Diversity and function of sulfur cycling microorganisms in sediments from Subglacial Lake Whillans, Antarctica

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Alicia Marie Purcell
December 2014

ACKNOWLEDGEMENTS

I would like to express gratitude to my advisor, Dr. Jill Mikucki. Her knowledge, dedication, and excitement regarding science, especially that in Antarctica is inspiring. With her guidance, I have gained many skills and confidence as a scientist. I am grateful for the opportunity she gave me, that expanded my interest in polar microbiology and for the opportunities that are now open. I would like to extend my acknowledgement to Dr. Dhritiman Ghosh for his encouragement and help, especially at a time when I needed it the most. I would also like to thank my thesis committee members, Dr. Steven Wilhelm and Dr. Karen Lloyd for their direction and advice regarding my research project.

I would like to thank my everlasting support system over my entire life, my parents, who are my two best role models. My work in the past two years would not have been possible without the encouragement of my family and friends. In particular, I would like to acknowledge my lab mate Michelle Chua, and friends, Lauren Krausfeldt, Robbie Martin, and Megan Silbaugh for their support and friendship. I would also like to thank the entire Department of Microbiology at the University of Tennessee through which I have had an enriching experience.

The research conducted in this thesis would not have been possible without the long-term aspiration of many Antarctic scientists that have pursued the knowledge, funds, and logistical efforts for accessing subglacial Antarctica, one of the last frontiers on Earth. I would also like to acknowledge the work performed by the WISSARD science team (and associated support), that made my research project possible. The research

conducted in this thesis was made possible through the financial support by the National Science Foundation's (NSF) Office of Polar Programs (OPP).

ABSTRACT

There is a growing consensus that metabolically and phylogenetically diverse assemblages of microorganisms mediate subglacial nutrient and elemental cycling. Subglacial Lake Whillans (SLW), located under 801 m of glacial ice, was recently penetrated using environmentally clean protocols. SLW is a permanently dark, cold (-0.5 °C [degrees Celsius]), and shallow (~2.2 m) freshwater lake beneath the West Antarctic Ice Sheet. The presence and diversity of key functional genes involved in dissimilatory sulfur oxidation and reduction were examined at various depths in two sediment cores taken from SLW. Our data show a diversity of sulfur transformation genes throughout the top 34 cm of SLW sediments, which changes with depth. The surficial sediments appear dominated by genes related to known sulfur-oxidizing chemoautotrophs. Sequences encoding the adenosine-5'-phosphosulfate (APS) reductase gene, involved in both dissimilatory sulfate reduction and sulfur oxidation were present in all samples and clustered into 16 distinct Operational Taxonomic Units (OTUs). The majority (74%) of APS reductase sequences clustered with known chemoautotrophic sulfur oxidizers including the "Sideroxydans" and *Thiobacillus* genera, but members of the genera *Thermodesulfovibrio*, *Desulfobacterium*, and *Desulfotomaculum* were also observed. Detection of sequences encoding for reverse-acting dissimilatory sulfite reductase (rDSR) were consistent with 16S rRNA gene data which indicated phylotypes of "Sideroxydans" and *Thiobacillus* were abundant in the top 2 cm of SLW sediments. Low rates (1.4 pmol [picomoles] cm^{-3} [cubed centimeter] d^{-1} [day]) of biologically-mediated sulfate reduction occurred in samples from 0-8 cm in bulk anaerobic sediment incubations. To our

knowledge, these are the first reported measurements of sulfate reduction from an Antarctic subglacial environment. Sequences encoding for the dissimilatory sulfite reductase gene (DSR) from known sulfate-reducing prokaryotes (SRP) indicates that the sediment microbial community has the genetic potential to reduce sulfate. The contribution of microbial activity to mineral weathering via sulfur driven chemosynthesis is relevant for understanding the ecology of subglacial lake ecosystems and their role as solute and nutrient sources to the Southern Ocean.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

- *aprA* - adenosine-5'-phosphosulfate reductase alpha subunit
- APS – adenosine-5'-phosphosulfate reductase
- bp – basepair
- DNA – Deoxyribonucleic acid
- DSR – Dissimilatory sulfite reductase
- *dsrA*(*B*) – Dissimilatory sufite reductase alpha (beta) subunit
- MC-2B – Sediment multicore '2B'
- MC-3C – Sediment multicore '3C'
- OTU – Operational Taxonomic Unit
- Q-PCR – Quantitative polymerase chain reaction
- rDSR – Reverse dissimilatory sulfite reductase
- *rdsrA*(*B*) – Reverse dissimilatory sufite reductase alpha (beta) subunit
- rRNA – ribosomal ribonucleic acid
- SALE – Subglacial Antarctic Lake Exploration
- SLW – Subglacial Lake Whillans
- SOP – Sulfur-oxidizing prokaryotes
- SRP – Sulfate-reducing prokaryotes
- SRR – Sulfate reduction rate
- WAIS – West Antarctic Ice Sheet
- WIS – Whillans Ice Stream
- WISSARD – Whillans Ice Stream Subglacial Access Research Drilling

I. INTRODUCTION AND RESEARCH AIMS

Introduction

Subglacial aquatic environments

Ice covers ~10% of the Earth's terrestrial surface, yet only recently with the detection of microorganisms above, within, and below glaciers, has ice been identified as a portion of the biosphere (Anesio and Laybourn-Parry, 2011; Priscu and Christner, 2004; Priscu, 2008; Edwards et al., 2014). Glaciers and ice sheets are not described as biomes on Earth in most textbooks, yet it is estimated that 1×10^{26} microbial cells exist within the Antarctic Ice Sheet and subglacial lakes alone (Priscu and Christner, 2004). Recent compilations of geophysical surveys have identified expansive systems of liquid water beneath the Antarctic Ice Sheet (Priscu et al., 2008; Fricker and Scambos, 2009; Wright and Siegert, 2012) and in Arctic regions (Fahnestock et al., 2001; Andersen et al., 2004; Gaidos et al., 2004). The focus of this thesis is on Antarctic subglacial environments, which remain largely unexplored due to their remoteness and seclusion beneath the thick (between 0.3-5 km) Antarctic Ice Sheet (Fretwell et al., 2013).

Subglacial Antarctic Lake Exploration (SALE) motivations

The Scientific Committee on Antarctic Research (SCAR) organized an international group of researchers to establish an initiative called Subglacial Antarctic Lake Exploration (SALE). This group developed a roadmap for large-scale projects that would enable the study of subglacial Antarctica (Priscu et al., 2003). SALE established priorities to determine how and which Antarctic subglacial lakes should be studied by assessing necessary technology, environmental considerations, and research goals (Priscu

et al., 2003). This organization recognized the challenges in studying subglacial lakes and promoted an interdisciplinary approach so that the study of the geology, biology, glaciology, and geochemistry of these subglacial systems would be integrated (Priscu et al., 2003). SALE acknowledges the importance of environmental stewardship with regards to pristine Antarctic ecosystems and that potential contaminates (such as chemicals or non-native organisms) could compromise the environment as well as future scientific analyses. SALE aims to promote the study of many subglacial lakes over time to survey the varying environments that exist beneath the large Antarctic continent. Like on other continents, each Antarctic subglacial lake is unique with physical and biological characteristics influenced by bed topography, elevation, and potential interconnectivity. Therefore sampling just one lake is not necessarily representative of all. Studying many of these subglacial lakes will allow for numerous scientific goals to be addressed, e.g. determining climatic information, understand the subglacial hydrological system, the origin of subglacial lakes, and the structure, function, and evolution of microbial life in these ecosystems (Priscu et al., 2003). The long-term goals established by SALE laid the framework for the long-term vision of subglacial access. This planning has led to the retrieval of the samples analyzed for this thesis.

The Antarctic subglacial water system

The water present beneath Antarctica is dynamic; ~130 of the 379 subglacial lakes identified in Antarctica (Wright and Siegert, 2012) are considered temporary storage reservoirs for liquid water (Fricker et al., 2007; Smith et al., 2009). These lakes were identified from ice surface elevation changes and are considered 'active lakes' because they drain and refill their contents sub-decadally (Fricker et al., 2007; Smith et

al., 2009). These ‘active lakes’ promote water movement, and likely sediment movement that influences ice flow rates. Understanding the physical activity of these systems is important for determining ice sheet stability, to predict potential ice sheet collapse that has occurred in the past (Scherer et al., 1998). Some of these ‘active’ lakes are connected along hydrological flow paths, creating subglacial estuaries that lead to the Southern Ocean (Carter and Fricker, 2012; Horgan et al., 2013).

The Whillans Ice Stream (WIS) and Subglacial Lake Whillans (SLW)

The Whillans Ice Stream (WIS) is one of six ice streams that drain the Siple Coast of West Antarctica into the Ross Sea (Hughes, 1977). These ice streams have unpredictable movement that is affected by melt water, geothermal flux, and water saturated sediment (Alley et al., 1986). This area of Antarctica is an important focus because if the ice covering West Antarctica were to melt, global sea level could rise by 4.3 meters (Fretwell et al. 2013). The WIS overlies a system of connected ‘active’ subglacial lakes (Fricker et al., 2007; Fricker and Scambos, 2009). One of these lakes, Subglacial Lake Whillans, is a small (0.59 km^2) and shallow (2.2 m), freshwater lake 801 m beneath the lower portion of the WIS (Christianson et al., 2012; Tulacyzk et al., 2014; Christner et al., 2014). The National Science Foundation funded an interdisciplinary project to study this lake, The Whillans Ice Stream Subglacial Access Research Drilling (WISSARD) Project. WISSARD is multi-disciplinary, multi-institutional initiative aiming to understand the water system, ice and sediment movement, stability, ocean interactions, geochemistry, and microbiology beneath the WIS (Fricker et al., 2011).

Water and sediment samples from Subglacial Lake Whillans were collected in January 2013 (Tulaczyk et al., 2014). A clean access protocol was employed for

environmental stewardship including an extensive melt water filtration system, UV and hydrogen peroxide treated instruments and cables as described by Priscu et al. (2013). The biology portion of the WISSARD project aimed to understand the microbial metabolic and phylogenetic diversity and associated mineral weathering reactions in a subglacial environment by analyzing sediment and water samples collected directly from an Antarctic subglacial lake (Fricker et al., 2011).

Subglacial microbial metabolic activity

Subglacial environments are defined as the interface between glaciers, ice sheets, and the underlying bedrock material, where ice, water, and mineral surfaces interact. Microorganisms have been detected in all sampled subglacial aquatic environments including a subglacial lake beneath the Grimsvötn Glacier, Iceland (Gaidos et al., 2004), sediments beneath Robertson Glacier, Canada (Hamilton et al., 2013), a brine outflow from beneath the Taylor Glacier in the McMurdo Dry Valleys, East Antarctica (Mikucki et al., 2004), and sediments beneath the Kamb Ice Stream, West Antarctica (Lanoil et al., 2009). The abundance of microorganisms in subglacial water range from 1×10^3 to 10^7 cells ml^{-1} (Christner et al., 2006; Mikucki and Priscu, 2007; Gaidos et al., 2004; Miteva et al., 2004; Sharp et al., 1999; Christner et al., 2014) and 5×10^4 to 5×10^7 cells g^{-1} (Gaidos et al., 2004; Lanoil et al., 2009; Pearce et al., 2013) in subglacial sediments.

Microbial metabolic activity beneath Arctic and Alpine glaciers and Antarctic Ice Sheet support both heterotrophic activity and chemoautotrophic production (e.g. Mikucki et al., 2004; Christner et al., 2006; Boyd et al., 2014). A combination of 16S rRNA gene surveys, geochemical data, and activity measurements have revealed diverse metabolic activities are likely to occur in these ecosystems, including sulfur oxidation, sulfate

reduction, iron oxidation and reduction, nitrate reduction, ammonia oxidation, and methanogenesis (Skidmore et al., 2000; Bottrell and Tranter, 2002; Wadham et al., 2004; Christner et al., 2006; Mikucki and Priscu, 2007; Mikucki et al., 2009; Lanoil et al., 2009; Christner et al., 2014).

SLW microbial community

Chemoautotrophic and heterotrophic activity were measured in the SLW water column, using ^{14}C-bicarbonate (32.9 ng C l^{-1} d^{-1}), and ^{3}H-leucine (2.9 ng C l^{-1} d^{-1}), respectively (Christner et al., 2014). 16S rRNA gene analyses (99.5% sequencing coverage) revealed phylotypes related to the nitrite-oxidizing *Candidatus Nitrotoga* were the most abundant (13%; Christner et al., 2014). Relatives of the ammonia oxidizing *Candidatus Nitrosoarchaeum* (2.5%) were also abundant (Christner et al., 2014). These phylotypes supported reported values of $\Delta^{17}O$ of NO_3 which indicates that primary production in the water column may be driven by nitrification (Christner et al., 2014). Dominant phylotypes identified in the SLW surficial sediments (0-2 cm) included clones related to sulfur and iron oxidizers including "Sideroxydans" (12%) and *Thiobacillus* (6%) (Christner et al., 2014).

The sulfur cycle

The element sulfur is essential for all life. The largest reservoir for biologically available sulfur on Earth is sulfate in the ocean, but an abundance of sulfur is contained in bedrock sulfide and sulfate minerals (Madigan, 2005). Sulfur transformations occur abiotically and biotically and sulfur exists in many oxidation states from -2 (sulfide) to +6 (sulfate), which participate in various redox reactions. The sulfur cycle is influenced by microbial metabolic activity and tightly linked to other element cycles including

carbon, iron, oxygen, and nitrogen. All organisms assimilate sulfur, for production of the amino acids cysteine and methionine and components of enzymes. Conversely, diverse groups of prokaryotes utilize sulfur compounds as energy sources for cell growth, carbon fixation, and anaerobic respiration. The sulfur-oxidizing prokaryotes (SOP) are represented in many phylogenetic groups including the anoxygenic phototrophic Chlorobi phylum and the *Gammaproteobacteria* order *Chromatiales*, within the chemolithoautotrophic members of *Betaproteobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*, and the archaeal phyla Crenarchaeota (Friedrich et al., 2001; Friedrich et al., 2005). SOP can oxidize a variety of sulfur compounds, including elemental sulfur, polysulfide, thiosulfate, sulfite, and sulfide in redox reactions with a variety of electron acceptors including O_2, NO_3^-, $Mn^{+3/+4}$, and Fe^{+3}. Reduced sulfur compounds are either contained in reduced minerals or generated by the sulfate reducing prokaryotes (SRP) that utilize sulfate as a final electron acceptor for anaerobic respiration. Sulfate reduction is an important process in anaerobic marine sediments, where it has been estimated that SRPs are responsible for half of organic carbon oxidation globally (Canfield et al., 1993; Thullner et al., 2009; Bowles et al., 2014). SRP have also been shown in syntrophic relationships with anaerobic methane oxidizing archaea (Schink, 2006) where these syntrophic consortiums can be abundant in some marine sediments (Boetius et al., 2000; Hinrichs and Boetius, 2003).

The pathway of microbial sulfate reduction to hydrogen sulfide has been well characterized and is a three step reaction (Wagner et al., 1998; Rabus et al., 2006; Meyer and Kuever, 2007b), however, not all SRP perform all steps (Rabus et al., 2006). Sulfate reduction begins with the activation of sulfate via sulfate adenyltransferase (sat), forming

adenosine-5'-phosphosulfate (APS). APS is then reduced via adenosine-5'-phosphosulfate reductase to AMP and sulfite. Sulfite is subsequently reduced to hydrogen sulfide via the dissimilatory sulfite reductase (DSR) enzyme system. A homologue of this pathway is found in some groups of SOP, where it is proposed to work in the reverse direction (Hipp et al., 1997; Dahl et al., 2005; Meyer and Kuever, 2007a) and has been found to be responsible for the oxidation of sulfur globules (Pott and Dahl, 1998; Dahl et al., 2005; Holkenbrink et al., 2011) that are formed by some groups of anoxygenic phototrophic and chemolithoautotrophic sulfur oxidizers. Other SOP contain a multi-enzyme SOX system capable of oxidizing reduced sulfur compounds (Meyer et al., 2007).

Sulfur transformations in subglacial environments

Multiple lines of evidence support the presence of microbially mediated sulfur transformations in subglacial environments. Beneath Arctic glaciers, 16S rRNA gene surveys have identified phylotypes related to chemoautotrophs including those capable of sulfur and iron oxidation (Skidmore et al., 2005; Hamilton et al., 2013). Beneath an alpine glacier in Switzerland, low ^{18}O values of SO_4, signified sulfur oxidation likely coupled to ferric iron reduction (Bottrell and Tranter, 2002). The importance of sulfur driven chemosynthesis in sediments beneath Robertson Glacier, Canada, has been confirmed via geochemical and molecular analyses of microcosm experiments (Boyd et al., 2014). Boyd et al. (2014) reported a dominant phylotype related to "Sideroxydans lithotrophicus", a known iron and sulfur oxidizer (Emerson et al., 2013), was likely responsible for dark carbon fixation based on increased sulfate production, uptake of bicarbonate, and analysis of RuBisCo gene sequences. Sulfate reducers have been

enriched from samples beneath John Evans Glacier, Canada and geochemical analysis based on $\delta^{18}O$ and $\delta^{34}S$ values of sulfate showed that sulfate reduction occurred in meltwater beneath Findsterwalderbreen Glacier in Svalbard (Wadham et al., 2004).

Few samples have been obtained from Antarctic subglacial environments, however 16S rRNA gene analysis from accretion ice above Lake Vostok and sediments beneath the Kamb Ice Stream have indicated phylotypes related to known sulfur oxidizers (Christner et al., 2006; Lanoil et al., 2009). In the brine outflow from beneath the Taylor Glacier, Blood Falls, 16S rRNA genes related to sulfur oxidizers and reducers were identified (Mikucki and Priscu, 2007). These phylotypes supported a microbially driven sulfur cycling based on abundances of $\delta^{18}O$ and $\delta^{34}S$ of sulfate and the presence of the sulfate reduction functional gene APS reductase in Blood Falls (Mikucki et al., 2009).

While these environments show evidence of microbially driven sulfur transformations, little is known about the structure, diversity, and abundance of sulfur transforming microbes in these vast ecosystems and what their impact to other elemental cycles including nitrogen, carbon, and iron, and might be. The work presented in this thesis provides evidence of a diverse sulfur cycling microbial community present in sediments from SLW.

Research objectives and hypotheses

Little is known about the presence and function of the microbial communities in Antarctic subglacial ecosystems. It is unknown what the impact of microbial metabolic activity beneath the Antarctic Ice Sheet may have on a global scale, however it is predicted that a methane reservoir exists beneath the Antarctic Ice Sheet (Wadham et al., 2012) and that ice sheets can be a significant source of iron into the oceans (Hawkings et al., 2014). Studying subglacial community function can determine the importance of elemental cycling that potentially influences primary production in the Southern Ocean. Accordingly, how the function of microbial communities evolve and sustain themselves in permanently cold subglacial environments isolated from photosynthetic energy is not known.

The main objective of this study was to assess the diversity and abundance of microorganisms that transform sulfur compounds in SLW sediments, addressing the following overarching hypothesis and sub-hypotheses:

The microbial community residing in SLW sediments metabolizes sulfur compounds

H1: Sulfate supports heterotrophic metabolism in the SLW sediment microbial community.

Rationale: Sulfate is a favorable electron acceptor upon depletion of oxygen, nitrate, iron (III), and manganese (IV) for the oxidation of organic compounds. Sulfate reduction has been shown to be responsible for the oxidation of half the organic carbon in marine sediments globally (Jørgensen, 1982). In subglacial environments, the potential for sulfate reduction has been identified via 16S

rRNA gene analysis, enrichment experiments, geochemical data, and APS reductase analysis (Skidmore et al., 2000; Wadham et al., 2004; Mikucki et al., 2009). We propose that sulfate is utilized for the oxidation of organic carbon in SLW sediments.

Objectives to address H1:

1. Extract DNA from SLW sediments and PCR amplify two key genes involved in the only known dissimilatory sulfate reduction pathway, adenosine-5'-phosphosulfate (APS) reductase and dissimilatory sulfite reductase (DSR)
2. Measure activity of sulfate reduction via hydrogen sulfide production in ^{35}S sulfate incubation experiments

H2: Microbial communities in SLW sediment gain energy via sulfur oxidation

Rationale: SLW is a permanently dark environment; therefore sulfur oxidation could be the mechanism in which energy is generated for carbon fixation in this system. Mineral weathering via sulfur oxidation has been found to occur beneath Alpine glaciers (Bottrell and Tranter, 2002). A sulfur driven community could be present in this ecosystem dependent on chemoautotrophic primary production.

Objective to address H2:

1. PCR amplify genes involved in sulfur oxidation including, adenosine-5-phosphosulfate (APS) reductase and reverse dissimilatory sulfite reductase (rDSR)

H3: Sulfur-oxidizing microbial abundance is great than sulfate-reducing microbes at the sediment surface in SLW

Rationale: Evidence for chemosynthesis is prevalent in subglacial environments via measurements of bicarbonate incorporation (Gaidos et al., 2004; Mikucki and Priscu, 2007; Boyd et al., 2014; Christner et al., 2014) and 16S rRNA gene sequences related to sulfur oxidizing chemolithoautotrophic phylotypes (Bottrell and Tranter, 2002; Christner et al., 2006; Mikucki and Priscu, 2007; Lanoil et al., 2009; Hamilton et al., 2013; Boyd et al., 2014). Sulfur-oxidizing microbes present in SLW would likely reside in the surface sediments where oxygen is present. While SOP could also utilize alternative electron acceptors under anaerobic conditions, like nitrate or ferric iron, a higher abundance of SOP would probably take advantage of oxygen which is more energetically favorable.

Objectives to address H3:

1. Analyze the diversity of APS reductase among the depths of two sediment cores taken from SLW
2. Perform Q-PCR to quantify copies of the prokaryote 16S rRNA gene and APS reductase among the depths of two sediment cores taken from SLW

Disclosure Statement

The research presented in this thesis was conducted under the scope of the large, multi-disciplinary, multi-institutional, highly collaborative, Whillans Ice Stream Subglacial Access Research Drilling (WISSARD) project (www.wissard.org). The contents of this thesis have been accepted for publication in a peer-reviewed journal under the citation:

Purcell A. M., Mikucki, J. A., Achberger A. M., Alekhina I. A., Barbante C., Christner B. C., Ghosh D., Michaud A. B., Mitchell A. C., Priscu J. C., Scherer R., Skidmore M. L., Vick-Majors T. J., and the WISSARD Science Team. (2014). Microbial sulfur transformations in sediments from Subglacial Lake Whillans. *Front. Microbiol*. 5:594. doi: 10.3389/fmicb.2014.00594

The publication included below was revised following submission. Sections of this thesis that were not included in the publication include the Appendix "Microbial Cell Enumeration". The data collection, methods, and analyses presented in sections "^{35}S-Sulfate incubation experiments", "Sediment characteristics", and "Activity of sulfate-reducing prokaryotes" were not performed by Alicia Purcell. Alicia Purcell was also not present for sample collection in the field. This large logistical feat was made possible by the team effort that is represented in the author list of this manuscript.

II. MICROBIALLY MEDIATED SULFUR TRANSFORMATIONS IN SUBGLACIAL LAKE WHILLANS

Introduction

It is now recognized that a diversity of subglacial aquatic environments exists beneath the Antarctic Ice Sheet, including lakes, streams, marine brines, and water-saturated sediments (Priscu et al., 2008; Fricker and Scambos, 2009; Skidmore, 2011; Wright and Siegert, 2012; Mikucki et al., 2009). Recently, the Whillans Ice Stream Subglacial Access Research Drilling (WISSARD) project explored Subglacial Lake Whillans (SLW), one of 379 subglacial lakes identified beneath the Antarctic Ice Sheet (Wright and Siegert, 2012). SLW is the first Antarctic subglacial lake to be directly sampled for bulk water and sediment (Tulaczyk et al., 2014). Initial analyses of samples collected from SLW show the presence of an active community of diverse heterotrophic and autotrophic microorganisms in the water column and surficial sediments (Christner et al., 2014).

In subglacial environments, interactions between physical drivers, including the presence of liquid water, glacial ice, and underlying bedrock, control the availability of essential elements and energetic substrates for microbial metabolism. As glaciers grind bedrock they expose minerals (Tranter et al., 2005; Anderson, 2007), increasing available reactive surfaces for direct microbial interaction. Subglacial environments are permanently dark and resident microorganisms must obtain energy for growth from redox active compounds derived from legacy material in pre-glacial sediments, minerals from underlying bedrock, or deposits from basal ice melt. The availability of oxygen for respiration in these systems is largely dependent on the balance between metabolic and

chemical demand, entrapment in accretion ice, and sources such as subglacial advective flow and release from the overlying ice sheet. Variations in oxygen concentration and availability of organic carbon can determine microbial community structure and function in subglacial environments, leading to alternative electron acceptors for anaerobic respiration (Tranter et al., 2005).

Sulfate and sulfide minerals in bedrock represent a large reservoir of sulfur on Earth (Schlesinger, 2013). This reservoir is in constant flux via weathering reactions that transfer sulfur to the atmosphere and ocean (Schlesinger, 2013). All microorganisms require sulfur for cellular components, such as the amino acids cysteine and methionine, however some microorganisms utilize sulfur compounds in dissimilatory, energy yielding metabolic processes. Microbial sulfur metabolism can influence mineral dissolution and precipitation indirectly via production of acidic metabolic byproducts, or directly via electron transfer (Ehrlich, 1996; Banfield, 1999). Organisms that oxidize reduced sulfur compounds, the sulfur-oxidizing prokaryotes (SOP) are metabolically and phylogenetically diverse (Friedrich et al., 2001; 2005), and can utilize a variety of electron acceptors including O_2, NO_3^-, $Mn^{+3/+4}$, and Fe^{+3}. Other organisms, sulfate-reducing prokaryotes (SRP), respire organic material using sulfate as an electron acceptor when oxygen is absent (Jørgensen, 1982; Jørgensen and Postgate, 1982). Sulfate reduction is widely recognized as an important process in anaerobic marine sediments, where it contributes to greater than 50% of total organic carbon oxidation globally (Canfield, 1993; Thullner et al., 2009; Bowles et al., 2014). Reduced sulfur compounds generated by sulfate reduction in turn can provide energy for SOP, although a larger fraction of reduced sulfur for microbial oxidation may come from mineral sources.

Measurements of metabolic substrate concentrations, enrichment cultures, and molecular surveys indicate sulfate reduction and sulfide oxidation occur beneath Arctic glaciers (Bottrell and Tranter, 2002; Wadham et al., 2004; Skidmore et al., 2000), although, less is known about Antarctic subglacial communities. 16S rRNA gene sequence surveys support the presence of the SOP *Gallionella* and *Thiobacillus* in sediments beneath the Kamb Ice Stream in West Antarctica, which neighbors the WIS (Lanoil et al., 2009). Wadham et al. (2012), predicted conditions favoring methane accumulation beneath the Antarctic Ice Sheet where sulfate reduction ceases (Wadham et al., 2012). Mikucki et al. (2009) described a catalytic sulfur cycle below the Taylor Glacier in the McMurdo Dry Valleys, Antarctica based on the natural isotopic abundance measurements of sulfate and sulfate reduction functional genes. Data on subglacial microbial processes to date all indicate that microorganisms enhance subglacial weathering (e.g., Montross et al., 2013) and may explain some observed diagenetic effects noted on subglacial mineral grains (Tulaczyk et al., 1998).

Analyzing genes involved in dissimilatory sulfur transformations in environmental samples can inform putative *in situ* metabolic activity. Here we analyzed the presence and diversity of three dissimilatory sulfur cycling genes (APS, DSR, and rDSR in SLW sediments). APS reductase is a conserved enzyme among both SRP and SOP (Meyer and Kuever, 2007c) and the alpha subunit of APS reductase, *aprA*, is a common marker for both metabolic groups (Meyer and Kuever, 2007c). Dissimilatory sulfite reductase (DSR) is found in all SRP and catalyzes the final energy-yielding step of sulfite reduction to hydrogen sulfide (Wagner et al., 1998; Rabus et al., 2006; Zverlov et al., 2005). A homologue of DSR, reverse-acting DSR (rDSR), is a marker for some

sulfur-storing and oxidizing members of the phyla Chlorobi and Proteobacteria and is thought to be involved in the oxidation of intracellular stored elemental sulfur compounds (Pott and Dahl, 1998; Loy et al., 2008; 2009).

Results presented here show that prokaryotes in SLW sediments mediate sulfur transformations and support growing evidence for the importance of chemosynthesis in SLW (Christner et al., 2014) and other cold, dark, subglacial environments. Our data elucidate a potential energetic strategy of the microbial communities in this subglacial ecosystem.

Materials and Methods

Site description and sample collection

SLW is located beneath the downstream portion of the WIS (S 84.237°, W 153.614°) (Christianson et al., 2012), ca. 100 km from the grounding zone, where the ice sheet transitions into the Ross Ice Shelf (Figure 1). SLW is a shallow lake located in what appears to be a large wetland along the Siple Coast of West Antarctica (Priscu et al., 2010; Fricker et al., 2011). SLW drains and refills on a sub-decadal time scale discharging water towards the Ross Sea (Fricker et al., 2007; Carter and Fricker, 2012; Siegfried et al., 2014). In January 2013, the WISSARD Project (www.wissard.org) used hot water drilling to penetrate 801±1 m of glacial ice to access SLW. Details of drilling operations are described elsewhere (Tulaczyk et al., 2014). A clean access protocol (Priscu et al., 2013) was followed to maintain both sample integrity and environmental stewardship under the Antarctic Treaty Code of Conduct. Briefly, drilling water was passed through two filtration units (2.0 and 0.2 μm) to remove large particulates and

microbial cells. Water was then subjected to two wavelengths of ultraviolet irradiation, 185 nm for organic matter destruction and germicidal 254 nm. Finally, drilling water was pressurized and heated to 90 °C and used to melt an access borehole. Drilling water was chemically and microbially tested throughout the operations. Instruments were cleaned with 3% hydrogen peroxide and cables and hoses were deployed through a UV collar during deployment down the borehole (Priscu et al., 2013).

The SLW water column was 2.2 m deep at the time of sampling (Tulaczyk et al., 2014; Christner et al., 2014). Sediments were collected using a gravity driven multi-corer (Uwitec) that was built to allow deployment through a 30 cm diameter borehole. The coring device was designed to simultaneously recover three 50 cm long x 6 cm diameter cores of undisturbed sediment and water from the sediment-water interface. The multi-corer was successful in recovering ~40 cm of sediment and 20 cm of basal water in most deployments. Sediment cores analyzed in this study were collected from the 2nd deployment of the multi-corer (identified as core 'MC-2B') and the 3rd deployment (core 'MC-3C'; Tulaczyk et al., 2014). The cores contained conspicuous bubbles when brought to the surface, suggesting possible degassing during core retrieval. The ice above the lake moved ~5 cm during the 2nd and 3rd multi-core casts, thus samples may represent overlapping locations (Tulaczyk et al., 2014). Approximately two thirds of the sediments from MC-3C slipped out of the core tube, leaving the top ~16 cm of sediment, which appeared structurally undisturbed. Cores were vertically extruded and serially sectioned using a core stand and cutter (Uwitec) in a Class 100 laminar flow hood. Sediments were sampled from three depth intervals in each core. MC-2B was sampled at depths of 0-4, 4-8, and 28-34 cm and MC-3C was sampled at depths of 2.0-3.5, 3.5-8.0, and 8-16 cm

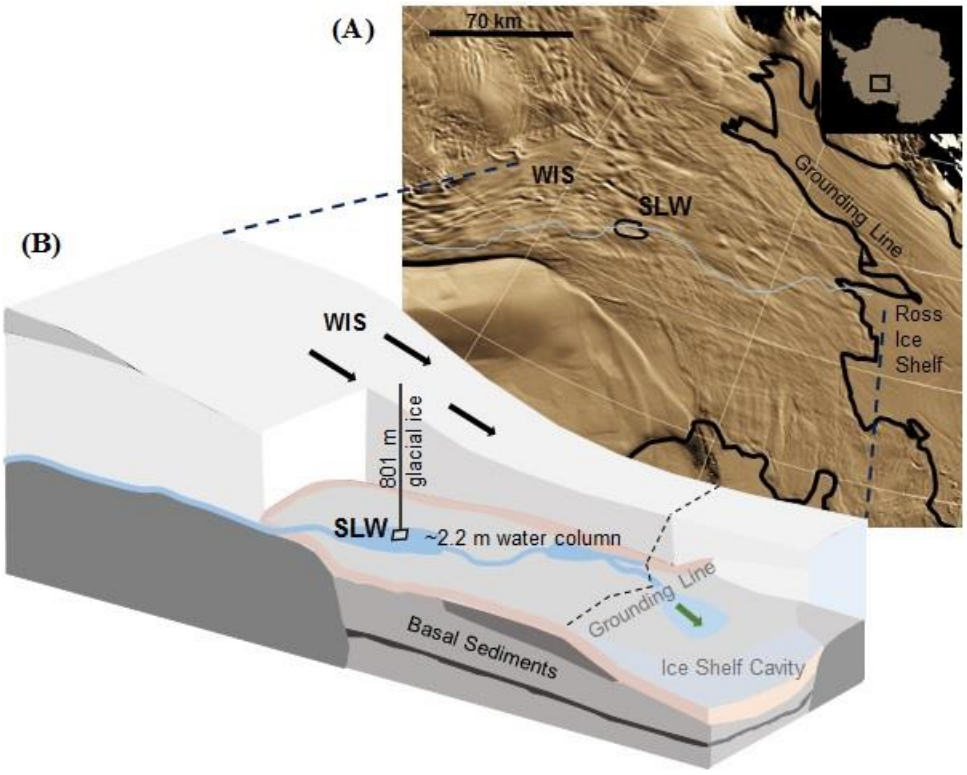

Figure 1. Location of Subglacial Lake Whillans (SLW) and schematic of the Whillans Ice Stream (WIS). (A) Satellite image of the Siple Coast with SLW labeled (after Fricker and Scambos, 2009); the blue line indicates the proposed subglacial water flow path towards the grounding line (Carter and Fricker, 2012). Background satellite image from MODIS Mosaic of Antarctica (Haran et al., 2005). (B) Cross-sectional cartoon of the WIS indicating the borehole through 801 m of ice. Sediment cores were collected through ~2.2 m of water. The black arrows indicate the direction of ice movement; the green arrow indicates predicted dispersal of subglacial water into the marine cavity beneath the Ross Ice Shelf. Cross-sectional cartoon of the WIS adapted from Fricker et al. (2011).

Table 1. Subglacial Lake Whillans sediment samples used in this study, gene amplifications, sulfate reduction rates (SRR), and Q-PCR gene quantification. Gene amplifications from SLW sediments: "+" indicates positive amplification. "-" indicates no amplification.

[a] At least one of the primer combinations amplified (Table 4)

[b] p value calculated by one tailed unpaired t test to determine significance differences when compared with kills; * = significant ($p < 0.05$); ** = highly significant ($p < 0.01$); The water column was NS. ND = Not Determined.

[c] p value calculated by two tailed unpaired t test assuming equal variance to determine significant differences between copies of bacterial and archaeal 16S rRNA gene copies. * = significant ($p < 0.05$); ** = highly significant ($p < 0.01$)

[d] First number is % *aprA* with respect to total prokaryote 16S rRNA gene copies and second is to total bacterial 16S rRNA from Q-PCR quantification.

Sample		**Gene**				**Sulfate Reduction Rates (SRR) (pmol cm^{-3} d^{-1})[b]**		**Gene quantification (copies g^{-1} wet sediment)**			
Core	**Depth (cm)**	***aprA* (384bp)**	***dsrAB* (1.9kb)**	***dsrA* (221bp)**	***rdsrAB*[a] (1.9kb)**	**Without formate**	**With formate**	**Bacterial 16S rRNA[c]**	**Archaeal 16S rRNA[c]**	***aprA***	**% *aprA* of 16S rRNA[d]**
MC-2B	0-4	+	-	+	+	0.42**	0.41**	3.9×10^{6}**	2.4×10^{6}**	9.12×10^{5}	14.5/23.6
MC-2B	4-8	+	-	+	-	ND	ND	2.6×10^{4}*	5.8×10^{5}*	9.60×10^{3}	1.6/36.6
MC-2B	28-34	+	+	+	-	ND	ND	1.2×10^{5}*	1.4×10^{6}*	8.42×10^{3}	0.6/7.3
MC-3C	2-3.5	+	-	+	+	1.67*	1.29*	8.5×10^{6}	4.4×10^{6}	9.58×10^{5}	7.4/11.3
MC-3C	3.5-8	+	+	+	+	1.20*	1.84**	2.2×10^{5}*	9.0×10^{5}*	2.47×10^{4}	2.2/11.1
MC-3C	8-16	+	+	+	-	ND	ND	2.0×10^{4}**	4.0×10^{5}**	3.67×10^{3}	0.9/18

(Table 1). Samples are referred to by core name with the depth in subscript throughout this manuscript, for example MC-2B$_{(0\text{-}4\ cm)}$. Sediments for activity experiments were processed on site. Samples for nucleic acid extraction were stored in sterile whirl-pak (Nasco) bags at -10 °C at the field site and then shipped to the University of Tennessee in the dark at -20 °C.

^{35}S-Sulfate incubation experiments

Biologically mediated sulfate reduction was measured using the passive extraction method (Ulrich et al., 1997) following incubation with $^{35}SO_4^{2-}$ tracer. Approximately 5 g of sediment from selected depths (Table 1) was aseptically transferred using a sterile spatula into pre-weighed, pre-combusted, N_2-gassed serum vials. These depths were selected because they corresponded to the lowest reduction potential in both cores (at ~3.5 cm) (Mitchell and Mikucki, unpublished data). MC-2B$_{(0\text{-}4\ cm)}$ corresponded to the surficial sediments selected for extensive biogeochemical characterization (i.e. Christner et al., 2014). SLW lake water (5 ml) was also tested. All solutions and vials used in this experiment were N_2-flushed. One ml of sterile DNA-free water (Fisher) was added to the sediments to make a slurry to minimize issues caused by potential isotope diffusion within the sediments. Small test tubes containing 2.5 ml of 10% zinc acetate (sulfide traps) flushed with N_2 gas were added to each serum vial. Blank serum vials containing sterile water were incubated along with all samples to correct for possible background transfer of the radiolabel to the traps. 2.75 µCi ^{35}S-SO_4^{2-} (specific activity ~ 1490 Ci/mmol) was added to the serum vials with a sterile syringe. We calculated that this injection added only 25 nM of sulfate to the porewater. Each sediment sample depth included 3 live and 3 killed controls whereas bulk SLW water included 4 live and 4 killed samples (kills = 2%

paraformaldehyde, final concentration). Because two organic carbon atoms are oxidized for every sulfate ion reduced, formate (50 mM, final concentration) was added to a parallel set of sediment samples to ensure that that organic carbon was present at saturating levels during the incubation period. Samples were incubated at 1-2 °C for 9 days. Experiments were terminated by the addition of 6 M HCl (8 ml) and 1M $CrCl_2$ in 0.5 M HCl (8 ml) via syringe. Vials were mixed at 125 RPM for 48 hrs to insure all total reactive inorganic S (TRIS) was liberated as H_2S and precipitated in the zinc traps. This passive extraction method has been shown to efficiently extract TRIS as FeS, FeS_2 and S^{2-} but has low efficiency for the extraction of S° thus it may underestimate total sulfate reduction (Ulrich et al., 1997). Zinc traps were then removed and the contents added to scintillation cocktail (Cytoscint ES) and the activity was measured using standard liquid scintillation spectrometry in the Crary Lab at McMurdo Station. Sulfate reduction rates (SRR; pmol SO_4^{-2} cm^{-3} d^{-1}) were estimated according to the equation (Fossing and Jørgensen, 1989)

$$SRR = \frac{a}{A+a} * \frac{[SO_4{}^{2-}]}{t} * 1.06$$

Where *a* is the radioactivity (dpm_{live}-dpm_{kills}) in the TRIS fraction, *A* is the radioactivity (dpm) added to the sample as ^{35}S-SO_4^{2-}. [SO_4^{2-}] is the concentration of sulfate (pmol cm^{-3}) in the sample, *t* is the incubation time (days), and 1.06 is a correction factor for enzymatic isotope discrimination. Sulfate reduction rates presented represent the mean (±SD) of three replicates. Density, porosity, and sulfate concentrations in sediment porewater for the experimental depths were based on values collected from a replicate core (MC-2A) obtained during the second multi-corer cast (Michaud and Priscu, in prep).

Porosity and density in core MC-2A were measured as described by Riedinger et al. (2010).

Microbial cell enumeration

Cells were enumerated in one sediment sample. This sample was collected from the exterior of an instrument that penetrated into the sediments no deeper than 20 cm. Since this sample was not obtained from a discrete depth of the stratified sediment core, we describe our methods here and report our results as an estimate of cell density in SLW sediments. Cells were extracted using a physical and chemical method. Slurries were prepared in triplicate by homogenizing sediments (2-4 g) with 1X PBS buffer (final ratio 1:2). Slurries were fixed with paraformaldehyde (2% final concentration) for ~ 16 hrs, then methanol and a 1% Tween80 solution were added (10% final concentration) to detach cells from sediments; this chemical extraction step was modified from Kallmeyer et al. (2008). Slurries were vortexed at medium-high speed at 4 °C for 30 min. then centrifuged at 50 x g for at least 1 hr at 4 °C, or until majority of sediment particles appeared settled. Three ml of supernatant was collected onto a 0.2 µm polycarbonate filter and stained with 25X SYBR Gold nucleic acid stain (Invitrogen™) for 15 min. (Ball and Virginia, 2014). Filters were rinsed with 1 ml 0.2 µm filtered nanopure water and enumerated using epi-fluorescence microscopy (Leica DM5500B with an excitation filter set BP 480/40). A procedure blank of solutions was processed alongside the sample replicates and quantified to rule out contamination. Three ml of autoclaved 0.2 µm filtered nanopure water was processed through the filtration towers prior to each sample replicate and was quantified and subtracted from total sample counts.

DNA extraction and PCR amplification

DNA was extracted in triplicate from 0.3-0.4 g of sediment in a class II type A2 clean hood (LabConco model #3460001) using the FastDNA™ SPIN Kit (MP Biomedicals) according to the manufacturer's protocol. Eluent containing DNA from each extraction from the same depth was pooled. Kit solutions were extracted simultaneously as a control for methodological contamination. A fragment (384-396 bp) of the alpha subunit of adenosine-5'-phosphosulfate reductase (*aprA*) was amplified using the forward primer AprA-1-FW and the reverse primer AprA-5-RV (Meyer and Kuever, 2007c). A short fragment (221 bp) of the alpha subunit of dissimilatory sulfite reductase (*dsrA*) was amplified using forward primer DSR1F+ and the reverse primer DSR-R (Kondo et al., 2004). The dissimilatory sulfite reductase alpha and beta subunits (*dsrAB*) were amplified (1.9 kb) using forward primer DSR1F and the reverse primer DSR4R (Wagner et al., 1998). Reverse dissimilatory reductase alpha and beta subunits (*rdsrAB*) were amplified with all published forward and reverse *rdsrAB* primer combinations (Loy et al., 2009; Lenk et al., 2011). All primer sequences are listed in Table 2. REDTaq® ReadyMix™ PCR Reaction Mix (Sigma Aldrich) was used with each primer combinations according to the manufacturer's protocol. PCR reactions contained 25 µl RedTaq, 4 µl of template (< 15 ng DNA ul^{-1}), 1 µl of each forward and reverse primer (final primer concentration 200 nM), and 19 µl of nuclease-free water for a final volume of 50 µl. Amplification of *aprA* was initiated at 94 °C for 2 min., then proceeded for 40 cycles (Green-Saxena et al., 2012) of 1 min. 94 °C denaturation; 1 min. 48 °C annealing; and 1 min. 72 °C extension, with a final elongation 7 min. at 72 °C. Amplification of *aprA* was increased to 43 cycles for core MC-$2B_{(4\text{-}8\ cm)}$ because no amplification was observed after 40 cycles. Annealing

temperature was increased to 57 °C and repeated for 41 cycles for *dsrA* amplification. *dsrAB* and *rdsrAB* amplification was performed as described for *aprA*, but included 42 cycles, 2 min. extension, with a final elongation of 7 min. Extracts (4 µl) from kit blanks and sterile water reagent blanks were processed for PCR controls for all amplifications. No amplification products were detected in the controls without template or the extraction kit blanks.

Quantitative PCR

Gene copy abundances of bacterial and archaeal 16S rRNA genes and *aprA* were measured in triplicate (technical replicates) using an iQ^{TM}5 Multicolor Real-Time PCR Detection System (Bio Rad). Standards for bacterial and archaeal 16S rRNA genes were constructed using DNA extracted from pure cultures of *Escherichia coli* and *Methanococcus jannaschii*, respectively. 16S rRNA genes were amplified, gel purified using Wizard PCR clean up (Promega), and cloned using the TOPO® TA cloning kit (Life Technologies) with One Shot® TOP10 Chemically Competent *E. coli* and the pCR^{TM}4 cloning vector. Plasmids were purified using the PureYield^{TM} Plasmid Miniprep System (Promega) and starting gene copy abundances in extracted plasmid was calculated according to Ritalahti et al. (2006). Plasmids were serially diluted to concentrations of 1×10^{1} to 1×10^{9} copies µl^{-1}. *aprA* gene standards were made using plasmids extracted from an *aprA* clone from this study and were diluted to concentrations of 1×10^{1} to 1×10^{7} copies µl^{-1}. A two-step protocol described by Lloyd et al. (2011) was used to quantify amplification under the following conditions: 95 °C for 5 min. and 40 cycles of 95 °C for 1 min. and 60 °C for 30 sec. Melting curves were performed at 0.5 °C steps from 55 °C to 95 °C and analyzed after each quantification to check for primer dimer formation and

Table 2. DNA oligonucleotide primers used in this study.

Primer and Use	*Sequence (5'-3')*	*Reference*
PCR Amplification and cloning		
AprA-1-FW Forward	TGGCAGATCATGATYMAYGG	Meyer and Kuever, 2007c
AprA-5-RV Reverse	GCGCCAACYGGRCCRTA	Meyer and Kuever, 2007c
DSR1F+ Forward	ACSCACTGGAAGCACGGCGG	Kondo et al., 2004
DSR-R Reverse	GTGGMRCCGTGCAKRTTGG	Kondo et al., 2004
DSR1 Forward	ACSCACTGGAAGCACG	Wagner et al., 1998
DSR4 Reverse	GTGTAGCAGTTACCGCA	Wagner et al., 1998
rDSR1Fa	AARGGNTAYTGGAARG	Loy et al., 2009
rDSR1Fb	TTYGGNTAYTGGAARG	Loy et al., 2009
rDSR1Fc	ATGGGNTAYTGGAARG	Loy et al., 2009
rDSR4Ra	CCRAARCAIGCNCCRCA	Loy et al., 2009
rDSR4Rb	GGRWARCAIGCNCCRCA	Loy et al., 2009
rDSRA240F	GGNTAYTGGAARGGNGG	Lenk et al., 2011
rDSR808R	CCDCCNACCCADATNGC	Lenk et al., 2011
Sequencing		
T3	ATTAACCCTCACTAAAGGGA	
T7	TAATACGACTCACTATAGGG	
Q-PCR		
Bac340 Forward	TCCTACGGGAGGCAGCAGT	Nadkarni et al., 2002
Bac515 Reverse	CGTATTACCGCGGCTGCTGGCAC	Nadkarni et al., 2002
Arc915 Forward	AGGAATTGGCGGGGGAGCAC	Takai and Horikoshi., 2000
Arc1059 Reverse	GCCATGCACCWCCTCT	Yu et al., 2005

amplification specificity. All samples were quantified on the same Q-PCR run and samples from the same DNA extraction were used to quantify all genes. 16S rRNA primer targets and coverage were checked in silico using TestPrime 1.0 application (Klindworth et al., 2012) using the SILVA SSU r119 RefNR database (Quast et al., 2013; http://www.arb-silva.de). 16S rRNA gene primer sets in this study (Table 2) cover 73% of the domain Bacteria (0% Archaea) and 73% of the domain Archaea (0% Bacteria). Primers from this study (Table 2), have successfully been used for *aprA* quantification in Peru margin sediment samples (Blazejak and Schippers, 2011). All reactions had a final volume of 25 µl, and included 12.5 µl of QuantiFast SYBR Green PCR mastermix (Qiagen, Valencia, CA), and 2 µl of template. Bacterial and archaeal 16S rRNA gene primer concentrations (80 nM final) were used as described by Lloyd et al. (2011). Final *aprA* primer concentrations were 200 nM. All standards were only thawed once and run in triplicate. Threshold cycles from each replicate were averaged to make a standard curve. Starting quantities were calculated from a log-linear standard curve (R^2 value ≥ 0.98). The detection limit based on the standard curves for archaeal 16S rRNA genes were 1×10^3 copies μl^{-1}, bacterial 16S rRNA genes were 1×10^2 copies μl^{-1}, and *aprA* was 1×10^1 copies μl^{-1}. Controls included DNA extraction blanks and Q-PCR reagents without template. Controls amplified more than 2 threshold cycles later than samples or fell below the quantification limit for each gene.

Clone library construction

Amplicons of the *aprA*, *dsrA*, *dsrAB*, and *rdsrAB* genes were purified by gel extraction using the Wizard® SV Gel and PCR Clean-Up System (Promega) following the manufacturer's protocol. The products from 7 primer sets for *rdsrAB* were pooled; for all

other genes, only one primer set was used (Table 2). Clone libraries were constructed using TOPO® TA cloning kit (Invitrogen™) with One Shot® TOP10 Chemically Competent E. coli and the pCR™4 cloning vector. Approximately 50 colonies were randomly picked after growth on Luria-Bertani (LB) agar plates with kanamycin and cultured in LB. Plasmids were extracted using the PureYield™ Plasmid Miniprep System (Promega) following the manufacturer's protocol. Sanger sequencing was performed on the extracted plasmids using the T3 and T7 primers (Table 2). ABI Big-Dye v3.1 cycle sequencing mix was used for reactions run on an ABI 3130 analyzer (Applied Biosystems) at the University of Tennessee, Knoxville Molecular Biology Resource Facility and the Clemson University Genomics Institute.

Phylogenetic and diversity analyses

Nucleotide sequences were imported into BioEdit version 7.2.3 (Hall, 1999; http://www.mbio.ncsu.edu/bioedit/bioedit.html). Vector sequence was removed, and nucleotide sequences were checked for possible chimeric artifacts using the program Bellerophon (Huber et al., 2004) or manually by BLASTn alignment analysis as was used previously (Antony et al., 2010; Nilsson et al., 2010). Nucleotide sequences were translated into amino acid sequences and aligned using ClustalW (Larkin et al., 2007) in BioEdit (Hall, 1999). Numerous functional gene diversity studies of environmental samples studies use an amino acid sequence identity cut off 90-97% to describe distinct operational taxonomic units or OTUs (Loy et al., 2009; Lenk et al., 2011; Leloup et al., 2009). Here we define a unique OTU for *aprA*, *dsrA*, and *rdsrA* sequences as clusters of sequences having an amino acid sequence identity of 90% or greater (Loy et al., 2009; Lenk et al., 2011). All functional gene sequences in this study were grouped into OTUs

using the BlastClust tool (http://toolkit.tuebingen.mpg.de/blastclust). Only the alpha subunit portion of the 1.9 kb *dsrAB* and *rdsrAB* (255 amino acids) was used for cluster analyses. Amino acid sequences were searched against the NR database in NCBI using BLASTp (http://blast.ncbi.nlm.nih.bov/Blast.cgi).

Phylogenetic analysis included aligning *aprA* sequences from reference strains and sequences of highest amino acid identity to cultured and uncultured *aprA* sequences in the NR database. *aprA* OTUs were then functionally classified as SRP or SOP based on lineages defined by Meyer and Keuver (2007a). These lineage designations are supported by amino acid indels that are unique to the lineages as described by Meyer and Keuver (2007a; 2007b). Sequences that did not fall within either the SRP or SOP *aprA* lineages were referred to as sequences of uncertain function. After designating *aprA* sequences into OTUs, one representative sequence from each OTU was selected for tree construction. A neighbor-joining tree was constructed using MEGA version 6 (Tamura et al., 2013) and Jones-Thornton-Taylor (JTT) substitution model and a bootstrap analysis of 1000 replicates.

Statistical tests (Table 3) were calculated to evaluate *aprA* diversity including, Shannon-Weaver diversity index (*H´)* and Simpon's index (*D*). Sampling coverage was evaluated using the Chao1 richness estimator, Good's coverage, and rarefaction. Good's coverage (*C*) was calculated using the equation $C = 1-(n_i/N)$, where n_i is the number of single unique clones, and *N* is the total number of clones in the library (Good, 1953; Singleton et al., 2001). Simpsons' indices, Shannon-Weaver, and Chao1 richness estimator were calculated using standard equations in Hill et al. (2003). Chao1 richness estimator was calculated to estimate the OTU abundance expected in each clone library

using standard equations in Hill et al. (2003). Rarefaction curves were generated to estimate the thoroughness of sequencing. Curves were generated using R version 2.15.0 (R Development Core Team, 2008) and the script (http://www.jennajacobs.org/R/rarefaction.html) to evaluate our clone library size.

Nucleotide sequence accession numbers

aprA, *dsrA*, and *rdsrA* gene sequences were deposited in GenBank under the accession numbers KM589857-KM590347.

Results

Sediment characteristics

SLW sediments were water-lain and homogeneous fine-grained diamicton with no evidence of particle size sorting (Powell and Hodson, 2014). The absence of silt or sand lags indicated slow basal water flow, even during the episodic lake draining events described by Fricker and Scambos (2009). Porosity and water content is notably higher in the upper 40 cm than the more consolidated till below. All analyses in this study are in the upper, higher water content unit (0-34 cm) (Powell and Hodson, 2014). Fossil diatoms and other marine sediment derived particulates are rare and poorly preserved in all SLW sediments (Scherer et al., 2014), despite the fact that the source rocks that make up the till include a significant marine component (Scherer et al., 1991). The relative lack of diatoms in the SLW sediments analyzed in this study when compared with tills further upstream, beneath the WIS (Scherer, 1991; Scherer et al., 1998), is largely a result of mechanical degradation, from both subglacial shear strain in deforming till (Scherer et al., 2004; 2005) and long distance transport from their upstream source rocks. As the opaline silica of diatom frustrales fragments, their surface area increases, exposing encased residual

Table 3. Estimates of *aprA* diversity, richness, and clone library coverage in SLW Sediments.

Sediment Sample	***All***	***MC-2B*** *(0-4 cm)*	***MC-2B*** *(4-8 cm)*	***MC-2B*** *(28-34 cm)*	***MC-3C*** *(2-3.5 cm)*	***MC-3C*** *(3.5-8 cm)*	***MC-3C*** *(8-16 cm)*
Total # clones	275	45	28	39	45	40	39
Total # OTUs	16	6	3	8	4	6	8
Good's Coverage	0.98	0.93	0.96	0.92	0.98	0.93	0.92
Simpson's Index (D)	0.40	0.61	0.5	0.24	0.68	0.45	0.16
Shannon-Weaver Index (H')	1.50	0.83	0.77	1.62	0.63	1.09	1.82
Chao1 Richness Estimator	34	11	3	13	5	6	8

organic matter which can be available to microbial colonization which would accelerate dissolution of remaining siliceous residues. Many of the fossil siliceous sponge spicules in SLW sediments, which are much denser than diatoms, have dissolution pitting that is likely influenced by microbial colonization. The SLW water column was -0.5 °C at the time of sampling with a pH of 8.1. Sediments from 0-2 cm depth had a pH of 7.3 (Christner et al., 2014). Sulfate concentration in the water column and surficial sediments was 0.56 and 0.62 mM, respectively (Christner et al., 2014).

Activity of sulfate-reducing prokaryotes

$^{35}SO_4^{2-}$ amended incubation experiments of sediment slurries indicated that SRR were statistically significant in all three of the SLW sediments tested, albeit at low rates (average = 1.14 pmol $cm^{-3}d^{-1}$ ±0.60). There was no significant stimulation in sulfide production with the addition of formate (Figure 2). Activity in SLW water column samples was not detected (i.e., live samples were not statistically greater than kills). Analysis of 16S rRNA gene libraries indicated that known sulfate-reducing taxa were also not abundant members of the water column (0.1% OTUs) or the $MC\text{-}2B_{(0\text{-}2\ cm)}$ (0.02%) sediment community (Christner et al., 2014).

Quantification of biomass and total 16S rRNA and *aprA* genes

Numerous protocols have been developed for quantification of microbial cells in sediments using fluorescent nucleic acid stains (Klauth et al., 2004; Kallmeyer et al., 2008; Morono et al., 2013). However, quantification remains challenging due to auto-fluorescent properties of sediment particles, non-specific binding of nucleic acid stain, or particle blocked microbial cells (Kepner and Pratt, 1994). Our extraction method required at least 6 grams of sediment, which limited the number of samples analyzed in this study.

Therefore, a Q-PCR approach was used to estimate abundance despite known caveats, including PCR inhibitors in environmental samples, and DNA extraction and primer biases (Smith and Osborn, 2009).

Copies of bacterial and archaeal 16S rRNA genes were similarly abundant in the surficial sediments (sample MC-3C$_{(2\text{-}3.5\ cm)}$; two-tailed unpaired t-test p value >0.05) and copy numbers of all three genes decreased with depth (Table 1; Figure 3). Abundance of total 16S rRNA genes decreased from 6.3×10^{6} copies g^{-1} in MC-2B$_{(0\text{-}4\ cm)}$ and 1.3×10^{7} copies g^{-1} in MC-3C$_{(2\text{-}3.5\ cm)}$ (the two top depths) to 1.5×10^{6} copies g^{-1} in MC-2B$_{(28\text{-}34\ cm)}$ and 4.2×10^{5} copies g^{-1} in MC-3C$_{(8\text{-}16\ cm)}$ (the lower depths) (Figure 3; Table 1). Gene copy numbers of *aprA* decreased from 9.1×10^{5} copies g^{-1} in MC-2B$_{(0\text{-}4\ cm)}$ and 9.6×10^{5} copies g^{-1} in MC-3C$_{(2\text{-}3.5\ cm)}$ to 8.4×10^{3} copies g^{-1} in MC-2B$_{(28\text{-}34\ cm)}$ and 3.7×10^{3} copies g^{-1} in MC-3C$_{(8\text{-}16\ cm)}$ (Figure 3; Table 1).

While 16S rRNA gene copy number cannot be directly converted into biomass, by accounting for average 16S rRNA copy number within sequenced genomes, gene copy number can be used as a proxy for total cells. A survey of the currently finished microbial genomes in JGI IMG indicate an average of 4.04 copies per bacteria cell and 1.64 copies per archaeal cells (Markowitz et al., 2014). Although this estimate may not be representative of the 16S rRNA gene copies of microbes in SLW, based on these values and averaging the Q-PCR results for all depths, we estimate a microbial abundance of 1.6×10^{6} cells g^{-1} wet sediment. DNA-containing cells in the sediment sample quantified by microscopy were 2.0×10^{5} ($\pm5.1\times10^{4}$) cell g^{-1} wet sediment.

***aprA* gene**

Amplification of *aprA* was detected in all samples (Table 1) and a total of 275 *aprA* clones were sequenced from SLW sediments. Primers used for *aprA* amplification target both SRP and SOP (Meyer and Keuver, 2007c) and both putative functional types (as defined by Meyer and Keuver, 2007a) were present in SLW. The SLW sequences formed 16 distinct operational taxonomic units (OTUs) (Table 4). Their relationships to each other and their closest relatives were described by constructing a phylogenetic tree (Figure 4). *aprA* sequences related to SOP comprised 74% of total *aprA* sequences (Table 4). The most abundant *aprA* OTU, 1A, represented 61% of the total sequences and was found in all samples analyzed. OTU1A sequences were affiliated with SOP lineage I, and were 97-94% identical to the *aprA* found in the Betaproteobacterium, "Sideroxydans lithotrophicus" ES-1 (Table 4; Figure 4). Other SOP-related sequences (OTUs 3A and 13A; combined 6% of total sequences) fell within SOP lineage II and were 95-92% related to *Thiobacillus* spp., including *T. denitrificans* and *T. plumbophilus*, and *Thiodictyon* sp. f4 (Table 4; Figure 4).

Five OTUs (9A, 11A, 12A, 14A, 15A) represented *aprA* sequences (4% of total *aprA* sequences) related to known SRP in samples MC-2B$_{(28\text{-}34\ cm)}$ and MC-3C$_{(8\text{-}16\ cm)}$ (Table 4). This included sequences most closely related to *Desulfobacterium anilini* (95-86%), *Desulfatitalea tepidiphila* (95%), and *Desulfotomaculum kuznetsovii* (88-80%) (Table 4). *aprA* OTUs 9A, 14A, and 15A (3% of sequences) are most closely related to *Desulfobacterium indolicum* (95-94% identity) and the Deltaproteobacterium strain NaphS2 (94-91% identity). Both of these organisms are anaerobic sulfate reducers isolated from marine sediments (Bak and Widdel, 1986; Galushko et al., 1990). However, the true

diversity of *aprA* sequences in the samples deeper than 8 cm, where SRP-related sequences were detected, is likely higher than reported as rarefaction did not reach the asymptote and a higher number of unique OTUs was estimated by Chao1 (Figure 5; Table 3).

Four *aprA* OTUs (2A, 4A, 6A, and 7A) represented 27% of total *aprA* sequences from SLW sediments and were 83-70% identical to the *aprA* found in *Thermodesulfovibrio* spp. and 79-77% identical to the *aprA* found in the Chlorobi members including *Pelodictyon clathratiforme* and *Chlorobium phaeobacteroides* (Table 4). *T. yellowstonii and T. islandicus* are known SRP, but their APS gene is thought to have been horizontally transferred to the sulfur-oxidizing anoxygenic phototrophic members of *Chlorobiacaea* (Meyer and Keuver, 2007b). These sequences of uncertain function identified from SLW also contain an amino acid insertion sequence (data not shown) at position 311(numbering after *aprA* in *Allochromatium vinosum*) that is unique to the *Thermodesulfovibrio* and *Chlorobiacaea aprA* sequences (Meyer and Keuver, 2007b). For these reasons, these *aprA* sequences cannot be designated to a specific function. The second most abundant OTU, 2A, also of uncertain function, represents 11% of total *aprA* sequences in SLW sediments. This OTU has an 83-81% amino acid identity to *Thermodesulfovibrio yellowstonii*, an anaerobic, heterotrophic, sulfate-reducer originally isolated from hydrothermal water (Henry et al., 1994).

***dsrA* and *rdsrA* genes**

The primer set targeting a short (221 bp) fragment of *dsrA* amplified in all sediment samples. The longer *dsrAB* (1.9 kb) fragment only amplified in the deeper sediments; MC-2B$_{(28\text{-}34\ cm)}$ and MC-3C$_{(3.5\text{-}8,\ 8\text{-}16\ cm)}$ (Table 1) and sequences related to SRP

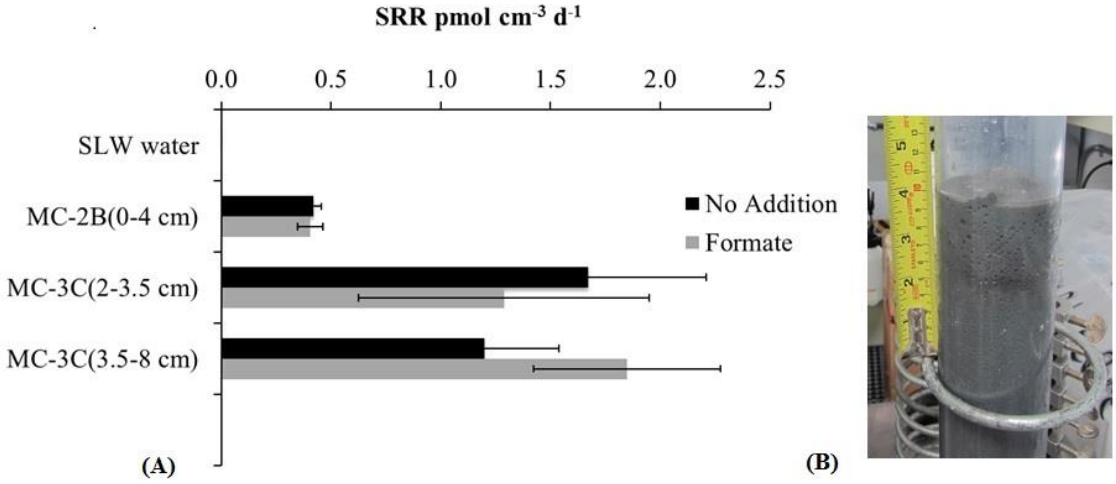

Figure 2. Sulfate reduction rates (SRR) in SLW sediment samples. (A) SRR from killed controls were subtracted from each sample replicate. Black bars represent sediment incubations with no carbon addition; Gray bars represent sediment incubations with 50mM formate addition (±SD of triplicates). **(B)** Image of SLW sediment core MC-2B.

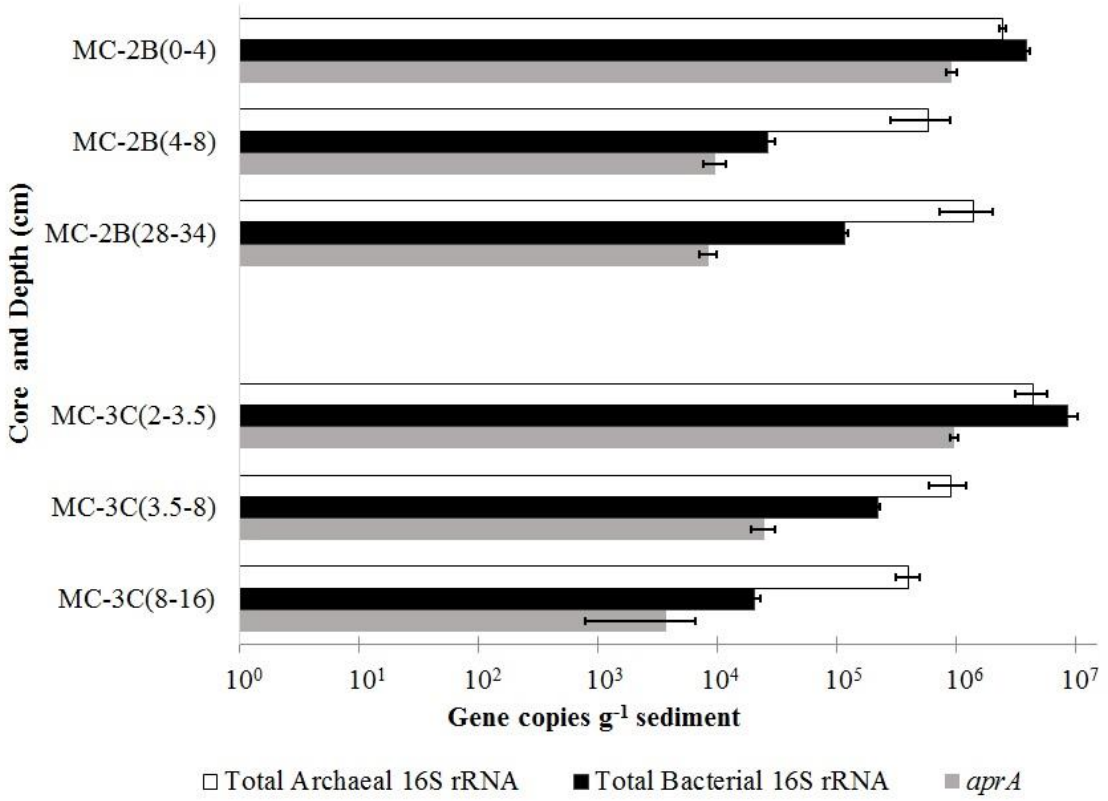

Figure 3. Q-PCR quantification of bacterial and archaeal 16S rRNA and *aprA* gene copies. Total bacterial (black bars) and archaeal (white bars) 16S rRNA and *aprA* (gray bars) gene copies from all sediment depths from SLW MC-2B and MC-3C (±SD of technical replicates).

Figure 4. Phylogenetic tree of SLW sediments *aprA* OTUs. Neighbor-joining reconstruction of 16 *aprA* sequences from SLW sediments and the most identical *aprA*-containing cultured organisms and environmental sequences. Values at nodes indicate bootstrap support from 1000 replicates. One representative *aprA* sequence from each of the 16 OTUs was randomly selected and included. SLW *aprA* OTUs are in bold and the total number of sequences obtained within that OTU are in parentheses. Lineage designations on the right are from Meyer and Kuever (2007c). *Pyrobaculum aerophilum* was used as an outgroup reference. Scale bar indicates the branch length corresponding to 0.1 substitutions per amino acid position.

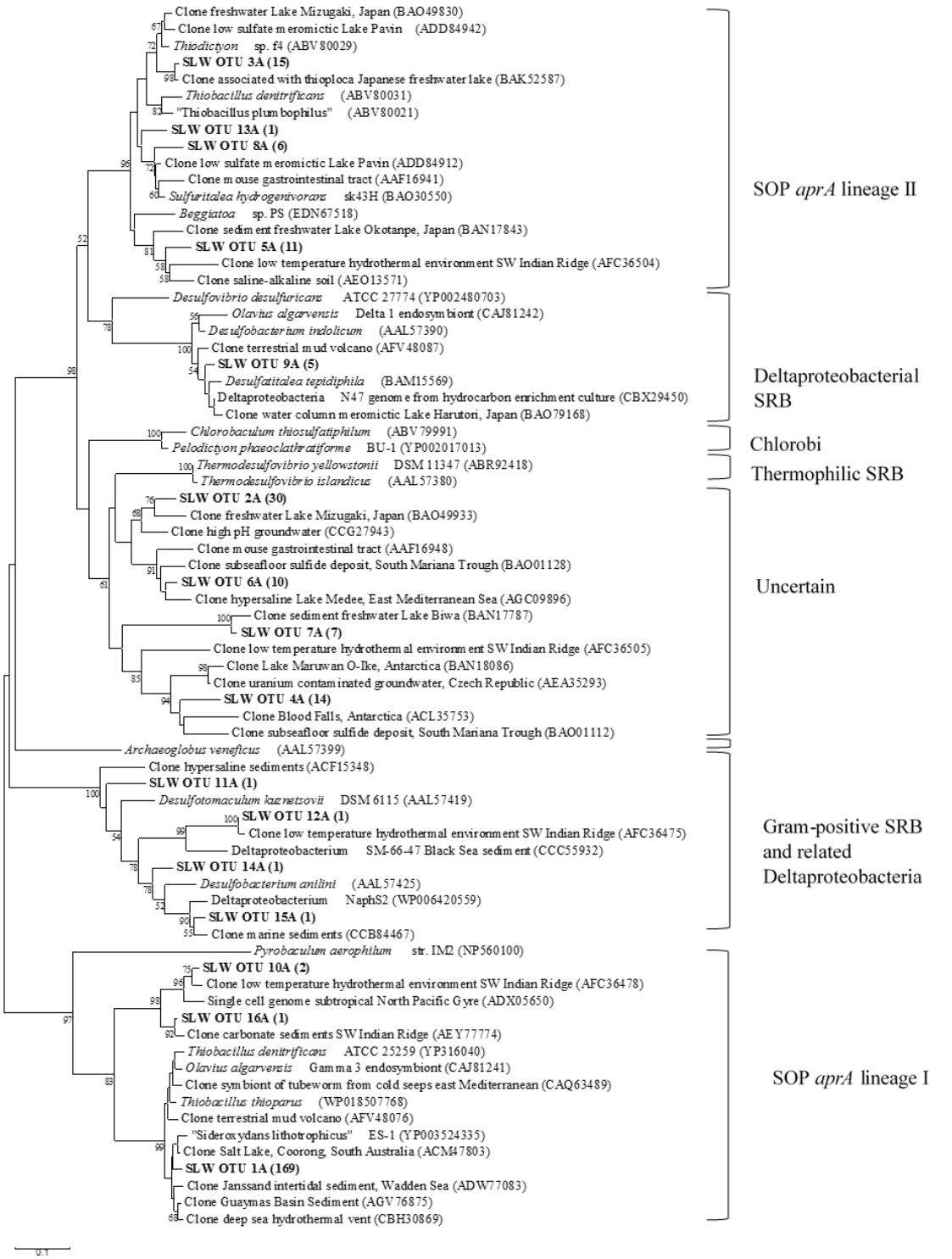

Figure 4. Continued

Table 4. Description of the closest cultured relatives related to SLW *aprA* OTUs and putative sulfur cycle function. SOP lineages and SRP related *aprA* are defined by Meyer and Kuever (2007a).

OTUs	*% total sequences*	*Sediment Depths Observed*	*Closest cultured representative*	*% AA identity*	*Characteristics*	*Reference*
			Sulfur oxidizing (SOP) - SOP lineage I			
1A	61	All	"Sideroxydans lithotrophicus" ES-1	97-93	Neutrophilic, iron and sulfur oxidizer	Emerson et al., 2013
10A, 16A	1	MC-2B (0-4, 28-34 cm), MC-3C (3.5-8 cm)	Single cell genome	93-89	N. Pacific and S. Atlantic Subtropical Gyre at 770m and 800m water depth	Swan et al., 2011
			Sulfur oxidizing (SOP) - SOP lineage II			
3A, 13A	6	MC-2B (0-4 cm), MC-3C (2-3.5, 3.5-8 cm)	"Thiobacillus plumbophilus"	95-92	Mesophilic, aerobic, hydrogen and sulfur oxidizer	Drobner et al., 1992
			Thiodictyon sp.f4	94-93	Photoautotrophic, iron oxidizer	Croal et al., 2004
5A, 8A	6	MC-2B (0-4, 28-34 cm), MC-3C (2-3.5 cm)	*Sulfuritalea hydrogenivorans*	93-86	Facultative anaerobic autotroph, sulfur oxidizer	Kojima and Fukui, 2011
			Sulfate- reducing (SRP)			
9A	2	MC-3C (8-16 cm)	*Desulfobacterium indolicum*	95-94	Anaerobic sulfate reducer from marine sludge	Bak and Widdel, 1986
11A, 12A	1	MC-3C (8-16 cm)	*Desulfotomaculum kuznetsovii*	88-80	Thermophilic anaerobic heterotrophic sulfate reducer	Visser et al., 2013
14A, 15A	1	MC-2B (28-34 cm); MC-3C (8-16 cm)	*Deltaproteobacterium* NaphS2	94-91	Anaerobic sulfate reducer, aromatic compound degradation, from marine sediments	Galushko et al., 1999
			Uncertain Function			
2A	11	MC-2B (4-8, 28-34 cm), MC-3C (3.5-8, 8-16 cm)	*Thermodesulfovibrio yellowstonii*	83-81	Thermophilic heterotrophic, obligate anaerobe, sulfate reducer	Henry et al., 1994
7A	3	MC-2B (0-4 cm); MC-3C (2-3.5, 3.5-8, 8-16 cm)	*T. yellowstonii*	71	Thermophilic heterotrophic, obligate anaerobe, sulfate reducer	Henry et al., 1994
			T. yellowstonii	78-69	Thermophilic heterotrophic, obligate anaerobe, sulfate reducer	Henry et al., 1994
4A, 6A	9	MC-2B (4-8, 28-34 cm); MC-3C (3.5-8, 8-16 cm)	*Thermodeulfovibrio islandicus*	78-69	Thermophilic sulfate reducer, isolated from hot spring in Iceland	Sonne-Hansen and Ahring, 1999
			Pelodictyon phaeoclathratiforme	78-69	Anoxygenic phototrophic sulfur oxidizer, isolated from a meromictic freshwater lake	Overmann and Pfennig, 1989

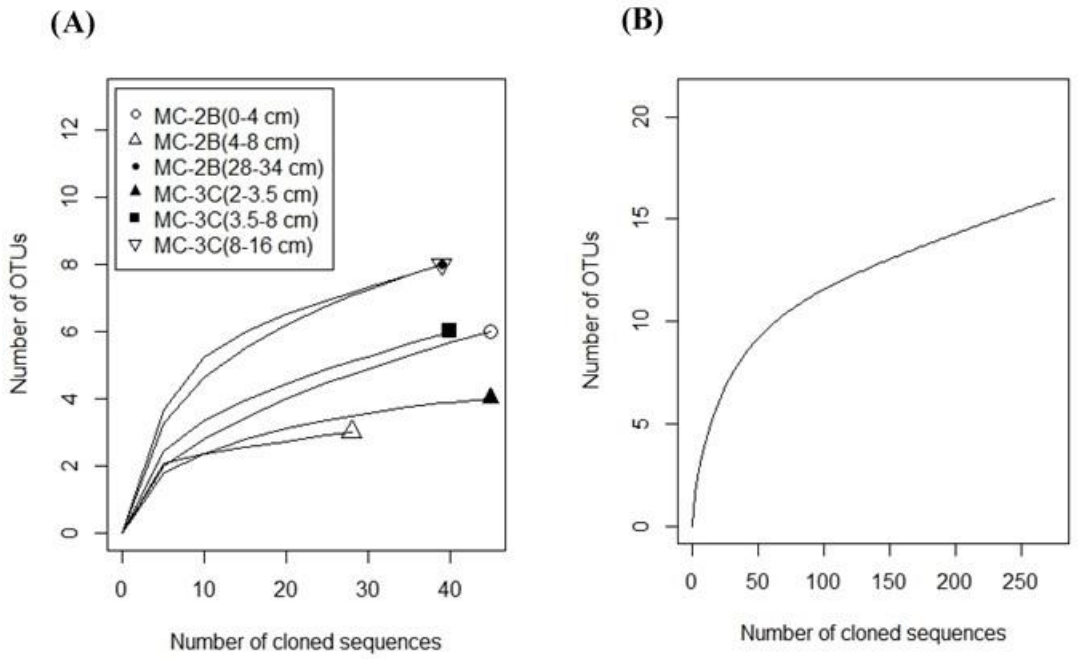

Figure 5. Rarefaction curves of *aprA* in SLW Sediments. (A) Individual depths from sediment cores MC-2B and MC-3C. **(B)** Total *aprA* sequences from all depths and cores.

aprA were only detected in MC-2B$_{(28\text{-}34\ cm)}$ and MC-3C$_{(8\text{-}16\ cm)}$. Fifty-five *dsrA* sequences from samples MC-2B$_{(0\text{-}4\ and\ 4\text{-}8\ cm)}$ and MC-3C$_{(2\text{-}3.5,\ 3.5\text{-}8,\ 8\text{-}16\ cm)}$ formed eight distinct OTUs. The majority of these OTUs (80% total sequences) were 78-73% identical to *dsrA* from characterized species in the genera *Desulfotomaculum* and *Carboxydothermus*. The remaining sequences were 88-78% identical to *dsrA* within the Deltaproteobacteria orders *Desulfovibrionales* and *Desulfobacterales* and 99-95% identical to clones from marine sediments (Blazejak and Schippers, 2011).

To increase the phylogenetic resolution of SRP diversity, the alpha subunit (255 amino acids) of the *dsrAB* gene from 36 clones collected from MC-2B$_{(28\text{-}34\ cm)}$ and MC-3C$_{(3.5\text{-}8\ and\ 8\text{-}16\ cm)}$ were analyzed. Six distinct OTUs were detected. OTU1D (84% of sequences) represented a deeply branching *dsrA* cluster, with 65-64% sequence identity to the *dsrA* from members of the *Desulfotomaculum* genus including *D. alkaliphilum* and the sulfate-reducing archaeon, *Archeoglobus veneficus*. OTU1D was 77-75% identical to a *dsrA* environmental clone obtained from various cold marine sediment environments (de Rezende et al., 2013; Harrison et al., 2009). OTU3D (4% of sequences) was most closely related (84% identity) to *dsrA* from the sulfate-reducing Deltaproteobacteria species *Desulfatibacillum alkenivorans,* an organic carbon oxidizer capable of chemolithoautotrophic growth (Callaghan et al., 2012) and *Desulfosalsimonas propionica,* a halophilic propionate oxidizer isolated from Great Salt Lake sediments (Kjeldsen et al., 2010). OTUs 2D, 5D, 6D (10% of *dsrA* sequences) were 69-64% identical to Firmicute sequences, including *Desulfurispora thermophila, Desulfotomaculum carboxydivorans,* and *Pelotomaculum propionicum*. *dsrA* OTU4D was 76-72% identical to *Desulfotomaculum carboxydivorans* and *Desulfurispora*

thermophila. These organisms are also members of the Firmicutes phyla and are known spore-forming, sulfate-reducers (Parshina et al., 2005; Kaksonen et al., 2007). *D. carboxydivorans* is a moderately thermophilic, chemolithoheterotroph capable of respiring with and without sulfate (Parshina et al., 2005).

Amplification of *rdsrAB* was detected in MC-2B$_{(0\text{-}4\ cm)}$ and MC-3C$_{(2\text{-}3.5\ and\ 3.5\text{-}8\ cm)}$, but not in MC-2B$_{(4\text{-}8\ and\ 28\text{-}34\ cm)}$, or MC-3C$_{(8\text{-}12\ cm)}$. Seven unique *rdsrA* OTUs were detected among the 111 *rdsrA* sequences retrieved from SLW sediments. The most abundant OTU, 1R (60% of sequences), was most closely related (83-78% identity) to members of the *Chromatiaceae* family including *Thiorhodococcus drewsii* and *Marichromatium purpuratum* which are anoxygenic phototrophs capable of oxidizing hydrogen sulfide (Zaar et al., 2003). OTU2R (28% of sequences) was 91-88% identical to *Thiobacillus denitrificans* and *Thiobacillus thioparus*. Three OTUs (3R, 4R, 6R), represented 12% of total *rdsrA* sequences and were 91-80% identical to "Sideroxydans lithotrophicus" ES-1. The remaining OTUs (5R and 7R) represented 2% of total *rdsrA* sequences and were 84-83% identical to *Sulfuritalea hydrogenivorans* sk43H, a facultative anaerobe and mixotroph that can oxidize sulfur (Kojima and Fukui, 2011).

Discussion

Abundance of 16S rRNA and *aprA* gene copies

Our bacterial and archaeal 16S rRNA gene copies indicate SLW sediments are heterogeneous, showing variable, but decreasing abundances with depth. These findings are consistent with observations from marine sediments, where abundance decreases with depth (Kallmeyer et al., 2012). Lloyd et al. (2013) compiled cell density data (based on both Q-PCR and fluorescence *in situ* hybridization) from 65 studies of marine sediments

and showed that abundance of archaea and bacteria varies and they dominate at different sites throughout the global ocean. Abundances of 16S rRNA gene copies of both archaea and bacteria were similar in the SLW surficial sediment depth sample (MC-3C$_{(2\text{-}3.5\ cm)}$; two-tailed unpaired t-test, p value >0.05) (Table 1; Figure 3). However, archaeal 16S rRNA gene copies were higher than bacterial 16S rRNA gene copies in all other samples; MC-2B$_{(4\text{-}8,\ 28\text{-}34\ cm)}$ and MC-3C$_{(3.5\text{-}8,\ 8\text{-}16\ cm)}$ (Table 1; Figure 3). Christner et al. (2014) reported low archaeal 16S rRNA gene abundance in the SLW water column and surficial (0-2 cm) sediments (3.6 and 0.3%, respectively). This discrepancy could be due to primer bias; the primers used in our Q-PCR analysis detect a wider range of archaea (according to in silico analysis using TestPrime 1.0 application (Klindworth et al., 2012) and the SILVA SSU r119 RefNR database (Quast et al., 2013; http://www.arb-silva.de). However, higher archaeal 16S rRNA gene abundance has been reported in the oligotrophic and organic matter poor North Pond marine sediments (e.g., Breuker and Schippers, 2013).

In SLW sediments, the abundance of *aprA* gene copies also decreased with depth (Table 1; Figure 3). Functional gene abundances relative to total 16S rRNA gene copies have been used to estimate population densities. For example, denitrifiers in soil have been estimated to represent 0.1-5% of bacterial 16S rRNA gene copies (Henry et al., 2006). Copies of *aprA* in SLW sediments represented 7.3% (MC-2B$_{(0\text{-}4\ cm)}$) and 14.5% (MC-3C$_{(2\text{-}3.5\ cm)}$) of total 16S rRNA gene copies in the top depths. These percentages decreased to 1.6% (MC-2B$_{(4\text{-}8)}$) and 2.2% (MC-3C$_{(2\text{-}3.5)}$) in the middle depths, and 0.6% (MC-2B$_{(28\text{-}34\ cm)}$) and 0.9% (MC-3C$_{(8\text{-}16\ cm)}$) in the deeper depths (Table 1). While the

abundance of *aprA* containing cells vary in our samples, they appear to be a large portion of the community.

The *aprA* primers used for this study are universal for bacteria and archaea, however no archaeal *aprA* were detected. If we look at *aprA* abundance relative to total bacterial 16S rRNA, sulfur cyclers represent a high portion (18% ±9.9) of the bacterial population (Table 1). Our *aprA* abundance is supported by reported 16S rRNA gene data from 0-2 cm where a "Sideroxydans"-like organism represented 12.7 % of total 16S rRNA gene sequences (Christner et al., 2014). Our results are higher than reports from 40 m below the Peru Margin seafloor, where *aprA* represented 0.5-1% of bacterial 16S rRNA gene copies (Blazejak and Schippers, 2011). Our *aprA* sequence data, in combination with previously published 16S rRNA gene data strongly support the notion that sulfur oxidation is a dominant metabolic process in SLW sediments, largely facilitated by a "Sideroxydans"-like organism.

Community structure, function, and diversity in SLW sediments

The presence of *aprA*, *dsrAB*, and *rdsrAB* in sediments from SLW indicates the microbial community has the genetic potential to transform sulfur compounds (Table 1). The functional lineages of *aprA* (e.g. oxidation, reduction, or uncertain function) varied with depth in both sediment cores (Figure 6) although sulfur-oxidizing *aprA* was present in all depths analyzed. SOP-like sequences were all related to autotrophs or facultative autotrophs suggesting sulfur driven chemosynthesis may occur in the top 34 cm of SLW sediments (Table 4; Figure 6).

Diversity measurements can be used to compare depth profiles or among different environments. The Shannon-Weaver diversity index (*H´*) varied from 0.63 to 1.82

suggesting heterogeneity in the sediment samples. Sulfur-oxidizing *aprA* sequences were dominant in the top sediment depths (98% of total *aprA* sequences; Figure 5), and had low diversity (Table 3). The abundance of *aprA* sequences related to sulfur oxidizers decreased with depth (Figure 6), but overall *aprA* sequence diversity was higher at deeper depths; MC-2B$_{(28\text{-}34cm)}$ and MC-3C$_{(8\text{-}16cm)}$, *H'* values, 1.62 and 1.82, respectively. SRP-related *aprA* sequences were only detected in MC-2B$_{(28\text{-}34\ cm)}$ and MC-3C$_{(8\text{-}16\ cm)}$ (Table 4; Figure 6), suggesting a change in the sulfur cycling community structure with depth. The true diversity of SRP was likely not sampled by the use of only one primer set for this gene and Chao1 and rarefaction analyses indicate *aprA* was not sampled to saturation (Table 3; Figure 5). The Chao1 richness estimates predicted a range of *aprA* containing species (3-13) with an overall richness estimate of 34 species for all samples (Table 3).

We have likely underestimated the total number of SOP in SLW since the alternative sulfur oxidation pathways (e.g., the SOX enzyme system), present in many SOP (Meyer et al., 2007), were not addressed in this study.

The presence of sulfur-oxidizing prokaryotes

The most abundant *aprA* sequences were related to SOP lineages (Table 4; Figure 6). Seven OTUs, representing 74% of total *aprA* sequences fall within the two SOP *aprA* lineages (SOP lineages I and II) as defined by Meyer and Kuever (2007a). SOP lineage I represents genes in SOP that have been vertically transferred and lineage II represents genes most closely related to SRP *aprA* which is thought to have been laterally transferred (Meyer and Keuver, 2007a). Combined *aprA* and *rdsrA* analyses indicated that the dominant sulfur oxidizer in SLW was related to "Sideroxydans lithotrophicus"

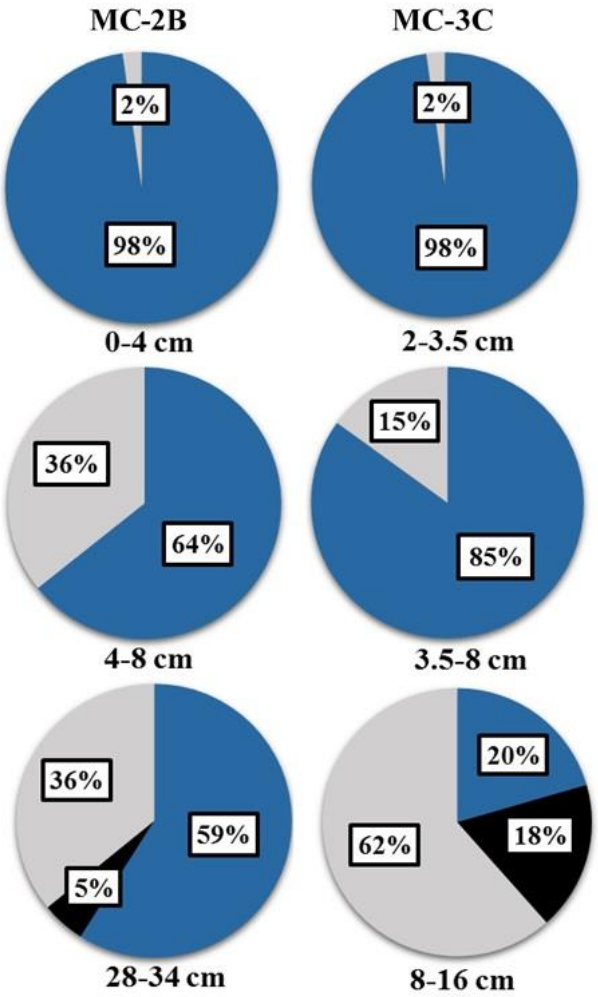

Figure 6. Distribution of *aprA* sequences from SLW sediment cores MC-2B and MC-3C among putative sulfur-cycling lineages. Sulfur cycling lineages as functional categories defined by Meyer and Kuever (2007a). Blue represents sulfur-oxidizing prokaryote lineages I and II, black represents sulfate-reducing lineages, and gray represents sequences of uncertain function. Percentages represent the number of sequences within the designated lineage out of the total *aprA* sequences obtained from each depth. The total number of sequences obtained for each depth are listed in Table 3.

ES-1 (93-97% and 89-91% amino acid identity, *aprA* and *rdsrA*, respectively). Strain ES-1 was originally isolated from groundwater in Michigan and is characterized as a neutrophilic, microaerophilic, iron oxidizer also capable of oxidizing reduced sulfur compounds such as thiosulfate and iron sulfide (Emerson et al., 2013; Emerson and Moyer, 1997). *Thiobacillus* spp. including *T. Thioplumbophilis* and *Sulfuritalea hydrogenivorans* sk43H were also detected in the *aprA* and *rdsrA* clone libraries. These results correspond with the 16S rRNA gene survey of MC-2B$_{(0\text{-}2\ cm)}$ sediments from SLW where a "Sideroxydans"-like phylotype was the most abundant (12.7% total sequences), and a *Thiobacillus*-like phylotype (6.1% total sequences) were detected (Christner et al., 2014). Our data provide functional gene evidence that supports the important ecological role of sulfur oxidation by "Sideroxydans"-like and *Thiobacillus*-like organisms in SLW sediments. Our results are consistent with findings from other subglacial systems. For example, the 16S rRNA gene sequence identified as "S. lithotrophicus" from SLW was 99% identical to a clone from sediments beneath the Kamb Ice Stream (Christner et al., 2014) and a "Sideroxydans" sp. comprised 12% of total 16S rRNA gene sequences in samples from Robertson Glacier in the Canadian Rockies (Hamilton et al., 2013). Perhaps the function of "Sideroxydans"-like organisms play a critical role in subglacial microbial ecosystems.

The rDSR pathway may facilitate a chemosynthetic lifestyle for some SOP in SLW. rDSR is necessary for the oxidation of intracellular elemental sulfur globules, or the temporary storage reservoirs that are formed during the oxidation of sulfides in many SOP (Dahl et al., 2005; Holkenbrink et al., 2011). *rdsrA* OTUs from SLW sediments were most closely related to cultured organisms including phototrophs which is likely due

to limited environmental surveys of rDSR diversity, in comparison to APS and DSR (Loy et al., 2009) and limited physiological studies of the rDSR gene in chemoautotrophic microorganisms (Loy et al., 2008). However, a recent study suggests rDSR may, for example, play an important role in energy gain from elemental sulfur in the dark ocean (Anantharaman et al., 2014). rDSR is present in SOP that lack the *SoxCD* gene which codes for sulfur dehydrogenase involved in thiosulfate oxidation (Friedrich et al., 2005; Frigaard and Dahl, 2008). Lenk et al. (2012) showed the presence of both the rDSR gene and the complete SOX pathway in members of the coastal marine *Roseobacter* clade, suggesting that the presence of two pathways increases metabolic versatility. It has been suggested that the rDSR/reverse APS reductase pathway for sulfur oxidation is more efficient in environments with low sulfide concentrations (Frigaard and Bryant, 2008; Holkenbrink et al., 2011; Gregersen et al., 2011). Energetic efficiency might convey a competitive advantage to microorganisms in SLW sediments making the rDSR pathway preferred for the oxidation of sulfur compounds.

Sulfate reduction in SLW sediments

Multiple lines of evidence indicate that SRP are present in SLW sediments, however, sulfate reduction is not likely a dominant process. Biological sulfate reduction was extremely low, but measurable in all three sediment samples incubated with ^{35}S-labeled sulfate (Table 1; Figure 2). Sulfate reduction activity was not detected in the SLW water column, likely due to the presence of oxygen. Sulfate reduction was detected in MC-2B$_{(0\text{-}4\ cm)}$ and MC-3C$_{(2\text{-}3.5\ cm)}$, where the *dsrA* fragment was amplified, even though the *dsrAB* gene was not amplified. *dsrAB* could be in low abundance or not detectable with the primer set used. While no *aprA* sequences related to SRP were detected in MC-

$2B_{(0\text{-}4 \text{ and } 4\text{-}8 \text{ cm})}$ and MC-$3C_{(2\text{-}3.5 \text{ and } 3.5\text{-}8 \text{ cm})}$, the small amounts of reduced sulfur detected in our SRR experiments from these depths could have been generated by organisms carrying the *aprA* sequences of uncertain function, (i.e. those related to both *Thermodesulfovibrio* and members of *Chlorobiacaea). aprA* OTU 4A, was 83% identical to a clone from Blood Falls (Mikucki et al., 2009), and both were 74-72% identical to *Thermodesulfovibrio* spp, which are known SRP.

Detection of *dsrAB* and measureable rates of sulfate reduction were obtained for MC-$3C_{(3.5\text{-}8 \text{ cm})}$. The highest rates of sulfate reduction were measured in unamended samples from MC-$3C_{(2\text{-}3.5 \text{ cm})}$ (avg = 1.7 pmol cm^{-3} d^{-1} +/- 0.54) and formate amended MC-$3C_{(3.5\text{-}8 \text{ cm})}$ (avg = 1.8 pmol $cm^{-3}d^{-1}$ +/- 0.43). Lower rates were measured in MC-$2B_{(0\text{-}4 \text{ cm})}$ (avg = 0.4 pmol cm^{-3} d^{-1} +/- 0.04). MC-3C was stored at 4 °C in its core tube for ~24 hrs while MC-2B was processed; however the sediments collected from MC-2B were likely exposed to oxygen during processing. Unlike the MC-3C samples for SRR, MC-2B sediments were not immediately transferred to N_2-gassed serum vials due to logistical constraints. Thus, variations in rates between depths could be affected by limitations of our field laboratory. SRR did not show a significant difference with formate addition (Figure 2). SRR in SLW are low compared to surface marine sediments (0-4.5 cm) where rates have ranged 20-38 nmol cm^{-3} d^{-1} (Holmkvist et al., 2011). However, similar rates (0.2-1.0 pmol cm^{-3} d^{-1}) to SLW have been measured in deeper marine sediments (300-500 cm; Holmkvist et al., 2011). The low measured rates might also be due to rapid reoxidation of reduced sulfur. If reduced sulfur generated by SRP activity is reoxidized before it can be scavenged by the zinc trap, our rates may be an underestimation; FeS, FeS_2 and S^{2-} are not released from solution until the experiment is terminated by passive

extraction. A similar process, where reduced sulfur was quantitatively reoxidized to sulfate, described as a catalytic sulfur cycle, was observed in the Blood Falls subglacial brine (Mikucki et al., 2009). This type of sulfur cycle has also been detected in marine oxygen-minimum zones and is referred to as a cryptic sulfur cycle (Canfield et al., 2010). Rapid turnover of reduced sulfur may be an economical strategy for energy gain in deep subsurface environments.

Three *aprA* OTUs (9A, 14A, 15A) and one *dsrA* OTU (3D) were detected in MC-$2B_{(28\text{-}34\ cm)}$ and MC-$3C_{(8\text{-}16\ cm)}$ sediment samples that were closely related to Deltaproteobacteria members of the family *Desulfobacteraceae* which are all known SRP. Most *Desulfobacteraceae* isolates have been from marine and hypersaline sediments (Foti et al., 2007; Kjeldsen et al., 2007), however *aprA* sequences related to this group have also been detected in freshwater lakes (Biderre-Petit et al., 2011), including ice-covered lakes Oyako-Ike and Skalle O-Ike in Antarctica (Watanabe et al., 2013). These OTUs, which comprise 3% of total *aprA* sequences and represent 15% of *aprA* sequences in MC-$3C_{(8\text{-}16\ cm)}$, could provide reduced sulfur compounds to SOP at deeper sediment depths. SOP found at this depth may use alternative electron acceptors such as nitrate or ferric iron in the absence of oxygen. The concentration of nitrate was higher in the upper 2 cm of SLW sediments (9.1 µM) compared to the water column (0.8 µM) (Christner et al., 2014), suggesting nitrate is available for microbial reduction. Sulfide oxidation can also be coupled to ferric iron reduction (Schink et al., 2006), although ferric iron concentrations for SLW have not been processed.

MC-$2B_{(28\text{-}34\ cm)}$ and MC-$3C_{(8\text{-}16\ cm)}$ contain *aprA* and *dsrA* sequences related to *Desulfotomaculum* spp. and the family *Desulfobacteraceae*. Two SRP-like *aprA* OTUs

(11A and 12A) had 88-80% identity and one *dsrA* OTU (4D) had 76-72% to *Desulfotomaculum* spp. However, the majority of *dsrA* sequences from SLW sediments were more distantly related (65% identity) to *Desulfotomaculum* spp. *dsrA*, making reliable inference about physiology difficult. It has been argued that *Desulfotomaculum* spp. play an important ecological role in subsurface environments because they are metabolically plastic. They have been shown to grow under a range of sulfate concentrations, can use diverse organic substrates, are capable of autotrophy, have been identified as syntrophic partners with methanogens, and have the ability to form endospores (Aüllo et al., 2013; Imachi et al., 2006). SLW *dsrA* OTU1D is 77-75% identical to clones detected in Aarhus Bay sediments (de Rezende et al., 2013). *dsrA* sequences similar to those from SLW have been found in other low sulfate environments including estuarine sediments (Kondo et al., 2007). Thus the *dsrA* sequences from SLW sediments could represent active microorganisms adapted to freshwater, relatively low sulfate environments. Or, given the low sequence similarity to characterized SRP, the *dsrA* detected in this study could also represent novel SRP lineages.

Conclusion

Combined analyses of the functional genes *aprA*, *dsrA*, and *rdsrA*, in concert with measureable rates of sulfate reduction revealed a diverse sulfur cycling community and identified potential microorganisms participating in sulfate reduction and sulfur oxidation in SLW sediments. Functional gene OTUs in this study represent groups that encompass a broad range of physiological traits. While some OTUs are related to previously documented species from environments including marine sediments, groundwater, and freshwater lakes, many of the OTUs represent novel lineages whose function is not yet

known. Our results further support the fact that Antarctic subglacial aquatic environments host a diverse microbial ecosystem that remains inadequately studied. These data provide new insight into the structure of microbial communities in subglacial environments.

The presence of chemosynthetic sulfur oxidizers in SLW surface sediments reinforces previous reports of sulfur oxidation at subglacial sediment-water interfaces (e.g., Tranter et al., 2002; Skidmore et al., 2005; Lanoil et al., 2009; Hamilton et al., 2013) and supports the importance of dark CO_2 fixation in subglacial environments (Boyd et al., 2014). We provide the first estimate of sulfate reduction rates below the West Antarctic ice sheet. Model simulations of subglacial methanogenesis in Antarctica, an important unknown in the global methane budget, require an understanding of microbially-mediated sulfur transformations, including rates of sulfate reduction (Wadham et al., 2012). Rapid retreat of the grounding line and eventual collapse of the Ross Ice Shelf and West Antarctic ice sheet will expose the subglacial ecosystem to marine conditions, as has happened in the past (Mercer, 1978; Scherer et al., 1998). Understanding the structure and function of subglacial microbial communities can help predict the ecological impact of ice sheet thinning or retreat to the proglacial ecosystem. Upcoming WISSARD studies at the grounding zone, where the stream draining SLW enters the Ross Ice Shelf cavity will provide new information on how subglacial systems interact with the global ocean.

LIST OF REFERENCES

Alley, B., Bentley, R. (1986). Deformation of till beneath Ice Stream B, West Antarctica. *Nature*. 322, 57-59. doi:10.1038/322057a0

Anantharaman, K., Duhaime, M. B., Breier, J. A., Wendt, K. A., Toner, B. M., and Dick, G. J. (2014). Sulfur oxidation genes in diverse deep-sea viruses. *Science*. 344:6185, 757-760. doi: 10.1126/science.1252229

Anderson, S. P. (2007). Biogeochemistry of glacial landscape systems. *Annu. Rev. Earth Pl. Sc*. 35: 375-399. doi: 10.1126/science.1140766

Andersen, K. K., Azuma, N., Barnola, J. M., Bigler, M., Biscaye, P., Caillon, N., Chappellaz, J., and White, J. W. C. (2004). High-resolution record of Northern Hemisphere climate extending into the last interglacial period. *Nature*. 431:7005, 147-151. doi:10.1038/nature02805

Anesio, A. M. and Laybourn-Parry, J. (2011). Glaciers and ice sheets as a biome. *Trends Ecol. Evol*. 27(4), 220–226. doi:10.1016/j.tree.2011.09.012

Antony, C. P., Kumaresan, D., Ferrando, L., Boden, R., Moussard, H., Scavino, A. F., et al. (2010). Active methylotrophs in the sediments of Lonar Lake, a saline and alkaline ecosystem formed by meteor impact. *ISME J*. 4:11, 1470-1480. doi: 10.1038/ismej.2010.70

Aüllo, T., Ranchou-Peyruse, A., Ollivier, B., and Magot, M. (2013). *Desulfotomaculum* spp. and related gram-positive sulfate-reducing bacteria in deep subsurface environments. *Front. Microbiol*. 4:362. doi: 10.3389/fmicb.2013.00362

Bak, F. and Widdel, F. (1986). Anaerobic degradation of phenol and phenol derivatives by *Desulfobacterium phenolicum* sp. nov. *Arch. Microbiol*. 146:2, 177-180. doi: 10.1007/BF00402347

Ball, B. A. and Virginia, R. (2014). Microbial biomass and respiration responses to nitrogen fertilization in a polar desert. *Polar Biol*. 37:4, 573-585. doi: 10.1007/s00300-014-1459-0

Banfield, J. F., Barker, W. W., Welch, S. A., and Taunton, A. (1999). Biological impact on mineral dissolution: application of the lichen model to understanding mineral weathering in the rhizosphere. *Proc. Natl. Acad. Sci. U.S.A*. 96:7, 3404-3411. doi: 10.1073/pnas.96.7.3404

Biderre-Petit, C., Jézéquel, D., Dugat-Bony, E., Lopes, F., Kuever, J., Borrel, G., and Peyret, P. (2011). Identification of microbial communities involved in the methane cycle of a freshwater meromictic lake. *FEMS Microbiol Ecol*. 77:3, 533-545. doi: 10.1111/j.1574-6941.2011.01134.x

Blazejak, A., and Schippers, A. (2011). Real-time PCR quantification and diversity analysis of the functional genes *aprA* and *dsrA* of sulfate-reducing prokaryotes in marine sediments of the Peru continental margin and the Black Sea. *Front. Microbiol.* 2:253. doi: 10.3389/fmicb.2011.00253

Bottomley, P. J. (1994). "Light microscopic methods for studying soil microorganisms", in *Methods of Soil Analysis: Part 2—Microbiological and Biochemical Properties*, eds. J. S. Angle and R. W. Weaver, 81-105.

Bottrell, S. H. and Tranter, M. (2002). Sulphide oxidation under partially anoxic conditions at the bed of the Haut Glacier d'Arolla, Switzerland. *Hydrol. Process.* 16:12, 2363-2368. doi: 10.1002/hyp.1012

Bowles, M. W., Mogollón, J. M., Kasten, S., Zabel, M., and Hinrichs, K. U. (2014). Global rates of marine sulfate reduction and implications for sub–sea-floor metabolic activities. *Science*. 344:6186, 889-891. doi: 10.1126/science.1249213

Boyd, E. S., Hamilton, T. L, Havig, J. R., Skidmore M., Shock, E. L. (2014). Chemolithotrophic primary production in a subglacial ecosystem. *Appl. Environ. Microbiol*. 80:19, 6146-6153. doi: 10.1128/AEM.01956-14

Breuker, A., and Schippers, A. (2013). Data report: total cell counts and qPCR abundance of Archaea and Bacteria in shallow subsurface marine sediments of North Pond: gravity cores collected during site survey cruise prior to IODP Expedition 336. In *Proc. IODP| Volume* (Vol. 336, p. 2). doi: 10.2204/iodp.proc.336.201.2013

Callaghan, A. V., Morris, B. E. L., Pereira, I. A. C., McInerney, M. J., Austin, R. N., Groves, J. T., et al. (2012). The genome sequence of *Desulfatibacillum alkenivorans* AK-01: a blueprint for anaerobic alkane oxidation. *Environ. Microbiol*. 14:1, 101–113. doi: 10.1111/j.1462-2920.2011.02516.x

Canfield, D. E., Jørgensen, B. B., Fossing, H., Glud, R., Gundersen, J., Ramsing, N. B., et al. (1993). Pathways of organic carbon oxidation in three continental margin sediments. *Mar. Geol.* 113:1, 27-40. doi: 10.1016/0025-3227(93)90147-N

Canfield, D. E., Stewart, F. J., Thamdrup, B., De Brabandere, L., Dalsgaard, T., Delong, E. F., et al. (2010). A cryptic sulfur cycle in oxygen-minimum–zone waters off the Chilean coast. *Science*. 330:6009, 1375-1378. doi: 10.1126/science.1196889

Carter, S. P. and Fricker, H. A. (2012). The supply of subglacial meltwater to the grounding line of the Siple Coast, West Antarctica. *Ann. Glaciol*. 53:60, 267-280. doi: 10.3189/2012AoG60A11

Christianson, K., Jacobel, R. W., Horgan, H. J., Anandakrishnan, S., and Alley, R. B. (2012). Subglacial Lake Whillans—Ice-penetrating radar and GPS observations of a shallow active reservoir beneath a West Antarctic ice stream. *Earth Planet. Sc. Lett*. 331, 237-245. doi: 10.1016/j.epsl.2012.03.013

Christner, B. C., Royston-Bishop, G., Foreman, C. M., Arnold, B. R., Tranter, M., Welch, K. A., et al. (2006). Limnological conditions in subglacial Lake Vostok, Antarctica. *Limnol. Oceanogr*. 51:6, 2485-2501.

Christner, B. C., Priscu, J. C., Achberger, A. M., Barbante, C., Carter, S. P., Christianson, K., et al. (2014). Subglacial Lake Whillans: A microbial ecosysytem beneath the West Antarctic Ice Sheet. *Nature*. 512:7514, 310-313. doi: 10.1088/nature13667

Croal, L. R., Johnson, C. M., Beard, B. L., and Newman, D. K. (2004). Iron isotope fractionation by Fe (II)-oxidizing photoautotrophic bacteria. *Geochim. Cosmochim. Ac*. 68:6, 1227-1242. doi: 10.1146/annurev.genet.38.072902.091138

Dahl, C., Engels, S., Pott-Sperling, A. S., Schulte, A., Sander, J., Lübbe, Y., et al. (2005). Novel genes of the *dsr* gene cluster and evidence for close interaction of Dsr proteins during sulfur oxidation in the phototrophic sulfur bacterium *Allochromatium vinosum*. *J. Bacteriol*. 187:4, 1392-1404. doi: 10.1128/JB.187.4.1392

de Rezende, J. R., Kjeldsen, K. U., Hubert, C. R., Finster, K., Loy, A., and Jørgensen, B. B. (2013). Dispersal of thermophilic *Desulfotomaculum* endospores into Baltic Sea sediments over thousands of years. *ISME J*. 7:1, 72-84. doi: 10.1038/ismej.2012.83

Drobner, E., Huber, H., Rachel, R., and Stetter, K. O. (1992). *Thiobacillus plumbophilus* spec. nov., a novel galena and hydrogen oxidizer. *Arch. Microbiol*. 157:3, 213-217. doi: 10.1007/BF00245152

Edwards, A., Irvine-Fynn, T., Mitchell, A. C., and Rassner, S. M. (2014). A germ theory for glacial systems? *WIREs Water*. 1(4), 331-340. doi: 10.1002/wat2.1029

Ehrlich, H. L. (1996). How microbes influence mineral growth and dissolution. *Chem. Geol*. 132:1, 5-9. doi: 10.1016/S0009-2541(96)00035-6

Emerson, D., and Moyer, C. (1997). Isolation and characterization of novel iron-oxidizing bacteria that grow at circumneutral pH. *Appl. Environ. Microbiol*. 63:12, 4784-4792.

Emerson, D., Field, E. K., Chertkov, O., Davenport, K. W., Goodwin, L., Munk, C., et al. (2013). Comparative genomics of freshwater Fe-oxidizing bacteria: implications for physiology, ecology, and systematics. *Front. Microbiol*. 4:254. doi: 10.3389/fmicb.2013.00254

Fahnestock, M., Abdalati, W., Joughin, I., Brozena, J., and Gogineni, P. (2001). High geothermal heat flow, basal melt, and the origin of rapid ice flow in central Greenland. *Science*. *294*(5550), 2338-2342.

Foti, M., Sorokin, D. Y., Lomans, B., Mussman, M., Zacharova, E. E., Pimenov, N. V., et al. (2007). Diversity, activity, and abundance of sulfate-reducing bacteria in saline and hypersaline soda lakes. *Appl. Environ. Microbiol.* 73:7, 2093-2100. doi: 10.1128/AEM.02622-06

Fossing, H. and Jørgensen, B. B. (1989). Measurement of bacterial sulfate reduction in sediments: evaluation of a single-step chromium reduction method. *Biogeochemistry*. 8:3, 205-222. doi: 10.1007/BF00002889

Fretwell, P., Pritchard, H. D., Vaughan, D. G., Bamber, J. L., Barrand, N. E., Bell, R., Casassa, G. (2013). Bedmap2: improved ice bed, surface and thickness datasets for Antarctica. *Cryosphere*. 7(1), 375–393. doi:10.5194/tc-7-375-2013

Fricker, H. A., Scambos, T., Bindschadler, R., Padman, L. (2007). An active subglacial water system in West Antarctica mapped from space. *Science*. 315, 1544. doi: 10.1126/science.1136897

Fricker, H. A., and Scambos, T. (2009). Connected subglacial lake activity on lower Mercer and Whillans Ice Streams, West Antarctica, 2003–2008. *J. Glaciol.* 55:190, 303-315. doi: 10.3189/002214309788608813

Fricker, H. A., Powell, R., Priscu, J., Tulaczyk, S., Anandakrishnan, S., Christner, B., et al. (2011). Siple coast subglacial aquatic environments: The Whillans Ice Stream subglacial access research drilling project. *Geoph. Monog. Series*. 192, 199-219. doi: 10.1002/9781118670354.ch12

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*. 67:7, 2873-2882. doi: 10.1128/AEM.67.7.2873-2882.2001

Friedrich, C. G., Bardischewsky, F., Rother, D., Quentmeier, A., and Fischer, J. (2005). Prokaryotic sulfur oxidation. *Curr. Opin. Microbiol*. 8:3, 253-259. doi: 10.1128/AEM.67.7.2873

Frigaard N.-U. and Bryant D. A. (2008). "Genomics insights into the sulfur metabolism of phototrophic green sulfur bacteria," in *Advances in Photosynthesis and Respiration*, eds. R. Hell, C. Dahl, D. B. Knaff, and T. Leustek. (Heidelberg: Springer), 337–355. doi: 10.1007/978-1-4020-6863-8_17

Frigaard, N. U., and Dahl, C. (2008). Sulfur metabolism in phototrophic sulfur bacteria. *Adv. Microb. Physiol*. 54, 103-200. doi: 10.1016/S0065-2911(08)00002-7

Gaidos, E., Lanoil, B., Thorsteinsson, T., Graham, A., Skidmore, M., Han, S., and Popp, B. (2004). A Viable Microbial Community in a Subglacial Volcanic Crater Lake, Iceland. *Astrobiology*. 4:3, 327-344. doi: 10.1089/ast.2004.4.327

Galushko, A., Minz, D., Schink, B., and Widdel, F. (1999). Anaerobic degradation of naphthalene by a pure culture of a novel type of marine sulphate-reducing bacterium. *Environ. Microbiol*. 1:5, 415-420. doi: 10.1046/j.1462-2920.1999.00051.x

Green-Saxena, A., Feyzullayev, A., Hubert, C. R., Kallmeyer, J., Krueger, M., Sauer, P., et al. (2012). Active sulfur cycling by diverse mesophilic and thermophilic microorganisms in terrestrial mud volcanoes of Azerbaijan. *Environ. Microbiol*. 14:12, 3271-3286. doi: 10.1111/1462-2920.12015

Good, I. J. (1953). The population frequencies of species and the estimation of population parameters. *Biometrika*. 40:3-4, 237-264. doi: 10.1093/biomet/40.3-4.237

Gregersen, L. H., Bryant, D. A., and Frigaard, N. U. (2011). Mechanisms and evolution of oxidative sulfur metabolism in green sulfur bacteria. *Front. Microbiol*. 2:116. doi: 10.3389/fmicb.2011.00116

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* 41:95-98.

Hamilton, T. L, Peters, J. W., Skidmore, M. L., and Boyd, E. S. (2013). Molecular evidence for an active endogenous microbiome beneath glacial ice. *ISME J*. 7, 1402-1412. doi: 10.1038/ismej.2013.3

Haran, T., Bohlander, J., Scambos, T., and Fahnestock, M. (2005). MODIS mosaic of Antarctica (MOA) image map. NSIDC Digital Media. http://nsidc.org/data/nsidc-0280

Harrison, B. K., Zhang, H., Berelson, W., and Orphan, V. J. (2009). Variations in archaeal and bacterial diversity associated with the sulfate-methane transition zone in continental margin sediments (Santa Barbara Basin, California). *Appl. Environ. Microbiol*. 75:6, 1487-1499. doi:10.1128/AEM.01812-08

Hawkings, Jon R., Jemma L. Wadham, Martyn Tranter, Rob Raiswell, Liane G. Benning, Peter J. Statham, Andrew Tedstone, Peter Nienow, Katherine Lee, and Jon Telling. (2014). Ice sheets as a significant source of highly reactive nanoparticulate iron to the oceans. *Nature communications* 5.

Henry, E. A., Devereux, R., Maki, J. S., Gilmour, C. C., Woese, C. R., Mandelco, L., et al. (1994). Characterization of a new thermophilic sulfate-reducing bacterium. *Arch. Microbiol*. 161:1, 62-69. doi: 10.1007/BF00248894

Henry, S., Bru, D., Stres, B., Hallet, S., and Philippot, L. (2006). Quantitative detection of the *nosZ* gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, *narG*, *nirK*, and *nosZ* genes in soils. *Appl. Environ. Microbiol*. 72:8, 5181-5189. doi: 10.1128/AEM.00231-06

Hill, T. C., Walsh, K. A., Harris, J. A., and Moffett, B. F. (2003). Using ecological diversity measures with bacterial communities. *FEMS Microbiol. Ecol*. 43:1, 1-11. doi: 10.1111/j.1574-6941.2003.tb01040.x

Hinrichs, K. U. and Boetius, A. (2003). The anaerobic oxidation of methane: new insights in microbial ecology and biogeochemistry. *Ocean margin systems*. Springer Berlin Heidelberg. 457-477.

Holkenbrink, C., Barbas, S. O., Mellerup, A., Otaki, H., and Frigaard, N.U. (2011). Sulfur globule oxidation in green sulfur bacteria is dependent on the dissimilatory sulfite reductase system. *Microbiol*. 157:4, 1229-1239. doi: 10.1099/mic.0.044669-0

Holmkvist, L., Ferdelman, T. G and Jørgensen, B. B. (2011). A cryptic sulfur cycle driven by iron in the methane zone of marine sediment (Aarhus Bay, Denmark). *Geochim. Cosmochim. Ac*. 75:12, 3581-3599. doi: 10.1016/j.gca.2011.03.033

Horgan, H. J., Alley, R.B., Christianson, K., Jacobel, R.W., Anandakrishnan, S., Muto, A., Beem, L.H., and Siegfried, M. R. (2013). Estuaries beneath ice sheets. *Geology*. 41:11. 1159-1162.

Huber, T., Faulkner, G., and Hugenholtz, P. (2004). Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. *Bioinformatics*. 20:14, 2317-2319. doi: 10.1093/bioinformatics/bth226

Hughes, T. (1977). West Antarctic ice streams. *Reviews of Geophysics*. 15(1), 1-46.

Imachi, H., Sekiguchi, Y., Kamagata, Y., Loy, A., Qiu, Y. L., Hugenholtz, P., et al. (2006). Non-sulfate-reducing, syntrophic bacteria affiliated with *Desulfotomaculum* cluster I are widely distributed in methanogenic environments. *Appl. Environ. Microbiol*. 72:3, 2080-2091. doi: 10.1128/AEM.72.3.2080-2091.2006

Jørgensen, B. B. (1982). Mineralization of organic matter in the sea bed—the role of sulphate reduction. *Nature*. 296: 643-645. doi: 10.1038/296643a0

Jørgensen, B. B., and Postgate, J. R. (1982). Ecology of the bacteria of the sulphur cycle with special reference to anoxic-oxic interface environments. *Philos. T. Roy. Soc. B.* 298:1093, 543-561. doi: 10.1098/rstb.1982.0096

Kaksonen, A. H., Spring, S., Schumann, P., Kroppenstedt, R. M., and Puhakka, J. A. (2007). *Desulfurispora thermophila* gen. nov., sp. nov., a thermophilic, spore-forming sulfate-reducer isolated from a sulfidogenic fluidized-bed reactor. *Int. J. Syst. Evol. Microbiol*. 57:5, 1089-1094. doi: 10.1099/ijs.0.64593-0

Kallmeyer, J., Smith, D. C., Spivack, A. J., and D'Hondt, S. (2008). New cell extraction procedure applied to deep subsurface sediments. *Limnol. Oceanogr. Methods*. 6, 236-245. doi: 10.4319/lom.2008.6.236

Kallmeyer, J., Pockalny, R., Adhikari, R. R., Smith, D. C., and D'Hondt, S. (2012). Global distribution of microbial abundance and biomass in subseafloor sediment. *Proc. Natl. Acad. Sci. U.S.A.* 109:40, 16213-16216. doi: 10.1073/pnas.1203849109

Kelly, D. P. (1982). Biochemistry of the chemolithotrophic oxidation of inorganic sulphur. *Philos. T. Roy. Soc. B.* 298:1093, 499-528. doi: 10.1098/rstb.1982.0094

Kepner, R. L., and Pratt, J. R. (1994). Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. *Microbiol. Reviews*. 58:4, 603-615.

Kjeldsen, K. U., Loy, A., Jakobsen, T. F., Thomsen, T. R., Wagner, M., and Ingvorsen, K. (2007). Diversity of sulfate-reducing bacteria from an extreme hypersaline sediment, Great Salt Lake (Utah). *FEMS Microbiol. Ecol*. 60:2, 287-298. doi: 10.1111/j.1574-6941.2007.00288.x

Kjeldsen, K. U., Jakobsen, T. F., Glastrup, J., and Ingvorsen, K. (2010). *Desulfosalsimonas propionicica* gen. nov., sp. nov., a halophilic, sulfate-reducing member of the family *Desulfobacteraceae* isolated from a salt-lake sediment. *Int. J. Syst. Evol. Microbiol*. 60:5, 1060-1065. doi: 10.1099/ijs.0.014746-0

Klauth, P., Wilhelm, R., Klumpp, E., Poschen, L., and Groeneweg, J. (2004). Enumeration of soil bacteria with the green fluorescent nucleic acid dye Sytox green in the presence of soil particles. *J. Microbiol. Methods*. 59:2, 189-198. doi: 10.1016/j.mimet.2004.07.004

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., et al. (2012). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res*. 41:1. doi: 10.1093/nar/gks808

Kojima, H. and Fukui, M. (2011). *Sulfuritalea hydrogenivorans* gen. nov., sp. nov., a facultative autotroph isolated from a freshwater lake. *Int. J. Syst. Evol. Microbiol*. 61:7, 1651-1655. doi: 10.1099/ijs.0.024968-0

Kondo, R., Nedwell, D. B., Purdy, K. J., and Silva, S. Q. (2004). Detection and enumeration of sulphate-reducing bacteria in estuarine sediments by competitive PCR. *Geomicrobiol. J*. 21:3, 145-157. doi: 10.1080/01490450490275307

Kondo, R., Purdy, K. J., Silva, S. D. Q., and Nedwell, D. B. (2007). Spatial dynamics of sulphate-reducing bacterial compositions in sediment along a salinity gradient in a UK estuary. *Microbes Environ*. 22:1, 11-19. doi: 10.1264/jsme2.22.11

Lanoil, B., Skidmore, M., Priscu, J. C., Han, S., Foo, W., Vogel, S. W., et al. (2009). Bacteria beneath the West Antarctic Ice Sheet. *Environ. Microbiol*. 11:3, 609-615. doi: 10.1111/j.1462-2920.2008.01831.x

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., et al. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*. 23:21, 2947-2948. doi: 10.1093/bioinformatics/btm404

Leloup, J., Fossing, H., Kohls, K., Holmkvist, L., Borowski, C., and Jørgensen, B. B. (2009). Sulfate-reducing bacteria in marine sediment (Aarhus Bay, Denmark): abundance and diversity related to geochemical zonation. *Environ. Microbiol*. 11:5, 1278-1291. doi: 10.1111/j.1462-2920.2008.01855.x

Lenk, S., Arnds, J., Zerjatke, K., Musat, N., Amann, R., and Mußmann, M. (2011). Novel groups of *Gammaproteobacteria* catalyse sulfur oxidation and carbon fixation in a coastal, intertidal sediment. *Environ. Microbiol*. 13:3, 758-774. doi: 10.1111/j.1462-2920.2010.02380.x

Lenk, S., Moraru, C., Hahnke, S., Arnds, J., Richter, M., Kube, M., et al. (2012). *Roseobacter* clade bacteria are abundant in coastal sediments and encode a novel combination of sulfur oxidation genes. *ISME J*. 6:12, 2178-2187. doi: 10.1038/ismej.2012.66

Li, J., Peng, X., Zhou, H., Li, J., and Sun, Z. (2013). Molecular evidence for microorganisms participating in Fe, Mn, and S biogeochemical cycling in two low-temperature hydrothermal fields at the Southwest Indian Ridge. *J. Geophys. Res: Biogeosciences*. 118:2, 665-679. doi: 10.1002/jgrg.20057

Lloyd, K. G., Alperin, M. J., and Teske, A. (2011). Environmental evidence for net methane production and oxidation in putative ANaerobic MEthanotrophic (ANME) archaea. *Environ. Microbiol*. 13:9, 2548-2564. doi: 10.1111/j.1462-2920.2011.02526.x

Lloyd, K. G., May, M. K., Kevorkian, R. T., and Steen, A. D. (2013). Meta-analysis of quantification methods shows that Archaea and Bacteria have similar abundances in the subseafloor. *Appl. Environ. Microbiol*. 79:24, 7790-7799. doi: 10.1128/AEM.02090-13

Loy, A., Duller, S., and Wagner, M. (2008). "Evolution and ecology of microbes dissimilating sulfur compounds: insights from siroheme sulfite reductases," in *Microbial Sulfur Metabolism*, eds. C. Dahl and C. G. Friedrich (Heidelberg: Springer), 46-59. doi: 10.1007/978-3-540-72682-1_5

Loy, A., Duller, S., Baranyi, C., Mußmann, M., Ott, J., Sharon, I., et al. (2009). Reverse dissimilatory sulfite reductase as phylogenetic marker for a subgroup of sulfur-oxidizing prokaryotes. *Environ. Microbiol*. 11:2, 289-299. doi:10.1111/j.1462-2920.2008.01760.x

Lunau, M., Lemke, A., Walther, K., Martens-Habbena, W., and Simon, M. (2005). An improved method for counting bacteria from sediments and turbid environments by epifluorescence microscopy. *Environ. Microbiol*. 7(7), 961-968.

Madigan, M. T. (2005). Brock Biology of Microorganisms, 11th edn.

Markowitz V. M., Chen I. M., Palaniappan K., Chu K., Szeto E., Pillay M., et al. (2014). IMG 4 version of the integrated microbial genomes comparative analysis system. *Nucleic Acids Res*. 42: D560-D567.

Mattes, T. E., Nunn, B. L., Marshall, K. T., Proskurowski, G., Kelley, D. S., Kawka, O. E., et al. (2013). Sulfur oxidizers dominate carbon fixation at a biogeochemical hot spot in the dark ocean. *ISME J*. 7:12, 2349-60. doi: 10.1038/ismej.2013.113

Mercer, J. H. (1978). West Antarctic ice sheet and CO_2 greenhouse effect: a threat of disaster. *Nature*. 271: 321-325.

Meyer, B. and Kuever, J. (2007a). Molecular analysis of the distribution and phylogeny of dissimilatory adenosine-5′-phosphosulfate reductase-encoding genes (*aprBA*) among sulfur-oxidizing prokaryotes. *Microbiology*. 153:10, 3478-3498. doi: 10.1099/mic.0.2007/008250-0

Meyer, B. and Kuever, J. (2007b). Phylogeny of the alpha and beta subunits of the dissimilatory adenosine-5′-phosphosulfate (APS) reductase from sulfate-reducing prokaryotes–origin and evolution of the dissimilatory sulfate-reduction pathway. *Microbiology*. 153:7, 2026-2044. doi: 10.1099/mic.0.2006/003152-0

Meyer, B. and Kuever, J. (2007c). Molecular analysis of the diversity of sulfate-reducing and sulfur-oxidizing prokaryotes in the environment, using *aprA* as functional marker gene. *Appl. Environ. Microbiol*. 73:23, 7664-7679. doi:10.1128/AEM.01272-07

Meyer, B., Imhoff, J. F., and Kuever, J. (2007). Molecular analysis of the distribution and phylogeny of the *soxB* gene among sulfur-oxidizing bacteria–evolution of the Sox sulfur oxidation enzyme system. *Environ. Microbiol*. 9:12, 2957-2977. doi: 10.1111/j.1462-2920.2007.01407.x

Mikucki, J. A., Foreman, C. M., Sattler, B., Lyons, W. B., and Priscu, J. C. (2004). Geomicrobiology of Blood Falls : An Iron-Rich Saline Discharge at the Terminus of the Taylor Glacier , Antarctica. 199–220.

Mikucki, J. A. and Priscu, J. C. (2007). Bacterial diversity associated with Blood Falls, a subglacial outflow from the Taylor Glacier, Antarctica. *Appl. Environ. Microbiol*. 73:12, 4029-4039. doi: 10.1128/AEM.01396-06

Mikucki, J., Pearson, A., Johnston, D. T., Turchyn, A. V., Farquhar, J., Schrag, D. P., et al. (2009). A contemporary microbially maintained subglacial ferrous ocean. *Science*. 324:5925, 397-400. doi: 10.1126/science.1167350

Miteva, V. I., Sheridan, P. P., and Brenchley, J. E. (2004). Phylogenetic and Physiological Diversity of Microorganisms Isolated from a Deep Greenland Glacier Ice Core Phylogenetic and Physiological Diversity of Microorganisms Isolated from a Deep Greenland Glacier Ice Core. 70(1). doi:10.1128/AEM.70.1.202

Montross, S. N., Skidmore, M., Tranter, M., Kivimäki, and Parkes, R. J. (2013). A microbial driver of chemical weathering in glaciated systems. *Geology*. 41:2, 215-218. doi: 10.1130/G33572.1

Morono, Y., Terada, T., Kallmeyer, J., and Inagaki, F. (2013). An improved cell separation technique for marine subsurface sediments: applications for high-throughput analysis using flow cytometry and cell sorting. *Environ. Microbiol*. 15:10, 2841-2849. doi: 10.1111/1462-2920.12153

Muyzer, G. and Stams, A. J. M. (2008). The ecology and biotechnology of sulphate-reducing bacteria. *Nature reviews. Microbiology*. 6(6), 441–54. doi:10.1038/nrmicro1892

Nadkarni, M. A., Martin, F. E., Jacques, N. A., and Hunter, N. (2002). Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology*. 148:1, 257-266.

Nilsson, R. H., Abarenkov, K., Veldre, V., Nylinder, S., De Wit, P., Brosché, S., et al. (2010). An open source chimera checker for the fungal ITS region. *Mol. Ecol. Resour*. 10:6, 1076-1081. doi: 10.1111/j.1755-0998.2010.02850.x

Overmann, J., and Pfennig, N. (1989). Pelodictyon phaeoclathratiforme sp. nov., a new brown-colored member of the *Chlorobiaceae* forming net-like colonies. *Arch. Microbiol*. 152:4, 401-406. doi: 10.1007/BF00425181

Parshina, S. N., Sipma, J., Nakashimada, Y., Henstra, A. M., Smidt, H., Lysenko, A. M., et al. (2005). *Desulfotomaculum carboxydivorans* sp. nov., a novel sulfate-reducing bacterium capable of growth at 100% CO. *Int. J. Syst. Evol. Microbiol*. 55:5, 2159-2165. doi: 10.1099/ijs.0.63780-0

Pearce, D., Hodgson, D., Thorne, M., Burns, G., & Cockell, C. (2013). Preliminary Analysis of Life within a Former Subglacial Lake Sediment in Antarctica. *Diversity*. 5(3), 680–702. doi:10.3390/d5030680

Pikuta, E., Lysenko, A., Suzina, N., Osipov, G., Kuznetsov, B., Tourova, T., Akimenko, V., Laurinavichius, K. (2000). *Desulfotomaculum alkaliphilum* sp. nov., a new alkaliphilic, moderately thermophilic, sulfate-reducing bacterium. *Int. J. Sys. Evol. Microbial.*. 50(1), 25-33. doi: 10.1099/00207713-50-1-25

Pott, A. S. and Dahl, C. (1998). Sirohaem sulfite reductase and other proteins encoded by genes at the *dsr* locus of *Chromatium vinosum* are involved in the oxidation of intracellular sulfur. *Microbiology*. 144:7, 1881-1894. doi: 10.1099/00221287-144-7-1881

Powell, R. and Hodson, T. (2014) Bed Conditions of Subglacial Lake Whillans, West Antarctica. Geophysical Research Abstracts Vol. 16, EGU2014-3212, 2014, EGU General Assembly 2014

Priscu, John C., Robin E. Bell, Sergey A. Bulat, Cynan J. Ellis-Evans, Mahlon C. Kennicutt, Valery V. Lukin, Jean-Robert Petit, Ross D. Powell, Martin J. Siegert, and Ignazio Tabacco. (2003). An international plan for Antarctic subglacial lake exploration. *Polar Geography*. 27, 1: 69-83.

Priscu, J. C., and Christner, B. C. (2004). Earth's icy biosphere. *Microbial Diversity Prospecting*, 130-145.

Priscu, J. C., Tulaczyk, S., Studinger, M., Kennicutt II, M. C., Christner, B. C., and Foreman, C. M. (2008). "Antarctic subglacial water: origin, evolution and ecology", in *Polar Lakes Rivers*, eds. W. F. Vincent and J. Laybourn-Parry (Oxford, UK; Oxford Univ. Press), 119-136.

Priscu, J. C., Powell, R. D., and Tulaczyk, S. (2010). Probing subglacial environments under the Whillans Ice Stream, *Eos T. Am. Geophys. Un*. 91:29, 253. doi: 10.1029/2010EO290002.

Priscu, J. C., Achberger, A. M., Cahoon, J. E., Christner, B. C., Edwards, R. L., Jones, W. L., et al. (2013). A microbiologically clean strategy for access to the Whillans Ice Stream subglacial environment. *Antarct. Sci*. 25:5, 637-647. doi: 10.1017/S0954102013000035

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res*. 41:D1, D590-D596. doi: 10.1093/nar/gks1219

R Development Core Team. (2008). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Rabus, R., Hansen, T. A., and Widdel, F. (2006). "Dissimilatory sulfate-and sulfur-reducing prokaryotes", in *The Prokaryotes*, eds. M. Dworkin, S. Falkow, E. Rosenburg, K. Schleifer, and E. Stackebrandt (New York: Springer), 659-768. doi: 10.1007/0-387-30742-7_22

Riedinger, N., Brunner, B., Lin, Y. S, Voßmeyer, A., Ferdelman, T. G., and Jørgensen, B. B. (2010). Methane at the sediment-water transition in Black Sea sediments. *Chem. Geol*. 274:1, 29-37. doi: 10.1016/j.chemgeo.2010.03.010

Ritalahti, K. M., Amos, B. K., Sung, Y., Wu, Q., Koenigsberg, S. S., and Löffler, F. E. (2006). Quantitative PCR targeting 16S rRNA and reductive dehalogenase genes simultaneously monitors multiple *Dehalococcoides* strains. *Appl. Environ. Microbiol*. 72:4, 2765-2774. doi: 10.1128/AEM.72.4.2765

Scherer, R.P. (1991) Quaternary and Tertiary microfossils from beneath Ice Stream B: Evidence for a dynamic West Antarctic Ice Sheet history. *Global and Planetary Change*, 4(4), 395-412. doi: 10.1016/0921-8181(91)90005-H

Scherer, R. P., Aldahan, A., Tulaczyk, S., Possnert, G., Engelhardt, H., and Kamb, B. (1998). Pleistocene collapse of the West Antarctic ice sheet. Science, 281(5373), 82-85.

Scherer, R. P., Sjunneskog, C. M., Iverson, N. R., and Hooyer, T. S. (2004). Assessing subglacial processes from diatom fragmentation patterns. *Geology.* 32(7), 557-560.

Scherer, R. P., Sjunneskog, C. M., Iverson, N. R., and Hooyer, T. S. (2005). Frustules to fragments, diatoms to dust: how degradation of microfossil shape and microstructures can teach us how ice sheets work. *J. nanoscience and nanotechnology*. 5(1), 96-99.

Scherer, R., Coenen, J., and Warny, S. (2014) Subglacial sediment provenance and transport in West Antarctica from micropaleontological analysis of Subglacial Lake Whillans and the upstream sectors of the Whillans and Kamb ice streams. Geophysical Research Abstracts, Vol. 16, EGU2014-8817, 2014, EGU General Assembly 2014.

Schlesinger, W. H. (2013). *Biogeochemistry: an analysis of global change*. Academic press.

Schink, B. (2006). Microbially driven redox reactions in anoxic environments: Pathways, energetics, and biochemical consequences. *Engineer. Life Sci*. 6:3, 228-233. doi: 10.1002/elsc.200620130

Sharp, M., Parkes, J., Cragg, B., Fairchild, I. J., Lamb, H., and Tranter, M. (1999). Widespread bacterial populations at glacier beds and their relationship to rock weathering and carbon cycling. *Geology*. *27*(2), 107. doi:10.1130/0091-7613(1999)027<0107:WBPAGB>2.3.CO;2

Shively, J. M., Van Keulen, G., and Meijer, W. G. (1998). Something from almost nothing: carbon dioxide fixation in chemoautotrophs. *Annual Rev. Microbiol*. 52(1), 191-230.

Siegfried, M. R., Fricker, H. A., Roberts, M., Scambos, T. A., and Tulaczyk, S. (2014). A decade of West Antarctic subglacial lake interactions from combined ICESat and CryoSat-2 altimetry. *Geophys. Res. Lett*. 41:3, 891-898. doi: 10.1002/2013GL058616

Singleton, D. R., Furlong, M. A., Rathbun, S. L., and Whitman, W. B. (2001). Quantitative comparisons of 16S rRNA gene sequence libraries from environmental samples. *Appl. Environ. Microbiol*. 67:9, 4374-4376. doi: 10.1128/AEM.67.9.4374

Skidmore, M. L., Foght, J. M., and Sharp, M. J. (2000). Microbial life beneath a high Arctic glacier. *Appl. Environ. Microbiol*. 66:8, 3214-3220. doi: 10.1128/AEM.66.8.3214-3220.2000

Skidmore, M., Anderson, S. P., Sharp, M., Foght, J., and Lanoil, B. D. (2005). Comparison of microbial community compositions of two subglacial environments reveals a possible role for microbes in chemical weathering processes. *Appl. Environ. Microbiol*. 71:11, 6986-6997. doi: 10.1128/AEM.71.11.6986

Skidmore, M., Tranter, M., Tulaczyk, S. and Lanoil, B., (2010). Hydrochemistry of ice stream beds – evaporitic or microbial effects? *Hydrol. Process.* 24:4, 517–523. doi: 10.1002/hyp.7580

Skidmore, M. (2011). "Microbial communities in Antarctic subglacial aquatic environments", In *Antarctic Subglacial Aquatic Environ*, eds. M. Siegert, M. C. Kennicutt, and R. A. Bindschadler (Washington DC: American Geophysical Union Press), 192, 61-81. doi: 10.1029/2010GM000995

Smith, B. E., Fricker, H. A., Joughin, I. R., & Tulaczyk, S. (2009). An inventory of active subglacial lakes in Antarctica detected by ICESat (2003–2008). *J. Glaciol*. 55(192), 573-595.

Smith, C. J. and Osborn, A. M. (2009). Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology. *FEMS Microbiol. Ecol*. 67:1, 6-20. doi: 10.1111/j.1574-6941.2008.00629.x

Sonne-Hansen, J., and Ahring, B. K. (1999). *Thermodesulfobacterium hveragerdense* sp. nov., and *Thermodesulfovibrio islandicus* sp. nov., two thermophilic sulfate reducing bacteria isolated from a Icelandic hot spring. *Syst. Appl. Microbiol*. 22:4, 559-564. doi: 10.1016/S0723-2020(99)80009-5

Swan, B. K., Martinez-Garcia, M., Preston, C. M., Sczyrba, A., Woyke, T., Lamy, D., et al. (2011). Potential for chemolithoautotrophy among ubiquitous bacteria lineages in the dark ocean. *Science*. 333:6047, 1296-1300. doi: 10.1126/science.1203690

Takai, K. and Horikoshi, K. (2000). Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. *Appl. Environ. Microbiol*. 66:11, 5066-5072. doi: 10.1128/AEM.66.11.5066-5072.2000

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol*. 30:12, 2725-2729. doi: 10.1093/molbev/mst197

Thullner, M., Dale, A. W., and Regnier, P. (2009). Global-scale quantification of mineralization pathways in marine sediments: A reaction-transport modeling approach. *Geochem. Geophys. Geosyst*. 10:10. doi: 10.1029/2009GC002484

Tranter, M., Sharp, M. J., Lamb, H. R., Brown, G. H., Hubbard, B. P., and Willis, I. C. (2002). Geochemical weathering at the bed of Haut Glacier d'Arolla, Switzerland—a new model. *Hydrol. Process*. 16:5, 959-993. doi: 10.1002/hyp.309

Tranter, M., Skidmore, M., and Wadham, J. (2005). Hydrological controls on microbial communities in subglacial environments. *Hydrol. Process*. 19:4, 995-998. doi: 10.1002/hyp.5854

Tulaczyk, S., Mikucki, J. A., Siegfried, M. R., Priscu, J. C., Barcheck, C. G., Beem, L. H., et al. (2014). WISSARD at Subglacial Lake Whillans, West Antarctica: scientific operations and initial observations. *Ann. Glaciol*. 55:65. doi: 10.3189/2014AoG65A009

Ulrich, G. A., Krumholz, L. R., and Suflita, J. M. (1997). A rapid and simple method for estimating sulfate reduction activity and quantifying inorganic sulfides. *Appl. Environ. Microbiol*. 63:4, 1627-1630.

Varon-Lopez, M., Dias, A. C. F., Fasanella, C. C., Durrer, A., Melo, I. S., Kuramae, E. E., et al. (2014). Sulphur-oxidizing and sulphate-reducing communities in Brazilian mangrove sediments. *Environ. Microbiol*. 16:3, 845-855. doi: 10.1111/1462-2920.12237

Visser, M., Worm, P., Muyzer, G., Pereira, I. A., Schaap, P. J., Plugge, C. M., et al. (2013). Genome analysis of *Desulfotomaculum kuznetsovii* strain 17T reveals a physiological similarity with *Pelotomaculum thermopropionicum* strain SIT. *Stand. Genomic Sci*. 8:1, 69-87. doi: 10.4056/sigs.3627141

Wadham, J. L., Bottrell, S., Tranter, M., and Raiswell, R. (2004). Stable isotope evidence for microbial sulphate reduction at the bed of a polythermal high Arctic glacier. *Earth Planet. Sc. Lett*. 219:3, 341-355. doi: 10.1016/S0012-821X(03)00683-6

Wadham, J. L., Tranter, M., Skidmore, M., Hodson, A. J., Priscu, J., Lyons, W. B., et al. (2010). Biogeochemical weathering under ice: size matters *Global Biogeochem. Cycles*. 24:3. doi: 10.1029/2009GB003688.

Wadham, J. L., Arndt, S., Tulaczyk, S., Stibal, M., Tranter, M., Telling, J., et al. (2012). Potential methane reservoirs beneath Antarctica. *Nature*. 488:7413, 633-637. doi: 10.1038/nature11374

Wagner, M., Roger, A. J., Flax, J. L., Brusseau, G. A., and Stahl, D. A. (1998). Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. *J. Bacteriol*. 180:11, 2975-2982.

Watanabe, T., Kojima, H., Takano, Y., and Fukui, M. (2013). Diversity of sulfur-cycle prokaryotes in freshwater lake sediments investigated using *aprA* as the functional marker gene. *Syst. Appl. Microbiol*. 36:6, 436-443. doi: 10.1016/j.syapm.2013.04.009

Wright, A. and Siegert, M. (2012). A fourth inventory of Antarctic subglacial lakes. *Antarctic Sci*. 24:06, 659-664. doi: 10.1017/S095410201200048X

Yu, Y., Lee, C., Kim, J., and Hwang, S. (2005). Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnol. Bioengin*. 89:6, 670-679. doi: 10.1002/bit.20347

Zaar, A., Fuchs, G., Golecki, J. R., and Overmann, J. (2003). A new purple sulfur bacterium isolated from a littoral microbial mat, *Thiorhodococcus drewsii* sp. nov. *Arch. Microbiol*. 179:3, 174-183. doi: 10.1007/s00203-002-0514-3

Zverlov, V., Klein, M., Lücker, S., Friedrich, M. W., Kellermann, J., Stahl, D. A., et al. (2005). Lateral gene transfer of dissimilatory (bi) sulfite reductase revisited. *J. Bacteriol*. 187:6, 2203-2208. doi: 10.1128/JB.187.6.2203

APPENDIX

Microbial cell enumeration optimization

Three protocols for microbial cell enumeration of low biomass samples were tested using a glacial till sediment sample taken from the terminus of the Taylor Glacier in the McMurdo Dry Valleys, East Antarctica. The first method is, designated in this thesis as "Minimally Manipulated LTER" and is similar to methods described by Bottomley (1994) and Ball and Virginia (2014). Sediment slurries were prepared in a 2:5 ratio of sediment mass to 0.2 µm filtered 1X PBS and fixed with 2% paraformaldehyde (final concentration). Slurries were shaken at 200 rpms for 1 hr and were let physically settle until a sediment free layer was formed. The sediment free layer (~2 mls) was filtered onto a 0.2 µm black polycarbonate filter and stained with 1 ml of 25X SYBR Gold and counted using epifluorescence microscopy. Since this method may not be rigorous enough to detach cells from sediment particles, a different method modified from Kallmeyer et al. (2008) was used. This method incorporated chemical (acetate buffer, detergent mix (containing 10% Tween80), and methanol treatment), physical (vortexing), and density gradient (Nycodenz) treatments aiming to separate microbial cells from sediment particles (Kallmeyer et al., 2008). The third method, called, 'Modification Method', was a combination of the 'Minimally Manipulated LTER' and Kallmeyer et al. (2008) protocols. This method forwent the density gradient steps of the Kallmeyer et al. (2008) protocol, yet still incorporating the chemical treatment described (acetate buffer, detergent mix, and methanol), a physical treatment (vortexing), and a low speed centrifugation to settle out majority of coarse sediment particles.

To initially compare cell quantification with the 'Minimally Manipulated LTER' and Kallmeyer et al. (2008) methods, the 'Taylor Uplift' sediment sample and a known

amount of a pure culture of the bacterium *Shewanella frigidimarina* was spiked into autoclaved sand and were processed as described using the 'Minimally Manipulated LTER' method and the Kallmeyer et al. (2008) method, modified to not include the sonication step.

The order of magnitude decrease in cell enumeration with the Kallmeyer et al. (2008) protocol determined that the 'Minimally Manipulated LTER' method was more reliable than the Kallmeyer protocol with both 'Taylor Uplift' and spiked *Shewanalla* sand (Table 5; Figure 7). Certain steps of the Kallmeyer et al. (2008) method were tested with a pure culture of the bacterium *Shewanella frigidimarina* to determine which steps caused the cell loss. The chemical treatment step included an acetate buffer to dissolve carbonates in sediments and a detergent mix containing 10% Tween80 aiming to release cells from sediment particles. The effect of the carbonate step was tested on the fixed pure culture dilution of *S. frigidimarina* spiked into autoclaved sand and the Taylor Uplift sample. One ml of each fixed sample was added to 5 mls of acetate buffer for 2 hrs, and were subsequently stained with 1 ml of 25X SYBR Gold. The cell abundances were compared to the 'Minimally Manipulated LTER' method and determined there was no statistically significant change in cell abundance (Table 5; Figure 8).

The pure culture of diluted *S. frigidimarina* was used to independently test the density gradient step of the Kallmeyer et al. (2008) protocol. Two mls of 50% nycodenz (% w/v) were placed in a 15 ml falcon tube beneath one ml of diluted *S. frigidimarina* using a sterile syringe and needle, careful to not mix layers. The tube was then centrifuged at 3000×g for 10 minutes and the top layer (cell containing layer) was

decanted with a sterile needle and syringe, then stained with 25X SYBR Gold and quantified. A significant decrease of cell abundance was observed (Table 5; Figure 9).

To determine if low speed centrifugation could be an alternative to separate sediment particles from cells, the lowest centrifugation speeds and times that visibly settled 'Taylot Uplift' sample coarse sediment particles (100 ×g and 200 ×g) were tested to determine if *S. frigidimarina* cells would also settle at that those speeds. Centrifugation and times (200×g for 5 min, 200 ×g for 2 min, and 100 ×g for 5 min) did not result in significant changes in cell abundance (Table 5; Figure 10)

To determine if the remaining steps of the Kallmeyer et al. (2008) method caused a loss in cell abundance, a third method that combined results of optimization steps so far, included preparation of sediment slurries of 'Taylor Uplift', fixed with 2% paraformaldehyde in a 15 ml falcon tube. Methanol and the detergent mix (Kallmeyer et al., 2008) were added to the slurry to a 10% final concentration and were inverted to mix thoroughly. Slurries were vortexed on medium high at 4 °C for 30 min. Slurries were then low speed centrifuged at 200×g for 5 min. or until majority of sediment particles settled. No significant difference in cell abundance were observed (Table 5; Figure 11).

Table 5. Comparisons of cell extraction protocols and certain steps among 'Taylor Uplift' and *S. frigidimarina* samples. Two sided t-tests were performed assuming unequal variance. *p < 0.05 = significant

Protcol/Step Comparison	Sample(s)	p value
'MM LTER'	*S. frigidimarina* + Sand	0.0038
Kallmeyer et al. (2008)		
'MM LTER'	'Taylor Uplift'	0.0012
Kallmeyer et al. (2008)		
Kallmeyer et al. (2008) Carbonate diss. step (with or without)	*S. frigidimarina*	0.6079
Kallmeyer et al. (2008) Carbonate diss. step (with or without)	'Taylor Uplift'	0.2054
Kallmeyer et al. (2008) Nycodenz step (with or without)	*S. frigidimarina*	0.0005
Low speed centrif. (200×g 5 min) vs. no centrif.	*S. frigidimarina*	0.5123
Low speed centrif. (200×g 2 min) vs. no centrif.	*S. frigidimarina*	0.2054
Low speed centrif. (200×g 2 min)	*S. frigidimarina*	0.8722
'MM LTER'	'Taylor Uplift'	0.2641
Modified Method		

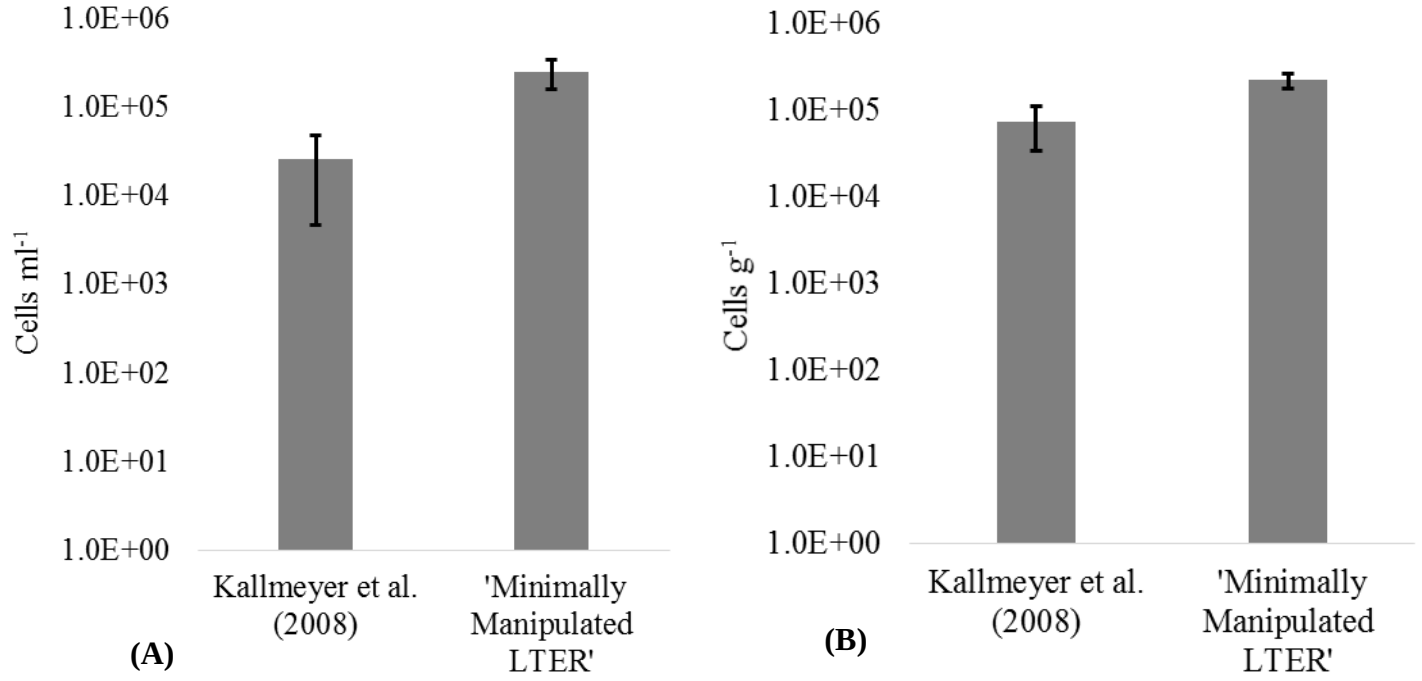

Figure 7. Comparison of Kallmeyer et al. (2008) and 'Minimally Manipulated LTER' extraction methods. Comparison between a pure culture dilution of the bacterium *S. frigidimarina* (A) and 'Taylor Uplift' sediment sample (B).

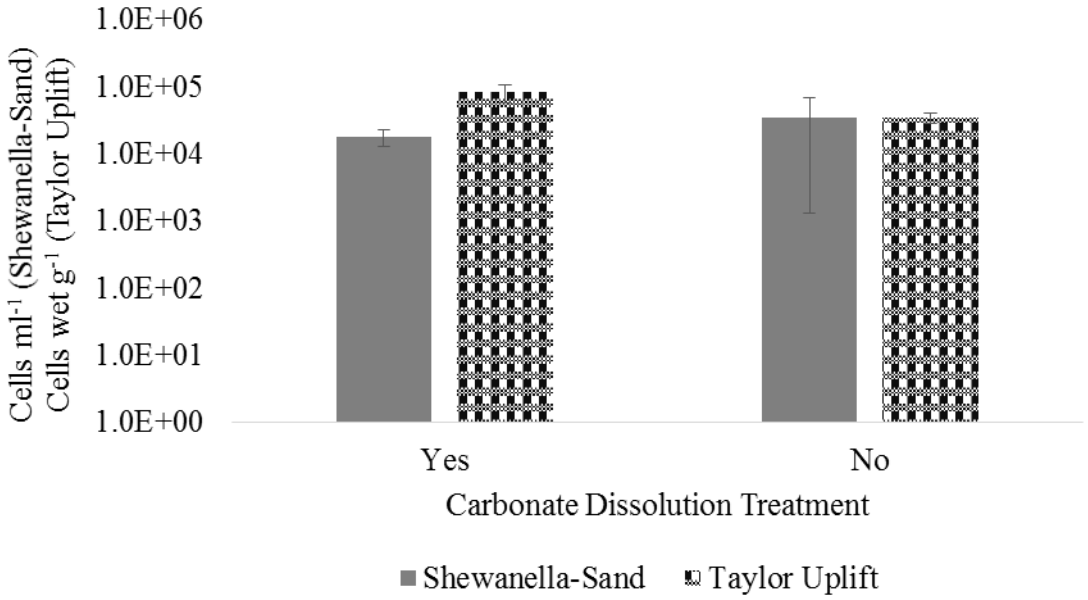

Figure 8. Comparison of treatment of a *S. frigidimarina* dilution spiked into autoclaved sand '*Shewanella*-Sand' and 'Taylor Uplift' sediment sample with and without the carbonate dissolution treatment as described by Kallmeyer et al. (2008).

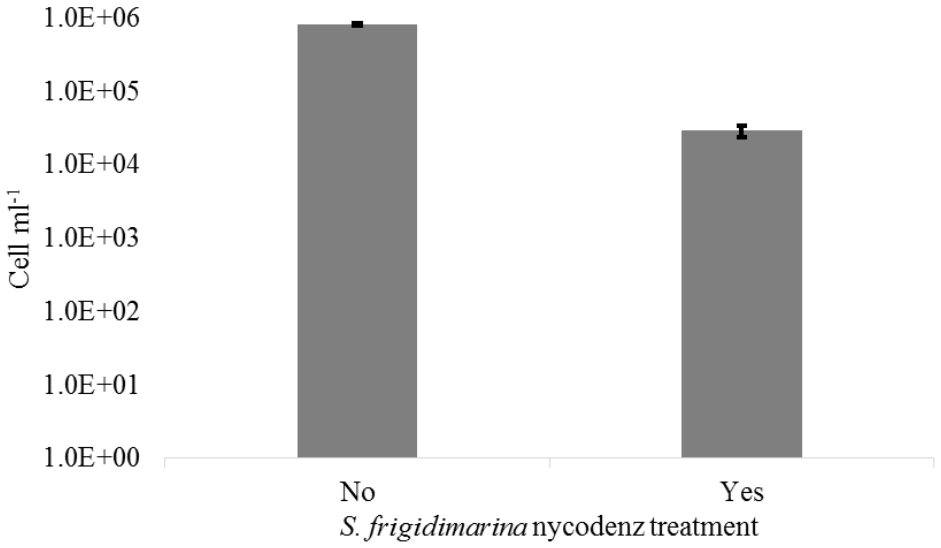

Figure 9. Quantification of diluted *S. frigidimarina* without or with nycodenz and centrifugation treatment.

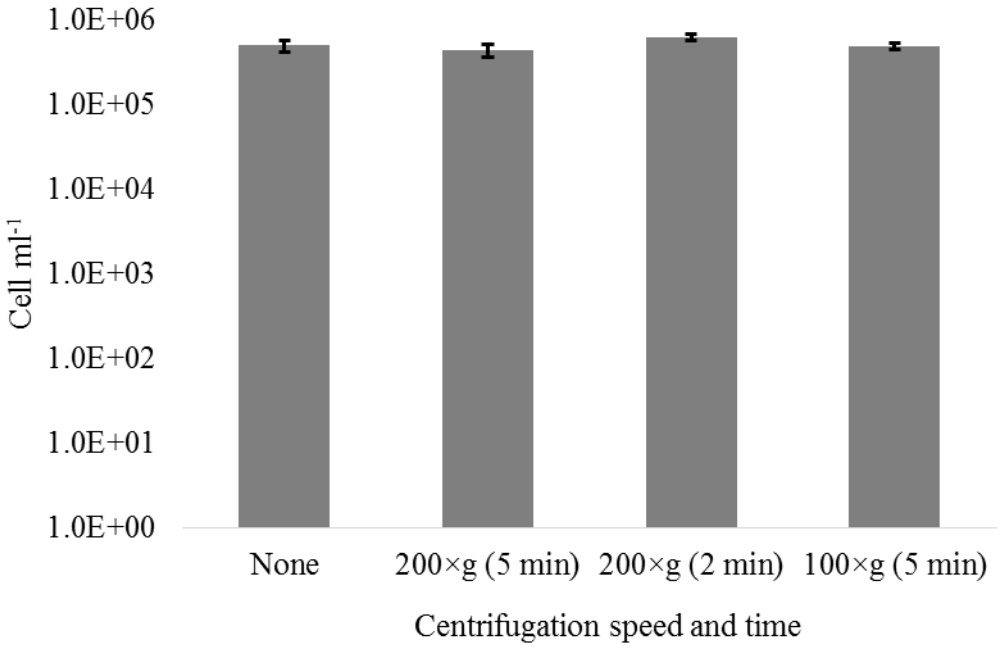

Figure 10. Quantification of diluted *S. frigidimarina* centrifuged at various speeds and times.

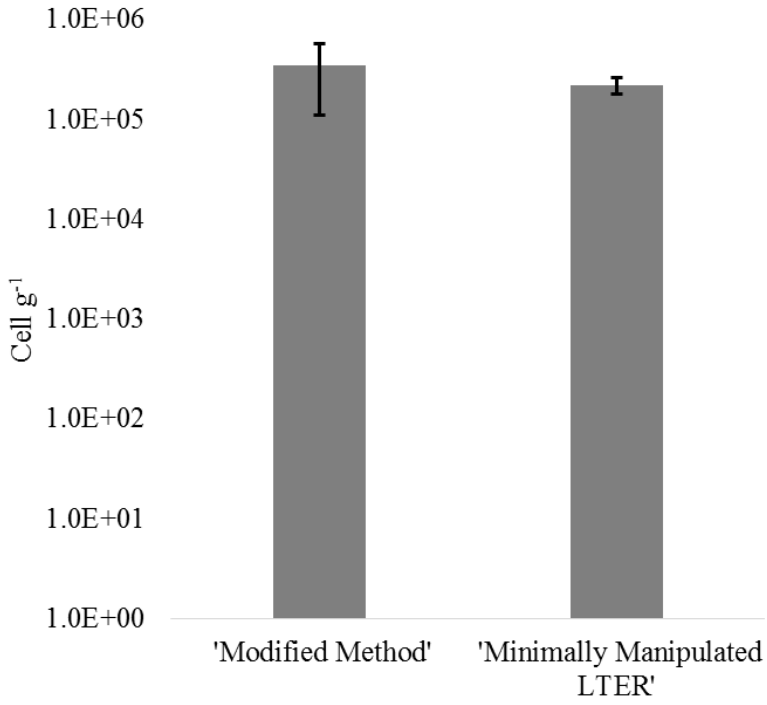

Figure 11. Comparison of 'Taylor Uplift' sediment cell extraction methods. The two methods compared are 'Modified Method' versus 'Minimally Manipulated LTER'.

Optimization of cell counting from low biomass sediment

Quantification of microbial cells in low biomass sediments requires optimization depending on sediment sample types of varying biomass. It appears to be essential to optimize cell extraction protocols for different sediment samples as shown by many methods aiming to optimize cell extraction (Klauth et al., 2004; Lunau et al., 2005; Morono et al., 2013). For the 'Taylor Uplift' and '*S. frigidimarina*' model samples, the Kallmeyer method density gradient step seemed to have been the cause of cell loss. Out of the initial two extraction methods tested for epifluorescence microscopy cell quantification ('Minimally Manipulated' and 'Kallmeyer et al. (2008)'), a combination of methods that forwent addition of the density gradient and supernatant retrieval was decided on. Even though no significant cell increase was obtained using the combined 'Modified Method', since no cell loss was observed, we used this method to quantify cells in sediment samples from SLW. Since we did not have an abundance of SLW sediment to optimize with, we still employed this 'Modified Method' since it incorporated extra steps that have been previously shown to increase cell loss in other sediment samples (Kallmeyer et al. 2008). Cell quantification using a cell extraction method and epi-fluorescence microscopy includes many variables that can be modified so in the future, careful attention to this manipulation needs to occur in a systematic fashion.

VITA

Alicia Marie Purcell was born in Manchester, Connecticut on January 15th 1990. She graduated from Cumberland County High School in Crossville, Tennessee. She received her Bachelor of Science degree with a major in Biology, concentration in Biochemistry, Cellular, and Molecular Biology from the University of Tennessee, Knoxville in May 2012. In January 2013, she entered the graduate program in Microbiology at the University of Tennessee - Knoxville in the lab of Dr. Jill Mikucki. She completed the requirements for the Masters of Science degree in December 2014.