2009

Soil Nutrient Changes Following a Typha X Glauca Invasion in a Great Lakes Coastal Wetland

Lane Marie Vail
Loyola University Chicago

Recommended Citation
http://ecommons.luc.edu/luc_theses/552

This Thesis is brought to you for free and open access by the Theses and Dissertations at Loyola eCommons. It has been accepted for inclusion in Master's Theses by an authorized administrator of Loyola eCommons. For more information, please contact ecommons@luc.edu.

This work is licensed under a Creative Commons Attribution-Noncommercial-No Derivative Works 3.0 License.
Copyright © 2009 Lane Marie Vail
LOYOLA UNIVERSITY CHICAGO

SOIL NUTRIENT CHANGES FOLLOWING
A TYPHA X GLAUCA INVASION
IN A GREAT LAKES COASTAL WETLAND

A THESIS SUBMITTED TO
THE FACULTY OF THE GRADUATE SCHOOL
IN CANDIDACY FOR THE DEGREE OF
MASTER OF SCIENCE

PROGRAM IN BIOLOGY

BY
LANE M. VAIL

CHICAGO, IL
DECEMBER 2009
ACKNOWLEDGEMENTS

I have received enormous support and guidance from several people that I must thank. First, I would like to thank my thesis advisor, Nancy Tuchman, who took me into her lab, though I did not yet have a background in Biology. She mentored me as I transitioned into this field and continues to mentor me as I grow in my career. I would also like to thank the members of my committee, John Kelly, Marty Berg and Eric Grimm, for their guidance, comments and advice as this thesis project developed. I would like to thank all the members of Team Typha, without whom this work would not have been completed. In particular, Pam Geddes and Dan Larkin gave me endless support and guidance and were always available when I had questions or just need someone to talk to. Special thanks to Kathi Jo Jankowski, who developed and trained me on many of the methods I used in this study; Mike Grant, who spent hours teaching and training me on proper chemistry techniques; and Pietra Mueller for helping me process all of my pollen samples. I must thank undergraduate student Pat Grucelski, who volunteered his entire summer to this research and all other undergraduate students who helped me in the field and lab. Many thanks to the faculty and staff at Loyola, especially Terry Grande, Audrey Berry and Pam Bradley, who always took time to answer my administrative questions. Lastly, I must give thanks to my husband, Tim, who supported me endlessly through three years as a student at Loyola, including two summers when I left him to conduct this research. He helped me to stay focused on my goal and finish this thesis. This research
was supported by a Henry Allan Gleason Research Fellowship, a Joel T. Heinen 
Endowment Fund Fellowship and a CUERP Research Fellowship.
To my husband and friend Tim.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS iii

LIST OF FIGURES viii

LIST OF TABLES ix

ABSTRACT x

CHAPTER ONE: LITERATURE REVIEW AND THESIS MOTIVATION 1
- Literature Review 1
  - Defining wetlands 1
  - Great Lakes coastal wetlands 2
  - Threats to coastal wetlands 3
  - Eutrophication 3
  - Invasive plant species 3
  - *Typha x glauca* 7
    - *Typha x glauca*: response to nutrients and hydrology 8
- Thesis Motivation: Effect of *Typha x glauca* on nutrients 9

CHAPTER TWO: INCREASED RATES OF NITROGEN FIXATION IN A FRESHWATER MARSH INVADED BY *TYPHA X GLAUCA* 12
- Introduction 12
- Materials and Methods 15
  - Patterns of nitrogen fixation in a *Typha x glauca*-invaded coastal wetland 15
    - Field site 15
    - Transect description 2006 16
    - Transect description 2007 16
    - Plant community composition and T. x glauca density 17
    - Nitrogen ($N_2$) fixation rates 17
    - Soil organic matter 19
    - Soil nitrogen ($NO_3^-, NH_4^+$) 19
    - Redox potential, temperature and moisture 19
  - The effect of water level on rates of nitrogen fixation 20
    - Species composition 21
    - Nitrogen ($N_2$) fixation rates 21
    - Soil carbon, moisture and nitrogen 22
    - Statistical analyses 22
- Results 22
  - 2006 Field study: Patterns of nitrogen fixation in a *Typha*-invaded coastal wetland 22
    - Preliminary results 2006 22
2007 Field study: Patterns of nitrogen fixation in a *Typha*-invaded coastal wetland

- Plant community composition and *Typha* density
- Physical, chemical and biogeochemical characteristics of vegetation zones
- Soil organic matter, nitrate \((\text{NO}_3^-)\) and ammonium \((\text{NH}_4^+)\)
- Redox potential, soil temperature and moisture
- Nitrogen \((\text{N}_2)\) fixation rates
- Patterns in the *Typha* zone

Mesocosm study: Effects of water level on rates of nitrogen fixation

- Species composition
- Soil carbon, nitrogen and moisture
- Nitrogen \((\text{N}_2)\) fixation

Discussion

Field study

Mesocosm experiments

Conclusion

CHAPTER THREE: RECONSTRUCTING THE VEGETATION HISTORY OF A GREAT LAKES COASTAL WETLAND WITH POLLEN ANALYSIS

Introduction

Materials and Methods

- Study site
- Sample collection
- Loss-on-ignition and lithology
- Lead-210 and cesium-137
- Pollen sample processing
- Pollen counting and identification
- Aerial photographs and water level data

Results

- Loss-on-ignition and lithology
- Lead-210 and cesium-137 dating
- Pollen analysis
- Aerial photograph interpretation and water level

Discussion

Conclusion

REFERENCES

VITA
LIST OF FIGURES

Figure 1.1. Conceptual diagram

Figure 2.1. Nitrogen fixation rates

Figure 2.2. Comparison of nitrogen fixation rates measured in Cheboygan Marsh

Figure 2.3. Mean Typha x glauca density (n=3) in each vegetation zone

Figure 2.4. Differences between the three vegetation zones

Figure 2.5. Correlations with Typha x glauca stem density

Figure 2.6. Mean nitrogen fixation rates

Figure 2.7. Correlation between nitrogen fixation and Typha x glauca stem density

Figure 2.8. Correlations of N₂-fixation rate with the measured variables

Figure 2.9. Mean differences among months in the Typha zone

Figure 2.10. Correlations of N₂-fixation rate in the Typha zone

Figure 2.11. Total plant aboveground biomass in experimental mesocosms

Figure 2.12. Total plant aboveground biomass (native vegetation only) in the experimental mesocosms

Figure 2.13. Shannon-Wiener diversity in the experimental mesocosms

Figure 2.14. Comparison of measured variables

Figure 2.15. Nitrogen fixation rates in experimental mesocosms

Figure 3.1. Map showing location of Cheboygan Marsh

Figure 3.2. Percentage pollen, organic carbon and lithology for core B

Figure 3.3. Aerial photographs of coring locations in Cheboygan Marsh

Figure 3.4. Hydrograph of yearly average water levels for Lake Huron/Michigan
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table Reference</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>2x2 full-factorial design of the mesocosm treatments</td>
<td>21</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Mean percent cover</td>
<td>26</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>Comparisons of all measured variables</td>
<td>28</td>
</tr>
<tr>
<td>Table 2.4</td>
<td>Comparisons of the measured variables</td>
<td>35</td>
</tr>
<tr>
<td>Table 2.5</td>
<td>Comparisons of the measured variables</td>
<td>36</td>
</tr>
<tr>
<td>Table 2.6</td>
<td>Comparisons of the measured variables</td>
<td>37</td>
</tr>
<tr>
<td>Table 2.7</td>
<td>Comparison of nitrogen fixation rates</td>
<td>45</td>
</tr>
<tr>
<td>Table 2.8</td>
<td>Comparison of field data</td>
<td>50</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>Percent abundance</td>
<td>60</td>
</tr>
</tbody>
</table>
ABSTRACT

Invasive species are one of the major threats to the integrity and health of Great Lakes coastal wetlands. Plant invaders, such as the hybrid cattail *Typha x glauca*, threaten wetlands, as they can cause shifts in ecosystem structure and function and modify biogeochemical cycles and nutrient availability. Cheboygan Marsh on the coast of Lake Huron is currently undergoing invasion by *T. x glauca*, and soils in *T. x glauca*-dominated areas of the marsh have greater soil carbon and nitrogen concentrations than in areas dominated by native vegetation. This study investigated whether *T. x glauca* is affecting the accumulation of carbon and nitrogen in Cheboygan Marsh soils. A pollen study revealed that *T. x glauca* became the dominant species in Cheboygan Marsh by the late 1950s, and approximately two decades later, soil organic matter rose dramatically and reached current levels by the 1980s. A field study to test whether increased nitrogen fixation was responsible for increases in soil nitrogen found significantly higher nitrogen fixation rates in *T. x glauca* soils than in native or newly-invaded soils. A controlled mesocosm experiment designed to test the effects of water level on nitrogen fixation rates were inconclusive, because soil carbon, which fuels microbial-mediated nitrogen fixation, remains relatively low in the mesocosms and may take several years to decades to accumulate to field levels.
CHAPTER ONE
LITERATURE REVIEW AND THESIS MOTIVATION

LITERATURE REVIEW

Defining wetlands

Over the past 100 years the definition of a wetland has been somewhat fluid. Because wetlands vary greatly from one another in their hydrology, soils and vegetation communities, an all-encompassing definition that allows for consistent wetland classification and identification has been difficult to attain. In 1956 the U.S. Fish and Wildlife Service defined wetlands in a publication referred to as Circular 39 as “lowlands covered with shallow and sometimes temporary or intermittent waters” (Shaw and Fredine 1956). Circular 39 emphasized the importance of wetlands as waterfowl habitat as well as classified 20 distinct types of wetlands.

Circular 39 was used by scientists and managers to classify wetlands until 1979, when a more comprehensive definition of wetlands was released in another report by the U.S. Fish and Wildlife Service entitled Classification of Wetlands and Deepwater Habitats of the United States (Cowardin et al. 1979). This new report was significant in that it included characteristics of soils and vegetation that have adapted to periodic flooding. In the USFWS report wetlands were “defined by plants (hydrophytes), soils (hydric soils), and frequency of flooding.”
Generally, wetlands are defined by the presence of water and having species that have adapted to that water. The most recent and complex definition was provided by the wetland characterization committee from the National Academy of Sciences, defining wetlands in this manner:

“The minimum essential characteristics of a wetland are recurrent, sustained inundation or saturation at or near the surface and the presence of physical, chemical, and biological features reflective of recurrent, sustained inundation or saturation. Common diagnostic features of wetlands are hydric soils and hydrophytic vegetation” (National Research Council 1995).

In the U.S. alone, more than half of all wetlands have been lost since European settlement in the 1700s owing to drainage, in-fill or other development (Mitsch and Gosselink 2007). These formalized definitions of wetlands have served an important function in wetland protection. If a piece of land supports hydric soils and wetland plants, it is protected from development under Section 404 of the Clean Water Act (1977).

**Great Lakes coastal wetlands**

Great Lakes coastal wetlands are among North America’s most productive ecosystems (Environment Canada 2005), providing a unique combination of environmental functions and human services. First, Great Lakes wetlands support a large diversity of plants and animals including many listed as threatened or endangered by the U.S. Fish and Wildlife Service (2007). They provide essential habitat for hundreds of birds, mammals, fish, amphibians, reptiles and invertebrates (Environment Canada 2005). Fish, amphibians and reptiles use Great Lakes wetlands for breeding and rearing, while birds use them year round or as stopover sites during spring and fall migration.
(Environment Canada 2005, Mitsch and Gosselink 2007). Additionally, coastal wetlands mitigate floods by intercepting storm runoff and absorbing wave energy (Mitsch and Gosselink 2007). Lastly, healthy-functioning wetlands filter water pollution by taking up excess nutrient loads from runoff and groundwater. This function is particularly important in Great Lakes coastal wetlands given their proximity to urban and agricultural areas that contaminate runoff with high nutrient inputs (Carpenter et al. 1998). Coastal wetlands intercept agricultural and urban runoff, removing nutrients and contaminants before the surface runoff empties into the Great Lakes.

**Threats to coastal wetlands**

*Eutrophication*

Eutrophication caused by excess nitrogen and phosphorous inputs is the leading cause of degradation to freshwater systems (Carpenter et al. 1998). Sources of pollution leading to eutrophication include point source and nonpoint source. Point-source pollution, as the name implies, is pollution discharged from a known location, such as municipal sewage treatment plants. Point-source pollution tends to be relatively easy to monitor and regulate due to its fixed position in the landscape. Nonpoint-source pollution is pollution released from many unknown, scattered sources. Non-point inputs are not as easily controlled, making them the largest source of water pollution in the United States (U.S. Environmental Protection Agency 1996). Sources of non-point pollution include atmospheric nitrogen deposition, agriculture and urban runoff.

*Invasive plant species*
Invasive plant species are one of the major threats to the biodiversity, structure and function of Great Lakes coastal wetlands. The National Invasive Species Council defines invasive species as: “a species that is non-native to the ecosystem under consideration and whose introduction causes or is likely to cause economic or environmental harm or harm to human health” (www.invasivespeciesinfo.gov).

The introduction of invasive plants species in wetlands often leads to shifts in species composition or complete replacement of native vegetation by one or more invader (Galatowitsch et al. 1999, Werner and Zedler 2002). Invasive species are the second greatest threat to biodiversity, following only habitat loss (Wilcove et al. 1998), and seem to disproportionately invade wetlands. That is, nearly a quarter of the most invasive plant species are wetland plants, though wetlands only cover about 5 to 8 percent of the Earth’s land surface (Zedler and Kercher 2004, Mitsch and Gosselink 2007). The susceptibility of wetlands to plant invasion may be a result of their role as landscapes sinks; because wetlands are typically transitional areas between upland areas and bodies of water, they tend to receive surface water with materials such as water-dispersed seeds, sediments, debris and nutrients which facilitate invasions (Zedler and Kercher 2004)

The number of species that have been introduced to new ranges has increased dramatically over the past 200 years as a result of expanding human transportation and commerce (Mack et al. 2000). This increased human travel has resulted in both intentional and unintentional introductions of non-native species, many of which have become nuisance or invasive species. Some intentional introductions of non-native species include: plantings by early European settlers, nostalgic for familiar “old world”
garden plants (i.e. *Alliaria petiolata* (garlic mustard), *Rhamnus cathartica* (European buckthorn)); release of fish for predation, angling or algae control (i.e. *Salmo trutta* (brown trout), *Hypophthalmichthys molitrix* (silver carp)); dumping of aquaria plant species in local waterway (i.e. *Hydrilla verticillata* (hydrilla), *Myriophyllum spicatum* (Eurasian water-milfoil)). Most plant and animal invasions are the result of unintentional actions. Invasive species may “hitch a ride” on cargo ships or in ballast water (i.e. *Boiga irregularis* (brown tree snake), *Dreissena polymorpha* (zebra mussel)) or arrive as contaminants among crop seeds (Mack et al. 2000).

Extensive research has focused on understanding the patterns of plant invasions (Mills et al. 1993, Galatowitsch et al. 1999, Mack et al. 2000, Hager 2004, MacDougall and Turkington 2005, Crowl et al. 2008, Tuchman et al. 2009), and several hypotheses have been developed to explain how certain life history traits make invasive species more successful than natives they displace (Levine et al. 2003). Of the proposed hypotheses, only five have been tested experimentally in wetlands and were described in detail by Zedler and Kercher (2004). The five hypotheses are *Enemy Release, Broader Tolerance, Efficient Use, Hybrid Vigor* and *Allelopathy* and are not necessarily mutually exclusive of one another.

The *Enemy Release Hypothesis* predicts that when certain new species are introduced to new ranges, they are no longer subject to predators or herbivores of their old ranges and are “released” from those enemies or constraints. Because they no longer need to devote resources and nutrients to building chemical defenses, they can redirect these resources to other areas such as productivity and reproduction. The *Broader
Tolerance Hypothesis predicts that invasive species are successful because they are capable of tolerating broader environmental conditions, such as variable hydrology, nutrient flux, sedimentation or salinity (Galatowitsch et al. 1999, Werner and Zedler 2002, Miller and Zedler 2003, Angeloni et al. 2006, Boers et al. 2007). The Efficient Use Hypothesis states that invasive species use resources, such as nutrients and light, more efficiently than do native species. The Hybrid Vigor Hypothesis describes a phenomenon discussed by Ellstrand and Schierenbeck (2000) whereby introduced species hybridize with local species (28 documented), and the resulting hybrid offspring become invasive, out-competing both parent species in growth and reproduction. Finally, the Allelopathy Hypothesis describes how certain invasive species become dominant in a new area because they release biochemical toxins in the surrounding soils and hinder the germination and growth of native species.

Once invasive plant species move in to an area and become dominant, they decrease native species diversity as well as modify other important wetland functions and processes. In addition to altering native plant diversity, several studies have demonstrated how invasive species can alter food web dynamics (Zedler and Kercher 2004), sedimentation and decomposition rates (Werner and Zedler 2002, Freyman 2008) and hydrology (Kercher et al. 2007). In recent years, several studies have focused on how invasive species affect native soil nutrient pools and fluxes. In 2003, Joan Ehrenfeld reviewed data from 79 papers involving 56 invasive plant species, focusing on how they affect soil-associated processes involving carbon and nitrogen. Overall, Ehrenfeld found that invasive plants, when compared to native plants, have greater net primary production
(carbon sequestration) and growth rates, and have associated soils with more inorganic nitrogen, increased nitrogen mineralization and (in some instances) increased rates of nitrogen fixation (Ehrenfeld 2003). In another, more recent review, Liao et al. performed a meta-analysis on 94 experimental studies of plant invasions to quantify the changes of carbon (C) and nitrogen (N) fluxes and availability (2008). They found plant invasions tended to increase both C and N fluxes and pools in invaded ecosystems relative to native ecosystems (Liao et al. 2008). These soil modifications by invasive plants suggest positive feedback mechanisms that accelerate invasions or stabilize them once they have begun (Ehrenfeld 2003, Liao et al. 2008). For example, if an invader is either capable of fixing atmospheric nitrogen or facilitating species that do, then the resulting soil nitrogen pools not only benefit the invader itself but may make reversal of invasion all the more difficult (Corbin and D'Antonio 2004).

**Typha x glauca**

One of the most common plant invaders found in Great Lakes wetlands is the cattail *Typha x glauca*, a hybrid of *Typha latifolia* and *Typha angustifolia* (Green and Galatowitsch 2001, Kercher et al. 2007). *Typha latifolia* is native to the Great Lakes region, whereas the origins of *Typha angustifolia* remain unclear. *Typha angustifolia* has traditionally been considered an introduced species to North America, but recent studies suggest that the cattail is native to the northeastern United States, as it has been found in the pollen record pre-dating European settlement (Pederson et al. 2005, Shih and Finkelstein 2008). By the early 1900s, *T. angustifolia* had reportedly migrated to Michigan wetlands, where it hybridized with *T. latifolia* (Stuckey and Salamon 1987).
*T. x glauca* possesses several traits that contribute to its success as an invader. Similar to many plant invaders, *Typha x glauca* reproduces clonally, allowing it to persist year round and spread more rapidly than native species or seed-dispersed species. As a hybrid, *T. x glauca* has successful traits of both parent species. For example, the hybrid contains abundant aerenchyma tissue, making it more tolerant of high water conditions like *T. angustifolia* but retains a high capacity for biomass production like *T. latifolia* (Grace and Wetzel 1981). *T. x glauca* is also capable of tolerating a wide range of salinity, a trait conferred by *T. angustifolia*, which is historically a salt-marsh species. Its large stature enables *T. x glauca* to capture more light for increased primary production, while rapid nutrient uptake enables it to out-compete native species. In addition, *T. x glauca* can stimulate rates of soil nitrogen-fixation that are higher than either parent species (Eckardt and Biesboer 1988).

**Typha x glauca: response to nutrients and hydrology**

Similar to many invasive species, all *Typha* species thrive in areas of high nutrient input (i.e. nitrogen and phosphorous), mainly because of their fast growth rates and ability to take up nutrients rapidly (Newman et al. 1996, Miao and Sklar 1997, Mack et al. 2000). Woo and Zedler (2002) conducted controlled fertilizer experiments with *T. x glauca* and native sedge-meadow species to determine whether or not additions of nitrogen and phosphorous accelerated the expansion of monospecific stands of *T. x glauca* into native wetland habitat. They found that *T. x glauca* responded to nutrient additions through increased above ground biomass, stem density and height, whereas native sedge species showed no response to nutrient additions (Woo and Zedler 2002).
Similar to many species of invasive wetland plants, the physiology of *T. x glauca* is well-adapted to high fluctuations in water levels (Harris and Marshall 1963, Wilcox et al. 1985, Miller and Zedler 2003, Wilcox et al. 2008). Abundant aerenchyma tissue enables *T. x glauca* to survive during periods of high water by transporting oxygen to its root (Mitsch and Gosselink 2007). Wilcox et al. (2008) suggested that historical Great Lakes water levels may have encouraged the cattail’s dominance, as native sedge species were pushed beyond their threshold of water-level tolerance during high water periods and *T. x glauca* was not. Moreover, if the high water conditions also carry nutrients, *T. x glauca* maintains a competitive advantage over native species, as it takes up nutrients at a higher rate (Wilcox et al. 1985, Miao and Sklar 1997).

**THESIS MOTIVATION: EFFECT OF TYPHA X GLAUCA ON NUTRIENTS**

*T. x glauca* responds well to wetland systems with high nutrients (Woo and Zedler 2002); however, recent evidence suggests that *T. x glauca* thrives in systems with low nutrients and may even cause nutrient shifts in the systems it invades (Corbin and D'Antonio 2004, Angeloni et al. 2006, Jankowski 2007). In their study of a Lake Huron coastal wetland, Cheboygan Marsh, Angeloni et al. (2006) found that in areas invaded by *T. x glauca* soil carbon and soil inorganic nitrogen were significantly higher than in native soils. In addition, Jankowski (2007) found that these increases in organic carbon and inorganic nitrogen in Cheboygan Marsh followed a gradient that corresponded with increased densities of *T. x glauca*. The increased nutrients in Cheboygan Marsh did not appear to be coming from groundwater, surface runoff or Lake Huron (Jankowski 2007).
The following two chapters of this thesis present studies aimed at investigating the origin of *T. x glauca*-associated soil carbon and nitrogen and whether *T. x glauca*-derived organic carbon in soils is affecting soil nitrogen accumulation. I will attempt to answer the following questions: 1) Does *Typha x glauca* and its contribution to soil organic carbon increase rates of soil microbial nitrogen fixation relative to native plant communities? 2) How long has *Typha x glauca* been the dominant plant in Cheboygan Marsh, and is it responsible for the thick layer of organic carbon deposited in the soil record?

The conceptual model in Figure 1.1a demonstrates a positive feedback mechanism that may be operating in Cheboygan Marsh, which is tested in Chapter 2. In this model *T. x glauca* fixes large quantities of CO₂ which contributes to soil organic carbon. This soil organic carbon stimulates soil microbial N₂ fixers, which in turn benefits the invader and completes the feedback loop.

The conceptual model in Figure 1.1b demonstrates how *T. x glauca* may be responsible for the accumulation of soil organic matter in Cheboygan. In Chapter 3, pollen is used as a marker to determine whether or not *T. x glauca* is responsible for this organic matter accumulation.
Figure 1.1 Conceptual diagram a) positive feedback mechanism in which CO$_2$ and N$_2$ fixation contribute to elevated soil nutrients in Cheboygan Marsh, and b) soil organic matter has accumulated over time with the presence of *T. x glauca*. 
CHAPTER TWO
INCREASED RATES OF NITROGEN FIXATION IN A FRESHWATER MARSH INVADED BY *TYPHA X GLAUCA*

INTRODUCTION

The Laurentian Great Lakes comprise one-fifth of the world’s freshwater supply and provide the United States with 95% of its economic, recreational and potable water (Great Lakes Information Network, 2006). The health of the Great Lakes relies heavily on the coastal wetlands that line their shores. Wetlands provide habitat for wildlife, including fish, amphibians and migratory birds and perform critical functions such as buffering floods, impeding erosion along streams and lakeshore and acting as nutrient sinks along agricultural streams and other areas of high nitrogen and phosphorus loads that would otherwise flow into and contaminate adjacent waters (Hammer 1989, Schultz et al. 1995, Zedler 2003, Fisher and Acreman 2004, Mitsch and Gosselink 2007).

Invasive plant species are a major threat to wetlands (Mills et al. 1993, Pimentel et al. 2005, Zedler and Kercher 2005). Invasive species often reduce biodiversity by displacing native vegetation with dense, monospecific stands that fundamentally alter ecosystem functioning (Mack et al. 2000, Zedler and Kercher 2004). They can modify biogeochemical cycling and alter the capacity of wetlands to store and release much of the excess carbon, nitrogen and phosphorous inputs to our waterways (Ehrenfeld 2003). Invasive species, including invasive wetland plants, also have adverse economic effects.
In the United States they have caused an estimated $120 billion per year in damages due to losses and active management expenses (Pimentel et al. 2005).

The ability of invasive plant species to outcompete natives through exploiting areas of disturbed hydrology and increased nutrient inputs has been well-documented (Green and Galatowitsch 2001, Woo and Zedler 2002, Kercher et al. 2007). However, more recent studies suggest that invasive plants may possess the capacity to drive ecosystem change themselves (Levine et al. 2003, MacDougall and Turkington 2005). They can dominate native vegetation through higher growth rates and biomass production and shifting historical nutrient cycling and biogeochemical processes (Welsh 2000, Ehrenfeld 2003, Callaway et al. 2004, Corbin and D'Antonio 2004). For example, plant invaders can increase levels of soil organic matter through the production of leaf litter and leaching of root exudates, fueling microbial activity associated with the nitrogen cycle and nitrogen fixation (Jones et al. 2003, Liao et al. 2008). Increased soil nitrogen concentrations may give invasive plants a competitive advantage over native plants that are better competitors in low-nitrogen soils (Ehrenfeld and Scott 2001, Evans et al. 2001, Corbin and D'Antonio 2004).

One particularly aggressive invader in Great Lakes wetlands is *Typha x glauca* (hereafter *T. x glauca*), a hybrid of *Typha latifolia* and *Typha angustifolia* (Green and Galatowitsch 2001, Angeloni et al. 2006). Typically, plant invaders such as *T. x glauca* exploit high-nutrient areas, including road-side ditches, urban areas and agricultural streams (Zedler and Kercher 2004); but newer evidence suggests these cattail invaders
may be *responsible* for the functional changes that occur in the wetlands they invade (Angeloni et al. 2006, Tuchman et al. 2009).

Cheboygan Marsh in northern Michigan is a coastal wetland on Lake Huron undergoing invasion by *T. x glauca*. *T. x glauca* is capable of growing quickly and spreading aggressively and can have up to 300x more aboveground biomass than its native counterparts. The extensive growth of *T. x glauca* has increased production of leaf litter and root exudates, which has probably promoted a 4-fold increase in organic matter and a 900-fold increase in inorganic nitrogen, compared to nutrient-poor soils under native vegetation (Angeloni et al. 2006).

In 2005, Jankowski (2007) investigated the high soil nitrogen by testing whether rates of denitrification were also altered by *T. x glauca* in Cheboygan Marsh soils. Rates of denitrification were measured along a gradient of increasing *T. x glauca* density to determine whether soils under high *T. x glauca* densities had lower rates of denitrification, resulting in the accumulation of soil nitrogen. To the contrary, *T. x glauca* was found to increase rates of denitrification, ruling out this pathway as the explanation for elevated soil nitrogen (Jankowski 2007).

Nitrogen fixation (*N*_2*-fixation) has since become the leading hypothesis to explain why nitrogen concentration is high in *T. x glauca*-invaded soils. Certain plant invaders are known to increase rates of *N*_2*-fixation in the soils they invade (Vitousek 1989, Liao et al. 2008), subsequently increasing inorganic soil nitrogen and giving invaders a competitive advantage over native plants that are better suited to low-nitrogen soils. In addition, *T. x glauca* has root-associated bacteria that fix nitrogen at higher rates than
either of its parent species (Eckardt and Biesboer 1988). In mineral soils that are low in organic carbon, as historically occurring in Cheboygan Marsh, the organic carbon enrichment produced by *T. x glauca* could stimulate the nitrogen fixation process.

The purpose of this study was to examine whether soil microbial nitrogen fixation is a mechanism producing elevated nitrogen concentrations in Cheboygan Marsh soils invaded by *T. x glauca*. The following question was addressed: Does *Typha x glauca* and its contribution to soil organic carbon increase rates of soil microbial nitrogen fixation relative to native plant communities? To answer this question, measurements were made in the field and in a parallel controlled mesocosm experiment. Because soil organic matter, light availability, litter depth and water level fluctuation all vary in Cheboygan Marsh, wetland mesocosms designed to simulate Cheboygan Marsh were used to measure N₂-fixation under conditions where these variables could be held constant.

**MATERIALS AND METHODS**

**Patterns of nitrogen fixation in a *T. x glauca*-invaded coastal wetland**

*Field site*

This study was conducted in Cheboygan Marsh, a freshwater coastal wetland located in Northern Lower Michigan on Lake Huron during summers 2006 and 2007. Cheboygan Marsh was chosen because it has a large stand of invasive *T. x glauca* and remnant native wetland vegetation. The marsh is open to nutrient exchange with Lake Huron through seiche activity (wave action caused by strong winds). The marsh does not
receive significant amounts of exogenous nutrients from either groundwater or oligotrophic (low nutrient) Lake Huron. *T. x glauca* probably invaded in the 1950s (L. Vail, unpublished data presented in Chapter 3) and has since taken over more than 2/3 of the marsh area.

**Transect description 2006**

In 2006, a preliminary field study in Cheboygan Marsh was conducted. Soil samples were collected from a 92-meter long transect that ran along a gradient of increasing *T. x glauca* density. N$_2$-fixation rates and other variables (described below) were measured along this transect with three replicates (n=3) at each of 12, 1-meter diameter plots along the transect.

**Transect description 2007**

A power analysis of the 2006 data suggested a dramatic increase in replication was needed. Thus, in 2007 several new transects were established to cover an area larger than the single transect used in 2006, and the marsh was divided into three distinct vegetation zones: a *Typha* zone (no native plant species present, only *T. x glauca*), a Transition zone (the zone of active invasion with about 35% *T. x glauca* cover, with the remaining native plant cover) and a Native zone (no *T. x glauca* present, only native plants). In each of the three zones, three 10-meter long transects were placed running parallel to the invasion front (to reduce vegetation gradient effects), for a total of 9 transects.
**Plant community composition and T. x glauca density**

To characterize differences in plant community composition in the three vegetation zones of Cheboygan Marsh, percent cover of all plant species in 0.25m$^2$ quadrats was measured based on plant-species cover-classes 0-7, where 0=0% cover; 1=0-1% cover; 2=2-5% cover; 3=6-25% cover; 4=26-50% cover; 5=51-75% cover; 6=76-95% cover and 7=96-100% cover (Daubenmire 1959). Twice during the summer 2007, once in the beginning and once toward the end of the growing season, percent cover was measured at three replicate quadrats along each of the three transects in the *Typha*, Transition and Native zones. In the Transition and *Typha* zones, *Typha* stem density and height were recorded three times in June, July, and August.

**Nitrogen ($N_2$) fixation rates**

$N_2$-fixation rates were measured using the acetylene reduction method for aquatic sediments (Odonohue et al. 1991, Capone 1993). The acetylene reduction method measures nitrogenase activity, the enzyme responsible for the reduction of atmospheric nitrogen ($N_2$) into ammonia ($NH_3$). Nitrogenase is capable of reducing acetylene ($C_2H_2$) to ethylene ($C_2H_4$) at the stoichiometric ratio of 3:1. Thus, ethylene production served as a proxy for rates of $N_2$-fixation.

Sediment cores were collected from 7 plots that were regularly placed 1-2 meters apart on each randomly-placed transect, for a total of 21 samples in each vegetation zone, in June, July and August. Sediments in each vegetation zone vary greatly in their structure and composition, making consistent sampling difficult. In the *Typha* zone, soils are peaty and full of dense roots, which prohibit the use of traditional coring devices, as
they can not cut through the root matrix. In the Native zone, soils are sandy and completely water saturated, with a slurry-like consistency. Thus, all soil cores were “cut out” using a compass saw, with a diameter of 10 cm and a depth ranging from 8-17 cm, to capture soils from at least 50% of the depth of the rooting zone. These “large sediment cores” were placed in bags in the field and taken back to the laboratory on ice.

Because soil microbial communities are easily disturbed by destructive sampling, care was taken to maintain the integrity of the soils. In the lab, 60 ml soil subsamples were cut from the center of each “large core” and placed in 230 ml canning jars. To avoid measuring N$_2$-fixation by cyanobacteria, the top 2 cm of soil were removed from the sample core, which was where cyanobacteria were observed. The canning jars were covered with a gastight lid fitted with a rubber septum for needle injections. Pure acetylene gas, made by combining calcium carbide with deionized water, was injected into the jars at volumes of 15% of the headspace and allowed to incubate for 24 hours at room temperature. After incubation, 100 µl subsamples were extracted from the headspace with a Hamilton gastight microsyringe and analyzed immediately for ethylene production on a Finnigan Trace Gas Chromatograph fitted with a flame-ionization detector. A HayeSep T stainless-steel packed column (Restek, Inc., Bellefonte, PA) was used with helium as the carrier gas (flow rate of 25 ml/min), oven temperature at 60 °C and detector temperature at 180 °C.
Soil organic matter

After soil samples were analyzed for N\textsubscript{2}-fixation, soil organic matter for each was determined as loss on ignition in a muffle furnace at 550 °C for 2 hours and calculated as percent of dry mass (APHA 2005).

Soil nitrogen (NO\textsubscript{3}, NH\textsubscript{4}+)

Leftover soils from the N\textsubscript{2}-fixation “large cores” taken at the beginning, middle and end of each transect were combined, creating three composite samples from each transect that were analyzed for concentrations of soil nitrate and ammonium. When samples could not be run immediately, they were stored in the refrigerator at 4 °C and processed within 24 hours. Samples were then sieved using a 2 mm mesh sieve to remove rocks and root materials. Ten grams of wet soil were placed in a 50 ml centrifuge tube, extracted with 40 ml of 2M KCl and shaken for 1 hour. Samples were centrifuged at 1000 rpm for 5 minutes and the supernatant filtered through a G8 glass fiber filter into acid-washed glassware. Samples were then stored at -5 °C until analysis. NO\textsubscript{3} was determined in the extractant using the automated cadmium reduction method (APHA 2005) on a Bran Luebbe Auto-Analyzer 3. NH\textsubscript{4}+ was determined in the extractant using the automated phenate method (APHA 2005) on the same Auto-Analyzer.

Redox potential, temperature and moisture

Platinum-tipped redox electrodes (Wafer et al. 2004) were constructed and deployed in the marsh in early July. There were six randomly placed replicate electrodes in the Native zone, six in the Transition zone and four in the Typha zone. The platinum tip was placed in the soil 10 cm below the soil surface and allowed to equilibrate for 48
hours before the first measurements were made. Redox potential was measured using a volt meter as the voltage difference between the platinum probes and an Accumet Epoxy body calomel reference electrode. Within a 0.5 meter radius of each probe, water temperature was measured with a standard field thermometer at the water-soil interface and soil temperature was measured at 10 cm below the soil surface. Redox potential and temperature were measured in mid July, early August and late August. Soil moisture was measured as the soil water content after drying the soils at 105 °C for 24 hours (Jarrell et al. 1999).

The effect of water level on rates of nitrogen fixation

$\text{N}_2$-fixation rates were also measured in a controlled mesocosm experiment to control for water depth differences between $\text{Typha}$ and Native vegetation zones in Cheboygan Marsh. In 2003, 20 artificial wetlands (2 m$^3$ mesocosms) were constructed at the University of Michigan Biological Station, approximately 30 km from Cheboygan Marsh. These outdoor mesocosms were constructed with a wood frame (2 meter long x 1 meter wide x 1 meter deep), lined with pond liner and filled with a mixture of 80% rubicon sand and 20% hydrosoils from a local wetland. They were planted with the 12 most abundant native wetland species from Cheboygan Marsh, in densities similar to those in the native vegetation zone. $T. x$ glauca and its litter was added to half of the mesocosms in 2004 at a density of 8 stems/m$^2$, to mimic the invasion within the Transition zone. The mesocosms have been maintained for the past 5 years with treatments following a 2x2 full-factorial design of plus and minus Typha treatment and high and low water treatments (Table 2.1). High water level was defined as constant
standing water throughout the growing season. Low water level was defined as low to no
standing water throughout the growing season. Water level treatments were maintained
by watering high water mesocosms for 30 minutes per week and watering low water
mesocosms for 10 minutes per week. These treatments allowed me to isolate and
quantify the effects of *T. x glauca* and water level on rates of N$_2$-fixation.

<table>
<thead>
<tr>
<th>+ <em>Typha</em></th>
<th>- <em>Typha</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>High water</td>
<td>High water</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>+ <em>Typha</em></td>
<td>- <em>Typha</em></td>
</tr>
<tr>
<td>Low water</td>
<td>Low water</td>
</tr>
</tbody>
</table>

*Table 2.1. 2x2 full-factorial design of the mesocosm treatments.*
There were five replicate mesocosms per treatment.

*Species composition*
Species composition was measured in the mesocosms to assess diversity and to
obtain *T. x glauca* densities. Stem densities and heights of each species were counted on
10 cm wide and 180 cm long transects, covering 50% of each mesocosm area. Biomass
of each plant was calculated using previously-determined length-to-biomass regressions
and diversity of each mesocosm was determined using the Shannon-Wiener index.

*Nitrogen (N$_2$) fixation rates*
Nitrogen fixation was measured in each mesocosm using the acetylene reduction
method described above for field sampling. However, samples were extracted from the
mesocosms using a 9 cm-long, 22 mm-diameter cork borer. This sampling device was
used because soils were consistent in texture among mesocosms. Two soil cores totaling 60 mL in volume were composited into one sample per mesocosm. Nitrogen fixation was measured once in July at the peak of the growing season.

**Soil carbon, moisture and nitrogen**

Separate soil samples were collected from the mesocosms, with the same corkscrew used for N$_2$-fixation samples, for measurements of soil carbon, moisture, nitrate and ammonium. The field methods described above were used.

**Statistical analyses**

Data from the field were analyzed using one-way ANOVA ($\alpha=0.05$) and Pearson product-moment correlation analysis with changes in variables as a function of *T. x glauca* stem density and N$_2$-fixation rates. The water level experiment data were analyzed using a 2-way ANOVA ($\alpha=0.05$). Data that did not meet the assumptions of ANOVA were transformed. All analyses were run using SyStat 11 statistical package.

**RESULTS**

**2006 Field study: Patterns of nitrogen fixation in a *Typha*-invaded coastal wetland**

**Preliminary results 2006**

Results from the preliminary field study suggested Cheboygan Marsh soils under invasive *T. x glauca* had higher N$_2$-fixation rates than soils under native vegetation (Figure 2.1). The highest rates were measured in *T. x glauca*-invaded soils, though with considerable variability and no statistical significance. N$_2$-fixation rates were measured at each of 12 plots along the transect, with three replicates per plot (n=3). With so few
replicates, I was able to identify patterns of increased N$_2$-fixation rates with increased *T. x glauca* density but was unable to capture the soil heterogeneity with any statistical significance.

![Graph showing nitrogen fixation rate vs. typha density](image)

**Figure 2.1. Nitrogen fixation rates** (±1 standard error) measured in Cheboygan Marsh, July 2006 along a transect of increasing *T. x glauca* density and decreasing native plant density. Samples were collected at four or eight meter intervals, with highest resolution taken in newly-invaded areas.

The study from 2006 also revealed an additional layer of complexity: N$_2$-fixing cyanobacteria residing in the top layer of soil in the marsh’s native plant community, where water depth is approximately 10 cm and light levels are high with little shading from the small and sparse plants. These benthic cyanobacteria were not present in the *T. x glauca*-invaded areas where standing water is not present and leaf litter minimizes light penetration to the soils. Thus, considerable rates of N$_2$-fixation were measured in the top 1cm of soil in areas of native vegetation (Figure 2.2), likely from the cyanobacterial
nitrogen fixers identified to be *Nostoc, Oscillatoria, Anabaena, Lyngbya, Epithemia* and *Rivularia*.

![Figure 2.2. Comparison of nitrogen fixation rates measured in Cheboygan Marsh, August 2006 in the upper 1 cm of soil core versus lower 2-5 cm of soil core.](image)

**2007 Field study: Patterns of nitrogen fixation in a Typha-invaded coastal wetland**

*Plant community composition and Typha density*

*T. x glauca* density was significantly different (p<0.001) among the three vegetation zones, with an average of 13.2 stems/m² in the Transition zone, 40.4 stems/m² in the Typha zone and no *T. x glauca* present in the Native zone (Figure 2.3). Species richness was highest in the Native and Transition zones, with 9 species present in the Native zone and 11 species (including *T. x glauca*) present in the Transition zone (Table 2.2). Of the 11 species in the Transition zone, *T. x glauca* had the greatest percent cover (7.5 ± 2.0%). Percent cover of six of the nine species that were present in the Native zone declined in the Transition zone. In the *Typha* zone, *T. x glauca* was the only species present, with an average percent cover of 49.4 ± 5.8%.
Figure 2.3. Mean *Typha x glauca* density (n=3) in each vegetation zone. The Native zone is characterized by the absence of *Typha x glauca* stems and thus has a zero value for *Typha* density. Error bars represent ± 1 standard error.
Physical, chemical and biogeochemical characteristics of vegetation zones

Of the soil physical, chemical and biogeochemical variables measured (soil organic matter, soil NO$_3^-$ and NH$_4^+$, soil moisture, redox potential, temperature and nitrogen fixation), all but redox potential had significant differences between vegetation zones (Table 2.3). Soil organic matter, inorganic nitrogen, soil moisture and nitrogen fixation were all highest in the *Typha* zone and highly positively correlated with *Typha* stem density (Figures 2.4, 2.5, 2.6 and 2.7). Temperature was lowest in the *Typha* zone and strongly negatively correlated with *Typha* density (Figures 2.4e and 2.5e).

Table 2.2. Mean percent cover (n=3, ± 1 standard error) of each species present in each of the three vegetation zones, as well as standing dead, litter and ground.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Native</th>
<th>Transition</th>
<th><em>T. x glauca</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. x glauca</em></td>
<td>0</td>
<td>7.5 ± 2.0</td>
<td>49.4 ± 5.8</td>
</tr>
<tr>
<td><em>Eleocharis smallii</em></td>
<td>11.4 ± 4.4</td>
<td>2.5 ± 0.6</td>
<td>0</td>
</tr>
<tr>
<td><em>E. erythropoda</em></td>
<td>1.7 ± 0.5</td>
<td>7.0 ± 2.2</td>
<td>0</td>
</tr>
<tr>
<td><em>Cicuta bulbifera</em></td>
<td>0</td>
<td>7.1 ± 2.6</td>
<td>0</td>
</tr>
<tr>
<td><em>Schoenoplectus americanus</em></td>
<td>5.2 ± 2.0</td>
<td>0.5 ± 0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>S. acutus</em></td>
<td>3.5 ± 0.0</td>
<td>1.7 ± 0.5</td>
<td>0</td>
</tr>
<tr>
<td><em>Carex viridula</em></td>
<td>0.5 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ca. aquatilis</em></td>
<td>2.8 ± 0.5</td>
<td>2.0 ± 0.6</td>
<td>0</td>
</tr>
<tr>
<td><em>Juncus alpinus</em></td>
<td>4.0 ± 1.9</td>
<td>4.4 ± 1.7</td>
<td>0</td>
</tr>
<tr>
<td><em>J. balticus</em></td>
<td>18.1 ± 4.8</td>
<td>2.2 ± 0.5</td>
<td>0</td>
</tr>
<tr>
<td><em>J. nodosus</em></td>
<td>4.8 ± 1.3</td>
<td>6.9 ± 2.4</td>
<td>0</td>
</tr>
<tr>
<td>Litter</td>
<td>52.2 ± 9.7</td>
<td>69.9 ± 8.7</td>
<td>98.0 ± 0.0</td>
</tr>
<tr>
<td>Ground</td>
<td>24.4 ± 6.1</td>
<td>20.4 ± 8.9</td>
<td>0.5 ± 0.0</td>
</tr>
<tr>
<td>Standing Dead</td>
<td>1.7 ± 0.6</td>
<td>2.3 ± 0.5</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>1.5 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>0</td>
</tr>
</tbody>
</table>
Soil organic matter, nitrate (NO$_3^-$) and ammonium (NH$_4^+$)

Soil organic matter was highest in the *Typha* zone with a mean of 75.3% of dry mass and was significantly higher than in both the Native and Transition zones, where SOM averaged 2.9% and 3.8% respectively, but there was no difference between the Native and Transition zones (Figure 2.4a, Table 2.3). Soil organic matter was highly and positively correlated with *Typha* stem density (Figure 2.5a).

Soil NO$_3^-$ and NH$_4^+$ concentrations were significantly higher in the *Typha* zone compared to the Native and Transition zones (Table 2.3), with a *Typha* zone summer mean of 10.9µg N/g dry soil in the form of NO$_3^-$ and 34.5µg N/g dry soil in the form of NH$_4^+$ (Figure 2.4b and 2.4c). Little to no NO$_3^-$ or NH$_4^+$ was present in soils of the Native and Transition zones. Similar to organic matter, NO$_3^-$ and NH$_4^+$ were highly and positively correlated with *T. x glauca* stem density (Figure 2.5b and 2.5c). In the *Typha* zone, soil NH$_4^+$ gradually increased throughout the summer (Figure 2.9b), with the highest concentrations in August that were significantly higher than June concentrations (Table 2.6).

Redox potential, soil temperature and moisture

No significant difference in redox potential existed between the three zones (Figure 2.4d, Table 2.3), and redox potential was not correlated with *Typha* density (Figure 2.5d). However, significant differences in redox potential did occur throughout the summer, between all successive measurement dates in each zone (Tables 2.4, 2.5, 2.6). The overall general trend for all zones was an increase in redox potential from July...
to August, with redox potential being significantly higher on August 2, 2007 than July 18, 2007.

<table>
<thead>
<tr>
<th>Depend. Variable</th>
<th>N</th>
<th>Source</th>
<th>Df</th>
<th>F-ratio</th>
<th>ANOVA p-value</th>
<th>Zone</th>
<th>Tukey p-value</th>
<th>Tukey p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOM</td>
<td>27</td>
<td>Zone</td>
<td>2</td>
<td>1213.0</td>
<td>&lt;0.001*</td>
<td>Native and Transition</td>
<td>0.451</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Native and Typha</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Transition and Typha</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>27</td>
<td>Zone</td>
<td>2</td>
<td>681.8</td>
<td>&lt;0.001*</td>
<td>Native and Transition</td>
<td>0.154</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Native and Typha</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Transition and Typha</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redox</td>
<td>27</td>
<td>Zone</td>
<td>2</td>
<td>0.9</td>
<td>0.416</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp</td>
<td>27</td>
<td>Zone</td>
<td>2</td>
<td>10.2</td>
<td>0.001*</td>
<td>Native and Transition</td>
<td>0.141</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Native and Typha</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Transition and Typha</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil Moisture</td>
<td>27</td>
<td>Zone</td>
<td>2</td>
<td>342.3</td>
<td>&lt;0.001*</td>
<td>Native and Transition</td>
<td>0.998</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Native and Typha</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Transition and Typha</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depend. Variable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typha density</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N₂ fixation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₃⁻</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.3. Comparisons of all measured variables.** Analyses above are summaries of one-way factorial ANOVAs and Tukey multiple comparison tests by zone. NH₄⁺ was log transformed and SOM and soil moisture were arcsine square-root transformed. Analyses below are summaries of non-parametric Kruskal-Wallis tests and Wilcoxon tests by zone.
Figure 2.4. Differences between the three vegetation zones for the measured variables, a) soil organic matter, b) nitrate, c) ammonium, d) redox potential, e) temperature and f) soil moisture. n=3, and error bars represent ± 1 standard error.
Figure 2.5. Correlations of *Typha x glauca* stem density with a) Soil OM, b) nitrate, c) ammonium, d) redox potential e) temperature and f) soil moisture. ▲ = samples from Native zone, ○ = samples from Transition zone, □ = samples from the *Typha* zone.
When averaged over all dates, mean soil temperatures were significantly lower in the *Typha* zone compared to the Native and Transition zones (Figure 2.4e, Table 2.3), with a mean of 22.0 ± 0.9 °C in the Native zone, 20.0 ± 0.7 °C in the Transition zone and 17.4 ± 0.6 °C in the *Typha* zone. Soil temperature was significantly and negatively correlated with *Typha* density (Figure 2.5e). In all zones, soil temperature increased throughout the growing season and was significantly higher on August 16, 2007 that on July 18, 2007 (Tables 2.4, 2.5, 2.6).

Averaged over all months, soil moisture was significantly higher in the *Typha* zone (88.5 ± 0.8%) than in the Native zone (24.0 ± 2.0%) and Transition zone (24.2 ± 2.3%) (Figure 2.4f, Table 2.3). Moisture was also significantly and positively correlated with *T. x glauca* density (Figure 2.5f). In the Transition and *Typha* zone, soil moisture was significantly lower in August compared to June or July (Tables 2.5 and 2.6).

*Nitrogen (N₂) fixation rates*

N₂-fixation followed the same trends as the other variables measured. Rates of N₂-fixation were significantly higher in the *Typha* zone compared to the Native and Transition zones (Figure 2.6). In the Native zone, the mean summer rate of N₂-fixation was 0.016 µg-N₂ fixed/day/gram of dry soil and did not differ significantly among months (Table 2.4). In the Transition zone, the average rate of N₂-fixation was 0.011 µg-N₂ fixed/day/gram of dry soil and rates were significantly different between all months (Table 2.5). In the *Typha* zone, the mean summer rate of N₂-fixation was 0.330 µg-N₂ fixed/day/gram of dry soil, and showed a pattern of decreasing throughout the summer,
with August N$_2$-fixation rates being significantly lower than June rates (Table 2.6, Figure 2.9a).

![Figure 2.6. Mean nitrogen fixation rates (n=3) in each vegetation zone for June, July and August 2007. Error bars represent ± 1 standard error.](image)

In the *Typha* zone, N$_2$-fixation rates decreased dramatically from June to August. The average rate of N$_2$-fixation in the *Typha* zone was 0.492, 0.254 and 0.068 µg-N$_2$ fixed/day/gram of dry soil for June, July and August respectively. This was a 48.4% decrease from June to July and a 73.2% decrease from July to August. This pattern was significant only between June and August (Table 2.6), when N$_2$-fixation decreased by 86.2%.
N_{2}-fixation was significantly and positively correlated with *T. x glauca* stem density (Figure 2.7), as well as with soil organic matter, NH_{4}^{+} and soil moisture (Figure 2.8a, 2.8c and 2.8d).

![Figure 2.7. Correlation between nitrogen fixation and *Typha x glauca* stem density. ▲ = samples taken from Native zone, ○ = samples taken from Transition zone, □ = samples taken from the *Typha* zone.](image-url)
Figure 2.8. Correlations of N\textsubscript{2}-fixation rate with the measured variables a) soil OM, b) nitrate, c) ammonium, d) soil moisture e) redox potential and f) temperature. ▲ = samples taken from Native zone, ○ = samples taken from Transition zone, □ = samples taken from the Typha zone.
<table>
<thead>
<tr>
<th>Analysis (within Native zone)</th>
<th>N</th>
<th>Source</th>
<th>Df</th>
<th>F-ratio</th>
<th>ANOVA p-value</th>
<th>Month/Date</th>
<th>Tukey p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOM</td>
<td>27</td>
<td>Month</td>
<td>2</td>
<td>0.1</td>
<td>0.902</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>27</td>
<td>Month</td>
<td>2</td>
<td>13.6</td>
<td>&lt;0.001*</td>
<td>June and July</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>June and August</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>July and August</td>
<td>0.991</td>
</tr>
<tr>
<td>Soil Moisture</td>
<td>9</td>
<td>Month</td>
<td>2</td>
<td>1.8</td>
<td>0.243</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Temp.</td>
<td>18</td>
<td>Date</td>
<td>2</td>
<td>26.3</td>
<td>&lt;0.001*</td>
<td>18-Jul-07 and 2-Aug-07</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18-Jul-07 and 16-Aug-07</td>
<td>0.043*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2-Aug-07 and 16-Aug-07</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analysis (within Native zone)</th>
<th>Group/ count</th>
<th>Rank sum</th>
<th>Df</th>
<th>Kruskal-Wallis Test Statistic</th>
<th>p-value</th>
<th>Month/Date</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₂⁻ fixation</td>
<td>June/3</td>
<td>15</td>
<td>2</td>
<td>0.8</td>
<td>0.670</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td>July /3</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>August/3</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>June/9</td>
<td>151</td>
<td>2</td>
<td>2.7</td>
<td>0.277</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td>July /8</td>
<td>91.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>August/9</td>
<td>108.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redox Potential</td>
<td>18-Jul-07/6</td>
<td>37</td>
<td>2</td>
<td>7.3</td>
<td>0.026*</td>
<td>18-Jul-07</td>
<td>0.028*</td>
</tr>
<tr>
<td></td>
<td>2-Aug-07/6</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
<td>2-Aug-07</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>16-Aug-07/6</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
<td>16-Aug-07</td>
<td>0.028*</td>
</tr>
</tbody>
</table>

**Table 2.4. Comparisons of the measured variables** in the Native zone by month. Analyses above are summaries of one-way factorial ANOVAs and Tukey multiple comparison tests by month or date. Analyses below are summaries of non-parametric Kruskal-Wallis tests and Wilcoxon tests by month or date.
Table 2.5. **Comparisons of the measured variables** in the Transition zone by month. Analyses above are summaries of one-way factorial ANOVAs and Tukey multiple comparison tests by month or date. NO$_3^-$ data were transformed to meet the assumptions of ANOVA. Analyses below are summaries of non-parametric Kruskal-Wallis tests and Wilcoxon tests by month or date.
Table 2.6. **Comparisons of the measured variables** in the *Typha* zone by month. Analyses are summaries of one-way factorial ANOVAs and Tukey multiple comparison tests by month or date. N\textsubscript{2}-fixation is log transformed and SOM is arcsine square root transformed to meet the assumptions of ANOVA

### Patterns in the Typha zone

Most of the measured variables were dramatically different in the *Typha* zone compared to the Native and Transition zones. To elucidate patterns in the *Typha* zone only, separate ANOVAs were run on *Typha* zone samples between months (Table 2.6 and Figure 2.9). N\textsubscript{2}-fixation rates and soil moisture decreased throughout the summer and were significantly lowest in August. Soil NH\textsubscript{4}\textsuperscript{+} increased throughout the summer and was significantly highest in August.
Variables that were significantly correlated with N₂-fixation rates for all zones (soil OM, NH₄⁺ and soil moisture) were analyzed separately in the *Typha* zone. In the *Typha* samples, soil OM had no correlation with N₂-fixation rates; NH₄⁺ was negatively correlated with N₂-fixation rates, though not significantly; and soil moisture was strongly, positively correlated with N₂-fixation rates (Figure 2.10).
Figure 2.9. Mean differences among months in the *Typha* zone for the variables a) N$_2$-fixation rate, b) ammonium and c) soil moisture. n=9, and error bars represent ± 1 standard error.
Figure 2.10. Correlations of N₂-fixation rate in the *Typha* zone with the variables a) soil OM, b) ammonium and c) soil moisture.
Mesocosm study: Effects of water level on rates of nitrogen fixation

Species composition

Total aboveground plant biomass differed significantly between the plus Typha treatment mesocosms and minus Typha control mesocosms (ANOVA, df =3, F-ratio=9.3, p <0.001). However, plant biomass did not differ significantly between high and low water treatments (Figure 2.11). For native vegetation, total aboveground plant biomass did not vary significantly between treatments (Figure 2.12). In addition, the Shannon-Wiener index of diversity did not differ significantly among any of the mesocosm treatments (Figure 2.13).

**Figure 2.11.** Total plant aboveground biomass in the experimental mesocosms. Error bars represent ± 1 standard error.

**Figure 2.12.** Total plant aboveground biomass (native vegetation only) in the experimental mesocosms. Error bars represent ± 1 standard error.
Soil carbon, nitrogen and moisture

Neither water level nor *Typha* treatment had a significant effect on soil organic matter, NH$_4^+$ or moisture in the mesocosms in 2008 (Figure 2.14a, 2.14b and 2.14c). Soil organic matter increased slightly in the *T. x glauca* treatment mesocosms, but this difference was not significant. Soil NO$_3^-$ concentrations were below detection limits in all mesocosms.
Figure 2.14. Comparison of measured variables a) soil OM, b) ammonium and c) soil moisture in experimental mesocosms. Error bars represent ± 1 standard error.
Nitrogen (N$_2$) fixation

Neither *Typha* nor water treatment significantly affected rates of N$_2$-fixation in the mesocosms (Figure 2.15).

![Figure 2.15. Nitrogen fixation rates in experimental mesocosms. Error bars represent ± 1 standard error.](image)

DISCUSSION

Field study

The purpose of this study was to investigate whether the increase in nitrogen found in *T. x glauca*-invaded soils was due to increased rates of nitrogen fixation. The question was asked: Does *T. x glauca* and its contribution to soil organic carbon increase
rates of soil microbial nitrogen fixation relative to native plant communities? In Cheboygan Marsh, the answer to this question is “yes.” In the field, N₂-fixation rates were significantly higher in soils invaded by *T. x glauca*, with average rates ~20-30% greater than in native or newly invaded (i.e., Native and Transition zones) wetland soils. Moreover, the rates measured in this study are within the range of N₂-fixation rates reported by Eckardt and Biesboer (1988) in Minnesota *Typha* stands as well as other wetland studies (Table 2.7).

<table>
<thead>
<tr>
<th>Wetland Type</th>
<th>Location</th>
<th>N₂-fixation rate (mg N₂/m²/day)</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt marsh</td>
<td>Virginia, USA</td>
<td>16.3-49.5</td>
<td>Tyler et al. (2003)</td>
</tr>
<tr>
<td>Cattail marsh</td>
<td>Minnesota, USA</td>
<td><em>T. latifolia</em>: 6.1 <em>T. angustifolia</em>: 13.7 <em>T. x glauca</em>: 15.0</td>
<td>Eckardt and Biesboer (1988)</td>
</tr>
<tr>
<td>Freshwater marsh</td>
<td>Massachusetts, USA</td>
<td>1.1-5.5</td>
<td>Kanna and Tjepkema (1978)</td>
</tr>
<tr>
<td>Freshwater marsh</td>
<td>Michigan, USA</td>
<td>June 2007: <strong>0.40-27.4</strong> July 2007: <strong>0.98-22.9</strong> August 2007: <strong>0.03-21.0</strong></td>
<td>This study (2007)</td>
</tr>
</tbody>
</table>

Table 2.7. Comparison of nitrogen fixation rates in this study with that of aquatic ecosystems in other published studies.

This trend of increased N₂-fixation associated with the invasion of *T. x glauca* is consistent with the hypothesis that copious amounts of carbon introduced into the soils by high production of *T. x glauca* produces a shift in the soil microbial community. *T. x glauca* is known to grow considerably larger and produce up to 300x more aboveground biomass than its native counterparts (Tuchman et al. 2009). This abundant *T. x glauca*
litter production is likely contributing to the carbon content of the soil, as soils under *T. x glauca* stands have ~16x more organic matter than native wetland soils. Thus, once a stand of *T. x glauca* has had enough time to establish itself and increase the carbon content of the soils it invades, carbon-fueled, soil microbes may be affected. In particular, if nitrogen fixers are supported by *T. x glauca*-derived carbon, N$_2$-fixation could subsequently increase, elevating nitrogen soil pools and ultimately benefiting the invading plant itself. This positive feedback mechanism could drive the invader to dominance.

According to the conceptual model in Figure 1.1, inorganic nitrogen and soil carbon are the two most important components controlling N$_2$-fixation rates. In this study, though NH$_4^+$ and SOM were positively correlated with N$_2$-fixation when all variables were analyzed, they were not positively correlated with N$_2$-fixation when *Typha* zone samples were analyzed separately. It seems that the presence of high SOM is important to the function of N$_2$-fixation, but because SOM remains relatively stable over the short term, it may not be an important control of N$_2$-fixation. Soil NH$_4^+$ was positively correlated with N$_2$-fixation when all samples were analyzed, but showed a different pattern when *Typha* zone samples were analyzed separately. Though not significant, there appears to be a slight negative correlation between soil NH$_4^+$ and N$_2$-fixation, where N$_2$-fixation rates decrease as soil NH$_4^+$ increases. Similarly, other studies have found that soil nitrogen (i.e. ammonium) is one of the most important controls in regulating nitrogenase activity, and organic carbon acts as a necessary energy source for most diazotrophic, N$_2$-fixing bacteria (Hill 1992).
Thus, such soil interactions and microbial communities may have important implications for wetland restoration efforts that frequently target the removal of invasive plants over removal of invaded, nutrient-enriched soils. A study by Perry et al. (2004) found that native wetland *Carex* species suppressed the invasive plant *Phalaris arundinacea* when soil carbon and nitrogen were manipulated in a restored wetland. However, this study and others use the application of carbon amendments (i.e. sucrose, sawdust) as a means of lowering nitrogen concentrations in invaded soils (Morghan and Seastedt 1999). In cases where the invasive plant is responsible for large inputs of nitrogen via carbon-fueled N$_2$-fixation, carbon amendments may not be a suitable solution. This study has shown that N$_2$-fixation rates are higher under *T. x glauca*-dominated soils, but nitrogen inputs may not be solely due to nitrogen fixation. External inputs of nitrogen in Cheboygan Marsh have been assessed and appear to be negligible (Tuchman et al. 2009), but a more thorough assessment may be warranted to rule out potential external inputs via groundwater or point-source pollution.

Within the *Typha* zone, soil moisture emerged as the only variable positively correlated with N$_2$-fixation. This finding suggests that while organic carbon is necessary for nitrogen fixing bacteria, it is not the main limiting factor when soil moisture is high. Organic carbon may be more of a limiting factor in the Native and Transition zone, where soil moisture is low, and less limiting in the *Typha* zone where organic carbon is abundant in the soil. Rather, soil organic matter may be serving two purposes: one being to provide an energy source for microbes and the other being its ability to retain moisture and ammonium.
The strong correlation between N\textsubscript{2}-fixation and soil moisture in the *Typha* zone may be related to differences in dissolved oxygen gradients in samples of different soil moisture content. Both N\textsubscript{2}-fixation and soil moisture were highest in the *Typha* zone and steadily decreased as summer progressed. Nitrogen fixation can occur under both aerobic and anaerobic conditions, as nitrogen fixing microbes are capable of existing within a gradient of oxygen levels (Stacey et al. 1992). Dissolved oxygen was not measured in this study, but redox potential measurements suggest that oxygen levels increased throughout the summer, and this increase in oxygen may have played a role in limiting rate of N\textsubscript{2}-fixation.

When all samples were included in analyses, N\textsubscript{2}-fixation rates and NH\textsubscript{4}\textsuperscript{+} were significantly positively correlated. However, when data from the *Typha* zone were analyzed separately, a different pattern emerged. N\textsubscript{2}-fixation decreased as the concentration of ammonium increased throughout the summer, and these two variables showed a negative correlation, though not significant. This pattern is consistent with other N\textsubscript{2}-fixation studies, in which a mechanism termed “ammonium turn-off” causes rates of N\textsubscript{2}-fixation to decline (Stacey et al. 1992). For example, when Laane et al. (1980) added ammonium chloride to cultures of several free-living N\textsubscript{2}-fixers, nitrogenase activity was halted. This inhibition of NH\textsubscript{4}\textsuperscript{+} was reversible, however, indicating that the nitrogenase was only temporarily rendered inactive.

**Mesocosm experiments**

The mesocosm experiments were used to examine how the addition of *T. x glauca* at high and low water levels affects native wetland plant biodiversity, soil nutrient
concentrations and nitrogen fixation rates. Because water levels differ in the field, the controlled high and low water treatments were a key advantage to using the mesocosms. Of all the variables measured in the mesocosms in summer 2008, only plant aboveground biomass was found to be significantly different among treatments. The *Typha* mesocosms had significantly greater total above ground biomass than control mesocosms, with no differences in biomass between water level treatments, but this was the case only when *Typha* plant biomass was included in the analysis. Freyman (2008) also measured plant biomass in the mesocosms in 2005 and found similar results in control mesocosms, with total plant biomass ~500 g/m$^2$. Three years after Freyman’s measurements, however, plant biomass has increased significantly in the *Typha* mesocosms, from ~500 g/m$^2$ in 2005 to between 1000 and 1250 g/m$^2$ in 2008. This increase represents nearly an order of magnitude and is a result of the increase in *T. x glauca* stem density over three years.

Overall, soil nutrient and N$_2$-fixation data collected from the mesocosm experiment were inconclusive. Differences between mesocosms were likely undetected because soils have not had sufficient time to accumulate nutrients at levels observed in the field. Only five growing seasons have passed since the *Typha* treatments began in the mesocosms, whereas in Cheboygan Marsh, *T. x glauca* has been established in the marsh for approximately five decades (L. Vail, unpublished data). Thus, mesocosms mimic the nutrient dynamics of the Transition zone rather than those of the *Typha* zone. The levels of SOM and nitrogen in the mesocosms are most similar to field measurements in the Native and Transition zones, and no mesocosm treatments simulate the *Typha* zone.
where high levels of SOM, \( \text{NH}_4^+ \) and soil moisture are correlated with \( \text{N}_2 \)-fixation rates (Table 2.8).

<table>
<thead>
<tr>
<th>Soil variable</th>
<th>Native zone</th>
<th>Transition zone</th>
<th>Typha zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mesocosm</td>
<td>Field</td>
<td>Mesocosm</td>
</tr>
<tr>
<td>Mean ( \text{NO}_3^- ) ug/gDM</td>
<td>0</td>
<td>0.086</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Field</td>
<td></td>
<td>0.109</td>
</tr>
<tr>
<td>Mean ( \text{NH}_4^+ ) ug/gDM</td>
<td>0.901</td>
<td>0.208</td>
<td>1.367</td>
</tr>
<tr>
<td></td>
<td>Field</td>
<td></td>
<td>0.542</td>
</tr>
<tr>
<td>Mean % SOM</td>
<td>2.5</td>
<td>2.8</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Field</td>
<td></td>
<td>3.798</td>
</tr>
<tr>
<td>Mean ( \text{N}_2 )-fixed ug/day/gDM</td>
<td>0.253</td>
<td>0.016</td>
<td>0.265</td>
</tr>
<tr>
<td></td>
<td>Field</td>
<td></td>
<td>0.011</td>
</tr>
</tbody>
</table>

Table 2.8. Comparison of field data to the corresponding mesocosm treatment data.

All mesocosm treatments had average rates of \( \text{N}_2 \)-fixation that were relatively high and more similar to rates in the Typha zone. Fixation from cyanobacteria is probably the reason. \textit{Nostoc} colonies occurred on the surface of several mesocosm soils. Mesocosms are excellent sites for the proliferation of cyanobacteria as sunlight can easily penetrate to the soils, and each of the mesocosms were subject to standing water for at least one day per week. These conditions mimic those of the Native zone, where cyanobacteria were also present. As for the field samples, the upper 2 cm of soil were removed to reduce the affect of cyanobacteria on soil root-associated \( \text{N}_2 \)-fixation.

However, \textit{Nostoc} may have remained suspended in mesocosm soil samples, as the samples were mixed to produce soil slurries.
CONCLUSION

The results of this study show that soil-associated nitrogen fixation is driving the observed accumulation of nitrogen in soils of Cheboygan Marsh. This finding has implications for the management of Great Lakes wetlands that are invaded by *Typha x glauca*. Currently, the most common methods for control of invasive cattail species are mechanical removal, fire, and herbicides. Although these methods are useful for seasonal management of the plant, they do not address the edaphic modifications observed following invasion and do not prevent repeated annual return of invaders. By not managing invaded soils and the nutrient legacy left behind by invasive plant species, the long-term success of wetland restoration efforts may be compromised.
CHAPTER THREE

RECONSTRUCTING THE VEGETATION HISTORY OF A GREAT LAKES COASTAL WETLAND WITH POLLEN ANALYSIS

INTRODUCTION

Since European settlement of the Great Lakes region in the early 1800’s, approximately 70% of Great Lakes coastal wetlands have been lost due to anthropogenic land-use changes, hydrological alterations and the introduction of invasive species (Moser et al. 1996). Coastal wetlands provide important ecological, recreational and economic services to the Great Lakes region. In recent decades heightened public awareness of the functional benefits of wetlands has resulted in increased research and management aimed at restoring them. Numerous studies have addressed hydrology (Wilcox 2004, Boers et al. 2007, Hausman et al. 2007), nutrient cycling (Jordan et al. 1989, Doren et al. 1997, Tyler et al. 2003) and invasive species dynamics (Rickey and Anderson 2004, Herr-Turoff and Zedler 2005) of degraded Great Lakes coastal wetlands; however, studies that address the ecological and vegetation history of wetlands and the different phases of their development through time are scarce.

Our knowledge of Laurentian Great Lakes development stems from paleoecological studies that reconstruct vegetation histories through fossil pollen analysis (Janssen 1967, Jackson et al. 1988, Finkelstein and Davis 2006). These studies tend to examine changes in vegetation at the centennial or millennial timescales, with assessment
of anthropogenic impacts focusing on changes that occurred at the time of European
settlement in the mid-nineteenth century (Bunting et al. 1997, Pan and Brugam 1997,
Finkelstein et al. 2005). However, in a wetland restoration context, ecologists and
conservation biologists may be more concerned with recent vegetation trends at the
decadal timescale (Lynch and Saltonstall 2002). To that end, paleoecological methods
may serve as a useful analytical tool in wetlands that have undergone more recent
invasion (50-100 ybp) by invasive plant species (Jackson 1997).

Invasive plant species are one of the major threats to the integrity and health of
wetlands as they reduce biodiversity and cause often irreversible, edaphic modifications
Huron in northern Michigan is currently undergoing invasion by the cattail *Typha x
glauca* (hereafter *T. x glauca*). *T. x glauca* is one of several aggressive plant invaders in
the Great Lakes region that displaces native wetland vegetation in the form of large,
nearly monospecific stands (Galatowitsch et al. 1999). In Cheboygan Marsh, *T. x glauca*
produces copious amounts of leaf litter (>14x more than native plant litter production per
year), which over time has probably enriched the soil with organic carbon (Jankowski
2007). A thick layer of black soil occurs wherever *T. x glauca* is present in the marsh,
over 40 cm above the wetland’s sandy soil base in some areas. This pattern of increasing
organic matter appears to be a function of *T. x glauca* density.

Invasive plant species have the capacity to modify soils in the ecosystems they
invade (Ehrenfeld 2003, Corbin and D’Antonio 2004), and an understanding of how they
have altered historical soil processes should be a key component in any restoration
attempt. In this study, paleoecological methods were used to reconstruct the history of invasion by *T. x glauca* in Cheboygan Marsh. Using pollen and organic matter analyses, aerial photography, isotopic soil dating and historical Lake Huron water level data, the following questions were addressed: 1) When did invasion by *T. x glauca* begin in Cheboygan Marsh? 2) Is there a correlation between organic matter accumulation and *T. x glauca* pollen appearance/abundance in the soil profile? 3) What role did historical Lake Huron water levels play in the invasion of Cheboygan Marsh by *T. x glauca*?

**MATERIALS METHODS**

**Study site**

Cheboygan Marsh is a freshwater coastal wetland located in northern Lower Michigan on Lake Huron (N 45.65, W -84.47, Fig. 2a). In the marsh, there are areas of remnant wetland with native vegetation and areas that have been completely invaded by *T. x glauca*. Roughly 2/3 of the marsh is dominated by *T. x glauca*; the remainder is native vegetation. The marsh is open to seiche activity from Lake Huron and has a beach ridge running northwest to southeast. The ridge is lined intermittently with willows (*Salix* spp.).

**Sample collection**

A typical soil profile in the *Typha* zone of Cheboygan Marsh has three layers, a top highly organic, peaty layer (approximately 20-25 cm), a middle layer of sand (approximately 15-20 cm) and a base layer of clay. The organic layer of soil contains a dense matrix of coarse and fine roots. Because roots and compaction limited the utility of
common coring devices (i.e. Livingston corer, Russian peat borer), a large pit (0.5 meter diameter) was excavated with a shovel, exposing the soil profile. A compass saw was used to cut through the peat on the face of the excavation pit and remove an intact soil section.

Two “cores” were extracted from Cheboygan Marsh (Fig. 3.1), one in August 2006 (core A) and the other in August 2007 (core B). Their coordinate locations were recorded with a Garmin GPS unit. Both core A and B were extracted near the center of large monospecific stands of $T. x$ glauca, believed to be the oldest stands of $T. x$ glauca, approximately 200 meters apart. The two coring sites were assumed to have received similar histories of invasion, based on their parallel proximity to the invasion front of the $T. x$ glauca stand, and soil organic matter at these sites was 4 x greater than native wetland soils (Chapter 2). Core A was 32 cm deep, which was the depth of the water table in 2006. When core B was extracted in 2007, the water table was lower and a 40-cm deep soil core was removed. Core A was processed and analyzed for lead-210 and cesium-137 dating. Core B was processed for pollen analysis.
Figure 3.1 Map showing location of Cheboygan Marsh in northern Michigan (top). Map showing Cheboygan Marsh area and location of study cores A and B (bottom).

**Loss-on-ignition and lithology**

To correlate the accumulation of organic carbon with the accumulation of *T. x glauca* pollen over time, soil organic matter content was analyzed in core B. Subsamples were taken at 1 cm intervals throughout the length of the core, dried at 105 °C for 24 hours, weighed, then placed in a muffle furnace at 550 °C for 2 hours. The non-volatile ash remaining was subtracted from the initial dry mass and organic matter was calculated
as percent of dry mass (APHA 2005). Lithology of core B was determined visually and by texture.

**Lead-210 and cesium-137**

The Lead-210 ($^{210}\text{Pb}$) dating method is applicable to relatively recent sediments in aquatic environments. $^{210}\text{Pb}$ is part of the uranium-238 radioactive decay series and has a half-life of 22.3 years, thus suitable for dating changes over the last century. $^{210}\text{Pb}$ is deposited from the atmosphere at a presumed constant rate, and its half-life is then used to determine an accumulation rate in the sediments. The constant flux: constant sedimentation rate CF:CS (Robbins 1978) assumes a constant flux of excess $^{210}\text{Pb}$ from the atmosphere and was used to calculate a constant dry-mass sedimentation rate. From this accumulation rate, the age of sediments from a particular depth in a soil core can be estimated.

In a sediment column, a marked peak of $^{137}\text{Cs}$ can be detected in soils from the nuclear testing in the early 1960s. For this study $^{210}\text{Pb}$ and $^{137}\text{Cs}$ were used together to determine the approximate sediment accumulation rate. MicroAnalytica LLC performed all the radiometric dating $^{210}\text{Pb}$ and $^{137}\text{Cs}$ for this study. From core A (described above), 5 cm-long subsections were homogenized, dried at 105 ºC for 48 hours and sent to MicroAnalytica for analysis.

**Pollen sample processing**

Pollen samples were prepared according to standard protocol (Faegri et al. 1989) for removal of pollen from aquatic sediments. Subsamples (1 cc) were taken from the core for pollen analysis at 2 cm intervals. A higher sampling resolution of 1 cm was used
near the peat-sand interface. A 0.5ml microsphere spike of known concentration was added initially to each subsample for calibration of pollen grain densities. All samples were prepared in 50 ml polystyrene centrifuge tubes. Samples were washed with deionized (DI) water, centrifuged at 3000 rpm for 4 minutes (Hettich Rotina 420 bucket centrifuge) and the supernatant decanted between each of the following chemical treatments.

To remove soil carbonates, 15 ml of 10% HCl was added to each sample until effervescence ceased. Samples were then topped off with DI water for dilution, spun down and decanted. To break up humic acid particulates in the soil, 20 ml of 10% KOH was added to the samples, heated in a hot water bath (90 ºC) and stirred for 10 minutes. Samples with high peat content were passed through a 500 µm sieve, and sandy samples were passed through a 250 µm sieve. Any material that did not pass the sieves was discarded. Samples were washed several times after this step with deionized water until the supernatant was clear. To remove silicates, 15 ml of 49% HF was added to each sample, placed in the hot water bath and stirred every five minutes for one hour. Sample tubes were then topped off with 95% ethanol, spun down and decanted into a waste container filled with sodium carbonate, which neutralizes HF. Acetolysis was used to remove organic matter. A 40 ml 9:1 mixture of acetic anhydride to concentrated sulfuric acid was added to the samples and placed in a hot water bath for 2 minutes. Following acetolysis, 40 ml of concentrated glacial acetic acid was added to the samples before washing three more times with DI water. Samples were topped off with 5 ml DI water and the suspension passed through a 7 µm Nitex mesh screen. The Nitex mesh is fine
enough to prevent the passage of pollen grains, while removing fine clay particles. The material that did not pass the sieve was washed and spun down. Samples were transferred from centrifuge tubes to shell vials, and concentrated tert-butyl alcohol was added. Silicone oil was added to the samples, in volumes equal to the volume of the remaining pollen residues, then allowed to dry at 40 °C in a dust free environment for 24 hours.

Pollen counting and identification

*Typha x glauca* is the hybrid cross between *Typha latifolia* and *Typha angustifolia*. *T. latifolia* produces tetrad pollen grains and *T. angustifolia* produces monads. When *T. x glauca* pollen is present in the soil record, tetrads and monads are observed as well as dyads and triads, the latter two forms indicative of *T. x glauca* (Finkelstein 2003). Finkelstein (2003) assigns percent abundance ranges to each *Typha* pollen type when the source is *T. x glauca* (monads 47-92%; dyads 7-30%; triads 0-10%; tetrads 0-14%), and the percent abundances of *Typha* pollen in this study fall within these ranges (Table 3.1). Thus, all four types of *Typha* pollen grains were counted (monads, dyads, triads and tetrads) in each sample, as well as native sedge pollen (Cyperaceae) and pine (*Pinus*) pollen. Pollen grains from other species were identified as “other.” A minimum of 300 pollen grains were counted under 400x magnification and recorded with the counting and inventory software package PCount (© Grimm 1994). Pollen diagrams were produced using TGView and Tilia (© Grimm 2004).
Table 3.1. Percent abundance of monads, dyads, triads and tetrads from this study as compared to Finkelstein (2003) study. Depths above 22 cm were chosen, because all four types of Typha grains had appeared and increased by that depth.

<table>
<thead>
<tr>
<th>Typha pollen type</th>
<th>This study (depth 0-22 cm)</th>
<th>Finkelstein paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monads</td>
<td>41-88 (73)</td>
<td>47-92 (75)</td>
</tr>
<tr>
<td>Dyads</td>
<td>1-8 (3)</td>
<td>7-30 (17)</td>
</tr>
<tr>
<td>Triads</td>
<td>0-1 (0)</td>
<td>0-10 (3)</td>
</tr>
<tr>
<td>Tetrads</td>
<td>1-5 (2)</td>
<td>0-14 (5)</td>
</tr>
</tbody>
</table>

Aerial photographs and water level data

Aerial photographs of Cheboygan Marsh from 1938-2005 were obtained from the Michigan Department of Information Technology’s Center for Geographic Information spatial data library and edited using Google Earth. Water level data were downloaded from the U.S. Army Corps of Engineers Detroit District website (www.lre.usace.army.mil/) for historic water levels of Lake Huron and Lake Michigan, which are hydrologically one lake.

RESULTS

Loss-on-ignition and lithology

Organic matter was very low (≤1%) at the base of the soil core for depths 22-40 cm. From depths 16-21 cm organic matter began to increase slowly and ranged from 1-8%. Organic matter increased sharply to 23% at 15 cm and continued to increase gradually upwards. The highest organic matter reached was 80% at the 2 cm depth (Figure 3.2).
Three stratigraphic layers were evident. The lower stratum (23-40 cm) consisted of mostly sand and clay particles; the middle stratum (16-22 cm) consisted of a mixture of sand and dark peaty soil; the upper stratum (1-15 cm) consisted of all dark, peaty soil containing some fine root material. These strata corresponded nicely with shifts in organic matter content (Figure 3.2).

**Lead-210 and cesium-137 dating**

The rate of deposition as determined by $^{210}\text{Pb}$ dating of core A was 2.5 yr/cm. $^{137}\text{Cs}$ peaked in the soil horizon at 17 cm, indicating an age of AD 1963 ±2 years. Both the $^{137}\text{Cs}$ and the $^{210}\text{Pb}$ data assign the same age to depth 17 cm, which further supports the deposition time of 2.5 yr/cm. This rate was applied to the soils of core B under the assumption that both cores were taken from an area of the marsh with similar sedimentation histories. Because the sediments in core A were not dated below 22 cm, the deposition time was not applied to core B soils below that depth, nor were years assigned.

**Pollen analysis**

For sediments below 22 cm, dates were not assigned. A clear break in pollen spectra occurs around AD 1952 (Fig. 3.2), which corresponds to a high peak in Lake Huron water levels. After 1952, overall pollen concentrations gradually increased and reached a high in mid-1990. *Typha* monads increased markedly after 1952, as did tetrads, and *Typha* dyads and triads appeared in the record. An obvious peak in Cyperaceae pollen occurred at AD 1952, after which it declined, disappearing completely
around mid-1990. Pine was a dominant pollen type before 1952, along with “other pollen,” but both decreased dramatically when Typha pollen types increased.

Figure 3.2. Percentage pollen, organic carbon and lithology for core B. Years are assigned based on $^{210}$Pb analysis of core A. The dashed line represents the $^{137}$Cs horizon in core A, corresponding to 1963 ± 2 years.

Aerial photograph interpretation and water level

Six aerial photographs of Cheboygan Marsh from 1938, 1952, 1963, 1980, 1987 and 2005 were obtained and are shown in Figure 3.3. Water level data (Figure 3.4) show that Lake Huron levels have fluctuated over the past 85 years with a range of 2 meters. Three of the images (1938, 1963 and 2005) were taken during years of relatively low water levels. The other three images (1952, 1980 and 1987) were from years of relatively high water levels. The water-level data is consistent with aerial photograph
interpretation: during high water years, the marsh was inundated and during low-water years, it appeared to be dryer. The high-water phase was especially evident in 1952, when water covered more than 75% of the marsh (Fig 3.3).

*Figure 3.3. Aerial photographs of coring locations in Cheboygan Marsh. Left, top to bottom (1938, 1952, 1963), right, top to bottom (1980, 1987, 2005). Images are of the same location as shown in the bottom image of Figure 3.1.*
Figure 3.4. *Hydrograph of yearly average water levels for Lake Huron/Michigan.* Points in bold black (•) correspond with the years of aerial photographs in Figure 3 (U.S. Army Corps of Engineers 2009).

**DISCUSSION**

The purpose of this study was to address three questions: 1) When did invasion by *Typha x glauca* begin in Cheboygan Marsh? 2) Is there a correlation between organic matter accumulation and *Typha x glauca* pollen appearance and abundance in the sediment? 3) What role did historical Lake Huron water levels play in the invasion of Cheboygan Marsh by *Typha x glauca*?

*Typha x glauca* became the dominant plant in Cheboygan Marsh by the early 1960s. After a peak in 1952, Lake Huron water levels steadily declined, reaching an 85-year low by 1965. According to the pollen record, it was during this lake-lowering
period that Typha monads increased dramatically. During this time native Cyperaceae pollen increased slightly as well, but it then decreased as Typha dominated. Typha dyads, triads and tetrads increased simultaneously with Typha monads. Finkelstein (2003) found the presence of dyads and triads in the pollen record, even at low levels, to be diagnostic for T. x glauca (Table 3.1). Thus, it can be inferred that the increase in abundance of all four Typha pollen types in the years after 1952 (starting at depth 22 cm) originated from T. x glauca.

Prior to the increase in dyad and triad abundance in the early 1950s, Typha monads and tetrads, presumably from T. angustifolia and T. latifolia respectively, were present in the pollen record with less evidence of hybridization (note a few dyad grains at 32 cm). At many of these lower depths (22-40 cm), T. angustifolia greatly exceeded T. latifolia, suggesting that T. angustifolia was present at greater abundances before T. latifolia in Cheboygan Marsh, though the latter species is native to the Great Lakes region. However, pollen data in other studies have found T. latifolia produces far less pollen than T. angustifolia and has much lower dispersability (Clark and Patterson III 1985, Finkelstein and Davis 2005). Therefore, the greater numbers of Typha monads at lower depths may be more indicative of greater pollen production and increased extralocal dispersal rather than actual greater species abundance of T. angustifolia over T. latifolia.

Approximately 10-15 years after T. x glauca became the dominant plant in Cheboygan Marsh, organic matter accumulation began to increase dramatically. From depth 16 cm to 15 cm (approximately 1968), percent organic matter sharply increased
from 5.7% to 23.1% and continued to climb steadily to 80.3% at depth 2 cm. These results indicate that it took more than a decade for \textit{T. x glauca} to increase soil organic matter in the study area. This evidence is further supported by a \textit{Typha} litter transplant study conducted in Cheboygan Marsh by Freyman (2008), in which she found it takes approximately 3.8 years for \textit{Typha} litter to reach 50% decay. Such a slow rate of decay implies that build-up of organic matter resulting from \textit{T. x glauca} production would have taken decades.

The results of this study point to 1952 as being a critical year for the advancement of \textit{T. x glauca} in Cheboygan Marsh. According to the hydrograph of Lake Huron (Fig. 3.4) and aerial photographs (Fig. 3.3), 1952 was one of the wettest years for the marsh in the past 100 years. In the 1952 image of Cheboygan Marsh, >75% of the marsh is covered by standing water. Interestingly, a drainage ditch (lower middle section of all images) that terminates in the marsh, and is currently dry, is completely full of water in 1952. I hypothesize that this ditch originally supplied high nutrient water to the marsh, facilitating dominance of \textit{T. x glauca} in the early 1950s.

During the early 1950’s expansion of \textit{T. x glauca}, the pollen diagram also shows a peak in Cyperaceae pollen (between 20-24 cm), indicating native vegetation may have briefly expanded its range during this time as well. However, the sedge pollen peak declines and reaches low levels by the late 1950s. This decline suggests that native wetland sedges were exposed to water levels well beyond their optimum, likely hindering their capacity to rebound, especially when in competition with \textit{T. x glauca}. This finding is supported by other studies of wetlands in which hydrology drives shifts in wetland
vegetation. In particular, sedges and certain grasses were found to decline or die out completely following flooding or high water levels (Farney and Bookhout 1982, Sjoberg and Danell 1983, Mauer 2009), whereas cattails were found to invade wetlands the year following flooding (Millar 1973, Farney and Bookhout 1982, Wilcox et al. 1985).

**CONCLUSION**

Paleoecology can be a useful method for studying recent changes in vegetation in Great Lakes coastal marshes. The sediment profile from Cheboygan Marsh revealed information about the history and dynamics of a *T. x glauca* invasion. Following a peak in water level in 1952, when Lake Huron was approximately 120 cm higher than today, the marsh appears to have shifted toward a *T. x glauca*-dominant wetland community, replacing most other species by the late 1950s. This suggests that Great Lakes water level periodicity may play an important role in the establishment and proliferation of invasive plant species. Additionally, invasion by *T. x glauca* appears to have preceded the accumulation of soil organic matter by approximately 10-15 years, suggesting that *T. x glauca* is responsible for the increase in soil organic matter in Cheboygan Marsh.
REFERENCES


Jankowski, K. J. 2007. Effects of an invasive cattail (Typha x glauca) on nitrogen cycling in a Great Lakes coastal marsh. M.S. Loyola University Chicago, Chicago.


Moser, M., C. Prentice, and S. Frazier. 1996. A global overview of wetland loss and degradation. in Conference of the Contracting Parties (Ramsar), Brisbane, Australia.


VITA

Lane Vail received a Bachelor of Arts in Art History from the University of Illinois at Chicago in 2002. From 2004-2006, she attended the City Colleges of Chicago and Northeastern Illinois University, taking science courses in preparation for graduate school in Biology. In summer 2006, she received a fellowship from Loyola’s Center for Urban Environmental Research and Policy (CUERP) to conduct research at the University of Michigan Biological Station. She went on to work at CUERP for one year as a GIS assistant, while continuing to take Biology prerequisite courses. She received an assistantship and entered the Biology Graduate Program at Loyola in 2007. She presented her research at the North American Benthological Society Annual Meeting in 2008 and at the Society of Wetland Scientists Annual Conference in 2009. Currently, she works at CUERP as a research associate, conducting research on the effects of pharmaceuticals and personal care products on stream quality and function.