Coupled reductive and oxidative sulfur cycling in the phototrophic plate of a meromictic lake

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ABSTRACT

Mahoney Lake represents an extreme meromictic model system and is a valuable site for examining the organisms and processes that sustain photic zone euxinia (PZE). A single population of purple sulfur bacteria (PSB) living in a dense phototrophic plate in the chemocline is responsible for most of the primary production in Mahoney Lake. Here, we present metagenomic data from this phototrophic plate – including the genome of the major PSB, as obtained from both a highly enriched culture and from the metagenomic data – as well as evidence for multiple other taxa that contribute to the oxidative sulfur cycle and to sulfate reduction. The planktonic PSB is a member of the Chromatiaceae, here renamed Thiohalocapsa sp. strain ML1. It produces the carotenoid okenone, yet its closest relatives are benthic PSB isolates, a finding that may complicate the use of okenone (okenane) as a biomarker for ancient PZE. Favorable thermodynamics for non-phototrophic sulfide oxidation and sulfate reduction reactions also occur in the plate, and a suite of organisms capable of oxidizing and reducing sulfur is apparent in the metagenome. Fluctuating supplies of both reduced carbon and reduced sulfur to the chemocline may partly account for the diversity of both autotrophic and heterotrophic species. Collectively, the data demonstrate the physiological potential for maintaining complex sulfur and carbon cycles in an anoxic water column, driven by the input of exogenous organic matter. This is consistent with suggestions that high levels of oxygenic primary production maintain episodes of PZE in Earth’s history and that such communities should support a diversity of sulfur cycle reactions.

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INTRODUCTION

Euxinic conditions have been an intermittent feature of global oceans throughout Earth’s history. They accompanied significant events such as Phanerozoic biotic crises (e.g., Pancost et al., 2004; Grice et al., 2005) and perhaps were common in the Mesoproterozoic (Canfield, 1998; Brooks et al., 2005; Reinhard et al., 2013). Although rare today, persistently euxinic environments serve as modern analogues to ancient water columns and their associated sedimentary basins. Of these modern analogues, meromictic lakes have been of intense interest due to their
phototrophic chemocline communities, large geochemical gradients, and laminated sedimentary records that can preserve bioignatures (Meyer & Kump, 2008). Mahoney Lake (ML) is a small meromictic lake in British Columbia, Canada, that represents an extreme end-member of such euxinia. The hypolimnion of the lake contains high levels of sulfide (30–35 mM). ML has a pH in the range 7–9 and thus is not a soda lake (Northcote & Hall, 1983). Its dominant anion is sulfate (400–500 mM), which is supplied by local surface waters draining alkaline lavas rich in Mg$^{2+}$, Ca$^{2+}$, Na$^+$, and SO$_4^{2–}$ (Northcote & Hall, 1983). The hypolimnion is anoxic, eutrophic, and very saline (~74 g L$^{-1}$ total dissolved solids (TDS)), while the epilimnion is oxic, oligotrophic, and of lower salinity (~28 g L$^{-1}$ TDS) (Northcote & Hall, 1983).

Mahoney Lake (ML) is stable year-round due to density stratification, and a 10–20 cm-thick chemocline occurs at a depth of about 7 m. This layer supports a dense (~10$^8$ cells mL$^{-1}$) layer of anoxygenic phototrophs, limiting the mixing of nutrients to the epilimnion and absorbing all visible light (Overmann et al., 1991, 1996a, 1996b). Primary productivity has largely been attributed to a single purple sulfur bacterium (PSB), which was originally classified as a Thiocapsa sp. based on cell morphology (Northcote & Halsay, 1969). However, cultivation studies yielded an okenone-producing member of the family Chromatiaceae, denoted strain ML1. Okcenone, a biomarker for some species of PSB (e.g., Brocks & Schaeffer, 2008), is abundant in the lake sediments (Overmann et al., 1993; Coolen & Overmann, 1998). Based on morphological and physiological properties, strain ML1 was reclassified as Amoebobacter purpureus (Overmann et al., 1991) and was subsequently reclassified again to the genus Lamprocystis (Imhoff, 2001). More recently, 16S rRNA and psfLM gene sequencing revealed that strain ML1 is only distantly related to the type strain of Lamprocystis purpurea (Coolen & Overmann, 1998; Tank et al., 2009).

While small numbers of green sulfur bacteria (GSB) are also present in and below the chemocline, the total sulfide oxidation rate cannot be attributed to PSB and GSB alone (Overmann et al., 1996a). Chemoautotrophic sulfide oxidation has been observed near the oxic–anoxic interfaces of other stratified systems and by analogy may be expected in Mahoney Lake (Hadas et al., 2001; Bühiring et al., 2011). Analysis of 16S rRNA gene sequences supports this, showing the presence of Epsilonproteobacteria that are likely to be sulfide and/or sulfur oxidizers (Klepac-Ceraj et al., 2012). Sulfate reduction also occurs in the chemocline, and in situ generation is thought to supply much of the sulfide necessary to support anoxygenic photosynthesis (Overmann et al., 1991, 1996a).

Collectively, these data indicate that complex cycling of carbon and sulfur occurs in the Mahoney Lake chemocline. The ML chemocline maintains a stable ecosystem in which sulfate-reducing bacteria supply sulfide for both phototrophic and chemolithoautotrophic primary productivity. These primary producers in turn supply some of the organic carbon necessary to support the heterotrophic sulfate reducers, resulting in a coupled reductive and oxidative sulfur cycle. Seasonal analyses of primary productivity and sulfur oxidation and reduction rates (Overmann et al., 1996a), and modeling of the carbon cycle in the chemocline (Overmann, 1997), indicate that the system must depend on additional organic carbon or other electron donors to sustain the cycle. Examining the microbial taxonomic and functional potential of such systems is critical to understanding the links between aerobic and anaerobic biogeochemical feedbacks (Johnston et al., 2009).

Here, we evaluated the metabolic potential of the organisms in this community using genomic and metagenomic sequencing. High-throughput DNA sequencing provides opportunities to obtain nearly complete genomes in systems of moderate complexity, or in systems with low strain diversity of a specific ecotype (e.g., Tyson et al., 2004; Iverson et al., 2012). In accordance with this, we were able to analyze several nearly complete genomes. We confirmed that strain ML1 (Chromatiaceae) is the dominant PSB in the Mahoney Lake chemocline and that its closest taxonomic relatives belong to the genus Thiobacilalops. In addition, there is a single major taxon of Epsilonproteobacteria (Sulfurimonas sp.); eight putative sulfate-reducing Deltaproteobacteria; other phototrophs including Cyanobacteria, Alphaproteobacteria, and GSB; and a diverse array of other organisms from the phyla Firmicutes, Mollicutes, Bacteroidetes, Actinobacteria, and Deinococcus. The results suggest that a high degree of functional redundancy exists within a system that nonetheless possesses limited taxonomic diversity within the major populations. The data also point to specific interpretations about the energy balance of sulfide-driven autotrophy and – via the taxonomic placement of genes for okenone synthesis – address the continuity between planktonic and benthic low-oxygen ecosystems.

**MATERIALS AND METHODS**

**Chromatiaceae** strain ML1: DNA Extraction, genomic sequencing, and annotation

A sample for enrichment and isolation of strain ML1 was collected from the chemocline of Mahoney Lake at a depth of 7 m on October 1, 1988 (Fig. 1A; J. Overmann, unpubl. data), and has been maintained since as an enrichment culture (Fig. 1B) using Peffnig’s medium as described previously (Overmann & Peffnig, 1989). Genomic DNA was extracted according to the cetyltrimethylammonium bromide (CTAB) protocol for bacterial DNA isolation of the Joint Genome Institute [http://my. jgi.doe.gov/general/]. Purified DNA was sequenced with
A 454 pyrosequencer (GS FLX+; Roche, Branford, CT, USA) in the Schuster Lab at The Pennsylvania University. A total of 278 972 211 bases in 750 635 reads, with an average read length of 372 bp were generated and assembled by Newbler assembler version 2.6 (Roche) into 867 contiguous reads (contigs) of 500 bp or larger with an average read depth of ~40×. Contigs belonging to strain ML1 were assigned by their GC-content and verified with BLAST (Altschul et al., 1990); 137 contigs (6 305 354 bp in total) with content of >53% were assigned to strain ML-1 in this way (Table 1). Contigs were annotated using RAST (http://rast.nmpdr.org; Aziz et al., 2008).

Environmental sample collection, DNA extraction, and metagenomic sequencing

Samples were collected from the phototrophic plate at 7 m from Mahoney Lake, British Columbia (49°17′N, 119°35′W) in July 2008. Samples were frozen on dry ice for transport to Harvard University, where they were stored at −80 °C until further processed. Samples were thawed and centrifuged, and DNA was extracted from the resulting pellet with an e.Z.N.A SP Plant Maxi Kit (Omega Biotek, Norcross, GA, USA) according to the instructions of the manufacturer. The yield and quality of the extracted DNA was assessed using gel electrophoresis and spectrophotometry (NanoDrop, Wilmington, DE, USA). Fragmentation and library preparation were performed by the North Carolina State University Genomic Sciences Laboratory. Paired-end 150-bp Illuma (HiSeq 2500; Illumina, San Diego, CA, USA) sequencing was performed at the Harvard Center for Systems Biology and resulted in ~300 000 000 high-quality reads.

Metagenome assembly, binning, and annotation

Illumina sequencing reads were trimmed with Trimmomatic 0.20 (Lohse et al., 2012). Only sequences with at least 50 base pairs in both the forward and reverse direction were retained and assembled into contigs with IDBA-UD 1.09 (Table S1), using eight threads with default parameters (Peng et al., 2012). Coverage was determined by aligning raw reads to contigs with BWA 0.5.9 (Li & Durbin, 2009).

The assembled contigs were binned using emerging self-organizing maps (ESOM) based on tetranucleotide frequency, which resulted in clusters corresponding to taxonomically sorted tetranucleotide usage patterns (Dick et al., 2009). A custom Python script (available at http://github.com/bovee/Ochre) was used to calculate tetranucleotide frequency of all contigs ≥2500 bp. Corresponding reverse-complement tetranucleotides were combined as described (Dick et al., 2009). Contigs were split into 5000-bp segments, clustered into taxonomic groups [or ‘genomic bins’ (Voorhies et al., 2012)] by tetranucleotide frequency and visualized with Databionic-ESOM (http://databionic-esom.sourceforge.net) – again using parameters from Dick et al. (2009) (Fig. S1). Bins were inspected for coverage. (Multimodal distribution could indicate the bin contained scaffolds derived from multiple genomes, requiring that the bin be further split, but no such instances were found.) Following manual inspection and further curation by BLAST, phylum-level taxonomic assignment was performed using Phylotox (Shah et al., 2010) and MEGAN (Huson et al., 2011). Contigs larger than 2500 bp were annotated with an in-house annotation pipeline as described in the supporting online material (SOM).

The two highest-coverage bins in the metagenome, a Gammaproteobacterium referred to here as ML-Gamma and an Epsilonproteobacterium referred to as ML-Epsilon, plus four Deltaproteobacteria bins (ML-Delta 1-4) (Figs S1 and S2), were selected for in-depth characterization and taxonomic assignment of their predicted genes. For the ML-Gamma and ML-Epsilon bins, paired reads mapping to scaffolds from each bin were reassembled

Table 1 Statistics for the strain ML1 draft genome and the ML-Gamma and ML-Epsilon genomic bins

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Strain ML1</th>
<th>ML-Gamma</th>
<th>ML-Epsilon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scaffolds</td>
<td>137</td>
<td>485</td>
<td>72</td>
</tr>
<tr>
<td>Longest (bp)</td>
<td>332 978</td>
<td>105 972</td>
<td>114 097</td>
</tr>
<tr>
<td>General information</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total bp</td>
<td>6 305 354</td>
<td>4 442 835</td>
<td>2 742 932</td>
</tr>
<tr>
<td>Average length (bp)</td>
<td>46 024</td>
<td>12 597</td>
<td>29 155</td>
</tr>
<tr>
<td>N50 (bp)</td>
<td>114 369</td>
<td>17 120</td>
<td>38 096</td>
</tr>
<tr>
<td>Characteristics</td>
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<tr>
<td>G + C content</td>
<td>68.3</td>
<td>65.9</td>
<td>38.6</td>
</tr>
<tr>
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<td>5433</td>
<td>2732</td>
</tr>
<tr>
<td>tRNA genes</td>
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<td>1</td>
<td>0</td>
</tr>
<tr>
<td>23S</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>rRNA genes</td>
<td>42</td>
<td>57</td>
<td>48</td>
</tr>
</tbody>
</table>

Fig. 1 Phase contrast photomicrograph of the Mahoney Lake chemocline community (A) and of strain ML1 in culture with visible intracellular sulfur globules (B).
using Velvet (Zerbino & Birney, 2008) as previously described (Hug et al., 2013). Scaffolds of each re-assembly were annotated as described in the SOM. Genome completeness was evaluated using a suite of 76 genes selected from a set of single-copy marker genes that show no evidence for lateral gene transfer (Sorek et al., 2007; Wu & Eisen, 2008) (Tables S2 and S3). The ML-Gamma genomic bin was annotated by RAST for comparison to the strain ML1 (Chromatiaceae) genome. The number of genomes per bin assigned to the Deltaproteobacteria was estimated using 35 single-copy orthologous groups (Raes et al., 2007; Handley et al., 2012; Table S4). Genome coverage was estimated by assuming that the genome size of each phylotype was approximately the same as its closest relative (Whitaker & Banfield, 2006; Jones et al., 2012).

**Taxonomic assignments and phylogenetic analyses**

16S rRNA sequences often fail to assemble into larger contigs and scaffolds in metagenomic studies, and independent assemblies of these genes (e.g., Phyloshop (Shah et al., 2010) or EMIRGE (Miller et al., 2011)) are difficult to assign to bins separated by genomic signatures such as %GC-content or tetranucleotide frequency. To overcome these obstacles, single-copy ribosomal proteins were analyzed as described in the SOM (Table S5) to make genus-level assignments of the ML-Gamma genomic bin and the ML-Epsilon genomic bin. Such analyses yield resolution comparable to that for 16S rRNA phylogenetic trees (Hug et al., 2013).

The phylogenetic position of bacterial 16S rRNA genes was evaluated by approximate likelihood-ratio tests (Anisimova & Gascuel, 2006) as implemented in PhyML. Sequences were aligned and the best evolutionary model was determined using JMODELTEST (version 2.1.1, Darriba et al., 2012). Maximum-likelihood reconstructions used the General Time Reversible substitution model and gamma-distributed rate variation with a proportion of invariable sites as recommended by JMODELTEST. Phylogenograms were rate-smoothed using the multidimensional version of Rambaut’s parameterization as implemented in PAUP v. 4.0 (Swofford, 2001) as previously described (Meuser et al., 2013). Methods for gene-specific phylogenetic analyses (e.g., suxB, crnO; Table S6) are provided in the SOM.

**Nucleotide sequence accession numbers**

Metagenome bin sequences are deposited at CAMERA (http://camera.calit2.net/), and the assembled sequences can be accessed via IMG/M (http://img.jgi.doe.gov). Metagenome bin sequences—ML-Gamma and ML-Epsilon—are deposited at DDBJ/EMBL/GenBank under the accession numbers AZMQ01000000 and AZMQ010000000, respectively; and the versions described in this paper are AZMQ01000000 and AZMQ0100000000, respectively. The draft genome sequence of *Thiohalocapsa* sp. strain ML1 (Chromatiaceae) is deposited at DDBJ/EMBL/GenBank under the accession JABX00000000. The version described in this paper is version JABX0100000. Gene IDs reported in Table S6 represent the assembled gene sequences available on the IMG/M Web site.

**RESULTS**

Genomic sequencing of the highly enriched culture of strain ML1 resulted in a draft genome of strain ML1, a Chromatiaceae sp. Contigs of the draft genome of strain ML1 were easily resolved from a contaminant organism belonging to the class Mollicutes by %GC (68% vs. ~30%) and verified by BLAST. The draft genome assembled into 137 scaffolds containing 6.3 Mbp (Table 1).

*De novo* assembly of the metagenomic data from 7 m water depth resulted in 452 140 contigs containing ~640 Mbp. The vast majority of contigs could be assigned to bacteria, while smaller numbers of eukaryotic, archaean, and viral sequences were recovered (Fig. 2A). Most of the bacterial contigs (>60%) were affiliated with Proteobacteria, but sequences affiliated with Firmicutes, Bacteroidetes, and Actinobacteria were also abundant (Fig. 2B). Tetranucleotide frequency analysis of the *de novo* metagenomic assembly resolved 26 distinct taxonomic bins (Fig. S1), including well-defined genomic bins for a Gammaproteobacterium (ML-Gamma) and an Epsilonproteobacterium (ML-Epsilon). These two organisms represent the dominant members associated with the oxidative arm of the sulfur cycle. Four distinct bins of sulfate-reducing Deltaproteobacteria (ML-Delta), including Desulfobacteria and Desulfovibrionales, were also resolved, and these bins collectively contained at least eight genomes (Fig. S2). As observed in other studies employing similar binning strategies (e.g., Handley et al., 2012), the coverage, GC-content, and phylogenetic similarity of scaffolds within the Deltaproteobacteria bins precluded further resolution of individual genomes. In contrast, the Gamma- and Epsilon-proteobacteria bins each contained a single genome. We also recovered six low coverage bins affiliated with the Rhyodobacteriales of the Alphaproteobacteria, three bins of Actinobacteria and Bacteroidetes, two other (non-phototrophic) Gammaproteobacteria bins (Xanthomonadales and Thiootricales), and single bins affiliated with Betaproteobacteria (Burkholderiales), Cyanobacteria, Spirochetes, and Mollicutes, (Fig. S1). Based on %GC and BLAST results, the Mollicutes bin recovered from the metagenome is not the same Mollicutes organism present in the co-culture with strain ML1 (data not shown). These results reveal a diverse capacity for sulfur and carbon cycling in the Mahoney Lake chemocline.
Here, we will focus on genomic insights as they relate to sulfur and carbon cycling catalyzed by the high-coverage Gammaproteobacterium, the Epsilonproteobacterium, and the members of the Deltaproteobacteria. Together, these taxa are sufficient to demonstrate a complete oxidative, reductive, and phototrophic sulfur cycle. We also discuss the correspondence between the high-coverage Gammaproteobacterium and isolate strain ML1, and we address implications for the presence of the carotenoid pigment, okenone, in the geologic record.

Oxidative sulfur cycle

Strain ML1 (Chromatiaceae) and the ML-Gamma genomic bin

The highest-coverage organism in the metagenome, ML-Gamma (Fig. 2C), contains a 16S rRNA gene identical to strain ML1 (Coolen & Overmann, 1998; EMBL acc. no. AJ006212). Phylogenetic analysis of 19 concatenated phylogenetic marker genes (Table S5) indicates that the ML-Gamma genome bin is strain ML1 or a very closely related ecotype (Fig. S3). Alignment of single-copy genes between strain ML1 and the ML-Gamma bin (Table S2) shows >99% identity across these phylogenetic markers. The ML-Gamma genome bin is similar in size (5.7 Mbp) to the draft genome (6.3 Mbp) of strain ML1 and contains 73 of these 76 marker genes (Table S2), indicating that it is >90% complete.

Taxonomic assignment places strain ML1 into the order Chromatiales and the family Chromatiaceae. In contrast to the initial assignment based on morphological and physiological characteristics, our 16S rRNA gene sequence analysis indicates that this strain is most closely related to Thiohalocapsa spp. and Halochromatium spp. (Fig. 3). The 16S rRNA gene of strain ML1 is 98% identical to that of Thiohalocapsa halophila 4270T (DSM 6210T), which is its closest cultured relative. In contrast, phylogenetic analysis
based on concatenated sequences of phylogenetic marker genes shows that strain ML1 is most closely affiliated with *Thiorhodovibrio* spp., including *Thiorhodovibrio* sp. 970 and the Mahoney Lake shoreline isolate *Thiorhodovibrio winogradsky* (Overmann et al., 1992) (Fig. S3); however, this analysis is restricted to available complete genomic sequences, which currently includes no members of the genera *Thiohalocapsa* and *Halochromatium*.

Strain ML1 and the ML-Gamma metagenomic bin both encode the genes necessary for photoautotrophic growth utilizing reduced sulfur compounds as the electron source for carbon dioxide reduction. These genomes contain a *psulf* operon encoding a type-2 photochemical reaction center comprised of the subunits PufL, PufM, and PufH, which are identical in arrangement and sequence to the previously published *psulf* operon of the isolate (Tuschak et al., 2005); bacteriochlorophyll *a* biosynthesis genes; and genes (e.g., *csoS*) for alpha-carboxysomes. Both genomes contain *cbbLS* and *cbbM* genes, which encode Forms I and II of RuBisCO, the key enzyme in the Calvin–Benson–Bassham (reductive pentose phosphate) cycle. Genes encoding the large and small subunit of RuBisCO form I, *cbbL* and *cbbS*, are clustered with *cso* genes encoding carboxysome shell proteins; while the Form II gene *cbbM* is located elsewhere in the genome. The *CbbL* and *CbbM* sequences of strains ML1 and ML-Gamma are closely related to the large and small subunit of RuBisCO form I, *CbbL* and *CbbS*, respectively. Scaffold numbers refer to the location of predicted genes in the draft genome of strain ML1. Proteins and the reactions they catalyze in the pathway for the biosynthesis of okenone, from Vogl & Bryant, 2011, are summarized.

\[ \text{Okenone} \]

\[ \gamma \text{-ring of okenone, and the unique CruO 4/4' ketolase protein (Vogl & Bryant, 2011, 2012) (Fig. S6). Importantly, the genes encoding CruO and the associated CruS enzyme in the ML-Gamma genome bin are the only copies of these diagnostic genes detected in the entire metagenome, establishing that this PSB taxon is probably the only source of okenone in the chemocline.} \]

The genomes of strain ML1 and ML-Gamma encode thiosulfate oxidation enzymes (Sox), a flavocytochrome *c*-sulfide dehydrogenase (*Fcc*), and a *D*-type sulfide:quinone oxidoreductase (*Sqr*) (Fig. 5, Fig. S7) (Gregersen et al., 2011). SoxA, B, X, Y, and Z comprise a multi-enzyme complex of SoxXA, a heterodimeric *c*-type cytochrome; SoxB; and SoxYZ, a heterodimeric thiosulfate-binding protein necessary for oxidation of thiosulfate to sulfate. As in other purple sulfur bacteria, no *ssoCD* genes were found in either the isolate genome or the genome bin. In other *Chromatiaceae* that lack *ssoCD* – for example, *Allochromatium vinosum* – sulfur/polysulfide globules form in the periplasm as oxidation intermediates (Hensen et al., 2006; Frigaard & Dahl, 2009; Dahl et al., 2013). In *Alc. vinosum*, reverse dissimilatory sulfite reductase (*DsrAB*) and other related *Dsr* proteins – including the transmembrane complex *DsrMKJOP*, the *DsrEFH* complex, and *DsrC* – are necessary for oxidation of the...
stored sulfur to sulfite (Dahl et al., 2008; Oliveira et al., 2008; Grein et al., 2010). The DsrEFH complex, involved in sulfur transfer reactions, is encoded in genomes of sulfur-oxidizing prokaryotes that also encode DsrAB but is absent in sulfate-reducing organisms (Sander et al., 2006; Grimm et al., 2008). Genes encoding the reverse Dsr proteins, including DsrEFH, are present in strain ML1 (and ML-Gamma), and their gene context is similar to the genomic context that is observed in *Alc. vinosum* (Grein et al., 2010).

Genes encoding homologues of key enzymes involved in dissimilatory oxidation of reduced sulfur and assimilatory sulfate reduction are present (Fig. 5), including an adenosine phosphosulfate reductase (APS reductase, ApsAB) and a sulfate adenylyltransferase (ATP-sulfurylase, Sat). In some PSB, indirect oxidation of sulfite in the cytoplasm results from the activity of APS reductase and ATP-sulfurylase; however, these enzymes are not required for sulfite oxidation and are not universally found in sulfur-oxidizing prokaryotes (Dahl et al., 2013). The genomes also encode homologues of the SocABC sulfite-oxidizing enzyme complex, which is believed to oxidize sulfite presumably by transferring electrons to the electron transport chain via the quinone pool (Dahl et al., 2013), as well as enzymes involved in assimilatory sulfate reduction including adenylly sulfite kinase (CysC), phosphoadenosine phosphosulfate reductase (CysH), and an NADPH-dependent sulfate reductase (CysJI) (Fig. 5).

The ML-Epsilon genomic bin
The ML 7-m metagenome contains a well-defined genomic bin identified as an Epsilonproteobacterium closely related to *Sulfurimonas autotrophica* (Fig. 6), a chemo-lithoautotrophic, sulfur-oxidizing aerobe that can use elemental sulfur, sulfide, and thiosulfate as electron donors (Inagaki et al., 2003). Based on the presence of phylogenetic marker genes, the assembled genome is estimated to be >90% complete, and it is similar in size (~2.7 Mbp) (Table 1) to that of several closely related species, including the genome of *S. autotrophica* (~2.1 Mbp). Similar to characterized chemolithoautotrophic Epsilonproteobacteria, the ML-Epsilon genome encodes the enzymes necessary for carbon fixation via the reverse TCA (rTCA) cycle, including ATP-dependent citrate lyase (*acl*), pyruvate:ferrodoxin oxidoreductase (*parAB*), and 2-oxoglutarate:ferrodoxin oxidoreductase (*vorAB*) (Campbell et al., 2006), but does not contain RuBisCO genes (*cbbLS* or *cbbM*). Unlike *S. autotrophica*, the ML-Epsilon genome encodes enzymes for the reduction of nitrate to N₂, including a periplasmic nitrate reductase (*NapAB*), a cytochrome *cd*₁-dependent nitrite reductase (*NirS*), a nitric oxide reductase (*NorBC*), and nitrous oxide reductase (*NosZ*). These genes, together with the *sox* and *sqr* genes, suggest that nitrate-dependent sulfide oxidation can occur under anoxic conditions (Simon & Klotz, 2013). Similar to other *Sulfurimonas* spp., the ML-Epsilon genomic bin encodes a Mo-dependent nitrogenase and an ammonium transporter (*Amt*). It also contains cytochrome *cbb₃* oxidase and cytochrome *bd* quinol oxidase genes, both of which are high-affinity terminal oxygen reductases capable of functioning under micro-oxic conditions; formate dehydrogenase (*fdhABC* genes); and two [NiFe]-hydrogenases, one cytoplasmic and one membrane-bound—suggesting this organism may use H₂ and formate as electron donors.

Fig. 6 Maximum-likelihood phylogenetic tree of 14 concatenated single-copy ribosomal proteins (Table S5) showing the taxonomic placement of the ML-Epsilon genomic bin. Bootstrap support values based on 1000 bootstrap samplings were >85 for each node.
thiosulfate to sulfate (Fig. 5). The genome also encodes a membrane-bound polysulfide reductase (psr-encoded), which may enable the use of polysulfide as an electron acceptor (Hedderich et al., 1998); a sulfite cytochrome c oxidoreductase (Sor), possibly involved in the inorganic disproportionation of reduced sulfur compounds (Finster, 2008); and a rhodanese-related sulfurrtransferase presumably involved in the oxidation of thiosulfate (Ghosh & Dam, 2009).

**Other organisms putatively involved in the oxidative sulfur cycle**

The 7-m metagenome contains multiple copies of key enzymes involved in sulfur oxidation that are assigned to other taxonomic bins. These include genes encoding sulfide:quinone oxidoreductase (Sqr), flavocytochrome c (Fcc), components of dissimilatory sulfite reductase (DsrAB, DsrEFH, DsrC, and the DsrMKJOP complex), the Sox multi-enzyme complex, and components of assimilatory sulfate reduction (CysND, CysC, CysH, CysJL, Sat, and Sir) (Fig. 5; Table S7). The sow-encoded enzymes of thiosulfate oxidation are most abundant among these, while six copies each of homologs of DsrE, F, and H, specific to sulfur-oxidizing organisms are present. Full-length SoxB sequences affiliated with the Alpha-, Beta-, and Gammaproteobacteria were recovered from other tetranaucleotide-resolved genomic bins (Fig. 7). More putative sequences for SoxA and SoxB were present (31 and 33 copies, respectively) than the sow-encoded proteins C, D, X, Y, Z (Table S7), and the majority are affiliated with Alphaproteobacteria. Several sow and sgr genes affiliated with GSB, which use the Sox system for thiosulfate oxidation (Ogawa et al., 2008; Gregersen et al., 2011), were also observed (Table S7), despite the low number of reads assigned to the phylum Chlorobi. The translated sgr sequences cluster near the SqrD-type of Chlorobium tepidum TLS (Gregersen et al., 2011; Holkenbrink et al., 2011) (Fig. S7; Table S8).

**Reductive arm of the sulfur cycle**

**ML Deltaproteobacteria community genomic bins**

The Mahoney Lake chemoline contains multiple taxa of Deltaproteobacteria, including at least eleven distinct genotypes (Fig. S8A) based on ribosomal protein S3 sequences (Hug et al., 2013). At least six genomes of Desulfurobacteriales spp. (present in genomic bins designated Delta 1, 3, and 4) and at least two Desulfuvibionales spp. genomes (genomic bin designated Delta 2) (Table S9, Figs S1 and S2) could be resolved. The high degree of phylogenetic similarity, GC-content, and coverage among the Deltaproteobacteria spp. precludes the resolution of single genomes. The recovery of multiple sulfate-reducing bacteria (SRB) genomes across several tetranaucleotide bins indicates organismal redundancy is also present within the reductive arm of the sulfur cycle.

The genomic bins assigned to the Desulfurobacteriales and Desulfuvibionales encode the enzymes necessary for dissimilatory sulfate reduction, including a sulfate adenyltransferase (ATP-sulfurylase, Sat), an APS reductase (AprAB), and the dissimilatory sulfite reductase complex (DsrAB, DsrC, and DsrMKJOP), which catalyzes the reduction of SO\(_{4}^{2-}\) to S\(^{2-}\) (Fig. S8B). The genomic bins also encode Mn-dependent, inorganic pyrophosphatase (PpaC) and abundant ferredoxins (predominantly Ferredoxin I). The genomes contain homologues of D-lactate dehydrogenase (gene ldlA) and pyruvate dehydrogenase (gene pdh) for conversion of lactate to acetyl-CoA, as well as carbon monoxide dehydrogenase/acetyl-CoA synthetase (CODH/ACS). Many acetotrophic SRBs employ a CODH/ACS pathway to cleave acetyl-CoA, and yet the presence of CODH/ACS genes in SRBs closely related to autotrophic species (i.e., Desulfovibrio spp.; Fig. S8A) also suggests at least facultative autotrophy may occur (Schauer et al., 1989). The ML-Desulfuvibionales genomic bins also encode fumarate reductases, indicating that these organisms may be capable of fumarate respiration; a periplasmic [FeFe]-hydrogenase (HydA); and heterodisulfide reductase-like proteins, specifically HdrA and HdrD-related proteins, which are thought to be involved in sulfite reduction through DsrC or reduction of the menaquinone pool (Pereira et al., 2011). The SRB genomic bins also encode Mo-dependent nitrogensases (nifHDK).

**Other organisms putatively involved in the reductive sulfur cycle**

Genes from other species involved in reducing sulfur compounds include 21 unique copies of dsrD. Although the exact function of DsrD remains unknown, it is postulated to be involved in sulfite reduction (Grein et al., 2013) and is typical of SRB, but it is not found in sulfur-oxidizing organisms (Grimm et al., 2008). The metagenome also contains tetrathionate reductase genes (ttt), which catalyze the reduction of tetrathionate to thiosulfate and are widely distributed in the Enterobacteriaceae and other facultative anaerobes (Zopfi et al., 2004). Dimethyl sulfoxide (DMSO) reductase (DmsA), also common in anaerobic heterotrophic bacteria, was identified. The majority of ttt and dmsA sequences were affiliated with the Firmicutes, Deltaproteobacteria, and the Alpha- and Gammaproteobacteria (Table S7).

**Phylogenetic diversity of dsr**

The 7-m metagenome contains eleven distinct copies of dsrAB, encoding dissimilatory sulfite reductase, the enzyme necessary for sulfite reduction or sulfur oxidation (Fig. 8). Concatenated amino acid sequence alignments of full-
Fig. 7 Phylogenetic diversity of SoxB in the Mahoney Lake chemocline. Maximum-likelihood phylogenetic tree constructed from translated SoxB sequences identified in the ML 7-m metagenome. Red, Alphaproteobacteria; orange, Betaproteobacteria; purple, Gammaproteobacteria; green, green sulfur bacteria; black, Acidithiobacillia; yellow, Epsilonproteobacteria; blue, Aquificales. The strain ML1 genome and ML-Gamma genomic bin contain identical SoxB sequences represented here as ML1. Abbreviations: SRB, sulfate-reducing bacteria; SOB, sulfur-oxidizing bacteria. Bootstrap support values based on 1000 bootstrap samplings \( \geq 85 \) are shown for each node.
length alpha and beta subunits (DsRA and DsRB, respectively) confirm the presence of multiple sulfate-reducing Deltaproteobacteria-affiliated sequences and several sulfur-oxidizing Alpha- and Gammaproteobacteria-affiliated sequences. Several of these SRB-affiliated sequences are not represented in the genome bins discussed above and presumably derive from other less abundant taxa in the metagenome. Several of these SRB-affiliated sequences are not oxidizing Alpha- and Gammaproteobacteria-affiliated Deltaproteobacteria-affiliated sequences and several sulfur-oxidizing members of the Alpha- and Gammaproteobacteria, RLP are involved in 5-methylthioadenosine-metabolism (Hanson & Tabita, 2003), while in some Alpha-proteobacteria, RLP are involved in in situ sulfur metabolism (Hanson & Tabita, 2003), while in some Alpha-proteobacteria, RLP are involved in 5-methylthioadenosine-dependent growth (Tabita et al., 2007). The remaining sequences all branch with characterized RuBisCO proteins, indicating they are involved in CO2 assimilation in situ. In addition to the Type I and Type II RuBisCO identified in the ML-Gamma genomic bin, the metagenome contains three sequences affiliated with Type I RuBisCO (similar to Thioalkalivibrio spp. and members of the phyla Cyanobacteria and Chloroflexi) and two affiliated with Type II RuBisCO (similar to sequences of Sulfuricella denitrificans, a
sulfur-oxidizing Betaproteobacterium, and *R. rubrum* (Fig. S9). Two additional sequences branch with the methanogen Type III RuBisCO and two sequences group together with the novel bacterial–archaeal hybrid Type II/III sequences (Fig 8), which are putatively functionally analogous to Type III (Tabita *et al.*, 2007); these enzymes are thought to fix CO₂ and contribute to adenosine monophosphate (AMP) recycling (Wrighton *et al.*, 2012).

**DISCUSSION**

**Identification of the dominant PSB in Mahoney Lake**

The enrichment culture strain ML1 (*Chromatiaceae; Overmann *et al.*, 1991) is very closely related to the highest-coverage population in the 7-m metagenome, indicating that strain ML1 is representative of the major PSB population. This PSB is believed to be the most abundant organism in the Mahoney Lake chemocline (Overmann *et al.*, 1991), and the similarity between our metagenome (sampled in 2008) and strain ML1 (enriched in 1989) confirms decadal stability of this population. The initial classification of strain ML1 as *Amoebobacter purpureus* was based on cell morphology, the presence of gas vesicles, okenone production, and its ability to use glucose, pyruvate, and acetate (Overmann *et al.*, 1991). Due to the re-classification of *A. purpureus* as a *Lamprocystis* species (Guyoneaud *et al.*, 1998), strain ML1 has been referred to as ‘*Lamprocystis purpurea* ML1’ (EMBL acc. nos. AJ006212, AY177752, Klepac-Ceraj *et al.*, 2012). However, initial analyses of the 16S SSU rRNA sequence suggested that the isolate had been misclassified (Coolen & Overmann, 1998), an observation bolstered by more recent analyses of the 16S rRNA and the *psbLM* genes, both of which indicated that strain ML1 is affiliated with the genus *Thiohalocapsa* (Tank *et al.*, 2009). Phylogenetic analyses presented here and elsewhere (Coolen & Overmann, 1998; Tank *et al.*, 2009) show this organism is most closely related to marine mat-forming members of the *Chromatiaceae*, such as *Thc. halophila* as well as *Halochromatium roseum* and Hch. glycolicum. The 16S rRNA sequences from *Thc. halophila* (formerly *Thiocapsa halophila*; Caumette *et al.*, 1991) and strain ML1 are 98% identical. Although some characteristics of strain ML1—such as the presence of gas vesicles and production of okenone—are also characteristic of *Lamprocystis* spp., strain ML1 exhibits higher salt tolerance than freshwater species (Imhoff, 2005). Moreover, gas vesicles and okenone are not taxonomically diagnostic. Gas vesicles have been observed in multiple genera of *Chromatiaceae*, including *Thiocapsa*, *Thiodictyon*, *Thiolamprovum*, *Thiopedia*, *Lamprocystis*, *Lamprobacter*, and *Halochromatium* (Kumar *et al.*, 2007). Okenone production is similarly irregularly distributed; it is mainly found in the gas vesicle containing genera *Thiocapsa*, *Thiodictyon*, *Lamprocystis*, *Lamprobacter*, and *Thiolamprovum*, but it is also produced by the halophilic species *Hch. roseum* (Kumar *et al.*, 2007) and the non-gas vacuolate relative of strain ML 1, *Thc. halophilum*. Based on the robust 16S rRNA gene analyses presented here, the clade containing strain ML1 and *Thc. halophilum* is distinct from other major clades of planktonic PSB (e.g., *Lamprocystis* spp., *Chromatiaceae* spp.) (Fig. 3), including ‘Ca. Thiodictyon syntrophicum’ Cad16, an abundant PSB that occurs in the compact chemocline of Lake Cadagno (Storelli *et al.*, 2013). As a result, we
suggest that strain ML1 is a potentially new species within the genus *Thiohalocapsa* and propose that it be renamed *Thiohalocapsa* sp. strain ML1.

A coupled sulfur cycle in a stable planktonic community

Metagenomic sequencing of the Mahoney Lake chemocline indicates this sulfur-rich ecosystem harbors functional redundancy in both the oxidative and reductive arms of the sulfur cycles (Fig. 10A). This functional redundancy could be a key factor in maintaining the apparent stability of the Mahoney Lake chemocline over the past ~30 years, or it may have developed as a result of such stability. The recovery of aerobic taxa such as Cyanobacteria and Epsilonproteobacteria may suggest fine-scale niche separation within the narrow phototrophic plate, the top of which is bounded by the oxic epilimnion. Alternatively, these taxa may represent biomass from the oxygenated layer, which settled and was captured by the dense phototrophic plate. Both Cyanobacteria and Epsilonproteobacteria are capable of living in micro-oxic as well as sulfidic conditions (Jorgensen *et al.*, 1986; Campbell *et al.*, 2006). Conversely, the bottom of the plate intersects the highly sulfidic (35 mM) hypolimnion, an environment also rich in polysulfides, elemental sulfur, and other sulfur intermediates that may be amenable to sulfur disproportionation. Single genome bins (single populations) of sulfur oxidizers recovered in the present study contain multiple pathways for oxidation of sulfur compounds, implying flexibility and/or redundancy in their sulfur metabolism, a genomic characteristic observed in other sulfidic systems (Jones *et al.*, 2012). Isotope values of sulfur species in the chemoclines of other meromictic lakes, such as Fayetteville Green Lake, indicate that multiple processes contribute to sulfur oxidation and that these vary seasonally (Zerkle *et al.*, 2010). A similar seasonal variation may also occur in the

![Fig. 10](image-url) Genomic models of the strain ML1/ML-Gamma, ML-Epsilon and the ML-Delta spp. (A) and putative model of the sulfur cycle in the Mahoney Lake chemocline (B). The loss of biomass (organic carbon and reduced sulfur, purple arrow) from the chemocline due to rafting, external organic carbon as a significant source of carbon to SRB in the chemocline (TOC, brown arrow) and loss of S0 to the lower layers of the lake (yellow arrow with dashes) are depicted. Further results and discussion of strain ML1/ML-Gamma, ML-Epsilon, and the ML-Delta spp. are provided in the SOM. AcIB, ATP-dependent citrate lyase; Amt, ammonia/ammonium transporter; Apr, adenylyl sulfate (APS) reductase; cbb3, cytochrome c oxidase; Cyt3, cytochrome c3; Dsr, dissimilatory sulfite reductase; Fdh, formate dehydrogenase; Gvp, gas vesicle proteins; Hyd, hydrogenase; Hme, heterodisulfide reductase-like menaquinol-oxidizing enzyme complex; LH, light-harvesting complex; Ldh, lactate dehydrogenase; NapAB, NO3/C02 reductase; NirS, NO2/C02 reductase; NorBC, NO reductase; NosZ, N2O reductase; Nqr, Na+-translocating NADH:quinone oxidoreductase; RC, photosynthetic reaction center; Pdh, pyruvate dehydrogenase; Psr, polysulfide reductase; rTCA, reductive TCA cycle; Omo, quinone-interacting membrane-bound oxidoreductase complex; Qrc, quinone reductase complex; Rhd, rhodanese-related sulfurtransferase; Rnf, Rnf-electron transport complex; Sat, ATP-sulfurylase; Sox, sulfur oxidation proteins; Sdh, succinate dehydrogenase/fumarate reductase; Soe, sulfate-oxidizing enzyme complex; SQR, sulfide:quinone oxidoreductase; Sqr, sulfide:quinone oxidoreductase; SOB, sulfur-oxidizing bacteria; SRB, sulfate-reducing bacteria.
chemocline of Mahoney Lake, supporting functional redundancy in sulfur oxidation pathways and a diversity of sulfur-oxidizing species.

Historical data from Mahoney Lake show that the chemocline is relatively stable in both thickness and depth, although seasonal variation (e.g., autumnal die-off) affects the total cell abundance of *Thiohalocapsa* sp. strain ML1 (Overmann *et al.*, 1994). Other anaerobic phototrophic bacteria, including *Thiocapsa* sp. and GSB (Overmann *et al.*, 1991) as well as other autotrophs (e.g., ML-Epsilon; this study) and a large number of Alphaproteobacterial sequences (this study), are observed in the Mahoney Lake chemocline. However, in past studies, *Thiohalocapsa* sp. strain ML1 was responsible for >98% of primary productivity and, on average, 75% of the sulfide oxidation (Overmann *et al.*, 1991, 1996a). In contrast, the narrow chemocline of Lake Cadagno, a meromictic lake in Switzerland, hosts multiple populations of PSB, including *Chromatium*, *Lamprocytis*, *Thiocysit*, and *Thiodictyon* spp., as well as GSB of the genus *Chlorobium* (Bosshard *et al.*, 2000). These multiple species of PSB persist and remain active despite a recent increase in the abundance of *Chlorobium clathratiforme* (Decristophris *et al.*, 2009; Gregersen *et al.*, 2009). In particular, ‘Ca. Thiodictyon syntrophicum’ strain Cad16 accounts for over 25% of primary production and is also a prominent source of CO₂ assimilation in the dark (Storelli *et al.*, 2013).

It is unclear what factors support the limited diversity among photoautotrophs in the Mahoney Lake chemocline. Several lines of evidence indicate that of the total *Thiohalocapsa* sp. strain ML1 biomass, a relatively small proportion (~15%), is physiologically active (Overmann *et al.*, 1991, 1994). The formation of buoyant aggregates of *Thiohalocapsa* sp. causes high cell numbers to accumulate within the chemocline (Overmann & Pfennig, 1992), and the high salinity of the hypolimnion and gas vacuolation of the cells inhibit sedimentation of these aggregates (Overmann *et al.*, 1991). The elevated levels of sulfate and especially sulfide (35 mM) in Mahoney Lake may create a very narrow niche space that precludes the majority of anoxygenic photoautotrophs. Specific adaptations including elevated salt tolerance and adaptation to low light (Overmann *et al.*, 1994) may enable *Thiohalocapsa* sp. strain ML1 to persist, albeit with very slow growth rates (Overmann *et al.*, 1991, 1994).

Phylogenetic analyses of the ribosomal protein S3 indicates multiple SRB spp. are present (Fig. S8A) in the Mahoney Lake chemocline. This record of SRB is consistent with the observation that sulfate reduction provides the majority of reducing equivalents for PSB-dominated primary production, and more specifically, that some of the sulfide is supplied in situ rather than by vertical mixing (Overmann *et al.*, 1996a). Redox oscillations at the oxic-anoxic boundary, as is common in sediment systems (Aller, 1998), are also observed both diurnally and seasonally in Mahoney Lake. The associated small vertical excursions of the chemocline (Hall & Northcote, 1990) may help maintain a diverse population of SRBs. Alternatively, varying levels of oxygen or sulfide tolerance among taxa could result in increased diversity (Baumgartner *et al.*, 2006).

Encompassing both of these ideas, the metagenomic data presented here contain genetic evidence for both heterotrophic and autotrophic SRB, implying that fluctuations in carbon supply play a role in maintaining multiple SRB spp. in the chemocline. SRB utilize organic compounds as electron sources for cellular metabolism. However, autotrophic growth of hydrogen-utilizing SRB via the Wood-Ljungdahl pathway has been demonstrated in some species, including close relatives of some of those recovered in the present study, that is, *Desulfobacteriaciae* (Rabus *et al.*, 2013). The strong density gradient in Mahoney Lake inhibits much of the in situ organic matter of the chemocline from entering the layers below (Overmann *et al.*, 1991), implying it is consumed by heterotrophs within the chemocline or lost to vertical mixing and/or buoyancy. During the autumnal die-off and upwelling of PSB biomass (Overmann *et al.*, 1994), however, there is minimal concomitant change in net activity in the chemocline. This implies that the total chemocline sulfate reduction rate is relatively stable, even if the loss of this organic matter affects the fractional balance of processes (autotrophic vs. heterotrophic carbon supply). This seasonal variation in organic carbon supply may support the multiple SRB spp. observed in the Mahoney Lake chemocline.

**The energetic maintenance of euxinia**

Although sulfate reduction and sulfide oxidation both occur in the Mahoney Lake chemocline, such a system is not self-sustaining. To satisfy the basic principles of thermodynamics and maintain a coupled oxidative and reductive sulfur cycle, exogenous input of electrons is a stoichiometric requirement (Overmann, 1997). All of the sulfide in Mahoney Lake must be derived from sulfate reduction, with the electrons provided by respiration of organic matter. However, oxygenic primary production in the epilimnion is not sufficient to supply these electrons in the form of phytoplanktonic carbon fixed in situ. Instead, most of the needed organic matter must come from the surrounding landscape (Overmann *et al.*, 1996a; Overmann, 1997).

Flux balance analysis confirms this principle. From data in Overmann *et al.* (1996a), we calculate an average diffusive vertical mixing of 9.2 mmol H₂S m⁻² day⁻¹ and 23 mmol SO₄²⁻ m⁻² day⁻¹ into the chemocline (described in the SOM). The H₂S flux is only sufficient to supply ~40% of measured oxidation rates (Overmann *et al.*, 2014 John Wiley & Sons Ltd.
1991), implying that SO$_4^{2-}$ must be reduced in situ in the chemocline to supply the remainder of the H$_2$S. This is consistent with suggestions that input of allochthonous – and therefore necessarily oxygenic – organic matter is required to supply the extra flux of electrons needed to sustain sulfate reduction (Overmann, 1997). The question then remains: where does all the upwelled sulfur go? The epilimnion maintains a net SO$_4^{2-}$ deficit (and zero H$_2$S) relative to the hypolimnion, while sediment trap data show that <0.5% of the total upward sulfur flux sinks out of the chemocline as S$^0$ (Overmann et al., 1994). To close mass balance, the remainder must be rafted to the shoreline in association with the annual partial die-off and buoyant dispersal of the PSB from the chemocline (Overmann et al., 1994)(Fig. 10B). This relationship implies that the net sulfur cycle in the lake primarily is SO$_4^{2-}$ → S$^2-$ (H$_2$S) → S$^0$, that is, the terminal sink for sulfur is significantly more reduced relative to its oxidized input (SO$_4^{2-}$ from surrounding rocks). The ability to sustain net sulfate reduction in such a euxinic system, both in the chemocline and in the deeper hypolimnion, at first appears remarkable. However, simple free energy calculations under in situ conditions ($\Delta G = \Delta G^0 + RT \ln Q$) based on collated carbon and sulfur species concentrations from the literature (Northcote & Hall, 1983; Overmann et al., 1991, 1996a, b) show that while Mahoney Lake represents an extreme end-member of euxinia with regard to sulfide concentrations, the H$_2$S:SO$_4^{2-}$ ratio in this system still is not high enough to thermodynamically inhibit sulfate reduction (Amend & Shock, 2001; Table S10, Fig. S10). The persistence of sulfate reduction under the extreme euxinic conditions Mahoney Lake implies that – at least under oxygenated atmospheres – SRB should be active in anoxic systems throughout Earth history, regardless of the degree of euxinia.

The redox stability and stable, poised conditions of Mahoney Lake likely result from its unique combination of high salinity, high sulfur concentrations, and high allochthonous carbon loading. The more dilute conditions of Lake Cadagno – another analogue for ocean euxinia – result in greater seasonal changes in fluxes, redox variability, and microbial community succession (Bosshard et al., 2000; Del Don et al., 2001; Decristophiris et al., 2009; Grgersen et al., 2009). More generally, the concentration of free sulfide in any water column should scale directly with the extent of organic matter overloading, and the extreme case of Mahoney Lake undoubtedly is a factor in its biogeochemical and ecological stability.

Implications for biosignatures of photic zone euxinia

The chemocline of Mahoney Lake provides valuable insight into the geologic record of anoxicogenic phototrophy. Because the habitat of phototrophic sulfur bacteria is restricted – requiring micro-oxic or anoxic conditions, free sulfide, and sunlight – diageneric products of the carotenoid okenone have been invoked as markers for shallow photic zone euxinia (Brocks et al., 2005; Brocks & Schaef-fer, 2008). However, the synthesis of okenone is irregularly distributed among Chromatiaceae, both taxonomically and physiologically. Therefore, it is still debated whether diageneric okenone in geologic sediments is of planktonic origin, or if it also may be derived from benthic mat communities (Meyer et al., 2011) in which PSB are widespread and diverse (Imhoff, 2005). Mat- or benthic-derived isolates that produce okenone include Halochromatium roseum JA134 (Kumar et al., 2007), Thiocapsa marina (Caumette et al., 1985, 2004), and the closest relative of Thiohalo- capsap sp. strain ML1, Th. halophila (Caumette et al., 1991; Kumar et al., 2007).

Okenone in ML sediments (Overmann et al., 1993; Colen & Overmann, 1998) has been linked to the presence of Thiobahalocapsap sp. strain ML1 in the water column (Overmann et al., 1991). The metagenomic sequencing and phylogenetic analyses presented here show that the only detectable okenone producer in the water column is indeed this species. It is not only closely related to Thc. halophila, but it also appears to be more closely related to the Trv. winogradskyi isolated from the shoreline mats (Overmann et al., 1992) than to typical planktonic members of the Chromatiaceae, such as ‘Ca. Thiodicycton syntrophicum’. The taxonomic similarity between Thiobhalocapsap, Halochromatium, and Thiorhodovibrio genera (Tank et al., 2009; this work) suggests that a more general continuity between planktonic and benthic PSB communities exists, especially where oxygen minimum zones impinge on sediments within the photic zone. Similar to Mahoney Lake, the okenone-rich chemocline of meromictic Fayetteville Green Lake in New York hosts a diverse population of PSB, and phylogenetic analyses of clones from benthic and planktonic habitats in that lake suggest these niches host distinct PSB populations (Meyer et al., 2011). Okenone-rich sediments derived from benthic mats underlying the oxic water column at Fayetteville Green Lake have challenged the current interpretation of okenone as an exclusive biomarker for planktonic PSB. In Mahoney Lake, the example of okenone production in the water column by a PSB strain that is a close relative of benthic species further highlights the difficulty in linking okenone uniquely to water column processes. While it remains assured that the recovery of okenone from ancient sediments is strong evidence for sunlit, sulfidic environments inhabited by PSB, the presence of okenone alone may not be sufficient evidence for photic zone euxinia in the open water column.

At the same time, in environments such as Mahoney Lake where planktonic PZE clearly is present, chemotrophic sulfide oxidation and sulfate reduction also should be suspected as co-occurring reactions in an active sulfur
cycle, mediated by a wide variety of micro-organisms. This is distinct from a cryptic sulfur cycle in which no net sulfur cycle redox processes occur (Canfield et al., 2010), but rather is an expected consequence of the water column anoxia associated with high organic carbon fluxes. Under this model, both PZE and water column sulfate cycling necessarily depend on oxygenic photosynthesis to supply electrons. This implies that PZE is only possible in high productivity, low and moderate $p$O$_2$ oceans (including ocean basins with restricted circulation). Episodes of euxinia in Earth history should require a confluence of all of these conditions, perhaps helping to explain their apparent rarity (e.g., Meyer & Kump, 2008; Johnston et al., 2009; Canfield, 2013; Leavitt et al., 2013).

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**SUPPORTING INFORMATION**

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

**Data S1** Supplementary methods and results.

**Table S1** Metagenome summary statistics.

**Table S2** Marker genes used to evaluate genome completion of the isolate genome, strain ML1, and the ML-Gamma and ML-Epsilon genomic bins.

**Table S3** Marker genes used to evaluate genome completion of the Delta-proteobacteria community genome bins.

**Table S4** Orthologous group markers and their abundance used to estimate the number of complete genomes in the Deltaproteobacteria community genome bins.

**Table S5** Single-copy phylogenetic marker genes used for taxonomic assignment of the ML-Gamma and the ML-Epsilon genomic bins.

**Table S6** List of genes from the Mahoney Lake 7-m metagenome identified and included in functional gene phylogenetic analyses.

**Table S7** Number and taxonomic affiliation of key genes involved in the sulfur cycle.

**Table S8** Accession numbers of the Sqr sequences included in Fig. S6.

**Table S9** Summary of community genomic bins of Deltaproteobacteria spp.

**Table S10** Calculations for \( \Delta G = \Delta G^0 + RT \ln Q \) at 18°C for selected sulfur redox reactions in situ.

**Table S11** List of organisms from which NifH/VnfH/AnfH were identified and included in the phylogenetic analysis of NifH/VnfH/AnfH identified from the Mahoney Lake 7-m metagenome.

**Fig. S1** ESOM (repeating-tiled view) bins of the tetranucleotide frequency signatures of the 7-m metagenome with bins of interest indicated.

**Fig. S2** The estimated number of SRB genomes recovered from the Mahoney Lake metagenome.

**Fig. S3** Maximum likelihood phylogenetic tree of 19 concatenated single-copy ribosomal proteins showing the placement of the strain ML1 and ML-Gamma compared to closely related Gammaproteobacteria spp. for which genome sequences are available.

**Fig. S4** Phylogenetic diversity of RubisCO in the Mahoney Lake chemocline. Maximum likelihood phylogenetic trees constructed from CbbL and CbbM sequences mined from the ML 7-m metagenome and the NCBI and IMG/M database.

**Fig. S5** Phylogenetic diversity of the nitrogenase Fe protein in the Mahoney Lake chemocline. Maximum likelihood phylogenetic trees constructed from CbbL and CbbM sequences mined from the ML 7-m metagenome and the NCBI and IMG/M database.

**Fig. S6** Phylogenetic diversity of RubisCO in the Mahoney Lake chemocline. Maximum likelihood phylogenetic tree of translated genes encoding okenone C-4/4’ ketolase (CrOS), okenone accessory ketolase (CrUS), carotenoid desaturase (CrtD) and methoxynaphtorospine dehydrogenases (CrtD/CrtI family homologs).

**Fig. S7** Maximum likelihood phylogenetic tree of the 7-m metagenome (Table S6) and the NCBI and IMG/M database.

**Fig. S8** Taxonomic diversity of SRB-affiliated SP3 proteins and the Predicted role of the ML SRB spp. in the sulfur cycle.

**Fig. S9** Phylogenetic diversity of RubisCO in the Mahoney Lake chemocline. Maximum likelihood phylogenetic trees constructed from CbbL and CbbM sequences mined from the ML 7-m metagenome and the NCBI and IMG/M database.

**Fig. S10** Calculated values for \( \Delta G = \Delta G^0 + RT \ln Q \) at 18°C for selected sulfur redox reactions in situ, at depths above, within, and below the purple chemocline.