# A comparative study of cryo-pelagic coupling of protist communities in the Arctic and Southern Ocean

## Master Thesis

by

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# Thesis Summary

Protists are single-celled organisms, which are very sensitive to changes in environmental parameters. They show a high diversity and occur under a huge variety of environmental conditions – also in polar regions. They live in and on the ice flows, as well as in the water column beneath. The knowledge about the interchange of marine protists between sea ice and the water surface is still insufficient, whereas more and more studies pay attention to the cryo-pelagic coupling of these microorganisms. Recently in the context of global change, where sea ice minima are observed more frequently - especially in the Arctic Ocean.

The central hypothesis of this thesis refers to the coupling of the protist communities in the sea ice and the water column. During the freezing process, the salt leaves the ice through a channel system ("brine channels"), which contains high salinities and offers many habitats for different organisms to coexist on small scales. Therefore, we assume a higher diversity in the sea ice than in the under-ice water. Although the distance between both habitats is relatively small, results of other studies in the Arctic Ocean showed already differences in the community composition. To address this hypothesis, a molecular approach has been chosen. The protist community in the ice and the water shows a similarity of ~ 60-70%. This result indicates, that the exchange between ice and water is relatively high, which confirms former studies about cryo-pelagic coupling.

The second part of this thesis is about the comparison of the cryo-pelagic coupling between the Arctic and the Southern Ocean, to get insides into potentially different mechanisms in both polar regions. Data for the Southern Ocean are still scarce in this context. Therefore, we include Antarctic samples from the equivalent season. A taxonomic overlap of ~ 60-70% between the sea ice and the under-ice water is remarkable. Therefore, we conclude similar mechanisms like in the Arctic Ocean. In total, ~ 60% of the taxa are found in both, the Arctic and the Southern Ocean. Consequently, a global exchange of marine protists is imaginable, but true bipolarity has to be proven by sampling in latitudes between both poles.

The focus of the last part is on freshwater taxa and especially the comparison between the land-surrounded Arctic Ocean and the ocean-surrounded Southern Ocean. The Arctic Ocean is influenced by a higher amount of freshwater input (e.g. rivers), and our results confirm more freshwater taxa in the Arctic samples than in the samples of the Southern Ocean. The results of this study bring inside into a variety of aspects of cryo-pelagic coupling in the Arctic and Southern Ocean.

The high exchange of taxa between the sea ice and under-ice water, as well as the occurrence of one taxon at both poles, might be more common than assumed by previous studies and need to get more attention in the future, when a further impact of climate change on ice extension takes place.

# Zusammenfassung

Protisten sind einzellige Organismen, die sehr sensitiv auf Veränderungen von jeglichen Umweltparametern reagieren. Sie treten in großer Vielfalt auf und sind unter sämtlichen Umweltbedingungen vertreten. So zum Beispiel auch in den Polarregionen. Sie befinden sich im und am Eis, sowie in der Wassersäule. Die Wechselbeziehung zwischen Eis und Oberflächenwasser ist für marine Protisten immer noch nicht gänzlich geklärt, obwohl in mehr und mehr Studien die Bedeutung des Austausches der Microorganismen zwischen beiden Habitaten untersucht wird - gerade in Zeiten des Klimawandels. Hierbei treten immer häufiger Minima in der Meereis- Ausdehnung vor allem im Arktischen Sommer auf.

Die zentrale Hypothese in dieser Abschlussarbeit bezieht sich auf die Kopplung der im Meereis lebenden Protistengemeinschaften und denen in den Wassermassen darunter. Da das Eis mit seinen sog. "Brine Channels" und verschiedenen Salzgehalten auf engem Raum einen anderen Lebensraum darstellt, als das homogen vermischte Oberflächenwasser darunter, nehmen wir eine höhere Protisten Diversität im Meereis an. Obwohl die Distanz zwischen beiden Habitaten relativ gering ist, konnte bereits in vorhergehenden Studien in der Arktis ein Unterschied festgestellt werden. Um diese Hypothese zu testen, wird ein molekularer Ansatz zur Bestimmung der Protistengemeinschaft gewählt. Unsere Ergebnisse für die Arktis zeigen, dass sich die Protisten Gemeinschaften in beiden Habitaten zu ca. 60-70% ähneln. Wir können damit die Befunde vorheriger Studien bestätigen.

Im zweiten Teil dieser Arbeit wird ein Vergleich zwischen dem Arktischen und Antarktischen Ozean aufgestellt, um einen Einblick in generelle Mechanismen der kryo-pelagischen Kopplung in beiden Polen zu erhalten. Für den Südozean ist die Datenlage bisher sehr begrenzt. Daher werden in dieser Studie auch Proben der Antarktis aus der equivalenten Saison miteinbezogen. Hierbei kann ebenfalls eine Überschneidung von 60-70% der Taxa zwischen Eis und Wasser festgestellt werden. Die Austauschmechanismen scheinen demnach ähnlich zu sein wie in der Arktis. Insgesamt können ca. 60% der Taxa in der Arktis auch in den Proben des Südozeans nachgewiesen werden. Ein globaler Austausch von marinen Protisten zwischen beiden Polen ist daher denkbar, tatsächliche Bipolarität von Taxa muss jedoch mit Hilfe von Probennahmen in den zwischenliegenden Breitengraden untersucht werden.

Der Fokus des letzten Teiles der Thesis liegt auf dem Vergleich von Süßwasser Protisten im Eis und der Wassersäule zwischen dem von Land umgebenen Arktischen Ozean und den von

Ozean umgebenen Antarktischen Kontinent. Aufgrund des höheren Süßwassereintrages durch Flüsse und Schmelzwasser in den Arktischen Ozean, wird ein höheres Aufkommen von Süßwasser Protisten erwartet. Diese Hypothese kann ebenfalls bestätigt warden.

Die Ergebnisse der vorliegenden Arbeit geben Einsicht in die kryo-pelagische Kopplung im Arktischen und Südozean. Der starke Austausch von Taxa zwischen Eis und Wasser, sowie das Vorkommen von Taxa an beiden Polen, ist möglicherweise stärker ausgeprägt als bisher angenommen. Weitere Untersuchungen zu diesem Thema sollten in der Zukunft in Betracht gezogen werden, vor Allem im Kontext des fortschreitenden Klimawandels.

# Abbreviations

| | |
|---|---|
| ACC | Antarctic circumpolar current |
| AW | Atlantic water |
| AZ | Antarctic zone |
| Chl a | Chlorophyll a |
| DCM | Deep-chlorophyll maximum layer |
| DMS | Dimethyl sulfide |
| MYI | Multi-year ice |
| NGS | Next-generation sequencing |
| OMZ | Oxygen minimum zone |
| OTU | Operational taxonomic unit |
| PF | Polar front |
| PSU | Practical salinity unit |
| PSW | Polar surface water |
| UIW | Under-ice water |

# 1. Introduction

## 1.1  Polar ecosystems

### 1.1.1  The Arctic Ocean

The Arctic Ocean is defined as the area in the northern hemisphere, from 66°N to 90°N.  The relatively small ocean with broad shelves is landlocked, but connected to the Pacific Ocean via the Bering Strait between Alaska and Russia, and to the Atlantic Ocean via many channels to the Baffin Bay in north Canada, the Norwegian Sea, as well as the Fram Strait (between Svalbard and Greenland). The latter is the deepest and most significant connection (Thomas & Dieckmann, 2009). The Arctic ecosystem is influenced many different currents and circulations in the water column, drifting ice aggregates and a relatively high fresh water inflow by rivers from the surrounding land masses. The light regime shows a strong variability of seasonal magnitude, which leads to several biotic and abiotic responses (Matrai et al., 2013).

The Arctic Ocean plays a very important role in Earth´s climate as it affects global thermohaline circulation: Warm surface water containing a high salt content (Atlantic Water [AW]) arrives through the Fram Strait via the Gulf Stream. These water masses meet colder ones with a lower salinity in the Arctic (Polar Surface Water [PSW]). Sinking down, it passes the Arctic halocline (located in depths between 50 and 200m), which represents also a strong density gradient/pycnocline (Thomas & Dieckmann, 2009; Carmack et al., 2016). It flows back as deep water current in southern direction (Aagaard et al., 1985; Thomas & Dieckmann, 2009). Sea ice extension increases during the winter and decreases in the summer time. Under global warming conditions, Arctic sea ice extent is declining rapidly. Since the late 1970´s, the sea ice extent in the summer declined ~40% (e.g. (Tilling et al., 2015). Forecasts based on models show, that in the mid-century the sea ice might disappear at least during the summertime (Kerr, 2007; Mahoney et al., 2008). This will have lots of consequences for flora and fauna in this area, but may influence the whole global system. For example, the shrinking sea ice could lead to a higher primary production, which influences the whole ecosystem and climate, too (Arrigo et al., 2008). In addition, the chemical equilibrium and the elemental

cycling in the surface ocean might alter due to ocean acidification, which all together will have consequences for the biogeochemistry and ecology of the Arctic system.

### 1.1.2 The Southern Ocean

In contrast to the Arctic Ocean, which is surrounded by land, the Southern Ocean is an Ocean surrounding a continent. It is deeper and larger than the Arctic Ocean and with properties, which are only found there, e.g. the permanent thermocline which reaches partly to the surface (Tomczak & Godfrey, 2013). It is directly connected to the Atlantic, Pacific and Indian Oceans and is surrounded by the Antarctic Circumpolar Current [ACC] between 45°S and 65°S. This is the unique, dominant circulation feature and represents a strong barrier (front) in the water column.

The annual ice cover in the Arctic doubles from the summer to the winter period. In the Southern Ocean the sea ice extent is five times higher in the winter (Gloersen et al., 1992; Parkinson et al., 1999; Zwally et al., 2002). Thus, the impact on the global atmospheric system is significant due to e.g. a high albedo (reflection of solar radiation from the surface back to the atmosphere) and therefore lower heat flow between water surface and atmosphere. All these properties make the Southern Ocean a very unique environment – although also being a polar region – with very different conditions for life compared to the Arctic Ocean.

An important common feature in both systems is the increasing salt content in the water layers below areas of ice formation.

## 1.2 Marine protists

### 1.2.1 Introduction to marine protists

Marine protists are single-celled eukaryotes and the major primary producers both in the Arctic and the Southern Ocean. They numerically dominate the planktonic community in the sea ice and surface water masses (Adl et al., 2005; Caron et al., 2012; E. Kilias et al., 2014; Torstensson et al., 2015; Vanzan et al., 2015).

Their cell sizes range from pico- [0.2-2 μm, e.g. *Micromonas pusilla*] over nano- [2-20 μm, e.g. many flagellates] to micro- [20-200 μm, e.g. *Melosira arctica*] plankton (Beans et al.,

2008; Brewin et al., 2010; Massana, 2011; Thomsen et al., 2014) and they have various strategies to cover their nutritional needs: autotrophy, heterotrophy and mixotrophy (Caron et al., 2012).

Although Arrigo (2005) proposes, that prokaryotic microorganisms play the most important role in biogeochemical cycles, marine protists are an important control mechanism due to predation on -, symbioses with - and parasitism of several microbial organisms (Taylor, 1982; Guillou et al., 2008; Edgcomb et al., 2011; Orsi et al., 2012).

The importance of their role in the ecosystem is important as they modify their environment by nutrient conversion and organic matter production (photosynthesis or even climate active trace gases like dimethyl sulfide [DMS]) (Walsh et al., 2009; Zaikova et al., 2010; Webb, 2015).

On the other hand, these organisms themselves are highly dependent on environmental conditions, such as light and/or nutrient availability as well as dissolved oxygen [$O_2$] concentrations or ice properties (Diaz & Rosenberg, 2008; Lee et al., 2011; Orsi et al., 2012; Peeken et al., 2016). Despite this dependency on environmental conditions, microbial eukaryotes are also able to deal with various stressors like oxygen minimum zones [OMZ] and reach a high diversity (Stoeck et al., 2009). Due to their high abundances, they are the most important food source to higher tropic levels (Brett et al., 2009).

Marine protists are a very diverse group of organisms and community composition is highly dependent on environmental conditions and interactions with other organisms.


### 1.2.2  Marine protists in polar regions

In general, protist community composition in the polar oceans is manly characterized by Haptophytes, Chlorophytes, Stramenopiles, Alveolates (incl. Dinoflagellates and Syndiniales) and Ciliates, which all occur in varying (relative) abundances (Thiele et al., 2014; Metfies et al., 2016). Sea-ice communities include micro- and nanoplankton species, such as diatoms, dinoflagellates and ciliates, but can also contain picoeukaryotes (Medlin & Priddle, 1990; Thomas & Dieckmann, 2002; Piwosz et al., 2013).

Protists in the polar regions live in the deep sea, the water column, within ice channels and in melt ponds on top of the ice, where environmental conditions vary significantly between these habitats (E. S. Kilias et al., 2014). Organisms must deal with special situations, e.g. autotrophic species in the water column are exposed to high variability in light regimes due to the ice cover. Snow cover on the ice affects the primary production of ice associated algae by

reducing the light availability as well (Nicolaus et al., 2012; Lange et al., 2015). Temperatures, the sea ice concentration and ice extension change seasonally in high magnitudes. Certain adaptations for nutrient uptake are needed to live under these conditions, especially in the context of global change (Li et al., 2009). The communities are characterized or even dominated by different taxa and structured by both, the biotic and abiotic conditions.

# 1.3   Molecular approach & its advantages

In this study, Next generation sequencing [NGS, in particular MiSeq/Illumina system] was used to identify the protist community in water and ice samples from three RV *Polarstern* expeditions in 2014/15. NGS offers the opportunity to implement parallel high-throughput sequencing of certain nucleic acid molecules or even whole genome sequences within a relatively short time period.

In contrast to traditional Sanger sequencing millions of sequencing reactions of shorter read lengths (~50-500bp) are possible. The Illumina platform determines the sequences of each sample by generating a complementary DNA strand for each target strand ("SBS", sequencing-by-synthesis technology) – parallel for all samples. It can produce 2 x 300 paired-end reads in a single run. In addition, just 0.2 ng/µl DNA are needed per sample for the sequencing process. This is very important for environmental samples, which can contain low amounts of DNA. The MiSeq integrates cluster generation, sequencing and pre-analysis of the data (MiSeq System User Guide, Illumina 2014; Illumina web page 2016).

In the past, small organisms like pico-eukaryotic species were restricted in their characterization because of morphological, technical and therefore also temporal limitations (Bolte & Cordelieres, 2006). DNA sequencing/barcoding can help to achieve reliable statements on both, individual and population level. Additionally, since everyone can learn the molecular methods quickly, also non-professional taxonomists are able to achieve their goals. Finally, small variations in the techniques offer a broad spectrum of application fields.

In phylogenetic studies, especially for investigation of protist community composition, gene fragments of the hypervariable V4 region of the 18S rDNA (**Supplementary Fig. S2**) are often used (Ebenezer et al., 2012; Hugerth et al., 2014; Metfies et al., 2016). The information of the 18S rDNA is encoding the 18S rRNA, a component of the small eukaryotic 40S ribosomal RNA subunit in the cytoplasm (Moon-van der Staay et al., 2001). In general,

sequencing of rRNA genes is relatively simple because they are flanked by highly conserved regions and universal primers can be used and the repetitive structure offers a sufficient amount of DNA template for further investigation steps (Meyer et al., 2010). 18S ribosomal sequences provided new insights into the phylogenetic tree of life already in the 80´s and at present, is extensively used (Field et al., 1988; Cleary & Durbin, 2016).

## 1.4  Cryo- pelagic coupling in polar ecosystems

In the Arctic Ocean, sea ice extent, ice thickness and net ice growth rates are expected to further decline until the end of the 21st century - sea ice minimum observed in 2012 - due to an increasing amount of atmospheric greenhouse gases (Zhang, 2007; Massonnet et al., 2012). Loss of multi-year ice [MYI], acidification and warming of water masses are some of the major effects of global change on polar ecosystems. Sea ice deformation and drifting properties may change as well (Rampal et al., 2009; Spreen et al., 2011).

During sea ice melting, ice associated organisms and particular matter are released into the water column and affect the protist community (Boetius et al., 2013). Declining sea ice coverage on the ocean, due to faster melting or a lower extension rates, - and therefore, increased solar radiation on the water surface - might result in a greater abundance of autotrophic cells in the water column. This may have impacts for deeper habitats as well, in terms of higher sedimentation rates and changes in carbon fluxes (Wassmann & Reigstad, 2011). Recent results suggest, that these changes could also lead to northward movement of species (plankton, fish & marine mammals) which have not been observed in northern regions before (Hollowed et al., 2013; Soltwedel et al., 2014; C. D. Hamilton et al., 2015).

In contrast, the Antarctic region experienced a markedly different climatic trend in the recent decades. While e.g. the sea ice extension in the Arctic Ocean declined rapidly due to rising greenhouse gas levels, it actually increased regionally in the Southern Ocean (Zhang, 2007; J. O. Turner, J., 2009; Bintanja et al., 2013).

Since the marine food web dynamics in the Southern Ocean are also strongly depending on the sea ice coverage and extension (Flores et al., 2011), the contrary development in both polar regions might lead at each to more different communities. This assumption can be supported by higher nutrient availability, the circum-polar currents, and deeper shelves in the topographically isolated Southern Ocean (J. Turner et al., 2014). As overall diversity in the

Southern Ocean seems to be much higher than in the Arctic Ocean (Ocean Biogeographic Information System, OBIS 2016), impacts of global change might be different, especially in terms of resistance towards introduction of non-native species etc.

Polar microbes in the water column are influenced by the structure (e.g. ice thickness) and properties of the sea ice, as is the sea ice community (Hegseth & Sundfjord, 2008; Moran et al., 2012; E. Kilias et al., 2014). This strongly implies a potential connectivity between the bottom of sea ice and the water beneath, which was shown already by various studies (Arrigo et al., 2003; Søreide et al., 2013) (Hardge et al., unpublished). The release of ice associated taxa into the water column affects not only the community composition and therefore the whole food web in the pelagic system, but also the benthic community due to sinking particles. At the same time, distinct taxa can only be found either in the sea ice or in the water column, with a strong dependency on their habitat properties (Booth & Horner, 1997; Ardyna et al., 2011; E. S. Kilias et al., 2014). Less sea ice due to melting processes might alter the input of taxa into the water column and in total lead to less diverse and less complex protist communities (Hardge et al., unpublished). However, controls on cryo-pelagic coupling are still deficient. Therefore, more studies are necessary in the context of global warming.

There is an ongoing debate about the global distribution of especially small marine organisms (like protists). The question, whether they are more endemic or cosmopolitan, is still not definitively answered (Whitaker et al., 2003; Fenchel & Finlay, 2004; Azovsky & Mazei, 2013). Thus, additionally to new insides into the cryo-pelagic coupling at one pole, this comparative thesis should contribute new knowledge about protist exchange under global change conditions in both, the Arctic and Southern Ocean.

Ghiglione et al. (2012) found a clear difference between bacterial communities in the Arctic and the Southern Ocean (~70% to 80% dissimilarity). They assumed, that differences between the poles are based on different environmental conditions and geographical separation. Furthermore, they explain their findings with different selection mechanisms, which control the surface and deep ocean community. Nevertheless, there were still some OTUs present in both polar regions.

On the other hand, it was already shown, that some species occur at both poles, but not in water masses in between (Sul et al., 2013). Scientists argue, that both polar regions indeed show very similar environmental conditions like the summer-winter light regimes (Wolf et al., 2015). Long-range dispersion via the Conveyor belt, wind and globalization (in this case mainly shipping) are potential drivers for global distribution (especially for organisms which build resistant resting spores) and could prevent species diversification.

If there is a clear separation, very slow genetic divergences between species at both poles can be another or additional explanation for overlaps (Jungblut et al., 2012). First insights into the potential global distribution of marine protists by Wolf et al. (2015) showed, that mostly autotrophic organisms were restricted to the polar regions, whereas heterotrophic organisms showed more similarities to organisms in warmer water masses.

In summary, overlaps in species occurrence are astonishing due to the huge geographical separation. But one could also ask the question, why the overlap is so small, since there are some very similar environmental conditions, (and over long time scales) the connectivity between all world oceans. All these findings indicate, that more than one explanatory factor for (marine) species distribution is conceivable and different species might be subject to different factors. Therefore, this study includes also a comparison between the Arctic and Antarctic protist communities, with one focus on the freshwater-saltwater species ratio, as e.g. (Ghiglione et al., 2012) proposed this as the main environmental difference between both polar regions. Since this study does not include samples from regions between both poles, an overall conclusion about true bipolarity cannot be made.

Based on this information, this Master thesis addresses the following hypotheses:

1) In each, the Arctic and Southern Ocean, taxa in the sea ice differ significantly from those in the water column, as both habitats offer different environmental conditions for organisms (e.g. brine channels in the sea ice).

2) The Arctic and Southern Ocean differ in both, the sea ice and the under-ice water protist community composition, due to the geographical separation and different environmental conditions of the two polar regions.

3) In the land surrounded Arctic Ocean, protist communities are more characterized by freshwater taxa than the Antarctic communities, since the Antarctic continent is isolated and surrounded by the open Ocean.

# 2. Material & Methods

## 2.1 Sample collection

The samples used in this study were collected during two Arctic cruises onboard RV *Polarstern* in 2015 PS92 (19.05.-28.06.2015) and PS94 (17.08.-16.10.2015; **Fig. 1 A**) and the Antarctic expedition PS89 (02.12.2014 – 01.02.2015; **Fig. 1 B**).
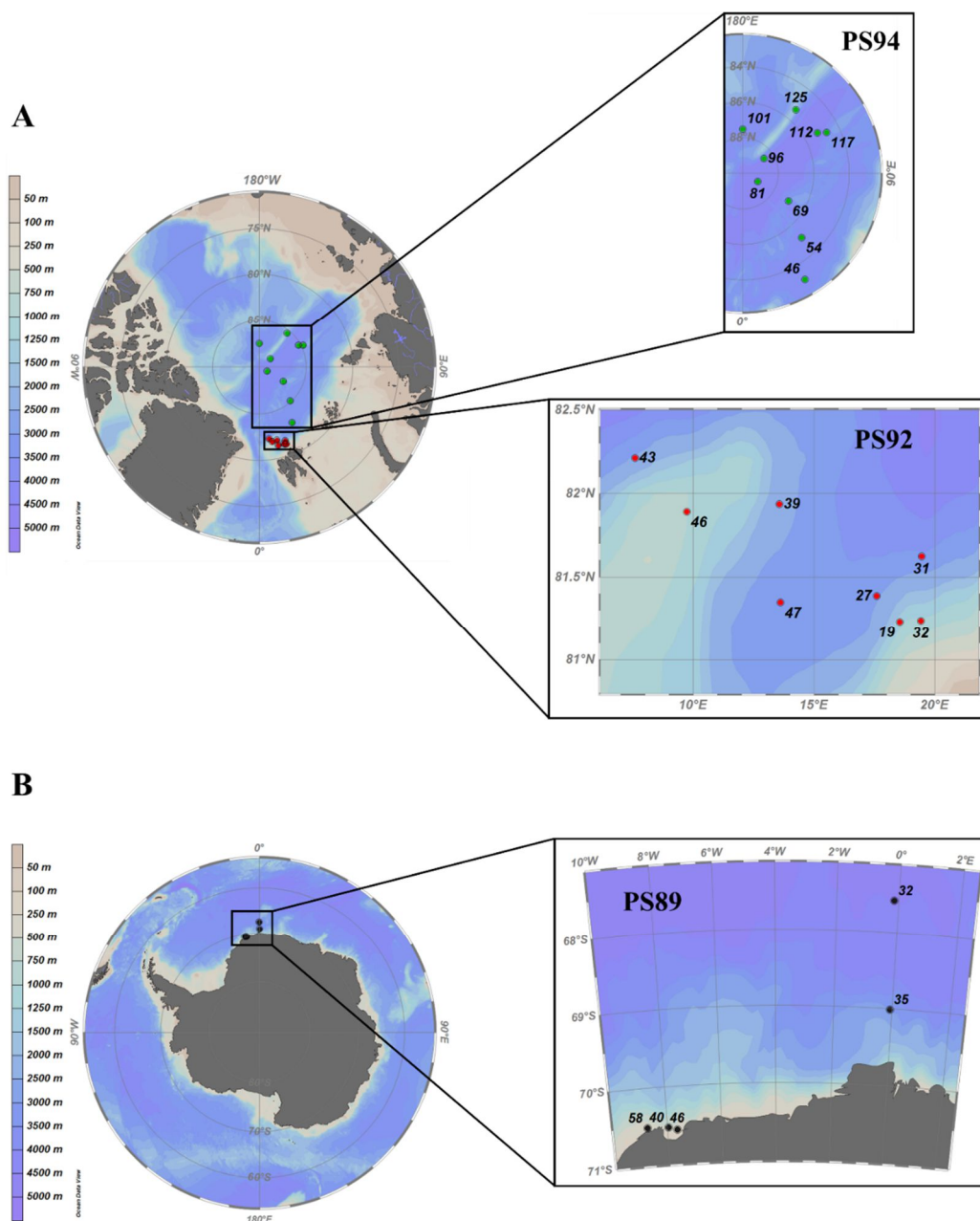


**Figure 1.** Station maps of the Arctic (**A**) and Southern Ocean samples (**B**) in an overview (left) and more detailed version (right). Green dots indicate stations of PS94, red dots indicate stations of PS92.

In the Arctic, from both expeditions 15 under-ice water (depth = 0.5 m) and 17 ice core samples were collected. Additionally, we collected 8 samples from the chlorophyll maximum layer in the water column during PS94. In the Antarctic region, 5 under-ice water samples and 5 ice cores were taken (**Tab. 1** and **Supplementary Tab. S1**).
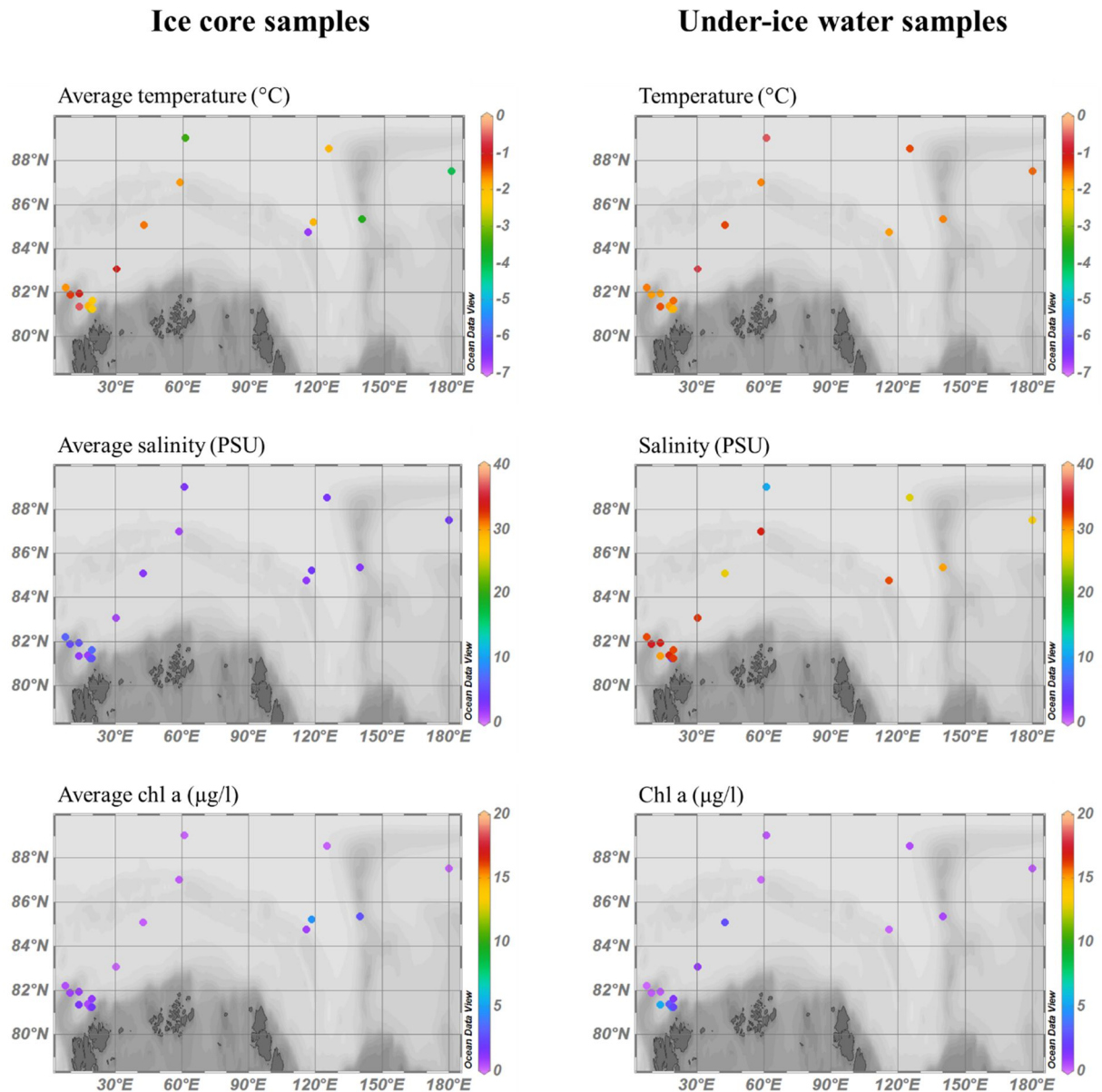


**Figure 2A.** Environmental conditions at the Arctic stations for the ice core (left) and under-ice water samples (right).

Environmental conditions (surface temperature, salinity and fluorescence) were measured at each station, separately for the ice cores and water samples (**Fig. 2A, B**). For each station one under-ice water sample and one ice core were taken. The under-ice water samples were collected by a Niskin and 0.5 – 2.0 l of each sample filtered through 47 mm Isopore Membrane Filters (Millipore, Billerica, MA, USA) with three different pore sizes (10 µm,

3 μm and 0.4 μm). The ice cores were collected with a corer (Kovacs Enterprise, Roseburg, USA, ⌀ = 9 cm). Temperature and salinity of each 5-10 cm of the core were measured, before the ice cores were melted and the meltwater was then filtered in the same manner as the under-ice water samples. Each filter was stored in a 1.5 ml Eppendorf tube at -80°C until further processing in the laboratory.
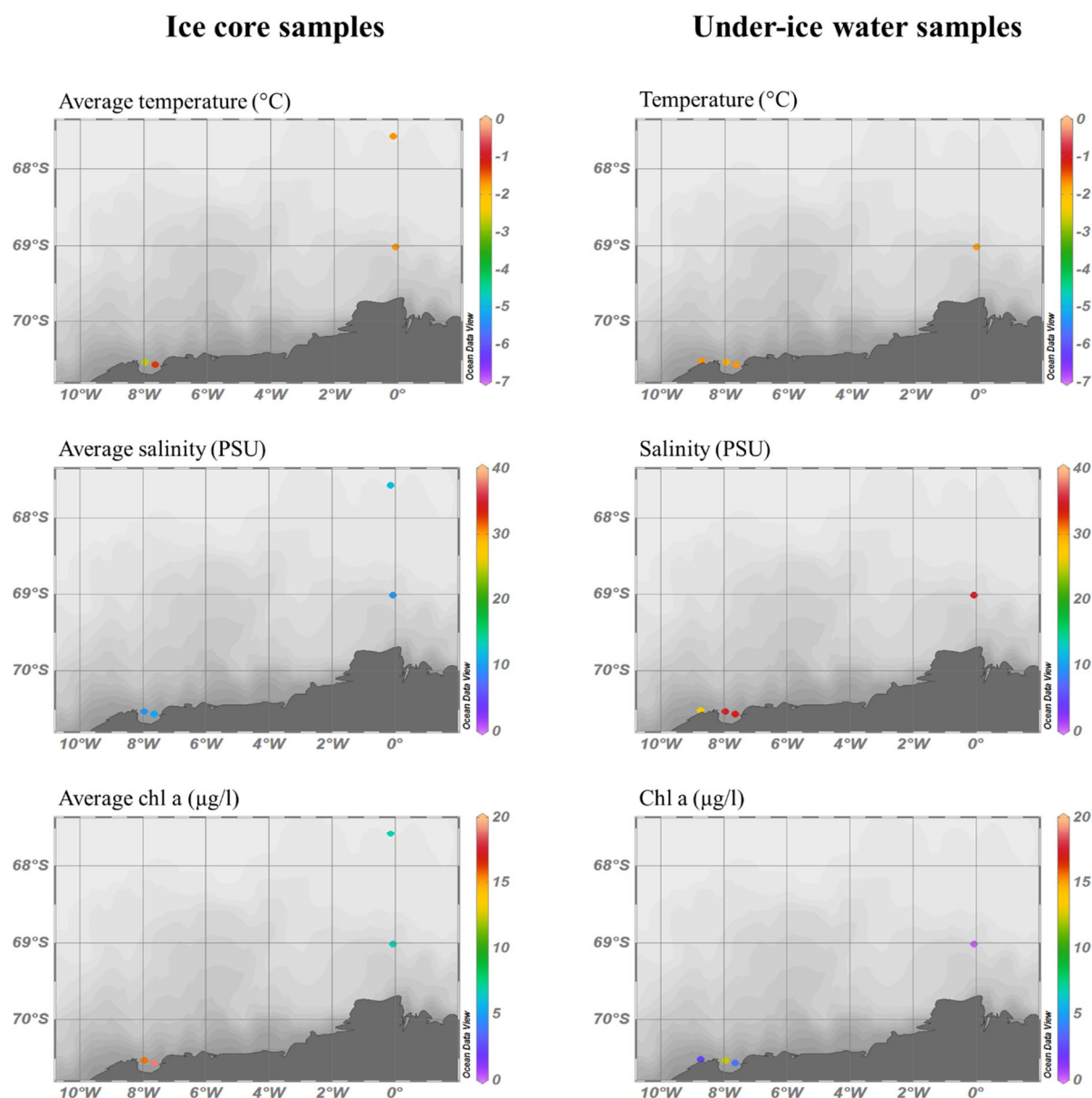


**Figure 2B.** Environmental conditions at the Southern Ocean stations for the ice core (left) and under-ice water samples (right). Temperature, salinity and chlorophyll in ice core samples were not available for station 58 and in under-ice water samples not available for station 32.

## 2.2  DNA isolation & PCR amplification

DNA isolation was performed with the NucleoSpin Plant II kit (Macherey-Nagel) according to the manufacturer´s instructions. This process includes scratching cells from the filters, cell disruption by chemical lysis, RNA degradation by RNase A, and sequential purification and elution by the elution buffer of the kit. To verify successful DNA isolation, 1-2 µl of each sample were quantified with the NanoDrop (ND-1000 Spectrophotometer, Thermo Fisher Scientific).

To increase the amount of target DNA fragments (V4 region on protist 18S rDNA; **Supplementary Fig. S2**), a PCR amplification was performed using a Mastercycler (Eppendorf, Germany). The 50 µl PCR reaction mixes contained 40.55 µl PCR clean water, 5 µl HotMaster Taq buffer (2.5 mM $Mg^{2+}$), 1 µl of each primer (forward primer: Illumina NextV4 F 5´-GCGGTAATTCCAGCTCC-3´, reverse primer: Illumina NextV4 R 5´-GGCAAATGCTTTCGC-3´, Eurofins Genomics; V4 region on the 18S rDNA), 1 µl of dNTP mix (10 mM each dNTP; Prime, USA), 1 µl of BSA (Bovines Serumalbumin 2%), 0.25 µl HotMaster Taq DNA polymerase (5U/µl; Prime, USA) and 0.2 µl DNA template. PCR was performed under the following conditions: Initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 20 s, annealing at 58°C for 30 s, extension at 68°C for 30 s followed by a final extension at 68°C for 10 min.

For each sample, 5 µl of the PCR products were used for visualization by gel electrophoresis in 1% TAE agarose (LE Agarose, Biozym; 80 Volt) and watched under UV light to check the amplification success (picture taken with VILBER LOURMAT; Software BioCapt, Version 11.02). After testing amplification success, 40 µl of each sample was run on another agarose gel at 60 Volts for adequate fragment separation. Products with the correct size (~300 bp) were purified using the DNA, RNA and protein purification kit (Macherey-Nagel) following the included protocol. The three fractions per sample (0.4 µm, 3 µm and 10 µm) were pooled with an equal amount and double-stranded DNA concentration, which was measured using the Quantus Fluorometer and QuantiFluor dsDNA System (Promega) according the manufacturer´s protocol. The recommended DNA concentration for Illumina library preparation is 0.2 ng/µl per sample, so the pooled samples were diluted with PCR clean $H_2O$.

DNA quantity and quality were controlled again for the Antarctic samples using the 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany), because some of the DNA concentrations were below the recommended 0.2 ng/µl. The samples were stored at -20°C until further analysis.

## 2.3  Sequencing & sequence processing

The library preparation was performed following the Nextera XT DNA Library Preparation Guide (Illumina) for all samples and within the same run in terms of comparability (MiSeq System User Guide, Illumina 2014).

The raw data (one forward and one reverse file per sample) created by the MySeq (output in FASTQ format, ".fastq") had to be unpacked and further analyzed by the open-source bioinformatics pipeline QIIME (Quantitative Insights Into Microbial Ecology; version: 1.8) for performing microbiome analysis from raw DNA sequencing data. This included quality filtering, taxonomic assignment and phylogenetic reconstruction as described as follows:

Forward and reverse reads were trimmed and sequences with less than 100 bp (base pairs) were removed. Forward and reverse reads per sample were joined using the *join_paired_ends.py* function with a minimum overlap of 200 bp. The resulting sequences were cut to a maximum length of 400 bp as the amplified primer pair in the PCR leads (theoretically) to a 380 bp long dsDNA fragment. After this quality check, 56.4% of the Arctic sample reads and 85.8% of the Antarctic sample reads were left (total in/out ratio).

The SILVA (www.arb-silva.de, version119) sequence database was used as a reference database for sequence analysis (Pruesse et al., 2007; Quast et al., 2013). The percentage identity level for USEARCH (The USEARCH algorithm allows searching and clustering of biological sequences much faster than the BLAST algorithm by Altschul et al. (1990); Edgar (2010))  OTU clustering was set to 97%, as this similarity level seems to be the best estimation of the original diversity and compensates sequencing errors relatively well (Kunin et al., 2010; Behnke et al., 2011). OTUs (Operational Taxonomic Units) are the most commonly used microbial diversity unit (Koeppel & Wu, 2013). According to Bokulich et al. (2013) the threshold for the minimum amount of sequences per OTU was set to 0.005% of the whole OTU abundance. Thus, OTUs without a sufficient number of sequences, Metazoa and Fungi OTUs were excluded before further statistical analysis. Additionally, for comparability of all samples, a random subsampling to a minimum amount of 3515 high-quality reads was performed and 465 different OTUs were identified.

## 2.4 Statistical analyses

The relative abundance of the protist OTUs per sample was illustrated by bar charts. Higher taxonomic groups were selected and proportions statistically compared between habitats using the two-samples t-test. In the case of differences between standard deviations, Welch´s t test was applied.

The Mantel test was performed with 999 permutations to test for potential correlations of OTU occurrence with environmental parameters, where Jaccard distances were measured for protist values and Euclidean distances for environmental parameters. Similarity patterns of protist community composition were illustrated using non-metric multidimensional scaling (NMDS) based on the Jaccard index, implemented in the R package "vegan" (R Development Core Team, 2008). Results of the cluster analyses are presented with dendrograms in the appendix. Venn´s diagrams for OTU overlap visualization were performed with the online tool "Venny 2.1" (Oliveros, 2007-2015).

Canonical correspondence analysis (CCA, R package "vegan") was performed to test, whether OTU distribution patterns depend on distinct environmental factors. Temperature values were squared transformed. Starting with a full cca model (including snow coverage, ice thickness, temperature, salinity and chlorophyll content), a backward selection using the *ordistep* function (based on AIC comparison) was performed to choose the parameters with the highest explanatory power. The significance of the chosen factors was checked again using the anova.cca function, also implemented in R.

Using NGS, an estimation of biodiversity with diversity indices was not adequate, because OTU based analyses in general do not represent single organisms since gene copy numbers differ among species (Zhu et al., 2005; Větrovský & Baldrian, 2013).

# 3. Results

## 3.1  Illumina sequencing

In total, 555 different OTUs were found after sequencing. After excluding Metazoa and Fungi, 465 OTUs were left for further analyses. In total, 219124 high-quality reads passed quality control steps. After the subsampling process, the mean amount of OTUs per sample (over all 50 samples) was 160 (± 44) (**Tab. 1**). A more detailed summary of the sequencing results is shown in **Supplementary Tab. S3**.

**Table 1.** Average number of different OTUs per samples type and sample gained by Illumina sequencing.

| Expedition | Region | Sample type | No. of samples | Average no. of different OTUs per sample |
|---|---|---|---|---|
| PS94 2015 | Arctic Ocean | Ice core | 9 | 227 ±18.628 |
| | | Under-ice water | 8 | 162 ±27.917 |
| | | Chlorophyll max. | 8 | 155 ±29.267 |
| PS92 2015 | Arctic Ocean | Ice core | 8 | 160 ±25.389 |
| | | Under-ice water | 7 | 144 ±23.267 |
| PS89 2014/15 | Southern Ocean | Ice core | 5 | 113 ±35.436 |
| | | Under-ice water | 5 | 111 ±20.873 |

Rarefaction curves indicated, that the sequencing process was successful and with good quality, as each sample nearly reached its saturation (**Fig. 3**). However, there were huge differences in diversity, since the saturation curves reached their maximum at levels between 50 and 240 OTUs.
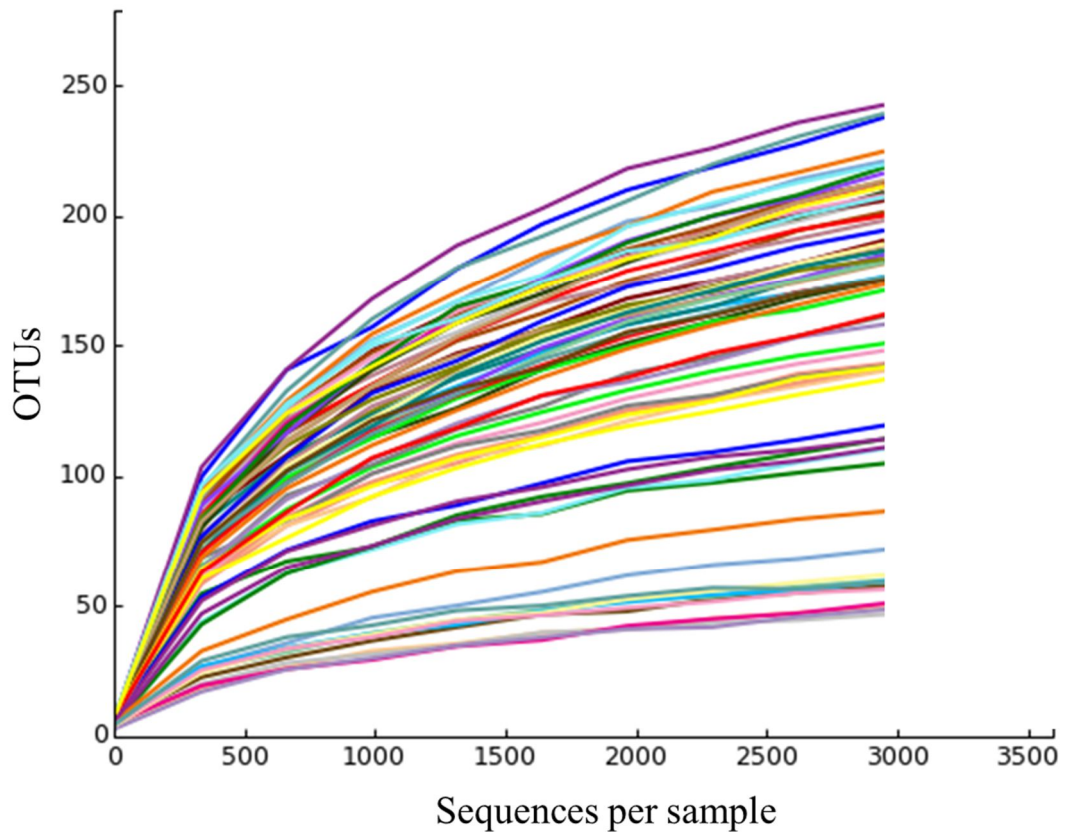
**Figure 3.** Rarefaction analysis for each sequenced sample after quality check and subsampling. OTU clustering based on a 97% similarity level.

## 3.2 Protist community composition in the Arctic Ocean

In total, 18600 OTUs were found within the Arctic samples, 6975 OTUs from PS92 (ice core and under-ice water) and 11625 OTUs from PS94 (ice core, under-ice water and chlorophyll max. layer). Subsequently, results of the analyses are presented separately by Arctic cruises.

### 3.2.1 Arctic cruise PS92

Since the taxonomic sequencing depth is not the same for each OTU, OTUs were not classified beyond the genus level and 13 major taxonomic protist groups of interest were assigned. These groups were applied and relative abundances of sequences per sample are shown (**Fig. 4**).

In the PS92 samples, the most dominant taxa were dinoflagellates (mostly Gymnodiniphycidae and Peridiniphycidae), representing more than 50% (in PS92_St.27 ~ 85%) of the relative sequence abundances in the sea ice and UIW. Chlorophyceae instead, occurred significantly more often in the ice core samples (4-41%) than in the UIW ($p < 0.05$), where they did not occur (stations 19 and 27) or at most with a maximum proportion of 3% on station 46. The Chlorophyceae were exclusively containing *Carteria* and *Chlamydomonas*. Ciliates were found in all samples, varying from 2% (UIW, St.39) to 15% (UIW, St.32), with greater amounts in the UIW ($p < 0.05$). Haptophytes (Pavlovophyceae and Prymnesiophyceae) occurred in amounts from 2% (ice station 19) to 9% (ice station 31). Other alveolates represented another significant proportion, ranging from 1% (UIW station 39) to 15% (ice station 43), with a higher occurrence in the sea ice than in the UIW ($p < 0.05$).
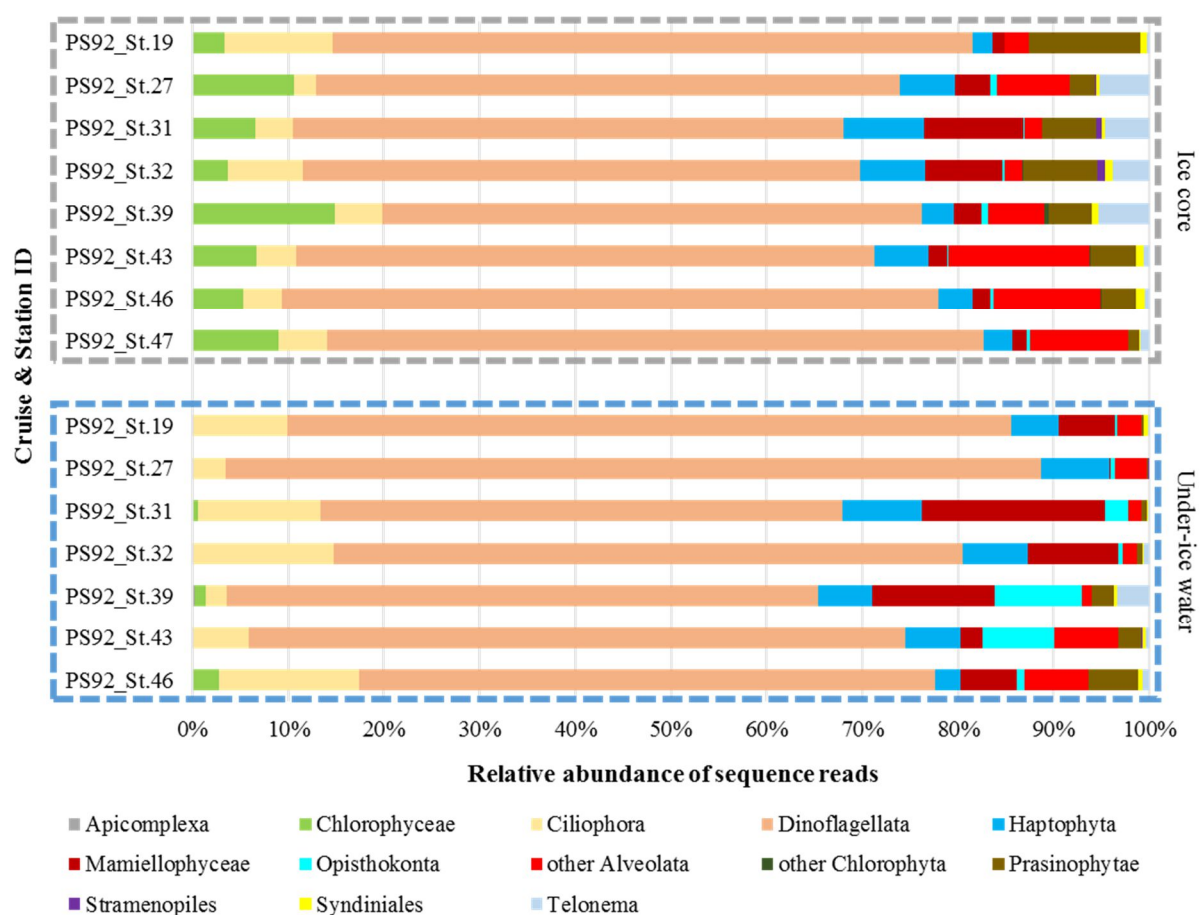


**Figure 4.** Relative abundance of sequence reads for the Arctic stations of PS92. Ice core samples are represented on the top (grey box) and under-ice water samples are presented in the middle part (blue box).

From our data, it seems that they occurred in contrary proportions to the Mamiellophyceae. The latter ones showed a high variability with maximum values on stations 31 (10% in the

ice, 18% in the UIW), 32 (8% in the ice, 10% in the UIW) and 39 (12% in the UIW). The UIW contained a slightly higher proportion than the sea ice (p < 0.05). Prasinophytae showed the highest read abundance in the ice core samples (up to 12% at PS92_St.19), which differed significantly from UIW samples (p < 0.05). Opisthokonts showed only high proportions in the UIW stations 39 and 43, leading to a significant difference between sea ice and UIW (p < 0.05). *Telonema* showed a greater read abundance in the ice core samples, than in the UIW samples (p < 0.05). Syndiniales occurred in all samples, but with differences between habitats. More Syndiniales were found in the ice core samples than in the UIW samples (p < 0.05). Stramenopiles were generally found in very small amounts in the ice cores and UIW. In general, only a few sequences of Apicomplexa were found in the PS92 samples. They did not occur at all in the sea ice, leading to a significantly higher proportion in the UIW (p < 0.05).

Non-metric multidimensional scaling (NMDS) was performed for the OTUs of the PS92 samples (**Fig. 5**). Based on the cluster analysis, corresponding colored boxes are inserted to highlight the four clusters (**Supplementary Fig. S4**).
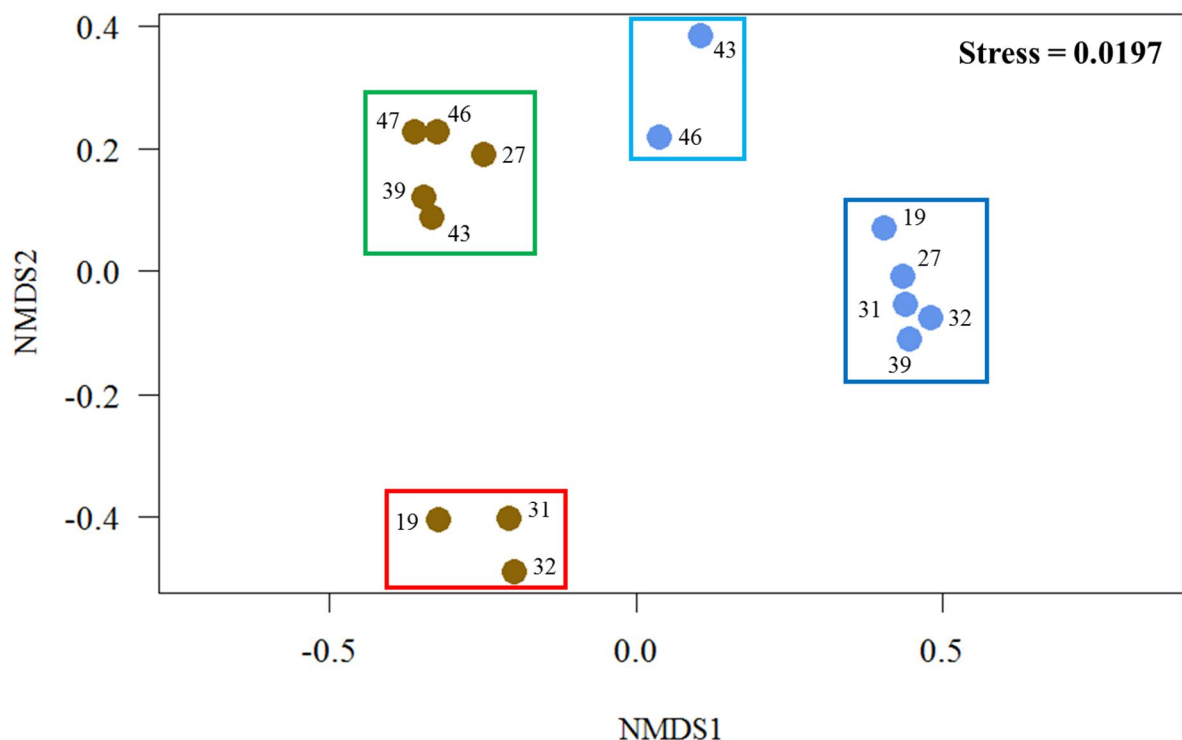


**Figure 5.** Non-metric multidimensional scaling (NMDS) based on Jaccard distances of OTU profiles found in the 15 PS92 samples. Dot colors indicate the 2 habitats: Ice cores (brown) and under-ice water (blue). Colored boxes around stations represent own clusters, indicated by the cluster analysis (**Supplementary Fig. S4**).

In general, the 8 ice core and 7 under-ice water sample clusters separated from each other. Two clusters were found for each, the ice core and the UIW samples. Stations 19, 31 and 32 of the ice core samples (red box) seemed to be different from the others (green box), indicated by the distance between clusters. To find possible reasons, the mean OTU overlap between all ice stations was calculated. Results revealed that only 41.9 ± 3.6% of the OTUs of these three ice core samples overlap with the remaining ice core samples, which had a significantly greater overlap among themselves (52.3 ± 1.9%). This difference appeared to be a result of the absence of certain OTUs in these three sea ice samples. This included for example *Crustomastix*, *Bathycoccus* (both Mamiellophyceae), *Haptolina* (Haptophyta), *Archaeperidinium* (Dinoflagellata) and, most influencing, many ciliates OTUs (*Uronychia*, *Neourostylopsis*). What made the three ice core samples unique, were the colder temperatures in combination with a higher salinity measured within the ice. They were below -2°C, whereas all the other ice cores had a higher temperature. Furthermore, the salinity was high (ranging from 5-7 PSU), whereas most of the other samples had a lower salinity. Stations 43 and 46 of the UIW samples (turquoise box) differed in terms of OTU composition from the other UIW samples (blue box) and seemed to be more similar to the majority of the ice core samples.

The OTU overlap between the PS92 sea ice and under-ice water is shown in Venn diagrams (**Fig. 6**). The number of OTUs are shown for each of both habitats, with the percentage share of these numbers on the OTU entirety in brackets.



**PS92 ICE**   **PS92 UIW**

95
(25.5%)

194
(52.2%)

83
(22.3%)
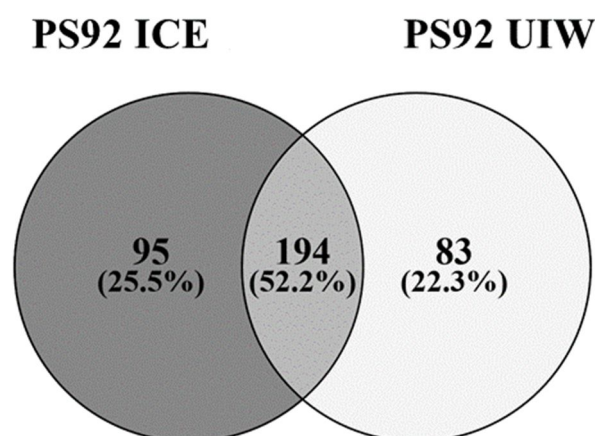
**Figure 6.** Venn diagrams of the OTU overlap between the sea ice and under-ice water (UIW) of the Arctic cruise PS92. OTUs were generated with a threshold of 97% identity.

In total, 372 OTUs were found, 95 (25.5%) of these only in the ice. These were *Histiobalantium*, *Homalogastra*, *Phialina*, *Xystonella*, *Holosticha*, *Peritromus*, *Wilbertomorpha*, *Symbiodinium*, *Crustomastix*, *Haptolina*, *Archaeperidinium*, *Lagenoeca*, *Neourostylopsis*, *Uronychia*, Protalveolata, Pavlovophyceae, Ulvophyceae, as well as the marine diatom taxa *Thalassiosira*. 83 OTUs (22.3%) were unique in the under-ice water, which were *Prasinoderma*, *Strombidinopsis*, *Peridiniopsis*, *Helicosphaera*, *Laboea*, *Pseudotontonia*, *Cochlodinium*, *Chaunocanthida* and *Neroceratium*. The remaining OTUs belonged to unassigned haptophytes and Syndiniales. 194 OTUs (52.2%) were shared between both habitats. These were mainly OTUs belonging to chlorophytes (like *Chlamydomonas*, *Micromonas*, *Bathycoccus* and *Pyramimonas*) but also haptophytes (like *Phaeocystis*, *Chrysochromulina* and *Imantonia*), some ciliates and dinoflagellates (*Gymnodinium* and *Gyrodinium*), as well as the only freshwater taxa in PS92, *Carteria* and *Woloszynskia*.

The OTU overlap matrix was also calculated for the UIW samples, since most of the PS92 UIW samples clustered separately. $40.6 \pm 5.9\%$ of the OTUs of the outstanding cluster were also found in the remaining UIW samples. Among themselves, they overlapped with approximately $45.1 \pm 6.0\%$ of the OTUs, which was a significantly higher value.

Canonical Correspondence Analysis (CCA) was performed to explain community compositions by environmental parameters (**Fig. 7A&B**). The environmental factors temperature and chlorophyll content showed a significant impact on the model outcome for the sea ice samples (**A**). Samples on station 31 and 32 were characterized by low temperature and intermediate chlorophyll levels, stations 27, 39, 43, 46 and 47 showed higher temperatures and a lower chlorophyll content and station 19 had the lowest temperature and chlorophyll level.

In contrast, UIW communities (**B**) were defined by differences in chlorophyll content and ice thickness. Stations 19, 27, 31 and 32 built a cluster, explained by high chlorophyll levels. Stations 39 and 46 were displayed separated from all others by lower chlorophyll levels, but ice thickness seemed to have an impact on community structure. Station 43 was determined by low chlorophyll levels and ice thickness.

However, no significant model could be reached with the available environmental parameters ($p = 0.309$). Thus, the model did not explain the OTU distributions properly.
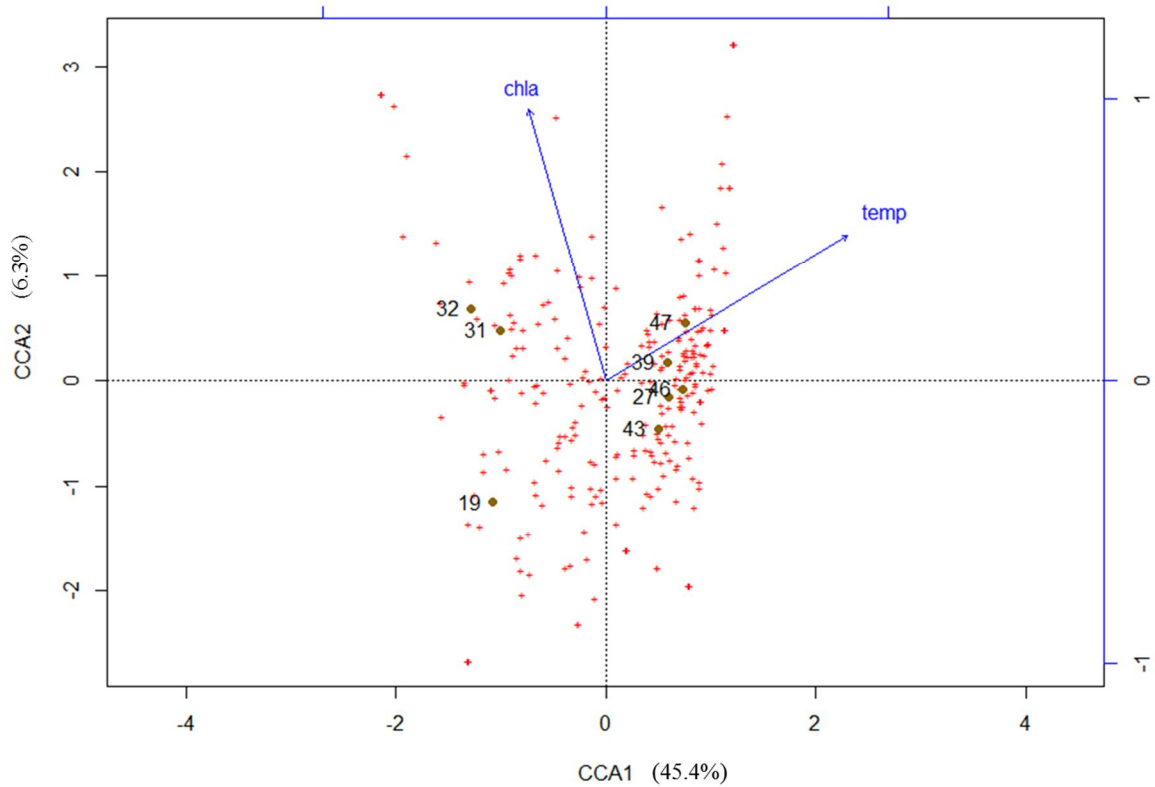
**Figure 7A.** Canonical Correspondence Analysis (CCA) of the PS92 ice samples. Proportion of the total variance, which is explained by all environmental factors: 59.1% (p < 0.05). Arrows represent explanatory variables (temperature and chlorophyll) with arrowheads indicating their direction of increase. Red crosses represent response variables (OTU abundance) and stations are color coded due to their habitat type (sea ice samples = brown).
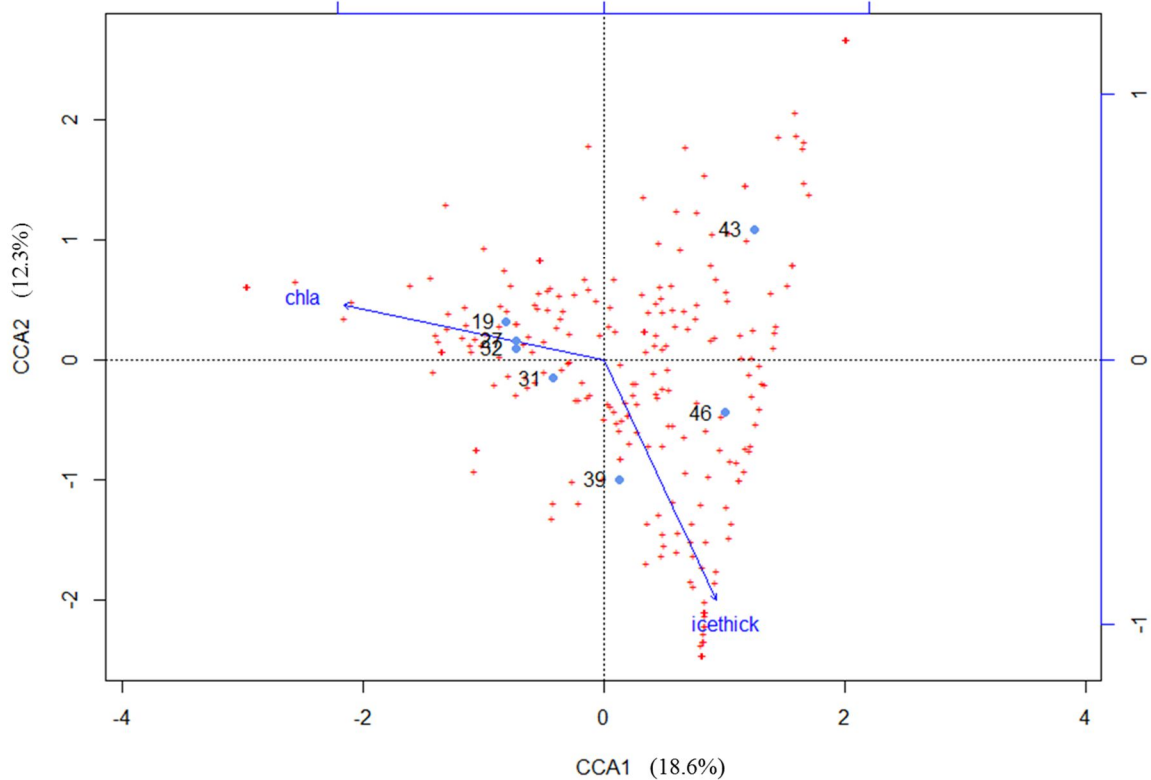


**Figure 7B.** Canonical Correspondence Analysis (CCA) of the PS92 UIW samples. Proportion of the total variance, which is explained by all environmental factors: 30.9% (p = 0.309). Arrows represent explanatory variables (chlorophyll and ice thickness) with arrowheads indicating their direction of increase. Red crosses represent response variables (OTU abundance) and stations are color coded due to their habitat type (UIW samples = blue).

## 3.2.2 Arctic cruise PS94

Like in the PS92 samples, the most dominant taxa in PS94 were dinoflagellates (**Fig. 8**). An exception was UIW samples on stations 101, 112 and 117, where their proportion was reduced mainly by Chlorophyceae (to approximately 30% at St.117).
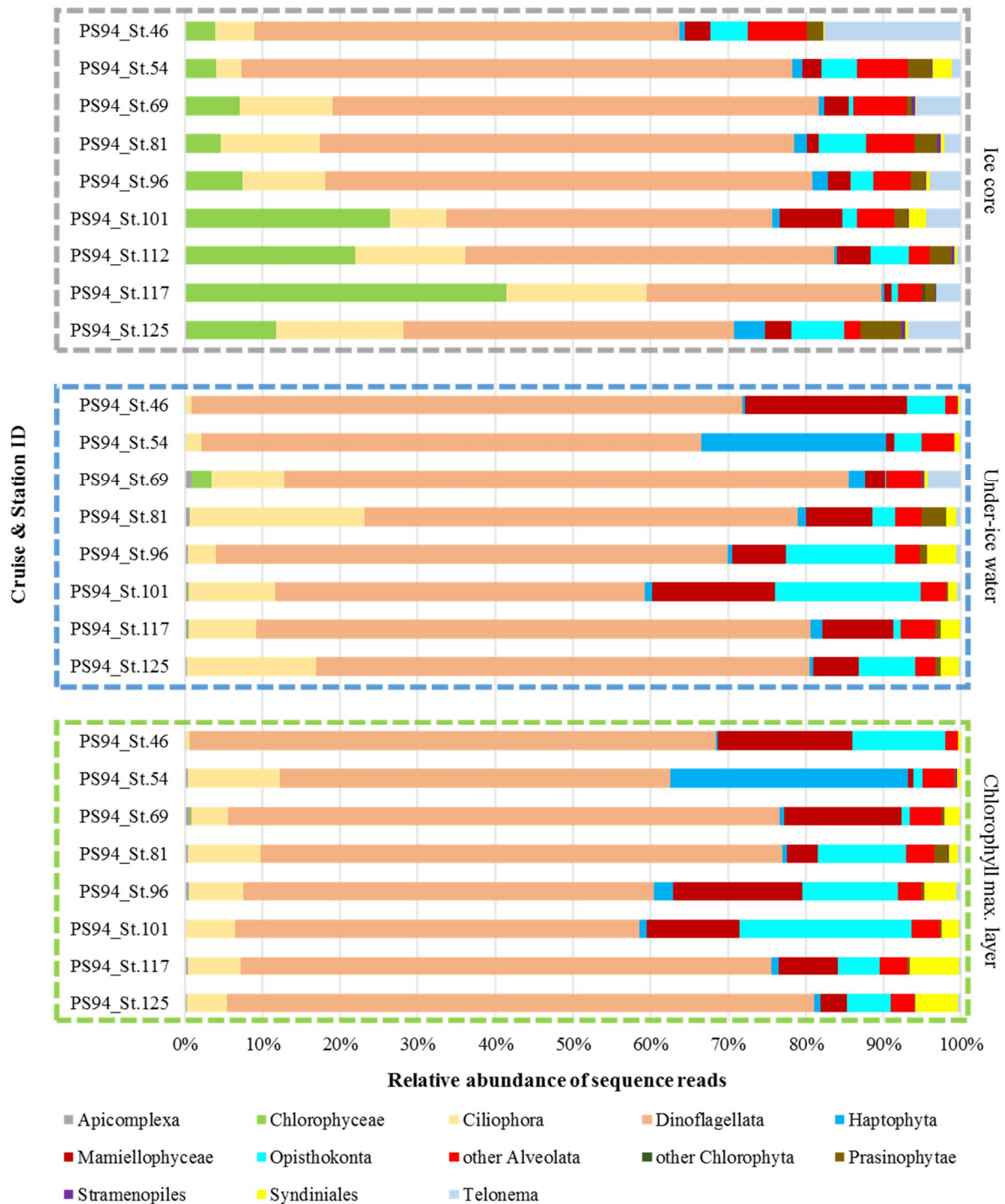


**Figure 8.** Relative abundance of sequence reads for the Arctic stations of PS94. Ice core samples are represented on the top (grey box), under-ice water samples are presented in the middle part (blue box) and chlorophyll samples are shown at the bottom (green box).

In the UIW and DCM samples on station 54 as well, due to the remarkable high amount of haptophytes (Pavlovophyceae and Prymnesiophyceae), which seem to have experienced a blooming phase in this area. In the remaining samples, they occurred in much lower amounts (0.5-5%). Chlorophyceae again showed significantly higher proportions in the sea ice samples, than in the UIW or DCM samples ($p < 0.05$). Only in the UIW sample at station 69 we found a relatively high amount of Chlorophyceae (3%) compared to the other UIW and DCM samples. Ciliates were found in all samples in approximately same amounts, varying from 1% (DCM PS94_St.46) to 23% (UIW PS94_St.81). Mamiellophyceae represented a significant proportion, especially in UIW and DCM samples (up to 21% on the UIW station 46), whereas in ice cores they were less abundant ($p < 0.05$). The UIW and DCM showed similar proportions. On station 54 Mamiellophyceae were less abundant, probably related to the high proportion of haptophytes on this station. OTUs, belonging to other alveolates taxa, occurred in significantly higher proportions in the sea ice than in the UIW or DCM ($p < 0.05$), which did not differ among themselves. Like during the PS92 cruise, prasinophytes and *Telonema* occurred significantly more often in the sea ice, than in the UIW or DCM ($p < 0.05$). On the other side, opisthokonts and Syndiniales were more abundant in the UIW and DCM than in the sea ice ($p < 0.05$). All things considered, the UIW and DCM showed a very similar OTU composition and both differed significantly from the ice core samples.
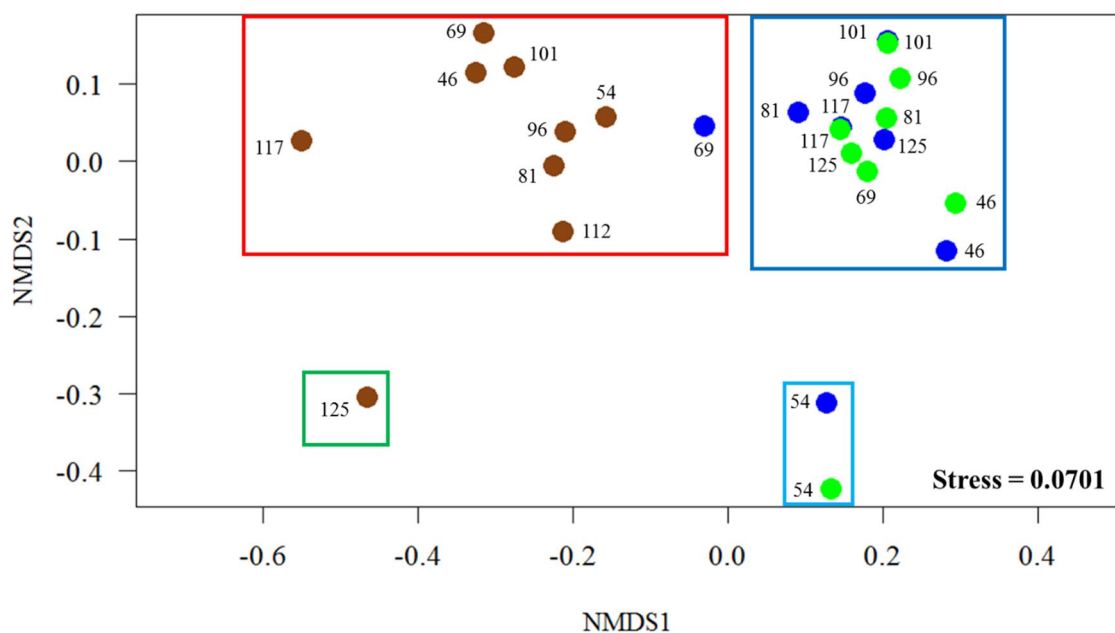


**Figure 9**. Non-metric multidimensional scaling (NMDS) based on Jaccard distances of OTU profiles found in the 25 PS94 samples. Dot colors indicate the 3 habitats: Ice cores (brown), under-ice water (blue) and chlorophyll maximum layer (green). Colored boxes around stations represent own clusters, indicated by the cluster analysis (**Supplementary Fig. S5**).

Cluster analysis revealed 4 different clusters from the PS94 samples (**Supplementary Fig. S5)**. Like for the PS92 samples, ice core samples clustered apart from the UIW samples, which showed a high similarity with their related DCM samples. The NMDS analysis (**Fig. 9**) confirmed this result, showing one cluster with most of the sea ice samples (red box) and an individually cluster of the sea ice sample on station 125 (green box). The UIW and DCM samples clustered together within a third cluster (blue box), with an exception of station 54 representing the fourth cluster with both, the UIW and DCM sample (turquoise box). The UIW sample on station 69 clustered together with the majority of the sea ice samples.

A Venn diagram for overlapping OTUs between habitats of PS94 is presented in **Fig. 10** to include information about the potential coupling between sea ice, under-ice water and the chlorophyll maximum layer. 20.5% of the OTUs in PS94 were exclusively found in the ice core samples. The genera *Pavlova*, *Phialina*, *Hypotrichia*, *Pelagodinium*, *Symbiodinium*, *Prorocentrum*, *Aporthotrochilia*, *Neourostylopsis*, *Uronychia*, *Archaeperidinium*, *Euplotes*, *Peritromus*, *Wilbertomorpha*, *Chromera*, *Salpingella*, *Trithigmostoma*, *Holosticha*, the freshwater genus *Choricystis* and opisthokonts, as well as the diatom genera *Melosira* and *Fragilariopsis* were observed. Only 8 OTUs (1.8%) were unique in the DCM, belonging to *Prasinoderma*. The UIW did not contain any particular OTU.
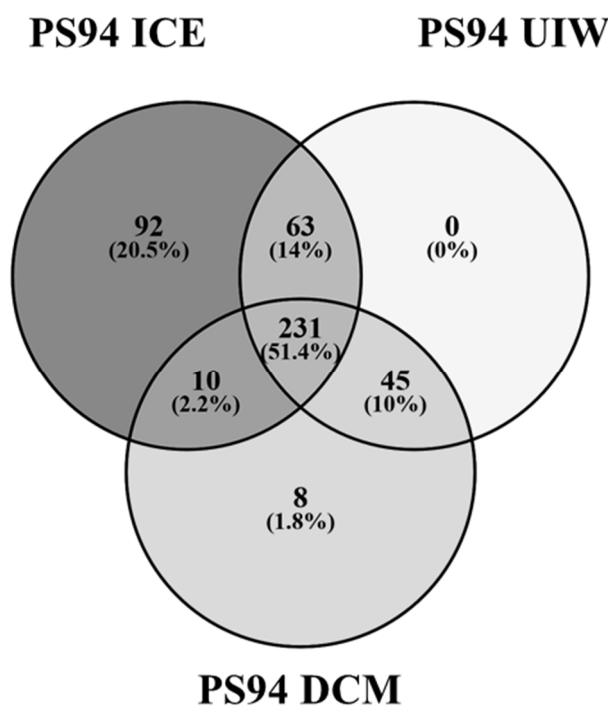


**Figure 10.** Venn diagrams of the OTU overlap between the sea ice, under-ice water (UIW) and chlorophyll maximum layer (DCM) of the Arctic cruise PS94. OTUs were generated with a threshold of 97% identity.

Sea ice and under-ice water shared 14% of the OTUs, which represent *Crustomastix*, *Homalogastra* and *Cryptocaryon* beside some unassigned Chlorophyceae and Chrysophyceae. Only 2.2% of the OTUs were found in both, the sea ice samples and the DCM. The subclasses Scuticociliatia and Haptoria belonged exclusively to these, as well as *Isochrysis spp*. The identity between UIW and DCM was intermediate (10%). Both habitats shared *Neoceratium*, *Lagenoeca*, some unassigned haptophytes and the freshwater dinoflagellate genus *Wolozynskia*. Approximately half of the OTUs in PS94 (51.4%) were shared between all three habitats. Like in the PS92 samples, the shared proportions belonged to a high variety of taxa, but mostly chlorophytes.

The CCA of the PS94 ice core samples revealed temperature and chlorophyll content as explanatory parameters for the OTU distribution (**Fig. 11**). Stations 46, 54, 69, 81 and 96 clustered closely together at intermediate temperatures and chlorophyll levels. Stations 101 and 117 were separated at even lower temperatures. Stations 112 and 125 at higher chlorophyll levels.
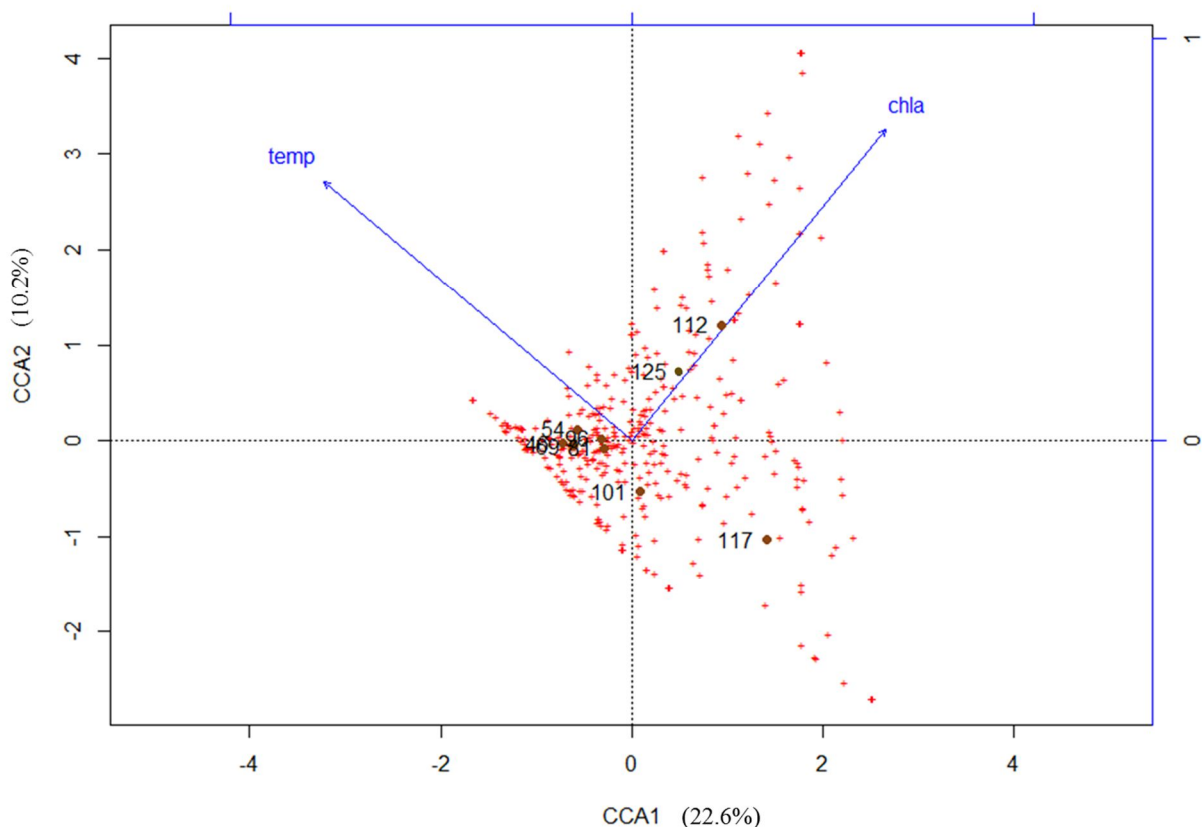


**Figure 11.** Canonical Correspondence Analysis (CCA) of the PS94 sea ice samples. Proportion of the total variance, which is explained by all environmental factors: 32.7% (p < 0.05). Arrows represent explanatory variables (temperature and chlorophyll) with arrowheads indicating their direction of increase. Red crosses represent response variables (OTU abundance) and stations are color coded due to their habitat type (sea ice samples = brown).

A CCA was also performed for the UIW samples of PS94 (**Fig. 12**). Again, temperature and chlorophyll represented the most powerful explanatory variables in the model. Stations 69, 96, 101, 117 and 125 clustered together at low temperatures and chlorophyll levels. Station 54 was determined by low temperature and high chlorophyll values, station 81 by low chlorophyll and high temperature values and station 46 by as well high temperature as also high chlorophyll values.
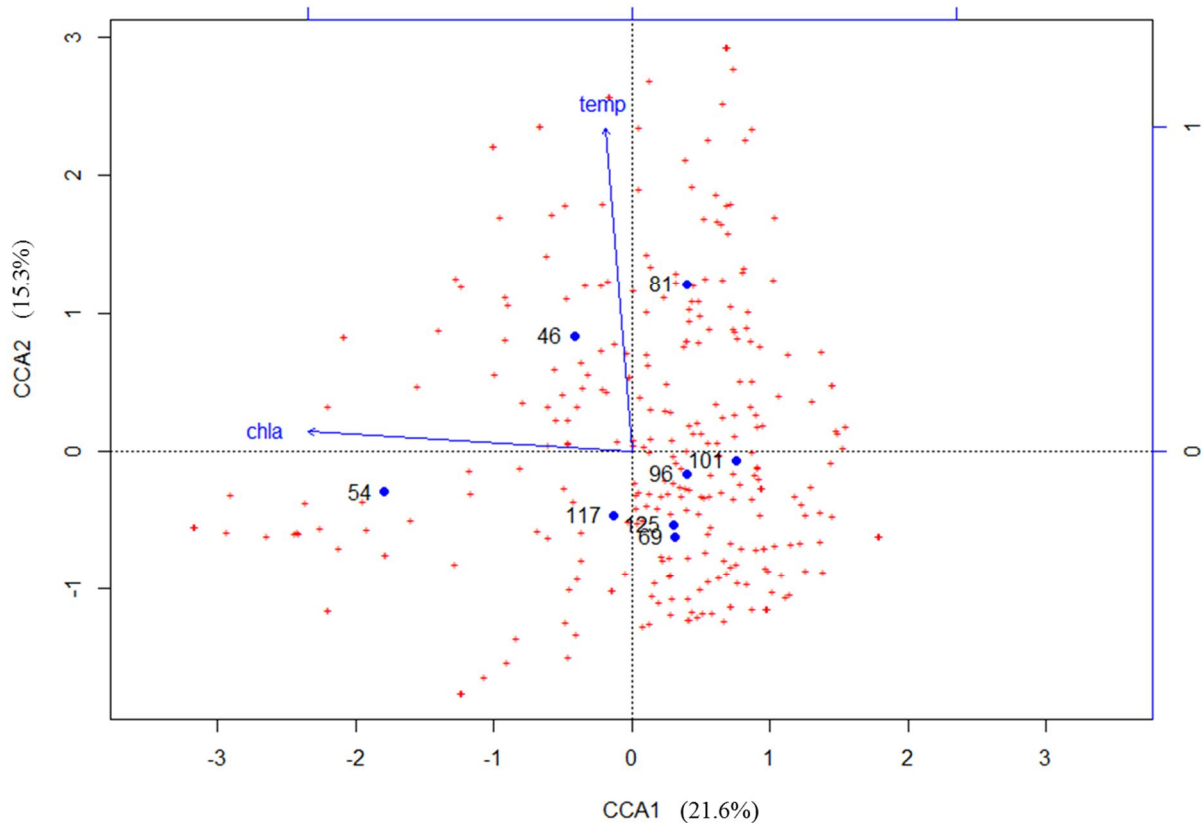


**Figure 12.** Canonical Correspondence Analysis (CCA) of the PS94 UIW samples. Proportion of the total variance, which is explained by all environmental factors: 36.9% (p < 0.05). Arrows represent explanatory variables (temperature and chlorophyll) with arrowheads indicating their direction of increase. Red crosses represent response variables (OTU abundance) and stations are color coded due to their habitat type (UIW = blue).

For PS94, also DCM samples were available. The CCA output for these habitats is presented in **Fig. 13**. Ice thickness, temperature, and chlorophyll values determined the explanatory power of this model. Stations 125, 117, 69, 96, 101 and 46 followed an increasing temperature and chlorophyll gradient, whereas they showed decreasing ice thickness values. As it was already shown in the CCA plot of the UIW samples, the DCM sample on station 54 was separated from the other samples and much more influenced by chlorophyll and ice thickness values.
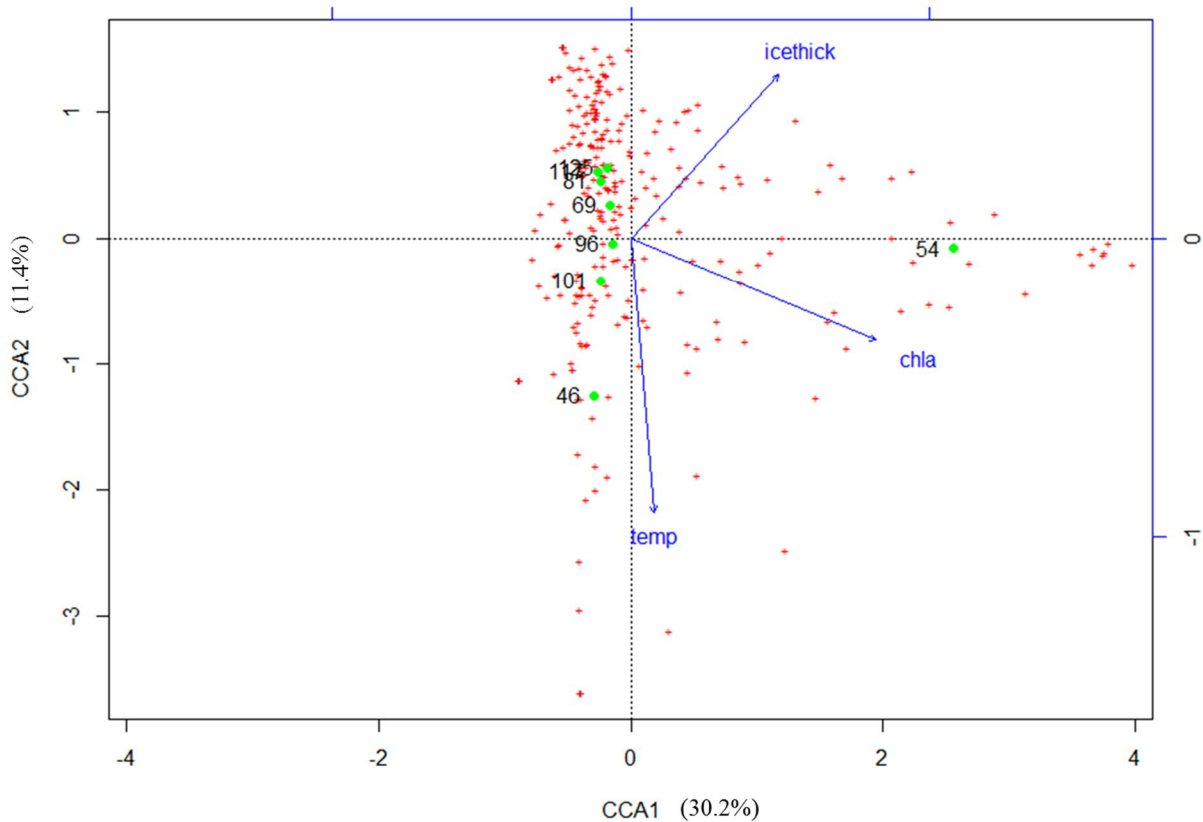
**Figure 13.** Canonical Correspondence Analysis (CCA) of the PS94 UIW samples. Proportion of the total variance, which is explained by all environmental factors: 50.1% (p < 0.05). Arrows represent explanatory variables (temperature, chlorophyll and ice thickness) with arrowheads indicating their direction of increase. Red crosses represent response variables (OTU abundance) and stations are color coded due to their habitat type (DCM = green).

### 3.2.3 Time shift in the Arctic: Comparison between PS92 and PS94

The two Arctic cruises PS92 and PS94 took place in different times and also different areas. This temporal and spatial shift can be expected to lead to different protist communities. Therefore, we compared also the OTU composition between both cruises to investigate temporal and spatial variability in marine protist communities in the Arctic Ocean.

Venn diagrams (**Fig. 14**) demonstrated that only 2.2% of the Arctic OTUs occurred exclusively in the sea ice samples of PS92 (**A**). These OTUs represented *Prasinoderma*, *Strombidinopsis*, *Woloszynskia* and some unassigned haptophytes. 28.6% of the OTUs were found only in the sea ice of PS94, containing *Crustomastix*, *Haptolina*, some Acanthoecidae, *Homalogastra*, *Phialina*, *Symbiodinium*, Chrysophyceae, *Histiobalantium*, *Aporthotrochilia*, *Neourostylopsis*, *Uronychia*, *Archaeperidinium*, Syndiniales_Group_I, *Euplotes*, *Melosira*, *Choricystis*, freshwater opisthokonts, choanoflagellates, *Xystonella*, *Peritromus*, *Wilbertomorpha*, *Chromera*, *Salpingella*, *Trithigmostoma*, *Holosticha* and *Fragilariopsis*.

The majority of OTUs (69.1%) was shared between ice core samples of PS92 and PS94, containing mostly members of Chlorophyceae and dinoflagellates.
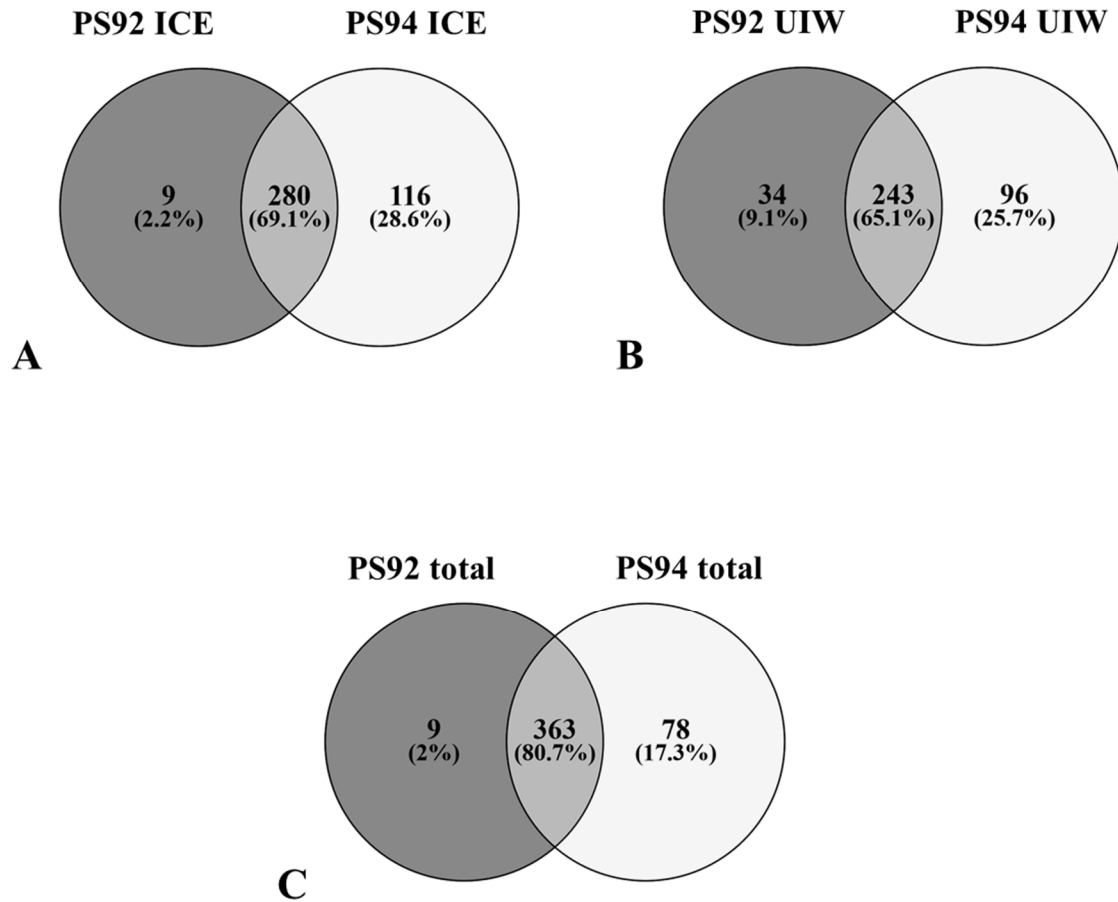


**Figure 14.** Venn diagrams of the OTU overlap between the sea ice, under-ice water (UIW) of the two Arctic cruises PS92 and PS94. OTUs were generated with a threshold of 97% identity.

A similar pattern of the OTU ratio was found in the UIW samples between both Arctic cruises (**B**). Less OTUs occurred in the PS92 samples (9.1%), 25.7% only in the UIW of PS94 samples and an overall overlap of 65.1%. In total (**C**), samples of both cruises shared over 80% of the OTUs, 17.3% belonging to PS94 and only a few OTUs (2%) were exclusively found in the PS92 samples.

# 3.3 Protist community composition in the Southern Ocean

## 3.3.1 Antarctic cruise PS89

Proportions of 13 major taxonomic groups of the Southern Ocean per samples are presented in **Fig. 15**. The same classification was chosen like for the Arctic samples. As observed in the Arctic samples, dinoflagellates were the groups with the highest overall proportion. The UIW samples contained a significantly higher amount of dinoflagellates than the sea ice samples ($p < 0.05$). Haptophytes were another dominant group and occurred in high proportions, in the UIW sample on station 46 even higher than the dinoflagellates (up to 40%). The UIW contained more (but statistically not significant) haptophytes than the ice cores ($p = 0.063$). Mamiellophyceae were present in every sample, with very high variability between the samples, ranging from ~1% (St.40, UIW) to ~55% (St.58, sea ice).
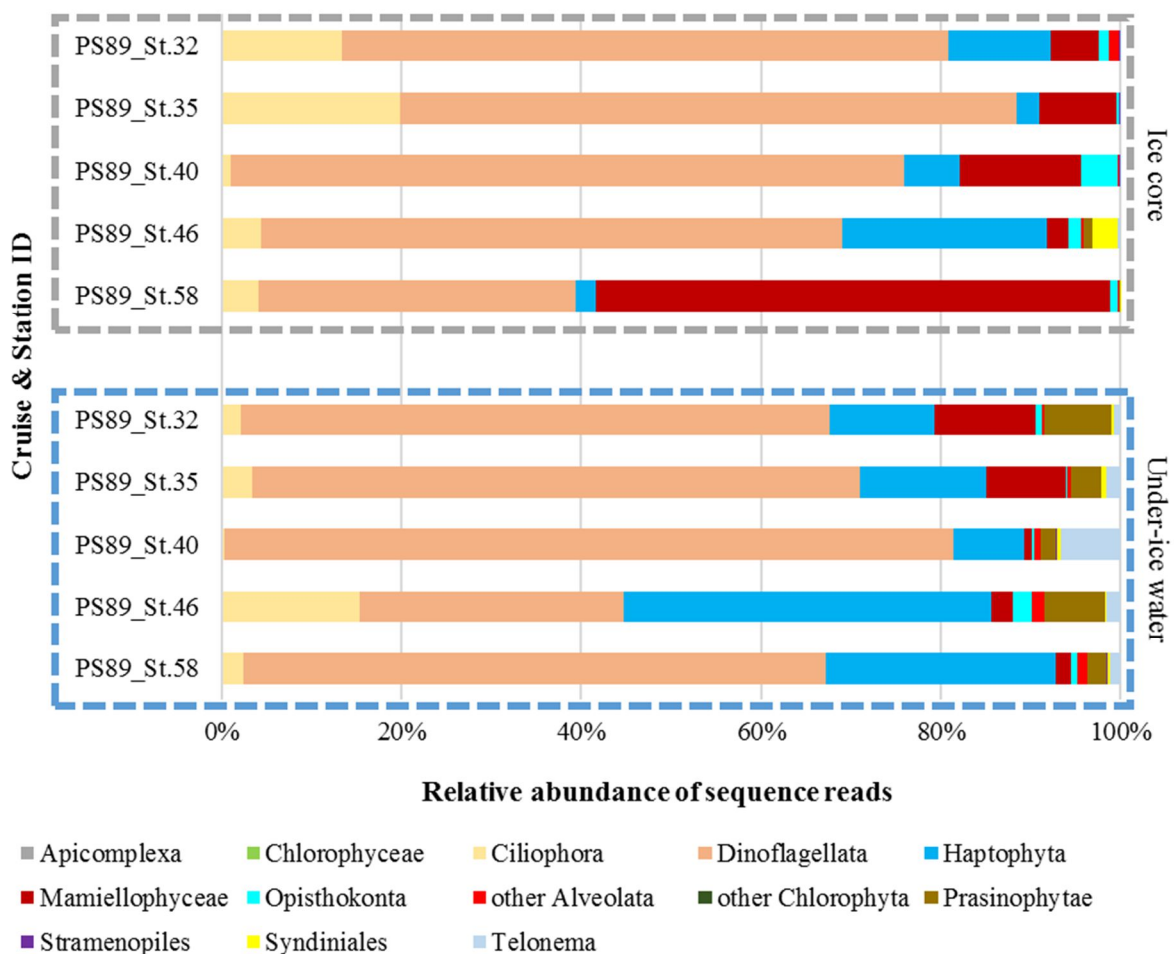


**Figure 15.** Relative sequence abundances for the Southern Ocean stations of PS89. Ice core samples are represented at the top (grey box) and under-ice water samples are presented at the bottom (blue box).

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Ciliates were present in all the samples with higher proportions in the sea ice at station 32 and 35, as well as at station 46 of the UIW samples. Overall, there was no significant difference between sea ice and under-ice water concerning ciliates ($p = 0.085$). Apicomplexa occurred only at station 32 in both, sea ice and under-ice water, but very rarely. Chlorophyceae were only found at station 35, also in both habitats and in very low amounts as well. Statistically, no difference could be observed between sea ice and UIW samples ($p = 0.163$).

From a visual inspection, it looks like the Mamiellophyceae have contrarily occurrence with the haptophytes (e.g. sea ice vs. UIW at St.46). Opisthokonts occurred in all samples with very few proportions and the proportion of opisthokonts were very similar between sea ice and UIW. One exception is station 40, where a relatively high proportion showed up in the sea ice, but almost none in the UIW. Other alveolates occurred generally in few amounts with a maximum of 2% in the UIW on station 46. Prasinophytes were significantly more abundant in the under-ice water than in the sea ice ($p < 0.039$). Although stramenopiles appeared in relatively small amounts in all samples, they represented a higher proportion in the sea ice than in the under-ice water ($p < 0.05$). Syndiniales were not present in the sea ice stations 35 and 58, whereas they occurred in all UIW stations. *Telonema* OTUs were significantly more abundant in the UIW, ranging from 1-8% proportion.

NMDS and cluster analyses were performed for the Southern Ocean samples (**Fig. 16** & **Supplementary Fig. S8**).
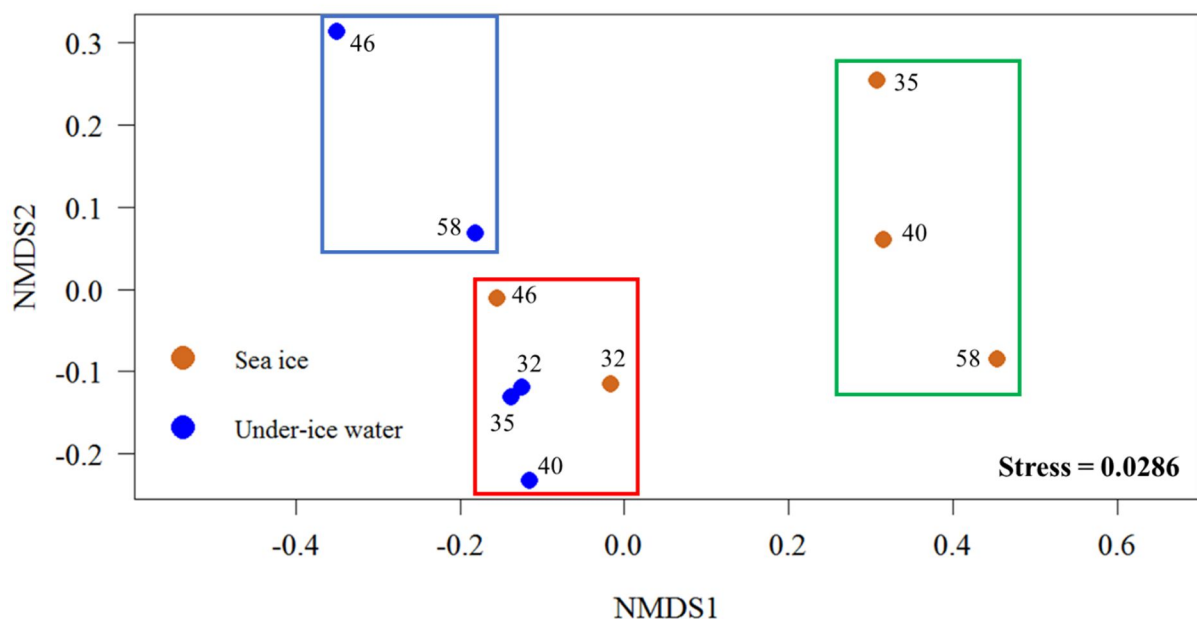


**Figure 16.** Non-metric multidimensional scaling (NMDS) based on Jaccard distances of OTU profiles found in the 10 samples from the Southern Ocean. Dot colors indicate the 2 habitats: Ice cores (brown) and Under-ice water (blue). Colored boxes around stations indicate own clusters, indicated by the cluster analysis (**Supplementary Fig. S8**).

Three clusters were observed in the dataset, highlighted with colored boxes (blue, green, red) in the NMDS plot. Sea ice stations 35, 40 and 58 (green box) clustered apart from the stations 32 and 46, which clustered closer to the UIW samples of the stations 32, 35 and 40 (test). The UIW stations 46 and 58 formed the 3$^{rd}$ cluster. Both, the ice core from station 32 and its corresponding UIW sample, were the only station overlap within one cluster. All other habitat pairs were found in separated clusters. All things considered, no general difference between the sea ice and UIW was apparent from the cluster analysis.

The overall OTU overlap between the ice core and UIW samples in the Southern Ocean is shown in **Fig. 17**. 151 OTUs (53%) were found in both habitats. With 78 (27.4%) OTUs, there were more OTUs exclusively found in the sea ice than in the UIW samples (56 OTUs; 19.6%). Taking a closer look at the taxonomic composition, *Isochrysis*, *Homalogastra*, *Cryptocaryon*, *Loxophyllum*, *Codonella*, *Holosticha*, *Neourostylopsis*, *Uronychia*, *Polarella* and *Melosira* occurred exclusively in the sea ice, whereas *Chrysochromulina*, *Salpingella*, *Laboea*, *Codonellopsis*, Syndiniales (Group III), *Cochlodiniumm* and *Karlodinium* were found only in the under-ice water samples.
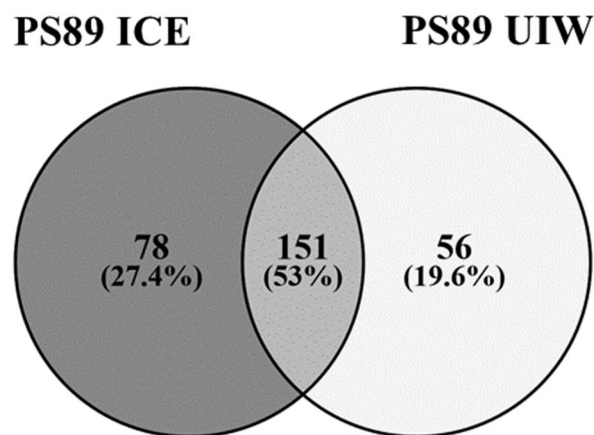


**Figure 17.** Venn diagrams of the overlap of OTUs between the sea ice (left) and under-ice water (right) samples during PS89. OTUs were generated with a threshold of 97% identity.

On the basis of the CCA, the environmental factors snow coverage and chlorophyll content were discovered as having the strongest explanatory power for the OTU proportions within the ice core samples (**Fig. 18**). As no environmental measurement were available for the sea ice sample on station 58 and the UIW sample on station 32, these stations were excluded from this analysis.
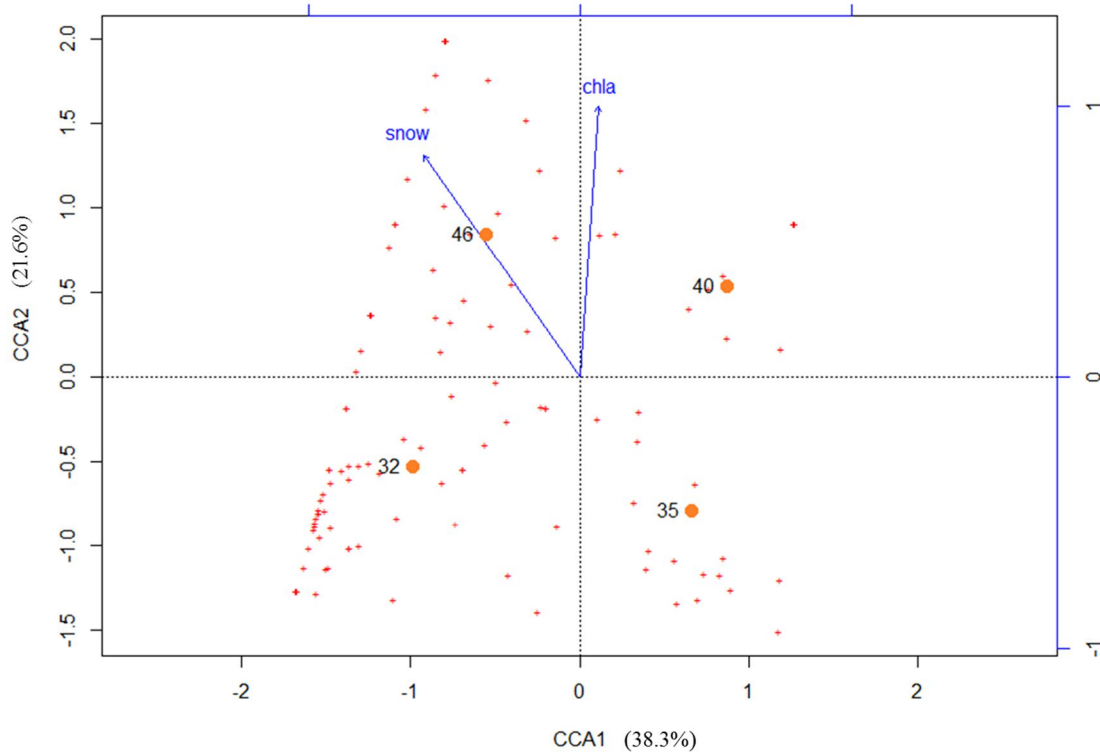
**Figure 18.** Canonical Correspondence Analysis (CCA) of the PS89 sea ice samples. Proportion of the total variance, which is explained by all environmental factors: 59.9% (p = 0.667). Arrows represent explanatory variables (chlorophyll and snow coverage) with arrowheads indicating their direction of increase. Red crosses represent response variables (OTU abundance) and stations are color coded due to their habitat type (sea ice samples = brown).
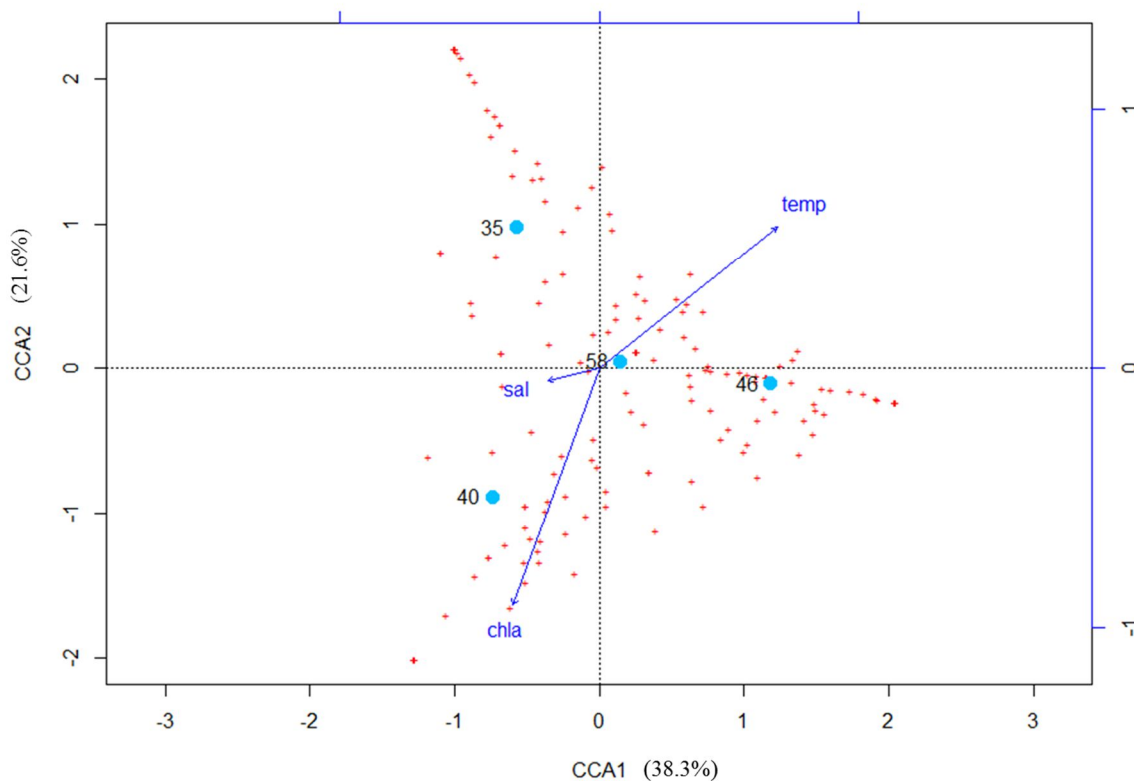


**Figure 19.** Canonical Correspondence Analysis (CCA) of the PS89 UIW samples. Proportion of the total variance, which is explained by all environmental factors: 59.9% (p = 0.667). Arrows represent explanatory variables (chlorophyll, temperature and salinity) with arrowheads indicating their direction of increase. Red crosses represent response variables (OTU abundance) and stations are color coded due to their habitat type (UIW samples = blue).

The OTU distribution of sea ice station 35 was determined by less snow coverage and chlorophyll content. Stations 32 and 40 followed an intermediate amount of snow coverage and chlorophyll. Station 46 was influenced the most by high snow coverage and chlorophyll values. However, with the given environmental parameters, no significant CCA model could be achieved. Also for the UIW samples of PS89, no significant model was reached with the parameters (**Fig.19**).

## 3.4  Protist communities: Arctic vs. Southern Ocean

Boxplots of the environmental parameters, separated for cruise and habitat type, are shown in **Fig. 20 A, B**. We performed an ANOVA or alternatively the non-parametric Kruskal Wallace test to test for potential differences between the habitats of the three cruises. Snow coverage and ice thickness at the sample stations did not differ significantly between cruises (Kruskal Wallace test: p = 0.119).
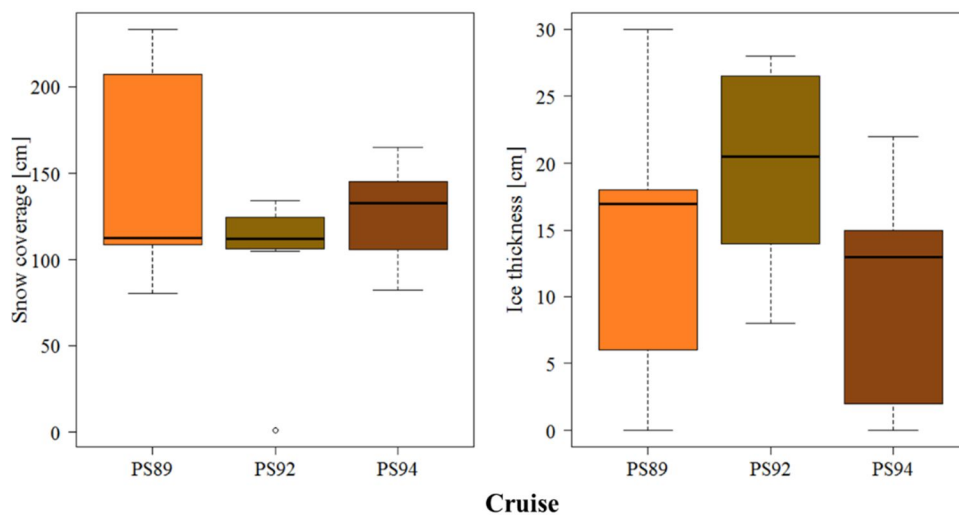


**Figure 20A.** Boxplot of snow coverage and ice thickness data from the samples of PS89 (n = 5), PS92 (n = 8) and PS94 (n = 9). Median abundance is represented by the horizontal bar. The 1$^{st}$ and 3$^{rd}$ quantiles are indicated by the upper and lower end of the boxes.
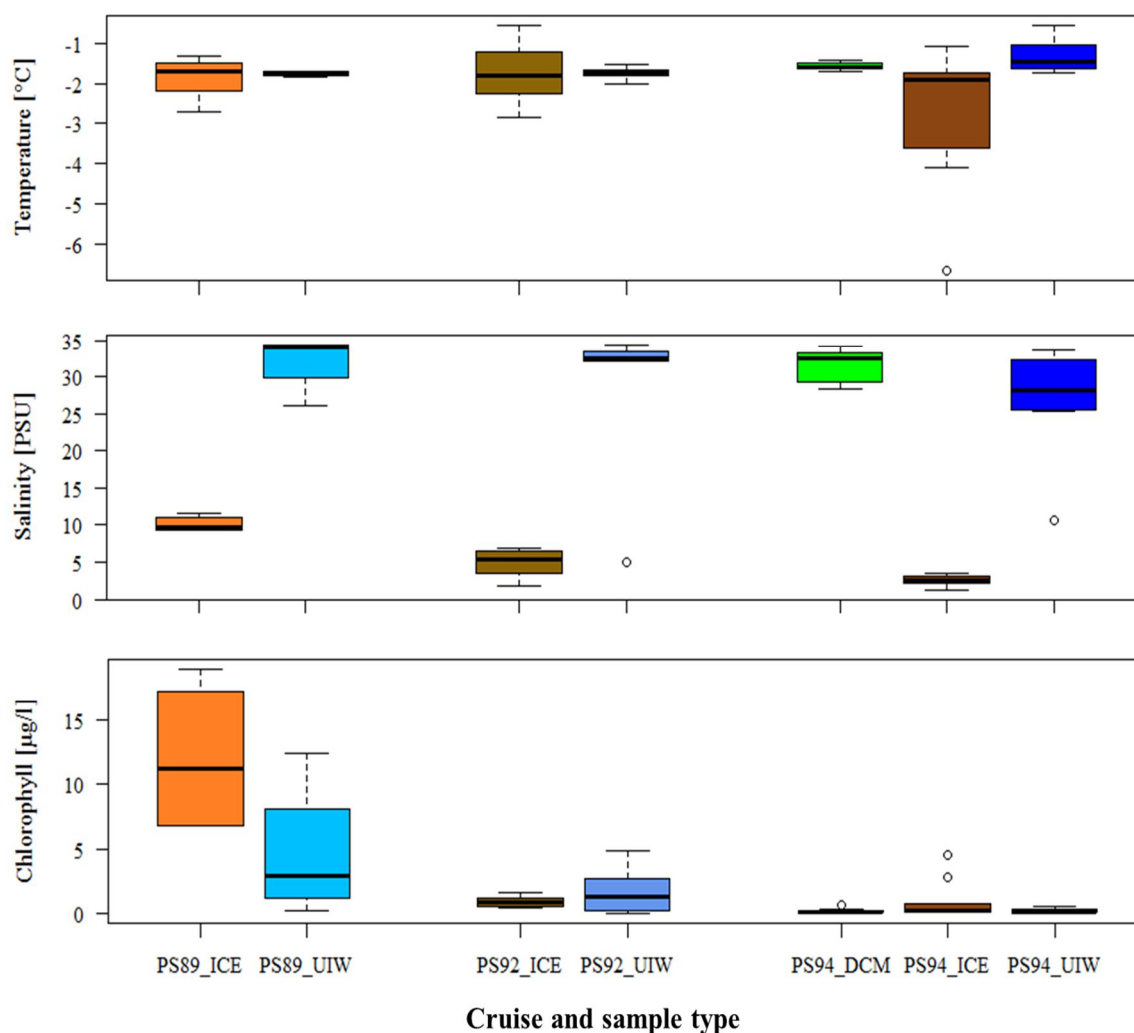
**Figure 20B.** Boxplot of environmental parameters at both polar regions according to the different habitat types (sea ice, under-ice water and chlorophyll maximum layer) for PS89 (n = 8), PS92 (n = 17) and PS94 (n = 16). Median abundance is represented by the horizontal bar. The 1st and 3rd quantiles are indicated by the upper and lower end of the boxes. The ice core sample of station 58 and the UIW sample of station 32 in the Southern Ocean were excluded, since the temperature, salinity and chlorophyll values were not available.

In general, the temperatures between sea ice and the UIW did not differ during PS89 and PS92. In PS94 instead, the sea ice was significantly colder than the DCM or UIW (ANOVA: $p < 0.05$). Between the three cruises, no significant difference of the salinity level could be observed neither for the ice cores nor for the UIW/DCM measurements. The chlorophyll concentration was higher in the Antarctic sea ice samples than in the Arctic ones (Kruskal Wallace test: $p < 0.001$), with higher amounts in the Antarctic sea ice than in the Antarctic UIW (ANOVA: $p < 0.01$). The Antarctic UIW samples instead, did not contain a higher chlorophyll concentration than the Arctic UIW samples, which showed similar concentrations among themselves.

Proportions of the ten most abundant taxonomic groups are presented in **Fig. 21**, to visualize potential differences in terms of main community-influencing taxa in both polar regions. In general, the two Arctic cruises showed very similar patterns concerning the OTU proportions and PS89 samples differed a lot from the other ones.

Dinoflagellates were the most abundant taxa in both polar regions, displaying around 60% of the OTUs in each cruise. The UIW contained slightly more dinoflagellates than the ice cores. Haptophytes showed higher proportions in the Antarctic samples, especially in the UIW (20%; Sea ice: 9%). In the Arctic samples, the UIW contained slightly more haptophytes (PS92: 6%; PS94: 4%) than the ice core (PS92: 5%; PS94: 1%), the Southern Ocean samples showed higher proportions (Sea ice: 9%; UIW: 20%). Mamiellophyceae occurred more often in the Antarctic sea ice (18%) than in the Arctic sea ice (PS92: 4%; PS94: 3.5%), whereas the Arctic UIW contained more Mamiellophyceae (PS92: 8%; PS94: 9%) than the Antarctic UIW samples (5%). Chlorophyceae were almost exclusively found in the Arctic ice cores (PS92: 8%; PS94: 15%). The Arctic UIW and both Antarctic habitats contained much less (< 1%). For PS89 and PS94 Ciliates showed lower proportions in the UIW (PS89: 5%; PS94: 10%) than in the ice (PS89: 9%; PS94: 11%). For PS92 it was the other way round, the UIW (9%) contained more OTUs belonging to Ciliates than the sea ice (6%). Other alveolates showed a higher proportion in the Arctic sea ice samples (PS92: 7%; PS94: 6%) than in the UIW samples (both: 3%). The Antarctic samples showed less proportions in both habitats (< 1%). Opisthokonts showed higher proportions in the Arctic UIW samples (PS92: 3%; PS94: 7%) than in the sea ice (PS92: < 1%; PS94: 4%). The Antarctic ice samples instead had more opisthokonts in the sea ice (1.5%) than in the UIW (< 1%). Finally, Syndiniales showed slightly higher proportions in the Arctic sea ice (PS92: 5%; PS94: 2%) than in the UIW (PS92: 2%; PS94: < 1%). In the Southern Ocean instead, Syndiniales occurred more often in the UIW (4%) than in the sea ice (< 1%).

As it was done individually for the Arctic and Southern Ocean, a non-metric multidimensional scaling (NMDS) of the OTU compositions was performed also for the combined data set (**Fig. 22**). The colors correspond to those of the separated NMDS plots of the different cruises and the hulls connect the individual cruises and sample types (sea ice, under-ice water and chlorophyll maximum layer). A cluster analysis was carried out for all 50 samples (**Supplementary Fig. S9**) and the 5 obtained clusters are imported into the NMDS plot, indicated by colored boxes.
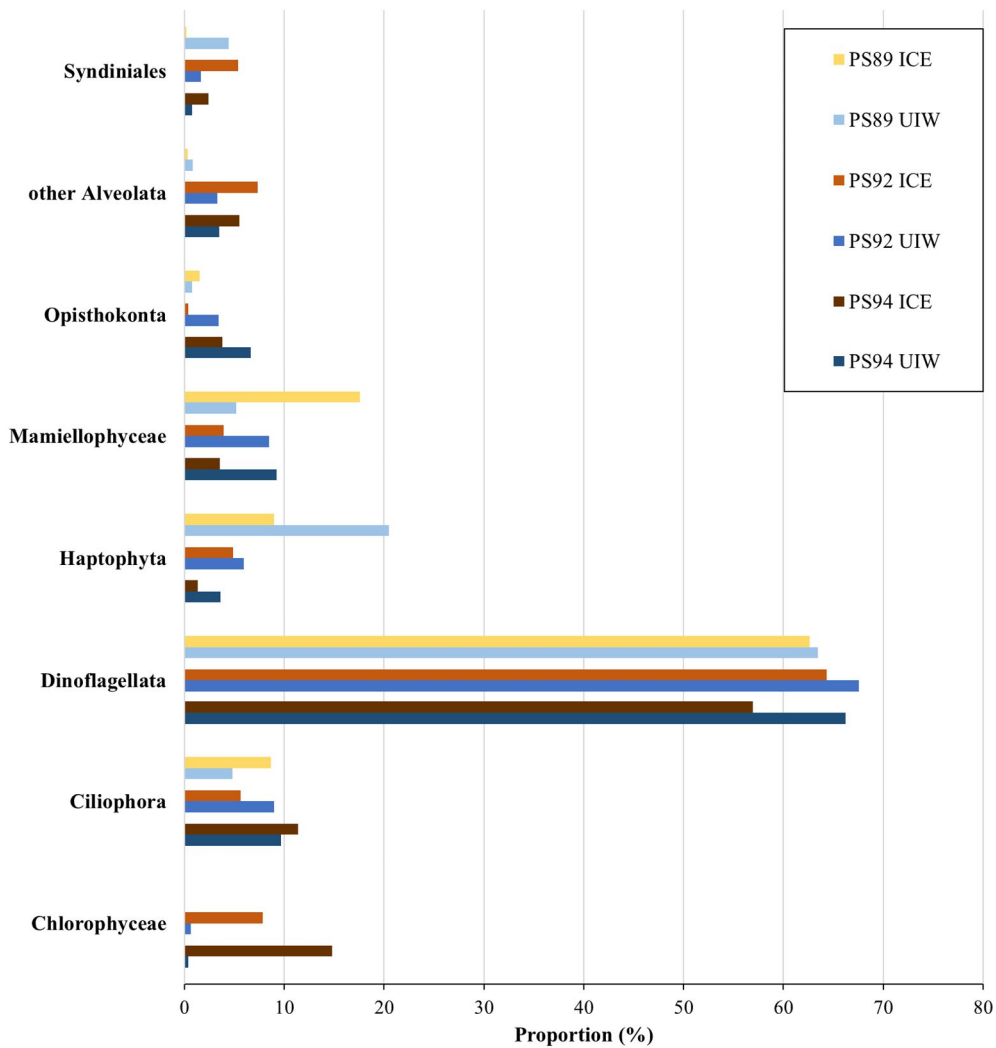
**Figure 21.** OTU proportions (%) of high taxonomic groups within the two habitat types (sea ice and UIW) for PS89, PS92 and PS94.
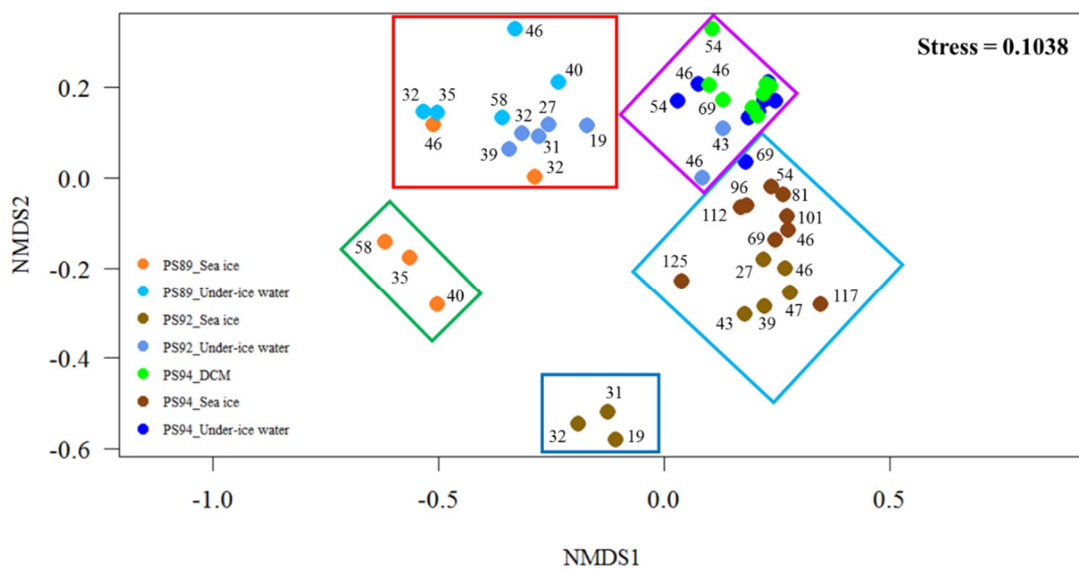


**Figure 22.** Non-metric multidimensional scaling (NMDS) based on Jaccard distances of OTU profiles found in 40 Arctic samples and the 10 samples from the Southern Ocean. Colors indicate the cruise and habitats: Ice cores (brown), UIW (blue) and DCM (green). Colored boxes around stations indicate own clusters, calculated by the cluster analysis (**Supplementary Fig. S9**).

Like in the individual cluster analysis of the Arctic Ocean samples, the ice core samples (blue boxes) clustered separated from the UIW and DCM samples, which clustered close together (purple box). An exception was again the PS94 UIW sample of station 69, which showed a more similar OTU composition with the sea ice samples. Stations 19, 31 and 32 formed an own cluster (dark blue box). Notable was the OTU composition overlap of the 5 PS92 UIW stations 19, 27, 31, 32 and 39 with a part of the Southern Ocean samples (red box). This cluster contained all UIW samples of PS89 and additionally the Antarctic ice core samples on station 32 and 46, which clustered also together in the Southern Ocean NMDS plot. Sea ice samples on station 35, 40 and 58 formed an own cluster (green box).

Venn diagrams in **Fig. 23** show the OTU overlap between the Arctic and both Southern Ocean cruises. Sea ice samples of PS89 and PS92 (**A**) showed an OTU similarity of 49.7%. 16.8% occurred only in the sea ice samples of PS89 and 33.8% only in the samples of PS92. The overlap of the UIW samples (**B**) was higher (57.1%) than between the sea ice samples for these two cruises. The proportion of exclusive OTUs in the UIW of PS92 (32.8%) was similar to the proportion in the PS92 sea ice samples. Less OTUs were observed in the UIW of PS89 (10.1%) than in the sea ice samples (16.5%).

The comparison of the OTU proportions in the sea ice between PS89 and PS94 (**C**) revealed an equal overlap of 47.1% like in the comparison of PS89 and PS92 ice core samples. However, the proportion of unique OTUs in the PS94 sea ice was higher (46.1%).

A very similar pattern was found for the PS89 – PS94 UIW comparison (**D**). The overlap of 49.6% between the UIW samples of both cruises was lower than between PS89 and PS92 (57.1%). But the amount of unique OTUs in the PS94 was higher (43.3%).

The total congruence of sea ice OTUs between both polar regions is shown in **Fig. 23 E**. From 429 OTUs found in the sea ice, 205 OTUs (47.8%) occurred in both polar regions, 24 (5.6%) were only observed in the Southern Ocean samples and 200 (46.6%) were exclusively found in the Arctic Ocean. Compared to the sea ice samples from the Arctic, the ice cores from the Southern Ocean contained all defined central groups (**Fig. 21**). When looking at deeper taxonomic levels, exceptions were *Carteria*, freshwater opisthokonts, "other Chlorophyta" (*Choricystis* & Ulvophyceae), Pavlovophyceae, *Imantonia*, *Chrysochromulina*, or "other Chlorophyceae". Two ciliate taxa (*Frontonia* & *Aspidisca*) were only found in ice of the Southern Ocean. However, most of the OTUs, which occurred only in the Arctic sea ice, were ciliates.

The OTU overlap between the Arctic and Antarctic (**F**) revealed that from 387 OTUs in total, 193 (49.9%) occurred in both regions, 14 (3.6%) only in the Southern Ocean and 180 (46.5%)

only in the Arctic samples. Diatoms (*Fragilariopsis* & *Thalassiosira*), some protalveolates and ciliates were exclusively found in the Southern Ocean samples. Other OTUs occurred exclusively in the Arctic water samples: *Carteria*, *Crustomastix* (Mamiellophyceae); *Prasinoderma* (Prasinophytae); *Imantonia*, *Isochrysis* and *Haptolina* (Prymnesiophyceae); *Pavlova* (Pavlovophyceae); *Homalogastra*, *Cryptocaryon*, *Loxophyllum*, *Codonella*, *Strombidinopsis* and *Xystonella* (Ciliates); *Pelagodinium*, *Polarella*, *Woloszynskia*, *Neoceratium* and *Peridiniopsis* (Dinoflagellata); Chaunocanthida (Rhizaria) and Chrysophyceae (Stramenopiles).

After a comparison of all 464 different OTUs in this study (**G**), 14 (3%) were found only in the Antarctic. These included: Oligohymenophorea, *Cryptocaryon*, *Codonellopsis*, *Aspidisca*, *Frontonia* and other Haptoria (Ciliates); *Diaphanoeca* (Choanomonada); other Mamiellophyceae; one *Phaeocystis* OTU (Prymnesiophyceae); some Peridiniphycidae OTUs (Dinoflagellata); one *Amoebophrya* OTU (Syndiniales).

179 (38.6%) OTUs occurred exclusively in the Arctic samples and represented the following taxa: Ulvophyceae; Pavlovophyceae; *Crustomastix* (Mamiellophyceae); *Haptolina*, *Imantonia* (Prymnesiophyceae); *Peridiniopsis*, *Archaeperidinium*, *Neoceratium*, *Pelagodinium* and *Symbiodinium* (Dinoflagellata); *Chromera* (Protalveolata); Chaunocanthida (Rhizaria); Chrysophyceae; *Prasinoderma* (Prasinophytae); *Histiobalantium*, *Trithigmostoma*, *Euplotes*, *Peritromus*, *Wilbertomorpha*, *Strombidinopsis*, *Xystonella* and *Phialina* (Ciliates). Particularly noteworthy, the 4 fresh water taxa found in this study (*Carteria*, *Choricystis*, *Woloszynskia* and freshwater-opisthokonts) did only occur in the Arctic samples.

271 (58.4%) were found in both, the Arctic and Antarctic samples. These OTUs were all already mentioned taxa, so we refrain from mentioning each again. Following just a noteworthy pattern: All diatom taxa found in this study (*Melosira*, *Fragilariopsis*, *Thalassiosira*, Bacillariophytina and some unassigned diatoms) occurred in both polar regions, except one strain referring to (marine or freshwater) taxa of Naviculales, which only occurred in the Arctic (**Tab. 2**). In PS89 and PS94, more diatoms were found in the sea ice than in the water column, whereas for PS92 it reversed. The PS94 UIW samples contained no diatoms at all.
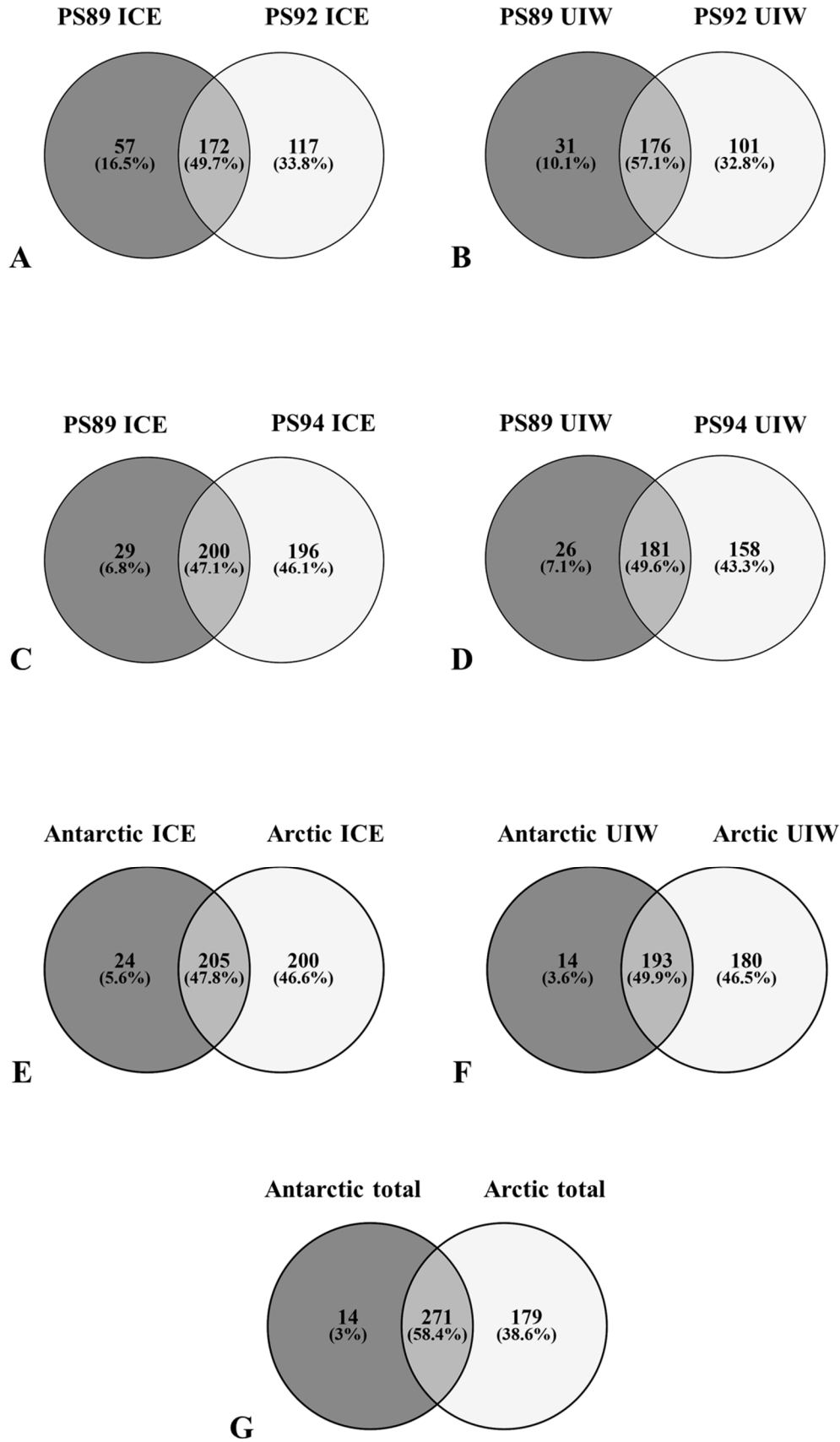
**Figure 23.** Venn diagrams of the overlap of OTUs between **A** the sea ice samples of PS89 and PS92 **B** the UIW samples of PS89 and PS92 **C** the sea ice samples of PS89 and PS94 **D** the UIW samples of PS89 and PS94 **E** all sea ice samples from the Arctic and the Antarctic **F** all UIW samples from the Antarctic and Arctic **G** all Antarctic and Arctic samples. OTUs were generated with a threshold of 97% identity.

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**Table 2.** Qualitative occurrence of diatom taxa in samples oft he three cruises PS89, PS92 and PS94.

| Taxon / Habitat | PS89 Sea ice | PS89 UIW | PS92 Sea ice | PS92 UIW | PS94 Sea ice | PS94 UIW |
|---|---|---|---|---|---|---|
| Unassigned Diatom | ■ | | | | ■ | |
| Bacillariophytina | ■ | | ■ | ■ | ■ | |
| *Fragilariopsis* | ■ | ■ | | | ■ | |
| *Melosira* | | ■ | | | ■ | |
| *Thalassiosira* | ■ | ■ | | ■ | | |
| Naviculales | | | | ■ | ■ | |

The mean OTU overlap between several habitat combinations is shown in the **Supplementary Tab. S10**. The ice core samples of PS92 shared on average 51.2% of the different OTUs, and the UIW samples shared 49.1%. Ice core samples of PS94 shared more taxa (57.8%) than the UIW samples (44.5%). PS89 ice samples overlapped with on average 41.0%, the UIW samples with 46.7%.

Comparing the mean overlap between the sea ice and UIW samples of PS92, on average 31.3% of the different OTUs were found in both habitats. For PS94, the mean overlap was a bit higher (35.5%) and for PS89 it was intermediate (33.2%).

For the comparison of both polar regions, the sea ice samples of PS89 showed a mean overlap of 25.7% with PS92 sea ice samples and 27.7% with PS94 ice core samples. The UIW samples of PS89 had a mean overlap of 37.8% with PS92 UIW samples and 32.0% with PS94 sea ice samples.

The heatmap (**Fig. 24**) shows the proportions of taxa per sample in the abundant (>1% of the OTUs) biosphere (**A**) and the rare (<1% of the OTUs) biosphere (**B**). Herewith we reveal again, which taxa dominated the individual samples. But furthermore, the division of the abundant and rare biosphere allowed to explain the OTU overlap between different habitats and cruises, indicated by the Venn diagrams. For example, *Ostreococcus* occurred in the abundant biosphere of PS89, but not in the abundant biosphere of any other samples of PS89, PS92 and PS94. However, *Ostreococcus* was found in most of the rare biosphere samples during all cruises and also in higher proportions in the ice than in the UIW.

In fact, the strong OTU overlap between the Arctic and Antarctic was assumed to be determined more by the rare biosphere than by abundant taxa.

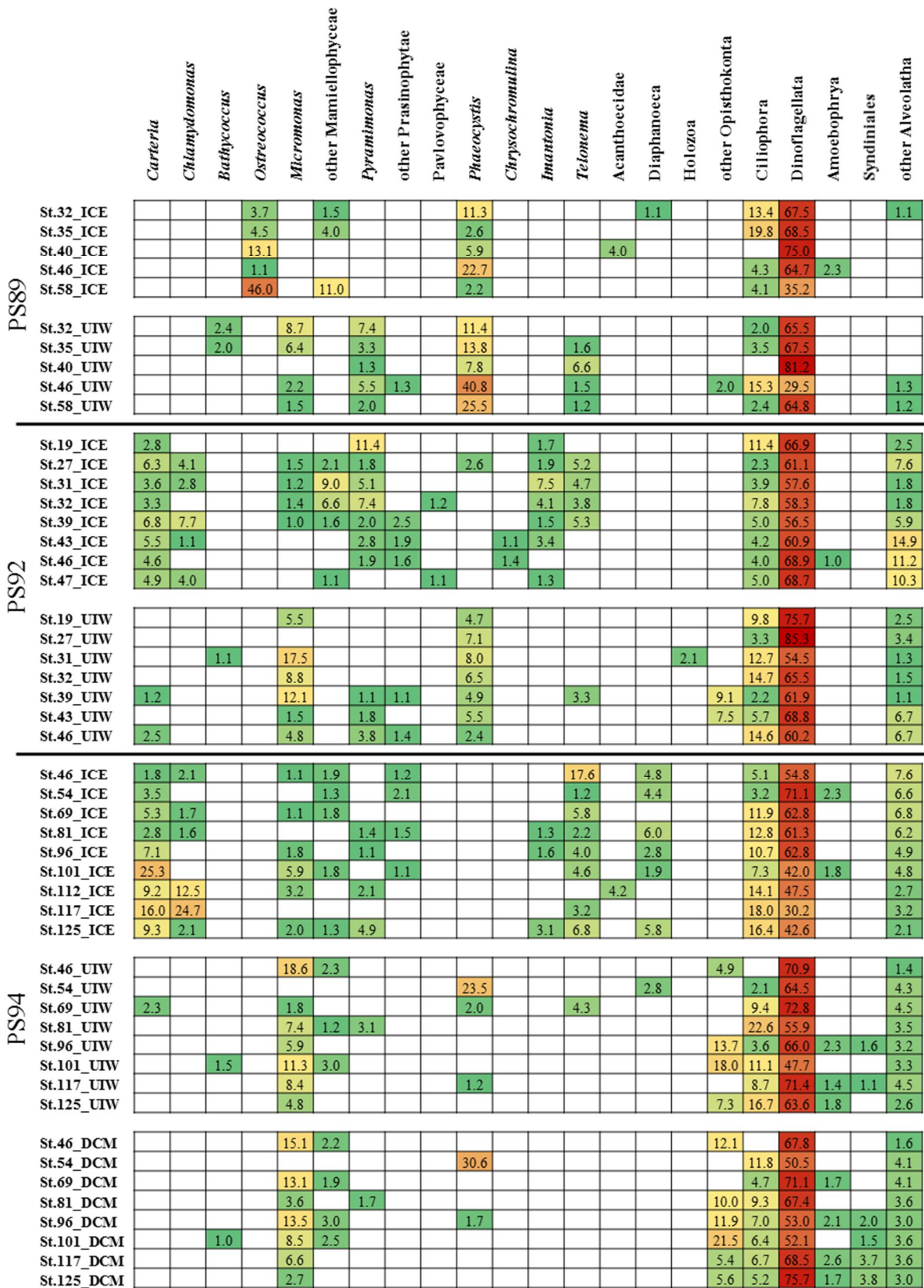| Cruise | Sample | Carteria | Chlamydomonas | Bathycoccus | Ostreococcus | Micromonas | other Mamiellophyceae | Pyramimonas | other Prasinophytae | Pavlovophyceae | Phaeocystis | Chrysochromulina | Imantonia | Telonema | Acanthoecidae | Diaphanoeca | Holozoa | other Opisthokonta | Ciliophora | Dinoflagellata | Amoebophrya | Syndiniales | other Alveolatha |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PS89 | St.32_ICE | | | | 3.7 | | 1.5 | | | | 11.3 | | | | 1.1 | | | | 13.4 | 67.5 | | | 1.1 |
| | St.35_ICE | | | | 4.5 | 4.0 | | | | | 2.6 | | | | | | | | 19.8 | 68.5 | | | |
| | St.40_ICE | | | | 13.1 | | | | | | 5.9 | | | | | 4.0 | | | | 75.0 | | | |
| | St.46_ICE | | | | 1.1 | | | | | | 22.7 | | | | | | | | 4.3 | 64.7 | 2.3 | | |
| | St.58_ICE | | | | 46.0 | 11.0 | | | | | 2.2 | | | | | | | | 4.1 | 35.2 | | | |
| | St.32_UIW | | | 2.4 | | 8.7 | | 7.4 | | | 11.4 | | | | | | | | 2.0 | 65.5 | | | |
| | St.35_UIW | | | 2.0 | | 6.4 | | 3.3 | | | 13.8 | | | 1.6 | | | | | 3.5 | 67.5 | | | |
| | St.40_UIW | | | | | | | 1.3 | | | 7.8 | | | 6.6 | | | | | | 81.2 | | | |
| | St.46_UIW | | | | | 2.2 | | 5.5 | 1.3 | | 40.8 | | | 1.5 | | | | 2.0 | 15.3 | 29.5 | | | 1.3 |
| | St.58_UIW | | | | | 1.5 | | 2.0 | | | 25.5 | | | 1.2 | | | | | 2.4 | 64.8 | | | 1.2 |
| PS92 | St.19_ICE | 2.8 | | | | | | 11.4 | | | | | | 1.7 | | | | | 11.4 | 66.9 | | | 2.5 |
| | St.27_ICE | 6.3 | 4.1 | | | 1.5 | 2.1 | 1.8 | | | 2.6 | | | 1.9 | 5.2 | | | | 2.3 | 61.1 | | | 7.6 |
| | St.31_ICE | 3.6 | 2.8 | | | 1.2 | 9.0 | 5.1 | | | | | | 7.5 | 4.7 | | | | 3.9 | 57.6 | | | 1.8 |
| | St.32_ICE | 3.3 | | | | 1.4 | 6.6 | 7.4 | | 1.2 | | | | 4.1 | 3.8 | | | | 7.8 | 58.3 | | | 1.8 |
| | St.39_ICE | 6.8 | 7.7 | | | 1.0 | 1.6 | 2.0 | 2.5 | | | | | 1.5 | 5.3 | | | | 5.0 | 56.5 | | | 5.9 |
| | St.43_ICE | 5.5 | 1.1 | | | | | 2.8 | 1.9 | | | 1.1 | | 3.4 | | | | | 4.2 | 60.9 | | | 14.9 |
| | St.46_ICE | 4.6 | | | | | | 1.9 | 1.6 | | | | 1.4 | | | | | | 4.0 | 68.9 | 1.0 | | 11.2 |
| | St.47_ICE | 4.9 | 4.0 | | | | | 1.1 | 1.1 | | | | | 1.3 | | | | | 5.0 | 68.7 | | | 10.3 |
| | St.19_UIW | | | | | 5.5 | | | | | 4.7 | | | | | | | | 9.8 | 75.7 | | | 2.5 |
| | St.27_UIW | | | | | | | | | | 7.1 | | | | | | | | 3.3 | 85.3 | | | 3.4 |
| | St.31_UIW | | | 1.1 | | 17.5 | | | | | 8.0 | | | | | | 2.1 | | 12.7 | 54.5 | | | 1.3 |
| | St.32_UIW | | | | | 8.8 | | | | | 6.5 | | | | | | | | 14.7 | 65.5 | | | 1.5 |
| | St.39_UIW | 1.2 | | | | 12.1 | | | 1.1 | 1.1 | 4.9 | | | 3.3 | | | | 9.1 | 2.2 | 61.9 | | | 1.1 |
| | St.43_UIW | | | | | 1.5 | | 1.8 | | | 5.5 | | | | | | | 7.5 | 5.7 | 68.8 | | | 6.7 |
| | St.46_UIW | 2.5 | | | | 4.8 | | 3.8 | 1.4 | | 2.4 | | | | | | | | 14.6 | 60.2 | | | 6.7 |
| PS94 | St.46_ICE | 1.8 | 2.1 | | | 1.1 | 1.9 | | 1.2 | | | | | 17.6 | 4.8 | | | | 5.1 | 54.8 | | | 7.6 |
| | St.54_ICE | 3.5 | | | | | 1.3 | | 2.1 | | | | | 1.2 | 4.4 | | | | 3.2 | 71.1 | 2.3 | | 6.6 |
| | St.69_ICE | 5.3 | 1.7 | | | | 1.1 | 1.8 | | | | | | | 5.8 | | | | 11.9 | 62.8 | | | 6.8 |
| | St.81_ICE | 2.8 | 1.6 | | | | | 1.4 | 1.5 | | | 1.3 | | 2.2 | 6.0 | | | | 12.8 | 61.3 | | | 6.2 |
| | St.96_ICE | 7.1 | | | | | 1.8 | 1.1 | | | | 1.6 | | 4.0 | 2.8 | | | | 10.7 | 62.8 | | | 4.9 |
| | St.101_ICE | 25.3 | | | | | 5.9 | 1.8 | 1.1 | | | | | 4.6 | 1.9 | | | | 7.3 | 42.0 | 1.8 | | 4.8 |
| | St.112_ICE | 9.2 | 12.5 | | | | 3.2 | 2.1 | | | | | | | 4.2 | | | | 14.1 | 47.5 | | | 2.7 |
| | St.117_ICE | 16.0 | 24.7 | | | | | | | | | | | 3.2 | | | | | 18.0 | 30.2 | | | 3.2 |
| | St.125_ICE | 9.3 | 2.1 | | | | 2.0 | 1.3 | 4.9 | | | | 3.1 | 6.8 | | 5.8 | | | 16.4 | 42.6 | | | 2.1 |
| | St.46_UIW | | | | | 18.6 | 2.3 | | | | | | | | | | | 4.9 | | 70.9 | | | 1.4 |
| | St.54_UIW | | | | | | | | | | 23.5 | | | | | 2.8 | | | 2.1 | 64.5 | | | 4.3 |
| | St.69_UIW | 2.3 | | | | 1.8 | | | | | 2.0 | | | | 4.3 | | | | 9.4 | 72.8 | | | 4.5 |
| | St.81_UIW | | | | | 7.4 | 1.2 | 3.1 | | | | | | | | | | | 22.6 | 55.9 | | | 3.5 |
| | St.96_UIW | | | | | 5.9 | | | | | | | | | | | | 13.7 | 3.6 | 66.0 | 2.3 | 1.6 | 3.2 |
| | St.101_UIW | | | | 1.5 | 11.3 | 3.0 | | | | | | | | | | | 18.0 | 11.1 | 47.7 | | | 3.3 |
| | St.117_UIW | | | | | 8.4 | | | | | 1.2 | | | | | | | | 8.7 | 71.4 | 1.4 | 1.1 | 4.5 |
| | St.125_UIW | | | | | 4.8 | | | | | | | | | | | | 7.3 | 16.7 | 63.6 | 1.8 | | 2.6 |
| | St.46_DCM | | | | | 15.1 | 2.2 | | | | | | | | | | | 12.1 | | 67.8 | | | 1.6 |
| | St.54_DCM | | | | | | | | | | 30.6 | | | | | | | | 11.8 | 50.5 | | | 4.1 |
| | St.69_DCM | | | | | 13.1 | 1.9 | | | | | | | | | | | | 4.7 | 71.1 | 1.7 | | 4.1 |
| | St.81_DCM | | | | | 3.6 | | | | 1.7 | | | | | | | | 10.0 | 9.3 | 67.4 | | | 3.6 |
| | St.96_DCM | | | | | 13.5 | 3.0 | | | | 1.7 | | | | | | | 11.9 | 7.0 | 53.0 | 2.1 | 2.0 | 3.6 |
| | St.101_DCM | | | | 1.0 | 8.5 | 2.5 | | | | | | | | | | | 21.5 | 6.4 | 52.1 | | 1.5 | 3.6 |
| | St.117_DCM | | | | | 6.6 | | | | | | | | | | | | 5.4 | 6.7 | 68.5 | 2.6 | 3.7 | 3.6 |
| | St.125_DCM | | | | | 2.7 | | | | | | | | | | | | 5.6 | 5.2 | 75.7 | 1.7 | 3.8 | 3.0 |

**Figure 24A**. Heatmap of taxa, which represent OTUs contributing to the abundant biosphere (>1% of sequences in a sample), for all samples during the three cruises PS89, PS92 and PS94. Numbers and colors indicate the proportions of certain taxa of the whole OTU abundance per sample.

**Figure 24B**. Heatmap of taxa, which represent OTUs contributing to the rare biosphere (<1% of sequences in a sample), for all samples during the three cruises PS89, PS92 and PS94. Numbers and colors indicate the proportions of certain taxa of the whole OTU abundance per sample.

**Fig. 25** shows the results of the CCAs for the sea ice (**A**) and UIW (**B**) samples of both, the Arctic and Southern Ocean. In both models, temperature, salinity and chlorophyll content had the highest power for explaining the OTU compositions.

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The composition of the PS94 sea ice samples was influenced rather more by a temperature gradient, than by salinity and chlorophyll values. Stations 46, 54, 69 and 96 clustered closer together at intermediate temperature values. Stations 81, 101, 112, 117 and 125 were determined by lower temperatures.

OTU distribution on stations 27, 39, 43, 46 and 47 of PS92 clustered separately at a higher temperature level. However, stations 19, 31 and 32 showed an equal OTU composition due to intermediate temperatures and slightly higher salinity and chlorophyll levels than on the other stations.

Finally, ice core samples of PS89 showed a wider distribution due to intermediate temperatures and higher salinities and chlorophyll levels. The pack ice stations 32 and 35 clustered closely together, while the fast ice stations 40 and 46 laid apart.
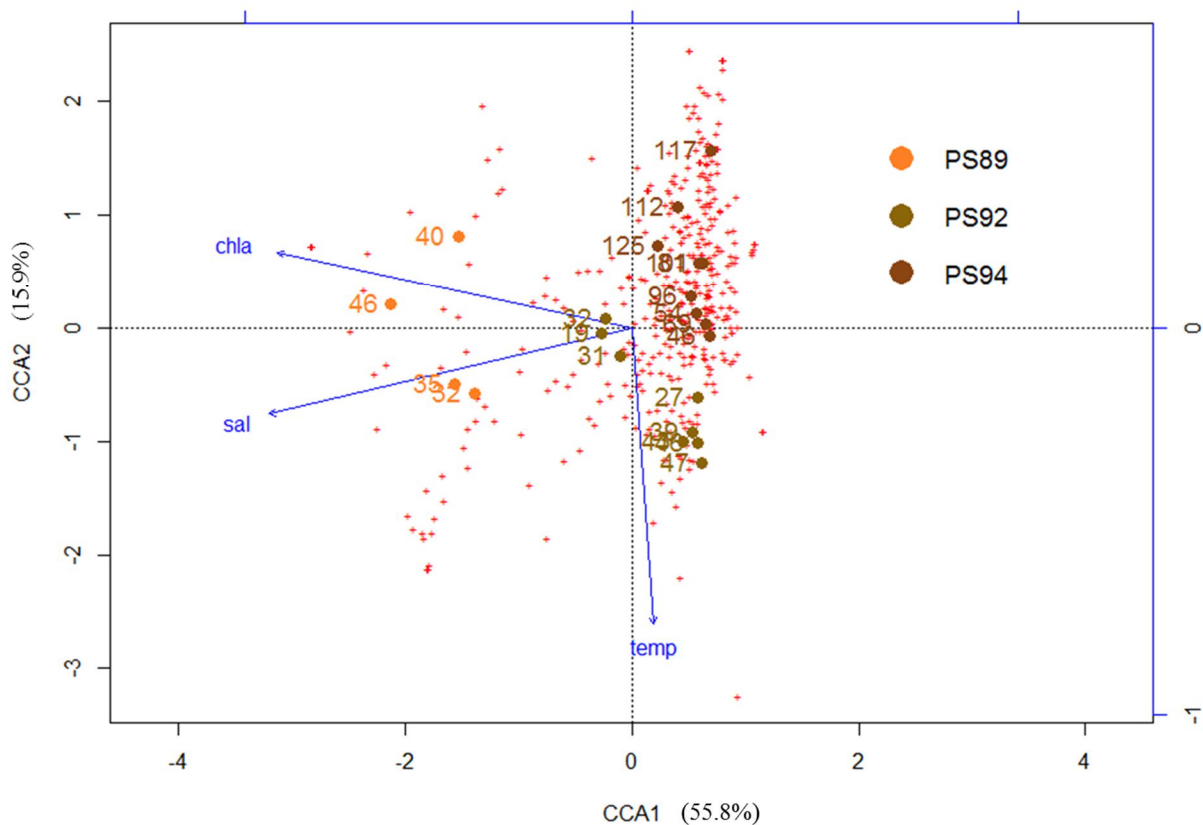


**Figure 25A.** Canonical Correspondence Analysis (CCA) of sea ice samples from PS89, PS92 and PS94. Proportion of the total variance, which is explained by all environmental factors: 84.8% (p < 0.05). Arrows represent explanatory variables (chlorophyll, temperature and salinity) with arrowheads indicating their direction of increase. Red crosses represent response variables (OTU abundance) and stations are color coded due to their habitat type (sea ice samples = brown).

Concerning the dependency of OTU distribution of the UIW samples on environmental parameters, the separation of the three cruises was not that sharp, like it was for the sea ice

samples. Especially, samples of PS89 and PS92 clustered together, determined by high chlorophyll levels and intermediate salinity and temperature levels. Moreover, the UIW stations of PS94 clustered at lower chlorophyll levels, and the OTU distributions on stations 46, 54 and 81 seemed to be also influenced by higher temperatures. Station 43 and 46 of PS92 clustered together with the UIW samples of PS94 than with the other PS92 samples.
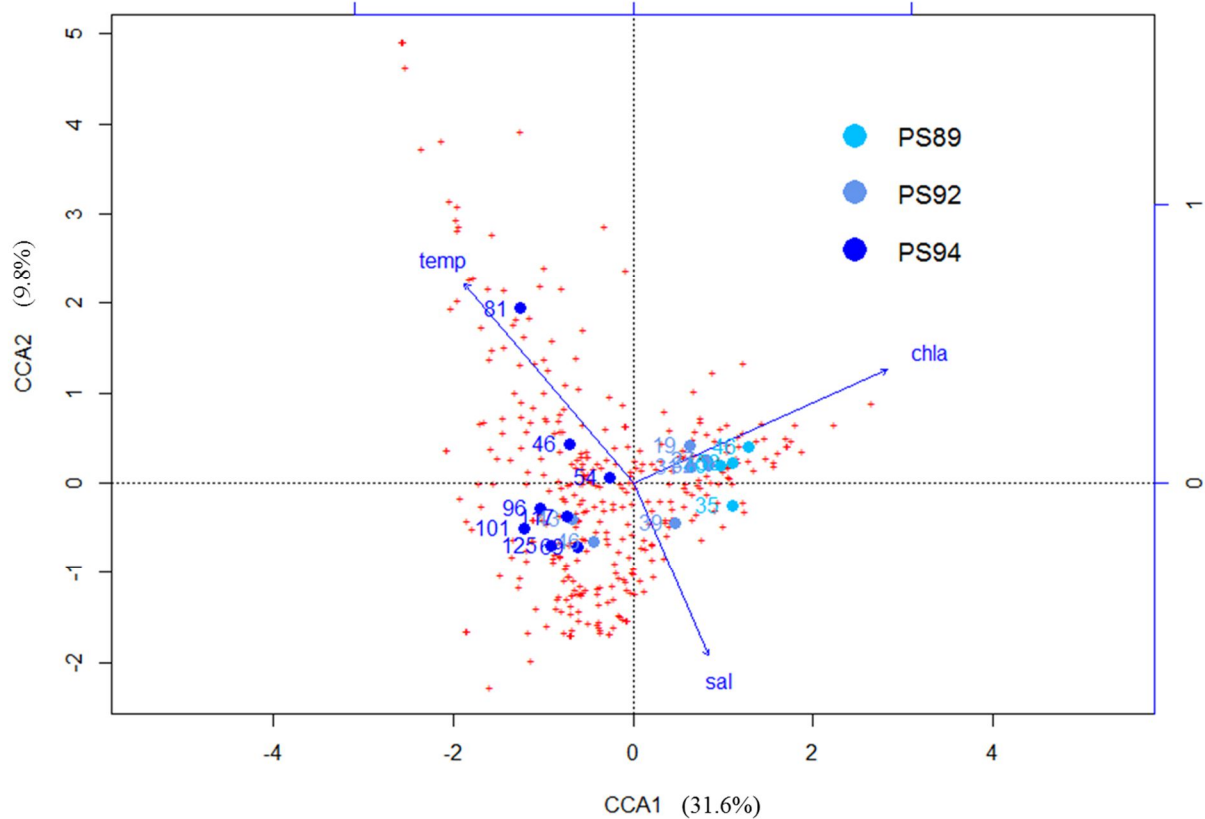


**Figure 25B.** Canonical Correspondence Analysis (CCA) of sea ice samples from PS89, PS92 and PS94. Proportion of the total variance, which is explained by all environmental factors: 47.4% (p < 0.05). Arrows represent explanatory variables (chlorophyll, temperature and salinity) with arrowheads indicating their direction of increase. Red crosses represent response variables (OTU abundance) and stations are color coded due to their habitat type (UIW samples = blue).

# 4. Discussion

In this Master thesis, the coupling of the marine protist community composition between sea ice and under-ice water, as well as between both polar oceans was investigated. Samples were taken in the respective seasonal period, where two seasons (spring and fall) are covered for the Arctic samples. Thus, this study provides insights into the spatial, but - in the case of the Arctic samples - also temporal resolution. To address this topic, three research hypotheses were developed and will be discussed in this chapter.

The focus of the first hypothesis was on the potential organism exchange between sea ice and the water beneath, because both habitats offer different conditions.

Whether the protist communities differ between both polar regions due to their geographical separation, was the central question of the second hypothesis.

Finally, we hypothesized that the amount of fresh water taxa in the Arctic is greater than in the Southern Ocean, because of a greater influence of riverine waters on the Arctic Ocean.

## 4.1 The connection between sea ice & under-ice water

The sea ice, as well as the water beneath, offers a completely different habitat setup for marine protists (Mock & Thomas, 2005). The thickness of the sea ice is highly variable due to its age and origin (Peeken et al., 2016). It drifts away from the area, where it was formed, encloses different organisms while floating and hence transports them to new locations. Due to its inclusion of taxa, it is expected to contain a high variety and heterogeneity of taxa. Sea ice contains several habitats (snow cover, brine channels, bottom surface) with different physical conditions, which allow different taxa to coexist (Krembs et al., 2000). Our results in this study confirm this hypothesis, since we observed a greater amount of different OTUs in sea ice than in the UIW and therefore, a relatively high overlap (~50%) between both habitats was found, in the Arctic as well in the Southern Ocean. This finding matches former studies by Majaneva et al. (2012) and Hardge et al. (unpublished). Additionally, heterotrophic organisms like ciliates and dinoflagellates represent the OTU-richest groups, which confirmed also findings of other researchers (Bachy et al., 2011; Majaneva et al., 2012).

Gulliksen and Lønne (1989) separated organisms within the sea ice into two groups: autochthonous forms, which are regularly found in the ice, and allochthonous forms, which only occur occasionally. Here, we found more opisthokonts, dinoflagellates and Chlorophyceae but less Syndiniales and haptophytes in the ice samples of the fall cruise PS94 than during the spring cruise PS92. This led to an OTU great overlap of ~70% between the sea ice of the Arctic spring and the fall samples and confirm the classification by Gulliksen and Lønne (1989). Since microbial community composition strongly follows physical and chemical constraints, which vary temporally and spatially (Eicken, 2003), slight differences between PS92 and PS94 OTU compositions can be explained by seasonal shifts in environmental conditions, which corresponds with results of former studies in the Arctic Ocean (Mikkelsen et al., 2008). However, this could also be the result of sampling in two different regions and thus we cannot make a proper statement here.

Chlorophyceae, stramenopiles, prasinophytes, *Telonema* and some other alveolates preferred the sea ice more than the UIW or DCM, which is a typical finding (Hasle & Heimdal, 1998). Whereas the cold-adapted chlorophyte *Micromonas* for example was found more often and in higher amounts in the UIW than in the sea ice, which confirms former studies, too (Lovejoy et al., 2007; Metfies et al., 2016).

Freshwater organisms, several ciliates and diatoms displayed unique taxa, too. Diatoms contribute significantly to the primary production in the Arctic Ocean (Gradinger et al., 1999; Arrigo et al., 2003), but were underestimated in this study according to the chosen primers, which did not amplify these unicellular algae sequences very well. Nevertheless, the qualitative occurrence mainly in the sea ice was remarkable. The great amount of autotrophic OTUs in the ice compared to the water column indicated the importance of this habitat for photosynthetic active organisms. They show special adaptations like specific pigment compositions (e.g. Fucoxanthin), which are able to absorb energy poor wavelengths to carry out photosynthesis even during winter time (Dickinson et al., 2016). Kaartokallio (2004) observed that autotrophic producers dominate the biomass of the ice-associated communities and sea ice has the potential to serve as a biodiversity source for the water column, where cells could be dispersed.

Bachy et al. (2011) found that the deepest sea ice layer displays an intermediate microbial community composition compared to the upper ice layers and the UIW, suggesting that this layer acts as a bridge for cryo-pelagic protist exchange. However, since ice cores in this study were taken as complete units, these previous findings could not be explored within this study.

Furthermore, physical properties of the sea ice determine not only the biodiversity within sea ice but also the interactions of the ice with the underlying water column. For example, FYI is more connected to the under-ice water via brine channels than MYI, which leads to a higher degree of exchange of nutrients and protists between the two habitats (R. Gradinger & Ikävalko, 1998).

Spatial variation in community compositions could be observed in the three ice stations St.19, St.31 and St.32 (PS92), which showed huge differences compared to the other ice core samples of the Arctic Ocean. Since no studies about temperature and salinity optima of missing taxa in these samples exist, we can only assume that these physical parameters might be responsible for the separation. The PS92 station St.27 clustered more closely to the other sea ice samples because of its higher core temperature, although it was geographically close to the three outlier stations. This became also apparent in the CCA. Therefore, temperature could be one main driver (beside salinity) for taxa occurrence in general.

Differences in physical properties between water masses can lead to fronts, which cannot be easily overcome by passively drifting organisms. But within one water mass, the protist community was assumed to be more homogeneous (Hamilton et al., 2008). Indeed, for the Southern Ocean sample collection, the mean OTU overlap between all UIW samples was significantly higher than between the sea ice samples. But for both Arctic cruises, this was not the case. Hardge et al. (unpublished) found, that the sea ice community in 2011 was more diverse and spatially variable compared to the UIW community. Additionally, the protist communities in 2012 were more similar in both, the sea ice and the UIW, compared to 2011. They assumed that this was a result of increased exchange of protists between sea ice and the water column due to thinning sea ice during this year.

10% of all OTUs found in the Arctic were ubiquitous in the water column. These were on the one hand some dinoflagellates, ciliates and prasinophytes, which did not occur in the sea ice. But in fact, Mamiellophyceae and opisthokonts were the significant representatives in the water column compared to the sea ice. Additionally, parasitic syndiniales occurred also more likely in the water column, and even more in the DCM. These findings correspond with those of Hardge et al. (unpublished). Many OTUs belonging to syndiniales were found in this study, probably according to the high number of dinoflagellates, on which syndiniales parasite (Kim et al., 2008; Majaneva et al., 2012).

For PS94, samples from the chlorophyll maximum layer were included. Interestingly, the community composition there was very similar to that from the under-ice water, giving evidence to a connectivity between at least the upper 0-30m.

Looking to the group proportions of the Southern Ocean, the number of haptophytes seemed to influence the difference between the sea ice and UIW community the most, where the UIW samples in general contained more haptophytes than their appropriate sea ice sample. Different water masses might be responsible for differences between station 32 and the others, as this station was located at the most northern position, divided from the others by oceanographic fronts.

The uniqueness of OTUs in the surface waters and the sea ice was not mainly determined by higher taxonomic groups. They occurred qualitatively in both habitats. Instead, it was a mixture of single OTUs belonging to several taxa of the rare biosphere.

All in all, we can summarize that the sea ice and the UIW harbor distinct protist communities, with a higher diversity in the ice, which thus might act as a biodiversity source for the water column. Thus, partly exchange between both habitats could be observed. Sea ice plays an important role for marine protists for both, sea ice and water column.

## 4.2  Geographical separation vs. similar physical conditions

It is an ongoing discussion, whether microbial organisms are in general more globally distributed or endemic species (Staley & Gosink, 1999; Fenchel & Finlay, 2004; Ghiglione et al., 2012). Baas-Becking (1934) proposed his famous hypothesis "everything is everywhere, but the environment selects" and the same idea was more recently repeated by Finlay (2002) in the so-called Ubiquity hypothesis, which suggests (microbial) organisms with a huge population size as well as very good dispersal abilities are globally distributed. Problematic for investigations and reliable conclusions of this issue are certain criteria, listed by Vyverman et al. (2010): "[…] uneven and/or incomplete sampling over large geographical scales by the lack of standardization in the delineation of taxonomical units on the basis of morphological criteria, and more recently, also in the molecular markers used to delimit OTUs (operational taxonomic units) […].".

Insights into the (protist) diversity of the Southern Ocean are still superficial, even more than for the Arctic Ocean. Several studies have already been made, giving snap shots of different locations and years: "So why should one cosmopolitan morpho-species have a global common gene pool and others fragmented populations. We must assume that we are looking at difference snapshots in species evolution. As new species evolve, become genetically and

physiologically diverse, they could be distributed globally with a common gene pool. At this point in evolutionary time, everything is everywhere, but as time progresses; the common gene pool becomes fragmented and isolated as local populations become locally adapted. Eventually gene flow is reduced such that populations can no longer interbreed; even become locally extinct. Eventually morphological/physiological differentiation occurs, and new species evolve, and the process begins all over again." (Medlin, 2007).

The Arctic and the Southern Ocean are far away from each other (~18000 km) and connected by water masses, which require years to move from one pole to the other one. The results of this study showed, that ~60% of the different OTUs were found in both poles. Alveolates were the most abundant group among all samples. This result fits to findings by Bachy et al. (2011). On the other hand, there were no Chlorophyceae worthy of remark in both, the sea ice and UIW within the the Southern Ocean samples. This finding is contrary to a study by Vyverman et al. (2010), which proposed a high grade of endemism of cyanobacteria and Chlorophyceae in the Antarctic.

However, occurrence of taxa in both polar regions does not automatically mean global distribution or true bipolarity, thus we cannot differentiate between them, which is still a common issue in science (Wolf et al., 2015).

Nevertheless, we are able to come up with the hypothesis that both polar regions are connected or at least share a high amount of global distributed taxa. In general, rare taxa contributed much more to the large overlap between polar regions, which provides information about connection between both polar regions. So we hypothesize, that despite the huge distance, if taxa make it to the other pole, they have the opportunity to survive there if the conditions are suitable, but probably occur in different proportions. This assumption has to be proven by e.g. intensive transect work between the poles.

Furthermore, the comparison of both, the spring and the fall Arctic cruise with the Antarctic one, revealed, that the bipolar overlap of taxa is also dependent on seasonal changes. The main drivers during the season are differences in radiation/day-night cycles and nutrient availability (Gradinger, 2009). In general, nutrient levels in the water column increase during winter, because assimilation rates by autotrophic organisms are low, because light availability is too low for photosynthesis. For diatoms, we found that more taxa occurred in the sea ice of the Southern Ocean (PS89) and the fall cruise of the Arctic (PS94) than in the sea ice of the Arctic spring cruise (PS92). Here we found more OTUs in the UIW. This pattern was not responsible for the higher OTU overlap between PS89 and PS92, but might also be another hint for seasonal changes in the community composition.

All things considered, our data showed, that the Arctic and the Southern Ocean share a great number of taxa. Additionally, we found that protist community complexity, composition and diversity can change between seasons, which matches with former studies (Hardge et al., unpublished). Therefore, the poles could be strongly connected, but also other mechanism like coevolutionary processes might be responsible. Based on our data we cannot conclude one single explanation.

## 4.3  Fresh water taxa in the Arctic & Southern Ocean

The Southern Ocean, especially the deep water, is connected to all of the world's oceans, as it represents a junction within the conveyor belt. However, the Antarctic continent is isolated from water masses beyond the ACC, which appears as a strong front (PF) between the Antarctic Zone (AZ) and the outer areas. Therefore, organismic exchange (especially for small, more or less passively drifting taxa) is minimized and different protist communities were found before and behind fronts (Wolf et al., 2014). These findings imply also, that the only input of freshwater taxa into the water column is possible via wind, resting stadiums or input from the Antarctic continent itself.

A contrary assumption can be made for the Arctic Ocean. Freshwater input by rivers of the surrounding land masses is omnipresent. Organisms and their resting stadiums drift with the water currents and can be enclosed into the ice.

Because of this principle difference between both polar ecosystems, the hypothesis was made, that the Arctic Ocean samples will contain more freshwater taxa compared to the Southern Ocean samples.

In fact, freshwater taxa (*Choricystis*, *Carteria*, *Woloszynskia* and some opisthokonts) only occurred in the Arctic samples. *Carteria* were found in both, in the ice and the UIW. The freshwater dinoflagellate genus *Woloszynskia* was found in the UIW and DCM samples of PS94, but not in the ice, whereas this genus occurred in the sea ice and UIW of PS92. *Choricystis* (Chlorophyceae) is a globally distributed genus. Several ways are imaginable and discussed, how freshwater species find their way into the sea ice: Either through wind, enclosure during ice formation near the shore or offshore transportation of resting cells via ocean currents and subsequently enclosure during ice formation (Thomas & Dieckmann, 2008, 2009; Harding et al., 2011). It can be assumed, that their occurrence was due to the high

freshwater input in the Arctic Ocean, whereas the Southern Ocean is not affected by main freshwater inflows. Naviculales (diatoms) were only found in the Arctic samples. In general, this taxon contains marine and freshwater species. If it was a freshwater taxon in this study (which cannot be affirmed due to the sequencing depth), this would underline the hypothesis even more.

But based on the results, that no freshwater OTUs were found in the Southern Ocean samples, the 3rd hypothesis could be confirmed.

# Conclusion & future perspectives

Protist diversity in polar regions has been already studied in sediments, water, sea ice and snow by several scientists (Luo et al., 2009; Terrado et al., 2009; Piquet et al., 2010; Harding et al., 2011). But all of them concluded, that diversity and vertical distribution of microbial eukaryotic communities remains to be studied furthermore. The results in this study suggest, that sea ice is a biodiversity hot spot, but also strongly connected to the water column. Sea ice (thickness, extension and duration of persistence) in the Arctic is predicted to decline rapidly due to raising temperatures and therefor melting processes (Stroeve et al., 2014) and (microbial) biodiversity could suffer because of the diminishing ice cover (Majaneva et al., 2012). Accordingly, massive ecological consequences can be expected. Melting means also favoring the input of sea ice species into the water column where they might influence the food composition and availability for other trophic levels (Post et al., 2013).

Gast et al. (2004) made the first molecular survey of the microbial eukaryotes from sea ice in the Southern Ocean, showing a large genetic diversity. The sample size of the Southern Ocean in this study is relatively small, but it is even more surprising, that so many taxa were found in both polar regions. However, Hardge et al. (unpublished) found a lower protist diversity in the pelagial of the Arctic Ocean in 2012 than in 2011, despite they analyzed a larger sample size.

The assumption is supported, that there is more microbe exchange between the Arctic and the Southern Ocean than assumed before. In fact, in the Antarctic the sea ice coverage is expected to increase. Saying that both polar regions might develop in different directions. Hence, long term studies of highly environment-depending organisms like marine protists in the polar oceans are more than ever necessary for detecting global change. The molecular approach chosen in this study offers great opportunities for the determination of the protist community, compared with the determination with microscopy for example. Nevertheless, certain issues have to be taken into account. The sequencing process is a balance between low sequence quality coupled with diversity overestimation, or high sequence quality coupled with diversity underestimation. Additionally, finding an optimal primer set to get as most taxa as possible is still a challenge. Here, we underestimated for example diatom taxa in their abundance, this can be improved in further studies.

All things considered, we were able to show a strong coupling of marine protists between sea ice and under-ice water in the Arctic and Southern Ocean. Global warming might change the mechanisms behind it in the future. Thus, further studies are necessary to understand global processes.

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1. MiSeq System User Guide, Illumina 2014
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# Statutory declaration

I hereby affirm, that the present work was written independently by me and I didn´t use any other aids - particularly internet sources - than those specified in the list of sources. The work has not been submitted by me another examination. The submitted written version corresponds to that on the electronic storage medium. I agree that the thesis will be published.

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Stephan Wietkamp

# Appendix

**Table S1a.** Sample overview including environmental properties for PS89.

## PS89

| Station ID | Habitat type | Latitude °N | Longitude °E | Temperature (°C) | Salinity (PSU) | Chlorphyll (µg/l) | Snow cover (cm) | Ice thickness (cm) |
|---|---|---|---|---|---|---|---|---|
| 32 | ICE | -67.578 | -0.148 | -1.70 | 11.65 | 6.84 | 18 | 113 |
| 35 | ICE | -69.014 | -0.067 | -1.70 | 9.29 | 6.80 | 6 | 80 |
| 40 | ICE | -70.526 | -7.957 | -2.70 | 9.37 | 215.57 | 17 | 207 |
| 46 | ICE | -70.559 | -7.643 | -1.30 | 10.19 | 18.90 | 30 | 109 |
| 58 | ICE | -70.515 | -8.732 | n.d. | n.d. | n.d. | 0 | 233 |
| 32 | UIW | -67.578 | -0.148 | n.d. | n.d. | n.d. | 18 | 113 |
| 35 | UIW | -69.014 | -0.067 | -1.74 | 34.30 | 0.21 | 6 | 80 |
| 40 | UIW | -70.526 | -7.957 | -1.84 | 34.40 | 12.39 | 17 | 207 |
| 46 | UIW | -70.559 | -7.643 | -1.71 | 33.57 | 3.76 | 30 | 109 |
| 58 | UIW | -70.515 | -8.732 | -1.69 | 26.01 | 2.16 | 0 | 233 |

**Table S1b.** Sample overview including environmental properties for PS92.

## PS92

| Station ID | Habitat type | Latitude °N | Longitude °E | Temperature (°C) | Salinity (PSU) | Chlorphyll (µg/l) | Snow cover (cm) | Ice thickness (cm) |
|---|---|---|---|---|---|---|---|---|
| St.19 | ICE | 81.230 | 18.551 | -2.84 | 6.53 | 0.53 | 13 | 1 |
| St.27 | ICE | 81.386 | 17.594 | -1.92 | 1.76 | 0.60 | 24 | 113 |
| St.31 | ICE | 81.623 | 19.449 | -2.27 | 6.94 | 1.01 | 28 | 117 |
| St.32 | ICE | 81.236 | 19.425 | -2.27 | 5.33 | 1.65 | 27 | 111 |
| St.39 | ICE | 81.937 | 13.570 | -1.09 | 5.47 | 0.92 | 17 | 132 |
| St.43 | ICE | 82.212 | 7.595 | -1.69 | 6.53 | 0.48 | 26 | 108 |
| St.46 | ICE | 81.891 | 9.733 | -1.32 | 4.92 | 0.81 | 8 | 134 |
| St.47 | ICE | 81.348 | 13.613 | -0.56 | 2.03 | 1.44 | 15 | 105 |
| St.19 | UIW | 81.230 | 18.551 | -2.00 | 5.00 | 4.87 | 13 | 1 |
| St.27 | UIW | 81.386 | 17.594 | -1.74 | 33.25 | 3.05 | 24 | 113 |
| St.31 | UIW | 81.623 | 19.449 | -1.52 | 32.04 | 1.31 | 28 | 117 |
| St.32 | UIW | 81.236 | 19.425 | -1.87 | 32.42 | 2.35 | 27 | 111 |
| St.39 | UIW | 81.937 | 13.570 | -1.73 | 33.66 | 0.31 | 17 | 132 |
| St.43 | UIW | 82.212 | 7.595 | -1.59 | 32.01 | 0.04 | 26 | 108 |
| St.46 | UIW | 81.891 | 9.733 | -1.75 | 34.34 | 0.27 | 8 | 134 |

**Table S1c.** Sample overview including environmental properties for PS94.

## PS94

| Station ID | Habitat type | Latitude °N | Longitude °E | Temperature (°C) | Salinity (PSU) | Chlorphyll (µg/l) | Snow cover (cm) | Ice thickness (cm) |
|---|---|---|---|---|---|---|---|---|
| St.46 | ICE | 83.072 | 30.357 | -1.08 | 1.31 | 0.13 | 2 | 82 |
| St.54 | ICE | 85.087 | 42.591 | -1.56 | 2.38 | 0.20 | 2 | 165 |
| St.69 | ICE | 86.998 | 58.715 | -1.72 | 1.37 | 0.27 | 9 | 133 |
| St.81 | ICE | 89.030 | 61.071 | -3.42 | 2.79 | 0.13 | 13 | 133 |
| St.96 | ICE | 88.546 | 125.191 | -1.90 | 3.02 | 0.17 | 18 | 151 |
| St.101 | ICE | 87.531 | 179.943 | -4.09 | 3.45 | 0.18 | 22 | 135 |
| St.112 | ICE | 85.215 | 118.395 | -1.90 | 3.50 | 4.58 | 0 | 86 |
| St.117 | ICE | 84.753 | 115.988 | -6.67 | 2.14 | 0.78 | 15 | 106 |
| St.125 | ICE | 85.343 | 140.099 | -3.62 | 2.52 | 2.78 | 13 | 145 |
| | | | | | | | | |
| St.46 | UIW | 83.072 | 30.357 | -0.71 | 32.56 | 1.01 | 2 | 82 |
| St.54 | UIW | 85.087 | 42.591 | -1.38 | 25.77 | 2.61 | 2 | 165 |
| St.69 | UIW | 86.998 | 58.715 | -1.63 | 33.56 | 0.08 | 9 | 133 |
| St.81 | UIW | 89.030 | 61.071 | -0.56 | 10.64 | 0.35 | 13 | 133 |
| St.96 | UIW | 88.546 | 125.191 | -1.40 | 25.33 | 0.49 | 18 | 151 |
| St.101 | UIW | 87.531 | 179.943 | -1.51 | 26.42 | 0.34 | 22 | 135 |
| St.117 | UIW | 84.753 | 115.988 | -1.74 | 31.95 | 0.12 | 15 | 106 |
| St.125 | UIW | 85.343 | 140.099 | -1.62 | 29.93 | 0.61 | 13 | 145 |
| | | | | | | | | |
| St.46 | DCM | 83.072 | 30.357 | -1.43 | 33.45 | 0.36 | 2 | 82 |
| St.54 | DCM | 85.087 | 42.591 | -1.54 | 34.09 | 0.70 | 2 | 165 |
| St.69 | DCM | 86.998 | 58.715 | -1.65 | 33.12 | 0.10 | 9 | 133 |
| St.81 | DCM | 89.030 | 61.071 | -1.62 | 32.10 | 0.14 | 13 | 133 |
| St.96 | DCM | 88.546 | 125.191 | -1.51 | 28.58 | 0.09 | 18 | 151 |
| St.101 | DCM | 87.531 | 179.943 | -1.47 | 28.35 | 0.03 | 22 | 135 |
| St.117 | DCM | 84.753 | 115.988 | -1.69 | 32.66 | 0.22 | 15 | 106 |
| St.125 | DCM | 85.343 | 140.099 | -1.63 | 29.98 | 0.12 | 13 | 145 |
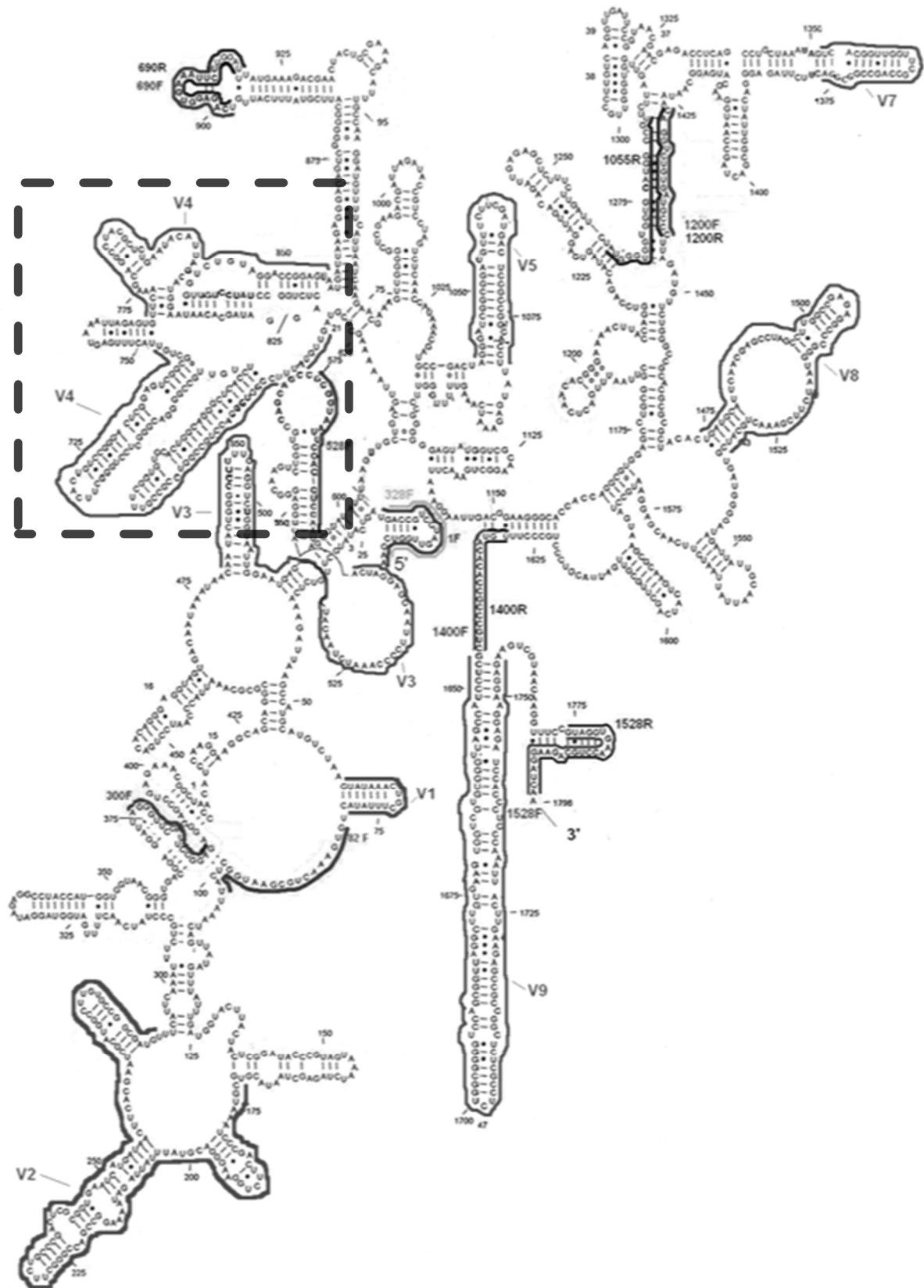
**Figure S2.** Structure and primer-map of the 18S locus (SSU rRNA gene) highlighting the V4 region (dashed box).

**Table S3a.** Sequencing results oft he PS89 samples.

| PS89 | Ice core samples | | | | | | Under-ice water samples | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Station ID | 32 | 35 | 40 | 46 | 58 | | 32 | 35 | 40 | 46 | 58 |
| Total reads | 36744 | 205241 | 187374 | 244693 | 350751 | | 78319 | 95981 | 33891 | 102166 | 107275 |
| Quality filtering | | | | | | | | | | | |
| < 200 bp | 2377 | 11828 | 55490 | 22011 | 29765 | | 5330 | 805 | 14521 | 0 | 22886 |
| > 400 bp | 10627 | 59 | 526 | 3866 | 2931 | | 14 | 3440 | 119 | 48950 | 2967 |
| Final read number | 23740 | 193354 | 131358 | 218816 | 318055 | | 72975 | 91736 | 19251 | 53216 | 81422 |
| Subsampling | 3531 | 3531 | 3531 | 3531 | 3531 | | 3531 | 3531 | 3531 | 3531 | 3531 |
| Total OTUs | 3195 | 3253 | 3207 | 3057 | 3162 | | 3357 | 3304 | 3430 | 3350 | 3285 |
| Different OTUs | 158 | 91 | 81 | 144 | 90 | | 83 | 102 | 106 | 134 | 129 |

**Table S3b,c.** Sequencing results of the PS92 and PS94 samples.

**PS92**

| Station ID | Ice core samples | | | | | | | | Under-ice water samples | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 19 | 27 | 31 | 32 | 39 | 43 | 46 | 47 | 19 | 27 | 31 | 32 | 39 | 43 | 46 |
| Total reads | 104814 | 92661 | 66306 | 71749 | 56039 | 72618 | 74748 | 80218 | 72766 | 82393 | 81625 | 69173 | 79961 | 95653 | 57958 |
| Quality filtering | | | | | | | | | | | | | | | |
| <200 bp | 54750 | 0 | 12864 | 22511 | 10108 | 3894 | 25880 | 0 | 20038 | 0 | 25505 | 4367 | 670 | 964 | 0 |
| >400 bp | 101 | 34276 | 1217 | 51 | 3173 | 10197 | 10444 | 42631 | 2564 | 21004 | 81 | 7542 | 7546 | 22516 | 18841 |
| Final read number | 49963 | 58385 | 52225 | 49187 | 42758 | 58527 | 38424 | 37587 | 50164 | 61389 | 56039 | 57264 | 71745 | 72173 | 39117 |
| Subsampling | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 |
| Total OTUs | 2669 | 2571 | 2253 | 2715 | 2997 | 2754 | 2740 | 2612 | 2695 | 2187 | 2981 | 2716 | 3346 | 3192 | 2569 |
| Different OTUs | 147 | 181 | 139 | 118 | 183 | 166 | 171 | 177 | 134 | 102 | 152 | 128 | 150 | 169 | 176 |

**PS94**

| Station ID | Ice core samples | | | | | | | | | Under-ice water samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 46 | 54 | 69 | 81 | 96 | 101 | 112 | 117 | 125 | 46 | 54 | 69 | 81 | 96 | 101 | 117 | 125 |
| Total reads | 175518 | 180449 | 164466 | 93940 | 49430 | 74881 | 41268 | 228718 | 64128 | 25503 | 80615 | 107725 | 64618 | 74168 | 60747 | 97900 | 62177 |
| Quality filtering | | | | | | | | | | | | | | | | | |
| <200 bp | 29696 | 20178 | 55717 | 8383 | 6776 | 19646 | 11046 | 85228 | 4991 | 1010 | 37592 | 16990 | 0 | 0 | 5993 | 41446 | 0 |
| >400 bp | 64525 | 81102 | 32621 | 16317 | 10262 | 6973 | 4074 | 48055 | 19036 | 14424 | 520 | 30031 | 40565 | 41655 | 26662 | 13 | 34140 |
| Final read number | 81297 | 78969 | 76128 | 69240 | 32392 | 48262 | 26148 | 95435 | 40101 | 10069 | 42503 | 60704 | 24053 | 32513 | 28092 | 56441 | 28037 |
| Subsampling | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 |
| Total OTUs | 3179 | 3116 | 3200 | 3011 | 2982 | 2922 | 2758 | 2854 | 1846 | 3370 | 2683 | 3276 | 3129 | 3156 | 2794 | 3066 | 3175 |
| Different OTUs | 210 | 231 | 218 | 230 | 241 | 245 | 257 | 216 | 198 | 117 | 135 | 156 | 207 | 177 | 182 | 162 | 158 |

**Table S3d.** Sequencing results of the PS94 chlorophyll samples.

| PS94 | Chlorophyll maximum layer samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Station ID | 46 | 54 | 69 | 81 | 96 | 101 | 117 | 125 |
| Total reads | 134128 | 109536 | 114005 | 27881 | 68714 | 74992 | 77383 | 77644 |
| Quality filtering | | | | | | | | |
| < 200 bp | 4002 | 62953 | 8686 | 16034 | 2156 | 0 | 32815 | 8876 |
| > 400 bp | 67488 | 2154 | 47584 | 1 | 38871 | 35688 | 2278 | 34175 |
| Final read number | 62638 | 44429 | 57735 | 11846 | 27687 | 39304 | 42290 | 34593 |
| Subsampling | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 | 3531 |
| Total OTUs | 3376 | 1903 | 3253 | 3184 | 3031 | 2997 | 3020 | 2991 |
| Different OTUs | 103 | 124 | 145 | 173 | 187 | 153 | 180 | 171 |
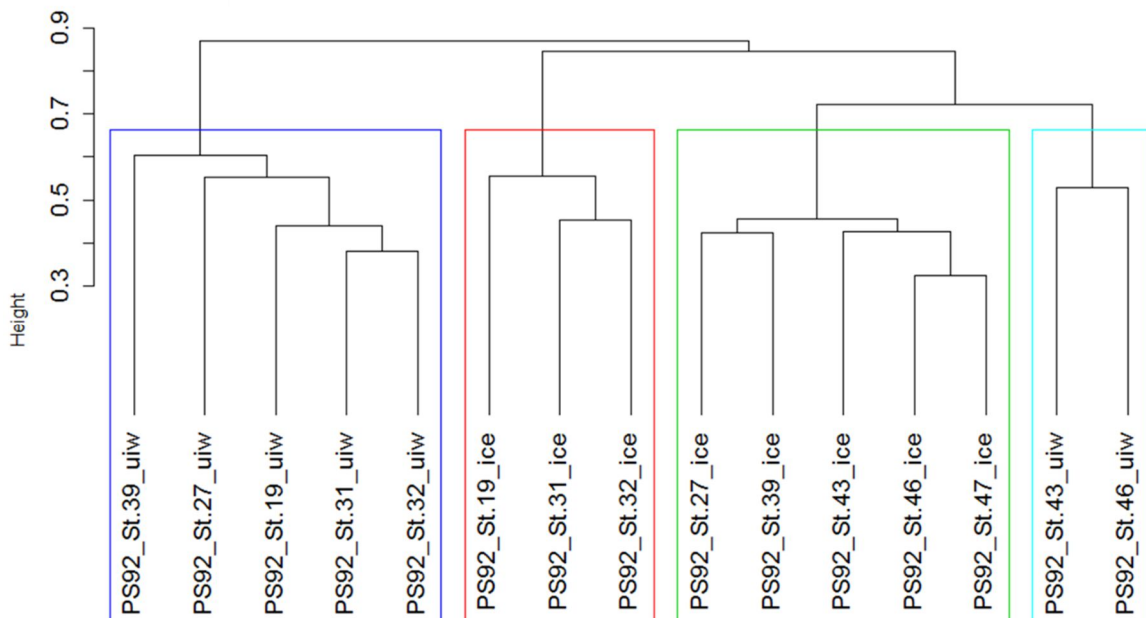


**Figure S4.** Cluster analysis of the Arctic samples (PS92) using the *hclust* function ("complete" linkage, based on Cophonic correlation and Gower distance comparison) implemented in the R package *vegan* (Oksanen et al., 2012). 4 clusters (marked with colored hulls) were gained based on Pearson correlation.
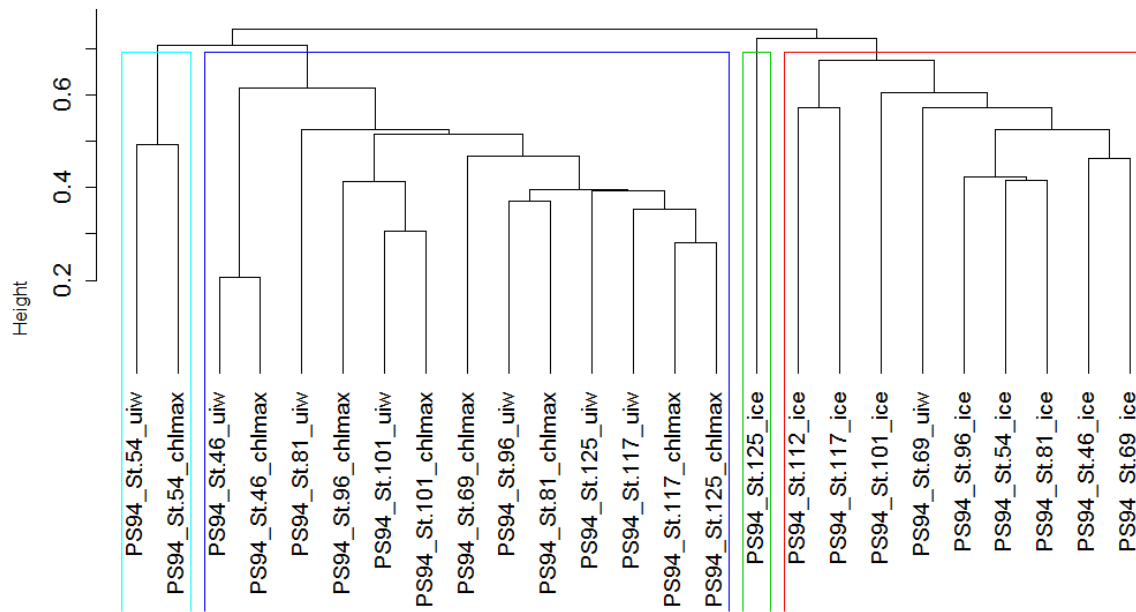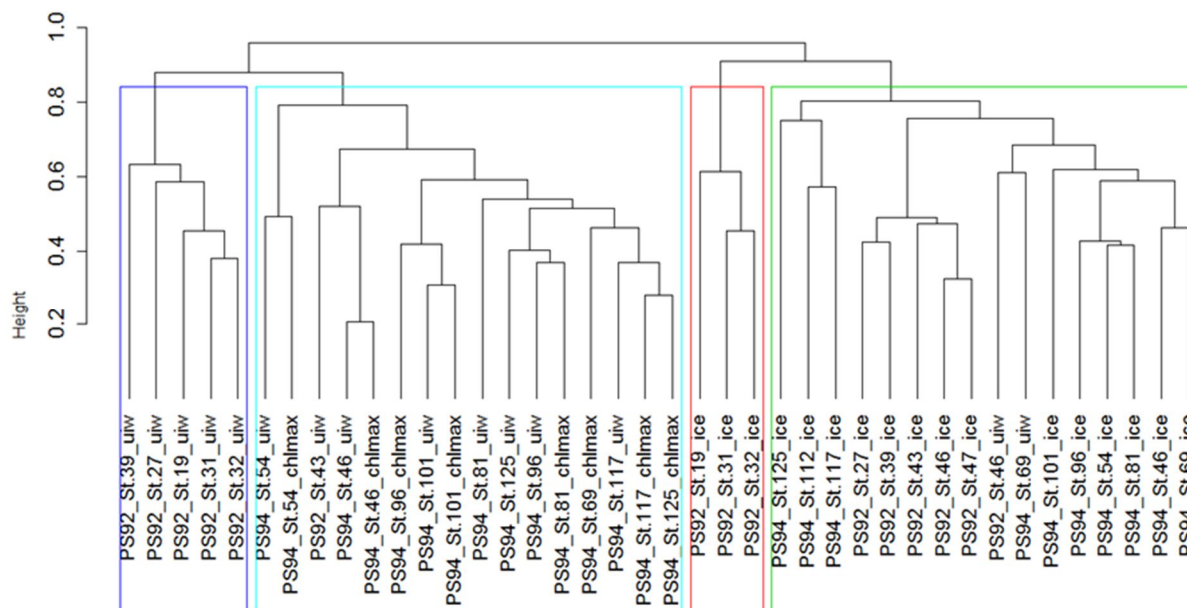
**Figure S5.** Cluster analysis of the Arctic samples (PS94) using the *hclust* function ("complete" linkage, based on Cophonic correlation and Gower distance comparison) implemented in the R package *vegan* (Oksanen et al., 2012). 4 clusters (marked with colored hulls) were gained based on Pearson correlation.
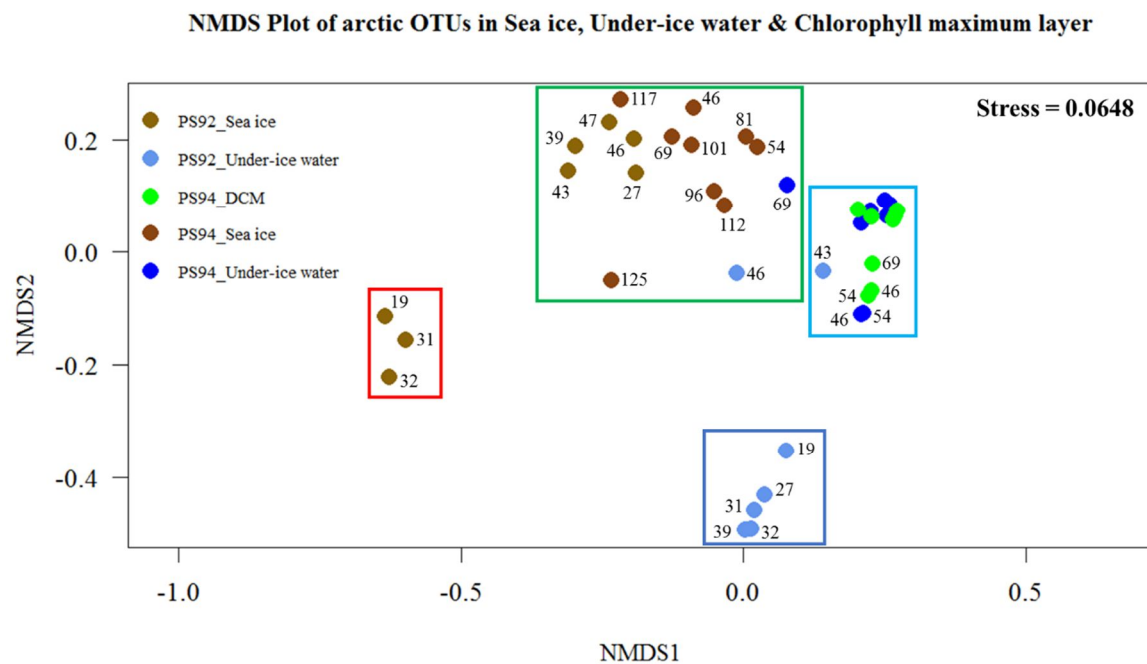


**Figure S6.** Non-metric multidimensional scaling (NMDS) based on Jaccard distances of OTU profiles found in the 15 PS92 samples. Dot colors indicate the 3 habitats: Ice cores (brown), under-ice water (blue) and chlorophyll maximum layer (green). Colored boxes around stations represent own clusters, indicated by the cluster analysis (**Supplementary Fig. S6**).
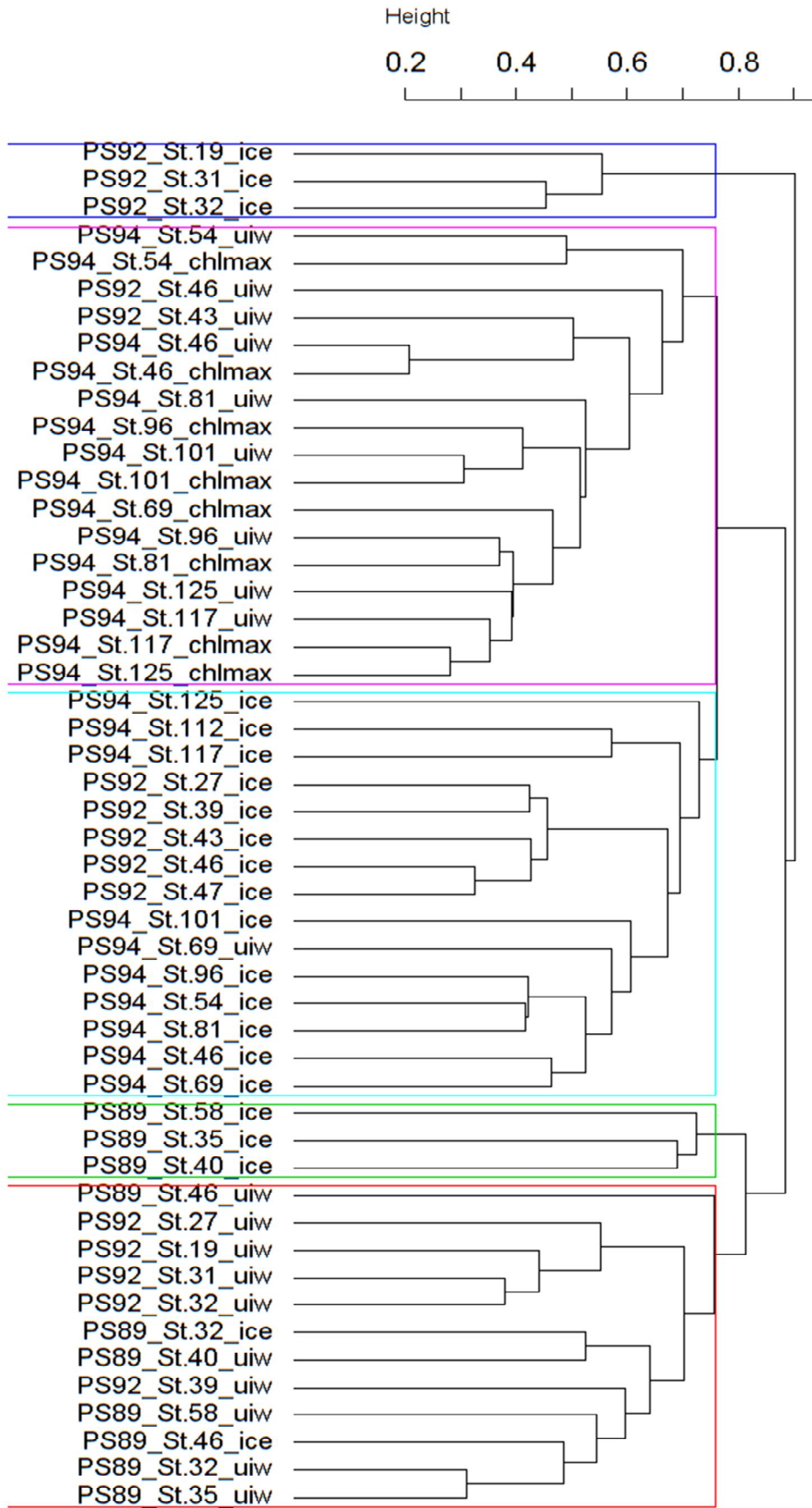
**Figure S7.** Cluster analysis of the Arctic samples using the *hclust* function ("complete" linkage, based on Cophonic correlation and Gower distance comparison) implemented in the R package *vegan* (Oksanen et al., 2012). 4 clusters (marked with colored hulls) were gained based on Pearson correlation.
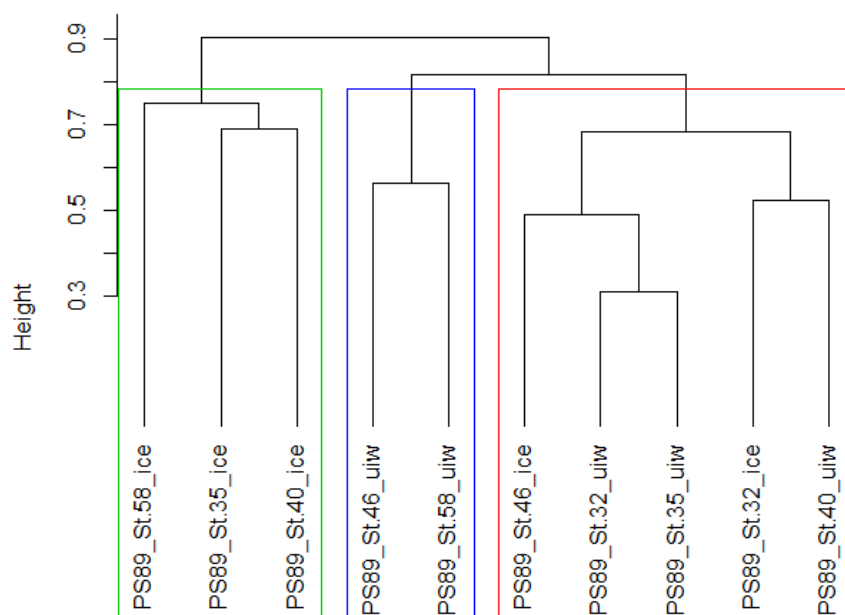


**Figure S8.** Cluster analysis of the Southern Ocean samples using the *hclust* function ("complete" linkage, based on Cophonic correlation and Gower distance comparison) implemented in the R package *vegan* (Oksanen et al., 2012). 3 clusters (marked with colored hulls) were gained based on Pearson correlation.

**Figure S9**. Cluster analysis of the Southern and Arctic Ocean samples using the *hclust* function ("complete" linkage, based on Cophonic correlation and Gower distance comparison) implemented in the R package *vegan* (Oksanen et al., 2012). 5 clusters (marked with colored hulls) were gained based on Pearson correlation.

**Table S10.** Average OTU overlap (%) between several habitat combinations.

| Region | Habitat & Cruise | Mean ± SD (%) |
|---|---|---|
| Arctic Ocean | ICE PS92 | 51.2 ± 7.9 |
| | ICE PS94 | 57.8 ± 4.7 |
| | UIW PS92 | 49.1 ± 6.7 |
| | UIW PS94 | 44.5 ± 6.9 |
| | ICE PS92 vs UIW PS92 | 31.3 ± 5.0 |
| | ICE PS94 vs UIW PS94 | 35.4 ± 5.8 |
| | UIW PS92 vs UIW PS94 | 40.8 ± 5.2 |
| | ICE PS92 vs ICE PS94 | 46.6 ± 7.5 |
| Southern Ocean | ICE PS89 | 41.0 ± 4.3 |
| | UIW PS89 | 46.7 ± 8.6 |
| | ICE PS89 vs UIW PS89 | 33.2 ± 6.8 |
| Arctic vs. Southern Ocean | ICE PS89 vs ICE PS92 | 25.7 ± 4.6 |
| | ICE PS89 vs ICE PS94 | 27.7 ± 5.8 |
| | UIW PS89 vs UIW PS92 | 37.8 ± 6.7 |
| | UIW PS89 vs UIW PS94 | 32.0 ± 5.9 |