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Olivia Johnson

*DePaul University College of Science and Health*, [ojohns10@mail.depaul.edu](mailto:ojohns10@mail.depaul.edu)

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## Greenhouse Gas Flux Response to Harvest of *Typha x glauca* in a Great Lakes Coastal Wetland

Olivia Johnson\*

Department of Environmental Science and Studies

Beth Lawrence, PhD; Faculty Advisor

Department of Environmental Science and Studies

**ABSTRACT** Although harvesting invasive species can promote biodiversity during wetlands restoration, there is little known about this mechanical treatment's impacts on greenhouse gas flux, a significant biosphere-atmosphere interaction. We quantified greenhouse gas flux response to experimental harvest of invasive hybrid cattail (*Typha x glauca*) at Cheboygan Marsh in Northeastern Michigan during the 2015 growing season. During each sampling campaign (July 15, July 31, August 12) we collected gas samples from static PVC chambers at 6 harvest and adjacent *Typha* control plots. Using gas chromatograph analysis, we found no significant difference in CH<sub>4</sub> or CO<sub>2</sub> flux between harvest and control plots on any date. Average CH<sub>4</sub> flux rates for harvest and *Typha* control were 56.0 and 36.0 mg C m<sup>-2</sup> h<sup>-1</sup> respectively. Average CO<sub>2</sub> flux rates for harvest and *Typha* control were 35.7 and 43.2 mg C m<sup>-2</sup> h<sup>-1</sup> respectively. From hourly I-Button temperature measurements, we found harvest plots had a higher average maximum daily temperature than *Typha* control plots. We found a positive linear relationship between reduction-oxidation potential and greenhouse gas flux on harvested plots. *While our hypothesis of decreased greenhouse gas flux in harvest plots was not supported by our results, limitations in our experimental design indicate need for improved instrumentation and sampling procedure. Further, trends in temperature and redox data support need for more comprehensive inquiry into the interaction between temperature, harvest of Typha, and microbial production of greenhouse gases.*

### INTRODUCTION

In anoxic wetland environments, organic carbon is transformed to inorganic forms of methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) -

through microbial activity. Both CH<sub>4</sub> and CO<sub>2</sub> are radiatively active greenhouse gases (GHGs), and CH<sub>4</sub> has a global warming potential 25 times higher than CO<sub>2</sub> (Robertson, 1999, Forster et al., 2007).

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\* Corresponding Author

[ojohns10@mail.depaul.edu](mailto:ojohns10@mail.depaul.edu)

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Measurements of GHG flux are important in

quantifying the trade-off between long-term ecosystem services (flood protection, local nutrient retention, and carbon storage) and short-term release of CH<sub>4</sub> and CO<sub>2</sub> that might occur during wetland restoration.

Invasive species can alter a suite of biotic and abiotic factors that impact greenhouse gas (GHG) flux, including hydrological conditions, substrate composition, soil temperature, and vegetation community (Bridgham et al., 2013). Specifically, *Typha x glauca* (hybrid cattail of native species *Typha angustifolia* and *Typha latifolia*) is a problematic invasive plant throughout eastern North America. *Typha x glauca* (hereafter *Typha*) expands and sustains dominance in the Great Lakes region because of its higher rates of primary productivity and more efficient use of nutrients than native species (Galatowitsch et al., 1999, Lishawa et al., 2010). Further, the accumulation of dense *Typha* litter shades the soil surface and reduces native seed germination (Vaccaro et al., 2009).

Due to its invasive tenacity and tough rhizomial root system, *Typha* has historically been managed by application of glyphosate-based herbicides (Morton, 1975, Linz et al., 2010). However, excessive nutrient input from herbicide could promote re-spread of *Typha*, given the species' efficient utilization of nutrients such as phosphorus, as mentioned above. Further, the negative effects of herbicides on nutrient cycling and water quality have sparked significant research interest in mechanical management methods. Mowing and harvesting both involve cutting *Typha* at the sediment surface, with removal of biomass during harvest because biomass is a potential source of biofuel.

Specifically, the results of a recent study highlight mechanical methods as positive alternatives to herbicide; Lawrence et al. (2015) established mesocosms of *Typha* and applied herbicides to a group of mesocosms, harvested *Typha* from another group, and left the third group as a *Typha*-dominated

control. They observed increased light penetration and plant diversity along with decreased phosphate pore water concentration in the harvested mesocosms, reduction in *Typha* cover in all three groups of mesocosms, but increased phosphate pore water concentration in the herbicide mesocosms. Further, a field study found direct positive relationships between harvest of *Typha*, light penetration to the soil, and species diversity and richness (Lishawa et al., 2015). These studies support harvest of *Typha* in Northeastern Michigan as a sustainable land management practice. Our study's objective was to fill a gap in knowledge and quantify the effect of harvest on GHG flux.

The effect of biomass harvest on GHG flux is uncertain, as similar studies of aerenchymatic plant harvests have yielded mixed results. Plots of *Scirpus lacustris* (bulrush) and *Phragmites australis* (reed) that were clipped weekly in tidal freshwater marshes had significantly lower CH<sub>4</sub> emissions than adjacent control plots (Van der Nat et al., 2000). After cultivating mesocosms of reed canary grass, Karki et al. (2015) found a significant increase in CH<sub>4</sub> and CO<sub>2</sub> emission compared to pre-harvest measurements of the mesocosms. Gunther et al. (2014) observed one cut per year of separate stands of *Typha latifolia*, *Phragmites australis*, or *Carex acutiformis* did not significantly alter annual CH<sub>4</sub> nor CO<sub>2</sub> balance in a peatland where a mix of these species was present.

We proposed its dense litter as a mechanism by which *Typha* can alter GHG flux. Plant detritus is a significant source of organic carbon, an important electron donor during anaerobic decomposition. Excess biomass is a prime resource for methanogens (eg: organisms that produce methane), which are able to thrive after other microbes consume soil substrates in their carbon-limited respiration (Megonigal et al., 2004). In a mesocosm experiment of soil and plant community similar to our study-site, the addition of litter increased CH<sub>4</sub> production potential (Valentine et al., 1994). Further,

Van den Pol-Van Dasselaar (1999) et al. tested soil samples from a fen and found 90% of CH<sub>4</sub> produced from soil organic matter mineralization came from particle size of >2mm, typical of recent plant detritus.

Under anaerobic conditions, CO<sub>2</sub> is produced by respiration of microbial functional groups in processes such as denitrification, iron reduction, and sulfate reduction. These functional groups utilize different electron acceptors from each other and from methanogens. As stated above, they rely on carbon as an electron donor, but they are usually carbon-limited (Meronigal et al., 2004). Thus, plant detritus impacts CO<sub>2</sub> production to a certain extent.

In our study, we compared GHG flux between *Typha*-harvest plots and *Typha*-dominated control plots during the summer of 2015. We accounted for litter depth and abiotic factors of temperature and reduction-oxidation (redox) potential. We expected CH<sub>4</sub> and CO<sub>2</sub> flux to increase with increased temperature. We expected CH<sub>4</sub> to increase and CO<sub>2</sub> to decrease with increasingly reduced (lack of oxygen) conditions. Ultimately, we tested the null hypothesis that neither CH<sub>4</sub> nor CO<sub>2</sub> flux will differ between harvested and control plots. Our alternative hypothesis was that harvested plots would have lower CH<sub>4</sub> and CO<sub>2</sub> flux because litter removal decreased a source of labile carbon for the microbial community.

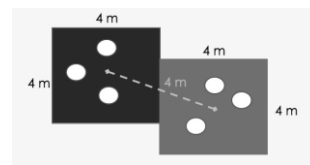
## METHODS

Cheboygan Marsh is a Great Lakes lacustrine open-embayment wetland on northern Lake Huron near the city of Cheboygan, Michigan (lat 45°39'N, long 84°28'W). *Typha* is estimated to have invaded the marsh between 1953 and 1963, and now makes up 99% of the above ground biomass (Tuchman et al. 2009). In 2011, 4x4 m experimental plots were harvested by cutting all stems at the sediment surface with an aquatic weed-whacker, followed by removal of biomass and standing litter. These plots were re-harvested in 2012. (Lishawa et al. 2015). In

July 2015, we located the center of six of these “harvest plots,” and paired them with unmanipulated- and *Typha*-dominated “control plots” that were located within 4 m of the harvest plots.

We made static gas chambers using 6-in diameter PVC pipe cut to heights ranging 20-45 cm to account for varying water depth, and constructed caps according to design recommendations by Holland et al. (1999). We installed 3 chambers within each harvest and control plot, randomly placing each chamber ~1 m from the plot center (Fig 1.1) In total we installed 36 greenhouse gas flux chambers (2 treatments x 6 paired plots x 3 replicates/plot). They were placed in unvegetated locations (between plant stems), and hammered 8-10 cm into the soil with a rubber mallet to create a seal (Fig 1.2).

At the center of each harvested and control plot (n = 6 each for harvest *Typha*-dominated control) we installed pore water sampling tubes to 10 cm depth that consisted of 1-in diameter PVC pipe with deep cuts on the side covered with fiber glass screen to reduce sediment contamination. In addition, I-Button temperature probes were placed in Nalgene bottles and buried 10 cm into the soil. They were programmed to collect temperature readings every hour.



**Figure 1.1:** 4x4 meter harvest and adjacent *Typha* control plots. 3 GHG flux chambers were randomly placed 1 meter from center of each plot.



**Figure 1.2:** Example of chamber installation, pictured here is a *Typha* control plot. Photo credit: Olivia Johnson.

We sampled GHG on July 15, July 31, and August 12, 2015. At each plot, we recorded soil temperature and air temperature then capped the 3 chambers. At time 0, 10, 20, and 30 minutes, we flushed the head space of the chamber 3 times using a needle and syringe (air transfer remained between chamber and syringe, not outside air), then drew 30mL of gas from each chamber using the needle and syringe and transferred samples to sealed-glass vials (Fig 1.3). We recorded the capped chamber height from sediment surface, then removed the cap and recorded final air temperature.



**Figure 1.3:** Example of capped chamber and gas collection tools, pictured here is a *Typha* control plot. Photo credit: Olivia Johnson.

During the second sampling campaign, we measured *Typha* litter depth in each chamber. Litter depth included both detritus from the sediment surface to the surface of the water and dead vegetation above the water surface. Because litter in the chambers was relatively undisturbed throughout the summer, this single measurement was judged to be an adequate representation of litter depth across sampling dates.

Due to initial instrument error and time constraints, we measured redox potential at each plot only once, on Aug 16, 4 days after

the last sampling campaign. Following the procedure and materials of Vepraskas (2000), we had inserted one Platinum electrode (lab-tested using Zobell's solution) in the center of each harvest and control plot adjacent to the porewater tube on Aug 12. To measure redox, we created a circuit using a voltmeter, field electrode and Calomel reference electrode submerged in a flow cell. Because water was too deep to insert reference electrode directly in the soil, we drew water from the pore water sampling tube using syringe and tubing, and immediately flushed it past the electrode in a stopcock-sealed flow cell, which consisted of an electrode bottle cemented in short piece of PVC pipe.

We transported sample vials to the University of Michigan Biological Station, where within 3 days of each campaign, we analyzed samples using a SRI 8610C Gas Chromatograph equipped with Flame Ionization Detector (FID) and connected to a laptop with Peak Simple 426 software. The FID measures CH<sub>4</sub> and CO<sub>2</sub> concentrations based on the speed at which their respective charged particles travel (within nitrogen carrier gas) through a sensor after the total sample is initially heated in a temperature-controlled oven (lit by a hydrogen flame) and passed through a series of coils that separates the sample. We exported CH<sub>4</sub> and CO<sub>2</sub> concentrations to an Excel document, in which we converted the concentrations (ppm) to mass volume/concentration (mg/m<sup>3</sup>) using molecular weight of respective gas, and corrected to field conditions (initial air temperature, atmospheric pressure) using the Ideal Gas Law. We calculated flux rates as the linear change of concentration over soil area and time (mg C m<sup>-2</sup> h<sup>-1</sup>), as outlined in Holland et al. (1999). The r<sup>2</sup> value of the flux rate as calculated by the "RSQ" function in Excel is the absolute value of the Pearson Product-Moment Correlation Coefficient for two supplied sets of values. Our two supplied sets of values were the range of time (0-30 minutes) and the respective range of GHG flux rates for each chamber.

Some individual samples and total flux rates were omitted based on the following set of guidelines, which were informed by knowledge of gas behavior and sampling error, with consideration of instrument variability and with considerations of similar calculation in the literature (Yates et al., 2006, Morse et al., 2012).

- 1) If  $r^2$  of the flux calculation was greater than 0.75, we considered it a linear flux.
- 2) If  $r^2$  was less than 0.75, we accounted for accuracy of instrument using the Minimum Detectable Concentration Difference (MDCD) as outlined in Yates et al. (2006) based on standard gas (Scotty Standard CH<sub>4</sub>:CO<sub>2</sub>:N<sub>2</sub>O mix) concentrations, and:
  - a) If an individual measurement (or measurements) indicated plateau, we removed the value(s) and re-calculated the flux. We justified this removal with the assumption that the air space of the chamber was saturated and interfering with diffusion of gas from soil to atmosphere.
  - b) If difference between sample measurements were below MDCD, we determined flux was 0.
  - c) When we observed significant jumps that indicated significant sampling error in the time frame, we omitted the entire flux rate. We attributed these jumps to ebullition, local bubbling of GHG caused by disturbance to soil surface during sampling.

Our experimental unit was at the plot level ( $n = 6$  each for *Typha*-dominated control and harvest). Therefore, we averaged the flux rates of the three chambers within each plot for GHG statistical analysis. In R, we ran a linear-mixed effect model to test the effect of treatment, sampling date, and their interaction on GHG flux. We included plot as a random factor to account for our paired (harvest and adjacent control) sample design, given that GHG flux may vary spatially throughout the marsh, and additionally because we repeatedly measured GHG flux at the plot level.

We were interested in maximum daily temperature because this value is related to light penetration, as higher light penetration to the soil can result in higher maximum soil temperature. Further, soil temperature can influence microbial activity, which drives the breakdown of litter and consequent GHG flux. Due to loss of I-Buttons at two plots and interest in general comparison between harvest and control plots, we averaged the max daily temperature data (control  $n = 6$ , harvest  $n = 4$ ) from remaining sets of I-Buttons. We ran a 2-tailed paired t-test (pairing average max daily temperature of harvest and control for each date across the sampling season, July 15th-August 12<sup>th</sup>,  $n=29$  pairs) in R to explore daily differences between average maximum temperature of harvest and control plots. Then we ran a 1-tailed t-test between harvest and *Typha*-control with alternative="greater" to specifically explore the statistical significance of harvest plots exhibiting a higher temperature than *Typha*-control.

We graphically examined in Excel the linear relationship between litter depth and average GHG flux (across 3 dates) at each chamber to test our hypothesis that labile carbon from decomposing litter could be driving GHG flux. In addition, we examined linear relationships between redox potential from each plot to average plot flux rates from Aug 12, the sampling campaign closest to the redox measurement date.

## RESULTS

We found no significant differences in CO<sub>2</sub> or CH<sub>4</sub> flux between harvest and *Typha* control plots (Table 1; Fig. 2). Flux rates did not differ significantly among sampling dates, and there was no significant variation between dates, as indicated by  $p$  values  $> 0.05$  for Treatment: Date interaction (Table 1). There was no statistical reason to separate flux rates by date, so we averaged values across the three sampling dates (6 plot averages  $\times$  3 sampling dates,  $n=18$ ). Average CH<sub>4</sub> flux rates for *Typha* control and harvest were 36.0 and 56.0 mg C m<sup>-2</sup> h<sup>-1</sup>

respectively (Fig 2A). Standard deviations of our data sets of CH<sub>4</sub> values (n=18 for each) for *Typha* control and harvest were 46.7 and 79.6 respectively. Average CO<sub>2</sub> flux rates for harvest and *Typha* control were 43.2 and 35.7 mg C m<sup>-2</sup> h<sup>-1</sup> (Fig 2B). Standard deviations of our data sets of CO<sub>2</sub> values (n=18 for each) for *Typha* control and harvest were 21.6 and 18.1 respectively.

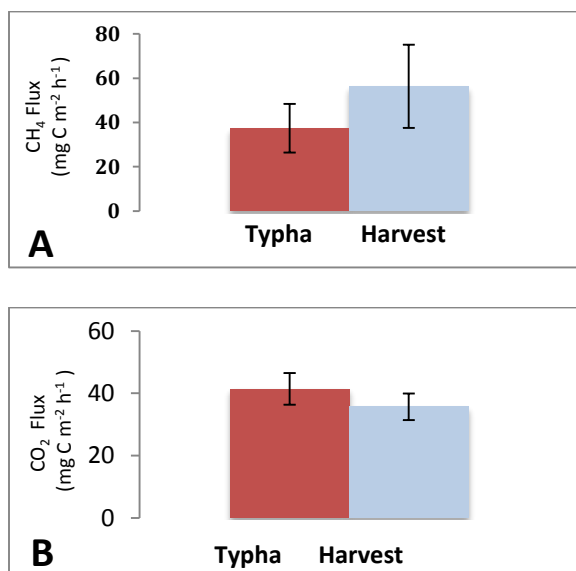
**Table 1. ANOVA Table of type III with Satterthwaite approximation for degrees of freedom for A) CH<sub>4</sub> flux and B) CO<sub>2</sub> flux.**

A linear-mixed effect model tested the effect of treatment (harvest and *Typha* control) on GHG flux with consideration of separate sampling campaigns (Jul 15, Jul 31, Aug 12).

A) CH <sub>4</sub> flux	df	F	P
Treatment	1, 25	0.423	0.530
Date	2, 25	0.331	0.722
Treatment: Date	2, 25	1.670	0.213

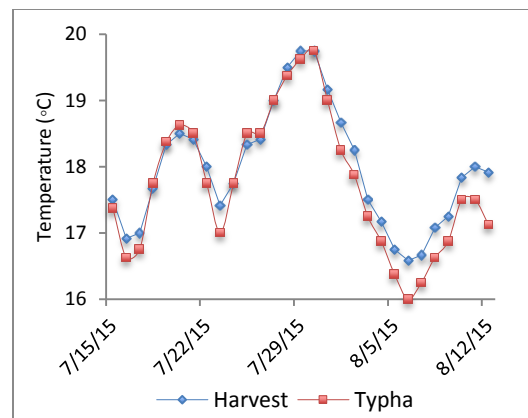
  

B) CO <sub>2</sub> flux	df	F	P
Treatment	1, 25	0.903	0.359
Date	2, 25	1.178	0.320
Treatment: Date	2, 25	0.812	0.452



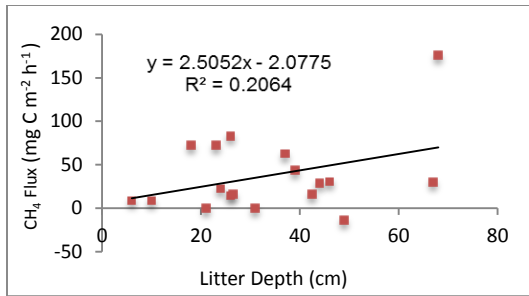
**Fig 2: Average of GHG flux rates from harvest and *Typha* control plots during summer 2015 for A) CH<sub>4</sub> B) CO<sub>2</sub>.** Because there was no statistical variation across date, we presented total average, with n=18 (6plots/campaign over 3 campaigns) for each treatment. Error bars = +/- SE.

Soil temperatures varied throughout the 2015 sampling period, with the maximum daily temperature of harvest plots typically exceeding *Typha* control plots for most days (Fig 3). Our 2-tailed paired t-test yielded a p-value < 0.05. To more strongly confirm the significance of this increased temperature, we ran a 1-tailed paired t-test in R and found a p-value of  $1.698 \times 10^{-5}$ .



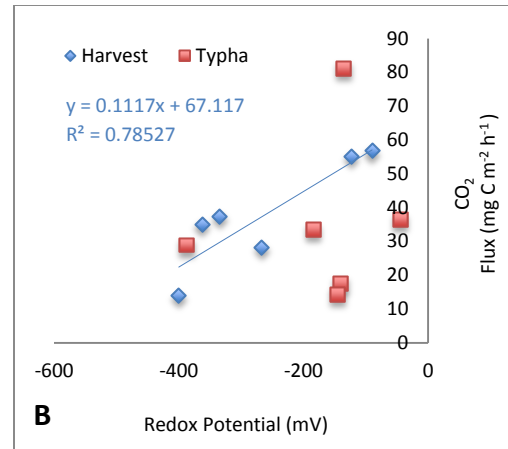
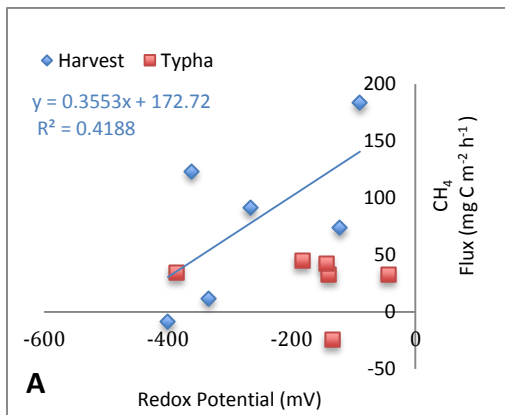
**Fig 3: Average of maximum daily temperatures for harvest and *Typha* control plots.** Based on I-Button temperature probes in the center of each harvest and *Typha* control plot. We averaged the maximum daily temperatures across plots for a collective comparison of harvest and *Typha* control plots. A 1-tailed paired t-test (alternative = greater,  $t = 4.926$ ,  $df = 28$ ) in R yielded a p-value =  $1.698 \times 10^{-5}$ .

Within the harvest plots we found no measurable *Typha* litter, as it was removed in 2011 with minimal accumulation in the intervening years. For the *Typha* control plots, we observed a slight positive correlation between litter depth and average CH<sub>4</sub> flux. In this linear graphical model, an R<sup>2</sup> value of 0.2064 indicates that 20.64% of variation CH<sub>4</sub> flux could be attributed to litter depth (Fig 4). We found no relationship between litter depth and CO<sub>2</sub> flux (R<sup>2</sup> = 0.01413, graph not included).



**Fig 4: Litter depth and average CH<sub>4</sub> flux for each chamber.** (6 *Typha* plots, 3 chambers/plot, n=18) Based on mid-season (Jul 31) litter depth measurements and average flux values (across Jul 15, Jul 31, Aug 12) for each chamber. Linear trend line and R<sup>2</sup> displayed on chart.

We observed a slight positive correlation between redox potential and flux rates for both CH<sub>4</sub> and CO<sub>2</sub> on the harvest plots. In this linear graphical model, R<sup>2</sup> values of 0.4188 and 0.78527 indicate that 41.88% of variation in CH<sub>4</sub> flux and 78.527% of variation in CO<sub>2</sub> flux could be attributed to redox potential. We observed no relationship for CH<sub>4</sub> (R<sup>2</sup>=0.027) or CO<sub>2</sub> (R<sup>2</sup>= 0.233) on *Typha* control plots (Fig 5.).



**Fig 5: Redox potential and Aug 12 flux rates at harvest and *Typha* control plots for A) CH<sub>4</sub> flux B) CO<sub>2</sub> flux.** We measured redox potential at center of each plot on Aug 16, and we present it here in relation to values from closest sampling date, Aug 12. Linear trend line and R<sup>2</sup> for Harvest plot values displayed on charts. (R<sup>2</sup> for *Typha* control plots not included, both < 0.1)

## DISCUSSION

Our objective was to quantify the effect of mechanical harvest of invasive *Typha* on GHG flux, a missing component of restoration considerations at Cheboygan Marsh and an understudied ecosystem function in restored wetlands globally. We found average CH<sub>4</sub> flux was 55% higher in harvested plots than in *Typha*-dominated control plots. We found average CO<sub>2</sub> flux was 35% lower in harvested plots than in *Typha*-dominated control. However, statistical analysis indicated no significant difference for either of these flux rates between harvest and *Typha*-dominated control plots during the summer of 2015, which is contrary to our predictions.

Higher standard deviations indicate larger variation in our set of CH<sub>4</sub> flux (SD = 46.7 and 79.6) data than in our sets of CO<sub>2</sub> flux data (SD = 21.6 & 18.1) for both *Typha* control and harvest plots. This variability is important because if there were smaller variability, than our hypothesis of increased GHG flux would have been supported by our results for CO<sub>2</sub>. Further, our results would



have indicate the opposite of our hypothesis for CH<sub>4</sub>. We continue our discussion in the context of this consideration.

Our hypothesis reasoned that removal of litter would decrease a source of labile carbon for the microbial community, and thus reduce gaseous carbon emissions. While we did not find treatment differences, we observed litter depth and CH<sub>4</sub> flux within control plots to be positively related (Fig 4), which suggests that abundant litter may be partially driving differential CH<sub>4</sub> emissions. However, this relationship is largely driven by an outlier, and therefore should not be taken as concluding support for a connection between litter and GHG flux.

Our results align with those of Gunther et al. (2014), who found one harvest (cut and removal) per year of *Typha latifolia*, *Phragmites australis*, or *Carex acutiformis* did not significantly alter annual CH<sub>4</sub> or CO<sub>2</sub> flux in a peatland. They attributed these results to *Typha*'s ability to transport oxygen via connective flow in the rhizosphere and to variation in water table level. Similar to our study, Gunther et al. installed cylindrical collars as gas chambers 10 cm into the soil. Their use of boardwalks to access chambers throughout the sampling season leads to discussion of a limitation in our own study.

Despite careful maneuvering at our sample sites, we occasionally noticed CH<sub>4</sub> ebullition (i.e., bubbles) from the soil when we stepped near the chambers. We accounted for these "artificial" emissions by omitting non-linear values that were present in otherwise linear flux observations. While we were confident in our static chamber design and consistent in our flux analysis guidelines, we may consider other instruments/methodologies that would allow for continuous measurement of emissions and improve our estimates of flux rates.

Our results do not align with those of Karki et al. (2014), who found an increase in CH<sub>4</sub>

and CO<sub>2</sub> emissions after harvest of reed canary grass. They suggested influence of plant community, specifically respiration by and exodus from root systems. Unlike our study, Karki et al. was a mesocosm study.

Van der Nat et al. (2000) found lower CH<sub>4</sub> emissions at plots of weekly-clipped bulrush and reed than at unmanipulated adjacent plots. They used a similar paired harvest-control design to our study, and also proposed removal of carbon rich litter as mechanism, but their discussion highlighted that the processes reducing emission (oxidation of methane in the rhizosphere) were likely outweighed by those enhancing emission (transport through cut plant stems and stimulation of methanogenesis by soil substrates). As we found with our slight, but ultimately outlier- driven relationship between litter and GHG flux, the direct relationship between detritus material and GHG flux may be minimally relevant to understanding the impact of harvest on GHG flux.

In comparing our results to Gunther et al. (2014), Karki et al. (2014), and Van der Nat et al. (2000), we can suggest the priority of future studies should include inquiry on how harvest alters the physical and chemical structure of the rhizosphere for a more direct inquiry on the impact of harvest on GHG flux. To consider other future study suggestions, we look at our temperature and redox potential results.

As emphasized by the studies above, GHG flux is influenced by a number of interacting factors. We observed greater average maximum daily temperatures in soils from harvest than *Typha* control plots on most days, a relationship statistically confirmed by a 1-tailed paired t-test (Fig 3). This is likely due to the increased light penetration to the soil with biomass removal, as observed in Lishawa et al. (2015) and Lawrence et al. (2015). Methanogenesis increases with increasing temperature, and is more sensitive to temperature change than other biological processes. Megonning et al. (2004) explains

this trend relates to the time required for methanogen competitors to consume alternative electron acceptors.

This relates to our findings of higher CH<sub>4</sub> flux in the harvested plots than control plots. Higher temperatures in the harvest plots may facilitate quicker consumption of alternative electron acceptors, and thus allow for increased CH<sub>4</sub> production by methanogens. Thus it is possible that despite less carbon substrates in harvest plots, elevated temperatures could have increased CH<sub>4</sub> production and resulted in no net difference between harvest and control emissions. Here again as mentioned above, future use of instruments/methodologies that would allow for continuous measurement of emissions could prove useful to further explore the relationship between harvest treatment, soil temperature, and GHG flux because we have continuous (hourly) temperature measurements.

The negative values of our redox potential confirm the reduced (i.e., oxygen poor) conditions of our study wetland. We observed decreased CH<sub>4</sub> flux as conditions became more reduced, which is contrary to our expected trend. CO<sub>2</sub> flux increased with redox potential, as expected, and the strength of this relationship was stronger than that of redox potential and CH<sub>4</sub> flux. Our redox values ranged from -386.8 to -44.2 mV (Fig 5). Our results indicated more reduced conditions than in Karki et al. (2014), who reported -115, -27, and 40 mV for 0, -10, -20 cm respectively in harvest plots and -118, -51, 151 mV for 0, -10, -20 cm respectively in bare soil treatments.

A number of factors including time of sampling (we took redox measurements 4 days after last sampling campaign), spatial variability, sensor equilibrium, and small sample size could explain the unexpected trend in CH<sub>4</sub>. However, it is also possible that in extremely reduced conditions, a linear trend is no longer an appropriate model. As emphasized by Megonigal et al. (2004), the production of CH<sub>4</sub> occurs as the final step in a thermodynamic sequence for

transformation of inorganic substances by organic matter at -244 mV. In other words, below -244 mV, the relationship between reduction oxidation potential and GHG flux may be less predicatable. As mentioned in the introduction, CO<sub>2</sub> is produced in the series of denitrification, iron reduction, and sulfate reduction that energetically proceed methanogenesis. Thus, below -200mV, we should also be critical of the relationship we found between redox potential and CO<sub>2</sub>.

What this means for our future inquiry is that redox potential may not be an adequate parameter to explore in direct relation to GHG flux. What are needed instead are direct measurements of the site's nutrient content and microbial community (the carbon dynamics of the rhizosphere, as mentioned above) in order to explore how these parameters and consequent GHG flux are affected by harvest of *Typha*.

Our initial measurements of these abiotic factors of temperature and redox potential highlight the need for a more comprehensive understanding of the underlying nutrient and microbial environment. This need is supported by the findings of a mesocosm study by Brooker et al. (2014), that CH<sub>4</sub> emissions are similar across microsites in absence of other field scale effects, such as redox boundaries, vegetation, or hydrologic fluctuations. In other words, as we highlighted in this discussion, further connections between harvest, microsite effects of plant community, soil temperature nutrient content, and microbial community activity may offer a more sufficient conclusion on the relationship between harvest and GHG flux than other field effects and than GHG flux measurements alone. Ultimately, this may offer a more direct connection between the biogeochemistry of our site and how it is impacted by harvest of *Typha*, and how this relates to more continuous GHG measurements.

Finally, this combination of new measurements may allow for an exploration of the interacting factors that may contribute to

the variability of our data set. The body of literature on GHG flux behavior and its underlying factors constitutes an evolving framework that is necessary to understand the

response of wetland dynamics to restoration treatments such as harvest of invasive species such as *Typha x glauca*.

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