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Groundwater nitrogen processing in Northern Gulf of Mexico restored marshes

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ABSTRACT

Groundwater nitrogen processing was examined in a restored black needlerush (*Juncus roemerianus*) marsh to assess its potential for removing land-derived nitrogen pollution. Two restoration designs, one initially planted at 50% cover (half density plots) and the other one at 100% cover (full density plots), were compared with non-vegetated controls. The introduction via groundwater of a NO_3^- solution with a conservative tracer (Br^-) and labeled isotopically (^{15}N) allowed calculation of nitrogen removal in the plots following two methods. The first method used changes in the ratio $[NO_x]:[Br^-]$ as the groundwater plume traveled through the plot, and the second method relied on balancing ^{15}N input with ^{15}N export. Both methods showed ≈97% of the N from the simulated groundwater plume was removed (i.e. not delivered to the open waters of the adjacent estuary) in vegetated plots and ≈86% was removed in non-vegetated controls. The most dominant routes of N removal from the introduced solution were N_2 production and assimilation into macrophyte biomass, which were similar in magnitude for the vegetated plots, whereas N_2 production dominated in the unvegetated plots. The majority of N removed from the introduced solution occurred in the first 30 cm the solution traveled in the vegetated treatments. In addition, ambient porewater concentrations of dissolved inorganic nitrogen (DIN) were similar between full and half density plots, but lower than the non-vegetated control (≈8.5× and 7.5×), suggesting full and half density plots removed more DIN than non-vegetated plots. These results suggest that restoring marshes by planting 50% of the area may be a more cost-effective restoration design in terms of mitigating land-derived nutrient pollution than planting 100% of the area since it requires less effort and cost while removing similar quantities of N.

1. Introduction

Human development of coastal watersheds is expanding exponentially world-wide (UNEP, 2006). Coastal development has caused and continues to cause large losses of marshland acreage in many areas of the U.S., with the most dramatic losses occurring in the northern Gulf of Mexico (nGOM; Gagliano et al., 1981; Lotze et al., 2006). Marshes play important ecological roles that sustain human well-being such as carbon sequestration (Chmura et al., 2003), shoreline stabilization (King and Lester, 1995; Moeller et al., 1996), provision of food and shelter for commercially-important organisms (Beck et al., 2001; Boesch and Turner, 1984; Cai et al., 2000; Phillips, 1987; Turner, 1977), and removal of land-derived nutrient pollution prior to entering coastal waters (Tobias et al., 2001a,b; Valiela et al., 2000; Valiela and Cole, 2002). Thus, there is strong pressure to restore marshland to offset losses due to development (Bromberg-Gedan et al., 2009).

The financial cost of most restoration efforts, combined with little knowledge of the effectiveness and success of those efforts, hinder the application of restoration for coastal environmental management (Chapman and Underwood, 2000). This is particularly the case for small-scale projects typically sought by municipalities and private landowners, as the majority of coastal property corresponds to privately-owned small tracts of land. Thus, research on the cost-effectiveness of small-scale restoration designs is needed.

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Sparks et al. (2013) demonstrated that planting marsh vegetation on 50% of a restoration area filled out the area completely (i.e., to 100% cover) within 2 years since planting. In addition, plants initially covering 50% of the marsh restoration area performed similarly, in terms of growth, photosynthesis and nutrient content, throughout the 2 years compared to plants in a companion restored marsh initially planted with 100% cover. On this basis, Sparks et al. (2013) suggested restoring marshes by initially planting them with 50% cover is more cost-effective than planting them with 100% cover. Nevertheless, marsh ecological functions, such as habitat provision, carbon sequestration or nutrient removal, were not compared among these two restoration designs.

Nutrient removal is considered one of the most monetarily valuable ecosystem services provided by marshes (Costanza et al., 1997). Increases in human population in coastal watersheds invariably results in increased nutrient loading into adjoining estuaries and bays (Jackson et al., 2000; Nixon, 1995; Valiela et al., 1992). In turn, increased nutrient loading frequently leads to degraded environmental quality (Cebrian et al., 2014; Hauxwell et al., 2003, 2001). Non-point sources are often a major contributor to anthropogenic nutrient pollution (Howarth et al., 2000; Lehrter and Cebrian, 2010). Prior work in the Mid-Atlantic US has suggested *Spartina alterniflora* marshes may remove large quantities of non-point nutrient inputs from discharging groundwater before reaching coastal waters (Tobias et al., 2001a,b), but it is not known if such potential exists for the black needlerush (*Juncus roemerianus*) marshes that dominate in the nGOM and US South Atlantic coast.

The interest of such question is heightened by our current need for *J. roemerianus* marsh restoration. The largest loss of US marshlands is occurring in the nGOM (Brown et al., 2011; Dahl, 2005; Turner, 1990) and *J. roemerianus* is the dominant marsh plant along this coast (Eleuterius, 1976). *J. roemerianus* can grow in a wide range of environmental conditions (Lin and Mendelssohn, 2009; Woerner and Hackney, 1997) and is expected to have a competitive advantage over C_4 plants, given rising atmospheric CO_2 levels, due to its C_3 photosynthetic pathway (Ainsworth and Long, 2005; Erickson et al., 2007; Lenssen et al., 1993; Rozema et al., 1991). Thus, *J. roemerianus* is commonly a target species for marsh restoration in the nGOM. Marsh restoration efforts with *J. roemerianus* have occurred (LaSalle, 1996; Lewis, 1982; Turner and Streever, 2002); however, evaluations of ecosystem functional enhancement, such as increased removal of land-derived nutrient pollution, brought about by these efforts rarely occur.

Here we compare two *J. roemerianus* marsh restoration designs that differ in cost and effort required to complete for effectiveness in reducing land-derived nutrient loading. Both of these restoration designs are tailored for small-scale projects usually carried out by private owners and municipalities. Thus, this research may inform managers and private property owners interested in maximizing effectiveness of restoration efforts focused on reducing nutrient pollution of coastal waters.

2. Materials and methods

2.1. Experimental setup and sampling

Intertidal *J. roemerianus* marsh was restored at the Grand Bay National Estuarine Research Reserve (GBNERR) in Mississippi, USA (30°24′29″ N, 88°24′10″ W) in April 2006 and the experiment was conducted during the summer of 2008. The restoration site construction, marsh planting, and experimental layout are described in Sparks et al. (2013). A randomized block design with 3 blocks, 3 treatments, and a replicate of each treatment per block was used (Fig. 1). The treatments were full density (100% initial planting density; F), half density (50% initial planting density; H) and control (0% initial planting density and kept unvegetated throughout the experiment; C). Each experimental plot was 1.5 m long (perpendicular to the shoreline) and 0.5 m wide (parallel to the shoreline; Fig. 1). Within each plot, subsurface flumes were constructed to minimize lateral dispersion of the simulated groundwater solution (SGW). PVC flume walls were buried alongside the plots to the depth of the pre-existing clay layer. A diffuser plate was buried at the upland edge of the plots to ensure dispersal of the SGW throughout the plot (Fig. 2). Five porewater collection wells were evenly spaced within the plots and labeled A–E, with A being the most upland well and E the most downland well. Wells A, C and E were screened from 10 cm to 20 cm below the sediment surface while B and D were screened 20–30 cm below the sediment surface.

The SGW contained 150 μM $^{15}KNO_3^-$, 7.5 mM KBr and 240 nM SF_6 with an isotopic concentration of 10 at% ($\delta^{15}N \approx 30{,}500$) for ^{15}N. Sulfur hexafluoride (SF_6) was dissolved in the SGW by filling 40 L Tedlar bags with freshwater and SF_6 in the headspace over the freshwater, allowing the SF_6 to diffuse and dissolve into the water prior to introduction into the plots (Tobias et al., 2009). Using metering pumps the solution was continuously pumped through each plot at a rate of 28.8 L day^{-1} for 31 days during June–July 2008.

Sampling of target wells occurred over a period of 31 days. Using a peristaltic pump, wells were purged to dryness or of three well volumes and allowed to refill before samples were taken. Injectate samples (INJ) were taken directly from the solution prior to being introduced into the marsh at the diffuser plate. Porewater samples were taken from each well (A–E) and injectate (INJ) on days 5, 9, 13, 20, 24 and 31 of the experiment.

Porewater samples were analyzed for Br^-, dissolved inorganic nitrogen (DIN), $^{15}NO_3^-$ and $^{15}NH_4^+$ isotopes, N_2, N_2O, $^{15}N_2$ and $^{15}N_2O$

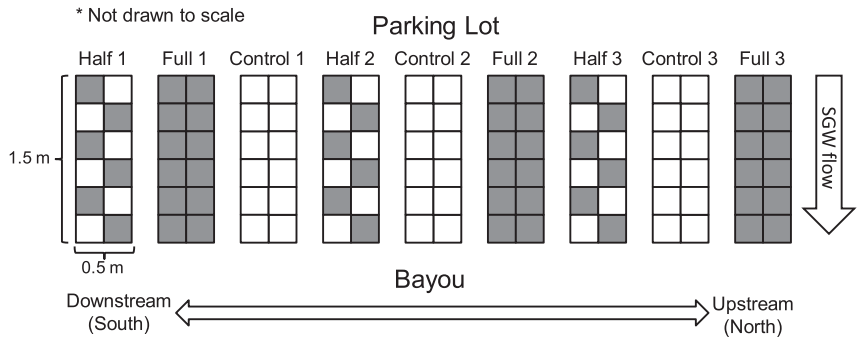

Fig. 1. Experimental layout schematic. Each shaded square represents one transplanted sod with dimensions of 25 cm × 25 cm and a depth of 30 cm. SGW flow direction went from the parking lot toward the bayou and this flow is depicted by the arrow on the right side of the figure.

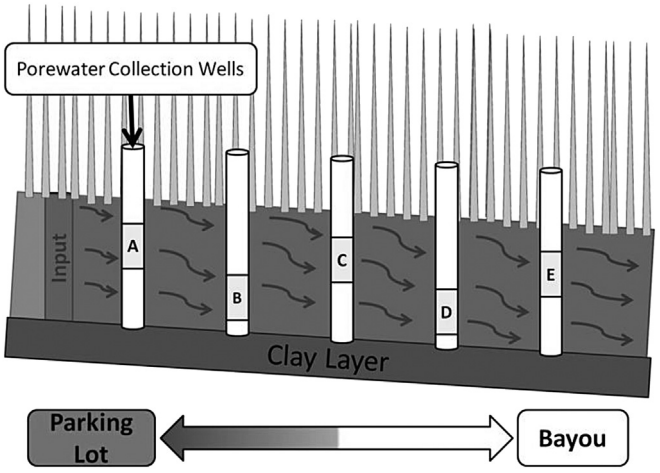

Fig. 2. Cross sectional view of one plot. The diffuser plate where the SGW was introduced is the input; the solution traveled down the plot (parking lot to bayou) as indicated by the black arrows. Wells were buried to the impermeable clay layer and screened at set distances below the sediment surface. The labeled region on each well represents the area that was screened (10–20 cm for A, C and E and 20–30 cm for B and D).

isotopes, and SF_6. DIN includes $NO_3^- + NO_2^-$ (NO_x) and NH_4^+ concentrations. Br^- was analyzed using an ion specific electrode (Tobias et al., 2001b). NH_4^+ and NO_x concentrations were analyzed using the phenolhypochlorite and cadmium reduction azo dye assays, respectively (Maynard and Kalra, 1993; Solorzano, 1969). Isotopic analysis for $^{15}NO_3^-$ followed the devardas reduction, diffusion method of Sigman et al. (1997). $^{15}NH_4^+$ for isotope analysis was isolated using alkaline/acid trap diffusion (Holmes et al., 1998). Isotope analysis for both $^{15}NO_3^-$ and $^{15}NH_4^+$ was performed using continuous flow isotope ratio mass spectrometry (IRMS) using an elemental analyzer interface. All isotopic analyses ($^{15}NO_3^-$, $^{15}NH_4^+$, $^{15}N_2$, $^{15}N_2O$, plants, and sediment) were performed at the University of North Carolina–Wilmington (UNCW) isotope lab. N_2 samples were collected by pumping porewater into vacuum sealed serum vials flushed with He and preserved with KOH. The samples were analyzed for $^{15}N_2$ analysis following the methodology described in Böhlke et al. (2004). N_2O samples were collected on days 20 and 31 of the experiment in all wells except B, and processed for N_2O concentration and isotopic composition ($^{15}N_2O$) using the methodology in Tobias et al. (2001a). Samples for SF_6 were collected by pumping porewater into pre-weighed serum vials that were pre-flushed with N_2. The headspace was analyzed for SF_6 using a Shimadzu GC-8A equipped with an electron capture detector (Cole and Caraco, 1998). Headspace SF_6 concentrations were converted to aqueous concentrations based on SF_6 solubility and sample to headspace volumes.

Plant (*J. roemerianus*) above- and belowground samples for internal nitrogen and ^{15}N concentration analysis were taken on days 5, 13, 20 and 31 of the experiment at three locations in each plot (upland, middle, and lowland along the elevation gradient). Upland samples were collected between wells A and B, middle samples were near well C and downgradient samples were between wells D and E. Three living shoots were clipped at the sediment surface for aboveground analysis. For belowground analysis, three 5 cm long sections of rhizomes were excavated by hand and clipped. All samples were rinsed followed by separation of the roots from the rhizome for belowground samples. Samples were dried and ground prior to analyses on an IRMS for nitrogen and ^{15}N concentrations. Additional plant samples for biomass determination were taken at the end of the experiment by harvesting 15 cm × 15 cm sods at the same three plot locations as the plant isotopic samples for each restored plot and in three nearby natural marsh sites where transplanted plugs were collected. These sods were harvested to a depth of 30 cm to completely encompass all roots and rhizomes. Biomass samples were separated into living and dead portions for both above- and belowground compartments and dried at 80 C prior to weighing.

Cores for sediment analysis were taken on the final day (31) of the experiment at the same locations as the plant samples. Cores were taken to a depth of 30 cm using a 5.08 cm diameter corer. All plant material was removed from the cores prior to drying at 80 C. After drying, cores were sectioned by depth intervals of 2 cm, ground and homogenized for nitrogen content and ^{15}N analysis on an IRMS.

2.2. Calculations

The amount of N removal from SGW was calculated two ways: First by using changes in the $[NO_x]$:$[Br^-]$ ratio to yield a net removal rate; and second by using a ^{15}N tracer.

2.2.1. N removal from SGW using the NO_x:Br^- ratio

NO_x is biologically active, i.e. actively taken up and removed, while Br^- served as a conservative tracer with similar transport characteristics to highly reactive NO_x; therefore, changes in the $[NO_x]$:$[Br^-]$ ratio were used to indicated net biological removal of NO_x during transit through the plots. The change in the $[NO_x]$:$[Br^-]$ in the most downland well (E) relative to the injectate (INJ) permits the calculation of NO_x loss from SGW that is corrected for dilution of the SGW via precipitation or tidal water. A potential caveat to the $[NO_x]$:$[Br^-]$ method is that it is a net NO_x removal measurement; therefore, any NO_x added through nitrification or other external sources can confound some measurements of NO_x removed from a target solution. For the aforementioned reasons, the ratio of $[NO_x]$:$[Br^-]$ method for estimating NO_x removal from a known solution is ideally used in areas with relatively low background $[NO_x]$ (Tobias et al., 2001a). This study site had low $[NO_x]$ of 0–3.8 μM (unpublished data). The $[NO_x]$:$[Br^-]$ approach is useful to estimate removal of NO_x from a known solution, whereas isotopic measurements can be used to determine individual routes of removed NO_x.

2.2.2. ^{15}N mass balance approach to estimate N removal from SGW

A ^{15}N isotopic mass balance approach was also used to estimate N removed from the SGW. An advantage to isotopic mass balance measurements is the ability to quantify routes of N removal as well as overall N removal. The following equations describe the methodology to calculating N removal from the SGW using isotopic mass balance approach. First we calculated the total mass of $^{15}NO_3^-$ removed (moles ^{15}N day^{-1}) from the SGW in each plot according to:

$$^{15}NO_{3\ \text{removed}} = \{MFE^{15}NO_{3\ INJ} \times [NO_{3\ INJ}] \times Q\} - \{MFE^{15}NO_{3\ E} \times [NO_{3\ E}] \times Q\} \quad (1)$$

$MFE^{15}NO_{3\ INJ}^-$ and $MFE^{15}NO_{3\ E}^-$ are the ^{15}N mole fraction excesses of the NO_3^- in the injectate (INJ) and most downland well (well E), $[NO_{3\ INJ}^-]$ and $[NO_{3\ E}^-]$ is NO_3^- concentration (μM), and Q is the pump rate through the experimental plots (28.8 L day^{-1}). MFE is defined as the ^{15}N mole fraction measured minus the ^{15}N natural abundance mole fraction (0.003663). Rates were converted to total masses using the duration of the experiment. The amount of N derived from the SGW introduced NO_3^- that was converted to ammonium (either through uptake and mineralization and/or via DNRA was calculated from the second term of eq. (1) by substituting the $MFE^{15}NH_{4\ E}^+$ and $[NH_{4\ E}^+]$ for that of $MFE^{15}NO_{3\ E}$

and [NO_{3E}]. This type of calculation is possible since the only substantial flux of ^{15}N into the plots was from the $^{15}NO_3^-$ in the SGW.

Production of $^{15}N_2$ or $^{15}N_2O$ (moles ^{15}N day^{-1}) from the SGW $^{15}NO_3^-$ solution (i.e., denitrification) was calculated from the steady state mass balance of $^{15}N_2$ production, $^{15}N_2$ outgassing and dissolved gas export via drainage according to eq. (2):

$$^{15}N_2,{}^{15}N_2O\text{production} = k_{N_2,N_2O}(MFE^{15}N_2,{}^{15}N_2O \times [N_2,N_2O] \times V) + MFE^{15}N_2,{}^{15}N_2O \times [N_2,N_2O] \times Q \quad (2)$$

The constant k_{N_2,N_2O} is the gas-specific aeration coefficient (day^{-1}), $MFE^{15}N_2$, $^{15}N_2O$ is the mole fraction excess measured for each dissolved gas. [N_2O] is the measured dissolved N_2O concentrations, [N_2] N_2 concentrations were calculated from air equilibrium values using temperature and salinity as described in Weiss (1974) and V is the porewater volume (L). Use of the equilibrium N_2 concentrations was justified based on past experience in discharge zones where N_2 subsidies are small relative to large dissolved N_2 gas inventories even under conditions of high denitrification (Tobias et al., 2001a). The $^{15}N_2$ production was calculated for the interval between each well and summed to yield whole plot values ($n = 9$). Rates were converted to total mass using the duration (days) of the experiment. The reaeration coefficient (k_{N_2}, k_{N_2O}) in equation (2) was calculated from the SF_6 concentrations in the injectate and in individual wells as:

$$k_{N_2,N_2O} = \left\{ -\mathrm{Ln}\left[\frac{\frac{SF6}{Br}\,\mathrm{INJ}}{\frac{SF6}{Br}\,\mathrm{well}} \right] \times \frac{1}{t} \right\} \times Sc \quad (3)$$

SF_6 and Br^- are the dissolved SF_6 and Br^- concentrations, respectively, and t is the travel time from the injection point to the well. The travel time (t) in equation (3) is the time required for the Br^- solution to reach the most downland portion of the plot (well E) was found to be 5 days and was assumed to be linear from the input to well E (i.e., 3 days for well C). All Br^- values were corrected for Br^- contributions from salinity, Sc is a correction factor to convert the aeration coefficient derived from SF_6 to N_2 or N_2O based upon the ratio of the Schmidt numbers for each gas to that of SF_6 (Tobias et al., 2009).

^{15}N from the SGW assimilated into above- and belowground macrophyte biomass (moles ^{15}N day^{-1}) was calculated as:

$$\text{Macrophyte}\,{}^{15}N_{uptake} = \frac{\text{Biomass}_{live} \times \text{N content} \times MFE^{15}N}{15} \times \frac{1}{\text{days}} \quad (4)$$

Biomass_{live} in eq. (4) is living biomass and N content is the fractional N content (i.e. %N/100) in each individual sample where 15 is the molar mass of ^{15}N. Days in the second term of eq. (4) is number of days the solution had been continuously introduced at the time of sampling.

^{15}N from the SGW bound in the sediment (moles ^{15}N day^{-1}) was calculated similarly to macrophyte ^{15}N uptake:

$$\text{Sediment}\,{}^{15}N\,\text{uptake} = \frac{\text{Sed mass} \times \text{N content} \times MFE^{15}N}{15} \times \frac{1}{\text{days}} \quad (5)$$

Sed mass in equation (5) is the mass of the sediment within the plot, extrapolated from the core subsections using plume dimensions, core slab thickness, and sediment bulk density. ^{15}N uptake for macrophytes and sediments was calculated for the upland, middle, and downland portion of each plot and summed.

Unaccounted ^{15}N removal (moles ^{15}N day^{-1}), i.e. the portion of ^{15}N removed not accounted for by the processes listed above, was calculated as:

$$^{15}N\,\text{unaccounted} = {}^{15}NO_{3\ \text{removed}} - {}^{15}N_2O, N_{2\ \text{production}} - {}^{15}N_{\text{macrophytes}} - {}^{15}N_{\text{sediments}} \quad (6)$$

All of the values derived from the above equations were converted into percentage ^{15}N removed relative to the total ^{15}N input ($\approx$465 μmols ^{15}N $plot^{-1}$ day^{-1}) for presentation in Table 2 and Fig. 7.

2.3. Statistical analysis

Biomass values were analyzed using an ANOVA (block × plot location × treatment) with post-hoc Tukey tests to compare treatments individually. Significant values were considered at $p \leq 0.05$. Biomass did not vary with block or location in the plot, thus values from the three locations (upland, middle, lowland) were averaged across the plot and these mean values compared among treatments ($n = 3$). There was no plant colonization of control plots at the time of the experiment (2.1 years after the marsh was created); therefore, biomass values are only reported for F and H plots. The SGW quickly expanded throughout all plots with consistent concentrations of the conservative tracer (Br^-) sampling dates ($p = 0.349$; Fig. 3a), treatments ($p = 0.848$; Fig. 3a). Interestingly, the ratio of [NO_x]:[Br^-] also was consistent across all dates ($p = 0.316$), but was lower for the vegetated treatments ($p < 0.001$; Fig. 3b). Since there was no difference in [Br^-] or [NO_x]:[Br^-] across dates, we averaged all nutrient concentration and uptake data across dates for subsequent figures and analyses. Uptake values derived for different wells or locations in the same plot were summed to obtain whole plot values ($n = 9$). We then statistically

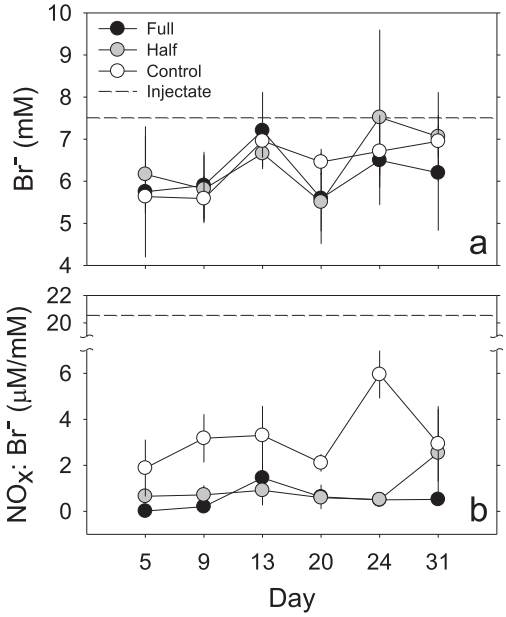

Fig. 3. Mean porewater chemical concentrations in well E through time. Black circles represent the full density treatment, gray circles are the half density treatment, open circles are the control (non-vegetated) treatment, and the dashed line is the injectate. Panel a is Br^-, and b is the ratio of NO_x:Br^-. The x-axis is not to scale. Error bars indicate ±1S.E.

tested this data using an ANOVA with post-hoc Tukey tests. Concentration data was analyzed with a two way ANOVA (treatment × well), whereas, uptake data was analyzed with a one way ANOVA (treatment).

3. Results

3.1. Biomass

At the time of the experiment, F plots had significantly more living aboveground *J. roemerianus* biomass than H plots ($p = 0.013$; Fig. 4), but belowground living biomass was not significantly different between the two types of plots ($p = 0.316$; Fig. 4). F plots contained significantly higher dead belowground biomass than H plots ($p = 0.009$; Fig. 4), but this was not the case for dead aboveground biomass ($p = 0.477$; Fig. 4). Biomass values in F plots were not significantly different from those found at the natural marsh ($p = 0.689$; Fig. 4).

3.2. Porewater DIN concentrations

Porewater [NO_x], [NH_4^+] and DIN was higher in C than in F and H plots (Table 1), which were not significantly different from each other (Fig. 5a–c). However, there were some significant interactions between treatment and well for NO_x and NH_4^+ (Table 1), thus treatments were compared at individual wells. Only well A showed treatment effects for NO_x with the F and H treatments having significantly lower [NO_x] than the C treatment ($p < 0.001$; Fig. 5a). For all treatments, there was a sharp decline in [NO_x] between the injectate and well A. The most pronounced declines were observed in the vegetated treatments. In the C and H treatments, there was a sharp decline in [NO_x] after well A, whereas the F treatment had low concentrations throughout all wells (Fig. 5a). Porewater [NH_4^+] concentrations were significantly higher in C than in F and H plots, except at well A ($p = 0.214$ at A; Fig. 5b). Combining the [NO_x] and [NH_4^+] data resulted in the C treatment having significantly higher DIN concentrations than the F and H treatments (Table 1; Fig. 5c).

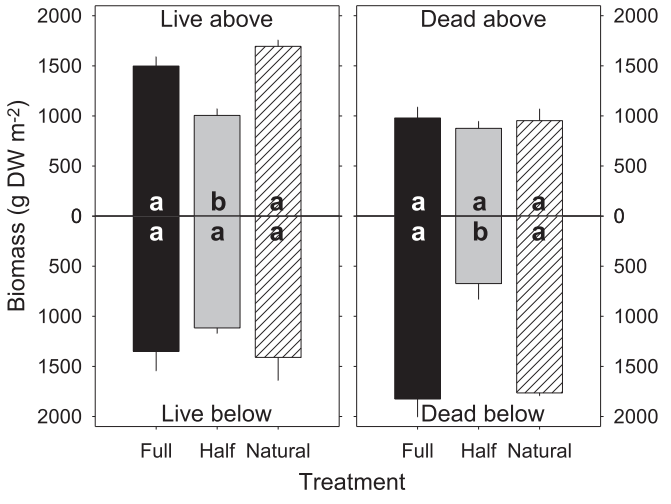

Fig. 4. Mean biomass for full density, half density and natural marsh treatments. The top left panel represents living aboveground biomass, top right is dead aboveground, bottom left is living belowground and bottom right is dead belowground. The full density treatment is represented by black bars, half density treatment by gray bars and natural marsh treatment by white crosshatched bars. Pairwise comparisons were conducted for data in each panel with different lower case letters (a or b) representing significant differences between treatments. Significance was considered at $p < 0.05$ and error bars indicate 1S.E.

Table 1
Results of ANOVA for treatment, well, and interaction of treatment × well for porewater nutrient concentrations. Bold p indicates significance ($p < 0.05$).

Variable	Treatment		Well		Treatment × well	
	F value	p Value	F value	p Value	F value	p Value
NO_x	16.261	**<0.001**	10.223	**<0.001**	5.416	**<0.001**
NH_4^+	34.668	**<0.001**	2.380	0.074	2.749	**0.021**
DIN	49.801	**<0.001**	0.983	0.432	1.332	0.266
Br^-	0.075	0.928	3.024	**0.033**	0.994	0.461
NO_x:Br^-	12.951	**<0.001**	5.566	**0.002**	3.144	**0.010**

3.3. N removal from SGW

3.3.1. [NO_x]:[Br^-] method

Bromide concentrations showed no treatment effect (Table 1), but did have a well effect caused by lower concentrations at well B (Table 1; Fig. 5d). There was a maximum dilution of Br^- of 15% from the diffuser plate to the downland edge of the plot (well E; Fig. 5d), indicating ≈85% of the porewater in well E was from the SGW. There was a rapid reduction of [NO_x]:[Br^-] between the injectate (INJ) and well A for the F and H treatment (≈21 to ≈3), while there was less of a reduction for the C treatment (≈21 to ≈15; Fig. 5e). There was a general reduction of [NO_x]:[Br^-] for the C treatment as

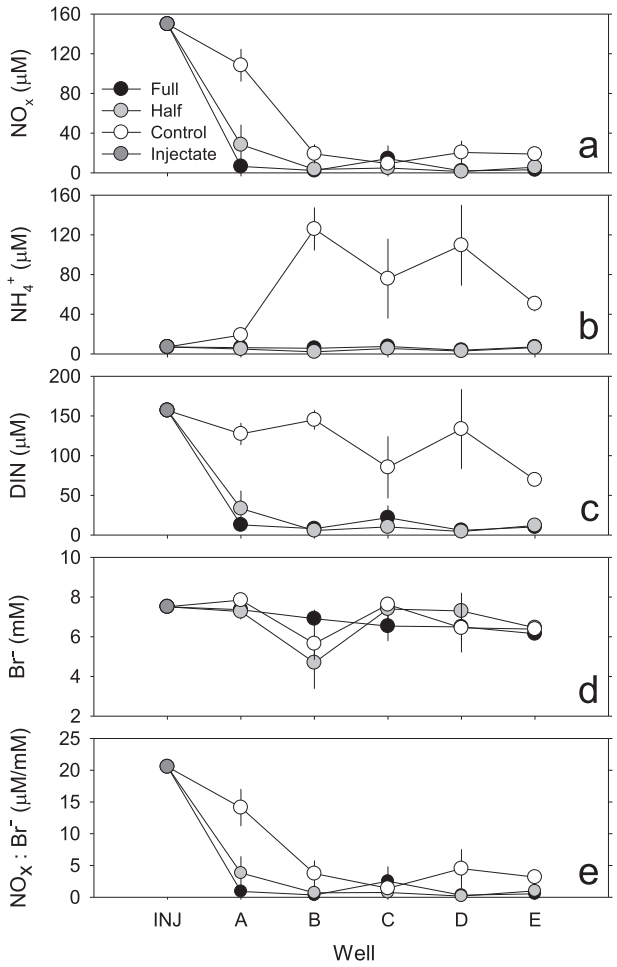

Fig. 5. Mean porewater chemical concentrations in the injectate (INJ) and each porewater collection well. Black circles represent the full density treatment, gray circles are the half density treatment, open circles are the control (non-vegetated) treatment, and the dark gray circle is the injectate concentration. Panel a is NO_x, b is NH_4^+, c is DIN, d is Br^- and e is the ratio NO_x:Br^-. Error bars indicate ±1S.E.

the SGW traveled through the plot, whereas the F and H treatments had constantly low values (≈2) throughout all wells. The ratio of $[NO_x]$:$[Br^-]$ at well E was higher in C than in F and H plots ($p = 0.028$), which did not differ ($p = 0.728$; Fig. 5e). Estimations of percent removal of N using $[NO_x]$:$[Br^-]$, as the difference from INJ to well E, was found to be 97.38 ± 1.22, 95.12 ± 1.91 and 84.56 ± 1.88 for F, H and C plots respectively.

3.3.2. ^{15}N mass balance method

Isotopic enrichment for all measured nitrogen species show similar trends of sharp decreases in the upland portions of all treatments (Fig. 6). When calculating the removal of N from our SGW, we find F and H treatments removed similar quantities of nitrogen ($p = 0.208$) and significantly more nitrogen than the C treatment ($p < 0.01$; Table 2). Isotopic concentrations of $\delta^{15}NO_3^-$ and $\delta^{15}NH_4^+$ ranged from ≈5000 to 30,000‰ for well A to ≈500–6000‰ in well E (Fig. 6a,b). Production of $^{15}N_2$ comprised the largest fate of the N removed from the SGW (Table 2). Enrichments for $\delta^{15}N_2$ reached as high as ≈1100‰ at well A for the C treatment and dropping down to as low as ≈50‰ for the vegetated treatments as well E (Fig. 6c). The C treatment had more measured $^{15}N_2$ production than the F treatment ($p = 0.044$; Table 2) while the H treatment had statistically similar $^{15}N_2$ production as both the F and C treatment. N_2O isotopic enrichments were high ($\delta^{15}N_2$ of ≈15,000–30,000‰ at well A; Fig. 6d), effectively equivalent to MFE $^{15}NO_{3\ INJ}^-$, demonstrating that all N_2O was derived from denitrification of the $^{15}NO_3^-$ from the SGW; however N_2O concentrations were low relative to dissolved N_2 concentrations (<0.01 μM for N_2O and ≈450 μM for N_2), thus N_2O accounted for <1% of ^{15}N from the SGW found in the dissolved gas phase.

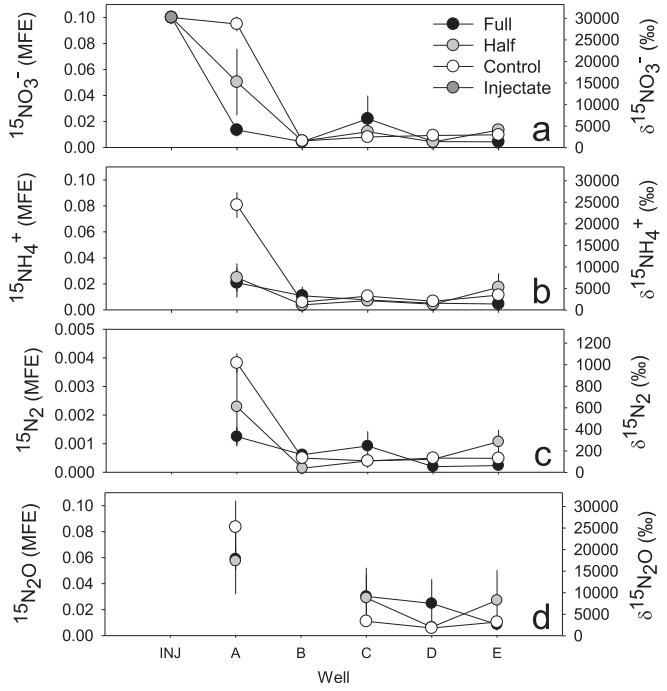

Fig. 6. Mean isotopic concentrations for all measured nitrogen species, expressed as mole fraction excess (MFE) and $\delta^{15}N$ (‰), in the injectate (INJ) and each porewater collection well. Black circles represent the full density treatment, gray circles are the half density treatment, open circles are the control (non-vegetated) treatment, and the dark gray circle is the injectate concentration. Panel a only contains injectate values since the only isotopically labeled nitrogen species introduced was $^{15}NO_3^-$. Panel b is $^{15}NH_4^+$, c is $^{15}N_2$ and d is $^{15}N_2O$. No samples were taken in well B for $^{15}N_2O$ (panel d). Error bars indicate ±1S.E.

J. roemerianus was responsible for the next highest quantity of nitrogen uptake from the SGW (18.76–33.07%). Isotopic enrichment in plant tissues increased through time with mean $\delta^{15}N$ values for ranging from ≈250‰ at day 5 to ≈1450‰ at day 31. Interestingly, macrophytes in the H treatment removed marginally more nitrogen from our solution than macrophytes in the F treatment ($p = 0.081$; Table 2). Most of the nitrogen from our solution assimilated by macrophytes over the duration of the study was allocated into the belowground portion (roots and rhizomes) of the plants in both F and H treatments (Fig. 7). SGW derived nitrogen storage in bulk sediments accounted for similar ($p = 0.223$) and relatively small proportion of nitrogen removal across all treatments (Table 2).

There was also a sizable portion of N from our SGW that was removed (based on isotopic input–export calculations) that we were not able to account for in our sampling (Table 2). The treatment with the largest unaccounted nitrogen was the F treatment, followed by C and H treatments respectively (Table 2).

4. Discussion

The use of SGW containing a conservative tracer (Br^-) and isotopically labeled N ($^{15}NO_3^-$) paired with porewater [DIN] measurements led to four principal findings: 1) restored *J. roemerianus* marshes are highly effective at removing N from porewater; 2) $[NO_x]$:$[Br^-]$ ratios can be as effective as an isotopic approach at estimating removal of NO_3^- from a groundwater plume, although it cannot provide information on the fate of the N; 3) marsh macrophytes and denitrification are similarly important routes of N removal from discharging groundwater; 4) 50% initial planting density (H design) is as effective as 100% initial planting density (F design) at removing N at 2 years after planting.

4.1. Restored *J. roemerianus* marshes are highly effective at removing N from porewater

Our study demonstrated that restored *J. roemerianus* is effective at removing N from porewater through analyzing NO_3^- removal from the SGW and overall [DIN]. From the SGW, vegetated treatments removed ≈96% of the incoming NO_3^- while the non-vegetated treatment removed ≈86% of the incoming NO_3^- over the entire plot. Interestingly, the vast majority of N removal from the SGW in the vegetated treatments occurred in the most landward portion of the plot (i.e., the 30 cm between the input and well A). This N removal pattern is evident through sharp declines in nutrient concentrations between the injectate and upland wells (Figs. 5 and 6). Rapid N removal at the upper marsh edge has been similarly observed in natural groundwater–marsh discharge settings (Tobias et al., 2001b), and is wholly consistent with denitrification and plant uptake being NO_3^- limited in marshes (Böhlke et al., 2009; Kaplan et al., 1979; Tobias and Neubauer, 2009). We found the percentage $^{15}NO_3^-$ removed from the SGW between the input and most upland well (A) was ≈95%, 84%, and 35% for F, H, and C treatments respectively. The observed high N removal rate over a short distance demonstrates that narrow strips (≈30 cm) of marsh can be planted to process moderate NO_3^- inputs via groundwater. While the other ecosystem services provided by marshes, such as habitat and sediment stabilization, would be dampened with decreased initial planting areas, our results suggest processing of moderate nutrient loads can be accomplished with narrow strips of planted marsh.

The entire plot difference in NO_3^- removal from the SGW of ≈10% between the vegetated (≈96%) and non-vegetated (≈86%) treatments may seem marginal; however, these estimates do not account for the processing of N derived from sources other than the

Table 2
Total nitrogen removed from the SGW and the contribution of individual N removal categories to total N removal. Values are expressed as percentage removal and removal rate (mmols N m^{-2} day^{-1}).

	Treatment					
	Full density		Half density		Control	
Category	% N removed	mmols N m^{-2} day^{-1}	% N removed	mmols N m^{-2} day^{-1}	% N removed	mmols N m^{-2} day^{-1}
Total removal	99.49 (±0.06)	5.78 (±0.01)	98.48 (±0.67)	5.72 (±0.04)	88.67 (±2.04)	5.15 (±0.12)
N_2 production	26.96 (±5.27)	1.57 (±0.31)	44.95 (±6.64)	2.61 (±0.39)	58.87 (±11.11)	3.42 (±0.65)
Macrophytes	18.76 (±4.69)	1.09 (±0.27)	33.07 (±4.00)	1.92 (±0.23)	NA	NA
Bulk sediment	7.37 (±2.16)	0.43 (±0.13)	12.19 (±2.34)	0.71 (±0.14)	8.52 (±0.45)	0.50 (±0.03)
Unaccounted	46.40 (±11.16)	2.69 (±0.65)	8.27 (±7.24)	0.48 (±0.42)	21.28 (±9.04)	1.23 (±0.53)

Error is calculated as standard error with $n = 3$.

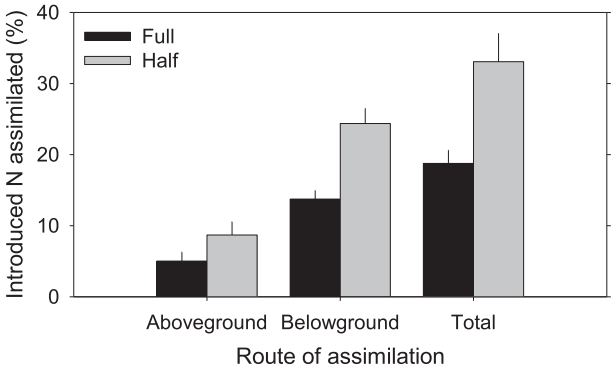

Fig. 7. Mean percentage of N removed from the SGW through macrophyte assimilation. Aboveground is the percentage of N assimilated into the leaves, belowground is the percentage of N assimilated into the roots and rhizomes and total is the sum of above- and belowground assimilation. The full density treatment is represented by black bars and half density treatment by gray bars. Error bars indicate ±1S.E.

SGW. The most dominant source of N to porewater in this marsh is NH_4^+ flux from mineralization, which was more evident as distance from the SGW input increased (Figs. 5b and 6b). In all treatments there was evidence of $^{15}NH_4^+$ enrichment in well A (i.e., closest to the SGW input; Fig. 6b) most likely from dissimilatory nitrate reduction to ammonium (DNRA) or preferential uptake and mineralization by benthic microalgae of SGW derived NO_3^- (Tobias et al., 2003, 2001b). At the upland edge of the non-vegetated plots, $^{15}NO_3^-$ contributed roughly 80% of the NH_4^+; for the vegetated plots, this was less than 20% (Fig. 6b). This labeled $^{15}NH_4^+$ was isotopically diluted to near background $\delta^{15}NH_4^+$ enrichment levels in all treatments as the plume transited downgradient (i.e., not derived from the SGW; Fig. 6b). The mineralization rates required to deplete isotopic concentrations of our $^{15}NH_4^+$ are within previously documented rates for salt marsh mineralization (Anderson et al., 1997; van Wijnen et al., 1999). Therefore, we consider the bulk of the DIN (predominantly NH_4^+) in the plots as a whole to be not derived from SGW, but rather the mineralization of organic N already present within the marsh.

While the restored vegetated treatments did attenuate only 10% more of the NO_3^- from the SGW, overall porewater [DIN] (i.e., the dominant pool of N) was much lower in the vegetated treatments than non-vegetated (8.5× and 7.5× less DIN in the F and H treatment respectively; Fig. 5c). The principal difference for the vegetated vs. non-vegetated treatments was the ability of the vegetation to decrease the total DIN pool available for export through the system. Thereby, providing more evidence for the effectiveness of vegetated areas at removing excess N from porewater. The maintenance of low porewater [DIN] in the vegetated plots provided strong limitations on advective N flux from the marsh to adjacent waters from tidal infiltration and drainage or by Fickian diffusive flux from the marsh to overlying water during flooding (Chambers et al., 1992; Gardner et al., 1987; Morris, 1991; Rozema et al., 2000).

NH_4^+ can accumulate in soils for many reasons, including lack of oxygen and organic matter. Macrophytes oxygenate soils through the leaking of oxygen from roots and rhizomes and provide a large source of organic matter through decaying plant material, thereby, increasing the chances of the aerobic process of nitrification occurring. When this newly derived NO_3^- encounters prevalent anoxic conditions in marsh sediments, the process of denitrification can occur and is likely the dominant route of N removal from marsh systems (Tobias and Neubauer, 2009). This coupled nitrification–denitrification aided by the presence of macrophytes (Hammersley and Howes, 2005) and direct uptake by the macrophytes is the most plausible reason we observed drastically lower [DIN] in our vegetated plots. The isotope calculations we used account only for direct denitrification of the $^{15}NO_3^-$ and do not include this coupled nitrification–denitrification. Depending upon the amount isotopic dilution of the $^{15}NO_3^-$ from nitrification (observed primarily in the vegetated treatments) the denitrification estimate based on the measured $^{15}N_2$ (equation (2)) should be considered a conservative denitrification estimate.

Other studies have shown the presence of macrophytes can increase N removal in marshes (Hammersley and Howes, 2005). Our study offers more suggestive evidence that marsh macrophytes enhance N removal from porewater. The marsh macrophytes, in our study, did this through the combination of direct uptake and facilitating bacterial N removal processes, leading the vegetated treatments to have lower porewater [DIN] than unvegetated treatments.

4.2. [NO_x]:[Br^-] ratios vs. isotopic approach at estimating removal of NO_3^- from a groundwater plume

From the SGW, there were similar nutrient removal capability results from [NO_x]:[Br^-] and isotopic import–export measurements with differences of <2% (Fig. 5e and Table 2). These findings demonstrate a more economical measurement of nutrient to conservative tracer ratios may yield similar results to isotopic import–export measurements in projects similar to this where only bulk removal is concerned. However, it is important to consider that using the [NO_x]:[Br^-] is associated with limitations and precautions.

First, the [NO_x]:[Br^-] can only estimate NO_3^- removal from a known source of NO_3^- (e.g. SGW). When DNRA is prevalent, the use of this ratio to estimate N removal is hindered since the input NO_3^- changes form to NH_4^+. Therefore, the [NO_x]:[Br^-] would show a reduction in NO_3^- when there was no net DIN removal, simply a chemical transformation to NH_4^+. DNRA of the input NO_3^- did occur in our plots, primarily in the upland edge of the C treatment; however, we paired our measurements with porewater DIN measurements. Pairing the [NO_x]:[Br^-] with porewater

DIN measurements (NO_3^-, NO_2^-, and NH_4^+) minimizes the chance of mistaking DNRA for NO_3^- removal, especially if background DIN is determined in addition to analyzing DIN throughout the experiment.

Second, this [NO_x]:[Br^-] ratio cannot discern the fate of the removed N (e.g. denitrification, plant uptake, etc.). The fate of removed N is important to consider when determining N removal capabilities. Most nitrogen assimilated into plant biomass is only temporarily stored until the plants senesce and decompose, whereas outgassing of N is a more permanent removal of N from the system. Therefore, if permanent N removal is the primary goal of a restoration effort, outgassing should be considered the primary success indicator. The use of isotopes allows for fates and retention times of the attenuated N to be determined, whereas when using [NO_x]:[Br^-] alone these calculations are impossible.

Furthermore, [NO_x]:[Br^-] has long been used in freshwater systems (Bowmer, 1987; Kessavalou et al., 1996; Schuh et al., 1997) because freshwater has little to no Br^- present. In saline water [Br^-] is ≈0.8 mM, so robust calculations for introduced concentration and estimated dilution should be conducted when using the [NO_x]:[Br^-] in saline environments. We added approximately 10× the seawater concentration of Br^-, were working in a brackish system with [Br^-] lower than seawater, and had very little dilution of the SGW. Therefore, our [Br^-] data is robust. However, it would have been more difficult to discern background Br^- from SGW derived Br^- if large dilutions of the SGW with seawater had occurred.

4.3. Marsh macrophytes and denitrification are similarly important routes of N removal from SGW

Through the use of isotopic tracer, macrophytes and N_2 production (predominately denitrification) were determined to be the dominant routes of NO_3^- removed from the SGW and similarly important. N_2 production measurements indicate the C treatment produced more $^{15}N_2$ from the NO_3^- in the SGW, largely driven by differences at well A, than the F or H treatment. However, this finding does not necessarily imply the C treatment can remove the same quantity of N through N_2 production as the F and H treatments. First, macrophytes in the F and H treatments competed with bacteria (responsible for N_2 production) for the N from the SGW while this competition did not exist in the C treatment. Therefore, more of the traceable N from the SGW was available to be denitrified in the C treatment. Second, the $^{15}NO_3^-$ enrichment in the H and F treatments were ≈53% and ≈15% that measured in the C treatment, respectively. We have attributed this to enhanced nitrification in the vegetated plots. When $^{15}N_2$ production is adjusted for this differential $^{15}NO_3^-$ MFE, the total (direct denitrification of SGW $^{15}NO_3^-$ + coupled nitrification–denitrification of ambient N) denitrification rates were ≈1.5× and ≈2.7× higher in H and F treatments, respectively. Thus, from a purely denitrification perspective, the vegetated treatments retain a higher NO_3^- buffering capacity against higher acute loads, and against chronic NO_3^- loads of long duration.

Unaccounted portions of N removed from the SGW for the F and H treatments can be attributed to a rapid removal of isotopically labeled N derived from the SGW between the injectate and first well (A; Fig. 6). Uptake of this SGW derived ^{15}N would decrease the mass of ^{15}N reaching well A, thereby, causing the initial SGW isotopic signal to be heavily depleted when mixed with background N. Samples for isotopic concentrations in macrophytes and porewater for the top portion of the plot were taken at well A. However, the SGW was heavily depleted of N and ^{15}N prior to arriving at well A for the F and H treatments (Figs. 5 and 6). The lack of macrophyte and porewater samples between the INJ and well A leads to an underestimation of N removal. Given that macrophytes and denitrification accounted for similar proportions of measureable N removal from the SGW, we can assume that they would be responsible for similar proportions of removal between the injectate and well A.

4.4. H restoration design is as effective as F design at removing N at 2 years after planting

This study is the first to our knowledge to evaluate nutrient removal effectiveness of multiple *J. roemerianus* restoration designs and compare those designs to adjacent non-vegetated plots. Another study was conducted on these same plots comparing plant performance between restored designs and an adjacent natural marsh as well as cost-effectiveness of the restoration designs for increases in vegetated area over time (Sparks et al., 2013). Their findings indicated no differences among treatments with regards to plant morphology, physiology, and growth (i.e. potential for similar functionality). With regards to vegetative area over time, they found higher cost-effectiveness for the H treatment. Interestingly, we did not find any differences in nutrient removal capacity between the two restoration designs in this study. Our findings complement the previous research on this restoration site and indeed show similar functionality for nutrient removal between both restoration designs.

Given higher cost-effectiveness of the H design at 2 years after planting for vegetated area (Sparks et al., 2013) and similar nutrient removal capabilities between F and H designs, the H design must also be more cost-effective with regards to nutrient removal. While we did not run this same experiment in a natural marsh, it is highly likely the F treatment mimics natural marsh conditions due to the sod transplantation planting method used and complete planting of the plot. Assuming the F treatment and a natural marsh perform similarly with regards to nutrient removal, our results for the H treatment are even more interesting. Over a relatively short time scale (2 years), an area planted at half the planting density of another area can achieve similar levels of nutrient removal, which has been suggested as the most monetarily valuable ecosystem service for salt marshes (Costanza et al., 1997). Other studies have demonstrated a disparity between ecosystem services provided in restored and natural marsh sites over short time scales (Zedler and Callaway, 1999). While we cannot assume other ecosystem functions and services, such as faunal habitat, are equivalent between our restored marsh and a natural marsh, it is important to note the macrophyte performance (Sparks et al., 2013) and nutrient removal capabilities are likely similar.

5. Conclusion

In summary, these results suggest small-scale restored marshes are effective at removing nitrogen pollution. Additionally, planting marshes at 100% planting density and 50% planting density show similar levels of nitrogen removal capacity 2 years after planting, suggesting the 50% planting density may be a more cost-effective planting design in certain scenarios when nutrient removal is the target goal of a restoration project. Due to the small-scale designs, both managers and private landowners can utilize these results for future restoration projects. To broaden the applicability of this research to a global audience, similar testing of additional restoration techniques, designs, and plant species should be undertaken.

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