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LETTER

Organic matter supply and bacterial community composition predict methanogenesis rates in temperate lake sediments

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Scientific Significance Statement

Lake sediment microbial communities play an important role in carbon cycling and influence ecosystem function. Despite this, there are still gaps in our understanding of how sediment communities vary across freshwater lakes and whether variation in community composition influences sediment function. Using a survey of 14 freshwater lake sediments, we provide evidence that pH and sediment organic matter content are environmental determinants of microbial community composition across a range of lakes. Further, we provide support for a conceptual model of lake methanogenesis, which suggests that variation in methanogenesis is related to the supply of organic matter to anoxic lake sediments and the bacterial community responsible for generating the precursors of methanogenesis from this organic matter.

Abstract

The objective of our study was to identify environmental conditions that structure lake sediment microbial communities and determine whether community composition explained inter-lake variation in potential methanogenesis rates. We performed a comparative analysis of microbial communities and methanogenesis rates in 14 lake sediments along gradients of pH and primary productivity. Variation in methanogen community composition and non-methanogen microbial community composition was best explained by pH and sediment organic matter content. However, these regulators of methanogen community structure were not associated with differences in methanogenesis rates. Instead, variation in lake methanogenesis rates was best explained by proxies for organic matter supplied to sediments (lake chlorophyll *a* concentration and sediment pore-water total phosphorus) and the composition of the non-methanogen microbial community. Our results suggest a role for sediment bacterial community in influencing methanogenesis via the supply of growth substrates.

In freshwater lakes, sediments are an important component of carbon processing and mineralization. Specifically, anoxic sediments are the primary contributor to methane (CH_4) production and emissions in lake ecosystems (Borrel et al. 2011). CH_4 production from sediments influences a number of in-lake processes, including carbon mineralization rates

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Additional Supporting Information may be found in the online version of this article.

(Tranvik et al. 2009) and trophic dynamics (Jones and Grey 2011). Additionally, CH_4 emissions from freshwater lakes are a substantial component of the global CH_4 cycle and can account for up to 16% of total natural CH_4 emissions (Bastviken et al. 2011). However, lake CH_4 production and emission are incredibly variable across ecosystems, and a comprehensive understanding of the mechanisms that contribute to this variation has yet to be achieved.

In lakes, CH_4 production predominantly occurs in the anoxic sediments, through the metabolism of methanogenic Archaea (hereafter referred to as "methanogens") (Liu and Whitman 2008). This biological methanogenesis occurs via three unique metabolic pathways—aceticlastic, hydrogenotrophic, and methylotrophic—each characterized by its electron donor and terminal acceptor (Liu and Whitman 2008; Fig. 1). In lake sediments, the aceticlastic and hydrogenotrophic pathways are predicted to generate CH_4 at a ratio of 2:1 (Conrad 1999). However, empirical estimates of the relative dominance of each pathway vary across lake ecosystems (Nusslein et al. 2001; Kotsyurbenko et al. 2004; Conrad et al. 2010), and, owing to the difficulties involved in partitioning these pathways, it is often unclear which microbial transformations are responsible for emergent rates of CH_4 production.

Methanogens exist in diverse communities of microorganisms and are intricately associated with syntrophic and homoacetogenic bacteria that supply them with growth substrates (Liu and Whitman 2008; Fig. 1). While variation in these communities has been documented across various aquatic ecosystems (Chan et al. 2002; Xiong et al. 2012), these studies have been primarily limited to a single location or habitat type. Consequently, the contributions of environmental variation to sediment microbial community composition across lake sediments are not well understood. Furthermore, while CH_4 production is a direct byproduct of microbial metabolism, a quantitative link between the composition of methanogen or non-methanogen sediment microbial assemblages and CH_4 production has yet to be achieved.

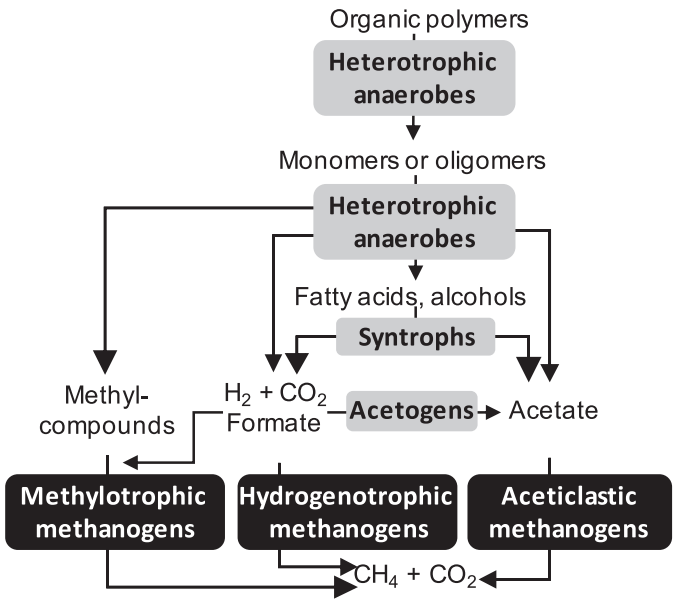

Fig. 1. Anaerobic conversion of organic matter to methane and carbon dioxide, adapted from Liu and Whitman (2008). Major microbial groups catalyzing the reactions are denoted in boxes. Gray boxes denote the non-methanogen community, and black boxes denote the methanogen community.

To understand variation in methanogenesis rates across lakes ecosystems, recent work has provided support for the *methanogenesis-substrate supply* model (West et al. 2016), which predicts that variation in methanogenesis is driven by the supply of methanogenesis substrates derived from settling autochthonous organic matter (OM). Lakes with greater rates of primary productivity are anticipated to have greater rates of carbon sedimentation into anoxic sediments, allowing for higher rates of OM conversion to CH_4. Experiments confirm that CH_4 production from lake sediments increases with algal biomass input (West et al. 2012; Grasset et al. 2018), and field surveys have reported positive relationships between lake primary production and CH_4 production (West et al. 2016) and emissions (Deemer et al. 2016). However, the magnitude of the response of CH_4 production to substrate supply can vary substantially across lakes, and the sources of this variation remain unaccounted for.

The objectives of our study are (1) to identify the potential roles of environmental conditions in structuring lake sediment microbial communities and (2) to determine whether variation among sediment microbial communities could explain variation in methanogenesis rates among lakes. We hypothesized that pH and ecosystem primary productivity represent important environmental controls over microbial community structure and function. To test these hypotheses, we performed a comparative analysis of microbial communities and methanogenesis rates in 14 freshwater lake sediments along gradients of pH and primary productivity. We determined how methanogen community composition (MCC) and non-methanogen microbial community composition (non-MCC) vary across lake sediments and then tested whether and how observed methanogenesis rates covary with microbial community composition.

Methods

To determine variation in sediment MCC, non-MCC, and methanogenesis rates across north temperate lakes, we sampled 14 lakes in northwest Michigan at the University of Notre Dame Environmental Research Center from May to July 2012. Data, metadata, and code are available at https://github.com/brittnibertolet/MicrobeRegCH4/tree/v1.0.0 (Jones et al. 2019).

Microbial community composition

To determine bacterial and archaeal community composition, we performed 16S rRNA paired-end sequencing on sediment collected from the deepest point of each lake. Sediments

were collected from the top 15 cm of the sediment surface using an Ekman dredge. The sample was homogenized and stored at −80°C until DNA extraction. A single DNA extraction was performed with 0.5 g of sediment using a MoBio PowerSoil DNA Isolation kit (Mo Bio) following the manufacturer's instructions. The V4 region of the 16S rRNA gene was amplified in a 25 μL PCR reaction according to the Department of Energy Joint Genome Institute (DOE JGI) iTag sample amplification protocol with amplicon primers 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 805R (5′-GGACTACNVGGGTWTCTAAT-3′). Amplified DNA served as template for high-throughput paired-end (2 × 150 bp) sequencing on an Illumina MiSeq at the DOE JGI (DOE JGI). Sequencing produced 3,851,982 sequence reads across the 14 lake sediment samples. Raw sequence reads are available online at DOE JGI Genome Portal under Project ID 1041357.

To quantify community membership, operational taxonomic units (OTUs) were defined at 97% similarity using the QIIME (version 1.9.1) bioinformatics pipeline for merging of paired-end reads, quality filtering, and OTU picking with the *pick_open_reference_otu.py* command (Caporaso et al. 2010; Jones et al. 2019). Representative sequences for each OTU were aligned and classified against the Greengenes database (version 13.5) (McDonald et al. 2012). OTUs with a single read count were discarded. We then identified methanogen OTUs by the presence of "Methano" in taxonomic assignments, which encompasses all previously identified methanogen taxa. These OTUs were used for MCC analyses and excluded from the non-MCC analyses.

Lake environmental conditions

We measured a number of environmental covariates at the time of sediment collection to determine biotic and abiotic factors that may influence sediment MCC, non-MCC, and potential rates of sediment methanogenesis (Additional Supporting Information Table 1). pH and dissolved oxygen (DO) were recorded at the sediment–water interface using a YSI Professional Plus Multiparameter meter (Yellow Springs Instruments). Both pH and DO vary within the water column of stratified lakes, so to approximate surface sediment conditions, we measured these variables 0.25 m above the sediment (referred hereafter as the sediment–water interface) in each lake. An integrated sample of epilimnetic lake water was collected for analysis of lake water column chlorophyll *a* (Chl *a*) concentrations. Particles from 450 mL of epilimnion lake water were captured onto a 0.7 μm glass fiber filter for analysis, and analyzed using methanol extraction and fluorometry (Welschmeyer 1994). Epilimnetic Chl *a* concentrations were used as proxies for supply rates of settling autochthonous carbon.

Surface sediments were also collected to determine sediment OM content and pore-water total nitrogen (TN) and total phosphorus (TP) concentrations. Percent OM was determined using loss on ignition measurements of dried sediment samples (Heiri et al. 2001). To determine nutrient concentrations, 30 mL of sediment was centrifuged for 10 min to extract sediment pore water. Each sediment sample produced at least 10 mL of pore water, which was diluted to 20 mL and used for determination of pore-water TN and TP concentrations. TN and TP concentrations were quantified as described in West et al. (2016).

Methanogenesis potential

During each sampling event, surface sediments were also collected to measure potential lake methanogenesis rates. Methanogenesis rates were determined using sediment incubations as described in West et al. (2016). Incubations were conducted in the laboratory in 300 mL sealed serum bottles containing 50 mL of lake sediment and 50 mL of hypolimnetic lake water. Bottles were flushed with N_2 gas to maintain anoxia and stored at in situ lake temperature in the dark for 9 d. Methanogenesis rates were then estimated by sampling the headspace three times over 9 d and fitting a linear regression to the time course. CH_4 concentrations were measured using gas chromatography as described in West et al. (2016).

Statistical analyses

All statistical analyses were conducted in R (R Core Team 2018) using the *vegan* (Oksanen et al. 2017) and *ggplot2* (Wickham 2009) packages.

To assess differences in MCC and non-MCC across lakes, we first standardized both OTU matrices to relative abundance matrices. We then generated pairwise Bray–Curtis dissimilarity matrices using the *vegdist* function. To relate variation in MCC and non-MCC to each of the measured environmental conditions, we conducted permutational multivariate ANOVA (PERMANOVA) tests using the *adonis* function in R (Anderson 2001). Significance of the PERMANOVA statistic was evaluated using 1000 permutations of the dissimilarity matrices for each community. Variation in community composition was visualized by performing principle coordinates analysis (PCoA) ordinations using the Bray–Curtis dissimilarity matrices. We determined relationships between the first two PCoA axes and measured environmental conditions using linear regression. Finally, we determined whether the two dissimilarity matrices were correlated using a Mantel test.

To determine whether differences in sediment microbial communities explained variation in lake methanogenesis rates, we first identified metrics of composition: PCoA axes of MCC and non-MCC and total methanogen relative abundance (number of methanogen reads divided by total, quality-controlled sample reads). We used a multiple model comparison approach to determine whether these metrics explained variation in methanogenesis using linear regression, both on their own and in addition to the variation explained by environmental conditions. The relative support for candidate models was then assessed using likelihood ratio tests.

Results

MCC in lake sediments

We detected 425 methanogen 97% OTUs from five different orders (Methanobacteriales, Methanocellales, Methanomassiliicoccales, Methanomicrobiales, and Methanosarcinales)

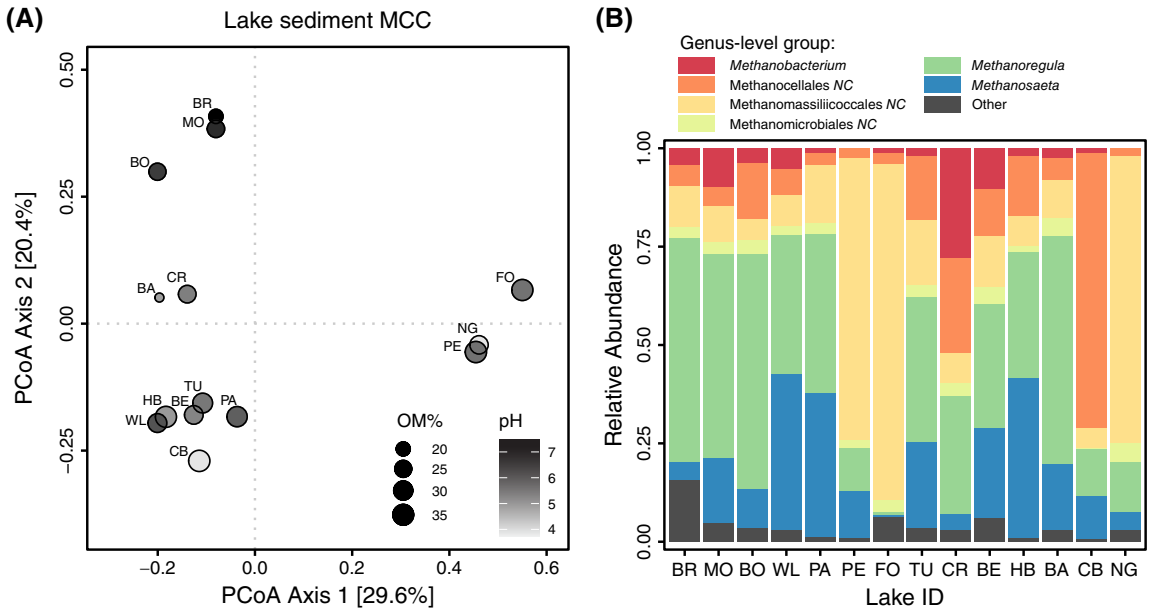

Fig. 2. (A) First two principal coordinates of MCC in 14 lake sediments. Points denote individual lake methanogen assemblages. MCC significantly differed across sediment OM content (point size; $F_{13,1} = 2.06$, $p < 0.05$) and pH at the sediment–water interface (point shading; $F_{13,1} = 1.95$, $p < 0.05$). Bracketed values indicate the percentage of variation explained by each axis. (B) Relative abundance of methanogen genus-level groups in 14 lake sediments, ordered by pH. OTUs not classified at the genus-level were aggregated into unclassified members of a given order (i.e., Methanomicrobiales *NC*). Genera that represented less than 1% of total methanogen DNA sequences are denoted as Other (*see* Fig. S1 for their relative abundances).

across our 14 lake sediment samples. These OTUs represented 2.1% of the total number of 16S sequences that passed quality control filtering. Samples had an average of 41,097 (± 19,054 SD) high-quality 16S sequences, and total sequence counts were not correlated with any measured environmental variable. Similarly, total methanogen relative abundance ranged from 0.4% to 5.6% across samples and was not correlated with any measured environmental variable.

Sediment methanogen communities were dominated by the genus *Methanoregula* (41.1%), unclassified members of the order Methanocellales (20.8%), the genus *Methanosaeta* (19.7%), unclassified members of the order Methanomassiliicoccales (15.8%), and the genus *Methanobacterium* (4.5%). These groups also represented the most abundant genus-level group in each of the five methanogen orders detected in the sampled lake sediments (Fig. 2).

As hypothesized, variation in sediment MCC could be explained by environmental conditions (Fig. 2A). PERMANOVA tests indicated that MCC significantly differed across sediment OM content ($F_{13,1} = 2.06$, $p < 0.05$) and pH ($F_{13,1} = 1.95$, $p < 0.05$). Both sediment OM content and pH were correlated with PCoA axis 2 scores (OM content: $R^2 = 0.38$, $p < 0.05$; pH: $R^2 = 0.48$, $p < 0.01$). Although pH and sediment OM were correlated with similar components of MCC (PCoA axis 2), they were not significantly correlated. Neither water column Chl *a* concentration nor sediment pore-water nutrient content explained significant variation in MCC.

Non-MCC in lake sediments

The non-methanogen community of lake sediments was largely dominated by the phyla Proteobacteria and Chloroflexi, but communities also exhibited marked variation in non-MCC across lakes (Fig. 3). Similar to the variation observed in MCC, PERMANOVA tests indicated that non-MCC also differed across pH ($F_{13,1} = 1.97$, $p < 0.05$) and sediment OM content ($F_{13,1} = 1.8$, $p < 0.01$). Correlations between PCoA axes scores and environmental conditions supported these results; PCoA axis 1 was negatively correlated with pH ($R^2 = 0.47$, $p < 0.01$) and positively correlated with sediment OM ($R^2 = 0.47$, $p < 0.01$). Additionally, PCoA axis 2 scores were positively correlated with Chl *a* concentrations ($R^2 = 0.33$, $p < 0.05$), but neither sediment pore-water TN nor TP were correlated with any metric of non-MCC. Finally, a Mantel test comparing the two dissimilarity matrices indicated that non-MCC and MCC were significantly correlated ($r = 0.63$, $p = 0.001$).

Environmental variables and sediment microbial community composition correlate with potential methanogenesis rates

Lake Chl *a* concentrations and sediment pore-water TP concentrations were both significantly correlated with methanogenesis rates (Table 1). Metrics of MCC (PCoA axes of MCC, total methanogen relative abundance, relative abundance of methanogen genera) did not explain significant variation in methanogenesis rates across lake ecosystems, neither

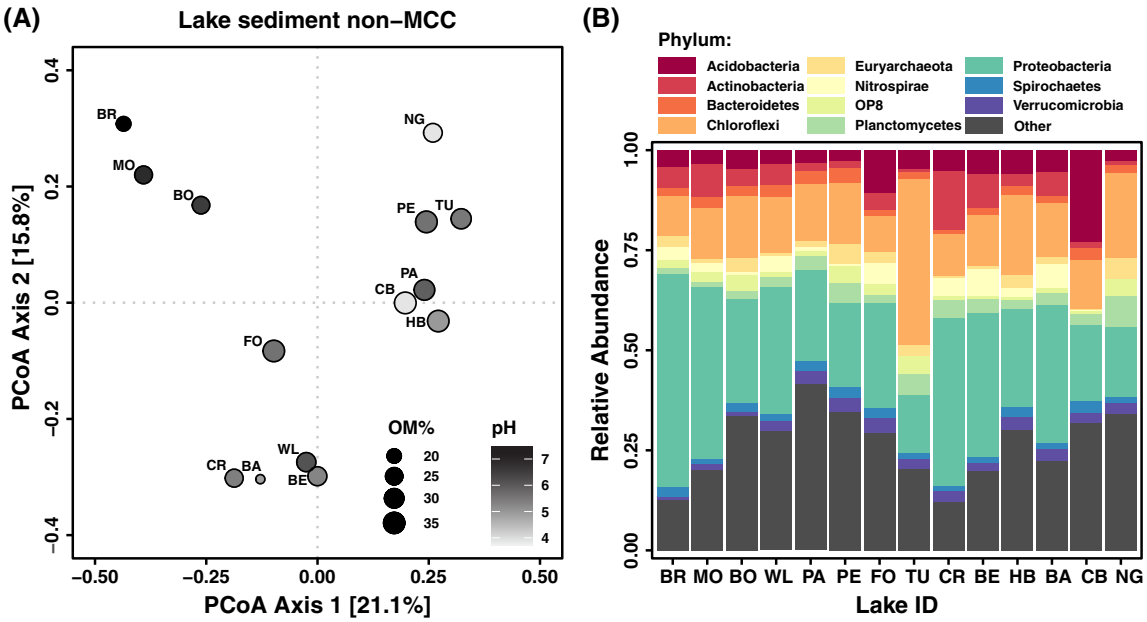

Fig. 3. (A) First two principal coordinates of non-MCC in 14 lake sediments. Points denote individual lake non-methanogen assemblages. Non-MCC significantly differed across pH at the sediment–water interface (point shading; $F_{13,1} = 1.97$, $p < 0.05$) and sediment OM content (point size; $F_{13,1} = 1.8$, $p < 0.01$). Bracketed values indicate the percentage of variation explained by each axis. (B) Relative abundance of bacterial phyla in 14 lake sediments. Phyla that represented less than 2% of total bacterial DNA sequences are denoted as Other (*see* Fig. S2 for their relative abundance).

alone nor together with environmental factors. Instead, a model including sediment pore-water TP concentration, lake Chl *a* concentration, and non-MCC PCoA 1 explained the most amount of variation in methanogenesis rates ($R^2 = 0.74$, $p < 0.05$; Table 1). Likelihood ratio tests indicated this TP + Chl *a* + non-MCC PCoA 1 model to be significantly better than the TP + Chl *a* model ($\chi^2 = 4.8$, $p < 0.05$) and the Chl a + non-MCC PCoA 1 model ($\chi^2 = 8.4$, $p < 0.05$).

The PCoA axis 2 of the non-MCC was also positively correlated with both lake Chl *a* concentration ($R^2 = 0.33$, $p < 0.05$) and lake methanogenesis ($R^2 = 0.37$, $p < 0.05$), but because the two predictors were correlated, PCoA axis 2 did not explain significant additional variation when included in a model with Chl *a* (likelihood ratio test comparing model to Chl *a*-only model: $\chi^2 = 2.6$, $p = 0.10$). No other metrics of MCC or non-MCC were directly correlated with methanogenesis.

Discussion

Lake sediment microbial communities play an important role in carbon cycling and ecosystem functioning. Despite this, there are still gaps in our understanding of how sediment communities vary across lake ecosystems and whether variation in community composition influences sediment function. Our study identifies environmental factors contributing to variation in MCC and non-MCC across a diverse set of north temperate freshwater lakes and motivates the potential for microbial compositional influences on lake ecosystem function. We encountered marked variation in MCC and non-MCC across both pH and sediment OM contents of our study lakes. However, neither these abiotic factors nor MCC were associated with differences in potential lake methanogenesis rates. Instead, variation in methanogenesis rates was best explained by sediment pore-water TP concentration, lake Chl *a* concentration, and the composition of non-methanogens observed in the local habitat. This result suggests a potential link between bacterial community composition and variation in sediment methanogenesis rates via the supply of metabolic byproducts.

pH and sediment OM content influence MCC and non-MCC

An improved understanding of factors that influence microbial community assembly may be important in identifying the role of microbial community structure in dictating function (Hall et al. 2018). In the present study, both pH and sediment OM content influenced MCC (Fig. 2) and non-MCC (Fig. 3) across lakes; however, the two environmental conditions were not correlated. Because our data are observational, it is difficult to determine the mechanisms behind these responses, but previous research has highlighted the importance of both pH (Lindström et al. 2005; Fierer and Jackson 2006; Xiong et al. 2012) and OM (Findlay et al. 2003; Ward et al. 2019) as drivers of microbial community composition and activity across terrestrial and aquatic ecosystems.

In support of the relationship between pH and microbial community composition, laboratory (Phelps and Ziekus 1984), field (Chan et al. 2002), and simulation (Jin and Kirk 2018) experiments have found pH to regulate microbial

Table 1. Coefficient of determination (R^2) and *p*-values for regression models relating lake methanogenesis rates to environmental and microbial predictor variables. For multiple regression models, likelihood ratio test *p*-values are reported, see footnotes for competing models. Models that significantly out-performed the null or other specific competing model are bolded.

Type	Predictors	R^2	*p*-value
Environmental	**Chl *a***	**0.35**	**<0.05**
	pH	0.001	0.89
	OM%	0.005	0.8
	TN	0.17	0.13
	TP	**0.54**	**<0.05**
	TP+Chl *a*	0.64	0.058*
Microbial	Methanogen relative abundance	0.02	0.61
	MCC PCoA 1	0.05	0.43
	Non-MCC PCoA 1	0.08	0.31
Environmental	Chl *a*+methanogen relative abundance	0.39	0.33†
+ Microbial	Chl *a*+MCC PCoA 1	0.38	0.34†
	Chl *a*+non-MCC PCoA 1	**0.54**	**<0.05**†
	TP+methanogen relative abundance	0.54	0.82*
	TP+MCC PCoA 1	0.55	0.61*
	TP+non-MCC PCoA 1	0.58	0.25*
	TP+Chl *a*+non-MCC PCoA 1	**0.74**	**<0.05**‡
			<0.05§

*Compared to TP-only model.
†Compared to Chl *a*-only model.
‡Compared to TP + Chl *a* model.
§Compared to Chl *a* + non-MCC PCoA 1 model.

community assembly and function, especially in anaerobic environments. pH is hypothesized to influence energy yields of metabolism (Jin and Kirk 2018), which have direct consequences for competition in the community. However, few other studies have compared the effects of pH to other environmental factors or considered the range of pH (pH 4.1–8.3) encompassed by our study. Xiong et al. (2012) found that sediment microbial communities differed across pH in a set of alkaline lakes along a gradient of salinity. Our results provide additional evidence that pH is a strong environmental filter on sediment microbial communities in north temperate freshwater lakes and that community responses to pH are evident across a diverse set of lake ecosystems.

Similarly, it could be hypothesized that sediment OM content acts as an environmental filter on microbial communities by influencing the quantity and quality of available substrates. It is well supported in lake bacterioplankton communities that the source and quality of OM influences microbial community composition (Kritzberg et al. 2006; Jones et al. 2009). This is hypothesized to be due to the effect of varying resource compositions selecting for microbial taxa with specific resource affinities. However, few studies have documented similar connections in sediment communities (Ward et al. 2019), which may be due to difficulty in characterizing the quality of sediment OM.

In the present study, the relationship between sediment OM quantity and quality is still uncertain, and differences in the supply of methanogenesis precursors (acetate concentrations, H_2/CO_2 concentrations, etc.) between lakes were not quantified. It is thus possible that an unmeasured metric of sediment OM quality may be driving these patterns between OM quantity and community composition. There was a large amount of variation in community composition that was not explained by any measured environmental driver, and further research, specifically with directed experimental tests, is still needed to understand the process by which community composition responds to both pH and sediment OM.

Relationships between community structure and methanogenesis rates

Advances in sequencing technology have greatly increased our ability to determine variation in microbial community composition across space, but we still lack a predictive process-based understanding of how variation in community structure influences ecosystem function (Hall et al. 2018). Contrary to our expectations, methanogenesis rates were not related to variation in MCC. Similarly, neither pH at the sediment–water interface nor sediment OM content—which were significant environmental drivers of microbial community structure—explained variation in methanogenesis. Instead, a model that included proxies for OM supply to sediments (water column Chl *a* concentrations and sediment pore-water TP concentrations) and the composition of the non-methanogen microbial community was the best predictor of methanogenesis rates (Table 1).

Rates of methanogenesis in lake sediments are influenced by ecosystem primary productivity via the sedimentation of organic material that fuels the anaerobic microbial loop (West et al. 2016; DelSontro et al. 2016; Fig. 1). Chlorophyll *a* is a commonly used proxy for algal biomass, but sediment TP has also been related to lake trophic status and both served as proxies of sediment OM supply in our study (Carey and Rydin 2011). In fact, a previous study observed that sediment pore-water phosphorus was related to OM mineralization and was correlated with methanogenesis (Sinke et al. 1990). In our study, both Chl *a* and sediment pore-water TP were positively correlated with methanogenesis, consistent with these previous studies.

Interestingly, our best-fit model for methane production suggests that bacterial community composition explains some variation in methanogenesis rates. The mechanism for how bacterial composition may influence rates of methanogenesis is uncertain, and further research is needed to determine whether

there is a direct effect. Methanogens are intricately, and often obligately, associated with syntrophic and other heterotrophic bacteria (Liu and Whitman 2008), and it is hypothesized that these bacteria may influence rates of methanogenesis by controlling the supply of metabolic substrates (Ye et al. 2014). In our study, Proteobacteria and Chloroflexi were the dominant bacterial phyla, but varied across lakes (Fig. 2B). These bacterial phyla contain many known syntrophic and acetogenic bacteria, including the GIF9 group (Hug et al. 2013). However, fermentation and acetogenesis are not strongly taxonomically constrained and therefore our data cannot directly identify the bacterial groups most likely responsible for substrate supply to methanogens (Drake et al. 2002). Differences in lake sediment MCC and non-MCC were also significantly correlated, indicating the potential for non-MCC to influence MCC. However, it is also possible that MCC and non-MCC are responding concomitantly to differences in pH and sediment OM, and composition does not have a direct effect on sediment function.

While MCC did vary across both pH and sediment OM, our data did not reveal systematic responses of putative methanogen functional groups to these environmental variables nor any correlation of MCC with methanogenesis rates. It is worth noting that many of the detected sequences have not been characterized and metabolic function is not always conserved within methanogen orders, so it is difficult to identify patterns across functional groups. However, among the genera that can be classified functionally, the hydrogenotrophic methanogens of the genus *Methanoregula* were the most abundant across all lakes, and aceticlastic methanogens of the genus *Methanosaeta* were less abundant than previously hypothesized (Conrad 1999), accounting for less than 16% of detected methanogens.

Although much work remains to elucidate the complex ecology that dictates the composition of lake sediment Bacteria and methanogenic Archaea and sediment methanogenesis rates, our results are consistent with methanogenesis rates being driven by supply rate of complex OM and the bacterial community that converts this complex OM into precursors of methanogenesis. Methanogens utilize only a limited number of substrates (Liu and Whitman 2008), and this restricted substrate use by methanogens may explain the lack of a correlation between MCC and methanogenesis rates in our lakes. We hypothesize that upstream processes (Fig. 1), such as OM supply and generation of methanogenesis precursors, act as the major controls on rates of methanogenesis. However, further research, including manipulative experiments, is needed to directly test this hypothesis and solidify our understanding of the primary drivers of methanogenesis in lake sediments.

Conclusion

Looking forward, an improved understanding of links between local environmental characteristics, microbial communities, and carbon processing rates may allow us to better predict shifts in CH_4 production rates under environmental change. We note that community composition is not always indicative of microbial activity, and measures of other microbial transformations (such as the production of acetate or CO_2) would greatly increase our understanding of the role of non-methanogen communities. This connection between community composition and function is a critical next step for incorporating community traits into ecosystem models, and we encourage further research that directly identifies links between abundances of microbial functional groups and rates of OM degradation and CH_4 production. We conclude that pH and sediment OM are important environmental determinants of bacterial and methanogenic archaeal assemblages, but that lake primary production remains the best predictor of methanogenesis and that interactions between methanogens and other syntrophic bacteria are potentially key for linking microbial community composition to lake methanogenesis rates.

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