Matthew Joshua Medina

Genomic and transcriptomic evidence for niche partitioning among sulfate-reducing bacteria in redox-stratified cyanobacterial mats of the Middle Island Sinkhole

submitted in partial fulfillment of the requirements for the degree of
Master of Science in Earth and Environmental Sciences
Department of Earth and Environmental Sciences
The University of Michigan

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Acknowledgements

I thank my parents, my mentors, teachers, and my University of Kansas family for supporting me through my education and academic career. I also thank the Geomicrobiology lab and sinkhole team for their support: Greg Dick, Keven Meyer, Judith Klatt, Sunit Jain, Sharon Grim, Derek Smith, Chien Tan, Katy Rico, Nathan Sheldon, and Dack Stuart.

The research presented in this thesis was supported, in part, by Wiebke Ziebis (sulfate-reduction rates) and Lauren Kinsman-Costello (pore water chemistry).

Russ Green, Tane Casserly, Stephanie Gandulla, Wayne Lusardi, John Bright, and Phil Hartmeyer at the NOAA Thunder Bay National Marine Sanctuary assisted the research with sampling at the Middle Island Sinkhole.

I am grateful for receiving the Rackham Merit Fellowship (RMF), which supported me as a graduate student at the University of Michigan.

The research presented here was supported by the National Science Foundation (EAR 1035955 and EAR 1035957) and the University of Michigan CCMB Pilot Grant.

Abstract

Sulfate-reducing bacteria (SRB) often shape the biogeochemistry of cyanobacterial mats through metabolic interactions and production of sulfide. Nevertheless, the ecology and physiology of sulfate-reducers inhabiting microbial mats remains poorly understood. For instance, in cyanobacterial mats inhabiting the Middle Island Sinkhole (MIS) in Lake Huron, some of the largest knowledge gaps regarding SRB involve the controls of their distribution, diversity, and metabolic activities with respect to a changing geochemical profile of oxygen and sulfide during diel cycles. Metagenomic and metatranscriptomic approaches offer high potential to close such knowledge gaps and to understand the genomic potential and activity of SRB better. Through genomic binning of metagenomic data from MIS cyanobacterial mats, I identified 8 draft genomes of SRB. Transcriptomic reads were recovered from day and night microbial mat samples and mapped to the SRB genomes, revealing gene transcripts associated with use of electron donors such as H_2 and various organic carbon compounds and use of electron acceptors such as nitrate and sulfate. The data presented here show patterns of niche partitioning in SRB, which is likely an important factor in controlling SRB diversity in cyanobacterial mats. This research enhances our understanding of microbial ecology and biogeochemistry in cyanobacterial mats and has implications for geobiology of both the modern and ancient Earth.

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Introduction

Sulfate-reducing bacteria (SRB) play important roles in photosynthetic microbial mat ecosystems (Wagner *et al.* 1998; Canfield & Raiswell 1999), mediating key transformations of sulfur compounds and carbon in sediments and microbial mats (Canfield & Des Marais 1993; Jørgensen 1994; Teske *et al.* 1998; Baumgartner *et al.* 2006). As producers of sulfide through dissimilatory sulfate reduction, SRB can regulate the metabolic switch between oxygenic and anoxygenic photosynthesis in cyanobacteria (Klatt *et al.* 2015). Therefore, SRB may have influenced biogeochemistry and oxygen production in photosynthetic microbial mats that were widespread and played key geobiological roles in the Precambrian (Bosak et al. 2013; Grotzinger and Knoll 1999).

Although SRB are acknowledged to drive critical biogeochemical processes such as those described above, there remain considerable knowledge gaps about their ecology and physiology in microbial mat ecosystems. For example, (1) what controls their distribution, abundance, diversity, and activity microbial mats and sediments? (2) What are their interactions with

cyanobacteria? (3) How does their energy metabolism, both in terms of electron donors/acceptors and metabolic rates, change along with changing geochemistry throughout a diel cycle?

Given limitations in the geological record and in geochemical and paleontological tools for studying ancient microbial ecosystems, the study of modern analogues is critical for understanding the functioning of cyanobacterial mat ecosystems. One such potential analogue for ancient microbial mat ecosystems is the cyanobacterial mats that inhabit the Middle Island Sinkhole (MIS). The MIS is a submerged karst feature in Lake Huron that was formed by dissolution of Silurian-Devonian sedimentary bedrock over geologic time (Biddanda *et al*. 2006; Ruberg *et al*. 2008; Biddanda *et al.* 2009) (**Figure 1**). The MIS hosts cyanobacterial mats that occur at a water depth of 23 meters as either flat microbial mat or methane inflated "fingers" (**Figure 2**). These mats thrive under low-oxygen and high sulfate (SO_4^{2-}) conditions, which is the effect of groundwater intrusion into the sinkhole through multiple conduits (Baskaran *et al*. 2016). This groundwater is distinct in chemical and physical properties compared to Lake Huron freshwater (**Figure 3**) (Biddanda *et al*. 2006; Ruberg *et al.* 2008; Biddanda *et al.* 2009). Generally, MIS groundwater has low O_2 and high SO_4^{2-} concentrations, whereas Lake Huron is well oxygenated and contains lower concentrations of SO_4^{2-} (Kinsman-Costello *et al.* 2017). Cyanobacteria in MIS mats can switch between oxygenic and anoxygenic photosynthesis (Biddanda *et al*. 2009; Nold *et al.* 2010, Voorhies *et al*. 2012 Nold *et al*. 2013; Kinsman-Costello *et al*. 2017). Taken together, the low-O_2 and high SO_4^{2-} conditions and presence of cyanobacteria that carry out anoxygenic photosynthesis make the MIS a valuable modern analogue of Precambrian microbial mat ecosystems (Biddanda *et al*. 2009; Voorhies *et al*. 2012; Nold *et al*. 2013; Lyons *et al*. 2014; Fischer *et al*. 2016; Kinsman-Costello *et al*. 2017).

The earliest research on the microbial ecology of SRB and cyanobacteria in MIS mats indicated a tight symbiotic relationship through the byproducts of their energy metabolism—sulfate reduction and anoxygenic photosynthesis, respectively (**Figure 3**) (Biddanda *et al*. 2009). SRB use SO_4^{2-} as a terminal electron acceptor (a "breathable") with organic carbon or hydrogen (H_2) as an electron donor ("edible") and generate carbon dioxide (CO_2) and S^{2-} as byproducts. The sulfide generated by SRB is used as an electron donor by cyanobacteria during anoxygenic photosynthesis, producing elemental sulfur (S^0) as a product. S^0, along with other sulfur intermediates, may be used as an electron donor for SRB in dissimilatory sulfate reduction. Research outside the MIS has also explored this type of cross-feeding symbiotic relationship between SRB and cyanobacteria in terms of transfer of organic carbon compounds, showing that SRB use glycolate, which is produced and excreted from cyanobacteria, as an energy and carbon source (Nold and Ward 1996; Bateson *et al*. 1998; Stal & Caumette 2013; Kim *et al.* 2015). Given such interactions, cyanobacteria and SRB in MIS microbial mats are likely metabolically linked through electron donors and acceptors they make available for one another (Biddanda *et al*. 2009; Nold *et al*. 2010). Nevertheless, such interactions, and the biogeochemical gradients in cyanobacterial mats, change on a diel cycle along with light availability and photosynthesis (Jørgensen *et al*. 1994; Teske *et al.* 1998).

Genetic methods have enhanced our knowledge on the community of SRB that inhabit the MIS microbial mats and sediments. 16S rRNA gene sequencing methods on MIS microbial mats and sediments revealed 6 putative Deltaproteobacteria operational taxonomic units (OTUs) exhibiting differential relative abundance within the MIS microbial mats and underlying sediments (Kinsman-Costello *et al*. 2017). This study suggested that certain members of *Desulfonema*, *Desulfocapsa*, and *Desulfatirhabdium* genera prefer to inhabit the cyanobacterial

mat layer, given that OTUs assigned to those genera were detected in high abundances in the mat. Different members of the Desulfobacteraceae family and the *Desulfatirhabdium* genus were more abundant at ~5–10 cm depth in the sediment (Kinsman-Costello *et al.* 2017). Although this research provided valuable information about SRB species distribution throughout cyanobacterial mats and sediments, questions regarding the controls on such distributions of SRB remain unanswered. Studying the genomes and metatranscriptomes SRB recovered directly from the environment can help to fill in the knowledge gaps with respect to SRB and cyanobacterial mat ecology.

Here, I present eight draft genomes of SRB recovered from 15 MIS cyanobacterial mat samples collected over five years, and show that these SRB are metabolically active through measurements of sulfate-reduction rates. I also analyzed metatranscriptomic datasets derived from night and day conditions in order to evaluate the impact of light, photosynthesis, oxygen production, and changing redox gradients on SRB metabolism.

Methods

Sample collection, molecular preparation and sequencing

Fifteen environmental samples were collected at the MIS during multiple field campaign seasons over a period of five years as described previously by Voorhies (2014) and depicted in the appendix (**Table A-1**). Samples were collected in Plexiglas® cylinder cores (radius = 7.5 cm and height = 30 cm) by inserting into the sediment and closing off each end with rubber stoppers. One 2011 sample and three of the 2012 samples were collected during night under dark conditions (01:00) and the remaining were collected during the day during light conditions. Three 2012 samples in which metatranscriptome samples were collected were collected at 13:00.

The distinction between day and night samples is indicated by D/N notation in the Sample ID shown in Table A-1. Immediately upon retrieval of intact cores shipboard, portions of the microbial mats were dissected from the cores and stored in RNAlater® Stabilization Solution (1:1). Samples were later stored at -80˚C until DNA extraction.

DNA and RNA was extracted as described previously in Voorhies (2014) and Voorhies *et al.* (2016). Briefly, DNA was extracted from 1 g of microbial mat sample using the FastDNA™ Spin Kit for soil from MP Biomedicals LLC by following the company's protocol with the following amendment: 0.3 g of beads were used for bead beating instead of the company's recommendation. A FastPrep®-24 Instrument (MP Biomedicals LLC) was used during the bead beating step during DNA extraction. DNA Clean & Concentrator™ from Zymo Research was used for clean-up and to concentrate the extracted DNA. Finally, DNA concentration was quantified using Invitrogen™ Quant-iT™ PicoGreen™ dsDNA Assay Kit. Extracted DNA from all fifteen microbial mat samples were shotgun sequenced using Illumina Hi Seq 2000 technology at the University of Michigan DNA Sequencing Core. RNA was extracted from all six samples collected in 2012 using the mirVana™ miRNA Isolation Kit and following the manufacture's protocol. 5 µL of extracted RNA was amplified using the Invitrogen™ Ambion™ MessageAmp™ II-Bacteria RNA Amplification Kit by following the company's protocol. RNA was converted into complementary DNA (cDNA) using the SuperScript® Double-Stranded cDNA Synthesis Kit. 2 µg of purified cDNA was used for sequencing at the University of Michigan DNA Sequencing Core on an Illumina Hi Seq 2000 machine (Frias-Lopez *et al.* 2008).

Metagenomic assembly

All genomic DNA reads from each MIS microbial mat sample were initially co-assembled with IDBA-UD (Peng *et al.* 2012). Following binning (see below), genomic bins were extracted and individually re-assembled, again using IDBA-UD. Scripts used for assembly are published in the Michigan Geomicrobiology Lab GitHub repository (https://github /Geo-omics/scripts) in the following location: Geo-omics/scripts/wrappers/Assembly.

Genomic binning

Initially, contigs greater than 4 kb were clustered into genomic bins by tetranucleotide frequencies with emergent self-organizing maps (ESOM; Dick *et al.* 2009). From this map, SRB genomic bins were extracted and clustered again with Anvi'o (version 1.2.2; Eren *et al.* 2015) to utilize coverage information in addition to tetranucleotide frequencies. Recovered SRB genomic bins were quality checked using CheckM (version v1.0.4; Parks *et al*. 2015) and taxonomic assignments were carried out with PhyloSift (version 1.0.1; Darling *et al.* 2014).

Identification of contigs containing dsrAB genes and annotation

Contigs containing sequences for dissimilatory sulfate reductase alpha and beta subunits (DsrAB) were identified via BLAST (Version 2.2.30+) sing the a publically available DsrAB database described in Müller *et al*. (2015).

Draft genomes were submitted for annotation and gene calling through the Joint Genome Institute-integrated Microbial Genomes Expert Review portal (Huntemann *et al.* 2015).

Metatranscriptomic pipeline and analysis

cDNA forward and reverse reads for the six 2012 samples were mapped and aligned to concatenated SRB contigs using Burrows-Wheeler Aligner (BWA) software package (Li &

Durbin 2009). The scripts used for this step are published in the Michigan Geomicrobiology Lab GitHub repository (https://github /Geo-omics/scripts) in the following location: Geo-omics/scripts/wrappers/BamTools. The script, bamTools.pl, was used to calculate the number of reads mapped onto genes and those values were normalized using the following formula:

$$Normalized\ No.of\ Mapped\ reads\ = \frac{Raw\ No.\ of\ mapped\ reads\ to\ each\ gene}{Total\ cDNA\ counts\ per\ genomic\ bin}.$$

DsrA Phylogenetics

A Phylogenetic tree of the DsrA protein amino acid sequences was constructed using the PROTGAMMAGTR algorithm in RAxML with a bootstrap of 1000 (version 8.1.15; Stamatkis *et al*. 2014). Amino acid sequences for DsrA of non-MIS SRB included on this tree were collected from the National Center for Biotechnology Information (NCBI).

Sulfate-reduction rates

Sulfate reduction rates were measured using $^{35}SO_4^{2-}$ radiotracer according to Røy *et al.* (Røy *et al.* 2014). In brief, upon collection of 15 cm long cores from the field, cores in the lab were injected with 10 µL of 150 kBq $^{35}SO_4^{2-}$ at 1 cm intervals. After injection, cores were incubated in a water bath set at *in situ* temperature (~9° C) in the dark for 20 mins. After incubation, cores were sectioned at 1 cm intervals. Each section collected was homogenized in 20% ice-cold zinc acetate solution in a 50 mL Falcon™ Conical tube to stop SRR and fix sulfides ($H^{35}S^-$), the product of sulfate reduction. SRRs were determined using the cold single-step distillation method, modified from Kallmeyer *et al*. (Kallmeyer *et al*. 2004). Sulfate concentrations were determined from pore water from a separate set of cores collected at the same site with Thermo Scientific™ Dionex™ membrane-suppression ion chromatography with a detection limit of SO_4^{2-} concentration of 0.015 ppm. The average percent recovery is 10% for a 50 ppm SO_4^{2-}

quality control standard. For duplicate run samples ranging from <1-700 ppm SO_4^{2-}, the average relative percent difference is 4.6%.

Results and discussion

Sulfate-reduction rates in a cyanobacterial mat

From measurements of sulfate reduction rates using $^{35}SO_4^{2-}$ radio-tracer incubations in five MIS cores, the highest sulfate-reduction rates were found to occur in the cyanobacterial mat in the upper cm of the core, where the average sulfate-reduction rate was 1.8 ± 0.4 μmol cm^{-3} d^{-1} (**Figure 4**). No sediment was visible in this upper layer. These results suggest that cyanobacterial mat are hotspots for sulfate reduction and SRB are active in regions where they are regularly exposed to oxygenated conditions. Multiple studies have reported rapid bacterial sulfate reduction rates under oxic conditions in cyanobacterial mats (Canfield *et al.* 1991; Fründ & Cohen 1992). Additionally, SRB have been found to inhabit oxygenated micro-niches in sediments (Jørgensen 1977). Sulfate reduction rates detected in such oxygenated environments has been shown to be enhanced by type of carbon species available for SRB to use as electron donors—mainly glycolate. Ethanol and lactate, on the other hand, have been shown to enhance sulfate reduction in anoxic environments (Fründ & Cohen 1992). The work presented here reinforces the view that SRB are not always strict anaerobes and they are detectable through measurements of sulfate reduction rates in micro-niches where oxygenic photosynthesis takes place.

Beneath the cyanobacterial mat from sediment depths of 2–3 cm sulfate reduction rates decreased sharply before increasing again to a local maximum at 6 cm sediment depth, where the average rate was 0.8 ± 0.2 μmol cm^{-3} d^{-1} (**Figure 4**). It is unclear what the control is for

supporting sulfate reduction at this depth, ~6 cm. One possibility is that the lateral or vertical advection of sulfate or electron donors for sulfate reduction from subsurface fluids rich in sulfate is supporting microbial sulfate respiration at ~ 6 cm at depth. Patterns of lateral advection of methane (CH_4) has been shown in models (Chatterjee *et al*. 2011 and Sultan *et al.* 2016) and pore water measurements for sulfate concentration from two MIS cores collected at the same site as the sulfate-reduction cores show this pattern, but at a depth of 10–11 cm (**Figure 5**).

Genomes of sulfate reducing bacteria (SRB) recovered from the MIS cyanobacterial mat

To identify and understand the organisms responsible for sulfate reduction in the MIS cyanobacterial mat better, metagenomic datasets (Voorhies *et al.* 2016) were used to reconstruct genome sequences of sulfate reducing bacteria. As a marker gene of SRB, I used the reductive bacterial type dissimilatory sulfite reductases (*dsrAB*). These genes encode for a heterotetramer protein (DsrAB) responsible for catalyzing the reduction of sulfite (SO_3^{-2}) to sulfide (S^{2-}) during microbial sulfate respiration. From the co-assembled DNA sequence reads from 15 MIS microbial mat samples, nine contigs were identified as having *dsrAB* genes (**Table A-2**). These nine contigs were then used to identify the genomic bins from a previous study of this dataset (Voorhies *et al.* 2016) that carry the *dsrAB* genes (**Figure 6**). However, quality checking of these genomic bins via CheckM (completion, strain heterogeneity, and redundancy based on universal single copy marker genes) showed that they were highly redundant (**Table A-3**). The high redundancy reaffirms the difficulty in separating bins of closely related species, or strains, by clustering contigs solely by tetranucleotide frequency measurements.

To separate bins of closely related strains and reduce the redundancy of the nine putative SRB bins, I used the alternative method of binning—taking advantage of both tetranucleotide frequencies and coverage information (Hess *et al.* 2011; Albertsen *et al.* 2013, Alneberg *et al.*

2014), following the Anvi'o workflow (Eren *et al.* 2015). Binning through this method produced eight SRB genomic bins using *dsrAB* as a marker gene of sulfate reduction (**Table 1**). Three additional putative SRB genomic bins were missing *dsrAB genes* but assigned as putative SRB due to these bins possessing other genes involved in dissimilatory sulfate-reduction taxonomically ranking to Deltaproteobacteria (**Table A-4**). These draft genomes extracted from the metagenome had various ranges of completion. The most complete genomic bin was bin 30 (67% complete), which was identified as *Desulfotalea psychrophila* with PhyloSift, followed by bin 2 (48 complete; *Desulfobacula toluolica* Tol2), and bin 36 (43% complete; Desulfobacteraceae family). Other bins were less than 30% complete (**Table 1**). The incomplete nature of these bins should be considered when interpreting results; the absence of genes does not necessarily imply their absence from the genomes, thus focus should be placed on the genes that are present rather than absent.

The relative abundance of each SRB genomic bin was estimated across all samples based on average mean coverage of contigs (**Figure 7**). These results showed that the abundance of SRB was highly dynamic, differing in some cases even between cores collected at the same time.

Phylogenetic relationships

To investigate the phylogenetic relationships of the MIS SRB with each other and with previously studied SRB, I constructed a phylogenetic tree of DsrA amino acid sequences (**Figure 8**). Only eight of the eleven MIS SRB genomes are represented on this tree because bins 3, 15, and 32 are missing DsrAB sequences. Bin 35 is represented twice on the DsrA tree because this genome has two copies of dsrA on separate contigs.

DsrA sequences from bin 24 (*D. sulfexigens*), bin 25 (unclassified), and bin 30 (*D. psychrophila*) cluster closely in a clade of sequences from Desulfobulbaceae, including representatives of the genera Desulforhopalus, Desulfofustis, Desulfopila, Desulfotalea, Desulfobulbus, and Desulfocapsa. DsrA sequences from bin 24 and bin 25 are closely related to each other, sharing 98% amino acid identity, but only 87% amino acid identity with the top hit in the NCBI Genbank nr protein database (from *Desulfocapsa sulfexigens*). The DsrA sequence of bin 30 is most closely related to *Desulfofustis glycolicus*, which was isolated from a marine anoxic sediment, and was described as a strict anaerobe that uses glycolate and glycoxylate as electron donors with sulfite and elemental sulfur as terminal electron acceptors (Friedrich & Schink 1995 and Friedrich *et al*.1996). The potential for use of glycolate is noteworthy because Teske *et al.* (1998) reported glycolate, which is excreted by cyanobacteria during photorespiration and under high O_2 and low CO_2 conditions (Han & Eley 1973; Bergman *et al*. 1984; Fründ & Cohen1992; Canfield & Des Marais 1993), as having a strong stimulatory impact on sulfate reduction in a cyanobacterial mat (Teske *et al*. 1998),

The DsrA sequences from Bin 35 (*Desulfococcus oleovorans*), and Bin 2 (*Desulfotalea toluolica* Tol2) cluster together in a clade of sequences from Desulfobacteraceae. Bin 35 contains two different DsrA sequences, hereby referred to as 35a and 35b. The low completeness of this bin (**Table 1**) makes it difficult to determine whether these two sequences truly represent two different copies of DsrA within the same genome. This has been observed previously for sulfur-oxidizing bacteria (Beller *et al.* 2006), but not for sulfate-reducing bacteria to our knowledge. The most immediate members of the smaller sub-clade containing bin 35a and bin 2 had ~97% amino acid identity to the DsrA sequence from bin 35a and were retrieved from a metagenome of aquifer sediments and groundwater (accession number: OGR21700.1; Anantharaman *et al.* 2016). These sequences are also closely related (89–91% amino acid identity) to *Desulfobacula*

spp. isolated from marine sediments (Finster *et al.* 1997), an oil-contaminated tidal flat (Kim *et al.* 2014), and a water-oil separation system (Lien & Beeder 1997). The DsrA sequence from bin 35b was more divergent, being nearly equidistant between the Desulfobacula clade (82% amino acid identity) and a clade of sequences from Desulfosarcina sequences (**Figure 8**).

Bin 36 and bin 4—both members of the Desulfobacteraceae family—share a clade with a SRB genome recovered through shotgun sequencing of groundwater from the Alpena city fountain (Michigan, U.S.A.). This fountain is located near the Alpena County George N. Fletcher Public Library and is fed by the same groundwater that intrudes into the MIS system (Sharrar *et al.* 2017). The SRB genome recovered from this fountain has 98.56% identity to Desulfobacteraceae based on 16S BLAST hit (Sharrar *et al.* 2017).

Bin 1 (classified by PhyloSift as *Desulfatibacillum alkenivorans*) shares a clade with Isolated Sinkhole Lake Huron genomic bin 3, which is a SRB recovered through shotgun sequencing of microbial mat from another, deeper submerged sinkhole in Lake Huron—The Isolated Sinkhole (Sharrar *et al.* 2017). This Isolated Sinkhole SRB genomes shares 97.1% identity to *Desulfonema* Sp. Based on 16S BLAST hits.

Overall, these results confirm previous inferences that there are diverse sulfate-reducing bacteria in the MIS cyanobacterial mat (Kinsman-Costello *et al.* 2016). Phylogenetically, their sequences are widespread across the tree of reductive type DsrA sequences of the Deltaproteobacteria. Only a few SRB from the MIS microbial mats are closely related to SRB genomes recovered from the Alpena city fountain and Isolated Sinkhole.

Content and expression of genes within genomes of MIS sulfate-reducing bacteria

To understand the metabolic capabilities of MIS mat SRB better, I used metagenomic and metatranscriptomic approaches. Particularly, I sought to investigate potential metabolic explanations for why multiple operational taxonomic units (OTUs) of SRB are present in the cyanobacterial mat. Although previous 16S rRNA gene based approaches identified these OTUs, they provide little information about the metabolisms used by these groups. Metagenomics and metatranscriptomics can provide a culture-independent view of which genes are present and expressed in each group of SRB, enhancing our understanding of the physiological potential and activity of these organisms. Specifically, metatranscriptomics yields information about how microorganisms sense and respond to their environment over short time scales (Moran *et al*. 2013). Although such approaches do not always conclusively or quantitatively demonstrate metabolic processes, they provide valuable insights that can be used to generate hypotheses, particularly for uncovering metabolic interactions in complex microbial mat communities. Here I focused on the presence and expression of genes involved in key biogeochemical cycles within cyanobacterial mats (sulfur, carbon, oxygen, and nitrogen) during the day and night.

Of the genes considered here, the most abundantly represented genes in the metatranscriptome encode for enzymes associated with dissimilatory sulfate reduction pathway, mainly *dsrAB*, dissimilatory adenylylsulfate reductase alpha and beta subunits (*aprAB*), and sulfate adenylyltransferase (*sat*) (**Figure 9**). These genes are considered essential for sulfate reduction. Bin 36, which is the most complete genome, and bin 4 also have high abundances of transcripts for adenylylsulfate reductase-associated electron transfer proteins (QmoABC). QmoABC is known to participate in electron transfer to dissimilatory adenosine-5'-phosphosulfate reductase (APS) (Zane *et al.* 2010; Pereira *et al.* 2011; Ramos *et al.* 2012).

Another striking feature is that bins 1, 2, 4, 30, and 36 have genes for heterodisulfide reductase (Hdr). The heterodisulfide reductases found in methanogens and are known to be homologs to QmoAB; however, little is known about the function of these enzymes in SRB and more research needs to be completed to understand their function (Mander *et al.* 2004; Hedderich *et al.* 2005; Pereira *et al.* 2011). There were no clear or strong differences in transcript levels of sulfate reduction gene in the day versus the night (**Figure 9**). These results indicate that the SRBs are metabolically active during the photosynthetic period, when oxygen is produced, suggesting that they are likely tolerant to O_2, as observed previously (Canfield and Des Marais 1991). On the other hand, these results suggest that sulfate reduction is not restricted to the photosynthetic period, when cyanobacteria excrete carbon compounds that may support sulfate reduction.

To investigate potential mechanisms for tolerance to O_2, I searched the SRB genomes for cytochrome c oxidases, which have been implicated in reducing and detoxifying O_2 in SRB (Hardy & Hamilton 1981; Dilling & Cypionka 1990; Dannenberg *et al.* 1992). Genes for all four subunits of cytochrome c oxidase were identified in the genome of bin 30, but not in any of the other bins. In bin 30, no evidence of active transcription was detected for cytochrome c oxidases. Although there is no evidence of MIS SRB using cytochrome c oxidases to reduce O_2, it is important to consider the reports describing the ability of other SRB, from culture, to reduce O_2 coupled with hydrogen, organic carbon, sulfite, and thiosulfate as electron donors (Dilling & Cypionka 1990; Dannenberg *et al.* 1992). Whether SRB reduce oxygen for energy or detoxification purposes is very much debated.

The metagenome contained evidence that bins 30 and 35 possess the ability to conduct nitrate reduction to ammonium though the nitrite reductase enzyme (cytochrome; ammonia-forming) (**Figure 9**). The cytochrome nitrite reductases have been observed before in SRB

isolated from culture (Liu & Peck 1981 and Almeida *et al.* 2003), and in the SRB genomes recovered from the Alpena city fountain (Sharrar *et al.* 2017). However, no metatranscriptomic reads were mapped to these genes.

Bins 1, 2, and 36 were found to contain hydroxylamine reductase (Hao), which is an enzyme that catalyzes the second step in the transformation of nitrate to ammonium in the nitrate reduction to ammonium by the reverse hydroxylamine:ubiquinone reductase module (reverse-HURM) pathway. However, these bins were missing genes encoding an enzyme catalyzing the first step in the reverse-HURM pathway where nitrate is first transformed to hydrooxyalamine (reverse hydroxylamine:ubiqinone reductase). Both enzymes involved in the reverse-HURM pathway have been detected in SRB genomes from the Alpena city fountain and Isolated Sinkhole in Lake Huron (Sharrar *et al.* 2017). Although these genes hint that the MIS SRBs may be capable of dissimilatory nitrate reduction, the lack of key genes in the metagenome (perhaps due to incomplete genomes) and metatranscriptome (perhaps due to low-coverage of the SRB transcriptomes) makes this uncertain. However, the lower representation of these genes in the metatranscriptome relative to those for sulfate reduction suggests that sulfate is the primary electron acceptor for these SRB populations.

To understand the processes driving sulfate reduction, I analyzed the SRB genomes for genes encoding enzyme for oxidation of electron donors and transport of potential carbon sources. Bins 1, 4, 24, 30, 36 all have genes associated with hydrogen oxidation, and transcripts for most these genes were more abundant during the day than at night (**Figure 9**). Transcripts for glycolate oxidase are also more abundant during the day than during the night, which supports previously reported research of glycolate enhancing sulfate-reduction in SRB during conditions in which oxygen production is high in similar microbial mat ecosystems (Teske *et al*. 1998).

Such conditions are expected during the day when light levels are high and cyanobacteria are conducting photosynthesis. Additionally, I report that MIS SRB likely utilize formate, fatty acids, and pyruvate as electron donors given the evidence of genes responsible for the transformation of formate to CO2 (*fdoG, fdoH, fbhB, fdhA1, and fnuoG*) in bins 1, 2, and 36 (**Figure 9**). Bin 2 possesses nearly every gene in the metagenome and metatranscriptome to complete pyruvate fermentation to acetate pathway (**Figure 9**). Finally, bins 1,4, and 36 hold genes that are used in fatty acid alpha oxidation (aldehyde dehydrogenase and ALDH7A1) (**Figure 9**).

In summary, I identified a number of genes involved in various critical biogeochemical pathways in SRB associated with cyanobacterial mats at MIS (**Figure 9**). Only some of the genes and pathways were represented in metatranscriptomic datasets. This may reflect that those genes were not transcriptionally active at the time of sampling. However, given that samples were collected only at two time points (13:00 and 01:00), and that geochemical conditions such as oxygen, sulfate, sulfide water concentrations vary dramatically across the diel cycle, such genes could very well be active at another stage of the diel cycle.

Concluding remarks

Investigating the microbial ecology of SRB in MIS cyanobacterial mats is key to understanding transformations of sulfur and carbon species in microbial mats and sediments, the controls on photosynthesis, and the diversity and ecology of SRB in microbial mat systems. Understanding such subject matter enhances our understanding of microbial mat ecology, particularly interactions between SRB and cyanobacteria. A central question driving this research involves the diversity of SRB in MIS mats—why, within a single mat sample, are there multiple species of sulfate reducing bacteria? I sought to answer this question with metagenomic and

metatranscriptomic analysis of the eight genomes I recovered to find differences and similarities with respect to their metabolic capabilities.

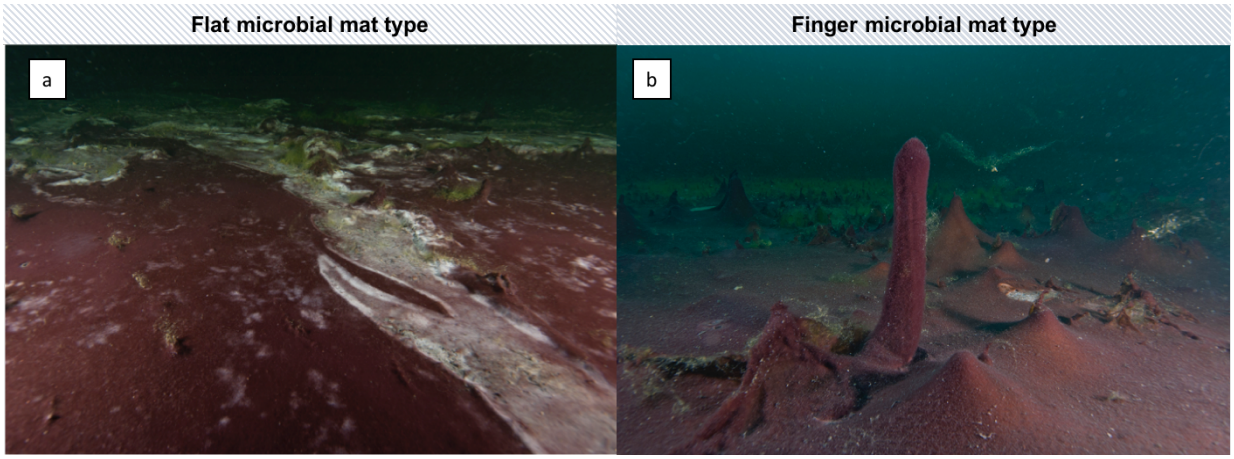
These results from this research show patterns of niche partitioning, which is likely an important control on the diversity of SRB in the MIS cyanobacterial mats and explains why multiple different SRB in the same microbial mat can be recovered. In the MIS mats, niche partitioning may be promoted by vertical migration of the redox front through the microbial mat system on a diurnal cycle. Consistent with this conclusion, the results from this research provide evidence for SRB in cyanobacterial mats possessing versatility in use of electron acceptors, which may be an adaptation to diel shifts in geochemistry and the availability of electron acceptors.

Figures and tables

Figure 1

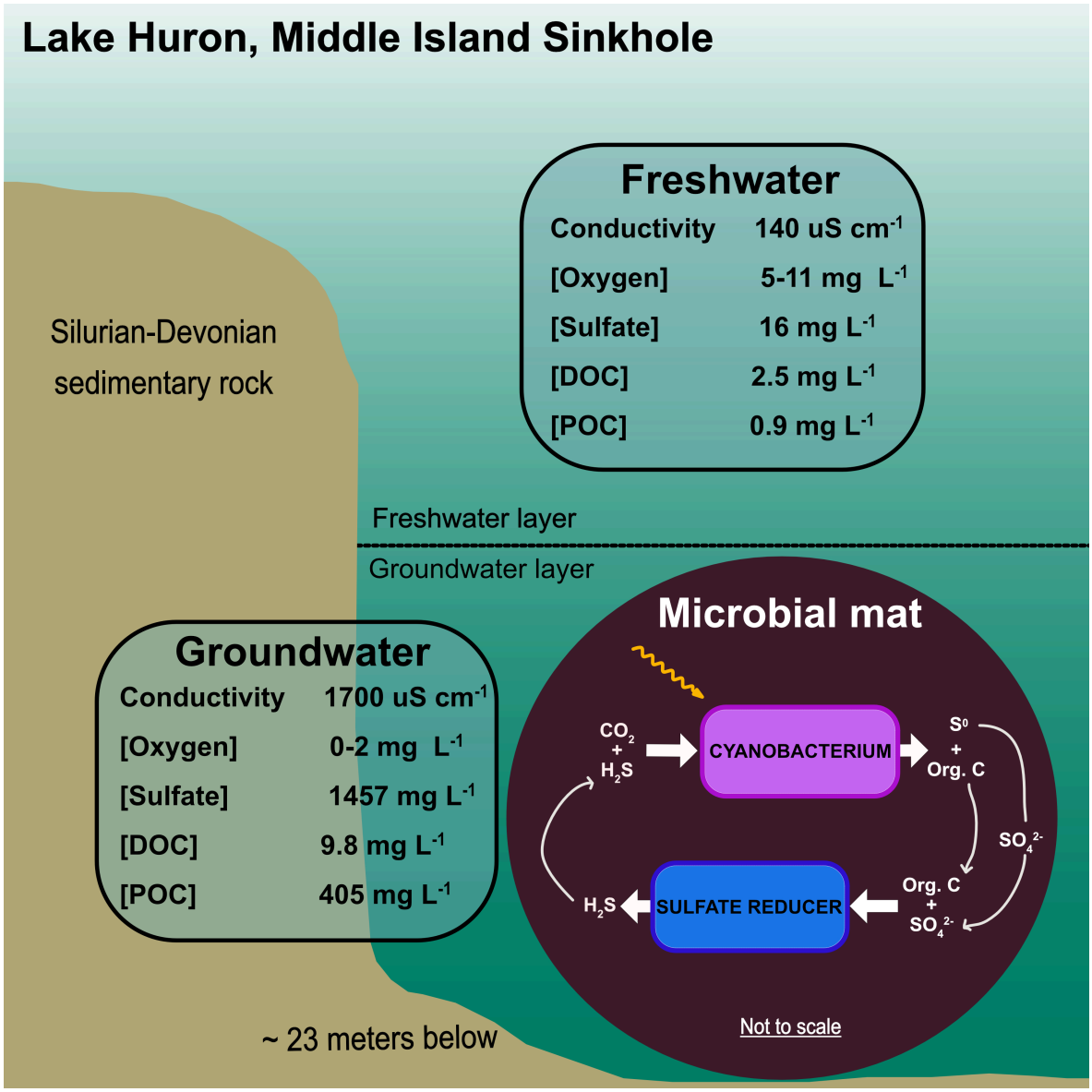
Map of the state of Michigan, U.S.A., and Lake Huron displaying the location of the Middle Island Sinkhole (MIS) near the city Alpena, MI. A closer view of the Middle Island, lies in the top right corner. The MIS lies NE of the Middle Island. This figure was rendered with Google Earth Pro (7.1.5.1557) and Affinity Designer (1.5.5).

Figure 2



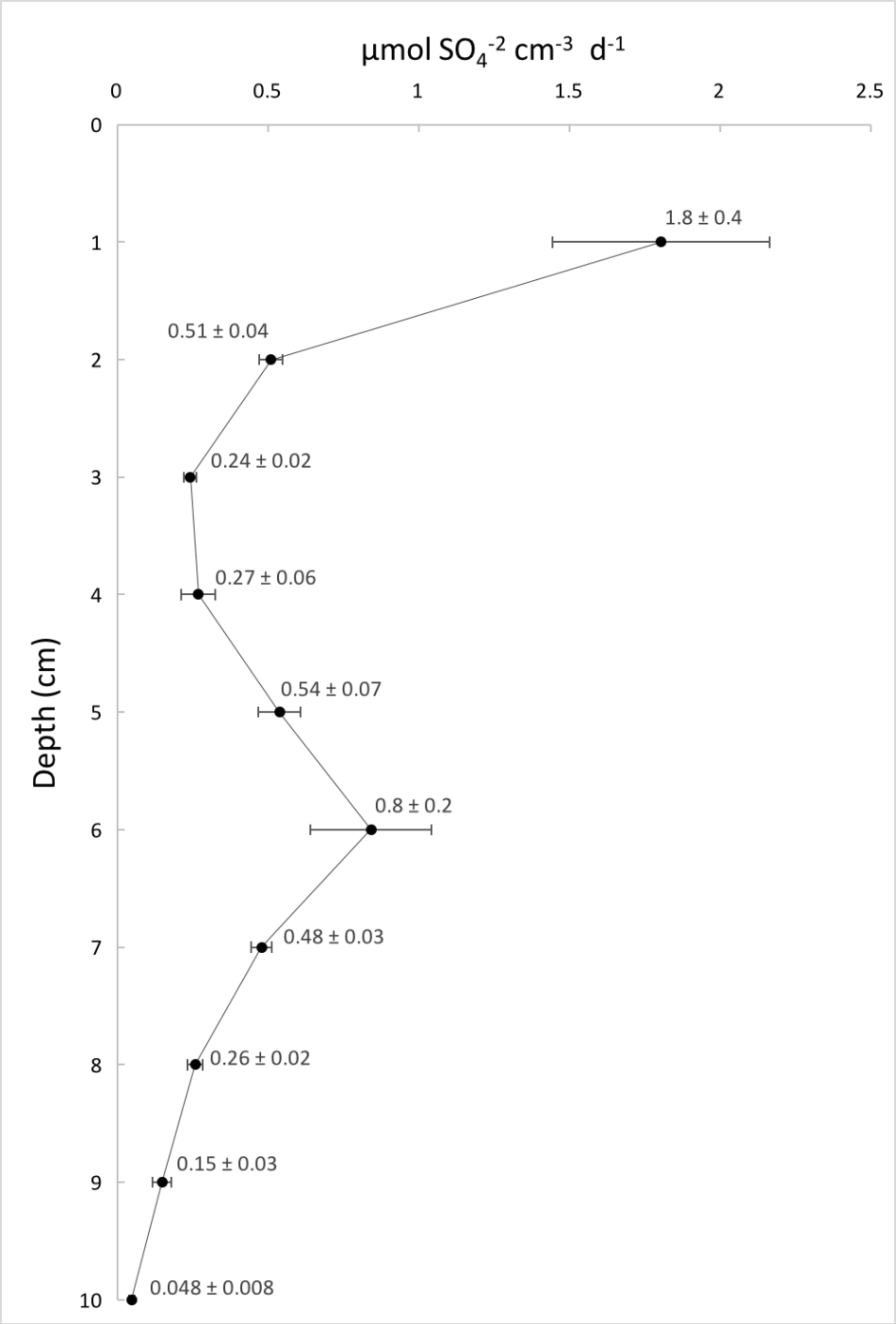
Two types of cyanobacterial microbial mats at MIS: flat (a) and finger (b). White colors at the edges of the flat microbial mat in (a) are sulfur-oxidizing bacteria. Finger mats average 10—15 cm in height while flat mats are generally 0.2 cm thick (Voorhies *et al.* 2012). Images courtesy of Joe Hoyt, NOAA Thunder Bay National Marine Sanctuary.

Figure 3



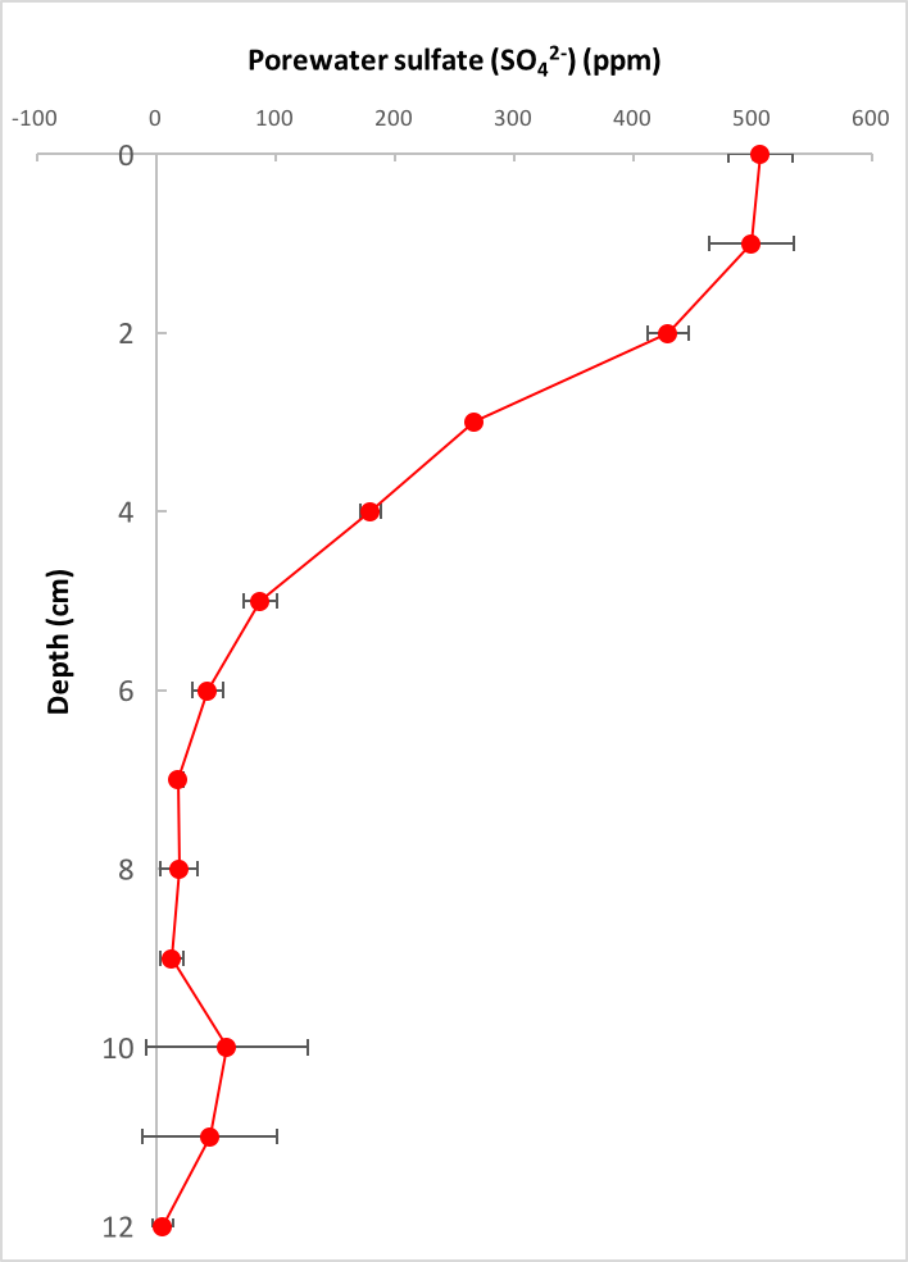
Schematic of water chemistry in the MIS groundwater versus freshwater in Lake Huron and metabolic interactions between dissimilatory sulfate reduction by sulfate-reducing bacteria and anoxygenic photosynthesis by cyanobacteria. Water chemistry data is from Ruberg *et al.* (2005).

Figure 4



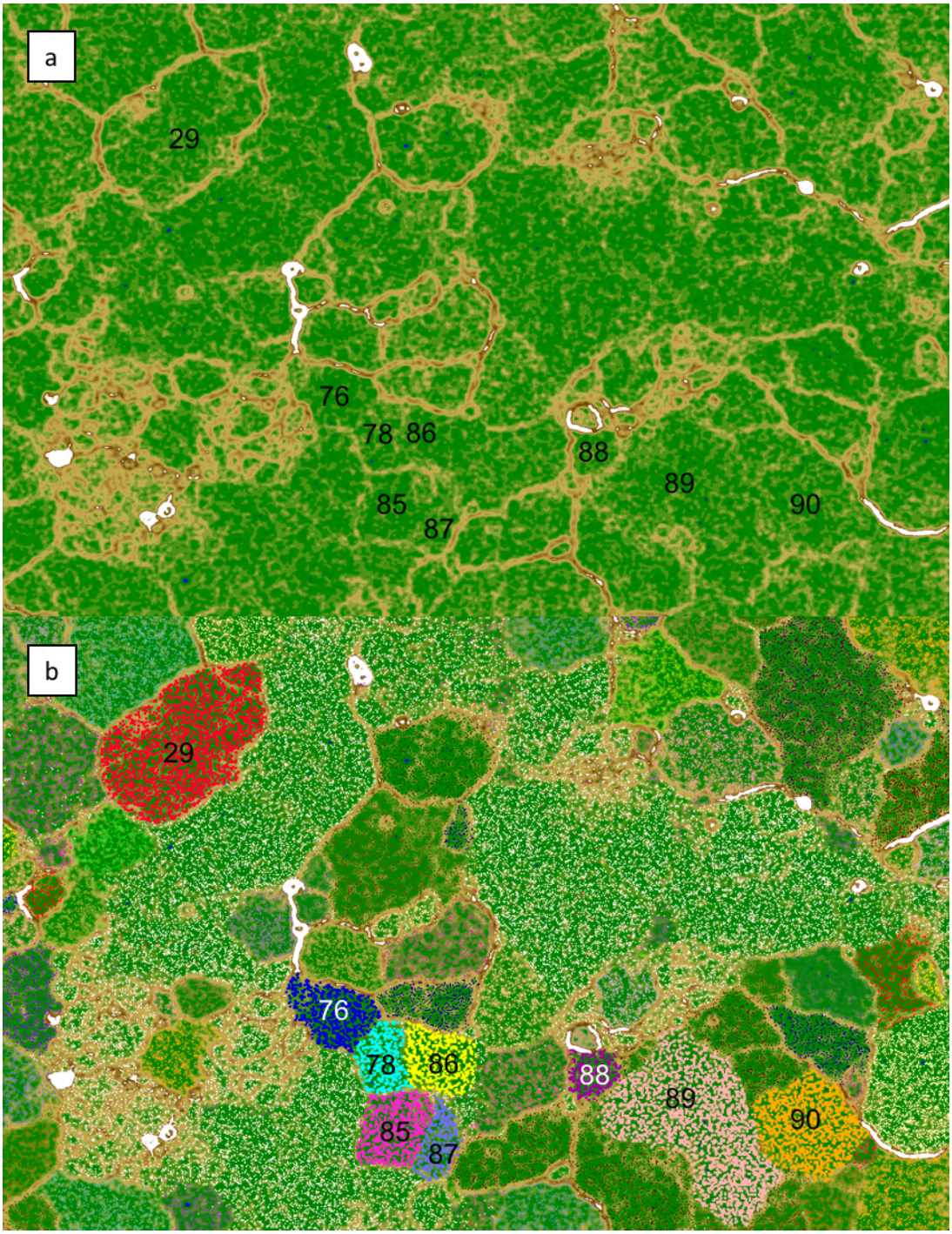
Average sulfate reduction rates among five cores collected from the MIS. The highest rates occur at 1 cm where the cyanobacterial mat layer lies and at 6 cm in depth in sediment. Error was calculated with standard deviation.

Figure 5



Average sulfate concentrations (ppm) from two MIS cores collected at the same time and same site as the cores collected for sulfate-reduction experiments (Figure 4). Sulfate concentrations were determined with membrane-suppression ion chromatography. Error was calculated with standard deviation.

Figure 6



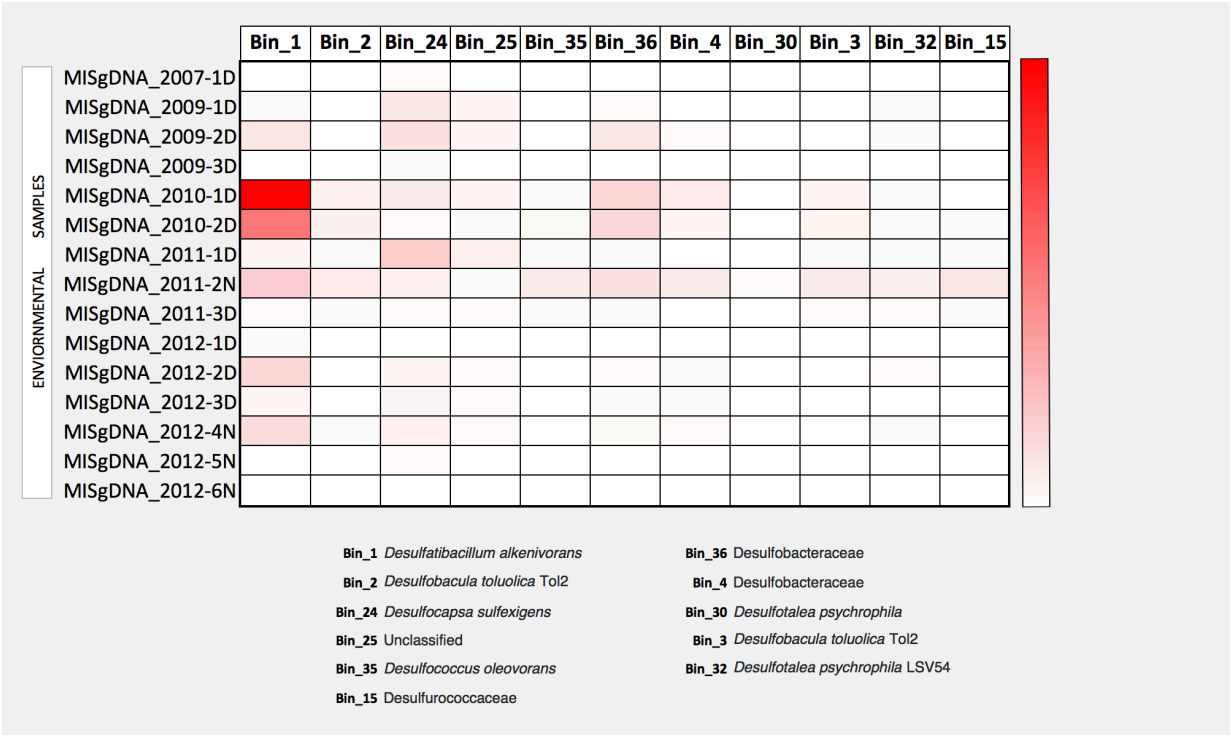
Initial SRB bins (highlighted and numbered) clustered in ESOM using tetranucleotide frequencies.

Table 1

Bin ID	Taxanomic assignment (PhyloSift)	Completeness (CheckM,%)	Redundancy (CheckM)	Total Length (bp)	No. of Contigs	No. of Genes
Bin_30	*Desulfotalea psychrophila*	67	0.00	2,803,052	215	2,718
Bin_2	*Desulfobacula toluolica* Tol2	48	0.00	2,010,011	92	1,954
Bin_36	Desulfobacteraceae	43	1.16	2,547,705	435	2,563
Bin_1	*Desulfatibacillum alkenivorans*	26	0.65	1,460,998	268	1,748
Bin_4	Desulfobacteraceae	20	0.00	1,819,358	317	1,631
Bin_24	*Desulfocapsa sulfexigens*	19	0.58	727,052	164	782
Bin_35	*Desulfococcus oleovorans*	7	0.00	638,467	123	660
Bin_25	Unclassified	0	0.00	627,324	108	641

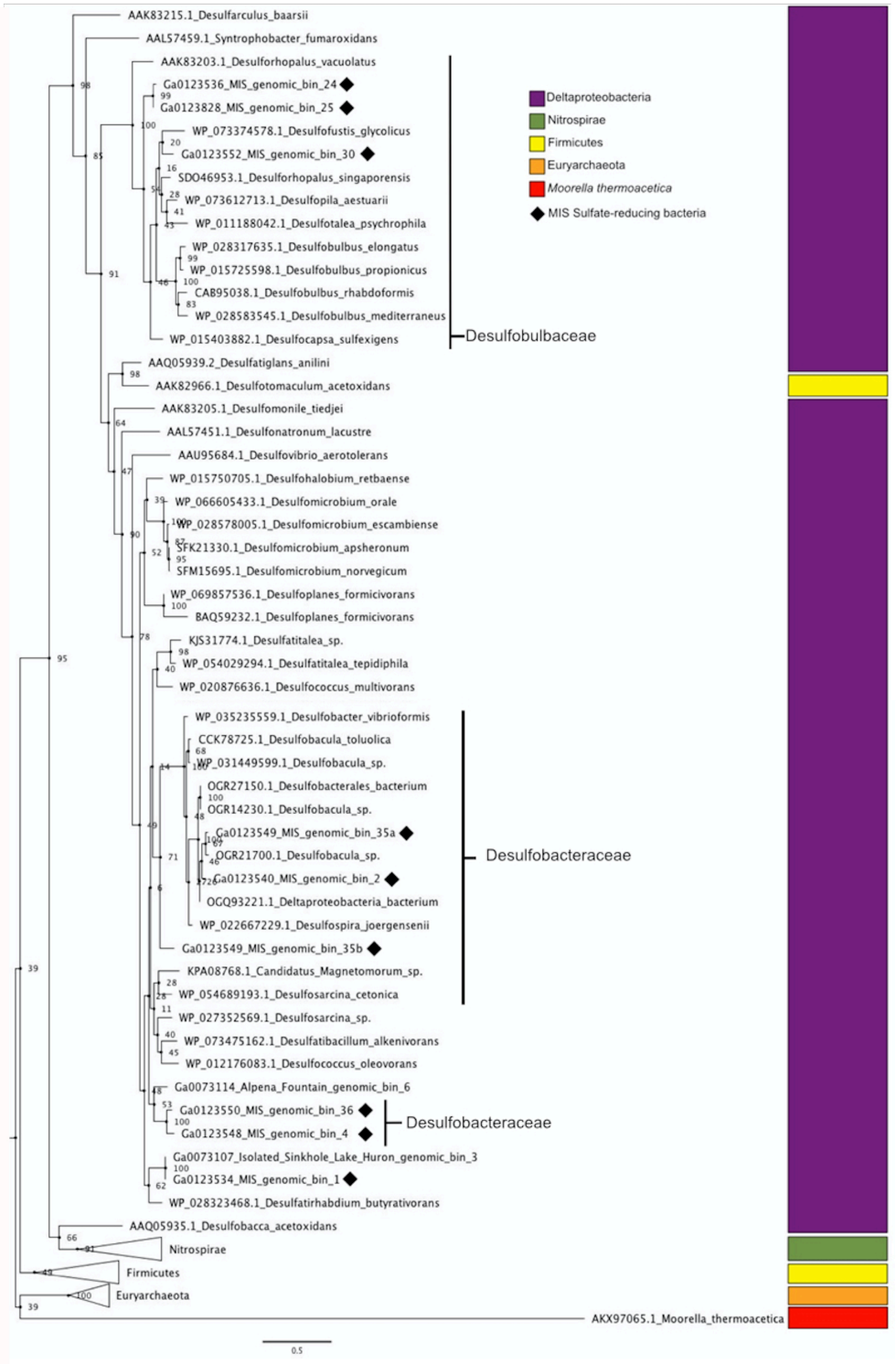
Final SRB MIS genomes recovered from MIS mat samples and clustered using both tetranucleotide frequencies and coverage information of contigs. DsrA sequence from Bin 25 is closely related to that of bin 24; however, bin 25 is not complete enough for PhyloSift to assign this genome to a taxonomic group.

Figure 7



Relative abundance of SRB genomes recovered from MIS cyanobacterial mat based on mean coverage. On the spectrum, the darkest red indicates highest coverage (119 X) whereas white indicates lower coverage values—the lowest being 0.009 X.

Figure 8



Phylogenetic tree of MIS SRB and other sulfate-reducing bacteria based on DsrA amino acid sequences.

Figure 9

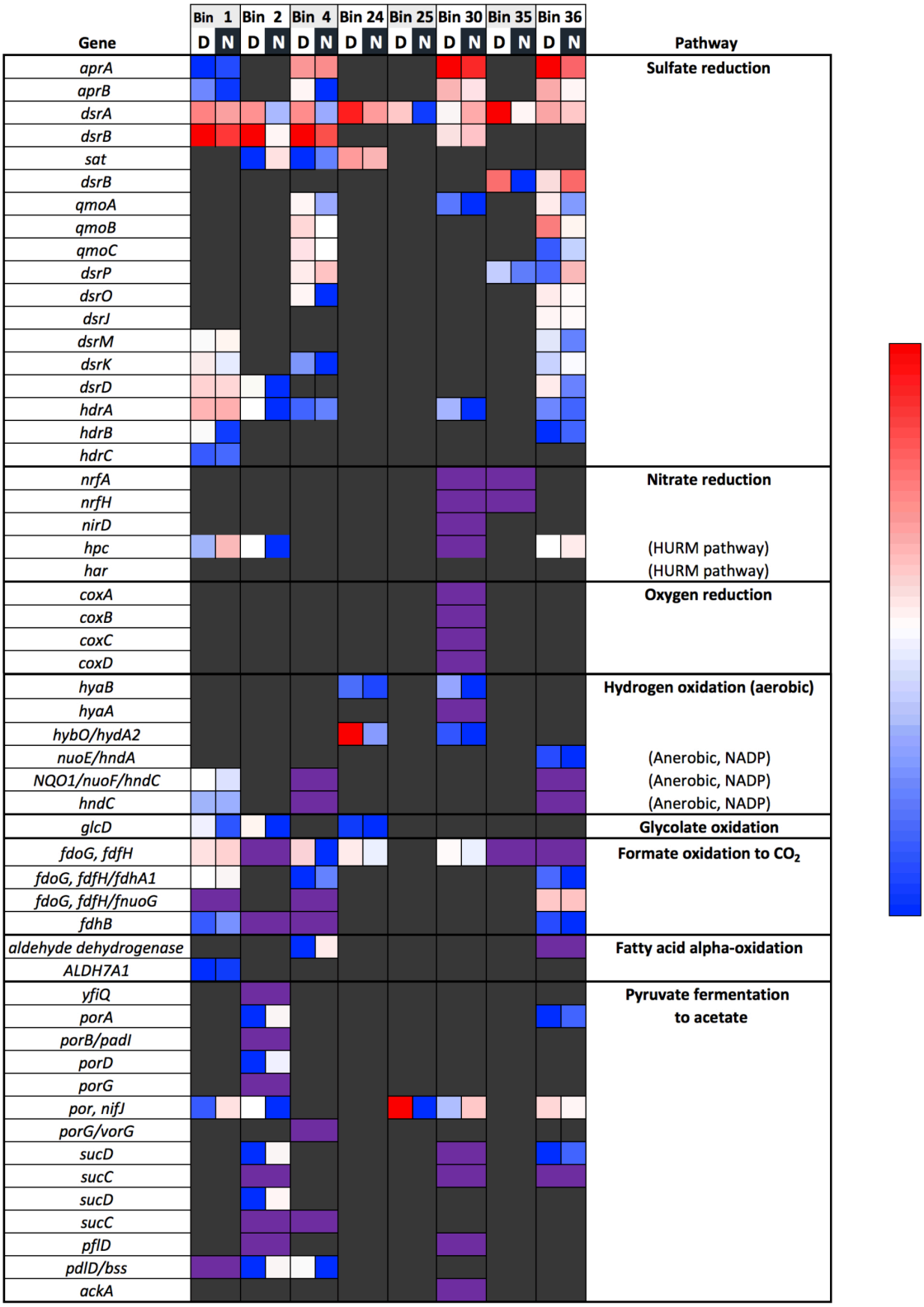

Bin 1
Bin 2
Bin 4
Bin 24
Bin 25
Bin 30
Bin 35
Bin 36
D
N
Gene
Pathway
aprA
aprB
dsrA
dsrB
sat
dsrB
qmoA
qmoB
qmoC
dsrP
dsrO
dsrJ
dsrM
dsrK
dsrD
hdrA
hdrB
hdrC
Sulfate reduction
nrfA
nrfH
nirD
hpc
har
Nitrate reduction
(HURM pathway)
(HURM pathway)
coxA
coxB
coxC
coxD
Oxygen reduction
hyaB
hyaA
hybO/hydA2
nuoE/hndA
NQO1/nuoF/hndC
hndC
Hydrogen oxidation (aerobic)
(Anerobic, NADP)
(Anerobic, NADP)
(Anerobic, NADP)
glcD
Glycolate oxidation
fdoG, fdfH
fdoG, fdfH/fdhA1
fdoG, fdfH/fnuoG
fdhB
Formate oxidation to CO2
aldehyde dehydrogenase
ALDH7A1
Fatty acid alpha-oxidation
yfiQ
porA
porB/padI
porD
porG
por, nifJ
porG/vorG
sucD
sucC
sucD
sucC
pflD
pdlD/bss
ackA
Pyruvate fermentation to acetate

Figure 9 continued.

Normalized read transcripts for genes associated with metabolic pathways. The heat map depicts abundances of transcripts for genes during the day (D) and night (N) for each genomic bin. Reds indicate high abundances of transcripts for the corresponding gene, while blue indicates low abundances of transcripts. Violet cells indicate that the gene in each pathway is in the organism's genome, but no transcripts were detected. A list of genes with their respective enzyme products is presented in the appendix (**Table A-5**).

References

Albertsen, M., Hugenholtz, P., Skarshewski, A., Nielsen, K. L., Tyson, G. W., & Nielsen, P. H. (2013). Genome sequences of rare, uncultured bacteria obtained by differential coverage binning of multiple metagenomes. Nature biotechnology, 31(6), 533-538.

Almeida, M. G., Macieira, S., Gonçalves, L. L., Huber, R., Cunha, C. A., Romão, M. J., Costa, C., Lampreia, J., Moura, J. J., & Moura, I. (2003). The isolation and characterization of cytochrome c nitrite reductase subunits (NrfA and NrfH) from Desulfovibrio desulfuricans ATCC 27774. *The FEBS Journal*, *270*(19), 3904-3915.

Alneberg, J., Bjarnason, B. S., De Bruijn, I., Schirmer, M., Quick, J., Ijaz, U. Z., Lahti, L., Loman, N. J., Anderson, A. F., & Quince, C. (2014). Binning metagenomic contigs by coverage and composition. Nature methods, 11(11), 1144-1146.

Anantharaman, K., Brown, C. T., Hug, L. A., Sharon, I., Castelle, C. J., Probst, A. J., Thomas, B. C., Singh, A., Wilkins, M. J., Karaoz, U., Brodie, E. L., Williams, K. H., Hubbard, S. S., & Banfield, J. F. (2016). Thousands of microbial genomes shed light on interconnected biogeochemical processes in an aquifer system. Nature Communications, 7.

Baskaran, M., Novell, T., Nash, K., Ruberg, S. A., Johengen, T., Hawley, N., Klump, J.V., & Biddanda, B. A. (2016). Tracing the seepage of subsurface sinkhole vent waters into Lake Huron using radium and stable isotopes of oxygen and hydrogen. Aquatic Geochemistry, 22(4), 349-374.

Bateson, M. M., & Ward, D. M. (1988). Photoexcretion and fate of glycolate in a hot spring cyanobacterial mat. Applied and Environmental Microbiology, 54(7), 1738-1743.

Baumgartner, L. K., Reid, R. P., Dupraz, C., Decho, A. W., Buckley, D. H., Spear, J. R., Przekop, K. M., & Visscher, P. T. (2006). Sulfate reducing bacteria in microbial mats: changing paradigms, new discoveries. Sedimentary Geology, 185(3), 131-145.

Beller, H. R., Chain, P. S., Letain, T. E., Chakicherla, A., Larimer, F. W., Richardson, P. M., Coleman, M. A., Wood, A. P., & Kelly, D. P. (2006). The genome sequence of the obligately chemolithoautotrophic, facultatively anaerobic bacterium Thiobacillus denitrificans. Journal of bacteriology, 188(4), 1473-1488.

Bergman B., Codd G. A., and Hallbom L. (1984) Glycolate excretion by Ns-fixing cyanobacteria treated with photorespiratory inhibitors. 2. Pflanzenphysiol. 113,45 l-460.

Biddanda, B. A., Nold, S. C., Ruberg, S. A., Kendall, S. T., Sanders, T. G., & Gray, J. J. (2009). Great lakes sinkholes: a microbiogeochemical frontier. Eos, 90(8), 61-62.

Biddanda, Bopaiah A., et al. "Exploration of a submerged sinkhole ecosystem in Lake Huron." Ecosystems 9.5 (2006): 828-842.

Black, T. J. (1983). Selected views of the tectonics, structure and karst in Northern Lower Michigan, Michigan. In Tectonics, structure and karst in Northern Lower Michigan. Michigan Basin Geological Society Field Conference Proceedings. p (pp. 11-35).

Bosak, T., Knoll, A. H. & Petroff, A. P. 2013. The meaning of stromatolites. Annual Review of Earth and Planetary Sciences 41: 21-44.

Canfield, D. E., & Des Marais, D. J. (1991). Aerobic sulfate reduction in microbial mats. Science, 251(5000), 1471.

Canfield, D. E., & Des Marais, D. J. (1993). Biogeochemical cycles of carbon, sulfur, and free oxygen in a microbial mat. Geochimica et Cosmochimica Acta, 57(16), 3971-3984.

Canfield, D. E., & Raiswell, R. (1999). The evolution of the sulfur cycle. American Journal of Science, 299(7-9), 697-723.

Chatterjee, S., Dickens, G. R., Bhatnagar, G., Chapman, W. G., Dugan, B., Snyder, G. T., & Hirasaki, G. J. (2011). Pore water sulfate, alkalinity, and carbon isotope profiles in shallow sediment above marine gas hydrate systems: A numerical modeling perspective. *Journal of Geophysical Research: Solid Earth*, *116*(B9).

Dannenberg, S., Kroder, M., Dilling, W., & Cypionka, H. (1992). Oxidation of H2, organic compounds and inorganic sulfur compounds coupled to reduction of O2 or nitrate by sulfate-reducing bacteria. Archives of Microbiology, 158(2), 93-99.

Darling, A. E., Jospin, G., Lowe, E., Matsen IV, F. A., Bik, H. M., & Eisen, J. A. (2014). PhyloSift: phylogenetic analysis of genomes and metagenomes. PeerJ, 2, e243.

Dick, G. J., Andersson, A. F., Baker, B. J., Simmons, S. L., Thomas, B. C., Yelton, A. P., & Banfield, J. F. (2009). Community-wide analysis of microbial genome sequence signatures. Genome biology, 10(8), R85.

Dilling, W., & Cypionka, H. (1990). Aerobic respiration in sulfate-reducing bacteria. FEMS Microbiology Letters, 71(1-2), 123-127.

Duarte, A. G., Santos, A. A., & Pereira, I. A. (2016). Electron transfer between the QmoABC membrane complex and adenosine 5′-phosphosulfate reductase. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1857(4), 380-386.

Eren, A. M., Esen, Ö. C., Quince, C., Vineis, J. H., Morrison, H. G., Sogin, M. L., & Delmont, T. O. (2015). Anvi'o: an advanced analysis and visualization platform for 'omics data. PeerJ, 3, e1319.

Finster, K., Liesack, W., & Tindall, B. J. (1997). Desulfospira joergensenii, gen. nov., sp. nov., a new sulfate-reducing bacterium isolated from marine surface sediment. Systematic and applied microbiology, 20(2), 201-208.

Fischer, W. W., Hemp, J., & Johnson, J. E. (2016). Evolution of oxygenic photosynthesis. Annual Review of Earth and Planetary Sciences, 44, 647-683.

Frias-Lopez, J., Shi, Y., Tyson, G. W., Coleman, M. L., Schuster, S. C., Chisholm, S. W., & DeLong, E. F. (2008). Microbial community gene expression in ocean surface waters. Proceedings of the National Academy of Sciences, 105(10), 3805-3810.

Friedrich, M., & Schink, B. (1995). Isolation and characterization of a desulforubidin-containing sulfate-reducing bacterium growing with glycolate. Archives of microbiology, 164(4), 271-279.

Friedrich, M., Springer, N., Ludwig, W., & Schink, B. (1996). Phylogenetic Positions of Desulfofustis glycolicus gen. nov., sp. nov. and Syntrophobotulus glycolicus gen. nov., sp. nov., Two New Strict Anaerobes Growing with Glycolic Acid. International Journal of Systematic and Evolutionary Microbiology, 46(4), 1065-1069.

Fründ, C., & Cohen, Y. (1992). Diurnal cycles of sulfate reduction under oxic conditions in cyanobacterial mats. Applied and Environmental Microbiology, 58(1), 70-77.

Grotzinger, J. P. & Knoll, A. H. 1999. Stromatolites in Precambrian carbonates: Evolutionary mileposts or environmental dipsticks? Annual Review of Earth and Planetary Sciences, 27, 313-358

Han T. W. & Eley J. H. (1973) GlycoIate excretion by Anacystis nidulans: Effect of HCO, concentration, oxygen concentration, and light intensity. Plant Cell Physiol. 14, 285-291.

Hardy, J. A., & Hamilton, W. A. (1981). The oxygen tolerance of sulfate-reducing bacteria isolated from North Sea waters. Current Microbiology, 6(5), 259-262.

Hedderich, R., Hamann, N., & Bennati, M. (2005). Heterodisulfide reductase from methanogenic archaea: a new catalytic role for an iron-sulfur cluster. Biological chemistry, 386(10), 961-970.

Hedderich, R., Hamann, N., & Bennati, M. (2005). Heterodisulfide reductase from methanogenic archaea: a new catalytic role for an iron-sulfur cluster. Biological chemistry, 386(10), 961-970.

Hess, M., Sczyrba, A., Egan, R., Kim, T. W., Chokhawala, H., Schroth, G., Luo, S., Clark, D. S., Chen, F., Zhang, T., Mackie, R. I., Pennacchio, L. A., Tringe, S. G., Visel, A., Woyke, T., Wang, Z., & Rubin, E. M. (2011). Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. Science, 331(6016), 463-467.

Huntemann, M., Ivanova, N. N., Mavromatis, K., Tripp, H. J., Paez-Espino, D., Palaniappan, K., Szeto, E., Pillay, M., Chen, I. A., Pati, A., Nielsen, T., Markowitz, V. M. & Kyropides, N. C. (2015). The standard operating procedure of the DOE-JGI Microbial Genome Annotation Pipeline (MGAP v. 4). Standards in genomic sciences, 10(1), 86.

Jørgensen, B. B. (1977). Bacterial sulfate reduction within reduced microniches of oxidized marine sediments. Marine Biology, 41(1), 7-17.

Jørgensen, B. B. (1994). Sulfate reduction and thiosulfate transformations in a cyanobacterial mat during a diel oxygen cycle. FEMS Microbiology Ecology, 13(4), 303-312.

Jørgensen, B. B. (1994). Sulfate reduction and thiosulfate transformations in a cyanobacterial mat during a diel oxygen cycle. FEMS Microbiology Ecology, 13(4), 303-312.

Kallmeyer, J., Ferdelman, T. G., Weber, A., Fossing, H., & Jørgensen, B. B. (2004). A cold chromium distillation procedure for radiolabeled sulfide applied to sulfate reduction measurements. Limnol. Oceanogr. Methods, 2, 171-180.

Kim, S. J., Park, S. J., Jung, M. Y., Kim, J. G., Min, U. G., Hong, H. J., & Rhee, S. K. (2014). Draft genome sequence of an aromatic compound-degrading bacterium, Desulfobacula sp. TS, belonging to the Deltaproteobacteria.

Kim, Y. M., Nowack, S., Olsen, M. T., Becraft, E. D., Wood, J. M., Thiel, V., Klapper, I., Kühl, M., Fredrickson, J. K., Bryant, D. M., Ward, D. M., & Metz, T. O. (2015). Diel metabolomics analysis of a hot spring chlorophototrophic microbial mat leads to new hypotheses of community member metabolisms. Frontiers in microbiology, 6, 209.

Kinsman-Costello, L. E., Sheik, C. S., Sheldon, N. D., Allen Burton, G., Costello, D. M., Marcus, D., Den Uyl, P. A. & Dick, G. J. (2017). Groundwater shapes sediment biogeochemistry and microbial diversity in a submerged Great Lake sinkhole. Geobiology, 15, 225-239.

Klatt, J. M., Haas, S., Yilmaz, P., Beer, D., & Polerecky, L. (2015). Hydrogen sulfide can inhibit and enhance oxygenic photosynthesis in a cyanobacterium from sulfidic springs. Environmental microbiology, 17(9), 3301-3313.

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics, 25(14), 1754-1760.

Lien, T., & Beeder, J. (1997). Desulfobacter vibrioformis sp. nov., a sulfate reducer from a water-oil separation system. International Journal of Systematic and Evolutionary Microbiology, 47(4), 1124-1128.

Liu, M. C., & Peck, H. D. (1981). The isolation of a hexaheme cytochrome from Desulfovibrio desulfuricans and its identification as a new type of nitrite reductase. *Journal of Biological Chemistry*, *256*(24), 13159-13164.

Lyons, T. W., Reinhard, C. T., & Planavsky, N. J. (2014). The rise of oxygen in Earth/'s early ocean and atmosphere. Nature, 506(7488), 307-315.

Mander, G. J., Pierik, A. J., Huber, H., & Hedderich, R. (2004). Two distinct heterodisulfide reductase-like enzymes in the sulfate-reducing archaeon Archaeoglobus profundus. European journal of biochemistry, 271(6), 1106-1116.

Mander, G. J., Pierik, A. J., Huber, H., & Hedderich, R. (2004). Two distinct heterodisulfide reductase-like enzymes in the sulfate-reducing archaeon Archaeoglobus profundus. European journal of biochemistry, 271(6), 1106-1116.

Moran, M. A., Satinsky, B., Gifford, S. M., Luo, H., Rivers, A., Chan, L. K., Meng, J., Durham, B. P., Shen, C., Varaljay, V. A., Smith, C. B., Yager, P. L., & Hopkinson, B. M. (2013). Sizing up metatranscriptomics. The ISME journal, 7(2), 237-243.

Müller, A. L., Kjeldsen, K. U., Rattei, T., Pester, M., & Loy, A. (2015). Phylogenetic and environmental diversity of DsrAB-type dissimilatory (bi) sulfite reductases. The ISME journal, 9(5), 1152-1165.

Nold, S. C., & Ward, D. M. (1996). Photosynthate partitioning and fermentation in hot spring microbial mat communities. Applied and Environmental Microbiology, 62(12), 4598-4607.

Nold, S. C., Bellecourt, M. J., Kendall, S. T., Ruberg, S. A., Sanders, T. G., Klump, J. V., & Biddanda, B. A. (2013). Underwater sinkhole sediments sequester Lake Huron's carbon. Biogeochemistry, 115(1-3), 235-250.

Nold, S. C., Pangborn, J. B., Zajack, H. A., Kendall, S. T., Rediske, R. R., & Biddanda, B. A. (2010). Benthic bacterial diversity in submerged sinkhole ecosystems. Applied and environmental microbiology, 76(1), 347-35

Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P., & Tyson, G. W. (2015). CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome research, 25(7), 1043-1055.

Peng, Y., Leung, H. C., Yiu, S. M., & Chin, F. Y. (2012). IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics, 28(11), 1420-1428.

Pereira, I. A. C., Ramos, A. R., Grein, F., Marques, M. C., Da Silva, S. M., & Venceslau, S. S. (2011). A comparative genomic analysis of energy metabolism in sulfate reducing bacteria and archaea. Frontiers in microbiology, 2.

Ramos, A. R., Keller, K. L., Wall, J. D., & Pereira, I. A. C. (2012). The membrane QmoABC complex interacts directly with the dissimilatory adenosine 5-phosphosulfate reductase in sulfate reducing bacteria. Frontiers in microbiology, 3, 137.

Røy, H., Weber, H. S., Tarpgaard, I. H., Ferdelman, T. G., & Jørgensen, B. B. (2014). Determination of dissimilatory sulfate reduction rates in marine sediment via radioactive 35S tracer. *Limnol. Oceanogr. Methods*, *12*, 196-211.

Ruberg, S. A., Coleman, D. F., Johengen, T. H., Meadows, G. A., Van Sumeren, H. W., Lang, G. A., & Biddanda, B. A. (2005). Groundwater plume mapping in a submerged sinkhole in Lake Huron. Marine Technology Society Journal, 39(2), 65-69

Ruberg, S. A., Kendall, S. T., Biddanda, B. A., Black, T., Nold, S. C., Lusardi, W. R., Wayne, R., Green, R., Casserly, T., Smith, E., Sanders, G. T., Lang, G. A., & Constant, S. A. (2008). Observations of the Middle Island Sinkhole in Lake Huron–a unique hydrogeologic and glacial creation of 400 million years. Marine Technology Society Journal, 42(4), 12-21.

Sharrar A.M., Flood B.E., Bailey J.V., Jones D.S., Biddanda B.A., Ruberg A., Marcus D.N., Dick G.J. (2017). Novel Large Sulfur Bacteria in the Metagenomes of Groundwater-Fed Chemosynthetic Microbial Mats in the Lake Huron Basin. Frontiers in Microbiology,8, 791.

Stal, L., & Caumette, P. (Eds.). (2013). Microbial mats: structure, development and environmental significance (Vol. 35). Springer Science & Business Media.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30(9), 1312-1313.

Sultan, N., Garziglia, S., & Ruffine, L. (2016). New insights into the transport processes controlling the sulfate-methane-transition-zone near methane vents. *Scientific reports*, *6*, 26701.

Teske, A., Ramsing, N. B., Habicht, K., Fukui, M., Küver, J., Jørgensen, B. B., & Cohen, Y. (1998). Sulfate-reducing bacteria and their activities in cyanobacterial mats of Solar Lake (Sinai, Egypt). Applied and Environmental Microbiology, 64(8), 2943-2951.

Voorhies, A. A. (2014). Investigation of Microbial Interactions and Ecosystem Dynamics in a Low O2 Cyanobacterial Mat. University of Michigan, Ann Arbor, Michigan.

Voorhies, A. A. (2014). Metabolic function and microbial mediation of geochemical cycling revealed by community genomic analysis and gene expression of low-O2 cyanobacterial mat. Doctoral dissertation, University of Michigan.

Voorhies, A. A., Biddanda, B. A., Kendall, S. T., Jain, S., Marcus, D. N., Nold, S. C., Sheldon, N. D., & Dick, G. J. (2012). Cyanobacterial life at low O2: community genomics and function reveal metabolic versatility and extremely low diversity in a Great Lakes sinkhole mat. Geobiology, 10(3), 250-267.

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

Zane, G.M., Yen, H. C. B., & Wall, J. D. (2010). Effect of the deletion of qmoABC and the promoter-distal gene encoding a hypothetical protein on sulfate reduction in Desulfovibrio vulgaris Hildenborough. Applied and environmental microbiology, 76(16), 5500-5509.

Appendix

Table A-1

Sample ID	Date (mm/yyyy)	Mat type	Illumina gDNA reads	Illumina cDNA reads
MISgDNA_2007-1D	06/2007	Finger	55,742,528	-
MISgDNA_2009-1D	06/2009	Flat	56,610,020	-
MISgDNA_2009-2D	06/2009	Finger	58,811,950	-
MISgDNA_2009-3D	06/2009	Finger	25,542,490	-
MISgDNA_2010-1D	08/2010	Finger	69,716,858	-
MISgDNA_2010-2D	08/2010	Flat	67,190,598	-
MISgDNA_2011-1D	06/2011	Flat	98,430,946	-
MISgDNA_2011-2N	06/2011	Flat	211,878,358	-
MISgDNA_2011-3D	06/2011	Finger	59,608,800	-
MISg/cDNA_2012-1D	05/2012	Finger	25,666,608	11,997,534
MISg/cDNA_2012-2D	05/2012	Finger	41,595,684	14,577,110
MISg/cDNA_2012-3D	05/2012	Finger	41,318,538	11,524,300
MISg/cDNA_2012-4N	05/2012	Finger	42,876,682	12,125,414
MISg/cDNA_2012-5N	05/2012	Finger	38,335,454	12,100,438
MISg/cDNA_2012-6N	05/2012	FInger	28,207,614	13,079,448

List of environmental samples with respective Illumina genomic (gDNA) and complementary (cDNA) DNA. The date when collected and classification of mat type presented for each sample.

Table A-2

Query ID	Bin ID	Subject ID	Percent identity	e value	Bit score	Query subject	Environment
scaffold_259_MIS_10000260.50	85	entry578	83.67	0	1079	Marine microbial community metagenome	Marine
scaffold_712_MIS_10000713.88	86	entry74y	80.14	0	937	Freshwater microbial community metagenome	Freshwater
scaffold_13184_MIS_10013185.17	29	AtcSed69	78.51	2x10^-147	520	Arctic sediment clone AH2 19	Marine
scaffold_19845_MIS_10019846.37	29	entry92y	76.8	4x10^-180	628	Marine metagenome	Marine
scaffold_20572_MIS_10020573.1	29	FR695872	86.87	0	1123	Desulfobacterium sp. N47	Marine
scaffold_33212_MIS_10033213.16	76	DcpSulf5	79.39	0	782	Desulfocapsa sulfexigens	Marine
scaffold_40931_MIS_10004798.5	87	JN798924	85.41	0	1170	Deep sea sediment clone	Marine
scaffold_43226_MIS_10036537.5	29	OlaAlg18	78.13	0	822	Olavius algarvensis Delta 1 symbiont	Symbiotic
scaffold_46672_MIS_10037061.17	78	DcpSulf5	80.4	0	970	Desulfocapsa sulfexigens	Marine

List of contigs that have sequences for reductive type dsrAB.

Table A-3

Bin ID	Genome size	No. of contigs	No. of predicted genes	Completetion %	Contamination	Strain heterogeneity
ESOM_bin29	10,487,020	1525	10269	96	78.9	11.28
ESOM_bin85	3,652,853	277	3,623	95	3.87	28.57
ESOM_bin90	6,730,400	659	6,922	91	92.4	45.56
ESOM_bin89	12,577,677	1,323	12789	79	208.8	17.24
ESOM_bin86	2,832,954	246	2713	67	0	78.95
ESOM_bin88	1,301,656	165	1,448	56	3.45	0
ESOM_bin76	3,014,718	366	2,924	54	0.66	0
ESOM_bin78	1,903,878	281	1,871	23	5.26	100
ESOM_bin87	1,119,725	183	1,108	11	0	0

Bin statistics for SRB bins recovered from ESOM and depicted in Figure 5.

Table A-4

Bin ID	Taxanomic assignment (PhyloSift)	Completeness (CheckM, %)	Redundancy (CheckM)	Total Length (bp)	No. of Contigs	No. of Genes	DSR genes
Bin_3	*Desulfobacula toluolica* Tol2	30	0.00	637,603	28	635	aprAB
Bin_32	*Desulfotalea psychrophila* LSV54	28	0.60	559,853	85	561	dsrJKMO
Bin_15	Desulfurococcaceae	15	0.00	977,728	105	1,003	sat, aprAB

Putative SRB recovered from the MIS cyanobacterial mat. These genomes are missing dsrAB; however, there is evidence of other genes involved in dissimilarity sulfate reduction.

Table A-5

Pathway	*Gene*	Enzyme
Sulfate reduction	*aprA*	dissimilatory adenylylsulfate reductase alpha subunit
	aprB	dissimilatory adenylylsulfate reductase beta subunit
	dsrA	dissimilatory sulfite reductase alpha subunit
	dsrB	dissimilatory sulfite reductase beta subunit
	sat	sulfate adenylyltransferase
	dsrB	sulfite reductase beta subunit
	qmoA	putative adenylylsulfate reductase-associated electron transfer protein QmoA
	qmoB	putative adenylylsulfate reductase-associated electron transfer protein QmoB
	qmoC	putative adenylylsulfate reductase-associated electron transfer protein QmoC
	dsrP	putative sulfite reductase-associated electron transfer protein DsrP
	dsrO	putative sulfite reductase-associated electron transfer protein DsrO
	dsrJ	putative sulfite reductase-associated electron transfer protein DsrJ
	dsrM	putative sulfite reductase-associated electron transfer protein DsrM
	dsrK	putative sulfite reductase-associated electron transfer protein DsrK
	dsrD	Dissimilatory sulfite reductase D (DsrD)
	hdrA	heterodisulfide reductase subunit A
	hdrB	heterodisulfide reductase subunit B
	hdrC	heterodisulfide reductase subunit C
Nitrate reduction	*nrfA*	respiratory nitrite reductase (cytochrome; ammonia-forming) precursor
	nrfH	respiratory nitrite reductase specific menaquinol--cytochrome-c reductase (NrfH) precursor
	nirD	nitrite reductase (NADH) small subunit
(HURM pathway)	*hpc*	hydroxylamine reductase
(HURM pathway)	*har*	Reverse hydroxylamine:ubiquinone reductase
Oxygen reduction	*coxA*	cytochrome c oxidase subunit 1
	coxB	cytochrome c oxidase subunit 2
	coxC	cytochrome c oxidase subunit 3
	coxD	cytochrome c oxidase subunit 4
Hydrogen oxidation (aerobic)	*hyaB*	hydrogenase large subunit
	hyaA	hydrogenase small subunit
	hybO/hydA2	hydrogenase small subunit/[NiFe] hydrogenase small subunit
(Anerobic, NADP)	*nuoE/hndA*	NADH-quinone oxidoreductase subunit E/NADP-reducing hydrogenase subunit HndA
(Anerobic, NADP)	*NQO1/nuoF/hndC*	NADH-quinone oxidoreductase subunit F/NAD(P)H dehydrogenase (quinone)/NADP-reducing hydrogenase subunit HndC
(Anerobic, NADP)	*hndC*	NADP-reducing hydrogenase subunit HndB
Glycolate oxidase	*glcD*	Glycolate oxidase
Sulfite oxidation	*aprA*	dissimilatory adenylylsulfate reductase alpha subunit precursor
	aprB	dissimilatory adenylylsulfate reductase beta subunit
	sat	sulfate adenylyltransferase
Formate oxidation to CO2	*fdoG, fdfH*	formate dehydrogenase major subunit
	fdoG, fdfH/fdhA1	formate dehydrogenase major subunit/formate dehydrogenase alpha subunit
	fdoG, fdfH/fnuoG	formate dehydrogenase major subunit/NADH-quinone oxidoreductase subunit G
	fdhB	formate dehydrogenase subunit beta
Fatty acid alpha-oxidation	aldehyde dehydrogenase	aldehyde dehydrogenase (NAD+)
	ALDH7A1	aldehyde dehydrogenase (NAD+)/aldehyde dehydrogenase family 7 member A1
Pyruvate fermentation to acetate	*yfiQ*	acetyl-CoA synthetase (ADP-forming)/acetyltransferase
	porA	pyruvate ferredoxin oxidoreductase alpha subunit
	porB/padI	pyruvate ferredoxin oxidoreductase beta subunit/phenylglyoxylate dehydrogenase beta subunit
	porD	pyruvate ferredoxin oxidoreductase delta subunit
	porG	pyruvate ferredoxin oxidoreductase gamma subunit
	por, nifJ	pyruvate-ferredoxin/flavodoxin oxidoreductase
	porG/vorG	pyruvate ferredoxin oxidoreductase gamma subunit/2-oxoisovalerate ferredoxin oxidoreductase gamma subunit
	sucD	succinyl-CoA synthetase (ADP-forming) alpha subunit
	sucC	succinyl-CoA synthetase (ADP-forming) beta subunit
	sucD	succinyl-CoA synthetase alpha subunit
	sucC	succinyl-CoA synthetase beta subunit
	pflD	formate C-acetyltransferase
	pdlD/bss	formate C-acetyltransferase/benzylsuccinate synthase
	ackA	acetate kinase

Table of the genes associated with a given metabolic pathway and the corresponding enzymes encoded for by each gene.