Characterization of sulfur metabolizing microbes in a cold saline microbial mat of the Canadian High Arctic

Raven Comery

Master of Science

Department of Natural Resource Sciences

Unit: Microbiology

McGill University, Montreal

July 2015

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master in Science

Abstract/Résumé

The Gypsum Hill (GH) spring system is located on Axel Heiberg Island of the High Arctic, perennially discharging cold hypersaline water rich in sulfur compounds. Microbial mats are found adjacent to channels of the GH springs. This thesis is the first detailed analysis of the Gypsum Hill spring microbial mats and their microbial diversity. Physicochemical analyses of the water saturating the GH spring microbial mat show that in summer it is cold (9°C), hypersaline (5.6%), and contains sulfide (0-10 ppm) and thiosulfate (>50 ppm). Pyrosequencing analyses were carried out on both 16S rRNA transcripts (i.e. cDNA) and genes (i.e. DNA) to investigate the mat's community composition, diversity, and putatively active members. In order to investigate the sulfate reducing community in detail, the sulfite reductase gene and its transcript were also sequenced. Finally, enrichment cultures for sulfate/sulfur reducing bacteria were set up and monitored for sulfide production at cold temperatures. Overall, sulfur metabolism was found to be an important component of the GH microbial mat system, particularly the active fraction, as 49% of DNA and 77% of cDNA from bacterial 16S rRNA gene libraries were classified as taxa capable of the reduction or oxidation of sulfur compounds. Major components of the bacterial libraries are *Gamma*-, *Epsilon*-, and *Deltaproteobacteria*, *Flavobacteriia*, and *Actinobacteria*. The GH microbial mat community appears to be highly influenced by the microorganisms present in the upstream GH spring sediment. Close investigation of archaeal species and sulfate reducing bacteria within the mat revealed the presence of enigmatic phyla for which no representatives have yet been isolated. In addition to sulfate reducing bacteria, a variety of elemental sulfur reducing bacteria were detected from the GH microbial mat, including *Desulfuromusa*, *Desulfuromonas*, and *Sulfurospirillum*. As well, cultures enriched with either elemental sulfur, thiosulfate, or sulfate, and inoculated with sediments from Axel Heiberg sulfidic spring sediments generally showed sulfide at low temperatures (5°C and 10°C), indicating that many different sulfur compounds may be metabolised within the springs.

Le système de source de Gypsum Hill (GH) est situé sur l'île d'Axel Heiberg dans le Haut-Arctique, et évacue perpétuellement de l'eau hypersaline, froide et riche en composés soufrés. Les tapis microbiens se trouvent à côté des canaux de source GH. Cette thèse est la première analyse détaillée des tapis microbiens de source Gypsum Hill et leur diversité microbienne. Des analyses physico-chimiques de l'eau saturant le tapis microbiens de source GH montrent que durant l'été il est froid (9 ° C), hypersalin (5,6%), et contient du sulfure (0-10 ppm) et du thiosulfate (> 50 ppm). Des analyses en pyroséquençage ont été effectuées sur les transcriptions ARNr 16S (c.-à-ADNc) et sur des gènes (c.-à-ADN) pour enquêter sur la composition des communautés du tapis, de sa diversité, et des membres présumés actifs. Afin d'étudier la communauté réductrice de sulfate en détail, le gène de la réductase sulfite et sa transcription ont également été séquencés. Enfin, des cultures d'enrichissement pour les bactéries réductrices de soufre/sulfate ont été mises en place et suivies pour la production de sulfure à des températures froides. Dans l'ensemble, le métabolisme du soufre s'est trouvé être un élément important du système de tapis microbien GH, en particulier la fraction active, étant donne que 49% de l'ADN et de 77% de l'ADNc de banques bactériennes de gènes ARNr 16S ont été classés comme étant un taxon capable de la réduction ou l'oxydation des composés de soufre. Les principaux éléments des banques bactériennes sont *Gamma-, Epsilon*, et *Deltaproteobacteria, Flavobacteriia* et *Actinobacteria.* La communauté de tapis microbien GH semble être fortement influencée par les micro-organismes présents en amont de sédiments des sources GH. Des enquêtes fermées d'espèces d'archées et des bactéries réductrices de sulfate dans le tapis microbien ont révélé la présence d'embranchements énigmatique pour laquelle aucun représentant n'a encore été isolé. En plus de bactéries sulfato-réductrices, une variété de bactéries réductrices de soufre élémentaire ont été détectés à partir du tapis microbien GH, y compris *Desulfuromusa, Desulfuromonas*, et *Sulfurospirillum.* Aussi, des cultures enrichies avec soit du soufre élémentaire, thiosulfate, ou du sulfate, et inoculé avec les sédiments provenant des sédiments des sources sulfurées d'Axel Heiberg montrent généralement du sulfure à basse température (5 ° C et 10 ° C), ce qui indique que de nombreux différents composés soufrés peuvent être métabolisés à l'intérieur des ressorts.

Acknowledgements

I would like to thank my supervisor, Dr. Lyle Whyte, for giving me to opportunity to pursue research in the exciting field of polar microbiology. His support and guidance throughout the course of my studies and the writing of this thesis have been invaluable. I'm also very grateful to my friends and colleagues in the Whyte laboratory for their advice, assistance, emotional support, and some memorable lunchtime conversations. In particular, I would like to thank Dr. Boswell Wing and André Pellerin for the chance to dip my toes into the world of biogeochemistry, and Guillaume Lamarche-Gagnon for his mentorship during the start of my research. Thanks to the members of my committee – Dr. Lyle Whyte, Dr. Brian Driscoll, and Dr. Charles Greer – for their useful comments on the direction of my research. Thank you also to those who assisted in the field during two memorable expeditions to Axel Heiberg Island, namely Isabelle, Jen, Jesse, Dr. Chris Omelon and Miles Ecclestone. I would also like to acknowledge the funding institutions which provided financial support during my thesis: the Fond Québécois de Recherche Nature et Technologies (FRQNT), the Northern Science Training Program (NSTP), and the Natural Sciences and Engineering Research Council of Canada (NSERC), in particular the Canadian Astrobiology Training Program (CATP). Thanks to McGill University for additional assistance, and the logistical support of the Polar and Continental Shelf Program (PCSP). Finally, thanks to my friends and family. This thesis could not have been accomplished without their patience, understanding, and willingness to listen to me blather on about science stuff they don't understand. Thank you.

Table of Contents

List of Tables

List of Figures

Nomenclature and Abbreviations

16S rRNA gene: Gene that codes for the small subunit of ribosomal RNA; commonly used as a phylogenetic marker.

BLAST: Basic Local Alignment Search Tool

Discontiguous MegaBLAST: BLAST search optimized for somewhat dissimilar sequences.

DNA: Deoxyribonucleic acid

cDNA: complementary DNA; DNA that has been generated via reverse transcription of RNA

dsr gene: Gene that codes for dissimilatory sulfite reductase, used in the sulfate reduction pathway of SRBs; commonly used as a phylogenetic marker for sulfate reducing microorganisms.

EPS: Extracellular polymeric substances

Halophile: A microorganism capable of metabolism in hypersaline conditions

MegaBLAST: BLAST search optimized for very similar sequences.

NCBI: National Centre for Biotechnology Information

OTU: Operational Taxonomic Unit

PCR: Polymerase Chain Reaction

Psychrophile: Cold adapted microorganism that has optimum growth below 20°C.

rRNA: ribosomal Ribonucleic Acid

SRB: Commonly, Sulfate Reducing Bacteria. In this thesis, is used to refer to any bacteria that can reduce a sulfur compound, including elemental sulfur, thiosulfate, and sulfate.

SOB: Sulfur Oxidizing Bacteria. Refers to any bacteria that can oxidize a sulfur compound, this may include thiosulfate, sulfide, or elemental sulfur.

Sulfidogenesis: The generation of sulfide, either biologically or chemically.

Chapter 1: Introduction

1.1. Microbial life in cold ecosystems

Cold environments are an important but under-researched ecosystem on Earth. Most of the planet exists at low temperatures; ~25% of Earth's landmass is either permanently frozen or covered by ice and ~90% of its ocean water never rises above 5°C (Margesin and Miteva, 2011). Approximately 85% of the biosphere permanently exists at temperatures below 5°C. These include settings such as the deep ocean, formations within polar and alpine regions such as glacial ice, ice shelves, cold soils and lakes, as well as seasonally cold habitats. Despite these harsh conditions, cold environments are host to a wide array of microorganisms, and are an important source of novel taxa for microbiological study (Margesin and Miteva, 2011).

Beyond exposure to intense cold, microorganisms in cold environments also face challenges such as reduced enzymatic reaction rates, increased molecular stability, changes in protein conformation, limited bioavailability of water and/or nutrients, and often extremes in pH and salinity (Bakermans, 2008). The main constraint to life in subzero settings (called cryoenvironments) is the availability of liquid water. Solutes depress the freezing point of water, allowing it to remain liquid at subzero temperatures. For this reason, many psychrophiles have evolved to tolerate high salt concentrations (i.e. are halophiles and/or halotolerant). Adaptation to osmotic stress can occur by the accumulation of compatible solutes within the cell. Other common cold adaptations include: the maintenance of membrane fluidity by changes in fatty acid structure, the production of cold-adapted enzymes and antifreeze proteins, a greater cold-shock response, and the production of exopolysaccharides (Margesin and Miteva, 2011; Goordial et al., 2013). The study of psychrophiles (microbes that have growth optima below 20°C) has expanded our views regarding the limits of terrestrial habitability and the habitability of other settings within our solar system, past or present.

1.2. Implications for astrobiology

Astrobiology is a broad field of research focused on the study of the origin, evolution and distribution of life throughout the universe. Two major aspects study are the search for life within the solar system and the origins and early evolution of life on Earth. Current

astrobiological targets of interest within the solar system include Mars, Jupiter's moon Europa, and Saturn's moon Enceladus. Despite the extreme cold experienced by these planetary bodies (-60°C, -160°C and -190°C, respectively), they all offer evidence of the presence of past or present liquid water. The NASA Cassini mission observed geyser-like jets of water vapor and salt crystals emerging from the south polar region of Enceladus (Hansen et al., 2006). Recent research has found that these plumes originate from a large (10-km-deep), regional ocean capped by 30-40 km of ice (Iess et al., 2014). Similar water vapour plumes emerging from Europa's icy crust have been observed by the Hubble Space Telescope (Roth et al., 2014). Scientific consensus is that Europa possesses a 100-km-deep subsurface ocean of liquid water beneath a 10-30-km thick ice layer. This ocean is likely rich in dissolved salts, including sulfates and carbonates (McCord et al., 1998).

Mars is arguably the most promising target in the search for extraterrestrial life due to mounting evidence that liquid water was once abundant on Mars, and may even exist today. This may have taken the form of a surface ocean, or groundwater seepage in the form of springs (Di Achille and Hynek, 2010; Ehlmann et al., 2011). Much of the evidence of past liquid water on Mars is from geomorphic observations of ancient river valley networks, deltas, and lakebeds (Di Achille and Hynek, 2010). Other evidence includes the presence of hydrated minerals which form due to chemical weathering. Unlike on Earth, these sedimentary deposits are dominated by sulfate minerals such as gypsum, as opposed to carbonate minerals (Gaillard et al., 2012). The observation of recurring slope lineae raises the question of whether liquid brines may continue to exist on Mars today (McEwen et al., 2011; Levy, 2012). Overall, due to its cold, saline, anoxic properties, and its abundance of sulfur compounds, GH spring shows great similarity to current astrobiological targets such as Mars. By studying the microbial populations present at this terrestrial analogue site, we can gain a better understanding of what potential extra-terrestrial life could resemble. Furthermore, a fossilized microbial mat would be a convincing biosignature if found elsewhere in the solar system. Unlike other forms of microbial growth, microbial mats are visible, distinctive and require no specialized equipment to detect. As well, macroscopically visible microbial life such as a biofilms are easily identified via microscopic staining and would therefore be an excellent target for astrobiological investigation (Nadeau et al., 2008).

Astrobiology as a field investigates not only on the possibility of life on other planetary bodies, but also the development of early life on Earth. Microbial mats are modern analogues to Precambrian stromatolites. Stromatolite fossils have been found that date to 3.5 billion years ago (Margulis et al., 1980) and thus are one of the oldest known forms of life on Earth. Stromatolites are made of laminated rock, formed by microbial mats which became lithified due to calcification or silicification. Layers form as *Cyanobacteria* grow near the surface where photosynthesis can occur. It is thought that the *Cyanobacteria* within stromatolites and other environments were largely responsible for oxygenating Earth's atmosphere, paving the way for the evolution of complex eukaryotic life. As well, the study of microbial life in cryoenvironments has relevance to what life may have existed during glaciation events on early Earth. Approximately 2.4 Gya and again 550-880 Mya a cooling trend occurred due to a decrease in greenhouse gas and solar radiation levels, exacerbated by runaway ice-albedo effects (Donnadieu et al., 2004; Vincent et al., 2005). The exact condition of the Earth during these periods is highly debated. Some studies suggest that all of Earth's oceans were frozen up to a depth of 1 km, while others think an equatorial band of ocean may have remained liquid or under thin ice (the "snowball" and "slushball Earth" hypotheses) (Lubick, 2002). Microbial mats may have been an important form of life during these periods due to their ability to form in frozen environments (such as ice shelves), and may have provided a unique refuge for Eukaryotic organisms (Vincent et al., 2000). By studying modern microbial mats, we can gain a better understanding of their ancient relatives.

1.3. Research objectives

The overall objective of this research project was to characterize the microbial community of a microbial mat located adjacent to Gypsum Hill springs, on west-central Axel Heiberg Island of the Canadian High Arctic. The microbial mats at GH springs were only briefly described in the initial survey of the site and otherwise have remained entirely unstudied (Pollard et al., 1999). These microbial mats are, to our knowledge, unique due to their presence by a sulfide spring that is also cold and saline. The combination of these factors makes the GH springs an excellent analogue to other planetary bodies within the solar system, and thus these microbial mats are particularly interesting to study from an astrobiology perspective.

The current research is intended to complement previous microbiological research on the GH spring source sediments and filaments (Perreault et al., 2007, 2008; Niederberger et al., 2009). The general goal of the present study is to evaluate the prokaryotic community composition of the GH microbial mats through a combination of culture-dependent and culture-independent methods, with a focus on the sulfur metabolizing component. Specific research objectives were as follows:

i. To identify potentially active bacteria and archaea within a GH springs microbial mat via pyrosequencing of the 16S rRNA genes (DNA) and transcripts (RNA)
ii. To measure the diversity and richness of the GH spring microbial mat community
iii. To compare the microbial community of the GH microbial mat to that of other GH spring sites (sediment and filaments)
iv. To identify potentially active sulfate-reducing bacteria of the GH springs microbial mat via pyrosequencing of the *dsrB* genes (DNA) and transcripts (RNA)
v. To determine the sulfidogenic activity of potential halophilic and cryophilic sulfur compound-reducing bacteria by the incubation of sulfidic spring sediments in targeted media

Chapter 2: Literature Review

2.1. Overview of literature review

The beginning of this literature review introduces the concept of sulfur metabolism by microorganisms – its importance to life on Earth, the mechanisms by which it occurs, and the microbial taxa which are known to oxidize or reduce sulfur compounds. The focus then shifts to the environmental requirements for the formation of microbial mats, and their typical microbial composition. Particular attention is paid to microbial mats found in locations similar to the study site (i.e. cold, saline, or sulfide-rich environments). Finally, an overview is presented of the study site (GH springs), and other Axel Heiberg Island sulfidic springs (CP and LH springs). This includes an examination of their physicochemical parameters, their proposed origins, and the microbial diversity previously reported from them.

2.2. Sulfur metabolism

2.2.1. The sulfur cycle

Sulfur is one of the most abundant elements on earth, present mainly as pyrite (Fe_2S) and gypsum ($CaSO_4$) in mineral form, and as inorganic sulfate in seawater. Sulfur compounds are an important source of energy for life on earth, with many possible transformations due to sulfur's broad range of valence states (Fig. 2.1). These range from -2 as sulfide (completely reduced) to +6 as sulfate (completely oxidized), with a variety of intermediate states including elemental sulfur (0), sulfite (+4), and thiosulfate (-1 and +5). Sulfur compounds can act as either electron donors or acceptors, depending on valence state and (in the case of intermediate compounds) environmental conditions.

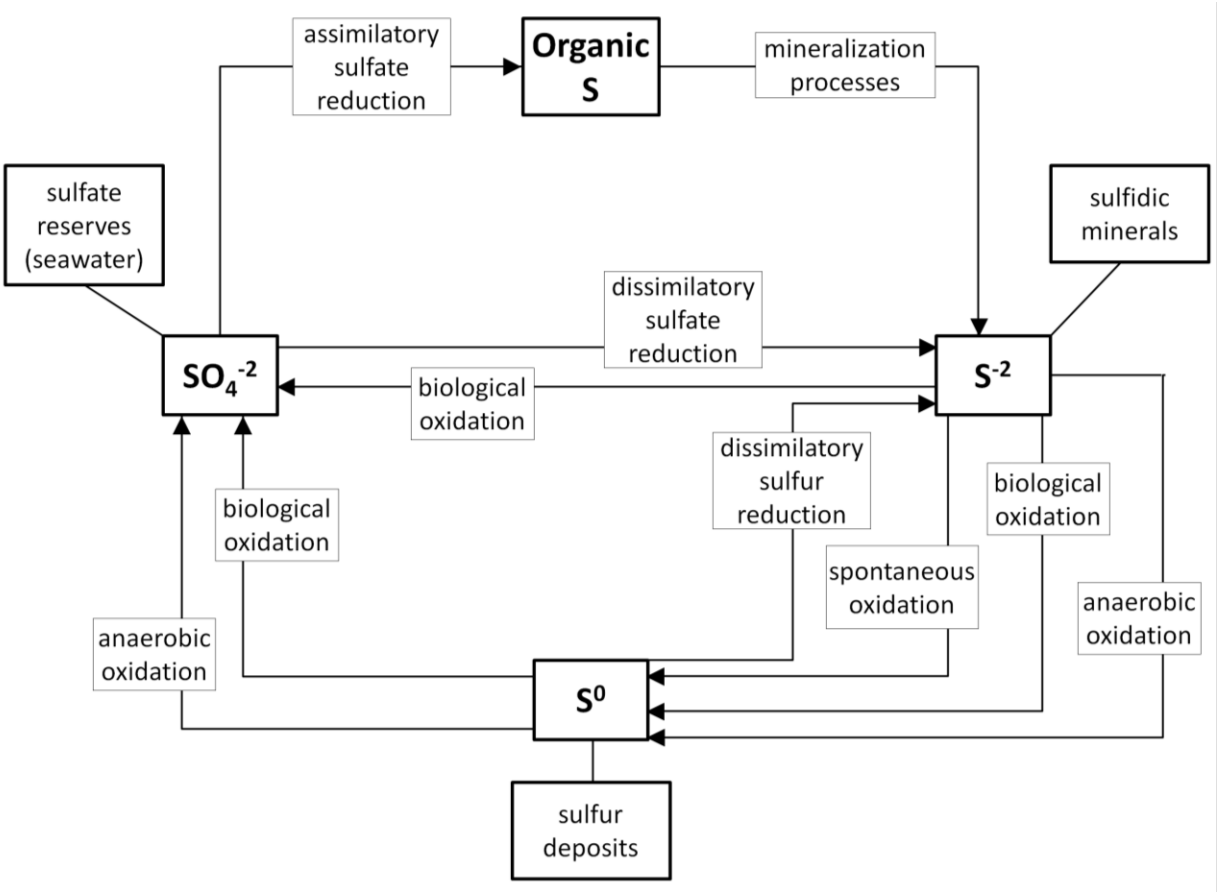

Fig. 2.1. Transformations of the sulfur cycle (adapted from Robertson and Kuenen, 2006).

2.2.2. Sulfur compound reducing microorganisms

Sulfate can be transformed either chemically or biologically. Abiotic sulfate reduction occurs at high temperatures (100-180°C) over long time periods (tens of thousands to a few million years) (Machel, 2001). The sulfide produced emerges in locations such as hydrothermal vents and cold seeps. More common, however, is biological sulfate reduction, which is thought to account for 50% of organic carbon mineralization in marine sediments (Jørgensen, 1982). There are two types of biological reduction of sulfate: assimilatory and dissimilatory sulfate reduction. Assimilatory sulfate reduction is performed in small amounts by many plants, fungi, and bacteria for the production of sulfur-containing molecules such as amino acids and proteins. The type of sulfate metabolism of interest to this research is dissimilatory sulfate reduction, which refers to the use of sulfate as the terminal electron acceptor during respiration. During this

process, adenosine triphosphate (ATP) is created which subsequently provides energy for microbial growth, and sulfide is released.

Enzymes involved in dissimilatory sulfate reduction are ATP sulfurylase (Sat), adenylyl-sulfate reductase (Apr), and dissimilatory sulfite reductase (Dsr), which are conserved amongst all cultivated sulfate reducing microbes. The *dsr* operon encodes two subunits, *dsrA* and *dsrB*, which make up the enzyme. As well as sulfate reducing bacteria (SRB), *dsr* is present in organisms such as sulfite-reducing bacteria (e.g. *Desulfitobacterium*, *Desulfitibacter*, *Pyrobaculum*), sulfur-disproportionating bacteria (e.g. *Desulfocapsa sulfexigens*), or in a reversed form in some sulfur oxidizing bacteria (within the *Proteobacteria* and *Chlorobi* phyla) (Müller et al., 2014). With a few exceptions due to lateral gene transfer, phylogenies constructed using the *dsr* gene are largely congruent with those using the 16S rRNA gene (Müller et al., 2014). For this reason, the *dsr* gene is often used as a target in sequencing-based phylogenetic studies. It should be noted that some microbes which reduce other sulfur compounds, such as *Desulfuromonas* species and some *Epsilonproteobacteria*, can do so without the possession of a *dsr* gene.

Sulfate reducing microbes are distributed amongst four bacterial phyla (*Proteobacteria*, *Nitrospirae*, *Firmicutes*, and *Thermodesulfobacteria*) and two archaeal phyla (*Euryarchaeota*, *Crenarchaeota*). SRB are found in a wide variety of environments where sulfate is present, including marine sediments, hydrothermal vents, hydrocarbon seeps, and mud volcanoes. Extreme environments they are present at include hypersaline microbial mats, acid-mine drainage sites, and soda lakes. Traditionally, it was believed that SRB were strictly anaerobic, however this belief has been challenged by evidence such as the discovery of SRB that can survive aeration, and even use aerobic respiration for energy (*Desulfovibrio oxyclinae*) (Dilling and Cypionka, 1990; Sigalevich et al., 2000). Despite their name, SRB are known to use many other electron acceptors for growth, including other sulfur compounds (thiosulfate, sulfite, and elemental sulfur), nitrogen compounds (nitrate and nitrite), iron, selenate, chromate, arsenate, and (as previously mentioned) oxygen (Muyzer and Stams, 2008). As well, SRB can sometimes ferment organic compounds in the absence of inorganic electron acceptors. Therefore, the occurrence of SRB in an environment should not be taken as direct evidence of sulfate reduction, particularly in freshwater environments that are low in sulfate.

In addition to SRB that also reduce sulfur, there are some taxa which can only reduce sulfur (and not sulfate). These include: anaerobes within the *Deltaproteobacteria* (genera: *Desulfuromonas*, *Desulfuromusa*, *Desulfurella*, *Dethiosulfovibrio*, *Hippea*, *Geobacter*, and *Pelobacter*), anaerobic chemolithoautotrophs within the phylum *Aquificae* (family: *Desulfurobacteriaceae*), and microaerophiles within the *Gamma-* and *Epsilonproteobacteria* (genera: *Wolinella*, *Campylobacter*, *Shewanella*, *Sulfos*, *Sulfurospirillum*, *Geospirillum, Sulfurimonas*) (Finster et al., 1998; Rabus et al., 2006). Sulfur-reducing archaea are hyperthermophilic and include the orders *Desulfurococcales*, *Thermococcales, Thermoproteales, Pyrodictales, Sulfolobales, Pyrodictales*, and *Sulfolobales* (Rabus et al., 2006). Many sulfur-reducers are also able to reduce ferric iron, nitrate, and/or thiosulfate. All of the above-mentioned microbes use sulfur compounds as electron acceptors, but other organisms may reduce sulfur for other purposes, such as in a facilitated fermentation process or as a by-reaction. Examples of such sulfur-reducing microbes include some *Beggiatoa*, *Chromatium*, and *Oscillatoria* species (Rabus et al., 2006). Sulfur reducing bacteria tend to occupy many of the same habitats as SRB, and have been isolated from acidic hot springs, hydrothermal vents, hypersaline lakes, submarine thermal springs, marine sediments, and freshwater sulfur spring sediments (Pfennig and Biebl, 1976; Belkin and Jannasch, 1985; Finster et al., 1997; Alain et al., 2009; Sorokin and Muyzer, 2010; Yoneda et al., 2012).

2.2.3. Sulfur compound oxidizing microorganisms

Sulfide and other reduced sulfur compounds produced by SRB can be used as electron donors for sulfur oxidizing microbes. Typically, sulfide is oxidized completely to sulfate, which can be used by SRB to complete the sulfur cycle. Sulfide oxidizing microbes are a phylogenetically diverse group of organisms that can undergo anaerobic phototrophic or aerobic/anaerobic chemotrophic growth. Sulfide is the most common substrate for these microbes, but others may include thiosulfate, elemental sulfur, tetrathionate, or sulfite. Henceforth, for simplicity, all sulfur-compound oxidizing microbes will be referred to as sulfide oxidizing bacteria (SOB).

SOB can use a variety of mechanisms to oxidize sulfur compounds. One major system involves the *sox* genes (found in members of the *Proteobacteria*), which in combination allow the oxidation of sulfide, thiosulfate, sulfur, and sulfite (Dahl et al., 2008). Another method

utilizes the *sqr* and *dsr* genes for anaerobic phototrophic oxidation of sulfur compounds (performed by the purple sulfur bacteria). This pathway involves the formation of elemental sulfur as an intermediate, which may be stored as globules in the periplasm or outside the cell. SOB living in extreme environments (such as members of *Acidithiobacillus*, *Thermithiobacillus*, and *Halothiobacillus* of the *Beta-* and *Gammaproteobacteria*) utilize the *dox* genes to catalyze the oxidation of thiosulfate, with tetrathionate as an intermediate (Dahl et al., 2008). Hybrid systems which use combinations of these genes are also possible. Sulfur oxidation by archaea is largely uncharacterized, but it is known that some archaea use the SOR enzyme to produce sulfite, thiosulfate, and sulfide via the aerobic disproportionation of elemental sulfur (Friedrich et al., 2005). The *sor* genes have been found in genomes of *Acidianus*, *Picrophilus*, *Ferroplasma*, and *Sulfolobus* species.

SOB are very phylogenetically diverse and include many taxonomic clades. They have been traditionally grouped into the purple sulfur bacteria (anaerobic/microaerophilic photolithoautotrophs of the *Chromatiaceae* and *Ectothiorhadospiraceae*), the green sulfur bacteria (anaerobic photoautotrophs of the phylum *Chlorobi*), and the colourless sulfur bacteria (aerobic chemolithoautotrophs of the class *Proteobacteria*) (Friedrich et al., 2001). As well, there are chemoheterotrophic and mixotrophic (able to grow autotrophically or heterotrophically) SOB. Heterotrophic sulfur oxidizers are taxonomically diverse, including *Loktanella, Roseobacter*, and *Sulfitobacter* of the *Rhodobacteraceae* family, *Spirochaeta*, and some *Marinobacter* species. Examples of mixotrophic SOB are some *Salinisphaera, Thiobacillus,* and *Paracoccus* species. Unlike the vast diversity found amongst the sulfide oxidizing bacteria, sulfide oxidizing archaea are almost entirely restricted to members of the order *Sulfolobales*, which are thermoacidophiles that may use a variety of metabolisms (heterotrophy, chemolithoautotrophy, or mixotrophy) (Dahl et al., 2008). Sulfur oxidizing microbes occur in a wide range of aquatic habitats, including extremely acidic and alkaline environments (pH 0.7 – 10.5), hot geothermal vents, cold marine sediments, and hypersaline lakes/springs (Dahl et al., 2008). Due to the rapid spontaneous oxidation of sulfide when exposed to oxygen, aerobic oxidation by chemotrophs often occurs in the thin zone where sulfide and oxygen meet.

2.3. Microbial mats

2.3.1. Formation of microbial mats

Microbial mats are vertically laminated benthic communities of micro-organisms that grow on solid surfaces such as sand, rock, or sediment. They typically possess physicochemical gradients of compounds such as sulfide and oxygen that provide micro-environments for different functional groups of micro-organisms, which in turn strengthen and maintain these gradients. This is what causes the characteristic multilayered structure of most microbial mats (van Gemerden, 1993).

Microbial mats develop over time due to a combination of microbial growth/activity and sedimentation. There are several environmental parameters that have been proposed to control whether or not a microbial mat will develop. These are: grain size of the sediment, capillary attraction of water, penetration of light, and grazing pressure (Walter, 1976). Mats generally persist on sediments that have a uniform grain size (Stal and Moezelaar, 1997). This size should be such that it allows for the effective capillary attraction of water. Light penetration should be enough that anaerobic phototrophic purple sulfur bacteria can grow beneath a layer of oxygenic phototrophs. This is aided by development on quartz sediment, which can reflect light to deeper layers (Jorgensen and Marais, 1986; Lassen et al., 1992). The presence of grazing meio- and macrofauna would cause the destruction of the microbial mat, thus it is thought that microbial mats only develop in environments that mostly exclude them (Fenchel, 1998). These include extreme environments such as hypersaline and hyperthermal environments. Intertidal microbial mats undergo periodic grazing by nematodes, snails, or fish during flooding (Feazel et al., 2008).

Microbial mats are typically dominated by a few functional groups of microbes: photosynthetic cyanobacteria or algae, colourless sulfur bacteria, purple sulfur bacteria, sulfate reducing bacteria, and aerobic heterotrophs (van Gemerden, 1993). The driving force of most microbial mats are primary producers, which may be either phototrophic *Cyanobacteria* or *Eukarya*. These will use light for energy to fix CO_2, leading to the generation of organic matter as well as extracellular polymers (EPS). EPS is an important component of microbial mats, lending strength to the mat structure by acting as a type of glue within which microbes are embedded (Grant and Gust, 1987). In addition, EPS can protect the vulnerable top layer of the

mat from the stresses of UV radiation and dehydration. Oxygenation of the mat does not remain constant, but fluctuates depending on time of day. In the dark, respiration by cyanobacteria and aerobic heterotrophs overtakes photosynthesis, leading to depletion of oxygen within the mat (Marshall, 1989). Under anoxic conditions, phototrophs may ferment their carbon reserves, leading to the release of small organic acids and alcohols (Stal and Moezelaar, 1997). These, in turn, can be utilized by sulfate reducing (and in low-sulfate environments, methanogenic) bacteria. Sulfate reducing bacteria produce sulfide, which can be oxidized by S-oxidizing bacteria including purple sulfur bacteria and colourless sulfur bacteria.

Purple sulfur bacteria are anaerobic/microaerophilic phototrophs which can oxidize sulfide and other reduced sulfur species in order to fix CO_2 as cellular carbon. The purple sulfur bacteria are of the order *Chromatiales* with the exception of the *Halothiobacillaceae* which are functionally different, being chemotrophic obligate aerobes instead of phototrophic anaerobes. The purple sulfur bacteria often form internal or external elemental sulfur granules as a byproduct of S-oxidation. The purple sulfur bacteria normally form a layer beneath the cyanobacteria/algae (van Gemerden, 1993). Colourless sulfur bacteria are chemotrophs which are generally aerobic, but are sometimes able to reduce nitrate instead. These bacteria are found throughout the mat (Visscher et al., 1992). The term "colourless sulfur bacteria" is a general term used to designate bacteria that use reduced sulfur compounds as a source of energy (instead of light), and does not refer to one particular taxonomic group. Families which show this property include the *Thiobacteriaceae*, *Beggiatoaceae*, and *Achromatiaceae*.

2.3.2. Microbial mat diversity

Levels of diversity and taxonomic composition of microbial mats differ depending on their environment. Bolhuis *et al.* investigated the differences between intertidal, hypersaline, and hyperthermal microbial mats (Bolhuis et al., 2014). One of their major findings is that, as general ecological principles state, mats in more extreme conditions (hyperthermal and hypersaline environments) show less diversity and richness than those in intertidal and moderately saline microbial mats (Frontier, 1985; Bolhuis et al., 2014). They also found that microbial mats are dominated by *Bacteria*, even in extreme environments that otherwise consist mostly of *Archaea* (Casamayor et al., 2013). For instance, in hypersaline waters Archaea can make up 80% of the total biomass, but are not found in nearby microbial mats in nearly the same numbers (Ley et al.,

2006; Armitage et al., 2012; Harris et al., 2013). And finally, though they are often the most visually abundant organisms, the green *Cyanobacteria* are often not numerically dominant (Bolhuis et al., 2014).

To our knowledge, the current study is the first investigation of a microbial mat associated with a High Arctic spring. Four low temperature sulfide springs hosting macroscopically visible microbial growth (mats or streamers) have been previously studied – Flybye springs in the Northwest Territories, the Sippenauer Moor marsh in Germany, and the Ancaster and Great Sulphur springs in Ontario (Douglas, S. Douglas, 2001; Moissl et al., 2002; Bonny and Jones, 2007; Chaudhary et al., 2009). All of these locations have source water which is cold (8.5°C to 11°C) and non-saline, with high concentrations of sulfide and low levels of oxygen. All four locations show the presence of white filamentous growth, and all but the Sippenauer Moor marsh also possess microbial mats of various colours. Microscopy techniques were used on the Flybye spring filaments to determine that the major components are the S-oxidizing *Thiothrix* and *Beggiatoa* species (Bonny and Jones, 2007). Flybye spring's dysoxic brown-green microbial mats are composed of diatoms, *Oscillatoria* (a cyanobacterium), and *Beggiatoa*. Oxygenated floating green-orange mats also possess these species, but additionally include other cyanobacterial species and *Chromatium* (a purple sulfur bacterium). Precise taxonomies were not determined for the Ancaster sulfur spring growth, but microscopy revealed that the oxygenated channel mats were formed by a diverse group of nonphotosynthetic S-oxidizers and the source mats consisted of green sulfur bacteria, purple sulfur bacteria, and a sulfide-utilizing cyanobacterium (Douglas, S. Douglas, 2001). The white filaments from the Sippenauer Moor marsh have a distinctive string-of-pearls morphology. These were found to be formed by an association between a bacterial *Thiothrix* species and a novel euryarchaeon (Rudolph et al., 2001; Moissl et al., 2002). A clone library study of Great Sulphur Spring microbial mats found that *Epsilonproteobacteria* and *Cyanobacteria* sequences were particularly abundant (Chaudhary et al., 2009). Other major bacterial players include sulfur-metabolizing or chemolithotrophic taxa of *Beta-*, *Gamma-*, and *Deltaproteobacteria*. Archaeal sequences showed similarity with uncultivated clades of *Euryarchaeota* and *Crenarchaeota*.

Microbial mats may also form in locations that lack a geochemical source of sulfide, but possess sulfate. Sulfate can support the establishment of sulfate reducing bacteria, which produce

sulfide that is utilized by other members of the community. Two such environments that exist at low temperatures are ice shelves and Antarctic lakes. The Ward and Markham ice shelves are areas of grounded perennial sea ice which are host to abundant microbial mat communities. These microbial mats are subject to changes in salinity, which ranges from freshwater during the summer melt (0.5 mS cm^{-1}) to saline during freeze up (>10 mS cm^{-1}) (Mueller et al., 2005). A metagenomic analysis of the Ward and Markham ice shelf microbial mats was performed, building on previous work using clone library studies (Bottos et al., 2008; Varin et al., 2010). Both studies found that the communities were dominated by *Proteobacteria*, especially *Alpha-* and *Betaproteobacteria*. Other important phyla include the *Bacteroidetes* and *Actinobacteria*. The clone library work found no *Cyanobacteria*, but metagenomics analysis revealed their presence as 12.4-22.7% of the total bacterial coding sequences. Archaea were rare, consisting of mostly *Euryarchaeota* followed by *Crenarchaeota*.

One of the most studied Antarctic lakes is Lake Fryxell, which is permanently ice covered and highly stratified. Salinity ranges from freshwater to 1% NaCl at the sediment surface where the microbial mats are found (Sattley and Madigan, 2010). At the bottom, Lake Fryxell water is cold (3°C) and anoxic with very high levels of sulfide (1.3 mM) (Karr et al., 2005). Abundant sulfate is detected in the water column with a maximum of 1.7 mM, but this level drops off to only 0.34 mM at the lake bottom, likely due to high rates of sulfate reduction in the sediment. A clone library study showed a microbial composition quite different from mats in other environments (Brambilla et al., 2001). A large portion of clones consisted of anaerobic fermenters within the *Clostridia* and *Bacilli* classes. Other important taxa include *Cytophaga*, *Flavobacteria*, *Verrucomicrobiales*, and *Proteobacteria*. Unlike other mats, the most abundant of the *Proteobacteria* were *Betaproteobacteria*, followed by *Alphaproteobacteria*, with less *Delta-* and *Gammaproteobacteria*. An isolation approach by van Trappen *et al.* (2002) targeting microbial mats from 10 Antarctic lakes yielded 746 heterotrophic isolates belonging to the following taxa: *Alpha-*, *Beta-*, and *Gammaproteobacteria*, and the *Cytophaga-Flavobacterium-Bacteroides* branch.

Another type of sulfide-rich environment is the cold seep, an area of the ocean floor where sulfide and methane seep out of fissures caused by tectonic activity. Cold seeps range in temperature and salinity depending on the characteristics of the surrounding sea water. Some

cold seeps which show microbial mat growth include those at: the Gulf of Mexico, the Milano mud volcano, the Japan Sea, and the Mediterranean Sea (Mills et al., 2004; Heijs et al., 2005; Arakawa et al., 2006; Grünke et al., 2011). Due to high concentrations of methane, which other sulfide-rich environments lack, methane oxidation is an important energy source in these environments, particularly anaerobic methane oxidation, which is performed by the ANME group of *Archaea*. Sequences are often not closely related to any cultured representative of a phylum. Similarly to microbial mats in other environments, *Gamma-, Delta-, and Epsilonproteobacteria* often play a key role in the mat community at cold seeps due to their ability to oxidize/reduce inorganic sulfur compounds. Cold seep microbial mats have been specifically associated with *Beggiatoa* species – white/yellow/orange-coloured sulfide oxidizing filamentous bacteria (Mills et al., 2004; Zhang et al., 2005; Lloyd et al., 2010).

2.4. **Axel Heiberg Island sulfidic spring sites**

2.4.1. Location and site description

Perennial springs are rare in locations with deep continuous permafrost, such as is typical of the Canadian High Arctic, due to limited opportunities for connection between sub- and supra-permafrost groundwater systems. Axel Heiberg Island is a part of the Canadian High Arctic archipelago of Nunavut, west of Ellesmere Island and slightly north of the magnetic north pole. It is home to several perennial saline discharges, among the only known cold springs in thick permafrost on Earth (Andersen et al., 2002). These springs are unique because they flow through deep permafrost (~400-600 m), unlike other deep Arctic springs, which flow through thinner (~100-400 m) warmer permafrost (Taylor and Judge, 1976; Lauritzen and Bottrell, 1994).

To date, geomorphology and microbiology research has focused on three sets of Axel Heiberg Island springs: the Gypsum Hill (GH), Colour Peak (CP), and Lost Hammer (LH) (sometimes called "Wolf spring") springs. GH springs is located ~7 km upstream from the head of Expedition Fjord, present on west-central Axel Heiberg Island. The perennial GH spring system consists of approximately forty springs and seeps located at 79°24'30"N, 90°43'05"W, discharging into Expedition River ~7 km upstream from the head of Expedition Fjord (Pollard et al., 2009). The CP springs are located ~11 km from Gypsum Hill springs on the north side of Expedition Fjord (79°22'48"N, 91°16'24"W) (Fig. 2.2). They consist of ~30 springs which

discharge from the steep south-facing slope of Colour Peak. LH spring consists of one outlet from a salt tufa structure, and is located in a valley south of Strand Fjord (79°07'N, 90°21'W) ().

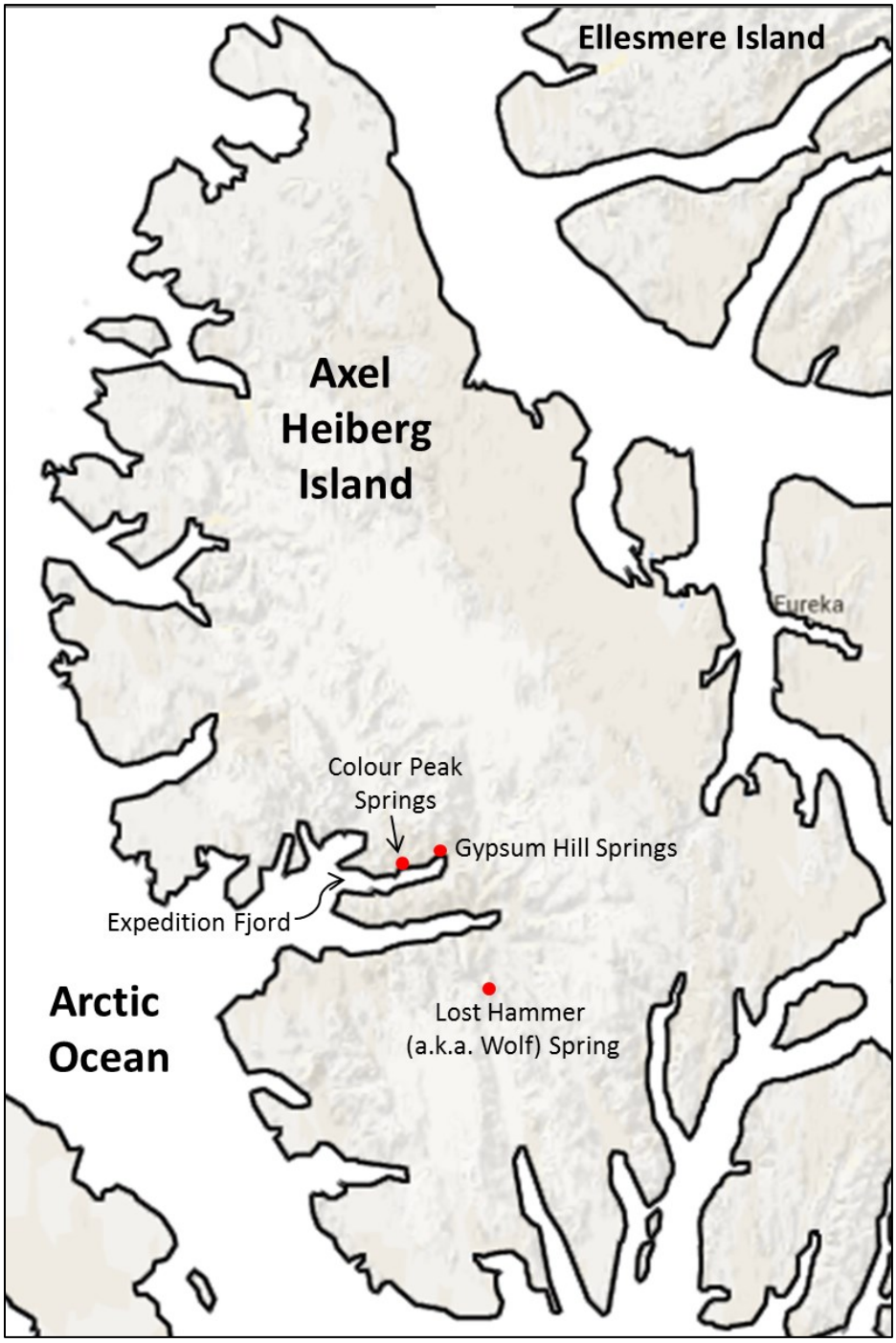

Fig. 2.2. Approximate locations of three Axel Heiberg Island sulfidic saline spring sites: Gypsum Hill Springs, Colour Peak Springs, and Lost Hammer spring (adapted from Battler et al., 2013).

2.4.2. Physicochemical parameters

The Canadian High Arctic archipelago is considered a polar desert, characterized by very cold dry winters and cool wetter summers. A weather station located at Eureka (120 km northeast of Expedition Fjord) records mean monthly temperatures of 5.4°C for July and -36.1°C for January. A long-term automatic meteorological station at Colour Lake (approximately 3 km north of GH springs) indicates a mean average annual air temperature of -15°C. Despite these low temperatures, the GH, CP, and LH springs flow year-round, maintaining a constant temperature. LH spring is colder (~-5°C) than GH and CP springs, which maintain temperatures of -0.5 to 6.9°C and 3.1 to 5.7°C, respectively, depending on the outlet (Perreault et al., 2007; Pollard et al., 2009; Lay et al., 2012). LH spring also has a higher salinity (22-26%) than GH and CP springs (7.5 to 7.9% and 15.5 to 17%, respectively) (Pollard et al., 1999; Perreault et al., 2007). All three of the springs are near-neutral, reduced, nearly anoxic, and rich in sulfate and sulfide.

As well, all three springs discharge gases. GH and CP springs emit gas that consists mainly of N_2 (>98%), with minor contributions of CH_4 (<1%), and CO_2 (<1%) (Pollard et al., 1999). LH spring discharges a mix of thermogenic CH_4 (50%), N_2 (35%), CO_2 (10%), He, H_2, and other hydrocarbons (Niederberger et al., 2010). GH and CP springs have high concentrations of dissolved ions such as Na^+, Ca^{2+}, $SO4^{2-}$, and Cl^-, which when normalized to Na^+ displays the same signature as seawater (Pollard et al., 1999). A comprehensive mineralogical study of the GH spring site found that the most abundant minerals there are, in decreasing order, gypsum ($CaSO_4*2H_2O$), halite (NaCl), elemental sulfur (S), quartz (SiO_2), calcite ($CaCO_3$), and plagioclase/clays (Battler et al., 2013). Elemental sulfur is found majorly in downstream yellow-gray terraces and channels, as well as in association with gypsum in wet sediment.

2.4.3. Origin of the springs

The origin of the GH and CP spring waters are not definitively known, but several hypotheses have been proposed. One model suggests that the water source is a combination of subglacial meltwaters from the Müller Ice Cap and the glacially-dammed Phantom Lake in contact with evaporitic piercement structures (Andersen et al., 2002). Another possibility is that

GH springs has a similar origin as the Ellesmere Island supraglacial sulfur spring, Borup Spring (Battler et al., 2013). This model states that a proximal glacier (such as White and/or Thompson glaciers) creates an area of unfrozen subsurface, within which microbial sulfate reduction produces sulfide gas. This gas is then oxidized by other microbes to produce sulfuric acid, which dissolves subsurface limestone rock to form spring pipes (Grasby et al., 2012). Recent evidence from an ancient network of hydrothermal veins suggests that GH springs may have originated as a high temperature (100-300°C) deeply-circulating basinal fluid system (Zentilli et al., 2013). Due to its position far from any glaciers or glacially-dammed lakes, the above models cannot apply to LH spring (Battler et al., 2013). The source of LH spring is therefore currently unknown.

2.4.4. Microbial diversity at Gypsum Hill springs

Previous microbiological work performed at GH springs focused on the largest of the spring outlets, GH-4, and used clone library or isolation techniques (Perreault et al., 2007, 2008). Perreault *et al.* constructed a bacterial clone library from DNA extracted from GH-4 spring sediment (Perreault et al., 2007). This library consisted of 155 clones representing 46 phylotypes. The major families found include *Proteobacteria* (63%), *Firmicutes* (14%), and *Bacteroidetes* (8%). The majority of the sequences were found to belong to one species, the chemolithoautotrophic *Thiomicrospira psychrophila* (19%). 30% of reads belonged to S-oxidizing bacteria, and 26% were S-reducers. S-oxidizing bacteria included *Epsilonproteobacteria* such as *Sulfuricurvum*, and *Sulfurimonas*, as well as *Gammaproteobacteria* like *Thiobacillus*, *Halothiobacillus*, and the aforementioned *Thiomicrospira*. S-reducers were mainly *Deltaproteobacteria* classified as either *Desulfobacteriaceae* or *Desulfurimonaceae*. The archaeal clone library showed a similar number of clones (156) that grouped into 18 archaeal phylotypes. 79% of the library was made up of *Crenarchaeota*, and the remaining clones were classified as *Euryarchaeota*. Classification beyond this level was not possible at the time. Isolation techniques were also applied to GH-4 sediment using a minimal thiosulfate medium and postgate B, an anaerobic sulfate-reducing bacteria medium (Perreault et al., 2008). GH-4 isolates include *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Halothiobacillus* and *Thiomicrospira*. The most common isolates were bacteria of the genera *Gillisia*, *Psychrobacter*, *Marinobacter*, *Sporosarcina*,

Halothiobacillus. All isolates grew at 5°C, and were generally salt-tolerant facultative anaerobes. GH spring sediment slurries were positive for anaerobic heterotrophic sulfur oxidation and aerobic autotrophic sulfur oxidation.

Other investigations have focused on the whitish-coloured microbial filaments that form during the winter months in GH spring run-off channels (Nadeau et al., 2008; Niederberger et al., 2009; Rogers et al., 2010). Microscopy revealed that the filament material is made up of a globular substructure composed of EPS embedded with bacterial cells and crystallites of gypsum. Further microscopy using fluorescent nanoparticule quantum dot probes showed bacterial cells primarily in association with elemental sulfur crystals, with lesser amounts of gypsum, aluminum silicate, and calcium carbonate (Clarke et al., 2010; Rogers et al., 2010). Clone libraries, FISH, and DGGE analyses indicate that the bacteria consist almost entirely of a *Thiomicrospira* species 99% genetically identical to one found previously from the GH-4 sediments (Niederberger et al., 2009). All but one of the archaeal clone sequences were identical to an unclassified *Crenarchaeota* from the GH-4 source sediment. Both the streamers and the *Thiomicrospira* strain alone were confirmed to be capable of oxidizing sulfide/thiosulfate and fixing CO_2 under cold, saline conditions.

2.4.5. Microbial diversity at Colour Peak and Lost Hammer springs

A clone library study of the CP spring sediment microbial community found that it was similar to that of the GH springs, being dominated by *Proteobacteria* (82% of the library), with lesser amounts of *Firmicutes* (9%) and *Bacteroidetes* (6%) (Perreault et al., 2008). Other similarities include the predominance of *Thiomicrospira psychrophila* (45%) and a focus on sulfur metabolism; 74% of the library belonged to putative S-oxidizers, and 2% to putative S-reducers. The LH spring source and channel sediment have been the subject of several microbiological studies (Niederberger et al., 2010; Lay et al., 2012, 2013; Lamarche-Gagnon et al., 2015). One 16S rRNA profile of the LH spring source sediment suggests that the most active microbial taxa are *Gammaproteobacteria*, followed by *Actinobacteria*, *Bacteroidetes*, *Betaproteobacteria*, and *Alphaproteobacteria* (Lay et al., 2013). However, this contrasts with a separate 16S rRNA pyrosequencing study, which found a high proportion of *Chloroflexi* group T78 and lesser numbers of *Synergistetes*, *Betaproteobacteria*, and *Deltaproteobacteria* (Lamarche-Gagnon et al., 2015). Metagenomic data has found that important types of

metabolism within the LH spring community include sulfate reduction, nitrogen cycling, hydrogenotrophy, and short-chain alkane degradation (Lay et al., 2013; Lamarche-Gagnon et al., 2015).

Chapter 3: Materials and Methods

3.1. Site sampling and physicochemical characterization

Gypsum Hill (GH) microbial mat samples were collected in July, 2013, from a mat located at approximately 79°24'149"N, 90°44'203"W. The microbial mat and underlying sediment was sampled up to a depth of ~5 cm and stored in LifeGuard Soil Preservation Solution (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) in a sterile, nuclease-free conical tube. Samples were kept cold (<10°C) during transport and immediately stored at -80°C upon arrival to the laboratory. Physicochemical parameters of two GH microbial mats were measured in July, 2014 using a YSI 556 Multi Probe System in the water 1-5 cm below the surface. Measurement of soluble/ferrous iron, total iron, sulfide, and thiosulfate were tested using CHEMetrics kits (unpublished data, Raymond-Bouchard).

3.2. Nucleic acid extraction and 454-Pyrosequencing

Nucleic acids were extracted using the MoBio RNA PowerSoil total RNA isolation kit and RNA PowerSoil DNA elution accessory kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). The Mo Bio protocol was modified to optimize DNA and RNA yield in the following ways. The sample in LifeGuard solution was allowed to thaw then centrifuged at 2500g for 5 minutes and the supernatant removed. The sample was homogenized in liquid nitrogen with a mortar and pestle and three replicate preparations of 2 g of mat were added to the bead tubes. Acidic, not neutral, phenol:chloroform:isoamyl alcohol was used for nucleic acid extraction. Bead tubes were vortexed for 10 minutes then left to incubate for 10 minutes on ice before centrifuging for 10 minutes at 2500 x g. The rest of the MoBio protocol was followed unchanged with two exceptions: preparations were pooled by adding them to the same column in the RNA capture step, and the final RNA and DNA pellets were resuspended in 50 µl of solution SR7 instead of 100 µl. RNase OUT Recombinant Ribonuclease Inhibitor (Invitrogen, Carlsbad, CA, USA) (2.5 µl) was added to the final RNA solution. cDNA was generated from RNA using the

iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA) using the random primers included in the reaction mix.

DNA and cDNA samples were sequenced at Genome Quebec (Montreal, QC, Canada) using a Roche 454 GS-FLX sequencer with Titanium chemistry (Roche, Branford, CT, USA). Primers used for sequencing are as seen in Table 3.1.

Before being sent for sequencing, DNA and cDNA was checked for its ability to amplify by the Polymerase Chain Reaction (PCR) with the below primers (Table 3.1). PCR reactions were performed using the HotStarTaq Master Mix kit (Qiagen, Venlo, Netherlands). The recipe used was: 12.5 µl master mix buffer, 2 µl forward primer (0.2 µM), 2 µl reverse primer (0.2 µM), and 2 µl template DNA/cDNA, made to 25 µl with sterile distilled water. The following program was used with the Mastercycler Pro thermocycler (Eppendorf, Hamburg, Germany): an initial 15-min denaturation at 95°C; 10 cycles with a 1-min 94°C denaturation step, a 1-min 48°C annealing step, and a 1-min 72°C extension step; 30 cycles with a 1-min 94°C denaturation step, a 1-min 55°C annealing step, and a 1-min 72°C extension step; then a final extension of 72°C for 7-min. Both DNA and cDNA showed good amplification with all primer pairs, with the exception of mat DNA in combination with archaeal 16S rRNA primers, which showed a very faint band of a slightly larger size than expected. Negative and positive (DNA from *Escherichia coli*) controls were performed alongside and showed expected results.

Table 3.1. Primers used for amplicon sequencing of DNA and cDNA extracted from GH microbial mats.

Primer	Primer sequence	Target	Reference
27F	5'-AGRGTTTGATCMTGGCTCAG-3'	Bacterial 16S rRNA gene	Rogers et al., 2014
519R	5'-GTNTTACNGCGGCKGCTG-3'		
A344F	5'- ACGGGGTGCAGCAGGCGCGA-3'	Archaeal 16S rRNA gene	Perreault et al., 2007
A934R	5'- GTGCTCCCCCGCCAATTCCT-3'		
dsr2060F	5'- CAACATCGTYCATACMCAGGG-3'	*dsrB* gene	Oakley et al., 2012
dsr4R	5'-GTGTAGCAGTTACCGCA-3'		

3.3. Classification and analysis of 16S rRNA sequences

16S rRNA sequences were analyzed using the program Mothur v.1.34.4 following the processing pipeline described in Schloss et al., 2011. Alterations to this protocol are outlined below. Sequences in .sff file format were extracted and processed using the "sff.multiple" command with the following options: pdiffs=2, sdiffs=2, bdiffs=2, order=B, maxhomop=8, minflows=360, and maxflows=720. Bacterial and archaeal reads were processed separately by using the "remove.groups" command. Sequences were aligned to the Silva v119 reference database then screened as according to the protocol using "screen.seqs" with an optimized start position and criteria=85. After chimera removal using UChime, sequences were classified using the Silva v119 alignment reference and taxonomy databases with a cutoff of 60%. Any sequences classified as Mitochondria, Eukaryota, or unknown were removed. When processing sequences amplified with the archaeal primers, those classified as Bacteria were also removed.

Operational Taxonomic Units (OTUs) were defined and analyzed as outlined in the Schloss et al., 2011 processing pipeline at an identity level of 97%. Graphs of relative abundance of phyla were generated in Excel from data output from the "make.shared" and "classify.otu" commands in Mothur. Representative sequences for OTUs were determined using the "get.oturep" command. Representative sequences of major and deltaproteobacterial OTUs were classified using MegaBLAST searches against the GenBank nt database (http://www.ncbi.nlm.nih.gov/GenBank/) (McGinnis and Madden, 2004). Non-rare OTUs were designated putative SOBs or SRBs via manual literature searches of individual taxa. An OTU was considered a putative SOB if its Mothur classification is of a clade that has been shown in the literature to be capable of oxidizing one or more inorganic sulfur compounds. An OTU was considered a putative SRB if its Mothur classification is of a clade that has been shown in the literature to be capable of reducing one or more inorganic sulfur compounds.

Close BLAST relatives and other reference sequences were compared to representative sequences of deltaproteobacterial OTUs consisting of more than one or two sequences to create a phylogenetic tree. Reference sequences for orders other than *Desulfobacterales* and *Desulfuromonadales* were determined by using discontinuous MegaBLAST to compare representative sequences against a database consisting of only the order of interest. In the case of the archaeal phylogenetic tree, in addition to close BLAST hits, reference sequences included

those identified as belonging to enigmatic groups of interest (DHVEG-6 and the *Nitrosphaera* group), as well as clones from previous studies performed at Axel Heiberg Island sulfidic springs.

Sequences were aligned using the Muscle algorithm within the program MEGA6 (Edgar, 2004; Tamura et al., 2013). Sequences were trimmed then aligned again. MEGA6 was used to determine the best DNA model to use to construct a maximum likelihood tree. For the deltaproteobacterial sequences, the Kimura 2 parameter substitution model was used with rates among sites set to gamma distributed with invariant sites. For the archaeal phylogenetic tree, the same options were used, but without invariant sites. The maximum likelihood phylogenetic trees were created with MEGA6 using these settings, as well as complete deletion of gaps/missing data and a bootstrap test of phylogeny with 1000 replications.

3.4. Classification and analysis of *dsrB* gene sequences

The *dsrB* sequences were analyzed using a combination of the programs Mothur, MEGA6, and the FrameBot and USearch tools of the Fungene Pipeline. DNA and cDNA *dsrB* sequences were processed separately via the start of the Fungene Pipeline (Fish et al., 2013). Reads were first dereplicated, then checked for chimeras using USEARCH 6.0 (Edgar et al., 2011). In order to determine the proper frameshift for translation, the FrameBot tool was used (Wang et al., 2013). Reads were then translated and aligned with the ClustalW algorithm with default parameters and primers, barcodes, and spacers were manually trimmed within MEGA6 (Thompson et al., 1994; Tamura et al., 2013). Frameshift was checked again using FrameBot, then files were expanded using FunGene's Expand Mapping tool in order to include replicated sequences in downstream analyses. DNA and cDNA .fasta files were combined then dereplicated in Mothur using the "unique.seqs" command to create a .names file. DNA and cDNA sequences were translated within MEGA6 and aligned again using ClustalW. Overhanging edges were trimmed within MEGA6, and sequences were reverted to nucleotides. All subsequent analyses were performed based on the nucleotide sequences of the *dsrB* gene.

In Mothur, the data were dereplicated once more then the "make.group" command was used to create a .groups file compatible with Mothur for downstream analyses. Sequences were preclustered with "pre.cluster", using a difference parameter of 2. A distance matrix was

generated using “dist.seqs”, which was then used to cluster the sequences with “cluster”. Samples were subsampled by the number of sequences present in the group with the smallest abundance of reads (in this case, 1538).

A nucleotide identity level of 90% was chosen for *dsrB* OTU analysis based on evidence presented in Müller et al., 2014 that this corresponds to species-level 16S rRNA gene OTUs. Representative sequences for OTUs were determined using the “get.oturep” command in Mothur. Representative sequences for OTUs were classified using discontiguous MegaBLAST searches against the GenBank nt database (http://www.ncbi.nlm.nih.gov/GenBank/), excluding uncultured/environmental sample sequences (McGinnis and Madden, 2004).

Maximum likelihood phylogenetic trees were constructed using representative nucleotide sequences from OTUs consisting of more than 1 or 2 reads. Other sequences comprising the trees include: the closest discontiguous MegaBLAST hits, the *dsrB* gene from closest MegaBLAST hits to 16S rRNA gene representative sequences, and representatives from other orders of *Deltaproteobacteria* that have a *dsr* gene.

Sequences of interest were first aligned with the Muscle algorithm within the program MEGA6, not accounting for codons (Edgar, 2004; Tamura et al., 2013). Reference sequences were trimmed to the correct coding frame then re-aligned using Muscle, this time accounting for codons. The optimal model for the maximum likelihood tree was found to be Kimura 2 with substitution model with gamma distributed sites. These settings were used to construct the phylogenetic tree, completely deleting gaps and using a bootstrap test of phylogeny with 1000 repetitions.

3.5. Biodiversity and activity analyses

Alpha and Beta diversity analyses were carried out on 16S rRNA gene and *dsrB* gene data. The “rarefaction.single” command in Mothur was used to create rarefaction curves with a sampling frequency of 25 (or 10 for Archaeal samples). Diversity indices and corresponding collector’s curves were calculated using “summary.single” and “collect.single”, respectively. Subsampling was performed to the size of the smallest group, and collector’s curves were generated by calculating the diversity index every 25 sequences sampled. The following indices were calculated: the Shannon, Simpson, and Good’s coverage indicators of community diversity,

the Chao1 estimator of community richness, and the Shannon index-based measure of evenness. The dissimilarity between DNA and cDNA data was described with the Sorensen index, calculated with the "summary.shared" command. No indices were calculated for the Archaeal 16S rRNA gene cDNA data, as it consists of only 3 sequences.

Venn diagrams of OTUs shared between DNA and cDNA were constructed using Mothur's "venn" command. In order to look at only the common biosphere, OTUs consisting of only one sequence (singletons) or two sequences (doubletons) were removed using the "remove.rare" command with nseqs=2. Afterward, the non-rare sequence data were subsampled to the size of the smallest group (863 16S rRNA sequences and 1533 *dsrB* sequences), and this data was used to graph the relative abundances of RNA to DNA, as performed in Campbell et al., 2011. cDNA:DNA ratios were calculated by comparing the fraction of an OTU's reads from the cDNA library, divided by the fraction of that OTU's reads from the DNA library.

3.6. Enrichment of sulfidic spring source sediments with targeted media

Enrichments were performed using sediments from three Axel Heiberg sulfidic springs – GH spring, CP spring, and the LH spring – with the intent of culturing sulfate/sulfur reducing bacteria. One set of enrichments was set up in the laboratory with sediments from two GH spring outlets (GH-2 and GH-6) and the LH spring, which had been kept sealed anaerobically and frozen at -20°C since the time of sampling (2011 for the former, 2012 for the latter). In sealed vials, media targeting *Dethiosulfovibrio* and *Desulfohalobium* species were bubbled with N_2 and reduced with 0.05% Cysteine*HCl (final concentration) to ensure anaerobicity before being inoculated with sediment slurry (DSMZ 499, Surkov et al., 2001). Sediment slurry was prepared within an anaerobic chamber and subsequently sampled using wide-gauge needles (Coy Laboratory, Grass Lake, MI, USA). Incubations proceeded at either 5°C or 10°C, with vial headspace of either pure N_2 or H_2:CO_2 (80:20). A second set of media were inoculated on site at CP and GH springs during May, 2014. Media used were those targeting *Desulfuromonas*, *Desulfohalobium*, *Desulfobacter*, *Desulfobulbus*, and *Dethiosulfovibrio* species, as well as Postgate B and Knoblauch media, with NaCl concentrations modified to 7.5% (DSMZ 148; DSMZ 499; DSMZ 195; DSMZ 195b; DSMZ 906; Widdel and Bak, 1992; Knoblauch and Jorgensen, 1999; Surkov et al., 2001). Vials were inoculated in duplicate with GH-4 and CP-2 sediment slurry without negative controls and left to incubate at 5°C. All enrichments were set

up with a H_2:CO_2 headspace with the exception of the *Desulfobacter* vials, which had N_2:CO_2. Table 3.2 is a list of the media used for enrichment of S-reducing bacteria and a description of their contents.

Sulfide production was periodically evaluated using an SRI 8610C gas chromatograph equipped with a flame photometric detector (FPD) and 60M MXT-1 column (SRI Instruments, Torrance, CA, USA). PMT volts was set to 400 V, the oven and FPD temperatures were 150°C with a temperature program of sustained 60°C. Nitrogen was used as the carrier gas at 25 psi and the GC was calibrated using 100 ppm hydrogen sulfide standards (Gasco, Cairo, Egypt). In order to measure sulfide levels, vials were shaken for 2 minutes then 100 µl of headspace was injected into the GC.

Table 3.2. List of media used to enrich for SRBs and important ingredients.

Media	Carbon sources*	Electron donors	Electron acceptors	Reference
Dethiosulfovibrio I	Organic (Y.E.), CO_2	H_2, acetate, citrate	Elemental Sulfur	Surkov et al., 2001
Dethiosulfovibrio II	Organic (Y.E.), CO_2	H_2, citrate	Thiosulfate	DSMZ 906
Desulfohalobium	Organic (Y.E.), CO_2	H_2, acetate, lactate	Sulfate	DSMZ 499
Desulfuromonas	Organic, CO_2, HCO_3	H_2, acetate, fumarate	Sulfate	DSMZ 148
Desulfobacter	Organic, CO_2, HCO_3	Acetate	Sulfate	DSMZ 195
Desulfobulbus	Organic, CO_2, HCO_3	H_2, propionate	Sulfate	DSMZ 195b
Postgate B	Organic (Y.E.), CO_2	H_2, lactate, thioglycolate	Sulfate	Widdel and Bak, 1992
Knoblauch + lactate	Organic, CO_2, HCO_3	H_2, lactate	Sulfate	Knoblauch and Jorgensen, 1999

*Y.E. = Organic carbon sources include yeast extract

Chapter 4: Results and Discussion

4.1. Site description and chemical characterization of the GH springs microbial mat

Microbial mats of mixed red/orange/green colouring are adjacent to outflow channels from GH springs (Fig. 4.3). No vertical stratification of the mats was visible by macroscopic observation. The microbial mat sampled in this study was located at approximately 79°24'149"N, 90°44'203"W (± 3 m). It was wet throughout, but mostly uncovered by water. There was no observable flow of water through the mats to Expedition River. At GH springs, changes in outflow patterns have been observed that lead to variability in the wetness of the microbial mats from one year to the next. The mats are covered completely by snow in the winter.

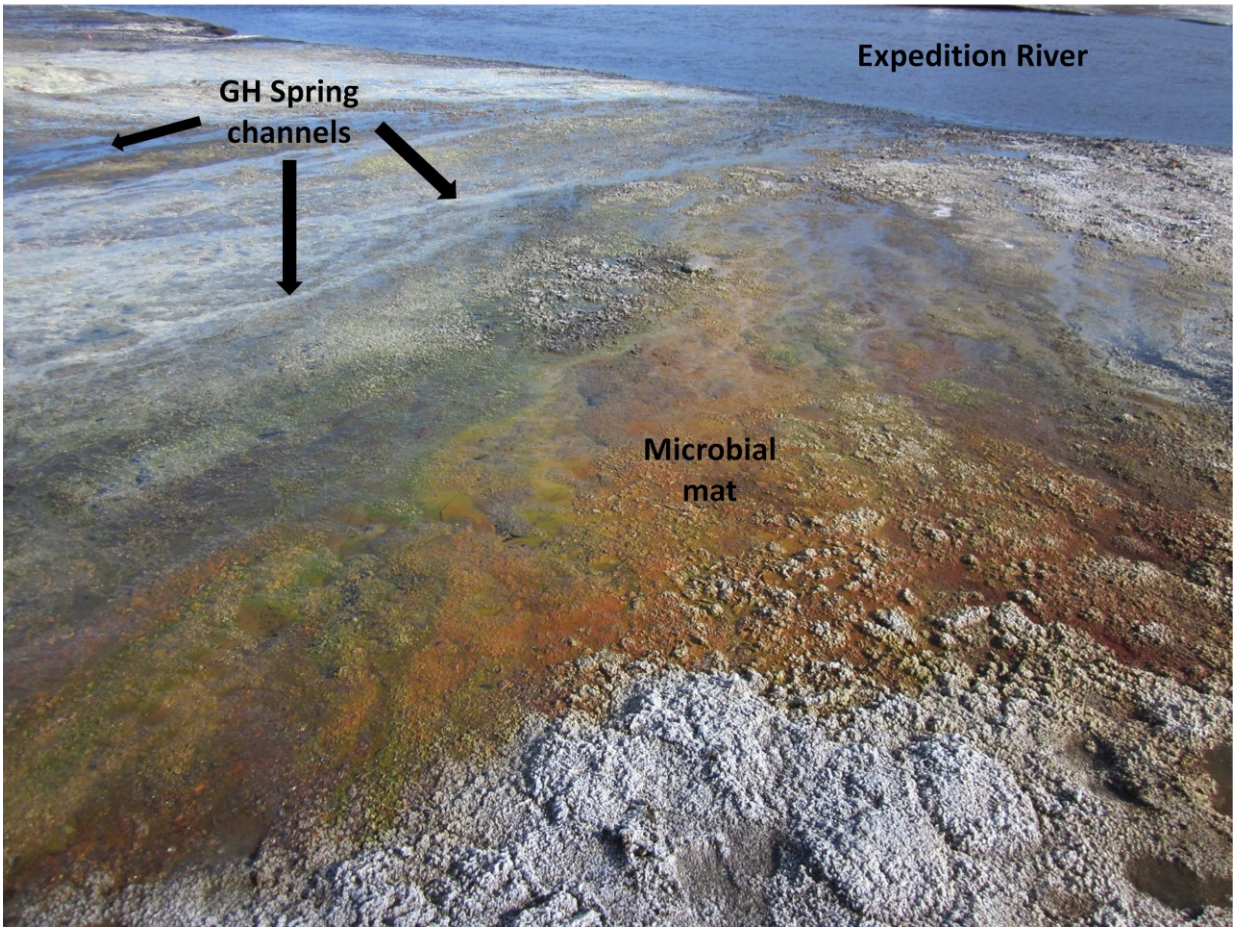

Fig. 4.3. Location of one of the GH microbial mats in relation to GH spring channels, which flow south towards Expedition River. GH-4 outlet, not shown, is east of the microbial mats.

Physicochemical parameters of two microbial mats found adjacent to GH spring outlets were measured in July, 2014 (Table 4.3). The differences between these two mats may be due to water level (the western mat was visibly drier than the eastern mat), location (separated by at least 50 meters), or a differing microbial community. Overall, it appears likely that the chemical properties of the microbial mats vary over time due to the changeable nature of the GH springs outflow channels. This, however, should be confirmed with additional readings in the future. It should be noted that the sample used for sequencing analysis was obtained from the western mat the year before these chemical measurements were taken, in 2013.

Table 4.3. Physicochemical parameters of two GH microbial mats in July, 2014 (unpublished data, Raymond-Bouchard).

	Western mat (this study) 79°24'149"N, 90°44'203"W	**Eastern mat**
Temperature	9.25°C	9.13°C
Salinity	5.65%	2.68%
Dissolved oxygen	2.94 ppm	0.14 ppm
Oxidation/Reduction Potential	-367.0	-363.8
pH	6.74	8.13
Soluble/ferrous iron	~3-4 ppm	1-2 ppm
Total iron	~3-4 ppm	>10 ppm
Sulfide	0-10 ppm	---
Thiosulfate	>50 ppm	---

4.2. Sequencing coverage and biodiversity indices for the GH spring microbial mat

4.2.1. Sequencing coverage and biodiversity indices

The nucleic acids were extracted from a composite sample of the first 5 cm of the GH spring microbial mat and the underlying sediment. rRNA genes and rRNA transcripts (henceforth referred to as DNA and cDNA, respectively) were sequenced and subjected to

analyses in order to determine the biodiversity of the microbial mat and the efficiency of the sequencing process. 16S rRNA gene OTUs were clustered at a 97% nucleotide identity level, commonly associated with the species level (Stackebrandt and Goebel, 1994). At this level, bacterial and archaeal rarefaction curves do not appear to approach a plateau as sequences are sampled (Fig. 4.4). Curves are especially steep in the case of the bacterial DNA and cDNA libraries, indicating that there may be a large number of bacterial OTUs that were not detected with the current sampling effort. The archaeal cDNA curve is also notably steep and short, due to it only consisting of 3 reads and 3 OTUs. Overall, the rarefaction curves give the impression that the 16S rRNA gene sequencing effort does not appear to have covered the full diversity of the GH spring microbial mats.

This assertion is supported by the Chao1 richness estimates calculated for these communities, which found the theoretical minimum number of OTUs significantly higher than the number of OTUs actually recorded (28%, 45%, and 16% more for bacterial DNA, bacterial cDNA, and archaeal DNA, respectively). However, it is important to note that collector's curves for the Chao1 estimate (Fig. 4.6) show that the index does not normalize as the number of sequences sampled approaches the total number of sequences. Specifically, the collector's curves were found to show an instantaneous change of $\geq 2.5\%$ over the last 300 sequences sampled, indicating possible inaccuracy according to the definition established by Schloss and Handelsman, 2005. The Good's coverage values range from 95.63% to 98.59% suggest that the proportion of uncaptured phylotypes is not as high as the Chao1 estimator would indicate (Table 4.4).

dsrB gene OTUs were clustered at a 90% nucleotide identity level, which has been found to roughly correspond to a 16S rRNA gene identity of 97% (Müller et al., 2014). Unlike the 16S rRNA rarefaction curves, the graphs for DNA and cDNA *dsrB* gene libraries appear to plateau (Fig. 4.4). However, they continue to increase with a linear slope (albeit a small one) after their initial flattening, indicating that some rare phylotypes may remain undetected. Good's coverage as calculated for the *dsrB* gene libraries showed that the vast majority of the total phylotypes had been sequenced (99.67% and 99.74% for DNA and cDNA, respectively). In contrast, the Chao1 indices estimate a minimum richness of at least 20 or 25 OTUs for DNA or cDNA *dsrB* gene libraries, while the number of observed OTUs is only 10 for both (Table 4.4). It should be noted

that pyrosequencing error can result in spurious singletons which could adversely affect the determination of true values for richness and diversity. Schloss et al. recommend performing a mock community analysis, wherein a defined group of DNA sequences are sequenced in parallel to environmental samples in order to assess sequencing errors (Schloss et al., 2011). In the present study, however, a mock community analysis was not performed, and thus the sequencing error rate is unknown and could not be accounted for.

Venn diagrams showinng the OTUs shared between DNA and cDNA libraries further provide evidence that not all OTUs present in the environment were accounted for by the pyrosequencing results. Theoretically, the DNA pyrosequencing results should include all the species present in the microbial mat while the cDNA results should account for the active portion of those species. However, it was found that 53% of all bacterial OTUs, and 37.5% of all *dsrB* OTUs were present in the cDNA but not in the DNA library (Fig. 4.5). When only the non-rare biosphere (herein defined as OTUs that consist of more than one or two sequences) is considered in order to minimize the impact of potential sequencing errors, 29% of non-rare bacterial OTUs are still found to be present in the cDNA but not in the DNA library (Fig. 4.5). However, in terms of abundance, a full 97% of sequences belong to OTUs which are present in both the DNA and cDNA. This gives creedence to the notion that most of the non-rare microbial species were, in fact, able to be sampled. The Venn diagram for non-rare *dsrB* gene OTUs shows that all common species are shared between DNA and cDNA, with the exception of one OTU that is found in the DNA but not in the cDNA library (Fig. 4.5B). Overall, it appears that the sulfate reducing community of the GH springs microbial mat is fairly well represented.

In general, due to the non-plateauing rarefaction curves, Chao1 values indicating the presence of unidentified OTUs, and discrepancies between species present in the DNA and cDNA results, it is likely that the GH microbial mat community was not completely sampled. The high Good's coverage estimator values and the large percentage of total sequences that are shared between DNA and cDNA libraries, however, indicate that the unsampled portion likely represents only less abundant OTUs present in the environment. For these reasons, the interpretation of phylogenetic information from the GH microbial mat pyrosequencing data should be constrained to the non-rare biosphere.

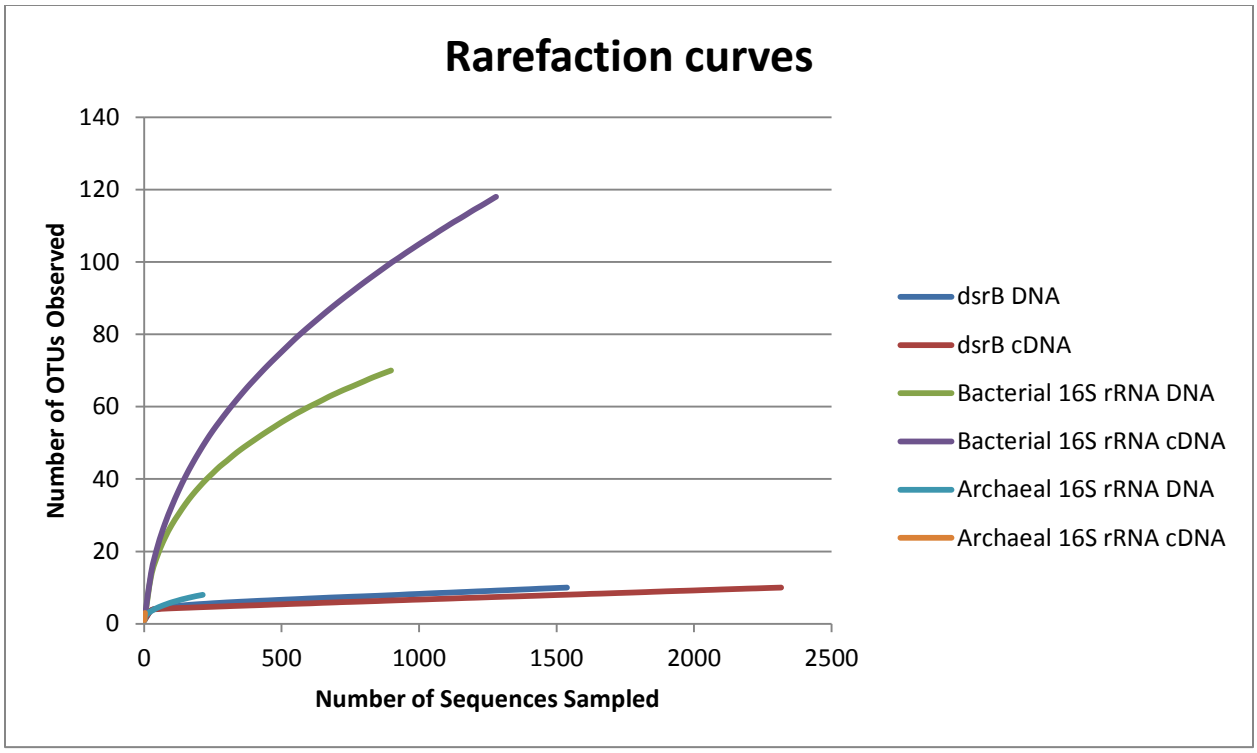

Fig. 4.4. Rarefaction curves for Bacterial and Archaeal 16S rRNA gene libraries at an OTU cutoff of 97% nucleotide identity and for the *dsrB* gene libraries at an OTU cutoff of 90% nucleotide identity.

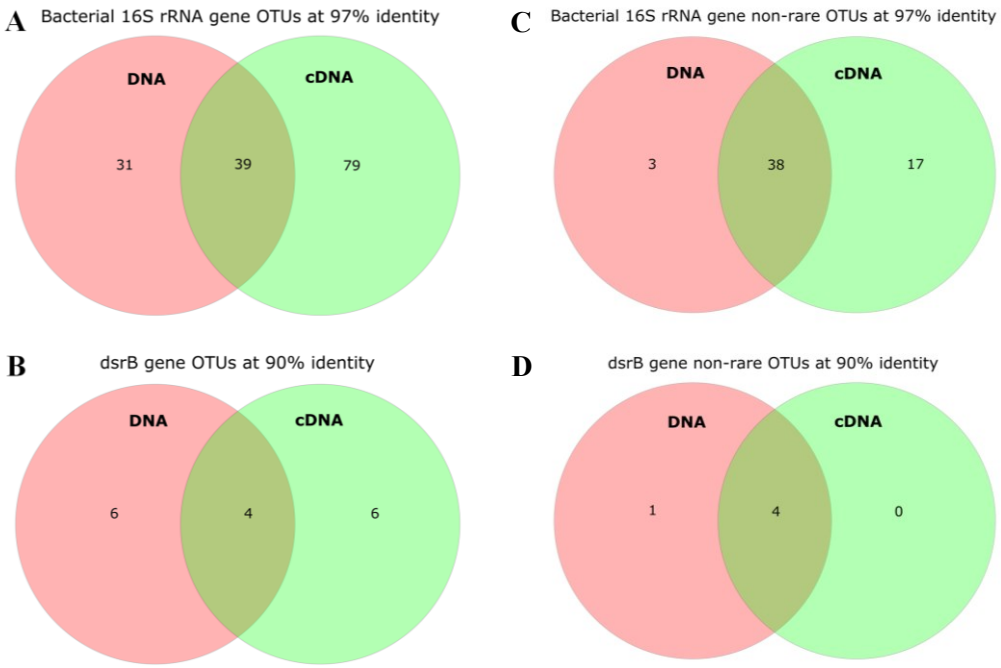

Fig. 4.5. Venn diagrams showing the number of OTUs shared between the DNA and cDNA pyrosequencing libraries of the A) bacterial 16S rRNA gene clustered at a 97% identity level, and the B) *dsrB* gene clustered at a 90% identity level. C) and D) show the number of shared non-rare OTUs (not singletons or doubletons) for the bacterial 16S rRNA gene and *dsrB* gene, respectively.

Table 4.4. Summary of the diversity indices for the Gypsum Hill microbial mat pyrosequencing libraries.

		# Sequences	# OTUs	Coverage (%)	Shannon (H')	Inverse Simpson (1/D)	Chao1	Evenness (E)	Sorensen similarity
Bacterial 16S rRNA gene	**DNA**	898	70	97.1	3.04	12.27	97.1*	0.71	0.55
	cDNA	1280	118	95.6	3.23	10.60	214.3*	0.68	
Archaeal 16S rRNA gene	**DNA**	213	8	98.6	0.90	1.91	9.5*	0.43	---
	cDNA	3	3	---	---	---	---	---	
***dsrB* gene**	**DNA**	1538	10	99.7	1.12	2.55	20*	0.49	0.58
	cDNA	2317	10	99.7	1.10	2.61	25	0.48	

*Diversity indices which show a collector's curve with at least 2.5% instantaneous change over the last 300 sequences sampled, indicating possible inaccuracy due to insufficient sampling, as discussed in Schloss et al. 2005. See Fig. 4.6 for collector's curves.

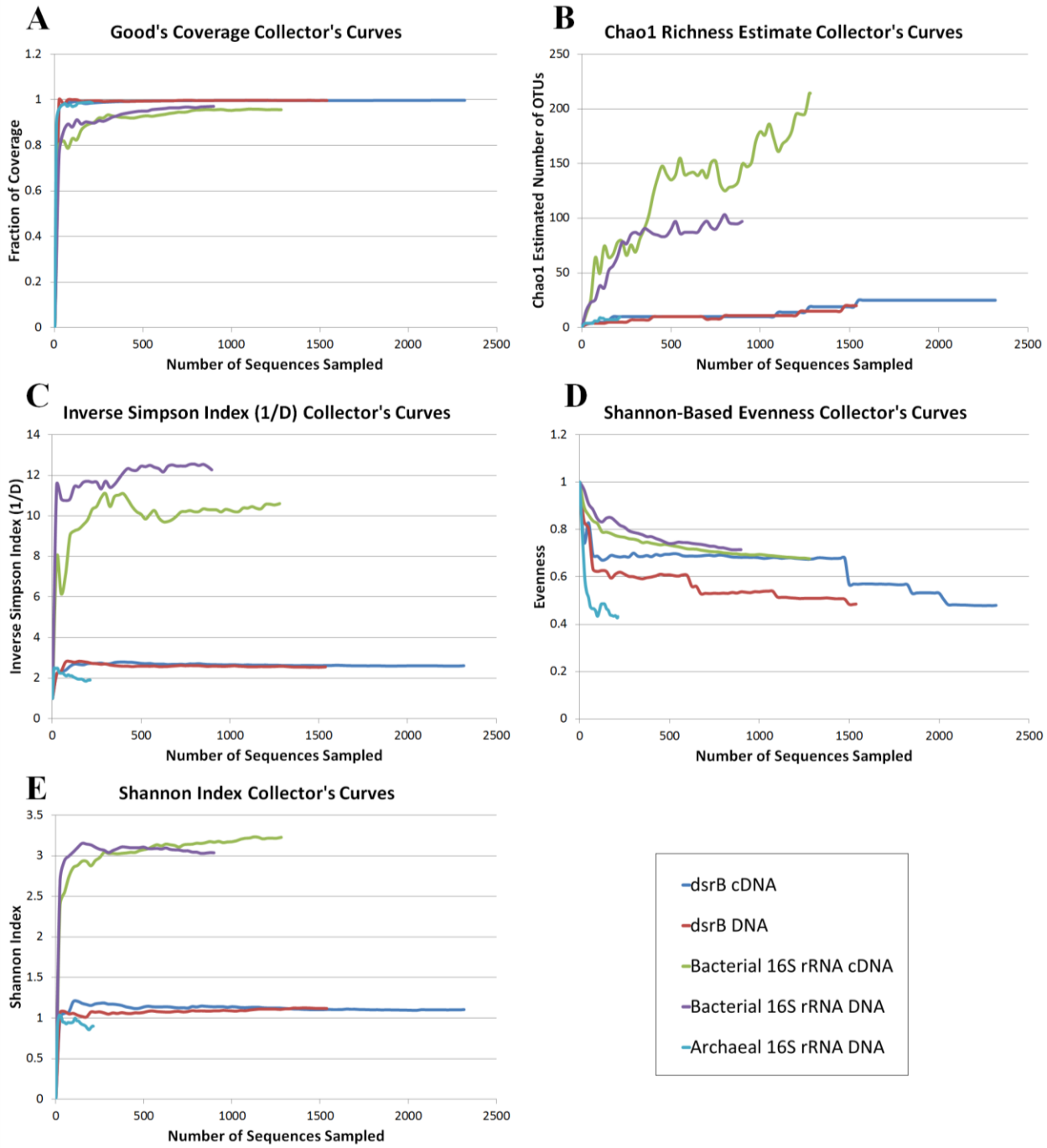

Fig. 4.6. Collector's curves for A) Good's coverage, B) the Chao1 richness estimate, C) the Inverse Simpson index, D) the Shannon-Based Evenness index, and the E) Shannon index, as described in Schloss and Handelsman, 2005. All collector's curves were generated by Mothur's diversity index calculator using the "collect.single" command.

4.2.2. Comparison of biodiversity indices to those for other environments

A comparison of the biodiversity indices measured in this study to those measured from other varied environments can be seen in Table 4.5 (*Bacteria*) and Table 4.6 (*Archaea*). The sequenced bacterial community of the GH microbial mat has generally greater evenness than other arctic environments (Table 4.5). The mat's bacterial diversity measures were similar to those for the GH spring source, but greater than those found for other Axel Heiberg saline sulfidic springs (CP-1 spring and LH spring). This is in accordance with general ecological principles, which state that biodiversity will be lower in more extreme conditions – a trend which can be observed when examining aquatic hypersaline and thermal ecosystems (Frontier, 1985; Bolhuis et al., 2014). The GH microbial mat is less saline (5.65% NaCl), with a more moderate temperature (9.25°C), and greater oxygen levels (2.94 ppm) than CP and LH springs, and thus would be expected to have higher diversity. Microbial mats in temperate climates generally have very high species richness and diversity (Bolhuis et al., 2014); due to their layered nature, microbial mats possess a large amount of biogeochemical heterogeneity that result in a large number of potential ecological niches. In addition, they may experience fluctuations in environmental conditions such as water level, which can further generate niches in a temporal manner as well. However, in this study we found the GH microbial mats' bacterial diversity to be lower than that of other Arctic microbial mats and Arctic soils (Table 4.5).

The archaeal diversity in the GH spring mat was very low (H' = 0.90 and 1/D = 1.91) in comparison to most other environments. Even the GH-4 spring source, which was found to share many of the same species, possessed greater archaeal diversity than the GH spring mat (Table 4.6). Though the diversity values reported here are particularly low, this result appears to be typical of most microbial mats. Recent studies indicate that, despite the extreme conditions in which microbial mats sometimes form, *Archaea* actually account for only a small part of microbial mat communities (S M Sievert et al., 2000; López-López et al., 2013). Diversity of *Archaea* also tends to be lower than that of *Bacteria*, with one study finding that overall archaeal diversity is approximately 2.1-fold lower than for the corresponding bacterial fraction (Schneider et al., 2013). Similarly, studies of other cold environments found their archaeal community to almost always have lower Shannon index values than the bacterial community (Table 4.5, Table 4.6).

Table 4.5. Summary of diversity indices for bacterial populations, from this study and others, based on sequencing of the 16S rRNA gene.

Microbial community		# Sequences	# OTUs	Coverage (%)	Shannon (H')	Inverse Simpson (1/D)	Chao1	Evenness (E)
GH Spring Mat	**DNA**	898	70	97.1	3.04	12.27	97.1*	0.71
	cDNA	1280	118	95.6	3.23	10.60	214.3*	0.68
GH-4 Spring Source[a]		155	46	84	3.17	14.82	71	0.52
CP-1 Spring Source[b]		174	30	91	2.16	4.25	50	0.33
LH Spring Source[c]		61	9	95.1	1.65	---	---	---
LH Spring Channel[d]		80-236	16-76	68-80	1.69-3.80	3-25	44-104	0.34-0.59
Mesophilic Sulfide Spring Source[e]		116	66	---	3.84	---	---	0.65
Mesophilic Sulfide Spring Mat[e]		96	40	---	2.95	---	---	0.81
Markham Arctic Ice Shelf Mat[f]		189	105	63.5	4.41	95.52	243	---
Ward Arctic Ice Shelf Mat[f]		128	52	76.6	3.50	25.52	106	---
Mediterranean sea cold seep mat[g]		44-46	---	---	2.83-3.45	---	---	---
High Arctic active layer[h]		139	86	38	4.14	54.5	246	---
High Arctic permafrost[h]		134	75	44	4.00	51.5	205	---

References: this study[a]; Perreault et al., 2007[b]; Niederberger et al., 2010[c]; Lay et al., 2012[d]; Elshahed et al., 2003[e]; Bottos et al., 2008[f]; Heijs et al., 2005[g]; Wilhelm et al., 2011[h].

Table 4.6. Summary of diversity indices for archaeal populations, from this study and others, based on sequencing of the 16S rRNA gene.

Microbial community		# Sequences	# OTUs	Coverage (%)	Shannon (H')	Inverse Simpson (1/D)	Chao1	Evenness (E)
GH Spring Mat	**DNA**	213	8	98.6	0.90	1.91	9.5*	0.43
	cDNA	3	3	---	---	---	---	---
GH-4 Spring Source[a]		156	18	96	2.12	5.94	23	0.47
CP-1 Spring Source[b]		164	29	92	2.77	12.18	68	0.61
LH Spring Source[c]		66	7	98.5	1.39	---	---	---
LH Spring Channel[d]		24-80	3-6	88-95	0.78-1.37	2-4	3-6	0.36-0.98
Mediterranean sea cold seep mat[e]		14-31	---	---	1.03-1.24	---	---	---
High Arctic active layer[f]		59	12	80	1.82	2.24	17	---
High Arctic permafrost[f]		134	75	90	1.54	3.49	13	---

References: this study[a]; Perreault et al., 2007[b]; Niederberger et al., 2010[c]; Lay et al., 2012[d]; Heijs et al., 2005[e]; Wilhelm et al., 2011[f].

4.3. Classification and relative abundance of gene sequences

4.3.1. Classification and relative abundance of bacterial 16S rRNA gene sequences

Fig. 4.7 shows the presumed total (DNA) and active (cDNA) bacterial community profiles of the GH microbial mat at the class level. *Proteobacteria* make up the majority of both the DNA (62%) and cDNA libraries (82%), with *Gammaproteobacteria* composing a full 50% and 64% of the DNA and cDNA libraries, respectively. Other major classes contributing to the GH microbial mat communities (DNA and cDNA) include *Actinobacteria* (15%, 0.5%), *Flavobacteriia* (15%, 6.1%), *Deltaproteobacteria* (6.2%, 11%), and *Epsilonproteobacteria* (4.0%, 4.8%). Interestingly, *Cyanobacteria* comprise only 1.1% and 2.8% of the DNA and cDNA sequences.

Fig. 4.8A shows the relative abundance of sequences classified as *Deltaproteobacteria* by Mothur with the Silva v119 database. The major genera identified are *Desulforhopalus* (8.9% of total *Deltaproteobacteria* from the DNA library, 34% from the cDNA), *Desulfuromusa* (64%, 6.1%), and *Desulfuromonas* (3.6%, 21%), as well as two OTUs of unclassified *Desulfobacteraceae* (14%, 16% for one OTU; 0%, 8.8% for the other). Not included in Fig. 4.8A are taxa which make up ≤ 1% of the total number of reads. These genera include *Desulfocapsa* (0%, 1.4%), *Desulfopila* (0%, 0.7%), and *Desulfotalea* (0%, 1.4%). Other minor components were unable to be identified past the family level, including *Desulfobulbaceae* (0%, 4.8%), *Desulfuromonadaceae* (1.8%, 0%), and bacteria of the proposed group GR-WP33-58 (7.1%, 4.8%).

Epsilonproteobacteria are made up entirely of sequences classified as *Sulfurimonas* and *Sulfurospirillum* (Fig. 4.8B). OTUs are more evenly distributed than in other classes, with *Sulfurospirillum* sequences divided into two non-rare OTUs (i.e. those with >2 reads), and *Sulfurimonas* into four OTUs. Two of the *Sulfurimonas* OTUs are present in the cDNA, but not the DNA, indicating that these species may be particularly active in the microbial mat.

The *Gammaproteobacteria* make up most of the GH microbial mat sequences, and correspondingly contain the largest number of OTUs (Fig. 4.8C). Major components are *Thiomicrospira* (36% of DNA, 41% of cDNA), unclassified *Piscirickettsiaceae* (9.4%, 15%), *Halothiobacillus* (13%, 2.1%), *Marinomonas* (3.1%, 2.5%), and *Saccharospirillum* (0.9%,

2.5%), each of which are made up of only 1 non-rare OTU. *Rhodanobacter* (22%, 7.7%), and *Marinobacter* (16%, 26%) are more heterogeneous, consisting of two and four non-rare OTUs, respectively. Minor components not included in Fig. 4.8C include: unclassified *Gammaproteobacteria* (0%, 0.9%), *Salinisphaera* (0%, 0.5%), *Hydrogenovibrio* (0%, 0.4%), unclassified *Thiotrichales* (0%, 0.4%), *Glaciecola* (0%, 0.3%), proposed Marine Methylotrophic Group (0%, 0.1%), unclassified *Xanthomonadaceae* (0%, 0.1%), and unclassified *Oceanospirillales* (0%, 0.1%).

Other bacterial taxa of interest not shown in the below figures include *Demequina* and *Rhodoglobus*, which make up most of the *Actinobacteria* (87% DNA, 100% cDNA; and 12% DNA, respectively). The *Flavobacteriia* are mainly made up of *Psychroflexus* (86%, 64%), *Cryomorpha* (0.7%, 5.1%), *Owenweeksia* (2.9%, 6.4%), *Gillisia* (8.8%, 1.3%), and unclassified *Flavobacteraceae* (0%, 22%). All the *Spirochaetes* were classified to the genus *Spirochaeta*. Other potential sulfur-metabolizing microbes present in small abundances include: *Sulfitobacter* (an alphaproteobacterium), *Dethiosulfatibacter*, and *Fusibacter* (genera within the class *Clostridia*).

Most MegaBLAST results of representative sequences from major OTUs correlate well to the Mothur classifications (Table 4.7). OTUs which may have been misclassified by Mothur include the *Demenquina* OTU, which shares 99% of its sequence with a *Cellulomonas* strain, and the *Rhodanobacter* OTU, which when MegaBLASTed was found to be 99% identical to a species of the genus *Frateuria*. Other OTUs which were classified with MegaBLAST include two OTUs classified by Mothur as *Cyanobacteria* at the phylum level, and "Chloroplasts" at the class level. The first OTU, which consists of 25 sequences in the cDNA library, was classified as *Oophila amblystomatis* with 97% identity. The second OTU, which consists of 10 sequences in both the DNA and cDNA libraries, was found to share 99% nucleotide similarity with the chloroplast of *Durinskia baltica*. Organisms of the genera *Oophila* and *Durinskia* are actually eukaryotes, not bacteria, indicating that the primers may have amplified some non-bacterial species. *Oophila* are single-celled algae of the division *Chlorophyta* that grow in symbiosis with the eggs of the spotted salamander. *Durinskia* are photosynthetic dinoflagellates which may contain endosymbiotic diatoms and are usually found in marine environments (Pienaar et al., 2007).

Notably, of the nine most abundant OTUs, five were classified with 99-100% identity as belonging to species that had been previously isolated from the nearby GH springs (Table 4.7). Other isolation locations for close relatives to the major OTUs are mainly cold and/or saline environments (ex. haloalkaline soil, glacial fjord, and ocean sediments). MegaBLAST uncultured hits similarly show mostly salty (saltern pond, marine sources) or cold (permafrost) sources.

The most abundant deltaproteobacterial OTUs were also classified using MegaBLAST to examine the S-reducing community with greater focus (Table 4.8). Those OTUs which were able to be classified by Mothur to the genus level were found to closely relate to species of the same genera using MegaBLAST. Both the OTUs identified as unclassified *Desulfobacteraceae* by Mothur were found to share the most similarity to a *Desulfatiferula* strain isolated from estuary sediment, though only with a below-species-level of identity (94% and 93%). These OTUs, when used to construct a phylogenetic tree, cluster more closely with each other than with *Desulfatiferula* species, within the *Desulfobacteraceae* family (Fig. 4.11). The unclassified *Desulfobulbaceae* sequences relate most closely to the species [*Desulfobacterium*] *catecholicum* (97% identity). It is important to note that this species was named before it was found to belong to the *Desulfobulbaceae* family, not the *Desulfobacteraceae*. It has been proposed that this "*Desulfobacterium*" species should in fact be renamed as a novel genus within the *Desulfobulbaecae* family (Kuever et al., 2005). As expected, this OTU clusters within the *Desulfobulbaceae* in the *Deltaproteobacteria* phylogenetic tree. The deltaproteobacterial representative sequence with the lowest similarity to any isolated representative was identified by Mothur to belong to the proposed GR-WP33-58 group, and by MegaBLAST to be 89% similar to *Pelobacter venetianus*. The Silva database includes GR-WP33-58 and *Pelobacter* as parts of the *Desulfuromonadales* order; however, the paper proposing the group considers it a novel order itself, one that clusters between *Desulfuromonadales* and *Myxococcales* (Moreira et al., 2006). Though the bootstrap values are low, this is what the corresponding phylogenetic tree shows for this OTU – it does not cluster within any of the known deltaproteobacterial orders, but between the *Desulfuromonadales* and *Myxococcales* (Fig. 4.11). This OTU is therefore likely to belong to this proposed enigmatic group, called by Moreira et al. the myxobacteria-related group (MRG). Unsurprisingly, most close MegaBLAST hits to deltaproteobacterial OTUs show a marine sediment source (Table 4.8). Other sources include an oil-polluted microbial mat, and a sulfur-reducing/acetate-oxidizing enrichment culture from a hydrothermal vent microbial mat.

Two OTUs show 100% identity with uncultured clone sequences – one of these had been previously isolated from GH-4 spring outlet, and the other is from the abovementioned enrichment culture.

Overall, 42% of DNA and 63% of cDNA bacterial 16S rRNA gene libraries were classified by Mothur as taxa capable of the oxidation of sulfur compounds. A further 7.3% of DNA sequences and 14% of cDNA sequences were classified as genera capable of sulfate or sulfur reduction. In general, it appears that sulfur metabolism is an important energy source for the GH microbial mat bacteria, especially the active community.

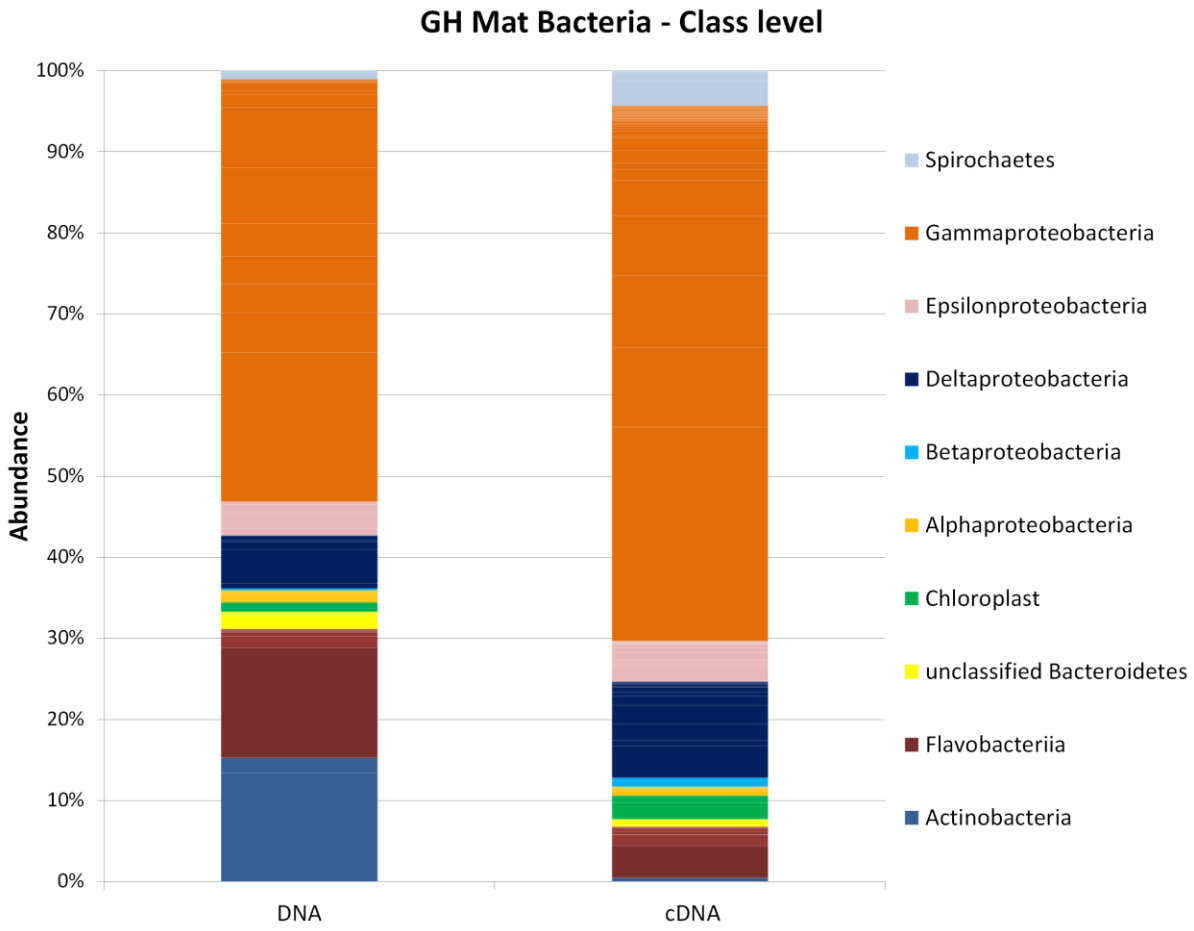

Fig. 4.7. Relative abundance of bacterial 16S rRNA sequences in the Gypsum Hill microbial mat pyrosequencing library. Phyla, as classified by Mothur against the Silva v119 reference database, are indicated based on colour. OTU divisions within phyla are indicated by the faint lines bisecting the bars. Only phyla which consist of >1% of the total number of reads are shown.

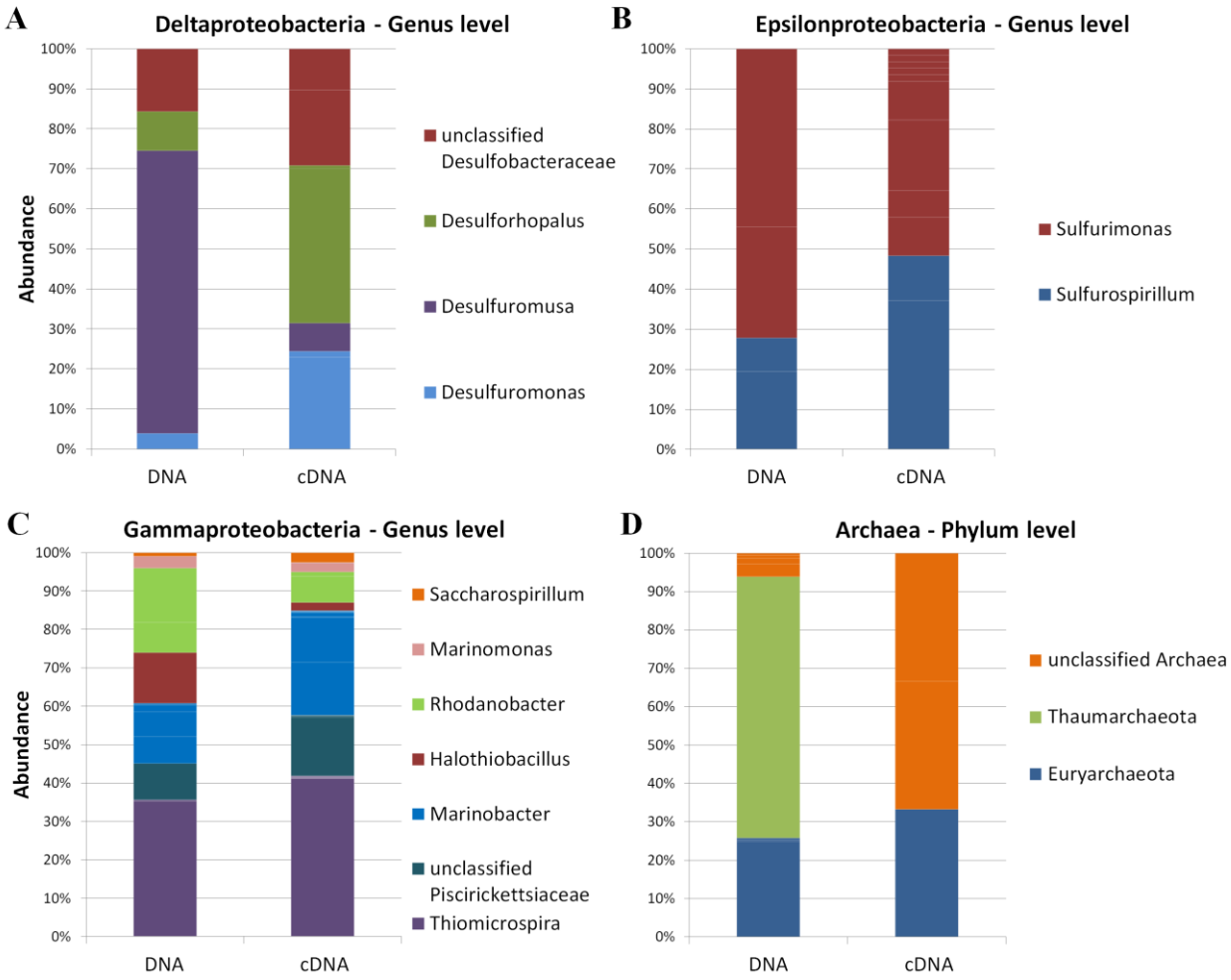

Fig. 4.8. Relative abundance of A) Deltaproteobacterial, B) Epsilonproteobacterial, C) Gammaproteobacterial, and D) Archaeal 16S rRNA sequences in the Gypsum Hill microbial mat pyrosequencing libraries. Phyla, as classified by Mothur against the Silva v119 reference database, are indicated based on colour. OTU divisions within phyla are indicated by the faint lines bisecting the bars. Only phyla which consist of >1% of the total number of reads are shown.

Table 4.7. Taxonomic information of representative sequences from the most abundant OTUs of the bacterial 16S rRNA gene libraries.

# (DNA)	# (cDNA)	Mothur classification	MegaBLAST best hits*	Accession #	E-value	Identity	Isolation source of best hits
157	326	*Thiomicrospira*	*Thiomicrospira* sp. NP51	EU196304.1	5e-180	100%	GH-4 spring sediment
			Uncultured *Thiomicrospira*	EU430112.1	6e-180	100%	GH-2 filament
116	49	*Psychroflexus*	*Psychroflexus sediminis* strain YIM C238	NR_044410.1	4e-166	98%	Haloalkaline soil
			Clone IC21-C1758	KJ588890.1	4e-171	99%	Saltern pond
42	121	Unclassified *Piscirickettsiaceae*	*Thiomicrospira* sp. Tms-MPN/6-8 mm depth	AJ011069.1	6e-150	95%	Coastal sea sediments
			Clone S3-41	EF491422.2	3e-178	99%	Marine steel structure
31	109	*Marinobacter*	*Marinobacter* sp. NP40	EU196314.1	7e-179	100%	GH-4 spring sediment
			Clone C139300058	JX528447.1	4e-172	99%	Marine water
115	6	*Demequina*	*Cellulomonas* sp. NP4	EU196315.1	5e-165	99%	GH-4 spring sediment
			Clone BJGMM-1s-338	JQ800802.1	1e-166	99%	River delta soil
29	92	*Marinobacter*	*Marinobacter* sp. KJF8-10	JQ800125.1	7e-179	100%	Subarctic glacial fjord
			Clone sz-269	JQ279010.1	3e-177	99%	Groundwater
35	54	*Rhodanobacter*	*Frateuria* sp. WJ64	AY495957.1	7e-174	99%	Mine drainage wetland
			Clone oNE05	EF034245.1	7e-179	100%	Permafrost soil
59	17	*Halothiobacillus*	*Halothiobacillus* sp. NP37	EU196318.1	7e-179	100%	GH-4 spring sediment
			Clone oE02	EF034178.1	7e-179	100%	Permafrost soil
63	9	*Rhodanobacter*	*Rhodanobacter* sp. NP47	EU196307.1	7e-179	100%	GH-4 spring sediment
			Clone SBS-RV-086	HQ326340.1	7e-179	100%	SWRO membrane biofilm

*First line: isolated organism. Second line: uncultured organism.

Table 4.8. Taxonomic information of representative sequences from OTUs from the 16S rRNA gene libraries classified as *Deltaproteobacteria*.

# (DNA)	# (cDNA)	Mothur classification	MegaBLAST best hits*	Accession #	E-value	Identity	Isolation source of best hits
5	49	*Desulforhopalus*	*Desulforhopalus singaporensis* strain S'pore T1	NR_028742.1	2e-145	96%	Marine mud
			Proteobacterium FM1C-A9	LM656153.1	1e-171	98%	Oil-polluted microbial mat
36	9	*Desulfuromusa*	*Desulfuromusa kysingii* strain Kysw2	NR_029275.1	3e-157	97%	Anoxic marine mud
			Bacterium	EU430107.1	7e-174	100%	GH-4 spring sediment
8	24	Unclassified *Desulfobacteraceae*	*Desulfatiferula* sp. BE2801	HE613444.1	2e-139	94%	Estuary sediment
			Clone Ca1-C31	FJ875169.1	5e-166	96%	Marine sediment
2	29	*Desulfuromonas*	*Desulfuromonas svalbardensis* strain 103	AY835391.1	2e-179	98%	Arctic marine sediments
			Clone GAE_28	KF623783.1	0.0	100%	Enrichment from hydrothermal mat**
0	13	Unclassified *Desulfobacteraceae*	*Desulfatiferula* sp. BE2801	HE613444.1	2e-134	93%	Estuary sediment
			Clone H00065	JX391105.1	8e-164	96%	Marine sediment
4	7	Unclassified GR-WP33-58	*Pelobacter venetianus* strain Gra PEG 1	NR_044779.1	1e-91	89%	Anoxic channel mud
			Proteobacterium FA350	KM034744.1	1e-162	98%	Marine sediment
0	7	Unclassified *Desulfobulbaceae*	[*Desulfobacterium*] *catecholicum* strain NZva20	NR_028895.1	7e-164	97%	Anoxic bay mud
			Bacterium YS-UMF5_120	DQ901617.1	1e-176	99%	Intertidal sediment

*First line: isolated organism. Second line: uncultured organism.

**Sulfur-reducing, acetate-oxidizing enrichment.

4.3.2. Analysis of bacterial 16S rRNA gene sequences

Culture-independent methodologies provide a way to examine an environment's microbial diversity without the necessity of culturing each organism. Nucleic acids can be extracted from environmental samples and either sequenced wholesale (metagenomics/transcriptomics) or used to sequence a chosen gene through amplification by the polymerase chain reaction (PCR). There are, however, drawbacks to culture-independent methods of study. Primarily, if the nucleic acid extraction is not effective, rare members of the community may not be represented in the final data. As well, results may be skewed due to biases in primers used to amplify the gene of interest. If primers do not bind with equal efficiency to the DNA of all taxa, particular species will appear to be more or less represented than is true, or may be missing entirely. This especially is problematic when investigating largely unstudied organisms, as primers can only be developed to target genetic sequences of which we have prior knowledge. The problem is further exacerbated when attempting to target non-16S rRNA genes; functional genes have been less researched and thus have less comprehensive databases of sequences.

Primer efficacy can be assayed using tools such as RDP's ProbeMatch, which finds sequences in the RDP database that match given primer pairs (Cole et al., 2014). When the bacterial 16S rRNA gene primers are analyzed using this tool, looking only at good quality sequences covering the target region, their limitations can be identified and analyzed. Allowing a primer difference of one nucleotide, 70% of the database's bacterial sequences were able to be targeted by the 27F/519R primer pair. All taxa show representation to the order level. Generally, the coverage of the bacterial primers is good, as they target a wide array of bacteria with similar efficacy.

Microbial mats are typically dominated by a few functional groups of microbes: photosynthetic cyanobacteria or algae, colourless sulfur bacteria, purple sulfur bacteria, sulfate reducing bacteria, and aerobic heterotrophs (van Gemerden, 1993). The overall composition of the GH microbial mat (primarily *Proteobacteria*, with major contributions from *Bacteroidetes* and *Actinobacteria*) is typical of many microbial mats, though they will almost always also possess a considerable *Cyanobacteria* and *Chromatiales* (purple sulfur bacteria) component (Bolhuis et al., 2014), which the GH mat is missing. While the GH microbial mat shares many

traits with other, non-polar microbial mats, it may be driven by chemolithoautotrophic S-oxidizers of the colourless sulfur bacteria instead of photoautotrophic cyanobacteria as the primary producer of this ecosystem. By abundance, the most important clade within the GH microbial mat is undoubtedly *Thiomicrospira*, which accounts for 18% of the DNA and 26% of the cDNA libraries. If the unclassified *Piscirickettsiaceae* is included in that number, it increases this S-oxidizing chemolithoautotrophic component to a whole 22%/36% of the DNA/cDNA dataset. In contrast, the Cyanobacterial OTUs were found to account for only 1.1% and 2.8% of the DNA and cDNA sequences.

It should be noted that the mat's green colour indicates a more important role for chlorophyll-containing photoautotrophs than is represented in the pyrosequencing data. As well, *Cyanobacteria* are a major component of both temperate and polar microbial mats, including those present in ice-covered lakes, Arctic rivers, and ice shelf ponds (Vincent, 2007). One possible explanation for the small cyanobacterial component to the sequencing library is due to inefficient extraction of their DNA and RNA. The EPS which coats filamentous *Cyanobacteria* can cause less efficient lysis of cyanobacterial cells than others in the same community (Bolhuis et al., 2014). These bacterial may require a combination of lysis techniques to properly sample, including chemical, enzymatic, and mechanical approaches. Though an effort was made to combine several of these techniques (a liquid nitrogen homogenization step, mechanical bead-beating, and using a chemical lysis solution), it is possible that *Cyanobacteria* nucleic acids were extracted less efficiently than those of other bacteria. Sequencing of filamentous *Cyanobacteria* is further complicated by the fact that even after lysis nucleic acids can become trapped and inaccessible within the EPS. Another potential explanation for the low *Cyanobacteria* counts is that the green colour of the mat is due to the presence of eukaryotic algae which would not be amplified efficiently by the bacterial 16S rRNA primers. This explanation is supported by the fact that the two OTUs classified by Mothur as *Cyanobacteria* were found by MegaBLAST to actually be eukaryotes (an algae and a dinoflagellate) with high levels of identity (97% and 99%). This aligns with a microscopic investigation by Rogers et al. in 2010, which found the presence of autofluorescing algae and diatoms downstream of GH-4 spring. Alternatively, the nature of the sampling (taking the top 5 cm of mat and underlying soil) may have led to only a thin layer of *Cyanobacteria* being present, resulting in the low amounts recorded here.

Interestingly, besides its low abundance of *Cyanobacteria*, the GH spring microbial mat also differs from other mats in its lack of purple sulfur bacteria, another photoautotrophic group. These anaerobic/microaerophilic bacteria use light for energy like *Cyanobacteria*, but oxidize sulfide and other reduced sulfur species instead of water. All purple sulfur bacteria are found within the order *Chromatiales*. The only GH spring mat sequences found within this order belong to the genus *Halothiobacillus*, which while genetically similar to the purple sulfur bacteria, are not known to perform phototrophy. The only other potential phototrophs present in the GH spring microbial mat data are 6 sequences in the DNA and 10 sequences in the cDNA that belong to the family *Rhodobacteraceae*, part of the purple non-sulfur bacteria. However, this family consists entirely of heterotrophs, and hence could not contribute to primary production within the mat ecosystem. These results are in line with what was found by Perreault et al., who found in their gene survey of CP and GH spring sediment only a single phototrophic clone, which belonged to a *Cyanobacteria* (Perreault et al., 2007).

While photoautotrophs are an important component of microbial mat communities due to their usual role as primary producers, it is not unprecedented for sequencing analyses of microbial mats to return low amounts or the absence of photoautotrophic species. For example, a clone library study of Antarctic ice shelves found no *Cyanobacteria* or *Chromatiales* sequences at all (Bottos et al., 2008). However, a review article examining several metagenomics studies found that, on average, 10-20% of a microbial mat will be made up of *Cyanobacteria* (Bolhuis et al., 2014). Microbial mats lacking *Cyanobacteria* are more commonly found in deep sea environments that lack the sunlight necessary for phototrophy to occur, such as at cold seeps and hydrothermal vents. In these environments, chemolithoautotrophs are the primary producers which drive the development of the microbial mat (Grünke et al., 2011). Due to its high arctic location, the GH spring microbial mats are only exposed to direct sunlight during the summer months when the snowfall covering them melts. For this reason, it is not unreasonable to expect that chemoautotrophic growth, which could occur year-round, may be an important source of organic carbon for the GH microbial mat system, in addition to photoautotrophy.

It is interesting to consider the orange/red component of the GH spring microbial mat's colouring. Such colouring may be indicative of the presence of iron oxides on the surface of the mat which may be formed abiotically, by iron oxidizing microorganisms, or a combination of the

two processes. Soluble ferrous iron is present in the spring water saturating the microbial mats in high enough concentrations (3 to 4 ppm) to match those of iron seeps that sustain iron oxidizing microbial populations (0.6 to 11 ppm) (Emerson et al., 2010). Two low-abundance OTUs have been identified from the GH spring microbial mat as potential iron oxidizing bacteria (unclassified *Rhodobacteraceae* and *Gallionella*). *Gallionella* is a genus containing only one defined species, which is capable of iron oxidation, while *Rhodobacteraceae* is a highly diverse family, of which only a few species show evidence of iron oxidation. Often, iron oxidizing bacteria may be able to use sulfur compounds such as thiosulfate, sulfide, and elemental sulfur as alternative electron donors (Hedrich et al., 2011). Soluble iron in the water may also be oxidized abiotically as it is exposed to oxygen on the surface of the microbial mat, where it then settles as a red precipitate. Oxygen levels of the water saturating the GH microbial mat were measured to be 2.94 ppm (53 μM) – above the concentration at which abiotic iron oxidation dominates and biotic iron oxidation becomes undetectable (Druschel et al., 2008). Iron oxide precipitates have been known to contribute to the formation of microbial mats, often in conjunction with sulfur metabolizing microbes (Emerson et al., 2010). Another possible source of the microbial mats' orange/red colouring is microbial pigmentation. Dinoflagellates of the genus *Durinskia* are known to form characteristic red-orange blooms in tidal pools (Pienaar et al., 2007); however, *Durinskia* were only detected as a small proportion of the sequencing data and were all classified as *Durinskia baltica*, a species which has not yet been observed to form these blooms. Diatoms and green algae were microscopically observed in the GH-4 spring channel; however, these had green, not red, colouring (Rogers et al., 2010). Orange or white colouring in microbial mats is often caused by the presence of *Beggiatoa* species, but these were not detected in the GH microbial mat 16S rRNA sequence libraries. Thus, precipitated iron oxide is a more likely source of the GH microbial mats' orange/red colouring than the presence of pigmented microbes.

Overall, chemolithotrophic sulfur metabolism appears to be the major source of energy for the GH microbial mat, while other types of chemolithotrophy (ex. energy from inorganic carbon or nitrogen compounds) are less important. GH spring and the downstream mats are rich in sulfur compounds such as sulfide, thiosulfate, and sulfate, so it's logical that forms of metabolism which utilize these compounds would dominate the communities here. Though the GH spring discharges gas containing N_2 (>98%) and CH_4, no bacteria were detected in either the

GH-4 spring source or at the GH microbial mat which could utilize these compounds (i.e. nitrogen fixing or methanotrophic bacteria) (Perreault et al., 2007, 2008).

Many different species contribute toward sulfur cycling within this system. The most abundant chemolithoautotrophic putative sulfur oxidizers present in the GH mat include *Thiomicrospira*, *Halothiobacillus*, and *Sulfurimonas*. Chemoheterotrophic putative sulfur oxidizing bacteria include *Marinobacter*, and *Spirochaeta*. Putative SRBs are almost completely *Deltaproteobacteria* and *Epsilonproteobacteria*; clades of sulfate reducing bacteria include unclassified *Desulfobacteraceae*, unclassified *Desulfobulbaceae*, and, within that family, *Desulforhopalus*. As well, several taxa that perform elemental sulfur reduction were detected, including *Desulfuromusa*, *Desulfuromonas*, and *Sulfurospirillum*. Overall, 42% of DNA and 63% of cDNA bacterial 16S rRNA gene libraries were found to consist of putative sulfur oxidizing bacteria, and a further 7.3% of DNA sequences and 14% of cDNA sequences were classified as putative SRBs. The majority of the species found within the GH microbial mat have been previously found in cold and/or saline environments such as marine sediments, hydrothermal vents, and hypersaline ponds/springs – a trend which can also be seen with close uncultured MegaBLAST hits (Table 4.7).

4.3.3. Comparison of the 2014 GH microbial mat archaea with GH spring outlet and GH filament community profiles

Only abundant OTUs from the current study show a close level of identity with species previously found in GH spring sediments. Other than the five OTUs described in Table 4.7 and the *Desulfuromusa* OTU in Table 4.8, all of which were found to be 99-100% identical to GH-4 spring sediment isolates, only one OTU of more than 1 or 2 sequences was most related to a sequence previously taken from the GH springs. Specifically, this small *Bacteroidetes* OTU (7 sequences) was identified by MegaBLAST as being closely related to a clone library sequence from a GH-2 filament (EU430108.1 with 99% identity). No other non-rare OTUs show their top BLAST hits to be to GH spring sequences. The species which are closely related to GH spring sediment samples include: three putative S-oxidizers (*Halothiobacillus*, *Thiomicrospira*, *Marinobacter*) and one sulfur-reducer (*Desulfuromusa*). *Halothiobacillus* and *Thiomicrospira*

are chemolithoautotrophs, while *Desulfuromusa* and the remaining related OTUs are heterotrophs (*Marinobacter*, *Demequina/Cellulomonas*, *Rhodanobacter*, unclassified *Bacteroidetes*). By abundance, 43% of the bacterial GH microbial mat community has very close genetic similarity to microbes previously described elsewhere in the GH spring system (either GH-4 sediment or GH-2 filaments) (Perreault et al., 2007; Niederberger et al., 2009). It should be noted that this earlier work was done using clone library techniques, not the higher-coverage pyrosequencing techniques used in the present study. As well, different bacterial primers were used for amplification of the sequences, which could lead to differing amplification of particular taxa within each sample. For these reasons, caution should be exercised when directly comparing the results obtained in this thesis to earlier work in the GH spring system. Overall, however, it appears that a large portion of the GH microbial mat community may have originated from the GH spring sediment and/or the S-oxidizing filaments present in the GH spring channels. This seems especially to be the case with the most predominant species within the mat.

Other than the aforementioned OTUs, GH-4 spring sediment S-oxidizers were composed of *Thiobacillus*, *Sulfurimonas*, and *Spirochaeta* (Perreault et al., 2007). Bacteria of the *Sulfurimonas* and *Spirochaeta* genera were also found in the GH microbial mat in this study. Sulfur- and sulfate-reducers from the GH spring sediments include *Desulfobulbus*, *Desulfobacula,* and *Desulfuromonas* as well as the previously mentioned *Desulfuromusa* OTU. Of these, the latter two were also found, to the genus level, in the GH microbial mat data. Major contributors to both the GH spring sediment and the GH microbial mat sequencing libraries were *Bacteroidetes* and *Firmicutes*, though different genera make up each library.

There appear to be functional similarities, as well, between the GH microbial mat and GH-4 spring sediment (Perreault et al., 2007). Both environments possess a large population of S-metabolizing microbes, suggesting that the cycling of sulfur compounds plays an important role in the maintenance of these microbial communities. Perreault et al. found that 30% of the GH spring sediment clone library belonged to putative S-oxidizers, and 26% were putative S-reducers. The GH microbial mats appear to have a greater focus on S-oxidation, with 42% of DNA and 63% of cDNA sequences assigned to taxa capable of S-oxidation, and only 7.3% of DNA and 14% of cDNA sequences as putative SRBs. Considering that oxygen levels are higher in the mats (2.94 ppm) than at the spring source (0.05-0.20 ppm), it is logical that SRBs – which

are anaerobes – would be more abundant in the spring source sediment, and SOBs – which commonly use oxygen as an electron acceptor – more abundant within the microbial mats.

4.3.4. Classification and relative abundance of archaeal 16S rRNA gene sequences

Fig. 4.8D displays the Mothur classifications of archaeal 16S rRNA gene sequences. It is important to note that the cDNA library consists of only 3 sequences. 68% of the archaeal DNA sequences were classified as belonging to the proposed Soil Crenarchaeotic Group of the phylum *Thaumarchaeota*. 26% of reads were classified as *Eurychaeota*, almost all of which classified to the proposed Deep Sea Hydrothermal Vent Group 6 of the *Halobacteriales*. The remaining 6% archaeal reads, consisting of 2 non-rare OTUs, were unable to be classified past the kingdom level.

The cultured MegaBLAST results for the archaeal 16S rRNA gene OTUs have mostly low identity with the representative sequences (Table 4.9). This is likely due to the difficulty of isolating archaea, leading to a low number of cultured representatives in the database. The OTU classified by Mothur as belonging to the proposed Soil Crenarchaeotic Group (SCG), was identified by MegaBLAST as a *Nitrososphaera* species with 96% identity (also called Group I.1b in the literature). The SCG is, despite its name, a cluster within the new phylum *Thaumarchaeota*. Investigation of other sequences labelled by the Silva database as being of the SCG shows that many of them have more recently been clustered with *Nitrosphaera* species (Bartossek et al., 2012). The archaeal phylogenetic tree corroborates this classification with good bootstrap support (Fig. 4.9). This OTU is the largest by abundance (145 sequences) and shows very high similarity (100-99%) to a large number of environmental sequences taken from extremely varied environments including an air filter, wetland soils, a high-altitude desert, the floor of a hospital intensive care unit, human skin, estuary creek sediment, endolithic rocks, and agricultural soil (Table 4.9, Fig. 4.9). This archaeal OTU of the *Nitrosphaera* group clusters quite closely with the Colour Peak spring sediment clone CP-A146 from Perreault et al., 2007. Perreault et al. classified CP-A146 as part of an unidentified *Crenarchaeota* group, but it now can be placed with relative certainty into the *Nitrosphaera* cluster within the *Thaumarchaeota*.

Another taxon found in the GH springs microbial mat data was related to the Deep Sea Hydrothermal Vent Euryarchaeotic Group 6 (DHVEG-6). This group is reported to belong to the class *Halobacteria*; however, the closest MegaBLAST hit to this OTU is from a *Methanobacterium* species of the class *Methanobacteria* (though with only 77% similarity). There are many cultured isolates within the *Halobacteria*, so this result is not merely due to a lack of database targets, and raises concerns about the legitimacy of the classification. The phylogenetic tree supports the Mothur classification over the BLAST one, as the OTU clusters with other clones previously reported as belonging to the DHVEG-6, and not with the *Methanobacteria* (Fig. 4.9). The enigmatic group DHVEG-6, as the name suggests, was originally identified from a deep sea hydrothermal vent, but has since been found in the water of saline lakes, deep sea methane seep sediments, and the microbial mat of a hypersaline lake (Nunoura et al., 2012; Casamayor et al., 2013; Schneider et al., 2013). This DHVEG-6 OTU's representative sequence was 99% identical to a clone taken from a hydrothermal vent (Table 4.9), with other top environmental hits including sequences isolated from anoxic hypersaline lake sediments (97% identity), a hypersaline microbial mat (96%, 92%), and sea sediments (93%). Also within this cluster is a clone from the GH-4 spring sediment, GH-A171 (DQ521152.1) (Perreault et al., 2007).

The remaining two archaeal OTUs were unable to be categorized by Mothur beyond naming them *Archaea,* and show similarly poor matches via MegaBLAST (Table 4.9). Though clustered into separate OTUs, these representative sequences showed the most similarity to the same *Methanobrevibacter* strain, both with an identity measurement of 79%. These sequences were found to show 99% similarity to clones previously isolated from GH spring sediment (clone GHA8, GenBank #: DQ521152.1) and GH-2 filaments (EU430110.1), and 98% similarity to a sequence reported from Colour Peak spring (DQ521205.1) (Table 4.9). All of these sequences clustered closely together with 95% bootstrap support. The next closest environmental hits include 91%/92% similarity to sequences from anoxic marine sediment (AM980604.1/AM998391.1) and 90% similarity to a coral-associated microbial mat (JX021885.1). All other MegaBLAST hits with >90% query coverage show 89% or less similarity. The next best search results include sequences from environments such as a freshwater mesophilic microbial mat, a hypersaline microbial mat, deep sea hydrothermal vents, and aquifer groundwater. The phylogenetic tree shows that the Axel Heiberg OTUs cluster

together with these marine sequences, but not with cultured representatives from any of the current archaeal phyla, including the *Euryarchaeota* (*Methanobrevibacter flexile* and *Methanobacterium millerae*) (Fig. 4.9). This, and the fact that Mothur characterized them as unclassified *Archaea*, indicates that these sequences may be part of an as yet uncharacterized archaeal phylum.

Close MegaBLAST relatives have also remained mainly uncharacterized; a clone from sediments of the Mamara sea (AM980604.1) was clustered with a group of unidentified Archaea they called the Mamara Archaeal Cluster 1 (MAC1) (Kolukirik et al., 2011). Other studies classify clusters of related sequences as either unclassified *Crenarchaeota* (DQ521152.1 and DQ521205.1 from Perreault et al., 2007) or unclassified *Euryarchaeota* (EU731040.1 and EU731036.1 from Robertson et al., 2009; AF526960.1 from Nercessian et al., 2003), though examination of the phylogenetic trees from these studies shows that they do not cluster with any isolated representatives of these phyla. Furthermore, a sequence identity cutoff value of 85% has been proposed for distinguishing new candidate phyla, which the novel GH mat OTUs exceed considerably, with only 79% identity to their closest isolated MegaBLAST hit (Hugenholtz et al., 1998). Interestingly, an aquifer groundwater clone (KP308726.1) to which this group has good similarity (89%) was placed within a new candidate superphylum called DPANN, which includes the candidate phyla *Diapherotrites*, *Parvarchaeota*, *Aenigmarcheota*, *Nanoarchaeota*, and *Nanohaloarchaea* (Castelle et al., 2015). Of these phyla, so far only the *Nanoarchaeota* contains isolated representatives. As culture independent methods for studying microbial communities have advanced, our knowledge of archaeal taxonomy has drastically improved and resulted in the proposal of several new phylum-level lineages consisting solely of uncultured organisms. Further clarification on the true classification and physiological function of the enigmatic archaeal group described herein could be attained via future high throughput sequencing work to increase the knowledge of unknown archaeal species and their place in the overall *Archaea* phylogenetic tree.

Table 4.9. Taxonomic information of representative sequences from major OTUs of the archaeal 16S rRNA gene libraries

# (DNA)	# (cDNA)	Mothur classification	MegaBLAST best hits*	Accession #	E-value	Identity	Isolation source of best hits
145	0	Soil Crenarchaeotic Group (SCG)	*Nitrososphaera* sp. JG1	JF748724.1	9e-153	96%	Agricultural soil
			Clone 59-L046936-122-074-C08-unis	JQ249434.1	4e-176	100%	Air filter
53	0	Deep Sea Hydrothermal Vent Group 6 (DHVEG-6)	*Methanobacterium flexile* strain GH	NR_116276.1	8e-44	77%	Lake sediments
			Clone met35	DQ082982.1	3e-168	99%	Hydrothermal vent
7	1	Unclassified Archaea	*Methanobrevibacter millerae* strain ZA-10	NR_042785.1	7e-40	79%	Ovine rumen
			Archaeon	EU430110.1	6e-175	99%	GH-2 filament
3	0	Unclassified Archaea	*Methanobrevibacter millerae* strain ZA-10	NR_042785.1	3e-38	79%	Ovine rumen
			Archaeon	EU430110.1	1e-171	99%	GH-2 filament

*First line: isolated organism. Second line: uncultured organism.

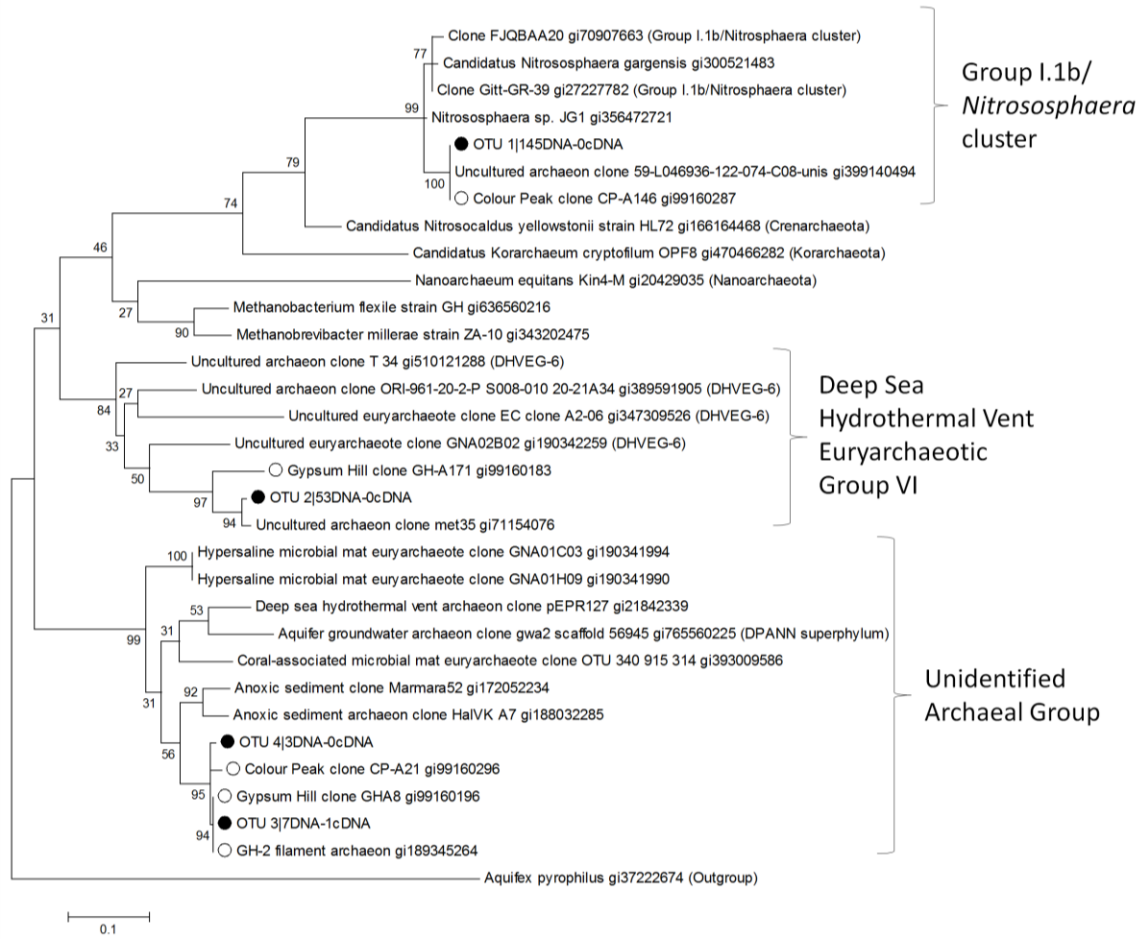

Fig. 4.9. Phylogenetic relationship of representative archaeal 16S rRNA sequences grouped into OTUs based on an identity cutoff of 97%. Representative sequences from this study are marked by a black circle and sequences from previous studies at Axel Heiberg Island sulfide springs are marked by a white circle. The maximum likelihood phylogenetic tree was constructed by aligning ~330 nucleotides of the archaeal 16S rRNA gene with 1000 bootstrap repetitions. The bar denotes the expected number of changes per nucleotide position.

4.3.5. Analysis of archaeal 16S rRNA gene sequences

If we investigate the archaeal 16S rRNA primers using ProbeMatch set to examine only good quality sequences within the target region, only 49% of sequences matched the primers with one difference allowed. The A344F/A934R primer pair appear to target preferentially *Euryarchaeota* (accounting for 87% of hits), which made up 26% of archaeal sequences of the microbial mat data. Conflictingly, ProbeMatch indicates that only 3.2% of *Thaumarchaeota* are targeted, but *Thaumarchaeota* of the Soil Crenarchaeotic Group (SCG) make up a full 67% of the GH spring mat dataset. Of the *Thaumarchaeota* that match the primers, though, almost all are of the genus *Nitrososphaera* (97%), which the SCG OTU was classified as via phylogenetic tree and MegaBLAST analyses (Fig. 4.9, Table 4.9). Phyla which showed no representation at all are the *Korarchaeota* and *Nanoarchaeota*. All other taxa show representation to the class level. In general, the archaeal primers' coverage is fair, but potentially biased toward amplification of *Euryarchaeota*.

Though definitive conclusions cannot be made due to the use of different primer sets for bacteria and archaea, it appears that archaea are less numerous than bacteria within the GH spring microbial mat. Recent studies indicate that, despite the extreme conditions in which microbial mats sometimes form, *Archaea* actually account for only a small part of microbial mat communities. For instance, in hypersaline mats, the surrounding water column may be dominated by archaea while nearby microbial mats are made up majorly of bacteria (Bolhuis et al., 2014). A ratio of 90:9:1 was calculated for the abundances of bacteria:archaea:eukarya within the Guerrero Negro hypersaline microbial mats (Robertson et al., 2009).

The archaeal communities share similarities in composition between the GH microbial mats, the GH-4 spring sediments, and the GH-2 spring filaments. Perreault et al. found a larger archaeal community in the GH-4 spring sediment, with 18 phylotypes represented in comparison to the 10 reported from the GH microbial mat. The 2007 study reported that 79% of the spring sediment clones were grouped into the *Crenarchaeota*, however this occurred before the addition of the *Thaumarchaeota* phylum to archaeal taxonomy in 2008, and thus some of these phylotypes may have been misidentified. This is especially the case due to the lack of isolated representatives found to cluster with the GH spring sediment *Crenarchaeota* groups. In their studies, Perreault et al. had found that clone GH-A171 grouped with GHA8 within an

unidentified *Crenarchaeota* group; however, evidence from this study suggests that these are more likely to be separate, with GH-A171 being a part of the DHVEG-6 and GHA8 as part of an unknown archaeal phylum.

Thaumarchaeota as a phylum is populated entirely by chemolithoautotrophic ammonia-oxidizing *Archaea*. The *Nitrososphaera* cluster of the *Thaumarchaeota* is mainly represented by sequences from soil environments, but they can also be found in other environments, such as hot springs and freshwater sediments (Zhalnina et al., 2014). Nitrogen metabolism doesn't appear to be an important form of metabolism for the GH microbial mat, considering the lack of other nitrogen metabolizing microbes within the mat. As well, the small number of cDNA sequences extracted from the mat suggests that the archaeal fraction of the community is not active. *Thaumarchaeota* have been previously found in CP spring source sediments and in both the source and channel of LH spring (Perreault et al., 2007; Lay et al., 2012, 2013; Lamarche-Gagnon et al., 2015). Dissolved and sediment ammonia concentrations at LH spring were moderate (381 μM and 2.55 mg/kg), but far above the levels required for ammonia oxidation by marine *Thaumarchaeota* species (<0.03-1 μM) (Könneke et al., 2005; Lay et al., 2012). Ammonia concentrations have not yet been measured at GH or CP springs because the presence of sulfide interferes with typical Nessler methods of ammonia quantification. While a metagenomic study of the LH spring source sediments found 16S rRNA sequences classified as *Thaumarchaeota*, it was unable to reconstruct the complete ammonia oxidation pathway. Nor has ammonia oxidation been detected in enrichment flasks of LH outlet and channel sediments. As well, evidence for *Thaumarchaeota* in these environments comes from the examination of DNA sequence libraries, not cDNA libraries which would give a better indication of activity. Overall, it is still uncertain whether the *Thaumarchaeota* found in Axel Heiberg sulfidic springs are active *in situ*. *Thaumarchaeota* are generally not present in microbial mats; *Archaea* present in temperate microbial mats are predominantly halophiles and/or methanogens (Bolhuis et al., 2014). Based on its similarity to species within the *Halobacteria* and *Methanobacteria*, it is likely that the OTU classified within the DHVEG-6 has one or both of these properties, but this cannot be said with certainty considering the lack of cultured representatives.

4.3.6. Classification and relative abundance of *dsrB* gene sequences

Though 16S rRNA-based analyses provide a good framework for determining the general diversity and identity of microbial community members, they cannot definitively prove the metabolic capabilities of those organisms. The expression of *dsrAB* gene transcripts has been directly correlated to sulfate reducing activity in sediments and in pure cultures, and appears to be more sensitive to environmental conditions than rRNA (Neretin et al., 2003; Chin et al., 2008; Lloyd et al., 2010). Therefore, by targeting the *dsrB* gene and its mRNA transcripts for sequencing, the actively sulfate reducing community can be examined and identified with greater certainty than by examining the presence of 16S rRNA genes belonging to putative SRBs. The *dsrAB* gene is found in a wide variety of sulfur metabolizing microbes including sulfate-reducing bacteria (*Deltaproteobacteria*, *Firmicutes* and *Nitrospirae*), sulfate-reducing archaea (*Archaeoglobus* and *Pyrobaculum*), and in a reversed form in some sulfur-oxidizing bacteria (*Proteobacteria* and *Chlorobi*).

All *dsrB* gene representative sequences were classified using discontiguous MegaBLAST, which is optimized for sequences which have a low degree of identity with those in the database (Table 4.10). The *dsrB* sequences show generally low similarity because they are protein-coding and thus conserved on the amino acid level instead of on the nucleotide level as 16S rRNA genes are. All *dsrB* gene OTUs from the GH mat sequence libraries were found to show the closest similarity to species that belong to either the *Desulfobacteraceae* or *Desulfobulbaceae*. The *Desulfobacteraceae* were made up of (similarity to MegaBLAST hits in parentheses): *Desulfobacterium* (OTU1: 92%) and *Desulfobacula* (OTU2: 83%, OTU5: 78%). The *Desulfobulbaceae* consisted of two OTUs that were found to have the greatest similarity to the same arctic *Desulfotalea* strain, though with differing identity levels (OTU3: 86%, OTU4: 96%). Overall, the MegaBLAST hits and phylogenetic tree show similar classifications, though the bootstrap values/similarity percentages are in most cases too low for identification to the genus level (Fig. 4.10). Exceptions to this are the clustering of OTU4 with *Desulfotalea* and OTU1 with *Desulfobacterium* with bootstrap support values of 98% and 91%, respectively. Similarity to environmental sequences was generally higher than to isolated representatives. Almost all close discontiguous MegaBLAST hits were taken from marine sources, including those in northern settings such as Aarhus Bay, Denmark (OTU1 and OTU2), and off the coast of

Svalbard, Norway (OTU3 and OTU4). The best hit was OTU2, which was found to share 99% nucleotide identity with the aforementioned Aarhus Bay clone sequence.

A thorough analysis of many commonly used *dsr* primers was conducted by Müller et al., including the *dsr4R* primer and a close variant of the *dsr2060F* primer (*dsrp2060F* with a sequence of 5'-CAACATCGTYCAYACCCAGG-3') that were used in this study. It was found that the *dsr4R* primer targeted 80% of the database's sequences when allowing for one weighted mismatch, or 28% without any mismatches. Examining *dsr4R*'s efficacy at amplifying different *dsr*-containing phylogenetic clusters, it was able to amplify the *Deltaproteobacteria* supercluster, the *Firmicutes* group, and the *Archaeoglobus* cluster (31%, 29%, and 20%, respectively, with no mismatches), but not the Environmental supercluster 1, or *Nitrospirae* supercluster. The *dsrp2060F* primer performs more poorly, able to amplify only 14% (zero mismatches) or 56% (one mismatch) of the curated database sequences. Similarly to *dsr4R*, it targets mainly the *Deltaproteobacteria* supercluster and the *Firmicutes* group, but with a small amplification of Environmental supercluster 1 (23%, 39%, and 8%, respectively). The *Nitrospirae* and *Archaeoglobus* clusters are not represented well by this primer. Overall then, it appears that the *dsrB* primers used in this study may preferentially target bacteria within the *Deltaproteobacteria* and the *Firmicutes*.

Therefore, this potential bias with primer selectivity must be considered in the interpretation of the *dsrB* data. Fortunately, the major groups less represented by the chosen primers (the *Nitrospirae* and *Archaeoglobus*) weren't found in the 16S rRNA data, despite good coverage of those taxa by the bacterial and archaeal primers. Therefore, these groups don't appear to be present within the GH spring mat, and so the *dsrB* primers aren't likely to have missed an important component of the community. *Firmicutes* and *Deltaproteobacteria* are present in the 16S rRNA sequence data; however, S-reducers of the former group are only present in small abundances (3 sequences total of the genera *Dethiosulfatibacter* and *Fusibacter*). The *dsrB* gene is also present in a reversed form in some SOBs; however, none of these particular bacteria were present in the microbial mat according to the 16S rRNA gene libraries (Müller et al., 2014). Therefore, the fact that only *Deltaproteobacteria* were detected by analysis of the *dsrB* gene data was unsurprising and likely representative of the community as a whole.

Table 4.10. Taxonomic information of representative sequences from major OTUs of the *dsrB* gene libraries.

# (DNA)	# (cDNA)	Label	Discontiguous MegaBLAST best hits	Accession #	E-value	Identity	Isolation source of best hits
845	1081	OTU1	*Desulfobacterium vacuolatum* strain DSM 3385	AF418203.1	5e-125	92%	Marine mud
			Clone OTU13	AB857201.1	5e-157	96%	Marine sediment
32	914	OTU2	*Desulfobacula toluolica* Tol2	FO203503.1	4e-94	83%	Anoxic marine mud
			Clone OTU12	AB857200.1	2e-168	99%	Marine sediment
369	119	OTU3	*Desulfotalea arctica* strain DSM 12342	AY626032.2	3e-109	86%	Arctic marine sediments
			Clone PIMO1D07	AY741558.1	4e-107	87%	Estuary sediments
277	197	OTU4	*Desulfotalea arctica* strain DSM 12342	AY626032.2	8e-154	96%	Arctic marine sediments
			Clone DB_dsr8	EU350968.2	1e-106	86%	Marine aquaculture biofilter
10	0	OTU5	*Desulfobacula toluolica* Tol2	FO203503.1	4e-69	78%	Anoxic marine mud
			Clone mRNA 25	FR689706.1	1e-82	83%	Marine steel structure

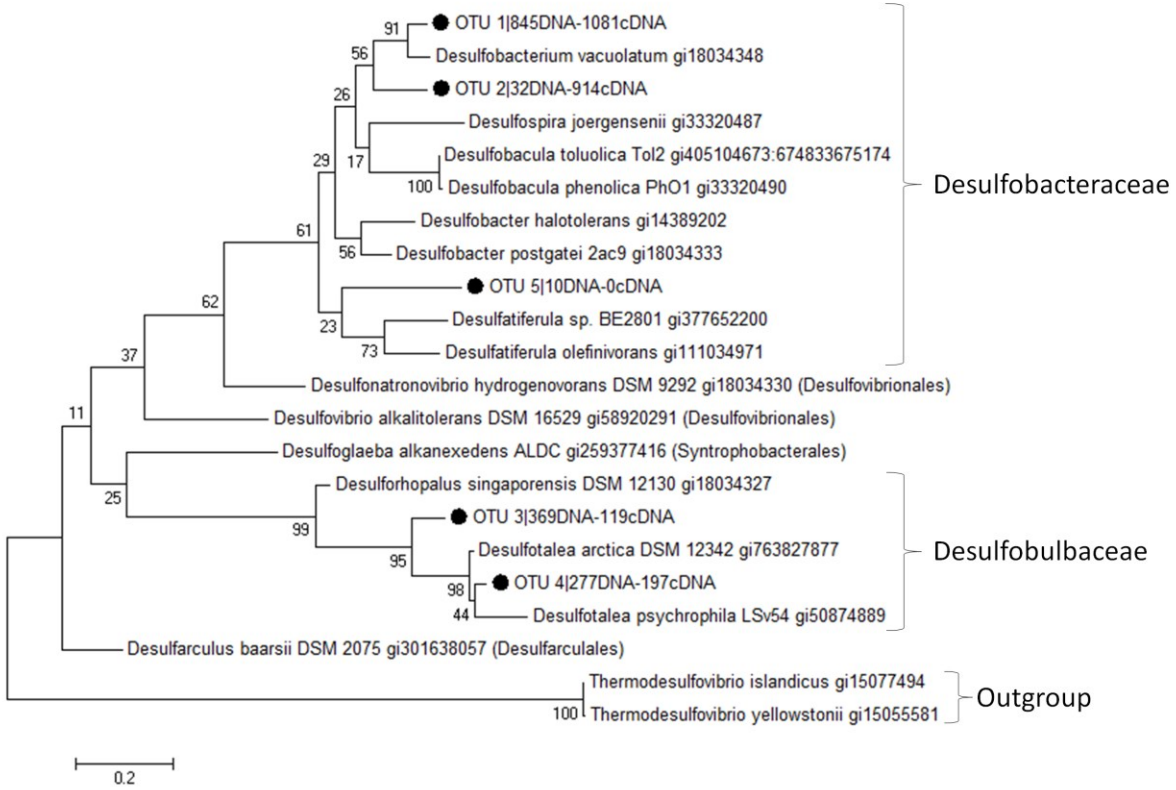

Fig. 4.10. Phylogenetic relationship of representative *dsrB* sequences grouped into OTUs based on an identity cutoff of 90%. The maximum likelihood phylogenetic tree was constructed by aligning ~340 nucleotides of the *dsrB* gene with 1000 bootstrap repetitions. The bar denotes the expected number of changes per nucleotide position.

4.3.7. Comparison of dsrB gene sequences and deltaproteobacterial 16S rRNA gene sequences

Phylogenetic trees of *dsrB* and deltaproteobacterial 16S rRNA gene OTUs generally support the MegaBLAST classifications, though low bootstrap values limit their interpretations in some cases (Fig. 4.10, Fig. 4.11). It is important to note that the *Deltaproteobacteria* include sequences classified as *Desulfuromonadaceae*; however, as sulfur- not sulfate -reducing bacteria this class does not possess the *dsrB* gene and thus is not present in the *dsrB* sequence data. With the exception of the *Desulfuromonadaceae* branch, the 16S rRNA deltaproteobacterial tree shows similarity to the *dsrB* tree at the family level. Examining the *Desulfobulbaceae*, both trees show two OTUs within this family. However, on the genus level these OTUs cluster quite differently, as both *dsrB* OTUs cluster closely with *Desulfotalea* species, while the 16S rRNA OTUs cluster with *Desulforhopalus singaporensis* and [*Desulfobacterium*] *catecholicum*. The 16S rRNA gene phylogenetic tree shows 2 OTUs within the *Desulfobacteraceae*, which group with each other but not with any previously studied genus. These may correspond to OTU5 of the *dsrB* results, which clusters in a similar location between the *Desulfatiferula* and other *Desulfobacteraceae*, with similar low identity to known genera. The *dsrB* tree's other *Desulfobacteraceae* OTUs are very well represented in the *dsrB* data, making up 57% of DNA and 86% of cDNA sequences, but not at all in the 16S rRNA data. One of these, OTU1, clusters closely with a *Desulfobacterium* species, while the other (OTU2) is less well characterized due to low bootstrap values.

It has previously been shown that *dsrB* phylogenies are largely congruent with 16S rRNA phylogenies at a family level using curated databases, but the differences between the 16S rRNA and *dsrB* phylogenetic trees shows that experimental data is not so simple (Müller et al., 2014). Discrepancies between the 16S rRNA and *dsrB* phylogenetic trees mean that no conclusions can be made as to whether or not individual OTUs for each gene actually correspond to the same organism within the environment. Nevertheless, certain patterns can be discerned; not counting singletons or doubletons, both datasets show that *dsrB* gene-possessing bacteria within the microbial mat belong to either the *Desulfobacteraceae* (2 16S rRNA OTUs and 3 *dsrB* OTUs) or *Desulfobulbaceae* (2 16S rRNA OTUs and 2 *dsrB* OTUs). Of the *Desulfobacteraceae*, the 16S rRNA Mothur classifications and *dsrB* phylogenetic trees agree that 2 OTUs present in the mat cannot be classified beyond the family level. The last OTU within the *dsrB* dataset was classified

as a *Desulfobacterium* species. The *Desulfobulbaceae* appear to be a mixture of *Desulfotalea* (by *dsrB* classification), *Desulforhopalus* (by 16S rRNA Mothur, MegaBLAST, and phylogenetic classification methods), and a novel genus represented by [*Desulfobacterium*] *catecholicum* (by 16S rRNA MegaBLAST and phylogenetic tree methods). It is possible that the unidentified *dsrB* OTU3 is in fact a part of this novel genus, as no *dsr* sequences belonging to [*Desulfobacterium*] are publicly available for comparison. The 16S rRNA data also includes one OTU that exists outside of the *Desulfobacteraceae*, *Desulfobulbaceae*, and *Desulfuromonadales*; this OTU may be part of a novel order of SRBs that also would include the environmental sequence GR-WP33-58 (Moreira et al., 2006). It is thought that this order, named the myxobacteria-related group (MRG), may be able to reduce sulfate and oxidized metallic compounds, but whether it possesses the *dsrB* gene is currently unknown. Overall, it appears that sequencing both the *dsrB* gene and 16S rRNA gene revealed populations of sulfate reducing bacteria that sequencing of only one gene would not have. Classification of taxa using functional genes such as *dsrB* will likely improve over time as increased high-throughput sequencing leads to more comprehensive reference databases.

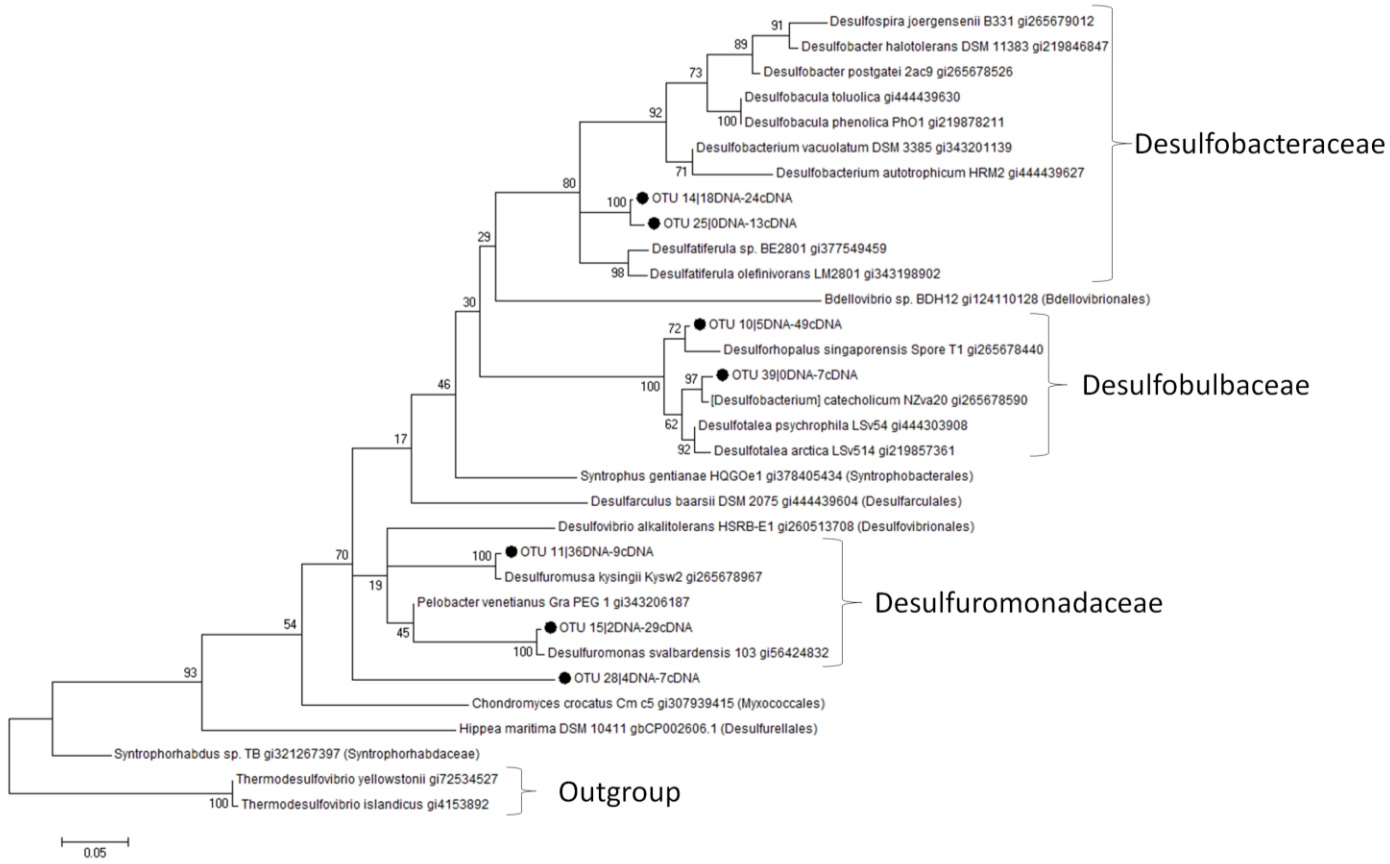

Fig. 4.11. Phylogenetic relationship of representative Deltaproteobacterial 16S rRNA sequences grouped into OTUs based on an identity cutoff of 97%. The maximum likelihood phylogenetic tree was constructed by aligning ~365 nucleotides of the 16S rRNA gene with 1000 bootstrap repetitions. The bar denotes the expected number of changes per nucleotide position.

4.5. Potentially active taxa at Axel Heiberg sulfidic springs

4.5.1. Potentially active taxa within the GH microbial mats

It has been shown that the abundance of a species does not necessarily correlate to the activity of that species, or its importance to biogeochemical fluxes (Röling, 2007). The sequencing of RNA in addition to DNA has the advantage of providing information on the activity of microbes within a community, not just the overall abundances. In pure cultures, rRNA quantity correlates positively with bacterial growth rates and a variety of measures of cellular activity including cellular protein levels, chlorophyll a content, ^{14}C-fixation rates, bromodeoxyuridine uptake, 3H-adenine incorporation, and oxygen consumption (Nomura et al., 1984; Lloyd et al., 2010; Campbell et al., 2011). Microbial growth rate corresponds to the number of ribosomes (indicated by the number of 16S rRNA transcripts) per cell (indicated by the number of 16S rRNA genes), and thus this ratio can be used as an index of the growth rate for particular taxa within a microbial community (Jones and Lennon, 2010; Campbell et al., 2011; Gaidos et al., 2011).

Though the use of rRNA as a way to infer microbial activity has been well established in microbial ecology, recent evidence suggests that caution should be exercised in interpretation of this data. A review by Blazewicz et al. found several limitations of rRNA as an indicator of current microbial activity. These include the fact that the relationship between rRNA and growth rate may differ between taxa and due to changes in environmental conditions, and that dormant cells can contain high numbers of ribosomes (Blazewicz et al., 2013). Despite these limitations, the use of RNA in addition to DNA measurements is very valuable, especially in the context of researching microbial mat diversity. Due to the EPS matrix within microbial mats, they contain high amounts of extracellular DNA that is stable and protected from nucleases (Lorenz and Wackernagel, 1994). It is thought that extracellular DNA could make up as much as 90% of the total DNA recovered from marine sediments (Dell'Anno and Danovaro, 2005). For these reasons, the examination of RNA rather than DNA can be a much better indicator of the active species in a microbial mat environment.

Though cDNA:DNA ratios are calculated on a relative basis, due to the need to account for variation in sequencing coverage, statistical models by Campbell et al. have shown that

OTUs with a ratio above one are consistent with higher than average growth rates (Campbell et al., 2011). Hence, we will define 16S rRNA OTUs with cDNA:DNA ratios above one as being 'active'.

All but one of the 16S rRNA OTUs classified as putative SRBs were found to be active (ratio of cDNA:DNA in parenthesis), particularly the *Desulforhopalus* (6.9), *Desulfuromonas* (10), *Sulfurospirillum* (2.3, 1.6), and unclassified *Desulfobacteraceae* (2.1) and *Deltaproteobacteria* (2.3) (Fig. 4.12A). The only non-active SRB OTU was *Desulfuromusa* (0.18). Similarly, a greater number of putative SOB OTUs are shown to be active than not, and many of these have a high abundance. High abundance putative SOBs include: *Thiomicrospira* (1.5), unclassified *Piscirickettsiaceae* (2.0), *Marinobacter* (2.5, 2.2), and *Spirochaeta* (6.7). As well, there are several potential sulfur-metabolizing OTUs which are present in the cDNA library, but not at all in the DNA library (*Salinisphaera*, *Sulfurimonas*, *Gallionella*, *Spirochaeta*, unclassified *Desulfobacteraceae* and *Desulfobulbaceae*). Putative SOBs that were found to have a low growth rate are OTUs classified as *Halothiobacillus* (0.20), *Sufurimonas* (0.18, 0.42, 0), and *Marinobacter* (0.18). Heterotrophs show a mix of active and slow growing species. Major heterotrophic OTUs by abundance include *Psychroflexus* (0.30), *Demequina/Cellulomonas* (0.037), *Rhodanobacter* (1.1, 0.10), *Marinomonas* (0.95), and *Saccharospirillum* (3.5). Of the two non-rare *Cyanobacteria* OTUs, one had a cDNA:DNA ratio of 0.7 and the other was found only in the cDNA and not at all in the DNA.

Unlike the 16S rRNA gene SRB OTUs, most of the *dsrB* gene OTUs are found to have cDNA:DNA ratios of less than one (Fig. 4.12). There is one very active OTU (OTU2 with a cDNA:DNA ratio of 19), which was previously found to share 83% nucleotide similarity with *Desulfobacula toluolica* and clustered within the *Desulfobacteraceae* (Table 4.10, Fig. 4.10). It is important to note that, due to the need to account for different sequencing efficiencies, the cDNA:DNA ratios utilized here are only a measure of relative activity, not absolute activity. Unlike 16S rRNA transcripts and genes which have been directly correlated to growth rate and cell number, respectively, the physiological basis for using an mRNA:DNA ratio is less certain. As well, unlike for the 16S rRNA gene, no modelling analyses have been performed for the *dsrB* gene to determine what ratio value corresponds to higher than average growth rates. Nonetheless, the use of mRNA:DNA ratios are a useful tool to visualize what components of a community are

most functionally active and have been previously used in the field of microbial ecology, usually by comparing metagenomic to metatranscriptomic data (Shi et al., 2011; Yu and Zhang, 2012). Thus, all of the sulfate reducing bacteria present may, in fact, be actively reducing sulfate within the environment, but most do so to a far lesser extent than OTU2 of the *Desulfobacteraceae*.

Overall, there are many more OTUs that are present in the cDNA data but not the DNA data than vice versa (Fig. 4.5A). And when examining the average cDNA:DNA ratio for abundant OTUs (>1% of community) in comparison to non-abundant OTUs (<1% of community), abundant OTUs have a lower ratio (0.96) than non-abundant species (1.17). Such a result isn't unusual; studies utilizing similar methods in marine and lake waters found that rare taxa were, in general, more active than abundant taxa (Jones and Lennon, 2010; Campbell et al., 2011; Campbell and Kirchman, 2013).

OTUs of taxa belonging to soil bacteria such as *Actinobacteria* (ex. *Demequina/Cellulomonas*) and *Bacteroidetes* (ex. *Psychroflexus*) tended to be much less abundant in the cDNA than the DNA (**Error! Reference source not found.**). Other studies have found this to be the case as well; in LH spring sediment *Actinobacteria*, *Firmicutes*, and *Bacteriodetes* were much more present in cDNA libraries than DNA ones, and this is the case for *Bacteroidetes* within photosynthetic microbial mats (Burow et al., 2013; Lamarche-Gagnon et al., 2015). *Bacteroidetes*, *Actinobacteria*, and *Firmicutes* are phyla typically associated with permafrost due to their ability to form spores, but they are also commonly abundant within microbial mat environments as well (Wagner, 2008; Bolhuis et al., 2014). Whether or not they are active within these environments is largely unknown; further research using sequencing approaches targeting both RNA and DNA could provide insight into whether our results are typical of microbial mat communities and/or cold sulfidic spring environments. Perhaps the functional niche of chemoorganoheterotrophy has been primarily filled by the *Marinobacter*, which tend to be more active (1.5, 2.2, 0.18) and are almost as abundant (16% DNA/26% cDNA in comparison to 30% DNA/6.6% cDNA) as the *Bacteroidetes* and *Actinobacteria* combined. Based on cDNA:DNA ratios, the putative SOB and SRB populations (as determined by their 16S rRNA sequences) appear to be, in general, more active than heterotrophs. Notable exceptions to this rule are the *Desulfuromusa* (0.18) and *Halothiobacillus* (0.20). The *Desulfuromusa* share a similar niche to the much more active *Desulfuromonas* OTU (10); both genera can reduce

elemental sulfur and iron but not sulfate, are obligately anaerobic, and are typically found in marine mud/sediments (Vandieken et al., 2006). Similarly, *Halothiobacillus* fills the same role as the more active and abundant *Thiomicrospira* (1.5) within the community; they are both halophilic obligate aerobes that grow chemolithoautotrophically by oxidizing a variety of sulfur compounds, and are often isolated from marine and hydrothermal vent sediments (S. M. Sievert et al., 2000; Knittel et al., 2005). It is possible that the vast differences in activity level of these OTUs are the product of competition within the GH microbial mat for particular niches.

4.5.2. Potentially active taxa at LH spring: sulfur isotope fractionation

Another way of measuring activity on a longer time scale is to examine the isotope fractionation of elements present in rock or sediment. There are four stable sulfur isotopes, of which the most abundant are ^{32}S (95%) and ^{34}S (4.2%). During the process of dissimilatory sulfate reduction, light isotopes are preferentially utilized over heavy isotopes such that over time the reaction product (sulfide) will become enriched in ^{32}S and depleted in ^{34}S, and vice versa for the reactant (sulfate) (Canfield, 2001). Isotopic composition ($\delta^{34}S$) is expressed as per mil differences relative to the isotopic composition of the Canyon Diablo iron-nickel meteorite, which is thought to be the primordial value for the Earth's crust and mantle ($\delta^{34}S = [\{(^{34}S/^{32}S)_{sample}/(^{34}S/^{32}S)_{std}\} - 1] \times 1000$). The isotopic fractionation is the difference between the isotopic compositions of the reactant and the product, in this case sulfate and sulfide in the form of pyrite ($\Delta_{sulfate\text{-}pyrite} = \delta^{34}S_{sulfate} - \delta^{34}S_{pyrite}$). In this study, preliminary results were obtained on the sulfur isotope fractionation of sulfate from the LH spring water and pyrite from the LH spring sediments (Table 4.11). Acid-volatile sulfide composition was not measured due to low yield. As an Axel Heiberg sulfidic spring with saline, cold, anoxic water, LH spring shares many features in common with GH spring.

Preliminary results suggest that the LH spring water may have a marine source, as the $\delta^{34}S_{sulfate}$ value of ~22.18‰ is only slightly more enriched in ^{34}S than modern seawater, which has an average value of 21.24 ‰ (±0.88‰) (Tostevin et al., 2014). In comparison, modern values for the isotopic composition of pyrite within marine sediments varies greatly; one study found $\delta^{34}S_{pyrite}$ measurements ranging from ~-12.5 ‰ (from a marine tidal flat), to ~-48.7 ‰ (from Black Sea sediments) (Johnston et al., 2008). Furthermore, pyrite isotope differences can vary greatly even over short distances within sediment (Canfield, 2001). Therefore, the

difference in $\delta^{34}S_{pyrite}$ values between the two replicates ($\delta^{34}S_{pyrite}$ = -19.10 ‰ and -27.32 ‰) are not surprising. Such heterogeneity is a marker of biogenic sulfate reduction (Canfield, 2001). The fractionation values (41.32 ‰ and 49.71 ‰) also support the presence of an active sulfur cycle at the time of sulfide deposition as pyrite, as they indicate that the pyrite is significantly enriched in ^{32}S and depleted in ^{34}S in comparison to sulfate. Considering the similar conditions of the LH spring and GH springs, these results support the conclusion that an active sulfur metabolizing community exists within the GH spring system.

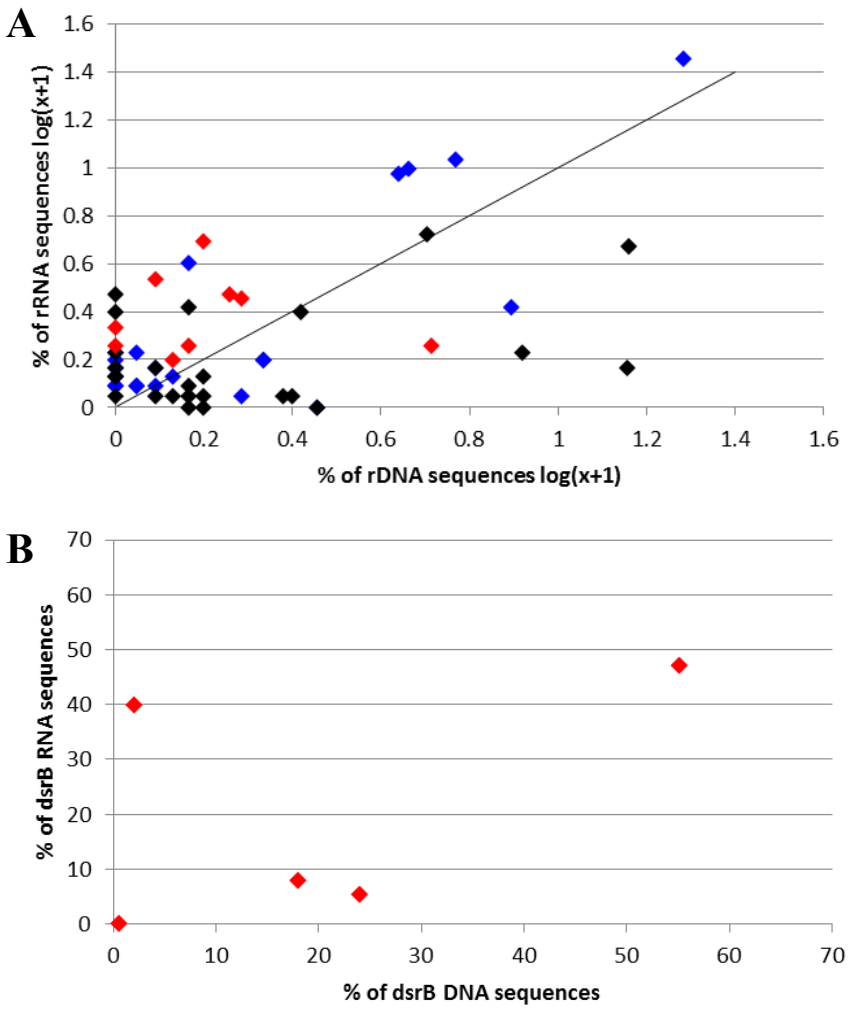

Fig. 4.12. Relationship between relative frequency of RNA and DNA sequences for each non-rare OTU, as defined by A) 97% similarity for the bacterial 16S rRNA gene, and B) 90% similarity for the *dsrB* gene. Red datapoints indicate putative SRB OTUs, and blue datapoints indicate putative SOB OTUs. Data were subsampled in order to account for differences in sequencing effort. Bacterial sequences are shown using a log scale for improved clarity of low-abundance OTUs. The black line is the 1:1 ratio line.

Table 4.11. Isotopic fractionation of sulfur compounds from Lost Hammer spring sediment/water, collected in summer 2013. Samples were fixed with 20% zinc acetate and stored at -20°C. Cr-reducible sulfide was extracted and purified as according to Scott et al., 2014. Dissolved sulfate was extracted and isotope mass ratios were determined in accordance with the protocol outlined in Pellerin et al., 2015.

	Replicate 1	Replicate 2
Dissolved sulfate $\delta^{34}S$	22.22 ‰	22.12 ‰
Cr-reducible sulfide (pyrite) $\delta^{34}S$	-19.10 ‰	-27.32 ‰
Fractionation $\Delta_{sulfate - pyrite}$	41.32 ‰	49.71 ‰

4.7. Assessment of sulfide production from sulfidic spring sediments enriched with targeted media

In order to determine the metabolic capabilities of the Axel Heiberg sulfidic spring sediment communities, enrichments cultures were established using media containing different electron donors and acceptors. Due to the opaque nature of the inoculating sediment slurries, turbidity could not be used to measure growth. Instead, sulfide production was monitored over time. Enrichment of LH, GH-2, and GH-6 sediments with media targeting *Dethiosulfovibrio* and *Desulfohalobium* species resulted in sulfidogenesis (the production of sulfide) in some conditions and not in others (Table 4.12). Certain patterns can be seen: sulfide production was seen in all vials amended with GH-2 sediments, but only in GH-6 sediment vials with a H_2:CO_2 headspace. Sulfidogenesis is only observed from LH sediment-amended vials that have both a H_2:CO_2 headspace and media containing elemental sulfur as an electron acceptor. Enrichment cultures set up on site at GH-4 and CP-2 springs also showed sulfidogenic potential (Table 4.12). All media inoculated with CP-2 sediments showed production of sulfide, regardless of electron acceptor present (sulfate, elemental sulfur, or thiosulfate). Sulfidogenesis was observed in most GH-4 sediment-amended vials, including those which contained sulfate, sulfur or thiosulfate as electron acceptors.

It is important to note that enrichments were performed with the intent of culturing and isolating SRBs and SOBs, and therefore no negative controls and limited replicates were established. Hence, the above results should be interpreted with care. However, overall, the production of sulfide by sediments enriched with a variety of electron acceptors indicates that

sulfur and thiosulfate reduction, not just sulfate reduction, may be important types of metabolism within the microbial communities of Axel Heiberg sulfide springs. This conclusion is supported by the variety of elemental sulfur reducing bacteria detected within the GH microbial mat in surprising abundances that are generally active. This includes OTUs classified as *Desulfuromusa*, (which make up 55% of the DNA reads for putative SRBs and 5% of the cDNA, with a cDNA:DNA ratio of 0.18), *Desulfuromonas* (3% DNA, 17% cDNA, ratio of 10), and *Sulfurospirillum* (15% DNA, 17% cDNA, ratios of 2.3 and 1.6). Overall, of the sequences classified as sulfur compound-reducing bacteria, 73% of DNA and 39% of cDNA were found to be bacteria capable of elemental sulfur reduction.

Isolation of SRBs from enrichment cultures were attempted using the Hungate roll tube technique (Hungate, 1969). In this technique, the enrichment media with agar is mixed with a reducing agent and any necessary vitamin solutions then rolled to coat the inside surface of a Hungate tube while still molten. In order to avoid killing the putative psychrophiles, inoculum was added to the agar's surface after the tubes were cooled instead of mixed with the agar itself. Media used in these attempts (headspace in parentheses) include: Knoblauch + lactate (H_2:CO_2), Knoblauch + lactate (N_2), Knoblauch + acetate (H_2:CO_2), and Postgate C (H_2:CO_2) (Table 3.2). Only one culture showed the production of colonies (Knoblauch + lactate inoculated with GH-4 sediments with an H_2:CO_2 headspace, incubated at 5°C). Partial sequencing of the 16S rRNA gene showed that it was 98% similar to *Marinobacter* sp. ZS1-18 (FJ889643.1). Later attempts to grow the organism in liquid media failed, and the culture was subsequently lost.

Attempts to culture SOBs from enrichment cultures met with more success. Aerobic media used included a thiosulfate media as described in Perreault et al., 2008, and MJ media (DSMZ 1011) with either thiosulfate or sulfide as the electron donor. Inocula included LH sediments, GH-4 sediments, GH filaments, and three microbial mat samples (yellow, green, and orange/yellow). In many cases, colonies were obtained and partial 16S rRNA gene sequencing was performed, but contamination by slow-growing *Pseudomonas* and fungal strains was endemic and subculturing proved difficult, so further attempts at isolation were not made. Table 4.13 is a summary of culture conditions and partial 16S rRNA gene sequencing results.

Table 4.12. Sulfide production after enrichment of Axel Heiberg sulfidic spring sediments with various media after incubation for 141 or 166 days.

			5°C					10°C		
Media	**e^- acceptors**	**Headspace**	**LH**	**GH-2**	**GH-6**	**GH-4**	**CP-2**	**LH**	**GH-2**	**GH-6**
Dethiosulfovibrio I media	S^0	H_2:CO_2	+	+	+	+	+	+	+	+
		N_2						-	+	-
Desulfohalobium media	SO_4	H_2:CO_2	-	+	+	-	+	-	+	+
		N_2						-	+	-
Dethiosulfovibrio II media	S_2O_3	H_2:CO_2				+	+			
Desulfuromonas media	SO_4	H_2:CO_2				+	+			
Desulfobulbus media	SO_4	H_2:CO_2				+	+			
Postgate B	SO_4	H_2:CO_2					+			
Knoblauch + lactate	SO_4	H_2:CO_2				-	+			
Desulfobacter media	SO_4	N_2:CO_2				-	+			

*-: no sulfide release, +: increase in sulfide concentration over 141 or 166 days of incubation.

Table 4.13. Culture conditions and partial 16S rRNA gene matches for putative SOBs from various Axel Heiberg Island sulfidic springs.

Inoculum	**Medium**	**Temperature**	**Closest BLAST match**
GH streamers	MJ + Na_2S	RT*	*Pelagicola* sp. YM-20/*Roseovarius* sp. H50
LH sediment	MJ + Na_2S	RT	*Halomonas* sp. 5(2010)
LH sediment	MJ + Na_2S	RT	*Halomonas* sp. SYOJ52
LH sediment	MJ + thiosulfate	RT	*Halomonas* sp. NP35
GH streamers	Thiosulfate	RT	*Marinobacter* sp. D3-2M
GH streamers	Thiosulfate	10°C	*Thiomicrospira* sp. NP51
GH streamers	Thiosulfate	10°C	*Psychrobacter* sp. NP45
GH streamers	Thiosulfate	10°C	*Halothiobacillus* sp. NP37
CP-2 sediment	Thiosulfate	10°C	*Thiomicrospira* sp. NP51
CP-2 sediment	Thiosulfate	10°C	*Psychrobacter* sp. NP45

*RT = Room temperature

Chapter 5: Conclusions

This thesis investigates the microbial diversity and activity of a microbial mat found near the Gypsum Hill springs. It is the first detailed analysis of any of the GH microbial mats, and to our knowledge, the first description of a microbial mat associated with a High Arctic spring, as well as the first description of a microbial mat associated with a cold saline sulfidic spring. This work has revealed that the major components of the mat's bacterial community are *Gamma-*, *Epsilon-*, and *Deltaproteobacteria*, *Flavobacteriia*, and *Actinobacteria*. A large proportion of the active and total bacteria were composed of sulfur-compound oxidizers and reducers. Of the putative SRBs, both sulfate and elemental sulfur reducers were detected and may be active *in situ*, as enrichment cultures provided with these electron acceptors showed sulfidogenesis at low temperatures. Chemolithoautotrophy and photoautotrophy seem to be important sources of organic material within the GH microbial mat ecosystem. Bacterial biodiversity indices and OTU identities were very similar to that of GH spring sediment, indicating that the composition of the GH microbial mats may have been highly influenced by the spring sources which feed into them. In contrast to the bacterial community, archaeal diversity within the GH microbial mat is quite low. Of the archaeal OTUs present within the mat, however, only one was classified as a taxon that has an isolated representative. Other archaeal OTUs are found to belong to uncharacterized groups of *Archaea*, including one cluster which may belong to a novel phylum. Similarly, an investigation of SRBs via analysis of the 16S rRNA and *dsrB* genes reveals the presence of OTUs which are currently unclassified, most likely due to lack of isolated representatives of these taxa. This includes one putative SRB OTU which clusters with sequences of a candidate order called the myxobacteria-related group, and two archaeal OTUs which might be placed within the new candidate superphylum DPANN (Moreira et al., 2006; Castelle et al., 2015). Many of the microorganisms detected in this study were genetically similar to sequences taken from other cold, saline environments, often marine in nature. Further research of the GH microbial mats is recommended to clarify the roles of photoautotrophs in this ecosystem. The pyrosequencing approach used herein can only give an indication of the relative abundance of various taxa, not the absolute abundance present in the environment. Bacteria and archaea within the GH microbial mats could be quantified using Fluorescent In Situ Hybridization (FISH) and quantitative PCR (qPCR) methods. Though little lamination was observed with the GH mats, a

cross-sectional study of the mat could provide more information on the location and biochemical roles of particular taxa detected in this work. As reference databases become more comprehensive due to greater sequencing and isolation efforts, the function and taxonomy of unidentified OTUs from this study may become clearer.

The present thesis emphasizes the importance of cold saline environments as sources of unexpected microbial diversity. The current work is highly relevant to the field of astrobiology, which aims to investigate the possibility of life on other planets. Current astrobiological targets of interest such as Mars, Europa, and Enceladus, all offer evidence of past or present liquid water, experience extremely low temperatures, and have very low levels of oxygen. Often there are indications that the liquid water present on these worlds may be/have been saline and rich in sulfates. Due to similarly cold, saline, anoxic, and sulfur compound rich environments, the sulfidic springs of Axel Heiberg Island are excellent analogue sites for astrobiological study. Another aspect of astrobiological study which is informed by the results of this study is the study of the development of early life on Earth. Due to its ability to survive at low temperatures, the GH microbial mat may be a good analogue to ancient microbial mats formed during glaciation events of Earth's past. Overall, the current study shows that the GH spring microbial mat hosts a wealth of bacterial diversity, particularly amongst the sulfur-compound metabolizing community.

List of References

Di Achille, G. and Hynek, B.M. (2010) Ancient ocean on Mars supported by global distribution of deltas and valleys. *Nat. Geosci.* **3**: 459–463.

Alain, K., Callac, N., Guégan, M., Lesongeur, F., Crassous, P., Cambon-Bonavita, M.-A., et al. (2009) Nautilia abyssi sp. nov., a thermophilic, chemolithoautotrophic, sulfur-reducing bacterium isolated from an East Pacific Rise hydrothermal vent. *Int. J. Syst. Evol. Microbiol.* **59**: 1310–5.

Andersen, D.T., Pollard, W.H., McKay, C.P., and Heldmann, J. (2002) "Cold springs in permafrost on Earth and Mars." *J. Geophys. Res.* **110**: 5015.

Arakawa, S., Sato, T., Sato, R., Zhang, J., Gamo, T., Tsunogai, U., et al. (2006) Molecular phylogenetic and chemical analyses of the microbial mats in deep-sea cold seep sediments at the northeastern Japan Sea. *Extremophiles* **10**: 311–9.

Armitage, D.W., Gallagher, K.L., Youngblut, N.D., Buckley, D.H., and Zinder, S.H. (2012) Millimeter-scale patterns of phylogenetic and trait diversity in a salt marsh microbial mat. *Front. Microbiol.* **3**: 293.

Bakermans, C. (2008) Psychrophiles: from Biodiversity to Biotechnology. In, Margesin,R., Schinner,F., Marx,J.-C., and Gerday,C. (eds), *Psychrophiles: from Biodiversity to Biotechnology*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 17–28.

Bartossek, R., Spang, A., Weidler, G., Lanzen, A., and Schleper, C. (2012) Metagenomic analysis of ammonia-oxidizing archaea affiliated with the soil group. *Front. Microbiol.* **3**: 208.

Battler, M.M., Osinski, G.R., and Banerjee, N.R. (2013) Mineralogy of saline perennial cold springs on Axel Heiberg Island, Nunavut, Canada and implications for spring deposits on Mars. *Icarus* **224**: 364–381.

Belkin, S. and Jannasch, H.W. (1985) A new extremely thermophilic, sulfur-reducing heterotrophic, marine bacterium. *Arch. Microbiol.* **141**: 181–186.

Blazewicz, S.J., Barnard, R.L., Daly, R.A., and Firestone, M.K. (2013) Evaluating rRNA as an indicator of microbial activity in environmental communities: limitations and uses. *ISME J.* **7**: 2061–8.

Bolhuis, H., Cretoiu, M.S., and Stal, L.J. (2014) Molecular ecology of microbial mats. *FEMS Microbiol. Ecol.* **90**: 335–50.

Bonny, S.M. and Jones, B. (2007) Barite (BaSO 4) biomineralization at Flybye Springs, a cold

sulphur spring system in Canada's Northwest Territories. *Can. J. Earth Sci.* **44**: 835–856.

Bottos, E.M., Vincent, W.F., Greer, C.W., and Whyte, L.G. (2008) Prokaryotic diversity of arctic ice shelf microbial mats. *Environ. Microbiol.* **10**: 950–66.

Brambilla, E., Hippe, H., Hagelstein, A., Tindall, B.J., and Stackebrandt, E. (2001) 16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antarctica. *Extremophiles* **5**: 23–33.

Burow, L.C., Woebken, D., Marshall, I.P.G., Lindquist, E.A., Bebout, B.M., Prufert-Bebout, L., et al. (2013) Anoxic carbon flux in photosynthetic microbial mats as revealed by metatranscriptomics. *ISME J.* **7**: 817–29.

Campbell, B.J. and Kirchman, D.L. (2013) Bacterial diversity, community structure and potential growth rates along an estuarine salinity gradient. *ISME J.* **7**: 210–20.

Campbell, B.J., Yu, L., Heidelberg, J.F., and Kirchman, D.L. (2011) Activity of abundant and rare bacteria in a coastal ocean. *Proc. Natl. Acad. Sci. U. S. A.* **108**: 12776–81.

Canfield, D.E. (2001) Biogeochemistry of Sulfur Isotopes. *Rev. Mineral. Geochemistry* **43**: 607–636.

Casamayor, E.O., Triadó-Margarit, X., and Castañeda, C. (2013) Microbial biodiversity in saline shallow lakes of the Monegros Desert, Spain. *FEMS Microbiol. Ecol.* **85**: 503–18.

Castelle, C.J., Wrighton, K.C., Thomas, B.C., Hug, L.A., Brown, C.T., Wilkins, M.J., et al. (2015) Genomic expansion of domain archaea highlights roles for organisms from new phyla in anaerobic carbon cycling. *Curr. Biol.* **25**: 690–701.

Chaudhary, A., Haack, S.K., Duris, J.W., and Marsh, T.L. (2009) Bacterial and archaeal phylogenetic diversity of a cold sulfur-rich spring on the shoreline of Lake Erie, Michigan. *Appl. Environ. Microbiol.* **75**: 5025–36.

Chin, K.-J., Sharma, M.L., Russell, L.A., O'Neill, K.R., and Lovley, D.R. (2008) Quantifying expression of a dissimilatory (bi)sulfite reductase gene in petroleum-contaminated marine harbor sediments. *Microb. Ecol.* **55**: 489–99.

Clarke, S., Mielke, R.E., Neal, A., Holden, P., and Nadeau, J.L. (2010) Bacterial and mineral elements in an arctic biofilm: a correlative study using fluorescence and electron microscopy. *Microsc. Microanal.* **16**: 153–65.

Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., et al. (2014) Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res.* **42**: D633–42.

Dahl, C., Friedrich, C., and Kletzin, A. (2008) Sulfur Oxidation in Prokaryotes. In, *Encyclopedia of Life Sciences*. John Wiley & Sons, Ltd, Chichester, UK.

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

Dilling, W. and Cypionka, H. (1990) Aerobic respiration in sulfate-reducing bacteria*. *FEMS Microbiol. Lett.* **71**: 123–127.

Donnadieu, Y., Goddéris, Y., Ramstein, G., Nédélec, A., and Meert, J. (2004) A "snowball Earth" climate triggered by continental break-up through changes in runoff. *Nature* **428**: 303–6.

Douglas, S. Douglas, D.D. (2001) Structural and Geomicrobiological Characteristics of a Microbial Community from a Cold Sulfide Spring. *Geomicrobiol. J.* **18**: 401–422.

Druschel, G.K., Emerson, D., Sutka, R., Suchecki, P., and Luther, G.W. (2008) Low-oxygen and chemical kinetic constraints on the geochemical niche of neutrophilic iron(II) oxidizing microorganisms. *Geochim. Cosmochim. Acta* **72**: 3358–3370.

Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**: 1792–7.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., and Knight, R. (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**: 2194–200.

Ehlmann, B.L., Mustard, J.F., Murchie, S.L., Bibring, J.-P., Meunier, A., Fraeman, A.A., and Langevin, Y. (2011) Subsurface water and clay mineral formation during the early history of Mars. *Nature* **479**: 53–60.

Elshahed, M.S., Senko, J.M., Najar, F.Z., Kenton, S.M., Roe, B.A., Dewers, T.A., et al. (2003) Bacterial Diversity and Sulfur Cycling in a Mesophilic Sulfide-Rich Spring. *Appl. Environ. Microbiol.* **69**: 5609–5621.

Emerson, D., Fleming, E.J., and McBeth, J.M. (2010) Iron-oxidizing bacteria: an environmental and genomic perspective. *Annu. Rev. Microbiol.* **64**: 561–83.

Feazel, L.M., Spear, J.R., Berger, A.B., Harris, J.K., Frank, D.N., Ley, R.E., and Pace, N.R. (2008) Eucaryotic diversity in a hypersaline microbial mat. *Appl. Environ. Microbiol.* **74**: 329–32.

Fenchel, T. (1998) Formation of laminated cyanobacterial mats in the absence of benthic fauna. *Aquat. Microb. Ecol.* **14**: 235–240.

Finster, K., Coates, J.D., Liesack, W., and Pfennig, N. (1997) Desulfuromonas thiophila sp. nov.,

a new obligately sulfur-reducing bacterium from anoxic freshwater sediment. *Int. J. Syst. Bacteriol.* **47**: 754–8.

Finster, K., Liesack, W., and Thamdrup, B. (1998) Elemental sulfur and thiosulfate disproportionation by Desulfocapsa sulfoexigens sp. nov., a new anaerobic bacterium isolated from marine surface sediment. *Appl. Environ. Microbiol.* **64**: 119–25.

Fish, J.A., Chai, B., Wang, Q., Sun, Y., Brown, C.T., Tiedje, J.M., and Cole, J.R. (2013) FunGene: the functional gene pipeline and repository. *Front. Microbiol.* **4**: 291.

Friedrich, C.G., Bardischewsky, F., Rother, D., Quentmeier, A., and Fischer, J. (2005) Prokaryotic sulfur oxidation. *Curr. Opin. Microbiol.* **8**: 253–9.

Friedrich, C.G., Rother, D., Bardischewsky, F., Quentmeier, A., and Fischer, J. (2001) Oxidation of reduced inorganic sulfur compounds by bacteria: emergence of a common mechanism? *Appl. Environ. Microbiol.* **67**: 2873–82.

Frontier, S. (1985) Diversity and structure in aquatic ecosystems. *Oceanogr. Mar. Biol.* **23**: 253–312.

Gaidos, E., Rusch, A., and Ilardo, M. (2011) Ribosomal tag pyrosequencing of DNA and RNA from benthic coral reef microbiota: community spatial structure, rare members and nitrogen-cycling guilds. *Environ. Microbiol.* **13**: 1138–52.

Gaillard, F., Michalski, J., Berger, G., McLennan, S.M., and Scaillet, B. (2012) Geochemical Reservoirs and Timing of Sulfur Cycling on Mars. *Space Sci. Rev.* **174**: 251–300.

van Gemerden, H. (1993) Microbial mats: A joint venture. *Mar. Geol.* **113**: 3–25.

Goordial, J., Lamarche-Gagnon, G., Lay, C.-Y., and Whyte, L.G. (2013) Left Out in the Cold: Life in Cryoenvironments. In, Seckbach,J., Oren,A., and Stan-Lotter,H. (eds), *Polyextremophiles*, Cellular Origin, Life in Extreme Habitats and Astrobiology. Springer Netherlands, Dordrecht, pp. 335–363.

Grant, J. and Gust, G. (1987) Prediction of coastal sediment stability from photopigment content of mats of purple sulphur bacteria. *Nature* **330**: 244–246.

Grasby, S.E., Beauchamp, B., and Bense, V. (2012) Sulfuric acid Speleogenesis associated with a glacially driven groundwater system-paleo-spring “pipes” at Borup Fiord Pass, Nunavut. *Astrobiology* **12**: 19–28.

Grünke, S., Felden, J., Lichtschlag, A., Girnth, A.-C., De Beer, D., Wenzhöfer, F., and Boetius, A. (2011) Niche differentiation among mat-forming, sulfide-oxidizing bacteria at cold seeps of the Nile Deep Sea Fan (Eastern Mediterranean Sea). *Geobiology* **9**: 330–48.

Hansen, C.J., Esposito, L., Stewart, A.I.F., Colwell, J., Hendrix, A., Pryor, W., et al. (2006) Enceladus' water vapor plume. *Science* **311**: 1422–5.

Harris, J.K., Caporaso, J.G., Walker, J.J., Spear, J.R., Gold, N.J., Robertson, C.E., et al. (2013) Phylogenetic stratigraphy in the Guerrero Negro hypersaline microbial mat. *ISME J.* **7**: 50–60.

Hedrich, S., Schlömann, M., and Johnson, D.B. (2011) The iron-oxidizing proteobacteria. *Microbiology* **157**: 1551–64.

Heijs, S.K., Damsté, J.S.S., and Forney, L.J. (2005) Characterization of a deep-sea microbial mat from an active cold seep at the Milano mud volcano in the Eastern Mediterranean Sea. *FEMS Microbiol. Ecol.* **54**: 47–56.

Hugenholtz, P., Pitulle, C., Hershberger, K.L., and Pace, N.R. (1998) Novel Division Level Bacterial Diversity in a Yellowstone Hot Spring. *J. Bacteriol.* **180**: 366–376.

Hungate, R.E. (1969) A roll tube method for cultivation of strict anaerobes. In, Norris,R. and Ribbons,D.W. (eds), *Methods in Microbiology, vol. 3B*. Academic Press Inc., New York, pp. 117–132.

Iess, L., Stevenson, D.J., Parisi, M., Hemingway, D., Jacobson, R.A., Lunine, J.I., et al. (2014) The gravity field and interior structure of Enceladus. *Science* **344**: 78–80.

Johnston, D.T., Farquhar, J., Habicht, K.S., and Canfield, D.E. (2008) Sulphur isotopes and the search for life: strategies for identifying sulphur metabolisms in the rock record and beyond. *Geobiology* **6**: 425–35.

Jones, S.E. and Lennon, J.T. (2010) Dormancy contributes to the maintenance of microbial diversity. *Proc. Natl. Acad. Sci. U. S. A.* **107**: 5881–6.

Jørgensen, B.B. (1982) Mineralization of organic matter in the sea bed—the role of sulphate reduction. *Nature* **296**: 643–645.

Jorgensen, B.B. and Marais, D.J. (1986) Competition for sulfide among colorless and purple sulfur bacteria in cyanobacterial mats. *FEMS Microbiol. Lett.* **38**: 179–186.

Karr, E.A., Sattley, W.M., Rice, M.R., Jung, D.O., Madigan, M.T., and Achenbach, L.A. (2005) Diversity and distribution of sulfate-reducing bacteria in permanently frozen Lake Fryxell, McMurdo Dry Valleys, Antarctica. *Appl. Environ. Microbiol.* **71**: 6353–9.

Knittel, K., Kuever, J., Meyerdierks, A., Meinke, R., Amann, R., and Brinkhoff, T. (2005) Thiomicrospira arctica sp. nov. and Thiomicrospira psychrophila sp. nov., psychrophilic, obligately chemolithoautotrophic, sulfur-oxidizing bacteria isolated from marine Arctic sediments. *Int. J. Syst. Evol. Microbiol.* **55**: 781–6.

Knoblauch, C. and Jorgensen, B.B. (1999) Effect of temperature on sulphate reduction, growth rate and growth yield in five psychrophilic sulphate-reducing bacteria from Arctic sediments. *Environ. Microbiol.* **1**: 457–467.

Kolukirik, M., Ince, O., Cetecioglu, Z., Celikkol, S., and Ince, B.K. (2011) Spatial and temporal changes in microbial diversity of the Marmara Sea Sediments. *Mar. Pollut. Bull.* **62**: 2384–2394.

Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B., and Stahl, D.A. (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **437**: 543–6.

Kuever, J., Rainey, F.A., and Widdel, F. (2005) Class IV. Deltaproteobacteria class nov. In, Brenner,D.J., Krieg,N.R., Staley,J.T., and Garrity,G.M. (eds), *Bergey's Manual of Systematic Bacteriology*. Springer, New York, pp. 922–1144.

Lamarche-Gagnon, G., Comery, R., Greer, C.W., and Whyte, L.G. (2015) Evidence of in situ microbial activity and sulphidogenesis in perennially sub-0 °C and hypersaline sediments of a high Arctic permafrost spring. *Extremophiles* **19**: 1–15.

Lassen, C., Ploug, H., and JÃ¸rgensen, B.B. (1992) A fibre-optic scalar irradiance microsensor: application for spectral light measurements in sediments. *FEMS Microbiol. Lett.* **86**: 247–254.

Lauritzen, S.-E. and Bottrell, S. (1994) Microbiological activity in thermoglacial karst springs, south spitsbergen. *Geomicrobiol. J.* **12**: 161–173.

Lay, C.-Y., Mykytczuk, N.C.S., Niederberger, T.D., Martineau, C., Greer, C.W., and Whyte, L.G. (2012) Microbial diversity and activity in hypersaline high Arctic spring channels. *Extremophiles* **16**: 177–91.

Lay, C.-Y., Mykytczuk, N.C.S., Yergeau, E., Lamarche-Gagnon, G., Greer, C.W., and Whyte, L.G. (2013) Defining the functional potential and active community members of a sediment microbial community in a high Arctic hypersaline subzero spring. *Appl. Environ. Microbiol.*

Levy, J. (2012) Hydrological characteristics of recurrent slope lineae on Mars: Evidence for liquid flow through regolith and comparisons with Antarctic terrestrial analogs. *Icarus* **219**: 1–4.

Ley, R.E., Harris, J.K., Wilcox, J., Spear, J.R., Miller, S.R., Bebout, B.M., et al. (2006) Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. *Appl. Environ. Microbiol.* **72**: 3685–95.

Lloyd, K.G., Albert, D.B., Biddle, J.F., Chanton, J.P., Pizarro, O., and Teske, A. (2010) Spatial structure and activity of sedimentary microbial communities underlying a Beggiatoa spp.

mat in a Gulf of Mexico hydrocarbon seep. *PLoS One* **5**: e8738.

López-López, A., Richter, M., Peña, A., Tamames, J., and Rosselló-Móra, R. (2013) New insights into the archaeal diversity of a hypersaline microbial mat obtained by a metagenomic approach. *Syst. Appl. Microbiol.* **36**: 205–14.

Lorenz, M.G. and Wackernagel, W. (1994) Bacterial gene transfer by natural genetic transformation in the environment. *Microbiol. Rev.* **58**: 563–602.

Lubick, N. (2002) Snowball fights. *Nature* **417**: 12–3.

Machel, H.. (2001) Bacterial and thermochemical sulfate reduction in diagenetic settings — old and new insights. *Sediment. Geol.* **140**: 143–175.

Margesin, R. and Miteva, V. (2011) Diversity and ecology of psychrophilic microorganisms. *Res. Microbiol.* **162**: 346–61.

Margulis, L., Barghoorn, E.S., Ashendorf, D., Banerjee, S., Chase, D., Francis, S., et al. (1980) The microbial community in the layered sediments at Laguna Figueroa, Baja California, Mexico: Does it have Precambrian analogues? *Precambrian Res.* **11**: 93–123.

Marshall, K.C. (1989) Cyanobacterial-heterotrophic bacterial interactions. In, Cohen,Y. and Rosenberg,E. (eds), *Microbial Mats: Physiological Ecology of Benthic Microbial Communities*. ASM, Washington, DC, pp. 239–245.

McCord, T.B., Hansen, G.B., Fanale, F.P., W., R., Carlson, Matson, D.L., et al. (1998) Salts on Europa's Surface Detected by Galileo's Near Infrared Mapping Spectrometer. *Science (80-.).* **280**: 1242–1245.

McEwen, A.S., Ojha, L., Dundas, C.M., Mattson, S.S., Byrne, S., Wray, J.J., et al. (2011) Seasonal flows on warm Martian slopes. *Science* **333**: 740–3.

McGinnis, S. and Madden, T.L. (2004) BLAST: at the core of a powerful and diverse set of sequence analysis tools. *Nucleic Acids Res.* **32**: W20–5.

Mills, H.J., Martinez, R.J., Story, S., and Sobecky, P.A. (2004) Identification of members of the metabolically active microbial populations associated with Beggiatoa species mat communities from Gulf of Mexico cold-seep sediments. *Appl. Environ. Microbiol.* **70**: 5447–58.

Moissl, C., Rudolph, C., and Huber, R. (2002) Natural Communities of Novel Archaea and Bacteria with a String-of-Pearls-Like Morphology: Molecular Analysis of the Bacterial Partners. *Appl. Environ. Microbiol.* **68**: 933–937.

Moreira, D., Rodríguez-Valera, F., and López-García, P. (2006) Metagenomic analysis of

mesopelagic Antarctic plankton reveals a novel deltaproteobacterial group. *Microbiology* **152**: 505–17.

Mueller, D.R., Vincent, W.F., Bonilla, S., and Laurion, I. (2005) Extremotrophs, extremophiles and broadband pigmentation strategies in a high arctic ice shelf ecosystem. *FEMS Microbiol. Ecol.* **53**: 73–87.

Müller, A.L., Kjeldsen, K.U., Rattei, T., Pester, M., and Loy, A. (2014) Phylogenetic and environmental diversity of DsrAB-type dissimilatory (bi)sulfite reductases. *ISME J.*

Muyzer, G. and Stams, A.J.M. (2008) The ecology and biotechnology of sulphate-reducing bacteria. *Nat. Rev. Microbiol.* **6**: 441–54.

Nadeau, J.L., Perreault, N.N., Niederberger, T.D., Whyte, L.G., Sun, H.J., and Leon, R. (2008) Fluorescence microscopy as a tool for in situ life detection. *Astrobiology* **8**: 859–74.

Nercessian, O., Reysenbach, A.-L., Prieur, D., and Jeanthon, C. (2003) Archaeal diversity associated with in situ samplers deployed on hydrothermal vents on the East Pacific Rise (13oN). *Environ. Microbiol.* **5**: 492–502.

Neretin, L.N., Schippers, A., Pernthaler, A., Hamann, K., Amann, R., and J��rgensen, B.B. (2003) Quantification of dissimilatory (bi)sulphite reductase gene expression in Desulfobacterium autotrophicum using real-time RT-PCR. *Environ. Microbiol.* **5**: 660–671.

Niederberger, T.D., Perreault, N.N., Lawrence, J.R., Nadeau, J.L., Mielke, R.E., Greer, C.W., et al. (2009) Novel sulfur-oxidizing streamers thriving in perennial cold saline springs of the Canadian high Arctic. *Environ. Microbiol.* **11**: 616–29.

Niederberger, T.D., Perreault, N.N., Tille, S., Lollar, B.S., Lacrampe-Couloume, G., Andersen, D., et al. (2010) Microbial characterization of a subzero, hypersaline methane seep in the Canadian High Arctic. *ISME J.* **4**: 1326–39.

Nomura, M., Gourse, R., and Baughman, G. (1984) Regulation of the synthesis of ribosomes and ribosomal components. *Annu. Rev. Biochem.* **53**: 75–117.

Nunoura, T., Takaki, Y., Kazama, H., Hirai, M., Ashi, J., Imachi, H., and Takai, K. (2012) Microbial diversity in deep-sea methane seep sediments presented by SSU rRNA gene tag sequencing. *Microbes Environ.* **27**: 382–90.

Oakley, B.B., Carbonero, F., Dowd, S.E., Hawkins, R.J., and Purdy, K.J. (2012) Contrasting patterns of niche partitioning between two anaerobic terminal oxidizers of organic matter. *ISME J.* **6**: 905–14.

Pellerin, A., Anderson-Trocmé, L., Whyte, L.G., Zane, G.M., Wall, J.D., and Wing, B.A. (2015) Sulfur isotope fractionation during the evolutionary adaptation of a sulfate-reducing

bacterium. *Appl. Environ. Microbiol.* **81**: 2676–89.

Perreault, N.N., Andersen, D.T., Pollard, W.H., Greer, C.W., and Whyte, L.G. (2007) Characterization of the prokaryotic diversity in cold saline perennial springs of the Canadian high Arctic. *Appl. Environ. Microbiol.* **73**: 1532–43.

Perreault, N.N., Greer, C.W., Andersen, D.T., Tille, S., Lacrampe-Couloume, G., Lollar, B.S., and Whyte, L.G. (2008) Heterotrophic and autotrophic microbial populations in cold perennial springs of the high arctic. *Appl. Environ. Microbiol.* **74**: 6898–907.

Pfennig, N. and Biebl, H. (1976) Desulfuromonas acetoxidans gen. nov. and sp. nov., a new anaerobic, sulfur-reducing, acetate-oxidizing bacterium. *Arch. Microbiol.* **110**: 3–12.

Pienaar, R.N., Sakai, H., and Horiguchi, T. (2007) Description of a new dinoflagellate with a diatom endosymbiont, Durinskia capensis sp. nov. (Peridiniales, Dinophyceae) from South Africa. *J. Plant Res.* **120**: 247–58.

Pollard, W., Haltigin, T., Whyte, L., Niederberger, T., Andersen, D., Omelon, C., et al. (2009) Overview of analogue science activities at the McGill Arctic Research Station, Axel Heiberg Island, Canadian High Arctic. *Planet. Space Sci.* **57**: 646–659.

Pollard, W., Omelon, C., Andersen, D., and McKay, C. (1999) Perennial spring occurrence in the Expedition Fiord area of western Axel Heiberg Island, Canadian High Arctic. *Can. J. Earth Sci.* **36**: 105–120.

Rabus, R., Hansen, T.A., and Widdel, F. (2006) Dissimilatory Sulfate- and Sulfur-Reducing Prokaryotes. In, Dworkin,M., Falkow,S., Rosenberg,E., Schleifer,K.-H., and Stackebrandt,E. (eds), *The Prokaryotes*. Springer New York, New York, NY, pp. 659–768.

Robertson, C.E., Spear, J.R., Harris, J.K., and Pace, N.R. (2009) Diversity and stratification of archaea in a hypersaline microbial mat. *Appl. Environ. Microbiol.* **75**: 1801–10.

Robertson, L.A. and Kuenen, J.G. (2006) The Colorless Sulfur Bacteria. In, Dworkin,M., Falkow,S., Rosenberg,E., Schleifer,K.-H., and Stackebrandt,E. (eds), *The Prokaryotes*. Springer New York, New York, NY, pp. 985–1011.

Rogers, G.B., Kozlowska, J., Keeble, J., Metcalfe, K., Fao, M., Dowd, S.E., et al. (2014) Functional divergence in gastrointestinal microbiota in physically-separated genetically identical mice. *Sci. Rep.* **4**: 5437.

Rogers, J.D., Perreault, N.N., Niederberger, T.D., Lichten, C., Whyte, L.G., and Nadeau, J.L. (2010) A life detection problem in a High Arctic microbial community. *Planet. Space Sci.* **58**: 623–630.

Röling, W.F.M. (2007) Do microbial numbers count? Quantifying the regulation of

biogeochemical fluxes by population size and cellular activity. *FEMS Microbiol. Ecol.* **62**: 202–10.

Roth, L., Saur, J., Retherford, K.D., Strobel, D.F., Feldman, P.D., McGrath, M.A., and Nimmo, F. (2014) Transient water vapor at Europa's south pole. *Science* **343**: 171–4.

Rudolph, C., Wanner, G., and Huber, R. (2001) Natural communities of novel archaea and bacteria growing in cold sulfurous springs with a string-of-pearls-like morphology. *Appl. Environ. Microbiol.* **67**: 2336–44.

Sattley, W.M. and Madigan, M.T. (2010) Temperature and nutrient induced responses of Lake Fryxell sulfate-reducing prokaryotes and description of Desulfovibrio lacusfryxellense, sp. nov., a pervasive, cold-active, sulfate-reducing bacterium from Lake Fryxell, Antarctica. *Extremophiles* **14**: 357–66.

Schloss, P.D., Gevers, D., and Westcott, S.L. (2011) Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. *PLoS One* **6**: e27310.

Schloss, P.D. and Handelsman, J. (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl. Environ. Microbiol.* **71**: 1501–6.

Schneider, D., Arp, G., Reimer, A., Reitner, J., and Daniel, R. (2013) Phylogenetic analysis of a microbialite-forming microbial mat from a hypersaline lake of the Kiritimati atoll, Central Pacific. *PLoS One* **8**: e66662.

Scott, C., Wing, B.A., Bekker, A., Planavsky, N.J., Medvedev, P., Bates, S.M., et al. (2014) Pyrite multiple-sulfur isotope evidence for rapid expansion and contraction of the early Paleoproterozoic seawater sulfate reservoir. *Earth Planet. Sci. Lett.* **389**: 95–104.

Shi, Y., Tyson, G.W., Eppley, J.M., and DeLong, E.F. (2011) Integrated metatranscriptomic and metagenomic analyses of stratified microbial assemblages in the open ocean. *ISME J.* **5**: 999–1013.

Sievert, S.M., Heidorn, T., and Kuever, J. (2000) Halothiobacillus kellyi sp. nov., a mesophilic, obligately chemolithoautotrophic, sulfur-oxidizing bacterium isolated from a shallow-water hydrothermal vent in the Aegean Sea, and emended description of the genus Halothiobacillus. *Int. J. Syst. Evol. Microbiol.* **50**: 1229–1237.

Sievert, S.M., Ziebis, W., Kuever, J., and Sahm, K. (2000) Relative abundance of Archaea and Bacteria along a thermal gradient of a shallow-water hydrothermal vent quantified by rRNA slot-blot hybridization. *Microbiology* **146 (Pt 6**: 1287–93.

Sigalevich, P., Meshorer, E., Helman, Y., and Cohen, Y. (2000) Transition from anaerobic to

aerobic growth conditions for the sulfate-reducing bacterium Desulfovibrio oxyclinae results in flocculation. *Appl. Environ. Microbiol.* **66**: 5005–12.

Sorokin, D.Y. and Muyzer, G. (2010) Desulfurispira natronophila gen. nov. sp. nov.: an obligately anaerobic dissimilatory sulfur-reducing bacterium from soda lakes. *Extremophiles* **14**: 349–55.

Stackebrandt, E. and Goebel, B.M. (1994) Taxonomic Note: A Place for DNA-DNA Reassociation and 16S rRNA Sequence Analysis in the Present Species Definition in Bacteriology. *Int. J. Syst. Bacteriol.* **44**: 846–849.

Stal, L.J. and Moezelaar, R. (1997) Fermentation in cyanobacteria. *FEMS Microbiol. Rev.* **21**: 179–211.

Surkov, A. V, Dubinina, G.A., Lysenko, A.M., Glöckner, F.O., and Kuever, J. (2001) Dethiosulfovibrio russensis sp. nov., Dethosulfovibrio marinus sp. nov. and Dethosulfovibrio acidaminovorans sp. nov., novel anaerobic, thiosulfate- and sulfur-reducing bacteria isolated from "Thiodendron" sulfur mats in different saline environments. *Int. J. Syst. Evol. Microbiol.* **51**: 327–37.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* **30**: 2725–9.

Taylor, A.E. and Judge, A.S. (1976) Canadian Geothermal Data Collection— Northern Wells 1975 Ottawa.

Thompson, J.D., Higgins, D.G., and Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**: 4673–80.

Tostevin, R., Turchyn, A. V., Farquhar, J., Johnston, D.T., Eldridge, D.L., Bishop, J.K.B., and McIlvin, M. (2014) Multiple sulfur isotope constraints on the modern sulfur cycle. *Earth Planet. Sci. Lett.* **396**: 14–21.

Van Trappen, S., Mergaert, J., Van Eygen, S., Dawyndt, P., Cnockaert, M.C., and Swings, J. (2002) Diversity of 746 heterotrophic bacteria isolated from microbial mats from ten Antarctic lakes. *Syst. Appl. Microbiol.* **25**: 603–10.

Vandieken, V., Mussmann, M., Niemann, H., and Jørgensen, B.B. (2006) Desulfuromonas svalbardensis sp. nov. and Desulfuromusa ferrireducens sp. nov., psychrophilic, Fe(III)-reducing bacteria isolated from Arctic sediments, Svalbard. *Int. J. Syst. Evol. Microbiol.* **56**: 1133–9.

Varin, T., Lovejoy, C., Jungblut, A.D., Vincent, W.F., and Corbeil, J. (2010) Metagenomic

profiling of Arctic microbial mat communities as nutrient scavenging and recycling systems. *Limnol. Oceanogr.* **55**: 1901–1911.

Vincent, W.F. (2007) Cold Tolerance in Cyanobacteria and Life in the Cryosphere. In, Seckbach,J. (ed), *Algae and Cyanobacteria in Extreme Environments*, Cellular Origin, Life in Extreme Habitats and Astrobiology. Springer Netherlands, Dordrecht, pp. 287–301.

Vincent, W.F., Gibson, J.A.E., Pienitz, R., Villeneuve, V., Broady, P.A., Hamilton, P.B., and Howard-Williams, C. (2000) Ice Shelf Microbial Ecosystems in the High Arctic and Implications for Life on Snowball Earth. *Naturwissenschaften* **87**: 137–141.

Vincent, W.F., Mueller, D., Van Hove, P., and Howard-Williams, C. (2005) Glacial periods on early Earth and implications for the evolution of life. In, Seckbach,J. (ed), *Origins*, Cellular Origin, Life in Extreme Habitats and Astrobiology. Springer Netherlands, Dordrecht, pp. 481–500.

Visscher, P.T., Prins, R.A., and Gemerden, H. (1992) Rates of sulfate reduction and thiosulfate consumption in a marine microbial mat. *FEMS Microbiol. Ecol.* **9**: 283–294.

Wagner, D. (2008) Microbial Communities and Processes in Arctic Permafrost Environments. In, Dion,P. and Nautiyal,C.S. (eds), *Microbiology of Extreme Soils*, Soil Biology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 133–154.

Walter, M.R. (1976) Stromatolites Elsevier.

Wang, Q., Quensen, J.F., Fish, J.A., Lee, T.K., Sun, Y., Tiedje, J.M., and Cole, J.R. (2013) Ecological patterns of nifH genes in four terrestrial climatic zones explored with targeted metagenomics using FrameBot, a new informatics tool. *MBio* **4**: e00592–13.

Widdel, F. and Bak, F. (1992) Gram-Negative Sulfate-Reducing Bacteria. In, Balows,A., Trüper,H.G., Dworkin,M., Harder,W., and Schleifer,K.-H. (eds), *The Prokaryotes*. Springer New York, New York, NY, pp. 3352–3378.

Wilhelm, R.C., Niederberger, T.D., Greer, C., and Whyte, L.G. (2011) Microbial diversity of active layer and permafrost in an acidic wetland from the Canadian High Arctic. *Can. J. Microbiol.* **57**: 303–15.

Yoneda, Y., Yoshida, T., Kawaichi, S., Daifuku, T., Takabe, K., and Sako, Y. (2012) Carboxydothermus pertinax sp. nov., a thermophilic, hydrogenogenic, Fe(III)-reducing, sulfur-reducing carboxydotrophic bacterium from an acidic hot spring. *Int. J. Syst. Evol. Microbiol.* **62**: 1692–7.

Yu, K. and Zhang, T. (2012) Metagenomic and metatranscriptomic analysis of microbial community structure and gene expression of activated sludge. *PLoS One* **7**: e38183.

Zentilli, M., Hanley, J., LeFort, D., and Omelon, C. (2013) Fluid inclusion study of salt-dome related hydrothermal development on Axel Heiberg Island, Canadian Arctic Archipelago. In, *Atlantic Geoscience Society Colloquium*.

Zhalnina, K. V, Dias, R., Leonard, M.T., Dorr de Quadros, P., Camargo, F.A.O., Drew, J.C., et al. (2014) Genome sequence of Candidatus Nitrososphaera evergladensis from group I.1b enriched from Everglades soil reveals novel genomic features of the ammonia-oxidizing archaea. *PLoS One* **9**: e101648.

Zhang, C.L., Huang, Z., Cantu, J., Pancost, R.D., Brigmon, R.L., Lyons, T.W., and Sassen, R. (2005) Lipid biomarkers and carbon isotope signatures of a microbial (Beggiatoa) mat associated with gas hydrates in the gulf of Mexico. *Appl. Environ. Microbiol.* **71**: 2106–12.