Phosphorus and nitrogen effects on algal growth and subsequently methane production

BIOS 35502: Practicum in Environmental Field biology

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Abstract:

Since the industrial revolution anthropogenic greenhouse gas emissions have increased at alarming rates. Methane is a potent greenhouse gas that holds as much as 21x the solar radiation in earth's atmosphere than carbon dioxide (CO_2). Agricultural runoff into the few freshwater ecosystems may be contributing more methane gases into our atmosphere then the world's entire oceanic system. The nutrient rich soils that end up in aquatic systems increase algal production which is then used by methanogens and turned into methane in lake sediments. We created twelve mesocosms, six were left as controls and six were amended with nitrogen and phosphorus then methane production was measured in the limnocorral sediments weekly. Our results were inconclusive possibly due to time constraints.

Introduction:

Methane (CH_4) is a greenhouse gas 21x more effective at trapping heat in the atmosphere than carbon dioxide (CO_2). Greenhouse gases hold solar radiation that has reached earth's surface and is reflected back into the atmosphere. This is necessary for earth's life harboring climate. Greenhouse gases keep the temperatures on earth livable at 15℃ - 33℃ higher than it would be without these gases. This is no doubt a benefit which has enabled animal and plant life to thrive. It was not until the industrial revolution that anthropogenic greenhouse gas emissions increased significantly (Anderson *et al.* 2010). Since the 1970's warming has occurred at about 2℃ annually also rain and snow fall runoff declined 50% from the mid 1970's into the 1980's (Schindler *et al.* 1996). Ice core records show that the 650,000 years prior to the industrial revolution CH_4 concentrations ranged from 350 to 800 parts per billion and post industrial revolution atmospheric CH_4 concentrations are as high as 1774 parts per billion (Loulergue *et al.* 2008). Freshwater lakes cover a mere 0.9% of the earth's surface (Downing *et al.* 2006) but could account for 6-16% of the overall CH_4 cycles (Bastviken *et al.* 2004) but at this point are not included in the global greenhouse gas emission budget. Since freshwater sources are not being accounted for in the CH_4 budget, and anthropogenic sources may be driving increases in greenhouse gases from freshwater ecosystems; lakes should be included as a source of CH_4 in the global budget (West *et al.* 2012).

Specific anthropogenic sources are deforestation, the burning fossil fuels and may be a result of agricultural practices resulting in drainage of nitrogen (N) and phosphorus (P) into aquatic ecosystems. It is necessary that farmers have fertile soil as well as water in order to produce the quantity and quality of products in which to make suitable profits. This fertile soil must contain N and P , these nutrients occur naturally until crops are produced on the same plot of land annually then these nutrients become depleted and must be artificially replaced. The nutrients can be replaced with natural animal fertilizers or synthetic fertilizers both have high concentrations of N and P which enable the producers to produce a large quantity of their selected crop. Precipitation, eolian and other natural processes move these nutrient rich soils into the near bodies of freshwater causing increased primary productivity called eutrophication (Vitousek *et al.* 1997). And much like on land the nutrient rich soils enable abundant production of crops. In the bodies of fresh water this addition of concentrated nutrients allows for abundant algal production. Upon death the algal blooms secrete labile carbon such as acetate and other volatile fatty acids (citation). This fermented labile carbon becomes H_2 and CO_2 which can be utilized by methanogens, anaerobic archaea (Borrel *et al.* 2011).

Since the industrial revolution the world's population has grown at exponential rates due to advanced medical practices, the ability to transport goods far distances in short periods and our ability to produce food on a mass scale. In terms of mass food production agriculture has expanded as to meet the needs of the multitudes people. Recently, the EPA approved the use of E15 in motor vehicles. Current estimates of ethanol production in the U.S. is approximately 14 billion gallons per year (Bringezu et al. 2009). However, with the passing of the Energy Independence Act and Security Act of 2007, the United States has set production goals of 35 billion gallons of corn-based ethanol by 2020 (Osborne, 2007). In light of increased agricultural based ethanol production; understanding the relationship between nutrient runoff into aquatic ecosystems and methane emissions from lakes has become quite apparent.

Our goal is to determine the relationship between methane production, nutrient loading, and subsequent eutrophication in lakes. More specifically we will look to see if methane production and emissions are best predicted by phosphorus concentrations and algal abundance in lakes. We added liquid fertilizer to induce algal growth within in lake mesocosms to determine if this relationship exists. We predict that methane production and emissions will increase as a result of higher phosphorous and subsequent algal biomass available to methanogens in lakes.

Methods:

Sampling

Twelve limnocorrals were built and placed in Morris Lake on the University of Notre Dame Environmental Science Research Center (UNDERC) property in the Upper Peninsula of Michigan (46◦15' 22" N 89◦31' 16" W) in the beginning of June 2012. Each limnocorral had a depth of approximately 3.5 to 4 meters and were 1 m x 1 m square columns. The limnocorrals were made up of PVC pipe structures wrapped in plastic sheeting. Each one was held in place with cement cinder blocks and styrofoam noodles placed around the top perimeter to insure isolation.

Once in place there were six unenriched control corrals, and six with nutrients (N and P) added. The nutrients added equaled 10 ml P per liter of water for the nutrient modified corrals. The nutrients were added to a final concentration of 10 mg/L P and a final ratio of 25 to 1 N:P by weight. Each corral was sampled weekly for six weeks. Measurements of temperature, dissolved oxygen (DO), acidity (pH) were taken with a YSI and visibility was taken with a Secki Disk each week. With a Van Dorn we took samples of the hypolimnion layer and sediments were taken with an Ekman dredge from each corral each week.

Subsamples from the sediment samples collected from each limnocorral were taken, 25 ml of the sediment were injected with 25 ml of hypolimnion water into a glass bottle with 69 ml

of headspace flushed to anoxia with nitrogen (N_2) gas and refrigerated at 4℃ simulating the hypolimnion layer for 22 to 24 hours. After 24 hours the sediment CH_4 production samples were used to take 10 ml of air out and insert the 10 ml into a GC vial. The 10 ml of air that was taken out of the sediment bottle has to be replaced and 10 ml of air is inserted to the bottles and then they are put back in the fridge for another 3 days, these are day four samples and we take the 10 ml of air from within the bottle and inject them into the GC vial to be analyzed by the GC and then we are done with that weeks sediment CH_4 production sample.

Gas Chromatograph

The samples were taken back to the Hank Laboratory where a Agilent 6890 Gas Chromatograph (GC) equipped with a flame ionization detector and a thermal conductivity detector (TCD) was used to analyze the sediment samples for CH_4 and CO_2, respectively. (Agilent Technologies 2000)

Statistical Analysis

The methane production rate was calculated in excel by way of total sediment collection volume, hours lapsed, subsample volume, and a dilution factor between day one and day four samples and square root normalized. A simple one-way unpaired t-test was used to determine if CH4 production was significantly different in the nutrient amended limnocorrals.

Results:

We ran two simple unpaired t-test on the last two weeks of CH_4 production data to compare nutrient amended limnocorral samples with unamended samples. Week three's CH_4 production samples taken on 30 June 2012 showed no significant CH_4 production in the sediments ($p=0.6968$). Week four's CH_4 production samples taken on 6 July 2012 also showed

no significant CH_4 production in the sediments (p=0.3923).

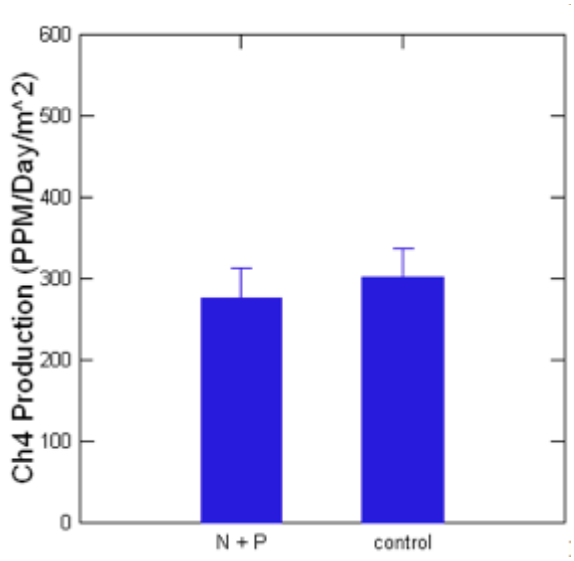

Fig.1: Mean CH_4 production rates in part per million from mesocosm sediments in Morris Lake from the last two weeks of data collection; enriched with nitrogen and phosphorus compared to unamended limnocorrals. Standard deviation error bars.

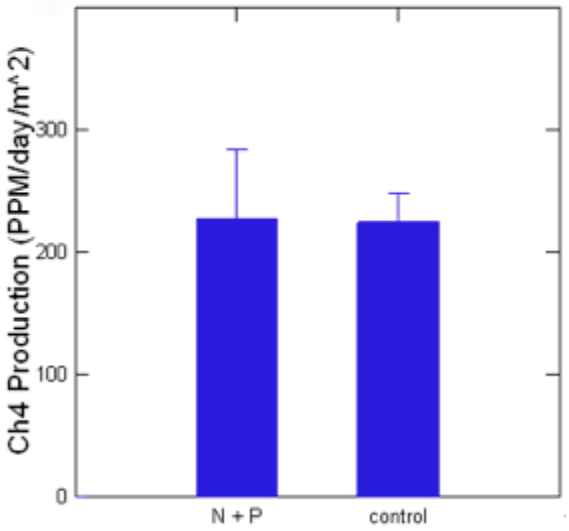

Fig. 2: Mean CH_4 production rates in parts per million from mesocosm sediments in Morris Lake from week three of data collection; enriched with N+P compared to unamended limnocorrals(p=0.6968). Standard error bars.

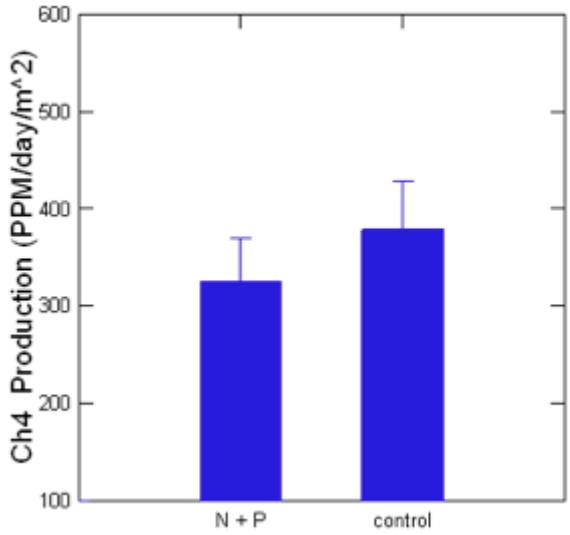

Fig.3: Mean CH_4 production rates in parts per million from mesocosm sediments in Morris Lake from week four of data collection; enriched with N+P compared to unamended limnocorrals(p=0.3923). Standard error bars.

Discussion:

The purpose of this study was to determine if nutrient runoff into lakes enhances CH_4 production. Aquatic ecosystems enriched with N + P are thought to increase CH_4 production by way of increased algal biomass secreting labile carbon that is reduced in the sediments by anaerobic archaea into CH_4 (Hanson and Hanson 1996). Our results show very little difference in CH_4 production between the control limnocorrals and nutrient enriched limnocorrals (figure 1). Between week three (figure 2) and four (figure 3) there are some interesting comparisons that were observed in the results. Week three showed little difference in CH_4 production between the control and enriched limnocorrals. We found that in week four there was slightly more CH_4 production in the control limnocorrals then the nutrient enriched limnocorrals. In addition from week three to week four we found an overall CH_4 parts per million increase of about 100 ppm.

In order for significant amounts of CH_4 to be produced a significant amount of algae must be built up through a turnover process. This turnover process includes an abundance of algal mass formed at the surface of the water limiting the light reaching to the layers of algae underneath the surface. The layers of algae underneath the surface layer die due to lack of the

ability to photosynthesize. New algal blooms form near the surface where there is an abundance of light, subsequently the first surface layer of algal blooms sink and a new layer of algae forms at the surface. Our assumption is that four weeks was not enough time to produce a cycle of algal turnover. Without the algal production cycle CH_4 production will continue to be stagnant across mesocosms.

Although our results were inconclusive, previous studies such as West *et al.* 2012 have proven that an abundance of algal biomass increases CH_4 production. Once the algal biomass dies the organic algal carbon sinks to the sediments where methanogenesis takes place and archaea produce methane. Methanogenesis is an important process to understand fully, to realize the overall greenhouse gas budget. Methane emissions from aquatic ecosystems will be properly understood when CH_4 production in freshwater sediments is fully understood.

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