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# A decadal-scale nutrient loading study in a coastal wetland: Impacts on soil microbial processes

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ABSTRACT

Fertilizer use in agricultural lands and treated wastewater disposal have increased bioavailable nitrogen (N) and phosphorus (P) loading to wetlands and aquatic systems with potential implications for nutrient cycling and soil microbial processes. Recently, conflicting results of N loading impacts on microbial-mediated organic matter decomposition in coastal wetland soils have been reported. However, to date, the majority of studies have investigated short-term nutrient loading effects while few have reported long-term integrated impacts. Here, we present results from an 11-year soil nutrient loading study in an oligohaline coastal wetland treated with 0, 50, 200, or 1200 kg N $ha^{-1}$ $yr^{-1}$ in combination with 0 or 131 kg P $ha^{-1}$ $yr^{-1}$. We measured soil (0–10 cm) physiochemical characteristics, soil microbial biomass N, and measures of biogeochemical cycling of N, including potentially mineralizable nitrogen and denitrification rates. Our results show that soil total P increased with P additions, but no significant differences in measures of soil microbial biomass or activity occurred with N, P, or N × P loading. We conclude that long-term N loading at rates equivalent to Mississippi River diversions had no persistent lasting effects on total N or examined soil microbial measures.



## 1. Introduction

Agricultural activities necessary to feed an ever growing human population have resulted in world-wide deleterious environmental impacts, including eutrophication of lakes, streams, and coastal systems. The combination of both fixing atmospheric nitrogen (N) via the Haber-Bosch process and phosphorus (P) mining for fertilizer production has led to a doubling of bioavailable N and increased bioavailable P by 75% in the biosphere globally (Vitousek et al., 1997; Bennett et al., 2001). However, fertilizer-applied nutrients are not completely retained in the agricultural landscape, and consequently discharge into adjacent and downstream aquatic ecosystems are pervasive (Carpenter et al., 1998). Coastal areas can therefore become the sites of persistent eutrophication as rivers empty into the sea. For example, the Mississippi River watershed encompasses 31 of the contiguous 48 U.S. states and two Canadian provinces, discharging 1.4 million metric tons of N and 140,000 t of P to the northern Gulf of Mexico annually (USEPA, 1993). This large bioavailable nutrient load catalyzes a spring phytoplankton bloom followed by bacterial decomposition of the resulting plankton biomass, triggering the formation of an annual summer hypoxic zone that can exceed 20,000 $km^2$ (Turner and Rabalais, 1991).

The Mississippi River historically deposited a portion of its nutrient load into surrounding wetlands rather than into open waters of the Northern Gulf of Mexico (DeLaune and White, 2012). In addition, the sediment influx from the River, concomitant with organic matter accretion, was vital for allowing coastal wetlands to keep pace with the high (~1 cm) rates of relative sea level rise caused by subsiding deltaic sediments (DeLaune and White, 2012). However, the current hydrologic disconnect between the coastal wetlands and the Mississippi River through levee construction prevents the annual deposition of fluvial sediment into adjacent wetlands accompanying spring floods. As a result of these and other factors, Louisiana's coastal wetlands are being lost at an unprecedented rate, accounting for ~80% of the wetland loss that occurs in the conterminous United States (Boesch et al., 1994). In an effort to counteract these high wetland loss rates, the state of Louisiana has proposed the construction of Mississippi River water and sediment diversions. Diversions are conveyances constructed within the levees of the Mississippi River that will reconnect the River with surrounding wetlands during high river water events. While freshwater influx can restore historic salinity regimes of the surrounding estuaries, combating salt water intrusion (LA DNR, 2003) and sediment can help slow coastal wetland loss, the addi-

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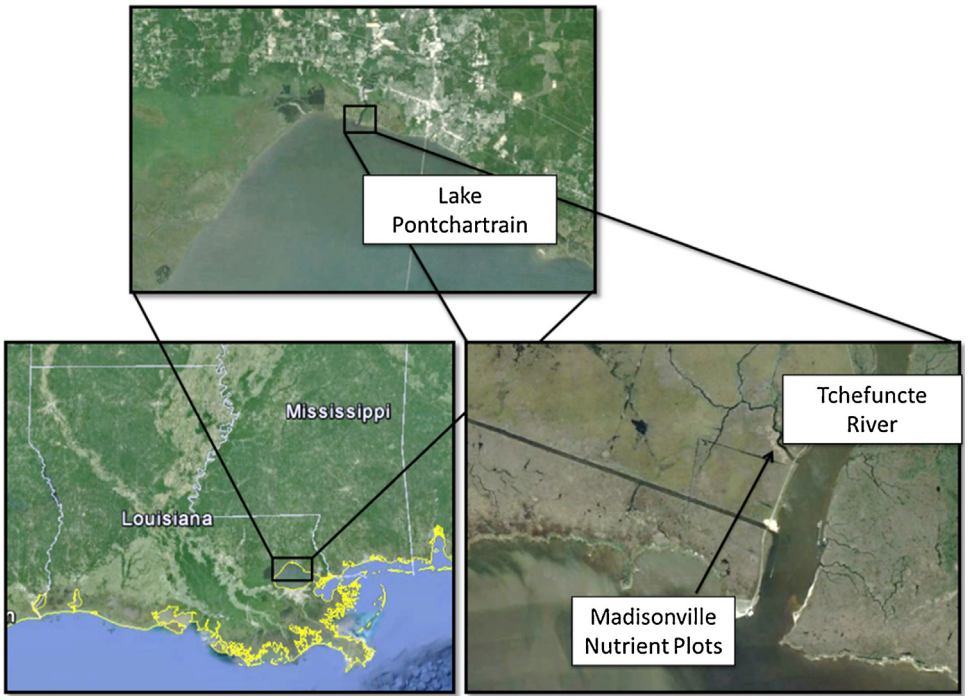


**Fig. 1.** The location of nutrient loading plots utilized in this experiment, adjacent to the Tchefuncte River, along the northern rim of Lake Pontchartrain in coastal Louisiana.

tion of nutrient-laden freshwater to the surrounding wetlands is an unintended consequence of diversion projects with potentially important implications to coastal wetlands (LA DNR, 2003).

Implementation of the first few large-scale diversions, the first completed in 1992, has sparked vigorous research into the effects of increased nutrient loading, particularly the belowground effects of N enrichment, on Louisiana's coastal wetlands. Addition of both N and P fertilizer to wetland systems has been shown to reduce belowground biomass in the coastal marshes (Darby and Turner, 2008a,b,c; Graham and Mendelssohn, 2013, 2016). In the receiving basin of a freshwater diversion, Swarzenski et al. (2008) reported a higher rate of decomposition and loss of soil shear strength. Similarly, a geographically disparate study by Deegan et al. (2012) in New England showed that nitrate, dissolved in tidal creek floodwater, increased microbial decomposition of organic matter, which the authors attributed to contributing to marsh destabilization. However, VanZomeren et al. (2012) reported in a controlled greenhouse study that nitrate loaded in surface water at concentrations mimicking the Mississippi River had no significant impact on belowground biomass while determining ~1/3 of the N was incorporated into the plant and 2/3 of the N was denitrified. Similarly, Graham and Mendelssohn (2013) found no effect of nutrient enrichment on organic matter decomposing in litterbags. Furthermore, Morris et al. (2014) reported that refractory organic matter should increase and contribute to sediment accretion, and additionally concluded that rhizome biomass would not decline with nutrient addition.

A number of issues have been raised regarding the appropriateness of the design of several of these studies. Many studies have been temporally limited, with only a few investigating the long term effects (Graham and Mendelssohn, 2010, 2013, 2016). In some, N and P have been added to wetland environments at very high loading rates not representative of nutrient loading for a river diversion setting. Therefore, our research goal was to determine the decadal effects of nutrient loading on an oligohaline coastal wetland soil by examining soil microbial processes, specifically focusing on N transformations. We hypothesized that over the 11-year fertilization period, soil N and P content would increase with N and P loading, with a corresponding increase in soil microbial activity.

## 2. Materials and methods

### 2.1. Study site

The study site was located along the west bank of the Tchefuncte River, along the northern rim of the Lake Pontchartrain Estuary, near the town of Madisonville, Louisiana (30°23.205′N, 90°09.551′W) (Fig. 1). Adjacent to a river, the site is characterized as oligohaline marsh, with microtidal (10 cm) pulses from the Lake Pontchartrain Estuary and discharge from the Tchefuncte River. The average surface water salinity was recorded at 1.6 practical salinity units (Graham and Mendelssohn, 2010). Soils are in the Kenner series, specifically Fluvaquentic Medisaprist, which are poorly drained organic soils with permeable underlying material (Graham and Mendelssohn, 2010). Surrounding vegetation is dominated by the presence of *Sagittaria lancifolia*, *Polygonum punctatum*, and *Eleocharis fallas*, all of which are common in Gulf Coast oligohaline marshes (Visser et al., 1998).

### 2.2. Experimental design

Sampling sites were established in 2002, consisting of three locations (i.e., statistical blocks), spaced 5–10 m apart and parallel to a small drainage canal adjacent to the Tchefuncte River. At each location, 1 m × 1 m plots received one of 4 levels of N (0, 50, 200, 1200 kg ha$^{-1}$ yr$^{-1}$) applied via granulated slow-release methylene urea 40-0-0 paired with one of 2 P levels (0, 131 kg ha$^{-1}$ yr$^{-1}$) applied as Humaphos 0-5-0, yielding 8 treatment combinations in a randomized complete block design (n = 3 per treatment). Each fertilization treatment was applied in both April and July during the growing season of each year for 11 years. Fertilizer application levels were chosen to bracket the concentrations of N and P flowing into coastal wetlands via the Caernarvon freshwater diversion (Lane et al., 1999; Slocum and Mendelssohn, 2008).

### 2.3. Soil sampling

One core was collected from each plot (n = 24 total) in late February 2014, seven months after the most recent fertilizer application. Cores, 25 cm long × 7.62 cm diameter, were collected with a sharpened metal tube using the push-coring method. All cores were immediately sealed to ensure no loss of moisture and transported back to the lab where each was extruded and sectioned into a 0–10 cm soil interval. The 10 cm soil interval is representative of ~10 years of wetland soil accretion in this marsh (Graham and Mendelssohn, 2013). Each segment was placed in a polyethylene container, large rhizomes removed, then the soil was homogenized, and stored at 4 °C.

### 2.4. Soil physiochemical properties

Soil samples were analyzed for bulk density, moisture content, percent organic matter, and total C, N, and P. Gravimetric moisture content was determined by homogenizing field-moist soil samples and placing them in a forced air oven at 70 °C until constant weight. Bulk density was then calculated and expressed on a dry-weight basis. Percent organic matter was determined by loss on ignition, using the weight of the ashed sample divided by the soil sample prior to combustion. Dried, ground subsamples of soil were also used to determine total C and N concentrations using a CNS Elemental Analyzer 4010 with a detection limit of 0.005 g $kg^{-1}$ (Costech Analytical Technologies, Inc. Valencia, California). Solid-phase total soil P analysis was performed by combusting dried, ground subsamples at 550 °C for 4 h in a muffle furnace. Following combustion, the resulting ash was then dissolved in 6M HCl on a hot plate and filtered through Whatman #40 filters (Andersen, 1976). Phosphorus concentrations were then measured colorimetrically on an AQ2 Discrete Analyzer (Seal Analytical Inc., Mequon, Wisconsin) following Method 351.2 (USEPA, 1993).

### 2.5. Microbial biomass nitrogen

Microbial biomass N was determined by using the chloroform-fumigation extraction method after Brookes et al. (1985) with modifications by White and Reddy (2000). A non-fumigate and a fumigate for each sample were prepared: non-fumigated samples were extracted with 25 mL of 0.5M $K_2SO_4$, while fumigate samples underwent 24 h of chloroform-fumigation in a vacuum-sealed glass desiccator after which they were also extracted with 25 mL of 0.5M $K_2SO_4$. Both sets of samples were centrifuged for 10 min at 4000*g*, vacuum filtered through Supor 0.45 μm membrane filters, and analyzed using a Shimadzu TOC-V Analyzer with a Total Nitrogen Measuring Unit (White and Reddy, 2003). The difference in N between fumigate and non-fumigate samples represents microbial biomass N.

### 2.6. Potentially mineralizable nitrogen (PMN)

The potentially mineralizable N assay determines the rate of net mineralization of N under anaerobic conditions typical of wetland soils (White and Reddy, 2000). N Mineralization is the process of decomposition of organic matter releasing ammonium, a bioavailable form of N (Reddy and Delaune, 2008). Soil subsamples were first analyzed for extractable ammonium by extraction with 25 mL of 2M KCl, which served as time zero controls. In a 160 mL glass serum bottle, 2.5 g of field-moist soil was added along with 10 mL of $N_2$-purged saline solution (2.58) to form a slurry, approximating the salinity of the field sites at collection. The headspace of each bottle was purged with $N_2$ gas. Samples were incubated at 40 °C to amplify microbial activity while being continuously agitated at 100 rpm to eliminate diffusion gradients. The temperature increase and continuous shaking ensured that the mineralization rates were potential measurements representative of the maximum N mineralization rates. Samples were extracted by addition of 20 mL of 2M KCl after the ten day incubation period, placed on a longitudinal shaker for 30 min, centrifuged at 4000*g* for 10 min, and filtered through Supor 0.45 μm membrane filters via vacuum filtration. Samples were acidified to a pH of <2 using concentrated $H_2SO_4$, and stored at 4 °C until $NH_4$-N colorimetric analysis using an AQ2 Automated Discrete Analyzer (Seal Analytical Inc., Mequon, Wisconsin) (Method 351.2; USEPA, 1993).

### 2.7. Potential denitrification

Potential denitrification rates were determined using the acetylene block method (Yoshinari and Knowles, 1976), only on samples at all N levels and 0 P to investigate N loading effects. Potential denitrification was measured to determine the maximum attainable rate of denitrification under site conditions. While potential rates are overestimates of actual field denitrification rates, they are representative of the available pool of denitrifying microbes. One hundred and sixty mL glass serum bottles containing 5 g of field-moist soil were sealed with 20 mm septa and aluminum crimps and purged with 99.99% $O_2$-free $N_2$ gas. Ten mL of $N_2$-purged, deionized water was added to each sample, along with 16 $cm^3$ of acetylene gas to inhibit the conversion of nitrous oxide to $N_2$ gas. Five mL of a 10 ppm $NO_3$-N and glucose-C solution, which was prepared by using potassium nitrate ($KNO_3$) and glucose monohydrate ($C_6H_{12}O_6 \cdot H_2O$). This solution was added to eliminate nitrate and carbon constraints on denitrification. The use of potassium nitrate created the appropriate salinity conditions of the site (Marks et al., 2016). Samples were agitated continuously in the dark on a longitudinal shaker at room temperature (~25 °C). Gas headspace samples were extracted at 2, 4, 8, 12, 20, and 24 h by syringe, and $N_2O$ concentrations were measured on a Shimadzu (Kyoto, Japan) gas chromatograph GC-8A with an electron capture detector (detection limit of 0.006 mg $N_2O$-N $kg^{-1}$ $h^{-1}$). The mass of $N_2O$ dissolved in the aqueous phase was calculated using a Bunsen adsorption coefficient of 0.544 (White and Reddy, 1999). Denitrification rates were obtained by linear regression of $N_2O$ concentrations over time.

### 2.8. Statistical analysis

Data analyses were performed using a mixed model ANOVA in SAS (version 9.1.4, SAS Institute, Cary, NC) to determine effects of N, P, and N × P loading. Prior to analysis, normality and homoscedasticity were verified using Shapiro-Wilks and Levene's tests, respectively, at $\alpha = 0.05$. The Type 3 Tests of Fixed Effects were used to determine the significant main effects of each variable separately at a probability of $p < 0.05$. When ANOVA identified significance, Tukey's HSD was used for post-hoc comparisons among treatment-means.

## 3. Results

### 3.1. Soil properties

Mean bulk density (0–10 cm) was 0.15 ± 0.004 g $cm^{-3}$ and was negatively correlated to moisture content ($r = -0.88$, $p < 0.001$, Table 1). The average weight percent organic matter was 41.3 ± 1.04% (Table 2). Soil bulk density, moisture content, organic matter percent were not significantly affected by enrichment with N, P or their interaction. Total C and total N concentrations averaged 177 ± 4.9 g $kg^{-1}$ and 13.7 ± 0.6 g $kg^{-1}$, respectively (Table 2). The average total soil P content was 3123 ± 644 mg P $kg^{-1}$ across both P treatment levels (Table 2). Total soil P was the only soil

**Table 1**
Results from ANOVA test for each variable. Significance is denoted by bold, underlined values. N/A indicates analysis not performed. Num. and Den. are the abbreviations for numerator and denominator, respectively. d.f. stands for degrees of freedom.

| | N Loading | | | | P Loading | | | | N * P Interaction | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Num d.f. | Den. d.f. | F-ratio | p-value | Num. d.f. | Den. d.f. | F-ratio | p-value | Num. d.f. | Den. d.f. | F-ratio | p-value |
| Organic Matter Content | 3 | 16 | 0.720 | 0.552 | 1 | 16 | 1.030 | 0.325 | 3 | 16 | 0.590 | 0.633 |
| Moisture Content | 3 | 14 | 0.150 | 0.925 | 1 | 14 | 1.950 | 0.185 | 3 | 14 | 0.990 | 0.428 |
| Bulk Density | 3 | 14 | 0.000 | 1.000 | 1 | 14 | 2.080 | 0.171 | 3 | 14 | 1.030 | 0.411 |
| Total P | 3 | 16 | 0.480 | 0.702 | 1 | 16 | 13.47 | **0.002** | 3 | 16 | 0.170 | 0.915 |
| Total C | 3 | 14 | 0.590 | 0.633 | 1 | 14 | 0.240 | 0.634 | 3 | 14 | 2.430 | 0.108 |
| Total N | 3 | 14 | 0.800 | 0.514 | 1 | 14 | 1.440 | 0.251 | 3 | 14 | 1.600 | 0.234 |
| Extractable $NH_4^+$ | 3 | 16 | 0.790 | 0.518 | 1 | 16 | 1.340 | 0.264 | 3 | 16 | 0.380 | 0.770 |
| Microbial Biomass N | 3 | 16 | 1.140 | 0.364 | 1 | 16 | 0.000 | 0.950 | 3 | 16 | 0.800 | 0.511 |
| Potentially Mineralizable N | 3 | 16 | 0.520 | 0.673 | 1 | 16 | 2.230 | 0.155 | 3 | 16 | 3.590 | **0.037** |
| Potential Denitrification | 3 | 8 | 1.940 | 0.202 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |

**Table 2**
Soil physiochemical characteristics and microbial measures (mean ± 1 standard error) for each N treatment level averaged over P treatment (n = 3 for denitrification rates; n = 6 for all other measures) and the overall mean (n = 12 for denitrification rates; n = 24 for all other measures).

| | N Levels (kg $ha^{-1}$ $yr^{-1}$) | | | | Overall Mean |
|---|---|---|---|---|---|
| | 0 | 50 | 200 | 1200 | |
| Organic Matter Content | 39.4 ± 1.45 | 39.9 ± 2.23 | 42.0 ± 2.47 | 44.0 ± 2.88 | 41.3 ± 1.15 |
| Moisture Content | 0.847 ± 0.008 | 0.842 ± 0.012 | 0.845 ± 0.007 | 0.844 ± 0.009 | 0.845 ± 0.004 |
| Bulk Density | 0.148 ± 0.007 | 0.149 ± 0.010 | 0.151 ± 0.008 | 0.148 ± 0.011 | 0.150 ± 0.004 |
| Total C (g $kg^{-1}$) | 181 ± 12.4 | 165 ± 6.50 | 172 ± 6.21 | 180 ± 16.2 | 174 ± 5.38 |
| Total N (g $kg^{-1}$) | 13.4 ± 0.749 | 13.5 ± 1.41 | 13.1 ± 0.468 | 15.2 ± 1.45 | 13.8 ± 0.544 |
| Total P (mg $kg^{-1}$) | 2620 ± 1200 | 3280 ± 1350 | 2980 ± 937 | 3620 ± 894 | 3120 ± 522 |
| Microbial N (mg N $kg^{-1}$) | 136 ± 24.1 | 214 ± 34.3 | 178 ± 35.4 | 203 ± 36.0 | 183 ± 16.5 |
| Extractable $NH_4^+$ (mg N $kg^{-1}$) | 92.4 ± 17.2 | 87.8 ± 13.4 | 74.9 ± 10.8 | 72.8 ± 22.0 | 82.0 ± 7.85 |
| Potentially Mineralizable N (mg N $kg^{-1}$ $d^{-1}$) | 53.4 ± 20.3 | 30.3 ± 2.37 | 32.3 ± 4.70 | 39.4 ± 9.22 | 38.8 ± 5.67 |
| Potential Denitrification Rates (mg $N_2ON$ $kg^{-1}$ $d^{-1}$) | 7.02 ± 2.91 | 16.5 ± 2.12 | 8.37 ± 2.15 | 9.14 ± 1.52 | 10.3 ± 1.46 |

physiochemical variable that was significantly affected by treatment, specifically P loading (p = 0.002, Table 1). The average total soil P content for the 0 P level was 913 ± 39 mg P $kg^{-1}$ compared to 6000 ± 1950 mg P $kg^{-1}$ for the 131 P level (Table 2). Both total C and total N showed no significant change with treatment levels. However, total C was positively correlated with total N in the soil (r = 0.76, p < 0.001).

### 3.2. Nitrogen cycling processes

Extractable ammonium was not significantly different among nutrient fertilization levels, averaging 82.0 ± 7.85 mg N $kg^{-1}$ regardless of treatment (Table 2). In addition, no significant differences in soil microbial biomass N (183 ± 16.5 mg N $kg^{-1}$) or potential denitrification rates (10.3 ± 1.46 mg $N_2O$-N $kg^{-1}$ $d^{-1}$) were detected with N, P, or N*P loading (Table 2). Although PMN rates were also not affected by N or P loading independently, they were significantly different due to an N × P interaction at the 0 and 200 kg N $ha^{-1}$ $yr^{-1}$, depending on P treatment level (Table 1, Fig. 2).

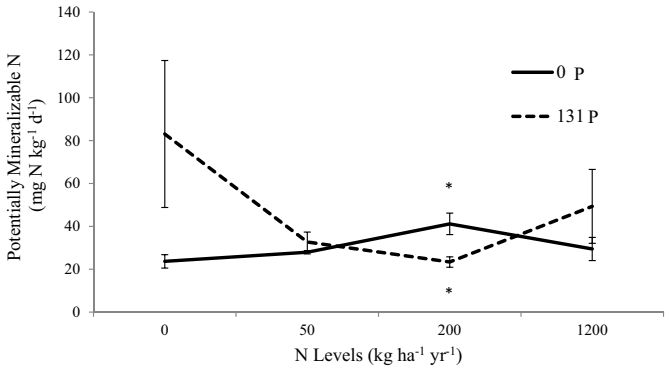


**Fig. 2.** Relationship between potentially mineralizable N concentrations and N and P treatment levels. Data shown as mean ± 1 standard error (n = 3). Asterisks denote significance.

## 4. Discussion

A number of studies have demonstrated the effect of nutrient fertilization on wetland plant biomass and community responses, but relatively few have focused on the response of both the soil microbial pool and soil microbial activity. Of the fertilization studies that have, the majority were short-term, lasting from a period of several months to a year (VanZomeren et al., 2012). In contrast to previous investigations, our study investigated the decadal loading of N and P at levels bracketing the loading found in the diversion receiving basins on select N cycling processes and microbial biomass.

In the present study, increased N loading had no significant effect on total soil N or extractable ammonium concentrations, suggesting that coupled nitrification-denitrification played a major role in effectively removing excess bioavailable N from the system which was contrary to our original hypothesis (Morris et al., 2014; Lane et al., 1999; Mitsch et al., 2001; DeLaune et al., 2005). It is highly likely that any nitrate diffusing into the soil was denitrified within the top 10 cm of the soil profile as has been documented in a number of studies (Gardner and White, 2010; Roy and White, 2012). Previous research has indicated that 25% of ammonium is lost through rapid nitrification-denitrification after fertilization (White and Howes, 1994). The coupled process of nitrification-denitrification is a direct loss mechanism for N loaded to this system. Additional N fertilizer was taken up by plants, which serve as a major sink for ammonium in wetlands (Morris, 1991). A study in a New England saltmarsh showed 81% of N isotopes added in the form of ammonium was taken up by plants three days after loading (Hammersley and Howes, 2005). A published companion study, focused on plant effects, found that aboveground primary

productivity and tissue N increased with N treatment at this study site (Graham and Mendelssohn, 2010), indicating that the macrophytes were utilizing the newly bioavailable N. However, this N enrichment of the macrophyte material was not detected in the resulting soil that formed in just over a decade. Consequently, plant uptake likely serves as an indirect loss mechanism for N. As the plants senesced, N was released into the soil in the form of organic compounds, which eventually were mineralized and lost through coupled nitrification-denitrification resulting in no N enrichment of the soil. In contrast, total soil P increased with P loading because there is no gaseous removal pathway and consequently P can continue to leak out of the soil over time (Reddy et al., 2011). Also, because plant productivity in this marsh is N-limited (Slocum and Mendelssohn, 2008; Graham and Mendelssohn, 2010), P application in excess of plant requirements would likely be stored in the soil, although some plant luxury consumption of P will occur. Similar results have been reported in other wetland systems, such as Blue Cypress marsh in the Upper St. John's River Basin (Bostic and White, 2007) and the Florida Everglades (Fisher and Reddy, 2001).

Nitrogen and P addition to wetland soils has been documented to have a significant effect on microbial populations and associated microbial activity in a number of P-limited wetland systems (Ogram et al., 2011; DeBusk and Reddy, 1998; Wright and Reddy, 2001a,b; Rejmankova et al., 2008). For example, in the Florida Everglades, a primarily P-limited system, N and P loading increased microbial biomass (Corstanje et al., 2007; DeBusk and Reddy, 1998; Qualls and Richardson, 2000). In contrast to these studies, microbial biomass and activity in the present study did not change with nutrient loading, which can most likely be attributed to nutrient limitation status (i.e., N vs. P limitation). Our results show that for all N treatments, the average N:P ratio was 10.3 at the 0 kg P $ha^{-1}$ $yr^{-1}$ P treatment compared to 3.08 in the 131 kg P $ha^{-1}$ $yr^{-1}$ treatment, suggesting N-limited growing conditions as found by other investigations in this marsh (Graham and Mendelssohn, 2010, 2016) and coastal marsh systems elsewhere (e.g., Sundareshwar et al., 2003).

Concern over nutrient loading in river diversion projects has focused on nitrate in the Mississippi River water. Roy et al. (2013) reported that 95% of inorganic, bioavailable N in the Mississippi River is present as nitrate. Diversion projects would therefore create an influx of nitrate into their receiving basins, which include coastal wetland systems. Adding nitrate to wetlands with high soil organic matter has also been suspected of increasing decomposition rates and resulting in marsh deterioration (Swarzenski et al., 2008; Deegan et al., 2012). VanZomeren et al. (2012) provided a stoichiometric calculation of carbon loss via denitrification for a receiving basin of a Mississippi River diversion and reported that for 90 days of continuous maximum denitrification, 0.33% of the C stored in the top 30 cm would be oxidized under these condition. This calculation did not include the ~1 cm of newly accreted soil added each year (DeLaune and White, 2012). Thus, soil C oxidation through denitrification is likely a minor, insignificant constituent of C loss in these coastal wetland basins.

In a companion study within the same contiguous marsh, Graham and Mendelssohn (2013) found that N added at the same rate and in the same form as the present study had no effect soil organic matter decomposing in litterbags for two years. However, the form of nitrogen used in this study and the companion study was methylene urea, which is metabolized to ammonium. Ammonium can potentially persist in wetlands longer because it is not immediately removed via denitrification, whereas nitrate can be immediately reduced to $N_2$ gas via denitrification in wetland soils (Reddy and DeLaune, 2008). Nitrification converts ammonium to nitrate, and occurs only under aerobic conditions. After nitrification, denitrification can convert nitrate to $N_2$ gas under anaerobic conditions. Both oxygen regimes must be present to directly remove N as $NH_4^+$ via coupled nitrification-denitrification.

The timescale of this study allows for integration of nutrients into the soil matrix and therefore provides a more clear understanding of the long-term impacts of N and P loading to coastal wetlands. Numerous studies have established that nutrient loading increases aboveground biomass with corresponding reductions in belowground biomass (Darby and Turner, 2008a,b,c; Graham and Mendelssohn, 2014, 2016). Results from this long-term study further indicate that added N (as ammonium), is eventually lost from the coastal marsh system via denitrification, though two pathways containing intermediate steps: (1) ammonium can be directly lost through coupled nitrification-denitrification, or (2) ammonium can be taken up by the macrophytes and subsequently lost from the system via the more indirect pathway (i.e, senescence, mineralization, and coupled nitrification-denitrification). The lack of significant differences in soil N or activity of microbial processes related to N cycling with long-term N loading suggests no lasting effect of N loading at these levels on this coastal wetland soil.

## 5. Conclusion

The implementation of Mississippi River diversion projects in coastal Louisiana has raised important questions regarding the role of nutrient loading, specifically N, on coastal wetland soil physical, chemical, and microbiological properties. In particular, N loading has been implicated in leading to wetland destabilization by accelerating decomposition via coupled microbial N and C cycling. Results from our study indicate that there was no significant difference in soil N content, microbial biomass, or microbial activity after more than a decade of N and P loading to an oligohaline wetland compared to control plots. Thus, we conclude that excess bioavailable N is rapidly removed from the system over the long-term via direct (denitrification) or indirect (plant uptake-senescence-decomposition-coupled nitrification-denitrification) pathways. In sum, the soil showed no long-term impacts from the N loading and therefore, measured microbial activity is likely not a soil-destabilizing mechanism in this or similar coastal marshes.

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