Spatial biodiversity patterns of bacterio- and picoplankton communities in Arctic fjords

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1 Summary

Marine microbial plankton drive global biogeochemical cycles and are therefore pivotal to the ecosystem functioning of the biosphere. In particular marine picoplankton harbour a vast biodiversity on which their community dynamics and functioning are based. Because they function collectively as a community, it is crucial to understand the underlying diversity patterns of microbial assemblages and identify their drivers. The data set I investigated here allows insights into surface water bacterio- and picoplankton communities of Arctic and subarctic coastal waters and fjord systems. To infer their diversity with a metabarcoding approach, I amplified and sequenced the V4 regions of the prokaryotic 16S and eukaryotic 18S ribosomal DNA which serve as molecular markers. The resulting amplicons were arranged into amplicon sequence variants (ASVs) which I used as a substitute for species. In comparing prokaryotic and picoeukaryotic alpha and beta diversity across space, I unveiled profound differences between the domains, the investigated regions and the respective drivers. Picoeukaryotes appeared to vastly exceed prokaryotes in their richness and are thus hypothesized to comprise a large rare biosphere ensuring community stability. They are more strongly influenced by fjord structures and glaciers than prokaryotes and I found spring bloom conditions to induce a drastic decrease in picoeukaryotic richness. Prokaryotes appeared to be more strongly influenced by nutrient availability and environmental conditions than picoeukaryotes, resulting in a higher spatial turnover through more efficient taxa sorting. I found no distance-decay relationship in prokaryotic and picoeukaryotic communities on the scales observed here. I assume a functional coupling and mutual dependence of the prokaryotic and eukaryotic communities based on co-varying alpha diversity measures, which were fundamentally restructured by spring bloom conditions. I observed a pronounced compositional turnover in both space and time. Seasonal succession and change across years appeared to shape picoplankton communities equally strong as spatially differing influences, stressing the need to control for time in future spatial analyses. In contributing to a better understanding of the basic patterns and their drivers underlying picoplankton diversity, this study may also contribute to a better understanding of the impact climate change will have on the planet. Spatial dynamics across environmentally differing sites can deliver indications to the influence of environmental changes in time. Thus, they allow to anticipate changes in microbial plankton dynamics and therefore the functioning of the global biosphere in the face of climate change.

2 Introduction

Oceans are the cradle of evolution which created the biodiversity on earth we know today. It harbours the vast diversity of marine microbial plankton, who drive carbon and nutrient cycles and provide half of the global primary production (Field et al., 1998). Because of their huge abundances and large genetic biodiversity, those minute organisms impact processes on a global scale and are flexible in their response to fluctuating environmental conditions. Not only the evolutionary roots of the contemporary global biodiversity, but its vast majority in the sense of a genetic diversity can be found in the as of yet largely unexplored microbial world.

Microbes comprise organisms of all domains of life – prokaryotes, i. e. bacteria and archaea and unicellular eukaryotes (protists) – which possess different traits and functions. However, all of them have the following features in common which distinguish microorganisms sharply from macroorganisms: Being unicellular, characterized by small cell sizes, short life cycles and generally large population sizes. These features evoke the need for different concepts to study their diversity and distribution patterns. Common approaches to assess the biodiversity and distribution of terrestrial macrobial life, which is shaped by dispersal barriers leading to biological speciation (Mayr, 1948), endemism and extinction, cannot be applied so easily to microbial life. Even more so, if the marine biosphere is addressed: At first sight, the world´s oceans appear to be a continuous space, lacking physical dispersal barriers and being globally connected by freely flowing ocean currents. Because of their size, microbes are passively and widely dispersed within them (Finlay & Clarke, 1999) – the passive dispersal in fact is the feature plankton is defined by. Therefore, it has long been thought that in principle “everything is everywhere” and through selection by environmental factors certain species dominate distinct ecological regions (Baas Becking, 1934). However, the limitless dispersal of all marine microbial species in the absence of dispersal barriers as proposed by Baas Becking has increasingly been doubted (Martiny et al., 2006; Spatharis et al., 2019). Despite the occurrence of widely distributed, diluted microbes (Farooq Azam & Malfatti, 2007), global sampling efforts have been revealing clearly differing community compositions across spatial and temporal scales (e. g. Galand et al., 2009; Massana et al., 2015). These findings indicate that marine microbial species, just as all other organisms, exhibit a distinct biogeography that is shaped by both historical contingencies, i. e. dispersal limitations, as well as by current environmental influences (Martiny et al., 2006). Density differences between water masses for example can act as physical boundaries restricting distribution (Galand et al., 2009).

With the knowledge we have today, the notion of a cosmopolitan distribution of all marine microbial species solely shaped by environmental selection seems highly unlikely as a universal rule. All the more interesting it becomes to address the patterns of local marine diversity.

In contrast to the concept of biodiversity, which refers to the entirety of contemporary species, i. e. the global gene pool, the term diversity describes the local selection of taxa of a specific region or site (Margalef, 1994). Global microbial biodiversity can only be roughly estimated, while the local diversity of one community or region can be tackled more easily. In order to characterize diversity, it can be partitioned into two subcommunities: 1) the few abundant and 2) the many rare taxa constituting every microbial community, which have substantially different characteristics. Usually, taxa with abundances >1% are considered abundant species and taxa with abundances <0.01% rare (Galand et al., 2009; Logares et al., 2014).

The abundant subcommunity comprises few species but most individuals. They are abundant because the environmental conditions in that time and place are well suited for them, resulting in increased

growth rates and abundances. At the same time, they suffer high losses from predation and viral lysis, thus contributing to the carbon and energy flow of the respective ecosystem (Pedrós-Alió, 2006).

The proposed rare biosphere (Sogin et al., 2011) consists of a number of taxa vastly exceeding that of the abundant community, while being present at extremely low abundances. Its members are defined solely by their abundance at a given time and place under specific environmental conditions, in fact, it is an ever changing assortment. Low abundance protects microbes from predation, competition and viral lysis, which is one of the aspects fundamentally distinguishing micro- from macroorganisms: competitively superior taxa can never completely extinguish inferior taxa in the microbial world, because they survive even at almost undetectable abundances. In the case of the rare biosphere, this is a great advantage to the functioning of the whole community, because they serve as a seed bank (Campbell et al., 2011). Most rare species are thought to be functionally redundant, but in the current environmental regime competitively inferior. However, because of the short microbial life cycles, they can quickly increase in number in response to environmental changes in favour of their optima, replacing former abundant taxa and providing the same ecological functions. Thus, flexible and constant taxonomic reassembles are thought to maintain stable ecological processes such as nutrient cycling and carbon flow on the community level over a broad range of environmental changes (Caron & Countway, 2009).

However, being rare doesn´t necessarily mean being inactive. While some taxa switch between being rare and abundant, that is active and inactive, in accordance to the surrounding conditions, others permanently remain rare while being metabolic active (Campbell et al., 2011). Among prokaryotes, dormancy and low metabolic activity is comparatively more common than among protists (Massana & Logares, 2013), but for both activity can even increase with decreasing abundance (Logares et al., 2014). The composition and richness of the rare biosphere can strongly influence an ecosystems and a communitys robustness and resilience, i. e. their ability to recover from and maintain their functions despite of disturbances or changes. The more genetically divers the rare community is, the more possible responses it contains and the wider the range of change it can buffer (Caron & Countway, 2009). Both the rare and the abundant marine subcommunities can differ greatly in community composition in time and space and thus show a clear biogeography (Galand et al., 2009; Logares et al., 2014). These findings argue once more against a ubiquitous distribution of marine microbes, because otherwise the rare biosphere would be the same wherever investigated. While the species composition of both the rare and abundant communities are subject to strong fluctuations, they were observed to have strikingly similar and temporally constant relative proportions and species turnover, i. e. beta-diversity, across sites, indicating self-perpetuation possibly linked to interactions across the two spheres (Logares et al., 2014).

Despite being the most ancient forms of life, quantifying microbial diversity remains a challenging task to accomplish. Traditional approaches to assign individuals to species or genus are deficient for pro- and eukaryotic unicellular organisms, who can hardly be distinguished according to their shape or other morphological features. The scarcity of sexual reproduction among unicellular eukaryotes and the absence among prokaryotes make Ernst Mayrs biological species concept (1948) challenging or even unfeasible for microbes. What is more, in order to be assigned to a species, microbes need to be cultivated and described first. Present cultivation methods manage to cultivate only a small percentage of microbial taxa which creates the need for a different approach to group individuals into biological entities (Amann et al., 1995). Nowadays, improved DNA sequencing methods allow an additional approach that is often applied in combination with cultivation efforts (e. g. Siegesmund et al., 2008). Molecular analyses for species identification are based on the comparison of specific genomic regions. These regions serve as molecular markers who reveal genetic variation among different individuals. The most widely used markers in microbial research are the ubiquitous gene of the small subunit (SSU) of the ribosomal RNA, the 16S for prokaryotes and 18S for eukaryotes. While the whole marker is used for clonal and culture characterisation, only a specific variable region (e. g. V4 or V9) is used for bulk

or field sample analyses (e. g. in Massana et al., 2015). These specific regions within the SSU are chosen to simultaneously amplify and parallel sequence the molecular marker for all organisms in one community sample, resulting in so called metabarcode amplicons. When resolving these amplicons into exact sequences, nucleotide polymorphism allows clustering them into so called operational taxonomic units (OTUs) or arranging them into amplicon sequence variants (ASVs; Callahan et al., 2017), i. e. groups of individuals with a similar genetic code. ASVs are less biased than OTUs because reads are not fit into a predefined pattern, but rather sorted according to the genetic variation among them. Still, they are no equivalent to species, as they only represent molecules. However, they can serve as "phylospecies" to tackle ecological questions such as describing the environmental distribution of microorganisms. It is a powerful approach that facilitates exploring the temporal and spatial patterns of marine microbial diversity (e. g. Vargas et al., 2015).

Diversity lacks a clear definition but rather is a broad concept composed of several different components that can be weighted and interpreted according to purpose (Swingland, 2001). The most intuitive component is species richness, which refers to the total number of different species within a given sample. However, species richness is prone to the sampling problem. Observing only species richness might lead to underestimation of the correct number of species, due to insufficient sampling effort that overlooks in particular rare species. Moreover, species richness masks the different proportions of the species present. Another parameter of biodiversity is therefore species evenness, which describes how evenly the individuals are distributed across the different species. In order to combine both components of diversity and express them quantitatively, they are used to calculate diversity indices which allow comparison on a spatial or temporal scale. These diversity indices are (1) the Shannon index, which quantifies the uncertainty of predicting the identity of a given organism in a sample and (2) the Simpson index, which describes the probability that two individuals chosen randomly from a community belong to the same species or ASV. However, these two indices are (unlike species richness) non-linearly connected to diversity and rather a measure for the entropy within a community from which the effective number of species can be deduced (Jost, 2006). The effective number of species indicates the number of equally common species in a community that would result in the same index value and is provided by the Hill Numbers (Chao et al., 2014; Hill, 1973). The Hill Numbers calculate the Shannon entropy and the Simpson concentration instead of the raw indices, thus using the information given by the indices while effectively tackling the abundance and the sampling problem mentioned above. Hill Numbers are a class of diversity measures varying in their order q. They include species richness ($q = 0$), the Shannon entropy ($q = 1$, the exponential of the Shannon index) and the Simpson concentration ($q = 2$, the Simpson index subtracted from unity and taken its reciprocal). They value species evenness proportionally higher than species richness the higher their order q, and with increasing q samples become less susceptive to the sampling problem. While the Shannon entropy, like any other index of order one, weighs each species according to its abundance and therefore reflects the diversity of typical species, the Simpson concentration can be thought of as the diversity of the dominant species since, as an index of order two, it is mostly based on species evenness and little on species richness. Species richness itself, in turn, disproportionately reflects rare species by neglecting frequencies altogether. A comprehensive acquisition of the diversity of a community can only be achieved in incorporating these different aspects into the analysis.

The presented indices describe the alpha diversity of a community and can be used to measure the difference in alpha diversity between sites by comparing its magnitude and statistical significance. Across multiple samples, diversity can furthermore be explored by assessing species compositional similarity. This level of diversity is called beta diversity, a term first introduced by Whittaker (1960), which describes dissimilarity in community composition between samples on a spatial or temporal scale. It is shaped by two different processes: (1) species replacement, i. e. species turnover, and (2) species

gain or loss, resulting in richness difference (Legendre, 2014). The latter can also lead to nestedness of communities, meaning the community of one site is a subset of another. The spatial turnover and nestedness components of beta diversity are additive (Baselga, 2010) and in combination provide indications on how ecological processes differ across sites and along environmental gradients and hence shape community assemblage (Loiseau et al., 2017). A changing environment, similar to a spatial environmental gradient, may be reflected in species turnover and hence in a changing beta diversity (Hillebrand et al., 2010).

Taken together, the different components of alpha- and beta-diversity constitute fundamental descriptors of ecology since they form the basis for further exploration of the ecological factors shaping species distribution. They allow insights into interactions between individuals, because the more evenly individuals are distributed across species, the higher the possible variety of interactions among different individuals. Furthermore, high evenness generally indicates an improved resistance to environmental changes and thus a higher stability of ecosystems (Shade et al., 2012). A larger variety of functionally redundant species allows to buffer changes by maintaining overall community functions, despite structural changes. Keeping this in mind it becomes clear why understanding the ecological potential of the manifold pro- and eukaryotic microbial interactions can only be approached through an understanding of the underlying patterns of diversity.

Microbial interactions, functions and diversity patterns are strongly influenced by the surrounding environmental regime. The Arctic coastal waters are a unique habitat characterized by the formation of sea ice during winter, the resulting low light availability during the periods of ice cover, and strong stratification resulting from meltwater, i. e. fresh water, input during summer. Temperature, the primary metabolic rate driver, is known to significantly influence both microbial communities (Sunagawa et al., 2015; Ward et al., 2017) and diversity (Fuhrman et al., 2008) as well. Therefore, global warming in the course of climate change affects the spatial distribution of microbes and thereon the functioning of ecosystems all over the world (Thomas et al., 2012). Specifically, it impacts the polar regions more severely than any other part of the planet because of an accelerated warming rate (IPCC, 2014). Sea ice diminishment (Screen & Simmonds, 2010) and surface water warming (Steele et al., 2008) will alter Arctic ecosystems profoundly, as will thawing of permafrost and glaciers (Garcia-Lopez et al., 2019; Müller et al., 2018).

Arctic fjords are influenced by glaciers and permafrost in their inside, especially in their tips, and by the open ocean in their mouth regions. The freshwater input from permafrost and glacier runoffs creates an environmental gradient along the fjord with lower salinity and an additional nutrient input in the tips of the fjords, as well as an increased stratification of the water column. Melting glaciers transport terrestrial and englacial organic matter and nutrients along their runoffs into the fjords (Kim et al., 2020; Müller et al., 2018). Additionally, it acts as a vehicle for glacier inhabiting microbes, thus shaping the fjord microbial communities (Garcia-Lopez et al., 2019). Tidewater glaciers, who terminate at the ocean margin, discharge freshwater at depth which upwells along with entrained fjord water, pumping nutrients from the depth to the surface (Cape et al., 2019). If tidewater glaciers decline because of global warming, so does the nutrient input and its turnover. At the same time, the increased freshwater inflow into the Arctic Ocean surface waters via runoffs of melting glaciers leads to increased stratification, which even more diminishes nutrient input into surface waters through upwelling. While intensifying the vertical stratification of the ocean surface, melting glaciers and permafrost are not the only cause for this global phenomenon. Freshening of the surface caused by increased precipitation in higher latitudes is another cause associated with climate change (Sarmiento et al., 1998).

Microbes can rapidly respond to changing environmental conditions due to their short generation time and large population sizes and thus both indicate and amplify change (Vincent, 2010). Thus, the

warming climate has been observed to alter microbial community structure in general and favour smaller microbes in marine surface waters in particular (Li et al., 2009), especially picoeukaryotes and bacterioplankton.

Picoplankton consists of both pico-sized eukaryotes, who have cell sizes of 0.2 to 3 µm, and prokaryotes. Due to their minuteness, picoplankton is characterized by an even higher dispersal potential, larger abundances and a higher specific activity than the microbial world in general (Massana & Logares, 2013). Their large surface-to-volume-ratio allows them to deal better with low nutrient availability, such as nitrogen (Li et al., 2009), and some of them may be more efficient in absorbing radiant energy (Fogg, 1986; González-Olalla et al., 2017), which makes them suited for life in polar regions. Despite belonging to different domains of life, picoeukaryotes and prokaryotes share those size-specific features. The surface communities remain in the upper ocean layer because they sink extremely slowly and they have a limited variability in abundance as a community, unlike bigger protists (Massana & Logares, 2013). Thus, they form a stable global ocean veil (Smetacek, 2002). Among picoeukaryotes, an unexpected diversity is increasingly being discovered (Farrant et al., 2016; Moon-Van Der Staay et al., 2001). In fact, they have repeatedly been found to be not only more abundant, but also more diverse than bigger sized protists (Elferink et al., 2017; Fenchel & Finlay, 2004; Vargas et al., 2015). Also, they appear to be more ubiquitously distributed across seas than eukaryotes of larger size fractions, so contemporary environmental conditions may indeed shape their biogeography more than historical events do (Massana & Logares, 2013), as originally proposed by Baas Becking (1934) for microbes in general.

Thus, picoplankton may be of special significance when observing the response of marine ecosystems to environmental changes. Shifts in the size structure of microbial communities in favour of picoplankton are particularly interesting to observe because of their metabolic interactions which influence ecosystem functions.

Picoplanktonic communities form a major part of the marine microbial loop, a system of production and recycling of organic matter (F. Azam et al., 1983; Pomeroy et al., 2007). The microbial loop is vital for biomass production, its turn-over and biogeochemical cycling. Phytoplankton, which comprises photoautotrophic eukaryotes and prokaryotes, fix dissolved inorganic carbon from the atmosphere via photosynthesis, produce organic material and form hence the base of the marine food web. They release dissolved organic matter (DOM; e. g. carbon, lipids and amino acids) into the ocean via different processes such as passive leakage along a concentration gradient or active exudation of info chemicals (Thornton, 2014). The DOM released by phytoplankton constitutes a major energy source for the heterotrophic picoplankton community. Other sources of DOM include sloppy feeding by zooplankton grazing on protists (Møller et al., 2003), phagotrophy, microbial cell lysis through lytic viral infections as well as excretion of waste products on all levels of the trophic food web during the carbon flux towards larger organisms. DOM production occurs directly or via an intermediate stage of particulate organic matter (POM), i. e. organic matter of a bigger size fraction, that results from the same sources via the same processes. POM is either dissolved by bacterial enzymes to DOM or exported to the deep sea via the biological carbon pump for long term storage facilitated by its higher sinking ability compared to dissolved matter (Buchan et al., 2014).

The outlined production of DOM is the first part and the driver of the microbial loop – the reincorporation into the food web by heterotrophic bacteria the second. During secondary production, the largest fraction of the available organic matter is rapidly respired and thereby turned into bacterial biomass or released as CO_2 back into the atmosphere. The carbon uptake during this process is an important step of the global carbon cycle (Azam & Malfatti, 2007). While DOM itself is not accessible by most eukaryotic marine organisms, after incorporation into bacterial biomass the organic matter can be returned into the marine food web, following the classic food chain: eukaryotic grazers such as

mixotrophic and heterotrophic flagellates and microzooplankton who prey on bacteria are in turn grazed upon by larger zooplankton, which eventually feeds fish and marine mammals. Phagotrophic eukaryotes who ingest bacteria and phytoplankton are another effective channel for the products from the base of the food web to reach higher trophic levels (Sherr & Sherr, 2002). The microbial loop therefore is a self-sustained cycle with high significance especially in the global carbon cycle (Kirchman et al., 2009).

Within the microbial loop, the cycling of organic matter is tightly coupled with the flow of mineral nutrients (Pomeroy et al., 2007). Nutrients such as nitrogen, phosphorus and silicate are remineralized by bacteria and thereby kept within the upper mixed layer of the ocean where it remains available for reusage by phytoplankton (Sigman & Hain, 2012). Phytoplankton rely on the bacterial nutrient supply for growth because bacteria can recapture nutrients from various sources, more efficiently and from lower concentrations due to their unique metabolic potential resulting from a high surface to volume ratio (Pomeroy et al., 2007).

Besides nutrient supply, bacteria also affect the amount and composition of the DOM released and thus the phytoplanktonic community composition. Phytoplankton in turn influences the bacterial community composition, drives secondary production via DOM release and determines their numbers and biomass through the rate of primary productivity (Bell et al., 1974; Thornton, 2014). The complex coupling of the communities constituting the microbial loop is furthermore shaped by higher levels of the food web as well as by abiotic environmental influences: Mixotrophic and heterotrophic flagellates and microzooplankton control the bacterial densities by feeding on them. Through POM and DOM release while sloppy feeding, protist grazing on bacteria provides the very basis their prey subsists on (Sherr & Sherr, 2002). The more complex the described interactions are, the more ecological niches are created and the more divers microbial communities tend to be. A niche for heterotrophic bacteria, for example, can be determined by the algal release of specific organic carbon (Sarmento & Gasol, 2012) while for protists, competition for nutrients and other resources influences diversity. Pro- and eukaryotes are also interlinked in their diversities through the food web: The richness and evenness of heterotrophic and mixotrophic protists can be influenced by their prey´s richness, while heterotrophic bacterial productivity and richness can be influenced by their predator´s abundance (Saleem et al., 2013).

The partitioning of prokaryotic and eukaryotic marine microbes is not only visible in their respective functions within the microbial loop and biogeochemical cycles, but these distinctions led to the development of different strategies of complexity during evolution: eukaryotes display a large array of morphological diversity resulting in a wide variety regarding structure and behaviour, such as locomotion, feeding and reproduction. They show a higher phenotypic plasticity (Keeling & Campo, 2017). Bacteria have been evolving a complexity on the level of molecules, which is visible in an unprecedented metabolic diversity. Because of their different strategies to occupy ecological niches, prokaryotic and eukaryotic microbes develop diversity at different levels (Keeling & Campo, 2017).

3 Outline of the Thesis

1.1 Diversity can be understood as a combination of species richness and evenness. To quantify and compare the alpha diversity across the study area, I infer the Hill Numbers of the Shannon und Simpson Indices and taxonomic richness of the prokaryotic and picoeukaryotic communities for each sample. I distinguish between the samples taken from the six regions North Norway, South Norway (including one Swedish fjord), Svalbard, Iceland, East Greenland and West Greenland and between the prokaryotic and picoeukaryotic communities. I depict the indices clustered per region to observe differences between the ecologically distinct regions of the study areas and use the Student´s t-test to evaluate the significance

of the differences between them. For the same six regions, I compare the main oceanographic parameters temperature and salinity and the nutrient concentrations of nitrate, phosphate and silicate to observe possible links to alpha diversity.

Across all investigated samples, I expect alpha diversity to differ among the main regions. Since they are distinct in latitudinal position, ice influence and water mass history, I expect them to have different pro- and eukaryotic diversities in adaption to different water temperatures, stratification and nutrient availability. Furthermore, I expect eukaryotic and prokaryotic diversities to co-vary, because they are ecologically strongly coupled, e. g. via the microbial loop, and provide ecological niches for each other.

1.2 I observe both geographical distance and dissimilarity of environmental parameters as potential drivers of community dissimilarity among samples (beta diversity). I will perform a pairwise Mantel test to evaluate the correlations between the pro- and eukaryotic ASV variance (genetic divergence as provided by Bray-Curtis-dissimilarity), the environmental variance (differences in temperature and salinity) and the spatial variance (geographic distance), and plot the respective distances.

I hypothesize that geographical distance is the dominant parameter influencing prokaryotic and eukaryotic ASV turnover among the main areas, while environmental parameters have comparatively less influence on this scale. Despite their high dispersal ability, I expect community turnover to increase with distance, especially since fjords provide habitats that are less well connected with each other than the open ocean.

2.1 On a regional scale, within the main regions, I also assess the influence of environmental parameters and geographical distance on beta diversity. To examine this relationship, I run a pairwise Mantel test as described in 1.2 and plot the respective dissimilarities with the samples of each region separately, both for prokaryotes and picoeukaryotes.

On this smaller spatial scale, I expect environmental dissimilarities to have a stronger influence on beta diversity than geographical distance. Nutrient concentrations and oceanographic parameters can vary widely across spatially close sites due to the characteristics of different fjords, e. g. regarding glacial influence. Glaciers shape sea surface temperatures, stratification and upwelling processes within fjords. As a consequence, nutrient availability will also vary along with these parameters. For instance, I expect that within the Svalbard region, the more Atlantic Water influenced west fjords will differ from the rather polar northern fjords.

2.2 I furthermore want to explore if the species turnover, i. e. beta diversity, is higher across or within the different regions. I implement non-metric multidimensional scaling (NMDS) analysis, which allows to depict the variance in diversity (based on Bray-Curtis-dissimilarity) across and within regions for prokaryotes and picoeukaryotes, respectively. The resulting ordination plots visualize the extent of compositional difference among all samples in relation to each other.

I expect that samples from the different regions will cluster together and be distinct from each other. Beta diversity reflects differences in the environmental regimes. Picoplankton in particular can flexibly and rapidly respond to even minor differences in the environmental regime because of their short generation time and large population sizes. The samples from the ecologically distinct regions observed here might therefore be reflected in the degree of taxonomic turnover. For example, the temperate climate of southern Norway may be separated by a higher ASV turnover from the polar influenced fjord waters of Greenland and Svalbard.

3.1 Finally, I will explore the beta diversity across the tip and mouth fjord communities of the different regions. By employing NMDS analysis, as described in 2.2, I visualize how the different samples cluster together in their compositional similarity.

Among the stations inside of each fjord, I expect the community turnover to be higher than among the stations outside (in its mouth). Along the mouth regions of the fjords, ocean streams mix water masses more effectively than in fjord tips. Furthermore, the environmental conditions inside of each fjord are more unique than in the fjord mouth regions, dependent on possible glacial or anthropogenic influence. I presume that fjords with glacier estuaries in their tips will show more pronounced biodiversity differences between tip and outer station compared with fjords without glaciers.

4 Materials and Methods

4.1 Study Area

The samples analysed in my study were taken during five expeditions in Arctic and subarctic coastal waters and fjord waters in the Arctic and northern hemisphere spring and summer. The expeditions took place between 2012 and 2019 with the research vessels RV Maria S. Merian (MSM) and RV Heincke (HE). These studied areas provide a habitat shaped by a strong seasonality: ice cover and low light availability during winter and freshwater influence from thawing glaciers and permafrost and the resulting strong stratification during summer. Two main water masses influence most sampled locations: cold and low salinity polar water and warmer and more saline Atlantic Water. The samples cluster together in six spatially and ecologically distinct regions: North Norway, South Norway, Svalbard, Iceland, East Greenland and West Greenland (Fig. 1). In my analysis I will investigate differences and similarities between these regions and their different fjord systems. In the following, I will introduce each region individually.

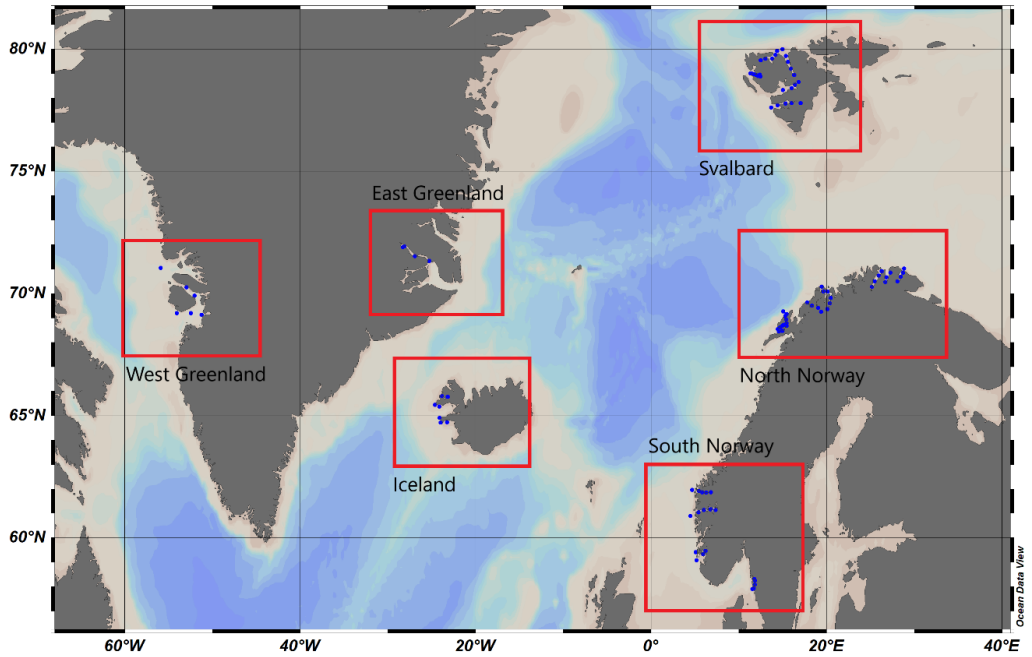

Fig. 1: *Study area, partitioned into regions, sites marked blue. Regions are indicated with red boxes and labelled accordingly.*

4.1.1 North Norway

The studied field of North Norway includes the five fjords Balsfjord, Lyngenfjord, Porsangerfjord, Laksefjord and Tanafjord as well as the Lofoten archipelago (Fig. 2). The fjords are subglacial with no glaciers in their tips, but receive surface freshwater input from rivers and freeze during the winter period. The fjords are largely influenced by the Barents Sea and partly by the Norwegian Sea. The three northernmost fjords have the strongest polar influence from the Barents Sea within North Norway. The Lofoten are not a fjord system, but open towards the ocean and strongest influenced by the Norwegian Sea. They have a more temperate climate and higher water temperatures than the fjords in this region. Because of the different environmental settings within North Norway, it serves as an interesting example to explore the diversity of prokaryotes and picoeukaryotes in a (compared to all samples) spatially close but environmentally differing location. North Norway was sampled on the expedition HE533 between 23.05.2019 and 04.06.2019. The Lofoten were additionally sampled on the expedition HE431 between 24. and 25.08.2014.

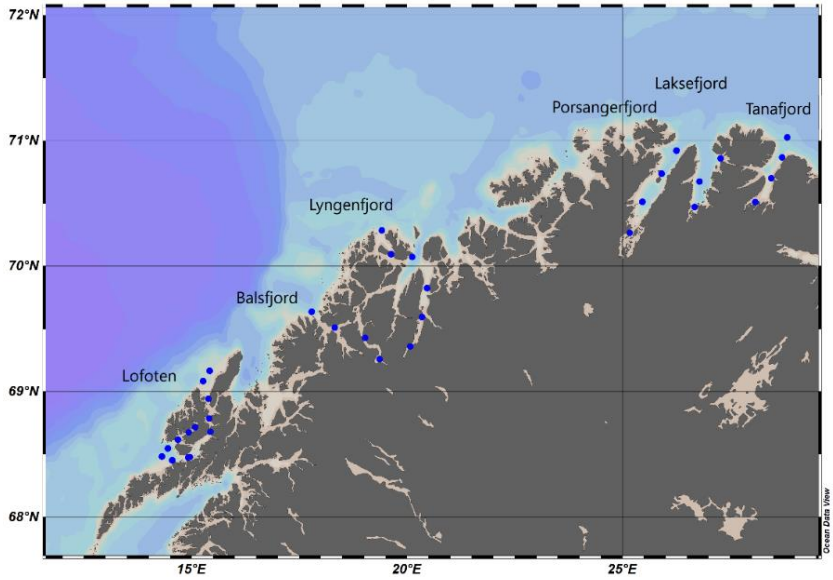

Fig. 2: *Sampled stations of North Norway. Samples are indicated by blue dots and fjords are labelled accordingly.*

4.1.2 South Norway

To the South Norwegian fjords Nordfjord, Sognefjord and Boknafjord and the Swedish Orust-Tjörn fjord system sampled, I will collectively refer to as South Norway hereafter (Fig. 3). The fjords in this region are characterized by a temperate climate and a moderate seasonality, climatically resembling the Lofoten. Nordfjord and Sognefjord have a clear fjord structure and are the deepest fjord systems within this study. Including this region into this comparative analysis might highlight the typical polar features of the Arctic and subarctic regions in this study. Furthermore, it allows to reveal the influence on diversity the fjord structure itself has, independently from the climatic setting. South Norway was sampled on the expedition HE431 between 28.08.2014 and 06.09.2014

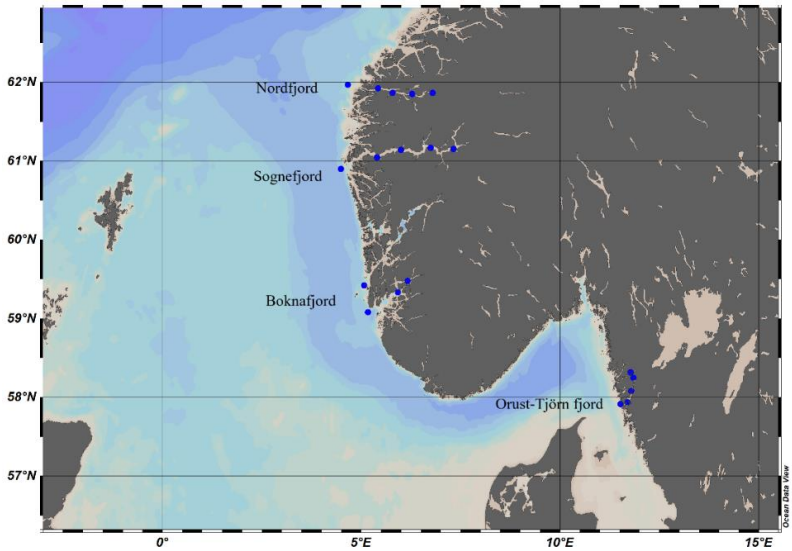

Fig. 3: *Sampled stations of South Norway. Samples are indicated by blue dots and fjords are labelled accordingly.*

4.1.3 Svalbard

Within the Svalbard archipelago, Van Mijen Fjord, Isfjord, Kongsfjord, Woodfjord and Wijdefjord were sampled (Fig. 4). Among the regions investigated in this data set, Svalbard comprises the most northern and most strongly polar-influenced fjords. The fjords are influenced by melting sea ice and melting and calving glaciers at their tips in summer, creating surface stratification but also upwelling through subglacial freshwater discharge (Svendsen et al., 2002). Along the western coast of Svalbard, Atlantic (West Spitsbergen Current) and Arctic Water flow. In the course of the summer, Atlantic Water increasingly intrudes into the fjords (Cottier et al., 2005). Among the different fjords of Svalbard, the northern Woodfjord and Wijdefjord are stronger influenced by Arctic Water than the western Kongsfjord, Isfjord and Wijdefjord. Svalbard serves in this analysis as a typical Arctic habitat, with more and less polar influenced fjords within it. Samples were taken on the expedition HE492 between 03.08.2017 and 16.08.2017. The Kongsfjord was additionally sampled on the expedition MSM56 on 03. and 04.07.2016.

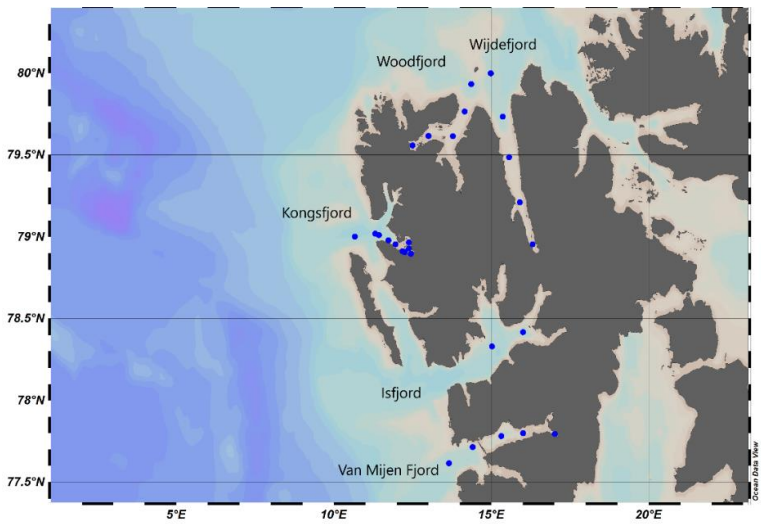

Fig. 4: *Sampled stations of Svalbard. Samples are indicated by blue dots and fjords are labelled accordingly.*

4.1.4 Iceland

In Iceland, samples were taken in Arnarfjörður, Breiðafjörður and Faxaflóe (Fig. 5). The coastal structure of Iceland is very open and thus resembles more the Lofoten area of North Norway than other northern regions of this study. The investigated fjords are open towards the open ocean and strongly influenced by Atlantic currents flowing northwards. Therefore, the water temperature and salinity is most similar to the South Norwegian region, despite being located almost on the polar circle. Iceland was sampled on the expedition MSM21/3 between 05. and 08.08.2012.

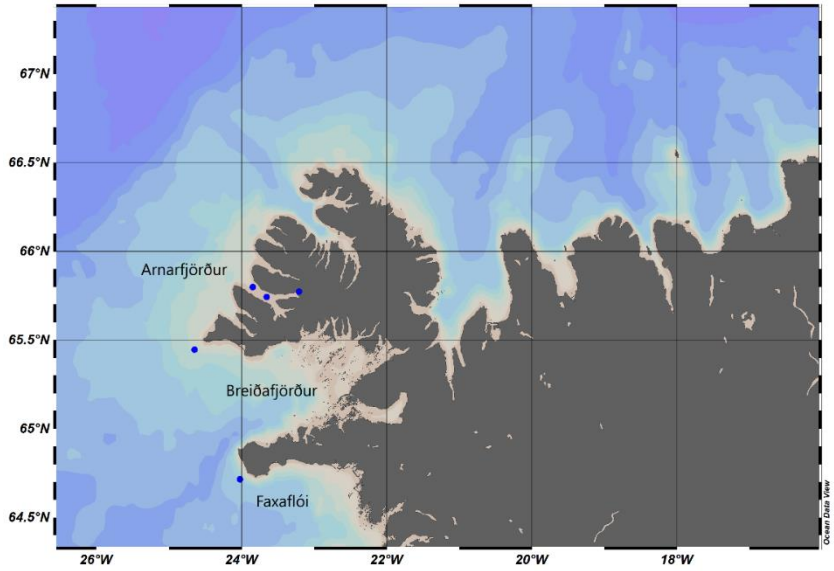

Fig. 5: *Sampled stations of Iceland. Samples are indicated by blue dots and fjords are labelled accordingly.*

4.1.5 East Greenland

In my study, only one fjord in eastern Greenland, the Nordvestfjord within Scoresby Sund, was sampled (Fig. 6). The Scoresby Sund fjord system is the largest in the northern hemisphere. The Nordvestfjord receives large amounts of meltwater from the inland glaciers, both into the surface and through subglacial discharge. From the open ocean both Atlantic and polar water intrude into the fjord system. East Greenland was sampled on board MSM56 between 12. and 17.07.2016.

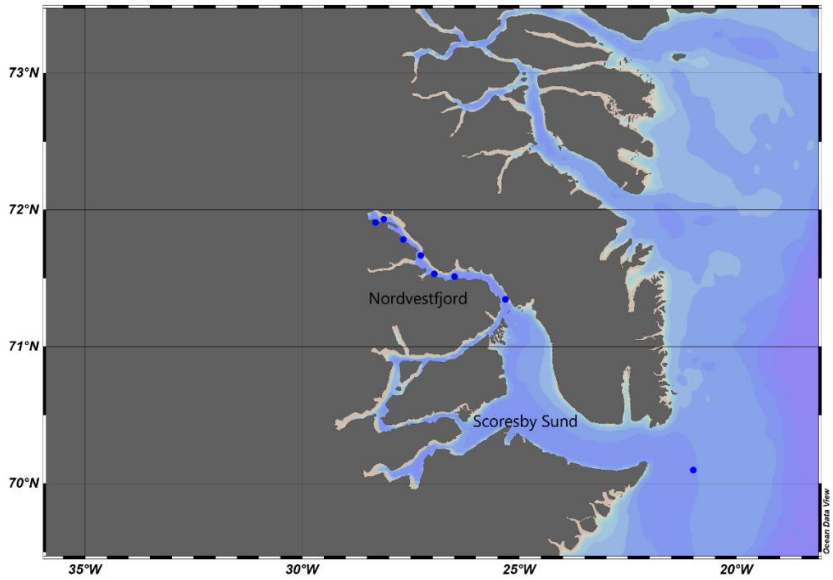

Fig. 6: *Sampled stations in East Greenland. Samples are indicated by blue dots and fjords are labelled accordingly.*

4.1.6 West Greenland

In West Greenland, samples were taken in Disko Bay, Sullorsuaq Strait and Baffin Bay (Fig. 7). The sampled waters surrounding Disko Island are strongly influenced by meltwater from the Greenland ice shield, especially from the Ilulissiat glacier (Meire et al., 2017), and due to their exposure to the open ocean also by Atlantic and polar waters. The Ilulissiat glacier is a tidewater glacier terminating into these coastal waters. It is probably the most active polar glacier on the planet, causing strong upwelling and thus turning the Disko Bay waters into a very productive area supporting huge fish, bird and whale populations. West Greenland was sampled on board MSM21/3 on 27. and 28.07.2012.

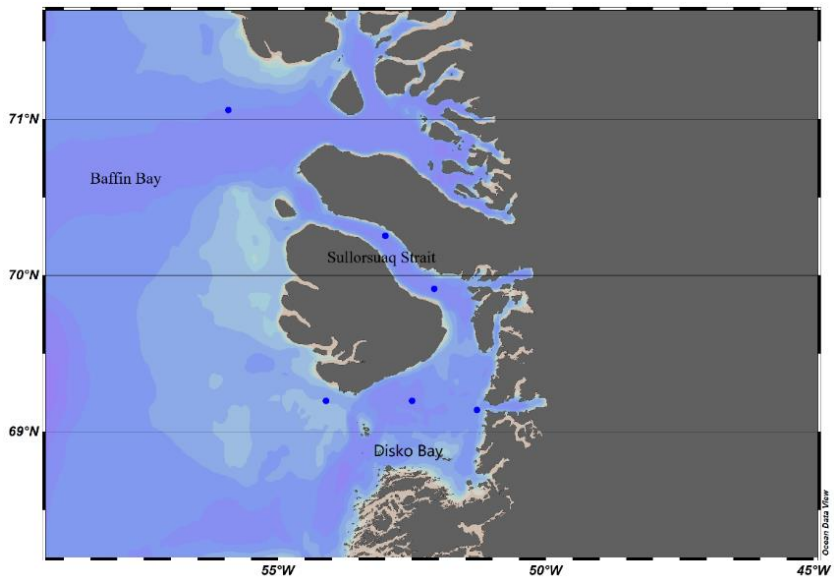

Fig. 7: *Sampled stations of West Greenland. Samples are indicated by blue dots and fjords are labelled accordingly.*

4.2 Sampling

Samples were taken during five different expeditions between 2012 and 2019. Sea surface water (3m to 30m below surface) was pooled, a depth corresponding to the photosynthetically active layer. On the expedition HE533 water from 0m - 40m below surface was used. Water samples were filtered with a peristaltic pump through a 3µm sized filter comprising a total volume of 20 L during expedition HE492, 15 L during expedition MSM56 and 12 L during expeditions HE431 and HE533, respectively. All water samples followed a second filtration step through a 0,2 µm filter to obtain the picoplankton biomass. The filters were then immersed into lysis buffer and ceramic beads from the DNA extraction kit (see below) were added. After being treated with liquid nitrogen for a few seconds, the samples were stored at -80 °C until further processing. The samples taken on the expedition HE533 in North Norway were taken as triplicates which were treated as independent samples in all subsequent processing and analyses. Overall, 149 prokaryotic and 156 eukaryotic samples were analysed, including the triplicates.

4.3 DNA Extraction and Quantification

4.3.1 DNA extraction

To analyse the genetic diversity within the samples, the DNA has to be isolated from the filters first. This is done during the DNA extraction. I performed the DNA extraction only for prokaryotes and eukaryotes of the expedition HE533, since for all other samples DNA extracts or already sequenced samples were provided. For DNA extraction, I used the „Genomic DNA from soil“ (NucleoSpin® Soil) kit following the manufacturer´s protocol (https://github.com/CoraHoerstmann/Arctic_picos/blob/master/DNA_extraction_protokoll.pdf) with minor modifications. For the sample lysis step I didn´t deploy a vortexer for breaking up the cells with the beads, but used a bead beater (MagNA Lyser, Roche).

During the silica based extraction procedure, the cells were (1) eluted from the filters and broken up with ceramic beads to access the DNA. (2) The nucleic acid component was bound to a silica membrane by adding chaotrophic salts, which remove the hydrate shell of the DNA molecules. This allows the sugar phosphate backbone of the DNA to bind to the silica membrane instead. (3) With the DNA securely attached to the membrane, non-nucleic-acid components such as proteins, polysaccharides and PCR inhibitors (e.g. humic acids) can be removed from the sample. The purification is done in several washing steps in the presence of salt and alcohol, who prevent the reformation of the hydrate shell and hence the DNA from being washed off the membrane. (4) Then the alcohol is removed as to not interfere with the subsequent processing and the isolated and purified DNA is eluted off the silica membrane under low salt conditions.

4.3.2 DNA Quantification with Spectrophotometry

I quantified the extracted DNA in the samples regarding purity and concentration with a NanoDrop™ 1000 UV-VIS spectrophotometer, which measures the light absorbance of a given sample. If the measured absorbance curve shows an absorption maximum at 260 nm and an absorption minimum at 230 nm, which is typical for pure DNA, the presence of DNA in the sample can be verified (Supplement Fig. 33). If the absorbance spectrum differs, like in my analysis, the samples may be contaminated with proteins or humic acids, indicating an insufficient purification during the extraction (Fig. 8). Also, the DNA concentration may be too low to be detected by the device. The NanoDrop measures the DNA concentration in ng/µl based on the absorbance at 260 nm. Naturally, this calculation will deliver misleading results if contaminants contribute to a higher absorbance at this wavelength than the pure

DNA would show. The NanoDrop therefore calculates two purity ratios. (1) The $A_{260/230}$ value is above 2 for pure DNA. If the sample is contaminated, there is no absorption minimum at 230 nm, thus the $A_{260/230}$ value is too low and it can be assumed that the DNA concentration measured by the NanoDrop is likely higher than it actually is. (2) The $A_{260/280}$ purity ratio indicates protein contamination if below 1.8, since proteins absorb at 280 nm.

According to the concentrations measured by the NanoDrop (Supplement Table 1), I normalized all samples to DNA concentrations of 5ng/µl. Since the absorption curves and purity ratios in my analysis suggested very low DNA concentrations and possible contaminations (Fig. 8), I decided to verify the presence and estimate the yield of DNA additionally with agarose gel electrophoresis.

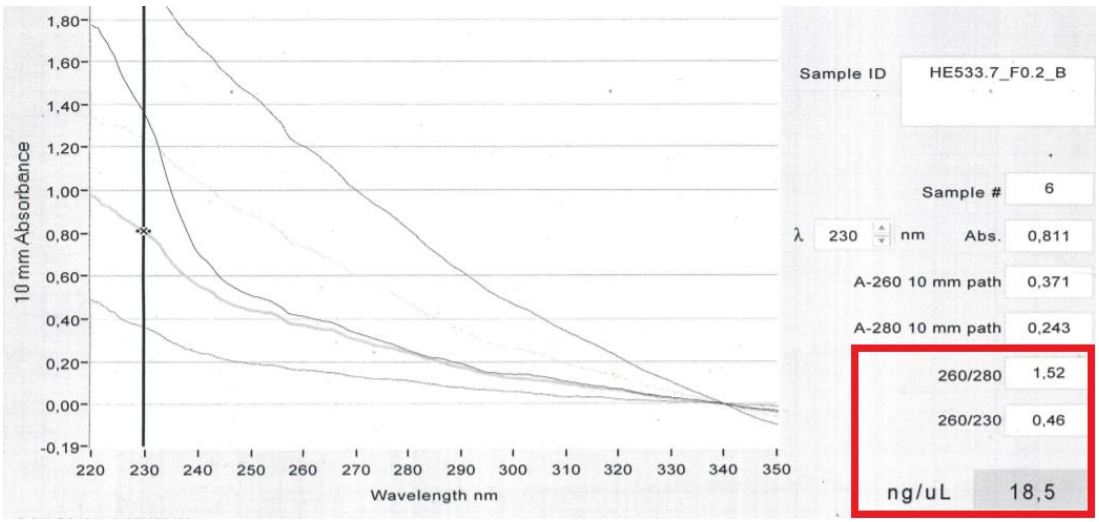

Fig. 8: *Spectrophotometric absorption curves, DNA concentration and purity ratios of five exemplary samples, as measured by the NanoDrop. Ratios and final concentration are indicated with a red box.*

4.3.3 DNA quantification with gel electrophoresis

I ran the extracted DNA on a 1% agarose gel to validate the successful extraction (Supplement Fig. 34-36), an exemplary result is depicted in Fig. 9. During the process, an electric field is applied to the gel which causes the slightly negative charged DNA fragments to move through the gel towards the cathode. Thereby, the DNA fragments are being sorted according to their length, since shorter fragments move faster through the pores of the gel than larger ones. To assess the size spectrum of the DNA in the sample, a mix of fragments of known length, i. e. a "ladder", is run alongside the samples. As loading dye, ethidium bromide was added to the gel which intercalates in the DNA molecules and makes the bands visible by fluorescing in UV-light afterwards.

For observing the gel electrophoresis results, I used a gel electrophoresis documentation system with an integrated camera (type B-1393-3U7N), which didn´t function properly and didn't reliably deliver good pictures. Later on, I preferred looking at the gels on a transillumination UV-table, taking pictures with a smartphone camera. Since neither technique provided adequate pictures, I only relied on the notes in my lab book for further processing, where I classified every band as either "no", "weak" or "yes". Therefore, the pictures shown here are not complete and may not always conform to the actual results, since weak bands were often undetectable.

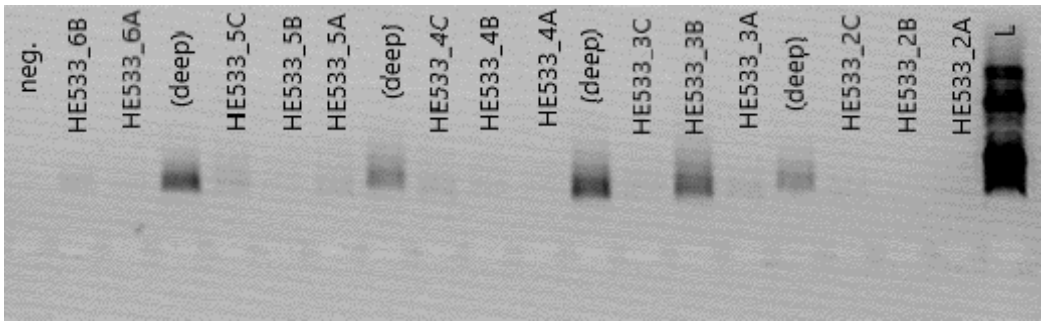

Fig. 9: *Gel bands of the extracted DNA of 19 exemplary samples of the HE533 cruise. The "L" indicates the ladder.*

4.4 Library Preparation and Sequencing

To determine the genetic diversity of the different samples, I targeted specific regions of the extracted gene sequences as molecular markers. These amplicons allow the comparison of different sites regarding their compositional diversity. Here, I use the hypervariable V4 region in the gene of the small subunit of the 16S ribosomal RNA for prokaryotes and 18S ribosomal RNA for eukaryotes. The V4 region has highly evolutionary conserved parts and can be targeted with the same primers in most taxa, but also includes highly variable sections allowing a high taxonomic differentiation and resolution. To unravel the taxonomic diversity within an environmental sample, the V4 region of all present genetic material is selected with specific primers, isolated and amplified during the library preparation for sequencing to obtain the exact genetic code of the amplicons. I did the library preparation for prokaryotes of the expeditions MSM21/3, MSM56, HE431 and HE533 and for eukaryotes of the expeditions MSM21/3, HE431 and HE533.

Library preparation for both 16S and 18S rDNA was conducted using the manual „Metagenomic sequencing library preparation. Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System" (Illumina Technology, https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf) using the following primers to obtain both prokaryotic and eukaryotic V4 amplicon libraries: Primers were chosen in accordance with the Earth Microbiome Project (https://earthmicrobiome.org/) to ensure comparability of my results with the results of other studies. I used the primers M5-V4_806R-1 (Apprill et al., 2015) and M5-V4_515F-N (Parada et al., 2016) for the 16S library and V4R and V4F for the 18S library (Stoeck et al., 2010) with slight modifications (Supplement Fig. 37, Geisen et al., 2019; Piredda et al., 2017)

The library preparation consists of two polymerase chain reaction (PCR) steps. In the first PCR the prokaryotic and eukaryotic V4 regions are targeted by specific primers and amplified. In the second PCR sample specific indices are attached to the amplicons while amplification to tag sample origination and attach illumina specific primers. The prepared 16S and 18S rDNA libraries are ready for sequencing.

4.4.1 Amplicon PCR

The aim of the Amplicon PCR is the isolation and amplification of the V4 regions of the 16S and 18S rDNA, respectively, resulting in the so-called amplicons. During the PCR, the sequences of the primers tailored to complement the forward and reverse strands bordering the desired region bind to all V4 gene

sequences present in the sample to mark the segments to be amplified. Attached to the 5´ end of the primer sequences are overhang adaptor sequences that will be needed during the Index PCR.

For the Amplicon PCR I used the undiluted extracted DNA, after all, because a first test Amplicon PCR with samples diluted according to the concentration measured by the NanoDrop in step 3.3.2 showed positive amplification as indicated by a gel electrophoresis result only in 2 out of 6 samples (Supplement Fig. 38). The NanoDrop is known to measure samples with a concentration below 50 ng/µl inaccurately which is why I presumably ended up with falsely normalized aliquots. From now on, samples with NanoDrop concentrations above 50 ng/µl were diluted 1:5 to exclude inhibitors and avoid a rapid decline in primer and dNTP concentration during PCR. Samples with NanoDrop concentrations below 50ng/µl were used undiluted. Now the Amplicon PCR worked well. All PCR products were checked on a gel to ensure a positive result (Supplement Fig. 39-43), as is exemplarily depicted in Fig. 10. For the samples without a PCR result, I repeated the amplification with 30 cycles instead of the 25 cycles as suggested in the protocol and used the undiluted samples in addition to a PCR with an aliquot diluted 1:10 to reduce the concentration of inhibitors, resulting in successful amplification (Fig. 10). The PCR products were cleaned up according to the protocol, using magnetic beads (AMPure XP beads), to remove the PCR reactants.

The PCR has an inherent bias that can distort the result. The performance of the PCR depends strongly on primer choice, since most primers don´t amplify all taxonomic groups equally readily, resulting in over- or underrepresentation of some groups (Parada et al., 2016). For example, the primers used here were found to underrepresent haptophytes in their original form and were modified accordingly (Geisen et al., 2019).

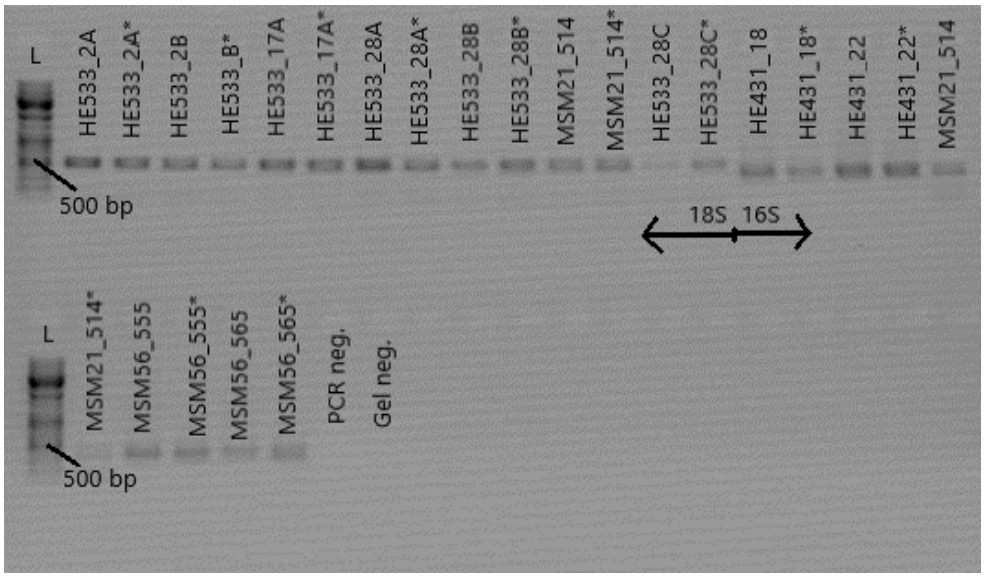

Fig. 10: *Gel bands of Amplicon PCR products of 18S and 16S samples. The ladder (L), the PCR negative control (PCR neg.) and the gel electrophoresis negative control (Gel neg.) are indicated. Each sample was PCR amplified twice: undiluted and * diluted 1:10.*

4.4.2 Index PCR and Quantification

During the Index PCR, multiplexing indices are attached to the overhang adapter sequences on the primers enclosing the amplicons. Thus, the fragments of each sample are tagged differently (Supplement Table 2). The indices are sequenced along with the amplicon, so each amplicon sequence can later be

assigned to the sample it originated from. I completed the Index PCR according to the protocol, followed by another clean-up step to remove the PCR reactants.

In preparation for the final pooling step, DNA concentration was determined in [ng/µl] with LabChip® GX Touch HT™ (Perkin Elmer. Supplement Table 2). The LabChip® also identifies the average length of the DNA fragments, which was 600 base pairs for the 18S amplicons and 463 base pairs for the 16S amplicons, including the overhang adapters and multiplexing indices. I verified the successful index PCR by comparing for six exemplary samples the fragment length of the Index PCR product to the fragment length of the Amplicon PCR product. It clearly displayed a difference in fragment length before and after the Index PCR, thus with and without indices (Supplement Fig. 44).

For sequencing, the samples of a library need to be pooled into one sample so they can be sequenced simultaneously. To make the samples and the read proportions within them comparable afterwards, they have to be normalized to equal DNA concentrations before being pooled. I prepared 4nM aliquots based on the DNA concentrations in [nM], which were calculated with the following formula:

$$\frac{DNA\ conc.\ [ng/\mu l]}{\frac{660g}{mol} * fragment\ length\ [bp]} * 10^6 = DNA\ conc.\ [nM]$$

To calculate the sample volume needed to arrive at 20 µl aliquots with a concentration of 4 nM, I used the following formula:

$$\frac{4\ [nM] * 20[\mu l]}{DNA\ con.\ [nM]} = sample\ volume\ [\mu l]$$

10µl of the 4nM aliquots were combined to a prokaryotic and a eukaryotic pool, respectively.

4.5 Sequencing

The amplicon libraries were sequenced using a MiSeq Sequencer (Illumina). The 16S rDNA amplicon libraries were sequenced at the Alfred-Wegener-Institute in Bremerhaven, Germany, and 18S rDNA PCR products were sequenced at the Leibniz Institute on Aging (FLI) in Jena, Germany using the MiSeq Reagent Kit v3 (600-cycle) MS-102-3003, respectively. They were sequenced with the paired-end approach, meaning that each amplicon is sequenced from both ends simultaneously. The forward and reverse reads generated for each amplicon are later aligned and allow a more precise result than in single read sequencing. In each direction, 300 base pairs are sequenced which includes an overlap of approximately 100 bp in eukaryotes and 40 bp in prokaryotes. Raw sequences will be submitted along with the metadata to https://www.ebi.ac.uk/ena.

4.6 ASV Table

From here on, all subsequent analyses are conducted in RStudio (R Core Team, 2019; version 3.6.2, RStudio version 1.2.5033). Code is deposited at https://github.com/CoraHoerstmann/Arctic_picos.git.

Samples were demultiplexed, i. e. the reads belonging to one sample sorted according to their indices, and transferred into fasta files. For each independent sequencing run (including previously sequenced 16S samples from the expeditions HE492, MSM21/3, MSM56 and HE431 expeditions and samples from the MSM56 and HE492 expeditions for eukaryotes, respectively), sequencing runs were individually processed as follows.

The raw reads were quality filtered to remove non-DNA characters such as primer sequences, adapters and PCR artefacts. Primers were removed using Cutadapt version 1.18 (Martin, 2011). Sequence reads were dereplicated and forward and reverse reads were merged with a minimum overlap of 20 bp. ASV tables were constructed and potential chimeras were de-novo identified (removeBimeraDenovo command) and removed. For prokaryotes and eukaryotes, ASV tables from different expeditions were merged using the mergeSequencetable command, resulting in single ASV tables for 16S and 18S, respectively, with ASVs as rows and samples as columns.

4.7 Rarefaction

As part of a quality control of sample sequencing, I made rarefaction curves for all sites using the *rarecurve* function in the package *vegan* (Oksanen et al., 2019). Rarefaction curves show if enough reads were sequenced to retrieve the majority or all taxa present, i. e. if the sequencing depth was sufficient to be representative of the microbial community. The sample size based curve displays the number of detected taxa as it grows with growing sample size. Ideally, it eventually reaches an asymptote when taking into account more reads doesn´t anymore deliver new taxa or, in this case, ASVs. Comparisons between samples or groups of samples are only meaningful, if similar proportions of the microbial community are taken into account. Most samples of my data set showed a sufficient sequencing depth to deliver relevant results in the further analyses (Supplement Fig. 45-57). Exceptions (eukaryotic sample HE533_13B and prokaryotic samples MSM21_540, HE431_15, HE431_24 because of insufficient sampling depth) were removed from the analysis.

4.8 Diversity Measures and Nutrient Concentrations

I aim to compare the alpha diversity, i. e. species richness and evenness, of the pro- and eukaryotic communities in the investigated regions. To make it comparable, diversity can be quantified by calculating certain indices that reflect different aspects of community structure. To characterize the communities and regions in my study, I chose richness, i. e. total ASV numbers, the Shannon index and the Inverse Simpson index. These diversity measures can be expressed by the corresponding Hill numbers ${}^{q}D$ of different orders of q: ASV richness (q = 0), Shannon diversity (q = 1, effective number of common species) and the reciprocal of the Simpson index (q = 2, the effective number of dominant species).

$$ {}^{q}D = \left(\sum_{i=1}^{S} p_i^q\right)^{1/(1-q)} $$ (Hsieh et al., 2016).

I calculated the Hill numbers with the R package *iNEXT* (Hsieh & Chao, 2020) Hereafter, I will use the richness estimates and the Inverse Simpson for further analysis and supply the Shannon diversity supplementary. To depict the variation of the alpha diversity measures among regions, I plotted them with the function *ggplot* (Wickham, 2009) in R.

The nutrient concentrations and environmental parameters were provided from the cruise reports and the corresponding PANGEA depositories. To compare them among regions and observe correlations with the diversity measures, I plotted them with the function *ggplot* (Wickham, 2009) in R and with Microsoft Excel 2003.

4.9 T-Test

To determine if the difference in alpha diversity between regions and between prokaryotes and eukaryotes is statistically significant, I implemented the independent two group t-test, i. e. Student´s t-test. It compares the means of the diversity measures of two groups. The Student´s t-test is the statistical test of choice, if the two groups compared are unrelated and all samples only belong to either group, which applies to my data. The hypothesis H_0 was that there is no effective difference between the respective two groups tested. The H_0 can be rejected, i. e. a significant difference between groups can be assumed, if the p-value calculated by the t-test is below 0.05. To run the test, I used the function *t.test* in R. The results can be found in the Supplement Table 3-5.

4.10 Dissimilarity Calculations and Mantel Test

To determine what influences beta diversity, i. e. ASV turnover, between sites and regions, I aimed to compare differences in compositional variance, geographical distances and environmental dissimilarity. Before testing for statistical correlation between those dissimilarities, they have to be quantified.

ASV turnover refers to the differences in community composition between two sites, including addition and removal of species and changes in the absolute species number and relative abundances. The Bray-Curtis-Dissimilarity coefficient takes these aspects into account and calculates for a given pair of sites a value between 0 (identical composition) and 1 (no shared taxa). It is based on raw abundance data, that is absolute counts. For each ASV it adds up the differences between its counts in each site and divides it by its overall count sum (Goslee, 2010). The resulting value can be used to compare the turnover between multiple pairs of sites and to compare compositional turnover with changes in other parameters between sites. I calculated Bray-Curtis-Dissimilarities in R with the function *vegdist* in the package *vegan* (Oksanen et al., 2019).

The environmental dissimilarity is metric and can therefore be calculated as Euclidean distance between all pairs of sites with the *vegdist* function of the package *vegan* (Oksanen et al., 2019). For the geographic distance, I used the function *geodist* in the package *geodist* (Padgham, 2019). It transforms the geographic coordinates of the sites into geodesic distances that represent distances on the Earth´s surface taking into account its curvature.

The derived distance matrices can be tested for correlations between them with the Mantel test. The Mantel test uses the Pearson product-moment correlation to assess the statistical significance of the correlation. It permutes rows and columns of the distance matrices to calculate how well the given variables fit a linear regression when plotted against each other. It expresses the correlation with the Pearson correlation coefficient *r* which ranges between 0 (no association between the variables) and 1 (perfect fit) and delivers a *p*-value.

4.11 NMDS Analysis

Nonmetric-multidimensional scaling (NMDS) visualizes beta diversity among sites. It ranks each site according to its compositional similarity in relation to all other sites. Unlike the multidimensional arrangement when theoretically considering the relationships between all sites at the same time, the calculated rank orders can be collapsed into a two-dimensional space, where sites cluster closer together the more similar their taxonomic composition is. The technique only visualizes ordinations of variables and no true distances of a particular unit.

Before visualizing the samples´ compositional similarity with NMDS, I normalized the abundance data with the Hellinger transformation to account for compositionality of sequencing data (Gloor et al., 2017). The transformation prepares the ASV counts for ordination by changing each total count to the percentage of the overall counts of the respective ASV it presents. Then, the square root is taken for each value. Thus, less weight is given to rare ASVs which prevents the result from being distorted by samples whose rare proportion has been retrieved less thoroughly. As a cut off for low abundance ASVs, a row sum of 5 was chosen for prokaryotes, and 1 for eukaryotes, respectively. A higher cut off for eukaryotes made the NMDS plot meaningless (and the stress too low), because they have more rare species that account for a significant proportion of the overall counts and can´t be left out of the analysis.

I performed NMDS with the transformed abundance data using the function *metaMDS* from the package *vegan* (Oksanen et al., 2019) in R. The analysis is based on Bray-Curtis-Distances which are calculated during the process. The function then calculates different configurations in multiple runs to fit the rank ordinations in a two dimensional space until the stress is minimized, i. e. the solution best preserving the original rank orders is reached.

5 Results

5.1 Alpha Diversity

Alpha diversity is determined by species richness and evenness. To assess the variation of alpha diversity across regions, I depicted ASV richness, i. e. total ASV numbers per sample, and the Inverse Simpson index as boxplots for comparison and used the Student´s t-test to assess if the differences are statistically significant. Additionally, the Shannon index is supplied (Supplement Fig. 58), but not included into the analysis since the aim is to relate the total ASV numbers to the heterogeneity of dominant ASVs, which is best covered by richness and the Inverse Simpson.

5.1.1 Alpha Diversity across regions: Richness

Richness clearly varies in total ASV numbers and in its range among regions and among prokaryotes and eukaryotes (Fig. 11). Among eukaryotes, the North Norway and West Greenland samples have an overall lower richness that is significantly different from all other regions (Student´s t-test, $p < 0.05$), but not from each other (Fig. 11). South Norway eukaryotes have a significantly higher richness than all regions but Iceland (Student´s t-test, $p < 0.05$). Eukaryotic richness ranges from numbers as low as 15 in North Norway up to 1224 in South Norway. Among prokaryotes, all Norwegian samples have a significantly higher prokaryotic richness than the other regions (Student´s t-test, $p < 0.05$) and the Svalbard prokaryotic richness is the lowest among all samples, though not statistically significantly different (Fig. 11).

Within North Norway and Svalbard there are sites that differ fundamentally from the majority in ASV richness: In Svalbard, where prokaryotic richness is lowest of all regions, most samples differ only slightly within a particularly narrow range (183-332), especially considering the high number of stations. However, I found an exceptionally high prokaryotic richness in the Kongsfjord stations sampled on the expedition MSM56 (Supplement Fig. 59) compared to the Kongsfjord samples taken on the expedition HE492 (shown as outliers in Fig. 59). In North Norway, I identified outliers in eukaryotic richness within four stations of the Lofoten showing exceptionally high eukaryotic ASV numbers of up to 1062, thus ranging among the richness observed in South Norway (visible as outliers in Fig. 11). An exceptionally low eukaryotic richness (<30 different ASVs) is observed in one station inside Balsfjord and two of the stations in the coastal archipelago outside Lyngenfjord (visible as outliers in Fig. 11).

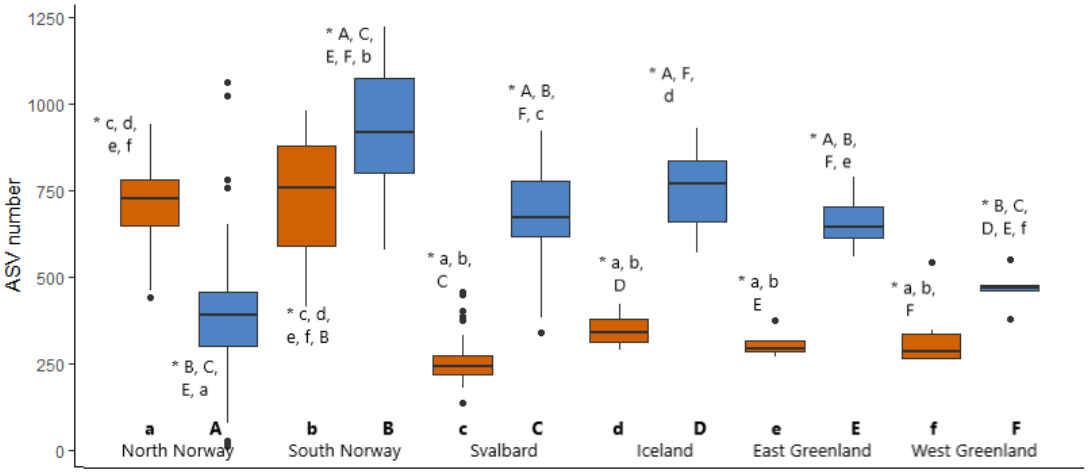

Fig. 11: *Ranges in ASV richness of pro- and eukaryotic communities per region. Lower case letters and orange colouring mark prokaryotic and capital letters and blue colouring mark eukaryotic results. *The letters indicate to which other boxplot the respective boxplot is statistically significantly different, as calculated with the Student´s t-test.*

To explore possible determinants of ASV richness, I related ASV richness to the environmental parameters temperature and salinity and to the nutrient concentrations of phosphate, silicate and nitrate and performed a regression analysis. For salinity, nitrate and silicate no or minor correlations to richness were observed (Fig. 14, Supplement Fig. 60-61). Temperature correlates significantly with eukaryotic (regression analysis, R^2 = 0.38) and prokaryotic richness (regression analysis, R^2 = 0.25, Fig. 12). Phosphate also correlates significantly, though weaker than temperature, with prokaryotic richness (regression analysis, R^2 = 0.37, Fig. 13). Nitrate correlates significantly with prokaryotic richness (regression analysis, R^2 = 0.23, Fig. 14).

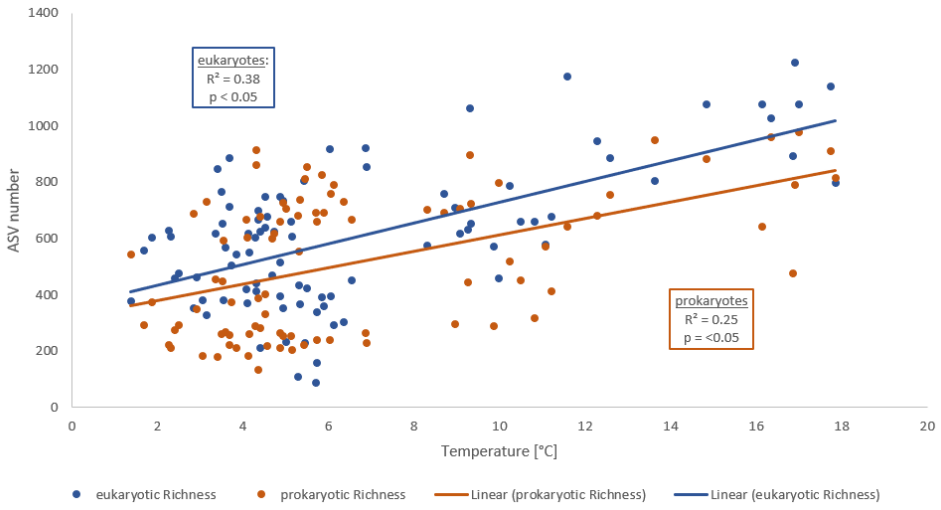

Fig. 12: *Temperature versus eukaryotic and prokaryotic richness. R^2 and p-value are indicated.*

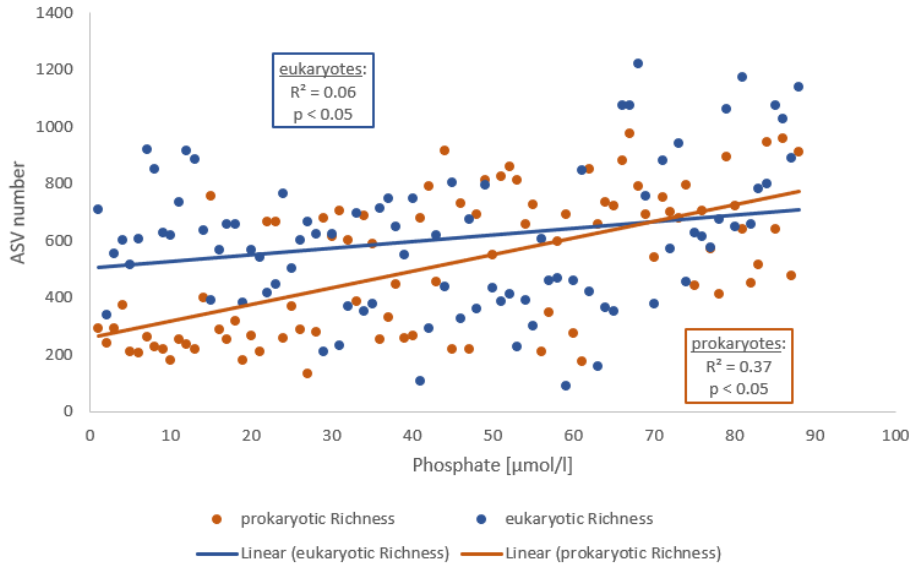

Fig. 13: *Phosphate concentration versus eukaryotic and prokaryotic richness. R^2 and p-value are indicated.*

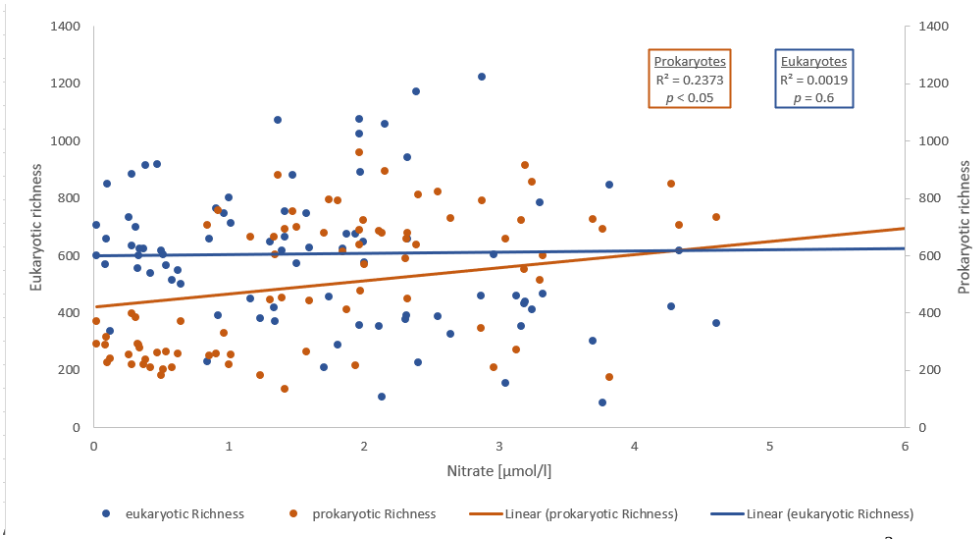

Fig. 14: *Nitrate concentration versus eukaryotic and prokaryotic richness. R^2 and p-value are indicated.*

5.1.2 Alpha Diversity across regions: Diversity

To further characterize the regions regarding their alpha diversity, I calculated the Inverse Simpson index, which is strongly influenced by ASV evenness. I depicted the values as boxplots for comparison and used the Student´s t-test to assess if the differences are statistically significant.

Not only richness, but also Simpson index values vary across regions (Fig. 15). Among eukaryotes, Iceland has the highest evenness (up to a value of 127), which is significantly distinct from North Norway, Svalbard and East Greenland (Student´s t-test, $p < 0.05$). These three regions in turn showed the lowest Inverse Simpson. Iceland displayed the widest range in eukaryotic Inverse Simpson (28-270), despite the low number of samples included, while East Greenland has the most narrow range (5-33; Fig. 15). Among prokaryotes, South Norway, which also has the highest richness, has the highest evenness (up to 85) and is significantly distinct from all other regions except Iceland (Student´s t-test,

$p < 0.05$). The Greenland regions have the lowest Inverse Simpson. North Norway has the highest variability of Inverse Simpson and West Greenland the most narrow range (Fig. 15).

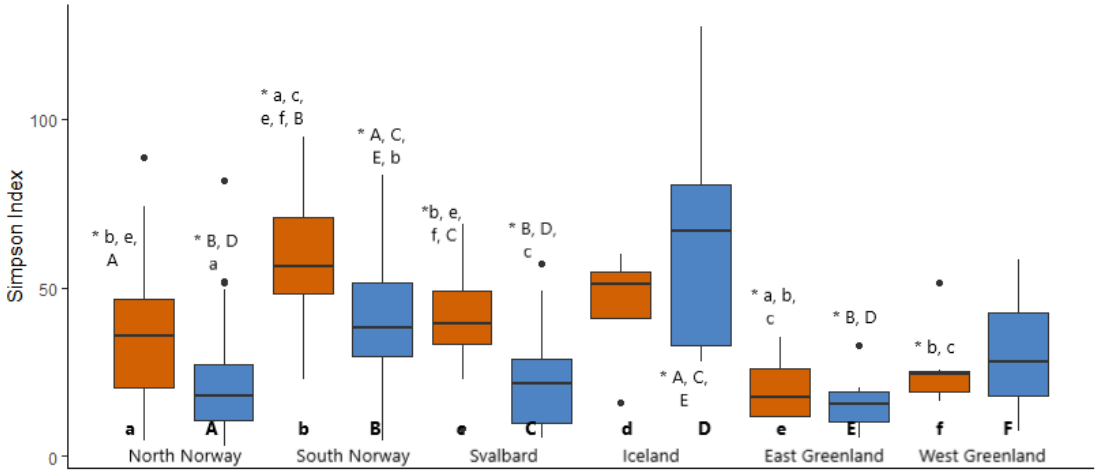

Fig. 15: *Ranges of the Inverse Simpson index for prokaryotes and eukaryotes per region, respectively. Lower case letters and orange colouring mark prokaryotic and capital letters and blue colouring mark eukaryotic results. *The letters indicate to which other boxplot the respective boxplot is statistically significantly different, as calculated with the Student´s t-test.*

To explore possible influences on the Inverse Simpson, I performed a regression analysis of richness and Inverse Simpson within prokaryotes and eukaryotes, respectively (Supplement Fig. 65 and Fig. 17). The correlation is significant in both prokaryotes and eukaryotes (regression analysis, $p < 0.05$), though it becomes much stronger in prokaryotes when excluding North Norway from the analysis (Fig. 16).

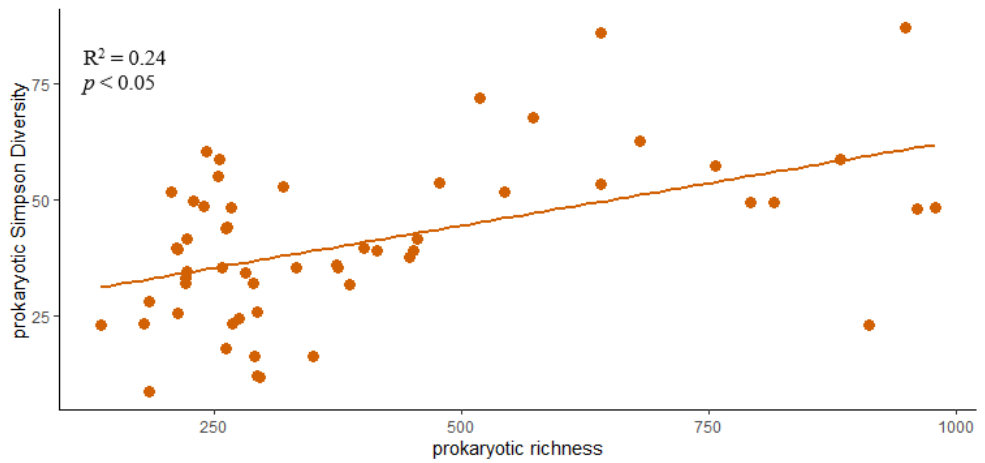

Fig. 16: *Prokaryotic Simpson diversity versus richness without North Norway. R^2 and p-value are indicated.*

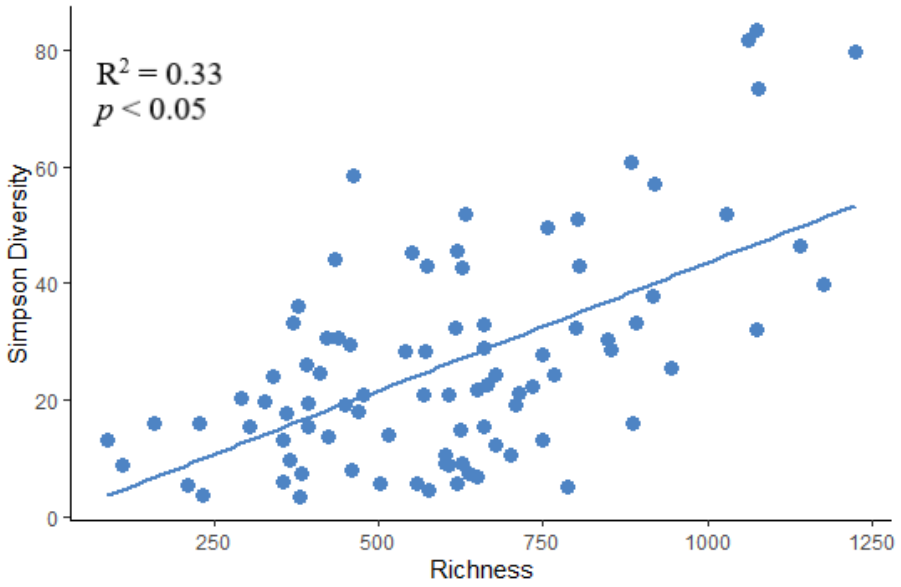

Fig. 17: *Eukaryotic Simpson diversity versus richness. R^2 and p-value are indicated.*

To explore further determinants of the Inverse Simpson, I related it to the environmental parameters temperature and salinity and to the nutrient concentrations of phosphate, silicate and nitrate. For salinity, silicate and nitrate I observed no or minor correlations to the Inverse Simpson (Supplement Fig. 62-64). Temperature correlates significantly with the eukaryotic (regression analysis, $R^2 = 0.30$) and prokaryotic Inverse Simpson index (regression analysis, $R^2 = 0.25$, Fig. 18). Phosphate also correlates significantly, though weaker than temperature, with eukaryotic (regression analysis, $R^2 = 0.12$) and prokaryotic Inverse Simpson index (regression analysis, $R^2 = 0.18$, Fig. 19).

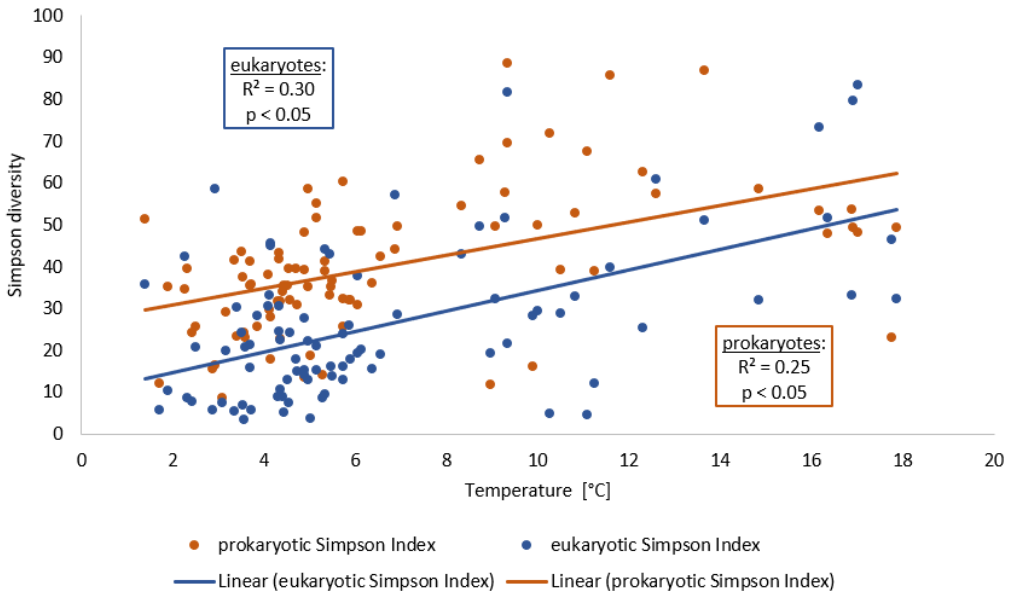

Fig. 18: *Temperature versus eukaryotic and prokaryotic Inverse Simpson. R^2 and p-value are indicated.*

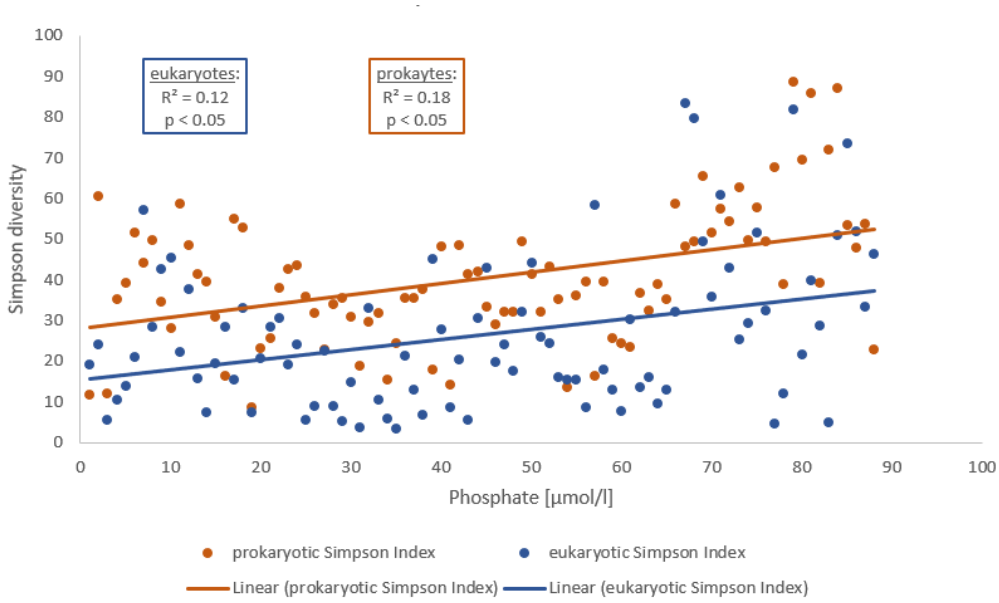

Fig. 19: *Phosphate versus eukaryotic and prokaryotic Inverse Simpson. R^2 and p-value are indicated.*

My results so far indicate a co-variation of as well as differences between prokaryotes and eukaryotes in alpha diversity (Fig. 11 and Fig. 15). However, North Norway is distinct from the general pattern observed between the other regions. To resolve differences in diversity patterns, I continued with analysing North Norway and all other regions independently.

5.1.3 Alpha Diversity across prokaryotes and eukaryotes: co-variation

To observe possible correlations between pro- and eukaryotic alpha diversity, I performed a regression analysis with the richness and Inverse Simpson across the prokaryotic and eukaryotic communities, respectively. It appeared, that eukaryotic richness is positively correlated to prokaryotic richness in all regions, excluding North Norway (regression analysis, $R^2 = 0.38$, $p < 0.05$; Fig. 20, Supplement Fig. 66). In contrast, eukaryotic Inverse Simpson was only within North Norway positively correlated to prokaryotic evenness (regression analysis, $R^2 = 0.62$, $p < 0.05$; Fig. 21, Supplement Fig. 67).

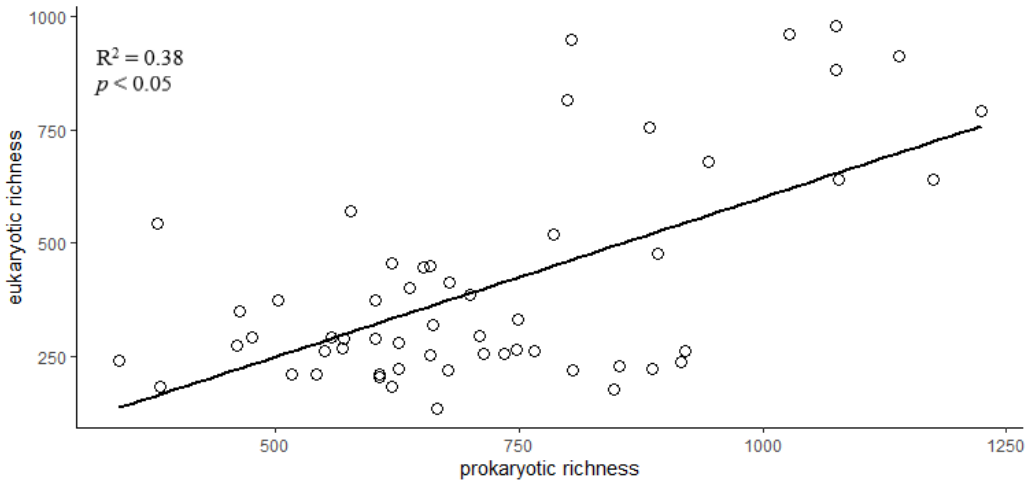

Fig. 20: *Eukaryotic versus prokaryotic richness without North Norway. R^2 and p-value are indicated.*

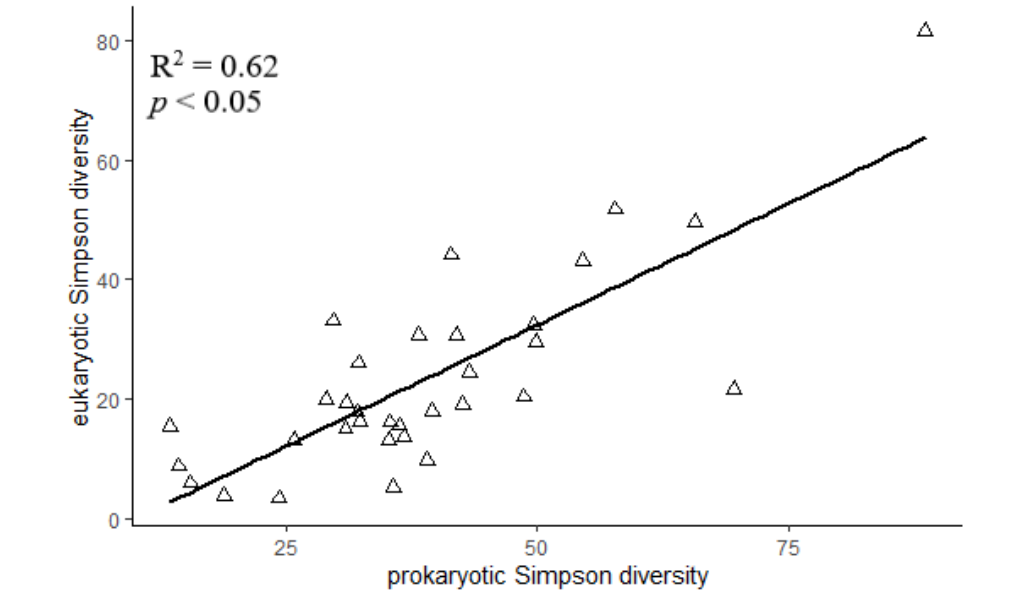

Fig. 21: *Eukaryotic versus prokaryotic Inverse Simpson in North Norway. R^2 and p-value are indicated.*

Despite the presented co-variations of prokaryotic and eukaryotic alpha-diversity, there are also pronounced differences. Except in the North Norwegian samples, the eukaryotic richness is significantly higher in absolute numbers than the prokaryotic richness within all regions (Student´s t-test, $p < 0.05$, Fig. 11). In North Norway, in contrast, the prokaryotic even significantly exceeds the eukaryotic richness (Student´s t-test, $p < 0.05$, Fig. 11). In prokaryotes, richness within each region varies within a smaller range than among eukaryotes.

As an overall tendency, the more evenness is weighed in the Hill numbers, the higher the diversity measure for prokaryotes compared to eukaryotes (Fig. 11, Fig. 15, Supplement Fig. 58). While eukaryotes (except in North Norway) exceed prokaryotes vastly in ASV richness, the prokaryotic Simpson diversity is significantly (South Norway) or slightly (East Greenland) higher than the eukaryotic (Student´s t-test, $p < 0.05$, respectively) or at least there is no obvious difference anymore

between the communities (Iceland, West Greenland, Fig. 15). In North Norway, however, prokaryotes display both higher richness and Inverse Simpson than prokaryotes (Student´s t-test, $p < 0.05$, respectively).

Overall, eukaryotic Simpson diversity is positively correlated with prokaryotic Simpson diversity in North Norway. Everywhere else, prokaryotic and eukaryotic richness are significantly correlated. Eukaryotic richness is significantly higher than prokaryotic richness. In prokaryotes, richness within each region varies within a smaller range than among eukaryotes.

5.2 Beta Diversity

5.2.1 Beta Diversity across regions

Furthermore, I explored the spatial diversity patterns of the pro- and eukaryotic communities in the investigated regions by means of beta diversity, i. e. ASV turnover. I evaluated if the ASV turnover is higher across or within the different regions and how it is structured within regions and their fjords. I approached the beta diversity investigation with two different methods that look from different angles on ASV turnover: NMDS ordination plots rank all sites based on their compositional similarity without indicating total counts or percentages, thus providing an overall degree of similarity. “Upset”-plots depict total numbers of ASVs that are shared between or unique to different sites, thus allowing first insights into what causes the similarity.

The turnover across regions is larger than within regions in prokaryotes and eukaryotes, indicated by the clustering of sites per region (Fig. 22). An exception are the Lofoten samples taken on a different expedition than the other North Norway samples and the Kongsfjord samples taken on a different expedition than the other Svalbard samples. For better visualization, they are coloured separately Fig. 22). Among eukaryotes, some North Norway stations who clustered far apart from all others had to be removed first, because they distorted the image (Supplement Fig. 68).

Among prokaryotes, the samples mostly cluster together per region (Fig. 22). Only the two Greenland regions are not entirely distinct from each other. North Norway and Svalbard are visually separated according to the two different expeditions they were sampled on, because they cluster separately. Clustering per region can be observed as a tendency among eukaryotes as well, though if sampled at different expeditions, the samples cluster according to expedition instead of region. Among eukaryotes, West Greenland and North Norway were arranged with an overlap during the scaling process while in prokaryotes, the two Greenland regions cluster together and are distinct from North Norway.

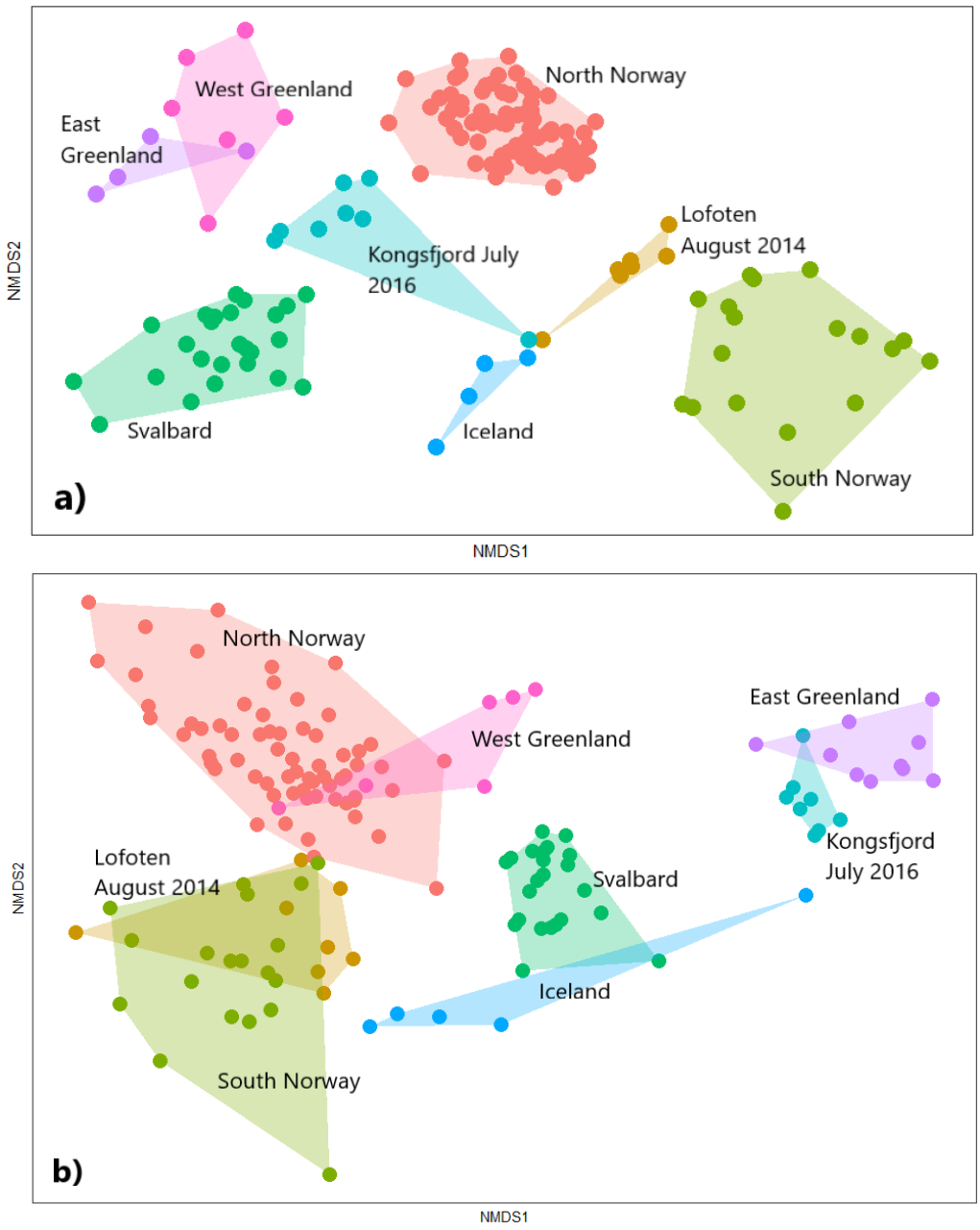

Fig. 22: *NMDS of the **a)** prokaryotic and **b)** eukaryotic samples across all regions, as they cluster together according to their compositional similarity. The samples are coloured per region of origin. North Norway and Svalbard are additionally separated into the two expeditions they were sampled on.*

Among prokaryotes and eukaryotes, most ASVs are either unique to one region or shared among all (Fig. 23). Among prokaryotes, North Norway, South Norway and Svalbard have the biggest proportions of unique ASVs with North Norway vastly exceeding the other two. Among eukaryotes, the same three regions have the highest number of unique ASVs, though more evenly distributed among them. The proportions shared between respectively two of these three regions also are substantial. The number of ASVs ubiquitously distributed across all regions is considerable, too, in prokaryotes and eukaryotes. In general, the more ASVs are present in a region in total, the more ASVs are unique to that region.

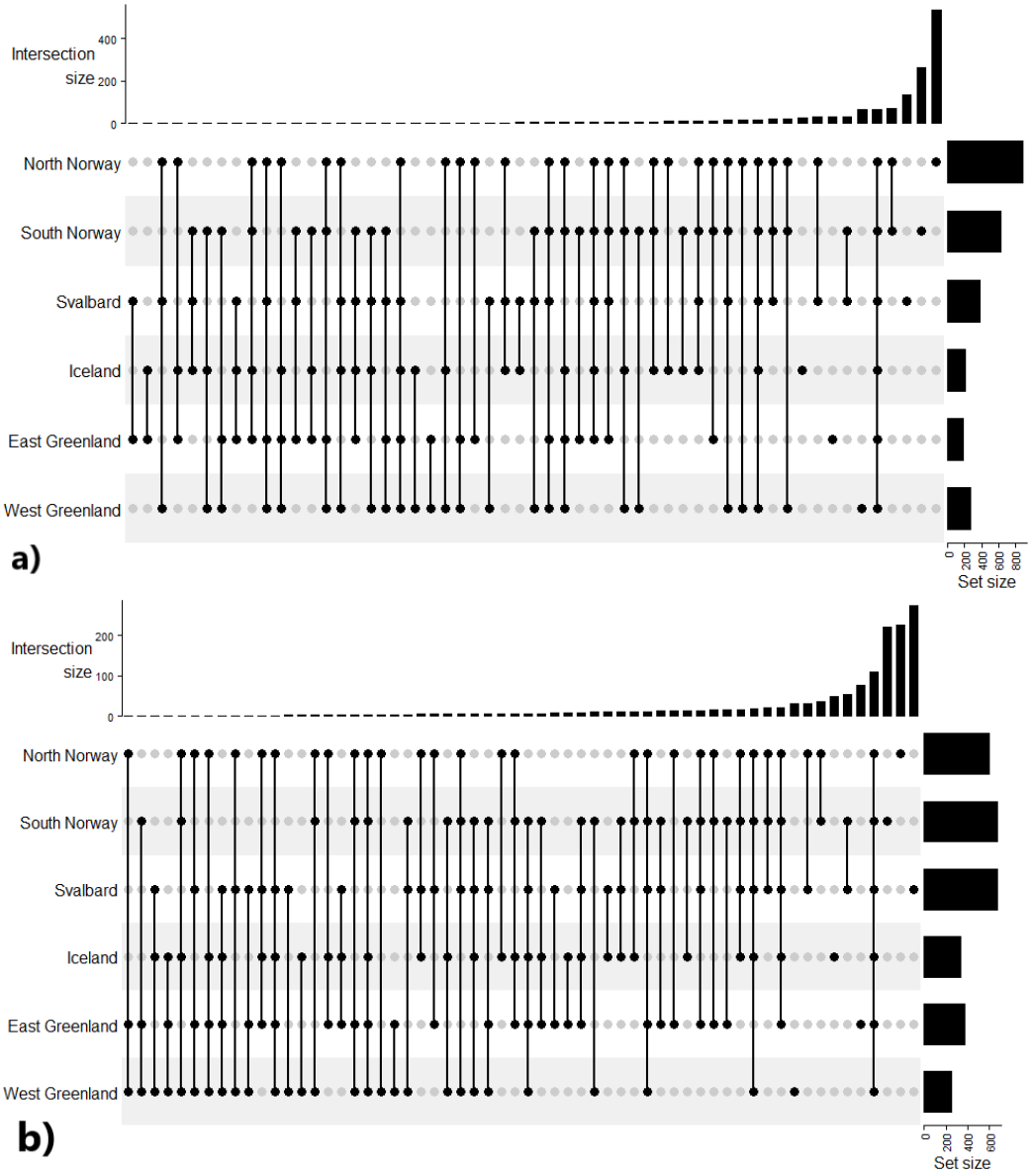

Fig. 23: *Proportions of unique **a)** prokaryotic and **b)** eukaryotic ASVs per region and the number of ASVs shared by multiple regions, as indicated by the intersection size. The set size depicts the number of different ASVs each region comprises in total.*

5.2.2 Beta Diversity across regions: Drivers

I aimed to determine spatial distance and environmental dissimilarity, as indicated by temperature and salinity, as possible drivers of ASV turnover across regions. I calculated distance matrices regarding these variables between all sites paired with all other sites and depicted the distance measures for all pairs of sites between regions. To assess statistical significant correlations, I performed Mantel tests using the Pearson product-moment correlation.

Environmental and compositional dissimilarity of both prokaryotic (Pearson, $R^2 = 0.52$, $p < 0.05$) and eukaryotic (Pearson, $R^2 = 0.1$, $p < 0.05$) communities are positively correlated, though stronger in prokaryotes (Fig. 24). The pairs of stations with low environmental dissimilarity include pairs with all levels of ASV variance. With growing environmental dissimilarity between stations, there are fewer up to no pairs of stations with a small turnover. The described trend is more marked in prokaryotes which

show all ranges of ASV variance at a low environmental dissimilarity, but display a steady decrease in station pairs with low ASV turnover with growing environmental dissimilarity. The decrease in pairs of stations with a small turnover described for prokaryotes can be observed for eukaryotes as well, though less pronounced. ASV turnover plateaus above a value of environmental dissimilarity of approximately 9.

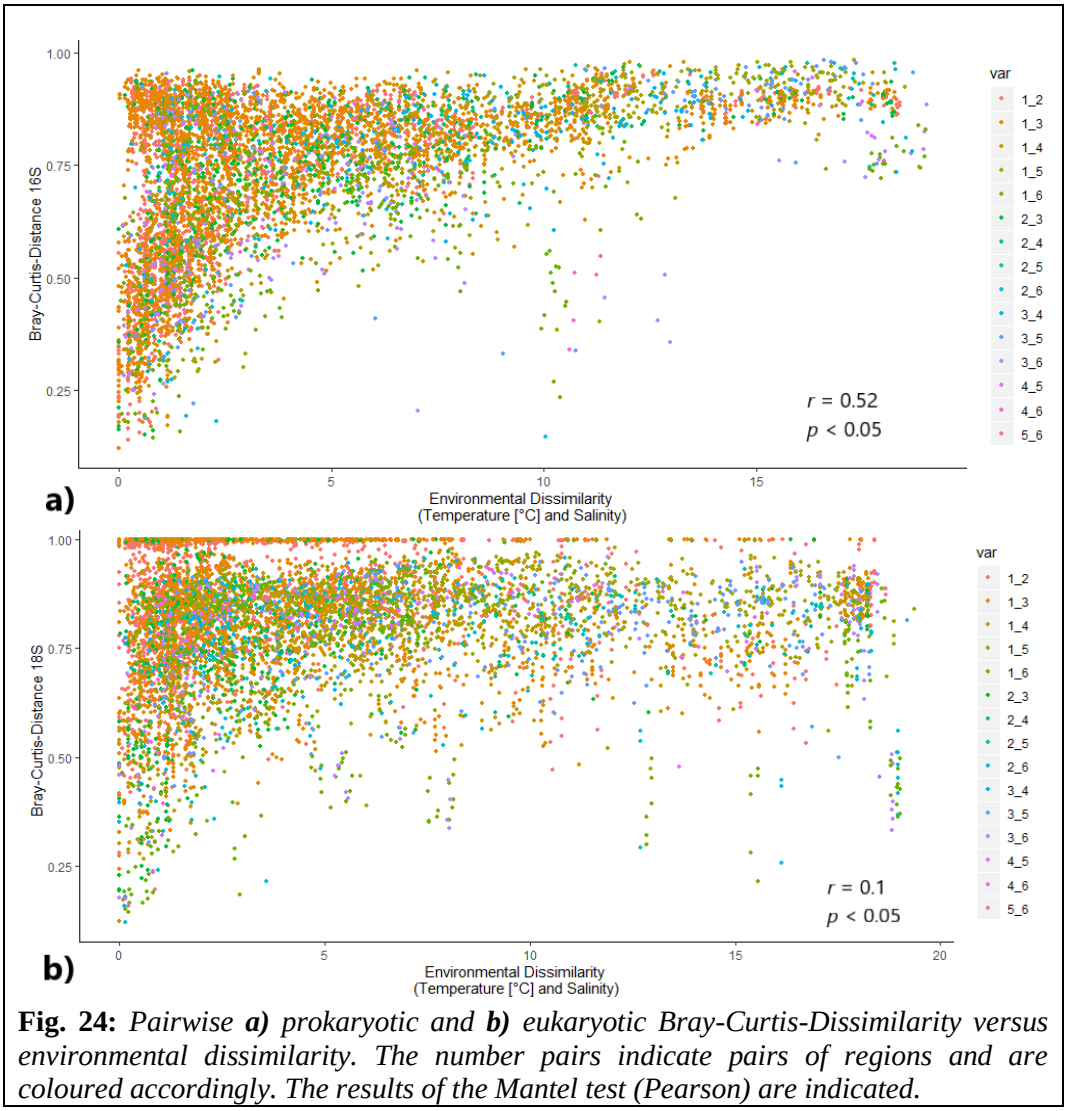

Fig. 24: *Pairwise **a)** prokaryotic and **b)** eukaryotic Bray-Curtis-Dissimilarity versus environmental dissimilarity. The number pairs indicate pairs of regions and are coloured accordingly. The results of the Mantel test (Pearson) are indicated.*

I observed a minor distance-decay of similarity in prokaryotic (Pearson, $R^2 = 0.59$, $p < 0.05$) and slightly less pronounced in eukaryotic (Pearson, $R^2 = 0.23$, $p < 0.05$) communities (Fig. 25). Within all magnitudes of distance, a high variance in community similarity is apparent. Only among the station pairs that are farthest away from each other there are fewer pairs with a low turnover. Among eukaryotes, some station pairs from within North Norway showed a turnover of nearly 100% (Fig. 24 and Fig. 25). These are pairs between stations comprising less than 100 individuals.

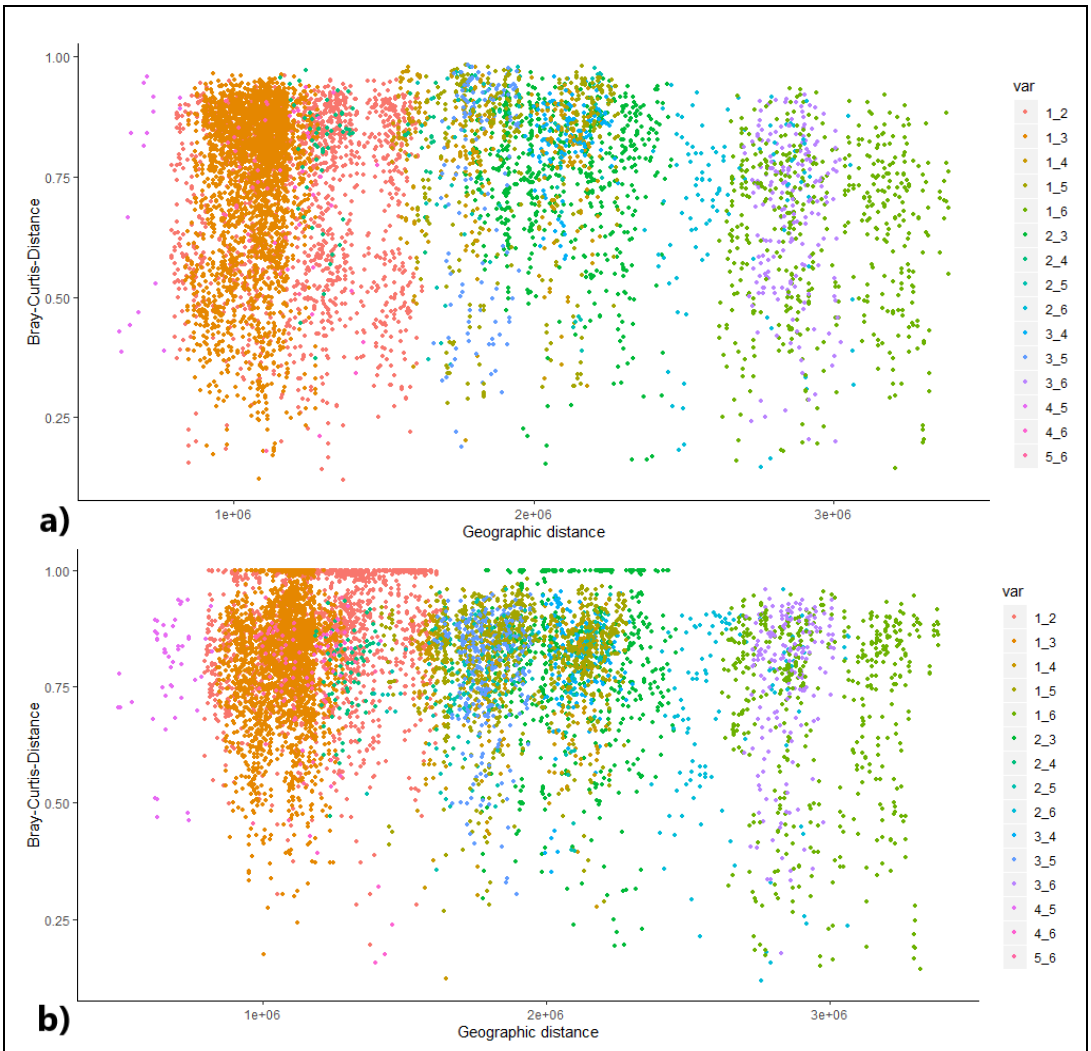

Fig. 25: *Pairwise **a)** prokaryotic and **b)** eukaryotic Bray-Curtis-Dissimilarity versus geographic distance. The number pairs indicate pairs of regions and are coloured accordingly. The results of the Mantel test (Pearson) are indicated.*

5.2.3 Beta Diversity within regions: North Norway

To assess beta diversity within regions, I deployed the same methods as for the beta diversity analysis across regions. I exemplary analysed North Norway and Svalbard, since these regions include many sampled fjords and fjords with distinct environmental influences.

In North Norway the fjords cluster in the ordination plots according to three groups that can be assigned to the influence of three different water masses: the Barents Sea influenced fjords Tanafjord, Laksefjord and Porsangerfjord cluster as one group, Lyngenfjord and Balsfjord, who are less influenced by the Barents Sea and more by the Norwegian Sea and are well in the Tromsø archipelagic fjord system cluster together, and the Norwegian Sea influenced Lofoten without a pronounced fjord structure cluster separately and are additionally separated by expedition (Fig. 26). This pattern is more pronounced among prokaryotes. Among eukaryotes, Lyngenfjord shows no clear distance to any group.

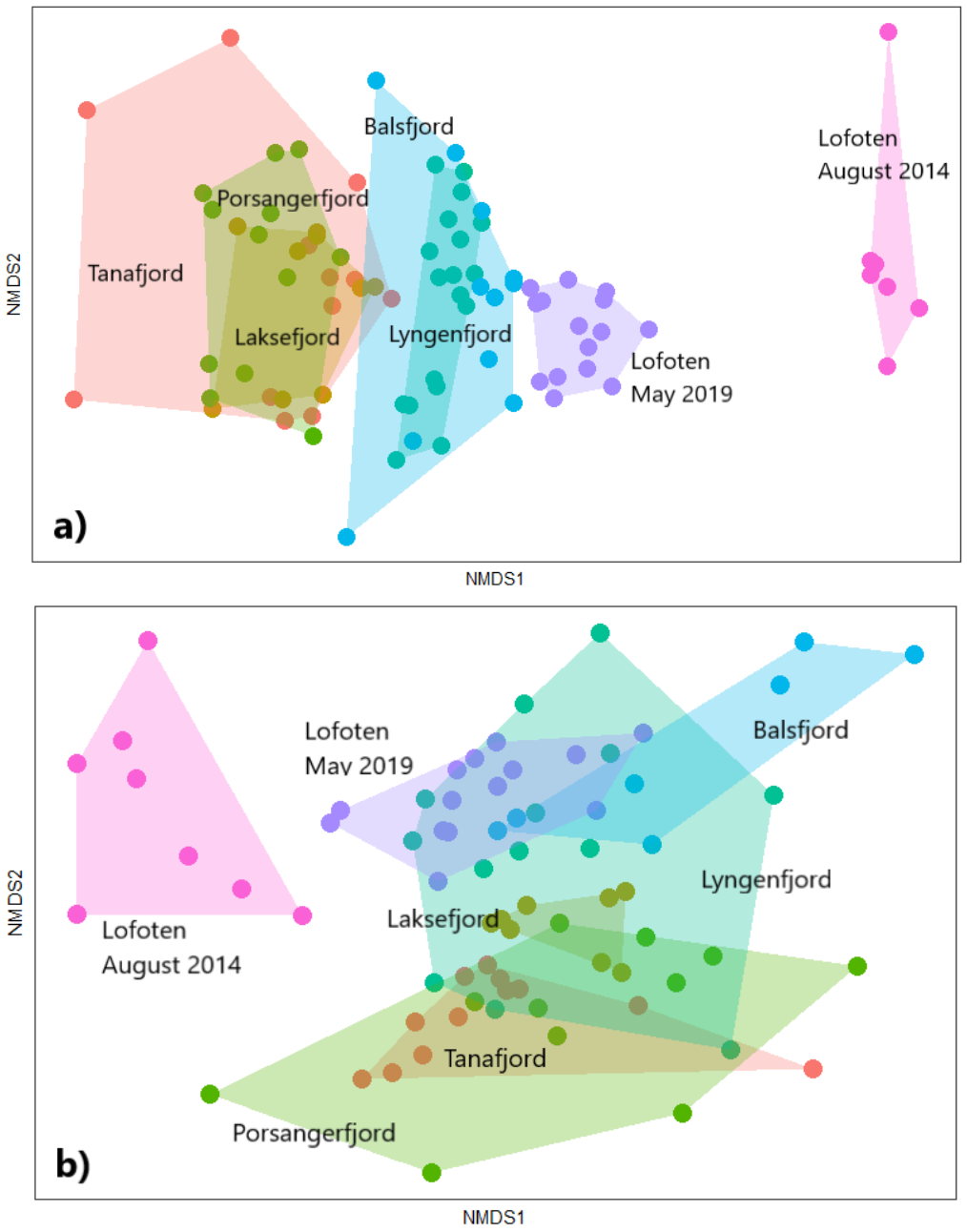

Fig. 26: *NMDS of the **a)** prokaryotic and **b)** eukaryotic samples of North Norway, as they cluster together according to their compositional similarity. The samples are coloured per fjord, except for the Lofoten, which are additionally separated into the two expeditions they were sampled on.*

The influence of environmental dissimilarity and geographic distance within North Norway is similar to the influence on all samples: the environmental influence is stronger than geographic distance and both act stronger on prokaryotes than eukaryotes (Supplement Fig. 69-72).

Analogous to the division across all regions, most ASVs within North Norway are either unique to one fjord or shared among all (Fig. 27). The Lofoten show the highest proportion of unique ASVs in prokaryotes and eukaryotes, in eukaryotes only exceeded by Porsangerfjord. Especially in prokaryotes, the numbers of unique ASVs is similar between fjords.

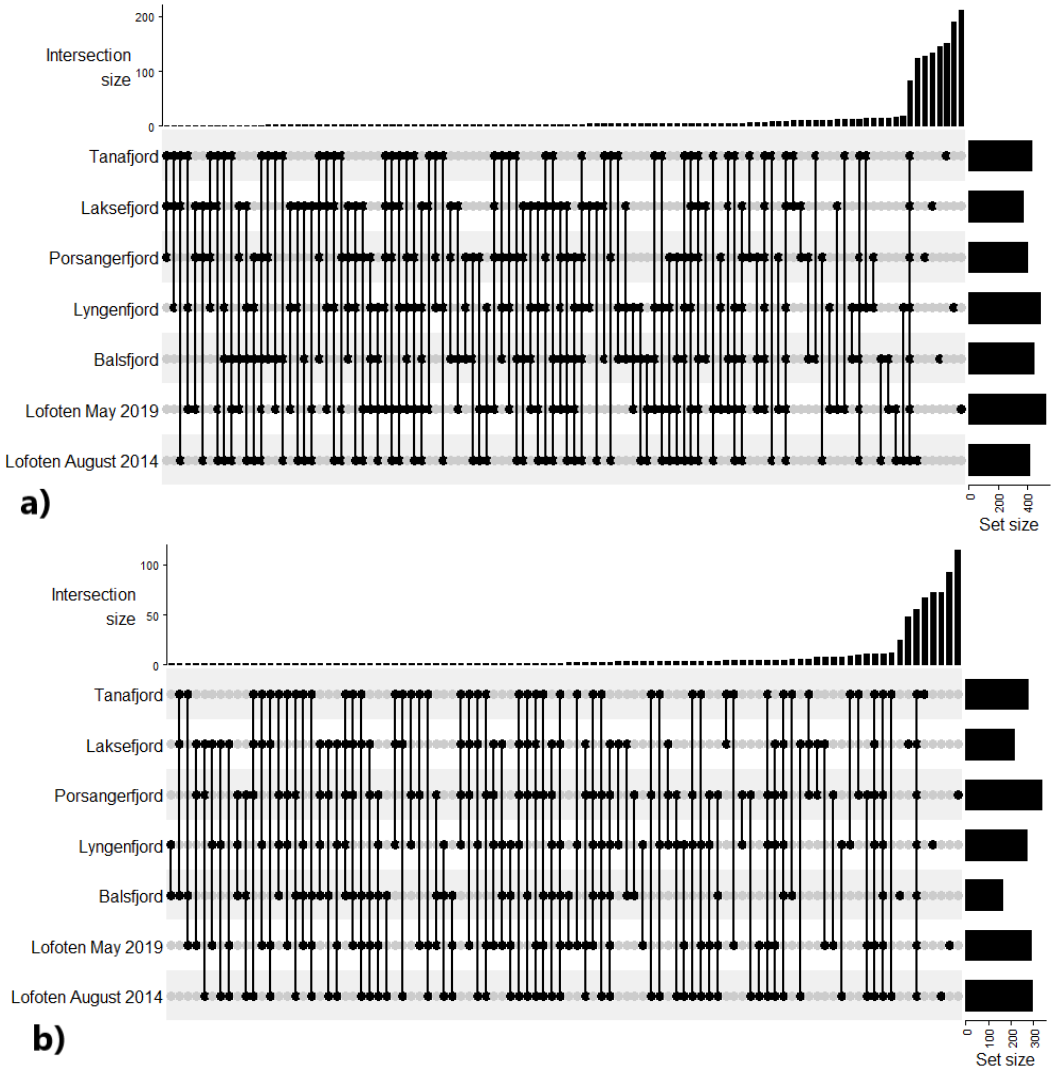

Fig. 27: *Proportions of unique **a)** prokaryotic and **b)** eukaryotic ASVs per fjord in North Norway and the number of ASVs shared by multiple fjords, as indicated by the intersection size. The Lofoten were sampled on two different expeditions and are depicted accordingly. The set size depicts the number of different ASVs each fjord comprises in total.*

5.2.4 Beta Diversity within regions: Svalbard

The Svalbard fjords cluster more distinct per fjord than the North Norwegian fjords (Fig. 28). Woodfjord and Wijdefjord cluster together in both prokaryotes and eukaryotes, though stronger in eukaryotes. For Kongsfjord, the samples taken on different expeditions cluster far apart. Within the fjords, a gradual differentiation of eukaryotic communities along the fjords transects is visible as lined up data points (Fig. 28).

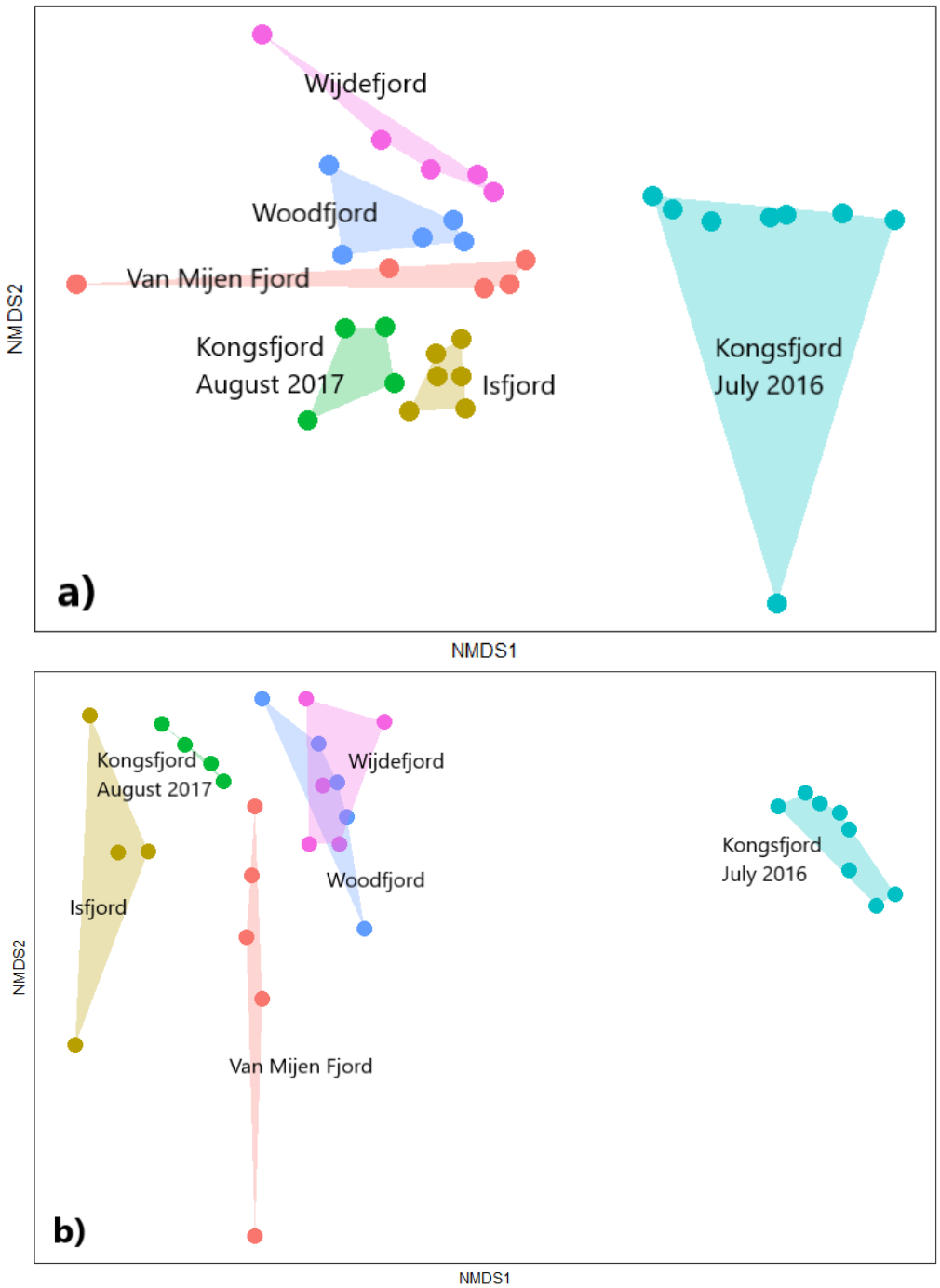

Fig. 28: *NMDS of the a) prokaryotic and b)eukaryotic samples of Svalbard, as they cluster together according to their compositional similarity. The samples are coloured per fjord, except for the Kongsford, who is additionally separated into the two expeditions it was sampled on.*

Svalbard shows the same pattern as it was visible across all regions and in North Norway (Fig. 29): the vast majority of ASVs is either unique to one fjord or shared between all. Among prokaryotes, the positive correlation of set size and unique ASVs per fjord or sampling campaign is particularly pronounced.

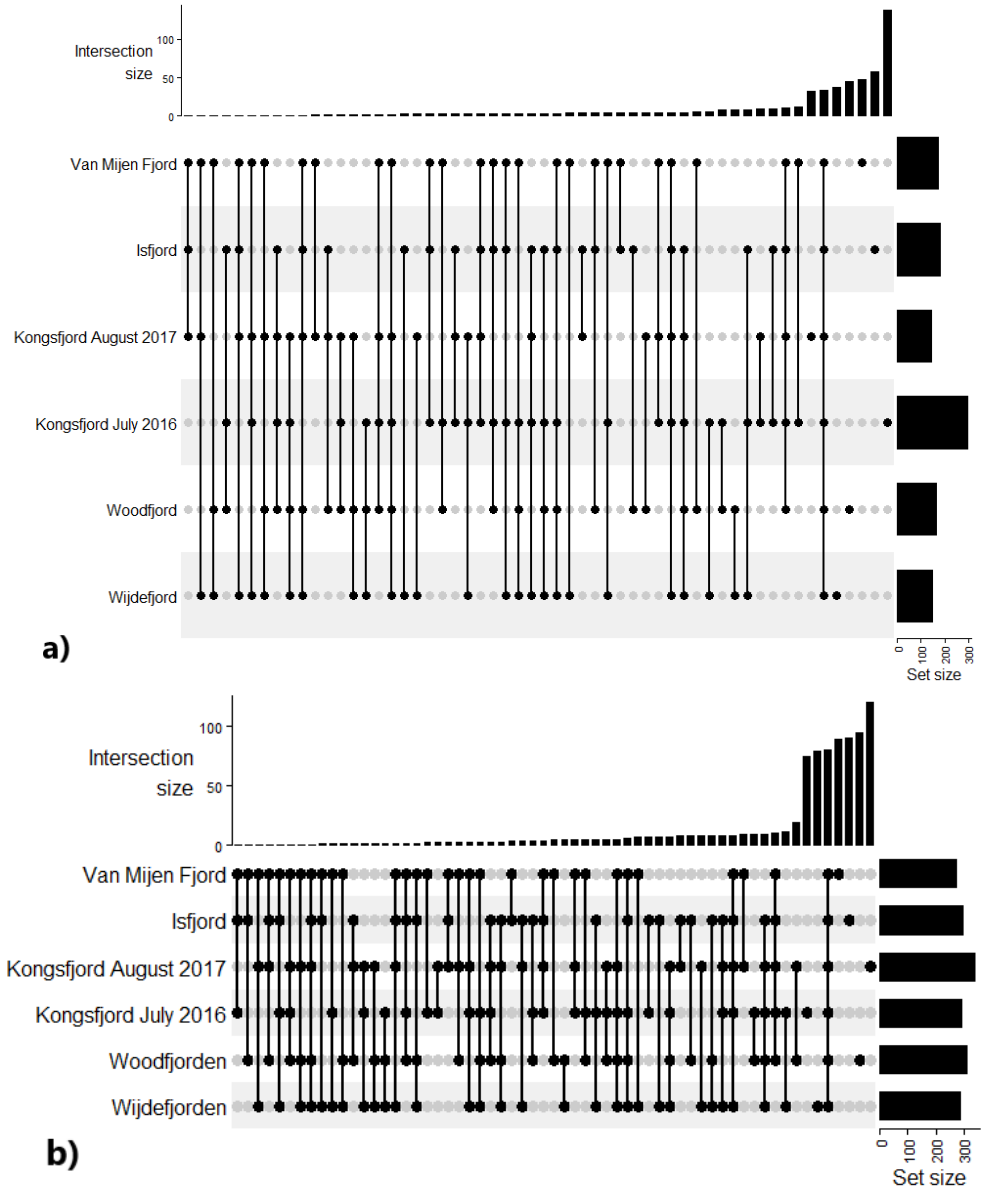

Fig. 29: *Proportions of unique **a)** prokaryotic and **b)** eukaryotic ASVs per fjord within Svalbard and the number of ASVs shared by multiple fjords, as indicated by the intersection size. Kongsfjord was sampled on two different expeditions and is depicted accordingly. The set size depicts the number of different ASVs each fjord comprises in total.*

5.2.5 Beta Diversity across tip and mouth stations: fjords and glaciers

Furthermore, I investigated if beta diversity is higher across inner than across outer fjord stations, distinguishing fjords with and without glacial influence.

I observed a clear distinction in eukaryotes between the tip and mouth stations, which is almost absent in prokaryotes (Fig. 30). Mouth stations cluster closer together, indicating a more similar community composition, while tip stations cluster far apart from each other. Furthermore, eukaryotic samples from fjords with and without glacial influence are very clearly separated and more similar within groups. Prokaryotes cluster strongly per region and not according to the parameters investigated here.

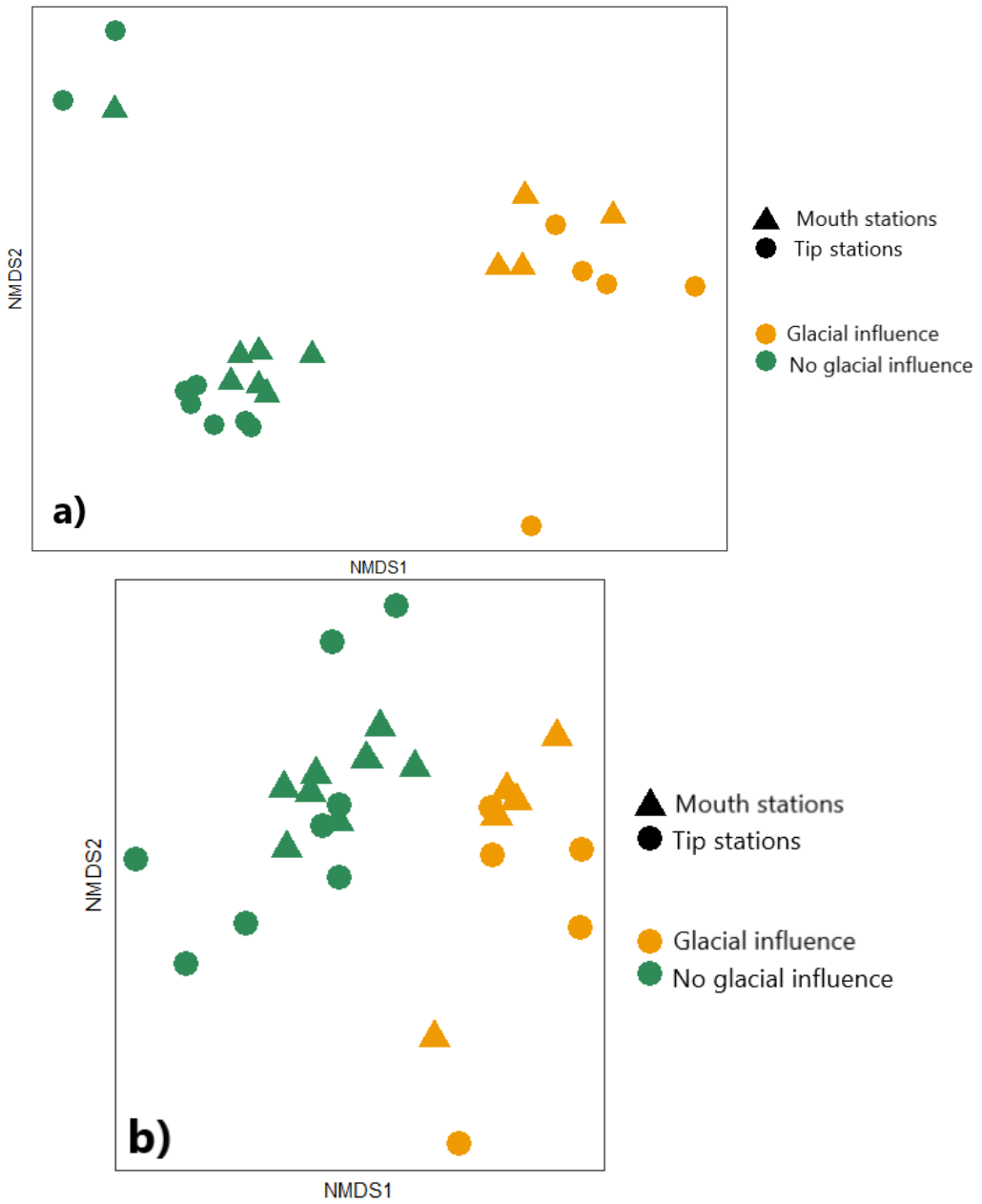

Fig. 30: *NMDS of the **a)** prokaryotic and **b)** eukaryotic tip and mouth stations of fjords with and without glacial influence, as they cluster together according to their compositional similarity. Each stations location within the fjord is indicated by shape and the glacial influence by colour.*

Shared and unique ASVs are displayed for the four groups tip station with and without glacial influence and mouth stations with and without glacial influence (Fig. 31). Among eukaryotes most ASVs found in these groups are unique to only one group or shared by all, with mouth stations having a larger number of unique ASVs than tip stations. Among prokaryotes, most ASVs are unique to the groups without glacial influence, shared by only these two or shared among all groups (Fig. 31). Glacial influenced fjords display a rather small number of unique prokaryotic ASVs, regardless whether tip or mouth.

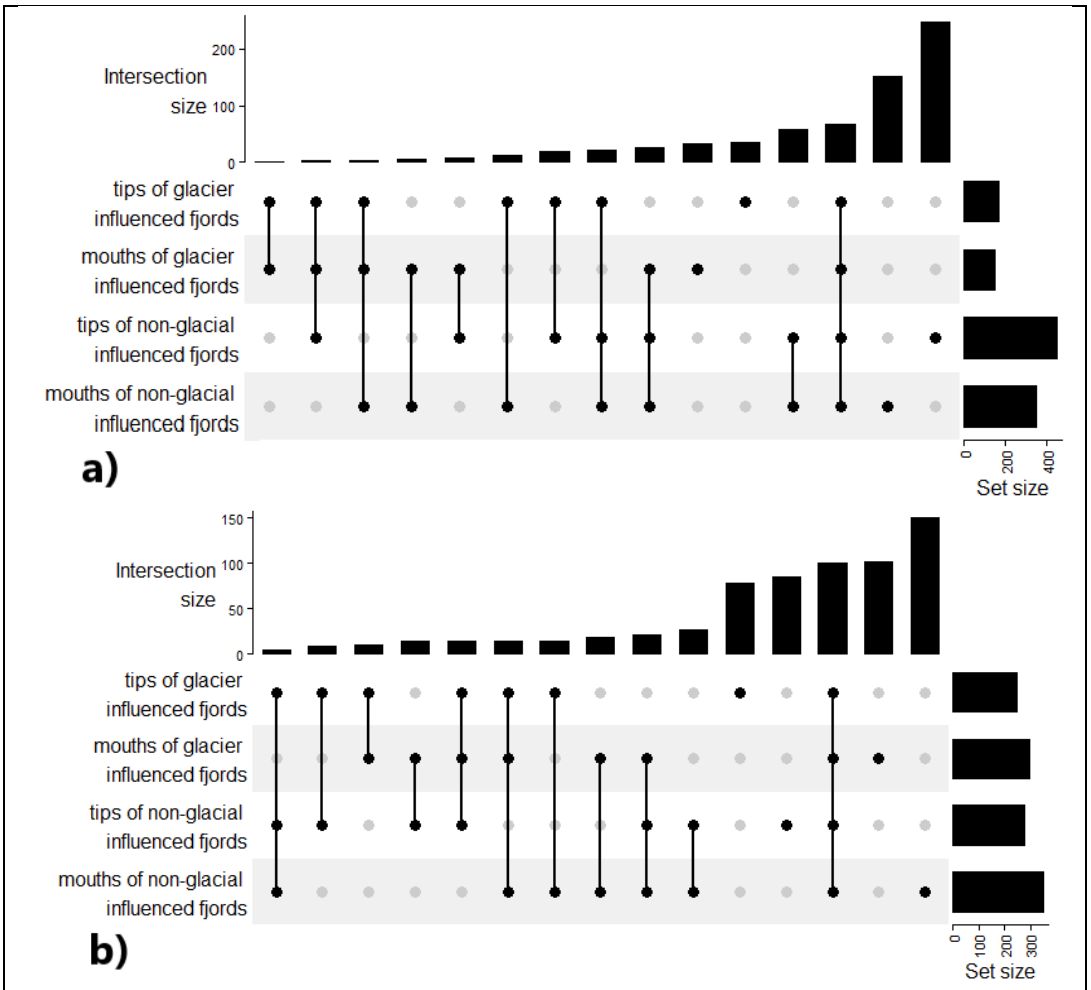

Fig. 31: *Proportions of unique **a)** prokaryotic and **b)** eukaryotic ASVs for tip and mouth stations with and without glacial influence. The plot also shows the number of ASVs shared by multiple groups, as indicated by the intersection size. The set size depicts the number of different ASVs each fjord comprises in total.*

6 Discussion

The aim of my project was to explore the spatial diversity patterns of picoplankton inhabiting Arctic Ocean surface waters and investigate the differences they reveal between prokaryotic and picoeukaryotic communities. I show differences in alpha diversity, i. e. taxonomic richness and evenness, and beta diversity, i. e. species turnover, across and within the different regions and their respective fjords. I evaluate differences in diversity in relation to geographical distance between microbial communities and the prevailing environmental parameters, particularly with regard to fjords and glaciers.

6.1 Methodological Approach

In this study, I use amplicon sequence variants (ASVs) as a substitute for species. ASVs allow the least artificial delineation of taxonomic units while there is no unified species concept for microorganisms. In order to define microbial species, cultivation experiments are necessary, since they reveal the specific features and characteristics based on which species can be defined. Considering the magnitude of

microbial biodiversity, only a small proportion of the presumed species has been described as such (Pedrós-Alió, 2016). The proportion is even smaller among extremophiles, such as microbes inhabiting polar environments, since it is more difficult to culture them (García-López et al., 2016). To assess diversity patterns of whole communities, like in my study, an amplicon sequencing approach using genetic markers is least biased by our current ability to identify species based on proceeding culturing efforts. The spatial or temporal distribution of molecular markers delivers indications on overarching diversity patterns and allows comparisons between sites in space and time, making it a suitable tool to approach my research questions. From here on, I will use the technical term "ASV" and "taxon" interchangeable, since ASV is a proxy for a taxonomic unit of yet unknown classification.

6.2 Alpha Diversity

6.2.1 Alpha Diversity across regions: Richness

One aim of this study was to evaluate differences in picoplankton alpha diversity across different regions in Arctic and subarctic waters. Species richness is an important component of alpha diversity to explore if the studied regions differ in their diversity. I compared regional species richness as measured in total ASV numbers per sample. When related to abiotic environmental parameters, such as temperature, salinity and nutrient concentrations, differences in richness across regions can indicate how the environment shapes microbial assemblages. Thus, it can be an indicator for the number and the range of available ecological niches, as created by biotic and abiotic factors. Biotic factors are mostly species interactions and will be discussed below. Abiotic factor shaping niches are for example environmental gradients of oceanographic parameters or nutrients who select for a specific range of optima in organisms towards these parameters. In addition to quantity, the quality, i. e. the kind of source for nutrients, can favour differently specialised species. The more diverse the composition of organic and inorganic matter and the wider the range of environmental parameters is within the regions, the more niches are provided and the higher the observed richness (Passow, 2002).

I found a variation in richness across regions (Fig. 11) and a positive correlation between microbial richness and water temperature (Fig. 12). In particular, South Norwegian prokaryotic and eukaryotic richness as well as the Lofoten outliers of the North Norwegian eukaryotic richness were associated with higher temperatures (Fig. 11). Temperature has repeatedly been found to be positively related to taxa richness (Gran-Stadniczeñko, 2018, Sunagawa, 2015), as is also reflected in a proposed (Fuhrman et al., 2008), though controversial (Ghiglione et al., 2012; Ladau et al., 2013), latitudinal microbial diversity gradient. A warmer environment increases the metabolic rate in living organisms, which influences the pace of reproduction, mutation, speciation, interactions and productivity (Thomas et al., 2012). With increasing productivity larger populations can be sustained, which creates more niches through more possible interspecific interactions and, consequently, could lead to a higher taxa richness, as was vividly rephrased by Fuhrman et al. (2008) as ‘‘the larger pie can be divided into more pieces". Yet, the observed correlation could also be a result of autocorrelation with different environmental parameters such as oxygen concentration or other unmeasured water mass characteristics.

Similarly, prokaryotic richness was high in South Norway, despite much lower water temperatures. A common feature South and North Norway share, but is absent in the other regions, is a high level of human impact (Halpern et al., 2008) due to e. g. pollution, commercial fishing and tourism (e. g. cruise ships). High human caused perturbation was repeatedly linked to an elevated bacterial richness (Ladau et al., 2013; Nogales et al., 2011) which may be reflected in my data as well.

Among nutrient concentrations, I found a positive correlation of taxa richness with phosphate

concentration. This observation is in agreement with the findings of (Ladau et al., 2013) and (Elferink et al., 2017), whose studies included polar and subpolar regions. Sunagawa et al. (2015), in contrast, analysed a global sampling campaign that did not include the polar regions and found no correlation between phosphate concentration and taxa richness. My observation and the literature indicate that phosphorus plays a more important role as a limiting nutrient in the coastal waters of polar regions than in oceans of lower latitude. Prokaryotic richness was furthermore influenced by nitrate, indicating nutrient availability to more strongly shape prokaryotic and picoeukaryotic richness.

The observed richness may not reflect the actual number of available niches, if the community harbours a large number of taxa in the rare biosphere, i. e. in the part of the microbial community that is composed of many different taxa at very low abundances (Pedrós-Alió, 2007). Furthermore, the pro- and eukaryotic communities may harbour different proportions of rare taxa. Thus, from their richness alone it is not possible to draw conclusions about the number of niches available to compare both communities in this regard. Among the rare biosphere, some taxa may be resting stages only rising in abundance if environmental conditions become favourable, i. e. niches become available. Until then they contribute to the richness measure without being an active part of the community. Other members of the rare biosphere may be specialists that are active despite being rare. Hence it is important to include other measures of diversity into the analysis before being able to compare the pro- and eukaryotic microbial communities of the different regions investigated.

6.2.2 Alpha Diversity across regions: Diversity

To reveal differences in diversity across regions, I furthermore compared species diversity, considering both species richness and species evenness as another descriptor of alpha diversity. As a measure of diversity I decided to use the Inverse Simpson because its value is strongly shaped by taxa evenness. Although it reflects both richness and evenness, I will use the term Inverse Simpson interchangeable with evenness as it serves here to compare richness and evenness as revealed by Inverse Simpson. When related to environmental parameters, differences in community evenness across regions can indicate how the environment shapes microbial assemblages and provide indications on community stability.

Generally, I found a positive coupling of richness and evenness within prokaryotes and eukaryotes, respectively, with the exception of prokaryotic communities in North Norway (Fig. 16 and Fig. 17). The positive coupling of richness and evenness as a general pattern is in agreement with other studies (e. g. Pommier et al., 2010). If more different taxa are present and if the seed bank constituted by the rare biosphere is large, a community has larger pool of ecotypes and can occupy more provided niches. It thus becomes more divers and more abundant, i. e. more competition and top down control (e. g. via grazing and coupling through the microbial loop) is exerted (Prowe et al., 2012). For instance, functionally redundant taxa inhabiting slightly different niches may control each other through competition. This prevents numerical domination of few taxa but instead leads to an increased evenness. A high evenness, in turn, leads to more possible interactions between taxa. The more different and the more complex interactions there are, the more ecological niches are created and the more divers microbial communities tend to be. Evenness and richness are therefore tightly coupled and generally co-vary, which is reflected in my data.

Similar as for taxa richness, evenness was influenced by temperature and phosphate availability (Fig. 18 and Fig. 19). The fact that most abiotic parameters explained no or small proportions of alpha diversity measures indicates that biotic factors, i. e. interactions between organisms, may strongly define the complexity of available niches, as proposed by (Connell & Orias, 1964) and Piredda et al. (2017).

- *I found a coupling of community richness and evenness within prokaryotes and eukaryotes, respectively, indicating that more niches are created in more diverse communities.*

- *I found prokaryotic and picoeukaryotic diversity to be positively influenced by temperature and phosphate concentration. In referring my observations to literature, I assume phosphorus to play an increased role as limiting nutrient in polar environments and human impact to elevate prokaryotic richness.*

6.2.3 Alpha Diversity across prokaryotes and eukaryotes: co-variation

The main objective of this study is to evaluate similarities and differences between the prokaryotic and eukaryotic diversity of picoplankton communities. Not only abiotic influences shape spatial diversity patterns, but also biotic factors, i. e. species interactions. By testing for correlations between prokaryotic and eukaryotic alpha diversity measures across the different regions, possible co-variations and dependencies can be revealed.

I observed a significant difference between prokaryotic and eukaryotic richness within all regions (Fig. 11), except in North Norway. In North Norway, picoeukaryotes vastly exceed prokaryotes in their taxa numbers, which supports the presumed existence of a vast undiscovered picoeukaryotic diversity (Farrant et al., 2016; Moon-Van Der Staay et al., 2001). Thus, either in the investigated habitats at the time of sampling more niches were available to picoeukaryotes than to prokaryotes or eukaryotes can divide into more taxa due to their larger and more complex genomes (Lynch & Conery, 2003) and hence are intrinsically able to occupy more niches. Eukaryotes evolutionary developed diversity at a structural and behavioural level including a high phenotypic plasticity (Keeling & Campo, 2017). Higher plasticity proves to be an advantage over the prokaryotic metabolic diversity in occupying a wider niche space (Poole et al., 2003). For example, while a prokaryotic taxon might already have disappeared at an environmentally changing site when conditions become unfavourable, eukaryotic taxa from the former might still be present in the current environmental regime, resulting in a higher observed richness (Massana & Logares, 2013). The prokaryotic may thus rise disproportionately more than the eukaryotic richness when observed over time, as it is strongly influenced by e. g. season (Wilson et al., 2017).

In North Norway, the pattern of a higher eukaryotic than prokaryotic richness was reversed. This may be explained by the season of sampling: while in all other regions the sampling took place between July and September, North Norway was sampled at the end of May / beginning of June. In the Lofoten, Balsfjord, Lyngenfjord and Tanafjord indications to a beginning or ongoing spring bloom were evident. Sørensen et al. (2011) could relate a significant decrease in picoeukaryotic richness to high productivity and Linacre et al. (2010) observed a decreased contribution of picoautotrophs to total phytoplankton biomass during high productivity. While having an advantage over larger cells under low nutrient conditions because of their high surface to volume ratio, upwelling events and blooms seem to lead to decreased richness and abundance in pico-sized eukaryotes, as opposed to larger eukaryotes and prokaryotes (Sørensen et al., 2011).

Three of the four upper Lofoten outliers of eukaryotic richness in North Norway (Fig. 11) were sampled on the expedition HE431 in August 2014, corresponding with both the elevated temperatures in the

Lofoten in late summer compared to spring and with post spring bloom sampling. Increased productivity might not only explain the low eukaryotic richness in North Norway, but also in West Greenland (Fig. 11), which is a highly productive area with strong upwelling because of marine terminating glaciers (Meire et al., 2017). The higher nitrate concentrations in North Norway and West Greenland compared to most other regions (Supplement Fig. 73) support the assumption of high productivity in these regions at the time of sampling. A decreased richness during periods of high primary production was also observed for marine bacteria by Storesund et al. (2015) and Hodges et al. (2005). However, I didn´t find these patterns in my study. It also contradicts the observed increase in bacterial richness in areas with high human impact (Ladau et al., 2013; Nogales et al., 2011) which is usually accompanied by an elevated nutrient input.

Prokaryotes comprise overall fewer taxa, but they occur similarly (Iceland and both Greenland regions) or significantly more (Svalbard and Norwegian regions) evenly distributed in their respective abundances within sites as indicated by a higher Simpson diversity (Fig. 15). A higher evenness may indicate a higher resistance to environmental changes and thus a higher stability within the prokaryotic communities of Norway and Svalbard compared to eukaryotes (Shade et al., 2012). A higher initial evenness in bacterial communities was found to improve quick responses to perturbations or environmental changes (Wittebolle et al., 2009). The equal or higher evenness in prokaryotes may also suggest that among eukaryotes, fewer dominant taxa are needed to maintain ecosystem functions than among prokaryotes (Vargas et al., 2015). This could be traced back to the high specificity of prokaryotic metabolism, so more taxa are needed to perform all required nutrient and organic matter turnover. However, considering the greater picoeukaryotic richness, a lower evenness in eukaryotes may still result in the same total number of dominant taxa while the rare biosphere is large. Within the scope of this study it is not possible to draw further conclusions.

Notably, the otherwise observed coupling between richness and evenness was only minor within prokaryotes in North Norway (Supplement Fig. 74), but strong in eukaryotes (Supplement Fig. 75). Considering the wide range of Inverse Simpson for prokaryotes in North Norway (Fig. 15), this may indicate a current transition in community structure related to the spring bloom situation that was progressing everywhere except in Porsangerfjord and Laksefjord, where it had not even started. At the time of sampling, the prokaryotic communities may have been at different stages of the process of restructuring their communities according to differing stages of bloom in different fjords (Sørensen et al., 2011). A wide range in evenness as an indicator for structural changes can also be observed in the eukaryotic communities of Iceland and West Greenland. Both regions have open coastal structures, where different water masses meet in contrast to the fjord systems in the other regions. Eukaryotic communities may therefore be constantly reassembling due to immigration of new eukaryotic taxa or in response to the immigration of new prokaryotic taxa from the currents. This is also reflected in a high range in eukaryotic richness within Iceland, especially considering the low number of stations. Apart from the exceptions discussed above, prokaryotes generally show a narrower range in richness and evenness. They seem to maintain a more stable community structure than eukaryotes (Curtis & Sloan, 2004). This may be because of their narrower ecological niche space, so the environment more quickly selects for a well-adapted community .

A mutual dependence of pro- and eukaryotes, reflected in co-variation of alpha diversity patterns, can generally be explained by their tight coupling of metabolic and predator-prey interactions. For instance, bacterial communities can be shaped according to their substrate preferences by specific exometabolites (Baran et al., 2015). Saleem et al. (2013) found, that increased bacterial prey richness can enhance evenness and production of bacteriovorous eukaryotes. Through various interactions like these, prokaryotic and eukaryotic communities provide niches for each other. An increased richness and

evenness in one community leads to more possible interspecific interactions and thus to an elevated richness and evenness in the other community. However, the different patterns in North Norway and the other regions indicate a fundamentally different structuring of prokaryotic and eukaryotic interactions during these two distinct phases. During the onset and process of spring blooms, i. e. as represented by North Norway in this study, the evenness of the prokaryotic diversity is coupled to the evenness of the eukaryotic communities (Fig. 21). Later in plankton succession, a positive correlation can be found between prokaryotic and eukaryotic richness, as represented by the other regions (Fig. 20). As described above, picoeukaryotes decrease in richness and abundance during spring blooms (Sørensen et al., 2011). This may lead to a greater importance of a more even structure of the remaining community and in response also in the prokaryotic community to maintain their interaction based functions. Perhaps the transition from a coupled richness to a coupled evenness during spring bloom buffers the effect of the eukaryotic taxon loss.

- *I found a greater richness in picoeukaryotes than prokaryotes, except during spring bloom, indicating that picoeukaryotic community stability may be based on a larger rare biosphere. I found a narrower range in richness within regions and a narrower range in evenness across regions in prokaryotes than eukaryotes, indicating a higher stability through more efficient taxa sorting.*

- *I found spring blooms/high productivity to profoundly decrease richness in picoeukaryotes and restructure prokaryotic communities as well as induce a transition from positively coupled richness to evenness across domains.*

6.3 Beta Diversity

6.3.1 Beta Diversity across regions

I aimed to assess how prokaryotic and eukaryotic community composition changes across regions and what drives the turnover by means of beta diversity measures. Beta diversity, i. e. species turnover, extends the structural description of a community as provided by alpha diversity to a description of compositional dissimilarity across communities in space or time. By comparing community dissimilarities to geographic distance and differences in temperature and salinity between sites, I assessed possible drivers of species turnover.

Across all sites, taxa turnover is greater between regions than within them in both prokaryotes and eukaryotes, as indicated by the partitioning of sites according to their respective regions in the ordination plots (Fig. 22). This pattern is supported by Fig. 23, which reveals that most taxa are unique to only one region, if not ubiquitously dispersed among all. Geographic distance between the regions is likely not the reason for the partitioning according to regions, since a distance-decay relationship was almost imperceptible in prokaryotes and eukaryotes, though slightly higher in prokaryotes (Fig. 25). My findings contrast with those of Logares et al. (2018), who observed a distance-decay in both prokaryotes and picoeukaryotes. However, these findings result from a global sampling campaign, highlighting the influence of spatial scale on the observed patterns. My study indicates that within a spatial range like the one investigated here, distance may not be of great importance for community turnover. This is in

accordance with Sunagawa et al. (2015), who only found a minor influence of distance on prokaryotic communities below 5000 km. The absence of a distance-decay may reflect a high connectivity of the investigated regions via ocean currents or the dominance of environmental influences who overrule the effect of distance.

Indeed, I found environmental conditions to be strong drivers of taxa turnover (Fig. 24). A strong influence of environmental conditions on community turnover was also observed by Milici et al. (2016) and Pinhassi et al. (2003). It may be even enhanced in polar environments, where strong seasonal changes in the environmental conditions shape microbial assemblages (Wilson et al., 2017). The great differences in the influence of ice cover (temperature), meltwater (salinity) and solar radiation create environmental fluctuations within the year that may have an even stronger effect on community composition than in lower latitudes, where microbial communities are exposed to less strong environmental fluctuation. The extremely high picoplankton turnover described here is further supported by the observation, that a high taxa turnover was equally observed across all magnitudes of spatial distance and environmental dissimilarity, while the influence of environmental dissimilarity was only evident by a decrease in low turnover.

Furthermore, the separation per region appears to be even more pronounced in prokaryotes than in eukaryotes, whose regional clusters partly overlap (Fig. 22). This observation may be explained by the higher correlation of environmental dissimilarity with prokaryotic than with eukaryotic community dissimilarity. Additionally, the slightly bigger impact of distance on prokaryotes than eukaryotes may contribute to this effect. In contrast to my findings, Logares et al. (2018) didn´t only find a distance-decay in prokaryotic and picoeukaryotic community composition, but also a higher dispersal limitation among picoeukaryotes than prokaryotes. Despite their similar size based characteristics when compared to the whole microbial biosphere, the dispersal potential of prokaryotes was presumed to be even higher than of picoeukaryotes because of their even smaller cell sizes and the believed higher capacity for dormancy and resting stages in eukaryotic microbes (Jones & Lennon, 2010). My data, however, shows no indication to a more restricted dispersal in picoeukaryotes compared to prokaryotes but rather the opposite tendency, since there are more compositionally similar stations across regions among eukaryotes than prokaryotes (Fig. 22). The environmental influences obviously overrule potential effects of distance or dispersal limitations in my study, a phenomenon also observed by Milici et al. (2016).

While prokaryotic community composition varies more, their evenness varies less strongly across regions then in eukaryotes (Fig. 15). This indicates a higher structural stability in prokaryotic than in picoeukaryotic diversity in response to environmental pressures. On the other hand, having a higher richness implies a bigger rare biosphere among eukaryotes, which may result in more resilience to change through different processes. For example, taxa for whom conditions become unfavourable can be substituted with functionally redundant taxa from the rare biosphere which is serving as a seed bank, thus maintaining functions over a broader range of environmental conditions.

Among eukaryotes, some regions cluster together in the NMDS plot (Fig. 22) which can be explained either by many shared taxa or by similar degrees of alpha diversity. The overlap between Norway and West Greenland eukaryotic communities, for example, may reflect the similar and low eukaryotic richness (Fig. 11). West Greenland has the lowest number of total eukaryotic taxa and few shared with North Norway (Fig. 23), so compositional similarity does not seem to set these regions apart from the others. Therefore, the structural difference in response to the high productivity in these regions at the time of sampling, as described above, may cause the close rank orders.

A temporal influence is evidently not only exerted via season, but also across years. Within Svalbard, the Kongsfjord samples taken in July 2016 on the expedition MSM56 and the samples taken in August 2017 on the expedition HE492 differ greatly in their compositional dissimilarity. Similarly, the Lofoten

samples of North Norway taken in August 2014 on the expedition HE431 and the samples taken in May 2019 on the expedition HE533 cluster separately. Among eukaryotes, samples cluster even stronger per expedition than per region, while among prokaryotes, all groups cluster equally according to region and expedition (Fig. 22). The observed pattern indicates to a strong seasonality changing on an at least monthly basis as well as largely differing environmental conditions between years. The temporal aspect seems to select stronger on eukaryotes than environmental differences, which in turn influence prokaryotic turnover equally strong as the time of sampling. An extreme seasonality in Arctic microbial eukaryotic communities was also observed by Marquardt et al. (2016). In Svalbard, in the course of the summer, sea ice and glaciers melt increase so a continuous input of fresh water stratifies the water column. The surface water temperatures rise during summer through solar radiation. What is more, the amount of sea ice, the duration of ice cover and the time of melting in spring can differ greatly between years (Belchansky et al., 2004). This influences the succession of changes in environmental conditions, for example, at what time of the year the Atlantic current starts intruding into the west Svalbard fjords. The higher temperature and lower salinity measured in August 2017 compared to July 2016 (Supplement Fig. 76-77) indicate a larger amount of Atlantic water present in August 2017. Within the Lofoten it is mainly the temperature difference distinguishing the seasons while salinity is less distinct between the sampling times (Supplement Fig. 78-79). It is evident, that meaningful comparisons between fjords or regions should ideally be based on samples taken at the same time. Even then, they would only allow insights into the specific patterns prevailing at that time of the year and cannot be extrapolated to other seasons.

Besides the observed high turnover across regions and the large number of taxa that are unique to each region, there is a considerable proportion of taxa found in all regions (Fig. 23). The observed strong environmental influence on community composition makes this rather big proportion even more noteworthy. Hence, this study supports the proposition of Massana & Logares (2013), who suggested that marine picoplankton may to some extend conform to the dogma of a ubiquitously dispersed species pool on which the environment selects (Baas Becking, 1934). The high numbers of unique taxa per region, on the other hand, support the assumed biogeography for marine microbes (Pommier et al., 2007; Whitaker et al., 2003). My study thus supports the proposition of Van Der Gucht et al. (2007), who assumed that both ubiquitous dispersal as well as biogeographical patterns shape marine microbial communities. This may be explained by different processes such as selection, drift, dispersal and mutation acting differently on different phylogenetic groups (Hanson et al., 2012). This in turn further highlights the need to not treat microorganisms as one uniform group, but distinguish within them (Keeling & Campo, 2017). This may lead to more meaningful conclusions, if additionally done on the basis of traits or phylogenetic groups than merely on the basis of size fractions.

- *I found a higher spatial turnover across than within regions in both prokaryotes and eukaryotes and identified environmental dissimilarities as the main driver of turnover. Geographic distance had a negligible influence. In referring my observations to literature, I assume distance-decay to depend on spatial scale.*

- *I found most taxa to be unique to only one region or ubiquitously dispersed among all, indicating that both wide dispersal as well as biogeographical patterns driven by environmental influences shape picoplankton communities.*

- *I found prokaryotes to cluster more distinctly per region than eukaryotes, highlighting the stronger influence of environment and distance on prokaryotes that may overrule potential size based differences in dispersal ability.*

- *I observed strong seasonality and heterogeneity in community composition across years that are particularly pronounced in picoeukaryotes, stressing the importance of controlling for time in spatial comparisons.*

6.3.2 Beta Diversity within regions

I aimed to evaluate, if community turnover within regions is shaped by different processes than across regions. Comparing prokaryotic and eukaryotic taxa turnover within and across the fjords of Svalbard and North Norway allowed insights into geographic distance and dissimilarity in temperature and salinity as possible drivers of compositional differences.

Like across regions, I also observed a pronounced influence of the environmental parameters temperature and salinity as drivers of community turnover within North Norway. It is much stronger than the influence of geographic distance and affects prokaryotes more than eukaryotes. With the exception of the eukaryotic community in Lyngenfjord, the fjords cluster in the ordination plots according to three groups that can be assigned to the influence of three different water masses. Furthermore, the three northern fjords are covered by sea ice during winter and have thus a stronger seasonality than the other North Norwegian fjords and the Lofoten. A strong influence of water mass on differences in community composition was also observed by Galand et al. (2009) and is reflected in the positive coupling of salinity and eukaryotic richness (Supplement Fig. 60). Microbes may travel with currents that don´t mix because of density differences. Being passively dispersed organisms, plankton may thus stick with their water mass of origin over large distances (Kalenitchenko et al., 2019).

Within the areas sampled on two different expedition (Lofoten and Kongsfjord), eukaryotes clustered across regions stronger per expedition than prokaryotes did, which is similarly visible in this smaller spatial scale within North Norway. The eukaryotic Lofoten samples from May 2019 cluster together with the Lyngenfjord and Balsfjord samples from the same cruise. With increasing distance and environmental dissimilarity to the northern fjords, however, the effect seems to level off. The northern fjords are distinct in both the progress of the spring bloom, which had mostly not yet started, and the influencing ocean currents, which is mainly the Barents Sea, and the sea ice coverage during winter. Among prokaryotes, the Lofoten are equally separated by fjord and time of sampling. Apparently, the time of sampling influences the retrieved picoeukaryotic community more than the prokaryotic on both spatial scales observed in this study. Within eukaryotes, the temporal effect decreases with increasing distance and environmental dissimilarity.

Svalbard reveals some distinct characteristics in its beta diversity pattern. Here, samples cluster strongly per fjord and expedition in prokaryotes and eukaryotes equally, except for the eukaryotic communities of Woodfjord and Wijdefjord who are not distinct from each other. The observation that eukaryotes are mainly separated per expedition in North Norway and across all regions, but per expedition and fjord within Svalbard, may be explained by the glacial and sea ice influence in Svalbard. As described above, the strong fluctuations across years in the seasonal succession of sea ice coverage, sea ice and glacier melting and the intrusion of Atlantic water into the western Svalbard fjords make the environmental fluctuations in the Svalbard region more intense than in the subarctic region of North Norway. This is

reflected in an exceptionally high community turnover in Kongsfjord across years, since the samples taken in July 2016 cluster particularly far apart from the samples in August 2017 (Fig. 28).

What is more, the polar Svalbard fjords seem to strongly select for a gradual community turnover along the environmental gradients unfolding within the fjords. The Kongsfjord stations, though separated per expedition, show the same differentiation within each cluster. The same pattern was vaguely indicated in eukaryotes in the mostly temperate North Norway fjords, but only clearly expressed in the Svalbard eukaryotes and will be discussed below.

Notably, sampling the same area at different times evidently brings as many new taxa to light as sampling different areas at the same time. The extreme compositional variability over time is further highlighted by a surprisingly small proportion of taxa shared between samples of the same location at different times. This seconds Nolte et al. (2010), who observed 25% unique eukaryotic OTUs in each of the observed samples that were taken in three weeks intervals. The effect may be enhanced in my study because the different expeditions sampling Kongsfjord and the Lofoten didn´t always sample the exact same stations. The high spatial turnover is emphasized by the fact that the vast majority of taxa is unique to only one fjord (or ubiquitously dispersed among all) and samples belonging to one fjord sometimes cluster farther apart from each other than between fjords. The high number of taxa unique to each region and fjord explains, why across and within regions the number of unique taxa is related to set size. While sampling depth in my study appeared to be sufficient to cover the majority of taxa present at that time and place, expanding the sampling effort even slightly in space or time would evidently bring many more taxa to light.

While the environmental conditions observed here explain taxa turnover to some extent, there obviously have to be additional factors selecting on community composition that change more intensely across space and time. Similarly, I found few limiting nutrients influencing alpha diversity, ruling them out as main niche provider for the observed vast taxa diversity. Huisman & Welssing (1999) suggested internal community dynamics based on competition as multiplier of niche space. Constant oscillations of abundances among taxa caused by competitive interactions may lead to chaotic and unpredictable reassembling of microbial communities, independent of the environmental conditions. This model may explain the vast diversity and the extreme turnover in space and time observed here better than the different environmental settings of the studied area.

- *I found environmental and temporal influences to shape community dissimilarity within North Norway and Svalbard. Glacier influence evidently causes a higher taxa turnover across fjords and within fjords across years, than fjord structure alone.*
- *I found most taxa within regions to be unique to only one fjord or ubiquitously dispersed among all, repeating the pattern observed on a larger spatial scale across regions.*
- *I suggest niches created by community intrinsic chaotic fluctuations of taxa abundances across time to partly explain the observed diversity patterns, in addition to the predictable external influences observed in this study.*

6.3.3 Beta Diversity: Influences of fjord structures and glaciers

Finally, I was interested in how fjords as habitats shape microbial diversity, especially if they are influenced by glaciers. I compared tip and mouth stations of all fjords regarding their community turnover, expecting a higher turnover across tip station than across mouth station. In distinguishing glacial influenced fjords from not glacial influenced fjords, the influence of fjord structures themselves and of glaciers can be disentangled.

A compositional partitioning along fjord transects in eukaryotes became already apparent in Svalbard and North Norway highlighting fjords as strongly structuring habitats. This is supported by the higher community turnover across tip than across mouth stations in eukaryotes, though not in prokaryotes (Fig. 30). While I found that temperature and salinity generally shape the community turnover of prokaryotic stronger than the turnover of eukaryotic communities, there are evidently gradients along fjord transects that create a pronounced differentiation only in picoeukaryotic communities. One possible gradient may be created by the meltwater of thawing glaciers and permafrost and the water of rivers terminating into the fjord tips. They release terrestrial derived organic matter into the fjord tips, which can alter microbial community composition (Garcia-Lopez et al., 2019; Sipler et al., 2017). They furthermore transport microorganisms into the fjords (Garcia-Lopez et al., 2019), which may lead to distinct tip and mouth communities as well. The capability of eukaryotes to occupy a wider niche space, as assumed above, may explain why prokaryotes appear only separated by region, while eukaryotes are markedly partitioned by tip and mouth samples. The eukaryotes originating from glaciers and rivers may still be viable in the fjords, thus altering the local community composition, while a more efficient taxa sorting selects the prokaryotic community quickly according to their more narrow environmental optima, resulting in more homogenous communities within fjords.

The turnover across tip stations is higher than across mouth stations (Fig. 30). This may be caused by stronger mixing of the mouth stations who are connected by water currents flowing along the coast lines. More unique communities can develop in the tips according to the distinct characteristics of each fjord. For example, tidewater glaciers that terminate with a calving front into the ocean differ in the amount and composition of the organic matter they release from glaciers that terminate inland and release meltwater via river runoffs into the fjords (Garcia-Lopez et al., 2019). Additionally, different rivers and glaciers discharge different microorganisms into the fjords. The response of community composition to different environmental settings is furthermore visible in the clear partitioning of glacial and non-glacial influenced fjords (Fig. 30).

The pronounced pattern of most taxa being unique to only one spatial unit or dispersed among all is also visible when grouping stations according to qualitative feature, i. e. the mouth and tip stations with and without glacial influence, though stronger pronounced in eukaryotes (Fig. 31). This suggests that a considerable number of taxa may be even unique to each station.

- *I found picoeukaryotic communities to be partitioned along fjord transects, suggesting environmental gradients within fjords as drivers. Based on literature research, I propose organic matter and microorganism input from rivers and meltwater into the fjord tips as possible influences.*

- *The turnover across mouth stations is higher than across tip stations, suggesting unique conditions inside the fjord tips and mixing along the coast line.*

7 Conclusion

In my study, I found similarities as well as pronounced differences in microbial picoplankton diversity between regions and between prokaryotic and eukaryotic communities and identified possible drivers.

Picoplankton communities changed both in spatial and temporal scales in Arctic and subarctic surface waters, while at the same time, a considerable proportion of the pro- and eukaryotic communities was ubiquitously dispersed across the studied areas. This suggests both a wide dispersal ability and selection by environmental conditions, both across and within regions, thus rejecting the hypothesized stronger influence of distance on the diversity patterns across regions.

I found picoeukaryotes to vastly exceed prokaryotes in their richness, which may reflect inherent differences between the two domains of life. I suggest a larger rare biosphere as driver of community stability in eukaryotes, while prokaryotic communities may be stabilized through more efficient taxa sorting by the environment, as indicated by a higher evenness despite a higher taxa turnover across space than eukaryotes.

The picoeukaryotic community was stronger influenced by the time of sampling, while prokaryotes were stronger influenced by environmental parameters and geographic distance (Fig. 32), resulting in a higher spatial turnover. Among environmental influences, temperature was a strong driver of alpha and beta diversity and phosphorus and nitrogen appeared to be weakly limiting nutrients influencing prokaryotes (Fig. 32). Fjord structures – and even more so glacial influence – appeared to strongly shape picoeukaryotic communities along fjord transects, thus confirming the hypothesized difference between the tip and mouth communities for picoeukaryotes (Fig. 32). I observed seasonal succession to strongly influence community composition, for instance spring blooms progression, which affected particularly the picoeukaryotic richness and induced shifts in community structures of both prokaryotes and picoeukaryotes (Fig. 32). Furthermore, communities from the same location sampled in different years showed little resemblance, highlighting the need to eliminate time as a confounding factor in future analyses of the marine microbial biosphere. While I could identify some environmental factors shaping picoplankton communities, a large proportion of variance and turnover in community composition remains unexplained and may be shaped by as of yet unpredictable community intrinsic dynamics.

In conclusion, my study contributes to a better understanding of the general patterns and their drivers underlying picoplankton community dynamic across different scales and fjord systems in Arctic and subarctic surface waters. My findings can be used as groundwork for urgently needed future explorations of microbial community successions across space and time. Given their vital contribution to global biogeochemical cycles, advancing our understanding of marine microbial community dynamics is pivotal to assess the impact of climate change on the planet and its biodiversity.

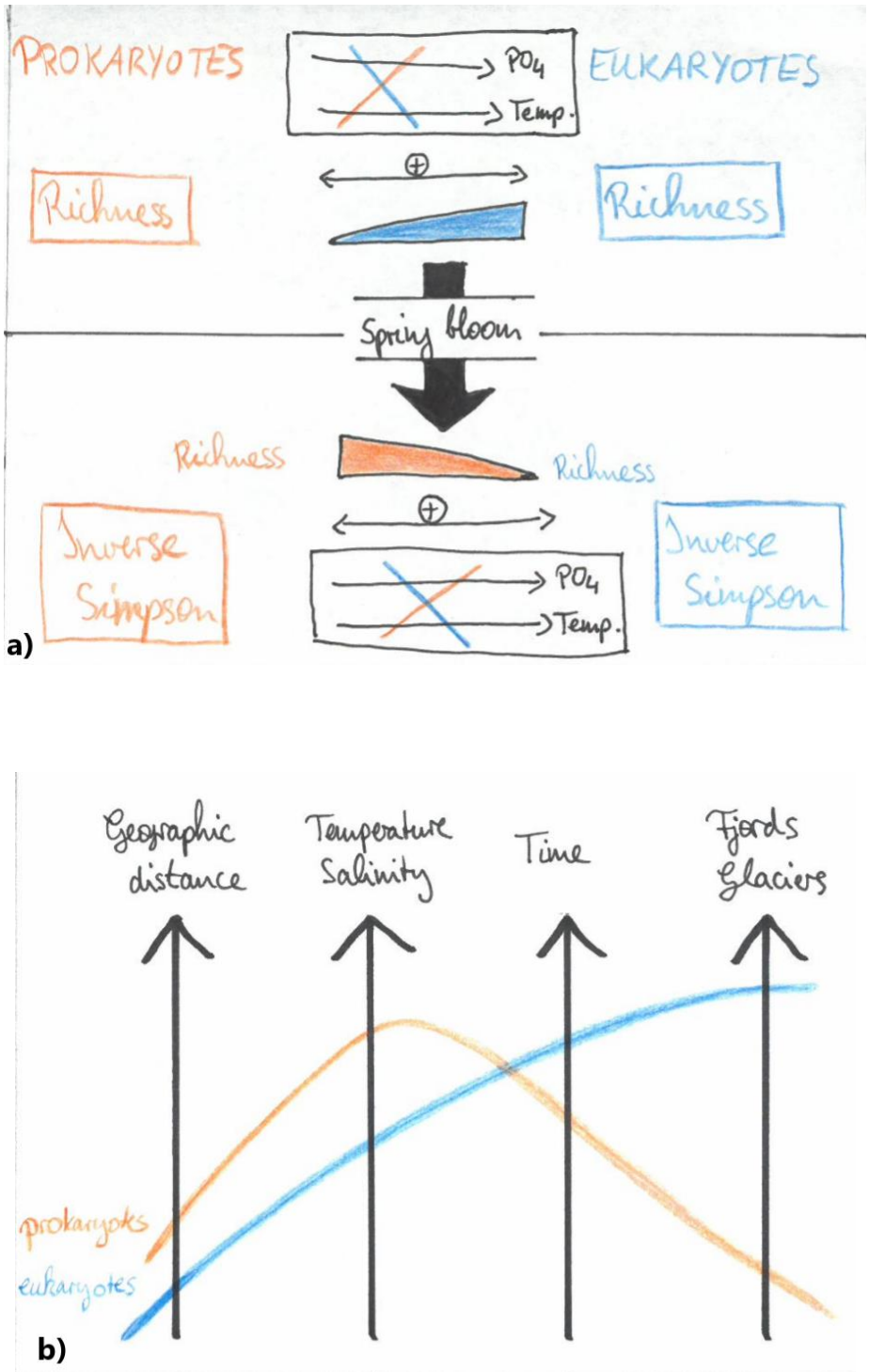

Fig. 32: *Synthesis figure.* ***a)*** *Alpha diversity: Depicted as arrows is the influence of phosphate and temperature on pro- and eukaryotic richness and Inverse Simpson, respectively. The intersection of the curves with the arrows indicates the strength of the influence on pro- in relation to eukaryotic alpha diversity measures. Depicted is also the coupling of richness and Inverse Simpson across domains as they change during spring bloom, along with the relation of prokaryotic to eukaryotic richness.* ***b)*** *Beta diversity: The environmental parameters geographic distance, temperature/salinity, time and fjords/glaciers are depicted, their influence increases along the arrows. The intersection of the curves with the arrows indicates the influence on pro- in relation to eukaryotic community turnover, respectively.*

8 Outlook

In my study I only scratched the surface of the taxonomic analyses that would be possible with the data set at hand. It may be interesting to extend the structural diversity analysis I did to a qualitative analysis. Resolving the picoplankton communities not only by domain but also by phylogenetic group, i. e. on the phylum or class level, may provide further insights into the stability of prokaryotic and picoeukaryotic communities. Resistance to environmental changes, for example, may be based on community stability within higher taxonomic levels, masked in my study by the high turnover on ASV level.

Using the same approach, the pro- and eukaryotic communities could be grouped by trophic mode, i. e. autotrophs, heterotrophs and mixotrophs. It could be explored, if the relative proportions of and the spatial turnover within trophic groups differs between prokaryotes and eukaryotes. This may allow insights into their functional coupling. i. e. within the microbial loop, in different environmental conditions. It could also be explored, if different environmental parameters shape the trophic groups differently. This may reveal indications on how community functions may change in the course of the climate change, using differences in environmental conditions in space as a proxy for change in time.

Furthermore, the data used here allows investigations of the rare biosphere to observe spatial community turnover and its drivers in more detail. Partitioning the pro- and picoeukaryotic communities into their rare and abundant subcommunities may reveal, if concepts such as the distance-decay relationship apply differently to the rare and abundant community proportion. In comparing their respective spatial turnover it could furthermore be explored, if the ubiquitously distributed and the subcommunity underlying high turnover rates, as observed in this study, comprise different proportions of the rare biosphere. This may reveal further insights into community stability.

The observed pronounced changes of microbial diversity with time indicate, that controlling for time would increase the significance of spatial comparisons. Furthermore, universal conclusions about microbial communities can evidently only be derived from exploration across both space and time. If setting up a new sampling campaign, samples would therefore ideally be all taken at the same time and the same stations would be sampled at multiple times per year and at the same times of the year across different years. A thus derived data set, though hardly feasible in reality, may allow to disentangle community intrinsic chaotic oscillations in abundance from diversity patterns reflecting different environmental settings, seasons or inherent differences between prokaryotes and eukaryotes. With this knowledge, it would be possible to further anticipate changes in the marine microbial biosphere and its ecosystem functions in the face of climate change.

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Supplement

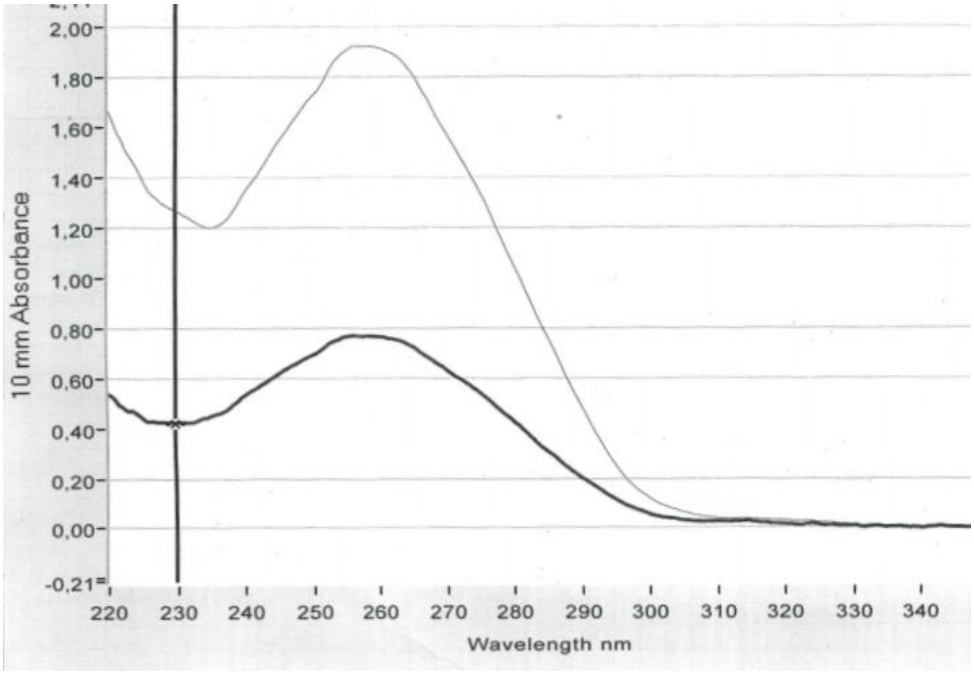

Fig. 33: *Typical absorbance curves of pure DNA, as measured with spectrophotometry. Exemplary depicted for two samples*

Table 1: *DNA concentrations measured with spectrophotometry of all HE533 samples.*

Station	replicate	depth	Nanodrop_conc
HE533_2	A	surface_pooled	40.43
HE533_2	B	surface_pooled	19.18
HE533_2	C	surface_pooled	12.76
HE533_2	deep	deep	16.9
HE533_3	A	surface_pooled	8.56
HE533_3	B	surface_pooled	32.5
HE533_3	C	surface_pooled	13.68
HE533_3	deep	deep	29.4
HE533_4	A	surface_pooled	26.86
HE533_4	B	surface_pooled	16.93
HE533_4	C	surface_pooled	11.05
HE533_4	deep	deep	19.9
HE533_5	A	surface_pooled	10.07
HE533_5	B	surface_pooled	26.29
HE533_5	C	surface_pooled	9.66
HE533_5	deep	deep	19.25
HE533_6	A	surface_pooled	66.25
HE533_6	B	surface_pooled	43.48
HE533_6	C	surface_pooled	6.31

HE533_6	deep	deep	86.71
HE533_7	A	surface_pooled	36.77
HE533_7	B	surface_pooled	50.67
HE533_7	C	surface_pooled	18.54
HE533_7	deep	deep	17.55
HE533_8	A	surface_pooled	20.63
HE533_8	B	surface_pooled	60.24
HE533_8	C	surface_pooled	8.13
HE533_8	deep	deep	9.08
HE533_9	A	surface_pooled	2.8
HE533_9	B	surface_pooled	2.53
HE533_9	C	surface_pooled	6.56
HE533_9	deep	deep	61.04
HE533_10	A	surface_pooled	11.89
HE533_10	B	surface_pooled	39.15
HE533_10	C	surface_pooled	1.43
HE533_10	deep	deep	37.29
HE533_11	A	surface_pooled	16.01
HE533_11	B	surface_pooled	14.91
HE533_11	C	surface_pooled	6.14
HE533_11	deep	deep	9.81
HE533_12	A	surface_pooled	12.77
HE533_12	B	surface_pooled	6.69
HE533_12	C	surface_pooled	12.08
HE533_12	deep	deep	10.44
HE533_13	A	surface_pooled	39.89
HE533_13	B	surface_pooled	71.21
HE533_13	C	surface_pooled	40.63
HE533_13	deep	deep	7.39
HE533_14	A	surface_pooled	38.13
HE533_14	B	surface_pooled	49.09
HE533_14	C	surface_pooled	10.82
HE533_14	deep	deep	78.47
HE533_15	A	surface_pooled	12.88
HE533_15	B	surface_pooled	10.82
HE533_15	C	surface_pooled	39.89
HE533_15	deep	deep	96.13
HE533_16	A	surface_pooled	58.53
HE533_16	B	surface_pooled	133.73
HE533_16	C	surface_pooled	40.22
HE533_16	deep	deep	23.72
HE533_17	A	surface_pooled	62.71
HE533_17	B	surface_pooled	37.59
HE533_17	C	surface_pooled	44.61
HE533_17	deep	deep	18.19
HE533_18	A	surface_pooled	27.18

HE533_18	B	surface_pooled	65.53
HE533_18	C	surface_pooled	31.77
HE533_18	deep	deep	25.17
HE533_19	A	surface_pooled	74.26
HE533_19	B	surface_pooled	27.24
HE533_19	C	surface_pooled	46.93
HE533_19	deep	deep	34.47
HE533_20	A	surface_pooled	28.97
HE533_20	B	surface_pooled	12.07
HE533_20	C	surface_pooled	36.18
HE533_20	deep	deep	29.18
HE533_21	A	surface_pooled	3.21
HE533_21	B	surface_pooled	66.48
HE533_21	C	surface_pooled	17.39
HE533_21	deep	deep	16.59
HE533_22	A	surface_pooled	26.23
HE533_22	B	surface_pooled	87.57
HE533_22	C	surface_pooled	119.23
HE533_22	deep	deep	11.54
HE533_23	A	surface_pooled	33.51
HE533_23	B	surface_pooled	9.64
HE533_23	C	surface_pooled	22.77
HE533_23	deep	deep	133.39
HE533_25	A	surface_pooled	12.5
HE533_25	B	surface_pooled	32.89
HE533_25	C	surface_pooled	3.1
HE533_25	deep	deep	8.97
HE533_26	A	surface_pooled	8.01
HE533_26	B	surface_pooled	71.37
HE533_26	C	surface_pooled	43.33
HE533_26	deep	deep	51.48
HE533_27	A	surface_pooled	16.11
HE533_27	B	surface_pooled	32.01
HE533_27	C	surface_pooled	12.08
HE533_27	deep	deep	41.92
HE533_28	A	surface_pooled	16.92
HE533_28	B	surface_pooled	16.16
HE533_28	C	surface_pooled	10.41
HE533_28	deep	deep	47.37

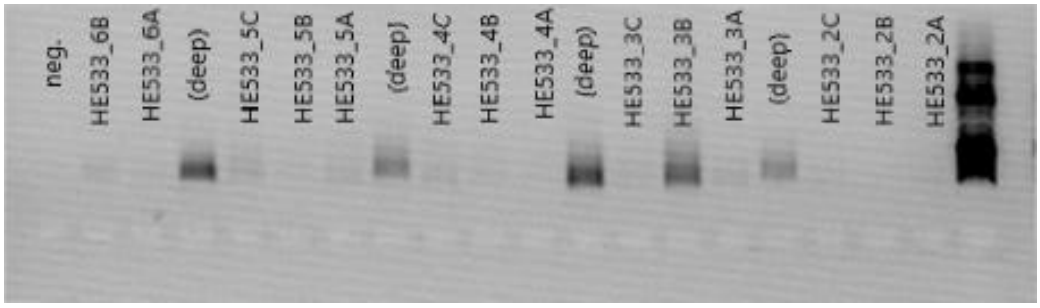

Fig. 34: *Gel electrophoresis of the extracted DNA of HE533 samples 1-20. The samples include non-surface ("deep") samples that were not analysed in the scope of this study.*

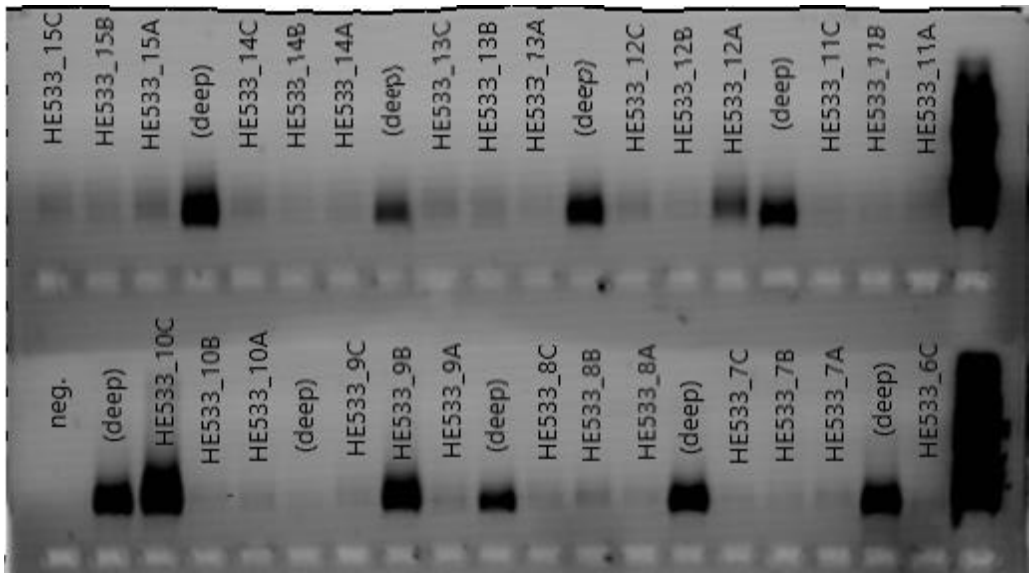

Fig. 35: *Gel electrophoresis of the extracted DNA of HE533 samples 21-60. The samples include non-surface ("deep") samples that were not analysed in the scope of this study.*

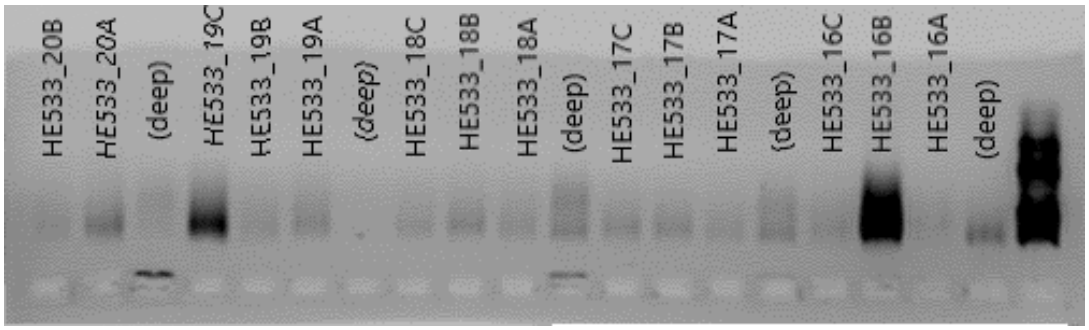

Fig. 36: *Gel electrophoresis of the extracted DNA of HE533 samples 61-80. The samples include non-surface ("deep") samples that were not analysed in the scope of this study.*

Target	Primer	Illumina tail	Sequence (5′ - 3′)
18S	V4F_illumina	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	CCAGCASCYGCGGTAATTCC
18s	V4R_illumDiv+Hapto	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	ACTTTCGTTCTTGAT
V4 Bakterien	MS_v4_515F_N	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	GTGCCAGCMGCCGCGGTAA
V4 Bakterien	MS_v4_806R_1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	GGACTACHVGGGTWTCTAAT

Fig. 37: *Forward and reverse primer for 16S and 18S rDNA to target the V4 region during the Amplicon PCR..*

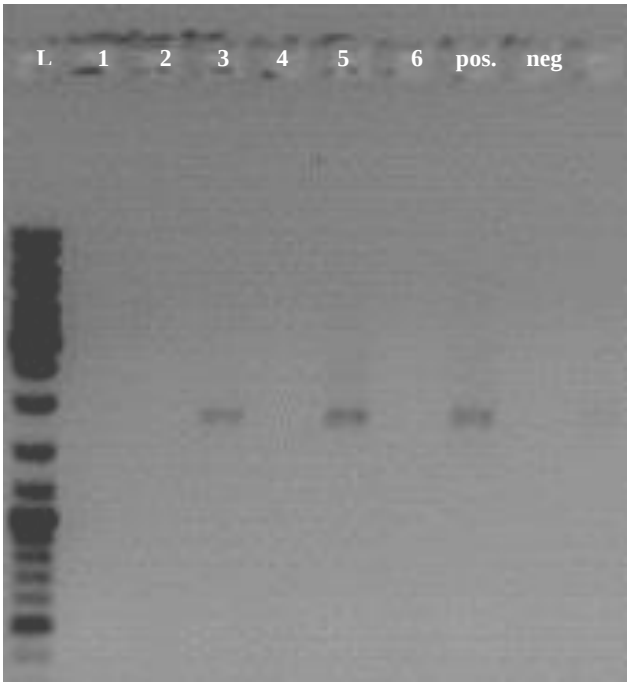

Fig. 38: *Gel electrophoresis of a test 18S Amplicon PCR. Indicated are the ladder (L), the tested samples 1-6 and the positive and negative control ("pos" and "neg").*

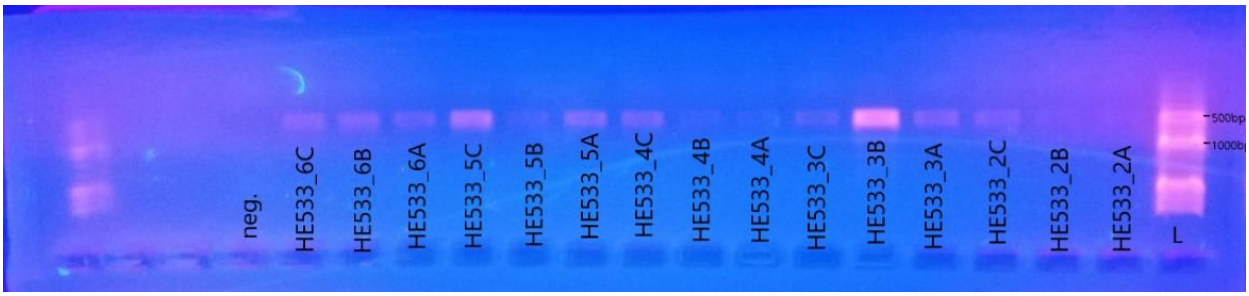

Fig. 39: *Gel electrophoresis of the 18S Amplicon PCR products of the HE533 stations 2-6. The ladder (L) and the negative control (neg.) are indicated.*

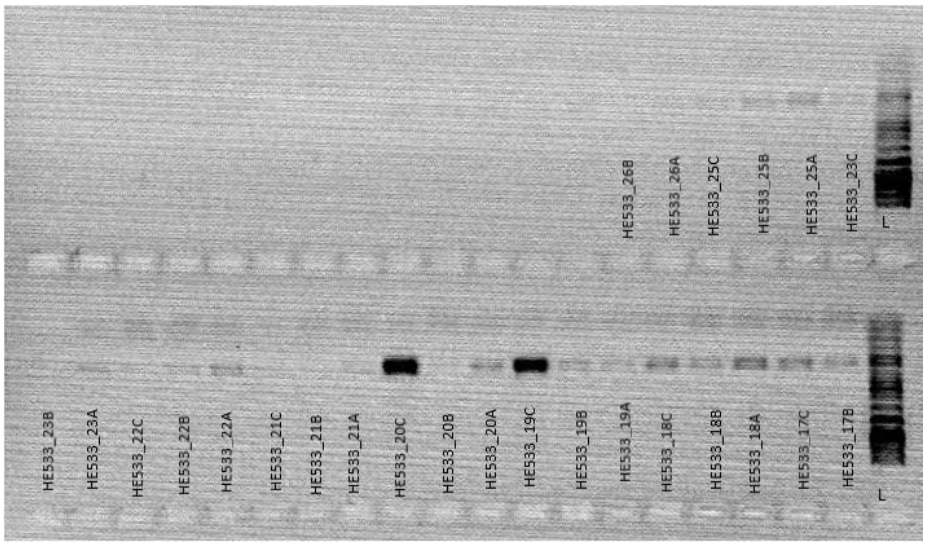

Fig. 40: *Gel electrophoresis of the 18S Amplicon PCR products of the HE533 stations 17-28 (not all are visible in the picture due to technical difficulties). The ladder is indicated (L).*

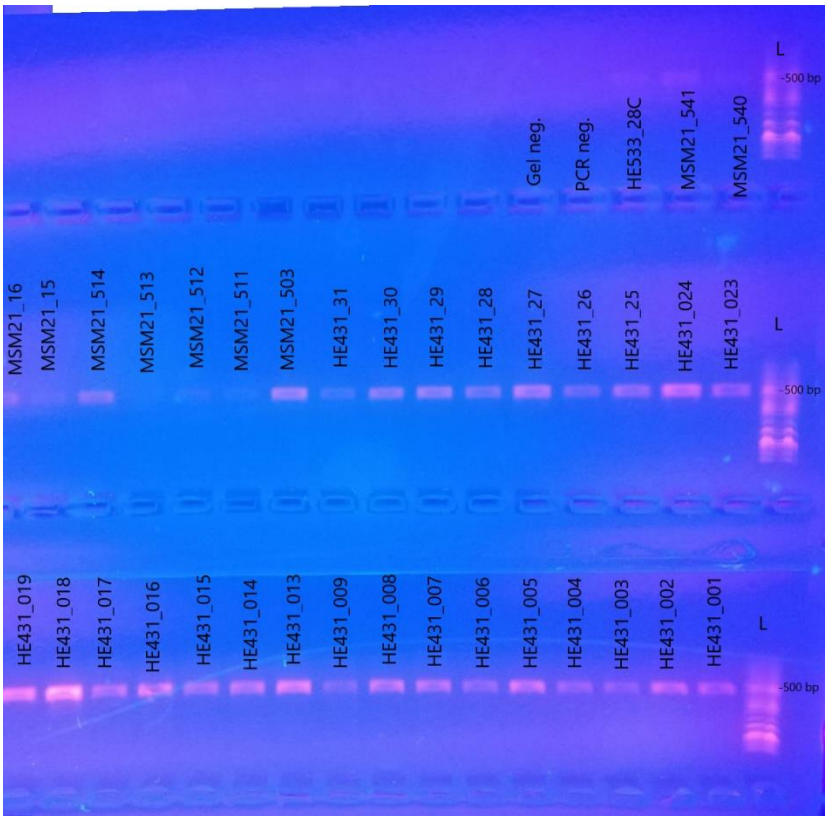

Fig. 41: *Gel electrophoresis of the 18S Amplicon PCR products of HE431 and MSM21/3. The ladder (L), the PCR negative control (PCR neg.) and the gel electrophoresis negative control (Gel neg.) are indicated.*

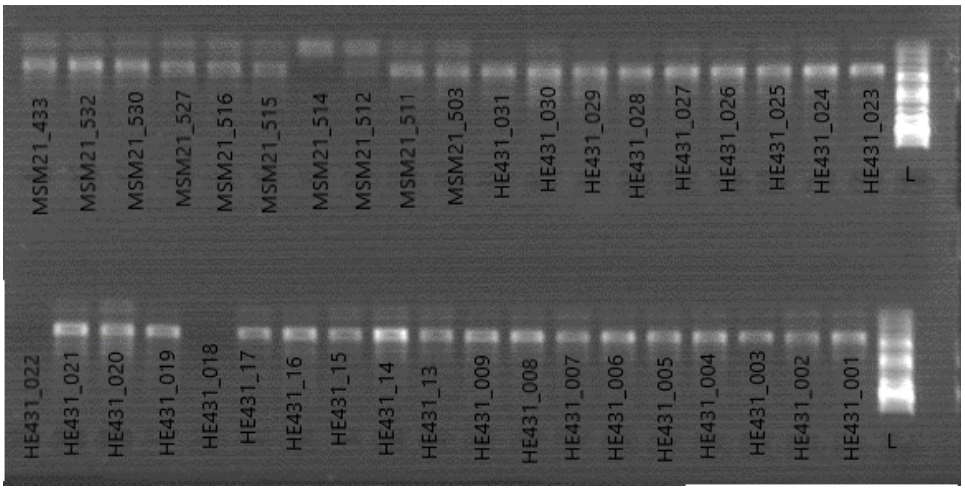

Fig. 42: *Gel electrophoresis of the 16S Amplicon PCR products of HE431 and MSM21/3. The ladder (L) is indicated.*

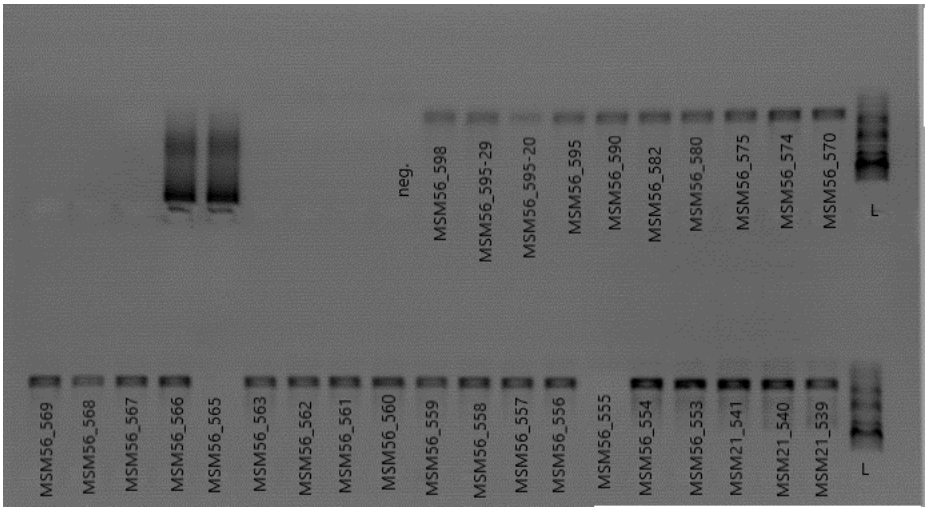

Fig. 43: *Gel electrophoresis of the 16S Amplicon PCR products of MSM21/3 and MSM56. The ladder (L) and the negative control (neg.) are indicated.*

Table 2: *LabChip® quantification. Illumina indices, peak counts, DNA concentrations and average fragment length as calculated by the LabChip®*

Sample name	**Indices**	**Peak Count**	**Total Conc. (ng/µl)**	**LabChip_conc**	**average_length [bp]**
HE533_Euk_F02_2A	S502 N701	12	2.095492472	41.90984944	600
HE533_Euk_F02_2B	S502 N702	11	1.019900727	20.39801453	600
HE533_Euk_F02_2C	S502 N703	3	0.764007598	15.28015197	600
HE533_Euk_F02_3A	S502 N704	3	0.434655458	8.693109151	600
HE533_Euk_F02_3B	S502 N705	13	2.254696727	45.09393453	600

HE533_Euk_F02_3C	S502 N706	3	0.55483203	11.09664059	600
HE533_Euk_F02_2A_Amp	control	1	0.446184101	8.923682025	
HE533_Euk_F02_2B_Amp	control	1	0.329304507	6.586090135	
HE533_Euk_F02_2C_Amp	control	2	0.069154649	1.383092983	
HE533_Euk_F02_3A_Amp	control	1	0.140504062	2.810081235	
HE533_Euk_F02_3B_Amp	control	5	0.496479378	9.92958757	
HE533_Euk_F02_3C_Amp	control	1	0.069723953	1.394479057	
HE533_Euk_F02_04A	S502 N707	4	0.410727133	8.214542666	600
HE533_Euk_F02_04B	S502 N710	4	0.349679385	6.993587702	600
HE533_Euk_F02_04C	S502 N711	6	1.542269323	30.84538646	600
HE533_Euk_F02_05A	S502 N712	9	4.704753888	94.09507776	600
HE533_Euk_F02_05B	S502 N714	1	0.394677941	7.893558819	600
HE533_Euk_F02_05C	S502 N715	18	5.724880056	114.4976011	600
HE533_Euk_F02_06A	S503 N701	3	0.710797719	14.21595438	600
HE533_Euk_F02_06B	S503 N702	15	2.090936362	41.81872724	600
HE533_Euk_F02_06C	S503 N703	5	1.695685678	33.91371357	600
HE533_Euk_F02_07A	S503 N704	3	0.243319116	4.866382315	600
HE533_Euk_F02_07B	S503 N705	4	0.020470603	0.40941206	600
HE533_Euk_F02_07C	S503 N706	1	0.141415141	2.828302813	600
HE533_Euk_F02_08A	S503 N707	3	0.280412494	5.608249886	600
HE533_Euk_F02_08B	S503 N710	0	0	0	600
HE533_Euk_F02_08C	S503 N711	3	0.051250723	1.025014456	600
HE533_Euk_F02_09A	S503 N712	3	0.041566935	0.831338696	600
HE533_Euk_F02_09B	S503 N714	2	0.017244179	0.344883585	600
HE533_Euk_F02_09C	S503 N715	2	0.170292116	3.405842325	600
HE533_Euk_F02_10A	S505 N701	2	0.080851138	1.617022755	600
HE533_Euk_F02_10B	S505 N702	7	0.295263723	5.905274459	600

HE533_Euk_F02_10C	S505 N703	2	0.041516774	0.830335473	600
HE533_Euk_F02_11A	S505 N704	2	0.051123013	1.02246025	600
HE533_Euk_F02_11B	S505 N705	2	0.052512009	1.050240174	600
HE533_Euk_F02_11C	S505 N706	3	0.041674623	0.833492455	600
HE533_Euk_F02_12A	S505 N707	4	0.349277003	6.985540058	600
HE533_Euk_F02_12B	S505 N710	3	0.016978669	0.339573375	600
HE533_Euk_F02_12C	S505 N711	2	0.041230728	0.824614566	600
HE533_Euk_F02_13A	S505 N712	4	0.334366276	6.687325513	600
HE533_Euk_F02_13B	S505 N714	0	0	0	600
HE533_Euk_F02_13C	S505 N715	24	3.032303598	60.64607196	600
HE533_Euk_F02_14A	S506 N701	5	0.394748761	7.894975227	600
HE533_Euk_F02_14B	S506 N702	2	0.055362049	1.107240972	600
HE533_Euk_F02_14C	S506 N703	7	1.402371998	28.04743997	600
HE533_Euk_F02_15A	S506 N704	5	0.328142022	6.562840431	600
HE533_Euk_F02_15B	S506 N705	3	0.121233797	2.42467595	600
HE533_Euk_F02_15C	S506 N706	3	0.305434413	6.108688262	600
HE533_Euk_F02_16A	S506 N707	2	0.024595971	0.491919413	600
HE533_Euk_F02_16B	S506 N710	2	0.150119765	3.002395292	600
HE533_Euk_F02_16C	S506 N711	2	0.368540527	7.370810538	600
HE533_Euk_F02_17A	S506 N712	17	5.895427872	117.9085574	600
HE533_Euk_F02_17B	S506 N714	2	0.767113203	15.34226405	600
HE533_Euk_F02_17C	S506 N715	2	0.421633098	8.432661963	600
HE533_Euk_F02_18A	S507 N701	15	3.790947338	75.81894677	600
HE533_Euk_F02_18B	S507 N702	5	0.891712418	17.83424837	600
HE533_Euk_F02_18C	S507 N703	5	1.007626663	20.15253326	600
HE533_Euk_F02_19A	S507 N704	9	1.213062083	24.26124165	600
HE533_Euk_F02_19B	S507 N705	10	1.566627492	31.33254985	600

HE533_Euk_F02_19C	S507 N706	16	4.994617246	99.89234493	600
HE533_Euk_F02_20A	S507 N707	4	0.895956923	17.91913846	600
HE533_Euk_F02_20B	S507 N710	5	0.453222087	9.064441748	600
HE533_Euk_F02_20C	S507 N711	15	3.256386408	65.12772817	600
HE533_Euk_F02_21A	S507 N712	4	0.603068843	12.06137686	600
HE533_Euk_F02_21B	S507 N714	4	0.254956754	5.099135074	600
HE533_Euk_F02_21C	S507 N715	2	0.738348407	14.76696815	600
HE533_Euk_F02_22A	S508 N701	4	0.827950678	16.55901356	600
HE533_Euk_F02_22B	S508 N702	5	0.302166634	6.043332686	600
HE533_Euk_F02_22C	S508 N703	2	0.060724811	1.214496213	600
HE533_Euk_F02_23A	S508 N704	2	0.167900027	3.358000549	600
HE533_Euk_F02_23B	S508 N705	2	0.23812998	4.762599604	600
HE533_Euk_F02_23C	S508 N706	4	0.937523717	18.75047435	600
HE533_Euk_F02_25A	S508 N707	5	1.578311051	31.56622102	600
HE533_Euk_F02_25B	S508 N710	5	0.507821034	10.15642069	600
HE533_Euk_F02_25C	S508 N711	5	0.565838716	11.31677432	600
HE533_Euk_F02_26A	S510 N701	3	0.542089612	10.84179225	600
HE533_Euk_F02_26B	S510 N702	6	0.442591897	8.85183794	600
HE533_Euk_F02_26C	S510 N703	5	0.529914611	10.59829223	600
HE533_Euk_F02_27A	S510 N704	3	0.507496262	10.14992524	600
HE533_Euk_F02_27B	S510 N705	4	1.028449751	20.56899501	600
HE533_Euk_F02_27C	S510 N706	2	0.905897707	18.11795413	600
HE533_Euk_F02_28A	S510 N707	10	1.609973257	32.19946513	600
HE533_Euk_F02_28B	S510 N710	3	0.536833637	10.73667275	600
HE533_Euk_F02_28C	S510 N711	10	1.778061221	35.56122441	600
HE431_Euk_F02_01	S510 N712	9	1.003871038	20.07742075	600
HE431_Euk_F02_02	S510 N714	18	9.239288975	184.7857795	600

HE431_Euk_F02_03	S510 N715	18	3.565563753	71.31127506	600
HE431_Euk_F02_04	S511 N701	9	1.725557723	34.51115446	600
HE431_Euk_F02_05	S511 N702	16	1.222251076	24.44502153	600
HE431_Euk_F02_06	S511 N703	12	4.661070734	93.22141467	600
HE431_Euk_F02_07	S511 N704	3	0.336638903	6.732778064	600
HE431_Euk_F02_08	S511 N705	16	2.137785976	42.75571953	600
HE431_Euk_F02_09	S511 N706	1	0.352147288	7.042945761	600
HE431_Euk_F02_13	S511 N707	5	0.681369284	13.62738567	600
HE431_Euk_F02_14	S511 N710	21	1.766168296	35.32336593	600
HE431_Euk_F02_15	S511 N711	3	0.972400061	19.44800121	600
HE431_Euk_F02_16	S511 N712	20	2.304591142	46.09182284	600
HE431_Euk_F02_17	S511 N714	6	0.733053001	14.66106001	600
HE431_Euk_F02_18	S511 N715	16	2.456129581	49.12259163	600
HE431_Euk_F02_19	S502 N716	16	3.152081747	63.04163494	600
HE431_Euk_F02_20	S502 N718	14	1.580808596	31.61617192	600
HE431_Euk_F02_21	S502 N719	17	4.711180105	94.2236021	600
HE431_Euk_F02_22	S502 N720	29	6.193839921	123.8767984	600
HE431_Euk_F02_23	S502 N721	14	4.194289717	83.88579435	600
HE431_Euk_F02_24	S502 N722	19	8.192771039	163.8554208	600
HE431_Euk_F02_25	S502 N723	21	3.855225275	77.10450551	600
HE431_Euk_F02_26	S502 N724	15	2.07987514	41.5975028	600
HE431_Euk_F02_27	S502 N726	16	2.599431641	51.98863281	600
HE431_Euk_F02_28	S502 N727	23	2.088194686	41.76389372	600
HE431_Euk_F02_29	S502 N728	17	1.449627642	28.99255285	600
HE431_Euk_F02_30	S502 N729	9	0.666072727	13.32145454	600
HE431_Euk_F02_31	S503 N716	16	5.175194961	103.5038992	600
MSM21_Euk_F02_503	S503 N718	19	2.028934661	40.57869323	600

MSM21_Euk_F02_511	S503 N719	5	0.767640852	15.35281703	600
MSM21_Euk_F02_512	S503 N720	8	1.156533503	23.13067006	600
MSM21_Euk_F02_514	S503 N721	16	4.181239431	83.62478862	600
MSM21_Euk_F02_515	S503 N722	17	3.0757171	61.51434201	600
MSM21_Euk_F02_516	S503 N723	14	1.075135794	21.50271589	600
MSM21_Euk_F02_527	S503 N724	6	1.017228834	20.34457669	600
MSM21_Euk_F02_530	S503 N726	8	0.895405543	17.90811086	600
MSM21_Euk_F02_532	S503 N727	5	0.804013768	16.08027537	600
MSM21_Euk_F02_540	S503 N728	19	1.516537026	30.33074052	600

Index PCR products of 6 samples (1-6a)

Amplicon PCR products of the same 6 samples (1-6b). They moved further in the gel.

← →

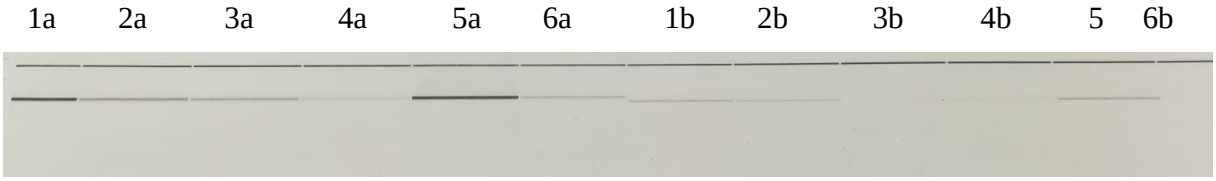

Fig. 44: *Verification of successful index attachment during the Index PCR for six exemplary samples.*

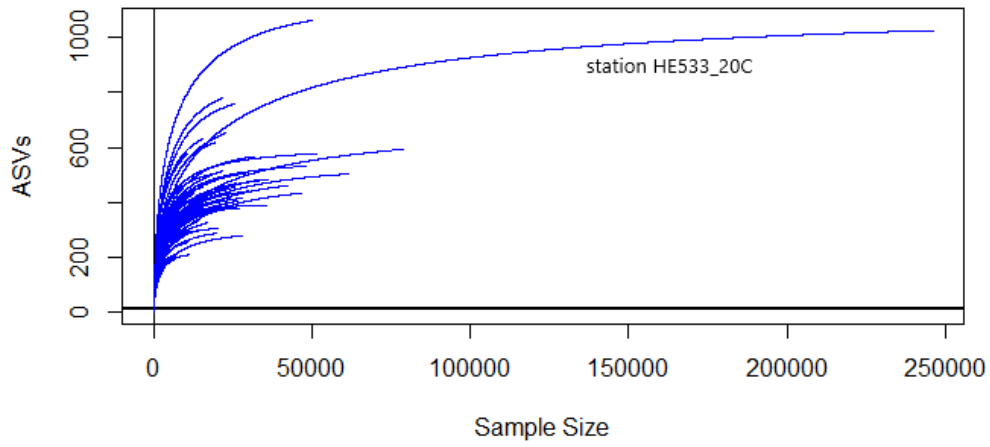

Fig. 45: *Rarefaction curves for all eukaryotic North Norwegian samples.*

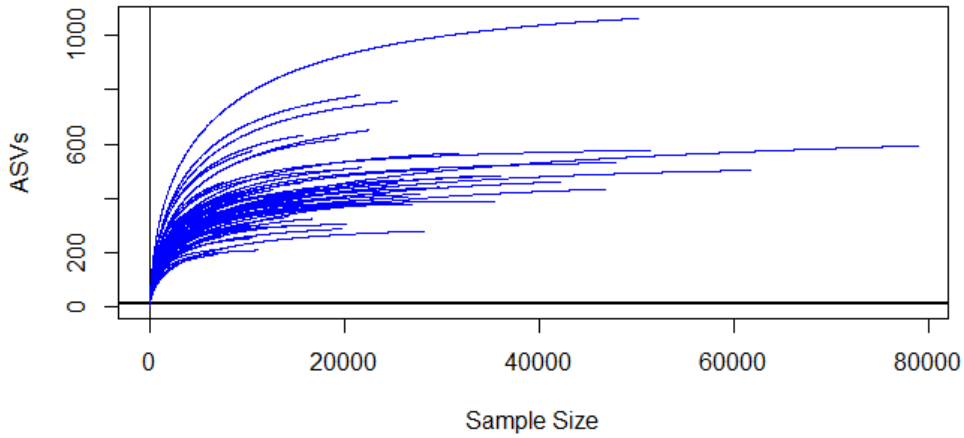

Fig. 46: *Rarefaction curves for eukaryotic North Norwegian samples without HE533_20C, which distorted the image.*

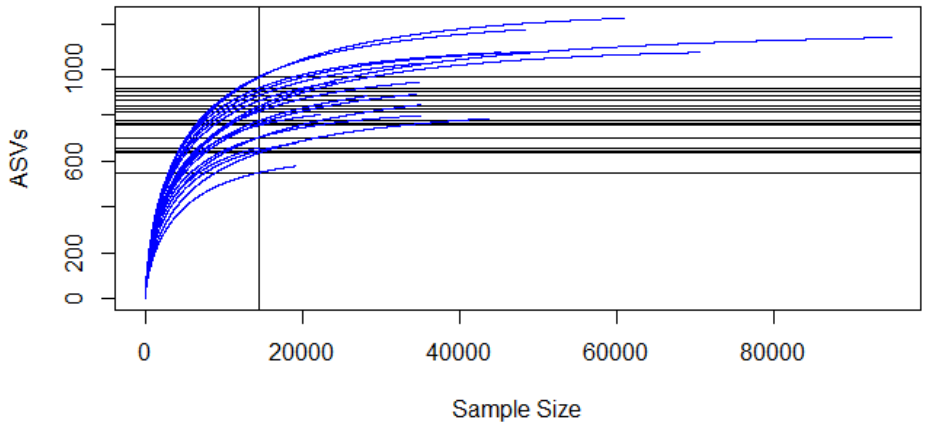

Fig. 47: *Rarefaction curves for eukaryotic South Norwegian samples.*

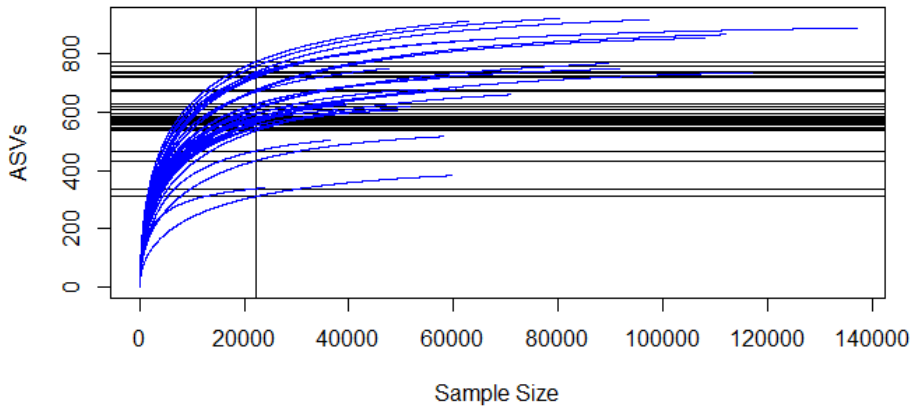

Fig. 48: *Rarefaction curves for eukaryotic Svalbard samples.*

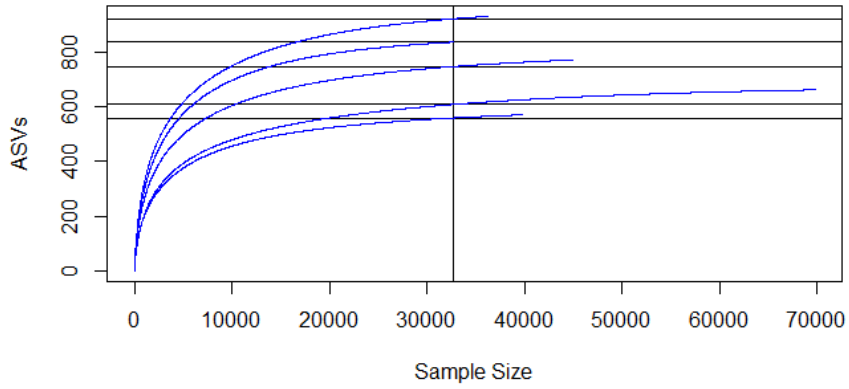

Fig. 49: *Rarefaction curves for eukaryotic Iceland samples.*

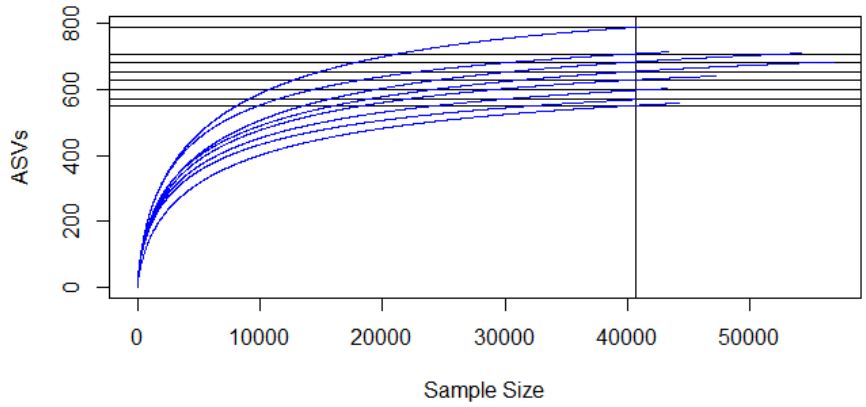

Fig. 50: *Rarefaction curves for eukaryotic East Greenland samples.*

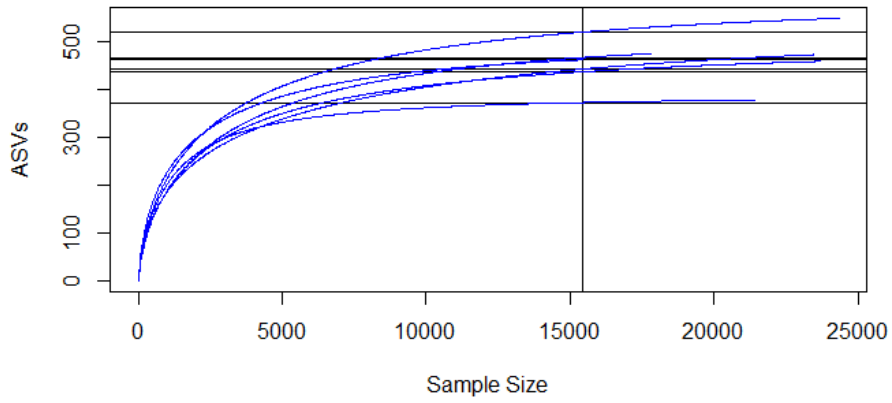

Fig. 51: *Rarefaction curves for eukaryotic West Greenland samples.*

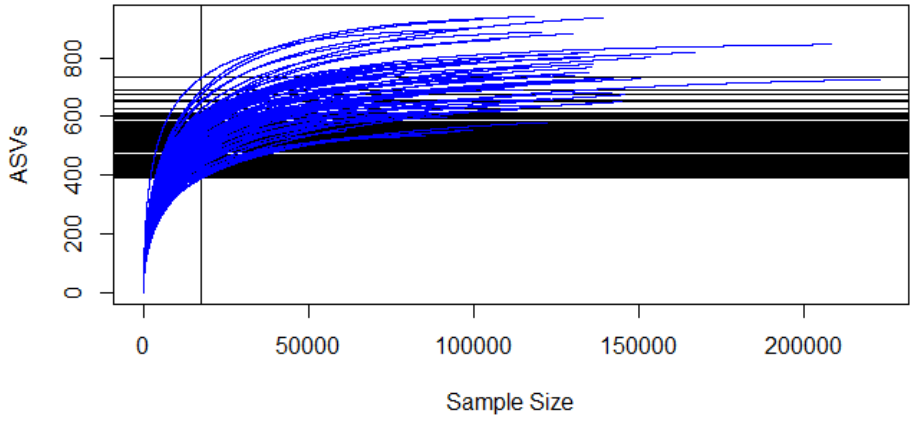

Fig. 52: *Rarefaction curves for prokaryotic North Norwegian samples.*

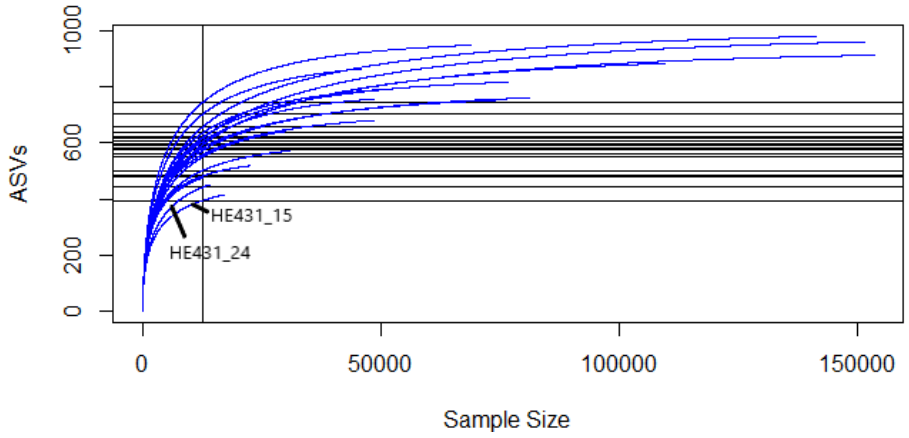

Fig. 53: *Rarefaction curves for prokaryotic South Norwegian samples.*

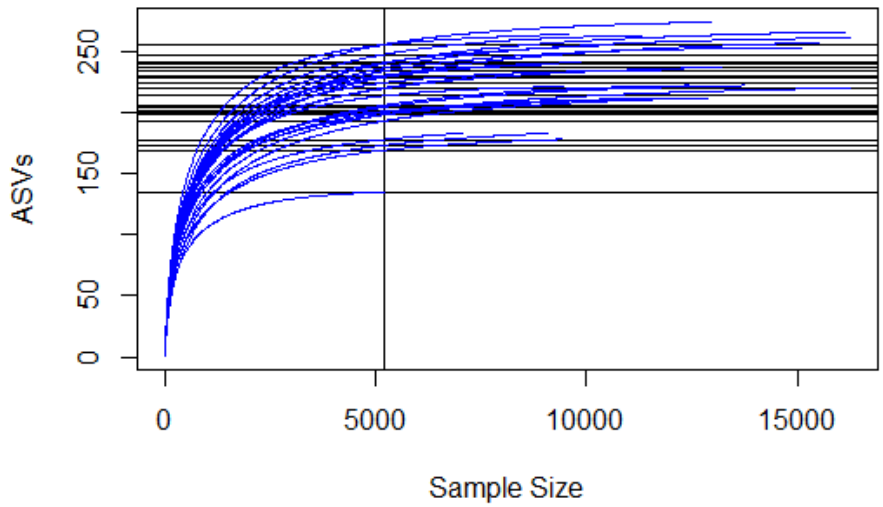

Fig. 54: *Rarefaction curves for prokaryotic Svalbard samples.*

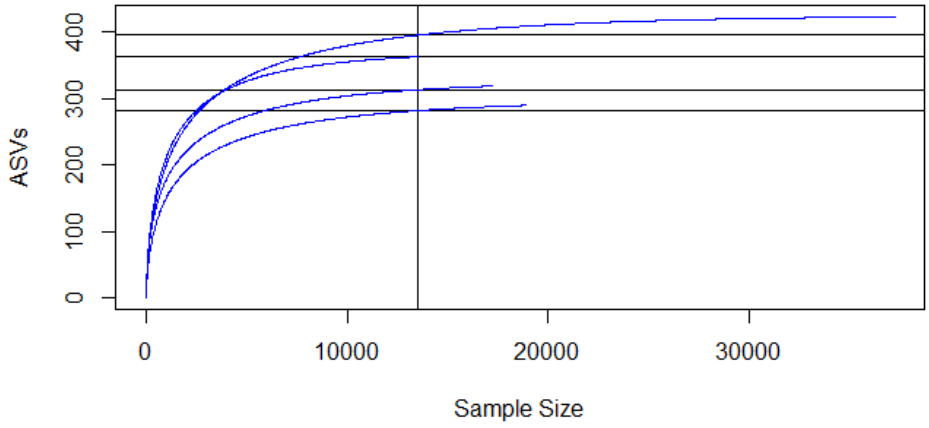

Fig. 55: *Rarefaction curves for prokaryotic Iceland samples.*

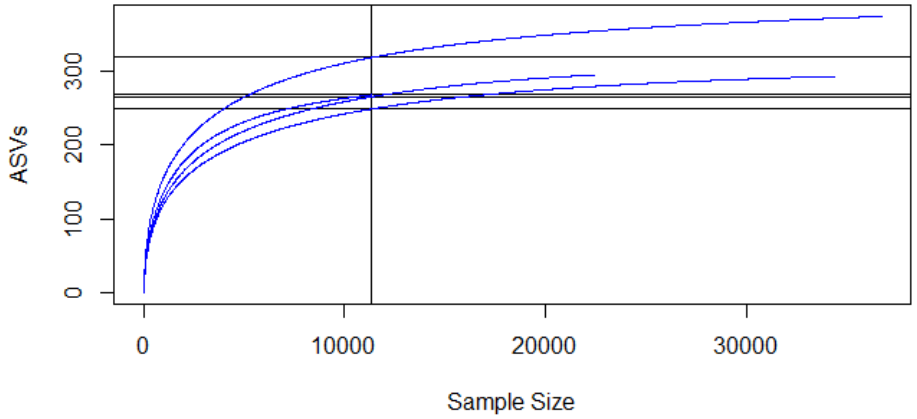

Fig. 56: *Rarefaction curves for prokaryotic East Greenland samples.*

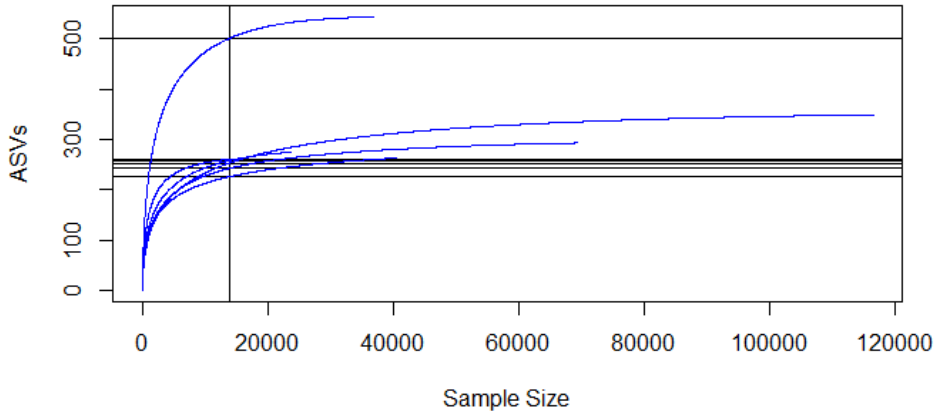

Fig. 57: *Rarefaction curves for prokaryotic West Greenland samples.*

Table 3: *T-test results of eukaryotic alpha diversity measures, compared across regions.*

EUK	**Richness**		**Shannon**		**Simpson**	
t-test	t	*p*	t	*p*	t	*p*

nnor-snor	-8.72		-5.03	<0.05	-3.13	<0.05
nnor-sval	-6.02	<0.05	-0.42	0.6	0.17	0.8
nnor-ice	-3.51	<0.05	-4.14	<0.05	-4.47	<0.05
nnor-egreen	-3.19	<0.05	0.28	0.7	1.04	0.3
nnor-wgreen	-0.48	0.6	-1.04	0.3	-0.97	0.3
snor-sval	4.98	<0.05	5.12	<0.05	3.4	<0.05
snor-ice	1.88	0.07	-0.74	0.4	-1.9	0.06
snor-egreen	3.84	<0.05	3.55	<0.05	2.77	<0.05
snor-wgreen	5.83	<0.05	2.09	<0.05	0.96	0.3
sval-ice	-0.95	0.3	-4.5	<0.05	-4.84	<0.05
sval-egreen	0.54	0.5	0.6	0.5	1.06	0.2
sval-wgreen	3.6	<0.05	-0.97	0.3	-1.1	0.2
ice-egreen	1.5	0.1	3.6	<0.05	3.4	<0.05
ice-wgreen	4.6	<0.05	2.28	<0.05	1.9	0.07
egreen-wgreen	5.02	<0.05	-1.68	0.1	-1.8	0.09

Table 4: *T-Test result of prokaryotic alpha diversity measures compared across regions.*

PROK	**Richness**		**Shannon**		**Simpson**	
t-test	t	*p*	t	*p*	t	*p*
nnor-snor	-0.3	0.7	-5.2	<0.05	-3.8	<0.05
nnor-sval	20.04	<0.05	2.9	<0.05	-0.26	0.7
nnor-ice	6.21	<0.05	0.4	0.6	-0.07	0.9
nnor-egreen	7.9	<0.05	2.5	<0.05	2.19	<0.05
nnor-wgreen	8.5	<0.05	2.2	<0.05	1.7	0.09
snor-sval	11.9	<0.05	10.12	<0.05	7.5	<0.05
snor-ice	3.3	<0.05	3.03	<0.05	1.6	0.1
snor-egreen	4.3	<0.05	5.7	<0.05	4.01	<0.05
snor-wgreen	4.7	<0.05	5.5	<0.05	3.9	<0.05
sval-ice	-1.7	0.08	-1.01	0.3	0.02	0.9
sval-egreen	-1.1	0.2	2.6	<0.05	4.89	<0.05
sval-wgreen	-1.8	0.06	1.4	0.1	2.37	<0.05
ice-egreen	0.8	0.4	1.8	0.1	1.6	0.1
ice-wgreen	0.19	0.8	1.03	0.3	1.2	0.2
egreen-wgree	-0.3	0.7	-0.6	0.5	-0.7	0.4

Table 5: *T-Test result of pro- and eukaryotic alpha diversity measures compared across regions and domains.*

EUK vs PROK	Richness		Shannon		Simpson	
t-test	t	*p*	t	*p*	t	*p*
nnor	9.22	<0.05	5.01	<0.05	6.1	<0.05
snor	-3.1	<0.05	0.03	0.9	2.3	<0.05
sval	-14.43	<0.05	0.37	0.7	5.02	<0.05
ice	-4.6	<0.05	-1.7	0.1	-1.09	0.3
egreen	-8.02	<0.05	-0.88	0.3	0.55	0.5
wgreen	-2.7	<0.05	-1.3	0.1	-0.44	0.6

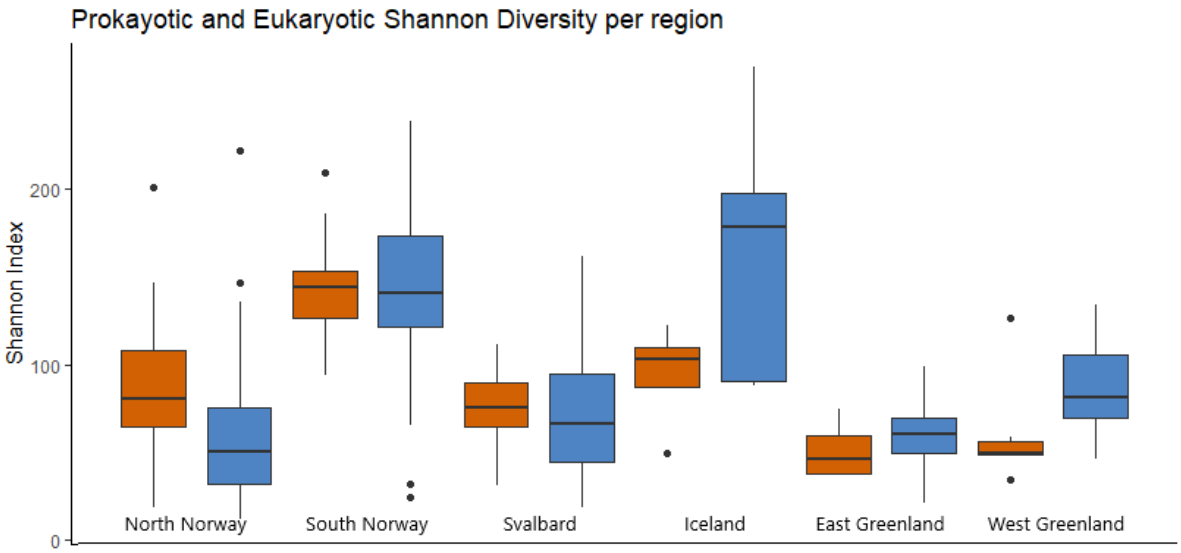

Fig. 58: *Ranges in Shannon diversity of the pro- and eukaryotic communities per region. Orange colouring marks prokaryotic and blue colouring marks eukaryotic results.*

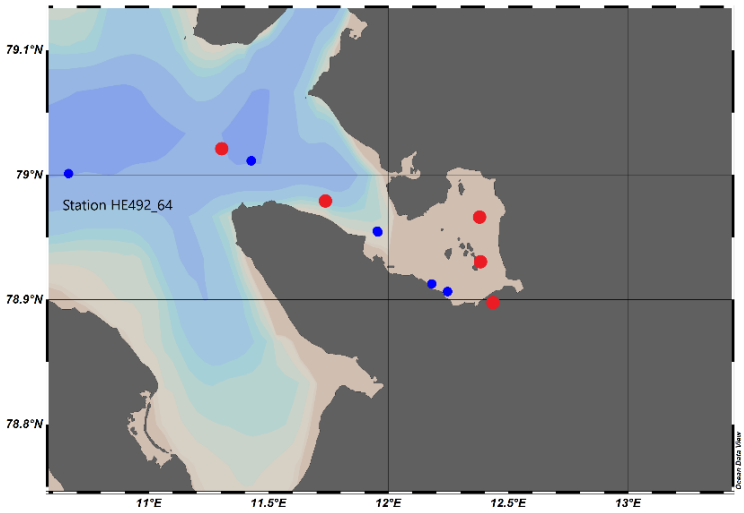

Fig. 59: *Sites in Kongsfjord, Svalbard. Stations marked red were sampled on expedition MSM56. Stations marked blue were sampled on expedition HE492.*

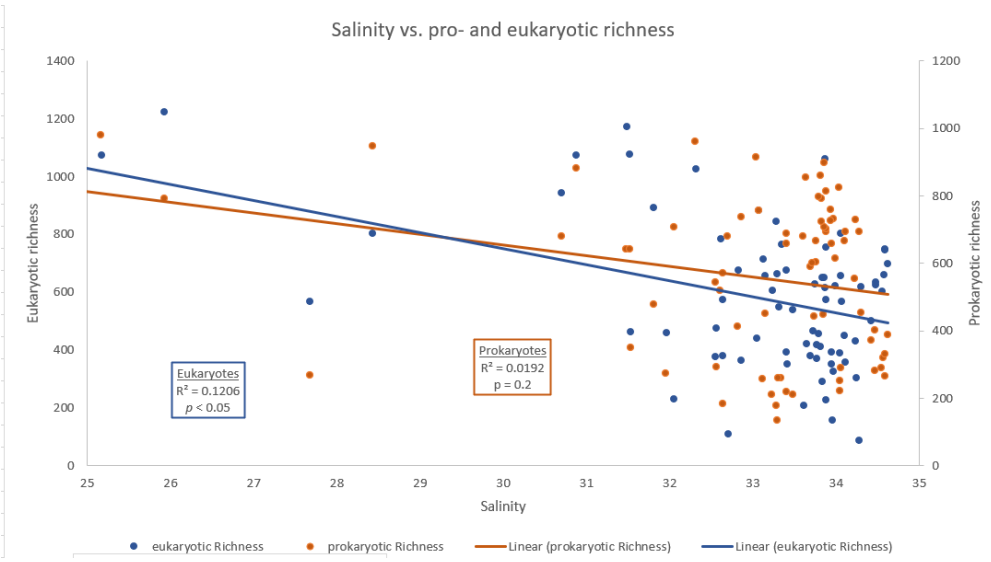

Fig. 60: *Salinity versus prokaryotic and eukaryotic richness. R^2 and p-value are indicated.*

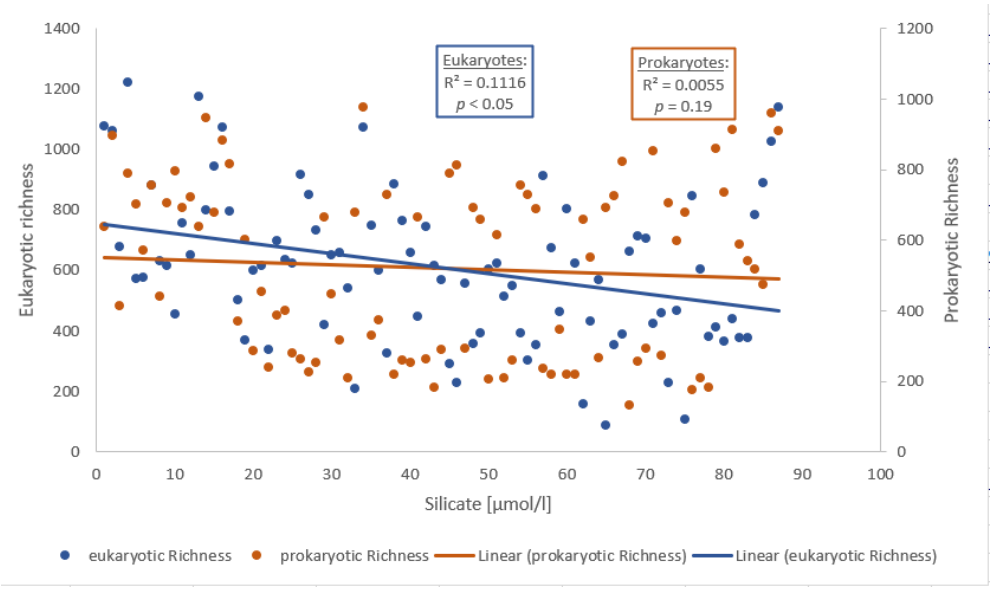

Fig. 61 *Silicate concentration versus prokaryotic and eukaryotic richness. R^2 and p-value are indicated.*

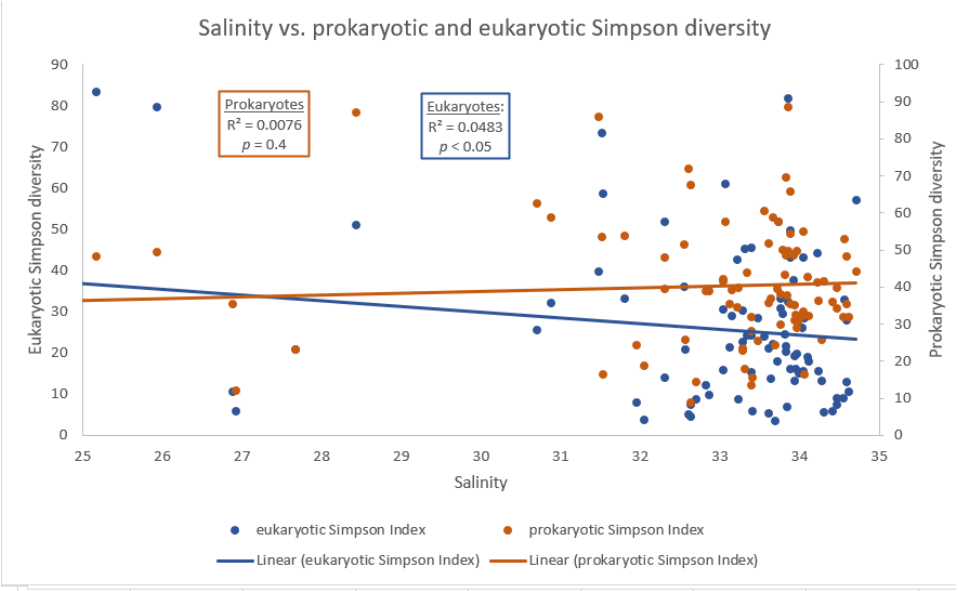

Fig. 62: *Salinity versus prokaryotic and eukaryotic Simpson diversity. R^2 and p-value are indicated.*

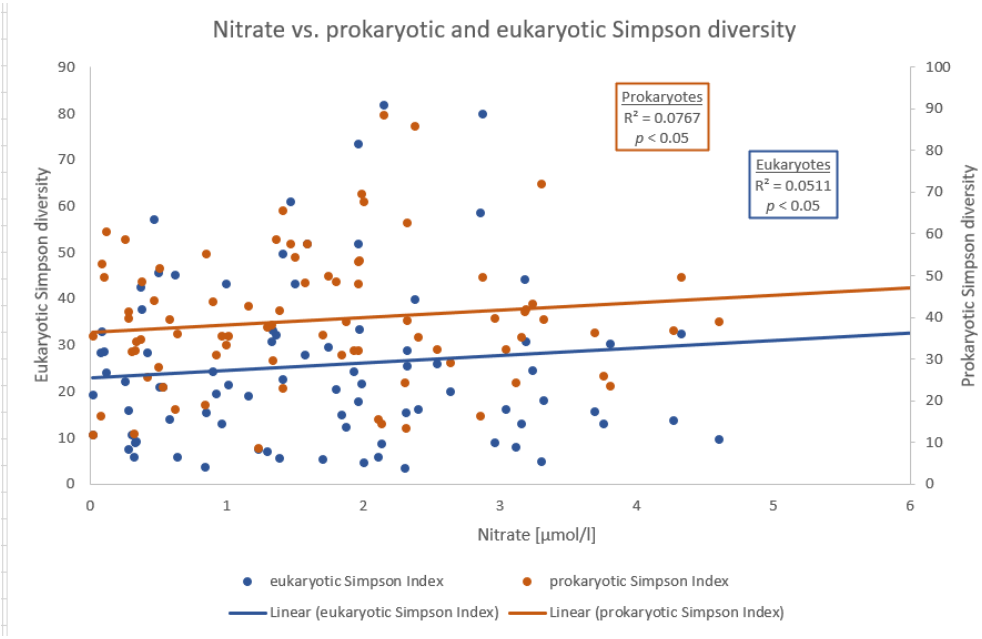

Fig. 63: *Nitrate concentration versus prokaryotic and eukaryotic Simpson diversity. R^2 and p-value are indicated.*

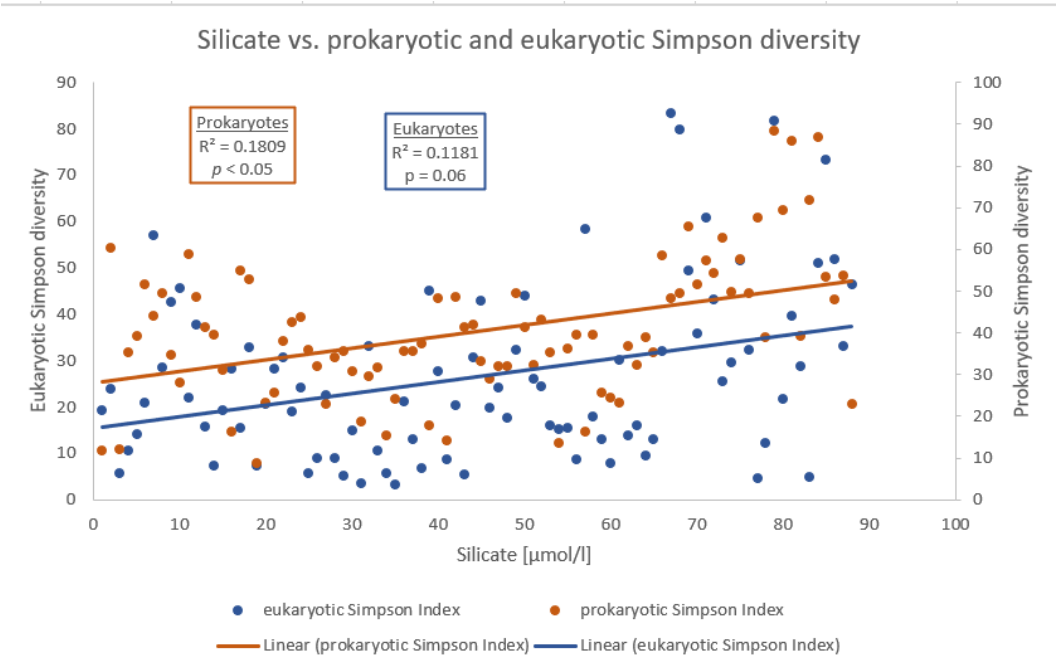

Fig. 64: *Silicate versus prokaryotic and eukaryotic Simpson diversity. R^2 and p-value are indicated.*

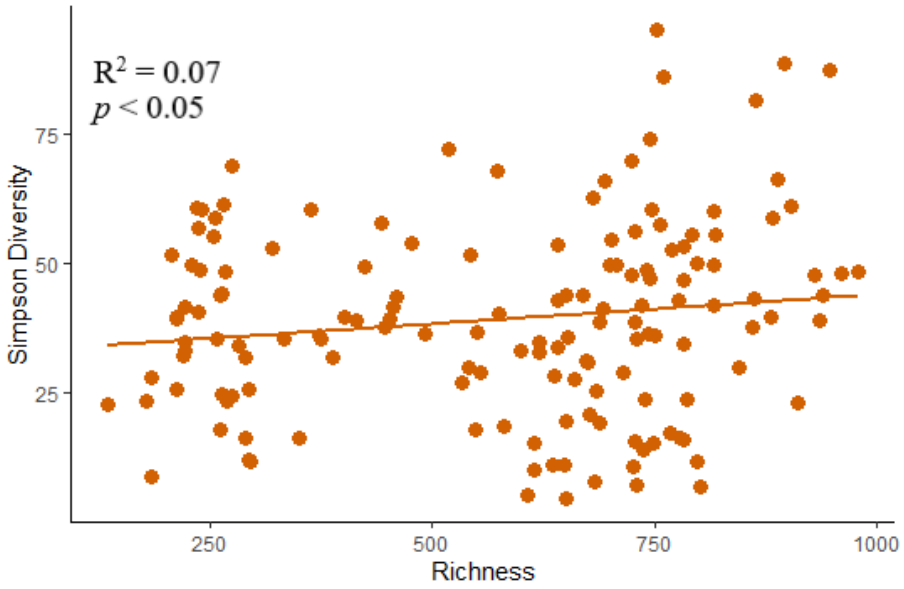

Fig. 65: *Prokaryotic Simpson diversity versus richness. R^2 and p-value are indicated.*

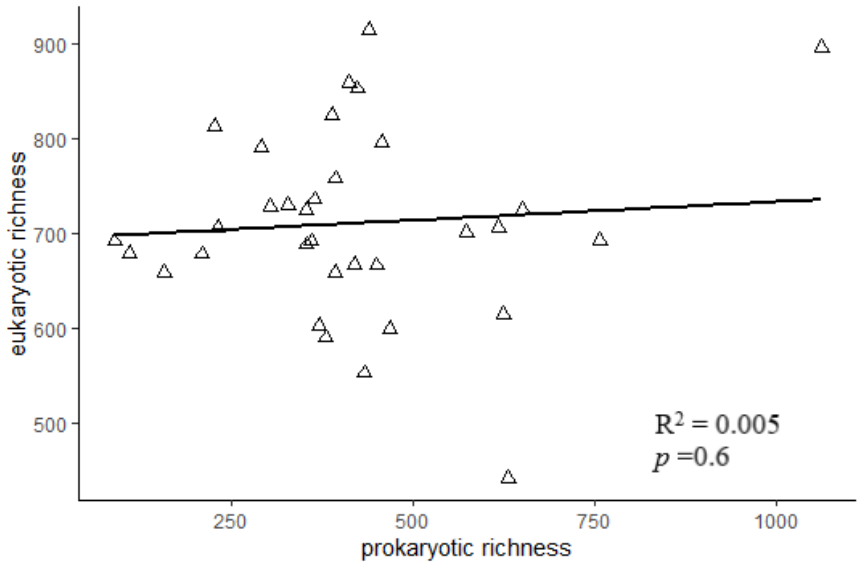

Fig. 66: *Eukaryotic versus prokaryotic richness in North Norway. R^2 and p-value are indicated.*

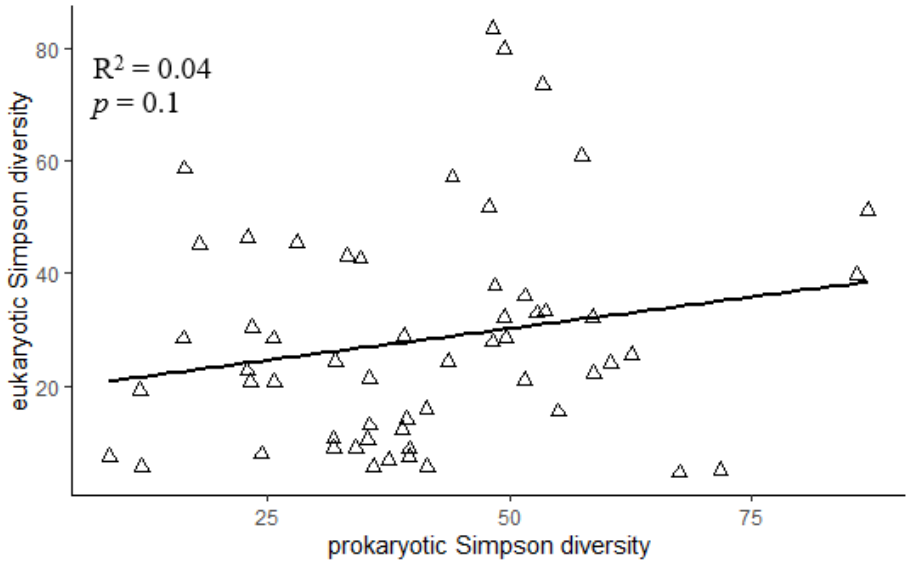

Fig. 67: *Eukaryotic versus prokaryotic Simpson diversity without North Norway. R^2 and p-value are indicated.*

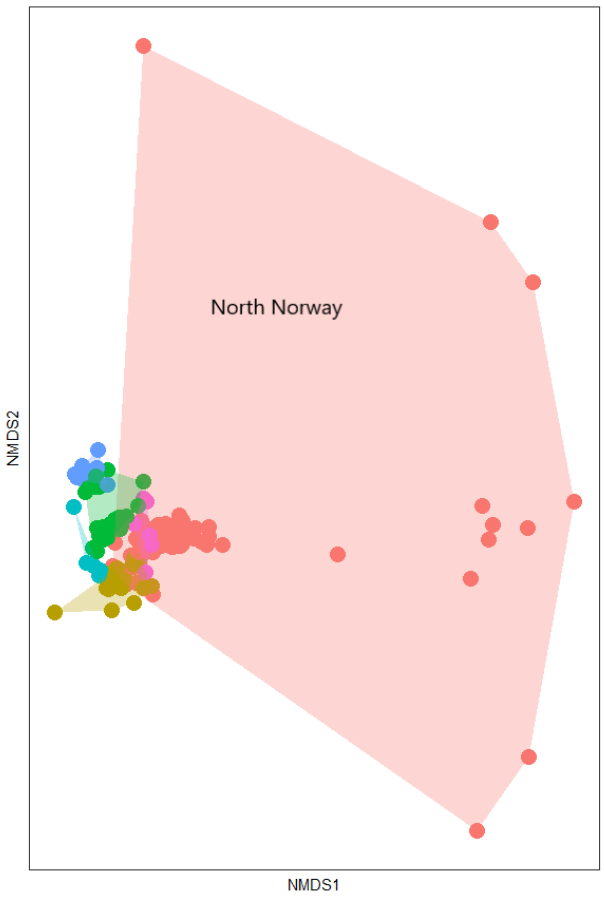

Fig. 68: *NMDS of all eukaryotic samples per region.*

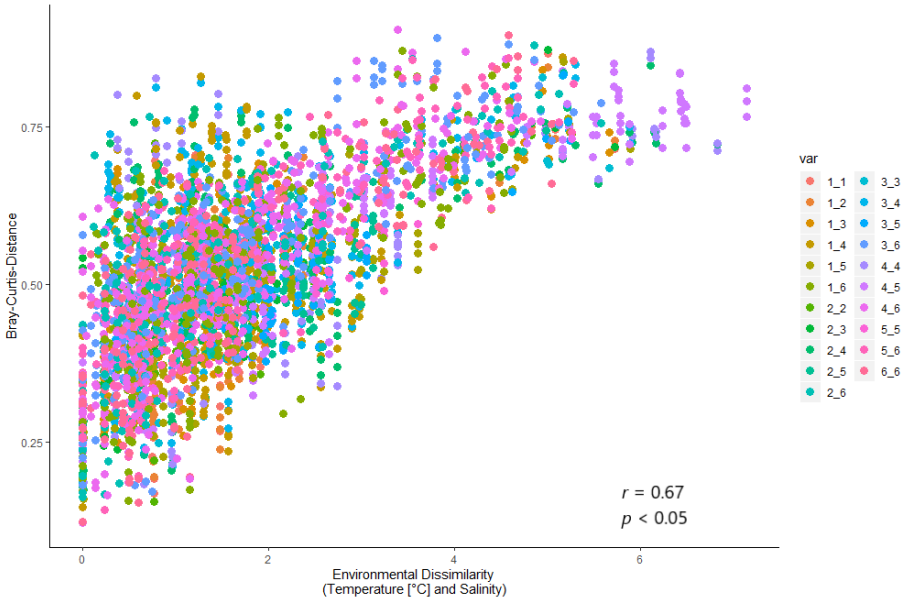

Fig. 69: *Influence of environmental dissimilarity on prokaryotic ASV turnover in North Norway. All data points that represent pairs of sites from the same two fjords are coloured accordingly. The results of the Mantel test (Pearson) are indicated.*

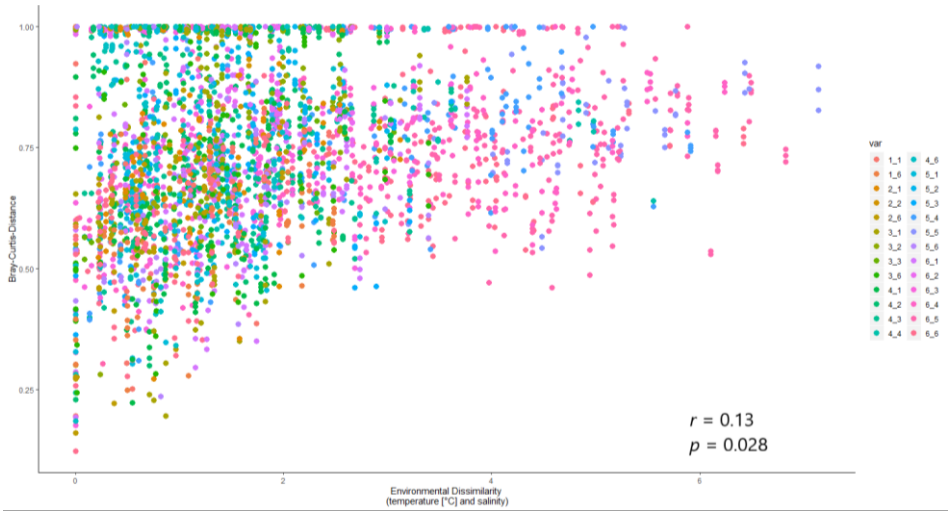

Fig. 70: *Influence of environmental dissimilarity on eukaryotic ASV turnover in North Norway. All data points that represent pairs of sites from the same two fjords are coloured accordingly. The results of the Mantel test (Pearson) are indicated.*

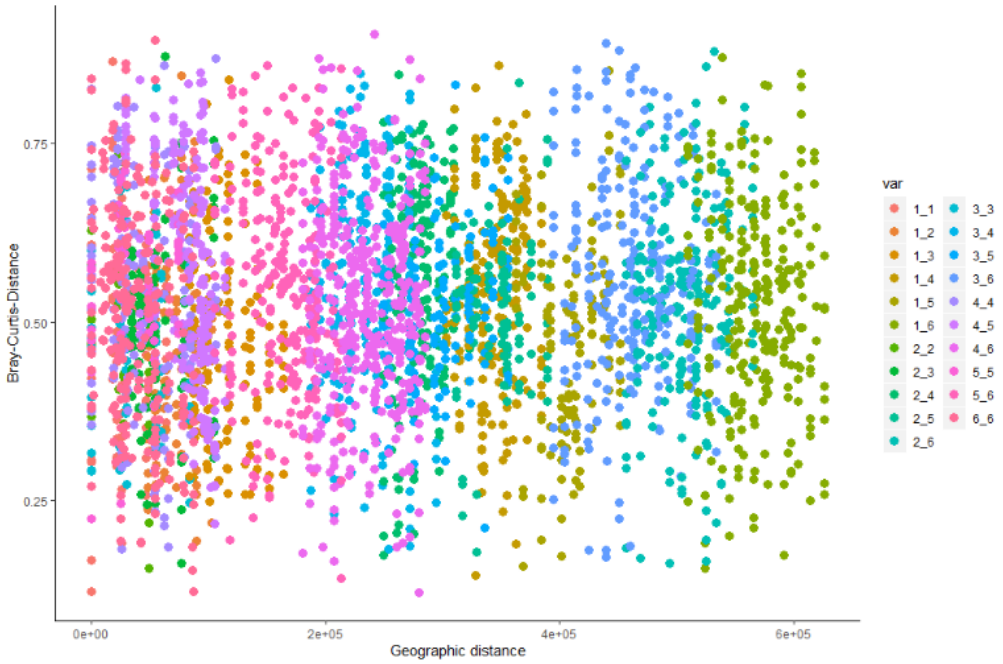

Fig. 71: *Influence of geographic distance on prokaryotic ASV turnover in North Norway. All data points that represent pairs of sites from the same two fjords are coloured accordingly. The results of the Mantel test (Pearson) are indicated.*

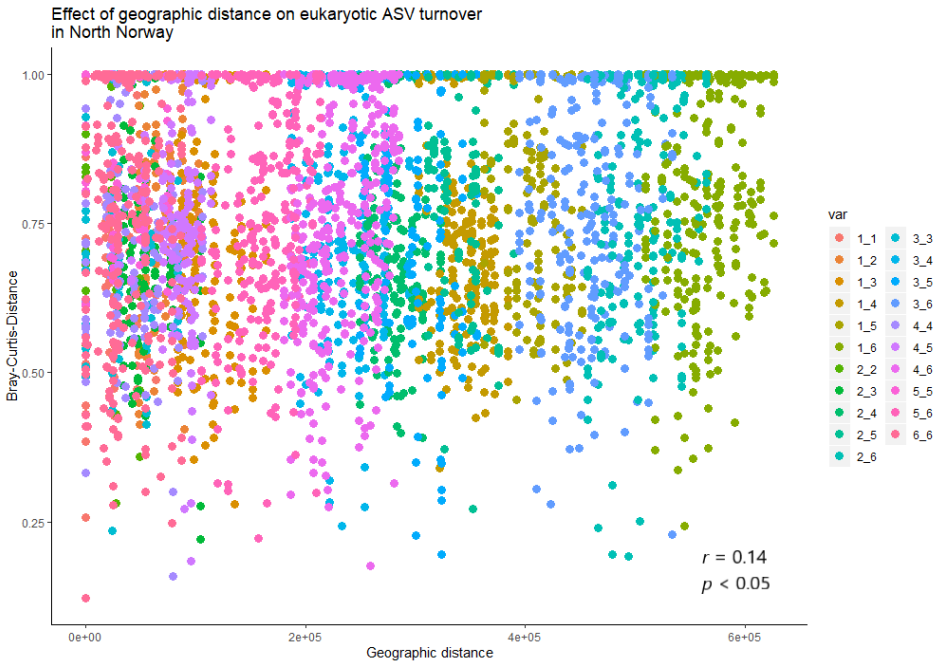

Fig. 72: *Influence of geographic distance on eukaryotic ASV turnover in North Norway. All data points that represent pairs of sites from the same two fjords are coloured accordingly. The results of the Mantel test (Pearson) are indicated.*

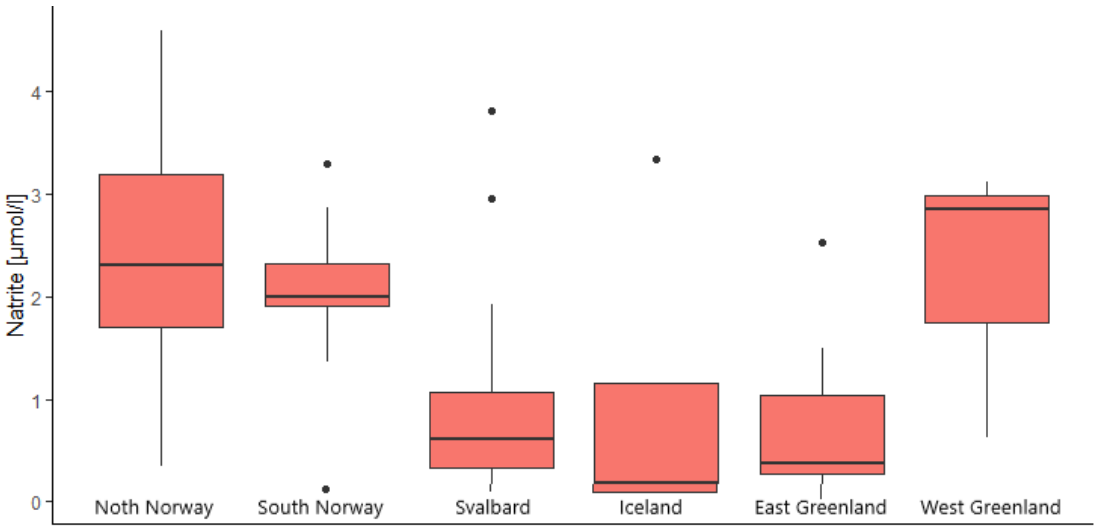

Fig. 73: *Ranges in nitrate concentration per region.*

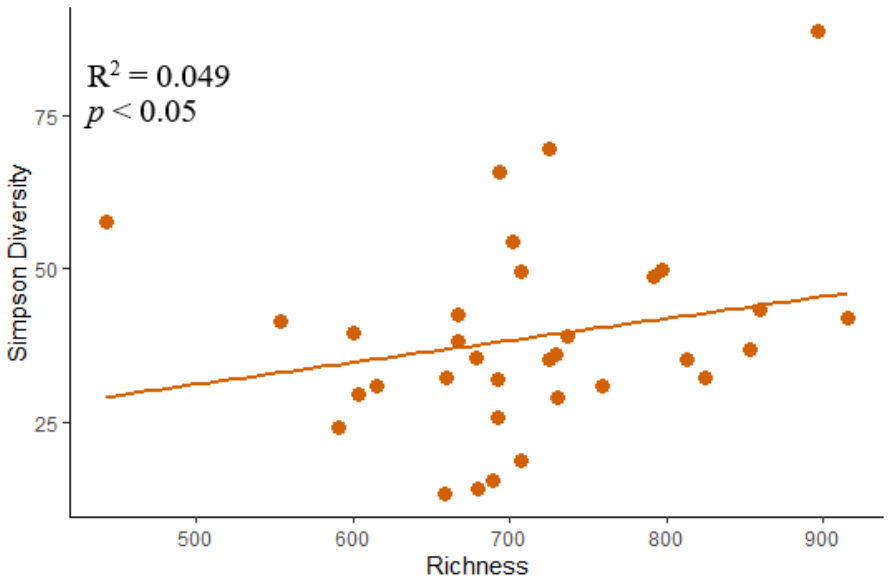

Fig. 74: *Prokaryotic Simpson Diversity versus richness in North Norway. R^2 and p-value are indicated.*

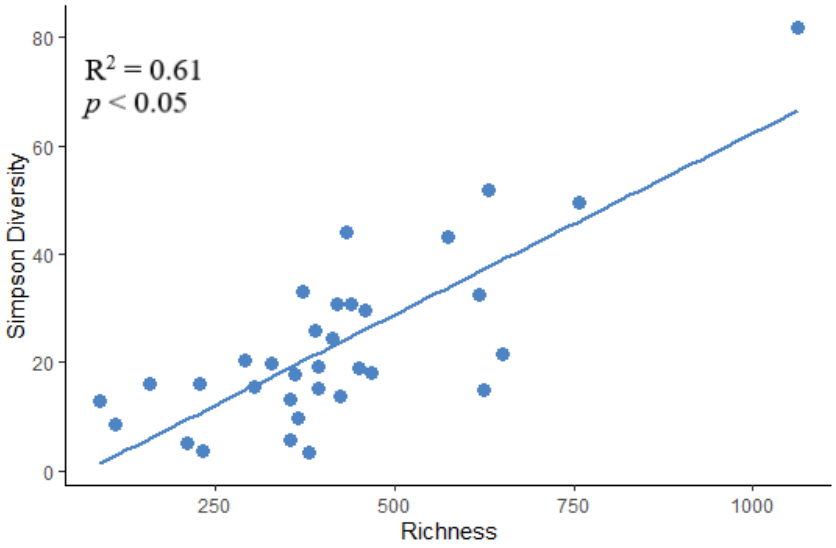

Fig. 75: *Eukaryotic Simpson Diversity versus richness in North Norway. R^2 and p-value are indicated.*

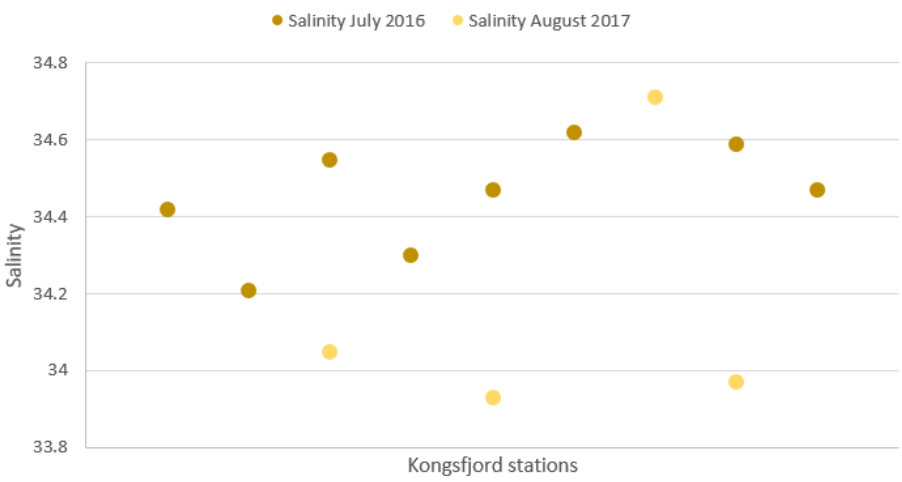

Fig. 76: *Salinity in Kongsfjord, Svalbard, as measured on two different expeditions.*

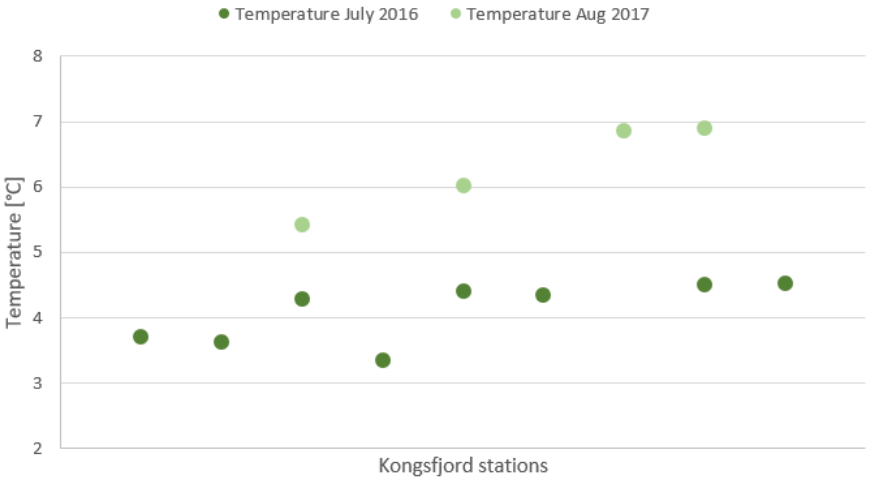

Fig. 77: *Temperature in Kongsfjord, Svalbard, as measured on two different expeditions.*

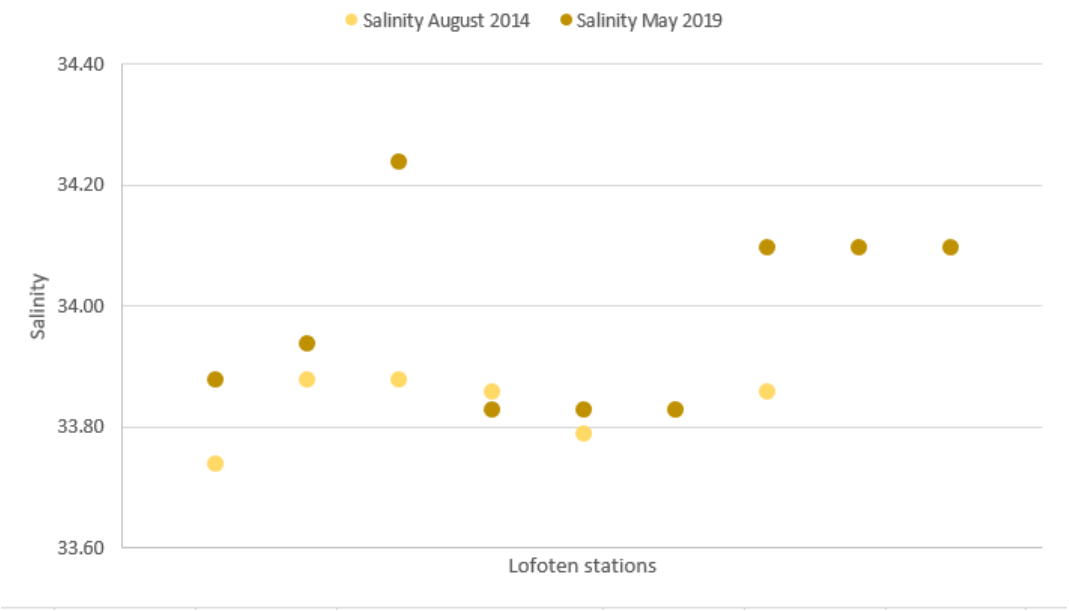

Fig. 78: *Salinity in the Lofoten, Norway, as measured on two different expeditions.*

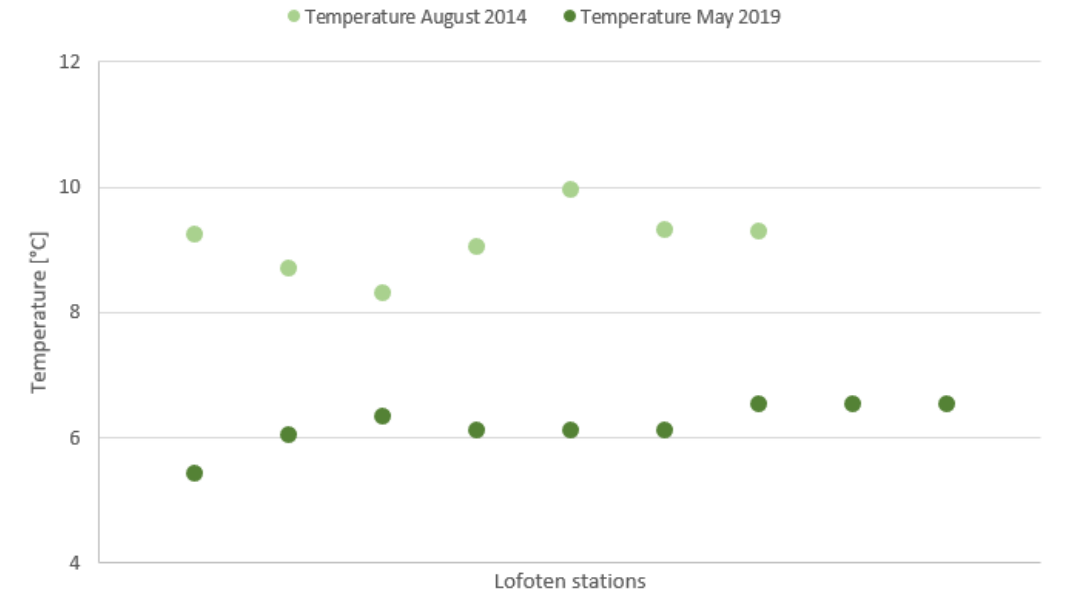

Fig. 79: *Temperature in the Lofoten, Norway, as measured on two different expeditions.*

Nachweis über die Prüfungsanmeldung in FlexNow

Name: Frau Pauline Thomé
Matrikel-Nr.: 21550954

Semester: SoSe20
Studiengang: Biologie (Bachelor of Science)
Modul: Bachelorarbeit
Prüfung: Bachelorarbeit (Erstgutachter)
Dozent: Prof. Dr. Thomas Friedl

Erklärung

Ich versichere hiermit, dass ich die vorliegende Arbeit ohne fremde Hilfe selbstständig verfasst und nur die von mir angegebenen Quellen und Hilfsmittel verwendet habe.

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Mir ist bewusst, dass bei Verstoß gegen diese Grundsätze die Prüfung mit nicht bestanden bewertet wird.

Göttingen, den 28.05.2020

Pauline Thomé

Nachweis über die Prüfungsanmeldung in FlexNow

Name: Frau Pauline Thomé
Matrikel-Nr.: 21550954

Semester: SoSe20
Studiengang: Biologie (Bachelor of Science)
Modul: Bachelorarbeit
Prüfung: Bachelorarbeit (Zweitgutachter)
Dozent: externer Gutachter

Erklärung

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P. Thomé

Göttingen, den 28.05.2020

Pauline Thomé