Effects of *Typha x glauca* on Methane Emissions in Freshwater Ecosystems: Implications of Invasive Species Effects on Global Climate Change

Buck Tanner Castillo

University of Michigan Biological Station

1933 Biological Road

Pellston, MI 49769

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ABSTRACT

Across the globe, freshwater ecosystems play vital roles in global nutrient and emission cycles and provide key ecosystem services. Great Lakes wetlands have increasingly been dominated by an invasive cattail hybrid? *Typha x glauca*. Cheboygan Marsh, a Great Lake marsh formed by Lucustrine deposits, was invaded by *Typha* in the 1950's. Resent surveys indicate *Typha* now dominates two-thirds of the ~150 ha marsh. *Typha* has the propensity to alter freshwater ecosystems by forming dense stands of live and dead biomass. As *Typha* biomass accumulates and decomposes in the soil it can act as a fuel for methanogenesis when coupled with anaerobic conditions often found in these ecosystems. As tipping points in global climate change are quickly being approached research on both anthropogenic and natural emissions are warranted. In this study we used both a field and mesocosm experiment to test for the effect *Typha* invasion is having through its leaf litter on methane emissions. We found soil organic carbon to be in much higher concentrations in *Typha* zones than Native vegetation zones dominated by sedges and rushes in Cheboygan Marsh. In the mescocom array we also found a positive correlation between higher soil organic carbon and increased methane flux rates. These finding may suggest that the possibility exists for *Typha* invasion to play a significant role in future climate change.

INTRODUCTION

Globally, freshwater wetlands are important ecosystems that serve many ecosystem functions. Freshwater wetlands buffer inland habitats from flooding, store storm waters, provide habitat for both plant species and wildlife, and can act as a sink for organic carbon, nitrogen, and phosphorus (Angeloni et al. 2006, Charles & Dukes 2007; Kercher & Zedler 2004). Wetlands can prevent eutrophication from occurring downstream by storing and filtering out nutrients (Mitch and Gosselink 200**0**). Wetlands affect nutrient dynamics through the interrelationships between soil microbial communities, hydroperiods, and wetland plants (Angeloni et al. 2006; Boers et al. 2007; Frieswyk et al. 2007; Lishawa et al. 2010; Zedler et al. 2004). For example, denitrifying bacterial communities are important since they drive the conversion of nitrates in the soils into inert N_2.

While freshwater wetlands offer many beneficial ecosystem services, they can also play important roles in global cycles as sources for greenhouse gas emissions. Globally, freshwater wetlands are responsible for 1/3 of annual methane emissions (IPCC 2007). These emissions are especially significant when considering the global warming potential of methane, which is 25 times that of CO2 (IPCC 2007). Methanogenesis is the process by which methane is produced and requires an extremely reduced amount of oxygen in the soils. Redox, or oxidation-reduction potential, is used to determine the amount of oxygen in the soil (Mitsch & Gosselink 2000) and can predict the reactions occurring in the soils (DeLaune & Reddy 2005). Typically, CH4 production occurs when redox potentials are below -200mV (Mitsch & Gosselink 2000). Organic carbon accumulation and anaerobic (low redox) conditions typical of many freshwater wetland ecosystems are the main drivers for methanogenesis in wetland soils. Exotic species that invade freshwater wetlands can promote organic matter accumulation (Tuchman et al. 2009, Rooth et al. 2003, Kercher and Zedler 2004) and potentially alter methane dynamics.

Wetland ecosystems are delicate bionetworks that are highly susceptible to invasions by exotic species. Less than 6% of the earth's surface is covered in wetlands

yet up to 24% of the worlds most invasive plant species are found in wetlands (Zedler and Kercher 2004). There are several reasons that wetlands are so susceptible to invasion such as possible seed dispersal into wetland ecosystems from terrestrial ecosystems and through seeds being carried in from water currents. Wetlands are often low-lying lands where seeds come to rest. Wetlands that have experienced hydrological disturbances are also particularly prone to invasions by exotics (Zedler and Kercher 2004).

Invasive plant species alter ecosystems both directly and indirectly. They directly outcompete native plants for available nutrients and sunlight. Invasive plant species are often superior competitors than the native species of the ecosystems they invade(Angeloni et al. 2006). They can lead to alterations indirectly by causing changes in the microbial communities associated with the plants (Angeloni et al. 2006). Another mechanism through which invasive plant species alter ecosystems is through their leaf litter (Farrer & Goldberg 2010). Invasive plant species are often larger than the native species they displace, thus having greater biomass that accumulates on the soil surface over time. As this biomass accumulates it can lead to various changes in community structure and functionality. An accumulation of biomass through leaf litter legacy can decrease temperatures at the soil surface, reduce the amount of light that reaches the soil, and can act as a carbon sink over time (Freyman 2008, Farrer & Goldberg, 2010, Larkin et al. 2011). Various community changes can accompany invasions including reduced biodiversity, changes in hydrology, and loss of ecosystem functions (Charles and Dukes 2007, Ehrenfeld 2003, Larkin 2009).

Typha x glauca, a hybrid cattail formed when *Typha latifolia* (broad-leaved cattail) and *Typha angustifolia* (narrow-leaved cattail) cross, is an invasive plant species that has been associated with changes in community composition and ecosystem function in freshwater wetlands in the Great Lakes region (Angeloni 2006; Larkin 2009; Tuchman et al. 2009; Woo and Zedler 2002). The main driver by which *Typha x glauca* (hereafter referred to as *Typha*) alters community composition and ecosystem functions is the legacy effect of its leaf litter. *Typha* is typically greater than native species in stature; stem density, biomass, and litter production (Angeloni et al. 2006, Boers et al. 2007, Farrer & Goldberg, 2010, Larkin et al. 2009). Dense litter blocks sunlight from reaching

the soil surface, reduces temperatures at the soil surface, and creates a barrier that native plants must grow through (Farrer & Goldberg, 2010). *Typha* has also been associated with changes in microbial communities in wetland soils that it invades (Angeloni et al. 2006). An increase in both microbial community biomass and diversity have been correlated with *Typha* invasion (Angeloni et al. 2006) These changes in microbial communities, accompanied by the addition of organic carbon in the form of *Typha* litter, could be leading to greater fluxes of greenhouse gas emissions, particularly CH_4, in inundated wetlands.

Cheybogan Marsh offers an ideal study site to test the effects of *Typha* invasion. Using an ongoing 7-year mesocosm experiment, I will test the effect of *Typha* litter addition on methane emissions in native and *Typha*-invaded communities while controlling for water and soil variability often seen in the field. We hypothesize that addition of organic carbon coupled with anaerobic conditions will result in greater CH_4 emissions from *Typha*-invaded communities than in native plant communities. We predict that *Typha'*s leaf litter legacy is the mechanism by which organic carbon builds up in the soils.

METHODS

In order to determine the effects of *Typha* and its litter on CH_4 fluxes in anaerobic wetland soils, gas samples were collected in the field at Cheboygan Marsh in Cheboygan, Michigan, and as part of a controlled experiment conducted at the University of Michigan Biological Station in Pellston, Michigan. Gas samples were collected following the procedures of the bucket method of Millar and Kahmark from the Kellogg Biological Station of Michigan State University.

Field Site

Cheboygan Marsh is a 150 ha freshwater wetland on the coast of Lake Huron in northern Michigan. The soils in Cheboygan Marsh are predominately nutrient-poor sandy soils. Daily seiche events occur from Lake Huron and approximately one third of the marsh is continuously inundated with water (Tuchman et al. 2009). The marsh has 3 distinct vegetation zones: 1) native zone, a mixture of sedges and rushes dominated by

several species of *Juncus, Scheonoplectus,* and *Eleocharis*; 2) a *Typha* zone that was invaded by *Typha* over 40 years ago and is now almost completely a monoculture of *Typha*; 3) and a transition zone between the native and *Typha* zone that has a mixture of native plant species and *Typha* (Angeloni, 2006; Tuchman, 2010). Over 2/3 of the marsh is now occupied by the *Typha* zone (Tuchman 2010). The *Typha* zone advances on average 3-5 m per year (Angeloni, 2006).

Field Sampling

Gas samples were collected using the methods of Millar and Kahmark in order to estimate methane flux rates from two vegetation zones: 1) native emergent marsh and 2) Typha-dominated stands. A total of 10 gas-sampling chambers were placed in the field. In order to randomize plots 2 transects at a 90° angle from another. A randomized set of x and y coordinates for each were determined by taking 2 random angles from a compass. Plots in the *Typha* zone were plots also used for an EPA project. We chose 5 of the EPA plots located in a young *Typha* zone where soils were saturated. Five buckets were placed in a portion of the marsh that contained only native species. An additional 5 buckets were placed in the *Typha* zone. Gas collection chambers were constructed out of 18.93L (5 gallon) buckets containing an airtight sealing lid. Any vegetation growing in the bucket was clipped below the surface of the water. The buckets were inserted 10cm deep in the soil.

To determine CH4 flux rates during two sampling campaigns in July and August 2011, samples were taken every ten minutes for forty minutes. The lids were only placed on during the sampling trials. Each lid was drilled with 2 holes. One hole was permanently filled in with a rubber septa. The other hole was for a rubber stopper that was placed in the hole once the lid had been placed on the bucket to prevent gas from escaping. The stopper was placed in the hole after the lid has been placed on the bucket to allow pressure and gas stabilization in the buckets headspace before samples were taken. A 1.5m length of tubing was connected through the stopper and sealed, allowing for the samples to be taken while standing away from the bucket. This reduced the potential for human-caused ebulation in the chamber during sampling campaigns.

After the lids had been on the buckets for 1 minute the rubber stopper was placed in the hole and the time zero sample was taken.

Samples were drawn from the chamber by inserting a hypodermic needle attached to a syringe into each septa and drawing out 30ml of gas. Each syringe was pumped three times before the sample was taken from the headspace in order to mix the gas in the chamber thoroughly. A 10ml vile was then flushed with 15ml of the sample from the syringe, at which point the filter needle was removed from the septum of the vile and the final 15ml of sample left in the syringe were pumped into the vile.

Sampling was conducted after 7pm in the evening so as to minimize any temperature change in the headspace of the buckets during sampling. Ambient temperature was taken next to the buckets at time zero and at the final sampling of the forty-minute period (final time). Redox measurements were also taken at three randomly selected plots per vegetation zone using platinum tipped redox electrodes in order to estimate the redox potential. The electrodes were calibrated with an Accumet Epoxy body calomel reference electrode in a solution of KCl (Zoballs solution).

Soil samples were also taken from each bucket location in order to determine total organic carbon and the C:N ratio within the soils. Samples were collected using a Xcm diameter soil corer and a cork borer. Three 10cm deep samples were randomly taken from each plot and homogenized.

Study site/Experimental Design

In order to control for the variability of conditions in the field a controlled experiment was conducted in 20 mesocosms located at the University of Michigan Biological Station in Pellston, Michigan. The mesocosms were constructed in 2002. Each mesocosm is 2 meters long, 1 meter wide, and 1 meter deep. The frames were lined with 1.0 mm thick rubber pond liner and filled with hydric soil from a nearby wetland and sand (Larkin et al. 2011 for details).

The mesocosm communities were planted based on plant sampling that was conducted in Cheboygan marsh. All communities were planted with a representative mixture of species that occurred in the native habitats in Cheboygan Marsh. Each

mesocosm was planted with 16 stems of *Typha* in 2002. Soils for the mesocosms were a homogeneous mixture taken from a wetland in a nearby area.

We estimated methane flux from four treatments (n = 5); 1) "native"- only native plant species from Cheboygan marsh, 2) "Typha"- native plant species plus *Typha* , 3) "native + litter" only native plant species from Cheboygan Marsh plus an annual addition of *Typha* leaf litter collected from Cheboygan Marsh 4) "Typha + litter"- native plant species plus *Typha* plus an annual addition of *Typha* leaf litter collected from Cheboygan Marsh.

The mesocosms were constructed in order to control water levels and soil conditions that were confounding variables in the field. All 20 mesocosms were constantly inundated with water for the duration of the experiment (June 19th- Aug 23rd, 2011?) with approximately 8-9 cm above the soil surface to promote anaerobic conditions.

Gas Sampling/Mesocosms

The same methods that were used in the field were also used in the mesocosms. Gas collection chambers in the mesocoms were constructed out of 1 gallon buckets instead of the 5 gallon buckets used in the field. One gallon buckets were used in the mesocosms in order to minimize the amount of disturbance to the communities and to avoid clipping more plants than necessary. Each bucket was placed near the center of the mesocosm avoiding as much vegetation growth from growing within the bucket as possible. Any vegetation growing in the bucket was clipped below the surface of the water.

As in the field, sampling was conducted after 7pm in the evening so as to minimize any temperature change in the headspace of the buckets during sampling. Ambient temperature was taken at time zero and at the final sampling. Redox measurements were also taken in the mesocosms. Soil samples were also taken from each mesocosm in order to get total organic carbon and the C:N ratio within the soils.

Soil Analysis

Soil organic carbon was determined using standard ash-free dry weight (AFDW) methods (Robertson 1999). Soil samples were dried at 60°C, then sieved with a #10 Newark standard sieve. Ten grams of each sieved sample were weighed and cooked in a muffle furnace for 2 hours at 550°C. The samples were then reweighed and the final weight was divided by the original weight to determine percent organic matter. A one gram subsample was used to measure the C:N ratio on a Carlo-Erba elemental analyzer. Each sample was pulverized using a ball mill before being processed.

Statistical analysis

To test the hypothesis that organic carbon and CH4 flux were greater in the Typha than the native zone at Cheboygan Marsh, we used independent t-tests to compare means. We used linear regression to test the linear relationship between CH4 flux and AFDW. A one-way ANOVA was used to test for variance in CH4 flux and AFDW among treatments in the mesocosms. Linear regression was also used to determine how much variability in CH4 flux was due to soil organic carbon. Any plot, in either the field or mesocosms, that showed a negative flux was identified as an outlier and the same tests were run removing them from the sample set. We were able to identify these plots as outliers do to the anaerobic conditions of the soils.

RESULTS

Cheboygan Marsh *Typha* stand soils had significantly greater organic carbon (p<0.001) than Native stand soils, offering support for our hypothesis (Figure 1). The *Typha* zones had more than 6 times the amount of organic carbon in the soils than the native stands. An independent t-test ran when outliers were removed revealed an equally significant difference (p<0.001) between treatments (Figure 2). When an independent t-test was run on the full field sample set to determine variance between the *Typha* and native zones mean CH4 flux the data revealed no significant differences between the treatments (Figure 3). The data also revealed an extremely large standard error in both treatments. When the outliers were removed the t-test revealed that, while

there was still no significant difference between treatments (p=0.414), *Typha* stand soils had slightly greater mean CH4 fluxes than the native zones (Figure 4). Neither regression revealed a significant relationship between ash-free dry weight and CH4 flux (Figure 5 and 6).

We observed no significant difference in ash-free dry weight between the different treatments in the mesocosms. While neither the full data set nor the set with outliers removed showed a statistically significant difference between treatments, (p=0.281, p=0.379) respectively, both suggest a trend indicating the litter treatments have higher mean ash-free dry weights. This offers support for our hypotheses that treatments with *Typha* litter will have higher amounts of organic carbon in the soils. CH4 flux in the mesocosms varied marginally (p=0.087) between the *Typha* + litter treatments and the native treatments (Figure). When the outliers were removed the results again indicated a marginally significant difference (p=0.060) difference between the *Typha* + litter treatments and the native treatments (Figure). Linear regressions that were run on both the full data set (p=.031, R2=234)) and the data set with outliers removed (p=0.006, R2=0.476) both revealed a statistically significant relationship between ash-free dry weight and CH4 flux. These findings offer support for our hypothesis that as organic carbon increases in soils where anaerobic conditions are present the CH4 flux will also increase.

CONCLUSIONS/DISCUSSION

Our results offer several insights into the effects of *Typha x glauca* on the freshwater wetlands they invade. Previous research has revealed that *Typha* has the capacity to alter community structure through its leaf litter legacy (Angeloni et al. 2006). Our field data support the work of others that *Typha* stands have significantly greater amounts of organic carbon in their soils relative to native stands. Due to *Typha*'s robust size and high productivity (Angeloni et al. 2006) compared with native species, annual inputs of biomass into the soils are much higher than pre-invasion by *Typha*. While the data in the field didn't show a statistically significant relationship between percent organic carbon in the soil and methane flux, the mesocosm data may suggest that a

relationship between the two does exist. These data suggest that the leaf litter legacy of *Typha* may be acting as a fuel for methanogenesis. While an increase in methanogenesis occurring in these wetlands doesn't directly lead to higher methane emissions, it does bring to light the importance of understanding these systems and the effects that invasion by *Typha* may have on them.

These results may have important implications due to the high global warming potential of methane in the atmosphere. *Typhas* highly competitive nature and propensity to spread 4-5 meters per year make it important to fully understand the relationship between methane emissions and invasion by *Typha*. Furthermore, since *Typha* may have the capacity to terrestrialize ecosystems through it's leaf litter legacy (citation), further studies are needed to determine what changes might occur in fluxes in N2O and CO2 emissions. As terrestrialization occurs the soils begin to get oxidized, driving up the redox levels, and creating conditions conducive for N_2O production. It is extremely important to understand these processes since the ramifications can be quite drastic. Nitrous oxide is an even stronger greenhouse gas with an atmospheric lifespan of 120 years and a global warming potential 310 times that of CO2 (IPCC 2007). As *Typha* continues to invade wetlands and terrestrialize, there may be potential for drastic increase in N2O production occurring in natural freshwater wetlands.

While there may be negative implications to *Typha* invasion, such as reduced community diversity and terrestrialization (Farrer & Goldberg 2009, Tuchman 2009), it may also be contributing to promote carbon sequestration. We saw no significant difference between *Typha* and Native CH_4 fluxes but a statistically significant difference between soil organic carbon in the two. *Typha* fixes a high amount of carbon that is stored in its biomass. The biomass decomposes slowly in the often anoxic conditions of the marsh. This accumulation of carbon in the soils over time can result in net carbon storage and make the wetland act as a sink. The complex interactions between *Typha*, the native community, soils, biogeochemical processes, and greenhouse gas emissions warrant the necessity for further research of this invasive species and the delicate ecosystems it inhabits.
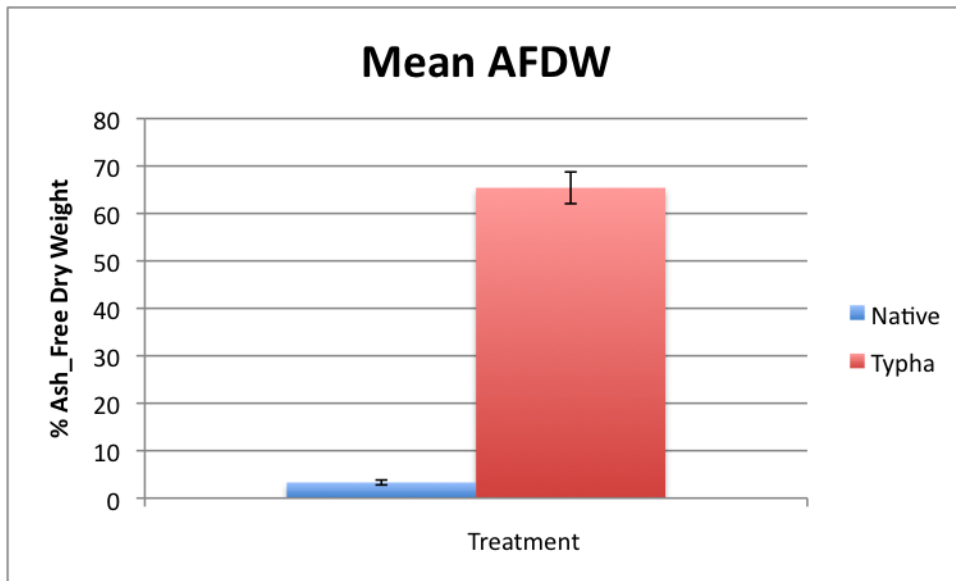
Appendix

Figure 1. Mean ash-free dry weight of field treatments in Cheboygan Marsh collected in July 2011. There were five replicates for each treatment. Ash-free dry weight serves as an index for the percent organic carbon in soils. Bars indicate standard error.
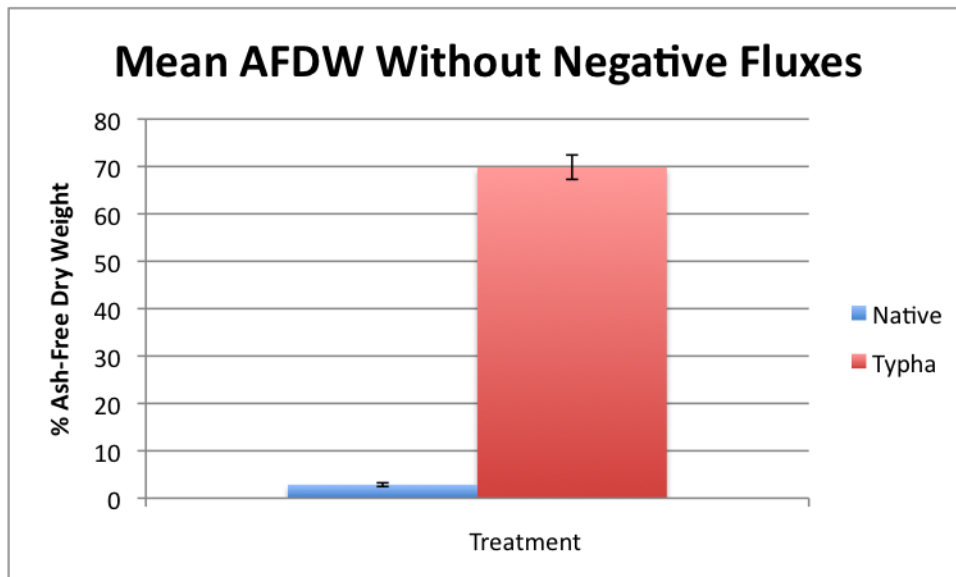

Figure 2. Mean ash-free dry weight (% organic carbon) of field treatments in Cheboygan Marsh collected in July 2011. Outliers identified by negative CH_4 fluxes were removed. There were three replicates for each treatment. Bars indicate standard error.
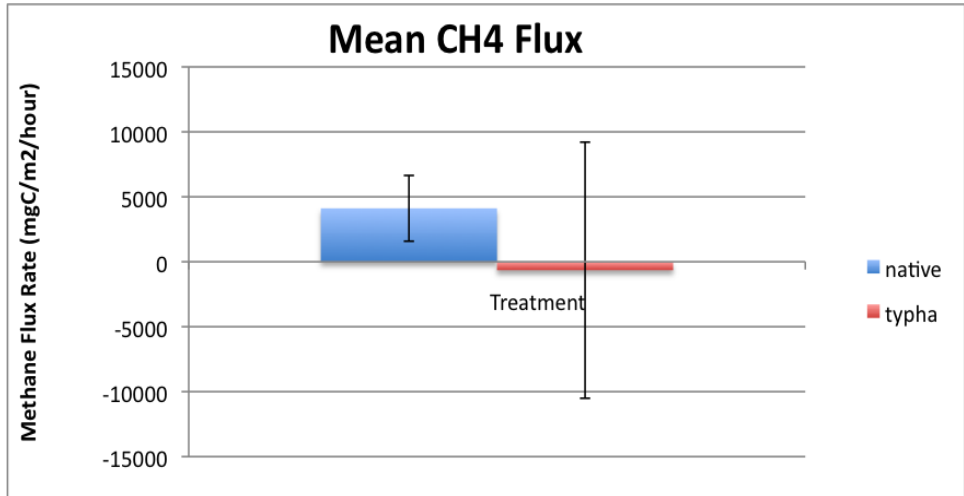
Figure 3. Mean CH_4 flux for field treatments in Cheboygan Marsh. Samples collected July 31st summer 2011. 5 replicates per treatment. Bars indicate standard error.
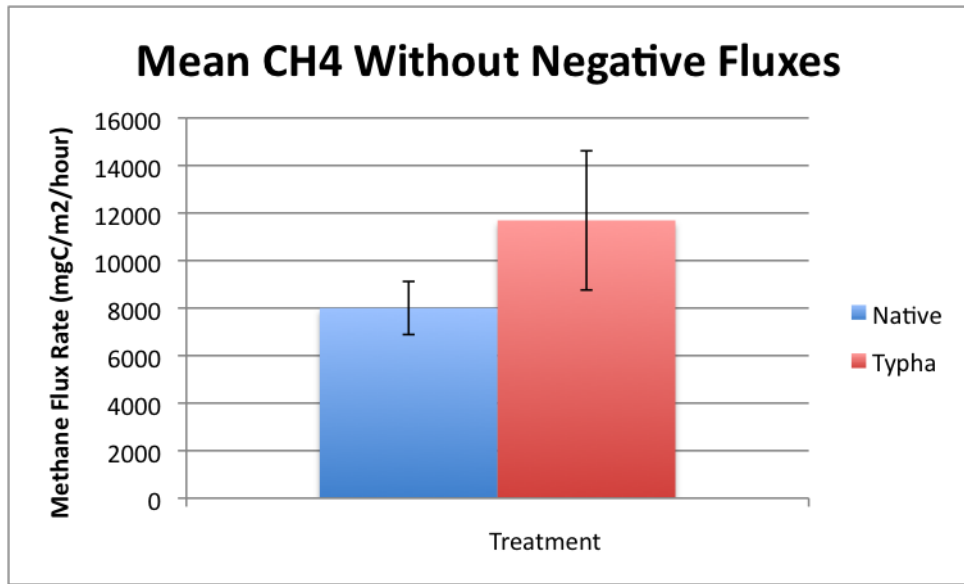
Figure 4. Mean methane flux for field treatments in Cheboygan marsh. Samples collected July 31st, 2011. Outliers were identified by negative CH_4 fluxes and removed. 3 replicates per treatment. Bars indicate standard error.
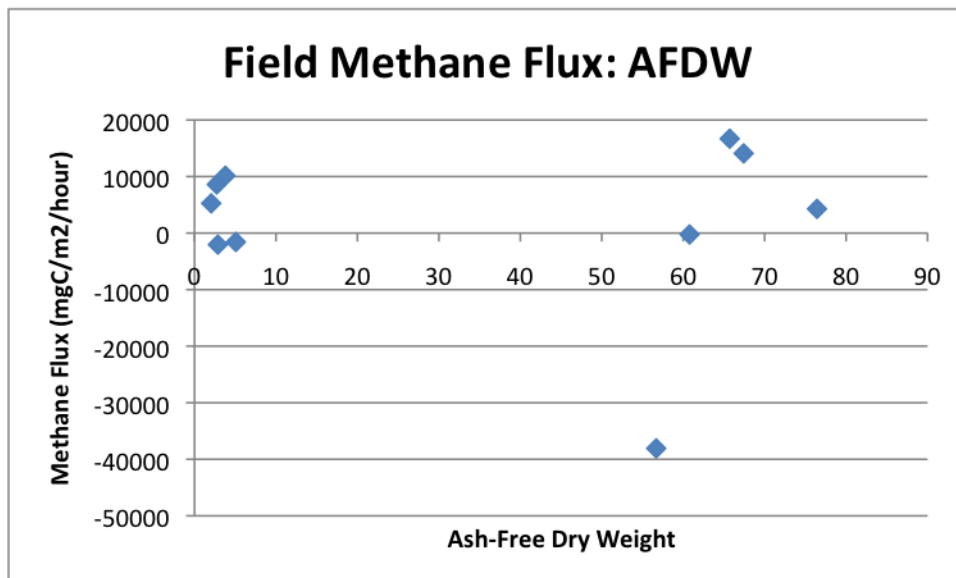
Figure 5. Relationship between CH_4 flux and ash-free dry weight (% organic carbon in soils) in field treatments of Cheboygan Marsh. Both treatments are included, n=5.
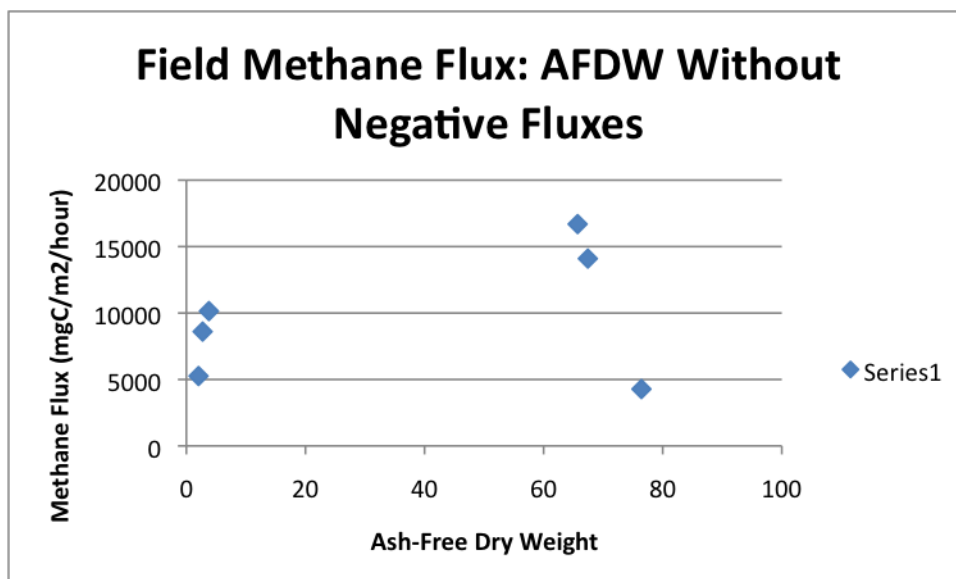
Figure 6. Relationship between CH_4 flux and ash-free dry weight (% organic carbon in soils). Outliers were identified by negative CH_4 fluxes and removed. Both treatments are included, n=3.
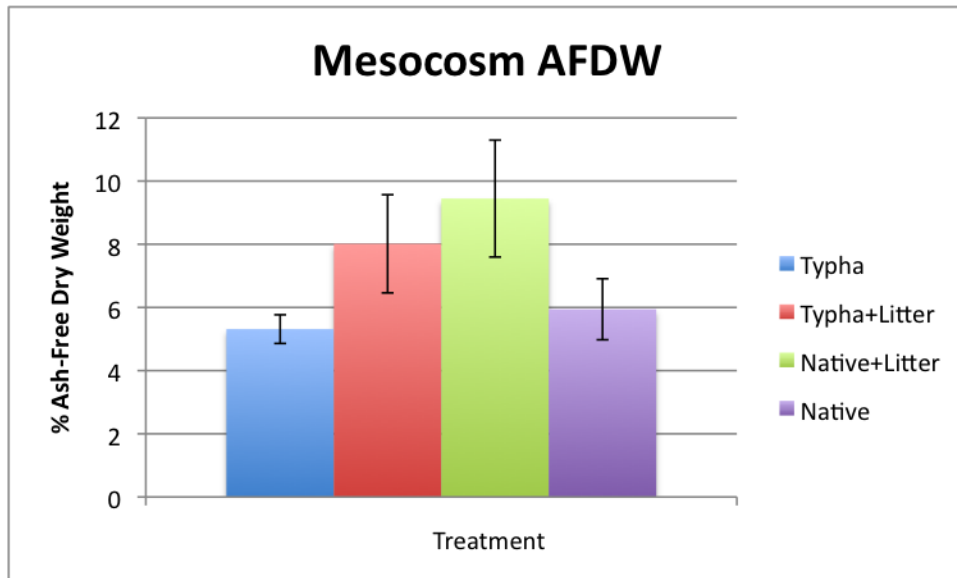
Figure 7. Mean ash-free dry weight (% organic carbon) of mesocosm treatments at UMBS Hill, collected in Aug 2011. N=5 for each treatment. Bars indicate standard error.
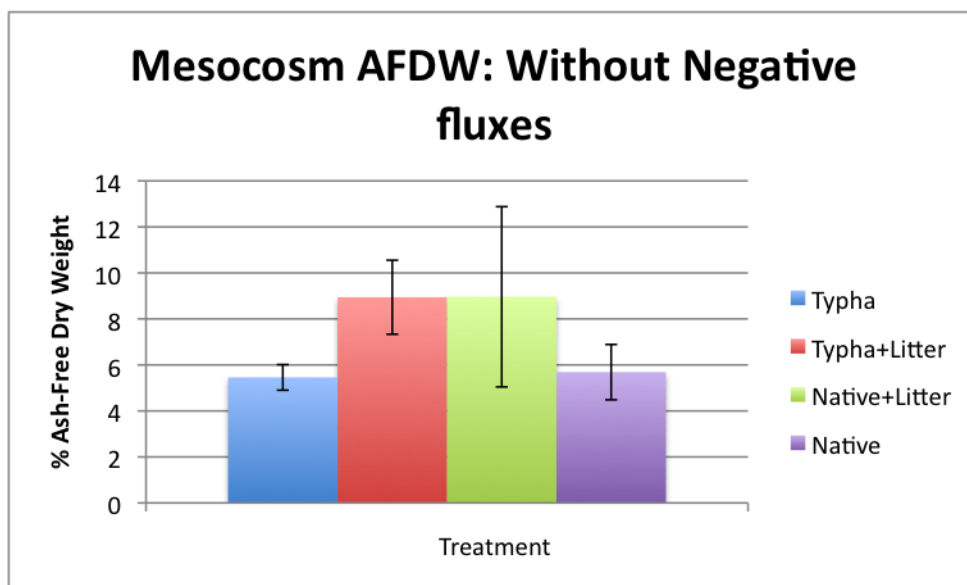
Figure 8. Mean ash-free dry weight (% organic carbon) of mesocosm treatments at UMBS Hill, collected in Aug 2011. Outliers were identified by negative CH_4 fluxes and removed. Typha n=4, Typha+litter n=4, Native+litter n=2, Native n=4. Bars indicate standard error.
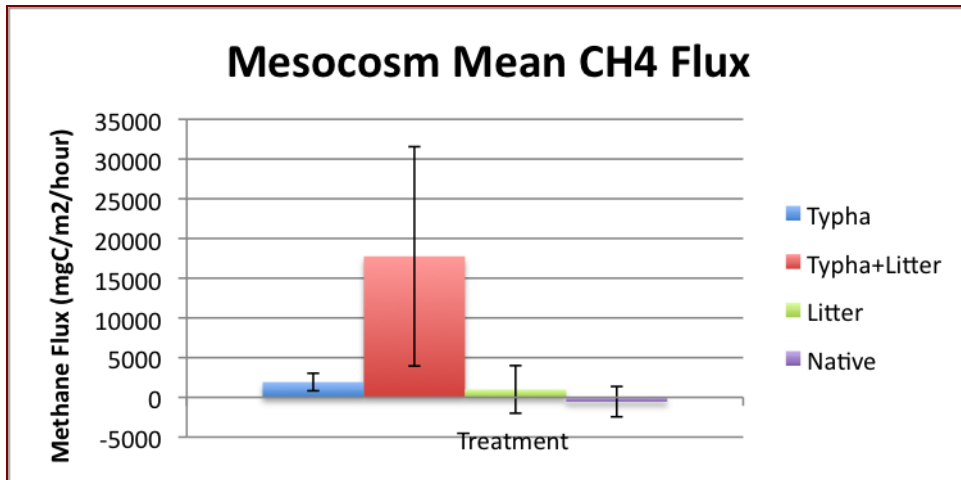
Figure 9. Mean CH$_4$ flux of mesocosm treatments at UMBS Hill, collected on Aug 4th and 5[th,] 2011. N=5 for each treatment. Bars indicate standard error.
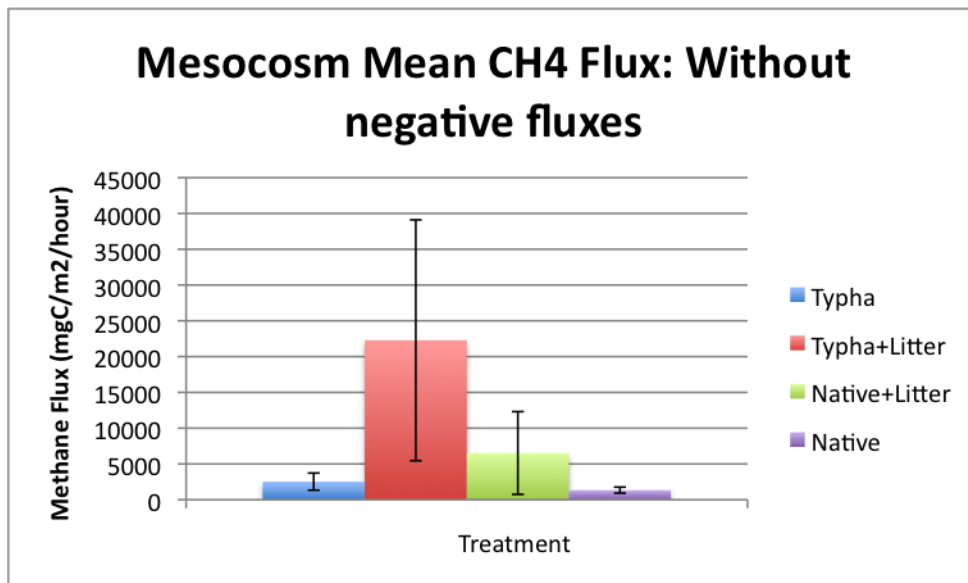

Figure 10. Mean CH$_4$ flux of mesocosm treatments at UMBS Hill, collected on Aug 4th and 5[th,] 2011. Outliers were identified by negative CH$_4$ fluxes and removed. Typha n=4, Typha+litter n=4, Native+litter n=2, Native n=4. Bars indicate standard error.
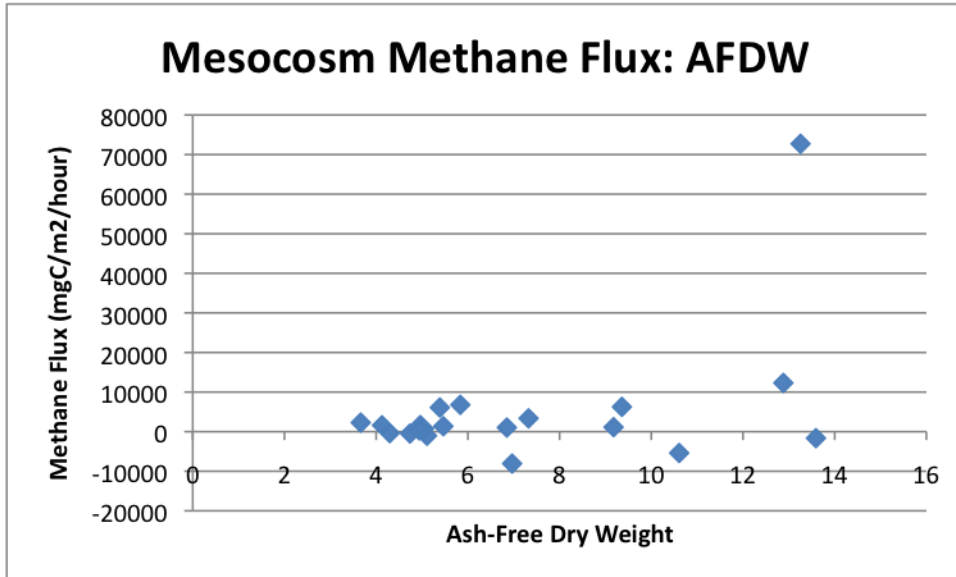
Figure 11. Relationship between CH$_4$ flux and ash-free dry weight (% organic carbon in soils) in mesocosm experiment at UMBS Hill. Four treatments, n=5.
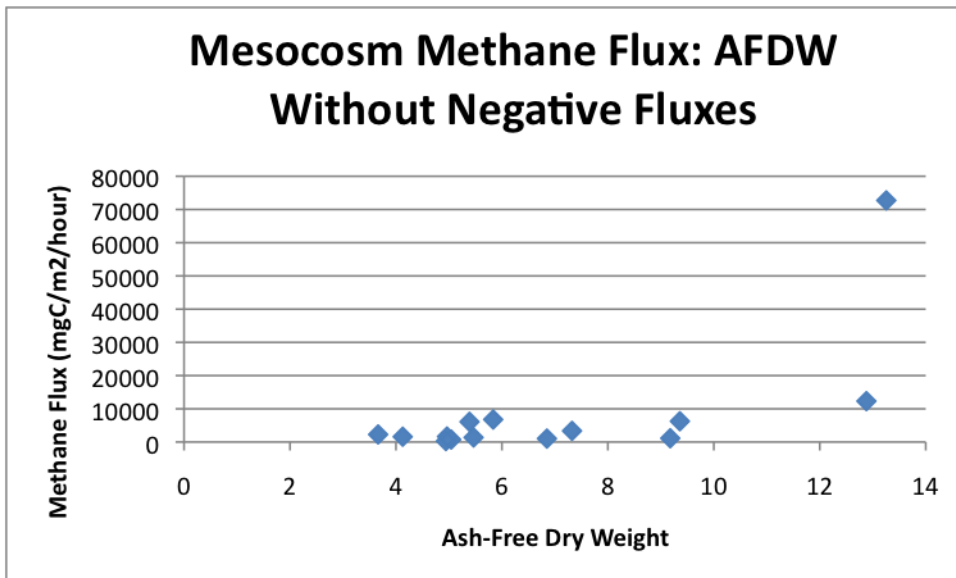

Figure 12. Relationship between CH$_4$ flux and ash-free dry weight (% organic carbon in soils) in mesocosm experiment at UMBS Hill. Outliers were identified by negative CH$_4$ fluxes and removed. Typha n=4, Typha+litter n=4, Native+litter n=2, Native n=4.

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