

## ORIGINAL ARTICLE

# Vertical structure of the bacterial diversity in meromictic Fayetteville Green Lake

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**Abstract**

The permanently stratified water columns in euxinic meromictic lakes produce niche environments for phototrophic sulfur oxidizers and diverse sulfur metabolisms. While Green Lake (Fayetteville, New York, NY) is known to host a diverse community of ecologically important sulfur bacteria, analyses of its microbial communities, to date, have been largely based on pigment analysis and smaller datasets from Sanger sequencing techniques. Here, we present the results of next-generation sequencing of the eubacterial community in the context of the water column geochemistry. We observed abundant purple and green sulfur bacteria, as well as anoxygenic photosynthesis-capable cyanobacteria within the upper monimolimnion. Amidst the phototrophs, we found other sulfur-cycling bacteria including sulfur disproportionators and chemotrophic sulfur oxidizers, further detailing our understanding of the sulfur cycle and microbial ecology of euxinic, meromictic lakes.

**KEYWORDS**

bacterial diversity, freshwater, meromictic lake, sulfur cycling

## 1 | INTRODUCTION

Meromictic lakes are permanently stratified lakes, with anoxic bottom waters that accumulate reduced chemical species like sulfide or ferrous iron. In these lakes, complete circulation is prevented by a higher density layer beneath the circulation on the surface, called the mixolimnion, which can act similar to a holomictic lake sitting atop this bottom, unmixing layer, called a monimolimnion. Meromixis can be caused by salt intrusions from outside the lake or via groundwater through saline springs within the lake. For example, Hot Lake (Oroville, WA) is a meromictic lake formed through a flood of highly saline mine drainage, with spring melt flow forming a fresher surface mixolimnion (Anderson, 1985). It can also be caused via *biogenic* meromixis, as in the small kettle lake Devil's Bath tub (Henrietta, NY), which was formed through glacial melt processes and became meromictic through biogenic salts powered by organics loaded from

the surrounding forest, primarily via fall leaves (Stewart, 1997). The permanent stratification of these lakes can lead to a stable vertical redox structure through the oxygenic mixolimnion through the *chemocline* and into the monimolimnion (e.g., Havig et al., 2015). As a result, microbial communities are heavily influenced by physical and geochemical gradients of light, dissolved oxygen, reduced compounds, and so on (Danza et al., 2018; Hamilton et al., 2014; Phillips et al., 2021). In meromictic lakes with euxinic (anoxic and sulfide-rich) monimolimnia, the bacterial communities above, within, and below the chemocline contribute to complex carbon and sulfur cycling, most active in lakes where the chemocline is within the photic zone, in turn supporting robust populations of anoxygenic phototrophs (Avetisyan et al., 2019; Danza et al., 2017; di Nezio et al., 2021; Pjevac et al., 2019; Savvichev et al., 2017).

Sulfur-cycling bacteria provide strong connections between biogeochemical cycles in meromictic lakes, particularly sulfur and

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carbon cycles (Zadereev et al., 2017). Anoxygenic photosynthesis plays an important role not only in sulfur cycling but in carbon fixation. Depending on depth and conditions, dark carbon fixation by sulfide-oxidizing chemolithoautotrophs (Camacho et al., 2001; Zadereev et al., 2017) or even purple sulfur bacteria (PSB) (Berg et al., 2019) can be equal or greater. Likewise, anaerobic respiration of organic matter via sulfate reduction or methanogenesis are also important contributors to biogeochemical cycles in these lakes (Canfield et al., 2010; Hamilton et al., 2014; Havig et al., 2018; Zerkle et al., 2010). In addition to physical and geochemical controls, interactions with other taxa through competition or syntrophy may control the activity and distribution of these groups in the water column. Thus, meromictic lakes are ideal aquatic ecosystems in which to study the interplay between limnological conditions, biogeochemistry, and microbial ecology.

Green Lake (Fayetteville, NY; FGL) is a meromictic lake, formed via a glacial plunge pool during the Wisconsin glaciation. FGL has been studied extensively in the past (Culver & Brunskill, 1969; Havig et al., 2015; Takahashi et al., 1968), including the active sulfur cycling in the euxinic monimolimnion (Zerkle et al., 2010). In the mixed layer, the photic and oxic waters allow for oxygenic photosynthesis by both eukaryotic and prokaryotic phytoplankton (Thompson et al., 1990). Where the photic zone transitions to euxinic conditions, the dominant phototrophs can include PSB, green sulfur bacteria (GSB), and, to a lesser degree, the cyanobacterium *Synechococcus* (Culver & Brunskill, 1969; Meyer et al., 2011; Thompson et al., 1990), highlighting the important biogeochemical interaction between sulfur and carbon cycling in FGL.

So, despite extensive research into the biogeochemistry of FGL, to date, there has been no published high-throughput sequencing with respect to the identity and distribution of sulfur-cycling bacteria, anoxygenic phototrophs, and cyanobacteria in the lake. The distribution of these and other microbes may give insight into the microbial sulfur and carbon biogeochemical cycles of the lake (Øvreås et al., 1997; Scholten et al., 2005). In other meromictic lakes, characterization of the microbial communities throughout the water column has been useful in identifying important microbial groups in major biogeochemical cycles (Diao et al., 2018; Kojima et al., 2014; Vigneron et al., 2021). Here, we report the physicochemical characteristics and the results of 16S rRNA gene sequencing through the chemocline of FGL and compare our results to similar meromictic lakes.

## 2 | MATERIALS AND METHODS

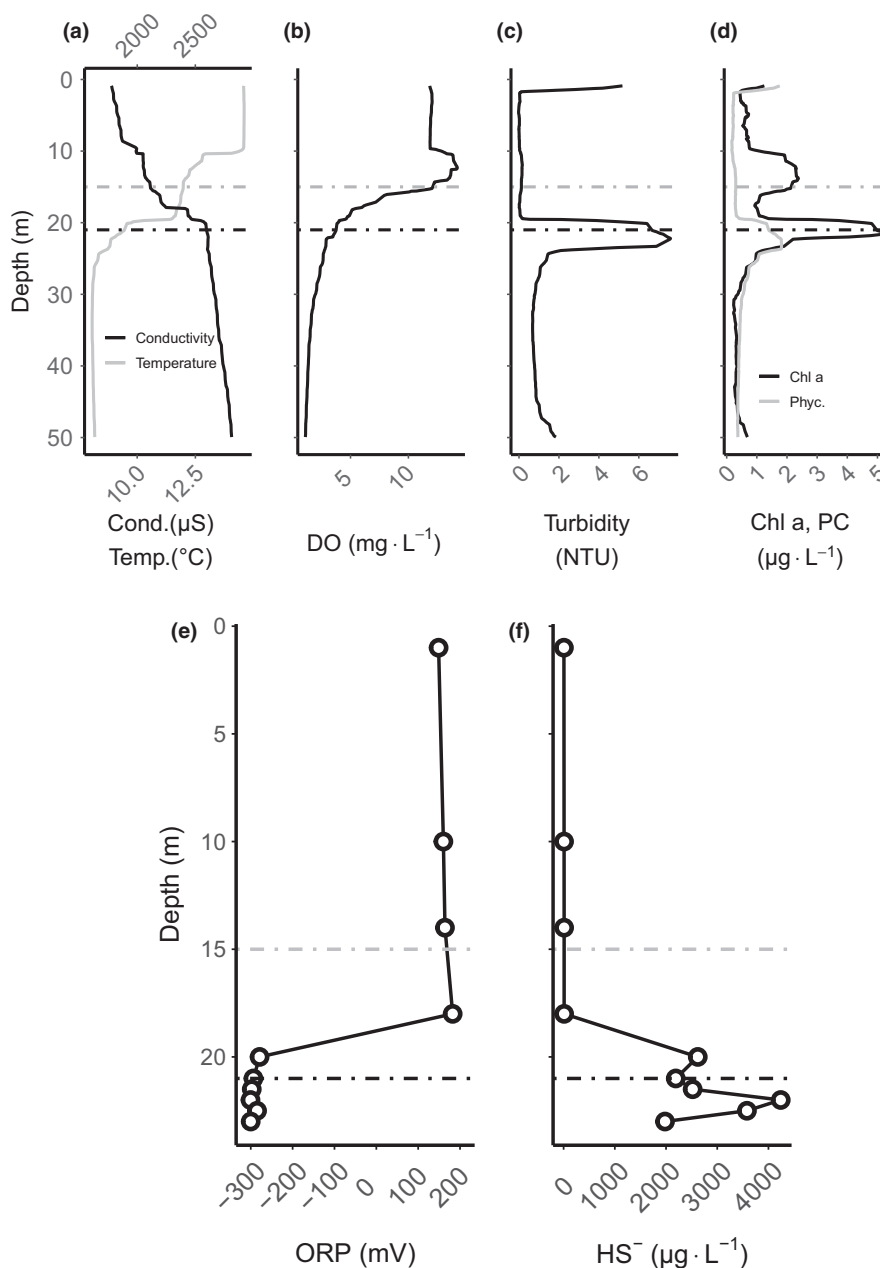
Samples were taken in situ at FGL on 20 June 2018 (43.051389 N 75.965 W). We used a SonTek CastAway CTD to collect conductivity, temperature, and depth data to approximately 50 m, reaching the lakebed. Turbidity, chlorophyll, dissolved oxygen (DO), and phycocyanin (PC) data were collected using the EXO1 Multiparameter Sonde (Yellow Springs Instruments) from depths 0 m to 50 m. All sensors were calibrated at Niagara University before the field

expedition, except pH and depth which were calibrated on-site. The probes were lowered at approximately 1 m per 5 s, which was adequate to vertically resolve most parameters. Despite the EXO using a fast response oxygen optode, the micro-oxic conditions required additional response time, creating the tail in the oxygen profile observed in Figure 1b. Using the CTD and EXO profiles, we selected van Dorn water collection depths corresponding to water masses of similar density: at the top and bottom of the wind-mixed layer of the mixolimnion (1 m, 9 m), at the bottom of the mixolimnion above the chemocline (18 m), and then across the chemocline and microbial plate (22 m, 23 m, 23.5 m, 24 m, 24.5 m and 25 m). The van Dorn (2.2 L) was lowered on a nylon line from an unanchored rowboat, causing drift and line stretch to make these collection depths approximations. Because of this, the additional samples were necessary to ensure sampling above, in, and below the microbial plate observed in the EXO data, with depths measured from the surface, rather than from a stable reference. The surface waters (mixolimnion) vary over a season, while the chemocline and microbial plate are extremely stable making our measurements approximations of the more robust depths of the microbial plate and chemocline reported in Havig et al. (2015), Havig et al. (2018). Samples were stored in two sterile 1 L amber Nalgene bottles, one for geochemical analyses and the other for microbial analyses and sterilized briefly with 70% ethanol between samples and allowed to dry before the next sample collection. Geochemical analyses were performed on-site. Water samples for microbial analyses were stored on ice in the dark for a few hours when they were filtered. Filters were stored on ice until returned to the laboratory for  $-20^{\circ}\text{C}$  storage and subsequent processing. Visual remotely operated underwater vehicle (ROV) observations were made using a Blue Robotics BlueROV attached to a shore laptop and recorded to an internal SSD. The ROV was deployed from shore with a 300 m cable, navigated via compass and visual surfacing to the center of the lake, and then descended vertically through the water column.

A Hanna Instruments HI98194 multiparameter probe was used to obtain oxidation-reduction potential (ORP) measurements for each van Dorn water sample and sulfide measurements for the water samples at each depth were obtained by using a Hach DR 1900 portable spectrophotometer. We used the Hach Sulfide Reagent set for the methylene blue method according to manufacturer instructions, diluting as necessary.

Water samples for each depth were filtered through a 73 mm diameter, 0.22  $\mu\text{m}$  polyethersulfone filter in a MilliporeSigma (Darmstadt, Germany) Stericup sterile vacuum filter unit until the 1 L of the sample had passed or the filter had clogged. Filters were aseptically removed from their casings, aseptically sliced into sections with approximately 20% of the filter used with a DNeasy PowerSoil Kit (Qiagen) according to the manufacturer's instructions. Following extraction, DNA was quantified on a NanoDrop 1000 spectrophotometer (Thermo Fisher and stored at  $-20^{\circ}\text{C}$  until shipped for Illumina MiSeq sequencing of the 16S rRNA gene V4-V5 region at Dalhousie University's Integrated Microbiome Resource (IMR; Halifax, Nova Scotia, Canada).

**FIGURE 1** Vertical depth profiles of (a) temperature ( $^{\circ}\text{C}$ ), conductivity ( $\mu\text{S}$ ), (b) dissolved oxygen ( $\text{DO}$ ;  $\text{mg}/\text{L}$ ), (c) turbidity ( $\text{NTUs}$ ), (d) chlorophyll A and phycocyanin ( $\mu\text{g}/\text{L}$ ), (e) oxidation-reduction potential ( $\text{ORP}$ ;  $\text{mV}$ ), and (f) hydrogen sulfide ( $\text{HS}^-$ ;  $\mu\text{g}/\text{L}$ ) in FGL from June 2018. The gray horizontal line represents the upper bound of the chemocline, and the black horizontal line represents the lower bound of the chemocline. Data points for panels (e) and (f) represent locations where samples were taken for 16S sequencing. Note: the tail of  $\text{DO}$  below the chemocline in anoxic water is due to the response time of the oxygen optode relative to the micro-oxic conditions



**TABLE 1** The number of reads and diversity measures for each sampled depth

Depth (m)	Reads	Good's coverage	Shannon index
1	17,958	0.958	3.14
9	21,217	0.958	3.88
18	21,947	0.954	4.30
22	47,383	0.940	4.47
23	38,182	0.937	5.08
23.5	28,620	0.930	5.14
24	30,198	0.934	5.26
24.5	36,123	0.925	5.47
25	44,390	0.934	5.38

We used *mothur* and the MiSeq SOP (Kozich et al., 2013; Schloss et al., 2009) to clean, align, and trim sequences, as well as to remove chimeras (UCHIME) and non-bacterial sequences. Sequences were aligned to the SILVA database (v132), and the database was also used to classify OTUs following clustering at 97% similarity. Subsequent analyses were also performed using *mothur*.

### 3 | RESULTS AND DISCUSSION

The number of reads for each sample ranged from 17,958 to 47,383 and generally increased with depth (Table 1). The 16S sequencing for the 14 m sample failed, which we attribute to a peak in eukaryotes, based on 18S sequence data (Figure A1). Good's coverage ranged from 92.5% to 96% and the Shannon Index of diversity increased

with depth (Table 1). Archaeal sequences made up a very small number of reads and were excluded from subsequent analysis. We found the chemistry and corresponding microbial community can broadly be divided into three layers.

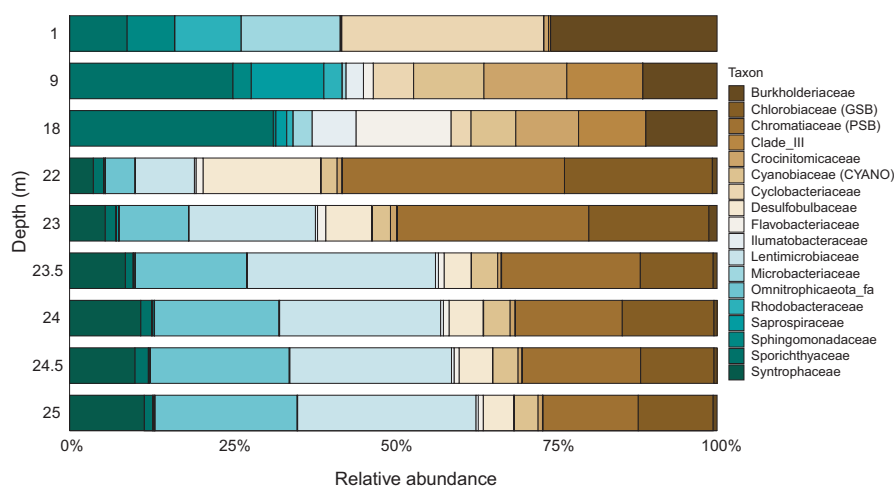
### 3.1 | 1–18 m

The mixolimnion was 14°C, with a three-degree thermocline and 11°C below the wind-mixed layer. Conductivity gradually increased from 1700  $\mu\text{S}$  near the surface to 2250  $\mu\text{S}$  at 18 m (Figure 1). ORP remained a constant 100 mV with no detectable  $\text{HS}^-$  through 18 m, consistent with data reported in Havig et al. (2015). These surface waters had high turbidity (4.0 NTU), with chlorophyll and phycocyanin values of 1.0  $\mu\text{g L}^{-1}$  and 1.5  $\mu\text{g}$ , respectively; all three parameters sharply decreased across the top meter. This near-surface maximum visually corresponded to buoyant cyanobacteria and a white snow-like calcium carbonate flocculant/marl. Similar formations, bioherms, are associated with thrombolytic reef-building around the edge of FGL (McCormick, 2012). A chlorophyll maximum of approximately 2  $\mu\text{g L}^{-1}$  was reached between 10 to 14 m, without a corresponding peak in phycocyanin, which we attribute to cryptomonad algae based on eukaryotic 18S sequences (Figure A1). This peak is at a similar depth to the 4.3  $\mu\text{g L}^{-1}$  peak observed by Butler (2017) in August and the 14.3  $\mu\text{g L}^{-1}$  peak in October of 2016.

Heterotrophic aerobic bacteria are abundant from the surface of the lake to the chemocline. The most abundant families in the water column, Cyclobacteriaceae, Burkholderiaceae, and Sporichthyaceae were well represented from 1 to 18 m (Figure 2). Cyclobacteriaceae, which are common in freshwater and soil environments (Pinnaka & Tanuku, 2014), were the predominant group at the surface, representing 27.3% of the sequences at the 1 m sampling depth. Cyclobacteriaceae are aerobic, chemo-organotrophic, pigmented microorganisms found in marine sediments, haloalkaline soda lakes, microbial mats, and freshwater bodies (Anil Kumar et al., 2012; Pinnaka & Tanuku, 2014; Van Trappen et al., 2002; Ying et al., 2006). Also abundant at the 1 m sampling depth were Burkholderiaceae (22.5%), which are also aerobic chemoorganotrophs (Coenye, 2013).

While Cyclobacteriaceae and Burkholderiaceae are the most abundant at the surface (1 m), they also made up >2.5% and >4.9% of the reads, respectively, at 9 m and 18 m. Sporichthyaceae, however, were the most abundant family at the 9 and 18 m sampling depths (19.8% and 25.5%, respectively) and were still abundant at 1 m (7.8%). Sporichthyaceae are members of the Frankiales, which generally consist of slow-growing aerobic bacteria that are often capable of nitrogen fixation (Normand & Fernandez, 2020). Though many Sporichthyaceae strains were originally isolated from soil, they have also been found in freshwater ranging from arctic dimictic lakes to the Osterseen Lakes in Bavaria (Zwirgmaier et al., 2015). Like the Sporichthyaceae, Crocinitomaceae were also abundant at 9 and 18 m but essentially absent from the 1 m sampling depth. Genera within the Crocinitomaceae are known freshwater heterotrophic bacteria (Bowman et al., 2003; Munoz et al., 2016; O'Sullivan et al., 2005) and members have been isolated in association with eukaryotic algae (Bowman et al., 2003; Shi et al., 2017), perhaps explaining their abundance at 9 and 18 m, where we attributed pigment fluorescence to algae. These and the other abundant aerobic, heterotrophic families likely subsist on oxygen and organic carbon from phytoplankton. Chemoorganotrophic bacteria in the surface waters may link the surface productivity to the sulfur-cycling bacteria below through the transformation and transport of carbon. Dissolved inorganic carbon (DIC) concentrations in the shallow mixolimnion of FGL are elevated above a best-fit model of DIC sources and sinks (Havig et al., 2018). The mineralization of organic carbon by chemoorganotrophs in this layer may explain this phenomenon in part. Indeed,  $\delta^{13}\text{C}$  values from FGL suggest rapid cycling of labile dissolved organic carbon (DOC) in the water column, with the remaining recalcitrant DOC serving as inputs to the sediment (Havig et al., 2018).

There was also a notable population of Cyanobiaceae between 9 and 18 m (Figure 2). Many of these sequences in this family were identified as either *Cyanobium* or *Synechococcus* (97.34% identity to *Synechococcus rubescens* strain SAG 3.81, NR\_125481.1), the latter of which is the former genus assigned to *Cyanobium* PCC-6307 (Komárek et al., 1999). *Cyanobium* and *Synechococcus* are part of a monophyletic group of non-marine picocyanobacteria (Sánchez-Baracaldo et al., 2019). Members of the *Synechococcus* genera have



**FIGURE 2** Relative abundance of bacteria in Fayetteville Green Lake at the Family level. Relative abundances are based on families that made up at least 5% of the sequences in at least one sampling depth

previously been observed in microaerobic and sulfidic environments (Bhatnagar et al., 2020; Koizumi et al., 2004), and *Synechococcus* sp. strain PCC7002 has recently been demonstrated to detoxify sulfide using sulfide:quinone oxidoreductase (SQR) for anoxygenic photosynthesis (Liu et al., 2020). While there is a low concentration of sulfide at these depths, the proximity of the Cyanobiaceae to the euxinic waters below may necessitate metabolic flexibility to deal with the entrainment of sulfide through the chemocline. This metabolic versatility has been observed in other cyanobacteria. Notably, oxygenic photosynthesis in the sinkhole mat isolate *Leptolyngbya* sp. strain hensonii is inhibited by very little H<sub>2</sub>S and, with no induction time, begins anoxygenic photosynthesis, suggesting constitutively expressed sulfide-oxidizing genes, that is, *sqr* (Hamilton et al., 2018). However, once sulfide is depleted, there is a light-dependent return to oxygenic photosynthesis within ~30 min (Hamilton et al., 2018). In other anoxygenic photosynthesis-capable cyanobacteria, both oxygenic and anoxygenic photosynthesis can be performed simultaneously over a wide range of light levels and sulfide concentrations (Klatt et al., 2016). While these dynamics have not been studied in FGL, they may be active in the Cyanobiaceae populations where sulfide is present, and the Cyanobiaceae may therefore participate in sulfur cycling in the chemocline (Figure 4).

### 3.2 | 18–23 m

Between 18 and 20 m, the temperature remained a constant 12°C, while conductivity increased from 2250 µS to 2550 µS through the chemocline (Figure 1). At 20 m, ORP decreases sharply to -278.9 mV corresponding with the increase in HS<sup>-</sup> to 2620 µg L<sup>-1</sup> (Figure 1), consistent with the ORP values and detectable HS<sup>-</sup> reported in Havig et al., 2015. There was a large turbidity maximum, 6.5 NTU, from 20 to 23 m corresponding to the sulfur bacteria (Figure 1c). Chlorophyll a fluorescence reached a water column maximum of 5.0 µg L<sup>-1</sup> at 21 m with a large but lower phycocyanin maximum of 1.8 µg L<sup>-1</sup> at 22 m, corresponding to a distinct change in the bacterial community structure. Members of the sulfur-cycling families Chromatiaceae, Chlorobiaceae, and Desulfobulbaceae made up the majority of the community at 22 and 23 m (Figure 2), making a dense plate of pink water visible via ROV inspection, and with notably less light below this depth.

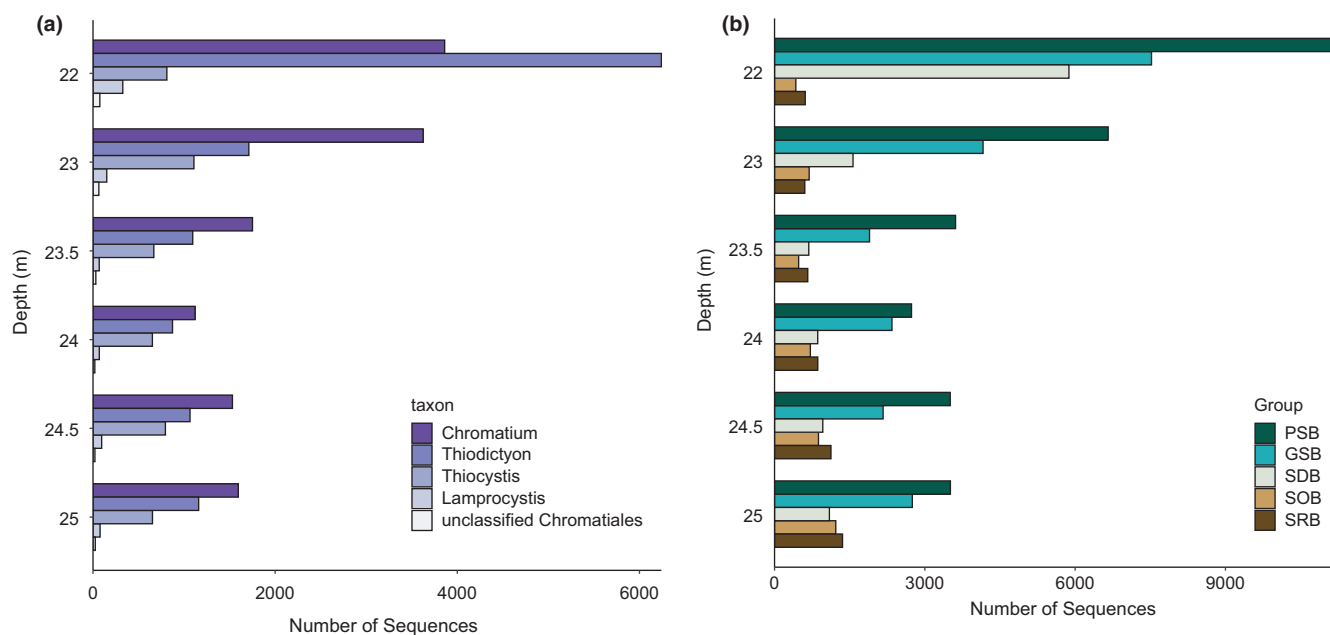
The Chromatiaceae, PSB, and the Chlorobiaceae, GSB, are anoxygenic phototrophs that normally use sulfide as an electron donor for anoxygenic photosynthesis. The dominant GSB OTU in FGL is most closely related to *Chlorobium phaeobacteroides* (99.51% identity, accession number NR\_074352.1), a brown-colored member of the GSB. Similar strains have been found in other meromictic environments such as the Black Sea, Lake Blankvann, Lake Cadagno, Lake Ciso, and others (Bergstein et al., 1979; Borrego et al., 1999; Danza et al., 2018; Grouzdev et al., 2019; Llorens-Marès et al., 2016; Montesinos et al., 1983; Overmann et al., 1992; Pfennig, 1968). Despite the presence of both GSB and PSB in the microbial plate,

we expect PSB to be more active in the shallower waters with GSB more active with increasing depth due to their respective light preferences (Biebl & Pfennig, 1978). The distribution and activity of these taxa are a focus of ongoing work.

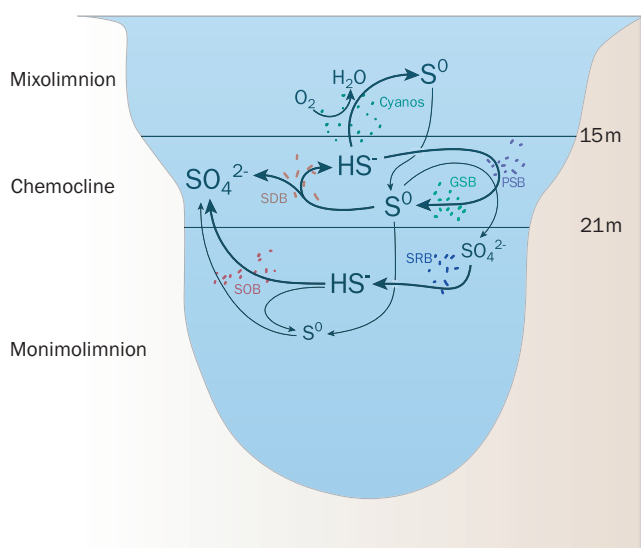
PSB are more abundant, overall, than GSB. The PSB sequences identified in FGL are affiliated with OTUs in the *Chromatium*, *Thiodictyon*, *Thiocystis*, and *Lamprocystis* genera (Figure 3a). PSB such as *C. okenii*, *Thiocystis*, and *Thiodictyon syntrophicum* all show some degree of tolerance to micro-oxic conditions (Berg et al., 2019; Kampf & Pfennig, 1980; Luedin, Storelli, et al., 2019). The ability to tolerate and grow in micro-oxic conditions may provide an advantage to the PSB in the shallower depths, which may be intermittently oxygenated, a condition that GSB are much less likely to tolerate.

The *Chromatium* found in FGL, most closely related to *Chromatium okenii* (99.27% identity, accession number NR\_025315.1), was the most represented taxa between depths of 23–25 m. The only existing isolate of *Chromatium okenii* was recently obtained from meromictic Lake Cadagno, Switzerland (Luedin, Liechti, et al., 2019). In Lake Cadagno, the large-celled *C. okenii* are responsible for bioconvection (Sommer et al., 2017), and in FGL, these organisms may contribute to mixing the isothermal conditions of the upper monimolimnion and, perhaps, sulfur transport.

While *Chromatium* were still abundant at 22 m, this was the only depth sampled where another PSB OTU—*Thiodictyon*—was more abundant (Figure 3a). The closest relative of the *Thiodictyon* OTU is *Thiodictyon syntrophicum* (99.52% identity, accession number NR\_114886.1), an isolate also from Lake Cadagno (Luedin et al., 2018; Peduzzi et al., 2012). Additionally, *Desulfocapsa* were also abundant at 22 m. *Desulfocapsa* sp. and *T. syntrophicum* have been found to grow in compact clumps of cells in Lake Cadagno, which was attributed to a syntrophic relationship, where PSB provide a necessary sulfide sink for sulfur disproportionation (Peduzzi et al., 2012). In FGL, sulfur-cycling models showed that isotopic data are best explained with the inclusion of sulfur compound disproportionation (Zerkle et al., 2010). Sulfur disproportionators can use a variety of intermediate sulfur compounds, including elemental sulfur. Extracellular sulfur globules produced by GSB (Marnocha et al., 2016) or cyanobacteria performing anoxygenic photosynthesis (Castenholz & Utkilen, 1984; Cohen et al., 1975; Hamilton et al., 2018; Klatt et al., 2020) could be used for sulfur disproportionation in FGL (Figure 4), and a peak of zero-valent sulfur at 22 m (Zerkle et al., 2010) aligns with our observed maximum abundance of sulfur-disproportionating bacteria (Figure 3b). The byproducts of sulfur disproportionation are sulfide and sulfate (Finster et al., 1998), the former of which can provide an additional source of electron donors for sulfide-oxidizing bacteria, and the latter another source of electron acceptors for sulfate reducers found in the monimolimnion (Figure 4). Moreover, abiotic interactions between sulfide and sulfur may also produce soluble polysulfides, which could theoretically provide electron donors to both GSB and PSB (Marnocha et al., 2016). Interactions such as these likely facilitate dynamic sulfur cycling in the chemocline.



**FIGURE 3** (a) Number of sequences assigned to genera of PSB from 22 to 25 m. (b) Number of sequences assigned to bacterial genera with known sulfur-based metabolisms from 22–25 m. Purple sulfur bacteria (PSB), green sulfur bacteria (GSB), sulfur-disproportionating bacteria (SDB), sulfur-oxidizing bacteria (SOB), and sulfate-reducing bacteria (SRB)



**FIGURE 4** A simple model of sulfur cycling in Fayetteville Green Lake by Cyanobacteria (Cyanos), purple sulfur bacteria (PSB), green sulfur bacteria (GSB), sulfur-oxidizing bacteria (SOB), sulfur-disproportionating bacteria (SDB), and sulfate-reducing bacteria (SRB)

### 3.3 | 23–25 m

Chlorophyll a and phycocyanin fluorescence both decreased sharply in the bottom waters of the lake but then remained constant between  $0.3 \mu\text{g L}^{-1}$  and  $0.5 \mu\text{g L}^{-1}$ , respectively (Figure 1d). Turbidity ranged from 1 to 2 NTU (Figure 1c), much higher than what was

observed in the epilimnion, which ROV observation indicated was more of the white flocculant observed in the surface waters. At 23 m, the temperature of FGL decreased to a constant  $8^\circ\text{C}$  throughout the entire monimolimnion, while conductivity gradually increased from  $2550 \mu\text{S}$  to  $2800 \mu\text{S}$  from 20 m to 50 m, consistent with measurements reported by Brunskill and Ludlam (1969).

Abundant within these samples were members of the Omnitrphicaeota, Lentimicrobiaceae, and Syntrophaceae (Figure 2). Recent work suggests that members of the uncultivated phylum Omnitrphica may be able to reduce sulfite but not sulfate (Anantharaman et al., 2018), and the sulfide produced by this process may feed the chemolithoautotrophic sulfur-oxidizing bacteria found in low but increasing abundances in this layer (Thiovalaceae make up  $\sim 2.5\%$  of sequences between 23–25 m; Figure 4). Additional molecular analysis in the deeper waters may better clarify the roles of groups like Omnitrphicaeota in the monimolimnion.

The type strain of the Lentimicrobiaceae, *Lentimicrobia saccharophilum*, cannot utilize sulfate, sulfite, elemental sulfur, or thiosulfate, although the genus has only recently been described (Sun et al., 2016). Instead, members of the Lentimicrobiaceae are characterized as strict anaerobic fermenters and have been found in bedrock groundwater, chlorinated aquifers, and the sediments of Lake Baikal (Bukin et al., 2018; Maturro et al., 2018; Purkamo et al., 2018). Likewise, Syntrophaceae are also known to be strict anaerobes with limited fermentative and respiratory metabolisms and without any known ability for dissimilatory sulfate reduction (McInerney et al., 2007; Plugge et al., 2011). In FGL, the organic carbon used by these microorganisms likely comes from the upper water column, as suggested by  $\delta^{13}\text{C}$ -DOC values (Havig et al., 2018). The organic carbon could then be recycled by Lentimicrobiaceae in the monimolimnion,

before becoming further degraded by *Syntrophaceae* and later methanogens. The byproducts from Lentimicrobiaceae may also support the coexistence of sulfate reducers and methanogens, which might otherwise compete for substrates (Dalcin Martins et al., 2017; Dolfig et al., 2008; Morris et al., 2013; Oremland & Polcin, 1982; Sela-Adler et al., 2017). Organic carbon mineralization in the monimolimnion by fermentation or sulfate reduction is supported by  $\delta^{13}\text{C}$  values, along with methane of biogenic origin diffusing into the water column from the sediment (Havig et al., 2018). Chemotrophic sulfur-cycling bacteria likely play an important role with increasing depth, though our samples extend only to 25 m in FGL. Nevertheless, sulfate-reducing bacteria (SRB), sulfur-disproportionating bacteria (SDB), and sulfur-oxidizing bacteria (SOB) are all observed between 22 and 25 m depths (Figure 3b).

The bacterial diversity of FGL shares similarities with what has been observed in other meromictic lakes, particularly with respect to chemocline phototrophic sulfur oxidizers. Lake Cadagno, for example, hosts several PSB genera (including *Chromatium*, *Thiocystis*, and *Thiodictyon*) and GSB related to *Chlorobium phaeobacteroides* (Danza et al., 2018; Gregersen et al., 2009; Peduzzi et al., 2012; Tonolla et al., 1999, 2005, 2017). Additionally, there appears to be a strong seasonal component to the dominance of anoxygenic phototrophs in Lake Cadagno; while our study focuses on a single point in time, other work at FGL has suggested seasonal changes based on and around whiting events (Thompson et al., 1990; Zerkle et al., 2010). Shifts in the dominant anoxygenic phototrophs caused by chemocline depth changes have also been observed in Lake Cadagno, extending for years (Gregersen et al., 2009; Tonolla et al., 2005).

Meromictic Mahoney Lake in British Columbia, Canada, is more saline, sulfate-rich, and sulfide-rich (Klepac-Ceraj et al., 2012; Overmann et al., 1996) than either FGL or Lake Cadagno. Though its shallow depth (15 m) is more similar to Lake Cadagno (18 m) than to FGL (53 m), similar dominant phototrophs can be observed between Mahoney Lake and FGL, distributed similarly with respect to each lake's chemocline. For example, *Synechococcus* is the dominant cyanobacterium, found primarily above the lake's 7 m PSB plate (Klepac-Ceraj et al., 2012). This plate is composed of multiple PSB genera, including *Lamprocystis* and *Thiocapsa*, though notably, few GSB are found in the lake (Hamilton et al., 2014; Klepac-Ceraj et al., 2012).

In the deeper Framvaren Fjord, Norway (180 m), PSB are found directly beneath the chemocline but within the redox transition zone, and GSB are found just below (Behnke et al., 2006, 2010; Sorensen, 1988), similar to FGL. In contrast, in the Black Sea (maximum depth >2200 m), GSB are observed, but the presence of PSB in the water column has not been confirmed (Cabello-Yeves et al., 2021; Overmann & Manske, 2006). Like FGL, the Black Sea GSB consist largely of *Chl. phaeobacteroides* and are found beneath the chemocline, and *Synechococcus* are the dominant cyanobacterium above the chemocline (Cabello-Yeves et al., 2021).

Stratified euxinic water bodies are valuable analog environments for geobiological studies of the Earth's ancient oceans (Hamilton et al., 2017; Klepac-Ceraj et al., 2012; Rico & Sheldon, 2019; Xiong et al., 2019). There is strong evidence in the rock record that the

Earth's oceans were at least intermittently euxinic during the Proterozoic era (2–0.5 Gyr), particularly at ocean margins (Lyons et al., 2014; Meyer & Kump, 2008). To better interpret the rock record and reconstruct the paleoenvironment of these early Earth oceans, contextual insights from modern analogs are necessary. During the mid-Proterozoic, when oxygen began to accumulate, the stratification of the ocean would have had a similar structure with photic zone euxinia to meromictic lakes like FGL, thus making FGL an excellent analog for the study of the Proterozoic oceans (Havig et al., 2015; Johnston et al., 2009; Meyer & Kump, 2008).

Photosynthetic pigments from GSB and PSB (e.g., isorenieratene and okenone), and the diagenetic products of those pigments are key biomarkers used to interpret paleoconditions of ancient sediments (Brocks et al., 2005; Meyer & Kump, 2008). Biomarkers like these have been found at a small number of sites older than 1 Gyr (e.g., Blumenberg et al., 2012; Brocks et al., 2005; Gueneli et al., 2018; Jarrett et al., 2019; Luo et al., 2015). Recently, GSB and/or cyanobacterial biomarkers and possible PSB biomarkers were discovered in the 1.73 Ga Wollongorang Formation in Australia (Vinnichenko et al., 2020). Carotenoids in association with extant GSB and PSB have been previously studied in FGL, with their spatial distribution coinciding with the PSB plate (Hunter, 2012; Meyer et al., 2011). Thus, by better understanding the predominant phototrophs and the controls on their distribution in modern-day analog environments like FGL, more robust interpretations of biomarkers in the rock record are possible.

In this work, we expand our understanding of the bacterial community structure of the upper 25 m of the FGL water column, with corresponding limnological and geochemical context. We found a complex, vertically stratified bacterial community, corresponding to a vertical gradient in density and redox potential in the water column. A range of bacteria capable of sulfide oxidation was abundant at and below the chemocline, including cyanobacteria capable of anoxygenic photosynthesis, GSB, and multiple genera of PSB. Sequences of sulfur-disproportionating, sulfide-oxidizing, and sulfate-reducing bacteria were also recovered. Beneath this phototrophic bacterial plate, chemoheterotrophs and fermenters became abundant and likely play a role in carbon and sulfur cycling in the monimolimnion. We provide a simple model of the bacterial community structure and sulfur-cycling occurring in the upper half of the FGL water column (Figure 4).

Overall, our results can be used as a framework for understanding the interplay between the physicochemical characteristics of a meromictic lake and the corresponding microbial ecology. In particular, the data provide better resolution of the bacterial taxa that are likely playing an important role in FGL's biogeochemical cycles. These results will serve as the foundation for our future work investigating gene expression of sulfur-cycling bacteria, and the dynamics of carbon recycling between bacteria and microscopic eukaryotes.

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## CONFLICT OF INTEREST

None declared.

## AUTHOR CONTRIBUTIONS

**Kaleigh R. Block:** Formal analysis (equal); investigation (equal); visualization (equal); writing-original draft (equal); writing-review & editing (supporting). **Joy M. O'Brien:** Formal analysis (equal); investigation (equal); writing-original draft (equal); writing-review & editing (supporting). **William J. Edwards:** Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); supervision (equal); visualization (equal); writing-original draft (equal); writing-review & editing (equal). **Cassandra L. Marnocha:** Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); supervision (equal); visualization (equal); writing-original draft (equal); writing-review & editing (equal).

## ETHICS STATEMENT

None required.

## DATA AVAILABILITY STATEMENT

All data are provided in full in this paper, except the 16S rRNA gene amplicon data, which are available at the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject PRJNA641339: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA641339>.

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## APPENDIX A

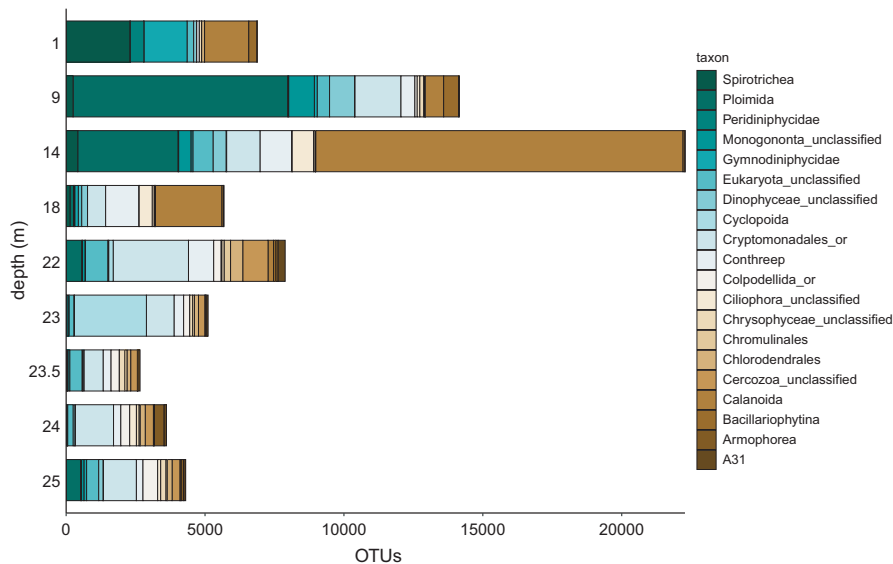


Figure A1 Order-level community structure of eukaryotes based on sequencing of the V4 region of the 18S gene. DNA was collected using the same methods as described in the text for bacterial sequencing, and sequencing was performed at Dalhousie University's Integrated Microbiome Resource (Halifax, Nova Scotia, Canada). Shown in this figure are the top 20 most abundant orders across all depths