Influence of Microbial Community Composition and Organic Matter Supply on Methane Production in Anoxic Lake Sediments

Alexandra M. Lugo-Arroyo, Brittni Bertolet

Abstract

Freshwater lakes are a significant site for biological methane production and its subsequent emission into the atmosphere as a potent greenhouse gas. Methane in freshwater lakes is mostly produced in anoxic sediments by archaea from the taxon *Euryarchaeota*. The archaea metabolize substrates derived from the decomposition of organic matter by anaerobic bacteria. This indicates that both organic matter supply and microbial community composition are drivers behind methanogenesis. However, the interaction between these two factors and the extent of their role remains mostly unexplored. We sampled from Morris Lake, a mesotrophic lake situated at the University of Notre Dame Environmental Research Center, and constructed sediment incubations treated with algal additions and antibiotic additions in full factorial. The rates of methane and carbon dioxide were estimated and used to determine the influence of organic matter supply and microbial community composition. We hypothesized that treatments with algal additions and without antibiotics would have the highest production rates. Accordingly, we found a significant effect and interaction between algal additions and a lack of antibiotics. This indicates that microbial community composition and organic matter supply are significant factors in the production of methane in anoxic lake sediments, as well as being intricately dependant on each other.

Keywords: biological methanogenesis, bacterial substrates, organic matter supply, eutrophication, *Euryarchaeota*, lakes, anoxic, freshwater, sediments

Introduction

The biological production of methane is a significant source of methane emission to the atmosphere, accounting for approximately 74% of the emitted methane globally (Liu & Whitman, 2008). This is driven by the metabolization of microbial substrates by obligate anaerobic archaea from the taxon *Euryarchaeota* (Liu & Whitman, 2008). These archaea have been commonly found in temperate freshwater sediments, marine sediments, flooded soils, among other anoxic and lightless environments (Liu & Whitman, 2008). As such, anoxic freshwater lake sediments are a significant source of biological methane production and can account for 30%-80% of the anaerobic carbon mineralization in water reservoirs (Bastviken et al., 2003). Consequently, it has a significant role in the global carbon cycle and influences atmospheric concentrations of methane. Globally, freshwater ecosystems are estimated to contribute anywhere from 6%-16% of global methane emissions (West et al., 2012). Previous research suggests that the biological production of methane by *Euryarchaeota* is driven by the supply of organic matter and, to a less defined degree, the microbial community composition (West et al., 2012; Grasset et al., 2018).

Methanogenic archaea have an inherent dependence on anaerobic organisms and eukaryotes. They are unable to directly metabolize complex organic matter and require other organisms to break down complex molecules- such as carbohydrates, fatty acid chains, and alcohols- into simpler molecules that they can then convert into methane (Liu & Whitman, 2008). Methanogenic archaea are known to utilize three substrates; namely, carbon dioxide, methyl containing groups, and acetate (Liu & Whitman, 2008). However, in freshwater sediments, carbon dioxide and acetate are the most abundant substrates (Liu & Whitman, 2008). Furthermore, non methanogenic microbes also aid in the fate of the methane in the ecosystem. Methane can either be oxidized by methanotrophic bacteria or evade this process and be emitted into the atmosphere

(West et. al., 2016). Thus, bacterial metabolization of methane is an alternate pathway to methane emission. This metabolic dependency is indicative of an intricate relationship between methanogenic organisms and non methanogenic organisms, where the microbial community composition plays a key role in biological methanogenesis.

Understanding how biological methanogenesis is influenced by organic matter supply and microbial community composition is significant in the face of anthropogenic eutrophication of water reservoirs. This is especially important in the context of ongoing climate change and the effect of greenhouse emissions, including methane gas. Eutrophication is commonly defined as an increase in primary productivity- namely, algae- caused by excess supply of limiting nutrients. In many places, eutrophication of inland waters is primarily due to anthropogenic causes, specifically, agriculture. Increased sewage waste and agricultural fertilizers residue coupled with increased runoff and severe precipitation patterns represent important pathways for nutrient discharges from agricultural farms into inland water reservoirs (Beaulieu et al., 2019). This trophic status is characterized by having a poor phytoplankton biomass, causing a shift in secondary production and, thus, microbial community composition (Haukka et al., 2006). The shift in microbial composition caused by eutrophication is significant given that previous research has found that increased quantity of algal substrate has a strong positive correlation to increased methanogenesis rates in anoxic lake sediments (West et al., 2012, 2015; Beaulieu et al., 2019). This highlights the importance and interaction of organic matter supply in the context of microbial community composition.

The goal of this investigation was to determine the effect of microbial diversity on the availability of substrates for the archaea to metabolize- and, thus, perform methanogenesis- as well as how this compared to the effect of organic matter supply. To do so, incubation experiments will

be designed with algal additions and antibiotic additions in full factorial. We hypothesize that antibiotic treatments will negatively influence methanogenesis rates, via the reduced supply of growth substrates, and that algal additions will positively influence methanogenesis. Thus, the treatments without antibiotics and with algal additions were expected to have the highest rates of methanogenesis. The differences in the results were used to determine whether microbial community composition or organic matter supply had a greater effect in driving methanogenesis in anoxic lake sediments.

Methods

Experimental Design

To determine the influence of organic matter supply and microbial community composition on sediment methane production, sediment incubation experiments were conducted in which they were treated with antibiotics and algal additions in full factorial with five replicates each. Sediments samples were obtained from Morris Lake to construct laboratory incubations. We chose Morris Lake because previous research has shown a marked positive effect when treated with algal additions (West et al., 2015). Additionally, there is extensive physical, chemical, and biological characterization on the lake available which provided useful background for the experiments (Solomon et al., 2018). Morris lake is approximately 6 ha and has a maximum depth of approximately 6.7 meters (Figure 1). The limnological profile of Morris Lake detailing dissolved oxygen levels and temperature is depicted in Figure 2. The thermocline is at an approximate depth of 2 meters (Figure 2). The dissolved oxygen levels are 0% at the bottom of the lake, ensuring the anoxic conditions of the sediments sampled (Figure 2).

To collect construct sediment incubations that mimic the conditions in the sediments of Morris Lake, anoxic sediments were sampled from the deepest point in the pelagic region. Hypolimnion water was collected from above the sediment surface. Incubations were constructed in 125 mL sealed serum bottles containing 25 mL of sediment and 25 mL of hypolimnion water. To ensure anoxic conditions, the headspace of the serum bottle was purged with N_2 gas. Bottles were incubated at 4 °C in the dark for 25 days.

There were five replicates for each treatment for a total of 20 incubations distributed as follows: 5 control, 5 with algal additions, 5 with antibiotic treatments, and 5 with both algal and antibiotic additions. The phytoplankton *S. obliquus* was cultured in 50% Bold's Basal Medium (BBM) and used for algal additions. Incubations assigned with algal addition treatments received 25 mg of dried *S. obliquus* biomass at the beginning of the incubation. To manipulate the microbial community, rifampicin and ampicillin were added to serum bottles to final concentrations of 100 ug/mL.

Quantification of CO_2 and CH_4 Production

Carbon dioxide and methane were sampled from the headspace of the sediment incubations 6 times over 26 days, or approximately once every 5 days. The gas samples were collected in a sealed GC vials and analyzed with gas chromatography as described in West et al. 2012. To estimate rates of methane and carbon dioxide production, a linear regression was fitted to the time series and the slope used to infer the rate of production.

Statistical Analyses

To determine whether algal additions or microbial community composition had a greater effect in driving methanogenesis in anoxic lake sediments, we used a two-way Analysis of Variance (ANOVA) test to determine if there were any significant differences between the

sediment incubation treatments. The rates of methane and carbon dioxide production were the response, or dependent, variables; whereas the different treatments- algal additions, antibiotics, both, or none- were the independent variables.

Results

Overall, the treatments with the most methane production were the sediment incubations with algal additions and without antibiotics (Figure 4). Treatments without antibiotics consistently presented low rates of production, regardless of the presence of algal additions (Figure 4). The treatments with the lowest production rates were those without algal or antibiotic additions, as expected. Carbon dioxide production rates showed a similar trend (Figure 6). The linear regressions for methane and carbon dioxide production depicted in Figure 3 and 5, respectively, show a marked increase in methane and carbon dioxide production in the absence of antibiotics.

In the case of methane production (Figure 4), both algal ($F_{16,1} = 34.40$, $p < 0.05$) and antibiotic ($F_{16,1} = 12.71$, $p < 0.05$) treatments were significant, but the interaction of the treatments was also significant ($F_{16,1} = 26.64$, $p < 0.05$). This indicates a dampened effect of algal additions in the presence of antibiotics. Likewise, in the case of carbon dioxide production (Figure 6), both algal ($F_{16,1} = 29.52$, $p < 0.05$) and antibiotic ($F_{16,1} = 33.01$, $p < 0.05$) treatments were also significant in influencing carbon dioxide production, as well as the interaction of the treatments was also significant ($F_{16,1} = 12.49$, $p < 0.05$). Carbon dioxide production only increased relative to the controls in the populations that had algal additions, but no antibiotic additions. The data indicates that microbial community composition does have a direct effect on biological methane production.

Discussion

Biological methane production is influenced by organic matter supply and microbial community composition (West et al., 2012; Grasset et al, 2018). The sediment incubation treatments with algal additions and without antibiotics had the highest rates of methane production, while those with antibiotics had consistently low production rates (Figure 4). The treatments with antibiotics presented low production rates regardless of algal additions. This could indicate that the effect of algal additions is suppressed in the presence of antibiotics. The antibiotics only have adverse effects on the bacterial community present, excluding the methanogenic archaea. Thus, the antibiotics affect the microbes who are decomposing the organic matter supply and providing substrate for the archaea to then metabolize into methane. The repression of the bacterial population by the antibiotics inhibits biological methane production by cutting off the substrate supply. This is supported by previous studies conclusions (Bertolet et al., in press; West et al., 2016). In other words, the algal additions effect is nullified because the organic matter supply on its own is not enough to drive methanogenesis. This implies that biological methane production is to a notable extent dependant on the microbial community to process an organic matter additions.

Similarly, the rates of carbon dioxide production follow the same trend. Carbon dioxide is a common substrate for methanogenic archaea (Liu & Whitman, 2008). The treatments without antibiotics and with algal additions had the highest rates of carbon dioxide, while the treatments with antibiotics had the lowest rates (Figure 6). In the case of the treatments with antibiotics and with algal additions, there was no bacterial community available to decompose the algae added and had the second lowest rate- next to the control treatment (Figure 6). A lack of a bacterial community is conducive to a lack of substrate production, as demonstrated by the low carbon dioxide in the treatments with antibiotics (Figure 6). This, as well as the methane production rates (Figure 4), also highlights the relationship between the non methanogenic microbes and the

methanogenic microbes as a crucial network for the production of methane in anoxic lake sediments.

Notably, in the methane production rates, negative trends are seen in all of the treatments except the ones without antibiotics and with algal additions (Figure 4). This could be due to the concentration becoming diluted over time, given that after sampling we added nitrogen gas to ensure the anoxic conditions and it not being actively produced. It could also be due to human introduced error if all or most of the sample bottles were leaky or defective. However, it still stands that the treatments without antibiotics and with algal additions showed the highest rates of both methane and carbon dioxide production, indicating that both organic matter supply and microbial community composition are potentially drivers for methanogenesis in anoxic lake sediments.

This study presented several limitations that could be amended in future research investigations. Firstly, we only sampled from Morris Lake as a representative mesotrophic site, due to time constraints. Although we had replicates for the treatments conducted, we did not sample other mesotrophic lakes. Consequently, it is possible that Morris Lake is not representative enough of all temperate mesotrophic lakes and, thus, results might not be accurate enough to be able to be extrapolated in order make general statements and conclusions about methane production in temperate lakes. For future investigations, we recommend the experimental design include a more comprehensive selection of lakes. Secondly, for the antibiotic additions in the treatments, we used the same standard concentration for all of them. A future project could incorporate different concentrations of antibiotics within the treatments to quantify how dependent methane production is on organic matter supply decomposition by anaerobic bacteria. Conclusively, microbial community composition does significantly influence biological methane

production in anoxic lake sediments as well as organic matter supply. However, the exact mechanisms and degree of influence is still to be explored.

Acknowledgments

Firstly, I would like to sincerely thank my mentor Brittni Bertolet for her integral role in the development of this project, providing the template, knowledge, guidance and resources to complete it. As well as my research partner Elizabeth Hargis, for helping with the execution of the experiment and entrainment during sampling sessions. I would also like to thank Hunter Wojtas, Alejandra Porras-Maturana, and Carolina Quiles-Bengochea for all the helpful comments, company during late nights, and, most importantly, emotional support during this entire process. Moreover, an extended thanks to UNDERC director Gary Belovsky, UNDERC assistant director Michael Cramer, teaching assistant Matt Gregory and Jasper Leavitt, and lab technician Shannon Jones for providing the resources and equipment needed. Finally, I would like to acknowledge the Bernard J. Hank Family Endowment and the José Enrique Fernandez Endowment for funding this research project.

Figure appendix

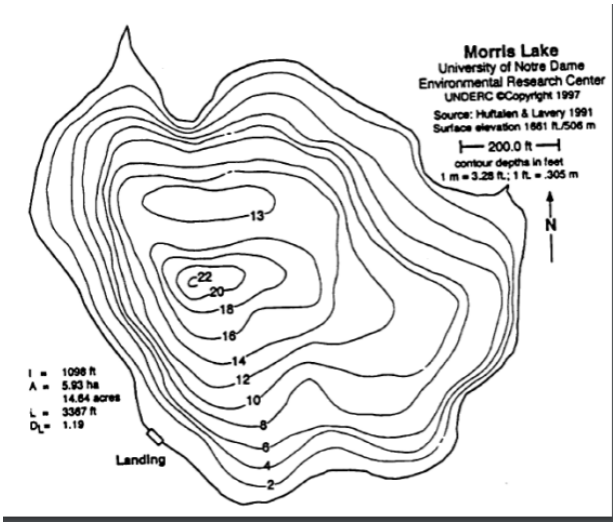

Figure 1. Bathymetric Map of Morris Lake obtained from UNDERC records ("Lake Information", n.d.).

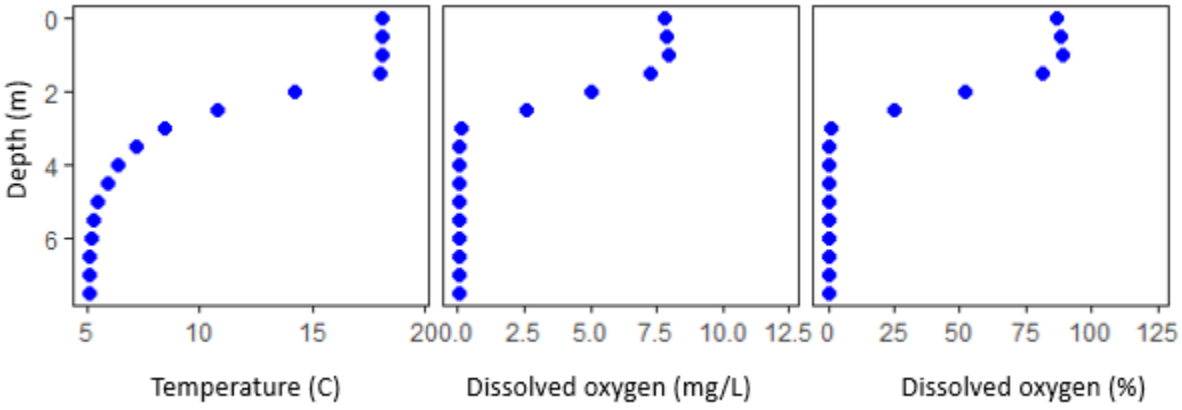

Figure 2. Limnological profile of Morris Lake measuring temperature (C) and dissolved oxygen levels in mg/L and percentage.

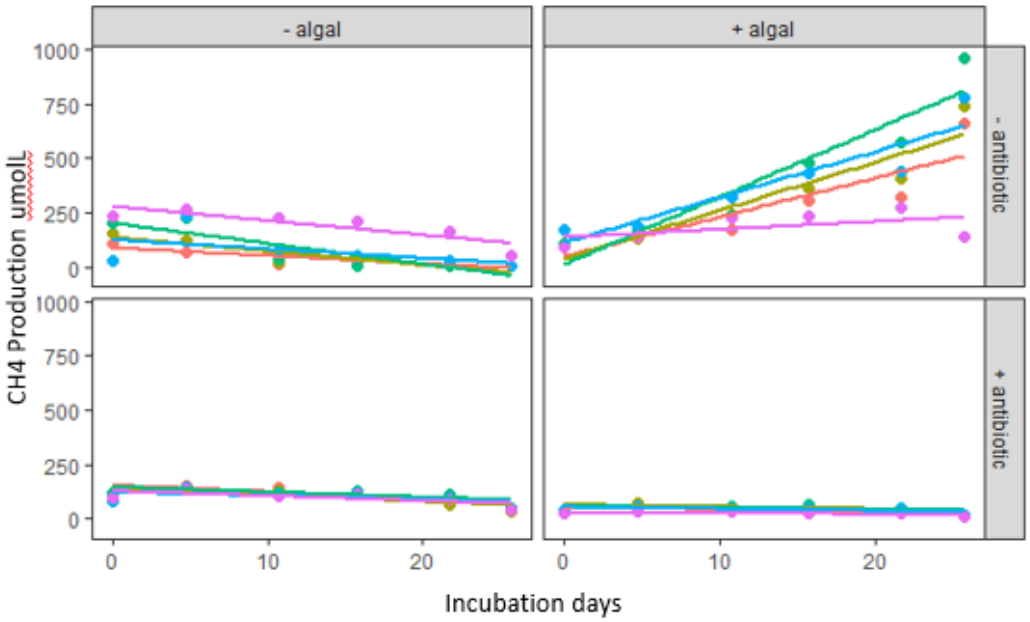

Figure 3. Linear regression of methane production (µmolL) per replicate per treatment plotted against the incubation days. The slope was used to infer the production rate of methane for each incubation. Treatments without antibiotics and with algal additions had the highest production rates, indicating that both factors are drivers in methanogenesis. Treatments with antibiotics had low methane production regardless of algal additions.

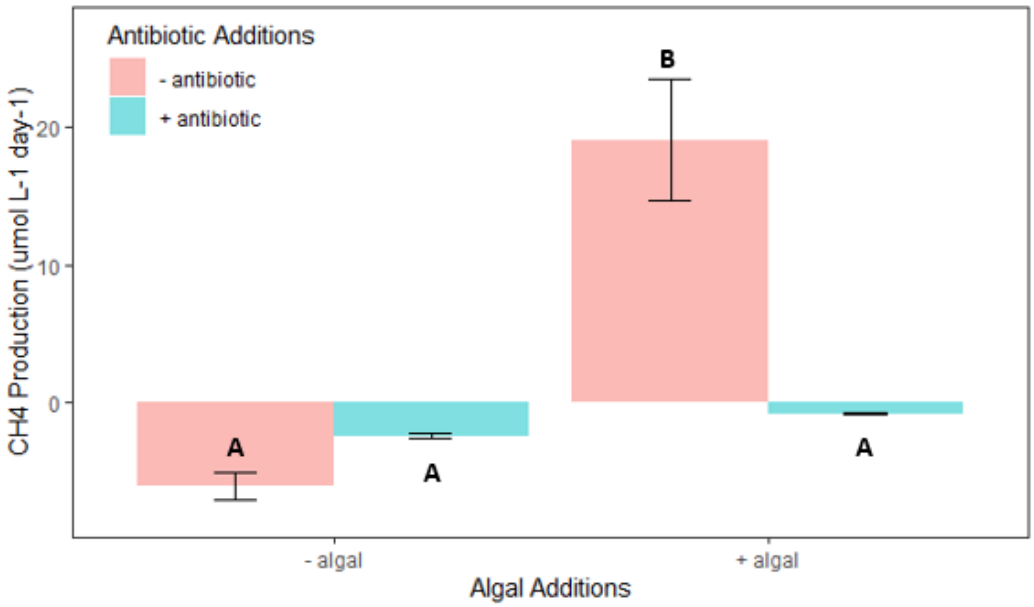

Figure 4. Methane production rate (µmol L-1 day-1) plotted against the different treatments of antibiotics and algal additions. Both algal ($F_{16,1} = 34.40$, $p < 0.05$) and antibiotic ($F_{16,1} = 12.71$, $p < 0.05$) treatments were significant, but the interaction of the treatments was also significant ($F_{16,1} = 26.64$, $p < 0.05$). This indicates a dampened effect of algal additions in the presence of antibiotics.

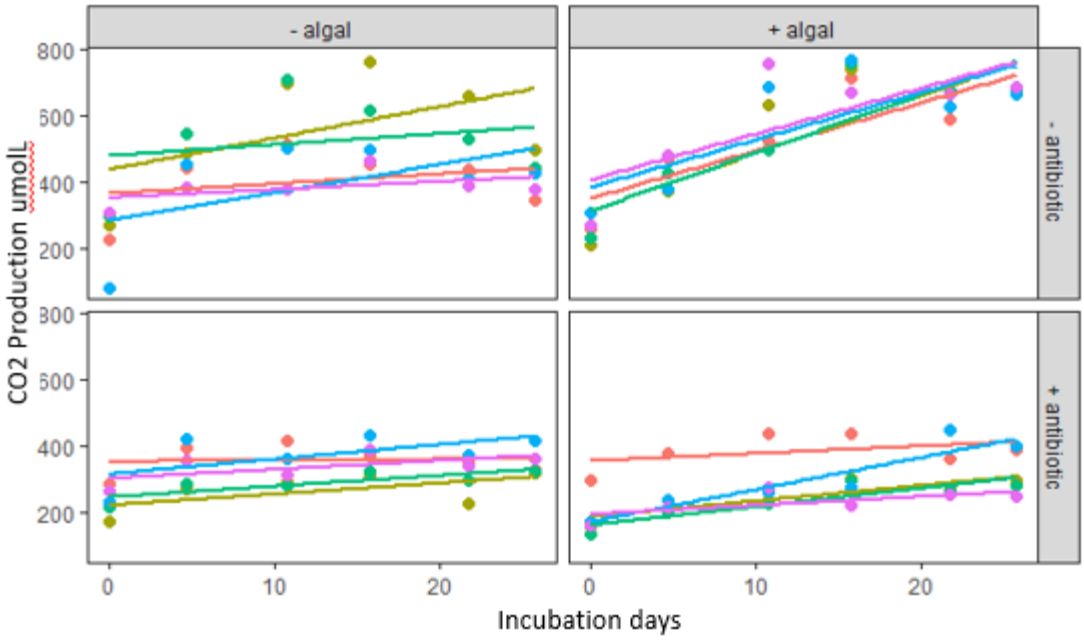

Figure 5. Linear regression of carbon dioxide production (µmol L) per replicate per treatment. The mean slope was used to estimate the production rate of carbon dioxide. Treatments without antibiotics and with

algal additions showed the highest methane production. Treatments with antibiotics showed the lowest rates of methane production, regardless of algal additions.

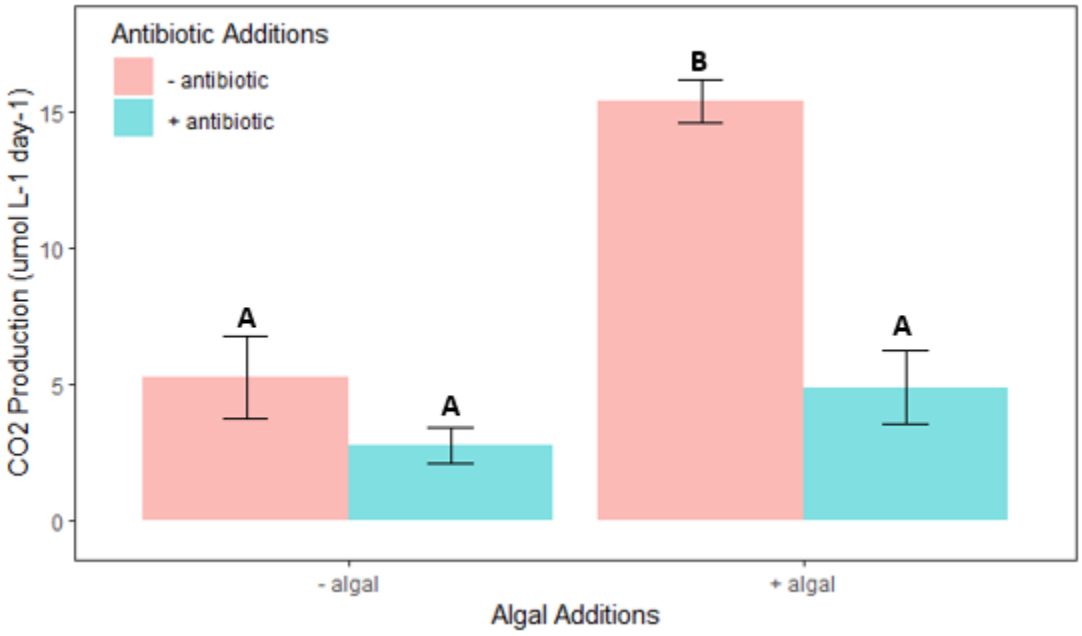

Figure 6. Carbon dioxide production rate (µmol L-1 day-1) plotted against the different treatments of antibiotics and algal additions. Both algal ($F_{16,1} = 29.52$, $p < 0.05$) and antibiotic ($F_{16,1} = 33.01$, $p < 0.05$) treatments were also significant in influencing CO2 production, as well as the interaction of the treatments was also significant ($F_{16,1} = 12.49$, $p < 0.05$). CO2 production only increased relative to the controls in the populations that had algal additions, but no antibiotic additions.

References

Bastviken, D., et al. 2003. Methane as a source of carbon and energy for lake food webs. Ecology 84: 969-981.

Beaulieu, J. J., Delsontro, T., & Downing, J. A. (2019). Eutrophication will increase methane emissions from lakes and impoundments during the 21st century. *Nature Communications, 10*(1). doi:10.1038/s41467-019-09100-5

Bertolet, B., et al. in review. Microbial predictors of methanogenesis in temperate lake sediments.

Bertolet BL, West WE, Armitage DW, and Jones SE. Organic matter supply and bacterial community composition predict methanogenesis rates in temperate lake sediments. *Limnology and Oceanography Letters.* In press.

Grasset, C., et al. 2018. Large but variable methane production in anoxic freshwater sediment upon addition of allochthonous and autochthonous organic matter. Limnology and Oceanography 63: 1488-1501.

Haukka, K., Kolmonen, E., Hyder, R., Hietala, J., Vakkilainen, K., Kairesalo, T., . . . Sivonen, K. (2006). Effect of Nutrient Loading on Bacterioplankton Community Composition in Lake Mesocosms. *Microbial Ecology, 51*(2), 137-146. doi:10.1007/s00248-005-0049-7

Liu & Whitman 2008. Metabolic, phylogenetic, and ecological diversity of the Methanogenic Archaea. Ann. N.Y. Acad. Sci. 1125: 171-189.

Solomon, Chris; Jones, Stuart; C. Weidel, Brian; Bertolet, Brittni; Bishop, Chelsea; Coloso, Jim; et al. (2018): MFE database: Data from ecosystem ecology research by Jones, Solomon, and collaborators on the ecology and biogeochemistry of lakes and lake organisms in the Upper Midwest, USA. figshare. Dataset.

University of Notre Dame. (n.d.). Lake Information // UNDERC // University of Notre Dame. Retrieved from https://underc.nd.edu/underc-east/the-environment/bathymetric-maps/

West, W., et al. 2012. Effect of algal and terrestrial carbon on methane production rates and methanogen community structure in a temperate lake sediment. Freshwater Biology 57: 949-955.

West, W., et al. 2015. Phytoplankton lipid content influences freshwater lake methanogenesis. Freshwater Biology 60: 2261-2269.

West, W., et al. 2016. Productivity and depth regulate lake contributions to atmospheric methane. Limnology and Oceanography 61: S51-S61.

Raw Data Appendix:

lakeID	treatment	algal	antibiotic	replicate	CH4Prod_umolLday	CH4Intercept_umolL	CO2Prod_umolLday	CO2Intercept_umolL
MO	control	- algal	- antibiotic	1	-3.607911483	85.72656415	2.934003518	364.7495951
MO	control	- algal	- antibiotic	2	-6.315053415	133.6851511	9.443528023	437.6434781
MO	control	- algal	- antibiotic	3	-9.361462322	199.950942	3.275394038	478.8181688
MO	control	- algal	- antibiotic	4	-4.198222451	122.9909211	8.382858565	284.5399714
MO	control	- algal	- antibiotic	5	-6.568884437	276.7972305	2.372078149	353.0058572
MO	algal	+ algal	- antibiotic	1	17.92344096	49.61030839	14.35690882	351.1328323
MO	algal	+ algal	- antibiotic	2	22.10451732	39.29221415	17.30169349	314.6181686
MO	algal	+ algal	- antibiotic	3	30.88926533	13.19642981	17.44279818	314.2297146
MO	algal	+ algal	- antibiotic	4	20.88248874	112.071288	14.22263076	384.9542867
MO	algal	+ algal	- antibiotic	5	3.612770184	134.7444775	13.75283311	407.0622701
MO	antibiotic	- algal	+ antibiotic	1	-2.828385025	153.7406778	0.325571015	352.4196514
MO	antibiotic	- algal	+ antibiotic	2	-2.910513915	138.0744259	3.291676531	221.8536522
MO	antibiotic	- algal	+ antibiotic	3	-2.257616439	146.0931374	3.224104546	245.734045
MO	antibiotic	- algal	+ antibiotic	4	-1.668322203	123.0145844	4.409907356	315.1409653
MO	antibiotic	- algal	+ antibiotic	5	-2.219777768	128.1506622	2.659508734	301.8096248
MO	algalAntibiotic	+ algal	+ antibiotic	1	-0.908826353	54.16456731	2.19940067	354.3348769
MO	algalAntibiotic	+ algal	+ antibiotic	2	-0.940042309	65.20528915	4.646230535	187.4611346
MO	algalAntibiotic	+ algal	+ antibiotic	3	-0.717436225	57.98427526	5.504856883	161.6493262
MO	algalAntibiotic	+ algal	+ antibiotic	4	-0.850264269	55.37672357	9.630131572	172.5117558
MO	algalAntibiotic	+ algal	+ antibiotic	5	-0.463968574	31.22288662	2.660913184	193.9734691

Table 1. Raw data summary table. Rates of methane and carbon dioxide production for each of the replicates for each of the treatments.

lakeID	treatment	replicate	CH4_prod	CH4_Intercept	CH4_Rsquared	CH4_pval	CO2_prod	CO2_Intercept	CO2_Rsquared	CO2_pval
MO	algal	1	17.92344096	49.61030839	0.7700136563	0.02158878631	14.35690882	351.1328323	0.7346047815	0.02917520607
MO	algal	2	22.10451732	39.29221415	0.8699628705	0.006638012629	17.30169349	314.6181686	0.6968191991	0.03870188654
MO	algal	3	30.88926533	13.19642981	0.8912362947	0.004607521596	17.44279818	314.2297146	0.7762755472	0.02037709931
MO	algal	4	20.88248874	112.071288	0.8528485332	0.008554970612	14.22263076	384.9542867	0.5827463652	0.07736056523
MO	algal	5	3.612770184	134.7444775	0.2630188029	0.2981646861	13.75283311	407.0622701	0.5745743308	0.08075540735
MO	algalAntibiotic	1	-0.9088263532	54.16456731	0.4240145402	0.1613055166	2.19940067	354.3348769	0.1673277648	0.4206375621
MO	algalAntibiotic	2	-0.940042309	65.20528915	0.2508850248	0.3115058135	4.646230535	187.4611346	0.7172389894	0.03336526826
MO	algalAntibiotic	3	-0.7174362247	57.98427526	0.178787781	0.4035491284	5.504856883	161.6493262	0.7865958393	0.01846328621
MO	algalAntibiotic	4	-0.8502642689	55.37672357	0.3415379988	0.2231807553	9.630131572	172.5117558	0.8482481315	0.009114099201
MO	algalAntibiotic	5	-0.4639685735	31.22288662	0.3116116369	0.2496414335	2.660913184	193.9734691	0.448874059	0.1453975037
MO	antibiotic	1	-2.828385025	153.7406778	0.4850574741	0.1242207133	0.3255710149	352.4196514	0.004768025843	0.8965882785
MO	antibiotic	2	-2.910513915	138.0744259	0.4939691653	0.1193439262	3.291676531	221.8536522	0.3199366496	0.2420386503
MO	antibiotic	3	-2.257616439	146.0931374	0.4716157585	0.1318250849	3.224104546	245.734045	0.6402555899	0.05591377763
MO	antibiotic	4	-1.668322203	123.0145844	0.2196061856	0.3485237284	4.409907356	315.1409653	0.3474926167	0.2181932846
MO	antibiotic	5	-2.219777768	128.1506622	0.4602447593	0.1384979891	2.659508734	301.8096248	0.3768178107	0.1948734092
MO	control	1	-3.607911483	85.72656415	0.7311662605	0.02997986843	2.934003518	364.7495951	0.08045727706	0.5859359702
MO	control	2	-6.315053415	133.6851511	0.7988908858	0.01631667451	9.443528023	437.6434781	0.270869911	0.2898098828
MO	control	3	-9.361462322	199.950942	0.7614523077	0.02330782246	3.275394038	478.8181688	0.05184818965	0.6643500044
MO	control	4	-4.198222451	122.9909211	0.2512714606	0.3110726313	8.382858565	284.5399714	0.2789188351	0.2814608731
MO	control	5	-6.568884437	276.7972305	0.7249946126	0.03145495542	2.372078149	353.0058572	0.22076953	0.3470737437

Table 2. Raw data summary table. P values and r squared values for methane and carbon dioxide production rates.