Geobiology

Geobiology (2012), **10**, 250–267 DOI: 10.1111/j.1472-4669.2012.00322.x

Cyanobacterial life at low O_2: community genomics and function reveal metabolic versatility and extremely low diversity in a Great Lakes sinkhole mat

A. A. VOORHIES,[1] B. A. BIDDANDA,[2] S. T. KENDALL,[2] S. JAIN,[1,3] D. N. MARCUS,[1] S. C. NOLD,[4] N. D. SHELDON[1] AND G. J. DICK[1,3,5]

[1]*Deptartment of Earth and Environmental Sciences, University of Michigan, Ann Arbor, MI, USA*
[2]*Annis Water Resources Institute, Grand Valley State University, Muskegon, MI, USA*
[3]*Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI, USA*
[4]*Biology Department, University of Wisconsin-Stout, Menomonie, WI, USA*
[5]*Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA*

ABSTRACT

Cyanobacteria are renowned as the mediators of Earth's oxygenation. However, little is known about the cyanobacterial communities that flourished under the low-O_2 conditions that characterized most of their evolutionary history. Microbial mats in the submerged Middle Island Sinkhole of Lake Huron provide opportunities to investigate cyanobacteria under such persistent low-O_2 conditions. Here, venting groundwater rich in sulfate and low in O_2 supports a unique benthic ecosystem of purple-colored cyanobacterial mats. Beneath the mat is a layer of carbonate that is enriched in calcite and to a lesser extent dolomite. *In situ* benthic metabolism chambers revealed that the mats are net sinks for O_2, suggesting primary production mechanisms other than oxygenic photosynthesis. Indeed, ^{14}C-bicarbonate uptake studies of autotrophic production show variable contributions from oxygenic and anoxygenic photosynthesis and chemosynthesis, presumably because of supply of sulfide. These results suggest the presence of either facultatively anoxygenic cyanobacteria or a mix of oxygenic/anoxygenic types of cyanobacteria. Shotgun metagenomic sequencing revealed a remarkably low-diversity mat community dominated by just one genotype most closely related to the cyanobacterium *Phormidium autumnale*, for which an essentially complete genome was reconstructed. Also recovered were partial genomes from a second genotype of *Phormidium* and several *Oscillatoria*. Despite the taxonomic simplicity, diverse cyanobacterial genes putatively involved in sulfur oxidation were identified, suggesting a diversity of sulfide physiologies. The dominant *Phormidium* genome reflects versatile metabolism and physiology that is specialized for a communal lifestyle under fluctuating redox conditions and light availability. Overall, this study provides genomic and physiologic insights into low-O_2 cyanobacterial mat ecosystems that played crucial geobiological roles over long stretches of Earth history.

Received 26 May 2011; accepted 11 January 2012

Corresponding author: G. J. Dick. Tel.: 734 763 3228; fax: 734 763 4690; e-mail: gdick@umich.edu

INTRODUCTION

Cyanobacteria mediated Earth's oxygenation and thus played a central role in geochemical and biological evolution. They are widely recognized as the innovators of oxygenic photosynthesis, in which water provides electrons for photosynthesis and O_2 is released as a by-product (Blankenship *et al.*, 2007). This cyanobacterial metabolism is thought to have driven a significant increase in atmospheric O_2 concentration ~2.4 billion years ago known as the great oxidation event (GOE) (Bekker *et al.*, 2004). Conversely, recent work also suggests that cyanobacteria capable of anoxygenic photosynthesis may have subsequently perpetuated an extended low-O_2 phase of Earth's history (Johnston *et al.*, 2009). This intermediate stage of Earth's redox history lasted for at least a billion years and was characterized by a low-O_2 atmosphere and redox-stratified oceans, where sulfide–O_2 interfaces would have been prevalent (Johnston *et al.*, 2009; Lyons *et al.*, 2009). Despite the large portion of cyanobacterial evolution that occurred while O_2 was scarce, and the critical geobiological turning points that occurred under such conditions (Falkowski *et al*., 2008; Johnston *et al*., 2009), little is known about the genetic or physiological characteristics of cyanobacteria that thrive under persistent sulfide-rich and/or O_2-limited conditions.

Modern cyanobacteria exhibit a range of physiologies in the presence of sulfide. Most are highly sensitive to sulfide because of irreversible blockage of the H_2O-splitting component of photosystem II (Cohen *et al*., 1986; Miller & Bebout, 2004). However, cyanobacteria inhabiting anoxic or hypoxic environments that are regularly exposed to sulfide have developed strategies for sulfide tolerance and even utilization (Castenholz, 1976, 1977; Garlick *et al*., 1977; Oren *et al*., 1977; Bühring *et al*., 2011). Cohen *et al*., (1986) described several adaptations to sulfide, ranging from sulfide-resistant oxygenic photosynthesis to the ability to utilize sulfide as the electron donor for anoxygenic photosynthesis. Different cyanobacterial species sharing the same oxic/anoxic interfacial environment often exhibit distinct sulfide physiologies, with different sulfide optima and tolerance (Garlick *et al*., 1977; Jorgensen *et al*., 1986). Such cyanobacteria that are capable of tolerating sulfide or using it for anoxygenic photosynthesis are phylogenetically diverse and spread throughout the phylum Cyanobacteria (Miller & Bebout, 2004).

Despite the phylogenetically widespread nature of anoxygenic photosynthesis among the cyanobacteria, the biochemical mechanisms of this process and its genetic underpinnings have been studied in just a few cyanobacterial strains, primarily *Geitlerinema* sp. PCC 9228 (formerly *Oscillatoria limnetica*). When confronted with sulfide in the presence of light, this organism rapidly switches from oxygenic to anoxygenic photosynthesis by an inducible process that requires protein synthesis (Cohen *et al*., 1975b; Oren & Padan, 1978). Photosystem I receives electrons from sulfide and transfers them to the electron transport chain to drive proton pumping (Belkin & Padan, 1978), and extracellular globules of elemental sulfur are generated as an end-product (Cohen *et al*., 1975a). Biochemical and genetic methods have identified genes encoding the enzyme that oxidizes sulfide, sulfide quinone reductase (SQR), which transfers electrons from sulfide to the quinone pool (Arieli *et al*., 1994; Schütz *et al*., 1997; Bronstein *et al*., 2000). In *Geitlerinema* sp. 9228, these sulfide-derived electrons are used for photosynthesis or nitrogen fixation, whereas in the cyanobacterium *Aphanothece halophytica* and the yeast *Schizosaccharomyces pombe*, SQR is thought to oxidize sulfide for the purpose of detoxification (Bronstein *et al*., 2000).

Investigation of modern cyanobacteria inhabiting low-O_2 environments can provide insights into the biological processes that influenced Earth's oxygenation. Particularly relevant to understanding the rise of O_2 on Earth are questions surrounding the evolution of anoxygenic photosynthesis in the cyanobacteria, mechanisms by which versatile cyanobacteria regulate oxygenic vs. anoxygenic photosynthesis, and factors that affect competition between versatile cyanobacteria and other anoxygenic bacteria. Many studies have focused on stratified cyanobacterial mat communities where O_2 and sulfide concentrations fluctuate on diel cycles; often cyanobacteria are exposed to sulfidic conditions at night and oxic conditions during the day (Richardson & Castenholz, 1987). However, few studies have focused on cyanobacteria that thrive under persistent low-O_2 conditions.

Here, we investigate cyanobacterial mats inhabiting such a persistently low-O_2 environment, the submerged Middle Island Sinkhole (MIS) in Lake Huron (Fig. 1). Groundwater that gently vents into the MIS bottom (water depth 23 m) has significantly different physical and chemical properties than Lake Huron water, with a lower temperature (7–9 vs. 4–25 °C), lower pH (~7.1 vs. 8.3), lower concentrations of dissolved oxygen (0–2 vs. 5–11 mg L^{-1}), lower oxidation–reduction potential (−134 vs. 500 mV), and higher specific conductivity (~2.3 vs. 0.3 mS cm^{-1}) (Biddanda *et al*., 2009). The high conductivity of venting groundwater is attributable to high concentrations of dissolved sulfate (1250 mg L^{-1}), carbonate (48 mg L^{-1}), and chloride (25 mg L^{-1}) ions derived from interactions with subsurface Devonian evaporites (Black, 1983; Ruberg *et al*., 2008). This dense groundwater forms a thin (~1 m), visibly stratified benthic layer that persists perennially except for brief disruptions because of major storms (Ruberg *et al*., 2008). The low-O_2 conditions of the groundwater inhibit typical Lake Huron biological communities, favoring purple-colored cyanobacterial mats with finger-like protrusions (Fig. 2) that have not been found elsewhere in the Great Lakes (Biddanda *et al*., 2009). Beneath the mats are stratified microbial layers of sulfide-oxidizing bacteria, sulfate-reducing bacteria, and methanogens (Nold *et al*., 2010a). Molecular diversity studies (Nold *et al*., 2010a) have revealed that they are dominated by cyanobacteria remarkably similar (>98% 16S rRNA gene sequence identity) to *Phormidium autumnale* isolates from the Arctic and Antarctic (Taton *et al*., 2006a,b; Comte *et al*., 2007), and host to distinctive archaeal and eukaryotic communities (Nold *et al*., 2010b). Furthermore, carbon, nitrogen, and sulfur stable isotopic signatures of sinkhole chemistry have been detected in the surrounding environment and food web (Sanders *et al*., 2011).

Little attention has been paid to the potential for the MIS mats as analogs for ancient microbial mat ecosystems. Microbial mats are widespread throughout the Precambrian rock record, often as carbonate-associated stromatolites (Grotzinger & Knoll, 1999). In addition to their low-O_2 habitat and facultatively anoxygenic metabolism (Biddanda *et al*., 2009), several other features of the MIS mats make them excellent and novel analogs of Precambrian cyanobacterial mats (Biddanda *et al*., in press). The MIS mats are bathed in groundwater that is constantly cold (7–9 °C year-long) and thus representative of low-temperature stromatolite settings that were common in the Paleoproterozoic (Walter & Bauld,

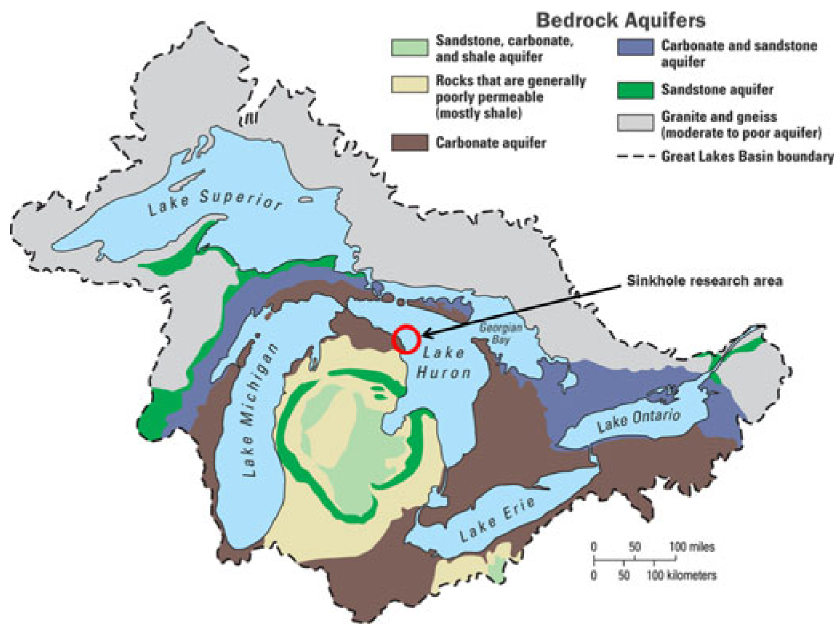

Fig. 1 Location map of the study area and geologic map of bedrock aquifers of the Great Lakes Basin. The focus of this study is the Middle Island Sinkhole, one of many submerged karst sinkholes in the Thunder Bay National Marine Sanctuary, Lake Huron (modified from Ruberg *et al.*, 2008; Biddanda *et al.*, 2009).

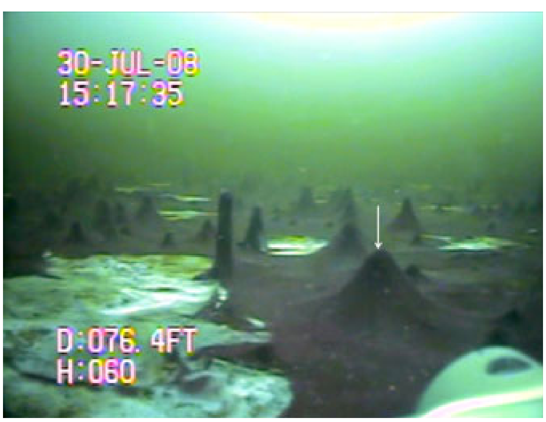

Fig. 2 Remotely operated vehicle image of the sinkhole bottom, showing cyanobacterial mats, including purple-colored prostrate mat and raised conical structures we refer to as 'fingers', and exposed white areas that have lost cyanobacterial mat cover. 'Fingers' average 10–15 cm in height; an example is indicated in the figure by a white arrow. Photo credit: R. Paddock and V. Klump, University of Wisconsin-Milwaukee, WI, USA.

1983; Kopp *et al.*, 2005). Sulfate concentrations are intermediate between freshwater and seawater (Ruberg *et al.*, 2008), similar to those of the Proterozoic oceans (Shen *et al.*, 2003). Underlying the cyanobacterial mat layer is a mineral layer thought to be rich in carbonate (Nold *et al.*, 2010a). Finally, the raised finger-like mat features at MIS (Fig. 2) are similar to the conical mat structures produced by *P. autumnale* in an Antarctic lake, which were recently highlighted as analogs of stromatolites (Andersen *et al.*, 2011). Here, we present genomic and functional insights into the MIS cyanobacterial mats and highlight their value as novel analogs of ancient anoxygenic phototrophic ecosystems.

METHODS

Field work and sampling

Field work was conducted in the spring, summer, and fall from May 2007 to June 2009 at the MIS (N 45.19843°N, W 083.32721°W) near Alpena, MI (Fig. 1). Water samples were collected from above and below the near-bottom chemocline (lake water and groundwater, respectively) by divers using Niskin bottles and then dispensed into 10 L collapsible poly cubitainers. Sediment cores with intact mats, underlying sediments, and overlying water were hand collected by divers using plexiglas tubes (7.5 cm dia. × 20 cm tall). Cores were capped with rubber stoppers and kept upright in a core rack that was raised to the surface. Water and cores were kept in iced coolers in the field and then refrigerated in the laboratory. Water was stored at 4 °C and cores were maintained at *in situ* temperatures of ~9.5 °C. A subset of sediment cores were extruded and sectioned for microscopy, isotopic, and mineralogical analysis, according to visual cues in the sediment profile, including thin (0–0.2 cm) prostrate cyanobacterial mat, the underlying mineral-rich layer (0.2–0.5 cm), and sections of the thick organic-rich sediment. Sediment samples were stored in 2-mL plastic tubes. The 'finger' used for metagenomics was collected on June 14, 2007, separated from the sediment, and transferred to a 50-mL polypropylene tube. Once collected, all samples were stored frozen at −20 °C until processing.

Microscopic studies of mat structure and composition

Filamentous cyanobacteria from the surface of the mats were gently suctioned into eye droppers, fixed with 2% formaldehyde, imaged by differential interference contrast (DIC) with a Nikon eclipse 80i microscope (Nikon Metrology Inc., www.nikonmetrology.com), and photographed with a QIClick digital camera (Qimaging, Surrey, BC, Canada). To obtain a cross-sectional image of the mat-sediment continuum, intact mats were carefully peeled from the surface of sediment cores and placed on a Petri dish over a thin layer of groundwater. Portions of the mat were sectioned and photographed using a Nikon SMZ-2T Binocular Microscope equipped with a Micropublisher 5.0 RTV QImaging digital camera.

Benthic chamber studies of dissolved oxygen

Benthic metabolic studies were performed as time series using custom diver-deployed acrylic benthic chambers to evaluate potential for *in situ* net O_2 production via oxygenic photosynthesis and net O_2 consumption via respiration. Each chamber consisted of a tube (21 cm dia. × 50 cm length), adjustable plastic collar, and a removable cap equipped with an YSI 6920 sonde (Fig. 3). Divers first pushed the tube/collar through the water column and into the mat/sediments, ensuring the tube contained representative groundwater and not any overlying lake water. The collar allowed precise control over chamber volume and sensor position before clamping the cap onto the chamber tube. These procedures allowed us to measure metabolic processes *in situ* without disturbing the intact microbial communities with overlying groundwater and underlying sediments. Sondes were configured to record data every hour for each sensor including temperature, conductivity, pH, oxidative–reductive potential, and O_2. From the measured changes in dissolved O_2, estimates of photosynthesis and respiration of carbon were made using either a photosynthetic quotient of 1.0 or a respiratory quotient of 1.0, respectively (Biddanda *et al.*, 1994). Typically, triplicate light and dark (covered with opaque dark plastic sheets) chambers were deployed for a period of 24–48 h and changes in dissolved oxygen tracked as an index of net carbon metabolism. During chamber studies (June 14–15, 2007; July 24–26, 2007;

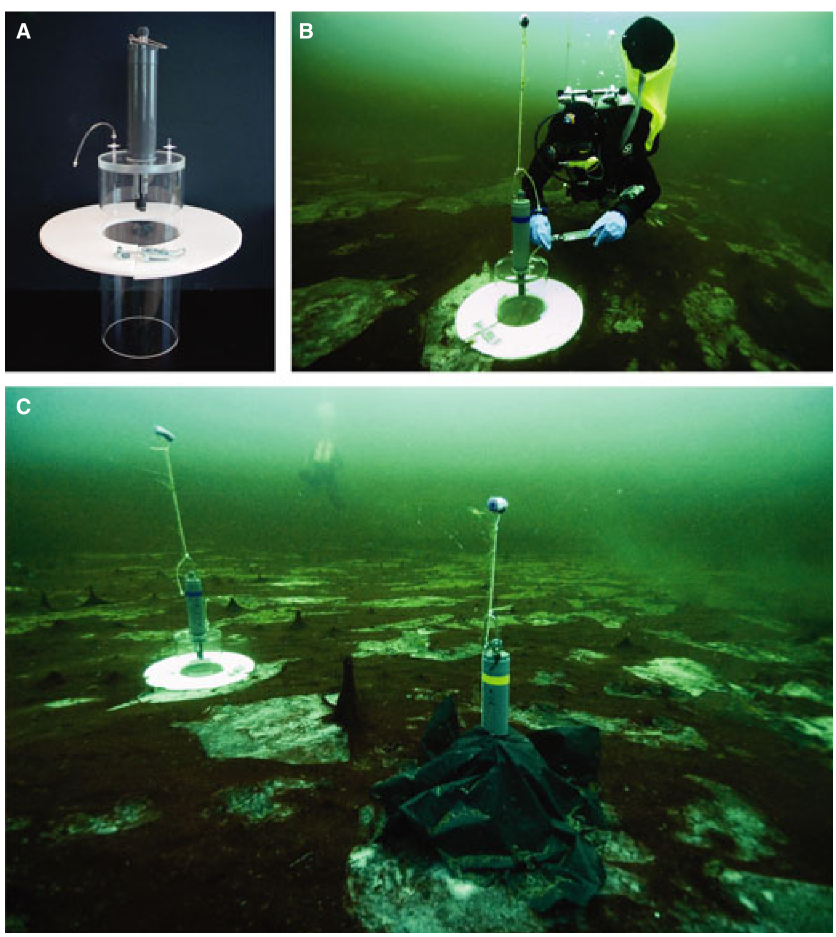

Fig. 3 (A) Custom benthic metabolism chamber equipped with YSI-sonde sensors for dissolved oxygen, temperature, and conductivity. (B) Light and (C) light and dark chamber deployments at the Middle Island Sinkhole (Photo credit: T. Casserly, NOAA, USA).

August 14–15, 2007; September 18–19, 2008), hourly PAR measurements at the mat surface were recorded from a LICOR LS-193 spherical bulb to a Nexsens SDL-500 data logger. Chamber deployment studies were conducted five times during 2007 (May 17–18; June 14–15; July 24–26; August 11–14; August 14–15), twice during 2008 (June 18–19; September 3–5), and once during 2009 (June 3–5 2009).

Autotrophic process measurements by ^{14}C bicarbonate uptake

Laboratory ^{14}C-bicarbonate uptake experiments were conducted on mats from sediment cores within 24 h of collection using two methods: (i) mats were left intact with sediments in cores to simulate processes occurring at the mat/sediment interface in the sinkhole (e.g., sulfide production), and (ii) mats were peeled away from the surface of the sediment cores and homogenized in groundwater prior to incubation to simulate conditions of oxic groundwater. ^{14}C-sodium bicarbonate was added at a final specific activity of 2 μCi mL^{-1}, and *in vitro* experiments were performed under simulated *in situ* conditions of temperature and light for 6–8 h under the following conditions: (i) light vs. dark treatments to distinguish between photosynthesis and dark chemosynthesis, and (ii) treatments with and without DCMU, an inhibitor of photosystem II and hence oxygenic photosynthesis (Pedros-Alio *et al.*, 1993; Biddanda *et al.*, 2006). Each treatment had a parallel 'killed' treatment where the samples were pre-treated with 2% formaldehyde for 3 h prior to label spike and incubation. At the end of the incubations, any unassimilated inorganic carbon was liberated from the samples by acidification with 1N HCL for 16 h, and the radioactivity of the remaining assimilated organic alone was determined in a LS6500 Liquid Scintillation Counter (Beckman Coulter, Brea, CA, USA). Killed controls accounted for 2–10% of the radiolabel found in live samples. All autotrophic production estimates (oxygenic photosynthesis, anoxygenic photosynthesis, and chemosynthesis) were performed after correcting for the radioactivity in parallel killed controls (Biddanda *et al.*, 2006; Casamayor *et al.*, 2008). Incubations were conducted in a temperature-controlled dry incubator at ~9.5 °C, the average temperature of the groundwater at MIS. Light source was provided by a 75 watt 120 volt Sylvania Halogen lamp with a light output of 1100 lumens and regulated by one layer of blue film and one neutral density filter to approximate the light climate available at the bottom of the MIS (~5% of surface irradiance) (Ruberg *et al.*, 2008; Biddanda *et al.*, 2009).

Stable isotope analyses

Samples (n = 5) collected from a microbial 'finger' were placed in a freeze-dryer for 24 h and then decarbonated in weak HCl (2% solution) and re-dried. The resulting powders were weighed on a microbalance (~150 μg) and placed in tin capsules. The capsules were combusted in a Costech elemental analyzer attached to a Delta V+ isotope-ratio mass spectrometer for isotopic analysis. Results were calibrated using standards IAEA600 and IAEA-CH-6 and are reported as $\delta^{13}C_{org}$ values in per mil notation relative to the VPDB scale. Analytical precision was maintained at better than 0.1‰ during the run.

X-ray diffraction (XRD)

Samples (n = 18) were collected as a depth profile through a cyanobacterial mat into the underlying sediments to a depth of 23.5 cm. X-ray diffraction (XRD) spectra were measured on powders using a 2.2 kW Cu-Kα Rigaku Ultima IV XRD (40 kV, 44 mA beam) with a Theta/Theta wide angle goniometer from 2 to 70° with a 0.05 2θ step size. Measurements of peak position were made using PDXL software (Rigaku Americas, The Woodlands, TX, USA).

DNA extraction, genome sequencing, annotation, and phylogenetic analyses

DNA was extracted from 1 g of mat material using the Fast DNA spin kit for soil (MP Biomedicals), a Fastprep-24 Bead Beater (MP Biomedicals, Solon, OH, USA), and a DNA Clean and Concentrator-5 kit (Zymo Research, Irvine, CA, USA) according to the manufacturer, except that only 0.3 g of beads were used for bead beating. DNA was quantified using the Quant-IT PicoGreen dsDNA reagent and kit (Invitrogen, Grand Island, NY, USA) and submitted to the University of Michigan DNA Sequencing Core for one plate of 454 Titanium pyrosequencing. Genomic assembly was performed using MIRA (Chevreux *et al.*, 2004), and contigs binned using emergent self-organizing maps (ESOM) of tetranucleotide frequency patterns, whereby contiguous sequences were chopped into 5 kb sequences for which tetranucleotide frequencies were calculated and then clustered by ESOM (Dick *et al.*, 2009). Further binning was performed manually using BLAST. Annotation was performed through the Joint Genome Institute's (JGI) Integrated Microbial Genomes Expert Review (IMG-ER) portal (http://img.jgi.doe.gov/cgi-bin/w/main.cgi), where gene and protein sequences are publicly available (see Table S2 for accession numbers). Nucleotide sequences were submitted to GenBank under BioProject ID PRJNA72255.

Unless noted otherwise, all BLAST analyses were performed using an e-value cutoff of 1e-5. Genes for sulfur oxidation were identified through BLAST with queries described in detail in Table S1 (Supporting Information). Universally conserved genes used to evaluate genome completeness were identified via BLAST with cutoffs of 1e-30 and 60% sequence identity.

Sequences for phylogenetic analysis were aligned with ClustalW (Larkin *et al.*, 2007). Phylogenetic analysis was performed with MEGA 4 (Tamura *et al.*, 2007) for minimum evolution and maximum parsimony trees and RAxML

(Stamatakis, 2006) for maximum likelihood trees. All three approaches were used and found to yield consistent results for each phylogenetic analysis. All trees were bootstrapped 5000 times; only bootstrap values >70 are reported on the trees.

RESULTS AND DISCUSSION

Mat structure and microscopy

The benthic environment of the MIS is impacted by hypoxic, saline groundwater where dense cyanobacterial mats thrive (Fig. 2). In addition to prostrate purple mats, there are occasional white patches where the cyanobacteria are not growing, exposing sediment or white layers of the microbial mat, as well as variably shaped features (conical to columnar) of raised cyanobacterial mat, which we designate 'fingers' (Fig. 2). These fingers contain gas bubbles of methane and sulfide derived from microbial metabolism in underlying sediments. The structures are similar to those observed in ice-covered Antarctic lakes, where they have been observed to 'lift-off' because of buoyant microbial gases (Wharton *et al.*, 1983; Hawes & Schwarz, 1999; Cowan & Tow, 2004; Andersen *et al.*, 2011).

Microscopic examination of the MIS mats showed predominately filamentous cells with straight, unbranched trichomes lacking heterocysts that fall into two main groups on the basis of trichome width: ~12- to 16-μm-thick trichomes and ~6-μm-thin trichomes (Fig. 4). Sheaths have been observed in both types but their presence appears to be variable. Thick trichomes are consistently observed with rectangular cells that are shorter than one-half cell width and terminate with rounded apical cells (Fig. 4C), common of the genus *Oscillatoria*. Thin trichomes have cell shapes that are less consistent but are typically longer than their width and terminate with rounded apical cells (Fig. 4B) common in the genus *Phormidium* (Vincent, 2000; Komarek *et al.*, 2003). Despite advances made using modern genotypic and phenotypic approaches, there remains considerable uncertainty regarding the classification of these cyanobacteria at the species level (Palinska, 2007; Strunecky *et al.*, 2010). Although trichomes of *Phormidium* sp. and *Oscillatoria* sp. were the most abundant, we have observed other types of less common cyanobacterial filaments that are spiral-shaped and tall-celled (Fig. 4A). Their identities are unclear because of lack of coverage in both the metagenome and clone libraries performed previously (Nold *et al.*, 2010a). Clearly, additional molecular and taxonomic studies are needed.

Under the fluorescence microscope, the filamentous cyanobacteria autofluoresce purple–red when exited by green light, suggesting the presence of phycobiliproteins. Vertical cross-section of the mat and sediment revealed a thick layer of woven cyanobacterial trichomes over a white crystalline sediment matrix interspersed with unidentified white filamentous bacteria (Fig. 4D). Trichomes exhibited remarkable motility under light, being able to rapidly re-aggregate or climb over small pebbles in minutes or hours. When placed in darkness, the white filaments were observed to migrate to the mat surface, consistent with behavior of mat-associated sulfur-oxidizing chemosynthetic bacteria. Determining whether such diel migrations occur *in situ* at MIS and the biogeochemical consequences of any such spatio-temporal dynamics is worthy of future investigation.

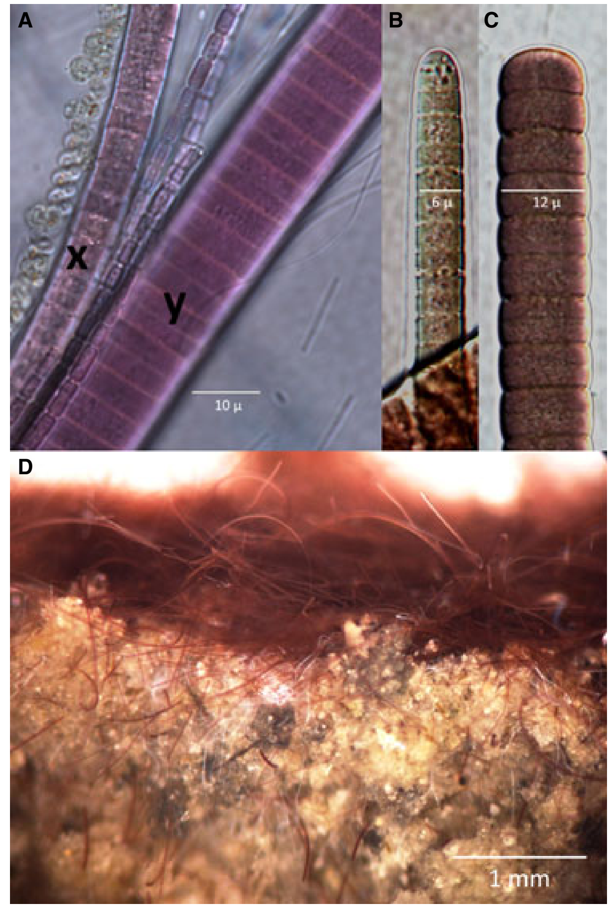

Fig. 4 (A) Bright-field microscope images of dominant thin (x) and thick (y) cyanobacterial trichomes of *Phormidium* sp. and *Oscillatoria* sp., respectively. The spiral-shaped filaments and tall-celled filaments are far less common and have not been identified. (B) and (C) show details of rounded apical cells in *Phormidium* sp. and *Oscillatoria* sp. trichomes, respectively. (D) Cross-sectional stereo microscopic image of purple microbial mat at the Middle Island Sinkhole showing layers of motile purple cyanobacterial trichomes on top and motile white filamentous sulfur-oxidizing bacteria and carbonate crystals below. Underlying dark organic sediment is not pictured.

Carbon metabolism and respiration

Two approaches were taken to investigate carbon metabolism in the MIS mats. First, benthic metabolism chambers were deployed *in situ* under light and dark conditions (Fig. 3) to measure changes in dissolved O_2 as an index of O_2 production/respiration. Second, laboratory experiments were performed on mat/sediment cores to track autotrophic

^{14}C-bicarbonate incorporation into biomass. Five benthic chamber studies conducted in 2007 and 2008 consistently showed there was net consumption of O_2, with dissolved O_2 decreasing similarly in both light and dark treatments (Fig. 5). However, the rates of net O_2 consumption were significantly different (paired t-test, $P = 0.025$), being ~30% greater in the dark (~17 mg C m^{-2} day^{-1}) than in the light (~12 mg C m^{-2} day^{-1}) (Table 1). This net consumption of O_2 shows that any O_2 produced via oxygenic photosynthesis is quickly consumed either by aerobic respiration or by reduction with sulfide (chemical or chemosynthetic) (Fig. S2). Such tight coupling of O_2 production and consumption has been observed in cyanobacterial mats previously (Canfield & Des Marais, 1993). Calculations based on these observed rates of O_2 consumption and using a respiratory quotient of 1.0 suggest that ~17 mg C m^{-2} day^{-1} of autotrophic carbon synthesis is required to balance the system (Table 1). These results also indicate an excess of carbon synthesis relative to O_2 production and suggest that primary production mechanisms that do not produce O_2, such as anoxygenic photosynthesis and chemosynthesis, play significant roles in the carbon balance of the MIS habitat (Fig. S2).

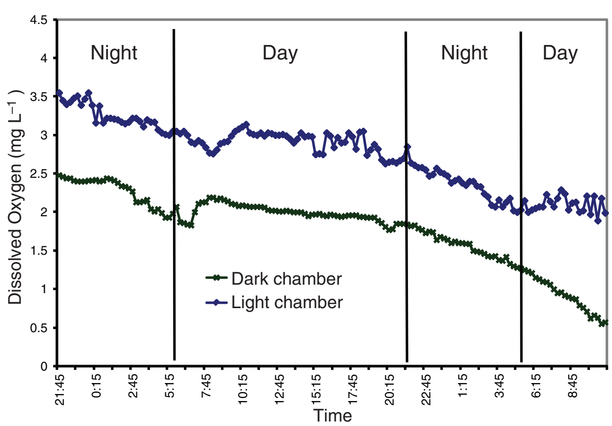

Fig. 5 Dissolved O_2 concentration measured in benthic metabolism chambers over a 36-h period (July 24–26, 2007). Changes in dissolved O_2 concentration in light and dark chambers (both decreasing at nearly identical slopes) suggest that alternative production mechanisms such as anoxygenic photosynthesis and chemosynthesis must be prevalent at sinkholes to explain how such a prolific mat community could sustain itself. Nearly identical rates and trends were measured in different years from 2006 to 2009.

Table 1 Carbon consumption estimated from decline in dissolved oxygen in benthic metabolism chambers

Date of benthic chamber study (mg C m^{-2} day^{-1})	Light chambers (mg C m^{-2} day^{-1})	Dark chambers
June 14–15, 2007	7	11
July 24–26, 2007	11	14
August 11–14, 2007	12	17
August 14–15, 2007	11	22
September 18–19, 2008	19	22
Mean (SD)	12.0 (4.4)	17.2 (4.8)

^{14}C-bicarbonate incorporation studies of mats in laboratory incubations were used to further assess contributions to primary production from oxygenic photosynthesis, anoxygenic photosynthesis, and chemosynthesis. Experiments were conducted under two conditions designed to assess the effect of sulfide and O_2 on autotrophic production processes by the mats: (i) mats were left intact with sediments (sulfide present; low O_2), and (ii) mats were removed from the sediment and suspended in groundwater (sulfide absent; high O_2). For the intact cores, primary production was dominated by anoxygenic photosynthesis and chemosynthesis, and no oxygenic photosynthesis was detected (Table 2). In contrast, oxygenic photosynthesis was the main mode of primary production in the oxygenated mat suspension. Interestingly, if we take the difference in net O_2 consumption between day and night benthic chambers (5.2 mg C m^{-2} day^{-1}) as a rough estimate of oxygenic photosynthesis (Table 1), this rate is approximately matched by the measured rate of oxygenic photosynthesis in ^{14}C tracer studies (4.0 mg C m^{-2} day^{-1}) – albeit under oxygenated and sulfide-free conditions (Table 2). Based on these findings, we draw the following conclusions. First, the mats are metabolically versatile, being capable of significant primary production through oxygenic or anoxygenic photosynthesis or chemosynthesis. Second, the balance of oxygenic vs. anoxygenic photosynthesis depends on the presence of sulfide and/or O_2. Third, measured rates of anoxygenic photosynthesis and chemosynthesis of ~17 mg C m^{-2} day^{-1} from ^{14}C tracer studies of intact sediment cores are collectively sufficient to satisfy the carbon-deficit of ~17 mg C m^{-2} day^{-1} estimated from O_2 consumption observed in the benthic chamber studies (Tables 1 and 2) – suggesting that the carbon cycle in the MIS mat is well balanced (Fig. S2). In terms of the quantity of primary production, rates of anoxygenic photosynthesis and chemosynthesis measured in the MIS mat–sediment complex are comparable to those made for aquatic microbial mat communities in Solar Lake, Israel, and other similar mat-dominated habitats (Jorgensen *et al.*, 1979, 1983; Revsbech *et al.*, 1983; Cohen *et al.*, 1986; Overmann *et al.*, 1991; Stal, 2000; Bachar *et al.*, 2007; Casamayor *et al.*, 2008; Fontes *et al.*, 2011).

Microbial 'finger' $\delta^{13}C_{org}$ values ranged from −27.25 to −28.43‰, with a mean of −27.88‰ (±0.47 1σ). The C wt. %

Table 2 Autotrophic production processes in intact MIS cyanobacterial mat with sediment (cores) and mat trichomes in groundwater (suspension)

Production process	Intact cores (low/no O_2) (mg C m^{-2} day^{-1})	Mat suspension (oxygenated) (mg C L^{-1} day^{-1})
Oxygenic PS	Not detected	4.0 (2.3)
Anoxygenic PS	8.4 (2.4)	0.9 (0.4)
Chemosynthesis	8.6 (2.1)	0.2 (0.03)
Total autotrophic production	17	5.1

Mean ± (1 SD) n = 3.
MIS, Middle Island Sinkhole.

was variable within a relatively narrow range and averaged 44.88%. Groundwater in the MIS system has a DIC isotopic composition of −4.1‰ (Sanders *et al.*, 2011), so fractionation of carbon by the mat because of photosynthesis was about −24‰. These $\delta^{13}C_{org}$ values are consistent with typical autotrophic carbon fixation by the Calvin-Benson cycle (which is present in the MIS-Ph1 genome – see below). While cyanobacterial systems in nature may display a wide array of $\delta^{13}C_{org}$ values, both the mean value of the Lake Huron mats themselves and the degree of C fractionation are consistent with previous results from both marine and freshwater cyanobacterial systems (Schidlowski, 2000). Thus, while there are relatively few Precambrian lacustrine systems that have been recognized, results from this system are also relevant for understanding similar marine settings with photic-zone benthic microbial mats. The MIS mat $\delta^{13}C_{org}$ values are consistent with reported terrestrial Precambrian organic matter (Imbus *et al.*, 1992; Horodyski & Knauth, 1994; Retallack & Mindszenty, 1994; Rye & Holland, 2000). Most Precambrian kerogen (fossilized organic matter that is insoluble in solvents) ranges between −25 and −40‰ (Pavlov *et al.*, 2001), where the lightest values are from systems thought to have been influenced by methanotrophy. Microbial decomposition of C in sediments typically changes $\delta^{13}C_{org}$ values by +2‰ or more relative to the original value of the organic matter (Walter *et al.*, 2007), so if organic matter from a Precambrian system similar to the MIS ($\delta^{13}C_{org}$ value of −27.9‰) was buried, the preserved signal would be near the heavy end of the Precambrian kerogen range, appropriately representing photosynthetic systems without significant methanotrophic influence. Precambrian microbial mats are most often recognized on the basis of gross morphological features (e.g., stromatolites) or of microbially induced sedimentary structures (Noffke, 2009; Sheldon, in press), neither of which are obvious in the MIS system. Our results showing an anoxygenic cyanobacterial mat with $\delta^{13}C_{org}$ values that are indistinguishable from those of oxygenic cyanobacteria highlight the difficulty in relating $\delta^{13}C_{org}$ values to specific metabolic or biogeochemical function. Further work is needed on the isotopic and elemental composition of the carbonates in the system, and on the $\delta^{15}N$ values of the mats, to determine whether the combined isotopic results will provide a biosignature for similar systems in the geologic record.

Identification of minerals associated with mats and underlying sediments

The unique geochemical setting of the MIS mats presents opportunities to investigate cyanobacterial calcification under conditions relevant to the Precambrian, which may inform long-standing questions regarding the distribution of calcified stromatolites through geologic time (Grotzinger & Knoll, 1999; Riding, 2006). The MIS mats are not lithified, but a carbonate-rich layer just beneath the mat has been observed (Nold *et al.*, 2010a). XRD identified quartz, calcite, and dolomite as the three major mineral phases associated with the mat and underlying sediments. For the purposes of comparing normalized abundance, we present the ratio of XRD primary peak intensity of calcite ($2\theta = 29.5°$) and dolomite ($2\theta = 31°$) to quartz ($2\theta = 26.7°$). This calcite/quartz ratio (C/Q) ranged with depth in mat/sediment from 0.39 to 2.31, with a mean of 0.57 (±0.12 1σ; excluding the high value). The layer immediately beneath the mat is characterized by a > 14σ increase in the C/Q ratio, indicating a prominent enrichment of calcite and confirming the presence of a carbonate-rich sedimentary layer immediately beneath the cyanobacterial mat (Nold *et al.*, 2010a). The dolomite/quartz ratio (D/Q) ranged from 0.58 to 1.12 with a mean of 0.79 (±0.13 1σ). The D/Q ratio was elevated both deep in the core and near the surface, including a > 2 σ enrichment immediately underneath the cyanobacterial mat, at the same level as the calcite enrichment.

There are several possible mechanisms by which the microbial mat and/or sediment communities may influence carbonate precipitation in this environment. First, cyanobacteria produce extracellular polymeric substances (EPS), which can nucleate carbonate mineralization and influence the type of mineral produced (Riding, 2006, 2011). Heterotrophic bacteria have also been shown to catalyze carbonate formation through heterogeneous nucleation (Bosak & Newman, 2003). Second, carbonate precipitation can be promoted by increases in alkalinity driven by photosynthesis or heterotrophic sulfate reduction (Dupraz *et al.*, 2009). In particular, carbon concentration mechanisms possessed by the dominant MIS organism (see 'carbon acquisition and metabolism' section below) are thought to induce calcification (Riding, 2011). Sulfate reduction observed in the carbonate-rich layer of MIS has been linked to sulfate-reducing bacteria (Nold *et al.*, 2010a), and interestingly, the cyanobacteria themselves also show some evidence for sulfur reduction (see below) and thus could play a role in carbonate precipitation through this mechanism. However, it is unclear whether sulfur/sulfate metabolism is directly involved in carbonate precipitation in the MIS sinkhole, and the relevance of this process under Precambrian conditions is also questionable (Bosak & Newman, 2003). The formation of dolomite in modern, freshwater, low-temperature settings is rare, but microbial mediation of dolomite precipitation has also been linked to sulfate reduction (Vasconcelos *et al.*, 1995; Warthmann *et al.*, 2000; Van Lith *et al.*, 2003) and as well as methanogenesis (Kenward *et al.*, 2009). Both of these metabolisms are active in MIS sediments but further investigation is required to determine the specific chemical and biological factors that influence the formation of the observed calcite and dolomite.

Metagenomic sequencing, assembly, and binning

To explore the genetic diversity and metabolic capability of the MIS microbial mat, community genomic DNA of a mat finger was shotgun sequenced, producing 827 593 DNA sequencing reads that assembled into 19 463 contiguous sequences (contigs) (Table 3). 16S rRNA gene sequences in this metagenome were dominated by two genera of cyanobacteria, *Phormidium* and *Oscillatoria*. The dominant 16S rRNA gene sequence (38× average coverage) is closely related to *P. autumnale*-like sequences retrieved previously from prostrate MIS mats and from a variety of Arctic and Antarctic environments (Fig. 6) (Nold *et al*., 2010a). Of the 45 sequences obtained from the MIS mat previously, 31 came from the same dominant *Phormidium* operational taxonomic unit (OTU), indicating that this organism dominates both prostrate and raised mats in the MIS. Also present in the MIS metagenome were four contigs (2–13 × coverage) with 16S rRNA gene sequences that cluster tightly with *Planktothrix rubescens* CCAP 1459-14, *Oscillatoria agardhii*, and *Oscillatoria* sp. 49. A partial *Bacteroidetes*-like 16S rRNA (2× genomic coverage) was also recovered.

To better understand the genetic potential underpinning the physiology and metabolism of the different MIS mat community members, contigs were assigned to taxonomic groups, or 'genomic bins', based on ESOM of tetranucleotide frequency (Dick *et al*., 2009). Two bins were apparent, one containing contigs with the *Phormidium* 16S rRNA gene (MIS-Ph1), and the other containing the *Oscillatoria* 16S contigs. Contigs in the *Phormidium* bin showed a bimodal distribution of genomic coverage with approximately half at 38× coverage and half at <10× coverage; we designate these as two separate bins, MIS-Ph1 and MIS-Ph2, respectively. The MIS-Ph2 bin contains a partial 16s rRNA gene that is identical to the 16S rRNA gene from MIS-Ph1; thus, we infer that these bins represent two closely related but distinct genotypes, one high abundance and one low abundance. The *Oscillatoria* bin contains contigs from at least four different genotypes that are present at similar abundance (2–10 × coverage); we refer to these populations collectively as MIS-Os. The 4.6 Mb of DNA recovered in this bin represent partial genomes of the *Oscillatoria* populations. Seventy percent of the total DNA sequence reads assembled into the dominant MIS-Ph1 genotype, and just 17% of DNA sequence reads fell outside of the dominant *Phormidium* and *Oscillatoria* organisms (Table 3). These results show that MIS mats have remarkably low species and genomic diversity, containing just a few dominant cyanobacteria and several lower abundance bacteria.

Putative genes for anoxygenic photosynthesis

To investigate the potential for anoxygenic photosynthesis among members of the MIS mat community, we searched the metagenome for genes known to be involved in sulfur oxidation. Genes with sequence homology to SQR were found in the genomic bins of all three dominant mat cyanobacteria as well as in unassigned contigs (Table 4; accession numbers and annotations of all genes discussed in the text are provided in Table S2). The *Oscillatoria* bin contains an SQR with 56% amino acid identity to the SQR from the cyanobacterium *Geitlerinema* sp. PCC 9228, which is involved in sulfide-dependent anoxygenic photosynthesis (Bronstein *et al*., 2000). The *Phormidium* and unassigned bins contain homologs of SQR that are much more divergent. Four genes with limited sequence similarity to SQR in the unassigned contigs are most closely related to oxidoreductases of unknown function from *Oscillatoria* sp. 6506 and *Lyngbya* sp. PCC 8106 (15–25% amino acid identity). These unassigned SQR genes are on contigs with very low genomic coverage (1–2×) and thus derive from very low abundance organisms, so it is highly unlikely that they contribute significantly to the anoxygenic photosynthesis reported in Table 2. A phylogenetic tree of SQR shows that the MIS-Os SQR falls into a well-resolved cluster of SQRs including two with experimentally verified sulfide-oxidizing activity (*Geitlerinema* sp. PCC 9228 and *A. halophytica*) and eight others that are present in sequenced cyanobacterial genomes (Fig. 7). Putative SQR sequences from MIS-Ph1 and MIS-Ph2 fall outside of the main clade of cyanobacterial SQR sequences but within the broader family, which includes an SQR from the eukaryote *Arenicola marina* that oxidizes sulfide for the purpose of detoxification (Bronstein *et al*., 2000).

While the MIS mat SQR sequences that we report do indeed likely encode functional sulfide-oxidizing enzymes, the actual physiological role that this sulfide oxidation plays is difficult to discern based solely on sequence similarity. *Geitlerinema* sp. PCC 9228 and *A. halophytica* encode SQR enzymes that have been experimentally shown to oxidize sulfide (Bronstein *et al*., 2000), but they perform different physiological functions. The SQR from *Geitlerinema* sp. PCC 9228 can be used for sulfide-dependent anoxygenic photosynthesis, whereas the SQR from *A. halophytica* is used for detoxification of sulfide and is not linked to cell growth (Oren

Table 3 Summary of metagenomic assembly

Bin	No. of reads	No. of contigs	Avg. contig length (bp)	Avg. coverage	Avg. %GC content	Total consensus sequence (MB)
Total	827 593	19 463	1357	4.4×	43	26.6
MIS-Ph1	577 920	555	11 251	38.4×	45.1	5.3
MIS-Ph2	14 400	254	3991	5.9×	44.4	1.0
MIS-Os	94 238	748	6210	7.9×	38.9	4.6
Unassigned	141 035	17 906	819	3.2×	43.1	14.7

MIS, Middle Island Sinkhole.

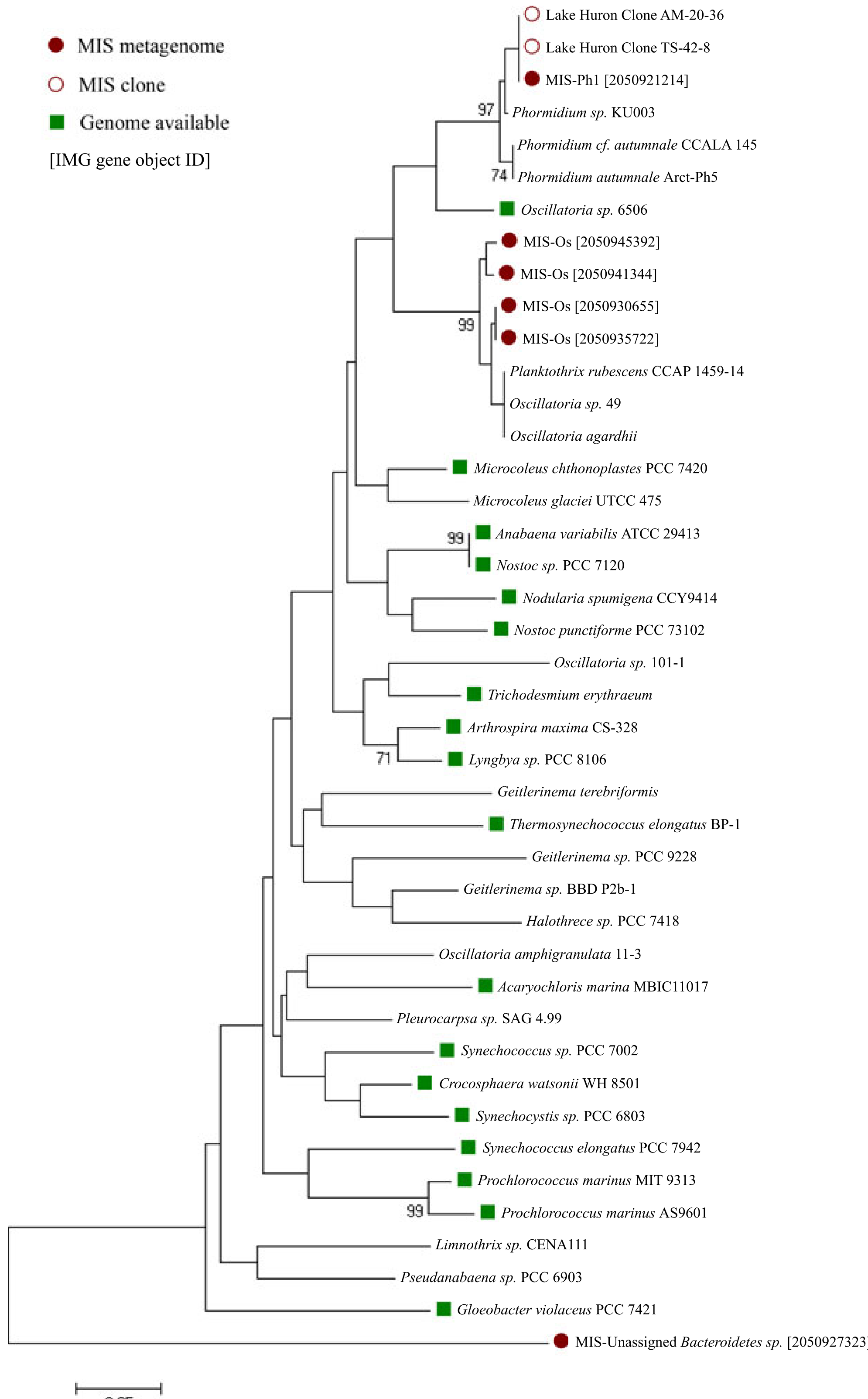

Fig. 6 Phylogenetic tree of the 16S rRNA gene of selected cyanobacteria. Metagenomic contigs from this study (closed red circles), clones from Nold *et al.* (2010a) (open red circles), and species for which genome sequences are available (green squares) are indicated. Bootstrap values are the results of 5000 iterations; values <70 are not shown.

Table 4 Occurrence of dissimilatory sulfur metabolism genes in the MIS finger metagenome. BLAST survey after Frigaard *et al.* (2008), using parameters and queries described in Methods

Organism	SQR	fccA	fccB	sox	dsr	sorA	sorB	aprA	aprB	qmoA	qmoB	qmoC
LHS-Ph1	+	–	–	–	+	–	–	+	–	–	+	–
LHS-Ph2	+	–	+	–	+	–	–	–	–	+	+	+
LHS-Os	+	–	+	–	+	+	–	–	–	–	–	–
Unassigned	+	–	+	–	+	+	–	–	–	–	+	+

MIS, Middle Island Sinkhole; SQR, sulfide quinone reductase.
Note that the dsr column represents the presence of any of the 13 dsr genes (*dsrABCEFHLNMKJOP*). None of the bins has a full set of dsr genes, but all bins contain BLAST hits to some *dsr* genes.

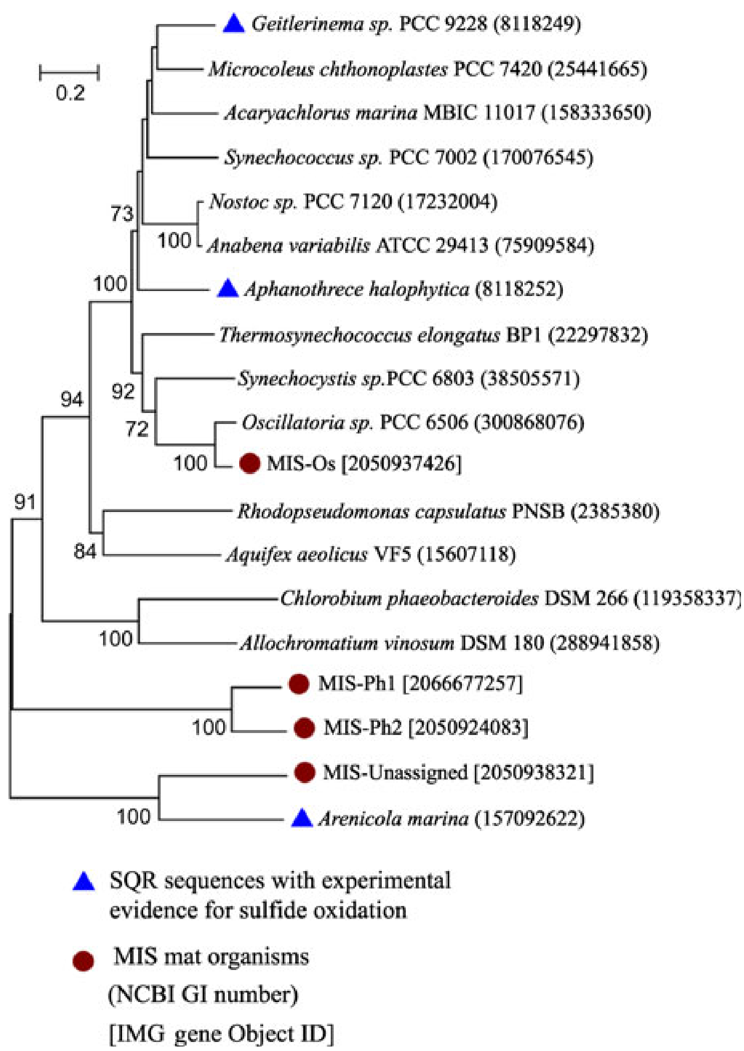

Fig. 7 Phylogenetic tree of sulfide quinone oxidoreductase. Sequences from the Middle Island Sinkhole mat are indicated with red circles. Genes that have been experimentally verified to oxidize sulfide are shown with a blue triangle.

& Shilo, 1979; Bronstein *et al*., 2000). Further complicating the functional role of this enzyme in the cyanobacteria, the SQR from *Geitlerinema* sp. PCC 9228 can also be involved in anaerobic respiration (Oren & Shilo, 1979). Clearly, there is much that remains to be learned about the function of SQR-like genes in the cyanobacteria.

Although SQR is the only enzyme that has been implicated in anoxygenic photosynthesis in the cyanobacteria to date, sulfide-dependent anoxygenic photosynthesis is phylogenetically widespread throughout the cyanobacteria (Miller & Bebout, 2004). The vast majority of this diversity has not been explored with genetic or biochemical tools; therefore, it is feasible that there are novel sulfur oxidation pathways in the cyanobacteria. Homologs of several genes known to be involved in sulfur oxidation in the MIS metagenome were identified (Table 4), including flavocytochrome c (*fccB*), various dissimilatory sulfur reductase (*dsr*) genes, sulfite:cytochrome c oxidoreductase (*sor*), adenosine-5′-phosphosulfate reductase (*apr*), and the quinone-interacting membrane bound oxidoreductase (*qmo*) (Frigaard *et al.*, 2008). Many of these genes are only distantly related (<30% AA identity) to known sulfur-oxidizing enzymes, and in many cases, only a subset of subunits are present, and thus, we cannot ascribe function or taxonomy (in the case of unassigned contigs) to these genes with confidence.

Also notable was the absence of certain sulfur-oxidizing pathways typically prevalent in bacterial sulfur oxidation, including anoxygenic photosynthesis. No *sox* genes (Friedrich *et al.*, 2005; Ludwig *et al.*, 2006) were identified, and while some *dsr* genes were found in the metagenome, they were typically quite divergent from known genes, and no bin had a full complement. Finally, SQR is the only gene implicated in anoxygenic photosynthesis associated with sulfide oxidation found in MIS-Ph1 (Frigaard *et al.*, 2008). Taken together, our results suggest that the anoxygenic photosynthesis observed in the MIS mat is conducted by cyanobacteria via genes and biochemical pathways that are not yet well characterized.

Evidence for a complete genome of *Phormidium* sp. MIS-Ph1

The MIS-Ph1 genome has ~40× genomic coverage and few polymorphisms (Fig. S1), suggesting an essentially complete genome from a near-clonal population. To evaluate genome completeness, we searched the MIS-Ph1 genome for 40 universally conserved housekeeping genes that are not often duplicated or horizontally transferred (Raes *et al.*, 2007). All 40 genes are present in the MIS-Ph1 genome; 36 are present in one copy and four were present in two copies (Table S3). The occurrence of multiple copies of these genes is not uncommon among sequenced cyanobacteria; every cyanobacterial genome available on the JGI Integrated Microbial Genomes website (60 total as of May, 2011) has at least two of these 40 genes in multiple copies, and eight of the genomes are missing at least one of the 40 genes. The presence of all 40 universal housekeeping genes suggests that the gene content of the MIS-Ph1 genome is very close to complete. The completeness of the MIS-Ph1 genome is also supported by the presence of genes encoding complete photosynthetic machinery and pathways of energy and carbon metabolism; we identified genes for biosynthesis of chlorophyll, for proteins of photosystems I and II, the cytochrome b6/f complex, a complete ATP synthase, NADH dehydrogenase complex, and a Heme-Cu-type cytochrome/quinol oxidase.

A total of 5824 genes were identified in the *Phormidium* sp. MIS-Ph1 genome, including 5774 protein coding genes and 50 RNA (rRNA and tRNA) coding genes. 1095 of these genes are present in the vast majority of cyanobacteria genomes sequenced to date (>59 of 62), whereas 602 of the MIS-Ph1 genes are not present in any other cyanobacterial genomes. Overall, the majority of MIS-Ph1 genes could not be assigned specific functions (Fig. 8). Genes that are absent or uncommon in previously sequenced cyanobacterial genomes are especially poorly defined in terms of function (Fig. 8).

Carbon acquisition and metabolism

The MIS-Ph1 genome encodes a complete Calvin-Benson cycle, consistent with the $\delta^{13}C_{org}$ values (−27.25 to −28.43‰) reported above and its role as the major primary producer of the finger community. The genome also has genes for carboxysome shell proteins and carbonic anhydrase, which constitute a carbon-concentrating mechanism (CCM) and suggest that CO_2 can become limiting in the mat environment. This CCM is also thought to induce carbonate precipitation by cyanobacteria (Riding, 2006).

Genes for glycogen synthase and carbohydrate branching and debranching enzymes reveal a mechanism of carbon and energy storage and subsequent utilization. The complete genes for glucose degradation via the pentose phosphate pathway are present, as are two genes that allow *Phormidium* to perform acetate fermentation, pyruvate:ferredoxin oxidoreductase and acetate kinase (Stal & Moezelaar, 1997). Fermentation may sustain MIS-Ph1 at night when sunlight is no longer available for photosynthesis. During the day, mat-forming cyanobacteria store carbon and energy in polyglucose reserves and ferment those reserves at night (Stal, 1995; Nold & Ward, 1996), creating fermentation products that are cross-fed to heterotrophic community partners.

In addition to genes for autotrophy, the MIS-Ph1 genome also contains genes for the acquisition and utilization of organic carbon (carbohydrates and amino acids) from the environment (Table S2). Interestingly, genes encoding beta-galactosidase/beta-glucuronidases are not found in any of the other 60 cyanobacteria genomes sequenced to date except the recently sequenced closest relative, *Oscillatoria* sp. PCC 6506 (Mejean *et al.*, 2010). There are close homologs of these proteins (>50% amino acid ID) in other phyla such as Proteobacteria, Verrucomicrobia, Firmicutes, and Lentisphaerae, raising the intriguing possibility of a horizontal gene transfer origin of these genes in the lineage shared by MIS-1 and *Oscillatoria* sp. PCC 6506.

Oxygen sensing, regulation, and respiratory metabolism

The MIS-Ph1 genome contains a host of genes dedicated to sensing and metabolizing O_2 and regulating O_2-sensitive

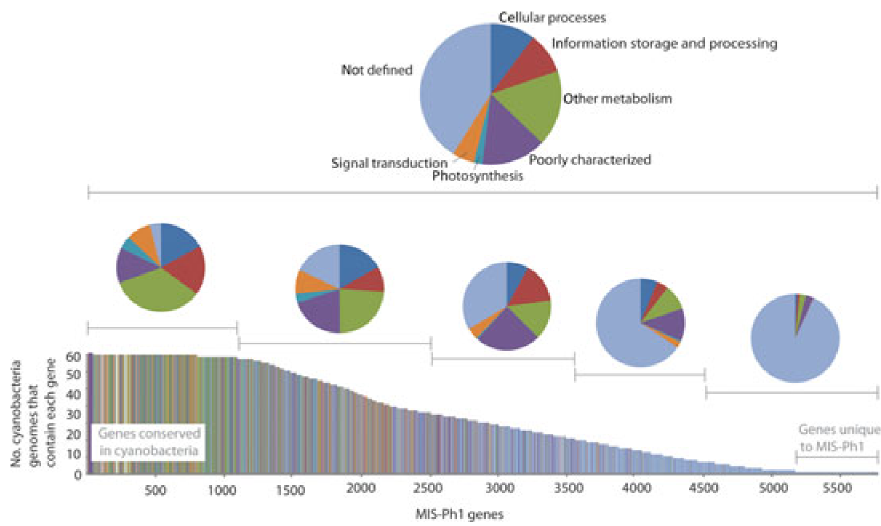

Fig. 8 Function and distribution of *Phormidium* sp. Middle Island Sinkhole (MIS)-Ph1 genes across the 62 cyanobacteria genome sequences currently publicly available. The top pie chart shows COG functional categories for the entire MIS-Ph1 genome. Pie graphs below show composition of COG functional categories for the indicated fractions. 'Cellular Processes' includes genes for cell cycle control, cell division, and cell motility. 'Poorly Characterized' indicates that the genes are of unknown function.

pathways. There are two different electron transport chain terminal reductases: a cytochrome c oxidase (subunits I, II, and III) that is present in nearly all currently available cyanobacteria genomes, and a cytochrome bd plastoquinol oxidase (subunits 1 and 2) that is less widely distributed in cyanobacteria and which is known to operate under low-O_2 conditions (Kana *et al.*, 2001). There are also hints of anaerobic respiration; the SQR described above has been reported to be involved in reduction of elemental sulfur, and there is also a gene annotated as a sulfite reductase. The presence of these genes raises the intriguing possibility that MIS-Ph1 could be involved in dissimilatory sulfur reduction that has been observed in the MIS mat (Nold *et al.*, 2010a). However, these genes are distantly related to genes of known function; hence, experimental evidence is required to test this possibility. Although anaerobic respiration of elemental sulfur has been observed in cyanobacteria, it remains poorly understood (Stal & Moezelaar, 1997), and dissimilatory reduction of sulfite or sulfate by cyanobacteria has not been described. The presence of two terminal O_2 reductases as well as a putative mechanism for sulfur reduction may provide versatility in the stratified redox environment in which MIS-Ph1 thrives. This versatility would also be important in a microbial mat ecosystem where oxygen and sulfide concentrations likely vary on a diel cycle (Stal, 2000).

MIS-Ph1 is also well equipped for detoxification of reactive oxygen species (ROS) with genes for superoxide dismutase, cytochrome c peroxidase, glutathione peroxidase, and peroxiredoxin. These genes likely play important roles in protecting the MIS-Ph1 biomolecules, including the photosynthetic apparatus, from ROS that are commonly produced at both photosynthetic reaction centers (He & Hader, 2002).

Hopanoid biosynthesis

Genes for squalene-hopene cyclase (*shc*) and a radical SAM methylase (*hpnP*) (Welander *et al.*, 2010) have been implicated in biosynthesis of 2-methylhopanoids, which have been used as a biomarker of cyanobacteria and oxygenic photosynthesis in the geologic record (Summons *et al.*, 1999). However, recent evidence that 2-methylhopaoids and their biosynthetic genes are not present in all cyanobacteria and are present in certain non-cyanobacteria questions the reliability of this biomarker (Rashby *et al.*, 2007; Welander *et al.*, 2009, 2010). We were unable to detect *shc* genes within the MIS-Ph1 genome, and homologs of *hpnP* are present but only at such low similarity (<35% amino acid identity) that their function is uncertain. The absence of hopanoid biosynthesis genes in a cyanobacterium that thrives under persistent low-O_2 conditions, which were likely common in cyanobacterial habitats through certain periods of the Precambrian, casts further doubt on the utility of hopanoids as a biomarker of cyanobacteria in the geologic record.

Genomic insights into interactions of MIS-Ph1 with the mat community

The MIS-Ph1 genome contains genes that reflect a communal lifestyle in which interactions with other community members are prevalent. First, there are many genes reflective of viral predation pressure (Table S2), including seven CRISPR sequences, which are thought to provide adaptive immunity to genetic elements such as viruses and plasmids (Makarova *et al.*, 2011). Second, there are many genes that appear to be involved in antagonistic chemical interactions with other community members including the toxins colicin D and hemolysin, non-ribosomal peptide synthetases (NRPS) commonly involved in the production of bioactive compounds, and antibiotic synthesis and drug resistance. The function of genes for antibiotics is uncertain given the diverse physiological functions recently attributed to them such as electron transfer, signaling, and community development (Dietrich *et al.*, 2008; Wang *et al.*, 2010). Third, we detected a large number of genes involved in communication and mat construction (Table S2). Overall, the communal lifestyle encoded by the MIS-Ph1 genome offers significant benefits including antibiotic resistance, predation deterrence, and facilitation of nutrient acquisition (Blenkinsopp & Costerton, 1991).

Environmental sensing, regulation, and nutrient acquisition

The MIS-Ph1 genome includes many genes dedicated to interfacing with the environment. Genes encoding a number of light antenna proteins, including allophycocyanin, phycoerythrin, and phycocyanin/phycoerythrocyanin, indicate an ability to efficiently capture energy over a wide spectrum of light (Deruyter & Fromme, 2008) which would be useful over daily and seasonal light fluctuations. There are also four genes for bacteriophytochromes, which are used for sensing light and regulating light-dependent cellular processes. Thus, the MIS-Ph1 genome is well equipped to optimize photosynthetic machinery according to prevailing light availability. There are many more sensing and regulatory genes for light and motility for which only general functional predication can be made (Table S2).

The MIS-Ph1 genome encodes numerous transport systems for efficient acquisition of nutrients from the environment. Genes are present for high-affinity transporters of iron (Fe^{2+}, Fe^{3+} and heme), cobalt, nickel, manganese, phosphate, nitrate, and sulfate and for regulation of cellular processes governing homeostasis of these nutrients. The only authentic nitrogen fixation gene identified is present on an unassigned contig from a low abundance member of the community. An exceptional number of genes (5) for cobalt-containing cobalamin (Vitamin B_{12}) biosynthesis are present; this micronutrient limits primary production in pelagic marine environments (Bertrand *et al.*, 2007) but its significance in mats is unknown.

Finally, genes encoding biosynthesis of betaine and trehalose suggest that these organic compatible solutes are used to maintain cellular turgor pressure in the face of osmotic stress. The presence of both trehalose, which has been associated with halotolerance in freshwater cyanobacteria, and betaine, which has been associated with halotolerance in hypersaline cyanobacteria (Stal, 2000), suggests that MIS-Ph1 is prepared to survive a broad range of salinities.

CONCLUSIONS

The MIS hosts cyanobacterial mats that thrive under persistent low-O_2 conditions, thus providing a novel modern system for investigating geobiological processes that were critical in geochemical and biological evolution. Cyanobacterial mat systems are typically considered as a source of O_2, for example, for the great oxidation event (Holland, 2006) or for O_2 oases that fostered development of early animal life (Gingras *et al.*, 2011). In contrast, we find that the MIS mats can be sinks for O_2 because of significant primary production of carbon via anoxygenic photosynthesis and chemosynthesis. Recognition of this modern microbial mat community that is dominated by cyanobacteria and has $\delta^{13}C_{org}$ values indistinguishable from oxygenic cyanobacteria, yet functions as a sink for O_2, underscores the need for caution in inferring metabolic functions of filamentous cyanobacteria (or of sedimentary structures putatively made by cyanobacteria) preserved in the geologic record. More broadly, these results suggest that the biogeochemical function of cyanobacterial communities through Earth history should be considered more carefully, for example, as performed by Johnston *et al.* (2009).

Remarkably, the MIS mat community is dominated by just one genotype of the cyanobacterium *Phormidium* sp. MIS-Ph1, which enabled cultivation-independent insights through genomic reconstruction. This genome sequence reveals many adaptations for life in the hostile MIS environment, including metabolic versatility (autotrophy and heterotrophy) and the ability to facultatively switch between oxygenic and anoxygenic photosynthesis. In addition, it also possesses systems to sense environmental conditions and optimize cellular machinery according to light and redox conditions. Also encoded in the genome are tools to sustain a dense mat community while contending with other microorganisms, grazers, and viruses.

Although these findings provide the first genomic insights into life within cyanobacterial mats under low O_2 concentrations, the preponderance of novel genes of unknown function also highlights critical gaps in understanding of the genetic underpinnings of these systems, especially with regard to anoxygenic photosynthesis. Further insights into the relationship between genetic diversity and community function await deeper sequencing and tracking of physiology and gene expression over gradients of light and redox chemistry. The metagenome also holds clues to nutrient requirements that will guide cultivation of the key organisms so that genome-generated hypotheses can be tested experimentally. Overall, the MIS mat system offers a promising natural laboratory for determining the factors that control processes of geobiological interest such as O_2 production, and for evaluating mineralogical, isotopic, and organic signatures relevant to interpretation of the geologic record.

The dominance of the MIS mats by one genotype points to exceptionally low microbial diversity; such unevenness of microbial community structure is rivaled by just a few microbial communities in extreme subsurface environments (Chivian *et al*., 2008; Denef *et al*., 2010). On the one hand, this lack of diversity likely reflects the incredible versatility of MIS-Ph1; through its ability to thrive under a wide range of conditions, it appears to have simultaneously occupied several key niches in the MIS environment. On the other hand, the uneven, low-diversity nature of the community indicates a lack of redundancy that could signal a fragility of the MIS community (Wittebolle *et al*., 2009). This potential fragility should be considered in efforts to protect and preserve these unique ecosystems in the face of environmental change.

ACKNOWLEDGMENTS

We gratefully acknowledge the NOAA Thunder Bay National Marine Sanctuary (particularly Jeff Grey, Russ Green, Wayne Lusardi, Joe Hoyt and Tane Casserly) for their assistance in field operations, the University of Michigan DNA Sequencing Core for DNA sequencing, Anja Schleicher for assistance with XRD, Steve Long and Michael Snider for assistance with microscopy and microphotography, and Ryan Lesniewski for assistance with metagenomic assembly. The authors also thank students in the BIO 370 *Biotechnology* course at UW-Stout who identified and described genes unique to the *P. autumnale* MIS-Ph1 genome as part of the course requirements. This work was supported by NSF grants MCB0603944 and MCB0604158 to BB and SN, and EAR1035955 to GJD, NDS, and BB.

REFERENCES

Andersen DT, Sumner DY, Hawes I, Webster-Brown J, Mckay CP (2011) Discovery of large conical stromatolites in Lake Untersee, Antarctica. *Geobiology* **9**, 280–293.

Arieli B, Shahak Y, Taglicht D, Hauska G, Padan E (1994) Purification and characterization of sulfide-quinone reductase, a novel enzyme driving anoxygenic photosynthesis in *Oscillatoria limnetica*. *Journal of Biological Chemistry* **269**, 5705–5711.

Bachar A, Omoregie E, De Wit R, Jonkers HM (2007) Diversity and function of Chloroflexus-like bacteria in a hypersaline microbial mat: phylogenetic characterization and impact on aerobic respiration. *Applied and Environment Microbiology* **73**, 3975–3983.

Bekker A, Holland HD, Wang PL, Rumble D, Stein HJ, Hannah JL, Coetzee LL, Beukes NJ (2004) Dating the rise of atmospheric oxygen. *Nature* **427**, 117–120.

Belkin S, Padan E (1978) Hydrogen metabolism in the facultative anoxygenic Cyanobacteria (Blue-Green Algae) *Oscillatoria limnetica* and *Aphanothece halophytica*. *Archives of Microbiology* **116**, 109–111.

Bertrand EM, Saito MA, Rose JM, Riesselman CR, Lohan MC, Noble AE, Lee PA, Ditullio GR (2007) Vitamin B-12 and iron colimitation of phytoplankton growth in the Ross Sea. *Limnology and Oceanography* **52**, 1079–1093.

Biddanda BA, Opsahl S, Benner R (1994) Plankton respiration and carbon flux through bacterioplankton on the Louisiana Shelf. *Limnology and Oceanography* **39**, 1259–1275.

Biddanda BA, Coleman DF, Johengen TH, Ruberg SA, Meadows GA, Vansumeran HW, Rediske RR, Kendall ST (2006) Exploration of a submerged sinkhole ecosystem in Lake Huron. *Ecosystems* **9**, 828–842.

Biddanda BA, Nold SC, Ruberg SA, Kendall ST, Sanders TG, Gray JJ (2009) Great Lakes sinkholes: a microbiogeochemical frontier. *Eos, Transactions, American Geophysical Union* **90**, 61–62.

Biddanda BA, Nold SC, Dick GJ, Kendall ST, Vail JH, Ruberg SA, Green CM (in press) Rock, water, microbes: underwater sinkholes in Lake Huron are habitats for ancient microbial life. *Nature Education*.

Black TJ (1983) Selected views of the tectonics, structure and karst in northern lower Michigan. In *Michigan Basin Geological Society Field Conference Proceedings* (ed. Kimmel RE). Michigan Basin Geological Society, Lansing, MI, pp. 11–35.

Blankenship R, Sadekar S, Raymond J (2007) The evolutionary transition from anoxygenic to oxygenic photosynthesis. In *Evolution of Primary Producers in the Sea* (eds Falkowski PG, Knoll AH). Elsevier, Philadelphia, PA, pp. 21–35.

Blenkinsopp SA, Costerton JW (1991) Understanding bacterial biofilms. *Trends in Biotechnology* **9**, 138–143.

Bosak T, Newman DK (2003) Microbial nucleation of calcium carbonate in the Precambrian. *Geology* **31**, 577–580.

Bronstein M, Schutz M, Hauska G, Padan E, Shahak Y (2000) Cyanobacterial sulfide-quinone reductase: cloning and heterologous expression. *Journal of Bacteriology* **182**, 3336–3344.

Bühring SI, Sievert SM, Jonkers HM, Ertefai T, Elshahed MS, Krumholz LR, Hinrichs KU (2011) Insights into chemotaxonomic composition and carbon cycling of phototrophic communities in an artesian sulfur-rich spring (Zodletone, Oklahoma, USA), a possible analog for ancient microbial mat systems. *Geobiology* **9**, 166–179.

Canfield DE, Des Marais DJ (1993) Biogeochemical cycles of carbon, sulfur, and free oxygen in a microbial mat. *Geochim Cosmochim Acta* **57**, 3971–3984.

Casamayor EO, Garcia-Cantizano J, Pedros-Alio C (2008) Carbon dioxide fixation in the dark by photosynthetic bacteria in sulfide-rich stratified lakes with oxic-anoxic interfaces. *Limnology and Oceanography* **53**, 1193–1203.

Castenholz RW (1976) Effect of sulfide on blue-green algae of hot springs.1. New-Zealand. *Journal of Phycology* **12**, 54–68.

Castenholz RW (1977) Effect of sulfide on blue-green algae of hot springs.2. Yellowstone National Park. *Microbial Ecology* **3**, 79–105.

Chevreux B, Pfisterer T, Drescher B, Driesel AJ, Muller WEG, Wetter T, Suhai S (2004) Using the miraEST assembler for reliable and automated mRNA transcript assembly and SNP detection in sequenced ESTs. *Genome Research* **14**, 1147–1159.

Chivian D, Brodie EL, Alm EJ, Culley DE, Dehal PS, Desantis TZ, Gihring TM, Lapidus A, Lin LH, Lowry SR, Moser DP, Richardson PM, Southam G, Wanger G, Pratt LM, Andersen GL, Hazen TC, Brockman FJ, Arkin AP, Onstott TC (2008) Environmental genomics reveals a single-species ecosystem deep within Earth. *Science* **322**, 275–278.

Cohen Y, Jorgensen BB, Padan E, Shilo M (1975a) Sulphide-dependent anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. *Nature* **257**, 489–492.

Cohen Y, Padan E, Shilo M (1975b) Facultative anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. *Journal of Bacteriology* **123**, 855–861.

Cohen Y, Jorgensen BB, Revsbech NP, Poplawski R (1986) Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. *Applied and Environment Microbiology* **51**, 398–407.

Comte K, Sabacka M, Carre-Mlouka A, Elster J, Komarek J (2007) Relationships between the Arctic and the Antarctic cyanobacteria;

three *Phormidium*-like strains evaluated by a polyphasic approach. *FEMS Microbiology Ecology* **59**, 366–376.
Cowan DA, Tow LA (2004) Endangered antarctic environments. *Annual Review of Microbiology* **58**, 649–690.
Denef VJ, Mueller RS, Banfield JF (2010) AMD biofilms: using model communities to study microbial evolution and ecological complexity in nature. *Isme Journal* **4**, 599–610.
Deruyter YS, Fromme P (2008) Molecular structure of the photosynthetic apparatus. In *The Cyanobacteria, Molecular Biology, Genomics, and Evolution* (eds Herrero A, Flores E). Caister Academic Press, Norfolk, UK, pp. 217–269.
Dick GJ, Andersson AF, Baker BJ, Simmons SL, Thomas BC, Yelton AP, Banfield JF (2009) Community-wide analysis of microbial genome sequence signatures. *Genome Biology* **10**, R85.
Dietrich LEP, Teal TK, Price-Whelan A, Newman DK (2008) Redox-active antibiotics control gene expression and community behavior in divergent bacteria. *Science* **321**, 1203–1206.
Dupraz C, Reid RP, Braissant O, Decho AW, Norman RS, Visscher PT (2009) Processes of carbonate precipitation in modern microbial mats. *Earth-Science Reviews* **96**, 141–162.
Falkowski PG, Fenchel T, Delong EF (2008) The microbial engines that drive Earth's biogeochemical cycles. *Science* **320**, 1034–1039.
Fontes MLS, Suzuki MT, Cottrell MT, Abreu PC (2011) Primary production in a subtropical stratified coastal lagoon-contribution of anoxygenic phototrophic bacteria. *Microbial Ecology* **61**, 223–237.
Friedrich CG, Bardischewsky F, Rother D, Quentmeier A, Fischer J (2005) Prokaryotic sulfur oxidation. *Current Opinion in Microbiology* **8**, 253–259.
Frigaard N-U, Dahl C, Robert KP (2008) Sulfur metabolism in phototrophic sulfur bacteria. In *Advances in Microbial Physiology* (ed Robert KP). Academic Press, London, UK, pp. 103–200.
Garlick S, Oren A, Padan E (1977) Occurrence of facultative anoxygenic photosynthesis among filamentous and unicellular cyanobacteria. *Journal of Bacteriology* **129**, 623–629.
Gingras M, Hagadorn JW, Seilacher A, Lalonde SV, Pecoits E, Petrash D, Konhauser KO (2011) Possible evolution of mobile animals in association with microbial mats. *Nature Geoscience* **4**, 372–375.
Grotzinger JP, Knoll AH (1999) Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? *Annual Review of Earth and Planetary Sciences* **27**, 313–358.
Hawes I, Schwarz AM (1999) Photosynthesis in an extreme shade environment: benthic microbial mats from Lake Hoare, a permanently ice-covered Antarctic lake. *Journal of Phycology* **35**, 448–459.
He Y-Y, Hader D-P (2002) Reactive oxygen species and UV-B: effect on cyanobacteria. *Photochemical & Photobiological Sciences* **1**, 729–736.
Holland HD (2006) The oxygenation of the atmosphere and oceans. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences* **361**, 903–915.
Horodyski RJ, Knauth LP (1994) Life on land in the Precambrian. *Science* **263**, 494–498.
Imbus SW, Macko SA, Elmore RD, Engel MH (1992) Stable isotope (C, S, N) and molecular studies on the Precambrian Nonesuch Shale (Wisconsin-Michigan, USA) – evidence for differential preservation rates, depositional environment and hydrothermal influence. *Chemical Geology* **101**, 255–281.
Johnston DT, Wolfe-Simon F, Pearson A, Knoll AH (2009) Anoxygenic photosynthesis modulated Proterozoic oxygen and sustained Earth's middle age. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 16925–16929.
Jorgensen BB, Revsbech NP, Blackburn TH, Cohen Y (1979) Diurnal cycle of oxygen and sulfide microgradients and microbial photosynthesis in a cyanobacterial mat sediment. *Applied and Environmental Microbiology* **38**, 46–58.
Jorgensen BB, Revsbech NP, Cohen Y (1983) Photosynthesis and structure of benthic microbial mats – microelectrode and SEM studies of 4 cyanobacterial communities. *Limnology and Oceanography* **28**, 1075–1093.
Jorgensen BB, Cohen Y, Revsbech NP (1986) Transition from anoxygenic to oxygenic photosynthesis in a *Microcoleus chthonoplastes* cyanobacterial mat. *Applied and Environment Microbiology* **51**, 408–417.
Kana BD, Weinstein EA, Avarbock D, Dawes SS, Rubin H, Mizrahi V (2001) Characterization of the cydAB-Encoded cytochrome bd oxidase from *Mycobacterium smegmatis. Journal of Bacteriology* **183**, 7076–7086.
Kenward PA, Goldstein RH, Gonzalez LA, Roberts JA (2009) Precipitation of low-temperature dolomite from an anaerobic microbial consortium: the role of methanogenic Archaea. *Geobiology* **7**, 556–565.
Komarek J, Kling H, Komarkova J (2003) Filamentous cyanobacteria. In *Freshwater Algae of North America: Ecology and Classification* (eds Wehr JD, Sheath R). Elsevier Science, San Diego, CA, USA, pp. 117–196.
Kopp RE, Kirschvink JL, Hilburn IA, Nash CZ (2005) The paleoproterozoic snowball Earth: a climate disaster triggered by the evolution of oxygenic photosynthesis. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 11131–11136.
Larkin MA, Blackshields G, Brown NP, Chenna R, Mcgettigan PA, Mcwilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947–2948.
Lyons TW, Anbar AD, Severmann S, Scott C, Gill BC (2009) Tracking euxinia in the ancient ocean: a multiproxy perspective and Proterozoic case study. *Annual Review of Earth and Planetary Sciences* **37**, 507–534.
Ludwig KA, Kelley DS, Butterfield DA, Nelson BK, Früh-Green G (2006) Formation and evolution of carbonate chimneys at the Lost City Hydrothermal Field. *Geochimica et Cosmochimica Acta* **70**, 3625–3645.
Makarova KS, Haft DH, Barrangou R, Brouns SJ, Charpentier E, Horvath P, Moineau S, Mojica FJ, Wolf YI, Yakunin AF, Van Der Oost J, Koonin EV (2011) Evolution and classification of the CRISPR-Cas systems. *Nature Reviews Microbiology* **9**, 467–477.
Mejean A, Mazmouz R, Mann S, Calteau A, Medigue C, Ploux O (2010) The genome sequence of the cyanobacterium *Oscillatoria* sp. PCC 6506 reveals several gene clusters responsible for the biosynthesis of toxins and secondary metabolites. *Journal of Bacteriology* **192**, 5264–5265.
Miller SR, Bebout BM (2004) Variation in sulfide tolerance of photosystem II in phylogenetically diverse cyanobacteria from sulfidic habitats. *Applied and Environment Microbiology* **70**, 736–744.
Noffke N (2009) The criteria for the biogenicity of microbially induced sedimentary structures (MISS) in Archean and younger, sandy deposits. *Earth-Science Reviews* **96**, 173–180.
Nold S, Ward D (1996) Photosynthate partitioning and fermentation in hot spring microbial mat communities. *Applied and Environment Microbiology* **62**, 4598–4607.
Nold SC, Pangborn JB, Zajack HA, Kendall ST, Rediske RR, Biddanda BA (2010a) Benthic bacterial diversity in submerged sinkhole ecosystems. *Applied and Environment Microbiology* **76**, 347–351.
Nold SC, Zajack HA, Biddanda BA (2010b) Eukaryal and archaeal diversity in a submerged sinkhole ecosystem influenced by

sulfur-rich, hypoxic groundwater. *Journal of Great Lakes Research* **36**, 366–375.
Oren A, Padan E (1978) Induction of anaerobic, photoautotrophic growth in the cyanobacterium *Oscillatoria limnetica*. *Journal of Bacteriology* **133**, 558–563.
Oren A, Shilo M (1979) Anaerobic heterotrophic dark metabolism in the cyanobacterium *Oscillatoria limnetica*: sulfur respiration and lactate fermentation. *Archives of Microbiology* **122**, 77–84.
Oren A, Padan E, Avron M (1977) Quantum yields for oxygenic and anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 2152–2156.
Overmann J, Beatty JT, Hall KJ, Pfennig N, Northcote TG (1991) Characterization of a dense, purple sulfur bacterial layer in a meromictic Salt Lake. *Limnology and Oceanography* **36**, 846–859.
Pavlov AA, Kasting JF, Eigenbrode JL, Freeman KH (2001) Organic haze in Earth's early atmosphere: source of low-13C late Archean kerogens? *Geology* **29**, 1003–1006.
Palinska K, Marquardt J (2007) Genotypic and phenotypic analysis of strains assigned to the widespread cyanobacterial morphospecies *Phormidium autumnale* (Oscillatoriales). *Archives of Microbiology* **189**, 325–335.
Pedros-Alio C, Garcia-Cantizano J, Calderon J (1993) Bacterial production in anaerobic water columns. In *Handbook of Methods in Aquatic Microbial Ecology* (eds Kemp P, Sherr B, Sherr B, Cole J). Lewis Publishers, Boca Raton, FL, pp. 519–530.
Raes J, Korbel JO, Lercher MJ, Von Mering C, Bork P (2007) Prediction of effective genome size in metagenomic samples. *Genome Biology* **8**, R10.
Rashby SE, Sessions AL, Summons RE, Newman DK (2007) Biosynthesis of 2-methylbacteriohopanepolyols by an anoxygenic phototroph. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 15099–15104.
Retallack GJ, Mindszenty A (1994) Well preserved late Precambrian Paleosols from Northwest Scotland. *Journal of Sedimentary Research* **64**, 264–281.
Revsbech NP, Jorgensen BB, Blackburn TH, Cohen Y (1983) Microelectrode studies of the photosynthesis and O_2, H_2S, and pH profiles of a microbial mat. *Limnology and Oceanography* **28**, 1062–1074.
Richardson LL, Castenholz RW (1987) Diel vertical movements of the cyanobacterium *Oscillatoria terebriformis* in a sulfide-rich hot spring microbial mat. *Applied and Environment Microbiology* **53**, 2142–2150.
Riding R (2006) Cyanobacterial calcification, carbon dioxide concentrating mechanisms, and Proterozoic-Cambrian changes in atmospheric composition. *Geobiology* **4**, 299–316.
Riding R (2011) Calcified cyanobacteria. In *Encyclopedia of Geobiology* (eds Reitner J, Thiel V). Springer, Heidelberg, pp. 211–223.
Ruberg SA, Kendall ST, Biddanda BA, Black T, Nold SC, Lusardi WR, Green R, Casserley T, Smith E, Sanders TG, Lang GA, Constant SA (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**, 12–21.
Rye R, Holland HD (2000) Life associated with a 2.76 Ga ephemeral pond?: evidence from Mount Roe #2 paleosol. *Geology*, **28**, 483–486.
Sanders JTG, Biddanda BA, Stricker CA, Nold SC (2011) Stable isotope analysis reveals benthic macroinvertebrate and fish communities linked to submerged groundwater vents in Lake Huron. *Aquatic Biology* **12**, 1–11.
Schidlowski M (2000) Carbon isotopes and microbial sediments. In *Microbial Sediments* (eds Riding RE, Awramik SM). Springer-Verlag, Berlin, pp. 84–95.
Schütz M, Shahak Y, Padan E, Hauska G (1997) Sulfide-quinone reductase from *Rhodobacter capsulatus*. Purification, cloning, and expression. *Journal of Biological Chemistry* **272**, 9890–9894.
Sheldon N (in press) Microbially induced sedimentary structures in the ca. 1100 Ma terrestrial Midcontinental Rift of North America. In *Microbial Mats in Siliciclastic Depositional Systems Through Time* (eds Noffke N, Chafetz H). SEPM Special Publication No. 11. (ISBN: 978-1-56576-314-2).
Shen Y, Knoll AH, Walter MR (2003) Evidence for low sulphate and anoxia in a mid-Proterozoic marine basin. *Nature* **423**, 632–635.
Stal LJ (1995) Tansley Review No. 84. Physiological ecology of cyanobacteria in microbial mats and other communities. *New Phytologist* **131**, 1–32.
Stal LJ (2000) Cyanobacterial mats and stromatolites. In *The Ecology of Cyanobacteria* (eds Whitton B, Potts M). Kluwer Academic Publishers, Dordrecht/London/Boston, pp. 61–120.
Stal LJ, Moezelaar R (1997) Fermentation in cyanobacteria. *FEMS Microbiology Reviews* **21**, 179–211.
Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690.
Strunecký O, Elster J, Komárek J (2010) Phylogenetic relationships between geographically separate Phormidium cyanobacteria: is there a link between north and south polar regions? *Polar Biology* **33**, 1419–1428.
Summons RE, Jahnke LL, Hope JM, Logan GA (1999) 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature* **400**, 554–557.
Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596–1599.
Taton A, Grubisic S, Balthasart P, Hodgson DA, Laybourn-Parry J, Wilmotte A (2006a) Biogeographical distribution and ecological ranges of benthic cyanobacteria in East Antarctic lakes. *FEMS Microbiology Ecology* **57**, 272–289.
Taton A, Grubisic S, Ertz D, Hodgson DA, Piccardi R, Biondi N, Tredici MR, Mainini M, Losi D, Marinelli F, Wilmotte A (2006b) Polyphasic study of Antarctic cyanobacterial strains. *Journal of Phycology* **42**, 1257–1270.
Van Lith Y, Warthmann R, Vasconcelos C, Mckenzie JA (2003) Sulphate-reducing bacteria induce low-temperature Ca-dolomite and high Mg-calcite formation. *Geobiology* **1**, 71–79.
Vasconcelos C, Mckenzie JA, Bernasconi S, Grujic D, Tien AJ (1995) Microbial mediation as a possible mechanism for natural dolomite formation at low-temperatures. *Nature* **377**, 220–222.
Vincent WF (2000) Cyanobacterial dominance in the polar regions. In *The Ecology of Cyanobacteria* (eds Whitton BA, Potts M). Kluver Academic Publishers, Amsterdam, 321–340.
Walter MR, Bauld J (1983) The association of sulphate evaporites, stromatolitic carbonates and glacial sediments: examples from the Proterozoic of Australia and the Cainzoic of Antarctica. *Precambrian Research* **21**, 129–148.
Walter LM, Ku TCW, Muehlenbacks K, Patterson WP, Bonnell L (2007) Controls on δ13C of dissolved inorganic carbon in marine pore waters: an integrated case study of isotope exchange during syndepositional recrystallization of biogenic carbonate sediments (South Florida Platform, USA). *Deep Sea Research Part II: Topical Studies in Oceanography* **54**, 1163–1200.
Wang Y, Kern SE, Newman DK (2010) Endogenous phenazine antibiotics promote anaerobic survival of *Pseudomonas aeruginosa* via extracellular electron transfer. *Journal of Bacteriology* **192**, 365–369.

Warthmann R, Van Lith Y, Vasconcelos C, Mckenzie JA, Karpoff AM (2000) Bacterially induced dolomite precipitation in anoxic culture experiments. *Geology* **28**, 1091–1094.
Welander PV, Hunter RC, Zhang L, Sessions AL, Summons RE, Newman DK (2009) Hopanoids play a role in membrane integrity and pH homeostasis in *Rhodopseudomonas palustris* TIE-1. *Journal of Bacteriology* **191**, 6145–6156.
Welander PV, Coleman ML, Sessions AL, Summons RE, Newman DK (2010) Identification of a methylase required for 2-methylhopanoid production and implications for the interpretation of sedimentary hopanes. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 8537–8542.
Wharton RA, Parker BC, Simmons GM (1983) Distribution, species composition and morphology of algal mats in Antarctic dry valley lakes. *Phycologia* **22**, 355–365.
Wittebolle L, Marzorati M, Clement L, Balloi A, Daffonchio D, Heylen K, De Vos P, Verstraete W, Boon N (2009) Initial community evenness favours functionality under selective stress. *Nature* **458**, 623–626.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1 A typical MIS-PH1 contig viewed through the program consed.

Fig. S2 Schematic of oxygen and carbon metabolic processes and measurements.

Table S1 Genes used as queries for sulfur oxidation genes.

Table S2 List of MIS genes of interest with IMG identifier numbers.

Table S3 Single copy COG genes used to assess genome completeness.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.