Effect of invasive *Typha x glauca* on denitrification potential and quality in Cheboygan Marsh, Northern Michigan

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Introduction

Wetland ecosystems are important hotspots of biodiversity that have critical ecological functions. Wetlands play a significant role in improving water quality through the removal of nutrients from the surface water runoff before it reaches lakes and rivers (Mitsch and Gosselink, 2000). As the nutrient load introduced into wetlands increases through anthropogenic processes such as agriculture and urbanization, the ability of wetlands to remove excess nutrients becomes more critical. However, this ecosystem service is compromised by wetland destruction and degradation which can lead to eutrophication in surrounding waterways (Mitsch and Gosselink, 2000). Denitrification (DN) is a critical wetland process undertaken by soil microbes under anaerobic conditions involving the conversion of inorganic nitrogen (NO_3-) to N_2 and N_2O (Burgin and Hamilton, 2007). Invasion of wetlands by exotic plant species, however, can change a wetland's ability to cycle nitrogen and carbon (Ehrenfeld, 2003). This is significant because wetlands are extremely susceptible to invasions by exotic plants (Galatowitsch et al, 1999, Ehrenfeld, 2003). Although wetlands occupy less than 6% of the world's land mass, they harbor 24% of the world's most invasive plant species (Zedler and Kercher, 2004). It is important, therefore, to understand how such invasions affect critical nutrient cycling processes.

Typha x glauca (hereafter referred to as *Typha*) is an aggressive wetland exotic in North America that is a hybrid between the endemic *Typha latifolia* and the non-native

Typha angustifolia. *Typha's* invasiveness is attributed in part to hybrid vigor which enhances its ability to take advantage of increased nutrient loads, broadens its tolerance to variable hydrologic conditions, and increases its growth rate (Galatowitsch et al, 1999, Zedler and Kercher, 2004). In Cheboygan Marsh, Northern Michigan, *Typha* invasion has been coupled with a loss of wetland biodiversity (forming monotypic stands), an increase in aboveground biomass, an increase in soil organic matter (SOM), and an increase in soil nutrient loads (Angeloni et al, 2006, Tuchman unpublished data). Furthermore, similar invasions in wetlands by *Phragmites australis* causing increased biomass and SOM through the accumulation of litter have led to a process known as terrestrialization, by which wetlands become filled in and subsequently dried out over many years (Rooth et al, 2003). Filling in and drying of wetlands is of concern because drier conditions lead to increased oxygenation of the soil and higher redox potentials, which have been coupled with decreases in denitrification potential and quality (Kralova et al, 1992). DN quality is defined as the ratio of N_2O gas produced from incomplete DN to N_2 gas from complete DN. Several studies have found a significant correlation between decreases in DN potential with lowering water levels in wetlands (Hunter and Faulkner, 1999, Baker and Vervier, 2004), but little research has been done to measure how changing hydrologic conditions affect DN quality. N_2O emission is of special concern because it is a known greenhouse gas that is 300 times more potent than CO_2. Furthermore, 80% of N_2O evolution is attributed to biotic sources, with just under half of that total coming from soil microbial processes (Olivier et al, 1998).

Wetland restoration efforts in invaded ecosystems are often targeted towards recovering lost biodiversity. However, restoration of biodiversity in *Typha* infested wetlands is costly and very difficult due to the aggressive nature of the plant and its ability to reproduce clonally through underground rhizomes. An alternative investigated by this study is to consider restoration of ecosystem services provided by wetlands.

By collecting soil sample data from *Typha* invaded plots in the Cheboygan Marsh along with controlled mesocosms, this study aims to further clarify the relationship between wetland water levels and DN potential and quality. This study will additionally examine the correlation between time since *Typha* invasion and DN quality and potential in order to further understand the terrestrialization process and its effect on DN.

Specific objectives of this study include:

1. To investigate how water level affects redox potential and DN quality within established *Typha* stands
2. To investigate how time since *Typha* invasion correlates to *Typha* density, terrestrialization of wetlands, redox potential, and DN potential and quality
3. To compare our field DN data with data from controlled mesocosm experiments

Materials and Methods

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Field Study

The Cheboygan Marsh is a freshwater coastal marsh located in Northern Michigan on Lake Huron that is exposed to daily sieche activity (Angeloni et al, 2006). Over 80% of the marsh is covered in invasive *Typha* that has been present since at least the 1950's (Vail, unpublished data). This study investigated two distinct sections of the marsh.

In order to investigate our first objective of study, we considered how water level affects DN quality in the "*Typha* bowl," which contains a homogeneous stand of densely growing and well established monocultures of *Typha* that have been compounding litter for decades resulting in the production of a deep organic layer of soil. Since this area is bowl shaped, water levels vary significantly from the high water in the middle of the bowl to the dryer peripheral areas. Five 1x1 m plots were established in areas with water levels well below the soil surface and five 1x1 m plots were established with levels at or above the soil surface.

The second part of our field study addressed our second objective of correlating time since invasion to DN quality. To accomplish this, an area coined "*Typha* harbor," was investigated along a transect from the center of a long established *Typha* stand out towards Lake Huron where younger, newly established *Typha* is spreading. Five 1x1 m plots were established for each *Typha* age class..

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A 10 cm soil core was collected from all 20 field sites and analyzed for DN potential and quality, SOM, NH_4+, and NO_3-. Measurements of redox potential for each plot were taken and monitored using a redox probe and a voltmeter. Organic layer (belowground organic material), litter (aboveground dead organic material), and water depth were also measured using a graduated metal pipe. All of these measurements were taken during two intensive DN campaigns in July and August.

Controlled Study

Our field study was coupled with a controlled study that will investigate our first and third objectives. 20 1x 2 m wetland mesocosms were constructed in 2003 to mimic the plant community compositions found in the Cheboygan Marsh. Each mesocosm is 1 m deep, and all began the experiment with the same soil types. Five mesocosms containing *Typha* monocultures were maintained at water levels 20 cm below the soil surface. Five more mesocosms containing *Typha* monocultures were maintained at water levels 10 cm above the soil surface. Five additional mesocosms for each water depth were planted with a representative mix of native species mimicking those found in Cheboygan marsh to serve as control plots. A 10 cm soil core was collected from all 20 mesocosms and analyzed for DN potential and quality, SOM, NH_4+, and NO_3-. Measurements of redox potential were taken and monitored for each mesocosm using a redox probe and a voltmeter.

Measuring DN Potential and Quality

Direct measurement of N_2 evolved off of soil samples would be difficult as N_2 gas makes up 78% of Earth's atmosphere. This would make detection of trace amounts of N_2 produced during DN challenging when compared to atmospheric levels. An alternative method, known as the acetylene block method, was used. This method involves the use of acetylene to inhibit the DN process at the N_2O production step, disallowing N_2 emission.

N_2O levels will be analyzed using an electron capture detecting gas chromatograph-2014 (Tiedje et al., 1989).

Each soil core was placed in a plastic bag and homogenized. Two samples were taken from each bag and placed in one of 80 ball jars fitted with air-tight septa on their lids. The airspace in all jars were flushed with inert He gas. In 40 of the samples (one sample from each bag), acetylene were injected and mixed into the jar to create a 90:10 ratio of He to acetylene while the other 40 samples will contain only He. Gas samples were collected from the jars and sent to the ECD-GC 2014 at 30, 90, and 180 minutes after the addition of acetylene and tested for N_2O levels. After each gas sample collection, the 90:10 ratio of He: acetylene was restored via injection. A detailed description of this method can be found in Jankowski (2003). The N_2O detected from the acetylene jars represents total DN potential, while the N_2O collected from the He only jars represents incomplete DN. The ratio between N_2O levels collected from these two treatments resulted in an indication of DN quality.

Other Soil Testing

Depth of the soil organic layer was determined in the field using a metal probe. Redox potential measurements were collected and monitored in the field and controlled experiments using a redox probe and a voltmeter. Soil sample NH_4+ analysis was carried out using the Cadmium Reduction Method NO_3- was analyzed using the automated phenate method (APHA, 2005). Soil organic matter was determined by loss on ignition in a muffle furnace at 550°C for two hours (APHA, 2005).

Statistical Analysis

Data from the field and controlled experiments were analyzed using a multiple regression analysis, independent samples t-tests, and linear regression tests using SPSS v. 17.0, Chicago, Il, 8/23/2008.

Results and Discussion

Field Study

Typha legacy data

In our investigation of the effect of time since invasion on environmental factors in Cheboygan marsh, we found a significant increase (p=.009) in *Typha* density between the young (<5 year old) and old (approx. 60 year old) transects in the *Typha* harbor. Along with an increase in *Typha* density, we also found significant increases in litter depth (p<.001), organic layer depth (p<.001), and SOM (p<.001) (Figure 1a). We also found a significant increase in NO_3- and NH_4+ from young to old *Typha* in the harbor (p=.008, .017 respectively). These data indicate that *Typha* invasion is correlated with an increase in organic matter and available nitrogen in soils over time, which supports our hypothesis that long-term *Typha* invasion impacts wetland environments by causing a buildup of organic material thereby increasing the total available carbon for microbial processes. These results are conclusive with data found in previous investigations (Jankowski, 2006, Angeloni, 2006). Such an increase in organic carbon availability reflects our observed (but not statistically significant) increase in total DN rates between the young compared to the old *Typha* in the harbor and the significant increase (p=.009) in total DN rate between the young *Typha* in the harbor and the old *Typha* in the bowl (Figure 2).

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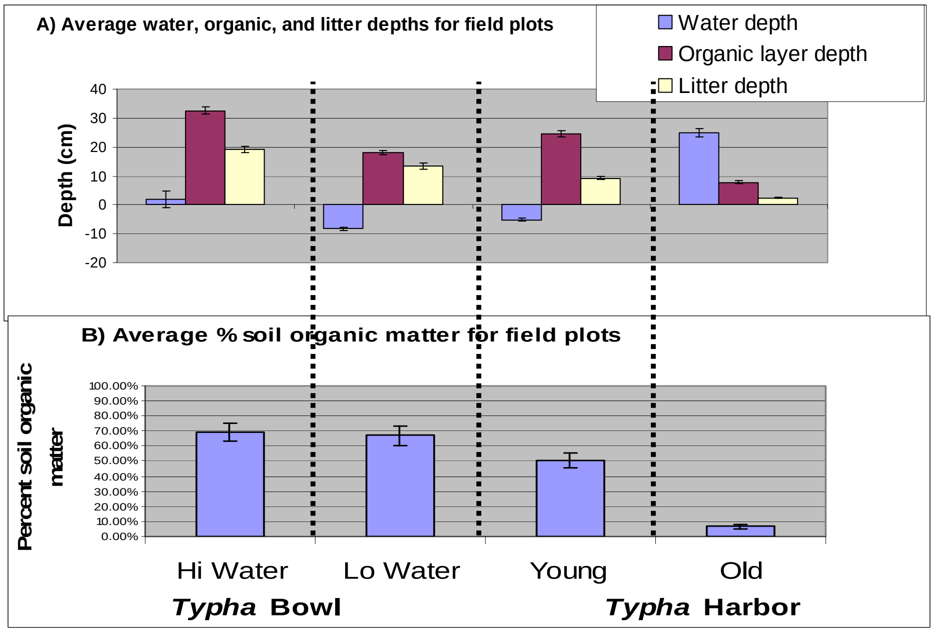

Figure 1: A) Water, organic, and litter depths in cm for all four field plots, B) Percent soil organic matter. Bars represent mean values for each transect (n=5) and error bars represent standard error.

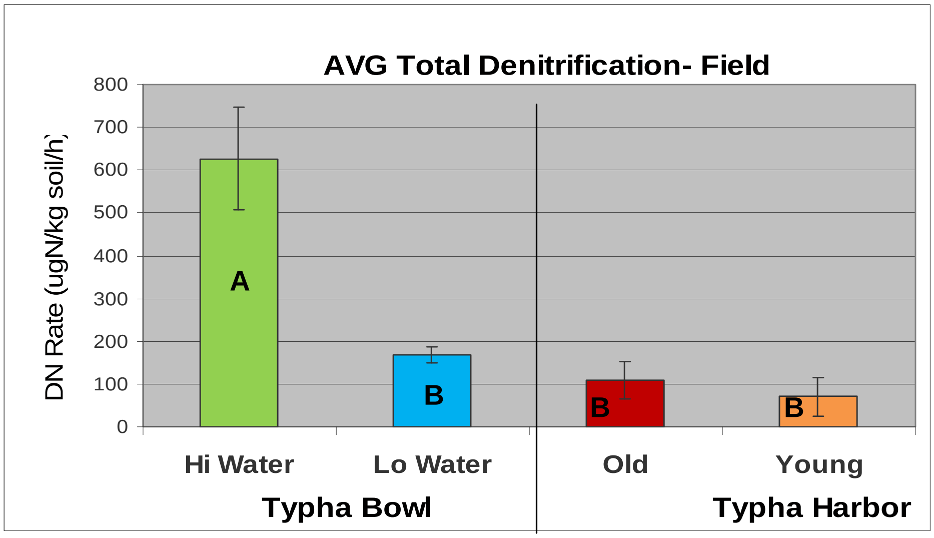

Figure 2: Total DN rate for all four field plots measured in ugN/ Kg soil/h. Bars represent mean values for each transect (n=5) and error bars represent standard error. Values with different letters are significantly different based on independent samples t-tests (P<.05).

Water Level Data

In our investigation of the effects of time since invasion on water level factors and DN potential and quality, we found a significant decrease (p<.001) in water level between the old and young *Typha* in the harbor (Figure 1). Such a decrease, when combined with the increase in organic soil layer depth, supports our hypothesis that long term *Typha* invasion contributes to the terrestrialization of wetland soils, which supports studies done on *Phragmites australis*, a wetland invasive that has a similar impact on wetlands (Rooth et al., 2003).

The effect of water level on *Typha* was examined by comparing the established, old, *Typha* in the high and low water plots in the *Typha* bowl. These plots were found to have significantly different water levels (p=.024) while having similar soil organic depths and percent SOM (Figure 1). We found that total DN rates in the high water *Typha* bowl

Comment [SL4]: Find and replace *Typha* with *Typha* plots were significantly higher (p=.017) than rates in the low water *Typha* bowl (Figure 2). Redox potential was also lower in the high water bowl plots than the low water ones, which correlated with the differences in water level. When a linear regression analysis was carried out comparing water level's effect on total DN rates in the old *Typha* plots in the *Typha* bowl and harbor, a linear increase (R^2=.75) in DN with increasing water depths was found (Figure 3). We also did a regression comparing redox potential and total DN rates in the *Typha* bowl, where a representative variance in redox compared to water depths was observed, and found a linear decrease in total DN as redox potential increased (R^2=.623) (Figure 4). These findings support our hypothesis that decreasing water levels and increasing redox potentials can cause increases oxygenation of wetland soils that may lead to a decrease in total DN rates due to inhibition of the DN process by increasing oxygen levels.

As redox potential increased along the water level gradient in the *Typha* bowl, we expected to find decreasing DN quality because of a higher proportion of incomplete DN due to increased oxygenation of soils. However, no significant differences in DN quality were found between *Typha* stands along the water level gradient in the bowl due to variability of data. We are still awaiting the results from an additional DN campaign that was conducted in August which may be significant to total DN rates and quality.

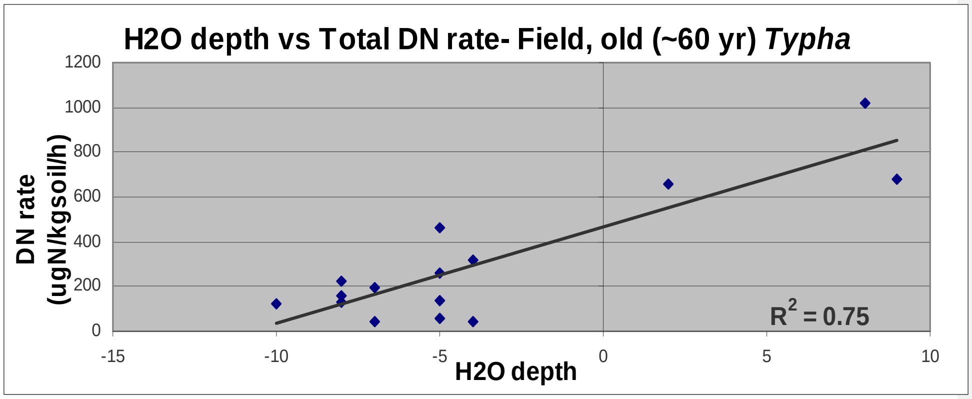

Figure 3: Linear regression model comparing water depth (cm) and total DN rate (ugN/kgSoil/h) in established (~60 yr old) *Typha* in the bowl and harbor field transects (R^2= 75)(n=15)

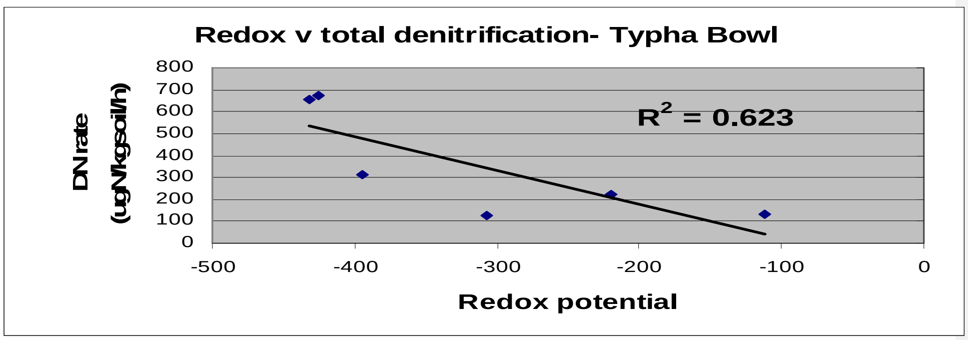

Figure 4: Linear regression model comparing redox potential (mV) to total DN rate (ugN/kgSoil/h) among established (~60 yr old) *Typha* in the high and low water bowl transects (n=6)

Model Summary				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.773[a]	.597	.557	178.990
2	.905[b]	.819	.779	126.477
3	.962[c]	.924	.896	86.623
4	1.000[d]	1.000	1.000	.000
5	1.000[e]	1.000	1.000	.000
6	1.000[f]	1.000	1.000	.000

a. Predictors: (Constant), litter depth

b. Predictors: (Constant), litter depth, H2O Level

c. Predictors: (Constant), litter depth, H2O Level, Redox (7/12)

d. Predictors: (Constant), litter depth, H2O Level, Redox (7/12), Organic depth

e. Predictors: (Constant), litter depth, H2O Level, Redox (7/12), Organic depth, % Organic

f. Predictors: (Constant), litter depth, H2O Level, Redox (7/12), Organic depth, % Organic, *Typha* density- stems/ m^2

Figure 5: Multiple regression analysis considering the effect of litter depth, water level, redox potential on July 12, organic depth, % soil organic matter, and *Typha* density on variability in total DN rate.

There are multiple factors that strongly covary with *Typha* density. In order to consider which of these factors has the strongest influence on variation total DN rates in the field, a multiple regression analysis was conducted considering water depth, redox potential, litter depth, % SOM, organic depth, and *Typha* density (Figure 5). We found litter depth to have the greatest contribution to variation in total DN, accounting for 56% of the variation (p=.003). Litter depth and water depth in tandem accounted for 78% of the variation in total DN (p<.001). Litter depth, water depth, redox potential, and organic depth combined accounted for 100% of the variation in total DN (p<.001). These results show how total DN is affected by both environmental factors controlled by *Typha* legacy and water level factors. The interaction of these two categories strongly explains variation in total DN, suggesting that processes such as terrestrialization by which increase in organic matter leads to a decrease in water levels are critical when considering the effect of *Typha* invasion on total DN.

Controlled Study

In our mesocosm study, we found no significant difference between total DN rates in *Typha* invaded and native control mesocosms (Figure 6). These findings could possibly be due to the fact that the mesocosms are only seven years old, and this time period is likely too short to observe a *Typha* legacy effect on biogeochemical processes. When examining water level effects on total DN in the mesocosms, we found low water mesocosms to have higher rates of total DN than the high water treatments. This trend (though not statistically significant) was opposite to our hypothesized trend that we had observed in the field. It is important to note that the low water mesocosms were drained from water levels 10 cm below the soil surface to levels 20 cm below the soil surface less than two weeks before the initial soil cores that produced our DN data were collected. It has been suggested in the literature that events of water level flux may cause an increase in DN activity in wetland soils (Olde Venterink et al., 2002). Recent draining of the mesocosms may have lead to a temporary increase in DN rates; however, we would expect these levels to decrease significantly in our next round of DN sampling in August. Results from our soil NH_4+ assay indicate a trend toward increased levels of NH_4+ in the low water mesocosms (p=.07 for control treatments, p=.089 for *Typha* treatments), which may be increasing total DN through the availability of nitrogen for microbes to denitrify.

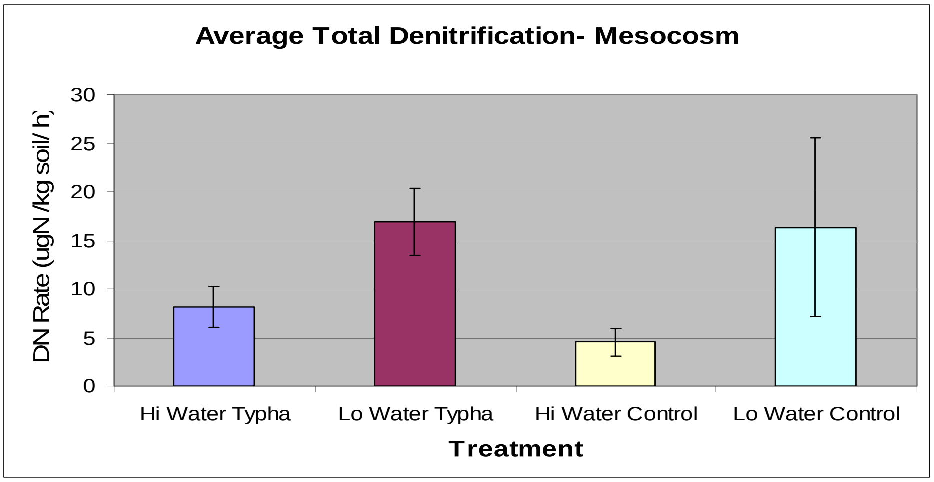

Figure 6: Total DN rate for all four mesocosm treatments measured in ugN/ Kg soil/h. Bars represent mean values for each treatments (n=5) and error bars represent standard error.

In our analysis of DN quality, we found increased quality (measured as portion of N_2 produced/Total DN) in high water mesocosms compared to low water mesocosms (Figure 7). These data support our prediction that DN occurring in more oxic soils leads to a higher proportion of incomplete DN. This was predicted on the basis of differences in redox potentials, and low water redox was found to be significantly higher (p=.008) than high water redox. N_2O evolution is known to peak at redox potentials between 0 and +100 mV (Kralova et al., 1991), which represents the range of redox potentials present in the low water mesocosms. These results confirm that more oxic soils inhibit complete DN and lead to the increased emission of N_2O, a greenhouse gas, from wetland soils.

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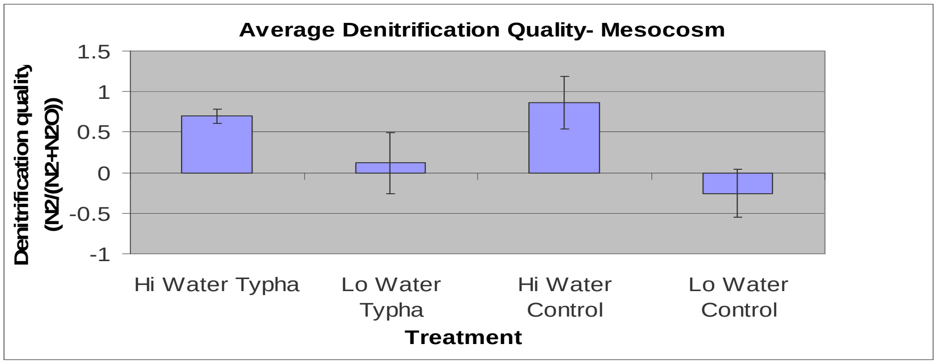

Figure 7: DN quality for all four mesocosm treatments measured as a ratio of $N_2/(N_2+N_2O)$ gas detected in DN. Bars represent mean values for each treatment (n=5) and error bars represent standard error.

Conclusions

The results of this study show the strong impact of *Typha* legacy and water level factors on total DN. As *Typha* invades, the buildup of organic matter from accumulation and subsequent decomposition of litter leads to an overall increase in available organic carbon to denitrifying soil microbes that increases their potential total DN rates. The concomitant drying of wetland soils due to excess buildup of organic matter and subsequent terrestrialization, however, has the capability to significantly reduce a wetland's ability to denitrify (Figure 8). From these findings, we propose the flooding of invaded and terrestrialized wetlands as a possible wetland management strategy for the restoration of DN potential. Such an event would restore the conditions under which DN is optimized: high organic carbon, high inorganic nitrogen, and low redox potential.

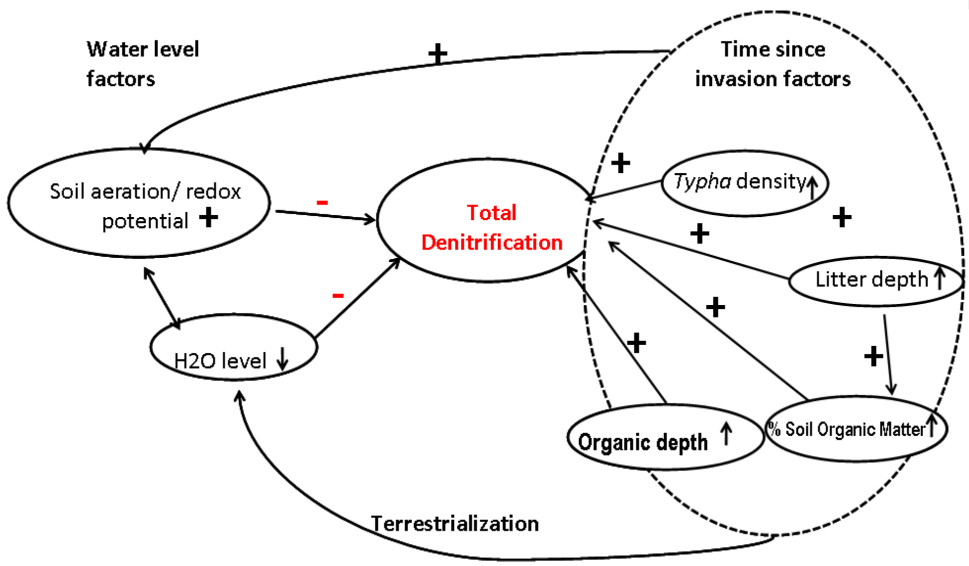

Figure 8: Flow chart summarizing interactions between factors that affect total DN.

Future research into flooding as a wetland management strategy must investigate the effect of wetting and drying cycles on nitrogen cycling (Olde Venterink et al., 2002). Also to be considered when flooding wetland soils is the production of CH_4 gas through methanogenesis which is known to occur at low redox potentials below -200 mV (Mitsch and Gosselink, 2000) which commonly occur under prolonged flooded conditions. CH_4 is a known greenhouse gas, so efforts to maximize wetland biogeochemical activity must consider CH_4 emissions and their impact on global climate change in addition to the release of N_2O through incomplete DN.

Although mesocosm data failed to confirm our findings in the field, trends in overall DN quality indicate that the drying of wetland soils may lead to increased N_2O emissions through incomplete DN. More research must be done to strengthen our knowledge on the relationship between terrestrialization in wetlands and N_2O emissions.

Further research should be done considering the impact of global climate change on wetland water levels, especially in the Great Lakes region. Most climate change models predict that increasing temperatures will lead to increased evaporation and decreased ice cover in the Great Lakes basin, leading to a decrease in water levels (Lofgren et al., 2002). Such a decrease may accelerate the drying out of wetland soils which would severely impact wetland biogeochemical processes.

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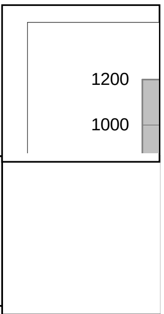

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