REVIEW ARTICLE

Key microbial drivers in Antarctic aquatic environments

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Abstract

Antarctica is arguably the world's most important continent for influencing the Earth's climate and ocean ecosystem function. The unique physico-chemical properties of the Southern Ocean enable high levels of microbial primary production to occur. This not only forms the base of a significant fraction of the global oceanic food web, but leads to the sequestration of anthropogenic CO_2 and its transport to marine sediments, thereby removing it from the atmosphere; the Southern Ocean accounts for ~ 30% of global ocean uptake of CO_2 despite representing ~ 10% of the total surface area of the global ocean. The Antarctic continent itself harbors some liquid water, including a remarkably diverse range of surface and subglacial lakes. Being one of the remaining natural frontiers, Antarctica delivers the paradox of needing to be protected from disturbance while requiring scientific endeavor to discover what is indigenous and learn how best to protect it. Moreover, like many natural environments on Earth, in Antarctica, microorganisms dominate the genetic pool and biomass of the colonizable niches and play the key roles in maintaining proper ecosystem function. This review puts into perspective insight that has been and can be gained about Antarctica's aquatic microbiota using molecular biology, and in particular, metagenomic approaches.

Introduction

Low temperature: a key factor controlling the evolution of life on Earth

Microorganisms have evolved to be able to colonize environmental niches that span a broad temperature range, from below −10 °C to above 120 °C. However, individual types of microorganisms have evolved a capacity to grow within a defined temperature range, typically 40 °C or less (Williams *et al.*, 2011). As a result, hyperthermophiles that can thrive at 100 °C cannot grow at 4 °C. Even mesophiles that grow well at 37 °C can struggle to grow at temperatures below 10 °C. The reason for this thermal effect on microorganisms pertains by and large to the effect of temperature on kinetics (Feller & Gerday, 2003; Cavicchioli, 2006; Siddiqui & Cavicchioli, 2006; Siddiqui *et al.*, 2013). In effect, an enzyme or molecular machine that functions properly within its temperature range will experience insufficient or excessive movement within its molecular structure at temperatures beyond that range to be able to function properly. In low-temperature environments, the reduced kinetic energy imposes many constraints on cellular activity; for example, rates of enzyme-catalyzed reactions are reduced, membranes become rigid, solute transport become compromised, and nucleic acids acquire inhibitory secondary structures that impinge on gene expression and protein synthesis (Feller & Gerday, 2003; Cavicchioli, 2006; Siddiqui & Cavicchioli, 2006; Margesin & Miteva, 2011; Siddiqui *et al.*, 2013).

In view of the imposition that low temperature has on cellular processes, it is a striking realization that the majority of microbial biomass resides in cold environments. On the order of 85% of the Earth's biosphere is permanently below 5 °C, dominated by ocean depths, glacier, alpine, and polar regions (Margesin & Miteva, 2011). Given the apparent irony that cold environments constrain growth rates yet represent the largest proportion of the biosphere, many questions can be raised about the characteristics of the indigenous psychrophilic microbial communities. Three obvious questions arise: which microorganisms are present in cold environments; what

biogeochemical processes do microorganisms in cold environments perform; and how have microorganisms evolved and adapted to growth in the cold? This review addresses the first two questions, focusing on the Antarctic polar environment and reviewing discoveries made with the aid of metagenomic and other molecular biology techniques. The review does not address molecular mechanisms of cold adaptation, and reviews of this topic are available (e.g. Feller & Gerday, 2003; Cavicchioli, 2006; Siddiqui & Cavicchioli, 2006; Casanueva *et al.*, 2010; Margesin and Miteva, 2011; Siddiqui *et al.*, 2013).

Antarctica: a brief overview

Ninety percent of the Earth's ice is present in Antarctica, equating to 60–70% of the Earth's freshwater supply (AASSP, 2011). As a frozen deep mass of ice, Antarctica reflects the sun's radiation to buffer against global warming trends. By maintaining freezing temperatures, cold water masses sink near the Antarctic continent and drive thermohaline circulation of global ocean currents. While temperatures in the interior of Antarctica have been recorded as low as −89 °C, Antarctica's coastal and surrounding Southern Ocean regions experience seasonal temperatures that rise above 0 °C.

As a result of the annual freeze/thaw cycle, the growth (up to 19×10^6 km^2) and melting of Antarctic sea ice represent one of the Earth's most significant seasonal events (AASSP, 2011). The scale of the Antarctic sea ice (up to 1.5 × the area of the Antarctic continent) places into context the influence of anthropogenic climate change on the Antarctic region. Although satellite data suggest a small overall increase in sea ice extent since observations started in the 1970s, this masks the highly variable nature of sea ice extent around the continent. The effects of global warming have been particularly noted for the Antarctic Peninsula and most of West Antarctica (Meredith & King, 2005; Murray and Grzymski, 2007; Reid *et al.*, 2009; Steig *et al.*, 2009), extending to South Georgia where some of the fastest warming waters on Earth exist (Whitehouse *et al.*, 2008; Hogg *et al.*, 2011) and a corresponding decrease in sea ice extent has been noted (e.g. Bellingshausen/Amundsen Seas; ~ 5% decrease per decade; Cavalieri & Parkinson, 2008). Conversely, while other sectors of the continent are displaying small but significant increases in sea ice extent since the 1970s (Zhang, 2007; Cavalieri & Parkinson, 2008), there is compelling ice-core evidence based on historical levels of methanesulfonic acid that this apparent increase masks an ~ 20% decrease in sea ice extent since the early 1950s (Curran *et al.*, 2003; Liu & Curry, 2010).

Anthropogenic climate change has been particularly associated with reducing the capacity of the Southern Ocean to absorb CO_2 (Le Quéré *et al.*, 2007). In addition to the threat to marine biota caused by ocean acidification (Kintisch & Stoksta, 2008; McNeil & Matear, 2008; Falkowski, 2012), nutrient supply in surface waters is expected to decrease, particularly at higher latitudes as a result of increased stratification caused by ocean warming (Sarmiento and LeQuéré, 1996; Wignall & Twitchett, 1996; Matear & Hirst, 1999).

A general problem with forecasting the impact of global climate change on Antarctic biota is the lack of physical and particularly biological records for Antarctica (Gille, 2008; McNeil & Matear 2008; Rosenzweig *et al.*, 2008; Steig *et al.*, 2009; AASSP, 2011). Scientific records are available for only a tiny fraction of the land and surrounding waters, and the biodiversity in East Antarctica in particular is the largest under-sampled region of the continental shelf around Antarctica making it essentially impossible to apply spatial management to area protection (AASSP, 2011). This issue is further compounded by the increasing risk associated with direct anthropogenic impact to the Antarctic environment. Since humans first visited Antarctica in 1821, the number of tourists (and research activities) has increased greatly, and it is both challenging to prevent non-indigenous microorganisms from being introduced into Antarctica (Cowan *et al.*, 2011) and to remediate sites that have become polluted (AASSP, 2011). Science plans of many nations (e.g. AASSP, 2011) articulate the need for science to deliver information suitable for policy development to meet the challenges of global change, natural resource sustainability, biodiversity conservation, and management of human impacts on the Antarctic continent. While the integration of molecular information (e.g. genomics, proteomics) with ecological principles and physical, chemical, and earth sciences represents one of the biggest challenges in modern ecology, it also provides the greatest opportunities for facilitating effective ecosystem management (DeLong & Karl, 2005), particularly for the Antarctic environment (Hoag, 2003; Clark *et al.*, 2004; Cavicchioli, 2007; Murray & Grzymski, 2007; Cavicchioli & Lauro, 2009; Lauro *et al.*, 2011; Yau *et al.*, 2011; Yau & Cavicchioli, 2011).

The following sections review knowledge of microbial communities present in Antarctic lake and marine environments, emphasizing where available information that has been obtained using metagenomics.

Antarctic lakes

Antarctica: unique lake systems that are sentinels for climate change

Only 0.4% of the total ice area of Antarctica (12.3×10^6 km^2) is seasonally ice free, comprising exposed

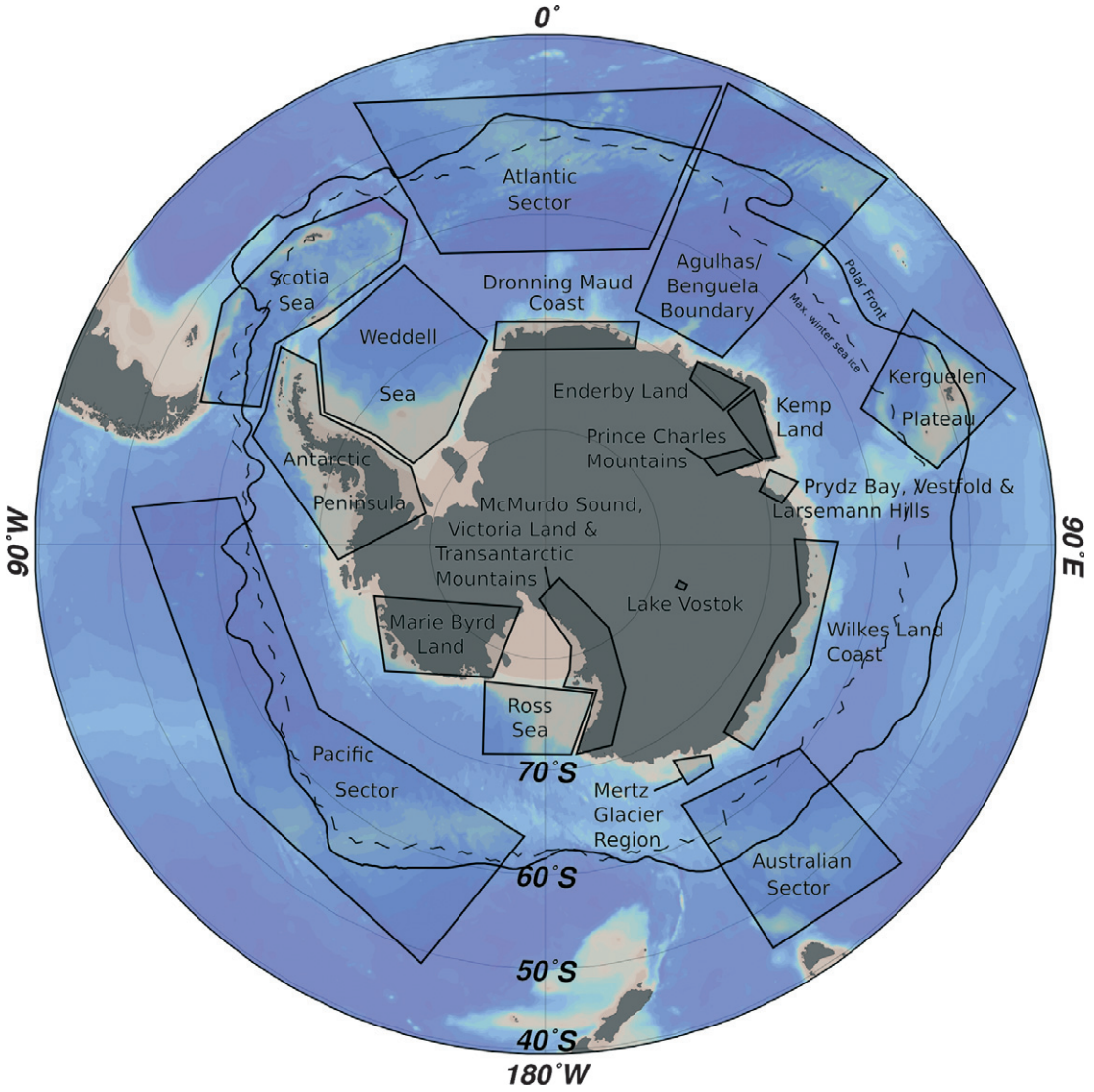

Fig. 1. Antarctica and the Southern Ocean. Polar Front (circumpolar full line); Maximum sea ice extent (circumpolar dotted line). Regions of Antarctica shown, with lakes in the regions listed in alphabetical order. Dronning Maud Coast: Dronning Maud Land; Schirmacher Oasis. Enderby Land: Syowa Oasis; Thala Hills. Kemp Land: Framnes Mountains; Stillwell Hills; Ufs Island. Marie Byrd Land: Coleman Ridge; Mt Richardson. McMurdo Sound, Victoria Land, and Transantarctic Mountains: Beardmore Glacier; Darwin Glacier region; McMurdo Dry Valleys; Ross Island; Terra Nova Bay. Mertz Glacier Region: Commonwealth Bay. Prince Charles Mountains: Amery Oasis; Else Platform; Jetty Peninsula; Prince Charles Mountains. Prydz Bay, Vestfold, and Larsemann Hills: Bollingen Islands; Gillock Island; Larsemann Hills; Rauer Islands; Vestfold Hills. Wilkes Land Coast: Bunger Hills; Haswell Island; Obruchev Hills; Windmill Islands. Southern Ocean regions shown, with references for studies from the regions listed in alphabetical order. Agulhas/Benguela Boundary: Simon *et al.* (1999); Selje *et al.* (2004); Giebel *et al.* (2009). Antarctic Peninsula: DeLong *et al.* (1994); Massana *et al.* (1998); Murray *et al.* (1998); García-Martínez & Rodríguez-Valera (2000); Guixa-Boixereu *et al.* (2002); Hollibaugh *et al.* (2002); Short & Suttle (2002); Brinkmeyer *et al.* (2003); Church *et al.* (2003); Short & Suttle (2005); Grzymski *et al.* (2006); Murray & Grzymski (2007); Kalanetra *et al.* (2009); Manganelli *et al.* (2009); Straza *et al.* (2010); Bolhuis *et al.* (2011); Ducklow *et al.* (2011); Piquet *et al.* (2011); Grzymski *et al.* (2012); Williams *et al.* (2012a). Atlantic Sector: Giebel *et al.* (2009); Weinbauer *et al.* (2009). Australian Sector: Oliver *et al.* (2004); Abell & Bowman (2005a, b); Evans *et al.* (2009); Evans *et al.* (2011); Wilkins *et al.* (2012); Williams *et al.* (2012b). Kerguelen Plateau: Christaki *et al.* (2008); West *et al.* (2008); Obernosterer *et al.* (2011); Ghiglione & Murray (2012). McMurdo Sound, Victoria Land, and Transantarctic Mountains: Murray *et al.* (1998). Mertz Glacier Region: Bowman & McCuaig (2003); Bowman *et al.*, (2003); Wilkins *et al.* (2012). Pacific Sector: Hollibaugh *et al.* (2002). Ross Sea: Murray *et al.* (1998); Hollibaugh *et al.* (2002); Gast *et al.* (2004); Gowing *et al.* (2004); Gast *et al.* (2006); Gentile *et al.* (2006); Koh *et al.* (2010, 2011); Cowie *et al.* (2011); Ghiglione & Murray (2012); Lo Giudice *et al.* (2012). Scotia Sea: Murray *et al.* (1998); García-Martínez & Rodríguez-Valera (2000); López-García *et al.* (2001); Topping *et al.* (2006); Giebel *et al.* (2009); Manganelli *et al.* (2009); Murray *et al.* (2010); Jamieson *et al.* (2012). Weddell Sea: García-Martínez and Rodríguez-Valera (2000); Brinkmeyer *et al.* (2003). Wilkes Land Coast: Bowman *et al.* (1997a); Brown & Bowman (2001); Piquet *et al.* (2008); Patterson & Laybourn-Parry (2012).

mountains (e.g. Ellsworth and Transantarctic Mountains, the North Victoria Land mountains, and the nunataks) and coastal areas (e.g. lower-latitude Antarctic Peninsula in West Antarctica, and the Vestfold Hills and McMurdo Dry Valleys) (Poland *et al.*, 2003; Cary *et al.*, 2010) (Fig. 1). Within this ice-free 50 850 km^2 area of Antarctica, the geographic distribution and diversity of types of aquatic systems are considerable, ranging from fresh to hypersaline, permanently ice covered to perennially ice free, and mixed to stratified (dimictic, polymictic, amictic, meromictic). Lake biota is microbially dominated with generally reduced diversity and few or no metazoans present (Laybourn-Parry, 1997), and distinct communities inhabit specific niches including ice, the water column, sediment, and microbial mats.

The age of water within Antarctic lakes varies considerably, with the subglacial outflow from Blood Falls estimated to be 1.5 million years old (Mikucki *et al.*, 2009), the water in Ace Lake about ~ 5000 years old (Rankin *et al.*, 1999), and Lake Miers water < 300 years old (Green *et al.*, 1988). Subglacial and epiglacial lakes also appear to be prevalent in Antarctica with at least 145 subglacial lakes identified (Siegert *et al.*, 2005; Gibson, 2006; Cavicchioli, 2007; Pearce, 2009). Epiglacial lakes form as a result of glacier ice melt, on the down-ice flow sides of mountains that penetrate the polar ice cap, and organisms inhabiting the lakes could be recent (postglacial) or ancient inhabitants of Antarctica (Gibson, 2006; Cavicchioli, 2007).

In the Vestfold Hills (68°33′S, 78°15′E), more than 300 lakes and ponds from freshwater (< 0.1%) to hypersaline (32%) have been described, including ~ 20% of the world's meromictic lakes (Gibson, 1999). The Australian Antarctic Data Centre describes more than 3000 water bodies (fresh and saline) mapped in the Vestfold Hills, ranging in area from 1 to 8 757 944 m^2. Fjords also cut across the Vestfold Hills. Some of these are large, such as Ellis Fjord, which is 10 km long, up to 100 m deep, and has become a stratified system due to its restricted opening (< 4 m deep) to the ocean (Burke & Burton, 1988).

Table 1. Main groups of microorganisms associated with key metabolic processes in Antarctic lakes and mats

Microorganism	Key functions	Detected in mats?	Comments
Cyanobacteria	Oxygenic photosynthesis and N_2 fixation	Yes	More prevalent in lower salinity environments, generally dominate mats
Green sulfur bacteria (GSB) (*Chlorobi*)	Anaerobic anoxygenic phototrophy, N_2 fixation, and H_2S/S oxidation	No	Prevalent, predominant in saline lakes of the Vestfold Hills
Green nonsulfur bacteria (*Chloroflexi*)	Anoxygenic phototrophy and heterotrophy	No	Mainly detected in saline lakes; limited distribution
Alphaproteobacteria	Heterotrophy and aerobic anoxygenic phototrophy	Yes	Metabolically diverse generalists with wide distribution; some groups linked to algal blooms (e.g. *Roseobacter* clade), others oligotrophic (SAR11 clade)
Betaproteobacteria	Heterotrophy, nitrification, and anoxygenic phototrophy	Yes	Dominate freshwater lakes; some associated with algal blooms (OM43)
Gammaproteobacteria	Heterotrophy, anoxygenic phototrophy, nitrification, and nitrate/DMSO/metal reduction	Yes	Includes heterotrophs (e.g. *Alteromonadales*/*Oceanospirillales*), anoxygenic phototrophs (purple sulfur bacteria = *Chromatiales*), sulfur oxidizers (*Thiotrichales*); wide distribution
Deltaproteobacteria	Dissimilatory sulfur/sulfate reduction	Yes	Predominant sulfate reducers, found in anoxic waters
Firmicutes	Fermentation and sulfate/nitrate/metal reduction	Yes	Most prevalent in anoxic water and sediment
Actinobacteria	Heterotrophy	Yes	Wide distribution, may increase in dominance with trophic status in freshwater lakes
Cytophaga–Flavobacterium –Bacteroides (CFB) group	Aerobic heterotrophy	Yes	Wide distribution, linked with algal blooms, prefer HMW carbon
Methanogenic *Archaea*	Hydrogenotrophic, acetoclastic, and methylotrophic methanogenesis	Yes	Limited to anoxic waters, usually where sulfate and nitrate are depleted
Haloarchaea	Heterotrophy	No	Limited to hypersaline lakes
Diatoms	Oxygenic photosynthesis	Yes	Can dominate mat communities, wide distribution, species vary with salinity
Phytoflagellates	Oxygenic photosynthesis and mixotrophy	Yes	Can be dominant primary producers in lakes; wide distribution
Flagellates and ciliates	Aerobic heterotrophy	Yes	May occupy highest trophic level

Many of the coastal lake systems, including a large number in the Vestfold Hills, are marine derived, having been separated from the marine environment ~ 3000–7000 years ago by isostatic rebound of the Antarctic continent (Gibson, 1999). The marine-derived systems provide unique opportunities for studying the evolution of marine microbial populations to chemically, physically, and temporally defined ecosystem changes (e.g. Lauro *et al.*, 2011). Due to global warming, by being exposed annually to longer periods of temperatures above 0 °C, the lake systems are at an increasing risk of being physically perturbed (e.g. ice cover melt exposing the water column to longer periods of wind turbulence, and increasing surface temperatures and irradiation penetrating deeper and for longer).

The reduced biodiversity of Antarctic lakes makes them ideal model systems to examine microbial influence on geochemistry as it is possible to encompass a large proportion of the diversity present and relate taxa to particular processes (Laybourn-Parry & Pearce, 2007; Lauro *et al.*, 2011). Through the use of molecular methods, new insights are being derived on how microorganisms have adapted to the Antarctic environment under a diverse array of chemical and physical conditions. By being especially sensitive to climatic influences, these Antarctic lakes function as sentinels for monitoring climate change. By understanding plausible functional relationships between ecosystem components in these lakes, there is capacity to monitor ecosystem health. By providing key sentinel indicators, managers of human activities, policy makers, scientists, and the general public can become better informed about the causes and effects and consequences of human activities, including anthropogenic climate change.

The topics in this Antarctic lakes section describe findings linked to important members of the *Bacteria*, *Archaea*, *Eucarya* (Table 1), and viruses that inhabit the water column, assessments of lake microbial mat communities, and 'omic'-based studies that have led to an integrated view of ecosystem function.

Molecular approaches used in Antarctic lake systems

The majority of molecular-based studies of Antarctic aquatic microbial communities have made use of PCR amplification of small subunit ribosomal RNA sequences to survey the diversity of *Bacteria* and in some cases *Archaea* and *Eucarya* (Supporting Information, Tables S1 and S2). Microbial composition has been determined by cloning and sequencing of rRNA gene amplicons exclusively (Bowman *et al.*, 2000a; Bowman *et al.*, 2000b; Gordon *et al.*, 2000; Christner *et al.*, 2001; Purdy *et al.*, 2003; Karr *et al.*, 2006; Matsuzaki *et al.*, 2006; Kurosawa *et al.*, 2010; Bielewicz *et al.*, 2011), although most studies have also made use of denaturing gradient gel electrophoresis (DGGE) to provide a molecular 'fingerprint' of the community (Pearce, 2003; Pearce *et al.*, 2003; Karr *et al.*, 2005; Pearce, 2005; Pearce *et al.*, 2005; Unrein *et al.*, 2005; Glatz *et al.*, 2006; Mikucki & Priscu, 2007; Mosier *et al.*, 2007; Schiaffino *et al.*, 2009; Villaescusa *et al.*, 2010). Functional genes have also been targeted using PCR amplification to assess the potential of biochemical processes occurring, such as nitrogen fixation (Olsen *et al.*, 1998), ammonia oxidation (Voytek *et al.*, 1999), anoxygenic photosynthesis (Karr *et al.*, 2003), and dissimilatory sulfate reduction (Karr *et al.*, 2005; Mikucki *et al.*, 2009).

Empowered by technological advances in DNA sequencing, the value of applying metagenomics to microbial communities in Antarctic lakes has been realized (Cavicchioli, 2007; Yau & Cavicchioli, 2011). Metagenomic studies have assessed both the taxonomic composition and genetic potential of lake communities, and in some cases have linked function to specific members of the community (López-Bueno *et al.*, 2009; Ng *et al.*, 2010; Lauro *et al.*, 2011; Yau *et al.*, 2011; Varin *et al.*, 2012). When coupled with functional 'omic' techniques (to date, metaproteomics has been applied, but not metatranscriptomics or stable isotope probing), information has also been gained about the genetic complement that has been expressed by the resident populations (Ng *et al.*, 2010; Lauro *et al.*, 2011; Yau *et al.*, 2011).

Bacterial diversity: adaptation to unique physical and chemical conditions

The vast majority of molecular studies of Antarctic lakes have focused on bacteria. Consistent with the wide range of physical and chemical properties of Antarctic lakes, a large variation in species assemblages have been found. While exchange of microorganisms must be able to occur between lakes that are in close vicinity to each other, the picture that has emerged from the data to date is that microbial populations are relatively unique to each type of isolated system. Nonetheless, certain trends in bacterial composition are also apparent. Focusing on the similarities, lakes of equivalent salinities tend to have similar communities. Hypersaline lakes from the Vestfold Hills (Bowman *et al.*, 2000b) and McMurdo Dry Valleys (Glatz *et al.*, 2006; Mosier *et al.*, 2007) were all dominated by *Gammaproteobacteria* and members of the CFB group (= *Bacteroidetes*) as well as harboring lower abundance populations of *Alphaproteobacteria*, *Actinobacteria*, and *Firmicutes*. The surface waters of saline lakes resemble marine communities dominated by CFB, *Alphaproteobacteria*, and *Gammaproteobacteria*, but divisions such as *Actinobacteria* and specific clades of *Cyanobacteria* have

been found to be overrepresented compared to the ocean (Lauro *et al.*, 2011). Sediments from saline lakes in the Vestfold Hills (Bowman *et al.*, 2000a) and Nuramake-Ike in the Syowa Oasis (Kurosawa *et al.*, 2010) were very similar, containing in addition to the surface clades, *Deltaproteobacteria*, *Planctomycetes*, *Spirochaetes*, *Chloroflexi* (green nonsulfur bacteria), *Verrucomicrobia*, and representatives of candidate divisions. Plankton from freshwater lakes were characterized by an abundance of *Betaproteobacteria*, although *Actinobacteria*, CFB group, *Alphaproteobacteria*, and *Cyanobacteria* were also prominent (Pearce *et al.*, 2003; Pearce, 2005; Pearce *et al.*, 2005; Schiaffino *et al.*, 2009).

Differences in bacterial community structure are also influenced by nutrient availability. In studies of freshwater lakes in the Antarctic Peninsula and the South Shetland Islands, cluster analysis of DGGE profiles grouped together lakes of similar trophic status (Schiaffino *et al.*, 2009; Villaescusa *et al.*, 2010). Most of the variance in community structure could be explained by related chemical parameters such as phosphate and dissolved inorganic nitrogen. Similarly, three freshwater lakes, Moss, Sombre, and Heywood on Signy Island, are alike except that Heywood Lake is enriched by organic inputs from seals. Bacterial composition in each lake changed from winter to summer, and this was again correlated to variation in physico-chemical properties (Pearce, 2005). The bacterial population of Heywood Lake had shifted from a dominance of *Cyanobacteria* toward a greater abundance of *Actinobacteria* and marine *Alphaproteobacteria* (Pearce *et al.*, 2005). This hints at a link between a copiotrophic lifestyle in the Heywood Lake *Actinobacteri*a and inhibition of Antarctic freshwater *Cyanobacteria* by eutrophication. This type of study exemplifies how inferences can be made about taxa and function by examining population changes over time and over gradients of environmental parameters.

Inferring functional potential from taxonomic surveys can be problematic due to species- or strain-level differences in otherwise related bacteria. For example, the majority of the *Gammaproteobacteria* in hypersaline lakes were relatives of *Marinobacter* suggesting that this genus is particularly adapted to hypersaline systems (Bowman *et al.*, 2000b; Glatz *et al.*, 2006; Matsuzaki *et al.*, 2006; Mosier *et al.*, 2007). Nonetheless, *Marinobacter* species from different lakes appeared biochemically distinct as isolates from hypersaline lake Suribati-Ike were all able to respire dimethylsulfoxide (DMSO) but not nitrate (Matsuzaki *et al.*, 2006). In contrast, those from the west lobe of Lake Bonney were all able to respire nitrate (Ward & Priscu, 1997). Interestingly, in the east lobe of the same lake, nitrate respiration was inhibited although a near-identical *Marinobacter* phylotype was present; it was speculated that the inhibition may have been caused by an as yet unidentified chemical factor (Ward *et al.*, 2005; Glatz *et al.*, 2006).

The relative isolation and diverse chemistries of the lakes facilitate biogeographical studies. The anoxic and sulfidic bottom waters of some meromictic lakes form due to a density gradient that precludes mixing. Although sedimentation from the upper aerobic waters may occur, there is little opportunity for interchange of species with the bottom water of lakes allowing for greater divergence in community composition as nutrients can become depleted and products of metabolism can accumulate. As a result, distinct distributions of bacterial groups can inhabit these strata, and different types of microorganisms can be found in equivalent strata in different lakes. A good example of this is the presence of common types of purple sulfur bacteria (*Chromatiales*) and (GSB = *Chlorobi*) in some meromictic lakes and stratified fjords in the Vestfold Hills (Burke & Burton, 1988), compared to diverse 'purple nonsulfur bacteria' in Lake Fryxell in Victoria Land (Karr *et al.*, 2003).

In Lake Bonney, the east and west lobes harbor overlapping but distinct communities in the suboxic waters (Glatz *et al.*, 2006). The east lobe was dominated by *Gammaproteobacteria* and the west lobe by CFB, illustrating how divergent communities can form from the same seed population. In contrast, ice communities are more readily dispersed by wind, aerosols, and melt water, and 16S rRNA gene probes designed from bacteria trapped in the permanent ice cover of Lake Bonney hybridized to microbial mat libraries sourced up to 15 km away (Gordon *et al.*, 2000). This demonstrates how a single lake may encompass microorganisms that are biogeographically dispersed, while also harboring others that have restricted niches and are under stronger selection pressure.

Subglacial systems, such as Lake Vostok, have been isolated from the open environment for hundreds of thousands to millions of years (Siegert *et al.*, 2001). As a result, they provide a reservoir of microorganisms that may have undergone significant evolutionary divergence from the same seed populations that were not isolated by the Antarctic ice cover. The uniqueness of these types of systems also creates a conundrum for studying them. Lake Vostok is approximately 4 km below the continental ice sheet making it extremely difficult to determine suitable means for accessing the lake without inadvertently contaminating it with biological (e.g. surface microorganisms) or chemical (e.g. drilling fluid) matter (Inman 2005; Wingham *et al.*, 2006; Lukin & Bulat 2011; Gramling, 2012; Jones, 2012). To date, molecular microbial studies have concentrated on the accretion ice above the ice–water interface (Priscu *et al.*, 1999; Christner *et al.*,

2001). Accretion ice has been found to contain a low density of bacterial cells from *Alphaproteobacteria*, *Betaproteobacteria*, *Actinobacteria*, and CFB divisions closely allied to other cold environments. Molecular signatures of a thermophilic *Hydrogenophilus* species were also identified in accretion ice raising the possibility that chemoautotrophic thermophiles were delivered to the accretion ice from hydrothermal areas in the lake's bedrock (Bulat *et al.*, 2004; Lavire *et al.*, 2006). However, interpretation of results from samples sourced from the Lake Vostok bore hole is very challenging as it is difficult to differentiate contaminants from native Vostok microorganisms. From a study that assessed possible contaminants present in hydrocarbon-based drilling fluid retrieved from the Vostok ice core bore hole, six phylotypes were designated as new contaminants (Alekhina *et al.*, 2007). Two of these were *Sphingomonas* phylotypes essentially identical to those found in the accretion ice core (Christner *et al.*, 2001), which raises question about whether bacterial signatures identified from the ice cores are representative of Lake Vostok water, and further highlights the ongoing problem of causing forward contamination into the lake.

Archaea: methanogens and haloarchaea

Archaea have been detected mainly in anoxic sediments and bottom waters from lakes that range in salinity from fresh to hypersaline, and those with known isolates are affiliated with methanogens or haloarchaea (Bowman *et al.*, 2000a, b; Purdy *et al.*, 2003; Kurosawa *et al.*, 2010; Lauro *et al.*, 2011). Anoxia allows for the growth of methanogenic archaea that mineralize fermentation products such as acetate, and H_2 and CO_2 into methane, thereby performing an important step in carbon cycling. The acetoclastic methanogens thrive in environments where alternative terminal electron acceptors such as sulfate and nitrate have been depleted. One example of this is Lake Heywood where methanogenic archaea were found to comprise 34% of the total microbial population in the freshwater sediment, the majority of which were *Methanosarcinales*, which include acetate and C1-compound utilizing methanogens (Purdy *et al.*, 2003). Both $H_2 : CO_2$ (*Methanogenium frigidum*) and methylamine/methanol (*Methanococcoides burtonii*) utilizing methanogens were isolated from Ace Lake (Franzmann *et al.*, 1992, 1997) providing opportunities for genomic analyses (Saunders *et al.*, 2003; Allen *et al.*, 2009) and a host of studies addressing molecular mechanisms of cold adaptation (e.g. Cavicchioli, 2006; Williams *et al.*, 2011).

In general, archaeal populations appear to be adapted to their specific lake environment. Sediments from saline lakes of the Vestfold Hills were inhabited by members of the *Euryarchaeota* typically found in sediment and marine environments with the phylotypes differing between the lakes examined (Bowman *et al.*, 2000a). While a phylotype similar to *Methanosarcina* was identified, the majority were highly divergent. Similarly, *Methanosarcina* and *Methanoculleus* were detected in Lake Fryxell but other members of the *Euryarchaeota* and *Crenarchaeota* (a single sequence) were divergent, clustering only with marine clones (Karr *et al.*, 2006). Based on the lake chemical gradients and the location of these novel phylotypes in the water column, the authors speculated these *Archaea* may be have alternative metabolisms such as anoxic methanotrophy or sulfur utilization. In sediments from Lake Nurume-Ike in the Langhovde region, 205 archaeal clones grouped into three phylotypes, with the predominant archaeal clone being related to a clone from Burton Lake in the Vestfold Hills, while the other two did not match to any cultivated species (Kurosawa *et al.*, 2010). Consistent with these observations, from a metagenomic study that involved the analysis of ~ 9 million genes, a high level of divergence was found for the *Archaea* present in the bottom waters of Ace Lake, the majority of which did not match to known methanogens including *M. frigidum* and *M. burtonii* that were isolated from the lake (Lauro *et al.*, 2011). However, high levels of methane are present in Ace Lake bottom waters, and this is likely to have been produced gradually by the methanogenic community, and retained in the lake due to the very low potential for aerobic methane oxidation and the apparent absence of anaerobic methane-oxidizing *Euryarchaeota* (Lauro *et al.*, 2011).

In hypersaline lakes where bottom waters do not become completely anoxic, methanogens are not present and archaea have extremely low abundance. For example, only two archaeal clones of the same phylotype were recovered from deep water samples from Lake Bonney (Glatz *et al.*, 2006), and Organic Lake in the Vestfold Hills had an extremely low abundance of archaeal clones related to *Halobacteriales* (Bowman *et al.*, 2000b). In contrast to these stratified hypersaline lakes, the microbial community in the extremely hypersaline Deep Lake is dominated by haloarchaea (Bowman *et al.*, 2000b). Many of the clones identified from Deep Lake are similar to *Halorubrum* (formerly *Halobacterium*) *lacusprofundi*, which was isolated from the lake (Franzmann *et al.*, 1988). The genome sequence of *H. lacusprofundi* has been completed, and ongoing metagenome studies are examining the microbial community composition and microbial ecology of the lake (R. Cavicchioli *et al.*, unpublished results).

Eucarya perform multiple ecosystem roles

Single-celled *Eucarya* are important members of Antarctic aquatic microbial communities. In many Antarctic systems,

eucaryal algae are the main photosynthetic organisms, and in others, only heterotrophic protists occupy the top trophic level. As eucaryal cells are generally large with characteristic morphologies, microscopic identifications have been used. However, microscopy is unable to classify smaller cells such as nanoflagellates with high resolution, although these may constitute a high proportion of algal biomass. For example, five morphotypes of *Chrysophyceae* evident in Antarctic lakes were unidentifiable by light microscopy but were able to be classified using DGGE and DNA sequencing (Unrein *et al.*, 2005). Consistent with this, molecular studies specifically targeting eucaryal diversity (Unrein *et al.*, 2005; Mosier *et al.*, 2007; Bielewicz *et al.*, 2011) have identified a much higher level of diversity than previously suspected, and the studies have discovered lineages not previously known to be present such as silicoflagellates (Unrein *et al.*, 2005) and fungi (Mosier *et al.*, 2007; Bielewicz *et al.*, 2011).

Most eucarya in Antarctic lakes are photosynthetic microalgae that are present in marine environments with a wide distribution including chlorophytes, haptophytes, cryptophytes, and bacillariophytes. Molecular methods have afforded deeper insight into the phylogenetic diversity within these broader divisions and have revealed some patterns in their distribution. Using 18S rRNA gene amplification and DGGE, the same chrysophyte phylotypes were identified in lakes from the Antarctic Peninsula and King George Island despite being 220 km apart (Unrein *et al.*, 2005) indicating these species may be well adapted to Antarctica or highly dispersed. Similarly, an unknown stramenopile sequence was detected throughout the 18S rRNA clone libraries of Lake Bonney demonstrating a previously unrecognized taxon occupied the entire photic zone in the lake (Bielewicz *et al.*, 2011). In contrast, other groups showed distinct vertical and temporal distributions with cryptophytes dominating the surface, haptophytes the midwaters, and chlorophytes the deeper layers during the summer while stramenopiles increased in the winter (Bielewicz *et al.*, 2011).

The influence of flagellates on ecosystem function is not necessarily clear-cut as they can simultaneously inhabit several trophic levels. For instance, in Ace Lake, the mixotrophic phytoflagellate *Pyramimonas gelidocola* derives a proportion of its carbon intake through bacterivory (Bell & Laybourn-Parry, 2003) but in the nearby Highway Lake, it uptakes dissolved organic carbon (DOC) (Laybourn-Parry *et al.*, 2005). This again illustrates potential limitations for deriving ecosystem-level functions from taxonomic studies alone, even with taxa that appear physiologically straightforward. A good illustration of this was the finding from shotgun metagenomics that a large proportion of the 18S rRNA gene sequences in the aerobic zone of Ace Lake were related to *Mantoniella*, a picoeucaryal member of the *Chlorophyta*, and that phycodnaviruses were likely to be controlling the seasonal population dynamics of this alga (Lauro *et al.*, 2011); previously, it was interpreted that the virus-like particles were likely to be bacteriophages infecting bacteria (Madan *et al.*, 2005). Further studies are necessary to determine the basis for apparent specific adaptations of some species to particular lakes or lake strata, and for the cosmopolitan distribution of others. Here, molecular-based research of the kind that has been applied to bacteria such as functional gene surveys will undoubtedly help answer these questions.

Antarctic virus diversity and top-down ecosystem control

In the absence of metazoan grazers, viruses are hypothesized to play an increased role in the microbial loop in Antarctic systems (Kepner *et al.*, 1998) and as drivers of microbial evolution (Anesio & Bellas, 2011). This idea has been supported by microscopy-based observations of viral density, virus-to-bacteria ratios, and infection rates that are different in Antarctic lakes than lower-latitude systems (Laybourn-Parry *et al.*, 2001; Madan *et al.*, 2005; Laybourn-Parry *et al.*, 2007; Säwström *et al.*, 2007). Morphological examination of virus-like particles only provides limited insight into viral physiology and diversity. Metagenomics has enabled unprecedented insight into viruses by permitting more precise classification, information on genetic content, and discovery of novel species.

Metagenomic analysis of the viriome of the freshwater Lake Limnopolar, Livingston Island, uncovered the greatest depth of viral diversity of any aquatic system to date (López-Bueno *et al.*, 2009). Representatives from 12 viral families were detected, but unlike the two previous viromes that had been published at that time using comparable techniques, ssDNA viruses, and large dsDNA viruses that putatively infect *Eucarya* were the dominant viral types rather than bacteriophages. The ssDNA viruses were related to circoviruses, geminiviruses, nanoviruses, and satellites; viruses previously only known to infect plants and animals indicating they are much more diverse than previously suspected and may constitute new viral families. Samples taken in summer showed a shift in the viral community composition toward phycodnaviruses similar to *Ostreococcus tauri* virus, OtV5. This shift potentially reflects an increase in the host algae that are stimulated to bloom by the increased light availability.

Metagenomic data from Ace Lake revealed a viral community comprising *Phycodnaviridae*, *Myoviridae*, *Siphoviridae*, *Podoviridae*, and unidentified viral families (Lauro *et al.*, 2011). Bacteriophages were abundant in the bottom

waters, whereas the surface summer community, similar to Lake Limnopolar (López-Bueno *et al.*, 2009), was dominated by phycodnaviruses (Lauro *et al.*, 2011). Most notably, no viral signatures were found at 12.7 m depth, which is a zone occupied by a near-clonal population of GSB. Mathematical modeling predicted that the absence of phage predation in the GSB could be due to an adaptation to longer cycles of growth and inactivity in response to the polar light regime. Phototrophs with faster growth rates, such as eucaryal algae and *Cyanobacteria* in the surface water, were predicted to be more susceptible to viral predation. The viral susceptibility of these phototrophs was predicted to lead to increased host genetic variation, and this was borne out in the analysis of the metagenome data (Lauro *et al.*, 2011).

Metagenome analysis also unveiled some unexpected findings about viral–host interactions in Organic Lake in the Vestfold Hills. In the metagenome, a relative of the newly described virophage family (La Scola *et al.*, 2008; Fischer & Suttle, 2011) was discovered (Yau *et al.*, 2011). A virophage depends upon a giant helper or 'host' virus to replicate but is detrimental to its helper (La Scola *et al.*, 2008). Termed the Organic Lake virophage (OLV), genomic evidence pointed toward OLV being a virophage of Organic Lake phycodnaviruses (OLPV) that infect eucaryal algae (Yau *et al.*, 2011). Modeling of the OLV–OLPV and algal host dynamics indicated that the presence of OLV may contribute to stability of the algal populations by increasing the frequency of algal blooms, and contributing to the carbon flux throughout the lake. The major capsid protein gene sequence was also detected in Ace Lake, and a number of temperate aquatic environments suggesting that virophages may have widespread ecological influence.

Understanding of Antarctic viral diversity and ecology is still in its early days, because a complete viral survey is problematic due to the lack of a universal viral gene or even universal genetic material. Furthermore, the enormous depth of viral diversity remains largely unsampled so most viral sequences have no significant similarity to sequence data repositories (López-Bueno *et al.*, 2009; Yau *et al.*, 2011). What is clear is that viruses perform a crucial role in shaping community structure, driving host evolution, contributing to the dissolved nutrient pool, and understanding them is essential to understanding ecosystem function (Danovaro *et al.*, 2011).

Microbial mats as microcosms of Antarctic life

Microbial mats are dense, vertically stratified communities typically dominated by cyanobacteria or algae in which microorganisms orientate themselves in response to micrometer-scale light and chemical gradients. The close association of organisms facilitates diverse metabolic processes, biogeochemical cycling, and niche differentiation, which affords greater microbial growth and survival than would be possible for individual species alone (Paerl *et al.*, 2000). Cyanobacterial mat systems are widespread in the lake environments of Antarctica, and they often dominate the total biomass and productivity in these ecosystems (Vincent, 2000; Moorhead *et al.*, 2005; Laybourn-Parry & Pearce, 2007).

Although microbial mats are common across Antarctica (Fig. 1, Table S2), the specific organisms present in a mat may vary considerably. Certain groups are regularly detected in Antarctic mats: *Cyanobacteria*, particularly members of the *Oscillatoriales* such as *Leptolyngbya* and *Phormidium*, and the *Nostocales* (Taton *et al.*, 2003; Jungblut *et al.*, 2005; Taton *et al.*, 2006; Fernández-Valiente *et al.*, 2007; Sutherland, 2009; Borghini *et al.*, 2010; Verleyen *et al.*, 2010; Anderson *et al.*, 2011; Callejas *et al.*, 2011; Fernandez-Carazo *et al.*, 2011), *Proteobacteria*, the CFB group, *Actinobacteria*, and *Firmicutes* (Brambilla *et al.*, 2001; Van Trappen *et al.*, 2002; Peeters *et al.*, 2011; Antibus *et al.*, 2012a; Varin *et al.*, 2012). Less commonly reported are *Deinococcus-Thermus*, *Planctomycetes*, *Verrucomicrobiales*, and others (Brambilla *et al.*, 2001; Antibus *et al.*, 2012b; Peeters *et al.*, 2012; Varin *et al.*, 2012). Diatoms may be absent (Anderson *et al.*, 2011), present at low levels alongside dominant *Cyanobacteria* (Sutherland, 2009; Hawes *et al.*, 2011), or in some cases present as the dominant phototrophs (Fernández-Valiente *et al.*, 2007). Other *Eucarya* such as green algae, fungi, and tardigrades have been noted in a wide range of mats (Verleyen *et al.*, 2010). Many studies report detection of novel bacterial species and genera (Brambilla *et al.*, 2001; Peeters *et al.*, 2011; Peeters *et al.*, 2012), and there is no doubt mat communities are a reservoir of considerable new biodiversity. In contrast, archaeal diversity in Antarctic mats is yet to be well studied, with only a few studies examining their presence (Brambilla *et al.*, 2001; Varin *et al.*, 2012), and viral populations in mats are almost completely undocumented.

Surprisingly, even some very recent studies of Antarctic mats have relied on microscopy, morphological taxonomy, and culturing rather than employing modern, culture-independent molecular methods for community characterization. A number of these studies focus on *Cyanobacteria* and their key roles in mat formation, where morphological taxonomy is possible because of the large and distinctive cell shapes (Sutherland, 2009; Sutherland & Hawes, 2009; Borghini *et al.*, 2010; Anderson *et al.*, 2011). Other studies focused on obtaining bacterial isolates, because of the advantages of having novel organisms in culture for future characterization and biotechnological exploitation (Brambilla *et al.*, 2001; Van Trappen

et al., 2002; Peeters *et al.*, 2011; Peeters *et al.*, 2012). Nevertheless, more widespread use of molecular methods is desirable for gaining greater resolution of species composition and community diversity. The advantage provided is illustrated by the cultivation of isolates from ancient algal mats yielding 15 types of cultivable bacteria (Antibus *et al.*, 2012b), compared to 215 operational taxonomic units (OTUs) for the same mats using clone libraries (Antibus *et al.*, 2012a). Similarly, in studies focused only on the presence of *Cyanobacteria*, 16 morphotypes were identified from five mats by microscopy compared to 28 OTUs by molecular methods (Taton *et al.*, 2006), and in a separate study of mats from the Transantarctic Mountains, six species were identified by microscopy and 15 by molecular methods (Fernandez-Carazo *et al.*, 2011).

Large-scale metagenomic studies provide a much greater level of information, and this is well illustrated by the yield of taxonomic and functional data that was obtained from an Antarctic microbial mat from fresh pond on the McMurdo Ice Shelf (Varin *et al.*, 2012). A total of 83 271 sequences were obtained from the mat, with ~ 25% having matches in the SEED phylogenetic profile database and 14% having matches to the SEED metabolic profile subsystems database. In addition to documenting the bacterial, archaeal, eucaryal, and viral taxa present (Table S2), the data enabled inferences about which taxa contributed what functional capacities, a boon for understanding the diverse mechanisms of adaptation present in this community. For cold adaptation, it was inferred that fatty acid desaturases were hallmarks of *Cyanobacteria*, while DNA gyrase A genes were present in *Betaproteobacteria*, *Planctomycetes*, *Alphaproteobacteria*, and CFB. Chaperones *dnaK* and *dnaJ* were distributed across *Cyanobacteria*, *Alpha-* and *Betaproteobacteria*, *Actinobacteria*, *Planctomycetes*, and others, while osmolyte (choline and betaine) uptake-related proteins were most common in *Alphaproteobacteria*, followed by *Betaproteobacteria*, *Cyanobacteria*, *Actinobacteria*, and CFB. Other genes involved in adaptive responses included the alternative sigma factor B, which was linked to *Cyanobacteria* and CFB, while carbon starvation response genes were present in *Planctomycetes*, *Beta-* and *Gammaproteobacteria*, and other bacteria. The ability to link taxonomic identities to specific functions that are important to individual and communal adaptation will be valuable for monitoring mat communities and forecasting how they may respond to ecosystem change.

The impact of a changing physico-chemical environment on microbial communities can be significant, and this was effectively described by a study of 13 ponds formed after an original larger pond (6712 m^2, 10 m deep) was drained by a crack in an ice dam (Sutherland, 2009). The newly created ponds have sizes ranging from 1.2 to 660 m^2, conductivities from 2.8 to 22.3 mS cm^{-1}, pH from 7.4 to 10.5, and DOC from 18.6 to 140 g m^{-3}. Two years after their formation, the cyanobacterial population distribution varied greatly among the new ponds (e.g. *Phormidium autumnale* ranged from 0% to 83% of total community in any given pond), while 60% of diatom species remained in common to all ponds. These community shifts have evolved from the original lake biota functioning as a seed inoculum, and can be related to the conductivity, nitrate, DOC, and desiccation profiles of the new ponds (Sutherland, 2009).

Antarctic lakes as models to understand whole ecosystem function

The relatively low diversity of Antarctic microbial food webs existing within effectively closed systems allows for an integrative understanding of the microbial community and biogeochemical cycling to be obtained. Studies of Blood Falls, an outflow of anoxic ferrous brine from the Taylor Glacier in the McMurdo Dry Valleys, revealed an unusual iron–sulfur cycle. The water is sulfate rich, exists in permanent darkness, and is estimated to have been isolated from external inputs for 1.5 million years (Mikucki *et al.*, 2009). 16S rRNA gene analysis showed the community was dominated by a close relative of *Thiomicrospira arctica*, an autotrophic sulfur-oxidizing gammaproteobacterium (*Thiotrichales*), as well as sequences related to *Delta-* and *Gammaproteobacteria* capable of iron and/or sulfur compound reduction, and CFB capable of heterotrophic growth on complex organic compounds (Mikucki & Priscu, 2007). A large proportion of adenosine 5′-phosphosulfate reductase genes related to those involved in dissimilatory sulfate metabolism were detected. However, dissimilatory sulfite reductase (*dsrA*) was not present, and radioisotope data indicated sulfide is not produced. The implication of this is that sulfate reduction does not proceed to sulfide as typically occurs in other aquatic systems, and instead, sulfate is expected to be regenerated via an alternative cycle with Fe(III) acting as the terminal electron acceptor (Mikucki *et al.*, 2009). This is a fascinating example of how a closed system has adapted to sustain life in the absence of light energy through the use of atypical chemical cycling, a pathway that was speculated to have possibly occurred in the ancient Neoproterozoic ocean (1000–500 mya) (Mikucki *et al.*, 2009).

A whole systems level approach was employed to piece together ecosystem functioning of a moderately complex lake system (Ng *et al.*, 2010; Lauro *et al.*, 2011). Ace Lake is a marine-derived meromictic saline lake in the Vestfold Hills that was isolated from the ocean ~ 5000 ya. Utilizing

both metagenomics and metaproteomics, a taxonomic profile of all three domains of life and viruses throughout the water column was determined as well as carbon, nitrogen, and sulfur cycles. The upper oxic waters resemble a simplified marine planktonic community, below which a dense layer of GSB resides at the oxycline, while the anoxic bottom waters host a diverse assemblage of anaerobic bacteria and archaea. By adopting a whole ecosystem perspective, it was apparent that community structure and resource partitioning defined nutrient cycling. For example, a strictly interdependent sulfur cycle was found to exist between the GSB and sulfate-reducing bacteria at the oxycline. The GSB were able to convert sulfide to sulfate but lacked assimilatory sulfate reduction capability (Ng *et al.*, 2010; Lauro *et al.*, 2011). It was also apparent that short circuiting of the nitrogen cycle was occurring. Nitrogen fixation proteins were not found in the metaproteome, likely due to preferential assimilation of ammonia (Ng *et al.*, 2010; Lauro *et al.*, 2011). Genes involved in nitrification and denitrification were underrepresented indicating the system had shifted away from nitrogen mineralization consistent with a mechanism to conserve bioavailable nitrogen (Lauro *et al.*, 2011). It is noteworthy that these findings are in contrast to lakes in the Dry Valleys where ammonia monooxygenase genes were present in the aerobic zone of six lakes of various salinities and extents of stratification (Voytek *et al.*, 1999).

The Ace Lake study also identified the importance of the loss of pathways including nitrification, the expansion of gene families (such as transposons), and the selection of key species (in particular GSB) as important steps in transiting the microbial population from marine to a meromictic lake ecosystem (Lauro *et al.*, 2011). To attempt to describe and predict the effects of ecosystem change on the lake, a mathematical model was developed that described the impact of fluctuations in environmental parameters on key microbial populations in the lake. One outcome of the model was the prediction that invasion (e.g. by introduction) by GSB-infecting phage would eliminate the population and leave the lake in an unstable condition, in terms of microbial processes, and unable to recover for many years into the future (Lauro *et al.*, 2011).

The biological responses to intra-annual and inter-annual variability in lake systems can only be interpreted effectively if associated environmental data are collected. When historical data are not available, the capacity of benthic microbial communities to form cumulative sedimentary laminae can be extremely valuable. Organic and carbonate (or other mineral) layers may be formed on annual (Sutherland & Hawes, 2009) or decadal timescales (Anderson *et al.*, 2011), and through careful interpretation can reveal a record of biological and environmental change. For example, concentrations of organic matter and carbonate in growth layers from microbial mats from Lake Hoare in Victoria Land have been linked with irradiation levels within the lake, and also to summer air temperatures (Sutherland & Hawes, 2009). In Lake Joyce, the lake level has risen 7 m between 1973 and 2009 due to increased meltwater influx, at least partly due to an increase in solar radiation and a gradual summer warming trend in the McMurdo Dry Valleys (Bomblies *et al.*, 2001). New microbial mats are forming at the margins of Lake Joyce at a rate of millimeters per year, while established mats and microbialites, which formed in shallow water but are now located below 13–16 m, are no longer receiving sufficient irradiance for growth (Hawes *et al.*, 2011). Thus, the larger microbial structures in deep water of this lake now represent legacies of previously favorable environmental conditions for phototrophic growth. In contrast, active microbial mats, pinnacles, and conical stromatolites are present to $\sim$ 100 m depth in Lake Untersee, where photoautotrophic growth is facilitated by light penetration to greater depths (Anderson *et al.*, 2011).

The responses of mats in Lakes Hoare, Joyce, and Untersee will be particular to those environments, and other systems will have different characteristics and therefore provide different information about their history. By integrating the fluctuations in environmental parameters over time with spatial and physico-chemical factors, it will be possible to infer the causes and effects of ecosystem change on microbial community diversity, structure, and function.

The Southern Ocean

Antarctica: marine microorganisms and the global importance of the Southern Ocean

Marine microorganisms play a critical role in maintaining the planet in a habitable state for several reasons. The oceans have the highest cellular production rate of any ecosystem, and despite having low levels of nutrients maintain microbial numbers on the order of 1×10^5 cells mL^{-1} (Schut *et al.*, 1997; Whitman *et al.*, 1998). In the open ocean, bacteria and archaea dominate in terms of biomass and play an essential role in regulating the accumulation, export, re-mineralization, and transformation of the world's largest pool of organic carbon (Cole *et al.*, 1988; Schut *et al.*, 1997; Azam, 1998; Azam & Malfatti, 2007). Bacteria can contribute up to 90% of the cellular DNA (Paul *et al.*, 1985), 40% of the planktonic carbon (Azam, 1998; Azam & Malfatti, 2007), perform up to 80% of the primary production (Ducklow, 1999), and have nutrient uptake potentials around 100 times faster than that of eucaryal phytoplankton (Black-

burn *et al.*, 1998). The fixation of carbon, nitrogen, and phosphorus by microorganisms and their conversion into particulate matter form the basis of the food web in the oceans. There are global consequences for these microbial processes since the downward flow of microbial particles is the most efficient means of transporting CO_2 fixed by primary production to marine sediments, thus sequestering it from the atmosphere. The balance between particle degradation, regenerating CO_2 via respiration, and burial directly impacts the trajectory of climate change.

The functions of the Southern Ocean are dependent on the existence of different water body masses, which can be distinguished based on the distribution of physicochemical parameters and ocean currents (Whitworth, 1980; Orsi *et al.*, 1995; Sokolov & Rintoul, 2002; Sokolov & Rintoul, 2009a, b). The nutrient-rich upper and lower Circumpolar Deep Waters is formed from the mixing of the deep waters from the Atlantic and Indian oceans with the cold sinking Antarctic Surface Waters (Fig. 2). Upwelling of Circumpolar Deep Waters returns nutrients to surface waters at lower latitudes, resulting in 75% of global primary production occurring north of 30°S (Sarmiento *et al.*, 2004). The Antarctic Bottom Water, which originates in the Weddell and Ross seas, sinks and is pushed north becoming the driving force of the global conveyor belt. The Polar Front separates the cold Antarctic Surface Waters from the warmer waters of the Subantarctic Zone. Across the Polar Front, the water temperature abruptly changes by 1.5–2 °C over a distance of 30–50 km, marking the most pronounced biogeographic boundary in Antarctic waters (Chiba *et al.*, 2001; Hunt *et al.*, 2001; Esper and Zonneveld, 2002; Ward *et al.*, 2003; Selje *et al.*, 2004; Abell & Bowman, 2005a; Giebel *et al.*, 2009; Weber & Deutsch, 2010; Jamieson *et al.*, 2012; Wilkins *et al.*, 2012.

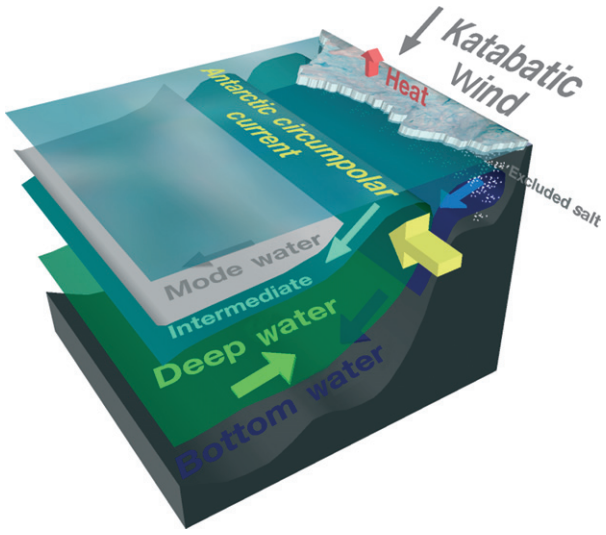

Fig. 2. Depiction of the main water bodies near Antarctica, and the Antarctic Circumpolar Current. Figure adapted from Rintoul (2000).

Bounded by the Polar Front and the Antarctic continent, the Antarctic Circumpolar Current recirculates surface waters around the Antarctic continent and is integral to the seasonal cycles of sea ice formation and loss. Seasonal variation not only causes extreme changes in sea ice cover, but controls light levels and day length. As a result, phototrophic growth and biomass production are high in summer and very low in winter. Overall, bacteria are abundant in the Southern Ocean despite the low temperatures and seasonal variability in productivity and are a major pathway for carbon flow (Delille, 2004; Hessen *et al.*, 2004).

It is expected that microbial communities that are unique to each water body (Esper & Zonneveld 2002; Ward *et al.*, 2003; Selje *et al.*, 2004; Abell & Bowman, 2005a) will be affected in a unique way by climate change and anthropogenic impact (Brown *et al.*, 2012). Anthropogenic climate change appears to be pushing the Antarctic Circumpolar Current poleward (Fyfe & Saenko, 2005; Gille, 2008; Biastoch *et al.*, 2009) due to warming and fresh water inputs (Böning *et al.*, 2008). The Southern Ocean covers ~ 10% of the earth's surface area (Aronson *et al.*, 2011), and due to the important role it plays in sequestering anthropogenic CO_2 (Sabine *et al.*, 2004; Mikaloff Fletcher *et al.*, 2006) through both physiochemical processes and the 'biological pump' of CO_2 fixation and export from the euphotic zone to the ocean depths (Thomalla *et al.*, 2011), the changes taking place in Antarctic waters could have major global ecological consequences (Liu & Curry, 2010). This is emphasized by the fact that changes to ecosystems in the south polar region (e.g. Ross Sea) are likely to have similarities to those occurring in the north (e.g. western Arctic Ocean) (Kirchman *et al.*, 2009). Predictions are that polar systems are generally very sensitive to environmental changes (Moline *et al.*, 2004; Murray & Grzymski, 2007) with global warming expected to cause increased microbial activity and lower availability of energy and food for organisms higher up the food chain (Kirchman *et al.*, 2009).

Many of the Antarctic marine zones (Fig. 1) have been sampled for the purpose of characterizing microbial communities using molecular approaches. A few transects have also been performed, comparing communities present in water bodies that represent different currents, temperatures, nutrient load, etc. A limited analysis of Southern Ocean sediment and deep water has been performed, but the majority of studies have been on surface waters. Sea ice communities have also been studied. However, the Southern Ocean is expansive with many environmental variables that may affect community composition. As a result, studies are still at the stage of establishing baseline values for diversity and community

Table 2. Southern Ocean studies that used metagenomics, metaproteomics, or tag pyrosequencing

Location	Methods	Notes	Reference	Fig. 1 map location
Kerguelen Islands and Antarctic Peninsula	Tag pyrosequencing of 16S rRNA genes	Sampled at multiple time points over an annual cycle	Ghiglione & Murray (2012)	Kerguelen Plateau; Antarctic Peninsula
Australian sector of Southern Ocean	Metagenomics (454 shotgun pyrosequencing)	Sampled along a longitudinal transect including multiple oceanic provinces	Wilkins *et al.* (2012)	Australian Sector
Australian sector of Southern Ocean	Metaproteomics and metagenomics (454 shotgun pyrosequencing)	Samples mainly coastal and metaproteomics performed on *Flavobacteria*-enriched sample	Williams *et al.* (2012b)	Australian Sector
Global, including samples from the Southern Ocean and Antarctic coastal waters	Metagenomics (454 shotgun pyrosequencing)	Global study of SAR11 biogeography including the Southern Ocean	Brown *et al.* (2012)	Australian Sector; Wilkes Land Coast; Prydz Bay; Vestfold and Larsemann Hills; Ross Sea
Palmer Station, Antarctic Peninsula	Metaproteomics	Complementary to metagenomic study of Grzymski *et al.* (2012)	Williams *et al.* (2012a)	Antarctic Peninsula
Palmer Station, Antarctic Peninsula	Metagenomics (shotgun library)	Complementary to metaproteomic study of Williams *et al.* (2012a)	Grzymski *et al.* (2012)	Antarctic Peninsula

dynamics. Moreover, large-scale shotgun sequencing metagenomic surveys and associated functional studies (metaproteomics, metatranscriptomics) have only commenced recently (Table 2). Topics in this Southern Ocean section describe the main marine pelagic bacterial, archaeal, eucaryal, and viral taxa, inferring where known, the functional capacities and roles they play in biogeochemical cycles. The characteristics of sea ice communities are also described.

Alphaproteobacteria: SAR11: highly abundant with distinct biogeography

The SAR11 clade of *Alphaproteobacteria* is probably the most abundant class of marine microorganism worldwide (Morris *et al.*, 2002). *Candidatus* 'Pelagibacter ubique' strain HTCC1062, the first and most intensively studied SAR11 isolate, has one of the smallest genomes and gene complements of any known free-living cell, and has a very small cell volume (Giovannoni *et al.*, 2005). The small cell volume, streamlined genome, and use of ATP-binding cassette (ABC) nutrient-uptake transporter genes are all consistent with an oligotrophic lifestyle, scavenging a wide range of substrates using high-affinity, broad-specificity transporters (Giovannoni *et al.*, 2005; Lauro *et al.*, 2009; Sowell *et al.*, 2009). SAR11 cells probably preferentially utilize low over high molecular weight (HMW) dissolved organic matter (DOM) (Malmstrom *et al.*, 2005), and their relative contribution to uptake of DOM may decrease as substrate concentration increases (Alonso & Pernthaler, 2006). A consequence of this oligotrophic strategy is that SAR11 members are probably unable to take advantage of sudden nutrient influxes, such as during phytoplankton blooms to rapidly increase cell density (Tripp *et al.*, 2008).

SAR11 has been consistently detected at high abundances in molecular surveys of the Southern Ocean. The surveys include open ocean regions as well as at depth and in coastal waters, and SAR11 is usually the dominant alphaproteobacterial, if not bacterial, group (García-Martínez and Rodríguez-Valera, 2000; López-García *et al.*, 2001; Murray & Grzymski, 2007; Giebel *et al.*, 2009; Murray *et al.*, 2010; Straza *et al.*, 2010; Piquet *et al.*, 2011; Ghiglione & Murray, 2012; Jamieson *et al.*, 2012), and appears to be more abundant in the epipelagic zone than at depth (Giebel *et al.*, 2009).

SAR11 exhibits biogeographic partitioning in the Southern Ocean and is represented by two major phylotypes with a temperature-driven boundary in the region of the Polar Front (Brown *et al.*, 2012). The clade appears to be more abundant in the Subantarctic and polar frontal zones than in the Antarctic Zone (Giebel *et al.*, 2009; Ghiglione & Murray, 2012; Wilkins *et al.*, 2012). This may be related to a competitive advantage of the oligotrophic SAR11 in the high nutrient-low chlorophyll (HNLC) Subantarctic Zone relative to the Antarctic Zone, where blooming phytoplankton lead to increased concentrations of HMW DOM and particulate organic matter.

SAR11 was found to account for the largest fraction of leucine uptake among all bacterial groups in continental shelf waters off the West Antarctic Peninsula, but a comparatively small fraction of protein uptake, consistent with a role as a low molecular weight (LMW) DOM specialist (Straza *et al.*, 2010). In a 16S rRNA gene survey on the Kerguelen Plateau (Subantarctic Zone), SAR11 was found to be a dominant group in HNLC waters outside a phytoplankton bloom but less abundant within the bloom (West *et al.*, 2008). A separate study of the same bloom found SAR11 had a markedly smaller relative contribution to bulk leucine incorporation within the bloom, suggesting it was not a major contributor to DOM degradation (Obernosterer *et al.*, 2011). Interestingly, SAR11 did dominate in abundance and leucine incorporation at an additional site where a transient phytoplankton bloom had taken place, implying that a time lag occurred during the succession between the baseline HNLC and bloom populations. It was also reported that the SAR11 abundances at the bloom station began to climb toward nonbloom levels once the bloom had peaked and begun to decline. Consistent with this, the relative abundance of SAR11 has been found to weakly inversely correlate with chlorophyll *a* fluorescence levels in Australian Antarctic waters (Williams *et al.*, 2012b).

In a coastal Antarctic Peninsula metaproteome, the SAR11 component was dominated by ABC transport proteins for the capture and uptake of labile substrates, especially taurine, polyamines and amino acids, and also included dimethylsulfoniopropionate (DMSP) demethylase (Williams *et al.*, 2012a). In another study, despite an apparently negative correlation between SAR11 and blooming phytoplankton, only small seasonal changes in abundance were reported during an annual cycle at the Antarctic Peninsula and Kerguelen Island (Ghiglione and Murray, 2012). All of these above studies of SAR11 are consistent with the interpretation that SAR11 is a nonopportunistic oligotroph specializing in LMW DOC.

An interesting physiological feature of SAR11 is the expression of the retinal-binding pigment proteorhodopsin, which has been shown to act as a proton pump when exposed to light (Béjà *et al.*, 2000) and has been implicated in photoheterotrophy. Proteorhodopsin may also perform nonenergetic functions such as photoregulatory sensing (Fuhrman *et al.*, 2008), and constitutive expression may facilitate the phototrophic ability to immediately respond to cellular energy deficits caused by carbon starvation (Steindler *et al.*, 2011). These latter characteristics may explain the unanticipated finding that, despite very low light levels in Antarctic waters during austral winter, proteorhodopsin was detected in the metaproteome throughout the annual cycle (Williams *et al.*, 2012a).

Alphaproteobacteria: *Roseobacter* clade: Antarctic phylotypes and a role in phytoplankton bloom turnover

The *Roseobacter* clade is an abundant and ecologically significant group of marine *Alphaproteobacteria*, found at high (> 15%) abundance in most marine surface environments (Buchan & Moran, 2005 and references therein). Unlike some other major proteobacterial groups which are strongly associated with a particular ecological niche (e.g. the SAR11 clade), the *Roseobacter* clade has diverse metabolic abilities, with members capable (for example) of aerobic anoxygenic phototrophy (Béjà *et al.*, 2002; Biebl *et al.*, 2005), degradation of DMSP by at least two pathways (Moran *et al.*, 2003; Miller & Belas, 2004), carbon monoxide oxidation (King, 2003) and heterotrophic utilization of a broad range of organic substrates (reviewed in Brinkhoff *et al.*, 2008). Members of the *Roseobacter* clade are found in the planktonic fraction as well as in commensal association with phytoplankton and metazoans (reviewed in Buchan & Moran, 2005). These characteristics are reflected in the broad diversity of genomic traits exhibited by related members of the *Roseobacter* clade (Lauro *et al.*, 2009) and the significant differences in gene content present between the cultured and uncultured members of the clade (Luo *et al.*, 2012).

Several 16S rRNA gene-based studies have identified the *Roseobacter* Clade Affiliated (RCA) subgroup as ubiquitous and abundant in Southern Ocean surface waters and to a depth of at least 2200 m. They composed ~ 10–30% of surface bacteria (and the majority of the *Roseobacter* clade) in the Subantarctic and Antarctic Zones (Selje *et al.*, 2004; Murray & Grzymski, 2007; Giebel *et al.*, 2009; Manganelli *et al.*, 2009; Ghiglione & Murray, 2012; Wilkins *et al.*, 2012) and were a major fraction of the population in coastal waters (Murray & Grzymski, 2007; Koh *et al.*, 2011). Two major RCA phylotypes appear to be present in the Southern Ocean and form the majority of the *Roseobacter* population. The phylotypes are strictly segregated by the Polar Front, coexisting only within the Polar Frontal Zone (Selje *et al.*, 2004; Giebel *et al.*, 2009) where they may outnumber even the SAR11 clade. There is some evidence that the Antarctic Zone RCA phylotype originates from the North Atlantic. North Atlantic Deep Water is formed by the sinking of dense, saline waters in the surface north Atlantic, and is transported to the Southern Ocean via global thermohaline circulation to become Circumpolar Deep Water (Callahan, 1972). Consistent with the upwelling of Circumpolar Deep Water in the Antarctic Zone south of the Polar Front, a global study of specifically amplified RCA 16S rRNA gene fragments found that the surface phylotype south of the Polar Front was identical to that found in

the Arctic Ocean, while differing by 3 bp from the phylotype north of the Polar Front (Selje *et al.*, 2004).

Little is known about the functional capabilities of RCA as only two isolated representatives have been described. *Candidatus* 'Planktomarina temperata' was isolated from the North Sea, where it was the dominant phylotype (Giebel *et al.*, 2010). The identification of the *pufM* gene encoding a bacteriochlorophyll a subunit suggests this member of the RCA is capable of performing aerobic anoxygenic photosynthesis, a function that is potentially ecologically important (Giebel *et al.*, 2010). An apparently heterotrophic RCA member was isolated from subtropical waters (Mayali *et al.*, 2008). This strain was found to colonize and increase mortality of blooming dinoflagellates, but its photosynthetic potential was not investigated (Mayali *et al.*, 2008).

Members of the *Roseobacter* clade, and particularly the RCA, have been strongly associated with phytoplankton blooms in the Southern Ocean. Two separate 16S rRNA gene-based studies of a naturally fertilized bloom at the Kerguelen Islands (Obernosterer *et al.*, 2011; West *et al.*, 2008) found that RCA and the *Roseobacter* NAC11-7 and NAC11-6 clusters were dominant bacterial OTUs within the bloom, suggesting they play a role in heterotrophic degradation of bloom products. However, unlike the other clusters, RCA representatives were also relatively abundant and metabolically active outside of the bloom. In Southern Ocean vertical profiles RCA abundances have been found to peak at the deep chlorophyll maximum, again suggesting an association with phytoplankton (Giebel *et al.*, 2009; Obernosterer *et al.*, 2011).

RCA abundance may follow seasonal cycles in the Southern Ocean. In the coastal current and Weddell Sea, RCA phylotypes have been found to reach a maximum of ~ 8% of all bacterial 16S rRNA gene sequences during winter but up to 36% during autumn (Giebel *et al.*, 2009). In coastal waters off the Antarctic Peninsula and around the Kerguelen Islands, the proportion of RCA peaked in January and February (summer) (Ghiglione & Murray, 2012).

A metagenomic study of Southern Ocean waters off West Antarctica found that *Roseobacter* clade 16S rRNA gene sequences were much more abundant in summer than in winter, with *Sulfitobacter* sequences the most abundant within this clade (Grzymski *et al.*, 2012). This is consistent with the association of the *Roseobacter* clade with phytoplankton (Moran *et al.*, 2007). Nevertheless, *Roseobacter* clade representatives in these polar waters appear to be metabolically active in both seasons. Metaproteomic analysis of coastal Antarctic Peninsula waters revealed an emphasis on high-affinity uptake systems such as ABC and tripartite ATP-independent periplasmic (TRAP) systems for capturing labile nutrients such as sugars, polyamines, amino acids, and oligopeptides (Williams *et al.*, 2012a).

Alphaproteobacteria: SAR116: marine generalists

The SAR116 clade of *Alphaproteobacteria* has been detected throughout the global ocean. SAR116 has been detected in molecular studies of the Southern Ocean, in both the Subantarctic Zone (Topping *et al.*, 2006; West *et al.*, 2008) and at lower abundance in the Antarctic Zone (Wilkins *et al.*, 2012). Estimates of relative abundance vary depending on the method used; fluorescence *in situ* hybridization (FISH) estimates were obtained for ~ 13% and ~ 32% of total bacterioplankton in the West and East regions of the Scotia Sea, respectively (Topping *et al.*, 2006), whereas metagenomic estimates indicated relative abundances of ~ 0.7% and ~ 0.4% of the total picoplankton in the Subantarctic Zone and Antarctic Zone, respectively (Wilkins *et al.*, 2012).

The only isolated SAR116 representative, *Candidatus* 'Puniceispirillum marinum', has been reported to have a versatile repertoire of genes for aerobic carbon monoxide oxidation, C1 metabolism, and DMSP degradation, suggesting it may occupy a 'marine generalist' niche similar to that of SAR11 and some members of the *Roseobacter* clade (Oh *et al.*, 2010a). Proteins for ABC and TRAP transport and C1 metabolism with high matches to SAR116 bacteria were detected in both the summer and winter metaproteomes of Antarctic Peninsula coastal waters, consistent with a preference for labile compounds and C1 substrates (Williams *et al.*, 2012a).

Betaproteobacteria: roles in ammonia oxidation and C1 turnover

The *Betaproteobacteria* are a large and cosmopolitan class with a range of ecological roles in the global ocean (reviewed in Kirchman, 2008). While not found at high abundance (Gentile *et al.*, 2006; Ghiglione & Murray, 2012; Lo Giudice *et al.*, 2012; Jamieson *et al.*, 2012), there is evidence that *Betaproteobacteria* perform significant ecological functions. Most known ammonia-oxidizing bacteria belong to the *Betaproteobacteria* (Head *et al.*, 1993; Teske *et al.*, 1994). *Nitrosospira*-like 16S rRNA gene sequences were detected in Ross Sea and Antarctic Peninsula surface waters, and the ribotype appeared to be similar to one found in the Arctic (Hollibaugh *et al.*, 2002). A metagenomic survey also detected OTUs for *Nitrosomonas europaea*, *Nitrosomonas eutropha*, and *Nitrosospira multiformis* strains in Southern Ocean surface waters (Wilkins *et al.*, 2012). However, ammonia-oxidizing archaea outnumbered ammonia-oxidizing bacteria at most sites (Wilkins *et al.*, 2012), consistent with the view that the former are the major nitrifiers in the marine environment (Wuchter *et al.*, 2006). Metagenomic and metaproteomic analyses of surface coastal waters off the Antarctic

Peninsula also showed evidence for ammonia-oxidizing *Betaproteobacteria* performing Calvin cycle carbon fixation and ammonia oxidation during winter (Grzymski *et al.*, 2012; Williams *et al.*, 2012a).

The OM43 clade of *Betaproteobacteria* is associated with coastal phytoplankton blooms (Morris *et al.*, 2006) and appears to be an obligate methylotroph capable of utilizing methanol and formaldehyde as carbon and energy sources (Giovannoni *et al.*, 2008). As it has one of the smallest reported genomes for a free-living cell, OM43 seems to be highly specialized for this unusual niche (Mira *et al.*, 2001). OM43 has been detected in a 16S rRNA gene library in a bloom in the Subantarctic Zone, where it was the only betaproteobacterial representative (West *et al.*, 2008), and in a metaproteomic survey of Antarctic Peninsula coastal waters where methanol dehydrogenase from OM43 was detected (Williams *et al.*, 2012a). Although the source of methanol in the marine environment is not known, it may be a byproduct of phytoplankton growth (Heikes *et al.*, 2002), which would be consistent with the association of OM43 with coastal blooms. The capacity for methylotrophic growth is also consistent with the identification of methyltransferases from oligotrophic marine *Gammaproteobacteria* (OMG) and methanogenic *Archaea* in an Antarctic coastal water sample taken during a phytoplankton bloom (Williams *et al.*, 2012b). Alternative sources of methanol are atmospheric deposition (Sinha *et al.*, 2007) or photochemical degradation of organic material (Dixon *et al.*, 2011). The latter is of particular relevance for Antarctic waters, given the high levels of solar irradiation during the austral summer. These possibilities indicate that OM43, and perhaps other C1 specialists, may play important roles in the Southern Ocean microbial loop.

Gammaproteobacteria: SAR86: no competition with SAR11

The gammaproteobacterial SAR86 clade is an abundant group in the surface ocean, being, for example, the most abundant genome for an uncultured organism in the GOS dataset (Dupont *et al.*, 2011). While it has been detected in the Southern Ocean (Abell & Bowman 2005a; Topping *et al.*, 2006; West *et al.*, 2008; Obernosterer *et al.*, 2011), little is known about its distribution or ecological role. Based on FISH estimates of activity, SAR86 cells composed ~ 8% and ~ 18% of total bacterioplankton in the western and eastern Scotia Sea, respectively, suggesting that at least in the Subantarctic Zone it is a major component of the surface community (Topping *et al.*, 2006). Genomic analysis of partial SAR86 genomes assembled from metagenomic datasets found the clade members had streamlined genomes and appear specialized for utilizing lipids and carbohydrates. These functional differences suggest minimal competition between SAR86 and SAR11 for DOC (Dupont *et al.*, 2011) and appear consistent with the high abundance and activity of SAR11 and SAR86 in the HNLC waters of the Subantarctic Zone (Obernosterer *et al.*, 2011).

Gammaproteobacteria: GSO-EOSA-1: dark carbon fixation, even at the surface

The GSO-EOSA-1 complex of sulfur-oxidizing *Gammaproteobacteria*, which includes the uncultivated ARCTIC96BD-19 and SUP05 lineages and cultivated chemoautotrophic clam symbionts, has been reported in global mesopelagic waters (Swan *et al.*, 2011) and oxygen minimum zones (Walsh *et al.*, 2009; Canfield *et al.*, 2010). The GSO-EOSA-1 complex appears to be affiliated with the *Thiotrichales* branch of the *Gammaproteobacteria* (Williams *et al.*, 2010). Four studies have recently identified GSO-EOSA-1 representatives at high abundance in coastal and Antarctic Zone waters (Ghiglione and Murray, 2012; Grzymski *et al.*, 2012; Williams *et al.*, 2012a; Wilkins, *et al.*, 2012). A metagenomic survey of coastal waters at Palmer station (Antarctic Peninsula) found winter bacterioplankton to be dominated by *Gammaproteobacteria* (~ 20% of the winter library compared to ~ 3% of the summer library), falling into five closely related taxa that were affiliated with the GSO-EOSA-1 complex (Grzymski *et al.*, 2012). Metaproteomic analysis of the same sites confirmed the abundance and seasonal pattern, and indicated that GSO-EOSA-1 appeared to be metabolically active at the surface in both summer and winter. In a latitudinal study from Hobart, Australia, to the Mertz Glacier, OTUs for GSO-EOSA-1 were found to be more abundant in the Antarctic Zone than in the Subantarctic Zone (Wilkins *et al.*, 2012).

Genomic and metagenomic analyses of GSO-EOSA-1 representatives, particularly SUP05, have revealed their potential for carbon fixation using the Calvin cycle and sulfur oxidation, even in well-oxygenated waters (Walsh *et al.*, 2009; Swan *et al.*, 2011; Grzymski *et al.*, 2012). Based on the characteristics of taxa identified, it was estimated that 18–37% of the winter bacterioplankton community has the potential to perform chemolithoautotrophy, including GSO-EOSA-1, suggesting that winter chemolithoautotrophy may contribute significantly to Southern Ocean carbon fixation (Grzymski *et al.*, 2012).

Gammaproteobacteria: OMG: contributors to phytoplankton bloom remineralization

The OMG are physiologically diverse heterotrophs that belong to previously detected clades (OM60, BD1-7, KI89A, OM182, SAR92) (Cho & Giovannoni, 2004). Cultured OMG isolates appear to be oligotrophic (Cho &

Giovannoni, 2004), although SAR92, a member of the OMG group, has been observed in nutrient-rich waters with high phytoplankton abundances (Pinhassi *et al.*, 2005; Stingl *et al.*, 2007). In the Southern Ocean, OTUs for SAR92 were far more abundant inside a bloom in the Kerguelen Islands and plateau region than in Subantarctic Zone waters outside the bloom, with abundance also declining with bloom age (West *et al.*, 2008; Obernosterer *et al.*, 2011). This is consistent with the growth of SAR92 being carbon limited (Stingl *et al.*, 2007) and suggests that the OMG group may play an important role in degradation of organic carbon produced by phytoplankton blooms in the Southern Ocean.

The OMG group has also been detected in coastal Antarctic Peninsula and Kerguelen Islands waters (Ghiglione & Murray, 2012), and metagenome and metaproteome analyses of coastal waters at Palmer station where OMG were found to be more abundant in summer than in winter (Grzymski *et al.*, 2012; Williams *et al.*, 2012a). TonB-dependent receptor systems matching the OMG group were highly abundant in the Palmer metaproteome, indicating that this is the preferred uptake system for ambient substrates (Williams *et al.*, 2012a). Certain OMG strains encode proteorhodopsin (HTCC2207, Stingl *et al.*, 2007; HTCC2143, Oh *et al.*, 2010b), and matches to these proteins were also identified in the metaproteome of both the summer and winter samples (Williams *et al.*, 2012a).

Other *Gammaproteobacteria*: contributors to DOM turnover

Ant4D3, an uncultured gammaproteobacterium, was identified in fosmids from nearshore waters at Palmer station (Grzymski *et al.*, 2006) and has since been reported as one of the most abundant proteobacterial groups in the Southern Ocean (West *et al.*, 2008; Murray *et al.*, 2010; Straza *et al.*, 2010; Ghiglione & Murray, 2012). In waters off the western Antarctic Peninsula, Ant4D3 represented 10% of the total and 50% of the gammaproteobacterial community, and 68% of cells incorporating amino acids (Straza *et al.*, 2010). Based on rRNA gene sequence data, the clade appeared to have low diversity (Straza *et al.*, 2010). Similar to SAR86, Ant4D3 cells were more active in HNLC Subantarctic waters than in bloom conditions on the Kerguelen Plateau (West *et al.*, 2008). In contrast, ~17% of excised 16S-DGGE bands from summer Antarctic Peninsula waters matched Ant4D3, dominating the *Gammaproteobacteria* and outnumbering those from winter Antarctic Peninsula and from Kerguelen Island waters (Ghiglione & Murray, 2012). Ant4D3 sequences were also abundant in a 16S rRNA gene library from waters in the vicinity of Antarctic icebergs (Murray *et al.*, 2010). Little is known about the function and ecological role of Ant4D3, although it has been detected in Antarctic waters associated with a phytoplankton bloom (Williams *et al.*, 2012b), and Arctic waters where it appeared to occupy a DOM utilization niche different from that of other major heterotrophs such as SAR11 (Nikrad *et al.*, 2012).

Various other gammaproteobacterial groups (e.g. *Oceanospirillales*, *Alteromonadales*) have been detected in Southern Ocean waters, including bacteria with best matches to *Neptuniibacter caesariensis*, *Marinomonas* spp., *Marinobacter aquaeolei*, *Colwellia psychrerythraea*, and *Pseudoalteromonas haloplanktis* (Murray & Grzymski, 2007; Grzymski *et al.*, 2012; Wilkins *et al.*, 2012; Williams *et al.*, 2012a). These are motile chemoorganotrophs that target labile substrates such as simple sugars, amino acids, organic acids, or (in the case of *M. aquaeolei*) hydrocarbons (Médigue *et al.*, 2005; Methé *et al.*, 2005; Arahal *et al.*, 2007; Espinosa *et al.*, 2010; Singer *et al.*, 2011). Some marine *Oceanospirillales* possess genes for both carbon fixation (Calvin cycle) and sulfur oxidation (Swan *et al.*, 2011).

Deltaproteobacteria: a chemoautotrophic role in deep waters

Deltaproteobacteria have rarely been detected in abundance in surface waters (e.g. Venter *et al.*, 2004), and this is also the case for the Southern Ocean (Murray & Grzymski 2007; West *et al.*, 2008, Murray *et al.*, 2010; Ducklow *et al.*, 2011; Ghiglione & Murray 2012; Jamieson *et al.*, 2012; Wilkins *et al.*, 2012). However, numbers may be higher in mesopelagic waters (Wright *et al.*, 1997; Zaballos *et al.*, 2006; Pham *et al.*, 2008). At a 3000-m-deep site at the Polar Front in the Drake Passage, several deltaproteobacterial 16S rRNA gene sequences were detected (López-García *et al.*, 2001), all of which clustered with the marine deltaproteobacterial clade SAR324 from the mesopelagic Sargasso Sea (Wright *et al.*, 1997). Whole-genome analysis of SAR324 indicates an ecology that includes carbon fixation via the Calvin cycle and sulfur oxidation, as well as oxidation of methylated compounds (Swan *et al.*, 2011). The SAR324 group may therefore be a significant contributor to chemoautotrophy in the dark ocean (Swan *et al.*, 2011).

CFB: algal detritus degradation

The CFB group is cosmopolitan and abundant in the global ocean (Glöckner *et al.*, 1999). The abundance of CFB may be underrepresented in 16S rRNA gene libraries and FISH analyses due to probe specificity biased against CFB 16S rRNA (Cottrell & Kirchman, 2000; Eilers *et al.*, 2000; Kirchman, 2002), with better estimates being achievable

from shotgun metagenomic surveys (O'Sullivan *et al.*, 2004; Cottrell *et al.*, 2005).

The CFB class *Flavobacteria* appear to be abundant in both freshwater and marine environments (O'Sullivan *et al.*, 2004; Cottrell *et al.*, 2005) including the Southern Ocean (Abell & Bowman, 2005a; Williams *et al.*, 2012b). The *Flavobacteria* often form a major fraction of planktonic taxa (Fandino *et al.*, 2001) and are particularly prevalent in particle-attached communities (DeLong *et al.*, 1993) and in association with phytoplankton blooms (Pinhassi *et al.*, 2004). Isolated representatives have a well-described capacity to degrade HMW DOM, particularly biopolymers which may be recalcitrant to utilization by other bacterial heterotrophs (reviewed in Kirchman, 2002), suggesting they play an important role in remineralization of primary production products.

Flavobacteria in the Southern Ocean are strongly biogeographically partitioned. From 16S-DGGE analysis, the abundance and diversity of particle-attached *Flavobacteria* were higher in the nutrient- and phytoplankton-rich waters south of the Polar Front compared to HNLC Subantarctic waters (Abell & Bowman, 2005a). A large-scale metagenomic analysis that identified the Polar Front as a major biogeographic boundary found that CFB contributed a large fraction of the variance between the zones north and south of the Polar Front and that CFB were more abundant south of the front (Wilkins *et al.*, 2012). This difference in abundance may be largely attributable to the low iron availability in the Subantarctic, which probably limits primary production (Boyd *et al.*, 2007). Both natural and artificial iron fertilization events in the Subantarctic have resulted in high abundances of bacterial heterotrophs (Oliver *et al.*, 2004; Christaki *et al.*, 2008), and *Flavobacteria* have been identified as a major component of the bacterial response to blooms induced by natural iron input on the Kerguelen Plateau (West *et al.*, 2008).

The higher abundance of *Flavobacteria* in the Antarctic Zone may also relate to their prevalence in sea ice (Brown & Bowman, 2001; Brinkmeyer *et al.*, 2003), from which they would be released into Antarctic Zone waters during seasonal melting. Two groups, the uncultured agg58 cluster and the genus *Polaribacter*, appear to dominate flavobacterial populations and activity in the Southern Ocean (Abell & Bowman, 2005a, b; Murray & Grzymski 2007; West *et al.*, 2008; Straza *et al.*, 2010; Ducklow *et al.*, 2011; Obernosterer *et al.*, 2011; Ghiglione & Murray 2012). Members of the *Polaribacter* genus are gas-vacuolated, proteorhodopsin-containing *Flavobacteria* that are prevalent in Antarctic and Arctic seawater. Genomic analysis of *Polaribacter* sp. MED152 indicates it is genetically geared to utilize polymers obtained from algal detritus rather than labile exudates (González *et al.*, 2008). Metagenomic analysis of Antarctic Peninsula coastal waters found *Polaribacter*-related sequences to be dominant in summer, consistent with them being associated with phytoplankton blooms and/or being seeded from melting sea ice (Grzymski *et al.*, 2012). Flavobacterial proteins (including those with the best matches to *Polaribacter* spp.) were similarly much more abundant in the summer vs. winter metaproteome from the same sites, with components of TonB-dependent receptor systems predominating (Williams *et al.*, 2012a).

A comparative metagenomics study of coastal East Antarctica samples found that the relative abundance of *Flavobacteria* (dominated by *Polaribacter*) positively correlated with chlorophyll *a* fluorescence, and the relative abundance of SAR11 inversely correlated with fluorescence and *Flavobacteria* abundance (Williams *et al.*, 2012b). A metaproteomic assessment of the sample with highest relative abundance of *Flavobacteria* concluded that the *Flavobacteria* synthesized proteins for actively binding and exploiting algal-derived polymeric substrates (carbohydrates, polypeptides, lipids), while *Alphaproteobacteria* (SAR11, *Rhodobacterales*, SAR116) and *Gammaproteobacteria* (Ant4D3, OMG, *Oceanospirillales* + *Alteromonadales*) synthesized high-affinity uptake systems to utilize simple byproducts (sugars, acetate, ammonia) released from the degradation of the algal polymers (Williams *et al.*, 2012b).

There is some evidence that planktonic and particle-attached *Flavobacteria* may include specific phylotypes. In a mesocosm experiment using 16S-DGGE to examine colonization of diatom detritus in Southern Ocean seawater, a large proportion of flavobacterial phylotypes present in the planktonic phase were found not to colonize detrital particles (Abell & Bowman, 2005b). This suggested that these phylotypes may grow more slowly, perhaps comprising a secondary group of colonizers that dominate when the more accessible detrital nutrients have been exhausted and the primary colonizers have secreted useful secondary metabolites. Consistent with this hypothesis, the analysis of single-cell genome sequencing data indicates that the majority of uncultured marine *Flavobacteria* are adapted to specialized ecological niches, while also possessing the genomic capacity to attach to particles and degrade biopolymers (Woyke *et al.*, 2009). It is likely that size fractionating these communities will help to physically separate planktonic from particle-attached cells, and metagenomic analyses of the separate fractions should help to define phylogenetic and functional differences within the *Flavobacteria* (Williams *et al.*, 2012b); this approach has proven useful for Antarctic lake (e.g. Lauro *et al.*, 2011) and Southern Ocean (e.g. Wilkins *et al.*, 2012) communities.

Cyanobacteria: low abundance phototrophic survivors

Cyanobacteria are the most abundant photosynthetic organisms on Earth, dominated by the marine genera *Prochlorococcus* and *Synechococcus* (Scanlan *et al.*, 2009 and references therein), but little molecular research has been performed on their role in Southern Ocean ecosystems. This may be because it has been generally accepted that there are no *Cyanobacteria* in Antarctic waters (Zubkov *et al.*, 1998; Evans *et al.*, 2011; Ghiglione & Murray, 2012), although recent metagenomic (Wilkins *et al.*, 2012) and metaproteomic (Williams *et al.*, 2012a) results indicate they are present. *Cyanobacteria* survive at Antarctic temperatures, and both *Synechococcus* and *Prochlorococcus* strains have been identified in the water column of several marine-derived Antarctic lakes, including at sub-zero water temperatures (Bowman *et al.*, 2000b; Powell *et al.*, 2005; Lauro *et al.*, 2011), while filamentous *Cyanobacteria* dominate many benthic mat communities in Antarctic lakes (see Microbial mats as microcosms of Antarctic life above). Given their persistence from Subantarctic waters (Abell & Bowman, 2005a; Topping *et al.*, 2006; Wilkins *et al.*, 2012) across the Polar Front to the Antarctic continent (Wilkins *et al.*, 2012), albeit at low abundance, it will be valuable to assess their ecological role as survivors in the Southern Ocean (also see cyanophage in Virioplankton: crucial influence and much to be learned below).

Other bacteria: members of the community that may play important roles

The *Verrucomicrobia* is a recently described bacterial phylum that is ubiquitous in the marine environment and appears to be composed of several physiologically distinct lineages (Freitas *et al.*, 2012). A small number of representatives of *Verrucomicrobia* have been detected in the Southern Ocean (Gentile *et al.*, 2006; Murray & Grzymski, 2007; West *et al.*, 2008; Murray *et al.*, 2010). More recently, 16S rRNA gene analysis identified higher numbers of *Verrucomicrobia* at a Kerguelen Island site relative to a site near Palmer Station on the Antarctic Peninsula (Ghiglione & Murray, 2012). In contrast, a metagenomic survey identified a larger number of OTUs for the verrucomicrobium *Coraliomargarita akajimensis* in Antarctic Zone compared to Subantarctic Zone waters (Wilkins *et al.*, 2012).

Bacteria of the phylum *Planctomycetes* have been detected at low abundance in molecular surveys of the Southern Ocean (López-García *et al.*, 2001; Abell & Bowman, 2005a; Gentile *et al.*, 2006; Murray *et al.*, 2010; Jamieson *et al.*, 2012). *Planctomycetes* is emerging as a group of interest in marine microbial ecology, because they perform anaerobic ammonia oxidation (anammox) (Strous *et al.*, 1999), and metaproteomic analysis indicates they may be active in coastal Antarctic Peninsula waters (Williams *et al.*, 2012a). The metaproteome study also detected *Nitrospirae* proteins involved in nitrite oxidation and carbon fixation via the reductive tricarboxylic acid cycle (Williams *et al.*, 2012a). These metaproteome data support a role for members of the *Nitrospirae* and *Planctomycetes* in completing nitrification using nitrite generated by ammonia-oxidizing archaea and bacteria in Antarctic waters.

Other bacterial groups that have been reported at low abundance in the Southern Ocean include *Actinobacteria* (Brinkmeyer *et al.*, 2003; Abell & Bowman, 2005a; Gentile *et al.*, 2006; Murray & Grzymski, 2007; Murray *et al.*, 2010; Bolhuis *et al.*, 2011; Ghiglione & Murray, 2012; Jamieson *et al.*, 2012), *Epsilonproteobacteria* (Gentile *et al.*, 2006; Murray & Grzymski, 2007), and *Firmicutes* (Murray & Grzymski, 2007; Murray *et al.*, 2010; Lo Giudice *et al.*, 2012). Little is known about their respective ecological roles, although *Actinobacteria* are known to associate with marine aggregates (Grossart *et al.*, 2004), and their terrestrial counterparts have diverse HMW substrate degradation capabilities (reviewed in Kirchman, 2008). A strong negative correlation has been reported between actinobacterial abundance and latitude in a global survey using 16S rRNA gene libraries (Pommier *et al.*, 2007), with higher abundances in tropical and subtropical waters.

Archaea: high abundance and novel properties

Following the discoveries in 1992 of new clades of *Archaea* in the marine environment (DeLong, 1992; Fuhrman *et al.*, 1992), in 1994, *Archaea* were discovered in Antarctic coastal surface waters at high abundance (up to 34%) (DeLong *et al.*, 1994). These discoveries helped to establish that *Archaea* were ubiquitous members of the environment, and not just unusual extremophiles (Cavicchioli, 2011). The majority of rRNA gene sequences from Antarctic waters were affiliated with the Marine Group I *Crenarchaeota* (MGI; also called Thaumarchaeota), while the remainder represented Group II *Euryarchaeota* (DeLong *et al.*, 1994). Subsequent rRNA gene analyses verified that MGI are the most abundant *Archaea* in surface waters of coastal Antarctica, followed by Group II *Euryarchaeota* (Gerlache Strait, Massana *et al.*, 1998; near Anvers Island, Murray *et al.*, 1998). Further studies have demonstrated the widespread distribution of Antarctic marine *Archaea* both longitudinally and north and south of the Polar Front (Topping *et al.*, 2006; Kalanetra *et al.*, 2009; Jamieson *et al.*, 2012; Wilkins *et al.*, 2012). *Archaea* including MGI have also been identified in benthic

sediments on the Antarctic coast (Bowman & McCuaig, 2003; Bowman *et al.*, 2003).

In the Southern Ocean, total archaeal rRNA gene levels were found to decrease during spring (Massana *et al.*, 1998) and summer (Murray *et al.*, 1998) and to negatively correlate with chlorophyll *a* concentration (Murray *et al.*, 1998). Consistent with this, MGI abundance was found to increase by 44% during winter (Church *et al.*, 2003). MGI are able to perform ammonia-oxidizing chemolithoautotrophy (Ingalls *et al.*, 2006; Berg *et al.*, 2007, 2010). Ammonia-oxidizing MGI have been shown to be especially sensitive to photoinhibition (Merbt *et al.*, 2012), which might account for their decline during periods of extended illumination. It has also been speculated that the decline of *Archaea* during spring/summer represents competition with nonarchaeal microorganisms during phytoplankton blooms (Massana *et al.*, 1998) or that the majority of MGI are chemoautotrophic and therefore more competitive compared to heterotrophs during carbon-scarce winter conditions (Murray *et al.*, 1998).

Metaproteomic analysis of winter coastal Antarctic Peninsula samples revealed that MGI proteins (most with best matches to the ammonia-oxidizer *Nitrosopumilus maritimus*) represented 30% of all archaeal plus bacterial proteins, and no MGI proteins were detected in the summer metaproteome (Williams *et al.*, 2012a). The winter metaproteome included MGI proteins involved in the 3-hydroxypropionate/4-hydroxybutyrate cycle, the pathway used by ammonia-oxidizing MGI for carbon fixation (Berg *et al.*, 2007, 2010), and proteins for ammonium uptake and ammonia oxidation. Based on the metagenomic and metaproteomic analyses, it was proposed that chemolithoautotrophic ammonia oxidation was performed by MGI and sulfur oxidation by *Gammaproteobacteria* (see *Gammaproteobacteria*: GSO-EOSA-1: dark carbon fixation, even at the surface, above), suggesting that these communities were likely to be the major drivers of carbon fixation in Antarctic waters during winter (Grzymski *et al.*, 2012; Williams *et al.*, 2012a).

Marine Group II *Euryarchaeota* include motile, proteorhodopsin-containing photoheterotrophs that specialize in protein and lipid degradation (Frigaard *et al.*, 2006; Iverson *et al.*, 2012). Marine Group II *Euryarchaeota* have been found in higher abundance in surface waters than at depth (Massana *et al.*, 1998), and in waters off Anvers Island, numbers increased in autumn (Murray *et al.*, 1998). However, there are little molecular data for Marine Group II *Euryarchaeota*, particularly seasonal data, and their importance in Southern Ocean ecosystem function is not clear.

It is noteworthy that primer design can greatly impact on the ability to effectively evaluate the abundance of any taxa, and this has particularly impacted on the detection and enumeration of *Archaea*. A striking example relates to the discovery of the marine hydrothermal *Nanoarchaeum equitans*, which was unable to be detected using archaeal probes (Huber *et al.*, 2002), but when primers were designed from the 16S rRNA gene sequence present in the genome sequence, *Nanoarchaeum* species were identified in many environments around the globe (Casanueva *et al.*, 2008). In the Southern Ocean, a summer transect between the Polar Front and the ice edge failed to identify DAPI-positive archaeal cells when *Archaea*-specific probes ARCH334 and ARCH915 were used (Simon *et al.*, 1999). A similar outcome for samples from 3000 m depth at the Polar Front in the Drake Passage prompted the redesign of primers leading to the discovery of both a higher number and greater diversity of *Archaea* in the samples (López-García *et al.*, 2001). While shotgun metagenomic avoids this problem, the issue is relevant for the design of primers employed for pyrotag sequencing, a cost-effective means of baselining community composition. This is particularly the case for the design of universal primers suitable for detecting *Archaea*, *Bacteria*, and *Eucarya*. This issue is well illustrated by the analysis of an Antarctic lake community where archaeal representation increased by many fold when standard universal primers were redesigned to better represent *Archaea* (R. Cavicchioli *et al.*, unpublished results).

Virioplankton: crucial influence and much to be learned

The 'viral shunt', by which nutrients are released via lysis from marine microorganisms and returned to the dissolved and particulate pools, may mediate the flux of a quarter of all organic matter in the microbial loop (Wilhelm & Suttle, 1999), and the viral release of iron from bacterioplankton may be crucial for phytoplankton growth (Poorvin *et al.*, 2004). Viral production, and by inference the viral shunt, has been shown to be highly active in HNLC Subantarctic (Evans *et al.*, 2009), iron-fertilized Subantarctic (Weinbauer *et al.*, 2009), and coastal waters, where virus-mediated carbon flux may account for 50–100% of all heterotrophic production (Guixa-Boixereu *et al.*, 2002). Despite this crucial ecosystem role, molecular analysis of the diversity and function of Southern Ocean virioplankton is sparse.

Using probes for marker genes, both algal viruses and cyanophage have been detected in Southern Ocean waters (Short & Suttle, 2002, 2005). Cyanophage genes and proteins, and a major capsid protein from *Phaeocystis pouchetii* virus PpV01, were identified in coastal Antarctic Peninsula waters by metagenomic and metaproteomic analyses (Grzymski *et al.*, 2012; Williams *et al.*, 2012a). In a latitudinal study, OTUs for *Ostreococcus* viruses were

found to be more abundant in Antarctic compared to Subantarctic Zone waters, and cyanophage were detected in all Southern Ocean samples examined (D. Wilkins *et al.*, unpublished results). While preliminary, these studies suggest that the more abundant viruses in the Southern Ocean are predators of phytoplankton.

Antarctic sea ice communities: unique interactions between *Bacteria*, *Archaea*, *Eucarya*, and viruses

Sea ice is one of the largest and most climatically sensitive geophysical parameter on the planet. In the Antarctic and Southern Ocean region, sea ice ranges in extent from 3×10^6 km^2 in summer to 18×10^6 km^2 in winter (Parkinson, 2004). As resource availability in the Southern Ocean is strongly seasonal and related to the cycle of sea ice, the life history traits of the dominant macrofauna, including marine mammals, penguins, and various other seabirds, as well as benthic communities (Wing *et al.*, 2012), are tightly synchronized with the presence of sea ice. Predicted changes in sea ice extent threaten to unravel the current synchronicity and may impact all levels of the food web. Several recent studies have already identified declines in polar species related to zones of decreasing sea ice extent (Anisimov *et al.*, 2007; Schofield *et al.*, 2010; Trathan *et al.*, 2011).

The sea ice environment can be highly productive, despite being cold (0 to −35 °C) and highly saline (up to seven times seawater salinity). As an ecosystem, sea ice is characterized by a continuum of temperature, salinity, pH, light, and nutrient gradients, which arise due to the physical and chemical processes of ice formation, and it varies both spatially and temporally (Eicken, 2003; Mock & Thomas, 2005). Conditions are generally harshest at the ice surface where *in situ* temperatures are governed by air temperature (including wind regimes) and the extent of insulating snow cover. At the ice water interface, temperatures are stable at −2 °C buffered by the seawater, nutrient supply is constantly replenished through wave action, and UV radiation and photosynthetic light intensities are lower. This interface is the site of highest biological productivity. Exclusion of salt crystals during the freezing of seawater results in the formation of highly saline 'brine' channels within the ice matrix, and it is these channels that provide the habitat for organisms residing within the sea ice structure.

The first application of molecular biology methods to the sea ice microbial community (SIMCO) was the use of 16S rRNA gene sequencing to taxonomically characterize culture collections. This identified many novel stenopsychrophiles, including members of the *Gammaproteobacteria* genera *Colwellia*, *Shewanella*, *Marinobacter*, and *Glaciecola* (Bowman *et al.*, 1998a), the *Firmicutes* genus *Planococcus*, and the CFB genera *Psychroserpens* (Bowman *et al.*, 1997b), *Gelidibacter* (Bowman *et al.*, 1997b), and *Psychroflexus* (Bowman *et al.*, 1998b) (Fig. 1). Eurypsychrophiles isolated from sea ice include the *Gammaproteobacteria* genera *Pseudoalteromonas*, *Psychrobacter* (Bowman *et al.*, 1997c), *Halomonas*, and *Pseudomonas*, the *Alphaproteobacteria* genera *Hyphomonas* and *Sphingomonas* (related to the *Roseobacter* clade), the *Actinobacteria* genus *Arthrobacter*, and the *Firmicutes* genera *Planococcus* and *Halobacillus* (Bowman *et al.*, 1997a).

The use of cultivation-independent (16S rRNA gene sequencing of DNA extracted from sea ice) and cultivation-dependent methods led to the discovery that the majority of sea ice organisms are active (Brinkmeyer *et al.*, 2003) and the dominant organisms are culturable. These features distinguish SIMCOs from other pelagic marine microbial communities where generally between 0.1% and 15% of organisms (Donachie *et al.*, 2007) are readily cultivatable (Amann *et al.*, 1995).

The main organisms identified from culture-independent surveys in both Antarctic and Arctic sea ice are members of the *Alpha-* and *Gammaproteobacteria*, CFB, *Actinobacteria*, *Chlamydiales*, and *Verrucomicrobiales* (Brown & Bowman, 2001; Brinkmeyer *et al.*, 2003; Murray & Grzymski, 2007). Microbial communities are phylogenetically similar in sea ice in the southern and northern polar regions, highlighting that strong and common selection mechanisms take place during the development of SIMCOs (Brown & Bowman, 2001; Brinkmeyer *et al.*, 2003).

Bacteria in SIMCOs are taxonomically and physiologically different to the community inhabiting the water column from which ice is formed. However, this is not the case for *Archaea*. In contrast to sea water where *Archaea* may be abundant (see *Archaea*: high abundance and novel properties above), in sea ice, *Archaea* are either below detection (Brown & Bowman, 2001; Brinkmeyer *et al.*, 2003; Murray & Grzymski, 2007) or comprise a very low abundance (Junge *et al.*, 2004; Collins *et al.*, 2010; Cowie *et al.*, 2011). The most abundant archaeal taxa in sea ice are MGI that are similar to seawater members (Cowie *et al.*, 2011), and the relatively low abundance, diverse members of the *Euryarchaeota* are also closely related to pelagic members. Similar to *Bacteria*, *Archaea* from the Antarctic are phylogenetically similar to those from the Arctic (Collins *et al.*, 2010; Cowie *et al.*, 2011).

Proteorhodopsin-containing bacteria have been isolated from Antarctic sea ice (e.g. *Psychroflexus torquis*, Bowman *et al.*, 1998b), and the presence and *in situ* activity of photoresponsive genes for bacterial chlorophyll A (*puf*M) and proteorhodopsin have been confirmed (Koh *et al.*, 2010, 2011), suggesting that the strong seasonal light regimes may play a direct role in structuring sea ice bac-

terial communities. However, the major phototrophs in sea ice are *Eucarya*.

The sea ice eucaryal community has generally been examined using traditional microscopy techniques and is composed of a diverse range of phototrophic, heterotrophic, and mixotrophic organisms including *Stramenopiles* (mainly diatoms), *Alveolates*, and metazoans that are incorporated into the ice matrix by physical scavenging of cells during ice formation (Garrison *et al.*, 1983). Once incorporated, the community undergoes maturation based on the different physical and chemical properties it experiences in the sea ice. Hence, depending on factors such as position in the ice column, snow cover, light intensity, and nutrient supply, there is a large spatial heterogeneity, and habitat-specific communities develop (Gast *et al.*, 2004).

Seasonal changes in physical and chemical properties also play a large role in shaping the sea ice eucaryal community, and community composition can vary significantly. For example, postwinter/early spring communities have been observed to comprise dinoflagellates, ciliates, cercozoans, *Stramenopiles*, *Viridiplantae*, haptophytes, and metazoans, or a dinoflagellate-dominated community, or a diatom-dominated community that developed after sea ice breakup (Piquet *et al.*, 2008). Furthermore, at the end of winter, phototrophs may be essentially absent from sea ice, having been removed by extensive over-winter grazing (Bachy *et al.*, 2011), although spatial heterogeneity can result in significantly different postwinter communities (Kramer *et al.*, 2011). Evidence from Arctic sea ice suggests that communities inhabiting the ice/water interface are more similar to seawater communities than those entrained higher in the ice matrix, isolated from seawater intrusions (Bachy *et al.*, 2011). These ice–water interface communities, generally dominated by phototrophic pennate diatoms, are the focus of numerical models aimed at estimating sea ice contributions to climate active compounds such as dimethyl sulfide (Elliot *et al.*, 2012).

Although there are sea ice *Eucarya* such as *Fragilariopsis cylindrus*, which reside in the south and north polar regions (Lundholme & Hasle, 2008), the true nature of species bipolarity is unclear (Poulin *et al.*, 2010) as there is a lack of molecular data with sufficient resolution to establish biogeography and sea ice vs. sea water speciation differences (Gast *et al.*, 2004, 2006). The lack of taxonomic marker data for morphologically well-defined cultured *Eucarya* (e.g. Skvovgaard *et al.*, 2005) also hinders assessment of claims of highly novel diversity in marine eucaryal microorganisms (e.g. López-García *et al.*, 2001; Moon-van-de Staay *et al.*, 2001; Gast *et al.*, 2004).

Sea ice microorganisms produce compounds that interact with the ice matrix to enhance its habitability (Krembs *et al.*, 2011). Algae and bacteria produce extracellular polymeric substances (Mock & Thomas, 2005) that can display antifreeze activity that inhibits ice recrystallization and increases salt retention in the sea ice matrix (Raymond, 2011). Salt retention translates to greater retention of other source water impurities including iron and nutrients critical for photosynthetic activity (Krembs *et al.*, 2011). Genomic and transcriptomic analyses of the sea ice diatoms *F. cylindrus* (Krell *et al.*, 2008; Bayer-Giraldi *et al.*, 2010) and *Chaetoceros neogracile* (Gwak *et al.*, 2010) have revealed the importance of novel antifreeze proteins in responding to cold stress. Genes encoding antifreeze proteins appear to have undergone horizontal gene transfer into the dominant metazoan calanoid copepod in Antarctic sea ice, *Stephos longipes* (Kiko, 2010). The proteins have high identity to a group of (putative) antifreeze proteins from diatoms, bacteria, and a snow mold, in contrast to a lack of homologs in any known metazoan lineage.

Viruses are present and may be highly abundant in sea ice, although the factors driving their distributions are not clear. Viral abundances have been observed ranging from 10^5 to 10^9 in Antarctic sea ice (Gowing *et al.*, 2004; Patterson & Laybourn-Parry, 2012) and are greater than in the underlying seawater, suggesting active entrainment during ice formation or *in situ* production (Collins & Demming, 2011). When observed over a complete sea ice cycle, virus-to-bacterium ratios showed a clear seasonal pattern in Antarctica, with lowest values in winter (range 1.2–20.8) (Patterson & Laybourn-Parry, 2012). Temperature and salinity fluctuations within the brine channel system, the high abundance of viruses, and the predicted increased frequency of interactions between bacteria and viruses compared to seawater indicate that sea ice provides 'natural transformation' conditions and is a potential 'hot spot' for horizontal gene transfer (Kiko, 2010; Collins & Demming, 2011). Such conditions may also have promoted the transfer of antifreeze genes into copepods.

Antarctic microorganisms: a brief Southern Ocean perspective

The picoplankton in the surface waters of the Southern Ocean that encircle the Antarctic continent are dominated by *Alphaproteobacteria*, *Gammaproteobacteria*, *Flavobacteria*, and MGI. The *Alphaproteobacteria* consist mainly of the SAR11 and *Roseobacter* clades, with a metabolic preference for labile substrates, including those released by phytoplankton. *Flavobacteria* have metabolic preferences focused on more complex organic matter, although the relative roles of free-living vs. attached *Flavobacteria* in processing algal-derived organic matter are still poorly understood. Collectively, the *Gammaproteobacteria* have

diverse metabolic capabilities, with members of the GSO-EOSA-1 complex serving as potentially important contributors to carbon fixation. The ammonia-oxidizing MGI associate with the prolonged periods of minimal light exposure during the long polar winter and are inferred to be major contributors to carbon fixation during this season. In summer, the high light availability and intensity drive algal oxygenic photoautotrophic carbon assimilation, a process that appears to be greatly influenced by viral activity. Sea ice contributes importantly to phytoplankton growth and provides a dynamic environment for a range of typically culturable bacteria.

While many taxa present in the Southern Ocean are found in temperate or tropical waters, the accumulation of metagenome data is beginning to resolve differences in phylotypes, such as those defined for the SAR11 clade (Brown *et al.*, 2012). Large-scale metagenomics is also defining community-wide differences defined by major water body features, such as the Polar Front (Wilkins *et al.*, 2012), and combined metagenome/metaproteome analyses are discovering how specific events (e.g. phytoplankton blooms and seasonal changes) affect both community composition and function (Grzymski *et al.*, 2012; Williams *et al.*, 2012a,b). In view of the continuing improvements in cost effectiveness offered by DNA sequencing technologies, there is a bright outlook for the application of pyrotag-sequencing diversity surveys, shotgun metagenomics of size-fractionated samples, and single-cell genomics of important individuals (Stepanauskas, 2012). In association with the application of functional omics approaches (metaproteomics, metatranscriptomics, stable isotope probing), the next 10 years should see major advances being made about the microbial communities and their responses to ecosystem perturbation from an expanded range of Southern Ocean locations and conditions; the targets of these studies should include an examination of seasonal influences and communities present in distinct water bodies, currents, fronts, and in specific oceanic regions and features (e.g. surface vs. depth, distinct photic zones such as the deep chlorophyll maximum, coastal vs. island/plateau vs. pelagic, phytoplankton blooms, sea ice, polynyas, and in the vicinity of glaciers and icebergs).

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Authors' contribution

All authors contributed to the information gathering, ideas and concepts, construction of figures and tables, and/or writing of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Studies of Antarctic lakes that have utilized PCR amplification and sequencing of marker genes.
Table S2. Recent studies of Antarctic lacustrine microbial mats.