INVASIVE-SPECIES REMOVALS AND NITROGEN-REMOVAL ECOSYSTEM SERVICES IN FRESHWATER TIDAL MARSHES

A Final Report to the Tibor T. Polgar Fellowship Program

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Alldred, M. K. and S. B. Baines. 2012. Invasive-Species Removals and Nitrogen-Removal Ecosystem Services in Freshwater Tidal Marshes. Section IV: 1-23 pp. In S.H. Fernald, D.J. Yozzo and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2010. Hudson River Foundation

ABSTRACT

Two major management goals in the Hudson River Estuary are the control of invasive species such as *Phragmites australis* and the maintenance of water quality. Species invasions and invasive species removals have the potential to alter the fraction of nitrogen that is removed from wetland sediments via denitrification. Several small-scale *Phragmites australis* removals were performed in Ramshorn Marsh, a freshwater tidal marsh of the upper Hudson River, in late September 2010. This report includes data from two pre-removal sampling efforts in late August of 2009 and 2010, with the ultimate goal of characterizing the response of denitrification potential to the removal of an invasive plant community. Pre-removal data indicate that the selected reference sites, West Flats and Brandow Point, do not differ from Ramshorn Marsh in terms of denitrification potential or important sediment characteristics and will therefore be useful as reference sites for post-removal comparisons. Differences in denitrification potential were not detected between sediments dominated by native and invasive vegetation; however, significant interannual variations in denitrification potential and organic content of sediments were detected in Ramshorn Marsh. These results suggest that repeated sampling efforts throughout the growing season will be essential to understanding plant-mediated effects on sediment dynamics.

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INTRODUCTION

Two major management goals in the freshwater tidal marshes of the upper Hudson River are the control of invasive species and the maintenance of water quality. Nitrogen pollution from non-point sources is a major concern in the Hudson watershed (USACE 2009). Delivery of excess nitrogen to downstream salt marshes and estuaries can result in eutrophication, hypoxia, and harmful algal blooms, all of which can have severe consequences for the economy and human health (Vitousek et al. 1997; Bertness et al. 2002; Hinga et al. 2005). Marshes provide a valuable ecosystem service as sites of active denitrification, a microbial process in which nitrate is permanently removed from an ecosystem as nitrogen gas (Zedler 2003).

Marsh plants are generally understood to play an important role in nitrogen removal, either directly by assimilating nitrate and ammonium, or indirectly by altering sediment characteristics to favor denitrification (Caffrey et al. 2007). Denitrification requires that anoxic conditions (or very low oxygen concentrations) accompany the presence of organic carbon (as an energy source) and nitrate (as an oxidant) in sediments. Marsh plants may alter denitrification rates by directly or indirectly affecting the availability of any of these three components. The degree to which plants alter organic matter and nitrogen availability will depend on their own nutrient demands, their productivity, and the quality of the litter they produce. Denitrification could be depressed if plants have high nitrogen demands or if they produce low quality litter to promote immobilization of inorganic nitrogen by sediment microbes, as *Phragmites* has been suggested to produce (Meyerson et al. 2000b). Alternatively, plants that produce more easily decomposed litter with a high nitrogen content could enhance denitrification by increasing the availability of inorganic nitrogen and organic carbon substrates.

Plants and sediment microbes are also likely to influence denitrification through the daily cycle of oxygen concentrations in sediments. Because sediments that are saturated with water tend to become anoxic, the major mineralized form of inorganic nitrogen in marsh sediments tends to be ammonium (the reduced form of mineral nitrogen). Denitrification cannot occur unless nitrate diffuses into the anoxic sediments, or the ammonium in those sediments is converted to nitrate via nitrification, which requires the presence of oxygen. Therefore, denitrification tends to occur at maximum rates at oxic/anoxic boundaries, where nitrate can diffuse into anoxic sediments, or where sediments alternate between oxygenated and non-oxygenated states over time (Seitzinger et al. 2006). Near plant roots, oxygen increases during the day, while at night this oxygen is rapidly consumed by sediment organisms. The diel pumping of oxygen into plant roots increases the spatial and temporal variability of oxygen in sediments, thus maximizing the potential for denitrification to occur (Bodelier et al. 1996).

Because marsh plants differ in many of the key characteristics expected to affect denitrification rates (e.g. productivity, nutrient demands, and root-zone oxygenation) (Windham and Meyerson 2003), species invasions and invasive-species removals may be expected to modify nitrogen-removal ecosystem services that marshes provide. In freshwater tidal marshes of the Hudson, replacement of the native marsh grasses, predominantly *Typha angustifolia*, by the invader *Phragmites australis* has caused considerable decreases in the native diversity of plants, insects, birds, and other wildlife (Chambers et al. 1999; Meyerson et al. 2000a). Control and removal of *Phragmites* is thus an important management goal for the Hudson River Estuary (Kiviat 2006). Removal of *Phragmites* from small patches of freshwater marshes is often successful in promoting the regrowth of native plants and restoring native plant diversity (Farnsworth and Meyerson 1999; Meyerson et al. 2000a). However, the effects of *Phragmites* removals on nitrogen-removal ecosystem services have been less well-documented (Findlay et al. 2003).

A glyphosate-herbicide removal was performed in several small (<2 acre) patches of *Phragmites australis* in Ramshorn Marsh (also Catskill Marsh), a freshwater tidal marsh of the upper Hudson River. Here, the results of two pre-removal monitoring efforts are reported, which were conducted in late August of 2009 and 2010, with the purpose of characterizing sediment characteristics and denitrification potential of the site of future *Phragmites* removal, as well as two similar marshes not undergoing removal that will serve as reference sites. Data collected thus far will be used to answer three major questions:

1) Do West Flats and Brandow Point differ from Ramshorn Marsh in denitrification potential or any sediment characteristics, such that they would be inappropriate for use as reference sites?
2) Do denitrification potentials or sediment characteristics differ between *Phragmites*- and *Typha*-dominated sediments within Ramshorn Marsh?
3) Do denitrification potentials and sediment characteristics in Ramshorn Marsh differ interannually?

These results will lay the basis for a larger study that will examine the effects of *Phragmites australis* removal, and subsequent recolonization by the native vegetation, on denitrification potential, and thus the capacity of marsh sediments to act as a sink for nitrogen.

METHODS

STUDY DESIGN AND SITES

Figure 1: Study sites sampled in August 2010. Ramshorn Marsh (also Catskill Marsh) was the site of *Phragmites* removals, and West Flats and Brandow Point were sampled as reference *Phragmites* stands that will not undergo removal.

Ramshorn Marsh was first sampled in August 2009, with the goal of adequately assessing the variation in denitrification potential and sediment characteristics for each *Phragmites* patch prior to removal with herbicide. Due to a delay in herbicide treatment, the pre-removal assessment of Ramshorn Marsh was repeated in August 2010, and additional data were collected on two reference sites, West Flats and Brandow Point (Figure 1).

In August 2009, four locations were sampled within each *Phragmites* patch of Ramshorn Marsh (Fig. 2A). In August 2010, two locations were sampled within each *Phragmites* patch of West Flats (Fig. 2B) and Brandow Point (Fig. 2C). For the Ramshorn Marsh patches (Fig. 2A), one location was sampled in each *Phragmites* patch and in an adjacent patch of native vegetation (predominantly *Typha angustifolia*). For each sample location in 2009 and 2010, one nutrient profile was obtained using a PVC equilibrator, and duplicate sediment cores, each with a total depth of about 15 cm, were collected for organic-content and denitrification-potential measurements.

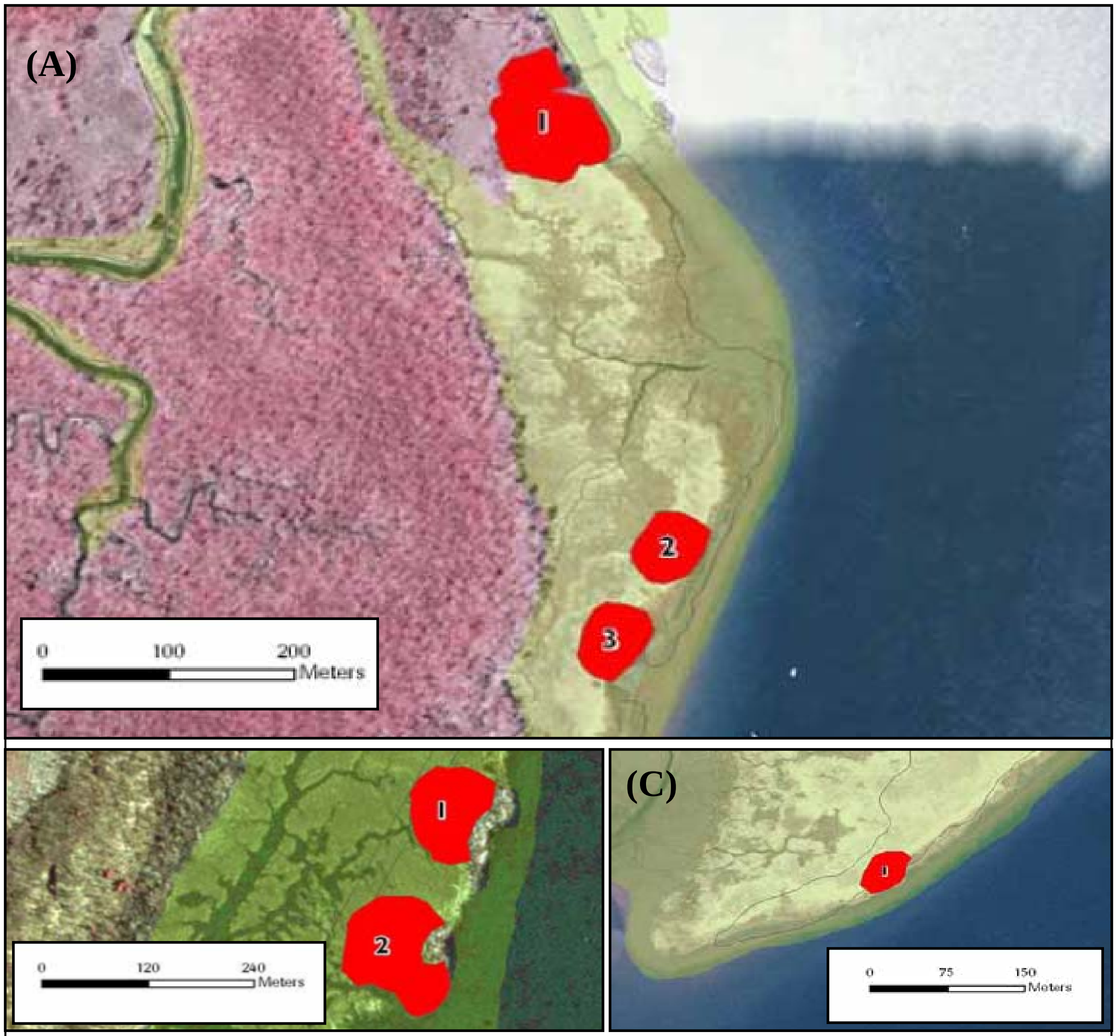

Figure 2: Extent of *Phragmites australis* stands sampled for this study in (A) Ramshorn Marsh, (B) West Flats, and (C) Brandow Point. Maps modified from Zimmerman and Shirer (2009).

SEDIMENT ANALYSES

Pore-water samples were collected at each sampling location using PVC equilibrators, each containing two 12 ml sampling cavities spaced vertically at 3 cm intervals. Equilibrators were prepared in the laboratory using deoxygenated water and Spectr-Por cellulose membranes and allowed to equilibrate in the vegetation sites for eight days. Pore water was collected immediately upon removal from sediments using a syringe, after which it was acidified and stored in 20 ml scintillation vials. In August 2009, samples were analyzed for nitrate and ammonium via ion chromatography using a Lachat Flow Injection Analyzer (Lachat, Loveland, CO). In August 2010, samples were analyzed for nitrate and ammonium using standard colorimetric techniques (Jones 1984; Parsons et al. 1984). Each duplicate sediment core was homogenized and a ~5.0 g subsample analyzed for sediment water content and total organic matter. Organic content was measured as loss after combustion at 450° C for 4 hours.

DENITRIFICATION POTENTIALS

Denitrification enzyme activity (DEA) measurements provide an estimate of the maximum potential of the microbial community to perform denitrification under ideal conditions (Smith and Tiedje 1979). Because these measurements are less sensitive to transient nutrient and oxygen availability in the field, they are useful in comparing responses in denitrification (or the denitrifier community) among field sites and experimental treatments (Groffman et al. 2006). For this analysis, sediment subsamples from duplicate sediment cores (~5.0 g) were amended with KNO_3, glucose, chloramphenicol, and acetylene, and incubated under anaerobic conditions for 90 minutes, with gas sampling at 30 and 90 minutes. Gas samples were stored in evacuated septum vials and analyzed for N_2O by electron-capture gas chromatography.

STATISTICAL ANALYSES

Organic content and denitrification potential were analyzed using two-level, nested ANOVAs with unequal samples size. The three independent variables analyzed were site (Ramshorn, West Flats, and Brandow Point), vegetation (native and invasive), and year (2009 and 2010). Patches were nested within these variables to determine whether variation was greater among the variables, or among patches within each variable. Percent organic matter was arcsine-square-root transformed prior to analysis to achieve homoscedasticity. Pore-water ammonium and nitrate were analyzed using three-level, nested ANOVAs with unequal sample size. The same independent variables were analyzed; however, depth within the sediment was added as an additional level, nested within sampling patch. Because the sampling design was necessarily unbalanced, Satterthwaite approximations were used to calculate error degrees of freedom and mean squares for "site" and "year." All analyses were performed in SAS (SAS 2002-2008).

RESULTS

TREATMENT AND REFERENCE SITES

Pore-water ammonium ranged between 0 and 0.5 mg/L and did not vary significantly among the three sites (Figure 3), nor among patches within sites (Table 1). Nitrate concentrations did not vary significantly among sites or patches (Table 2), and mean concentrations remained below detectable limits across all sampling locations.

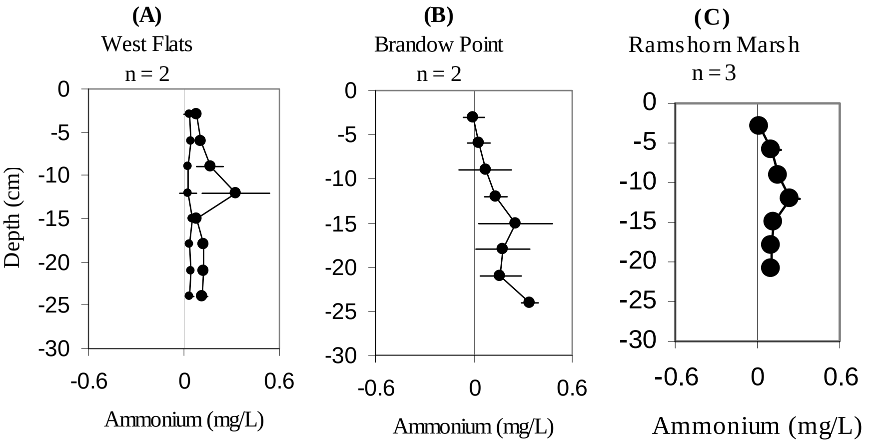

Figure 3: Ammonium sediment-pore-water profiles for (A) West Flats, (B) Brandow Point, and (C) Ramshorn Marsh in August 2010. Error bars show standard error.

Table 1: ANOVA Table: Pore-water ammonium in reference and treatment sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Site	2	0.019311	0.009655	0.21	0.8401
Error	0.8868	0.040126	0.045247		
Patch	1	0.039549	0.039549	3.06	0.0945
Depth	21	0.270955	0.012903	0.99	0.4924
Error	44	0.573220	0.013028		

Table 2: ANOVA Table: Pore-water nitrate in reference and treatment sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Site	2	0.053782	0.026891	0.31	0.8082
Error	0.5976	0.051727	0.086559		
Patch	1	0.092118	0.092118	1.44	0.2436
Depth	21	1.344206	0.064010	0.54	0.9341
Error	44	5.191765	0.117995		

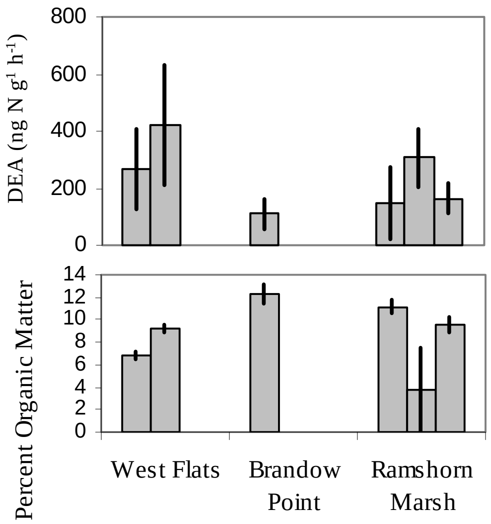

Figure 4: Percent organic matter and denitrification potential in reference and treatment patches (n_i = 4, 4, and 2).

Sediment organic matter did not differ among reference and treatment sites but did differ significantly among patches within sites (p = 0.021, Table 3). Though the F-value for sites appears large (F = 5.40, Table 4), no significant difference was detected in denitrification potential among sites. This result may be due to a lack of power. However, mean values for DEAs and organic matter in Ramshorn Marsh generally fall within the range of those measured at the two reference sites (Fig. 4).

Table 3: ANOVA Table: Sediment organic matter in reference and treatment sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Site	2	0.0222	0.0111	0.56	0.627
Error	2.75	0.0551	0.0200		
Patch	3	0.0501	0.0167	4.75	0.021
Error	12	0.0422	0.0035		

Table 4: ANOVA Table: Denitrification potential in reference and treatment sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Site	2	159276	79683	5.40	0.424
Error	0.57	8466	14741		
Patch	3	78000	26000	0.37	0.779
Error	12	852419	71035		

NATIVE AND INVASIVE SITES

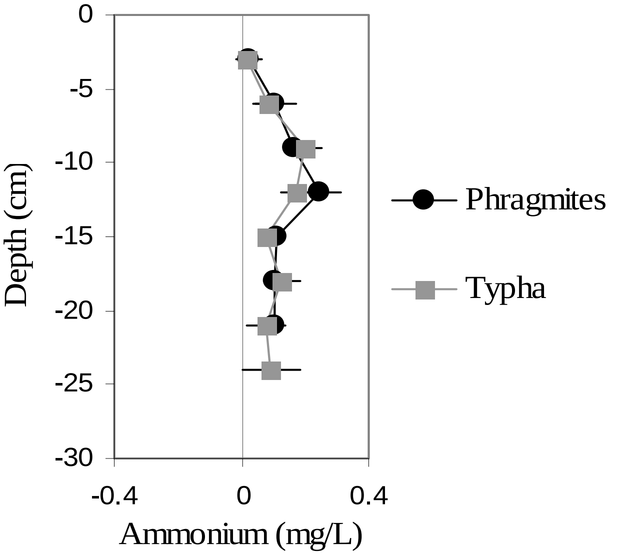

Figure 5: Ammonium sediment-pore-water profiles for *Phragmites*- and *Typha*-dominated patches in Ramshorn Marsh ($n_i = 3$).

Pore-water ammonium did not differ between *Phragmites*- and *Typha*-dominated locations in Ramshorn Marsh (Table 5); however, they did differ significantly by depth (p = 0.014, Table 5), with the largest ammonium concentrations occurring approximately 12 cm from the sediment surface (Fig. 5). Nitrate did not differ significantly between vegetation types, nor among patches or depths (Table 6).

Organic matter content and denitrification potential were similar across vegetation types (Fig. 6). Organic matter content (Table 7) and denitrification potential (Table 8) did not vary significantly between *Phragmites*- and *Typha*-dominated locations within Ramshorn Marsh, nor did they vary among patches within sites.

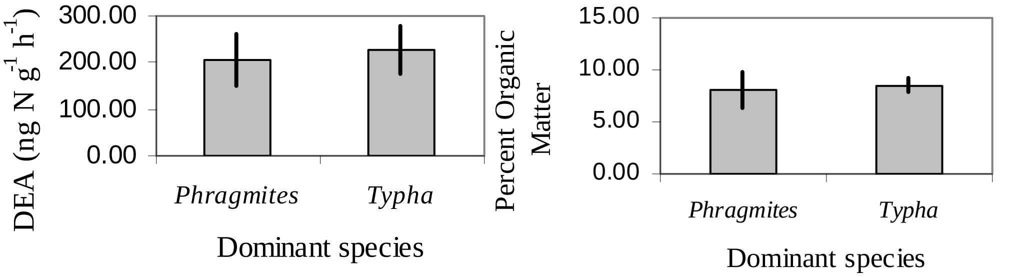

Figure 6: Denitrification potential and percent organic matter of *Phragmites*- and *Typha*- dominated patches in Ramshorn Marsh ($n_i = 6$).

Table 5: ANOVA Table: Pore-water ammonium in *Phragmites*- and *Typha*-dominated sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Vegetation	1	0.0014	0.0014	0.05	0.839
Error	2.00	0.0516	0.0258		
Patch	2	0.0517	0.0258	2.65	0.095
Depth	20	0.1944	0.0097	2.83	0.014
Error	19	0.0651	0.0034		

Table 6: ANOVA Table: Pore-water nitrate in *Phragmites* and *Typha*-dominated sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Vegetation	1	0.0132	0.0132	0.84	0.456
Error	2.00	0.0315	0.0158		
Patch	2	0.0316	0.0158	2.47	0.110
Depth	20	0.1279	0.0064	1.70	0.126
Error	19	0.0714	0.0038		

Table 7: ANOVA Table: Sediment organic matter in *Phragmites* and *Typha*-dominated sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Vegetation	1	0.0033	0.0033	0.27	0.630
Patch	4	0.0492	0.0123	1.80	0.247
Error	6	0.0409	0.0068		

Table 8: ANOVA Table: Denitrification potential in *Phragmites* and *Typha*-dominated sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Vegetation	1	1256	1256	0.14	0.728
Patch	4	36002	9000	0.41	0.797
Error	6	132021	22004		

INTERANNUAL VARIATION

Ammonium and nitrate concentrations did not differ between years in *Phragmites*-dominated patches of Ramshorn Marsh (Fig. 7), and ammonium concentrations were similar among replicate patches and across depths (Table 9). While nitrate concentrations differed significantly among replicate patches ($p = < 0.0001$, Table 10) and depths ($p = 0.046$, Table 10), average concentrations were near or below detectable limits (Fig. 7); however, nitrate was occasionally detected in individual samples, normally at shallow depths (Fig. 7). Percent organic matter ($p = 0.017$, Table 11) and denitrification potentials ($p = < 0.0001$, Table 12) differed significantly between sampling years, with an average 78% decrease in denitrification potential and 54% decrease in organic matter between 2009 and 2010 (Fig. 8). These large differences in denitrification potential and organic matter between sampling years were consistent across all *Phragmites*-dominated patches in Ramshorn Marsh (Tables 11 and 12).

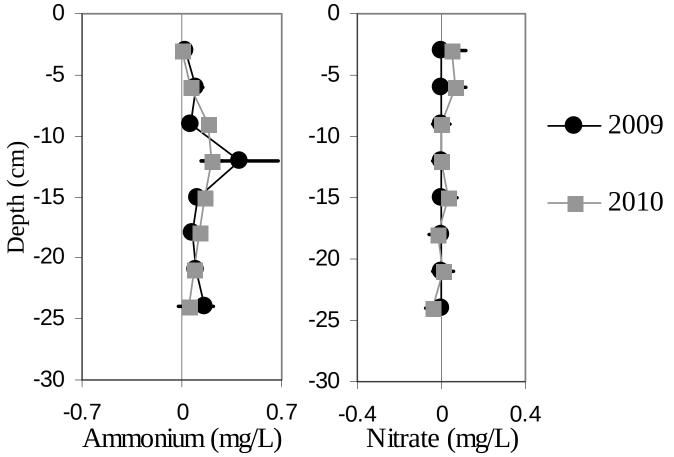

Figure 7: Ammonium profiles for *Phragmites*-dominated patches in Ramshorn Marsh in 2009 ($n_i = 12$) and 2010 ($n_i = 3$).

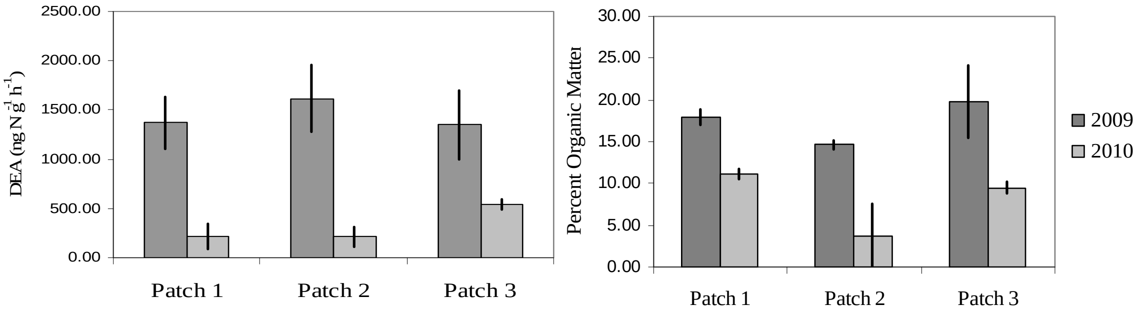

Figure 8: Denitrification potentials and organic matter content in Ramshorn Marsh in 2009 ($n_i = 8$) and 2010 ($n_i = 2$).

Table 9: ANOVA Table: Pore-water ammonium in *Phragmites*-dominated patches of Ramshorn Marsh in 2009 and 2010.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Year	1	0.0018	0.0018	0.53	0.536
Error	2.22	0.0076	0.0034		
Patch	2	0.0065	0.0033	0.03	0.969
Depth	21	2.1732	0.1034	0.89	0.609
Error	88	10.2830			

Table 10: ANOVA Table: Pore-water nitrate in *Phragmites*-dominated patches of Ramshorn Marsh in 2009 and 2010.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Year	1	0.0020	0.0020	0.09	0.788
Error	2.00	0.0423			
Patch	2	0.0423	0.0212	22.87	< 0.0001
Depth	21	0.0194	0.0009	1.70	0.046
Error	88	0.0479	0.0005		

Table 11: ANOVA Table: Percent organic matter in *Phragmites*-dominated patches of Ramshorn Marsh in 2009 and 2010.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Year	1	0.1192	0.1192	8.79	0.017
Error	8.28	0.1123	0.0136		
Patch	4	0.0579	0.0145	1.21	0.332
Error	24	0.2868	0.0120		

Table 12: ANOVA Table: Denitrification potential in *Phragmites*-dominated patches of Ramshorn Marsh in 2009 and 2010.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Year	1	7502020	7502020	22.98	<0.0001
Error	27.10	8849708	326526		
Patch	4	421485	105371	0.15	0.963
Error	24	17272550	719690		

DISCUSSION

Results confirm that West Flats and Brandow Point do not differ from Ramshorn Marsh in sediment nutrient concentrations, organic matter, or denitrification potential. Given the similarity among these sites, West Flats and Brandow Point will serve as useful reference sites for testing the response in denitrification potential and sediment characteristics to the *Phragmites*-removal treatment at Ramshorn Marsh next summer.

Additionally, the variation in pore-water ammonium and sediment organic content was found to be greater among the patches of a single site than among the different sites (Tables 1 and 3, respectively). This within-site variation will be useful in the future because it will allow for assessing variation in the response of sediment characteristics and denitrification potential, if any, to the removal of *Phragmites*, relative to native vegetation patches and intact *Phragmites* patches.

Despite differences between *Phragmites* and *Typha* in characteristics that may be expected to influence sediment characteristics and denitrification potential (Windham and Meyerson 2003), no differences in these variables between *Phragmites*- and *Typha*-dominated sediments were detected (Fig. 5-6). This lack of difference between *Phragmites*- and *Typha*-dominated sites may result from sufficient similarity between these two species, such that abiotic factors (temperature, sediment type, etc.) (Boyer et al. 2006) are more important drivers than plant-mediated factors in driving nutrient availability and denitrification rates. However, the lack of a difference between *Phragmites*- and *Typha*-dominated stands may also be an artifact of collecting field measurements at the end of August, which coincides with the end of growing season in these systems. Differences in plant characteristics (especially productivity, nutrient uptake, and root-zone oxygenation) may vary as biomass accumulates and as peak biomass is reached (Vitousek and Reiners 1975). Such seasonality has been observed in agricultural soils, in which vegetated soils experience peak denitrification rates during the early growing season, with these rates steadily decreasing as the plant community reaches peak biomass (Parsons et al. 1991).

Perhaps the most surprising result of this study was a very large difference in denitrification potential and sediment organic content in Ramshorn Marsh between 2009 and 2010 (Fig. 8). Given the consistency of this effect across all three patches (Tables 11-12), the similarity in organic-matter measurements across all sampling sites (Fig. 4), and the simplicity of organic-matter measurement methods, this interannual variation is probably a true difference and not a result of measurement error. One possible explanation for this difference is the large difference in summer weather between 2009 and 2010. In 2009, mean and maximum summer temperatures (May-August) were lower (19.97°C and 25.76°C, respectively), relative to mean and maximum summer temperatures in 2010 (21.78°C and 28.28°C, respectively) (NOAA 2009-2010). Total monthly precipitation during the summer of 2009 (174.1 cm/month) was nearly double that of 2010 (88.1 cm/month) (NOAA 2009-2010). Lower temperatures may have resulted in lower decomposition rates in the summer of 2009, leaving a larger amount of organic matter remaining at the end of August, providing more energy for sediment denitrifiers, and resulting in higher denitrification potentials in 2009, compared to 2010. Conversely, higher temperatures and drier conditions in 2010 would result in heating of the sediments and faster decomposition rates, leaving less organic matter for denitrifiers by the end of August.

In future summers, Ramshorn Marsh will be revisited to assess the response in denitrification potential and sediment characteristics to the removal of *Phragmites*, relative to the reference sites, as well as responses that occur as the native vegetation recolonizes devegetated patches. The hypothesis to be tested is that following the removal of vegetation, pore-water ammonium should accumulate due to lack of assimilation by plants, as well as decreased rates of nitrification in the absence of plant-mediated oxygenation of sediments. The resulting decrease in nitrate availability is expected to cause a decrease in denitrification potentials, relative to the reference sites. As vegetation recolonizes, a decrease in ammonium and a recovery in denitrification potential should be observed, and thus the ability of the wetland to act as a nitrogen sink should increase. Similar responses have been observed following *Phragmites* removals in Connecticut (Findlay et al. 2003).

Based on pre-removal results, repeating measurements throughout the growing season (May through August) will be essential to understanding the dynamics of plant-sediment interactions in freshwater tidal marshes. Differences in sediment characteristics and denitrification potential between *Phragmites*- and *Typha*-dominated sediments will also be revisited to assess important plant characteristics that could be responsible for differences in denitrification potential. Sediment and plant measurements will be repeated throughout the growing season to assess variation in denitrification, as well as variation among plant communities, over time. Differences among plant communities, if they exist, are expected to be more easily detected earlier in the growing season as biomass accumulates and plants are more physiologically active. This information will greatly assist efforts to predict responses in nitrogen-removal ecosystem services to invasive-species-management practices.

ACKNOWLEDGEMENTS

We would like to think our collaborators at the Cary Institute of Ecosystem Ecology, particularly Dr. Stuart Findlay and David Fischer, as well as Troy Weldy and Melissa Kalvestrand of The Nature Conservancy. Stony Brook University undergraduates Diana Lenis and Michael Tong assisted greatly with laboratory nutrient analyses. This research was supported by The Nature Conservancy and a grant from the Hudson River Foundation Tibor T. Polgar Fellowship program.

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