject to conservative interpretations of diversity because of the inherent PCR bias (see above), it is a powerful method for examination of bacterial community diversity on a much deeper scale than previously possible. The greater depth of sequencing allows detection of rare taxa, which are generally missing from datasets, retrieved by other molecular techniques (Pedros-Alio 2006, Galand et al. 2009). Other studies have reported an overestimation of taxa detected by this method due to sequencing artefacts (Quince et al. 2009, Reeder & Knight 2009). Continuous development and application of quality filters has greatly improved the reliability of sequence information obtained from pyrosequencing datasets (Huse et al. 2007).

For diversity measurements, pyrosequencing represents the best compromise of throughput and detection sensitivity, as diversity estimates are determined by both abundant and rare taxa. Clone libraries generated from small numbers of clones (< 100) (**Paper V**), albeit insufficient for precise diversity estimations, are still useful for rough predictions of diversity and for rapid examination of abundant bacterial taxa

present in environmental samples. The main interest of this thesis was to investigate major shifts in bacterial communities due to high nutrient load, therefore our choice of methods are aptly suited to examination of abundant bacterial taxa. It should be noted, however, that the rare microbial biosphere represents the majority of bacterial diversity in the oceans (Pedros-Alio 2006), and as such may contribute critical functionality to major biogeochemical processes in the ocean.

5.2 Experimental approaches for seawater experiments

Two different experimental approaches were used in this thesis to study bacterial communities: (1) field investigations using seawater mesocosm experiments and (2) a microcosm study in the laboratory. Microcosm experiments, also called bottle experiments, allow studying the bacterial community in a manageable water volume in the laboratory (fig. 5a, **Paper V**). Advantages of such experiments are easier handling and less space requirement than large-scale experiments. One disadvantage seems to be that the occurring living conditions in such a small volume are further away from real-life conditions than in large-scale experiments (mesocosms) or *in situ*. This "bottle effect" has been shown to affect bacterial abundance, activity and community composition, and is often discussed as a potential explanation for variability in experimental results (Zobell & Anderson 1936, Marrase et al. 1992). On the other hand, it has also been reported that the "bottle effect" does not affect bacterial abundance (Hammes et al. 2010).
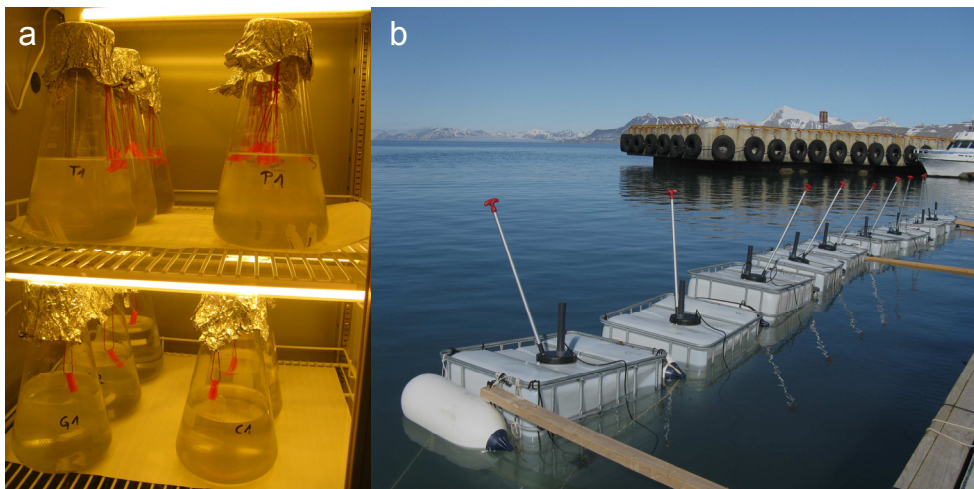
Fig. 5 Different approaches to study marine microbial communities: (a) Microcosm experiments in the laboratory and (b) mesocosm experiments on location (Kongsfjorden, Svalbard).

Mesocosm studies in marine environments have been performed since the early 1960's (McAllister et al. 1961, Antia et al. 1963), and have allowed examination of microbial communities on a larger scale and with a higher degree of realism than land-based constructions (Egge 1993) or bottle experiments. In this thesis, water volumes of approximately 700 L or more (fig. 5b, **Papers II**, **III** and **IV**) were used to study the bacterial community on location (Kongsfjorden and Fram Strait). Mesocosm experiments are, however, time- and resource-consuming, and therefore require greater investment in planning and logistics.

6. Conclusions and future perspectives

High organic carbon load, predicted for a high CO_2 environment, was shown to affect the structure of marine bacterial communities, which are the main consumers of DOC in the marine environment. The results of two different mesocosm experiments in the Arctic (**Papers II**, **III** and **IV**) and a laboratory experiment (**Paper V**) showed a strong effect of high organic carbon load on the outcome of bacterial competition for mineral nutrients (**Paper II**), on the bacterial community composition (**Papers III**, **IV** and **V**) and on bacterial diversity (**Papers IV** and **V**). The combined results of this thesis clearly reflect the complexity of marine microbial food webs and the factors that determine bacterial community structure and diversity. In summary, the most important findings of this thesis are:

- Under *in situ* conditions heterotrophic microorganism dominated in the Arctic Ocean, although the system was found to be net-autotrophic (**Paper I**). At the time of sampling, nitrogen was identified as the growth-limiting factor for bacterial proliferation.

- The effects of high organic carbon load, in the form of glucose addition, on mineral nutrient competition between bacteria and phytoplankton were demonstrated (**Paper II**). Promotion of bacterial growth was interpreted as an increased immobilisation of mineral nutrients by the bacterial community as a response to increased organic carbon load, leading to a shift from net-autotrophy to net-heterotrophy in the investigated system.

- The structure of bacterial communities, as determined by DGGE analysis of partial 16S rDNA amplicons, changed in response to glucose addition (**Papers III** and **V**).

- Artificial acidification of surface Arctic seawater affected the response of the bacterial community structure to high organic carbon load (**Paper IV**). Bacterial communities exposed to glucose in three-fold excess of the Redfield ratio shifted dramatically under ambient CO_2 conditions but not under artificially acidified conditions intended to approximate a future high-CO_2 environment.

- Both composition of carbon compounds (bottom-up regulation) and viral lysis (top-down regulation) are major factors whose interaction affect bacterial community composition and diversity (**Paper V**).

- A shift in dominance of bacterial communities by specific taxa in response to organic carbon addition was observed (**Papers IV** and **V**). Such shifts have the potential to affect vertical transport of bacterial biomass to higher trophic levels, as related bacterial taxa have been shown to increase in size in response to carbon addition, thereby changing their susceptibility to size-selective predation.

Evidence suggests that shifts in bacterial community structures in response to high organic carbon load may have consequences for other components of the marine food

web, although further research is clearly needed. Possibilities for further investigation from this thesis might include investigation of a more diverse carbon pool rather than glucose alone, as glucose addition consistently promotes the dominance of certain bacteria groups and shifts to lower bacterial diversity. Examination of the impact of an increasing complexity of carbon substrates on bacterial community dynamics might further bolster the positive correlation between carbon complexity and bacterial community diversity observed here. In addition, investigation of complex carbon substrate pools is obviously desirable as it most closely approximates the carbon environment experienced by bacteria in the marine environment.

Artificial acidification of seawater mesocosms revealed a strong effect of elevated $p\mathrm{CO}_2$ on the response of bacterial communities to high organic carbon loads. These results are, however, issued from one mesocosm experiment performed with one specific water mass taken from the Fram Strait, a fact requiring further investigations both in Arctic water masses in order to confirm our findings and in other marine environments in order to obtain a more holistic picture. As discussed in chapter 3.3, the observed differences in the bacterial community composition between the two $p\mathrm{CO}_2$ treatments may have been an indirect response of increased particle production due to manipulation of seawater chemistry, rather than a direct effect of ocean acidification on the bacterial community. Further investigation might include monitoring of particle production (e.g. TEP) and differentiation of free-living and particle-attached bacteria, as these two bacteria groups were reported to react differently to increased CO_2 (Allgaier et al. 2008).

Bacteria have different functions in the microbial community, e.g. nitrogen fixation or degradation of certain complex carbon compounds, e.g. mineral oil. Oil degrading bacteria might be of particular interest in Norway, as the oil industry is strongly represented along the whole Norwegian coast up to the Barents Sea. Further research to specifically investigate function-related changes in the bacterial community in response to ocean acidification and elevated organic carbon load is needed. This knowledge will contribute to a general understanding of the potential of the marine microbiome, as the base of the marine food web and driver of biogeochemical cycles, to respond to a changing marine environment.

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