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Methylated mercury (MeHg) is produced by sulfate reducing bacteria (SRB), iron reducing bacteria (FeRB), methane producing Archaea (MPA), and other microbes under anaerobic conditions. These microbial groups compete for substrates including hydrogen and acetate. SRB are able to outcompete other anaerobic microbes when sulfate is in excess. However, low concentrations of sulfate in streams are thought to reduce the metabolic importance of SRB in streams. Although SRB are regarded as the primary producers of MeHg in many aquatic environments, it may not be universal. MPA have been implicated as the primary producers of MeHg in periphyton in a Canadian fluvial lake. Suppression of methanogenesis by SRB and the potential contributions from SRB, MPA and other MeHg producing microbes (including FeRB) to the production of MeHg in stream sediments was evaluated. Lower methanogenesis rates occurred if SRB were not inhibited, but methane production significantly increased if SRB were inhibited. These data suggest SRB reduce methane production, and are potentially the primary producers of MeHg. Other MeHg producing microbes (i.e., FeRB) contributed less than SRB to MeHg production. MPA produced MeHg in negligible amounts. This suggests that Hg methylation in sediments examined in this study was mediated primarily by SRB. Availability of sulfate and electron acceptors likely determines the relative importance of these microbial groups and thus pathways of Hg methylation in natural stream conditions.

METHANE AND METHYLMERCURY PRODUCTION POTENTIALS IN NORTH CAROLINA PIEDMONT STREAM SEDIMENTS

by

Peter Wilson Blum, IV

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Approved by

_____
Committee Co-Chair

_____
Committee Co-Chair

To my family and friends who have continuously loved and supported me in all of my endeavors.

APPROVAL PAGE

This thesis written by Peter Wilson Blum, IV, has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

Committee Co-Chair _____

Anne E. Hershey

Committee Co-Chair _____

Martin Tsz-Ki Tsui

Committee Member_____

Parke A. Rublee

_____

Date of Acceptance by Committee

_____

Date of Final Oral Examination

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CHAPTER I

INTRODUCTION

Although streams are oxygenated systems, anaerobic metabolism is an important form of respiration in the hyporheic zone (Grimm and Fisher 1984; Muholland et al., 1997). The heterogeneity of stream sediments provides areas of low flow with small pore spaces and accumulations of organic matter (Nogaro et al., 2010). These characteristics create a 'mosaic' of anaerobic microsites in stream sediments (Baker et al., 1999). Aerobic biofilms can support microsites for anaerobic metabolism (Fenchel and Finlay 1995). Additionally, some anaerobes such as sulfate reducing bacteria (SRB) have adaptations to withstand oxic conditions (Cypionka 2000). Sulfate reduction and methanogenesis are important anaerobic biogeochemical processes in stream sediments (Baker et al., 2000). Streams can be 'sulfate sinks' and significant sources of methane (CH_4) (Hlavacova et al., 2005). Variations in temperature and availability of electron donors and acceptors drive the relative importance of anaerobic pathways in stream sediments (Takii and Fukui 1991).

Methane producing Archaea (MPA) are obligate CH_4 producers and use methanogenesis for most, if not all energy production (Liu and Whitman 2008). In freshwater ecosystems, methanogenesis is generally the primary anaerobic metabolic process for the decomposition of organic matter (Maerki et al., 2009). The majority of CH_4 production in freshwater is from acetoclastic and hydrogenotrophic methanogenesis.

The acetoclastic pathway utilizes acetate and is responsible for approximately 70% of freshwater methanogenesis (Whiticar 1999). In contrast, the hydrogenotrophic pathway uses hydrogen and carbon dioxide substrates, and accounts for approximately 30% of freshwater methanogenesis (Conrad 1999). Hydrogenotrophs are less active at cold temperatures (Shultz and Conrad 1996) and in acidic conditions (Phelps and Zeikus 1984). Additionally, availability of H_2 relative to acetate influences the importance of the two pathways in MPA communities (Liu and Whitman et al., 2008).

The production of CH_4 by MPA may have important implications on food webs and climate change. CH_4 derived carbon is known to provide carbon subsidies to food webs (Jones and Grey 2011). This is also important in some lotic systems, as stream macroinvertebrates are known to utilize CH_4 derived carbon in their diet (Trimmer et al., 2009). Further, 74% of CH_4 production is mediated by MPA (Whitman et al., 2006). Because CH_4 is a potent greenhouse gas, methanogenic activity in streams is potentially consequential to global climate change (Hedderich and Whitman 2006).

The competition for substrate and electron donor availability influences the ecology of anaerobic microbes. Interactions between SRB and MPA are driven by sulfate availability. SRB are known to outcompete MPA for H_2 and acetate when sulfate is in excess (Stams et al., 2003). Additionally, other anaerobic microbes compete with SRB and MPA for resources, including iron-reducing bacteria (FeRB) and acetogens (Muyzer and Stams 2008). FeRB may compete with SRB in environments rich in Fe(III) (Lovley and Philips 1986) and compete with MPA for acetate and H_2 (Roden and Wetzel 2003).

In natural freshwater systems sulfate is found at low concentrations (100 - 200 µM) (Capone and Kiene 1998) and is commonly thought to restrict SRB growth (Whitman et al., 2006). Low sulfate concentrations in streams may promote methanogenic conditions as MPA and are released from competition with SRB (Liu and Whitman 2008). Additionally, SRB are reliant on H_2 and propionate when concentrations of sulfate are low (Muyzer and Stams 2008). Under low sulfate conditions, competition between MPA and SRB may occur for hydrogen, but not for acetate (Muyzer and Stams 2008). This gives merit to the documentation of SRB outcompeting MPA at low sulfate concentrations in freshwater systems (Lovley and Klug 1983).

The concentration at which sulfate limits SRB growth is unclear. Models have predicted that methanogenesis suppression can occur at sulfate concentrations above 30 µM (Lovley and Klug 1986). However, the stoichiometry of electron donors and sulfate may be crucial for this relationship. In a bioreactor study, methanogenic conditions were created with a ratio of substrate (lactate) to sulfate at 20.9, and sulfate limiting conditions were created with a ratio of 1.94 (Dar et al., 2008). This suggests that the relative abundance of suitable substrates for sulfate reduction and concentration of sulfate drive competition of MPA with SRB, not sulfate concentration alone.

Several lineages of anaerobic prokaryotes are able to methylate inorganic Hg(II) into bioavailable and toxic MeHg (Gilmour et al., 2013). SRB have been implicated in production of MeHg in estuarine (Compeau and Bartha 1985) and freshwater ecosystems (Gilmour et al., 1992; Yu et al., 2012), and have been demonstrated to produce MeHg in laboratory culture (Parks et al., 2013). However, other microbial groups also produce

MeHg. FeRB are known to methylate Hg in freshwater sediment (Fleming et al., 2006; Kerin et al., 2006;Yu et al., 2012) and in pure culture (Gilmour et al., 2013). MPA also produce MeHg in culture (Yu et al., 2013; Gilmour et al., 2013) and have been implicated as the primary producer of MeHg in lake periphyton (Hamelin et al., 2011). A variety of other microbes including firmicutes and acetogens also are known to possess the genes (*HgcA* and *HgcB*) for MeHg production (Gilmour et al., 2013).

The ability to methylate Hg is not universal to all members of SRB, MPA and FeRB and is thought to depend on the presence and expression of the *HgcAB* gene cluster or ortholog (Parks et al., 2013). Additionally, the effectiveness of conversion of inorganic Hg(II) to MeHg is dependent on microbial species (Gilmour et al., 2013). SRB are thought of as the primary producers of MeHg in the environment (Poulain and Barkay 2013). Supporting this view, some SRB in pure culture have high conversion rates (>70 %) of inorganic Hg(II) to MeHg (Gilmour et al., 2013). FeRB also are known to play a significant role in the production of MeHg in natural systems (Yu et al., 2012). FeRB have been documented to methylate >30% of spiked Hg in culture (Gilmour et al., 2013). MPA have only recently been demonstrated to methylate Hg, converting low amount of inorganic Hg (1 - 4%) to MeHg in culture (Gilmour et al., 2013).

Observations of the competition of SRB with MPA have been facilitated by the use of specific microbial inhibitors (Lovley and Philips 1983). The terminal steps of methanogenesis are inhibited by 2-bromoethane sulfonic acid (BESA), rendering that metabolic pathway unusable for MPA (Bouwert and McCarty 1983). Thus, BESA has been used to inhibit methanogenic activity experimentally (Lovley and Klug 1983).

Molybdate has been similarly used to inhibit sulfate reduction. Sulfate and molybdate are structurally analogous, allowing SRB to reduce molybdate when concentrations of molybdate exceed those of sulfate. However, molybdate reduction inhibits sulfate metabolism and is toxic to SRB (Biswas et al., 2009). There is no known specific microbial inhibitor for FeRB.

The competition between SRB and MPA has been explored in some lake sediments (Lovley and Philips 1983), but to our knowledge has not been studied in streams. Additionally, the relative contributions of different Hg methylating microbial groups are not characterized in mid-order streams. Because MeHg contamination is an important concern in freshwater fishes that spend part or all of the life cycles in streams (Chasar et al., 2009), it is important to evaluate the importance of these groups of microbes in the production of MeHg.

Objectives and Hypotheses

Objective 1: To describe the potential of SRB to limit MPA metabolism in four North Carolina Piedmont streams.

I hypothesize that SRB has the potential to suppress MPA in stream sediments.

Objective 2: To compare potential production of MeHg from SRB, MPA and other MeHg producing microbes (including FeRB) in sediments of four North Carolina Piedmont streams.

I hypothesize that SRB are the major Hg methylating group, and MPA and other MeHg producing microbes play a minor role in the production of MeHg in North Carolina Piedmont stream sediment

CHAPTER II

MATERIALS AND METHODS

Study Sites and Sample Collection

Stream sediments were collected over three consecutive days from selected streams in Guilford County North Carolina in late January 2015. Streams used in the study were a part of the Cape Fear River Basin and included North Buffalo Creek, South Buffalo Creek, Little Alamance Creek, and Reedy Fork Creek (Figure 1). Discharge data was available via USGS monitoring stations for North Buffalo Creek, South Buffalo Creek and Reedy Fork Creek. Streams were sampled during base flow conditions (Table 1). At each site 35 sediment cores of 38 mm internal diameter and 5 cm depth were taken by driving acid-cleaned polycarbonate cores (length of 25 cm) into sediment along a reach of 75 meters. Water above the sediment-water interface was discarded on site, taking care to not disturb the sediment-water interface. Samples were pooled, transported to the laboratory on ice, and then refrigerated until subsequent processing.

Experimental Design

A microcosm experiment was conducted to compare the activities of SRB, MPA, and all other microbes in production of CH_4 and methylation of Hg from sediments in four study streams. In each microcosm the production of CH_4 and MeHg from sediments production were measured. Five treatments were used to compare microbial communities across streams, with four replicates for each treatment. The experimental treatments were

as follows: 1) control without inhibitors, 2) MPA inhibition treatment with 50 mM of BESA, 3) SRB inhibition treatment with 2 mM molybdate, 4) MPA and SRB co-inhibition treatment (50 mM BESA & 2 mM molybdate), and a general bacterial inhibition treatment (2 mM chloramphenicol) (Table 2). Inhibitor concentrations of were chosen based on preliminary experiments, which established effectiveness of those concentrations in the study sediments. This approach may allow uninhibited microbial groups to remain metabolically active.

Microcosm Construction

Microcosms consisted of acid cleaned 200 mL glass serum bottles (Zhang et al., 2014; Randall et al., 2013) containing 100.1 ± 0.1 g (± SE) of wet surficial sediment and 101.1 ± 0.2 mL (± SE) of argon-purged artificial soft water (U.S. E.P.A. 1991). Artificial soft water purged in argon was used to reduce chemical variation in microcosm water and to create anoxic conditions. Inhibitors were prepared separately in water used in their respective experimental treatments before being introduced to the microcosms.

Pooled sediments from each stream were manually homogenized for five minutes and large particles (e.g., stones and leaves) were removed before 100 g of wet sediment subsamples were distributed into microcosms. Microcosms were sealed with gas impermeable butyl rubber stoppers (Geo-Microbial Technologies) and crimped with aluminum seals. After sealing, microcosms were shaken vigorously for 30 seconds to equilibrate CH_4 between the water and headspace before evacuating gas and filling with high-purity N_2 for six 30-second cycles to create an anoxic headspace free of CH_4.

Microcosms were incubated at 22.5 ± 0.6 °C (± SD) for 18 days in the dark to prevent photoreductions of Hg(II), photodegradation of MeHg, and algal growth.

Sediment and Water Chemistry Analysis

Trace elements of interest (including Fe and Na) were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) for water, sediment, and extract of sediment. Extractible sulfur from sediment was measured using inductively coupled plasma atomic emission spectrometry (ICP-AES). Ash free dry mass (AFDM) was measured by weighing dried sediment before and after combustion in a muffle furnace for 2 hours at 500°C. The pH of sediment was measured using an H^+ ion-selective glass electrode (Sikora and Kissel 2014). Total sulfur (µg/g) in sediment was determined using a LECO S144-DR Sulfur Analyzer.

Methane Sampling

Acid cleaned 20 mL glass serum vials with crimped aluminum seals served as CH_4 sample vials. Before use, sample vials were evacuated and filled with high-purity N_2 for six 15 second cycles each ending with N_2, equalized to atmospheric pressure, treated with 0.1 mL of 10% HCl to prevent microbial activity, and then injected with 3 mL of N_2 purged deionized water. Purged water in sample vials provided gas tight seals with the vial's septa when inverted.

Microcosms were sampled for CH_4 over the course of 18 days. Before sampling, each microcosm was shaken for 30 seconds to release trapped CH_4 in the sediment slurry. Prior to withdrawing samples, the headspace gas was further mixed by the withdrawal and reintroduction of 5 mL headspace gas for ten times via syringe. 5 mL samples of gas

were taken from the microcosm headspace using a 22-gauge gas-tight syringe needle, and replaced with 5 mL of high purity N_2. Samples were injected into sample vials with butyl rubber stoppers, from which 5mL of gas had been previously evacuated. Sample vials were inverted during storage prior to processing.

Methane Analysis

Sample CH_4 concentrations were measured using a Shimadzu GC-8A gas chromatograph equipped with a flame ionization detection unit (GC-FID) operating with a 1/8" diameter X 1 m length molecular sieve 5A (60/80) column at 140°C, using ultra-high purity N_2 at 33 L min^{-1} flow rate for the carrier gas (Lofton et al., 2014). The GC-FID was calibrated using 2 points of NIST certified CH_4 standards before use. Output from the GC-FID was used to calculate concentrations of CH_4 from sample serum vials and then converted to nM concentrations in each microcosm. This was calculated by determining headspace volume, total water volume, accounting for temperature, atmospheric pressure, correcting for ratio of microcosm gas sample to non-sample gas volume in sample serum vials, and the equilibration between aqueous and gaseous phases of CH_4. Concentration values of CH_4 (with a unit of nM CH_4 g^{-1} dry weight of sediment) were normalized to the mass of sediment by dividing the measured CH_4 (nM) by the calculated dry weight of sediment (g). These values were plotted over time for each microcosm to establish the change in CH_4 production over time. The linear portion of the curve was used to calculate the daily production of CH_4, with a unit of nM CH_4 g^{-1} day^{-1}.

Methane Data Analysis

Before statistical analysis, CH_4 data were examined for outliers and conformation to heterogeneity and normality assumptions of ANOVA analysis by residuals. Significant outliers were detected with Cook's distance and removed. The data had a non-normal distribution with some very small negative values, so the data were rank transformed. A one-way ANOVA was used to assess treatment effects in ranked data, being analogous to non-parmetric analysis (Conover and Iman 1981). Comparison between rank transformed treatments were made using Tukey-Kramer post-hoc analysis.

Mercury Spiking and Sample Processing

Total Hg in sediment was determined from collected sediments from each stream using total Hg analysis. Concentrations of total Hg from wet sediment were determined with acid digestion, using stannous chloride to reduce Hg(II) into Hg(0) for collection by a trap with gold-coated glass beads. Hg collected on the traps was quantified by a cold vapor atomic fluorescence spectrometer (U.S. E.P.A. 2002). After incubation for 10 days, the natural abundance of Hg was doubled by injecting a solution of enriched stable isotope of inorganic Hg(II), $^{200}Hg(NO_3)_2$ (96.4% purity, Oak Ridge National Laboratory), via syringe, into the microcosm bottles. The amount of $^{200}Hg(NO_3)_2$ spike was determined for each stream with total Hg concentrations from a subsample of sediment used for microcosm creation. Microcosm bottles were incubated for 8 days after the spike and were shaken every 24 hours to ensure adequate mixing. Upon completion of incubation, supernatant water was collected and spun down via centrifuge (2000 rpm) to remove suspended particulate matter. Supernatant water from the control treatments was

analyzed for multiple elements by ICP-MS (i.e., Na, Al, Cr, Mn, Fe, Ni, Zn, As, Se, and Pb). Suspended particulate matter collected from the supernatant water was freeze-dried along with remaining sediment in microcosms. Freeze-dried particulate matter was combined with sediment from the same microcosm and then pulverized and homogenized by a mixer mill for subsequent analyses for $Me^{200}Hg$ and sediment chemistry.

Methylmercury Isotope Analysis

The amount of spiked $^{200}Hg(II)$ converted to $Me^{200}Hg$ was measured using isotope dilution ICPMS (Hintelmann and Evans 1997). Subsamples of lyophilized sediment were weighed into 50 mL centrifuge tubes, amended with $Me^{199}Hg$ internal standard, and extracted for MeHg with an acid and methylchloride technique (Bloom et al., 1997). Sodium tetraethylborate was used to bind to MeHg and allow for collection on Tenax resin traps. $Me^{200}Hg$ was quantified by ICP-MS coupled to gas chromatography (Hintelmann et al., 2000). The amount of measured $Me^{200}Hg$ was corrected for $^{200}Hg(II)$ introduced into the microcosm by dividing $Me^{200}Hg$ by the sum of $^{200}Hg(II)$ present in the total Hg in sediment from each stream as calculated from the ratio of isotopes in natural sources and the amount of $^{200}Hg(II)$ spiked into the microcosm. A rate was not used, because an unknown amount of microbial demethylation of MeHg may have occured simultaneously (Schaeffer et al., 2004). Percent of total Hg as MeHg (i.e, % $Me^{200}Hg$) in sediment samples was interpreted as net MeHg production potential in each microcosm.

Methylmercury Data Analysis

MeHg production potentials, represented as % $Me^{200}Hg$, were examined for outliers and conformation to heterogeneity and normality assumptions of ANOVA by residuals. Data were log transformed to correct for non-normality. One-way ANOVA was used to assess treatment effects, excluding stream as an explanatory variable. Comparison between treatments as made using Tukey-Kramer post-hoc analysis.

Methylmercury Data Regression Analysis

No outliers were found in the regression model and data transformation was necessary. To establish the relationship between % $Me^{200}Hg$ and methanogenesis, a linear regression model was created with data from control treatments. MPA inhibited treatments were excluded because there was very low production of CH_4 in those treatments. As percent MeHg (or methylation potential) represented the production of MeHg at the conclusion of the experiment, it was compared with the final concentrations of CH_4 in the control treatment. An examination of the relationship between total S (µg/g) and % $Me^{200}Hg$ was also made to explore if total S (µg/g) may serve as a potential indicator of SRB producing MeHg.

CHAPTER III

RESULTS

Abiotic Stream Conditions

North Buffalo Creek, South Buffalo Creek, and Little Alamance Creek had similar ion concentrations in water and sediment (Table 3 and Table 4). Reedy Fork Creek had the lowest concentrations of Na, Al, Fe, S, Mg, Zn, Pb, Ni and had the lowest total S and AFDM among study streams (Table 3 and 4).

Methane Production

The SRB inhibited treatment had the highest CH_4 production rate (0.879 nM/g/day ± SE 0.160). The second highest CH_4 production rate was found in the control treatment (0.143 nM/g/day ± SE 0.036) (Figure 2). The average value of the SRB inhibited treatment was 6.15 fold higher than the control average (Figure 2). MPA inhibited treatments (BESA, BESA and molybdate, and chloramphenicol) had very low CH_4 production rates under 0.03nM/g/day (Figure 2). Additionally, peak production of CH_4 was distinctive between treatments. Control treatments peaked within the first 10 days, leveling off after day 10 (Figure 3). SRB inhibited treatments peaked after day 3 to day 18 and did not decrease in production (Figure 4). The difference between treatments was significant, with a p-value of 0.001 as indicated by ANOVA (Table 5). Both SRB and control treatments were statistically different from each other and distinct from all

other treatments (Table 5). There was no statistical difference between CH_4 production rates between any treatments that inhibited MPA (Table 5).

While CH_4 production rate treatment effects were present in each stream sediment, the pattern varied among sites (Figure 5). Little Alamance Creek and North Buffalo Creek had the highest CH_4 production rates of the study (Figure 5). Both Reedy Fork Creek and South Buffalo Creek had lower CH_4 production rates, with Reedy Fork Creek having the lowest CH_4 production of the four streams (Figure 5).

Methylmercury Production

Both the control and MPA inhibited treatment had the highest % $Me^{200}Hg$ of treatments, with an average of 7.43 % $Me^{200}Hg$ and 7.27 % $Me^{200}Hg$, respectively (Figure 6). Although not statistically different from each other, control and MPA inhibited treatments were significantly different from all other treatments ($p<0.001$, Table 5). Lower production of MeHg was observed in the SRB inhibited treatment and the MPA and SRB inhibited treatment, 1.15 % $Me^{200}Hg$ and 1.74 % $Me^{200}Hg$, respectively (Figure 4). SRB inhibited treatments, and MPA and SRB co-inhibited treatments were not statistically different from each other (>0.05, Table 5), but were different from all other treatments. The chloramphenicol treatment had the lowest production of $Me^{200}Hg$ with 0.36 % $Me^{200}Hg$ (Figure 6) and was statistically different from all other treatments (Table 5).

All four streams followed a similar pattern of MeHg production observed in the control, although there was variation of % $Me^{200}Hg$ in control sediments among study streams (Figure 7). For example, Reedy Fork Creek had lower MeHg production in

control sediments than other study sediments, with the average % $Me^{200}Hg$ in the control treatment being 3.31 compared to 7.90 in the MPA inhibited treatment (Figure 7).

Regression Analysis on Methylmercury Data

The regression model between % $Me^{200}Hg$ and CH_4 was highly significant, $p < 0.0001$ (Table 5, Figure 8). The fit of the regression was strong ($R^2 = 0.7505$) (Table 5).

There was a positive relationship between total S and % $Me^{200}Hg$ within stream sediments ($R^2 = 0.5694$) (Figure 9).

CHAPTER IV

DISCUSSION

Suppression of Methanogenesis by Sulfate Reducers

Methanogenesis appears to have been controlled by SRB in this study. This study demonstrates that methanogenesis could be suppressed by SRB in stream sediments as previously observed in lake sediments (Lovley and Klug 1983). While methanogenesis occurred in the control, it was lower than SRB inhibited treatments (Figure 2). The increase in methanogenesis seen in the molybdate treatment likely was due to MPA being released from competition with SRB. Thus, SRB may indirectly regulate the potential for CH_4 emissions from streams by reducing MPA metabolic activity. SRB could provide an ecosystem service by reducing CH_4 production, which contributes to global warming (Boucher et al., 2009). The dynamics of these SRB-MPA interactions likely depend on sulfate concentrations and substrate availability (Takii and Fukui 1991; Lovley and Klug 1986).

Methanogenesis rates in stream sediments are variable across studies and difficult to compare due to differences in experimental approaches and units of measurement. Because wet sediment is denser than dried sediment, values reported as dry weight are generally higher than those for wet sediment. Similarly, production rates reported per ml sediment are higher than per g of sediment. At Coweeta long term ecological research station (LTER), in western North Carolina, potential CH_4 production rates in streams

were > 5000 nM g^{-1} sediment day^{-1} (Baker et. al 2000). CH_4 production rates in Guilford county streams ranged from 2.5 nM CH_4 ml^{-1} sediment day^{-1} in winter to 25 nM CH_4 ml^{-1} sediment $day^{-}1$ in early fall (A. E. Bullard UNCG unpublished data). The highest CH_4 production rate observed in this study was in the SRB inhibited treatment in Little Alamance Creek sediments (1.5 nM CH_4 g^{-1} dry sediment day^{-1}) (Figure 5), much lower than that reported by Baker et al., (2000) for Coweeta streams.

Methanogenesis rates observed in this study were likely influenced by experimental conditions. Because methanogenesis was measured in microcosms, rates are comparable to other treatments, but not necessarily indicative of in-stream CH_4 production. The homogenization and manipulation of sediment during microcosm creation allowed exposure to atmospheric oxygen. This potentially reduced populations of strict anaerobes, such as MPA (Liu and Whitman 2008). Additionally, sediment was collected during winter months, when there is reduced methanogenic activity (Jones et al., 1995). However, methanogenesis was observed in all streams in both the control and SRB inhibited treatments (Figure 5).

Without assessing if sulfate was in excess for SRB growth, it is difficult to determine how well the experimental conditions translate to controls on MPA activity in the streams. However, presence of sulfate may not have significantly impacted the competition of SRB with MPA. When sulfate is abundant it is thought that intra-guild competition for sulfate occurs between different SRB taxa (Muyzer and Stams 2008). The limiting concentration of sulfate for SRB is unclear, and SRB have been observed to

outcompete MPA for acetate and hydrogen substrates in freshwater conditions (Lovley and Klug 1983).

Concentrations of sulfate may have been sufficient to induce competition between MPA and SRB prior to introduction of artificial soft water. Guilford county streams have variable sulfate concentrations, ranging from 42 – 739 µM, with higher sulfate concentrations observed in urban streams (Davenport 1989). The concentration of sulfate used in the artificial soft water for the study, 425 µM, was within this observed range. These values are all above the reported 30 µM of sulfate needed for the suppression of methanogenesis (Lovley and Klug 1986). However, conclusions from this study are limited in scope, and should be cautiously applied outside this study. Further investigation into the applicability of artificial soft water used in the experiment is required.

Microbial Contributions to Methylmercury Production

SRB were the most important microbial group for the production of MeHg in this study, with SRB active treatments producing the majority of MeHg (Figure 6). If the control was assumed to account for 100% of MeHg produced, then SRB would account for ~76% (Figure 6). To the extent that experimental conditions simulate natural processes in local streams, these results suggest that SRB are the primary producers of MeHg in sediment in the study streams, and potentially other North Carolina Piedmont streams. The positive relationship between total S and % MeHg (Figure 9) may reveal information about SRB as methylators. Because sulfate would be proportional to the level of total S, the positive relationship may be indicative of a correlation of favorable

conditions for SRB activity and Hg methylation. However, the generality of SRB as the primary methylators of Hg (Gilmour et al., 1992; Yu et al., 2011) bears investigation in other stream sediments. In-stream concentrations of electron donors and acceptors likely influence SRB activity (Lovley and Klug 1986).

Other microbes than SRB and MPA contributed a significant amount of MeHg in microcosms in this study, providing the second highest source of MeHg, accounting for ~18% of the overall Hg methylation (Figure 6). Microbes that would have been active in this community may have had a variety of metabolic pathways, including iron, benzoate and fumarate reduction (Gilmour et al., 2013). Among these, FeRB are known to coexist with SRB in river sediment (Yu et al., 2012). Interestingly, when both MPA and SRB were co-inhibited there was more methylation than in the SRB inhibited treatment, although the difference was not statistically significant (Table 5). However, Reedy Fork Creek did not show an increase in Hg methylatation in the MPA and SRB inhibited treatments (Figure 6). Lower Fe concentrations at Reedy Fork Creek, may have reduced the importance of FeRB in Hg methylation.

The lack of statistical difference between SRB inhibited and MPA and SRB co-inhibited treatments shows that MPA were not important methylators of Hg in the study sediments. Although MPA are known to produce MeHg, only certain members of the class Methanomicrobia can methylate Hg (Gilmour et al., 2013). Potentially, the MPA that were active in sediment did not possess the *HgcAB* gene cluster needed for Hg methylation (Gilmour et al., 2013). Low production rates of MeHg by MPA (Gilmour et al., 2013) could have been responsible for the lack of discernable methylation by MPA.

Minimal MeHg production in general microbial inhibition treatment illustrates that microbes were the primary producers of MeHg in the experiment. Small amounts of Hg can be methylated abiotically (Celo et. al 2006), and could account for the trace amount of methylation, which occurred in the chloramphenicol treatments. Further investigation on the importance of abiotic Hg methylation in nature is warranted. In addition, it is possible that the chloramphenicol treatments used in this study did not fully suppress all microbial Hg methylation activities.

The positive relationship between CH_4 and % $Me^{200}Hg$ seemingly contradicts results from our experiments, implying MPA were methylating Hg(II) in excess of what was observed in MPA active treatments. Sulfate reduction using propionate, butyrate and lactate produces acetate (Muyzer and Stams 2008). The production of acetate by these SRB could promote acetate utilizing SRB and MPA. Further, CH_4 is also a byproduct of the breakdown of MeHg using *MerB* (Schaeffer et al., 2004). Accordingly, a portion of the CH_4 produced in the control treatment may have been the product of microbial demethylation, resulting in the observed relationship between % $Me^{200}Hg$ and CH_4 concentration observed in the control.

Conclusions

Some insights into anaerobic microbial ecology of stream sediments are gained from this study. By competing for substrates, SRB could reduce methanogenesis in stream sediments. However, additional studies on the role of sulfate limitation of SRB activity is needed to fully evaluate the interactions of SRB and MPA in streams. In some

streams with low sulfate concentrations, MPA may not compete for substrates with SRB. Quantifying the regulation of CH_4 production by SRB warrants investigation.

The majority of the MeHg production could be mediated by SRB even if methanogenic conditions are present, due to low rates of MeHg production by MPA (Gilmour et al., 2013). Further, other groups of stream microbes are also important for Hg methylation. MeHg producing microbes other than SRB or MPA had an important role in the production in MeHg in sediments (Figure 3; Figure 4). These microbes, which likely include FeRB, are the primary producers of MeHg when SRB are not active in these stream sediments. However, if sulfate availability is highly limited in natural conditions then these groups could become an important source of MeHg. Future research should be done to investigate anaerobic processes in streams. Further, the identity of important microbes in the cycling of Hg in streams should be investigated.

REFERENCES

Avramescu, M.-L., Yumvihoze, E., Hintelmann, H., Ridal, J., Fortin, D., & Lean, D. R. S. (2011). Biogeochemical factors influencing net mercury methylation in contaminated freshwater sediments from the St. Lawrence River in Cornwall, Ontario, Canada. *The Science of the Total Environment, 409*, 5, 968–978.

Baker, M. A., Dahm, C. N., & Valett, H. M. (2000). Anoxia, anaerobic metabolism biogeochemistry of the stream water-ground water interface. In J. B. Jones & P. J. Muholland (1st Ed.), *Streams and Ground Waters* (pp. 259-283). San Diego: Academic Press.

Baker, M. A., Dahm, C. N., & Valett, H. M. (1999). Acetate retention and metabolism in the hyporheic zone of a mountain stream. *Limnology & Oceanography*, *44*, 6, 1530–1539.

Biswas, K., Woodards, N., Xu, H., & Barton, L. (2009). Reduction of molybdate by sulfate-reducing bacteria. *Biometals*, 22, 131–139.

Bloom, N. S., Colman, J. A., & Barber, L. (1997). Artifact formation of methyl mercury during aqueous distillation and alternative techniques for the extraction of methyl mercury from environmental samples. *Fresenius' Journal of Analytical Chemistry, 358*, 3, 371-377.

Boucher, O., Friedlingstein, P., Collins, B., & Shine, K. P. (2009). The indirect global warming potential and global temperature change potential due to methane oxidation. *Environmental Research Letters, 4*, 4, 044007.

Bouwert, E. J., & McCarty, P. L. (1983). Effects of 2-Bromoethanesulfonic Acid and 2-Chloroethanesulfonic Acid on Acetate Utilization in a Continuous-Flow Methanogenic Fixed-Film Column. *Applied & Environmental Microbiology, 45*, 4, 1408–1410.

Buriánková, I., Brablcová, L., Mach, V., Hýblová, A., Badurová, P., Cupalová, J. & Rulík, M. (2012). Methanogens and methanotrophs distribution in the hyporheic sediments of a small lowland stream. *Fundamental & Applied Limnology, 181*, 2, 87–102.

Capone, D. G., & Kiene, R. P. (1988). Comparison of microbial dynamics in marine and freshwater sediments: Contrasts in anaerobic carbon catabolism. *Limnology & Oceanography, 33*, 4, 725-749.

Celo, V., Lean, D. R. S., & Scott, S. L. (2006). Abiotic methylation of mercury in the aquatic environment. *The Science of the Total Environment, 368,* 1, 126–137.

Chasar, L. C., Scudder, B. C., Stewart, A. R., Bell, A. H., & Aiken, G. R. (2009). Mercury cycling in stream ecosystems. 3. Trophic dynamics and methylmercury bioaccumulation. *Environmental Science & Technology, 43*, 8, 2733-2739.

Conover, W. J., & Iman, R. L. (1981). Rank transformations as a bridge between parametric and nonparametric statistics. *The American Statistician, 35*, 3, 124-129.

Conrad, R. (1999). Control of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. *FEMS Microbiology Ecology*, 28, 39–58.

Compeau, G., & Bartha, R. (1985). Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediment. *Applied & Environmental Microbiology, 50*, 2, 498–502.

Cypionka, H. (2000). Oxygen respiration by *Desulfovibrio* species 1. *Annual Reviews in Microbiology, 54,* 1, 827-848.

Davenport, Marjorie S. (1988) Water quality in Reedy Fork and Buffalo Creek basins in the Greensboro area, North Carolina, 1986-87.

Dar, S. A., Kleerebezem, R., Stams, A. J. M., Kuenen, J. G., & Muyzer, G. (2008). Competition and coexistence of sulfate-reducing bacteria, acetogens and methanogens in a lab-scale anaerobic bioreactor as affected by changing substrate to sulfate ratio. *Applied Microbiology & Biotechnology, 78,* 6, 1045–1055.

U.S. E.P.A. (1991). *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (No. 363.739463 M592m).* Retrieved from U.S. Environmental Protection Agency.

U.S. E.P.A. (2002). *Method 1631, Revision E: Mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry*. Retrieved from U.S. Environmental Protection Agency.

Fenchel, T., & Finlay, B. J. (1995). Ecology and evolution in anoxic worlds. Microbial mats. New York: Oxford University Press.

Fleming, E. J., Mack, E. E., Green, P. G., & Nelson, D. C. (2006). Mercury methylation from unexpected sources: molybdate-inhibited freshwater sediments and an iron-reducing bacterium. *Applied & Environmental Microbiology, 72,* 1, 457–464.

Gilmour, C. C., Podar, M., Bullock, A. L., Graham, A. M., Brown, S. D., Somenahally, A. C., Elias, D. A. (2013). Mercury Methylation by Novel Microorganisms from New Environments. *Environmental Science & Technology, 47*, 20, 11810–11820.

Grimm, N. B., & Fisher, S. G. (1984). Exchange between interstitial and surface water: Implications for stream metabolism and nutrient cycling. *Hydrobiologia, 111*, 3, 219–228.

Hamelin, S., Amyot, M., Barkay, T., Wang, Y., & Planas, D. (2011). Methanogens: principal methylators of mercury in lake periphyton. *Environmental Science & Technology, 45*, 18, 7693–7700.

Hintelmann, H., Keppel-Jones, K., & Evans, D. (2000). Constants of mercury methylation and demethylation rates in sediments and comparison of tracer and ambient mercury availability. *Environmental Toxicology & Chemistry, 19*, 9, 2204–2211.

Hintelmann, H., & Evans, R. D. (1997). Application of stable isotopes in environmental tracer studies - Measurement of monomethylmercury (CH 3 Hg +) by isotope dilution ICP-MS and detection of species transformation. *Fresenius' Journal of Analytical Chemistry, 358*, 3, 378–385.

Hlaváčová, E., Rulík, M., & Čáp, L. (2005). Anaerobic microbial metabolism in hyporheic sediment of a gravel bar in a small lowland stream. *River Research and Applications, 21*, 9, 1003–1011.

Jones, J. B., Holmes, R. M., Fisher, S. G., Grimm, N. B., & Greene, D. M. (1995). Methanogenesis in Arizona, USA Dryland Streams. *Biogeochemistry, 31*, 3, 155–173.

Jones, R. I., & Grey, J. (2011). Biogenic methane in freshwater food webs. *Freshwater Biology, 56*, 2, 213–229.

Jones, J. B., & Mulholland, P. J. (1998). Methane input and evasion in a hardwood forest stream: Effects of subsurface flow from shallow and deep flowpaths. *Limnology & Oceanography, 43*, 6, 1243–1250.

Kerin, E. J., Gilmour, C. C., Roden, E., Suzuki, M. T., Coates, J. D., & Mason, R. P. (2006). Mercury methylation by dissimilatory iron-reducing bacteria. *Applied and Environmental Microbiology, 72*, 12, 7919–7921.

Liu, Y., & Whitman, W. B. (2008). Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Annals of the New York Academy of Sciences, 1125*, 171–189.

Lofton, D. D., Whalen, S. C., & Hershey, A. E. (2014). Effect of temperature on methane dynamics and evaluation of methane oxidation kinetics in shallow Arctic Alaskan lakes. *Hydrobiologia, 721,* 1, 209–222.

Lovley, Derek R., & Phillips, E. J. P. (1986). Availability of ferric iron for microbial reduction in bottom sediments of the freshwater tidal Potomac River. *Applied and Environmental Microbiology, 52,* 4, 751-757.

Lovley, D. R., & Klug, M. J. (1986). Model for the distribution of sulfate reduction and methanogenesis in freshwater sediments. *Geochimica et Cosmochimica Acta, 50,* 11–18.

Lovley, D. R., Stolz, J. F., Nord, G. L., & Phillips, E. J. P. (1987). Anaerobic production of magnetite by a dissimilarity iron-reducing microorganism. *Nature, 330,* 252–254.

Lovley, D. R. & Klug, M. (1983). Sulfate reducers can outcompete methanogens at freshwater sulfate concentrations. *Applied and Environmental Microbiology, 45,* 1, 187–197.

Maerki, M., Müller, B., Dinkel, C., & Wehrli, B. (2009). Mineralization pathways in lake sediments with different oxygen and organic carbon supply. *Limnology & Oceanography, 54,* 2, 428–438.

Mulholland, P. J., Marzolf, E. R., Webster, J. R., Hart, D. R., & Hendricks, S. P. (1997). Evidence that hyporheic zones increase heterotrophic metabolism and phosphorus uptake in forest streams. *Limnology & Oceanography, 42,* 3, 443-451.

Muyzer, G., & Stams, A. J. M. (2008). The ecology and biotechnology of sulphate-reducing bacteria. *Nature Reviews. Microbiology, 6,* 6, 441–454.

Nogaro, G., Datry, T., Mermillod-Blondin, F., Descloux, S., & Montuelle, B. (2010). Influence of streambed sediment clogging on microbial processes in the hyporheic zone. *Freshwater Biology, 55,* 6, 1288–1302.

Parks, J. M., Johs, A., Podar, M., Bridou, R., Hurt, R. A., Smith, S. D., Tomanicek, S. J., Qian, Y., Brown, S. D., Brandt, C. C., Palumbo, A. V., Smith, J. C., Wall, J. D., Elias, D. A. and Liang, L. (2013). The genetic basis for bacterial mercury methylation. *Science, 339,* 6125, 1332-1335.

Phelps, T. J., & Zeikus, J. G. (1984) Influence of pH on terminal carbon metabolism in anoxic sediments from a mildly acidic lake. *Applied and Environmental Microbiology 48,* 6, 1088-1095.

Poulain, A. J., & Barkay, T. (2013). Cracking the mercury methylation code. *Science, 339*, 6125, 1280–1281.

Randall, P. M., Fimmen, R., Lal, V., & Darlington, R. (2013). In-situ subaqueous capping of mercury-contaminated sediments in a fresh-water aquatic system, Part I-Bench-scale microcosm study to assess methylmercury production. *Environmental Research, 125*, 30–40.

Hedderich, R., & Whitman, W. B. (2006). Physiology and biochemistry of the methane-producing Archaea. In M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt (3rd Ed.), *The Prokaryotes Vol. 2* (pp. 1050-1079). New York: Springer.

Schaefer, J. K., Yagi, J., Reinfelder, J. R., Cardona, T., Ellickson, K. M., Tel-Or, S., & Barkay, T. (2004). Role of the bacterial organomercury lyase (MerB) in controlling methylmercury accumulation in mercury-contaminated natural waters. *Environmental Science & Technology, 38*, 16, 4304–4311.

Schulz, S., & Conrad, R. (1996). Influence of temperature on pathways to methane production in the permanently cold profundal sediment of Lake Constance. *FEMS Microbiology Ecology, 20*, 1, 1–14.

Sikora, F.J. & Kissel, D.E. (2014) Soil pH. *Southern Cooperative Series Bulletin*, 419, 48-53.

Stams, A. J. M., Oude Elferink, S. J. W. H., & Westermann, P. (2003). Metabolic interactions between methanogenic consortia and anaerobic respiring bacteria. In T. Scheper. *Biomethanation I* (pp. 31-56). Berlin: Springer.

Takii, S., & Fukui, M. (1991). Relative Importance of Methanogenesis, in Sediments Sulfate Reduction and Denitrification of the Lowe Tama River. *Microbial Ecology, 6*, 1, 9–17.

Trimmer, M., Hildrew, A. G., Jackson, M. C., Pretty, J. L., & Grey, J. (2009). Evidence for the role of methane-derived carbon in a free-flowing, lowland river food web. *Limnology & Oceanography, 54*, 5, 1541–1547.

Whitman, W. B., Bowen, T. L., & Boone, D. R. (2006). The Methanogenic Bacteria. In M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt (3rd Ed.), *The Prokaryotes Vol. 3* (pp. 165-207). New York: Springer.

Whiticar, M. J. (1999). Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane." *Chemical Geology, 161*, 1, 291-314.

Yu, R.-Q., Reinfelder, J. R., Hines, M. E., & Barkay, T. (2013). Mercury methylation by the methanogen Methanospirillum hungatei. *Applied & Environmental Microbiology*, *79*, 20, 6325–6330.

Yu, R.-Q., Flanders, J. R., Mack, E. E., Turner, R., Mirza, M. B., & Barkay, T. (2012). Contribution of coexisting sulfate and iron reducing bacteria to methylmercury production in freshwater river sediments. *Environmental Science & Technology*, *46*, 5, 2684–2691.

Zhang, T., Kim, B., Levard, C., Reinsch, B. C., Lowry, G. V, Deshusses, M. a, & Hsu-Kim, H. (2012). Methylation of mercury by bacteria exposed to dissolved, nanoparticulate, and microparticulate mercuric sulfides. *Environmental Science & Technology*, *46*, 13, 6950–6958.

APPENDIX A

TABLES AND FIGURES

Table 1. Coordinates and Discharge of Study Streams.

Summary of location and discharge of streams used in study. NBC = North Buffalo Creek, SBC = South Buffalo Creek, LAC = Little Alamance Creek, RFC = Reedy Fork Creek.

Stream	Geographic Coordinates	Discharge* (m^3/s)
NBC	36º4’45.1446”, -79° 48' 46.0182"	0.0679604
SBC	36° 2' 57.3246", 79° 51' 17.7192"	0.0368119
LAC	36° 2' 37.5612", -79° 39' 54.7878"	N/A
RFC	36° 10' 22.008", -79° 57' 12.1572"	0.311485

*Discharge at the day of collection.

Table 2. Experimental Design.

SRB = sulfate-reducing bacteria, MPA = methane producing Archaea, Others = all other MeHg producing microbes.

Treatment	Inhibited Microbes	Active Microbes	Inhibitor conc. (mM)
Control	None	SRB, MPA, Others	N/A
BESA	MPA	SRB and Others	50
Molybdate	SRB	MPA and Others	2
BESA + Molybdate	MPA + SRB	Others	50, 2*
Chloramphenicol	MPA, SRB, others	None	2

*50 mM of BESA and 2 mM molybdate.

Table 3. Sediment Physiochemistry.

Elemental concentration was measured directly (Fe and NA) and as extractible forms (S). Total Hg units was derived per unit of wet sediment. All other sediment chemistry was calculated per gram of dry sediment. NBC = North Buffalo Creek, SBC = South Buffalo Creek, LAC = Little Alamance Creek, RFC = Reedy Fork Creek.

Stream	pH	Total S (ug/g)	AFDM (mg/g)	Total Hg (ng/g)	S (ug/g)	Fe (mg/g)	Na (ug/g)
NBC	7.35	190	3.03	3.79	7.5	17.46	553.26
SBC	7.45	195	2.92	3.87	7	25.29	661.46
LAC	7.05	175	4.76	4.56	8	20.34	277.77
RFC	7.15	150	2.67	1.13	4	11.4	119.57

Table 4. Water Chemistry.

NBC = North Buffalo Creek, SBC = South Buffalo Creek, LAC = Little Alamance Creek, RFC = Reedy Fork Creek.

Stream	Na (mg/L)	Al (ug/L)	Cr (ug/L)	Mn (ug/L)	Fe (ug/L)	Ni (ug/L)	Zn (ug/L)	As (ug/L)	Se (ug/L)	Pb (ug/L)
NBC	26.47	201.31	2.52	4443.66	4386.84	2.96	71.70	3.91	0.26	3.04
SBC	27.40	329.04	2.85	5107.30	4608.25	3.99	54.87	3.87	0.41	2.60
LAC	28.03	291.81	1.93	23510.78	4654.42	3.52	162.56	3.13	1.64	2.22
RFC	23.88	174.35	1.27	11499.10	903.43	2.45	49.38	0.52	0.62	0.71

Table 5. Statistical Analysis.

Summary of statistical analyses for potential methane production and MeHg production rates responses to experimental treatments. Pairwise contrasts were made using Tukey-Kramer post-hoc analysis. M = molybdate, CTL = control, B = BESA, B+M = BESA with molybdate, and C = chloramphenicol.

1-way ANOVA	F-value	df	p <	Pairwise Contrasts
CH_4 production	23.18	4	0.001	M > CTL > B = B + M = C
MeHg production	83.25	4	0.001	CTL = B > M = B + M > C
Simple Linear regression	**F-value**	**df**	**p <**	**R^2**
MeHg CH_4	39.11	13	0.001	0.7505

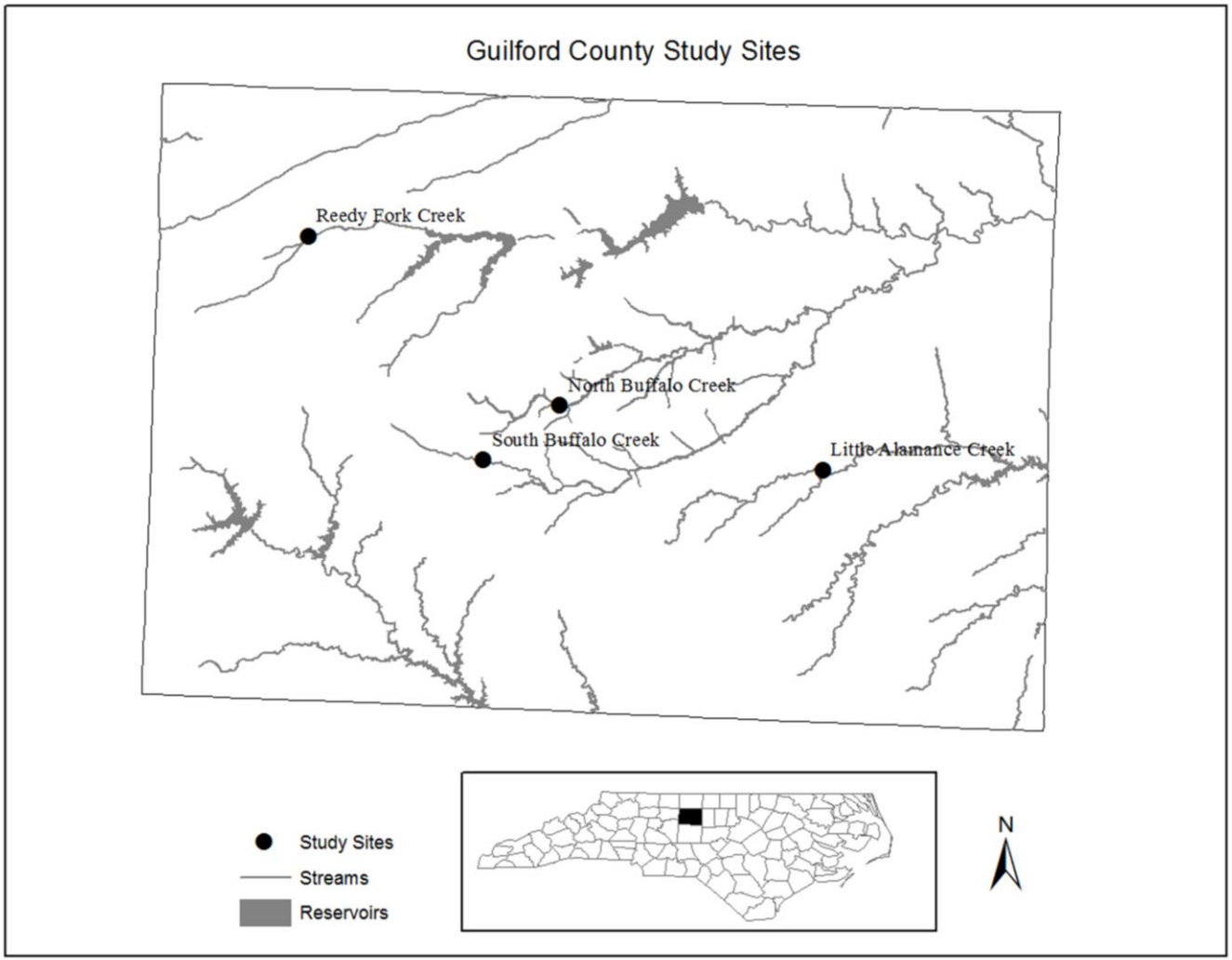

Figure 1. Study Sites.

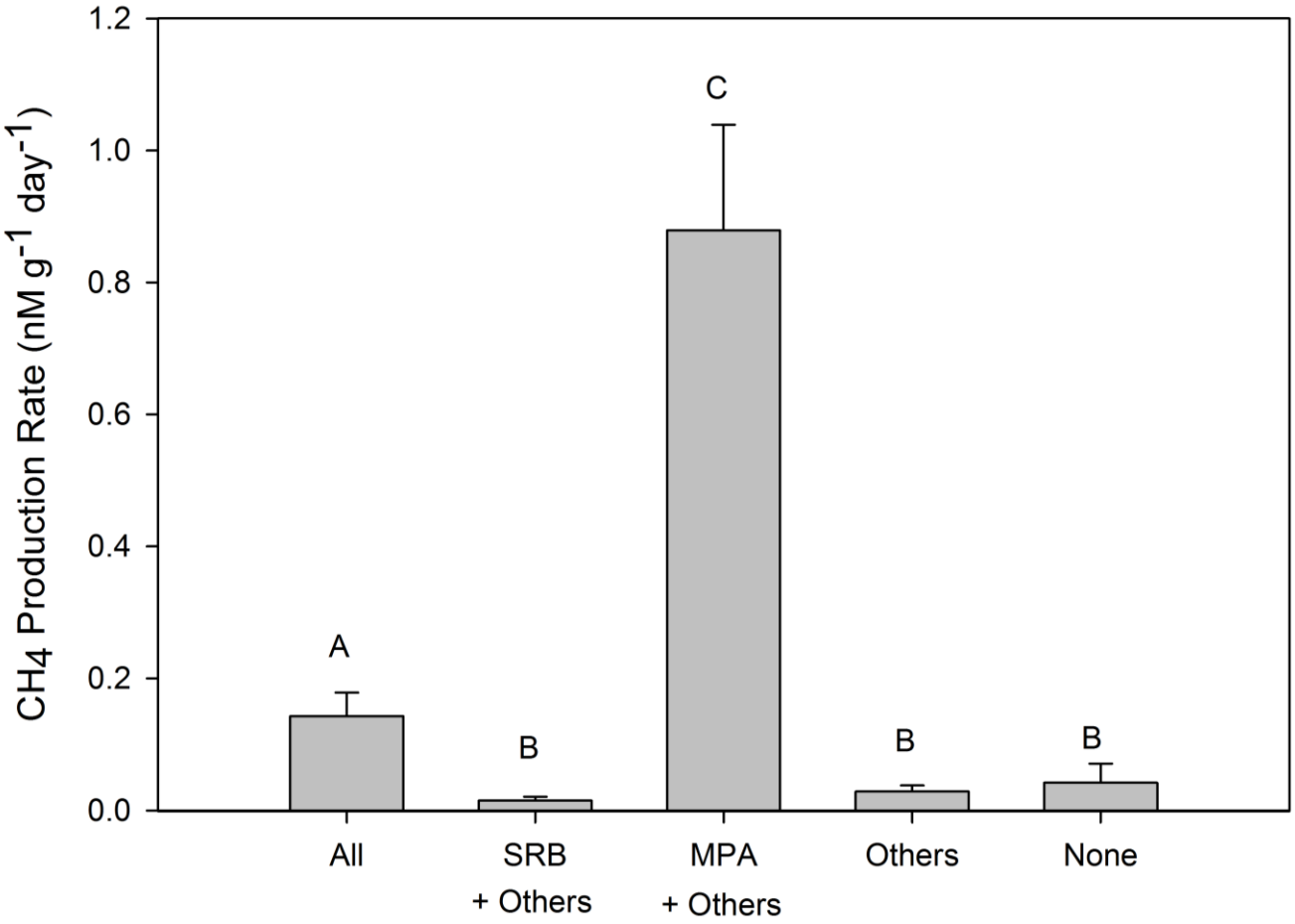

Figure 2. Treatment Methane Production.

Individual streams were not compared. Major active microbial groups are represented on the x-axis. Error bars represent standard error. Letters above bars represent Tukey-Kramer HSD post-hoc comparisons.

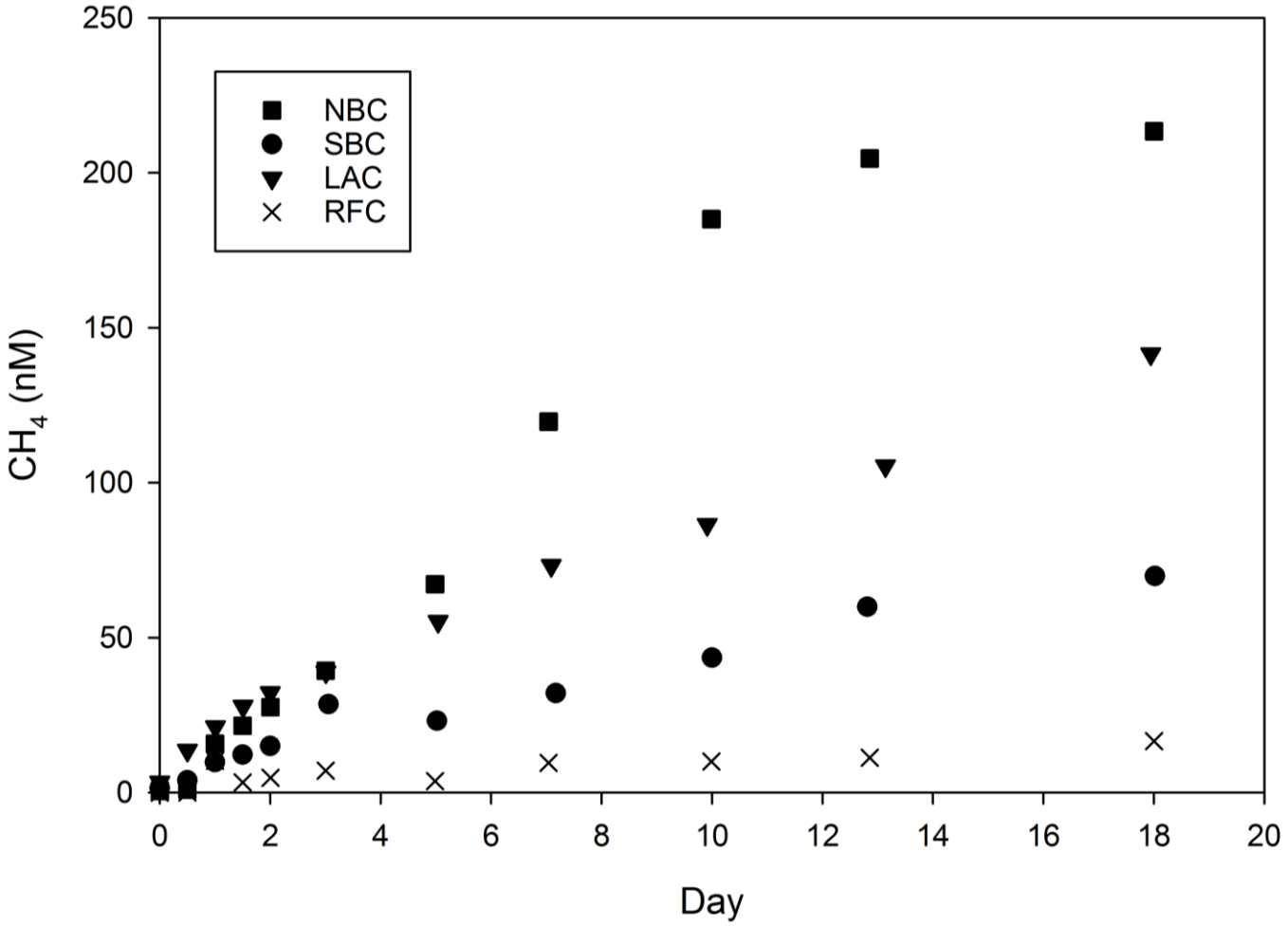

Figure 3. Methane Production in Control Treatments.

Average stream CH4 concentrations in streams plotted across day of sample collection. NBC = North Buffalo Creek, SBC = South Buffalo Creek, LAC = Little Alamance Creek, RFC = Reedy Fork Creek.

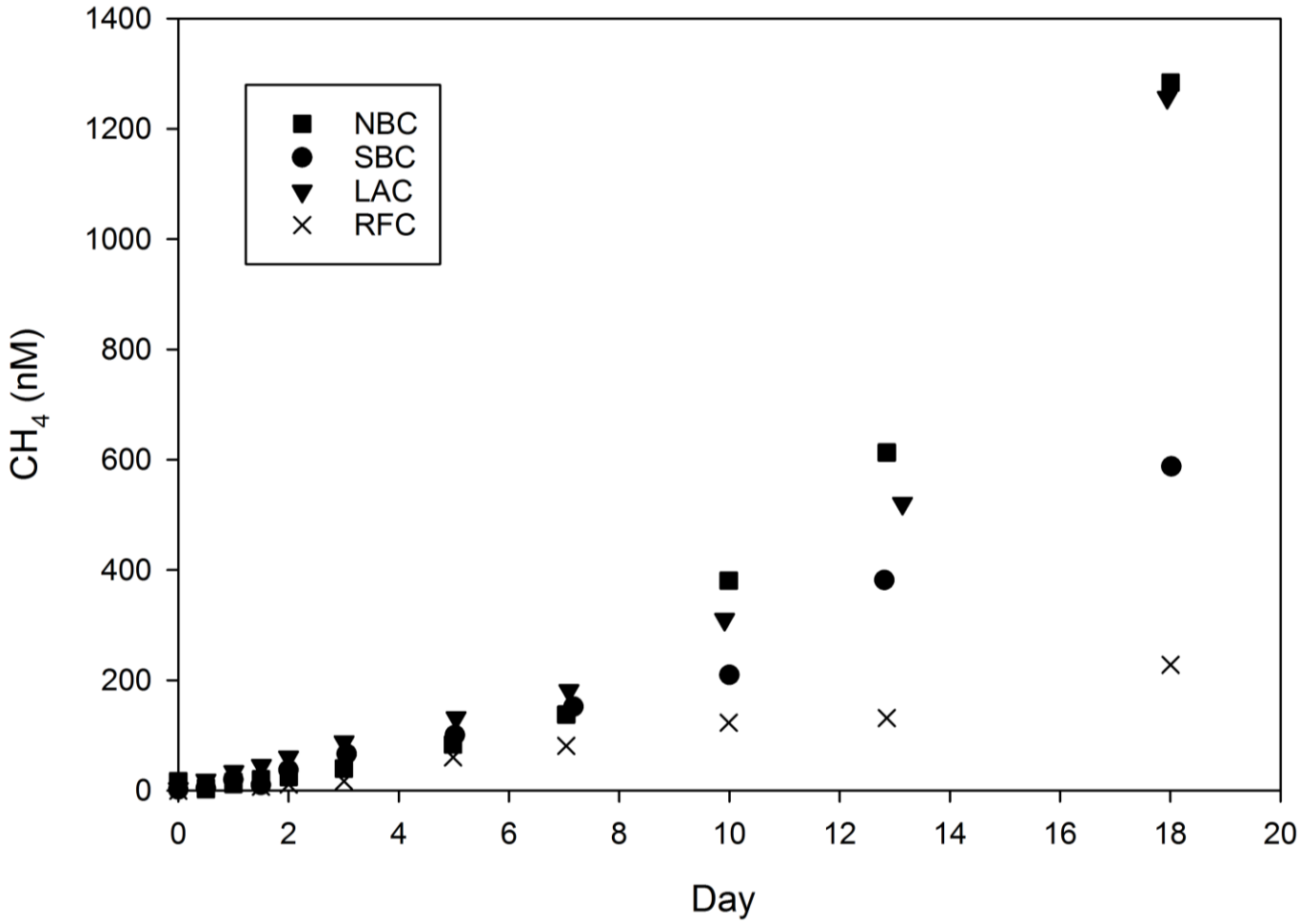

Figure 4. Methane Production in Molybdate Treatments.

Average stream CH4 concentrations in streams plotted across day of sample collection. NBC = North Buffalo Creek, SBC = South Buffalo Creek, LAC = Little Alamance Creek, RFC = Reedy Fork Creek

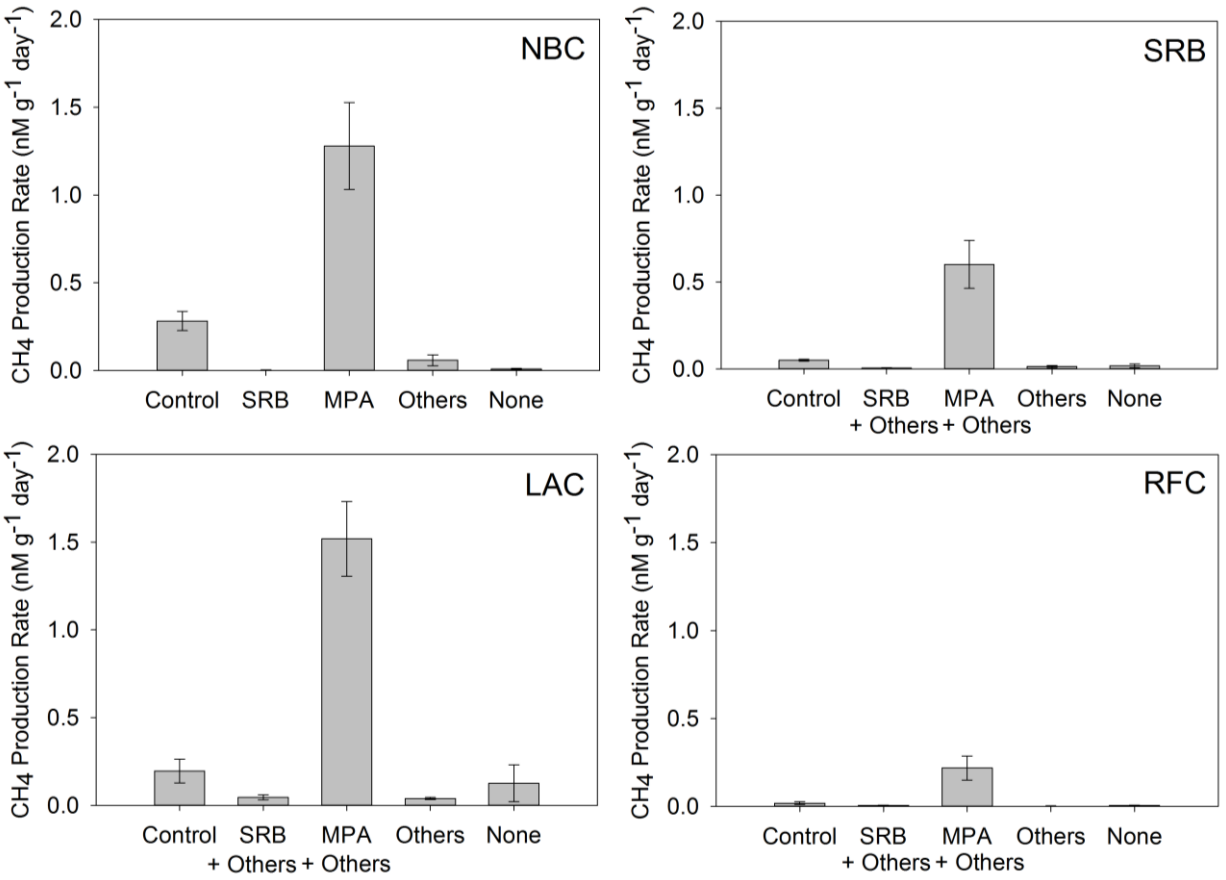

Figure 5. Methane Production across Streams.

Individual stream production of CH_4. NBC = North Buffalo Creek, SBC = South Buffalo Creek, LAC = Little Alamance Creek, RFC = Reedy Fork Creek. Major active microbial groups are represented on the x-axis. Error bars represent standard error.

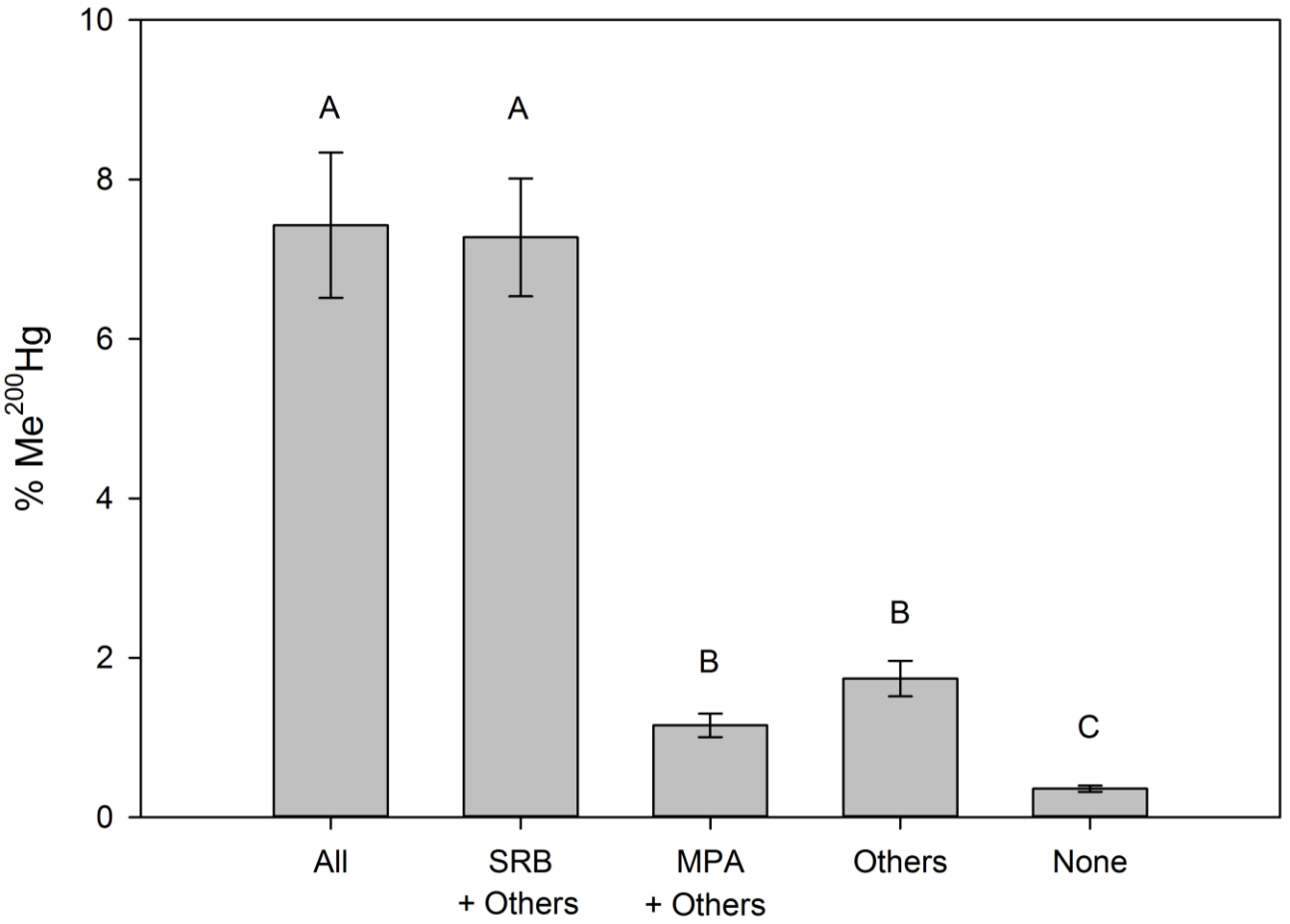

Figure 6. Treatment Methylmercury Production.

Individual streams were not compared. Major active microbial groups are represented on the x-axis. Error bars represent standard error. Letters above bars represent Tukey-Kramer HSD post-hoc comparisons.

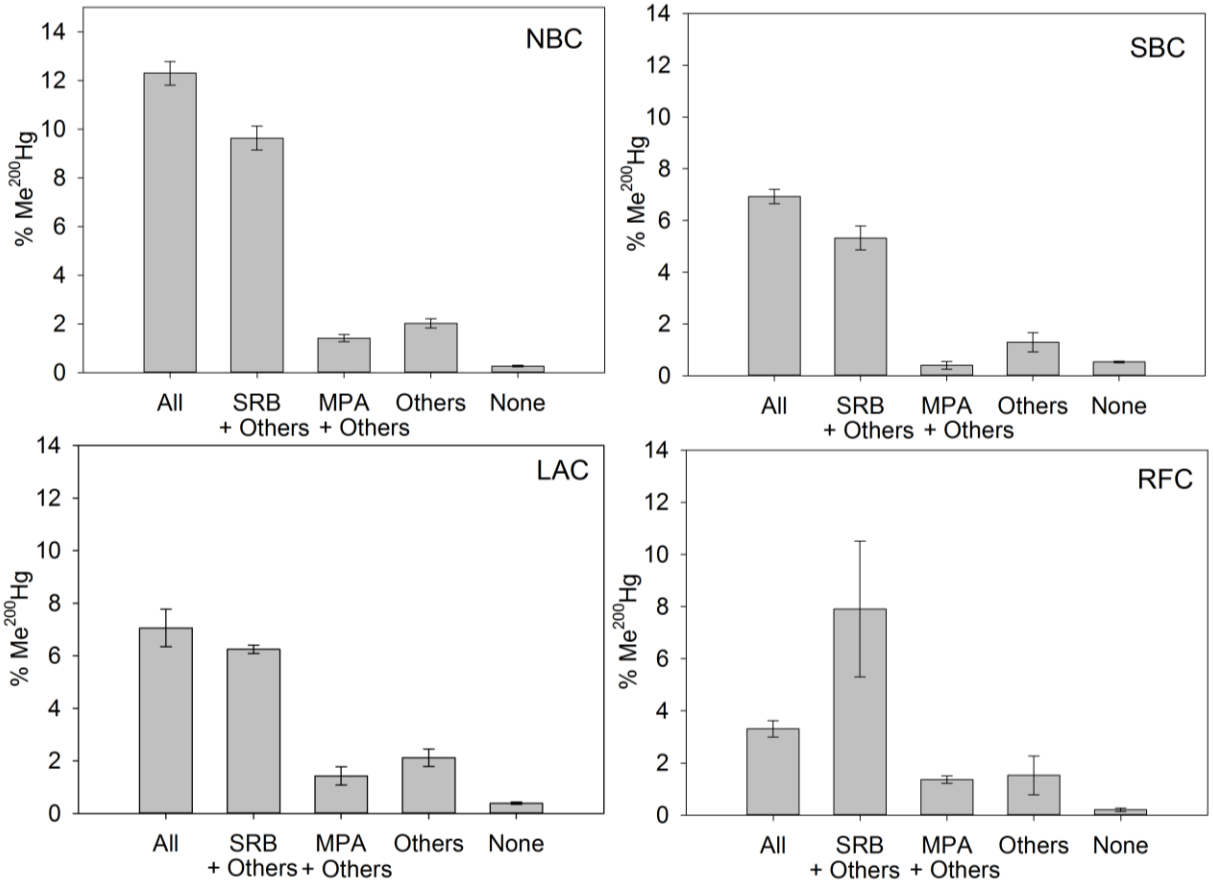

Figure 7. Methylmercury Production across Streams.

Individual stream production of MeHg. Major active microbial groups are represented on the x-axis. Error bars represent standard error. NBC = North Buffalo Creek, SBC = South Buffalo Creek, LAC = Little Alamance Creek, RFC = Reedy Fork Creek.

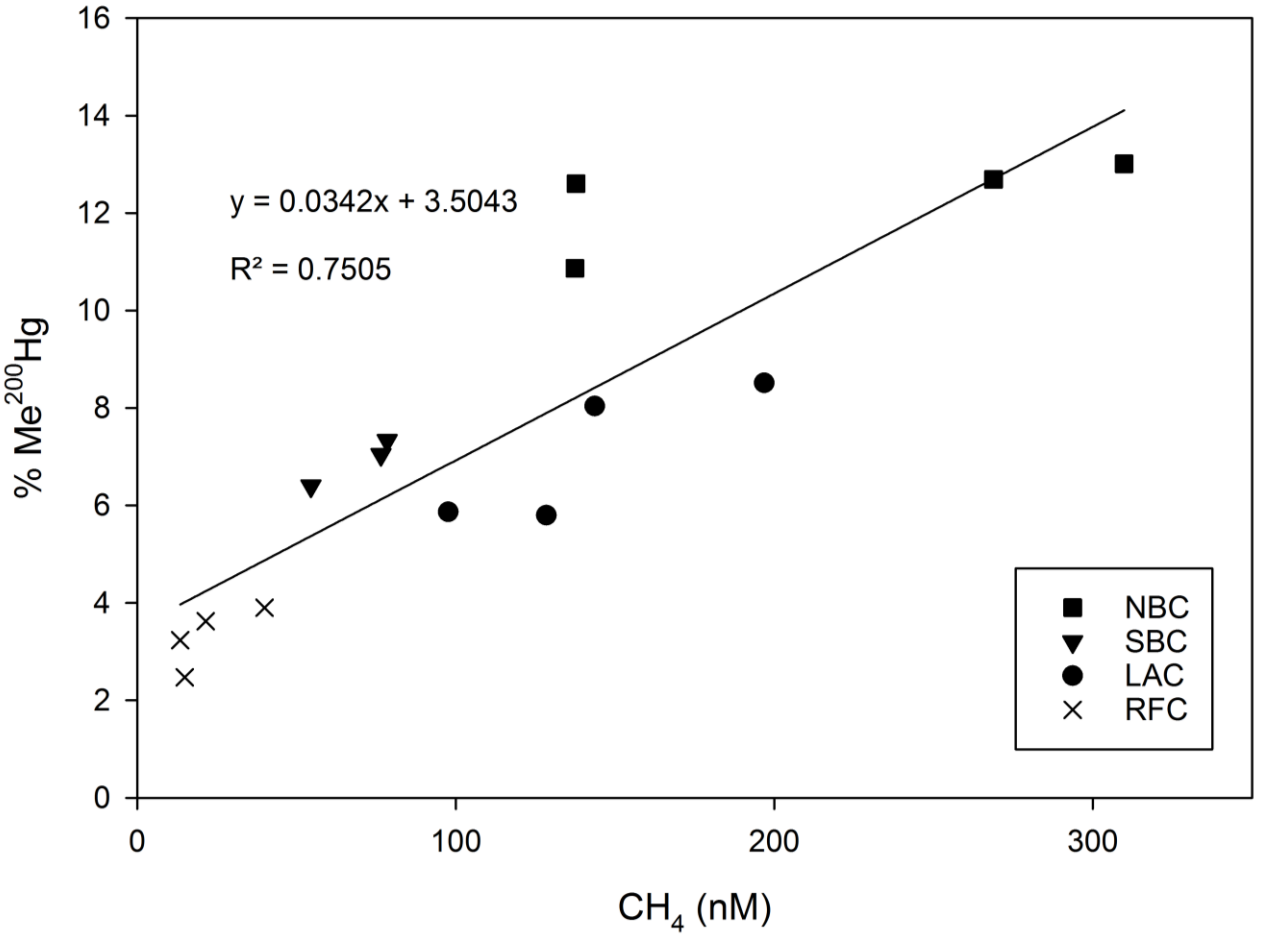

Figure 8. Control Methylmercury and Methane Regression.

NBC = North Buffalo Creek, SBC = South Buffalo Creek, LAC = Little Alamance Creek, RFC = Reedy Fork Creek.

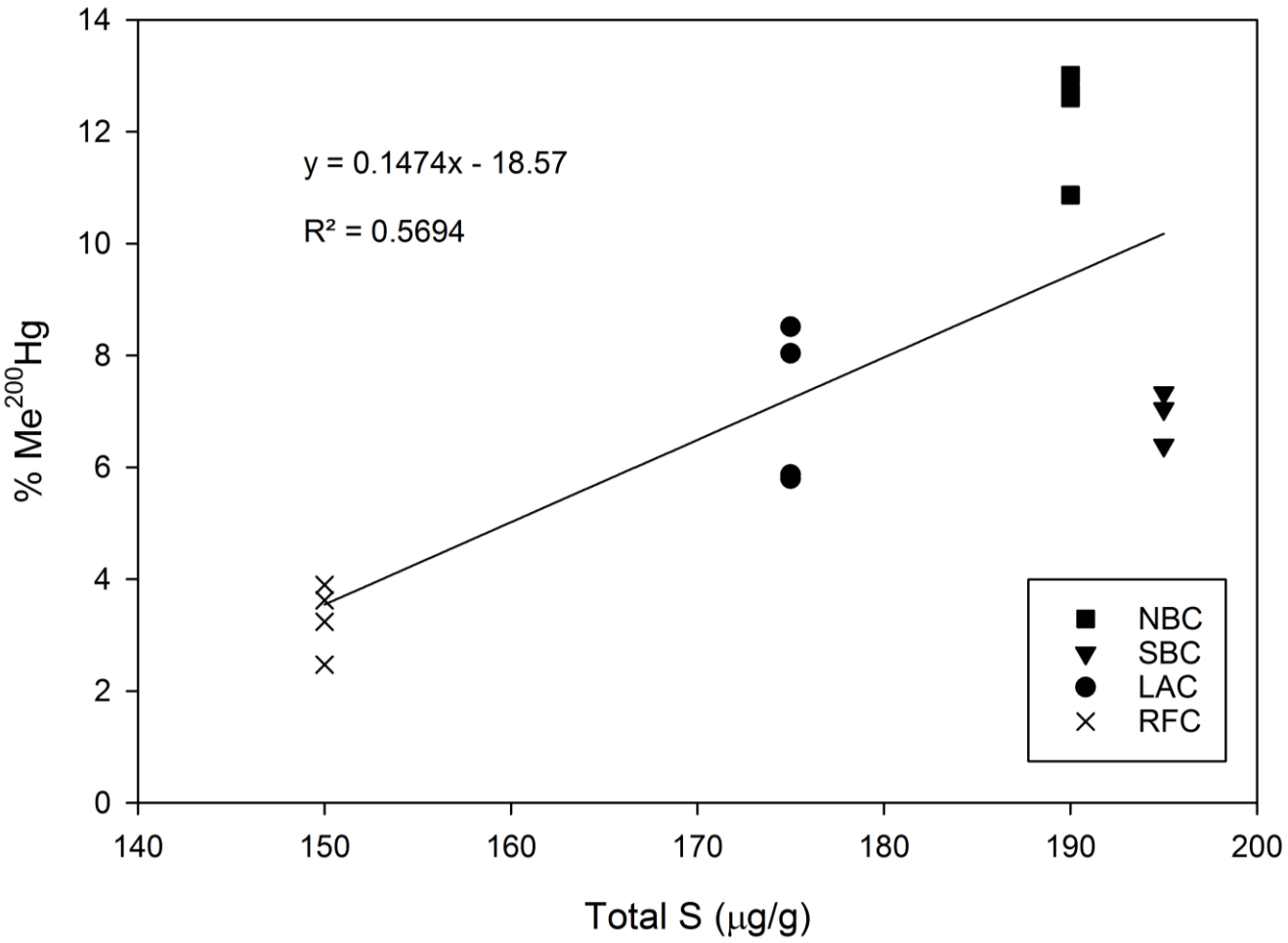

Figure 9. Control Total Sulfur and $Me^{200}Hg$ Regression.

NBC = North Buffalo Creek, SBC = South Buffalo Creek, LAC = Little Alamance Creek, RFC = Reedy Fork Creek.