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

Jeff S. Bowman\textsuperscript{1,2*}

\textsuperscript{1}Lamont-Doherty Earth Observatory, Palisades, New York, United States
\textsuperscript{2}Blue Marble Space Institute of Science, Seattle, Washington, United States
*bowmanjs@ldeo.columbia.edu

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 Summer Time 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
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 Ikaävalko, 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 cryoprotective 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 (McLlan, 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 measurable 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 Gammaproteobacteria 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 *Psychodoleromonas* (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 anoxicogenic 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 (Diez 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; Torstenson 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 Bacteriodetes 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 Glacieola agarilytica 4H37YE5, bacteriodetes Flavobacteriaceae spp., the betaproteobacterium Rhodobacter 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 2ii). 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...
Table 1. Definitions of key terms and abbreviations

| Term                              | Abbreviation | Definition                                                                                                                                 |
|-----------------------------------|--------------|-------------------------------------------------------------------------------------------------------------------------------------------|
| 5-cyano-2,3-dinitol tetrazolium   | CTC          | Dye that fluoresces under blue light excitation when reduced in actively respiring cells                                                   |
| Bacterial production              | BP           | The amount of carbon incorporated into bacterial biomass; typically estimated from the uptake of radiolabeled thymidine or leucine              |
| Clade                             |              | The descendants of an ancestral phylotype                                                                                                 |
| Community composition             |              | The taxonomic makeup of a microbial community                                                                                              |
| Community structure               |              | The proportional taxonomic makeup of a microbial community                                                                               |
| Copiotroph                        |              | A microorganism optimized for the rapid uptake of high concentrations of organic matter                                                  |
| Denatured gel gradient electrophoresis | DGGE         | 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 |
| Dissolved organic carbon          | DOC          | Typically operationally defined as carbon that can pass through a 0.7 µm filter                                                          |
| Fluorescent in-situ hybridization  | FISH         | Epifluorescent microscopy technique for identifying different microbial taxa; highly quantitative                                            |
| Genomic plasticity                |              | Genomic variability due to gene gain or loss                                                                                               |
| Microautoradiography              | MAR          | A microscopy technique that identifies radiolabeled substrate uptake by specific bacterial cells, based on the ability of a cell to expose a photographic emulsion |
| Metagenome                        |              | 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 |
| Metaproteome                      |              | The product of a mass spectrometry technique that identifies random peptide fragments from an environment, providing a quantitative overview of translated genes |
| Metatranscriptome                 |              | The product of a sequencing technique that sequences random fragments of mRNA from an environment, providing a quantitative overview of transcribed genes |
| Multiyear sea ice                 | MYI          | Sea ice that has survived one summer melt cycle                                                                                           |
| Next generation sequencing        | NGS          | Sequencing by any method of greater throughput than Sanger sequencing, here referring to 454 and Illumina sequencing                          |
| Node                              |              | 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). |
| Oligotrophic                     |              | An environment characterized by low concentrations of DOC                                                                                 |
| Particulate organic matter        | POM          | Typically operationally defined as the organic component of particles captured on a 0.7 µm filter                                            |
| Phylotype                         |              | 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) |
| Primary production                | PP           | The amount of inorganic carbon reduced to organic carbon by primary producers; typically estimated from the uptake of radiolabeled bicarbonate |
| Psychrophile                      |              | An organism optimized to grow at low temperatures; for bacteria, often operationally defined as $T_{\text{max}}$ of growth < 20 °C              |
| Young sea ice                     |              | Newly formed sea ice, with a microbial community that is functionally distinct from that found in mature sea ice.                           |

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 datasets 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,
Table 2. Sea-ice studies included in the phylogenetic analyses of Figure 2

| Study                   | Sample type | Sequencing approach | Notes                                      |
|------------------------|-------------|---------------------|--------------------------------------------|
| Bowman et al. (1997b)  | Isolates    | Sanger              |                                            |
| Brown and Bowman (2001)| Environmental | Sanger            |                                            |
| Petri and Imhoff (2001)| Environmental | Sanger            |                                            |
| Junge et al. (2002)    | Isolates    | Sanger              |                                            |
| Brinkmeyer et al. (2003)| Isolates    | Sanger              | Arctic/Antarctic comparison               |
| Groudieva et al. (2004)| Isolates    | Sanger              |                                            |
| Gerdes et al. (2005)   | Both types  | Sanger              | In situ crude oil addition                 |
| Brakstad et al. (2008) | Environmental | Sanger            | In situ crude oil addition                 |
| Kaartokallio et al. (2008)| Environmental | Sanger    |                                            |
| Collins et al. (2010)  | Environmental | Sanger            | Winter sea ice                             |
| Koh et al. (2010)      | Environmental | Sanger            | 16S and proteorhodopsin genes             |
| Bowman et al. (2012)   | Environmental | NGS (454)           |                                            |
| Cowie et al. (2011)    | Environmental | Sanger            | Archaea only                              |
| Martin et al. (2011)   | Environmental | Sanger            | In situ transplant experiment              |
| Maas et al. (2012)     | Environmental | Sanger            |                                            |
| Møller et al. (2011)   | Isolates    | Sanger              | Mercury resistant isolates                 |
| Cowie et al. (2014)    | Environmental | Sanger            |                                            |
| Okubo et al. (2014)    | Environmental | Sanger            |                                            |
| Hatam et al. (2014)    | Environmental | NGS (454)           |                                            |
| Eronen-Rasimus et al. (2015)| Environmental | Sanger |                                            |

do: 10.12952/journal.elementa.000072.t002

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. Alternately 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

| Study                      | BP (ng C l\(^{-1}\) h\(^{-1}\)) | PP (ng C l\(^{-1}\) h\(^{-1}\)) | Chlorophyll \(a\) (µg l\(^{-1}\)) |
|----------------------------|----------------------------------|----------------------------------|----------------------------------|
| Kottmeier et al. (1987)    | 257 ± 355                        |                                  |                                  |
| Kottmeier and Sullivan (1988) |                                  |                                  |                                  |
| Bunch and Harland (1990)   |                                  |                                  |                                  |
| Smith and Clement (1990)   | 68.9 ± 30.4                      |                                  |                                  |
| Grossman and Dieckmann (1994) | 5.63 ± 4.19                     | 950 ± 768                        | 3.21 ± 3.46                     |
| Grossmann (1994)           |                                  |                                  |                                  |
| Helmke and Weyland (1995)  | 44.4 ± 59.0                      |                                  |                                  |
| Grossmann et al. (1996)    |                                  |                                  |                                  |
| Mock et al. (1997)         | 690 ± 391                        |                                  |                                  |
| Haecy and Andersson (1999) |                                  |                                  |                                  |
| Guglielmo et al. (2000)    | 5.11 ± 3.60                      |                                  | 841 ± 1440                      |
| Guiglielmo et al. (2004)   |                                  |                                  |                                  |
| Junge et al. (2004)        |                                  |                                  |                                  |
| Kaartokallio et al. (2004) | 126\(^{a}\)                      |                                  |                                  |
| Kaartokallio et al. (2005) |                                  |                                  |                                  |
| Kuosa et al. (2006)        | 230 ± 138                        | 3270 ± 1520                     | 9.58 ± 6.36                     |
| Kaartokallio et al. (2007) |                                  |                                  |                                  |
| Koparinen et al. (2007)    |                                  |                                  |                                  |
| Kaartokallio et al. (2008) | 170 ± 106                        | 11.2 ± 10.5                     |                                  |
| Martin et al. (2009)       | 740\(^{c}\)                      |                                  |                                  |
| Pusceddu et al. (2009)     | 11.3 ± 10.5                      | 1810 ± 731                      |                                  |
| Sagaard et al. (2010)      | 11.5 ± 5.60                      | 12.5 ± 3.54                     | 0.25 ± 0.13                     |
| Koparinen et al. (2011)    |                                  |                                  |                                  |
| Nguyen and Maranger (2011) | 427 ± 272                        | 378 ± 785                       |                                  |
| Paterson and Laybourn-Parry (2011b) | 224 ± 271                        | 14.0 ± 13.4                     |                                  |
| Sagaard et al. (2013)      | 73.5 ± 65.8                      | 142 ± 240                       |                                  |
| Kaartokallio et al. (2013) | 361 ± 508                        |                                  |                                  |
| Cowie et al. (2014)        |                                  |                                  |                                  |
| Eronen-Rasimus et al. (2015) |                                  |                                  |                                  |
| Zhou et al. (2014)         |                                  |                                  |                                  |
| Baer et al. (2015)         | 13.9 ± 21.3\(^{c}\)             | 662 ± 1520                      | 73.6 ± 167                      |

\(^{a}\) 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 mean value was reported; 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.

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 nifH (Díez et al., 2012) gene sequences have been observed in Arctic MYI, and filamentous cyanobacterial morphologies have been observed by microscope (Díez et al., 2012), they may be of allochthonous origin (Walseron 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

| Phylotype/Clade                      | Sanger | Bowman et al. (2012) | Hatam et al. (2014) |
|--------------------------------------|--------|----------------------|---------------------|
| Psychromonas ingrahamii 37           | 0.011  | 0                    | 0                   |
| Pseudoalteromonas haloplanktis TAC125| 0.019  | 0                    | 0                   |
| Shewanella frigidimarina NCIMB400    | 0.010  | 0                    | 6.24E-05            |
| Acinetobacter haumanii strains       | 0.017  | 0                    | 0                   |
| Psychrobacter spp.                   | 0.017  | 0                    | 0                   |
| Stenotrophomonas maltophilia R551    | 0.010  | 0                    | 0                   |
| Cyanoacteria spp.                    | 0.041  | 0                    | 0.000249            |
| Nitrosopumilus maritimus SCM1        | 0.080  | 0                    | 6.24E-05            |
| Phycisphaera mikurensis NBRC102666  | 0.020  | 8.41E-05             | 0.000374            |
| Roseobacter spp.                     | 0.015  | 0                    | 0                   |
| Octadecabacter arcticus 238         | 0.023  | 0.000925             | 6.24E-05            |
| Candidatus Pelagibacter ubiqu HTCC1062| 0.023 | 0.00135              | 6.24E-05            |
| Calceolaria psychrophythusa 34H      | 0.050  | 0                    | 0.00343             |

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

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...
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 \textit{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.
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 (Guggisbro-Lmcino 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 (Bejä et al., 2000), is thought to aid bacteria during periods of starvation by maintaining ATP regeneration (Aksam 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 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 (Frip 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 (Haecky 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 reassimilated 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). Frip 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 archaeal nitrifiers discussed previously, potential bacterial nitrifiers have been observed in young (Barber et al., 2014) and mature (Baer et al., 2015) sea ice.
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 Bacteriodetes, and using the redox sensitive dye 5-cyano-2,3-ditolyl 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 recalcitrance 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, consensus genome, as seen for many of the abundant and widely shared genomes in Figure 2. Despite the fact that several sea ice associated gammaproteobacteria have had their genomes sequenced, including Colwellia psychrerythraea 34H, Psychromonas ingrahamii 37, Pseudoalteromonas spp., Marinomonas sp. Bsi20584, and Glaciecola psychrophila 170, many gammaproteobacteria sea ice reads still 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 gene 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), anaerobic 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 phylotypes 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 in situ biogeochemical analysis, including prokaryotic CO$_2$ fixation (but see Priscu et al., 1990), C$_3$ metabolism, halocarbon degradation, nitrogen fixation, choline degradation, glycine betaine production, and sulfite 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 phylotype 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 bacterioetes, 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 across them 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
### Sea ice bacteria and biogeochemistry

**Table 5. Pathways of special biogeochemical interest identified through metabolic inference**

| Function                  | Pathway                     | Sanger studies          | Hazam et al. (2014)         | Bowman et al. (2012)          |
|---------------------------|-----------------------------|-------------------------|-----------------------------|-------------------------------|
| CO₂ fixation              | CO₂ fixation into oxaloacetate (anaplerotic) | *Pseudalteromonas haloplanktis* TAC125 | *Polaribacter MED152, Acidimicrobiiales YM16-304 | *Polaribacter cryohalolentis K5, Polaribacter MED152* |
| Antibiotic resistance     | Triclosan resistance        | *Pelagibacter ubique HTCC1062, Polaribacter MED152* | *Polaribacter MED152, Leadbetterella cryosophila DSM17132, Thiomicrosps spp., Glaccapsa PCC7428, Acidimicrobiiales YM16-304, Jannichinobacterium spp.* | *P. cryohalolentis K5, Polaribacter MED152, GSOS* |
| C1 metabolism             | Formaldehyde oxidation II (glutathione-dependent) | *Colwellia psychrerythraea 34H* | *Glaccapsa PCC7428, Marinobacter BS-20148, Glacieola nitratireducens FR1064* | *Octadecabacter antarcticus 307* |
| Choline degradation       | Choline degradation I       | *C. psychrerythraea 34H* | *Acidimicrobiiales YM304* | *P. cryohalolentis K5, O. antarcticus 307* |
| Glycine betaine production| Glycine betaine biosynthesis I (Gram-negative bacteria) | *C. psychrerythraea 34H* | *Acidimicrobiiales YM304* | *P. cryohalolentis K5, O. antarcticus 307* |
| Halocarbon degradation    | 2-chlorobenzoate degradation | *P. cryohalolentis K5* | *Polaromonas naphthaleniowano CJ2* | *P. cryohalolentis K5* |
| Mercury conversion        | Phenylmercury acetate degradation | *Marinobacter BS-20148, P. haloplanktis TAC125, Octadecabacter arcticus 238* | *Bellellia ballica DSM15983, Bordetella petri* | *O. antarcticus 307* |
| Nitrogen fixation         | Nitrogen fixation           | *Coralimargarita akajimensis DSM45221* | *C. akajimensis DSM45221, Methylomonas methanica MC09, Aeromonas spp.* | *C. akajimensis DSM45221* |
| Sulfite oxidation         | Sulfite oxidation II/III    | *Pelagibacter ubique HTCC1062* | *Celledchiro japonicus UEDA107* | *GSOS* |
| Sulfate reduction         | Sulfate reduction IV/V      | *Halomonas elongata DSM2581, Psychrobacter arcticum 273* | *Viriod vulnificus YJ016* | *GSOS* |
| Denitrification           | Nitrate reduction I/VII     | *C. psychrerythraea 34H* | *C. japonicus UEDA107* | *-* |

*Taxonomy of the nodes contributing the pathways in each dataset are given in the respective columns.

Complete pathway names are according to the MetaCyc nomenclature.

doi: 10.12952/journal.elementa.000072.t005

---

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...
Sea ice bacteria and biogeochemistry

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 $B_12$ 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 inferplay 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.
Sea ice bacteria and biogeochemistry

References

Akram N, Lindell K, Pedersen A, Neutze R, Pinhasi J, et al. 2010. Proteorhodospin phototrophy promotes survival of marine bacteria during starvation. PLoS Biol 8(4):2–11. doi: 10.1371/journal.pbio.1000358.

Amin SA, Hmelo LR, van Tol HM, Durham BP, Carlson LT, et al. 2015. Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. Nature. doi: 10.1038/nature14488.

Arrigo KR, Thomas DN. 2004. Large scale importance of sea ice biology in the Southern Ocean. Antartic Sci 16(4):471–486. doi:10.1017/S0954102004002263.

Azam F, Graf JS. 1983. The ecological role of water-column microbes in the sea. Mar Ecol-Prog Ser 10:257–263.

Baer SE, Connelly TL, Bronk DA. 2015. Nitrogen uptake dynamics in landfast sea ice of the Chukchi Sea. Polar Biol. doi: 10.1007/s00300-014-1639-y.

Barber DG, Ehn JK, Pucho M, Rygsgaard S, Deming JW, et al. 2014. Frost flowers on young Arctic sea ice: The climatic, chemical and microbial significance of an emerging ice type. J Geophys Res-Atmos 119:11593–11612. doi:10.1002/2014JD021736.

Beja O, Aravind L, Koonin EV, Suzuki MT, Hadd A, et al. 2000. Bacterial rhodopsins: Evidence for a new type of phototrophy in the sea. Science 289(5486):1902–1906.

Bian F, Xie B-B, Qin Q-L, Shu Y-L, Zhang X-Y, et al. 2012. Genome sequences of six Pseudalteromonas strains isolated from Arctic sea ice. J Bacteriol 194(4):908–909. doi:10.1128/JB.06427-11.

Boe J, Hall A, Qiu X. 2009. September sea-ice cover in the Arctic Ocean projected to vanish by 2100. Nat Geosci 2(5):341–343. doi:10.1038/ngeo667.

Boetius A, Albrecht S, Bakker K, Bienhold C, Felden J, et al. 2013. Export of algal biomass from the melting Arctic sea ice. Science 339(6126):1430–1432. doi: 10.1126/science.1231346.

Bowman JP, Gosink JJ, McCammon SA, Skerratt H, Lewis TE, et al. 1997a. Colwellia demingiae sp. nov., Colwellia bornesi sp. nov., Colwellia eussenais sp. nov. and Colwellia psychrotropa sp. nov: Psychrophilic Antarctic species with the ability to synthesize docosahexaenoic acid. Int J Syst Bacteriol 48:1171–1180.

Bowman JP, McCammon SA, Brown MV, Nichols DS, McMeekin TA. 1997b. Diversity and association of psychrophilic bacteria in Antarctic sea ice. Appl Environ Microbiol 63(8):3068–3078.

Bowman JP, McCammon SA, Nichols PD, Skerratt JJ, Rea SM, et al. 1997c. Shewanella gelidimarina sp. nov. and Shewanella frigida-marina sp. nov., novel Antarctic species with the ability to produce eicosapentaenoic acid and grow anaerobically by dissimilatory Fe(III) reduction. Int J Syst Bacteriol 47(4):1040–1047.

Bowman JS, Berthiaume CT, Armbrust EV, Deming JW. 2014. The genetic potential for key biogeochemical processes in Arctic frost flowers and young sea ice revealed by metagenomic analysis. FEMS Microb Ecol 89(2):376–387. doi:10.1111/1574-6941.12331.

Bowman JS, Deming JW. 2014. Alkane hydroxylase genes in psychrophile genomes and the potential for cold active catalysis. BMC Genomics 15(1):1–11. doi:10.1186/1471-2164-15-1120.

Bowman JS, Ducklow HW. 2015. Microbial communities can be described by metabolic structure: A general framework and application to a seasonally variable, depth-stratified microbial community from the coastal West Antarctic Peninsula and application to a seasonally variable, depth-stratified microbial community from the coastal West Antarctic Peninsula. PLoS One in press.

Bowman JS, Rasmussen S, Blom N, Deming JW, Rygsgaard S, et al. 2012. Microbiological structure of Arctic multiyear sea ice and surface seawater by 454 sequencing of the 16S RNA gene. ISME J 6(11):11–20. doi:10.1038/ismej.2011.76.

Brakstad OG, Nonstad I, Faksness LG, Brandvik PJ. 2008. Responses of microbial communities in Arctic sea ice after contamination by crude petroleum oil. Microbial Ecol 55:540–552. doi:10.1007/s00248-007-9299-x.

Brinkmeyer R, Glockner F, Helmke E, Amann R. 2004. Predominance of Betaproteobacteria in summer melt pools on Arctic pack ice. Limol Oceanogr 49(4):1013–1021.

Brinkmeyer R, Knittl K, Ju J, Weyland H, Amann R. 2004. Predominance of Betaproteobacteria in summer melt pools on Arctic pack ice. Limol Oceanogr 49(4):1013–1021.

Brown MV, Bowman JP. 2001. A molecular phylogenetic survey of sea-ice microbial communities (SIMCO). FEMS Microbiol Ecol 35(3):267–275.

Bunch JN, Harland RC. 1990. Bacterial production in the bottom surface of sea ice in the Canadian subarctic. Can J Fish Aquat Sci 47:1986–1995. doi:10.1139/f90-223.

Collins RE, Deming JW. 2011. Abundant dissolved genetic material in Arctic sea ice through an Arctic winter. Environ Microbiol 12(7):1828–1841. doi:10.1111/j.1462-2920.2010.02179.x.

Collins RE, Deming JW. 2010. Persistence of bacterial and archaeal communities in sea ice through an Arctic winter. Environ Microbiol 12(7):1828–1841. doi:10.1111/j.1462-2920.2010.02179.x.

Collins RE, Deming JW. 2011. Antarctic sea-ice microbial communities show distinct patterns of zonation in response to algal-derived substrates. Aquat Microb Ecol 63(2):123–134. doi:10.3354/ame01710.

Deser C, Tomas R, Alexander M, Lawrence D. 2010. The seasonal atmospheric response to projected Arctic sea ice loss in the late twenty-first century. J Clim 23(2):333–351. doi:10.1175/2009JCLI3053.1.

Diaz B, Bergman B, Pedrós-Abad C, Ánto M, Snoeijis P. 2012. High cyanobacterial nifH gene diversity in Arctic seawater and sea ice brine. Environ Microbiol Rep 4(3):360–366. doi:10.1111/j.1758-2229.2012.00343.x.

Ducklow H, Fraser WR, Meredith MP, Stammerjohn SE, Doney SC, et al. 2013. West Antarctic Peninsula: An ice dependent coastal marine ecosystem in transition. Oceanography. doi:10/5670/oceanog.2013.62.

Ducklow HW. 2003. Seasonal production and bacterial utilization of DOC in the Ross Sea, Antarctica. Biogeochemistry of the Ross Sea 78: 143–158.

References
Sea ice bacteria and biogeochemistry

Ducklow HW, Carlson CA. 1992. Oceanic bacterial production, in Marshall KC, ed., Advances in Microbial Ecology. pp. 113–181.

Eronen-Rasimus E, Lyra C, Rintala J-M, Jürgens K, Ikonen V, et al. 2015. Ice formation and growth shape bacterial community structure in Baltic Sea drift ice. FEMS Microbiol Ecol 91.

Ewert M, Deming J. 2013. Sea ice microorganisms: Environmental constraints and extracellular responses. Biology 2(2): 603–628. doi: 10.3390/biology2020603.

Feng S, Powell SM, Wilson R, Bowman JP. 2013. Light-stimulated growth of protoctihodopsin-bearing sea-ice psychrophile Psychrobacter torquis is salinity dependent. ISME J 7(11): 2206–2213. doi: 10.1038/ismej.2013.97.

Feng S, Powell SM, Wilson R, Bowman JP. 2014. Extensive gene acquisition in the extremely psychrophilic bacterial species Psychrobacter torquis and the link to sea-ice ecosystem specialization. Genome Biol Evol 6(1): 133–148. doi: 10.1093/gbe/evu009.

Fernández-Méndez M, Wenzhöfer F, Peeken I, Sørensen HL, Glud RN, et al. 2014. Composition, buoyancy regulation and fate of algal aggregates in the Central Arctic Ocean. PLoS One 9(9): e107452. doi: 10.1371/journal.pone.0107452.

Forster J. 1887. Über einige Eigenchaften leuchtender Bakterien. Centr. Bakteriol. Parasitenk. 2: 337–340.

Fortier M, Fortier L, Michel C, Legendre L. 2002. Climatic and biological forcing of the vertical flux of biogenic particles under seasonal Arctic sea ice. Mar Ecol-Prog Ser 225: 1–16. doi: 10.3354/meps225001.

Fripiat F, Sigman DM, Massé G. 2015. High turnover rates indicated by changes in the fixed N forms and their stable isotopes in Antarctic landfast sea ice. J Geophys Res-Oceans 120: 3079–3097.

Garrison DL, Ackley SF, Buck KR. 1983. A physical mechanism for establishing algal populations in frazil ice. Nature 306(5941): 363–365. doi: 10.1038/306363a0.

Gerdes B, Brinkmeyer R, Dieckmann G, Helmkne H. 2005. Influence of crude oil on changes of bacterial communities in Arctic sea-ice. FEMS Microbiol Ecol 53: 129–139. doi: 10.1016/j.femsec.2004.11.010.

Giovannoni SJ, Britschgi TB, Moyer CL, Field KG. 1990. Genetic diversity in Sargasso Sea bacterioplankton. Nature 345: 60–63. doi: 10.1038/346183a0.

Golden KM. 1998. The percolation phase transition in sea ice. Science 282: 2238–2241. doi: 10.1126/science.282.5397.2238.

Gowing M, Garrison D, Gibson A, Krupp J, Jeffries M, et al. 2004. Bacterial and viral abundance in Ross Sea summer pack ice communities. Mar Ecol-Prog Ser 279: 3–12. doi: 10.3354/meps279003.

Gradinger R, Iakovlev J. 1998. Organism incorporation into newly forming Arctic Sea ice in the Greenland Sea. J Plankton Res 20(5): 871–886.

Grossmann S, Dieckmann GS. 1994. Bacterial activity in sea ice and open water of the Weddell Sea, Antarctica, a microautoradiographic study. Microbial Ecol 28: 1–18.

Grossmann S, Dieckmann GS. 1994. Bacterial standing stock, activity, and carbon production during formation and growth of sea ice in the Weddell Sea, Antarctica. Appl Environ Microbiol 60(8): 2746–2753.

Grossmann S, Loche K, Scharek R. 1996. Algal and bacterial processes in platelet ice during late austral summer. Polar Biol 16: 623–633. doi: 10.1007/s003000050097.

Groudeva T, Kambourova M, Yusef H, Royter M, Grote R, et al. 2004. Diversity and cold-active hydrolytic enzymes of culturable bacteria associated with Arctic sea ice, Spitzbergen. Extremophiles 8(6): 475–488. doi: 10.1007/s00792-004-0409-0.

Guglielmo L, Carrada GC, Catalano G, Cozzi S, Dell’Anno A, et al. 2004. Biogeochemistry and algal communities in the annual sea ice at Terra Nova Bay (Ross Sea, Antarctica). Polar Biol 27: 43–55. doi: 10.1007/s00300-005-0219-9.

Haecky P, Andersson A. 1999. Primary and bacterial production in sea ice in the northern Baltic Sea. Aquat Microb Ecol 17: 107–118. doi: 10.3354/ame201017.

Häkkinen E, Tuovinen TJ, Sunnucks P, Jacobsson M, Jämsä J, et al. 2015. Composition of Arctic sea-ice microbial communities and the link to sea-ice ecosystem specialism. GOSIB Ecol 22(1): 101–102.

Hauptmann AL, Stibal M, Balum J, Sithiritz-Pontén T, Brunak S, et al. 2014. Bacterial diversity in snow on North Pole ice. Extremophiles 18(6): 945–951. doi: 10.1007/s00792-014-0660-y.

Helmke E, Harder J, Kirst GO. 2008. Epiphytic bacteria on the Antarctic ice diatom Amphiprora kufferathii Manguin. Polar Biol 31(5): 623–633. doi: 10.1007/s00300-008-0219-7.

Helmke E, Weyland H. 1995. Bacteria in sea ice and underlying water of the eastern Weddell Sea in midwinter. Mar Ecol-Prog Ser 115(1): 231–242.

Helmke E, Weyland H. 1995. Bacteria in sea ice and underlying water of the eastern Weddell Sea in midwinter. Mar Ecol-Prog Ser 115(1): 231–242.
Sea ice bacteria and biogeochemistry

Junge K, Imhoff F, Staley T, Deming JW. 2002. Phylogenetic diversity of numerically important Arctic sea-ice bacteria cultured at subzero temperatures. Microbial Ecol 43(3): 315–328. doi: 10.1007/s00248-001-1026-4.

Karl DM. 2014. Solar energy capture and transformation in the sea. Elem Sci Anth 2: 800021. doi: 10.12952/journal.elementa.000021.

Kapraun RS, Mahaffey RD, Tsuji T, et al. 2016. Radiative forcing of natural Arctic sea ice communities: Evidence for nutrient stress during the spring bloom. Deep Sea Res Part I 102: 24–35. doi: 10.1016/j.dsr.2015.03.013.

Kershaw MJ, Kaila M, Kuosa H. 2014. Effect of salinity on microbial communities and nutrient cycling in Arctic and Subantarctic sea ice. Polar Biol 37: 1433–1444. doi: 10.1007/s00300-014-1667-x.

Karl DM, Rasmussen S, Chapman L, et al. 1995. Direct irradiance of natural sea ice communities. Mar Ecol-Prog Ser 116: 191–202. doi: 10.3354/meps116191.

Karl DM, Raukas OA, Kuosa H, et al. 1998. Microbial communities on Arctic sea ice surfaces: Evidence for nutrient stress during the spring bloom. Deep Sea Res Part I 45: 891–907. doi: 10.1016/S0967-0637(97)00010-4.

Karl DM, Stuckey DL, Healy AR, et al. 1990. Natural Arctic sea ice communities: Evidence for nutrient stress during the spring bloom. Deep Sea Res Part I 37: 1099–1113. doi: 10.1016/0149-0419(90)90132-E.
Sea ice bacteria and biogeochemistry

Michels J, Dieckmann GS, Thomas DN, Schnack-Schiel SB, Kreit A, et al. 2008. Short-term biogenic particle flux under late spring sea ice in the Weddell Sea. Deep Sea Res Part II 55: 1024–1039. doi: 10.1016/j.dsr2.2007.12.019.

Miller LA, Fripiat F, Else BGT, Bowman JS, Brown KA, et al. 2015. Methods for biogeochemical studies of sea ice: The state of the art, caveats, and recommendations. Elem Sci Anth 3(8): 1–53. doi: 10.12925/journal.elementa.000038.

Miller LA, Papakyriakou TN, Collins RE, Deming JW, Ehn JK, et al. 2011. Carbon dynamics in sea ice: A winter flux time series. J Geophys Res 116(C2): 1–20. doi: 10.1029/2009JC006058.

Mock T, Meiners KM, Giesenhausen HC. 1997. Bacteria in sea ice and underlying brackish water at 54 degrees 26'50"N (Baltic Sea, Kiel Bight). Mar Ecol-Prog Ser 158: 23–40.

Møller AK, Barkay T, Abu Al-Soud W, Sørensen SJ, Skov H, et al. 2011. Diversity and characterization of mercury-resistant bacteria in snow, freshwater and sea-ice brine from the High Arctic. FEMS Microb Ecol 75(3): 390–401. doi: 10.1111/j.1574-6941.2010.01016.x.

Møller AK, Barkay T, Hansen MA, Barkman A, Hansen LH, et al. 2014. Mercuric reductase genes (merA) and mercury resistance plasmids in High Arctic snow, freshwater and sea-ice brine. FEMS Microb Ecol 87: 52–63. doi: 10.1111/1574-6941.12189.

Møller AK, Saborg DA, Al-Soud WA, Sørensen SJ, Kroer N. 2013. Bacterial community structure in High-Arctic snow and freshwater as revealed by pyrosequencing of 16S rRNA genes and cultivation. Polar Res 32: 17390. doi: 10.3402/polar.v32i0.17390.

Monfort P, Demers S, Levasseur M. 2000. Bacterial dynamics in first year sea ice and underlying seawater of Saroma-ko Lagoon (Sea of Okhotsk, Japan) and resolute passage (High Canadian Arctic): Inhibitory effects of ice algae on bacterial dynamics. Can J Microbiol 46: 623–632. doi: 10.1139/w00-024.

Mumm N, Poulin M. 2010. A multiple biomarker approach to tracking the fate of an ice algal bloom to the sea floor. Polar Biol 34(1): 101–112. doi: 10.1007/s00300-010-0863-3.

Morgan-Kiss RM, Priscu JC, Pocock T, Gudynaire-Savitch L, Huner NPA. 2006. Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiol Mol Biol R 70(1): 222–252. doi: 10.1128/MMBR.70.1.222-252.2006.

Morris RM, Nunn BL, Frazier C, Goodlett DR, Ting YS, et al. 2010. Comparative metaproteomics reveals ocean-scale shifts in microbial nutrient utilization and energy transduction. ISME J 4(5): 673–85. doi: 10.1038/isemj.2010.4.

Mueller DR, Vincent WF, Bonilla S, Laurion I. 2005. Extremotrophs, extremophiles and broadband pigmentation strategies in a high arctic ice shelf ecosystem. FEMS Microb Ecol 53(1): 73–87. doi: 10.1016/j.femsec.2004.11.001.

Nguyen D, Maranger R. 2011. Respiration and bacterial carbon dynamics in Arctic sea ice. Polar Biol 34: 1843–1855. doi: 10.1007/s00300-011-1040-z.

Okubo T, Toyokawa Y, Sato T, Usui M, Nakajima C, et al. 2014. Bacterial diversity in sea ice from the Southern Ocean and the Sea of Okhotsk. J Appl Microbiol Bacterial Mol Biol 2(6): 266–272. doi: 10.12691/jjem-2-6-1.

Ottesen EA, Young CR, Gifford SM, Eppley JM, Marin R, et al. 2014. Multispecies diel transcriptional oscillations in open ocean heterotrophic bacterial assemblages. Science (345(6193): 207–212. doi: 10.1126/science.1252476.

Paterson H, Laybourn-Parry J. 2011a. Antarctic sea ice viral dynamics over an annual cycle. Polar Biol 35(4): 491–497. doi: 10.1007/s00300-011-1093-x.

Paterson H, Laybourn-Parry J. 2011b. Sea ice microbial dynamics over an annual ice cycle in Prydz Bay, Antarctica. Polar Biol. doi: 10.1007/s00300-011-1146-3.

Pearson GA, Lago-Leston A, Canoves F, Cos CJ, Verret F, et al. 2015. Metatranscriptomes reveal functional variation in diatom communities from the Antarctic Peninsula. ISME J. 1–15. doi: 10.1038/ismej.2015.40.

Petri R, Imhoff J. 2001. Genetic analysis of sea-ice bacterial communities of the Western Baltic Sea using an improved double gradient method. Polar Biol 24(4): 252–257. doi: 10.1007/s003000000205.

Pomeroy LR. 1974. The ocean's food web, a changing paradigm. BioScience 49: 504–509.

Priscu JC, Downes MT, Priscu LR, Palmisano AC, Sullivan CW. 1990. Dynamics of ammonium oxidizer activity and nitrous oxide within and beneath Antarctic sea ice. Mar Ecol-Prog Ser 62(1): 37–46.

Priscu JC, Michaud AB, Marie S. 2012. Cyanobacterial diversity across landscape units in a polar desert: Taylor Valley, Antarctica. FEMS Microb Ecol 82: 268–278. doi: 10.1111/j.1574-6941.2012.01297.x.

Pusceddu A, Dell’Anno A, Vezzulli L, Fabiano M, Saggioneto V, et al. 2009. Microbial loop malfunctioning in the annual sea ice at Terra Nova Bay (Antarctica). Polar Biol 32: 337–346. doi: 10.1007/s00300-008-0539-4.

Qin Q-L, Xie B-B, Shu Y-L, Long J-C, Zhao D-L, et al. 2012. Genome sequence of prochlorohodopin-containing sea ice bacterium Glaciecola punicea ACAM 611T. J Bacteriol 194(12): 2627. doi: 10.1128/JB.00463-12.

Reay DS, Nedwell DB, Priddle J, Ellis-Evans JC. 1999. Temperature dependence of inorganic nitrogen uptake: Reduced affinity for nitrate at suboptimal temperatures in both algae and bacteria. Appl Environ Microbiol 65(6): 2577–2584.

Riley M, Staley JT, Danchin A, Wang TZ, Brettin TS, et al. 2008. Genomics of extreme psychrophile, Psychromonas ingrahamii. BMC Genomics 9: 210. doi: 10.1186/1471-2164-9-210.

Röling WFM, van Bodegom PM. 2014. Toward quantitative understanding on microbial community structure and functioning: A modeling-centered approach using degradation of marine oil spills as example. Frontiers in Microbiology 5: 1–12. doi: 10.3389/fmicb.2014.00125.

Różkotiska M, Poulin M, Gosolin M. 2008. Prokaryote entrapment in newly formed sea ice in the Coastal Arctic Ocean. J Marine Syst 74(3–4): 887–901. doi: 10.1016/j.marsys.2007.11.009.

Rysgaard S, Glud RN. 2010. Anaerobic N production in Arctic sea ice. Limnol Oceanogr 49(1): 86–94.

Saba GK, Fraser WR, Saba VS, Iannuzzi RA, Coleman KE, et al., 2014. Winter and spring controls on the summer food web of the coastal West Antarctic Peninsula. Nature Communications 5: 4318. doi: 10.1038/ncomms5318.

Saenz BT, Arrigo KR. 2014. Annual primary production in Antarctic sea ice during 2005–2006 from a sea ice state estimate. J Geophys Res-Oceans 119: 3645–3678. doi: 10.1002/2013JC009677.

Schäfer H, Abbas B, Witte H, Muyzer G. 2002. Genetic diversity of “satellite” bacteria present in cultures of marine diatoms. FEMS Microb Ecol 42(1): 25–35. doi: 10.1111/j.1574-6941.2002.d600992.x.

Smith DJ, Timonen HJ, Jaffe DA, Griffin DW, Birmele MN, et al. 2013. Intercontinental dispersal of bacteria and archaea by transpacific winds. Appl Environ Microbiol 79(4): 1134–9. doi: 10.1128/AEM.03029-12.
Sea ice bacteria and biogeochemistry

Smith LM, Sanders JZ, Kaiser RJ, Hughes P, Dodd C, et al. 1986. Fluorescence detection in automated DNA sequence analysis. *Nature* **321**(June): 674–679.

Smith REH, Clement P. 1990. Heterotrophic activity and bacterial productivity in assemblages of microbes from sea ice in the high Arctic. *Polar Biol* **10**: 351–357. doi: 10.1007/BF00237822.

Smith REH, Clement P, Costa GF. 1989. Population dynamics of bacteria in Arctic sea ice. *Microbial Ecol* **17**(1): 63–76.

Søgaard DH, Kristensen M, Rysgaard S, Ghad RN, Hansen PJ, et al. 2010. Autotrophic and heterotrophic activity in Arctic first-year sea ice: Seasonal study from Malene Bight, SW Greenland. *Mar Ecol-Prog Ser* **419**: 31–45. doi: 10.3354/meps08845.

Søgaard DH, Thomas DN, Rysgaard S, Ghad RN, Norman L, et al. 2013. The relative contributions of biological and abiotic processes to carbon dynamics in subarctic sea ice. *Polar Biol* **36**(12): 1761–1777. doi:10.1007/s00300-013-1396-3.

Tang EPY, Tremblay R, Vincent WF. 1997. Cyanobacterial dominance of polar freshwater ecosystems: Are high-latitude mat-formers adapted to low temperature? *J Phycol* **181**: 171–181.

Taylor GT, Sullivan CW. 2008. Vitamin B₁₂ and cobalt cycling among diatoms and bacteria in Antarctic sea ice microbial communities. *Limnol Oceanogr* **53**(5): 1862–1877.

Teeling H, Fuchs BM, Becher D, Klockow C, Gardebrechta, et al. 2012. Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science* **336**: 608–611.

Torstensson A, Dinaasquet J, Chierici M, Fransson A, Riemann L, et al. 2015. Physicochemical control of bacterial and protist community composition and diversity in Antarctic sea ice. *Environ Microbiol*. doi: 10.1111/1462-2920.12865.

Toселand A, Daines SJ, Clark JR, Kirkham A, Strauss J, et al. 2013. The impact of temperature on marine phytoplankton resource allocation and metabolism. *Nat Clim Change* **3**(11): 979–984. doi: 10.1038/nclimate1899.

Underwood GJC, Fietz S, Papadimitriou S, et al. 2010. Distribution and composition of dissolved extracellular polymeric substances (EPS) in Antarctic sea ice. *Mar Ecol-Prog Ser* **404**: 1–19. doi: 10.3354/meps08557.

Vincent WF. 2010. Microbial ecosystem responses to rapid climate change in the Arctic. *ISME J* **4**(9): 1087–90. doi: 10.1038/ismej.2010.108.

Vollmer J, Vogel S, Dierich S, Gollnow K, Smits M, et al. 2013. Polar apart: Arctic and Antarctic *Octadeahexabacter* strains share high genome plasticity and a new type of xanthorhodopsin. *PLoS One* **8**(5): e63422. doi: 10.1371/journal.pone.0063422.

Waleron M, Waleron KE, Vincent WF, Wilmotte A. 2007. Allochthonous inputs of riverine picocyano-bacteria to coastal waters in the Arctic Ocean. *FEMS Microbiol Ecol* **59**(2): 356–65. doi: 10.1111/j.1574-6941.2006.00236.x.

Ward DM, Wellers R, Bateson MM. 1990. 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature* **345**: 63–65. doi: 10.1038/346183a0.

Wells LE, Deming JW. 2006. Modelled and measured dynamics of viruses in Arctic winter sea-ice brines. *Environ Microbiol* **8**(6): 1115–1121. doi: 10.1111/j.1462-2920.2005.00984.x.

Woese CR, Fox GE. 1977. Phylogenetic structure of the prokaryotic domain: The primary kingdoms. *P Natl Acad Sci USA* **74**(11): 5088–90.

Yin J, Chen J, Liu G, Yu Y, Song L, et al. 2013. Complete genome sequence of Glaciecola psychrophila strain 170T. *Genome Announcements* **1**(3): e00199–13. doi: 10.1128/genomeA.00199–13.

Zhou J, Delille B, Kaartokallio H, Kattner G, Kuosa H, et al. 2014. Physical and bacterial controls on inorganic nutrients and dissolved organic carbon during a sea ice growth and decay experiment. *Mar Chem* **166**: 59–69. doi: 10.1016/j.marchem.2014.09.013.

Acknowledgments

This work is part of a series on sea ice biogeochemistry and is a product of SCOR WG140 on Biological Exchange Processes at the Sea Ice Interface (BEPSII). I would like to thank the BEPSII membership and leadership for their many suggestions and discussions, and for warmly welcoming the participation of junior scientists. In particular I would like to thank Eeva Eronen-Raimus and Francoise Fripiat for numerous improvements to an early draft, and two anonymous reviewers for many helpful comments. A translation of Forster (1887) was provided by Jule Gust, MD PhD.

Funding information

JSB was funded by an EPA STAR graduate fellowship and a postdoctoral fellowship through the Lamont-Doherty Earth Observatory.

Competing interests

The authors have no competing interests to declare.

Copyright

© 2015 Bowman. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.