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Effect of Fluctuating Salinity on Potential Denitrification in Coastal Wetland Soil and Sediments

Brett M. Marks
Dep. of Oceanography & Coastal Sciences
College of the Coast & Environment
Louisiana State Univ.
Baton Rouge, LA 70808

Lisa Chambers
Department of Biology
University of Central Florida
Orlando, FL 32816

John R. White*
Dep. of Oceanography & Coastal Sciences
College of the Coast & Environment
Louisiana State Univ.
Baton Rouge, LA 70808

The coastal wetlands of southern Louisiana provide an ideal environment for removing nitrate (NO_3^-) from the Mississippi River before discharge into the Gulf of Mexico where it can contribute to hypoxia. However, denitrification, the primary mechanism of excess N removal, may be sensitive to the fluctuating salinities that characterize coastal wetlands. Salinity can shift from salt to fresh during river inflows, or from fresh to salt during storm surge events. This study investigated the impact of shifting salinity on potential denitrification rates in marsh soils and bayou sediments from the fresh and salt marshes of Brenton Sound estuary. Potential denitrification rates were quantified using bottle incubations for both short-term (2 d), and longer-term (11 d) studies. Fresh marsh sites had higher denitrification enzyme activity (DEA), but all soils and sediments achieved high denitrification when exposed to ideal conditions. Pulses of freshwater (0 ppt) in salt marsh soils decreased potential denitrification rates by 98% for an 11-d study, indicating the rapid opening of a freshwater diversion could significantly reduce that ability of coastal wetlands to provide significant nutrient-removal before discharge to the coastal ocean. Pulses of intermediate salinity (15 ppt) water stimulated denitrification rates in the fresh marsh soil by 75%, while full salinity seawater (35 ppt) suppressed potential denitrification by 73% for 11 d. This research demonstrates the sensitivity of the denitrifying microbial consortia to rapid shifts in salinity and this information can be used to guide water managers on maximizing NO_3^- removal in Mississippi River surface water diversions.

Abbreviations: DEA, denitrification enzyme activity; DI, deionized; DOC, dissolved organic carbon; MBC, microbial biomass carbon; TOC, total organic carbon.

Core Ideas

- **Fresh water pulse to salt marsh soil reduced denitrification.**
- **Salt water reduced denitrification by 73% in fresh marsh soil.**
- **Coastal storm surges can dramatically reduce denitrification.**
- **River flood pulses can reduce salt marsh soil denitrification.**

The coastal wetlands of southern, deltaic Louisiana represent the last line of defense for removing excess nitrate (NO_3^-) from the Mississippi River before discharge into the Gulf of Mexico. High river NO_3^- concentrations (~2 mg N L^{-1}) have resulted in eutrophication, harmful algal blooms (Bargu et al., 2011), and the subsequent development of a substantial hypoxic zone on the adjacent continental shelf (Turner and Rabalais, 1994; Rabalais et al., 1996). Denitrification, the reduction of NO_3^- by facultative anaerobic soil microbes, typically occurs at high rates in wetlands due to the combination of low O_2 availability and high organic C content (White and Reddy, 1999; 2003; Valiela et al., 2000). It is estimated that the restoration of approximately 10 million ha of wetland and riparian areas throughout the Mississippi River watershed could remove enough NO_3^- from the river, primarily through denitrification, to eliminate hypoxia in the Gulf of Mexico (Mitsch et al., 2001). While this magnitude of wetland restoration may not be feasible, a few large-scale river reconnection projects have been implemented in the Louisiana coastal zone, called diversion projects. For example, the Davis Pond freshwater diversion (completed in 2002 with a maximum discharge rate of 302 m^3 s^{-1}) was designed to combat saltwater intrusion and land loss in Barataria Basin (DeLaune et al., 2013). However, it has also effectively reduced surface wa-

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*Corresponding author (jrwhite@lsu.edu).

ter NO_3^- concentration by approximately 75% in the receiving wetland during low flow conditions, down from an input concentration of 2 mg N L^{-1} from the Mississippi River (Gardner and White, 2010). Likewise, studies conducted in wetland soils downstream of the Caernarvon freshwater diversion (completed in 1991 with a maximum discharge rate of 225 m^3 s^{-1}) indicated a 88 to 97% NO_3^- removal efficiency (Lane et al., 1999), with denitrification being the major pathway of loss (VanZomeren et al., 2012). The Bonnet Carré spillway, a flood release valve for the city of New Orleans, can divert up to 18% of the Mississippi River and potentially load more than 20,000 metric tonnes of nitrate N into the Lake Pontchartrain estuary in 1 mo. In this system, removal is primarily by phytoplankton uptake with only moderate reductions by denitrifications observed in this open water system (Roy and White, 2012; Roy et al., 2013).

Researchers agree coastal wetlands can serve as a buffer and sink for nutrients from upland sources that cause eutrophication in coastal marine systems (Hatton et al., 1982; Reddy et al., 1993; Lane et al., 1999; VanZomeren et al., 2013), but the physical conditions in coastal ecosystems can be highly dynamic, reducing the predictability of ecological functions like NO_3^- removal. In particular, pulsing events, such as river floods and coastal storm surges, are a natural attribute of coastal wetlands with some positive consequences, including promoting plant productivity, sediment deposition, and wetland soil accretion (Nyman et al., 1990; Day et al., 1995). However, the quick, dramatic shifts in salinity that often accompany coastal pulsing events can also significantly alter the physiochemical properties of the soil and impart osmotic stress to microorganisms.

Because soil microbes regulate many biogeochemical cycles in coastal wetlands, including denitrification, any temporary reduction in microbial activity could negatively impact their expressed ecological function. For example, the introduction of high salinity surface water into previously fresh marsh soils and sediments can cause rapid soil cation exchange that releases NH_4^+ and Fe^{2+} into the soil solution (Portnoy and Giblin, 1997; Weston et al., 2006) and enhances the adsorption of PO_4^{3-} (Jun et al., 2013), altering the chemical environment of soil microbes. Furthermore, soil microbial populations are typically adapted to either freshwater conditions (hyper-osmotic regulators) or saline conditions (hypo-osmotic regulators), and can experience osmotic stress during salinity pulses that interrupts cellular function, growth, or even lead to cell lysis (Frankenberger and Bingham, 1982; Hart et al., 1991; Saviozzi et al., 2011; Rath and Rousk, 2015). A laboratory incubation study demonstrated a temporary reduction in the metabolic activity of freshwater wetland soil microbial communities (as indicated by a decrease in the rate of CO_2 + CH_4 flux) when pulsed with salinities $\geq$ 14 ppt (Chambers et al., 2011). Other studies have demonstrated the longer-term resilience of microbial communities to salinity pulsing events. Rates of soil organic C cycling typically returned to pre-pulsed levels within 9 d of an event, even when the salinity difference was as much as 35 ppt higher (or lower) than the typical ambient salinity of the soil (Chambers et al., 2013). However, a more precise estimate of the lag period between a salinity shift and the re-establishment of the microbial functions and rates observed before the shift has not been determined, nor has research addressed the unique salinity concentration thresholds that elicit a change in the rate of biogeochemical processes for specific microbial consortia, such as denitrifiers.

Nitrogen cycling appears to be significantly impacted by salinity pulsing events, raising concern that the NO_3^- reduction efficiency of Louisiana's coastal wetlands could be diminished by fluctuating surface water salinities. Numerous studies have documented increased NH_4^+ availability in fresh marsh soil porewater following exposure to increased salinity. This increase has been attributed to abiotic processes, such as cation exchange, and biotic processes, including soil mineralization, reduced activity of nitrifying bacteria, and the inhibition of nitrifying enzymes by chloride (Cl^-) in saltwater (Roseberg et al., 1986; Portnoy and Giblin, 1997; Baldwin et al., 2006; Weston et al., 2006; Gennari et al., 2007; Seo et al., 2008; Chambers et al., 2013). Furthermore, there is evidence of a direct suppression of denitrifying bacteria due to salinity (Wu et al., 2008), with Cl^- toxicity suggested as the possible cause (Seo et al., 2008).

Previous studies indicate that pulses of freshwater into wetland brackish or saline soils can affect the rate of soil respiration (CO_2 flux) and overall soil organic C loss (Chambers et al., 2013). This effect may be stimulatory (e.g., increased microbial activity due to reduced osmotic stress and the flushing of deleterious compounds, such as sulfide), or inhibitory (e.g., decreased microbial activity as organisms adjust their internal osmotic potential to conform to the new environment; King and Wiebe, 1980; King et al., 1982; Hart et al., 1991; Oren, 2008). To our knowledge, the impact of freshwater pulses on potential denitrification rates in saltwater marshes and bayous has not been previously evaluated.

This study sought to quantify the effect of a dramatic decrease in salinity of salt marsh soil and sediments due to the opening of a freshwater river diversion, and an increase in salinity, such as due to a hurricane storm surge, on potential denitrification rates in freshwater and saline soils and sediments. We hypothesized that a significant (>15 ppt) change in water salinity, whether it is an increase or decrease from the ambient salinity condition, would cause a short-term decrease in denitrification rate due to osmotic stress. However, the suppression of denitrification would be greater with salinity increases due to Cl^- toxicity inhibiting denitrifying bacteria. We also sought to quantify, (i) how denitrification potential differs between salt marsh soil, salt bayou sediment, fresh marsh soil, and fresh bayou sediment, and (ii) the timescale at which denitrification rates recover following a salinity shift.

METHODS AND MATERIALS

Study Area and Sample Collection

Soils and sediments were collected in Breton Sound estuary, a large coastal marsh-bay complex located downstream of the Caernarvon freshwater diversion. The estuary consists of approximately 1100 km^2 coastal marshes, as well as abundant bayous,

lakes, and canals (Lane et al., 2006). The diversion is located at Mississippi River Mile 81.5 on the east bank, approximately 24 km downriver of New Orleans, LA, and was designed to reduce the effects of salt water intrusion in the estuary while also helping to combat land loss, enhance primary production, and stimulate the commercially and recreationally important fish and wildlife industries (Louisiana Department of Natural Resources, 2003). Salinity within the estuary can be highly variable depending on the flow rate through the diversions, but tends to be fresh (<0.5 ppt) in the upper estuary, and gradually increases to 35 ppt at the coastal ocean outer edge of the Breton Sound (Lane et al., 2007).

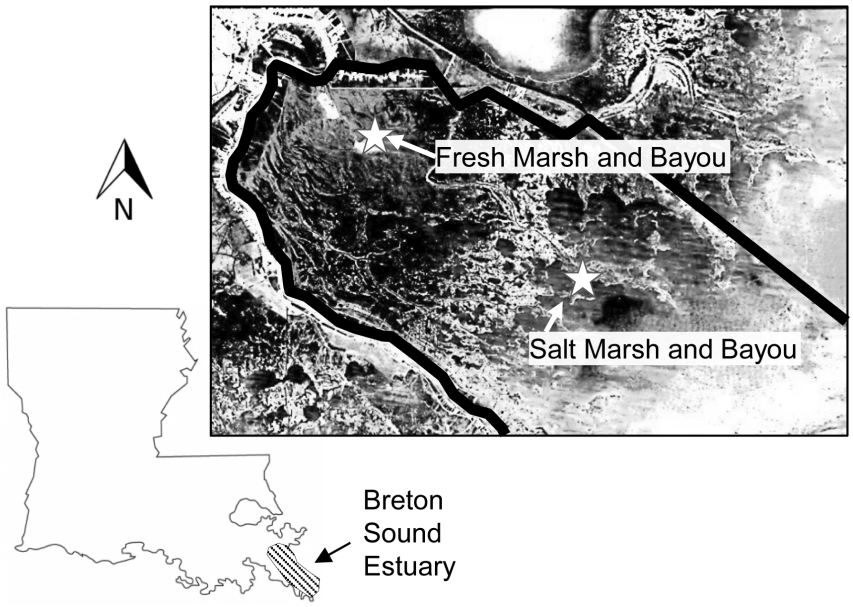

Fig. 1. Site location map for Benton Sound Estuary (Louisiana, USA) and the approximate freshwater and salt water sampling locations.

A total of 28 soil cores were collected from two distinct salinity regimes (salt marsh and fresh marsh) within the Breton Sound estuary in April 2009 (Fig. 1). At the salt marsh site (dominated by *Spartina alterniflora* and located 47 km southeast of the diversion), seven replicate soil cores were collected within a 3-m^2 area of the vegetated marsh (29°39¢52.4² N, 89°36′35.6² W) and the adjacent bayou (29°39¢52.0² N, 89°36′34.9² W). Likewise, seven replicate soil cores were also collected from a freshwater marsh site (29°48′21.8² N, 89°52′28.4² W) and the adjacent bayou (29°48'05.0" N, 89°52'23.7" W), located approximately 7.5 km southeast of the diversion structure and dominated by *Schoenoplectus americanus* (Fig. 1). During the spring of 2009, discharge rates from the Caernarvon diversion ranged from 14 to 28 $m^3 s^{-1}$ (Fig. 2). Soil samples were collected by push core method using a 7-cm diam. clear plexiglas tube. The top 10 cm of soil were extruded in the field, put in labeled zip-lock bags, and placed in coolers on ice. Upon return to the lab, the samples were hand mixed and any large root fragments were removed (henceforth referred to as the "unhomogenized" samples). Subsamples, 60 g wet weight from each core sample, were combined and homogenized in an electrical blender to create a slurry to represent the four sampling sites: salt marsh soil, fresh marsh soil, salt bayou sediment, and fresh bayou sediment (henceforth referred to as the "homogenized" samples). Unhomogenized samples were used to represent variability within the field site, while the homogenized samples were used to represent variability in the response to treatment conditions in the laboratory. The samples were stored in polyethylene containers and at 4°C until incubation.

Soil Property Analysis

Soil characterization (moisture content, bulk density, porewater salinity, organic matter content, total C, total N, extractable dissolved organic C [DOC], and microbial biomass C [MBC]) was conducted on the homogenized composite soils from each of the four sites, as well as on the bulk unhomogenized samples from each of the 28 soil cores to capture the field variability. Moisture content and bulk density were determined by oven drying wet weight subsamples at 70°C until constant dry weight in a forced air oven. Bulk density was determined for each of the 28 unhomogenized samples and was expressed as g dry wt cm^{-3}. Porewater salinity was measured in the supernatant of a 30-g subsample of homogenized soil from each of the four sites using a model 85–50 YSI sonde (YSI Environmental, Yellow Springs, OH) following centrifuging at 4000 × *g* for 10 min. Total C and N were measured on the dried, ground subsample using an Elemental Combustion System (Costech Analytical Technologies, Valencia, CA). The organic matter content was estimated by mass loss-on-ignition (LOI), where dry soils were combusted at 550°C for 4 h and the final weight was subtracted from the initial weight. The microbial biomass (MB) fumigate-extraction method after Vance et al. (1987) was used to determine MBC and extractable DOC. Triplicate 5-g wet weight subsamples from each of the four sites were prepared in duplicate, one set was fumigated with chloroform for 24 h and

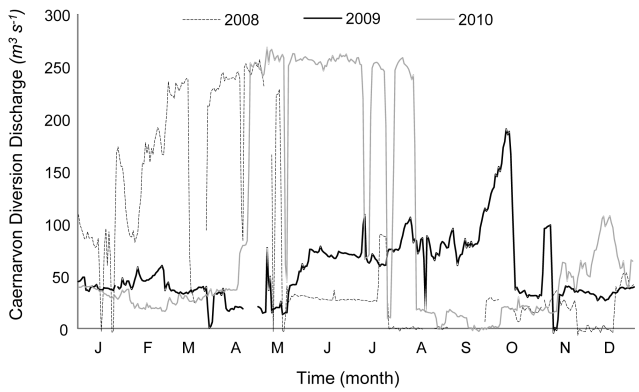

Fig. 2. Annual freshwater discharge hydrographs for the study year (2009), and the years before (2008) and after (2010) the study. Data retrieved at http://nwis.waterdata.usgs.gov/nwis/dv, USGS gauge 295124089542100.

the other set served as the unfumigated control. Following the chloroform treatment, both fumigated and unfumigated samples were extracted with 25 mL of 0.5 mol L^{-1} K_2SO_4, agitated for 30 min on a longitudinal shaker, and centrifuged 10 min at 4000 × *g*. Samples were vacuum filtered through a 0.45-μm filter and stored at 4°C until analyzed for dissolved total organic carbon (TOC) (Shimadzu Scientific Instrument TOC- VCSN, Columbia, MD). The dissolved TOC in the unfumigated control sample (μg kg^{-1}) represented extractable DOC, while the difference between the fumigated and unfumigated TOC represented MBC (White and Reddy, 2001).

Denitrification Enzyme Activity

Denitrification enzyme activity was determined for each of the 28 unhomogenized samples (seven from each site) as well as the homogenized samples (one from each site, analyzed in triplicate) using the methods developed by Tiedje (1982) and modified by White and Reddy (1999). Five-gram wet weight subsamples were placed in 70-mL glass serum bottles, sealed with a rubber septa and aluminum crimp cap, evacuated to −75 kPa, and purged with high purity N_2 gas (99.99% O_2–free) for 3 min. Soil slurries were created by adding 10 mL of N_2–purged deionized (DI) water and 15% of headspace was replaced with acetylene gas (C_2H_2) while maintaining atmospheric pressure within the bottle (Yoshinari and Knowles, 1976). Acetylene distribution was achieved by shaking on a longitudinal shaker for 30 min. Ten milliliters of DEA solution (56 mg KNO_3–N L^{-1}, 288 mg dextrose-C L^{-1}, and 500 mg chloramphenicol L^{-1}) were added to create a slight overpressure. The enzyme inhibitor, chloramphenicol, was used to prevent new enzyme synthesis during the 2-h incubation (Smith and Tiedje, 1979). Samples were incubated in the dark at 25°C on a longitudinal shaker. Headspace samples were collected at 30, 60, 90, and 120 min and analyzed on a Shimadzu GC-8A equipped with a 10 mCi ^{63}Ni electron capture detector operated at 300 (Shimadzu Scientific Instruments, Columbia, MD). A 1.8-m long by 2-mm i.d. stainless steel column packed with PoropakQ (0.177–0.149 mm; 80–100 mesh) was used (Supelco, Bellefonte, PA). The N_2O production was calculated as mg N_2O-N kg^{-1} soil using the Bunsen adsorption coefficient for 25°C (Tiedje, 1982). The detection limit was 0.006 mg N_2O–N kg^{-1} h^{-1}. The carrier gas (% CH_4 in Argon) flow rate was 30 mL min^{-1} with an injector temperature of 150°C and column temp of 30°C.

Short-Term Potential Denitrification

Triplicate subsamples from each of the four homogenized samples (salt marsh, salt bayou, freshwater marsh, and fresh bayou) were prepared by placing 5-g wet weight subsamples into 70-mL serum bottles. Each bottle was then sealed with septa and aluminum crimps, evacuated, and purged with N_2–gas to create an anoxic headspace, as described above. All bottles received 20 mL of a N_2–purged solution containing non-limiting C and N (14 g of glucose and 5 g of KNO_3 L^{-1}), but half received the solution prepared in a matrix of 0 ppt DI water, and half in a matrix of 35 ppt filtered seawater. An acetylene block was also utilized by replacing 15% of the headspace with C_2H_2 (Yoshinari and Knowles, 1976). The slurries were continuously agitated in the dark on a longitudinal shaker at 25°C and headspace gas samples were extracted at 2, 12, 24, and 48 h and analyzed on a Shimadzu GC-8A equipped with an ECD (Shimadzu Scientific Instruments, Columbia, MD). The N_2O production was calculated using a Bunsen adsorption coefficient of 0.544. Phase I (lag phase) was calculated from results between 0 and 24 h. Maximum denitrification rates (Phase II) from this short-term experiment were determined with data collected between 24 and 48 h.

Longer-Term Potential Denitrification

Triplicate subsamples from each of the four homogenized samples were prepared by placing 2-g wet weight subsamples into 160-mL serum bottles. Each bottle was then sealed, evacuated, and purged with N_2–gas for 5 min to create an anoxic headspace, as described above. For this study, three different salinity-matrices were prepared, 0 ppt DI water, 15 ppt mixed filtered saltwater and DI water, and 35 ppt filtered seawater. All solutions were added as 20 mL and contained 14 g L^{-1} of glucose and 5 g L^{-1} of KNO_3, were purged with N_2 gas. An acetylene block was used by replacing 15% of the headspace with C_2H_2 (Yoshinari and Knowles, 1976). The slurries were incubated in the dark at 25°C while being continuously agitated on a longitudinal shaker. Headspace gases were extracted every 24 to 36 h over 11 d and analyzed on a Shimadzu GC-8A equipped with an ECD (Shimadzu Scientific Instruments, Columbia, MD) and N_2O production was calculated using the Bunsen adsorption coefficient of 0.544. For this study, the 0 ppt treatment was considered the ambient/control condition for the freshwater soils/sediments, and the 15 ppt treatment was considered the ambient/control condition for the salt marsh soils/sediments.

Data Analysis

Differences in soil properties according to sampling site were analyzed using a one-way ANOVA. A two-way ANOVA was used to identify significant differences in DEA among soil/sediment type and homogenization. Differences in potential denitrification rates among soil type and salinity treatment were also analyzed using a two-way ANOVA model for both the short-term and longer-term studies. Significance was set at $p < 0.05$ and post-hoc ANOVA testing was conducted with Tukey's Studentized (HSD) test. Levene's Test and Bartlett's Test for Equality (both $p < 0.05$) were utilized to verify the homogeneity of variance and normality of distribution for experimental results, and data was transformed as needed. Denitrification enzyme activity, short-term potential denitrification, and longer-term potential denitrification data were log transformed before analysis to achieve the assumption of normality. Correlations were used to identify the relationships between soil properties (moisture content, bulk density, porewater salinity, organic matter content, total C, total N, extractable DOC, MBC, DEA).

SAS 9.1 was used to conduct all statistical analysis (SAS Institute Inc., Cary, NC).

RESULTS

Soil Properties

Porewater salinity ranged from 9.28 to 9.34 ppt at the salt marsh sites, and from 0.23 to 0.26 ppt at the fresh marsh sites (Table 1). Soil moisture content was higher in the fresh marsh soil than all other soil and sediment samples ($p < 0.05$), while bulk density was higher in the salt marsh soil and salt bayou sediment, and lowest in fresh bayou sediment. Soil moisture was negatively correlated with bulk density ($p < 0.001$; Table 2). Organic matter content, total C, and total N were more than 2.5 times greater in the fresh marsh soil than all other soil/sediment samples and all three parameters were positively correlated with soil moisture and with one another. The MBC was also greatest in the fresh marsh soil ($p < 0.05$) and showed a positive correlation with organic matter content, total C, and total N ($p < 0.001$). Extractable DOC was significantly different at all sampling locations, with the highest concentrations in the fresh marsh soil, followed by the salt marsh, salt bayou, and fresh bayou samples. Extractable DOC was positively correlated with other measures of soil quality (e.g., organic matter content, total C, total N, and MBC; Table 2).

Table 1. Soil and sediment properties (mean ± standard deviation) determined for the homogenized, composite samples (unless otherwise noted) from each of the four soil types. Different letters indicate significant differences ($p < 0.05$) based on a one-way ANOVA.

	Salt marsh	Salt bayou	Fresh marsh	Fresh bayou
Porewater salinity, ppt	9.28	9.34	0.26	0.23
Moisture, %	52.8 ± 8.6^a	56.3 ± 5.7^a	77.0 ± 2.0^b	60.4 ± 3.6^a
Bulk density, g cm^{-3}†	0.7 ± 0.1^a	0.6 ± 0.1^a	0.3 ± 0.1^b	0.5 ± 0.1^c
Organic matter, %	9.9	7.3	28.1	9.5
Total C, g C kg^{-1}	38.4	29.6	120.8	42.0
Total N, g N kg^{-1}	2.63	1.90	9.79	3.10
MBC, g kg^{-1} C	5.3 ± 0.3^a	4.7 ± 0.1^a	10.2 ± 0.5^b	5.2 ± 0.2^a
Extractable DOC, mg kg^{-1}	269 ± 1.4^a	132 ± 8.7^b	322 ± 5.1^c	103 ± 2.1^d

† Analyses performed on unhomogenized bulk samples. C = carbon; N = nitrogen; MBC = microbial biomass carbon; DOC = dissolved organic carbon.

Table 2. Product-moment correlation coefficients for soil properties.† For $n = 12$ (df = 10), $r \geq 0.576$ is significant at $p < 0.05$ and $r \geq 0.708$ is significant at $p < 0.01$, $r \geq 0.823$ is significant at $p < 0.001$.

	Moisture	Bulk density	Organic matter	Total C	Total N	MBC	DOC
	%	g cm^{-3}	%	g C kg^{-1}	g N kg^{-1}	g kg^{-1} C	mg kg^{-1}
Bulk Density, g cm^{-3}	-0.969						
Organic Matter, %	0.827	-0.835					
Total C, g C kg^{-1}	0.841	-0.851	0.998				
Total N, g N kg^{-1}	0.844	-0.855	0.997	1.000			
MBC, g kg^{-1} C	0.844	-0.837	1.000	0.998	0.997		
DOC, mg kg^{-1}	0.451	-0.450	0.765	0.730	0.719	0.757	
DEA, µg N_2O-N kg^{-1}	0.595	-0.618	0.579	0.600	0.607	0.577	0.235

† C = carbon; N = nitrogen; MBC = microbial biomass carbon; DOC = dissolved inorganic carbon; DEA = denitrification enzyme activity.

Denitrification Enzyme Activity

Both the salt marsh and salt bayou had significantly higher DEA rates when homogenized, compared with the unhomogenized samples, whereas the process of mechanical homogenization did not significantly alter rates of DEA in the fresh marsh soils or fresh bayou sediments (Fig. 3). The fresh marsh soil (24.26 ± 15.02 µg N_2O-N kg^{-1} h^{-1}) and fresh bayou sediment (14.25 ± 4.59 µg N_2O-N kg^{-1} h^{-1}) had higher mean rates of DEA than either of the salt marsh soil (2.12 ± 1.74 µg N_2O-N kg^{-1} h^{-1}) or salt bayou sediment (0.39 ± 0.66 µg N_2O-N kg^{-1} h^{-1}). Denitrification enzyme activity was positively correlated with soil moisture, organic matter content, total C, total N, and MBC (Table 2).

Short-Term Potential Denitrification

Short-term (2 d) potential denitrification exhibited a two-phase rate, with an initial lag phase (Phase I) observed from 0 to 24 h, and a maximum rate (Phase II) from 24 to 48 h. Both the Phase I and Phase II potential denitrification rates showed a significant interaction between soil type and salinity treatment ($F = 4.5$, $p = 0.02$, and $F = 13.11$, $p < 0.001$, respectively; Table 3). During Phase I, potential denitrification was significantly higher in the fresh marsh soils (mean = 16.11 ± 2.58 mg N_2O-N kg^{-1} d^{-1}) than all other soils, regardless of salinity treatment. During Phase II, denitrification potential of the fresh marsh soil remained higher than all others (mean = 217.95 ± 148.16 mg N_2O-N kg^{-1} d^{-1}), with the exception of the salt marsh soil treated with saltwater (35 ppt), which had a mean rate of 154.6 ± 10.31 mg N_2O-N kg^{-1} d^{-1} (Fig. 4; Table 3). The salt marsh soil treated with fresh (0 ppt) water was the only condition to have a negative (not significantly different from zero) rate during Phase II (Fig. 4; Table 3).

Longer-Term Potential Denitrification

Longer-term (11 d) potential denitrification also exhibited a two phase rate, but the length of the lag phase (Phase I) differed due to the combined effects of the soil/sediment type and salin-

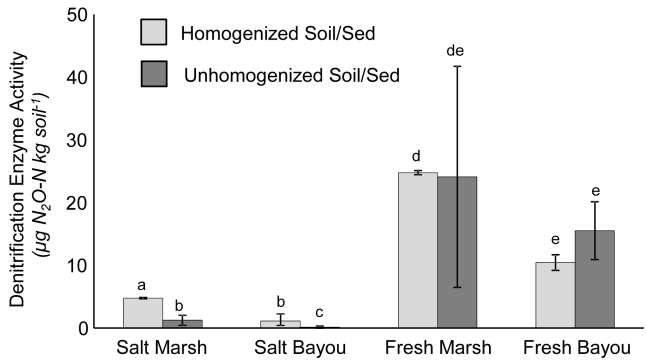

Fig. 3. Denitrification enzyme activity according to soil/sediment type and preparation procedure (n = 7 for unhomogenized, n = 3 for homogenized). Different letters represent significant differences ($p < 0.05$) according to a two-way ANOVA.

Table 3. Short-term potential denitrification rates (mean ± standard deviation) determined for the homogenized, composite samples according to soil type and salinity treatment (n = 3 for each). Rates are presented for two phases of denitrification, Phase I (lag phase, 0 to 24 h) and Phase II (maximum potential, 24 to 48 h). Different letters indicate significant differences between salinity treatments (down the column) within each phase; different numbers indicate significant differences between soil types (across the row) within each phase, according to a two-way ANOVA ($p < 0.05$).

	Salt marsh	Salt bayou	Fresh marsh	Fresh bayou
	mg N_2O-N $kg^{-1}d^{-1}$			
Phase I				
0 ppt	18.08 ± 1.98^{a,1}	6.23 ± 1.00^{a,2}	30.57 ± 12.04^{a,3}	6.53 ± 0.41^{a,4}
35 ppt	14.14 ± 1.02^{a,1}	2.64 ± 0.19^{b,2}	25.46 ± 3.71^{a,3}	4.19 ± 0.10^{b,4}
Phase II				
0 ppt	-8.08 ± 5.17^{a,1}	35.58 ± 14.06^{a,2}	210.20 ± 172.60^{a,3}	2.36 ± 0.35^{a,1}
35 ppt	154.62 ± 10.31^{b,1}	12.45 ± 7.79^{a,2}	225.70 ± 157.82^{a,1}	12.14 ± 5.27^{b,2}

ity treatment. Therefore, Phase I varied from 2 to 7 d in length, as determined by the shaped of the flux curve (Fig. 5). Longer-term potential denitrification rates for several treatment conditions exhibited a period of leveling-off, or even a decrease in flux rate, that typically occurred between Days 5 and 8 (Fig. 5). Both Phase I and Phase II longer-term potential denitrification rates differed due to the interaction of soil/sediment type and salinity treatment ($F = 18.9, p < 0.001$; $F = 37.1, p < 0.001$, respectively). In Phase I, increases in salinity above the control condition salinity (control defined as 15 ppt for salt marsh samples and 0 ppt for fresh marsh samples) resulted in a significant increase in potential denitrification rate for the salt and fresh marsh soils, while increases in salinity generally decreased the potential denitrification rate in the salt and fresh bayou sediments (Table 4). During Phase II, the potential denitrification decreased in the salt marsh soil when freshwater (0 ppt) was added, but 35 ppt water had no effect. Denitrification was stimulated by increased salinity in the salt bayou sediment, but was unaffected by freshwater additions. In both the fresh marsh soil and fresh bayou sediment, slight increases in salinity (15 ppt) enhanced denitrification rates, while large increases in salinity (35 ppt) lowered it (Table 4).

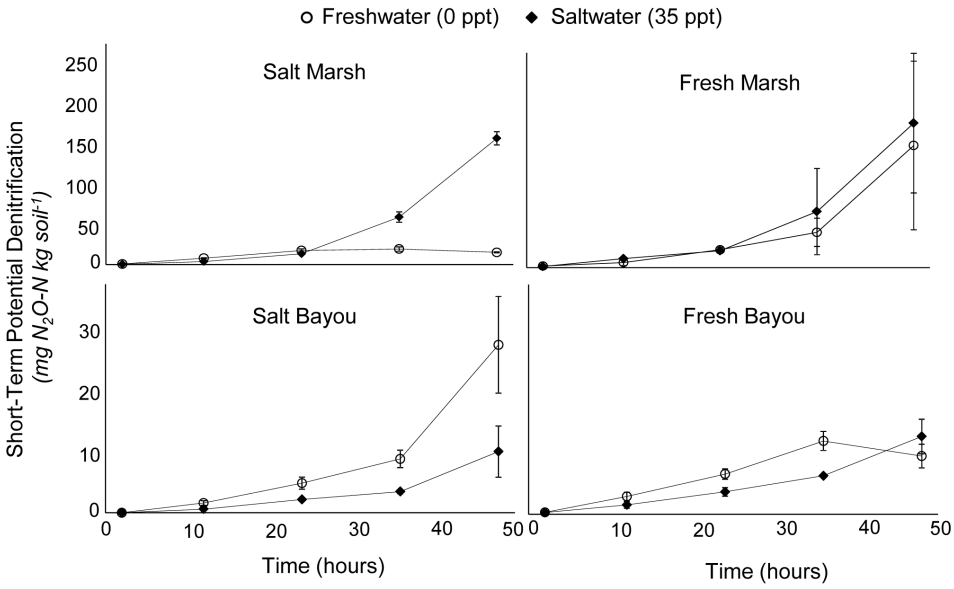

Fig. 4. Short-term (2 d) potential denitrification rates according to soil/sediment type and salinity treatment condition using homogenized soils (n = 3 for each condition). Error bars represent standard deviation.

DISCUSSION

Soil Properties and Denitrification Enzyme Activity

Soil characteristics and DEA varied greatly among the four sampling sites. When comparing the marsh soils, the fresh marsh had a higher moisture content, lower bulk density, and higher concentrations of organic matter, total C, total N, MBC, and DOC than the salt marsh site. Other studies have observed similar patterns when comparing freshwater and saltwater wetlands and have attributed the differences to higher plant diversity, greater net primary productivity, and lower rates of sediment deposition in freshwater coastal wetlands (Odum, 1988; Craft, 2007; Wieski et al., 2010). The bulk densities of the bayou sediments more closely resembled that of the salt marsh soil, with the fresh bayou sediment generally having higher soil quality (e.g., higher organic matter, total C, and total N) than the salt bayou sediment. This suggests the salt marsh soil and bayou sediments experience greater mineral sediment deposition, as to be expected due to a closer proximity and connectivity to the coast.

Denitrification enzyme activity assays, which utilize a short incubation time and an enzyme inhibitor to prevent *de novo* enzyme synthesis, can provide a snap shot of the in situ site conditions and relative rate of denitrification at the time of sampling (Smith and Tiedje, 1979; Groffman and Tiedje, 1989; Luo et al., 1996). Our study found DEA was highest in fresh marsh soil, followed by the fresh bayou sediment, and that DEA in both freshwater sites were significantly greater than in both salt marsh sites. In particular, the fresh marsh soil averaged approximately 8 times higher DEA than the salt marsh soil, and the fresh bayou sediment approximately 20 times higher than the salt bayou sediment. Previous studies conducted along natural coastal salinity gradients have found potential denitrification rates to be two to three times higher in freshwater wetlands, compared with salt marshes, and attributed this difference to the negative impacts of salinity on nitrifying bacteria, which provide the source of nitrate through nitrification (Joye and Hollibaugh, 1995; Craft et al., 2009). In this ecosystem, the proximity of the sites to the Caernarvon freshwater diversion is likely an even

more relevant explanation for the observed differences along the salinity gradient. The Mississippi River water discharged through the diversion serves as the dominant source of NO_3^- to Brenton Sound and previous studies have shown that most of this NO_3^- is rapidly removed and transformed on entering the estuary (Lane et al., 1999). Since DEA is known to directly correlate with surface water NO_3^- availability in anoxic, high organic matter environments (Schipper et al., 1993; Gardner and White, 2010), higher in situ denitrification potential at the freshwater end of the salinity gradient likely reflects greater exposure to NO_3^- enriched river water.

The DEA rates were also compared for the homogenized and unhomogenized soil and sediments from each site. This comparison revealed that the process of mechanical homogenization significantly decreased variance between samples collected at the same site, but also resulted in a slight overestimation of the DEA rates in the salt marsh soil and salt bayou sediment. The mechanical breaking-up of organic complexes in these soils and sediments may have mineralized a small amount of NO_3^- that stimulated new enzyme synthesis between the time of homogenization and the addition of the enzyme inhibitor. This demonstrates the sensitivity of the DEA assay to sample preparation in addition to environmental conditions and N availability.

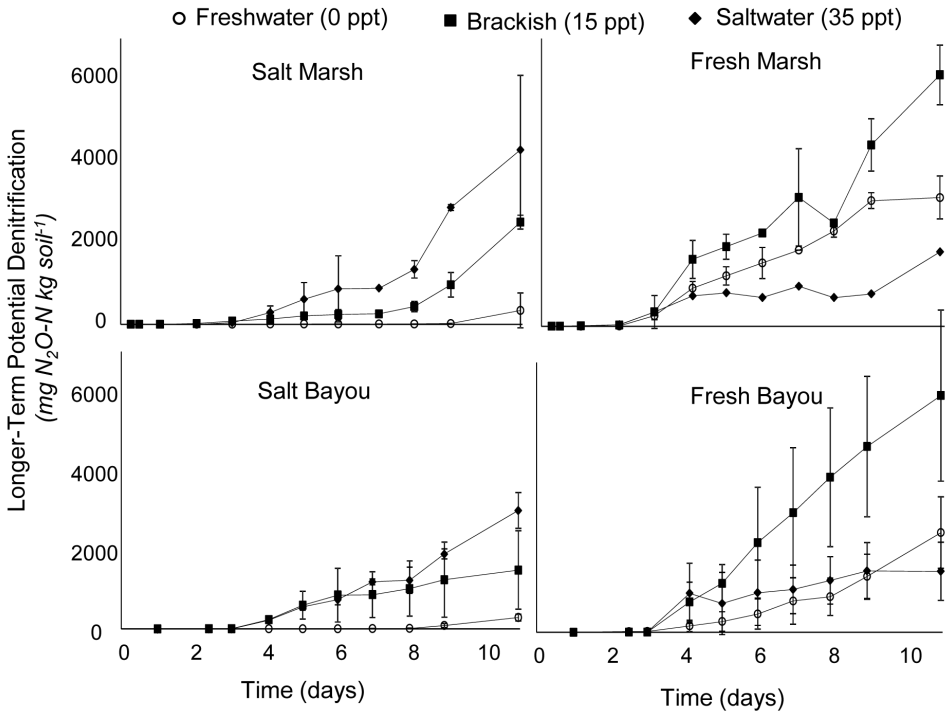

Fig. 5. Longer-term (11 d) potential denitrification rates according to soil/sediment type and salinity treatment condition using homogenized soils (n = 3 for each condition). Error bars represent standard deviation.

Impact of Freshwater Pulses on Potential Denitrification

The Caernarvon freshwater diversion was designed and is managed for the purpose of controlling salinity within Benton Sound estuary (Louisiana Department of Natural Resources, 2003). However, unlike a natural flood-pulse regime that involves seasonal overbank flooding and gradual shifts in the location of the tidal prism within the estuary, the magnitude and timing of freshwater inputs to Breton Sound through the Caernarvon diversion often exhibits a flashy hydroperiod and varies considerably from year to year (Fig. 2). High discharge events (i.e., fully opening the diversion structure) can decrease surface water salinities throughout the entire estuary, causing the systems to become completely fresh for a short period of time (<1 mo) (Lane, 2003; Lane et al., 2007).

In the short-term study, there was no measureable effect of salinity on denitrification for the first 24 h, but during Phase II, the rate of potential denitrification declined markedly to a negative flux (not significantly different from zero) in the salt marsh soil, but had no impact in the salt bayou sediment (Table 3). The differing effect of the freshwater pulse may be an artifact of the area's hydrology. The bayou sediments are continuously flooded and likely to receive some freshwater from the diversion even when it is only partially open, whereas the diversion must have a very high discharge rate in order for freshwater to overflow the bayou and flood the salt mash soils. If the bayou sediments typically experience greater fluctuations in salinity, especially exposure to freshwater, the microbial community may

Table 4. Longer-term potential denitrification rates (mean ± standard deviation) determined for the homogenized, composite samples according to soil type and salinity treatment (n = 3 for each). Rates are presented for two phases of denitrification, Phase I (lag phase, 0 to 2–7 d) and Phase II (maximum potential, 2–7 to 11 d). Different letters indicate significant differences between salinity treatments (down the column) within each phase; different numbers indicate significant differences between soil types (across the row) within each phase, according to a two-way ANOVA (p < 0.05). Italic values indicate the "control" condition based on the porewater salinity when the samples were collected.

	Salt marsh	Salt bayou	Fresh marsh	Fresh bayou
	mg N_2O-N $kg^{-1}d^{-1}$			
Phase I				
0 ppt	1.09 ± 0.68^{a,1}	1.1 ± 0.02^{a,1}	*4.95 ± 3.46^{a,2}*	*10.29 ± 3.57^{a,3}*
15 ppt	*35.06 ± 1.18^{b,1}*	*0.42 ± 0.18^{a,2}*	30.72 ± 6.15^{b,1}	0.85 ± 0.45^{b,3}
35 ppt	59.46 ± 26.85^{b,1}	0.18 ± 0.23^{b,2}	22.12 ± 5.71^{b,1}	3.28 ± 0.38^{a,3}
Phase II				
0 ppt	9.18 ± 5.67^{a,1}	94.31 ± 16.53^{a,2}	*373.08 ± 38.36^{a,3}*	*330.12 ± 39.88^{a,4}*
15 ppt	*507.32 ± 46.73^{b,1}*	*149.34 ± 88.65^{a,2}*	654.12 ± 157.38^{b,1}	917.21 ± 590.37^{b,1}
35 ppt	614.96 ± 315.38^{b,1}	373.42 ± 62.23^{b,1}	99.66 ± 36.54^{c,2}	44.76 ± 26.15^{c,3}

be better adapted to pulsing events than the salt marsh soil (Van Ryckegem and Verbeken, 2005). During the longer-term study, potential denitrification rate in the salt bayou sediment was similarly unimpacted by the freshwater pulse, but the salt marsh soil experienced, on average, a 97% lower denitrification rate during Phase I, and a 98% lower rate during Phase II (Table 4). If these freshwater effects translate to field conditions, the ability of Benton Sound's salt marshes to remove excess NO_3^- from the Mississippi River water may be significantly reduced when the diversion is fully opened for a time period sufficient to push the isohaline out past the salt marshes. Furthermore, the suppression of potential denitrification rates in a salt marsh soil exposed to a freshwater pulse is expected to exceed 11 d, as indicated by the fact that denitrification rates did not recover to that of the control salinity condition within the timeframe of the longer-term potential denitrification assay.

Impact of Saltwater Pulses on Potential Denitrification

Coastal Louisiana marshes are exposed to numerous saltwater flooding events driven by storms, high winds, waves, and extreme tides. The expansive low-lying deltaic topography increases the ability of storm surges to propagate landward, resulting in rapid inundation of large areas of the coastal zone wetlands with high-salinity ocean water for several days (Flather, 2001; Westerink et al., 2008; Li et al., 2009; Rego and Li, 2009). In the short-term study, the fresh bayou sediment showed a greater sensitivity to the saltwater (35 ppt) pulse than the fresh marsh soil, producing an initial decrease in denitrification potential (Phase I), followed by an increase (Phase II). A similar pattern of initial suppression by increased salinity, followed by a stimulatory effect (at least at moderate salinities) was mimicked in the longer-term study for fresh bayou sediments. We suspect the sensitivity of the fresh bayou sediment to saltwater pulses may be related to a smaller microbial community in these sediments, where MBC was approximately 50% less than that observed in the fresh marsh soil (Table 1). Microbial community biomass and diversity tend to be correlated (Córdova-kreylos et al., 2006), suggesting microbial communities with lower biomass may require more significant shifts in community structure to adapt to new conditions, temporality reducing other functions such as denitrification.

Interestingly, the maximum potential denitrification rate (Phase II of the longer-term study) for both freshwater sites occurred at the intermediate salinity (15 ppt), with the mean denitrification rates increasing by 75% in the marsh soils and 178% in the bayou sediments. Meanwhile, the highest salinity treatment (35 ppt) resulted in a substantial decline in denitrification in these same sediments (73 and 86% reduction in the mean relative to the 0 ppt control in the fresh marsh soil and bayou sediment, respectively). Activity maximums at intermediate salinities have been observed for nitrifiers, and have been attributed to low salt concentrations leading to NH_4^+ to desorption from the cation exchange complex and stimulate nitrification (Pathak and Rao, 1998; Magalhaes et al., 2005). Although nitrification and denitrification are often tightly coupled in coastal soils and sediments (Giblin et al., 2010), this is an unlikely explanation for the present study due to the fact the denitrifiers were already given with a non-limiting supply of NO_3^- as part of the assay. The suppression of denitrification at the highest salinity is consistent with previous work suggesting physiological stress inhibits the activity of denitrifiers at high salt concentrations (Rysgaard et al., 1999; Seo et al., 2008; Wu et al., 2008; Edmonds et al., 2009). This stress could be osmotic, and/or a consequence of high Cl^- and HS^- concentration in saline environments affecting the denitrification pathway (Roseberg et al., 1986; Joye and Hollibaugh, 1995; Seo et al., 2008). Other research has found a reduction in denitrification rates at high salinities may be due to a shift to the dissimilatory nitrate reduction to ammonium pathway (DNRA), which is favored by the high rates of sulfate reduction in saline environments, and could also result in lower N_2O-N flux rates (Giblin et al., 2010).

The addition of an intermediate salinity treatment (15 ppt) during the longer-term study provided a closer analog to the ambient salinity observed in the salt marsh sites at the time of sampling (Table 1), allowing for an investigation of how pulses of higher salinities (35 ppt) impacted potential denitrification in the salt marsh soils and salt bayou sediments. Similar to the findings from the freshwater sites, the salt bayou sediments were more sensitive to salinity changes than the salt marsh soils (Table 4), exhibiting an initial decrease in denitrification rate, followed by an increase. This result is again, likely related to microbial communities shifting to better adapted species, which could include changes in the structure and/or composition of the active microbial community. Further evidence of potential microbial community shift is suggested by slight lag periods in the N_2O-N flux observed in the longer-term assays, especially in the higher salinity treatment conditions (Fig. 5). Certain microbial species are stenohaline and better adapted to freshwater conditions, others to saline conditions, and still others are euryhaline and can tolerate large fluctuations in salinity (Hart et al., 1991; Oren, 2008). Studies indicate the lag time for salt-sensitive nitrifiers to be replaced by more salt-tolerant species can range from 14 to 24 d (Helder and De Vries, 1983; Coci et al., 2005). In this study, there appears to be a temporary reduction in microbial activity between Days 5 and 8 of the longer-term incubations, after which denitrification rates recovered or even accelerated, suggesting the lag period for these denitrifying communities to adapt to salinity change may be within a 5 to 8 d window of time and not generally observed in many shorter term incubation studies.

Implications for Management and the Response to Storm Surges

These results have important management and ecological implications. The current management of the Caernarvon freshwater diversion focuses primarily on the stage of the Mississippi River and the salinity regime within Brenton Sound estuary. Our study suggests the rapid opening of the diversion to the ex-

tent a pulse of freshwater from the Mississippi River inundates previously salt marsh sites could result in a dramatic (97–98%) decline in potential denitrification rates in salt marsh soils that can persist 11 or more days. This reduction of denitrification potential could increase the likelihood excess NO_3^- will reach the Gulf of Mexico where it may be assimilated by phytoplankton and contribute to hypoxia. The salt bayou sediments had generally lower rates of potential denitrification and appeared less sensitive to freshwater pulses than the salt marsh soil, possibly due to more frequent exposure to low salinity conditions.

This work also provides new information regarding the possible ramifications of saltwater pulses (e.g., hurricane storm surge) on denitrification within the estuary. While pulses of full-salinity water (35 ppt) are not expected to significantly alter the function of denitrifiers in the salt marsh soils, the maximum potential denitrification rate in fresh marsh soil exhibited a stimulatory effect at moderate salinities (75% higher at 15 ppt than under the freshwater control) and a significant suppression effect at full salinity (73% lower at 35 ppt than under the freshwater control). Severe storm surges (i.e., 35 ppt) can therefore be expected to diminish the denitrification capacity of the freshwater portions of the estuary for 11 or more days following a saltwater pulse, while intermediate saltwater pulses (i.e., 15 ppt) may actually enhance the denitrification capacity of the upper estuary. The ability to translate the results of these bottle incubations to field conditions requires further study, but this research provides preliminary evidence that salinity pulses can negatively impact the denitrification capacity of coastal soils and sediments and should be considered in diversion management decisions and when assessing the ecological impacts of storms on coastal nutrient dynamics.

CONCLUSIONS

In situ denitrification, as indicated by DEA, was highest in the fresh marsh soil, followed by the fresh bayou sediment, both of which exhibited a significantly greater denitrification rates than the salt marsh sites. This spatial pattern in denitrification was positively correlated with several indicators of soil quality (% OM, total C, total N, and MBC) and is also hypothesized to reflect the spatial pattern of high NO_3^- loading from the Mississippi River in the upper reaches of the estuary. This study indicates that given ample NO_3^- and labile C, all soils and sediments had a high potential capacity to support denitrification, establishing the Brenton Sound estuary a potentially critical buffer between the NO_3^-–enriched Mississippi River and the N-limited Gulf of Mexico.

Our original hypothesis that a salinity increase or decrease of >15 ppt would result in a temporary suppression in denitrification rate was only partially supported by the experiment. Indeed, a pulse of 35 ppt saltwater in the freshwater soils did reduce denitrification, while the intermediate salinity pulse (15 ppt) did not (and actually stimulated denitrification). However, the salt marsh sites had an ambient soil porewater salinity of only ~9 ppt at the time of sampling, yet a pulse of fresh water (0 ppt) was enough to almost completely cease the activity of denitrifiers during an 11-d incubation, suggesting the denitrifiers were close to their lower limit of salinity tolerance in the field. Our second hypothesis that the suppression of denitrification would be greater with salinity increases due to Cl^- toxicity was rejected. Based on the percentage change in denitrification rate relative to the control treatment, the freshwater pulse in the salt marsh soil actually reduced the activity of denitrifiers ~25% more than the saltwater pulse in the fresh marsh soil. Future work should focus on salinity effects on area denitrification rates and linking microbial community shifts to expressed rates of denitrification.

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