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Characterizing the Link Between Algal Bloom Biomass and Methane Production in Ohio Reservoirs

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1. Summary

In this study, we examined the link between eutrophication and methane production in Ohio Reservoirs, with a focus on sediment microbial processes. Algal blooms and the associated negative environmental impacts caused by nutrient enrichment have been identified as a leading cause of impairment of surface waters in Ohio. One of the understudied negative consequences of eutrophication is the increased potential for in-lake production and emissions of methane (CH_4), a potent greenhouse gas with a global warming potential >20 times that of CO_2. Lab-scale studies have indicated the potential for increased methane production in lake/reservoir sediments with labile algal organic matter added, but it is not known whether this relationship translates to the field scale. This is an important question for Ohio water resource management, as Ohio has a large number of reservoirs, many of which suffer from eutrophication due to high nutrient inputs, mainly from agricultural landscapes. The *overall objective* of this project was to determine the relationship between the occurrence of algal blooms and sediment CH_4 production rates in Ohio reservoirs, as mediated by sediment organic matter quantity and quality and the sediment microbial community. We examined these relationships at two spatial scales, highlighting both within-reservoir and among reservoir variation.

To date we have carried out the analysis for the within-reservoir variability in Harsha Lake, and those results are presented here; in the coming year we will continue to compile results from the among-reservoir Ohio-wide sampling as well as finishing off lab analyses. For Harsha Lake, areal CH_4 production rates were highest in the riverine portion of the reservoir below the main inlet where sediment organic matter (OM) quantity was greatest, presumably due to high OM sedimentation rates. The pattern of high CH_4 production rates in the riverine portion of the reservoir persisted even when rates were normalized to OM quantity, suggesting that not only was OM more abundant in the riverine zone, it was more readily utilized by methanogens. Sediment stable isotopes and elemental ratios indicated a greater proportion of allochthonous (terrestrial) OM in the riverine zone than other areas of the reservoir, suggesting that watershed derived OM is an important driver of CH_4 production in the system. Methanogens were abundant at all sampling sites but the functional diversity of methanogens was highest in the riverine zone, likely reflecting differences in decomposition processes or OM quality across the reservoir. Notably, in contrast to our expectation that water column algal productivity would be a key predictor of CH_4 emission rates, we found that the highest production rates occurred at sites with a strong contribution of terrestrial OM – indicating that both algal OM and terrestrial OM may be important substrates for CH_4 production in this eutrophic reservoir.

2. Problem and Research Objectives

Increased nutrient loading to lakes and reservoirs can cause eutrophication and an associated suite of environmental impacts including toxic algal blooms, water column anoxia, fish kills, and taste and odor problems. As such, nutrient enrichment has been identified as a leading cause of impairment of surface water ecosystems in Ohio (Ohio EPA, 2014). Eutrophication also has other less well-known negative consequences, notably the potential for in-lake production and emissions of methane (CH_4), a potent greenhouse gas (GHG) with a global warming potential 21 times that of CO_2. Research in southwest Ohio has shown that GHG emissions from reservoirs draining agricultural watersheds can be exceptionally high (Beaulieu et al. 2014) and a recent review study indicates that reservoir productivity is the best predictor of CH_4 emissions (Deemer et al. 2016). Methanogenesis (microbial methane production) in aquatic ecosystems is an important source of methane to the atmosphere, and there is compelling evidence that CH_4 emissions from reservoirs is underestimated and thus not adequately quantified in the global GHG budget (Bastviken 2011, Deemer et al. 2016). Meanwhile, lab studies have demonstrated that the addition of fresh algal material to lake/reservoir sediments can stimulate CH_4 production under anoxic conditions by providing a labile carbon source (West et al. 2012, Schwarz et al. 2008). These data suggest that increased algal blooms resulting from nutrient loading may stimulate *in situ* methanogenesis, leading to increased CH_4 emissions from eutrophic reservoirs. This would be a significant environmental disservice, but there is a critical knowledge gap regarding the relationship between algal bloom occurrence and CH_4 production at the scale of whole ecosystems. A better understanding of this process is required to evaluate the extent of negative impacts caused by nutrient loading, and to properly place value on nutrient loading reductions resulting from regulatory or water quality trading approaches (US EPA, 2003).

The **objective** of this project was to quantify the relationship between algal bloom formation, sediment organic matter quality, and sediment microbial CH_4 production in Ohio reservoirs. We highlight reservoirs rather than natural lakes, because (1) reservoirs are understudied relative to natural lakes, yet cover more area than natural lakes (aside from Erie) within Ohio and many regions, and are growing in prevalence globally (Downing et al., 2006); (2) reservoirs typically have a high watershed area:lake area ratio, thus tend to receive high sediment and nutrient loads and are prone to eutrophication (Jones et al. 2004, Dodds and Whiles 2010); (3) recent measurements in a SW Ohio reservoir have revealed CH_4 emissions among the highest ever reported for freshwaters (Beaulieu et al., 2014, Beaulieu et al., 2016).

Our *central hypothesis* was that sediment CH_4-production rates would be higher and methanogens more abundant in reservoirs (and at locations within a given reservoir) where sediment characteristics reflect large contributions of algal-derived organic matter, that in turn reflect spatial variation in algal blooms. We approached our overall objective by testing the central hypothesis at two different spatial scales, with the following specific aims:

Specific Aim #1: **Determine the relationship between algal-derived organic matter, CH_4 production and microbial community structure in sediments from Harsha Lake, over a within-reservoir spatial gradient in nutrient and algal organic matter loading.** Our working hypothesis is that sediment CH_4 production rates and the abundance of methanogens will co-vary with sediment organic matter quantity and quality, which will in turn co-vary with algal productivity and deposition rates, and will thus be highest near the sediment delta formed downstream of the inlet of this well-studied reservoir in SW Ohio.

Specific Aim #2: **Determine the relationship between algal-derived organic matter, CH_4 production and microbial community structure in sediments from 18 Ohio (and nearby) reservoirs, varying across a gradient of watershed land-use/land-cover and predicted trophic status.** Our working hypothesis is that sediment CH_4 production rates and the abundance of methanogens will co-vary with sediment organic matter quantity and quality, which will in turn vary among reservoirs as a function of watershed agricultural cover.

We addressed these aims by determining sediment CH_4 production rates, methanogen abundance and microbial community structure, and labile organic matter content along a transect of 15 sites in Harsha Lake, and for two sediment locations (one deep, one near the main river inlet) in each of 18 Ohio reservoirs varying across a gradient of land-use.

3. Specific Aim #1: Within-Reservoir Spatial Variability in Methane Production

3.1 Methodology

Site description. William H. Harsha Lake (Figure 1) 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 km^2, is seasonally stratified, and reaches 32.8 m at its maximum depth. Harsha Lake's watershed is 882 km^2, with agriculture (including corn, soybean and pasture) as the dominant land-use (Beaulieu et al. 2016).

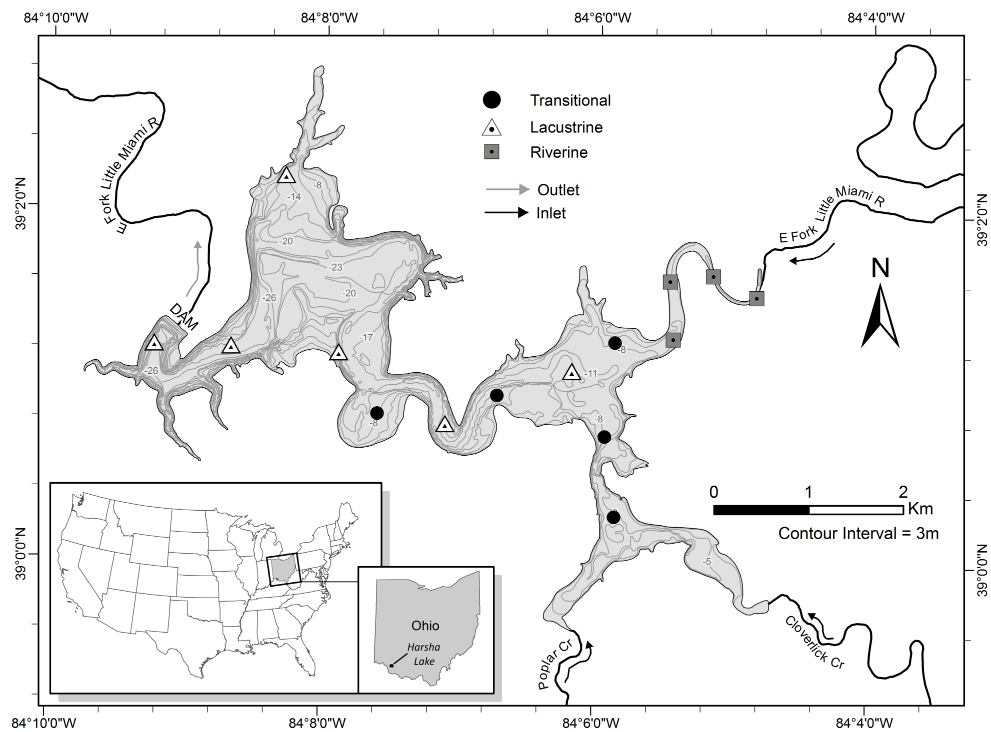

Figure 1. Harsha Lake sampling sites and site categories. The legend indicates which sites were assigned to the riverine, transitional, and lacustrine zones.

Sample collection. Triplicate sediment cores, water samples, and water column measurements were collected from 15 sites across the reservoir (Figure 1) within a 7-day window in late May. 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, when stratification was expected to be strongest. On each date, three sediment cores (Figure 2) were collected from each of five sites distributed across the three reservoir zones using a K-B Corer. Epilimnion (0.1 m) and hypolimnion (0.5-2 m above sediment) water samples were collected at each site using a Niskin bottle. Depth profiles of water temperature, pH, dissolved oxygen, specific conductivity were measured using a YSI ProDSS multiparameter sonde, and depth profiles of chromophoric dissolved organic matter (CDOM), in vivo chlorophyll a, and turbidity were measured using a Turner C3 Submersible Fluorometer at each site. Secchi depth and depth profiles of photosynthetically active radiation (PAR) were also measured.

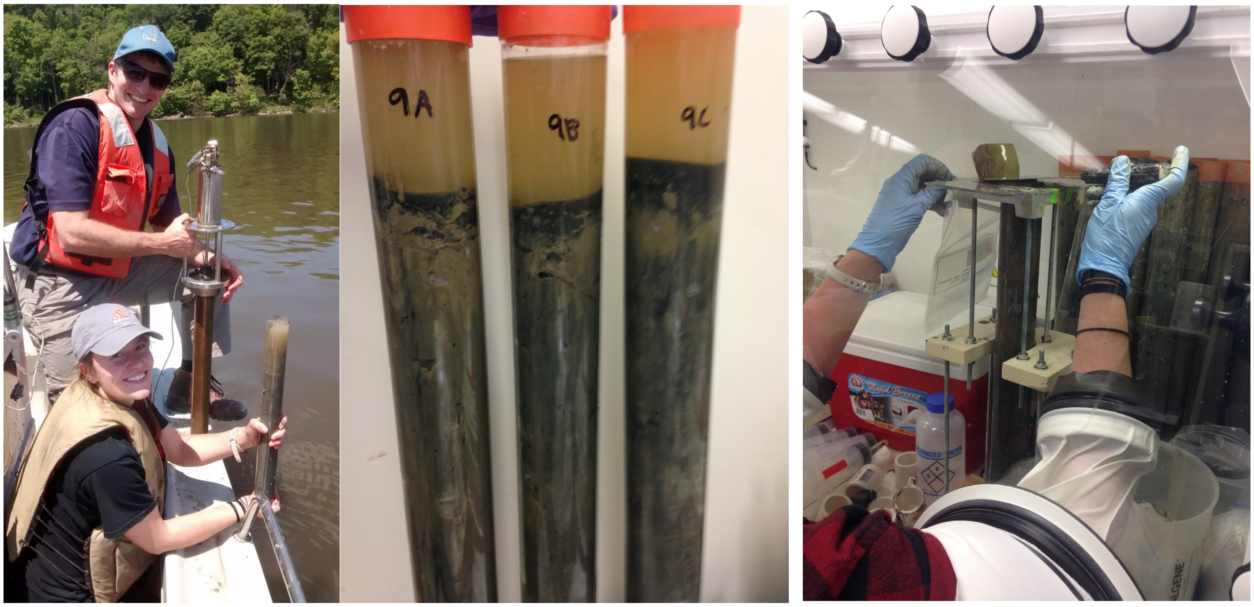

Figure 2. Left: Dr. Jake Beaulieu and Megan Berberich collecting sediment cores with K-B corer. Middle: Triplicate cores from a single site in Harsha Lake. Right: Core extrusion under N2 atmosphere in glove box to retain anaerobic conditions while subsampling.

Water sample processing. Water samples collected from each site were stored on ice or refrigerated until they were processed (within 24 hours). Water samples were analyzed for chlorophyll *a*, dissolved nutrients, and total suspended solids (TSS). The spectrophotometric method was used to measure chlorophyll *a* following acetone extraction (APHA 2012).

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 N_2 atmosphere (Figure 2). Subsamples of sediment were used for CH_4 potential production rate assays, sediment characterization, porewater collection and nucleic acid extraction. Sediment slurries 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. Slurries were capped with a rubber stopper, crimp sealed, and wrapped in aluminum foil.

Sediment slurries – potential CH_4 production rates. 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. After taking the gas sample, 11 mL of N_2 gas was returned to the serum bottle to maintain the same pressure. Gas samples were analyzed on a gas chromatograph (Bruker 450, Massachusetts, U.S.A.) equipped with a flame ionization detector (FID). Methane production rates for the slurries were calculated by accounting for dilution during sampling, then determining the change of moles of methane over time. Methane production rates were expressed either normalized to sediment volume, sediment dry mass, or mass of sediment organic matter.

Sediment characterization. Sediment was dried at 60˚C for 3 days or until constant weight, and then ground to a fine powder before further analysis. Elemental analysis and stable isotope analysis (^{13}C and ^{15}N) were conducted using an elemental analyzer connected to an isotope ration mass spectrometer (EA-IRMS). Sediment was fumigated with hydrochloric acid prior to C and ^{13}C analysis to removed carbonates (Harris et al. 2001). Density was calculated from the 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 autochthonous and allochthonous organic matter (see *Mixing model*).

Porewater analyses. Porewater was extracted from a subsample of sediment for determination of volatile fatty acids (VFA), dissolved organic carbon (DOC), and for excitation emission matrix (EEMs) fluorescence. We extracted porewater by centrifuging sediment in 50 mL tubes at 7800 rpm. The porewater was filtered at 0.45 µm prior to measurement of optical absorbance and fluorescence using a scanning spectrofluorometer. The optical data were used to calculate the fluorescence index (FI), biological index (BIX), relative fluorescence efficiency (RFE), and the specific ultraviolet absorbance at 254 nm ($SUVA_{254}$), which provide information about the composition and source of dissolved organic matter. A summary of these and other optical properties is described by Hansen et al (2016).

Sediment traps. Sediment traps (Figure 3) were deployed at 3 of the 15 sampling sites (one per reservoir zone) to determine sedimentation rates and composition of the water column particulate matter. Sediment traps were deployed for seven weeks during summer, with the initial deployment in early June. Traps were constructed using 2” PVC pipe and caps for a final 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. Traps were sampled weekly to minimize OM decomposition. 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 (^{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 above.

Figure 3. Sediment trap deployment in Harsha Lake.

DNA extraction and analysis. DNA was extracted from ~500 mg (wet weight) of sediment from each core using a MoBio PowerSoil ® DNA Isolation Kit following the manufacturer’s protocol. DNA concentration was determined using a Qubit dsDNA HS Assay kit and a Qubit 3.0 Fluorometer. 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). ***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). Further details of the *Microbial community analysis* and *qPCR* methods, including quality control approach, can be found in Berberich 2017.

Mixing model for determining contribution of autochthonous OM to sediment. A mixing model was generated to estimate the proportion of autochthonous (aquatic) vs. allochthonous (terrestrial) organic matter found in each sediment sample, using the MixSIAR package version 3.1.7 (Stock & Semmens 2013) in R. The model used as input the C/N ratios and $\delta^{15}N$ isotopic signature of terrestrial and aquatic end-members (sources) of OM. 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 in late May.

Statistical methods and data analysis. All statistical analyses were performed using R version 3.4.0 (R Core Team 2017). One-way nested ANOVAs were used to evaluate differences in sediment characteristics, methane production, and microbial community characteristics among reservoir zones. Partial least square (PLS) regression analysis with the R package 'plsdepot' was used to investigate which measured parameters best explained variation in methane production rates (Gaston Sanchez 2012). One response variable, methane production rates (µmol CH_4 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).

3.2 Principal Findings

In summary, we found distinct differences among reservoir zones in Harsha Lake sediments for physicochemical parameters including organic matter (OM) quantity and source, and for the methane production rates. Methanogens were abundant at all sampling sites but the functional diversity of methanogens was highest in the riverine zone. Variation in functional diversity of methanogens likely reflects differences in decomposition processes or OM quality across the reservoir. Methane production rate was highest in the shallow riverine zone near the inlet, as hypothesized as this was also the area of highest surficial methane emissions and an area of extensive algal blooms. Methane production was also correlated with both sediment organic matter quantity and source. As hypothesized, higher overall OM content gave rise to higher CH_4 production rates. However, the relationship with OM source was not a simple positive correlation with sediment algal OM in the way that we had initially hypothesized. In fact, the zone with the highest methane production rates, the shallow riverine zone, also had the highest proportion of terrestrial (as opposed to algal-derived) organic matter in the sediment. Thus, overall sediment methane production rates were negatively correlated with algal-derived OM proportion in the solid sediment, at the same time positively correlated with algal-derived OM proportion in the dissolved phase (porewaters) of the sediment.

We have several working hypotheses for why this may be the case, and continue to explore this as we develop this project. One possibility is that it is the dissolved fraction of OM that is primarily driving methane production, therefore the relationship that is seen between methane production rates and the solid fraction of OM is of less consequence. More likely, both terrestrial and algal-derived OM may be important for CH_4 production, and the algal sources are rapidly degraded but the terrestrial OM is degraded over both short and longer time scales (see Guillemette et al. 2017; Grasset et al. 2018). Overall, the riverine zone has more OM, and definitely more terrestrial OM than the other zones. Thus, the terrestrial OM source in this zone may be degraded at a more consistent rate over time and contribute to CH_4 production along with the algal OM, leading to an overall higher CH_4 production rate.

Key results from each component of the Harsha Lake study are detailed below.

Site characteristics. 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, chlorophyll *a* was highest in the riverine zone (47.6 µg/L). Secchi depth increased from the riverine zone (average 0.33 m) to the lacustrine zone (average 0.78 m).

Methane production among reservoir zones. Potential CH_4 production rates normalized by slurry volume and by grams of organic matter were greater in riverine zone compared to the transitional or lacustrine zones (Figure 4), which is consistent with previous reports of higher CH_4 emission rates in the riverine zone compared to other areas of this reservoir (Beaulieu et al. 2014, 2016). Published areal surface CH_4 emission rates from the Harsha Lake riverine zone (Beaulieu et al. 2016) and the average of our measured riverine zone sediment CH_4 production rates from this study were in remarkable agreement (33.0 mg CH_4 m^{-2} h^{-1} and 30.8 mg CH_4 m^{-2} h^{-1}, respectively). However, CH_4 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. 2014) higher than other areas of the reservoir, while sediment CH_4 production rates were only two-fold higher in the riverine zone compared to other zones. This contrast is likely due to methane oxidation in lacustrine and transitional zones where the water column is deeper and more strongly stratified.

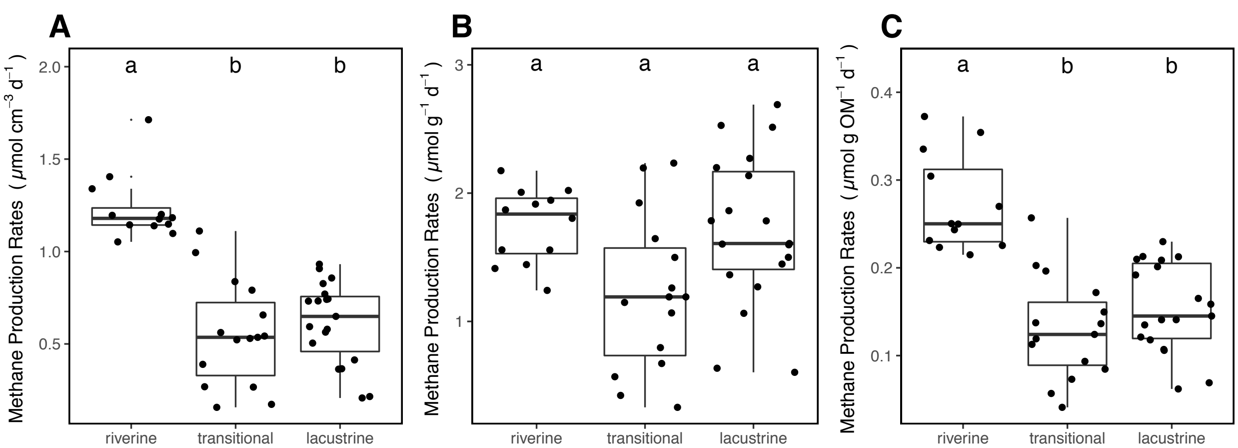

Figure 4. Potential methane production rates from sediment slurries in each of the reservoir zones, normalized to sediment volume (A), sediment mass (B), and sediment organic matter (C). Dots represent the potential methane production rate calculated from each sediment core by a sediment slurry assay. Different lowercase letters indicate significant differences between reservoir zones.

Sediment characteristics among reservoir zones. Sediment was the densest in the riverine zone, intermediate in the transitional zone, and the least dense in the lacustrine zone. The quantity of organic matter was greatest in the riverine zone for both the solid fraction of OM (g OM cm^{-3}) and the dissolved fraction of OM from the sediment porewater (DOC, g mL^{-1}) (Figure 5), but the differences among zones for the solid fraction of OM were not significant. The dissolved fraction of OM was greater in the riverine zone than either the transitional or lacustrine zones. Stable isotope and elemental composition data indicated that the source of OM varied significantly among reservoir zones in the solid fraction (Figure 6). The proportion of autochthonous solid-fraction OM (calculated from the mixing model) was lowest in the riverine zone, intermediate in the transitional zone, and highest in the lacustrine zone (Figure 6). Optical properties of the dissolved OM showed some evidence of differences in DOM source across reservoir zones. FI values of DOM were significantly higher in the riverine zone than the lacustrine or transitional zones, indicating a higher proportion of DOM derived from microbial sources (e.g. bacteria and algae) relative to terrestrial OM (McKnight et al. 2001; Cory et al. 2010).

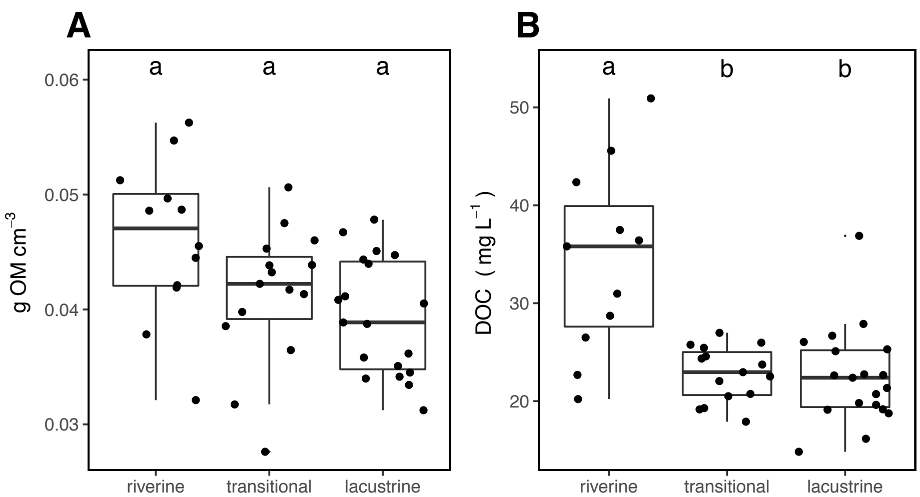

Figure 5. Comparison of the quantity of OM among reservoir zones. Panel A represents the amount of OM found in the bulk sediment, normalized to sediment volume. Panel B represents the concentration of the dissolved fraction of OM found in the sediment porewater, measured as DOC. One riverine value with an exceptionally high DOC concentration was excluded from the plot for readability. Different lowercase letters indicate significant differences between reservoir zones.

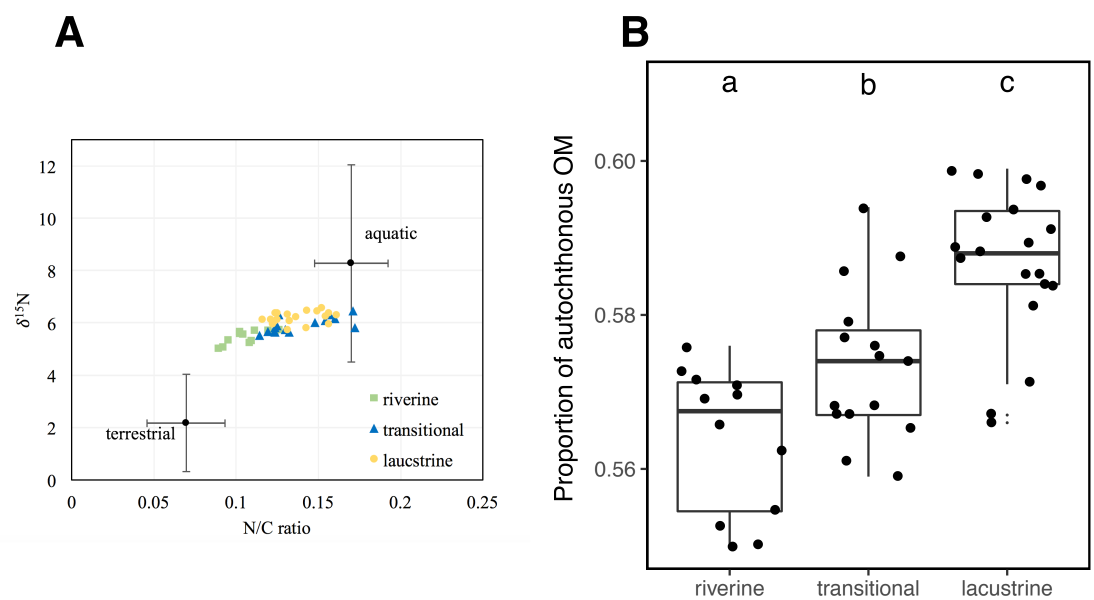

Figure 6. Plot of δ15N vs. N/C elemental ratios of terrestrial and aquatic OM sources, and the sediment mixtures for each core across the three reservoir zones (A). The center dots for the two sources represent the mean values (n = 13 for terrestrial sources, n = 16 for aquatic sources), and error bars represent the standard deviation. Panel B depicts the proportion of autochthonous (aquatic) OM found in each core grouped by reservoir zones calculated from the stable isotope mixing model. Different lowercase letters indicate significant differences among reservoir zones.

Methanogen communities among reservoir zones. There were no differences in copy number for either the *mcrA* gene (an indicator of methanogen community abundance in the sediments) or the archaeal 16S rRNA gene (an indicator of total archeal abundance in the sediments) among reservoir zones. On average, methanogens represented 34% of total archaea. Representative operational taxonomic units (OTUs) from the order Methanomicrobiales accounted for 53%, 77% and 76% of total methanogen sequences from the riverine, transitional, and lacustrine zones, respectively. The order Methanosarcinales comprised 34%, 16% and 17% of riverine, transitional and lacustrine methanogens. The most notable difference in methanogen communities among reservoir zones was the abundance of *Methanosarcina*, a genus of the Methanosarcinales order, which composed 15% of methanogens at the riverine sites, but < 1% of transitional and lacustrine methanogens.

Similar to results of other studies in freshwater systems, we found no correlation between methanogen abundance and CH_4 production rates, likely because methanogen activity, rather than abundance, determines rates of methanogenesis (West et al. 2012; Chaudhary & Blaser 2017). We observed higher methanogen functional diversity in the zone with the highest methane production rates and high OM diversity—the riverine zone where the water does not stratify. The elevated rates of CH_4 production in the riverine zone could be due to higher processing of OM in the presence of oxygen before it reaches the anoxic sediment layers, resulting in high substrate availability for methanogenesis.

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 1). Similar to the benthic sediment, the proportion of organic matter of the deposited sediment was lowest in the riverine site and highest in the lacustrine site. Despite this, the rate of chlorophyll deposition in the lacustrine sediment trap over the 6-week period averaged less than that of the riverine and transitional sites. In short, the quantity and source of OM observed in the sediment traps, matched well with the patterns observed in the bottom sediments for the respective reservoir zones.

Table 1. Average sedimentation rates, and %OM for the three sites that were sampled regularly.

Site (Zone)	**Sedimentation rates (mg/cm2/day)**	**OM percent**	**Sedimentation rates (mg OM/cm2/day)**
EFL (Lacustrine)	1.53	22.57	0.33
ENN (Transition)	4.66	18.91	0.82
EUS (Riverine)	53.07	19.37	6.52

Predicting CH_4 production rates from all measured variables. PLS analysis confirmed that the three reservoir zones have distinct sediment, water column, and microbial community characteristics, and that the triplicate cores show tight grouping within zones, with a few exceptions (Figure 7). The first and second components from PLS regression explained 70.0% and 15.8% of the variance among cores, respectively. Axis 1 depicts the main gradient in the data, which shows the transition from lacustrine to terrestrial (riverine) sites across many co-varying variables, and is reflected in methane production rates which increase across the gradient from lacustrine to terrestrial/riverine.

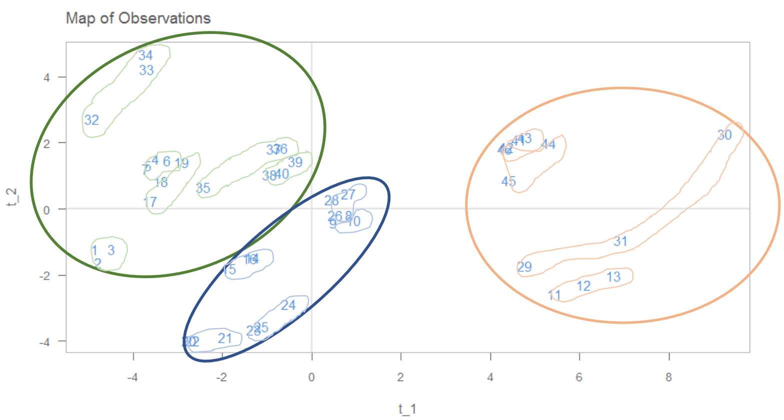

Figure 7. Results of Partial Least Squares Regression (PLS) multivariate analysis relating sediment methane production rates to characteristics of the sediments, water column, and microbial community at each core site in Harsha Lake. Each number represents a single core. The x-axis is the first PLS component, and the y-axis the second PLS component. The orange oval includes all cores from riverine sites, the blue oval includes all cores from the transitional sites, and the green oval includes all cores from the lacustrine sites. Smaller shapes are drawn around each set of three replicate cores from a given location.

The riverine zone is characterized by denser sediments with lower % OM, but a greater overall amount of OM per unit area due to the higher density. The riverine zone is also characterized by a higher proportion of terrestrially-derived OM, a higher apparent functional diversity of archeal methanogen communities, and a higher CH_4 production rates. In short, the riverine zone is an area of active organic matter production, deposition, and decomposition, and consequently, CH_4 production. Our results show evidence for high sedimentation rates of OM of both algal and terrestrial origin, and both likely contribute to the high sediment CH_4 production rates. Because of the shallow nature of this zone, the CH_4 produced here is also likely able to quickly evade to the atmosphere, with little removal by CH_4 oxidation in the water column. Further work is needed to more fully partition the algal vs. terrestrial source of the carbon used to produce the CH_4, and to determine whether similar patterns are seen in riverine vs. lacustrine zones of other reservoirs.

4. Specific Aim #2: Methanogens and sediment characteristic variation among Ohio reservoirs

4.1 Methodology

In conjunction with a regional assessment of reservoir CH_4 emissions undertaken by the US EPA, our collaborator Dr. Jake Beaulieu provided our group with sediment samples from 18 reservoirs in the states of Ohio, Kentucky, and Indiana collected during the summer of 2016 (Figure 8). The reservoirs were selected to span gradients of agriculture/forest land-use (7 – 83% forested) and water depth (max depth: 3 - 35 m). Ebullitive CH_4 emissions were measured using 14 – 28 hour inverted funnel deployments, and diffusive emissions via 5 minute floating chamber deployments. On average, 65% of total CH4 emissions were derived from ebullition, with diffusion constituting the balance, and emissions were positively correlated with agricultural land use (Waldo and Beaulieu, unpublished data) and chlorophyll-a, in keeping with our overarching hypothesis.

>62% Ag	25-42% Ag
1. Paint Creek	9. Apple Valley
2. Lake Loramie	10. Waynoka
3. Harsha Lake	11. M.J. Kirwan
	12. Wingfoot
	13. Atwood
43-62% Ag	**<25% Ag**
4. Brookville	14. Senecaville
5. Dillon Lake	15. Mohawk
6. Rocky Fork	16. Cave Run
7. Lake Milton	17. Buckhorn
8. Roaming Rock	18. Carr Creek

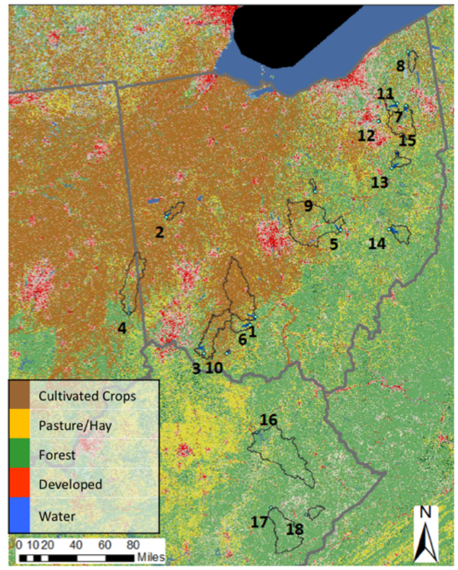

Figure 8. Watersheds of 18 sampled reservoirs in OH, IN and KY, and their % agricultural landcover.

Regional Reservoirs Sediment Sampling. Sediment was collected from the 18 regional reservoirs using a ponar grab sampler. Both deep locations (lacustrine zone) and shallow (riverine zone) sediment samples were taken (2 sediment samples total), except for a few lakes where this was not logistically possible and only 1 sample was taken. Upon retrieval, sediment was collected and stored in 500 mL amber Nalgene bottles without headspace on ice (~4˚C) until processed.

Sediment Analysis. Sediment samples were processed and analyzed for methane production rates, sediment characteristics including organic matter source and quantity. A subset of samples were also analyzed for microbial community with a focus on methanogens. All analyses are carried out as described in the Methodology for Specific Aim #1 above.

$\delta^{13}C$ in CO_2 and CH_4 – CH_4 production pathway. Stable isotope ratios of carbon ($\delta^{13}C$) in CO_2 and CH_4 were measured from gas sampled from ebullition bubbles collected from a subset of the reservoir locations to discern between dominant CH_4 production pathways. Analysis was carried out at the UC Davis Stable Isotope Facility. The apparent fractionation factor (α_C) of $\delta^{13}C$ in CO_2 and CH_4 was used to estimate the dominant methanogenesis pathway (Whiticar et al. 1986; Conrad 2005) (Eq. 1):

$$\alpha_C = \frac{(\delta CO_2 + 10^3)}{(\delta CH_4 + 10^3)} \qquad \text{Eq. 1}$$

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

4.2 Principal Findings

The measured water column and sediment characteristics varied considerably among the different reservoirs in our survey (Table 2), indicating that the study sampling design succeeded in capturing a broad range of reservoirs with different trophic status – in particular, chlorophyll-a ranged from 1 $\mu g\ L^{-1}$ (oligotrophic) to 77 $\mu g\ L^{-1}$ (hypereutrophic). For the multi-reservoir samples we were not able to get consistent CH_4 production rate measurements which passed our QA/QC requirements, thus we focus our analysis on the variation in methanogen communities and their association with sediment characteristics.

Table 2. Water column and sediment summary characteristics from the multi-reservoir survey.

Parameter (units)	**Median**	**Min**	**Max**
Water column pH	8.7	8.0	9.4
Water column specific conductivity (uS/cm)	401	207	705
Water column chlorophyll-a (ug/L)	11	1	77
Sediment bulk density (g/cm^3)	1.3	1.0	1.9
Sediment organic matter content (%)	8	2	36
Sediment organic carbon content (%)	2.3	0.7	19
Sediment N content (%)	0.24	0.1	1.7
$\delta 13C_{org}$ (sediment)(per mil)	-27.0	-32.0	-21.4
δ15N (sediment)(per mil)	6.0	3.3	9.7
Sediment C:N	9.3	6.3	14.4

Methanogens and methane production pathway associated with sediment OM source. Methanogens belonging to several different taxa were present in the sediments of all reservoirs, but in varying proportions (Table 3). Genera *Methanoregula* and *Metanosaeta* had the highest relative abundances of methanogens at most reservoirs, with the exception of Lake Loramie, Lake Milton, and Wingfoot Lake.

Several methanogen taxa showed clear relationships with variables indicating the relative proportion of terrestrial-derived vs. autochthonous (algal) organic matter in the sediments. For example, *Methanoregula* and *Methanosaeta* had different relationships with the OM source of the sediment; relative abundance of *Methanoregula* was negatively correlated to C:N, while *Methanosaeta* showed the opposite trend (Figure 10). This indicates that sediments with OM that had higher terrestrial signature (higher C:N) also had a higher relative abundance of *Methanosaeta*, a specialist genus that can only utilize acetate as a substrate.

	Apple Valley Lake	Atwood Lake	Brookville Lake	Buckhorn Lake	Carr Fork Lake	Cave Run Lake	Dillon Lake	Lake Loramie	Lake Milton	Lake Mohawk	Lake Roaming Rock	Lake Waynoka	Michael J Kirwin Reservoir	Paint Creek Lake	Rocky Fork Lake	Senecaville Lake	William H Harsha Lake	Wingfoot Lake
Methanomicrobiales; Methanoregula	46.3	42.2	68.1	30.1	28.5	58.3	60.5	46	54.9	69.6	66.7	43.8	62.6	53	69.2	51	61.8	12.3
Methanosarcinales; Methanosaeta	33.7	45.8	21.1	52.7	44.4	27.9	18.2	13	10.7	16.9	19.5	29.3	28.4	19.2	15	34.1	25.6	32.4
Methanomicrobiales; Methanolinea	9.2	6	9.5	0.4	0	9.2	12.9	31.5	17.4	9	4.3	2.7	3	9	10.8	6.4	5.4	26.4
Methanobacteriales; Methanobacterium	8.2	3.3	1.6	3	19.6	0.4	0.3	0.8	16.7	2.4	2.2	4.3	3.2	14.3	1.5	0.3	2.5	0.5
Methanomicrobiales; Methanomicrobiales	1.5	0	2.5	6.1	0	0.9	7.9	0.1	0.3	0	0.5	26.8	1.1	1.3	1.3	0.1	4.4	0.5
Methanomicrobiales; f__Methanomicrobiaceae_Otu00535	0.5	5	0	0.7	0	2.6	1.7	6.1	0.2	0	6.3	0.7	0.9	0	2.7	0.5	0.1	6
Methanomicrobiales; Rice	0	0	0	0	0	0	0	0.4	0	1	0	0	0	0	0	0	0	25.1
Methanobacteriales; Methanobrevibacter	0	0	0.5	0	0	0	0	0	16.7	0.3	0.4	0	0.2	0	0	0	0	0
Methanocellales; Rice	0.7	0	0	0.5	10.7	0.4	0.2	0.8	0	0.5	0.2	1.1	0.2	3	0.2	0	0.1	0
Methanosarcinales; Candidatus	0	0	0	0.8	0.7	0.3	0	0.6	0	0.1	0	0	0	0	0	6.7	0	0
Methanomicrobiales; Methanospirillum	0	0	0.1	0.9	0	0	0	0	0	0	0	0.1	0	0	0.2	0	0.6	0
Methanosarcinales; Methanolobus	0	0	0	0.8	0.8	0	0	0	0	0	0	0	0	0	0	0	0.1	0
Methanocellales; Methanocella	0	0	0	0	0.8	0	0	0	0	0.2	0	0	0.3	0	0	0	0	0
Methanomicrobiales; f__Methanomicrobiaceae_Otu29105	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0
Methanomicrobiales; Methanocorpusculum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0

Table 3. Heat map showing relative abundance of different methanogen taxa in sediments, by reservoir name. 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).

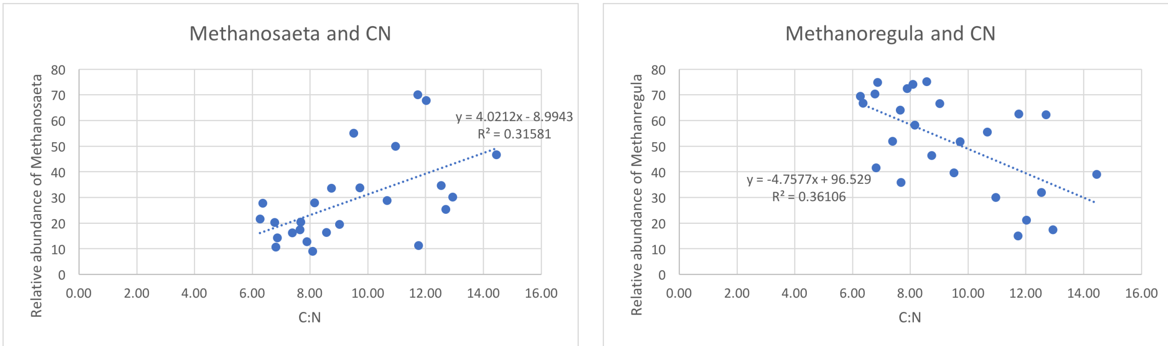

Figure 10. Relationship between sediment C:N ratio and the relative sediment abundance of two different genera of methanogens, for 1-2 cores in each of 18 reservoirs.

These results are supported by the relationship between C:N and the dominant methane production pathway (Figure 11), as indicated by the apparent fractionation factor of $\delta\ ^{13}C$ in CO_2 and CH_4. Higher C:N sediment values tended to be associated with lower apparent fractionation factors; with fractionation factors under about 1.05 indicating that acetoclastic methanogenesis is the dominant pathway (Whiticar et al. 1986; Conrad 2005).

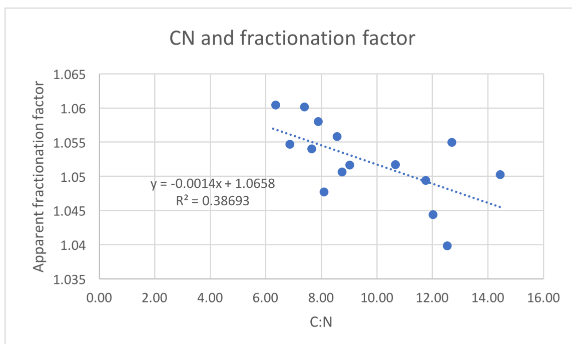

Figure 11. Relationship between sediment C:N ratio and the apparent isotopic fractionation factor of CH_4 production from CO_2.

5. Significance

We found that within the eutrophic Harsha Lake reservoir, the riverine zone was an area of particularly active organic matter production, deposition, and decomposition, and consequently, CH_4 production. Our results show evidence for high sedimentation rates of OM of both algal and terrestrial origin in this zone, and both likely contribute to the high sediment CH_4 production rates. Because of the shallow nature of this riverine zone, the CH_4 produced here is also likely able to quickly evade to the atmosphere, with little removal by CH_4 oxidation in the water column. Based on the high degree of spatial variation in CH_4 production rates, studies of reservoirs as well as natural lakes with substantial riverine inputs, would do well to take a spatially-aware sampling approach to determine CH_4 production and emissions, rather than sampling only at a single deep location. We recommend placing special attention on the riverine zone, as this zone is important in carbon biogeochemistry of reservoirs.

In the survey of 18 midwestern U.S. reservoirs varying in watershed nutrient enrichment and trophic status, we found as predicted a strong gradient of organic matter quality indicated by sediment OM content and C:N ratio. Interestingly, the sediment methanogen archaea communities responded to this gradient, with taxa abundances shifting towards those utilizing acetate for the reservoirs with a higher apparent terrestrial OM contribution (higher C:N). This suggests that the eutrophic reservoirs with high nutrient loading and high algal production are giving rise to different sediment methanogen communities, and different methanogen production pathways, than the more oligotrophic reservoirs.

Another finding of significance was that source and quantity of organic matter are both important for methane production rates in reservoir sediments. This has important implications for global carbon cycling, as the amount of sediment OM transported to reservoirs is expected to increase as a function of land use change and with the number of reservoirs globally increasing. Terrestrial OM may play an important role in fueling methane production in the zone with the highest potential rates. However, to verify this, this needs to be tested more directly. Future work should focus on specifically evaluating the role of terrestrial OM in methane production, particularly in depositional/riverine zones. A follow-up study would be useful to more fully partition the algal vs. terrestrial source of the carbon used to produce the CH_4.

6. Publication Citations

Journal Articles:

One submitted article, awaiting review in the peer-reviewed journal *Limnology & Oceanography*:

Berberich, M.E., J. Beaulieu, T. L. Hamilton, S. Waldo, and I. Buffam. *Submitted*. Spatial variability of methane production and methanogen communities within a reservoir: importance of organic matter source and quantity.

Theses/Dissertations:

Berberich, M.E. 2017. Spatial variability of methane production and methanogen communities in a reservoir: importance of organic matter source and quantity. *MS Thesis*, Department of Biological Sciences, University of Cincinnati.

Presentations at Conferences:
*Indicates student presenter

Buffam, I., *M. Berberich, J.J. Beaulieu, T.L. Hamilton, and S. Waldo. November 2018. Spatial variation in sediment OM quantity and quality in a eutrophic reservoir: can CH4 production rate be explained? Conference: Predicting the interactivity of dissolved organic matter across terrestrial and aquatic ecosystems, Lund University, Lund, Sweden. (*Keynote speaker*)

*Berberich, M., J. Beaulieu, T. Hamilton, and I. Buffam. June 2018. Spatial variability of methane production and methanogen communities within a eutrophic reservoir: evaluating the importance of organic matter source and quantity. American Society for Limnology and Oceanography (ASLO) 2018 Summer Meeting, Victoria, BC, Canada (*oral presentation*).

*Berberich, M., I. Buffam, J. Beaulieu, T. Hamilton, S. Waldo, and X. Li. December 2016. Sediment characteristics and microbial communities associated with methane production in a eutrophic reservoir. American Geophysical Union Fall 2016 Conference, San Francisco, CA, USA *(poster)*.

7. Students Supported by the Project

This project provided funding and research opportunities for 1 graduate student and 1 undergraduate student. Megan Berberich's MS research was directly supported by this grant, and Nick Willse was hired using grant funding to serve as a research technician on the project.

Student name	Degree sought	Period on project	Association/role of student
Megan Berberich	MS, Biological Sciences	2017-2018	MS research directly funded by grant
Nick Willse	BS, Environmental Studies	2017-2019	Undergraduate lab technician, funded by grant

8. Professional Placement of Students

- Megan Berberich successfully defended her MS thesis in Oct. 2017, and was supported by the grant as a graduate student at UC as she continued to analyze samples, work up data, and write on the project through Dec. 2017. Since graduating, Megan worked full-time as a research technician in a molecular biology lab at the U.S. EPA research lab in Cincinnati, OH for 5 months; and for the past year she has worked as a full-time research assistant at the Tropical Responses to Altered Climate Experiment (TRACE), an NSF/DOE/USFS funded large-scale experimental climate-change project in tropical forest ecosystems in Puerto Rico (http://www.forestwarming.org).

- Nick Willse, undergraduate research technician analyzing samples in the lab, recently graduated with a BS in Environmental Studies and a minor in Biological Sciences from the University of Cincinnati. He is currently seeking work in the environmental field.

9. Notable Awards and Achievements

- Megan Berberich – J. Robie Vestal Award for Outstanding MS Student, University of Cincinnati Biological Sciences Department (2017)
- Nick Willse – Ohio EPA Environmental Fellowship (2018)
- Nick Willse – University of Cincinnati McMicken STEM Summer Fellowship (2017)
- Nick Willse – STEM Fellowship Award, Environmental Studies, University of Cincinnati (2018)
- Ishi Buffam - Faculty Development Council Grant, University of Cincinnati: *Presenting Research and Establishing Collaborations on Carbon Connectivity of Watersheds, Lakes and Streams at the American Water Resources Association Conference (2017)*
- *Ishi Buffam – Research Director, Greater Ohio Living Architecture Center (www.golacenter.org)*

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