The relationship between sea ice bacterial community structure and biogeochemistry: A synthesis of current knowledge and known unknowns

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Abstract

Sea ice plays an important role in high latitude biogeochemical cycles, ecosystems, and climate. A complete understanding of how sea ice biogeochemistry contributes to these processes must take into account the metabolic functions of the sea ice bacterial community. While the roles of sea ice bacteria in the carbon cycle and sea ice microbial loop are evidenced by high rates of bacterial production (BP), their metabolic diversity extends far beyond heterotrophy, and their functionality encompasses much more than carbon turnover. Work over the last three decades has identified an active role for sea ice bacteria in phosphate and nitrogen cycling, mutualistic partnerships with ice algae, and even prokaryotic carbon fixation. To better understand the role of sea ice bacteria in the carbon cycle the existing sea ice BP and primary production data were synthesized. BP in sea ice was poorly correlated with primary production, but had a strong, variable relationship with chlorophyll \textsubscript{a}, with a positive correlation below 50 mg chlorophyll \textsubscript{a} m\textsuperscript{-3} and a negative correlation above this value. These results concur with previous work suggesting that BP can be inhibited by grazing or the production of bacteriostatic compounds. To extend existing observations and predictions of other community functions a metabolic inference technique was used on the available 16S rRNA gene data. This analysis provided taxonomic support for some observed metabolic processes, as well as underexplored processes such as sulfur oxidation and nitrogen fixation. The decreasing spatial and temporal extent of sea ice, and altered timing of ice formation and melt, are likely to impact the structure and function of sea ice bacterial communities. An adequate modeling framework and studies that can resolve the functional dynamics of the sea ice bacterial community, such as community gene expression studies, are urgently needed to predict future change.

Introduction

Sea ice is a vast and dynamic habitat between the atmosphere and ocean. In addition to the roles it plays in the chemistry and physics of polar marine environments, topics explored elsewhere in this special feature on Biogeochemical Exchange Processes at Sea-Ice Interfaces (and in two other special features, on the Amundsen Sea Polynya International Research Expedition in the Antarctic and on Marginal Ice Zone Processes in the Summertime Arctic), sea ice plays a central role in marine ecosystems at high latitudes. Spring and summer sea ice in both the Arctic and Antarctic hosts an impressive amount of biomass in the form of ice algae, which form thick mats on the underside of the ice. Because ice algae are particularly energy rich, and much more concentrated than phytoplankton in the water column, they are an essential food source for many consumers in the polar water column (Arrigo and Thomas, 2004) and at the seafloor (McMahon et al., 2006), accounting for as much as 33\% of annual depth-integrated primary production (Legendre et al., 1992; Arrigo and Thomas, 2004; Saenz and Arrigo, 2014). Sharing the sea ice environment with ice algae are heterotrophic protists and a community of bacteria that, during and after the spring ice algal bloom, are taxonomically and
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functionally distinct from the open ocean bacterial assemblage. Despite a large body of work on autotrophic and heterotrophic community dynamics within sea ice, and the close physical association between sea ice bacteria and algae noted in several studies (Grossi et al., 1984; Smith et al., 1989; Hünken et al., 2008), there is little information available on the specific interactions between sea ice algae, heterotrophic protists, and bacteria. As observed in other environments, however, sea ice bacteria and algae are assumed to interact both synergistically and antagonistically, depending on resource availability and the unique stressors of the sea ice environment.

The sea ice microbial community inhabits pore spaces within sea ice and can colonize the underside of the ice at the sea ice-seawater interface. During the fall and winter period of sea ice growth, bacteria and phytoplankton in the water column are entrained within the (downward) advancing sea ice front, and within frazil ice deeper in the water column (Garrison et al., 1983). Although this process is selective for eukaryotic cells and other particles of similar size (Gradinger and Ikävalko, 1998; Różańska et al., 2008), the composition of the bacterial community in new autumn sea ice reflects the composition of the source seawater (Collins et al., 2010). As the ice cools in winter, bacteria, phytoplankton, and the dissolved components of the source seawater are sequestered and concentrated into increasingly smaller pore spaces. Initially able to exchange brine, nutrients, and other dissolved material, these pore spaces lose their connectivity at approximately −5 °C (Golden, 1998). Despite low temperature and high salinity, the sea ice bacterial assemblage remains relatively unchanged through the winter compared to the seawater assemblage (Collins et al., 2010), a phenomenon that may be attributable to the high concentration of cytoprotective exopolymers (EPS) commonly found within sea ice (Krembs et al., 2002; Underwood et al., 2010). The onset of ice algal photosynthesis in the spring is accompanied by a dramatic shift in bacterial community composition, from taxa reflective of the source seawater to those reflective of the unique ecological opportunities of spring and summer sea ice.

A brief history of sea ice microbial ecology

Despite their ubiquity in sea ice, and although the first reports of bacteria cultured from sea ice go back to at least 1918 (McLan, 1918), sea ice bacteria were not studied in detail until some decades later. Some of this delay can be attributed to the temperature sensitive nature of indigenous sea ice bacteria, a large number of which are psychrophiles that stop growth well below room temperature (Junge et al., 2002). Although the concept of cold-adapted bacteria appeared in the literature (Forster, 1887) well before the first study of sea ice bacteria, there was a delay in translating the concept of temperature sensitivity from industrial settings to the sea ice environment. Some of the earliest studies of sea ice bacteria, for example, involved culturing bacteria from sea ice at 25 °C (Iizuka et al., 1966) even as psychrophiles were being isolated from seawater and fish at near in-situ temperatures (Colwell and Morita, 1964).

During the 1970s and early 1980s, the works of Pomeroy (1974), Azam and Graf (1983), and others brought forward the role that heterotrophic bacteria play in the marine carbon cycle. The “microbial loop,” wherein dissolved organic carbon (DOC) is recycled via bacterial assimilation and predation by bacterivores, became recognized as an important component of the marine food web. Sullivan and a host of co-authors transferred this concept to the sea ice ecosystem in a pivotal series of papers in the 1980s (Grossi et al., 1984; Sullivan and Palmisano, 1984; Kottmeier et al., 1987; Kottmeier and Sullivan, 1988). Their work confirmed that sea ice bacteria are not only abundant and active within sea ice but also closely coupled to the occurrence of ice algae. These observations, all on mature land-fast ice within McMurdo Sound, Antarctica, were extended to more variable sea ice types by Grossmann and Dieckmann (1994) and Helmke and Weyland (1995). Working on newly formed drift ice in autumn, Grossmann and Dieckmann (1994) observed measureable bacterial growth rates even under relatively oligotrophic conditions, as well as bacterial production (BP) rates exceeding those observed in seawater. Helmke and Weyland (1995) extended such work to winter pack ice with observations of high rates of activity and bacterial biomass relative to the underlying water column; at times the ATP concentration, a measure of biomass that implies activity (Karl, 2014), in a single meter of sea ice exceeded the 100 m depth-integrated value for the underlying water column. The comparison was even more marked, however, as the high levels of activity were limited to the bottom-most, warmest horizon of the sea ice.

By the mid-1990s, it was clear that sea ice bacterial communities were composed of physiologically distinct, often psychrophilic bacteria, capable of surviving conditions of severe environmental stress, and of responding rapidly to new inputs of carbon. The taxonomic and functional diversity of this community, however, was almost entirely unknown, except for the phenotypic and morphology-based classifications of a few isolates for the former (Iizuka et al., 1966) and the limited observations of extracellular enzyme activity for the latter (Helmke and Weyland, 1995). Concurrent with the growing appreciation of sea ice bacteria as important components of the polar marine ecosystem came major advances in understanding taxonomic diversity within microbial communities. In a groundbreaking paper, Woese and Fox (1977) used 16S and 18S rRNA gene sequences to classify life into three broad domains. Improvements in sequence technology nearly a decade later (Smith et al., 1986) opened the door for more wide-spread sequencing of 16S rRNA genes from environmental samples and led to a rapid shift in the existing paradigm of prokaryotic diversity (Giovannoni et al., 1990; Ward et al., 1990). These methods were eventually applied to sea ice, first to identify
isolates (e.g., Bowman et al., 1997a, 1997b, 1997c) and ultimately to identify sequences from an environmental
sea ice clone library (e.g., Brown and Bowman, 2001). These and later studies established that while most
genera observed in sea ice have members common to other environments, there are 16S rRNA phylotypes
that appear to be unique or strongly favored within sea ice.

Although the studies of the 1990s began to elucidate the composition of the sea ice bacterial community,
they did not address its functional roles. In some cases function could be inferred from specific experiments.
Gerdes et al. (2005) and Brakstad et al. (2008), for example, used diesel and crude oil perturbation experiments
to explore the ability of the sea ice bacterial community to respond to these inputs of carbon. While both
studies observed a loss in diversity in sea ice samples incubated with crude oil, the members of the class
Gamma-proteobacteria were dominant in crude oil and abundant in clean incubations, with co-occurring denatured
gel gradient electrophoresis (DGGE) bands suggesting that some of the same gammaproteobacteria, including
members of the genera Marinobacter and Glaciecola, were present in both. These findings illustrate that some
members of the sea ice bacterial community are copiotrophic (adapted to high substrate concentrations) and
capable of metabolizing a wide range of organic compounds.

Additional insight into community function has come from the few bacterial isolates associated with
sea ice to have their genomes sequenced. The first of these was Colwellia psychrerythraea 34H (Methé et al.,
2005). Although the sequenced strain was isolated from Arctic marine sediment (Huston et al., 2000),
16S rRNA gene sequences associated with Colwellia spp., including C. psychrerythraea, have been observed
repeatedly in sea ice (Bowman et al., 1997a, 1997b; Junge et al., 2002; Brinkmeyer et al., 2003; Barber et al.,
2014). Since publication of the C. psychrerythraea 34H genome, published genomes have been sequenced
from a variety of sea ice bacteria, including Psychromonas ingrahamii 37 (Riley et al., 2008), multiple species
of Glaciecola (Qin et al., 2012; Yin et al., 2013), Octadecabacter (Vollmers et al., 2013), and Pseudoalteromonas
(Bian et al., 2012), Psychroflexus torquis ATCC 700755 (Feng et al., 2014), and Marinomonas sp. BS120584
(Liao et al., 2015). Considering that at the time of writing there were over 22,000 draft and 2,700 finished
genomes within Genbank, it is clear that sea ice bacteria are genetically undersampled with respect to other
ecologically significant environments. The existing genome sequencing efforts, however, have enabled a small
number of genome-genome comparison studies (Vollmers et al., 2013; Bowman and Deming, 2014) that
have identified features unique to and common among sea ice bacteria.

The adoption of more advanced techniques to probe bacterial community function within sea ice has been slow. Despite the intriguing ecology of the sea ice environment, the analysis of specific functional genes
within sea ice – the easiest way to confirm that a microbial community has the genetic capacity for a metabolic
function – has been surprisingly limited. Koh et al. (2010) identified proteorhodopsin genes and genes for
anoxygenic photosynthesis (Koh et al., 2011) within Antarctic sea ice, which suggests that bacterial energy
acquisition in sea ice is not limited to chemotrophy. The merA (Møller et al., 2014) and nifH (Díez et al.,
2012) genes have been found in Arctic sea ice, suggesting the capacity for mercury reduction and nitrogen
fixation, respectively. Metagenomics, an approach that yields the sequences of a random subsample of all of
the genetic material (and thus metabolic potential) within a sample, has been applied only to limited, unique
samples of young sea ice (Bowman et al., 2014). Transcriptomics, an approach that identifies the mRNA
products of genes being actively transcribed, has only been applied to the analysis of proteorhodopsin within
sea ice (Koh et al., 2010). No studies using metatranscriptomics or metaproteomics (an approach that yields
all of the proteins transcribed in a sample) have been reported for the prokaryotic sea ice community, despite
the sharp spatial and temporal gradients present within sea ice, broad application of these techniques to other
marine environments (e.g., Morris et al., 2010; Ottesen et al., 2014), and to ice algal communities (Toseland
et al., 2013; Pearson et al., 2015).

Known knowns

Community structure

Much of what is known about bacterial community composition and structure in sea ice comes from studies
using the 16S rRNA taxonomic marker gene (Figure 1). Typically these studies have employed Sanger
sequencing technology, which, depending on the resolve and resources of the investigator, produces at best
a semi-quantitative profile of the bacterial community. Recent studies (Bowman et al., 2012; Hatam et al.,
2014; Barber et al., 2014; Torstensson et al., 2015) have begun to employ so-called next generation sequencing
technology to sea ice and peripheral habitats, including snow on top of sea ice (Hauptmann et al., 2014). These
analyses are valuable in that by sequencing more deeply they provide a more complete, and more quantitative
(in terms of relative abundance), view of bacterial community structure.

To identify which taxonomic groups of bacteria and archaea are consistently reappearing in sea ice 16S
rRNA gene studies, and thus might constitute indigenous sea ice phylotypes, I conducted a meta-analysis of
the existing sea ice datasets (Table 2). This analysis included 16S rRNA gene sequences from sea ice isolates,
but not strains or environmental sequences from experiments where ice was grown artificially or manipulated
experimentally away from the sample site. Because of the shallow depth of gene sampling in the Sanger
sequencing studies, the results from each of these studies were considered as a single dataset. Sequences from the only deeper sequencing studies of Bowman et al. (2012) and Hatam et al. (2014), both conducted on multiyear sea ice (MYI), were analyzed separately. Using the metabolic inference pipeline PAPRICA (Bowman and Ducklow, 2015), all sea ice sequences were phylogenetically placed (Matsen et al., 2010) on a non-redundant reference tree of full length 16S rRNA gene sequences obtained from all completed genomes in Genbank (Figure 2). Placement to a terminal node on the reference tree indicates that a sea ice sequence is most similar to that reference sequence. Placement to an internal node on the reference tree suggests that the read belongs to the clade diverging from the node, but that a more precise placement within the clade could not be made. Because the reference sequences originated from completed genomes, the idea of phylogenetic relatedness was extended to genomic relatedness and the placements are referred to as “genomes”.

The earliest studies of sea ice bacterial community composition recognized select gammaproteobacteria and alphaproteobacteria and members of the division Cytophaga-Flavobacteria-Bacteriodes (CFB, referred to more recently as the phylum Bacteriodetes) as dominant sea ice taxa (Brown and Bowman, 2001; Junge et al., 2002; Brinkmeyer et al., 2003). Typical seawater phylotypes, including marine archaea and the ubiquitous SAR11 clade, were conspicuously rare in clone libraries of mature sea ice, though they were observed to be abundant in young and winter sea ice samples (Collins et al., 2010; Barber et al., 2014). The meta-analysis (Figure 2) confirmed this early view. Although there was considerable variability between the Sanger and deep sequencing studies, members of the Gammaproteobacteria and Bacteriodes were overwhelmingly dominant across all three datasets. Other predominant taxa included the Alphaproteobacteria and members of the phyla Actinobacteria and Verrucomicrobia.

The most abundant genomes in any one dataset were often present in all three datasets, but at varying relative abundances. Among the deep sequencing datasets the most abundant genomes were associated with the gammaproteobacteria Psychrobacter arcticum 273, Ruthia magnifica, and Glaciecola agarilytica 4H37YE5, bacteriodes Flavobacteriaceae spp., the betaproteobacterium Rhodovulum ferrireducens, strains of the actinobacterium Clavibacter michiganensis, and Chlamydia spp. in the phylum Chlamydiae. An indication of sea ice specialism might be the presence of an abundant genome in all three datasets, whether abundant in each case or not. This presence applied to the genomes from P. arcticum 273, R. magnifica, Flavobacteriaceae spp., and G. agarilytica 4H37YE5. The remaining abundant genomes were all reported by Hatam et al. (2014), but not by Bowman et al. (2012) or any of the studies using Sanger sequencing. This difference reflects the overall higher richness and diversity of the sea ice in Hatam et al. (2014).

An important difference between Hatam et al. (2014) and Bowman et al. (2012) is that the former study targeted specific horizons within MYI while the latter integrated across the entire core. Although some Sanger sequencing studies (Collins et al., 2010; Cowie et al., 2014) have included analyses of different horizons in young and first year sea ice, the age of MYI may add to the complexity of the bacterial community. Hatam et al. (2014) observed that betaproteobacteria were more prevalent in the upper ice horizons, likely the result of melt pond influence from the previous summer (Brinkmeyer et al., 2004). The genus Clavibacter may represent a similar case of bacteria not necessarily indigenous to sea ice, as actinobacteria, common to soil, may have arrived at the ice surface with dust or snowfall (Smith et al., 2013; Hauptmann et al., 2014).

In general, genomes that were abundant in the Sanger sequencing studies made up a significant fraction of the deep sequencing studies, although the relative abundance of these nodes varied considerably between datasets (Figure 2i). In some cases abundant genomes in the Sanger sequencing studies were not present, or were present at low abundance, within the deep sequencing datasets. These genomes could represent shifts

Figure 1
Geographic location of activity based and community composition studies in sea ice.

Studies around the Antarctic continent are shown in the left frame; the right frame shows studies from the Arctic and sub-Arctic (Baltic Sea and Sea of Okhotsk). Community structure studies are listed in Table 2 and metabolic activity studies in Table 3. The orange points show the 30 year median maximum sea ice extent, obtained from the NSIDC website at http://nsidc.org/data/gis/data.htm. Black arrows show the location of the two deep sequencing studies that describe community structure in addition to community composition.

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### Table 1. Definitions of key terms and abbreviations

<table>
<thead>
<tr>
<th>Term</th>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-cyano-2,3-dinitol tetrazolium</td>
<td>CTC</td>
<td>Dye that fluoresces under blue light excitation when reduced in actively respiring cells</td>
</tr>
<tr>
<td>Bacterial production</td>
<td>BP</td>
<td>The amount of carbon incorporated into bacterial biomass; typically estimated from the uptake of radiolabeled thymidine or leucine</td>
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<tr>
<td>Clade</td>
<td></td>
<td>The descendants of an ancestral phylotype</td>
</tr>
<tr>
<td>Community composition</td>
<td></td>
<td>The taxonomic makeup of a microbial community</td>
</tr>
<tr>
<td>Community structure</td>
<td>-</td>
<td>The proportional taxonomic makeup of a microbial community</td>
</tr>
<tr>
<td>Copiotroph</td>
<td>-</td>
<td>A microorganism optimized for the rapid uptake of high concentrations of organic matter</td>
</tr>
<tr>
<td>Denatured gel gradient electrophoresis</td>
<td>DGGE</td>
<td>A method for identifying small differences in gene sequence based on the point of denaturation; can resolve a complex assemblage as individual bands on a polyacrylamide gel</td>
</tr>
<tr>
<td>Dissolved organic carbon</td>
<td>DOC</td>
<td>Typically operationally defined as carbon that can pass through a 0.7 µm filter</td>
</tr>
<tr>
<td>Fluorescent in-situ hybridization</td>
<td>FISH</td>
<td>Epifluorescent microscopy technique for identifying different microbial taxa; highly quantitative</td>
</tr>
<tr>
<td>Genomic plasticity</td>
<td>-</td>
<td>Genomic variability due to gene gain or loss</td>
</tr>
<tr>
<td>Microautoradiography</td>
<td>MAR</td>
<td>A microscopy technique that identifies radiolabeled substrate uptake by specific bacterial cells, based on the ability of a cell to expose a photographic emulsion</td>
</tr>
<tr>
<td>Metagenome</td>
<td>-</td>
<td>The product of a sequencing technique that sequences small, random fragments of DNA from an environment, providing a quantitative overview of metabolic potential and community structure</td>
</tr>
<tr>
<td>Metaproteome</td>
<td>-</td>
<td>The product of a mass spectrometry technique that identifies random peptide fragments from an environment, providing a quantitative overview of translated genes</td>
</tr>
<tr>
<td>Metatranscriptome</td>
<td>-</td>
<td>The product of a sequencing technique that sequences random fragments of mRNA from an environment, providing a quantitative overview of transcribed genes</td>
</tr>
<tr>
<td>Multiyear sea ice</td>
<td>MYI</td>
<td>Sea ice that has survived one summer melt cycle</td>
</tr>
<tr>
<td>Next generation sequencing</td>
<td>NGS</td>
<td>Sequencing by any method of greater throughput than Sanger sequencing, here referring to 454 and Illumina sequencing</td>
</tr>
<tr>
<td>Node</td>
<td>-</td>
<td>The branch tip on a phylogenetic tree associated with a single sequence (terminal node), or the ancestral branch point for a collection of homologous sequences (internal node).</td>
</tr>
<tr>
<td>Oligotrophic</td>
<td>-</td>
<td>An environment characterized by low concentrations of DOC</td>
</tr>
<tr>
<td>Particulate organic matter</td>
<td>POM</td>
<td>Typically operationally defined as the organic component of particles captured on a 0.7 µm filter</td>
</tr>
<tr>
<td>Phylotype</td>
<td>-</td>
<td>Analogous to the concept of species in higher organisms and observed as a collection of very similar 16S rRNA gene sequences, which may include considerable variability in ecology; phylogenetic placements to branch tips here referred to as phylotypes (and placements to internal branches as clades)</td>
</tr>
<tr>
<td>Primary production</td>
<td>PP</td>
<td>The amount of inorganic carbon reduced to organic carbon by primary producers; typically estimated from the uptake of radiolabeled bicarbonate</td>
</tr>
<tr>
<td>Psychrophile</td>
<td>-</td>
<td>An organism optimized to grow at low temperatures; for bacteria, often operationally defined as $T_{max}$ of growth &lt; 20 °C</td>
</tr>
<tr>
<td>Young sea ice</td>
<td>-</td>
<td>Newly formed sea ice, with a microbial community that is functionally distinct from that found in mature sea ice.</td>
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In community composition by geography or ice type, or they may represent methodological biases associated with culturing or clone library construction. Nodes over-represented within the Sanger sequencing dataset were defined as those accounting for more than 1% of the total Sanger sequences but present within both deep sequencing datasets at below 10% of their relative abundance within the Sanger dataset. Thirteen genomes met these criteria (Table 4). Because some of these genomes were selected for sequencing based on their association with sea ice (e.g., *P. ingrahamii* 37 and *O. arcticus* 238), their over-representation in the Sanger sequencing dataset may indicate a bias in our assessment of sea ice bacterial genomics. The available sea ice bacterial genomes may reflect the phylotypes most amenable to culturing, or most abundant within clone libraries, but not necessarily the most abundant in the sea ice environment. Of the six most abundant genomes across all datasets only *G. psychrophila* 170 was represented by a genome obtained from sea ice. Similarly,
although culture and Sanger sequencing based studies have done a remarkable job of identifying the major taxa present within sea ice, these studies may not accurately describe community structure. Conversely, while deep sequencing studies are beginning to describe community structure within sea ice, the extremely limited spatial and temporal extent of the available datasets means that they reflect an incomplete picture of sea ice bacterial diversity and community structure.

Less abundant genomes that were consistently present within sea ice were identified with a ternary plot (Figure 2iii). Genomes located near the center of the ternary plot were similarly represented across sample groups. The ubiquity of bacteriodetes in sea ice is evident in this analysis; genomes associated with the families *Cyclobacteriaceae* and *Flavobacteriaceae* were broadly represented across sample groups. Surprisingly, only a single gammaproteobacterium was similarly shared across datasets, although different gammaproteobacteria were abundant in different datasets. These included genomes related to the gammaproteobacterial sulfur-oxidizing symbionts (GSOS). The close homology between the sea ice and GSOS 16S rRNA gene sequences was confirmed by a blastn analysis of select 16S rRNA reads from Bowman et al. (2012). These blastn and phylogenetic placement analyses, however, could be biased by poor phylogenetic coverage of a larger clade of gammaproteobacteria encompassing both the GSOS and a separate, ecologically distinct group. Alternatively different ecologies, such as particle or host associated and free living, may be associated with these similar 16S rRNA gene sequences. A genome associated with *Akkermansia muciniphila* ATCC BAA835 of the phylum *Verrucomicrobia* was also consistently present across datasets. *A. muciniphila* ATCC BAA835 is closely related to *Coraliomargarita akajimensis* DSM45221 (originally isolated from coral), which was found in some abundance in both of the deeper sequencing datasets (Figure 2ii). Because of the close similarity in 16S rRNA genes between *A. muciniphila* ATCC BAA835 and *C. akajimensis* DSM45221 these reference genomes may represent the same environmental genome within sea ice.

None of the abundant nodes was widely shared between datasets, although in two cases closely related genomes were together abundant and widely shared. *Polaribacter* MED152 of the family *Flavobacteriaceae* was abundant in both deep sequencing datasets while genomes associated with the genus *Flavobacterium* were among the most evenly shared. Similarly, within the *Verrucomicrobia*, *C. akajimensis* DSM45221 was highly abundant while *A. muciniphila* ATCC BAA835 was widely shared.

This analysis has confirmed that the prototypical sea ice bacterial taxa are bacteriodetes and gammaproteobacteria, although a diverse assemblage of other taxa is either abundant in sea ice, occurs frequently in this environment, or both. In the marine environment bacteriodetes and gammaproteobacteria encompass a wide range of ecologies; however, bacteriodetes are notable for their close physical association with phytoplankton.
Table 3. Sea ice studies of microbial activity included in Figures 1 and 3

<table>
<thead>
<tr>
<th>Study</th>
<th>BP (ng C l(^{-1}) h(^{-1}))</th>
<th>PP (ng C l(^{-1}) h(^{-1}))</th>
<th>Chlorophyll a (µg l(^{-1}))</th>
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<tbody>
<tr>
<td>Kottmeier et al. (1987)</td>
<td>257 ± 355</td>
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<tr>
<td>Kottmeier and Sullivan (1988)</td>
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<td>Bunch and Harland (1990)</td>
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<tr>
<td>Smith and Clement (1990)</td>
<td>68.9 ± 30.4</td>
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<td>Grossman and Dieckmann (1994)</td>
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<tr>
<td>Grossmann (1994)</td>
<td>5.63 ± 4.19</td>
<td>950 ± 768</td>
<td>3.21 ± 3.46</td>
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<td>Helmke and Weyland (1995)</td>
<td>44.4 ± 59.0</td>
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<tr>
<td>Grossmann et al. (1996)</td>
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<tr>
<td>Mock et al. (1997)</td>
<td>690 ± 391</td>
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<td>Haecy and Andersson (1999)</td>
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<tr>
<td>Guglielmo et al. (2000)</td>
<td>5.11 ± 3.60</td>
<td>841 ± 1440</td>
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<tr>
<td>Guglielmo et al. (2004)</td>
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<td>Junge et al. (2004)</td>
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<tr>
<td>Kaartokallio et al. (2004)</td>
<td>126(^a)</td>
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<tr>
<td>Kuparinen et al. (2007)</td>
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<tr>
<td>Kaartokallio et al. (2008)</td>
<td>170 ± 106</td>
<td>11.2 ± 10.5</td>
<td></td>
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<tr>
<td>Martin et al. (2009)</td>
<td>740(^c)</td>
<td></td>
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<tr>
<td>Pusceddu et al. (2009)</td>
<td>11.3 ± 10.5</td>
<td>1830 ± 731</td>
<td></td>
</tr>
<tr>
<td>Søgaard et al. (2010)</td>
<td>11.5 ± 5.60</td>
<td>12.5 ± 3.54</td>
<td>0.25 ± 0.13</td>
</tr>
<tr>
<td>Kuparinen et al. (2011)</td>
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<tr>
<td>Nguyen and Maranger (2011)</td>
<td>427 ± 272</td>
<td>378 ± 785</td>
<td></td>
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<tr>
<td>Paterson and Laybourn-Parry (2011b)</td>
<td>224 ± 271</td>
<td>14.0 ± 13.4</td>
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<tr>
<td>Søgaard et al. (2013)</td>
<td>73.5 ± 65.8</td>
<td>142 ± 240</td>
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<tr>
<td>Kaartokallio et al. (2013)</td>
<td>361 ± 508</td>
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<td>Cowie et al. (2014)</td>
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<td>Eronen-Rasimus et al. (2015)</td>
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<td>Zhou et al. (2014)</td>
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<tr>
<td>Baer et al. (2015)</td>
<td>13.9 ± 21.3(^d)</td>
<td>662 ± 1520</td>
<td>73.6 ± 167</td>
</tr>
</tbody>
</table>

\(^a\) All studies listed appear in Figure 1; studies used in Figure 3, and data included in that figure, are noted by values (mean ± S.D.) reported in the columns for BP, PP, and Chlorophyll a.

\(^b\) Only the mean value was reported.

\(^c\) Only the final melt salinity of 33 ‰ was used.

\(^d\) These values reflect the uncorrected values in Baer et al. (2015); see discussion in that work.

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(Schäfer et al., 2002; Jasti et al., 2005), including ice algae (Brown and Bowman, 2001), and as copiotrophs, performing best when DOC concentrations are high (Teeling et al., 2012). Many gammaproteobacteria associated with sea ice, including members of the genera *Psychrobacter* and *Glaciecola*, are also known to associate with ice algae (Bowman et al., 1997b; Staley and Gosink, 1999; Brown and Bowman, 2001). The copiotrophic nature of sea ice bacteria has been used to explain their relatively high culturability in comparison with bacteria from the water column (Junge et al., 2002).

Appearing only in low abundance in sea ice sequence libraries are archaea and cyanobacteria, important groups elsewhere in the marine environment. Although Bowman et al. (2012) observed some archaeal 16S rRNA genes in MYI, neither that study nor Hatam et al. (2014), which did not report any archaeal reads in MYI, used primers optimized to amplify archaeal 16S rRNA genes. Cowie et al. (2011) and Collins et al. (2010) used primers specific to the domain *Archaea* to amplify DNA for clone library construction. Archaea were relatively abundant in young, winter sea ice (reflecting the composition of the source seawater; Collins et al., 2010), but comprised only a minor component of mature Antarctic (Cowie et al., 2011) and...
Arctic (Baer et al., 2015) first year sea ice. Brinkmeyer et al. (2003) also did not identify archaea in samples of Arctic and Antarctic sea ice using FISH. This temporal distribution of archaea in sea ice (and polar surface seawater) matches the spatial distribution of marine crenarchaeota in the water column at lower latitudes. In the tropical Pacific, marine crenarchaeota are most abundant below the photic zone (Karner et al., 2001), where a reduced concentration of DOC favors a chemoautotrophic lifestyle. In this way early winter sea ice is analogous to the mesopelagic zone. In late winter and spring, photosynthesis and DOC concentrations begin to rise and heterotrophy is favored over chemoautotrophy.

While cyanobacteria are common in other cold aquatic environments (Jungblut et al., 2010), there is little evidence for indigenous cyanobacteria in first year sea ice (Koh et al., 2012). Although cyanobacterial 16S rRNA (Bowman et al., 2012) and *nif*H (Diez et al., 2012) gene sequences have been observed in Arctic MYI, and filamentous cyanobacterial morphologies have been observed by microscope (Diez et al., 2012), they may be of allochthonous origin (Walron et al., 2007; Hauptmann et al., 2014), or, in the case of the Bowman et al. (2012), chloroplasts. The (presumed) absence of cyanobacteria from surface melt ponds (Brinkmeyer et al., 2004) is surprising given the relative stability, low salinity, and low eukaryotic productivity of melt ponds disconnected from the water column. Cyanobacteria seem less able to optimize to low temperature than their heterotrophic counterparts, and polar cyanobacteria often grow best at temperatures above 20 °C (Tang et al., 1997). Despite slow growth at in situ temperatures, cyanobacteria are a significant part of the microbial community in many other aquatic polar environments, including melt ponds on ice shelves (Jungblut et al., 2005; Mueller et al., 2005) and glacial streams (Priscu et al., 2012). What ecological characteristics distinguish low salinity melt ponds on the surface of sea ice from those on ice shelves remains to be determined.
Table 4. Relative abundance in NGS datasets of phylotypes or clades over-represented in sea ice studies that used Sanger sequencing technology.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Psychromonas ingrahamii 37</td>
<td>0.011</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pseudalteromonas haloplanktis TAC125</td>
<td>0.019</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Shewanella frigidimarina NCIMB400</td>
<td>0.010</td>
<td>0</td>
<td>6.24E-05</td>
</tr>
<tr>
<td>Acinetobacter baumannii strains</td>
<td>0.017</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Psychrobacter spp.</td>
<td>0.017</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia R551</td>
<td>0.010</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cyano bacteri a spp.</td>
<td>0.041</td>
<td>0</td>
<td>0.000249</td>
</tr>
<tr>
<td>Nitrosopumilus maritimus SCM1</td>
<td>0.080</td>
<td>0</td>
<td>6.24E-05</td>
</tr>
<tr>
<td>Psychi phera mikurensis NBRC102666</td>
<td>0.020</td>
<td>8.41E-05</td>
<td>0.000374</td>
</tr>
<tr>
<td>Roseobacter spp.</td>
<td>0.015</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Octadecabacter arcticus 238</td>
<td>0.023</td>
<td>0.00025</td>
<td>6.24E-05</td>
</tr>
<tr>
<td>Candidatus Pelagibacter ubique HTCC1062</td>
<td>0.023</td>
<td>0.00135</td>
<td>6.24E-05</td>
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<tr>
<td>Candidatus Pelagibacter ubique HTCC1062</td>
<td>0.050</td>
<td>0</td>
<td>0.00343</td>
</tr>
</tbody>
</table>

* Bowman et al. (2012) and Hatam et al. (2014) used next generation sequencing (NGS).

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Bacterial production in sea ice

The sea ice bacterial community is largely heterotrophic and assimilates DOC to produce biomass and energy. The rate of assimilation can be assessed by the uptake of radioactive organic compounds, typically $^3$H-leucine or $^3$H-thymidine, and converted to a measure of BP. Although some studies have noted a decoupling of BP and primary production (PP) in sea ice (Grossmann et al., 1996; Monfort et al., 2000; Pusceddu et al., 2009), with low BP values under conditions of high PP (and presumably high DOC), bacterial carbon turnover

![Figure 3](https://example.com/image3.png)

The relationship between bacterial production (BP) and other biological parameters.

Values were taken from the studies listed in Table 3. Only data where values were published in units of C, or where BP was measured by the uptake of $^3$H-leucine, were used. In the latter case a conversion of 1.5 kg C mol$^{-1}$ $^3$H-leucine was applied (Ducklow, 2003). Units for chlorophyll $a$ and for BP and primary production, not included on the axes for clarity, are mg chlorophyll $a$ m$^{-2}$ and ng C l$^{-1}$ h$^{-1}$.

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Sea ice bacteria and biogeochemistry

is often high in sea ice during the spring and summer. In a study from Prydz Bay, Antarctica, Paterson and Laybourn-Parry (2011b) observed BP values as high as 27.8 µg C l$^{-1}$ d$^{-1}$ in early summer sea ice (the highest value indicated in Figure 3). This value is high for a marine setting and comparable to a coastal temperate environment (Ducklow and Carlson, 1992). Generally BP is low through the winter (Bunch and Harland, 1990), though sea ice bacteria remain metabolically active (Junge et al., 2004) with minor contributions to wintertime accumulations of CO$_2$ in sea ice (Miller et al., 2011). Respiration increases with the earliest onset of photosynthesis in the spring, and can reach a high rate well before BP increases (Nguyen and Maranger, 2011). BP increases in late spring (Søgaard et al., 2010; Nguyen and Maranger, 2011) as carbon is directed to bacterial biomass (Figure 4).

In most marine and aquatic environments BP is correlated with chlorophyll a concentration and PP (Cole et al., 1988). Relatively few studies have measured PP or chlorophyll a concurrently with BP in sea ice; however, in some of the available studies BP is not positively correlated with PP or chlorophyll a (Grossmann, 1994; Guglielmo et al., 2000; Pusceddu et al., 2009). Combining the available data (Table 3) reveals a weak relationship between BP and PP, but two significant and opposing relationships are observed between BP and chlorophyll a (Figure 3). For values below 50 mg chlorophyll a m$^{-3}$, chlorophyll a and BP were positively correlated ($R^2 = 0.18, p = 5.39 \times 10^{-6}$). BP and chlorophyll a were negatively correlated ($R^2 = 0.53, p = 0.003$) for chlorophyll a concentrations above 50 mg m$^{-3}$. The disconnect between high BP and high chlorophyll a concentration could be the result of undersaturation of the $^3$H-leucine tracer; Kaartokallio et al. (2013) proposed that sea ice BP measurements should use tracer concentrations as high as those used for biofilms, rather than the low concentrations used for seawater. Alternatively it has been proposed that the production of bacteriostatic compounds by ice algae may be responsible for suppressing BP (Monfort et al., 2000; Pusceddu et al., 2009).

The physical stressors in sea ice, reviewed by Ewert and Deming (2013), may also play a role in suppressing BP. Martin et al. (2009) reported that osmotic stress and UV-B radiation significantly reduced single-cell activity levels in Antarctic sea ice. Although in the Antarctic the period of maximal sea ice melt (late summer) is temporally decoupled from the time of maximum UV-B flux (late winter), environmental stress could play a role in BP/PP decoupling if ice algae and sea ice bacteria are differentially affected by these stressors. Further confusing the issue are methodological differences in BP estimates imposed by the semi-solid nature of the ice matrix (Miller et al., 2015). Some studies evaluating BP in the context of chlorophyll a concentration have worked around this issue by focusing on the liquid brine fraction of the sea ice (Grossmann, 1994), while others have employed a variety of melt techniques (Pusceddu et al., 2009; Søgaard et al., 2010; Paterson and Laybourn-Parry, 2011b; Baer et al., 2015), or evaluated crushed ice slurries (Guglielmo et al., 2000; Kuosa and Kaartokallio, 2006; Kaartokallio et al., 2008). These methods result in varying, and as yet unquantified, differences between the in situ and experimental salinities. Although there is no obvious relationship between the method used and the degree of inhibition, possible methodological biases must be kept in mind.

**Figure 4** Conceptual sketch of sea ice community dynamics.

Values for bacterial production (BP) and respiration (R) were taken from Nguyen and Maranger (2011) and are given in µg C l$^{-1}$ d$^{-1}$. Bacterial growth efficiency (BGE) was calculated as BGE = BP/(R + BP). The x-axis scale was kept deliberately ambiguous to emphasize the conceptual nature of this plot and the geographical control of season. Although the illustration is representative of the Arctic, Antarctic, and subpolar sea ice, the exact timing of these events will vary with region. The top frame shows the hypothetical relative dominance of the sea ice assemblage by clades typically associated with seawater and clades typically associated with mature sea ice.

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Sea ice bacteria and biogeochemistry

A sea ice microbial loop?

The canonical microbial loop identifies heterotrophic bacteria (and archaea) as the means by which dissolved organic matter (DOM) can be re-packaged for consumption by higher trophic levels, starting with bacterivorous single-celled eukaryotes. Consistent with the observed high rates of BP, sea ice bacteria are known to associate with and stay active around particulate organic matter (POM) within sea ice (Junge et al., 2004; Meiners et al., 2008), and to contain the enzymatic machinery necessary to convert POM to DOM (Huston et al., 2000; Cowie et al., 2014). Protist grazing of sea ice bacteria, however, and the subsequent incorporation of ice-associated protists into larger organisms are understudied phenomena in sea ice. In very cold sea ice the geometry of brine channels should limit access to prey. In relatively warm basal ice, large brine channels may facilitate bacterivory. While heterotrophic protists (Kaartokallio, 2004; Gowling et al., 2004; Torstensson et al., 2015) and viruses (Gowing et al., 2004; Collins & Deming, 2011; Paterson & Laybourn-Parry, 2011a), the dominant predators of bacteria, are observed in sea ice, they are not known to exert a strong control on bacterial cell abundance (Gowing et al., 2004) (but see Wells and Deming, 2006; Torstensson et al., 2015).

Despite the uncertainty of a link between heterotrophic bacteria in sea ice and their consumers, a prerequisite for the microbial loop, sea ice bacteria play a significant role in the polar carbon cycle by enhancing sea ice primary production, a considerable amount of which is exported to the water column (Michels et al., 2008; Fernández-Méndez et al., 2014) and seafloor (Morata et al., 2010). Active phosphate (Helmke and Weyland, 1995; Cowie et al., 2014) and nitrogen (Guglielmo et al., 2000, 2004; Pusceddu et al., 2009; Baer et al., 2015) remineralization by sea ice bacteria helps to support regenerated sea ice primary production. Excess cobalamin (vitamin B12) production by sea ice bacteria has been hypothesized to support ice algal growth (Taylor and Sullivan, 2008), and epiphytic bacteria, observed to colonize sea ice diatoms abundantly (Grossi et al., 1984; Smith et al., 1989), have been hypothesized to mitigate oxygen stress (Hünken et al., 2008).

Other bacterial energy and carbon conversions in sea ice

Although heterotrophy is the dominant means of bacterial energy acquisition in sea ice, there is evidence for other energy conversions. Proteorhodopsin genes and gene transcripts have been observed in sea ice (Koh et al., 2010) and in the genomes of sea ice bacteria (Feng et al., 2013; Vollmers et al., 2013; Feng et al., 2014). In bacteria adapted to the open ocean, proteorhodopsin, which functions as a light-driven proton pump (Béjà et al., 2000), is thought to aid bacteria during periods of starvation by maintaining ATP regeneration (Akram et al., 2010). Because primary production is generally robust in spring and summer sea ice, and thus DOC should not be limiting, proteorhodopsin may serve a different function in sea ice bacteria. One possible function is the maintenance of osmotic balance; the psychrophilic sea ice isolate P. torquis was shown to utilize proteorhodopsin during salinity stress under carbon-replete conditions (Feng et al., 2013). It is worth noting, however, that energy acquisition from light is generally less temperature-limited than the process of catabolism (Morgan-Kiss et al., 2006); thus proteorhodopsin could also aid bacterial survival during periods when low temperatures may limit access to the energy stored in organic carbon. Though less common than proteorhodopsin-containing bacteria, aerobic anoxygenic phototrophs have also been observed in sea ice (Koh et al., 2011). Like proteorhodopsin-based energy acquisition, aerobic anoxygenic phototrophy is an accessory metabolism and may play a similar ecological role in sea ice.

Although sea ice is generally considered to be an oxygenated environment, the rate of heterotrophic activity can exceed the rate of oxygen production, particularly during the melting of low oxygen ice crystals in summer (Rysgaard and Glud, 2010). Under these conditions alternative oxidants to O2 can be used for metabolism, although field observations of the use of other oxidants are sparse. Kaartokallio (2001) observed denitrification in the interior of Baltic sea ice, while Rysgaard and Glud (2010) observed measurable rates of denitrification and anammox at two Arctic sites. Denitrification rates were estimated to be as up to 50% of sediment activity, suggesting that sea ice bacteria can be a major sink for nitrate.

Other nitrogen transformations that have been observed in sea ice include ammonium oxidation (Priscu et al., 1990; Baer et al., 2015) and the parent process of nitrification (Fripiat et al., 2015). These aerobic processes are significant because nitrification is a major source of energy for chemoautotrophic carbon fixation in the marine environment. Ammonia, often found at high concentration in sea ice (Haedecy and Andersson, 1999; Søgaard et al., 2010), is produced by ammonification during the heterotrophic degradation of proteins and other nitrogen-containing molecules, and is exuded by phytoplankton during periods of stress when nitrogen is not limiting (Lomas et al., 2000). How much of this ammonia is reasimilated by ice algae is not clear, though phytoplankton have been shown to increase their dependence on ammonia over nitrate at low temperatures (Reay et al., 1999). Fripiat et al. (2015) inferred from isotopic evidence that the rates of nitrate assimilation and nitrification in early spring sea ice are nearly equal (but see Baer et al., 2015), suggesting a strong coupling between ice algae and prokaryotic nitrifiers. When photosynthesis becomes light or nutrient limited, this system should become decoupled, with continued bacterial degradation of eukaryotic biomass fueling a period of carbon fixation by nitrifiers. In addition to the archael nitrifiers discussed previously, potential bacterial nitrifiers have been observed in young (Barber et al., 2014) and mature (Baer et al., 2015) sea ice.

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Known unknowns

Connecting community structure with activity

Remarkably few sea ice studies have attempted rate measurements or measurements of biogeochemical properties (other than chlorophyll a, EPS, and bacterial abundance) in parallel with data on bacterial community structure (Figure 1). Exceptions include studies by Kaartokallio et al. (2008) and Eronen–Rasimus et al. (2015) on bacterial community structure and BP in Baltic sea ice, and Cowie et al. (2014) on bacterial community structure and enzyme activity within Antarctic sea ice. Cowie et al. (2014) found that variable enzyme activity rates corresponded with different bacterial taxa in top, middle, and bottom sea ice horizons, but stopped short of implicating specific bacteria in the production of the identified enzymes. Kaartokallio et al. (2008) observed a clear shift in community composition and increase in BP during the transition from late winter to early spring; however, as in Cowie et al. (2014), the community members responsible for the enzyme activity are not known. Eronen–Rasimus et al. (2015) observed a similar shift in community composition and BP in the transition from young to mature Baltic sea ice. An additional consideration is the location of these latter two studies in the Baltic Sea, a region that has produced a wealth of knowledge on sea ice microbial ecology. While analyses of community composition have shown that Baltic sea ice bacterial communities are compositionally similar to polar sea ice communities (Kaartokallio et al., 2008; Eronen–Rasimus et al., 2015), the temperature regime, dynamics of ice formation, and seasonality of sea ice in the Baltic Sea differ from high latitude sea ice. The timing of sea ice formation and melt with respect to light conditions, for example, is much different at the lower latitudes of the Baltic Sea.

Two additional studies, by Junge et al. (2004) on Arctic wintertime sea ice and Baer et al. (2015) on Arctic summer sea ice, should be noted for the simultaneous evaluation of high level taxa and metabolic activity. Using FISH to identify the domains Bacteria and Archaea and members of the phylum Bacteroidetes, and using the redox sensitive dye 5-cyano-2,3-ditoyl tetrazolium (CTC) to identify aerobically respiring cells, Junge et al. (2004) noted that the proportion of bacteriodetes and CTC-labeled cells associated with particles increased with decreasing temperature, but did not report on the contribution of the different bacterial groups to the CTC-stained population. Similarly Baer et al. (2015) used FISH probes to identify the domain Bacteria, the archaeal phylum Crenarchaeota, and the bacterial classes Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, and Flavobacteria, finding a distribution consistent with a typical sea ice bacterial community. They could not, however, associate activity with these different groups, though the authors noted a potential link between betaproteobacteria and the observed nitrification.

Despite the paucity of studies evaluating both community structure and activity, it is apparent that community respiration is high within first year sea ice (Nguyen and Maranger, 2011) before the composition of the bacterial community is expected to shift from one dominated by seawater bacteria to one dominated by putatively indigenous sea ice bacteria, based on the seasonal period observed by Collins et al. (2010). This timing makes sense in the context of the ecology of typical seawater and sea ice bacteria. In the water column, motile bacteria preferentially seek out high DOC microenvironments around phytoplankton cells and aggregates (Stocker, 2012). Slower growing, non-motile bacteria passively await the onset of high-carbon conditions. During the process of sea ice formation, a highly favorable carbon regime is imposed on water column bacteria as they are sequestered into pore spaces containing high concentrations of POM (Meiners et al., 2004, 2008), though this bonanza is offset by the physical challenges of low temperature and high salinity, and the potential recalitrance of sea ice POM (Underwood et al., 2010). High rates of BP have been observed in young sea ice (Grossmann and Dieckmann, 1994; Paterson and Laybourn-Parry, 2011b), however, these conditions do not persist through the winter. The onset of photosynthesis opens a new ecological niche for sea ice specialists capable of rapidly incorporating fresh DOC into bacterial biomass (Figure 4).

Connecting community structure with function

The disconnect between observations of activity and community structure, combined with the relatively limited number of sequenced sea ice isolates, has made it difficult to connect bacterial community structure with biogeochemical function. The phylogenetic placement of sea ice 16S rRNA gene reads on a tree of 16S rRNA genes from completed genomes provides a convenient, if incomplete, view of which reads are poorly represented by published genomes. When a read cannot be definitely placed with one of the published genomes, it is placed to an ancestral genome. Such placement was also observed for bacteriodetes reads, many of which placed to ancestral genomes in the families Flavobacteriaceae and Cyclobacteriaceae, and for betaproteobacteria reads, many of which placed to the order Burkholderiales. Even placement to a genome representing a terminal node is, however, no guarantee that the reference genome is a good representation of the corresponding genome.
in sea ice. For parts of the reference tree where phylogenetic resolution is poor, the best reference genome could be a distant relative to the sea ice strain. A good example of such distance is the verrucomicrobium Coraliomargarita akajimensis DSM45221, the best matching genome for a large number of sea ice reads. Although it is clear that strains closely related phylogenetically to C. akajimensis DSM45221 are present in sea ice, and while they likely share certain genomic and ecological features, there may be significant differences as well. These clades are good targets for isolation and sequencing, and for genome reconstruction from environmental sequencing efforts.

The problem of connecting community structure to function is exasperated by the limited number of functional genes studies available for sea ice. Targeted functional gene studies have identified genes involved in mercury transformations (Møller et al., 2014), nitrogen fixation (Díez et al., 2012), anoxygenic aerobic photosynthesis (Koh et al., 2011), and proteorhodopsin (Koh et al., 2010). At the time of writing only two metagenomes were available for any sea ice environment and they were for young sea ice and frost flowers in the Arctic (Bowman et al., 2014). These metagenomes contained a variety of biogeochemically interesting genes, including genes coding for haloperoxidases and others involved in sulfur cycling, but are not representative of mature sea ice. Two investigations have used metatranscriptomics to evaluate metabolic function among the ice algal communities along the West Antarctic Peninsula (Toseland et al., 2013; Pearson et al., 2015). Although the methods employed by these studies were not exclusive of prokaryotic mRNA (i.e., there was no selection for transcripts with poly-A tails), the subsequent analyses and annotation were specific to eukaryotic gene transcripts, and the studies did not highlight bacterial community functions.

To bridge the gap between bacterial community structure and metabolic function in the absence of direct observations, I made an inference of likely metabolisms from the available 16S rRNA genes using PAPRICA (Bowman and Ducklow, 2015). In brief, metabolic pathways were predicted (Karp et al., 2010) on the genomes previously assigned by phylogenetic placement to reads from sea ice 16S rRNA gene libraries. For placements to internal nodes on the reference tree, metabolic pathways were predicted for a consensus genome defined as all genes shared by all clade members.

The three study sets used to describe community structure contained a combined 798 metabolic pathways, with a subset of high biogeochemical or ecological interest highlighted in Table 5. Nearly all of these pathways were associated with genomes represented in all three datasets, suggesting that they are broadly distributed in the sea ice environment. The exception is denitrification, a metabolic process that was not associated with any phylogenotypes found in Bowman et al. (2012). This absence could be due to a lack of anaerobic environments within that ice, or could result from the conservative nature of the metabolic inference technique; a false negative can be achieved through incomplete depth of sequencing, incomplete or improper genome annotation, or the absence of a strain that is closely related phylogenetically with a sequenced genome. Thus a negative result does not constitute evidence for the absence of a process in sea ice.

Metabolic inference predicted several processes that have been underreported in situ biogeochemical analysis, including prokaryotic CO\textsubscript{2} fixation (but see Priscu et al., 1990), C\textsubscript{3} metabolism, halocarbon degradation, nitrogen fixation, choline degradation, glycine betaine production, and sulfate and sulfate oxidation. Among these processes nitrogen fixation has particular significance, given the high nitrogen demand of both eukaryotic and prokaryotic primary producers. Although nifH genes from a variety of taxonomic groups have been reported in sea ice (Díez et al., 2012), the recurring C. akajimensis DSM45221 related phylogotype was not recognized as a potential diazotroph by Bowman et al. (2012) or Hatam et al. (2014). Because C. akajimensis DSM45221 was isolated in association with hard coral, the potential associative ecology of a sea ice Coraliomargarita phylotype fits well with bacteriodes, gammaproteobacterial GSOS, and other sea ice associated groups.

**Temporal and spatial variation**

The sea ice environment is defined by strong spatial and temporal gradients. Microbiological sampling of sea ice, however, has often ignored these gradients or integrated them across by necessity. Extracting enough DNA or RNA for sequence analysis often requires a liter or more of seawater, equivalent to a 15.7 cm section of ice from a standard 9 cm diameter core. Thus the maximum resolution of microbial community structure within sea ice might be considered as 15.7 cm. Recent work has resolved differences in bacterial community structure at multiple horizons within multiyear (Hatam et al., 2014) and first year (Cowie et al., 2014) sea ice, but observations are lacking at finer spatial scales. Microscopic analysis of sea ice algae (Kottmeier and Sullivan, 1988) and pore spaces (Krembs et al., 2002; Junge et al., 2004) suggest that microbial communities are structured in sea ice at micrometer scales. More advanced techniques are now being applied to other environments to make observations at these spatial scales, including MAR-FISH, NANO-SIMS, micro-RAMAN, and single-celled sequencing. While there will be significant technical challenges in transferring this technology to sea ice, these and related techniques will ultimately link sea ice community structure and function on the scales most relevant to the microbial community.

Temporal gradients present an additional challenge to understanding sea ice microbial ecology. While it is clear that the young sea ice bacterial community present in the fall and winter is considerably different from the bacterial community present within spring and summer first year sea ice (Collins et al., 2010), the
dynamics of this transformation have not been observed in detail. One study (Kaartokallio et al., 2008) that involved sampling Baltic sea ice during the months of January, February, and March showed a clear shift in the bacterial community (and a corresponding shift in BP). While that study showed some recognizable features of the expected sea ice succession, with members of the SAR11 clade appearing only in January, bacteriodetes appearing in February and March, and gammaproteobacteria appearing only in March, both a greater taxonomic and temporal resolution is desired – along with an extension to different latitudes and regions. The authors also noted that the coastal nature of the sampling site, with a water column bacterial assemblage abundant in bacteriodetes, might represent an atypical scenario.

An overarching temporal gradient at high latitudes is the warming atmosphere and ocean. This warming has an effect on the duration and extent of sea ice in the Arctic and Antarctic, although the specific response of sea ice to changing climate varies regionally. In the Arctic multiyear sea ice is being replaced by first year sea ice, with major reductions in the overall age of sea ice and in its minimal summertime extent (Boé et al., 2009). In northern regions of the West Antarctic the ice season has decreased dramatically since 1981 (Ducklow et al., 2013), though warming has been less pronounced further south (including the East Antarctic). While numerous studies have explored the implications of reduced ice-associated primary production on the marine ecosystem (e.g., Boetius et al., 2013; Saba et al., 2014), there are few data on the ramifications of a reduction in ice-associated bacteria, or a functionally or compositionally altered sea ice bacterial community.

Reduced nutrient regeneration in the surface ocean is one possible consequence of a reduction in the duration and extent of the sea ice bacterial community. As noted previously the sea ice ecosystem is strongly syntrophic, with sea ice bacteria and algae exhibiting strong symbiotic partnerships. These partnerships can involve the exchange of macronutrients (such as phosphate and nitrogen) and micronutrients (such as iron and vitamin B₁₂) for fixed carbon. A major unknown is whether sea ice is a net source or sink of these nutrients.
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across the annual cycle. During periods of peak growth ice algae can exhibit nutrient stress (Lizotte and Sullivan, 1992; McMinn et al., 1999), indicating that sea ice is a net sink (demand exceeds production). Post bloom, however, when PP has decreased but BP might remain high (Paterson and Laybourn-Parry, 2011b), excess vitamin B12 production (and the remineralization of other nutrients) could support continued or future PP in the water column. The flux of nutrients to the polar photic zone could be reduced if late summer sea ice bacterial growth is limited by a loss of ice; however, this reduction could increase the quality and quantity of sea ice derived POM that is released into the water column during ice melt (Fortier et al., 2002). The idea of reduced in situ remineralization, analogous to a weakened microbial loop, can be extended to the period of ice formation as well. Because the winter sea ice bacterial community reflects the composition of the source seawater (Collins et al., 2010), the timing of sea ice formation can have an impact on the functions encapsulated within winter sea ice. Later formation from more oligotrophic surface waters could inoculate sea ice with bacteria less optimized to POM remineralization, leaving spring sea ice less nutrient-rich.

A warming climate could also change the exogenous contributions to the sea ice bacterial community. One possible implication of reduced sea ice cover in the Arctic is increasing snowfall (Deser et al., 2010). Greater snowfall could increase the flux of actinobacteria, bacteriodetes, cyanobacteria, and other snow-associated bacteria (Møller et al., 2013) to the upper horizons of sea ice while at the same time decreasing ice freeboard. This change could result in ice with a weaker thermal gradient that nonetheless has a more strongly stratified bacterial community. Increased glacial melt in both the Arctic and Antarctic could similarly alter the surface seawater community that is incorporated into newly forming sea ice (Vincent, 2010). Good models for both of these scenarios can probably be found in the contemporary sea ice of different regions. Antarctic sea ice, for example, generally receives more snowfall than Arctic sea ice, giving rise to surface flooding and algal blooms at the ice surface, phenomena that are not often observed in the Arctic. The analysis of differences in structure and function between Arctic and Antarctic sea ice bacterial communities, and between communities at finer regional scales, could provide important insights into future change. Currently our ability to make these comparisons is hampered by the low geographic diversity of favored sample sites (Figure 1), imposed in many cases by logistical constraints, and by limited cross-site coordination.

Unknown unknowns

A goal of the sea ice biogeochemical and microbial ecology communities is to develop a holistic, mechanistic understanding of the interplay between the biological, chemical, and physical components of the sea ice system. Models can be a powerful tool for developing a system-level understanding because they serve as a convenient inventory of the components of a system and their interactions. One example is the widely hypothesized but rarely observed exchange of metabolites between sea ice bacteria and algae. Future studies will need to avoid the temptation to study these groups as discrete units and consider the coupled sea ice bacteria–algae system; however, it is methodologically challenging to observe specific metabolic exchanges. A conceptual model, such as recently published by Amin et al. (2015), can help by identifying what exchanges are likely to occur, while a quantitative model can provide support for their occurrence. The complexity of models, however, scales with the number of components and their interactions. As a result, modelers are often reluctant to reproduce a sufficient number of interactions to provide a realistic experimental framework for observationalists, while observationalists are often reluctant to reduce the complexity of their system so that it is amenable to modeling (Steiner et al., 2015, under review for this Special Feature). Developing a framework that can accommodate both the known knowns and the known unknowns of sea ice biogeochemistry, and that can be used to experimentally identify unknown unknowns, will require a close and creative coordination between sea ice biogeochemical modelers and observational microbial ecologists. This effort will be aided by future improvements in our understanding of bacterial community function.

Underlying bacterial community function is community genetics, with the link between these features mediated by the complex (and poorly understood) process of gene expression. The close coupling between genetics and function provides an opportunity for mechanistic modeling, informed by community gene expression and biogeochemical observations, that is only beginning to be explored in other systems (Röling and van Bodegom, 2014). Although the genetic capacity of sea ice isolates and the in situ sea ice bacterial community is underexplored, adding challenge to the analysis of metatranscriptomic and metaproteomic datasets, the spatially constrained nature of the sea ice environment means that it is more amenable to a complete, systems level characterization than the water column. Such a characterization would be aided by the strong spatial and temporal gradients present within sea ice (e.g., seasonal succession), which serve as naturally repeating experimental treatments. These features should be taken advantage of not only to further our understanding of sea ice, a critical component of high latitude marine ecosystems, but also to develop a framework for better understanding all environments within the Earth system.
References


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Competing interests

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