**Heterotrophic resourcefulness and unusual sulfur biogeochemistry in a hypersaline Antarctic lake**

Sheree Yau1, Federico M. Lauro1, T.J. Williams1, Matthew Z. DeMaere1, Mark V. Brown1,2, John Rich3, John A.E. Gibson4 and Ricardo Cavicchioli1

1 School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, New South Wales, Australia.

2 Evolution and Ecology Research Centre, The University of New South Wales, Sydney, New South Wales, Australia.

3 \*\*\*\*\*\*\*\*\*\*\*\*\* Albany, Western Australia, Australia.

4 Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, Australia

**Running title:** Heterotrophic resourcefulness and unusual sulfur cycling

**Keywords:** Metagenomics, Organic Lake, Antarctic microbial ecology, nutrient cycle, dimethylsulfide

# Abstract

**Organic Lake is a shallow marine-derived hypersaline lake in the Vestfold Hills, Antarctica that has the highest reported concentration of dimethylsulfide (DMS) in a natural body of water (Franzmann *et al.*, 1987b). To determine the composition and functional potential of the microbial community and learn about the unusual sulfur chemistry in Organic Lake, shotgun metagenomics (2.4 Gbp titanium 454) was performed on size fractionated samples (3.0, 0.8 and 0.1 µm) collected along a depth profile. Eucaryal phytoflagellates were the main photosynthetic organisms. Bacteria were dominated by the globally distributed heterotrophic lineages *Marinobacter*, *Roseovarius* and *Psychroflexus.* Candidate division RF3 was overrepresented at the oxycline and associated with fermentation. The dominance of heterotrophic degradation coupled with low fixation potential indicates possible net carbon loss. However, abundant marker genes for aerobic anoxygenic phototrophy, CO oxidation, rhodopsins and facultative chemoautotrophy were also linked to the dominant heterotrophic bacteria and may be indicative of mechanisms for conserving carbon. Similarly, a high genetic potential for the recycling of nitrogen compounds likely functions to retain fixed nitrogen in the lake. Dimethylsulfoniopropionate (DMSP) lyase genes (*dddD, dddL and dddP*) were abundant indicating DMSP is a significant carbon and energy source. Unlike marine environments, DMSP demethylases (*dmdA*) were less abundant than DMSP lyases indicating that DMSP cleavage is the likely source of the high DMS concentration. Strategies of nutrient resourcefulness such as DMSP cleavage and carbon and nitrogen remineralization in dominant Organic Lake bacteria are potentially important adaptations to nutrient constraints. This study sheds light on how microbial communities and the functional processes they perform evolve in response to unusual environmental conditions.**

# Introduction

Life in the Antarctic is constrained by low temperature, and water, nutrient and light availability. In the Antarctic frozen desert, ice-free regions containing liquid water in lakes and ponds are rare oases for life (Wilkins *et al.,* 2012). The Vestfold Hills, on the eastern shore of Prydz Bay, East Antarctica (Figure S1), is a unique region where hundreds of lakes are present. The lakes were formed from seawater, trapped less than 10 000 BP when the continental ice-sheet receded and the land rose above sea-level (Zwartz *et al*., 1998; Gibson, 1999). Differing local conditions has led each lake to develop unique physical and chemical properties, and life in the lakes tends to be entirely microbial with low levels of diversity (Bowman *et al.*, 2000b; Wilkins *et al.,* 2012). The Vestfold Hills contains the highest density of meromictic (permanently stratified) water bodies in Antarctica (Gibson, 1999). By providing strong physicochemical stratification within a single, largely closed system, these meromictic lakes provide the opportunity to investigate the ways in which microbial communities and ecosystem processes have evolved in the cold and in response to gradients of nutrients, oxygen, salinity and solar irradiance.

Molecular biology approaches have proven useful for describing the diversity and gene content of microorganisms in Antarctic lakes and for inferring the functional roles of the taxa present (Laybourn-Parry & Pearce, 2007). However to date, only a few large scale shotgun metagenome studies have been performed on the Antarctic continent and in the surrounding Southern Ocean (reviewed in Wilkins *et al.,* 2012). In the Vestfold Hills, metagenomics and metaproteomics have been used to study Ace Lake (68.4731 S, 78.1891 E) and Organic Lake (68° 27’ 23.4” S, 78° 11’ 22.6” E) (Ng *et al.*, 2010; Lauro *et al.*, 2011; Yau *et al.*, 2011). For Ace Lake, a comprehensive assessment of the community structure, biogeochemical fluxes and responses to resource limitation have been described (Lauro *et al.*, 2011). The metabolism of abundant green sulfur bacteria (Ng *et al.*, 2010) was found to play a central role in nutrient cycling and a mathematical model was developed that showed its dominance was dependent on synchronicity with the polar light cycle leading to absence of phage predation (Lauro *et al*., 2011). For Organic Lake, a member of the virophage virus family was discovered that potentially regulates microbial loop dynamics (Yau *et al*., 2011). The Organic Lake virophage (OLV) likely depends on phycodnaviruses (algal viruses) and it was predicted that OLV would reduce infective phycodnaviruses leading to an increased frequency of algal blooms and thus carbon flux (Yau *et al.*, 2011). OLV-like sequences were also identified in coastal marine, hypersaline and freshwater metagenomes revealing that virophages are likely to play ecologically important roles in many aquatic systems (Yau *et al.*, 2011). These studies on Ace and Organic lakes both used shotgun metagenomics, and the unanticipated nature of the discoveries (e.g. OLV) serve to illustrate the value of adopting a “look and see” metagenomics approach for learning about microbial ecology in Antarctic environments.

Organic Lake is shallow (6.8 m) and has variable surface water temperatures (−14 to +15 °C) while remaining sub-zero throughout most of its depth (Franzmann *et al.,* 1987; Gibson *et al.,* 1991; Roberts *et al.,* 1993; Gibson, 1999). The lake has a high organic load generated from autochthonous production and input from penguins and terrestrial algae, and nutrient turnover is slow due to the constraints imposed on microbial activity by the lake’s hypersalinity (≈230 g L−1 maximum salinity) and low temperature (Franzmann *et al.,* 1987; Gibson *et al.,* 1991; Roberts *et al.,* 1993; Gibson, 1999). The salt and marine biota in the lake originate from seawater that was trapped in a basin about ca. 3 000 y B.P. (Zwartz *et al.,* 1988; Bird *et al.,* 1991). The bottom waters of Organic Lake are unusual due to the high concentration of the volatile gas dimethylsulfide (DMS) (Deprez *et al*., 1986; Franzmann *et al.*, 1987; Gibson *et al.*, 1991; Roberts & Burton 1993a; Roberts *et al.*, 1993b). Concentrations of DMS as high as 5 000 nM have been recorded in Organic Lake (Gibson *et al*., 1991), 100 times the maximum concentration recorded from seawater in the adjacent Prydz Bay and at least 1000 times that of the open Southern Ocean (Curran & Jones, 1998).

Over forty years ago, atmospheric DMS was proposed to have a regulatory effect on global cloud cover as it forms cloud condensation nuclei (Lovelock & Maggs, 1972; Charlson *et al.*, 1987). However, the first enzymes involved in DMS production were only identified in the last five years (Todd *et al.*, 2007). Rapid progress has been made in this short period and the pathways and organisms involved in DMS transformations have been extensively reviewed (Johnston *et al.*, 2008; Schäfer *et al.*, 2010; Curson *et al.*, 2011b; Reich *et al.*, 2011b; Moran *et al.*, 2012). The main source of DMS in the marine environment is from the breakdown of DMSP. Eucaryal phytoplankton, in particular, diatoms, dinoflagellates and haptophytes produce large quantities of DMSP, which is thought to function principally as an osmolyte. DMSP is released due to cell lysis, grazing or leakage and follows two known fates: DMSP cleavage by DMSP lyases (DddD, -L, -P, -Q, -W and -Y) or demethylation by DMSP demethylase (DmdA). Both pathways are associated with diverse microorganisms that can utilize DMSP as a sole carbon and energy source. However, it is only the cleavage pathway that releases volatile DMS that can lead to sulfur loss through ventilation to the atmosphere.

The very high levels of DMS in Organic Lake make it an ideal system for identifying the microorganisms and the processes they perform that lead to high levels of DMS accumulation. The previous Organic Lake metagenome study examined viruses from the 0.1 µm fraction of surface water that was collected from Organic Lake in December 2006, and November and December 2008 (Yau *et al.*, 2011). In the present study we focused on the cellular population rather than viruses, and examined the microbial community throughout the entire lake. Metagenomic analyses were performed on biomass captured by sequential filtration through a 20 µm pre-filter onto 3.0, 0.8, 0.1 µm filters, from a depth profile (1.7, 4.2, 5.7, 6.5 and 6.7 m) taken in November 2008 from the deepest point in the lake. This filtration and shotgun sequencing approach was originally adopted by the Global Ocean Sampling (GOS) expedition (Rusch *et al*., 2007) and has proven to be a powerful approach for studying Antarctic aquatic microbial communities (Ng *et al*., 2010; Lauro *et al*., 2011; Yau *et al*., 2011; Brown *et al*., 2012; Williams *et al*., 2012b; Wilkins *et al.,* 2012ab). By taking this approach our study determined the composition and functional potential of Organic Lake microbiota and, in conjunction with historic and contemporary physico-chemical data, generated an integrative understanding of the whole lake ecosystem.

# Materials and Methods

## *Characteristics of the lake and samples taken*

The water level of Organic Lake was measured by surveying as +1.886 m relative to the survey mark (NMV / S / 53) located at S 6827’28.3” E07811’20.9”. Water was collected from Organic Lake on 10 November 2008 through a 30 cm hole in the 0.8 m thick ice cover above the deepest point in the lake. The sampling hole (S 6827’22.2” E 7811’23.9”) was established following bathymetry measurements constructed on a metric grid (Figure \*\*\*\*). Samples were collected for metagenomics, microscopy and chemical analyses at 1.7, 4.2, 5.7, 6.5 and 6.7 m depths (maximum depth 6.8 m). For metagenomics, lake water was passed through a 20 µm pore size pre-filter, and microbial biomass captured by sequential filtration onto 3.0 µm, 0.8 µm and 0.1 µm pore size 293mm polyethersulfone membrane filters, and samples immediately preserved in buffer and cryogenically frozen in liquid nitrogen, as described previously (Ng *et al.*, 2010; Lauro *et al.*, 2011). Between 1–2 L of lake water was sufficient to saturate the holding capacity of the filters. DNA was extracted from the filters, samples sequenced using the Roche GS-FLX titanium sequencer, and reads processed to remove low quality bases, assembled and annotated, as previously described (Ng *et al.*, 2010; Lauro *et al.*, 2011).

## *Physical and chemical analyses*

An *in situ* profile of pH, conductivity, turbidity, dissolved oxygen (DO) and pressure was measured using a submersible probe (YSI sonde model V6600). A temperature profile was measured using a maximum-minimum mercury thermometer (the YSI probe did not have a capacity to record temperature below −10ºC). The 5.7 m sample corresponded to the turbidity maximum and the 6.5 m sample to the turbidity minimum. Conductivity at *in situ* temperature was converted to conductivity at 15ºC as described previously (Gibson, 1999). The adjusted conductivity brings the temperature to within a range suitable for estimating practical salinity using the formula of Fofonoff and Millard (1983). Salinity was likely to have been underestimated as it is higher than the range (2–42) for which the conductivity–salinity relation holds. However, the relative difference in salinity between the samples would be accurate. Density was calculated from the *in situ* conductivity and temperature using the equations described by Gibson *et al.* (1990) and expressed at temperature T as:

σT = (1000 − density) kg/m3

Ammonia, nitrate, nitrite, total nitrogen (TN), total dissolved nitrogen (TDN), dissolved reactive phosphorus (DRP), total phosphorus (TP), total dissolved phosphorus (TDP), total organic carbon (TOC), total dissolved carbon (DOC), total sulfur (TS) and total dissolved sulfur (TDS) were determined by American Public Health Associations Standard Methods at the Analytical Services, Tasmania. Values for dissolved nutrients were measured after filtration through a 0.1 µm pore size membrane filter. All other nutrients were measured from water collected after filtration through the on-site 20 µm pore size pre-filter. Ammonia, nitrate, nitrite, DRP, TN, TDN, TP and TDP were measured in a Flow Injection Analyser (Lachat Instruments, Colorado, USA). TOC and DOC were determined in the San++ Segmented Flow Analyser (Skalar, Breda, Netherlands). TS and TDS were analyzed in the 730ES Inductively Coupled Plasma–Atomic Emission Spectrometer (Agilent Technologies, California, USA). Principal Component Analysis (PCA) was performed using the PRIMER Version 6 statistical package (Clarke & Gorley, 2006) on the normalized physical and chemical parameters.

## *Epifluorescence microscopy*

Water samples collected for microscopy were preserved in formaldehyde (1% v/v). Cells and virus-like particles (VLPs) were vacuum filtered onto 25 mm polycarbonate 0.015 µm pore-size membrane filters (Nuclepore Track-etched, Whatman, GE Healthcare, USA) with a 0.45 µm pore-size backing filter. The 0.015 µm filter was mounted onto a glass slide with ProLong® Gold anti fade reagent (Invitrogen, Life Technologies, NY, USA) and 2 µl (25 × dilution in sterile filtered milliQ water <0.015 µm) SYBR® Gold nucleic acid stain (Invitrogen, Life Technologies, NY, USA). Prepared slides were visualized in an epifluorescence microscope (Olympus BX61, Hamburg, Germany) under excitation with blue light (460–495 nm, emission 510–550 nm). Cell and VLP counts were performed on the same filter over 30 random fields of view.

### *Cellular diversity analyses*

Diversity of *Bacteria, Archaea* and *Eucarya* was assessed using ribosomal small subunit (SSU) gene sequences. Metagenomic reads that matched the 16S and 18S rRNA genes were retrieved using Metaxa (Bengtsson *et al.*, 2011). Only sequences longer than 200 bp were accepted for downstream analysis. The Quantitative Insights Into Microbial Ecology (QIIME) pipeline (version 1.4.0) (Caporaso *et al*., 2010) implementing UCLUST, was used to group SSU sequences into operational taxonomic units (OTUs) at 97% percent identity against the SILVA SSU reference database (release 108). SSU sequences that did not cluster with sequences from SILVA were allowed to form new OTUs (no suppression). A representative sequence from each OTU was chosen and classified to the genus level using QIIME implementing the RDP classifier (Wang*et al*., 2007) trained against SILVA (release 108) sequences (www.arb-silva.de). Assignments were accepted to the lowest taxonomic rank with bootstrap value ≥85%. To allow comparison of the relative abundance of taxa, the number of SSU matches per sample filter was normalized to the average number of reads (403 577). Statistical analysis on the relative SSU abundances was performed using the PRIMER Version 6 package (Clarke & Gorley, 2006). The SSU counts of each sample filter were aggregated to the genus level and square root transformed to reduce the contribution of highly abundant taxa. A resemblance matrix was computed using Bray-Curtis similarity. The upper mixed zone (1.7, 4.2 and 5.7 m) and deep zone (6.5 and 6.7 m) samples were designated as separate groups and an analysis of similarity (ANOSIM) performed to test for difference between the two groups. BEST analysis was performed with the abiotic variables: conductivity, temperature, turbidity, DO, pH, TOC, TN, TP, TS, total C:N, total C:P, total N:P, cell counts and VLP counts. The Bio-Env procedure in BEST looks at all the abiotic variables in combination and finds a subset sufficient to best explain the biotic structure. A heat map with bi-clustering dendogram was generated using R and the package ‘seriation’ (Hahsler *et al*., 2008) on the normalized square-root transformed SSU counts.

## *Analysis of functional potential*

Open reading frames (ORFs) were predicted from trimmed metagenomic reads using MetaGene (Noguchi *et al*., 2006) accepting those >90 bp in length. ORFs were translated using the standard bacterial/plastid translation table and compared to protein sequences from the Kyoto Encyclopedia of Genes and Genomes (KEGG) GENES database (release 58) using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1990). KEGG GENES is a collection of genes from all complete genomes from public resources. The BLAST output was processed using KEGG Orthology Based Annotation System (KOBAS) version 2.0 (Xie *et al.*, 2011) accepting assignments to KEGG Orthology (KO) groups with e-value <1e−05 and rank >5. Matches to KO that are functional markers for carbon, nitrogen and sulfur conversions (Table S1) were normalized to 100 000 reads per sample and counted. Normalized frequencies of markers from the same pathway were averaged and those from different pathways were summed. Marker enzymes were assigned to taxonomic groups based on the species of origin of the best KEGG GENES BLASTp match. Marker genes not represented in KO were retrieved by alternative strategies. Organic Lake rhodopsin homologs were retrieved if they had a top BLAST match to any in a list of 139 entries in the KEGG GENES database affiliated with bacteriorhodopsin, xanthorhodopsin, halorhodopsin or proteorhodopsin. The DMSP lyases and demethylases with experimentally confirmed function (Table S2) were retrieved from the National Center for Biotechnology Information (NCBI) ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) sequence databases. These sequences were used to query a BLAST database of translated ORFs predicted from Organic Lake metagenomic reads. Matches were accepted if the e-value was <1e−10 and sequence identity was within the range shared by the query enzymes of the same family (Table S2). BLAST matches to the single copy gene *recA* of *E. coli* K12 (Table S2) was retrieved as described for DMSP lyases and demethylases, except a lower e-value cut-off of <1e-20, as established by Howard *et al.* (2008), was applied and no identity cut-off. The same process was used to determine the frequency of homologs of these genes in metagenome data from Ace Lake and the GOS expedition. Counts of *dddD*, *dddL*, *dddP* and *dmdA* genes were comparable to that obtained by Howard *et al.* (2008) (\*Table S\*\*).

Frequencies of genes of interest were normalized to 100 Mb in order to allow comparison between Antarctic lake and GOS metagenomes, which were sequenced using different DNA sequencing technologies. The number of total base pairs from each GOS sample was estimated by multiplying the number of reads from each sample by the average read length (822 bp) (Rusch *et al.*, 2007). The percentage of genomes containing a gene of interest in a sample was estimated by dividing the normalized count of each gene of interest by the *recA* count.

Marker gene sequences for phylogenetic analysis were clustered using the CD-HIT web server (Huang *et al*., 2010) at 90% global amino acid identity. A representative sequence from the clusters that resided within a desired conserved region and homologs from NR were used in phylogenetic analyses performed in MEGA 5.05 (Tamura *et al.* 2011). Sequences were aligned with MUSCLE (Robert, 2004) using default parameters (gap opening penalty: −a2.9, gap extension penalty: 0). Neighbor-joining was used to compute the phylogenies with a Poisson substitution model, uniform rates of change and complete deletion of alignment gaps. Node support was tested with bootstrap analysis (500 replicates).

# Results and discussion

## *Abiotic properties and water column structure*

*In situ* physico-chemical profiles (Figure S2) measured over the deepest point in the lake (Figure S3) determined the existence of two zones: an upper mixed zone above 5.7 m and a suboxic deep zone below 5.7 m (Figure 1A). The separation of the two zones was indicated by a pycnocline and oxycline starting at 5.7 m. The pH also decreased with DO, likely due to fermentation products such as acetic, formic and lactic acids that have previously been recorded in the bottom waters (Franzmann *et al*., 1987b; Gibson *et al*., 1994). The deep zone was not completely anoxic, consistent with previous records (Franzmann *et al.*, 1987b; Gibson *et al.*, 1991). Oxygen may be episodically introduced to bottom waters as a result of currents of cold dense water sinking during surface ice-formation (Ferris *et al*., 1999). In comparison to meromictic lakes such as Ace Lake which have strong pycnoclines and a steep salt gradient in the anoxic zone, Organic Lake is shallow and has relatively weak stratification (Gibson, 1999). Samples were collected from the upper mixed (1.7, 4.2 and 5.7 m) and deep (6.5 m and 6.7 m) zones.

All nutrients, except for nitrate and nitrite reached maximum concentrations at 6.5 m (Table 1) suggestive of a layer of high biological activity above the lake bottom. Consistent with this, cell and VLP counts were highest at 6.5 m. However, turbidity was lowest at this depth demonstrating turbidity was not principally determined by cell density (Figure 1B). Microscopy images did not show a shift in cell morphology that could account for the large drop in turbidity (\*Figure S4), which suggests particulate matter primarily contributed to turbidity readings. The low turbidity and peak in cell counts and nutrients in the microaerophilic zone at 6.5 m may be caused by an active microbial community degrading particulate matter. This inference is supported by the report of high concentrations of dissolved short chain fatty acids (SCFA) and free amino acids in the deep zone (Gibson *et al.*, 1994) as these nutrients are indicative of the breakdown of high molecular weight carbohydrates, lipids and proteins. Furthermore, the C:N and C:P ratios throughout the lake were high compared to the Redfield ratio (Redfield *et al.*, 1963) except at 6.5 m indicating this was the only depth where dissolved N and P were not relatively limited (Table 1). PCA analysis of physico-chemical parameters showed all samples, except the 6.5 m sample, separated with depth along the PC1 axis (Figure S5). Accordingly, turbidity, TS and cell density were the strongest explanatory variables for the separation of the 6.5 m sample from the other deep sample, indicating that increased activity at 6.5 m was related to breakdown of particulate matter and sulfur chemistry.

## *Overall microbial diversity*

SSU genes (3 959 reads) that were retrieved from the metagenome data (Table S3) grouped into 983 OTUs. OTUs for *Bacteria* comprised 76.2%, *Eucarya* 16.3% and 7.5% of SSU sequences could not be classified. Only 2 reads, assigned to a deep sea hydrothermal clade of *Halobacteriales* (Table S4) indicating *Archaea* were rare in Organic Lake. Microbial diversity was low, consisting of 15 bacterial phyla and 6 eucaryal superkingdom divisions. Of these, only 7 bacterial phyla and 4 eucaryal phyla were predominant. (\*diversity indices)

The most abundant bacterial classes, *Gammaproteobacteria*, *Alphaproteobacteria* and *Flavobacteria*, were represented by OTUs on all filter sizes at all depths (Figure 2A) and each consisted of one dominant genus, *Marinobacter*, *Roseovarius* and *Psychroflexus*, respectively (Figure 2C). Essentially all OTUs for *Cyanobacteria*/chloroplasts were classified as chloroplasts (Figure 2A), except for three reads that could not be assigned to any lower rank (Table S4) indicating free-living *Cyanobacteria* were rare or absent. OTUs for moderately abundant bacterial classes were *Actinobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, and candidate divisions OD1 and RF3. Lower abundance divisions included OTUs for *Bacilli*, *Clostridia*, *Spirochaetes*, *Lentisphaera*, TM7, *Opitutae*, *Verrucomicrobia*, Bhi80-139, Bd1-5, SR1 and *Chlamydiae* (Figure 2A). The dominant eucaryal OTUs were for photosynthetic *Chlorophyta* (green algae) and *Dictyochophyceae* (silicoflagellate algae) (Figure 2B) principally assigned to the genus *Dunaliella* and the order *Pedinellales*, respectively (Table S4)*.* Lower abundance eucaryal OTUs included *Bacillariophyta* (diatoms), *Dinophyceae*, *Fungi* and heterotrophic *Choanoflagellida* and *Ciliophora* (see Table S4 for lower taxonomic rank assignments).

## *Variation of microbial composition according to size and depth*

Community composition varied with size fraction and depth. This was supported by seriation analysis that showed samples clustered according to size fraction, and those clusters further separated into upper mixed and deep zone groups (Figure 3). A significant difference in genus-level composition between mixed and deep zone samples was supported by ANOSIM test (Rho: 0.53, significance: 0.1%). Differential vertical distribution of taxa is consistent with partitioning of ecological functions in the lake and in association with the physical and chemical data, described functional roles of those taxa.

### *20–3.0 µm fraction community composition*

The upper mixed zone samples had a relatively high OUT abundance of *Dunaliella* chloroplasts and chlorophyte algae consistent with large active photosynthetic organisms concentrating near surface light. They are likely the main source of primary production in Organic Lake and have previously been reported to be the dominant algae (Franzman *et al.*, 1987b). The SSU sequences for these algae at the bottom of the lake are likely to be due to sedimentation of dead cells or resting cysts.

*Psychroflexus* OTUs were overrepresented in the surface and 6.7 m samples. Consistent with enrichment on the 3.0 µm filters, *Psychroflexus gondwanensis* (previously *Flavobacterium*) isolated from Organic Lake (Franzmann *et al*., 1987b) had cells 1.5–11.5 µm in length (Dobson *et al*., 1991). *Flavobacteria* associate with phytoplankton blooms in the Southern Ocean (Abell & Bowman 2005a; Abell & Bowman 2005b; Williams *et al.,* 2012\*\*), and have specialized abilities to degrade polymeric substances from algal exudates and detritus (reviewed in Kirchman *et al.*, 2009; Williams *et al.,* 2012\*\*). It is likely that Organic Lake *Psychroflexus* fills a similar ecological role. In support of this, *Psychroflexus* OTUs cluster with *Dunaliella* chloroplasts in the seriation analysis (Figure 3) and *P. gondwanese* abundance in Organic Lake has been correlated with average hours of sunshine per day indicating population dynamics that is related to summer algal blooms (James *et al.*, 1994). The *Psychroflexus* OTUs in the deep zone are most likely due to sedimentation as *P. gondwanense* is non-motile and strictly aerobic (Dobson *et al.*, 1991).

*Roseovarius* OTUs were enriched at 4.2 m and 6.5 m suggesting different ecotypes may be present in the upper mixed zone compared to the deep zone. *Roseovarius tolerans*, an isolate from Ekho Lake in Antarctica has a cell size (1.1–2.2 μm; Labrenz *et al*., 1999) consistent with *Roseovarius* capture on the 3 µm filter. A strain from Ekho Lake is capable of microaerophilic growth (Labrenz *et al.*, 1999). Overrepresentation at 6.5 m may therefore be indicative of growth at that depth rather than sedimentation to the bottom of the lake. *Roseovarius* is a member of the *Roseobacter* clade whose diverse metabolic capabilities include DMSP degradation, aerobic anoxygenic phototrophy (AAnP) and CO oxidation (reviewed in Wagner-Döbler & Biebl, 2006). All of these capabilities should facilitate growth in both the upper mixed and deep zones of Organic Lake (see *Carbon resourcefulness in dominant heterotrophic bacteria*below).

### *3–0.8 µm size fraction community composition*

On the 0.8 µm filter, OTUs for *Marinobacter* dominated at all depths except 6.5 m. Their capture on this size fraction is consistent with the cell size of isolates (1.2–3 µm) (Gauthier *et al.*, 1992). The genus is extraordinarily metabolically versatile and described as an “opportunitroph” (Singer *et al.*, 2011). Some isolates are capable of interacting with diatoms (Gärdes *et al*., 2010) and dinoflagellates (Green *et al.*, 2006). Others are metal-oxidizing autotrophs (Edwards *et al.*, 2003; Wang *et al.*, 2011) or are capable of unusual redox cycling (Handley *et al*., 2009). *Marinobacter* isolates from Antarctic lakes are capable of anaerobic respiration using dimethyl sulfoxide (DMSO) (Matsuzaki *et al*., 2006) or nitrate (Ward & Priscu, 1997). Analysis of functional potential linked to *Marinobacter* revealed which of these capabilities was related to its dominance in Organic Lake (see *Carbon resourcefulness in dominant heterotrophic bacteria*below).

OTUs for RF3 and *Halomonas* were overrepresented at 6.5 m, and RF3 sequences were more abundant (Figure 2 and 3). Their relative abundance in the deep zone indicates a role in microaerophilic processes. The majority of RF3 sequences to date are from anaerobic environments including mammalian gut (Tajima *et al.*, 1999; Ley *et al*., 2006; Samsudin *et al*., 2011), sediment (Yanagibayashi *et al.*, 1999; Röske *et al.*, 2012), municipal waste leachate (Huang *et al.*, 2005), anaerobic sludge (Chouari *et al.*, 2005; Goberna *et al*., 2009; Rivière *et al.*, 2009; Tang *et al*., 2011), a subsurface oil well head (Yamane *et al.*, 2011), and the anaerobic zone of saline lakes (Humayoun *et al*., 2003; Schmidtova *et al*., 2009) including an Antarctic lake (Bowman *et al.*, 2000b). However, some members have been found in surface waters (Demergasso *et al*., 2008; Xing *et al.*, 2009; Yilmaz *et al.*, 2012) suggesting not all members are strict anaerobes. Several *Halomonas* isolates have been sourced from Organic Lake including two described species *Halomonas subglaciescola* and *Halomonas meridiana*, both of which grow as rods with dimensions consistent with capture on this size fraction (Franzmann *et al.*, 1987a; James *et al.*, 1990). Despite these isolates being aerobic, *Halomonas* has been reported to be enriched at the oxycline in Organic Lake (James *et al.*, 1994), indicating *Halomonas* in the lake play an ecological role in the suboxic zone. This capacity may be linked to the ability of free amino acids and organic acids (which are abundant in the deep zone) to stimulate the growth of isolates (Franzmann *et al.*, 1987a).

### *0.8–0.1 µm size fraction community composition*

A large number of eucaryal sequences were evident in the 0.1 µm size fraction. The upper zone was overrepresented by OTUs for *Pedinellales* (silicoflagellate algae) that co-varied with chloroplasts (Figure 2 and 3). *Pedinellales* have only been detected in Antarctic lakes from molecular studies (Unrein *et al.*, 2005; Lauro *et al.*, 2011) including Organic Lake (Yau *et al.*, 2011), with subsequent light microscopy analyses reporting cells resembling *Pseudopedinella* that were 5–8 µm in diameter (Unrein *et al.*, 2005). It is possible that in Organic Lake small (0.8–0.1 µm) free-living members or chloroplast containing cyst forms (Thomsen, 1988) of this eucaryal class exist. However, without evidence to support this (*e.g.* microscopy-based) it seems more likely that the lake sustains a relatively small number of active photosynthetic cells and the sequences detected arise from cysts or degraded cellular material.

OTUs for *Candidatus* “Aquiluna”, in the Luna-1 cluster of *Actinobacteria* (Hahn *et al.*, 2004; Hahn *et al.*, 2009) were most abundant at 1.7 m. The genus has small cells (<1.2 µm; Hahn *et al.*, 2009), accounting for their concentration on this size fraction. Although originally described in freshwater lakes, the same clade was detected in abundance in Ace Lake (Lauro *et al.*, 2011) and surface Artic seawater (Kang *et al*., 2012) demonstrating they play ecological roles in polar saline systems. In Ace Lake surface waters they were associated with utilization of labile C and N substrates (Lauro *et al.*, 2011), and in Organic Lake surface waters probably perform similar functions. The presence of this clade in the deep zone implies a facultative anaerobic lifestyle or sedimented cells.

The bottom of the water column was distinguished by the presence of OTUs for candidate divisions OD1 and TM7. OD1 was more abundant, and its prevalence on this size fraction is consistent with similar findings for size fractionation of ground water (Miyoshi *et al.*, 2005). OD1 is consistently associated with anoxic environments (Harris *et al*., 2004). Genomic analyses identified OTUs for OD1 in the anoxic zone of Ace Lake (Lauro *et al.,* 2011), and OD1 from Zodletone Spring, Oklahoma was reported to possess oxygen sensitive enzymes related to those from anaerobic bacteria (Elshahed *et al.*, 2005). In the marine environment OD1 has been associated with reduced conditions with high sulfur (Harris *et al*., 2004; Elshahed *et al.*, 2005). The distribution of OD1 in Organic Lake is consistent with an anaerobic metabolism and potential involvement in sulfur chemistry. (\*TM7).

## *Organic Lake functional potential*

To determine the functional processes occuring in Organic Lake, gene markers for C, N and S conversions (Figure 4) were retrieved from metagenomic reads. BEST analysis showed that variation in the population structure was significantly correlated (Rho: 0.519, significance: 0.3%) with the abiotic parameters, DO, temperature, TS and TN. The DO gradient has an obvious effect of separating aerobic from anaerobic taxa, and functions to allow oxygen sensitive N and S processes to occur in the deep zone. Functional potential, taxonomic composition and the physico-chemical data were integrated to infer the C, N and S cycles in Organic Lake.

## *Carbon resourcefulness in dominant heterotrophic bacteria*

In both the upper mixed and deep zones, potential for C fixation was much lower than for degradative processes, indicating potential for net C loss (Figure 4A). Potential for aerobic carbon fixation (Figure 4A) via the oxygen-tolerant Calvin-Benson-Basham (CBB) cycle was assessed by presence of the marker genes ribulose-bisphosphate carboxylase (RuBisCO) and phosphoribulokinase (PRK) (Hügler & Sievert, 2011). The majority of RuBisCO homologs were related to *Chlorophyta* (Table 2, Figure S6A) supporting the ecological role of green algae as the principle photosynthetic organisms. The majority of PRK genes had best matches to *Gammaproteobacteria*, predominantly *Marinobacter* (Table 2, Figure S6A). RuBisCO was only associated with a small proportion of *Gammaproteobacteria* (Figure S6A), principally from sulfur-oxidizing *Thiomicrospira*, indicating some *Gammaproteobacteria* are *bona fide* autotrophs. However, all genomes reported for *Marinobacter* have PRK but lack RuBisCO, and only one of these *Marinobacter* is known to oxidize manganese and is thus possibly capable of autotrophy (Wang *et al.*, 2011) and iron-oxidizing autotrophic members of the genus have been isolated (Edwards *et al.*, 2003). It is therefore possible that PRK is functioning as part of the CBB cycle in association with an unknown functional analogue of RuBisCO, or serving another phosphotransferase function that is not linked to carbon fixation. The majority of respiration potential in Organic Lake was linked to *Proteobacteria* (Table 2), specifically to *Marinobacter*, indicating any autotrophic potential would likely be facultative. (\*possible use as electron dump?)

Anaerobic C fixation was represented by some potential for the Wood-Ljungdahl (WL) pathway, but mostly by the reverse tricarboxylic acid (rTCA) cycle (Figure S6A). WL-mediated carbon fixation was linked to *Deltaproteobacteria* that are known to grow autotrophically using this pathway (Hügler & Sievert, 2011). ATP citrate lyase, which is the most definitive marker for rTCA, was linked to sulfur-oxidizing chemolithoautotrophic *Epsilonproteobacteria* (Figure S6A, Table S4) indicating some anaerobic C fixation does proceed by this pathway. However, the majority of rTCA cycle potential was assigned to *Clostridia* (Figure S6A) including the genera *Ammonifex*, *Chitinophaga*, *Halothermothrix* and *Thermoanaerobacter* due to the presence of 2-oxogluterate:ferreoxidin oxidase genes linked to these organisms*.* Some of these genera are known to fix carbon anaerobically by an unknown mechanism that involves the rTCA cycle (Hügler & Sievert, 2011).

In the deep zone, potential for fermentation and CO oxidation was greatest at 6.5 m (Figure 4A) and likely the main biological activity that was occurring at that depth. Fermentation marker genes were linked to *Mollicutes* (Table 2), but as no *Mollicutes* were detected, they most likely originated from the related candidate division RF3 (Tajima *et al.*, 1999) which has relatively high abundance in this zone (see *0.8–3.0 µm size fraction* *community composition* above). This would be the first data indicating that RF3 possesses fermentative metabolism and may play an important ecological role in Organic Lake by degrading high molecular weight compounds to SCFA that other organisms could utilize. Assimilation of fermentation products may play a greater role in Organic Lake rather than complete anaerobic oxidation as typically the end of the anaerobic food chain involves methanogens or sulfate-reducing bacteria; the former were absent and the latter were present in low abundance (Figure 2A, 2C). CO oxidation is a lithoheterotrophic process involving the oxidation of CO to generate energy and metabolism of organic carbon for growth (Moran & Miller, 2007), although CO oxidation may also be involved in anaplerotic C fixation (Moran *et al*., 2007). CO oxidation genes matched to *Alphaproteobacteria* (Table 2), predominantly the *Roseovarius* genus (Figure 2C). The CO oxidation capacity at 6.5 m is therefore associated with the deep-zone ecotype of Organic Lake *Roseovarius*. CO oxidation may allow SCFA to be directly assimilated rather than oxidized, and for some CO2 to be fixed in the deep zone (Figure 4A) thereby addressing the overall carbon shortfall in the lake.

Photoheterotrophy generates energy from light (organic carbon is still required for growth) by two bacterial processes: aerobic anoxygenic phototrophy (AAnP) mediated by bacteriochlorophyll A (BchlA) and associated photosynthesis reaction centers, or rhodopsin mediated phototrophy (Moran& Miller, 2007). AAnP genes are abundant in the ocean and related to diverse *Proteobacteria* (Béjà *et al.*, 2002), and proteorhodopsins (PR) are widely distributed in the surface ocean (Rusch *et al*., 2007) in diverse bacterial clades (de la Torre *et al.*, 2003; Venter *et al.*, 2004). The ecological function of rhodopsins may be diverse and are hypothesized to include light or depth sensing (Fuhrman *et al.*, 2008). However, PRs of marine *Flavobacteria* and *Vibrio* have been linked to light-dependent energy generation, particularly during C limitation (Gómez-Consarnau *et al*., 2007; Gómez-Consarnau *et al*., 2010).

Both AAnP and rhodopsin genes were abundant in Organic Lake (Figure 4A). AAnP genes were linked to *Roseobacter* clade *Alphaproteobacteria* (Table 2), consistent with the known metabolic potential of *R. tolerans* from Ekho Lake which produces BchlA (Labrenz *et al.*, 1999). Organic Lake rhodopsins were associated with all the dominant Organic Lake aerobic heterotrophic lineages. Phylogenetic analysis revealed six well-supported Organic Lake rhodopsin groups (Figure S7). All groups had an L or M residue corresponding to position 105 in the SAR86 PR, denoting tuning to surface green light (Man *et al.*, 2003; Gomez-Consarnau *et al.*, 2007), and a characteristic of oceanic coastal samples (Rusch *et al.*, 2007). Four of the groups clustered with homologs of genera detected in the lake, namely *Marinobacter*, *Psychroflexus*, *Octadecabacter* and “*Candidatus* Aquiluna” (Figure S7, Table S4). In the SAL-R group, Xanthorhodopsin originates from the sphingomonad *Salinibacter ruber* (Balashov *et al.*, 2005). It is therefore likely that *Sphingobacteria* (Table S4) are the likely origin of the SAL-R group. The most abundant group, OL-R1 (Figure S7), had no close homologs from GENBANK but it was abundant on the 3.0 µm fraction and has a distribution (Figure 4A) suggesting it originates from member of the *Roseobacter* clade (\*link to scaffoldsto establish taxonomic origin definitively).

(Table comparing frequencies of rhodopsin, AAnP, DMSP lyases and Dmd genes to other marine environments).

The contribution of light-driven energy generation processes to the carbon budget is difficult to infer from genetic potential alone. For example, the abundance of AAnP and PR genes in Arctic bacteria has been reported to be the same in winter and summer (\*Cottrell *et al.*, 2009), and BchlA in *R. tolerans* was shown to be expressed in cells grown in the dark but inhibited by continuous dim light (Labrenz *et al.*, 1999). However, it is possible that the apparent negative balance in carbon conversion potential could be augmented by photoheterotrophy performed by bacterial groups that are abundant in Organic Lake. In particular, the Organic Lake *Psychroflexus* could play a particular role as it has a PR related to *Dokdonia* that was shown to function under C-limitation (Gómez-Consarnau *et al*., 2007).

## *Regeneration is predominant in the nitrogen cycling*

N cycling potential throughout the lake was dominated by assimilation and mineralization/uptake pathways (Figure 4B) linked to *Proteobacteria* (Table 2, Figure S6). Assimilatory nitrite reductase was not abundant but genes involved in reduced N uptake were (Figure S6B). The presence of glutamate dehydrogenase genes indicated the potential for mineralization to ammonia, and also, by functioning in reverse, a mechanism for ammonium uptake (\*ref). The high ammonia concentration in the deep zone would result from a higher rate of mineralization than assimilation (Table 2, Figure S6B). In addition, a capacity for dissimilatory nitrate reduction to ammonia (DNRA) was linked to *Sphingobacteria* and other anaerobic bacteria (Table 2, Figure S6B), and Stickland fermentation (\*figure) to *Clostridia*.

Potential for nitrogen conversions typically found in other aquatic environments was greatly reduced in Organic Lake. There was a very low potential for N fixation that was confined to the deep zone (Figure 2B) and principally linked to anaerobic *Epsilonproteobacteria* (Table 2, Figure S6B). Potential for aerobic ammonia oxidation was not detected, nor were ammonia-oxidizing bacteria or archaea. Organic Lake microorganisms therefore have a very low capacity to perform nitrification. Anaerobic ammonia oxidation (anammox) potential, indicated by hydroxylamine/hydrazine oxidase-like proteins (HAO/HZO), was also very low and the small number of genes were linked to sulfate-reducing *Deltaproteobacteria* (Table 2, Figure S6). All known anammox organisms are from the order *Brocardiales* (Niftrick & Jetten, 2012), which were not detected in the lake. HAO/HZO genes have been noted in non-ammonia oxidizing bacteria and proposed to be related to NrfA heme cytochrome C nitrite reductase that functions in DNRA (Bergmann *et al*., 2005). Collectively these data indicate an inability for nitrification to occur in the upper mixed zone and likely no potential for ammonia loss in the deep zone.

Denitrification genes were present throughout the water column (Figure 4B) and were linked primarily to *Gammaproteobacteria* (Table 2, Figure S6). Low nitrate and nitrite in the deep zone (Figure 1B, Table 1) indicates that depletion by dissimilatory reduction has contributed to the establishment of N-limitation in the lake. Denitrification genes are phylogenetically widespread and usually induced by low oxygen or oxidized N species (Kraft *et al*., 2011) and thus expected to be active in the deep zone or oxycline. However, denitrification may be inhibited even if conditions appear appropriate. For example, in Lake Bonney, Antarctica, denitrification occurs in the west lobe, but not in the east lobe of the lake despite the presence of anoxia, nitrate and denitrifying *Marinobacter* species (Ward & Priscu, 1997; Ward *et al*., 2005). Moreover, in the absence of nitrification, denitrification would be limited by the lack of potential to re-form oxidized N. The preponderance of assimilation/mineralization pathways geared towards reduced N appears to reflect a “short circuit” of the typical N cycle that would conserve N in a largely closed system. Hence, the predominant N source is regenerated fixed N. Similar findings were also made for Ace Lake, although in this system the presence of a dense layer of green sulfur bacteria with the potential to fix nitrogen augments the N cycle (Lauro *et al*., 2011). (\*DMSP can inhibit N2O reductase Magalhaes *et al.*, 2012)

## *Molecular basis for unusual sulfur chemistry*

Organic Lake differs markedly from other meromictic Antarctic lakes (Ng *et al.*, 2010; Lauro *et al*., 2011, \*others) in possessing a low potential for dissimilatory sulfur cycling (Figure 4C). Sulfur oxidation by the Sox multienzyme system was linked to *Alphaproteobacteria* (Table 2). These genes were most abundant in the upper mixed zone indicating that sulfur oxidation was restricted to where terminal electron acceptors, most likely oxygen, were available. Although sulfur-oxidizing *Epsilonproteobacteria* (Figure 2A, 2C) were present in the deep zone, no potential for sulfur oxidation was linked to them (Figure S6C) and polysulfide reductase (PSR) genes were not detected; both genes are known to be possessed by deep-sea sulfur-oxidizing *Epsilonproteobacteria* (Yamamoto & Takai, 2011). This suggests that rather than S, Organic Lake *Epsilonproteobacteria* make use of alternate electron donors such as SCFA or hydrogen (\*check). Importantly, it is therefore likely that appreciable S oxidation cannot occur in the deep zone as the known terminal electron acceptors, oxygen and nitrate are deplete.

In the deep zone, dissimilatory sulfate reduction (DSR) potential was extremely low (Figure 4C) as was the abundance of sulfate-reducing *Deltaproteobacteria* (Figure 2A,C). The reason for the limited DSR potential is unclear, although it is possible that the high salinity, transient oxygenation or positive electro-potential inhibits microorganisms from performing DSR, and hence colonizing the deep zone of the lake. It is also likely that the lack of dissimilatory sulfur cycling contributes to the accumulation of DMS and DMSP in Organic Lake in the deep zone. In the upper mixed zone, DMS could potentially be oxidized as a carbon and energy source or utilized as an electron donor by sulfur-oxidizing autotrophs (Schäfer *et al.*, 2010). In anoxic zones, methanogenic *Archaea* or sulfate-reducing bacteria are the main organisms known to break down DMS (\*Scholten *et al.*, 2003 or Schäfer *et al.*, 2008). However, the very low dissimilatory sulfur conversion potential in the deep zone coupled with the relatively stagnant waters would likely minimize DMS oxidation and loss by ventilation. DMS would therefore be expected to accumulate in the deep zone.

To determine the source of high DMS in the bottom waters of Organic Lake, the genes involved in DMS formation were surveyed. Genes for DMSP lyases *dddD*, *dddL* and *dddP*, were detected in Organic Lake at levels comparable to other dominant processes such as respiration and fermentation (Figure 4C) indicating DMSP is an important carbon and energy source in Organic Lake. *dddD* was the most abundant of the Organic Lake DMSP lyases (\*Table 3) and comprised two main types: MAR-dddD and OL-dddD (Figure S8). Neither of these types clustered with the non-functional *Dinoroseobacter shibae* DFL 12 and *Ruegeria pomeroyi* DSS-3 *dddD* homologs (Todd *et al.*, 2011) or carnitine coenzyme A transferase outgroups, thereby providing support for their proposed role as functional DMSP lyases. The MAR-dddD type includes the *Marinobacter* sp. ELB17 *dddD* homolog, and MAR-dddD sequences were most abundant on the 0.8 µm fraction where *Marinobacter* OTUs were also more abundant, indicating MAR-dddD derives from Organic Lake *Marinobacter* (Figure S8). OL-dddD did not have a close relative from cultured bacteria making its taxonomic origins uncertain. The abundance of OL-dddD on the 3.0 µm fraction suggests it originates from *Alphaproteobacteria*, *Bacteroidetes* or *Dunaliella* (\*link ddd gene to scaffolds to determine taxonomic orgin).

Two *dddL* groups were detected in Organic Lake: SUL-dddL and MAR-dddL (Figure S9). The former includes the *Sulfitobacter* sp. EE-36 *dddL* and the latter the *Marinobacter manganoxydans* MnI7-9 homolog indicating they originate from *Roseobacter*-clade and *Gammaproteobacteria*, respectively. *Sulfitobacter* sp. EE-36 has demonstrated DMSP lyase activity and the *dddL* gene alone is sufficient for DMS generation (Curson *et al.*, 2008). These functional data indicate that the Organic Lake members of the SUL-dddL group perform the same functional role. The MAR-dddL clade appears to be a new branch of the *dddL* family. *dddP* was detected as the least abundant of the DMSP lyases (\*Table 3). Phylogenetic analyses showed Organic Lake *dddP* likely originate from *Roseovarius* (Figure S10). The Organic Lake sequences formed a clade with the functionally verified *Roseovarius nibinhibens* ISM *dddP* (Todd *et al.*, 2009).

A single type of DMSP demethylase, *dmdA* was identified. It clustered with *Roseobacter*-clade *dmdA* (Figure S11), corresponding to the marine clade A (Howard *et al.*, 2006), and includes the functionally verified *R. pomeroyi* DSS-3 homolog. These data indicate that the Organic Lake sequences correspond to true DMSP demethylases and not related glycine cleavage T proteins or aminomethyltransferases (Howard *et al.*, 2006).

DMSP cleavage appears to be a significant source of DMS in Organic Lake. DMSP likely originates from *Bacillariophyta* or *Dinoflagellida* as Organic Lake *Dunaliella* have been reported not to produce DMSP in culture (Franzmann *et al.*, 1987b) and marine *Dunaliella tertiolecta* is known to cleave DMSP extracellularly (\*Seymour *et al.*, 2010). Based on the abundance of marker genes, DMSP cleavage is predicted to occur at highest levels in the deep zone (Figure 4C) where the DMS concentration has been measured to be highest (Deprez *et al*., 1986; Franzmann *et al.*, 1987; Gibson *et al.*, 1991; Roberts & Burton 1993a; Roberts *et al.*, 1993b). DMS can also be produced in anoxic environments from the reduction of DMSO, degradation of sulfur containing amino acids, and sulfide methylation (Schäfer *et al.*, 2010). Our data indicates that reduction of DMSO was not a major pathway (Figure 4C; \*figure S), and the potential for the other DMS yielding processes could not be determined because the pathways have not been established. When cultivated, *Halomonas* isolates from Organic Lake produced DMS from cysteine (Franzmann *et al.*, 1987b) providing some evidence that DMS production from anaerobic degradation of amino acids can occur.

The potential for DMSP cleavage to occur is more than twice that of DMSP demethylation (Figure 4C, Table 3\*). This ratio differs from estimates from the marine environment that place demethylation potential as up to two orders of magnitude higher than cleavage (Howard *et al.*, 2008; Todd *et al.*, 2009; Todd *et al.*, 2011b; Reisch *et al.*, 2011). The frequency of DMSP lyase genes in Organic Lake far exceeded those of the GOS expedition or from nearby Ace Lake (Table 3). It has been proposed that the cleavage pathway may be underrepresented in the ocean environment because 1) ecologically relevant Ddd enzymes may not have been discovered 2) larger or particle-attached bacteria have not been sampled or 3) that DMSP cleavage is not performed principally by bacteria (Moran *et al.,* 2012). Organic Lake is a marine-derived system where known DMSP lyase genes are highly abundant and linked to close relatives of marine bacteria. These genes were more abundant on the larger size fraction (Table 3) indicating some of the DMSP lysis potential may be under-sampled in the marine metagenomes due to bias against large or particle attached communities. However, the DMSP lyase genes were still clearly abundant in the 0.1 µm fraction in Organic Lake suggesting the dominance of DMSP lysis potential in Organic Lake is related to the high density of DMSP degrading bacteria and not due to sampling larger size fractions. There is evidence that the prevalence of DMSP cleavage may be the rule in hypersaline systems, as was observed in the Punta Cormorant hypersaline lagoon (Todd *et al.*, 2009) and saltern ponds (Raina *et al*., 2010). This provides some hints of the conditions that may favor the relatively wasteful lysis pathway, where both sulfur and carbon is lost to the organism performing the DMSP lysis, over the more ‘thrifty’ demethylation pathway; particularly in the *Roseobacter* lineages that can also perform either process. One possibility that has been proposed is that when sulfur is in excess and the organism can easily assimilate alternative sulfur sources, the lysis pathway may be competitive (Johnston *et al.,* 2008). This is in contrast to SAR11 for which a dependence on assimilation of reduced sulfur would favour demethylation.

## Conclusion

Through the use of shotgun metagenomics and size partioning of samples, we discovered that the lake system is dominated by remineralization and not net C and N fixation. The microbial community is characterized by organic material degradation, nutrient uptake and heterotrophy that occurs greatly in excess of fixation. However, the most active layer in the lake exists below the pycnocline/oxycline where cell and VLP numbers and nutrients are highest, turbidity is lowest due to microbial degradation of particulate matter, and processes occur, such as CO oxidation that may lead to carbon conservation. While the upper mixed zone is characterized by *Dunaliella* and chlorophyte algae performing primary production and polymeric algal material is remineralized by *Psychroflexus*, the deep zone is characterized by facultative anaerobic autotrophy and CO oxidationby *Marinobacter* and *Roseovarius*. In the deep zone, particularly the active layer, *Marinobacter* and *Roseovarius* play key roles in DMS formation by synthesizing DMSP lyases to catalyse DMSP cleavage of the DMSP generated by upper mixed zone phototrophic algae. The low potential for dissimilatory sulfur cycling (both S oxidation and DSR) and relatively stable waters of the deep zone, combined with the generation of DMS from DMSP, facilitate the accumulation of a high level of DMS in the lake.

In addition to being able to answer targeted questions about the biology of the unusual lake sulfur chemistry, the shotgun metagenomics approach provided insight into possible functional capacities and ecological importance of poorly understood classes of bacteria (*e.g.* RF3, *Ca.* “Aquiluna” *Actinobacteria*, OD1 and TM7), and the potential importance of poorly understood microbial processes occurring in the lake performed by a broad range of types of lake bacteria (*e.g.* photoheterotrophy by *Alphaproteobacteria*).

In view of the organic richness, including high levels of DMS in Organic Lake, we did not anticipate the extent to which the lake microbial community is orientated towards a net negative C and N balance. In contemplating this we examined what input the lake may have received throughout its relatively brief ~3 000 year history. The volume of the lake is relatively small (\*\*\*\*\* ). It is possible that the C and N balance is sporadically readdressed by exogenous input from guano deposited in a small penguin rookery nearby the lake, through Giant Petrel or Skua grazing and defecation, and/or by decaying animal carcasses such as elephant seals which can weigh on the order of 1 ton and therefore contribute substantial organic material. It is also possible that during isolation from the ocean, the base of the water column in the marine basin that formed the lake may have acted as a sump for organic material. Phytoplankton blooms and benthic mats tend to make marine basins very productive, and organic matter will become trapped in the denser bottom layers (REF). Retention of captured organic matter in the lake may also have been facilitated by Organic Lake having become highly saline quickly (REF). Studies in the future experimentally determining exogenous input and historical lake dynamics (*e.g.* stable isotope and biomarker analyses of lake sediment), and metaproteogenomic analyses of interannual community composition and function, will provide improved knowledge of the unusual biogeochemistry of Organic Lake and better enable predictions to be made about how the lake may be affected by ecosystem changes.

## Acknowledgements

## References

Gibson JAE, Garrick RC, Franzmann PD, Deprez PP, Burton HR (1991) Reduced sulphur gases in saline lakes of the Vestfold Hills, Antarctica. Palaeogeogr Palaeoclimatol Palaeoecol 84:131–140;

Roberts NJ, Burton HR, Pitson GA (1993) Volatile organic compounds from Organic Lake, an Antarctic, hypersaline, meromictic lake. Antarct Sci 5:361–366;

Franzman PD, Deprez PP, Burton HR, van den Hoff J (1987) Limnology of Organic Lake, Antarctica, a meromictic lake that contains high concentrations of dimethyl sulfide. Aust J Freshwater Res 38:409–417;

Gibson JAE (1999) The meromictic lakes and stratified marine basins of the Vestfold Hills, East Antarctica. Antarct Sci 11:175–192.

Zwartz D, Bird M, Stone J, Lambeck K (1998) Holocene sea-level change and ice-sheet history in the Vestfold Hills, East Antarctica. Earth Planet Sci Lett 155:131–145;

Bird MI, Chivas AR, Radnell CJ, Burton HR (1991) Sedimentological and stable-isotope evolution of lakes in the Vestfold Hills, Antarctica. Palaeogeogr Palaeoclimatol Palaeoecol 84:109–130.

Abell GCJ and Bowman JP. (2005a) Colonization and community dynamics of class *Flavobacteria* on diatom detritus in experimental mesocosm based on Southern Ocean seawater. *FEMS Microbiol Ecol* **53**: 379–391.

Abell GCJ and Bowman JP.(2005b) Ecological and biogeographic relationships of class Flavobacteria in the Southern Ocean.*FEMS Microbiol Ecol* **51**: 265–277.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. (1990) Basic Local Alignment Search Tool.*J Mol Biol* **215**: 403–410.

Antón J, Oren A, Benlloch S, Rodríguez-Valera F, Amann R, Roselló-Mora R. (2002) *Salinibacter ruber* gen. nov., sp. nov., a novel extremely halophilic member of the *Bacteria* from saltern crystallizer ponds. *Int J Syst Evol Microbiol* **52**: 485–491.

Balashov SP, Imasheva ES, Boichenko VA, Antón J, Wang JM, Lanyi JK. (2005) Xanthorhodopsin: a proton pump with a light-harvesting carotenoid antenna. *Science* **309**: 2061–2064.

Béjà O, Aravind L, Koonin EV, Suzuki MT, Hadd A, Nguyen LP, Jovanovich SB *et al*. (2000) Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. *Science* **289**: 1902–1906.

Béjà O, Suzuki MT, Heidelberg JF, Nelson WC, Preston CM, Hamada T, Eisen JA *et al.* (2002) Unsuspected diversity among marine aerobic anoxygenic phototrophs. *Nature* **6872**: 630–633.

Bengtsson K, Eriksson KM, Hartmann M, Wang Z, Shenoy BD, Grelet G-A *et al*. (2011) Metaxa: a software tool for automated detection and discrimination among ribosomal small subunit (12S/16S/18S) sequences of archaea, bacteria, eukaryotes, mitochondria, and chloroplasts in metagenomes and environmental sequencing datasets.*Antonie Van Leeuwenhoek***1 00**: 471–475.

Bergmann DJ, Hooper AB, Klotz MG. (2005) Structure and sequence conservation of *hao* cluster genes of autotrophic ammonia-oxidizing bacteria: evident for their evolutionary history. *Appl Environ Microbiol* **71**: 5371–5382.

Bird MI, Chivas AR, Radnell CJ, Burton HR. (1991) Sedimentological and stable-isotope evolution of lakes in the Vestfold Hills, Antarctica. *Palaeogeogr Palaeoclimatol Palaeoecol* **84**: 109–130.

Bowman JP, McCammon SA, Lewis T, Skerratt JH, Brown JL, Nichols DS, McMeekin TA. (1998) *Psychroflexus torquis* gen. nov., sp. nov., a psychrophilic species from Antarctic sea ice, and reclassification of *Flavobacterium gondwanense* (Dobson et al. 1993) as *Psychroflexus gondwanense* gen. nov., comb. nov. *Microbiology* **144**: 1601–1609.

Bowman JP, McCammon SA, Rea SM, McMeekin TA. (2000b)The microbial composition of three limnologically disparate hypersaline Antarctic lakes. *FEMS Microbiol Lett* **183**: 81–88.

Burke CM and Burton HR. (1988) Photosynthetic bacteria in meromictic lakes a stratified fjords of the Vestfold Hills, Antarctica. *Hydrobiologia* **165**: 13–23.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK *et al*. (2010) QIIME allows analysis of high-throughput community sequence data. *Nat Methods* **7**: 335–336.

Charlson RJ, Lovelock JE, Andreae MO, Warren SG. (1987) Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. *Nature* **326**: 655–661.

Chen YG, Cui XL, Wang YX, Tang SK, Zhang YQ, Li WJ, Liu JH *et al*. (2009) *Psychroflexus sediminis* sp. nov., a mesophilic bacterium isolated from salt lake sediment in China. *Int J Syst Evol Microbiol* **59**: 569–573.

Chouari R, Le Paslier D, Daegelen P, Ginestet P, Weissenbach J, Sghir A. (2005) Novel predominant archaeal and bacterial groups revealed by molecular analysis of an anaerobic sludge digester. *Environ Microbiol* **7**: 1104–1115.

Clarke KR and Gorley RN. (2006) PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.

Curran MAJ and Jones GB. (1998) Spatial distribution of dimethylsulfide and dimethylsulfonioproprionate in the Australasian sector of the Southern Ocean. *J Geophys Res* **103**: 16 677–16 689.

Curson ARJ, Rogers R, Todd JD, Bearley CA, Johnston AWB (2008) Molecular genetic analysis of a dimethysulfonioproprionate lyase that liberates the climate-changing gas dimethylsulfide in several marine α-proteobacteria and *Rhodobacter sphaeroides*. *Environ Microbiol* **10**: 757–767.

Curson ARJ, Sullivan MJ, Todd JD, Johnston AWB.(2010) Identification of genes for dimethyl sulfide production in bacteria in the gut of Atlantic Herring (*Clupea harengus*). *ISME J* **4**: 144–146.

Curson ARJ, Sullivan MJ, Todd JD, Johnston AWB. (2011a) DddY, a periplasmic dimethylsulfonioproprionate lyase found in taxonomically diverse species of Proteobacteria. *ISME J* **5**: 1191–1200.

Curson ARJ, Todd JD, Sullivan MJ, Johnston AWB. (2011b) Catabolism of dimethylsulphonioproprionate: microorganisms, enzymes and genes. *Nat Rev Microbiol* **9**: 849–859.

de la Torre JR, Christianson LM, Béjà O, Suzuki MT, Karl DM, Heidelberg J *et al.* (2003) Proteorhodopsin genes are distributed among divergent bacterial taxa. *PNAS* **100**: 12830–12835.

DeSantis Jr. TZ, Hugenholtz P, Keller K, Brodie EL, Larsen N, Piceno YM *et al*. (2006) NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. *Nucleic Acids Res* **34**:W394–399.

Demergasso C, Escudero L, Casamayor EO, Chong G, Balagué V, Pedrós-Alió. (2008) Novelty and spatio-temporal heterogeneity in the bacterial diversity of hypersaline Lake Tebenquiche (Salar de Atacama). *Extremophiles* **12**: 491–504.

Demergasso C, Dorador C, Meneses D, Blamey J, Cabrol N, Escudero L, Chong G. (2010) Prokaryotic diversity pattern in high-altitude ecosystems of the Chilean Altiplano. *J Geophys Res* **115**: G00D09

Deprez PP, Franzmann PD, Burton HR. (1986) Determination of reduced sulfur gases in Antarctic lakes and seawater by gas chromatography after solid adsorbent preconcentration.*J Chromatogr* **362**: 9–21.

Dobson SJ, James SR, Franzmann PD, McMeekin TA. (1991) A numerical taxonomic study of some pigmented bacteria isolated from Organic Lake, an antarctic hypersaline lake. *Arch Microbiol* **156**: 56–61.

Dobson SJ, Colwell RR, McMeekin TA, Franzmann PD. (1993) Direct sequencing of the polymerase chain reaction-amplified 16S rRNA gene of *Flavobacterium gondwanense* sp. nov. and*Flavobacterium salegens* sp. nov., two new species from a hypersaline Antarctic lake. *Int J Syst Bacteriol* **43**: 77–83.

Donachie SP, Bowman JP, Alam M. (2005) *Psychroflexus tropicus* sp. nov., an obligately halophilic *Cytophaga-Flavobacterium-Bacteroides* group bacterium from an Hawaiian hypersaline lake. *Int J Syst Evol Microbiol* **54**: 935–940.

Edgar RC. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nuc Acids Res* **32**: 1792–1797.

Edwards KJ, Rogers DR, Wirsen CO, McCollom TM.(2003) Isolation and characterization of novel psychrophilic, neutrophilic, Fe-oxidizing, chemolithoautotrophic α- and γ-*Proteobacteria* from the deep sea. *Appl Environ Microbiol* **69**: 2906–2913.

Ferris JM, Gibson JAE, Burton HR. (1991) Evidence of density currents with the potential to promote meromixis in the ice-covered saline lakes. *Palaeogeogr Palaeoclimatol Palaeoecol* **84**: 99–107.

Franzmann PD, Burton HR, McMeekin TA. (1987a) *Halomonas subglaciescola*, a new species of halotolerant bacteria isolated from Antarctica. *Int J Syst Bacteriol* **37**: 27–34.

Franzmann PD, Deprez PP, Burton HR, van den Hoff J*.* (1987b) Limnology of Organic Lake, Antarctica, a meromictic lake that contains high concentrations of dimethyl sulfide. *Aust J Mar Freshw Res* **38**:409–417.

Fofonoff NP and Millard RC Jr. (1983) Algorithms for computation of fundamental properties of seawater. *UNESCO Technical Papers in Marine Science*, no.**44**.

Fuhrman JA, Schwalbach MS, Stingl U. (2008) Proteorhodopsins: an array of physiological roles? *Nat Rev Microbiol* **6**: 488–494.

Gärdes A, Kaeppel E, Shehzad A, Seebah S, Teeling H, Yarza P, Glöckner FO *et al*. (2010) Complete genome sequence of *Marinobacter adhaerens* type strain (HP15), a diatom-interacting marine microorganism. *Stand Genomic Sci* **3**: 97–107.

Gauthier MJ, Lafay B, Christen R, Fernandez L, Acquaviva M, Bonin P, Betrand JC. (1992) *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. *Int J Syst Bacteriol* **42**: 568–576.

Ghai R, Pašić L, Fernández AB, Martin-Cuadrado A-B, Mizuno CM, McMahon KD, Papke RT *et al.* (2011) New abundant microbial groups in aquatic hypersaline environments. *Sci Rep* **1**: srep00135.

Gibson JAE, Ferris JM, Burton HR. (1990) Temperature density, temperature conductivity and conductivity-density relationships for marine-derived saline lake waters. *ANARE Research Notes*, No. 78.

Gibson JAE, Garrick RC, Franzmann PD, Deprez PP, Burton H. (1991) Reduced sulfur gases in saline lakes of the Vestfold Hills, Antarctica. *Palaeogeo Palaeoclimatol Palaeoecol* **84**:131–140.

Gibson JAE, Qiang XL, Franzmann PD, Garrick RC, Burton HR. (1994) Volatile fatty and dissolved free amino acids in Organic Lake, Vestfold Hills, East Antarctica. *Polar Biol* **14**: 545–550.

Gibson JAE, Burton HR, Gallagher JB.(1995) Meromictic Antarctic lakes as indicators of local water balance: structural changes in Organic Lake, Vestfold Hills 1978–1994. *ANARE Research Notes*, No.94, 16pp.

Gibson JAE. (1999) The meromictic lakes and stratified marine basins of the Vestfold Hills, East Antarctica. *Antarct Sci* **11**:175–192.

Glatz RE, Lepp PW, Ward BB, Francis CA. (2006) Planktonic microbial community composition across steep physical/chemical gradients in permanently ice-covered Lake Bonney, Antarctica. *Geobiology* **4**: 53–67.

Goberna M, Insam H, Franke-Whittle IH. (2009) Effect of biowaste sludge maturation on the diversity of thermophilic bacteria and archaea in an anaerobic reactor. *Appl Environ Microbiol* **75**: 2566–2572.

Gómez-Consarnau L, González JM, Coll-Lladó M, Gourdon P, Pascher T, Neutze R, Pedrós-Alió C, Pinhassi J. (2007) Light stimulates growth of proteorhodopsin-containing marine Flavobacteria. *Nature* **445**: 210–213.

Gómez-Consarnau L, Akram N, Lindell K, Pedersen A, Neutze R, Milton DL, González JM *et al.* (2010) Proteorhodopsin phototrophy promotes survival of marine bacteria during starvation. *PLoS Biol.* **8**: e1000358.

Gosink JJ, Herwig RP, Staley JT. (1997) *Octadecabacter articus* gen. nov., sp. nov., and *O. antarcticus*, sp. nov., nonpigmented, psychrophilic gas vacuolate bacteria from polar sea ice and water. *System Appl Microbiol* **20**: 356–365.

Hahn MW, Stadler P, Wu QL, Pöckl. (2004) The filtration–acclimatization method for isolation of an important fraction of the not readily cultivable bacteria. *J Microbiol Methods* **57**: 379–390.

Hahn MW. (2009) Description of seven candidate species affiliated with the phylum *Actinobacteria*, representing planktonic freshwater bacteria. *Int J Syst Evol Microbiol* **59**: 112–117.

Hahsler M, Hornik K, Buchta C. (2008) Getting things in order: an introduction to R package seriation. *J Stat Softw* **25**:1–34.

Huang L, Zhu S, Zhou H, Qu L. (2005) Molecular phylogenetic diversity of bacteria associated with the leachate of a closed municipal solid waste landfill. *FEMS Microbiol Lett* **242**: 297–303.

Huang Y, Niu B, Gao Y, Fu L, Li W. (2010) CD-HIT Suite: a web server for clustering and comparing biological sequences. *Bioinformatics* **26**: 680–682.

Hügler M and Sievert SM. (2011) Beyond the Calvin cycle: autotrophic carbon fixation in the ocean. *Annu Rev Mar Sci* **3**: 261–289.

Humayoun SB, Bano N, Hollibaugh JT. (2003) Depth distribution of microbial diversity in Mono Lake, a meromictic soda lake in California. *Appl Environ Microbiol* **69**: 1030–1042.

James SR, Dobson SJ, Franzmann PD, McMeekin TA.(1990) *Halomonas meridiana*, a new species of extremely halotolerant bacteria from Antarctic saline lakes. *System Appl Microbiol* **13**: 270–278.

James SR, Burton HR, McMeekin TA, Mancuso CA. (1994) Seasonal abundance of *Halomonas meridiana*, *Halomonas subglaciescola*, *Flavobacterium gondwanense* and *Flavobacterium salegens* in four Antarctic Lakes. *Antarctic Sci* **6**: 325–332.

Johnston AWB, Todd JD, Sun L, Nikolaidou-Katsaridou MN, Curson ARJ, Rogers R. (2008) Molecular diversity of bacterial production of the climate changing gas, dimethyl sulphide, a molecule that impinges on local and global symbioses. *J Exp Bot* **59**: 1059–1067.

Kang I, Lee K, Yang S-J, Choi A, Kang D, Lee YK, Cho J-C. (2012) Genome sequence of “*Candidatus* Aquiluna” sp. strain IMCC13023, a marine member of the *Actinobacteria* isolated from an Artic Fjord. *J Bacteriol* **194**: 3550–3551.

Kirchman DL. (2002) The ecology of *Cytophaga-Flavobacteria* in aquatic environments. *FEMS Microbiol Eco l***39**: 91–100.

Kraft B, Stous M, Tegetmeyer HE. (2011) Microbial nitrate respiration – genes, enzymes and environmental distribution. *J Biotechnol* **155**: 104–117.

La Scola B, Desnues C, Pagnier I, Robert C, Barrassi L, Fournous G, Merchat C *et al.* (2008) The virophage as a unique parasite of the giant mimivirus. *Nature* **455**: 100–105.

Labrenz M, Collins MD, Lawson PA, Tindall BJ, Schumann P, Hirsch P. (1999) *Roseovarius tolerans* gen. nov., sp. nov., a budding bacterium with variable bacteriochlorophyll *a* production from hypersaline Ekho Lake. *Int J Syst Bacteriol* **49**: 137–147.

Lauro FM, DeMaere MZ, Yau S, Brown MV, Ng C, Wilkins D*et al.* (2011) An integrative study of a meromictic lake ecosystem in Antarctica. *ISME J* **5**:879–895.

Ley RE, Turnbaugh PJ, Klein S, Gordon JI. (2006) Human gut microbes associated with obesity. *Nature* **444**: 1022–1023.

Lovelock JE and Maggs RJ.(1972) Atmospheric dimethyl sulfide and the natural sulphur cycle.*Nature* **237**: 452–453.

Laybourn-Parry J and Pearce D. (2007) The biodiversity and ecology of Antarctic lakes: models for evolution. *Phil Trans R Soc B* **364**: 2273–2289.

Lee ZM, Bussema C 3rd, Schmidt TM. (2009) rrnDB: documenting the number of rRNA and tRNA genes in bacteria and archaea. *Nucleic Acids Res* **37** (Database issue): D489–D493.

Man D, Wang W, Sabehi G, Aravind L, Post AF, Massana R *et al*. (2003) Diversification and spectral tuning in marine proteorhodopsins. *EMBO J* **22**:1725–1731.

Matsuzaki M, Kubota K, Satoh T, Kunugi M, Ban S, Imura S. (2006) Dimethyl sulfoxide-respiring bacteria in Suribati Ike, a hypersaline lake, in Antarctica and the marine environment. *Polar Biosc i***20**: 73–87.

McCammon SA and Bowman JP. (2000) Taxonomy of Antarctic *Flavobacterium* species: description of *Flavobacterium gillisiae* sp. nov., *Flavobacterium tegetincola* sp. nov.and*Flavobacterium xanthum* sp.nov., nom. rev. and reclassification of [*Flavobacterium*] *salegens* as *Salegentibacter salegens* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **50**: 1055–1063.

Miyoshi T, Iwatuski T, Naguma T. (2005) Phylogenetic characterization of 16S rRNA gene clones from deep-groundwater microorganisms that pass through 0.2 µm-pore-size filters. *Appl Environ Microbiol* **71**: 1084–1088.

Moran MA, Belas R, Schell MA, González JM, Sun F, Binder BJ, Edmonds J *et al.* (2007) Ecological genomics of marine Roseobacters. *Appl Environ Microbiol* **73**: 4559–4569.

Moran MA and Miller WL. (2007) Resourceful heterotrophs make the most of light in the coast ocean. *Nat Rev Microbiol* **5**: 792–799.

Moran MA, Reisch CR, Kiene RP, Whitman WB.(2012) Genomic insights into bacterial DMSP transformations.*Ann Rev Marine Sci* **4**: 523–542.

Naganuma T, Hua PN, Okamoto T, Ban S, Imura S, Kanda H. (2005) Depth distribution of euryhaline halophilic bacteria in Suribati Ike, a meromictic lake in East Antarctica. *Polar Biosci* **28**: 964–970.

Ng C, DeMaere MZ, Williams TJ, Lauro FM, Raftery M, Gibson JAE *et al.* (2010) Metaproteogenomic analysis of a dominant green sulfur bacterium from Ace Lake, Antarctica. *ISME J* **4**: 1002–1019.

van Niftrick L and Jetten MSM. (2012) Anaerobic ammonium-oxidizing bacteria: unique microorganisms with exceptional properties. *Micobiol Mol Biol Rev* **76**: 585–596.

Noguchi H, Park J, Takagi T. (2006) MetaGene: prokaryotic gene finding from environmental genome shotgun sequences. *Nucleic Acids Res* **34**: 5623–5630.

Pagaling E, Wang H, Venables M, Wallace A, Grant WD, Cowan DA, Jones BE *et al.* (2009) Microbial biogeography of six salt lakes in Inner Mongolia, China and a Salt Lake in Argentina. *Appl Environ Microbiol* **75**: 5750–5760.

Powell LM, Bowman JP, Skerratt JH, Franzmann PD, Burton HR. (2005) Ecology of a novel *Synechococcus* clade occurring in dense populations in saline Antarctic lakes. *Mar Ecol Prog Ser* **291**: 65–80.

Raina J-P, Dinsdale EA, Willis BL, Bourne DG. (2010) Do the organic sulfur compounds DMSP and DMS drive coral microbial associations? *Trends Microbiol* **3**: 101–108.

Redfield AC, Ketchum BH, Richards FA. (1963) The influence of organisms on the composition of seawater, In: Hill MN (ed). The sea. John Wiley and Sons: New York, pp 26–77.

Reisch CR, Moran MA, Whitman WB. (2011) Bacterial catabolism of dimethylsulfonioproprionate (DMSP). *Front Microbiol* **2**: 1–12.

Rivière D, Desvignes V, Pelletier E, Chaussonnerie S, Guermazi S, Weissenbach, Li T *et al.* (2009) Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. *ISME J* **3**: 700–714.

Roberts NJ and Burton HR. (1993a) Sampling volatile organics from a meromictic Antarctic lake. *Polar Biol* **13**: 359–361.

Roberts NJ, Burton HR, Pitson GA. (1993b) Volatile organic compounds from Organic Lake, an Antarctic hypersaline, meromictic lake. *Polar Biol* **13**: 361–366.

Röske K, Sachse R, Scheerer C, Röske I. (2012) Microbial diversity and composition of the sediment in the drinking water reservoir Saidenbach (Saxonia, Germany). *Syst Appl Microbiol* **35**: 35–44.

Rusch DB, Halpern AL, Sutton G, Heidelberg KB, Williamson S, Yooseph S *et al.* (2007) The *Sorcerer II* Global Ocean Sampling expedition: northwest Atlantic through eastern tropical Pacific. *PLoS Biol* **5**: 398–431.

Sabehi G, Loy A, Jung K-H, Partha R, Spudich JL, Isaacson T, Hirschberg J *et al*. (2005) New insights into metabolic properties of marine bacteria encoding proteorhodopsins. *PLoS Biol* **3**: e273.

Samsudin AA, Evans PN, Wright AG, Al Jassim R. (2011) Molecular diversity of the foregut bacteria community in the dromedary camel (*Camelus dromedariusi*). *Environ Microbiol* **13**: 3024–3035.

Schmidtova J, Hallam SJ, Baldwin SA. (2009) Phylogenetic diversity of transition and anoxic zone bacterial communities within a near-shore anoxic basin: Nitinat Lake. *Environ Microbiol* **11**: 3233–3251.

Sharma AK, Zhaxybayeva O, Papke RT, Doolittle WF. (2008) Actinorhodopsins: proteorhodopsin-like gene sequences found predominantly in non-marine environments. *Environ Microbiol* **10**: 1039–1056.

Sharma AK, Sommerfeld K, Bullerjahn GS, Matteson AR, Wilhelm SW, Jezbera J, Brandt U *et al*. (2009) Actinorhodopsin genes discovered in diverse freshwater habitats and among cultivated freshwater *Actinobacteria*. *ISME J* **3**: 726–737.

Singer E, Webb EA, Nelson WC, Heidelberg JF, Ivanova N, Pati A, Edwards KJ.(2011) Genomic potential of *Marinobacter aquaeoli*, a biogeochemical “opportunitroph”. *Appl Environ Microbiol* **77**: 2763–2771.

Swan BK, Martinez-Garcia M, Preston CM, Sczyrba A, Woyke T, Lamy D, Reinthaler T *et al.* (2011) Potential for chemolithoautotrophy among ubiquitous bacteria lineages in the dark ocean. *Science* **333**: 1296–1300.

Tajima K, Aminov RI, Nagamine T, Ogata K, Nakamura M, Matsui H *et al*. (1999) Rumen bacterial diversity as determined by sequence analysis of 16S rDNA. *FEMS Microbiol Ecol* **29**: 159–169.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**: 2731–2739.

Tang Y, Ji P, Hayashi J, Koike Y, Wu X, Kida K. (2011) Characteristic microbial community of a dry thermophilic methanogenic digester: its long-term stability and change with feeding. *Appl Microbiol Biotechnol* **91**: 1477–1461.

Thomsen HA. (1988) Ultrastructural studies of the flagellate and cyst stages of Pseudopedinella tricostata (Pedinellales, Chrysophyceae). *British Phycological Journal* **23**: 1–16.

Tian F, Yu Y, Chen B, Li H, Yao Y-F, Guo X-K. (2009) Bacterial, archaeal and eukaryotic diversity in Artic sediment as revealed by 16S rRNA and 18S rRNA gene clone libraries analysis. *Polar Biol* **32**: 93–103.

Todd JD, Rogers R, Li YG, Wexler M, Bond PL, Sun L, Curson ARJ *et al.* (2007) Structural and regulatory genes required to make the gas dimethyl sulfide in bacteria. *Science* **315**: 666–669.

Todd JD, Curson ARJ, Dupont CL, Nicholson P, Johnston AWB. (2009) The*dddP* gene, encoding a novel enzyme that converts dimethylsulfonioproprionate into dimethyl sulfide, is widespread in ocean metagenomes and marine bacteria and also occurs in some Ascomycete fungi. *Environ Microbiol* **11** :1376–1385.

Todd JD, Curson ARJ, Nikolaidou-Kataraidou N, Brearley CA, Watmough NJ, Chan Y, Page PCB *et al.* (2010) Molecular dissection of bacterial acrylate catabolism – unexpected links with dimethylsulfonioproprionate catabolism and dimethyl sulfide production. *Environ Microbiol* **12**: 327–343.

Todd JD, Curson ARJ, Kirkwood M, Sullivan MJ, Green RT, Johnston AWB. (2011) DddQ, a novel, cupin-containing, dimethylsulfonioproprionate lyase in marine roseobacters and in uncultured marine bacteria. *Environ Microbiol* **13** :427–438.

Todd JD, Kirkwood M, Newton-Payne S, Johnston AWB.(2012) DddW, a third DMSP lyase in model Roseobacter marine bacterium, *Ruegeria pomeroyi* DSS-3. *ISME J* **6** :223–226.

Unrein F, Izaguirre I, Massana R, Balagué V, Gasol JM. (2005) Nanoplankton assemblages in maritime Antarctic lakes: characterisation and molecular fingerprinting comparison. *Aquat Microb Ecol* **40**: 269–282.

Van Trappen S, Mergaert J, Van Eygen S, Dawyndt P, Cnockaert MC, Swing J. (2002) Diversity of 746 heterotrophic bacteria isolated from microbial mats from ten Antarctic lakes. *System Appl Microbiol* **25**: 603–610.

Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, Eisen JA *et al*. (2004) Environmental genome shotgun sequencing of the Sargasso Sea. *Science* **304**: 66–74.

Wagner-Döbler I and Biebl H. (2006) Environmental biology of the marine *Roseobacter* lineage. *Ann Rev Microbiol* **60**: 255–280.

Wang Q,Garrity GM, Tiedje JM, Cole JR. (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into new bacterial taxonomy. *Appl Environ Microbiol* **73**: 5261–5267.

Wang H, Li H, Shao Z, Liao S, Johnstone L, Rensing C, Wang G. (2011) Genome sequence of deep-sea Manganese-oxidizing bacterium *Marinobacter manganoxydans*. *J Bacteriol* **194**: 899–900.

Ward BB and Priscu JC. (1997) Detection and characterization of denitrifying bacteria from a permanently ice-covered Antarctic lake. *Hydrobiologia* **347**: 57–68.

Ward BB, Granger J, Maldonado MT, Casciotti KL, Harris S, Wells ML. (2005) Denitrification in the hypolimnion of permanently ice-covered Lake Bonney, Antarctica. *Aquat Microb Ecol* **38**: 295–307.

Xie C, Mao X, Huang J, Ding Y, Wu J, Dong S, Kong L, Gao G, Li CY, Wei L. (2011) KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. *Nucleic Acids Res* **39**: W316–W322.

Xing P, Hahn MW, Wu QL. (2009) Low taxon richness of bacterioplankton in high-altitude lakes of the eastern Tibetan Plateau, with a predominance of *Bacteroidetes* and *Synechoccocus* spp. *Appl Environ Microbiol* **75**: 7017–7025.

Yamamoto M and Takai K. (2011) Sulfur metabolisms in epsilon- and gamma-*Proteobacteria* in deep-sea hydrothermal fields. *Front Microbiol* **2**: 1–8.

Yamane K, Hattori Y, Ohtagaki H, Fujiwara K. (2011) Microbial diversity with dominance of 16S rRNA genes sequences with high GC contents at 74 and 98°C subsurface crude oil deposits in Japan. *FEMS Microbiol Ecol* **76**: 220–235.

Yanagibayashi M, Nogi Y, Li L, Kato C. (1999) Changes in the microbial community in Japan Trench sediment from a depth of 6292 m during cultivation without decompression. *FEMS Microbiol Lett* **170**: 271–279.

Yau S, Lauro FM, DeMaere MZ, Brown MV, Thomas T, Raftery MJ *et al.* (2011) Virophage control of antarctic algal host-virus dynamics. *Proc Natl Acad Sci USA* **108**: 6163–6168.

Yilmaz P, Iversen MH, Hankeln W, Kottman R, Quast C, Glöckner FO.(2012) Ecological structuring of bacterial and archaeal taxa in surface ocean waters. *FEMS Microbiol Ecol* **81**: 373–385.

Yoon JH, Kang SJ, Jun YT, Oh TK. (2009) *Psychroflexus salinarum* sp. nov., isolated from a marine solar saltern. *Int J Syst Evol Microbiol* **59**: 2404–2407.

Zhang H, Hosoi-Tanabe S, Nagata S, Ban S, Imura S. (2010) *Psychroflexus* lacisalsi sp. nov., a moderate halophilic bacterium isolated from a hypersaline lake (Hunazoko-Ike) in Antarctica. *J Microbiol* **48**: 160–164.

Zwartz D, Bird M, Stone J, Lambeck K. (1998) Holocene sea-level change and ice-sheet history in the Vestfold Hills, East Antarctica. *Earth Planet Sci Lett* **155**: 131–145.