Omics-Inferred Partitioning and Expression of Diverse Biogeochemical Functions in a Low-O$_2$ Cyanobacterial Mat Community

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ABSTRACT Cyanobacterial mats profoundly influenced Earth’s biological and geochemical evolution and still play important ecological roles in the modern world. However, the biogeochemical functioning of cyanobacterial mats under persistent low-O$_2$ conditions, which dominated their evolutionary history, is not well understood. To investigate how different metabolic and biogeochemical functions are partitioned among community members, we conducted metagenomics and metatranscriptomics on cyanobacterial mats in the low-O$_2$, sulfidic Middle Island sinkhole (MIS) in Lake Huron. Metagenomic assembly and binning yielded 144 draft metagenome assembled genomes, including 61 of medium quality or better, and the dominant cyanobacteria and numerous Proteobacteria involved in sulfur cycling. Strains of a Phormidium autumnale-like cyanobacterium dominated the metagenome and metatranscriptome. Transcripts for the photosynthetic reaction core genes psaA and psbA were abundant in both day and night. Multiple types of psbA genes were expressed from each cyanobacterium, and the dominant psbA transcripts were from an atypical microaerobic type of D1 protein from Phormidium. Further, cyanobacterial transcripts for photosystem I genes were more abundant than those for photosystem II, and two types of Phormidium sulfide quinone reductase were recovered, consistent with anoxygenic photosynthesis via photosystem I in the presence of sulfide. Transcripts indicate active sulfur oxidation and reduction within the cyanobacterial mat, predominately by Gammaproteobacteria and Deltaproteobacteria, respectively. Overall, these genomic and transcriptomic results link specific microbial groups to metabolic processes that underpin primary production and biogeochemical cycling in a low-O$_2$ cyanobacterial mat and suggest mechanisms for tightly coupled cycling of oxygen and sulfur compounds in the mat ecosystem.

IMPORTANCE Cyanobacterial mats are dense communities of microorganisms that contain photosynthetic cyanobacteria along with a host of other bacterial species that play important yet still poorly understood roles in this ecosystem. Although such cyanobacterial mats were critical agents of Earth’s biological and chemical evolution through geological time, little is known about how they function under the low-oxygen conditions that characterized most of their natural history. Here, we performed sequencing of the DNA and RNA of modern cyanobacterial mat communities under low-oxygen and sulfur-rich conditions from the Middle Island sinkhole in Lake Huron. The results reveal the organisms and metabolic pathways that are responsible for both oxygen-producing and non-oxygen-producing photosynthesis as well as...
interconversions of sulfur that likely shape how much O2 is produced in such ecosystems. These findings indicate tight metabolic reactions between community members that help to explain the limited amount of O2 produced in cyanobacterial mat ecosystems.

**KEYWORDS** cyanobacteria, geomicrobiology, metagenomics, metatranscriptomics, photosynthesis, biogeochemistry, mats, oxygen, sulfur

Cyanobacterial mats host communities of microorganisms that are linked through metabolic interactions in which the products of one metabolism are the substrate for another (1–4). These metabolic interactions underpinned critical biogeochemical processes throughout Earth’s history (5–7) and continue to do so in the modern world (2, 4). Cyanobacterial mats have been a prevalent feature of the biosphere for billions of years and strongly influenced the composition of the atmosphere (7, 8). Most prominently, cyanobacteria mediated the oxygenation of Earth’s surface by producing O2 via oxygenic photosynthesis, thus catalyzing a cascade of geobiological transitions that set the stage for complex life (9).

Modern microbial mats have long served as analogs for studying their ancient equivalents, and recent work has made great progress in elucidating cyanobacterial mat processes, organisms, and their interactions (10–12). However, relatively little work has been devoted to cyanobacterial mats that inhabit persistently low-O2 and/or sulfidic environments. This is a critical gap in knowledge, because low-O2, sulfidic phototrophic habitats were likely common in the Precambrian (13) and thus prevailed for much of the evolutionary history of cyanobacteria (6, 14). Further, cyanobacteria were likely anoxygenic phototrophs prior to evolving oxygenic photosynthesis (15–18), and anoxygenic cyanobacteria may have delayed Earth’s oxygenation during ~2 billion years of low-O2 conditions in the Proterozoic (6, 19, 20).

Sulfide is a key control of the physiology of cyanobacteria and the biogeochemical cycling of elements in cyanobacterial mats (6). Cyanobacteria typically conduct oxygenic photosynthesis, which is inhibited by sulfide because it blocks photosystem II (PSII) (21). However, some cyanobacteria can tolerate sulfide through a variety of mechanisms, including sulfide-resistant oxygenic photosynthesis, simultaneous operation of oxygenic and anoxygenic photosynthesis, and a complete switch to anoxygenic photosynthesis using sulfide as the electron donor (21). In some strains, sulfide can either inhibit or enhance oxygenic photosynthesis, depending on light availability and sulfide conditions (22). Sulfide-quinone reductase (SQR) is the key enzyme for anoxygenic photosynthesis by cyanobacteria; it oxidizes sulfide and transfers electrons to PSII through the quinone pool, effectively bypassing PSII (23–25). SQR is a diverse protein family that has also been linked to sulfide detoxification in cyanobacteria and other phototrophs (6, 24). Although studies have elucidated the physiological responses of cyanobacteria to sulfide and the role of SQR in anoxygenic photosynthesis (21, 26, 27), little is known about transcriptomic controls on cyanobacterial anoxygenic photosynthesis within cyanobacterial mats.

The Middle Island sinkhole (MIS) in Lake Huron, MI, hosts cyanobacterial mats in low-O2, intermittently sulfidic conditions (28). The mats sit atop anoxic, organic-rich sediments in which microbial methanogenesis and sulfate reduction produce methane and sulfide, leading to sharp redox gradients (29–32). The mats are metabolically versatile, having the ability to conduct oxygenic photosynthesis, anoxygenic photosynthesis, and chemoautotrophy (30, 33, 34). Despite this metabolic versatility, early 16S rRNA gene and metagenomic studies suggested that the mats have low taxonomic diversity, being dominated by just one cyanobacterial genotype, an organism closely related to *Phormidium autumnale* (29, 30, 34). However, deep 16S rRNA gene sequencing of the mat and underlying sediments revealed a taxonomically diverse microbial community, including numerous groups of sulfate-reducing and sulfur-oxidizing bacteria that are suggested to mediate key biogeochemical processes within and beneath the mat (31).
Further, diurnal vertical migration of sulfur-oxidizing bacteria and diatoms exerts a strong influence on the biogeochemistry of the systems and on light availability and photosynthesis in the mats (33, 35).

In order to investigate how different metabolic and geochemical functions are partitioned among community members and expressed over time, we conducted metagenomic analysis on 15 samples collected at seven time points between 2007 and 2012 and metatranscriptomic analysis on six samples taken during day and night in 2012. The Phormidium species was found to dominate transcriptional activity in the MIS mat community and displays gene expression patterns consistent with a mixture of oxygenic and anoxygenic photosynthesis. We also recovered genomes and transcripts of diatoms, sulfate-reducing bacteria, and sulfur-oxidizing bacteria, providing insights into the microbial groups that mediate key biogeochemical processes within the mat.

RESULTS AND DISCUSSION

Environmental setting and conditions. The environmental and geological setting of the MIS was described previously (28). In May 2012, at the time of collection of samples for metatranscriptomic and metagenomic sequencing (Table S1), the groundwater layer ~1 m immediately above the mat in the sinkhole had substantially elevated specific conductivity (1,813 μS cm⁻¹ versus 226 μS cm⁻¹ in the ambient lake water), lower and temporally consistent temperature (7 to 9°C), and an average dissolved O₂ level of 3.37 mg L⁻¹.

Community composition and function. Assembly and binning produced 16 high-quality draft metagenome-assembled genomes (MAGs) (>90% completion, <5% redundancy), 45 medium-quality (>50% completion, <10% redundancy), and 79 low-quality draft MAGs (<50% completion, <10% redundancy) according to estimates based on single-copy genes expected to be present (36) (Table S2). In addition, four MAGs had high redundancy (>10%), including three of the most abundant MAGs (Bin_4_1, Bin_1, Bin_235_243; Rhodotherax, Phormidium, and Planktothrix, respectively), which had high coverage and moderate completion (Table S2). For example, the dominant MAG in most samples, Phormidium (Bin_1), had high redundancy (56%) and moderate completeness (70%). Single-copy genes in the Phormidium MAG were on small contigs, consistent with fragmentation of contigs due to high coverage and strain heterogeneity (37, 38), and they were classified taxonomically as various cyanobacteria, as expected based on the lack of available Phormidium genomes (Fig. S1). Thus, these MAGs likely contain contigs from multiple strains of Phormidium.

While community membership was dynamic across time and space, Phormidium was consistently the dominant organism in the MIS mats (Fig. S2). Other cyanobacteria were also abundant in the mat, including Planktothrix (formerly referred to as Oscillatoria in previous studies of MIS, but its 16S rRNA genes are most similar to those of Planktothrix agardhii and Planktothrix rubescens [39]), Pseudanabaena, and Spirulina. MAGs were also recovered for various Bacteroidetes, Betaproteobacteria, Chloroflexi, Delta proteobacteria, Epsilonproteobacteria, Firmicutes, Gammaproteobacteria, and Spirochaetes (Table S2). In most cases there were multiple MAGs recovered for each of these taxonomic groups. Many of these groups are commonly found in anoxic or hypoxic sediments (40, 41), and several are enriched in sediments below the mats at the MIS (29, 31).

To investigate which community members have metabolic pathways for mat biogeochemical processes, we searched the MAGs for key genes involved in carbon metabolism, nitrogen and sulfur cycling, oxygenic and anoxygenic photosynthesis, and other energy metabolisms. Cyanobacteria were the dominant phototrophs in terms of genomic abundance; Phormidium had a mean genomic coverage of over 200x, though based on high redundancy (56%), multiple strains are present (Table S2). While two putative diatom MAGs (Bin_3_1 and Bin_3_3) had low average coverages (0.31 and 6.19x), their chloroplasts were very abundant (up to 230x coverage), likely reflecting their high copy number per cell and easier assembly than the nuclear genome (Fig. S3). Marker genes of anoxygenic photosynthesis, including photosynthetic reactions center (pufM and pufL) and bacteriochlorophyll synthesis (bchB and bchL), were
present in *Rhodoferax* (Bin_4_1) and *Chloroflexi* (Bin_120) MAGs (Table S2). Diatoms and *Chloroflexi* are often associated with cyanobacterial mats; migratory diatoms play important roles in nitrogen cycling in MIS mats and sediments (35), and *Chloroflexi* engage in tight metabolic interactions with cyanobacteria (2, 3, 10, 42).

The MAGs of various proteobacteria revealed organisms involved in sulfur cycling. Key genes for dissipatory sulﬁte reductase (dsrA) involved in sulfate reduction were present in deltaproteobacterial genomic bins, including one unclassiﬁed Desulfovibrioles member, one unclassiﬁable Desulfobulibacteriales member, and one Desulfooccus member (Table S2). Based on their relatively high abundance, we infer that these sulfate-reducing bacteria were present directly in the cyanobacterial mat (43, 44) rather than the alternative that the sequences could be due to contamination of the mat by underlying sediments. Related sulfate-reducing bacteria are associated with anoxicogenic bacteria in Lake Mahoney (45), with cyanobacterial mats at Guerrero Negro (46, 47), and in nearby mats of chemolithotrophic sulfur oxidizers inﬂuenced by the same groundwater as MIS (48).

Potential for oxidation of elemental sulfur using reverse dissimilatory sulﬁte reductase (rdsrA) was detected in genomic bins of Arcobacter (Epsilonproteobacteria), several Betaproteobacteria, Thiobrix, and Thioploca and in unbinned scaffolds putatively belonging to Beggiatoa (Table S2). Thiobrix, Thioploca, and Beggiatoa are likely the white ﬁlamentous bacteria observed directly underneath the cyanobacterial mat (29) and in a nearby artesian fountain fed by the same groundwater (48). They can migrate on diel cycles and inﬂuence the balance of oxygenic versus anoxicogenic photosynthesis by modulating light available to phototrophs when covering the mat (33). These large sulﬁur-oxidizing bacteria likely contribute to the substantial rates of chemosynthesis measured previously (30) and likely inﬂuence cyanobacterial photosynthesis by consuming sulﬁde. Potential for thiosulfate oxidation, indicated by the presence of soxA, was observed in betaproteobacterial, deltaproteobacterial, and gammaproteobacterial bins (Table S2). Finally, the mmoC gene, for methane oxidation, was identiﬁed within a MAG classiﬁed as Methylococcales (Table S2).

Whole-community transcriptomics. Metatranscriptomic sequencing was conducted to investigate the *in situ* metabolic activity of the MIS mat community members. Although transcript abundance is not directly proportional to protein abundance or enzymatic activity, transcriptomics provides valuable insights into which community members and metabolic pathways are active at the time of sampling and their response to environmental conditions (49). In order to evaluate the inﬂuence of light availability on gene expression in MIS mats, three samples collected in 2012 at 1 p.m. and three collected at 1 a.m. were studied. In terms of relative abundance, transcripts mapped to MAGs from *Phormidium, Bacteroidetes, Thiiricaceae*, and the putative diatom dominated the metatranscriptome (Fig. S2; Table S2). Other signiﬁcant contributors (>1× mean coverage) to the transcript pool were bins from Paludibacter and other Bacteroidetes members, Rhodoferax (Beta-proteobacteria), Chloroflexii, Planktothrix, and a variety of unidentiﬁed bins (Table S2). Mapping of metatranscriptomic data to marker genes and MAGs provided a picture of the organisms responsible for metabolic/biogeochemical processes within the mat in day and night (Fig. 1; Table S2).

Transcripts involved in phototrophy. We next focused our transcriptomic analysis on key genes for photosynthesis. Core components of the reaction centers of PSI and PSII, encoded by *psaA* and *psbA* genes, respectively, are degraded at an enhanced rate compared to other proteins due to absorption of excess light energy from photosynthesis (50, 51). This leads to higher cellular demand for protein and likely explains the high abundance of transcripts we observed for these genes. The most abundant transcripts for *psaA* and *psbA* genes were from *Phormidium* and the diatom, with minor contributions from *Planktothrix, Spirulina*, and *Pseudanabaena* (Fig. 2). Included in our analyses were multiple versions of the cyanobacterial *psbA* genes, encoding the D1 subunit of PSII, which are expressed according to light and redox conditions (52, 53) and have been suggested to be involved in sulﬁde tolerance and/or anoxicogenic photosynthesis in cyanobacteria (54). *Phormidium* contained three of the four *psbA* types, and type 3 had the most transcripts (Fig. 2). This type of *psbA* is expressed during
**FIG 1** Detection of transcripts from marker genes of key metabolic/biogeochemical processes in MAGs (see Table S2 for details). Symbols are colored according to the time of day at which transcripts were detected: white, day; black, night; gray, both night and day. "X" indicates that the gene was observed in the MAG, but no transcripts were detected.

| MAG bin | C metabolism | O metabolism | N metabolism | Oxidative pathways | S cycling |
|---------|--------------|--------------|--------------|--------------------|-----------|
| Bacteria (Bin_7_3-contigs) | X | X | | | |
| Bacteria (bin_80) | X | X | X | X | |
| Bacteria (bin_182) | X | X | X | X | |
| Bacteria (bin_118) | | | | | |
| Betaproteobacteria (bin_91) | X | X | | | |
| Betaproteobacteria (bin_127) | X | X | | | |
| Candidatus Accumulibacter (bin_122) | X | X | X | X | |
| Rhodobacter (bin_229) | X | X | | | |
| Collimonas (bin_131) | X | X | | | |
| Hydrogenophaga (Bin_A-4-contigs) | X | X | | | |
| Methylosaccharitale (bin_45) | | X | X | X | |
| Desulfobacteraceae (bin_101) | X | X | | | |
| Halobacteriaceae (bin_209) | X | X | | | |
| Desulfofusca (bin_26) | X | X | X | X | |
| Sulfuricurvum (bin_49) | X | X | | | |
| Arcobacter (Bin_6_9-contigs) | X | X | | | |
| Sulfitomonas (bin_6_3-contigs) | X | X | | | |
| Spirulina (bin_256) | X | X | | | |
| Pseudomonas (bin_148) | X | X | | | |
| Planctomycetis (bin_253_243) | X | X | | | |
| Promidium (bin_11) | X | X | | | |
| Bacteroidetes (bin_69) | X | X | | | |
| Paludibacter (bin_13) | X | X | | | |
| Bacteroidetes (bin_92) | X | X | | | |
| Flavobacterium (bin_227) | X | X | | | |
| Bacteroidetes (bin_57) | X | X | | | |
| Bacteroidetes (bin_228) | X | X | | | |
| Bacteroidetes (bin_207) | X | X | | | |
| Bacteroidetes (bin_82) | X | X | | | |
| Bacteroidetes (bin_133) | X | X | | | |
| Bacteroidetes (bin_9) | X | X | | | |
| Spirochaetes (bin_199) | X | X | | | |
| Bacillariophyceae (Bin_3_3-contigs) | X | X | X | | |
| Bacillariophyceae (Bin_3_1-contigs) | X | X | X | X | |

**Period of observed activity**

- ○: Day
- ●: Night
- ■: Both
- ×: Inactive

**Function**

- Autotrophy (photoautotrophy)
- Phototrophy (photoautotrophy)
- Phototrophy (chemoheterotrophy)
- Nitrogen fixation (nifH)
- Methane oxidation (methanosarcina)
- Sulfate reduction (sulfate)
- Thiosulfate reduction (thiosulfate)
- Oxygen respiration (bchD, bchC, ammO, sod)
- Hydrogen oxidation (hox, mtr, sfo, hup)
- Methane oxidation (methanococcus,甲烷)
- Methane oxidation (methanobacter,甲烷)
- Sulfate reduction (sulfate)
- Thiosulfate reduction (thiosulfate)

**Biochemical Processes**

- C metabolism
- O metabolism
- N metabolism
- Oxidative pathways
- S cycling

**Microbial Geochemistry in a Low-O2 Cyanobacterial Mat**

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microaerobic and/or dynamic redox conditions (55, 56). The other cyanobacterium that was abundant in metagenomic data sets, *Planktothrix*, had type 3 and psbA genes, with similar relative abundance of transcripts in day and night but at much lower levels than *Phormidium*.

Twenty-one of 32 psaA and psbA genes had transcripts that were more abundant at night than in the day, although these differences were not statistically significant (Fig. 2). When normalized by the number of transcript reads recruited to each bin, which removes effects of transcriptomic variability across the whole community on transcript counts for genes within each bin, 9 of 21 genes had transcripts that were more abundant at night (Fig. S4). These patterns contrast those in several laboratory studies of cyanobacterial transcription, which found highest expression of photosynthesis reaction core genes during the day (57–62). Several field studies have also shown highest expression of PSII genes during the day (63–65). One possible explanation is that whereas many previous studies focused on oxygenic unicellular cyanobacteria that typically undergo rapid cell division (59, 66), *Phormidium* species are filamentous and typically much slower growing (0.07 to 0.5 day⁻¹, depending on light and nutrient availability) (67, 68). There is a precedent for high transcription of photosynthetic genes in the dark and lower transcription in the light; in *Synechococcus* sp. strain PCC 7002, transcript levels of several psbA genes were constant across several conditions, including light and dark (69).

Photosynthetic genes were among the most highly expressed genes in marine surface waters collected 3 h before sunrise (70). Photosystem I genes in thrombolites were constitutively expressed, with nearly even transcript abundance at midday and

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**FIG 2** Relative abundance of transcripts for photosystem genes normalized by total number of sequences in each sample. Log-transformed transcript abundance in the day (white) and night (gray) of genes encoding photosystem I (psaA) and photosystem II (psbA) is shown for each MAG (top) with box-and-whisker plots. Boxes represent the 25th to 75th percentiles, the inside line indicates the median, and whiskers extend to minimum and maximum values. Observations are overlaid as points. The x-axis labels “psbA_2,” “psbA_3,” and “psbA_4” refer to psbA types (see the text).
Anoxygenic phototrophs such as *Chloroflexi* and *Chlorobi* also express structural components of the photosynthetic apparatus at night (11, 71). Finally, our results could also be explained in part by decreased rates of afternoon photosynthesis that have been observed in cyanobacterial mats, including sharp drops at midday (72–74), which have been attributed to limitation of dissolved inorganic carbon (2). It should also be noted that transcript levels do not necessarily reflect protein abundance; under some circumstances, *psbA* messages accumulate without synthesis of the D1:2 protein (75, 76).

The abundance of transcripts obtained from *Phormidium* PSII genes indicates that genes for oxygenic photosynthesis were transcriptionally active at the time of sampling. However, *Phormidium* had on average more than two times higher abundance of transcripts for PSI genes than PSII genes (*psbA*-to-*psaA* ratio < 0.5) (Fig. 3). In contrast, the diatom chloroplast recruited more than 25 times more transcripts to PSII genes (*psbA*) than PSI genes (*psaA*). However, likely due to high variability of the abundance of diatom transcripts for these genes, this large difference in the ratio of transcript abundance from PSII and PSI genes was not statistically significant.

Other MIS cyanobacteria exhibited an intermediate ratio, with 2 to 7 times more PSII than PSI transcripts. A high ratio of PSII to PSI transcripts was also found in *Prochlorococcus*, a unicellular marine planktonic cyanobacterium (59). We infer that the higher relative abundance of PSI transcripts in *Phormidium* (and perhaps other MIS cyanobacteria) reflects transcriptional regulation, either via downregulation of PSII genes or upregulation of PSI genes, to conduct anoxygenic photosynthesis in the presence of sulfide. Although to our knowledge these are the first transcriptional data from anoxygenic cyanobacteria, they are consistent with the physiological shift toward PSII-independent anoxygenic photosynthesis that was reported previously (21, 27), with a decrease in the stoichiometry of PSII-PSI in response to sulfide (77), and with genes for anoxygenic photosynthesis being inducible via transcriptional regulation (24, 78, 79).

The stoichiometry of PSII-PSI can also be regulated according to light levels (80); the PSII/PSI ratio is lower at lower light levels, which also favors anoxygenic photosynthesis in the MIS mat system (33).
Sulfi de quinone oxidoreductase (SQR) transfers electrons from sulfi de to PSI during anoxygenic photosynthesis (24). Of the fi ve cyanobacterial sqr homologs recovered from the MIS community, the Phormidium SQR had the highest abundance of transcripts, from both its type I and II sqr genes (Fig. 4). The bin-normalized transcripts-per-million (TPM) value of Phormidium’s type II SQR was signifi cantly higher than that for SQRI ($P < 0.05$). While MAGs of Planktothrix and Pseudanabaena have SQRs (6), transcripts for these genes were not observed. The Phormidium SQRs showed transcript abundance comparable to that of the PSI genes psaL and psaX (Fig. S5). Little is known about how anoxygenic photosynthesis and sulfi de tolerance are regulated at the genetic level in cyanobacteria. Expression of type II SQR for sulfi de detoxifi cation in Synechocystis sp. strain PCC6803 (79) and type I SQR for anoxygenic photosynthesis in Geitlerinema sp. strain 9228 (24, 81, 82) is inducible by sulfi de. Both constitutive expression (25, 83, 84) and inducible expression (85, 86) of sqr have been observed in anoxygenic bacteria. There was little metatranscriptomic evidence of anoxygenic photosynthesis by anoxygenic bacteria (i.e., Chloroflexi or Betaproteobacteria). Genes for photosynthetic reaction cores (pufM and pufL) and bacteriochlorophyll (bchB and bchl) were not highly expressed, with 0 or 1 read mapped in all samples. Overall, these results suggest that the cyanobacteria are largely responsible for anoxygenic photosynthesis previously measured in MIS mats (30, 33).

The most highly expressed terminal oxidase for respiration in Phormidium was a cytochrome bd-type oxidase (Fig. S6), which has exceptionally high aﬃ nity for $O_2$, with a $K_m$ of 3 to 8 nM (87). The high transcriptional activity of this low-$O_2$ respiratory oxidase is consistent with adaptation to low-$O_2$ conditions for extended time periods.

**Transcripts involved in sulfur cycling and carbon fi xation.** Transcripts of seven diﬀ erent dsrA genes were observed, and the presence of dsrD on the same scaffold (Fig. S7) was used to confi rm inclusion of these genes in the dissimilatory sulfate reductase pathway (dsr genes). dsrD is useful a marker of sulfi de reduction because it is absent from organisms that use homologous rdsrA genes for sulfur oxidation (88, 89). Transcriptionally active reductive dsrA genes were present in seven MAGs representing
six genera of *Deltaproteobacteria*, with most transcripts coming from *Desulfococcus* (**Deltaproteobacteria**; **Desulfobacterales**), followed by *Desulfomicrobium* (Fig. 5). These results reveal the organisms responsible for active sulfate reduction within the cyanobacterial mat, which has been measured at high rates by $^{35}$SO$_4^{2-}$ tracer studies (32). For most sulfate-reducing MAGs, *dsrA* transcripts were detected and even more abundant during the day, suggesting sulfate reduction during the photosynthetic period and likely metabolic interactions with cyanobacteria via the cycling of sulfur and/or carbon (6, 90). These sulfate-reducing bacteria are also present in sediments underlying the cyanobacterial mat (31).

Seven *rdsrA* genes for sulfur oxidation were observed, including those in MAGs from three *Betaproteobacteria* (two unclassified and one member of the *Hydrogenophilales*), one classified only as proteobacteria, and one *Thiobacillus* organism (Fig. 5). Two unbinned genes most similar to *Thiolithrix* and *Beggiatoa* (*Gammaproteobacteria*) were also recovered. With the exception of the unbinned proteobacterial gene, all of these *rdsrA* genes had more transcripts at night. Transcripts from *soxA* genes for thiosulfate oxidation were detected, with those from *Rhodoferax* (*Betaproteobacteria*) and unbinned representatives of the *Campylobacterales* (*Epsilonproteobacteria*) having the highest abundance of transcripts (Fig. S8). Transcripts of genes for sulfide oxidation via flavocytochrome c sulfide dehydrogenase (fcc) were also observed in bins from the *Betaproteobacteria* and *Gammaproteobacteria*, though their sample-normalized transcript abundance was nearly an order of magnitude lower than those of *dsrA*.

To assess sources of primary production at MIS, we measured abundance of transcripts encoding key genes of four autotrophic pathways: ribulose-1,5-bisphosphate carboxylase/
oxygenase (RuBisCO) for the Calvin cycle (rbcL), ATP citrate lyase (aclB) for the reverse tricarboxylic acid cycle, CO dehydrogenase/acetyl coenzyme A (acetyl-CoA) synthase (acsB) for the Wood-Ljungdahl pathway, and malyl-CoA/(S)-citramalyl-CoA lyase (mcl), malonyl-CoA reductase/3-hydroxypropionate dehydrogenase, and 3-hydroxypropionyl-CoA dehydratase (mcr) for the 3-hydroxypropionate cycle (Fig. 6). RuBisCO had the highest transcript abundance of any autotrophic pathway. Phormidium, Planctothrix, and a diatom chloroplast actively transcribed rbcL, and the cyanobacteria especially were active at night. Thiotrichaceae also expressed rbcL but at substantially lower levels than the phototrophs. Several Desulfobacterales (Deltaproteobacteria) MAGs expressed the Wood-Ljungdahl genes, with higher expression
during the day. We did not observe transcriptional activity of aclB, mcl, or mcr genes in any samples.

Conclusions. Our data on taxon-specific genomic content and gene expression patterns provide insights into the microbial players and pathways that mediate biogeochemistry in anoxic cyanobacterial mats, which likely underpinned critical aspects of Earth’s geobiological evolution but have been understudied in the modern world. The metatranscriptomic data revealed that a *Phormidium autumnale*-like cyanobacterium previously found to be the dominant community member (29, 30) is also responsible for the majority of transcripts for photosynthesis, underscoring its essential role in the MIS ecosystem. Taken together, low expression ratios of PSII genes to PSI genes and expression of SQR indicate that *Phormidium* within MIS mats conducts anoxic photosynthesis with sulfide as the electron donor, consistent with previous geochemical measurements at the MIS (30, 33) as well as studies of other members of the Oscillatoriales (24, 91). Hence, the *Phormidium* population appears to be metabolically versatile, capable of both oxygenic and anoxic photosynthesis. It is not clear whether this phototrophic versatility stems from niche adaptation among closely related ecotypes (e.g., differential activity of strains that are oxygenic and anoxic specialists) or true cellular versatility in which *Phormidium* cells switch pathways depending on sulfide concentration. Further, our bulk sampling of mats was not sensitive to the vertical microgradients of sulfide concentration, so we are unable to evaluate potential vertical stratification of oxygenic/anoxicogenic photosynthesis within the mat.

This study provides a picture of how metabolisms encoding specific biogeochemical functions are partitioned among mat community members (Fig. 1). Primary production occurs via oxygenic photosynthesis by cyanobacteria and diatoms, anoxic photosynthesis by cyanobacteria (with minor contributions potentially from Chloroflexi and Betaproteobacteria), and chemosynthesis via sulfur oxidation by Thiotrichales and Chromatiales (Gammaproteobacteria) and Epsilonproteobacteria. Sulfide sets the stage for these metabolisms and is produced via sulfate and/or sulfur reduction by several different groups of Deltaproteobacteria both within the cyanobacterial mat and in underlying sediments. Bacteroidetes are the major heterotrophs, consuming organic carbon released as photosynthetic exudate or via viral lysis (92). Although the spatial arrangement of these processes within the mat remains unresolved, sulfide, oxygen, and organic carbon are likely rapidly cycled between the organisms producing and consuming them (1–4). Such tightly coupled interactions would help explain why there is little mat-derived carbon sequestered in the sediments (93). Tight coupling of O₂ production and consumption metabolisms, together with substantial primary production by anoxicogenic photosynthesis, also helps explain the limited net O₂ production by the cyanobacterial mat when measured in bulk (30). Overall, these findings emphasize the importance of microbial metabolic interactions in shaping biogeochemical processes in cyanobacterial mats under low-O₂ conditions, which dominated the long evolutionary history of cyanobacteria and played key but poorly understood roles in Earth’s major geobiological turning points.

MATERIALS AND METHODS

Sample collection and sequencing. This study used samples and metagenomic and metatranscriptomic sequence data produced as described by Voorhies et al. (92). Fifteen mat samples were collected by scuba divers from the R/V Storm between 2007 and 2012 from within a 100-m area of the Middle Island sinkhole (45.1984°N, 83.32721°W) by hand push cores of sediments, mat, and overlying groundwater (Table S1). Cores were rapidly transferred to the surface, and mats were separated from underlying sediments and submerged in RNAlater immediately shipboard. Less than 5 min elapsed between collection and preservation. Before preservation, mat samples were quickly washed with groundwater to remove as much sediment as possible. Mat structure ranged from conical structures we refer to as “fingers” (30) to prostrate mat. In May 2012, at the time of metagenomic and metatranscriptomic sequencing, conductivity, temperature, and dissolved O₂ in the overlying lake water as well as the groundwater above the mats were measured by a YSI 6600 multiparameter sonde.

DNA was extracted and processed for shotgun metagenomic sequencing as previously described (30). Samples were sequenced using an Illumina Hi Seq 2000 (paired end, 100 bp) instrument at the University of Michigan DNA Sequencing Core. In 2012, three samples of mat were collected at approximately 1 p.m. (day) and 1 a.m. (night) from within a 9-m² sampling area. RNA was extracted from these
six samples, randomly amplified with the MessageAmp II-Bacteria kit (Ambion), and converted to cDNA using the SuperScript double-stranded-cDNA synthesis kit (Invitrogen), as previously described (70). In the interest of cost efficiency and to minimize sample handling, rRNA was not removed (94, 95). cDNA was sequenced at the University of Michigan DNA Sequencing Core on an Illumina Hi Seq 2000 instrument producing paired-end reads.

Assembly and genomic analysis. In order to optimize assembly of genomes from low abundance members, a total of 922 million sequence reads from all 15 genomic DNA samples were combined and coassembled and binned by two different methods. We used IDBA-UD (96) for assembly and checked the results against previous assemblies of MIS mats that used multiple sequencing platforms and assembly programs, including a previously published metagenome based on 454 (28, 30), and assemblies of Illumina data from individual samples performed with Velvet (97). Specifically, the assembly was checked by verifying recovery of key genes and MAGs that were observed in the previous assemblies and by manual curation using the Integrated Genome Viewer (98) and Geneious (99) to visualize reads mapped to contigs and genes by BWA (Burrows-Wheeler aligner) (100) and look for signs of misassembly (e.g., discontinuities in coverage). Multiple strategies and software were used to generate metagenome-assembled-genome (MAG) bins. The first strategy used CONCOCT (101) to automate binning by differential coverage and tetranucleotide frequency for the subset of contigs that were 5 kb and larger. The resulting bins were refined manually in anv’i’o (102, 103) and assigned taxonomy via Centrifuge (104) and CheckM (105). Likely due to high coverage and putative strain heterogeneity (37, 38), the 12.5-Mbp MAG bin representing the dominant cyanobacterium *Phormidium* had high completion (95.0%) but poor contamination (97.1%) metrics (106) (Table S2). For this bin as well as 6 other cyanobacterial MAG bins from the initial refinement, 6,011 contigs that were previously unbinned due to their short length (1,000 bp to 4,999 bp) were assigned to bins on the basis of similar coverage, nucleotide composition (tetranucleotide frequencies), and taxonomy via manual refinement in anv’i’o.

We also employed a second, purely automated binning strategy for comparison. EukRep (107) removed euukaryotic contigs from the data set, and MetaBAT (108) used differential coverage and tetranucleotide frequency to generate MAGs from contigs 1,500 bp and longer. We again used CheckM to taxonomically identify MAG bins and tracked 16S rRNA, psbA, and *psbA* genes from the previously extracted cyanobacterial bins to identify their counterparts in the MetaBAT bins. Contigs previously assigned to *Phormidium* were poorly binned in this method. dRep (109) was used to pick the best representative bin from the two methods. Though 10 of the bins from CONCOCT+anv’i’o were retained through dRep, the MetaBAT-generated bins were more often picked because they generally had lower estimates of contamination and strain heterogeneity. Putative *Phormidium* scaffolds that were not binned by MetaBAT were manually evaluated in anv’i’o, and retained as the representative *Phormidium* bin in this analysis. Gene calling and functional annotation was performed by the Joint Genome Institute’s Integrated Microbial Genomes Expert Review portal (https://img.jgi.doe.gov/cgi-bin/main.cgi) (110).

Coverage of contigs by cDNA and DNA sequence reads from each sample was assessed by mapping reads to contigs using BWA (100) with default settings. Raw counts of cDNA reads (referred to here as counts) for each predicted protein-coding gene were determined using the IMG-derived coordinates of gene start and stop sites, along with the mapping information. rRNA genes erroneously called as protein-coding genes were identified by BLASTn against the SILVA SSU and LSU database, release 123 (111), and removed. The python script HTSeq.scripts.count from HTSeq (112) extracted transcript counts that unambiguously mapped to genes. In targeted searches for metabolic genes of interest, we identified 20 partial "genes" that were not suitable lengths when IMG-determined start and stop sites were used and were of appropriate lengths when partial "genes" were incorporated immediately upstream or downstream. Thus, for metatranscriptomic analyses, the counts of these partial genes were merged.

For analysis of metatranscriptomic data, only genes with at least two counts were considered. Two different normalization methods were used to analyze the metatranscriptomic data, depending on the question. First, transcript abundance was normalized by total mRNA reads recovered in each sample to calculate relative abundance of transcripts at the gene level. This metric is a function of both the organism abundance and expression per gene copy and provides a measure of total contribution to the transcript pool for each gene. Second, to compare relative gene expression within genomic bins (and remove the effect of dynamic community-wide transcript and organism abundance), we normalized relative abundance of transcripts by number of mRNAs mapped to each genome bin. To account for variability in sequencing effort between samples and for the impact of gene and read lengths, gene expression levels were normalized using TPM (113). To evaluate differences in expression levels between organisms of assembled metagenomic bins, TPM for bin-specific genes were also calculated with the denominator consisting of only reads recruited to the bin of interest. Statistical testing was conducted on the sample- and bin-normalized TPM of genes in RStudio using Kruskal-Wallis nonparametric tests and paired t tests, corrected with a Benjamin-Hochberg false discovery rate (q) of 0.05.

Data availability. Sequences from this study are available from NCBI under BioProject no. PRJNA72255. Reads from all 15 metagenomes and 6 metatranscriptomes are available in NCBI’s Sequence Read Archive (Table S1). Accession numbers for MAGs that passed NCBI quality filtering are provided in Table S2.

**SUPPLEMENTAL MATERIAL**

Supplemental material is available online only.

**FIG S1**, PDF file, 0.3 MB.

**FIG S2**, PDF file, 0.2 MB.
REFERENCES

1. Paerl HW, Pinckney JL, Steppe TF. 2000. Cyanobacterial-bacterial mat consortia: examining the functional unit of microbial survival and growth in extreme environments. Environ Microbiol 2:11–26. https://doi.org/10.1111/j.1462-2920.2000.00071.x.

2. Stal LJ. 2012. Cyanobacterial mats and stromatolites, p 65–125. In Whitten BA (ed), Ecology of cyanobacteria: II. their diversity in space and time. Springer, Heidelberg, Germany.

3. Nold S, Ward D. 1996. Photosynthate partitioning and fermentation in hot spring microbial mat communities. Appl Environ Microbiol 62: 4598–4607. https://doi.org/10.1128/aem.62.12.4598-4607.1996.

4. Stal LJ, Kirschvink JL, Fischer WW. 2016. Timescales of oxygenation following the evolution of oxygenic photosynthesis. Orig Life Evol Biosph 46:51–65. https://doi.org/10.1007/s11084-015-9460-3.

5. Dick GJ, Grim SL, Klett JM. 2018. Controls on O₂ production in cyanobacterial mats and implications for Earth’s oxygenation. Annu Rev Earth Planet Sci 46:123–147. https://doi.org/10.1146/annurev-earth-082517-010035.

6. Sanchez-Baracaldo P, Bianchini G, Wilson JD, Knoll AH. 2021. Cyanobacteria and biogeochemical cycles through Earth history. Trends Microbiol https://doi.org/10.1016/j.tim.2021.05.008.

7. Hoehler TM, Bebout BM, Des Marais DJ. 2001. The role of microbial mats in the production of reduced gases on the early Earth. Nature 412: 324–327. https://doi.org/10.1038/35085554.

8. Falkowski PG, Isozaki Y. 2008. The story of O₂. Science 322:540–542. https://doi.org/10.1126/science.1162641.

9. Burrow LC, Woebken D, Marshall JRG, Lindquist EA, Bebout BM, Prüft-Bebout L, Hoehler TM, Tringe SG, Pett-Ridge J, Weber PK, Spormann AM, Singer SW. 2013. Anoxic carbon flux in photosynthetic microbial mats as revealed by metatranscriptomic analysis. ISME J 7:817–829. https://doi.org/10.1038/ismej.2012.150.

10. Klett CG, Liu ZF, Klatt JM, Haas SL, Skidmore DJ, Bryant DA, Ward DM. 2013. Temporal metatranscriptomic patterning in phototrophic Chloroflexi inhabiting a microbial mat in a geothermal spring. ISME J 7:1775–1789. https://doi.org/10.1038/ismej.2013.52.

11. Harris JK, Caporaso JG, Walker JJ, Spear JR, Gold NJ, Robertson CE, Hugenholtz P, Goodrich J, McDonald D, Knights D, Marshall P, Tufo H, Knight R, Pace NR. 2013. Phylogenetic stratigraphy in the Guerrero Normal Hot Springs microbial mat. ISME J 7:50–60. https://doi.org/10.1038/ismej.2012.79.

12. Hansen TE, Luther GW, Findlay AJ, MacDonald DJ, Hess D. 2013. Photo- trophic sulfide oxidation: environmental insights and a method for kinetic analysis. Front Microbiol 4:382. https://doi.org/10.3389/fmicb.2013.00382.

13. Hamilton TL, Bryant DA, Macalady JL. 2016. The role of biology in planetary evolution: cyanobacterial primary production in low-oxygen Proterozoic oceans. Environ Microbiol 18:325–340. https://doi.org/10.1111/1462-2920.13118.

14. Olson JM. 2006. Photosynthesis in the Archean Era. Photosynth Res 88: 109–117. https://doi.org/10.1007/s11120-006-9040-5.

15. Blankenship R, Sadak R, Raymond J. 2007. The evolutionary transition from anoxygenic to oxygenic photosynthesis, p 21–33. In Falkowski PG, Knoll AH (ed), Evolution of primary producers in the sea. Elsevier, Amsterdam, The Netherlands.

16. Fischer WW, Hemp J, Johnson JE. 2016. Evolution of oxygenic photosynthesis. Annu Rev Earth Planet Sci 44:647–683. https://doi.org/10.1146/annurev-earth-060313-054810.

17. Hamilton TL. 2019. The trouble with oxygen: the ecophysiology of extant phototrophs and implications for the evolution of oxygenic photosynthesis. Free Radic Biol Med 140:233–249. https://doi.org/10.1016/j.freeradbiomed.2019.05.003.

18. Johnston DT, Wolfe-Simon F, Pearson A, Knoll AH. 2009. Anoxic photosynthesis modulated Proterozoic oxygen and sustained Earth’s middle age. Proc Natl Acad Sci U S A 106:16925–16929. https://doi.org/10.1073/pnas.0909248106.

19. Ozaki K, Thompson KJ, Simister RL, Crowe SA, Reinhard CT. 2019. Anoxygenic photosynthesis and the delayed oxygenation of Earth’s atmosphere. Nat Commun 10:3026. https://doi.org/10.1038/s41467-019-10872-x.

20. Cohen Y, Jørgensen BB, Revsbech NP, Poplawski R. 1986. Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. Appl Environ Microbiol 51:398–407. https://doi.org/10.1128/aem.51.2.398-407.1986.

21. Klatt JM, Haas S, Yilmaz P, Beer D, Polerecky L. 2015. Hydrogen sulfide can inhibit and enhance oxygenic photosynthesis in a cyanobacterium from sulfidic springs. Environ Microbiol 17:3301–3313. https://doi.org/10.1111/1462-2920.12791.

22. Arieli B, Shahak Y, Taglicht D, Hauska G, Padden E. 1994. Purification and characterization of sulfide-quinone reductase, a novel enzyme driving anoxygenic photosynthesis in Oscillatoria-Limnetica. J Biol Chem 269: 5705–5711. https://doi.org/10.1074/jbc.269.9.5705.

23. Bronstein M, Schutz M, Hauska G, Padden E, Shahak Y. 2000. Cyanobacterial sulfide-quinone reductase: cloning and heterologous expression. J Bacteriol 182:3336–3344. https://doi.org/10.1128/JB.182.13.3336-3344.2000.

24. Schütz M, Shahak Y, Padden E, Hauska G. 1997. Sulfide-quinone reductase from Rhodobacter capsulatus. Purification, cloning, and expression. J Biol Chem 272:9890–9894. https://doi.org/10.1074/jbc.272.15.9890.

25. Miller SR, Bebout BM. 2004. Variation in sulfide tolerance of photosystem II in phylogenetically diverse cyanobacteria from sulfidic habitats. Appl Environ Microbiol 70:736–744. https://doi.org/10.1128/AEM.70.2.736-744.2004.

26. Jørgensen BB, Cohen Y, Revsbech NP. 1986. Transition from anoxygenic to oxygenic photosynthesis in a Micrococcus chthonoplastes cyanobacterial mat. Appl Environ Microbiol 51:408–417. https://doi.org/10.1128/aem.51.2.408-417.1986.
Grim et al.

28. Ruberg SA, Kendall ST, Biddanda BA, Black T, Nold SC, Lusardi WR, Green R, Caserley T, Smith E, Sanders TG, Lang CA, Constant SA. 2008. Observations of the Middle Island sinkhole in Lake Huron—a unique hydrogeologic and glacial creation of 400 million years. Mar Technol Soc J 42:12–21. https://doi.org/10.4031/002533208787157633.

29. Nold SC, Pangborn JB, Zajack HA, Kendall ST, Rediske RR, Biddanda BA. 2010. Benthic bacterial diversity in submerged sinkhole ecosystems. Appl Environ Microbiol 76:347–351. https://doi.org/10.1128/AEM.01869-09.

30. Vorhies AA, Biddanda B, Kendall ST, Jain S, Marcus DN, Nold SC, Sheldon ND, Dick GJ. 2012. Cyanobacterial life at low O2: community genomics and function reveal metabolite versatility and extremely low diversity in a Great Lakes sinkhole mat. Geobiology 10:250–267. https://doi.org/10.1111/j.1462-2495.2012.00322.x.

31. Kinsman-Costello L, Sheik CS, Sheldon ND, Burton GA, Costello D, Marcus DN, Den Uyl PA, Dick GJ. 2017. Groundwater shapes sediment biogeochemistry and microbial diversity in a submerged Great Lake sinkhole. Geobiology 15:225–239. https://doi.org/10.1111/j.1462-2495.2012.001215.

32. Gomes ML, Klatt JM, Dick GJ, Grimal SL, Rico KI, Medina MJ, Ziebis W, Kinsman-Costello LE, Sheldon ND, Fike DA. 2021. Sedimentary pyrite sulfur isotopic compositions preserve signatures of the surface microbial mat environment in sediments underlying low-oxygen cyanobacterial mats. Geobiology https://doi.org/10.1111/j.1462-2495.2012.001246.

33. Klatt JM, Chennu A, Abic BK, Biddanda BA, Dick GJ. 2021. Possible link between Earth’s rotation rate and oxygenation. Nat Geosci 14:564–570. https://doi.org/10.1038/s41561-021-00969-3.

34. Snider MJ, Biddanda BA, Lindback M, Grimal SL, Dick GJ. 2017. Versatile photophysiology of compositionally similar cyanobacterial mat communities inhabiting submerged sinkholes of Lake Huron. Aquat Microb Ecol 79:63–78. https://doi.org/10.1139/ame01813.

35. Merz E, Dick GJ, de Beer D, Grimal S, Hubener T, Littmann S, Olsen K, Stuart D, Lavik G, Marchant HK, Klatt JM. 2021. Nitrate respiration and diel migration patterns of diatoms are linked in sediments underneath a microbial mat. Environ Microbiol 23:1432–1435. https://doi.org/10.1111/1462-2920.15345.

36. Bowers RM, Kyprides NC, Stepanauskas R, Harmon-Smith M, Doud D, Reddy TBK, Schulz F, Jaret J, Rivers AR, Eloe-Fadrosh EA, Tringe SG, Ivanova NN, Copeland A, Becraft ED, Malmstrom RR, Birren B, Gilbert JA, Desai N, Glass EM, Paczian T, Wilke A, Antonopoulos D, Ettema TJG, Tighe S, Konstantinidis KT, Liu WT, Baker BJ, Rattei T, Eisen JS, Sutton G, Glockner FO, Gilbert JA, Nelson WC, Hallam SJ, Jungbluth SP, Iwanov NN, Campioni A, Podar M, Bork P, Weinstock GM, Garrity GM, Dodsworth JA, Yooseph S, Reddy TBK, Schulz F, Jarett J, Rivers AR, Eloe-Fadrosh EA, Tringe SG, Cardona T. 2015. A fresh look at the evolution and diversification of cyanobacteria. Nat Genet 47:1298–1305. https://doi.org/10.1038/ng.3389.

37. Hug LA, Thomas BC, Sharon I, Brown CT, Sharon R, Hettich RL, Wilkins MJ, Williams KH, Singh A, Banfield JF. 2016. Critical biological functions in the subsurface are associated with bacteria from new phyla and functional gene sets. Environ Microbiol Rep 3:277–281. https://doi.org/10.1111/1758-2229.2016.00221.x.

38. Stockel J, Jacobs JM, Elvitigala TR, Liberton M, Welsh EA, Polpitiya AD, Jonkers HM, Ludwig R, Wit R, Pringault O, Muyzer G, Niemann H, Finke N, Beer D. 2003. Structural and functional analysis of a microbial mat ecosystem from a unique permanent hypersaline inland lake: ‘La Salada de Chiparana’ (NE Spain). FEMS Microbiol Ecol 44:175–189. https://doi.org/10.1016/S0168-6496(02)00464-6.

39. Camfield DE, Des Marais DJ. 1991. Aerobic sulfate reduction in microbial mats. Science 251:1471–1473. https://doi.org/10.1126/science.1153266.

40. Sharrar AM, Flood BE, Bailey JV, Jones DS, Biddanda BA, Ruberg SA, Marcus DN, Dick GJ. 2017. Novel large sulfate bacteria in the metagenomes of groundwater-fed chemosynthetic microbial mats in the Lake Huron basin. Front Microbiol 8:791. https://doi.org/10.3389/fmicb.2017.00791.

41. Marcus DN, Kemner KM, Meyer F. 2014. The complete genome sequence for putative Synechococcus sp. in community composition of sediment bacteria in large and shallow lake. Geobiology https://doi.org/10.1038/nbt.3893.

42. Marcus DN, Dick GJ. 2017. Novel large sulfur bacteria in the metagenomes of groundwater-fed chemosynthetic microbial mats in the Lake Huron basin. Geobiology 15:225–239. https://doi.org/10.1038/ismej.2008.39.

43. Fike DA, Finke N, Zha N, Blake G, Hoehler TM, Orphal VJ. 2009. The effect of sulfate concentration on (sub)millimeter-scale sulfide dS435 in hypersaline cyanobacterial mats over the diurnal cycle. Geochim Cosmochim Acta 73:6187–6204. https://doi.org/10.1016/j.gca.2009.07.006.

44. Zehr JP, Glibko WP, III, Lyons TW, Tomsho LP, Schuster SC, Overmann J, Bryant DA, Pearson A, Macalady JL. 2014. Coupled reductive and oxidative sulfur cycling in the phototrophic plate of a meromictic lake. Geobiology 12:451–468. https://doi.org/10.1111/gbi.12092.

45. Wright MA, Rector T, Steen R, McNulty N, Thompson LR, Chisholm SW. 2006. Microscope-scale mapping of sulfur cycling across the oxygen of a cyanobacterial mat: a paired nanoSIMS and CARD-FISH approach. ISME J 2:749–759. https://doi.org/10.1038/ismej.2008.39.

46. Fike DA, Finke N, Zha N, Blake G, Hoehler TM, Orphal VJ. 2009. The effect of sulfate concentration on (sub)millimeter-scale sulfide dS435 in hypersaline cyanobacterial mats over the diurnal cycle. Geochim Cosmochim Acta 73:6187–6204. https://doi.org/10.1016/j.gca.2009.07.006.

47. Marcus DN, Kemner KM, Meyer F. 2014. The complete genome sequence for putative Synechococcus sp. in community composition of sediment bacteria in large and shallow lake. Geobiology https://doi.org/10.1038/nbt.3893.
Microbial Geochemistry in a Low-O₂ Cyanobacterial Mat

79. Nagy CI, Vass I, Rakhely G, Vass IZ, Toth A, Duzs A, Peca L, Kruk J, Kos P. 2021. Regulation of photosystem I and II genes in light-dark- and continuous-light-grown cultures of the unicellular cyanobacterium Synechocystis sp. strain ATCC 51142. J Bacteriol 180:519–526. https://doi.org/10.1128/JB.180.3.519-526.1998

80. Fujiyama Y, Murakami A, Aizawa K, Ohki K. 1994. Short-term and long-term adaption of the photosynthetic apparatus: homeostatic properties of thylakoids, p. 677–692. In Bryant DA (ed), The molecular biology of Cyanobacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.

81. Arieli B, Binder B, Shahak Y, Padan E. 1989. Sulfide induction of synthesis of a periplasmic protein in the cyanobacterium Oscillatoria limnetica. J Bacteriol 171:699–702. https://doi.org/10.1128/JB.171.2.699-702.1989

82. Straub C, Quillardet P, Vergalli J, de Marsac NT, Humbert JF. 2011. A day in the life of Microcystis aeruginosa strain PCC 7806 as revealed by a transcriptomic analysis. PLoS One 6:e161208. https://doi.org/10.1371/journal.pone.0016120.

83. Jensen SL, Steunou AS, Bhaya D, Kuhl M, Grossman AR. 2011. In situ dynamics of O₂, pH and cyanobacterial transcripts associated with CCM, photosynthesis and detoxification of ROS. ISME J 5:317–328. https://doi.org/10.1038/ismej.2010.131.

84. Lousyakisa, AS, Gourle H, Casaburi G, Bonjawa RME, Duscher AA, Foster JS. 2018. A year in the life of a thylomblate: comparative metatranscriptomics reveals dynamic metabolic changes over diel and seasonal cycles. Environ Microbiol 20:842–861. https://doi.org/10.1111/1462-2920.14109.

85. Hornlein C, Confurius-Guns V, Stal LJ, Krumbein WE. 1990. Interactions between nitrogen-fixation activity and photosynthesis in the cyanobacterium Synechococcus sp PCC 7942. Physiol Plant 84:3430–3440. https://doi.org/10.1111/j.1399-3054.1990.tb01005.x

86. Straub C, Quillardet P, Vergalli J, de Marsac NT, Humbert JF. 2011. A day in the life of Microcystis aeruginosa strain PCC 7806 as revealed by a transcriptomic analysis. PLoS One 6:e161208. https://doi.org/10.1371/journal.pone.0016120.
103. Eren AM, Kieff E, Shaiber A, Veseli I, Miller SE, Schechter MS, Fink I, Pan JN, Yousef M, Fogarty EC, Trigodet F, Watson AR, Eisen OC, Moore RM, Clayssen Q, Lee MD, Kivenson V, Graham ED, Merrill BD, Karkman A, Blankenberg D, Eppley JM, Sjodin A, Scott JJ, Vazquez-Campos X, McKay LJ, McDaniel EA, Stevens SLR, Anderson RE, Fuessel J, Fernandez-Guerra A, Maignien L, Delmont TO, Willis AD. 2021. Community-led, integrated, reproducible multi-omics with anvi’o. Nat Microbiol 6:3–6. https://doi.org/10.1038/s41564-020-00834-3.

104. Kim D, Song L, Breitwieser FP, Salzberg SL. 2016. Centrifuge: rapid and sensitive classification of metagenomic sequences. Genome Res 26:1721–1729. https://doi.org/10.1101/gr.210641.116.

105. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055. https://doi.org/10.1101/gr.186072.114.

106. Campbell JH, Olm MR, Brown CT, Brooks B, Banfield JF. 2017. dRep: a tool for fast and accurate genomic comparisons that enables improved genome recovery from metagenomes through de-replication. ISME J 11:2864–2868. https://doi.org/10.1038/ismej.2017.126.

107. West PT, Probst AJ, Grigoriev IV, Thomas BC, Banfield JF. 2018. Genome-reconstruction for eukaryotes from complex natural microbial communities. Genome Res 28:569–580. https://doi.org/10.1101/gr.228429.117.

108. Kang DD, Froula J, Egan R, Wang Z. 2015. MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities. PeerJ 3:e1165. https://doi.org/10.7717/peerj.1165.

109. Quast C, Pflueger E, Pruesse E, Tiedje JM, Yilmaz P, Gerken J, Schleifer T, Harz S, Peplies J, Glockner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596. https://doi.org/10.1093/nar/gks1219.

110. Anders S, Pyl PT, Huber W. 2015. HTSeq—A Python framework to work with high-throughput sequencing data. Bioinformatics 31:166–169. https://doi.org/10.1093/bioinformatics/btu638.

111. Wagner GP, Kin K, Lynch VJ. 2012. Measurement of mRNA abundance using RNA-seq data: RPKM measure is inconsistent among samples. Theory Biosci 131:281–285. https://doi.org/10.1007/s12064-012-0162-3.