**Title:**

Spatial variability of sediment methane production and methanogen communities within a eutrophic reservoir: importance of organic matter source and quantity

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**ABSTRACT**

Freshwater reservoirs are an important source of the greenhouse gas methane (CH4) to the atmosphere, but global emission estimates are poorly constrained (13.3 – 52.5 Tg C yr-1), partially due to extreme spatial variability in emission rates within and among reservoirs. Spatial heterogeneity in the availability of organic matter (OM) for biological CH4 production by methanogenic archaea may be an important contributor to this variation. To explore this, we measured sediment CH4 potential production rates, OM source and quantity, and methanogen community composition at fifteen sites within a eutrophic reservoir in Ohio, USA. CH4 production rates were highest in the riverine portion of the reservoir near the main inlet. This pattern persisted even when rates were normalized to OM quantity, indicating that OM was more readily utilized by methanogens in the riverine zone. Sediment stable isotopes and C:N indicated a greater proportion of terrestrial OM in the bulk sediment of this zone. Methanogens were present at all sites, and while taxa were similar across zones, the riverine zone contained a higher relative abundance of methanogens capable of acetoclastic and methylotrophic methanogenesis, likely reflecting differences in decomposition processes or OM quality. While we found that methane production rates were negatively correlated with algal-derived carbon in bulk sediment OM, production rates were positively correlated with indicators of algal-derived carbon in the porewater dissolved OM. It is likely that both dissolved and bulk OM affect CH4 production rates, and that both terrestrial and aquatic OM sources are important in the riverine methane production hotspot.

**INTRODUCTION**

Lakes and reservoirs are a globally significant source of methane (CH4) (Bastviken et al. 2011; Holgerson & Raymond 2016; Stanley et al. 2016), a greenhouse gas (GHG) with over 25 times the warming potential of carbon dioxide (CO2) (Myhre et al. 2013). Global CH4 fluxes from reservoirs remain poorly constrained (13.3 – 52.2 Tg C year-1; Deemer et al. 2016), due in part to uncertainty in reported emission rate estimates for individual reservoirs. Methane emissions from reservoirs (impoundments created by damming rivers) are of particular interest due to the increasing number of these systems, their land coverage area, and their high biogeochemical processing rates (Downing et al. 2006; Harrison et al. 2009; Zarfl et al. 2015). For example, compared to natural lakes, reservoirs tend to have a high watershed area - to - surface area ratio which can result in high sediment and nutrient loading from the surrounding catchment (Thornton et al. 1990, Hayes et al. 2017). This can lead to higher production (Hayes et al. 2017) and C burial rates (Knoll et al. 2014), with the potential to increase methane generation in these systems.

Methane is the end-product of organic matter (OM) decomposition in anoxic freshwater sediments in the absence of more favorable electron acceptors such as sulfate. OM availability is therefore an important constraint on the production of CH4 in freshwater sediments, where sources of OM inputs include allochthonous material derived from the watershed that are transported to the waterbody and autochthonous material produced within the waterbody (i.e. algal biomass). Transport of carbon and nutrients from the surrounding watershed may facilitate high inputs of both allochthonous OM (OMterr) and autochthonous OM (OMaq) to reservoir sediments, either directly or via primary production fueled by incoming nutrients. In the sediments, fermenting bacteria and archaea convert complex OM molecules to smaller substrates such as acetate or C1 compounds (methanol, methyl-amines, etc.) which can be utilized by methanogenic archaea (methanogens) to produce CH4.

Laboratory studies have demonstrated that OM additions to lake sediments increase CH4 production rates (Schwarz et al. 2009; West et al. 2012, 2015a). In these studies, algal derived OM stimulated a greater increase in CH4 production than terrestrial OM (West et al. 2012). This is consistent with reports that sediment CH4 production rates across different lakes are positively correlated with lake productivity and sediment OM derived from algae (West et al. 2015b, Duc et al. 2010), and that reservoir productivity and water-air interface CH4 emissions are positively correlated at a global scale (Deemer et al. 2016, DelSontro et al 2018). Despite growing evidence of the importance of OMaq in CH4 production, another recent study demonstrated that additions of OMterr to lake sediments can stimulate CH4 production to the same degree as additions of OMaq (Grasset et al. 2018) and ancient terrestrial OM has been shown to support aquatic respiration and food webs (Guillemette et al. 2017a and McCallister & delGiorgio 2012). Given the mixed evidence in the literature, our understanding of the contribution of OMterr and OMaq to CH4 production rates at an ecosystem scale is not well constrained, but has important implications for the mitigation of aquatic CH4 emissions; CH4 production driven by OMterr suggests that management efforts should focus on watershed OM inputs, while CH4 production driven by OMaq suggest efforts should focus on management of algal production.

Reservoirs are ideal ecosystems for investigating the relationship between OM dynamics and CH4 production due to the predictable patterns of OM input across their length. Reservoirs are comprised of three functional zones – riverine, transitional, and lacustrine – characterized by differences in depth, thermal stratification, sediment composition, primary productivity, and water velocity (Thornton et al. 1990). The quantity of OM in the sediment is expected to vary across reservoir zones, as is the relative contribution of OMaq and OMterr to the sediments. Further, reservoir tributary inlets and depositional zones have been demonstrated to be hot spots for CH4 emissions across the air-water interface (DelSontro et al. 2011; Maeck et al. 2013; Beaulieu et al. 2014a; Beaulieu et al. 2015; Grinham et al. 2011), suggesting these depositional zones are hotspots for sediment CH4 production. These hotspots may be fueled by 1) OMterr that settles out in these transitional zones, 2) OMaq stimulated by the delivery of nutrients from the riverine input, or 3) a combination of both sources.

Methanogens play a significant role in the global carbon cycle yet their composition and activity (CH4 production) are rarely studied together in freshwater environments. OM can shape microbial communities in freshwater environments (Fagervold et al. 2014), but little is known about the response of the methanogenic community to variations in OM source or quantity in these ecosystems. Taxonomic groups of methanogens vary in their ability to utilize different substrates (Liu & Whitman 2008); thus, variation in substrate availability in freshwater sediment likely affects methanogen community structure and activity, including the pathway utilized by methanogens to generate CH4.

To address the role of carbon source (OMterr vs. OMaq) and quantity on sediment CH4 production, we investigated methanogenesis rates, sediment OM characteristics, and methanogen communities across 15 sites within Harsha Lake, a eutrophic reservoir located in the midwestern United States, in the late spring of 2016. The objectives of the study were to: (1) establish if the three functional reservoir zones (lacustrine, transitional, and riverine) exhibit distinct variation in CH4 production rates, bulk sediment characteristics, sediment OM composition, and sediment methanogen communities, (2) determine the relative influence of OM source, including OMterr vs. OMaq, and OM quantity on sediment CH4 production rates, and (3) identify the variables that best explain spatial variation in CH4 production rates from a suite of sediment, water column, and methanogen community measurements. We expected that sediment methanogenesis rates would be highest in the riverine zone, intermediate in the transition zone, and lowest in the lacustrine zone. Based on direct and indirect evidence of the mechanistic relationship between algal-derived carbon and methanogenesis rates, we hypothesized that the source of OM would best predict methanogenesis rates, with the highest rates associated with high sediment OMaq content. Methanogen abundance was expected to increase with high CH4 production rates, and methanogen community composition was expected to vary spatially along the riverine-lacustrine gradient. Collectively, the results presented in this study contribute to our understanding of environmental factors that may influence spatial variation in CH4 production and methanogen community composition in freshwater sediments.

**METHODS**

***Site description.*** William H. Harsha Lake is a reservoir in southwest Ohio that was built on the East Fork of the Little Miami River in 1978. The primary functions of this reservoir include flood control, drinking water supply, recreation, and wildlife habitat. It has a surface area of 7.9 km2, is seasonally stratified, and reaches 32.8 m at its maximum depth. Harsha Lake’s watershed is 882 km2, with agriculture (including corn, soybean and pasture) as the dominant land-use (Beaulieu et al. 2016). Harsha Lake was an ideal system for this study because the surface CH4 emission rates of this reservoir have been quantified previously, and emission hotspots have been identified (Beaulieu et al. 2014a; Beaulieu et al. 2016). Both CH4 emissions and summer chlorophyll *a* concentrations are typically highest in the riverine zone near the main tributary of the reservoir (Beaulieu et al. 2016; Beck et al. 2017). These data informed our sample design and conceptual framework.

***Sample collection.*** Triplicate sediment cores, water samples, and water column measurements were collected from 15 sites across the reservoir (Fig. 1). Sites were selected to span the length of the reservoir and encompass a range of water depths, temperature, oxygen, productivity, and inputs to the sediment. Sites were categorized into reservoir zones (riverine, transitional or lacustrine) based on thermal stratification from temperature-depth profiles measured at each site in July 2016, when stratification was expected to be strongest. Thermally stratified sites with a hypolimnion thickness >1 m were defined as lacustrine. Weakly stratified sites with a hypolimnion thickness <1 m were categorized as transitional, and sites that were not stratified were defined as riverine (Niffy 2008). Sediment and water sampling was conducted on three separate dates that occurred within a 7-day window in late May 2016. On each date, three 5 cm diameter sediment cores were collected from each of five sites distributed across the three reservoir zones using a K-B Corer (Wildco ®, Yulee, FL, U.S.A.). Epilimnion (0.1 m below water surface) and hypolimnion (0.5-2 m above sediment) water samples were collected at each site using a Niskin bottle. At each site, depth profiles of water temperature, pH, dissolved oxygen, specific conductivity were measured using a ProDSS multiparameter sonde (YSI, Yellow Springs, OH, U.S.A.), and depth profiles of chromophoric dissolved organic matter (CDOM), in vivo chlorophyll *a*, and turbidity were measured using a C3 Submersible Fluorometer (Turner Designs, Sunnyvale, CA, U.S.A.) at each site. Secchi depth and depth profiles of photosynthetically active radiation (PAR) (LiCor LI-250A) were also measured.

***Water sample processing.*** Water samples collected from each site for chlorophyll *a*, total nitrogen (TN) and total phosphorus (TP) analyses were stored on ice or refrigerated until they were processed (within 24 hours). The spectrophotometric method was used to measure chlorophyll *a* following filtration (0.45 µm pore size) and acetone extraction (APHA 2012). TP was measured following acid persulfate digestion (Prokopy 1992) and TN was measured following alkaline persulfate digestion (APHA 1995, followed by Wendt 1995) using automated colorimetry (Lachat Instruments QuickChem 8000 Flow Injection Autoanalyzer, Loveland, CO, USA).

***Sediment processing.*** Sediment cores were sectioned and processed within 24 hours of collection. The top 5 cm of each core was extruded, homogenized, and subsampled in a glove box under N2 atmosphere. Subsamples of sediment were used for CH4 potential production rate assays, sediment characterization, porewater collection and nucleic acid extraction. Sediment slurries for CH4 production assays were prepared in the glove box by adding 15 mL of sediment and 15 mL of lake water to a 120 mL serum bottle using syringes with the tips cut off. Slurries were then capped with a rubber stopper, crimp sealed, and wrapped in aluminum foil. After subsampling for DNA extraction and sediment characterization, sediment was frozen at -80˚C (for DNA extraction) or -20˚C (for sediment characterization) until further analysis. Samples for porewater collection were stored at 4˚C until the porewater was extracted (within 24 hours of core subsampling).

***Sediment slurries –CH4 potential production rates.*** After removing the capped slurries from the glove box, the bottles were shaken vigorously for 2 minutes and purged with N2 gas for 5 minutes to remove as much of the initial dissolved CH4 as possible; then the incubation period began. All slurries were stored in the dark at room temperature (~23˚C) during the 9-day incubation. 11 mL gas samples were taken on days 1, 2, 3, 5, 7 and 9. An equal volume of 11 mL of N2 gas was returned to the serum bottle after each sampling to maintain pressure during the incubation. Gas samples were analyzed on a gas chromatograph (Bruker 450, Massachusetts, U.S.A.) equipped with a flame ionization detector (FID). Methane potential production rates for the slurries were calculated by accounting for dilution during sampling, then determining the change of moles of CH4 over time. Methane potential production rates were expressed either normalized to sediment volume, sediment dry mass, or mass of sediment organic matter. Details of calculations are available at the Center for Open Science Open Science Framework (OSF) project page (https://osf.io/59hnj/).

***𝛿 13C in CO2 and CH4 – CH4 production pathway.*** Stable isotope ratios of carbon (𝛿13C) in CO2 and CH4 were measured from gas sampled on the last day (day 9) of a subset of the CH4 production assays to discern between dominant CH4 production pathways. Analysis was carried out at the UC Davis Stable Isotope Facility. 13C – CO2 was measured on a ThermoScientific GasBench system interfaced to a ThermoScientific Delta V Plus isotope ratio mass spectrometer (IRMS) (ThermoScientific, Bremen, Germany), and 𝛿13C – CH4 was measured on a ThermoScientific Precon concentration unit system interfaced to a ThermoScientific Delta V Plus IRMS (ThermoScientific, Bremen, Germany).

The apparent fractionation factor (αC) of 𝛿13C in CO2 and CH4 was used to estimate the dominant methanogenesis pathway (Whiticar et al. 1986; Conrad 2005) (Eq. 1):

Eq. 1

Apparent fractionation factor values greater than 1.065 indicate that hydrogenotrophic methanogenesis is the dominant pathway, while C < 1.055 are indicative of acetoclastic methanogenesis (Whiticar et al. 1986; Whiticar et al. 1999; Conrad 2005).

***Sediment characterization.*** Sediment was dried at 60˚C for 3 days or until constant weight, and the difference between the wet and dry sediment weight was used to determine water content. Dried sediment was ground with a ceramic mortar and pestle prior to all other analyses. Elemental analysis and stable isotope analysis (13C and 15N) were conducted using an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS). Sediment was fumigated with hydrochloric acid prior to C and 13C analysis to removed carbonates (Harris et al. 2001). Analysis with EA-IRMS was conducted at the UC Davis Stable Isotope Facility (Elementar Vario EL Cube, Elementar Analysensysteme GmbH, Hanau, Germany interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer, Sercon Ltd., Cheshire, UK) or the Stable Isotope Geochemistry Lab in the Department of Geology in the University of Cincinnati (Costech Instruments Elemental Analyzer periphery interfaced to a Thermo Scientific Delta V Advantage Isotope Ratio Mass Spectrometer). An analytical intercomparison between laboratories verified that the different instruments used gave consistent elemental and isotopic results. Density was calculated from the wet weight of a known volume of sediment. Organic matter content was calculated by determining weight loss after ignition (550˚C, 4 hours). Elemental and isotopic composition of sediment was used to determine the proportion of OMaq and OMterr (see *Mixing model*).

***Porewater analyses.*** Porewater was extracted from a subsample of sediment cores for determination of volatile fatty acids (VFA), dissolved organic carbon (DOC), and for excitation emission matrix (EEMs) fluorescence. Porewater was extracted by centrifuging sediment in 50 mL polyproplyene centrifuge tubes at 7800 rpm. The porewater was filtered at 0.45 µm prior to measurement of optical absorbance and fluorescence using a scanning spectrofluorometer (Horiba Aqualog UV-800). The optical data were used to calculate the fluorescence index (FI), biological index (BIX), relative fluorescence efficiency (RFE), humification index (HIX), and the specific ultraviolet absorbance at 254 nm (SUVA254), all of which provide information about the composition and source of dissolved organic matter. FI is calculated as the ratio of emission wavelengths 470 nm / 520 nm at excitation 370 (McKnight et al. 2001). BIX is the ratio of emission wavelengths 380 nm / 430 nm at excitation 310 nm (Huguet et al. 2009). RFE is the ratio of fluorescence at excitation 370 nm and emission 460 nm to the absorbance at 370 nm (Downing et al. 2009). HIX is defined as the area under the emission spectra between 435-480 nm divided by the sum of the peak area at 300-345 nm and 435-480 nm at excitation 254 nm (Ohno 2002). Practically, due to lack of available excitation emission pairs at every wavelength combination, we used a slight modification of the HIX calculation with all values within 2 nm of the published method (Ohno 2002). SUVA254 is the absorbance at 254 nm divided by DOC concentration (Weishaar et al. 2003). A summary of these and other optical properties is described by Hansen et al (2016).

***Sediment traps.*** Sediment traps were deployed at 3 of the 15 sampling sites (one per reservoir zone, Fig. 1) to determine sedimentation rates and composition of the water column particulates. Sediment traps were deployed for seven weeks during the summer of 2016, with the initial deployment in early June 2016. Traps were constructed using 5 cm PVC pipe, were capped at the bottom, had a height to diameter ratio of 5:1 (Bloesch & Burns 1980), and were deployed 2 m from the sediment surface for the transitional and lacustrine sites, and 1.5 m above the sediment for the riverine site due to the shallow water depth at this location. Preservatives to prevent degradation of the material in the sediment traps were not used, but traps were sampled weekly to minimize OM decomposition. During trap sampling, samples were stored on ice or at 4˚C in the dark until filtered (within 24 hours). If necessary, the tubes were scrubbed with a brush before redeployment of the trap. Sedimentation rates were determined by calculating the total solids (TS) per sediment trap area, and dividing by the number of days the trap was deployed. Organic matter, chlorophyll *a*, elemental composition (C, N) and stable isotope composition (13C and 15N) for a subset of the samples were measured to evaluate the composition of the sediment trap material, using the methods described above.

***DNA extraction.*** DNA was extracted from ~500 mg (wet weight) of sediment from each core using a MoBio PowerSoil ® DNA Isolation Kit (MoBio, Carlsbad, CA, U.S.A.) following the manufacturer’s protocol. DNA concentration was determined using a Qubit dsDNA HS Assay kit and a Qubit 3.0 Fluorometer (Invitrogen, Burlington, ON, Canada). DNA was used in *Microbial community analysis* and *qPCR*.

***Microbial community analysis.*** The V4 region of the 16S rRNA gene was amplified and sequenced using the paired-end Illumina MiSeq sequencing platform with the primers 515f and 806rB (Caporaso et al. 2012; Apprill et al. 2015). All sequencing was performed at the Center for Bioinformatics & Functional Genomics at Miami University (Oxford, OH, USA). The resulting sequences were subjected to quality control, alignment to the SILVA (v128) database, chimera check, and classification of operational taxonomic units (OTUs) at a sequence identity of 97% using the mothur software (v1.39.5) (Schloss et al. 2009) following the MiSeq SOP protocol from Kozich et al. 2013 (accessed: 2/28/17). All sequences from two sediment cores (one from site 5 and one from site 7) were removed during quality control steps in mothur and were not included in the results. One OTU from an unclassified methanogen order with only one identified sequence was excluded from analysis. Figures for visualizing community data were generated using phyloseq v.1.20.0 (McMurdie & Holmes 2013) and ampvis v.1.27.0. (Albertson et al 2015).

***qPCR.*** Quantitative polymerase chain reactions (qPCR) were performed to determine the abundance of *mcrA* (a biomarker for methanogens), and archaeal 16S rRNA genes on a StepOne Plus ™ Real-Time PCR System. *mcrA* encodes a protein necessary for methanogenesis and has been widely used as a marker for methanogens (Luton et al. 2002). *mcrA* was quantified using the degenerate primers mcrA-F(5’-GGTGGTGTMGGATTCACACARtAYGCWACAGC-3’) and mcrA-R (5’-TTCATTGCRTAGTTWGGRTAGTT-3’) (Luton et al. 2002). Each 20 µL SYBR qPCR reaction contained 2x SYBR Green PCR Universal Master Mix (Applied Biosystems), 2 µL of DNA (pre-diluted to 2 ng/µL), and 500 nM of the forward and reverse primers using the following cycling conditions: 40 cycles of 15s at 95˚C followed by 60s at 55˚C. A TaqMan probe and primer set developed by Yu et al. (2005) were used quantify total archaea. TaqMan qPCR assays (20 µL final volume) consisted of 2x TaqMan PCR Universal Master Mix (Applied Biosystems), 500 nM of the forward and reverse primers, 200 nM of the TaqMan probe, and 2 µL of DNA (pre-diluted to 2 ng/µL) and were performed using the following cycling conditions: 45 cycles of 15s at 95˚C followed by 60s at 60˚C. The standard curve for the SYBR green assay was constructed using an *mcrA* clone (Promega pGEM® -T Easy Vector with JM109 High Efficiency Competent Cells) from environmental DNA. Briefly, *mcrA* was PCR-amplified from a Harsha Lake sediment DNA sample, purified (Wizard® PCR Preps DNA Purification System, Applied Biosystems), inserted into a vector, transformed into JM109 High Efficiency Competent Cells, and then the vector was isolated (Wizard® Plus SV Minipreps DNA Purification System, Applied Biosystems). Manufacturer’s instructions were followed for each step. Standard curves for archaeal 16S rRNA genes were constructed from a PCR-amplified 16S rRNA gene from a pure archaeal culture and purified (Wizard® PCR Preps DNA Purification System). The vector and purified PCR product were quantified as described above. The copy number of each gene was normalized by the concentration of DNA and the grams of sediment (wet weight) used in the DNA extraction.

***Statistical methods and data analysis.*** All statistical analyses were performed using R version 3.4.0 (R Core Team 2017). Mixed effects (ME) linear models with Satterthwaite approximations for degrees of freedom (Luke 2017) were used to evaluate the effect of reservoir zone on CH4 potential production and sediment characteristics using the lme4 package (Bates et al. 2015), and differences among least squares means of the zones were compared using the lmerTest package (Kuznetsova et al. 2016). This model structure nests replicate cores within each of the 15 sampling sites, thereby accounting for the likelihood that measurements from replicate cores are more likely to be related to each other than to measurements from cores at other sites.

To evaluate the factors that best explain variation in CH4 potential production rates, an information-theory approach was used (Anderson 2008). ME linear models with site as a random factor were performed using the nlme package (Pinheiro et al. 2017) as described in Zuur et al. (2009). Mixed effects linear models were generated to represent the following working hypotheses: 1) CH4 production rates are best explained by OM source, 2) CH4 production rates are best explained by OM quantity, and 3) CH4 production rates are best explained by the combination of OM source and quantity. All models used CH4 potential production rates per unit volume as the response variable. Candidate predictor variables included a large suite of measurements of water column and sediment characteristics at each site, partitioned into variables indicating “OM quantity” or “OM source”. All predictors were considered in the “source and quantity model”. The full list of variables can be found in Table S1 or at the OSF repository (<https://osf.io/9br4q/)>. Correlation among predictor variables for each model was assessed using variance inflation factor (VIF) scores, and variables with scores higher than 3 were excluded from models (Zuur et al. 2010). Variable selection for each model was conducted using a backward selection approach (Diggle et al. 2002) and all models were compared against the "null" (intercept-only) model. The final model representing each hypothesis were ranked based on AIC score corrected for the number of estimated parameters (AICc). Akaike weights (*wi*) representing the relative likelihood of the model, evidence ratios (*Ei,j*), and AICc valueswere calculated as described by Anderson (2008).

In addition, partial least square (PLS) regression analysis was used to investigate which measured parameters best explained variation in CH4 potential production rates. PLS regression was conducted using the R package ‘plsdepot’ (Gaston Sanchez 2012). One response variable, CH4 potential production rates per unit sediment volume (µmol CH4 cm-3 day-1), was used. Explanatory variables included water column variables (water chemistry, temperature, dissolved oxygen, chlorophyll, etc.), sediment variables (density, organic matter content, organic matter source, etc.) and biological variables (percent of different methanogen genera). The full list of variables can be found in Table S2 and at the OSF repository (<https://osf.io/9br4q/)>.

***Mixing model for determining contribution of OMaq to sediment.*** To determine the proportion of OMaq and OMterr found in each sediment sample, a Bayesian mixing model was used. The mixing model was generated using the MixSIAR package version 3.1.7 (Stock & Semmens 2013) in R. Terrestrial and aquatic end members were collected, and elemental N/C ratios and 𝛿15N were analyzed along with sediment samples using EA-IRMS. Terrestrial samples were taken from the surrounding watershed and included stream-bank soil, leaf litter, corn field soil, and corn stalk litter from tributary streams and a field along the perimeter of the reservoir. Aquatic end members came from epilimnion water samples from each of the 15 sites collected at the same time of sediment sampling (collected as described above). Water samples were filtered through pre-ashed 0.7 µm Whatman GF/F glass fiber filters and dried at 55˚C before packing into capsules for elemental and isotopic analysis. Epilimnion water samples from all sites were included in the determination of N:C ratios and 𝛿15N values for the aquatic end member to account for potential compositional variation of algae and cyanobacteria across the reservoir. One-way ANOVAs did not reveal differences among zones in the epilimnion water column particulate isotopic signatures (𝛿15N; F2, 10 = 1.01, p = 0.28, and 𝛿13C; F2, 10 = 2.11, p = 0.17) or elemental ratios (C:N; F2, 10 = 2.60, p = 0.12, and N:C; F2, 10 = 3.11, p = 0.09). Further, chlorophyll *a* (as determined by both *in vivo* fluorescence and extracted pigment absorbance) was greater than 16.0 µg L-1 at all but one of the sites at the time of sampling, indicating that a substantial fraction of the OM was algal biomass.

**RESULTS**

***Site characteristics.*** The water column depth at the sampling sites ranged from 2.5 – 31 m (Table 1). The depth of the water column averaged 3.6 m in the riverine zone, 9.6 m in the transitional zone, and 20.3 m in the lacustrine zone. All sites were nutrient rich, with epilimnion total nitrogen (TN) concentrations ranging from 1210 – 2040 µg/L and total phosphorus (TP) concentrations ranging from 166 – 232 µg/L. The riverine zone had the highest average TN and TP concentrations (1745 and 210 µg/L, respectively), and the lacustrine zone had the lowest average concentrations (1380 and 180 µg/L, respectively). Similarly, average epilimnion chlorophyll *a*, measured by *in vivo* fluorescence and from extracted pigment absorbance was highest in the riverine zone (4128 RFUB and 47.6 µg/L, respectively), lowest in the transitional zone (1021 RFUB and 21.8 µg/L), and intermediate in the lacustrine zone (1877 RFUB and 24.3 µg/L). Secchi depth increased from the riverine zone (average 0.33 m) to the lacustrine zone (average 0.78 m). The full suite of chemical and physical measurements at each site can be found in the OSF repository SI (https://osf.io/9br4q/).

***Methane potential production rates among reservoir zones.*** Methane potentialproduction rates normalized by sediment volume and by quantity of organic matter were greater in the riverine zone as compared to the transitional or lacustrine zones (µmol CH4 cm-3 day-1; F2,12 = 12.89, = 0.001, and CH4 g OM dry sediment-1 day-1; F2,12 = 10.26, = 0.003) (Fig. 2A & C). The mean areal CH4 potential production rate for all sediment cores was 3.79 µmol CH4 cm-2 day-1 (range: 0.79 - 8.56 µmol CH4 m-2 day-1), assuming an active sediment depth of only 5 cm.As the areal CH4 potential production rate was arrived at simply by multiplying the volume-normalized rate by the sampled depth (5 cm for all cores), the spatial variation in areal rate was the same as for the volume-normalized rate, i.e. higher in the riverine zone than the other two zones (F2,12 = 12.88, = 0.001). Methane potential production rates normalized by dry weight of the sediment slurry were not statistically different among the zones (F2,12 = 2.03, = 0.2; Fig. 2B).

***Sediment characteristics among reservoir zones.*** Sediment density was greatest in the riverine zone, intermediate in the transitional zone, and lowest in the lacustrine zone (Table 2), and while the ME model indicated significant differences among zones (F2,12 = 12.3, *p* = 0.001), the differences between the lacustrine and transitional zone were not statistically significant (p = 0.24). The concentration of dissolved organic carbon (DOC) in the sediment porewaters was greatest in the riverine zone (F2,12 = 5.52, *p* = 0.02; Fig. 3). Bulk OM trended toward greater abundance in the riverine zone, but the pattern was not significant (F2,12 = 2.02, *p* = 0.18; Fig. 3). Stable isotope and elemental composition data indicated that the source of OM varied significantly among reservoir zones (Fig. 4). The ME linear model indicated that zone was significant in explaining variation in the proportion of OMaq (calculated from the mixing model) in the bulk sediment (F2,12 = 8.06, *p* = 0.006). OMaq was lowest in the riverine zone, intermediate in the transitional zone, and highest in the lacustrine zone, though the riverine and transitional zone were not significantly different (p = 0.13; Figure 4).

The ratio of elemental C:N in the bulk sediment can indicate OM source, with higher C:N ratios resulting from terrestrial OM sources due to high C content of structural plant material, and lower C:N ratios resulting from algal and microbial sources. The average C:N ratio of sediment from the riverine zone was 9.6 ± 1.1, while the transitional and lacustrine zones had slightly lower ratios of 7.5 ± 1.0 and 7.4 ± 0.7, respectively (Table 2).

Optical properties of the porewater dissolved OM showed some evidence of differences in DOM source across reservoir zones (Fig. 5; Table 3). Fluorescence index (FI) values are usually between 1.2 and 1.8 in natural waters (Hansen et al. 2016), with higher FI values indicating DOM derived from microbial sources (e.g. bacteria and algae) and lower values indicating DOM derived from terrestrial OM (McKnight et al. 2001; Cory et al. 2010). FI values were highest in the riverine zone, indicating a higher proportion of microbial sources as compared to the lacustrine and transitional zones (F2,12.2 = 14.02, *p* = 0.0007). The biological index (BIX) can indicate autotrophic productivity: a BIX of 0.6 - 0.7 indicates a low autochthonous component, 0.7 - 0.8 an intermediate autochthonous component, 0.8 - 1.0 a strong autochthonous component, and values >1.0 indicate aquatic bacterial origin (Huguet et al. 2009). BIX did not vary among reservoir zones (F2,12.1 = 1.53, *p* = 0.2), with site averages ranging from 0.59 to 0.71 (Table 3). HIX and SUVA254 are both representative of the amount of processing that the DOM has undergone, with higher values representing more humification and higher aromatic content of the OM, respectively (Hansen et al. 2016). The HIX values present in Harsha Lake sediment porewaters were explained by reservoir zone, with averages of 0.72, 0.83, and 0.82 for riverine, transitional and lacustrine sites (F2,12.2 = 6.51, *p* = 0.01). The riverine zone had lower HIX values than the transitional (p = 0.006) and lacustrine (p = 0.009) zones, while the transitional and lacustrine zones did not differ from each other (p = 0.73; Fig. 5). Differences in porewater SUVA254 values across all zones were statistically significant (F2,12.9 = 10.74, *p* = 0.002; Fig. 5). Mean SUVA254 was 2.7 L mg-C-1 m-1 in the riverine zone, 4.2 L mg-C-1 m-1 in the transitional zone, and 5.5 L mg-C-1 m-1 in the lacustrine zone.

***Sediment deposition rates and deposited sediment composition.*** Over the 6-week sediment trap deployment period, average sediment deposition rates were highest in the riverine sediment trap site, and lowest in the lacustrine sediment trap site (Table 4). Similar to the benthic sediment, the percent of organic matter of the deposited sediment was lowest in the riverine site (12.3%), intermediate in the transitional site (16.7%), and highest in the lacustrine site (22.5%), but due to the high sedimentation rates, the riverine sediment trap had the highest OM deposition rates. Further, the mean rate of chlorophyll deposition in the riverine sediment trap for the 6-week period exceeded that of the transitional and lacustrine sites (Table 4).

***Methanogen communities among reservoir zones.*** Abundance of the *mcrA* gene and a region of the 16S rRNA gene targeting archaea were determined using qPCR. Copies of *mcrA* ranged from 1.4 x 101 to 3.8 x 104 ng DNA-1 g sediment-1 (wet weight), with a mean of 3.7 x 103. There were no differences among reservoir zones in copy number for either the *mcrA* gene (F2,12.4 = 0.06, = 0.94) or the archaeal 16S rRNA gene (F2,12.1 = 1.28, = 0.31).

From 16S rRNA gene sequencing, 5708 archaeal operational taxonomic units (OTUs) were observed across 44 sediment cores. Of the 5708 archaeal OTUs, 939 were assigned to methanogens (16.5%). Sequences affiliated with four of the six known orders of methanogens were recovered from the Harsha Lake sediments: Methanomicrobiales (606 OTUs), Methanosarcinales (218 OTUs), Methanobacteriales (105 OTUs), and Methanocellales (9 OTUs). On average, methanogens represented 34.8% of total archaea when calculated from 16S rRNA sequence data, and 33.7% of total archaea when calculated from qPCR data. Representative OTUs from the order Methanomicrobiales accounted for 53%, 77% and 76% of total methanogen sequences from the riverine, transitional, and lacustrine zones, respectively (Fig. 6). The order Methanosarcinales comprised 34%, 16% and 17% of riverine, transitional and lacustrine methanogens. The two most abundant methanogen genera in all three zones were *Methanoregula* and *Methanosaeta*. *Methanobacterium* was relatively abundant in all three zones, whereas *Methanosarcina* and *Methanospirillum* composed a greater proportion of methanogen genera in the riverine than in other zones.

***Methane production pathway.*** 𝛿13C of CH4 ranged from -56.7 to -48.0‰, and values were not explained by reservoir zone (F2,7 = 4.52, = 0.06; Fig. 7A).Apparent fractionation factors (C)were consistent with acetoclastic methanogenesis across all zones (range 1.040 – 1.050). The isotope fractionation trended slightly higher in the lacustrine zone relative to the riverine zone (higher C; Fig. 7B), suggesting an increased contribution of hydrogenotrophic methanogenesis in the lacustrine zone compared to the other two zones.

***Influence of OM source and quantity on CH4 production rates.*** CH4 potential production rates were best explained by the combination of OM source and quantity, with a model probability of 0.989 and by far the highest marginal R2 (0.70, i.e. 70% of variance explained) of the three model categories tested (Table 5). The best predictor variables for the “OM source” hypothesis were: (1) the proportion of OMaq in the sediment and (2) BIX (biological index calculated from porewater DOM fluorescence). The best model for the “OM quantity” hypothesis included: (1) porewater DOC concentration, (2) g OM per unit volume of sediment, and (3) the interaction between these two variables. The best model for the “OM source + quantity” hypothesis included all four of the above variables. All variables, except for the proportion of OMaq, had positive correlations with CH4 potential production rates, regardless of the model. The proportion of OMaq was negatively correlated with CH4 potential production rates. Despite the “OM quantity” model having a slightly lower model probability and rank than the “OM source” model, the marginal R2 was higher for the “OM quantity” model, indicating more explained variance (48% for the OM quantity model vs. 33% for the OM quality model).

***Contextual parameters that explain spatial variation in CH4 production rates.*** PLS analysis confirmed that the three reservoir zones have distinct sediment, water column, and microbial community characteristics, and that the triplicate cores at a given location show tight grouping within their respective zones, with a few exceptions (Fig. 8). The first and second components from PLS regression explained 67.0% and 17.9% of the variance among cores, respectively, and CH4 production was strongly positively correlated with both of these components, especially axis 1. Axis 1 depicts the main gradient in the data, which shows the transition from terrestrial (riverine) to lacustrine sites across many co-varying variables, and is reflected in CH4 potential production rates which increase across the gradient from lacustrine to terrestrial/riverine (Fig. S1, Table S2). Variables with high positive loadings on axis 1 were indicative of strong terrestrial influence, and included sediment characteristics (high bulk density, OM density, C:N), porewater DOM characteristics (high FI, DOC), and water column characteristics (high turbidity, conductivity, and hypolimnion CDOM). Variables with strong negative loadings on axis 1 were indicative of deeper, lacustrine sites with minimal terrestrial influence, and included bulk sediment characteristics (high OM%, OMaq%, N%, 15N) as well as site physical and water column characteristics (deep secchi depth and site depth). Two methanogen taxa were also associated with this axis, with Methanosarcina associated with the “riverine” sites (positive on axis 1), and Methanoregula associated with the “lacustrine/transitional” sites (negative on axis 1). Axis 2 was also positively associated with high CH4 potential production rates, and appeared to represent a secondary gradient of in situ productivity, not associated with the terrestrial-lacustrine gradient. The lacustrine sites were discriminated from the transitional sites on this axis, with lacustrine sites tending to be positively associated with axis 2. High values on axis 2 seemed to indicate sites (or cores) with high productivity, as indicated by high positive loadings on this axis of water column chlorophyll concentrations, epilimnion pH, porewater BIX, and bulk sediment %Corg. Epilimnion nutrient concentrations (TRP, NH4+) were negatively associated with this axis, which could indicate a drawdown of soluble inorganic nutrients at high productivity sites. The methanogen taxa Linea was also negatively correlated with axis 2 of the PLS (see Fig. S1, Table S2).

**DISCUSSION**

***Spatial variability of sediment measurements across the reservoir.***

Harsha Lake exhibited strong spatial heterogeneity in sediment characteristics related to longitudinal gradients in reservoir morphology, water velocity, and water residence times. Suspended sediment in the inflowing river water rapidly settles out in the riverine zone, resulting in high sedimentation rates, primarily of dense inorganic materials (Table 4). Sediments in this zone also included OM derived from both autochthonous (OMaq, aquatic) and allochthonous (OMterr, terrestrial) sources, however, and the sediment contained the same amount of OM per unit volume, and higher porewater DOC concentrations, than sediment in downstream portions of the reservoir (Fig. 3). Sedimentation rates in the transition and lacustrine zones were lower and the deposited material contained a smaller proportion of inorganic material than in the riverine zone (Table 4). This was reflected in lower sediment density and higher sediment %OM in these downstream reservoir zones (Table 2). These differences in OM quantity and quality across the longitudinal gradient coincided with distinct patterns in CH4 production potential and microbial community composition (Fig. 8). Sediment CH4 potential production rates, expressed on an areal or volumetric basis, were higher in the riverine than transitional or lacustrine zones (Fig. 2), which is consistent with previous reports of higher CH4 emission rates in the riverine zone compared to other areas of this reservoir (Beaulieu et al. 2014a, 2016).

Published areal surface CH4 emission rates from the Harsha Lake riverine zone (Beaulieu et al. 2016) and the average of our measured riverine zone sediment CH4 potential production rates from this study were in remarkable agreement (33.0 mg CH4 m-2 h-1 and 41.2 mg CH4 m-2 h-1, respectively). However, CH4 surface emission rates in the riverine zone in Harsha can be six-fold (Beaulieu et al. 2016) to 1-2 orders of magnitude (Beaulieu et al. 2014a) higher than other areas of the reservoir, while sediment CH4 potential production rates were only two-fold higher in the riverine zone compared to other zones. This distinction is likely due to patterns in water mixing regimes and CH4 oxidation, which remove a much greater proportion of the produced CH4 in the deeper parts of the reservoir. The lacustrine zone, and to a lesser extent the transitional zone, were thermally stratified during the study and most of the CH4 that diffuses out of the sediment remains in the hypolimnion due to low rates of diffusion and advection across the thermocline (Beaulieu et al. 2014b), resulting in limited CH4 evasion across the air-water interface. Furthermore, in the stratified areas of the reservoir, approximately 25% of the small fraction of hypolimnetic CH4 that diffuses into the epilimnion is oxidized to CO2 by methanotrophic bacteria (Beaulieu et al. 2014a) rather than evading to the atmosphere (Beaulieu et al. 2014b).

Methane potential production rates were consistent with or higher than those reported in other lakes and reservoirs at similar sediment depths. Eller and colleagues (2005) found that in the top 6 cm of sediment of a dimictic lake in northern Germany, average CH4 potential production rates were 39.7 nmol CH4 g-1 dry weight h-1, and decreased with depth, while values for Harsha Lake averaged 64.8 (range 13.8 – 112.1) nmol CH4 g-1 dry weight h-1 in the top 5 cm of sediment. However, Harsha Lake incubations were conducted at a higher temperature than those reported by Eller and colleagues (2005). In an oligotrophic and a mesotrophic lake, CH4 potential production rates were lower than those in our eutrophic lake, producing 2.5 and 1.9 nmol CH4 g-1 dry weight h-1, respectively, but these incubations were also conducted at lower temperatures and evaluated deeper sediment depths (Fuchs et al. 2016). The average CH4 potential production rates from the top 5 cm of sediment from 8 northern temperate and boreal lakes was 14.3 nmol CH4 g-1 dry weight h-1 (range: 0.5 – 57.0 nmol CH4 g-1 dry weight h-1) for slurries incubated at 20˚C. These values were still lower than the average found at Harsha Lake, despite having a similar incubation temperature. Few studies evaluate differences in sediment production rates across a reservoir; however, Rodriguez et al. (2018) found that in un-amended sediment slurries from the top 10 cm of sediment from Itaparica, a large hydropower reservoir in Brazil, the profundal (lacustrine) site had the largest potential production rates (23.3 nmol CH4 g-1 dry weight h-1), the intermediate (transitional) site had intermediate rates (2.5 nmol CH4 g-1 dry weight h-1), and the littoral site had the lowest rates (1.3 nmol CH4 g-1 dry weight h-1). These data contrast our results where the riverine site (most comparable to the littoral site) had the highest rates.

Although areal and volumetric CH4 potential production rates were higher in the riverine than transitional or lacustrine zones, rates did not differ among reservoir zones when expressed per gram dry mass. This illustrates that differences in sediment density contribute to the spatial patterns in volumetric and areal CH4 potential production rates. Sediment density in the riverine zone was 18-25% greater than in the other reservoir zones and therefore contained more material per unit volume or area that might enhance CH4 production. While inorganic fractions of the sediment may provide surface area for microbiota attachment, the organic fraction is the source fueling methanogenesis. When normalized to sediment OM, the riverine zone had higher CH4 potential production rates than the other reservoir zones (Fig. 2C), indicating that OM is converted to CH4 more efficiently in the riverine zone than in the downstream reservoir zones. This could be due to numerous factors including differences among reservoir zones in the quality of the OM, the composition of microbial communities, or sediment temperature and redox status.

Based on satellite and field measurements indicating greater water column chlorophyll concentration in the riverine than transitional or lacustrine zones as a rule in Harsha Lake (Beck et al. 2017), we had predicted that OM in the riverine zone sediment would contain a greater proportion of algal derived carbon than elsewhere in the system. However, this prediction was not borne out by the data. In fact, although the riverine zone had the greatest water column chlorophyll *a* during the survey (Table 1) and chlorophyll *a* sedimentation rates were 6 – 13 times greater than the other reservoir zones, the proportion of autochthonous derived material in the riverine sediment OM was lower than other portions of the reservoir. This pattern reflects the large input of OMterr that enters the reservoir via the river inflow and is subsequently deposited in the riverine zone, effectively diluting the OMaq carbon signature in the sediment. The OMaq carbon signature in the riverine sediment could be further eroded by preferential microbial degradation of this labile material. The spatial pattern in OM content provides some evidence of preferential carbon mineralization. Despite having summer chlorophyll *a* sedimentation rates that are up to an order of magnitude greater than the other reservoir zones, the sediment OM content (g OM cm-3) in the riverine zone was no different than the other sites. This suggests that microbial activity rapidly mineralizes freshly deposited algal derived carbon, thereby limiting the autochthonous signature in the sediment.

***Characterization of methanogen communities and CH4 production pathways.***

Similar to results of other studies in freshwater systems, we found no correlation between methanogen abundance and CH4 potential production rates (West et al. 2012; Chaudhary & Blaser 2017). This indicates that CH4 production rates in Harsha Lake were not limited by methanogen abundance, but instead are linked to substrate quantity and/or quality. The variation in substrate availability likely affects methanogen activity, rather than abundance, and thus determines rates of methanogenesis.

The two methanogen genera with the highest relative abundances across all zones in Harsha Lake, *Methanoregula* and *Methanosaeta*, are the two genera of methanogens that are most frequently encountered in freshwater lakes (Borrel et al. 2011). Combined, sequences from these two genera accounted for 67.3% of total methanogen sequences in the riverine zone, 84.7% in the transitional zone, and 83.5% in the lacustrine zone. The riverine zone had 4 genera that were each responsible for 10% or more of total methanogen sequences (*Methanoregula, Methanosaeta, Methanosarcina, and Methanobacterium*), while the transitional and lacustrine zone only had 2 genera (*Methanoregula* and *Methanosaeta*) that were responsible for 10% or more of total methanogen sequences. *Methanoregula* are hydrogenotrophic methanogens, while *Methanosaeta* are acetoclastic methanogens.

The most notable differences in methanogen communities among reservoir zones were the relatively high abundance of *Methanosarcina*, a genus of the Methanosarcinales order capable of both acetoclastic and methylotrophic methanogenesis, and the presence of methylotrophic methanogens *Methanolobus* and *Methanomethylovorans,* in the riverine zone (Fig. 6). *Methanosarcina* comprised an average of 15% of methanogens at the riverine sites, but less than 1% of methanogens in the transition and lacustrine zones. *Methanosarcina* can utilize a diverse range of substrates to produce CH4, including acetate as well as methanol and methylamine (Liu & Whitman 2008), while *Methanolobus* and *Methanomethylovorans* can only utilize methylated compounds. These compounds are derived from the degradation of lignin-containing organic matter (Grey et al. 2010; Penger et al. 2012), as well as degradation of compounds such as pectin and cholin (Borrel et al. 2011). Although lignin compounds were not directly quantified in the sediment samples, coarse pieces of leaves and sticks were routinely observed in riverine zone sediment cores, whereas this material was absent from the more downstream reservoir zones. Thus, the high relative abundance of *Methanosarcina* in the riverine zone and the presence of *Methanolobus* and *Methanomethylovorans* could be a result of the terrestrial-derived plant OM that is present there. The implications of the high relative abundance of *Methanosarcina* are twofold, depending on their activity: they could be performing acetoclastic methanogenesis, which often indicates high-quality substrate is available (i.e. OMaq), or they could be performing methylotrophic methanogenesis with methyl compounds derived from OMterr.

Though our method for estimating the methanogenesis pathway only provides a coarse estimate of the production pathway, our results indicated that acetoclastic methanogenesis was dominant in Harsha Lake, despite the hydrogenotrophic methanogen *Methanoregula* being the most abundant methanogen in all zones. This is consistent with expectations for freshwater systems (Conrad 1999), but contradicts some reports indicating that hydrogenotrophic methanogenesis may play an important role in freshwater systems (e.g. Blair et al. 2018, Murase & Sugimoto 2001).

Combined, the isotope fractionation and methanogen community data indicate that acetoclastic methanogenesis may play a larger role in the riverine zone relative to the rest of the reservoir. This is supported by a higher relative abundance of acetoclastic methanogens (*Methanosaeta* and *Methanosarcina*) and the lower apparent fractionation factor (Fig. 7B) in the riverine zone than in other zones. Paired decreases in the contribution of acetoclastic methanogenesis to total methane production and relative abundance of acetoclastic methanogens have been observed with sediment depth, likely as a result of decreased availability of easily-degradable OM (Liu et al. 2016). This pattern is observed laterally in Harsha Lake, and could also be a result of spatial variation in OM quality, at that larger scale.

***Relationships between sediment characteristics and CH4 potential production rates.***

Methane potential production rates were positively correlated with the quantity of bulk and dissolved OM, both of which were greatest in the riverine zone. This relationship likely reflects greater substrate availability for methanogens in organic rich environments; it is consistent with reports of correlation between DOC concentration and diffusive CH4 fluxes from northern lake and ponds (Wik et al. 2016), and correlation between dissolved CH4 and DOC concentrations in a eutrophic lake in China (Zhou et al. 2018). Both Duc et al. (2010) and our study found a negative correlation between sediment organic carbon (%) and CH4 production, and water content and CH4 production; however Duc and colleagues (2010) report that lower %OC (and high CH4 production) corresponded with algal C:N sediment signatures, while sediment from Harsha Lake showed the opposite pattern.

The relationship between carbon source and CH4 potential production rates was different between the solid and dissolved fraction of OM. In the dissolved portion of carbon, potential production rates were positively correlated with BIX, an indicator of OMaq, which is consistent with several reports that algal biomass is a labile carbon source readily utilized by methanogens (Schwarz et al. 2008, West et al. 2012, Duc et al. 2010, Grasset et al. 2018). The CH4 rates were also negatively correlated with HIX and SUVA254, indices that represent humified and aromatic DOM. In the bulk sediment in our study, the estimated proportion of OMaq was negatively correlated to potential production rates. This relationship was driven by the high CH4 production rates and relatively low proportion of autochthonous material in the riverine zone sediment.

There are several potential explanations for these seemingly contradictory results:

1. There are high rates of mineralization in the riverine zone, so algal material is more readily and rapidly degraded and less is incorporated into the bulk sediment, shifting the proportion to indicate terrestrial origin. In the riverine zone, the labile algal material fuels high CH4 production rates, while at the same time the high sedimentation rates result in sediments with a higher proportion of terrestrial OM (e.g., Guillemette et al. 2017b).
2. The dissolved fraction of OM is what primarily drives CH4 production, therefore the relationship that is seen between CH4 potential production rates and the solid fraction of OM is less relevant. This would suggest that of the total bulk pool of OM in the sediments, the algal-derived portion is more readily solubilized and made available for methanogens.
3. Both algal and terrestrial derived OM may be important for CH4 production. The algal OM sources may be rapidly degraded while the terrestrial OM is degraded over both short and longer time scales (see Guillemette et al. 2017a; Grasset et al. 2018). Overall, the riverine zone receives more OM than the other zones, including substantially greater amounts of terrestrial OM. Therefore the high rates of CH4 potential production can be explained by a slow sustained breakdown of terrestrial OM over time, on top of the higher rates sponsored by algal-derived OM.

Without more direct evidence, such as 14C-CH4 which would discriminate between modern autochthonous C and older terrestrial C sources, we cannot definitively say which source of OM is fueling methanogenesis with our data set. However, based on our regression analysis, both organic matter source and quantity are substantially relevant for CH4 production rates, much more so than either the source or quantity of OM alone. The predictor variables that were included in the model with source and quantity included the proportion of OMaq in the sediment, BIX (biological index) of porewater DOM, porewater DOC concentration, and the mass of OM per volume of sediment. The inclusion of DOC concentration and the BIX optical property in the model indicates that the dissolved fraction of organic matter was important for CH4 production in terms of both quantity and source. Importantly, all of the models included variables that represented both the bulk and dissolved fractions of OM, suggesting that both pools play a role in CH4 production.

Our data indicate there may be a link between bulk terrestrial OM and CH4 production in Harsha Lake. C:N ratios from bulk sediment were positively correlated to CH4 potential production rates; higher C:N ratios were associated with higher potential production rates. These results contrast with another study of boreal and northern temperate lake sediments in central Sweden, which indicated a negative correlation between CH4 production rates and C:N ratios (Duc et al. 2010). The positive correlation between C:N and CH4 potential production in Harsha Lake aligns with the observation that the highest production rates occurred in the riverine zone, which also receives the highest terrestrial inputs and has the highest proportion of OMterr in the sediments. Thus, the riverine zone is an exceptionally active deposition zone for both algal and terrestrial OM, and this material together sponsors very high CH4 production rates relative to other parts of the reservoir.

***Summary and conclusion***

Sediment methane potential production varied spatially across reservoir zones. Categorizing sites into zones aided in conceptualizing the differences in methane-generating processes across the reservoir. Further, this approach provides a generalizable framework for comparison with other reservoirs. The riverine differed from the other areas of the reservoir, and was characterized by high CH4 potential production rates, high terrestrial carbon inputs, dense sediments, and fresh porewater DOM. Research on reservoir biogeochemistry should include a focus on this zone, as it has the potential to play a large role in carbon cycling relative to the entire reservoir. Both source and quantity of organic matter were important for CH4 potential production rates. This has important implications for global carbon cycling, as the amount of sediment OM transported to reservoirs is expected to increase and the number of reservoirs globally are increasing (Downing et al. 2006, Zarfl et al. 2015). Terrestrial OM appears to play a role in fueling CH4 production in the zone with the highest potential production rates. This is an important area to explore in future studies; in addition to the growing body of evidence that OMaq is a driver of methane production, OMterr may contribute to the CH4 production and emission “hotspots” that are found within systems.

Although methanogen abundances did not vary specifically among reservoir zones or with CH4 production rates, methanogen communities did vary among zones - largely due to one taxonomic group, *Methanosarcina*; this group added functional diversity to the riverine zone. The methanogen community composition may help explain differences in CH4 production rates and pathways between the riverine zone and the rest of the reservoir.

Cumulatively, our results indicate that both algal and terrestrial sources of OM are important in driving high methane production in the riverine zone of the main reservoir inlet, which may have implications for reservoir management. Both watershed-scale soil erosion control and nutrient reductions may promote decreased methane production. From this study we recognize the potential of terrestrial OM to contribute to “hotspots” of reservoir CH4 production, particularly in depositional/riverine zones – with many central questions remaining to be addressed on the topic.

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**TABLES**

**Table 1.** Physical and chemical water column properties for each of the sampling sites.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Site** | **Site Depth (m)** | **Reservoir Zone** | **Secchi Depth (m)** | **Sample Location** | | **Sample Depth (m)** | | **Temperature (˚C)** | | **Dissolved Oxygen (% Saturation)** | | **pH** | **Chlorophyll *a* (µg/L)** | | **Chlorophyll *a* (*in vivo)* (RFUBa)** | **CDOM (RFUBa)** | | **TN (µg/L)** | **TP (µg/L)** |
| R1 | 2.5 | riverine | 0.22 | epilimnion | | 0.1 | | 27.3 | | 76.1 | | 7.91 | - | | 457 | 1743 | | 1210 | 172 |
| hypolimnion | | 1.5 | | 26.7 | | 58.7 | | 7.56 | - | | 526 | 1994 | | 1488 | 210 |
| R2 | 3.0 | riverine | 0.38 | Epilimnion | | 0.1 | | 22.9 | | 90.3 | | 8.20 | 4.8 | | 346 | 2102 | | 2000 | 232 |
| hypolimnion | | 2.0 | | 16.3 | | 74.5 | | 7.81 | 3.2 | | 455 | 2176 | | 2240 | 244 |
| R3 | 4.0 | riverine | 0.33 | epilimnion | | 0.1 | | 24.1 | | 174.2 | | 8.98 | 74.2 | | 6958 | 2036 | | 2040 | 227 |
| hypolimnion | | 3.0 | | 19.1 | | 39.3 | | 7.58 | 1.8 | | 437 | 2170 | | 2280 | 287 |
| R4 | 5.0 | riverine | 0.40 | epilimnion | | 0.1 | | 22.7 | | 163.5 | | 8.90 | 63.9 | | 8750 | 1926 | | 1730 | 210 |
| hypolimnion | | 4.0 | | 21.9 | | 133.7 | | 8.47 | 31.6 | | 8054 | 2068 | | 1600 | 185 |
| T1 | 9.0 | transitional | 0.52 | epilimnion | | 0.1 | | 27.0 | | 139.5 | | 8.80 | - | | 430 | 2197 | | 1410 | 182 |
| hypolimnion | | 8.0 | | 17.2 | | 3.0 | | 7.45 | - | | 352 | 2133 | | 1475 | 216 |
| T2 | 9.0 | transitional | 0.68 | epilimnion | | 0.1 | | 26.7 | | 147.3 | | 8.83 | - | | 495 | 2182 | | 1400 | 201 |
| hypolimnion | | 8.0 | | 17.2 | | 3.8 | | 7.35 | - | | 346 | 2174 | | 1288 | 203 |
| T3 | 9.0 | transitional | 0.73 | epilimnion | | 0.1 | | 22.8 | | 84.0 | | 7.85 | 18.6 | | 779 | 1894 | | 1490 | 220 |
| hypolimnion | | 8.0 | | 17.1 | | 35.5 | | 7.39 | 1.7 | | 387 | 2076 | | 1510 | 211 |
| T4 | 10.0 | transitional | 0.53 | epilimnion | | 0.1 | | 20.4 | | 108.7 | | 8.29 | 30.4 | | 2333 | 2184 | | 1490 | 199 |
| hypolimnion | | 9.0 | | 16.8 | | 31.7 | | 7.39 | 5.4 | | 436 | 2091 | | 1490 | 216 |
| T5 | 11.0 | transitional | 0.80 | epilimnion | | 0.1 | | 19.8 | | 85.5 | | 8.12 | 16.4 | | 1068 | 1954 | | 1400 | 194 |
| hypolimnion | | 10.0 | | 16.9 | | 31.5 | | 7.36 | 1.8 | | 286 | 2140 | | 1520 | 220 |
| L1 | 12.0 | lacustrine | 0.60 | epilimnion | | 0.1 | | 21.4 | | 122.4 | | 8.78 | 29.2 | | 3620 | 1983 | | 1380 | 166 |
| hypolimnion | | 10.5 | | 16.7 | | 30.4 | | 7.15 | 0.8 | | 303 | 2146 | | 1710 | B.D. |
| L2 | 14.0 | lacustrine | 0.53 | epilimnion | | 0.1 | | 21.7 | | 143.0 | | 8.56 | 32.0 | | 3035 | 1951 | | 1330 | 167 |
| hypolimnion | | 12.0 | | 15.6 | | 12.0 | | 7.08 | 1.9 | | 288 | 2105 | | 1710 | 194 |
| L3 | 16.5 | lacustrine | 1.04 | epilimnion | | 0.1 | | 27.7 | | 232.7 | | 9.25 | - | | 418 | 1937 | | 1750 | 185 |
| hypolimnion | | 13.5 | | 14.7 | | 1.0 | | 7.28 | - | | 255 | 1910 | | 1249 | 215 |
| L4 | 22.5 | lacustrine | 0.87 | epilimnion | | 0.1 | | 26.5 | | 198.0 | | 9.25 | - | | 286 | 1995 | | 1250 | 188 |
| hypolimnion | | 19.0 | | 11.0 | | 8.2 | | 7.28 | - | | 237 | 1968 | | 4575 | 46 |
| L5 | 26.0 | lacustrine | 1.00 | epilimnion | | 0.1 | | 19.5 | | 82.7 | | 7.90 | 16.0 | | 1078 | 1881 | | 1320 | 195 |
| hypolimnion | | 24.0 | | 10.4 | | 9.5 | | 7.04 | 0.5 | | 225 | 1898 | | 1270 | 248 |
| L6 | 31.0 | lacustrine | 0.67 | epilimnion | | 0.1 | | 21.6 | | 133.3 | | 8.57 | 19.9 | | 2827 | 1831 | | 1250 | 177 |
| hypolimnion | | 24.5 | | 10.3 | | 0.9 | | 6.81 | 0.5 | | 232 | 1891 | | 1330 | 254 |
|  | **3.6** | **riverine** | **0.33** | **epilimnion** | |  | | **24.3** | | **126.0** | | **8.50** | **47.6** | | **4128** | **1952** | | **1745** | **210** |
| **hypolimnion** | |  | | **21.0** | | **76.6** | | **7.86** | **12.2** | | **2368** | **2102** | | **1902** | **231** |
|  | **9.6** | **transitional** | **0.65** | **epilimnion** | |  | | **23.3** | | **113.0** | | **8.38** | **21.8** | | **1021** | **2082** | | **1438** | **199** |
| **hypolimnion** | |  | | **17.0** | | **21.1** | | **7.39** | **3.0** | | **362** | **2123** | | **1457** | **213** |
|  | **20.3** | **lacustrine** | **0.78** | **epilimnion** | |  | | **23.1** | | **152.0** | | **8.72** | **24.3** | | **1877** | **1930** | | **1380** | **180** |
| **hypolimnion** | |  | | **13.1** | | **10.3** | | **7.11** | **0.9** | | **257** | **1986** | | **1974** | **191** |
| Cells with no values were not measured for the given parameter. | | | | | | | | |  | |  | | |  | | |  | | | |
| B.D. values were below detection. | | | | |  | |  | |  | |  | | |  | | |  | | | |
| aRFUB are blank-subtracted raw fluorescence units from a submersible fluorometer. | | | | | | | | | | | | | |  | | |  | | | |

**Table 2.** Bulk sediment characteristics for each of the 15 sites. Values are reported as mean ± SD. Averages for each zone are in bold.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Site | Reservoir Zone | CH4 production rates  (µmol day-1 cm3) | %Corg | δ13Corg (‰) | %N | δ15N (‰) | Corg:N(g:g) | OM (% dry weight) | Density (g/mL) | Proportion autochthonous OM |
| R1 | riverine | 1.10 ± 0.05 | 1.90 ± 0.11 | -26.11 ± 0.42 | 0.19 ± 0.01 | 5.35 ± 0.04 | 10.01 ± 0.79 | 6.02 ± 0.45 | 1.66 ± 0.26 | 0.54 ± 0.00 |
| R2 | riverine | 1.36 ± 0.30 | 2.22 ± 0.18 | -26.20 ± 1.10 | 0.22 ± 0.02 | 5.25 ± 0.34 | 10.03 ± 1.76 | 6.93 ± 0.44 | 1.36 ± 0.08 | 0.54 ± 0.01 |
| R3 | riverine | 1.31 ± 0.11 | 1.90 ± 0.26 | -25.44 ± 0.28 | 0.21 ± 0.03 | 5.68 ± 0.10 | 8.91 ± 0.76 | 5.23 ± 0.99 | 1.45 ± 0.06 | 0.56 ± 0.00 |
| R4 | riverine | 1.16 ± 0.02 | 2.37 ± 0.15 | -25.84 ± 0.52 | 0.25 ± 0.01 | 5.68 ± 0.05 | 9.34 ± 0.79 | 7.91 ± 0.16 | 1.35 ± 0.02 | 0.56 ± 0.00 |
| T1 | transitional | 0.97 ± 0.16 | 2.27 ± 0.22 | -25.44 ± 0.47 | 0.28 ± 0.02 | 5.75 ± 0.25 | 8.00 ± 1.00 | 9.02 ± 0.68 | 1.2 ± 0.03 | 0.56 ± 0.01 |
| T2 | transitional | 0.55 ± 0.01 | 2.16 ± 0.24 | -25.79 ± 0.51 | 0.29 ± 0.00 | 5.86 ± 0.22 | 7.54 ± 0.92 | 9.32 ± 0.12 | 1.2 ± 0.03 | 0.56 ± 0.01 |
| T3 | transitional | 0.36 ± 0.18 | 1.98 ± 0.40 | -25.72 ± 0.26 | 0.27 ± 0.01 | 6.01 ± 0.40 | 7.32 ± 1.30 | 8.41 ± 0.32 | 1.27 ± 0.01 | 0.56 ± 0.01 |
| T4 | transitional | 0.67 ± 0.16 | 2.41 ± 0.28 | -25.82 ± 0.41 | 0.34 ± 0.01 | 5.73 ± 0.10 | 7.04 ± 1.06 | 10.67 ± 0.58 | 1.21 ± 0.01 | 0.55 ± 0.01 |
| T5 | transitional | 0.23 ± 0.06 | 1.92 ± 0.23 | -26.54 ± 0.38 | 0.26 ± 0.00 | 6.23 ± 0.13 | 7.39 ± 0.95 | 7.73 ± 0.26 | 1.26 ± 0.01 | 0.57 ± 0.01 |
| L1 | lacustrine | 0.86 ± 0.1 | 2.29 ± 0.24 | -25.32 ± 0.49 | 0.29 ± 0.01 | 6.25 ± 0.18 | 7.86 ± 0.99 | 9.34 ± 0.11 | 1.29 ± 0.01 | 0.58 ± 0.00 |
| L2 | lacustrine | 0.72 ± 0.06 | 2.51 ± 0.3 | -25.38 ± 0.48 | 0.34 ± 0.01 | 6.12 ± 0.18 | 7.49 ± 1.16 | 10.85 ± 0.21 | 1.23 ± 0.03 | 0.57 ± 0.00 |
| L3 | lacustrine | 0.26 ± 0.09 | 2.46 ± 0.03 | -26.43 ± 0.46 | 0.31 ± 0.01 | 6.38 ± 0.02 | 7.89 ± 0.24 | 9.62 ± 0.38 | 1.08 ± 0.02 | 0.58 ± 0.00 |
| L4 | lacustrine | 0.65 ± 0.14 | 2.72 ± 0.19 | -25.95 ± 0.73 | 0.37 ± 0.01 | 6.14 ± 0.34 | 7.44 ± 0.62 | 11.49 ± 0.77 | 1.13 ± 0.03 | 0.57 ± 0.01 |
| L5 | lacustrine | 0.66 ± 0.22 | 2.90 ± 0.28 | -26.17 ± 1.14 | 0.44 ± 0.02 | 6.06 ± 0.30 | 6.63 ± 0.37 | 11.73 ± 1.12 | 1.17 ± 0.01 | 0.56 ± 0.01 |
| L6 | lacustrine | 0.54 ± 0.19 | 3.27 ± 0.11 | -26.24 ± 1.13 | 0.45 ± 0.00 | 6.24 ± 0.25 | 7.21 ± 0.20 | 12.90 ± 0.26 | 1.05 ± 0.02 | 0.57 ± 0.01 |
|  | **riverine** | **1.23 ± 0.18** | **2.1 ± 0.26** | **-25.9 ± 0.64** | **0.22 ± 0.03** | **5.49 ± 0.25** | **9.57 ± 1.07** | **6.52 ± 1.16** | **1.45 ± 0.18** | **0.55 ± 0.01** |
|  | **transitional** | **0.56 ± 0.29** | **2.15 ± 0.3** | **-25.86 ± 0.52** | **0.29 ± 0.03** | **5.92 ± 0.28** | **7.46 ± 0.95** | **9.03 ± 1.09** | **1.23 ± 0.03** | **0.56 ± 0.01** |
|  | **lacustrine** | **0.62 ± 0.22** | **2.7 ± 0.37** | **-25.91 ± 0.79** | **0.37 ± 0.06** | **6.19 ± 0.24** | **7.42 ± 0.73** | **11.02 ± 1.34** | **1.16 ± 0.09** | **0.57 ± 0.01** |

**Table 3.** Concentration and optical properties of the porewater DOM for each of the 15 sites. Values are reported as mean ± SD. Averages for each zone are in bold.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Site | Reservoir Zone | DOC (mg/L) | FI | BIX | RFE | HIX | SUVA254  (mg C-1 m-1) |
| R1 | riverine | 28.73 ± 2.24 | 1.73 ± 0.01 | 0.65 ± 0.00 | 1.33 ± 0.06 | 0.74 ± 0 | 2.89 ± 0.32 |
| R2 | riverine | 134.49 ± 152.23 | 1.73 ± 0.04 | 0.69 ± 0.07 | 1.09 ± 0.18 | 0.64 ± 0.22 | 1.59 ± 1.32 |
| R3 | riverine | 39.26 ± 5.47 | 1.72 ± 0.02 | 0.69 ± 0.02 | 0.7 ± 0.18 | 0.68 ± 0.03 | 3.01 ± 0.86 |
| R4 | riverine | 26.79 ± 9.34 | 1.74 ± 0.02 | 0.69 ± 0.01 | 0.9 ± 0.07 | 0.8 ± 0.01 | 3.21 ± 0.90 |
| T1 | transitional | 23.9 ± 1.47 | 1.69 ± 0.02 | 0.65 ± 0.01 | 1.28 ± 0.13 | 0.85 ± 0.02 | 3.31 ± 0.04 |
| T2 | transitional | 24.59 ± 2.21 | 1.66 ± 0.01 | 0.64 ± 0.01 | 0.82 ± 0.1 | 0.84 ± 0.03 | 3.84 ± 0.08 |
| T3 | transitional | 21.45 ± 2.77 | 1.65 ± 0.03 | 0.63 ± 0.02 | 0.89 ± 0.02 | 0.82 ± 0.04 | 4.37 ± 0.33 |
| T4 | transitional | 24.76 ± 2.04 | 1.66 ± 0.01 | 0.67 ± 0.00 | 0.63 ± 0.06 | 0.78 ± 0.01 | 5.04 ± 0.59 |
| T5 | transitional | 19.27 ± 1.42 | 1.64 ± 0.01 | 0.61 ± 0.02 | 0.89 ± 0.09 | 0.84 ± 0.02 | 4.56 ± 0.11 |
| L1 | lacustrine | 22.9 ± 3.56 | 1.69 ± 0.01 | 0.70 ± 0.00 | 0.51 ± 0.26 | 0.8 ± 0.01 | 6.08 ± 2.44 |
| L2 | lacustrine | 19.23 ± 0.53 | 1.66 ± 0.04 | 0.69 ± 0.00 | 0.57 ± 0.16 | 0.83 ± 0.03 | 6.52 ± 1.01 |
| L3 | lacustrine | 20.85 ± 1.75 | 1.60 ± 0.01 | 0.59 ± 0.01 | 0.83 ± 0.37 | 0.87 ± 0.02 | 5.03 ± 1.67 |
| L4 | lacustrine | 26.75 ± 7.04 | 1.61 ± 0.02 | 0.61 ± 0.01 | 1.08 ± 0.08 | 0.87 ± 0.02 | 3.49 ± 0.37 |
| L5 | lacustrine | 26.09 ± 1.56 | 1.65 ± 0.01 | 0.70 ± 0.00 | 0.38 ± 0.14 | 0.75 ± 0.03 | 5.96 ± 1.13 |
| L6 | lacustrine | 17.87 ± 4.17 | 1.66 ± 0.04 | 0.71 ± 0.05 | 0.38 ± 0.15 | 0.77 ± 0.02 | 6.85 ± 1.46 |
|  | **riverine** | **57.32 ± 80.16** | **1.73 ± 0.02** | **0.68 ± 0.04** | **1.01 ± 0.27** | **0.72 ± 0.11** | **2.67 ± 1.03** |
|  | **transitional** | **22.8 ± 2.8** | **1.66 ± 0.02** | **0.64 ± 0.02** | **0.9 ± 0.23** | **0.83 ± 0.03** | **4.22 ± 0.67** |
|  | **lacustrine** | **22.52 ± 4.92** | **1.64 ± 0.04** | **0.66 ± 0.05** | **0.65 ± 0.33** | **0.82 ± 0.05** | **5.54 ± 1.73** |

| **Table 4.** Sediment trap data. Sediment traps were deployed during the summer of 2016. | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Sediment deposition rates (g m-2 day-1)** | **Chlorophyll a deposition rates (mg m-2 day-1)** | **OM deposition rates (g OM m-2 day-1)** | **Sediment OM (%)** | **δ15N** | **δ13Corg** | **%N** | **%Corg** | **Corg:N (g:g)** |
| Riverine (R1) | 473.02 | 67.77 | 63.51 | 12.32 | 5.87 | -27.24 | 0.57 | 4.00 | 6.78 |
| Transitional(T1) | 60.34 | 10.05 | 9.46 | 16.70 | 6.03 | -27.81 | 0.69 | 4.71 | 6.71 |
| Lacustrine (L3) | 16.33 | 4.87 | 3.76 | 22.52 | 4.59 | -27.15 | 0.99 | 6.49 | 6.71 |

**Table 5.** Results of model hypothesis testing. The response variable was methane production rates (µmol CH4 cm-3 day-1). Arrows next to predictor variables indicate a positive (↑) or negative (↓) relationship with the response variable. *K* is the number of estimated parameters, AICc is a second order AIC score used to account for the number of estimated parameters. Δ*i* values are AICc differences (compared to the best model). Model probabilities (*wi*), otherwise called Akaike weights, are estimates of the probability of the model being the best K-L (Kullback-Leibler) model, given the data and set of competing models (see Anderson 2008). The evidence ratios (*Ei,j*) measure the strength of evidence of the hypotheses and represent the relative likelihood of hypothesis *i* vs. *j*. In this case they represent the likelihood of the best model (H3) relative to the other models. Marginal R2 describes the proportion of variance explained by fixed factors alone, and the conditional R2 describes the proportion of variance explained by both fixed and random effects. Note that ranks, model probabilities, and evidence ratios (*Ei,j*) are relative to the set of models and dependent on the data used to create the models.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **H** | **Hypothesis** | **Model** | ***K*** | **log(*L*)** | **AIC** | **AICc** | **Rank** | **∆*i*** | **Model probability *wi*** | ***Ei,j*** | **Marginal R2** | **Conditional R2** |
| H1 | source OM | autochthonous OM (↓) + BIX (↑) | 5 | 13.53 | -17.06 | -15.56 | 2 | 9.6 | 0.0080 | 123.8 | 0.33 | 0.85 |
| H2 | quantity OM | log(DOC) (↑) \* g OM slurry-1 (↑) | 6 | 13.76 | -15.52 | -13.37 | 3 | 11.8 | 0.0027 | 370.6 | 0.48 | 0.87 |
| H3 | source OM + quantity OM | autochthonous OM + log(DOC) \* g OM slurry-1  + BIX | 8 | 22.55 | -29.09 | -25.20 | 1 | 0.0 | 0.9893 |  | 0.70 | 0.89 |

**FIGURE LEGENDS**

**Figure 1.** Harsha Lake sampling sites and site categories. The legend indicates which sites were assigned to the riverine, transitional, and lacustrine zones, and where sediment traps were deployed.

**Figure 2.** Methane potential production rates from sediment slurries in each of the reservoir zones, normalized to sediment volume (A), sediment dry mass (B), and sediment organic matter (C). Dots represent the potential CH4 production rate calculated from each sediment core by a sediment slurry assay. Different lowercase letters indicate significant differences between reservoir zones.

**Figure 3.** Comparison of the quantity of OM among reservoir zones. Panel A represents the amount of OM found in the bulk sediment, normalized to sediment volume. Panel B represents the concentration of the dissolved fraction of OM found in the sediment porewater, measured as dissolved organic carbon (DOC). One riverine value with an exceptionally high DOC concentration was excluded from the plot for readability. Different lowercase letters indicate significant differences between reservoir zones.

**Figure 4.** (A) Plot of 𝛿15N vs. N/C elemental ratios of terrestrial and aquatic OM sources, and the sediment mixtures for each core across the three reservoir zones. The center dots for the two sources represent the mean values (n = 13 for terrestrial sources, n = 16 for aquatic sources), and error bars represent the standard deviation. (B) The proportion of autochthonous (aquatic) OM found in each core grouped by reservoir zones, as calculated from the stable isotope mixing model. Different lowercase letters indicate significant differences among reservoir zones.

**Figure 5.** Optical indices of dissolved organic matter. Higher FI values (A) indicate DOM from microbial sources, and higher BIX values (B) indicate a stronger OMaq signature. Higher HIX (C) and SUVA254 (D) values represent higher degrees of humification and aromatic content of the OM. Different lowercase letters indicate significant differences between reservoir zones.

**Figure 6.** Heat map showing relative abundance of methanogen genera in sediments, by reservoir zone. Row labels show order and genus (order; genus). Note that values are normalized to total number of sequences of all methanogens (not to total archaea), and that percentages are averages of all samples within a group (within the zone).

**Figure 7.** Carbon isotope signature in CH4 (A) and the apparent fractionation factors (B) across reservoir zones. The blue horizontal line in (B) indicates the cutoff between hydrogenotrophic (above) and acetoclastic (below) methanogenesis. Methane production pathway was estimated at 10 of the 15 sampling sites (n = 30 sediment cores). Different lowercase letters indicate significant differences between reservoir zones.

**Figure 8.** Multivariate ordination of each observation (core) taken in May of 2016 using the first two PLS components (T-components). Water column data, sediment composition, and methanogen taxonomic data were included in the PLS analysis. See Table S1 for full list of variables included in the PLS analysis. Overlapping core cores were removed from the figure for readability.

Figure 1



Figure 2



Figure 3



Figure 4



Figure 5



Figure 6



Figure 7



Figure 8

