

# How does time since invasion by hybrid cattail affect wetland soil microbial communities?

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## Abstract

Native plant species support biodiversity and organic substrates for microbe driven biogeochemical processes in Great Lakes coastal wetlands. Invasive species such as hybrid cattail (*Typha x glauca*) outcompete native plant species due to increased soil nutrients from human sources such as agricultural runoff and sewage. Soil microbial populations are responsible for key wetland processes, nutrient cycling, and greenhouse gas flux. Invasive plant species are predicted to impact on the microbial community in wetland sediments by changing the composition of plant community organic substrates. I hypothesize that microbial diversity decreases along a gradient of time since invasion by *Typha*. In Summer 2019, I collected soil cores from *Typha*-invaded Sand Island Marsh in northern Michigan and extracted DNA samples to analyze the bacterial community present in benthic organic sediments. To determine microbial diversity, I classified taxonomic differences in bacteria by sequencing 16S rRNA gene amplicons and identifying the presence of major taxonomic groups. My results will inform wetland restoration by relating soil microbial diversity to plant diversity and *Typha* age as a potential indicator for the impact of invasive macrophytes.

## Introduction

Over the past several decades, Great Lakes coastal wetlands have witnessed changing plant communities due to increasing dominance of the invasive hybrid cattail, *Typha x glauca*. When *Typha* invades wetlands, biodiversity and ecosystem services are reduced due to altered light penetration to the soil, increased soil organic matter, and altered nutrient cycles (Moseman et al. 2008). *Typha* is more effective than native species at recovering N from *Typha* biomass, so increased dominance by *Typha*



and the accumulation of litter can change both the plant community composition and the quantity of available N (Larkin et al. 2012). Furthermore, the ecological impacts of *Typha* in benthic sediments and plant communities increase with the length of time since invasion (Mitchell et al. 2011).

Changes in microbial community composition may accompany decreased plant diversity and altered soil characteristics after invasion by *Typha*. Microbes are largely responsible for driving biogeochemical processes; heterotrophic bacteria in wetland soil help control carbon (C) and N cycling. Nutrient addition, especially nitrogen, and changes in soil redox lead to changes in microbial community composition. Thus, *Typha* invasion can alter soil microbial communities as organic matter accumulates.

This research addresses two hypotheses: 1) *Typha*-dominated areas will have lower plant and microbial diversity than uninvaded areas, and 2) as *Typha* percent cover will increase, both plant and microbial diversity will decrease along a gradient of time since invasion.

## Methods

**Site Description:** I collected soil cores from Sand Island, a partially *Typha*-invaded wetland in northern Michigan (Figure X). My research analyzed aerial imagery from 1998 through 2015 to determine a gradient of time-since-invasion within the five *Typha* clones: 1) pre-1998, 1998-2008, and 3) 2008 (Lishawa et al., 2017). The irregular green shapes pictured in Figure Y represent an expanding *Typha* stand. Transects were established through unmanaged *Typha* clones that extended into native vegetation. Sample quadrats were placed within each *Typha* age category along each transect.

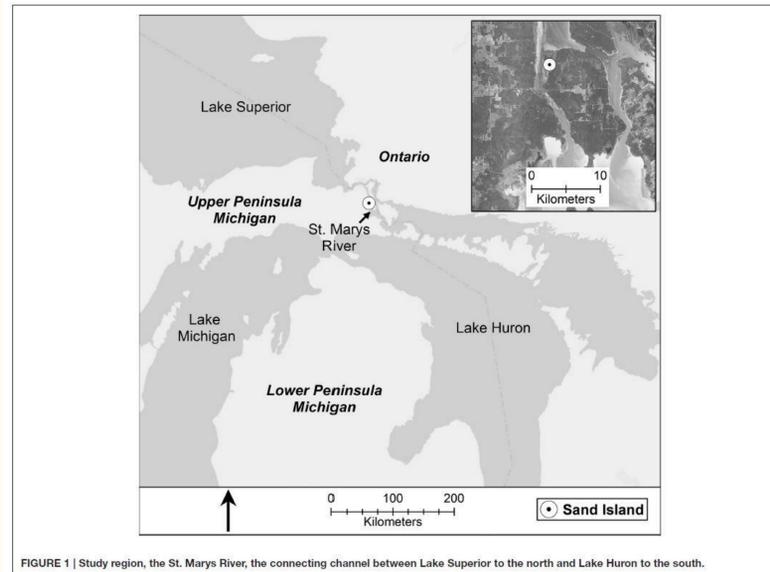


FIGURE 1 | Study region, the St. Marys River, the connecting channel between Lake Superior to the north and Lake Huron to the south.

Lishawa et al. 2017

**Sampling:** In July 2019, I collected one soil sample from 1m<sup>2</sup> plots in each *Typha* clone age class and one native vegetation plot (Total = 20 cores: 4 age classes x 5 clones). Soil cores were homogenized in the lab immediately upon return from the field, subsampled, and frozen for microbial analysis. For each soil core plot location, I recorded: 1) total plant percent cover, 2) individual plant species percent cover, 3) *Typha* height and 4) *Typha* stem count. Recorded environmental data included water depth, litter cover, soil organic depth, dissolved oxygen (DO), pH, and oxidation reduction potential (ORP).

**Microbial Assessment:** I used QIAGEN's DNeasy PowerSoil Kit to extract and purify DNA from soil subsamples. Extracted DNA was used to analyze the bacterial community composition via Illumina high-throughput sequencing of 16S rRNA gene amplicons. Extracted DNA was amplified with polymerase chain reaction. I used gel electrophoresis to ensure successful DNA extraction and amplification. Amplified 16S rRNA will be sequenced and the data analyzed with MOTHUR.

**Statistical Analysis:** I used ANOVA to test for statistical differences in DO, pH, ORP, plant species diversity (Shannon's diversity, H'), and plant richness between different age classes and clones. I used linear regression to assess the relationship of each independent variable against *Typha* percent cover. When required, data were logarithmically transformed to meet ANOVA assumptions. ORP data did not conform to normality and equal variance after transformation. A non-parametric Kruskal-Wallis and quantile regression test were employed. All statistical analyses were performed in the R version 4.0.4.

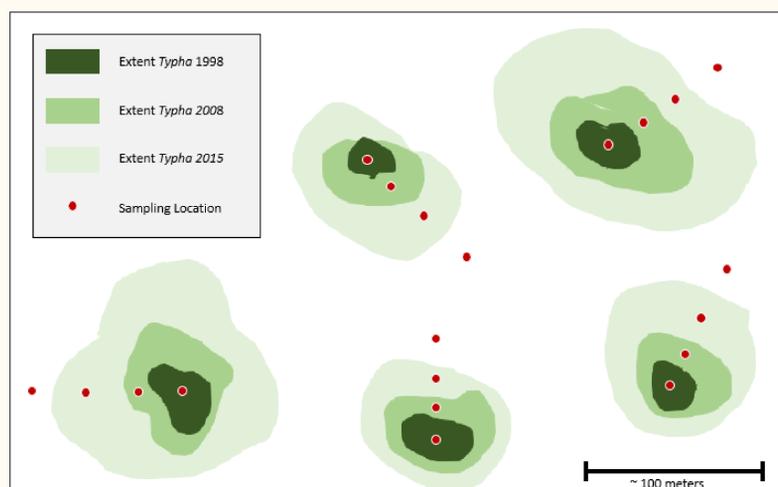


Figure 2 | A representation of five *Typha* clones at Sand Island Marsh. The irregular green shapes represent expanding stands of *Typha* assumed to be a collection of one or more individuals with multiple stems.

## Results and Conclusion

Significant effects were not detected between age ranges. DO ( $p = 0.002$ ) and pH ( $p < 0.001$ ) varied significantly by clones, thus I will look at as random effects in my future analyses. *Typha* percent cover against had a significant negative relationship with species richness ( $p = 0.015$ ), H' ( $p = 0.014$ , Figure 3), and ORP ( $p = 0.02$ , Figure 4). These results indicate that *Typha* cover may be better at predicting plant diversity than age of invasion, which would help validate the effectiveness of short-term studies (Figure 3). The higher ORP values indicate a more oxidizing environment which tends to favor bacterial activity, especially nitrification. Higher ORP occurs at lower levels of *Typha* cover (Figure 4).

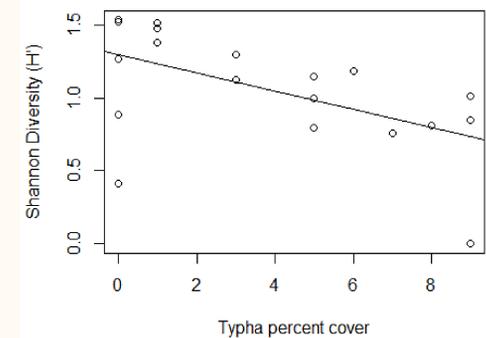


Figure 3 | Shannon diversity with increasing *Typha* cover. ( $p = 0.014$ ,  $r^2 = 0.25$ )

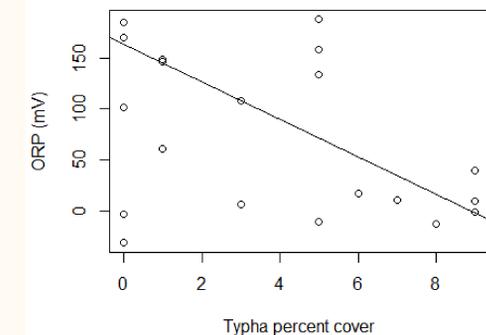


Figure 4 | Soil redox with increasing *Typha* cover. ( $p = 0.02$ ,  $r^2 = 0.25$ )

My next steps will analyze the microbial data collected from my DNA high throughput assessments. I plan to evaluate microbial richness and H' to elucidate any trends connected to plant community assemblage and environmental data. I predict higher microbial diversity and abundance of nitrifying bacteria in areas with low *Typha* cover.

Examining the time frame of potential changes in microbial and plant community composition will be useful when comparing invaded and uninvaded wetlands. Wetland restoration managers need to have a fundamental understanding regarding time-dependent invasive species impacts on plant and microbial communities. If time since invasion has no significant relationships to environmental variables, decisions for wetland management and restoration can be more accurately informed by percent cover of *Typha*.

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