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RESEARCH ARTICLE

Denitrification in a Laurentian Great Lakes coastal wetland invaded by hybrid cattail (*Typha* × *glauca*)

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Abstract Wetland ecosystems maintain and improve water quality through the process of denitrification, an increasingly important ecosystem service due to global N pollution. Invasive plants have the potential to disrupt denitrification by altering the environmental conditions that facilitate this process. Great Lakes coastal wetlands are experiencing widespread invasion by highly productive hybrid cattail with largely uncertain biogeochemical effects. Through field and controlled mesocosm studies, we sought to determine the effects of cattail invasion through time on denitrification rates and associated environmental factors in a Great Lakes coastal wetland. In the field, we found that cattail density correlated with increased denitrification and a suite of environmental and plant community characteristics and denitrification rates were positively correlated with NH_4^+, sediment organic matter, reduced water levels, and cattail stand age. Through our controlled mesocosm study, we documented conditions 1- and 5-year following invasion and found that denitrification rates and soil organic matter increased in year 5, and cattail and year-since-invasion altered plant communities and soil NH_4^+. Only a weak correlation between denitrification rates and cattail treatments was noted, however, owing to high replicate variability. Our results indicate that with increasing cattail residence time, one ecosystem service, biodiversity, was negatively impacted, while two other services, denitrification and sediment carbon accumulation, were enhanced. Thus, this highly invaded wetland still provides valuable services to aquatic ecosystems and to society. A holistic perspective is therefore critical when evaluating invasive species impacts in which *negative* impacts are weighed against other ecosystem services, which may be stimulated.

Keywords Denitrification · Invasive species · Temporal · Aquatic macrophyte · Ecosystem service · *Typha* × *glauca*

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Introduction

Modern civilization has fundamentally altered the global nitrogen (N) cycle and dramatically increased the quantity and distribution of biologically available N (Vitousek et al. 1997). The implications of these changes to global N cycling are myriad, including negative impacts on human health and biodiversity, and substantial increases in climate-disrupting greenhouse gases (Galloway et al. 2003). Further, increased N in the environment has detrimental impacts on aquatic and coastal ecosystems, such as stimulating invasive species dominance, eutrophication, hypoxia, biodiversity declines, and habitat loss (Vitousek et al. 1997; Carpenter et al. 1998; Camargo and Alonso 2006).

Wetlands are highly valued for their role in maintaining and improving water quality (Zedler 2003), and the ecosystem services provided by wetlands are increasingly important given the quantity of global N pollution. Denitrification, the biogeochemical process that converts nitrate into gaseous forms of nitrogen ($NO_3^- \rightarrow N_2$ and/or N_2O), is one of the most important mechanisms through which wetlands support aquatic ecosystem quality (Keddy 2000). Wetlands have all of the conditions necessary for denitrifying microbes to thrive: anoxic conditions (so that nitrate, rather than oxygen, is used as an electron acceptor), available nitrate (the substrate for denitrification, hereafter NO_3^-), and organic carbon (as a heterotrophic energy source) (Knowles 1982; Myrold and Tiedje 1985).

The environmental and biological conditions that control denitrification rates are affected by plant community composition (Bachand and Horne 1999; Lin et al. 2002; Angeloni et al. 2006) and therefore are likely to be impacted by dominant invasive plant species. Understanding the effects of invasive species on denitrification is of particular importance because wetlands are highly susceptible to plant invasions (Zedler and Kercher 2004). Invasive plant species often cause substantial structural changes in the ecosystems they invade (Mack et al. 2000; Ehrenfeld 2003), and can alter species composition and diversity by out-competing native species for resources such as light, nutrients or water (Crooks 2002; Larkin et al. 2012a). In addition, they have increasingly been found to alter other physical characteristics, such as hydrology and topography (Rooth et al. 2003; Gutierrez and Jones 2006). These structural changes often have cascading effects on ecosystem functions, such as primary productivity (Windham and Meyerson 2003), decomposition (Findlay et al. 2002; Freyman 2008), and nutrient cycling (Ehrenfeld 2003; Windham and Ehrenfeld 2003), though impacts on denitrification have not been adequately explored.

Laurentian Great Lakes wetlands are experiencing widespread invasion by invasive hybrid cattails (*Typha angustifolia* × *T. latifolia*: *T.* × *glauca*; hereafter *Typha*) (Galatowitsch et al. 1999; Tulbure et al. 2007; Lishawa et al. 2010; Tulbure and Johnston 2010; Mitchell et al. 2011). *Typha* outcompete native species directly through higher rates of primary productivity and more efficient nutrient utilization (Woo and Zedler 2002; Larkin et al. 2012b) and indirectly through the accumulation of partially decomposed litter which negatively affects native species while not limiting the growth of *Typha* itself (Farrer and Goldberg 2009; Tuchman et al. 2009; Vaccaro et al. 2009; Mitchell et al. 2011; Larkin et al. 2012a). Competitive dominance by *Typha* is especially pronounced in wetlands with exogenous nutrient inputs and/or stabilized water levels (Wilcox et al. 1985, 2008; Boers et al. 2007; Boers and Zedler 2008). In Great Lakes coastal wetlands, sediments within invasive *Typha* stands have greater carbon and N (Farrer and Goldberg 2009; Tuchman et al. 2009; Lishawa et al. 2010), as well as differences in denitrifying bacterial communities (Angeloni et al. 2006). Changes to sediment and plant community characteristics of *Typha*-invaded wetlands also increase with *Typha* residence time (Mitchell et al. 2011). Although *Typha* and other invasive wetland plants have been shown to affect nitrogen cycling, few studies have examined their effects on nitrogen loss through denitrification (Ehrenfeld 2003), and we know of no studies that have examined the effects of stand age on denitrification.

The goal of this study was to determine the effects of a *Typha* invasion on denitrification rates in a Great Lakes coastal wetland by asking the following questions: (1) How are denitrification rates, plant communities, and associated environmental characteristics impacted by *Typha* invasion, and (2) How does *Typha* stand-age influence these factors? To answer these questions, in 2005 and 2009 we conducted field studies in a coastal wetland with expanding *Typha* and remnant native marsh as well as controlled mesocosm experiments. These paired studies allowed us to evaluate the relationships between wetland plant community, *Typha* stand age, and denitrification rates, and the effects of *Typha* and water level on denitrification rates at two points in time following *Typha* establishment. We hypothesized that denitrification would be enhanced in a *Typha*-invaded wetland and would be positively correlated with increasing stand age due to predicted higher levels of the key substrates necessary for denitrification to occur: soil organic matter (SOM) and nitrate. Increased SOM and organic N would result from the accumulation and decomposition of *Typha* detritus; organic N is readily converted to nitrate through the processes of mineralization and nitrification in wetland soils.

Materials and methods

Field study

We conducted this study in Cheboygan Marsh, a lacustrine, open-embayment wetland (Albert et al. 2005) on Lake Huron in northern lower Michigan, during the summers of 2005 and 2009. We chose Cheboygan Marsh because of the presence of remnant native plant communities, actively spreading *Typha*, and *Typha* stands with a wide range of ages. *Typha* invaded the marsh in the 1940s and by 2009 dominated ~14 ha, over 60 % of the marsh area (Lishawa et al. 2013). Three distinct plant communities were identified and mapped in Cheboygan Marsh: a native emergent marsh community (native) dominated by *Schoenoplectus* spp., *Juncus* spp., *Carex* spp., and *Eleocharis* spp.; a

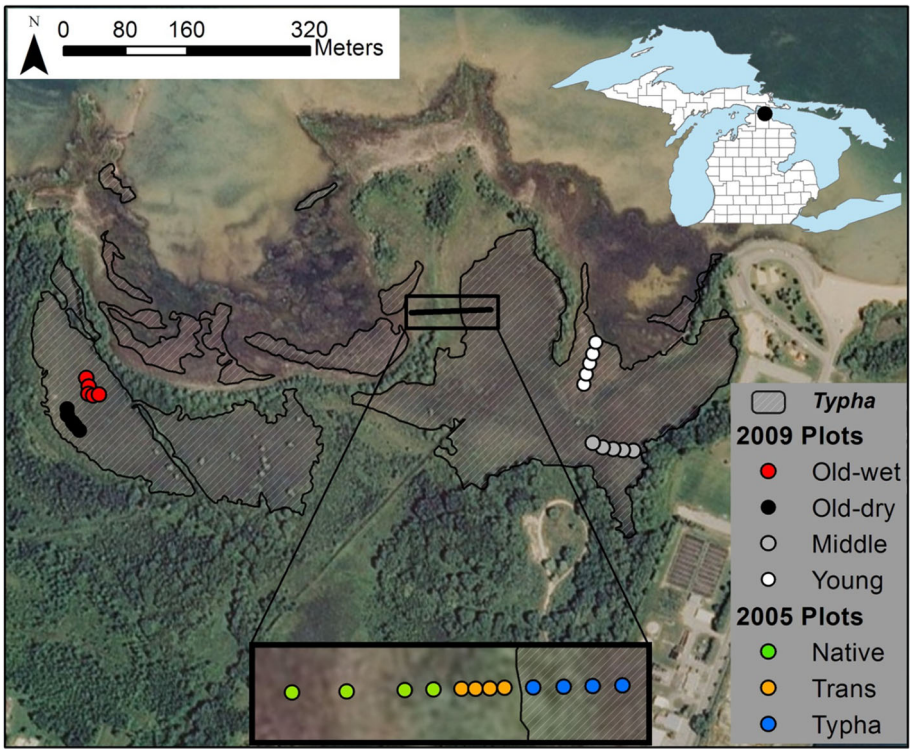

Fig. 1 Plot locations and extent of *Typha* (>50 % cover) in Cheboygan Marsh. Native = Uninvaded; Trans = Low *Typha* density; Typha = *Typha* dominated community

recently invaded community (transition) containing a mix of natives and *Typha*; and a *Typha*-dominated community (*Typha*). In 2005, we positioned one transect through these three community types, and established four replicate 1-m radius circular plots per community. The transition and *Typha* communities had been invaded by *Typha* for ~3 years and ~13 years, respectively. In 2009, we positioned transects within distinct portions of the *Typha*-dominated community representative of the variation in invaded marsh environmental conditions in order to evaluate how *Typha* stand-age and water level impacted denitrification: ~5 years (young; Y), ~40 years (middle; M), >46 years and wet (old wet; OW), and >46 years and dry (old-dry; OD) (Fig. 1). Each transect had five replicate 1-m^2 plots. We determined *Typha* stand age at each plot location from maps created through interpretation of a series of historical aerial photos between 1963 and 2010 (Lishawa et al. 2013).

Plant community structure

In 2005, we quantified plant density (stems m^{-2}) and aboveground biomass in late July; biomass was calculated by converting stem height measurements using species-specific height-biomass regressions based on 50 specimens of each species (unpublished data). We calculated Shannon–Weiner diversity (H′) at the plot scale using biomass values to account for large variability in biomass between stems of different species. We determined litter mass in 2005 plots by collecting, drying, and weighing all accumulated *Typha*-derived detritus at the end of the growing season (October 2005) from 0.25 m^2 sub-plots. In mid-July 2009, densities of all plant species were quantified and we used these data to calculate H′ and litter depth was recorded as the vertical distance from the sediment surface to the top of the litter layer.

Chemical and physical characteristics

We conducted physical and chemical analyses of sediments in July and August 2005 and in August 2009. From each plot, we collected four subsample (10-cm deep, 4-cm diameter) sediment cores for the measurement of SOM, moisture content, pH and N content (NO_3^-, NH_4^+). SOM was measured as mass loss on ignition in a muffle furnace at 550 °C for 2 h (APHA 2005). We extracted sediment inorganic nitrogen with 2 M KCl and measured NO_3^- concentrations using the cadmium reduction method, and NH_4^+ concentrations using the automated phenate method (Dahnke 1990; APHA 2005) on an Auto-Analyzer 3 (Bran-Luebbe, Farmington, MI).

We determined water depth in the field by installing 1-m deep × 10-cm diameter PVC wells and measuring positive or negative depth from the sediment surface by hand. We determined redox potential by measuring the voltage difference between platinum electrodes (Wafer et al. 2004) and a calomel reference electrode (Fisher Scientific, Pittsburgh, PA) inserted 10-cm below the sediment surface;

readings were taken with a standard volt meter (Fisher Scientific, Pittsburgh, PA). Organic matter depth was determined at each plot by measuring the distance from the top of the organic sediment horizon to the underlying mineral substrate.

Denitrification rate

We assessed denitrification potential (hereafter DNP) monthly from July to October 2005 and in August 2009 by measuring denitrification enzyme activity following standard acetylene inhibition methods (Yoshinari and Knowles 1976; Groffman et al. 1999). This approach provides a temporally-integrated measure of microbial community activity and generally correlates well with more direct measures of denitrification rates (Bruesewitz et al. 2006; Groffman et al. 2006). We collected four subsample sediment cores (10-cm deep, 4-cm diameter) from each plot on each sampling date, transferred them to the laboratory on ice, and processed them within 24 h. Sediment samples were homogenized and 25 mL subsamples were transferred into 230-mL air-tight glass jars fitted with butyl rubber septa. Five mL of a 140-mg L^{-1} KNO_3, 1,000-mg L^{-1} chloramphenicol solution, and 20 mL of distilled water were then added. KNO_3 was added to provide non-limiting conditions for denitrifying bacteria. Rates estimated from trials with both excess carbon substrate (glucose) and KNO_3 were not different than those with KNO_3 alone, therefore, results shown are from samples with KNO_3 only. Chloramphenicol stops *de novo* synthesis of denitrification enzymes, yielding linear production of N_2O over a short incubation time ($\sim$4 h) and closely approximates field rates (Murray and Knowles 1999; Bernot et al. 2003). Jars were sealed, purged with helium (He) to create anoxic conditions, and acetylene was added at 10 % of the headspace volume. Acetylene inhibits the conversion of N_2O to N_2 by nitrous oxide reductase and denitrification potential can then be calculated as N_2O production over time. Thus, we did not distinguish between the production of N_2 and N_2O by denitrification using this approach, but rather considered the total pool of denitrified N. We collected gas samples at 30, 90, 150, 210 and 270 min after acetylene addition and replaced withdrawn headspace volume with a mix of 90:10 He to acetylene. Samples were analyzed for N_2O within 1 month of collection on a Shimadzu MiniGC equipped with an ECD detector using a Poropak Q stainless steel column (Supelco Analytical, Bellefonte, Pennsylvania, USA).

Wetland mesocosm experiment

In order to control for *Typha* density, water depth, and stand age, which varied in the field, we conducted a mesocosm experiment to determine the effects of *Typha* and water level on DNP, plant communities, and environmental conditions 1- and 5-years after *Typha* establishment. We utilized 20, 2 $\times$ 1 m wetland mesocosms at the University of Michigan Biological Station (UMBS) in Pellston, Michigan, for this experiment. Mesocosms initially contained identical sediments. In 2003, the 11 most dominant native plant species in Cheboygan Marsh were transplanted into the mesocosms at field densities, and in 2004, 16 *Typha* plants (12-cm long rhizomes with 20-cm of senesced stem attached) were transplanted from the marsh into 10 mesocosms. Water levels were maintained at 10-cm above sediment surface (high) in 10 mesocosms and at 2-cm below sediment surface (low) in 10 mesocosms. A fully-crossed 2 $\times$ 2 factorial design was used with fivefold replication of all water level (high/low) and *Typha* (presence/absence) treatment combinations (see Larkin et al. 2012a). We surveyed plant community composition in August 2005 and 2009 in all mesocosms. The number, height, and species of all stems were measured, and heights were converted to biomass using mesocosm-specific biomass-to-height regressions for each species. Using biomass measurements, we calculated H' for each mesocosm. In August 2005 and August 2009, we measured sediment extractable inorganic nitrogen (NO_3^- and NH_4^+) and SOM. We measured DNP in July 2005 and August 2009. Measurement of nutrients and DNP was performed using the same methods described in the field study.

Data analyses

Multiple samples collected from the same site may not be statistically independent due to spatial autocorrelation. Thus, we tested for the independence of each variable $\times$ stand combination for all measures using the Moran's I test for spatial autocorrelation (Bivand et al. 2008). In cases where spatial autocorrelation was detected ($\alpha \leq 0.10$), we avoided pseudoreplication by using the variable mean by vegetation zone rather than data from each individual plot. We used linear regression analyses to evaluate correlations between spatially independent environmental variables and DNP and correlations between *Typha* density (stems m^{-2}) and measured environmental variables.

We evaluated differences among 2005 plant community types (native, transition, *Typha*) and among 2009 plant communities (OW, OD, M, and Y) using permutational multivariate analysis of variance (PERMANOVA; Anderson 2001). We used nonmetric multidimensional scaling (NMDS) ordination to characterize differences in plant communities and associated environmental variables. NMDS is a multivariate ordination method appropriate for both linear and non-linear responses and robust for non-normal data (McCune and Grace 2002). Each plot was

considered an individual community and plant data were transformed using Wisconsin double standardization to improve ordination quality (Oksanen 2006). Dissimilarity was based on Bray-Curtis distances, plots were constructed using two dimensions, and the following environmental variables were tested for significance as vectors by permutation procedure (10,000 replicate permutations): DNP (μg N_2O m^{-2} h^{-1}), water depth (cm), sediment NH_4^+ (μg N/g dry soil), sediment NO_3^- (μg N/g dry soil), SOM (%), organic depth (2009), sediment bulk density (2005), sediment pH (2005), litter (mass, 2005; depth 2009), *Typha* density (stems m^{-2}), H′, species richness, stem density (stems m^{-2}), redox potential (mV), and stand age (years).

Using mesocosm data from 2005 and 2009, we tested whether the presence of *Typha*, water level (high or low), and year (2005 or 2009) affected DNP, SOM, NH_4^+, total plant biomass, and non-*Typha* plant biomass using a two-factor repeated measures ANOVA with interaction terms; the between subject terms were *Typha* presence and water level, and the within subject factor was year. Additionally, we conducted PERMANOVA to determine differences between treatment plant communities. We conducted NMDS analyses with both years of mesocosm data to evaluate plant community response to treatments, correlation with environmental factors, and change between study years. Each mesocosm was considered to be a unique plant community and the following environmental variables were tested by permutation procedure as vectors: DNP (μg N_2O m^{-2} h^{-1}), SOM (%), sediment NH_4^+ (μg N/g dry soil), sediment NO_3^- (μg N/g dry soil), *Typha* density (stems m^{-2}), *Typha* biomass (g m^{-2}), native species biomass (g m^{-2}), and total biomass (g m^{-2}).

All statistical analyses were conducted using R 2.12.1 (R Development Core Team 2009) with the vegan and ape packages used for NMDS and Moran's I tests, respectively (Paradis et al. 2004; Oksanen et al. 2012).

Results

Field study

Patterns in plant communities, physical and chemical characteristics, and denitrification rates

In Cheboygan Marsh, 2005 and 2009 plant communities differed among vegetation zones (2005: $df = 2,9$, Pseudo $F = 5.812$, $P < 0.05$; 2009: $df = 3,16$, Pseudo $F = 31.133$, $P < 0.01$). *Typha* density varied between the Native ($X \pm$ SE; stems m^{-2}) (1.5 ± 1.5), Transition (15.5 ± 1.0), *Typha* (26.5 ± 4.3), Young (14.6 ± 3.3), Middle (29.8 ± 2.9), Old-wet (32.4 ± 2.2), and Old-dry (31.4 ± 2.8) vegetation zones. Plots located in *Typha* stands varied in age from 3 to >46 years. Denitrification rates also varied by vegetation zone (Fig. 2) and correlated significantly with spatially independent water depth, NH_4^+, stand age, SOM, and *Typha* density (Fig. 3). Several measured plant community variables, physical, and chemical conditions co-varied with *Typha* density (Table 1). Spatially independent field water depths were negatively correlated with SOM ($P < 0.001$; $R^2 = 0.40$) and NH_4^+ ($P < 0.01$; $R^2 = 0.30$), but there was no significant correlation with *Typha* stand age ($P = 0.14$), NO_3 ($P = 0.78$) or redox potential ($P = 0.24$).

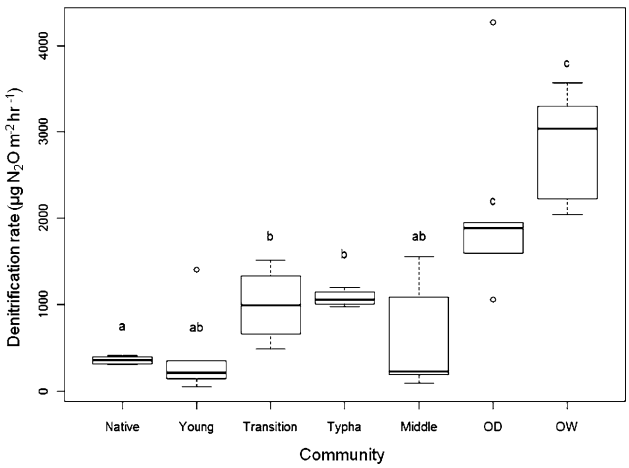

Fig. 2 Denitrification potential values from each plant community measured in 2005 (Native, Transition, and *Typha*) and 2009 (Young, Middle, Old-dry [OD], and Old-wet [OW]). Communities presented from left-right along a gradient of increasing *Typha* density (stems m^{-2}). Significant differences between communities, determined by ANOVA with Tukey *post-hoc* tests, represented by non-overlapping letters

The NMDS ordination of 2005 field plots highlighted community differences between the three sampled zones and zone × environmental variable associations. The *Typha* zone plots were tightly clustered and correlated positively with the significant vectors *Typha* density, SOM, NH_4^+, redox, age, DNP, litter mass, and NO_3^-, orthogonal to the H′ vector and negatively associated with pH, water depth, and sediment bulk density. The Native zone plots were also tightly clustered and positively correlated with pH, water depth, and sediment bulk density vectors. Transition zone plots were more widely dispersed and were intermediate between the *Typha* and Native zones in both species space and environmental factor association (Fig. 4a). The NMDS ordination of 2009 field plots revealed community differences between the four sampled portions of the *Typha* stand and zone × environmental variable associations. The Old-wet (OW) plots were tightly clustered and overlapping, showed the strongest positive association with the significant DNP vector, and related positively with stand-age, redox, *Typha* density, SOM,

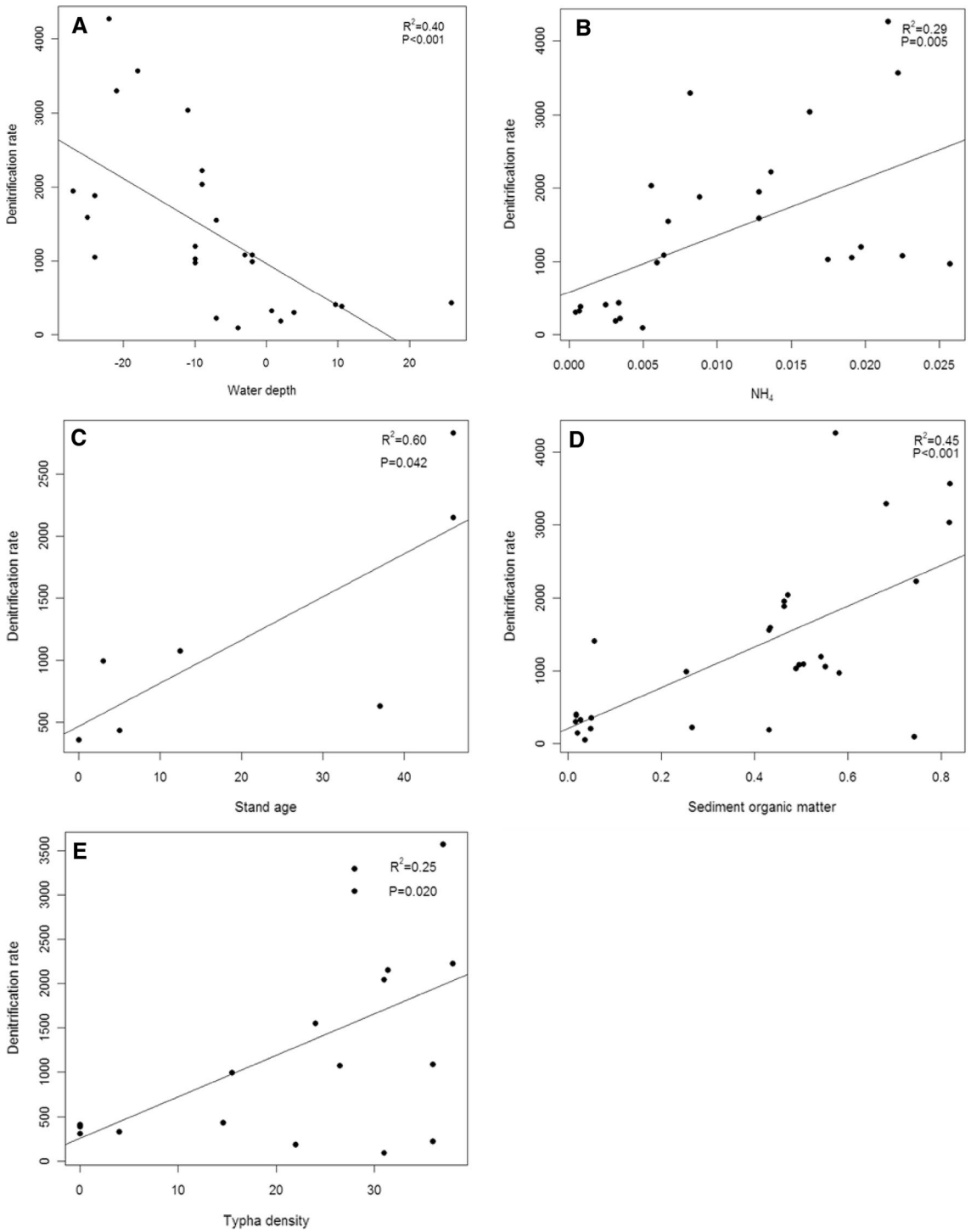

Fig. 3 Correlations between denitrification rates (μg N_2O m^{-2} h^{-1}) and water depth (cm) (**a**), NH_4^+ (μg/g dry soil (**b**), Stand age (years) (**c**), Sediment organic matter (proportion) (**d**), and *Typha* density (stems m^{-2}) (**e**) from spatially independent data points (determined with Moran's I test for spatial autocorrelation)

NH_4^+, and litter depth, were orthogonally-related to organic depth and stem density, and negatively related with H′, water depth, and species richness. Old-dry (OD) plots were proximal to the OW plots in species space, with related but somewhat weaker correspondence with the same environmental vectors. The Middle (M) plots were tightly clustered and related positively with organic depth and stem density, weakly with H′ and orthogonally related to all other variables. The Young (Y) plots were clearly clustered, most strongly related to water depth and species

Table 1 Correlations between *Typha* density and measured environmental variables of spatially independent field data from 2005 and 2009

Characteristic	*Typha* density (stems m^{-2})				
	N	F	R^2	*P*	Relationship
Denitrification rate ($\mu g\ N_2O\ m^{-2}\ h^{-1}$)	18	6.72	0.25	0.02	+
Stand age (years)	8	43.28	0.86	<0.001	+
Shannon–Weiner diversity	21	10.68	0.33	0.004	−
Species richness	21	89.76	0.82	<0.001	−
Total stem density	14	25.70	0.66	<0.001	−
NO_3^- (mg N/g dry soil)	14	1.39	0.03	0.26	
NH_4^+ (mg N/g dry soil)	21	6.01	0.20	0.02	+
SOM (% of dry mass)	18	29.81	0.63	<0.001	+
Water depth (cm)	21	10.91	0.33	0.004	−
Redox potential (mV)	21	7.14	0.23	0.02	+

richness, and were inversely related to stand age, redox, *Typha* density, SOM, NH_4^+, litter depth, and DNP (Fig. 4b).

Mesocosm experiment

Repeated measures ANOVA revealed that environmental variables varied by year in the mesocosms; DNP and SOM were greater in 2009 than in 2005, while NH_4^+ was lower in 2009 (Table 2). In 2005, DNP was significantly greater in low-water treatments than in high-water treatments, with the *Typha*-low water treatment exhibiting the greatest DNP (Table 3; $P < 0.001$). But in 2009, due in part to high variability among replicates, DNP was not significantly affected by *Typha*, water level, or *Typha* × water level treatments (Tables 2, 3). Similarly SOM was greater in 2009 than 2005, but there were no *Typha* or water level effects. In contrast, NH_4^+ was lower in 2009 than in 2005 and exhibited significant *Typha*, and year × *Typha* effects, but no water level effects; in 2005, *Typha* mesocosms had significantly greater NH_4^+ than native mesocosms, but in 2009 there was no difference in NH_4^+ concentrations. Several measures of mesocosm plant communities differed significantly between years and exhibited treatment effects. *Typha* biomass was greater in 2009 than in 2005 and was greater in the high water mesocosms in both years, while native biomass was lower in 2009, and showed a *Typha* treatment and year × *Typha* effect. H′ and species richness were lower in 2009 than 2005 but exhibited no other treatment effects. Total biomass was affected by *Typha* treatment, but did not show a year effect (Tables 2, 3).

Multivariate plant assemblages varied by year, and *Typha* and water treatments ($df = 7.32$; Pseudo $F = 5.812$; $P = 0.01$). NMDS ordination revealed community divergence in the +*Typha* treatment between 2005 and 2009 and

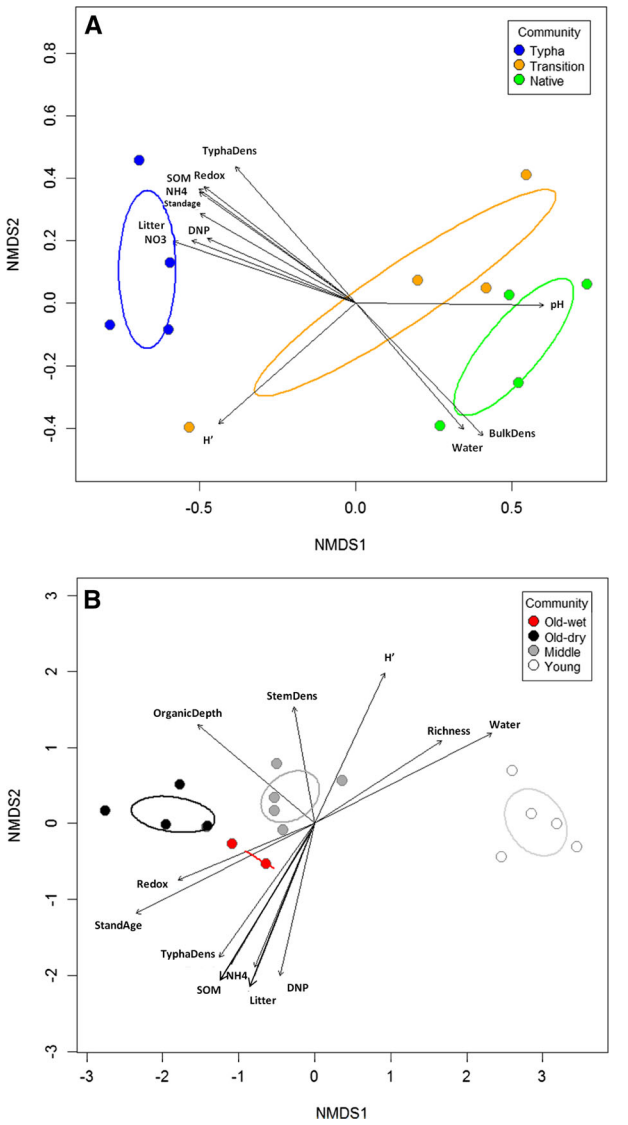

Fig. 4 Non-metric multidimensional scaling ordination of field plant community data from 2005 (**a**) and 2009 (**b**). *Fitted vector arrows* are significant ($P < 0.05$) with their length proportional to explanatory strength. Explanatory variables tested in 2005 (**a**) were: DN (denitrification potential; $R^2 = 0.57$; $P = 0.02$); water depth ($R^2 = 0.63$; $P = 0.01$); Litter mass (Litter; $R^2 = 0.69$; $P < 0.01$), NO_3^-($R^2 = 0.88$; $P < 0.001$); NH_4^+ ($R^2 = 0.88$; $P < 0.001$); SOM (Sediment organic matter; $R^2 = 0.84$; $P < 0.001$); TyphaDens (*Typha* stems m^{-2}; $R^2 = 0.73$; $P < 0.01$); H′ (Shannon-diversity; $R^2 = 0.73$; $P < 0.001$); Richness (Plant species richness; $R^2 = 0.21$; $P = 0.35$); Stems (Total plant stems m^{-2}; $R^2 = 0.31$; $P = 0.18$); Redox (mV; $R^2 = 0.86$; $P < 0.001$); pH ($R^2 = 0.82$; $P < 0.001$); Age (Years since *Typha* establishment; $R^2 = 0.71$; $P < 0.01$). Explanatory variables tested in 2009 (B) were: DN (Denitrification potential; $R^2 = 0.44$; $P < 0.01$); water depth ($R^2 = 0.92$; $P < 0.001$); Litter depth (Litter; $R^2 = 0.73$; $P < 0.001$), NO_3^- ($R^2 = 0.00$; $P = 0.99$); NH_4^+ ($R^2 = 0.51$; $P < 0.01$); SOM (Sediment organic matter; $R^2 = 0.65$; $P < 0.001$); TyphaDens (*Typha* stems m^{-2}; $R^2 = 0.59$; $P < 0.001$); H′ (Shannon-diversity; $R^2 = 0.62$; $P < 0.001$); Richness (Plant species richness; $R^2 = 0.52$; $P < 0.01$); Stems (Total plant stems m^{-2}; $R^2 = 0.44$; $P < 0.01$); Redox (mV; $R^2 = 0.52$; $P < 0.01$); Organic depth ($R^2 = 0.56$; $P < 0.001$); Age (Years since *Typha* establishment; $R^2 = 0.92$; $P < 0.001$)

clear correlations between measured environmental variables and year × *Typha* treatments. Native and *Typha* mesocosm treatments overlapped substantially in 2005, whereas in 2009, the two treatments were clearly separated in species space. The 2009 *Typha* mesocosms correlated with significant SOM ($P < 0.05$) and with weakly significant DNP vectors ($P = 0.08$), and correlated well with total biomass ($P < 0.01$), *Typha* biomass ($P < 0.001$), and *Typha* density ($P < 0.01$) environmental vectors (Fig. 5).

Discussion

This study was designed to examine patterns of DNP in a Great Lakes coastal wetland invaded by *Typha* and to determine relationships between *Typha* dominance, physical ecosystem factors, and DNP over the course of >46 years following *Typha* establishment. We were also able to evaluate the short-term impacts of *Typha* invasion on environmental factors, plant communities, and DNP by conducting a controlled mesocosm study that compared a native community and a *Typha* invaded community at 1 and 5 years after *Typha* introduction. We found that *Typha* and its residence time had significant negative impacts on biodiversity of plant communities in both the field and mesocosm studies, but measured concurrent increases in DNP and organic matter accumulation as *Typha* dominance increased. Thus, the capacity of this wetland to remove N from the watershed and to store C from the atmosphere increased as the diversity of its plant community decreased. Further, we found that the effects of this invasive species on ecosystem characteristics were not immediate but emerged over time. Our findings deepen our understanding of the interaction between invasion, invasive species residence time, environmental change, and biogeochemical processes and shed light on the importance of considering tradeoffs in how ecosystem changes, such as species invasions, influence the provision of multiple ecosystem services.

How are denitrification rates, plant communities, and associated environmental characteristics impacted by *Typha* invasion?

We expected to find higher DNP in locations with greater sediment C and N, the key substrates involved in denitrification; typically denitrification rates are positively correlated with increasing availability of each (Pfenning and McMahon 1997; Strong and Fillery 2002; Hernandez and Mitsch 2007). Further, we predicted finding greater C and N with increased *Typha* dominance (Farrer and Goldberg 2009; Tuchman et al. 2009; Lishawa et al. 2010). Our findings support these hypotheses; DNP in the field

Table 2 Results from repeated measures ANOVA model testing for effects of sampling year, presence of *Typha* (present, absent), and water level (high, low) on DNP, Total biomass, *Typha* biomass, Native biomass, NH_4^+, SOM, H′, and Richness in 2005 and 2009

Response variable	Source	*df*	*MS*	F	*P*
DNP (μg N_2O m^{-2} h^{-1})	*Typha*	1	483,634	2.48	0.14
	Water	1	67,777	0.35	0.56
	Typha × water	1	143,180	0.74	0.40
	Error[a]	15	194,605		
	Year	1	1,776,481	6.93	**0.02**
	Year × *Typha*	1	141,550	0.55	0.47
	Year × Water	1	422,709	1.65	0.22
	Error[b]	15	256,187		
Total biomass (g m^{-2})	*Typha*	1	234,587	7.08	**0.02**
	Water	1	69,428	2.10	0.17
	Typha × water	1	20,277	0.61	0.45
	Error[a]	16	33,132		
	Year	1	94,923	3.53	0.08
	Year × *Typha*	1	102,628	3.81	0.07
	Year × Water	1	10,555	0.39	0.54
	Error[b]	16	430,853		
Typha biomass (g m^{-2})	*Typha*	1	811,196	103.96	**<0.001**
	Water	1	48,456	6.21	**0.02**
	Typha × water	1	48,456	6.21	**0.02**
	Error[a]	16	7,803		
	Year	1	478,827	61.08	**<0.001**
	Year × *Typha*	1	478,827	61.08	**<0.001**
	Year × Water	1	32,961	4.20	0.06
	Error[b]	16	7,839		
Native biomass (g m^{-2})	*Typha*	1	173,324	7.63	**0.01**
	Water	1	2,880	0.08	0.78
	Typha × water	1	6,042	0.27	0.61
	Error[a]	16	22,721		
	Year	1	147,362	9.07	**<0.01**
	Year × *Typha*	1	138,099	8.50	**0.01**
	Year × Water	1	6,211	0.38	0.55
	Error[b]	16	16,256		
NH_4^+ (μg N/g dry soil)	*Typha*	1	27.00	54.32	**<0.001**
	Water	1	0.02	0.03	0.86
	Typha × water	1	0.27	0.54	0.47
	Error[a]	16	0.50		
	Year	1	9.66	9.41	**<0.01**
	Year × *Typha*	1	15.80	15.39	**<0.01**
	Year × water	1	0.81	0.79	0.39
	Error[b]	16	1.03		
SOM (% of dry mass)	*Typha*	1	<0.01	0.23	0.64
	Water	1	<0.01	0.69	0.42
	Typha × water	1	<0.01	0.18	0.67
	Error[a]	16	<0.01		
	Year	1	<0.01	15.42	**<0.01**
	Year × *Typha*	1	<0.01	0.06	0.81
	Year × water	1	<0.01	2.60	0.13
	Error[b]	16	<0.01		

Table 2 continued

Response variable	Source	*df*	*MS*	F	*P*
H′	*Typha*	1	0.28	0.61	0.45
	Water	1	0.01	0.12	0.73
	Typha × water	1	<0.001	<0.01	0.97
	Error[a]	16	0.046		
	Year	1	5.28	15.42	**<0.001**
	Year × *Typha*	1	0.12	0.06	0.11
	Year × water	1	<0.001	2.60	0.92
	Error[b]	16	0.04		
Richness	*Typha*	1	0.03	0.61	0.64
	Water	1	0.01	0.12	0.42
	Typha × water	1	<0.001	<0.01	0.67
	Error[a]	16	0.05		
	Year	1	5.28	122.70	**<0.001**
	Year × *Typha*	1	0.12	2.79	0.11
	Year × water	1	<0.001	0.01	0.92
	Error[b]	16	0.04		

Significance ($P < 0.05$) indicated in bold

[a] Between-subject error

[b] Within-subject error

was consistently higher where *Typha* was present and higher yet in the oldest stands of *Typha*; DNP in the *Typha*-dominated wetland was nearly three times higher than in the wetland dominated by native species, and nine times higher in the oldest *Typha* stands than in the native zone. These findings are largely consistent with studies in constructed wetlands, which have demonstrated that *Typha* can facilitate N removal more effectively than other wetland species (Bachand and Horne 1999). It is important, however, to consider the limitations of the acetylene inhibition methodological approach. First, DNP is only a measure of the potential for denitrification to occur given the optimum environmental conditions (i.e., excess availability of substrate, anoxic conditions), thus cannot be used as a direct estimate of how much denitrification is actually occurring in the field at a given time. Rather, it is an approximation of the potential in the microbial community present in a given sample to denitrify and gives an upper estimate of how much could occur. Thus, it is an especially useful technique in comparing long-term denitrification potential across environmentally distinct habitats such as between the *Typha* and native zones present in Cheboygan marsh. Second, as with other approaches that measure denitrification rates in the lab such as the *membrane inlet mass-spectrometer* method (Kana et al. 1994), measuring DNP involves disturbing natural conditions such as sediment temperature and moisture. However, the purpose of DNP is to give an estimate of possible denitrification rates under optimal conditions, thus these concerns are more minor for this approach than other laboratory approaches that aim to give actual field rates.

Typha stem density correlated with DNP (+), NH_4^+ (+), and SOM (+), as well as a suite of measured variables including plant diversity (−), water depth (−), and redox potential (+) (Table 1). No direct relationship between

Table 3 Mean environmental and plant community characteristics by experimental treatments (±*Typha*; ±High water) and sampling year (2005; 2009); significant differences ($P < 0.05$) within years and between treatments determined by ANOVA with Tukey *post-hoc* tests, and represented by non-overlapping superscript letters (2005, lowercase; 2009, uppercase)

		Means ± SE			
		Native-high	Native-low	*Typha*-high	*Typha*-low
DNP (μg N_2O m^{-2} h^{-1})	2005	188.4 ± 12.0^{a}	345.6 ± 54.3^{b}	152.1 ± 22.2^{a}	572.0 ± 28.2^{c}
	2009	440.5 ± 126.7^{A}	677.1 ± 394.8^{A}	1,151.6 ± 302.6^{A}	668.9 ± 269.8^{A}
Total biomass (g m^{-2})	2005	541.3 ± 98.7^{a}	460.7 ± 31.1^{a}	563.4 ± 14.6^{a}	542.3 ± 59.4^{a}
	2009	495.1 ± 76.0^{A}	499.1 ± 83.8^{A}	869.4 ± 105.3^{B}	633.7 ± 98.6^{AB}
Typha density (stems m^{-2})	2005	–	–	17.6 ± 1.5^{a}	19.8 ± 2.2^{a}
	2009	–	–	15.1 ± 2.1^{A}	16.1 ± 3.4^{A}
Typha biomass (g m^{-2})	2005	–	–	78.2 ± 6.0^{a}	53.8 ± 6.75^{b}
	2009	–	–	630.7 ± 90.6^{A}	376.6 ± 65.0^{B}
Native biomass (g m^{-2})	2005	541.3 ± 98.7^{a}	460.7 ± 31.1^{a}	485.2 ± 19.5^{a}	488.5 ± 60.0^{a}
	2009	495.1 ± 76.0^{A}	499.1 ± 83.8^{A}	238.7 ± 44.8^{B}	257.1 ± 41.2^{AB}
NH_4^+ (μg N/g dry soil)	2005	0.63 ± 0.07^{a}	0.56 ± 0.08^{a}	3.22 ± 0.52^{b}	3.78 ± 0.89^{b}
	2009	1.04 ± 0.12^{A}	0.70 ± 0.07^{A}	1.42 ± 0.25^{A}	1.10 ± 0.25^{A}
SOM (% of dry mass)	2005	3.20 ± 0.48^{a}	2.50 ± 0.16^{a}	3.04 ± 0.61^{a}	2.98 ± 0.48^{a}
	2009	3.77 ± 0.41^{A}	6.04 ± 2.04^{A}	5.07 ± 0.61^{A}	5.61 ± 0.27^{A}
Redox potential (mV)	2005				
	2009	−389.0 ± 4.3^{A}	191.9 ± 39.7^{B}	−383.9 ± 5.9^{A}	−26.7 ± 102.1^{A}

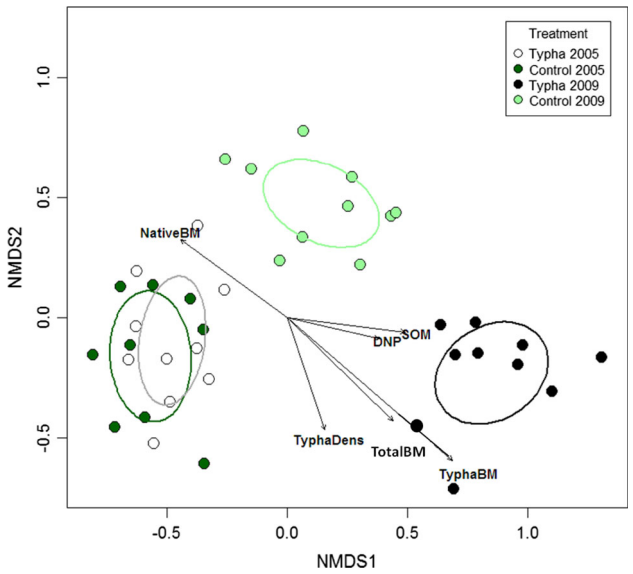

Fig. 5 Non-metric multidimensional scaling ordination of mesocosm plant community data from 2005 and 2009. Dissimilarity was based on Bray-Curtis distances and plots were constructed using two dimensions. Fitted vector arrows are significant ($P < 0.1$, by permutation procedure) and their length is proportional to their explanatory strength. Explanatory variables tested were: TyphaDens (*Typha* stems m^{-2}; $R^2 = 0.18$; $P < 0.05$); DNP (Denitrification potential; $R^2 = 0.13$; $P = 0.08$); NH_4^+ ($R^2 = 0.08$; $P = 0.20$); SOM (Sediment organic matter; $R^2 = 0.20$; $P < 0.01$); TotalBM (Total biomass; $R^2 = 0.30$; $P < 0.01$); *Typha* biomass ($R^2 = 0.69$; $P < 0.001$); Native biomass ($R^2 = 0.27$; $P < 0.01$)

NO_3^- and *Typha* density was observed in our study, however. This is not surprising given that in wetland environments NO_3^- concentrations are typically very low due to the anoxic conditions and low rates of nitrification (Keddy 2000) and available NO_3^- was likely rapidly denitrified leaving a small residual pool. Thus, NH_4^+ is an appropriate proxy for NO_3^- due to the potential for greater nitrification where NH_4^+ is abundant. Furthermore, while sediment NH_4^+ increased with *Typha* density in the field and was greater in the +*Typha* mesocosms in the first year of the study, sediment NH_4^+ levels in the +*Typha* mesocosms decreased from 2005 to 2009; in 2009 the NH_4^+ remaining was less than half that measured in 2005 and there was no difference between ±*Typha* treatments. These findings indicate that in the closed mesocosm systems, sediment N was likely lost to the atmosphere through denitrification.

Typha typically produces more biomass than the native plant species it displaces (Woo and Zedler 2002), leading to litter accumulation in the environment (Tuchman et al. 2009; Lishawa et al. 2010). Over time, litter decomposition will likely result in marsh sediment organic matter accumulation (Mitchell et al. 2011; Lishawa et al. 2013), thereby increasing labile carbon accessible to denitrifying microbes. We are confident that *Typha* is largely responsible for the deep organic sediment horizon present in Cheboygan Marsh due to a clear correlation between increasing *Typha* dominance and sediment organic matter content between 1945 and present, determined by aerial photography and sediment-core dating techniques (Lishawa et al. 2013).

Observed patterns of environmental associations with *Typha* dominance are also consistent with data from a survey of 14 upper Great Lakes coastal wetlands (Lishawa et al. 2010), which also showed positive correlations with SOM, and NH_4^+, and a negative correlations with water depth, and plant diversity measures and are largely consistent with observations in northern Illinois coastal wetlands (Mitchell et al. 2011), which showed increasing SOM and decreasing plant diversity. Ordinations from both 2005 and 2009 field data further demonstrated co-variation between DNP and these environmental and plant community characteristics (Fig. 4). However, we cannot assume a causative relationship between *Typha* density and DNP due to consistent co-variation with a suite of measured characteristics. Thus, the environmental factors most commonly associated with *Typha* dominance also correspond strongly with increased DNP and these changes, specifically increased carbon accumulation, most likely result from invasive *Typha*. Furthermore, we found an unexpected negative correlation between DNP and water level that contrasts with studies finding increased denitrification with deeper water (Song et al. 2012). The oldest, most dominant stands of *Typha* in Cheboygan Marsh tend to be at the wetland's upland edge (Lishawa et al. 2013) and thus, the suite of environmental variables that correlated with *Typha* dominance appeared to have stronger influence than water level variation on DNP.

How does *Typha* stand-age influence these factors?

Because invasive species impacts tend to vary temporally (Witkowski and Wilson 2001; Marchante et al. 2008), the importance of evaluating the ecological effects of invasive species over long time-scales is increasingly recognized (Strayer et al. 2006). In this study, we found that ecologically important plant community, environmental, and biogeochemical factors varied with *Typha* stand age; with increased age we found significant negative correlations with H′ and species richness and significant positive correlations with *Typha* density, sediment organic matter, and DNP. These findings echo Mitchell et al. (2011) who found a pattern of increasing *Typha* density and SOM and decreasing plant H′ with increasing stand age in southern Lake Michigan coastal wetlands. Furthermore, the effects of stand age on other measured variables were likely muted by low sample size in our analyses because the temporal resolution of *Typha* stand ages were limited by the availability of historical aerial photos (Lishawa et al. 2013); plot

stand-ages were not all spatially independent and data were aggregated for analyses, resulting in a low sample size (6–8) for age ~environmental comparisons.

Over a short time period, however, ecosystem changes resulting from *Typha* invasion were less apparent. Our mesocosm experiment controlled for water level, plant community, and date of *Typha* invasion. In the first year of the study, we documented a water level effect on DNP, but no differences between native and *Typha* treatments in plant communities or DNP (Fig. 5); 1 year appears to have been insufficient time for *Typha* to impact plant communities, sediment physical and microbial properties. By 2009, however, there was a clear plant community shift illustrated in the separation of *Typha* and native treatments in NMDS analyses (Fig. 5). We also documented a weak trend towards higher SOM and DNP in *Typha* compared to native treatments, providing measured support to our field findings. Thus, following invasion we found different time lags between *Typha* invasion and changes in ecosystem characteristics: *Typha* rapidly alters plant communities and more slowly impacts sediment chemistry and concomitant biogeochemical functions. As in the field, it appears that stand age may be driving changes in *Typha* density, overall biomass, and SOM accumulation, but 5-years following invasion may be an insufficient time period for broad changes in biogeochemical cycling to occur. Therefore, assessing how invasions affect these ecosystem characteristics depends on the point at which measurements occur.

Conclusions

The effects of invasive species on wetland ecosystems include reducing biodiversity and altering ecosystem function (Galatowitsch et al. 1999; Zedler and Kercher 2004). Compared with other ecosystem services, the impacts of invasive species on biodiversity have been particularly well documented. Influence on other ecosystem services, such as pollution abatement and carbon sequestration, have been less clearly demonstrated, partly resulting from poor spatial–temporal resolution of plant invasions. Our results suggest that documenting changes to biogeochemical properties of ecosystems requires longer time periods than the impacts on biodiversity. As Mitchell et al. (2011) and this study document, plant diversity and richness declined rapidly along a timeline of invasion whereas impacts on the ecological functions of carbon sequestration and DNP were just beginning to emerge after 5 years in a controlled invasion and were most apparent in stands invaded more than 46 years. In other words, we found that with increasing invasive species residence time, one ecosystem service, biodiversity, was negatively impacted, while two other ecosystem services, DNP and sediment carbon accumulation, were significantly enhanced. Thus in certain contexts, such as highly degraded and highly invaded ecosystems with high nutrient loads and low biodiversity, *Typha* dominated wetlands still can provide valuable services to aquatic ecosystems and to society. Further investigation into these relationships are warranted, however, because DNP is not a direct measure of denitrification rates, but is rather a measure of potential for denitrification to occur, and secondly because acetylene inhibition method for determining DNP in this study does not allow for differentiation between inert N_2 and the highly potent greenhouse gas N_2O (Yoshinari and Knowles 1976; Groffman et al. 1999; Jain et al. 2000). These results should be valuable to aquatic science and management decision making. A long-term, objective, and holistic perspective is critical when evaluating invasive species impacts to aquatic and wetland ecosystems in which *negative* ecological impacts should be measured against other ecosystem services, which may be stimulated by invasion.

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