Methanogenesis rates in acetate and nitrate amended anoxic slurries

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Abstract

With increasing urbanization and land use changes, pollution of lakes and wetland ecosystems is imminent. Any influx of nutrients, anthropogenic or natural, can have dramatic effects on lake gas production and flux. However, the net effect of simultaneous increase of both acetate and nitrate is unknown. Methane (CH_4) production was measured in anoxic sediment and water slurries amended with ammonium nitrate (NH_4NO_3), which has been shown to inhibit methanogenesis, and sodium acetate (CH_3COONa or NaOAc), which is known to increase methanogenesis. The addition of acetate significantly increased the methanogenesis rate, but the nitrate amendment had no significant effect. The simultaneous amendment of both acetate and nitrate showed no significant increase in CH_4 compared to the control, indicating that the presence of nitrate may have reduced the effect of acetate amendment.

Introduction

Methane, a greenhouse gas associated with global warming, continues to increase in concentration in our atmosphere. Global yearly flux of methane into the atmosphere is 566 teragrams of CH_4 per year, which is more than double pre-industrial yearly flux (Solomon *et al.* 2007). Increasing urbanization and land-use changes contribute significantly to increased gas levels (Anderson *et al.* 2010, Vitousek 1994). Nutrients travel from anthropogenic sources such as wastewater treatment facilities, landfills, and agricultural plots into nearby lakes, rivers, and wetlands, causing increased primary productivity in a process known as eutrophication (Vitousek *et al.* 1997). The increased nutrients and productivity lead to toxic algal blooms that create products such as acetate, H_2, and CO_2; a nutrient-rich anoxic environment suitable for anaerobic bacteria to produce unnaturally high levels of methane and other greenhouse gases (Davis and Koop 2006, West unpublished data). Yet, despite the imminent implications of increasing lake CH_4 levels, the US Environmental Protection Agency does not have enough information on methanogenesis in lakes to accurately estimate global lake methane production (Anderson *et al.* 2010).

It is vitally important to understand the impact of nutrient influx in lakes, which often occurs due to land-use change within a lake's watershed. Vitousek (1994) describes land-use

change as anthropogenic utilization or contamination of once-pristine land, often leading to reduced biodiversity and ecosystem pollution via habitat destruction and nutrient deposition. Algal blooms are increased due to an influx of limiting nutrients nitrogen (N) and phosphorus (P), however blooms overwhelm the limited light and oxygen supply, causing mass algal death and decomposition (Davis and Koop 2006). The algal matter, rich in labile autochthonous carbon, is quickly broken down through anaerobic digestion into H_2, CO_2, and acetate (CH_3COONa), the reactants in the biochemical processes of methanogenesis:

$$H_2 + CO_2 \rightarrow CH_4$$

$$H_2 + CH_3COONa \rightarrow CH_4 + CO_2$$

The digested algal matter and unused N and P, both products of eutrophication, provide the benthic anaerobic microbes, both methanogens and denitrifiers, with rich resources for maximal gas production.

Studies have shown that amendments of acetate, the byproduct of decaying algae and the direct precursor to methane, will significantly increase methanogenesis in wetlands and rice paddies (Baresi *et al.* 1978, Conrad 2002). Conflictingly, nitrate (NO_3^-), the precursor to algal growth, is found to inhibit methanogenesis by increasing denitrification levels (Kluber and Conrad 1998). However, there are few experiments examining methanogenesis rates in the presence of both of the nutrient nitrate, and the algal decomposition byproduct, acetate.

In this study, we sought to determine (1) how acetate and nitrate amendments independently affect the rate of methanogenesis in anoxic slurries of lake sediment and (2) the net effect on methanogenesis rates due to amendment of both acetate and nitrate. To do this, slurries were prepared from anoxic sediment and overlying water from Morris Lake in the Upper Peninsula of Michigan. Sodium acetate (CH_3COONa) and ammonium nitrate (NH_4NO_3)

solutions were amended to the slurries, which were incubated at 14 ºC for five days. Daily methane concentration was measured by gas chromatography (GC) and average daily methanogenesis rates were inferred.

Materials and Methods

Sample Site

Morris Lake is a stratified lake located in the Upper Peninsula of Michigan at the University of Notre Dame Environmental Research Center (46.258 ºN 89.521 ºW). Morris Lake is representative of a lake unaffected by land use, urbanization, and anthropogenic nutrient deposition. Throughout the summer, the Morris Lake was profiled at 6 meters for Temperature=5.83 ºC, pH= 6.89, Dissolved Oxygen (DO)=0.01 mg/L, and undetectable sulfide levels (Figure 1). In late June, three replicate hypolimnion (6 m) water and benthic sediment (~6.5 m) samples were collected with a 1 liter Van Dorn and an Eckman Dredge, respectively, and brought back to the lab.

Slurry Preparation and Incubation

Upon arrival at the lab, both the sediment and hypolimnion water were homogenized. We then added 25 ml of sediment and 45 ml of water to 125 ml serum bottles. The nitrate treatment slurries were then amended with ammonium nitrate for a final concentration of 1.422 mM nitrogen. Sodium acetate was added to the acetate treatment slurries for a final carbon concentration of 18.96 mM. Both NH_4NO_3 and CH_3COONa were added in their respective concentratons to the nitrate + acetate treatment for a final concentration of 1.422 mM N and 18.96 mM C. The control and each of the three treatments were done in triplicate and shaken vigorously to distribute the nutrients and suspend the sediment. Each bottle was sealed and

purged with ultra-pure helium for five minutes and incubated in a dark refrigerator at 14 ºC to recreate cold, dark, anoxic conditions of the bottom of the lake.

After the first twenty-four hours of incubation, the slurries were shaken to resuspend the sediment and evenly distribute the gas. We then took a 5 ml gas sample from the headspace of each serum bottle and transferred it into a helium-purged serum bottle. Thereafter, 5 ml of ultra-pure helium was replaced in each incubation bottle to maintain gas volume. Subsequently, gas samples were extracted every 24 hours for five days (120 hours) after the preparation of the slurry.

Gas Chromatography

The samples were measured on a Agilent 6890 gas chromatograph equipped with a flare ionizing detector, using a GS carbon plot column with a length, diameter, and filter size of 30 mm, 0.32 mm, and 3.0µm respectively. The GC yielded values in CH_4 ppmv for each daily gas sample.

Statistical Analysis

Using the proportion of 5 ml daily gas sample:51 ml headspace, I inferred the change in daily methane concentration for each slurry in order to determine the gross daily methane production for each treatment . I then computed the slope of the daily concentration to determine the average methanogenesis rate for each treatment over five days (Figure 2).

I used SYSTAT biostatistical analysis software (SYSTAT Software, Inc. 2009) and an $\alpha = 0.05$ for all statistical tests. A Shaprio-Wilk normality test confirmed that the data was normally distributed ($p=0.58$). I then ran a one-way analysis of variance with four treatments: control, nitrate amendment, acetate amendment, and nitrate + acetate amendment against the continuous dependent variable of average daily CH_4 production rate ($\mu g\ ml^{-1}\ day^{-1}$).

Results

ANOVA showed a significant difference between treatments (One-way ANOVA, $F_{3,8}$=4.868, p=0.0327). Tukey's Honestly Significant Different Test revealed that the acetate treatment, with an average methanogenesis rate of 0.109 ± 0.0028 µg CH_4 ml^{-1} day^{-1}, was significantly higher than the control treatment, with an average daily CH_4 production rate of 0.062 ± 0.0094 µg CH_4 ml^{-1} day^{-1} (Tukey's HSD, df=3, 8, p=0.025, Figure 3). Both the nitrate treatment methanogenesis rate of 0.079 ± 0.0073 µg CH_4 ml^{-1} day^{-1} and the nitrate + acetate treatment rate of 0.068 ± 0.0178 µg CH_4 ml^{-1} day^{-1} were not significantly different from the control incubation or the acetate treatment (Figure 3).

Drying (60 ºC, 5 days) and ashing (450 ºC, 4 hours) of 5 ml of each slurry revealed the sediment to be consistently 55.0 ± 0.002 % inorganic material and 45% organic material.

Discussion

In this study, we wanted to determine the effect of both acetate and nitrate amendments on methanogenesis and subsequently, the effect of their simultaneous addition. The addition of acetate significantly increased CH_4 production rates over the five day incubation. This is consistent with previous studies that indicate that increased concentrations of acetate are utilized by methanogens to produce methane as a byproduct (Zeikus *et al.* 1975). Previous studies have shown that methanogens may also be cation limited (Basiliko and Yavitt 2001) and the presence of the sodium cation (Na+) dissociating from acetate (CH_3COO^-) may further lead to the significant increase in methanogenesis rate in the sodium acetate amended slurry.

The amendment of ammonium nitrate showed no significant increase in methanogenesis compared to the control slurries. Many sources have suggested that NO, N_2O, nitrate, and other

NO_x^- amendments indirectly decrease methanogenesis rates through increased denitrification and reduction of intermediates formed during methanogenesis (Banihani *et al.* 2009, Clarens *et al.* 1998, Kluber and Conrad 1997). Despite these claims, the ammonium nitrate amended slurry showed no significant inhibitory effects compared to the control slurry, in fact, the daily methanogenesis rates were $0.062 \pm .010$ and $0.079 \pm .007$ μg ml^{-1} day^{-1} for the control and nitrate slurries, respectively.

The methanogenesis rates observed in the combined acetate and nitrate amended slurries were not significantly different from either the control or the nitrate amended slurries. This indicates that the significantly increased CH_4 production that we found in acetate slurries was negated by the added presence of nitrate. This effect may be explained by the electron tower theory, which suggests that there is a hierarchy, or tower, of certain chemical reactions and microbial processes which are more favorable in the presence of certain ions (Jorgensen 1989). According to B.B. Jorgensen's (1989) summary of the electron tower theory, in the presence of both methane precursors and nitrogen reactants, nitrate (NO_3^-) reduction is more energetically favorable than carbon dioxide reduction to methane; plainly, denitrification may outcompete methanogenesis in the presence of various nitrogen, nitrate, nitrite, and ammonium ions (Kluber and Conrad 1998). This theory of increased denitrification may explain why, in the presence of both acetate and nitrate, methanogenesis rates are decreased from their elevated acetate slurry rate and are more consistent with the rate of the slurries with only nitrogen amendments.

In future studies, it would be useful to utilize GC and high pressure liquid chromatography (HPLC) to obtain not only the methane, but the CO_2, N_2O, and VFA (volatile fatty acids, precursors to methanogenesis) values for each slurry to get a holistic picture of all microbial processes, including denitrification, occurring in the benthic sediment. With better

knowledge of in-vitro microbial interactions, we can better predict lake benthic gas production; however the EPA's current lack of in-vivo data may call for large-scale eutrophication experiments of lakes and wetlands to accurately assess the extensive ecosystem effects of nutrient input.

Increased eutrophication and subsequent algal growth lead to the production of acetate, which alone can increase methanogenesis up to almost double its regular levels. To minimize this unnatural production of methane and other greenhouse gases, nutrient deposition and its frequent precursor, land use change, need to be minimized. This process seems best addressed through watchful watershed management and responsible deposition of waste and fertilizer far from waterways. We must protect our lakes and wetlands vigilantly in order to preserve their pristine nature and reduce exorbitant flux of harmful greenhouse gases into the atmosphere.

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Figures

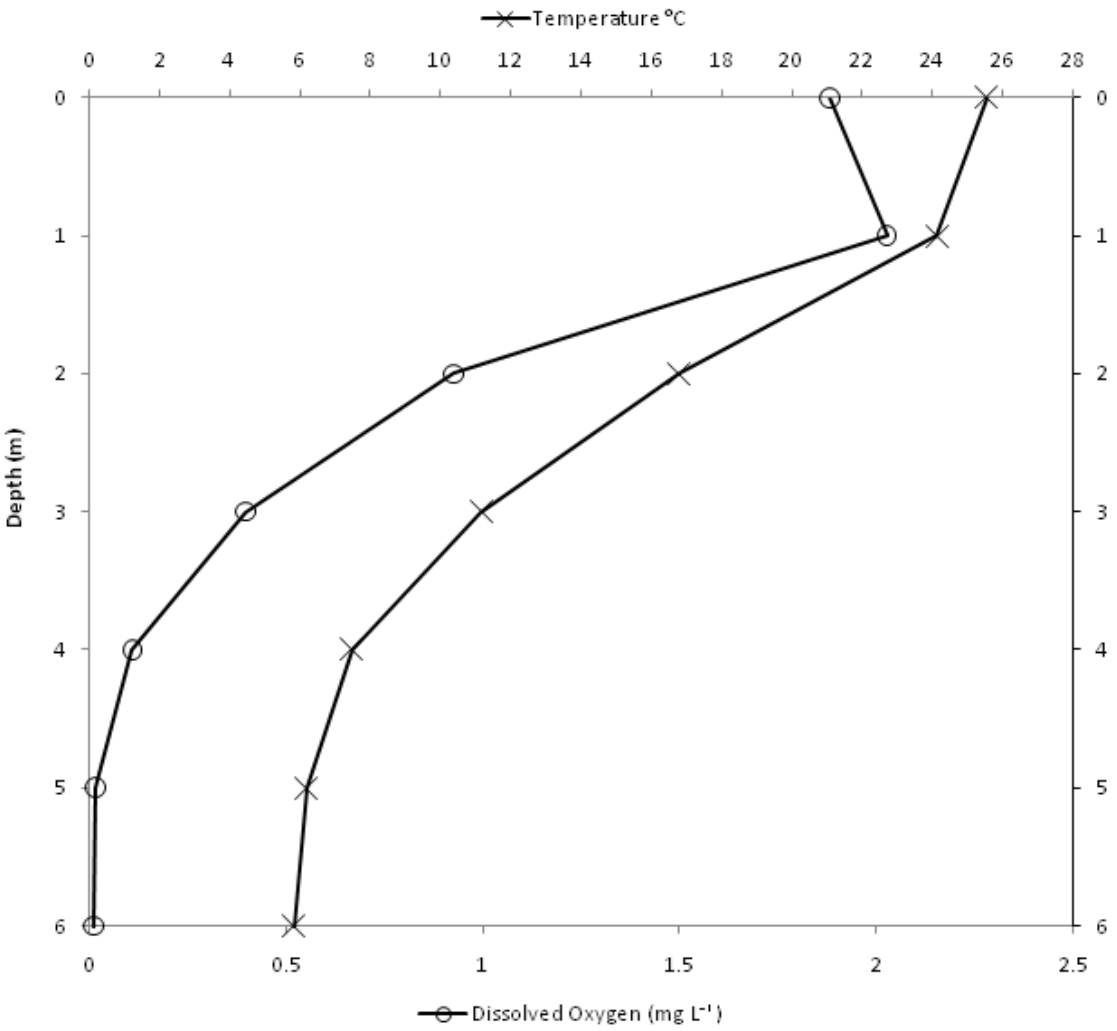

Figure 1. Morris Lake depth profile of temperature (°C), and dissolved oxygen (mg L^{-1}).

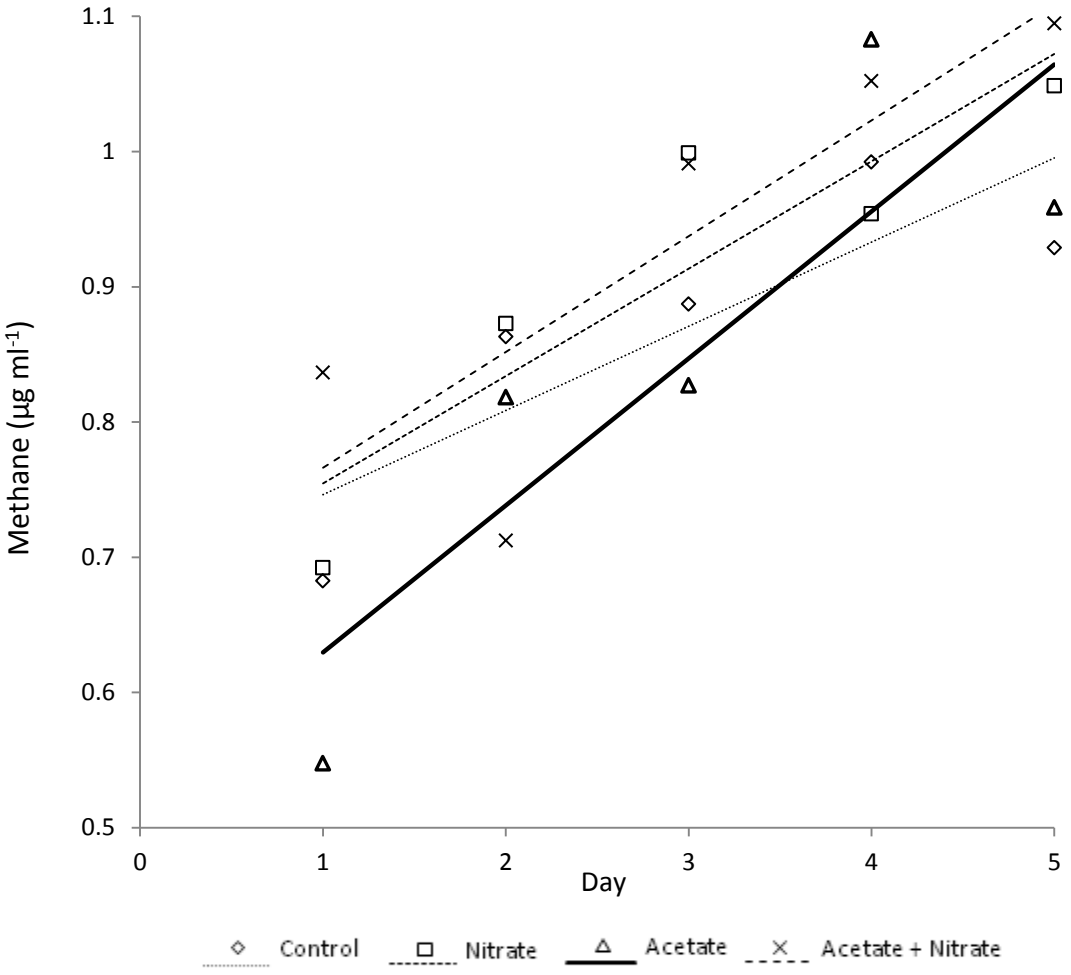

Figure 2. Daily methane concentration and linear trendline indicating CH_4 production rate in µg ml^{-1} day^{-1} for each treatment. All series show linear daily increase in methane production. Acetate amended slurries (triangle markers, solid trendline) have the steepest slope, indicating highest daily methanogenesis rate, 0.1087 µg ml^{-1} day^{-1}.

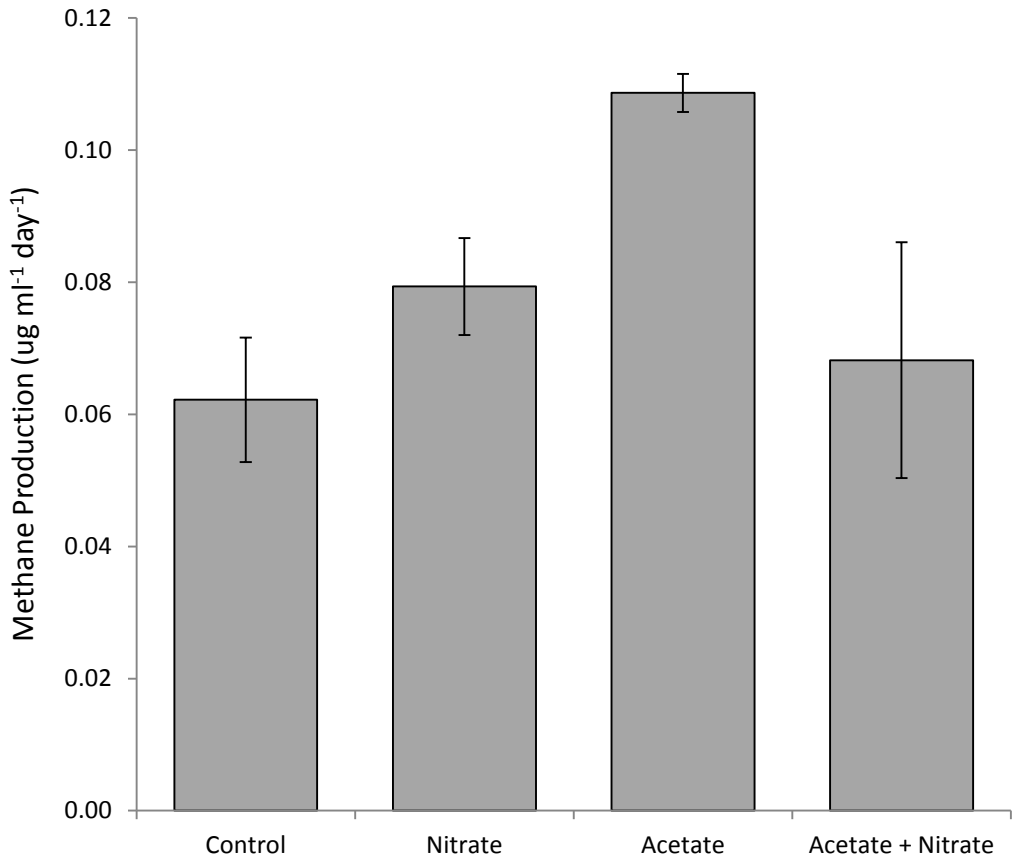

Figure 3. Barplot of mean daily CH_4 production rates and standard error of the mean in µg ml^{-1} day^{-1} for the five day incubation. Methane production rate in sodium acetate amended slurries is significantly higher than in control slurries. There is no significant difference in methanogenesis rates between control, ammonium nitrate, and sodium acetate + ammonium nitrate slurries. (One-way ANOVA, F=4.868, p=0.0327, df=3, 8, post-hoc: Tukey's HSD).