NUTRIENT POLLUTION IN HUDSON RIVER MARSHES: EFFECTS ON GREENHOUSE GAS PRODUCTION

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

The Hudson Valley has experienced rapid development and urbanization over the last century. As a result, the release of untreated sewage from Combined Sewer Overflows (CSOs) continues to be of concern as a nutrient loading vector to the environment. The majority of nutrient pollution in the Hudson River Estuary is transferred to the lower Hudson, including Iona Island Marsh, from New York City through tidal forcing. Wetland systems are primarily composed of anaerobic sediment that is regulated by different energy constraints than more well-studied terrestrial systems. A nutrient addition incubation experiment was performed with Iona Island wetland soil that measured the production of carbon dioxide and methane over a two-week period in response to different combinations of carbon and nitrogen additions. The addition of carbon, in the form of acetate, to incubated wetland soils was found to cause significantly increased production of carbon dioxide and methane, both potent greenhouse gases. In contrast, nitrogen only additions, in the form of nitrate or ammonium, did not result in significantly increased greenhouse gas production compared to the no nutrient addition control treatment. These results suggest that CSO releases into the lower Hudson River Estuary are likely to stimulate increased pulses of both carbon dioxide and methane from Hudson marshes and provide added rationale to more tightly manage anthropogenic carbon release into the estuarine environment.

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INTRODUCTION

Wetlands are being subjected to increasing pollution from anthropogenic phenomena (Bianchi 2011). The Hudson valley has seen rapid development and urbanization over the last century, and it hosts one of the largest population densities in the United States (US Census Bureau 2011). Restoration efforts following the Clean Water Act of 1972 have done much to improve water quality in the Hudson (Brosnan et al. 2006); however, the input of nutrient pollution in the form of carbon (C) and nitrogen (N) remains significant (Howarth et al. 2006). The majority of nutrient pollution to the lower Hudson, including Iona Island Marsh, originates in New York City and is transported through tidal forcing (Griffith and Raymond 2011; Yoon and Raymond 2012). In New York City, and many other local riverfront communities, street runoff and raw sewage are combined into a single sewer system. During periods of high precipitation, a portion of the water from sewers containing combined untreated sewage and street runoff are expelled directly into the Hudson as Combined Sewer Overflows (CSOs), a matter of ongoing concern as a vector of nutrient loading in the environment (Griffith and Raymond 2011).

Marshes are a major interface between terrestrial and aquatic habitats, providing crucial ecosystem services. For example, marshes are believed to play a critical role in filtering toxins from watersheds and providing a buffer to coastal flooding during major storm events (Barbier et al. 2011). Estuaries and associated marshes are also important fishery habitats and act as a nursery to many species of fish (Limburg et al. 2006). Therefore, the wetland loss observed in recent decades has been a matter of concern in coastal New York. For example, Jamaica Bay, NY, has experienced a steady increase in

salt marsh fragmentation and conversion to mud flats and pools since the 1950s (Hartig et al. 2002). Small plot – scale experimental manipulations (Deegan et al. 2002; Turner et al. 2009) and marsh-scale studies (Deegan et al. 2012) have shown that nutrient enrichment can lead to changes in plant physiology leading to decreased formation of below ground roots, consequently increasing erosion and wetland loss (Morris and Bradley 1999; Turner et al. 2009); however, less work has been conducted to describe the role microbial communities have in changing wetland dynamics following nutrient enrichment (Bowen et al. 2009).

Wetland systems are primarily composed of anaerobic sediment that is regulated by different energy constraints than more well-studied terrestrial systems (Reddy and DeLaune 2008). In anaerobic sediments, microbial communities are forced to use alternative electron acceptors, and subsequently alternative metabolic pathways to generate energy (Conrad 1996); however, these alternative acceptors have lower redox potentials than oxygen and their reduction generates less energy per mol of organic material degraded (Canfield et al. 2004; Thauer et al. 1977). The energy constraints of oxygen-deprived microbial communities lead to the buildup of recalcitrant C pools in anaerobic zones as organic C remains energetically unavailable (Bridgham et al. 2006). Nutrient additions to anaerobic soil could trigger the activation of microorganisms that promote increased greenhouse gas (GHG) emissions such as carbon dioxide (CO_2) and methane (CH_4) and possibly facilitate soil C loss (Blagodatskaya and Kuzyakov 2008). Therefore, elucidating the mechanistic interactions between nutrient additions, microbial activity, and GHG production is crucial in order to understand the consequences of sewage release into local waterways and more effectively manage estuaries.

The objective of this experiment was to quantify the microbial response to C and N addition by measuring the production of CO_2 and CH_4 in anaerobic soil slurries exposed to different C and N treatments. It was hypothesized that C additions to soil slurries would increase the decomposition of organic matter measured by an increase in both CO_2 and CH_4 rates, while N addition would not stimulate additional GHG production.

METHODS

Overview. The experiment aimed to quantify soil anaerobic microbial response to C and N sources, and the subsequent production of the GHGs; CO_2 and CH_4. Sediment cores and overlying water were collected from Iona Island Marsh, NY and utilized to created soil slurries in one-liter Mason jars. A nutrient addition incubation experiment was performed that measured the production of CO_2 and CH_4 in response to different combinations and forms of C and N over a two-week period. Following the incubation, units were broken down and extracted water and soil slurry samples were collected for future biogeochemical and molecular analysis (beyond the scope of this fellowship report).

Site description. Iona Island marsh (Figure 1), located 60 km north of New York City, was chosen for its proximity to Queens College, and its relatively low salinity concentrations. In World War II it served as a U.S Navy ammunition depot. In 1965, it was donated to the Palisades Interstate Park Commission. Today, it is one of four wetlands composing the Hudson River National Estuarine Research Reserve. It is a sanctuary for many bird species and marine and aquatic organisms like ducks and crabs, which were observed during field excursions there.

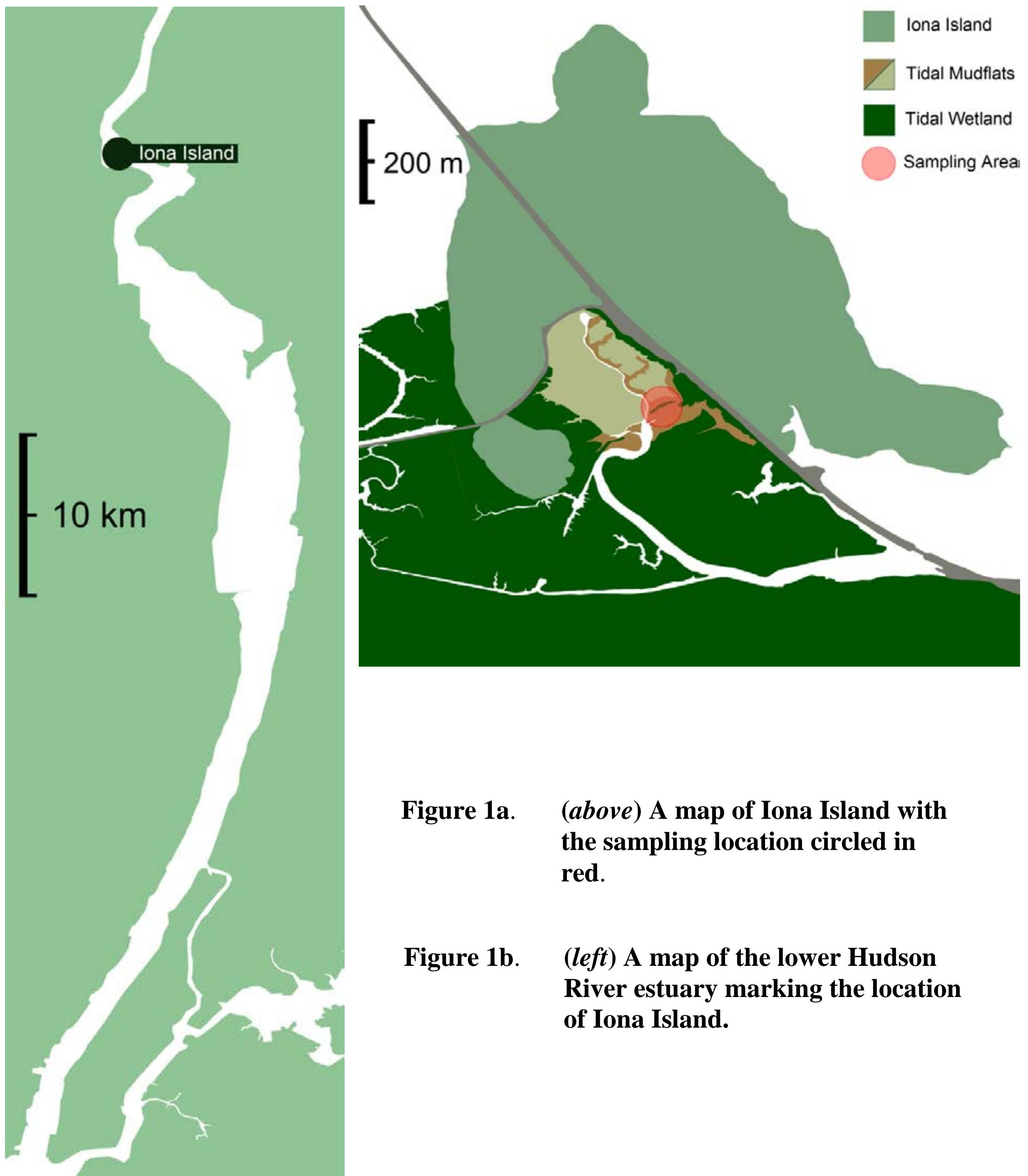

Figure 1a. (*above*) **A map of Iona Island with the sampling location circled in red**.

Figure 1b. (*left*) **A map of the lower Hudson River estuary marking the location of Iona Island.**

Field sampling phase. Soil samples were collected from eight sites with an average distance of 10 meters from each other. Overlying water (20 liters) was collected in close proximity to collection sites. Sediment cores were collected from each site with an AMS specialty wetland soil corer equipped with plastic core liners. The corer was driven into the ground, rotated clockwise 90 degrees, and slowly pulled up to extract a core. The cores varied from approximately 18 - 20 cm in length and were 5 cm in diameter. The core liners were then capped and stored in a collection bag at ambient temperature. Once all sites were visited, the soil cores were stored in an ice-cooled chest, while the overlying water was stored in containers at ambient temperature.

Preparatory phase. Upon arrival in the laboratory, the material collected was immediately processed and prepared for incubation. Soil cores were removed from liners and placed at 4°C in Ziploc bags. Before soil slurry unit fabrication, individual cores from each site were mixed together into homogenous slurry. The overlying water was filtered (0.22 micron Sterivex filter), autoclaved, degassed, and refrigerated (4°C). To prepare Mason jar units, approximately 105 g of wet soil was weighed. A small sample of approximately 5 g was removed from this mass, weighed, and stored for soil C and N analysis. The remainder (100 g) was weighed, and inserted into the Mason jar. The jar was then filled with approximately 200 g of processed overlying water and shaken at low rpm for 10 minutes. All the units were then flushed with nitrogen using a one hour procedure to remove oxygen from both the headspace and water of the experimental units. Seven replicate units from each site were constructed, for a total of 56 Mason jar units. A single replicate from each site was randomly assigned to one of seven treatment groups. The groups consisted of (Table 1): pre-treatment (PT); negative control (NT);

nitrate addition (NO_3); ammonia addition (NH_4); acetate addition (A); acetate + nitrate ($A+NO_3$); and acetate + ammonia addition ($A + NH_4$).

Pre-incubation phase. After preparing Mason jar units, a two–week incubation period was completed before nutrients were added. Units were stored in a temperature controlled incubation chamber at 25°C for both the pre-incubation and the incubation period following nutrient addition. CO_2 headspace concentrations were measured in the pre-incubation units to monitor microbial activity. At the conclusion of the pre-incubation, indicated by complete reduction in CO_2 production rate, nutrients were added as noted below (Table 1) to simulate expected nutrient loads following storm events (Griffith and Raymond 2011). The amount added was approximately ten-fold higher than the daily rate modeled by Griffith and Raymond 2011 simulating expected nutrient loading during storm events (Yoon and Raymond 2012).

Immediately following the nutrient addition, all units were flushed to eliminate any accumulated GHGs before the incubation began.

Treatment Type	**Vol (mL)**	**Nutrient Concentrations**
No Treatment (NT)	10	deionized water
Nitrate (NO_3)	10	potassium nitrate to final concentration of 3.57 mmol N
Ammonium (NH_4)	10	ammonium chloride to final concentration of 3.57 mmol N
Acetate (A)	10	sodium acetate to final concentration of 16.67 mmol C
Acetate + Nitrate ($A + NO_3$)	10	potassium nitrate + sodium acetate for final concentrations of 3.57 mmol N/16.67 mmol C
Acetate + Ammonium ($A + NH_4$)	10	ammonium chloride + sodium acetate for final concentrations of 3.57 mmol N/16.67 mmol C

Table 1. Nutrients added to treatment groups with C and N concentrations per L of volume (wet soil sample + overlying water volume.)

Nutrient phase. During the main incubation phase, both CO_2 and CH_4 headspace concentration were measured in regular time intervals, every 24 hours and every 48 hours, respectively. CO_2 was measured with an EGM-4 (IRGA) environmental

gas monitor and CH_4 was measured with a Hewlett-Packard 5890 Series plus II Gas Chromatograph (GC) installed with a Flame Ionization Detector. A 60 ml plastic BD syringe was used to extract gas samples from units. To extract a headspace sample, the syringe was inserted through the unit's septum, the headspace was then mixed by gently pulling the syringe's plunger up to 50 ml and back to 0 two times. The desired volume of headspace sample was then extracted. During days when both CO_2 and CH_4 were measured, 30 ml of headspace was extracted from the unit. From that 30 ml, 5 ml was initially expelled, 10 ml was injected into the GC and analyzed for CH_4, and the remaining 15 ml inserted into the EGM-4 and analyzed for CO_2. On days when only CO_2 was measured, only 15 ml of the unit's headspace sample was extracted and analyzed. The GC and EGM-4 were both flushed (air and N_2 respectively) with gas after each unit was sampled. The volume of gas extracted from the experimental unit's headspace was replaced with nitrogen after sampling.

Breakdown. At the end of the pre-nutrient (pre-treatment units only) and nutrient phases the Mason jar units being sampled were broken down, and slurry-water mix tested for pH, redox, and salinity (Sensorex reference electrodes) with the Micro Observatory sensor system (Analytical Instrument Systems). Each unit was first shaken for ten minutes. Before sampling, the unit slurries were inverted three times to homogenize the contents. The units were then opened, and probes inserted into the slurry mix to be analyzed. After probe-measurements, soil and water samples were vacuum filtered, collected, and frozen for future biogeochemical measurements.

Statistics. Data from the end point of experimental incubations were analyzed using software from the R project for statistical computing (www.r-project.org). Analysis

of Variance (ANOVA) was used to test for differences in the mean and if significant differences were detected among groups, a post-hoc Tukey's Range Test was used to adjust p-values for multiple comparisons to identify the pairs of experimental units with significantly different means.

RESULTS

Greenhouse gas production. CO_2 production rates increased in carbon-treated groups (A+NH_4, A+NO_3, A), most notably in days 8, 9, and 10, after which production rates leveled off (Figure 2). A temporary decrease in CO_2 accumulation was observed in the A+NO_3 group in the 2nd day of the experiment. Total CO_2 production differed significantly ($p < 0.01$) among treatments (Figure 3) with greater production found in C addition treatments. Only A ($p<0.01$), A+NO3 ($p<0.01$), and A+NH4 ($p<0.01$) treatment groups were significantly different from the control (NT) treatment. In contrast, N additions did not have a significant effect on CO_2 production when compared to the no addition control. In C–treated groups, an average of 460 µg C/ g of dry soil accumulated as CO_2 was produced. In contrast, an average of 229 µg C/ g of dry soil accumulated as CO_2 was produced in the NO_3, NH_4, and NT groups.

CH_4 production was observed in C addition treatments but was not measurable in N only and control treatments (Figure 4). Final CH_4 differed among groups (ANOVA, $p<0.01$) (Figure 5); however, only A ($p < 0.05$) and A+NH_4 ($p < 0.01$) treatments were significantly different from the no addition control (NT) treatment. In C–treated groups, an average of 206 µg C/ g of dry soil accumulated as CH_4 was produced. In contrast, an average of 3 µg C/ g of dry soil accumulated as CH_4 in the NO_3, NH_4, and NT groups.

In total, 66% percent (μg C / g of dry soil) of gaseous C measured was in the form of CO_2, while CH_4 made up 33% of gaseous C measured. C + N treatment groups accumulated 3.3 times as much C compared to treatments with N only addition. CO_2 comprised 51% of total C accumulation in acetate, 53% in A + NH_4, 73% in A + NO_3, and >99% for all other treatments. CH_4 comprised 49% of total C accumulation in acetate, 47% in A + NH_4, 27% in A + NO_3, and < 1% for all other treatments.

Probe measurements Redox measurements conducted at the end of the incubation period demonstrate that the most significant reduction of experimental units occurred in C treated groups (Figure 6). Significant differences were detected among groups (ANOVA, $p<0.01$). The A ($p<0.01$), A+NH_4 ($p<0.01$), A + NO_3 ($p<0.01$), and NO_3 ($p<0.01$) treatments differed significantly from the no addition control treatment.

There were significant differences in pH among groups ($p < 0.01$). The measured pH for the C–treated groups varied from 7 – 7.5. In contrast, the pH measured in N only and control groups varied from 6.5 to 7.0. Only A ($p < 0.01$), A+NO3 ($p < 0.01$) and A+NH4 ($p<0.01$) treatments significantly differed from the no addition control (NT). The salinity levels measured in the field varied from 0.5 to 2.0 ppt.

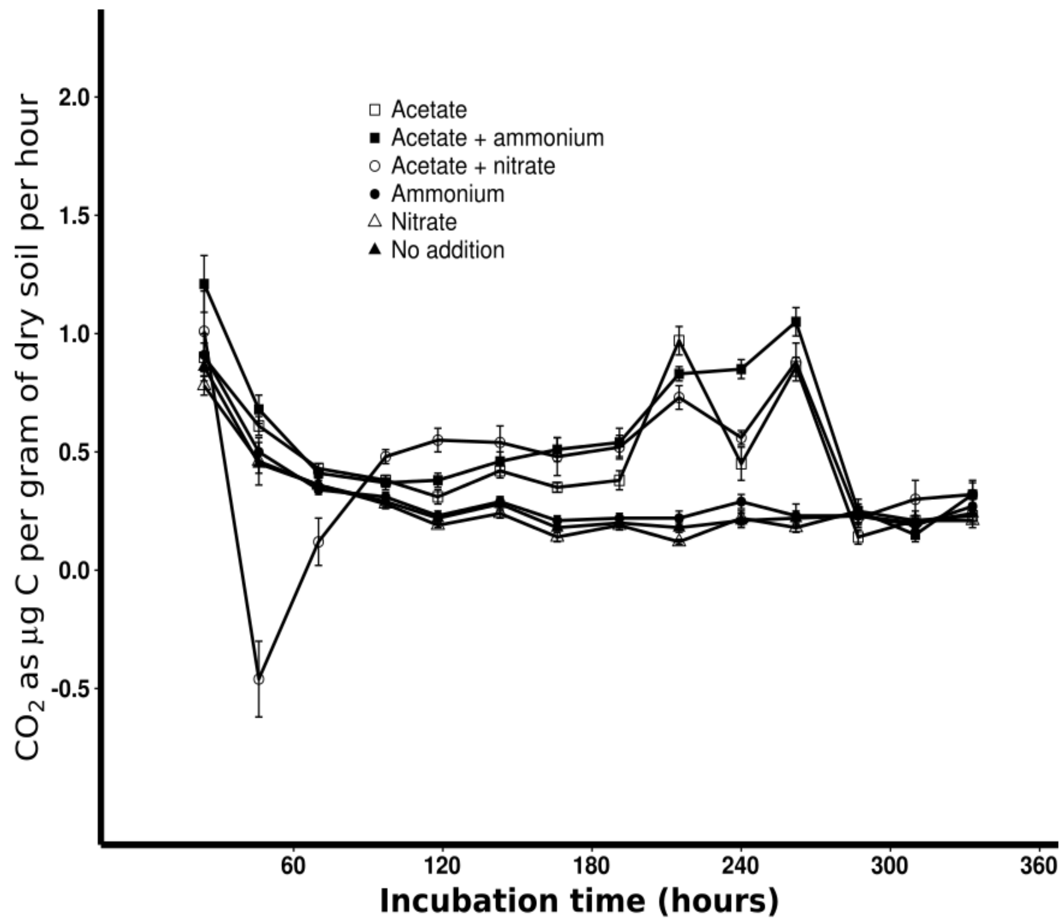

Figure 2. **CO_2 production rate for the treatment groups through the two – week incubation period. The rate is measured as the amount μg C produced as CO_2 since the last measurement point. The values were adjusted for slight variations in temperature and pressure, and normalized to the rate of C production per gram of dry soil weight present in the unit.**

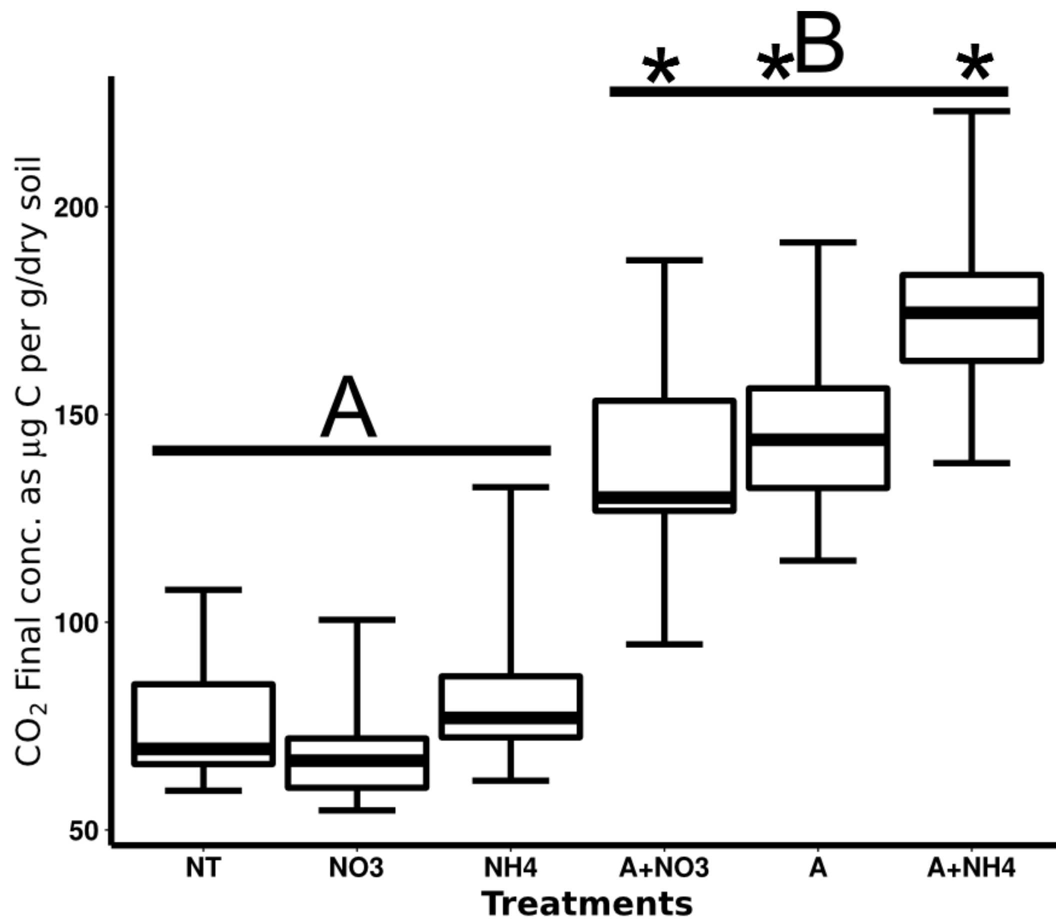

Figure 3. **Box plot demonstrating the amount of C mineralized as CO_2 at the end of the experiment. Concentrations were adjusted for slight temperature and pressure differences, and normalized to total µg C produced per gram of dry soil. Significant differences from the control are marked with an asterisk and groups that do not show significant differences among samples are designated with a letter.**

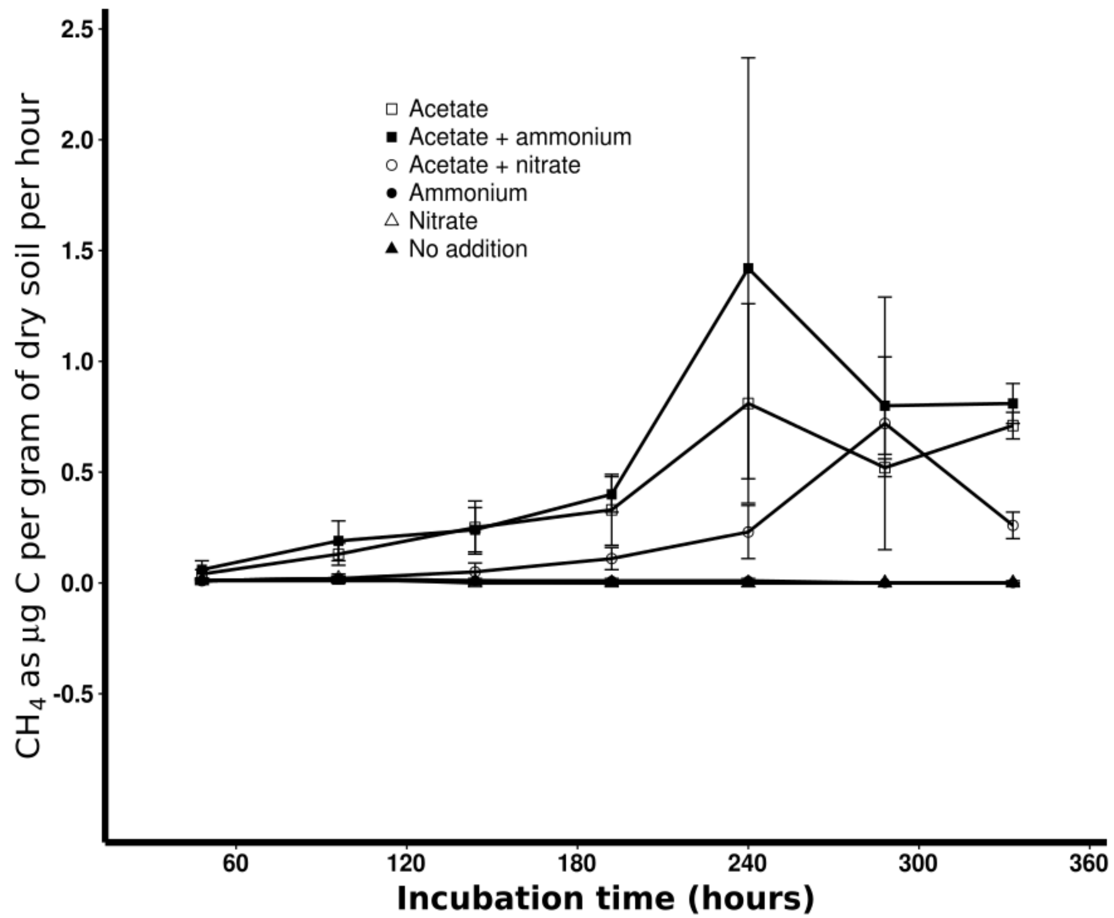

Figure 4. **CH_4 production rate from the treatment groups through the two – week incubation period. The rate is measured as the amount µg C produced as CH_4 since the last measurement point. The values were adjusted for slight variations in temperature and pressure, and normalized to the rate of C production per gram of dry soil weight present in the unit.**

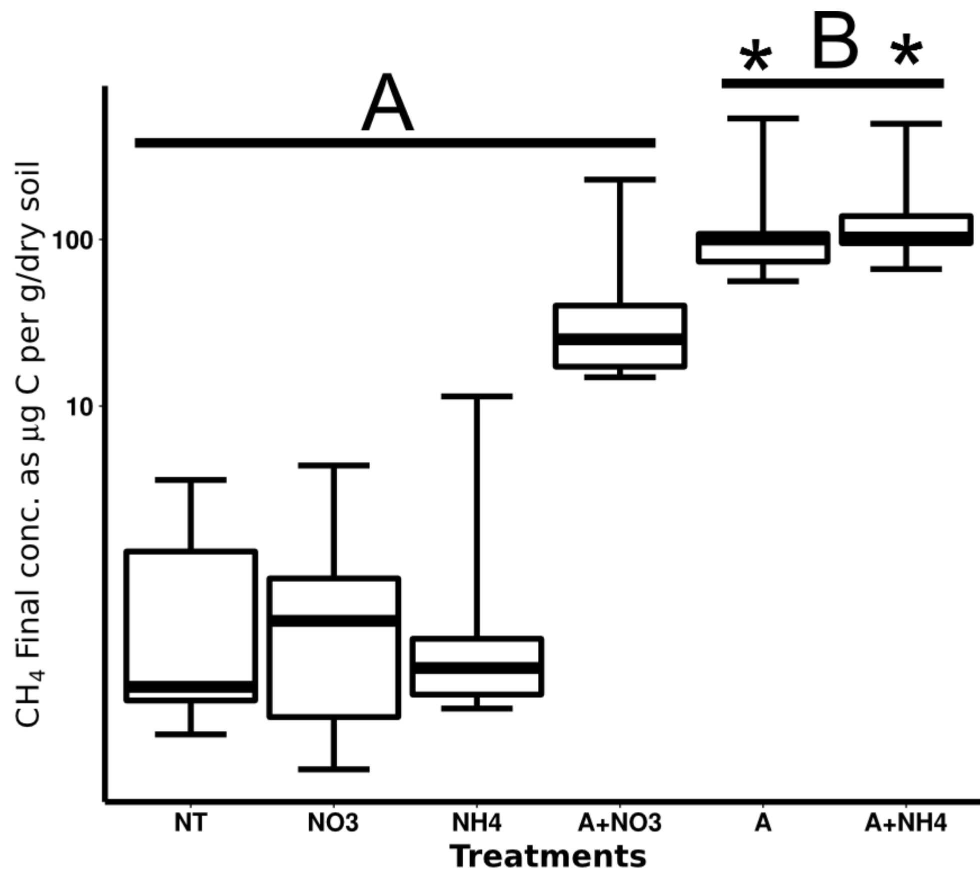

Figure 5. **Box plot demonstrating amount of C mineralized as CH_4 at the end of the experiment. Concentrations were adjusted for slight temperature and pressure differences, and normalized to total µg C produced per gram of dry soil. Note log scale of axis. Significant differences from the control are marked with an asterisk and groups that do not show significant differences among samples are designated with a letter.**

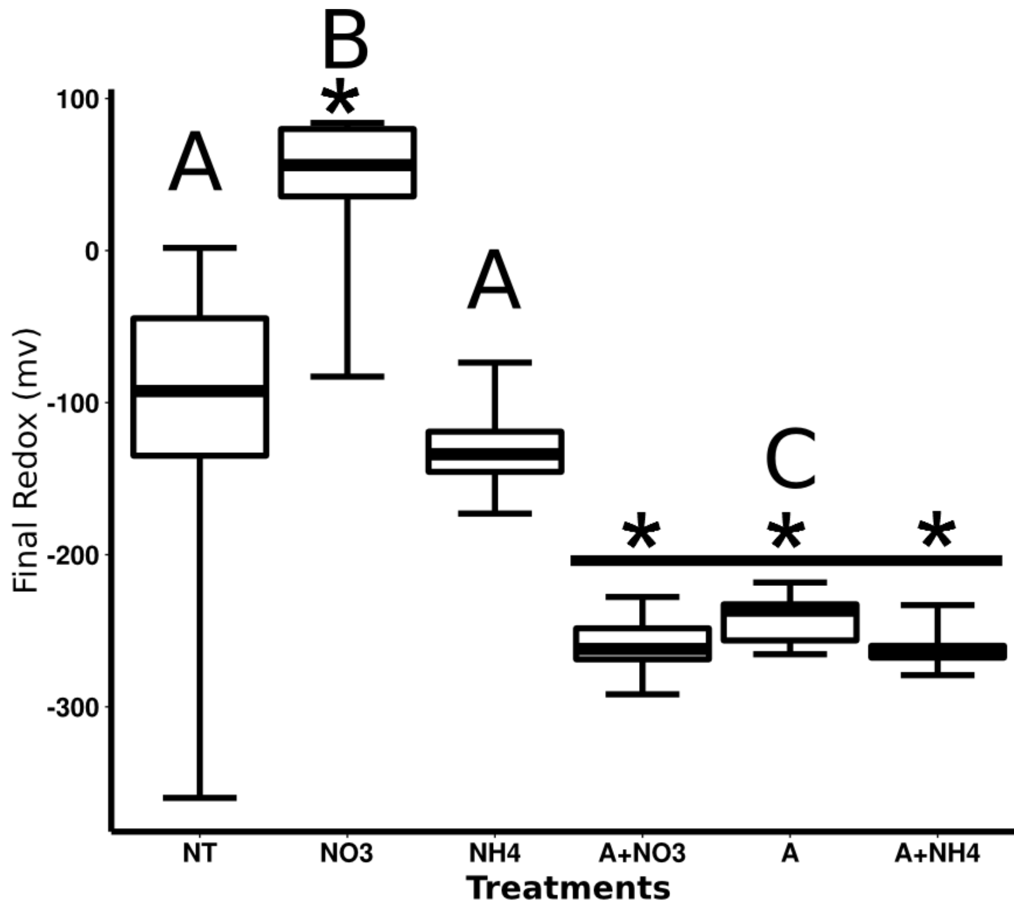

Figure 6. **Box plot showing redox condition at the end of experiment. Significant differences from the control are marked with an asterisk and groups that do not show significant differences among samples are designated with a letter.**

DISCUSSION

Nutrient additions, similar to concentrations that would be expected in post-storm nutrient pulses, were found to have variable influence on GHG production, depending on the chemical composition of the addition. The addition of acetate, with or without ammonium or nitrate, was found to cause significant increases in both CO_2 and CH_4 production in wetland soil slurries. Nutrient cycling in wetlands is generally thought to be controlled by the availability of C and N sources to produce energy for metabolism and growth (Conrad 1996). In the wetland system studied, anaerobic microbes use alternative electron acceptors in lieu of oxygen; however, the energy available through these compounds is very low in comparison to aerobic respiration (Thauer et al. 1977). As a consequence, microbial growth is often sluggish in anaerobic environments as they are starved for energy and unable to utilize the recalcitrant C pool present. It appears that microbial communities in these soils were limited mainly by energy in the form of easily degradable C. It is therefore not surprising that the redox potential in these treatments was also found to decrease and that, under these reduced redox conditions, CH_4 was produced in substantial quantities relative to the treatments lacking labile C additions and characterized by higher redox potential.

In contrast, the addition of nitrate or ammonium alone was not sufficient to increase GHG production from marshes as compared to the no addition control, suggesting that N limitation is not a major factor acting to suppress anaerobic metabolism, nor did it cause a reduction in redox potential of the experimental units by stimulating anaerobic metabolism. The addition of nitrate can also act as a favorable electron acceptor in anaerobic systems, and was observed to increase redox potential, as

expected, suggesting that denitrification might become a dominant energy producing pathway in this treatment. When adequate electron donors are available, microbial communities can be differentiated by their preference of electron acceptors that vary in metabolic efficiency. In the absence of aerobic respiration, due to the lack of oxygen, denitrification is the next most efficient catabolic process, followed by iron reduction, sulfate reduction, and lastly methanogenesis, which generally occurs only in highly reduced conditions (Conrad 1996). Microbial activity would be expected to be dominated by metabolic pathways providing the highest available energy yield, and as favorable electron acceptors become depleted, microbial activity would shift according to the redox condition. In saline environments, sulfate reduction has been found to produce ten times more energy than other metabolic activities (Howarth and Teal 1980) due to the high concentration of sulfate. Presence of sulfates has also been found to hamper methanogenic activities (Martens and Berner 1974; King and Wiebe 1980). Iona Island is typically fresh (0.5 - 2.0 ppt) suggesting that denitrification and methanogenesis would be expected to be important pathways utilizing available organic C; however, in this experiment the addition of N alone did not result in increased CO_2 or methane production, indicating that the system was primarily limited by availability of electron donors, not electron acceptors. In addition, it is expected that the lower Hudson area already receives ample N from wastewater sources (Brosnan et al. 2006), which would make N widely available in marshes such as Iona Island.

These findings are important to interpret in the context of sewage and other nutrient pollution sources. Sewage contains high levels of labile C that, based on these findings, would be expected to increase GHG production from marshes in both the form

of CO_2 and CH_4, while also reducing the redox potential in impacted marsh soils, causing an increase in the relative utilization of C for anaerobic respiration. N can be released into the estuary either from sewage or other sources such as fertilizer usage. It appears that the addition of N may have little direct impact on GHG production in Iona Marsh sediment; however, the N pollution sources could still have indirect impacts on marsh soils by stimulating primary production in the estuary and thereby resulting in the addition of labile C to anaerobic marsh soils (Bianchi 2011). Management activities, such as reduction in CSOs and improved efficiency of wastewater treatment, that reduce N pollution, and especially those that reduce C pollution, would be expected to reduce GHG production from Iona Island marsh and similar wetland systems.

CONCLUSIONS

These results highlight the importance of labile C as a mediator of GHG production. This is importantly noted, as previous studies in northeastern U.S.A have largely focused on the role of N impacts on wetland habitats (Deegan et al. 2002). There has been little previous work on the combined effects of C and N on microbial communities in the Hudson River Estuary. The results from this experiment show that C has a larger effect on microbial activity in Iona Island marsh than N. The results show that C should be a more closely monitored element in water quality research and steps should be taken to minimize watershed exposure to C from anthropogenic sources.

Methanogenesis was shown to be strongly influenced by C addition. This is important to consider as CH_4 is thought to have 25 times the warming potential of CO_2 over a hundred year period (IPCC 2007). Studying the ways CH_4 is produced and how

anthropogenic activities affect CH_4 production is vital to help mitigate the effects of climate change. CSO releases would be expected to stimulate pulses of CH_4 from Hudson marsh environments. Coupled with the increasing vulnerability of this fragile system to climate change and increased storm events, a closer look at the intricate relationship between anthropogenic pollution and the Hudson's health is warranted in order to better direct ecosystem conservation and restoration efforts.

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