Diversity and function of microbial communities in the Arctic Ocean

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“I live with these tiny beings in their separate universe, where they are born and die, generation after generation, where they pursue each other in the struggle for life, and carry on their love affairs with the same feelings, the same sufferings, and the same joys that permeate every living being, from these microscopic animalcules up to man – self-preservation and propagation, that is the whole story.”

Fridtjof Nansen, Farthest North - The Epic Adventure of a Visionary Explorer

SUMMARY

The Arctic Ocean ecosystem is rapidly changing in response to climate warming. The ongoing decline of its sea-ice cover has raised many questions as to the ecological consequences on biodiversity, primary productivity, and the biological carbon pump. The diversity and function of bacterial communities in the Arctic Ocean has been little explored, despite their often important role in biogeochemical cycling. One objective of this thesis was therefore to improve the current knowledge of microbial community diversity in the most understudied region of the Arctic Ocean, the deep central Eurasian basin. As sea-ice reduction is altering primary productivity and biological transport processes from the surface ocean to the deep sea, another focus of this thesis was the identification of bacterial groups associated to freshly formed, sinking and deposited particulate organic matter. Finally, the underlying genomic features that deep-sea surface sediment bacteria use for carbon turnover were analyzed, as the degradation of organic matter by heterotrophic bacteria in deep-sea sediments regulates the efficiency of CO_2 removal from the atmosphere over geological time scales.

Chapter I of this thesis is dedicated to the exploration of the diverse microbial life present in the Earth's cryosphere. This encompasses all environments where temperatures are so low that a substantial fraction of water freezes and is present in its solid form. **Chapter Ia** summarizes the current knowledge of the variety of cold-adapted microorganisms as revealed by high-throughput sequencing technologies, and reviews recent findings on the diverse metabolic strategies they employ to inhabit frozen environments. The microbial communities associated to its different components are distinct, but share similar adaptations to the cold. This synthesis also highlights that microbial life in ice generally differs from that in water, thereby raising questions on the ecological consequences of the rapidly declining cryosphere for microbial diversity and the maintenance of key ecological functions.

The synthesis of available sequence data from different components of the cryosphere stressed the heterogenous nature and patchiness of frozen environments. To ease comparability and integration of different datasets, and to allow inference of ecological patterns from sequence data, **Chapter Ib** thus proposes

minimal metadata requirements for the submission of microbial sequences from the cryosphere. In compliance with the Minimum Information about any (x) Sequence (MIxS) standard, established by the Genomic Standards Consortium, MIxS-cryo extension package provides a metadata description checklist to be collected and reported for each sequenced sample. Further, knowledge about cryosphere features was added to or updated in the Environmental Ontology (ENVO), and is featured in the cryosphere-dedicated release *PolarExpress*.

In **Chapter II**, the bacterial and microbial eukaryotic community composition of the central Arctic Eurasian basin in late summer 2012 were explored across a wide range of habitats from sea ice, surface waters to the seafloor. Eukaryotic communities were dominated by the sea-ice diatom *Melosira arctica*, which formed large aggregates in melt ponds and attached to the ice underside. Selected sea-ice heterotrophs, predominantly members of the *Flavobacteriia*, *Gamma- and Alphaproteobacteria,* were associated with the algae aggregates. Upon rapid ice melt, large amounts of ice-algal biomass sank to the deep-sea floor, transporting attached sea-ice bacteria with them. Unique bacterial communities established in association to the deposited aggregates, locally changing benthic community structure.

Benthic bacterial diversity and function is addressed in detail in **Chapter III**. Here, amplicon and 'omic sequencing of DNA and RNA along a water depth gradient was used to explore the underlying genomic features that deep-sea surface sediment bacteria use to degrade and take up deposited organic material, and to investigate whether communities residing at different water depths are taxonomically and functionally different. While communities exhibited high taxonomic turnover at the OTU level, the assigned heterotrophic potential along the water depth gradient remained similar, suggesting the presence of redundant functional types. However, first indications for distinct substrate utilization patterns at the level of different bacterial classes or phyla could be deduced.

ZUSAMMENFASSUNG

Die weltweite Klimaerwärmung führt zu einem rasanten Wandel des Ökosystems des arktischen Ozeans. Der stetig andauernde Rückgang der Meereisbedeckung wirft viele Fragen nach den Konsequenzen für Biodiversität, Primärproduktion und den Kohlenstoffkreislauf in der Arktis auf. Trotz der wichtigen Rolle von Bakterien für den Umsatz von Kohlenstoff und Nährstoffen, ist die Diversität und Funktion der bakteriellen Gemeinschaften im arktischen Ozean noch wenig erforscht. Ein wichtiger Bestandteil dieser Arbeit war es daher unser Verständnis der mikrobiellen Diversität besonders in dem bisher am wenigstens erforschten Gebiet des arktischen Ozeans, dem eurasischen Becken der Zentralarktis, zu verbessern. Da der Rückgang des Meereises die Primärproduktion und den Transport von biologischem Material von der Meeresoberfläche in die Tiefsee verändert, war ein weiterer Schwerpunkt der Thesis die Identifikation von Bakterien, die mit neu geformtem, sinkendem oder sedimentiertem organischen Material assoziiert sind. Zusätzlich wurden die genetischen Voraussetzungen zur Umsetzung von Kohlenstoff in Bakterien von Tiefseesedimenten analysiert. Ein besseres Verständnis ihrer Funktionsweise ist wichtig, da die Aktivität von heterotrophen Bakterien in marinen Sedimenten auf geologischen Zeitskalen reguliert, mit welcher Effizient Kohlenstoffdioxid (CO_2) im Sediment begraben und somit der Atmosphäre entzogen wird.

Kapitel I dieser Arbeit ist der Erforschung der Vielfalt von mikrobiellen Leben in der Kryosphäre der Erde gewidmet. Diese beinhaltet all jene Lebensräume, in denen die Temperaturen so niedrig sind, dass ein Großteil des vorhandenen Wassers gefriert. In **Kapitel Ia** wird der Wissensstand der aktuellen Forschung veranschaulicht und zusammengefasst welche Erkenntnisse mithilfe von Hochdurchsatz-Sequenzierungstechnologien über die Vielfalt von Kälte-adaptierten Mikroorganismen gewonnen wurden. Außerdem werden die verschiedenen Stoffwechselstrategien, die den Mikroorganismen das Leben in den verschiedenen gefrorenen Lebensräumen ermöglichen, erläutert. Es wird deutlich, dass sich die mikrobiellen Gemeinschaften, die die verschiedenen Lebensräume der Kryosphäre besiedeln, stark unterscheiden, jedoch ähnliche Anpassungen an die Kälte

aufweisen. Außerdem wird hervorgehoben, dass sich mikrobielles Leben im Eis sich wesentlich von dem im Wasser unterscheidet, was Fragen nach den ökologischen Konsequenzen der rasant zurückgehenden Kryosphäre für die mikrobielle Diversität und den Erhalt von ökologischen Schlüsselfunktionen aufwirft.

Die Zusammenführung der vorhandenen Sequenzdaten aus verschiedenen Teilen der Kryosphäre unterstrich die Heterogenität und ungleichmäßige Verteilung von mikrobiellen Lebensräumen in der Kryosphäre. Um die Vergleichbarkeit und Integration verschiedener Datensätze zu vereinfachen, und um die Interpretierbarkeit von ökologischen Mustern mithilfe von Sequenzdaten zu gewährleisten, wird in **Kapitel Ib** ein Standard an Minimalvoraussetzungen für die Bereitstellung von Metadaten vorgeschlagen, die für die Veröffentlichung von Sequenzdaten angewandt werden sollten. Nach Vorlage des Minimum Information about any (x) Sequence (MIxS) Standards des Genomic Standards Consortium, liefert das MIxS-cryo Erweiterungspaket eine Metadaten Checkliste, die während der Probennahme genommen und für jede sequenzierte Probe bereitgestellt werden sollten. Außerdem wurde das aktuelle Wissen über die verschiedenen Bestandteile der Kryosphäre in die Environmental Ontology (ENVO) eingepflegt oder aktualisiert. Die neuen Beiträge sind als eigene Rubrik, dem *PolarExpress*, in ENVO erschienen.

In **Kapitel II** wurde die Zusammensetzung der bakteriellen und eukaryotischen Gemeinschaften in verschiedenen Lebensräumen, von Meereis, über Oberflächenwasser bis hinab zum Meeresboden, des eurasischen Beckens der Zentralarktis im Spätsommer des Jahres 2012 untersucht. Die Meereis-Kieselalge *Melosira arctica* dominierte die eukaryotischen Gemeinschaften und bildete lange Aggregate in Schmelzwasser-Tümpeln und an der Unterseite des Meereises. Spezielle heterotrophe Bakterien aus dem Meereis, insbesondere Angehörige der *Flavobakterien*, *Gamma-* und *Alphaproteobakterien,* waren mit diesen Algenaggregaten assoziiert. Durch rasantes Schmelzen des Meereises sanken große Mengen der Eisalgen auf den Meeresboden und somit auch die mit ihnen assoziierten Meereis-Bakterien. In Folge dessen bildeten sich einzigartige bakterielle Gemeinschaften in Assoziation mit den gesunkenen Aggregaten, welche zu lokalen Veränderungen der benthischen Bakterienstruktur führten.

Die Diversität und Funktion von benthischen Bakterien in Tiefseesedimenten wurde in **Kapitel III** behandelt. Um die zugrundeliegenden genetischen Eigenschaften, welche Bakterien in den Tiefseesedimenten zum Abbau und der Aufnahme von gesunkenem organischen Material verwenden zu untersuchen, wurden Amplikon- und 'omics Sequenzierung von DNA und RNA kombiniert. Diese Analysen wurden entlang eines Tiefengradienten vorgenommen, um zu verstehen, ob sich die Gemeinschaften in verschiedenen Wassertiefen taxonomisch oder funktionell unterscheiden. Während sich die Gemeinschaften durch hohe taxonomische Fluktuation auf dem OTU Level auszeichneten, blieb das heterotrophe genetische Potential entlang des Tiefengradienten ähnlich, was auf das Vorkommen von funktionell redundanten Gruppen hindeutet. Allerdings wurden auch erste Hinweise auf die unterschiedliche Verwendung von Substraten auf dem Level von verschiedenen bakteriellen Klassen oder Stämmen entdeckt.

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Abbreviations

CAZymes	carbohydrate-active enzymes
CBM	carbohydrate-binding modules
CE	carbohydrate esterases
DNA	deoxyribonucleic acid
EPS	extracellular polymeric substances
FYI	first-year ice
GH	glycoside hydrolases
GT	glycosyltransferases
HMW	high-molecular-weight
LMW	low-molecular-weight
MYI	multi-year ice
OM	organic matter
OTU	operational taxonomic unit
PL	polysaccharide lyases
PML	polar mixed layer
PULs	Polysaccharide Utilization Loci
RNA	ribonucleic acid
SSU rRNA	small subunit ribosomal RNA

1 INTRODUCTION

1.1. The Arctic Ocean

The Arctic Ocean is the northernmost and smallest of the world's five oceans, accounting for only 4% of total ocean area (Jakobsson, 2002), and is often referred to as the Arctic Mediterranean, as it is nearly landlocked (Figure 1). It consists of two major deep basins, the Eurasian and the Amerasian Basin, separated by the Lomonosov Ridge, and surrounded by extensive continental shelves (Jakobsson, 2002). Together the shelves make up more than 50% of its total area, and as a consequence the Arctic Ocean is also the shallowest of all the oceans, with on average 1,200 m water depth (Jakobsson et al., 2003, 2004, 2012).

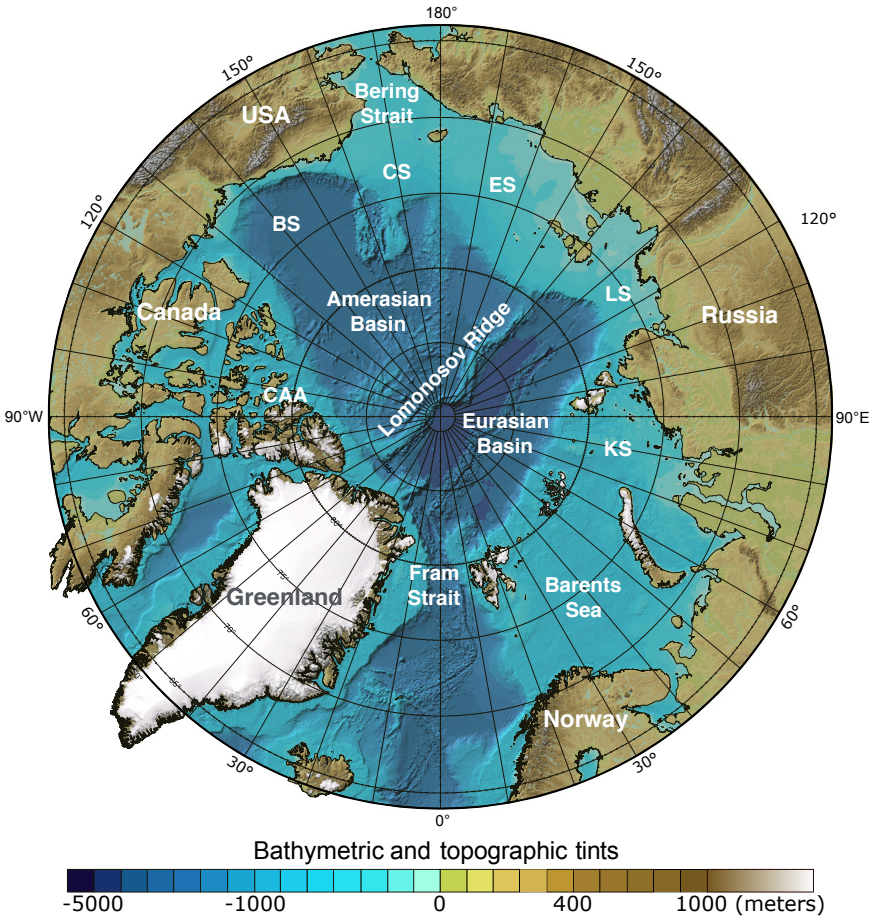


Figure 1 I Overview map showing the Arctic Ocean and its constituent seas; the broad continental shelves surrounding the deep Amerasian and Eurasian basin. The bathymetry depicts the latest IBCAO model. BS: Beaufort Sea; CS: Chukchi Sea; ES: East Siberian Sea; LS: Laptev Sea; KS: Kara Sea; CAA: Canadian Arctic Archipelago. Map was modified from Jakobsson et al., 2012.

Both geographic location and landlocked setting of the Arctic Ocean contribute to the emergence of several unique features, affecting its physicochemical environment and its biology:

Surrounded by some of the planet's largest river systems (Bring et al., 2017), the Arctic Ocean receives approximately 10% of the global river discharge, adding large amounts of freshwater and organic matter into the system (Dittmar and Kattner, 2003). As a result of the freshwater inflow, the Arctic Ocean is strongly stratified, with a low-density, fresh upper ocean overlying denser, more saline deep water (Carmack et al., 2016). River discharge therefore has implications for mixing, nutrient distribution, heat exchange and thus sea-ice formation (Carmack et al., 2016).

The strong stratification of the Arctic Oceans also structures and drives the exchange with the Pacific and Atlantic Ocean (Rudels and Friedrich, 2000; Rudels et al., 2013). Water from the Pacific enters the Arctic via Bering Strait (Roach et al., 1995), but largest water mass exchange occurs with the North Atlantic through Fram Strait and the Barents Sea (Figure1) (Fahrbach et al., 2001; Rudels et al., 2004, 2013). The Fram Strait is the only deep connection between the North Atlantic and Arctic Ocean and is characterized by two contrasting hydrographic patterns, which are separated by a frontal system (Paquette et al., 1985). While warm and nutrient-rich Atlantic water enters through the eastern part, the western Fram Strait is the major outflow area of cool, low-salinity Arctic water (Beszczynska-Möller et al., 2012) and sea ice (Kwok et al., 2004).

Situated mainly north of the Arctic Circle (66°34′ N), the most characteristic feature of the Arctic Ocean is its sea-ice cover. Sea ice plays an important role in the Arctic Ocean ecosystem, as it controls heat exchange with the atmosphere, determines albedo and, together with snow cover, governs light availability in the underlying water column (Perovich et al., 2002; Perovich and Polashenski, 2012). Its freezing and melting affects surface ocean temperature and salinity, which in turn impact ocean stratification, mixing and nutrient availability (Korhonen et al., 2013). Sea ice provides shelter and habitat for life on, in and under the ice (Gradinger, 2001; Bluhm and Gradinger, 2008; Arrigo, 2014) and can further act as a transport vehicle for material and organisms entrapped during its freeze-up (Nürnberg et al., 1994; Pfirman et al., 1997; Eicken et al., 2000; Wegner et al., 2017).

The whole Arctic system is subject to strong seasonality, with a long winter period, when the sun stays below the horizon for several months, and the spring, summer and autumn months, when the sun is above the horizon and the changing solar angle supports autotrophic life in sea ice and the upper ocean (Wassmann, 2011). Arctic sea ice grows during winter and attains its maximum seasonal extent in March, and melts and breaks up through spring and summer to a minimum extent in September (Polyak et al., 2010). Freshwater input to the system is therefore lowest in winter, when also river discharge is constrained by freezing, and stratification is weakened, allowing deep mixing and nutrient replenishment of the system (Korhonen et al., 2013).

1.2. Bacterial diversity and ecological function in the Arctic Ocean

1.1.1. Sea-ice ecosystem

The Arctic Ocean contains areas with a perennial and some with a seasonal ice cover. The perennial sea ice, which is present throughout the year, is centered around the deep basins, while large areas of the shelves and adjacent seas are covered by sea ice for a specific time of the year only (Polyak et al., 2010). Most of the ice builds up in open water over the shelves during autumn and winter and is then exported by wind and currents towards the central basins (Polyak et al., 2010). Here it can drift for several years before the majority of it melts and the remainder eventually exits the Arctic, primarily through Fram Strait (Koenigk et al., 2008; Kwok, 2009; Rudels et al., 2013; Smedsrud et al., 2017). Depending on the age of the ice, it is categorized into first-year ice (FYI), which represents a single year's growth, and ice that survived several years and melt seasons, then called multi-year ice (MYI) (Weeks and Ackley, 1986; Polyak et al., 2010). FYI and MYI can differ substantially in ice thickness, albedo, salinity and brine inclusion (Weeks and Ackley, 1986).

When sea ice freezes, salt is expelled and accumulates and concentrates as brine liquids in a network of pores and channels, which permeates sea ice from its surface to the bottom (Thomas and Dieckmann, 2002, 2010; Petrich and Eicken, 2010). The high salt concentration in these networks allows water to remain unfrozen despite subzero temperatures, and thus creates a habitable living space within the ice matrix

(Thomas and Dieckmann, 2002; Petrich and Eicken, 2010). Differences in ice morphology, as well as strong gradients in temperature, light, nutrients, salinity, brine volume and pore space add to the great spatial heterogeneity of this environment (Deming and Eicken, 2007; Petrich and Eicken, 2010; Thomas and Dieckmann, 2010; Arrigo, 2014).

Despite the challenging living conditions, the sea-ice matrix serves as habitat for a variety of organisms, including viruses, bacteria, algae, protists, diverse meiofauna and small crustaceans (Legendre et al., 1992; Thomas and Dieckmann, 2010; Bluhm et al., 2018). It sustains its own ice-associated food web, with photoautotrophic algae at its base and diatoms as the major biomass contributors. They accumulate in the bottom section to concentrations that can give the ice a brownish coloration, or grow long filamentous strands, which are attached to the ice underside (Melnikov and Bondarchuk, 1987; Mock and Gradinger, 1999; Mikkelsen et al., 2008; Arrigo, 2014; Katlein et al., 2014). When the ice melts, large amounts of sea-ice biomass can be released to the water column and sink to the seafloor (Ambrose et al., 2005; Tamelander et al., 2009; Boetius et al., 2013). Here it not only supports benthic life, but may also facilitate vertical dispersal of associated microorganisms, and thus promote microbial community connectivity.

Sea-ice biota are thought to originate mainly from the surface ocean, from where they are recruited during freeze-up, sticking to, or caught between newly formed ice crystals, and subsequently become trapped in the consolidating ice matrix (Gradinger and Ikävalko, 1998). They can, however, also originate from entrained sediments (Nürnberg et al., 1994; Pfirman et al., 1997; Wegner et al., 2017) or atmospheric deposition (Price et al., 2009). In their new habitat, organisms have to adjust to harsh abiotic changes, including changes in space availability, light intensities, salinity, and nutrient concentrations, as well as extremely low temperatures (Gradinger and Ikävalko, 1998). Many sea-ice inhabitants, thus, feature specific physiological or biochemical adaptations, i.e. specialized membrane composition, which maintains fluidity at low temperatures (Bayer-Giraldi et al., 2011; Feng et al., 2014), psychro- and halophilic enzymes (Pomeroy and Wiebe, 2001) or the potential for encystation to endure specific time intervals (Stoecker et al., 1998). All these features may also provide sea-ice microorganisms with the necessary

means to cope with sudden melt-out and relocation events, i.e. from sea ice to the water column, and the deep ocean.

Bacteria in sea ice can reach cell numbers of up to 10^7 cells per milliliter (Gosink et al., 1993; Deming and Eicken, 2007), and even higher, if associated to high ice algal biomass (Assmy et al., 2013; Fernández-Méndez et al., 2014). In the narrow brine channel system, they benefit from reduced grazing pressure by large metazoan predators (Krembs et al., 2000), the tight spatial association with ice algae (Krembs et al., 2000; Deming and Eicken, 2007), and high concentrations of dissolved organic matter produced by death and cell lysis or exudation of organic matter (Thomas et al., 2001; Krembs et al., 2002; Meiners et al., 2008; Collins and Deming, 2011). Both ice algae and bacteria are known to produce large amounts of extracellular polymeric substances (EPS) (Krembs et al., 2002; Decho and Gutierrez, 2017), consisting mainly of polysaccharides and glycoproteins (Verdugo et al., 2004). They enhance the stickiness of cell surfaces and are suspected to play a role in the selective incorporation of cells into the ice during freeze-up (Gradinger and Ikävalko, 1998; Riedel et al., 2007), as well as to provide potential attachment sites (Junge et al., 2004). EPS further serve as carbon-rich substrates for bacteria in sea ice (Meiners et al., 2004), and are also thought to act as cryoprotectants and buffer against the effects of strong and sudden changes in the chemical and physical environment, i.e. in pH or salinity (Collins et al., 2008). By producing EPS, bacteria are able to alter the ice's microstructure and thereby improve its habitability and permeability (Krembs et al., 2011), and affect the regeneration of nutrients (Riedel et al., 2007).

Current knowledge on the bacterial community structure in Arctic sea-ice has mostly been gathered during spring and summer months, when heterotrophic taxa, dominated by members of the *Flavobacteriia* and *Gammaproteobacteria*, exploit the high concentrations of organic material produced by algae (Bowman et al., 2012; Han et al., 2014; Hatam et al., 2014; Eronen-Rasimus et al., 2016). Interestingly, these spring and summer ice communities are distinct from the underlying source communities in seawater (Bowman et al., 2012; Han et al., 2014; Hatam et al., 2014), raising questions about selection processes or seasonal succession in sea ice, as well as on the connectivity between ice and water column communities. First results on the winter ice community structure and the early stages of sea-ice

community formation showed that the dominant bacterial members in winter ice, oligotrophic members of the *Alpha-* and *Gammaproteobacteria*, were also dominant in the underlying waters, speaking against selective incorporation of specific bacterial groups (Collins et al., 2010). Winter temperatures in the ice can drop to -35 °C (Deming, 2007; Deming and Eicken, 2007), which may gradually select for psychrophilic types with the capacity and metabolic traits to survive at these temperatures (Helmke and Weyland, 1995; Junge et al., 2004, 2011; Ewert and Deming, 2013; Feng et al., 2014). Further, winter conditions seem to select for types with the ability to attach to surfaces or particles, e.g. members of the *Bacteroidetes* (Junge et al., 2004).

Sea ice exhibits strong physical and chemical vertical gradients, which also transform over time as the ice ages (Deming and Eicken, 2007; Petrich and Eicken, 2010). Correspondingly, bacterial communities residing at different depth layers were found to be distinct (Hatam et al., 2014; Eronen-Rasimus et al., 2016), as were communities in MYI and FYI (Hatam et al., 2016). Higher abundances of presumed brackish- or freshwater groups, i.e. members of the *Actinobacteria* and *Betaproteobacteria*, in the ice surface layer have been linked to the presence of melt ponds on the sea ice (Brinkmeyer et al., 2004; Hatam et al., 2014). At the same time, dominance of copiotrophic members of the *Flavobacteriia*, *Alpha-*, *Gammaproteobacteria* and *Verrucomicrobia* was often linked to high algal biomass (Bowman et al., 2012; Eronen-Rasimus et al., 2016; Hatam et al., 2016). Algal-derived substrate availability has been identified as one key determinant in structuring sea-ice bacterial communities during the productive season (Bowman et al., 2012; Cowie et al., 2014; Eronen-Rasimus et al., 2016; Hatam et al., 2016), and potentially also during the dark winter months (Junge et al., 2004; Eronen-Rasimus et al., 2014, 2017; Bowman, 2015).

Heterotrophic bacteria play a crucial role for the sea-ice ecosystem (Figure 2), as their activity provides sea-ice algae with remineralized inorganic nutrients and thus supports the ice algal bloom (Kottmeier and Sullivan, 1990; Deming and Eicken, 2007). Their efficiency in utilizing organic matter present in the ice matrix also regulates the extent and state of matter, which is released to the water column during ice melt (Deming 2010). Despite this important interplay, our knowledge on

Arctic sea-ice bacteria is currently restricted to a small number of snapshots, mostly centered on the shelves or the Amerasian basin (see also 1.5). Also, which types of heterotrophic bacteria associate with sea-ice algae biomass, and whether these are specific associations have not been studied in detail and by then remain largely elusive (Krembs et al., 2000; Stewart and Fritsen, 2004; Collins et al., 2008; Cowie et al., 2014; Bertrand et al., 2015; Bowman, 2015; Eronen-Rasimus et al., 2017).

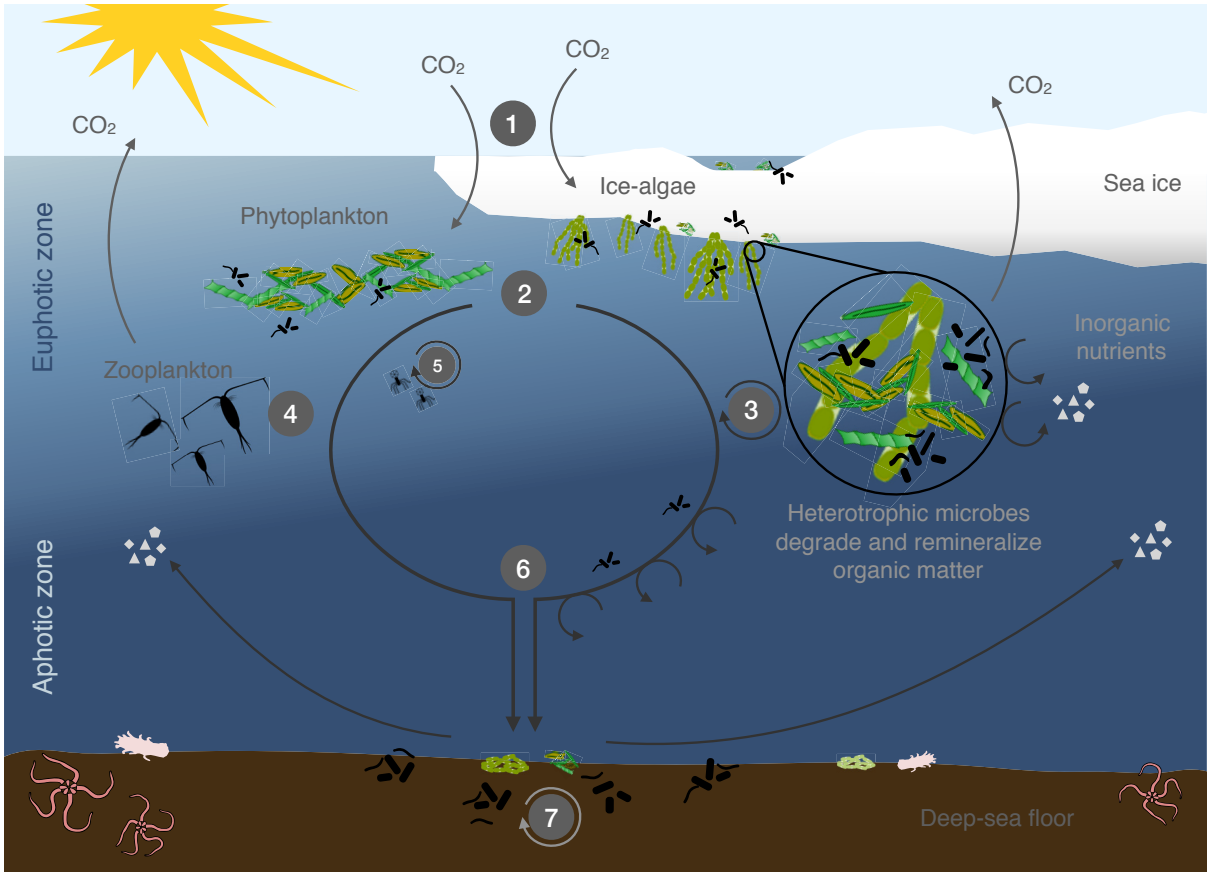


Figure 2 I Schematic illustration of the key processes of the biological pump in the Arctic Ocean. Photosynthetic primary producers in sea ice and the upper water column use energy from sunlight to fix carbon dioxide (CO_2) into biomass. (2) They produce a diverse pool of dissolved and particulate organic matter, most of which is directly respired by heterotrophs in sea ice and the surface ocean. (3) Heterotrophic microbes, mainly bacteria, degrade and remineralize the organic matter, recycling inorganic nutrients that can be used for primary production. (4) A fraction of the organic matter pool, as well as its associated heterotrophic microbes, is consumed and respired by zooplankton, and subsequently transferred to higher trophic levels. (5) Other processes, such as the viral shunt, may also contribute to the release of organic matter to the surrounding seawater or sea ice through cell lysis by viruses. The microbially mediated turnover and transfer of carbon, from its dissolved form to higher trophic levels via incorporation into microbial biomass, is called the microbial loop. (6) Through the interplay of these processes, most of the organic material is consumed and respired in the surface ocean, and only a small fraction sinks out into the deep ocean. Microbial degradation and grazing continue as the material sinks, resulting in a further reduction of export flux and a transformation of the material. (7) Only 1-3% of surface production sinks to the deep-sea floor where it fuels benthic

heterotrophic organisms. Their activity remineralizes most of the organic material, and consequently only a small fraction of approximately 0.1% is buried and contributes to the long-term sequestration of carbon from the atmosphere.

1.1.2. The Arctic water column

The water column of the Arctic Ocean exhibits a distinct layering of water masses, much of it owed to the large freshwater input it receives through river runoff and ice melt (Aagard and Carmack, 1989; Talley et al., 2011; Carmack et al., 2016), and it seems that distinct bacterial populations reside in each of the layers (Hamdan et al., 2013). The upper water mass is referred to as polar surface water, and can reach down to 200 m depth (Jones, 2001; Talley et al., 2011). It contains the Polar Mixed Layer (PML), extending from surface to between 25 and 50 m depth, characterized by a relatively low salinity of 27-34 and temperatures close to the freezing point, which vary strongly with season (Talley et al., 2011). The PML overlies a pronounced halocline of increasing salinity, which separates the surface layer from the intermediate Atlantic layer that carries warmer and more saline water, and limits exchange between deep and surface ocean (Aagard and Carmack, 1989; Talley et al., 2011; Rudels, 2012). Below that lies the deep water layer that carries cold and saline water (Jakobsson et al., 2004; Talley et al., 2011). There are, however, regional differences in water column properties as the water is modified during its transit through the Arctic, resulting in a characteristic vertical hydrography of Amerasian and Eurasian basin, and the wide shelf regions (Jakobsson et al., 2004; Talley et al., 2011; Rudels, 2012).

Most biological production takes place in the surface ocean, and here three major zones can be distinguished: (1) open ocean zones with a relatively deep euphotic zone (40-50 m), strong vertical mixing and high productivity, (2) seasonally ice-covered zones, where stratification is stronger, vertical mixing reduced and a shallower euphotic zone leads to lower primary productivity, but occasional high phytoplankton biomass events, and (3) permanently ice-covered zones with an extremely shallow euphotic zone and very low productivity and biomass (Wassmann, 2011; Wassmann and Reigstad, 2011). Thus, the ice-free or seasonally ice-covered shelf regions are typically more productive than the perennially ice-covered deep

central basins (Sakshaug, 2004; Carmack and Wassmann, 2006; Tremblay and Gagnon, 2009).

Reported bacterial numbers in the Arctic water column are in the range of 10^5 cells per milliliter, both for the shelf area and the central basins (Sherr *et al.,* 2003; Kirchman *et al.,* 2007). Studies that looked into the microbial diversity in Arctic surface waters consistently found oligotrophic *Alphaproteobacteria,* mostly classified as *Pelagibacter* or members of the SAR11 clade, to be the most dominant pelagic bacteria, independent of the study area (Alonso-Sáez et al., 2008; Kirchman et al., 2010; Bowman et al., 2012; Wilson et al., 2017). Yet, these results are based on a very limited number of studies, i.e. for the central Eurasian basin results have been reported only from a single study (Bowman et al., 2012) (see also 1.5). During and after algal bloom periods, the contribution of taxa that are known for their ability to exploit high biomass and nutrient-rich conditions increases, i.e. members of the *Bacteroidetes*, *Gammaproteobacteria* and certain *Alphaproteobacteria* (Alonso-Sáez et al., 2008; Teeling et al., 2012; Wilson et al., 2017). Thus, it seems that Arctic pelagic communities follow similar successional patterns as have been described for bacterial communities in more temperate oceans (Teeling et al., 2012, 2016; Bunse and Pinhassi, 2017).

In the deeper water column, the absence of light, higher inorganic nutrient concentrations, and overall lower seasonal variability create a more stable living space (Arístegui et al., 2009; Orcutt et al., 2011). Here, members of the chemolithotrophic *Thaumarchaeota*, as wells as members of the *Chloroflexi*-type SAR202 clade seem to dominate all year round (Wilson et al., 2017). While some previous results suggested little change in community composition between summer and winter months (Kirchman et al., 2010), others observed clear differences (Alonso-Sáez et al., 2008, 2014; Wilson et al., 2017), and reported the presence of deep water groups close to the surface in winter (Wilson et al., 2017). The degree of community connectivity, meaning the exchange and seeding of bacterial types, between the water column and sea ice is unknown. Whether it is the surface seawater community that seeds the sea-ice community during winter freeze-up, and if the melting and transport of sea-ice biomass affects surface water community composition in summer is not yet understood. Also, whether it is the same

heterotrophic bacterial groups that associate with algal biomass in ice and the water column, and thus contribute to upper ocean nutrient recycling has not been studied in detail.

1.1.3. The deep-sea floor ecosystem

The area of the oceans where the lack of sunlight prevents photosynthetic primary production is called deep sea. This typically encompasses the water column below 200 m water depth, which represents 95% of the oceans' volume, and its seafloor, which covers more than 65% of Earth's surface (Herring, 2002). Fine-grained sediments cover most of the deep-sea floor, with an often well-oxygenated upper sediment horizon (Wenzhöfer and Glud, 2002; Fischer et al., 2009; Wenzhöfer et al., 2016). These sediments are constantly exposed to high pressures, low temperatures between -1 and 4°C and typically low current velocities (Jørgensen and Boetius, 2007; Smith et al., 2008; Danovaro et al., 2014; Corinaldesi, 2015). With the exception of rare chemosynthetic production at cold seeps and hydrothermal vents, the deep-sea floor is characterized by the absence of *in situ* primary production, making this environment extremely energy-limited (Jahnke and Jackson, 1992; Glover and Smith, 2003; Klages et al., 2004; Jørgensen and Boetius, 2007; Smith et al., 2008; Ramirez-Llodra et al., 2010). As the majority of deep-sea benthic communities appear to be heterotrophic (Jørgensen and Boetius, 2007; Smith et al., 2008; Danovaro et al., 2014; Woolley et al., 2016; Snelgrove et al., 2017), they depend on the input of organic material that is either vertically exported from the productive surface ocean or laterally advected, e.g. from adjacent seas or from land (Jahnke and Jackson, 1992; Rowe et al., 1994; Schlüter et al., 2000; Buesseler et al., 2007; Smith et al., 2009; Lalande et al., 2016).

Microorganisms contribute a substantial fraction to benthic biomass and diversity (Rowe et al., 1991; Deming and Yager, 1992; Whitman et al., 1998; Danovaro, 2000; Jørgensen and Boetius, 2007; Danovaro et al., 2009), and several studies showed that bacteria typically dominate oxic deep-sea surface sediments (Jørgensen and Boetius, 2007; Danovaro et al., 2009; Giovannelli et al., 2013). Their cell numbers can be as high as 10^9 cells per gram of sediment (Rowe et al., 1991; Deming and Yager, 1992; Deming and Baross, 1993; Kröncke et al., 1994; Boetius et al., 1996;

Schewe et al., 1998; Schauer et al., 2010; Giovannelli et al., 2013), several orders of magnitude higher than in the overlying water column (Whitman et al., 1998; Glöckner et al., 1999; Eilers et al., 2000; Bano and Hollibaugh, 2002; Herndl et al., 2005; Kirchman et al., 2009), and in a similar range as cell numbers reported for coastal sediments (Llobet-Brossa et al., 1998; Sahm and Berninger, 1998; Ravenschlag et al., 2001). While the abundance and biomass of larger size classes, i.e. meiofauna, macrofauna and megafauna, steeply decline with increasing water depth and reduced input of organic matter to the seafloor (Rowe et al., 1991; Soltwedel, 2000; Rex et al., 2006; Soltwedel et al., 2009; Wei et al., 2010), bacterial standing stocks are less affected and do not show a similarly strong decline (Rowe et al., 1991; Deming and Baross, 1993; Rex et al., 2006). Their relative contribution therefore increases with depth, and they can account for up to 90% of total benthic biomass in abyssal surface sediments (Rowe et al., 1991; Rex et al., 2006; Rex and Etter, 2010).

Deep-sea surface sediments also host a high taxonomic diversity of bacterial communities (Schauer et al., 2010; Zinger et al., 2011; Jacob et al., 2013; Bienhold et al., 2016; Learman et al., 2016; Walsh et al., 2016), that appears to be substantially higher than estimates for the water column (Galand et al., 2009a; Amaral-Zettler et al., 2010; Kirchman et al., 2010; Zinger et al., 2011; Sunagawa et al., 2015). A global survey spanning samples from all major oceans further revealed a distinct core microbiome in deep-sea sediments, composed of a few cosmopolitan types, but also a high degree of endemism (Bienhold et al., 2016). Typically, *Proteobacteria*, affiliated with the classes *Gamma*-, *Alpha*- and *Deltaproteobacteria*, as well as *Actinobacteria* dominate these communities (Bowman and McCuaig, 2003; Schauer et al., 2010; Zinger et al., 2011; Bienhold et al., 2016; Learman et al., 2016; Walsh et al., 2016), and members of these groups seem to be predominant also in the Arctic (Tian et al., 2008; Teske et al., 2011; Bienhold et al., 2012; Jacob et al., 2013; Li et al., 2015). However, for the vast majority of benthic deep-sea bacteria no cultured representatives or genomic information are available, and thus their functional role remains unknown (Siezen and Wilson, 2009). Further, for large areas of the Arctic seafloor, i.e. the Eurasian basin, no information at all is yet available about benthic bacterial community composition.

Several studies reported correlations between sediment organic matter content and benthic bacterial biomass (Rowe et al., 1991; Deming and Yager, 1992; Pfannkuche, 1993; Turley and Dixon, 2002; Kanzog et al., 2008), activity (Turley and Lochte, 1990; Boetius and Lochte, 1994; Poremba and Hoppe, 1995; Boetius et al., 1996; Kanzog et al., 2008; Goffredi and Orphan, 2010; Hoffmann et al., 2017), and more recently also bacterial community structure (Austen et al., 2002; Goffredi and Orphan, 2010; Zinger et al., 2011; Bienhold et al., 2012, 2016; Jacob et al., 2013; Ruff et al., 2014; Learman et al., 2016; Hoffmann et al., 2017). Bacteria dominate the breakdown and recycling of organic matter in deep-sea surface sediments (Turley and Lochte, 1990; Deming and Baross, 1993; Pfannkuche, 1993; Boetius and Damm, 1998; Jørgensen and Boetius, 2007), and thereby play an essential role for carbon cycling, the regeneration of nutrients and the transfer of energy to higher trophic levels (Figure 2) (Nealson, 1997; Jørgensen and Boetius, 2007; Falkowski et al., 2008; Snelgrove et al., 2017). Their activity thus provides an integral ecosystem service, and yet we currently do not understand the underlying processes that deep-sea benthic bacteria use to interact with organic material.

1.2. The biological pump of the Arctic Ocean – Linking surface and the deep ocean

1.2.1. Primary production in the Arctic

Photoautotrophic primary producers that harvest light energy to turn carbon dioxide and water into organic compounds and oxygen constitute the basis of the sympagic, pelagic and benthic food webs. What limits primary production is often the availability of nutrients and light (Popova et al., 2012), which especially constrains productivity in the oligotrophic and perennially ice-covered central basins and limits the length of the productive season in the high Arctic from mid-May to mid-September, with a slightly earlier onset at lower latitudes (Leu et al., 2011).

Photoautotrophic communities in sea ice and in the sunlit water column contribute to annual production and both differ in composition and the timing of their bloom period. While the dominant phytoplankton members belong to the diatom genera *Chaetoceros*, *Thalassiosira*, *Cylindrotheca* and *Fragilariopsis*, dominant sea-ice algae are pennate diatoms of the genera *Navicula, Nitzschia, Entomoneis,*

Fragilariopsis, and the centric genera *Attheya* and *Melosira* (Poulin et al., 2010). *Melosira arctica* is known for building up great biomass in the form of long, filamentous strands, attached to the underside of ice floes, where it can scavenge nutrients directly from the water column (Melnikov and Bondarchuk, 1987; Ambrose et al., 2005; Poulin et al., 2014). Additionally, algae in fresh or brackish melt ponds, which form on top of the ice over the course of summer, can contribute to primary production in ice-covered areas (Figure 2) (Lee et al., 2012; Fernández-Méndez et al., 2014, 2015).

The sea-ice algae community is low-light adapted (Cota, 1985; Hancke et al., 2018), allowing it to start growing in and under the ice in early spring, as soon as the sun returns, despite the low solar angle (Leu et al., 2011). It usually reaches its productivity peak shortly before the onset of the phytoplankton bloom (Leu et al., 2011; van Leeuwe et al., 2018). When a higher solar angle allows more light to penetrate through the sea-ice and snow cover, phytoplankton starts blooming in the sunlit surface ocean and builds up very high biomass (Gosselin et al., 1997), even beneath the sea ice, if the ice is thin and ponded (Arrigo et al., 2012; Arrigo and van Dijken, 2015). Over the course of the season, nutrients become depleted near the surface and the decreased attenuation of light allows the formation of deep chlorophyll maxima at the deeper boundary of the euphotic zone (Hill and Cota, 2005; Martin et al., 2010; Arrigo et al., 2011).

Overall, annual integrated primary production is higher in the shelf seas, i.e. 100 to >1000 g C m^{-2} yr^{-1} in the Barents and Chukchi Seas, than in the central basins, where the lower solar angle, persistent sea-ice cover and strong nutrient limitation due to haline stratification result in average rates of less than 10 g C m^{-2} yr^{-1} (Hill et al., 2013; Tremblay et al., 2015a). The contribution of ice algal production to total production is not yet well understood, as their distribution is highly patchy and difficult to observe (Gosselin et al., 1997; Katlein et al., 2014; Fernández-Méndez et al., 2015). Estimates vary based on region and season, and range from 25% (Legendre et al., 1992) to up to 60% in MYI covered areas of the central Arctic basins (Gosselin et al., 1997; Fernández-Méndez et al., 2015). Despite their smaller annual production, ice algae are of great importance as they bloom asynchronous to phytoplankton and thus prolong the productive season (Leu et al., 2011). Further,

they play an important role for the food web and carbon cycling in the Arctic (Kohlbach et al., 2016), especially in the oligotrophic deep basins where they can contribute up to 90% of total primary production at a local scale (Fernández-Méndez et al., 2014).

1.2.2. Export of organic matter to the deep sea

Newly produced biomass is subject to grazing and heterotrophic activity by microbes in sea ice and the surface ocean, where the gross of the material (75-95%) is remineralized within days to months (Figure 2) (Cho and Azam, 1988; De La Rocha and Passow, 2007; Børsheim and Drinkwater, 2014; Turner, 2015; Moran et al., 2016). Algal cells, detritus and zooplankton fecal pellets constitute the majority of this export flux (Wassmann et al., 2004; De La Rocha and Passow, 2007; Turner, 2015), with carbohydrates, particularly polysaccharides, and proteins as their primary constituents (Pakulski and Benner, 1994; Amon and Benner, 1996; Emerson and Hedges, 2008; Kaiser and Benner, 2009). Grazing pressure, degradation efficiency and the amount of time the organic matter remains in the ice and in the surface ocean eventually control the extent and quality of material that sinks out and is exported to the seafloor (Wassmann, 1997; Olli et al., 2007; Wassmann and Reigstad, 2011; Tamelander et al., 2013). Microbial degradation throughout the water column further attenuates the organic matter flux, and, as a consequence, only 1-3% of surface production reaches the deep-sea floor (~1g C m^{-2} yr^{-1}) (Sakshaug, 2004; Wassmann et al., 2004; De La Rocha and Passow, 2007; Jørgensen and Boetius, 2007). With increasing water depth not only a decrease in total organic matter concentration is observed, but often also a shift from more labile towards more recalcitrant material (Soltwedel et al., 2005). This may be due to extended exposure to degradation processes during its descent (Smith et al., 1992, 2008; Azam, 1998), but may also reflect the gradual accumulation of refractory material through scavenging of other small suspended particles, or through the preferential microbial utilization of labile components (Bergauer et al., 2017). Several other factors can alter the nutritious quality and quantity of the exported material; some of its components sink faster than others, i.e. large and heavy diatom cells sink faster than smaller flagellates (Smetacek, 1985). Aggregation can increase the material's

sinking speed and thus reduce degradation and grazing pressure during its descent (Smetacek, 1985; Alldredge and Gotschalk, 1988; De La Rocha and Passow, 2007; Turner, 2015). Also fecal pellet production can accelerate the vertical flux rate of OM through the water column (Werner, 2000; De La Rocha and Passow, 2007; Turner, 2015), however, intense grazing activity may reduce its nutritious quality and quantity (Fortier et al., 2002; Olli et al., 2007; Turner, 2015). Timing mismatches between producers and consumers in the upper ocean can result in enhanced export rates (Wassmann, 1997; Leu et al., 2011; Ji et al., 2013) and a subsequent deposition of fresh organic carbon at the seafloor (Renaud et al., 2007; Tamelander et al., 2009).

The exported organic matter from the surface ocean constitutes the main source of energy for heterotrophic benthic organisms (Wassmann et al., 2004; Jørgensen and Boetius, 2007) and is a key determinant of benthic community structure and function (Corliss et al., 2009; Bienhold et al., 2012; Giovannelli et al., 2013). In turn, remineralization activity of the benthos releases nutrients to the water column (Jørgensen and Boetius, 2007; Falkowski et al., 2008; Moran et al., 2016) (see also 1.2.3). This coupling of surface and deep ocean, termed (sympagic)-pelagic-benthic coupling, is typically thought to be tighter over the shelves and adjacent seas than over the deep Arctic basins (Ambrose et al., 2005; Dunton et al., 2005; Grebmeier et al., 2006a; Wassmann and Reigstad, 2011; Tamelander et al., 2013). Recent observations of a widespread deposition of ice algae at the deep-sea floor of the Eurasian basin, however, indicate that rapid ice melt can tighten the coupling in the central Arctic Ocean (Boetius et al., 2013). Alterations of organic matter supply could have a profound influence on benthic microbial community structure (Bienhold et al., 2012; Ruff et al., 2014; Hoffmann et al., 2017) and potentially also on its functioning, yet have not been explored for the deep central Arctic basins.

1.2.3. Organic matter remineralization and burial at the seafloor

The activity of heterotrophic bacteria in deep-sea sediments plays a key role for the cycling of carbon and nutrients in the oceans (Jørgensen and Boetius, 2007; Falkowski et al., 2008; Moran et al., 2016). Through their involvement in the breakdown of exported organic matter and the subsequent regeneration of nutrients, they nourish higher trophic levels and replenish the water column with nutrients

needed for primary production (Azam and Malfatti, 2007; Mayor et al., 2012; Buchan et al., 2014). Some of the exported material escapes microbial processing at the seafloor and can thus contribute to long-term burial of carbon in the seabed (Middelburg and Meysman, 2007).

The initial step in bacterial organic matter utilization is often the extracellular hydrolysis of high-molecular-weight (HMW) compounds into smaller units (Arnosti, 2011), as bacteria can only transport sufficiently small substrates (around 600-800 Da) across their cell membranes (Weiss et al., 1991). Due to the often chemically and structurally complex features of organic matter, marine bacteria typically require multiple enzymes, which act synergistically, to attack it (Weiner et al., 2008). These enzymes are either released into the environment or attached to the cell membrane (Hoppe, 1991), and are categorized by the type of material they target. They comprise peptidases, lipases, nucleases or carbohydrate-active enzymes (CAZymes) (Azam and Malfatti, 2007), with the latter including glycoside hydrolases (GH), polysaccharide lyases (PL), carbohydrate esterases (CE) and glycosyltransferases (GT), as well as non-catalytic components, i.e. carbohydrate-binding modules (CBM), which foster substrate accessibility and contact between enzyme and substrate (Weiner et al., 2008; Lombard et al., 2014). Together with membrane-bound transport systems, these enzymes allow bacteria to interact, transform and uptake organic material and nutrients.

GHs, which catalyze the cleavage of glycosidic bonds between carbohydrates or between carbohydrates and non-carbohydrate components, play a central role in polysaccharide decomposition (Weiner et al., 2008; Lombard et al., 2014). Based on their structure and substrate specificities they are classified into GH families (Lombard et al., 2014; Berlemont and Martiny, 2016). Individual GHs typically hydrolyze a limited set of glycosidic bonds, and consequently, a bacterium either requires several GHs of different specificity to fully degrade complex carbohydrates or the degradation is a community effort with consortia of bacteria working in concert (Taylor et al., 2006; Teeling et al., 2012; Mann et al., 2013; Xing et al., 2014; Berlemont and Martiny, 2016). From several studies on bacteria-phytoplankton interactions in the water column we have learned that distinct clades seem to occupy different ecological niches and are specialized in the breakdown of dedicated

substrates (Teeling et al., 2012; Mann et al., 2013; Xing et al., 2014; Barbeyron et al., 2016). This specialization is also reflected in their gene profiles, with different clades showing pronounced differences in their expressed enzymes, binding modules and transport systems (Barbeyron et al., 2016; Teeling et al., 2016).

Members of the *Flavobacteriia* (*Bacteroidetes*), *Gammaproteobacteria* and Roseobacter clade within the class *Alphaproteobacteria* were shown to be most responsive to phytoplankton blooms (Teeling et al., 2012, 2016; Buchan et al., 2014; Klindworth et al., 2014). Changes in organic matter availability, as seen during bloom events, can therefore induce shifts in the bacterial community composition from being dominated by oligotrophic types, with low nutrient and energy requirements, to being dominated by those best adapted to utilize complex and fresh algae material, as well as their beneficiaries that take up already hydrolyzed products (Teeling et al., 2012; Bunse et al., 2016; Landa et al., 2016).

Experimental work on bacterial communities in deep-sea sediments revealed that these can quickly react to and process various types of input material (Turley and Lochte, 1990; Boetius and Lochte, 1994; Hoffmann et al., 2017), and utilize a wider range of potential target substrates than communities in the overlying water column from the same location (Arnosti, 2008; Teske et al., 2011). Also, hydrolysis rates in sediments are higher than in seawater (Hoppe et al., 2002) and were shown to be highest in the sediment surface layer (Meyer-Reil, 1986; Poremba and Hoppe, 1995; Teske et al., 2011), likely due to higher cell numbers and higher availability of fresh organic material (Boetius and Lochte, 1994; Arnosti, 2011).

The remineralization of organic carbon by benthic microbial communities is an important process, which determines nutrient regeneration, carbon turnover and burial on geological timescales (Figure 2). However, our current understanding of the underlying processes in Arctic deep-sea sediment is poor as we lack information on the identity and functional diversity of its dominant community members.

1.3. Climate change impacts on the Arctic Ocean ecosystem

Despite its remote location, the Arctic Ocean is the marine ecosystem most strongly affected by global warming (Vaughan et al., 2013), with temperatures currently warming two to three times faster than the global average (Overland et al., 2015).

Although sea-ice extent and thickness in the Arctic Ocean have varied considerably throughout the geological past of the Arctic, even with episodes of ice-free conditions during especially warm periods (Polyak et al., 2010), the observed rate of ice-loss over the last decades is unprecedented and does not compare to any historical record (Polyak et al., 2010). As a result of atmospheric warming, rising Arctic Ocean temperature is causing a reduction of sea-ice extent (Serreze and Rigor, 2007; Stroeve et al., 2007; Comiso et al., 2008; Comiso and Nishio, 2008; Cavalieri and Parkinson, 2012; Stroeve et al., 2012b), and a decrease in average ice thickness (Giles et al., 2008; Haas et al., 2008; Rothrock et al., 2008; Kwok and Rothrock, 2009; Laxon et al., 2013). The ice-loss is accompanied by a change of its physical properties (Figure 3), as also the average age of the ice is declining and transitioning towards a thinner and younger ice sheet (Kwok, 2007; Maslanik et al., 2007; Tschudi et al., 2016; Perovich et al., 2017). While thick multi-year ice dominated the spring ice cover in the 1980s, it has now been largely replaced by first-year ice (Nghiem et al., 2007; Stroeve et al., 2012b, 2012a), which makes the ice cover more vulnerable to melting during summer (Maslanik et al., 2007; Perovich et al., 2017) and causes a lengthening of the summer melt season (Figure 3), with an earlier onset and later freeze-up (Markus et al., 2009; Stroeve et al., 2014). Also, higher drift speed and increased ice export through Fram Strait have been observed and attributed to the thinning of Arctic sea ice (Rampal et al., 2009; Kwok et al., 2013; Smedsrud et al., 2017), and may also foster more frequent formation of leads and ridges (Assmy et al., 2017). At the same time, first indications for an increased melt pond fraction have been reported (Perovich et al., 2009; Rösel and Kaleschke, 2012), which can strongly reduce surface albedo (Fetterer and Untersteiner, 1998; Perovich et al., 2002; Perovich and Richter-Menge, 2009; Schröder et al., 2014) and increase light penetration into the underlying water column (Arrigo et al., 2012).

Overall, satellite records since 1979 show that the Arctic sea-ice extent in September is currently declining at a rate of about 13% per decade (Meier and Stroeve, 2018) and reached a record low of 3.41 million square kilometers in 2012, 44% below the 1981-2010 average. There is a linear relationship between the observed ice-loss and anthropogenic CO_2 emissions (Notz and Stroeve, 2016), and different model predictions estimate that the remainder of Arctic summer sea ice will be lost before

the middle of this century (Stroeve et al., 2007, 2012b; Snape and Forster, 2014; Notz and Stroeve, 2016), if emissions are not drastically reduced (Mahlstein and Knutti, 2012; Overland et al., 2014; Notz and Stroeve, 2016). However, most models are currently underestimating the rate of decline, and the observed loss is moving faster than the predictions (Winton and Winton, 2011; Mahlstein and Knutti, 2012; Stroeve et al., 2012a; Overland and Wang, 2013; Notz and Stroeve, 2016).

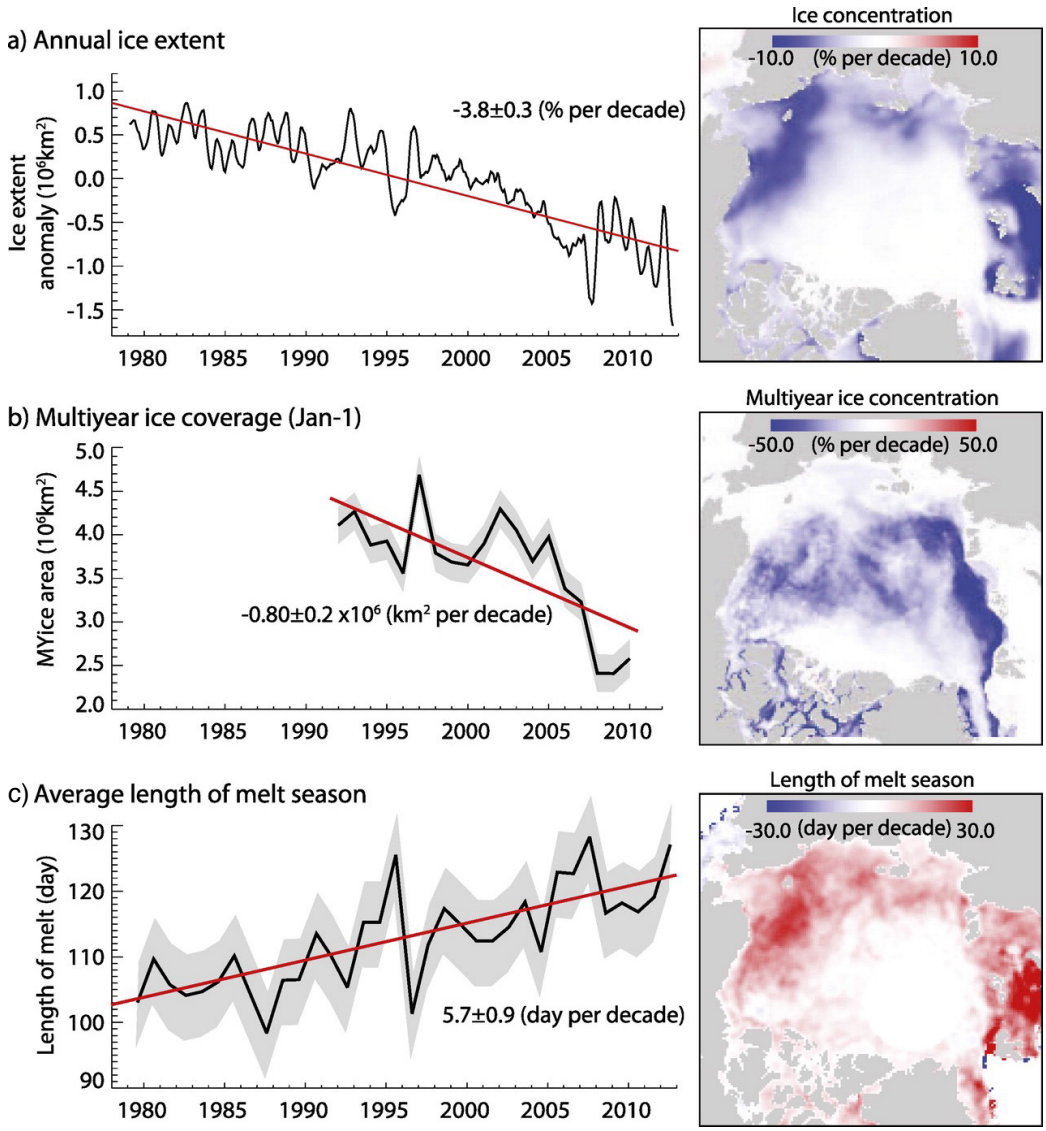


Figure 3 I Summary of linear decadal trends (red lines) and pattern of changes in: (a) Arctic sea ice extent [based on procedures in Comiso and Nishio (2008)]; (b) multi-year sea ice coverage on January 1st (Kwok 2009); gray band shows uncertainty in the retrieval; and (c) length of melt season [updated from Markus et al. (2009)]; gray band shows the basin-wide variability. Modified from Vaughan et al. (2013).

Along with the dramatic reduction in sea-ice cover (Figure 3), the Arctic Ocean is also freshening (McPhee et al., 2009; Rabe et al., 2011; Haine et al., 2015; Carmack

et al., 2016) and warming (McLaughlin et al., 2009; Polyakov et al., 2012; Steele and Dickinson, 2016). Interestingly, the described changes are tightly intertwined and are to a certain degree both cause and effect of each other, resulting in a complicated positive feedback loop termed Arctic amplification (Screen and Simmonds, 2010; Serreze and Barry, 2011; Alexeev and Jackson, 2013; Taylor et al., 2013).

1.4. Microbial responses to changes in the Arctic marine ecosystem

Rapid climate change is already affecting the ecology of Arctic organisms as has been documented mostly for larger sized marine biota, ranging from zooplankton to polar bears (reviewed in Wassmann et al., 2011), and has implications for food web structures and pelagic-benthic coupling (Grebmeier et al., 2006b). Microbial responses to ecosystem changes are not as well understood, but have been reported from different regions of the Arctic Ocean (Vincent, 2010; Wassmann, 2011; Barber et al., 2015).

Along with a change in physicochemical characteristics of the sea-ice cover in the Amerasian basin between the 1970s and the late 1990s, sea-ice algal community composition changed and declined in overall diversity and abundance, likely as a result of ice melt and upper ocean freshening (Melnikov et al., 2002). The disappearance of MYI may have further direct effects on sea-ice algal and bacterial community composition, as recent results underpinned its importance as a habitat for algae (Lange et al., 2017), and as a seed repository for specific pennate diatoms (Olsen et al., 2017), and revealed that the residing bacterial communities are distinct from FYI (Hatam et al., 2016). Recent changes in the duration of the melt season (Stroeve et al., 2014), and extent and thickness of the ice cover (Stroeve et al., 2012b), have prolonged phytoplankton growing season and increased light availability, thus boosting primary productivity in coastal waters of the Arctic (Arrigo et al., 2008, 2012; Pabi et al., 2008; Wassmann et al., 2010; Arrigo and van Dijken, 2011; Tremblay et al., 2011; Petrenko et al., 2013). Increasing water temperature and higher inflow of Atlantic and Pacific water masses (Spielhagen et al., 2011; Polyakov et al., 2012; Korhonen et al., 2013; Rudels et al., 2013) have allowed the intrusion of phytoplankton species characteristic of the North Atlantic and Pacific into the Arctic, e.g. the coccolithophore *Emiliania huxleyi*, the picocyanobacterium

Synechococcus and the diatom *Neodenticula seminae* (Reid et al., 2007; Hegseth and Sundfjord, 2008; Nöthig et al., 2015; Paulsen et al., 2016). For the first time in more than 800,000 years Pacific phytoplankton was able to migrate through the Arctic into the Atlantic Ocean, and more trans-Arctic exchange can be expected if warming continues (Reid et al., 2007; Miettinen et al., 2013).

Strengthening of upper ocean stratification due to increased freshwater input has been most pronounced in the oligotrophic deep basins, and may suppress nutrient supply to the surface (McLaughlin and Carmack, 2010), thus limiting primary production in the water column, despite increased light availability (Tremblay and Gagnon, 2009). A shift in phytoplankton composition has been observed, with an increase in small algae and bacteria, and a concomitant decrease in larger algae, potentially less effective in acquiring nutrients (Li et al., 2009b; Paulsen et al., 2016). Freshening of the ocean after the strong ice melt in 2007 also altered bacterial pelagic community structure in the Canadian Arctic, with a less diverse community after 2007 and a significant decrease of *Bacteroidetes* (Comeau et al., 2011).

All described changes in microbial biodiversity and community structure will likely alter the biological pump and carbon cycling in the Arctic. Indeed, first records of an effect on benthic-pelagic coupling were obtained from long-term observations in Fram Strait, where the continuing increase in water temperature and salinity of the North Atlantic inflow (Walczowski et al., 2017) was accompanied by a concomitant shift in phytoplankton community composition and export flux between 1998 and 2011, from being dominated by diatoms toward a predominance of the prymnesiophyte *Phaeocystis pouchetii* (Nöthig et al., 2015; Soltwedel et al., 2016). Even at the deep-sea floor, anomalies in organic matter supply, which were observed during the warm period from 2005-2007, were reflected in a shift in benthic bacterial community composition and decreased richness (Jacob, 2014; Soltwedel et al., 2016). In 2012, rapid ice melt in the central Arctic released large sub-ice filaments of the diatom *Melosira arctica*, which sank to the seafloor, resulting in a widespread deposition of fresh ice-algal material at 4400 m water depth (Boetius et al., 2013). Elevated rates of oxygen consumption in sediments with algal deposits indicated remineralization by bacteria as a response to elevated carbon flux rates (Boetius et al., 2013).

It therefore seems evident that the observed environmental changes in the Arctic affect microbial communities from the shelves to the deep basins, and from sea ice to the deep-sea floor.

1.5. Missing baseline knowledge on bacterial diversity and function from the central Arctic and the Arctic deep sea

Despite the important contribution of bacteria to total biomass and biodiversity, and their strong involvement in nutrient cycling and energy transfer, relatively little is known about the distribution, identity and function of dominant bacterial taxa in the Arctic.

We lack information on Arctic bacterial community composition and function both on spatial and temporal scales, which is mainly due to the difficult accessibility of the study region in general and the rareness of data collected outside the summer months, not acknowledging the strong seasonality of the system. Few year-round sampling efforts have given first insights into bacterial diversity and function across seasonal cycles in the Amerasian basin and off the coast of Svalbard (Sherr and Sherr, 2003; Sherr et al., 2003; Cottrell and Kirchman, 2009; Kirchman et al., 2010; Wilson et al., 2017).

When talking about spatial scales, this not only includes the lack of bacterial data from specific regions, i.e. the Eurasian basin, but also the paucity of data from specific environments, e.g. melt ponds. Most research on Arctic bacterial communities has focused on the shelf regions and the adjacent seas (i.e. Bienhold et al., 2012; Christman et al., 2011; Comeau et al., 2011; Cottrell and Kirchman, 2009; Galand et al., 2009, 2010; Han et al., 2014; Hatam et al., 2014, 2016; Jacob et al., 2013; Kirchman et al., 2007, 2009, 2010; Li et al., 2009b; Malmstrom et al., 2007; Sala et al., 2010; Suslova et al., 2012; Wilson et al., 2017), and the few available studies from the deep central basins have been centered around the Amerasian basin (i.e. Bano and Hollibaugh, 2000, 2002; Comeau et al., 2011; Ferrari and Hollibaugh, 1999; Galand et al., 2010; Kirchman et al., 2007, 2009; Sherr et al., 2003; Sherr and Sherr, 2003). The majority of research looked into pelagic bacterial communities. Much less is known about the bacterial diversity (Junge et al., 2002; Brinkmeyer et al., 2003; Bowman et al., 2012; Han et al., 2014; Hatam et al., 2014,

2016) and function (Huston et al., 2000) in Arctic sea ice and in deep-sea sediments (Li et al., 2009a, 2015; Bienhold et al., 2012; Jacob et al., 2013). Scarcity of data is greatest for the Eurasian basin of the central Arctic Ocean, where currently only a small number of studies investigated bacterial diversity in snow (Hauptmann et al., 2014), in sea ice and the underlying water column (Bowman et al., 2012), assessed the presence of nitrogen fixers in melt ponds, sea ice and water column (Fernández-Méndez et al., 2016), as well as the abundance of bacterial cells in deep-sea sediments (Kröncke et al., 1994; Schewe et al., 1998). As the two deep basins differ substantially in their water mass properties (McLaughlin and Carmack, 2010; Peralta-Ferriz and Woodgate, 2015; Carmack et al., 2016), more baseline information on bacterial community dynamics in the Eurasian basin are necessary for a comprehensive understanding of microbial ecology in the central Arctic Ocean. Further, a lot of our current understanding of bacterial diversity in these environments is based on results from molecular fingerprinting techniques, clone libraries or targeted gene assays (Bano and Hollibaugh, 2000, 2002, Brinkmeyer et al., 2003, 2004; Christman et al., 2011; Fernández-Méndez et al., 2016), and only over the course of the last years, the application of high-throughput sequencing allowed a deeper look into the alleged diversity and richness of bacterial life in the Arctic Ocean (Galand et al., 2009a, 2010; Kirchman et al., 2010; Comeau et al., 2011; Bienhold et al., 2012; Ghiglione et al., 2012; Jacob et al., 2013; Han et al., 2014; Hatam et al., 2014; Li et al., 2015; Hatam et al., 2016). As the identity of many bacterial groups remained elusive, and as the current knowledge of their presence is often based on snapshots from one distinct Arctic region, the loss or gain of species, as well as shifts in their distribution are difficult to detect.

There is growing evidence that bacterial diversity in the Arctic is just as tightly interlinked with phytoplankton bloom patterns and organic matter availability (Bienhold et al., 2012; Jacob, 2014; Wilson et al., 2017), as has been described for temperate oceans (Pinhassi et al., 2004; Teeling et al., 2012, 2016; Lima-Mendez et al., 2015; Sunagawa et al., 2015; Bunse et al., 2016). However, we currently do not understand whether this is due to specific interactions or associations between individual bacteria and phytoplankton groups. Given the strong changes in sea-ice and phytoplankton community composition and bloom dynamics in the Arctic

(Melnikov et al., 2002; Arrigo et al., 2008, 2012; Tremblay et al., 2011; Assmy et al., 2017), it becomes increasingly important to look deeper into bacteria-algae interactions and the potential effects of environmental changes on the biological pump and bacterial diversity and function. First indications for a propagation of biological climate change effects from the surface to the ocean interior and the benthos have been observed (Grebmeier et al., 2006b; Tamelander et al., 2009; Boetius et al., 2013; Soltwedel et al., 2016). The dramatic changes of the physical and chemical environment, but also in the biology of the Arctic ecosystem have led to the emergence of the term “New Arctic” (Jeffries et al., 2013; Wood et al., 2013). A comprehensive understanding of the present-day microbial biodiversity across different Arctic environments, the ubiquity or uniqueness of communities, and their individual roles in carbon and nutrient cycling will contribute important historic records from which change can be tracked and implications for ecosystem functioning can be deduced.

1.6. Molecular tools to study microbial community composition and function

Only a small fraction of viable marine microorganisms can be cultured under standard laboratory conditions, a phenomenon Staley and Konopka termed the “great plate count anomaly” in 1985 (Staley and Konopka, 1985), and thus, for many years the description of microbial communities was constrained by cultivation techniques (Eilers et al., 2000; Rappé and Giovannoni, 2003). Over the last decades, the development of a new molecular toolbox to assess microbial diversity has revolutionized the study of microbial communities (Lane et al., 1985; Amann et al., 1995; DeLong and Pace, 2001). Particularly the establishment of the small subunit ribosomal RNA (rRNA) gene as a molecular marker (Woese and Fox, 1977; Woese et al., 1990; Pace et al., 2012), and the advances in cost-effective sequencing technologies and computational means for data processing have greatly enhanced our means to understand the taxonomic composition of natural communities. Since then, high-throughput sequencing studies have given insights into the immense microbial diversity in the environment, and started to reveal the genomic composition and functional potential of whole microbial populations (Lane et

al., 1985; Pace, 1997, 2009; Hugenholtz, 2002; Rappé and Giovannoni, 2003; Karl, 2007; Morey et al., 2013; Reuter et al., 2015).

1.6.1.1. Amplicon sequencing of 16S and 18S ribosomal RNA

Today, targeted sequencing of specific genomic regions of interest, so called amplicon sequencing, has established itself as the most widely used method for exploring microbial diversity in environmental samples. Specifically, certain regions of the 16S or 18S rRNA genes are used for assessing either bacterial and archaeal, or eukaryotic diversity, respectively. These gene regions display properties that make them taxonomically and phylogenetically informative markers (Woese and Fox, 1977; Lane et al., 1985; Hugenholtz and Pace, 1996; Pace, 2009), namely ubiquity, extreme sequence conservation, and the presence of hypervariable regions (Tringe and Hugenholtz, 2008). After the extraction of DNA from all cells in a given sample, the genes or gene regions of interest are targeted using matching primer sequences, and amplified. The products of the amplification steps are then sequenced, and subsequent sequence processing and comparison to reference databases allow inferring community composition and structure of the input sample. Amplicon sequencing has been used to investigate the microbial biodiversity across a wide range of environments (e.g. Bik et al., 2012; Kopf et al., 2015; Lozupone and Knight, 2007; Pace, 1997; Rappé and Giovannoni, 2003), where it continues to reveal the often underexplored and underestimated richness and diversity of microbial taxa (Mikucki and Priscu, 2007; Galand et al., 2009b; Cowie et al., 2011; Marteinsson et al., 2013; Hauptmann et al., 2014; Walsh et al., 2016; Mestre et al., 2018), co-occurrence networks of organisms (e.g. Lima-Mendez et al., 2015; Milici et al., 2016), or spatial and temporal distribution patterns of microbial populations (Galand et al., 2010; Freitas et al., 2012; Jacob et al., 2013; Bienhold et al., 2016; Bunse et al., 2016; Salazar et al., 2016; Wilson et al., 2017).

However, amplicon sequencing also has several limitations and shortcomings, most evident in several technical biases related to the amplification and sequencing steps (Hong et al., 2009; Schloss et al., 2011; Wylie et al., 2012), as well as to the choice of the gene region (Liu et al., 2008; Schloss, 2010), all of which can alter the recovered biodiversity. Also, the method can only be applied to organisms, for which

a dedicated marker gene is known, but may neglect highly divergent or novel community members that cannot be targeted by the current primer sequences. Varying SSU rRNA gene copy numbers in different microbial genomes (Prokopowich et al., 2003; Zhu et al., 2005; Perisin et al., 2016), as well as the horizontal transfer of genes between taxa (Acinas et al., 2004) may further obscure the true community composition in a sample. Despite these drawbacks, amplicon sequencing of the SSU rRNA gene provides a cost- and time-efficient mean to study the diversity, community structure, and identity of individual members in large microbial datasets.

1.6.1.2. Meta'omic sequencing

Since amplicon sequencing does not allow inference about the biological functions of microbial communities in the environment, soon after so-called shotgun sequencing technologies emerged. Here, the entire genetic content of a community is randomly sequenced, and thereby returns both information on the taxonomic composition, but also on the functional potential encoded by its community members. Handelsman and colleagues proposed to coin the product "metagenome", as it consists of the collective genomes of all microorganisms in a given environment (Handelsman et al., 1998).

To go beyond functional prediction, the extraction of total community RNA, the so-called "metatranscriptome", allows insights on the expressed transcripts, and thus, provides information about active community members and actively used functions.

For shotgun sequencing, the extracted DNA or RNA is typically sheared into small fragments prior to sequencing, and the resulting sequence reads then need to be assembled into consensus sequences during downstream sequence processing. Doing so, it is sometimes possible to fully reconstruct genomes of individual community members (Tyson et al., 2004; Hugenholtz and Tyson, 2008). Despite its merits, the downstream processing and analysis of meta'omic sequencing also poses many new computational and analytical challenges, which rise depending on the complexity of the sampled microbial community. The more complex the community, the more sequences are required to ensure sufficient coverage of the genomic information during subsequent analyses or assembly. For example, it is often not possible to link function to identity, when genetic content was fragmented

and cannot be merged due to missing overlap. Also, conserved genetic regions from closely related, but distinct organisms may be falsely assembled into consensus sequences.

Nevertheless, the combined application of sequencing technologies has allowed unprecedented insights into the diversity, ecology and metabolism of multiple microbiomes of varying complexity (Tringe et al., 2005; Sharpton, 2014), including the human's (Qin et al., 2010; Human Microbiome Project Consortium, 2012; Methé et al., 2012; Integrative HMP (iHMP) Research Network Consortium, 2014), Earth's (Gilbert et al., 2014), and the global oceans' microbiomes (Venter et al., 2004; Nealson and Venter, 2007; Karsenti et al., 2011; Lima-Mendez et al., 2015; Sunagawa et al., 2015; Carradec et al., 2018).

2 OBJECTIVES of this thesis

The work presented in this thesis contributes to the ABYSS project - Assessment of bacterial life and matter cycling in deep-sea surface sediments, funded through an Advanced Investigator Grant (294757) of the European Research Council to Prof. Dr. Antje Boetius. The ABYSS project aimed to develop a systematic understanding of abyssal sediment bacterial community distribution, diversity, function and interactions.

The overall topic of this thesis was the taxonomic and functional diversity of bacterial communities across different environments in the Arctic Ocean, and the association and interactions of these communities with organic material. My aims were

I) to improve the current knowledge of microbial community diversity and connectivity in different Arctic environments (Chapter I & II)

II) to identify bacterial groups, which associate with freshly formed, sinking and deposited particulate organic matter (Chapter II & III)

and **III)** to analyze key functional genes, which deep-sea benthic bacteria use in organic matter degradation (Chapter III)

Which should later answer:

How does bacterial diversity vary across different Arctic environments and what are potential effects of environmental changes on the composition and distribution of bacterial communities?

Which bacteria associate with organic matter and what role does organic matter export play in connecting microbial communities across Arctic environments from surface to bottom?

What is the functional potential of benthic microbial assemblages to process organic matter in Arctic deep-sea sediments, and how does it compare with other marine environments? What is the taxonomic and functional variation of these assemblages across regional scales?

Together, the results from this thesis will help to develop a better understanding of how sea-ice melt and biological transport processes affect bacterial diversity in the Arctic from the surface ocean to the deep basins, and which types of deep-sea benthic bacteria are relevant in the remineralization of organic material.

Specific objectives

Building a knowledge base for Arctic Ocean microbial community data

The rapid retreat of sea ice has raised many questions as to the ecological consequences on primary productivity, the biological carbon pump and the composition of microbial communities. Yet, little is known about the diversity and function of microbial communities in different sea-ice habitats. A knowledge base, summarizing the available information on microbial ecology in frozen environments, is needed in order to assess the effect of sea-ice melt on microbial community composition and function in the future. In **chapter Ia** we therefore synthesized knowledge on sea-ice microbial communities as well as those from other compartments of the global cryosphere, and discussed community composition and their involvement in the key biogeochemical functions in these habitats, with a focus on bacteria.

Develop standardized metadata requirements to sustainably label and archive sequence data from cryosphere communities

The reporting of comprehensive metadata is essential for the interpretation of ecological patterns in microbial sequence data, as well as for the comparability and integration of different datasets. The high heterogeneity and patchiness of sea-ice microbial communities, as well as the multitude of different ice environments makes reliable metadata essential for data analysis. Within the framework of the Minimum Information about any (x) Sequence (MIxS) standard (Yilmaz et al., 2011b) established by the Genomic Standards Consortium (GSC, http://gensc.org; (Yilmaz et al., 2011a)), I developed an extension package for the submission of microbial sequences from the cryosphere, presented in **chapter Ib**. Compliance with the proposed Cryo-MixS standard extension should improve the quality and usability of submitted genomic, metagenomic and amplicon data in public repositories.

Assess microbial community turnover associated with the export of algae material from sea ice to the seafloor

To date, knowledge of the bacterial community composition in the central Arctic Ocean is scarce. The few existing studies report results from different regions, distinct sampling times and varying target environments, obscuring comprehensive insights into microbial community connectivity across habitat boundaries. In **chapter II**, I explored the diversity and community structure of bacteria and microbial eukaryotes from a wide range of habitats, including algal aggregates and their bacterial assemblages from sea-ice and melt-ponds, deposited algal detritus, seawater and deep-sea sediment communities. Using next-generation tag sequencing, I identified dominant community members, and assessed community turnover between environments associated with the export of algae from sea ice to the seafloor. Results from this study should give insights into potential effects of rapid sea-ice melt and subsequent particle export on benthic bacterial diversity in the future.

Investigate benthic bacterial community diversity and functions involved in organic matter processing

A major objective of this thesis was to investigate how deep-sea benthic bacterial communities degrade and interact with organic material. Using a combination of tag sequencing, metagenomic and -transcriptomic sequencing in **chapter III**, I elucidated total and active community composition, and explored the dominant functions involved in carbohydrate and protein degradation, as well as in substrate uptake. This study therefore provides a first look into the functional genomic profiles of benthic bacterial communities along a bathymetric transect in the Arctic Ocean, and generates new hypotheses on potential mechanisms of niche partitioning and the identity of key players involved in degradation. Understanding the underlying genomic features required for organic matter processing will be crucial to predict potential effects of climate change on carbon and nutrient cycling in the Arctic deep sea.

3 CONTRIBUTION to manuscripts

Chapter Ia – Full manuscript published in *Nature Reviews Microbiology* 2015

Microbial ecology of the cryosphere: sea ice and glacial habitats

doi:10.1038/nrmicro3522

Antje Boetius, Alexandre M Anesio, Jody W Deming, Jill Mikucki & Josephine Z Rapp

AB developed the review with input from all authors. All authors contributed to the collection of published data. JR synthesized the data, and prepared the figures and tables, with input from all co-authors. AB wrote the manuscript together with all authors.

Contribution of the candidate in % of the total work load

Experimental concept and design: 10%

Acquisition of published data and literature review: ca. 20%

Data analysis and interpretation: ca. 20%

Preparation of figures and tables: ca. 80%

Drafting of manuscript: ca. 20%

Chapter Ib – Full manuscript in preparation

MIxS-Cryo: Defining a minimum information standard for sequence data from the cryosphere

Josephine Z Rapp, Kai Blumberg & Marianne Jacob & Pier Luigi Buttigieg

JR, KB, MJ and PB designed the study. JR started the collection of metadata requirements; KB and PB implemented the addition into ENVO. JR and PB drafted the manuscript.

Contribution of the candidate in % of the total work load

Experimental concept and design: 30%

Acquisition of experimental data: not applicable

Data analysis and interpretation: not applicable

Preparation of figures and tables: 50%

Drafting of manuscript: 60%

Chapter II – Full manuscript published in *Frontiers in Microbiology* 2018

Effects of ice-algal aggregate export on the connectivity of bacterial communities in the central Arctic Ocean

Josephine Z Rapp, Mar Fernández-Méndez, Christina Bienhold & Antje Boetius

JR and MF-M designed the study, with contributions by CB and AB. MF-M, CB and AB collected the samples. JR performed laboratory molecular work and the bioinformatic analyses. JR and CB performed the statistical analysis. JR interpreted the data and all authors contributed to the discussion of the results. JR wrote the manuscript, with support and input from all-coauthors.

Contribution of the candidate in % of the total work load

Experimental concept and design: ca. 60%

Acquisition of experimental data: ca. 80%

Data analysis and interpretation: ca. 70%

Preparation of figures and tables: 100%

Drafting of manuscript: ca. 60%

Chapter III – Full manuscript in preparation

Deep-sea benthic bacteria form diverse, spatially variable communities with stable heterotrophic genome traits in the Arctic (1,500-5,500 m water depth)

Josephine Z Rapp, Pier Luigi Buttigieg, Christiane Hasemann, Pierre Offre, Halina E Tegetmeyer, Antje Boetius & Christina Bienhold

JR, CB and AB designed the study. JR, CH and CB collected the samples. CH performed the on-board analysis of biogenic sediment compounds. JR extracted DNA and RNA, and performed all subsequent laboratory analyses. HT performed all sequencing. JR conducted the data processing and bioinformatic analysis. JR

interpreted the data, with input from PL, PO and CB. JR prepared figures and tables. JR and CB drafted the manuscript with contributions from all co-authors.

Contribution of the candidate in % of the total work load

Experimental concept and design: ca. 70%

Experimental work/acquisition of experimental data: ca. 80%

Data analysis and interpretation: ca. 65%

Preparation of figures and tables: 100%

Drafting of manuscript: ca. 50%

3.1. Additional contributions

Expanding the world of marine bacterial and archaeal clades

Published in *Frontiers in Microbiology* **6**: 1524, January 2016,

DOI: https://doi.org/10.3389/fmicb.2015.01524

Pelin Yilmaz, Pablo Yarza, Josephine Z Rapp & Frank O Glöckner

Contribution: Reconstruction and curation of phylogenetic trees. Assignment and annotation of uncultivated clades. Preparation of figures.

Diazotroph diversity in the sea ice, melt ponds and surface waters of the Eurasian Basin of the Central Arctic Ocean

Published in *Frontiers in Microbiology* **7**: 1884, November 2016,

DOI: https://doi.org/10.3389/fmicb.2016.01884

Mar Fernández-Méndez, Kendra A Turk-Kubo, Josephine Z Rapp, Pier L Buttigieg, Thomas Krumpen, Jonathan P Zehr & Antje Boetius

Contribution: DNA extraction, Automated Ribosomal Intergenic Spacer Analysis (ARISA) and data analysis. Phylogenetic and statistical analysis.

Diversity and metabolism of the JTB255 clade (*Gammaproteobacteria*), a global member of deep-sea sediment communities

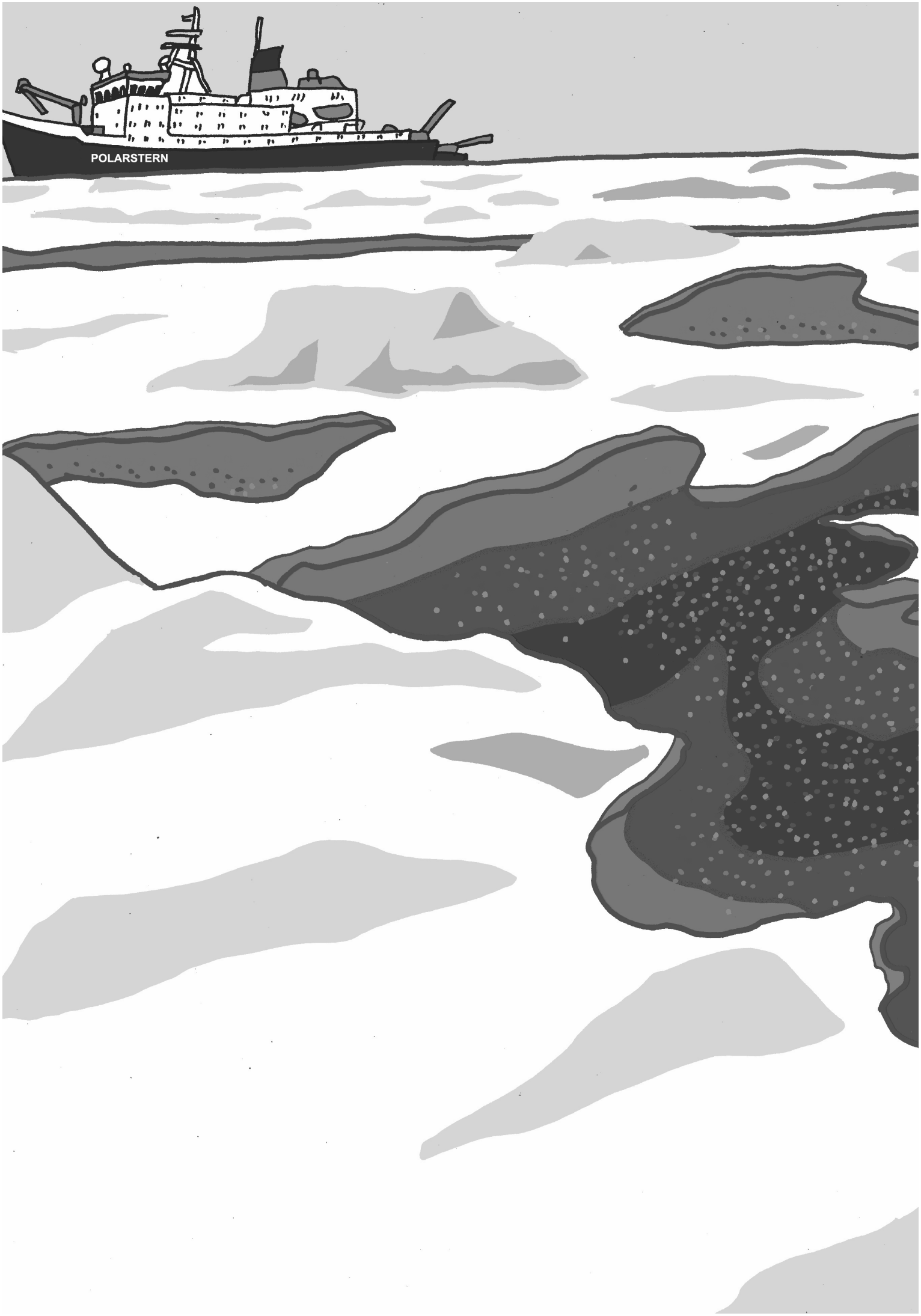
Manuscript in preparation for submission to *The ISME Journal*

Katy Hoffmann, Christina Bienhold, Pier L Buttigieg, Katrin Knittel, Rafael Laso-Pérez, Josephine Z Rapp, Antje Boetius & Pierre Offre

Contribution: Mining of 16S rRNA data for occurrence of JTB255. DNA extraction, metagenomic data processing, binning of target JTB255 contigs and quality control. Reconstruction and extraction of 16S and 23S rRNA genes from metagenomes for phylogenetic tree calculation.

More information on the additional contributions is provided in the **Appendix**.

4 THESIS CHAPTERS


POLARSTERN

Microbial ecology of the cryosphere: sea ice and glacial habitats

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Published in *Nature Reviews Microbiology* 13, 677–690 (2015)

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REVIEWS

Microbial ecology of the cryosphere: sea ice and glacial habitats

Antje Boetius[1,2], Alexandre M. Anesio[3], Jody W. Deming[4], Jill A. Mikucki[5] and Josephine Z. Rapp[1,2]

Abstract | The Earth's cryosphere comprises those regions that are cold enough for water to turn into ice. Recent findings show that the icy realms of polar oceans, glaciers and ice sheets are inhabited by microorganisms of all three domains of life, and that temperatures below 0 °C are an integral force in the diversification of microbial life. Cold-adapted microorganisms maintain key ecological functions in icy habitats: where sunlight penetrates the ice, photoautotrophy is the basis for complex food webs, whereas in dark subglacial habitats, chemoautotrophy reigns. This Review summarizes current knowledge of the microbial ecology of frozen waters, including the diversity of niches, the composition of microbial communities at these sites and their biogeochemical activities.

MIxS-Cryo: Defining a minimum information standard for sequence data from the cryosphere

Josephine Z. Rapp[1,2], Kai Blumberg[2], Marianne Jacob[1,2] & Pier Luigi Buttigieg[1,2]

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Manuscript in preparation

Standardizing sequence metadata for cryospheric investigations

The cryosphere comprises all environments on Earth where low temperatures result in a substantial fraction of water freezing into its solid form for at least one month per year (Fountain et al., 2012; Vaughan et al., 2013). This immense and multifaceted planetary system includes sea ice, ice sheets, glaciers, ice caps, ice on rivers and lakes, as well as snow and frozen earth (e.g. permafrost). The dynamic cryosphere thus covers a large fraction of Earth's surface, both on land and on sea (Vaughan et al., 2013). Far from being bleak and inactive, the cryosphere plays a vital role in regulating the planet's climate system by affecting both its physical environment, (e.g. by altering albedo and heat exchange) and its biology (e.g. by modulating primary production in and under the sea ice) (Nicolaus et al., 2012; Arrigo, 2014). While some of components of the cryosphere can persist for years, or even millennia (Yan et al., 2017; Kehrl et al., 2018), others are short-lived, seasonal features resulting in substantial variation in extent and volume over the course of a year. However, as frozen environments are particularly sensitive to temperature fluctuations, rising global temperatures have resulted in an overall decline in all of its components over the last few decades (Fountain et al., 2012; Vaughan et al., 2013; NSIDC[1]).

Despite their solid and often barren appearance, frozen environments can harbor diverse forms of life, as they are often permeated by a porous matrix of liquid inclusions. Current estimates suggest up to 10^{28} bacterial cells reside in Earth's frozen environments, a large fraction of which remains uncharacterized (e.g. Deming, 2010). Previous work applying sequencing technologies and accompanying multi-omic techniques revealed microbial communities with distinct structures occupying a wide range of ecological niches in different cryospheric environments (reviewed in Boetius et al., 2015). In the coming decade, omics-based approaches are likely to expand our understanding of biosphere-cryosphere interactions in the Earth's rapidly changing frozen ecosystems. Such investigations are particularly

[1]National Snow and Ice Data Center, University of Colorado, Boulder, USA, https://nsidc.org/cryosphere/allaboutcryosphere.html

urgent, as global warming rapidly melts and thaws away this chapter of our natural heritage.

Naturally, the high heterogeneity of the cryosphere renders the reporting of reliable and comprehensive metadata essential for the interpretation of ecological patterns in microbial sequence data, as well as for the comparability and integration of different datasets. Thus, within the framework of the Minimum Information about any (x) Sequence (MIxS) standard (Yilmaz et al., 2011b) established by the Genomic Standards Consortium (GSC, http://gensc.org), we have created MIxS-Cryo: an environmental extension to promote interoperability of cyrospheric 'omics data sets. Compliance with the proposed MIxS-Cryo standard extension will improve the quality and usability of submitted (meta)genomic, (meta)transcriptomic, and amplicon data in public repositories, and will enable scientists to mine data for community responses to changing environmental conditions in the rapidly changing cryosphere.

As with previous extensions (Glass et al., 2014) , the MixS-Cryo package is being developed in an interdisciplinary community effort, soliciting input from microbial ecologists, sea-ice physicists and other disciplines. The gathering of metadata terms was initialized at the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research and is now being opened to engage with other members of the GSC community, the GSC's MIxS developers, and researchers from polar sciences. In its current state, the extension includes 17 fields, which will be extended and defined in a community review (Table 1).

To advance the capacities of this MIxS extension and better align it to the FAIR principles (Wilkinson et al., 2016), we have substantially extended the cryospheric content of the Environment Ontology (EnvO) (Buttigieg et al., 2013b, 2016), collaboratively rehousing content from previously active ontologies (e.g. Duerr et al., 2015) and adding new content based on user needs. ENVO terms (more accurately, classes) connect MIxS-compliant records to a machine-readable and semantically consistent knowledge representation which links data and information resources across a multitude of domains (see Fig. 1 for an example). In a cryosphere-

dedicated release of the ontology (*PolarExpress*[2]), we established a basis for the progress of semantics working groups to be captured in this resource and thus become available to the users of the MIxS-cryo extension. As community review of our extension proceeds, we aim to link every descriptor (Table 1) to an ENVO class describing the parameter, rendering MIxS-cryo the first fully machine actionable component of the MIxS family of standards.

As with all MIxS extensions, development of MIxS-cryo is open, and a core working group will serve as custodians of the resource to ensure currency in the rapidly developing domain of cryospheric research. We invite all researchers in the domain to participate and join the development of this much-needed omic standard to help secure a FAIR future of multi-omic data sets from the planet's frozen reaches.

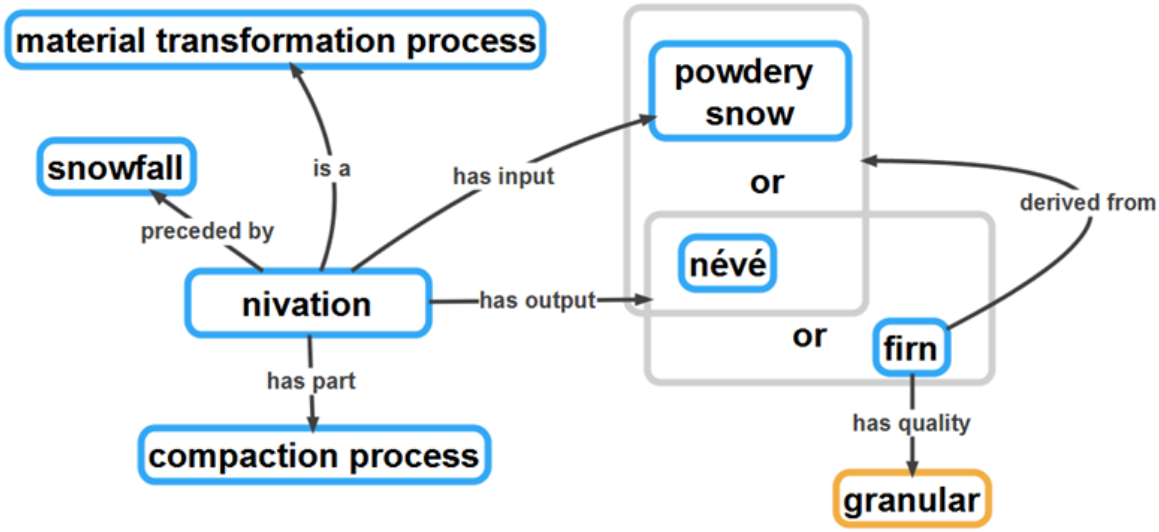


Figure 1 - Exemplary knowledge representation of cryosphere components and processes in the Environment Ontology (ENVO). ENVO is a semantically controlled, machine-readable representation of environmental processes. It represents knowledge as a graph where classes (~ terms) are defined by their relation to one another and to higher level, formal ontologies. Relations are controlled logical axioms which machine agents may reason over to ensure consistency, discover new resources, and use to perform inference. MIxS-cryo avails of a dedicated extension to the ENVO, which disambiguates and interlinks cryospheric entities such as nivation processes, during which snow is compacted into névé or firn, which possess structural qualities like granularity (defined in interoperable ontologies of physical qualities). With MIxS-cryo fields linked to web-resolvable ontology terms, it will be the first extension which machine agents can discover and "understand" through an

[2]https://github.com/EnvironmentOntology/envo/releases/tag/v2017-04-15

online knowledge graph, making the metadata it contains decidedly Findable Accessible Interoperable and Reusable (FAIR).

Table 1 | Proposed MIxS-cryo metadata package terms. This list will be reviewed and extended in a community effort.

Ice age	Freeboard
Sea ice concentration	Melt pond coverage
Sea ice drift	Temperature ice core
Ice extent	Temperature ice section
Ice floe history	Temperature underlying seawater
Ice horizon	Salinity Bulk
Ice permeability	Salinity Brine
Ice thickness	Salinity underlying water
Sea ice volume	

References

Arrigo, K. R. (2014). Sea ice ecosystems. *Ann. Rev. Mar. Sci.* 6, 439–467. doi:10.1146/annurev-marine-010213-135103.

Boetius, A., Anesio, A. M., Deming, J. W., Mikucki, J., and Rapp, J. Z. (2015). Microbial ecology of the cryosphere : sea ice and glacial habitats. *Nat. Rev. Microbiol.* 13, 677–690. doi:10.1038/nrmicro3522.

Buttigieg, P. L., Pafilis, E., Lewis, S. E., Schildhauer, M. P., Walls, R. L., and Mungall, C. J. (2016). The environment ontology in 2016: bridging domains with increased scope, semantic density, and interoperation. *J. Biomed. Semantics* 7, 57. doi:10.1186/s13326-016-0097-6.

Buttigieg, P., Morrison, N., Smith, B., Mungall, C. J., and Lewis, S. E. (2013). The environment ontology: contextualising biological and biomedical entities. *J. Biomed. Semantics* 4, 43. doi:10.1186/2041-1480-4-43.

Deming, J. W. (2010). "Sea ice bacteria and viruses" in *Sea Ice - An Introduction to its Physics, Chemistry, Biology and Geology*, eds. D.N. Thomas and G. S. Dieckmann (Oxford: Blackwell Science Ltd), 247–282.

Duerr, R. E., McCusker, J., Parsons, M. A., Khalsa, S. S., Pulsifer, P. L., Thompson, C., et al. (2015). Formalizing the semantics of sea ice. *Earth Sci. Informatics* 8, 51–62. doi:10.1007/s12145-014-0177-z.

Fountain, A. G., Campbell, J. L., Schuur, E. A. G., Stammerjohn, S. E., Williams, M. W., and Ducklow, H. W. (2012). The disappearing cryosphere: Impacts and ecosystem responses to rapid cryosphere loss. *Bioscience* 62, 405–415. doi:10.1525/bio.2012.62.4.11.

Glass, E. M., Dribinsky, Y., Yilmaz, P., Levin, H., Van Pelt, R., Wendel, D., et al. (2014). MIxS-BE: a MIxS extension defining a minimum information standard for sequence data from the built environment. *ISME J.* 8, 1–3. doi:10.1038/ismej.2013.176.

Kehrl, L., Conway, H., Holschuh, N., Campbell, S., Kurbatov, A. V., and Spaulding, N. E. (2018). Evaluating the duration and continuity of potential climate records from the Allan Hills Blue Ice area, East Antarctica. *Geophys. Res. Lett.* 45, 4096–4104. doi:10.1029/2018GL077511.

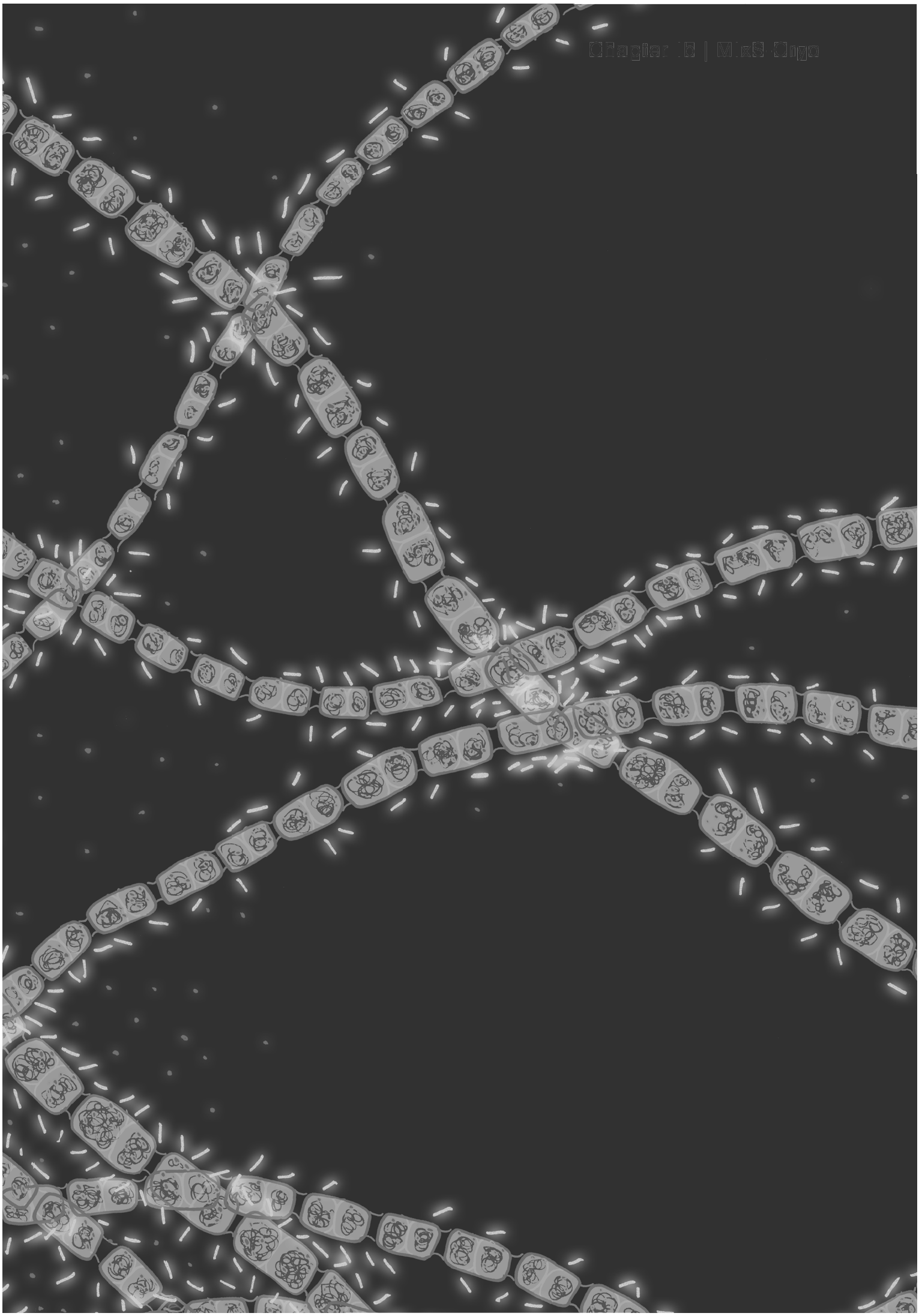
Nicolaus, M., Katlein, C., Maslanik, J., and Hendricks, S. (2012). Changes in Arctic sea ice result in increasing light transmittance and absorption. *Geophys. Res. Lett.* 39, L24501. doi:10.1029/2012GL053738.

Vaughan, D. ., Comiso, J. C., Allison, I., Carrasco, J., Kaser, G., Kwok, R., et al. (2013). Observations: Cryosphere, eds. T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, et al. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Wilkinson, M. D., Dumontier, M., Aalbersberg, Ij. J., Appleton, G., Axton, M., Baak, A., et al. (2016). The FAIR Guiding Principles for scientific data management and stewardship. *Sci. Data* 3, 160018. doi:10.1038/sdata.2016.18.

Yan, Y., Ng, J., Higgins, J., Kurbatov, A., Clifford, H., Spaulding, N., et al. (2017).

2.7-Million-year-old ice from Allan Hills Blue Ice areas, East Antarctica reveals climate snapshots since early Pleistocene. in *Goldschmidt Abstracts*, 4359. Available at: https://goldschmidtabstracts.info/abstracts/abstractView?id=2017004920 [Accessed June 8, 2018].



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Effects of Ice-Algal Aggregate Export on the Connectivity of Bacterial Communities in the Central Arctic Ocean

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In summer 2012, Arctic sea ice declined to a record minimum and, as a consequence of the melting, large amounts of aggregated ice-algae sank to the seafloor at more than 4,000 m depth. In this study, we assessed the composition, turnover and connectivity of bacterial and microbial eukaryotic communities across Arctic habitats from sea ice, algal aggregates and surface waters to the seafloor. Eukaryotic communities were dominated by diatoms, dinoflagellates and other alveolates in all samples, and showed highest richness and diversity in sea-ice habitats (~400–500 OTUs). *Flavobacteriia* and *Gammaproteobacteria* were the predominant bacterial classes across all investigated Arctic habitats. Bacterial community richness and diversity peaked in deep-sea samples (~1,700 OTUs). Algal aggregate-associated bacterial communities were mainly recruited from the sea-ice community, and were transported to the seafloor with the sinking ice algae. The algal deposits at the seafloor had a unique community structure, with some shared sequences with both the original sea-ice community (22% OTU overlap), as well as with the deep-sea sediment community (17% OTU overlap). We conclude that ice-algal aggregate export does not only affect carbon export from the surface to the seafloor, but may change microbial community composition in central Arctic habitats with potential effects for benthic ecosystem functioning in the future.

Keywords: sea-ice algae, deep-sea sediment, Illumina tag sequencing, microbial eukaryotes, sea-ice decline, microbial ecology, bacterial diversity

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INTRODUCTION

The Arctic Ocean is one of the marine regions most strongly affected by global climate change, with temperatures currently warming two to three times faster than the global average (Overland et al., 2015). As a result, thick multi-year sea ice is being replaced by thinner first-year sea ice, which only lasts for one melt season (Maslanik et al., 2007; Polyakov et al., 2012). An Arctic Ocean free of summer sea ice has been projected before the end of this century (Johannessen et al., 2004; Overland and Wang, 2013; Notz and Stroeve, 2016). First indications for planktonic community changes associated with Arctic change have been described, including a trend toward reduced eukaryote cell sizes with upper ocean freshening (Li et al., 2009) and shifts in species

composition associated with increasing water temperature (Nöthig et al., 2015). Sea-ice decline is likely to increase primary production on the Arctic shelves (Carmack and Chapman, 2003; Arrigo et al., 2008), in regions where enough nutrients are supplied (Tremblay and Gagnon, 2009; Arrigo and van Dijken, 2015; Tremblay et al., 2015a). Increasing light availability in and under thinning ice may also enhance ice-algal productivity and under-ice blooms in the deep ice-covered central basins, which have a lower nutrient availability than the shelves (Lalande et al., 2014; Arrigo and van Dijken, 2015; Fernández-Méndez et al., 2015). Furthermore, the extent of melt ponds in summer is increasing (Rösel and Kaleschke, 2012), opening up new habitats for sea-ice biota (Lee et al., 2011). Recent studies have suggested an important, previously underestimated, role of sea-ice algae in primary production and export flux in the central Arctic Ocean (Assmy et al., 2013; Boetius et al., 2013; Fernández-Méndez et al., 2015). Especially diatoms can form extensive blooms in sea ice, melt ponds and at the bottom of ice floes, and constitute the majority of sea-ice associated biomass (Arrigo, 2014 and references therein). Particularly *Melosira arctica*, which forms long filaments attached to the ice matrix, can build up patchy, but dense accumulations (Melnikov and Bondarchuk, 1987; Fernández-Méndez et al., 2014; Katlein et al., 2014; Poulin et al., 2014). During rapid ice melt, large pulses of these ice algae can be released from the ice (Tamelander et al., 2009) and sink out of the surface ocean, thereby significantly altering the magnitude and composition of organic matter reaching the seafloor (Ambrose et al., 2005; Boetius et al., 2013). Yet, the low number of documented observations of such large ice algae export events and the relatively poor knowledge of spatial and temporal variability of microbial community composition in the central Arctic currently impede predictions about the effects of such environmental changes on biodiversity and ecosystem functioning (Galand et al., 2010; Comeau et al., 2011; Wassmann, 2011; Ghiglione et al., 2012; Thaler and Lovejoy, 2015).

In the Arctic Ocean, in contrast to other ocean environments, temperatures close to the freezing point prevail across sea ice, water column and seafloor habitats, potentially permitting a close vertical connectivity of communities. Seed communities in transient environments such as sea ice are likely derived from directly connected environments (Ackley and Sullivan, 1994), i.e., surface ocean waters and sediments that are incorporated during ice formation on the shallow Arctic shelves (Wegner et al., 2017). In addition, the focused seasonal particulate organic carbon flux from the sea ice and surface ocean to the seafloor may transport viable microbes to the seafloor, where they could contribute to the turnover of carbon and nutrients, and potentially also become members of the benthic communities (Turley and Mackie, 1995; Ruff et al., 2014).

This study aimed at assessing the community composition and turnover of bacterial and eukaryotic microorganisms associated with ice-associated and sinking algal aggregates, as well as their similarity with potential source communities of sea ice, water and deep-sea sediments using next-generation sequencing. We hypothesized (i) that sea-ice algae aggregates select for specific members of eukaryotes and bacteria; (ii) that these originate from sea ice rather than the water column; (iii) that these are exported by rapidly sinking aggregates that transport sea-ice life to the deep sea, but (iv) get quickly overgrown by sediment microbes. Furthermore, this study contributes baseline knowledge of microbial diversity in the central Arctic Ocean in times of rapid sea-ice melt.

MATERIALS AND METHODS

Study Site and Environmental Conditions

Our samples were retrieved from nine sampling locations across the Eurasian Basin of the central Arctic Ocean during RV *Polarstern* expedition ARK-XXVII/3 (PS80) in August and September 2012 at the end of the productive season (**Figure 1**). Surface seawater temperatures were between −1.8 to −1.5°C and salinity ranged from 30.3 to 33.2 (Boetius et al., 2013). Nutrient inventories in the surface layer were already very low and nitrate generally limiting (Fernández-Méndez et al., 2015). First-year ice was the dominant ice type during the time of sampling (Supplementary Table S1), and ice thickness varied between 0.7 and 2 m (Fernández-Méndez et al., 2014). Salinity of the total sea-ice surface ranged between 0.1–2.9, and 1.0–3.6 in the bottom layer. Temperatures within surface sea ice varied from −8.5 to 0°C and were between −1.9 and −1.1°C in bottom ice (Hardge et al., 2017). Melt-pond coverage on ice floes varied between stations and ranged from 20 to 50%. Different types of melt ponds were observed, including closed (closed to the underlying seawater), and open ponds (directly connected to the underlying seawater). Some of them had a refrozen surface ice cover. Sea-ice algal aggregates of varying size and color were observed in all different types of ponds (Fernández-Méndez et al., 2014). Seafloor observations revealed the presence of deposited ice-algal aggregates on the sediment surface in the deep sea, with an especially high carbon deposition of 32 and 156 g C m^{-2} at stations Ice7 and Ice8 (**Figure 1**), when compared to the remaining stations where values ranged from 0 to 20 g C m^{-2} (Boetius et al., 2013). The presence of algae deposits at the seafloor often coincided with the presence of opportunistic holothurians of the species *Kolga hyalina*, which were attracted by the fresh food source (Boetius et al., 2013). Bottom water temperatures in the deep sea were stable at around −0.7°C (Rabe et al., 2013). Direct links to the original data on environmental conditions during sampling can be found via the PANGAEA database entry (Rapp et al., 2017).

Sampling

Sea-ice algal aggregates and melt pond water were sampled from three distinct melt ponds at stations Ice1 and Ice7 (**Figure 1** and Supplementary Table S1) using a manually operated vacuum pump (Model 6132-0010, Nalgene, Penfield, NY, United States). Deposited ice-algal aggregates from the deep-sea floor at stations Ice7 and Ice8 were retrieved using a TV-guided multicorer or a MultiGrab, and individually collected from the sediment surface with sterile plastic pipets or forceps. Sea-ice cores from all stations were taken with an ice corer (9 cm diameter) (Kovacs Enterprise, Roseburg, OR, United States), cut into two equal sections and melted in plastic containers (previously rinsed with ethanol and

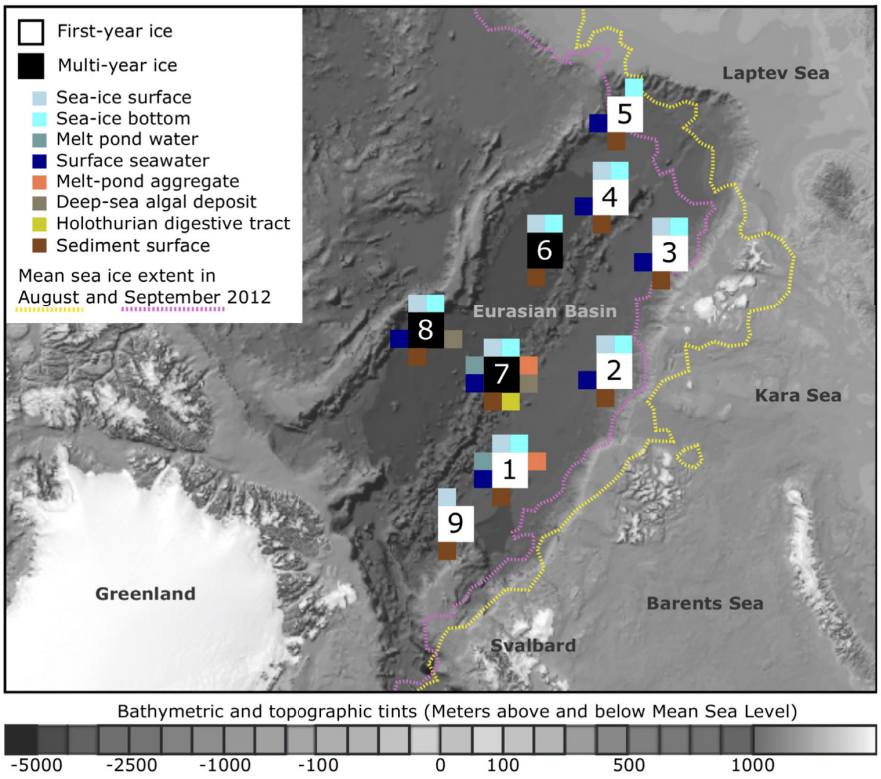

FIGURE 1 | Sampling locations and sampled environments. Map of the central Arctic Ocean, depicting sampling locations and mean sea-ice extent during RV Polarstern expedition ARK-XXVII/3 (PS80) in 2012. A total of nine stations were visited from August to the end of September, with the individual sampled environments indicated by color. The bathymetric map was modified from Jakobsson et al. (2012). For further details on sampling location and the conducted analyses see Supplementary Table S1.

ultrapure water) on a shaker in the dark at 4°C (Mikkelsen et al., 2008; Rintala et al., 2014). Ice core length ranged from 0.8 to 2.0 m (Supplementary Table S1). Four ice samples were not sectioned: two ice cores from station 9 (one of them from newly formed ice and the other one from very thin ice adjacent to it), as well as two cores from station 7 and 8, which showed a strong brownish coloration (indication of high algal biomass) (Supplementary Table S1). Melting of the ice took around 24 h and samples were immediately processed as soon as the last piece of ice melted, to avoid abrupt changes of temperature in the sample. Surface seawater was sampled either with a peristaltic pump through a hole in the sea ice (Masterflex® E/STM portable sampler, 115 VAC, Oldham, United Kingdom), or with a conductivity–temperature–depth (CTD) rosette sampler (Seabird SBE 911 plus, Bellevue, WA, United States) at 0–2 m depth. Between 0.5 and 2 L of melted sea ice or water were filtered through 0.22 µm polyethersulfone membranes (Millipore® Sterivex™, Merck KGaA, Darmstadt, Germany) with a multichannel peristaltic pump (Model PD 51; Heidolph, Schwabach, Germany). Three parallel deep-sea sediment cores were retrieved from each sampling site by a TV-guided multicorer and subsamples of the uppermost centimeter of each core were pooled per site. At station Ice1, we obtained a total of nine sediment cores, allowing us to prepare three replicate sediment samples (Supplementary Table S1). Holothurians feeding on the deposited algal aggregates were collected using an Agassiz trawl, digestive tracts were dissected and the content stored in plastic syringes. All Sterivex filters and deep-sea samples were stored at −20°C until further processing.

A total of 54 samples were taken for microbial community analysis, 52 of these were subject to bacterial community analysis, and 45 of these samples to eukaryotic community analysis (Supplementary Table S1).

DNA Extraction, PCR and Illumina Sequencing

Total community DNA was extracted from half Sterivex filters for sea-ice, melt-pond and seawater samples, from 0.5 g of algae aggregates and holothurian digestive tract content and from 1 g of sediment using the UltraClean™ Soil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, United States) following the manufacturer's instructions for maximum yields, with minor modifications. Instead of using the kit's enclosed

solution S5, DNA extracts were eluted in a final volume of 80 µl TE-buffer (10 mM Tris-Cl, pH 8.0; 1 mM EDTA). By using one standardized extraction kit across all sample types, we aimed to minimize potential biases toward differential extraction yields for individual microbial taxa and thus increase comparability. DNA concentration and purity were determined by fluorescence-based Qubit® quantitation assays (Life Technologies GmbH, Darmstadt, Germany). Amplicon libraries of the bacterial V4-V6 region of the 16S rRNA gene and the eukaryotic V4 region of the 18S rRNA gene were generated according to the protocol recommended by Illumina (16S Metagenomic Sequencing Library Preparation, Part #15044223, Rev. B). For selected sediment, sea-ice algae and deposited algae samples we obtained technical replicates, by preparing several sequencing libraries from the same DNA extract (Supplementary Table S1). For *Bacteria* we selected the S-D-Bact-0564-a-S-15 and S-*Univ-1100-a-A-15 primer pair based on a primer evaluation by Klindworth et al. (2013) and for *Eukaryota* the TAReukFWD1 and TAReukREV3 primers (Stoeck et al., 2010) (Supplementary Information Scripts bac and euk). Libraries were sequenced on an Illumina MiSeq platform in 2x300 cycles paired end runs. Raw paired-end sequences have been submitted to ENA under INSDC accession number PRJEB23005 using the data brokerage service of the German Federation for Biological Data (GFBio, Diepenbroek et al., 2014), in compliance with the Minimal Information about any (X) Sequence (MIxS) standard (Yilmaz et al., 2011). To corroborate the observed bacterial community patterns, we used Automated Ribosomal Intergenic Spacer Analysis in parallel to the tag sequencing approach for a wider set of in total 68 samples (Supplementary Information Material and Methods).

Sequence Processing, Taxonomic Assignment and Data Filtering

An initial 10,266,909 bacterial and 3,717,438 eukaryotic raw sequence pairs were generated (Supplementary Table S2). We used *cutadapt* (v. 1.8.1; Martin, 2011) for the removal of primer sequences and a custom *awk* script to ensure the correct orientation of reads prior to merging (Supplementary Information Scripts bac and euk). For merging forward and reverse reads we used *pear* (v. 0.9.5; Zhang et al., 2014) and trimmed and quality filtered all sequences using *trimmomatic* (v. 0.32; Bolger et al., 2014). 2,225,003 bacterial and 2,777,311 eukaryotic merged and quality trimmed sequences were retained after processing (Supplementary Table S2). We reassured correct formatting of the fastq files with bbmap (v. 34.00; B. Bushnell[1]) before clustering the reads into 613,270 bacterial and 59,853 eukaryotic OTUs by applying a local clustering threshold of $d = 1$ and the fastidious option in *swarm* (v. 2.1.1; Mahé et al., 2015). After alignment with the SINA aligner (v. 1.2.10; Pruesse et al., 2012) and classification of the seed sequence of each OTU with the SILVA SSU database release 123 (Quast et al., 2013), we removed all OTUs that were classified as chloroplasts, mitochondria, archaea, or those that could not be classified at domain level from further analysis. OTUs that were classified as bacteria within the eukaryotic dataset and vice versa, were removed as well. Furthermore, we removed all absolute singletons, i.e., OTUs that were only represented by a single sequence across the complete dataset (Supplementary Table S2). By doing so we reduced the effect of artificially inflated diversity potentially introduced through sequencing errors (Tremblay et al., 2015b). Filtering and removal of absolute singletons resulted in a final number of 8,869 bacterial and 7,627 eukaryotic OTUs (Supplementary Table S2). All further analyses were performed with these processed OTU abundance tables (Rapp et al., 2017). The full bioinformatic scripts are provided as supplementary information (Supplementary Information Scripts bac and euk).

[1]https://sourceforge.net/projects/bbmap

Community Comparison and Analysis of Abundant and Dispersed Taxa

We calculated OTU numbers, as well as the Chao1 richness estimate and the inverse Simpson diversity index per sample. To obtain reliable estimates independent of differences in sequencing depth between samples, we subsampled our input OTU tables (Rapp et al., 2017) 100-times to the lowest read number of each dataset (bacteria: 10,068 reads; eukaryotes: 16,372 reads) and used mean values across all iterations. Significant differences in OTU numbers between habitats were determined by analysis of variance using permutation tests as implemented in the function *aovp* from the *lmperm* package (Wheeler and Torchiano, 2016). *Post hoc* pairwise permutation tests were performed using the function *pairwisePermutationTest* from the *rcompanion* package (Mangiafico, 2017), and p-values were corrected for multiple testing using the Benjamini–Hochberg procedure (Benjamini and Hochberg, 1995). We generated species accumulation curves at different taxonomic resolution to assess the efficiency of our sampling effort to capture the present diversity in the individual environments (Supplementary Figures S1–S4). To assess overall differences in community structure we calculated Bray-Curtis dissimilarity matrices and visualized the results in non-metric multidimensional scaling (NMDS) plots. Due to the high variability in 18S gene copy numbers of microbial eukaryotes, which can vary by at least four orders of magnitude, depending on genome (Prokopowich et al., 2003) and cell (Zhu et al., 2005) size, we additionally assessed community structure using the Jaccard dissimilarity measure for presence/absence of eukaryotic taxa (Supplementary Figure S5). Correlation of both dissimilarity measures was examined by performing a mantel test with 999 permutations (Supplementary Figure S6). Concomitantly, we performed an analysis of similarity (ANOSIM) to test for the significance of differences between groups of samples from different environment types. All analyses were conducted in R (v. 3.1.2; R Core Team, 2014) using the *vegan* package (Oksanen et al., 2014) and custom R-scripts (Supplementary Information Scripts bac and euk; Gobet et al., 2010). For further community comparisons we chose a conservative presence-absence approach. We aimed to distinguish between abundant, widely dispersed groups of an environment and transient groups, which are present at a subset of locations (Shade and

Handelsman, 2012). Therefore, we applied a rule that would count a taxon (i.e., an OTU) as dispersed and abundant, if found in >50% of the samples of a habitat, and represented by at least 100 sequences (Supplementary Tables S3, S4). Next, we determined the proportion of community overlap (i.e., shared groups) between the sampled environments using Jaccard similarities. Further, we defined those OTUs that were shared across sea ice, water column, aggregates, as well as deep-sea surface sediments as generalists. Consequently, habitat-specific OTUs are members of only one environmental community.

RESULTS

Richness and Diversity of Microbial Communities in Central Arctic Ocean Habitats

We saw indications for higher eukaryotic and bacterial community richness in sea-ice algal aggregates than in the surrounding melt-pond water, and in the case of bacteria, also than in adjacent sea ice (**Table 1** and **Figure 2**). Only 11–12% of the OTUs associated with ice-algae aggregates met our definition of dispersed, abundant community members, i.e., they were present in >50% of aggregate samples and represented by at least 100 sequences. These OTUs represented the majority of sea-ice algae associated sequences (**Table 1**). Melt-pond waters showed the lowest eukaryote and bacterial OTU richness of the sampled ice habitats, but had a relatively large fraction of abundant bacterial types (23%). Bacterial communities in sea ice also exhibited relatively low richness, while eukaryotic communities showed highest richness in sea-ice samples (**Figure 2**).

Bacterial communities on sunken ice-algal aggregates deposited at the seafloor (4,400 m water depth) during the summer season were slightly richer than the communities on photosynthetically active aggregates in melt ponds, communities in sea ice and in surface seawater. They still shared 22% of their abundant OTUs with the original ice-algae associated community, but also 17% with the abundant, dispersed sediment bacteria (**Figure 3a**). We derived the relative contribution of these shared OTUs (Supplementary Table S3) to the total communities detected in deposits and sediments (Rapp et al., 2017). The OTUs shared between sediments and deposits accounted for on average 55% of the bacterial sequences in the algal deposits (up to 79% in individual aggregates), but only for 28% of sequences in the total sediment community. In contrast, eukaryotic communities of the deposited algae showed lowest richness and diversity compared to all other environments (**Table 1** and **Figure 2**). They shared only 29% with the algae-associated abundant types (**Figure 3b**), and 16% with the abundant sediment eukaryotes. Bacterial richness and diversity was lowest in the digestive tract content of holothurians feeding on the deposited aggregates on the seafloor (**Table 1** and **Figure 2**), and these bacteria were mostly unique to the holothurian digestive tract, with only 4% overlap with the abundant, dispersed types of the sediment community (**Figure 3**). Deep-sea surface sediments

TABLE 1 | Community alpha diversity and contribution of abundant and dispersed community members.

	Bacteria							Eukaryota						
	IceS	IceB	MPW	MPAGG	SW	DSAGG	HGC	Sed	IceS	IceB	MPW	MPAGG	SW	DSAGG	HGC	Sed
	n = 7	*n* = 7	*n* = 3	*n* = 3	*n* = 7	*n* = 7	*n* = 2	*n* = 9	*n* = 7	*n* = 8	*n* = 3	*n* = 3	*n* = 7	*n* = 8	*n* = 2	*n* = 3
nOTUs [m]	192	196	185	252	280	292	97	1,736	408	503	369	502	348	163	228	237
Total OTUs	1,374	1,457	657	1,016	874	1,446	173	5,612	2,576	3,545	2,095	1,742	1,730	1,413	435	497
SSOs [m] [%]	20	18	17	16	17	14	14	42	1	3	2	2	2	1	1	2
Chao1 [m]	265	307	269	343	369	441	111	2,415	684	916	747	833	449	239	277	242
invS [m]	9	9	4	10	22	10	3	154	9	15	17	5	11	3	9	7
A&D OTUs	149	138	148	117	204	190	33	413	208	284	240	184	228	109	111	61
A&D fraction	11	10	23	12	23	13	19	7	8	8	12	11	13	8	26	12
Total reads	249,151	216,935	103,504	62,740	271,203	194,048	36,786	171,767	400,528	462,135	239,671	207,779	391,252	398,697	132,294	102,951
A&D reads	235,827	197,078	98,772	52,935	258,915	184,516	35,105	118,626	377,330	422,583	224,213	194,520	357,325	371,597	128,930	72,378
A&D read fraction	95	91	95	84	96	95	95	69	94	91	94	94	91	93	98	70

nOTUs, OTUs present in an individual sample; total OTUs, the sum of unique OTUs detected in a given environmental grouping; SSOs, singleton OTUs, represented by only a single sequence across the total dataset, removed from analysis prior to alpha diversity calculation; Chao1, chao1 richness estimate; invS, inverse Simpson diversity index; A&D community, abundant and dispersed OTU present in >50% of the samples within an environmental grouping and represented by at least 100 sequences across the dataset; A&D fraction, proportion of abundant and dispersed OTUs relative to the total number of OTUs; Total reads, the sum of all reads after sequence processing. IceS, sea-ice surface; IceB, sea-ice bottom; MPW, melt-pond water; MPAGG, melt-pond aggregate; SW, surface seawater; DSAGG, deep-sea algae deposit; HDT, holothurian digestive tract content; Sed, deep-sea surface sediment. [m], median values per environmental grouping.

contained a significantly richer and more diverse bacterial community than sea ice, seawater and algae deposits, and showed almost no overlap with water column and sea-ice communities, while eukaryotic richness in sediments was at the lower end of the observed range across all sampled environments (**Figure 2**).

Non-metric multidimensional scaling (NMDS) of Illumina OTUs clustered the samples into five groups according to their habitat (**Figure 4** and Supplementary Figure S5), including communities from (i) sea-ice associated environments (sea-ice algae aggregates, melt ponds and sea ice), (ii) surface seawater, (iii) deep-sea sediment, (iv) deposited sea-ice algal aggregates and (v) holothurian digestive tract both for bacteria (ANOSIM: $R = 0.86$, $p = 0.001$) and eukaryotes (ANOSIM: $R = 0.63$, $p = 0.001$). In both datasets, the communities in sea-ice algal aggregates showed high within-group variation. Notably, eukaryotic community structure in deep-sea sediment and exported ice-algal aggregates showed a much larger dissimilarity than bacterial communities (**Figure 4b**). Overall, the dissimilarities of bacterial and eukaryotic community structures were significantly correlated across all habitats ($r = 0.76$; $p = 0.001$) (Supplementary Figure S7). Bacterial community patterns observed with Illumina tag sequences were supported by analyses of ARISA patterns, which built on a larger set of samples, i.e., from melt pond water, sea-ice algae aggregates and holothurian digestive tract (Supplementary Figure S8); dissimilarity matrices from both methods were significantly correlated (Supplementary Figure S9 and Supplementary Information Results).

Composition of Microbial Communities in Sea Ice, Surface Water and Sediments

At the time of sampling in late summer, contribution of diatom sequences in surface seawater was minor (**Figure 5a**). *Dinophyceae* were the predominant group in surface seawater, but the majority of its representatives could not be classified (**Figure 6**). One dominant genus was *Karlodinium*, which was also observed in individual deposited algae aggregates at the seafloor, and in very high sequence abundance in Ice7 deep-sea sediment, where we observed high algae deposition. Eukaryote community composition in sea ice comprised green-brown algae and *Dinophyceae*, mainly the genus *Scrippsiella,* members of the Gymnodinium clade and unclassified *Suessiaceae* in the upper part of the cores, and mostly unclassified diatoms at the bottom of the ice (**Figure 6**). The genera *Pseudo-nitzschia* and *Melosira* exhibited a patchy distribution with low relative abundance (<1%) at all stations except for Ice7. All of these groups were also present in melt-pond waters, in addition to other algae from the *Pelagophyceae* and a larger contribution of *Thecofilosea*, including the nanoflagellate *Cryothecomonas*. In deep-sea sediments, Labyrinthulomycetes, dinoflagellates and other flagellates from the *Imbricatea* dominated eukaryote communities (**Figures 5a**, **6**). At Ice7 we observed a relatively high contribution of the diatom *Chaetoceros*, the only diatom detected in the sediment outside of the algal deposits.

Flavobacteriia, *Gamma-* and *Alphaproteobacteria* dominated bacterial communities in sea ice, surface seawater and sediments (**Figure 5b**), yet with pronounced differences in their predominant representatives at the genus level (**Figure 6**). In sea ice and melt ponds, *Flavobacteriia* were mainly represented by the genus *Flavobacterium*, with reads of this single genus constituting 9–71% of the total community in the ice surface and 3–45% in the bottom of the ice. Also the genera *Polaribacter*, *Psychroflexus*, *Nonlabens,* and *Ulvibacter* were sequence-abundant in ice. Among these, *Polaribacter* was the only genus, which was also a dominant flavobacterial member in surface seawater. Here, the genera *Owenweeksia*, *Formosa* and the NS5 marine group were the most sequence-abundant *Flavobacteriia* (**Figure 6**). Microbial community composition of the deep-sea floor was quite distinct from the water column, and the contribution of *Flavobacteriia* was markedly reduced (**Figure 5b**). Most of its representatives in surface sediments could not be classified at genus level and clustered as unclassified *Flavobacteriaceae*.

Gammaproteobacteria in sea ice were dominated by *Paraglaciecola*, which displayed a similarly large variability in its contribution as *Flavobacterium*, ranging from 0 to 33% in sea-ice surface communities and 2–40% in bottom communities (**Figure 6**). Other important *Gammaproteobacteria* in sea ice belonged to the genera *Colwellia* and *Glaciecola*. In melt-pond waters *Gammaproteobacteria* representation was lower (**Figure 5b**), but *Paraglaciecola* and *Glaciecola* were also dominant members (**Figure 6**). In the water column, the most prominent *Gammaproteobacteria* taxa, *Balneatrix*, SAR86 clade and ZD0405 clade (**Figure 6**) showed only minor representation in sea ice, and were absent from the deep-sea samples. The most dominant *Gammaproteobacteria* in the sediment were members of the JTB255 marine benthic group, and these were restricted to deep-sea samples. Deep-sea surface sediment additionally showed a strong contribution of *Deltaproteobacteria* and *Acidimicrobiia* (**Figure 5b**).

Alphaproteobacteria contributed most to the communities in surface seawater, deep-sea sediment and the digestive tract content of holothurians, while their sequence abundance in sea ice and melt ponds was low (**Figure 5b**). While the genus *Planktomarina* and members of the SAR11 Surface1 clade predominated in the water column, *Rhodospirillaceae* were the most dominant representatives at the seafloor. The bacterial community in the holothurian digestive tract was very different from any other environment (**Figure 5b**) and almost entirely comprised of the alphaproteobacterial genus *Sedimentitalea* and the genus *Rubritalea* from the *Verrucomicrobiae* (**Figure 6**).

Based on OTU presence/absence, the microbial community associated with sea-ice algae aggregates showed highest community overlap with both melt-pond water and sea-ice communities. We found lower similarity in composition with the underlying community in surface seawater (**Figure 3**). Interestingly, the community overlap between ice-algal aggregates associated with melt ponds and the exported ice algae at the seafloor was comparable to or higher than the overlap between aggregates and surface seawater for both bacteria and eukaryotes (**Figure 3**), despite the >4000 m descent.

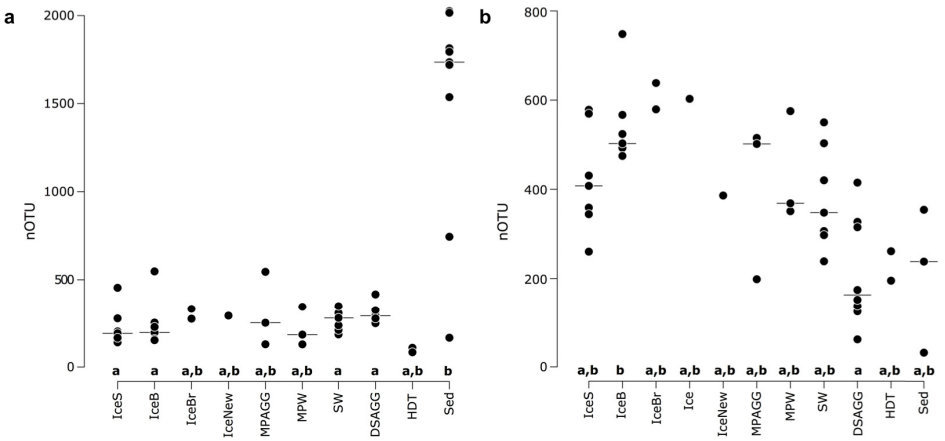

FIGURE 2 | Number of OTUs for **(a)** bacterial communities and **(b)** eukaryotic communities, with the horizontal black line indicating the median value per environmental grouping. Lower case letters at the bottom of the figure indicate environments that are significantly different from each other based on ANOVA permutation tests at a significance threshold of $p < 0.05$. IceS, sea-ice surface; IceB, sea-ice bottom; IceBr, brown sea ice; Ice, sea ice; IceNew, freshly formed sea ice; MPAGG, melt-pond aggregate; MPW, melt-pond water; SW, surface seawater; DSAGG, deep-sea algae deposit; HDT, holothurian digestive tract content; Sed, deep-sea surface sediment.

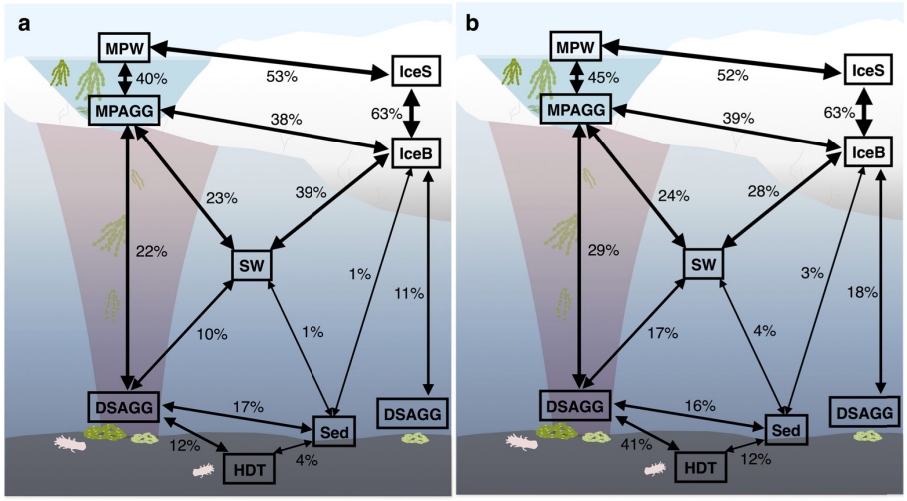

FIGURE 3 | Community overlap between environments for **(a)** bacteria and **(b)** eukaryotes. Shared proportions are based on presence-absence data for abundant and dispersed community OTUs only, including these OTUs that were present in at least 50% of the samples from one environment and represented by at least 100 sequences in the dataset. IceS, sea-ice surface; IceB, sea-ice bottom; MPAGG, melt-pond aggregate; MPW, melt-pond water; SW, surface seawater; DSAGG, deep-sea algae deposit; HDT, holothurian digestive tract content; Sed, deep-sea surface sediment.

At the seafloor, deposited ice-algal aggregate communities exhibited similar proportions of community overlap with the surrounding sediment as with the sea-ice bottom and surface seawater. A large overlap was also observed between eukaryotic communities in the deposited ice algae and the digestive tract content of the deep-sea dwelling holothurians (**Figure 3b**). Sea ice and sediment, as well as surface seawater and sediment communities showed lowest community overlap.

Only three bacterial and seven eukaryotic OTUs in the datasets fit our definition of generalists present across the Arctic habitats. These included members of the genera *Colwellia*, *Oleispira* (both *Gammaproteobacteria*) and *Lentimonas* (*Opitutae*) (Supplementary Information Discussion). Eukaryotic generalists included members of the genera *Cryothecomonas* (*Thecofilosea*), the NIF-3A7 and NW617 clades (*Thecofilosea*) and unclassified *Dinophyceae*, but also the photosynthetic *Micromonas* (*Mamiellophyceae*) and unclassified *Prasinophytae*.

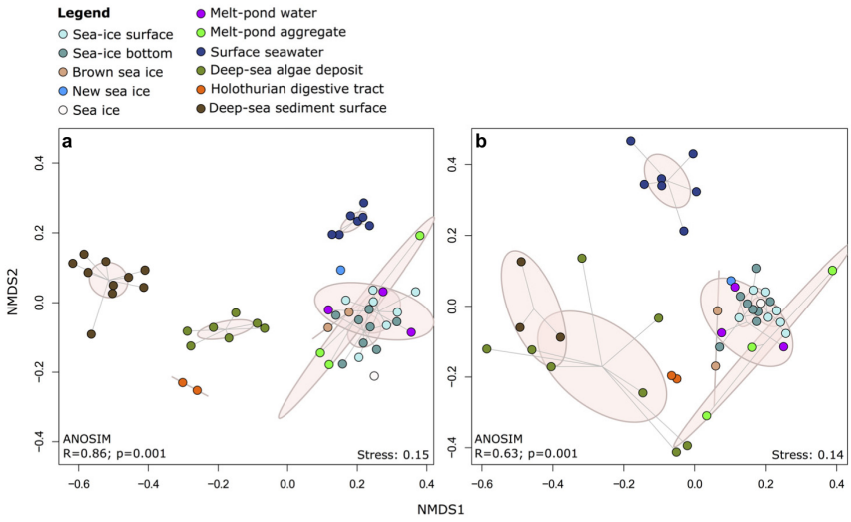

FIGURE 4 | Two-dimensional NMDS ordination of community dissimilarities for **(a)** bacteria and **(b)** eukaryotes. Dissimilarity matrices and ANOSIM were calculated using the Bray-Curtis dissimilarity measure on the basis of relative abundances of Illumina OTUs. Environments are depicted by color coding and points within each environmental grouping are connected to their group centroid through a spider diagram. Pink ellipses indicate the estimated 95% dispersion limits of each group.

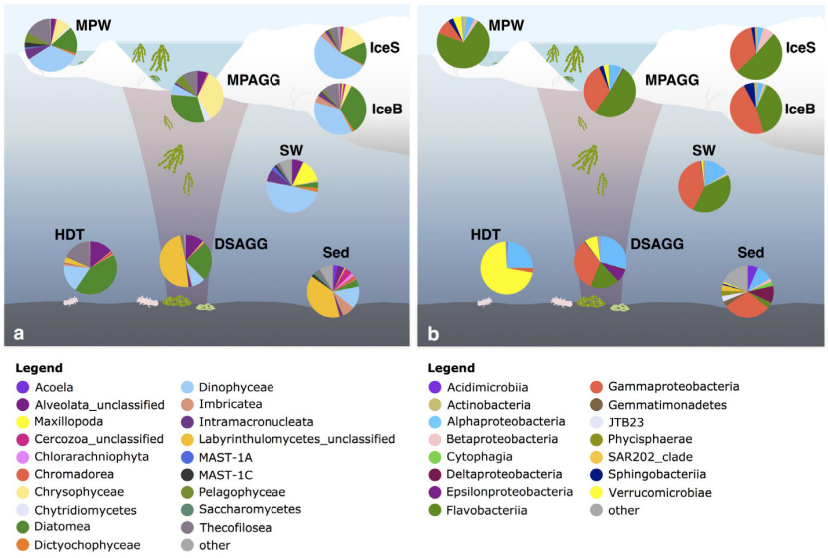

FIGURE 5 | Dominant **(a)** eukaryotic and **(b)** bacterial classes in the central Arctic Ocean. Shown are average values per environment, displaying classes with a relative sequence abundance of at least 2%. The remaining classes were grouped as 'other.' IceS, sea-ice surface; IceB, sea-ice bottom; MPAGG, melt-pond aggregate; MPW, melt-pond water; SW, surface seawater; DSAGG, deep-sea algae deposit; HDT, holothurian digestive tract content; Sed, deep-sea surface sediment. For details on the samples, see Supplementary Table S1.

We identified a larger number of OTUs in the bacterial and eukaryotic dataset that were shared members between sea-ice algae aggregates in melt ponds, sea ice, water column and deposited algae at the seafloor, but that could not be identified as abundant and dispersed members of the sediment community (Supplementary Tables S3, S4). Overall, a large fraction of community members were also associated with unclassified groups with no closely related cultured representatives, e.g., unclassified *Suessiaceae,* unclassified *Flavobacteriaceae,* SAR86 clade and ZD0405 clade, the JTB255 marine benthic group; their ecological role thus remains elusive.

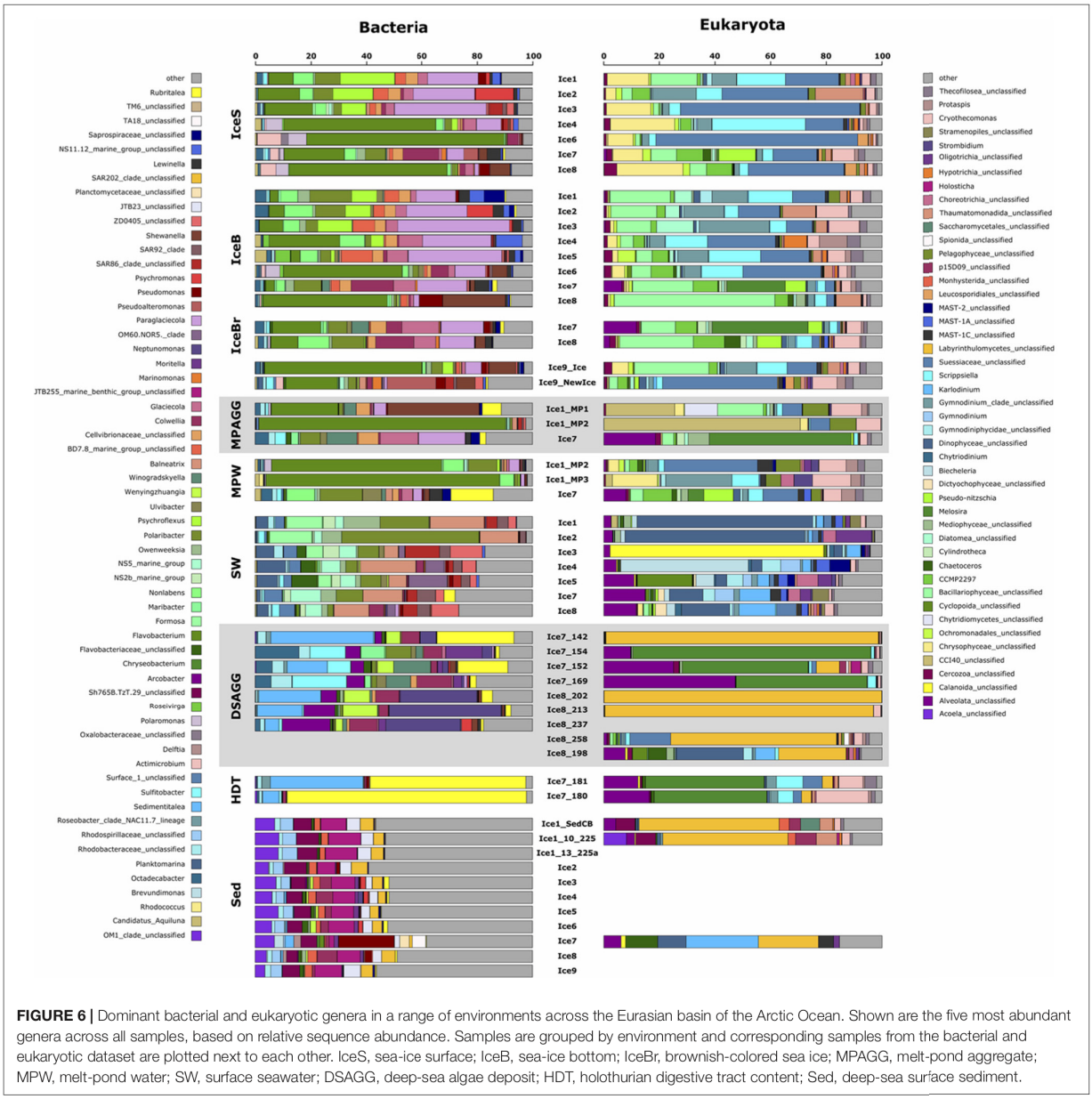

FIGURE 6 | Dominant bacterial and eukaryotic genera in a range of environments across the Eurasian basin of the Arctic Ocean. Shown are the five most abundant genera across all samples, based on relative sequence abundance. Samples are grouped by environment and corresponding samples from the bacterial and eukaryotic dataset are plotted next to each other. IceS, sea-ice surface; IceB, sea-ice bottom; IceBr, brownish-colored sea ice; MPAGG, melt-pond aggregate; MPW, melt-pond water; SW, surface seawater; DSAGG, deep-sea algae deposit; HDT, holothurian digestive tract content; Sed, deep-sea surface sediment.

Microbial Community Composition Associated With Sea-Ice Algal Aggregates

Visual inspection by light microscopy showed a predominance of *Melosira* in all sampled aggregates retrieved from the sea ice (Fernández-Méndez et al., 2014). Other diatoms observed under the microscope belonged to the pennate genera *Nitzschia*, *Fragilariopsis,* and *Cylindrotheca* (Supplementary Table S1). The ubiquitous presence of the genus *Melosira* (*Coscinodiscophyceae*) and of the above diatoms, as well as golden-brown algae (*Chrysophyceae*) was also revealed by sequencing (Supplementary Table S5). *Melosira* 16S rRNA gene contribution was <0.01% in the degraded aggregates of station Ice1 composed of empty *Melosira* frustrules (Supplementary Table S5), but dominated sequences in the fresh, green aggregate of station Ice7 (**Figure 6**).

Predominant bacterial classes associated with sea-ice algal aggregates were *Flavobacteriia* and *Gammaproteobacteria* (**Figure 5b**). The green aggregate from Ice7 included *Glaciecola* and *Paraglaciecola* (*Gammaproteobacteria*), and *Winogradskyella* (*Flavobacteriia*) (**Figure 6**). The genus *Flavobacterium*

(*Flavobacteriia*) was the most abundant genus in the highly degraded aggregates from Ice1, where it contributed up to 89% to the total bacterial sequences. *Flavobacterium* was barely detectable (<0.05%) in the much fresher *Melosira* aggregate from Ice7 (Supplementary Table S6).

Visual inspection of algal aggregates deposited at the seafloor also identified *Melosira* as the dominant component (Boetius et al., 2013; Fernández-Méndez et al., 2014). The eukaryotic sequences retrieved from the deposits fell into two groups: those dominated by diatom sequences, mainly *Melosira*, and unclassified alveolates, and others primarily composed of *Labyrinthulomycetes*, a group of stramenopile protists (**Figure 6**). The eukaryotic community in the holothurian digestive tract resembled the sequence composition of deposited algal aggregates, with a high contribution of the diatom *Melosira* and unclassified alveolates, but also a high contribution of *Cryothecomonas* and *Scrippsiella* observed in sea ice (**Figure 6** and Supplementary Table S5).

Alpha-, *Gammaproteobacteria,* and *Flavobacteriia* dominated all deposited algal aggregates (**Figure 5b**), and the overlap with the sea-ice aggregates was 22% at the OTU level. Yet, deposit-associated *Alphaproteobacteria* were dominated by the genera *Octadecabacter*, *Sedimentitalea* and *Sulfitobacter*, which were also detected in sea-ice algae aggregates, but in much lower relative abundances (**Figure 6** and Supplementary Table S6). The relative contribution of *Gammaproteobacteria* was similar in sea-ice algal aggregates and the deposited aggregates at the seafloor, but the predominant genera observed in the deposits, *Neptunomonas*, *Moritella,* and *Colwellia*, differed from the sea-ice aggregates. In contrast, *Winogradskyella*, the most prominent flavobacterial member associated with the deposits was also well represented in sea-ice aggregates. Overall, the contribution of *Flavobacteriia* associated with the deposited aggregates was lower than in the sea-ice aggregates. Instead, the contribution of other classes was higher, including *Epsilonproteobacteria* and *Verrucomicrobiae* (**Figure 5b**). *Epsilonproteobacteria* were entirely represented by the genus *Arcobacter*, which was not detected in any other environment, except for the surrounding surface sediments. *Verrucomicrobiae* were represented by the genus *Rubritalea*, which was also present in sea-ice algae aggregates. The two types of algae deposits that were differentiated by their eukaryotic community composition showed only minor differences in bacterial community composition. *Melosira*-dominated samples showed higher contributions of the genera *Octadecabacter* (on average by a factor of 30), *Sulfitobacter* (on average by a factor of 45), *Ulvibacter* (on average by a factor of 18) and *Winogradskyella* (on average by a factor of 21). Labyrinthulomycetes-dominated samples contained a higher proportion of *Neptunomonas* (on average by a factor of 12; **Figure 6**).

DISCUSSION

The Arctic Ocean sea-ice cover of the past 10 years shows minima both in extent as well as in thickness relative to the 1981–2010 mean[2]. In autumn 2012, at the time of this study, Arctic sea-ice extent reached a record minimum, a loss that has been unprecedented since the beginning of satellite records. The bottom of the relatively thin ice floes had been populated by diatoms, foremost the colonial *Melosira arctica* forming large filamentous aggregates. Strong melting resulted in a wide-spread export and rapid deposition of sea-ice algal aggregates at the deep-sea floor at around 4,000 m water depth (Boetius et al., 2013). Only few types of benthic fauna, primarily holothurians, were observed to feed on the exported algal material, but a substantial bacterial degradation of the algal deposits was recorded, leading to locally enhanced respiration rates and a depletion of oxygen in and under the algal food falls (Boetius et al., 2013). Other studies have confirmed recent high under-ice productivity and the high contribution of sea-ice algae to export fluxes (Comeau et al., 2013; Lalande et al., 2014; Poulin et al., 2014). Here we compared bacterial and eukaryote community composition on ice-algae aggregates and the surrounding habitats, to understand if the ice-algal aggregates would associate with specific kinds of bacterial groups, if these would be selected from sea ice rather than surrounding waters, and if these communities would get exported to the seafloor. Our analyses did not target archaea, and to our knowledge no data on archaeal community composition is currently available for the Eurasian basin, despite their presence in other Arctic regions (Galand et al., 2009; Comeau et al., 2011), and their potential importance in the biogeochemical cycling of nutrients and carbon (Kirchman et al., 2007). Archaea do not seem to be as tightly linked to phytoplankton biomass, as has been suggested for bacteria (Herfort et al., 2007; Kirchman et al., 2007; Comeau et al., 2011; Wilson et al., 2017), however, their ability to utilize algae-derived organic matter has been demonstrated (Alderkamp et al., 2006; Orsi et al., 2016). As Arctic archaeal communities appear to exhibit high spatio-temporal variability in composition, relative abundance, and heterotrophic activity (Bano et al., 2004; Kirchman et al., 2007; Alonso-Sáez et al., 2008; Galand et al., 2008, 2009; Wilson et al., 2017), future studies of the central Arctic should aim to include archaea in microbial community assessments.

Ice-Algal Aggregates Are Populated by Sea-Ice Bacteria

Sea-ice algal aggregates were mostly composed of healthy, green or degraded cells of the diatom *Melosira arctica*, but also included a variety of other sea-ice diatoms (Boetius et al., 2013). Microscopic analyses and sequence distribution of eukaryotes largely overlapped. Both eukaryotic and bacterial aggregate sequences showed highest community overlap with the surrounding melt-pond water and the sea ice (**Figure 3**), and the dominant members were also predominant members of the sea-ice and melt-water communities (**Figure 5**). The algal aggregates selected specifically for *Flavobacteriia*, particularly the genus *Flavobacterium*, as well as the gammaproteobacterial

[2]http://nsidc.org

Glaciecola and *Paraglaciecola* (**Figure 6**). Both are known to be tightly coupled to phytoplankton bloom dynamics (Teeling et al., 2012), and especially members of the *Flavobacteriia* exhibit the ability to hydrolyze complex polymers, such as polysaccharides found in plant and algal cell walls (Humphry et al., 2001; Knoll et al., 2001; Williams et al., 2013). Also for *Glaciecola* a key role in the breakdown of organic matter was suggested, and a specialization on diatom-derived material was observed (Beier et al., 2015). Most members of this genus seem to be psychrophilic, and recent experimental work suggests that cold-adapted *Glaciecola* can be the dominant consumers of algae material in low-temperature environments (von Scheibner et al., 2017). However, bacteria and eukaryotes associated with the ice-algal aggregates both exhibited high beta-diversity, reflecting substantial variations in community structure between individual aggregates. It has been observed before that differently composed phytoplankton blooms may lead to the establishment of distinct bacterial assemblages, due to differences in organic material released by individual algal taxa (Pinhassi et al., 2004; Grossart et al., 2005).

Previous studies observed a dominance of *Betaproteobacteria* in bacterial melt-pond communities (Brinkmeyer et al., 2004), while we detected a clear predominance of *Flavobacteriia* (**Figure 5b**). *Betaproteobacteria* are known as particle colonizers and were shown to play key roles in the decomposition of aggregates and nutrient cycling, especially in freshwater ecosystems (Knoll et al., 2001; Schweitzer et al., 2001; Lemarchand et al., 2006). However, while *Betaproteobacteria* were described as early colonizers of particles, *Flavobacteriia* contribution was shown to increase in the late phase of blooms (Knoll et al., 2001), when they potentially benefit from their capacity to break down refractory material. The high contribution of *Flavobacteriia* in the melt ponds and sea ice may therefore reflect a late or post bloom state of the system, indicated by the low nutrient inventories during the time of the sampling (Fernández-Méndez et al., 2015). Melt ponds, even when closed, can be directly connected to the surface ocean through a network of brine channels that penetrate the sea-ice matrix, allowing exchange of communities between environments (Boetius et al., 2015). However, the community in melt-pond water differed considerably from that of seawater, even at ice station 7, where the melt pond was open and salinity in the pond was comparable to the underlying water column (**Figure 6**). Our results, therefore, underpin a recruitment of bacterial groups mainly from sea ice, best adapted to the utilization of ice algal material in the ponds, which differed from the phytoplankton material in the surface ocean. We observed first indications for specific associations between bacterial groups and ice-algae aggregates, with 12 dispersed and abundant bacterial OTUs as exclusive members of ice-algae aggregates and algae deposits (Supplementary Table S3). These comprised mainly *Alphaproteobacteria*, including several members of the Roseobacter clade, e.g., *Sulfitobacter*. Examples of both mutualistic and pathogenic interactions have been described for Roseobacter-algae associations, with important implications especially for carbon and sulfur cycling in the environment (Ramanan et al., 2016). Roseobacter are known as rapid colonizers of algae surfaces, where they often outcompete other bacterial groups, probably facilitated by their ability to sense and utilize several compounds released by phytoplankton, including dimethylsulfoniopropionate (Buchan et al., 2014).

Ice-Algal Aggregates Transport Sea-Ice Microorganisms to the Deep-Sea Floor

Comparative analyses of microbial communities from upper ocean and deep sea showed that these communities barely overlap (Countway et al., 2007; Amaral-Zettler et al., 2010; Zinger et al., 2011; Walsh et al., 2016). Recent studies targeting the local overlap between surface water and surface sediment communities indicated a role of sinking particles and overlying water column properties in structuring benthic bacterial communities (Nagata et al., 2000; Hamdan et al., 2013; Ruff et al., 2014; Lindh et al., 2017). Here we investigated whether the rapidly sinking sea-ice algae deliver their associated bacteria to the deep-sea floor. *Melosira* filaments differ from other sinking particles in their large size and concomitant fast sinking rate (Alldredge and Gotschalk, 1988; Fernández-Méndez et al., 2014), with aggregates of a diameter of 3 cm potentially reaching the deep-sea floor within a single day after losing buoyancy at the surface (Katlein et al., 2014). We observed algae deposits, which were almost entirely composed of fresh *Melosira* and alveolates from the sea ice, with intact chloroplasts, presenting a fresh carbon source for benthic communities. These aggregates can potentially transport large numbers of microbial cells from the surface to the seafloor. In the most degraded *Melosira* aggregates, the dominant sequences belonged to members of the *Labyrinthulomycetes* (**Figure 6**), a group of heterotrophic protists that play important roles as saprobes of dead algal material and marine snow (Raghukumar, 2002; Bochdansky et al., 2017). *Labyrinthulomycetes* display an absorptive mode of nutrition and possess the ability to chemically alter and degrade algal detritus through the production of extracellular enzymes (Bahnweg, 1979; Raghukumar, 2002). Interestingly, in the investigated holothurian digestive tract *Melosira* sequences dominated largely over those of *Labyrinthulomycetes*, indicating a preference of the holothurians for fresh deposits (**Figure 6**).

We detected a large proportion of shared bacterial OTUs (22%) belonging to both the abundant types present on sea-ice algae aggregates and on deposited aggregates at the seafloor (**Figure 3a** and Supplementary Table S3). They included representatives of the genera *Paraglaciecola*, *Glaciecola* (both *Gammaproteobacteria*), *Octadecabacter* (*Alphaproteobacteria*), *Psychroserpens* and *Polaribacter* (*Flavobacteriia*), which have been previously described as psychrophiles, and were observed in sea ice and surface seawater (Bowman et al., 1997, 2012; Gosink et al., 1997; Junge et al., 2002; Brinkmeyer et al., 2003; Han et al., 2014; Hatam et al., 2014). While these OTUs represented 37% of the bacterial sequences associated to sea-ice aggregates and were also abundant members in sea ice, they contributed only 1% of the sequences in the deposits and were absent or represented by a minor fraction in sediments (Rapp et al., 2017). We therefore conclude that

they were exported with the aggregates, but overgrown by other bacteria.

Specific Sediment Microbes Overgrow Deposited Algal Aggregates

A considerable amount of bacteria can be exported to the deep sea attached to particles (Turley and Mackie, 1995), but their fate and biogeochemical roles remain elusive. Indigenous deep-sea bacteria are likely better adapted to *in situ* temperature and pressure of the deep-sea environment than surface-derived bacteria (Turley and Lochte, 1990; Turkey, 1993; Poremba, 1994; Tamburini et al., 2006, 2013). The results of this study suggest that the deposited algal aggregates were overgrown within a few weeks to months by specific bacterial groups of the surrounding sediment (**Figure 6**). Accordingly, the bacterial genera of highest relative sequence abundance (i.e., up to 37%) in the deposited ice-algae aggregates consisted of benthic groups that were absent from ice-associated environments and only of minor relative abundance in the surrounding sediment surface (**Figure 6** and Supplementary Table S6). Most prominent were members of the alphaproteobacterial Roseobacter clade, i.e., the genus *Sedimentitalea*, but also the genera *Arcobacter* (*Epsilonproteobacteria*) and *Neptunomonas* (*Gammaproteobacteria*) (**Figure 6**). The Roseobacter clade is a physiologically versatile group, known for its capability to utilize a wide range of organic and inorganic compounds and its tight interactions with phytoplankton, important for organic matter decomposition (Buchan et al., 2014 and references therein). *Arcobacter* has been recorded in a variety of sediment environments, including surface sediment from the Antarctic shelf (Bowman and McCuaig, 2003), the Wadden Sea (Llobet-Brossa et al., 1998) and the deep sea (Thamdrup et al., 2000). Its capacity to attach to surfaces (Assanta et al., 2002), denitrify (Heylen et al., 2006), fix nitrogen (Wirsen et al., 2002), as well as its ability to perform dissimilatory manganese reduction (Thamdrup et al., 2000) and recycle sulfur (Wirsen et al., 2002) indicate a potentially important role of *Arcobacter* in re-mineralizing nutrients from aggregates. Suboxic patches occurred under the algal aggregates in otherwise fully oxygenated sediments (Boetius et al., 2013), potentially selecting for *Arcobacter*. Members of the *Neptunomonas* genus have been isolated from sediments (Zhang et al., 2010), but were also found associated to unicellular eukaryotes (Frommlet et al., 2015) or in close vicinity to whale carcasses (Miyazaki et al., 2008). This genus is known for its capacity to degrade polycyclic aromatic hydrocarbons (Hedlund et al., 1999), indicating an involvement in the breakdown of recalcitrant carbon sources in the deep sea. The selection of rare sediment taxa best adapted to the utilization of the deposited algal detritus could on the long term induce shifts in the indigenous benthic bacterial communities, as has recently been observed in deep-sea surface sediments along the Antarctic Polar Front (Ruff et al., 2014). Overall, the algal deposits changed community composition locally at the deep-sea floor not only by introducing surface ice-bacteria, but also by selecting for specific sediment community members, which resulted in a unique community profile, distinct from any source community (**Figures 4a**, **6**). It remains to be further investigated whether this is a transient feature or whether such events, if reoccurring, will result in a gradual shift of deep-sea sediment communities.

As areas of strong summer ice melt are expanding in the central Arctic, it is expected that abrupt export events may become more frequent in areas covered by seasonal sea ice. This is supported by observations during recent expeditions in late summer 2016, where large areas of the central Arctic seafloor were again covered with *Melosira* deposits (Soltwedel, 2016; Boetius and Purser, 2017). Consequently, recurrent deposition of sea ice algae at the seafloor could facilitate the establishment of unique microbial assemblages as has been proposed for other discrete resource patches (Grassle and Morse-Porteous, 1987; Durán et al., 2013; Lindh et al., 2017) and would then likely lead to changes in bacterial community structure and in the biogeochemical cycling of carbon and nutrients in surface sediments.

Connectivity of Microbial Communities in the Central Arctic Ocean and Potential Effects of Climate Change

Bacteria in polar ice-associated environments need to be well-equipped to quickly adapt to changes and cope with rapid transitions of seasonal extremes, ranging from high salt concentration in brine inclusions to freshwater salinity in melt ponds (Deming, 2010), and protect themselves against sub-zero temperatures. Several studies showed the production of so-called compatible solutes by some sea-ice bacteria, which they can use as osmo- and cryoprotectants (Methe et al., 2005; Bowman, 2008). These specific adaptations to life around the freezing point of water may also facilitate the exchange of bacteria between habitats, e.g., after ice melt and export of attached bacteria in aggregates. We detected a high number of abundant OTUs shared between sea ice, melt ponds and aggregates, but only a low number of generalist OTUs (Supplementary Information Discussion and Supplementary Table S5), i.e., OTUs present in all sampled environments, despite the relatively uniform temperatures below zero degree.

Hence, this study confirms that sea-ice bacterial and eukaryotic communities are distinct from other Arctic habitats such as seawater and sediment (**Figure 4**). Consequently, ice dwelling organisms, and especially multi-year ice communities, which are distinct from those of seasonal ice (Leu et al., 2011; Bowman et al., 2012; Boetius et al., 2015; Hatam et al., 2016), may be lost from an Arctic devoid of summer sea ice, with unknown repercussions for productivity, organic matter cycling and other ecological functions. In addition, ice melt may contribute to upper water column freshening, potentially inducing shifts in the bacterioplankton composition, as has been observed for the phytoplankton community (Li et al., 2009). We saw first indications for a bacterial surface seawater community response to massive ice melt in the predominance of the gammaproteobacterial genus *Balneatrix*, which was previously described to thrive under freshwater conditions

(González and Whitman, 2006), and members of the genus *Planktomarina* of the Roseobacter clade (**Figure 6**), which was observed to correlate negatively with salinity (Giebel et al., 2011). Other than previous studies on bacterial community composition in the Arctic water column, we did not observe a predominance of the SAR11 clade (Kirchman et al., 2010; Bowman et al., 2012). This may be due to a primer bias (Apprill et al., 2015; Parada et al., 2016), but may also be ascribed to a lower representation of this oligotrophic clade during the post-bloom state of the system at the time of sampling. We observed a significant correlation between bacterial and eukaryote community patterns (Supplementary Figure S7), likely indicating similar drivers of diversification and potentially also biotic interactions, which remain largely unknown for polar organisms to date (Lima-Mendez et al., 2015). Future efforts should therefore aim to integrate bacterial, archaeal and eukaryotic community analyses, and expand the temporal resolution of sampling to better resolve seasonal dynamics.

AUTHOR CONTRIBUTIONS

JR and MF-M designed the study, with contributions by CB and AB. MF-M, CB, and AB collected the samples. JR performed the laboratory molecular and bioinformatic analysis. JR and CB performed the statistical analysis. JR interpreted the data and all authors contributed to the discussion of the results. JR wrote the manuscript, with support and input from all co-authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2018.01035/full#supplementary-material

REFERENCES

Ackley, S. F., and Sullivan, C. W. (1994). Physical controls on the development and characteristics of Antarctic sea ice biological communities- a review and synthesis. *Deep Res. Part I* 41, 1583–1604. doi: 10.1016/0967-0637(94)90062-0

Alderkamp, A., Sintes, E., and Herndl, G. (2006). Abundance and activity of major groups of prokaryotic plankton in the coastal North Sea during spring and summer. *Aquat. Microb. Ecol.* 45, 237–246. doi: 10.3354/ame045237

Alldredge, A. L., and Gotschalk, C. (1988). In situ settling behavior of marine snow. *Limnol. Oceanogr.* 33, 339–351. doi: 10.4319/lo.1988.33.3.0339

Alonso-Sáez, L., Sánchez, O., Gasol, J. M., Balagué, V., and Pedrós-Alio, C. (2008). Winter-to-summer changes in the composition and single-cell activity of near-surface Arctic prokaryotes. *Environ. Microbiol.* 10, 2444–2454. doi: 10.1111/j.1462-2920.2008.01674.x

Amaral-Zettler, L., Artigas, L. F., Baross, J., Bharathi, P. A. L., Boetius, A., Chandramohan, D., et al. (2010). "A global census of marine microbes," in *Life in the World's Oceans: Diversity, Distribution, and Abundance*, ed. A. D. McIntyre (Oxford: Blackwell Publishing Ltd.), 221–245. doi: 10.1002/9781444325508.ch12

Ambrose, W. G., Von Quillfeldt, C., Clough, L. M., Tilney, P. V. R., and Tucker, T. (2005). The sub-ice algal community in the Chukchi sea: large- and small-scale patterns of abundance based on images from a remotely operated vehicle. *Polar Biol.* 28, 784–795. doi: 10.1007/s00300-005-0002-8

Apprill, A., McNally, S., Parsons, R., and Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* 75, 129–137. doi: 10.3354/ame01753

Arrigo, K. R. (2014). Sea ice ecosystems. *Ann. Rev. Mar. Sci.* 6, 439–467. doi: 10.1146/annurev-marine-010213-135103

Arrigo, K. R., van Dijken, G., and Pabi, S. (2008). Impact of a shrinking Arctic ice cover on marine primary production. *Geophys. Res. Lett.* 35:L19603. doi: 10.1029/2008GL035028

Arrigo, K. R., and van Dijken, G. L. (2015). Continued increases in Arctic Ocean primary production. *Prog. Oceanogr.* 136, 60–70. doi: 10.1016/j.pocean.2015.05.002

Assanta, M. A., Roy, D., Lemay, M.-J., and Montpetit, D. (2002). Attachment of *Arcobacter butzleri*, a new waterborne pathogen, to water distribution pipe surfaces. *J. Food Prot.* 65, 1240–1247. doi: 10.4315/0362-028X-65.8.1240

Assmy, P., Ehn, J. K., Fernández-Méndez, M., Hop, H., Katlein, C., Sundfjord, A., et al. (2013). Floating ice-algal aggregates below melting Arctic sea ice. *PLoS One* 8:e76599. doi: 10.1371/journal.pone.0076599

Bahnweg, G. (1979). Studies on the physiology of Thraustochytriales. I. Carbon nutrition of *Thraustochytrium* spp., *Schizochytrium* sp., *Japonochytrium* sp., *Ulkenia* spp. and *Labyrinthuloides* spp. *Veröff. Inst. Meeresforsch. Bremerh.* 17, 269–274.

Bano, N., Ruffin, S., Ransom, B., and Hollibaugh, J. T. (2004). Phylogenetic composition of Arctic Ocean archaeal assemblages and comparison with Antarctic assemblages. *Appl. Environ. Microbiol.* 70, 781–789. doi: 10.1128/AEM.70.2.781-789.2004

Beier, S., Rivers, A. R., Moran, M. A., and Obernosterer, I. (2015). The transcriptional response of prokaryotes to phytoplankton-derived dissolved organic matter in seawater. *Environ. Microbiol.* 17, 3466–3480. doi: 10.1111/1462-2920.12434

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B* 57, 289–300. doi: 10.2307/2346101

Bochdansky, A. B., Clouse, M. A., and Herndl, G. J. (2017). Eukaryotic microbes, principally fungi and labyrinthulomycetes, dominate biomass on bathypelagic marine snow. *ISME J.* 11, 362–373. doi: 10.1038/ismej.2016.113

Boetius, A., Albrecht, S., Bakker, K., Bienhold, C., Felden, J., Fernández-Méndez, M., et al. (2013). Export of algal biomass from the melting Arctic sea ice. *Science* 339, 1430–1432. doi: 10.1126/science.1231346

Boetius, A., Anesio, A. M., Deming, J. W., Mikucki, J., and Rapp, J. Z. (2015). Microbial ecology of the cryosphere: sea ice and glacial habitats. *Nat. Rev. Microbiol.* 13, 677–690. doi: 10.1038/nrmicro3522

Boetius, A., and Purser, A. (2017). The expedition PS101 of the research vessel POLARSTERN to the Arctic Ocean in 2016. *Ber. Polar Meeresforsch.* 706, 1–236.

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. doi: 10.1093/bioinformatics/btu170

Bowman, J. P. (2008). "Genomic analysis of psychrophilic prokaryotes," in *Psychrophiles: From Biodiversity to Biotechnology*, eds R. Margesin, F. Schinner, J.-C. Marx, and C. Gerday (Berlin: Springer), 265–284. doi: 10.1007/978-3-540-74335-4_16

Bowman, J. P., McCammon, S. A., Brown, J. L., Nichols, P. D., and McMeekin, T. A. (1997). *Psychroserpens burtonensis* gen. nov., sp. nov., and *Gelidibacter algens* gen. nov., sp. nov., psychrophilic bacteria isolated from Antarctic lacustrine and sea ice habitats. *Int. J. Syst. Bacteriol.* 47, 670–677. doi: 10.1099/00207713-47-3-670

Bowman, J. P., and McCuaig, R. D. (2003). Biodiversity, community structural shifts, and biogeography of prokaryotes within Antarctic continental shelf sediment. *Appl. Environ. Microbiol.* 69, 2463–2483. doi: 10.1128/AEM.69.5.2463-2483.2003

Bowman, J. S., Rasmussen, S., Blom, N., Deming, J. W., Rysgaard, S., and Sicheritz-Ponten, T. (2012). Microbial community structure of Arctic multiyear sea ice and surface seawater by 454 sequencing of the 16S RNA gene. *ISME J.* 6, 11–20. doi: 10.1038/ismej.2011.76

Brinkmeyer, R., Glöckner, F.-O., Helmke, E., and Amann, R. (2004). Predominance of ß-proteobacteria in summer melt pools on Arctic pack ice. *Limnol. Oceanogr.* 49, 1013–1021. doi: 10.4319/lo.2004.49.4.1013

Brinkmeyer, R., Knittel, K., Ju, J., Weyland, H., Amann, R., Helmke, E., et al. (2003). Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. *Appl. Environ. Microbiol.* 69, 6610–6619. doi: 10.1128/AEM.69.11.6610-6619.2003

Buchan, A., LeCleir, G. R., Gulvik, C. A., and Gonzalez, J. M. (2014). Master recyclers: features and functions of bacteria associated with phytoplankton blooms. *Nat. Rev. Microbiol.* 12, 686–698. doi: 10.1038/nrmicro3326

Carmack, E., and Chapman, D. C. (2003). Wind-driven shelf/basin exchange on an Arctic shelf: the joint roles of ice cover extent and shelf-break bathymetry. *Geophys. Res. Lett.* 30:1778. doi: 10.1029/2003GL017526

Comeau, A. M., Li, W. K. W., Tremblay, J. -É., Carmack, E. C., and Lovejoy, C. (2011). Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum. *PLoS One* 6:e27492. doi: 10.1371/journal.pone.0027492

Comeau, A. M., Philippe, B., Thaler, M., Gosselin, M., Poulin, M., and Lovejoy, C. (2013). Protists in Arctic drift and land-fast sea ice. *J. Phycol.* 49, 229–240. doi: 10.1111/jpy.12026

Countway, P. D., Gast, R. J., Dennett, M. R., Savai, P., Rose, J. M., and Caron, D. A. (2007). Distinct protistan assemblages characterize the euphotic zone and deep sea (2500 m) of the western North Atlantic (Sargasso Sea and Gulf Stream). *Environ. Microbiol.* 9, 1219–1232. doi: 10.1111/j.1462-2920.2007.01243.x

Deming, J. W. (2010). "Sea ice bacteria and viruses," in *Sea Ice - An Introduction to its Physics, Chemistry, Biology and Geology*, eds D. N. Thomas and G. S. Dieckmann (Oxford: Blackwell Science Ltd.), 247–282.

Diepenbroek, M., Glöckner, F. O., Grobe, P., Güntsch, A., Huber, R., König-Ries, B., et al. (2014). "Towards an integrated biodiversity and ecological research data management and archiving platform: the German federation for the curation of biological data (GFBio)," in *Proceedings of the Informatik 2014 – Big Data Komplexität Meistern. GI-Edition: Lecture Notes in Informatics (LNI)*, eds E. Plödereder, L. Grunske, E. Schneider, and D. Ull (Bonn: Gesellschaft für Informatik eV), 1711–1724.

Durán, R., Canals, M., Lastras, G., Micallef, A., Amblas, D., Pedrosa-Pàmies, R., et al. (2013). Sediment dynamics and post-glacial evolution of the continental shelf around the Blanes submarine canyon head (NW Mediterranean). *Prog. Oceanogr.* 118, 28–46. doi: 10.1016/j.pocean.2013.07.031

Fernández-Méndez, M., Katlein, C., Rabe, B., Nicolaus, M., Peeken, I., Bakker, K., et al. (2015). Photosynthetic production in the central Arctic Ocean during the record sea-ice minimum in 2012. *Biogeosciences* 12, 3525–3549. doi: 10.5194/bg-12-3525-2015

Fernández-Méndez, M., Wenzhöfer, F., Peeken, I., Sørensen, H. L., Glud, R. N., and Boetius, A. (2014). Composition, buoyancy regulation and fate of ice algal aggregates in the central Arctic Ocean. *PLoS One* 9:e107452. doi: 10.1371/journal.pone.0107452

Frommlet, J., Guimarães, B., Sousa, L., Serôdio, J., and Alves, A. (2015). *Neptunomonas phycophila* sp. nov. isolated from a culture of *Symbiodinium* sp., a dinoflagellate symbiont of the sea anemone *Aiptasia tagetes*. *Int. J. Syst. Evol. Microbiol.* 65, 915–919. doi: 10.1099/ijs.0.000039

Galand, P. E., Casamayor, E. O., Kirchman, D. L., Potvin, M., and Lovejoy, C. (2009). Unique archaeal assemblages in the Arctic Ocean unveiled by massively parallel tag sequencing. *ISME J.* 3, 860–869. doi: 10.1038/ismej.2009.23

Galand, P. E., Lovejoy, C., Gv, Q. C., and Vincent, W. F. (2008). Microbial community diversity and heterotrophic production in a coastal Arctic ecosystem: a stamukhi lake and its source waters. *Limnol. Oceangr.* 53, 813–823. doi: 10.4319/lo.2008.53.2.0813

Galand, P. E., Potvin, M., Casamayor, E. O., and Lovejoy, C. (2010). Hydrography shapes bacterial biogeography of the deep Arctic Ocean. *ISME J.* 4, 564–576. doi: 10.1038/ismej.2009.134

Ghiglione, J.-F., Galand, P. E., Pommier, T., Pedrós-Alió, C., Maas, E. W., Bakker, K., et al. (2012). Pole-to-pole biogeography of surface and deep marine bacterial communities. *Proc. Natl. Acad. Sci. U.S.A.* 109, 17633–17638. doi: 10.1073/pnas.1208160109

Giebel, H.-A., Kalhoefer, D., Lemke, A., Thole, S., Gahl-Janssen, R., Simon, M., et al. (2011). Distribution of *Roseobacter* RCA and SAR11 lineages in the North Sea and characteristics of an abundant RCA isolate. *ISME J.* 5, 8–19. doi: 10.1038/ismej.2010.87

Gobet, A., Quince, C., and Ramette, A. (2010). Multivariate cutoff level analysis (MultiCoLA) of large community data sets. *Nucleic Acids Res.* 38:e155. doi: 10.1093/nar/gkq545

González, J., and Whitman, W. (2006). "Oceanospirillum and related genera," in *The Prokaryotes*, eds M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt (New York, NY: Springer), 887–915. doi: 10.1007/0-387-30746-x

Gosink, J. J., Herwig, R. P., and Staley, J. T. (1997). *Octadecabacter arcticus* gen. nov., sp. nov., and *O. antarcticus*, sp. nov., nonpigmented, psychrophilic gas vacuolate bacteria from polar sea ice and water. *Syst. Appl. Microbiol.* 20, 356–365. doi: 10.1016/S0723-2020(97)80003-3

Grassle, J. F., and Morse-Porteous, L. S. (1987). Macrofaunal colonization of disturbed deep-sea environments and the structure of deep-sea benthic communities. *Deep Sea Res. Part A Oceanogr. Res. Pap.* 34, 1911–1950. doi: 10.1016/0198-0149(87)90091-4

Grossart, H. P., Levold, F., Allgaier, M., Simon, M., and Brinkhoff, T. (2005). Marine diatom species harbour distinct bacterial communities. *Environ. Microbiol.* 7, 860–873. doi: 10.1111/j.1462-2920.2005.00759.x

Hamdan, L. J., Coffin, R. B., Sikaroodi, M., Greinert, J., Treude, T., and Gillevet, P. M. (2013). Ocean currents shape the microbiome of Arctic marine sediments. *ISME J.* 7, 685–696. doi: 10.1038/ismej.2012.143

Han, D., Kang, I., Ha, H. K., Kim, H. C., Kim, O. S., Lee, B. Y., et al. (2014). Bacterial communities of surface mixed layer in the pacific sector of the western Arctic Ocean during sea-ice melting. *PLoS One* 9:e86887. doi: 10.1371/journal.pone.0086887

Hardge, K., Peeken, I., Neuhaus, S., Lange, B. A., Stock, A., Stoeck, T., et al. (2017). The importance of sea ice for exchange of habitat-specific protist communities in the Central Arctic Ocean. *J. Mar. Syst.* 165, 124–138. doi: 10.1016/j.jmarsys.2016.10.004

Hatam, I., Charchuk, R., Lange, B., Beckers, J., Haas, C., and Lanoil, B. (2014). Distinct bacterial assemblages reside at different depths in Arctic multiyear sea ice. *FEMS Microbiol. Ecol.* 90, 115–125. doi: 10.1111/1574-6941.12377

Hatam, I., Lange, B., Beckers, J., Haas, C., and Lanoil, B. (2016). Bacterial communities from Arctic seasonal sea ice are more compositionally variable than those from multi-year sea ice. *ISME J.* 10, 2543–2552. doi: 10.1038/ismej.2016.4

Hedlund, B. P., Geiselbrecht, A. D., Bair, T. J., and Staley, J. T. (1999). Polycyclic aromatic hydrocarbon degradation by a new marine bacterium, *Neptunomonas naphthovorans* gen. nov., sp. nov. *Appl. Environ. Microbiol.* 65, 251–259.

Herfort, L., Schouten, S., Abbas, B., Veldhuis, M. J. W., Coolen, M. J. L., Wuchter, C., et al. (2007). Variations in spatial and temporal distribution of Archaea in the North Sea in relation to environmental variables. *FEMS Microbiol. Ecol.* 62, 242–257. doi: 10.1111/j.1574-6941.2007.00397.x

Heylen, K., Vanparys, B., Wittebolle, L., Verstraete, W., Boon, N., and De Vos, P. (2006). Cultivation of denitrifying bacteria: optimization of isolation conditions and diversity study. *Appl. Environ. Microbiol.* 72, 2637–2643. doi: 10.1128/AEM.72.4.2637-2643.2006

Humphry, D. R., George, A., Black, G. W., and Cummings, S. P. (2001). *Flavobacterium frigidarium* sp. nov., an aerobic, psychrophilic, xylanolytic and laminarinolytic bacterium from Antarctica. *Int. J. Syst. Evol. Microbiol.* 51, 1235–1243. doi: 10.1099/00207713-51-4-1235

Jakobsson, M., Mayer, L., Coakley, B., Dowdeswell, J. A., Forbes, S., Fridman, B., et al. (2012). The international bathymetric chart of the Arctic Ocean (IBCAO) version 3.0. *Geophys. Res. Lett.* 39:L12609. doi: 10.1029/2012GL052219

Johannessen, O. M., Bengtsson, L., Miles, M. W., Kuzmina, S. I., Semenov, V. A., Alekseev, G. V., et al. (2004). Arctic climate change: observed and modelled temperature and sea-ice variability. *Tellus Ser. A Dyn. Meteorol. Oceanogr.* 56, 328–341. doi: 10.1111/j.1600-0870.2004.00060.x

Junge, K., Imhoff, F., Staley, T., and Deming, J. W. (2002). Phylogenetic diversity of numerically important Arctic sea-ice bacteria cultured at subzero temperature. *Microb. Ecol.* 43, 315–328. doi: 10.1007/s00248-001-1026-4

Katlein, C., Fernández-Méndez, M., Wenzhöfer, F., and Nicolaus, M. (2014). Distribution of algal aggregates under summer sea ice in the Central Arctic. *Polar Biol.* 38, 719–731. doi: 10.1007/s00300-014-1634-3

Kirchman, D. L., Cottrell, M. T., and Lovejoy, C. (2010). The structure of bacterial communities in the western Arctic Ocean as revealed by pyrosequencing of 16S rRNA genes. *Environ. Microbiol.* 12, 1132–1143. doi: 10.1111/j.1462-2920.2010.02154.x

Kirchman, D. L., Elifantz, H., Dittel, A. I., Malmstrom, R. R., and Cottrell, M. T. (2007). Standing stocks and activity of Archaea and Bacteria in the western Arctic Ocean. *Limnol. Oceanogr.* 52, 495–507. doi: 10.4319/lo.2007.52.2.0495

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., et al. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* 41:e1. doi: 10.1093/nar/gks808

Knoll, S., Zwisler, W., and Simon, M. (2001). Bacterial colonization of early stages of limnetic diatom microaggregates. *Aquat. Microb. Ecol.* 25, 141–150. doi: 10.3354/ame025141

Lalande, C., Nöthig, E. M., Somavilla, R., Bauerfeind, E., Shevchenko, V., and Okolodkov, Y. (2014). Variability in under-ice export fluxes of biogenic matter in the Arctic Ocean. *Glob. Biogeochem. Cycles* 28, 571–583. doi: 10.1002/2013GB004735

Lee, S. H., McRoy, C. P., Joo, H. M., Gradinger, R., Cui, X. H., Yun, M. S., et al. (2011). Holes in progressively thinning Arctic sea ice lead to new ice algae habitat. *Oceanography* 24, 302–308. doi: 10.5670/oceanog.2011.81

Lemarchand, C., Jardillier, L., Carrias, J. F., Richardot, M., Debroas, D., Sime-Ngando, T., et al. (2006). Community composition and activity of prokaryotes associated to detrital particles in two contrasting lake ecosystems. *FEMS Microbiol. Ecol.* 57, 442–451. doi: 10.1111/j.1574-6941.2006.00131.x

Leu, E., Søreide, J. E., Hessen, D. O., Falk-Petersen, S., and Berge, J. (2011). Consequences of changing sea-ice cover for primary and secondary producers in the European Arctic shelf seas: timing, quantity, and quality. *Prog. Oceanogr.* 90, 18–32. doi: 10.1016/j.pocean.2011.02.004

Li, W. K., McLaughlin, F. A., Lovejoy, C., and Carmack, E. C. (2009). Smallest algae thrive as the Arctic Ocean freshens. *Science* 326, 539–539. doi: 10.1126/science.1179798

Lima-Mendez, G., Faust, K., Henry, N., Decelle, J., Colin, S., Carcillo, F., et al. (2015). Determinants of community structure in the global plankton interactome. *Science* 348:1262073. doi: 10.1126/science.1262073

Lindh, M. V., Maillot, B. M., Shulse, C. N., Gooday, A. J., Amon, D. J., Smith, C. R., et al. (2017). From the surface to the deep-sea: bacterial distributions across polymetallic nodule fields in the clarion-clipperton zone of the Pacific Ocean. *Front. Microbiol.* 8:1696. doi: 10.3389/fmicb.2017.01696

Llobet-Brossa, E., Rossello-Mora, R., and Amann, R. (1998). Microbial community composition of Wadden Sea sediments as revealed by fluorescence in situ hybridization. *Appl. Environ. Microbiol.* 64, 2691–2696.

Mahé, F., Rognes, T., Quince, C., de Vargas, C., and Dunthorn, M. (2015). Swarm v2: highly-scalable and high-resolution amplicon clustering. *PeerJ* 3:e1420. doi: 10.7717/peerj.1420

Mangiafico, S. (2017). *rcompanion: Functions to Support Extension Education Program Evaluation - R Package Version 1.11.1*. Available at: https://cran.r-project.org/package=rcompanion [accessed February 14, 2018].

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J.* 17:10. doi: 10.14806/ej.17.1.200

Maslanik, J. A., Fowler, C., Stroeve, J., Drobot, S., Zwally, J., Yi, D., et al. (2007). A younger, thinner Arctic ice cover: increased potential for rapid, extensive sea-ice loss. *Geophys. Res. Lett.* 34:L24501. doi: 10.1029/2007GL032043

Melnikov, I. A., and Bondarchuk, L. L. (1987). Ecology of mass accumulations of colonial diatom algae under drifting Arctic ice. *Oceanology* 27, 233–236. doi: 10.1594/PANGAEA.756627

Methe, B. A., Nelson, K. E., Deming, J. W., Momen, B., Melamud, E., Zhang, X., et al. (2005). The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. *Proc. Natl. Acad. Sci. U.S.A.* 102, 10913–10918. doi: 10.1073/pnas.0504766102

Mikkelsen, D. M., Rysgaard, S., and Glud, R. N. (2008). Microalgal composition and primary production in Arctic sea ice: a seasonal study from Kobbefjord (Kangerluarsunnguaq). West Greenland. *Mar. Ecol. Prog. Ser.* 368, 65–74. doi: 10.3354/meps07627

Miyazaki, M., Nogi, Y., Fujiwara, Y., and Kawato, M. (2008). *Neptunomonas japonica* sp. nov., an *Osedax japonicus* symbiont-like bacterium isolated from sediment adjacent to sperm whale carcasses off Kagoshima, Japan. *Int. J. Syst. Evol. Microbiol.* 58, 866–871. doi: 10.1099/ijs.0.65509-0

Nagata, T., Fukuda, H., Fukuda, R., and Koike, I. (2000). Bacterioplankton distribution and production in deep Pacific waters: large-scale geographic variations and possible coupling with sinking particle fluxes. *Limnol. Oceanogr.* 45, 426–435. doi: 10.4319/lo.2000.45.2.0426

Nöthig, E.-M., Bracher, A., Engel, A., Metfies, K., Niehoff, B., Peeken, I., et al. (2015). Summertime plankton ecology in Fram Strait — a compilation of long- and short-term observations. *Polar Res.* 34:23349. doi: 10.3402/polar.v34.23349

Notz, D., and Stroeve, J. (2016). Observed Arctic sea-ice loss directly follows anthropogenic CO_2 emission. *Science* 354, 747–750. doi: 10.1126/science.aag2345

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., et al. (2014). *vegan: Community Ecology Package - R Package Version 2.2-0*. Available at: http://cran.r-project.org/package=vegan

Orsi, W. D., Smith, J. M., Liu, S., Liu, Z., Sakamoto, C. M., Wilken, S., et al. (2016). Diverse, uncultivated bacteria and archaea underlying the cycling of dissolved protein in the ocean. *ISME J.* 10, 2158–2173. doi: 10.1038/ismej.2016.20

Overland, J., Hanna, E., Hanssen-Bauer, I., Kim, S.-J., Walsh, J. E., Wang, M., et al. (2015). *Surface Air Temperature*. Arctic Report Card 2015.

Overland, J. E., and Wang, M. (2013). When will the summer Arctic be nearly sea ice free? *Geophys. Res. Lett.* 40, 2097–2101. doi: 10.1002/grl.50316

Parada, A. E., Needham, D. M., and Fuhrman, J. A. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ. Microbiol.* 18, 1403–1414. doi: 10.1111/1462-2920.13023

Pinhassi, J., Sala, M. M., Havskum, H., Peters, F., Guadayol, Ò., Malits, A., et al. (2004). Changes in bacterioplankton composition under different phytoplankton regimens. *Appl. Environ. Microbiol.* 70, 6753–6766. doi: 10.1128/AEM.70.11.6753-6766.2004

Polyakov, I. V., Walsh, J. E., and Kwok, R. (2012). Recent changes of Arctic multiyear sea ice coverage and the likely causes. *Bull. Am. Meteorol. Soc.* 93, 145–151. doi: 10.1175/BAMS-D-11-00070.1

Poremba, K. (1994). Simulated degradation of phytodetritus in deep- sea sediments of the NE Atlantic (47° N, 19° W). *Mar. Ecol. Prog. Ser.* 105, 291–299. doi: 10.3354/meps105291

Poulin, M., Underwood, G. J. C., and Michel, C. (2014). Sub-ice colonial *Melosira arctica* in Arctic first-year ice. *Diatom Res.* 29, 213–221. doi: 10.1080/0269249X.2013.877085

Prokopowich, C. D., Gregory, T. R., and Crease, T. J. (2003). The correlation between rDNA copy number and genome size in eukaryotes. *Genome* 46, 48–50. doi: 10.1139/g02-103

Pruesse, E., Peplies, J., and Glöckner, F. O. (2012). SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 28, 1823–1829. doi: 10.1093/bioinformatics/bts252

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596. doi: 10.1093/nar/gks1219

R Core Team (2014). *R: A Language and Environment for Statistical Computing*. Available at: http://www.r-project.org/

Rabe, B., Wisotzki, A., Rettig, S., Somavilla Cabrillo, R., and Sander, H. (2013). *Physical Oceanography Measured on Water Bottle Samples During Polarstern Cruise ARK-XXVII/3*. Bremerhaven: Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research. doi: 10.1594/PANGAEA.819452

Raghukumar, S. (2002). Ecology of the marine protists, the Labyrinthulomycetes (Thraustochytrids and Labyrinthulids). *Eur. J. Protistol.* 38, 127–145. doi: 10.1078/0932-4739-00832

Ramanan, R., Kim, B.-H., Cho, D.-H., Oh, H.-M., and Kim, H.-S. (2016). Algae–bacteria interactions: evolution, ecology and emerging applications. *Biotechnol. Adv.* 34, 14–29. doi: 10.1016/J.BIOTECHADV.2015.12.003

Rapp, J. Z., Fernández-Méndez, M., Bienhold, C., and Boetius, A. (2017). Bacterial and eukaryotic operational taxonomic units (OTU) in sea ice, water and deep-sea sediment samples of the Central Arctic collected during POLARSTERN cruise ARK-XXVII/3 (IceArc) in 2012. *PANGAEA*. doi: 10.1594/PANGAEA.882580

Rintala, J. M., Piiparinen, J., Blomster, J., Majaneva, M., Müller, S., Uusikivi, J., et al. (2014). Fast direct melting of brackish sea-ice samples results in biologically more accurate results than slow buffered melting. *Polar Biol.* 37, 1811–1822. doi: 10.1007/s00300-014-1563-1

Rösel, A., and Kaleschke, L. (2012). Exceptional melt pond occurrence in the years 2007 and 2011 on the Arctic sea ice revealed from MODIS satellite data. *J. Geophys. Res. Oceans* 117:C05018. doi: 10.1029/2011JC007869

Ruff, E. S., Probandt, D., Zinkann, A.-C. C., Iversen, M. H., Klaas, C., Würzberg, L., et al. (2014). Indications for algae-degrading benthic microbial communities in deep-sea sediments along the Antarctic Polar Front. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 108, 6–16. doi: 10.1016/j.dsr2.2014.05.011

Schweitzer, B., Huber, I., Amann, R., Ludwig, W., and Simon, M. (2001). α- and β-Proteobacteria control the consumption and release of amino acids on lake snow aggregates. *Appl. Environ. Microbiol.* 67, 632–645. doi: 10.1128/AEM.67.2.632-645.2001

Shade, A., and Handelsman, J. (2012). Beyond the Venn diagram: the hunt for a core microbiome. *Environ. Microbiol.* 14, 4–12. doi: 10.1111/j.1462-2920.2011.02585.x

Soltwedel, T. (2016). The expeditions PS99.1 and PS99.2 of the research vessel POLARSTERN to the Fram strait in 2016. *Ber. Polar Meeresforsch.* 704:107.

Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M. D. M., Breiner, H. W., et al. (2010). Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Mol. Ecol.* 19, 21–31. doi: 10.1111/j.1365-294X.2009.04480.x

Tamburini, C., Boutrif, M., Garel, M., Colwell, R. R., and Deming, J. W. (2013). Prokaryotic responses to hydrostatic pressure in the ocean - a review. *Environ. Microbiol.* 15, 1262–1274. doi: 10.1111/1462-2920.12084

Tamburini, C., Garcin, J., Grégori, G., Leblanc, K., Rimmelin, P., and Kirchman, D. L. (2006). Pressure effects on surface Mediterranean prokaryotes and biogenic silica dissolution during a diatom sinking experiment. *Aquat. Microb. Ecol.* 43, 267–276. doi: 10.3354/ame043267

Tamelander, T., Reigstad, M., Hop, H., and Ratkova, T. (2009). Ice algal assemblages and vertical export of organic matter from sea ice in the Barents Sea and Nansen Basin (Arctic Ocean). *Polar Biol.* 32, 1261–1273. doi: 10.1007/s00300-009-0622-5

Teeling, H., Fuchs, B. M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C. M., et al. (2012). Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science* 336, 608–611. doi: 10.1126/science.1218344

Thaler, M., and Lovejoy, C. (2015). Biogeography of heterotrophic flagellate populations indicates the presence of generalist and specialist taxa in the Arctic Ocean. *Appl. Environ. Microbiol.* 81, 2137–2148. doi: 10.1128/AEM.02737-14

Thamdrup, B., Rosselló-Mora, R., and Amann, R. (2000). Microbial manganese and sulfate reduction in Black Sea shelf sediments. *Appl. Environ. Microbiol.* 66, 2888–2897. doi: 10.1128/AEM.66.7.2888-2897.2000

Tremblay, J. É., Anderson, L. G., Matrai, P., Coupel, P., Bélanger, S., Michel, C., et al. (2015a). Global and regional drivers of nutrient supply, primary production and CO_2 drawdown in the changing Arctic Ocean. *Prog. Oceanogr.* 139, 171–196. doi: 10.1016/j.pocean.2015.08.009

Tremblay, J., Singh, K., Fern, A., Kirton, E. S., He, S., Woyke, T., et al. (2015b). Primer and platform effects on 16S rRNA tag sequencing. *Front. Microbiol.* 6:771. doi: 10.3389/fmicb.2015.00771

Tremblay, J. -É., and Gagnon, J. (2009). "The effects of irradiance and nutrient supply on the productivity of Arctic waters: a perspective on climate change," in *Proceedings of the NATO Advanced Research Workshop on Influence of Climate Change on the Changing Arctic: Influence of Climate Change on the Changing Arctic and Sub-Arctic Conditions*, Liège, 73–93. doi: 10.1007/978-1-4020-9460-6_7

Turkey, C. M. (1993). The effect of pressure on leucine and thymidine incorporation by free-living bacteria and by bacteria attached to sinking oceanic particles. *Deep Res. Part I* 40, 2193–2206. doi: 10.1016/0967-0637(93)90098-N

Turley, C. M., and Lochte, K. (1990). Microbial response to the input of fresh detritus to the deep-sea bed. *Glob. Planet. Change* 3, 3–23. doi: 10.1016/0921-8181(90)90052-E

Turley, C. M., and Mackie, P. J. (1995). Bacterial and cyanobacterial flux to the deep NE Atlantic on sedimenting particles. *Deep Res. Part I* 42, 1453–1474. doi: 10.1016/0967-0637(95)00056-C

von Scheibner, M., Sommer, U., and Jürgens, K. (2017). Tight coupling of *Glaciecola* spp. and diatoms during cold-water phytoplankton spring blooms. *Front. Microbiol.* 8:27. doi: 10.3389/fmicb.2017.00027

Walsh, E. A., Kirkpatrick, J. B., Rutherford, S. D., Smith, D. C., Sogin, M., and D'Hondt, S. (2016). Bacterial diversity and community composition from seasurface to subseafloor. *ISME J.* 10, 979–989. doi: 10.1038/ismej.2015.175

Wassmann, P. (2011). Arctic marine ecosystems in an era of rapid climate change. *Prog. Oceanogr.* 90, 1–17. doi: 10.1016/j.pocean.2011.02.002

Wegner, C., Wittbrodt, K., Hölemann, J. A., Janout, M. A., Krumpen, T., Selyuzhenok, V., et al. (2017). Sediment entrainment into sea ice and transport in the Transpolar Drift: a case study from the Laptev Sea in winter 2011/2012. *Cont. Shelf Res.* 141, 1–10. doi: 10.1016/j.csr.2017.04.010

Wheeler, B., and Torchiano, M. (2016). *lmPerm: Permutation Tests for Linear Models - R Package Version 2.1.0*. Available at: https://cran.r-project.org/package=lmPerm [accessed February 14, 2018].

Williams, T. J., Wilkins, D., Long, E., Evans, F., DeMaere, M. Z., Raftery, M. J., et al. (2013). The role of planktonic *Flavobacteria* in processing algal organic matter in coastal East Antarctica revealed using metagenomics and metaproteomics. *Environ. Microbiol.* 15, 1302–1317. doi: 10.1111/1462-2920.12017

Wilson, B., Müller, O., Nordmann, E.-L., Seuthe, L., Bratbak, G., and Øvreås, L. (2017). Changes in marine prokaryote composition with season and depth over an Arctic polar year. *Front. Mar. Sci.* 4:95. doi: 10.3389/fmars.2017.00095

Wirsen, C. O., Sievert, S. M., Cavanaugh, C. M., Molyneaux, S. J., Ahmad, A., Taylor, L. T., et al. (2002). Characterization of an autotrophic sulfide-oxidizing marine *Arcobacter* sp. that produces filamentous sulfur. *Appl. Environ. Microbiol.* 68, 316–325. doi: 10.1128/AEM.68.1.316-325.2002

Yilmaz, P., Kottmann, R., Field, D., Knight, R., Cole, J. R., Amaral-Zettler, L., et al. (2011). Minimum information about a marker gene sequence (MIMARKS) and minimum information about any (x) sequence (MIxS) specifications. *Nat. Biotechnol.* 29, 415–420. doi: 10.1038/nbt.1823

Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A. (2014). PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* 30, 614–620. doi: 10.1093/bioinformatics/btt593

Zhang, X.-Y., Zhang, Y.-J., Yu, Y., Li, H.-J., Gao, Z.-M., Chen, X.-L., et al. (2010). *Neptunomonas antarctica* sp. nov., isolated from marine sediment. *Int. J. Syst. Evol. Microbiol.* 60, 1958–1961. doi: 10.1099/ijs.0.017756-0

Zhu, F., Massana, R., Not, F., Marie, D., and Vaulot, D. (2005). Mapping of picoeukaryotes in marine ecosystems with quantitative PCR of the 18S rRNA gene. *FEMS Microbiol. Ecol.* 52, 79–92. doi: 10.1016/j.femsec.2004.10.006

Zinger, L., Amaral-Zettler, L. A., Fuhrman, J. A., Horner-Devine, M. C., Huse, S. M., Welch, D. B. M., et al. (2011). Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. *PLoS One* 6:e24570. doi: 10.1371/journal.pone.0024570

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Supplementary Material

Effects of Ice-Algal Aggregate Export on the Connectivity of Bacterial Communities in the Central Arctic Ocean

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1 Supplementary Information

1.1 Material and Methods

1.1.1 Automated Ribosomal Intergenic Spacer Analysis (ARISA)

To corroborate the observed bacterial community patterns, we used Automated Ríbosomal Intergenic Spacer Analysis (Fisher and Triplett, 1999) in parallel to the tag sequencing approach. Following a similar approach, we covered all nine stations, with one sample from sea-ice surface and bottom, water column and pooled sediment samples. A larger number of samples from individual melt ponds, sea-ice algal aggregates and holothurian digestive tract content was available for ARISA, leaving us with a total number of 68 different samples.

The PCR mix for the ITS region contained 2.5 µl of 10x reaction buffer S (PEQLAB Biotechnologie GmbH, Erlangen, Germany), 1 µl of 25 mM MgCl2 (PEQLAB Biotechnologie GmbH, Erlangen, Germany), 0.625 µl of a 10 mM dNTP mix (PEQLAB Biotechnologie GmbH, Erlangen, Germany), 0.75 µl of 3 mg ml-1 bovine serum albumin (Sigma-Aldrich Chemie Gmbh; Munich, Germany), 0.25 µl of 40 µM universal forward primer ITSF (5'- GTCGTAACAAGGTAGCCGTA-3') (Biomers.net, Ulm, Germany), labeled with 6- carboxyfluorescein (FAM), 0.25 µl of 40 µM ITSReub reverse primer (5'- GCCAAGGCATCCACC-3') (Biomers.net, Ulm, Germany), 0.25 µl of 5 units µl-1 Taq polymerase (PEQLAB Biotechnologie GmbH, Erlangen, Germany) and approximately 10 ng environmental DNA. PCR water was added to each reaction mix to a final volume of 25 µl. Additionally, control reactions with either no template DNA (negative), or with DNA from sediment sampled on Sylt, Germany, (positive) were performed. All reactions were conducted in triplicates. The PCR was carried out in an Eppendorf MasterCycler (Eppendorf AG, Hamburg, Germany). After an initial denaturation for 3 min at 94°C, 30 cycles of first 94°C for 45 sec, 55°C for 45 sec and 72°C for 90 sec followed. Final extension time was 5 min at 72°C. PCR products were checked on a 1.5% agarose gel (3 g of LE agarose (Biozym Scientific GmbH, Hessisch Oldendorf, Germany) in 200 ml of 1x TAE (Tris-acetate-EDTA)-buffer), and PCR products were purified using SephadexTM G-50

Superfine (GE Healthcare, Bio-Sciences AB, Uppsala, Sweden) to remove low molecular substances and other contaminants.

Fragment analysis of the PCR products was done via capillary electrophoresis. A standardized amount of 100 ng amplified DNA was mixed with 0.5 µl of the internal size standard MapMarker® 1000, labeled with ROX (BioVentures, Inc. Murfreesboro, TN, USA), and 14 µl of deionized HiDiTM formamide (Applied Biosystems, California, USA). Samples were denatured at 95°C for 3 min (Eppendorf Mastercycler gradient, Eppendorf, Hamburg, Germany) and immediately put on ice for 5 min. Capillary electrophoresis was carried out on an ABI Prism 3130 XL - Genetic Analyzer (Applied Biosystems, California, USA).

1.1.1.1 ARISA Statistical analysis

Electropherograms were analyzed using the GeneMapper Software v3.7 (Applied Biosystems, California, USA). Subsequent binning of peaks was performed in R (R-Project; R Foundation for Statistical Computing Version 2.14.0; R Development Core Team, 2011, http://www.R-project.org), using a custom R script (Interactive Binner function, www.mpi-bremen.de/en/Software-4.html#section1549) (Ramette 2009) and a window size of 2 base pairs (bp) to compensate for slight technical shifts between profiles and fragment size calling imprecision. Only fragments with a length between 100 bp and 1000 bp and peaks with ≥50 relative fluorescence units and ≥0.09% relative fluorescence intensity (individual peak areas divided by the total peak area of the respective sample) were considered. Replicate PCR profiles were merged, using a custom R script (https://www.mpi-bremen.de/Binaries/Binary1658/replicate-merger-ALk-consensus-RFI-1.2.r) and OTU_{ARISA} were considered present if appearing in at least two of the three PCR replicates.

1.2 Results

1.2.1 ARISA Results

Analysis of the bacterial community structure revealed differences between the investigated environments. NMDS showed an apparent clustering of samples from the sea-ice environment (including all ice and melt pond samples), the water column (including all surface seawater and water under the ice samples) and the deep sea (including all samples from deep-sea sediments, holothurian digestive tract content and deposited aggregates), when grouping the data set a posteriori according to the different environments (Figure S8). ANOSIM confirmed significant differences of bacterial community structure between the sea-ice and the deep-sea environments (R=0.81; Bonferroni-corrected p=0.003), and even more pronounced structural differences between the water column and the deep-sea environment (R=0.99; Bonferroni-corrected p=0.003). Sea-ice bacterial community structure was highly dissimilar from any of the other investigated environments, but appeared host communities partially overlapping with those found in the water column (R=0.63; Bonferroni-corrected p=0.003) and in melt ponds (R= 0.41; Bonferroni-corrected p=0.003).

All, sea ice, melt ponds and surface seawater, showed strongest structural differences to the deep-sea environments. The overall observed bacterial community pattern using NMDS on the ARISA results resembled community patterns observed with Illumina tag sequencing (Figure 4 & Figure S8), and dissimilarity matrices from both methods were significantly correlated (Figure S9).

1.3 Discussion

The observed OTU richness in ice-associated environments and surface seawater, both for bacteria and eukaryotes (Figure 2), was in the range of results reported from previous Arctic molecular surveys based on next- generation sequencing of the SSU rRNA genes (Bowman et al., 2012; Kilias et al., 2014a, 2014b; Meshram et al., 2017; Stecher et al., 2016; Thaler and Lovejoy, 2015), but lower than numbers reported from Arctic coastal regions and marginal seas (Comeau et al., 2013; Ghiglione et al., 2012; Hatam et al., 2014, 2016). OTU richness in sediments was also comparable to, but at the lower end of OTU numbers reported for sediments from the Siberian continental margin (Bienhold et al., 2012) and the Fram Strait (Jacob et al., 2013). The wide Arctic shelves have a higher nutrient availability (Le Fouest et al., 2013; Garneau et al., 2006) and sustain higher productivity and microbial standing stocks (Tremblay et al., 2011), and it is therefore likely that they allow for higher diversity than the oligotrophic central basins (Horner-Devine et al., 2003).

1.3.1 Connectivity of microbial communities in the central Arctic Ocean and potential effects of climate change

Bacterial generalists, represented by members of the genera *Colwellia*, *Oleispira* and *Lentimonas*, showed very high sequence numbers in ice-associated environments, water column and ice-algae deposits, and low proportions in sediment. This may indicate that they also originate from surface environments, and are not indigenous benthic bacteria. The genus *Colwellia* exhibits distinct

adaptations to life at low temperatures (Huston et al., 2004) and has been found in a range of environments, including sea ice (Bowman et al., 1997), deep-sea sediment (Deming et al., 1988) and associated to particles or algae in seawater (Bowman et al., 1998). Its capability to degrade high-molecular-weight organic compounds suggests an important role of this genus in carbon and nutrient cycling in cold environments (Methe et al., 2005). Similarly, *Oleispira* contains several cold-adaptations in its genome (Kube et al., 2013) and its presence was reported from polar seawater and the deep sea (Li et al., 2015; Yakimov et al., 2003). Interestingly, this genus is part of an ecophysiologically unusual group of bacteria whose metabolism is restricted to the degradation of hydrocarbons, and its presence has so far always been attributed to environments contaminated with crude oil (Yakimov et al., 2003); their role in the Arctic environments sampled here thus remains to be determined. Very little is known about the genus *Lentimonas*. Its presence has been reported for seawater and sediment (Freitas et al., 2012), and its close relatives from the *Verrucomicrobia* phylum are known for their capacity to degrade highly complex polysaccharide substrates (Cardman et al., 2014; Martinez-Garcia et al., 2012), therefore suggesting a potential role of *Lentimonas* in organic matter cycling. Bacterial groups transported with the algal aggregates may thus locally influence carbon turnover of the aggregates at the seafloor, yet their persistence and contribution to the degradation and recycling of organic material remains unclear until temporal observations and measures of activity become available. The representation of surface-derived cells in the sediment may potentially increase after repeated deposition events or longer exposure of the aggregates at the seafloor (Kellogg and Deming, 2009).

References

Bienhold, C., Boetius, A., and Ramette, A. (2012). The energy-diversity relationship of complex bacterial communities in Arctic deep-sea sediments. *ISME J.* 6, 724–32. doi:10.1038/ismej.2011.140.

Bowman, J. P., Brown, M. V., and Nichols, D. S. (1997). Biodiversity and ecophysiology of bacteria associated with Antarctic sea ice. *Antarct. Sci.* 9, 134–142. doi:10.1017/S0954102097000175.

Bowman, J. P., Gosink, J. J., McCammon, S. A., Lewis, T. E., Nichols, D. S., Nichols, P. D., et al. (1998). Colwellia demingiae sp. nov., Colwellia hornerae sp. nov., Colwellia rossensis sp. nov. and Colwellia psychrotropica sp. nov.: psychrophilic Antarctic species with the ability to synthesize docosahexaenoic acid (22: 63). *Int. J. Syst. Bacteriol.* 48, 1171–1180. doi:10.1099/00207713-48-4-1171.

Bowman, J. S., Rasmussen, S., Blom, N., Deming, J. W., Rysgaard, S., and Sicheritz-Ponten, T. (2012). Microbial community structure of Arctic multiyear sea ice and surface seawater by 454 sequencing of the 16S RNA gene. *ISME J.* 6, 11–20. doi:10.1038/ismej.2011.76.

Cardman, Z., Arnosti, C., Durbin, A., Ziervogel, K., Cox, C., Steen, A. D., et al. (2014). Verrucomicrobia are candidates for polysaccharide-degrading bacterioplankton in an Arctic fjord of Svalbard. *Appl. Environ. Microbiol.* 80, 3749–3756. doi:10.1128/AEM.00899-14.

Comeau, A. M., Philippe, B., Thaler, M., Gosselin, M., Poulin, M., and Lovejoy, C. (2013). Protists in Arctic drift and land-fast sea ice. *J. Phycol.* 49, 229–240. doi:10.1111/jpy.12026.

Deming, J. W., Somers, L. K., Straube, W. L., Swartz, D. G., and Macdonell, M. T. (1988). Isolation of an Obligately Barophilic Bacterium and Description of a New Genus, Colwellia gen. nov. *Syst. Appl. Microbiol.* 10, 152–160. doi:10.1016/S0723-2020(88)80030-4.

Fisher, M. M., and Triplett, E. W. (1999). Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Appl. Environ. Microbiol.* 65, 4630–4636. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10508099 [Accessed November 5, 2017].

Le Fouest, V., Babin, M., and Tremblay, J. E. (2013). The fate of riverine nutrients on Arctic shelves. *Biogeosciences* 10, 3661–3677. doi:10.5194/bg-10-3661-2013.

Freitas, S., Hatosy, S., Fuhrman, J. A., Huse, S. M., Welch, D. B. M., Sogin, M. L., et al. (2012). Global distribution and diversity of marine Verrucomicrobia. *ISME J.* 6, 1499–1505. doi:10.1038/ismej.2012.3.

Garneau, M.-ève, Vincent, W. F., Alonso-sáez, L., Gratton, Y., and Lovejoy, C. (2006). Prokaryotic community structure and heterotrophic production in a river-influenced coastal arctic ecosystem. 42, 27–40.

Ghiglione, J.-F., Galand, P. E., Pommier, T., Pedrós-Alió, C., Maas, E. W., Bakker, K., et al. (2012). Pole-to-pole biogeography of surface and deep marine bacterial communities. *Proc. Natl. Acad. Sci. U. S. A.* 109, 17633–8. doi:10.1073/pnas.1208160109.

Hatam, I., Charchuk, R., Lange, B., Beckers, J., Haas, C., and Lanoil, B. (2014). Distinct bacterial assemblages reside at different depths in Arctic multiyear sea ice. *FEMS Microbiol. Ecol.* 90, 115–125. doi:10.1111/1574-6941.12377.

Hatam, I., Lange, B., Beckers, J., Haas, C., and Lanoil, B. (2016). Bacterial communities from Arctic seasonal sea ice are more compositionally variable than those from multi-year sea ice. *ISME J.* 10, 2543–2552. doi:10.1038/ismej.2016.4.

Horner-Devine, M. C., Leibold, M. A., Smith, V. H., and Bohannan, B. J. M. (2003). Bacterial diversity patterns along a gradient of primary productivity. *Ecol. Lett.* 6, 613–622. doi:10.1046/j.1461-0248.2003.00472.x.

Huston, A. L., Methe, B., and Deming, J. W. (2004). Purification, characterization, and sequencing of an extracellular cold-active aminopeptidase produced by marine psychrophile Colwellia psychrerythraea strain 34H. *Appl. Environ. Microbiol.* 70, 3321–3328. doi:10.1128/AEM.70.6.3321-3328.2004.

Jacob, M., Soltwedel, T., Boetius, A., and Ramette, A. (2013). Biogeography of Deep-sea benthic bacteria at regional scale (LTER HAUSGARTEN, Fram Strait, Arctic). *PLoS One* 8, e72779. doi:10.1371/journal.pone.0072779.

Kellogg, C., and Deming, J. (2009). Comparison of free-living, suspended particle, and aggregate-associated Bacterial and Archaeal communities in the Laptev Sea. *Aquat. Microb. Ecol.* 57, 1–

18. doi:10.3354/ame01317.

Kilias, E., Kattner, G., Wolf, C., Frickenhaus, S., and Metfies, K. (2014a). A molecular survey of protist diversity through the central Arctic Ocean. *Polar Biol.* 37, 1271–1287. doi:10.1007/s00300-014-1519-5.

Kilias, E. S., Peeken, I., and Metfies, K. (2014b). Insight into protist diversity in Arctic sea ice and melt-pond aggregate obtained by pyrosequencing. *Polar Res.* 33, 23466. doi:10.3402/polar.v33.23466.

Kube, M., Chernikova, T. N., Al-Ramahi, Y., Beloqui, A., Lopez-Cortez, N., Guazzaroni, M.-E., et al. (2013). Genome sequence and functional genomic analysis of the oil-degrading bacterium Oleispira antarctica. *Nat. Commun.* 4, 2156. doi:10.1038/ncomms3156.

Li, Y., Liu, Q., Li, C., Dong, Y., Zhang, W., Zhang, W., et al. (2015). Bacterial and archaeal community structures in the Arctic deep-sea sediment. *Acta Oceanol. Sin.* 34, 93–113. doi:10.1007/s13131-015-0624-9.

Martinez-Garcia, M., Brazel, D. M., Swan, B. K., Arnosti, C., Chain, P. S. G., Reitenga, K. G., et al. (2012). Capturing single cell genomes of active polysaccharide degraders: An unexpected contribution of verrucomicrobia. *PLoS One* 7, e35314. doi:10.1371/journal.pone.0035314.

Meshram, A. R., Vader, A., Kristiansen, S., and Gabrielsen, T. M. (2017). Microbial Eukaryotes in an Arctic Under-Ice Spring Bloom North of Svalbard. *Front. Microbiol.* 8, 1099. doi:10.3389/fmicb.2017.01099.

Methe, B. A., Nelson, K. E., Deming, J. W., Momen, B., Melamud, E., Zhang, X., et al. (2005). The psychrophilic lifestyle as revealed by the genome sequence of Colwellia psychrerythraea 34H through genomic and proteomic analyses. *Proc. Natl. Acad. Sci.* 102, 10913–10918. doi:10.1073/pnas.0504766102.

Stecher, A., Neuhaus, S., Lange, B., Frickenhaus, S., Beszteri, B., Kroth, P. G., et al. (2016). rRNA and rDNA based assessment of sea ice protist biodiversity from the central Arctic Ocean. *Eur. J. Phycol.* 51, 31–46. doi:10.1080/09670262.2015.1077395.

Thaler, M., and Lovejoy, C. (2015). Biogeography of heterotrophic flagellate populations indicates the presence of generalist and specialist taxa in the Arctic Ocean. *Appl. Environ. Microbiol.* 81, 2137–2148. doi:10.1128/AEM.02737-14.

Tremblay, J.-É., Bélanger, S., Barber, D. G., Asplin, M., Martin, J., Darnis, G., et al. (2011). Climate forcing multiplies biological productivity in the coastal Arctic Ocean. *Geophys. Res. Lett.* 38, n/a-n/a. doi:10.1029/2011GL048825.

Yakimov, M. M., Giuliano, L., Gentile, G., Crisafi, E., Chernikova, T. N., Abraham, W. R., et al. (2003). Oleispira antarctica gen. nov., sp. nov., a novel hydrocarbonoclastic marine bacterium isolated from Antarctic coastal sea water. *Int. J. Syst. Evol. Microbiol.* 53, 779–785. doi:10.1099/ijs.0.02366-0.

Supplementary Material

Effects of Ice-Algal Aggregate Export on the Connectivity of Bacterial Communities in the Central Arctic Ocean

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1 Supplementary Figures

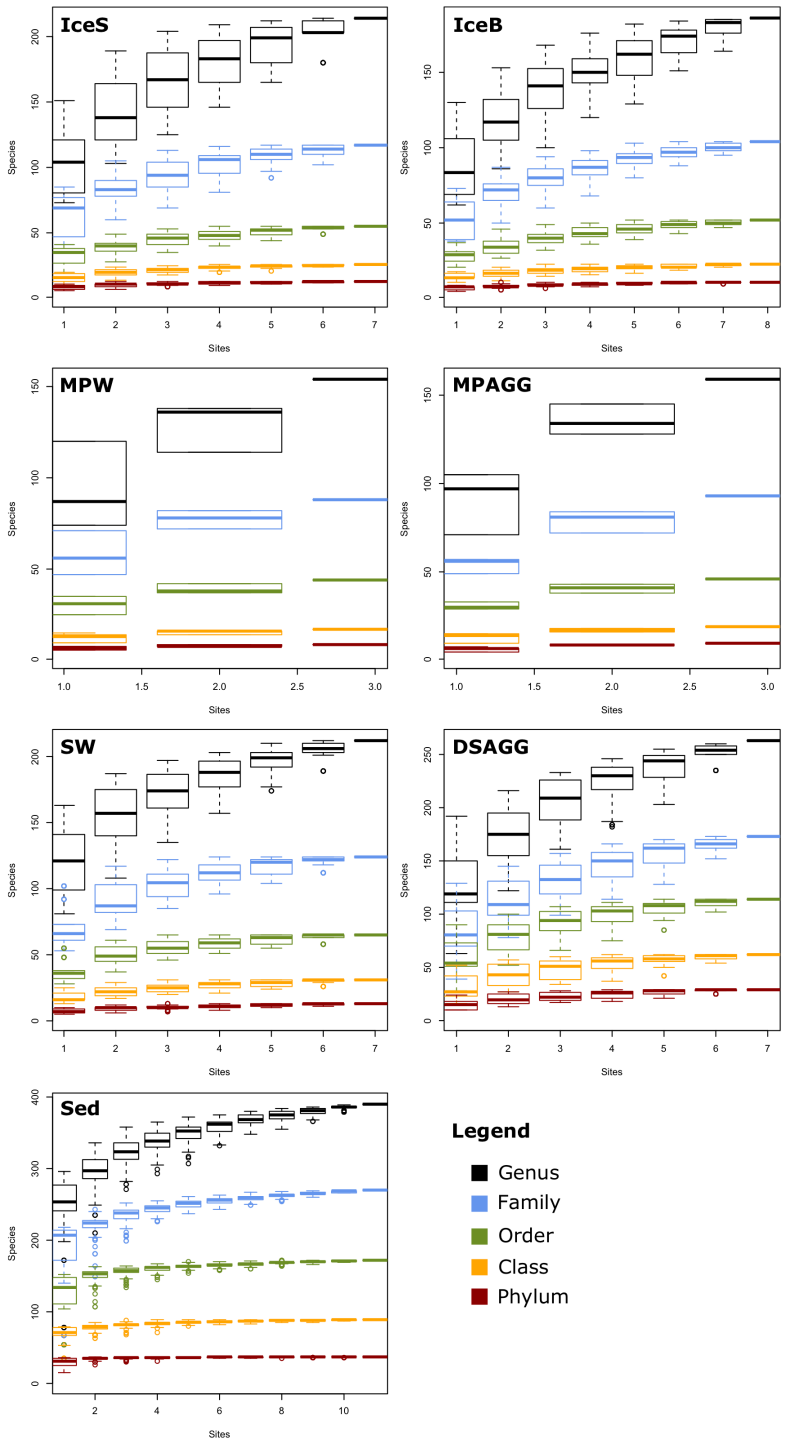


Figure S1 - Accumulation curves per taxonomic level for bacterial communities.
IceS: sea-ice surface; IceB: sea-ice bottom; MPW: melt-pond water; MPAGG: melt-pond aggregate; SW: surface seawater; DSAGG: deep-sea algae deposit; Sed: deep-sea surface sediment.

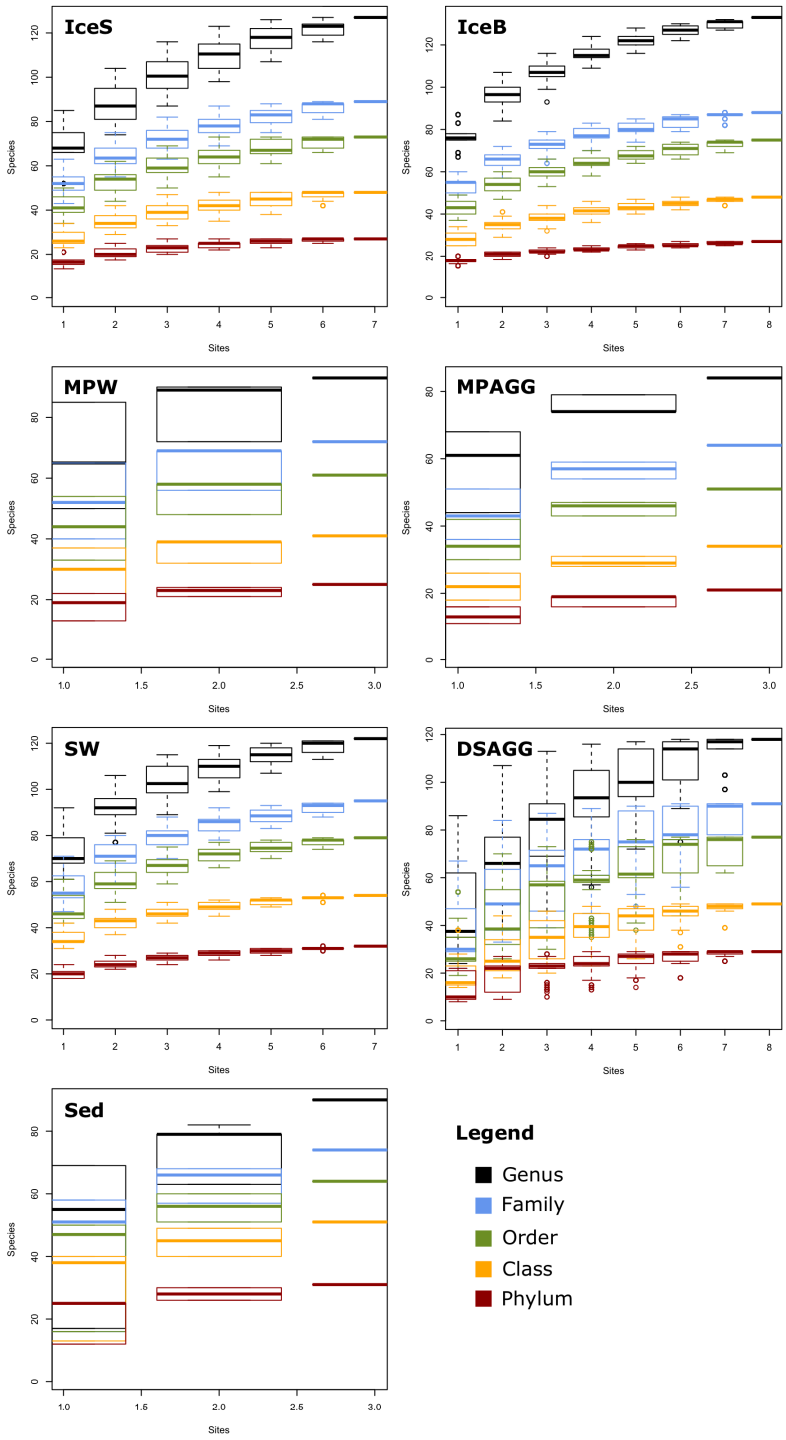


Figure S2 - Accumulation curves per taxonomic level for eukaryotic communities.
IceS: sea-ice surface; IceB: sea-ice bottom; MPW: melt-pond water; MPAGG: melt-pond aggregate; SW: surface seawater; DSAGG: deep-sea algae deposit; Sed: deep-sea surface sediment.

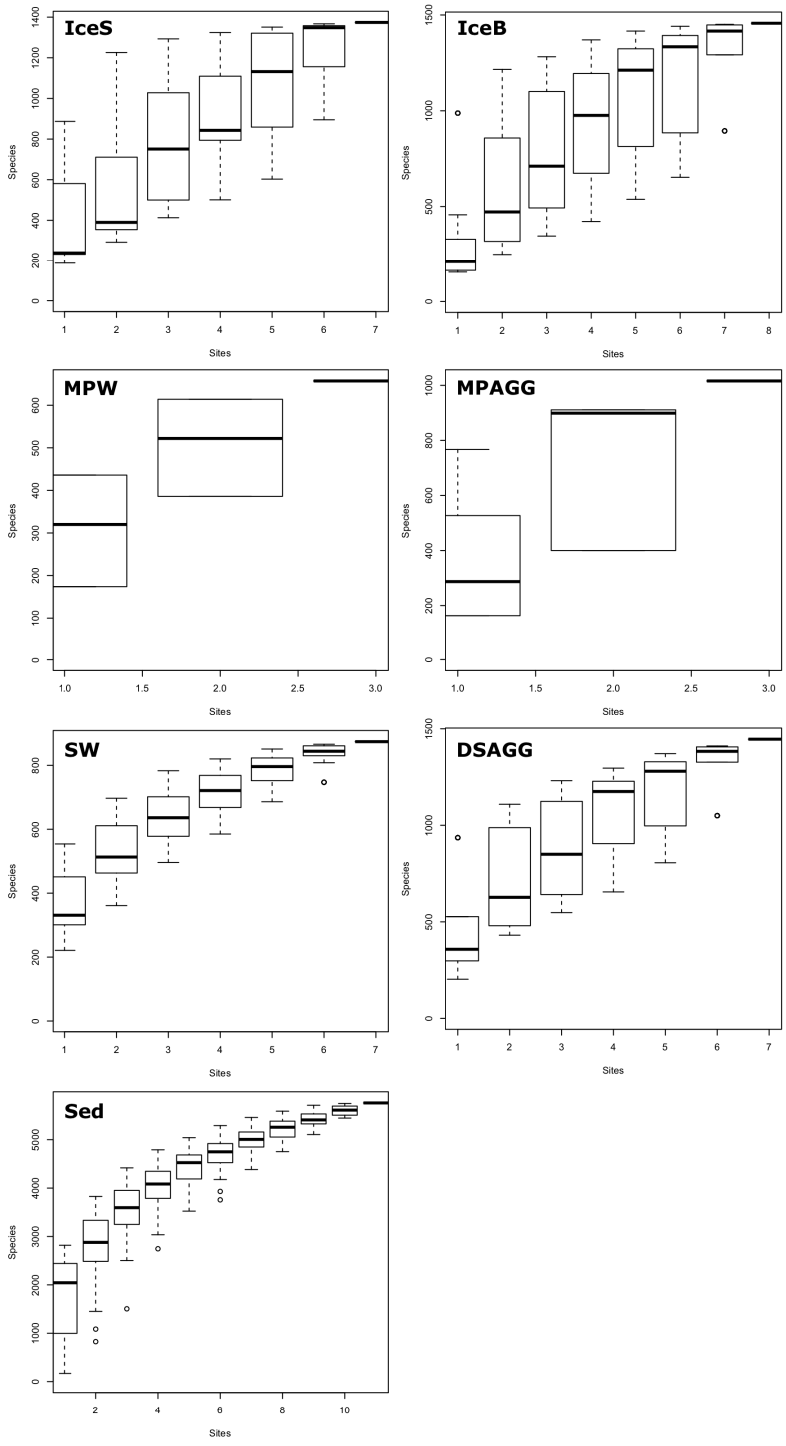


Figure S3 - OTU accumulation curves for bacterial communities.

IceS: sea-ice surface; IceB: sea-ice bottom; MPW: melt-pond water; MPAGG: melt-pond aggregate; SW: surface seawater; DSAGG: deep-sea algae deposit; Sed: deep-sea surface sediment.

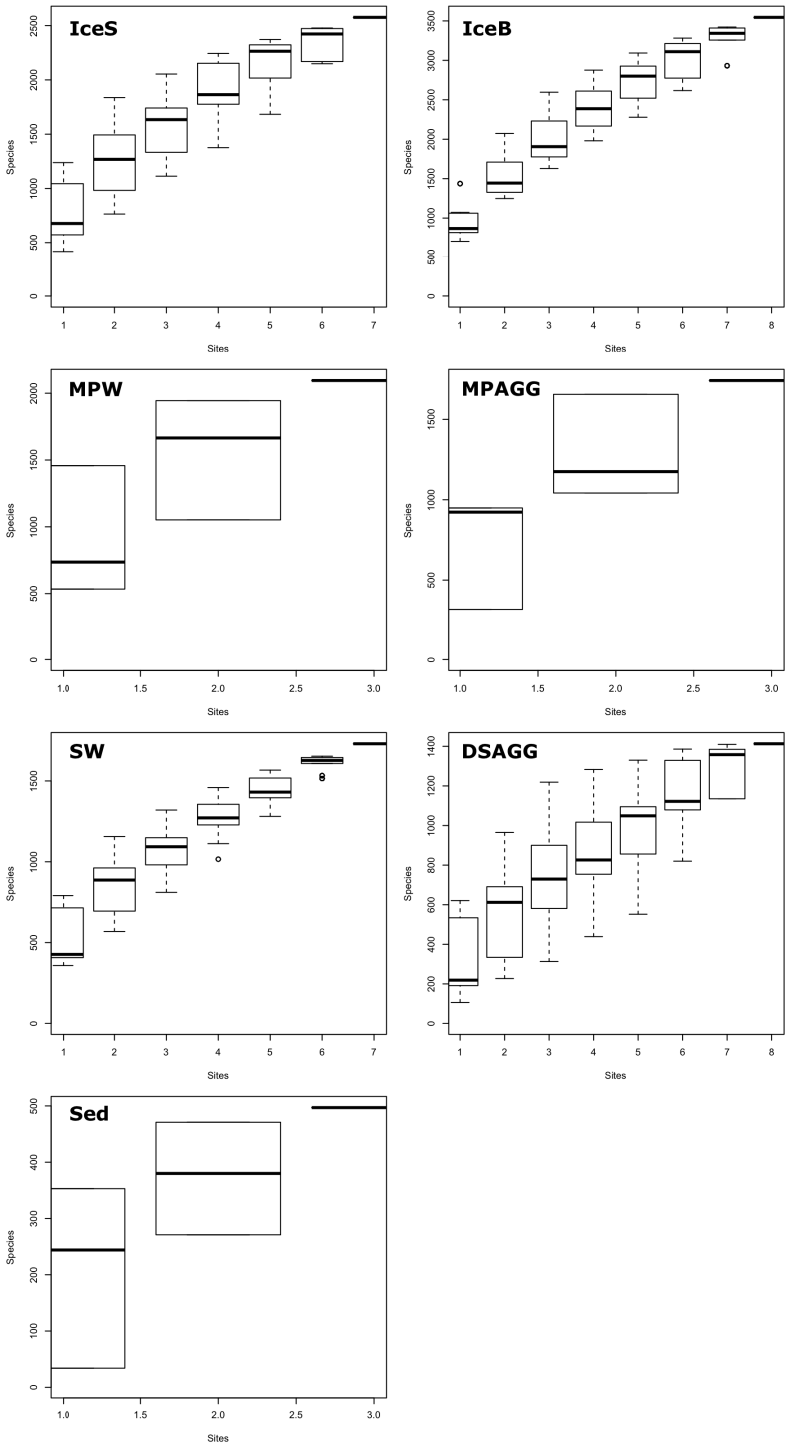


Figure S4 - OTU accumulation curves for eukaryotic communities.

IceS: sea-ice surface; IceB: sea-ice bottom; MPW: melt-pond water; MPAGG: melt-pond aggregate; SW: surface seawater; DSAGG: deep-sea algae deposit; Sed: deep-sea surface sediment.

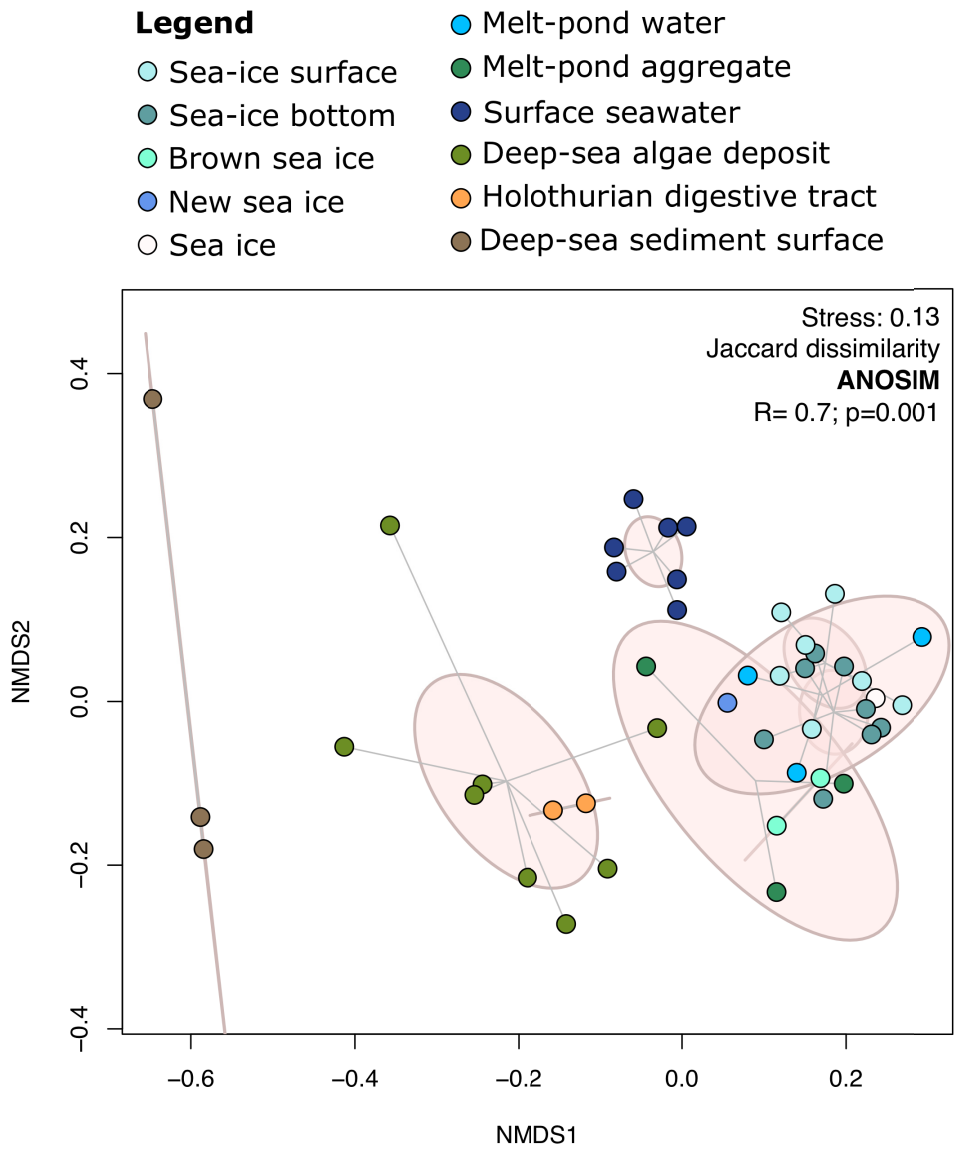


Figure S5 - Two-dimensional NMDS ordination of community dissimilarities for eukaryotes. Dissimilarity matrices and ANOSIM were calculated using the Jaccard dissimilarity measure on the basis of presence/absence of Illumina OTUs. Environments are depicted by color coding and points within each environmental grouping are connected to their group centroid through a spider diagram. Pink ellipses indicate the estimated 95% dispersion limits of each group.

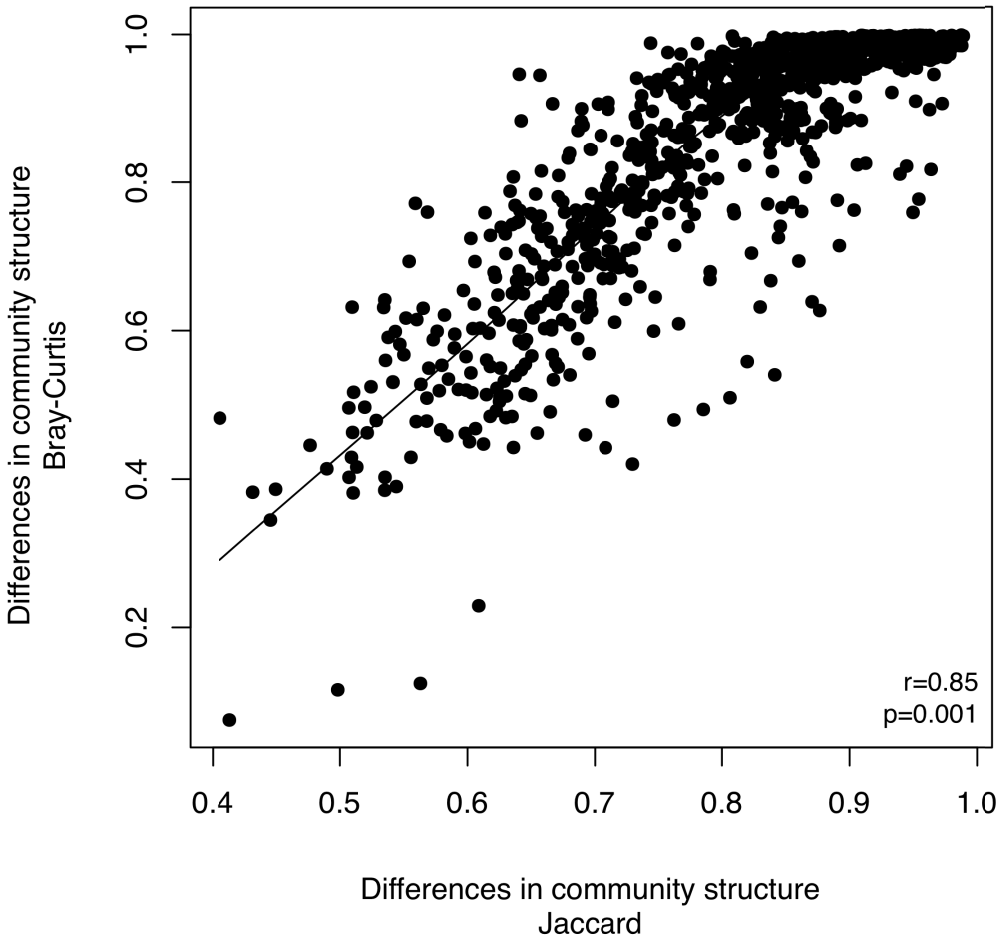


Figure S6 - Correlation of differences in eukaryotic community structure based on the Bray-Curtis and the Jaccard dissimilarity measure for the Illumina OTU dataset. Spearman's correlation as tested by a Mantel test with 999 permutations is indicated in the plot. The black line is a scatter smooth curve computed by LOESS.

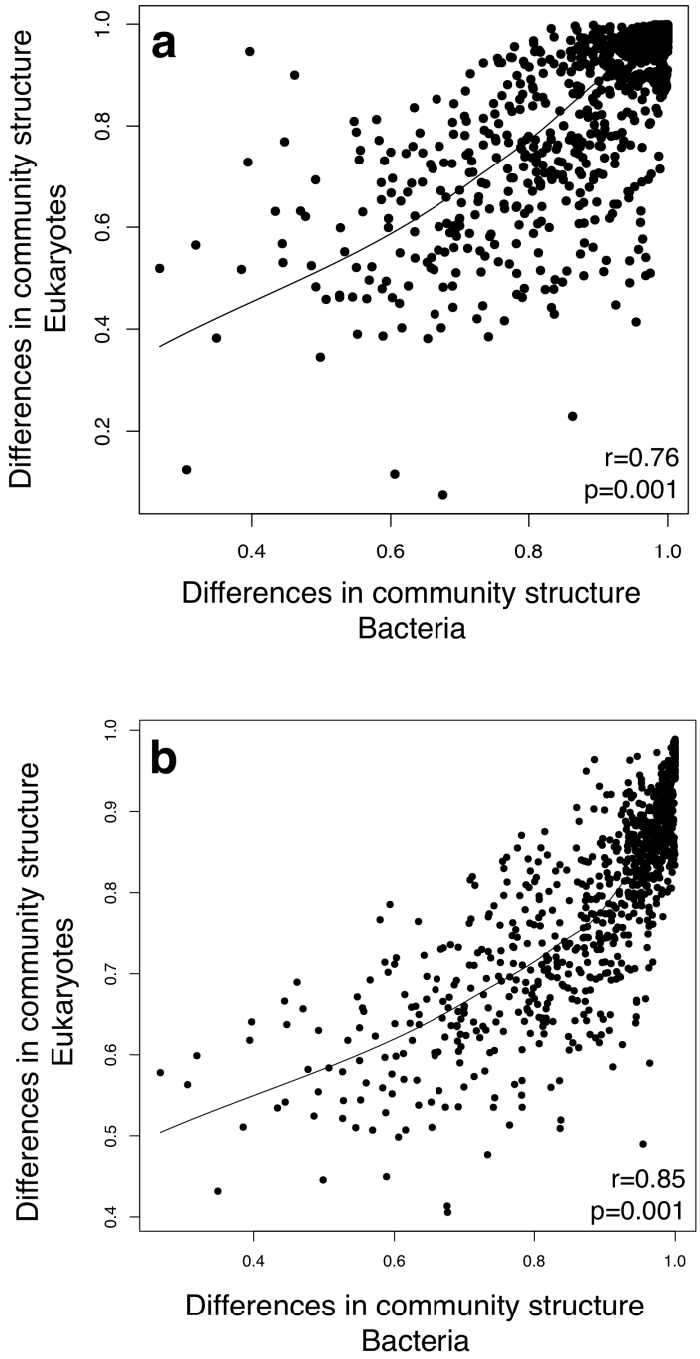


Figure S7 - Correlation of differences in bacterial and eukaryotic community structure based on Illumina OTU datasets. Dissimilarity matrices were calculated using (**a**) the Bray-Curtis dissimilarity measure for both bacteria and eukaryotes and (**b**) the Bray-Curtis dissimilarity measure for bacteria and the Jaccard dissimilarity measure for eukaryotes. Spearman's correlation as tested by a Mantel test with 999 permutations is indicated in the plot. The black line is a scatter smooth curve computed by LOESS.

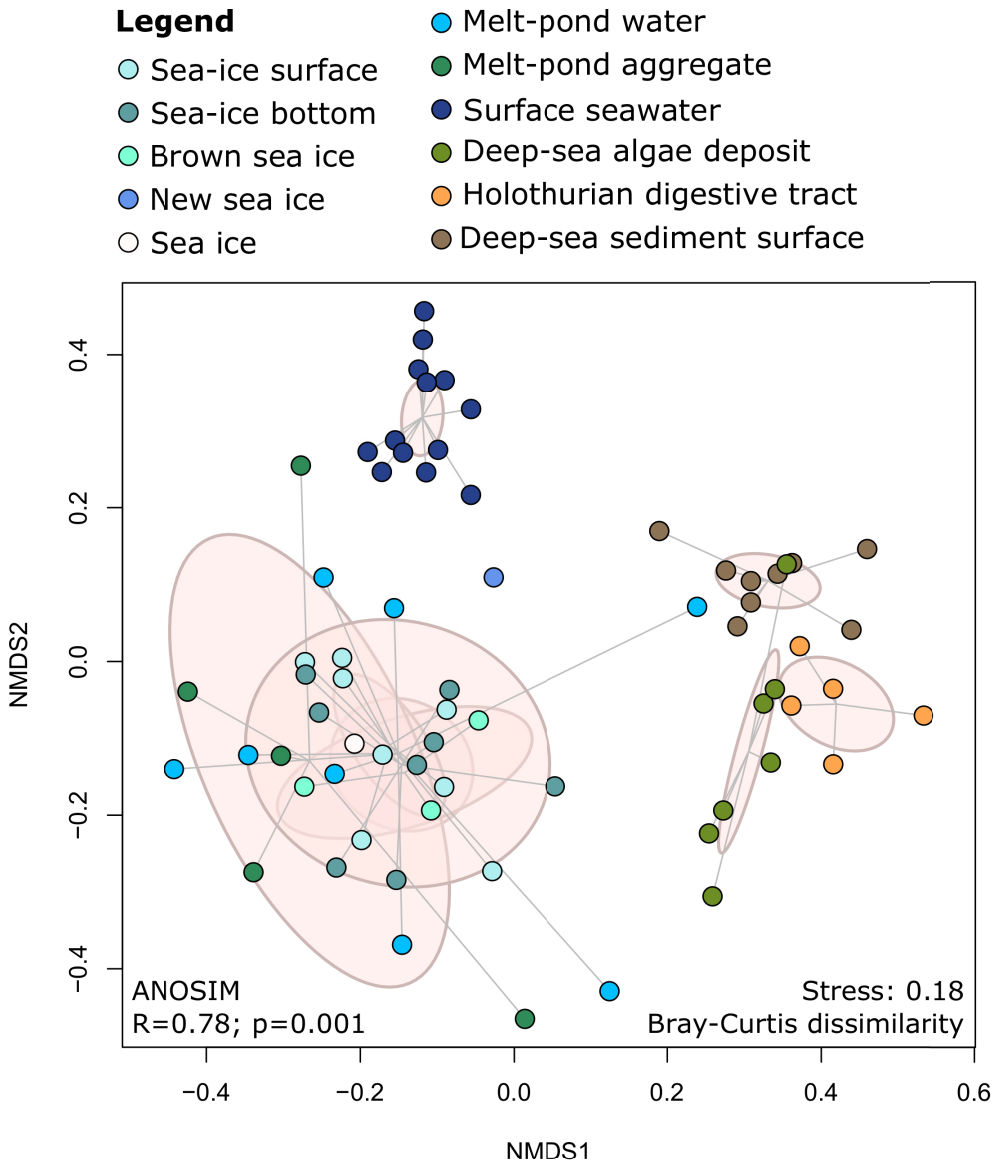


Figure S8 - Two-dimensional NMDS ordination of community dissimilarities for bacteria on the basis of ARISA OTUs. Dissimilarity matrices and ANOSIM were calculated using the Bray-Curtis dissimilarity measure. Environments are depicted by color coding and points within each environmental grouping are connected to their group centroid through a spider diagram. Pink ellipses indicate the estimated 95% dispersion limits of each group.

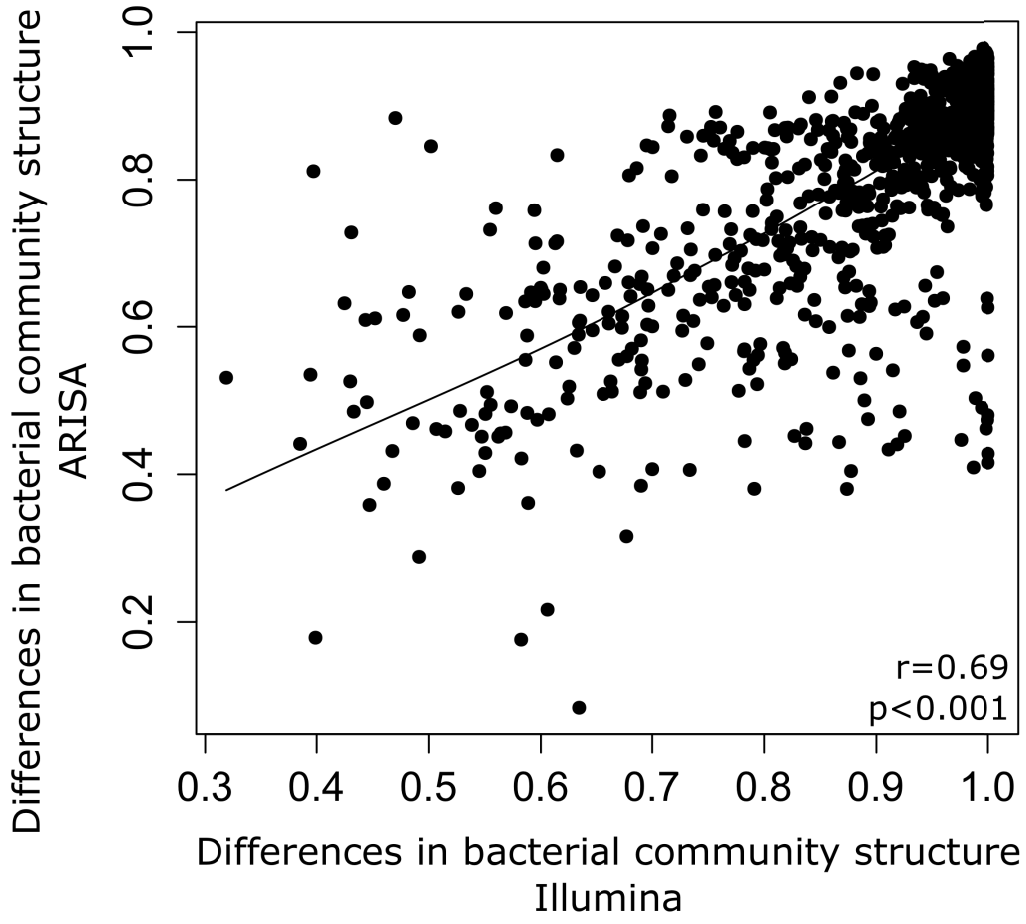


Figure S9 - Correlation of differences in bacterial community structure based on Illumina versus ARISA OTU datasets. Dissimilarity matrices for both datasets were calculated using the Bray-Curtis dissimilarity measure. Spearman's correlation as tested by a Mantel test with 999 permutations is indicated in the plot. The black line is a scatter smooth curve computed by LOESS.

Full bioinformatic scripts and supplementary tables are available at: https://www.frontiersin.org/articles/10.3389/fmicb.2018.01035/full#supplementary-material

Table S1: Overview of ice stations sampled during RV Polarstern expedition ARK-XXVII/3 (PS80) to the Central Arctic Ocean in 2012 in chronological order. Ice algal salinity and temperature values were taken from Fernández-Méndez et al. (2014); sea-ice salinity and temperature from Hardge et al. (2017); seawater salinity and temperature from Boetius et al. (2013); salinity and temperature values for deep-sea samples refer to bottom water measurements taken from Rabe et al. (2013). Bac: Bacteria; Euk: Eukaryotes; n.d.: no data.

Table S2: Sequence processing. Output of Illumina sequencing of the v4-v6 region of the 16S rRNA gene for bacteria and the v4 region of the 18S rRNA gene for eukaryotes. Shown are average values and standard deviation (STDEV) per environment from raw sequences to the final products of read processing and quality control, as well as subsequent filtering steps (i.e. removal of absolute singletons, chloroplast sequences and other domains). IceS: sea-ice surface; IceB: sea-ice bottom; other ice: brown sea ice, sea ice, freshly-formed sea ice; MPAGG: melt-pond aggregate; MPW: melt-pond water; SW: surface seawater; DSAGG: deep-sea algae deposit; HDT: holothurian digestive tract content; Sed: deep-sea surface sediment.

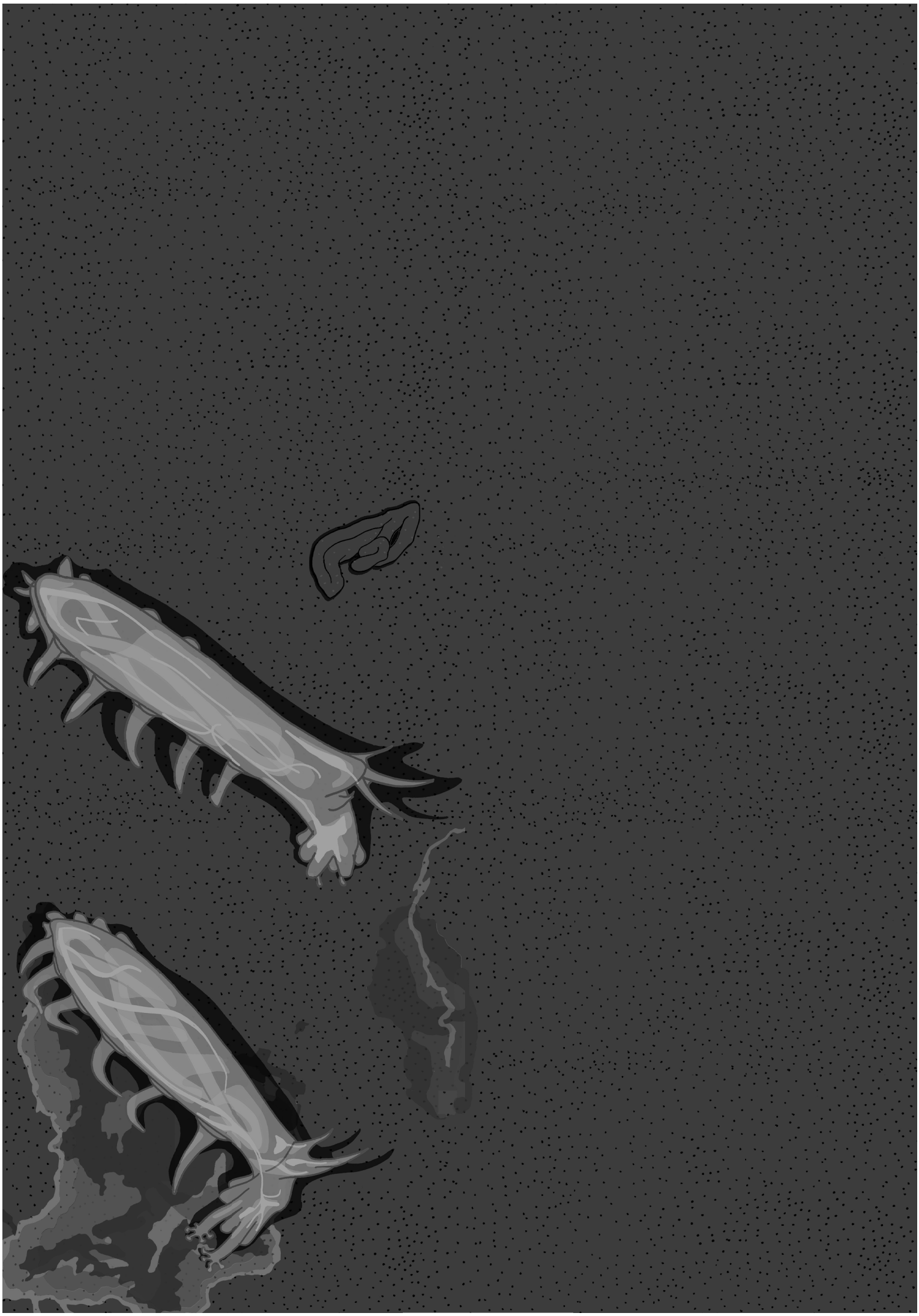
Table S3: Abundant and dispersed bacterial core community members. Abundant and dispersed members were defined as OTUs found in >50% of the samples of a habitat, and represented by at least 100 sequences. IceS: sea-ice surface; IceB: sea-ice bottom; MPW: melt-pond water; MPAGG: melt-pond aggregate; SW: surface seawater; DSAGG: deep-sea algae deposit; HDT: holothurian digestive tract content; Sed: deep-sea surface sediment. Note the three OTUs in bold, which we identified as generalists based on their occurrence patterns, as well as the 12 OTUs highlighted in grey, which represent exclusive members of ice-algae aggregates and

algae deposits. Taxonomic classification was assigned using the SILVA SSU database release 123.

Table S4: Abundant and dispersed eukaryotic community members. Abundant and dispersed members were defined as OTUs found in >50% of the samples of a habitat, and represented by at least 100 sequences. IceS: sea-ice surface; IceB: sea-ice bottom; MPW: melt-pond water; MPAGG: melt-pond aggregate; SW: surface seawater; DSAGG: deep-sea algae deposit; HDT: holothurian digestive tract content; Sed: deep-sea surface sediment. Note the seven OTUs in bold, which we identified as generalists based on their occurrence patterns. Taxonomic classification was assigned using the SILVA SSU database release 123.

Table S5: Relative sequence abundance of eukaryotic genera across all samples. For members that were unclassified at genus level, the next higher taxonomic rank is shown.

Table S6: Relative sequence abundance of bacterial genera across all samples. For members that were unclassified at genus level, the next higher taxonomic rank is shown.



Deep-sea benthic bacteria form diverse, spatially variable communities with stable heterotrophic genome traits in the Arctic (1,500-5,500 m water depth)

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Abstract

The degradation of organic matter in marine sediments is a key process in the global carbon cycle, regulating the efficiency of CO_2 removal from the atmosphere over geological time scales. The majority of marine sediments are located in the deep sea, where only a fraction of organic matter fixed by primary producers in the surface ocean comes to rest. This organic matter is primarily remineralized by the bacteria which dominate the surface of deep-sea sediments, yet knowledge of their metabolic capabilities and enzymatic machinery is limited. To gain insight into a) the potential of bacterial assemblages to process organic matter and b) the taxonomic and functional variation across these assemblages, we analyzed a collection of multi-omic data sets. We generated 16S rRNA gene amplicon libraries, as well as metagenomic and -transcriptomic datasets from samples collected along a benthic depth gradient from 1,200 m to 5,500 m at the Arctic long-term ecological research station HAUSGARTEN. We found that members of the *Gamma*-, *Delta*- and *Alphaproteobacteria* dominated communities at all depths. In the active fraction, representation of the *Flavobacteriia*, *Cytophagia* and SAR202 clade was enriched. Focusing on abundant community members (>100 16S rRNA tag sequences in the dataset), we observed high local diversification, with up to 51% turnover of operational taxonomic units (OTUs) between any two sites across the slope. Within our metagenomic data, we also observed a diversity of carbohydrate active enzymes, peptidases and transporters; however, we noted little variation between stations. This insignificant turnover and high functional redundancy of protein families between stations suggests that communities at all depths were equipped to utilize similar classes of organic compounds. However, we observed distinct substrate utilization patterns at the class or phylum level of the benthic bacteria. We conclude that local diversification in deep-sea sediments is likely to generate huge, largely unknown genetic diversity in digestive and transport enzymes.

Introduction

The deep sea, typically defined as those parts of the ocean below 200 m water depth, is the largest ecosystem on Earth, covering approximately 67% of the

planetary surface. Most of the deep seafloor is composed of clay sediments, which host microbial communities of immense population sizes with the highest cell densities observed in the uppermost sediment layers (Whitman et al., 1998). In these layers, bacteria dominate ecological communities in terms of biomass and diversity (Rowe et al., 1990; Jørgensen and Boetius, 2007; Bienhold et al., 2016). Through their involvement in the breakdown of organic material and subsequent regeneration of nutrients at the seafloor, heterotrophic benthic bacteria act as key mediators of carbon and nutrient cycling in the deep sea (Azam and Malfatti, 2007; Mayor et al., 2012; Buchan et al., 2014). These communities feed on organic matter fixed in the upper ocean, which has been stripped of labile compounds by heterotrophic pelagic organisms as it falls to the deep. As a result, deep-sea microbes endure energy-limited conditions and must be able to utilize a wide range of metabolic strategies to access substrates including particulate and dissolved refractory materials (Glover and Smith, 2003; Arnosti, 2008; Smith et al., 2008; Teske et al., 2011). Marine bacteria require multiple enzyme classes – including peptidases, lipases, nucleases and carbohydrate-active enzymes (CAZymes) – to synergistically break down chemically and structurally complex marine organic matter (Azam and Malfatti, 2007; Weiner et al., 2008; Arnosti, 2011). Aided by membrane-bound transport systems for low-molecular-weight (LMW) substrates, such as sugars and amino acids, and high-molecular-weight (HMW) substrates, such as polysaccharides, proteins, lipids and nucleic acids, these enzymes allow bacteria to interact, transform and take up organic material utilized for energy and nutrients. Previous studies of heterotrophic bacterioplankton have indicated that different groups of bacteria have different genetic capacities to degrade organic matter and to tap into a diverse array of diluted inorganic and organic resources (Mann et al., 2013; Xing et al., 2014; Berlemont and Martiny, 2015, 2016). However, next to nothing is known about the types of benthic bacteria and enzymatic machineries responsible for the breakdown of complex organic matter deposited in fine-grained deep-sea sediments. Previous work has suggested that some members of bacterial communities inhabiting the top few centimeters of benthic sediments are opportunistic heterotrophs that can quickly respond to the input of organic material from the surface ocean, while others appear to be slow growing and adapted to survive on refractory compounds (Gooday and

Turley, 1990; Turley and Lochte, 1990; Turley, 2000; Jørgensen and Boetius, 2007; Orsi et al., 2013; Hoffmann et al., 2017).

Recently, it has been found that bacterial diversity in deep-sea sediments exceeds that of the upper pelagic zones (Lozupone and Knight, 2007; Zinger et al., 2011; Ruff et al. 2015). Even when excluding the rare biosphere, deep-sea benthic bacterial diversity rivals that of soil bacteria, despite their habitat's energy limited conditions, high pressures, and near-freezing temperatures. The reliance of deep seafloor assemblages on surface input has led to the speculation that variations in organic matter flux to this ecosystem may be linked to variation in bacterial community structure (Goffredi and Orphan, 2010; Bienhold et al., 2012; Jacob et al., 2013; Ruff et al., 2014; Hoffmann et al., 2017; Rapp et al., 2018), biomass (Pfannkuche, 1993; Turley and Dixon, 2002; Kanzog et al., 2008) and activity (Boetius and Lochte, 1994; Poremba and Hoppe, 1995; Kanzog et al., 2008; Goffredi and Orphan, 2010; Hoffmann et al., 2017). Organic matter concentrations generally decrease with increasing water depth while its labile component is depleted (Soltwedel et al., 2005). Even so, high-throughput mass spectrometry has shown that sedimentary particulate and organic matter consists of an immense diversity of molecular components that may select for a wide range of organolytic enzymes (Schmidt et al., 2009, 2017; Rossel et al., 2016) and may result in diverse microbial assemblages.

Here, we studied Arctic deep-sea surface sediments at the LTER HAUSGARTEN (Fram Strait) along a water depth gradient ranging from 1,200 to 5,500 meters below sea level (mbsl). We investigated spatial trends in the total and active bacterial community composition, as well as in the community's functional gene repertoires. In the Arctic, substantial changes in the quantity and quality of export flux can result from the strong seasonality and interannual variation in ocean conditions, varying ice extent and planktonic composition (Dethleff et al., 2000; Hebbeln, 2000; Bauerfeind et al., 2009; Leu et al., 2011; Wassmann and Reigstad, 2011; Boetius et al., 2013; Arrigo, 2014). However, it is not known whether and how this is reflected in the genomic features of deep-sea surface sediment microbes (Herndl and Reinthaler, 2013; Techtmann et al., 2016). We assessed total and active bacterial community diversity using DNA and cDNA Illumina tag sequencing of the 16S rRNA gene, and generated metagenomes and metatranscriptomes to elucidate the capabilities of

these communities to degrade and take up organic material. To our knowledge, this is the first study to investigate the functional potential of bacterial communities in Arctic deep-sea sediments revealed through a multi-omic approach. We explored the taxonomic and functional variation in both the total and active components of these communities as they respond to the intertwined gradients across our study sites. As a result of the potentially variable quantity of organic matter at the seafloor, we anticipated that a key portion of ecologically relevant functional diversity would be evident in the hydrolytic enzymes and transporters which allow opportunistic utilization of exported biomass. We are confident that this work has established a basis for further exploration of the microbial ecology of fine-grained, deep-sea sediments.

Material & Methods

2.1 Site description

Sediment samples were collected at seven stations in the eastern Fram Strait during RV Polarstern expedition PS85 (ARK-XXVIII/2) in June 2014 (Table 1), which is the phase of the deposition of the annual spring bloom (Bauerfeind et al., 2009; Soltwedel et al., 2016). These stations form a bathymetric transect from approximately 1,200 to 5,500 mbsl along the Svalbard continental margin (Figure 1), and are part of the Long-Term Ecological Research (LTER) observatory HAUSGARTEN. The observatory is situated between northern Greenland and the Svalbard archipelago (Soltwedel et al., 2005). The deepest station of this transect is located in the Molloy Hole (5,500 mbsl), which is the deepest known seafloor depression in the Arctic Ocean. Located at around 79° N, the study area is exposed to seasonally varying sea-ice conditions, with mostly ice-free summer months, characterized by high productivity regimes along the marginal ice zone (Soltwedel et al., 2016).

2.2 Sampling procedure

We retrieved cores from undisturbed deep-sea sediments using a video-guided multiple corer (TV-MUC). We took subsamples from each core using syringes cut off

to expose the full diameter of the barrel for subsequent analyses. In this work, only the uppermost centimeter of sediment was processed. This layer was fully aerobic and subject to a flux of freshly deposited particles at an estimated rate of 2.7-6.3 g C m^{-2} yr^{-1} (Lalande et al., 2016). Samples for total community DNA and RNA were taken with 20 ml syringes, shock-frozen in liquid nitrogen and stored at -20°C or -80°C for up to eight months, respectively, prior to nucleic acid extraction in the laboratory. For microbial cell counts, two subsamples were taken with 5 ml syringes from two separate cores at each station. The uppermost centimeter of the subsamples were pooled and first fixed in formalin/seawater (FA/SW; 4% formaldehyde) and subsequently in a 1:1 mix of Ethanol (EtOH; 96 %) and 1x phosphate buffered saline (PBS) and stored at -20°C. Additionally, we measured biogenic sediment compounds, including chloroplastic pigment equivalents (CPE; the sum of chlorophyll a and phaeopigments) as a proxy for phytodetritus input (Yentsch and Menzel, 1963; Holm-Hansen et al., 1965), phospholipids as a proxy for total microbial biomass (Findlay et al., 1989; Boetius and Lochte, 1994), and sediment-bound particulate proteins as a proxy for living and dead biomass (Greiser and Faubel, 1988). As part of the regular long-term observation effort at HAUSGARTEN, the potential for extracellular enzymatic activity was assessed by measuring esterase activity via fluorescein-diacetate (FDA) cleaving rates (Köster et al., 1991). Three subsamples were analyzed for each of the parameters. All data will be made available in the PANGAEA database.

2.3 Total community DNA and RNA extraction

We extracted total DNA from 0.5 g of surface sediment (0-1 cm) using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA), according to the manufacturer's instructions. Total RNA was extracted from 5 g of surface sediment (0-1 cm) using the PowerSoil Total RNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA). Sediment for RNA extraction was still frozen when added to the first buffer solution. After resuspension of the extracted RNA in 50 µl RNase/DNase free water, we performed a DNase digest on the whole sample by adding 7 µl DNase buffer (10x) (Roche Diagnostics GmbH, Mannheim, Germany), 10 µl DNase I recombinant (Roche Diagnostics GmbH, Mannheim, Germany), and 2 µl RNAsin

RNase inhibitor (Promega Corporation, Madison, WI, USA). The mix was then incubated for 30 min at 37 °C and subsequently for 10 min at 56 °C before placing it on ice. Finally, RNA was cleaned and concentrated using the RNeasy MinElute Cleanup Kit (QIAGEN GmbH, Hilden, Germany) and the final product was eluted in 30 µl RNase-free TE-buffer. We used 15 µl of the eluate for cDNA synthesis by adding 1 µl of random hexamers (Peqlab Biotechnologie GmbH, Erlangen, Germany) and incubating the mix for 10 min at 70 °C, followed by 15 min on ice. Subsequently, 5 µl of RT 5x reaction buffer (Promega Corporation, Madison, WI, USA), 1.25 µl dNTPs (10 mM each), 2 µl reverse transcriptase (Promega Corporation, Madison, WI, USA) and 1 µl RNase inhibitor (Promega Corporation, Madison, WI, USA) were added and incubated for 10 min at room temperature, followed by 60 min at 45 °C. DNA and cDNA extracts were stored at -20°C until sequencing.

2.4 16S rRNA gene Illumina tag sequencing from DNA and cDNA

We generated amplicon libraries of the hypervariable v4-v6 region of the 16S rRNA gene for Illumina sequencing, following Illumina's recommended protocol (16S Metagenomic Sequencing Library Preparation, Part #15044223, Rev. B). We used the primers defined in (Klindworth et al., 2013), namely S-D-Bact-0564-a-S-15 and S-*Univ-1100-a-A-15. Amplicon libraries were sequenced on a MiSeq machine, in 2x300 cycles paired end runs. All amplicon sequences will be made available at ENA.

2.4.1 Read processing, OTU definition and analyses

We used *Cutadapt* v1.8.1 (Martin, 2011) to clip off primer sequences from the raw amplicon reads, allowing no indels and a maximum allowed error rate of 16%. Reads that passed the primer clipping were then merged using *Pear* v0.9.6 (Zhang et al., 2014), and only those kept that showed a minimum merging overlap of 10 bp and for which the final merged read length was between 450 and 570 bp. Subsequently, we used *Trimmomatic* v0.32 (Bolger et al., 2014) for quality filtering, scanning the reads with a 6-base wide sliding window, cutting when the average quality per base dropped below 12. We double-checked the quality of the remaining reads visually

using *Fastqc*[3] v0.11.4 and clustered the sequences into OTUs using *Swarm* v2.1.1 in the fastidious mode's default setting (Mahé et al., 2015). This method uses a agglomerative, unsupervised (*de novo*) single-linkage-clustering algorithm to delineate OTUs based on sequence differences and then refines the clusters using amplicon abundance information and the OTUs' internal structures (Mahé et al., 2014). Representative sequences of all OTUs were taxonomically classified against the Silva SSU Ref NR99 123 (Quast et al., 2013) using the *SINA* aligner v1.2.10 (Pruesse et al., 2012). We filtered the dataset and removed OTUs that were not classified within the bacterial domain, as well as OTUs assigned to chloroplast or mitochondrial sequences. Also, we removed all singleton sequences, i.e. OTUs that were represented by a single sequence across the complete dataset, to reduce potential bias introduced through sequencing errors (Tremblay et al., 2015b). Further data processing and plotting was conducted in *R* v3.2.2 (R Core Team, 2014) using the *vegan* package v2.2 (Oksanen et al., 2014). We calculated OTU number, Chao1 richness, and the inverse Simpson diversity index per sample using the customized SubsampleNGS[4] function with 100 iterations. The mean values of each iteration were used for further analyses. To assess beta diversity of the total and the active community between stations, we calculated Bray-Curtis dissimilarity matrices of the DNA and RNA OTU tables, and examined the correlation of both by performing a Mantel test with 999 permutations. We further calculated tables of fold changes across taxonomic levels using the taxapooler v1.4 script (Gobet et al., 2010) and performed a centered log-ratio (CLR) transformation of the data to attenuate the effect of compositionality when comparing communities across samples (Quinn et al., 2018). Bacterial community turnover, i.e. the number of OTUs which differ between two stations along the slope, was expressed as Jaccard dissimilarities, transformed into percentages. We excluded OTUs represented by less than 100 sequences in the whole dataset from this calculation.

2.6 Metagenomic and –transcriptomic library preparation and sequencing

[3]www.bioinformatics.babraham.ac.uk/projects/fastqc
[4]https://github.com/chassenr

Multiple extractions were pooled to generate a total of 1 to 2 μg DNA for the preparation of standard Illumina PCR-free TruSeq sequencing libraries. Libraries were sequenced on an Illumina MiSeq machine at CeBiTec, University of Bielefeld, generating metagenomes of 3-4 Mio. paired-end reads, with a length of 300 base pairs (bp) and an average insert size of 400-420 bp. For metatranscriptome sequencing, approx. 80 ng of total RNA extracts was used to generate illumina TruSeq stranded RNA libraries, following the TruSeq Stranded Total RNA Sample Preparation Guide, omitting the rRNA depletion step. Metatranscriptomic libraries were sequenced on a MiSeq instrument, yielding 4.5-8 Mio. single reads, with a length of 150 bp. The raw metagenomic and –transcriptomic libraries in ENA.

2.6.1 Read processing and analyses

We cropped sequencing adapters if present and discarded sequences if matching potential contaminants in all raw metagenomic and metatranscriptomic reads using *BBDuk* v35.68 (https://sourceforge.net/projects/bbmap) with a kmer size of 27 and visually checked per base sequence content, sequence length distribution and per base quality scores in all samples using *Fastqc* v0.11.4 (www.bioinformatics.babraham.ac.uk/projects/fastqc). Reads were then quality filtered using *Trimmomatic* v0.32 (Bolger et al., 2014) by clipping the 10 leading bases, scanning the remaining read with a 4-base wide sliding window, discarding entire reads when the average quality per base dropped below 15, and when they were shorter than 100 bp. The remaining metagenomic reads were then merged in *Pear* v0.9.6 (Zhang et al., 2014) using default settings and with a minimum overlap of 10 bp, while metatranscriptomic reads were kept as single reads. All reads, merged and non-merged, were sorted into those corresponding to rRNA and non-rRNA using *SortMeRNA* v2.0 (Kopylova et al., 2012). The rRNA and rRNA gene sequences were classified against the Silva SSU Ref NR99 123 (Quast et al., 2013) using *SINA* v1.2.11 (Pruesse et al., 2012). We predicted protein-coding regions in the merged and single reads using *FragGeneScan1*.20 (Rho et al., 2010), and used *UProC* v1.2 to annotate the detected regions referencing its *Pfam* v28.0 dictionary (Meinicke, 2015), both with default settings. We removed singleton protein families (Pfams) and performed a centered log-ratio (CLR) transformation of the Pfam data in

R v3.2.2 (R Core Team, 2014) using the *vegan* package v2.2 (Oksanen et al., 2014). This transformation mitigates compositional effects and variable sequencing depth and results in counts being expressed as fold changes relative to the (geometric) mean count per sample. We identified Pfams involved in carbohydrate and protein degradation, as well as in substrate uptake, by matching annotations in our data set against records in the Carbohydrate-Active enZymes (CAZy) Database (Lombard et al., 2014; accessed in August 2017), the Carbohydrate-Active enZymes encyclopedia (The CAZypedia Consortium, 2018), the *MEROPS* database release 12.0 (Rawlings et al., 2014), and the Transporter Classification Database (TCDB; accessed in September 2017) (Saier et al., 2016). For all Pfams of interest that could be detected both in the metagenomic and the metatranscriptomic dataset, we extracted the underlying metatranscriptomic reads and performed a BLASTx search against the NCBI database (https://blast.ncbi.nlm.nih.gov/, accessed in February 2018), applying an E-value cutoff of 1.0^{-5}. In cases where >100 sequences were mapped to a single Pfam model, we used *DIAMOND* v0.9.18 in sensitive mode with default parameters for the sequence alignment, which aligns sequences faster than BLAST without forfeiting much sensitivity (Buchfink et al., 2015),. We ordered annotation results by bitscore, then e-value, and retrieved taxonomic paths for all unique GenInfo Identifier (gi) accession numbers of the best hits using the *rentrez* package 1.2.1 (Winter, 2017) in *R* v3.2.2 (R Core Team, 2014).

Results & Discussion

High OTU turnover and differences in total and active bacterial community structure along water depth gradient

To investigate community responses to varying water depth and its covariates (e.g. organic matter concentrations), we generated >15,000 high-quality merged amplicon sequences per sample (SI Table 1). We retrieved an average of 2,340±529, and 2,496±609 bacterial OTUs for the total and active fraction, respectively (see methods for the definition of an OTU). Our results are comparable to previous reports of total bacterial community richness from Arctic deep-sea sediments in the Fram Strait (Jacob et al., 2013), the Siberian continental margin (Bienhold et al., 2012) and the

Antarctic Polar Front (Ruff et al., 2014), but on average 26% higher than numbers from central Arctic deep-sea sediments (Rapp et al., 2018). OTU rarefaction curves indicated that the sequencing depth was not sufficient to capture the full bacterial diversity detectable with our methods (SI Figure 3). Indeed, Chao1 estimates suggested a richness of >4,000 OTUs, both for total and active community members (SI Table 1).

Regarding the variation across the water-depth gradient, we observed only small differences of total and active OTU richness between 1,200 and 3,500 mbsl, but a steep drop of ~50% at the deepest station at 5,500 mbsl (SI Figure 4). This dynamic was not reflected in bacterial cell counts (SI Figure 2). Our OTU turnover results tracked the alpha diversity patterns, as the community at HGIX in the Molloy Hole was most divergent from any other station with an average 47±3% total, and 56±3% turnover in the active community. We can only speculate about the causes for such a large difference between the deepest station and the other sites, which all belong to one biogeochemical and oceanographic region in Fram Strait. Besides the substantially higher pressure prevailing here, it is known that the Molloy Hole receives a higher particle load than the shallower station due to accumulation processes in the funnel-shaped hole (Soltwedel et al., 2005; see SI Results & Discussion). Furthermore, it was previously found that this deepest station shows increased grazing pressure on microbial cells as a result of the high meiofauna densities (Soltwedel et al., 2003). Potentially this may reduce or simplify the available niche space, selecting for a smaller group of potentially piezophilic/tolerant and/or psychrophilic/tolerant microbes best adapted to exploit the relatively high organic matter load.

Between all other stations at the LTER HAUSGARTEN, only 6-13% of the total community and 14-26% of the active community were replaced with every 500 m difference in depth and ~5 nautical miles lateral distance (Figure 2). However, when including rare OTUs, turnover estimates increased to comparably high numbers (~70% with every 500 m depth difference; SI Table 2). If these rare OTUs are genuinely resident (as would be confirmed by sustained presence across repeated

samples), these dynamics would confirm that most of the detected community turnover is caused by local diversification forming a large rare biosphere (Jacob et al., 2013; Ruff et al. 2015). Taxonomic classification of the 16S rRNA gene tag sequences revealed that surface sediments at all depths were dominated by members of the *Gamma*-, *Delta*-, and *Alphaproteobacteria*, followed by *Acidimicrobiia* and *Flavobacteriia* (Figure 3; SI Figure 5). Overall, the total and active community structures were only weakly and not significantly correlated (Spearman's rho R=0.16; p=0.38; SI Figure 6). The representation of the dominant proteobacterial taxa was similar in both community fractions and featured taxa such as Sh765B-TzT-29 (*Deltaproteobacteria*), the JTB255 marine benthic group, the BD7-8 marine group (*Gammaproteobacteria*) and *Rhodospirillaceae* (*Alphaproteobacteria*) (SI Figure 5). Previous studies have also found these groups to be dominant at many different deep-sea sites (Siegert et al., 2011; Bienhold et al., 2016; Cerqueira et al., 2017; Lindh et al., 2017; Mußmann et al., 2017; Rasigraf et al., 2017), yet very little is known about their specific metabolic traits. Differences in functional diversity, as expressed in the metagenomic and metatranscriptomic profiles, were driven by the notably reduced representation of *Acidimicrobiia* (mainly composed of unclassified OM1 clade members) in the active fraction and the larger representation of *Flavobacteriia*, *Cytophagia* and the SAR202 clade (Figure 3). Such discrepancies in the total versus the active community structure may be explained by the realization of niches by functional guilds: Those that are more opportunistic and able to quickly respond to fresh particle inputs on a time scale of weeks will have different signatures relative to those that rely on a steady supply of refractory compounds. For example, members of the *Bacteroidetes* phylum, e.g. *Flavobacteriia* and *Cytophagia,* were overrepresented in the active fraction and are known for their involvement in degrading complex organic matter (Kirchman, 2002; Bauer et al., 2006; Xie et al., 2007; Angelov et al., 2011; Tang et al., 2012; Williams et al., 2013). Further, several members of this group have been linked with adaptations to life attached to particles or algal cells, as well as for the degradation of high molecular weight (HMW) compounds (Bauer et al., 2006; Thomas et al., 2011; Fernández-Gómez et al., 2013; Kabisch et al., 2014b; Barbeyron et al., 2016). Previous experimental work on deep-sea surface sediments from the same area has shown that members of the

Bacteroidetes were very responsive to phytodetritus additions and quickly increased in their proportional dominance (Hoffmann et al., 2017). Seemingly contrary to this reasoning, the contribution of the SAR202 clade was greater in the active fraction, although this group does not seem to be similarly responsive to fresh substrate (Hoffmann et al., 2017). However, it has been recently suggested that this clade plays a role in the oxidation of recalcitrant organic compounds (Landry et al., 2017), which may be a selective advantage in colonizing deep-sea environments. Similar to the SAR202 clade, the OM1 clade seemed to be non-responsive to detritus additions on short time scales of days to a few weeks (Hoffmann et al., 2017), and was linked to the degradation of refractory organics (Chen et al., 2016). Despite being a cosmopolitan member of deep-sea sediment communities (Bienhold et al., 2016), our results suggest low activity of the OM1 clade (Figure 3), and thus might indicate either a slow metabolism and low nutrient requirements, or dormancy. Both of these modes could be strategies to overcome energy limitation (Deming and Baross, 2000; Lennon and Jones, 2011).

Diversity of functional genes involved in the breakdown and uptake of organic matter

Fresh organic matter compounds such as carbohydrates like chitin, lipids and proteins are quickly reconfigured and metabolized during particle export (Wakeham et al., 1997b). By the time biomass is deposited on the seafloor, it is largely composed of refractory detrital matter such as cell wall components and fecal pellets (Azam and Malfatti, 2007; Lomstein et al., 2012; Turner, 2015). Extracellular enzymatic hydrolysis is the first step in the bacterial breakdown of these materials (Boetius and Lochte, 1994; Boetius, 1995; Poremba, 1995). Here we focused our metagenomic analyses on the diversity of carbohydrate-active enzymes (CAZymes), peptidases and transport systems (Azam and Malfatti, 2007; Weiner et al., 2008; Lombard et al., 2014), with the hypothesis that the observed high diversity and spatial variation in deep-sea bacteria would be reflected in the diversity of their key functional genes.

Raw metagenomes varied between 3.0-4.1 Mio. sequences per sample, and metatranscriptomes between 4.6-8.3 Mio. (SI Table 3). After quality control and sequence processing, we were able to assign protein family (Pfam) domain identifiers to ~54% of the metagenomic reads (SI Table 3). Approximately 90% of the metatranscriptomic reads represented ribosomal RNA, and only 0.5% of the metatranscriptomic reads were assigned to a Pfam domain. Out of the total 16,230 Pfam models in Pfam v28, we detected 8,032 in our data set. We note that this substantial fraction was present in only one small deep-sea area (60x60 nautical miles) sampled, confirming the immense diversity of enzymes present in deep-sea bacterial communities. More than 1,600 of these assignments (~ 20%) were to domains of unknown or putative function. Work on global ocean surveys, such as Tara Oceans and the Global Ocean Sampling (GOS) campaign, reported more than 40% of all detected functions to be unknown (Buttigieg et al., 2013a; Sunagawa et al., 2015), and observed that mainly singleton sequences contributed to novelty (Carradec et al., 2018). The fact that we detected a high proportion of Pfams of unknown function, despite the removal of singletons from analyses, stresses the extent of unexplored functional diversity in deep-sea ecosystems. We confined our analyses to Pfams that were detected in both our metagenomic and metatranscriptomic data sets, assuming that the presence of mRNA reflects to some extent processes occurring in the sediments at the time of sampling. This was intended to prevent an overestimation of effective benthic functional diversity, as inactive genetic material may have reached the system through the settling of organisms or material from the pelagic realm (Dell'Anno and Danovaro, 2005). Given the small percentage of transcripts belonging to functional genes in our data, we acknowledge that this may be a conservative position to take.

Despite the limited depth of sequencing, we detected 3,601 Pfams present in both the metagenomic and metatranscriptomic datasets. Of these, 77 were associated with CAZymes, 124 with peptidases, and 591 with transporters (SI Table 3 & SI Figure 7). While CAZyme and peptidase families composed less than 2% of the sequences in both metagenomes and metatranscriptomes, transporters accounted for 27-28% in the metagenomes and up to 31% of the sequences in the metatranscriptomes (SI Table 3), which is in a similar range as observed for uptake

systems in samples from 100 to 5,000 mbsl in the Atlantic water column (Bergauer et al., 2017).

Centered log-ratio (CLR) transformation of the number of reads matching individual Pfam domains revealed that the representation of CAZymes was on average 0.8-fold below the geometric mean of all detected Pfams. Peptidases and transporters, however, were enriched by 0.6 and 0.5-fold above the geometric mean, respectively (SI Table 4). In fact, of the ten most dominant Pfams in the dataset, eight were associated to transport functions (SI Table 4). Low-molecular-weight (LMW) substrate transporters, capable of importing the components of extracellularly degraded substrates, constituted the dominant type of transporter system in the system investigated. Adenosine triphosphate (ATP)-binding cassette (ABC) transporters were dominant at all depths (Figure 4 & SI Table 4). ABC transporters usually have a high substrate affinity and target multiple substrates, and were also identified as the dominant transport system for communities in other oligotrophic marine regions, where organisms need to thrive under energy limitation, i.e. for SAR11 from the Sargasso Sea (Sowell et al., 2009). Further, we detected Pfams related to tripartite ATP-independent periplasmic (TRAP) transporters, and a smaller number of tripartite tricarboxylate transporters (TTT) components (Figure 4). As TTTs have not been studied to the same degree as ABC and TRAP systems, lower representation of TTTs might potentially be an artifact of the lower number of reference sequences available in public databases. In contrast to ABC transporters, which require energy generated by ATP hydrolysis for the uptake of a wide range of substrates, including sugars, amino acids, polypeptides, vitamins and metal-chelate complexes (Davidson et al., 2008), TRAP and TTT transporters are fueled by energy derived from the thermodynamically favorable transport of counter-ions, usually H^+ or Na^+ (Mulligan et al., 2011), which may be advantageous in oligotrophic deep-sea settings. Uptake systems for HMW compounds such as TonB-dependent transporters (TBDT) were implicated in the uptake of substrates by bacterioplankton during phytoplankton blooms (Teeling et al., 2012). In addition to the transport of complex carbohydrates, amino acids, and organic acids, TBDT may also be involved in the uptake of aromatic compounds (Noinaj et al., 2010; Tang et al., 2012). In deep-sea sediment communities, their representation was on average 2-fold above

the mean, yet still lower than that of LMW systems (up to 7.5-fold enriched; Figure 4). This is in line with results from the water column, where LMW transporters outweighed TBDT at all depths (Bergauer et al., 2017), and supports the idea that only a few types of bacteria are specialized in HMW compound breakdown, while more are equipped to process its breakdown products and LMW material in the form of amino acids and sugars (Bauer et al., 2006; Weiner et al., 2008; Kabisch et al., 2014b; Xing et al., 2014; Berlemont and Martiny, 2015). Overall, the overrepresented genomic potential of deep-sea sediment microbes to take up various types of substrates may allow them to efficiently scavenge all available refractory sources for their nutrition, and to respond rapidly with growth once fresh organic material becomes available (e.g. after settling of the phytoplankton bloom as in this study). Further studies are needed to more accurately understand the dynamics of these communities, addressing potential seasonality and the lag time between the settlement of the spring bloom and the full expression of enzymes involved in particle degradation. This would necessarily include denser temporal sampling, ideally including a year-round observation of the benthic bacterial transcriptome.

We detected 124 Pfams related to peptidase activities, which included members of more than 20 peptidase clans in the MEROPS classification. Peptidase activities contained both endo- and exopeptidases of different catalytic type, yet the predominant type in deep-sea sediments were metallopeptidases, including aminopeptidases, carboxypeptidases, dipeptidases and tripeptidases (Figure 5). We identified several peptidases specialized in bacterial cell wall biosynthesis and lysis (the M15 and S13 families), and also detected peptidases in the M19 family, which are also likely involved in the degradation of bacterial cell walls as they target D-amino acids, the building blocks of peptidoglycan. Taken together, the variety of detected peptidases strongly suggests the ability of the communities to degrade diverse types of proteinaceous materials, including recalcitrant input, such as cell walls. The importance of proteinaceous matter as a source of nutrition has already been suggested in early publications on hydrolytic enzyme activities in deep-sea sediments. They reported a significantly higher peptidase activity, compared to other tested hydrolases, which was suggested to reflect the high availability of its substrates (Boetius, 1995; Poremba, 1995). Conversely, activities of CAZymes were

suggested to decline at great depth due to the decreasing lability of the targeted compounds (Boetius, 1995; Poremba, 1995). Correspondingly, the overall representation of CAZymes in our data was low (Table SI 3 & 4). However, the detected Pfams indicate the utilization of detrital matter of both algal and bacterial origin, including polymeric storage compounds (e.g. starch, glycogen), cell wall building blocks (cellulose, hemicellulose, peptidoglycan), and matter from decaying animals or fecal pellets (chitin). The dominant glycoside hydrolases (GHs) in the dataset were affiliated with the hydrolysis of oligosaccharide chains found in N- and O-linked glycoproteins, such as arabinogalactan proteins or chitin (GH109), other plant cell wall components including cellulose, hemicellulose (xylan, xyloglucan), pectin (GH3), mannose-containing glycans (GH130), as well as storage compounds including starch (GH13, GH15), glycogen and the related oligo- and polysaccharides (GH13), dextran and trehalose (GH15) (Figure 6). When comparing the set of GHs detected in Arctic deep-sea sediment to communities to other marine ecosystems, we found considerable overlap with enzymes detected in subsurface seawater from 1 mbsl during a North Sea phytoplankton bloom (Teeling et al., 2012). Indeed, 70% of GHs relevant for external carbohydrate degradation were also expressed in the sediment. Further, we found all of the GHs detected in a cross-section of the North Atlantic water column ranging from 100 to 5,000 mbsl (Bergauer et al., 2017) in our dataset. In comparison to non-marine environments, (e.g. the guts and rumen of different herbivores (Pope et al., 2010; Qi et al., 2011)) we found several of the same GHs targeting plant structural polysaccharides, and overlap was especially high for oligosaccharide-degrading GHs, where we detected >80% of those present in guts and rumen (Pope et al., 2010; Qi et al., 2011) also in the deep-sea communities. Further, the dominant carbohydrate esterase families (CE) were mainly associated with plant cell wall decomposition, targeting xylan (CE1, CE6), other xylo-oligosaccharides (CE6), cellulose and other polysaccharides (CE14). Pfams involved in adhesive protein-protein, and protein-carbohydrate interactions, such as the PKD domain, a large cell-surface glycoprotein, as well as carbohydrate binding modules (CBM) were also detected. The CBM found to be the most dominant in our data, CBM50, is active in the binding of peptidoglycan and chitin, and was also the predominant CBM found in bacterioplankton communities during decomposition of

algal-derived organic matter (Teeling et al., 2012). We detected only four other CBMs including those involved in the binding of xylan (CBM9 & 13), cellulose (CBM9), alpha-glucans (e.g. starch, glycogen) (CBM48) and other components of plant cell walls (CBM13), as well as for binding to eukaryotic glycans (CBM51).

As CAZymes often act on more than one substrate (Lombard et al., 2014; Berlemont and Martiny, 2015, 2016), similar profiles do not necessarily imply the utilization of the same set of substrates. Still, the coinciding detection of GHs in these various environments indicates similar mechanisms of organic matter breakdown, and further suggests that benthic communities have a similar functional potential for organic matter breakdown as the pelagic communities in the overlying water column, as the seafloor ultimately receives all material that escapes degradation during particle settlement.

Local diversification patterns

While we observed high bacterial turnover at the level of sequence-abundant OTUs along the water depth gradient of up to 51 (Figure 2a), there were no marked qualitative changes in the metagenomic Pfam signatures of CAZymes, peptidases, or transporters present at each depth (Figure 4-6). Similar observations were also obtained on much larger scale from functional analyses of the global ocean microbiome, where taxonomic variability across ocean depths and regions exceeded functional differences (Sunagawa et al., 2015; Bergauer et al., 2017). This suggests, at first sight, that a high degree of functional redundancy exists between different taxa. However, thousands of genes were assigned to each Pfam discussed, the largest containing up to 30,000 individual sequences. We thus expect that the high taxonomic diversity and spatial variation in community structure observed by 16S rRNA gene tags is tracked by the microdiversity within functional genes and their products. It is likely that such patterns of local diversification along the depth gradient would become apparent with deeper metatranscriptomic sequencing. Furthermore, when a higher proportion of genomic functions become known, this latent diversity will better contribute to a more comprehensive ecological understanding of the system. The highly diverse composition of sedimentary particulate and dissolved organic material – featuring thousands of compounds (Schmidt et al., 2009; Rossel

et al., 2016) – is likely to enhance local diversification of bacteria by substantially extending the range of ecological niches (Huisman and Weissing, 1999).

Novel insights into the identity of active substrate utilizers in deep-sea surface sediments

The potential to utilize polysaccharides has been reported for several marine bacterial phyla, including *Proteobacteria*, *Bacteroidetes*, *Verrucomicrobia* and *Planctomycetes*, and has been mostly studied in the context of bacterioplankton-algae interaction in the upper ocean (Martinez-Garcia et al., 2012; Cardman et al., 2014; Kabisch et al., 2014b; Lage and Bondoso, 2014; Teeling et al., 2016; Reintjes et al., 2017). Several studies indicated members of the *Flavobacteriia*, *Gammaproteobacteria* and Roseobacter clade within the class *Alphaproteobacteria* to be most responsive to phytoplankton blooms (Teeling et al., 2012, 2016; Buchan et al., 2014; Klindworth et al., 2014). The genomes of the individual bacterial groups exhibit different capacities for the hydrolysis of polysaccharides, resulting in trophic specialization (Teeling et al., 2012; Fernández-Gómez et al., 2013; Mann et al., 2013; Kabisch et al., 2014b; Xing et al., 2014; Berlemont and Martiny, 2015; Wietz et al., 2015; Barbeyron et al., 2016). Our BLASTx analysis of the transcript fragments we detected in deep-sea sediments showed that mRNA reads matching CAZymes involved in the hydrolysis of oligosaccharides, storage compounds (starch/glycogen) and chitin matched closely related sequences from numerous taxa. However, mRNA sequences encoding CAZyme fragments involved in the degradation of peptidoglycan, glycoproteins and plant polysaccharides had close relatives in only a limited number of bacterial taxa (Figure 7). Namely, these sequences matched records belonging to members of the *Bacteroidetes* and *Firmicutes,* sequences related to glycoprotein processing enzymes matched records associated with *Deltaproteobacteria*, *Bacteroidetes* and *Firmicutes*, and those matching peptidoglycan-hydrolyzing enzymes were linked to members of only five bacterial taxa, including *Gamma-* and *Alphaproteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Firmicutes* (Figure 7). Members of *Deltaproteobacteria* appear to possess genes encoding only some of the detected enzymes, e.g. those involved in the degradation of storage compounds and glycoproteins. Members of *Planctomycetes* encoded

those involved in the degradation of storage compounds and chitin (Figure 7). All of the detected groups are known to interact with algae-derived organic material (Goecke et al., 2013). Only recently, specific polymer utilization and uptake by members of the *Planctomycetes* was tested and visualized (Jeske et al., 2013; Lage and Bondoso, 2014; Faria et al., 2017; Reintjes et al., 2017). In contrast, close relatives of all detected CAZyme transcripts were encoded in members of *Bacteroidetes*, which therefore appear to process a variety of oligo- and polysaccharides. Our results, thus, support the specialized role of marine *Bacteroidetes* in polymer breakdown, as previous studies reported higher numbers of GH genes and the ability to process highly complex organic compounds for members of this taxon (Teeling et al., 2012; Fernández-Gómez et al., 2013; Klindworth et al., 2014). We did not detect a similar utilization pattern for the mRNA reads matching the 20 peptidase families most frequently detected in our datasets, as they had close relatives in a broad range of taxa (Figure 7).

Regarding transporter signatures, we detected components of ABC, TRAP and TTT transporters which had their closest homologs in members of the *Alphaproteobacteria.* mirroring high expression levels of transport systems for amino acids, sugars, carboxylic acids and compatible solutes in pelagic members of the *Alphaproteobacteria* (Sowell et al., 2009; Gifford et al., 2013). Identified components of TBDT systems had their closest homologs in the *Gammaproteobacteria,* consistent with the high expression of gammaproteobacterial TBDT in pelagic environments (Gifford et al., 2013). In marine surface waters, expression of gammaproteobacterial TBDT is second to that of *Bacteroidetes*-derived TBDT (Tang et al., 2012) and is associated with the uptake of complex carbohydrates, oligosaccharides, siderophores, heme, vitamin B12, and metals (Blanvillain et al., 2007; Schauer et al., 2008; Noinaj et al., 2010). Thus, *Gammaproteobacteria*, next to *Bacteroidetes* and *Alphaproteobacteria,* appear to be well-adapted to the uptake of these compounds in deep-sea sediments.

As our sequencing depth was shallow and read lengths were short, these results are only tentative and require further validation in the future, particularly reliant on long-term, repeated sampling to isolate stable patterns. However, they are able to suggest distinct substrate utilization patterns for different members of the deep-sea

sediment microbiome. While we found some functions to be affiliated with a large number of taxonomic groups in current reference databases, others were restricted to a smaller number of microbial taxa. This could reflect strategies of resource partitioning and niche differentiation, similar to what has been observed in bacterioplankton communities (Teeling et al., 2012; Gifford et al., 2013; Bombar et al., 2014; Ghylin et al., 2014; Xing et al., 2014; Sarmento et al., 2016), and has been suggested from microdiversity patterns along CPE gradients (Buttigieg and Ramette, 2014), This may facilitate coexistence of a rich and diverse sediment community, replete with functions tuned to maximize metabolic utilization of the ecosystems restrictive trophic landscape. This work makes clear that an improved understanding of microbial resource partitioning is needed in order to anticipate changes in carbon and nutrient remineralization in the immense sedimentary compartment of the rapidly changing Arctic Ocean (Gobeil et al., 2001; Grebmeier et al., 2006b; Forest et al., 2010; Wassmann, 2011; Wassmann et al., 2011; Lalande et al., 2013).

Concluding remarks

In this study, we have applied metagenomics, metatranscriptomics and 16S rRNA amplicon methods to show that the highly diverse bacterial communities of Arctic deep-sea floor are mostly composed of active taxa. Only some dominant groups, such as members of the *Flavobacteriia* and *Cytophagia*, or the SAR202 clade, were enriched in the active community, as would be expected when considering their adaptations for rapid particle degradation and likely roles in the turnover of recalcitrant matter in the deep ocean. For the majority of dominant groups, their representation was stable or lower in the active community relative to the total community. The combination of amplicon and metagenomic and metatranscriptomic analysis used here allowed early insights into the different ecological strategies employed by the deep-sea, sedimentary microbiome. Our focus on the capacities for organic matter uptake and metabolism, alongside their ecological consequences, suggests that this layered and specialized microbiome plays a multifaceted yet robust role in global biogeochemical cycling. This is supported by our detection of redundant functional types which suggest deep-sea sedimentary assemblages exploit a defined niche configuration driven by recalcitrant organic matter inputs from

the surface. Despite high local diversity at the OTU level, the heterotrophic traits at the protein family level remained similar along a water depth gradient from 1,200 to 5,500 mbsl, indicating that multiple taxa are functionally equipped to compete for the ecoregion's trophically defined niche space. Simultaneously, we observed early indications that distinct substrate utilization patterns can be linked to several bacterial classes or phyla, availing of both broad and narrow substrate spectra. Taken together, our results show that the Arctic, deep-sea, sedimentary microbiome is both stable and the host of fine-grained diversification processes which we are only beginning to understand. In this context, year-round observation coupled to increased spatiotemporal resolution and contextualized by global sampling schemes is needed to characterize this system and differentiate between regional variation, seasonal successional patterns and long-term shifts driven by external forcings.

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References

Angelov, A., Loderer, C., Pompei, S., and Liebl, W. (2011). Novel family of carbohydrate-binding modules revealed by the genome sequence of Spirochaeta thermophila DSM 6192. *Appl. Environ. Microbiol.* 77, 5483–9. doi:10.1128/AEM.00523-11.

Arnosti, C. (2011). Microbial extracellular enzymes and the marine carbon cycle. *Ann. Rev. Mar. Sci.* 3, 401–425. doi:10.1146/annurev-marine-120709-142731.

Arnosti, C. (2008). Functional differences between Arctic seawater and sedimentary microbial communities: contrasts in microbial hydrolysis of complex substrates. *FEMS Microbiol. Ecol.* 66, 343–351. doi:10.1111/j.1574-6941.2008.00587.x.

Arrigo, K. R. (2014). Sea ice ecosystems. *Ann. Rev. Mar. Sci.* 6, 439–467. doi:10.1146/annurev-marine-010213-135103.

Azam, F., and Malfatti, F. (2007). Microbial structuring of marine ecosystems. *Nat. Rev. Microbiol.* 5, 782–91. doi:10.1038/nrmicro1747.

Barbeyron, T., Thomas, F., Barbe, V., Teeling, H., Schenowitz, C., Dossat, C., et al. (2016). Habitat and taxon as driving forces of carbohydrate catabolism in marine heterotrophic bacteria: example of the model algae-associated bacterium *Zobellia galactanivorans* Dsij ^{T}. *Environ. Microbiol.* 18, 4610–4627. doi:10.1111/1462-2920.13584.

Bauer, M., Kube, M., Teeling, H., Richter, M., Lombardot, T., Allers, E., et al. (2006). Whole genome analysis of the marine Bacteroidetes “Gramella forsetii” reveals adaptations to degradation of polymeric organic matter. *Environ. Microbiol.* 8, 2201–2213. doi:10.1111/j.1462-2920.2006.01152.x.

Bauerfeind, E., Nöthig, E. M., Beszczynska, A., Fahl, K., Kaleschke, L., Kreker, K., et al. (2009). Particle sedimentation patterns in the eastern Fram Strait during 2000-2005: Results from the Arctic long-term observatory HAUSGARTEN. *Deep. Res. Part I Oceanogr. Res. Pap.* 56, 1471–1487. doi:10.1016/j.dsr.2009.04.011.

Bergauer, K., Fernandez-Guerra, A., Garcia, J. A. L., Sprenger, R. R., Stepanauskas, R., Pachiadaki, M. G., et al. (2017). Organic matter processing by microbial communities throughout the Atlantic water column as revealed by metaproteomics. *Proc. Natl. Acad. Sci. U. S. A.*, 201708779. doi:10.1073/pnas.1708779115.

Berlemont, R., and Martiny, A. C. (2016). Glycoside hydrolases across environmental microbial communities. *PLOS Comput. Biol.* 12, e1005300. doi:10.1371/journal.pcbi.1005300.

Berlemont, R., and Martiny, A. C. (2015). Genomic potential for polysaccharide deconstruction in bacteria. *Appl. Environ. Microbiol.* 81, 1513–1519. doi:10.1128/AEM.03718-14.

Bienhold, C., Boetius, A., and Ramette, A. (2012). The energy-diversity relationship of complex bacterial communities in Arctic deep-sea sediments. *ISME J.* 6, 724–

32. doi:10.1038/ismej.2011.140.

Bienhold, C., Zinger, L., Boetius, A., and Ramette, A. (2016). Diversity and biogeography of bathyal and abyssal seafloor bacteria. *PLoS One* 11, e0148016. doi:10.1371/journal.pone.0148016.

Blanvillain, S., Meyer, D., Boulanger, A., Lautier, M., Guynet, C., Denancé, N., et al. (2007). Plant carbohydrate scavenging through TonB-dependent receptors: A feature shared by phytopathogenic and aquatic bacteria. *PLoS One* 2, e224. doi:10.1371/journal.pone.0000224.

Boetius, A. (1995). Microbial hydrolytic enzyme activities in deep-sea sediments. *Helgoländer Meeresuntersuchungen* 49, 177–187. doi:10.1007/BF02368348.

Boetius, A., Albrecht, S., Bakker, K., Bienhold, C., Felden, J., Fernández-Méndez, M., et al. (2013). Export of algal biomass from the melting Arctic sea ice. *Science.* 339, 1430–1432. doi:10.1126/science.1231346.

Boetius, A., and Lochte, K. (1994). Regulation of microbial enzymatic degradation of organic matter in deep-sea sediments. *Mar. Ecol. - Prog. Ser.* 104, 299–307. doi:10.3354/meps104299.

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. doi:10.1093/bioinformatics/btu170.

Bombar, D., Heller, P., Sanchez-Baracaldo, P., Carter, B. J., and Zehr, J. P. (2014). Comparative genomics reveals surprising divergence of two closely related strains of uncultivated UCYN-A cyanobacteria. *ISME J.* 8, 2530–42. doi:10.1038/ismej.2014.167.

Buchan, A., LeCleir, G. R., Gulvik, C. A., and Gonzalez, J. M. (2014). Master recyclers: features and functions of bacteria associated with phytoplankton blooms. *Nat. Rev. Microbiol.* 12, 686–698. doi:10.1038/nrmicro3326.

Buchfink, B., Xie, C., and Huson, D. H. (2015). Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* 12, 59–60. doi:10.1038/nmeth.3176.

Buttigieg, P. L., Hankeln, W., Kostadinov, I., Kottmann, R., Yilmaz, P., Duhaime, M. B., et al. (2013). Ecogenomic perspectives on domains of unknown function: Correlation-based exploration of marine metagenomes. *PLoS One* 8, e50869. doi:10.1371/journal.pone.0050869.

Buttigieg, P. L., and Ramette, A. (2014). Biogeographic patterns of bacterial microdiversity in Arctic deep-sea sediments (HAUSGARTEN, Fram Strait). *Front. Microbiol.* 5, 660. doi:10.3389/fmicb.2014.00660.

Cardman, Z., Arnosti, C., Durbin, A., Ziervogel, K., Cox, C., Steen, A. D., et al. (2014). Verrucomicrobia are candidates for polysaccharide-degrading bacterioplankton in an Arctic fjord of Svalbard. *Appl. Environ. Microbiol.* 80, 3749–3756. doi:10.1128/AEM.00899-14.

Carradec, Q., Pelletier, E., Da Silva, C., Alberti, A., Seeleuthner, Y., Blanc-Mathieu, R., et al. (2018). A global ocean atlas of eukaryotic genes. *Nat. Commun.* 9, 373. doi:10.1038/s41467-017-02342-1.

Cerqueira, T., Pinho, D., Froufe, H., Santos, R. S., Bettencourt, R., and Egas, C. (2017). Sediment microbial diversity of three deep-sea hydrothermal vents southwest of the Azores. *Microb. Ecol.* 74, 332–349. doi:10.1007/s00248-017-0943-9.

Chen, P., Zhang, L., Guo, X., Dai, X., Liu, L., Xi, L., et al. (2016). Diversity, biogeography, and biodegradation potential of Actinobacteria in the deep-sea sediments along the Southwest Indian Ridge. *Front. Microbiol.* 7, 1340. doi:10.3389/fmicb.2016.01340.

Davidson, A. L., Dassa, E., Orelle, C., and Chen, J. (2008). Structure, function, and evolution of bacterial ATP-binding cassette systems. *Microbiol. Mol. Biol. Rev.* 72, 317–364. doi:10.1128/MMBR.00031-07.

Dell'Anno, A., and Danovaro, R. (2005). Extracellular DNA plays a key role in deep-sea ecosystem functioning. *Science.* 309, 2179–2179. doi:10.1126/science.1117475.

Deming, J. W., and Baross, J. A. (2000). "Survival, dormancy, and nonculturable cells in extreme deep-sea environments" in *Nonculturable microorganisms in the environment* (Boston, MA: Springer US), 147–197. doi:10.1007/978-1-4757-0271-2_10.

Dethleff, D., Rachold, V., Tintelnot, M., and Antonow, M. (2000). Sea-ice transport of riverine particles from the Laptev Sea to Fram Strait based on clay mineral studies. *Int. J. Earth Sci.* 89, 496–502. doi:10.1007/s005310000109.

Faria, M., Bordin, N., Kizina, J., Harder, J., Devos, D., and Lage, O. M. (2017). Planctomycetes attached to algal surfaces: Insight into their genomes. *Genomics*. doi:10.1016/J.YGENO.2017.10.007.

Fernández-Gómez, B., Richter, M., Schüler, M., Pinhassi, J., Acinas, S. G., González, J. M., et al. (2013). Ecology of marine Bacteroidetes: a comparative genomics approach. *ISME J.* 7, 1026–1037. doi:10.1038/ismej.2012.169.

Findlay, R. H., King, G. M., and Watling1, L. (1989). Efficacy of phospholipid analysis in determining microbial biomass in sediments. *Appl. Environ. Microbiol.* 55, 2888–2893. Available at: http://aem.asm.org/content/55/11/2888.full.pdf [Accessed May 21, 2018].

Forest, A., Wassmann, P., Slagstad, D., Bauerfeind, E., Nöthig, E. M., and Klages, M. (2010). Relationships between primary production and vertical particle export at the Atlantic-Arctic boundary (Fram Strait, HAUSGARTEN). *Polar Biol.* 33, 1733–1746. doi:10.1007/s00300-010-0855-3.

Ghylin, T. W., Garcia, S. L., Moya, F., Oyserman, B. O., Schwientek, P., Forest, K. T., et al. (2014). Comparative single-cell genomics reveals potential ecological niches for the freshwater acI Actinobacteria lineage. *ISME J.* 8, 2503–16. doi:10.1038/ismej.2014.135.

Gifford, S. M., Sharma, S., Booth, M., and Moran, M. A. (2013). Expression patterns reveal niche diversification in a marine microbial assemblage. *ISME J.* 7, 281–298. doi:10.1038/ismej.2012.96.

Glover, A. G., and Smith, C. R. (2003). The deep-sea floor ecosystem: current status and prospects of anthropogenic change by the year 2025. *Environ. Conserv.* 30, 219–241. doi:10.1017/S0376892903000225.

Gobeil, C., Sundby, B., Macdonald, R. W., and Smith, J. N. (2001). Recent change in organic carbon flux to Arctic Ocean deep basins' Evidence from acid volatile sulfide manganese and rhenium discord in sediments. *Geophys. Res. Lett.* 28, 1743–1746.

Gobet, A., Quince, C., and Ramette, A. (2010). Multivariate Cutoff Level Analysis (MultiCoLA) of large community data sets. *Nucleic Acids Res.* 38, e155. doi:10.1093/nar/gkq545.

Goecke, F., Thiel, V., Wiese, J., Labes, A., and Imhoff, J. F. (2013). Algae as an important environment for bacteria – phylogenetic relationships among new bacterial species isolated from algae. *Phycologia* 52, 14–24. doi:10.2216/12-24.1.

Goffredi, S. K., and Orphan, V. J. (2010). Bacterial community shifts in taxa and diversity in response to localized organic loading in the deep sea. *Environ. Microbiol.* 12, 344–63. doi:10.1111/j.1462-2920.2009.02072.x.

Gooday, A. J., and Turley, C. M. (1990). Responses by benthic organisms to inputs of organic material to the ocean floor: A Review. *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* 331, 119–138. doi:10.1098/rsta.1990.0060.

Grebmeier, J. M., Overland, J. E., Moore, S. E., Farley, E. V, Carmack, E. C., Cooper, L. W., et al. (2006). A major ecosystem shift in the northern Bering Sea. *Science.* 311, 1461–1464. doi:10.1126/science.1121365.

Greiser, N., and Faubel, A. (1988). "Biotic factors" in *Introduction to the study of meiofauna*, eds. R. P. Higgins and H. Thiel (Washington D.C./London: Smithsonian Institution Press), 79–114.

Hebbeln, D. (2000). Flux of ice-rafted detritus from sea ice in the Fram Strait. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 47, 1773–1790. doi:10.1016/S0967-0645(00)00006-0.

Herndl, G. J., and Reinthaler, T. (2013). Microbial control of the dark end of the biological pump. *Nat. Geosci.* 6, 718–724. doi:10.1038/ngeo1921.

Hoffmann, K., Hassenrück, C., Salman-Carvalho, V., Holtappels, M., and Bienhold, C. (2017). Response of bacterial communities to different detritus compositions in Arctic deep-sea sediments. *Front. Microbiol.* 8, 266. doi:10.3389/fmicb.2017.00266.

Holm-Hansen, O., Lorenzen, C. J., Holmes, R. W., and Strickland, J. D. H. (1965). Fluorometric determination of chlorophyll. *ICES J. Mar. Sci.* 30, 3–15. doi:10.1093/icesjms/30.1.3.

Jacob, M., Soltwedel, T., Boetius, A., and Ramette, A. (2013). Biogeography of deep-sea benthic bacteria at regional scale (LTER HAUSGARTEN, Fram Strait, Arctic). *PLoS One* 8, e72779. doi:10.1371/journal.pone.0072779.

Jeske, O., Jogler, M., Petersen, J., Sikorski, J., and Jogler, C. (2013). From genome

mining to phenotypic microarrays: Planctomycetes as source for novel bioactive molecules. *Antonie Van Leeuwenhoek* 104, 551–567. doi:10.1007/s10482-013-0007-1.

Jørgensen, B. B., and Boetius, A. (2007). Feast and famine - microbial life in the deep-sea bed. *Nat. Rev. Microbiol.* 5, 770–781. doi:10.1038/nrmicro1745.

Kabisch, A., Otto, A., König, S., Becher, D., Albrecht, D., Schüler, M., et al. (2014). Functional characterization of polysaccharide utilization loci in the marine Bacteroidetes 'Gramella forsetii' KT0803. *ISME J.* 8, 1492–1502. doi:10.1038/ismej.2014.4.

Kanzog, C., Ramette, A., Quéric, N. V., and Klages, M. (2008). Response of benthic microbial communities to chitin enrichment: an in situ study in the deep Arctic Ocean. *Polar Biol.* 32, 105–112. doi:10.1007/s00300-008-0510-4.

Kirchman, D. L. (2002). The ecology of Cytophaga–Flavobacteria in aquatic environments. *FEMS Microbiol. Ecol.* 39, 91–100. doi:10.1111/j.1574-6941.2002.tb00910.x.

Klindworth, A., Mann, A. J., Huang, S., Wichels, A., Quast, C., Waldmann, J., et al. (2014). Diversity and activity of marine bacterioplankton during a diatom bloom in the North Sea assessed by total RNA and pyrotag sequencing. *Mar. Genomics* 18, 185–192. doi:10.1016/j.margen.2014.08.007.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., et al. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* 41, e1. doi:10.1093/nar/gks808.

Kopylova, E., Noé, L., and Touzet, H. (2012). SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. *Bioinformatics* 28, 3211–3217. doi:10.1093/bioinformatics/bts611.

Köster, M., Jensen, P., and Meyer-Reil, L.-A. (1991). "Hydrolytic activities of organisms and biogenic structures in deep-sea sediments" in Microbial enzymes in aquatic environments (Springer, New York, NY), 298–310. doi:10.1007/978-1-4612-3090-8_19.

Lage, O. M., and Bondoso, J. (2014). Planctomycetes and macroalgae, a striking association. *Front. Microbiol.* 5, 267. doi:10.3389/fmicb.2014.00267.

Lalande, C., Bauerfeind, E., Nöthig, E.-M. M., and Beszczynska-Möller, A. (2013). Impact of a warm anomaly on export fluxes of biogenic matter in the eastern Fram Strait. *Prog. Oceanogr.* 109, 70–77. doi:10.1016/j.pocean.2012.09.006.

Lalande, C., Nöthig, E.-M. M., Bauerfeind, E., Hardge, K., Beszczynska-Möller, A., and Fahl, K. (2016). Lateral supply and downward export of particulate matter from upper waters to the seafloor in the deep eastern Fram Strait. *Deep. Res. Part I Oceanogr. Res. Pap.* 114, 78–89. doi:10.1016/j.dsr.2016.04.014.

Landry, Z., Swan, B. K., Herndl, G. J., Stepanauskas, R., and Giovannoni, S. J. (2017). SAR202 genomes from the dark ocean predict pathways for the oxidation of recalcitrant dissolved organic matter. *MBio* 8, e00413-17.

doi:10.1128/mBio.00413-17.

Lennon, J. T., and Jones, S. E. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nat. Rev. Microbiol.* 9, 119–130. doi:10.1038/nrmicro2504.

Leu, E., Søreide, J. E., Hessen, D. O., Falk-Petersen, S., and Berge, J. (2011). Consequences of changing sea-ice cover for primary and secondary producers in the European Arctic shelf seas: Timing, quantity, and quality. *Prog. Oceanogr.* 90, 18–32. doi:10.1016/j.pocean.2011.02.004.

Lindh, M. V., Maillot, B. M., Shulse, C. N., Gooday, A. J., Amon, D. J., Smith, C. R., et al. (2017). From the surface to the deep-sea: Bacterial distributions across polymetallic nodule fields in the clarion-clipperton zone of the Pacific Ocean. *Front. Microbiol.* 8, 1696. doi:10.3389/fmicb.2017.01696.

Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P. M., and Henrissat, B. (2014). The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res.* 42, D490–D495. doi:10.1093/nar/gkt1178.

Lomstein, B. A., Langerhuus, A. T., D'Hondt, S., Jørgensen, B. B., and Spivack, A. J. (2012). Endospore abundance, microbial growth and necromass turnover in deep sub-seafloor sediment. *Nature* 484, 101–104. doi:10.1038/nature10905.

Lozupone, C. A., and Knight, R. (2007). Global patterns in bacterial diversity. *Proc. Natl. Acad. Sci. U. S. A.* 104, 11436–40. doi:10.1073/pnas.0611525104.

Mahé, F., Rognes, T., Quince, C., de Vargas, C., and Dunthorn, M. (2015). Swarm v2: highly-scalable and high-resolution amplicon clustering. *PeerJ* 3, e1420. doi:10.7717/peerj.1420.

Mahé, F., Rognes, T., Quince, C., de Vargas, C., and Dunthorn, M. (2014). Swarm: robust and fast clustering method for amplicon-based studies. *PeerJ* 2, e593. doi:10.7717/peerj.593.

Mann, A. J., Hahnke, R. L., Huang, S., Werner, J., Xing, P., Barbeyron, T., et al. (2013). The genome of the alga-associated marine flavobacterium Formosa agariphila KMM 3901T reveals a broad potential for degradation of algal polysaccharides. *Appl. Environ. Microbiol.* 79, 6813–22. doi:10.1128/AEM.01937-13.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 17, 10. doi:10.14806/ej.17.1.200.

Martinez-Garcia, M., Brazel, D. M., Swan, B. K., Arnosti, C., Chain, P. S. G., Reitenga, K. G., et al. (2012). Capturing single cell genomes of active polysaccharide degraders: An unexpected contribution of verrucomicrobia. *PLoS One* 7, e35314. doi:10.1371/journal.pone.0035314.

Mayor, D. J., Thornton, B., Hay, S., Zuur, A. F., Nicol, G. W., McWilliam, J. M., et al. (2012). Resource quality affects carbon cycling in deep-sea sediments. *ISME J.* 6, 1740–8. doi:10.1038/ismej.2012.14.

Meinicke, P. (2015). UProC: tools for ultra-fast protein domain classification. *Bioinformatics* 31, 1382–1388. doi:10.1093/bioinformatics/btu843.

Mulligan, C., Fischer, M., and Thomas, G. H. (2011). Tripartite ATP-independent periplasmic (TRAP) transporters in bacteria and archaea. *FEMS Microbiol. Rev.* 35, 68–86. doi:10.1111/j.1574-6976.2010.00236.x.

Mußmann, M., Pjevac, P., Krüger, K., and Dyksma, S. (2017). Genomic repertoire of the Woeseiaceae/JTB255, cosmopolitan and abundant core members of microbial communities in marine sediments. *ISME J.* 11, 1276–1281. doi:10.1038/ismej.2016.185.

Noinaj, N., Guillier, M., Barnard, T. J., and Buchanan, S. K. (2010). TonB-dependent transporters: Regulation, structure, and function. *Annu. Rev. Microbiol.* 64, 43–60. doi:10.1146/annurev.micro.112408.134247.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., et al. (2014). vegan: Community ecology package - R package version 2.2-0. Available at: http://cran.r-project.org/package=vegan.

Orsi, W. D., Edgcomb, V. P., Christman, G. D., and Biddle, J. F. (2013). Gene expression in the deep biosphere. *Nature* 499, 205–8. doi:10.1038/nature12230.

Pfannkuche, O. (1993). Benthic response to the sedimentation of particulate organic matter at the BIOTRANS station, 47°N, 20°W. *Deep. Res. Part II Top. Stud. Oceanogr.* 40, 135–149. doi:10.1016/0967-0645(93)90010-K.

Pope, P. B., Denman, S. E., Jones, M., Tringe, S. G., Barry, K., Malfatti, S. A., et al. (2010). Adaptation to herbivory by the Tammar wallaby includes bacterial and glycoside hydrolase profiles different from other herbivores. *Proc. Natl. Acad. Sci. U. S. A.* 107, 14793–8. doi:10.1073/pnas.1005297107.

Poremba, K. (1995). Hydrolytic enzymatic activity in deep-sea sediments. *Fems Microbiol. Ecol.* 16, 213–221. doi:10.1111/j.1574-6941.1995.tb00285.x.

Poremba, K., and Hoppe, H.-G. (1995). Spatial variation of benthic microbial production and hydrolytic enzymatic activity down the continental slope of the Celtic Sea. *Mar. Ecol. Prog. Ser.* 118, 237–245.

Pruesse, E., Peplies, J., and Glöckner, F. O. (2012). SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 28, 1823–1829. doi:10.1093/bioinformatics/bts252.

Qi, M., Wang, P., O'Toole, N., Barboza, P. S., Ungerfeld, E., Leigh, M. B., et al. (2011). Snapshot of the eukaryotic gene expression in muskoxen rumen—a metatranscriptomic approach. *PLoS One* 6, e20521. doi:10.1371/journal.pone.0020521.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596. doi:10.1093/nar/gks1219.

Quinn, T. P., Erb, I., Richardson, M. F., and Crowley, T. M. (2018). Understanding sequencing data as compositions: an outlook and review. *Bioinformatics*. doi:10.1093/bioinformatics/bty175.

R Core Team (2014). R: A Language and Environment for Statistical Computing.

Available at: http://www.r-project.org/.

Rapp, J. Z., Fernández-Méndez, M., Bienhold, C., and Boetius, A. (2018). Effects of ice-algal aggregate export on the connectivity of bacterial communities in the central Arctic Ocean. *Front. Microbiol.* 9, 1035. doi:10.3389/fmicb.2018.01035.

Rasigraf, O., Schmitt, J., Jetten, M. S. M., and Lüke, C. (2017). Metagenomic potential for and diversity of N-cycle driving microorganisms in the Bothnian Sea sediment. *Microbiologyopen* 6, e00475. doi:10.1002/mbo3.475.

Rawlings, N. D., Waller, M., Barrett, A. J., and Bateman, A. (2014). *MEROPS*: the database of proteolytic enzymes, their substrates and inhibitors. *Nucleic Acids Res.* 42, D503–D509. doi:10.1093/nar/gkt953.

Reintjes, G., Arnosti, C., Fuchs, B. M., and Amann, R. (2017). An alternative polysaccharide uptake mechanism of marine bacteria. *ISME J.* 11, 1640–1650. doi:10.1038/ismej.2017.26.

Rho, M., Tang, H., and Ye, Y. (2010). FragGeneScan: predicting genes in short and error-prone reads. *Nucleic Acids Res.* 38, e191. doi:10.1093/nar/gkq747.

Rossel, P. E., Bienhold, C., Boetius, A., and Dittmar, T. (2016). Dissolved organic matter in pore water of Arctic Ocean sediments: Environmental influence on molecular composition. *Org. Geochem.* 97, 41–52. doi:10.1016/j.orggeochem.2016.04.003.

Rowe, G. T., Sibuet, M., Deming, J., Tietjen, J., and Khripounoff, A. (1990). Organic carbon turnover time in deep-sea benthos. *Prog. Oceanogr.* 24, 141–160. doi:10.1016/0079-6611(90)90026-X.

Ruff, E. S., Probandt, D., Zinkann, A.-C. C., Iversen, M. H., Klaas, C., Würzberg, L., et al. (2014). Indications for algae-degrading benthic microbial communities in deep-sea sediments along the Antarctic Polar Front. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 108, 6–16. doi:10.1016/j.dsr2.2014.05.011.

Saier, M. H., Reddy, V. S., Tsu, B. V., Ahmed, M. S., Li, C., and Moreno-Hagelsieb, G. (2016). The Transporter Classification Database (TCDB): recent advances. *Nucleic Acids Res.* 44, D372–D379. doi:10.1093/nar/gkv1103.

Sarmento, H., Morana, C., and Gasol, J. M. (2016). Bacterioplankton niche partitioning in the use of phytoplankton-derived dissolved organic carbon: quantity is more important than quality. *ISME J.*, 1–11. doi:10.1038/ismej.2016.66.

Schauer, K., Rodionov, D. A., and de Reuse, H. (2008). New substrates for TonB-dependent transport: do we only see the 'tip of the iceberg'? *Trends Biochem. Sci.* 33, 330–338. doi:10.1016/J.TIBS.2008.04.012.

Schmidt, F., Elvert, M., Koch, B. P., Witt, M., and Hinrichs, K.-U. (2009). Molecular characterization of dissolved organic matter in pore water of continental shelf sediments. *Geochim. Cosmochim. Acta* 73, 3337–3358. doi:10.1016/J.GCA.2009.03.008.

Schmidt, F., Koch, B. P., Goldhammer, T., Elvert, M., Witt, M., Lin, Y.-S., et al. (2017). Unraveling signatures of biogeochemical processes and the depositional

setting in the molecular composition of pore water DOM across different marine environments. *Geochim. Cosmochim. Acta* 207, 57–80. doi:10.1016/J.GCA.2017.03.005.

Siegert, M., Krüger, M., Teichert, B., Wiedicke, M., and Schippers, A. (2011). Anaerobic oxidation of methane at a marine methane seep in a forearc sediment basin off Sumatra, Indian Ocean. *Front. Microbiol.* 2, 249. doi:10.3389/fmicb.2011.00249.

Smith, C. R., De Leo, F. C., Bernardino, A. F., Sweetman, A. K., and Arbizu, P. M. (2008). Abyssal food limitation, ecosystem structure and climate change. *Trends Ecol. Evol.* 23, 518–528. doi:10.1016/j.tree.2008.05.002.

Soltwedel, T., Bauerfeind, E., Bergmann, M., Bracher, A., Budaeva, N., Busch, K., et al. (2016). Natural variability or anthropogenically-induced variation? Insights from 15 years of multidisciplinary observations at the arctic marine LTER site HAUSGARTEN. *Ecol. Indic.* 65, 89–102. doi:10.1016/j.ecolind.2015.10.001.

Soltwedel, T., Bauerfeind, E., Bergmann, M., Budaeva, N., Hoste, E., Jaeckisch, N., et al. (2005). HAUSGARTEN: Multidisciplinary investigations at a deep-sea, long-term observatory in the Arctic Ocean. *Oceanography* 18, 46–61. doi:10.5670/oceanog.2005.24.

Soltwedel, T., Miljutina, M., Mokievsky, V., Thistle, D., and Vopel, K. (2003). The meiobenthos of the molloy deep (5600 m), Fram Strait, Arctic Ocean. *Vie milieu* 53, 1–14.

Sowell, S. M., Wilhelm, L. J., Norbeck, A. D., Lipton, M. S., Nicora, C. D., Barofsky, D. F., et al. (2009). Transport functions dominate the SAR11 metaproteome at low-nutrient extremes in the Sargasso Sea. *ISME J.* 3, 93–105. doi:10.1038/ismej.2008.83.

Sunagawa, S., Coelho, L. P., Chaffron, S., Kultima, J. R., Labadie, K., Salazar, G., et al. (2015). Structure and function of the global ocean microbiome. *Science.* 348. doi:10.1126/science.1261359.

Tang, K., Jiao, N., Liu, K., Zhang, Y., and Li, S. (2012). Distribution and functions of TonB-dependent transporters in marine bacteria and environments: implications for dissolved organic matter utilization. *PLoS One* 7, e41204. doi:10.1371/journal.pone.0041204.

Techtmann, S. M., Fitzgerald, K. S., Stelling, S. C., Joyner, D. C., Uttukar, S. M., Harris, A. P., et al. (2016). Colwellia psychrerythraea strains from distant deep sea basins show adaptation to local conditions. *Front. Environ. Sci.* 4, 33. doi:10.3389/fenvs.2016.00033.

Teeling, H., Fuchs, B. M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C. M., et al. (2012). Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science.* 336, 608–611. doi:10.1126/science.1218344.

Teeling, H., Fuchs, B. M., Bennke, C. M., Krüger, K., Chafee, M., Kappelmann, L., et al. (2016). Recurring patterns in bacterioplankton dynamics during coastal

spring algae blooms. *Elife* 5, e11888. doi:10.7554/eLife.11888.

Teske, a, Durbin, a, Ziervogel, K., Cox, C., and Arnosti, C. (2011). Microbial community composition and function in permanently cold seawater and sediments from an arctic fjord of svalbard. *Appl. Environ. Microbiol.* 77, 2008–18. doi:10.1128/AEM.01507-10.

The CAZypedia Consortium (2018). Ten years of CAZypedia: a living encyclopedia of carbohydrate-active enzymes. *Glycobiology* 28, 3–8. doi:10.1093/glycob/cwx089.

Thomas, F., Hehemann, J.-H., Rebuffet, E., Czjzek, M., and Michel, G. (2011). Environmental and gut bacteroidetes: the food connection. *Front. Microbiol.* 2, 93. doi:10.3389/fmicb.2011.00093.

Tremblay, J., Singh, K., Fern, A., Kirton, E. S., He, S., Woyke, T., et al. (2015). Primer and platform effects on 16S rRNA tag sequencing. *Front. Microbiol.* 6, 771. doi:10.3389/fmicb.2015.00771.

Turley, C. (2000). Bacteria in the cold deep-sea benthic boundary layer and sedimentâ€“water interface of the NE Atlantic. *FEMS Microbiol. Ecol.* 33, 89–99. doi:10.1111/j.1574-6941.2000.tb00731.x.

Turley, C. M., and Dixon, J. L. (2002). Bacterial numbers and growth in surficial deep-sea sediments and phytodetritus in the NE Atlantic : Relationships with particulate organic carbon and total nitrogen. 49, 815–826.

Turley, C. M., and Lochte, K. (1990). Microbial response to the input of fresh detritus to the deep-sea bed. *Glob. Planet. Change* 3, 3–23. doi:10.1016/0921-8181(90)90052-E.

Turner, J. T. (2015). Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. *Prog. Oceanogr.* 130, 205–248.

Wakeham, S. G., Lee, C., Hedges, J. I., Hernes, P. J., and Peterson, M. J. (1997). Molecular indicators of diagenetic status in marine organic matter. *Geochim. Cosmochim. Acta* 61, 5363–5369. doi:10.1016/S0016-7037(97)00312-8.

Wassmann, P. (2011). Arctic marine ecosystems in an era of rapid climate change. *Prog. Oceanogr.* 90, 1–17. doi:10.1016/j.pocean.2011.02.002.

Wassmann, P., Duarte, C. M., Agustí, S., and Sejr, M. K. (2011). Footprints of climate change in the Arctic marine ecosystem. *Glob. Chang. Biol.* 17, 1235–1249. doi:10.1111/j.1365-2486.2010.02311.x.

Wassmann, P., and Reigstad, M. (2011). Future Arctic Ocean Seasonal Ice Zones and Implications for Pelagic-Benthic Coupling. *Oceanography* 24, 220–231. doi:10.5670/oceanog.2011.74.

Weiner, R. M., Taylor, L. E., Henrissat, B., Hauser, L., Land, M., Coutinho, P. M., et al. (2008). Complete genome sequence of the complex carbohydrate-degrading marine bacterium, Saccharophagus degradans strain 2-40T. *PLoS Genet.* 4, e1000087. doi:10.1371/journal.pgen.1000087.

Whitman, W. B., Coleman, D. C., and Wiebe, W. J. (1998). Prokaryotes: the unseen majority. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6578–83.

Wietz, M., Wemheuer, B., Simon, H., Giebel, H.-A., Seibt, M. A., Daniel, R., et al. (2015). Bacterial community dynamics during polysaccharide degradation at contrasting sites in the Southern and Atlantic Oceans. *Environ. Microbiol.* 17, 3822–3831. doi:10.1111/1462-2920.12842.

Williams, T. J., Wilkins, D., Long, E., Evans, F., Demaere, M. Z., Raftery, M. J., et al. (2013). The role of planktonic Flavobacteria in processing algal organic matter in coastal East Antarctica revealed using metagenomics and metaproteomics. *Environ. Microbiol.* 15, 1302–1317. doi:10.1111/1462-2920.12017.

Winter, D. J. (2017). rentrez: an R package for the NCBI eUtils API. *R J.* 9, 520–526. Available at: https://cran.r-project.org/package=rentrez.

Xie, G., Bruce, D. C., Challacombe, J. F., Chertkov, O., Detter, J. C., Gilna, P., et al. (2007). Genome sequence of the cellulolytic gliding bacterium Cytophaga hutchinsonii. *Appl. Environ. Microbiol.* 73, 3536–46. doi:10.1128/AEM.00225-07.

Xing, P., Hahnke, R. L., Unfried, F., Markert, S., Huang, S., Barbeyron, T., et al. (2014). Niches of two polysaccharide-degrading Polaribacter isolates from the North Sea during a spring diatom bloom. *ISME J.* doi:10.1038/ismej.2014.225.

Yentsch, C. S., and Menzel, D. W. (1963). A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. *Deep Sea Res. Oceanogr. Abstr.* 10, 221–231. doi:10.1016/0011-7471(63)90358-9.

Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A. (2014). PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* 30, 614–20. doi:10.1093/bioinformatics/btt593.

Zinger, L., Amaral-Zettler, L. A., Fuhrman, J. A., Horner-Devine, M. C., Huse, S. M., Welch, D. B. M., et al. (2011). Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. *PLoS One* 6, e24570. doi:10.1371/journal.pone.0024570.

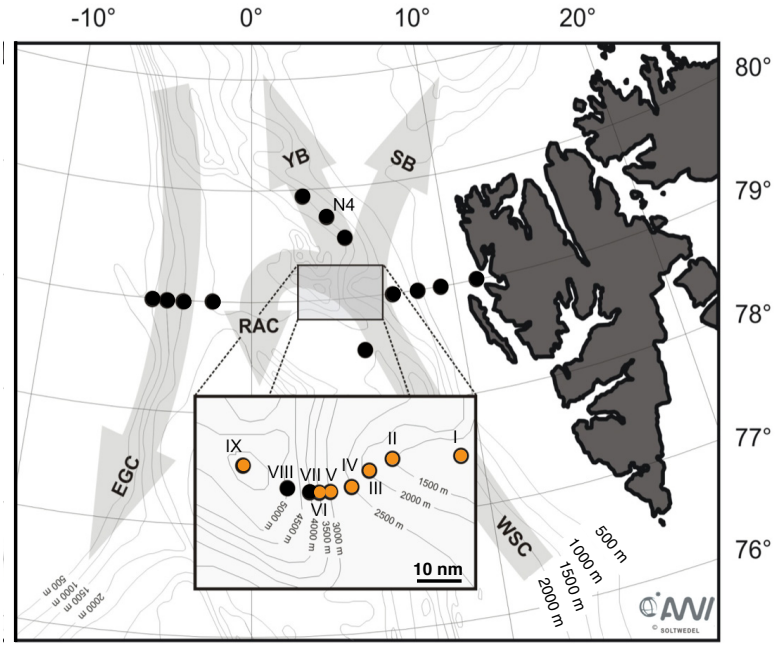

Figure III 1 - Map of the HAUSGARTEN Long-Term Ecological Research (LTER) observatory. Black dots indicate the 21 permanent research stations, orange dots indicate the selected stations that were part of this study. The box shows a close-up of the bathymetric transect ranging from 1,200 mbsl at HGI to 5,500 mbsl at HGIX. The scale bar indicates a 10 nautical miles distance. WSC: West Spitsbergen Current; EGC: East Greenland Current; RAC: Return Atlantic Current; YB: Yermak Branch, and SB: Svalbard Branch of the WSC. Map was modified from Soltwedel et al., 2016.

a

water depth [m] →

water depth [m] ↓	5,500	3,500	3,000	2,500	2,000	1,500	1,200
1,200	0.51	0.22	0.16	0.11	0.09	0.07	0.00
1,500	0.50	0.19	0.14	0.09	0.06	0.00	
2,000	0.49	0.17	0.11	0.07	0.00		
2,500	0.46	0.15	0.10	0.00			
3,000	0.44	0.13	0.00				
3,500	0.42	0.00					
5,500	0.00						

b

water depth [m] →

water depth [m] ↓	5,500	3,500	3,000	2,500	2,000	1,500	1,200
1,200	0.59	0.23	0.26	0.18	0.17	0.23	0.00
1,500	0.61	0.33	0.33	0.28	0.26	0.00	
2,000	0.56	0.19	0.21	0.15	0.00		
2,500	0.55	0.15	0.19	0.00			
3,000	0.51	0.14	0.00				
3,500	0.53	0.00					
5,500	0.00						

Figure III 2 - Community turnover along the water depth gradient as detected by 16S rRNA gene tag sequencing of (**a**) DNA and (**b**) RNA. Turnover represents the proportion of (**a**) total and (**b**) active OTUs differing between two stations and was calculated using the Jaccard dissimilarity measure and based on presence-absence data of abundant OTUs only (represented by at least 100 sequences in the dataset).

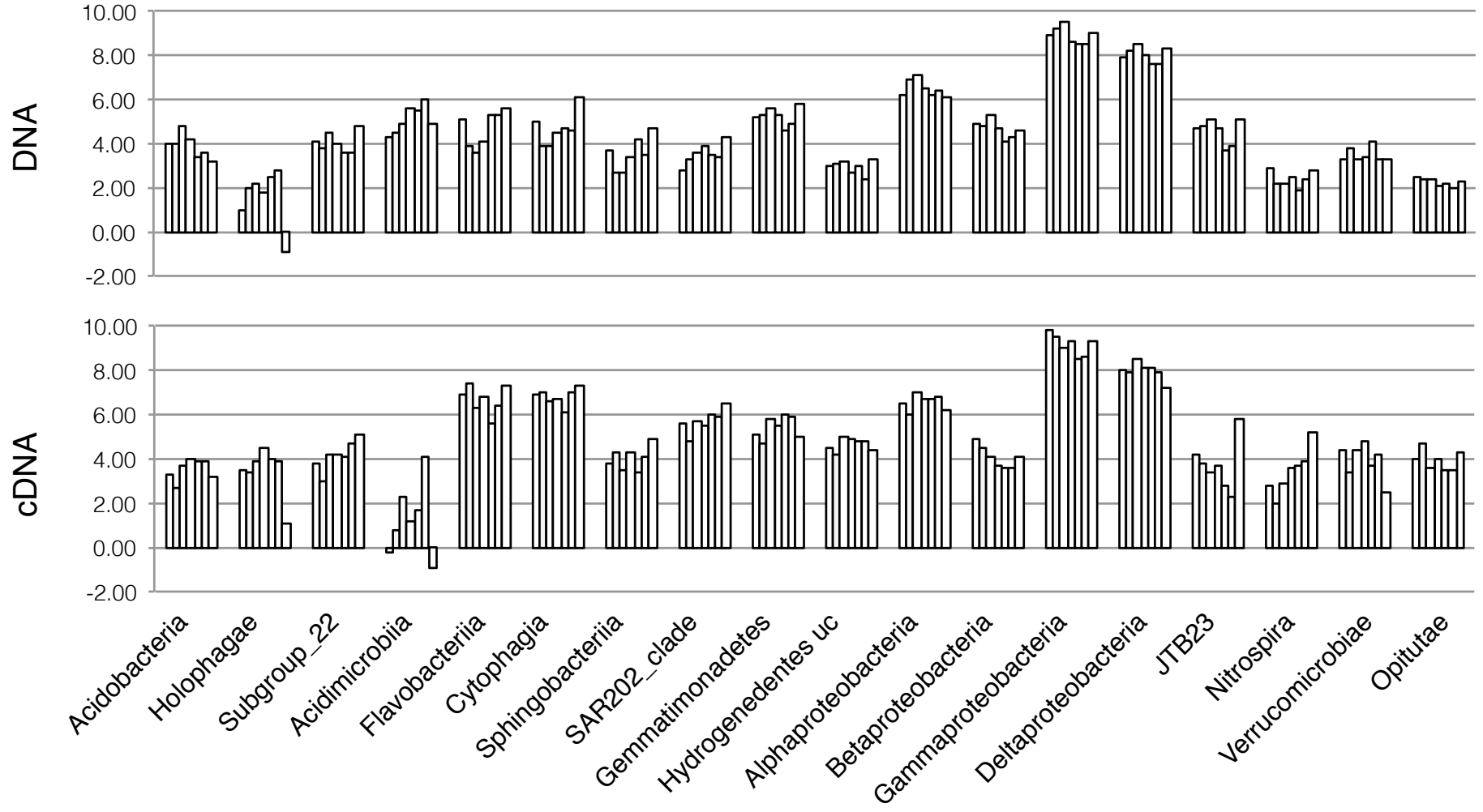

Figure III 3 - The top 10 most dominant bacterial classes across all samples in both DNA and cDNA tags. Data has been clr transformed and represents fold changes from the geometric mean in each sample. Bars for each class represent stations HGI (1,200 mbsl) to HGIX (5,500 mbsl) from left to right.

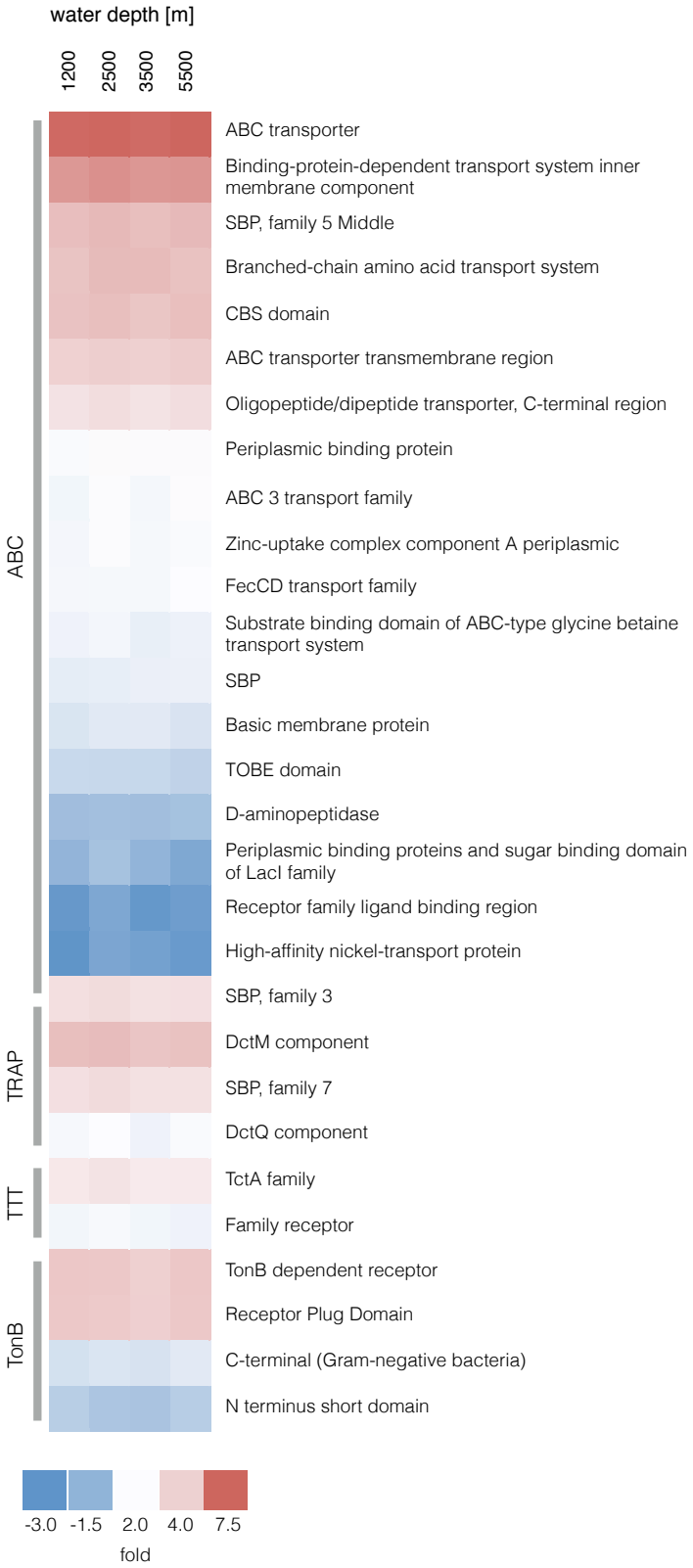

Figure III 4 - Over- and underrepresentation of protein families (Pfam) associated with ABC, TRAP, TTT and TonB transporter activities in deep-sea surface sediments (0-1 cm) at varying water depths. Heat map is based on centered log-ratio (CLR) transformed input data of metagenomic read counts associated with each Pfam and color coding indicates the fold-change from the geometric mean of all transport-associated Pfams. Pfams were assigned using UProC v1.2 according to Pfam database release 28.0.

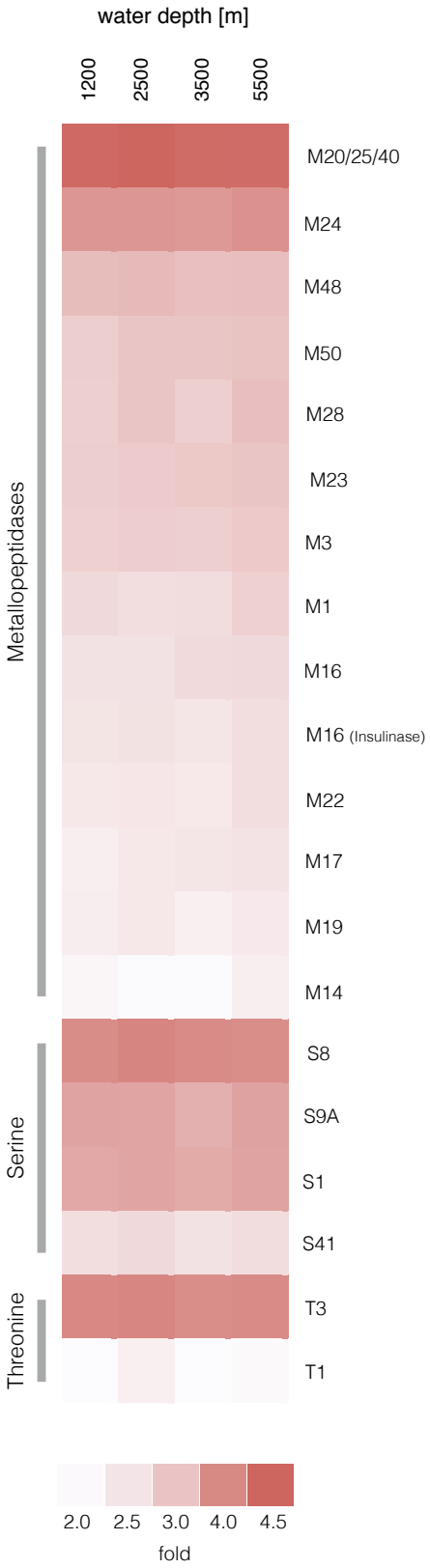

Figure III 5 - Overrepresentation of the top 20 protein families (Pfam) associated with peptidase activities in deep-sea surface sediments (0-1 cm) at varying water depths. Heat map is based on centered log-ratio (CLR) transformed input data of metagenomic read counts associated with each Pfam and color coding indicates the fold-change from the geometric mean of all peptidase-associated Pfams. Pfams were assigned using UProC v1.2 according to Pfam database release 28.0.

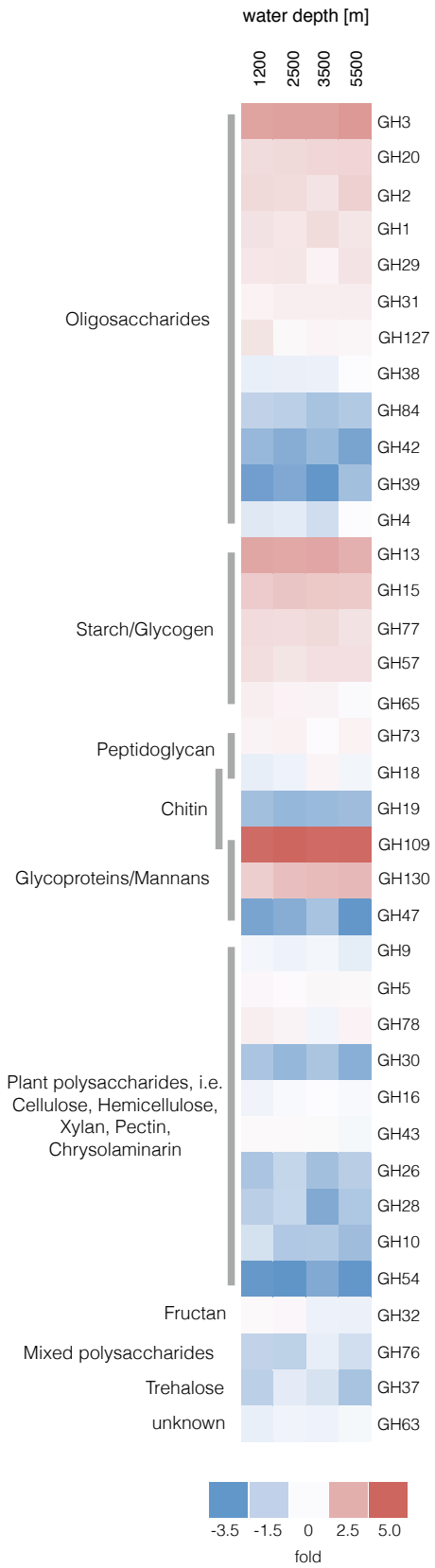

Figure III 6 - Over- and underrepresentation of protein families (Pfam) associated with glycoside hydrolase (GH) activities in deep-sea surface sediments (0-1 cm) at varying water depths. Heat map is based on centered log-ratio (CLR) transformed input data of metagenomic read counts associated with each Pfam and color coding indicates the fold-change from the geometric mean of all GH-associated Pfams. Pfams were assigned using UProC v1.2 according to Pfam database release 28.0.

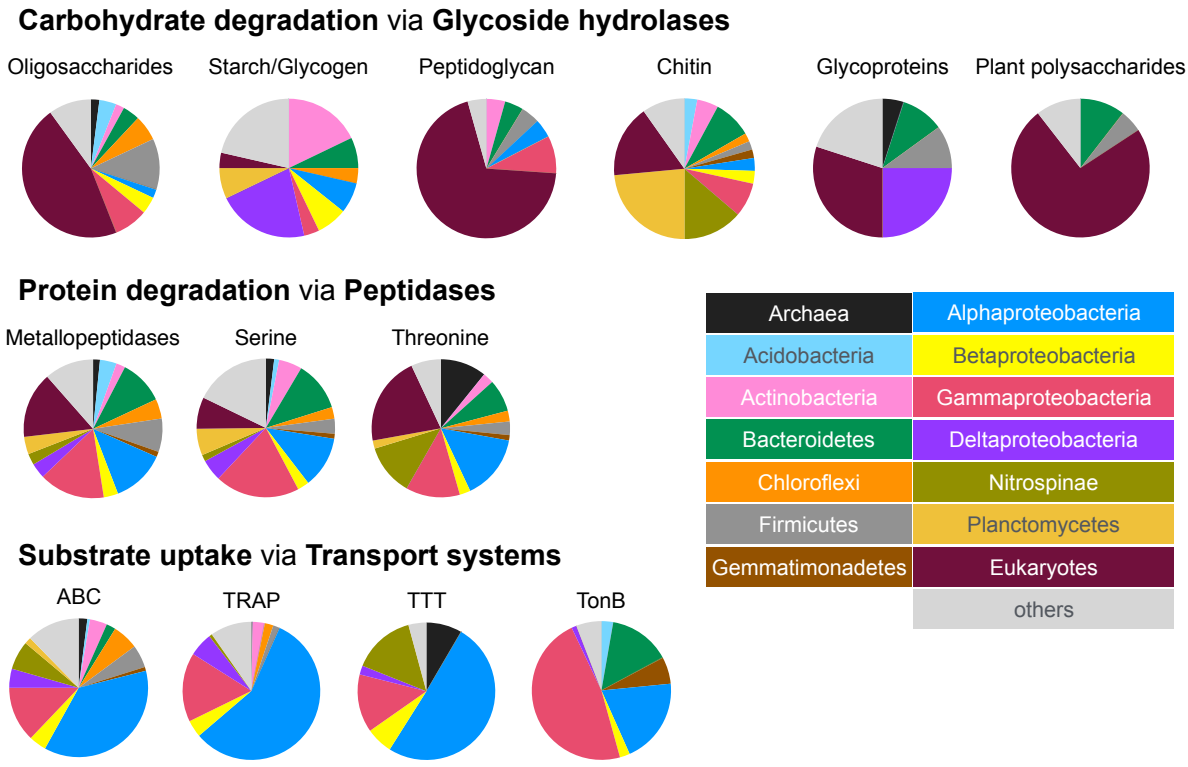

Figure III 7 - Blastp best hit output of metratranscriptomic reads associated with protein families identified as related to CAZymes, peptidases or transporters (SI Table 4).

Table III 1 - Overview of sediment sampling locations. Samples were taken in June 2014 during RV Polarstern expedition ARK-XXVIII/2 (PS85) to the Fram Strait. Gray-shaded stations are sampling locations for 'omics sequencing.

Date	Station name	Station ID	Latitude	Longitude	Water depth (m)
24.6.14	HGI	PS85/470-3	79° 8,01' N	6° 6,39' E	1,244
24.6.14	HGII	PS85/469-2	79° 7,93' N	4° 54,37' E	1,492
24.6.14	HGIII	PS85/468-1	79° 6,39' N	4° 35,10' E	1,905
22.6.14	HGIV	PS85/460-4	79° 3,91' N	4° 10,98' E	2,403
23.6.14	HGV	PS85/463-1	79° 3,14' N	3° 40,37' E	3,034
23.6.14	HGVI	PS85/464-1	79° 3,55' N	3° 34,37' E	3,531
24.6.14	HGIX	PS85/465-4	79° 9,50' N	2° 52,97' E	5,525

Supplementary material I Chapter III

High concentrations of organic material at the seafloor down to more than 5,500 m depth

Deep-sea sediments are typically considered energy-limited environments, receiving little input of exported organic material (Jørgensen and Boetius, 2007; Ramirez-Llodra et al., 2010; Danovaro et al., 2014; Corinaldesi, 2015). In the Arctic, strong seasonality results in elevated export pulses following the spring and summer phytoplankton blooms, and long periods of low export during the dark season (Bauerfeind et al., 1994, 2009; Lalande et al., 2011). A previous study in this region suggests that there was a declining phytoplankton bloom in surface waters during the time of sampling (Fadeev et al. in prep.) Measurements of POC flux in 300 and >2,000 m water depth, indicate that some of the photosynthetically produced organic matter may have already reached the seafloor (unpublished results, EM Nöthig, Alfred Wegener Institute), providing communities with a fresh input of organic material. In line with long-term observations in the Fram Strait (Soltwedel et al., 2016), sediment-bound CPE concentrations were relatively high compared to deep-sea surface sediments from other polar and temperate sites (Boetius et al., 1996; Boetius and Damm, 1998; Giovannelli et al., 2013), and hosted high numbers of microbial cells in the range of 10^9 cells cm^{-3} (SI Figure 2). While CPE concentrations decreased with increasing water depth from ~31 μg cm^{-3} at HGI to 13 μg cm^{-3} at HGVI, the contribution of chlorophyll a (used as a proxy for the “freshness” of this organic material) remained stable and accounted for ~10% of total CPE at all depths (SI Figure 2). This was surprising, as previous studies reported strong alterations of the chemical composition of export material during sinking (Amon and Benner, 1994; Wakeham et al., 1997a; Kiriakoulakis et al., 2001) with labile components being selectively lost and less available at greater depth (Wakeham et al., 1997a, 1997b; Boetius and Damm, 1998). Yet, fast-sinking, larger particles may escape heterotrophic activity during the descent through the water column and could therefore arrive at great depth without substantial losses of its labile constituents (Buesseler et al., 2007; De La Rocha and Passow, 2007; Turner, 2015). Similarly, the stable chlorophyll a ratio may also indicate that the sinking material was inaccessible for microbial degradation in the water column.

The deepest station, HGIX, situated at 5,500 mbsl in the Molloy Hole showed exceptions from the observed trends, as we measured high concentrations of sediment-bound CPE of 27 μg cm^{-3}, as well as high concentrations of phospholipids and proteins (SI Figure 2). The Molloy Hole was reported to accumulate organic material despite its great depth, due to its funnel-like topography, which appears to act as a particle trap (Soltwedel et al., 2005). Previous studies observed meiofauna abundances at this site, which were 10-times higher than those reported from comparably deep ocean regions, and were attributed to the higher food availability (Soltwedel et al., 2003). However, our assessment of microbial cell numbers and extracellular esterase activity did not match the comparably high organic matter content, and still showed lower bacterial standing stocks and enzyme activity than observed at the shallower stations. Increased grazing pressure on microbial cells as a result of the high meiofauna densities may explain this observation, as well as potentially reduced niche space due to the accumulation of specific organic material.
Interestingly, despite the more than 10-times higher organic matter concentrations measured in our study (SI Figure 2), rates of extracellular esterase activity, used as a proxy for the hydrolytic potential of the microbial community, were similar to those reported from sediments collected in comparable water depth at the Laptev Sea continental slope (Boetius and Damm, 1998) or in the Mediterranean deep sea (Boetius et al., 1996). A previous study that reported extremely high deposition of organic matter at the seafloor observed accelerated extracellular enzymatic activities, despite the extreme physical conditions of the deep-sea environment (Danovaro et al., 2003). However, it has been suggested that only a small fraction of the total organic matter pool in deep-sea sediments is enzymatically hydrolysable (on average ~15%) and thus accessible for microbial degradation and uptake (Dell'Anno et al., 2000), which could explain low enzymatic activity despite high organic matter concentrations.

References

Amon, R. M. W., and Benner, R. (1994). Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. *Nature* 369, 549–552. doi:10.1038/369549a0.

Bauerfeind, E., Bodungen, B. V., Arndt, K., and Koeve, W. (1994). Particle flux, and

composition of sedimenting matter, in the Greenland Sea. *J. Mar. Syst.* 5, 411–423. doi:10.1016/0924-7963(94)90005-1.

Bauerfeind, E., Nöthig, E. M., Beszczynska, A., Fahl, K., Kaleschke, L., Kreker, K., et al. (2009). Particle sedimentation patterns in the eastern Fram Strait during 2000-2005: Results from the Arctic long-term observatory HAUSGARTEN. *Deep. Res. Part I Oceanogr. Res. Pap.* 56, 1471–1487. doi:10.1016/j.dsr.2009.04.011.

Boetius, A., and Damm, E. (1998). Benthic oxygen uptake, hydrolytic potentials and microbial biomass at the Arctic continental slope. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 45, 239–275. doi:10.1016/S0967-0637(97)00052-6.

Boetius, A., Scheibej, S., Tselepides, A., and Thiel, H. (1996). Microbial biomass and activities in deep-sea sediments of the Eastern Mediterranean: trenches are benthic hotspots. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 43, 1439–1460. doi:10.1016/S0967-0637(96)00053-2.

Buesseler, K. O., Lamborg, C. H., Boyd, P. W., Lam, P. J., Trull, T. W., Bidigare, R. R., et al. (2007). Revisiting carbon flux through the ocean's twilight zone. *Science.* 316, 567–570. doi:10.1126/science.1137959.

Corinaldesi, C. (2015). New perspectives in benthic deep-sea microbial ecology. *Front. Mar. Sci.* 2, 17. doi:10.3389/fmars.2015.00017.

Danovaro, R., Della Croce, N., Dell'Anno, A., and Pusceddu, A. (2003). A depocenter of organic matter at 7800 m depth in the SE Pacific Ocean. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 50, 1411–1420. doi:10.1016/J.DSR.2003.07.001.

Danovaro, R., Snelgrove, P. V. R., and Tyler, P. (2014). Challenging the paradigms of deep-sea ecology. *Trends Ecol. Evol.* 29, 465–75. doi:10.1016/j.tree.2014.06.002.

De La Rocha, C. L., and Passow, U. (2007). Factors influencing the sinking of POC and the efficiency of the biological carbon pump. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 54, 639–658. doi:10.1016/J.DSR2.2007.01.004.

Dell'Anno, A., Fabiano, M., Mei, M., and Danovaro, R. (2000). Enzymatically hydrolysed protein and carbohydrate pools in deep-sea sediments: estimates of the potentially bioavailable fraction and methodological considerations. *Mar. Ecol. Prog. Ser.* 196, 15–23. doi:10.3354/meps196015.

Giovannelli, D., Molari, M., d'Errico, G., Baldrighi, E., Pala, C., and Manini, E. (2013). Large-scale distribution and activity of prokaryotes in deep-sea surface sediments of the Mediterranean Sea and the adjacent Atlantic Ocean. *PLoS One* 8, e72996. doi:10.1371/journal.pone.0072996.

Jørgensen, B. B., and Boetius, A. (2007). Feast and famine - microbial life in the deep-sea bed. *Nat. Rev. Microbiol.* 5, 770–781. doi:10.1038/nrmicro1745.

Kiriakoulakis, K., Stutt, E., Rowland, S. J., Annick Vangriesheim, Lampitt, R. S., and Wolff, G. A. (2001). Controls on the organic chemical composition of settling particles in the Northeast Atlantic Ocean. *Prog. Oceanogr.* 50, 65–87.

doi:10.1016/S0079-6611(01)00048-9.

Lalande, C., Bauerfeind, E., and Nöthig, E. M. (2011). Downward particulate organic carbon export at high temporal resolution in the eastern Fram Strait: Influence of Atlantic Water on flux composition. *Mar. Ecol. Prog. Ser.* 440, 127–136. doi:10.3354/meps09385.

Ramirez-Llodra, E., Brandt, A., Danovaro, R., De Mol, B., Escobar, E., German, C. R., et al. (2010). Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. *Biogeosciences* 7, 2851–2899. doi:10.5194/bg-7-2851-2010.

Soltwedel, T., Bauerfeind, E., Bergmann, M., Bracher, A., Budaeva, N., Busch, K., et al. (2016). Natural variability or anthropogenically-induced variation? Insights from 15 years of multidisciplinary observations at the arctic marine LTER site HAUSGARTEN. *Ecol. Indic.* 65, 89–102. doi:10.1016/j.ecolind.2015.10.001.

Soltwedel, T., Bauerfeind, E., Bergmann, M., Budaeva, N., Hoste, E., Jaeckisch, N., et al. (2005). HAUSGARTEN: Multidisciplinary investigations at a deep-sea, long-term observatory in the Arctic Ocean. *Oceanography* 18, 46–61. doi:10.5670/oceanog.2005.24.

Soltwedel, T., Miljutina, M., Mokievsky, V., Thistle, D., and Vopel, K. (2003). The meiobenthos of the molloy deep (5600 m), Fram Strait, Arctic Ocean. *Vie milieu* 53, 1–14.

Turner, J. T. (2015). Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. *Prog. Oceanogr.* 130, 205–248. Available at: https://www.sciencedirect.com/science/article/pii/S0079661114001281#b0780 [Accessed October 26, 2016].

Wakeham, S. G., Hedges, J. I., Lee, C., Peterson, M. L., and Hernes, P. J. (1997a). Compositions and transport of lipid biomarkers through the water column and surficial sediments of the equatorial Pacific Ocean. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 44, 2131–2162. doi:10.1016/S0967-0645(97)00035-0.

Wakeham, S. G., Lee, C., Hedges, J. I., Hernes, P. J., and Peterson, M. J. (1997b). Molecular indicators of diagenetic status in marine organic matter. *Geochim. Cosmochim. Acta* 61, 5363–5369. doi:10.1016/S0016-7037(97)00312-8.

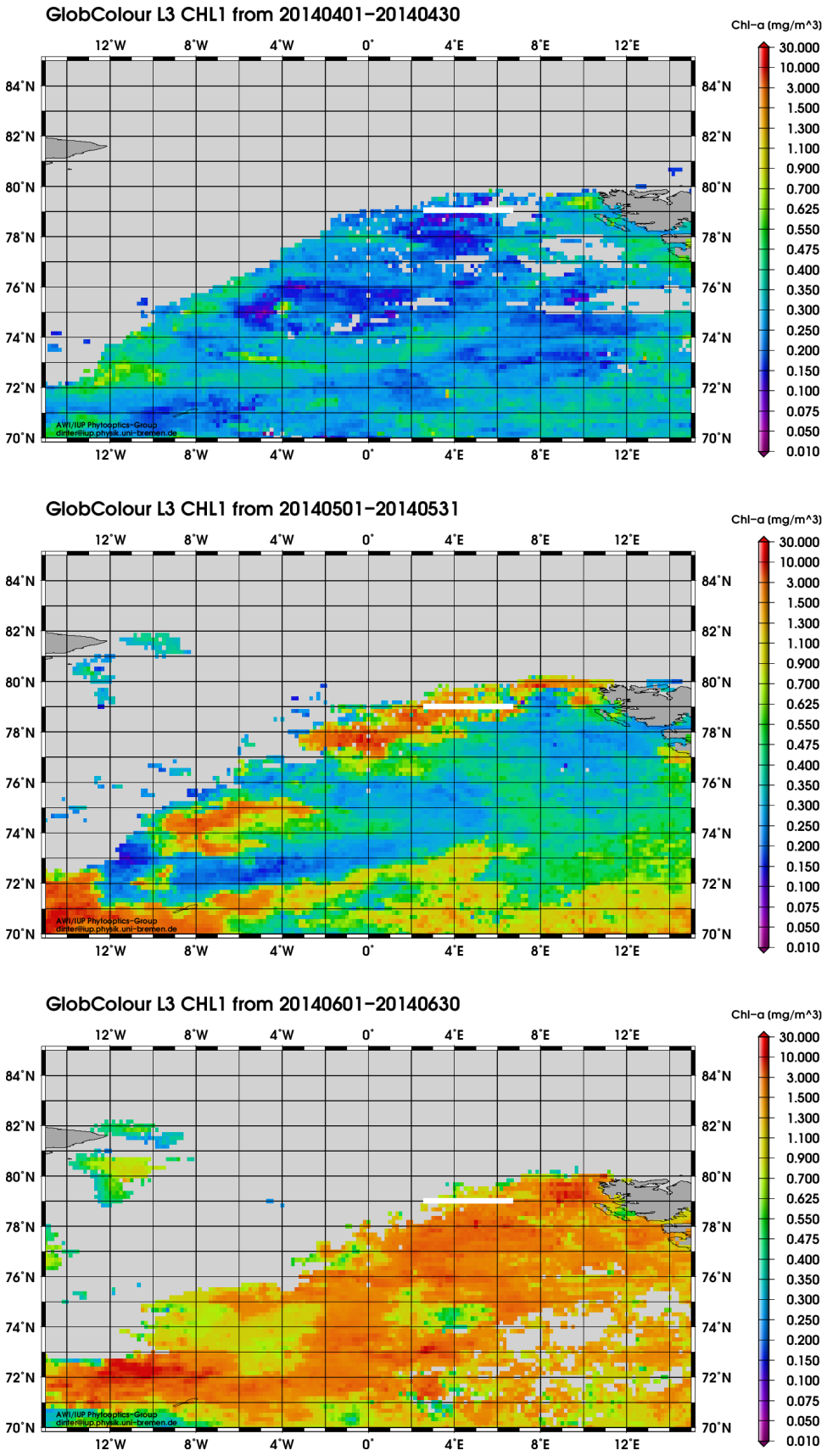

Figure III S1 - Average monthly surface ocean chlorophyll a concentration from April 2014 to June 2014 as derived from satellite measurements (courtesy Tilman Dinter, Alfred Wegener Institute). White bar indicates the location of the sampling area.

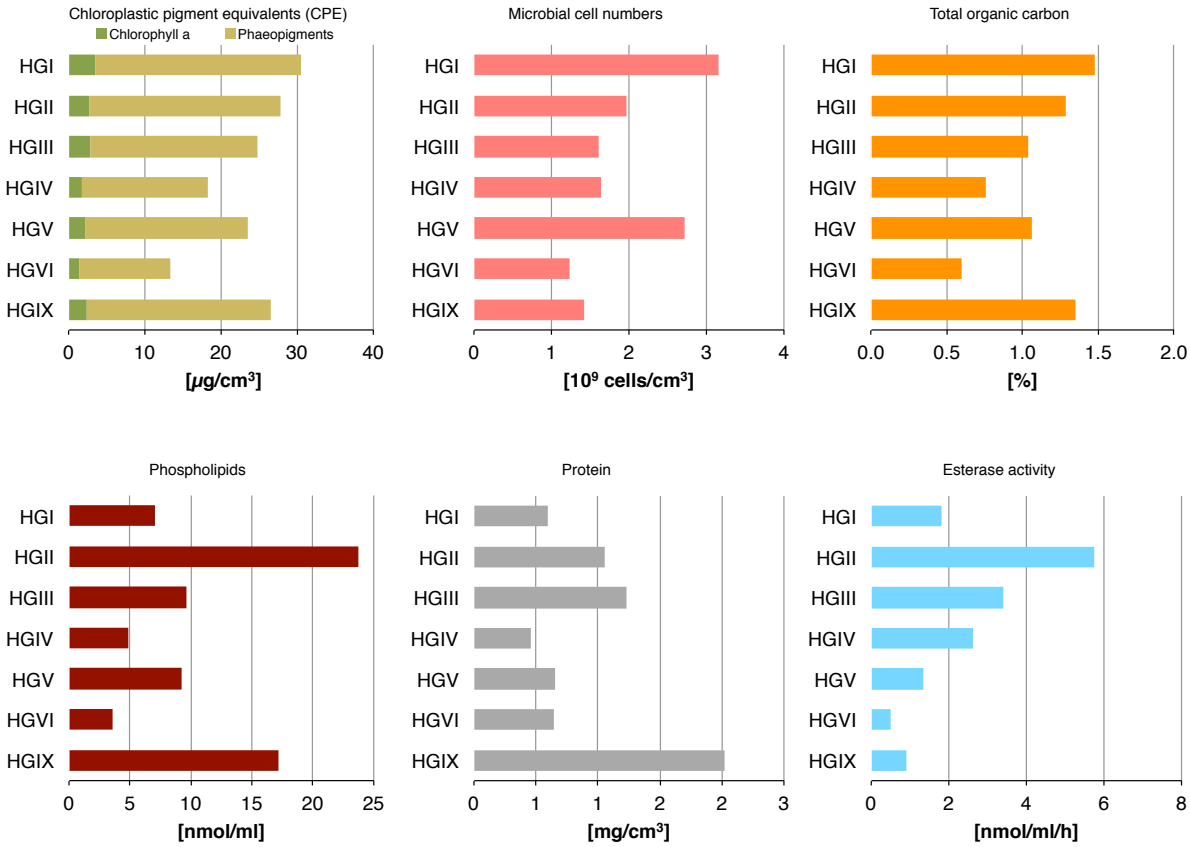

Figure III S2 - Biogenic sediment compounds measured in the uppermost sediment layer (0-1 cm) of deep-sea surface sediments along a depth gradient from station HGI (1,200 mbsl) to HGIX (5,500 mbsl).

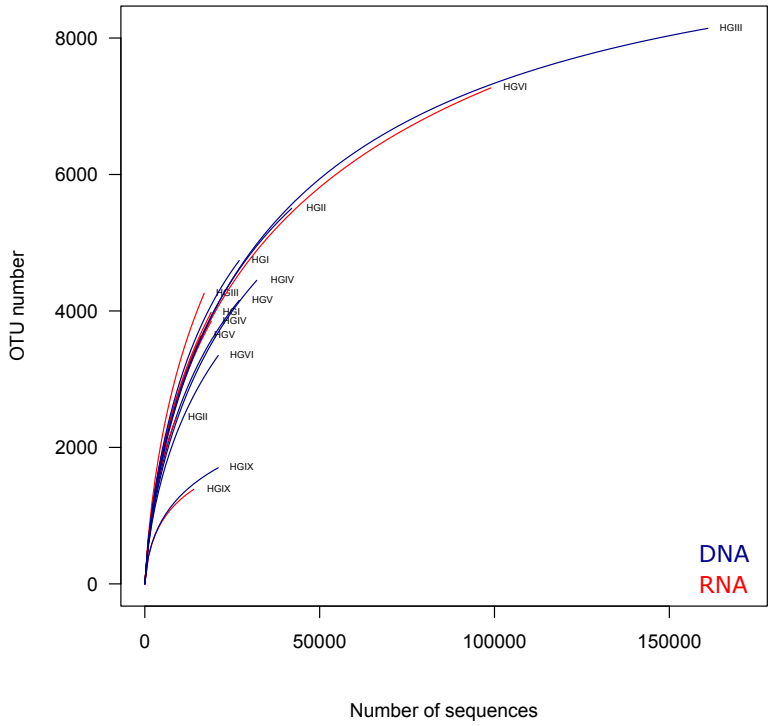

Figure III S3 - OTU rarefaction curve displaying the number of bacterial OTUs recovered from amplicon sequencing of the 16S rRNA gene at different sequencing depth.

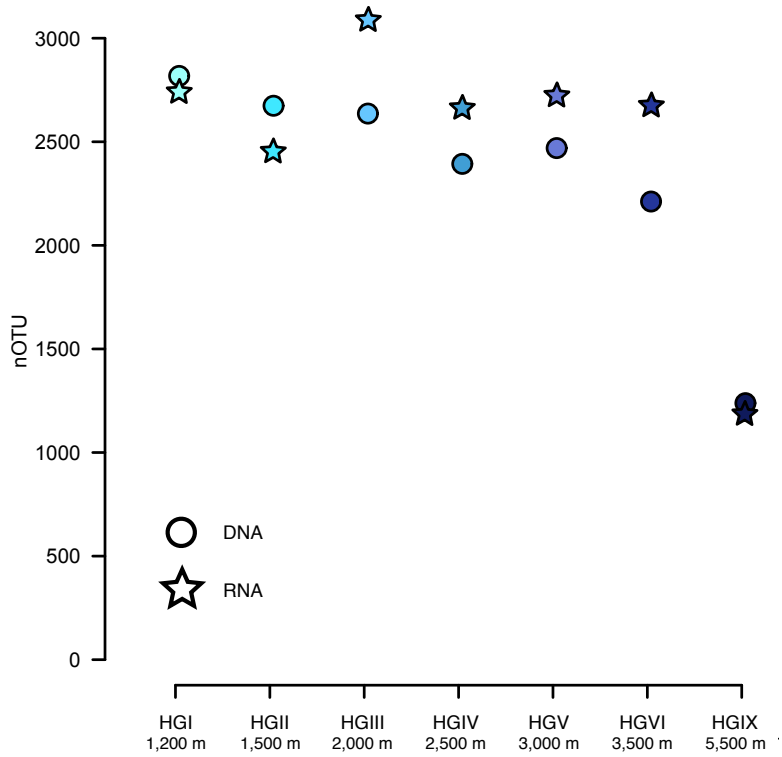

Figure III S4 - Community richness based on the number of observed OTUs for both total and active community fractions. Values are mean values of 100 randomly subsampled iterations.

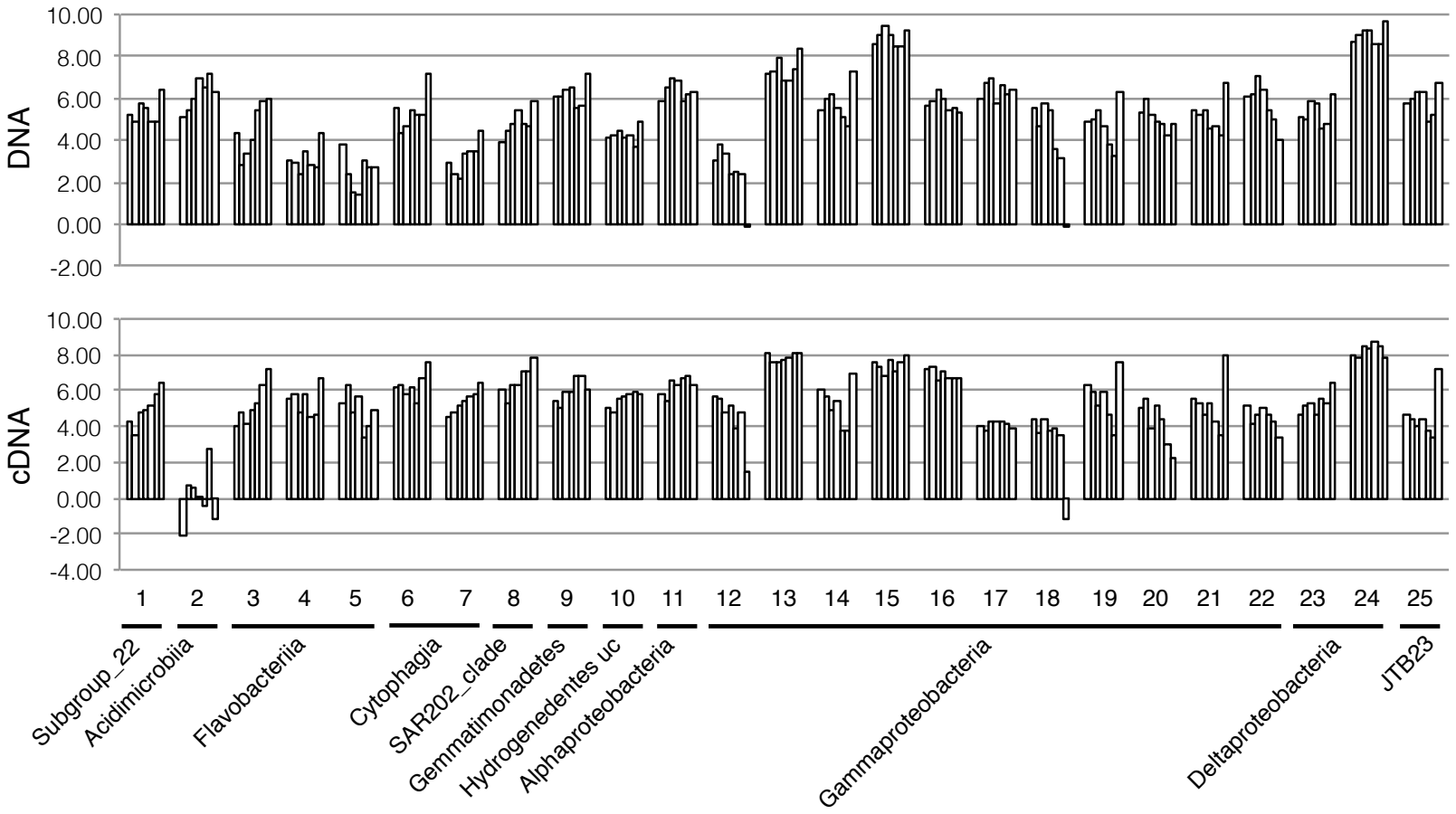

Figure III S5 - The top 10 most dominant bacterial genera across all samples in both DNA and cDNA tags. Data has been clr transformed and represents fold changes from the geometric mean in each sample. Bars for each class represent stations HGI (1,200 mbsl) to HGIX (5,500 mbsl) from left to right. 1: Subgroup 22 unclassified; 2: OM1 clade unclassified; 3: Aquibacter; 4: Owenweeksia; 5: Wenyingzhuangia; 6: Flammeovirgaceae unclassified; 7: Rhodothermaceae unclassified; 8: SAR202 clade unclassified; 9: BD2-11 terrestrial group unclassified; 10: Hydrogenedentes unclassified; 11: Rhodospirillaceae unclassified; 12: BD1-7 clade; 13: BD7-8 marine group unclassified; 14: Halieaceae unclassified; 15: JTB255 marine benthic group unclassified; 16: KI89A clade unclassified; 17: Marinicella; 18: Nitrosococcus; 19: OM182 clade unclassified; 20: OM60(NOR5) clade; 21: Pseudospirillum; 22: Xanthomonadales unclassified; 23: SAR324 clade(Marine group B) unclassified; 24: Sh765B-TzT-29 unclassified; 25: JTB23 unclassified.

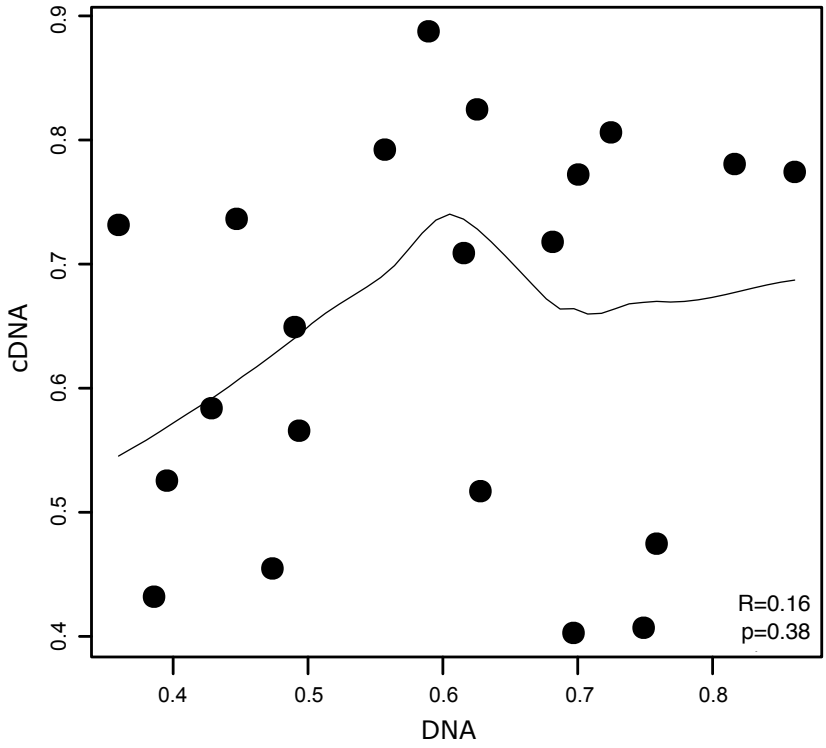

Figure III S6 - Differences in DNA community structure vs differences in cDNA community structure. Mantel test showed no significant correlation; R=0.16; p=0.38.

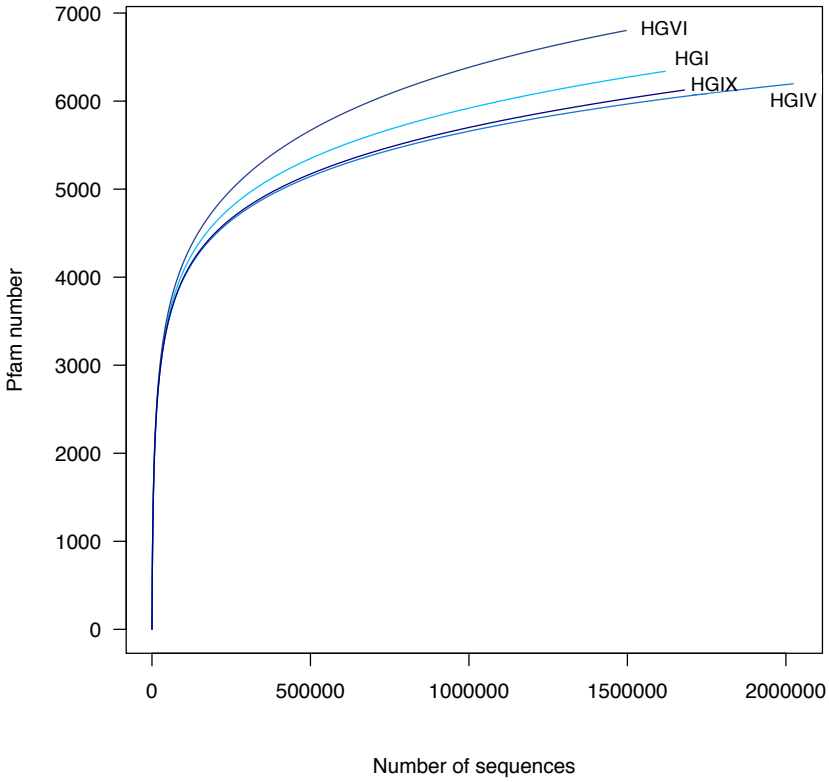

Figure III S7 - Functional rarefaction curve displaying the number of recovered Pfams per sequencing depth.

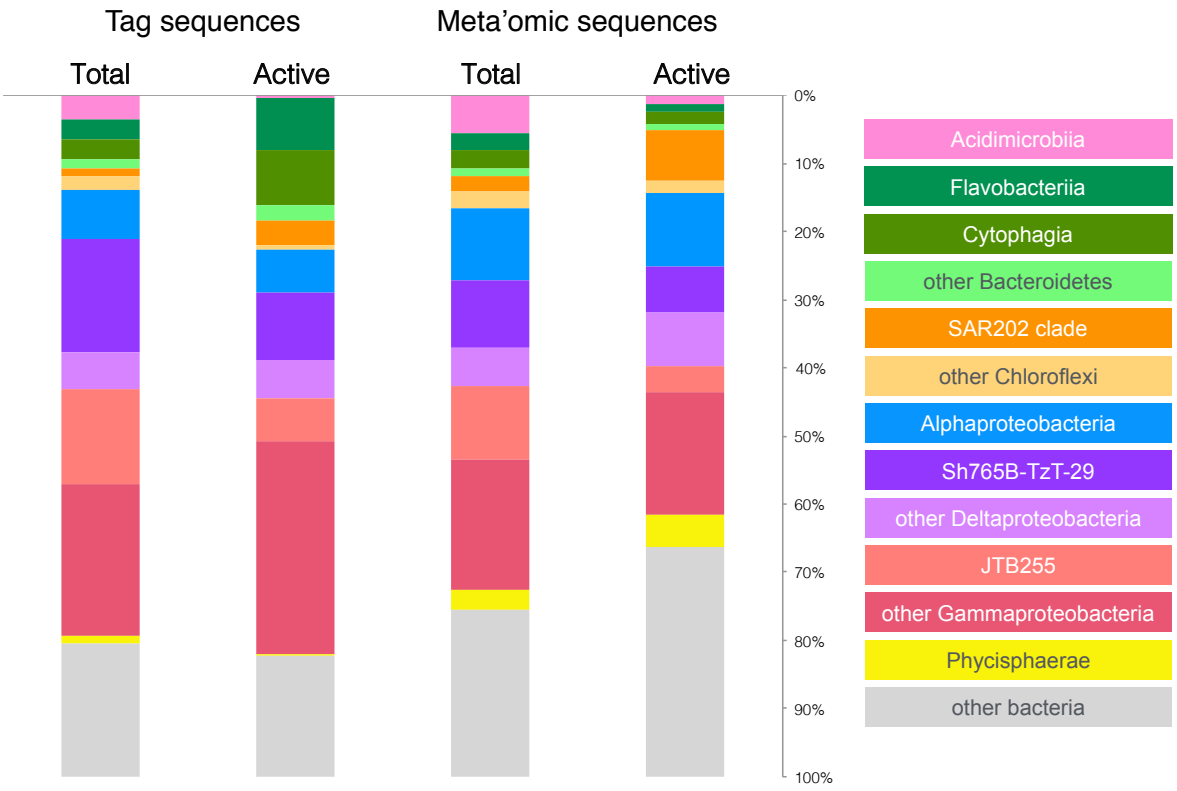

Figure III S8 - Average bacterial community composition as detected by tag sequencing of the 16S rRNA gene amplified from total extracted DNA (=total) and RNA (=active), as well as by shotgun metagenomic (=total) and metatranscriptomic (=active) sequencing. All data was classified against the SILVA SSU database release 123 (Quast et al.,2013) using the SINA aligner v1.2.10 (Pruesse et al., 2012).

Table III S1 - 16S rRNA gene tag sequence counts and community alpha diversity. QC: quality controlled; nOTU, OTUs present in an individual sample; Chao1, chao1 richness estimate; invS, inverse Simpson diversity index; ace: abundance-based coverage estimator; shannon: Shannon diversity index; SSOabs, singleton OTUs, represented by only a single sequence across the total dataset; SSOrel: singleton OTUs, represented by only a single sequence in at least one sample; DSOabs: double sequence OTUs.

	DNA HGIX	**DNA HGVI**	**DNA HGV**	**DNA HGIV**	**DNA HGIII**	**DNA HGII**	**DNA HGI**
Raw reads	544996	321837	322974	1027863	803075	279653	370255
QC reads	38286	37199	44451	51735	242536	68995	47295
nOTU	1230	2203	2461	2385	2628	2665	2809
chao1	1896	4124	4556	4598	5162	5114	5135
ace	1906	4414	4861	4928	5608	5453	5604
invS	129	256	297	222	231	305	342
shannon	6	7	7	7	7	7	7
SSOabs	4	7	7	7	13	9	10
SSOrel	38	47	47	49	45	47	46
DSOabs	7	9	9	8	11	10	11
	cDNA HGIX	**cDNA HGVI**	**cDNA HGV**	**cDNA HGIV**	**cDNA HGIII**	**cDNA HGII**	**cDNA HGI**
Raw reads	260376	569315	128282	264051	181106	155304	337916
QC reads	22297	197220	28973	30175	28969	15273	32639
nOTU	1183	2666	2712	2654	3079	2446	2735
chao1	1688	4923	4980	4883	5657	3858	5083
ace	1720	5196	5287	5189	6043	3992	5411
invS	120	401	418	488	604	419	369
shannon	6	7	7	7	7	7	7
SSOabs	5	8	7	6	7	5	7
SSOrel	34	46	48	49	49	42	48
DSOabs	8	9	9	7	9	9	9

Table III S 2 - Community turnover along the water depth gradient. Turnover represents Jaccard dissimilarity on presence-absence data of OTUs ≥2 reads.

.	DNA HGIX	cDNA HGIX	DNA HGVI	cDNA HGVI	DNA HGV	cDNA HGV	DNA HGIV	cDNA HGIV	DNA HGIII	cDNA HGIII	DNA HGII	cDNA HGII	DNA HGI	cDNA HGI
DNA HGIX	0	0.6	0.76	0.83	0.79	0.83	0.82	0.85	0.88	0.87	0.87	0.88	0.86	0.88
cDNA HGIX	0.6	0	0.83	0.84	0.85	0.82	0.88	0.84	0.92	0.87	0.91	0.87	0.9	0.88
DNA HGVI	0.76	0.83	0	0.68	0.62	0.72	0.67	0.76	0.76	0.79	0.75	0.84	0.76	0.81
cDNA HGVI	0.83	0.84	0.68	0	0.67	0.6	0.69	0.65	0.72	0.68	0.74	0.8	0.76	0.73
DNA HGV	0.79	0.85	0.62	0.67	0	0.7	0.63	0.72	0.71	0.75	0.69	0.8	0.7	0.77
cDNA HGV	0.83	0.82	0.72	0.6	0.7	0	0.71	0.63	0.78	0.67	0.77	0.76	0.78	0.72
DNA HGIV	0.82	0.88	0.67	0.69	0.63	0.71	0	0.7	0.65	0.73	0.65	0.8	0.67	0.76
cDNA HGIV	0.85	0.84	0.76	0.65	0.72	0.63	0.7	0	0.76	0.61	0.74	0.7	0.73	0.65
DNA HGIII	0.88	0.92	0.76	0.72	0.71	0.78	0.65	0.76	0	0.71	0.58	0.82	0.64	0.74
cDNA HGIII	0.87	0.87	0.79	0.68	0.75	0.67	0.73	0.61	0.71	0	0.7	0.68	0.7	0.59
DNA HGII	0.87	0.91	0.75	0.74	0.69	0.77	0.65	0.74	0.58	0.7	0	0.76	0.58	0.69
cDNA HGII	0.88	0.87	0.84	0.8	0.8	0.76	0.8	0.7	0.82	0.68	0.76	0	0.75	0.66
DNA HGI	0.86	0.9	0.76	0.76	0.7	0.78	0.67	0.73	0.64	0.7	0.58	0.75	0	0.67
cDNA HGI	0.88	0.88	0.81	0.73	0.77	0.72	0.76	0.65	0.74	0.59	0.69	0.66	0.67	0

		raw	quality trimmed		non rRNA	pfam annotation								CAZy pfams				Peptidase pfams				Transporter pfams			
	station	sequence count	sequence count	% of raw sequences	% of quality controlled sequences	sequence count	n pfams	% of quality controlled sequences	unknown function n pfams	unknown function %	putative function n pfams	putative function %	down-filtered data sequence count	n pfams	% of quality controlled sequences	sequences	npfams	% of all filtered sequences	sequences	npfams	% of all filtered sequences	sequences	npfams	% of all filtered sequences
Metagenomes	HGI	3368875	3057463	90.8	99.6	1619965	6339	53.0	1098	3.6	154	1.0	1581572	3546	97.6	26055	76	1.6	25894	123	1.6	439031	547	27.8
	HGIV	4108971	3781768	92.0	99.7	2023988	6197	53.5	1080	3.6	161	1.0	1977140	3525	97.7	32135	76	1.6	32277	123	1.6	537345	543	27.2
	HGVI	3009543	2775581	92.2	99.6	1496412	6802	53.9	1125	3.4	154	1.0	1459488	3576	97.5	23580	76	1.6	23247	122	1.6	393309	553	26.9
	HGIX	3399707	3133800	92.2	99.7	1680710	6126	53.6	1047	3.4	156	1.0	1640282	3513	97.6	26409	76	1.6	26606	123	1.6	435924	544	26.6
Metatranscriptomes	HGI	4610961	3348256	72.6	9.1	14814	2283	0.4	149	3.8	30	0.6	14682	2174	99.1	248	43	1.7	180	84	1.2	4290	373	29.2
	HGIV	4617970	3318396	71.9	9.2	16913	2640	0.5	158	3.2	35	0.4	16424	2342	97.1	264	49	1.6	215	96	1.3	4777	399	29.1
	HGVI	8326667	5948387	71.4	9.6	32307	2971	0.5	252	4.1	49	0.5	32110	2826	99.4	314	48	1.0	386	94	1.2	9402	453	29.3
	HGIX	4577698	3477151	76.0	11.6	18778	2381	0.5	173	3.7	39	0.6	18681	2318	99.5	187	41	1.0	207	89	1.1	5782	394	31.0

Table III S 3 | Metagenomic and -transcriptomic sequencing output and processing steps.

5 GENERAL DISCUSSION

The Arctic Ocean ecosystem is rapidly changing, most visible in the transformation of its sea-ice cover and the projected loss of MYI in the near future (Maslanik et al., 2007; Serreze and Rigor, 2007). While these changes are already strongly altering some of the physical and biological dynamics of the Arctic Ocean (e.g. Arrigo et al., 2012; Lee et al., 2011; Li et al., 2009b; Wassmann et al., 2011), we are still in the process of building ecological baseline knowledge about its microbial communities. High-throughput sequencing technologies do now allow the assessment of microbial diversity and functional potential of whole microbial populations (Hugenholtz, 2002; Rappé and Giovannoni, 2003; Pace, 2009; Morey et al., 2013; Reuter et al., 2015). We used these technologies to fill pressing knowledge gaps on microbial community composition and function in the Arctic Ocean:

Which are the dominant bacterial heterotrophs that associate with organic matter, and do they differ between different Arctic environments? As bacterial heterotrophs have key roles in the cycling of carbon and nutrients, understanding their diversity and distribution is necessary to predict implications of ice loss and changes in primary productivity regimes on biogeochemical cycling.

Does the ongoing sea-ice retreat have the potential to alter the distribution and composition of these heterotrophic communities? Sea-ice melt and subsequent biological transport processes may enhance the vertical exchange between sea ice, water column and benthos, with potential consequences for community composition and function.

What is the functional potential of deep-sea benthic microbial communities for organic matter turnover? An understanding of the genomic potential of benthic sediment bacteria to degrade various types of organic matter is needed to foresee the fate of carbon cycling, when productivity regimes and export fluxes in the Arctic continue to change.

5.1. Microbial diversity across different Arctic environments under climate change

All components of the cryosphere host microbial assemblages of distinct composition and metabolic potential (**Chapter Ia**). The few existing high-throughput studies that looked into the bacterial populations in sea ice (Bowman et al., 2012, 2013; Cowie et al., 2014; Han et al., 2014; Hatam et al., 2014, 2016; Torstensson et al., 2015; Eronen-Rasimus et al., 2016) prior to this thesis, suggest that individual sea-ice associated environments, i.e. FYI, MYI, melt ponds and frost flowers, represent heterogeneous microbial habitats, whose environmental parameters and available substrate sources select for populations of varying community structure (**Chapter Ia**). Results presented in this thesis extended our knowledge on sea-ice associated microbial communities by providing the first Eurasian basin wide survey of bacterial and eukaryotic community composition from the sea ice, melt ponds and the underlying water column. At the end of the productive season, in summer and fall, all investigated environments were dominated by distinct heterotrophic members of *Flavobacteriia* and *Gammaproteobacteria* (**Chapter II**), and thus revealed a different composition as previously reported from other Arctic Ocean regions (**Figure 4**). The main difference is the higher contribution of heterotrophic *Flavobacteriia* and *Gammaproteobacteria* in the Eurasian basin, as compared to a higher contribution of *Alphaproteobacteria* (almost exclusively comprised of the oligotrophic SAR11 clade) in melt ponds, sea ice and seawater in data mostly collected from the Canadian Arctic (**Chapter Ia**). Assuming that this is a true biological signal and not the result of a primer bias (Apprill et al., 2015; Parada et al., 2016), it would imply that the previous studies, albeit similar sampling time, recovered communities at a different time of seasonal succession, and underpins the need for reporting of standardized metadata to make sequence output more comparable (**Chapter Ib**).

The high representation of heterotrophs in our dataset was likely a direct response to the presence of high algal biomass. Their activity in the turnover of algal biomass releases regenerated nutrients, and thus may be of especially high importance in the stratified and nutrient limited central basins. Here, it could support primary productivity at the end of the productive season (Codispoti et al., 2013; Le Fouest et al., 2013; Fernández-Méndez et al., 2015), as has been suggested for oligotrophic

gyre systems (Letscher et al., 2015). In addition, several heterotrophic members displayed the potential for bacterial nitrogen fixation in sea-ice, melt-pond and seawater (Fernández-Méndez et al., 2016; Appendix I Additional contributions). which could provide an important source of nitrogen to the nutrient-limited algal communities in the central Arctic basin (LaRoche and Breitbarth, 2005; Fernández-Méndez et al., 2015; Tremblay et al., 2015a; Delmont et al., 2018). The projected increases in primary productivity due to increased light (Arrigo and van Dijken, 2015) will elevate the overall nitrogen demand in the Arctic. Our estimates suggest that diazotrophs could sustain up to 7% of the total annual production (Fernández-Méndez et al., 2016; Appendix I Additional contributions). However, several studies have also predicted increasing heterotrophic respiration rates with the warming climate, and projected an increase of more than 60% in Arctic surface waters (Vaquer-Sunyer et al., 2010), which would double the projected increases in primary productivity, and have important implications for the carbon cycle and the Arctic Ocean's role as a CO_2 sink (Duarte et al., 2012).

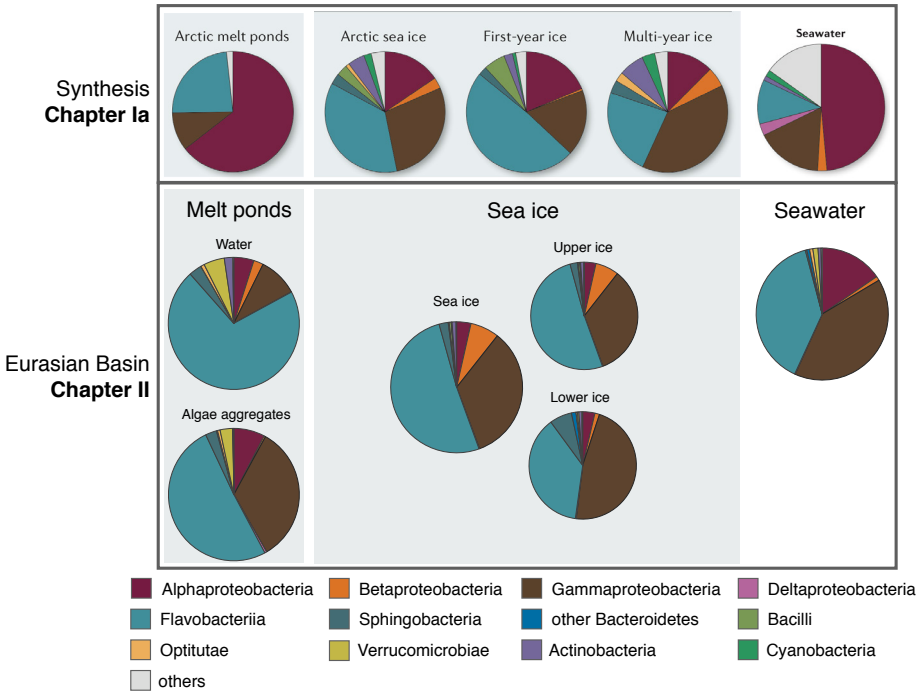

Figure 4 I Direct comparison of the bacterial community composition detected in melt ponds, sea ice and seawater as reviewed in Chapter Ia and as detected in the Eurasian basin in late summer of 2012 (Chapter II).

Our results suggest tight links between the bacterial and eukaryotic communities in sea ice. Indeed, community patterns of both groups were significantly correlated (**Chapter II** & Appendix Figures 6-8), indicating similar drivers of diversification. While ecological interdependencies and the substrate-controlled succession of pelagic microbes have received increasing attention over the past years (Chaffron et al., 2010; Gilbert et al., 2012; Teeling et al., 2012; Lima-Mendez et al., 2015; Sunagawa et al., 2015; Bunse and Pinhassi, 2017), much less is known about microbial networks in sea-ice environments. Specific interactions between protists and bacteria, e.g. diatoms and *Flavobacteriia*, or dinoflagellates and *Alphaproteobacteria*, have been reported from various marine environments (Lima-Mendez et al., 2015), and we observed the co-occurrence of distinct members of these groups also in sea ice, melt ponds and the underlying water column (**Chapter II**). The exclusive presence of individual bacterial community members, e.g. specific Roseobacters such as *Sulfitobacter*, in association with the investigated ice-algae aggregates provided first indications for potential biotic interactions in sea ice, the underlying water column and at the seafloor **(Chapter II**). Recently, high-throughput sequencing revealed that individual algae species can possess unique microbiomes, often including members of the Roseobacter clade (Behringer et al., 2018). Algal community composition in a future ice-free, fresher and nutrient limited Arctic Ocean has been projected to shift (Li et al., 2009b), and the disappearance of individual sea ice habitats, e.g. MYI, will also mean as loss of habitat for its associated microbial communities, and the seeding of specific algae communities (Olsen et al., 2017). A better understanding of the specific heterotrophic bacterial communities associated to the different algal assemblages will be necessary to assess potential consequences of algal community shift for biogeochemical cycling in the Arctic Ocean (Vancoppenolle et al., 2013).

5.2. Organic matter export and microbial community connectivity in light of sea-ice decline

The ecological consequences of the declining sea-ice cover are often times difficult to predict, as many components of the Arctic ecosystem are interdependent and can

thus experience indirect impacts (Vincent et al., 2011; Post et al., 2013). While sea-ice loss has direct effects on primary production in ice and the upper ocean, as it modulates light and nutrient availability, stratification, and algal community composition (Wassmann et al., 2006, 2011), it can indirectly affect the deep ocean by altering the physical, biogeochemical, and biological coupling between surface and deep sea (Vincent et al., 2011; Findlay et al., 2015). The biological carbon pump and the export of organic matter is a key link between the surface and the deep ocean. The large-scale release of ice algae in times of rapid ice melt affected microbial community composition at the seafloor despite being separated by several kilometers of water column (**Chapter II**). The deposited algae transported sea-ice life to the deep-sea benthos, and unique bacterial assemblages established in association to the fresh organic matter source. The role of sinking particles in mediating the vertical dispersal of microbial cells has been little explored (Novitsky, 1990). Previous work identified vertically migrating zooplankton as potentially important transport vectors of hitchhiking bacteria (Grossart et al., 2010), and only recently a fundamental role of large sinking particles in inoculating the deep sea with viable surface microbes has been postulated (Mestre et al., 2018). If this holds true, particle-attached surface microbes may at least partially shape deep sea community composition.

In our results, however, the relative contribution of sea-ice microbes to the total community associated to the exported material at the seafloor was minor (**Chapter II**). Instead, the deposition of the fresh material triggered the establishment of a bacterial community dominated by otherwise rare members of the surrounding sediments that could quickly react to the food pulse. Interestingly, the overlap between seawater and sediment, as well as seawater and export material was little, despite a certain residence time of sinking material during its fall through the water column. However, seawater communities in the analyzed dataset in **Chapter II** all came from surface waters, and lacked vertical resolution. The low connectivity between seawater and seafloor may have therefore resulted from the great spatial distance, lateral transport processes, and short residence time of the quickly sinking aggregates. In an ongoing study, we increased vertical resolution in the water column by including samples from near-surface (~10 m), the chlorophyll maximum

(~25 m) and below (50 m), sinking particles from various depths, as well as deep water samples, collected from several meters above the seafloor (**Appendix**).

Overall, we observed that the wide-spread deposition of ice-algal biomass had the potential to induce local changes in benthic bacterial community structure, which were accompanied by enhanced bacterial activity, as seen by higher oxygen consumption in sediments under the algae patches (Boetius et al., 2013). If reoccurring, this type of export event and its impact on benthic bacterial diversity might therefore be of relevance for carbon cycling and benthic remineralization at the Arctic deep-sea floor.

The fate of export fluxes in the future Arctic Ocean is, however, debated, as several climatic drivers could affect the strength of the biological pump (**Table 1**), and both increases in export flux, as well as longer retention times of organic matter in the water column and consequently a reduction of export fluxes (Piepenburg, 2006; Olli et al., 2007; Slagstad et al., 2011; Ji et al., 2013) were predicted. Results presented in **Chapter II** focused on ice-algal aggregates, dominated by the diatom *Melosira arctica*, and their associated bacteria. This chain-forming diatom can grow meter-long filaments, and its large size and high sinking speed can result in rapid export. Several studies have, however, suggested a gradual shift of the Arctic phytoplankton composition and instances of a northward intrusion of individual phytoplankton species (Reid et al., 2007; Hegseth and Sundfjord, 2008; Li et al., 2009b; Nöthig et al., 2015; Paulsen et al., 2016; Soltwedel et al., 2016). Preliminary results from ongoing work indicate that the bacterial communities associated with *Melosira arctica* and phytoplankton aggregates are distinct (**Appendix Figure 6**), and support the idea that ice algae growing attached to the ice underside or even floating in surface seawater, are preferentially associated with sea-ice microbes rather than with pelagic microbes (**Chapter II** & **Appendix Figure 6**). Hence, changes in the composition of algae and sinking particles could have direct effects on microbial community connectivity, as their associated bacteria will likely differ (Behringer et al., 2018), and their sinking rates could vary (Siegel et al., 2016).

Table 1 | Previously reported signs of climate change in the Arctic that could alter the biological pump through their observed or predicted effects on microbial communities.

Climatic driver	Effect on eukaryotic communities	Effects on bacterial communities
Sea-ice changes (including reduction, thinning, early break up, loss of MYI)	Increased primary production (Arrigo et al., 2008, 2012; Pabi et al., 2008; Slagstad et al., 2011; Arrigo and van Dijken, 2015)	Loss of MYI community members (Hatam et al., 2016)
	Loss of habitat for sea-ice organisms (Lange et al., 2017) Loss of seed bank for ice algal bloom (Olsen et al., 2017)	
	Shortening of ice-algae growth season (Wassmann, 2011; Wassmann and Reigstad, 2011)	
	Mismatch between primary and secondary producers (Wassmann, 1997; Olli et al., 2007; Leu et al., 2011; Ji et al., 2013)	
Freshening of water column	Shift from a dominance of diatoms to a dominance of picoplankton (Li et al., 2009b; Nöthig et al., 2015)	Decreased community diversity, community shift (Comeau et al., 2011)
Increasing water temperatures	Range shift and community changes (Reid et al., 2007; Hegseth and Sundfjord, 2008; Nöthig et al., 2015)	Range shift and community changes (Paulsen et al., 2016)
	Increased grazing activity (Piepenburg, 2006; Olli et al., 2007; Vernet et al., 2017)	Increased respiration by bacterial heterotrophs (Kritzberg et al., 2010; Vaquer-Sunyer et al., 2010)

On short term primary production is expected to increase, and some of the largest changes were predicted for the periphery of the currently low productive basins (Slagstad et al., 2011, 2015). Here, the contribution of sea-ice algae was suggested to increase with thinning ice (Wassmann, 2011). The future thinner icescape, will likely be more prone to early ice break up, potentially supporting more rapid export events of sea-ice biomass as observed in **Chapter II**. Early ice break up could also result in a mismatch between primary producers and the grazer community in the water column, and in the sinking of large quantities of fresh organic material to the seafloor (Wassmann, 1997; Wassmann et al., 2004; Olli et al., 2007; Leu et al.,

2011; Ji et al., 2013).

A better understanding of the implications of climate warming on the strength of the biological pump will be necessary to project the fate of carbon export and its role for benthic microbial diversity and biogeochemical cycling. Currently, the high degree of spatial heterogeneity in Arctic water mass properties, pelagic productivity regimes, benthic community structure, and the patchiness of ice algae contribution hamper predictions on the strength of the coupling between ocean surface and seafloor in the future (Klages et al., 2004; Carmack and Wassmann, 2006; Wassmann et al., 2010; Ardyna et al., 2013; Katlein et al., 2014). Better knowledge of the distribution of key Arctic primary producers, i.e. *Melosira arctica* (Katlein et al., 2014; Poulin et al., 2014), *Phaeocystis pouchetii* (Metfies et al., 2016; Assmy et al., 2017) and *Micromonas* (Kilias et al., 2014; Metfies et al., 2016), and their associated microbes could help to feed modelling approaches and predict the implications of change on coupling and export processes.

5.3. Microbial organic matter degradation at the deep-sea floor

The Arctic Ocean currently acts as a carbon sink (MacGilchrist et al., 2014), however, the fate of its role in the global carbon cycle is unclear, as climate warming is affecting benthic-pelagic coupling and potentially also the efficiency of its biological pump (Reid et al., 2007; Wassmann and Reigstad, 2011; Harada, 2016). Carbon turnover by deep-sea sediment bacteria is a key process in the carbon cycle, as only what passes their remineralization activity will be buried and sequestered from the atmosphere on geological timescales (Snelgrove et al., 2017).

The underlying genomic traits that deep-sea organisms use to degrade organic matter are, however, poorly understood, since for the vast majority of benthic deep-sea bacteria no cultured representatives or genomic information are available (Siezen and Wilson, 2009). Results of this thesis therefore provide first insights into the genomic potential of bacterial deep-sea surface sediment communities for organic matter breakdown and uptake (**Chapter III**). By combining amplicon sequencing, metagenomic and -transcriptomic sequencing, we were able to identify

benthic community members and functions that likely played an active role in deep-sea sediment carbon turnover.

The first step in the bacterial breakdown of organic material is the extracellular enzymatic hydrolysis (Boetius and Lochte, 1994; Boetius, 1995; Poremba, 1995), and we therefore mined the meta'omic data for genes involved in the hydrolysis of carbohydrates, namely carbohydrate-active enzymes (CAZymes), and proteins, namely peptidases, as well as for transport functions, involved in the take up of the various breakdown products (**Chapter III**).

The dominant community members belonged to the *Gamma*-, and *Deltaproteobacteria*, as well as to the *Alphaproteobacteria*, *Flavobacteriia*, *Cytophagia*, and members of the OM1 and SAR202 clades (**Chapter III**). Members of these groups seem to be typical deep-sea surface sediment community members, previously reported from other Arctic studies (Bienhold et al., 2012; Jacob et al., 2013; Li et al., 2015), but also on a global scale (Goffredi and Orphan, 2010; Zinger et al., 2011; Ruff et al., 2013; Bienhold et al., 2016). A comparison of community data obtained on the basis of DNA and RNA revealed that all dominant community members were active. The communities encoded diverse functions for the breakdown of detrital matter of both algal and bacterial origin, as well as for matter from decaying animals or fecal pellets (**Chapter III**). A high similarity in functional potential was found with bacterioplankton communities during the turnover of phytoplankton biomass, despite the strong differences in pelagic and benthic bacterial community structures (Zinger et al., 2011). The representation of functions involved in protein degradation in deep-sea sediment communities exceeded this of carbohydrates, in line with the idea that labile organic matter, such as algal storage compounds, are mostly remineralized during the export, leaving behind a larger pool of dissolved organic matter, proteins and lipids at depth (Wakeham et al., 1997b, 1997a; Kiriakoulakis et al., 2001; Aluwihare et al., 2005).

Export fluxes have been shown to strongly modulate benthic communities of all size classes (Smith et al., 1998; Danovaro et al., 2017; Snelgrove et al., 2017). Bacterial communities at the seafloor responded to variations in organic matter concentrations both in changes of cell abundance, composition, and activity (Boetius and Damm, 1998; Kanzog et al., 2008; Bienhold et al., 2012). Also, qualitative changes of

organic material have experimentally been shown to induce bacterial community changes, and revealed that dominant sediment members react differently to different types of substrates (Hoffmann et al., 2017), indicating the presence of distinct metabolic strategies and niche partitioning in sediments. To explore whether changes in organic matter are reflected in the functional capabilities of bacterial communities to degrade organic matter, we investigated community composition and genomic potential along a water depth gradient (**Chapter III**). Based on the assumption that the nutritious quality and total concentration of sinking material decreases the longer it is exposed to degradation processes in the water column (Wakeham et al., 1997a), we expected to observe an overall decrease of organic matter quality and quantity with increasing water depth. However, this simplified depiction neglected the multitude of processes that can alter sedimentary organic matter in the environment (**Figure 2**). Biogenic sediment compounds along the investigated water depth gradient did not show a linear decline of organic matter concentration with increasing depth, nor a reduced fraction of chlorophyll a (**Chapter III**). The deepest station along the transect accumulated high organic matter concentrations, due to its topography (Soltwedel et al., 2005), as has also been reported for several hadal trench systems. These can act as potential organic matter depocenters in the deep-sea (Rowe et al., 1994; Danovaro et al., 2003; Wenzhöfer et al., 2016), and sustain relatively high biological activity (Danovaro et al., 2003). Despite a steep drop in bacterial richness, and a ~50% difference in community composition at the OTU level compared to the other stations along the depth gradient, we detected a similar potential for the breakdown of organic matter along the whole slope. While this may suggest the occupation of full available niche space by functional redundant groups, it could also indicate that the current resolution of our functional analysis was not sufficient to reveal the full diversity of genomic variation. As a next step of analysis, microdiversity on the level of gene sequences will be explored.

Sequence similarity searches, allowed first tentative links between the detected community structure and the identified genomic traits (**Chapter III**). Our results are in line with previous findings that suggested distinct substrate utilization patterns of

bacterial taxa (Teeling et al., 2012; Xing et al., 2014; Hoffmann et al., 2017), which were shown to underlie the recurrent seasonal succession of heterotrophic bacterioplankton during phytoplankton blooms (Teeling et al., 2012, 2016). It remains an open question whether deep-sea sediment communities undergo similar seasonal dynamics driven by variations in organic matter export fluxes. We observed an enrichment of *Bacteroidetes* in the active bacterial community (**Chapter III**), which may be a response to the deposition of organic matter at the seafloor following the yearly phytoplankton spring bloom in Fram Strait (Bauerfeind et al., 1994). Previous experimental work on sediments from the same site showed that members of this group increased in relative abundance and displayed higher respiration activity after the addition of phytodetritus (Hoffmann et al., 2017). Heterotrophic members of this phylum have been recognized as key particle degraders in various marine and non-marine environments (Thomas et al., 2011; Fernández-Gómez et al., 2013), highly adapted to degrade complex organic matter (Mann et al., 2013; Kabisch et al., 2014a; Bennke et al., 2016; Unfried et al., 2018), and play an important role in the turnover of phytoplankton derived carbon (Teeling et al., 2012; Reintjes et al., 2017). Their capability to breakdown complex carbohydrates is encoded in dedicated polysaccharide utilization loci (PULs) in their genomes, where sequences of genes for polysaccharide degradation and uptake are located in operon-like structures (Sonnenburg et al., 2010). Our results indicated that *Bacteroidetes* in deep-sea sediments can potentially use a wider set of substrates than other sediment members (**Chapter III**). This could provide them with the versatility to quickly respond to various substrate types, as has been previously observed (Hoffmann et al., 2017), and could be advantageous if export composition is changing (Nöthig et al., 2015; Soltwedel et al., 2016).

All dominant community members were active, but interestingly the highest contribution to the active fraction came from sediment members, which were previously shown to be non-responsive to the addition of phytodetritus or chitin, the JTB255 marine benthic group (Hoffmann et al., 2017). This suggests that an active part of the community is not structured by the seasonal deposition of organic carbon, but could be using other sources of nutrition. Genomic analysis of this group suggested that JTB255 may grow on the vast pool of proteins and lipids present in

marine sediments (Aluwihare et al., 2005; Hoffmann et al. in prep, Appendix I Additional contributions).

The utilization of the multitude of sedimentary particulate and dissolved organic matter, containing a large fraction of refractory high-molecular-weight compounds (Schmidt et al., 2009; Rossel et al., 2016), could be a strategy to maintain an active metabolism year-round, independent from seasonal sedimentation pulses. This fits the finding that the majority of active transcripts for TonB-dependent transporters in our data was ascribed to *Gammaproteobacteria* (**Chapter III**). The known substrates for this transporter class include various types of dissolved organic material, including carbohydrates, amino acids, lipids, organic acid, and protein degradation products (Tang et al., 2012). The diversity of dissolved organic compounds that accumulate in marine sediments (Schmidt et al., 2009; Rossel et al., 2016), if accessible for bacterial degradation, brings the view of deep-sea sediments as an energy-limited environment into question (Jørgensen and Boetius, 2007; Danovaro et al., 2014), and gives wide room for potential niche partitioning (Huisman and Weissing, 1999).

The presented results give first genomic insights into potential niche partitioning strategies of highly diverse Arctic deep-sea sediment communities. The variety of genomic traits for the breakdown of organic matter suggests that these communities are able to turnover various types of export, ranging from fresh algae biomass to recalcitrant matter. The group most enriched in the active community, was also the group that showed the highest versatility in the choice of substrates. Long-term observations in the study area have revealed a gradual shift of phytoplankton community composition and the associated export flux (Nöthig et al., 2015; Soltwedel et al., 2016), and several studies further suggested that export flux in the Arctic will carry less labile components as a result of increased heterotrophic activity in the water column (Wassmann, 1997; Slagstad et al., 2011; Wassmann and Reigstad, 2011). Versatile community members, such as benthic *Bacteroidetes*, may then gain in importance for ecosystem functioning.

An interesting finding was that groups that appeared non-responsive to the addition of substrates, were still strongly represented in the active community. These groups

could be exploiting the rich substrate resource of sedimentary dissolved compounds that is not directly coupled to sedimentation patterns, and may thus be less affected by climatic changes.

Methodological considerations

A major challenge in the analysis of the meta'omic data was the high complexity of microbial communities in deep-sea sediments, and the richness of many closely related members of the dominant taxa, i.e. *Gamma*- and *Deltaproteobacteria*. Various tested assembly approaches yielded only a small number of relatively short contigs, typically well below 10,000 base pairs in length. Low read coverage prevented the unambiguous assignment of adjacent genomic regions. We therefore decided to follow a gene-centric analysis approach rather than aiming for the recovery of genomic bins of individual taxa. While this approach allowed the assessment of functional diversity, it did not allow direct linkage of detected functions and the identity of organisms involved. However, as the analyses of bacterial community structure in both amplicon sequences and 'omic sequences revealed highly comparable results (Supplementary material I Chapter III, Fig. S8), it suggests that functions associated to the dominant bacterial members in deep-sea sediments were also represented in the genomic data. Taxonomic assignments to functions of interest were further provided through protein sequence similarity searches, however, need to be interpreted with care, as these results can be biased by the overrepresentation of cultured organism in the databases. To be able to directly link function and microbial identify in deep-sea sediments in the future, much deeper sequencing efforts should be undertaken, potentially paired with long-read technologies, i.e. NanoPore sequencing (Madoui et al., 2015)

Conclusion & Perspective

The results presented in this thesis significantly enhance our understanding of present-day Arctic biodiversity, community connectivity and organic matter cycling. The various Arctic Ocean environments host distinct microbial communities that seem to be mainly structured by the association between algae and specific assemblages of bacterial heterotrophs. Their sinking and export increased the exchange of bacterial groups between surface and deep ocean and has the potential to alter benthic diversity and function given the projected environmental change scenarios. The benthic communities, however, seem to be equipped to mineralize a variety of organic compounds, by maintaining a diverse and active community, whose members use different nutritional strategies.

Our data provides the first report on bacterial diversity for some of the investigated environments, i.e. the deep-sea floor of the Eurasian Basin, and it is therefore not possible to assess whether these communities have already experienced changes in response to sea-ice reduction and warming. Results here can now be used to track changes in composition and function in the future. It remains, however, a major challenge to disentangle long-term community changes from signals of seasonal fluctuation or interannual variability. This is hampered as for large areas of the Arctic Ocean our knowledge on microbial communities is based on episodic snapshots. To achieve a comprehensive understanding of Arctic microbial diversity and function, as well as community responses to environmental changes, we need continuous monitoring approaches, on the basis of standardized sampling, analyses and metadata collection. The technological advances in the development of autonomous sampling platforms, although still challenging, now also allow year-round observations in areas that were previously hard to access, e.g. due to remoteness or harsh weather conditions. Coupled with the application of high-throughput sequencing technologies, this could give unprecedented insights into the microbial ecology of those Arctic regions less accessible by ship. This information will be especially important for the central Arctic basins, where most drastic changes are expected due to a switch from a permanent to a seasonal ice cover.

An emerging feature of this thesis was the potentially tight linkage between bacterial and eukaryotic community members. Future studies should investigate the microbiomes associated to different particles and their export efficiency, as they can be highly variable (Le Moigne et al., 2015; Behringer et al., 2018), and differently affected by sea-ice melt (Wollenburg et al., 2018). Further, microbial interactions and symbioses may play a key role for the habitability of cold environments, and could be of increasing importance in the nutrient limited central Arctic Ocean.

REFERENCES

Aagard, K., and Carmack, E. C. (1989). The role of sea ice and other fresh water in the Arctic circulation. *J. Geophys. Res.* 94, 14485–14498. doi:10.1029/JC094iC10p14485.

Acinas, S. G., Marcelino, L. A., Klepac-Ceraj, V., and Polz, M. F. (2004). Divergence and redundancy of 16S rRNA sequences in genomes with multiple rrn operons. *J. Bacteriol.* 186, 2629–35. doi:10.1128/JB.186.9.2629-2635.2004.

Alexeev, V. A., and Jackson, C. H. (2013). Polar amplification: is atmospheric heat transport important? *Clim. Dyn.* 41, 533–547. doi:10.1007/s00382-012-1601-z.

Alldredge, A. L., and Gotschalk, C. (1988). In situ settling behavior of marine snow. *Limnol. Oceanogr.* 33, 339–351. doi:10.4319/lo.1988.33.3.0339.

Alonso-Sáez, L., Sánchez, O., Gasol, J. M., Balagué, V., and Pedrós-Alio, C. (2008). Winter-to-summer changes in the composition and single-cell activity of near-surface Arctic prokaryotes. *Environ. Microbiol.* 10, 2444–2454. doi:10.1111/j.1462-2920.2008.01674.x.

Alonso-Sáez, L., Zeder, M., Harding, T., Pernthaler, J., Lovejoy, C., Bertilsson, S., et al. (2014). Winter bloom of a rare betaproteobacterium in the Arctic Ocean. *Front. Microbiol.* 5. doi:10.3389/fmicb.2014.00425.

Aluwihare, L. I., Repeta, D. J., Pantoja, S., and Johnson, C. G. (2005). Two Chemically Distinct Pools of Organic Nitrogen Accumulate in the Ocean. *Science.* 308, 1007–1010. doi:10.1126/science.1108925.

Amann, R. I., Ludwig, W., and Schleifer, K. H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* 59, 143–69. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7535888 [Accessed June 29, 2018].

Amaral-Zettler, L., Artigas, L. F., Baross, J., Bharathi P.A., L., Boetius, A., Chandramohan, D., et al. (2010). “A Global Census of Marine Microbes,” in *Life in the World’s Oceans: Diversity, Distribution, and Abundance*, ed. A. D. McIntyre (Oxford: Blackwell Publishing Ltd), 221–245. doi:10.1002/9781444325508.ch12.

Ambrose, W. G., Von Quillfeldt, C., Clough, L. M., Tilney, P. V. R., and Tucker, T. (2005). The sub-ice algal community in the Chukchi sea: Large- and small-scale patterns of abundance based on images from a remotely operated vehicle. *Polar Biol.* 28, 784–795. doi:10.1007/s00300-005-0002-8.

Amon, R. M. W., and Benner, R. (1996). Bacterial utilization of different size classes of dissolved organic matter. *Limnol. Oceanogr.* 41, 41–51. doi:10.4319/lo.1996.41.1.0041.

Apprill, A., McNally, S., Parsons, R., and Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* 75, 129–137. doi:10.3354/ame01753.

Ardyna, M., Babin, M., Gosselin, M., Devred, E., B??langer, S., Matsuoka, A., et al. (2013). Parameterization of vertical chlorophyll a in the Arctic Ocean: Impact of the subsurface chlorophyll maximum on regional, seasonal, and annual primary production estimates. *Biogeosciences* 10, 4383–4404. doi:10.5194/bg-10-4383-2013.

Arístegui, J., Gasol, J. M., Duarte, C. M., and Herndld, G. J. (2009). Microbial oceanography of the dark ocean's pelagic realm. *Limnol. Oceanogr.* 54, 1501–1529. doi:10.4319/lo.2009.54.5.1501.

Arnosti, C. (2011). Microbial Extracellular Enzymes and the Marine Carbon Cycle. *Ann. Rev. Mar. Sci.* 3, 401–425. doi:10.1146/annurev-marine-120709-142731.

Arnosti, C. (2008). Functional differences between Arctic seawater and sedimentary microbial communities: contrasts in microbial hydrolysis of complex substrates. *FEMS Microbiol. Ecol.* 66, 343–351. doi:10.1111/j.1574-6941.2008.00587.x.

Arrigo, K. R. (2014). Sea ice ecosystems. *Ann. Rev. Mar. Sci.* 6, 439–467. doi:10.1146/annurev-marine-010213-135103.

Arrigo, K. R., Matrai, P. A., and van Dijken, G. L. (2011). Primary productivity in the Arctic Ocean: Impacts of complex optical properties and subsurface chlorophyll maxima on large-scale estimates. *J. Geophys. Res.* 116, C11022. doi:10.1029/2011JC007273.

Arrigo, K. R., Perovich, D. K., Pickart, R. S., Brown, Z. W., Van Dijken, G. L., Lowry, K. E., et al. (2012). Massive phytoplankton blooms under arctic sea ice. *Science.* 336, 1408. doi:10.1126/science.1215065.

Arrigo, K. R., and van Dijken, G. L. (2015). Continued increases in Arctic Ocean primary production. *Prog. Oceanogr.* 136, 60–70. doi:10.1016/j.pocean.2015.05.002.

Arrigo, K. R., and van Dijken, G. L. (2011). Secular trends in Arctic Ocean net primary production. *J. Geophys. Res.* 116, C09011. doi:10.1029/2011JC007151.

Arrigo, K. R., van Dijken, G., and Pabi, S. (2008). Impact of a shrinking Arctic ice cover on marine primary production. *Geophys. Res. Lett.* 35, L19603. doi:10.1029/2008GL035028.

Assmy, P., Ehn, J. K., Fernández-Méndez, M., Hop, H., Katlein, C., Sundfjord, A., et al. (2013). Floating ice-algal aggregates below melting Arctic sea ice. *PLoS One* 8, e76599. doi:10.1371/journal.pone.0076599.

Assmy, P., Fernández-Méndez, M., Duarte, P., Meyer, A., Randelhoff, A., Mundy, C. J., et al. (2017). Leads in Arctic pack ice enable early phytoplankton blooms below snow-covered sea ice. *Sci. Rep.* 7, 40850. doi:10.1038/srep40850.

Austen, M. C., Lambshead, P. J. D., Hutchings, P. A., Boucher, G., Snelgrove, P. V. ., Heip, C., et al. (2002). Biodiversity links above and below the marine sediment–water interface that may influence community stability. *Biodivers. Conserv.* 11, 113–136. doi:10.1023/A:1014098917535.

Azam, F. (1998). Microbial Control of Oceanic Carbon Flux: The Plot Thickens. *Science.* 280.

Azam, F., and Malfatti, F. (2007). Microbial structuring of marine ecosystems. *Nat. Rev. Microbiol.* 5, 782–91. doi:10.1038/nrmicro1747.

Bano, N., and Hollibaugh, J. T. (2002). Phylogenetic Composition of Bacterioplankton Assemblages from the Arctic Ocean. 68, 505–518. doi:10.1128/AEM.68.2.505.

Bano, N., and Hollibaugh, J. T. (2000). Diversity and distribution of DNA sequences with affinity to ammonia-oxidizing bacteria of the beta subdivision of the class Proteobacteria in the Arctic Ocean. *Appl. Environ. Microbiol.* 66, 1960–9. doi:10.1128/AEM.66.5.1960-1969.2000.

Barber, D. G., Hop, H., Mundy, C. J., Else, B., Dmitrenko, I. A., Tremblay, J. E., et al. (2015). Selected physical, biological and biogeochemical implications of a rapidly changing Arctic Marginal Ice Zone. *Prog. Oceanogr.* 139, 122–150. doi:10.1016/j.pocean.2015.09.003.

Barbeyron, T., Thomas, F., Barbe, V., Teeling, H., Schenowitz, C., Dossat, C., et al. (2016). Habitat and taxon as driving forces of carbohydrate catabolism in marine heterotrophic bacteria: example of the model algae-associated bacterium *Zobellia galactanivorans* Dsij ^{T}. *Environ. Microbiol.* 18, 4610–4627. doi:10.1111/1462-2920.13584.

Bauerfeind, E., Bodungen, B. V., Arndt, K., and Koeve, W. (1994). Particle flux, and composition of sedimenting matter, in the Greenland Sea. *J. Mar. Syst.* 5, 411–423. doi:10.1016/0924-7963(94)90005-1.

Bayer-Giraldi, M., Weikusat, I., Besir, H., and Dieckmann, G. (2011). Characterization of an antifreeze protein from the polar diatom Fragilariopsis cylindrus and its relevance in sea ice. *Cryobiology* 63, 210–219. doi:10.1016/j.cryobiol.2011.08.006.

Behringer, G., Ochsenkühn, M. A., Fei, C., Fanning, J., Koester, J. A., and Amin, S. A. (2018). Bacterial Communities of Diatoms Display Strong Conservation Across Strains and Time. *Front. Microbiol.* 9, 659. doi:10.3389/FMICB.2018.00659.

Bennke, C. M., Krüger, K., Kappelmann, L., Huang, S., Gobet, A., Schüler, M., et al. (2016). Polysaccharide utilisation loci of Bacteroidetes from two contrasting open ocean sites in the North Atlantic. *Environ. Microbiol.* doi:10.1111/1462-2920.13429.

Bergauer, K., Fernandez-Guerra, A., Garcia, J. A. L., Sprenger, R. R., Stepanauskas, R., Pachiadaki, M. G., et al. (2017). Organic matter processing by microbial communities throughout the Atlantic water column as revealed by metaproteomics. *Proc. Natl. Acad. Sci. U. S. A.*, 201708779. doi:10.1073/pnas.1708779115.

Berlemont, R., and Martiny, A. C. (2016). Glycoside Hydrolases across

Environmental Microbial Communities. *PLOS Comput. Biol.* 12, e1005300. doi:10.1371/journal.pcbi.1005300.

Bertrand, E. M., McCrow, J. P., Moustafa, A., Zheng, H., McQuaid, J. B., Delmont, T. O., et al. (2015). Phytoplankton-bacterial interactions mediate micronutrient colimitation at the coastal Antarctic sea ice edge. *Proc. Natl. Acad. Sci. U. S. A.* 112, 9938–43. doi:10.1073/pnas.1501615112.

Beszczynska-Möller, A., Fahrbach, E., Schauer, U., and Hansen, E. (2012). Variability in Atlantic water temperature and transport at the entrance to the Arctic Ocean, 1997-2010. *ICES J. Mar. Sci.* 69, 852–863. doi:10.1093/icesjms/fss056.

Bienhold, C., Boetius, A., and Ramette, A. (2012). The energy-diversity relationship of complex bacterial communities in Arctic deep-sea sediments. *ISME J.* 6, 724–32. doi:10.1038/ismej.2011.140.

Bienhold, C., Zinger, L., Boetius, A., and Ramette, A. (2016). Diversity and Biogeography of Bathyal and Abyssal Seafloor Bacteria. *PLoS One* 11, e0148016. doi:10.1371/journal.pone.0148016.

Bik, H. M., Porazinska, D. L., Creer, S., Caporaso, J. G., Knight, R., and Thomas, W. K. (2012). Sequencing our way towards understanding global eukaryotic biodiversity. *Trends Ecol. Evol.* 27, 233–243. doi:10.1016/j.tree.2011.11.010.

Bluhm, B. A., and Gradinger, R. (2008). Regional variability in food availability for arctic marine mammals. *Ecol. Appl.* 18, S77–S96. doi:10.1890/06-0562.1.

Bluhm, B. A., Hop, H., Vihtakari, M., Gradinger, R., Iken, K., Melnikov, I. A., et al. (2018). Sea ice meiofauna distribution on local to pan-Arctic scales. *Ecol. Evol.* 8, 2350–2364. doi:10.1002/ece3.3797.

Boetius, A. (1995). Microbial hydrolytic enzyme activities in deep-sea sediments. *Helgoländer Meeresuntersuchungen* 49, 177–187. doi:10.1007/BF02368348.

Boetius, A., Albrecht, S., Bakker, K., Bienhold, C., Felden, J., Fernández-Méndez, M., et al. (2013). Export of algal biomass from the melting Arctic sea ice. *Science.* 339, 1430–1432. doi:10.1126/science.1231346.

Boetius, A., and Damm, E. (1998). Benthic oxygen uptake, hydrolytic potentials and microbial biomass at the Arctic continental slope. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 45, 239–275. doi:10.1016/S0967-0637(97)00052-6.

Boetius, A., and Lochte, K. (1994). Regulation of Microbial Enzymatic Degradation of Organic Matter in Deep-Sea Sediments. *Mar. Ecol. - Prog. Ser.* 104, 299–307. doi:10.3354/meps104299.

Boetius, A., Scheibej, S., Tselepides, A., and Thiel, H. (1996). Microbial biomass and activities in deep-sea sediments of the Eastern Mediterranean: trenches are benthic hotspots. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 43, 1439–1460. doi:10.1016/S0967-0637(96)00053-2.

Børsheim, K. Y., and Drinkwater, K. F. (2014). Different temperature adaptation in Arctic and Atlantic heterotrophic bacteria in the Barents Sea Polar Front region.

J. Mar. Syst. 130, 160–166. doi:10.1016/J.JMARSYS.2012.09.007.

Bowman, J. P., and McCuaig, R. D. (2003). Biodiversity, community structural shifts, and biogeography of prokaryotes within Antarctic continental shelf sediment. *Appl. Environ. Microbiol.* 69, 2463–2483. doi:10.1128/AEM.69.5.2463-2483.2003.

Bowman, J. S. (2015). The relationship between sea ice bacterial community structure and biogeochemistry: A synthesis of current knowledge and known unknowns. *Elem. Sci. Anthr.* 3, 000072. doi:10.12952/journal.elementa.000072.

Bowman, J. S., Larose, C., Vogel, T. M., and Deming, J. W. (2013). Selective occurrence of Rhizobiales in frost flowers on the surface of young sea ice near Barrow, Alaska and distribution in the polar marine rare biosphere. *Environ. Microbiol. Rep.* 5, 575–82. doi:10.1111/1758-2229.12047.

Bowman, J. S., Rasmussen, S., Blom, N., Deming, J. W., Rysgaard, S., and Sicheritz-Ponten, T. (2012). Microbial community structure of Arctic multiyear sea ice and surface seawater by 454 sequencing of the 16S RNA gene. *ISME J.* 6, 11–20. doi:10.1038/ismej.2011.76.

Bring, A., Shiklomanov, A., and Lammers, R. B. (2017). Pan-Arctic river discharge: Prioritizing monitoring of future climate change hot spots. *Earth's Futur.* 5, 72–92. doi:10.1002/2016EF000434.

Brinkmeyer, R., Glöckner, F.-O., Helmke, E., and Amann, R. (2004). Predominance of ß-proteobacteria in summer melt pools on Arctic pack ice. *Limnol. Oceanogr.* 49, 1013–1021. doi:10.4319/lo.2004.49.4.1013.

Brinkmeyer, R., Knittel, K., Ju, J., Weyland, H., Amann, R., Helmke, E., et al. (2003). Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. *Appl. Environ. Microbiol.* 69, 6610–6619. doi:10.1128/AEM.69.11.6610-6619.2003.

Buchan, A., LeCleir, G. R., Gulvik, C. A., and Gonzalez, J. M. (2014). Master recyclers: features and functions of bacteria associated with phytoplankton blooms. *Nat. Rev. Microbiol.* 12, 686–698. doi:10.1038/nrmicro3326.

Buesseler, K. O., Lamborg, C. H., Boyd, P. W., Lam, P. J., Trull, T. W., Bidigare, R. R., et al. (2007). Revisiting Carbon Flux Through the Ocean's Twilight Zone. *Science.* 316, 567–570. doi:10.1126/science.1137959.

Bunse, C., Bertos-Fortis, M., Sassenhagen, I., Sildever, S., Sjöqvist, C., Godhe, A., et al. (2016). Spatio-temporal interdependence of bacteria and phytoplankton during a baltic sea spring bloom. *Front. Microbiol.* 7, 517. doi:10.3389/fmicb.2016.00517.

Bunse, C., and Pinhassi, J. (2017). Marine Bacterioplankton Seasonal Succession Dynamics. *Trends Microbiol.* 25, 494–505. doi:10.1016/J.TIM.2016.12.013.

Carmack, E. C., Yamamoto-Kawai, M., Haine, T. W. N., Bacon, S., Bluhm, B. A., Lique, C., et al. (2016). Freshwater and its role in the Arctic Marine System: Sources, disposition, storage, export, and physical and biogeochemical

consequences in the Arctic and global oceans. *J. Geophys. Res. Biogeosciences* 121, 675–717. doi:10.1002/2015JG003140.

Carmack, E., and Wassmann, P. (2006). Food webs and physical-biological coupling on pan-Arctic shelves: Unifying concepts and comprehensive perspectives. *Prog. Oceanogr.* 71, 446–477. doi:10.1016/j.pocean.2006.10.004.

Carradec, Q., Pelletier, E., Da Silva, C., Alberti, A., Seeleuthner, Y., Blanc-Mathieu, R., et al. (2018). A global ocean atlas of eukaryotic genes. *Nat. Commun.* 9, 373. doi:10.1038/s41467-017-02342-1.

Cavalieri, D. J., and Parkinson, C. L. (2012). Arctic sea ice variability and trends, 1979-2010. *Cryosph.* 6, 881–889. doi:10.5194/tc-6-881-2012.

Chaffron, S., Rehrauer, H., Pernthaler, J., and von Mering, C. (2010). A global network of coexisting microbes from environmental and whole-genome sequence data. *Genome Res.* 20, 947–59. doi:10.1101/gr.104521.109.

Cho, B. C., and Azam, F. (1988). Major role of bacteria in biogeochemical fluxes in the ocean's interior. *Nature* 332, 441–443. doi:10.1038/332441a0.

Christman, G. D., Cottrell, M. T., Popp, B. N., Gier, E., and Kirchman, D. L. (2011). Abundance, Diversity, and Activity of Ammonia-Oxidizing Prokaryotes in the Coastal Arctic Ocean in Summer and Winter. *Appl. Environ. Microbiol.* 77, 2026–2034. doi:10.1128/AEM.01907-10.

Codispoti, L. a., Kelly, V., Thessen, a., Matrai, P., Suttles, S., Hill, V., et al. (2013). Synthesis of primary production in the Arctic Ocean: III. Nitrate and phosphate based estimates of net community production. *Prog. Oceanogr.* 110, 126–150. doi:10.1016/j.pocean.2012.11.006.

Collins, R. E., Carpenter, S. D., and Deming, J. W. (2008). Spatial heterogeneity and temporal dynamics of particles, bacteria, and pEPS in Arctic winter sea ice. *J. Mar. Syst.* 74, 902–917. doi:10.1016/j.jmarsys.2007.09.005.

Collins, R. E., and Deming, J. W. (2011). Abundant dissolved genetic material in Arctic sea ice Part I: Extracellular DNA. *Polar Biol.* 34, 1819–1830. doi:10.1007/s00300-011-1041-y.

Collins, R. E., Rocap, G., and Deming, J. W. (2010). Persistence of bacterial and archaeal communities in sea ice through an Arctic winter. *Environ. Microbiol.* 12, 1828–1841. doi:10.1111/j.1462-2920.2010.02179.x.

Comeau, A. M., Li, W. K. W., Tremblay, J.-É., Carmack, E. C., and Lovejoy, C. (2011). Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum. *PLoS One* 6, e27492. doi:10.1371/journal.pone.0027492.

Comiso, J. C., and Nishio, F. (2008). Trends in the sea ice cover using enhanced and compatible AMSR-E, SSM/I, and SMMR data. *J. Geophys. Res.* 113, C02S07. doi:10.1029/2007JC004257.

Comiso, J. C., Parkinson, C. L., Gersten, R., and Stock, L. (2008). Accelerated decline in the Arctic sea ice cover. *Geophys. Res. Lett.* 35, L01703.

doi:10.1029/2007GL031972.
Corinaldesi, C. (2015). New perspectives in benthic deep-sea microbial ecology. *Front. Mar. Sci.* 2, 17. doi:10.3389/fmars.2015.00017.
Corliss, B. H., Brown, C. W., Sun, X., and Showers, W. J. (2009). Deep-sea benthic diversity linked to seasonality of pelagic productivity. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 56, 835–841. doi:10.1016/j.dsr.2008.12.009.
Cota, G. F. (1985). Photoadaptation of high Arctic ice algae. *Nature* 315, 219–222. doi:10.1038/315219a0.
Cottrell, M. T., and Kirchman, D. L. (2009). Photoheterotrophic microbes in the Arctic Ocean in summer and winter. *Appl. Environ. Microbiol.* 75, 4958–66. doi:10.1128/AEM.00117-09.
Cowie, R. O. M., Maas, E. W., and Ryan, K. G. (2011). Archaeal diversity revealed in Antarctic sea ice. *Antarct. Sci.* 23, 531–536. doi:10.1017/S0954102011000368.
Cowie, R., Williams, G., Maas, E., and Voyles, K. (2014). Antarctic sea-ice microbial communities show distinct patterns of zonation in response to algal-derived substrates. *Aquat. Microb. Ecol.* 73, 123–134.
Danovaro, R. (2000). Viral Density and Virus-to-Bacterium Ratio in Deep-Sea Sediments of the Eastern Mediterranean. 66, 1857–1861.
Danovaro, R., Corinaldesi, C., Dell'Anno, A., and Snelgrove, P. V. R. (2017). The deep-sea under global change. *Curr. Biol.* 27, R461–R465. doi:10.1016/j.cub.2017.02.046.
Danovaro, R., Corinaldesi, C., Luna, G. M., Magagnini, M., Manini, E., and Pusceddu, A. (2009). Prokaryote diversity and viral production in deep-sea sediments and seamounts. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 56, 738–747. doi:10.1016/J.DSR2.2008.10.011.
Danovaro, R., Della Croce, N., Dell'Anno, A., and Pusceddu, A. (2003). A depocenter of organic matter at 7800 m depth in the SE Pacific Ocean. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 50, 1411–1420. doi:10.1016/J.DSR.2003.07.001.
Danovaro, R., Snelgrove, P. V. R., and Tyler, P. (2014). Challenging the paradigms of deep-sea ecology. *Trends Ecol. Evol.* 29, 465–75. doi:10.1016/j.tree.2014.06.002.
De La Rocha, C. L., and Passow, U. (2007). Factors influencing the sinking of POC and the efficiency of the biological carbon pump. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 54, 639–658. doi:10.1016/J.DSR2.2007.01.004.
Decho, A. W., and Gutierrez, T. (2017). Microbial Extracellular Polymeric Substances (EPSs) in Ocean Systems. *Front. Microbiol.* 8, 922. doi:10.3389/fmicb.2017.00922.
Delmont, T. O., Quince, C., Shaiber, A., Esen, Ö. C., Lee, S. T., Rappé, M. S., et al. (2018). Nitrogen-fixing populations of Planctomycetes and Proteobacteria are abundant in surface ocean metagenomes. *Nat. Microbiol.* 3, 804–813.

doi:10.1038/s41564-018-0176-9.

DeLong, E. F., and Pace, N. R. (2001). Environmental diversity of bacteria and archaea. *Syst. Biol.* 50, 470–8.

Deming, J. W. (2007). “Life in Ice Formations at Very Cold Temperatures,” in *Physiology and Biochemistry of Extremophiles* (American Society of Microbiology), 133–144. doi:10.1128/9781555815813.ch10.

Deming, J. W., and Baross, J. A. (1993). “The early diagenesis of organic matter: bacterial activity,” in *Organic Geochemistry* (Springer US), 119–144. doi:10.1007/978-1-4615-2890-6_5.

Deming, J. W., and Eicken, H. (2007). “Life in ice,” in *Planets and Life: The Emerging Science of Astrobiology*, eds. W. T. Sullivan and J. A. Baross (Cambridge University Press), 292–312.

Deming, J. W., and Yager, P. L. (1992). “Natural Bacterial Assemblages in Deep-Sea Sediments: Towards a Global View,” in *Deep-Sea Food Chains and the Global Carbon Cycle* (Dordrecht: Springer Netherlands), 11–27. doi:10.1007/978-94-011-2452-2_2.

Dittmar, T., and Kattner, G. (2003). The biogeochemistry of the river and shelf ecosystem of the Arctic Ocean: a review. *Mar. Chem.* 83, 103–120. doi:10.1016/S0304-4203(03)00105-1.

Duarte, C. M., Lenton, T. M., Wadhams, P., and Wassmann, P. (2012). Abrupt climate change in the Arctic. *Nat. Clim. Chang.* 2, 60–62. doi:10.1038/nclimate1386.

Dunton, K. H., Goodall, J. L., Schonberg, S. V., Grebmeier, J. M., and Maidment, D. R. (2005). Multi-decadal synthesis of benthic-pelagic coupling in the western arctic: Role of cross-shelf advective processes. *Deep. Res. Part II Top. Stud. Oceanogr.* 52, 3462–3477. doi:10.1016/j.dsr2.2005.09.007.

Eicken, H., Kolatschek, J., Freitag, J., Lindemann, F., Kassens, H., and Dmitrenko, I. (2000). A key source area and constraints on entrainment for basin-scale sediment transport by Arctic sea ice. *Geophys. Res. Lett.* 27, 1919–1922. doi:10.1029/1999GL011132.

Eilers, H., Pernthaler, J., Glöckner, F. O., and Amann, R. (2000). Culturability and In situ abundance of pelagic bacteria from the North Sea. *Appl. Environ. Microbiol.* 66, 3044–51. doi:10.1128/AEM.66.7.3044-3051.2000.

Emerson, S., and Hedges, J. (2008). *Chemical Oceanography and the Marine Carbon Cycle*. Cambridge University Press.

Eronen-Rasimus, E., Kaartokallio, H., Lyra, C., Autio, R., Kuosa, H., Dieckmann, G. S., et al. (2014). Bacterial community dynamics and activity in relation to dissolved organic matter availability during sea-ice formation in a mesocosm experiment. *Microbiologyopen* 3, 139–156. doi:10.1002/mbo3.157.

Eronen-Rasimus, E., Luhtanen, A.-M., Rintala, J.-M., Delille, B., Dieckmann, G., Karkman, A., et al. (2017). An active bacterial community linked to high chl-a

concentrations in Antarctic winter-pack ice and evidence for the development of an anaerobic sea-ice bacterial community. *ISME J.* 11, 2345–2355. doi:10.1038/ismej.2017.96.

Eronen-Rasimus, E., Piiparinen, J., Karkman, A., Lyra, C., Gerland, S., and Kaartokallio, H. (2016). Bacterial communities in Arctic first-year drift ice during the winter/spring transition. *Environ. Microbiol. Rep.* 8, 527–535. doi:10.1111/1758-2229.12428.

Ewert, M., and Deming, J. W. (2013). Sea ice microorganisms: environmental constraints and extracellular responses. *Biology (Basel).* 2, 603–28. doi:10.3390/biology2020603.

Fahrbach, E., Meincke, J., Østerhus, S., Rohardt, G., Schauer, U., Tverberg, V., et al. (2001). Direct measurements of volume transports through Fram Strait. *Polar Res.* 20, 217–224. doi:10.1111/j.1751-8369.2001.tb00059.x.

Falkowski, P. G., Fenchel, T., and Delong, E. F. (2008). The microbial engines that drive Earth's biogeochemical cycles. *Science.* 320.

Feng, S., Powell, S. M., Wilson, R., and Bowman, J. P. (2014). Extensive gene acquisition in the extremely psychrophilic bacterial species Psychroflexus torquis and the link to sea-ice ecosystem specialism. *Genome Biol. Evol.* 6, 133–148. doi:10.1093/gbe/evt209.

Fernández-Gómez, B., Richter, M., Schüler, M., Pinhassi, J., Acinas, S. G., González, J. M., et al. (2013). Ecology of marine Bacteroidetes: a comparative genomics approach. *ISME J.* 7, 1026–1037. doi:10.1038/ismej.2012.169.

Fernández-Méndez, M., Katlein, C., Rabe, B., Nicolaus, M., Peeken, I., Bakker, K., et al. (2015). Photosynthetic production in the central Arctic Ocean during the record sea-ice minimum in 2012. *Biogeosciences* 12, 3525–3549. doi:10.5194/bg-12-3525-2015.

Fernández-Méndez, M., Turk-Kubo, K. A., Buttigieg, P. L., Rapp, J. Z., Krumpen, T., Zehr, J. P., et al. (2016). Diazotroph diversity in the sea ice, melt ponds and surface waters of the Eurasian Basin of the Central Arctic Ocean. *Front. Microbiol.* 7, 1884.

Fernández-Méndez, M., Wenzhöfer, F., Peeken, I., Sørensen, H. L., Glud, R. N., and Boetius, A. (2014). Composition, buoyancy regulation and fate of ice algal aggregates in the central Arctic Ocean. *PLoS One* 9, e107452. doi:10.1371/journal.pone.0107452.

Ferrari, V. C., and Hollibaugh, J. T. (1999). "Distribution of microbial assemblages in the Central Arctic Ocean Basin studied by PCR/DGGE: analysis of a large data set" in *Molecular Ecology of Aquatic Communities* (Dordrecht: Springer Netherlands), 55–68. doi:10.1007/978-94-011-4201-4_5.

Fetterer, F., and Untersteiner, N. (1998). Observations of melt ponds on Arctic sea ice. *J. Geophys. Res. Ocean.* 103, 24821–24835. doi:10.1029/98JC02034.

Findlay, H. S., Gibson, G., Kedra, M., Morata, N., Orchowska, M., Pavlov, A. K., et

al. (2015). Responses in Arctic marine carbon cycle processes: Conceptual scenarios and implications for ecosystem function. *Polar Res.* 34. doi:10.3402/polar.v34.24252.

Fischer, J. P., Ferdelman, T. G., D'Hondt, S., Røy, H., and Wenzhöfer, F. (2009). Oxygen penetration deep into the sediment of the South Pacific gyre. *Biogeosciences* 6, 1467–1478. doi:10.5194/bg-6-1467-2009.

Fortier, M., Fortier, L., Michel, C., and Legendre, L. (2002). Climatic and biological forcing of the vertical flux of biogenic particles under seasonal Arctic sea ice. 225, 1–16.

Freitas, S., Hatosy, S., Fuhrman, J. A., Huse, S. M., Welch, D. B. M., Sogin, M. L., et al. (2012). Global distribution and diversity of marine Verrucomicrobia. *ISME J.* 6, 1499–1505. doi:10.1038/ismej.2012.3.

Galand, P. E., Casamayor, E. O., Kirchman, D. L., and Lovejoy, C. (2009a). Ecology of the rare microbial biosphere of the Arctic Ocean. 106.

Galand, P. E., Casamayor, E. O., Kirchman, D. L., Potvin, M., and Lovejoy, C. (2009b). Unique archaeal assemblages in the Arctic Ocean unveiled by massively parallel tag sequencing. *ISME J.* 3, 860–9. doi:10.1038/ismej.2009.23.

Galand, P. E., Potvin, M., Casamayor, E. O., and Lovejoy, C. (2010). Hydrography shapes bacterial biogeography of the deep Arctic Ocean. *ISME J.* 4, 564–76. doi:10.1038/ismej.2009.134.

Ghiglione, J.-F., Galand, P. E., Pommier, T., Pedrós-Alió, C., Maas, E. W., Bakker, K., et al. (2012). Pole-to-pole biogeography of surface and deep marine bacterial communities. *Proc. Natl. Acad. Sci. U. S. A.* 109, 17633–8. doi:10.1073/pnas.1208160109.

Gilbert, J. A., Jansson, J. K., and Knight, R. (2014). The Earth Microbiome project: successes and aspirations. *BMC Biol.* 12, 69. doi:10.1186/s12915-014-0069-1.

Gilbert, J. A., Steele, J. A., Caporaso, J. G., Steinbrück, L., Reeder, J., Temperton, B., et al. (2012). Defining seasonal marine microbial community dynamics. *ISME J.* 6, 298–308. doi:10.1038/ismej.2011.107.

Giles, K. A., Laxon, S. W., and Ridout, A. L. (2008). Circumpolar thinning of Arctic sea ice following the 2007 record ice extent minimum. *Geophys. Res. Lett.* 35, L22502. doi:10.1029/2008GL035710.

Giovannelli, D., Molari, M., d'Errico, G., Baldrighi, E., Pala, C., and Manini, E. (2013). Large-Scale Distribution and Activity of Prokaryotes in Deep-Sea Surface Sediments of the Mediterranean Sea and the Adjacent Atlantic Ocean. *PLoS One* 8, e72996. doi:10.1371/journal.pone.0072996.

Glöckner, F. O., Fuchs, B. M., and Amann, R. (1999). Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. *Appl. Environ. Microbiol.* 65, 3721–6.

Glover, A. G., and Smith, C. R. (2003). The deep-sea floor ecosystem: current status

and prospects of anthropogenic change by the year 2025. *Environ. Conserv.* 30, 219–241. doi:10.1017/S0376892903000225.

Goffredi, S. K., and Orphan, V. J. (2010). Bacterial community shifts in taxa and diversity in response to localized organic loading in the deep sea. *Environ. Microbiol.* 12, 344–63. doi:10.1111/j.1462-2920.2009.02072.x.

Gosink, J. J., Irgens, R. L., Staley, J. T., and Gosink, J. J. (1993). Vertical distribution of bacteria in arctic sea ice. *FEMS Microbiol. Ecol.* 102, 85–90. doi:10.1111/j.1574-6968.1993.tb05799.x.

Gosselin, M., Levasseur, M., Patricia, A., Horner, R. A., Booth, B. C., Wheeler, P. A., et al. (1997). New measurements of phytoplankton and ice algal production in the Arctic Ocean. *Deep. Res. Part II Top. Stud. Oceanogr.* 44, 1623–1644. doi:10.1016/S0967-0645(97)00054-4.

Gradinger, R., and Ikävalko, J. (1998). Organism incorporation into newly forming Arctic sea ice in the Greenland Sea. *J. Plankton Res.* 20, 871–886. doi:10.1093/plankt/20.5.871.

Gradinger, R. R. (2001). Adaptation of Arctic and Antarctic ice metazoa to their habitat. *Zoology* 104, 339–345. doi:10.1078/0944-2006-00039.

Grebmeier, J. M., Cooper, L. W., Feder, H. M., and Sirenko, B. I. (2006a). Ecosystem dynamics of the Pacific-influenced Northern Bering and Chukchi Seas in the Amerasian Arctic. *Prog. Oceanogr.* 71, 331–361. doi:10.1016/j.pocean.2006.10.001.

Grebmeier, J. M., Overland, J. E., Moore, S. E., Farley, E. V, Carmack, E. C., Cooper, L. W., et al. (2006b). A major ecosystem shift in the northern Bering Sea. *Science.* 311, 1461–1464. doi:10.1126/science.1121365.

Grossart, H.-P., Dziallas, C., Leunert, F., and Tang, K. W. (2010). Bacteria dispersal by hitchhiking on zooplankton. *Proc. Natl. Acad. Sci.* 107, 11959–11964. doi:10.1073/pnas.1000668107.

Haas, C., Pfaffling, A., Hendricks, S., Rabenstein, L., Etienne, J.-L., and Rigor, I. (2008). Reduced ice thickness in Arctic Transpolar Drift favors rapid ice retreat. *Geophys. Res. Lett.* 35, L17501. doi:10.1029/2008GL034457.

Haine, T. W. N., Curry, B., Gerdes, R., Hansen, E., Karcher, M., Lee, C., et al. (2015). Arctic freshwater export: Status, mechanisms, and prospects. *Glob. Planet. Change* 125, 13–35. doi:10.1016/J.GLOPLACHA.2014.11.013.

Hamdan, L. J., Coffin, R. B., Sikaroodi, M., Greinert, J., Treude, T., and Gillevet, P. M. (2013). Ocean currents shape the microbiome of Arctic marine sediments. *ISME J.* 7, 685–96. doi:10.1038/ismej.2012.143.

Han, D., Kang, I., Ha, H. K., Kim, H. C., Kim, O. S., Lee, B. Y., et al. (2014). Bacterial communities of surface mixed layer in the pacific sector of the western Arctic Ocean during sea-ice melting. *PLoS One* 9, e86887. doi:10.1371/journal.pone.0086887.

Hancke, K., Lund-Hansen, L. C., Lamare, M. L., Højlund Pedersen, S., King, M. D.,

Andersen, P., et al. (2018). Extreme Low Light Requirement for Algae Growth Underneath Sea Ice: A Case Study From Station Nord, NE Greenland. *J. Geophys. Res. Ocean.* 123, 985–1000. doi:10.1002/2017JC013263.

Handelsman, J., Rondon, M. R., Brady, S. F., Clardy, J., and Goodman, R. M. (1998). Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. *Chem. Biol.* 5, R245-9.

Harada, N. (2016). Review: Potential catastrophic reduction of sea ice in the western Arctic Ocean: Its impact on biogeochemical cycles and marine ecosystems. *Glob. Planet. Change* 136, 1–17. doi:10.1016/j.gloplacha.2015.11.005.

Hatam, I., Charchuk, R., Lange, B., Beckers, J., Haas, C., and Lanoil, B. (2014). Distinct bacterial assemblages reside at different depths in Arctic multiyear sea ice. *FEMS Microbiol. Ecol.* 90, 115–125. doi:10.1111/1574-6941.12377.

Hatam, I., Lange, B., Beckers, J., Haas, C., and Lanoil, B. (2016). Bacterial communities from Arctic seasonal sea ice are more compositionally variable than those from multi-year sea ice. *ISME J.* 10, 2543–2552. doi:10.1038/ismej.2016.4.

Hauptmann, A. L., Stibal, M., Bælum, J., Sicheritz-Pontén, T., Brunak, S., Bowman, J. S., et al. (2014). Bacterial diversity in snow on North Pole ice floes. *Extremophiles* 18, 945–951. doi:10.1007/s00792-014-0660-y.

Hegseth, E. N., and Sundfjord, A. (2008). Intrusion and blooming of Atlantic phytoplankton species in the high Arctic. *J. Mar. Syst.* 74, 108–119. doi:10.1016/J.JMARSYS.2007.11.011.

Helmke, E., and Weyland, H. (1995). Bacteria in sea ice and underlying water of the eastern Weddell Sea in midwinter. *Mar. Ecol. Prog. Ser.* 117, 269–288. doi:10.3354/meps117269.

Herndl, G. J., Reinthaler, T., Teira, E., van Aken, H., Veth, C., Pernthaler, A., et al. (2005). Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean. *Appl. Environ. Microbiol.* 71, 2303–9. doi:10.1128/AEM.71.5.2303-2309.2005.

Herring, P. (2002). *The Biology of the Deep Ocean*. Oxford, United Kingdom: Oxford University Press.

Hill, V., and Cota, G. (2005). Spatial patterns of primary production on the shelf, slope and basin of the Western Arctic in 2002. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 52, 3344–3354. doi:10.1016/J.DSR2.2005.10.001.

Hill, V. J., Matrai, P. a., Olson, E., Suttles, S., Steele, M., Codispoti, L. a., et al. (2013). Synthesis of integrated primary production in the Arctic Ocean: II. In situ and remotely sensed estimates. *Prog. Oceanogr.* 110, 107–125. doi:10.1016/j.pocean.2012.11.005.

Hoffmann, K., Hassenrück, C., Salman-Carvalho, V., Holtappels, M., and Bienhold, C. (2017). Response of bacterial communities to different detritus compositions in Arctic deep-sea sediments. *Front. Microbiol.* 8, 266.

doi:10.3389/fmicb.2017.00266.

Hong, S., Bunge, J., Leslin, C., Jeon, S., and Epstein, S. S. (2009). Polymerase chain reaction primers miss half of rRNA microbial diversity. *ISME J.* 3, 1365–1373. doi:10.1038/ismej.2009.89.

Hoppe, H.-G. (1991). "Microbial Extracellular Enzyme Activity: A New Key Parameter in Aquatic Ecology," in, 60–83. doi:10.1007/978-1-4612-3090-8_4.

Hoppe, H.-G., Arnosti, C., and Herndl, G. F. (2002). "Ecological significance of bacterial enzymes in the marine environment" in *Enzymes in the Environment. Activity, Ecology, and Applications*, eds. R. G. Burns and R. P. Dick (New York: Marcel Dekker), 73–107.

Hugenholtz, P. (2002). Exploring prokaryotic diversity in the genomic era. *Genome Biol.* 3, REVIEWS0003. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11864374 [Accessed July 4, 2018].

Hugenholtz, P., and Pace, N. R. (1996). Identifying microbial diversity in the natural environment: A molecular phylogenetic approach. *Trends Biotechnol.* 14, 190–197. doi:10.1016/0167-7799(96)10025-1.

Hugenholtz, P., and Tyson, G. W. (2008). Microbiology: Metagenomics. *Nat. 2008 4557212*.

Huisman, J., and Weissing, F. J. (1999). Biodiversity of plankton by species oscillations and chaos. *Nature* 402, 407–410. doi:10.1038/46540.

Human Microbiome Project Consortium (2012). Structure, function and diversity of the healthy human microbiome. *Nature* 486, 207–214. doi:10.1038/nature11234.

Huston, A. L., Krieger-Brockett, B. B., and Deming, J. W. (2000). Remarkably low temperature optima for extracellular enzyme activity from Arctic bacteria and sea ice. *Environ. Microbiol.* 2, 383–388. doi:10.1046/j.1462-2920.2000.00118.x.

Integrative HMP (iHMP) Research Network Consortium, T. I. H. (iHMP) R. N. (2014). The Integrative Human Microbiome Project: dynamic analysis of microbiome-host omics profiles during periods of human health and disease. *Cell Host Microbe* 16, 276–89. doi:10.1016/j.chom.2014.08.014.

Jacob, M. (2014). Influence of Global Change on microbial communities in Arctic sediments. Available at: https://elib.suub.uni-bremen.de/edocs/00103916-1.pdf [Accessed March 18, 2018].

Jacob, M., Soltwedel, T., Boetius, A., and Ramette, A. (2013). Biogeography of deep-sea benthic bacteria at regional scale (LTER HAUSGARTEN, Fram Strait, Arctic). *PLoS One* 8, e72779. doi:10.1371/journal.pone.0072779.

Jahnke, R. A., and Jackson, G. A. (1992). "The spatial distrubution of sea floor oxygen consumption in the Atlantic and Pacific Oceans" in *Deep-sea food chains and the global carbon cycle.* (Dordrecht: Springer Netherlands), 295–307. doi:10.1007/978-94-011-2452-2_18.

Jakobsson, M. (2002). Hypsometry and volume of the Arctic Ocean and its constituent seas. *Geochemistry, Geophys. Geosystems* 3, 1–18.

doi:10.1029/2001GC000302.

Jakobsson, M., Grantz, A., Kristoffersen, Y., and Macnab, R. (2003). Physiographic provinces of the Arctic Ocean seafloor. *Geol. Soc. Am. Bull.* 115, 1443. doi:10.1130/B25216.1.

Jakobsson, M., Grantz, A., Kristoffersen, Y., Macnab, R., MacDonald, R. W., Sakshaug, E., et al. (2004). "The Arctic Ocean: Boundary conditions and background information" in *The Organic Carbon Cycle in the Arctic Ocean* (Berlin, Heidelberg: Springer Berlin Heidelberg), 1–32. doi:10.1007/978-3-642-18912-8_1.

Jakobsson, M., Mayer, L., Coakley, B., Dowdeswell, J. A., Forbes, S., Fridman, B., et al. (2012). The International Bathymetric Chart of the Arctic Ocean (IBCAO) Version 3.0. *Geophys. Res. Lett.* 39, L12609. doi:10.1029/2012GL052219.

Jeffries, M. O., Overland, J. E., and Perovich, D. K. (2013). The Arctic shifts to a new normal. *Phys. Today* 66, 35–40. doi:10.1063/PT.3.2147.

Ji, R., Jin, M., and Varpe, Ø. (2013). Sea ice phenology and timing of primary production pulses in the Arctic Ocean. *Glob. Chang. Biol.* 19. doi:10.1111/gcb.12074.

Jones, E. P. (2001). Circulation in the Arctic Ocean. *Polar Res.* 20, 139–146. doi:10.1111/j.1751-8369.2001.tb00049.x.

Jørgensen, B. B., and Boetius, A. (2007). Feast and famine - microbial life in the deep-sea bed. *Nat. Rev. Microbiol.* 5, 770–781. doi:10.1038/nrmicro1745.

Junge, K., Christner, B., and Staley, J. T. (2011). "Diversity of psychrophilic bacteria from sea ice - and glacial ice communities" in *Extremophiles Handbook* (Tokyo: Springer Japan), 793–815. doi:10.1007/978-4-431-53898-1_39.

Junge, K., Eicken, H., and Deming, J. W. (2004). Bacterial activity at -2 to -20 degrees C in Arctic wintertime sea ice. *Appl. Environ. Microbiol.* 70, 550–557.

Junge, K., Imhoff, F., Staley, T., and Deming, J. W. (2002). Phylogenetic diversity of numerically important Arctic sea-ice bacteria cultured at subzero temperature. *Microb. Ecol.* 43, 315–28. doi:10.1007/s00248-001-1026-4.

Kabisch, A., Otto, A., König, S., Becher, D., Albrecht, D., Schüler, M., et al. (2014). Functional characterization of polysaccharide utilization loci in the marine Bacteroidetes "Gramella forsetii" KT0803. *ISME J.* 8, 1492–502. doi:10.1038/ismej.2014.4.

Kaiser, K., and Benner, R. (2009). Biochemical composition and size distribution of organic matter at the Pacific and Atlantic time-series stations. *Mar. Chem.* 113, 63–77. doi:10.1016/J.MARCHEM.2008.12.004.

Kanzog, C., Ramette, A., Quéric, N. V., and Klages, M. (2008). Response of benthic microbial communities to chitin enrichment: an in situ study in the deep Arctic Ocean. *Polar Biol.* 32, 105–112. doi:10.1007/s00300-008-0510-4.

Karl, D. M. (2007). Microbial oceanography: paradigms, processes and promise. *Nat. Rev. Microbiol.* 5, 759–69. doi:10.1038/nrmicro1749.

Karsenti, E., Acinas, S. G., Bork, P., Bowler, C., De Vargas, C., Raes, J., et al. (2011). A holistic approach to marine eco-systems biology. *PLoS Biol.* 9, e1001177. doi:10.1371/journal.pbio.1001177.

Katlein, C., Fernández-Méndez, M., Wenzhöfer, F., and Nicolaus, M. (2014). Distribution of algal aggregates under summer sea ice in the Central Arctic. *Polar Biol.* 38, 719–731. doi:10.1007/s00300-014-1634-3.

Kilias, E., Kattner, G., Wolf, C., Frickenhaus, S., and Metfies, K. (2014). A molecular survey of protist diversity through the central Arctic Ocean. *Polar Biol.* 37, 1271–1287. doi:10.1007/s00300-014-1519-5.

Kirchman, D. L., Cottrell, M. T., and Lovejoy, C. (2010). The structure of bacterial communities in the western Arctic Ocean as revealed by pyrosequencing of 16S rRNA genes. *Environ. Microbiol.* 12, 1132–1143. doi:10.1111/j.1462-2920.2010.02154.x.

Kirchman, D. L., Elifantz, H., Dittel, A. I., Malmstrom, R. R., and Cottrell, M. T. (2007). Standing stocks and activity of Archaea and Bacteria in the western Arctic Ocean. *Limnol. Oceanogr.* 52, 495–507. doi:10.4319/lo.2007.52.2.0495.

Kirchman, D. L., Hill, V., Cottrell, M. T., Gradinger, R., Malmstrom, R. R., and Parker, A. (2009). Standing stocks, production, and respiration of phytoplankton and heterotrophic bacteria in the western Arctic Ocean. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 56, 1237–1248. doi:10.1016/j.dsr2.2008.10.018.

Kiriakoulakis, K., Stutt, E., Rowland, S. J., Annick Vangriesheim, Lampitt, R. S., and Wolff, G. A. (2001). Controls on the organic chemical composition of settling particles in the Northeast Atlantic Ocean. *Prog. Oceanogr.* 50, 65–87. doi:10.1016/S0079-6611(01)00048-9.

Klages, M., Boetius, A., Christensen, J. P., Deubel, H., Piepenburg, D., Schewe, I., et al. (2004). “The benthos of Arctic seas and its role for the organic carbon cycle at the seafloor” in *The Organic Carbon Cycle in the Arctic Ocean* (Berlin, Heidelberg: Springer Berlin Heidelberg), 139–167. doi:10.1007/978-3-642-18912-8_6.

Klindworth, A., Mann, A. J., Huang, S., Wichels, A., Quast, C., Waldmann, J., et al. (2014). Diversity and activity of marine bacterioplankton during a diatom bloom in the North Sea assessed by total RNA and pyrotag sequencing. *Mar. Genomics* 18, 185–192. doi:10.1016/j.margen.2014.08.007.

Koenigk, T., Mikolajewicz, U., Haak, H., and Jungclaus, J. H. (2008). “Modelling the sea ice export through Fram Strait” in *Arctic–Subarctic Ocean Fluxes* (Dordrecht: Springer Netherlands), 171–191. doi:10.1007/978-1-4020-6774-7_9.

Kohlbach, D., Graeve, M., A. Lange, B., David, C., Peeken, I., and Flores, H. (2016). The importance of ice algae-produced carbon in the central Arctic Ocean ecosystem: Food web relationships revealed by lipid and stable isotope analyses. *Limnol. Oceanogr.* doi:10.1002/lno.10351.

Kopf, A., Bicak, M., Kottmann, R., Schnetzer, J., Kostadinov, I., Lehmann, K., et al.

(2015). The ocean sampling day consortium. *Gigascience* 4, 27. doi:10.1186/s13742-015-0066-5.

Korhonen, M., Rudels, B., Marnela, M., Wisotzki, A., and Zhao, J. (2013). Time and space variability of freshwater content, heat content and seasonal ice melt in the Arctic Ocean from 1991 to 2011. *Ocean Sci* 9, 1015–1055. doi:10.5194/os-9-1015-2013.

Kottmeier, S. T., and Sullivan, C. W. (1990). Bacterial biomass and production in pack ice of Antarctic marginal ice edge zones. *Deep Sea Res. Part A. Oceanogr. Res. Pap.* 37, 1311–1330. doi:10.1016/0198-0149(90)90045-W.

Krembs, C., Eicken, H., and Deming, J. W. (2011). Exopolymer alteration of physical properties of sea ice and implications for ice habitability and biogeochemistry in a warmer Arctic. *Proc. Natl. Acad. Sci. U. S. A.* 108, 3653–3658. doi:10.1073/pnas.1100701108.

Krembs, C., Eicken, H., Junge, K., and Deming, J. W. (2002). High concentrations of exopolymeric substances in Arctic winter sea ice: implications for the polar ocean carbon cycle and cryoprotection of diatoms. *Deep. Res. Part I Oceanogr. Res. Pap.* 49, 2163–2181. doi:10.1016/S0967-0637(02)00122-X.

Krembs, C., Gradinger, R., and Spindler, M. (2000). Implications of brine channel geometry and surface area for the interaction of sympagic organisms in Arctic sea ice. *J. Exp. Mar. Bio. Ecol.* 243, 55–80. doi:10.1016/S0022-0981(99)00111-2.

Kritzberg, E. S., Duarte, C. M., and Wassmann, P. (2010). Changes in Arctic marine bacterial carbon metabolism in response to increasing temperature. *Polar Biol.* 33, 1673–1682. doi:10.1007/s00300-010-0799-7.

Kröncke, I., Tan, T. L., and Stein, R. (1994). High benthic bacteria standing stock in deep Arctic basins. *Polar Biol.* 14, 423–428. doi:10.1007/BF00240263.

Kwok, R. (2009). Outflow of Arctic Ocean sea ice into the Greenland and Barent Seas: 1979-2007. *J. Clim.* 22, 2438–2457. doi:10.1175/2008JCLI2819.1.

Kwok, R. (2007). Near zero replenishment of the Arctic multiyear sea ice cover at the end of 2005 summer. *Geophys. Res. Lett.* 34. doi:10.1029/2006GL028737.

Kwok, R., Cunningham, G. F., and Pang, S. S. (2004). Fram Strait sea ice outflow. *J. Geophys. Res.* 109, C01009. doi:10.1029/2003JC001785.

Kwok, R., and Rothrock, D. A. (2009). Decline in Arctic sea ice thickness from submarine and ICESat records: 1958-2008. *Geophys. Res. Lett.* 36, L15501. doi:10.1029/2009GL039035.

Kwok, R., Spreen, G., and Pang, S. (2013). Arctic sea ice circulation and drift speed: Decadal trends and ocean currents. *J. Geophys. Res. Ocean.* 118, 2408–2425. doi:10.1002/jgrc.20191.

Lalande, C., Nöthig, E.-M. M., Bauerfeind, E., Hardge, K., Beszczynska-Möller, A., and Fahl, K. (2016). Lateral supply and downward export of particulate matter from upper waters to the seafloor in the deep eastern Fram Strait. *Deep. Res.*

Part I Oceanogr. Res. Pap. 114, 78–89. doi:10.1016/j.dsr.2016.04.014.

Landa, M., Blain, S., Christaki, U., Monchy, S., and Obernosterer, I. (2016). Shifts in bacterial community composition associated with increased carbon cycling in a mosaic of phytoplankton blooms. *ISME J.* 10, 39–50. doi:10.1038/ismej.2015.105.

Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L., and Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc. Natl. Acad. Sci. U. S. A.* 82, 6955–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2413450 [Accessed July 4, 2018].

Lange, B. A., Flores, H., Michel, C., Beckers, J. F., Bublitz, A., Casey, J. A., et al. (2017). Pan-Arctic sea ice-algal chl *a* biomass and suitable habitat are largely underestimated for multiyear ice. *Glob. Chang. Biol.* 23, 4581–4597. doi:10.1111/gcb.13742.

LaRoche, J., and Breitbarth, E. (2005). Importance of the diazotrophs as a source of new nitrogen in the ocean. *J. Sea Res.* 53, 67–91. doi:10.1016/J.SEARES.2004.05.005.

Laxon, S. W., Giles, K. A., Ridout, A. L., Wingham, D. J., Willatt, R., Cullen, R., et al. (2013). CryoSat-2 estimates of Arctic sea ice thickness and volume. *Geophys. Res. Lett.* 40, 732–737. doi:10.1002/grl.50193.

Le Fouest, V., Babin, M., and Tremblay, J. E. (2013). The fate of riverine nutrients on Arctic shelves. *Biogeosciences* 10, 3661–3677. doi:10.5194/bg-10-3661-2013.

Le Moigne, F. A. C., Poulton, A. J., Henson, S. A., Daniels, C. J., Fragoso, G. M., Mitchell, E., et al. (2015). Carbon export efficiency and phytoplankton community composition in the Atlantic sector of the Arctic Ocean. *J. Geophys. Res. Ocean.* 120, 3896–3912. doi:10.1002/2015JC010700.

Learman, D. R., Henson, M. W., Thrash, J. C., Temperton, B., Brannock, P. M., Santos, S. R., et al. (2016). Biogeochemical and microbial variation across 5500 km of Antarctic surface sediment implicates organic matter as a driver of benthic community structure. *Front. Microbiol.* 7, 284. doi:10.3389/fmicb.2016.00284.

Lee, S. H., McRoy, C. P., Joo, H. M., Gradinger, R., Cui, X. H., Yun, M. S., et al. (2011). Holes in progressively thinning Arctic sea ice lead to new ice algae habitat. *Oceanography* 24, 302–308. doi:10.5670/oceanog.2011.81.

Lee, S. H., Stockwell, D. a., Joo, H.-M., Son, Y. B., Kang, C.-K., and Whitledge, T. E. (2012). Phytoplankton production from melting ponds on Arctic sea ice. *J. Geophys. Res.* 117, C04030. doi:10.1029/2011JC007717.

Legendre, L., Ackley, S., Dieckmann, G., Gulliksen, B., Horner, R., Hoshiai, T., et al. (1992). Ecology of sea ice biota. *Polar Biol.* 12, 429–444. doi:10.1007/BF00243114.

Letscher, R. T., Knapp, A. N., James, A. K., Carlson, C. A., Santoro, A. E., and Hansell, D. A. (2015). Microbial community composition and nitrogen availability influence DOC remineralization in the South Pacific Gyre. *Mar. Chem.* 177, 325–

334. doi:10.1016/J.MARCHEM.2015.06.024.

Leu, E., Søreide, J. E., Hessen, D. O., Falk-Petersen, S., and Berge, J. (2011). Consequences of changing sea-ice cover for primary and secondary producers in the European Arctic shelf seas: Timing, quantity, and quality. *Prog. Oceanogr.* 90, 18–32. doi:10.1016/j.pocean.2011.02.004.

Li, H., Yu, Y., Luo, W., Zeng, Y., and Chen, B. (2009a). Bacterial diversity in surface sediments from the Pacific Arctic Ocean. *Extremophiles* 13, 233–46. doi:10.1007/s00792-009-0225-7.

Li, W. K. W., McLaughlin, F. A., Lovejoy, C., and Carmack, E. C. (2009b). Smallest algae thrive as the Arctic Ocean freshens. *Science.* 326, 539–539. doi:10.1126/science.1179798.

Li, Y., Liu, Q., Li, C., Dong, Y., Zhang, W. W., Zhang, W. W., et al. (2015). Bacterial and archaeal community structures in the Arctic deep-sea sediment. *Acta Oceanol. Sin.* 34, 93–113. doi:10.1007/s13131-015-0624-9.

Lima-Mendez, G., Faust, K., Henry, N., Decelle, J., Colin, S., Carcillo, F., et al. (2015). Determinants of community structure in the global plankton interactome. *Science.* 348, 1262073. doi:10.1126/science.1262073.

Liu, Z., DeSantis, T. Z., Andersen, G. L., and Knight, R. (2008). Accurate taxonomy assignments from 16S rRNA sequences produced by highly parallel pyrosequencers. *Nucleic Acids Res.* 36, e120–e120. doi:10.1093/nar/gkn491.

Llobet-Brossa, E., Rossello-Mora, R., and Amann, R. (1998). Microbial community composition of Wadden Sea sediments as revealed by fluorescence in situ hybridization. *Appl. Environ. Microbiol.* 64, 2691–2696.

Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P. M., and Henrissat, B. (2014). The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res.* 42, D490–D495. doi:10.1093/nar/gkt1178.

Lozupone, C. A., and Knight, R. (2007). Global patterns in bacterial diversity. *Proc. Natl. Acad. Sci. U. S. A.* 104, 11436–40. doi:10.1073/pnas.0611525104.

MacGilchrist, G. a., Naveira Garabato, a. C., Tsubouchi, T., Bacon, S., Torres-Valdés, S., and Azetsu-Scott, K. (2014). The Arctic Ocean carbon sink. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 86, 39–55. doi:10.1016/j.dsr.2014.01.002.

Madoui, M.-A., Engelen, S., Cruaud, C., Belser, C., Bertrand, L., Alberti, A., et al. (2015). Genome assembly using Nanopore-guided long and error-free DNA reads. *BMC Genomics* 16, 327. doi:10.1186/s12864-015-1519-z.

Mahé, F., Rognes, T., Quince, C., de Vargas, C., and Dunthorn, M. (2015). Swarm v2: highly-scalable and high-resolution amplicon clustering. *PeerJ* 3, e1420. doi:10.7717/peerj.1420.

Mahlstein, I., and Knutti, R. (2012). September Arctic sea ice predicted to disappear near 2°C global warming above present. *J. Geophys. Res. Atmos.* 117, n/a-n/a. doi:10.1029/2011JD016709.

Malmstrom, R. R., Straza, T. R. A. T., Cottrell, M. T. M., and Kirchman, D. L. D.

(2007). Diversity, abundance, and biomass production of bacterial groups in the western Arctic Ocean. *Aquat. Microb. Ecol.* 47, 45–55. doi:10.3354/ame047045.

Mann, A. J., Hahnke, R. L., Huang, S., Werner, J., Xing, P., Barbeyron, T., et al. (2013). The genome of the alga-associated marine flavobacterium Formosa agariphila KMM 3901T reveals a broad potential for degradation of algal polysaccharides. *Appl. Environ. Microbiol.* 79, 6813–22. doi:10.1128/AEM.01937-13.

Markus, T., Stroeve, J. C., and Miller, J. (2009). Recent changes in Arctic sea ice melt onset, freezeup, and melt season length. *J. Geophys. Res.* 114, C12024. doi:10.1029/2009JC005436.

Marteinsson, V. T., Rúnarsson, Á., Stefánsson, A., Thorsteinsson, T., Jóhannesson, T., Magnússon, S. H., et al. (2013). Microbial communities in the subglacial waters of the Vatnajökull ice cap, Iceland. *ISME J.* 7, 427–437. doi:10.1038/ismej.2012.97.

Martin, J., Tremblay, J. É., Gagnon, J., Tremblay, G., Lapoussière, A., Jose, C., et al. (2010). Prevalence, structure and properties of subsurface chlorophyll maxima in Canadian Arctic waters. *Mar. Ecol. Prog. Ser.* 412, 69–84. doi:10.3354/meps08666.

Maslanik, J. A., Fowler, C., Stroeve, J., Drobot, S., Zwally, J., Yi, D., et al. (2007). A younger, thinner Arctic ice cover: Increased potential for rapid, extensive sea-ice loss. *Geophys. Res. Lett.* 34, L24501. doi:10.1029/2007GL032043.

Mayor, D. J., Thornton, B., Hay, S., Zuur, A. F., Nicol, G. W., McWilliam, J. M., et al. (2012). Resource quality affects carbon cycling in deep-sea sediments. *ISME J.* 6, 1740–8. doi:10.1038/ismej.2012.14.

McLaughlin, F. A., and Carmack, E. C. (2010). Deepening of the nutricline and chlorophyll maximum in the Canada Basin interior, 2003-2009. *Geophys. Res. Lett.* 37, n/a-n/a. doi:10.1029/2010GL045459.

McLaughlin, F. A., Carmack, E. C., Williams, W. J., Zimmermann, S., Shimada, K., and Itoh, M. (2009). Joint effects of boundary currents and thermohaline intrusions on the warming of Atlantic water in the Canada Basin, 1993–2007. *J. Geophys. Res.* 114, C00A12. doi:10.1029/2008JC005001.

McPhee, M. G., Proshutinsky, A., Morison, J. H., Steele, M., and Alkire, M. B. (2009). Rapid change in freshwater content of the Arctic Ocean. *Geophys. Res. Lett.* 36, L10602. doi:10.1029/2009GL037525.

Meier, W., and Stroeve, J. (2018). National Snow and Ice Data Center (NSIDC), September Average Extents, 2002-2017. Available at: http://nsidc.org/cryosphere/sotc/sea_ice.html [Accessed March 16, 2018].

Meiners, K., Brinkmeyer, R., Granskog, M., and Lindfors, A. (2004). Abundance, size distribution and bacterial colonization of exopolymer particles in Antarctic sea ice (Bellingshausen Sea). *Aquat. Microb. Ecol.* 35, 283–296. doi:10.3354/ame035283.

Meiners, K., Krembs, C., and Gradinger, R. (2008). Exopolymer particles: Microbial hotspots of enhanced bacterial activity in Arctic fast ice (Chukchi Sea). *Aquat. Microb. Ecol.* 52, 195–207. doi:10.3354/ame01214.

Melnikov, I. A., and Bondarchuk, L. L. (1987). Ecology of mass accumulations of colonial diatom algae under drifting Arctic ice. *Oceanology* 27, 233–236. doi:10.1594/PANGAEA.756627.

Melnikov, I. A., Kolosova, E. G., Welch, H. E., and Zhitina, L. S. (2002). Sea ice biological communities and nutrient dynamics in the Canada Basin of the Arctic Ocean. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 49, 1623–1649. doi:10.1016/S0967-0637(02)00042-0.

Mestre, M., Ruiz-González, C., Logares, R., Duarte, C. M., Gasol, J. M., and Sala, M. M. (2018). Sinking particles promote vertical connectivity in the ocean microbiome. *Proc. Natl. Acad. Sci. U. S. A.*, 201802470. doi:10.1073/pnas.1802470115.

Metfies, K., von Appen, W.-J., Kilias, E., Nicolaus, A., and Nöthig, E.-M. (2016). Biogeography and photosynthetic biomass of Arctic marine pico-eukaroytes during summer of the record sea ice minimum 2012. *PLoS One* 11, e0148512. doi:10.1371/journal.pone.0148512.

Methé, B. A., Nelson, K. E., Pop, M., Creasy, H. H., Giglio, M. G., Huttenhower, C., et al. (2012). A framework for human microbiome research. *Nature* 486, 215–221. doi:10.1038/nature11209.

Meyer-Reil, L.-A. (1986). Measurement of hydrolytic activity and incorporation of dissolved organic substrates by microorganisms in marine sediments. *Mar. Ecol. - Prog. Ser.* 31, 143–149.

Middelburg, J. J., and Meysman, F. J. R. (2007). Burial at sea. *Science.* 316, 1294–1295. doi:10.1126/science.1144001.

Miettinen, A., Koç, N., and Husum, K. (2013). Appearance of the Pacific diatom Neodenticula seminae in the northern Nordic Seas - An indication of changes in Arctic sea ice and ocean circulation. *Mar. Micropaleontol.* 99, 2–7. doi:10.1016/j.marmicro.2012.06.002.

Mikkelsen, D. M., Rysgaard, S., and Glud, R. N. (2008). Microalgal composition and primary production in Arctic sea ice: A seasonal study from Kobbefjord (Kangerluarsunnguaq), West Greenland. *Mar. Ecol. Prog. Ser.* 368, 65–74. doi:10.3354/meps07627.

Mikucki, J. A., and Priscu, J. C. (2007). Bacterial diversity associated with blood falls, a subglacial outflow from the Taylor Glacier, Antarctica. *Appl. Environ. Microbiol.* 73, 4029–4039. doi:10.1128/AEM.01396-06.

Milici, M., Deng, Z. L., Tomasch, J., Decelle, J., Wos-Oxley, M. L., Wang, H., et al. (2016). Co-occurrence analysis of microbial taxa in the Atlantic ocean reveals high connectivity in the free-living bacterioplankton. *Front. Microbiol.* 7, 1–20. doi:10.3389/fmicb.2016.00649.

Mock, T., and Gradinger, R. (1999). Determination of Arctic ice algal production with a new in situ incubation technicrue. 177, 15–26.

Moran, M. A., Kujawinski, E. B., Stubbins, A., Fatland, R., Aluwihare, L. I., Buchan, A., et al. (2016). Deciphering ocean carbon in a changing world. *Proc. Natl. Acad. Sci.* 113, 3143–3151. doi:10.1073/pnas.1514645113.

Morey, M., Fernández-Marmiesse, A., Castiñeiras, D., Fraga, J. M., Couce, M. L., and Cocho, J. A. (2013). A glimpse into past, present, and future DNA sequencing. *Mol. Genet. Metab.* 110, 3–24. doi:10.1016/J.YMGME.2013.04.024.

Nealson, K. H. (1997). Sediment bacteria: Who's there, what are they doing, and what's new? *Annu. Rev. Earth Planet. Sci.* 25, 403–434. doi:10.1146/annurev.earth.25.1.403.

Nealson, K. H., and Venter, J. C. (2007). Metagenomics and the global ocean survey: what's in it for us and why should we care? *ISME J.* 1, 185–187. doi:10.1038/ismej.2007.43.

Nghiem, S. V., Rigor, I. G., Perovich, D. K., Clemente-Colón, P., Weatherly, J. W., and Neumann, G. (2007). Rapid reduction of Arctic perennial sea ice. *Geophys. Res. Lett.* 34, L19504. doi:10.1029/2007GL031138.

Nöthig, E.-M., Bracher, A., Engel, A., Metfies, K., Niehoff, B., Peeken, I., et al. (2015). Summertime plankton ecology in Fram Strait — a compilation of long- and short-term observations. *Polar Res.* 34, 23349. doi:10.3402/polar.v34.23349.

Notz, D., and Stroeve, J. (2016). Observed Arctic sea-ice loss directly follows anthropogenic CO2 emission. *Science.* 354, 747–750. doi:10.1126/science.aag2345.

Novitsky, J. A. (1990). Evidence for sedimenting particles as the orgin of the microbial community in a coastal marine sediment. *Mar. Ecol. Prog. Ser.* 60, 161–167.

Nürnberg, D., Wollenburg, I., Dethleff, D., Eicken, H., Kassens, H., Letzig, T., et al. (1994). Sediments in Arctic sea ice: Implications for entrainment, transport and release. *Mar. Geol.* 119, 185–214. doi:10.1016/0025-3227(94)90181-3.

Olli, K., Wassmann, P., Reigstad, M., Ratkova, T. N., Arashkevich, E., Pasternak, A., et al. (2007). The fate of production in the central Arctic Ocean – top–down regulation by zooplankton expatriates? *Prog. Oceanogr.* 72, 84–113. doi:10.1016/J.POCEAN.2006.08.002.

Olsen, L. M., Laney, S. R., Duarte, P., Kauko, H. M., Fernández-Méndez, M., Mundy, C. J., et al. (2017). The seeding of ice algal blooms in Arctic pack ice: The multiyear ice seed repository hypothesis. *J. Geophys. Res. Biogeosciences* 122, 1529–1548. doi:10.1002/2016JG003668.

Orcutt, B. N., Sylvan, J. B., Knab, N. J., and Edwards, K. J. (2011). Microbial ecology of the dark ocean above, at, and below the seafloor. *Microbiol. Mol. Biol. Rev.*

75, 361–422. doi:10.1128/MMBR.00039-10.

Overland, J. E., and Wang, M. (2013). When will the summer Arctic be nearly sea ice free? *Geophys. Res. Lett.* 40, 2097–2101.

Overland, J. E., Wang, M., Walsh, J. E., and Stroeve, J. C. (2014). Future Arctic climate changes: Adaptation and mitigation time scales. *Earth's Futur.* 2, 68–74. doi:10.1002/2013EF000162.

Overland, J., Hanna, E., Hanssen-Bauer, I., Kim, S.-J., Walsh, J. E., Wang, M., et al. (2015). "Surface air temperature," in *Arctic Report Card: Update for 2015*.

Pabi, S., van Dijken, G. L., and Arrigo, K. R. (2008). Primary production in the Arctic Ocean, 1998–2006. *J. Geophys. Res.* 113, C08005. doi:10.1029/2007JC004578.

Pace, N. R. (2009). Mapping the tree of life: progress and prospects. *Microbiol. Mol. Biol. Rev.* 73, 565–76. doi:10.1128/MMBR.00033-09.

Pace, N. R. (1997). A molecular view of microbial diversity and the biosphere. *Science.* 276, 734–40.

Pace, N. R., Sapp, J., and Goldenfeld, N. (2012). Phylogeny and beyond: Scientific, historical, and conceptual significance of the first tree of life. *Proc. Natl. Acad. Sci. U. S. A.* 109, 1011–8. doi:10.1073/pnas.1109716109.

Pakulski, J. D., and Benner, R. (1994). Abundance and distribution of carbohydrates in the ocean. *Limnol. Oceanogr.* 39, 930–940. doi:10.4319/lo.1994.39.4.0930.

Paquette, R. G., Bourke, R. H., Newton, J. L., and Perdue, W. F. (1985). The East Greenland Polar Front in autumn. *J. Geophys. Res.* 90, 4866–4882. doi:10.1029/JC090iC03p04866.

Parada, A. E., Needham, D. M., and Fuhrman, J. A. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ. Microbiol.* 18, 1403–1414. doi:10.1111/1462-2920.13023.

Paulsen, M. L., Doré, H., Garczarek, L., Seuthe, L., Müller, O., Sandaa, R.-A., et al. (2016). Synechococcus in the Atlantic gateway to the Arctic Ocean. *Front. Mar. Sci.* 3, 191. doi:10.3389/fmars.2016.00191.

Peralta-Ferriz, C., and Woodgate, R. A. (2015). Seasonal and interannual variability of pan-Arctic surface mixed layer properties from 1979 to 2012 from hydrographic data, and the dominance of stratification for multiyear mixed layer depth shoaling. *Prog. Oceanogr.* 134, 19–53. doi:10.1016/J.POCEAN.2014.12.005.

Perisin, M., Vetter, M., Gilbert, J. A., and Bergelson, J. (2016). 16Stimator: statistical estimation of ribosomal gene copy numbers from draft genome assemblies. *ISME J.* 10, 1020–4. doi:10.1038/ismej.2015.161.

Perovich, D. K., Grenfell, T. C., Light, B., Elder, B. C., Harbeck, J., Polashenski, C., et al. (2009). Transpolar observations of the morphological properties of Arctic sea ice. *J. Geophys. Res.* 114, C00A04. doi:10.1029/2008JC004892.

Perovich, D. K., Grenfell, T. C., Light, B., and Hobbs, P. V. (2002). Seasonal evolution of the albedo of multiyear Arctic sea ice. *J. Geophys. Res.* 107, 8044. doi:10.1029/2000JC000438.

Perovich, D. K., and Polashenski, C. (2012). Albedo evolution of seasonal Arctic sea ice. *Geophys. Res. Lett.* 39, L08501. doi:10.1029/2012GL051432.

Perovich, D. K., and Richter-Menge, J. a (2009). Loss of sea ice in the Arctic. *Ann. Rev. Mar. Sci.* 1, 417–41. doi:10.1146/annurev.marine.010908.163805.

Perovich, D., Meier, W., Tschudi, M., Farrell, S., Hendricks, S., Gerland, S., et al. (2017). Sea Ice. Arctic Report Card: Update for 2017.

Petrenko, D., Pozdnyakov, D., Johannessen, J., Counillon, F., and Sychov, V. (2013). Satellite-derived multi-year trend in primary production in the Arctic Ocean. *Int. J. Remote Sens.* 34, 3903–3937. doi:10.1080/01431161.2012.762698.

Petrich, C., and Eicken, H. (2010). "Growth, Structure and Properties of Sea Ice" in *Sea Ice: Second Edition* (Oxford, UK: Wiley-Blackwell), 23–77. doi:10.1002/9781444317145.ch2.

Pfannkuche, O. (1993). Benthic response to the sedimentation of particulate organic matter at the BIOTRANS station, 47°N, 20°W. *Deep. Res. Part II Top. Stud. Oceanogr.* 40, 135–149. doi:10.1016/0967-0645(93)90010-K.

Pfirman, S. L., Colony, R., Nürnberg, D., Eicken, H., and Rigor, I. (1997). Reconstructing the origin and trajectory of drifting Arctic sea ice. *J. Geophys. Res. Ocean.* 102, 12575–12586. doi:10.1029/96JC03980.

Piepenburg, D. (2006). The Seafloor fauna in a changing Arctic – a review on its past , present and future. 75, 63–76.

Pinhassi, J., Sala, M. M., Havskum, H., Peters, F., Guadayol, Ò., Malits, A., et al. (2004). Changes in bacterioplankton composition under different phytoplankton regimens. *Appl. Environ. Microbiol.* 70, 6753–6766. doi:10.1128/AEM.70.11.6753-6766.2004.

Polyak, L., Alley, R. B., Andrews, J. T., Brigham-Grette, J., Cronin, T. M., Darby, D. A., et al. (2010). History of sea ice in the Arctic. *Quat. Sci. Rev.* 29, 1757–1778. doi:10.1016/J.QUASCIREV.2010.02.010.

Polyakov, I. V., Walsh, J. E., and Kwok, R. (2012). Recent changes of Arctic multiyear sea ice coverage and the likely causes. *Bull. Am. Meteorol. Soc.* 93, 145–151. doi:10.1175/BAMS-D-11-00070.1.

Pomeroy, L., and Wiebe, W. (2001). Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. *Aquat. Microb. Ecol.* 23, 187–204. doi:10.3354/ame023187.

Popova, E. E., Yool, A., Coward, A. C., Dupont, F., Deal, C., Elliott, S., et al. (2012). What controls primary production in the Arctic Ocean? Results from an intercomparison of five general circulation models with biogeochemistry. *J. Geophys. Res.* 117, C00D12. doi:10.1029/2011JC007112.

Poremba, K. (1995). Hydrolytic enzymatic activity in deep-sea sediments. *Fems Microbiol. Ecol.* 16, 213–221. doi:10.1111/j.1574-6941.1995.tb00285.x.

Poremba, K., and Hoppe, H.-G. (1995). Spatial variation of benthic microbial production and hydrolytic enzymatic activity down the continental slope of the Celtic Sea. *Mar. Ecol. Prog. Ser.* 118, 237–245.

Post, E., Bhatt, U. S., Bitz, C. M., Brodie, J. F., Fulton, T. L., Hebblewhite, M., et al. (2013). Ecological Consequences of Sea-Ice Decline. *Science.* 341. doi:10.1126/science.1235225.

Poulin, M., Daugbjerg, N., Gradinger, R., Ilyash, L., Ratkova, T., and von Quillfeldt, C. (2010). The pan-Arctic biodiversity of marine pelagic and sea-ice unicellular eukaryotes: a first-attempt assessment. *Mar. Biodivers.* 41, 13–28. doi:10.1007/s12526-010-0058-8.

Poulin, M., Underwood, G. J. C., and Michel, C. (2014). Sub-ice colonial *Melosira arctica* in Arctic first-year ice. *Diatom Res.* 29, 213–221. doi:10.1080/0269249X.2013.877085.

Price, P. B., Rohde, R. A., and Bay, R. C. (2009). Fluxes of microbes, organic aerosols, dust, sea-salt Na ions, non-sea-salt Ca ions, and methanesulfonate onto Greenland and Antarctic ice. *Biogeosciences* 6, 479–486. doi:10.5194/bg-6-479-2009.

Prokopowich, C. D., Gregory, T. R., and Crease, T. J. (2003). The correlation between rDNA copy number and genome size in eukaryotes. *Genome* 46, 48–50. doi:10.1139/g02-103.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464, 59–65. doi:10.1038/nature08821.

Rabe, B., Karcher, M., Schauer, U., Toole, J. M., Krishfield, R. A., Pisarev, S., et al. (2011). An assessment of Arctic Ocean freshwater content changes from the 1990s to the 2006–2008 period. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 58, 173–185. doi:10.1016/J.DSR.2010.12.002.

Ramirez-Llodra, E., Brandt, A., Danovaro, R., De Mol, B., Escobar, E., German, C. R., et al. (2010). Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. *Biogeosciences* 7, 2851–2899. doi:10.5194/bg-7-2851-2010.

Rampal, P., Weiss, J., and Marsan, D. (2009). Positive trend in the mean speed and deformation rate of Arctic sea ice, 1979–2007. *J. Geophys. Res.* 114, C05013. doi:10.1029/2008JC005066.

Rappé, M. S., and Giovannoni, S. J. (2003). The uncultured microbial majority. *Annu. Rev. Microbiol.* 57, 369–394. doi:10.1146/annurev.micro.57.030502.090759.

Ravenschlag, K., Sahm, K., and Amann, R. (2001). Quantitative molecular analysis of the microbial community in marine Arctic sediments (Svalbard). *Appl. Environ. Microbiol.* 67, 387–395. doi:10.1128/AEM.67.1.387.

Reid, P. C., Johns, D. G., Edwards, M., Starr, M. I., Poulin, M., and Snoeijs, P. (2007). A biological consequence of reducing Arctic ice cover: Arrival of the Pacific diatom Neodenticula seminae in the North Atlantic for the first time in 800000 years. *Glob. Chang. Biol.* 13, 1910–1921. doi:10.1111/j.1365-2486.2007.01413.x.

Reintjes, G., Arnosti, C., Fuchs, B. M., and Amann, R. (2017). An alternative polysaccharide uptake mechanism of marine bacteria. *ISME J.* 11, 1640–1650. doi:10.1038/ismej.2017.26.

Renaud, P. E., Morata, N., Ambrose, W. G., Bowie, J. J., and Chiuchiolo, A. (2007). Carbon cycling by seafloor communities on the eastern Beaufort Sea shelf. *J. Exp. Mar. Bio. Ecol.* 349, 248–260. doi:10.1016/j.jembe.2007.05.021.

Reuter, J. A., Spacek, D. V., and Snyder, M. P. (2015). High-throughput sequencing technologies. *Mol. Cell* 58, 586–597. doi:10.1016/J.MOLCEL.2015.05.004.

Rex, M. A., and Etter, R. J. (2010). *Deep-sea biodiversity: pattern and scale*. Harvard University Press.

Rex, M. A., Etter, R. J., Morris, J. S., Crouse, J., McClain, C. R., Johnson, N. A., et al. (2006). Global bathymetric patterns of standing stock and body size in the deep-sea benthos. *Mar. Ecol. Prog. Ser.* 317, 1–8. doi:10.3354/meps317001.

Riedel, A., Michel, C., Gosselin, M., and LeBlanc, B. (2007). Enrichment of nutrients, exopolymeric substances and microorganisms in newly formed sea ice on the Mackenzie shelf. *Mar. Ecol. Prog. Ser.* 342, 55–67. doi:10.3354/meps342055.

Roach, A. T., Aagaard, K., Pease, C. H., Salo, S. A., Weingartner, T., Pavlov, V., et al. (1995). Direct measurements of transport and water properties through the Bering Strait. *J. Geophys. Res.* 100, 18443. doi:10.1029/95JC01673.

Rösel, A., and Kaleschke, L. (2012). Exceptional melt pond occurrence in the years 2007 and 2011 on the Arctic sea ice revealed from MODIS satellite data. *J. Geophys. Res. Ocean.* 117, C05018. doi:10.1029/2011JC007869.

Rossel, P. E., Bienhold, C., Boetius, A., and Dittmar, T. (2016). Dissolved organic matter in pore water of Arctic Ocean sediments: Environmental influence on molecular composition. *Org. Geochem.* 97, 41–52. doi:10.1016/j.orggeochem.2016.04.003.

Rothrock, D. A., Percival, D. B., and Wensnahan, M. (2008). The decline in arctic sea-ice thickness: Separating the spatial, annual, and interannual variability in a quarter century of submarine data. *J. Geophys. Res.* 113, C05003. doi:10.1029/2007JC004252.

Rowe, G., Sibuet, M., Deming, J., Khripounoff, A., Tietjen, J., Macko, S., et al. (1991). Total sediment biomass and preliminary estimates of organic carbon residence time in deep-sea benthos. *Mar. Ecol. Prog. Ser.* 79, 99–114. doi:10.3354/meps079099.

Rowe, G. T., Boland, G. S., Phoel, W. C., Anderson, R. F., and Biscaye, P. E. (1994). Deep-sea floor respiration as an indication of lateral input of biogenic

detritus from continental margins. *Deep. Res. Part II* 41, 657–668. doi:10.1016/0967-0645(94)90039-6.

Rudels, B. (2012). Arctic Ocean circulation and variability - Advection and external forcing encounter constraints and local processes. *Ocean Sci.* 8, 261–286. doi:10.5194/os-8-261-2012.

Rudels, B., and Friedrich, H. J. (2000). "The transformations of Atlantic water in the Arctic Ocean and their significance for the freshwater budget" in *The Freshwater Budget of the Arctic Ocean* (Dordrecht: Springer Netherlands), 503–532. doi:10.1007/978-94-011-4132-1_21.

Rudels, B., Jones, E. P., Schauer, U., and Eriksson, P. (2004). Atlantic sources of the Arctic Ocean surface and halocline waters. *Polar Res.* 23, 181–208. doi:10.1111/j.1751-8369.2004.tb00007.x.

Rudels, B., Schauer, U., Björk, G., Korhonen, M., Pisarev, S., Rabe, B., et al. (2013). Observations of water masses and circulation with focus on the Eurasian Basin of the Arctic Ocean from the 1990s to the late 2000s. *Ocean Sci* 9, 147–169. doi:10.5194/os-9-147-2013.

Ruff, E. S., Probandt, D., Zinkann, A.-C. C., Iversen, M. H., Klaas, C., Würzberg, L., et al. (2014). Indications for algae-degrading benthic microbial communities in deep-sea sediments along the Antarctic Polar Front. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 108, 6–16. doi:10.1016/j.dsr2.2014.05.011.

Ruff, S. E., Arnds, J., Knittel, K., Amann, R., Wegener, G., Ramette, A., et al. (2013). Microbial communities of deep-sea methane seeps at Hikurangi continental margin (New Zealand). *PLoS One* 8, e72627. doi:10.1371/journal.pone.0072627.

Sahm, K., and Berninger, U. G. (1998). Abundance, vertical distribution, and community structure of benthic prokaryotes from permanently cold marine sediments (Svalbard, Arctic Ocean). *Mar. Ecol. Prog. Ser.* 165, 71–80. doi:10.3354/meps165071.

Sakshaug, E. (2004). "Primary and Secondary Production in the Arctic Seas" in *The Organic Carbon Cycle in the Arctic Ocean* (Berlin, Heidelberg: Springer Berlin Heidelberg), 57–81. doi:10.1007/978-3-642-18912-8_3.

Sala, M. M., Arrieta, J. M., Boras, J. A., Duarte, C. M., and Vaqué, D. (2010). The impact of ice melting on bacterioplankton in the Arctic Ocean. *Polar Biol.* 33, 1683–1694. doi:10.1007/s00300-010-0808-x.

Salazar, G., Cornejo-castillo, F. M., Benítez-Barrios, V., Fraile-nuez, E., Álvarez-salgado, X. A., Duarte, C. M., et al. (2016). Global diversity and biogeography of deep-sea pelagic prokaryotes. *ISME J.* 10, 596–608. doi:10.1038/ismej.2015.137.

Schauer, R., Bienhold, C., Ramette, A., and Harder, J. (2010). Bacterial diversity and biogeography in deep-sea surface sediments of the South Atlantic Ocean. *ISME J.* 4, 159–170. doi:10.1038/ismej.2009.106.

Schewe, T. S. I., Soltwedel, T., and Schewe, I. (1998). Activity and biomass of the small benthic biota under permanent ice-coverage in the central Arctic Ocean. *Polar Biol.* 19, 52–62. doi:10.1007/s003000050215.

Schloss, P. D. (2010). The effects of alignment quality, distance calculation method, sequence filtering, and region on the analysis of 16S rRNA gene-based studies. *PLoS Comput. Biol.* 6, e1000844. doi:10.1371/journal.pcbi.1000844.

Schloss, P. D., Gevers, D., and Westcott, S. L. (2011). Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. *PLoS One* 6, e27310. doi:10.1371/journal.pone.0027310.

Schlüter, M., Sauter, E. J., Schäfer, A., and Ritzrau, W. (2000). Spatial budget of organic carbon flux to the seafloor of the northern North Atlantic (60°N - 80°N). *Global Biogeochem. Cycles* 14, 329–340. doi:10.1029/1999GB900043.

Schmidt, F., Elvert, M., Koch, B. P., Witt, M., and Hinrichs, K.-U. (2009). Molecular characterization of dissolved organic matter in pore water of continental shelf sediments. *Geochim. Cosmochim. Acta* 73, 3337–3358. doi:10.1016/J.GCA.2009.03.008.

Schröder, D., Feltham, D. L., Flocco, D., and Tsamados, M. (2014). September Arctic sea-ice minimum predicted by spring melt-pond fraction. *Nat. Clim. Chang.* 4, 353–357. doi:10.1038/nclimate2203.

Screen, J. A., and Simmonds, I. (2010). The central role of diminishing sea ice in recent Arctic temperature amplification. *Nature* 464, 1334–1337. doi:10.1038/nature09051.

Serreze, M. C., and Barry, R. G. (2011). Processes and impacts of Arctic amplification: A research synthesis. *Glob. Planet. Change* 77, 85–96. doi:10.1016/J.GLOPLACHA.2011.03.004.

Serreze, M. C., and Rigor, I. (2007). Perspectives on the Arctic's Shrinking Sea-Ice Cover. *Science.* 315, 120–125. doi:10.1002/9780470750636.ch23.

Sharpton, T. J. (2014). An introduction to the analysis of shotgun metagenomic data. *Front. Plant Sci.* 5, 209. doi:10.3389/fpls.2014.00209.

Sherr, B. F., and Sherr, E. B. (2003). Community respiration/production and bacterial activity in the upper water column of the central Arctic Ocean. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 50, 529–542. doi:10.1016/S0967-0637(03)00030-X.

Sherr, E. B., Sherr, B. F., Wheeler, P. A., and Thompson, K. (2003). Temporal and spatial variation in stocks of autotrophic and heterotrophic microbes in the upper water column of the central Arctic Ocean. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 50, 557–571. doi:10.1016/S0967-0637(03)00031-1.

Siegel, D. A., Buesseler, K. O., Behrenfeld, M. J., Benitez-Nelson, C. R., Boss, E., Brzezinski, M. A., et al. (2016). Prediction of the export and fate of global ocean net primary production: The EXPORTS Science Plan. *Front. Mar. Sci.* 3, 22. doi:10.3389/fmars.2016.00022.

Siezen, R. J., and Wilson, G. (2009). Genomics of deep-sea and sub-seafloor

microbes. *Microb. Biotechnol.* 2, 157–163. doi:10.1111/j.1751-7915.2009.00092.x.

Slagstad, D., Ellingsen, I. H., and Wassmann, P. (2011). Evaluating primary and secondary production in an Arctic Ocean void of summer sea ice: An experimental simulation approach. *Prog. Oceanogr.* 90, 117–131. doi:10.1016/J.POCEAN.2011.02.009.

Slagstad, D., Wassmann, P. F. J., and Ellingsen, I. (2015). Physical constrains and productivity in the future Arctic Ocean. *Front. Mar. Sci.* 2, 85. doi:10.3389/fmars.2015.00085.

Smedsrud, L. H., Halvorsen, M. H., Stroeve, J. C., Zhang, R., and Kloster, K. (2017). Fram Strait sea ice export variability and September Arctic sea ice extent over the last 80 years. *Cryosph.* 11, 65–79. doi:10.5194/tc-11-65-2017.

Smetacek, V. S. (1985). Role of sinking in diatom life-history cycles: ecological, evolutionary and geological significance. *Mar. Biol.* 84, 239–251. doi:10.1007/BF00392493.

Smith, C. R., Berelson, W., Demaster, D. J., Dobbs, F. C., Hammond, D., Hoover, D. J., et al. (1998). Latitudinal variations in benthic processes in the abyssal equatorial Pacific: control by biogenic particle flux. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 44, 2295–2317. doi:10.1016/S0967-0645(97)00022-2.

Smith, C. R., De Leo, F. C., Bernardino, A. F., Sweetman, A. K., and Arbizu, P. M. (2008). Abyssal food limitation, ecosystem structure and climate change. *Trends Ecol. Evol.* 23, 518–528. doi:10.1016/j.tree.2008.05.002.

Smith, D. C., Simon, M., Alldredge, A. L., and Azam, F. (1992). Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature* 359, 139–142. doi:10.1038/359139a0.

Smith, K. L., Ruhl, H. A., Bett, B. J., Billett, D. S. M., Lampitt, R. S., and Kaufmann, R. S. (2009). Climate, carbon cycling, and deep-ocean ecosystems. *Proc. Natl. Acad. Sci. U. S. A.* 106, 19211–8. doi:10.1073/pnas.0908322106.

Snape, T. J., and Forster, P. M. (2014). Decline of Arctic sea ice: Evaluation and weighting of CMIP5 projections. *J. Geophys. Res. Atmos.* 119, 546–554. doi:10.1002/2013JD020593.

Snelgrove, P. V. R., Soetaert, K., Solan, M., Thrush, S., Wei, C.-L., Danovaro, R., et al. (2017). Global carbon cycling on a heterogeneous seafloor. *Trends Ecol. Evol.* 33, 96–105. doi:10.1016/j.tree.2017.11.004.

Soltwedel, T. (2000). Metazoan meiobenthos along continental margins : a review. 46, 59–84.

Soltwedel, T., Bauerfeind, E., Bergmann, M., Bracher, A., Budaeva, N., Busch, K., et al. (2016). Natural variability or anthropogenically-induced variation? Insights from 15 years of multidisciplinary observations at the arctic marine LTER site HAUSGARTEN. *Ecol. Indic.* 65, 89–102. doi:10.1016/j.ecolind.2015.10.001.

Soltwedel, T., Bauerfeind, E., Bergmann, M., Budaeva, N., Hoste, E., Jaeckisch, N.,

et al. (2005). HAUSGARTEN: Multidisciplinary investigations at a deep-sea, long-term observatory in the Arctic Ocean. *Oceanography* 18, 46–61. doi:10.5670/oceanog.2005.24.

Soltwedel, T., Jaeckisch, N., Ritter, N., Hasemann, C., Bergmann, M., and Klages, M. (2009). Bathymetric patterns of megafaunal assemblages from the arctic deep-sea observatory HAUSGARTEN. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 56, 1856–1872. doi:10.1016/j.dsr.2009.05.012.

Sonnenburg, E. D., Zheng, H., Joglekar, P., Higginbottom, S. K., Firbank, S. J., Bolam, D. N., et al. (2010). Specificity of polysaccharide use in intestinal bacteroides species determines diet-induced microbiota alterations. *Cell* 141, 1241–1252. doi:10.1016/j.cell.2010.05.005.

Spielhagen, R. F., Werner, K., Sørensen, S. A., Zamelczyk, K., Kandiano, E., Budeus, G., et al. (2011). Enhanced modern heat transfer to the Arctic by warm Atlantic Water. *Science.* 331, 450–3. doi:10.1126/science.1197397.

Staley, J. T., and Konopka, A. (1985). Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annu. Rev. Microbiol.* 39, 321–346. doi:10.1146/annurev.mi.39.100185.001541.

Steele, M., and Dickinson, S. (2016). The phenology of Arctic Ocean surface warming. *J. Geophys. Res. Ocean.* 121, 6847–6861. doi:10.1002/2016JC012089.

Stewart, F. J., and Fritsen, C. H. (2004). Bacteria-algae relationships in Antarctic sea ice. *Antarct. Sci.* 16, 143–156. doi:10.1017/S0954102004001889.

Stoecker, D. K., Gustafson, D. E., Black, M. M. D., and Baier, C. T. (1998). Population dynamics of microalgae in the upper land-fast sea ice at a snow-free location. *J. Phycol.* 34, 60–69. doi:10.1046/j.1529-8817.1998.340060.x.

Stroeve, J. C., Kattsov, V., Barrett, A., Serreze, M., Pavlova, T., Holland, M., et al. (2012a). Trends in Arctic sea ice extent from CMIP5, CMIP3 and observations. *Geophys. Res. Lett.* 39, n/a-n/a. doi:10.1029/2012GL052676.

Stroeve, J. C., Markus, T., Boisvert, L., Miller, J., and Barrett, A. (2014). Changes in Arctic melt season and implications for sea ice loss. *Geophys. Res. Lett.* 41, 1216–1225. doi:10.1002/2013GL058951.

Stroeve, J. C., Serreze, M. C., Holland, M. M., Kay, J. E., Malanik, J., and Barrett, A. P. (2012b). The Arctic's rapidly shrinking sea ice cover: a research synthesis. *Clim. Change* 110, 1005–1027. doi:10.1007/s10584-011-0101-1.

Stroeve, J., Holland, M. M., Meier, W., Scambos, T., and Serreze, M. (2007). Arctic sea ice decline: Faster than forecast. *Geophys. Res. Lett.* 34, n/a-n/a. doi:10.1029/2007GL029703.

Sunagawa, S., Coelho, L. P., Chaffron, S., Kultima, J. R., Labadie, K., Salazar, G., et al. (2015). Structure and function of the global ocean microbiome. *Science.* 348. doi:10.1126/science.1261359.

Suslova, M. Y., Lipko, I. a., Mamaeva, E. V., and Parfenova, V. V. (2012). Diversity

of cultivable bacteria isolated from the water column and bottom sediments of the Kara Sea shelf. *Microbiology* 81, 484–491. doi:10.1134/S0026261712040157.

Talley, L. D., Pickard, G. L., Emery, W. J., Swift, J. H., Talley, L. D., Pickard, G. L., et al. (2011). Arctic Ocean and Nordic Seas. *Descr. Phys. Oceanogr.*, 401–436. doi:10.1016/B978-0-7506-4552-2.10012-5.

Tamelander, T., Reigstad, M., Hop, H., and Ratkova, T. (2009). Ice algal assemblages and vertical export of organic matter from sea ice in the Barents Sea and Nansen Basin (Arctic Ocean). *Polar Biol.* 32, 1261–1273. doi:10.1007/s00300-009-0622-5.

Tamelander, T., Reigstad, M., Olli, K., Slagstad, D., and Wassmann, P. (2013). New production regulates export stoichiometry in the ocean. *PLoS One* 8, e54027. doi:10.1371/journal.pone.0054027.

Tang, K., Jiao, N., Liu, K., Zhang, Y., and Li, S. (2012). Distribution and functions of TonB-dependent transporters in marine bacteria and environments: implications for dissolved organic matter utilization. *PLoS One* 7, e41204. doi:10.1371/journal.pone.0041204.

Taylor, L. E., Henrissat, B., Coutinho, P. M., Ekborg, N. A., Hutcheson, S. W., and Weiner, R. M. (2006). Complete cellulase system in the marine bacterium Saccharophagus degradans strain 2-40T. *J. Bacteriol.* 188, 3849–61. doi:10.1128/JB.01348-05.

Taylor, P. C., Cai, M., Hu, A., Meehl, J., Washington, W., Zhang, G. J., et al. (2013). A decomposition of feedback contributions to polar warming amplification. *J. Clim.* 26, 7023–7043. doi:10.1175/JCLI-D-12-00696.1.

Teeling, H., Fuchs, B. M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C. M., et al. (2012). Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science.* 336, 608–611. doi:10.1126/science.1218344.

Teeling, H., Fuchs, B. M., Bennke, C. M., Krüger, K., Chafee, M., Kappelmann, L., et al. (2016). Recurring patterns in bacterioplankton dynamics during coastal spring algae blooms. *Elife* 5, e11888. doi:10.7554/eLife.11888.

Teske, a, Durbin, a, Ziervogel, K., Cox, C., and Arnosti, C. (2011). Microbial community composition and function in permanently cold seawater and sediments from an arctic fjord of svalbard. *Appl. Environ. Microbiol.* 77, 2008–18. doi:10.1128/AEM.01507-10.

Thomas, D. N. (David N., and Dieckmann, G. (2010). *Sea ice*. Blackwell Publishing Ltd.

Thomas, D. N., and Dieckmann, G. S. (2002). Antarctic Sea ice - a habitat for extremophiles. *Science.* 295, 641–4. doi:10.1126/science.1063391.

Thomas, D. N., Kattner, G., Engbrodt, R., Giannelli, V., Kennedy, H., Haas, C., et al. (2001). Dissolved organic matter in Antarctic sea ice. *Ann. Glaciol.* 33, 297–303.

doi:10.3189/172756401781818338.

Thomas, F., Hehemann, J.-H., Rebuffet, E., Czjzek, M., and Michel, G. (2011). Environmental and gut bacteroidetes: the food connection. *Front. Microbiol.* 2, 93. doi:10.3389/fmicb.2011.00093.

Tian, F., Yu, Y., Chen, B., Li, H., Yao, Y.-F., and Guo, X.-K. (2008). Bacterial, archaeal and eukaryotic diversity in Arctic sediment as revealed by 16S rRNA and 18S rRNA gene clone libraries analysis. *Polar Biol.* 32, 93–103. doi:10.1007/s00300-008-0509-x.

Torstensson, A., Dinasquet, J., Chierici, M., Fransson, A., Riemann, L., and Wulff, A. (2015). Physicochemical control of bacterial and protist community composition and diversity in Antarctic sea ice. *Environ. Microbiol.* doi:10.1111/1462-2920.12865.

Tremblay, J.-É., Bélanger, S., Barber, D. G., Asplin, M., Martin, J., Darnis, G., et al. (2011). Climate forcing multiplies biological productivity in the coastal Arctic Ocean. *Geophys. Res. Lett.* 38, n/a-n/a. doi:10.1029/2011GL048825.

Tremblay, J.-É. É., Anderson, L. G., Matrai, P., Coupel, P., Bélanger, S., Michel, C., et al. (2015). Global and regional drivers of nutrient supply, primary production and CO2 drawdown in the changing Arctic Ocean. *Prog. Oceanogr.* 139, 171–196. doi:10.1016/j.pocean.2015.08.009.

Tremblay, J.-É., and Gagnon, J. (2009). The effects of irradiance and nutrient supply on the productivity of Arctic waters: a perspective on climate change. *Influ. Clim. Chang. Chang. Arct. Sub-Arctic Cond.*, 73–93. doi:10.1007/978-1-4020-9460-6_7.

Tringe, S. G., and Hugenholtz, P. (2008). A renaissance for the pioneering 16S rRNA gene. *Curr. Opin. Microbiol.* 11, 442–446. doi:10.1016/J.MIB.2008.09.011.

Tringe, S. G., Mering, C. von, Kobayashi, A., Salamov, A. A., Chen, K., Chang, H. W., et al. (2005). Comparative metagenomics of microbial communities. *Science.* 308, 554–557. doi:10.1126/science.1107851.

Tschudi, M., Stroeve, J., and Stewart, J. (2016). Relating the age of Arctic sea ice to its thickness, as measured during NASA's ICESat and IceBridge campaigns. *Remote Sens.* 8, 457. doi:10.3390/rs8060457.

Turley, C. M., and Dixon, J. L. (2002). Bacterial numbers and growth in surficial deep-sea sediments and phytodetritus in the NE Atlantic : Relationships with particulate organic carbon and total nitrogen. 49, 815–826.

Turley, C. M., and Lochte, K. (1990). Microbial response to the input of fresh detritus to the deep-sea bed. *Glob. Planet. Change* 3, 3–23. doi:10.1016/0921-8181(90)90052-E.

Turner, J. T. (2015). Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. *Prog. Oceanogr.* 130, 205–248.

Tyson, G. W., Chapman, J., Hugenholtz, P., Allen, E. E., Ram, R. J., Richardson, P. M., et al. (2004). Community structure and metabolism through reconstruction of

microbial genomes from the environment. *Nature* 428, 37–43. doi:10.1038/nature02340.

Unfried, F., Becker, S., Robb, C. S., Hehemann, J.-H., Markert, S., Heiden, S. E., et al. (2018). Adaptive mechanisms that provide competitive advantages to marine bacteroidetes during microalgal blooms. *ISME J.*, 1. doi:10.1038/s41396-018-0243-5.

van Leeuwe, M. A., Tedesco, L., Arrigo, K. R., Assmy, P., Campbell, K., Meiners, K. M., et al. (2018). Microalgal community structure and primary production in Arctic and Antarctic sea ice: A synthesis. *Elem Sci Anth* 6. doi:10.1525/elementa.267.

Vancoppenolle, M., Meiners, K. M., Michel, C., Bopp, L., Brabant, F., Carnat, G., et al. (2013). Role of sea ice in global biogeochemical cycles: Emerging views and challenges. *Quat. Sci. Rev.* 79, 207–230.

Vaquer-Sunyer, R., Duarte, C. M., Santiago, R., Wassmann, P., and Reigstad, M. (2010). Experimental evaluation of planktonic respiration response to warming in the European Arctic Sector. *Polar Biol.* 33, 1661–1671. doi:10.1007/s00300-010-0788-x.

Vaughan, D. ., Comiso, J. C., Allison, I., Carrasco, J., Kaser, G., Kwok, R., et al. (2013). Observations: Cryosphere. eds. T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, et al. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Venter, J. C., Remington, K., Heidelberg, J. F., Halpern, A. L., Rusch, D., Eisen, J. A., et al. (2004). Environmental genome shotgun sequencing of the Sargasso Sea. *Science.* 304.

Verdugo, P., Alldredge, A. L., Azam, F., Kirchman, D. L., Passow, U., and Santschi, P. H. (2004). The oceanic gel phase: a bridge in the DOM–POM continuum. *Mar. Chem.* 92, 67–85. doi:10.1016/J.MARCHEM.2004.06.017.

Vernet, M., Richardson, T. L., Metfies, K., Nöthig, E.-M., and Peeken, I. (2017). Models of plankton community changes during a warm water anomaly in Arctic waters show altered trophic pathways with minimal changes in carbon export. *Front. Mar. Sci.* 4, 1–19. doi:10.3389/fmars.2017.00160.

Vincent, W. F. (2010). Microbial ecosystem responses to rapid climate change in the Arctic. *ISME J.* 4, 1087–1090. doi:10.1038/ismej.2010.108.

Vincent, W. F., Callaghan, T. V., Dahl-Jensen, D., Johansson, M., Kovacs, K. M., Michel, C., et al. (2011). Ecological implications of changes in the Arctic cryosphere. *Ambio* 40, 87–99. doi:10.1007/s13280-011-0218-5.

Wakeham, S. G., Hedges, J. I., Lee, C., Peterson, M. L., and Hernes, P. J. (1997a). Compositions and transport of lipid biomarkers through the water column and surficial sediments of the equatorial Pacific Ocean. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 44, 2131–2162. doi:10.1016/S0967-0645(97)00035-0.

Wakeham, S. G., Lee, C., Hedges, J. I., Hernes, P. J., and Peterson, M. J. (1997b).

Molecular indicators of diagenetic status in marine organic matter. *Geochim. Cosmochim. Acta* 61, 5363–5369. doi:10.1016/S0016-7037(97)00312-8.

Walczowski, W., Beszczynska-Möller, A., Wieczorek, P., Merchel, M., and Grynczel, A. (2017). Oceanographic observations in the Nordic Sea and Fram Strait in 2016 under the IO PAN long-term monitoring program AREX. *Oceanologia* 59, 187–194. doi:10.1016/J.OCEANO.2016.12.003.

Walsh, E. A., Kirkpatrick, J. B., Rutherford, S. D., Smith, D. C., Sogin, M., and D'Hondt, S. (2016). Bacterial diversity and community composition from seasurface to subseafloor. *ISME J.* 10, 979–89. doi:10.1038/ismej.2015.175.

Wassmann, P. (2011). Arctic marine ecosystems in an era of rapid climate change. *Prog. Oceanogr.* 90, 1–17. doi:10.1016/j.pocean.2011.02.002.

Wassmann, P. (1997). Retention versus export food chains: Processes controlling sinking loss from marine pelagic systems. in *Hydrobiologia*, 29–57. doi:10.1023/A:1003113403096.

Wassmann, P., Bauerfeind, E., Fortier, M., Fukuchi, M., Hargrave, B., Moran, B., et al. (2004). Particulate Organic Carbon Flux to the Arctic Ocean Sea Floor. *Org. Carbon Cycle Arct. Ocean*, 101–138. doi:10.1007/978-3-642-18912-8_5.

Wassmann, P., Duarte, C. M., Agustí, S., and Sejr, M. K. (2011). Footprints of climate change in the Arctic marine ecosystem. *Glob. Chang. Biol.* 17, 1235–1249. doi:10.1111/j.1365-2486.2010.02311.x.

Wassmann, P., and Reigstad, M. (2011). Future Arctic Ocean seasonal ice zones and implications for pelagic-benthic coupling. *Oceanography* 24, 220–231. doi:10.5670/oceanog.2011.74.

Wassmann, P., Reigstad, M., Haug, T., Rudels, B., Carroll, M. L., Hop, H., et al. (2006). Food webs and carbon flux in the Barents Sea. *Prog. Oceanogr.* 71, 232–287. doi:10.1016/j.pocean.2006.10.003.

Wassmann, P., Slagstad, D., and Ellingsen, I. (2010). Primary production and climatic variability in the European sector of the Arctic Ocean prior to 2007: preliminary results. *Polar Biol.* 33, 1641–1650. doi:10.1007/s00300-010-0839-3.

Weeks, W. F., and Ackley, S. F. (1986). "The Growth, Structure, and Properties of Sea Ice" in *The Geophysics of Sea Ice* (Boston, MA: Springer US), 9–164. doi:10.1007/978-1-4899-5352-0_2.

Wegner, C., Wittbrodt, K., Hölemann, J. A., Janout, M. A., Krumpen, T., Selyuzhenok, V., et al. (2017). Sediment entrainment into sea ice and transport in the Transpolar Drift: a case study from the Laptev Sea in winter 2011/2012. *Cont. Shelf Res.* 141, 1–10. doi:10.1016/j.csr.2017.04.010.

Wei, C. L., Rowe, G. T., Fain Hubbard, G., Scheltema, A. H., Wilson, G. D. F., Petrescu, I., et al. (2010). Bathymetric zonation of deep-sea macrofauna in relation to export of surface phytoplankton production. *Mar. Ecol. Prog. Ser.* 399, 1–14. doi:10.3354/meps08388.

Weiner, R. M., Taylor, L. E., Henrissat, B., Hauser, L., Land, M., Coutinho, P. M., et

al. (2008). Complete genome sequence of the complex carbohydrate-degrading marine bacterium, Saccharophagus degradans strain 2-40T. *PLoS Genet.* 4, e1000087. doi:10.1371/journal.pgen.1000087.

Weiss, M. S., Abele, U., Weckesser, J., Welte, W., Schiltz, E., and Schulz, G. E. (1991). Molecular architecture and electrostatic properties of a bacterial porin. *Science.* 254, 1627–1630. doi:10.1126/science.1721242.

Wenzhöfer, F., and Glud, R. N. (2002). Benthic carbon mineralization in the Atlantic: a synthesis based on in situ data from the last decade. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 49, 1255–1279. doi:10.1016/S0967-0637(02)00025-0.

Wenzhöfer, F., Oguri, K., Middelboe, M., Turnewitsch, R., Toyofuku, T., Kitazato, H., et al. (2016). Benthic carbon mineralization in hadal trenches: Assessment by in situ O2 microprofile measurements. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 116, 276–286. doi:10.1016/J.DSR.2016.08.013.

Werner, I. (2000). Faecal pellet production by Arctic under-ice amphipods – transfer of organic matter through the ice/water interface. *Hydrobiologia* 426, 89–96. doi:10.1023/A:1003984327103.

Whitman, W. B., Coleman, D. C., and Wiebe, W. J. (1998). Prokaryotes: the unseen majority. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6578–83.

Wilson, B., Müller, O., Nordmann, E.-L., Seuthe, L., Bratbak, G., and Øvreås, L. (2017). Changes in marine prokaryote composition with season and depth over an Arctic polar year. *Front. Mar. Sci.* 4, 95. doi:10.3389/fmars.2017.00095.

Winton, M., and Winton, M. (2011). Do climate models underestimate the sensitivity of northern hemisphere sea ice cover? *J. Clim.* 24, 3924–3934. doi:10.1175/2011JCLI4146.1.

Woese, C. R., and Fox, G. E. (1977). Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proc. Natl. Acad. Sci. U. S. A.* 74, 5088–90.

Woese, C. R., Kandler, O., and Wheelis, M. L. (1990). Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc. Natl. Acad. Sci. U. S. A.* 87, 4576–9.

Wollenburg, J. E., Katlein, C., Nehrke, G., Nöthig, E.-M., Matthiessen, J., Wolf-Gladrow, D. A., et al. (2018). Ballasting by cryogenic gypsum enhances carbon export in a Phaeocystis under-ice bloom. *Sci. Rep.* 8, 7703. doi:10.1038/s41598-018-26016-0.

Wood, K. R., Overland, J. E., Salo, S. A., Bond, N. A., Williams, W. J., and Dong, X. (2013). Is there a “new normal” climate in the Beaufort Sea? *Polar Res.* 32, 19552. doi:10.3402/polar.v32i0.19552.

Woolley, S. N. C., Tittensor, D. P., Dunstan, P. K., Guillera-Arroita, G., Lahoz-Monfort, J. J., Wintle, B. A., et al. (2016). Deep-sea diversity patterns are shaped by energy availability. *Nature* 533, 393–396. doi:10.1038/nature17937.

Wylie, K. M., Truty, R. M., Sharpton, T. J., Mihindukulasuriya, K. A., Zhou, Y., Gao, H., et al. (2012). Novel bacterial taxa in the human microbiome. *PLoS One* 7,

e35294. doi:10.1371/journal.pone.0035294.

Xing, P., Hahnke, R. L., Unfried, F., Markert, S., Huang, S., Barbeyron, T., et al. (2014). Niches of two polysaccharide-degrading Polaribacter isolates from the North Sea during a spring diatom bloom. *ISME J.* doi:10.1038/ismej.2014.225.

Yilmaz, P., Gilbert, J. A., Knight, R., Amaral-Zettler, L., Karsch-Mizrachi, I., Cochrane, G., et al. (2011a). The genomic standards consortium: bringing standards to life for microbial ecology. *ISME J.* 5, 1565–1567. doi:10.1038/ismej.2011.39.

Yilmaz, P., Kottmann, R., Field, D., Knight, R., Cole, J. R., Amaral-Zettler, L., et al. (2011b). Minimum information about a marker gene sequence (MIMARKS) and minimum information about any (x) sequence (MIxS) specifications. *Nat. Biotechnol.* 29, 415–420. doi:10.1038/nbt.1823.

Zhu, F., Massana, R., Not, F., Marie, D., and Vaulot, D. (2005). Mapping of picoeucaryotes in marine ecosystems with quantitative PCR of the 18S rRNA gene. *FEMS Microbiol. Ecol.* 52, 79–92. doi:10.1016/j.femsec.2004.10.006.

Zinger, L., Amaral-Zettler, L. A., Fuhrman, J. A., Horner-Devine, M. C., Huse, S. M., Welch, D. B. M., et al. (2011). Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. *PLoS One* 6, e24570. doi:10.1371/journal.pone.0024570.

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APPENDIX

Appendix I Additional contributions

Expanding the World of Marine Bacterial and Archaeal Clades

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Abstract

Determining which microbial taxa are out there, where they live, and what they are doing is a driving approach in marine microbial ecology. The importance of these questions is underlined by concerted, large-scale, and global ocean sampling initiatives, for example the International Census of Marine Microbes, Ocean Sampling Day, or Tara Oceans. Given decades of effort, we know that the large majority of marine Bacteria and Archaea belong to about a dozen phyla. In addition to the classically culturable Bacteria and Archaea, at least 50 “clades,” at different taxonomic depths, exist. These account for the majority of marine microbial diversity, but there is still an underexplored and less abundant portion remaining. We refer to these hitherto unrecognized clades as *unknown*, as their boundaries, names, and classifications are not available. In this work, we were able to characterize up to 92 of these *unknown* clades found within the bacterial and archaeal phylogenetic diversity currently reported for marine water column environments. We mined the SILVA 16S rRNA gene datasets for sequences originating from the marine water column. Instead of the usual subjective taxa delineation and nomenclature methods, we applied the candidate taxonomic unit (CTU) circumscription system, along with a standardized nomenclature to the sequences in newly constructed phylogenetic

trees. With this new phylogenetic and taxonomic framework, we performed an analysis of ICoMM rRNA gene amplicon datasets to gain insights into the global distribution of the new marine clades, their ecology, biogeography, and interaction with oceanographic variables. Most of the new clades we identified were interspersed by known taxa with cultivated members, whose genome sequences are available. This result encouraged us to perform metabolic predictions for the novel marine clades using the PICRUSt approach. Our work also provides an update on the taxonomy of several phyla and widely known marine clades as our CTU approach breaks down these randomly lumped clades into smaller objectively calculated subgroups. Finally, all taxa were classified and named following standards compatible with the Bacteriological Code rules, enhancing their digitization, and comparability with future microbial ecological and taxonomy studies.

Keywords: marine, bacterioplankton, bacterial phylogeny, bacterial taxonomy, ecology, rare taxa

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Diazotroph Diversity in the Sea Ice, Melt Ponds, and Surface Waters of the Eurasian Basin of the Central Arctic Ocean

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Abstract

The Eurasian basin of the Central Arctic Ocean is nitrogen limited, but little is known about the presence and role of nitrogen-fixing bacteria. Recent studies have indicated the occurrence of diazotrophs in Arctic coastal waters potentially of riverine origin. Here, we investigated the presence of diazotrophs in ice and surface waters of the Central Arctic Ocean in the summer of 2012. We identified diverse communities of putative diazotrophs through targeted analysis of the nifH gene, which encodes the iron protein of the nitrogenase enzyme. We amplified 529 nifH sequences from 26 samples of Arctic melt ponds, sea ice and surface waters. These sequences resolved into 43 clusters at 92% amino acid sequence identity, most of which were non-cyanobacterial phylotypes from sea ice and water samples. One cyanobacterial phylotype related to *Nodularia* sp. was retrieved from sea ice, suggesting that this important functional group is rare in the Central Arctic Ocean. The diazotrophic community in sea-ice environments appear distinct from other cold-adapted diazotrophic communities, such as those present in the coastal Canadian Arctic, the Arctic tundra and glacial Antarctic lakes. Molecular fingerprinting of nifH and the intergenic spacer region of the rRNA operon revealed differences between the communities from river-influenced Laptev Sea waters and those from ice-related

environments pointing toward a marine origin for sea-ice diazotrophs. Our results provide the first record of diazotrophs in the Central Arctic and suggest that microbial nitrogen fixation may occur north of 77°N. To assess the significance of nitrogen fixation for the nitrogen budget of the Arctic Ocean and to identify the active nitrogen fixers, further biogeochemical and molecular biological studies are needed.

Keywords: nitrogen fixation, *nifH*, Arctic, non-cyanobacterial diazotrophs, sea ice, bacterial diversity

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Diversity and metabolism of the JTB255 clade (*Gammaproteobacteria*), a global member of deep-sea sediment communities

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Abstract

Surveys of 16S rRNA gene sequences derived from marine sediments have indicated that a widely distributed group of *Gammaproteobacteria*, named 'JTB255 marine benthic group', accounts for 1-22% of the retrieved sequences. Despite their ubiquity in seafloor communities, little is known about their distribution and specific ecological niches in the deep sea, which constitutes the largest biome globally. Here, we characterized phylo-environmental distribution patterns, abundance and metabolic potential of JTB255 bacteria with a focus on representatives from the deep sea. From a phylogenetic analysis of publicly available 16S rRNA gene sequences (≥1400 bp, n=994) we identified specific JTB255 lineages with greater prevalence in the deep sea than in coastal environments, a pattern corroborated by the distribution of 16S oligotypes recovered from 34 globally distributed sediment samples. Cell

counts revealed that JTB255 bacteria accounted for 5 ± 2% of all microbial cells in deep-sea surface sediments at 23 globally distributed sites. Comparative analyses of a genome, metagenome bins and single-cell genomes suggested that the investigated clades of JTB255 bacteria are likely to grow on proteinaceous and lipidic matter, potentially derived from detrital cell membranes, cell walls and other biological sources of proteins and lipids in marine sediments.

Keywords:
JTB255 Marine Benthic Group, *Woeseia oceani* XK5, marine sediments, deep sea, benthic bacteria

Appendix Figures

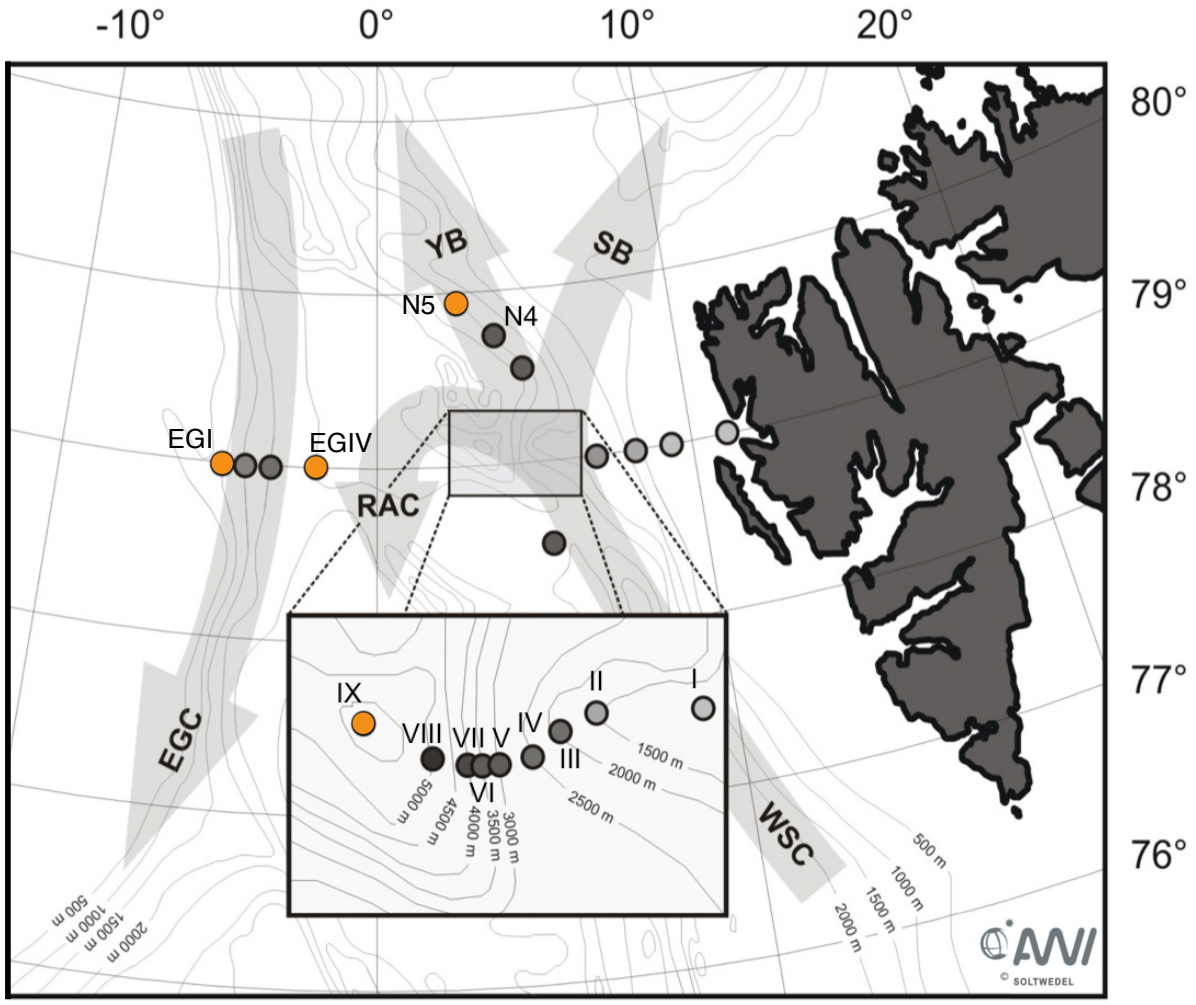

Figure A1 l Sampling sites from the PS99.2 Polarstern cruise in June-July 2016. Vertical sampling profiles were collected from four stations with sea-ice cover. Sampling included sea ice, upper water column, deep water column, and deep-sea sediment, together with sinking particles from within and below the chlorophyll max, as well as aggregates of the sea-ice algae *Melosira arctica*.

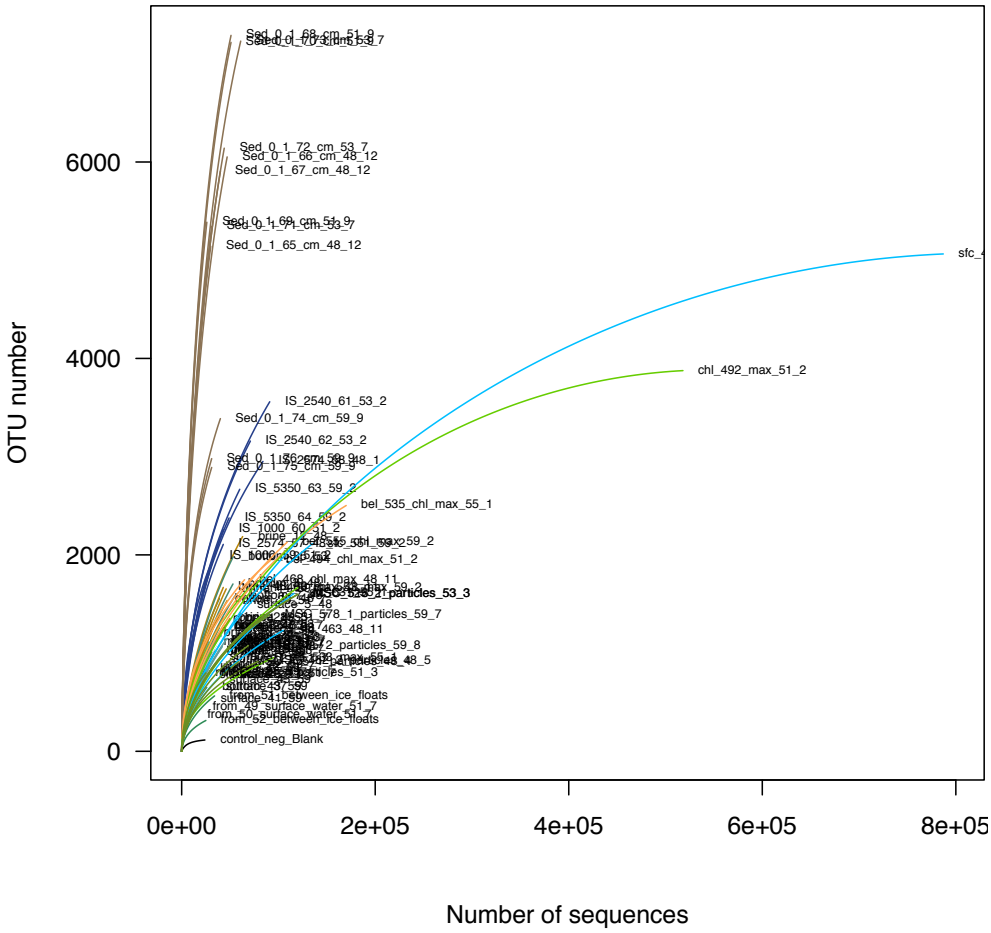

Figure A2 – Bacterial OTU rarefaction curves displaying the effect of sequencing depth on the recovery of the number of observed OTUs. Data represents *Swarm* OTUs from 16S rRNA gene amplicon sequencing (Mahé et al., 2015). For the majority of samples, the steep slope indicates that sequencing depth was not sufficient to recover the full bacterial diversity. Exceptions are two samples, one from surface seawater (light blue), the other from the chlorophyll a maximum depth (light green), for which the curves are reaching a plateau.

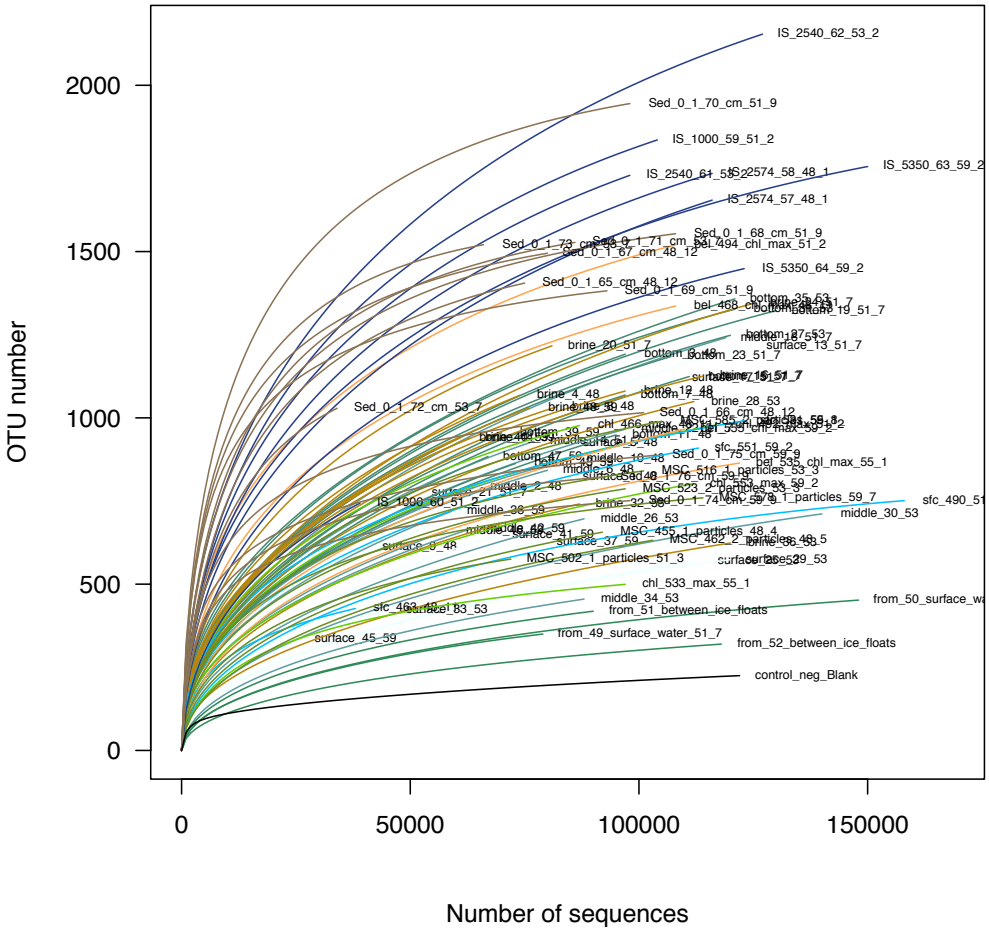

Figure A3 - Eukaryotic OTU rarefaction curves displaying the effect of sequencing depth on the recovery of the number of observed OTUs. Data represents *Swarm* OTUs from 18S rRNA gene amplicon sequencing (Mahé et al., 2015). The slope of the curves indicates that for most samples the sequencing effort was high enough to adequately represent the eukaryotic diversity in the environment. Exceptions are mostly seen for the deep-water samples (dark blue), where slopes are still steeply rising.

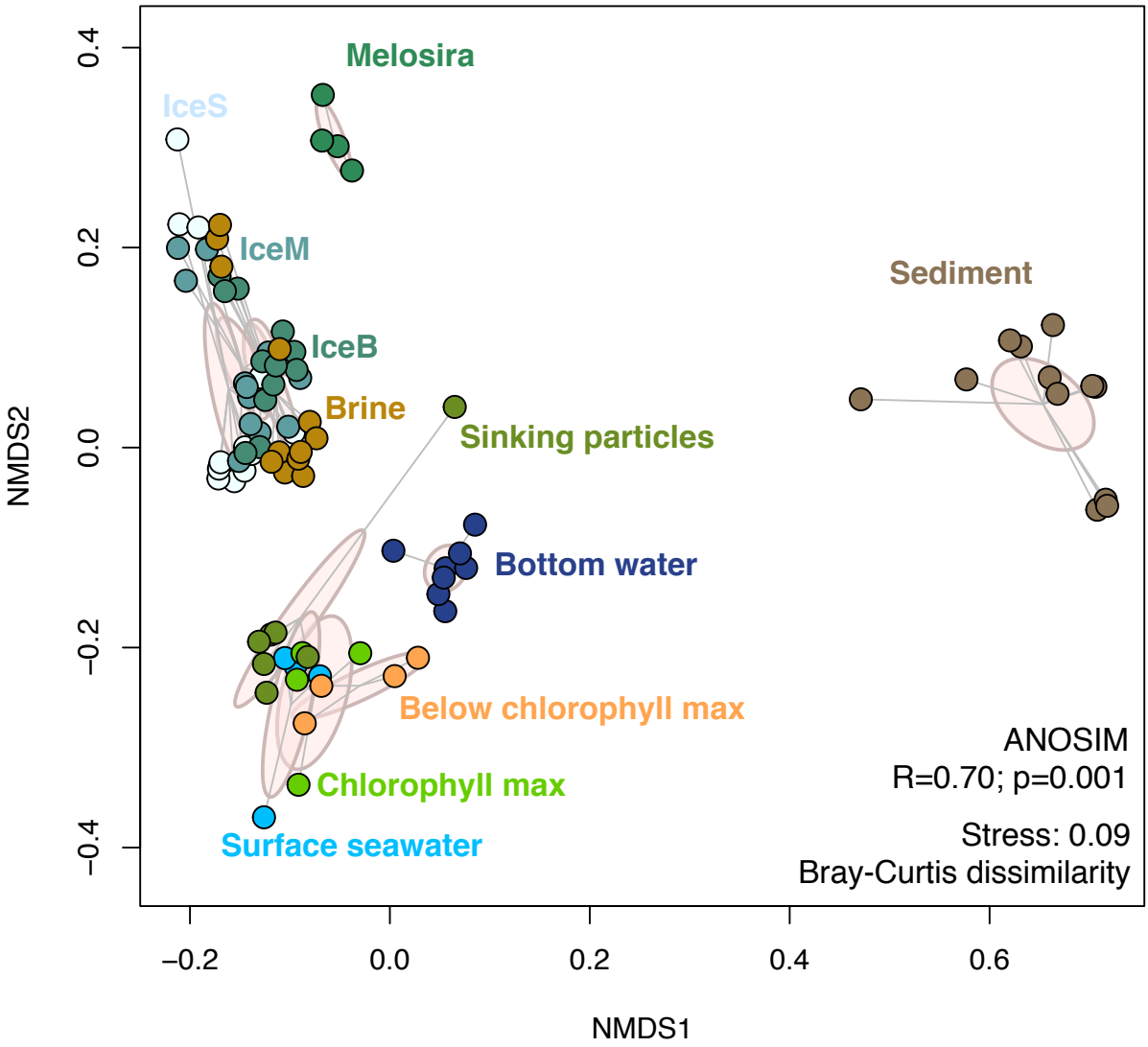

Figure A4 - Two-dimensional non-metric multidimensional (NMDS) scaling plot of bacterial community dissimilarities on the basis of 16S rRNA gene amplicons of the v3-v4 region. Dissimilarities and analysis of similarity (ANOSIM) were calculated on the basis of the Bray-Curtis dissimilarity measure. Pink ellipses around sample groupings indicate the estimated 95% dispersion limits of each environmental grouping. All points are connected to their group centroid through a spider diagram.

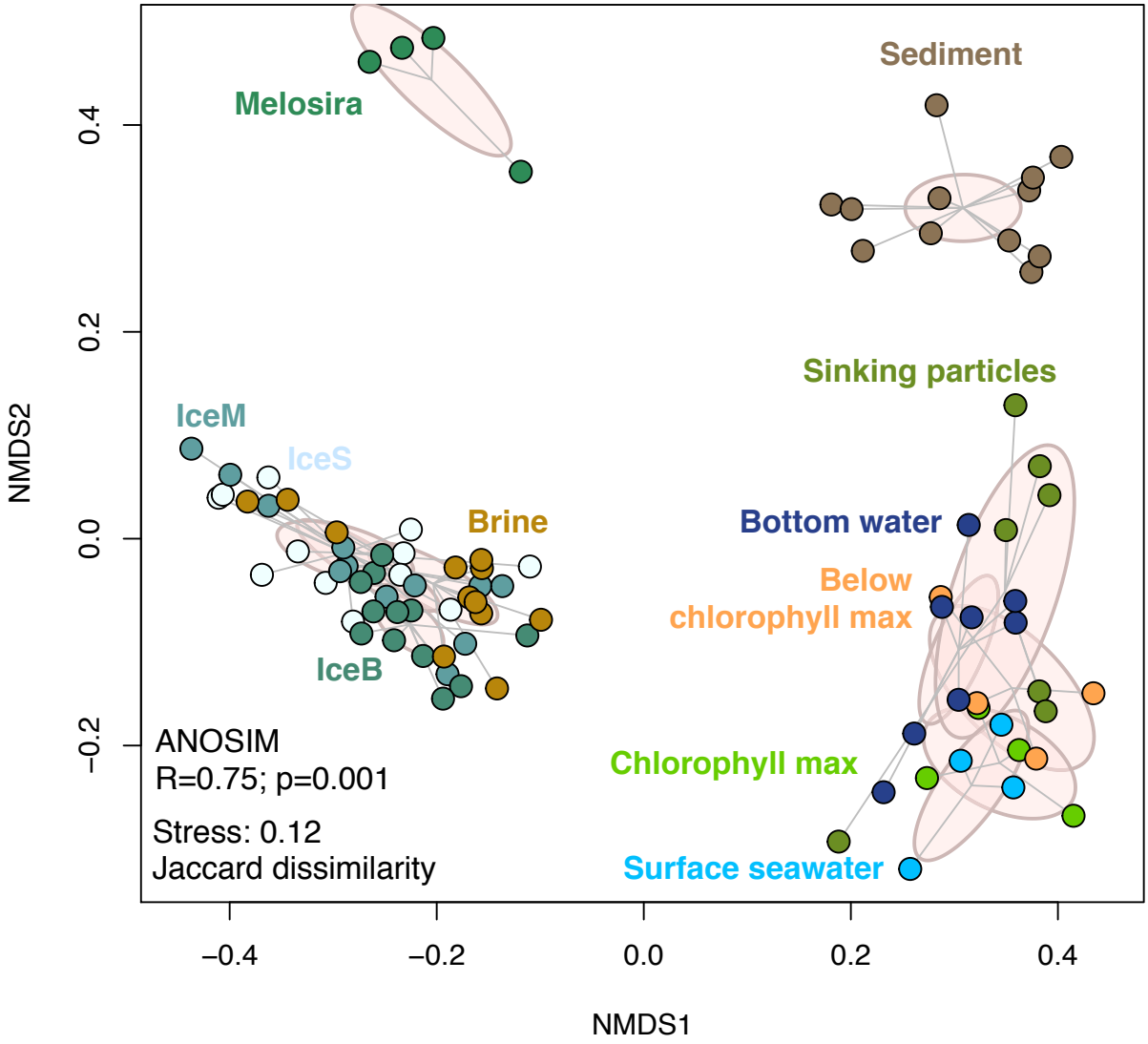

Figure A5 - Two-dimensional non-metric multidimensional (NMDS) scaling plot of eukaryote community dissimilarities on the basis of 18S rRNA gene amplicons of the v4 region. Dissimilarities and analysis of similarity (ANOSIM) were calculated on the basis of the Jaccard dissimilarity measure. Pink ellipses around sample groupings indicate the estimated 95% dispersion limits of each environmental grouping. All points are connected to their group centroid through a spider diagram.

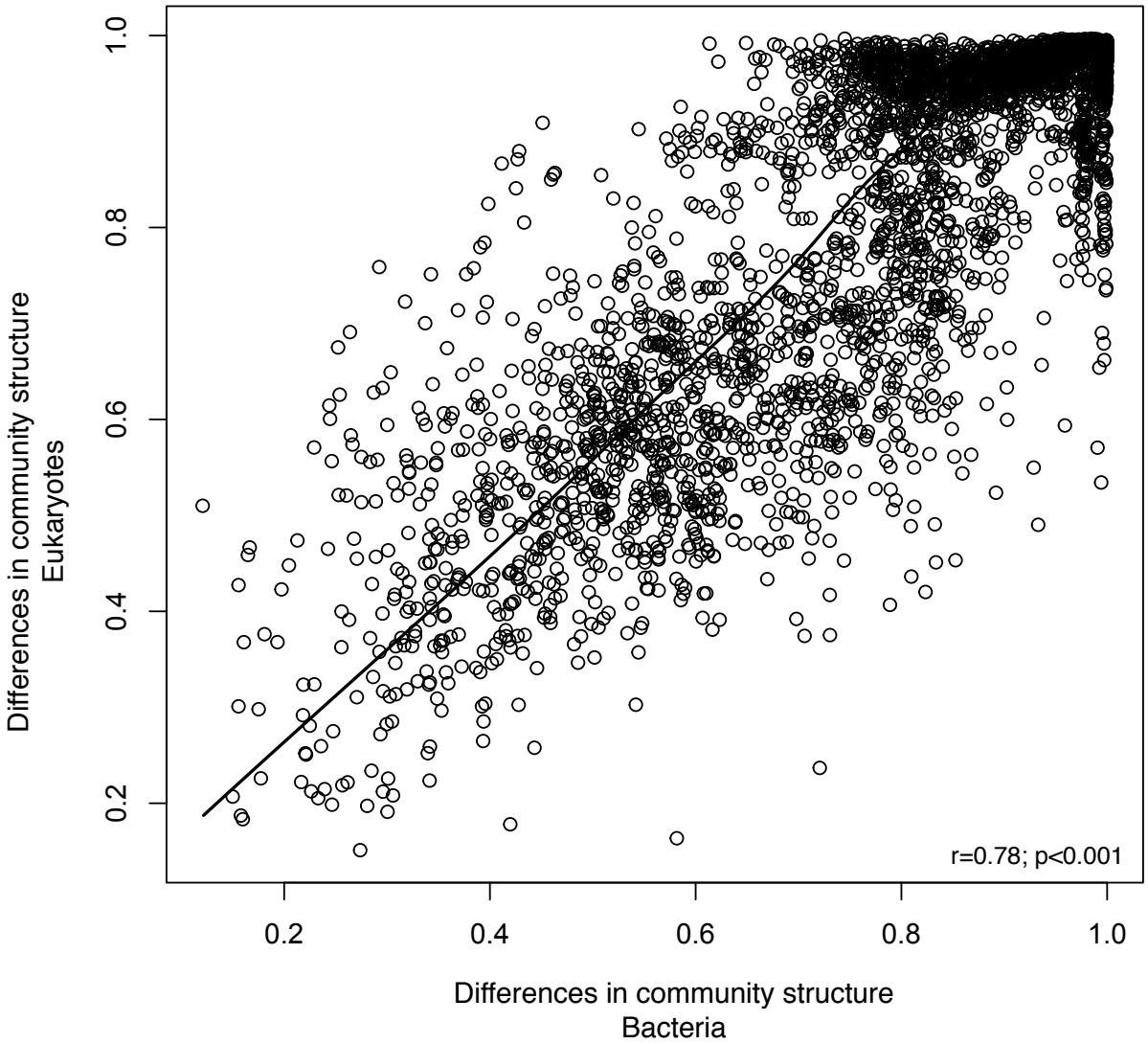

Figure A6 - Dissimilarity matrices were calculated using the Bray-Curtis dissimilarity measure for bacteria and the Jaccard dissimilarity measure for eukaryotes. Spearman's correlation as tested by a Mantel test with 999 permutations is indicated in the plot. The black line is a scatter smooth curve computed by LOESS.

Poster and oral presentations

Rapp JZ, Bienhold C, Tegetmeyer HE, Pala C, Offre P & Boetius A**:** Diversity of hydrolytic enzymes among Arctic deep-sea sediment bacteria. *Oral presentation* at Ocean Sciences Meeting 2018, Portland, OR, USA, February 2018

Rapp JZ, Bienhold C, Felden J, Hoffmann K, Jacob M, Soltwedel T, Wenzhöfer F & Boetius A: How microbial communities respond to a changing Arctic Ocean. *Oral presentation* at Polar regions And Coasts in the changing Earth System (PACES II) workshop, Bremen, Germany, October 2017

Rapp JZ, Bienhold C, Tegetmeyer HE, Pala C, Offre P & Boetius A: Diversity of hydrolytic enzymes among Arctic deep-sea sediment bacteria. *Poster presentation* at Symposium on High throughput methods for application in marine biodiversity time series, Hannover, Germany, October 2017

Rapp JZ: Functional diversity of Arctic deep-sea sediment bacteria. *Oral presentation* at Carbon seminar series, Alfred Wegener Institute, Bremerhaven, Germany, August 2017

Rapp JZ, Bienhold C, Offre P & Boetius A: Polysaccharide degradation potential of bacterial communities in Arctic deep-sea sediments (1000-5500 m water depth). *Poster presentation* at ISME16, Montreal, Canada, August 2016

Rapp JZ: From the frozen surface to the deep-sea floor: Microbial diversity in the Central Arctic Ocean during the 2012 sea-ice minimum. *Oral presentation* at the Max Planck Institute for Marine Microbiology, Bremen, Germany, January 2016

Rapp JZ: Functional potential of diverse and active microbial communities in Arctic deep-sea sediments. *Invited oral presentation* at the Genome Biology Program (GBP) Science meeting, Joint Genome Institute (JGI), Walnut Creek, California, September 2015

Rapp JZ, Fernández-Méndez M, Bienhold C & Boetius A: Journey to the deep sea: Do Arctic sea-ice bacteria hitchhike on ice-algal aggregates? *Oral presentation* at the General AWI PhD Student symposium/PhD Days, Sylt, Germany, June 2015

Winner “Best oral presentation” Award

Rapp JZ, Fernández-Méndez M, Bienhold C & Boetius A: Sea ice-pelagic-benthic links of bacterial diversity during the Arctic summer sea ice record minimum in 2012. *Poster presentation* at Arctic Frontiers 2015 - Climate & Energy, Tromsø, January 2015

Participation in fieldwork

June - July 2016	RV Polarstern expedition PS99.2 to Fram Strait, Greenland Sea, LTER HAUSGARTEN: sampling of sea ice, sinking particles, deep water column and sediment Chief scientist: Dr. Thomas Soltwedel, AWI
Mar - Apr 2016	AWIPEV base, Ny-Ålesund/Svalbard KOP 165: Black Carbon in Snow during the Arctic Haze Season (BlaCkSnow-AHS): snow sampling on glaciers PI: Hannes Schulz, AWI
July - Aug 2015	RV Polarstern expedition PS93.2 to Fram Strait, Greenland Sea, LTER HAUSGARTEN: sediment sampling & ROV-based experimental work Chief scientist: Dr. Thomas Soltwedel, AWI
June - July 2014	RV Polarstern expedition PS85 to Greenland Sea, LTER HAUSGARTEN: sediment sampling Chief scientist: Dr. Ingo Schewe, AWI

Teaching and tutoring

Oct - Nov 2016	**Course assistant** for the Microbial Oceanography practical course Topic: Measurements of extracellular enzymatic activity in deep-sea sediments PI: Prof. Dr. Antje Boetius, Marine Microbiology (MarMic) MSc Program, University of Bremen, Germany
Sep ‘16 - Mar 2018	**Supervision Bachelor thesis**: Biological contamination of sea ice through human-mediated dispersal of bacteria. Student: Linn Schmidtmann
Mar - Apr 2015	**Supervision Lab rotation**: Bacteria-Diatom interactions and diversity in sea-ice algae aggregates and cultures of *Melosira arctica.* Student: Tobias Vonnahme
Mar 2015	**Tutor** for the Marine Geology lecture series PI: Prof. Dr. Wolfgang Bach, Marine Microbiology (MarMIc) MSc Program, University of Bremen, Germany
Jan 2015	**Tutor** for the Molecular Ecology lecture series PI: Prof. Dr. Rudolf Amann, Marine Microbiology (MarMic) MSc Program, University of Bremen, Germany
Oct 2014	**Course assistant** for the Microbial Oceanography practical course Topic: Bacterial cell enumeration in deep-sea sediment PI: Prof. Dr. Antje Boetius, Marine Microbiology (MarMic) MSc Program, University of Bremen, Germany
Oct 2014	**Course assistant** for the Molecular Ecology practical course Topic: Field excursion to the island Sylt, Germany, for sampling of sediment and molecular lab work PI: Dr. Marc Mussmann, Marine Microbiology (MarMic) MSc Program, University of Bremen, Germany

„The greatest enemy of knowledge is not ignorance, it is the illusion of knowledge.“

- Steven Hawking

Universität Bremen

Name: _____ Ort, Datum: _____

Anschrift: _____

ERKLÄRUNG

Hiermit erkläre ich, dass ich die Doktorarbeit mit dem Titel:

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selbstständig verfasst und geschrieben habe und außer den angegebenen Quellen keine weiteren Hilfsmittel verwendet habe.

Ebenfalls erkläre ich hiermit, dass es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

_____
(Unterschrift)