## The following methods are from our paper entitled, “Geochemical Characterization of Two Ferruginous Meromictic Lakes in the Upper Midwest, USA” (Lambrecht, N., Wittkop, C., Katsev, S., Fakhraee, M., & Swanner, E. D. (2018). Geochemical Characterization of Two Ferruginous Meromictic Lakes in the Upper Midwest, USA. Journal of Geophysical Research: Biogeosciences, 123(10), 3403-3422.):

Brownie Lake (N44°5804.483′′, W93°19026.677′′) is located in Minneapolis, MN, and is the northernmost lake in the Minneapolis Chain of Lakes (Brownie, Cedar, Isles, Calhoun, and Harriet). The catchment area is mostly residential, with recreational paths surrounding the lake. It has an approximate surface area of 5 ha and a maximum depth of 14 m (Minneapolis Park and Recreation Board, 2013; Myrbo et al., 2011). Canyon Lake (N46°49058.069′′, W87°55014.858′′) lies in private lands belonging to the Huron Mountain Club (HMC) in the Upper Peninsula of Michigan. It is enclosed by deep canyon walls, which are surrounded by the pristine forests of the Huron Mountains. It has an approximate surface area of 1 ha and a maximum depth of 23 m (Anderson-Carpenter et al., 2011).

Sampling was performed from moored boats at the deepest locations in both lakes. Water was collected from a maximum depth of 13 m at Brownie Lake and 21 m at Canyon Lake (Figure 1). Each lake was sampled multi- ple times throughout 2015–2018 to assess seasonal change and confirm stable stratification. Sensors were lowered on depth-calibrated cables. All samples for nutrients, cations and anions, major and minor elements, redox species, and DIC were collected with a Proactive Mini Monsoon pump with low-flow controller attached to vinyl tubing and a cable marked with meter and half-meter depth increments. Water was filtered by attaching a syringe filter directly to the tubing, and where necessary to a needle via luer lock connections.

A Hydrolab Series 5 multiprobe (Hach) was deployed to collect conductivity/temperature/depth data, in addi- tion to dissolved oxygen (detection limit: 3 μM), pH, oxidation-reduction potential, and turbidity. Calibrations were performed after rinsing the sensors twice with deionized water. Conductivity and salinity were cali- brated with a two-point calibration utilizing buffers obtained from the manufacturer. The membrane-based dissolved oxygen probe was calibrated using a single point calibration with 100% air-saturated water. The pH sensor was calibrated by using commercially prepared buffers (pH 4, 7, 10). Light profiles were measured using a LI-COR 192SA (sensitivity of 4 μA per 1,000 μM·s1·m2) underwater quantum sensor on a lowering frame. From June until September of 2017, a vertical chain of Hobo temperature recorders (accuracy 0.2 °C, precision 0.02 °C) was deployed in Canyon Lake to characterize seasonal variations in stratification. Eight recorders were positioned in the water column at depths between 18.5 and 1 m; the recorders were pro- grammed to take temperature measurements every 5 min.

Dissolved sulfide was filtered using a 0.22-μm polyethersulfone (PES) filter. Nitrate and dissolved phosphate (also known as soluble reactive phosphorus) samples were filtered using a 0.45-μm PES filter. Samples for ammonium and total phosphorus quantification were preserved by acidifying unfiltered water with 5 N H2SO4 to pH < 2 on land after sample collection. All samples were stored on ice or at 4 °C until analysis, usually within 72 hr. Ammonium (detection limit: 5 μM; Weatherburn, 1967), nitrate (detection limit: 1 μM; Hood-Nowotny et al., 2010), total and dissolved phosphorus (detection limit: 0.1 μM; Wetzel & Likens, 2000), and dissolved sulfide (detection limit: 1 μM; Cline, 1969; Reese et al., 2011) were measured spectrophotometrically on an Epoch 2 Microplate Reader (Biotek).

Samples for cations and anions, which were collected simultaneously for each sampling trip, were filtered using 0.45 μm PES filters. Anions (Cl, Br, and SO42) were preserved on ice during transport and stored at 4 °C until analysis. Cation samples (Al, B, Ca, Cr, Cu, Fe, K, Mg, Mn, Si, Na, and Zn) were preserved with HNO3 (final acid concentration of 1%) and then transported on ice and stored at 4 °C until analysis. Cations were analyzed by ICP-OES (iCap 7600 Duo) at the University of Minnesota Department of Earth Sciences (2015 samples), the Minnesota Department of Health (2016 samples), or the U of MN Research Analytical Laboratory (2017 samples). Anions were analyzed at the same facilities using a Dionex 120 Ion Chromatograph following the Environmental Protection Agency method 300.0 (Pfaff, 1993). Ion detection limits can be found in supporting information Table S1. The significant digits reflect the quantification limit.

Samples for DIC analysis were filtered (0.45 μm) and stored in sealed headspace vials (2015 and 2016 sam- ples) or injected into evacuated, He-flushed exetainers containing 1 mL of concentrated phosphoric acid (2017 samples) and analyzed at the U of MN Stable Isotope Laboratory (2015 samples), and the UC-Davis Stable Isotope Facility (2016–2017 samples). Samples for methane were filtered (0.45 μm) and filled into evacuated exetainers with no headspace except for gas evolved from sample water. Methane samples were kept at 4 °C until analysis or preserved with HCl to pH < 2. We did not observe significant differences in CH4 concentrations between samples that were acidified or not acidified. Methane was analyzed at the UC-Davis Stable Isotope Facility by forcing dissolved gasses into headspace generated by injecting a known volume of He gas to the exetainer. The headspace gas was purified with a CO2 and H2O scrubber (Mg(ClO4)2) and a liquid nitrogen cold trap. Methane was separated using a GS-CarbonPLOT column and concentration was determined using a ThermoScientific Precon unit. Samples for water isotopes (δ2H and δ18O) were filtered (0.45 μm) and kept at 4 °C with no headspace until analyses. Water isotopes were analyzed by a Picarro L1102-i Isotopic Liquid Water Analyzer in the SIPERG Laboratory at Iowa State University.

Mineral saturation indices were calculated in Geochemist’s Workbench 12 (Bethke, 2007) using major dis- solved ion data, sonde measurements of O2 (O2 measurements were entered as zero values below the detec- tion limit), pH, and DIC concentrations. The saturation index (SI) for any mineral is defined as the log (Q/Ksp), where Q is the ion activity product, calculated using the measured data from species involved in mineral for- mation, and Ksp is the solubility product of the mineral. All calculations were made at ambient temperatures recorded by sonde readings. Where log (Q/Ksp) is positive, the mineral is thermodynamically favored to form, and where log (Q/Ksp) is negative, the mineral is undersaturated.

## The following methods are from a paper that is in press in Geobiology, “Biogeochemical and physical controls on methane fluxes from two ferruginous meromictic lakes” (citation to follow):

Brownie Lake is located on the Chain of Lakes in Minneapolis, Minnesota (Myrbo et al., 2011). It is an anthropogenically impacted, eutrophic lake with a surface area of 5 ha and a maximum depth of 14 m, with a relative depth of 5.6%. Brownie Lake became meromictic in the early 1900s due to lake-level lowering, which reduced its surface area and increased its relative depth to favor stratification, and further sheltered it from wind-mixing (Myrbo et al., 2011). The watershed area of Brownie Lake is approximately 150 ha, and the residence time for water within the lake is 2 years (Minneapolis Park and Recreation Board, 2013). Water sources likely include groundwater (Goudrealt, 1985) and storm sewer runoff (City of Minneapolis GIS Water Quality Model; Barr Engineering, 2019). Road salt, which has been in use since the mid-1900s (Swain, 1984), currently imparts additional stability against mixing (Lambrecht et al., 2018). Furthermore, the thermocline and chemocline are at the same relative location in the water column (~ 5 m). These profiles, along with water sampling methods, were previously described (Lambrecht et al., 2018). Water samples were collected from the deepest part of Brownie Lake (Fig. 1). Sampling campaigns were carried out in May 2017, July 2017, September 2017, and June 2018.

Canyon Lake is a pristine lake nestled in the Huron Mountains in the Upper Peninsula of Michigan. The maximum depth is 23 m and the approximate surface area is 1 ha (Anderson-Carpenter et al., 2011). Previous seasonal monitoring of Canyon Lake revealed a thermocline near the surface between 3 – 4 m and a persistent chemocline existing at ~ 17 m (Lambrecht et al., 2018). It is likely naturally meromictic and ferruginous, due to its great depth relative to its small surface area, along with wind protection from the surrounding 20 m high canyon walls (Smith, 1940; Lambrecht et al., 2018). Water sources to the lake are dominated by precipitation, with nearby seeps and springs supplying some water (Lambrecht et al., 2018). Canyon Lake was sampled in June 2017, September 2017, and May 2018. Additional details regarding sample collection can be found in Lambrecht et al. (2018).

Dissolved O2 (LDO model 1 luminescent sensor; detection limit of 3 µM) and chlorophyll a were measured by lowering a Hydrolab Series 5 multiprobe (Hach) through the water column of each lake. Sensors were rinsed with deionized water prior to calibration. The dissolved O2 probe was calibrated using 100% air-saturated water. The chlorophyll a sensor has a resolution of ± 0.01 μg L-1.

Samples for dissolved anions (NO3-, NO2- and SO42-; detection limits of 0.1 mg L-1) and cations (dissolved Fe and Mn; detection limits of 20 nmol) were filtered using 0.45 µm polyethersulfone (PES) filters (Sartorius). Dissolved cation samples were preserved with HNO3 at a final concentration of 1%. All samples were kept on ice or at 4 °C until analysis. Anions were analyzed using an ion chromatograph (IC) and cations were analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES) at the University of Minnesota Research Analytical Laboratory.

Samples for dissolved CH4 concentrations and isotopes were filtered using 0.45 µm PES filters and directly filled from the sampling line into evacuated Exetainers (Labco, U.K.) with no headspace using a needle attached to the syringe filter. Samples collected in 2018 were additionally preserved with 0.5 mL 6M HCl, with reported concentrations corrected for acid addition. No significant difference in dissolved CH4 concentration was observed between 2017-2018 samples. The field observation of exsolution in waters retrieved from depth was consistent with gas concentrations within the range of CH4 saturation (e.g. Molofsky et al., 2016) as displayed in our reported values, and also consistent with previous reports of a negligible impact of filtering on dissolved CH4 concentrations (e.g. Alberto et al., 2000). Dissolved inorganic carbon (DIC) concentrations and isotopes were filtered (0.45 µm PES) and injected into exetainers that were He-flushed and contained 1 mL of concentrated phosphoric acid. Methane and DIC concentrations and isotopes were analyzed at the UC-Davis Stable Isotope Facility compared against the Vienna Pee-Dee Belemnite international reference standard, with standard deviations of 0.2 and 0.1 ‰ respectively.

Methane gas fluxes from the lake surface to the atmosphere were measured with static flux chambers using a foam base for flotation. Chamber lids (acrylonitrile) and collars (polyvinyl chloride) had a diameter of 26 cm and a height of 22 cm. Chambers were vented using the design of Xu et al. (2006) to minimize pressure gradients between the chamber and the atmosphere and any wind-induced pressure perturbations due to the Venturi effect. Each flux measurement was calculated using a time series of five gas samples collected every five minutes following closure of the chamber over the collar. Samples were collected by extracting 20 ml of gas through a septum with a needle and gas-tight syringe, which was then injected into an evacuated 12 ml Exetainer vial. Five independent flux measurements per sampling campaign (five total) were made for the open water zone directly above the anoxic sediments. Methane concentrations were measured by gas chromatography at Iowa State University using a flame ionization detector, with a typical coefficient of variation < 2% for repeated analyses of standard gases with CH4 mole fractions between 2 and 10 ppm. Fluxes and standard deviation were calculated from time series of gas concentrations by selecting the optimum model (either a nonlinear diffusion model founded on Fick’s law or a linear trend) based on the estimated value of the concentrated least squares criterion fit using the HMR package in R (Pedersen, 2017). Using this approach, individual chamber flux measurements that exhibited CH4 spikes consistent with ebullition (Supporting Information Fig. 1) were automatically fit to a linear model for parsimony.

Vertical fluxes of CH4 through the water column by local (diffusional) processes were obtained from a geochemical reaction-transport model using data from May 2017 at Brownie Lake. Transport rates by turbulent eddy diffusion were determined by the balance between the rates of turbulent energy dissipation, which reflects wind forcing, and the strength of the density gradient, characterized by the Brunt-Vaisala stability frequency N (Osborn, 1980). In turbulent epilimnion, the vertical eddy diffusion coefficient (KZ, m2 s-1) often may be phenomenologically approximated (e.g. Katsev et al., 2010) as KZ=3x10-10 N-2. In the calm, stratified interior the less vigorous energy dissipation is expected to result in lower values of KZ, with turbulence being slightly higher in the bottom boundary layer (McGinnis and Wuest, 2005). In the absence of physical turbulence measurements, we calculated KZ in the epilimnion from the measurements-based N and approximated KZ below the thermocline by fitting the measured chemical profiles, using multiple species to better constrain the model (Supporting Information Fig. 2). As this approach necessarily relies on the chemical profiles being approximately steady, seasonal variations and mixing by storms can introduce uncertainty. We therefore restrict the analysis to Brownie Lake, where the range of the oxycline motion is more limited than in Canyon Lake. A one-dimensional reaction-transport model set up in MATLAB simulated vertical profiles of chemical species as steady-state solutions of a boundary-value problem. Decomposition of organic matter was considered throughout the water column at a fixed volume-specific rate RC (with a fitted value of 0.425 mmol m-3 hr-1) and in sediments with the average area-specific flux of Fsed (0.416 mmol m-2 hr-1). The sediment contribution was apportioned to the corresponding water column depths in accordance with the lake bathymetry. These rates and fluxes were used to calculate the corresponding rates for the consumption of O2 and the generation of DIC and NH4+ (with the C:N ratio of 17). Boundary conditions were prescribed-flux for DIC and NH4+ at the lake bottom, and fixed-concentration for O2 at the lake surface. The KZ(z) was adjusted as a function of depth to fit all the profiles simultaneously (Supporting Information Fig. 2). The turbulent diffusive fluxes of CH4 through the water column were then calculated from the CH4 concentration gradient as F=-KZ(d[CH4]/dz).

Microbial community composition was assessed using high throughput 16S rRNA gene sequencing. A total of 17 depths (13 whole meter and 4 half meter depths) were sampled from Brownie Lake in 2017. Sequencing samples was not collected at every depth in May (2.5, 9, and 13 m excluded) and September (2.5 and 3.5 m excluded). At Canyon Lake, 26 depths (21 whole meter and 5 half meter depths) were sampled in 2017. Samples for sequencing were not collected at 9.5 and 21 m in June and 13.5 and 15.5 m in September.

Water samples were collected from discrete depths using a 5 L Van Dorn bottle. Individual water samples were subsampled (volumes of ca. 250 mL) and transported in sterilized (10% bleach) high-density polyethylene plastic collection bottles. Samples were stored on ice in a dark cooler (max. two hours) to minimize microbial activity until on-shore filtration. A Masterflex portable peristaltic sampler (Cole-Parmer) was used to concentrate cellular biomass of particle-associated microbes, and planktonic microbes (3 and 0.22 µm PES filters; Millipore), respectively. Filters were submerged in a house-made RNA preservation solution (De Wit et al., 2012) in cryovials and stored on dry ice during transport and then a -80℃ freezer until DNA extraction.

Extraction of DNA from preserved filters was performed using modified steps from Lever et al. (2015). Filters were thawed and aseptically cut in half. Cellular lysis was performed using a lysis solution (30 mM tris hydrochloride, 30 mM EDTA, 800 mM guanidine hydrochloride, 0.5% Triton X-100) at pH 10, followed by a round of freeze-thawing. Nucleic acid extracts were purified using one volume of chloroform-isoamylalcohol (24:1). After purification, DNA was precipitated with one volume of PEG 6000 (30% v/v) and a 0.5 volume of 1.6 M NaCl. Two subsequent washes of the DNA pellet with 70% ethanol were used to remove the PEG-NaCl. DNA pellets were dissolved in PCR grade water.

The V4 region of the 16S rRNA gene was amplified and sequenced with the primer pair 515F (5’-GTGCCAGCMGCCGCGGTAA-3’) and 805R (5’-GACTACVSGGGTATCTAAT-3’) using a dual index approach (Kozich et al., 2013; Gohl et al., 2016). Illumina sequencing was performed on the amplicons at the University of Minnesota Genomics Center (Minneapolis, MN) using the MiSeq platform and 2x300 bp chemistry. Amplicon reads were processed using Mothur (v1.39) following the standard operating protocol (Schloss et al., 2009). Amplicon pairs were checked for quality, assembled, and aligned to the SILVA v132 database (Pruesse et al., 2012). Chimeras were checked with Uchime2 (Edgar, 2016), and operational taxonomic units (OTUs) clustered at 97% similarity using the opticlust method (Westscott and Schloss, 2017). Representative OTU sequences were taxonomically classified with the SILVA v132 database using the Naive Bayesian classifier (Wang et al., 2007). Graphs were built using the ggplot2 package in R (Wickham, 2016). All amplicon sequences were deposited to the National Center for Biotechnology Information (NCBI) Sequence Read Archive under Project Number PRJNA560450 and Accession numbers SRR9985080-SRR9985286.