Fate of Nitrate in Vegetated Brackish Coastal Marsh

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The Caernarvon Diversion meters Mississippi River water into coastal marshes of Breton Sound, Louisiana (29°51′40.15″ N, 89°54′43.62″ W). Elevated levels of N in river water have sparked concerns that nutrient-loading may affect marsh resilience and belowground biomass, as evidence from several marsh fertilization studies suggests. These concerns have resulted from casual observations that fresh and brackish Breton Sound marshes, closest to the Mississippi River levee suffered extensive damage from Hurricane Katrina. The goal of this study was to determine the fate of nitrate (the dominant inorganic N form in the Mississippi River) in Breton Sound Estuary marshes. We hypothesized that the majority of the nitrate will be removed by denitrification and that nitrate-loading will not affect belowground biomass over several months of loading. To test this hypothesis, a mass balance study was designed using ^{15}N-labeled nitrate. Twelve plant-sediment cores were collected from a brackish marsh and six cores received deionized water (control), while another six (treatment) received 2 mg L^{-1} of ^{15}N-labeled potassium nitrate twice a week for 3 mo. A set of three control and treatment cores were destructively sampled after 3 mo and analyzed for ^{15}N in the aboveground and belowground biomass and the soil. The N isotopic label allowed for a mass balance to distinguish N removal pathways, including denitrification, surface algae uptake, soil microbial uptake and incorporation into aboveground and belowground biomass of the macrophytes. Twelve hours after the addition of the 2 mg N L^{-1} water, nitrate levels were typically below detection. Approximately 64% of all added labeled nitrate was unaccounted for which suggests gaseous loss. The remaining ^{15}N was incorporated in plant and soil compartments, the majority being the aboveground component. There were no significant differences in belowground biomass production between the nitrate loaded and the control cores after 3 mo.

Wetlands are effective at removing N by assimilation into organic material (immobilization) or by transformation to gaseous forms (denitrification or ammonia volatilization). Under anaerobic conditions, nitrate is used as an alternate electron donor by facultative anaerobes producing N gas (White and Reddy, 1999). Ideal conditions for denitrification to occur are present in wetlands, including organic C supplied by high primary productivity, nitrate-loading, and a paucity of oxygen. Denitrification is an important component of the global N cycle as it facilitates removal of bioavailable N to the atmosphere. Denitrification can occur at high rates in wetland soils and can therefore regulate primary productivity as well as mediate possible adverse effects of eutrophication (Lane et al., 1999).

Stable isotopic techniques have been used extensively as important tools in determining the ultimate fate of N additions to a wide range of ecosystems, specifically allowing us to identify N transformation pathways including immobilization, nitrification, and denitrification processes (Barraclough, 1991). A number of studies have shown that denitrification tends to be the major nitrate removal

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mechanism in wetlands, ranging between 89 and 95% (Lund et al., 1999; Reinhardt et al., 2006). However, it is recognized that other possible removal pathways for added nitrate are phytoplankton uptake, dissimilatory nitrate reduction to NH_4^+ and anaerobic ammonium oxidation (ANAMMOX) (Reddy and DeLaune, 2008).

While nutrient abatement is a critical function for preventing coastal eutrophication, there has been some recent concern that nutrient-loading can have adverse effects on wetland resilience to storms (Darby and Turner, 2008a,b). The Mississippi River drains 41% of the continental United States. As a result of intense agricultural practices in the basin, the river water has elevated concentrations of nutrients, particularly N. Additionally, wastewater inputs along the river's course increase the N load, which under the prevailing well-mixed, aerobic water column conditions, leads to NO_3–N as the dominant bioavailable inorganic N form (White et al., 2009). This inorganic form of N is in high demand for use in biological processes such as plant assimilation, microbial immobilization, and denitrification.

A proposed restoration tool in the Mississippi River delta region is the reintroduction of river water into adjacent coastal wetlands through diversion control structures to simulate the annual spring flooding from the Mississippi River before levee construction. The placement of levees has hydrologically isolated the riparian marshes from the river during the past century. In 1991, the Caernarvon Diversion was completed to restore some annual freshwater loading, directing up to 226 m^3s^{-1} (8000 ft^3s^{-1}) of Mississippi River water into the Breton Sound estuary (29°51′40.15² N, 89°54¢43.62″W) in Louisiana during the spring, high-flow period (Lane et al., 2006). Discharge from the Caernarvon Diversion varies throughout the year and from year to year based on water levels in the Mississippi River as well as salinity in the receiving basin related to fisheries and wildlife. In response to these management concerns, the diversion discharges Mississippi River water on average 12 wk a year during the spring growing season (USGS, 2010). However, recent concerns about eutrophication have arisen after the large-scale disturbance (~100 km^2) of fresh and brackish marshes in the Breton Sound estuary observed after Hurricanes Katrina and Rita (Day et al., 2007). A number of studies have suggested that eutrophication of the marshes in Breton Sound from elevated nitrate from the Mississippi River was the underlying cause of the marsh destruction (Darby and Turner, 2008a,b; Swarzenski et al., 2008; Turner et al., 2009; Howes et al., 2010). This is an important concern because other diversions have been built or are being planned (Kral et al., 2012; Zhang et al., 2012).

Consequently, our study sought to examine the effects of elevated nitrate levels in surface water on *Spartina patens*-dominated coastal brackish wetlands using soil cores planted with *Spartina patens* plugs with ^{15}N labeled nitrate delivered in the water column on both aboveground and belowground biomass, as well as to determine the fate of the added N. This experiment was designed to mimic (i) the method of nutrient addition of river diversions into coastal wetlands by addition in the water column, (ii) the same N source (nitrate) similar to Mississippi River water, and (iii) concentrations of N documented in the Mississippi River (Lane et al., 1999) and average flood duration during the spring (12 wk; USGS 2010). We hypothesize that (i) belowground biomass, over the short term, will not be significantly different under elevated water column nitrate concentrations, and (ii) the majority of added nitrate will be removed by denitrification as opposed to plant uptake.

MATERIALS AND METHODS

Experiment Setup and Design

Vegetated soil plugs were collected from a brackish marsh located proximal to Delacroix, LA (29°45′12.72″N, 89°47′41.65″W) on 7 Apr. 2010. At the time of collection, water levels were sufficiently low that the marsh was not flooded. The plugs were collected from a typical area of the marsh colonized by near monotypic stands of *Spartina patens*. The vegetated soil plugs were transported to the Wetland and Aquatic Biogeochemistry Laboratory (WABL) at Louisiana State University (LSU) and were fitted into 12, 15.2-cm-diam. PVC tubes were sealed on the bottom and placed in a greenhouse the following day. The plants were watered for several weeks to allow for establishment.

An N-loading study utilizing ^{15}N labeled NO_3–N included 12 replicate cores that were randomly assigned to one of two groups, 0.0 (control) or 2.0 mg NO_3–N L^{-1} treatments. The treatment level of nitrate was chosen based on observed spring concentrations within the Mississippi River (Lane et al., 1999). Nitrate comprises more than 99% of soluble inorganic N in the Mississippi River water (White et al., 2009). The nitrate added was 99% atom ^{15}N (Cambridge Isotope Laboratory, Andover, MA). A 10-cm water column was maintained within each core for the duration of the experiment.

Nitrate or deionized (DI) water solution was added approximately twice a week for 12 wk for a total of 23 nutrient addition events. Twelve weeks of nutrient additions were chosen based on average diversion discharge into the Breton Sound estuary. We refrained from using a flow-through system, which would have perfectly mimicked the diversion delivery, so we could more accurately account for the total N (TN) removal rate from the water column. The treatments were imposed by removing the water column out of each core. Each core was then filled 10 cm above the soil surface with either DI water (control) or 2 mg NO_3–^{15}N L^{-1} solution (treatment) so that flooded conditions persisted for the duration of the experiment. We used DI water as the control so as not to introduce any other N sources, thereby isolating the nitrate effect. During the experiment, the water column pH was measured using an Accumet Research AR25 Dual Channel pH/Ion Meter (Fisher Scientific). Water column conductivity was monitored using an Accumet Basic AB30 Conductivity Meter (Fisher Scientific). Redox potential was taken at the 5- and 10-cm soil depth in six randomly selected cores, three control and three treatment cores. Redox potential was measured using a platinum working electrode and saturated calomel (SCE) reference electrode. A correction fac-

tor of +242 was applied to each redox potential measurement to correct for the potential of the calomel reference electrode (Land et al., 2011). The temperature was monitored in the greenhouse throughout the duration of the experiment.

At 6 wk, the aboveground biomass was harvested by clipping all stems approximately 5.08 cm above the soil surface to stimulate growth and maximize plant uptake. At the end of 12 wk, six cores (three each from control and treatment) were destructively harvested by clipping aboveground biomass and sectioned belowground biomass into 0- to 10- and 10- to 20-cm sections for the mass balance. To investigate the potential for surface algal N uptake, the surface of the soil cores were scraped to collect the top 2 mm of material containing attached algae.

Aboveground biomass was separated into live and dead biomass. Half of each soil section was used to determine assimilation of ^{15}N in belowground biomass by separating biomass into dead roots, live roots, and stem biomass. The other half of each soil section was used for soil samples by removing large roots and blending to a homogenous soil sample. Total weights of each component were recorded before separation. All samples were refrigerated in the dark at 4°C until analyzed.

Water Column, Plant, and Soil Characterization

Eight flood events out of 23 during the 12-wk study period were intensively monitored to document the decrease in water column nitrate. Twenty milliliters water column samples were collected at 0, 4, 8, 12, and 24 h after flooding and filtered through a 0.45-μm GHP membrane filter. Water samples were stored at 4°C until analysis for NO_3^-, NH_4^+, and soluble reactive phosphorus (SRP) using a Seal AQ2 Automated Discrete Analyzer (Seal Analytical). Method detection limits for NO_3–N, NH_4–N, and SRP were 0.014, 0.012, and 0.005 mg L^{-1} for USEPA methods 132-A Rev. 1, 103-A Rev. 4, 118-A Rev. 2, respectively. Aboveground biomass was analyzed for total C (TC), TN, total P (TP), and $\delta^{15}N$ by drying dead and live biomass at 70°C until constant weight. Belowground biomass was separated into live, dead, and stems; was dried at 70°C until constant weight; and was analyzed for TC, TN, TP, and $\delta^{15}N$. Roots categorized as live were gold in color, turgid, floated when placed in water and, had the presence of fine root hairs. Roots that were partially decomposed were considered dead.

Each soil core was sectioned into a 0- to 10- and 10- to 20-cm section and analyzed for moisture content, dry weight bulk density, TC, TN, TP, extractable NO_3–N, extractable NH_4–N, potentially mineralizable N (PMN), microbial biomass C (MBC), microbial biomass N (MBN), and $\delta^{15}N$ content. The extractable NO_3–N, extractable NH_4–N, PMN, MBC, and MBN were measured to assess possible effects of added water column N to several soil parameters important to N processing. Gravimetric moisture content was calculated by drying a soil subsample at 70°C until constant weight. Dry weight bulk density was determined from the total wet weight of each section, corrected for moisture divided by the volume of each core section. Total C and TN were measured on dried, ground subsamples of soil sections 0 to 10 and 10 to 20 cm using an Elemental Combustion System with a method detection limit of 0.005 g kg^{-1} (Costech Analytical Technologies, Valencia, CA).

Extractable NO_3–N and NH_4–N soil samples were measured using 25 mL of a 2 M KCl extract shaken on an end-to-end shaker for 1 h and analyzed for NO_3^- and NH_4^+ on the Seal AQ2 Discrete Analyzer using USEPA methods 132-A Rev. 1 and 103-A Rev. 4, respectively (USEPA, 1993). The PMN was determined from time zero, 3 d, and 10 d soil anaerobic incubations (White and Reddy, 2000) extracted with 2.0 M KCl. The PMN rate was calculated as the increase in NH_4–N over time by regression.

The MBC and MBN were calculated using the fumigation-extraction method (Brookes et al., 1985; Sparling et al., 1990) with modifications by White and Reddy (2001). Two sets of triplicate 5-g wet weight samples were placed in 25-mL centrifuge tubes. One set was fumigated for 24 h under a headspace of chloroform. Both sets were then extracted with 20 mL of 0.5 M K_2SO_4. Total organic C (TOC) and total dissolved N (TDN) were determined on the extracts using TOC analyzer (model TOC-VCSN, Shimadzu Scientific Instrument). Microbial biomass was calculated by subtracting the non-fumigate samples from the fumigate samples. The TP was determined using an ashing-HCl digestion method (Andersen, 1976) for aboveground plant biomass, belowground plant biomass, soil scrapings, and soil. Approximately 0.2 g of dried, ground sample were weighed in a 50-mL beaker. Samples were ashed in a muffle furnace (Barnstead Thermolyne 62700 Furnace, Barnstead Thermolyne Corp., Dubuque, IA) at 550°C for 4 h. Subsequently, samples were digested with 6 M HCl and analyzed for TP on a Seal AQ2 Discrete Analyzer using USEPA Method 119-A Rev. 3 (USEPA, 1993). The method detection limit for TP was 0.05 mg P L^{-1}.

Nitrogen-15 Analysis and Mass Balance Calculation

Live aboveground plant biomass and dead aboveground plant biomass for harvest at 6 and 12 wk, as well as live root biomass, dead root biomass, stem root biomass, soil scrapings, and soil samples harvested at 12 wk, were sent to the Ecosystem Center at the Stable Isotope Laboratory (Woods Hole, MA) for ^{15}N analysis. Analysis was done using a Europa 20–20 CF-IRMS (Sercon Ltd.) interfaced with the Europa ANCA-SL elemental analyzer (Sercon Ltd.). Stable isotope values were used in the mass balance calculation for this study.

The percentage of recovered ^{15}N for each component of a core was calculated by multiplying atom percent with total percentage of N and total dry weight (g), then dividing by the total added ^{15}N throughout the duration of the experiment using Eq. [1].

$$\%\ \text{Recovered}\ ^{15}N = \{[(\%\ ^{15}N/100)(\%\ TN/100)(\text{total dry weight})]/^{15}N\ \text{added}\}100 \quad [1]$$

The percentage of recovered ^{15}N was calculated for live aboveground biomass, dead aboveground biomass, soil scraping, live root biomass, dead root biomass, stem biomass, and soil for each

treatment core. Live roots, dead roots, stems, and soil percentage recovered ^{15}N were calculated for 0- to 10- and 10- to 20-cm sections. The values for all components of a core were added together to determine the total percentage recovered of added nitrate to each core. The mass added, minus the recovered, was assumed to be lost by gas. Because the water column pH had a mean of 6.8 ± 0.12, our assumption was that the loss of N occurred primarily through denitrification.

Data Analysis

The effect of nitrate addition between control and treatment cores for each soil section (either 0–10 cm or 10–20 cm) was determined using a student *t* test ($P < 0.05$). Data normality was determined using the Kolmogorov-Smirnov test (a = 0.01). Data were log-transformed to fit a normal distribution when necessary. Soil properties were compared, including bulk density, percentage moisture, TC, TN, TP, MBC, MBN, extractable NO_3–N, and extractable NH_4–N for each soil section by a student *t* test.

The effect of nitrate addition on aboveground and belowground biomass between control and treatment cores was also tested using a student *t* test ($P < 0.05$). Data normality was determined using the Kolmogorov-Smirnov test (a = 0.01) and log-transformed to fit a normal distribution when necessary.

Table 1. Soil characteristics for harvest at 12 wk for soil section 0 to 10 cm and 10 to 20 cm. Data are mean values (*n* = 3) ± sd.

Soil parameter	Units	Controls	Treatments
		0–10 cm	
Bulk Density	g cm^{-3}	0.24 ± 0.05	0.25 ± 0.03
% Moisture	%	67 ± 1.8	67 ± 2.9
TC	g kg^{-1}	67 ± 3.4	62 ± 25
TN	g kg^{-1}	4.87 ± 0.23	4.56 ± 1.33
TP	mg kg^{-1}	638 ± 33.4[a] †	502 ± 14.0[b]
MBC	g kg^{-1}	3.35 ± 0.22	3.71 ± 0.61
MBN	mg kg^{-1}	10.3 ± 12.3	11.7 ± 9.07
NO_3–N‡	mg kg^{-1}	2.40 ± 0.32	2.37 ± 0.76
NH_4–N‡	mg kg^{-1}	41 ± 21	39 ± 11
PMN	mg kg^{-1} d^{-1}	2.17 ± 2.19	3.33 ± 0.88
TC:TN		14	14
		10–20 cm	
Bulk Density	g cm^{-3}	0.33 ± 0.03	0.35 ± 0.08
% Moisture	%	63 ± 1.1[a]	60 ± 3.3[b]
TC	g kg^{-1}	57 ± 3.8	50 ± 7.0
TN	g kg^{-1}	4.32 ± 0.22	3.89 ± 0.50
TP	mg kg^{-1}	622 ± 104	510 ± 35.6
MBC	g kg^{-1}	2.81 ± 0.09	2.66 ± 0.24
MBN	mg kg^{-1}	7.50 ± 8.82	4.64 ± 5.54
NO_3–N‡	mg kg^{-1}	2.34 ± 0.25[a]	1.98 ± 0.14[b]
NH_4–N‡	mg kg^{-1}	90 ± 68	74 ± 25
PMN	mg kg^{-1} d^{-1}	2.42 ± 1.67	0.90 ± 0.90
TC/N		13	13

† a, b Different letters indicate significant differences between columns at $P = 0.05$.

‡ Indicates extraction by 2 M KCl.

RESULTS AND DISCUSSION

Soil Properties

Soil properties were compared in the control and treatment cores after 3 mo of nitrate additions. In general, there were few differences between control and treatment core soil properties in the 0- to 10-cm cores (Table 1). The MBC and MBN were similar in the control and treatment cores at 3.35 ± 0.22 g C kg^{-1}, 10.3 ± 12.3 mg N kg^{-1}, 3.71 ± 0.61 g C kg^{-1}, and 11.7 ± 9.07 mg N kg^{-1}, respectively, suggesting limited microbial assimilation. Mean extractable NH_4–N was not significantly different when comparing control and treatment cores at 41 ± 21 and 39 ± 11 mg N kg^{-1}, suggesting insignificant dissimilatory nitrate reduction to ammonium. Similarly, PMN was not significantly different between control and treatment cores, with 2.17 ± 2.19 and 3.33 ± 0.88 mg N kg^{-1} d^{-1}, which suggests the microbial activity was not significantly different. In the 0- to 10-cm soil interval, only TP was significantly different in the control and treatment cores (638 ± 33.4 and 502 ± 14.0 g P kg^{-1}, respectively; Table 1).

There were also few differences between the control and treatment cores for the 10- to 20-cm soil section (Table 1). The MBC and MBN values were also similar in treatment and control cores for both soil sections. The MBC and MBN values were similar to previous studies (Gardner and White, 2010; White and Reddy, 2003). Mean extractable NH_4-N and PMN were not significantly different between control and treatment cores in the 10 to 20-cm soil section (90 ± 68 mg N kg^{-1}, 74 ± 25 mg N kg^{-1}, 2.42 ± 1.67 mg N kg^{-1} d^{-1}, and 0.90 ± 0.90 mg N kg^{-1} d^{-1}, respectively). Similar PMN values have been observed in White and Reddy (2000). In the 10- to 20-cm soil interval, only percentage moisture was significantly different in the control and treatment cores (63 ± 1.1 and 60 ± 3.3%, respectively; Table 1).

Mean temperature in the greenhouse was 32.5 ± 4.8°C during the experimental time period. Redox potential was not statistically different between treatments and at each soil depth (5 and 10 cm) averaging -149.27 ± 27.04 mV. The average pH was 6.8 ± 0.12, and the average salinity was 0.83 ± 0.27 mS. The pH and salinity were not significantly different between control and treatment cores.

Plant Biomass

Differences in control and treatment aboveground plant biomass were compared as total biomass throughout the 3 mo, including clipping at 6 wk. There were no significant differences between control and treatment cores in the 0- to 10-cm depths of live aboveground biomass (5.86 ± 1.40 and 6.24 ± 1.51 g, respectively; Table 2) or dead aboveground biomass (0.50 ± 0.35 and 0.84 ± 1.08 g, respectively). This result suggests that the plants have a sufficient amount of N provided through mineralization from the soil organic matter. Live belowground biomass in the control and treatment 0- to 10-cm section did not have any measurable response to nitrate addition at 2.05 ± 0.34 and 1.82 ± 0.14 g, respectively (Table 2). Dead belowground biomass

Table 2. Dry weight in grams of live and dead aboveground and belowground components, and soil at 12 wk. Data are mean ± sd.

Experimental component	Control	Treatment
Live aboveground†	5.86 ± 1.40	6.24 ± 1.51
Dead aboveground†	0.50 ± 0.35	0.84 ± 1.08
Live roots 0–10 cm	2.05 ± 0.34	1.82 ± 0.14
Dead roots 0–10 cm	5.09 ± 0.24[a]‡	12.9 ± 1.09[b]
Stem roots 0–10 cm	4.33 ± 1.89	5.85 ± 0.83
Soil 0–10 cm	202 ± 42	202 ± 24
Live roots 10–20 cm	0.78 ± 0.21	0.79 ± 0.67
Dead roots 10–20 cm	9.70 ± 2.50[a]	24.3 ± 10.6[b]
Stem roots 10–20 cm	3.44 ± 1.02	3.22 ± 2.01
Soil 10–20 cm	286 ± 30	286 ± 68
Soil scraping†	3.15 ± 2.46	4.25 ± 3.86

† $n = 6$ for aboveground biomass, soil scraping; all other components $n = 3$.

‡a, b Different letters indicate significant differences between columns at $P = 0.05$.

was significantly different in the 0- to 10-cm section (5.09 ± 0.24 and 12.9 ± 1.09 g, respectively; Table 2), where the treatment core had higher dead belowground biomass. Stem biomass in the 0- to 10-cm soil section was not significantly different in the control and treatment cores.

Similarly to soil section 0 to 10 cm, there was no significant difference between live belowground biomass in the 10- to 20-cm soil section when comparing control and treatment cores (Table 2). There was no response to stem belowground biomass in the 10- to 20-cm soil section when comparing control and treatment cores. However, dead belowground biomass was significantly higher ($p = 0.05$) in the treatment core than in the control core in the 10- to 20-cm soil section (9.70 ± 2.50, and 24.3 ± 10.6 g, respectively; Table 2). While there was a significantly higher amount of dead roots in the nitrate treatment compared with the control, there was no significant difference in the amount of live belowground biomass between control and nitrate treatments or rooting distribution by depth interval. Live root distribution is important for coastal marsh resilience; however, dead roots do not contribute to marsh stability but do contribute to other important wetland functions, including C sequestration.

Water Column Nitrate Reduction

The nitrate treatment addition was 2 mg $K^{15}NO_3$–N L^{-1} of 99% atom ^{15}N. However, dilution by pore water and water associated with the surface floc layer occurred such that nitrate

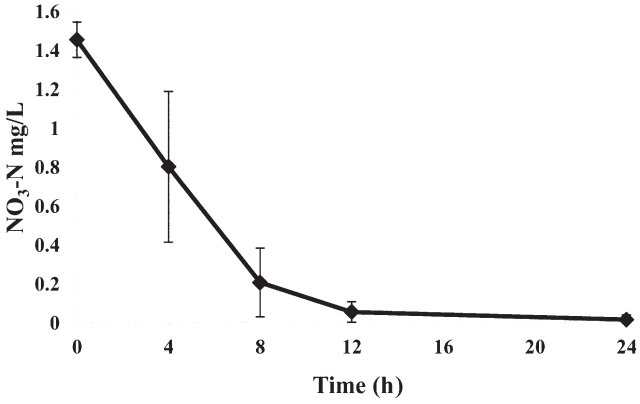

Fig. 1. Mean water column ± standard deviation of nitrate concentration for 8 flood events at 2 mg N L^{-1} over 12 wk ($n = 8$).

concentrations at time zero averaged 1.46 mg NO_3–N L^{-1} (Fig. 1). Presumably, a portion of the nitrate diffused into the soil. However, given that the water samples at time zero were taken 20 min after the water exchange, that loss at the time zero sampling was likely minimal. Complete loss of nitrate from the water column was documented within 12 h during eight intensively measured flood cycles during the 12 wk of 2 mg NO_3–N L^{-1} additions. Therefore, the coupled denitrification and plant uptake rates during the eight flood events spread across the 12 wk remained relatively constant, ranging from 167 to 191 mg N m^{-2} d^{-1} (Table 3).

Percentage of Nitrogen-15 Mass Balance

A total of 83.26 mg $^{15}NO_3$–N was added to each core in solution during the 12-wk experiment. Overall, 30 mg of $^{15}NO_3$–N (Table 4) were recovered for all components for an overall average of 36% recovery for all treatment cores. The average percentage recovery of added ^{15}N for individual components is shown in Fig. 2.

Soil scrapings, dead roots, and stems in the 0- to 10-cm soil section each recovered approximately 1% of the added ^{15}N. Live roots in the 0- to 10-cm soil section accounted for about 3% of the added labeled nitrate. Soil from the 0- to 10-cm soil section retained about 7% of the added ^{15}N. The 10- to 20-cm belowground biomass (live roots, dead roots, and stems combined) recovered <1%, and the 10- to 20-cm soil section recovered 1% of ^{15}N added to the treatment cores. Aboveground biomass (live + dead biomass) accounted for 24% of recovered ^{15}N delivered

Table 3. Maximum daily denitrification rate for 8 flood events over 12 wk with the addition of 2 mg N L^{-1}. Denitrification rate is the loss of nitrate (0–12 h) × 2 (for total of 24 h) and corrected for volume of water column, surface area of core, and ^{15}N loss by denitrification (Fig. 2).

Core number	Denitrification rate, mg N m^{-2} d^{-1}							
	6/9/2010	6/11/2010	6/14/2010	7/12/2010	7/15/2010	8/5/2010	8/9/2010	8/19/2010
1	157	179	158	139	183	109	132	119
2	200	193	195	223	202	186	173	185
3	152	149	137	198	194	192	183	207
4	205	192	208	214	213	183	222	200
5	215	215	193	162	181	165	192	167
6	189	178	169	174	171	168	181	145
Mean ± sd	186 ± 26	185 ± 22	177 ± 27	185 ± 32	191 ± 15	167 ± 30	180 ± 29	171 ± 34

Table 4. Labeled N derived from added nitrate vs. unlabeled N derived from mineralization over 12 wk for aboveground and belowground components in the 0- to 20-cm soil section. Data are mean values ± sd.

Experimental component	mg ^{15}N	mg ^{14}N
Live aboveground†	17.4 ± 3.28	247 ± 47.7
Dead aboveground†	1.72 ± 1.44	89.3 ± 27.9
Live roots 0–10 cm	2.2 ± 0.21	20 ± 1.5
Dead roots 0–10 cm	2.1 ± 0.70	128 ± 6.8
Stem roots 0–10 cm	1.3 ± 0.75	44 ± 6.9
Soil 0–10 cm	4.6 ± 1.4	901 ± 175
Live roots 10–20 cm	0.01 ± 0.02	5.8 ± 5.1
Dead roots 10–20 cm	0.25 ± 0.26	227 ± 101
Stem roots 10–20 cm	0.05 ± 0.04	21 ± 16
Soil 10–20 cm	0.45 ± 0.14	1123 ± 369
Soil scraping†	0.55 ± 0.21	38.8 ± 47.7
Total N	30	2845
Percentage of otal N	1	99

† $n = 6$ for aboveground biomass, soil scraping; all other components $n = 3$.

to the roots by diffusion and the transpiration stream. The largest component of added ^{15}N was unaccounted for and termed gaseous losses at 64%.

If dissimilatory reduction to ammonia were a major nitrate removal process in this experiment, we would expect higher ammonia concentrations in the treatment cores. An increase in ammonia was not found. Also, the pH of the water column was not within the range where ammonia volatilization is a significant process, and thus the N would have been conserved. If assimilatory nitrate reduction were a major process, then the total soil ^{15}N content would have accounted for more than the 8% of the amount recovered in the soil pools (Fig. 2). Therefore, we surmise that denitrification was the major nitrate removal process.

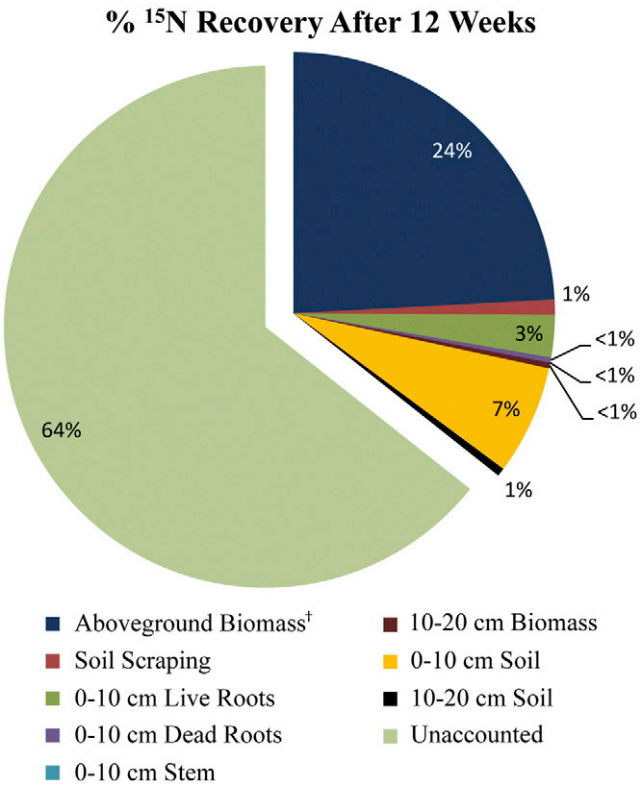

Fig. 2. Mass balance of labeled nitrate addition over 12 wk for aboveground and belowground components, represented as % of recovered ^{15}N in each component (†$n = 6$ for aboveground biomass, all other components $n = 3$).

External (labeled N) and internal (from mineralization of the soil) N sources were calculated for each core component to compare main N sources in the Breton Sound estuary. Added labeled nitrate represented external N sources, and N mineralization represented internal N sources. The plant biomass, both aboveground and belowground biomass, recovered 27.5% of the total added labeled N (Fig. 2). The remaining N in all of the plant components then came from the internal or soil-derived source. External N accounted for 30 mg N and internal N accounted for 2845 mg N in the 0- to 20-cm soil section (Table 4). External N from added labeled nitrate was only 1% of the TN recovered during the 12 wk.

All cores had similar soil physicochemical and microbial properties when comparing control and treatment cores in the 0- to 10-cm and 10- to 20-cm soil sections. Soil properties TC, TN, MBC, MBN, and extractable NO_3–N would be expected to be different with the addition of nitrate if the nitrate additions increased microbial biomass (higher MBC and MBN) or microbial activity (PMN), but this was not evident from our data (Table 1). The lack of significance between microbial biomass measures and denitrification is due to the fact that only a subset of the total microbial pool functionally moderates denitrification.

The rate of nitrate loss in the water column was consistent throughout the 12-wk experimental additions, with no measurable nitrate detected 12 h after addition, suggesting that denitrification was occurring. Ammonium concentrations in the water column during each flood event were at or below the detection limit (0.02 mg L^{-1}), and mean pH was 6.82 ± 0.12, suggesting ammonia volatilization was not a significant process for N removal. Redox conditions (-149 ± 27 mV) indicated that environmental conditions were suitable for denitrification to occur (Patrick et al., 1996). The presence of anaerobic conditions, nitrate, and high soil C advocate that the unaccounted-for nitrate was removed within 12 h by denitrification. Similar results have been reported by Yu et al. (2006), who showed in a field study at the Davis Pond Diversion, located two miles south of Luling, LA (29°55′52.59″N, 90°19′72″W), using ^{15}N-labeled nitrate that denitrification was the major mechanism for nitrate removal. Maximum denitrification rates in this study ranged from 167 to 191 mg N m^{-2} d^{-1} (Table 3) and are within the range of published denitrification rates (Table 5). These rates also underscore the role of macrophytes in driving nitrate flux into the soil when compared with rates for an adjacent, non-vegetated oligohaline estuary (Roy and White, 2012). Dissimilatory nitrate reduction to ammonium did not appear to play a major role in NO_3^- reduction in this study because the soil pore-water N was not significantly different between the treatment and control cores (data not shown).

Live roots in the 0- to 10-cm soil section assimilated only 3% of added labeled nitrate, which should have been higher if

significant root growth had occurred to surface roots. Also, there were no significant differences in weight of the live roots in either soil section when comparing control and treatment cores. The hypothesis that eutrophication causes lower live belowground biomass or increased shallower rooting in these coastal marsh soils receiving nitrate was not substantiated in this 12-wk study. Fox et al. (2012) and Anisfeld and Hill (2012) also measured belowground biomass and found that nutrient enrichment did not decrease live belowground biomass, corroborating results from our study and in contrast to other belowground biomass studies (Darby and Turner, 2008a,b; Swarzenski et al., 2008; Turner et al., 2009; Howes et al., 2010).

The percentage of recovered labeled nitrate in the aboveground and belowground biomass does show that assimilation of excess nitrate to aboveground and belowground biomass occurs in Breton Sound estuary. However, only 27.5% of labeled nitrate was recovered in all the aboveground and belowground plant biomass (Fig. 2). Therefore, the source of the other 72.5% of N in the plant biomass was from an internal source: N mineralization from the soil organic matter. Documentation of internal and external N sources support the conclusion that N mineralization is the primary source of plant bioavailable N in Breton Sound.

Darby and Turner (2008a,b) suggested that elevated nitrate in the Mississippi River led to lower belowground biomass that was more easily damaged from high-energy events like a hurricane storm surge. These studies (Darby and Turner, 2008a,b) and others (Howes et al., 2010; Laursen, 2004; Swarzenski et al., 2008; Turner et al., 2009; Turner, 2010) suggest that nutrient-loading decreases rooting depth, resulting in shallow roots and a marsh surface that is less resilient to storms. While these studies suggest that eutrophication of marshes located within the diversion area results in changes in belowground biomass and soil properties, there are some experimental design shortcomings in many of these studies that should be noted. The first potential problem is the use of granular fertilizers themselves, as granular fertilizers create a continuous supply of N at the soil surface, where application of fertilizers generally occurs (Darby and Turner, 2008a; Valiela et al., 1976). This surface broadcast has been shown in agronomic research to lead to the reduction of rooting depth as an artifact of the fertilization process itself, as deeper root biomass will decrease if available N is concentrated at the surface (Tilman and Wedin, 1991). This fertilization situation is dissimilar to the N-loading from the water column during intermittent flooding in the diversions from the Mississippi River (White et al., 2009).

A second problem is the use of ammonium or urea as the N source, because more than 95% of inorganic, bioavailable N in Mississippi River water is in the nitrate form (Gardner and White, 2010), while ammonium is a very small component of the river water. Ammonium and urea are not immediately reduced by denitrification as occurs with nitrate in wetlands (Reddy and DeLaune, 2008). Therefore, a greater proportion of N could potentially be available to the plant for a longer period of time, essentially artificially elevating the amount of N available to plants during the growing season and not accurately portraying the loading rate. It is also well-established that wetland soil provides a continuous flux of ammonium from the deeper soil (Reddy and DeLaune, 2008) with concentrations within the range of 1 to 3 mg NH_4–N L^{-1} for organic soils (Gardner and White, 2010). This flux of N from the deep soil is not a phenomenon seen in upland, agronomic settings because the soils are not continually flooded; therefore, agronomic studies are not appropriate to lend credence to the hypothesis that the N-loading in wetlands causes shallower rooting depth, because the systems are fundamentally dissimilar.

Additional problems from many fertilization studies are that N-loading rates are often much higher and, in other cases, an order of magnitude higher (Darby and Turner, 2008a,b), than the nitrate-loading rates in coastal marshes receiving diverted Mississippi River water. Additionally, some granular fertilizers contained SO_4^{2-}, which, under anaerobic conditions present in the soil, is transformed by sulfate reducers to H_2S, a compound that can be toxic to plants (Koch et al., 1990). Obviously, there should be some caution taken in extrapolating results from high-rate, surface-broadcast fertilization studies using alternate N sources in coastal wetland soils to wetlands receiving primarily nitrate in the water column delivered intermittently. Our study is the first study that investigated the fate of nitrate without using granular fertilizer but instead mimicked a diversion N source (NO_3–N) and delivery mechanism (dissolved in the surface water) in brackish coastal marshes.

Furthermore, long-term nutrient-enrichment studies (Fox et al., 2012) provide evidence that nutrient enrichment results in soil accretion, not loss of elevation, in salt marshes. Fox et al. (2012) found that nutrient enrichment shifted species composition in salt marshes to plant species that out-compete other species during high-nutrient conditions. The shift in dominant vegetation increased production and biomass, thus leading to accretion rates that could maintain elevation in the face of the current sea level rise in the Great Sippewissett salt marsh (41°35′18.26″N, 70°38′38.08″W) in Cape Cod, MA. This finding contradicts Turner et al. (2009), who found that marshes in the same area did not increase in elevation in response to nutrient additions. Fox et al. (2012) suggest that the use of ash

Table 5. Denitrification rates from published literature for coastal marine environments. Modified from Herbert (1999).

System	Denitrification rate, mg N m^{-2} d^{-1}	Reference
Chesapeake Bay, *Z. marina*	225–702	Caffrey and Kemp (1990)
Colne estuary	1–154	Ogilvie et al. (1997)
Great Ouse estuary	7–32	Trimmer et al. (1998)
Guadalupe estuary	15–116	Yoon and Benner (1992)
Patuxent River estuary	259–299	Jenkins and Kemp (1984)
Barataria Bay marsh	44–137	Gardner and White (2010)
Breton Sound estuary marsh	167–191	This study
Colne Point salt marsh	13–44	b Abd. Aziz and Nedwell (1986)
Torridge River marsh	8–198	Koch et al. (1992)

content in calculating marsh elevation as used by Turner et al. (2009) may not be appropriate in this system. In further support of Fox et al. (2012), Anisfeld and Hill (2012) also found that nutrient enrichment did not affect belowground root biomass or marsh surface elevation in a Long Island Sound, NY, tidal marsh (41°17′12.08″N, 72°43′49.90″W). Although these two studies are not in Louisiana coastal marshes, results from these studies agree with and support our findings.

No significant differences in TC or MBC between control and treatment cores indicate that an increase in soil metabolism did not occur during 12 wk of nutrient additions. This result is contrary to other studies that suggest the addition of nitrate-laden Mississippi River water is increasing soil metabolism and decreasing marsh stability (Howes et al., 2010; Turner, 2010; Turner et al., 2009). To explore this hypothesis, we have calculated the amount of C needed to denitrify all the unaccounted for ^{15}N in our experiment. Using an average C accumulation rate of 301 g C m^{-2} (DeLaune and White, 2012) for 20 yr, a 0- to 20-cm soil section has 6020 g C m^{-2}. The denitrification rate during the 12 wk was found by using the slope from Fig. 1 (see Table 3), so that the mean denitrification rate was 178 mg N m^{-2} d^{-1}. We can assume that 99% nitrate loss was to N_2 (Smith et al., 1981). We have calculated the TN loss of pulsed nitrate added; however, the uptake of soil mineralized N was continuous. Solving simultaneous stoichiometric equations for the conversion of nitrate to N_2 gas and glucose to CO_2 reveal that for every 4 mol of N, 5 moles of C are needed (Reddy and DeLaune, 2008).

Accordingly, for 30, 60, and 90 d of flooding, the amount of C required for denitrification to proceed is equivalent to 0.11, 0.22, and 0.33% of the TC (6020 g C m^{-2}) in the top 20 cm of the soil. These calculations also do not take into account additional annual C accumulation, which DeLaune and White (2012) report to be between 219 and 301 g C m^{-2} yr^{-1}. Clearly, the denitrification process is not an effective means of decomposing the soil organic matter, and this process plays no significant role on consumption of the organic marsh substrate.

CONCLUSIONS

Our results indicate that gaseous losses are the main removal mechanism for diverted Mississippi River nitrate, as near-complete loss of nitrate occurred within 12 h in vegetated, intact cores. Gaseous losses include denitrification and ammonia volatilization. Denitrification is the most likely removal pathway, given the circum-neutral pH. This study also confirms that assimilation of nitrate into live roots does occur; however, after 12 wk of labeled nitrate addition, only 3% of added labeled nitrate was assimilated into live root biomass. Also, no significant differences in TN live root biomass at either the 0- to 10-cm or 10- to 20-cm soil sections for nitrate-loaded and control cores indicate nitrate did not significantly affect the total amount of belowground biomass or rooting depth. Calculations of external and internal N sources to the experimental plant-soil core system, at 30 mg ^{15}N (1%) and 2845 mg ^{14}N (99%), support the conclusion that N mineralization from soil organic matter is the primary source of N for plant assimilation in the Breton Sound estuary. As a result, the hypothesis that loaded nitrate is affecting belowground root biomass in Breton Sound estuary was not confirmed in this experiment.

Furthermore, results indicate that although denitrification was the main removal mechanism for nitrate, soil C reserves were not significantly affected. This finding was indicated by the lack of significant differences in TC or MBC when comparing control and treatment cores, as well as stoichoimetric calculations, which demonstrated maximum rates of denitrification would require just 0.11, 0.22, and 0.33% of the 0- to 20-cm soil C for 30, 60, and 90 d of continuous nitrate-loading, respectively. Therefore, the hypothesis that tight coupling of denitrification and oxidation of soil C stores decreases soil strength and, hence, marsh stability, was not supported in this 12-wk study. Future research should include longer-term studies throughout several growing seasons using river nitrate concentrations in surface water to further evaluate NO_3–N effects on fresh and brackish coastal wetlands receiving diversion water.

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