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Effects of Storm Surge and Nutrient Loading on Coastal Wetland Soil Processes: Implications for Ecosystem Function

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EFFECTS OF STORM SURGE AND NUTRIENT LOADING ON COASTAL WETLAND SOIL PROCESSES: IMPLICATIONS FOR ECOSYSTEM FUNCTION

A Thesis
Submitted to the Graduate Faculty of the Louisiana State University and School of the Coast and Environment in partial fulfillment of the requirements for the degree of Master of Science

in

The Department of Oceanography and Coastal Sciences

by
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ABSTRACT

Coastal Louisiana is at risk from increases in salinity from storm surge and nutrient loading from the Mississippi River. Increased salinity causes plant death, decreases in microbial productivity, and shifts in biogeochemical processes. Eutrophication is linked to shifts in plant communities and changes in wetland biogeochemical properties. We hypothesized that 1) storm surge would increase soil porewater salinity and decrease extractable ammonium (NH$_4$), and 2) long-term nutrient loading would decrease soil extracellular enzyme activity and increase total nutrients. Intact soil cores from two sites in the Wax Lake Delta were continually flooded with 35 g L$^{-1}$ salt water for 1, 2, and 4 weeks. Salinity averaged 1.46 in the mudflat and 1.03 in the marsh soils. Salinity levels increased significantly in the top 8 cm of the marsh and top 6 cm of the mudflat cores after the first week of flooding. Approximately 53% and 80% of the salt that diffused into the mudflat and marsh, did so during the first week of flooding. Extractable NH$_4$ did not change significantly after the first week of flooding, but increased significantly from 4-12 cm in the mudflat soil and in the top 14 cm in the marsh cores at four weeks. This study found that even a short flood can significantly increase soil porewater salinity in a majority of the plant rhizosphere.

Intact soil cores from an ongoing fertilization experiment in Madisonville, LA were analyzed for extracellular enzyme activity. Mean activities in the control were 18058 nmol MUF g$^{-1}$ hr$^{-1}$, 2850 nmol MUF g$^{-1}$ hr$^{-1}$, and 330 nmol MUF g$^{-1}$ hr$^{-1}$ for phosphatase, β-glucosidase, and sulfatase, respectively. Enzyme activity was not significantly affected long term nutrient loading. Total N and C were not significantly affected by nutrient loading, but total P significantly increased with P loading. No differences in extracellular enzyme activities suggest that soil microorganisms have not been functionally affected by a decade of nutrient loading.
Our results indicated how salinity and nutrient loading impacted coastal wetland soil, and these results can be used to augment management techniques to help stem further coastal degradation.
CHAPTER 1: REVIEW OF LITERATURE

1.1 COASTAL WETLANDS OF LOUISIANA

The coast of Louisiana is impacted by both short- and long-term environmental factors; external forcings, such as short-term changes in salinity caused by a passing storm or nutrient loading from the Mississippi River can have significant effects on the coast. After large storm events, such as Hurricane Katrina, much of the Louisiana coast becomes inundated with salt water that takes a long period of time to drain back into the Gulf of Mexico. These elevated salinity levels have a variety of negative impacts on wetland plants and microorganisms, including decreases in biomass and changes in microbial assemblages. The coast receives large annual loads of nutrients from agricultural and urban areas in the Mississippi River watershed, which are washed off the land and into the river and eventually to the Gulf of Mexico. Eutrophic conditions negatively impact coastal waters and estuaries throughout coastal Louisiana by increase. Both short-term changes in salinity and long-term nutrient loading have significant consequences for the function and stability of coastal wetlands.

Coastal Louisiana is an extremely dynamic and unique system, heavily influenced by both the Gulf of Mexico and the Mississippi River, and has been in a constant state of flux for thousands of years. The Mississippi River has a drainage basin of approximately 3.22 million km\(^2\), and drains parts of 31 states in the continental United States and two provinces in Canada (US Environmental Protection Agency, 2011). The Louisiana coast, as it is known today, was built with the vast amount of sediments that used to flow through the Mississippi River. In the last 7500 years, the Mississippi River has flowed into the Gulf of Mexico via seven different
paths, creating distinct delta lobes, including the Atchafalaya Delta and Balize Delta that are still active today (Figure 1.1). Historically, the river would flow through a certain lobe and build land in that area, and then abandoned the delta once its flow gradient became inefficient (Roberts, 1997). After the river abandoned these lobes, the land that had accreted would subside and erode over time.

Figure 1.1 The Delta cycle of the Mississippi River (Day et al., 2007)

One of Louisiana’s most defining characteristics are the wetlands that are interwoven with the coast. Currently, more than 47% of Louisiana’s population lives within the coastal zone (US Census, 2011). These systems act as flood control mechanisms from hurricanes, potentially stopping billions of dollars’ worth of damage from occurring. The wetlands act as a wintering
area for migratory birds, a nursery for a variety of fish species, and support an extremely biodiverse native population (Chesney et al., 2000; Audobon Society, 2013). Louisiana’s wetlands also provide thousands of jobs to the state’s population. Both the state’s fishing industry and the fur trapping industry are linked to the coastal wetlands, and together generate billions of dollars of revenue per year (Coastal Wetlands Planning, Protection and Restoration Act, 1997). The oil and gas industry have over 200,000 km of pipeline in the coastal zone, and many of their refineries are located close to the coast (Scott, 2011). Estimations of the cost of annual land loss ranges from $77 to $544 million, depending on the initial value placed on the land (Costanza et al., 1989).

Levee building began in the early 1800s, with levees being built around a meter tall to protect farms from periodic flooding (Mississippi River Levee Board, 2008). These small privately built levees were not overly effective in stopping flooding during periods of high river flow. In the early 1880s, the United States government adopted a levees only policy to control flooding, and more sophisticated levees were built. After a flood in 1927, the Mississippi River almost switched delta lobes again, but was constrained and forced back into its predetermined path (Barry, 1998). As a result of this flood, a river control structure was built to divert 30% of the Mississippi River to the Atchafalaya Bay. The Atchafalaya River is one of the only areas in Louisiana that is currently building substantial land (Roberts, 1998). Today, most of the Mississippi River’s discharge is directed out through the Balize Delta, and the river is isolated from the wetlands that it used to nourish with sediment and water. Sediments that exit the river through the Balize delta are delivered off of the continental shelf where they cannot build any land.
The wetlands of Louisiana make up approximately 40% of the continental United States’ wetlands but account for 80% of the country’s wetland loss (Boesch et al., 1994). Subsidence rates in the Mississippi Delta and Chenier Plains exceed 100 km$^2$ per year (Figure 1.2), and these rates are expected to continue rising (Penland and Ramsey, 1990). A variety of factors contribute to this extremely high rate of land loss, including relative sea level rise, compaction of sediments, and the loss of a sediment source to build land. The International Panel on Climate Change (IPCC) has predicted that sea level will rise somewhere between 28 to 48 cm during the twenty first century. Relative sea level rise rates (RSLR) in Louisiana are significantly higher than global averages; RSLR was measured at 1.04 cm yr$^{-1}$ at Grand Isle, LA, close to ten times the global average of 0.12 cm yr$^{-1}$ (Penland and Ramsey, 1990). Compaction of the Holocene strata and glacial isostatic adjustment contribute significantly to higher relative sea level rise rates (Tornqvist et al., 2008). Additionally, a large majority of the coastal wetlands are isolated from the river, and therefore, their connection to a sediment source. Wetland success is dependent on the continued accretion of organic matter in the wetlands, which is given structure by the vast network of roots from macrophytes (Nyman et al., 2006). If organic matter accretion rates are unable to keep up with the rate of sea level rise, then several meters of organic matter can be lost once the root network holding the soil together begins to die (DeLaune and White, 2012).

The health of the soil microbial populations is inextricably linked with overall wetland health. Microorganisms mediate many biogeochemical processes that influence ecosystem function, such as denitrification and nitrogen (N) fixation (Gutknecht et al., 2006). Nitrogen-fixing bacteria form symbiotic relationships with some plants and provide inorganic N in exchange for a steady supply of bioavailable carbon (C) released from the plants as root
exudants. Microorganisms are also responsible for the decomposition of organic matter and subsequent release of bioavailable nutrients, which enhance primary production. Higher trophic levels, such as plants and animals, would be unable to obtain these nutrients without the soil microbial populations.

![Figure 1.2 Subsidence rates throughout southern Louisiana (US Geological Survey, 2000)](image)

1.2 SALINITY INUNDATION

Coastal systems experience two different types of salinity increases. Long-term increases are related to local and eustatic sea-level rise. Ecosystem changes related to sea-level rise are permanent in nature, as freshwater plants are unable to adapt to higher salinity levels, and are replaced by brackish and salt marsh species. Short-term changes in salinity are caused by storm surge events, such as cold-fronts or hurricanes. Ecosystem responses related to short-term salinity changes are more complex in nature as their effect depends on the duration of flooding and the stability of the system.
Louisiana’s coastal wetlands are extremely vulnerable to changes in salinity related to both sea-level rise and storm surge. Saltwater intrusion associated with hurricanes and tropical storms is a significant natural cause of land loss in Louisiana (Keddy et al., 2007). From 1950 to 2008, 18 hurricanes made landfall in Louisiana, 10 of which have been category 3 or greater (Figure 1.3), and this number is expected to increase as a result of global climate change (Roth, 2010). Coastal Louisiana also experiences storm surge from winter storms, which are typically more frequent, larger in size, and have more persistently changing winds than hurricanes and tropical storms (Feng and Li, 2010; Moeller et al., 1993). In the Chenier Plain, cold front passages appear to drive greater physical change than tropical storms (Roberts et al., 1989).

1.2.1 Salinity Effects on Coastal Wetlands

A short-term increase in salinity has a significant impact on ecosystem function, including macrophyte communities. All plant species are negatively impacted by salinity, but some species have a better tolerance for high salinities (Parida and Das, 2005). Research has shown that exposure to high salinity levels causes root hairs to wither and reduces the overall biomass of leaves, stems, and roots (Hill, 1908; Parida and Das, 2005). Salt-tolerant plants possess large vacuoles and thus, are able to more effectively regulate osmosis, but freshwater plants do not have these adaptations and quickly become dehydrated in the presence of high salinity levels (Bohnert and Jensen, 1996). Any loss of root biomass is extremely detrimental to coastal Louisiana because these roots provide structure to the highly organic soils in the Louisiana coast (Nyman et al., 2006).

Salinity effects on microorganisms are not as easily seen as those on plants but are just as important to overall wetland health. Microbial assemblages become more diverse after an increase in salinity as salt-tolerant species are able to now compete with the resident soil
populations (Zahran et al., 1997). With this change in assemblages, a 20% reduction in microbial extracellular enzyme activity has been recorded, which leads to a reduction of available nutrients to both plants and microorganisms (Jackson and Vallaire, 2009). Microbial biomass and activity both decrease with an increase in salinity (Joye and Hollibaugh, 1995; Tripathi et al., 2006).

An increase in salinity in a freshwater system significantly impacts the biogeochemical cycles in the system. An increase in salinity releases several compounds that are typically sorbed to the soil surface, such as extractable \( \text{NH}_4 \) and phosphate, into the soil porewater (Baldwin et al., 2006). Methane emissions tend to decrease with increasing salinity, but this relationship appears
to be only indirectly related, as studies have found sulfate and methane concentration in the soil to be inversely related, suggesting a negative coupling between methanogenesis and sulfide production (Bartlett et al., 1987).

Short-term salinity changes due to storm surge have significant impacts on wetland health. Despite the plethora of research related to salt effects on freshwater systems, there has been no research showing how salt moves through the soil during a short-term change in salinity. This type of research is important for the development of models designed to predict how coastal wetlands will be affected by short-term changes in salinity, and if anything should be done to try to mitigate this damage.

1.3 DIVERSIONS FOR SALINITY CONTROL

The use of river diversions has been suggested as a mechanism for protecting Louisiana’s coastal wetlands from both short- and long-term changes in salinity. Freshwater diversions would be opened periodically and would push freshwater into areas affected by high salinity levels. In the 2012 Coastal Master Plan, the Coastal Restoration and Protection Authority (CPRA) designated approximately $3.8 billion to the research and development of freshwater and sediment diversions in areas that were particularly hard hit by salinity changes and sediment loss (Coastal Protection and Restoration Authority, 2012). The building of freshwater diversions was originally authorized by the Flood Control Act of 1965, and both the Caernarvon and Davis Pond diversions were built as restoration projects designed to maintain specific salinities in their receiving basins.

The Caernarvon is a freshwater diversion located just down river and south of New Orleans and feeds into the Breton Sound Estuary. It was designed to divert a maximum flow of 227 m$^3$ s$^{-1}$ into Breton Sound. Construction on the diversion began in 1988 and it commenced...
operation in 1991. From 1991 to 2002, the average flow rate of the diversion was approximately 35 m$^3$s$^{-1}$, while remaining closed 42% of the time due to low river stage, tropical storms/hurricanes, and high tidal events. The Caernarvon was built to maintain the salinity from 5 to 15 ppt in Breton Sound (Louisana Department of Natural Resources, 2003). The Breton Sound estuary is isolated from the Mississippi River as a result of the building of levees, which prevents the estuary from receiving flood pulses from the river. As a result, the estuary has had a steadily increasing salinity that has led to a reduction in habitats and lower floral and faunal biodiversity (Louisana Department of Natural Resources, 2003).

The Davis Pond diversion is located just upriver west of New Orleans and feeds into Barataria Bay. Construction on Davis Pond began in 1997, and the project was completed in 2002. It was built to imitate historic spring floods and maintain salinity levels in the Barataria Basin from 2-10 ppt; this structure diverts approximately 300 m$^3$s$^{-1}$ from the Mississippi River at full capacity. The Army Corps of Engineers estimated that Davis Pond would help preserve around 130 km$^2$ in the first 50 years after it was built. From 2003-2004, the flow rate for Davis Pond was only 12 m$^3$s$^{-1}$, but it was closed 58% of that time because of back flow, tropical storms, and oil spills in the proximity of the diversion (Louisana Department of Natural Resources, 2005). Following design modifications in 2007, the Davis Pond diversion began operating more regularly (Gardner, 2006).

There has been a significant amount of controversy surrounding the effects of diversions on coastal wetlands. Lane et al. (2004) found that the salinity in the outer reaches of the areas affected by the Caernarvon diversion decreased significantly approximately two weeks after the diversion was opened. These data point to potential positive effects of the diversions, as the influx of freshwater could help protect freshwater tidal wetlands from the effects of hurricanes.
and other periods of short-term salinity increases. Turner (2011) found that when nutrients were added to a wetland, both the belowground biomass and the soil shear strength decreased while aboveground biomass increased. Because coastal Louisiana has such highly organic soils, the loss of belowground biomass equates to a loss in soil strength, which would lead to faster degradation of the soil. Davey et al. (2011) found that sites fertilized with N and P had significantly higher belowground biomass compared to the controls. The debate surrounding the issue of whether diversions would benefit the Louisiana coast or exacerbate land loss has divided both Louisiana lawmakers and the populace. Currently, no more freshwater diversions are going to be built in Louisiana, but planning has continued for sediment diversions (Figure 1.4). Construction on the first of ten major sediment diversions, which will alter salinity in coastal receiving basins and are planned to be bigger than both the Caernarvon and Davis Pond, is planned to begin in fall of 2015 (Schleifstein, 2014).

1.4 COASTAL NUTRIENT LOADING

The Mississippi River collects all of the pollutants from its watershed and transports them down to the Gulf of Mexico. Every year, coastal Louisiana receives 1.6 million metric tons of N and 180,000 metric tons of phosphorus (P) from the Mississippi and Atchafalaya Rivers (Hypoxia Task Force, 2011). Eutrophication causes many issues off of the Louisiana coast and has global consequences. The N cycle has been significantly altered by human interference; the amount of N applied to the land has more than doubled and is continually increasing (Vitousek et al., 1997). These loading rates have caused a number of problems including increased nitrous oxide concentrations in the atmosphere, soil acidification, loss of essential plant nutrients in the soil, and increased transport into lakes and rivers (Vitousek et al., 1997). One of the most notable results of nutrient loading in Louisiana is the large hypoxic zone that forms in the Gulf of
Mexico every summer (Figure 1.5). The low O₂ levels in the hypoxic zone lead to high mortality rates in benthic organisms and the forced migration of several species of fish (Rabalais et al., 2002). Closer to the coasts, high nutrient levels cause the degradation of water quality in estuaries and coastal wetlands and have been linked to a loss of biodiversity (Vitousek et al., 1997). Excess N and P are derived from a variety of both anthropogenic and natural sources.

Figure 1.4 A map of planned sediment diversions for the Mississippi River Delta (Schlefstein, 2013).

Natural sources of inorganic N include atmospheric deposition, fixation by microorganisms, and mineralization of organic N (Battaglin et al., 2001). Phosphorus is naturally derived from unweathered rocks and the mineralization of soil organic matter (Holtan et al., 1988). Anthropogenic loading of N and P is primarily derived from agricultural and urban uses (Chang
et al. 2002). Nonpoint sources contribute approximately 90% of the N and P loading to the Mississippi River basin (Carpenter et al., 1998; Chang et al., 2002). As the population of the United States has grown, fertilizer application has increased to ensure sufficient food production for the population. The N and P used in fertilizer are washed from the fields into local streams and rivers via either surface flow or irrigation pipes. Urban areas have large areas of impervious surface, so pollutants flow directly into receiving basins (Carpenter et al., 1998).

![Annual Spring Nutrient Load](image)

Figure 1.5 Nitrogen loading to the Gulf of Mexico and the corresponding hypoxic zone (US Environmental Protection Agency, 2010). This figure shows only loading during the month of May.

### 1.4.1 Nutrient Effects on Coastal Wetlands

Wetland plant species respond significantly to changes in nutrient loading. Species type can be heavily restricted by nutrient availability; many plants such as *Phragmites australis* are unable to compete with other species in low nutrient conditions, but are extremely competitive
once nutrient loading occurs. In the 1800s, *Phragmites* was reported as being “rare to occasional” in several states, but it has expanded to the point where it is now present in almost every state, with few exceptions (Chambers et al., 1999). Several studies have found that an increase in N loading causes a decrease in belowground biomass (Deegan et al., 2012; Turner, 2011). Deegan et al. (2012) found that long-term fertilization decreased belowground biomass of *Spartina alterniflora* located at the edges of tidal creeks in a New England salt marsh. Over time, the degradation of the roots led to the collapse of the tidal creeks, as the highly organic soil was no longer able to support itself. In a review of literature, Morse et al. (2014) found that most studies showed an increase in roots and shoots along with an increase in belowground macro-organic matter with nutrient loading. Overall, research on the effects of nutrient loading on wetland plants shows conflicting results based on region, fertilizer application rates, and fertilizers used, and illustrates the need for more research to provide concensus.

Soil microorganisms are a vital part of many biogeochemical cycles, and their activity can be strongly affected by changes in nutrient levels. There is a shift in microbial communities as a result of nutrient loading as diazotrophs and other microorganisms able to compete at low-nutrient concentrations are out-competed by other species (Kolb and Martin, 1988; Piceno and Lovell, 2000). Heterotrophic microbial activity increases with increased P loading, and the enhanced microbial activity contributes to a higher soil decomposition rate (Wright and Reddy, 2001a). Extracellular enzyme activity is another indicator of how microbial populations respond to nutrient loading; many extracellular enzymes liberate bioavailable nutrients from the soil organic matter. Typically, there is an inverse relationship between enzyme activity and nutrient availability because of the high energetic cost associated with enzyme production (Allison et al., 2007; Wright and Reddy, 2001b). There are very few studies looking at the long-term impact of
nutrient loading on soil microbial communities. Since microorganisms are such a critical driver of many ecosystem functions, it is important to understand how they will respond to long-term nutrient loading.

Coastal Louisiana is constantly at risk from both nutrient and salinity loading due to its positioning between the Gulf of Mexico and the Mississippi River. It is important to understand how these two drivers affect the coastal wetland soils to better inform management and restoration decisions. The opening of freshwater diversions may be able to mitigate salinity impacts. However, if the wetlands may be negatively affected by periodic long-term exposure to high nutrient water, then a lower frequency of events or a different approach may be necessary.

1.5 SYNOPSIS OF CHAPTERS

In Chapter 2, the movement of salinity into soil cores after exposure to high saline water is evaluated. Measurements were taken every two centimeters to establish high spatial resolution results of the movement of salinity. Additionally, extractable ammonium (NH$_4$) measurements were taken to try and examine a relationship between changing salinity and NH$_4$ production rates. We predicted that depth of salinity penetration would increase linearly with time. Based on literature that shows exchangeable NH$_4$ decreasing with increasing salinity, we expected that extractable NH$_4$ would decrease as salinity increased.

After discussion of the results on the impacts of salinity on a coastal wetland soil in Chapter 2, Chapter 3 investigates how soil characterization and microbial activity respond to a decade of nutrient loading in an oligohaline wetland. Extracellular enzyme activity, potentially mineralizable P, and total P concentrations were all investigated to see how they would be affected by the change in nutrients. We predicted that extracellular enzyme activity and potentially mineralizable P rates would decrease with nutrient loading while total P would
increase with P loading. The results can be used to determine if nutrient loading related to
diversion opening detrimentally affects soil microbial populations.
CHAPTER 2: EFFECTS OF SIMULATED STORM SURGE ON FRESHWATER COASTAL WETLAND SOIL POREWATER SALINITY AND EXTRACTABLE AMMONIUM LEVELS

2.1 INTRODUCTION

Between the early 1850s and 2008, approximately 106 tropical storm events have made landfall somewhere along the Louisiana coast (Stone et al., 1997; Roth, 2010), and cold-front passages occur even more often and affect larger swathes of coastline (Feng and Li, 2010; Moeller et al., 1993). Both hurricanes and storm fronts can cause major storm surge events that flood low-lying coastal wetlands with high salinity water (Moeller et al., 1993; Rego and Li, 2009). During hurricanes, storm surge can vary significantly based both on the Saffir-Simpson category and on the size of the hurricane (Irish et al., 2008). The resulting storm surge does not immediately exit the marshes after the passage of the storm, and high water levels persist (Li et al., 2010). As a result, a freshwater marsh may be exposed to more saline conditions for a significant length of time after the storm masses, and if there is more than one major storm in sequence, the effects of saltwater intrusion may be seen up to eight days after initial inundation occurs and may persist for longer periods of time in low lying depressions (Li et al., 2010).

Recently, concern over rising sea level has triggered a number of studies investigating the effects of salinity increases on wetland biogeochemical processes and ecosystem functions. Louisiana’s coastal deltaic plain is characteristically low relief and, as a consequence, is highly susceptible to inundation by frontal storm events or hurricanes and associated storm surge. A significant portion of the Mississippi River delta region is subsiding and in concert with increasing sea level, has led to significant coastal land loss over time (Barras et al., 2008).
Increased salinity is known to have a variety of effects on biogeochemistry, microorganisms, and plants. There is a clear change in biogeochemistry across a salinity gradient. Dausse et al. (2012) found that freshwater marshes exclusively exported dissolved organic carbon (DOC) whereas salt marshes imported DOC in the spring months, and nitrate was imported into the salt marshes and exported from freshwater marshes in the fall months. Chambers et al. (2011) found that short-term salinity pulses into freshwater wetlands affected carbon (C) cycling by increasing the rate of C mineralization. Salinity changes can reduce the rate of microbial processes in wetland soils such as denitrification (Giblin et al., 2010). Additionally, Jackson and Vallaire (2009) found that an increase in salinity decreased the activity of the extracellular enzymes phosphatase, β-glucodase, and NAGase. These enzymes are critical for ensuring nutrient availability. Research from an estuarine system receiving varying levels of salinity throughout the year has shown that salinity can penetrate the soil in response to seasonal changes in salinity, and that the shift in salinity can significantly decrease the availability of extractable ammonium (NH₄) (Weston et al., 2010). Short term changes in salinity reduces both the availability of nutrients to plants and the uptake rate of inorganic nutrients, such as NH₄ (Blood et al., 1991; Rysgaard et al., 1999). Research in a South African estuary showed that plant cover decreased by 15.2% and reed and sedge cover by 19.7% after a storm surge raised the salinity level from 22 g L⁻¹ to 31 g L⁻¹ (Riddin and Adams, 2010).

Many of Louisiana’s coastal freshwater wetlands are affected by storm surges associated with tropical storms and winter cold-front passages, but there is little documentation describing how these saltwater pulses cause vertical and temporal changes in the distribution of salinity and nutrients in the porewater of freshwater tidal wetlands. There have been published studies that model the effects of storm surges on coastal systems (Hubbert and McInnes, 1999; Temmerman
et al., 2012), but these models do not incorporate the change in soil porewater salinity with depth and time as a consequence of storm surge. This information is critical for predicting the extent to which short-term salinity changes can affect important wetland soil functions and ecosystem productivity. Therefore, the goal of this study was to measure changes in the vertical and temporal porewater salinity as a result of imposing a 35 g L\(^{-1}\) salinity water column over the coastal freshwater wetland soils collected from the Wax Lake delta, LA in a laboratory setting.

2.2 MATERIALS AND METHODS

2.2.1 Study Site

The study area is located at the southern terminus of the Atchafalaya Basin which receives approximately 30% of the Mississippi River through the old river control structure located just north of Baton Rouge, LA, in addition to contributions from the Red River. The Atchafalaya Basin has two primary outlets where active delta formation is currently underway with associated establishment of coastal freshwater marshes, the Atchafalaya and Wax Lake deltas (Figure 2.1). The wetlands located in the Wax Lake Delta are dominated by fresh marsh species as a consequence of continuous river flow, despite the location within the Gulf of Mexico as these deltas are progradational and consequently protrude into the coastal, saline environment. Newly accreting areas are a mixture of non-vegetated mudflats and early succession macrophyte species. Vegetation cover over the entire Wax Lake Delta is approximately 12%, and the vegetation is dominated by *Sagittaria latifoli Salix nigra*, and *Colocasia esculenta*. (Carle et al., 2013; Evers et al., 1998; Holm and Sasser, 2001; Johnson et al., 1985). The marsh sites are more established, and are dominated by *Eleocharis* sp., *Panicum hemitomon*, and *Typha*, but also contain a much more diverse group of species than the islands (Sasser, 2014).
2.2.2 Sample Collection

Intact soil cores were collected at two sites within the Wax Lake Delta, LA in mid-October 2013 (Figure 2.1). The first site, referred to as “mudflat” was located at one of the barren mudflat islands in the delta. The site was inundated to a depth of ~ 50 cm at the time of sampling where 12 cores were taken within a ~ 2 m² area. The second site, referred to as “marsh” was located along Hogs Bayou and was not flooded at the time of sampling. At the marsh site, all of the aboveground biomass was removed by cutting the stems at the soil surface before the 12 cores were collected within a two m² area. All cores were collected using 7 cm diameter pushcores to collect the top 16+ cm of soil/sediment.

Figure 2.1 Map of the Wax Lake Delta in relationship to the Gulf Coast; the magnified image shows both the Wax Lake Delta (left) and the Atchfalaya River Delta (right) Image from Google Earth
2.2.3 Experimental Design

Cores were capped on both ends with rubber stoppers and transported to the laboratory where they were placed in a polypropylene tank. Any surface water that remained was removed and triplicate cores from each site were randomly assigned to one of the four treatments; control, 1 week, 2 weeks and 4 weeks of inundation with salt water. A hole was drilled through the side of the core, 10 cm above the sediment, to allow water to flow out of the core in order to maintain a water column of 10 cm. Artificial seawater at 35 g L\(^{-1}\) was continually supplied to each core by peristaltic pumps and timers which allowed turnover of the water column 4 times per day. The cores were shielded from light by covering the tank sides and top with aluminum foil.

Triplicate cores were destructively sampled sectioning into 2-centimeter increments down to a maximum depth of 16 cm. All depth intervals were homogenized, transferred to polypropylene containers and stored at 4 C until analyzed. Each time interval had triplicate cores with a minimum of seven sections, for a total of approximately 190 samples. The triplicate control cores were sectioned immediately upon retrieval from the field.

2.2.4 Laboratory Analyses

Gravimetric moisture content was determined for all samples by drying 20 g subsamples at 70 °C in a forced air oven until constant weight. Bulk density was calculated using the moisture content, total wet weight, and volume of each sample. Total C and N were determined on dried, ground subsamples using an Elemental Combustion System (Costech Analytical Technologies, Valencia, CA). Total P was determined by ashing dried, ground subsamples at 550 °C followed by digestion with 6 M HCl (Andersen, 1976). Total P was measured colorimetrically (USEPA Method 350.2) using a SEAL AQ2 Automated Discrete Analyzer.
Weight loss on ignition (LOI) from the total P procedure was used as a measure of organic matter content.

Porewater salinity was determined on field moist subsamples which were prepared for analysis by adding a known amount of soil to polypropylene 30 ml polypropylene scintillation vials filled with 20 ml of de-ionized water. Samples were thoroughly shaken and then allowed to settle for 48 hours prior to salinity determinations using an Accumet AB30 electrical conductivity meter and probe (Cole-Palmer, Vernon Hills, IL). Electrical conductivity was converted to the practical salinity scale, a unitless measure of salinity.

Extractable NH$_4$ was measured by adding 20 mL of 2M KCl extractant to a 5 g subsample of moist soil in 50 mL centrifuge tubes. The tubes were capped, placed on a longitudinal shaker for 30 minutes and centrifuged in a Sorval refrigerated centrifuge for 10 minutes at 4000 G. Samples were then vacuum filtered through 0.45 µm membrane filter papers, acidified to a pH <2 with concentrated sulfuric acid and stored at 4 °C until analysis. Extractable NH$_4$ was analyzed colorimetrically (Method 350.1, US Environmental Protection Agency, 1993) using a SEAL AQ2 Automated Discrete Analyzer (West Sussex, England).

### 2.2.5 Statistical Analysis

The salinity and extractable NH$_4$ levels in the treatment cores were compared to the control cores using a Student’s T-test for each time step and by depth ($\alpha=0.05$). Characteristics between sites in the control cores were also compared to one another using the Student’s T-test ($\alpha=0.05$).
2.3 RESULTS

2.3.1 Control Characteristics

The moisture content at the mudflat site was 29 ± 3.0 %. In the marsh site, the moisture content was significantly higher at 48 ± 5.0 %. The marsh site had significantly lower bulk density of 0.91 ± 0.15 g cm⁻³ when compared with soils from the mudflat site which averaged 1.58 ± 0.27 g cm⁻³. The organic matter content, as determined by loss on ignition, was significantly higher at the marsh site compared with the mudflat site at 9.38 ± 2.40 and 2.66 ± 2.01 wt%, respectively. Total C, N and P concentrations were also significantly greater at the marsh site compared to the than the mudflat site (Table 2.1). The concentration of extractable NH₄-N was significantly lower at the marsh site- averaging 0.85 ± 1.72 mg kg⁻¹ than at the mudflat site, 1.47± 6.84 mg N kg⁻¹.

Table 2.1 Soil characteristics from the marsh and mudflat sites averaged over depth for all the control cores.

<table>
<thead>
<tr>
<th></th>
<th>Mudflat</th>
<th>Marsh</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD (g cm⁻³)</td>
<td>1.58 ± 0.23*</td>
<td>0.91 ± 0.1*</td>
</tr>
<tr>
<td>TN (g kg⁻¹)</td>
<td>0.02 ± 0.02*</td>
<td>0.66 ± 0.3*</td>
</tr>
<tr>
<td>TC (g kg⁻¹)</td>
<td>0.18 ± 0.05*</td>
<td>2.66 ± 0.65*</td>
</tr>
<tr>
<td>TP (mg kg⁻¹)</td>
<td>384 ± 52*</td>
<td>679 ± 36*</td>
</tr>
<tr>
<td>Organic Matter (wt%)</td>
<td>2.66 ± 1.47*</td>
<td>9.38 ± 2.03*</td>
</tr>
<tr>
<td>Salinity</td>
<td>1.46 ± 0.15*</td>
<td>1.03 ± 0.04*</td>
</tr>
<tr>
<td>NH₄-N (mg kg⁻¹)</td>
<td>4.75 ± 6.42</td>
<td>1.25 ± 0.8</td>
</tr>
</tbody>
</table>

Most soil characteristics did not change significantly with depth with the exception of extractable NH₄-N, which decreased significantly with depth. Extractable NH₄-N levels decreased from an average of 18.9 ± 9.6 mg kg⁻¹ in the top two cm down to an average of 0.52 ± 0.5mg N kg⁻¹ in the 14-16 cm interval of the mudflat soil cores. This decrease was not as pronounced in the marsh soil cores where the average concentration of extractable NH₄-N was 2.43 ± 2.91 mg N kg⁻¹ in the top two centimeters and 0.79 ± 0.04 mg kg⁻¹ at the 14-16 cm depth.
On average, the porewater salinity in the mudflat control cores was 1.46 ± 0.68. In the marsh cores, soil salinity was 1.03 ± 0.25. Soil salinity varied very little with depth in both the mudflat and marsh control cores, but porewater salinity was slightly elevated at the surface of the cores from both sites. In the mudflat cores, porewater salinity varied from an average of 1.74 ± 1.6 in the top two cm to 1.38 ± 0.4 g in the 14-16 cm depth. The marsh soil cores had a smaller difference in salinity between the top two cm of the soil cores and 14-16 cm. Porewater salinity was 1.04 ± 0.5 in the top two cm and 0.986 ± 0.04 in the 14-16 cm depth interval.

2.3.2 Salinity

During week 1, there was a significant increase in soil salinity in both sets of soil cores. In the mudflat soil cores seen in graph a in Figure 2.2, the soil porewater salinity increased over 30 in the top 2 cm of the mudflat cores. In the marsh cores seen in graph b of Figure 2.2, the marsh cores saw an increase of about 25 in the top two cm of soil after the first week of flooding. The effect of saltwater inundation diminished with depth. In the 14-16 cm interval in the mudflat cores average salinity levels increased to 2.35. In the marsh cores, average salinity levels in the 14-16 cm interval decreased to 1.98. In the mudflat soil cores, there was a significant increase in porewater salinity in the top 8 cm. In the marsh soil cores, soil porewater salinity increased significantly in the top 6 cm.

During the second week of flooding, significantly elevated levels of salinity were seen deeper in both the mudflat and marsh cores. In the mudflat cores, average salinity levels were 36.7 in the top two cm, and 2.20 in the 14-16 cm interval, as seen in graph a of Figure 2.2. Average salinity levels in the top two cm of the mudflat cores were 32.7, and in the 14-16 cm interval they were 2.43, as seen in graph b of Figure 2.2. In the mudflat cores, significantly
elevated salinity levels were seen in the top 8 cm of the soil cores. Significantly elevated salinity
levels were seen in the top 10 cm of the soil core in the marsh cores.

After the fourth week of flooding, significantly elevated salinity levels were seen in the
top 14 cm of both the marsh and mudflat cores. In the mudflat cores, the average salinity level in
the top two cm was 33.7, and in the 14-16 cm interval it was 1.19, as seen in graph a of Figure
2.2. In the marsh cores, the average salinity level of the top two cm was 25.0 and in the 14-16 cm

![Figure 2.2 Change in salinity with depth and time in the (a) mudflat and (b) marsh cores.](image)
interval it was 2.64, as seen in graph b of Figure 2.2. The average salinity in the mudflat and marsh cores increased to 12.24 and 10.03, respectively.

The mass of salt in each soil core was calculated using the moisture content and salinity in each 2 cm interval to compare the amount of salt that entered the core from week to week. In the mudflat cores, 53% of the salt that entered the cores during the entire experiment entered within the first week of exposure to saline waters. By the second week of flooding, 100% of the salt that entered the soil core during the experiment had diffused into the core. During the fourth week of flooding, the overall salt content of the core did not increase, but penetrated more deeply in the core. In the marsh cores, 80% of the salt that entered the soil during the entire experiment entered during the first week of flooding. At the end of the second week flooding, 96% of the salt that entered the soil core during the experiment had diffused into the core. The remaining 4% of salt entered during the fourth week of flooding.

2.3.3 Extractable NH₄-N

The concentration of extractable NH₄-N varied significantly between the marsh and mudflat cores after one week of flooding. In the mudflat cores, seen in graph a of Figure 2.3, the level of extractable NH₄-N after the first week of flooding ranged from 10.06 mg N kg⁻¹ in the top two centimeters to 1.87 mg kg⁻¹ in the 14-16 cm interval. In the marsh cores, seen in graph b of Figure 2.3, the level of extractable NH₄-N was 15.87 mg kg⁻¹ in the top two cm and to below the detection limit in the 14-16 cm interval. There was a significant increase in NH₄ in the top 2 cm of soil compared to the control.

Extractable NH₄-N levels generally increased in both the marsh and mudflat cores compared to the control after the second week of flooding. Level of extractable NH₄-N did not have any noticeable trends throughout the core, as seen in graph a of Figure 2.4. Ammonium
levels in the marsh cores as a whole were significantly higher than those seen in the the control, as seen in graph b of Figure 2.4. The level of extractable NH$_4$-N in the top two cm of the mudflat soil cores was 20.88 mg kg$^{-1}$, and 1.87 mg N kg$^{-1}$ in the 14-16 cm interval. In the marsh cores, the level of extractable NH$_4$-N was 16.51 mg N kg$^{-1}$ in the top two cm and 1.00 mg kg$^{-1}$ in the 14-16 cm interval, and was significantly higher than the control in the top 6 cm. Extractable NH$_4$-N
levels after the fourth week of flooding showed a showed a different pattern in the mudflat cores compared to the marsh cores. Extractable NH$_4$-N levels in the mudflat cores decreased compared to the control; concentrations were 5.89 mg kg$^{-1}$ in the top two cm and 1.87 mg kg$^{-1}$ in the 14-16 cm range. Levels in the marsh cores ranged from 12.27 mg kg$^{-1}$ in the top 2 cm to 4.13 mg kg$^{-1}$ in the 14-16 cm increment. Significantly elevated levels extended down to 14 cm in the marsh cores.

Figure 2.4 Extractable NH$_4$-N concentrations as a percentage of the control in the (a) mudflat and (b) marsh cores.

2.4 DISCUSSION

Soil properties for the marsh and mudflat soils were significantly different for most characteristics tested. These differences were likely due to the more complete colonization by
macrophytes and associated belowground biomass at the marsh sites. Initial extractable NH\textsubscript{4} levels were lower in the marsh cores than the mudflat cores likely due to the presence of vegetation at the time of sample collection. The lack of vegetation in the mudflat sites allowed for higher values of NH\textsubscript{4}, since mineralized NH\textsubscript{4} was not removed from the system via plant uptake.

Salinity levels increased significantly in the majority of the top 10 cm of soil in all treatments (Table 2.2). The highest salinity changes were seen at the surface of the soil column. In the top two centimeters, the salinity increased by a minimum of 25 for all treatments in both the marsh and mudflat soil cores. The highest salinity values were seen in the mudflat soil cores, but significantly elevated salinity levels were present deeper in the marsh cores compared to the mudflat after the second week of flooding. The presence of belowground biomass in the marsh soil cores may have created preferential flow path that allowed the salt to move through the soil more directly than in the mudflat soil cores. The results show that a majority of the salt that enters the soil column enters during the first week of flooding. In the mudflat cores, 53\% of the total mass of salt that entered during the entire experiment entered during the first week, and in the marsh cores 80\% of the salt that entered the cores during the experiment diffused into the cores during the first week.

The change in salinity had no significant effects on the concentration of extractable NH\textsubscript{4}. After the first week of flooding, there was no significant change in the amount of extractable NH\textsubscript{4} in the mudflat cores (Table 2.3). There was a marginally significant (p<0.1) increase in extractable NH\textsubscript{4} in 4-6 cm interval during the second week of flooding, but no significant change anywhere else in the profile. A significant increase was seen from the 4-12 cm, but no significant change at any other depths. In the marsh cores, there was a general increase in ammonium
throughout the experiment, likely because the microorganisms were still able to mineralize NH$_4$ due to higher organic matter content (Figure 2.5). After the first week of flooding there was a significant increase in NH$_4$ in the 14-16 cm interval and marginally significant increases in the 0-2 cm and 8-10 cm intervals. After the second week of flooding, there was a significant increase from 0-6 cm. After the final week of flooding there was a significant increase of extractable NH$_4$ in the top 14 cm of soil.

Table 2.2 Significant changes in salinity with depth and time. Upwards arrows indicate a significant increase in salinity (p=0.05), and N indicates no significant change compared to the control.

<table>
<thead>
<tr>
<th>Mudflat</th>
<th>Flood Time (weeks)</th>
<th>Marsh</th>
<th>Flood Time (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (cm)</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>0-2</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td>2-4</td>
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<td>4-6</td>
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<td>6-8</td>
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<tr>
<td>8-10</td>
<td>N</td>
<td>N</td>
<td>↑</td>
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<tr>
<td>10-12</td>
<td>↑</td>
<td>N</td>
<td>↑</td>
</tr>
<tr>
<td>12-14</td>
<td>N</td>
<td>N</td>
<td>↑</td>
</tr>
<tr>
<td>14-16</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

Short-term fluctuations in salinity can have a negative effect on both the plant and microorganisms if the organisms are unable to adapt to the shifting conditions. In coastal wetland soils, the majority of the plant roots are contained in the top 10 cm (Turner et al., 2004; VanZomeren et al., 2012). In this experiment, it took one week for the salinity levels to become elevated in the top 10 cm of the soil in both the marsh and mudflat soil cores. Therefore, a number of biogeochemical processes may also be affected, which can affect many wetland functions.

These data can be used to more accurately model the effects of storm surge and short-term fluctuations in salinity to better predict how storm surge will affect the soil of a flooded
coastal freshwater wetland. Additional work needs to be done to determine how long the porewater takes to return to previous conditions, once the salt has permeated down through the soil volume and freshwater conditions have returned.

Table 2.3 Significant changes in extractable NH$_4$-N with depth and time. Upwards arrows indicate a significant increase in extractable NH$_4$-N compared to the control (p=0.05), N indicates no significant change.

<table>
<thead>
<tr>
<th>Mudflat</th>
<th>Flood Time (weeks)</th>
<th>Marsh</th>
<th>Flood Time (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (cm)</td>
<td>1</td>
<td>2</td>
<td>4</td>
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<tr>
<td>0-2</td>
<td>N</td>
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<tr>
<td>2-4</td>
<td>N</td>
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<tr>
<td>4-6</td>
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<td>12-14</td>
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<tr>
<td>14-16</td>
<td>N</td>
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</table>

Figure 2.5 Change in extractable NH$_4$-N through the entire soil column with time.
2.5 CONCLUSION

Storm surge inundation is a global phenomenon and affects freshwater wetlands both in Louisiana and throughout the world. If the movement of saltwater in the soil column can be accurately modeled, then it will be possible to predict the extent of ephemeral shifts in salinity. This study is one of the first attempts to quantify how salinity changes with depth after a short-term increase in surface water salinity.

Our results showed that a significant portion of macrophyte roots would be exposed to high salinity levels after a very limited exposure time, and that the largest percentage of salt enters the soil cores during the first week of flooding. Plants could potentially be strongly affected by the changes in salinity associated with a weeklong flooding event. The concentration of extractable \(\text{NH}_4\) was not affected by the presence of salinity, and increased throughout the experiment in the marsh cores. The soil microorganisms were able to continue mineralizing organic matter to form \(\text{NH}_4\), which speaks positively for the resilience of wetland soil microorganisms, and implies the population may be adapted to short-term changes in salinity.
CHAPTER 3: EFFECTS OF LONG-TERM NUTRIENT LOADING ON SOIL MICROBIAL ACTIVITY

3.1 INTRODUCTION

Coastal eutrophication has become a major cause for concern both in the Mississippi River Delta and throughout the world (Rabalais et al., 2009). Nutrient inputs into the Gulf of Mexico causes a wide variety of environmental issues, including the degradation of water quality and the annual formation of a large hypoxic zone (Rabalais et al., 2002; Vitousek et al., 1997). A plethora of solutions have been suggested to help abate these problems, including using diversions to push nutrient laden water through coastal wetlands that do not currently receive water from the Mississippi River. The wetlands could potentially serve as a sink for nitrogen (N), phosphorus (P), and other nutrients/contaminants present in the Mississippi River. Lane et al. (1999) determined that Caernarvon marshes received nutrient loading at a rate of 234 kg ha\(^{-1}\) yr\(^{-1}\) of N and 20 kg ha\(^{-1}\) yr\(^{-1}\) of P from the Mississippi River. This study also found that N concentrations rapidly decreased via dentrification once the water entered the estuary, and that P was readily sorbed onto clay particles, thus significantly decreasing nutrients in the surface water. Another study completed in the Davis Pond Diversion confirmed these results, with the caveat that the ability of the marshes to remove N efficiently would decrease with increased discharge (Yu et al., 2006). These results indicate that freshwater diversions would be able to decrease nutrient loading to the Gulf of Mexico if they were managed primarily for this purpose. However, a number of concerns have been raised about the potential impacts of nutrient loading on coastal wetlands. Multiple studies have shown that high levels of nutrient loading decrease belowground biomass and soil shear strength while increasing decomposition rates, which is primarily a microbial process (Darby and Turner, 2008; Deegan et al., 2012; Turner, 2011).
combination of these changes to the soil would cause faster subsidence rates, and could lead to faster degradation of the Louisiana coast. In opposition to these studies, work done in the Breton Sound Estuary found that belowground biomass was either unaffected or actually increased as a result of receiving water from the Caernarvon Diversion (Moerschbaecher, 2008).

Soil microbial populations are diverse and play a pivotal role in a number of important ecosystem processes, such as coupled organic matter decomposition and nutrient cycling. Microbial processes are extremely sensitive to changes in environmental changes, and are therefore a good indicator of how a system will respond to both external and internal forcings. Research in the Florida Everglades has examined the effects of decades of P loading on microbial activity. The Everglades are traditionally a P limited system that has received significant P loading from surrounding agricultural and urban activities (Newman et al., 1998). Restoration efforts, including construction of some of the world’s largest treatment wetlands designed to remove P and increasing water storage to allow for the controlled release of water, have helped to decrease P loading to the system (Guardo et al., 1995; Perry, 2004). Unfortunately, the Everglades continue to be affected by elevated P values related to a legacy effect (Reddy et al., 2011). Soils from impacted sites had higher anaerobic populations than those from unimpacted sites, resulting in a higher rate of methane flux out of the soil (Drake et al., 1996). Castro et al. (2004) categorized the genera present in eutrophic and oligotrophic sites in the Everglades, and confirmed that sites affected by P loading had significantly higher populations of methanogens and consequently higher rates of methanogenesis. Microbial respiration, which reflects the activity of microorganisms, increased significantly with P loading to P limited soils P-limited soils (Amador and Jones, 1995). The increase in microbial activity has been linked to the large
release of nutrients from organic matter and shifts in the macrophyte community (Doren et al., 1997).

Extracellular enzyme activity is often used a proxy to gauge how soil microorganisms respond to external forcings and have historically been used to make inferences about a wide variety of soil processes. Enzyme activity can be used to gauge response to nutrient loading, analyze soil quality, and assess the response of soil microbial populations to xenobiotic compounds such as pesticides (Burns, 1982). Extracellular enzymes are found throughout the soil matrix. The total activity of any enzyme is comprised of a combination of eleven different activities (Figure 3.1) associated with soil constituents, living and dead cells, and the soil porewater (Burns et al., 2013). Extracellular enzymes can be broadly divided into two major categories, those that are attached to the cell and those that are excreted into the soil matrix. Enzymes that are directly associated with cells are able to provide bioavailable nutrients directly to their producing organism. These enzymes can either be within the perisplasmatic membrane or attached to the outside of the cell wall. However, they are less likely to come into contact with soil substrate as they can only capture substrate in close proximity to the cell. Enzymes released into the soil matrix are unlikely to provide nutrients to their original producer as they may become attached to the soil or move away from the cell. These enzymes are much more likely to come into contact with a usable substrate than enzymes attached to the cell, and release nutrients into the soil porewater. The concentration of extracellular enzymes excreted by the total soil microbial population is high enough that individual microorganisms are able to obtain nutrients required for survival.

Extracellular enzymes are not the only compounds used by microbial communities to obtain essential nutrients. In both aquatic and terrestrial systems, iron (Fe) deficiency is
alleviated by the release of extracellular molecules that solubilize and transport iron (Sandy and Butler, 2009). Siderophores are chelating agents that are used in a similar manner to extracellular enzymes, and are released into either the soil matrix or water column by organisms in need of bioavailable iron (Crowley et al., 1991; Miethke and Marahiel, 2007). Similar to extracellular enzymes, siderophores that have succeeded in solubilizing Fe do not necessarily return to the organism that produced them, but are produced and released by many organisms and are therefore abundant enough to maintain the microbial population (Sandy and Butler, 2009). Once there is sufficient Fe present for growth, siderophore production is stopped and resumes again once Fe levels are depleted, similar to extracellular enzyme production (Sandy and Butler, 2009).

Figure 3.1 Locations of enzymes in the soil matrix (Burns et al., 2013). Black arrows indicate how enzymes move through the soil matrix.
Understanding the response of extracellular enzymes to long-term nutrient loading may help researchers predict the response of siderophores and other extracellular compounds used for nutrient acquisition to long-term nutrient loading.

Hydrolyzing enzymes are important to the soil ecosystem, especially in systems where there are little to no external nutrient inputs. Enzymes are generated by both plants and microorganisms, and are the primary means of obtaining bioavailable nutrients. Once the enzymes come into contact with the soil substrate, the appropriate nutrient binds to the enzyme and is hydrolyzed from the organic complex. Enzymes production comes at a high energetic cost to microorganisms, and are therefore only produced when bioavailable nutrients in the soil are limited. As a result, enzyme activity can be used to gauge how microorganisms are responding to nutrient loading. Wright and Reddy (2001b) found that phosphatase activity in a wetland system was suppressed when bioavailable P was added to the soil, but that sulfatase and β-glucosidase were unaffected, suggesting both that not all enzymes are equally responsive to perturbations and that enzymes respond to a specific target nutrient.

The use of freshwater diversions would expose coastal wetlands to high levels of nutrient loading. There is little information looking at long-term effects of combined N and P loading on enzyme activity. There has been extensive work done at this site looking at changes in plant community structure and function but no work done on the effects of long-term nutrient loading on soil biogeochemistry (Graham, 2013; Graham and Mendelssohn, 2010). This information is critical for predicting the response of a wetland system to long-term annual nutrient loading. Therefore, the goals of this study were to analyze differences in soil chemistry, enzyme activity, and PMP rates resulting from changes in nutrient loading.
3.2 MATERIALS AND METHODS

3.2.1 Site Description

Lake Ponchartrain estuary is a brackish estuary located in southeastern Louisiana that is connected to the Gulf of Mexico via Chef Menteur Pass and the Rigolets strait, and covers an area of 1600 km². The watershed is composed of five rivers- Tangipahoa, Tchefuncte, Tickfaw, Amite, and Bogue Falaya rivers- and two bayous- Bayou Lacombe and Bayou Chinchuba, and encompasses over 25,000 km². Ponchartrain was formed approximately 2600 to 4000 years ago while the Mississippi River was flowing through Teche delta (US Geological Society, 2013). Salinity in the lake varies from 0 to 15, depending on the distance from fresh and salt water sources, precipitation, and tidal forces. The estuary is located within portions of six different parishes, including the Orleans parish where the city of New Orleans is located. There are currently 1.5 million people living around the lake, and is widely used for recreational and commercial purposes such as fishing, swimming, and boating.

The Tchefuncte River is located in southeastern Louisiana, and is approximately 110 km long (US Geological Survey, 2000). The Tchefuncte River begins in Tangipahoa parish and flows through both Washington and St. Tammany’s parish before emptying into Lake Ponchartrain. During the 19th century, the Tchefuncte was an important waterway and was used to transport building materials, such as lumber, down to New Orleans. At its mouth, the Tchefuncte experiences a microtidal regime of less than half a meter.

The study site was located on the northshore of the Lake Ponchartrain estuary near the Tchefuncte River in Madisonville, LA (Figure 3.2). The soil at the site is classified as a Fluvaquentic Medisaprist and is characterized as a “very poorly drained, rapidly permeable organic soil” (Trahan et al., 1990). The site is dominated by *Sagittaria lancifolia, Eleocharis*
fallax, and Polygonum punctatum. Average surface water salinity at the site from 1999 to 2006 was 1.6 g L\(^{-1}\) (Graham and Mendelssohn, 2010). Water levels at the site are affected by a 10 cm microtidal range, and fluctuations in either Lake Ponchartrain, driven by wind shifts during frontal passages, or changes in Tchefuncte River discharge related to precipitation.

3.2.2 Experimental Design

The Madisonville site was fertilized twice yearly from 2002 until 2013, during both the spring and summer. The experimental was set-up using factorial treatment arrangements of N and P with a randomized block design (Figure 3.3). There were four N treatments- 0, 50, 200, and 1200 kg N ha\(^{-1}\) yr\(^{-1}\) applied as Nutralene methylene urea 40-0-0 and two P treatments- 0 and
131 kg P ha\(^{-1}\) yr\(^{-1}\) applied as Humaphos 0-5-0. The control plots at the Madisonville site were considered to represent an unimpacted site that had not received any sort of nutrient loading from the Mississippi River. The next highest N fertilization rate was considered to be a site that received minimal loading. The 200 and 1200 kg ha\(^{-1}\) yr\(^{-1}\) plots were analogous with sites receiving Mississippi River water and extremely high N loading rates such as those that may be seen in a wetland near a sewage treatment plant respectively (Lane et al., 1999; Wigand et al., 2003). The highest P fertilization rate was more than five times higher than the expected loading rate of a site receiving water from the Caernarvon Diversion (Lane et al., 1999).

Figure 3.3 The layout of the Madisonville site. The blocks were placed parallel to a small canal. The four N treatments and two P treatments were factorially applied to each of the blocks (Graham and Mendelssohn, 2010)
3.2.3 Sample collection

Samples were collected in early February 2014 during the dormant season approximately seven months after the most recent fertilization event. Cores were collected from the marsh using pushcores with a 7.6 cm diameter. The soil was initially cut with a sharpened aluminum tube to avoid compaction, and then a 25 cm deep sample was taken. Cores were capped with rubber stoppers and transported to the lab at 4 °C for further processing. Soil cores were divided into two sections in the lab, 0-10 cm and 10-20 cm, placed into polypropylene sampling containers, and immediately weighed.

3.2.3 Laboratory Analyses

Extracellular enzyme activity was measured via fluorometric emission. Fluorescence is a property of some substances that absorb light at certain wavelength, called the excitation bandwidth, and then emit light at a longer, less energetic wavelength, called the emission bandwidth. When light is emitted, a microplate reader can measure the emission over a certain range above and below the emission bandwidth called the bandwidth of excitation. In this experiment, the emission bandwidth, excitation bandwidth, and bandwidth of excitation were 360, 460, and 20 nm, respectively.

Extracellular enzyme activity was determined within 48 hours of sample collection on a BioTek FLX800. Soils were kept at approximately 18 °C until they were analyzed. At the time of analysis, a standard and quench curves were prepared and soil and substrate controls were measured. Samples were incubated for approximately two hours before analysis. The rate of enzyme activity was calculated using the modified calculation presented in German et al. (2012). A more detailed method for determining extracellular enzyme activity can be found in the Appendix.
Gravimetric moisture content was determined for all samples by drying 15 g subsamples at 70 C in a forced air oven until constant weight. Bulk density was calculated using the moisture content, total weight of each sample, and volume of each sample. Total C and N were determined on dried, ground subsamples using an Elemental Combustion System (Costech Analytical Technologies, Valencia, CA). Total P was determined by ashing dried, ground subsamples at 550 C followed by digestion with 6 M HCl (Andersen 1976). The P was measured colorimetrically (USEPA Method 119-A) using a SEAL AQ2 Automated Discrete Analyzer (West Sussex, England). Weight loss on ignition (LOI) from the total P procedure was used as a measure of organic matter content.

Potentially mineralizable P (PMP) and extractable P were measured in the top 10 cm interval of the soil core and the two most extreme treatments in the 10-20 cm depth interval. Extractable P was determined by adding 20 mL of 2M KCl extractant to a 2 g subsample of moist soil in 50 mL centrifuge tubes. The tubes were capped, placed on a longitudinal shaker for 30 minutes and centrifuged in a Sorval refrigerated centrifuge for 10 minutes at 4000 G. Samples were then vacuum filtered through 0.45 µm membrane filter papers, acidified to a pH <2 with concentrated sulfuric acid and stored at 4 C until analysis. Approximately 2.5 g of field moist soil were used for potentially mineralizable P; the soil was placed in a glass serum bottle and purged with N2 gas. The samples had 10 mL of N2-purged water added to them, and were incubated in a Lab Companion IS-971R Floor Model Incubated Shaker for 5 days. At the end of the incubation time, the serum bottles were removed and 20 ml of 2M KCl was added to the serum bottle via syringe. The samples were then placed on a longitudinal shaker 30 minutes. Once shaking was completed, the septa were removed and the contents were placed in 50 mL centrifuge tubes and were centrifuged and filtered in the same fashion as the extractable P
samples. Extractable P was analyzed colorimetrically (USEPA Method 350.2) at 0 and 5 days using a SEAL AQ2.

3.2.4 Statistical Analysis

All data analyses were completed using a mixed model ANOVA in SAS (version 9.1.3, SAS Institute, Cary, NC) to determine effects of N, P, depth, and their interaction on all experimental variables individually (p<0.05). Model assumptions of normality and homogeneity of variance were determined using Shapiro Wilks and Lavene’s tests (α=0.05) respectively. When necessary logarithmic, square root, cube root, or square transformations were used to verify association. Significant main effects were determined using type 3 test for fixed effects with Tukey’s HSD for post hoc comparisons. Data that did not meet the assumptions for the model (normality and homogeneous variance) were analyzed using the Wilcoxon Rank-Sum Test (α=0.05).

3.3 RESULTS

3.3.1 Soil Properties

Soils properties were determined for both the 0-10 cm and 10-20 cm depth intervals (Table 3.1). The average moisture content in the upper 10 cm was 84.5 ± 1.8 wt% and 87.0 ± 2.2 wt% from 10-20 cm. The average bulk density was significantly higher in the top ten centimeters with an average of 0.149 ± 0.01 g cm⁻³ as opposed to 10-20 cm depth with an average of 0.133 ± 0.01 g cm⁻³. Total N ranged from 1.02 to 2.15 g kg⁻¹ across nutrient treatments. Total C ranged from 12.63 to 27.70 g kg⁻¹ and total P ranged from 728 to 4995 mg kg⁻¹.
Table 3.1 Soil characteristics from the top and bottom ten cm of the soil cores. Data are the mean values ± the 95% confidence interval.

<table>
<thead>
<tr>
<th></th>
<th>0-10 cm</th>
<th>10-20 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic Matter (wt%)</td>
<td>41.46±2.49</td>
<td>48.04±2.77</td>
</tr>
<tr>
<td>Bulk Density (g cm⁻³)</td>
<td>0.15±0.01</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td>Total N (g kg⁻¹)</td>
<td>1.37±0.08</td>
<td>1.62±0.07</td>
</tr>
<tr>
<td>Total C (g kg⁻¹)</td>
<td>17.72±0.80</td>
<td>21.74±1.09</td>
</tr>
<tr>
<td>Total P (mg kg⁻¹)</td>
<td>1868±435</td>
<td>1044±156</td>
</tr>
<tr>
<td>Extractable P (mg kg⁻¹)</td>
<td>1.48±0.82</td>
<td>0.03±0.02</td>
</tr>
</tbody>
</table>

3.3.2 Soil Properties Relationships

There was a significant correlation (r=0.909) between soil total C and total N concentrations of the soil. The relationships of total P to total C and total N were very weak; TC to TP correlation was r= -0.233 and the correlation between TN and TP was r= -0.166. Total N and TC both significantly increased with depth. Total P did not meet the assumptions for the statistical model, and significance was assessed using the Wilcoxon Rank-Sum Test. This test found that plots receiving higher nutrient loading had significantly higher TP concentrations (p<0.05), but was significant with depth at p<0.1.

3.3.3 Enzyme Activity

Enzyme measurements were taken for every treatment combination. Overall, there was no significant change in enzyme activity with depth or nutrient treatment and no significant treatment interaction.

Graph a in Figure 3.4 shows average phosphatase activity in the top 10 cm. Average phosphatase activity in the control nutrient treatments was 20391 ± 3783 nmol MUF g⁻¹ hr⁻¹. Phosphatase activity averaged 17808 ± 2090 nmol MUF g⁻¹ hr⁻¹ when P loading rates were 0 kg P ha⁻¹ yr⁻¹. Enzyme activity averaged from 17302 ± 2131 nmol MUF g⁻¹ hr⁻¹ when P loading rates were 131 kg P ha⁻¹ yr⁻¹. At 0 kg N ha⁻¹ yr⁻¹ loading, phosphatase rates averaged from 19059
± 2635 nmol MUF g⁻¹ hr⁻¹. Phosphatase activity averaged 14950 ± 3432 nmol MUF g⁻¹ hr⁻¹ at an N loading rate of 50 kg N ha⁻¹ yr⁻¹. Activity averaged 16682 ± 3169 nmol MUF g⁻¹ hr⁻¹ at a loading rate of 200 N kg ha⁻¹ yr⁻¹. At the highest loading rate of 1200 kg N ha⁻¹ yr⁻¹, phosphatase activity averaged 19530 ± 2695 nmol MUF g⁻¹ hr⁻¹.

Graph b in Figure 3.4 shows average β-glucosidase activity in the top 10 cm. Average β-glucosidase activity in the control nutrient treatments was 6018 ± 2612 nmol MUF g⁻¹ hr⁻¹. Activity averaged 3172 ± 746 nmol MUF g⁻¹ hr⁻¹ when there was no P fertilization. At a P loading rate of 131 kg P ha⁻¹ yr⁻¹, enzyme activity averaged 3562 ± 487 nmol MUF g⁻¹ hr⁻¹. At 0 kg N ha⁻¹ yr⁻¹ loading, β-glucosidase rates averaged 5040 ± 1485 nmol MUF g⁻¹ hr⁻¹.

B-glucosidase activity averaged 2069 ± 439 nmol MUF g⁻¹ hr⁻¹ at an N loading rate of 50 kg N ha⁻¹ yr⁻¹. Enzyme activity averaged 3809 ± 598 nmol MUF g⁻¹ hr⁻¹ at a loading rate of 200 kg N ha⁻¹ yr⁻¹. At the highest loading rate of 1200 kg N ha⁻¹ yr⁻¹ N, β-glucosidase activity averaged 2553 ± 298 nmol MUF g⁻¹ hr⁻¹.

Graph c in Figure 3.4 shows average sulfatase activity in the top 10 cm. Sulfatase activity in the control nutrient treatments averaged 553 ± 171 nmol MUF g⁻¹ hr⁻¹. Activity averaged 416 ± 79 nmol MUF g⁻¹ hr⁻¹ when there was no P fertilization. When P loading rates were 131 kg P ha⁻¹ yr⁻¹, enzyme activity averaged 526 ± 54 nmol MUF g⁻¹ hr⁻¹. Sulfatase rates averaged 472 ± 122 nmol MUF g⁻¹ hr⁻¹ when there was no N fertilization. At an N loading rate of 50 N kg ha⁻¹ yr⁻¹, sulfatase activity averaged 292 ± 108 nmol MUF g⁻¹ hr⁻¹. Activity averaged 428 ± 104 nmol MUF g⁻¹ hr⁻¹ at a loading rate of 200 kg N ha⁻¹ yr⁻¹. At the highest loading rate of 1200 kg N ha⁻¹ yr⁻¹, sulfatase activity averaged 692 ± 325 nmol MUF g⁻¹ hr⁻¹.
Comparisons in enzyme activity with depth were also made. Phosphatase, shown in graph a of Figure 3.5, averaged $13605 \pm 1611$ nmol MUF g$^{-1}$ hr$^{-1}$ in the 0-10 cm interval, and $21505 \pm 2228$ nmol MUF g$^{-1}$ hr$^{-1}$ in the 10-20 cm interval. β-glucosidase, shown in graph b of Figure 3.5, averaged $4010 \pm 809$ nmol MUF g$^{-1}$ hr$^{-1}$ in the top 10 cm and $2725 \pm 329$ nmol MUF g$^{-1}$ hr$^{-1}$ from 10-20 cm. Sulfatase, shown in graph c of Figure 3.5, averaged $671 \pm 168$ nmol MUF g$^{-1}$ hr$^{-1}$ from 0-10 cm, and from $271 \pm 66$ nmol MUF g$^{-1}$ hr$^{-1}$ in the 10-20 cm depth interval.

Figure 3.4 (a) Phosphatase activity, (b) β-glucosidase, and (c) sulfatase activity in the top 10 cm with nutrient treatment. Error bars show the standard error of each nutrient treatment combination.
3.3.4 Phosphorus Biogeochemistry

Extractable P varied significantly with P treatment, but not with depth or N treatment. Extractable P varied from below the detection limit to 0.342 mg kg\(^{-1}\) with 0 kg P ha\(^{-1}\) yr\(^{-1}\) and all N treatments. The average concentration of extractable P was 0.243 ± 0.18 mg kg\(^{-1}\) at the control P treatment. Extractable P ranged from below the detection limit to 10.5 mg kg\(^{-1}\) with the high P treatment and combination of N treatments, and average extractable P was 2.16 ± 0.87 mg kg\(^{-1}\).
Potentially mineralizable P rates did not significantly change with N treatment, but increased significantly with increased P loading (Table 3.2). The PMP rates averaged $6.43 \pm 1.50$ mg kg$^{-1}$ day$^{-1}$ with the 0 kg P ha$^{-1}$ yr$^{-1}$ treatment and $11.41 \pm 1.78$ mg kg$^{-1}$ day$^{-1}$ in the 131 P kg ha$^{-1}$ yr$^{-1}$ treatment.

Table 3.2 Potentially mineralizable P rates for all fertilization rates. PMP was only measured in the most extreme treatments for the 10-20 cm depth interval. Data shown are the mean values with 95% confidence interval.

<table>
<thead>
<tr>
<th>N (kg ha$^{-1}$ yr$^{-1}$)</th>
<th>P (kg ha$^{-1}$ yr$^{-1}$)</th>
<th>Depth (cm)</th>
<th>PMP (mg P kg$^{-1}$ day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1200</td>
<td>131</td>
<td>0-10</td>
<td>$13.68\pm10.91^1$</td>
</tr>
<tr>
<td>200</td>
<td>131</td>
<td>0-10</td>
<td>$11.84\pm4.50^1$</td>
</tr>
<tr>
<td>50</td>
<td>131</td>
<td>0-10</td>
<td>$15.60\pm5.53^1$</td>
</tr>
<tr>
<td>0</td>
<td>131</td>
<td>0-10</td>
<td>$12.46\pm10.38^1$</td>
</tr>
<tr>
<td>1200</td>
<td>0</td>
<td>0-10</td>
<td>$13.33\pm3.37^1$</td>
</tr>
<tr>
<td>200</td>
<td>0</td>
<td>0-10</td>
<td>$7.81\pm6.87^{1,2}$</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>0-10</td>
<td>$4.46\pm3.50^{1,2}$</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0-10</td>
<td>$0.96\pm0.31^2$</td>
</tr>
<tr>
<td>1200</td>
<td>131</td>
<td>10-20</td>
<td>$6.77\pm0.39^*$</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>10-20</td>
<td>$6.65\pm4.95^*$</td>
</tr>
</tbody>
</table>

$^{1,2}$ Indicates statistical significance; * indicates that this value was not used in statistical analysis

3.4 DISCUSSION

Soil characteristics between the top 10 cm and the 10-20 cm depth interval varied significantly in only a limited number of the characteristics tested (Table 3.3). Bulk density was found to vary significantly with depth, but contrary to many other systems, the highest bulk density was seen at the surface of the wetland rather than at the lower depths (Benscoter et al., 2011). This is similar to what other studies have found in coastal Louisiana, where wetlands are annually exposed to storm events that move significant amounts of inorganic sediments onto the soil surface thereby increasing the bulk density (Cahoon and Reed, 1995). Total C and N both increased significantly with depth, likely related to increased organic matter with depth. Total P did not vary significantly with depth, but there was a marginally significant increase (p<0.1).
None of the enzyme activities measure varied significantly with depth, but a general trend was
seen in both phosphatase and sulfatase. On average, phosphatase activity was higher at the
surface, likely related to increased microbial and plant activity at the surface. Sulfatase was
lower at the surface on average, implying that the salinity closer to the surface of the soil was
lower than at depth.

Overall, only three soil characteristics varied significantly with fertilization treatment
(Table 3.3). Neither total C nor total N varied significantly with changes in fertilization
treatment. The lack of significance suggests that the N that was loaded to the system was most
likely removed from the system via denitrification or incorporated into the organic pool without
significantly elevating the overall N level. In contrast, total P significantly increased with
increasing P treatment. Total P did not meet the assumptions for the SAS model, so was assessed
using the Wilcoxon Rank Sum test. Because there were more than two N treatments, significance
could not be assessed for either N loading or the NxP interaction effects. Extractable P was over
six times higher in the plots receiving P fertilization than those that were not, but was not
significantly affected by increases in N loading.

Table 3.3 Significant changes associated with depth, N treatment, P treatment, and
NxP interaction.

<table>
<thead>
<tr>
<th></th>
<th>Depth</th>
<th>N Treatment</th>
<th>P Treatment</th>
<th>NxP Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk Density</td>
<td>*</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Total Carbon</td>
<td>*</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Total Nitrogen</td>
<td>*</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Total Phosphorus</td>
<td>N</td>
<td>N/A</td>
<td>*</td>
<td>N/A</td>
</tr>
<tr>
<td>Phosphatase Activity</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>B-glucosidase Activity</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Sulfatase Activity</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Extractable P</td>
<td>N/A</td>
<td>N</td>
<td>*</td>
<td>N</td>
</tr>
<tr>
<td>Potentially Mineralizable P</td>
<td>N/A</td>
<td>N</td>
<td>*</td>
<td>N</td>
</tr>
</tbody>
</table>

* indicates significance at p<0.05
N indicates not significant
N/A indicates significance not assessed
Phosphatase, β-glucosidase, and sulfatase activity did not vary significantly with changing nutrient loading. The lack of significance in phosphatase activity implies that the microbial populations were not limited by P, since significantly higher levels of extractable P were found in the treatments receiving P loading. Past research has shown that β-glucosidase nor sulfatase activity responds to changes in elevated P levels (Wright and Reddy, 2001b). Sulfatase activity is more likely to be controlled by differing salinities than changes in nutrient loading, and is extremely low at this site since the site had low salinity levels, and therefore available sulfate. The response of β-glucosidase activity to nutrient loading is somewhat complex, and has been shown to require elevated levels of both N and P to see a significant effect (Allison and Vitousek, 2005).

Potentially mineralizable P rates provide an integrated measurement of how organic P is transferred to the bioavailable pool over time. Average PMP rates almost doubled with an increase in P fertilization, but there were no significant effects related to N loading. Data previously collected from the Madisonville site indicate that senesced aboveground biomass had significantly increased levels of P with increased P loading (Graham and Mendelssohn, unpublished data). After senescence, plants are decomposed by the soil microbial population (Figure 3.6), and the increased level of P in the plant materials has led to an increased PMP rate.

These data can be used to predict the response of microbial populations to long-term nutrient loading. The results from this experiment could be used to help inform management decisions regarding the use of freshwater and sediment diversions. Additional work needs to be done to determine how enzyme activity responds to nutrient loading at this site with time.
Figure 3.6 The cycling of P from the inorganic form to organic form. Phosphate is taken up by plants and accumulated in the aboveground biomass. After senescence the phosphate-rich plant matter is decomposed and accumulates in the organic matter. When the organic matter is mineralized by microorganisms, the P is made available for uptake again.

3.5 CONCLUSION

The Madisonville nutrient plots have been the site of an active plot fertilization experiment looking at the effects of long-term nutrient loading since 2002. During that time, changes in the plant community were monitored along with changes in above and belowground biomass. Overall, the plant study found that there were changes in dominant species related to N fertilization (Graham and Mendelssohn, 2010). There was a significant reduction (50%) in live belowground biomass but no significant change in either live rhizomes or total belowground biomass (Graham, 2013). The previous work done at this site did not include any measurements of the soil response to changes in nutrient loading.
Changes in the activity of the microbial population can be used to predict how a system
will respond to changes in nutrient loading. It has been shown that extracellular enzyme activity
responds to a change in nutrient loading (Allison and Vitousek, 2005; Wright and Reddy,
2001b). Our results found that enzyme activity was not significantly affected by changes in long-
term N and P fertilization. Total N levels in the system were not affected by nutrient loading, but
total P levels significantly increased. As a result of the increase in total P, increases in extractable
P and PMP rates were observed. This results implies that there was a shift in P biogeochemistry
related to P loading. Despite this shift, the lack of differences in to extracellular enzyme activity
suggests that this system is not affected once bioavailable nutrients are incorporated into the soil
organic pool.
CHAPTER 4: SUMMARY & CONCLUSIONS

4.1 SUMMARY & CONCLUSIONS

Coastal Louisiana experiences high rates of land loss as a result of both natural and anthropogenic forcings. Storm surge occurs naturally year round and affects the entire coast line. The movement of high saline waters into freshwater systems negatively impacts ecosystem function. Freshwater river diversions could potentially be used as a tool to minimize the effects of storm surge. However, there is significant controversy surrounding the effects of distributing the relatively high concentration of nutrients in the Mississippi River into coastal wetlands.

Changes in salinity cause a significant negative effects including shifts in microbial assemblages, decrease in plant productivity, and changes in biogeochemical cycling (Dausse et al., 2012; Jackson and Vallaire, 2009; Parida and Das, 2005). Storm surge events are frequent in Louisiana, and have the potential to cause a significant amount of ecological damage over a relatively short period of time. Past research has shown that storm surge will sit in an area for days before slowly draining out. Therefore, there is the potential for salt to move through the soil, but no research has been conducted confirming this until now. Past studies have shown that increases in salinity typically equate to a decrease in exchangeable NH$_4$ concentrations, causing a decrease in plant available nutrients. There is currently no information detailing how salt moves through the soil column, making it difficult to predict how severely a wetland will be affected by short-term salinity changes.

Overall, exposure of fresh marsh cores to ocean water indicated that storm surge significantly increased soil porewater salinity in a short period of time. Average salinity over the entire soil core in the controls was 1.46 and 1.03 in the mudflat and marsh cores, respectively.
After the first week of flooding, average salinity in the soil porewater was 9.09 in the mudflat cores and 8.47 in the marsh cores, with significant increases in the top 8 cm for the mudflat cores and in the top 6 of the marsh cores. In both the marsh and mudflat cores, a majority of the salt that entered the soil during the experiment diffused in during the first week of flooding. At the end of the experiment, the average salinity in the mudflat and marsh cores had increased on average to 12.24 and 10.03, respectively, with a significant increase in salinity from 0-14 cm in both the marsh and mudflat cores. Past studies have shown that increases in salinity as low as 3-10 g L⁻¹ have significant ecological consequences, implying that the increase seen in this experience could detrimentally affect ecosystem function (Blood et al., 1991, Brock et al., 2005, Jackson and Vallaire, 2009). Extractable NH₄-N levels were not significantly affected by changes in salinity in either the mudflat or marsh cores. No discernible pattern was present in the mudflat cores, but extractable NH₄-N levels increased every week compared to the control in the marsh cores. Therefore, the microbial communities in the marsh cores were able to continue mineralizing ammonium despite increasing salinity.

Eutrophication is known to have a plethora of negative effects on plant diversity, microbial populations, and water quality (Chambers et al., 1999; Kolb and Martin, 1988; Vitousek et al., 1997). The construction of diversions have allowed previously isolated coastal wetlands to receive Mississippi River water, and may be an effective tool for mitigating the influx of salt caused by storm surge. However, wetlands are also exposed to the relatively high levels of N from the Mississippi River. Some research in the Mississippi River Delta has shown that an increase in fertilization results in a significant reduction in belowground biomass and an increase in the rate of decomposition of the soil. However, this research has been contradicted by a number of other studies, and the issue has become lodged in controversy. There are limited
data available describing the effects of long-term nutrient loading on microbial functionality has been affected in deltaic coastal systems.

Phosphatase rates averaged 18059 nmol MUF g$^{-1}$ hr$^{-1}$ in the control plots and 15009 nmol MUF g$^{-1}$ hr$^{-1}$ in plots that received the highest nutrient loading, at 1200 kg N ha$^{-1}$ yr$^{-1}$ and 131 kg P ha$^{-1}$ yr$^{-1}$). B-glucosidase rates averaged 2850 nmol MUF g$^{-1}$ hr$^{-1}$ in the control plots and 1654 nmol MUF g$^{-1}$ hr$^{-1}$ in the plots receiving the highest fertilization rates. Sulfatase activity averaged 330 nmol MUF g$^{-1}$ hr$^{-1}$ in the control plots and 845 nmol MU g$^{-1}$ hr$^{-1}$ in the plots receiving the highest nutrient treatments. Long-term nutrient loading was found to have no significant effect on measured enzyme activity. Extractable P and PMP rates were positively correlated with P fertilization. Mean extractable P increased from 0.29 mg kg$^{-1}$ to 2.66 mg kg$^{-1}$ with increased P loading. Average PMP rates increased from 6.43 mg kg$^{-1}$ d$^{-1}$ to 12.28 mg kg$^{-1}$ d$^{-1}$. Total N and total C were not significantly affected by nutrient loading, but total P significantly increased with P loading. The results suggest that N was removed from the system as Total N did not change with fertilization.

Both increases in salinity and nutrients have the potential to negatively affect coastal wetlands. The first study found that short-term increases in salinity can significantly affect soil porewater salinity, but not extractable NH$_4$ mineralization. Increases in soil salinity would cause salt stress on freshwater macrophytes causing root shrinkage, dehydration, and a decrease in growth (Hill, 1908; Parida and Das, 2005). In the nutrient loading study designed to mimic loading caused by river diversions, we found that microbial enzyme activity was not significantly affected by nutrient loading. These results indicate that the operation of diversions in short pulses to help decrease salinity levels in areas affected by storm surge could be a potential tool to minimize the damage of storm surge associated salinization, and may assist in coastal restoration.
and preservation. Further research is needed to ascertain the optimal frequency of opening and magnitude of discharge.
REFERENCES


Coastal Restoration and Protection Authority. 2012. Louisiana's comprehensive master plan for a sustainable coast, Louisiana.


Coastal Wetlands Planning, Protection and Restoration Act, 1997. Louisiana coastal wetland functions and values., Baton Rouge, LA.


Graham, S.A., 2013. Decade-scale nutrient enrichment effects on wetland plant community structure and function, Oceanography and Coastal Sciences. Louisiana State University, Baton Rouge, LA.


Hill, T.G., 1908. Observation on the osmotic properties of root hairs of certain salt marsh plants. The New Phytologist 7, 133-14


Louisiana Department of Natural Resources, 2005. Davis Pond Freshwater Diversion annual report.


Moerschbaecher, M., 2008. The impact of the Caernarvon Diversion on above- and belowground marsh biomass of the Breton Sound Estuary after Hurricane Katrina, Enivornmentl Sciences. Louisiana State University, Baton Rouge, LA.


Sasser, C., Personal Comm. (e-mail) March 2014. Louisiana State University., Baton Rouge, LA


Scott, L.C., 2011. The energy sector: Still a giant economy engine for the Louisiana economy. Mid-Continent Oil and Gas Association


APPENDIX: DETAILED ENZYME ASSAY METHOD

Extracellular enzyme activity was determined within 48 hours of sample collection. Soils were kept at approximately 18 °C until they were analyzed. The stock solution used to make the standard was manufactured by adding 17.22 mg of MUF to a 100 mL volumetric flask to form a 0.1 M solution. The working solution formed by diluting the stock solution to 0.01 M with DI water. Standard were added to a 96-well microplate with 150 µL of water and 100 µL of the standard. The fluorescence emission standards were analyzed from 0 µM to 1.0 µM MUF. A sample was randomly chosen from each block/depth combination to serve as a representative sample for the quench curve, meant to show the masking properties of the soil. Approximately two grams of soil were diluted with 200 mL of water and shaken until the soil was completely broken up and distributed throughout the water column. The quench curves were analyzed using the same concentrations as the standards. To find the background fluorescence of the soil, 100 µL of DI water and 150 µL of each sample were added to 96-well plates. Background measurements were taken using a randomly selected sample from each plot/depth combination. A substrate control was measured to ascertain the background fluorescence of the enzyme substrates. Three different enzymes were analyzed: alkaline phosphatase, β-glucosidase, and sulfatase. A 0.1 M stock solution of each of these substrates was and then diluted to 0.05 M for the purpose of the experiment; 150 µL of each of the substrate was combined with 100 µL of the control and placed into a 96-well plate. Samples were prepared for analysis by mixing in the same fashion as the quench curves. Approximately 150 µL of sample were added to 100 µL of enzyme substrate and allowed to sit for two hours. The samples were analyzed after incubation. All measurements were taken on a BioTek flx800. Excitation bandwidths were set at 360 nm and emission bandwidths were 460 nm for all substrates, the bandwidth of excitation was set to 20
nm. All samples were poured into 50 mL reagent wells where they were pipetted into a 96-well microplate using an Eppendorf 300 μL multi-channel pipette with 8 channels. The rate of enzyme activity was calculated using the modified calculation presented in German et al. (2012).
VITA

Morgan McKee was raised in Brandywine, Maryland with her parents and two younger siblings. She was raised to love the outdoors, and her parents regularly took her and her siblings on camping trips across the country.

Morgan attended Virginia Polytechnic Institute in Blacksburg, VA where she received a degree in Environmental Science and a minor in Wetland Science. During her junior and senior years, Morgan participated in an undergraduate research project investigating alternative methods of identifying hydric soils in mountain wetlands. This research project inspired her to continue working in wetlands research. Upon graduation, Morgan accepted an offer to pursue a Master’s degree with Dr. John White at Louisiana State University.