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

Megan E. Berberich,1*,a Jake J. Beaulieu,2 Trinity L. Hamilton,1,b Sarah Waldo,2,c Ishi Buffam1

1Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio
2USEPA, Office of Research and Development, Cincinnati, Ohio

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 investigate this, we measured sediment CH4 potential production rates, OM source and quantity, and methanogen community composition at 15 sites within a eutrophic reservoir in Ohio, USA. CH4 production rates were highest in the shallow riverine inlet zone of the reservoir, even when rates were normalized to OM quantity, indicating that OM was more readily utilized by methanogens in the riverine zone than in the transitional or lacustrine zones. Sediment stable isotopes and C:N indicated a greater proportion of terrestrial OM in the particulate sediment of this zone. Methanogens were present at all sites, but 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 potential production rates were negatively correlated with autochthonous carbon in particulate sediment OM, rates were positively correlated with indicators of autochthonous carbon in the porewater dissolved OM. It is likely that both dissolved and particulate sediment OM affect CH4 production rates, and that both terrestrial and aquatic OM sources are important in the riverine methane production hot spot.

Lakes and reservoirs are a globally significant source of methane (CH4) (Bastviken et al. 2011; Holgerson and Raymond 2016; Stanley et al. 2016; DelSontro et al. 2018), a greenhouse gas (GHG) with 45 times the sustained-flux global warming potential of carbon dioxide (CO2) over a 100-year timescale (Neubauer and Megonigal 2015). Global CH4 fluxes from reservoirs remain poorly constrained (13.3–52.2 Tg C yr\(^{-1}\); 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 relative to lakes (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 that 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. Sediment OM is a complex mixture of autochthonous material, derived from in situ primary production within the waterbody (OM\(_{\text{aq}}\)), and allochthonous material, derived from terrestrial watershed sources transported to the waterbody (OM\(_{\text{ter}}\)), in different stages of decomposition.
Throughout the decomposition process, fermenting bacteria and archaea convert complex OM molecules to smaller substrates such as acetate or C1 compounds (methanol, methyl-amines, etc.) that can be utilized by methanogenic archaea (methanogens) in the terminal stages of decomposition to produce CH4 [see Liu and Whitman 2008].

The chemical composition of OM can be used to infer origin (i.e., OMterr vs. OMAq; e.g., McKnight et al. 2001; Stedmon et al. 2003; Hood et al. 2005; Mladenov et al. 2010) and determines, in part, the bioavailability of the material (e.g., Sun et al. 1997). For example, algae and aquatic microorganisms, components of OMAq are generally composed primarily of protein, and contain variable amounts of both lipids and carbohydrates (Bianchi and Canuel 2011). Higher plants, a component of OMterr, are composed mainly of cellulose and structural compounds, and contain a lower fraction of proteins (Bianchi and Canuel 2011). During biodegradation, aquatic OM, which is associated with aliphatic and peptide-like compounds (Kellerman et al. 2018), is generally considered to be more labile than terrestrial-derived OM (del Giorgio and Davis 2003) that can be comprised of complex structural polysaccharides and phenolic aromatic compounds such as those found in lignin. Therefore, OM source and OM quality are often discussed in conjunction.

Methanogens and the potential role OM plays in shaping microbial communities in freshwater environments (Fagervold et al. 2014) underlie the relationship between OM source and methane production. Little is known about the response of the methanogenic community’s composition and activity to variations in OM source or quantity in freshwater ecosystems, despite the significant role methanogens play in the global carbon cycle. Discrete taxonomic groups of methanogens vary in their ability to use different substrates (Liu and 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.

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; Grinham et al. 2011; Maca et al. 2013; Beaulieu et al. 2014a; Beaulieu et al. 2016), suggesting these depositional zones may be hot spots for sediment CH4 production.

OM additions to lake sediments in laboratory experiments have been shown to increase CH4 production rates (Schwarz et al. 2008; West et al. 2012, 2015a; Grasset et al. 2018), but our understanding of the contribution of OMterr and OMAq to CH4 production rates at an ecosystem scale is not well constrained. Specifically, it is unknown if, at an ecosystem scale, CH4 hot spots reflect inputs of presumed-labile OMAq or environmental conditions that facilitate the degradation of more complex molecules derived from OMterr into methanogenic substrates. In several lab studies, OMAq stimulated an increase in CH4 production (Schwarz et al. 2008; West et al. 2012, 2015a), including a greater increase in CH4 production compared to OMterr additions (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 OMAq, and that reservoir productivity and CH4 emissions from the air-water interface are positively correlated at a global scale (Deemer et al. 2016; DelSontro et al. 2018). In contrast, other recent studies demonstrate the importance of OMterr and ancient terrestrial OM to carbon cycling in aquatic systems. Grasset et al. (2018) showed that additions of OMterr to lake sediments can stimulate CH4 production to the same degree as additions of OMAq, and ancient terrestrial OM has been shown to support aquatic respiration and food webs (McCallister and del Giorgio 2012; Guillemette et al. 2017a).

Due to the complex nature of OM and OM degradation, the categorization of OM into source is a useful approach to address the major compositional differences expected in aquatic vs. terrestrial derived OM, and to categorize assumed lability. Further, for managed systems such as reservoirs, this grouping provides a framework to assess potential CH4 mitigation efforts. For example, if CH4 production is driven primarily by OMterr efforts aiming to reduce CH4 emissions should focus on the management of watershed OM inputs such as no-till agricultural practices and stream bank stabilization. On the other hand, if CH4 production is driven primarily by OMAq, efforts should focus on management of algal and cyanobacterial production, possibly through agricultural best management practices to reduce nutrient loading.

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 predictable patterns 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 relationship between aquatic-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 OMsag content due to its presumed greater lability as compared to OMterr. Methanogen abundance was expected to be highest in sites with high CH4 production rates, and methanogen community composition was expected to vary spatially along the riverine-lacustrine gradient.

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. Harsha Lake is located in the ancestral homelands of the Miami, Shawnee, Delaware, and Ojibwe people. 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 dimictic, 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 hot spots have been identified (Beaulieu et al. 2014a, 2016; Beaulieu et al. 2018). 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).

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 (Saji 2008). Sediment and water sampling was conducted on three separate dates 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®). Shallow (0.1 m below water surface) and deep (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), and depth profiles of chromophoric dissolved organic matter (CDOM), in vivo chlorophyll \( a \), and turbidity were measured using a C3 Submersible Fluorometer (Turner Designs). 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 at 5°C and analyzed within 24 h, or frozen and analyzed within 28 d. Water samples for dissolved CH\(_4\) were subsampled from the Niskin bottle—collected at both the shallow and deep water depths. Dissolved CH\(_4\) was sampled using the headspace equilibration method, as described by Beaulieu et al. (2018). The spectrophotometric method was used to measure chlorophyll \( a \) following filtration (0.45 \( \mu \)m pore size) and acetone extraction (APHA 2012). TP was measured following acid persulfate digestion (Boren 2001) and TN was measured following alkaline persulfate digestion (Smith and Bogren 2003) using automated colorimetry (Lachat Instruments QuickChem 8000 Flow Injection Autoanalyzer).

**Sediment processing**

Sediment cores were sectioned and processed within 24 h of collection. The top 5 cm of each core was extruded, homogenized, and subsampled in a glove box under \( \text{N}_2 \) atmosphere. Subsamples of sediment were used for CH\(_4\) potential production rate assays, sediment characterization, porewater collection, and nucleic acid extraction. The top sediment section was chosen for our study following similar sediment depths used for CH\(_4\) potential production assays in previous studies (e.g., Schwarz et al. 2008; Duc et al. 2010; Grasset et al. 2018), and after conducting a pilot study to determine sample depth (Supporting Information Table S1). One slurry was prepared from each sediment core, resulting in three slurries per site. Sediment slurries for CH\(_4\) potential production assays were prepared in the glove box by adding 15 mL of sediment and 15 mL of overlying core 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 h of core subsampling).

**Sediment slurries—CH\(_4\) potential production rates**

After removing the capped slurries from the glove box, the bottles were shaken vigorously for 2 min and purged with \( \text{N}_2 \) gas for 5 min to remove the initial dissolved CH\(_4\). All slurries were stored in the dark at room temperature (−23°C) during the 9-day incubation. Eleven milliliter gas samples were taken on days 1, 2, 3, 5, 7, and 9. An equal volume of 11 mL of \( \text{N}_2 \) 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) 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 in moles of CH\(_4\) over time. Methane potential production rates were expressed either normalized to sediment volume, sediment dry mass, or mass of sediment organic matter per slurry. Mass of sediment OM per slurry was calculated from the dry mass of the slurry multiplied by the percent OM determined from LOI of a sediment subsample taken from the core specifically for that purpose. Details of calculations are available at the Center for Open Science Open Science Framework (OSF) project page (https://osf.io/59hnj/).

### \( \delta^{13}\text{C} \) in CO\(_2\) and CH\(_4\)–CH\(_4\) production pathway

Stable isotope ratios of carbon (\( \delta^{13}\text{C} \)) in CO\(_2\) and CH\(_4\) were measured from gas sampled on the last day (day 9) of a subset of the CH\(_4\) production assays to discern between dominant CH\(_4\) production pathways. Analysis was carried out at the UC Davis Stable Isotope Facility. \( ^{13}\text{C}–\text{CO}_2 \) was measured on a ThermoScientific GasBench system interfaced to a ThermoScientific Delta V Plus isotope ratio mass spectrometer (IRMS) (ThermoScientific), and \( ^{13}\text{C}–\text{CH}_4 \) was measured on a ThermoScientific Preconcentration unit system interfaced to a ThermoScientific Delta V Plus IRMS (ThermoScientific). Precision of the \( ^{13}\text{C}–\text{CO}_2 \) measurement is 0.1‰, and of the \( ^{13}\text{C}–\text{CH}_4 \) measurement is 0.2‰ (stableisotopefacility.ucdavis.edu, accessed 01 September 2019).

The apparent fractionation factor (\( \alpha_c \)) of \( ^{13}\text{C} \) in CO\(_2\) and CH\(_4\) was used to estimate the dominant methanogenesis pathway (Whiticar et al. 1986; Conrad 2005) (Eq. 1):

\[
\alpha_c = \frac{(\delta\text{CO}_2 + 10^3)}{(\delta\text{CH}_4 + 10^3)}
\]

1339
Apparent fractionation factor values greater than 1.065 indicate that hydrogenotrophic methanogenesis is the dominant pathway, while $\alpha_{13} < 1.055$ are indicative of acetoclastic methanogenesis (Whiticar et al. 1986; Whiticar 1999; Conrad 2005).

**Sediment characterization**

Sediment was dried at 60°C for 3 d 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 ($^{13}$C and $^{15}$N) 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 $^{13}$C 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) interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer, Sercon Ltd.) 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. Precision of the $^{13}$C and $^{15}$N measurements are 0.2‰ and 0.3‰, respectively (see stabileisotopefacility.ucdavis.edu, accessed 01 September 2019). Reference standards USGS-40 and USGS-41 were used to calibrate $\delta^{13}$C and $\delta^{15}$N values and checked with previously quantified internal lab standards (glycine and organic wheat). NIST Standard 2710 was used for linearity corrections for both $\delta^{13}$C and $\delta^{15}$N. Sediment was dried at 60°C for 3 d or until constant weight; OM content was calculated by determining weight loss after ignition (550°C, 4 h). Elemental and isotopic composition of sediment was used to determine the proportion of OM$_{aq}$ and OM$_{terr}$ (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 polypropylene centrifuge tubes at 7800 rpm. The porewater was filtered at 0.45 μm prior to measurement of optical absorbance and fluorescence using a scanning spectrophuorometer (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 (SUVA$_{254}$), all of which provide information about the composition and source of DOM. 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 and 480 nm divided by the sum of the peak area at 300–345 nm and 435–480 nm at excitation 254 nm (Ohno 2002). 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). SUVA$_{254}$ 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 three 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 6 weeks during the summer of 2016, with the initial deployment in early June 2016. Traps were constructed using 5 cm PVC pipe that were capped at the bottom with a height to diameter ratio of 5:1 (following Bloesch and 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 h). 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. OM, chlorophyll $a$, elemental composition (C, N) and stable isotope composition ($^{13}$C and $^{15}$N) for a subset of the samples were measured to evaluate the composition of the sediment trap material, using the methods described earlier.

**DNA extraction**

DNA was extracted from ~500 mg (wet weight) of sediment from each core using a MoBio PowerSoil® DNA Isolation Kit (MoBio) following the manufacturer’s protocol. DNA concentration was determined using a Qubit dsDNA HS Assay kit and a Qubit 3.0 Fluorometer (Invitrogen). 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. 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 28 February 2017). All sequences from two sediment cores (one from site T2 and one from site T5) 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 and Holmes 2013) and ampvis v.1.27.0. (Albertsen et al. 2015). Raw sequence reads for each sample are available on NCBI Short Read Archive (SRA) under the BioProject number PRJNA532292.

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 StepOnePlus™ 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′-GGTGTTGTMGGATT CACACARTAYGCWACAGC-3′) and mcrA-R (5′-TCATTGCG RTAGTTWGGRTAGTT-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 (prediluted to 2 ng/μL), and 500 nM of the forward and reverse primers using the following cycling conditions: 40 cycles of 15 s at 95°C followed by 60 s at 55°C. A TaqMan probe and primer set developed by Yu et al. (2005) were used to 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 (prediluted to 2 ng/μL) and were performed using the following cycling conditions: 45 cycles of 15 s at 95°C followed by 60 s 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 earlier. 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 created 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 of sediment 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 Supporting Information Table S2 or at the OSF repository (https://osf.io/59hnj/). Multicollinearity 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 was ranked based on AICc 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 values were 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” (Sanchez 2012). One response variable, CH4 potential production rates per unit sediment volume (μmol CH4 cm−3 d−1), was used. Explanatory variables included water column variables (water chemistry, temperature, dissolved oxygen, chlorophyll, etc.), sediment variables (density, OM content, OM source, etc.), and additional variables (pH, redox potential, sediment characteristics, etc.).
Mixing model for determining contribution of OM$_{aq}$ and OM$_{terr}$ to sediment OM

A Bayesian mixing model was used to determine the proportion of OM$_{aq}$ and OM$_{terr}$ found in each sediment sample. The mixing model was generated using the MixSIAR package version 3.1.7 (Stock and Semmens 2013) in R. In this R package, estimation uncertainty of the means and variances for end-members is incorporated into the model (Stock et al. 2018). Uncertainty associated with each of the predicted values (proportion of OM$_{aq}$ for each core) is estimated, and the values presented here are mean proportion estimates. Terrestrial and aquatic end-members were collected, and elemental N:C ratios and $\delta^{15}$N were analyzed using EA-IRMS (see sediment characterization). Terrestrial end-member samples were collected 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 were from shallow water samples from each of the 15 sites collected at the same time of sediment sampling (collected as described earlier). Between 60 and 200 mL of water were filtered through preashed 0.7 µm Whatman GF/F glass fiber filters and dried at 55°C before packing into capsules for elemental and isotopic analysis. Shallow water samples from all sites were included in the determination of N:C ratios and $\delta^{15}$N values for the aquatic end-member to account for potential compositional variation of algae and cyanobacteria across the reservoir. 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 or cyanobacterial biomass.

RESULTS

Site characteristics

The water column depth at the sampling sites ranged from 2.5 to 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 surface TN concentrations ranging from 1210 to 2040 µg L$^{-1}$ and TP concentrations ranging from 166 to 232 µg L$^{-1}$. The riverine zone had the highest average TN and TP concentrations at the surface (1745 and 210 µg L$^{-1}$, respectively), and the lacustrine zone had the lowest average concentrations (1380 and 180 µg L$^{-1}$, respectively). Similarly, average surface 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$^{-1}$, respectively), lowest in the transitional zone (1021 RFUB and 21.8 µg L$^{-1}$), and intermediate in the lacustrine zone (1877 RFUB and 24.3 µg L$^{-1}$). 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.

Methane potential production rates among reservoir zones

Methane potential production rates normalized by sediment volume and by quantity of OM were greater in the riverine zone as compared to the transitional or lacustrine zones (µmol CH$_4$ cm$^{-3}$ d$^{-1}$; F$_{2,12}$ = 12.89, $p = 0.001$, and µmol CH$_4$ g OM$^{-1}$ d$^{-1}$; F$_{2,12}$ = 10.26, $p = 0.003$) (Fig. 2a,b). Methane potential production rates normalized by dry weight of the sediment slurry were not statistically different among the zones (F$_{2,12}$ = 2.03, $p = 0.2$; Fig. 2c). The rate most comparable to CH$_4$ emissions from the air-water interface, the areal CH$_4$ potential production rate, was 3.79 µmol CH$_4$ cm$^{-2}$ d$^{-1}$ (mean for all cores; range: 0.79–8.56 µmol CH$_4$ m$^{-2}$ d$^{-1}$), assuming an active sediment depth of only 5 cm. As the areal CH$_4$ potential production rate was determined 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, that is, higher in the riverine zone than the other two zones (F$_{2,12}$ = 12.89, $p = 0.001$).

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 (F$_{2,12}$ = 12.3, $p = 0.001$), the differences between the lacustrine and transitional zone were not statistically significant ($p = 0.24$). Particulate sediment OM trended toward greater abundance in the riverine zone, but the pattern was not significant (F$_{2,12}$ = 2.02, $p = 0.18$; Fig. 3a). The concentration of DOC in the sediment porewaters was greatest in the riverine zone (F$_{2,12}$ = 5.52, $p = 0.02$; Fig. 3b). 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 reservoir zone was significant in explaining variation in the proportion of OM$_{aq}$ (calculated from the mixing model) in the bulk sediment (F$_{2,12}$ = 8.06, $p = 0.006$). OM$_{aq}$ 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 ($p = 0.13$; Fig. 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).
| Site | Depth (m) | Reservoir zone | Secchi depth (m) | Sample depth (m) | Temperature (°C) | Dissolved oxygen (% saturation) | pH | Chlorophyll a (μg L⁻¹) | Chlorophyll a (in vivo) (RFUB*) | CDOM (RFUB*) | TN (μg L⁻¹) | TP (μg L⁻¹) | Dissolved CH₄ (μM) | SO₄²⁻ (mg L⁻¹) |
|------|-----------|----------------|-----------------|-----------------|-----------------|-------------------------------|----|-----------------------|-------------------------------|-------------|-------------|-------------|----------------|------------------|
| R1   | 2.5       | Riverine       | 0.22            | 0.1             | 27.3            | 76.1                          | 7.91| -                     | 457                           | 1743        | 1210        | 172         | 7.50           | -                |
|      | 1.5       |                |                 |                 | 26.7            | 58.7                          | 7.56| -                     | 526                           | 1994        | 1488        | 210         | 2.28           | 16.2             |
| R2   | 3.0       | Riverine       | 0.38            | 0.1             | 22.9            | 90.3                          | 8.20| 4.8                   | 346                           | 2102        | 2000        | 232         | 0.92           | -                |
|      | 2.0       |                |                 |                 | 16.3            | 74.5                          | 7.81| 3.2                   | 455                           | 2176        | 2240        | 244         | 1.09           | 21.7             |
| R3   | 4.0       | Riverine       | 0.33            | 0.1             | 24.1            | 174.2                         | 8.98| 74.2                  | 6958                          | 2036        | 2040        | 227         | 1.33           | -                |
|      | 3.0       |                |                 |                 | 19.1            | 39.3                          | 7.58| 1.8                   | 437                           | 2170        | 2280        | 287         | 3.19           | 18.3             |
| R4   | 5.0       | Riverine       | 0.40            | 0.1             | 22.7            | 163.5                         | 8.90| 63.9                  | 8750                          | 1926        | 1730        | 210         | 1.41           | -                |
|      | 4.0       |                |                 |                 | 21.9            | 133.7                         | 8.47| 31.6                  | 8054                          | 2068        | 1600        | 185         | 2.23           | 17.6             |

Cells with no values were not measured for the given parameter. B.D. values were below detection.

*RFUB are blank-subtracted raw fluorescence units from a submersible fluorometer.
Optical properties of the porewater dissolved OM showed some evidence of differences in DOM source across reservoir zones (Fig. 5; Table 3). Mean site FI values ranged from 1.60 to 1.74, which is on the upper end of the range found in natural waters (1.2–1.8; 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). BIX values, an indicator of autotrophic productivity, 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). A BIX of 0.6–0.7 indicates a low autochthonous component, 0.7–0.8 an intermediate autochthonous component, and higher values indicate increasing contribution of autochthonous sources (Huguet et al. 2009).

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), respectively. 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 OM 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 $1.4 \times 10^1$ to $3.8 \times 10^4$ ng DNA−1 g sediment−1 (wet weight), with a mean of $3.7 \times 10^2$. There were no differences among reservoir zones in copy number for either the mcrA gene (F2,12.4 = 0.06, p = 0.94) or the archaeal 16S rRNA gene (F2,12.1 = 1.28, p = 0.31).

From 16S rRNA gene sequencing, 5708 archaeal OTUs were observed across 44 sediment cores. Of the 5708 archaeal OTUs, 939 were assigned to methanogens (16%). 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 35% of total archaea when calculated from 16S rRNA sequence data, and 34% of total archaea when calculated from qPCR data. Representative OTUs from the order Methanomicrobiales accounted for 53%, 77%, and 76% of total

Fig. 2. Methane potential production rates from sediment slurries in each of the reservoir zones, normalized to sediment volume (a), sediment organic matter (b), and sediment dry mass (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.
Table 2. Bulk sediment characteristics for each of the 15 sites. Values are reported as mean ± SD. Mean values for each zone are in bold.

| Site | Reservoir zone | CH$_4$ production rates (µmol cm$^{-3}$ d$^{-1}$) | %C$_{org}$ | δ$^{13}$C$_{org}$ (‰) | %N | δ$^{15}$N (‰) | C$_{org}$:N (g:g) | OM (% dry weight) | Density (g mL$^{-1}$) | 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                                    | 2.2±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 |
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, respectively. 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**

$\delta^{13}C$ of CH$_4$ ranged from $-56.7$ to $-48.0\%$, and values were not explained by reservoir zone ($F_{2,7} = 4.52, p = 0.06$; Fig. 7a).

Apparent fractionation factors ($\alpha_C$) 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 $\alpha_C$; 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 CH$_4$ potential production rates**

The ME model relating CH$_4$ potential production rates to a combination of OM source and quantity indices was supported more strongly by the data than the models representing the “OM source” only or the “OM quality” only
hypotheses (model probability of 0.989, marginal $R^2 = 0.70$, Table 5). The best predictor variables for the "OM source" hypothesis were: (1) the proportion of OM$_{aq}$ in the sediment and (2) BIX (biological index calculated from porewater DOM fluorescence). The best model for the “OM quantity” hypothesis included a single interaction term between the variables:

Fig. 5. Optical indices of dissolved organic matter. Higher FI values (a) indicate DOM from microbial sources, and higher BIX values (b) indicate a stronger OM$_{aq}$ signature. Higher HIX (c) and SUVA$_{254}$ (d) values represent higher degrees of humification and aromatic content of the OM. Different lowercase letters indicate significant differences between reservoir zones.

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

| Site  | Reservoir zone | DOC (mg L$^{-1}$)  | FI     | BIX     | RFE     | HIX     | SUVA$_{254}$ (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 ± 0.01 | 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 d⁻¹) | Chlorophyll deposition rates (mg m⁻² d⁻¹) | OM deposition rates (g OM m⁻² d⁻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         |

Fig. 6. Heat map showing relative abundance of methanogen genera in sediments, by reservoir zone. Row labels show the genus and order abbreviation in parentheses. 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).

Fig. 7. Carbon isotope signature in CH₄ (a) and the apparent fractionation factors (b) across reservoir zones. The blue horizontal line in (c) 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.
ters, AICc is a second order AIC score used to account for the number of estimated parameters. ∆ values are AICc differences (compared to the best model). Model probabilities (\(w_i\)) otherwise called Akaik 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 (\(E_{ij}\)) 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 \(R^2\) describes the proportion of variance explained by fixed factors alone, and the conditional \(R^2\) describes the proportion of variance explained by both fixed and random effects. Note that ranks, model probabilities, and evidence ratios (\(E_{ij}\)) are relative to the set of models and dependent on the data used to create the models.

### Table 5: Results of model hypothesis testing. The response variable was methane production rates (\(\mu\)mol CH4 cm\(^{-3}\) d\(^{-1}\)). Arrows next to predictor variables indicate a positive (\(\uparrow\)) or negative (\(\downarrow\)) 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. ∆ values are AICc differences (compared to the best model). Model probabilities (\(w_i\)) otherwise called Akaik 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 (\(E_{ij}\)) 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 \(R^2\) describes the proportion of variance explained by fixed factors alone, and the conditional \(R^2\) describes the proportion of variance explained by both fixed and random effects. Note that ranks, model probabilities, and evidence ratios (\(E_{ij}\)) 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 | ∆ values | \(w_i\) | \(E_{ij}\) | Marginal \(R^2\) | Conditional \(R^2\) |
|----|---------------------|---------------------------------|-------|-------------|------|------|------|----------|---------|----------|----------------|------------------|
| H\(_1\) | Source OM          | Autochthonous OM \(+\) \(\beta\) \(\downarrow\) BIX \((\downarrow)\)          | 5     | 13.53       | −17.06 | −15.56 | 2    | 9.6      | 0.0080  | 123.8    | 0.33           | 0.85              |
| H\(_2\) | Quantity OM        | \(\log(DOC)\) \((\uparrow)\) \(*\) \(g\) OM slurry\(^{-1}\) \((\uparrow)\)           | 6     | 13.76       | −15.52 | −13.37 | 3    | 11.8     | 0.0027  | 370.6    | 0.48           | 0.87              |
| H\(_3\) | 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     | 0.70           | 0.89              |

(1) porewater DOC concentration and (2) g OM per unit volume of sediment. The best model for the “OM source + quantity” hypothesis included all four of the above variables. In each of the three mixed effects models, all terms were significant. The proportion of OM\(_{aq}\) was negatively correlated with CH\(_4\) potential production rates (univariate correlation: \(R^2 = 0.38, p < 0.0001\); Supporting Information Fig. S1). All other variables had positive correlations with CH\(_4\) potential production rates, regardless of the model (Supporting Information Fig. S1). Univariate correlations between the predictor variables and CH\(_4\) potential production rates were all significant (BIX: \(R^2 = 0.31, p < 0.0001\); DOC: \(R^2 = 0.25, p < 0.0001\); g OM per sediment volume: \(R^2 = 0.36, p < 0.0001\)). Despite the “OM quantity” model having a slightly lower model probability and rank than the “OM source” model, the marginal \(R^2\) 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 CH\(_4\) 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 CH\(_4\) 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 covarying variables and is reflected in CH\(_4\) potential production rates that increase across the gradient from lacustrine to terrestrial/riverine (Fig. 8b, Supporting Information Table S3). 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 CDOM from deep water samples). 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%, OM\(_{aq}\)%), N%, δ\(^{15}\)N) 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 CH\(_4\) 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 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, surface pH, porewater BIX, and bulk sediment %C\(_{org}\). Surface nutrient concentrations (TRP, NH\(_4^+\)) were negatively associated with this axis, which could indicate a drawdown of soluble inorganic nutrients at high productivity sites. The methanogen taxa *Methanolinella* was also negatively correlated with axis 2 of the PLS (see Fig. 8b, Supporting Information Table S3).
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 (Fig. 8; Supporting Information Table S3). Suspended sediment in the inflowing river rapidly settles out in the riverine zone, resulting in high sedimentation rates, primarily of dense inorganic materials (Table 4), although OM and chlorophyll a deposition rates were also much greater than in the transitional or lacustrine zones. Sediments in the riverine zone contained the same amount of OM per unit volume as sediment in downstream portions of the reservoir (Fig. 3a), reflecting...
the high rates of inorganic and organic deposition. Porewater DOC was higher in the riverine zone, possibly reflecting high rates of OM deposition in this zone relative to the other zones (Table 4). 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 a 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; Beaulieu et al. 2018).

The sediment slurry method used to generate CH4 potential production rates does not represent in situ production rates due to factors including incubation temperature that differs from environmental conditions and the restriction of our assays to the top 5 cm of sediment. Therefore, the data generated from these assays should not be indiscriminately used for upscaling. Acknowledging these limitations, we find it informative to compare results from this study with the published estimates of surface methane emission from the same reservoir, based on thorough spatial and temporal sampling (Beaulieu et al. 2014a, 2016, Beaulieu et al. 2018). The following discussion uses volumetric CH4 potential production rates that were converted to estimated areal rates by assuming an active sediment depth of 5 cm. Previously 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 close agreement; Beaulieu et al. (2016) estimated mean CH4 emissions from the tributary area to be 33.0 mg CH4 m−2 h−1 (95% CI 19.41–46.53 mg CH4 m−2 h−1) while our mean sediment CH4 potential production was 41.2 mg CH4 m−2 h−1 (range 35.2–57.2 mg CH4 m−2 h−1) in the riverine zone. However, CH4 surface emission rates in the riverine zone in Harsha can be sixfold (Beaulieu et al. 2016) to 2 orders of magnitude (Beaulieu et al. 2014a) higher than other areas of the reservoir, while sediment CH4 potential production rates were only twofold higher in the riverine zone compared to other zones. This difference between CH4 potential production rates and field-measured emission rates is likely due to methane processing that occurs in the reservoir, such as CH4 oxidation in the water column, water mixing regimes, and dissolution of ebullitive CH4 bubbles that increase with water depth (McGinnis et al. 2006). 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. For instance, Eller et al. (2005) found that in the top 6 cm of sediment of a dimictic lake in northern Germany, average CH4 potential production rates were 0.95 ± 0.10 μmol CH4 g dry weight−1 d−1, and decreased with depth, while values for Harsha Lake averaged 1.56 (range 0.33–2.69) μmol CH4 g dry weight−1 d−1 in the top 5 cm of sediment. However, Harsha Lake incubations were conducted at a higher temperature than those reported by Eller et al. (2005). In an oligotrophic and a mesotrophic lake, CH4 potential production rates were lower than those in our eutrophic lake, producing 0.062 ± 0.018 and 0.054 ± 0.024 μmol CH4 g dry weight−1 d−1, respectively. These incubations were also conducted at lower temperatures (12°C) and evaluated a deeper sediment depth of 15–20 cm, which the authors determined to be more active for methanogenesis in their systems (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 0.344 μmol CH4 g dry weight−1 d−1 (range: 0.012–1.369 μmol CH4 g dry weight−1 d−1) for slurries incubated at 20°C (Duc et al. 2010). 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 unamended sediment slurries from the top 10 cm of sediment from Itaparica, a large hydropower reservoir in Brazil, the littoral site had the lowest rates (0.032 μmol CH4 g dry weight−1 d−1; no range or SE available), the intermediate (transitional) site had intermediate rates (0.054 μmol CH4 g dry weight−1 d−1), and the profundal (lacustrine) site had the largest potential production rates (0.56 μmol CH4 g dry weight−1 d−1). These data contrast our results where the riverine (most comparable to the littoral site) and lacustrine (most comparable to the profundal site) zones had the highest rates of CH4 potential production when normalized to sediment dry weight (Fig. 2c). This qualitative difference in CH4 production in the riverine/littoral zone between studies could be due to the different nature of the “shallow” sites in the two studies. In our study, the shallow riverine sites are specifically in the path of the major tributary entering the reservoir, and thus experience very high sediment (and OM) deposition rates. In contrast, in the study of Itaparica by Rodriguez et al. (2018), the shallow site in the littoral zone may not be subject to the same depositional conditions.

Although volumetric CH4 potential production rates were higher in the riverine than transitional or lacustrine zones.
(Fig. 2a), rates did not differ among reservoir zones when expressed per gram dry mass (Fig. 2c). This illustrates that differences in sediment density contribute to the spatial patterns in volumetric CH$_4$ 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 that might enhance CH$_4$ production. While inorganic fractions of the sediment may provide surface area for microbiota attachment, the organic fraction is the energy source fueling methanogenesis. When normalized to sediment OM, the riverine zone had higher CH$_4$ potential production rates than the other reservoir zones (Fig. 2c), indicating that OM is more readily converted to CH$_4$ in the sediments of 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 or the composition of microbial communities.

Based on satellite and field measurements indicating greater water column chlorophyll concentration in the riverine than transitional or lacustrine zones in Harsha Lake (Beck et al. 2017), we had predicted that OM in the riverine zone sediment would contain a greater proportion of aquatic 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 at the survey (Table 1) and chlorophyll a sedimentation rates were 6–13 times greater than the other reservoir zones (Table 4), the proportion of aquatic derived material in the riverine sediment OM was lower than other portions of the reservoir. This pattern reflects the large input of OM in the river inflow and is subsequently deposited in the riverine zone, effectively diluting the OM$_{aq}$ carbon signature in the sediment. The OM$_{aq}$ carbon signature in the riverine sediment could also reflect preferential microbial degradation of this labile material. 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 sediment cores was no different than the other sites (Fig. 3a).

**Characterization of methanogen communities and CH$_4$ production pathways**

Similar to results of other studies in freshwater systems, we found no correlation between methanogen abundance and CH$_4$ potential production rates (West et al. 2012; Chaudhary et al. 2017). This indicates that CH$_4$ production rates in Harsha Lake were not limited by methanogen abundance, but instead are linked to other environmental factors such as substrate quantity and/or quality. The variation in substrate availability likely affects methanogen activity, rather than methanogen abundance, and thus determines rates of methanogenesis.

The two methanogen genera with the highest relative abundances across all zones in Harsha Lake, *Methanoregula* and *Methanoseta*, 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 four genera that were each responsible for 10% or more of total methanogen sequences (*Methanoregula*, *Methanoseta*, *Methanosarcina*, and *Methanobacterium*), while the transitional and lacustrine zone only had two genera (*Methanoregula* and *Methanoseta*) that were responsible for 10% or more of total methanogen sequences. *Methanoregula* are hydrogenotrophic methanogens, while *Methanoseta* 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 15.4% of methanogens at the riverine sites, but less than 1% of methanogens in the transition and lacustrine zones, possibly reflecting the metabolic diversity of this genera. *Methanosarcina* can generate CH$_4$ through acetoclastic and methylotrophic pathways, can utilize a diverse range of substrates to produce CH$_4$ and may be more abundant in the riverine zone due to the diverse mix of terrestrial and aquatic derived material present in this area of the reservoir.

*Methanolobus* and *Methanomethylovorans* can only utilize methylated compounds. These compounds are derived from the degradation of lignin-containing OM (Peng et al. 2012), as well as degradation of compounds such as pectin (Schink and Zeikus 1982) and cholin (Borrel et al. 2011). Though these compounds were not directly quantified in the sediment samples, lignin is found primarily in terrestrial vascular plants, and our mixing model results indicated a higher proportion of terrestrial derived OM in the riverine sediments. Pectin is a polymer that is found in both plant leaves, as well as cell walls of green algae and cyanobacterial sheaths (Kertesz 1951).

Thus, the high relative abundance of *Methanosarcina* in the riverine zone and the presence of *Methanolobus* and *Methanomethylovorans* could be a result of metabolism of methyl substrates originating from terrestrial plant OM, but could also be indicative of pectin metabolism from both terrestrial and aquatic sources. For example, the riverine zone had high overall OM deposition rates, but also had higher chlorophyll a deposition rates compared to the other zones (Table 4). The presence of these genera may indicate a higher functional diversity in terms of methanogenetic metabolism in this zone due to the likely presence of a diverse assortment of organic compounds derived from both terrestrial and aquatic sources.

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., Murase and Sugimoto 2001; Blair et al. 2018).

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. Concurrent 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. 2017). 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 particulate sediment OM and dissolved OM (Supporting Information Fig. S1), 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 negative correlations between sediment organic carbon (%) and CH4 production, and water content and CH4 production; however Duc et al. (2010) report that lower %OM (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; Duc et al. 2010; West et al. 2012; 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 OMaq material in the riverine zone sediment, despite high chlorophyll a deposition rates in this zone (Table 4). There are several potential explanations for these results:

1. There are high rates of mineralization in the riverine zone, so OMaq material is more readily and rapidly degraded and less is recovered from the bulk sediment, shifting the sediment OM to a more terrestrial signature. In the riverine zone, the labile OMaq 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 aquatic-derived portion is more readily solubilized and made available for methanogens.

3. Both aquatic and terrestrial derived OM may be important for CH4 production. OMaq sources may be rapidly degraded while 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 OMaq.

While the quantities of OMaq and OMterr (i.e., g OMaq or OMterr cm−3) can be calculated and both exhibit positive relationships with CH4 potential production rates (correlations not shown), the quantity of OM from a specific source includes measures of both OM source and quantity, and is therefore not as useful for describing source alone relative to the proportion of OMaq or OMterr. Without more direct evidence, we cannot definitively say what the relative influence of each source of OM is in contributing degradation products that fuel methanogenesis with our data set. However, based on our regression analysis, both OM source and quantity are important predictors of 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 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 OM was important for CH4 production in terms of both quantity and source. Importantly, all of the models included variables that represented both the particulate and dissolved fractions of OM, suggesting that both pools play a role in CH4 production.

Our data indicate there may be a link between particulate terrestrial OM and CH4 production in Harsha Lake. C:N ratios from bulk sediment were positively correlated to CH4 potential production rates. These results contrast with another study of boreal and northern temperate lake sediments in central
Sweden, which found a negative correlation between CH₄ production rates and C:N ratios (Duc et al. 2010). However, their study was an interlake comparison with a larger range of C:N ratios (8.6–23.4) than the C:N ratios found within Harsha Lake (range 6.6–10.0). Further, Duc et al. (2010) noted that the sediments in their study with the lowest methane production had high C:N ratios and were comprised of peat or flocculated humic matter. The positive correlation between C:N and CH₄ 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 OMₚ in the sediments. Thus, the riverine zone is an exceptionally active deposition zone for both OMₚ and OMₚterr, and this material 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 zone differed from the other areas of the reservoir, and was characterized by high CH₄ potential production rates, high terrestrial carbon inputs, high autotrophic production, dense sediments, and porewater DOM that was less humified, less aromatic, and more aquatic derived relative to other zones. Research on reservoir biogeochemistry should encompass this zone with a goal of understanding gradients and spatial variation across the reservoir continuum. Both source and quantity of OM appeared to be 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). In Harsha Lake, relatively recently produced and less aromatic DOM from aquatic and microbial sources appears to fuel CH₄ production in the zone with the highest potential production rates, though compounds derived from terrestrial OM may contribute to these high rates. While OMₚ is recognized as a key driver of high methane production rates, the role of the degradation of more complex compounds typically associated with OMₚterr in contributing to CH₄ production and emission “hot spots” in lacustrine systems warrants further study. These compounds may become bioavailable due to some combination of photodegradation or biological processing that could be occurring in the oxygenated water column.

Although methanogen abundance did not vary specifically among reservoir zones or with CH₄ production rates, methanogen communities did have some variation 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 aquatic and terrestrial sources of OM are important in driving high methane production in the riverine zone of the main reservoir inlet. This has implications for reservoir management, suggesting that both watershed-scale soil erosion control and nutrient reductions may help reduce reservoir methane production. From this study we recognize the potential of terrestrial OM to contribute to “hot spots” of reservoir CH₄ production, particularly in depositional/riverine zones—with many questions remaining to be addressed on the topic. The results presented here contribute to our understanding of environmental factors that may influence spatial variation in CH₄ production and methanogen community composition in freshwater sediments.

Data Availability Statement

Data, code, and supporting documentation for the results found in this publication are available at the Center for Open Science OSF project page: https://osf.io/59hnj/. Raw 16S rRNA gene sequence reads for each sample are also available on NCBI Short Read Archive (SRA) under the BioProject number PRJNA532292.

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication.

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1357
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Acknowledgments

This research was supported in part by a grant from the United States Geological Survey 104(b) program, sub-award #60059469 through the Ohio Water Resources Center. Megan Berberich was supported through the Graduate Research Traineeship program sponsored by the U.S. EPA and administered through the University of Cincinnati, in addition to support from the UC Department of Biological Sciences and the Wieman Wendel Benedict Fund. We thank Joel Allen for support in sediment trap deployment and sampling, Kit Daniels for key assistance with field sample collection, Madison Duke for sediment trap work and field and lab support, Mike Elovitz for advice and insights into DOC EEMs, Jeff Havig for stable isotope and elemental analysis of sediment, Xuan Li for field and laboratory support including EEMs, and Karen White for laboratory assistance; this research was made possible with their help. We are also grateful to Aaron Diefendorf for access to and use of the Stable Isotope Geochemistry Lab in the Department of Geology at the University of Cincinnati.

Conflict of Interest

The authors declare no conflicts of interest.

Submitted 28 April 2019
Revised 07 October 2019
Accepted 09 November 2019

Associate editor: Hans-Peter Grossart