# Anaerobic methanogenesis in wetland ecosystems: do cut emergent aerenchyma of *Typha × glauca* facilitate increased atmospheric methane emission?

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INTRODUCTION

Anaerobic methanogenesis by bacteria communities is a major source of global atmospheric methane and therefore an important area of concern regarding climate change (Dingemans et al. 2011). This is particularly relevant to wetland ecosystems whose warm, water-logged, oxygen-poor soil conditions are the ideal environment for the fermentation of methane (Reddy et al. 2000). Fermentation is the process microorganisms use to break down essential nutrients. In a process called acetoclastic methanogenesis, microorganisms from the Archaea domain ferment acetate and H_2-CO_2 into methane and carbon dioxide (Dingemans et al. 2011) :

H_3C-$COOH \rightarrow CH_4 + CO_2$
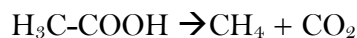
Depending on the specific wetland and type of microorganisms, hydrogenotrophic methanogenesis can also occur, in which Archaea oxidize hydrogen with carbon dioxide to yield methane and water:

$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$
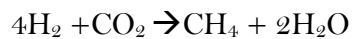
Because these processes depend on the input of organic matter, they are necessarily affected by the density and type of vegetation in the wetland (Angeloni et al. 2006). This primary productivity fuels methane emissions as plants provide most of the carbon needed for methanogenesis to take place. However, this is not the only influence plant communities have on methane emissions. In addition to relatively slow diffusion of methane through soil and water, and the sudden ebullition of trapped gas pockets, the aerenchyma (vessel-like tubes composed

of mostly airspace that transport essential gases throughout the plant) of plants provide a direct route for gases to reach the atmosphere (Dingemans et al. 2011). Essentially, the plant stem acts as a straw which allows methane to travel unhindered through the porous plant tissue. Hence, methane emissions bypass not only the soil and water, but also the methanotrophic, or methane consuming, bacteria in the substrate that would normally break down some portion of it before it reaches the atmosphere (Dingemans et al. 2011).

The wetland systems in the Great Lakes region are currently experiencing an aggressive invasion of cattail species, especially the mostly sterile hybrid (Annen 2007) *Typha × glauca*, of the exotic *T. angustifolia* and native *T. latifolia*. *Typha × glauca* is very adept at clonal reproduction via rhizomes and is able to outcompete and crowd out native wetland plant communities. The large amount of dead litter from *Typha × glauca* shades out other species and raises the soil level, which dries out the wetland and produces a more favorable environment for *Typha × glauca* proliferation. Additionally, this litter fuels methanogenesis by increasing organic matter input (Farrer and Goldberg, 2009).

Using the *Typha × glauca* stands of Cheboygan Marsh in Cheboygan County, MI, as a study site, this investigation compares the methane flux of areas where vegetation was cut below the water level to those where vegetation was cut above the water. The hypothesis is that methane flux will be higher when exposed directly to the atmosphere through the aerenchyma. The null hypothesis is that there will be no difference in methane flux between the two treatments. Because *Typha × glauca* is an invasive competitive dominant, it has become a target for removal by managers of protected areas. Previous studies of wetland hydrophytes, such as *Phragmites australis*, have found a significantly higher (up to 5x) methane flux from emergent shoots vs. submerged shoots (Dingemans et. al 2011). However, the effect of cattail aerenchyma on methane flux has yet to be determined. With management and restoration proposals ranging from burning and uprooting to clear cutting, these results may prove important to the development of best practices in the removal of invasive cattails.

METHODS

Study Site

Cheboygan Marsh is a wetland on the coast of Lake Huron in Cheboygan County, MI (45° 39' 29" N, 84° 28' 47" E). Native plant population consists mainly of *Juncus balticus*, *Schoenoplectus acutus*, *S. pungens*, and *Eleocharis spp.* However, much of the marsh is currently dominated by *Typha × glauca*, which has essentially formed a monoculture. The study site was located in an area only recently colonized by the invasive *Typha × glauca*. Within these stands, *Typha* grows well over two meters tall, towering over everything but nearby trees skirting the marsh. There are surprisingly few macroinvertebrates present in the densest *Typha* as compared to nearby

native communities, but frogs were abundant in both locations.

Field Measurements

The bottoms of ten 5-gallon plastic buckets were cut off and two holes cut into the lids—one hole for a rubber stopper and one hole plugged with a rubber syringe port. The buckets were taken to Cheboygan Marsh, positioned in pairs at 5 locations along a transect, and installed 10 cm deep into the substrate using a tree saw to cut through the rhizomes (*fig 1*). Each bucket contained 5 live stems of *Typha × glauca*. One week later, in each pair of buckets the vegetation was cut below the surface in one of the buckets. Immediately after cutting, the lids were placed on these 5 buckets without the rubber stopper inserted. The buckets were left to sit for 10 minutes to allow the gas that was released by disturbances to dissipate. Ambient air temperature in the bucket was recorded during the last minute of this waiting period. After the 10 minutes, the rubber stoppers were inserted into the lids and samples were taken. The syringe was inserted into the rubber syringe port and flushed to 60 mL three times before drawing 30 mL of sample from the bucket. Each sample was put into its own 15 mL vial with a rubber septum seal. One needle was inserted into the rubber septum seal of the vial as a vent so that the ambient air in the vial could be replaced by the sample. Another needle was placed on the syringe and inserted into the vial. Twenty mL of sample were flushed into the rubber syringe port of the vial before removing the vent. Two mL of extra sample were added to the vial to maintain positive pressure. This sample was designated time 0 minutes and the process was repeated at times 10, 20, 30. The temperature in the bucket was recorded again immediately after the time 30 sample. This entire process was then repeated for the other 5 buckets, but the *Typha* stems were cut above water instead of below water. After sampling, the headspace (distance between the top of the water and the top of the bucket) was measured for use in volume calculations.
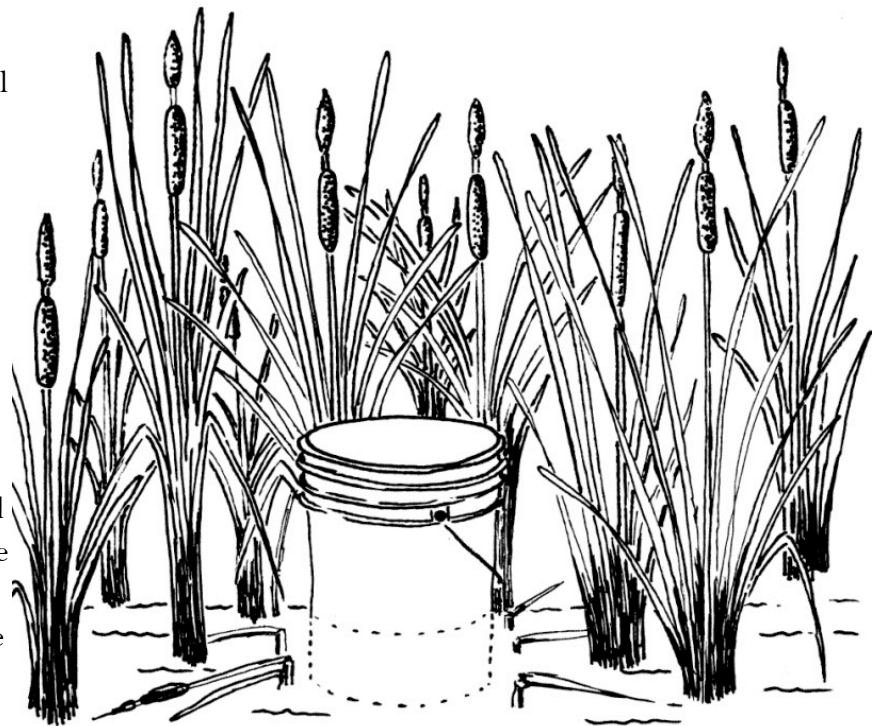
fig 1. Bucket installation
courtesy Brett Pedley

Chemical Analysis

A Trace Gas Chromatograph Ultra (hereafter referred to as GC) was used to identify and measure the collected gas samples (*fig 2*). 1 mL of the sample gas was injected with a gas-tight glass syringe through the septum of the GC. The gas then mixed with an unreactive nitrogen carrier gas. The function of the carrier gas is to force the molecules in the right direction and carry them through the column. The core of the column causes different compounds to leave the tubing at different times depending on their chemical and physical properties. The column was contained in an oven in which the temperature of the gases passing through was kept at 45° Celsius. As compounds exit the column, the Flame Ionization Detector (FID) counts carbon molecules. It is fueled by hydrogen gas, which oxidizes carbon molecules to ions. The ions then become attracted to an electrode, which measures the current flow of the ions. The current flow versus time is plotted electronically, where the area of the signal is proportional to the
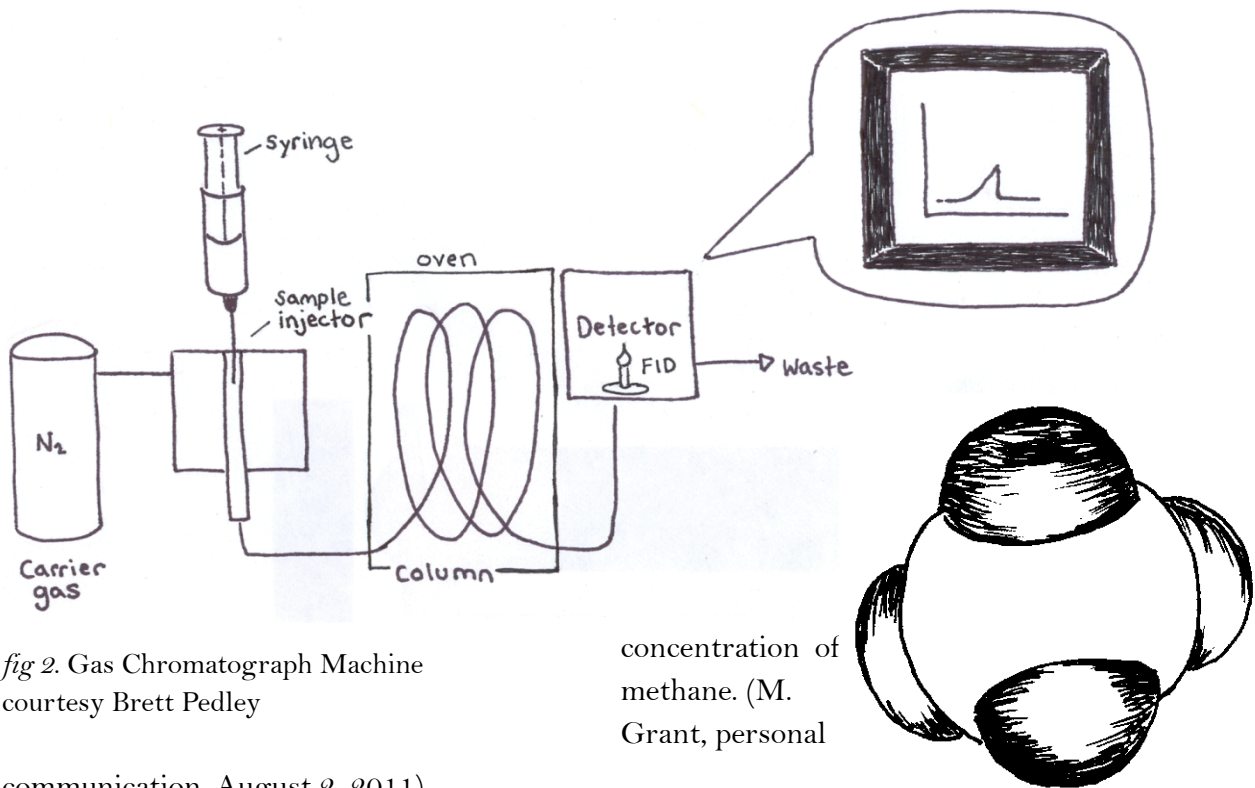

fig 2. Gas Chromatograph Machine
courtesy Brett Pedley

communication, August 2, 2011)

concentration of methane. (M. Grant, personal

fig 3. Methane Molecule
courtesy Brett Pedley

Concentration values computed with the GC were then converted from a volume/volume concentration (ppm) to mass/volume concentration (mg CH_4-C/m³ enclosure), using the following equation (Holland et al. 1999)

$$C_m = (C_v \times M \times P)/(R \times T)$$

where
C_m = the mass/volume concentration (mg CH_4-C/m³ enclosure)
C_v = the volume/volume concentration (ppm)
M = the molecular weight of the trace species
P = barometric pressure (in atm)
T = air temperature (in °K)
R = the universal gas constant (0.0820575 L atm x °K x mole)

Once converted, the flux of methane was calculated from the concentration values using the following equation (Holland et al. 1999):

$$f = V \times C_{rate}/A$$
where
f = gas flux as mg CH_4-C x m⁻² x h⁻¹
V = internal volume of the enclosure, including collar volume, expressed as m³
A = the soil area the enclosure covers, expressed as m²
C_{rate} = change in concentration of gas (C_m) over the enclosure period, expressed as mg CH_4-C x m⁻³ x h⁻¹

Statistical Analysis

To determine whether emergent *Typha* stands have a higher flux than submerged ones, a two-way ANOVA was run using SPSS. A two-way ANOVA was utilized because there were two treatments and samples were taken on two different days. A linear regression model was then run to determine how well our data points conform to a trend line.

RESULTS

[CH4] vs. Time

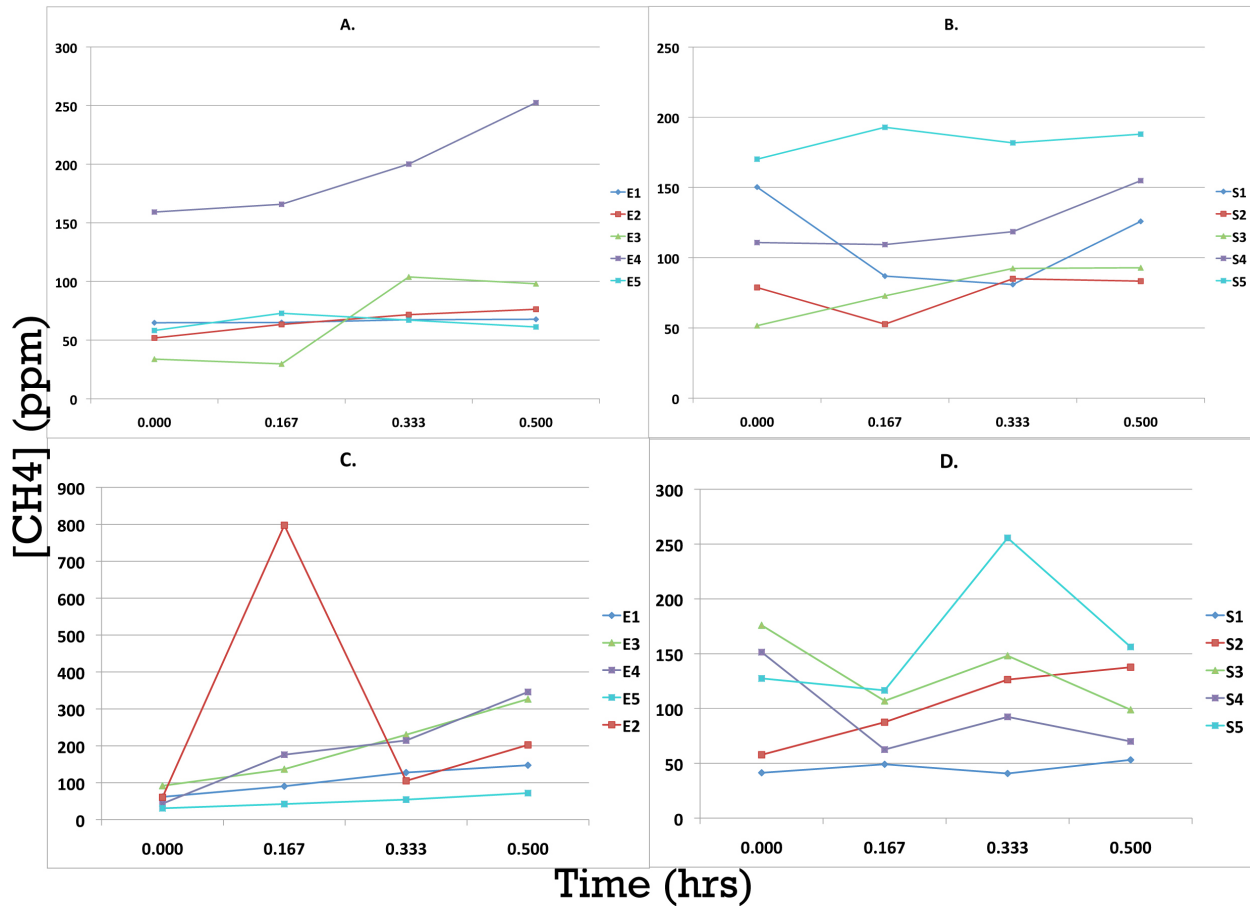**[CH4] (ppm)** *(y-axis)*

Time (hrs) *(x-axis)*

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fig 4.

Ch4 concentration vs time for trials over two days from a series of 10 buckets. Five emergent replicates (E1-E5) were cut above water (A,C) and five submerged replicates (S1-S5) were cut below water (B,D).

A: Day 1 emergent methane flux.

(R^2: E1 = .87651; E2 = .96536; E3 = .7403; E4 = .90335; E5 = .00385)

B: Day 1 submerged methane flux.

(R^2: S1 = .09652; S2 = .15491; S3 = .89307; S4 = .72973; S5 = .31148

C: Day 2 emergent methane flux. Contains a suspected outlier at time 0.167 hours.

(R^2: E1 = .98766; E2 = .0101; E3 = .97645; E4 = .96226; E5 = .98836)

D: Day 2 submerged methane flux.

(R^2: S1 = .3306; S2 = .96225; S3: .46103; S4: .47293; S5: .21071)

    The methane concentrations over time (*fig 4*) exhibit high variance, in some cases even representing a net loss of methane. However, the emergent plots consistently resulted in a better fitting linear regression, with the R^2 values ranging from 0.74 to 0.99 in 8 out of 10 trials. Emergent plot 5 on day 1 shows no significant slope, and emergent plot 2 on day 2 contains a suspected outlier, resulting in a poor R^2 value of 0.01. The data for the submerged treatments often did not show a strong linear correlation, with R^2 values ranging from 0.096 to 0.96, the majority of which are below 0.5.

Plot number	Emergent flux (mgC/ m2/hour)	Submerged flux (mgC/ m2/hour)
1	848.54	-5820.19
2	6356.83	3304.13
3	18806.77	10264.21
4	23189.53	10874.23
5	233.34	3043.77
Average	9887.00	4333.23
Standard error	4710.99	3031.30

Plot number	Emergent flux (mgC/ m2/hour)	Submerged flux (mgC/ m2/hour)
1	21830.52	2007.61
2	31811.12 (-19397.65)	20304.27
3	56748.36	-13492.84
4	69419.80	-16629.51
5	9553.64	16186.15
Average	37872.69 (27630.95)	1675.14
Standard error	11062.49 (16072.10)	7492.84

fig 5. Day 1 methane fluxes and descriptive statistics.

fig 6. Day 2 methane fluxes and descriptive statistics. One suspected outlier emitted from emergent plot 2, results with outlier shown in
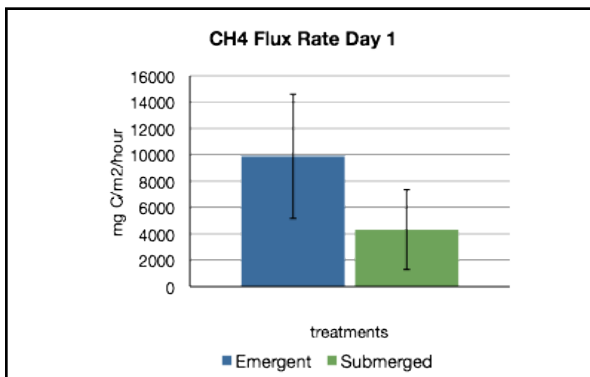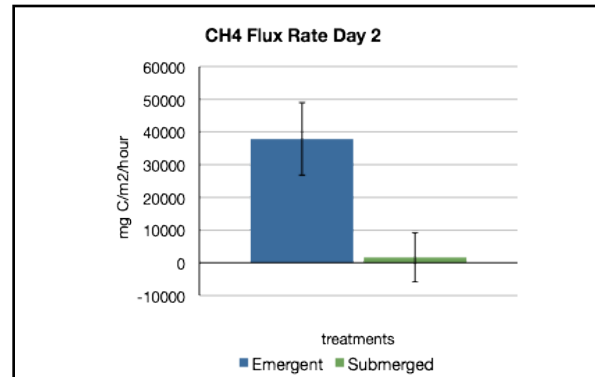

fig 7. Average methane flux, day 1.

fig 8. Average methane flux, day 2.

Despite high variation and three instances of a negative flux rate, the emergent treatment resulted in much higher flux rates, with mean flux 2.2 times greater on day one (*fig 7)* and 22.6 times greater on day two (*fig* 8). These results ignore the outlier called out in *figures* 4 and 6.

The two-way ANOVA test (*figures 9* and *10*) indicated that the difference between treatments alone is significant, with a p-value of 0.011,which is statistically significant given α= 0.05. The difference between sampling day one and day two was shown to be marginally significant (p = 0.100). The combination of treatment and sampling day also shows significance (p = 0.050), suggesting a strong interaction between day and treatment.
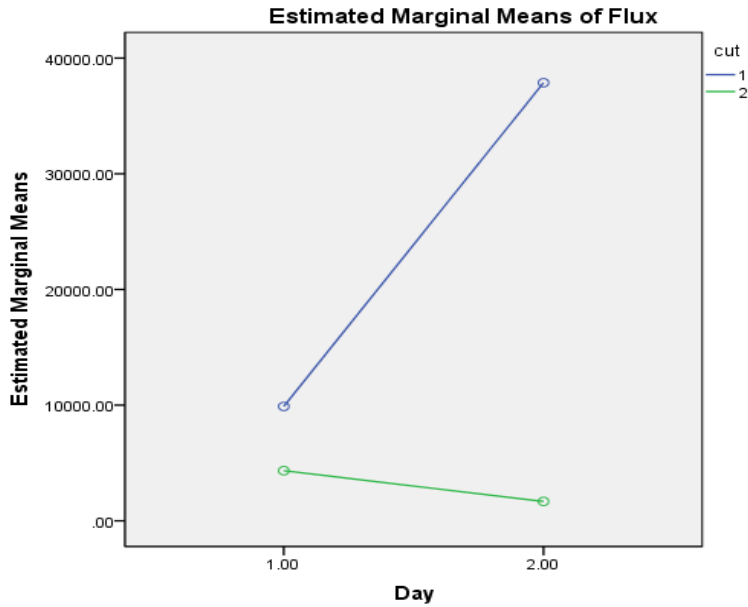
Estimated Marginal Means of Flux

fig 9. (left) Estimated marginal means of flux. Shows interaction between treatment and sampling day.
Cut 1= emergent
Cut 2= submerged

Statistical Results: Two way ANOVA					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	2.18E+09	1	2.18E+09	8.305	0.011
Day	8.02E+08	1	8.02E+08	3.056	0.100
Treatment * Day	1.17E+09	1	1.17E+09	4.474	0.050

fig 10. Test of between subject effects. Dependent variable= flux. Fixed variables= treatment, day.

DISCUSSION

As shown by the mean flux rate comparison, not only do plots with emergent *Typha* aerenchyma emit a greater net amount of methane, but also at a rate 2 to 22 times higher than submerged *Typha* plots. This supports our hypothesis that emergent *Typha* aerenchyma facilitate increased atmospheric methane emission. The analysis of variance strongly reinforces this claim, indicating a significant difference in flux rates between treatments. However, a marginally significant difference was shown between sampling day one and two, suggesting fairly large variation in methane production due to unknown, and presumably multiple, dynamic environmental factors. Temporal and spatial variations, including water level, make-up of plant and microbial communities, and nutrient content could largely affect methane production (Reddy et al. 2000). Water fluctuations, for example, are not uncommon in wetlands,

particularly along the shores of the Great Lakes (Keddy & Reznicek 1986). Despite the marginal significance of sampling day and the significant interaction between treatment and sampling day, our treatments still result in measurable differences. Hence, the aerenchyma of cut *Typha* stems do, in fact, act as a straws channelling methanogenesis emissions more directly to the atmosphere.

Different estimates claim that wetlands cover anywhere from 2.2% (Post et al. 1982) to 6% of the Earth's surface (Mitsch 1994), yet they are responsible for as much as 30% of annual methane emissions (Solomon et al. 2007). Although wetlands are one of the most important contributors of atmospheric methane, it is difficult to model exact mechanisms due to high variation. The inconsistencies in our data are forgivable when considering the huge variability found in wetlands. A large issue facing the project was unpredictable weather and soil conditions at our site, which caused several plots to dry up. The water level dropped below the substrate level, meaning that some emergent and submerged replicates could have been essentially the same treatment, with aerenchyma in both treatments emerging above the water level. However, the soil remained extremely moist in most plots, with submerged stems buried in soil and muck while emergent stems stayed high above the substrate. This still represents an important difference between treatments in most plots, and the effects of this drying-out can be seen and accounted for in our analysis. Trials resulting in negative flux rates (Day 1: S1 and Day 2: S3, S4) indicate that the water level in these plots dropped sufficiently to produce an oxidized soil environment, and therefore a methane sink. Trials with concentration slopes at or near zero may indicate an equilibrium between methane fermentation and methanotrophic activity due to relatively aerobic conditions.

Human inconsistency is certainly a large source of error in our study. The process of injecting and analyzing gas samples using a gas chromatograph can yield variable data unless great care and precision is used. Between five gas chromatograph operators testing methane standards, relative standard deviation ranged from 3% to 17%. Field methods were also abound with sources of error. The large spike in methane recorded at emergent plot 2 on day 2 is largely inexplicable. Previous studies have noted that too much movement around the plots during sampling can cause methane to ebullate out of the substrate and into the sampling buckets. An unusual increase in methane would show up in the results, however we would expect methane concentration levels to remain high for the remainder of the time trials at that bucket, which was not the case with emergent bucket 2. Although we are unable to explain this source of error, the datum was suspiciously different enough that we are confident in our decision to exclude it from our statistical analyses. Sampling chambers, needles, syringes and vials may not have been completely air tight. Due to the properties of methane, being such a small compound, vials in particular have been shown to leak gas over time (Castillo, forthcoming). Error from human imprecision is a likely source of error adding variation to the results and affecting how well the data fits a linear trend.

Despite numerous potential sources of error, our results support our hypothesis that *Typha* stems cut above the water act as a straw to release methane into the atmosphere at a rate that is significantly higher than *Typha* stems cut below the water level (p=.011). The affect of day on methane flux was also marginally significant (p= .100) and the interaction of cut treatment and day was also significant (p= .05). Because of limitations this project's design, We did not include treatments of uncut *Typha*, nor no *Typha* at all, so there is no telling how these flux rates compare to natural atmospheric gas exchanges in the plants or bare soil. This lack of control treatment results in no reference point for our results. The relative differences in methane flux are nevertheless meaningful in an invasive species management setting, as untouched *Typha* cannot be considered a management technique. Additionally, a bare soil treatment also seems irrelevant given the dense vegetative cover across all but the most peripheral areas of the marsh. While above water cutting of *Typha* x *glauca* may be the most economically feasible eradication method, management planners would do well to heed the mechanisms that this study explores. A more robust investigation into the effects of aerenchyma methane emission is necessary before definitive claims can be made, but this report presents an important starting point for further work on the subject.

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