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Cattail (Typha X Glauca) Invasion in Wetlands of the Great Lakes Region: Are Impacts Time-Dependent?

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CATTAIL (TYPHA X GLAUCA) INVASION IN WETLANDS OF THE GREAT LAKES REGION: ARE IMPACTS TIME-DEPENDENT?

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ABSTRACT

Some species introduced into new regions have the potential to greatly impact native diversity and ecosystem functioning. The invasive hybrid cattail, *Typha X glauca*, has established in wetlands across the Great Lakes region decreasing native plant diversity and altering soil and microsite characteristics. We utilized 80 years of historical aerial photographs from the Illinois Beach State Park wetland complex to map the spread and determine the age of *T. X glauca* stands. Floristic, edaphic, and environmental data were collected from plots across an invasion-age gradient to determine the impacts of *T. X glauca* and time since invasion on environmental variables. We also investigated the effect that *T. X glauca* has on the overall quantity of denitrification and the quality of the gases produced (N$_2$ vs. N$_2$O). As *T. X glauca* produces large quantities of biomass in the form of litter, we hypothesized that invasions will cause a decrease in water levels due to several decades of *T. X glauca* litter deposition and a subsequent reduction in the quantity and quality of denitrification. We found time since invasion to be an important component of *T. X glauca* invasions as it was strongly correlated with the ecological effects of *Typha*, including increases in litter mass and soil organic depth and decreases in plant diversity. However, *T. X glauca* had no significant impacts on total denitrification or denitrification quality.
CHAPTER ONE
LITERATURE REVIEW AND MOTIVATION

Literature Review

Invasive Species

Some species introduced into new regions have the potential to greatly impact native diversity and ecosystem functioning (Allendorf and Lundquist 2003). These species, commonly referred to as invasive species, proliferate after introduction and expand their range while damaging the environment (Mack et al. 2000). Invasion events have increased substantially in the last 200 years due to human migration and commerce (Mack et al. 2000). Currently, invasive species are considered one of the largest threats to conservation (Parker et al. 2003), right behind habitat loss and habitat fragmentation (Allendorf and Lundquist 2003). Species invasions can impact both biotic and abiotic ecosystem properties and thus have the potential to severely impact ecosystem functioning (Hooper et al. 2005, Hobbs and Huenneke 1992).

One of the most perplexing questions facing scientists is how a species becomes invasive upon introduction into a new region. Better understanding of the process of species invasions is important as it can lead to clues and research that may help to control or limit the introduction and spread of invasive species. Successful invaders tend to have short generation time, generalist diets, and broad native ranges while invadible habitats
tend to have a climate similar to the invader’s native range, a lack of predators, and low native diversity (Lozon and MacIsaac 1997).

The Intermediate Disturbance Hypothesis suggests that moderate levels of disturbance maintain the highest levels of diversity; however, disturbances can also facilitate the invasion of ecosystems by introduced species (Hobbs and Huenneke 1992). Of the 133 studies reviewed by Lozon and MacIsaac (1997), 56% indicated an association between disturbance and the establishment of introduced species. Disturbances can form small areas or patches within which introduced species can colonize and establish (Hobbs and Huenneke 1992). Scientists have observed that invaders frequently take hold in disturbed areas such as cultivated lands where they begin to spread (Lozon and MacIsaac 1997).

Novel competition mechanisms may also facilitate the success of introduced species. These invaders possess some competitive advantage that allows them to dominate the landscape. Cappuccino and Arnason (2006) reported in their review of invasive species that nearly half of the highly invasive plant species studied possessed novel secondary compounds to which native species within their introduced range have not been exposed. For instance, the invasive forb *Centaurea diffusa* (spotted knapweed), produces allelochemicals that species have adapted to within its native range. However, in its introduced range, these chemicals released in root exudates give the invasive species a strong competitive advantage over native species naïve to the allelochemicals (Callaway and Aschehoug 2000).
As species are transported to new environments, they may escape the competitive forces of herbivory, predation, parasitism, and pathogens that control them in their native ranges. In a study by Lake and Leishman (2004), invasive plant species experienced lower levels of herbivory than native species. However, exotic non-invasive species also experienced lower levels of herbivory than native species, indicating that escape from herbivory is not the only mechanism explaining invasive species success (Lake and Leishman 2004).

A proposed mechanism that facilitates the evolution of introduced species is the enemy-release hypothesis, which states that as introduced species are freed from natural enemies such as herbivores, they no longer have to allocate energy and resources to self-defense. The organisms’ energy and resources can instead be utilized for competition (i.e., increased growth rates and reproductive outputs) (Blossey and Notzold 1995). The invasive agricultural weed *Silene latifolia* (bladder campion) provides some evidence for this evolutionary mechanism. In its native range (Europe), *Silene latifolia* is assaulted by a fungus and both generalist and specialist herbivores. In its introduced range (North America), however, *Silene latifolia* faces little to no threat from specialist, generalist, or fungal enemies (Blair and Wolfe 2004). When Blair and Wolfe (2004) grew both the native and invasive *Silene latifolia* varieties, they found that the invasive North American variety germinates earlier, is larger, and has greater flower production and survival than the native European variety while investments by the invader towards defense are reduced. Blossey and Notzold (1995) found similar results with purple loosestrife (*Lythrum salicaria*), an invader of wetland habitats. When grown in a common garden,
the invasive variety had greater biomass and height than the native variety (Blossey and Notzold 1995).

In theory, invading populations should have lower potential for adaptation as only the genetic variability present in the founding, introduced population is available for selection to act upon (Dlugosch and Parker 2008). However, not all invasive species possess lower genetic variability in their introduced range. There may be sufficient genetic variation present in the founding population (Dlugosch and Parker 2008), or genetic variability and subsequent evolutionary changes in invader populations may arise from different evolutionary mechanisms which allow the introduced invaders to out-compete the native species.

Genetic bottlenecks can present a problem for evolving populations as genetic drift and high levels of inbreeding are common and can cause the fixation of deleterious alleles (Lambrinos 2004). However, under certain situations, genetic bottlenecks, or founding effects, can prove to be a mechanism favoring adaptation. Tsutsui et al. (2000) investigated the behavior and population genetics of the highly invasive Argentine ant (*Linepithema humile*). In their native region, Argentine ants form diverse communities with other ant species yet display high levels of intraspecific aggression. Reduced genetic variation, in this case, does not hinder evolution but is thought to instead operate as the mechanism for evolution and successful invasion. As the introduced ants have lower genetic diversity due to founder effects, they are more genetically similar and therefore display lower levels of intraspecific aggression than in their native range (Tsutsui et al. 2000). These low levels of aggression allow the ants to form “supercolonies” which can
dominate the landscape, excluding native ants and other arthropods (Holway et al. 1998, Tsutsui et al. 2000). While genetic bottlenecks here clearly represent a mechanism for the evolution of a successful invader, examples of such mechanisms are rare in the literature.

Invasive species can avoid genetic bottlenecks and increase genetic variability through multiple introductions. Multiple introductions may supply genetically depleted invading populations with genetic variability, providing the substrate for selection and evolution to act upon (Lambrinos 2004). Examples of multiple introductions fueling evolution are numerous. One well-known example concerns the fungus Phytophthora infestans, the cause of potato late blight disease and the Irish potato famine. Potato late blight is a devastating disease because it can infect the leaves, stems, and tubers of the potato plant, sometimes destroying whole fields of crops (Fry and Goodwin 1997). The initial outbreak was caused by a population of fungus possessing very low genetic diversity and was eventually managed by fungicides. However, when a drought struck Europe in 1976, potatoes from Mexico were shipped to Europe along with new populations of fungus. These multiple introductions of fungus likely increased the genetic diversity and provided the mechanism for evolving resistance to fungicides used to treat potato late blight, explaining the resurgence of the disease in the 1980’s and 1990’s (Fry and Goodwin 1997).

Another invasive species that has avoided genetic bottlenecks through multiple introductions is the brown anole lizard (Anolis sagrei) in Florida. These lizards were first introduced to the Florida Keys in the mid-to-late 1800’s and have since expanded throughout most of the state of Florida (Kolbe et al. 2004). Kolbe et al. (2004) sampled
native and introduced populations and determined that introduced populations actually have higher genetic diversity than native populations. They were also able to determine that at least eight introductions are responsible for the increased genetic variation. Haplotypes from eight different clades have been found in Florida that do not occur together in the lizard’s native range. The increased genetic diversity has also led to changes in morphology, including an increase in body size in their introduced range (Kolbe et al. 2004). While multiple introductions clearly help to limit founding effects for many invading populations, Dlugosch and Parker (2007) reviewed studies on 80 invasive species and found that many of these populations still possess low genetic diversity.

Hybridization can increase genetic variability and thus increase adaptive potential, often leading to rapid evolution of new plant species and range expansion (Vila et al. 2000). Hybridization between an invasive and a native species or between two invasive species can alleviate problems generated by genetic bottlenecks and founder effects. Hybridization not only increases genetic variability, but may also help to remove deleterious alleles which can become fixed in small, genetically isolated populations (Ellstrand and Schierenbeck 2000). Hybridization may also impact the adaptive potential of invasive species by generating new phenotypes that may be better adapted for the new environment than the parents (Ellstrand and Schierenbeck 2000, Abbott 2003). One example is the plant *Helianthus annuus* (common sunflower), which was most likely introduced by Native Americans from the Great Plains to California. In California, it has hybridized with the native *Helianthus bolanderi* (serpentine sunflower), forming hybrids which are very successful weeds (Vila et al. 2000). Another concern of hybridization is
that it can dilute the genetic variability of native species, sometimes leading to the extinction of the native species (Vila et al. 2000).

**Wetland invaders.** Wetlands are particularly prone to species invasions as they are impacted by high levels of direct (i.e., altered hydrology) and indirect (i.e., water pollution from upland areas) anthropogenic disturbances (Zedler and Kercher 2004). Species invasions, together with large anthropogenic disturbances, threaten wetland ecosystems and the services that they provide. Purple loosestrife (*Lythrum salicaria*) is a well-studied wetland invader that is now present throughout North America. It has been shown to reduce bird habitat and pore water phosphorus and alter rates of decomposition and evapotranspiration (Blossey et al. 2001). Other wetland invaders such as reed canary grass (*Phalaris arundinacea*) (Maurer et al. 2003), the common reed (*Phragmites australis*) (Marks et al. 1994), and the hybrid cattail (*Typha X glauca*) (Angeloni et al. 2006, Farrer and Goldberg 2009, Boers et al. 2007) have also decimated native wetland plant communities throughout the Midwest.

**Typha X glauca.** *Typha X glauca* (hybrid cattail), is an invasive hybrid between the native *Typha latifolia* and the invasive *Typha angustifolia* (Smith 1987). *Typha X glauca* and its parent species reproduce vegetatively via rhizomes, allowing them to spread throughout wetland communities (Gates 1912, Selbo and Snow 2004). The hybridization of native and invasive cattails is a main factor explaining cattail proliferation throughout North America (Travis et al. 2009). *Typha X glauca* is able to outperform both its parents (Travis et al. 2009) as it is more tolerant of a wide range of environmental conditions (Galatowitsch et al. 1999). Unlike *T. angustifolia*, the hybrid *T.
X _glauca_ can inhabit high water sites and is large in stature (Grace and Wetzel 1981). The hybrid _T. X glauca_ can also tolerate high salinity, a character that _T. latifolia_ does not possess (McMillan 1959). Previous studies have shown that _T. X glauca_ builds up biomass, decreases native plant diversity (Angeloni et al. 2006, Farrer and Goldberg 2009, Boers et al. 2007), and alters soil characteristics such as organic matter and nutrients (Tuchman et al. 2009). Changes to the soil are likely to impact microbial communities and processes such as denitrification (Angeloni et al. 2006, Windham and Ehrenfeld 2003). Denitrification rates in _Typha_-invaded areas are likely to increase due to increases in soil carbon and nitrogen (Jankowski 2006). However, biomass accumulation can, over time, drastically change ecosystem properties and has the potential to decrease rates and quality of denitrification.

**Wetlands and Their Services**

Ecosystems benefit humans by providing necessary resources and functions, which are collectively termed ecosystem services (Costanza et al. 1997). The world’s wetlands are estimated to provide 40% of the global renewable ecosystem services and are valued at $13.2 trillion per year (Costanza et al. 1997). Wetlands operate as reservoirs for flood control, support biodiversity, have high levels of productivity, and remove pollutants and nutrients from the ecosystem (Millennium Ecosystem Assessment, 2005). While these services are all vital, none are more crucial than the storage, cycling, and biogeochemical transformation of nutrients. Since 1950, large amounts of nitrogen have been used as fertilizer for agriculture (Mitsch et al. 2001). The excess nitrogen drains into groundwater, streams, rivers, and large bodies of water. Nitrate levels in the Mississippi
River have more than doubled since 1965 (Turner and Rabalais 1991) and have increased
two-fold in Norwegian lakes in less than a decade (Henriksen and Brakke 1988). As
nitrogen is frequently the limiting nutrient in coastal ecosystems (Day et al. 1989), these
inputs can lead to eutrophication (Martin and Reddy 1997). Wetlands can act as
important buffers between nutrient sources and aquatic ecosystems as they promote
sedimentation, uptake of nutrients via plants, and enhance denitrification (Fisher and
Acreman 2004).

**Denitrification.** The biogeochemical process of denitrification occurs when
denitrifying microbes reduce NO$_3^-$ to NO, N$_2$O, and N$_2$ gases (Figure 1; Groffman et al.
the reduced conditions and high levels of available carbon common in wetland
ecosystems (Fisher and Acreman 2004). The products of denitrification have different
consequences for the Earth’s atmosphere. N$_2$ is an inert gas that makes up the majority of
the atmosphere (Dobbie and Smith 2001), NO contributes to the ozone layer (Bollmann
and Conrad 1998), while N$_2$O depletes the ozone layer (Dobbie and Smith 2001) and is a
powerful greenhouse gas with 180 times
greater radiative forcing than CO$_2$ (Machefert
et al. 2002).

**Figure 1.** Nitrogen cycle processes (1 = nitrification; 2 =
denitrification; 3 = dissimilatory reduction of nitrate to
ammonium; 4 = chemo-denitrification; 5 = nitrogen
fixation). From Stevens and Laughlin 1998
As wetlands process large amounts of nitrogen from upland areas, it is likely that they release large amounts of N\textsubscript{2}O (Groffman et al. 2000). However, wetlands with high water conditions and chemically reduced soils release mostly N\textsubscript{2} as a product of denitrification (Machefert et al., 2002). Wetlands with low and intermediate soil moisture and more oxidized soils produce NO and N\textsubscript{2}O, respectively (Machefert et al. 2002). This is likely due to the relatively rapid synthesis of dissimilatory nitrate reductase under slightly oxic conditions while N\textsubscript{2}O reductase is only synthesized under anoxic conditions (Firestone and Tiedje 1979). Because the end products of denitrification are influenced by water levels, changes to a wetland’s hydrology can impact global climate.

Denitrification rates can be difficult to measure as a major product is N\textsubscript{2} gas, a major constituent of the atmosphere. However, N\textsubscript{2}O gas, another product of denitrification, is a minor constituent in the atmosphere and is easily detected with sensitive equipment (Groffman et al. 2006). Many methods have been established to measure or estimate denitrification rates. The most common is the acetylene inhibition technique (Groffman et al. 2006) in which acetylene is added to a soil slurry to impede reduction of N\textsubscript{2}O to N\textsubscript{2} during denitrification, emitting N\textsubscript{2}O as the final product. N\textsubscript{2}O concentrations can then be measured using gas chromatography. Another common technique employs isotopically labeled \textsuperscript{15}N to determine denitrification rates and the ratio of final gas products. Denitrifiers process \textsuperscript{15}N labeled nitrogen and convert it to labeled \textsuperscript{15}N\textsubscript{2} and \textsuperscript{15}N\textsubscript{2}O gases. As naturally occurring \textsuperscript{15}N is rare, it can be distinguished from background atmospheric N\textsubscript{2} gas, which is comprised almost completely of \textsuperscript{14}N, using a
trace gas unit coupled with a mass spectrometer (Stevens and Laughlin 1998, Yu et al. 2006).

**Thesis Motivation**

The process of denitrification is very important as it helps to remove nitrogen, high levels of which can cause eutrophication, from aquatic systems. Any alterations to denitrification or the products formed by this process, reported as denitrification quality (%N₂), caused by *T. X. glauca* could result in eutrophication of aquatic systems and may contribute to global climate change. *T. X. glauca* may alter denitrification and denitrification quality by increasing soil organic matter. Addition of carbon to the soil will likely fuel the microbially-mediated process of denitrification. However, organic matter inputs may build up the soil over time, decreasing localized water levels resulting in aeration of soils, increased redox potentials, and lower rates of denitrification and denitrification quality (%N₂). Few studies, however, have investigated *T. X. glauca* invasions and their impacts in a temporal context and thus little evidence is available to predict the temporal dynamics of species invasions.

Invaders can impact the environment in different ways through time while also adapting and evolving (Strayer et al. 2006). *Typha X. glauca* has the potential to greatly alter native habitat, diversity, and ecosystem functioning. Without management, *T. X. glauca* may continue to build up organic matter and completely dry out wetland communities (see Buffam et al. 2010, Kirschner et al. 2001, and Rooth et al. 2003). If we can determine when changes to diversity and organic matter occur following invasion, it may be possible to prevent or reduce losses of wetland habitat by informed management.
techniques. Studying invasive species such as *T. X glauca* may also indicate novel ways of controlling the spread and adaptation of invaders by controlling gene flow, enemy competition, hybridization, and disturbances.

In Chapter 2, I investigate impacts of *T. X glauca* on environmental variables through time following initial establishment to determine how and when significant changes to the ecosystem occur. In Chapter 3, I determine if and when *Typha X glauca* impacts rates and quality of denitrification and the potential consequences for global climate change. In Chapter 4, I present my overall conclusions derived from my findings in Chapters 2 and 3.
CHAPTER TWO

TIME-DEPENDENT IMPACTS OF CATTAIL (TYPHA X GLAUCA) INVASION IN GREAT LAKES COASTAL WETLANDS

Abstract

The invasive hybrid cattail, Typha X glauca, has become established in wetlands across the Great Lakes region decreasing native plant diversity and altering environmental conditions. We used 80 years of historical aerial photographs from a large Lake Michigan wetland complex to map the spread and determine the age of T. X glauca stands. Floristic, edaphic, and environmental data were collected from plots across an invasion-age gradient. Compared with reference uninvaded sites, litter mass more than doubled within 10 years of invasion (P < 0.001), plant diversity declined by more than 50% within 25 years of invasion (P < 0.05), and soil organic depth was more than 29 cm deeper in areas invaded for more than 35 years compared with areas invaded for 10 years or less (P < 0.05). These time-dependent changes in plant communities, soil, and environmental conditions fundamentally alter the structure of invaded wetlands, likely influencing a range of ecosystem services.
Introduction

Invasive species are a major threat to ecosystems worldwide (Wilcove et al. 1998) as they alter nutrient cycling, disrupt food webs, and decrease biodiversity (Zedler and Kercher 2004). Invasive plants are often more productive and thus produce large amounts of biomass compared to native species (Ehrenfeld 2003). As the invasive plants competitively exclude native species, this biomass can, over time, change the ecosystem’s soil composition and microbial community (Angeloni et al. 2006, Ehrenfeld 2003).

Invasive hybrid cattail, *Typha X glauca* (Smith 1987) (hereafter *Typha*) produces large amounts of biomass in the form of dead plant material (litter) due to its large size and high productivity. This litter accumulates, covering the soil surface, altering temperatures, excluding light, and decreasing native plant diversity (Angeloni et al. 2006, Farrer and Goldberg 2009, Boers et al. 2007, Tuchman et al. 2009, Vaccaro et al. 2009). Decomposition of *Typha* biomass should progressively increase amounts of soil organic matter (SOM). Tuchman et al. (2009) found decreases in species richness and water depth and increases in litter biomass and SOM where *Typha* had successfully invaded. These changes to the native plant community and soil will likely alter ecosystem services such as denitrification over time (Angeloni et al. 2006, Windham and Ehrenfeld 2003). While it is clear that *Typha* impacts wetland ecosystems, the ecological effects of *Typha* have not been thoroughly explored in a temporal context.

Many studies of species invasions focus on the impacts to native species without regard to how long an invader has been present (Strayer et al. 2006). After establishment
an invasive species can acclimate, evolve, and significantly change its surrounding environment (Strayer et al. 2006). For instance, one study of temporal invasion dynamics found more severe impacts to soil properties and ecosystem services due to biomass accumulation in older *Acacia longifolia* stands compared to both native and recently invaded areas (Marchante et al. 2008). A study of *Phragmites australis* found live biomass was 3 times greater in areas invaded for 20 years compared with areas invaded for 5 years (Rooth et al. 2003). Results of these studies indicate that temporal changes do occur and should be incorporated into future invasive species research. Developing an understanding of how invasive species change their environment over time is a critical component for understanding and managing species invasions.

Management of invasive species such as *Typha* is difficult and costly (Hall and Zedler 2010, Vitousek et al. 1996). Managing large areas of land is especially challenging, as portions of the landscape may have reached an “invasion threshold” where further management is impossible or at least impractical (Byers et al. 2002). A quick assessment tool is needed where managers can use geographic information systems (GIS) to find and assess invasions across large areas (Byers et al. 2002, Clewell and Rieger 1997). Paired with GIS technology, an invasion timeline may provide land managers with an assessment tool to determine a specific area’s invasion state and allow for the strategic selection of an appropriate and efficient management technique.

The goal of this study was to assess the temporal dynamics of *Typha* invasion and identify thresholds when ecologically significant changes occur. We hypothesized that the ecological impacts of *Typha* would increase with time, eventually leading to dramatic
changes to plant diversity and the local hydrology. We tested this hypothesis by measuring plant community and soil characteristics along a *Typha* invasion chronosequence and in paired uninvaded sites. We predicted that: 1) shortly after invasion by *Typha*, plant species richness and diversity would significantly decline; 2) over the short-term, as *Typha* density increased, so would the accumulation of biomass in the form of litter; 3) in the medium-term, the accumulated biomass would decompose and incorporate into the soil, increasing concentrations of SOM and gradually increasing the depth of the organic matter layer; and 4) in the long-term, increases in organic matter (OM) would elevate the marsh surface, leading to lower water levels and increasingly terrestrial habitats.

**Methods**

**Study Area and Experimental Design**

Research was conducted along the Illinois-Wisconsin border on the coast of Lake Michigan (Figure 2) in Chiwaukee Prairie (CP), Spring Bluff, and Illinois Beach State Park (IBSP). Together, these protected lands cover more than 2,357 ha (ICS 2007, SWRP 2004, Epstein et al. 2002), much of which is wetland. The study area is a dune and swale complex (Albert et al. 2005) comprised of dune, oak savannah, sedge meadow, calcareous fen, as well as dry and wet prairie communities (SWRP 2004). The presence of the hybrid *T. X glauca* was recorded in the Illinois Beach State Park study site in 1912 (Gates 1912).
In a previous study, Lishawa et al. (in prep) determined the age of *Typha* stands throughout the Illinois Beach dune and swale complex. Historical aerial photographs dating back to the 1930’s were obtained for photo interpretation. The presence and location of *Typha* in current images was determined from field data, and the characteristic shape, color and texture of sites invaded by *Typha* were used as models for delineating historical *Typha* patches. A trained interpreter delineated *Typha* patches in each historical
aerial photograph, beginning with the most recent, to determine the location and size of
*Typha* stands in each photo at each time period throughout the wetland complex.

Using the GIS aging data, we established transects within swales in CP and IBSP that encompassed a range of *Typha*-stand ages as well as uninvaded control sites. The control sites were sedge meadow communities dominated by *Carex lacustris*, *Carex aquatilis*, and *Carex stricta*. Environmental conditions within individual swales appeared relatively homogenous, minimizing non-*Typha* induced variability within transects. Within each transect, we established plots every 5, 10, or 25 m depending on the available area of wetland habitat. As uninvaded parcels were rare or diminished in all of our sites, plots in the native zones were spaced closer together than in the much more expansive invaded zones. Plots were then paired with GIS *Typha* aging data (Figure 3). A stratified random sample of field plots covering a broad range of times since *Typha* invasion were selected for detailed analysis. A total of 60 plots were chosen, spread across 5 transects.
Figure 3. Transect-within-a-swale study design showing plots (white dots) and *T. X glauca* invasion-age polygons (grayscale corresponds to different ages of *T. X glauca*) generated using historical aerial photographs (Lishawa et al. in prep). Plots not lying within an invasion-age polygon are native (control) plots. Transects were designed to run within a swale to minimize non-*T. X glauca* induced variability.
Data Collection

Vegetation sampling was performed in late August, 2009. Vascular plants within 1-m² plots were identified to species and the numbers of stems per species was recorded. These data were used to calculate species richness and Shannon-Weiner diversity.

Litter was collected in early May of 2009 before new growth emerged. Within 0.25 m² quadrats adjacent to each plot, all senesced biomass above the fibric organic soil surface was collected, dried for 60 days, and weighed.

SOM was measured using 10 cm soil cores taken approximately monthly from plots in IBSP and CP throughout the 2009 growing season. SOM was calculated as the percent of volatile dry mass lost on ignition at 550 °C in a muffle furnace (APHA 2005).

Soil organic matter depth was measured from the top of the fibric organic layer to the top of mineral soil using a 1.9 cm galvanized steel pipe. All initial depth measurements were confirmed by taking a soil sample at the intersection of the organic and mineral layers using a soil corer.

Standing water depth was recorded from the water surface down to the top of the fibric organic layer using a meter stick. Two measurements were taken adjacent to each plot and averaged. These measurements were taken approximately monthly from plots in IBSP and CP throughout the 2009 growing season.

Soil NH₄⁺ concentrations were measured using the phenol-hypochlorite method (Wetzel and Likens 1991) in 10 g soil samples from each plot. These measurements were taken approximately monthly in IBSP and CP throughout the 2009 growing season.
**Statistical Analyses**

All univariate statistical analyses were performed using SYSTAT statistical software version 11.0 (SYSTAT Software Inc., 2004). One-way randomized ANOVA, blocking for different transects, were used followed by Tukey post-hoc tests to analyze for differences in *Typha* stand-age groups. Non-parametric rank transformations were performed for *Typha* density, species richness, and Shannon-Weiner diversity as assumption of normality could not be met for these variables. We ran simple linear regressions for each variable using age (years) of *Typha* stands as the independent variable. Log transformations were used for both species richness and NH$_4^+$ data to normalize variance. Multivariate Principal Component Analysis (PCA) was used to develop a composite environmental variable including *Typha* density, litter mass, SOM, organic depth, soil NH$_4^+$, and water depth. The composite environmental variable was used for correlation analysis for *Typha* stand age and species richness. All multivariate analyses were performed using PC-ORD version 5 (McCune and Mefford 2006).

**Results**

*Typha* density increased significantly within the first 10 years of invasion, and then slowly increased with increasing age (Figure 4). Regression analyses showed that *Typha* density increased as the age of *Typha* stands increased ($R^2 = 0.19; P < 0.01$; Figure 5). The rank-transformed data showed the same patterns as the non rank-transformed data (Zar 2010).

Litter mass was also significantly greater in all ages of *Typha* stands than in uninvaded stands, more than doubling within the first 10 years following *Typha* invasion.
Litter mass did not significantly change following the initial increase during the first 10 years of *Typha* invasion (Figure 4). Regression analyses showed no significant changes in litter mass as time since invasion increased (R² = 0.05; P = 0.18; Figure 5).

Species richness significantly declined with increasing age of *Typha* stands (R² = 0.32; P < 0.001; Figure 5). However, significant differences in species richness were not observed until 15 to 25 years after invasion and then continued to decline with increasing age (Figure 4). Shannon-Weiner diversity followed a similar pattern, significantly decreasing with increasing age of *Typha* stands (R² = 0.26; P < 0.01; Figure 5) and differing significantly within *Typha*-invaded sites from the native sites 15 to 25 years following *Typha* invasion (Figure 4). Analyses using rank-transformed data confirmed these patterns (Zar 2010).

SOM increased significantly with increasing age of *Typha* stands (R² = 0.32; P < 0.001; Figure 5), increasing by 15% from sites invaded for 10 years or less (mean ± SE = 45% ± 3.1) to sites invaded for at least 35 years (mean ± SE = 60% ± 2.6). However, unexpectedly, SOM was not greater in sites invaded for more than 35 years (mean ± SE = 60% ± 2.6) compared with uninvaded control sites (mean ± SE = 47% ± 3.4; Figure 4) (P = 0.27). The same patterns were found for OM depth. There was a significant increase in OM depth with increasing age of *Typha* stands (R² = 0.22; P < 0.01; Figure 5). OM depth was >29 cm deeper in sites invaded for more than 35 years compared to sites invaded for 10 years or less (Figure 4), however, as with SOM, no other significant differences were observed. These results were used to develop a timeline of the impacts of *Typha* following invasion (Figure 6). There was a weak significant difference in NH₄⁺ (P = 0.09)
and no significant difference in mean water depths ($P = 0.32$) along an age of invasion gradient.

**Figure 4.** Mean values by time since *T. X glauca* invasion for *T. X glauca* density (a), litter mass (b), species richness (c), Shannon-Wiener diversity (d), soil organic matter (e), and organic matter depth (f). Error bars represent ±1 SE. Within each variable, treatment means with different uppercase letters differ significantly ($P < 0.05$) as determined by Tukey’s post-hoc test.
Figure 5. Simple linear regressions of *T. X glauca* stand age (years) and *T. X glauca* density (a; \( P = 0.006 \)), litter mass (b; \( P = 0.176 \)), species richness (log transformed) (c; \( P < 0.001 \)), Shannon-Wiener Diversity (d; \( P = 0.001 \)), soil organic matter (e; \( P < 0.001 \)), and organic matter depth (f; \( P = 0.003 \))
Figure 6. Timeline of *T. X glauca* invasion showing significant (P < 0.05) differences observed during each invasion age group. SOM = % Soil Organic Matter. Impacts on sites where *T. X glauca* invaded >55 years ago are hypotheses based on observed trends and literature.

Principal Components Analysis of *Typha* density, litter mass, SOM, organic depth, soil NH$_4^+$, and water depth was used to develop a composite environmental axis (PCA axis 1) that explained 34% of the variability in environmental data. PCA axis 1 was strongly and positively related with *Typha* density (Eigenvector = 0.53), litter mass (Eigenvector = 0.56), SOM (Eigenvector = 0.44), and organic depth (Eigenvector = 0.39). Soil NH$_4^+$ (Eigenvector = 0.20) was weakly positively correlated while water depth (Eigenvector = -0.15) had no relationship. Therefore, PCA axis 1 represents an environmental gradient with increasing *Typha* density, litter mass, SOM, and organic depth. PCA axis 1 explained 60% of the variability in *Typha* stand age (Figure 7) and 31% of the variability in species richness.
Figure 7. T. X. glauca stand age (P < 0.001) was correlated with the composite environmental axis (determined from PCA of plot water depth, litter mass, organic depth, T. X. glauca density, soil organic matter (%), and NH$_4^+$)

**Discussion**

This study aimed to further our understanding of the ecological impacts of *Typha* invasions by analyzing the changes over time in ecological characteristics such as plant diversity, litter accumulation, and SOM. Time since invasion was a very important component of *Typha* invasions and was strongly and positively correlated with an environmental gradient of increasing *Typha* density, litter mass, SOM and OM depth. However, some of these environmental changes occurred in less than 10 years following invasion while changes in others took more than 35 years.

Increasing litter mass and *Typha* density were the most rapid changes associated with *Typha* invasion. However, large changes to species richness and Shannon-Weiner diversity were not observed until 15 years after *Typha* establishment, suggesting that litter accumulation may have been a prerequisite for substantial plant-community changes. This underscores growing evidence that litter accumulation is a key driver of

Both SOM and OM depth increased with increasing age of *Typha* stands, providing evidence for the decomposition and incorporation of litter mass into the soil as organic matter and explaining the lack of observable differences in litter mass following *Typha* invasion. Furthermore, both SOM and OM depth increased considerably between newly invaded sites and sites invaded for more than 35 years. These increases follow the accumulation of litter mass, which occurred within the first 10 years following invasion. A delayed increase in SOM and OM depth likely resulted from the relatively slow rate of *Typha* litter decomposition compared to the plant species it replaces (Davis and Van der Valk 1978, Freyman 2008). *Typha* litter decomposition is further delayed by the persistence of standing-dead *Typha* stems, which remain erect for years following death, preserving senescing *Typha* litter in an above-ground canopy litter matrix (Davis and Van der Valk 1978). Dry conditions in the standing dead litter prevent rapid decomposition (Su et al. 2007). Eventually, precipitation, snow pack, deer bedding activity, and winds break down the standing dead, compressing it to the moist soil where it can more rapidly decompose and be incorporated into the organic layer (Davis and Van der Valk 1978, Welsch and Yavitt 2003).

Importantly, the native sites in this study were sedge-dominated (*Carex lacustris*, *Carex aquatilis*, and *Carex stricta*). Sedges can accumulate organic matter via dense aboveground tussocks and underground root systems (Brinson et al. 1981, Dwire et al. 2004, Moore et al. 2007). Because we did not measure belowground plant material, we
could not differentiate these organic matter inputs. In contrast, in a rush and bulrush-dominated emergent marsh in northern Michigan, Angeloni et al. (2006) saw a four-fold increase in SOM in areas where *Typha* had established compared to uninvaded areas.

Our timeline indicates that land managers should act within the first 10 years following *Typha* establishment to maintain species diversity before significant declines in habitat are observed. Managers should focus on areas that are newly invaded as the native community will be more readily reestablished in these sites. Managing young invasions will also limit the buildup of organic matter as the stand increases in age. Sites invaded for more than 35 years will require stronger interventions such as flooding (Leeds et al. 2009) or removal of organic material (White et al. 2008) in order to eliminate accumulated organic matter and reestablish the hydrologic regime. Even small hydrological changes can further alter habitat (Desgranges et al. 2006) and ecosystem services (see Fisher and Acreman 2004). Sites invaded by *Typha* for more than 55 years are likely to be drier as organic matter builds up, “terrestrializing” invaded sites (see Buffam et al. 2010, Kirschner et al. 2001, and Rooth et al. 2003). Habitat quality and ecosystem services are likely to decline as the water levels they depend on become lower and wetlands become less wet.

**Conclusions**

Time since invasion is an important component of biological invasions and was strongly correlated with the effects of *Typha* on wetland structure and function. This temporal context must be studied in order to fully understand invasion dynamics (Strayer et al. 2006). Future studies should investigate sites invaded for more than 55 years, as
changes to the environment are likely to further alter habitat and ecosystem services. This research may also shed light on the mechanisms behind an invader’s success. With improvements in the speed and efficiency of invasive stand aging, similar invasion timelines could be created for other species invasions resulting in improved landscape-scale management of invasive species.
CHAPTER THREE
IMPACT OF *TYPHA X GLAUCA* INVASION ON DENITRIFICATION RATES
AND QUALITY IN GREAT LAKES COASTAL WETLANDS

Abstract

Wetlands can limit nitrogen pollution in aquatic ecosystems by facilitating the process of denitrification, transforming NO$_3^-$ to N$_2$O, a potent greenhouse gas, or N$_2$, an inert gas abundant in the atmosphere. To produce the less harmful N$_2$ gas, wetlands must be inundated with water, creating anoxic conditions ideal for denitrification. We investigated the effect that the invasive cattail, *Typha X glauca*, has on the overall quantity of denitrification and the quality of the gases produced (N$_2$ vs. N$_2$O). As *Typha* produces large quantities of biomass in the form of litter, we hypothesized that invasions will cause a decrease in water levels due to several decades of *Typha* litter deposition and a subsequent reduction in the quantity and quality of denitrification. We collected soil cores from native sites and across an invasion-age gradient, incubating soils with and without acetylene to determine the amounts of N$_2$ and N$_2$O produced. Secondly, we conducted a laboratory study in which we manipulated redox levels to examine the effects on denitrification quality using labeled $^{15}$N. We found *Typha* had no significant impacts on water depth, total denitrification, or denitrification quality within the first 55 years of invasion. If, however, elevation changes become significant enough to impact water levels in the future, our $^{15}$N-labelling lab study indicates that *terrestrialized* areas
with elevated soil redox potential will produce significantly more harmful N$_2$O than flooded sites, which would most likely produce inert N$_2$ gas.

**Introduction**

Wetlands provide a vast array of ecosystem services and are frequently referred to as nature’s “kidneys” and “supermarkets” (Mitsch and Gosselink 2007) because they are highly productive, mitigate flooding and erosion, remove pollutants from water, influence climate, provide habitat for organisms, as well as supply aesthetic beauty to the landscape (Millennium Ecosystem Assessment 2005). While these services are all vital, none are more crucial than the ability to store, circulate, and remove inputs of nutrients. Since 1950, large amounts of nitrogen have been used as fertilizer for agriculture (Mitsch et al. 2001). The excess nitrogen drains into groundwater, streams, rivers, and large bodies of water. Nitrate levels in the Mississippi River have more than doubled since 1965 (Turner and Rabalais 1991) and have increased in Norwegian lakes by two-fold in less than a decade (Henriksen and Brakke 1988). As nitrogen is frequently the limiting nutrient in coastal ecosystems (Day et al. 1989), these inputs can lead to eutrophication (Martin and Reddy 1997). Wetlands can act as important buffers between nutrient sources and aquatic systems as they promote sedimentation, uptake of nutrients via plants, and high rates of denitrification (Fisher and Acreman 2004).

Wetlands in the Great Lakes region are experiencing unprecedented increases in invasive plant species establishment and dominance (Mills et al. 1994) which threaten ecosystems (Wilcove et al. 1998) as they alter nutrient cycling, disrupt food webs, and decrease biodiversity (Zedler and Kercher 2004). High levels of biomass production by
invasive plants can, over time, change the ecosystem’s soil composition and microbial community (Marchante et al. 2008). One of the most aggressive invaders in the region is *Typha X glauca* (Frieswyk and Zedler 2007). The invasive hybrid cattail, *Typha X glauca* (Smith 1987) (hereafter *Typha*) produces large amounts of biomass in the form of dead plant material (litter), resulting in competitive dominance over native species and reductions in plant species diversity (Farrer and Goldberg 2009, Vaccaro et al. 2009). Over time, as the substantial plant litter decomposes, wetland soils can be altered. The invaded portion of Cheboygan Marsh, a Great Lakes coastal wetland dominated by *Typha*, had significantly greater soil phosphate (PO$_4$), bacterial species richness, fourteen times the soil ammonium (NH$_4$), ten times the soil nitrate (NO$_3$), and four times the soil organic matter as compared to uninvaded portions of the wetland (Angeloni et al. 2006, Tuchman et al. 2009). The accumulation of organic matter associated with sites invaded by *Typha* may, over time, alter the elevation and hydrology of wetland soils.

Studies of invasion dynamics have shown an impact of biomass accumulation on wetland soils. In wetlands invaded by *Phragmites australis*, live biomass was 3 times greater in areas invaded for 20 years compared with areas invaded for 5 years (Rooth et al. 2003). Additionally, in *Phragmites* invaded sites, wetland elevation increases of 0.95 cm per year resulting from sediment and organic matter accretion through time have been observed, leading to *terrestrialization* (Rooth et al. 2003). Similarly, areas invaded by *Typha* for 35 to 54 years had accumulated 29 cm more organic matter than newly invaded areas while both % soil organic matter (SOM) and organic matter depth increased with increasing age of *Typha* stands (Mitchell et al. *in prep*). These significant alterations to
the ecosystem may also alter ecosystem services such as denitrification and are worthy of further study.

The process of denitrification is common in wetland ecosystems, allowing for removal of major nitrogen inputs from upland ecosystems. Due to the persistent anoxic conditions within wetland soils, communities of denitrifying microbes exploit forms of nitrogen for cellular respiration, reducing NO\textsubscript{3}\textsuperscript{-} to gaseous NO, N\textsubscript{2}O, and N\textsubscript{2} (Stevens and Laughlin 1998, Machefert et al. 2002, Groffman et al. 2006). This process is stimulated by carbon availability and water-logged sediments which help to supply microbes with available carbon and generate the reduced conditions required for denitrification (Fisher and Acreman 2004). However, the products of denitrification, N\textsubscript{2} and N\textsubscript{2}O, have different consequences for the Earth’s atmosphere; N\textsubscript{2} gas makes up the majority of our atmosphere and is a harmless, inert gas; whereas, N\textsubscript{2}O depletes the ozone layer (Dobbie and Smith 2001) and is a very potent greenhouse gas with 180 times greater radiative forcing than carbon dioxide (Machefert et al. 2002). Therefore, as the ratio of N\textsubscript{2}O to N\textsubscript{2} gas increases, denitrification quality is said to decrease. Factors affecting denitrification quality include soil moisture (Cai et al. 2001), temperature (Dobbie and Smith 2001), pH (Machefert et al. 2002), and redox potential (Kralova et al. 1992). Conditions of low soil moisture support production of NO, N\textsubscript{2}O production is favored under intermediate moisture conditions, and high moisture supports the formation of N\textsubscript{2} (Machefert et al. 2002). This is likely due to the relatively rapid synthesis of dissimilatory nitrate reductase under slightly oxic conditions while N\textsubscript{2}O reductase is only synthesized under anoxic conditions (Firestone and Tiedje 1979).
Denitrification can be difficult to measure as a major product is N\textsubscript{2} gas, a major constituent of the atmosphere. However, N\textsubscript{2}O gas, another product of denitrification, is easily detectable as it is a minor constituent in the atmosphere and sensitive detection equipment is available (Groffman et al. 2006). In the presence of acetylene, reduction of N\textsubscript{2}O to N\textsubscript{2} is impeded during denitrification, emitting N\textsubscript{2}O as the final product. N\textsubscript{2}O concentrations can then be analyzed using gas chromatography. Another common method for measuring denitrification employs \textsuperscript{15}N isotopically labeled nitrogen. Denitrifiers process the labeled nitrogen and convert it to labeled N\textsubscript{2} and N\textsubscript{2}O gases. As naturally occurring \textsuperscript{15}N is rare, it can be distinguished from background atmospheric N\textsubscript{2} gas in the form of \textsuperscript{14}N using a trace gas unit coupled with a mass spectrometer (Stevens and Laughlin 1998, Yu et al. 2006). In this way, the ratio of denitrification products can be determined and the quality of denitrification can be established.

While few studies have examined the effects of invasive plant species on denitrification rates (see Windham and Ehrenfeld 2003, Jankowski 2006), observed increases in NO\textsubscript{3}\textsuperscript{-} suggest that invasions may increase rates of denitrification (Ehrenfeld 2003). Increased rates of denitrification in \textit{Typha}-invaded areas are likely due to increased soil carbon and nitrogen resulting from invasion (Jankowski 2006). However, biomass accumulations can, over time, drastically change ecosystem properties and have the potential to impact denitrification rates and quality.

The goal of this study was to assess the impacts of \textit{Typha} stands of differing ages on denitrification rates and quality. We hypothesize that early on in the invasion, denitrification rates will be high, as nitrogen and organic carbon levels from root
exudates stimulate denitrifying microbial communities. However, denitrification rates and quality should decrease as changes in elevation as a result of organic matter accumulation cause soils to become less waterlogged and redox potentials to increase. We tested this hypothesis by measuring denitrification rates and quality ($%N_2$ vs. $%N_2O$) from soil cores collected throughout the growing season along a Typha invasion-age gradient and in paired uninvaded sites. We also performed a controlled lab microcosm study to compare wetland soil denitrification rates and quality under dry, high-redox, terrestrialized conditions with completely flooded, low-redox conditions. This study employed isotopically labeled $^{15}N$ amendments to assess the potential impacts of terrestrialization by invasive species such as Typha on total denitrification and denitrification quality.

**Methods**

**Study Area and Experimental Design**

Research took place along the Illinois-Wisconsin border on the coast of Lake Michigan (Figure 8). Chiwaukee Prairie, Spring Bluff, and Illinois Beach State Park are protected lands that together cover more than 5,825 acres along Lake Michigan (ICM 2007, SWRP 2004, Epstein et al. 2002), much of which is wetland. The study area is a dune and swale complex (Albert et al. 2005).
Using historical aerial photography, GIS software (ArcView 9.2, ESRI, Redlands, California), and ground truthing we determined the age of *Typha* stands throughout the Illinois Beach dune and swale complex. Together with ground-truthing, historical aerial photographs can be used to accurately determine the presence of vegetation types (Gottgens et al. 1998, Wei and Chow-Fraser 2006). Historical aerial photographs dating back to the 1930’s were obtained for photo interpretation. The presence and location of *Typha* in current images was determined from ground-truthing, and the characteristic
shape, color and texture of Typha-invaded sites were used as models for delineating historical Typha patches. A trained interpreter delineated Typha patches in each historical aerial photograph, beginning with the most recent, to determine the location and size of Typha stands in each photo at each time period throughout the wetland complex.

Using the GIS aging data, we established transects within swales in Chiwaukee Prairie and Illinois Beach State Park that encompassed a range of Typha stand ages as well as native control sites (Figure 9). Environmental conditions within individual swales were relatively homogenous, minimizing non-Typha induced variability within transects. Within each transect, we established plots every 5, 10, or 25 meters depending on the distribution of suitable plant communities. These plots were then paired with GIS Typha aging data. A stratified random sample of research plots representing a range of Typha stand ages were selected for detailed analysis. A total of 60 plots were chosen, spread across 6 transects.
Figure 9. Transect-within-a-swale study design showing plots (white dots) and *T. X glauca* invasion-age polygons (grayscale corresponds to different ages of *T. X glauca*) generated using historical aerial photographs (Lishawa et al. in prep). Plots not lying within an invasion-age polygon are native (control) plots. Transects were designed to run within a swale to minimize non- *T. X glauca* induced variability.

**Field Data Collection**

Water depths, soil redox potential, and soil samples were collected during the 2009 growing season on June 9th, July 13th, and August 10th from both Chiwaukee Prairie and Illinois Beach State Park transects and additionally on September 1st in Chiwaukee.
Prairie. Water depth was determined by measuring depth from the water surface to the top of the fibric organic layer. Two measurements were taken adjacent to each plot and averaged to generate a mean plot water depth. Redox potential was determined using platinum-tipped electrodes constructed by soldering 2.4 mm of exposed platinum to a metal wire (Vepraskas 2002). In a subsample of plots, 30 electrodes were installed 7 cm below the soil surface and were sheathed in PVC tubing to protect the exposed portion from the elements. Redox measurements were taken using a voltmeter attached to the electrode and a reference electrode, which was inserted into the soil near the probe (Vepraskas 2002). Soil cores were collected from each plot on each sample date using a bulb planter (Garden Plus® Bulb Planter). These cores were brought back to the lab and stored overnight in a 4°C refrigerator. Soil analyses were conducted the following day.

**Laboratory Soil Analyses**

Ten-gram soil samples were collected from each core to determine soil NH$_4^+$ using the phenol-hypochlorite method (Wetzel and Likens 1991).

Two 60 ml soil samples were collected from each homogenized core (one sample for acetylene incubation and the other for helium incubation). Each sample was placed in a 230 ml Ball® canning jar. To each sample we added 48 ml deionized water and 12 ml of DEA solution (made by mixing 1.01 g KNO$_3$, 0.30 g Dextrose, 1.00 g Chloramphenicol, and enough MQ H$_2$O to fill to 1 L) (Bartsch and Bruesewitz 2002). Jars were then swirled to ensure proper mixing. Each jar was sealed using lids with a 10 mm hole drilled in the center and fitted with a Wheaton® 20 mm butyl two legged septa.
Jars were then flushed with helium for 5 minutes and allowed to equilibrate with atmospheric pressure.

The denitrification rate or total denitrification (N\textsubscript{2} plus N\textsubscript{2}O) of each plot was determined using the Acetylene Inhibition Slurry technique (Yoshinari and Knowles 1976). We injected 10 ml of Acetylene through the septum into each jar, from which, after 30 minutes of incubation, 4 ml gas samples were collected and stored in Kendall® monject 3 ml vials evacuated for gas sample storage. Gas samples were also collected 90 and 180 minutes after addition of acetylene. Standards and gas samples were analyzed for N\textsubscript{2}O on a gas chromatograph (Shimadzu® GC 2014) equipped with an electron capture detector, a Hayesep Q column, and nitrogen as the carrier gas.

Denitrification quality was determined by calculating the %N\textsubscript{2} produced. Soil samples under acetylene incubation produced N\textsubscript{2}O gas. This measure represents total denitrification (N\textsubscript{2} + N\textsubscript{2}O) since acetylene blocks the conversion of N\textsubscript{2}O to N\textsubscript{2}. Soil samples under helium incubation produced both N\textsubscript{2} and N\textsubscript{2}O since no acetylene was present. Therefore, for each plot, we could calculate %N\textsubscript{2} produced ((N\textsubscript{2}O from acetylene treatment – N\textsubscript{2}O from helium treatment) / N\textsubscript{2}O from acetylene treatment) and thus determine the quality of denitrification occurring at each plot.

15\textsuperscript{N} Terrestrialization Study

A homogenized mixture of wetland soils was used to evaluate the impacts of redox potential on denitrification quality. On September 21, 2009, twenty soil cores were collected using a bulb planter (Garden Plus® Bulb Planter) within one meter of a Chiwaukee Prairie plot, which had high levels of denitrification throughout the growing
season. In the lab, one 180 ml soil subsample from each homogenized core was combined with subsamples taken from the other 19 cores. A nitrogen and carbon source solution was prepared to maintain microbial activity by mixing 1.01 g KNO$_3$ (a nitrate source) with 0.30 g Dextrose (a carbon source), in 1L of Milli-Q™ water (Bartsch and Bruesewitz 2002). A volume of 720 ml of solution was added to the soil and mixed to create a soil slurry. A 180 ml soil slurry sample was added to a 473 ml Ball Mason® canning jar and this process was repeated for the 20 replicates. To 10 of the jars, the high-water treatment, 180 ml of Milli-Q™ water were added. No additional water was added to the other 10 jars, the low-water treatment. Each jar remained open to the atmosphere and was fitted with a redox probe. Redox measurements were taken daily to determine water level influence on redox potential. Ten ml of Milli-Q™ water were added to each jar 9 and 17 days after the beginning of the experiment to replace water loss by evaporation. After 23 days, redox potential had diverged significantly between treatments (High water mean redox = -134.7 mV, SD= 44.2, Low water mean redox = 182.4, SD=134.9, p < 0.001). Jars were then sealed and purged with helium for five minutes. Afterwards, the interior pressure of each jar was allowed to equilibrate with atmospheric pressure.

We employed the $^{15}$N isotope pairing technique (Nielsen 1992, Nielsen and Glud 1996, Master et al. 2005) to determine amounts of N$_2$ and N$_2$O produced from both high water and low water treatments. A $^{15}$N isotopically labeled solution was added to each jar to obtain a final concentration of 76 µM $^{15}$N-KNO$_3$ (Nielsen and Glud 1996). Each jar was swirled to mix the solution throughout the soil sample in the jar. Gas samples for
analysis were then collected through the septum using a 5 ml syringe 3 and 5 hours after addition of the $^{15}$N solution. Four ml of gas were collected from each jar and injected into a Kendall® monoject 3 ml evacuated vial. These gas samples were sent to the University of California, Davis isotope facility to determine the amounts of N$_2$ and N$_2$O released as products of denitrification by Trace-gas Isotope Ratio Mass Spectrometry (IRMS).

**Statistical Analyses**

All univariate statistical analyses were performed using SYSTAT statistical software version 11.0 (SYSTAT Software Inc., 2004). One-way randomized block ANOVAS blocking for different transects were used followed by Tukey post-hoc tests to analyze for differences in *Typha* stand age groups. Simple linear regressions for each variable were run using age (years) of *Typha* stands. Log transformations were used for both redox and denitrification data as normality assumption could not be met for these variables. T-tests were performed to determine differences between native and *Typha* communities in the field study, as well as differences between high and low water treatments results in the $^{15}$N laboratory study. Multivariate Principal Component Analysis (PCA) was used to develop a composite environmental variable, which was then used for correlation analysis for total denitrification and denitrification quality. All multivariate analyses were performed using PC-ORD version 5 (McCune and Mefford 2006).

**Results**

**Impacts of *Typha* on Water Depth, Redox Potential, and Denitrification**

No significant differences were found between areas invaded by *Typha* and native control sites with regards to water depth ($P = 0.27$), redox potential ($P = 0.10$), total
denitrification (P = 0.41) and denitrification quality (%N₂) (P = 0.41) when averaged over all sampling dates. Water depth was, however, significantly greater (P = 0.01) in native sites in the June campaign but no significant differences were observed in subsequent campaigns. No significant differences were observed between native and Typha sites within sampling campaigns with regards to redox potential, total denitrification, or denitrification quality.

No significant changes in mean water depth, mean redox potential, mean total denitrification, or mean denitrification quality (%N₂) were observed with increasing age of invasion (Figure 10). Likewise, these variables did not differ significantly with age since Typha invasion(Figure 11). However, sites invaded for 35 years or more had significantly lower mean redox potential than newly invaded sites invaded for 10 years or less (Figure 11).
Water depth measurements did not significantly differ with age groups of *Typha* stands for the June, July, and August campaigns. In the September campaign, native sites had significantly higher water levels ($P = 0.03$) than sites invaded for more than 35 years. No significant within-campaign differences in redox potential were detected among age groups of *Typha* stands. No significant differences were found with regards to age groups of *Typha* stands and total denitrification for the June campaign ($P = 0.35$), July campaign ($P = 0.14$), August campaign ($P = 0.45$) or the September campaign ($P = 0.10$). A significant increase in total denitrification was observed with increasing stand age in the June campaign ($P = 0.02$, $R^2 = 0.09$) but no significant regressions were observed for the other campaigns. *Typha* stands present for 35 to 54 years did, however, have significantly higher denitrification quality ($\%N_2$) than native sites in the August campaign ($P = 0.04$).
Denitrification quality did not differ significantly among age groups in June (P = 0.25), July (P = 0.88) or September (P = 0.09). Linear regression analyses revealed no significant within campaign changes in denitrification quality with age of Typha stands.

Redox potential was not strongly related to water depth ($R^2 = 0.00$) and total denitrification decreased with increasing water depth ($R^2 = 0.14$). Denitrification quality was not strongly related to water depth ($R^2 = 0.02$).

Principal Components Analysis was used to develop a composite environmental axis (PCA axis 1) that explained 34% of the variability in the full suite of environmental data. PCA axis 1 was strongly and positively related with Typha density (Eigenvector = 0.53), litter mass (Eigenvector = 0.56), SOM (Eigenvector = 0.44), and organic depth
(Eigenvector = 0.39). Soil NH$_4^+$ (Eigenvector = 0.20) was weakly positively correlated while water depth (Eigenvector = -0.15) had no relationship. Therefore, PCA axis 1 represents an environmental gradient with increasing *Typha* density, litter mass, SOM, (P = 0.002; Figure 12). No trend was observed for denitrification quality ($R^2 = 0.00$; P and organic depth. PCA axis 1 explained 16% of the variability in total denitrification = 0.952; Figure 12).

**Figure 12.** Total denitrification (a; P = 0.002) and denitrification quality (b; P = 0.952) were correlated with the composite environmental axis determined from a multivariate Principal Component Analyses of plot water depth, litter mass, organic depth, *T. X glauca* density, % soil organic matter (SOM), and NH$_4^+$

**15N Terrestrialization Study**

The mean redox potential differed significantly between high (-134.7 mV) and low water treatments (182.4 mV) (P < 0.001). Total denitrification, however, was significantly greater in the low water treatment (467.9 umols/hr/ml) than the high water treatment (113.3 umols/hr/ml) (P = 0.03). However, denitrification quality (%$N_2$) was significantly lower (P < 0.001) in the low water treatment (99.35% $N_2$) than the high
water treatment (99.99% N₂). Total denitrification increased significantly (P = 0.005) while denitrification quality decreased significantly (P = 0.001) with increasing redox potential (Figure 13).

![Figure 13. Simple linear regressions of ¹⁵N study data showing significant (P = 0.005) increases in total denitrification with increasing redox potential (a) and significant (P = 0.001) decreases in denitrification quality (%N₂) with increasing redox potential (b). Points labeled white are high water treatments while points labeled black are low water treatments.]

**Discussion**

This study aimed to further our understanding of the impact of *Typha* on denitrification rates and the quality of the products (N₂ vs. N₂O). We expected to see reduced denitrification rates and quality in *Typha* invaded wetlands as compared to uninvaded wetlands and hypothesized that both total denitrification and quality would decline with increasing age of *Typha* stands. However, the presence of *Typha* did not significantly impact total denitrification or denitrification quality within 55 years of invasion compared to native control sites. Our results also indicate no change in
denitrification or denitrification quality with increasing time since invasion. However, our results do show a significant positive relationship of denitrification with our composite environmental variable. This weak positive relationship indicates that the *Typha* plant itself, litter mass, and organic matter may fuel denitrification rates.

A previous study in this location showed a 29 cm increase in organic matter in older *Typha* stands compared to newly invaded sites (Mitchell et al. *in prep*). We hypothesized that this increase in organic matter should increase the elevation of the site, thus drying out and oxidizing the upper portion of soil where water depth and redox potential measurements were taken. Water depth averaged for all campaigns did not change with age of *Typha* stands. Surprisingly, there was a nearly significant increase in water depth with increasing age (*P* = 0.07), but the explained variability was very low. Water depth was, however, significantly greater in native sites than *Typha* sites in the June campaign and was significantly greater than sites invaded by *Typha* for more than 35 years in the September campaign. Following the June campaign, most water depth measurements were zero cm above the soil and thus few differences could be detected. Nevertheless, contrary to our hypothesis, soils were significantly more reduced, over all, in older stands compared to newly invaded sites. Larger organic matter inputs in older stands and higher levels of available nitrogen (Ehrenfeld 2003) may fuel microbial respiration (D’Angelo and Reddy 1999), thus reducing soil conditions and possibly offsetting any effects of drying. Higher levels of organic matter in older *Typha* sites (Mitchell et al. *in prep*) likely retain moisture better than soils in newly invaded sites where less organic matter was present (Hudson 1994). These reduced conditions and
reservoirs of nitrogen and carbon in invaded regions may fuel the process of denitrification (Fisher and Acreman 2004), reducing any impacts of terrestrialization on denitrification.

While large accumulations of organic matter have been observed in our study sites invaded by Typha for 35 to 54 years (Mitchell et al. in prep), they do not yet appear large enough to significantly impact water levels and redox potentials. A study by Ballantine and Schneider (2009) found that organic matter levels in restored wetlands dominated by Typha sp. were 50% lower than reference sites 55 years after restoration. These slow rates of organic matter accrual may explain why we saw few significant changes even in our 54 year-old Typha stands. Older Typha stands may, however, expedite the terrestrialization process by facilitating the spread of Phragmites australis. Small increases in elevation may allow Phragmites to become established and then spread throughout the wetland (Tulbure et al. 2007). Wetlands invaded by Phragmites for 20 years following Typha invasion had lower levels of sediment water content than newly invaded Phragmites stands and existing Typha stands (Rooth et al. 2003).

To determine the potential impacts of terrestrialization on denitrification rates and quality, we performed a $^{15}$N lab microcosm study comparing total soil denitrification and denitrification quality ($\%N_2$) under dry, high-redox conditions with flooded, low-redox conditions. This study showed that water levels significantly impact redox potentials as well as denitrification rates and quality. Total denitrification increased significantly with increasing redox. This was unexpected as previous studies and literature have shown that denitrification dominates when redox is between -300 and
+100 mV (Gambrell and Patrick 1978). We observed the highest amount of total denitrification \( (N_2 + N_2O) \) when the redox potential was +363. Similarly, Kralova et al. (1992) observed that \( N_2O \) increased over time in soils maintained at +400 mV and suggested that this unexpected result may have been due to nitrification. Anaerobic pockets or short periods of anaerobiosis may also have allowed denitrification to occur at these high redox potentials (Kralova et al. 1992). Nevertheless, the potential ecosystem benefits of increased total denitrification may be offset by the increased release of \( N_2O \) in the low water, terrestrialized systems. The decrease in denitrification quality with increasing redox potential indicates that if terrestrialization does occur as a result of \textit{Typha} invasion, significantly more \( N_2O \) will likely be released from these wetlands.

**Implications and Conclusions**

If organic matter continues to increase with time since invasion (Mitchell et al. \textit{in prep}) and \textit{Phragmites australis} invades, changes to denitrification quality are likely to be observed as wetland areas become terrestrialized. Sites invaded for more than 55 years may contribute significantly more \( N_2O \) to the atmosphere. If large-scale terrestrialization does occur in areas invaded by \textit{Typha} throughout our study sites, extrapolation using our \(^{15}\text{N}\) study data shows that these areas could release 119 metric tons \( N_2O / \text{growing season} \) (the equivalent of 36,890 metric tons \( CO_2 \) (U.S. EPA)) compared to 0.4 metric tons \( N_2O / \text{growing season} \) (the equivalent of 124 metric tons \( CO_2 \) (U.S. EPA)) if invaded sites are flooded. If terrestrialization by \textit{Typha} and other invasives such as \textit{Phragmites} occurs on an even larger scale, the amount of \( N_2O \) produced is likely to significantly contribute to global climate change. In order to limit terrestrialization and subsequent \( N_2O \)
production, more drastic management techniques such as flooding (Leeds et al. 2009) and removal of organic material (White et al. 2008) must be performed. Terrestrialization may also cause large-scale habitat loss for wetland plants, amphibians, and waterfowl.

Our results, however, show that changes to denitrification and denitrification quality did not occur within the first 55 years following Typha invasion in these wetlands. Furthermore, the sum total environmental impacts of Typha (as represented by PCA) were significantly positively correlated with increased total denitrification. This indicates that Typha and other invasives may prove beneficial in some wetland areas for their ability to enhance soil denitrification while also storing large amounts of carbon (Rooth et al. 2003).
CHAPTER FOUR

SYNTHESIS

As wetlands continue to be degraded by pollutants and eliminated for agricultural and industrial land use, the few small high quality native regions still present are becoming increasingly important habitat for flora and fauna. However, even these high quality areas are threatened by species invasions.

*Typha X glauca* is a wetland invader that builds up large amounts of biomass immediately following invasion. Biomass in the form of litter, together with competition for light and nutrients, eventually hinders the survival of native plants. This loss of native habitat impacts macroinvertebrates, mammals, reptiles, and birds, which depend on the conditions and resources available in these disappearing wetland communities.

As biomass produced by *Typha* decomposes and incorporates into the soil, we begin to see changes to the organic makeup and increases in the depth of the soil layer. With continual buildup of organic matter over time, these changes will likely create drier conditions. Other invasive plant species such as *Phragmites australis* may move in, continuing the buildup of organic matter and terrestrialization of the site. These drastic changes may effectively remove the wetland from the landscape, completely eradicating
habitat and possibly altering denitrification, a very important ecosystem service with global consequences.

While *Typha* decimates native plant communities and alters the organic content of wetland soils, our results show that *Typha* does not impact denitrification rates or quality within 55 years of invasion. Denitrification is an important ecosystem service as it removes inputs of nitrogen from water that would otherwise cause eutrophication in aquatic ecosystems. While these older stands of invasives no longer provide high quality habitat for native species, they are able to process nitrogen at high rates while also sequestering large amounts of carbon. These two beneficial ecosystem services suggest that older *Typha* stands may play an important role in disturbed ecosystems. Our results also indicate that managers should focus on young *Typha* stands where native plant and animal communities can still be saved, leaving older *Typha* stands alone to continue processing nitrogen and sequestering carbon. However, results from the $^{15}$N terrestrialization study indicate that if wetland conditions are to severely dry out due to the increased biomass production of *Typha* and subsequent elevation increases, denitrification quality ($\%N_2$) will likely decline. These older *Typha* stands may become significant sources of $N_2O$, a very potent greenhouse gas, and may contribute to global climate change. If other wetlands undergo similar elevation and water level changes, large impacts to global climate change are likely.
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VITA

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