Effects of an invasive cattail species (*Typha* × *glauca*) on sediment nitrogen and microbial community composition in a freshwater wetland

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Abstract

Sediments from Cheboygan Marsh, a coastal freshwater wetland on Lake Huron that has been invaded by an emergent exotic plant, *Typha* × *glauca*, were examined to assess the effects of invasion on wetland nutrient levels and sediment microbial communities. Comparison of invaded and uninvaded zones of the marsh indicated that the invaded zone showed significantly lower plant diversity, as well as significantly higher aboveground plant biomass and soil organic matter. The sediments in the invaded zone also showed dramatically higher concentrations of soluble nutrients, including greater than 10-fold higher soluble ammonium, nitrate, and phosphate, which suggests that *Typha* × *glauca* invasion may be impacting the wetland's ability to remove nutrients. Terminal restriction fragment length polymorphism analyses revealed significant differences in the composition of total bacterial communities (based on 16S-rRNA genes) and denitrifier communities (based on *nirS* genes) between invaded and uninvaded zones. This shift in denitrifiers in the sediments may be ecologically significant due to the critical role that denitrifying bacteria play in removal of nitrogen by wetlands.

Introduction

Wetlands conduct critical ecosystem functions, including providing habitats for plants and wildlife, storing storm waters (Mitsch & Gosselink, 2000), and serving as sinks for terrestrially derived nutrients such as carbon and nitrogen, thus preventing the release of nutrients into adjacent surface waters, which could lead to eutrophication (Vitousek *et al*., 1996). Wetland nutrient uptake is driven by the activities of wetland plants and microbial communities (Mitsch & Gosselink, 2000), and denitrifying bacteria play an especially significant role in the nutrient removal function of wetlands due to their ability to convert nitrate to gaseous N_2 (Otto *et al*., 1999).

Throughout the United States, many ecosystems, including wetlands, are threatened by invasive species (Galatowitsch *et al*., 1999; Zedler & Kercher, 2004). In the Great Lakes region, over 162 exotic plant and animal species have become established to date, one-third of which were introduced within the last 30 years (Mills *et al*., 1993; Ricciardi 2001). Invasive plants can significantly reduce the diversity of native plant and animal communities by outcompeting native species (Detenbeck *et al*., 1999; Werner & Zedler, 2002; Zedler & Kercher, 2004), and they can also alter cycling of carbon and nitrogen (for a review, see Ehrenfeld, 2003).

Invasive plants can impact carbon and nitrogen cycles directly, as they typically show increases in net primary productivity and standing stock biomass compared with native plants (Ehrenfeld, 2003). Invasive plants can also impact nutrient cycling indirectly through their influence on microorganisms. Recent studies have shown that microbially driven nitrogen cycling processes in terrestrial ecosystems (Kourtev *et al*., 2003) and freshwater wetlands (Windham & Ehrenfeld, 2003) can be impacted by invasive plants. In addition, recent studies have demonstrated that invasive plants can alter microbial community structure in terrestrial soils (Kourtev *et al*., 2002, 2003; Duda *et al*., 2003) and brackish marsh sediments (Ravit *et al*., 2003). Such changes in microbial community structure may be significant, as Callaway *et al*. (2004) recently demonstrated that the invasive plant *Centaurea maculosa* cultivates a soil microbial community that aids its growth and thus may contribute to its invasive success. However, no studies to date have examined impacts of invasive plants on the composition of microbial communities in freshwater wetlands, and no studies have focused on denitrifiers.

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The objective of this study was to determine whether invasion of a freshwater wetland by an aggressive, exotic plant species would alter sediment nutrient content and microbial community composition. Sediment physical and chemical properties were analyzed in *Typha* × *glauca*-dominated and uninvaded zones of a coastal freshwater marsh, and terminal restriction fragment length polymorphism (T-RFLP) analysis was used to examine total sediment bacterial communities using 16S rRNA genes and to examine specifically denitrifier communities using the functional genes *nirS* and *nirK*, which code for two variants of nitrite reductase, a key enzyme in the denitrification pathway (Braker *et al*., 1998). The study focused on denitrifiers due to the significant role they play in wetland function, and *nirS* and *nirK* because they are effective targets for assessing denitrifier community composition via T-RFLP (Braker *et al*., 2001; Avrahami *et al*., 2002; Wolsing & Priemé, 2004).

Materials and methods

Study site

Cheboygan Marsh is a freshwater wetland located in Michigan on the northwestern shore of Lake Huron. The marsh covers *c*. 150 ha and experiences daily seiche activity, with approximately one-third of the marsh being continuously inundated. The native plant community of this marsh is a mixture of sedges, rushes, and bulrushes (Fig. 1). *Typha* × *glauca* (hereafter referred to as *Typha*), a hybrid of a native cattail species, *Typha latifolia*, and an exotic, *Typha angustifolia*, invaded the marsh 30–40 years ago (F. Cuthbert, pers. commun.) and now forms a monoculture covering more than 60% of the marsh. There are currently three distinct vegetation zones in the marsh: a *Typha* zone, which is composed almost entirely of *Typha*; a transition zone, which includes *Typha* and native plant species; and a native zone, which contains a diverse native plant community and no *Typha*. The *Typha* 'front' has been advancing at a rate of 3–5 m per year. All sampling for this study was conducted between July 15 and September 15, 2004.

Field measurements

Three 0.5 m^2 plots were established in each of the three vegetation zones (total of nine plots). Stem counts and heights of all emergent plants were measured within each plot. The total biomass of each plant species was determined by converting stem height measurements to biomass using species-specific height–biomass regressions based on 50 specimens of each species. The biomass of each specimen was measured after drying at 60 °C. Plant diversity was calculated using the Shannon–Weiner index (Krebs, 1989). Within each plot, water depth was measured, water temperature at the sediment–water interface was determined with a Hydrolab Scout 2 (Hach Environmental, Loveland, CO), and pH was determined with an Accumet AP61 (Fisher Scientific, Pittsburgh, PA).

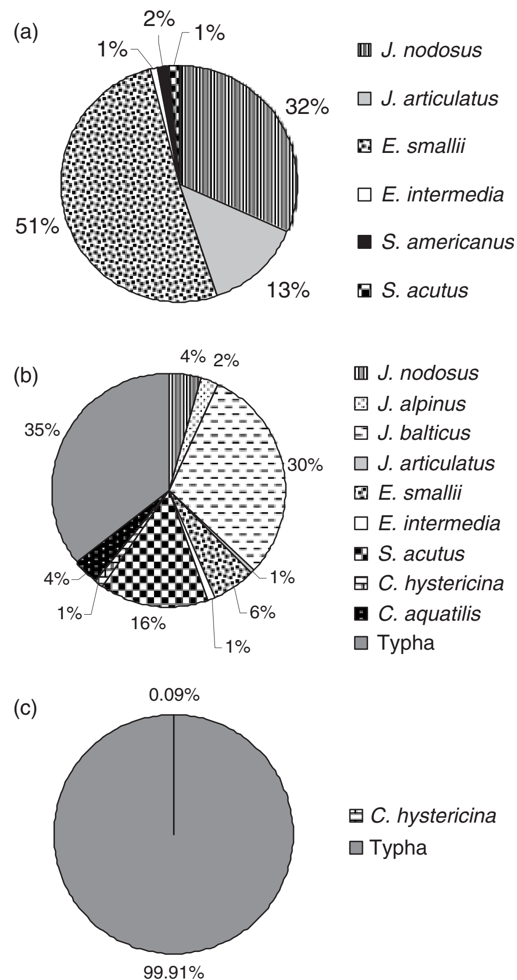

Fig. 1. Relative biomass of plant species in native zone (a), transition zone (b), and *Typha* zone (c). Genus names abbreviated as follows: J (*Juncus*), E (*Eleocharis*), S (*Schoenoplectus*), C (*Carex*).

Sediment physical and chemical analyses

Five replicate sediment samples were collected from each vegetation zone with a soil corer (4.7 cm diameter, 10 cm depth) (total of 15 cores). Cores were placed in ziploc bags and stored on ice for transport. Sediments were sieved using a 2-mm mesh sieve and stored at 4 °C. Physical and chemical analyses were completed within 24 h of sampling. Extraction for soluble NO_3^- and NH_4^+ was performed as follows: 10 g sediment was extracted with 40 mL 2 M KCl and centrifuged at 134 g for 5 min. The supernatant was filtered through G8 glass fiber filters (Fisher Scientific). Nitrate concentration in

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extracts was measured by the Automated Cadmium reduction method (APHA, 2005) on Auto-Analyzer 3 (Bran Luebbe, Farmington, MI), and ammonium concentration by the Automated Phenate method (APHA, 2005) on Auto-Analyzer 3. Extraction for soluble PO_4^{3-} was performed as described above but 2 M KCl was replaced with Truog's Extract (Mehlich, 1953). Phosphate concentration in extracts was determined by the automated Ascorbic Acid method (APHA, 2005) on Auto-Analyzer 3. Water content was determined by drying at 105 °C for 24 h and calculated as (wet weight − dry weight)/wet weight (Gardner, 1986). Organic matter content was determined by loss on ignition at 550 °C (Bear, 1955). Physical and chemical data were analyzed with ANOVA using SYSTAT version 11 (Systat Software Inc., Point Richmond, CA).

Microbial community analyses

Three replicate sediment samples were collected from each plot with a soil corer (4.7 cm diameter, 10 cm depth) (total of 27 cores, nine from each vegetation zone). Intact cores were placed in ziploc bags and stored on ice for transport. Within two hours of collecting, each individual core was homogenized and subsamples (0.5 g) from each were transferred to 2 mL microcentrifuge tubes and stored at −80 °C.

DNA was extracted from each sediment sample using the MoBio UltraClean Soil DNA Kit (MoBio Laboratories, Carlsbad, CA) and confirmed by agarose gel electrophoresis. 16S rRNA genes were amplified via PCR using bacterial domain primers 8F and 926R (Liu *et al*., 1997). 926R was obtained from Operon (Alameda, CA) and 8F (labeled at the 5′ end with IRD-800) from LI-COR Inc. (Lincoln, NE). PCR conditions and cycling parameters are described in Janus *et al*. (2005). Duplicate PCR reactions were run for each sample and pooled.

For *nirS*, primers nirS1F and nirS6R (Braker *et al*., 1998) were obtained from Operon. PCR reactions contained 0.4 μM of each primer, 200 μM deoxynucleoside triphosphates (Promega, Madison, WI), 1 × PCR buffer (Promega), 1.5 mM $MgCl_2$ (Promega), 1.5 U of Taq DNA polymerase (Promega), and 1.0 μL of DNA template. The cycling parameters were as follows: 5 min at 95 °C, followed by 30 cycles of 1 min at 95 °C, 1 min at 57 °C, and 3 min at 72 °C, followed by 4 min extension at 72 °C. For *nirS*, reamplification was used to increase product yield for digestion (Rösch & Bothe, 2005): 1.0 μL of the first PCR reaction was used as a template for a second round of PCR, which was identical to the first, with the exception that the forward primer was replaced by 0.04 μM of nirS1F labeled at the 5′ end with IRD-800 (LI-COR Inc.). Duplicate PCR reactions were run for each sample and pooled.

Amplification of *nirK* genes was attempted using primers nirK1F and nirK5R and the PCR cycling parameters specified by Braker *et al*. (1998). Although the *nirK* gene could be amplified from genomic DNA of reference strain *Achromobacter xylosoxidans* (ATCC 15173), *nirK* genes could not be amplified using DNA isolated from sediments despite repeated attempts to optimize PCR by modifying template concentrations, reagent concentrations, and cycling parameters (including using a touchdown protocol). The sensitivity of *nirK* amplification was determined by spiking environmental DNA extracts with *A. xylosoxidans* genomic DNA isolated with the MoBio DNA isolation kit (MoBio Laboratories).

16S and *nirS* PCR products from each sediment sample (total of 27 samples, nine from each vegetation zone) were purified with the UltraClean PCR Cleanup Kit (MoBio Laboratories) and analyzed by T-RFLP as described previously (Janus *et al*., 2005) with the following exceptions: for 16S amplicons, 30 ng of each sample was digested with *Msp*I, *Alu*I, and *Hae*III (New England BioLabs, Beverly, MA). For *nirS* amplicons, 30 ng of each sample was digested with *Msp*I, *Hha*I, and *Taq*I (New England BioLabs).

Resultant 16S and *nirS* T-RFLP data sets for all three digestions were analyzed by nonmetric multidimensional scaling (MDS) and analysis of similarity (ANOSIM) using PRIMER V.5 software package (Primer-E Ltd., Plymouth, UK). For a full description of MDS and ANOSIM procedures, see Clarke & Warwick (2001). In order to avoid potential biases introduced by reamplification, T-RFLP data were analyzed based on the presence/absence of each terminal restriction fragment (TRF) peak. In order to eliminate minor peaks from analysis, any TRFs present in less than 15% of the total samples were excluded from subsequent analyses. T-RFLP data were then imported into PRIMER V.5, and a similarity matrix was calculated using the Bray–Curtis coefficient (Bray & Curtis, 1957). MDS was used to ordinate the similarity data (after 100 random restarts), and ANOSIM was used to examine the statistical significance of differences between groups of samples.

Results and discussion

Typha invasion in Cheboygan Marsh has resulted in a clear shift in plant species composition between the three vegetation zones (Fig. 1), significantly decreasing plant diversity in the *Typha* zone compared with the native zone (Table 1). This decrease in plant diversity following invasion follows the trend seen for other invading exotic plants (Ehrenfeld, 2003). Invasive plants also frequently show higher levels of net primary productivity and standing stock biomass than native plants (Ehrenfeld, 2003), and in this study the *Typha* zone showed twice as much aboveground plant biomass as the native zone (Table 1). The *Typha* zone also exhibited a 14-fold increase in plant litter (Table 1) and a fourfold increase in soil organic matter compared with the native

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Table 1. Biological properties of 3 vegetation zones in Cheboygan Marsh*

Vegetation zone	Plant diversity (H')†	Aboveground plant biomass ($g\,m^{-2}$)	Plant litter biomass ($g\,m^{-2}$)	Bacterial species richness‡	*nirS* genotype richness§
Native	$0.82^{a¶} \pm 0.06$	$290.0^{a} \pm 7.13$	$177.3^{a} \pm 2.81$	$54^{a} \pm 3.36$	$26.4^{a} \pm 2.60$
Transition	$1.25^{b} \pm 0.13$	$354.8^{a,b} \pm 19.3$	$571.1^{b} \pm 27.7$	$67.5^{b} \pm 1.50$	$36.7^{b} \pm 1.74$
Typha	$0.14^{c} \pm 0.08$	$639.6^{b} \pm 29.3$	$2470.5^{c} \pm 40.5$	$68.4^{b} \pm 1.71$	$37.0^{b} \pm 1.83$

*Values given as mean ± standard error ($n = 9$).
†Shannon–Weiner Species Diversity Index.
‡Number of terminal restriction fragments produced by T-RFLP analysis using 16S rRNA gene primers.
§Number of terminal restriction fragments produced by T-RFLP analysis using *nirS* gene primers.
¶Values with different letters are significantly different as determined by ANOVA, followed by a Tukey Test for pairwise differences ($P < 0.05$) (SYSTAT v.11).

Table 2. Physical and chemical properties of three vegetation zones in Cheboygan Marsh*

Vegetation zone	pH	Water depth (cm)	Water temperature (°C)	Soil organic matter (%)	Soil water content (%)
Native	$8.15^{a†} \pm 0.04$	$30.3^{a} \pm 0.02$	$27.5^{a} \pm 0.13$	$1.98^{a} \pm 0.554$	$26.6^{a} \pm 2.03$
Transition	$7.73^{b} \pm 0.048$	$18.2^{b} \pm 0.016$	$26.6^{a} \pm 0.514$	$2.85^{a} \pm 0.811$	$33.9^{b} \pm 2.76$
Typha	$7.52^{c} \pm 0.044$	$9.2^{c} \pm 0.133$	$25.4^{b} \pm 0.496$	$8.08^{b} \pm 2.15$	$80.2^{c} \pm 4.51$

*Values given as mean ± standard error ($n = 5$).
†Values with different letters are significantly different as determined by ANOVA, followed by a Tukey Test for pairwise differences ($P < 0.05$) (SYSTAT v.11).

zone (Table 2). These dramatic increases in litter and soil organic matter are likely a function of high C-fixation and increased aboveground biomass of *Typha*. The increased aboveground biomass and litter mass have also likely led to the decreased water temperature in the *Typha* zone (Table 2) through increased shading.

Sediments associated with *Typha* demonstrated large and statistically significant increases in soluble nutrients compared with the native and transition zones, including a 14-fold increase in ammonium, a 10-fold increase in nitrate, and a 10-fold increase in phosphate (Fig. 2). This trend of elevated nutrients associated with *Typha* has been consistent at this site over eight samplings between 2003 and 2005 (data not shown). Previous studies by other groups have shown mixed results for impacts of invasive plants on soil nitrogen levels (Ehrenfeld, 2003). The increases in soluble nutrients observed in this study may have ecological significance, as one important function of wetlands is removal of terrestrially derived nutrients, including nitrogen and phosphorus, before these nutrients can enter adjacent surface waters and lead to eutrophication (Vitousek *et al.*, 1996). The data suggest that *Typha* invasion may be impacting the wetland's ability to remove nutrients from the water.

Sediment bacterial communities in the *Typha* zone were significantly different in composition from communities in the native zone based on MDS (Fig. 3a) and ANOSIM (Table 3) analyses. MDS was used for data analysis because it offers significant advantages over other, more widely used statistical methods (Clarke & Warwick, 2001) and is a powerful tool for analysis of T-RFLP data (Rees *et al.*, 2004). The *Typha* zone sediments also showed higher bacterial species richness, based on total number of TRFs, as compared with native zone sediments (Table 1). Although the T-RFLP assay generally provides an underestimate of biodiversity as it is biased toward the numerically dominant organisms, the number of TRFs produced for a set of samples is a useful indicator of changes in species richness (Klamer *et al.*, 2002). The difference in bacterial species composition between the *Tyhpa* and native zones was not surprising, given the significant differences in sediment physical and chemical characteristics (Table 2, Fig. 2), but the lower bacterial species richness in the native zone was surprising as the native zone had much higher plant diversity than the *Typha* zone (Fig. 1, Table 1). This result suggests that *Typha* invasion may be increasing the diversity of microniches available to the bacteria. The bacterial communities in the transition zone were intermediate in composition between the communities in the *Typha* and native zones (Fig. 3a, Table 3), but showed bacterial species richness levels that were equivalent to those associated with *Typha* (Table 1). This result again indicates a positive effect of *Typha* invasion on bacterial species richness.

Denitrifier communities in the *Typha* zone were significantly different in composition based on *nirS* gene sequences (Fig. 3b, Table 3) and showed higher *nirS* genotype richness as compared with the native zone (Table 1). Denitrifier communities in the transition zone were intermediate in composition between the communities in the *Typha* and native zones (Fig. 3b, Table 3), but showed *nirS* genotype richness levels that were equivalent to those in the

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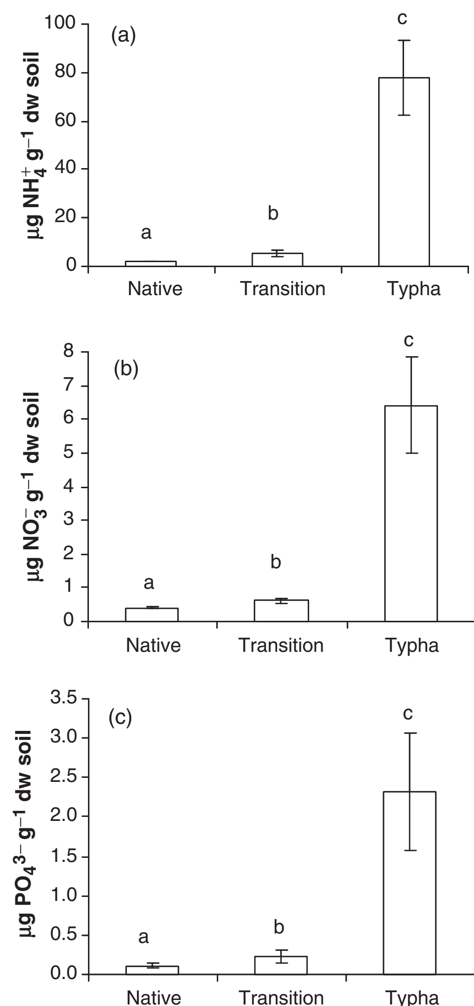

Fig. 2. Total soluble inorganic NH_4^+ (a), NO_3^- (b), and PO_4^{3-} (c) in soils of each vegetation zone. Bars represent means for each vegetation zone ($n = 5$) and error bars represent ± standard error. Values with different letters are significantly different based on ANOVA followed by a Tukey test for pairwise differences ($P < 0.001$) (SYSTAT v.11).

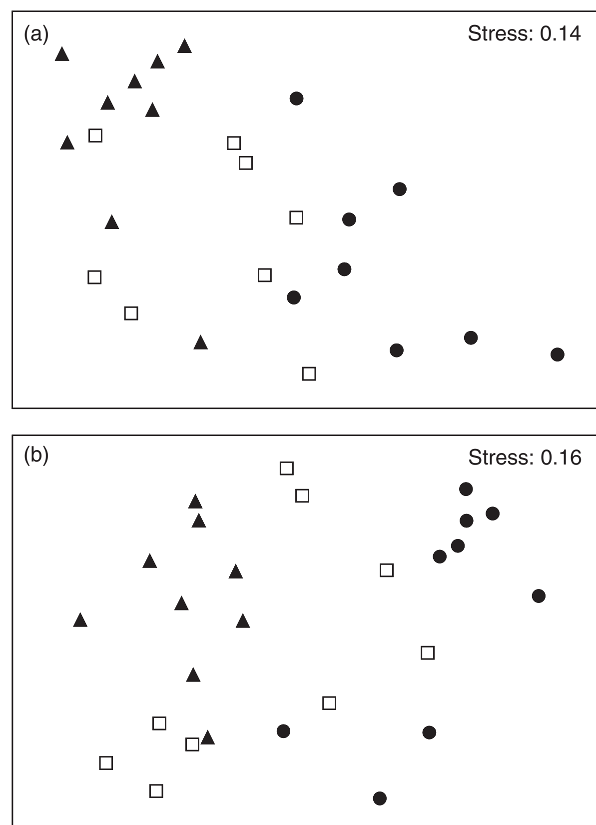

Fig. 3. Nonmetric multidimensional scaling (MDS) analysis of 16S T-RFLP data (a) and *nirS* T-RFLP data (b). ● (native zone), □ (transition zone), ▲ (*Typha* zone).

Table 3. ANOSIM *R* statistics and *p* values for 16S and *nirS* T-RFLP analyses

	16S		*nirS*	
Vegetation type	*R*	*P* <	*R*	*P* <
Native vs. *Typha*	0.795	0.001	0.738	0.001
Native vs. transition	0.291	0.005	0.465	0.005
Transition vs. *Typha*	0.364	0.004	0.452	0.001

Typha zone. This result indicates a positive effect of *Typha* invasion on *nirS* genotype richness.

In this study, *nirK* genes could not be amplified from DNA isolated from sediment samples, despite the fact that the same primer set has been used successfully by other researchers to amplify *nirK* genes from environmental samples (Prieme *et al*., 2002; Wolsing & Priemé, 2004). The failure to amplify *nirK* genes may have been caused by inhibition of PCR by inhibitory compounds in the sediment DNA extractions. However, when environmental DNA extracts were spiked with *A. xylosoxidans* genomic DNA, *nirK* genes were amplified from as few as 75 genome copies per reaction. Based on the DNA extraction protocol used, this corresponds to *c.* 4×10^3 *nirK* copies per gram of sediment. These data suggest that *nirK*-containing organisms were either not present in the Cheboygan Marsh sediments or were present at a level below 4×10^3 *nirK* gene copies per gram of sediment. As the two structurally different nitrite reductases that are encoded by *nirK* and *nirS* are found to be mutually exclusively among denitriers (Braker *et al*., 1998), these results suggest that *nirS* denitrifiers may have been dominant in Cheboygan Marsh. This conclusion is supported by several groups that have suggested that *nirK* denitrifiers are less abundant than *nirS* denitrifiers in estuarine sediments (Nogales *et al*., 2002) and marine sediments (Braker *et al*., 2000). However, Throback *et al*. (2004) demonstrated that the nirK1F/nirK5R primer

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set does not amplify all *nirK*-containing strains, so Cheboygan Marsh may have included *nirK* denitrifiers not targeted by this primer set.

The differences in denitrifier community composition that were observed based on *nirS* gene sequences may have ecological significance, as different denitrifying taxa are known to differ in their oxygen threshold, carbon requirements, and kinetic parameters (Tiedje, 1988), and the $N_2O : N_2$ ratio resulting from denitrication can be dependent on species composition of denitrifying communities (Munch, 1989). Few prior studies have examined the impacts of invasive plants on denitrification. Otto *et al.* (1999) found no change in denitrification rates in freshwater marsh sediments under invasive plants, while Bolton *et al.* (1990) found an increase in denitrification under an invasive plant in terrestrial soils. However, neither of these studies examined the impacts of invasive plants on the composition of denitrifier communities. The current study demonstrated that *Typha* supported a distinctly different community of denitrifiers with higher *nirS* genotype richness, as well as 10-fold higher levels of soluble nitrate, suggesting that *Typha* may be impacting the denitrification process.

Conclusions

The results of this study demonstrate that invasion of a Great Lakes freshwater wetland by an exotic plant species, *Typha* × *glauca*, had significant impacts on sediment physical, chemical, and biological characteristics. The significant increases in soluble ammonium, nitrate, and phosphorus in sediments associated with *Typha* suggest that *Typha* invasion is affecting the wetland's ability to remove nutrients from water. The shifts in total bacterial and denitrifier community composition and *nirS* genotype richness indicate that *Typha* is altering the microbial makeup of the wetland sediments, and the observed shift in denitrifiers in particular suggests that *Typha* may be affecting microbially catalyzed nutrient cycling processes such as denitrification. Further work is needed to determine whether *Typha* invasion has altered rates of microbially catalyzed nutrient cycling processes and whether *Typha* invasion has impacted the ecosystem's ability to prevent release of nutrients to the lake.

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