The Effect of Substrate Quality on Methane Production in Freshwater Lakes

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Abstract:

Nutrients in freshwater lakes can affect the activity of methanogens in the lake sediment. Increased eutrophication has been observed in lakes receiving run-off of phosphorus and nitrogen (Sharpley et al., 1994; Schindler et al., 2008). This leads to an environment that allows for the production of acetate and H_2/CO_2. These organic precursors can be anaerobically reduced to CH_4 by methanogens (Conrad, 1999).

We aimed to find the effect of substrate quality on methane production in freshwater lakes. We measured the rates of methane production of four lakes at the University of Notre Dame Environmental Research Center (UNDERC). Several nutrient and algal treatments were added to the sediment slurries from each of the four lakes. Every 4 to 6 days, methane concentrations were measured using gas chromatography and methane production rates were inferred. Volatile fatty acid concentration in the sediment was also measured.

We found that algal carbon, lipids, and acetate significantly increase methane production rates in freshwater lake sediment. We also observed that volatile fatty acids accumulated in sediment incubations treated with a methanogenesis inhibitor, indicating that a consortium of syntrophic bacteria are responsible for breaking down algae and lipids into more suitable substrates for methanogenesis.

Introduction:

As fossil fuel consumption causes CO_2 levels to rise, biofuels are being viewed as a way to combat climate change, (Demirbas, 2009). The increased agricultural production of biofuels, however, could have unintended consequences. The transport of excess nutrients and organic matter from agricultural fields to freshwater ecosystems is a major global concern (Edmondson

& Lehman, 1981). Excessive concentrations of nitrogen and phosphorous in bodies of water can be considered pollutants, as they cause some organisms to proliferate, which can be at the expense of others, (Davis and Masten, 2004). Increased eutrophication has been observed in lakes receiving run-off of nitrogen and phosphorus (Sharpley et al., 1994; Schindler et al., 2008). Eutrophication is characterized by proliferation of algae from surrounding agricultural watersheds, (Davis and Masten, 2004). Subsequent sinking and algal blooms provide increased nutrients in the water. This results in enhanced plant growth that can decrease dissolved oxygen content, (Pease *et al.,* 2010). The combination of these processes provides an environment that allows for fermentation and the production of acetate and $H_2/CO_{2.}$ These organic precursors can be anaerobically reduced to CH_4 by methanogens (Conrad, 1999).

Considering this information, the sinking of algae into the sediment could play a major role in the increasing methane levels by increasing CH_4 production in the sediment (West *et al.,* 2012). The process by which algae provides methanogens with acetate has multiple stages. Algae can produce high levels of lipids, which can be secreted into the surrounding environment, (Iwata, 1964). These lipids are then broken down further. In anaerobic bioreactors, complete long chain fatty acid degradation was shown to occur through the combined activity of syntrophic bacteria. These convert long chain fatty acids to acetate and hydrogen, (Schink,1997).

This experiment aimed to answer several questions regarding methanogenesis in lakes. First, we wanted to discover how carbon substrate quality affected CH_4 production rates. Beyond that, we wanted to see if lipids were inhibitory or could increase CH_4 production. We have known that volatile fatty acids can be converted into methane by methanogens, (Conrad, 1999). We were able to study this by chemically inhibiting methanogenesis and then analyzing

volatile fatty acid content in sediment.

With algae as a common component in lakes, we set out to examine substrate quality by comparing the influence of two algae species (a green algae Scenedesmus Obliquus, and a cyanobacteria, Microcystis Aeruginosa) on methanogenesis rates. In one study, 50% less methane was produced when an anaerobic digester was fed cyanobacterial biomass rather than the green macroalgae, (Zeng et al., 2010). Furthermore, we set out to examine the potential for algae growing under varying C:N ratios to effect lipid storage and subsequently how algae containing differing amounts of lipid would influence methanogenesis rates.

Finally, we examined if the effects of substrate quality were consistent across oligotrophic, mesotrophic, and eutrophic lakes. The experiment was performed using sediment collected from four different lakes located at the University of Notre Dame Environmental Research facility (UNCERC) in northern Wisconsin and Southern Michigan. The lakes at UNDERC provided an environment relatively sheltered from high levels of anthropogenic nutrient loading. The various lakes also provided several different aquatic environments. This allowed for the demonstration of the effects of labile carbon on sediment across lakes with different nutrient levels. Brown Lake, for example, has both high dissolved organic carbon (typically recalcitrant), high autochthonous carbon concentrations, and has one of the highest CH_4 sediment production rates at UNDERC. Bay lake, on the other hand, is noticeably clearer with less nutrients in the water.

Methods:

Sampling sites:

We selected four UNDERC lakes as sampling sites to collect lake sediment. These lakes included Crampton and Bay (oligotrophic), Morris (mesotrophic), and Brown (eutrophic). For

each lake, we collected sediment cores with an Ekman dredge, along with overlying water. These were returned to the lab and prepared for incubations within five hours of sampling.

Treatments, incubations, and CH_4 measurements

For each lake, sediment and water were placed into glass bottles. 50 ml of sediment and 50 ml of water were placed in each bottle, with 200 ml of headspace.

Various treatments were added to the bottles, with the control bottles receiving no additional treatment. Four replicates of each treatment were used. Treatments included 1.0% nitrogen *Scenedesmus Obliquus*, 0.03% nitrogen *Scenedesmus Obliquus,* and *Microcystis Aeruginosa.* For each bottle, 20 mg of algae were added. Other treatments included acetate (18.85 mg added in the form a 188.5 g/L solution) and Lipids (11 μL of peanut oil added). For many treatments, four additional replicates were created with an added 1.06 g of Sodium 2-bromo-ethanesulfonate (BES). BES was used as an agent to prevent methanogenesis, (Zinder *et al.,*1984).

The bottles were sealed and then flushed with nitrogen gas to ensure an anoxic environment conducive for methanogenesis. The incubations were stored in the dark at approximately 22 ^{o}C.

Beginning the first day after the sediment was obtained and bottles were sealed, the headspace of each incubation bottle was analyzed for CH_4 using a gas chromatograph with a flame-ionizing detector. This was repeated for each bottle every four to six days. A rate of CH_4 production was inferred from the slope of the CH4 concentrations measured through time.

Volatile Fatty Acid Concentration in Sediment:

Sediment and water samples were centrifuged for one hour. The water was collected from the centrifugation and analyzed for volatile fatty acids (acetate) concentrations on a Dionex IC5000.

Statistical analysis:

Regression was used to infer CH_4 production rates from the sampled concentrations. One-way ANOVA was used to compare the CH_4 production rates of the various treatments. Regressions and ANOVAs were analyzed using R (R Core Team, 2013).

Results:

CH_4 Production

Total CH_4 production varied amongst different lakes. However, the individual treatments had similar effects on each lake (Fig. 1). For each lake, significant differences were observed between the treatments (one-way ANOVA, $P<0.01$). Lipid treatment had greater CH_4 production than acetate treatment for Brown Lake, (one-way ANOVA, $P<0.05$). At Morris, the effect was opposite, and even more pronounced, as acetate resulted in greater CH_4 production, (one-way ANOVA, $P<0.01$). Bay and Crampton did not have significant differences between lipid and acetate treatment.

Morris serves as a representation of the effects that can be seen in the other lakes. (Fig. 2). Post-hoc analysis for Morris showed that each treatment, besides those with added BES, resulted in greater amounts of CH_4 production compared to the control ($P<0.01$). Acetate caused greater rates of methanogenesis than lipids, but was not statistically different than any of the algal carbon treatments. Microcystis and 0.03% N Scenedesmus produced greater amounts of CH^4 than 1.0% N Scenedesmus ($p<0.01$).

Volatile Fatty Acid Concentration in Sediment

Volatile fatty acid (VFA) concentrations in Morris Lake sediment were found to be different between treatments (Fig. 3), (one-way ANOVA, $P<0.01$). Post-hoc analysis revealed that only the 0.03%N Scenedesmus BES treatment had higher VFA from the control with a $p<0.01$. The 1.0%N Scenedesmus BES treatment differed from the control with a p-value of 0.042, showing significance at a 95% confidence interval. Both 1.0%N Scenedesmus and 0.03%N Scenedesmus had lower VFA concentrations than their respective BES treatments ($p<0.05$).

Discussion:

Nutrient and terrestrial carbon loading push lakes toward more eutrophic and humic states. (Vollenweider, 1989). Increased algal or terrestrial carbon should increase methane production rates in freshwater lakes (West et al. 2012). The goal of this experiment was to first observe the effect that different algal and nutrient treatments had on methanogenesis. Then, we wanted to discover possible mechanisms for any increase in methane production that we observed.

Our experiment showed that carbon from algae, acetate, and lipids clearly increases methanogenesis rates, (Fig. 2). We asked if substrate quality would affect methanogenesis. One study found that lipid content above 30% can have inhibitory effects on methanogenesis (Cirne et al., 2007). In our own experiment, however, both lipids and acetate increased methanogenesis. This provides a link to the effect that algae can have on methanogenesis. As algae produce and secrete lipids, the lipids can be used to increase methanogenesis.

We observed in Morris that the 0.03% N Scenedesmus (high lipid concentration)

produced more methane than the 1.0% N Scenedesmus. The lipid concentration of the 0.03% N Scenedesmus is not high enough for the inhibitory effect, so possibly the added carbon allows for more methane to be produced. As the lipid treatment illustrated, lipids can increase methanogenesis. As more lipids are produced by algae, more methane is produced.

Also, Microcystis treatment resulted in greater methane production than 1.0% N Scenedesmus, but no difference was observed with the 0.03% N Scenedesmus. Zeng *et al.,* 2010 found that 50% less methane was produced when an anaerobic digester was fed cyanobacterial biomass rather than the green macroalgae. Our results do not align with Zeng's finding. Further research, with more species of cyanobacteria and green macroalgae, will help to reveal the true difference.

The increased methanogenesis in the lipid treatment and the 0.03% N Scenedesmus treatment shows that lipids can increase methanogenesis. Whether or not methanogen use of lipids is direct or indirect is another question. Our analysis of volatile fatty acid (VFA) concentration in sediment helped to observe the effect. Methanogens can reduce VFAs to CH_4, (Conrad, 1999). Methanogenesis can be completely stopped using Sodium 2-bromo-ethanesulfonate (BES), (Zinder *et al.,*1984). We observed that after several weeks of incubation, VFA levels were higher than the control for 0.03% N Scenedesmus with BES and 1.0% N Scenedesmus with BES (Fig. 3). In fig. 3, the 0.03% N Scenedesmus appears to have a higher VFA concentration than the 1.0% N Scenedesmus, but no significant difference was observed. The 0.03% N Scenedesmus has a higher lipid concentration than the 1.0% N Scenedesmus. With more lipids to break down into to VFAs, the more VFAs would be expected. The lack of replicates for this analysis could explain to reason for not finding a significant difference

between the two. Sediment from the other lakes will be analyzed at a later date, which will give better results.

The effect of the BES treatments has interesting implications. Both algal BES treatments have significantly higher VFA concentrations (95% confidence interval) than their corresponding algal treatment without BES. BES inhibits methanogens, but the algal lipids seemingly continue to break down to VFAs, (Zinder *et al.,*1984). This indicates that methanogens have trophic relationships with other microorganisms, which are responsible for breaking down the lipids into VFAs that methanogens can use.

In this experiment, we observed that nutrients can increase methane production rates in freshwater lakes. Much more research can be done on the mechanisms for methanogenesis. During our incubations, we collected gas in containers, which will be analyzed at a later date. The gas in these containers was taken from the same bottles we used in this experiment, but a ^{13}C labeled algae was also used as a treatment. Analysis of a ^{13}C signature in the methane will tell us if algae has a priming effect on methanogenesis. This data will give us greater insight into the current questions that we ask.

Our data gives us new insights into greenhouse gas emissions. The support for biofuels has been high, but our results serve as a sign of caution for this expanding industry. As nutrient loading in agriculture continues, the increased methane emissions should not be ignored. Current agricultural methods, including nutrient loading, should be evaluated with our data taken into consideration.

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References cited:

Blagodatskaya, E., T. Yuyukina, S. Blagodatsky, Y. Kuzyakov. (2011). Three-source partitioning of microbial biomass and of CO2 efflux from soil to evaluate mechanisms of priming effects. Soil Biology and Biochemistry, 2011, Vol.43(4), pp.778-786

Cirne, D.G., Bjornsson, L., Alves, M., and Mattiasson, B. (2006) Effects of bioaugmentation by an anaerobic lipolytic bacterium on anaerobic digestion of lipid-rich waste. J.Chem. Technol.Biotechnol. 81 1745-1752.

Conrad R. (1999) Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. FEMS Microbiology Ecology, 28, 193–202.

Davis, M.L., S.J. Masten. (2004) Principles of Environmental Engineering and Science. McGraw-Hill Companies, Inc., New York, NY

Demirbas, B. 2009. Biofuels for internal combustion engines. Energy Edu. Sci. Technol. 22:117–132.

Loulergue L., Schilt A., Spahni R., Masson-Delmotte V., Blunier T., Lemieux B. et al. (2008) Orbital and millennial-scale features of atmospheric CH4 over the past 800,000 years. Nature, 453, 383–386.

Monteith D.T., Stoddard J.L., Evans C.D., de Wit H.A., Forsius M., Hogasen T. et al. (2007) Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. Nature, 450, 537–539.

Pease, Lyndon M. ; Oduor, P. ; Padmanabhan, G. Estimating sediment, nitrogen, and phosphorous loads from the Pipestem Creek watershed, North Dakota, using AnnAGNPS. Computers and Geosciences, 2010, Vol.36(3), pp.282-291

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Schink, B. (1997) Energetics of syntrophic cooperation in methanogenic degradation. Microbiol.Mol.Biol.Rev. 61 (2), 262-280.

Sharpley A.N., Chapra S.C., Wedepohl R., Sims J.T., Daniel T.C. & Reddy K.R. (1994) Managing agricultural phosphorus for protection of surface waters: issues and options. Journal of Environmental Quality, 23, 437–451.

Schindler D.W. (1974) Eutrophication and recover in experimental lakes: implications for lake management. Science, 184, 897–899

Schindler D.W., Hecky R.E., Findlay D.L., Stainton M.P., Parker B.R., Paterson M.J. et al. (2008)

Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37 year whole-ecosystem experiment. Proceedings of the National Academy of Sciences of the United States of America, 105, 11254–11258.

Vollenweider R.A. (1989) Global problems of eutrophication and its control. In: Conservation and Management of Lakes (Eds J. Salaćnki & S. Herodek). Symposium Biologica Hungarica, 38, 19–41.

West, W. E., J.J. Coloso, S.E. Jones. 2012. Effects of algal and terrestrial carbon on methane production rates and methanogen community structure in a temperate lake sediment. Freshwater Biology 57: 949–955

Zeng, X., Singh, S.R., Hou, D., Hou, S.X. (2010). Tumor suppressors Sav/scrib and oncogene ras regulate stem-cell transformation in adult Drosophila malpighian tubules. J. Cell. Physiol. 224(3): 766--774

Zinder S. H., Anguish T., Cardwell S. C. 1984. Selective inhibition by 2-bromoethanesulfonate of methanogenesis from acetate in a thermophilic anaerobic digestor. Appl. Environ. Microbiol. 47:1343–1345 [PMC free article] [PubMed]

Figures:

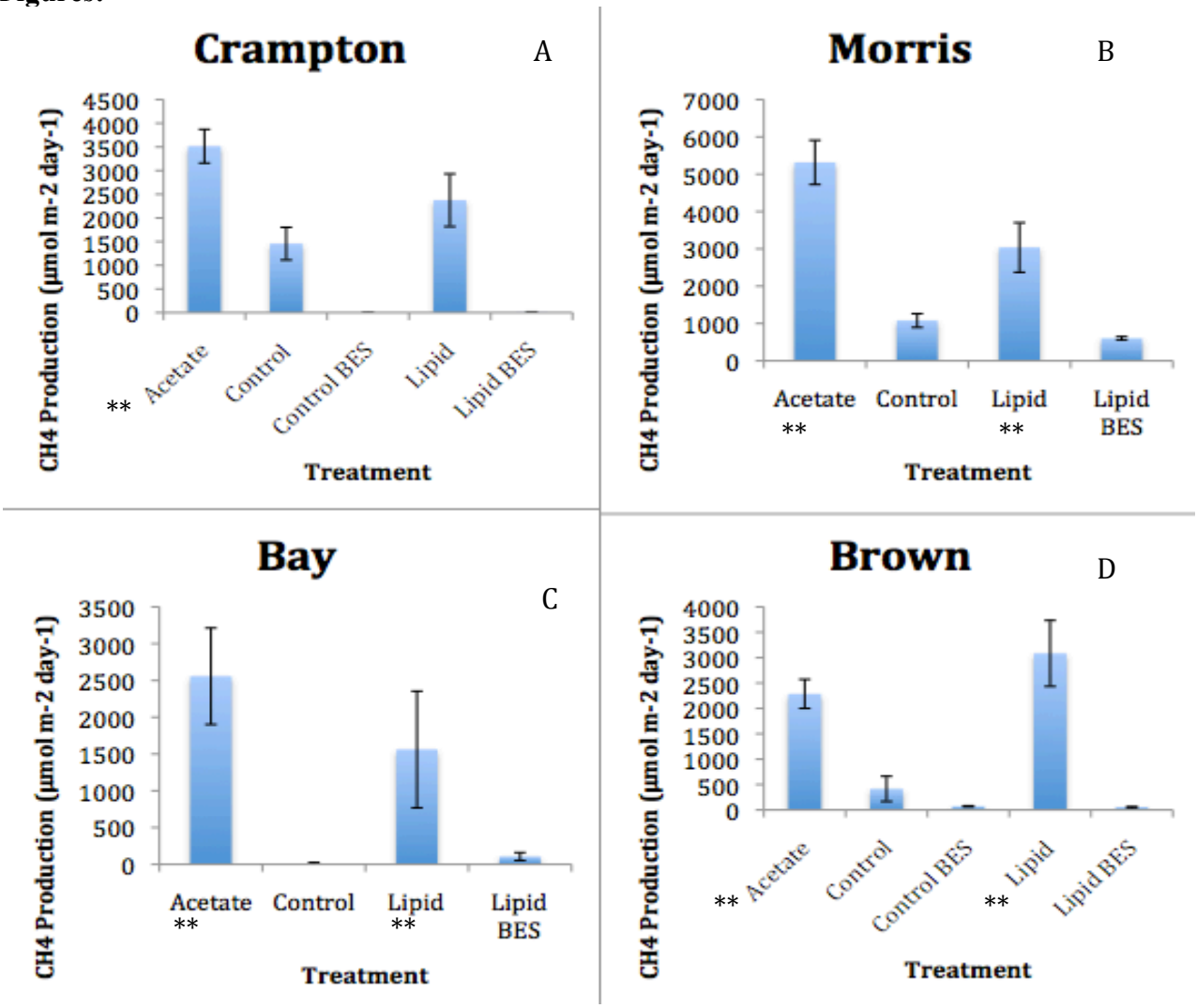

Figure 1. CH_4 production rates with added lipid and acetate for four lakes. CH_4 production rates are measured in (µmol m^{-2} day^{-1}). **A:** (One-way ANOVA, F = 24.88, P < 0.01, d.f. = 11,36). **B:** (One-way ANOVA, F = 143.7, P < 0.01, d.f. = 8,26). **C:** (One-way ANOVA, F = 17.62, P < 0.01, d.f. = 10,32). **D:** (One-way ANOVA, F = 64.84, P < 0.01, d.f. = 11,36). Error bars indicate one standard deviation. * indicates p<0.05 in post-hoc analysis between treatment and control. ** indicates p<0.01 in post-hoc analysis between treatment and control.

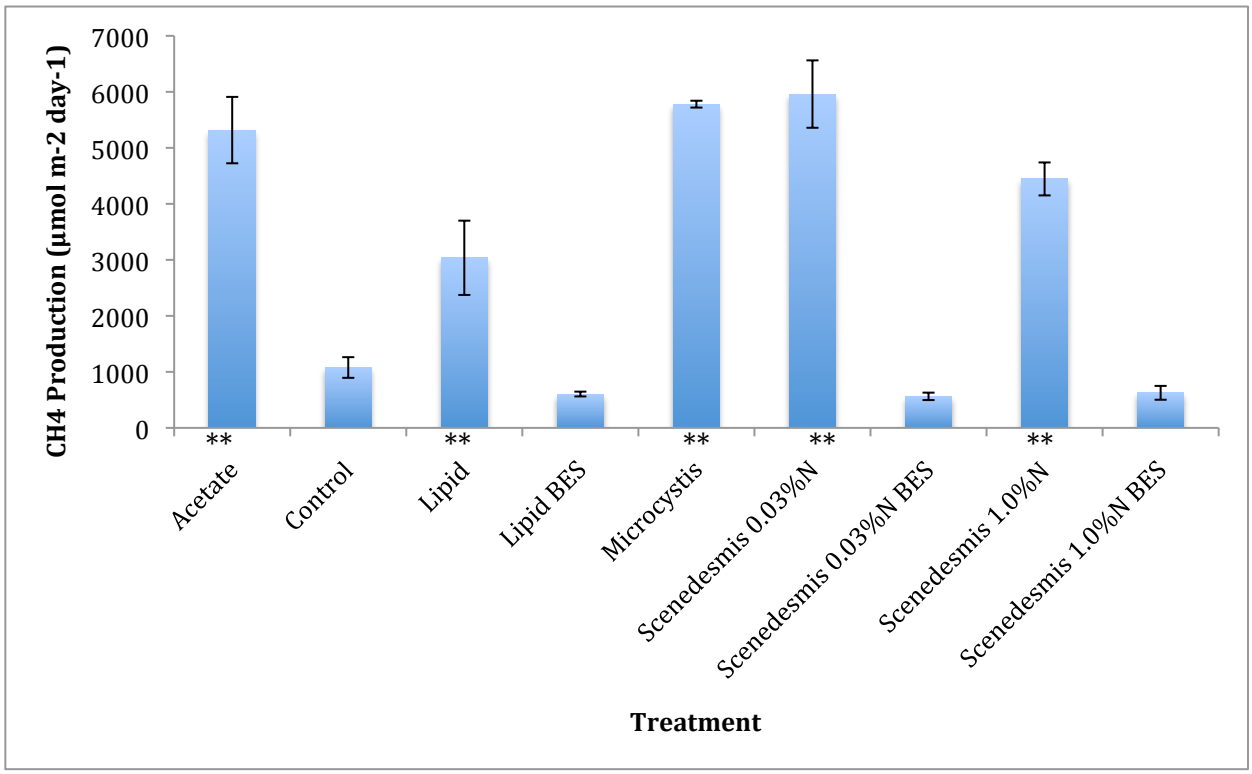

Figure 2. Morris CH_4 production rates for all treatments, including algal carbon. (One-way ANOVA, $F = 143.7$, $P < 0.01$, d.f. = 8,26). Error bars indicate one standard deviation. * indicates $p<0.05$ in post-hoc analysis between treatment and control. ** indicates $p<0.01$ in post-hoc analysis between treatment and control.

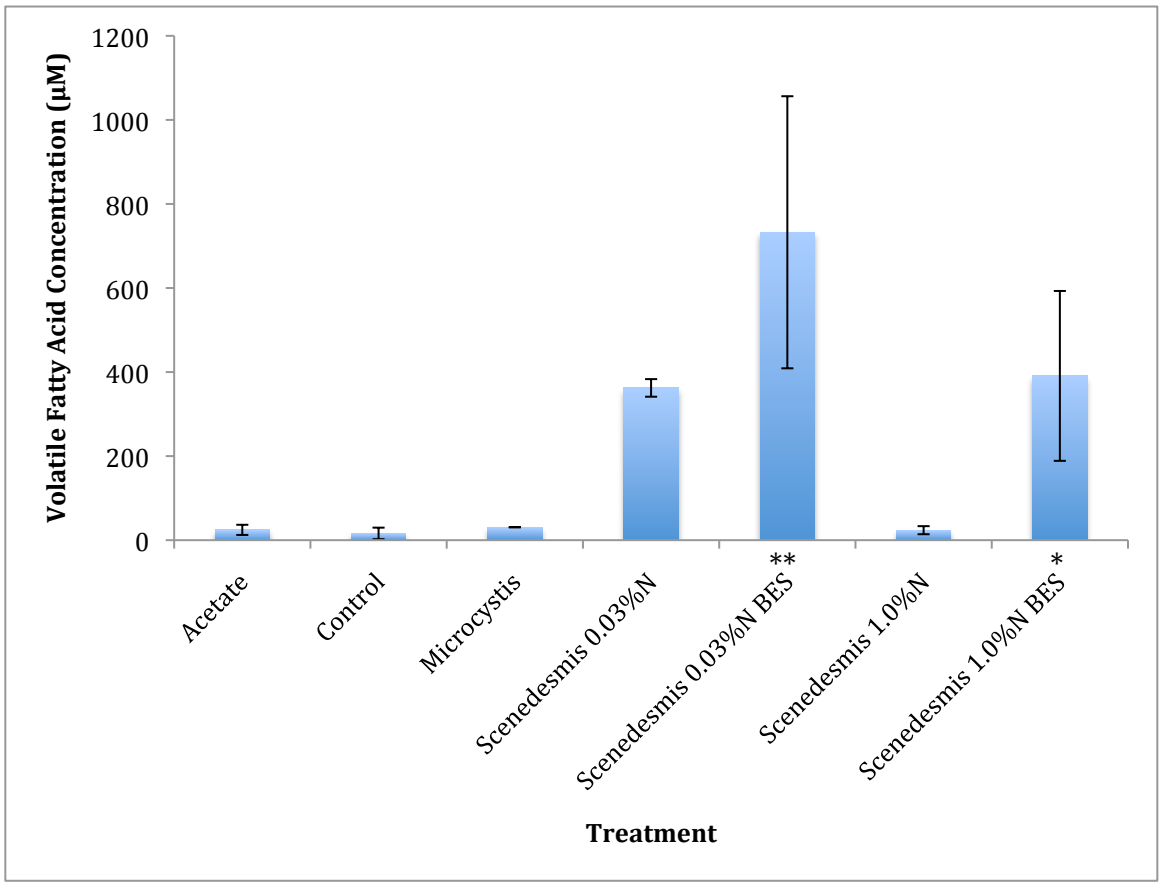

Figure 3. Concentration of volatile fatty acids for various treatments to Morris Lake sediment. Error bars indicate one standard deviation. * indicates $p<0.05$ in post-hoc analysis between treatment and control. ** indicates $p<0.01$ in post-hoc analysis between treatment and control.