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The effects of salinity on nitrogen cycling in wetland soils and sediments of the Breton Sound estuary, LA

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**THE EFFECTS OF SALINITY ON NITROGEN CYCLING IN WETLAND
SOILS AND SEDIMENTS OF THE BRETON SOUND ESTUARY, LA**

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Oceanography and Coastal Sciences

by

Brett Whitfield Marks
B.S., Louisiana State University, 2007
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ABSTRACT

Wetlands in the coastal zone are slowly becoming more saline under rising sea level over the long-term. However, there are a number of events in the coastal environment which lead to quick and temporary changes in the salinity of coastal marshes. Seawater driven inland from storm surge can significantly increase salinity in oligohaline wetlands over the short-term (weeks). Recent large-scale efforts to restore coastal wetlands in Louisiana have utilized Mississippi River surface water diversions to re-introduce freshwater into coastal marshes, decreasing the salinity of coastal marshes. We examined the effect of salinity changes on two important nitrogen cycling processes, potential denitrification and N-mineralization, in fresh and salt marsh soils/sediments in the Breton Sound estuary, LA. All soils/sediments were subjected to freshwater and saline treatments (0-35 ppt) simulating conditions within the soil that are caused by instantaneous flux of seawater due to storm surge events or high rates of freshwater flow directed by a surface water diversion. At 0 ppt potential denitrification in fresh and salt marsh soils reached 373 ± 22.2 and 9.18 ± 3.27 mg N₂O-N kg⁻¹ d⁻¹, respectively. At 35 ppt, the rates were 615 ± 182 in salt marsh and 99.7 ± 21.1 mg N₂O-N kg⁻¹ d⁻¹ in fresh marsh soils. Potentially mineralizable N rates in fresh marsh soils at 0 and 35 ppt averaged 28.6 ± 3.71 and 38.2 ± 4.31 mg-N kg⁻¹ d⁻¹, respectively. In salt marsh soils at 0 and 35 ppt, PMN rates were 12.3 ± 0.4 and 8.70 ± 0.32 mg-N kg⁻¹ d⁻¹, respectively. The effects of changing salinity on N-mineralization and potential denitrification will allow us to begin to discern the mechanisms of salinity-driven influences on overall nitrogen cycling and marsh biogeochemical function. Significance of these findings are applicable to large surface water diversion projects in the coastal Florida Everglades and Mississippi River Delta, where more saline sediments are exposed to freshwater and nitrogen pulses as well as impacts of increased salinity driven into the

fresh-brackish marsh from hurricanes. Sudden fluxes in salinity had short-term effects on N mineralization, while denitrification showed significant effects with sudden salinity changes in wetlands soils.

CHAPTER 1: REVIEW OF LITERATURE

1.1 The Mississippi River Delta and Louisiana Coast

Louisiana's coastal zone is an extensive collection of wetlands, coastal water bodies, and rivers. Approximately 30 percent of all coastal wetlands in the continental United States occur along Louisiana's coastline (Dahl, 2000). These highly valuable and productive systems are currently experiencing high rates of spatial reduction. Wetland areas within the Mississippi River Delta are suffering rapid shoreline retreat at rates exceeding 20 m yr^{-1} (Williams et al., 1997) and interior wetland loss of $51 \text{ km}^2 \text{ yr}^{-1}$ (Barras et al., 2003), accounting for 90% of coastal marsh lost in the lower 48 states (Field et al., 1991). Louisiana's coast is facing rapid land loss and rates of isostatic sea level rise much higher than any other area of the United States. Louisiana's mean rate of relative sea level rise is more than five times greater than the Gulf of Mexico coast average and ten times faster than the rest of the globe (Penland et al., 1988). This conversion of wetland habitat to open water is due to natural and anthropogenic processes.

The Louisiana coastline is the result of the Mississippi River's complex delta cycle (Fig. 1.1). Due to avulsion (Aslan et al., 2005), diversion of the main river channel, the Mississippi River has initiated and abandoned multiple deltaic lobes along the Southeast and South-central Louisiana coast over the past 7,500 years (Coleman and Gigliano, 1964). During the period of time that each of these deltas was actively used by the Mississippi River, accretion could occur due to the massive load of fluvial deposits carried by the river (Roberts and Coleman, 1996). Once these deltas were no longer used by the main channel of Mississippi River, the land would begin to subside over time. The finely grained sand, silt, and clay deposits composing the Louisiana coast are naturally susceptible to compaction, dewatering, and further consolidation (Gouw, 2007; Meckel et al., 2006). Tectonic influences, coupled with the gradual downwarping of these deltaic materials and the older geologic layers beneath are natural processes responsible

for significantly increasing the relative sea level rise observed along the Louisiana coast (Meckel et al., 2007; Dokka et al., 2006).

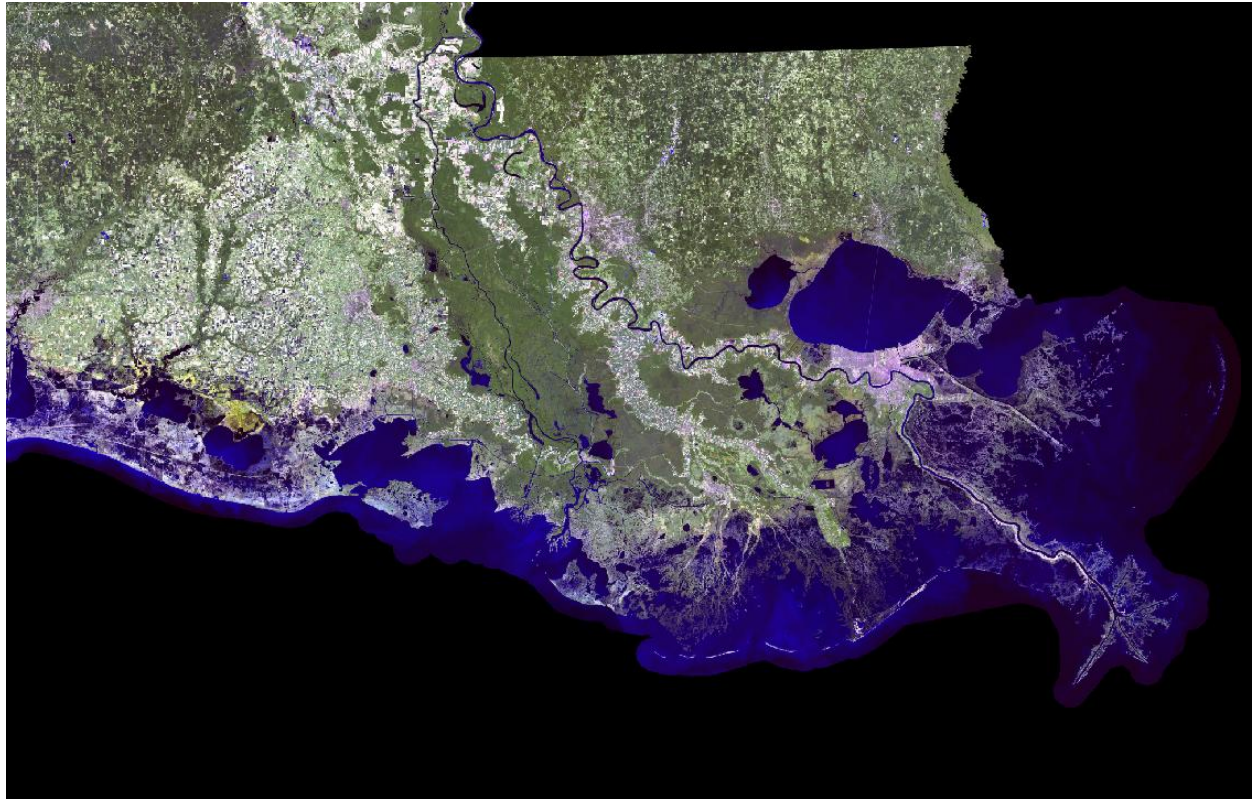


Figure 1.1 Louisiana's Coast and Mississippi River delta (ArcGIS 9.1, 2007).

Human activities have significantly impacted the natural processes responsible for shaping and maintaining the coast. The extensive system of levees built and maintained to control the flood waters of the Mississippi River is one example of human activities causing unbalance on the Louisiana coast. The levees and dams along the Mississippi River have halted the essential Delta-lobe switching process that is essential to accretion (Roberts, 1997; Hupp et al. 2009). Furthermore, due to the construction of dams and reservoirs along the Mississippi River's major tributaries, the sediment load carried by the River is severely diminished (Kesel, 1988). What remains of the Mississippi River's waters and sediment a load are forced through

the current Birdsfoot delta, directly out into the Gulf of Mexico without natural distribution into adjacent coastal wetlands where it would be available for land building. Damming at the confluence of the Mississippi River and Atchafalaya Rivers has prevented channel avulsion and establish of a new main river delta at the Wax Lake Estuary in Atchafalaya Bay (Aslan et al., 2005). The extensive levees and water control structures are preventing the regeneration of land in a new prograding delta-lobe. Even with the initiation of a new delta, much of the delta region would remain in a state of geologic degradation.

Louisiana's coastal zone is a working coast and due to its economical value, human influences in the coast are prevalent. Over 47% of Louisiana's population lives within the coastal zone (U.S. Census Bureau, 2007). Five of the top 15 largest ports in the United States are located here, and Louisiana waterborne commerce accounts for 18% of all U.S. waterborne commerce (USACE, 2007). Louisiana's coastal fisheries account for 21% of the total catch by weight in the lower 48 states (USDOC et al., 2007). Including Outer Continental Shelf production, Louisiana ranks 1st in crude oil and 2nd in natural gas production; excluding Outer Continental Shelf production, Louisiana ranks 4th in crude oil and 5th in natural gas production (LDNR, 2007). Many human intrusions associated with civil projects and commerce affect land loss and relative sea level rise. Direct wetland destruction by draining dredging, soil deposition, creation and subsequent widening of canals and navigation channels according to some estimates account for only 16% of wetland loss that has occurred in Louisiana over the past several decades (Boesch et al., 1994; Day et al., 2000). The indirect effects of these actions are potentially more significant. Dredging and the cutting of channels through the coastal wetlands have altered the natural hydrology of these systems. Canals and navigable waterways offer direct pathways for salinity intrusion into fresh marsh systems, and canal ways have been

connected with to reduction in adjacent marsh vegetation (Turner and Rao, 1990; Cahoon and Turner, 1989). Canals also reduced overland flow and sedimentation rates in coastal marshes (Ko and Day, 2004). Deposition of dredged materials and building of spoil banks have also imposed negative effects in coastal processes. Spoil banks have been shown to change flooding patterns in reduce accretion rates in associated wetlands (Cahoon and Turner, 1989; Swenson and Turner, 1987). The combined influences of natural and anthropogenic processes acting upon Louisiana's coastal zone have accelerated isostatic sea level rise within the Mississippi River Delta.

1.2 Fresh Water Diversions of the Mississippi River

Conservation and restoration of coastal wetlands within the Mississippi River Delta has recently become an issue of great social, economical, and environmental importance. These systems provide habitat to a plethora of diverse wildlife and economically important species. A significant portion of Louisiana's population lives within the coastal zone, and the oil and gas industry based here provides one of the largest sources of fossil fuel energy in the United States. Due to these essential functions and values attached to the Louisiana Coast, many restoration efforts are being focused here. The U.S. Army Corp. of Engineers (ACOE) is heading some of the largest and most ambitious projects in the Louisiana coastal zone. Construction of large scale water control structures are being used to reintroduce Mississippi River flow into a number of receiving basins. These projects will mimic historical overbank flooding events of the Mississippi River, in an attempt to reduce salinity and marsh subsidence. Four diversion areas were studied in the early planning stages, and within these areas 20 potential structure sites were identified. Upon further analysis of the engineering characteristics, potential environmental, cultural, economic and social effects, and the cost at each site, the Corps decided to construct

three diversions (ACOE, 1999). The Caernarvon, Davis Pond, and Bonnet Carre diversions are fully operational, and more projects are still in planning (Fig. 1.2).

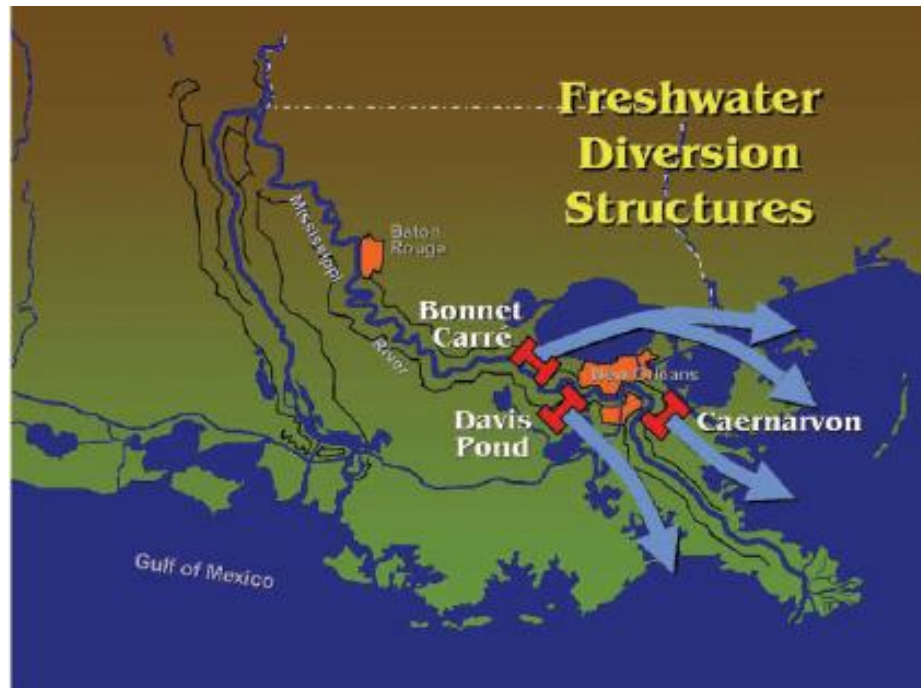


Figure 1.2 Operational freshwater diversion projects in southeast Louisiana (ACOE, New Orleans, LA).

These diversion projects were authorized by the Flood Control Act of 1965 (PL 89-298), the Water Resources Development Act (WRDA) 1974 (PL 93-251), and WRDA 1986 (PL 99-622) (LDNR, 2003). Surface water diversions function by gravity flow culverts fed by river waters. The flow rates of these three structures are managed to correspond to Mississippi River flood stages and control salinity regimes in the receiving basins. Caernarvon Diversion was the first project to begin operation in 1991. It is located south of New Orleans, on the east bank of the Mississippi River, and empties into the Breton Sound Estuary (Moerschbaeher, 2008). The Davis Pond Diversion is located west of New Orleans, on the west side of the river, and empties into the Barataria Basin (Gardner, 2008). Construction was completed in 2002, but it not fully

operational until 2007. The Bonnet Carre Spillway is located west (upstream) of New Orleans. Operation of this diversion is limited to approximately one large scale event every decade with the last two events occurring in 1997 and 2008. When the diversion is opened it discharges waters into Lake Pontchartrain and Lake Borne (ACOE, 2009).

1.3 Salinity Changes within Estuary Systems

The natural salinity regime of coastal systems, such as the Louisiana Breton Sound is under constant flux. Most in situ salinity fluctuations are in response to normal estuarine circulation and the combine effects of tidal and wind mixing and the freshwater discharges (Table 1.1). Seasonal freshwater inputs from rivers and streams can affect the salinity structure of estuaries annually (Wong, 1995; Sikora and Kjerfve, 1985). Within the Breton Sound estuary high freshwater discharge into the system, via the Caernarvon diversion, alters the salinity gradients occurring throughout the wetland (Lane et al., 2007). Storm surge, associated with Hurricanes and tropical storms can also alter local salinity regimes by pushing large volumes of sea water into the coastal wetlands (Edmiston et al., 2008).

Table 1.1 Major processes and events governing salinity regime shifts in wetland soils and sediments within the Breton Sound estuary, LA.

Cause	Salinity Change	Time Scale	Frequency
Sea Level Rise / Subsidence	Large	Decades	
Freshwater Diversion	Variable	Weeks, Months	Annually, Episodic
Hurricane / Storm Surge	Large	Days, Weeks	Seasonally, Annually

1.3.1 Diversion Effects on Salinity

Surface freshwater diversions of the Mississippi River were designed and managed to control salinity within their receiving basins (ACOE, 1995). Periods of high discharge into shallow coastal systems directly influences in situ salinity gradients. In the Breton Sound, high

discharge events during the fall and winter can decrease the salinities of the outer estuary (Lane, 2003). Large spring pulses directed through the control structure often cause the entire estuary to become fresh for a short period of time (<1 month) (Lane et al., 2007). This same study also measured a 2-week temporal lag in salinity influence to reach the lower estuary.

1.3.2 Hurricanes and Storm Surge

The land of South Louisiana was formed by slow accretion of fluvial deposits delivered by the Mississippi River (Roberts and Coleman, 1996). This delta system built land gradually extending out over the broad continental slope. The geography of this coast is defined by a low-lying coastal floodplain. This deltaic landscape is covered by interconnected sounds, bays, marshes, lakes, rivers, inlets, and rivers. Major reliefs in this region are geological features such as salt domes, barrier islands, river banks, and extensive networks of man-made levees and raised road embankments (Coleman et al., 1998). The ubiquitous water bodies and low-lying topography of Louisiana's coast make the region very susceptible to flooding caused by hurricane storm surge. Hurricane surges can push through coastal areas from many directions and the high relief features within the landscape can propagate flooding into areas far inland (Westerink et al., 2008). Flooding within Louisiana's low-lying coast is driven by wind, atmospheric pressure gradients, precipitation, river flow, and tides. Storm surge flooding events result in an instantaneous flux of high salinity sea water deep into coastal wetlands (Flather, 2001). The flooding stage of surges occur rapidly (5-12 hrs) while the receding stage alters salinity and hydrodynamics of low-lying coastal areas for longer (2-4 days) periods (Li et al., 2010; Rego and Li, 2009). These salinity changes that occur within coastal marsh systems can cause diverse shifts in biogeochemical cycles occurring within the soils and water column. The Breton Sound has been inundated by the storm surges of multiple hurricanes over the past

decade. In 2005, Hurricane Katrina made landfall over southeast Louisiana and pushed a 5 to 6 m surge over coastal marshes (Fritz et al., 2007). Salt water was deposited into all areas of the estuary. Fresh systems near the Caernarvon diversion were covered by as much a 4 m of salt water from the storm (Piazza and La Peyre, 2009). Storm surge results in altered soil dynamics by elevating soil salinity for extended periods of time (Blood et al., 1991).

1.4 The Nitrogen Cycle

Nitrogen is an essential nutrient found in a wide variety of forms throughout the biosphere (Fig. 1.3). Nitrogen occurs in many more chemical forms than most other elements. In nature, N is expressed in 5 oxidation states: +5, +3, +1, 0, and -3 (Reddy and Delaune, 2008). The N cycle is governed by main processes that oversee its transformation between molecular forms. Nitrogen Fixation is the conversion of elemental N (N_2) to organic N (Reddy and Delaune, 2008). This process can occur biologically by specialized plants and the process requires high energy inputs, specialized enzymes, and minerals. The resulting organic N is available to autotrophic organisms for assimilation in to organic matter. Organic N mineralization/ammonification is the microbial breakdown of organic N to ammonium (NH_4^+). Volatilization of ammonium to ammonia (NH_3) is a chemical process that can only occur in environments with a pH of more than 8. Nitrification is the microbial conversion of NH_3 to NO_2^- , then NO_3^- (Reddy and Delaune, 2008). Nitrification occurs as a two part process as each part is conducted by a specific bacterium (*Nitrosomas* spp. and *Nitrobacter* spp.). Finally, denitrification is the microbial-mediated reduction of nitrogen oxides to nitrous oxide (N_2O) or elemental nitrogen (N_2).

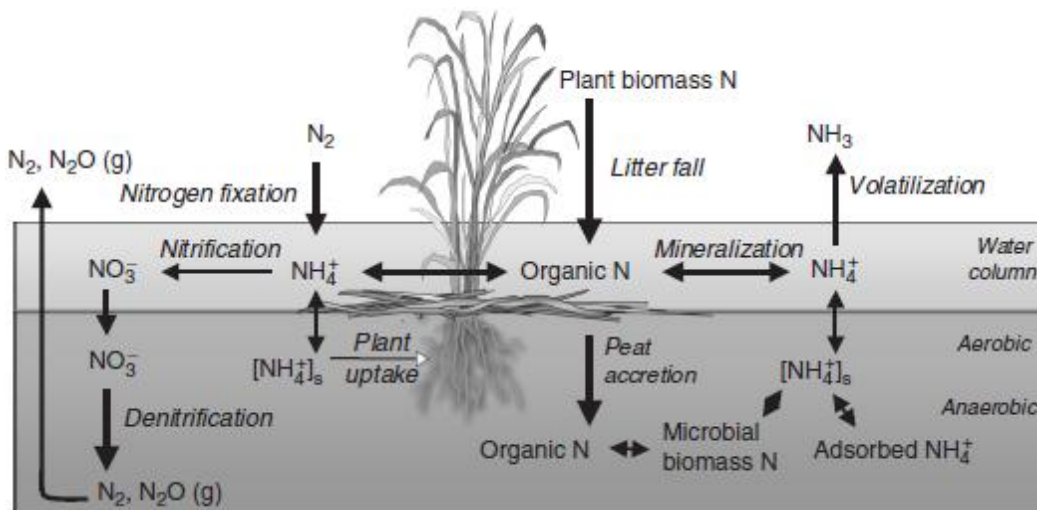


Figure 1. 3 Nitrogen transformations in wetlands (Reddy and Delaune, 2008).

1.4.1 Nitrogen in Coastal and Marine Systems

Nitrogen can be a limiting nutrient regulating primary productivity in terrestrial, wetland, and aquatic ecosystems. All these highly variable systems contain a complex mixture of nitrogen compounds (Gruber, 2008). The ability of wetlands to retain floodwaters originating from surface and subsurface inflows, results in a decreased rate of decomposition of organic matter. The net accumulating of organic matter, coupled with the low decomposition rate relative to upland ecosystems make wetlands sinks for organic carbon and nitrogen (Debusk et al. 2001). Most of the N compounds stored in wetland soils are in organic forms which are generally unavailable for uptake. The process of mineralization by heterotrophic bacteria slowly converts the biologically unavailable organic N compounds to ammonium. Due to the high biological demand for the inorganic nitrogen forms, inorganic nitrogen typically makes up 1% of the nitrogen pool found in wetlands (Debusk et al. 2001). These compounds, if released in high concentrations to marine and fresh aquatic systems can trigger eutrophication.

Recent concerns with eutrophication occurring off of Louisiana's coast have highlighted the role of wetlands as a buffer and sink for nutrients passing between upland and marine systems (Reddy et al. 1993; Hatton et al. 1982; Sharp et al., 1982). Dissolved nitrogen and phosphorous loads within river water have increased due to land development, agricultural and domestic fertilizer applications, and population growth with the Mississippi River's drainage basin (Turner and Rabalais, 1991; Mitsch et al., 2001). The discharge of relatively high concentrations of nutrient P and N directly into the Northern Gulf of Mexico cause eutrophication in coastal waters, and can result in initiating environmentally detrimental events.

Recent concerns with eutrophication off of Louisiana's coast have highlighted the function of wetlands as a buffer and sink for nutrients from the uplands (Reddy et al. 1993; Hatton et al. 1982; Sharp et al., 1982; Rabalais et al., 1994). The discharge of relatively high concentrations of nitrate (1.0 to 1.2 mg N L^{-1}) in Mississippi River waters directed into the Northern Gulf of Mexico is the main cause of eutrophication in coastal waters, and the consequent hypoxic zone that forms annually along the Louisiana and Texas coasts (Turner and Rabalais, 1991; Rabalais et al., 1994; Antweiler et al., 1995). The construction of the vast levee system in the early 1900's has largely isolated Louisiana's coastal wetlands from the Mississippi River's nutrient-laden flood waters and sediment (Mossa, 1996). One possible alternative to alleviate this nutrient loading into the coastal waters is to divert river water into the coastal wetlands. The nutrient load of the river water, especially the nitrate component, can be processed or removed by the wetlands. Nitrate can be recycled back into the atmosphere through denitrification, assimilated into organic matter, or reduced to ammonium by natural biochemical processes occurring simultaneously in the soil and water column (Seitzinger, 1988; Kaplan et al., 1979; Valiela et. al., 2000).

1.5 Denitrification

Denitrification is a key process regulating N cycling in natural environments. This process allows inorganic N, in the form of nitrate, to be removed from the system by facultative anaerobic bacteria present in wetland sediments (Knowles, 1982). Denitrifiers are known to exist in almost all soils and come from a wide range of genera, including *Pseudomonas* spp., *Alcalignes* spp., *Flavobacterium* spp., *Paracoccus* spp., and *Bacillus* spp. (Tiedje and Firestone 1981). When oxygen is unavailable for microbial metabolism, these organisms use nitrate as the terminal electron acceptor as they oxidize organic material in order to obtain energy (Seitzinger, 1988). The high primary productivity and high rate of organic matter sequestration coupled with anaerobic soil regimes of freshwater and coastal marshes provide ideal conditions for denitrification (Stefanson, 1972; Groffman, 1994; Nowicki et al., 1999). The end product of denitrification is nitrogen gas released into the atmosphere. Denitrification generally proceeds through some combination of the following intermediates:



The reduction of nitrate to N_2 gas effectively removes it from the environment and limits uptake by primary producers. Typically, high rates of microbial denitrification have been observed in coastal wetland soils (Seitzinger, 1988; Kaplan et al., 1979; Valiela et. al., 2000; Lindau et al., 2008; Lindau et al., 2009).

Denitrification has been found to be mediated by a variety of environmental factors. Many field and laboratory studies have indicated that due to high carbon content and low O_2 in wetland soils, nitrate supply becomes the limiting factor for denitrification (White and Reddy, 1999; Cooper, 1990). Increasing nitrate availability has also been linked to the overall increase of in situ denitrifying activity in riparian soils (Cooper, 1990; Gardner and White, 2010).

Variations in denitrification have also been positively correlated with increases in the nitrate concentrations in the overlying water in salt marshes (Thompson et al., 1995).

Organic matter in soils is also a limiting factor to denitrification. Mineralization of available C has been positively correlated with denitrification (Burford and Bremner, 1975; Reddy et al., 1982). Temperature effects on denitrification are complex because temperature influences microbial activity and oxygen solubility. Denitrification rates in terrestrial soils have been shown to increase with temperature (Stanford et al., 1975; Knowles, 1982). Seasonal variations in temperature have been shown to have significant influence over denitrification occurring in some salt marsh soils (Teal et al. 1979). In previous research involving estuarine sediments, oxygen availability, organic matter, nitrate supply, and temperature were reported to have the most significant influence over biological denitrification in wetland sediments (Wang et al., 2007).

1.5.1 Effects of Salinity on Denitrification

Studies investigating the effects of salinity on denitrification and they have produced a number of mixed results (Antheunisse et al., 2006; Magalhaes et al., 2005; Wu et al., 2008; Yu et al., 2008). Salinity changes on sediment from a surface freshwater diversion found that an increase in salinity caused decreased denitrification activity (Yu et al., 2008). Furthermore, by using separate salinity treatments of K₂SO₄, NaCl and seawater it was determined that the sulfate content of seawater had no significant effect on denitrification (Yu et al., 2008). In mangrove microcosms inundated with wastewater, salinity treatments between 0-30 ppt resulted in potential denitrification being reduced at higher salinities (Wu et al., 2008). Another study observed the effects of restored tidal movements and seawater incursion on soil nitrogen conversions in an outdoor mesocosm experiment (Antheunisse et al., 2006). The results of this

experiment however showed no significant correlation between the reintroduction of salt water to semi-natural and agricultural soils on denitrification enzyme activity or potential denitrification. Denitrification in sandy intertidal sediments and rocky bio-films showed no clear indications of influence due to raised salinity however increased nitrate loads delivered to each system showed a progressive linear increase of denitrification (Magalhaes et al., 2005). Further investigation of the influence of salinity on nitrogen cycling in wetland soils and sediments is needed.

1.6 Nitrogen Mineralization

Organic N mineralization is an important biogeochemical process occurring in wetland soils. Ammonification is the conversion of organic nitrogen within the water column and sediment to inorganic NH_4^+ available to macrophytes and other primary producers (Goldman and Dennett, 1991). Wetlands tend to accumulate organic matter over time, and therefore have an available supply of organic N for decomposers to act upon. Organic N mineralization results in the release of ammonium-N (NH_4^+). Most of this decomposition process is carried out by heterotrophic bacteria, which oxidize complex carbon compounds to CO_2 for energy. This sequential breakdown of organic N molecules into simpler compounds, and further microbial breakdown of amino acids releases ammonium (Goldman et al., 1987; Gardner et al., 1989).

Ammonification in wetlands systems is regulated by many environmental factors. The C:N ratio of soil organic matter may vary considerably in inundated soils and affect the N mineralization rate (Williams, 1972; Williams and Sparling, 1988). This ratio is critical in determining the efficiency, rate, and process by which organic matter is broken down (Waring and Bremner, 1964; Patrick and Tusneem, 1971; Williams and Cooper, 1976). Oxygen availability affects mineralization by determining the microbial assemblage present in the soil. Higher rates of organic matter reduction occur in aerobic soils largely due to the increased

efficiency of aerobic respiration over anaerobic respiration pathways (Reddy and Patrick, 1984; Updegraff et al., 1995; Hansen and Blackburn, 1991). The size and activity of the microbial pool control available inorganic N and, therefore, organic N mineralization (Amador and Jones, 1993; Perucci, 1991). The activity and magnitude of the microbial community has been found to be closely linked to the availability of limiting nutrients and soil organic matter (Amador and Jones, 1993; Schnurer et al., 1985; Anderson and Domsch, 1985; Damman, 1988).

1.6.1 Effects of Salinity on Nitrogen Mineralization

The effects of salinity on N mineralization rates have been observed in a variety of soil types and experimental settings. Biological stresses and altered chemical properties within the soil have been attributed to changes in salinity. A number of studies on upland and wetland soils have found that increased salinity resulted in reduced mineralization rates. Salinity induced biological stress to microbial assemblages resulted in smaller and less efficient microbial communities (Rietz and Haynes, 2003; Jackson and Vallaire, 2009). A diminished microbial biomass C: N ratio in soils incubated with higher salinities (0-12 ppt) has also been observed (Yuan et al., 2007). When floodplain soils were treated with increasing salinity, microbial biomass measurements had decreased and the community structure of bacteria had shifted to prokaryotes (Sardinha et al., 2003). Laura (1977) and Irshad et al. (2005) hypothesized that the observed reduction in N mineralization for agricultural soils was due to salinity inhibition of nitrification occurring in soils. Differences between strength of salt treatment and mineralized N over time showed a reduction at higher salinities (5.70-8.80 ppt). However, at the end of the 8 to 14 week incubation period, the concentrations of ammonium mineralized at the highest salinity were not significantly different from the lower salinity treatments, suggesting the effect of salinity on N mineralization was short-lived. Short term salinity effects were also observed in

studies involving wetland (Wu et al., 2008), agricultural soils (Pathak and Rao, 1998; Lodhi et al., 2009), and estuarine-bottom sediments (Khoi et al., 2006). An immediate suppression of mineralization rates was observed followed by a gradual recovery to rates similar to control soils. In two experiments, whole soil cores collected from fresh and salt tidal marshes were submerged in various salinity treatments (0-10, 1-33 ppt) and monitored over a month (Weston et al., 2006) and up to several years (Portnoy and Giblin, 1997). There was an overall increase in ammonification due to salt water inundation observed (Portnoy and Giblin, 1997; Weston et al., 2006). Further analysis of soils from the Weston et al. (2006) study showed no differences in the microbial communities resulting from the control and seawater amended treatment (Edmonds et al., 2009). The variability in salinity effects on N mineralization rates suppression in some cases and increases in others may be an indicator that influence of salinity on net N mineralization may be dependent on the biochemical attributes of the soil.

1.7 Site Description

The Breton Sound Estuary was formed thousands of years ago by the Mississippi River (Fig. 1.4), as part of the Plaquemines-St. Bernard delta complex (Coleman, 1988; Scruton, 1960; Roberts, 1997). Within the Breton of Sound Estuary, between the Gulf of Mexico and Caernarvon Diversion, there are approximately 1100 km² of fresh and saline wetlands (Lane, et al. 2006). Salt water intrusion within the Breton Sound Estuary was causing the conversion of fresh and intermediate marsh types to brackish and saline. These changes within the estuary can adversely affect economically important species such as waterfowl, alligators, furbearers, finfish and shellfish (ACOE and LDWF, 1994). The Caernarvon Freshwater Diversion Structure was designed to reduce the effects of salt water intrusion, and establish favorable salinity conditions in the area (ACOE, 1995). In addition to combating salt water intrusion, the diversion would

help reduce the loss of land, enhance vegetative growth, and stimulate production of commercially and recreationally important fish and wildlife (ACOE, 1995). Freshwater diversions have been shown to enhance marsh stability and accretion by lowering salinity and sediment input, which slow or reverse the rate of wetland loss (Delaune et al., 2003).

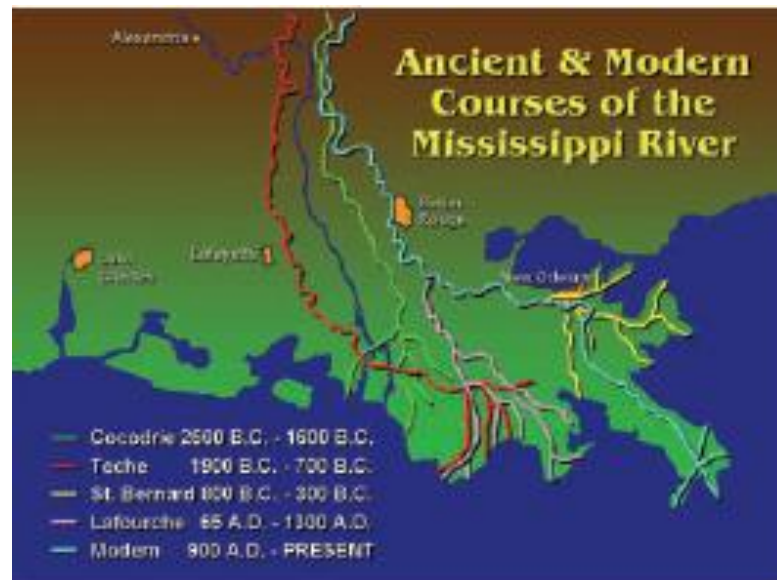


Figure 1.4 The St. Bernard delta complex and other historical courses of the Mississippi River (ACOE, New Orleans, LA).

The Caernarvon diversion is located near Caernarvon, LA in St. Bernard Parish. It redirects surface water into the Breton Sound Estuary from the 81.5 river mile on the East bank of the Mississippi River (Moerschbaeher, 2008). Construction of the diversion was carried out by the U.S. Corps of Engineers and is operated by the Louisiana Department of Natural Resources. The diversion structure has been in operation since fall of 1991, and floodwater is controlled by culvert gates which utilize gravity flow to allow for the passage of river water at a maximum rate of $8000 \text{ ft}^3\text{s}^{-1}$ (Lane et al. 2006). Year to year, the management of the flow of river water is dependent upon river flood stage as well as salinities within the basin. For

example, in 2008, the diversion was operating at new capacity from late March to mid May; while in 2009, discharges did not exceed $1000 \text{ ft}^3 \text{ s}^{-1}$ over the same period. Salinities in the upper estuary under normal flow are fresh and gradually increase to marine levels (35 ppt) at the outer edge of the Breton Sound (Lane et al., 2007).

Construction of the control structure began in June of 1988 and was completed in February 1991. The existing Caernarvon Control structure (Fig. 1.5) consists of five gated, box culverts which connect the Mississippi River to Big Mar and Bayou Mandeville, by an outflow channel approximately 1.5 miles long (ACOE, 1998). Each box culvert measures fifteen square feet, with each sluice gate constructed of cast iron. Each gate is operated by an electrical hoist motor that can open or close the gates in a half-hour, or $1 \frac{1}{2}$ hours if emergency power is used.



Figure 1.5 The Caernarvon Diversion structure at the Mississippi River, southeast of New Orleans, LA (ACOE, New Orleans, LA).

The diversion structure's total cost was \$25.9 million, with the federal government covering \$19.4 million of the expense, and Louisiana's share of the project, \$6.5 million (ACOE, 1999).

The Breton Sound Estuary's hydrological boundaries are defined by both natural and artificial structures (Fig. 1.6). The northern margin is defined by the natural levees of Bayou LaLoutre, and to the south the estuary extends into the northern Gulf of Mexico. The west boundary is the Mississippi River levee, and the eastern edge is made up of the Mississippi River Gulf Outlet's spoil banks (Moerschbaecher, 2008). Presently most the water delivered into the Gulf via this estuary flows through Lake Lery and Bayou Terre aux Bouef, and to a lesser degree Bayou Gentilly and Little Lake (Fig. 1.6). The discharge rate of the diversion structure, winds, and Gulf water levels commonly cause considerable marsh over-flow within the Breton Sound Estuary (Snedden et al., 2007).

The Breton sound is micro tidal and is made up of fresh, intermediate, brackish, and salt marsh dissected by numerous lakes, bayous, and canals. Bayou Terra aux Bouef, a relic Mississippi River distributary, separates the upper estuary into geographically and hydrologically isolated, eastward, and westward components. Dominant emergent vegetation of the brackish and salt marsh areas consists of *Spartina patens* (saltmeadow cordgrass), *Spartina alterniflora* (smooth cordgrass), and *Distichlis spicata* (saltgrass). *Spartina cynosuroides* (hog cane) were common on natural banks, elevated marsh sites, and along some spoil banks created by dredged material. *Juncus roemerianus* (black needlerush) is common in the interior marsh of both the saline and brackish zones. *Scirpus maritimus* (saltmarsh bulrush) is also found throughout most of the brackish and salt marsh but is not a dominant plant species. In fresh marsh systems of the upper Breton Sound dominant plant species are *Spartina patens* (saltmeadow cordgrass) and

Schoenoplectus americanus (chairmaker's bulrush) (Piazza and La Peyre, 2009; Visser et al., 1998).

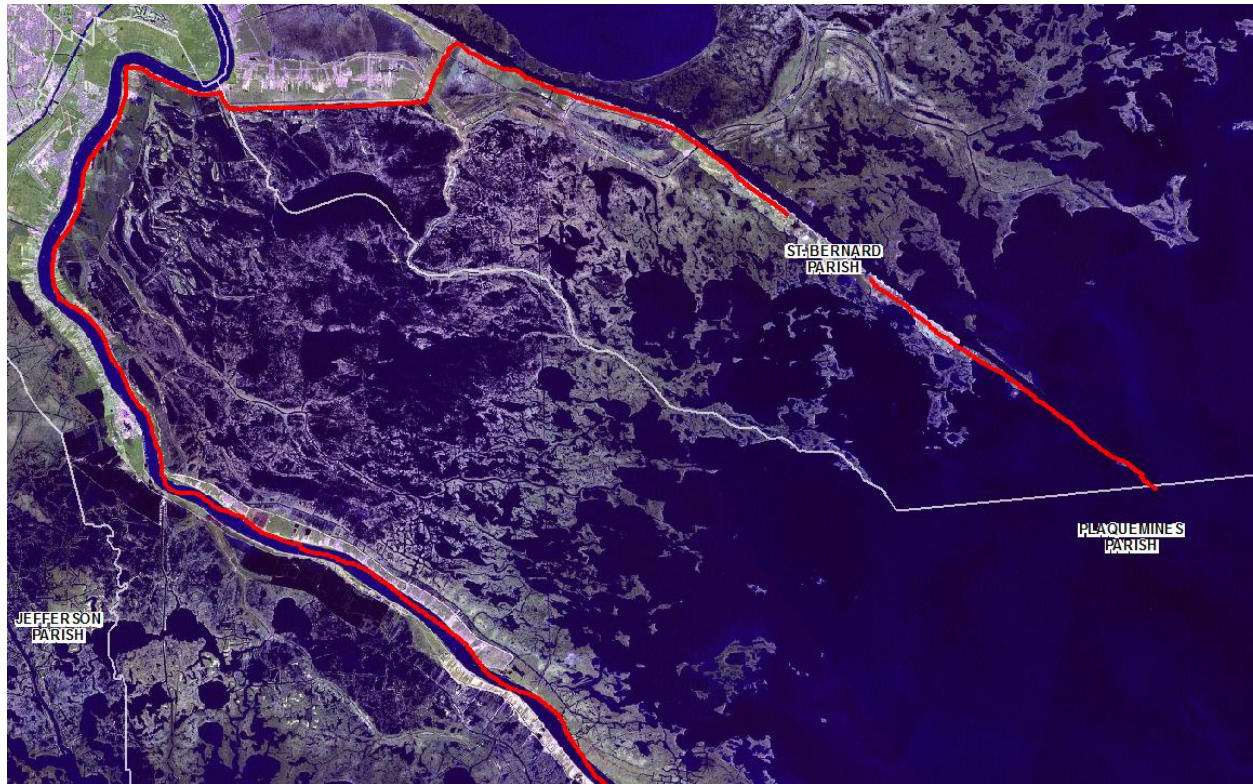


Figure 1.6 The Breton Sound Estuary and the Caernarvon Diversion in southeast Louisiana, U.S.A. (ArcGIS 9.1, 2007).

**CHAPTER 2: THE EFFECTS OF SALINITY ON DENITRIFICATION IN
WETLAND SOILS AND SEDIMENTS OF THE BRETON SOUND
ESTUARY, LA**

2.1 Introduction

Nitrogen is generally the limiting nutrient regulating productivity in coastal systems. Due to their position in the landscape, wetlands possess the ability to intercept floodwaters originating from upland sources resulting in a decreased rate of decomposition and, consequently, increased accumulation of organic matter. This organic carbon accumulation is related to the low decomposition rate relative to upland ecosystems which make wetlands natural sinks for organic carbon, nitrogen and phosphorous (Debusk et al., 2001). Inorganic forms of nitrogen such as ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-) are in high biological demand in all ecosystems. Unchecked anthropogenic loading of inorganic nitrogen compounds into coastal and marine systems can have potential detrimental effects on the coastal and marine ecosystems.

Recent concerns with eutrophication off of Louisiana's coast have highlighted the function of wetlands as a buffer and sink for nutrients from the uplands (Reddy et al. 1993; Hatton et al. 1982; Sharp et al., 1982; Rabalais et al., 1994). The discharge of relatively high concentrations of nitrate in Mississippi River waters directed into the Northern Gulf of Mexico is the main cause of eutrophication in coastal waters, and the consequent hypoxic zone that forms annually along the Louisiana and Texas coasts (Turner and Rabalais, 1991; Rabalais et al., 1994). The construction of the vast levee system in the early 1900's has largely isolated Louisiana's coastal wetlands from the Mississippi River's nutrient-laden flood waters and sediment (Mossa, 1996). One possible alternative to alleviate this nutrient loading into the coastal waters is to divert river water into the coastal wetlands. The nutrient load of the river water, especially the nitrate component, can be processed or removed by the wetlands. Nitrate can be recycled back into the atmosphere through denitrification, assimilated into organic matter,

or reduced to ammonium by natural biochemical processes occurring simultaneously in the soil and water column (Seitzinger, 1988; Kaplan et al., 1979; Valiela et. al., 2000; Lindau et al., 2008; Lindau et al., 2009).

Denitrification is a key process regulating nitrogen cycling in natural environments. This process allows inorganic nitrogen, in the form of nitrate, to be removed from the system by facultative anaerobic bacteria present in wetland sediments (Knowles, 1982). When oxygen is unavailable for microbial metabolism, these organisms use nitrate as the terminal electron acceptor as they oxidize organic material in order to obtain energy (Seitzinger, 1988). The high primary productivity and high rate of organic matter sequestration coupled with anaerobic soil regimes of freshwater and coastal marshes provide ideal conditions for denitrification (Stefanson, 1972; Groffman, 1994; Nowicki et al., 1999). The end product of denitrification is nitrogen gas released into the atmosphere. Denitrification generally proceeds through some combination of the following intermediates:



The reduction of nitrate to N_2 gas effectively removes it from the environment and limits uptake by primary producers. Typically, high rates of microbial denitrification have been observed in coastal wetland soils (Seitzinger, 1988; Kaplan et al., 1979; Valiela et. al., 2000).

Denitrification has been found to be mediated by a variety of environmental factors. Many field and laboratory studies have indicated that nitrate supply becomes the limiting factor for denitrification high carbon content and low O_2 in wetland soils (White and Reddy, 1999; Cooper, 1990). Increasing nitrate availability has also been linked to the overall increase of in situ denitrifying activity in riparian soils (Cooper, 1990; Gardner and White, 2010). Variations in

denitrification have also been positively correlated with increases in the nitrate concentrations in the overlying water in salt marshes (Thompson et al., 1995). Oxygen availability, organic matter, nitrate supply, and temperature are reported to have the most significant influence over biological denitrification in wetland sediments (Wang et al., 2007).

Studies investigating the effects of salinity on denitrification and they have produced a number of mixed results (Antheunisse et al., 2006; Magalhaes et al., 2005; Wu et al., 2008; Yu et al., 2008). Salinity changes on sediment from a surface freshwater diversion found that an increase in salinity caused decreased denitrification activity (Yu et al., 2008). Furthermore, by using separate salinity treatments of K_2SO_4 , NaCl and seawater it was determined that the sulfate content of seawater had no significant effect on denitrification (Yu et al., 2008). In mangrove microcosms inundated with wastewater, salinity treatments between 0-30 ppt resulted in potential denitrification being retarded at higher salinities (Wu et al., 2008). Another study observed the effects of restored tidal movements and seawater incursion on soil nitrogen conversions in an outdoor mesocosm experiment (Antheunisse et al., 2006). The results of this experiment however showed no significant correlation between the reintroduction of salt water to semi-natural and agricultural soils on denitrification enzyme activity or potential denitrification. Denitrification in sandy intertidal sediments and rocky bio-films showed no clear indications of influence due to raised salinity however increased nitrate loads delivered to each system showed a progressive linear increase of denitrification (Magalhaes et al., 2005). Further investigation of the influence of salinity on nitrogen cycling in wetland soils and sediments is needed.

The Breton Sound Estuary of southeast Louisiana is a system in constant flux as upland run off and Mississippi River discharge and a marine hydrologic regime alternatively exert an influence on the coastal zone. In the recent past, saltwater intrusion has accelerated due to canals

constructed that further link high and low salinity wetlands (Wang, 1988). The confinement of the Mississippi River by dams and levees has also altered the hydrology of estuary. High river discharge delivered to coastal wetlands through the main channels of the river and through surface water diversion structures quickly lower salinities within the estuary over the short term (Lane et al., 2007). Storm surge events associated with hurricanes result in the instantaneous flux of large volumes of high salinity seawater to adjacent marshes and can lead to longer term shifts in marsh salinity (Blood et al., 1991). Louisiana's coastal wetlands are also experiencing a slow but gradual increase in local salinity gradients due to a rise in relative sea-level brought on by the instability and compaction of fine grained sediments composing the continental shelf (Lane et al., 1999).

This study investigates salinity effects on denitrification in fresh marsh, salt marsh soils and adjacent shallow bayou sediments in the Breton Sound estuary. Short-term incubation experiments (2 days) were used to analyze the denitrifying potential of these soils under conditions of an instantaneous shift in the salinity regime to highly saline (35 ppt) or fresh conditions (0 ppt). Long-term incubation experiments were used to study the acclimation trends of the denitrifiers when exposed to variable salinity regimes (0, 15, 35 ppt) over extended periods (11 days). We hypothesize that a rapid change in salinity would negatively affect the potential denitrifying activity within all soils in the short term, and freshwater conditions would result in higher overall denitrification rates throughout the duration of the experiments. Furthermore, we propose that marsh soils will possess greater denitrifying potential than the shallow bottom sediments of the adjacent bayous.

2.2 Materials and Methods

2.2.1 Study Area

The Caernarvon diversion is located near Caernarvon, LA in St. Bernard Parish. It redirects surface water into the Breton Sound Estuary from the 81.5 river mile on the East bank of the Mississippi River (Moerschbaeche, 2008). Construction of the diversion was carried out by the U.S. Corps of Engineers and operated by the Louisiana Department of Natural Resources (Villarubia, 2006). The diversion structure has been in operation since fall of 1991, and its culvert gates utilize gravity flow in order to allow for the passage of river water at a maximum rate of $8000 \text{ ft}^3 \text{ s}^{-1}$ (Lane et al. 2006). The Breton Sound Estuary was formed thousands of years ago by the Mississippi River, as part of the Plaquemines-St. Bernard delta complex (Coleman, 1988; Scruton, 1960; Roberts, 1997). Within the Breton of Sound Estuary, between the Gulf of Mexico and Caernarvon Diversion, there are approximately 1100 km^2 of fresh and saline wetlands (Lane, et al. 2006). The Caernarvon Freshwater Diversion Structure was designed to reduce the effects of salt water intrusion, and establish favorable salinity conditions in the area (ACOE, 1995). In addition to combating salt water intrusion, the diversion would help reduce the loss of land, enhance vegetative growth, and stimulate production of commercially and recreationally important fish and wildlife (ACOE, 1995). Salinities in the upper estuary under normal flow are fresh and gradually increase to water of 35 ppt salinity at the outer edge of the Breton Sound (Lane et al., 2007).

The Breton sound is micro tidal and is made up of fresh, intermediate, brackish, and salt marsh dissected by numerous lakes, bayous, and canals. Bayou Terra aux Bouef, a relic Mississippi River distributary, separates the upper estuary into geographically and hydrologically isolated, eastward, and westward components. Dominant emergent vegetation of the brackish

and salt marsh areas consists of *Spartina patens* (saltmeadow cordgrass), *Spartina alterniflora* (smooth cordgrass), and *Distichlis spicata* (saltgrass). *Spartina cynosuroides* (hog cane) were common on natural banks, elevated marsh sites, and along some spoil banks created by dredged material. *Juncus roemerianus* (black needlerush) is common in the interior marsh of both the saline and brackish zones. *Scirpus maritimus* (saltmarsh bulrush) is also found throughout most of the brackish and salt marsh but is not a dominant plant species. In fresh marsh systems of the upper Breton Sound dominant plant species are *Spartina patens* (saltmeadow cordgrass) and *Schoenoplectus americanus* (chairmaker's bulrush) (Piazza and La Peyre, 2009; Visser et al., 1998).

2.2.2 Sample Collection and Preparation

Twenty-eight soil cores were taken from two distinct marsh sites within the Breton Sound estuary in April 2009 (Fig. 2.1). At each site sediment was also collected from the adjacent bayou. Site 1 consisted of a salt marsh area dominated by *Spartina alterniflora* located 29.4 mi southeast of the Caernarvon Diversion within Plaquemines parish (Fig. 2.2). Seven replicate soil cores were collected from a 3 m² area of the mid-marsh (29°39'52.4" N, 89°36'35.6" W) and from a 3 m² area of the adjacent bayou (29°39'52.0" N, 89°36'34.9" W) located adjacent to the marsh area. Site 2 was a freshwater marsh area located at the northeast edge of Lake Lery in Plaquemines parish and was located 4.65 miles southeast of the diversion structure (Fig 2.3). Seven replicate soil cores were taken from a 3 m² area of the mid-marsh (29°48'21.8" N, 89°52'28.4" W) another 7 cores from and the adjacent bayou (29°48'05.0" N, 89°52'23.7" W). Mississippi River discharge into the Breton Sound estuary during the spring of 2009 was low compared to previous years (Fig. 2.4), averaging 500-1000 ft³ s⁻¹ (max. discharge rate of 8000 ft³ s⁻¹).

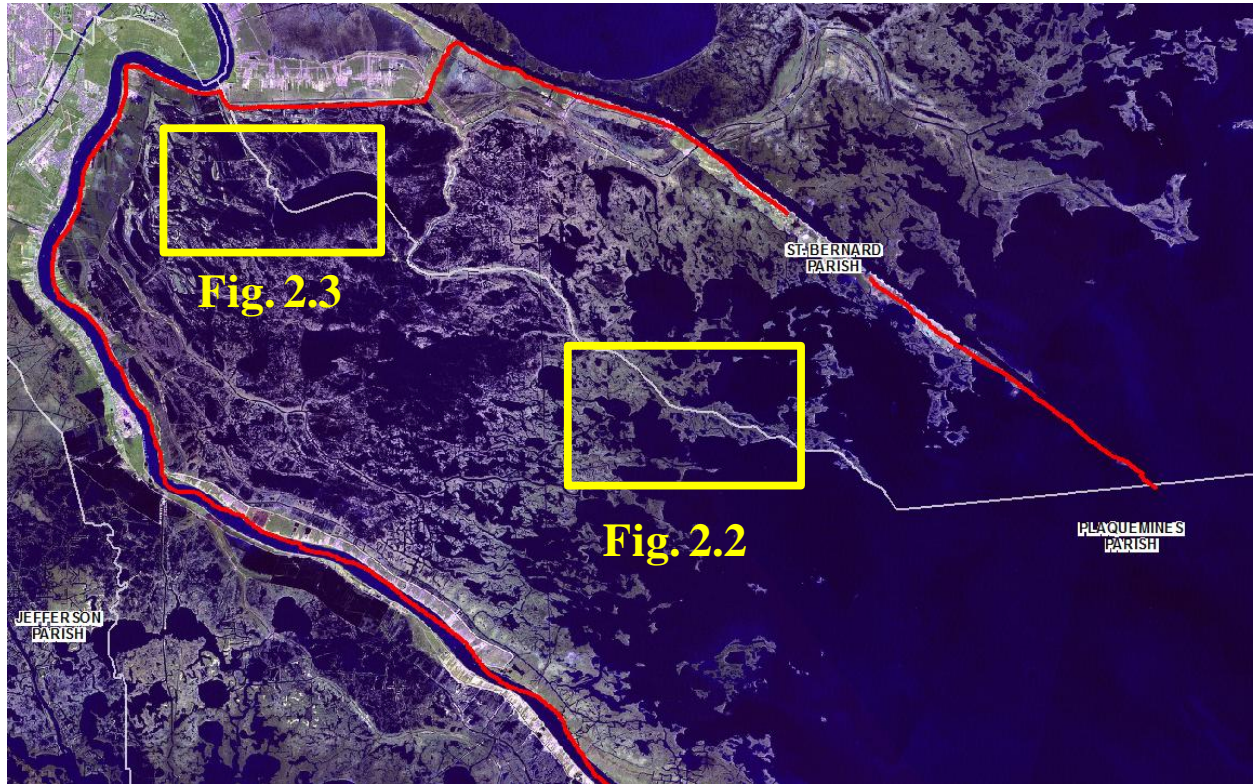


Figure 2.1 The Breton Sound estuary in southeast Louisiana (ArcGIS 9.1, 2007).

Soil samples were collected by push core technique using a 7 cm diameter, clear plexiglas tube. Sampling was in April, 2009. The top 10 cm of soil were extruded in the field, put in labeled zip-lock bags, and placed on ice. Upon return to the lab, the samples were homogenized by hand and any large root fragments were removed. Subsamples (mass=60 g) of each core sample were combined and homogenized in an electrical blender to create a slurry from the four sampling groups: salt marsh soil, fresh marsh soil, salt bayou sediment, and fresh bayou sediment. The samples were then stored in polyethylene containers, stored at 4° C until incubation.

2.2.3 Soil Characterization

Soil characterization analyses were conducted on the homogenized soils prepared in the lab and whole soil cores collected in the field. Moisture content, porewater salinity, bulk density, total carbon (TC), total nitrogen (TN), extractable DOC, microbial biomass C (MBC), and denitrification enzyme activity (DEA) of these soils were determined.

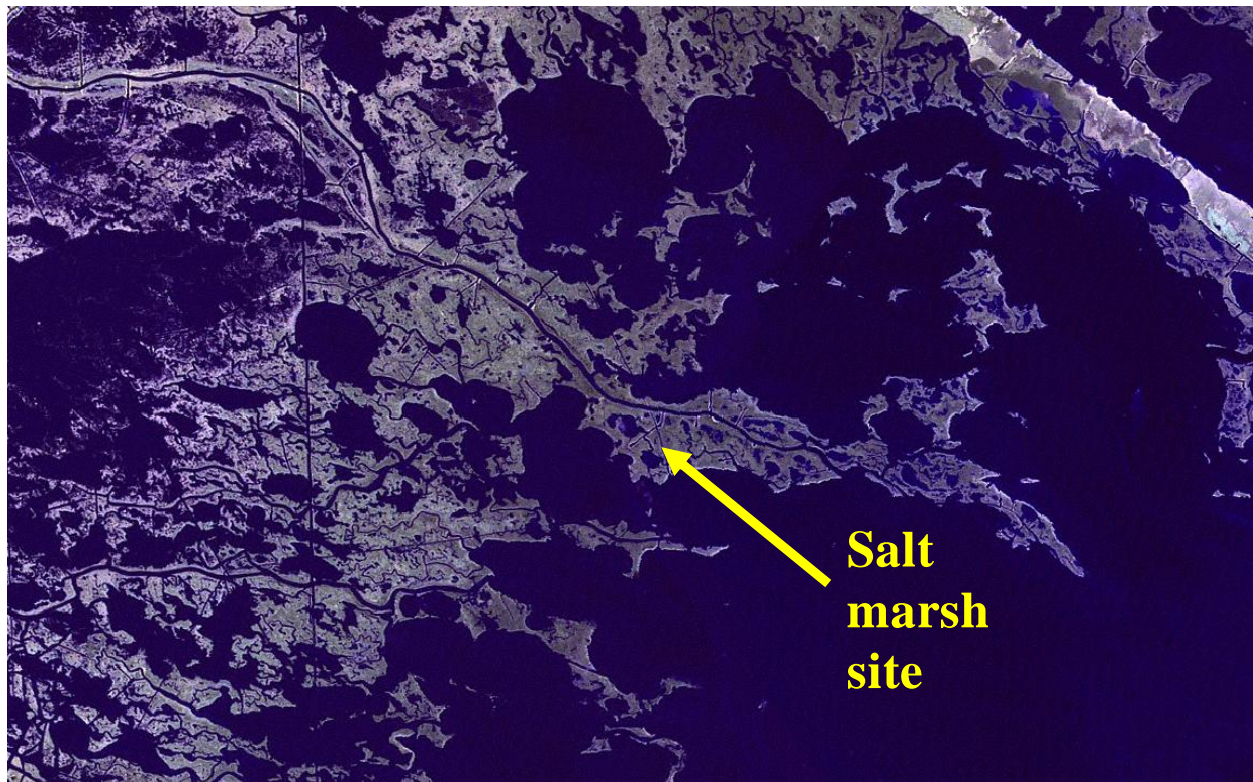


Figure 2.2 Salt marsh field site located in the lower Breton Sound estuary (ArcGIS 9.1, 2007).

Moisture content of homogenized soils and sediments was determined by drying wet weight subsamples at 70°C for 3 days, until constant dry weight. Bulk density (Blake and Hartge, 1986) was measured in whole soil cores (seven replicates for each soil type). Percent moisture of each whole soil subsample was calculated and used to determine the dry weight g of each soil subsample. Bulk density was calculated by dividing the dry soil weight by the volume

of the soil core used to collect the soil. Bulk density was expressed as dry weight g cm^{-3} .

Porewater salinity was measured from centrifuging 30 g of homogenized soil sample to separate the liquid from the soil. Salinity was measured on a YSI water sensor (YSI Environmental, Yellow Springs, OH).



Figure 2.3 Fresh marsh field site within the upper Breton Sound Estuary (ArcGIS 9.1, 2007).

Dried, ground subsamples were analyzed for TC and TN using an Elemental Combustion System with a detection limit of 0.005 g kg^{-1} (Costech Analytical Technologies, Inc., Valencia, CA).

The fumigate-extraction method (Vance et al., 1987) was utilized to determine microbial biomass C and extractable DOC. Fumigate and non-fumigate triplicate 5 g subsamples were placed in 25 ml centrifuge tubes. Fumigate samples had a one-half ml of pure chloroform added to each tube. All fumigate samples were placed in a vacuum desiccator, along with a beaker

containing approximately 50 ml of chloroform and 5-10 boiling stones. The desiccator was then evacuated using a vacuum pump and re-filled with room air three consecutive times.

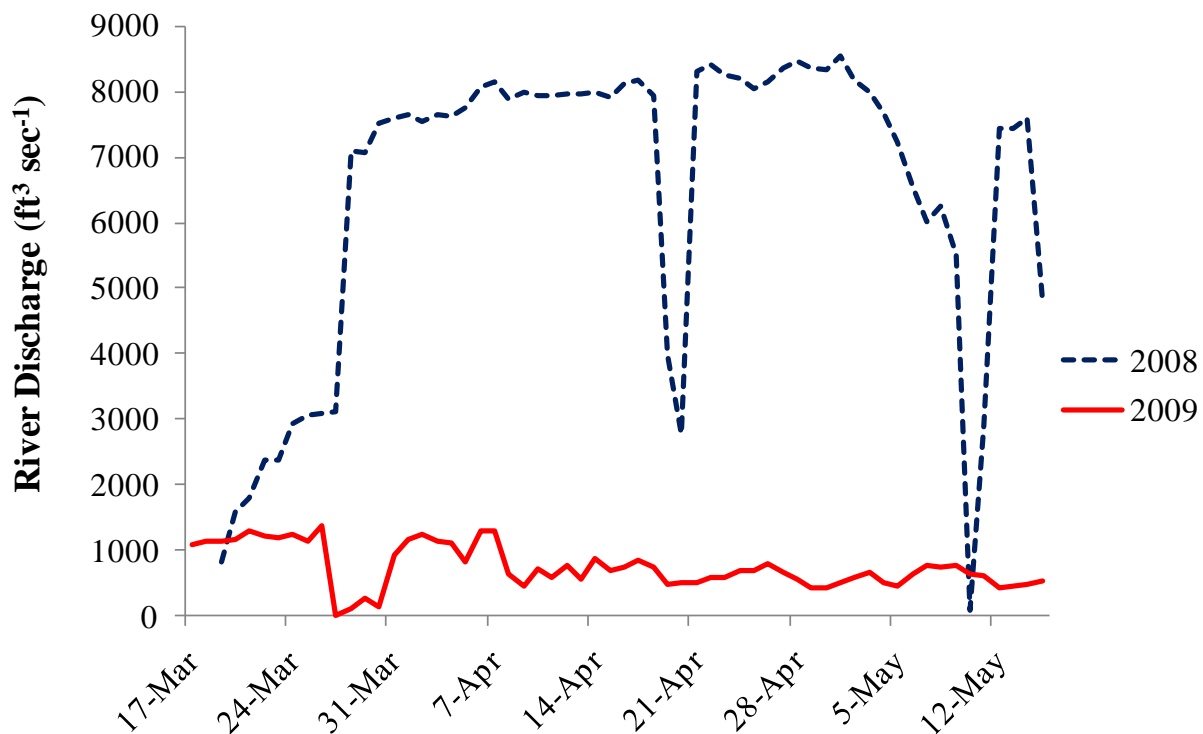


Figure 2.4 Caernarvon Diversion mean daily discharge rates from March 17- May 15, 2008 and 2009 (USGS, 2009).

Each time the air was vacuumed out of the desiccator until the chloroform in the beaker and samples would vigorously bubble. The desiccator was then sealed under high negative pressure and placed within the fume hood for 24 hours. The following day, the desiccator was opened and the glass beaker containing chloroform was removed. The desiccator was resealed and evacuated by vacuuming out the headspace to -87 kPa Hg and re-filling the container with room air 7 times. Both fumigate and non-fumigate samples then underwent the same extraction method. Twenty-five ml of 0.5 M K_2SO_4 were added to all samples, agitated on longitudinal

shaker for 30 min, then centrifuged for 10 min at 6000 rpm. Samples were vacuum filtered using 0.45 μm filter paper to isolate the supernatant, which was stored at 4°C until analyzed for dissolved organic carbon (TOC) (Shimadzu Scientific Instrument TOC- VCSN, Columbia, MD). The non-fumigate and fumigate masses (mg kg^{-1}) were determined by dividing the vial's total mass by dry soil weight (g). In order to calculate the microbial biomass C (MBC), the non-fumigate TOC measurement was subtracted from the corresponding fumigate TOC reading then divided by 1000. The extractable DOC data was obtained directly from the non-fumigate TOC (mg kg^{-1}).

Denitrification enzyme activity was determined using the methods developed by Tiedje (1982) with variations in procedure by White and Reddy (1999). Net five g wet weight subsamples of homogenized soils and sediments were placed in 70 ml glass serum bottles, and then sealed with a rubber septa and aluminum crimp cap. Headspace of each sample was evacuated to -75 kPa, and then purged with high purity N_2 gas (99.99% O_2 -free) for 3 min. Soil slurries were created by adding 10 ml of N_2 -purged DI water and 15% of headspace was replaced with acetylene gas (C_2H_2) while maintaining atmospheric pressure within the bottle (Yoshinari and Knowles, 1976). Acetylene distribution was achieved by shaking on a longitudinal shaker for 30 min. Ten ml of a solution composed of 56 $\text{mg KNO}_3\text{-N L}^{-1}$, 288 $\text{mg dextrose-C L}^{-1}$, and 2 $\text{mg chloramphenicol L}^{-1}$ was added to create a slight overpressure. The enzyme inhibitor, chloramphenicol, was added to prevent new enzymes from being synthesized during the 2 hr incubation (Smith and Tiedje, 1979). The 10 ml addition of DEA solution contains 0.56 mg of KNO_3 , 2.88 mg dextrose-C, and 0.02 mg chloramphenicol. Incubation of the samples occurred in darkness at 25°C on a longitudinal shaker. Headspace samples were pulled by syringe at 30, 60, 90, and 120 min time intervals. Gas samples were analyzed using a Shimadzu GC-8A ECD

with an ECD and N_2O production was calculated using the Bunsen adsorption coefficient (0.544) (Tiedje, 1982). DEA was calculated as the slope of the line when $\text{mg N}_2\text{O-N kg}^{-1}$ soil was plotted against time (min). All samples were measured in triplicate.

2.2.4 Short-Term Potential Denitrification (2 days)

Triplicate subsamples from each of the four homogenized soil types (Site 1: Salt marsh, Salt bayou bottom sediment and Site 2: Fresh Marsh, Fresh bayou bottom sediment) were prepared by placing 5 g wet weight subsamples into 70 ml serum bottles. Each bottle was then sealed using a rubber septa and aluminum crimp cap, and evacuated for 30 s to a pressure of -75 kPa. The bottles were then purged for another 5 min using 99.99% pure N_2 gas to create an anaerobic headspace. Two different salinity solutions were prepared for this experiment, using 140 g of glucose and 50 g of KNO_3 per liter to provide the non-limiting carbon and nitrogen source required for microbial denitrification. The carbon and nitrate were placed in two different salinity matrices; 0 ppt de-ionized water and 35 ppt filtered seawater. The resulting salinities of the 0 ppt and 35 ppt solutions were 25.8 ppt and 50.2 ppt, respectively, due to addition of the KNO_3 . The solutions were purged with 99.99% pure N_2 gas for 15 min to maintain the anaerobic conditions when 20 ml of solution (containing of 1000 mg KNO_3 and 3200 mg glucose) were added to each bottle. An acetylene block was also utilized by injecting acetylene gas (C_2H_2) equal to 15% of the bottles headspace (Yoshinari and Knowles, 1976). The serum bottle slurries were then continuously agitated in the dark on a longitudinal shaker at 25° C. Headspace gas samples were taken at 2, 12, 24, and 48 hrs in order to determine the short-term denitrification rates. Gas samples were extracted from bottle incubations by precision glass syringes and analyzed on a Shimadzu GC-8A equipped with an ECD (Shimadzu Scientific Instruments, Columbia, MD, detection limit $0.006 \text{ mg N}_2\text{O-N kg}^{-1} \text{ hr}^{-1}$). The N_2O production

was calculated with consideration for product in the aqueous phase using a Bunsen adsorption coefficient 0.544. The results were used to calculate rates of N_2O production and potential denitrification of the soil slurries in 0 ppt and 35 ppt water. Phase 1 (lag phase) were calculated from results between 0-24 hours. Maximum denitrification rates (phase 2) from this short-term experiment were determined by graphing results collected between 24-48 hours.

A set of control incubations were conducted for these short-term, potential denitrification soils inundated under two salinity regimes. Salt marsh and fresh marsh soils were inundated with both 35 and 0 ppt salinity treatment, while salt bayou was only incubated at 35 ppt and fresh bayou sediments only at 0 ppt salinity. These samples were analyzed using the same procedure as the experimental samples; however the two variable salinity solutions were produced using only glucose (140 g L^{-1}) in each solution. This incubate solution provided only non-limiting carbon for denitrification, and did not provide supplemental nitrate over the incubation period. Sampling and analysis of these samples followed the same procedure as outlined in the above experiment at 24, 48, 72, and 96 hrs.

2.2.5 Longer-Term Potential Denitrification (11 days)

Triplicate subsamples from each of the four homogenized soil types (salt marsh soil, salt bayou bottom sediment, fresh marsh soil, and fresh bayou bottom sediment) were prepared by placing 2 g wet weight subsamples into 160 ml serum bottles. Each bottle was then sealed using a rubber septa and aluminum crimp cap, and evacuated for 30 seconds to a pressure between -75 kPa. The bottles were then purged for another 5 min using 99.99% pure N_2 gas to create an anaerobic headspace. Three different salinity solutions were prepared for this experiment, and each included 140 g L^{-1} of glucose and 50 g L^{-1} of KNO_3 to provide the non-limiting carbon and nitrogen source required for microbial denitrification. Each solution also contained 0 ppt de-

ionized water, 15 ppt mixed saltwater, and 35 ppt filtered seawater. The resulting salinities of the 0 ppt, 15 ppt, and 35 ppt salinity/denitrification solutions were elevated to 25.8 ppt, 37.7 ppt, and 50.2 ppt, respectively, due to the addition of KNO_3 . The solutions were purged with 99.99% pure N_2 gas for 15 min to maintain the anaerobic conditions when 20 ml of the salinity solution (containing of 1000 mg KNO_3 and 3200 mg glucose) were added to each bottle. An acetylene block was also utilized by injecting acetylene gas (C_2H_2) equal to 15% of the bottles headspace (Yoshinari and Knowles, 1976). The serum bottle slurries were then continuously agitated in the dark on a longitudinal shaker, at 25°C. Headspace gas samples were taken at every 24-36 hrs over 11 days in order to record the long-term change in denitrification. Gas samples were extracted from bottle incubations by precision glass syringes. Collected gas samples were analyzed on a Shimadzu GC-8A equipped with an ECD (Shimadzu Scientific Instruments, Columbia, MD, detection limit $0.006 \text{ mg N}_2\text{O-N kg}^{-1} \text{ hr}^{-1}$) and N_2O production was calculated with consideration for product in the aqueous phase using the Bunsen Adsorption Coefficient 0.544. The results were used to calculate rates of N_2O production and potential denitrification of the soil slurries subjected to 0 ppt, 15 ppt, and 35 ppt water.

2.2.6 Data Analysis

The relationship between varying salinity treatment, soil type, and resulting rates of short-term and long-term potential denitrification were analyzed using a three-way ANOVA model ($P < 0.05$). Post-hoc ANOVA testing was conducted by Tukey's Studentized (HSD) test. Levene's Test and Bartlett's Test for Equality (both $P < 0.05$) were utilized to verify the homogeneity of variance and normality of distribution for experimental results. Correlations and linear regressions were used to identify the relationships between soil properties (Moisture content, Porewater salinity, bulk density, total carbon (TC), total nitrogen (TN), extractable

DOC, microbial biomass C (MBC), denitrification enzyme activity (DEA), and potential denitrification). SAS 9.1 was used to conduct all statistical analysis (SAS Institute Inc., Cary, NC).

Table 2.1 Soil characterization and analysis performed on homogenized marsh and bayou soils and whole core soils. Data are reported as mean \pm 1 std dev (n=3).

Soil Parameter	Units	Salt Marsh	Salt Bayou	Fresh Marsh	Fresh Bayou
Moisture	%	52.8 \pm 8.55 ^a	56.3 \pm 5.69 ^a	77.0 \pm 2.03 ^b	60.4 \pm 3.64 ^a
Salinity	ppt	9.28	9.34	0.26	0.23
Bulk Density	g cm ⁻³	0.65 \pm 0.12 ^c	0.62 \pm 0.09 ^c	0.51 \pm 0.07 ^b	0.27 \pm 0.03 ^a
Loss on Ignition	%	9.90	7.30	28.1	9.48
Total Carbon	g C kg ⁻¹	38.4	29.6	121	42.0
Total Nitrogen	g N kg ⁻¹	2.63	1.90	9.79	3.10
Microbial Carbon	mg kg ⁻¹ C	5.25 \pm 0.25 ^a	4.71 \pm 0.09 ^a	10.2 \pm 0.46 ^b	5.16 \pm 0.20 ^a
Extractable DOC	mg kg ⁻¹	270 \pm 1.40 ^c	132 \pm 8.67 ^b	323 \pm 5.11 ^d	104 \pm 2.12 ^a
DEA	μ g N ₂ O-N kg ⁻¹ hr ⁻¹	4.80 \pm 0.10 ^b	1.10 \pm 0.11 ^a	2.48 \pm 0.30 ^d	1.04 \pm 1.30 ^c
Extractable NH ₄ ⁺	mg N kg ⁻¹	12.1 \pm 0.93 ^a	19.2 \pm 0.18 ^a	39.8 \pm 0.28 ^b	56.8 \pm 0.38 ^c
Short-term Pot. DeN	mg N ₂ O-N kg ⁻¹ d ⁻¹	155 \pm 5.95 ^b	12.5 \pm 4.50 ^a	210 \pm 99.7 ^c	9.74 \pm 3.54 ^a
Long-term Pot. DeN	mg N ₂ O-N kg ⁻¹ d ⁻¹	615 \pm 182 ^b	373 \pm 35.9 ^a	373 \pm 22.2 ^a	330 \pm 23.0 ^a

DOC, Dissolved Organic Carbon; DEA, Denitrification Enzyme Activity; Short-term Pot. DeN, Short-term Potential Denitrification; Long-term Pot. DeN, Long-term Potential Denitrification.

a,b,c,d different letters indicate significant differences between soil/sediment types (across).

2.3 Results

2.3.1 Soil Properties

The mean moisture (n=3) taken from the salt marsh, salt bayou, fresh marsh, and fresh bayou were 52.8 \pm 8.55%, 56.3 \pm 5.69%, 77.0 \pm 2.03%, and 60.4 \pm 3.64%, respectively (Table 2.1). The moisture content of the fresh marsh soils was significantly higher than the other soils (P<0.05). Bulk density measurements were significantly higher (P<0.05) in the salt marsh and salt bayou soils (0.65 \pm 0.12 g cm⁻³ and 0.62 \pm 0.09 g cm⁻³) when compared with the fresh marsh and bayou (0.51 \pm 0.07 g cm⁻³ and 0.27 \pm 0.03 g cm⁻³).

Porewater salinity measurements in the salt marsh, saline bayou, fresh marsh, and fresh bayou at the time of sampling were 9.28 ppt, 9.34 ppt, 0.26 ppt, and 0.23 ppt, respectively (Table 2.1). Loss on ignition measured 9.90% and 7.30% in saline marsh and bayou. Loss on Ignition for fresh marsh and bayou soils were 28.1% and 9.48% (Table 2.1). Fresh marsh soils had the highest total C and total N (121 g C kg^{-1} and 9.79 g N kg^{-1}) and salt bayou sediment had the lowest overall (29.6 g C kg^{-1} and 1.90 g N kg^{-1}). Total C and N measured 41.0 C kg^{-1} and 3.10 g N kg^{-1} fresh bayou sediment and 38.4 C kg^{-1} and 2.63 g N kg^{-1} in saline marsh soil (Table 2.1).

Microbial biomass ranged from $10.2 \pm 0.46 \text{ g kg}^{-1} \text{ C}$ in fresh marsh to $4.71 \pm 0.09 \text{ g kg}^{-1} \text{ C}$ in the salt bayou sediments (Table 2.1). In salt marsh and fresh bayou soils microbial biomass C averaged $5.25 \pm 0.25 \text{ g kg}^{-1} \text{ C}$ and $5.16 \pm 0.20 \text{ g kg}^{-1} \text{ C}$. The microbial biomass C in fresh marsh soils was significantly higher than the other soils ($P < 0.05$).

Mean extractable DOC was determined to be highest in fresh marsh ($323 \pm 5.11 \text{ mg kg}^{-1}$) and lowest in fresh bayou samples ($104 \pm 2.12 \text{ mg kg}^{-1}$). In marsh and bayou soils from the saline site the extractable DOC measured $270 \pm 1.40 \text{ mg kg}^{-1}$ and $132 \pm 8.67 \text{ mg kg}^{-1}$, respectively (Table 2.1). All mean extractable DOC values determined from the four soil types were significantly different from one another ($P < 0.05$).

Average DEA rates in salt marsh ($4.80 \pm 0.10 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ hr}^{-1}$) and salt bayou ($1.10 \pm 0.11 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ hr}^{-1}$) were lower than those observed in fresh marsh ($2.48 \pm 0.30 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ hr}^{-1}$) and fresh bayou soils ($1.04 \pm 0.13 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ hr}^{-1}$). All DEA rates evaluated differed significantly between soils and sediments ($P < 0.05$).

The mean extractable ammonium in salt marsh and saline bayou soils were $12.1 \pm 0.93 \text{ mg N kg}^{-1}$ and $19.2 \pm 0.18 \text{ mg N kg}^{-1}$. In fresh marsh and bayou soil the extractable ammonium

averaged $39.8 \pm 0.28 \text{ mg N kg}^{-1}$ and $56.8 \pm 0.38 \text{ mg N kg}^{-1}$, respectively (Table 2.1). The extractable ammonium measured in fresh bayou sediment was significantly higher ($P < 0.05$) than all other soils and the extractable ammonium in both saline wetland soils was significantly lower than both fresh marsh soils and sediments ($P < 0.05$).

Table 2.2 Product-moment correlation coefficients for soil properties. For $n=12$, $r > 0.58$ is significant at $P < 0.05$.

	Moisture	Bulk Density	Loss on Ignition	Total C	Total N	MBC	Extractable DOC	DEA	Short Pot. DeN
Bulk Density	-0.95								
Loss on Ignition	0.81	-0.82							
Total C	0.82	-0.83	0.99						
Total N	0.83	-0.84	0.99	1.00					
MBC	0.80	-0.82	0.99	0.99	0.99				
Extractable DOC	0.40	-0.42	0.72	0.68	0.67	0.71			
DEA	0.85	-0.88	0.95	0.96	0.97	0.94	0.55		
Short Pot. DeN	0.39	-0.39	0.62	0.60	0.59	0.56	0.72	0.52	
Long Pot. DeN	-0.05	-0.03	0.24	0.22	0.22	0.21	0.59	0.25	0.51

MBC, Microbial biomass C; DOC, Dissolved Organic C; DEA, Denitrification Enzyme Activity; Short Pot. DeN, Short-term Potential Denitrification; Long Pot. DeN, Long-term Potential Denitrification.

2.3.2 Soil Properties Relationships

As expected, soil bulk density decreased ($r = -0.95$, $n=12$) with increasing moisture content (Table 2.1). Loss on ignition (LOI) decreased as soil bulk density increased ($r = -0.82$, $n=12$). Loss on ignition was greater in marsh soils than bayou sediments from both field sites most likely due to the presence of wetland vegetation in the marsh soils. Both total C and total N were significantly correlated with one another ($r = 1.00$, $n=12$). Total C and total N also were significantly correlated to loss on ignition ($r = 0.99$, $n=12$). Therefore, LOI is an effective predictor of total C and N in these soils. Microbial biomass C demonstrated a high positive correlation with LOI, total C and total N ($r = 0.99$, $r = 0.99$, and $r = 0.99$, respectively, [for $n=12$]).

Extractable dissolved organic C (DOC) increased with LOI and microbial biomass C ($r = 0.72$ and $r = 0.71$, $n=12$). DEA exhibited a close positive correlation with total C and total N ($r = 0.96$ and $r = 0.97$, $n=12$). DEA also significantly increased with increasing LOI ($r = 0.95$, $n=12$) and microbial biomass C ($r = 0.94$).

Table 2.3 Average rates of short-term potential denitrification and standard error calculated for each soil type and salinity during phase 1 (0-24 hrs).

Salinity treatment	Salt Marsh	Salt Bayou	Fresh Marsh	Fresh Bayou
	mg N ₂ O-N kg ⁻¹ d ⁻¹	mg N ₂ O-N kg ⁻¹ d ⁻¹	mg N ₂ O-N kg ⁻¹ d ⁻¹	mg N ₂ O-N kg ⁻¹ d ⁻¹
0 ppt	18.1 ± 1.14 ^c	2.51 ± 0.16 ^a	30.6 ± 6.95 ^d	6.53 ± 0.24 ^b
35 ppt	14.1 ± 0.59 ^b	6.23 ± 0.58 ^a	25.5 ± 2.14 ^c	7.53 ± 3.33 ^a

a,b,c,d different letters indicate significant differences between soil/sediment types (across).

2.3.3 Short-term Potential Denitrification

In the short-term potential denitrification experiment, the maximum rate of denitrification was achieved after a period of 24 hrs (Fig. 2.5, Fig. 2.6). The initial lag phase (phase 1) of denitrification was observed in all soil incubations during the first 24 hrs. The average phase 1 rates in salt marsh and salt bayou soils incubated in 35 ppt water was 14.1 ± 0.59 mg N₂O-N kg⁻¹ d⁻¹ and 6.23 ± 0.58 mg N₂O-N kg⁻¹ d⁻¹, respectively (Table 2.3). The maximum rate of denitrification (phase 2) observed in all soils and sediments in this experiment, occurred between 24-48 hrs. Salt marsh and bayou soils with 35 ppt water showed maximum rates averaging 155 ± 5.95 mg N₂O-N kg⁻¹ d⁻¹ and 12.5 ± 4.50 mg N₂O-N kg⁻¹ d⁻¹ (Table 2.4). For both the short-term and potential denitrification the salt marsh denitrification rates were 7.2 times and 12.4 times, respectively, greater than the bayou sediment rates. The phase 1 and phase 2 rates for these saline soils under the 35 ppt treatment differed significantly from one another ($P < 0.05$).

Phase 1 rates measured in fresh marsh and fresh bayou soils in 0 ppt solution were significantly different ($P < 0.05$). These initial rates averaged 30.6 ± 6.95 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ in fresh marsh and 6.53 ± 0.24 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ in fresh bayou sediments (Table 2.3). Mean phase 2 rates of potential denitrification in fresh marsh and bayou samples under 0 ppt regimes were 210 ± 99.6 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ and 9.74 ± 3.54 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$, respectively (Table 2.4). These two rates were significantly different from one another ($P < 0.05$). Fresh marsh phase 1 and phase 2 denitrification rates were 4.7 times and 21.5 times greater than rates observed in fresh bayou sediments.

Table 2.4 Average rates of short-term potential denitrification and standard error calculated for each soil type and salinity treatment at the maximum rate of denitrification measured during the incubation (phase 2; 24-48 hrs).

Salinity treatment	Salt Marsh	Salt Bayou	Fresh Marsh	Fresh Bayou
	mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$	mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$	mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$	mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$
0 ppt	$-8.08 \pm 2.99^{\text{a},1}$	$35.6 \pm 8.12^{\text{c},2}$	$210 \pm 99.6^{\text{d},1}$	$9.74 \pm 3.54^{\text{b},1}$
35 ppt	$155 \pm 5.95^{\text{b},2}$	$12.5 \pm 4.50^{\text{a},1}$	$226 \pm 91.1^{\text{b},1}$	$12.1 \pm 3.04^{\text{a},1}$

a,b,c,d different letters indicate significant differences between soil/sediment types (across). 1,2 different numbers indicate significant differences between salinity treatments (down).

2.3.4 Short-term Effects of Salinity on Potential Denitrification

Denitrification rates observed in marsh soils from the two field sites in the Breton Sound Estuary displayed were varied when exposed to the two salinity treatments for short-term effects. The fresh and salt marsh phase 1 rate under the 0 ppt salinity regimes averaged 30.6 ± 6.95 and 18.1 ± 1.14 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$, respectively. The rate of phase 1 denitrification with 35 ppt salinity were 25.5 ± 2.14 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ in fresh marsh soil which was significantly higher ($P < 0.05$) than the salt marsh at 14.1 ± 0.59 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ (Table 2.3). The phase 1 rates

observed in fresh bayou sediment in 0 ppt ($6.53 \pm 0.24 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$) were significantly higher ($P < 0.05$) than in saline bayou sediments ($2.51 \pm 0.16 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$; Table 2.3).

When treated with 35 ppt water the phase 1 denitrification rates in fresh and saline bayou bottom sediments were $7.53 \pm 3.33 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ and $6.23 \pm 0.58 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$, respectively (Table 2.3). These rates were not significantly different from one another ($P < 0.05$).

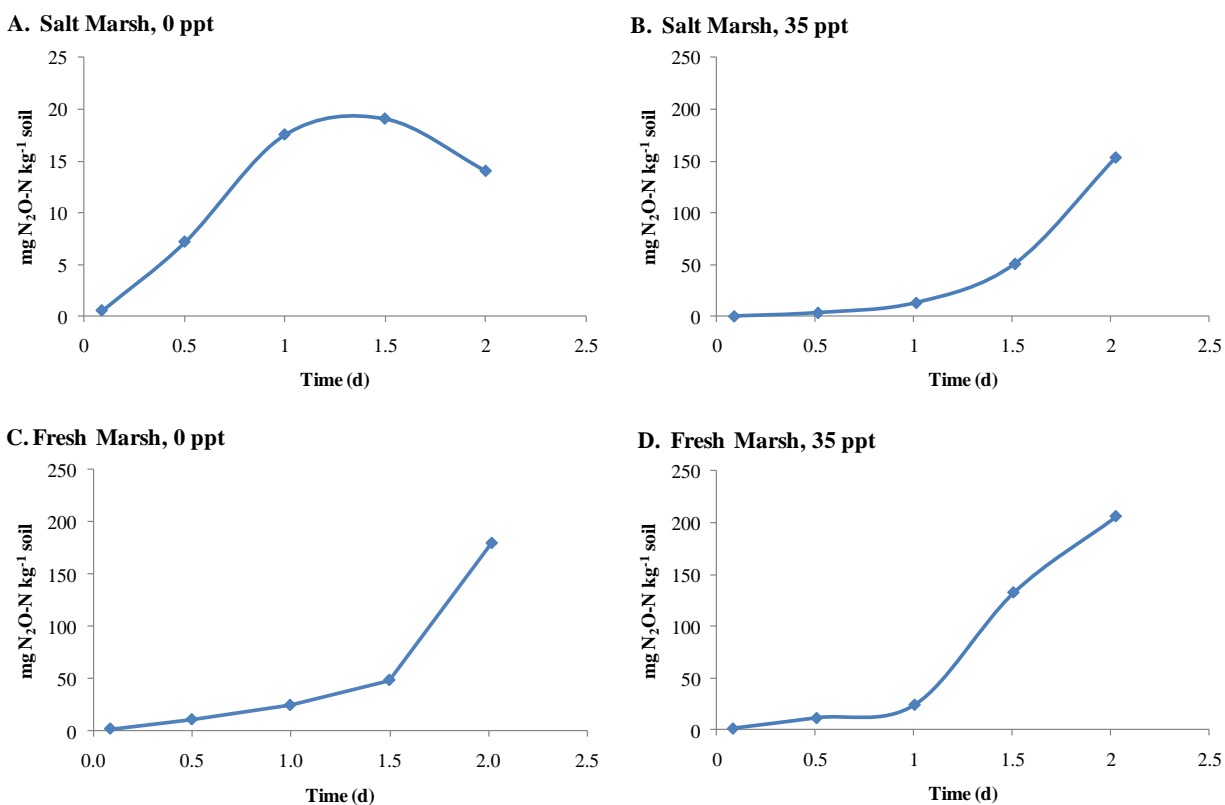


Figure 2.5 The relationship between mg of N₂O kg⁻¹ soil d⁻¹ produced over two days for marsh soils in two salinity regimes. A) salt marsh, 0 ppt salinity B) salt marsh, 35 ppt salinity C) fresh marsh, 0 ppt salinity D) fresh marsh, 35 ppt salinity.

The phase 2 rates of denitrification in fresh marsh soil averaged $210 \pm 99.6 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ and $226 \pm 91.1 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ when treated with 0 ppt and 35 ppt water (Table 2.4). Salt marsh soils incubated in 0 ppt and 35 ppt treatments produced mean rates of $-8.08 \pm 2.99 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ and $155 \pm 5.95 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$, respectively (Table 2.4). At 0 ppt, the rate of

denitrification in fresh marsh soils is significantly greater than saline marsh, but at 35 ppt there is no significant difference between the rates produced by the marsh soils ($P < 0.05$). Phase 2 rates of denitrification in fresh bayou soils (0 ppt, $9.74 \pm 3.54 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$; 35 ppt, $12.1 \pm 3.04 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$) are significantly lower than adjacent fresh marsh soils ($P < 0.05$; Table 2.4). Maximum rates in saline bayou bottom sediments averaged $35.6 \pm 8.12 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ in 0 ppt water and $12.5 \pm 4.50 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ when treated with 35 ppt water (Table 2.4). In saline marsh and bayou soils, the mean phase 2 rates observed under both salinity treatments were significantly different ($P < 0.05$). The saline bayou potential denitrification rate produced under 0 ppt conditions was significantly higher than fresh bayou productivity under the same treatment ($P < 0.05$).

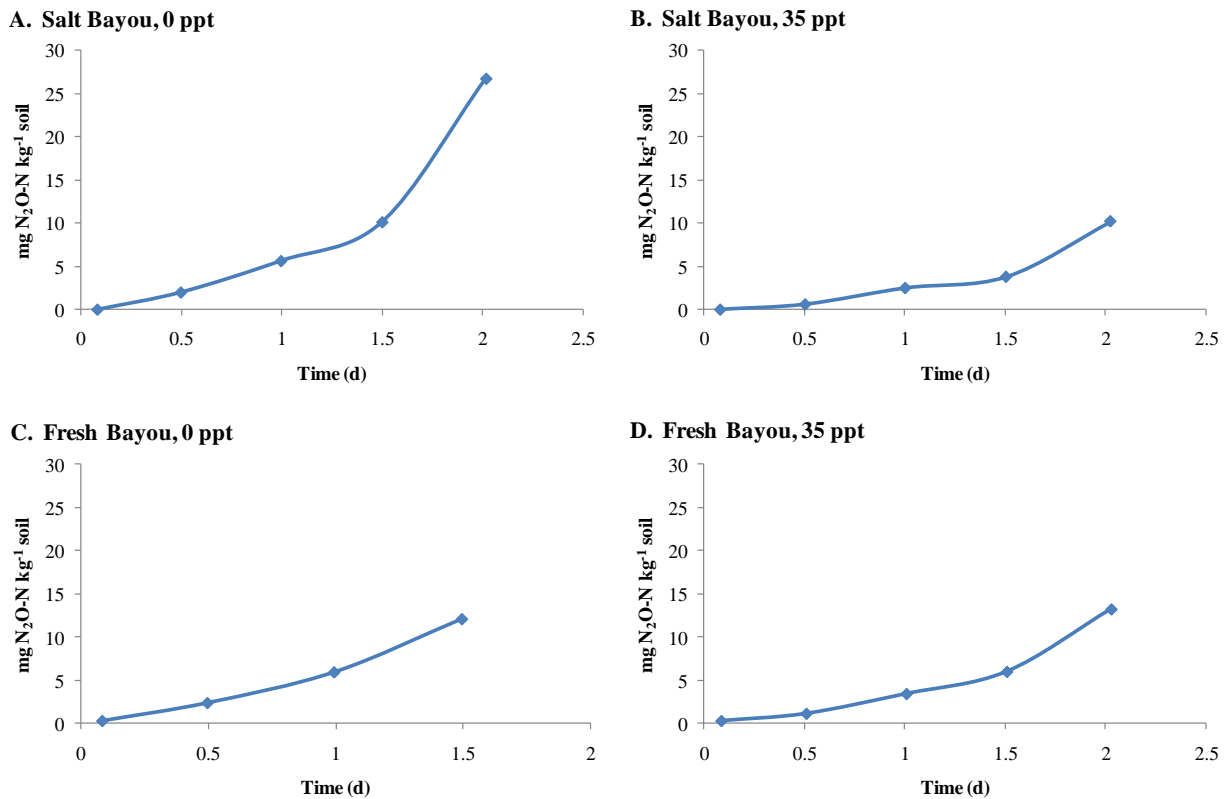


Figure 2.6 The relationship between mg of $\text{N}_2\text{O kg}^{-1} \text{ soil d}^{-1}$ produced over two days for bayou bottom sediments in two salinity regimes. A) salt bayou, 0 ppt salinity B) salt bayou, 35 ppt salinity C) fresh bayou, 0 ppt salinity D) fresh bayou, 35 ppt salinity.

Upon exposure to the 35 ppt treatment, the phase 1 and phase 2 rates of denitrification in fresh marsh soil were 3.4 and 18.7 times greater than the rate in fresh bayou sediments. Salt marsh soil, under 0 ppt treatment, had a phase 1 denitrification rate 7.2 times greater than the saline bayou sediments. The phase 2 rate observed in salt marsh soils in 0 ppt was a negative rate ($-8.08 \pm 2.99 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$) while the saline bayou rate ($35.6 \pm 8.12 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$; Table 2.4) was positive and significantly greater ($P < 0.05$).

2.3.5 Long-term Potential Denitrification

The long-term potential denitrification was determined for fresh marsh, salt marsh, fresh bayou, and saline bayou soils under incubation in three different salinities (0, 15, and 35 ppt) over 11 days. At the time of sampling in the field, saline marsh and bayou pore water salinity measured 9.28 ppt and 9.34 ppt, respectively (Table 2.1). In this experiment the 15 ppt treatment most closely compares to the natural salinity regime in the salt marsh site samples. Phase 1 rates of potential denitrification represent a lag period before maximum denitrification rates were observed in these incubations. Mean phase 1 denitrification rates were determined from sampling linear N_2O production occurring over 2, 3, 5, or 7 days (Table 2.5). Salt marsh and bayou soils under 15 ppt treatments had mean rates of denitrification measuring $35.1 \pm 0.68 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ and $0.42 \pm 0.10 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ (Table 2.5). The salt marsh denitrification rate was 83.7 times greater than the rate for the adjacent bayou sediment. Under experimental salinity conditions closest to the natural salinity regime, the salt marsh denitrification rates were significantly higher than the salt bayou sediment ($P < 0.05$). The porewater salinity measurements taken in the freshwater marsh site was 0.26 ppt in the fresh marsh and 0.23 ppt (Table 2.1) in the adjacent fresh bayou at the time of sampling. The 0 ppt salinity treatment used in this experiment closely mimics the in-situ salinity conditions for these soils. The phase 1 rates of

fresh marsh and fresh bayou soils while incubated in 0 ppt solution were 4.95 ± 2.00 mg N₂O-N kg⁻¹ d⁻¹ and 10.3 ± 2.06 mg N₂O-N kg⁻¹ d⁻¹ (Table 2.5). The fresh bayou potential denitrification rate was significantly higher (2 times greater) than the adjacent marsh (P<0.05).

Table 2.5 Mean rates (mg N₂O-N kg⁻¹ d⁻¹) of long-term potential denitrification and standard error in each soil type and salinity treatment during phase 1 of denitrification (time period varies with each soil and salinity treatment).

	Fresh Marsh	Fresh Bayou	Salt Marsh	Salt Bayou
0 ppt	* $4.95 \pm 2.00^{a,b,1}$	† $10.3 \pm 2.06^{b,3}$	‡ $1.09 \pm 0.39^{a,1}$	¶ $1.10 \pm 0.01^{a,2}$
15 ppt	† $30.7 \pm 3.55^{b,2}$	† $0.85 \pm 0.26^{a,1}$	‡ $35.1 \pm 0.68^{b,2}$	† $0.42 \pm 0.10^{a,1}$
35 ppt	* $22.1 \pm 3.30^{c,2}$	† $3.28 \pm 0.22^{b,2}$	‡ $59.5 \pm 15.5^{c,2}$	† $0.18 \pm 0.14^{a,1}$

* 0-2 days, † 0-3 days, ‡ 0-5 days, ¶ 0-7 days.

a,b,c letters indicate significant differences between soil/sediment types (across).

1,2,3 numbers indicate significant differences between salinity treatments (down).

The rates of potential denitrification (phase 2) measured in soils from the fresh and salt marsh sites occurred later in the incubation, following the lag phase of denitrification (Table 2.6). The maximum rate of denitrification in salt marsh soils under 15 ppt treatment (507 ± 27.0 mg N₂O-N kg⁻¹ d⁻¹) was significantly higher than saline bayou sediments (149 ± 51.2 mg N₂O-N kg⁻¹ d⁻¹) at 15 ppt (Table 2.6; P<0.05). The rate of potential denitrification in salt marsh soil was 3.4 times greater than denitrification in the saline bayou sediments. The mean phase 2 rate of denitrification produced by fresh marsh soils incubated in 0 ppt solution was 373 ± 22.2 mg N₂O-N kg⁻¹ d⁻¹ (Table 2.6). The rate of fresh bayou sediments under fresh treatment averaged 330 ± 23.0 mg N₂O-N kg⁻¹ d⁻¹ (Table 2.6) and was not significantly different than the rate observed in the fresh marsh samples (P<0.05).

Table 2.6 Mean rates ($\text{mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$) of long-term potential denitrification and standard error in each soil type and salinity treatment during phase 2 of denitrification (time period varies with each soil and salinity treatment).

	Fresh Marsh	Fresh Bayou	Salt Marsh	Salt Bayou
0 ppt	* $373 \pm 22.2^{c,2}$	† $330 \pm 23.0^{c,2}$	‡ $9.18 \pm 3.27^{a,1}$	‡ $94.3 \pm 9.55^{b,1}$
15 ppt	† $654 \pm 90.9^{b,c,3}$	† $917 \pm 341^{c,3}$	‡ $507 \pm 27.0^{b,2}$	† $149 \pm 51.2^{a,1}$
35 ppt	* $99.7 \pm 21.1^{a,1}$	† $131 \pm 86.4^{a,1}$	† $615 \pm 182^{c,2}$	† $373 \pm 35.9^{b,2}$

* 3-11 days, † 4-11 days, ‡ 6-11 days, § 9-11 days.

a,b,c letters indicate significant differences between soil/sediment types (across).

1,2,3 numbers indicate significant differences between salinity treatments (down).

2.3.6 Long-term Effects of Salinity on Potential Denitrification

Salt marsh soils under 0 ppt and 35 ppt treatment displayed phase 1 rates averaging $1.09 \pm 0.39 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ and $59.5 \pm 15.5 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$, respectively (Table 2.5). The lag phase rate produced by saline bayou sediment in 0 ppt and 35 ppt were $1.10 \pm 0.01 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ and $0.18 \pm 0.14 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ (Table 2.5). The phase 1 rate in salt marsh soil was significantly higher than the denitrification activity in saline bayou sediment treated with 35 ppt solution ($P < 0.05$). The phase 1 rate resulting from 35 ppt treatment in salt marsh soil was 330 times greater than denitrification in the saline bayou. Phase 1 denitrification rates in fresh marsh soil averaged $30.7 \pm 3.55 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ at 15 ppt and $22.1 \pm 3.30 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ in 35 ppt water (Table 2.5). Fresh bayou bottom sediments treated with 15 ppt and 35 ppt produced phase 1 rates averaging $0.85 \pm 0.26 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ and $3.28 \pm 0.22 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$, which were both significantly lower than those observed in the fresh marsh soil ($P < 0.05$). For both the 15 ppt and 35 ppt treatments of fresh marsh soils, the phase 1 denitrification were 36 and 6.7 times greater than the fresh bayou sediments, respectively. Lag phase denitrification rates in fresh bayou sediments under 0 and 35 ppt treatments were significantly higher than rates produced by saline bayou incubations ($P < 0.05$).

Phase 2 denitrification activity utilizing 0 ppt and 35 ppt water treatments averaged $9.18 \pm 3.27 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ and $615 \pm 182 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ in salt marsh soils, and in saline bayou sediments rates measured 94.3 ± 9.55 and $373 \pm 35.9 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ (Table 2.6). Phase 2 rates resulting from salt marsh denitrification in 0 ppt and 35 ppt water were 10.3 times less than and 1.6 times greater than the rates in saline bayou sediments. The maximum potential denitrification rates observed in these two soils were significantly different for both salinity regimes ($P < 0.05$). Fresh marsh soils incubated in 15 ppt and 35 ppt salinity treatments resulted in rates of phase 2 denitrification averaging $654 \pm 90.9 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ and $99.7 \pm 21.1 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$, respectively (Table 2.6). The rates in fresh and saline marsh soils under 0 and 35 ppt salinity regimes were significantly different ($P < 0.05$). The potential denitrification rates observed in fresh bayou sediments were $917 \pm 341 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ and $131 \pm 86.4 \text{ mg N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ at 15 ppt and 35 ppt, respectively (Table 2.6). Average potential denitrification rates in fresh bayou sediments were 1.4 times greater than fresh marsh soils in 15 ppt treatments and 1.3 times greater in 35 ppt water. Saline and fresh bayou soils under all three treatment levels produced phase 2 rates of denitrification that differed significantly from one another ($P < 0.05$).

Salt marsh and brackish bayou soils in 35 ppt water generate the highest N_2O concentration at the end of the incubation period. The 0 and 15 ppt treated salt marsh only produced 0% and 34%, respectively, of the maximum measured N_2O concentration measured (Table 2.7). Saline bayou sediments under 0 and 15 ppt regimes produce only 11% and 77% of the N_2O produced at 35 ppt (Table 2.7). After 11 days incubation the fresh marsh soils under 15 ppt treatment demonstrate the highest production of N_2O ($\text{mg N}_2\text{O-N kg}^{-1}$). Soils at 0 ppt represent 48% of the maximum production and 35 ppt soils only 28% (Table 2.7). Fresh bayou soils at 0 ppt and 35 ppt had rates that were 59% and 40% of the rate at 15 ppt (Table 2.7).

The rates of potential denitrification can also be derived using bulk densities of all 4 marsh and bayou soils. Using bulk densities to determine rates of denitrification allows for analysis of activity occurring in soils and sediments by volume. Rates of denitrification in fresh marsh soils at 0, 15, and 35 ppt were $0.24 \text{ mg N}_2\text{O-N cm}^{-3} \text{ d}^{-1}$, $0.43 \text{ mg N}_2\text{O-N cm}^{-3} \text{ d}^{-1}$, and $0.06 \text{ mg N}_2\text{O-N cm}^{-3} \text{ d}^{-1}$. In salt marsh soil the rates at 0 ppt ($0.01 \text{ mg N}_2\text{O-N cm}^{-3} \text{ d}^{-1}$) and 15 ppt ($0.26 \text{ mg N}_2\text{O-N cm}^{-3} \text{ d}^{-1}$) were less than the fresh marsh rates, however at 35 ppt ($0.31 \text{ mg N}_2\text{O-N cm}^{-3} \text{ d}^{-1}$) the saline marsh rate was greater. Fresh bayou sediment rates at 0 ppt and 15 ppt ($0.20 \text{ mg N}_2\text{O-N cm}^{-3} \text{ d}^{-1}$ and $0.57 \text{ mg N}_2\text{O-N cm}^{-3} \text{ d}^{-1}$, respectively) were greater than the saline bayou sediments (0 ppt, $0.03 \text{ mg N}_2\text{O-N cm}^{-3} \text{ d}^{-1}$; 35 ppt, $0.04 \text{ mg N}_2\text{O-N cm}^{-3} \text{ d}^{-1}$) under similar salinity treatments. At 35 ppt the rate of potential denitrification in saline bayou sediment ($0.10 \text{ mg N}_2\text{O-N cm}^{-3} \text{ d}^{-1}$) was slightly greater than the fresh bayou ($0.08 \text{ mg N}_2\text{O-N cm}^{-3} \text{ d}^{-1}$).

Table 2.7 Percentage of $\text{N}_2\text{O-N}$ ($\text{mg N}_2\text{O-N kg}^{-1} \text{ soil}$) produced by each soil at each salinity after 11 days.

	0 ppt	15 ppt	35 ppt
Fresh Marsh	48%	100%	28%
Fresh Bayou	59%	100%	40%
Salt Marsh	0%	34%	100%
Salt Bayou	11%	77%	100%

2.3.7 Nitrogen Limitation Effects on Potential Denitrification

Soils and sediments were incubated in two salinity treatments (0 ppt and 35 ppt) for 4 days. The denitrification solution provided no additional nitrate to the soils but still provided an available carbon source to microbial denitrifiers. Fresh marsh and salt marsh soils were incubated over 4 days in 0 ppt and 35 ppt salinity regimes. All gas samples extracted for analysis over the incubation period produced below detection readings by GC. The fresh bayou (0 ppt) and saline bayou (35 ppt) sediments under incubation also resulted in below detection

readings by GC. All soils and sediments incubated in this experiment produced no measurable rate of denitrification over the 4 days.

2.3.8 Potential Denitrification Relationships with Soil Properties

Potential denitrification rates in fresh marsh, salt marsh, fresh bayou, and saline bayou soils were analyzed for correlation relationships with the measured soils properties: moisture content, bulk density, loss on ignition, total C, total N, extractable DOC, microbial biomass C, and denitrification enzyme activity (Table 2.2). Rates from the short-term experiment had a significant positive correlation to loss on ignition ($r=0.62$, $n=12$; [Table 2.2]). Dissolved organic C data displayed a significant positive correlation to short-term denitrification ($r=0.72$, $n=12$; [Table 2.2]). Total C and N values increased with increasing short-term denitrification rates ($r=0.60$, $r=0.59$, $n=12$). The rates of potential denitrification produced by the long-term experiment only showed a significant positive correlation to measured extractable dissolved organic C ($r=0.59$, $n=12$; [Table 2.2]).

2.4 Discussion

Normal salinity conditions for the fresh water site were considered to be 0 ppt salinity treatment. This low salinity regime is similar to environmental conditions observed in the upper Breton Sound Estuary where diverted Mississippi River water is the main source of water (Lane et al., 2007). The saline setting considered for the saltwater site was the 35 ppt salinity treatment. This salinity treatment simulates conditions in marshes of the lower Breton Sound due to its close proximity to the ocean allowing for tidal and wind driven flushing with seawater (Lane et al., 2007). The rate of potential denitrification in the short-term produced no significant difference in 0 and 35 ppt treatments for both fresh marsh and fresh bayou soil (Table 2.4; Fig. 2.5; Fig. 2.6). These results are similar to those found when salinity incursion and restored tidal

influences effects of denitrification were observed in outdoor mesocosm studies using fresh semi-natural grassland/marsh soils and agricultural soils (Antheunisse et al., 2007). The rates of phase 2 denitrification in saline bayou sediments treated with 35 ppt were significantly lower than rates under the 0 ppt regime (Table 2.4; Fig. 2.6). Potential denitrification was also found to be negatively affected by high salinity in mangrove soil microcosm studies by Wu et al. (2008). Potential denitrification rates of fresh marsh soils in the Everglades (518 ± 48.0 – 192 ± 33.6 mg N kg⁻¹ d⁻¹; White and Reddy, 2003) were very similar to rates observed in fresh marsh soil under 0 ppt treatment (373 ± 22.2 mg N₂O-N kg⁻¹ d⁻¹). Denitrification rates derived from Louisiana's Barataria Basin fresh and salt marsh soils (24.1 ± 2.98 mg N kg⁻¹ d⁻¹, 2.02 ± 0.31 mg N kg⁻¹ d⁻¹; Dolda et al., 2008) were considerably lower than rates seen in control Breton Sound fresh and salt marsh soils (373 ± 22.2 mg N₂O-N kg⁻¹ d⁻¹, 507 ± 27.0 mg N₂O-N kg⁻¹ d⁻¹).

Storm surge due to hurricanes and tropical storms can push large volumes of sea water farther inland (Edmiston et al., 2008). This rapid pulse of water can introduce highly saline conditions to fresh wetland systems (Rogers et al., 2009; Piazza and La Peyre, 2009). Soils collected from fresh marsh and bayou sites were incubated under two elevated saline regimes (15 and 35 ppt), in order to observe the long-term effect of salinity on potential denitrification. The long-term denitrification curves for fresh marsh soils under various salinity regimes shows that microbial activity continuously increased in the 0 and 15 ppt treatments (Fig. 2.7).

Fresh marsh soil inundated by 35 ppt water shows a distinct negative rate of activity occurring between 7-8 days. The long-term potential denitrification curves of fresh bayou soils under 3 salinity regimes are displayed in Fig. 2.8. The curve of these bayou soils treated with 15 ppt salinity shows a shift to a negative rate of denitrification at days 6-7, before the curve resumes a positive trend. The fresh bayou soils incubated in 35 ppt also displays a possible

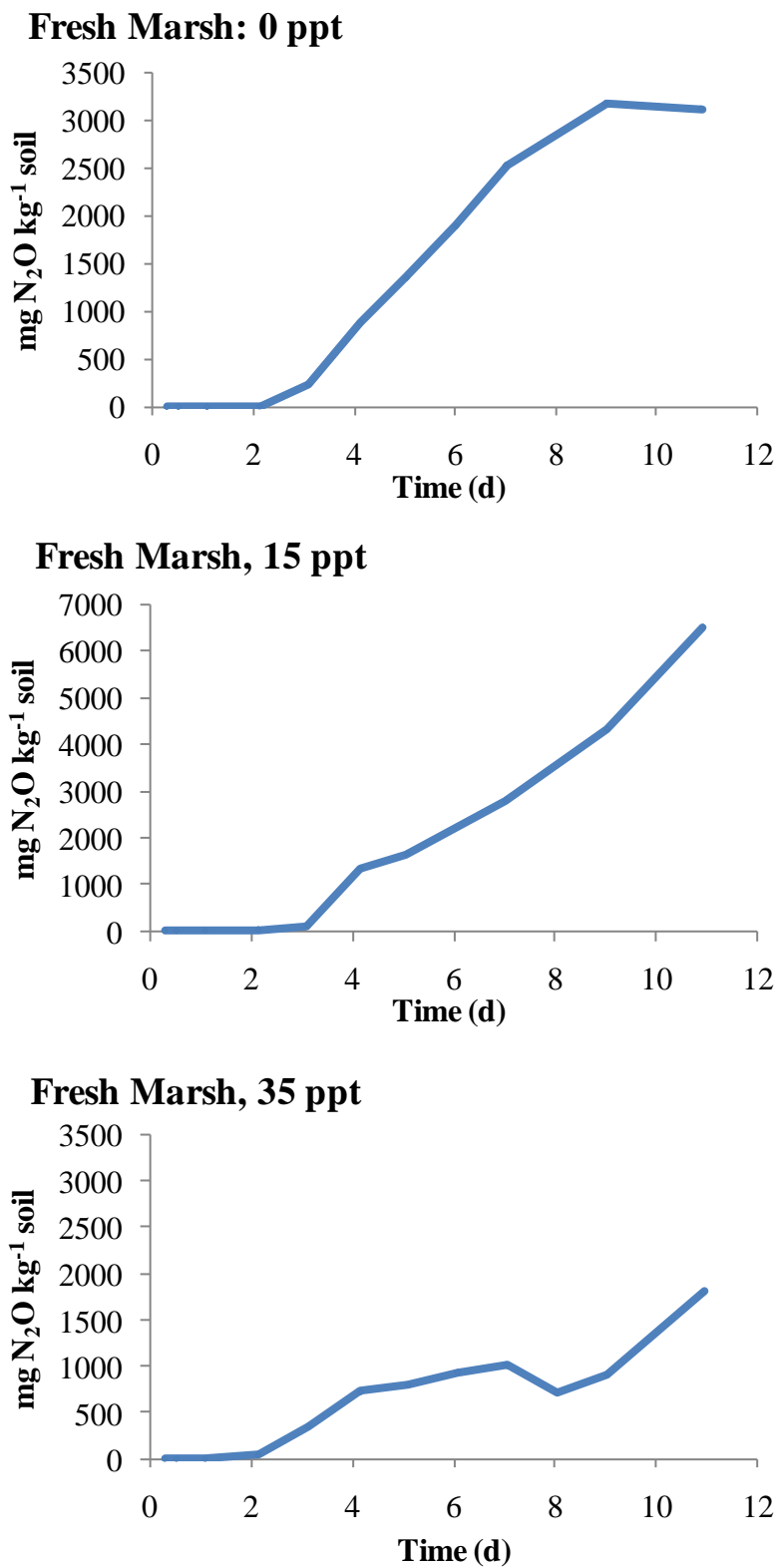


Figure 2.7 Production of mg N₂O-N kg⁻¹ soil over the 11 day period of long-term potential denitrification in fresh marsh soils in 3 salinity solutions (0, 15, 35 ppt).

salinity affect on microbial activity within the soils (Fig. 2.8). The angle of the denitrification curve plateaus from 9-11 days. The long-term treatment of salt marsh soil with 15 ppt results in a short negative salt affect on denitrification (Fig. 2.9). Saline bayou sediments under the influence of 15 ppt treatment also displayed a negative slope in the curve showing denitrification activity (Fig. 2.10). The temporary drop in activity occurs between days 6-7. Exposure of fresh wetland soils to elevated salinity depresses microbial function and changes the sediment microbial assemblage (Jackson and Vallaire, 2009). The brief reductions in long-term denitrification occurring in fresh marsh and bayou bottom soils may be caused by effects on the microbes due to experimental salinization.

When the Mississippi River carrying capacity is reaching its upper flood stages, the Caernarvon Diversion often is opened to discharge massive amounts of fresh water into the Breton Sound estuary. During these events, the diverted river water becomes the main hydrological influence acting on the estuary, even southern parts, adjacent to the coast (Hyfield et al., 2008). Salt marsh soils under treatment with fresh water (0 ppt) also showed a prolonged negative trend of denitrification from day 5-9 days (Fig. 2.9). A short-term salinity affect was also observed in the 0 ppt incubation of salt marsh soils during the short-term denitrification experiment. A negative rate of denitrification was displayed between 1.5-2.0 days (Fig. 2.5). These two experiments support the idea that salt marsh soils incubated in favorable conditions for denitrification (anaerobic environment, unlimited N and C) treated with 0 ppt water, will experience temporary negative effects in microbial activity. The prolonged exposure of wetland soils to saline influence leads to increased diversity of microbial communities within soils (Jackson and Vallaire, 2009). The lowering of salinity levels within brackish and salt wetland

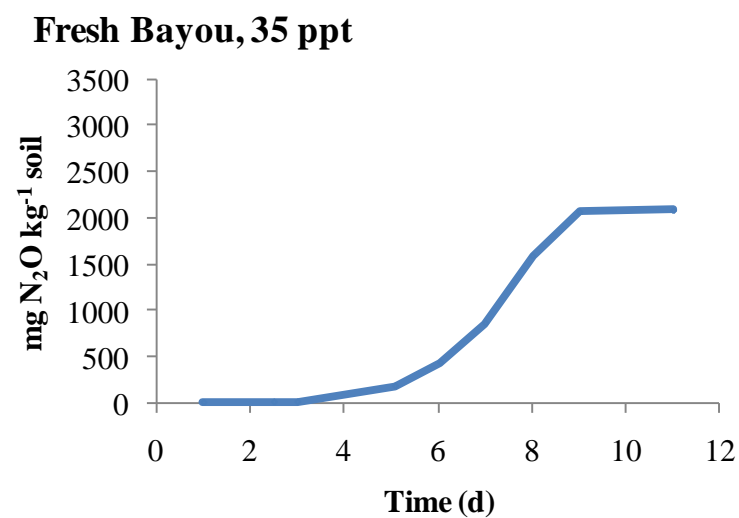
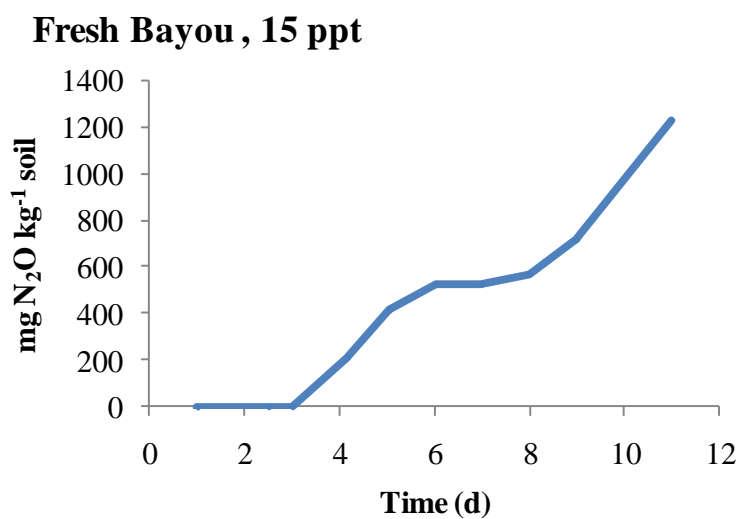
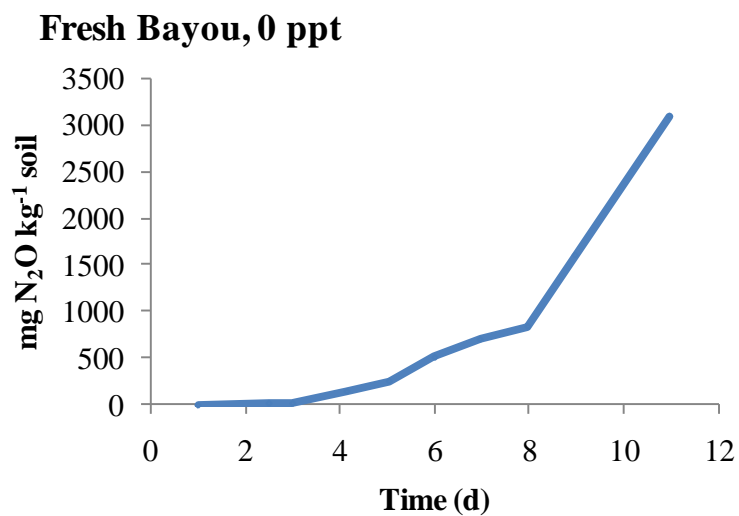


Figure 2.8 Production of mg N₂O-N kg⁻¹ soil over the 11 day period of long-term potential denitrification in fresh bayou sediments in 3 salinity solutions (0, 15, 35 ppt).

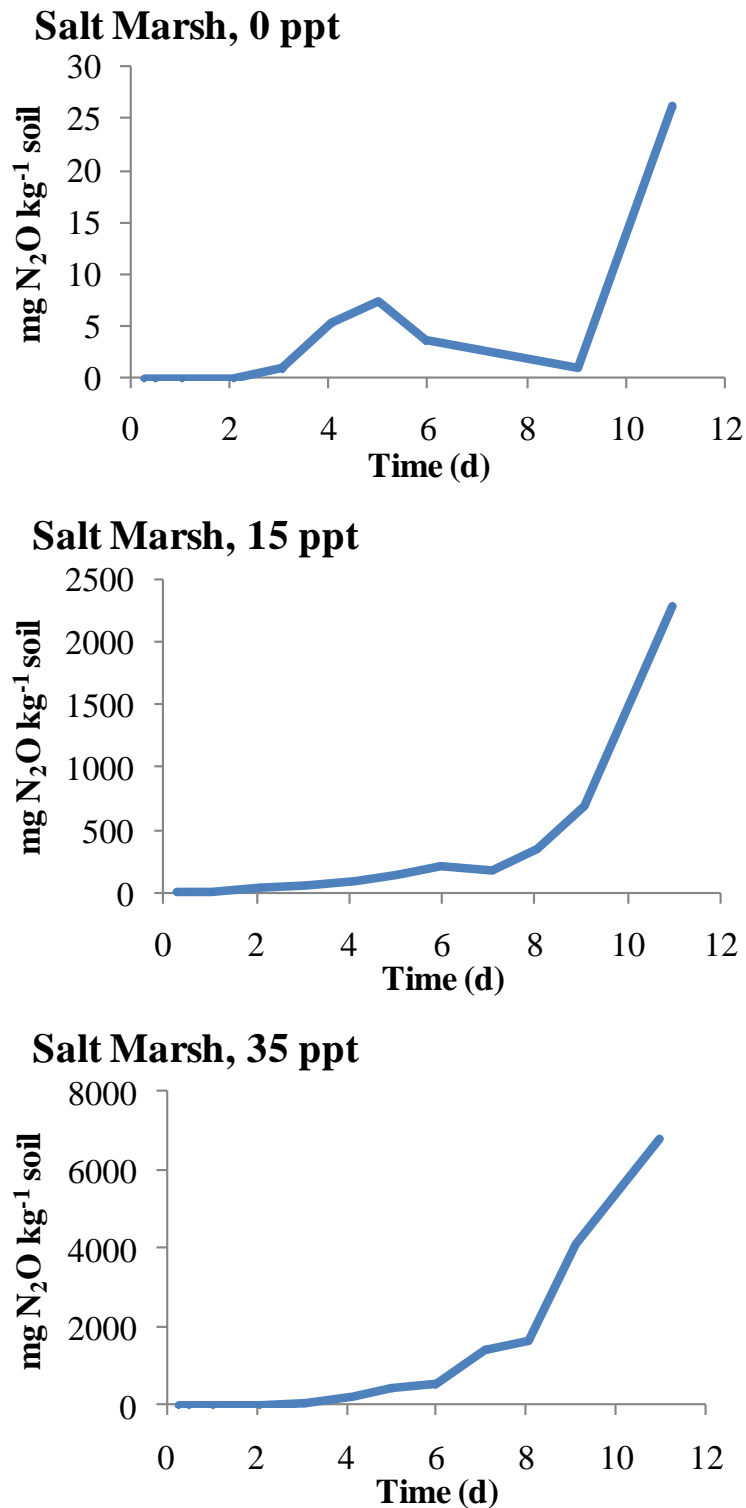


Figure 2.9 Production of mg N₂O-N kg⁻¹ soil over the 11 day period of long-term potential denitrification in salt marsh soils in 3 salinity solutions (0, 15, 35 ppt).

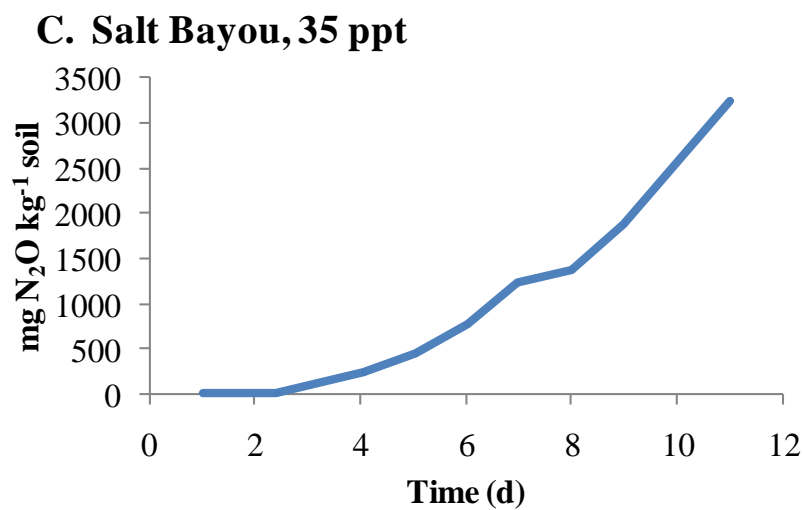
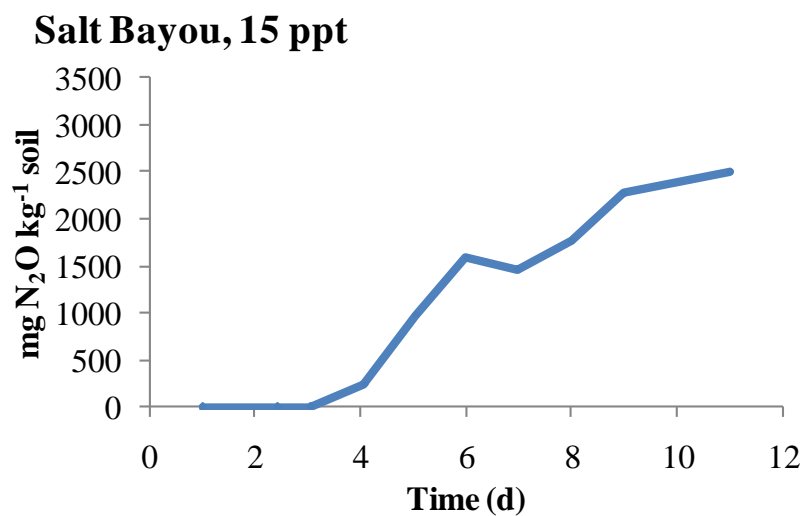
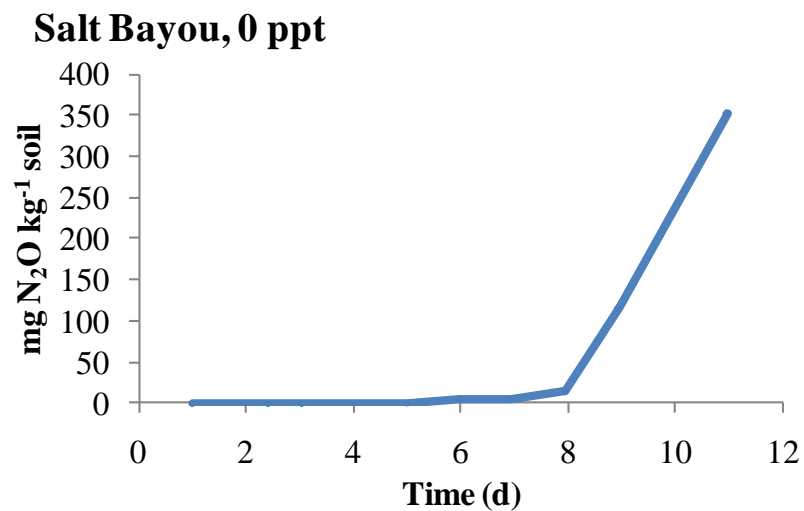


Figure 2.10 Production of mg N₂O-N kg⁻¹ soil over the 11 day period of long-term potential denitrification in saline bayou sediments in 3 salinity solutions (0, 15, 35 ‰).

soils may briefly alter microbial activity as the conditions support the existence of a less diverse make-up of microbes in the soil.

Following the completion of the long-term potential denitrification incubation on fresh and salt marsh soils the remaining concentration of available nitrate-N within the incubate solution was measured. The concentration of available nitrogen ($37.3 \pm 0.81 \text{ mg NO}_3^- \text{-N L}^{-1}$) to microbial communities was not a present limiting factor for denitrification occurring in these bottle incubations. Previous science has pointed out that under longer incubation periods the denitrifying community may shift to more halo-tolerant species and due to salinity, a difference in denitrification potential between salinity regimes may be observed (Magalhaes et al, 2005). The troughs and plateaus displayed in the graphs of potential denitrification imply that microbial activity may be responding to salinity effects and not a nitrate limitation. The limitations of nitrate availability on denitrification were further analyzed by measuring the denitrification activity of soils incubated in 0 ppt and 35 ppt solutions without supplemental nitrate. No detectable denitrification was observed in any of the soil incubations over the duration of the analysis. Previous work has shown that high nitrate concentrations in Mississippi River waters diverted into Louisiana coastal marshes will control the denitrification potential of wetlands given that the soils are inundated, anaerobic and contain high carbon (Gardner and White, 2010). These results may indicate that wetland soils and sediments within the Breton Sound estuary still retain the ability to treat nitrate in Mississippi River waters delivered to the Breton Sound estuary via the Caernarvon Diversion.

2.5 Conclusions

Potential denitrification rates occurring in marsh and bayou sediments were typically significantly different. In the short-term experiment, potential denitrification occurring in fresh

and saline marsh soils was greater than rates of adjacent bayou sediments (Table 2.4). Marsh soils in estuarine settings have been shown to have higher denitrifying potential than non-vegetated tidal flats (Wang et al., 2007).

In the long-term experiment, rates of potential denitrification occurring in the fresh marsh and bayou soils through all three treatments did not differ significantly (Table 2.6). In these soils, sufficient denitrifying activity is able to occur in order to aid in removal of nitrate from water flowing through the wetlands. Rates of denitrification in saline marsh soils and bayou sediments were significantly different under salinity treatments and the freshwater treatment, however, neither soil was consistently more productive than the other. Both soils from this saltwater area, showed high rates under particular conditions (Table 2.6). The ability of all four soils to achieve high rates of denitrification under varying salinity regimes demonstrates the potential value of coastal wetlands as a buffer and sink for nutrients from upland sources that can cause eutrophication in coastal marine systems (Reddy et al. 1993; Hatton et al. 1982; Sharp et al., 1982; Rabalais et al., 1994).

Fresh marsh and fresh bayou soils at 0 ppt and 15 ppt salinity had some of the highest observed rates in the long-term experiment. At 35 ppt, the rates in these freshwater wetland soils were lower than the other two salinities. Hurricane induced storm surge events are able to push vast volumes of seawater far into estuarine complexes, such as the Breton Sound (Piazza and La Peyre, 2009; Fritz et al., 2007; Flather, 2001). The high salinity water introduced into the upper regions of the estuary can take long periods of time to drain out of the area, leading to longer time frames for vegetation and soils to be exposed to a high salinity regime (Li et al., 2010; Rego and Li, 2009).

Salt marsh and saline bayou soils achieved the highest denitrification rates at the 35 and 15 ppt salinities (Table 2.6). The microbial assemblages in these soils are most likely acclimated to varying salinity regimes due to this areas close proximity to the ocean, and the many natural processes that constantly flush the lower estuary with seawater (Lane et al., 2007). The potential denitrification in 0 ppt water was significantly lower in these soils and sediments. Seasonal inputs of freshwater from streams and rivers can deliver high volumes of water to coastal systems (Wong, 1995; Sikora and Kjerfve, 1985). The delivery of Mississippi River water to the Breton Sound estuary by the Caernarvon Diversion is capable of altering the salinity regimes throughout the estuary. This shift of saline systems to fresher conditions negatively impacted the denitrification occurring in the soils from the salt marsh area.

**CHAPTER 3: THE EFFECTS OF SALINITY ON NITROGEN
MINERALIZATION IN WETLAND SOILS AND SEDIMENTS OF THE
BRETON SOUND ESTUARY, LA**

3.1 Introduction

Nitrogen can be a limiting nutrient regulating primary productivity in terrestrial, wetland, and aquatic ecosystems. All these highly variable systems contain a complex mixture of nitrogen compounds (Gruber, 2008). The ability of wetlands to retain floodwaters originating from surface and subsurface inflows, results in a decreased rate of decomposition of organic matter. The net accumulating of organic matter, coupled with the low decomposition rate relative to upland ecosystems make wetlands sinks for organic carbon and nitrogen (Debusk et al. 2001). Most of the nitrogen compounds stored in wetland soils are in organic forms which are generally unavailable for uptake. The process of mineralization by heterotrophic bacteria slowly converts the biologically unavailable organic N compounds to ammonium. Due to the high biological demand for the inorganic N forms, inorganic N typically make up 1% of the Nitrogen pool found in wetlands (Debusk et al. 2001). These compounds, if released in high concentrations to marine and fresh aquatic systems can trigger eutrophication.

Organic N mineralization is an important biogeochemical process occurring in wetland soils. Ammonification is the conversion of organic nitrogen within the water column and sediment to inorganic NH_4^+ available to macrophytes and other primary producers (Goldman and Dennett, 1991). Wetlands tend to accumulate organic matter over time, and therefore have an available supply of organic N for decomposers to act upon. Organic N mineralization results in the release of ammonium-N (NH_4^+). Most of this decomposition process is carried out by heterotrophic bacteria, which oxidize complex carbon compounds to CO_2 for energy. This sequential breakdown of organic N molecules into simpler compounds, and further microbial breakdown of amino acids releases ammonium (Goldman et al., 1987; Gardner et al., 1989).

Ammonification in wetlands systems is regulated by many environmental factors. The C:N ratio of soil organic matter may vary considerably in inundated soils and affect the N mineralization rate (Williams, 1972; Williams and Sparling, 1988). This ratio is critical in determining the efficiency, rate, and process by which organic matter is broken down (Waring and Bremner, 1964; Patrick and Tusneem, 1971; Williams and Cooper, 1976). Oxygen availability affects mineralization by determining the microbial assemblage present in the soil. Higher rates of organic matter reduction occur in aerobic soils largely due to the increased efficiency of aerobic respiration over anaerobic respiration pathways (Reddy and Patrick, 1984; Updegraff et al., 1995; Hansen and Blackburn, 1991). The size and activity of the microbial pool control available inorganic N and, therefore, organic N mineralization (Amador and Jones, 1993; Perucci, 1991). The activity and magnitude of the microbial community has been found to be closely linked to the availability of limiting nutrients and soil organic matter (Amador and Jones, 1993; Schnurer et al., 1985; Anderson and Domsch, 1985; Damman, 1988).

The coastal zone is a region in constant flux as freshwater from upland run off and saltwater from the sea come together leading to dynamic shifts in salinity regime. Saltwater intrusion can be accelerated due to construction of canals that increase hydraulic conduction between high and low salinity wetlands (Wang, 1988). The confinement of the Mississippi River by dams and levees has also altered the hydrology of estuary. High river discharge delivered to coastal wetlands through surface water diversion structures lower salinities within the estuary over the short term (Lane et al., 2007). Storm surge events associated with hurricanes result in the instantaneous flux of large volumes of seawater to the marshes and can lead to long term shifts in soil salinity (Blood et al., 1991). Louisiana's coastal wetlands are also experiencing a slow but gradual increase in salinity due to a rise in relative sea-level brought on by the

instability and compaction of fine grained sediments (subsidence) in concert with eustatic sea level rise (Lane et al., 1999).

The effects of salinization on N mineralization rates have been observed in a variety of soil types and experimental settings. Biological stresses and altered chemical properties within the soil have been attributed to changes in salinity. A number of studies on upland and wetland soils have found that increased salinity resulted in reduced mineralization rates. Salinity induced biological stress to microbial assemblages resulted in smaller and less efficient microbial communities (Rietz and Haynes, 2003; Jackson and Vallaire, 2009). A diminished microbial biomass C: N ratio in soils incubated with higher salinities (0-12 ppt) has also been observed (Yuan et al., 2007). When floodplain soils were treated with increasing salinity, microbial biomass measurements had decreased and the community structure of bacteria had shifted to prokaryotes (Sardinha et al., 2003). Laura (1977) and Irshad et al. (2005) hypothesized that the observed reduction in N mineralization for agricultural soils was due to salinity inhibition of nitrification occurring in soils. Differences between strength of salt treatment and mineralized N over time showed a reduction at high salinities (5.70-8.80 ppt). However, at the end of the 8 to 14 week incubation period, the concentrations of ammonium mineralized at the highest salinity were not significantly different from the lower salinity treatments, suggesting the effect of salinity on N mineralization was short-lived. Short term salinity effects were also observed in studies involving wetland (Wu et al., 2008), agricultural soils (Pathak and Rao, 1998; Lodhi et al., 2009), and lake-bottom sediments (Khoi et al., 2006). An immediate suppression of mineralization rates was observed followed by a gradual recovery to rates similar to control soils. In two experiments, whole soil cores collected from fresh and salt tidal marshes were submerged in various salinity treatments (0-10, 1-33 ppt) and monitored over a month (Weston et al., 2006)

and up to several years (Portnoy and Giblin, 1997). There was an overall increase in ammonification due to salt water inundation observed (Portnoy and Giblin, 1997; Weston et al., 2006). Further analysis of soils from the Weston et al. (2006) study showed no differences in the microbial communities resulting from the control and seawater amended treatment (Edmonds et al., 2009). The variability in salinity effects on N mineralization rates suppression in some cases and increases in others may be an indicator that influence of salinity on net N mineralization may be dependent on the biochemical attributes of the soil.

The goal of this study was to determine the net rates of N mineralization in response to salinity regime shifts in coastal wetland soils and sediments. Salinity treatments mimicking the introduction of seawater (35 ppt) and Mississippi River water (0 ppt) were added to 4 distinct soil/sediment types from the Breton Sound estuary: salt marsh, fresh marsh soils, fresh bayou, and saline bayou bottom sediments. Storm surge, associated with hurricanes and tropical storms pushes seawater into the upper freshwater regions of the Breton Sound estuary, altering the salinity regimes in wetland soils to higher in-situ salinity levels over extended periods of time (Edmiston et al., 2008; Li et al., 2010; Li and Rego, 2009). The Caernarvon surface freshwater diversion was designed to manage and control the longer term increases in salinity concentrations throughout the Breton Sound estuary (ACOE, 1995). When Mississippi River waters are diverted at high rates (up to $8000 \text{ ft}^3 \text{ s}^{-1}$) for extended periods of time, the brackish and salt marshes comprising the mid and outer regions of the estuary will experience shifts toward a fresh water salinity regime.

3.2 Materials and Methods

3.2.1 Study Area

The Breton Sound Estuary was formed thousands of years ago by the Mississippi River, as part of the Plaquemines-St. Bernard delta complex (Coleman, 1988; Scruton, 1960; Roberts, 1997). Within the Breton of Sound Estuary, between the Gulf of Mexico and Caernarvon Diversion, there are approximately 1100 km² of fresh and saline wetlands (Lane, et al. 2006). The Caernarvon Freshwater Diversion Structure was designed to reduce the effects of salt water intrusion, and establish favorable salinity conditions in the area (ACOE, 1995). In addition to combating salt water intrusion, the diversion would help reduce the loss of land, enhance vegetative growth, and stimulate production of commercially and recreationally important fish and wildlife (ACOE, 1995). Salinities in the upper estuary under normal flow are fresh and gradually increase to marine levels (35 ppt) at the outer edge of the Breton Sound (Lane et al., 2007).

The Breton sound is micro tidal and is made up of fresh, intermediate, brackish, and salt marsh dissected by numerous lakes, bayous, and canals. Bayou Terre aux Bouef, a relic Mississippi River distributary, separates the upper estuary into geographically and hydrologically isolated, eastward, and westward components. Dominant emergent vegetation of the brackish and salt marsh areas consists of *Spartina patens* (saltmeadow cordgrass), *Spartina alterniflora* (smooth cordgrass), and *Distichlis spicata* (saltgrass). *Spartina cynosuroides* (hog cane) were common on natural banks, elevated marsh sites, and along some spoil banks created by dredged material. *Juncus roemerianus* (black needlerush) is common in the interior marsh of both the saline and brackish zones. *Scirpus maritimus* (saltmarsh bulrush) is also found throughout most of the brackish and salt marsh but is not a dominant plant species. In fresh marsh systems of the

upper Breton Sound dominant plant species are *Spartina patens* (saltmeadow cordgrass) and *Schoenoplectus americanus* (chairmaker's bulrush) (Piazza and La Peyre, 2009; Visser et al., 1998).

The Caernarvon diversion is located near Caernarvon, LA in St. Bernard Parish. It redirects surface water into the Breton Sound Estuary from the 81.5 river mile on the East bank of the Mississippi River (Moerschbaeche, 2008). Construction of the diversion was carried out by the U.S. Corps of Engineers and is operated by the Louisiana Department of Natural Resources. The diversion structure has been in operation since fall of 1991, and floodwater is controlled by culvert gates which utilize gravity flow to allow for the passage of river water at a maximum rate of $8000 \text{ ft}^3 \text{ sec}^{-1}$ (Lane et al. 2006). Year to year, the management of the flow of river water is dependent upon river flood stage as well as salinities within the basin. For example, in 2008, the diversion was operating at new capacity from late March to mid May; while in 2009, discharges did not exceed $1000 \text{ ft}^3 \text{ s}^{-1}$ over the same period (Fig. 3.1).

3.2.2 Sample Collection and Preparation

Twenty-eight soil cores were taken at two sites within the Breton Sound estuary in April 2009 (Fig. 3.2). The salt marsh site was dominated by *Spartina alterniflora*. It was located 29.4 miles southeast of the Caernarvon Diversion (marsh site: $29^{\circ}39'52.4''$ N, $89^{\circ}36'35.6''$ W, bayou site: $29^{\circ}39'52.0''$ N, $89^{\circ}36'34.9''$ W) within Plaquemines parish (Fig. 3.3). Seven replicate soil cores were collected from a 3 m^2 area of the mid-marsh and an additional seven cores were taken from a 3 m^2 area of the bayou bottom adjacent to the marsh area. The freshwater marsh site was located at the northeast edge of Lake Lery in Plaquemines Parish and was located 4.65 mi southeast of the diversion structure (Fig. 3.4). Seven replicate soil cores were taken from a 3 m^2

area of the marsh (29°48'21.8" N, 89°52'28.4" W) and another seven from the adjacent bayou bottom (29°48'05.0" N, 89°52'23.7" W).

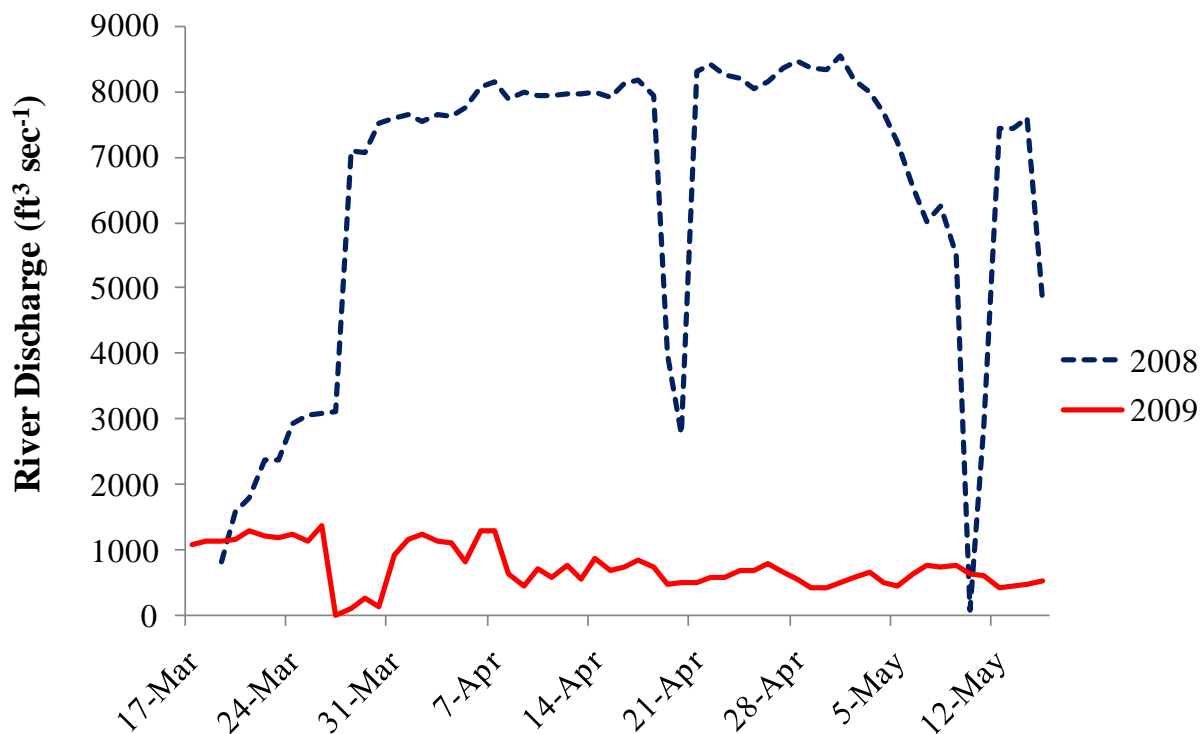


Figure 3.1 Caernarvon Diversion mean daily discharge rates from March 17- May 15, 2008 and 2009 (USGS, 2009).

Each soil core was collected by push core technique using a 7 cm diameter, clear plexiglas tube. The top 10 cm of each core were extruded in the field, placed in labeled zip-lock bags, and stored on ice. Upon return to the lab, the samples were homogenized by hand and any large root fragments were removed. Subsamples (mass=60 g) of each core sample were combined and homogenized in an electrical blender to create a slurry from the four sampling groups: salt marsh soil, fresh marsh soil, salt bayou sediment, and fresh bayou sediment. The samples were then stored in polyethylene containers, stored at 4°C until incubation.

3.2.3 Soil Characterization

Soil characterization was conducted on the combined homogenized soils as well as individual soils from each whole core collected in the field. Moisture content, porewater salinity, bulk density, total carbon (TC), total nitrogen (TN), extractable DOC, microbial biomass C (MBC), and potentially mineralizable N (PMN) of these soils were determined.

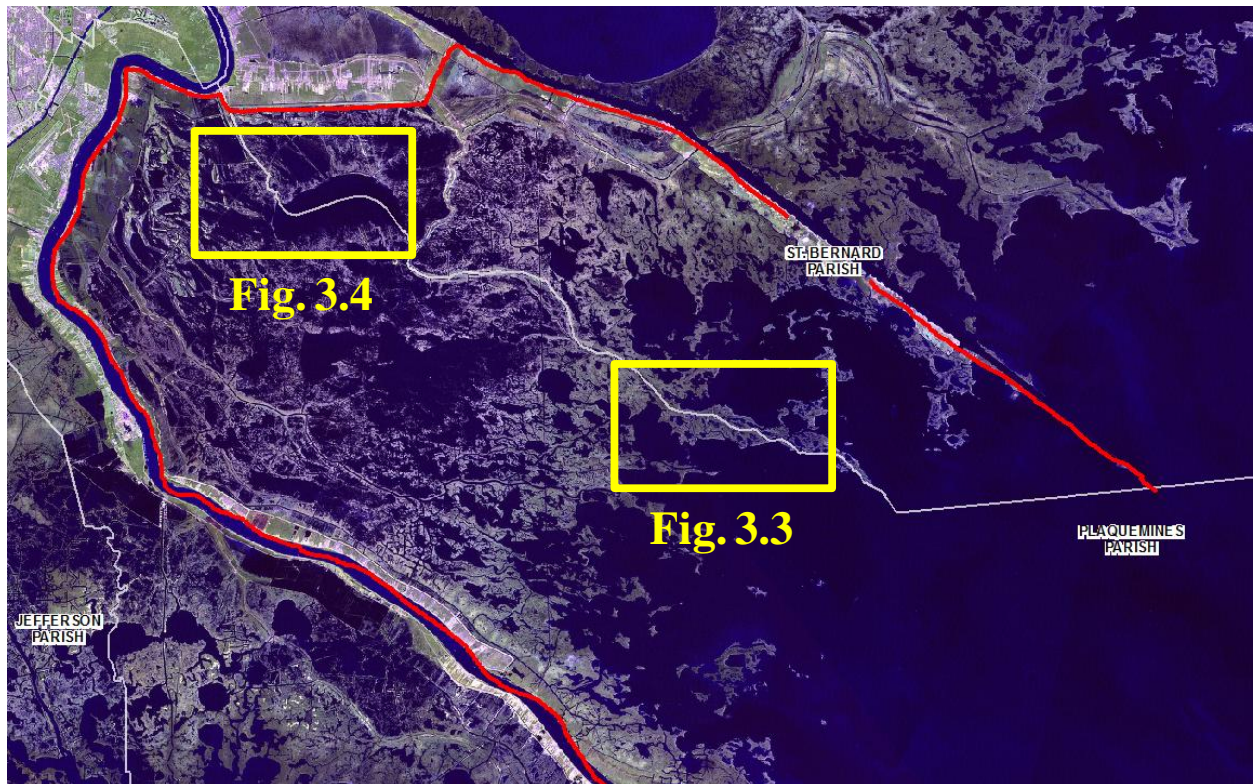


Figure 3.2 The Breton Sound estuary in southeast Louisiana (ArcGIS 9.1, 2007).

Moisture content of homogenized soils and sediments was determined by drying wet weight subsamples at 70°C for 3 days, until constant dry weight. Bulk density (Blake and Hartge, 1986) was measured in whole soil cores (seven replicates for each soil type). Percent moisture of each whole soil subsample was calculated and used to determine the dry weight g of

each soil subsample. Bulk density was calculated by dividing the dry soil weight by the volume of the soil core used to collect the soil. Bulk density was expressed as dry weight g cm^{-3} .

Porewater salinity was measured from centrifuging 30 g of homogenized soil sample to separate the liquid from the soil. Salinity was measured on a YSI water sensor (YSI Environmental, Yellow Springs, OH). Dried, ground subsamples were analyzed for TC and TN using an Elemental Combustion System with a detection limit of 0.005 g kg^{-1} (Costech Analytical Technologies, Inc., Valencia, CA).

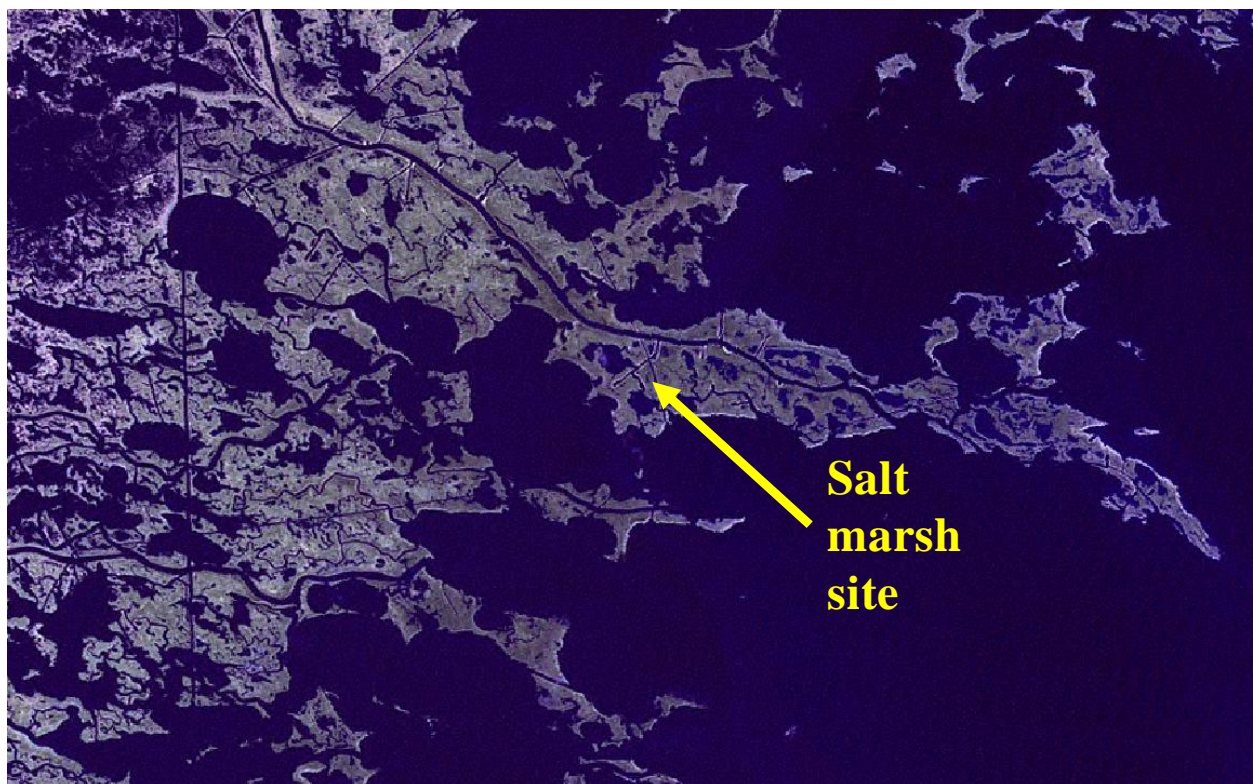


Figure 3.3 Salt marsh sampling site in the lower Breton Sound estuary (ArcGIS 9.1, 2007).

Loss on ignition analysis was performed on homogenized soil and sediment samples to estimate organic matter content (Dean, 1974). Soil samples that were dried in order to measure moisture content were pulverized to powder using mortar and pestle. Dried subsamples ($\sim 0.3 \text{ g}$)

were put in 40 ml beakers and burned at 550°C for 5 hrs in a muffle furnace. The post-burn sample weights were compared with the pre-burn sample weights in order to determine loss on ignition.

The fumigate-extraction method (Vance et al., 1987) was utilized to determine microbial biomass C and extractable DOC. Fumigate and non-fumigate triplicate 5 g subsamples were placed in 25 ml centrifuge tubes. Fumigate samples had a one-half ml of pure chloroform added to each tube. All fumigate samples were placed in a vacuum desiccator, along with a beaker containing approximately 50 ml of chloroform and 5-10 boiling stones. The desiccator was then evacuated using a vacuum pump and re-filled with room air three consecutive times.



Figure 3.4 Fresh marsh sampling site in the upper Breton Sound estuary (ArcGIS 9.1, 2007).

Each time the air was vacuumed out of the desiccator until the chloroform in the beaker and samples would vigorously bubble. The desiccator was then sealed under high negative pressure and placed within the fume hood for 24 hours. The following day, the desiccator was opened and the glass beaker containing chloroform was removed. The desiccator was resealed and evacuated by vacuuming out the headspace to -87 kPa and re-filling the container with room air 7 times. Both fumigate and non-fumigate samples then underwent the same extraction method. Twenty-five ml of 0.5 M K_2SO_4 were added to all samples, agitated on longitudinal shaker for 30 min, then centrifuged for 10 min at 6000 rpm. Samples were vacuum filtered using 0.45 μm filter paper to isolate the supernatant, which was stored at 4°C until analyzed for dissolved organic carbon (TOC) (Shimadzu Scientific Instrument TOC- VCSN, Columbia, MD). The non-fumigate and fumigate masses ($mg\ kg^{-1}$) were determined by dividing the vial's total mass by dry soil weight (g). In order to calculate the microbial biomass C (MBC), the non-fumigate TOC measurement was subtracted from the corresponding fumigate TOC reading then divided by 1000. The extractable DOC data was obtained directly from the non-fumigate TOC ($mg\ kg^{-1}$).

3.2.4 Potentially Mineralizable Nitrogen (PMN)

The PMN assay allows for the investigation of the net N mineralization rates in soils and detritus by anaerobic bottle incubation (Waring and Bremner, 1964). The method determines mineralization rates by measuring the release of ammonium (NH_4^+) over time. The incubation is conducted at an elevated temperature (40°C) to increase the N release rate and decrease the required length of incubation. The anaerobic conditions maintained within the incubation bottle are relevant to flooded or saturated soil conditions which dominate wetland soils.

Four replicate subsamples from each of the four homogenized soil types (salt marsh soil, saline bayou bottom sediment, fresh marsh soil, and fresh bayou bottom sediment) were prepared

by placing 2.5 g wet weight subsamples into 70 ml acid-washed glass serum bottles. Each bottle was sealed using a rubber septa and aluminum crimp cap, and the headspace evacuated. Five ml of filtered 35 ppt salinity seawater collected offshore in the Northern Gulf of Mexico, 9 ppt water (diluted seawater), or 0 ppt de-ionized water were added to each set of bottles after 15 min of purging with 99.99 % pure N₂ gas. The addition of 5 ml salinity treatment water with the wet weight subsample (1.33-1.93 ml moisture) soil diluted the actual salinity occurring within the bottle incubation. The bottles were then purged for another 5 min using 99.99% pure N₂ gas to create an anaerobic headspace. The serum bottles were placed in the dark within an incubator set at 40°C for 2, 5, and 10 days. Upon completion of the incubation period, bottles were removed and extracted with 25 ml of 2 M KCl. Extractable controls (0 days) included 2.5 g soil samples extracted with 25 ml of 2 M KCl. Bottles were shaken for 2 hrs on a longitudinal shaker, caps removed and samples transferred to centrifuge tubes for centrifugation for 10 min at 6000 rpm. The supernatant was collected by vacuum filtration through a 0.45 µm membrane filter, placed in 25 ml scintillation vials, acidified to pH<2, and refrigerated at 4°C for subsequent analysis by AQ2 Automated Discrete Analyzer for ammonia (SEAL Analytical Inc., Mequon, Wisconsin) (EPA Method 351.2, 1983). Rates of PMN (mg-N kg⁻¹ d⁻¹) are defined as the slope of the line when NH₄-N concentrations were plotted against incubation time (0, 2, 5, and 10 days).

3.2.5 Data Analysis

The relationships between salinity treatment, soil/sediment type, and PMN were investigated using a three-way ANOVA model (P<0.05). Post-hoc ANOVA testing was conducted by Tukey's Studentized (HSD) test. Levene's Test and Bartlett's Test for Equality (both P<0.05) were utilized to verify the homogeneity of variance and normality of distribution for experimental results. Correlations and linear regressions were used to identify the

relationships between soil properties (moisture content, bulk density, LOI, TC, TN, extractable DOC, MBC, and PMN assay). SAS 9.1 was used to conduct all statistical analysis (SAS Institute Inc., Cary, NC).

3.3 Results

3.3.1 Soil Properties

The mean moisture ($n=3$) taken from the salt marsh, salt bayou, fresh marsh, and fresh bayou were $52.8 \pm 8.55\%$, $56.3 \pm 5.69\%$, $77.0 \pm 2.03\%$, and $60.4 \pm 3.64\%$, respectively (Table 2.1). The moisture content of the fresh marsh soils was significantly higher than the other soils ($P<0.05$). Bulk density measurements were significantly higher ($P<0.05$) in the salt marsh and salt bayou soils ($0.65 \pm 0.12 \text{ g cm}^{-3}$ and $0.62 \pm 0.09 \text{ g cm}^{-3}$) when compared with the fresh marsh and bayou ($0.51 \pm 0.07 \text{ g cm}^{-3}$ and $0.27 \pm 0.03 \text{ g cm}^{-3}$).

Porewater salinity measurements in the salt marsh, saline bayou, fresh marsh, and fresh bayou at the time of sampling were 9.28 ppt, 9.34 ppt, 0.26 ppt, and 0.23 ppt, respectively (Table 3.1). Loss on ignition measured 9.90% and 7.30% organic matter composition in saline marsh and bayou. Fresh marsh and bayou soils were 28.1% and 9.48% organic matter (Table 3.1). Loss on ignition for Fresh marsh soils had the highest total C and total N (121 g C kg^{-1} and 9.79 g N kg^{-1}) and salt bayou sediment had the lowest overall (29.6 g C kg^{-1} and 1.90 g N kg^{-1}). Total C and N measured 41.0 C kg^{-1} and 3.10 g N kg^{-1} fresh bayou sediment and 38.4 C kg^{-1} and 2.63 g N kg^{-1} in saline marsh soil (Table 3.1).

Microbial biomass ranged from $10.2 \pm 0.46 \text{ g kg}^{-1} \text{ C}$ in fresh marsh to $4.71 \pm 0.09 \text{ g kg}^{-1} \text{ C}$ in the salt bayou sediments (Table 3.1). In salt marsh and fresh bayou soils microbial biomass

C averaged 5.25 ± 0.25 g kg⁻¹ C and 5.16 ± 0.20 g kg⁻¹ C. The microbial biomass C in fresh marsh soils was significantly higher than the other soils (P<0.05).

Table 3.1 Soil characterization and analysis performed on homogenized marsh and bayou soils and whole core soils. Data are reported as mean \pm 1 std dev (n=3).

Soil Parameter	Units	Salt Marsh	Salt Bayou	Fresh Marsh	Fresh Bayou
Moisture	%	52.8 ± 8.55^a	56.3 ± 5.69^a	77.0 ± 2.03^b	60.4 ± 3.64^a
Salinity	ppt	9.28	9.34	0.26	0.23
Bulk Density	g cm ⁻³	0.65 ± 0.12^c	0.62 ± 0.09^c	0.51 ± 0.07^b	0.27 ± 0.03^a
Loss on Ignition	%	9.90	7.30	28.1	9.48
Total Carbon	g C kg ⁻¹	38.4	29.6	121	42.0
Total Nitrogen	g N kg ⁻¹	2.63	1.90	9.79	3.10
Microbial Carbon	mg kg ⁻¹ C	5.25 ± 0.25^a	4.71 ± 0.09^a	10.2 ± 0.46^b	5.16 ± 0.20^a
Extractable DOC	mg kg ⁻¹	270 ± 1.40^c	132 ± 8.67^b	323 ± 5.11^d	104 ± 2.12^a
Extractable NH ₄	mg N kg ⁻¹	12.1 ± 0.93^a	19.2 ± 0.18^a	39.8 ± 0.28^b	56.8 ± 0.38^c
Potentially Mineralizable N	mg N kg ⁻¹ d ⁻¹	8.70 ± 1.68^b	3.79 ± 0.30^a	28.6 ± 3.71^c	4.52 ± 0.09^a

DOC, Dissolved Organic Carbon

a,b,c,d letters indicate significant differences between soil/sediment types (across)

Mean extractable DOC was determined to be highest in fresh marsh (323 ± 5.11 mg kg⁻¹) and lowest in fresh bayou samples (104 ± 2.12 mg kg⁻¹). In marsh and bayou soils from the saline site the extractable DOC measured 270 ± 1.40 mg kg⁻¹ and 132 ± 8.67 mg kg⁻¹, respectively (Table 3.1). All mean extractable DOC values determined from the four soil types were significantly different from one another (P<0.05).

Table 3.2 Product-moment correlation coefficients for soil properties. For n=12, $r > 0.58$ is significant at $P < 0.05$.

	Moisture	Bulk Density	Loss on Ignition	Total C	Total N	MBC	Extractable DOC
Bulk Density	-0.96						
Loss on Ignition	0.80	-0.82					
Total C	0.82	-0.83	0.99				
Total N	0.82	-0.84	0.99	1.00			
MBC	0.80	-0.81	0.99	0.99	0.99		
Extractable DOC	0.45	-0.44	0.76	0.73	0.72	0.75	
PMN	0.80	-0.82	0.99	0.99	0.99	0.99	0.74

MBC, Microbial biomass C; DOC, Dissolved Organic C; PMN, Potentially mineralizable N.

3.3.2 Soil Properties Relationships

As expected, bulk density decreased ($r = -0.96$, $n=12$) with increasing moisture content of soils (Table 3.2). Loss on ignition (LOI) increased as moisture content increased within soils increased ($r = 0.80$, $n=12$) and decreased as soil bulk density increased ($r = -0.82$). The percent lost on ignition was greater in marsh soils than bayou sediments from both field sites most likely due to high vegetative content of surface marsh soils. Both total C and total N were significantly correlated with one another ($r = 1.00$). Total C and N also increased significantly with loss on ignition ($r = 0.99$, $r = 0.99$, $n=12$) which demonstrates the loss on ignition is an effective predictor of the total C and N content for these soils (Table 3.2). Microbial biomass C increased significantly with moisture content ($r = 0.80$, $n=12$). Microbial biomass C was significantly correlated with loss on ignition, total C and total N ($r = 0.99$, $r = 0.99$, and $r = 0.99$, respectively, [Table 3.2]). Extractable dissolved organic C was correlated with loss on ignition and microbial biomass C ($r = 0.76$ and $r = 0.75$, $n=12$).

3.3.3 Potentially Mineralizable Nitrogen (PMN)

The porewater salinity measurements taken from salt marsh and saline bayou soils showed a salinity regime of 9.28 and 9.34 ppt (parts per thousand), respectively, at the time of the sampling (Table 3.1). The salt marsh soil incubated in 9 ppt salt water (control treatment) had the mean PMN rate of $8.70 \pm 1.68 \text{ mg-N kg}^{-1} \text{ d}^{-1}$, and the salt bayou sediment PMN under the same treatment was significantly ($P < 0.05$) lower at $3.79 \pm 0.30 \text{ mg-N kg}^{-1} \text{ d}^{-1}$ (Table 3.3). Fresh marsh and bayou bottom soil collected were characterized by very low porewater salinity measurements (Marsh, 0.26 ppt; Bayou, 0.23 ppt; Table 3.1). The mean rate of PMN measured in the fresh marsh soil under 0 ppt (control) treatment ($28.6 \pm 3.71 \text{ mg-N kg}^{-1} \text{ d}^{-1}$) was significantly higher ($P < 0.05$) than the rate measured in the bayou sediments, $4.52 \pm 0.09 \text{ mg-N kg}^{-1} \text{ d}^{-1}$ (Table 3.3). Potentially mineralizable N was well correlated with moisture content, loss on ignition, total C, total N, microbial biomass, and extractable DOC ($r = 0.80$, $r = 0.99$, $r = 0.99$, $r = 0.99$, $r = 0.99$, and $r = 0.73$, respectively), for $n = 12$ (Table 3.2).

Table 3.3 Mean rates of potentially mineralizable N for each soil type and salinity treatment.

Salinity treatment	Salt Marsh	Salt Bayou	Fresh Marsh	Fresh Bayou
	$\text{mg-N kg}^{-1} \text{ d}^{-1}$	$\text{mg-N kg}^{-1} \text{ d}^{-1}$	$\text{mg-N kg}^{-1} \text{ d}^{-1}$	$\text{mg-N kg}^{-1} \text{ d}^{-1}$
0 ppt	12.3 ± 0.44^b	4.58 ± 0.24^a	28.6 ± 3.71^c	4.52 ± 0.09^a
9 ppt	$8.70 \pm 1.68^{b,c}$	3.79 ± 0.30^a	30.1 ± 1.07^d	$6.01 \pm 0.23^{a,b}$
35 ppt	8.70 ± 0.32^b	3.69 ± 0.12^a	38.2 ± 4.31^c	5.75 ± 0.3^a

a,b,c,d different letters indicate significant differences between soil/sediment types (across).

3.3.4 Effects of Salinity on PMN

The PMN assay results from composite fresh marsh soil incubations at 0, 9, and 35 ppt treatments were significantly higher than all rates produced by salt marsh soils and adjacent fresh

bayou sediments ($P < 0.05$). The rates of N mineralization in fresh marsh soil at 0, 9, and 35 ppt were 28.6 ± 3.71 , 30.1 ± 1.07 , 38.2 ± 4.31 mg-N kg⁻¹ d⁻¹, respectively (Table 3.3). The mean PMN rates measured in salt marsh soils were 12.3 ± 0.4 mg-N kg⁻¹ d⁻¹ in 0 ppt water, 8.70 ± 1.68 mg-N kg⁻¹ d⁻¹ in 9 ppt water, and 8.70 ± 0.32 mg-N kg⁻¹ d⁻¹ in 35 ppt water (Table 3.3). Fresh bayou bottom sediments with 0 ppt, 9 ppt, and 35 ppt water produced mean PMN rates of 4.52 ± 0.09 mg-N kg⁻¹ d⁻¹, 6.01 ± 0.23 mg-N kg⁻¹ d⁻¹, and 5.75 ± 0.3 mg-N kg⁻¹ d⁻¹ (Table 3.3). Potentially mineralizable N in salt bayou sediment with in 0 ppt, 9 ppt, and 35 ppt water, was significantly ($P < 0.05$) lower than the activity observed in salt marsh soils, and averaged 4.58 ± 0.24 mg-N kg⁻¹ d⁻¹, 3.79 ± 0.30 mg-N kg⁻¹ d⁻¹, and 3.69 ± 0.12 mg-N kg⁻¹ d⁻¹ (Table 3.3). There was no significant differences ($P < 0.05$) between any of the PMN rates produced in the fresh and saline bayou sediments under any of the salinity treatments which suggests the bayou sediments contain a microbial consortium adapted for salinity fluctuations (Table 3.3).

The PMN rates for in saline marsh and bayou soils at 9 ppt represent the control treatments. Ammonification measured in salt marsh and saline bayou soil incubated in 0 ppt water was higher (salt marsh, 141%; salt bayou, 121%) than the control treatment, however the difference was not significant (Table 3.4; $P < 0.05$). The rate of N mineralization in salt bayou soil inundated in 35 ppt water was 100% of the 9 ppt rate. Saline bayou sediments under the 35 ppt treatment displayed 97% PMN activity of the control, but this difference was not significantly less than the rates under 9 ppt or 0 ppt (Table 3.4; $P < 0.05$). Fresh marsh and adjacent bayou soils were exposed to 0 ppt water for the control treatment. The 9 ppt and 35 ppt salt water incubations produced higher rates of N mineralization. Potentially mineralizable N in 9 ppt water produced 105% of the control in fresh marsh soil and 133% in the fresh bayou sediments (Table 3.4) but these differences were insignificant ($P < 0.05$). The PMN rate for fresh

marsh soils at 35 ppt was 134% greater than the control rate. A similar trend was seen for the fresh bayou sediment which had a PMN rate of 127% of the 0 ppt at 35 ppt (Table 3.4). Though the PMN in fresh water soils increased under elevated salinities (9 ppt and 35 ppt), the change in rates was not significantly higher than that of the 0 ppt treatment (Table 3.3).

Table 3.4 PMN ($\text{mg-N kg}^{-1} \text{ d}^{-1}$) in soils incubated for 10 days as a percentage of N produced at native salinities (fresh, 0 ppt and salt, 9 ppt).

	0 ppt	9 ppt	35 ppt
Salt Marsh	141%	100%	100%
Salt Bayou	121%	100%	97%
Fresh Marsh	100%	105%	134%
Fresh Bayou	100%	133%	127%

3.4 Discussion

Salinity regimes within the lower Breton Sound estuary are highly influenced by seawater flushing due to winds and tides (Lane et al., 2007). Salt marsh soils ammonification rates reflected no significant salinity effect between the 35 ppt and 0 ppt treatments (Table 3.3). Net N mineralization rates remain positive over the incubation period for all three treatments (Fig. 3.5). In saline marsh and bayou soils, the mean PMN rates under 0 ppt salinity were higher than 9 ppt and 35 ppt treatments, though not significantly higher (Table 3.3).

Previous studies have indicated that reduced mineralization, may be due to high salinity, induced biological stress to microbial assemblages in soils and sediments (Rietz and Haynes, 2003). These biological stresses lead to a diminished microbial biomass C: N ratio (Yuan et al., 2007) and less diverse community structure of bacteria responsible for N mineralization (Sardinha et al., 2003). Net N mineralization activity in both salt marsh and bayou soils shows a plateau effect from days 2-5 for the 35 ppt treatment (Fig. 3.5). After this temporary reduction of N mineralization, the rates of activity once again rebound to match the mean rate of PMN for the

control (9 ppt; Table 3.3). Wu et al. (2008) also found short-term salinity effects in wetland soils that caused a suppression of mineralization, followed by a gradual recovery to produce rates similar to the control treatment.

In the Breton Sound estuary, high freshwater discharge events, via the Caernarvon Diversion, can drastically reduce the existing salinity in the lower estuary during the spring (Lane et al, 2007). The 0 ppt treatment in these lower estuary soils simulated these high diversion flow conditions. Both soils from the lower estuary produced no significant difference in observed net N mineralization due to salinity (Table 3.3) which may have been due to the salt tolerant heterotrophic microorganisms responsible for ammonification (Sandhu et al., 1975). The lack of differences in saline soils due to freshwater inundation suggests that the diversion's operation does not appear to stimulate the release of inorganic N from the salt marsh soils, and hence does not contribute a source of inorganic N to coastal eutrophication.

Nitrogen mineralization rates occurring in Everglades fresh marsh soils averaged 35.8 mg-N kg⁻¹ d⁻¹ (White and Reddy, 2000). In another Everglades study, fresh marsh soil taken from high and low phosphorus sites produced PMN rates ranging from 46–26 mg-N kg⁻¹ d⁻¹ (White and Reddy, 1997). The PMN rates in fresh marsh soils in the 3 salinity treatments ranged from 28.6 to 38.2 mg-N kg⁻¹ d⁻¹ in this study. These fresh marsh soil rates were comparable with rates occurring in some other fresh marsh systems from previous studies. N mineralization rates measured in Mekong River delta bottom sediments ranged from 7.3–1.3 mg NH₄-N kg⁻¹ d⁻¹, similar to control salt bayou PMN rates in this study, which averaged 3.79 ± 0.30 mg-N kg⁻¹ d⁻¹.

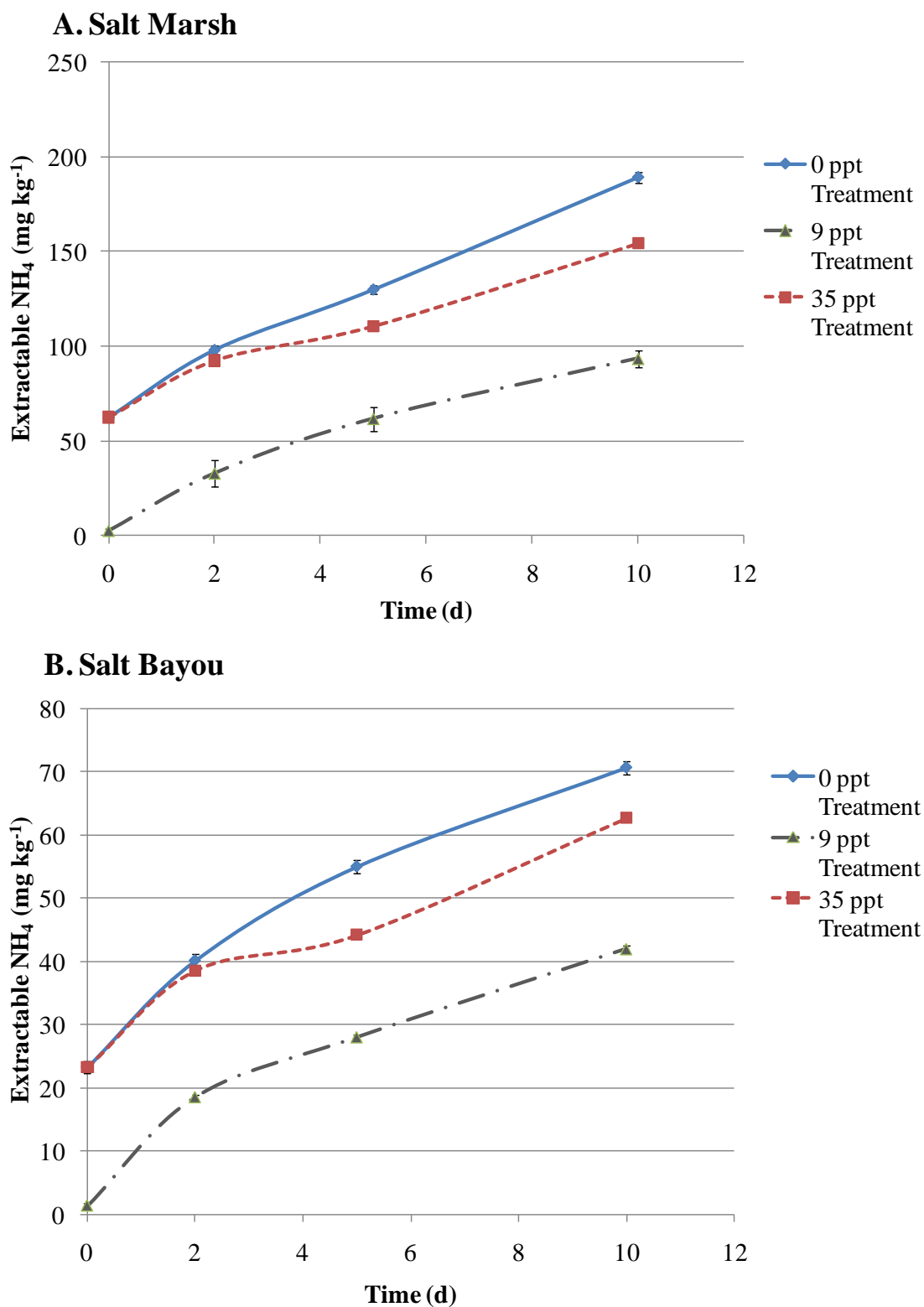


Figure 3.5 Net N mineralization in soils from the salt marsh site.

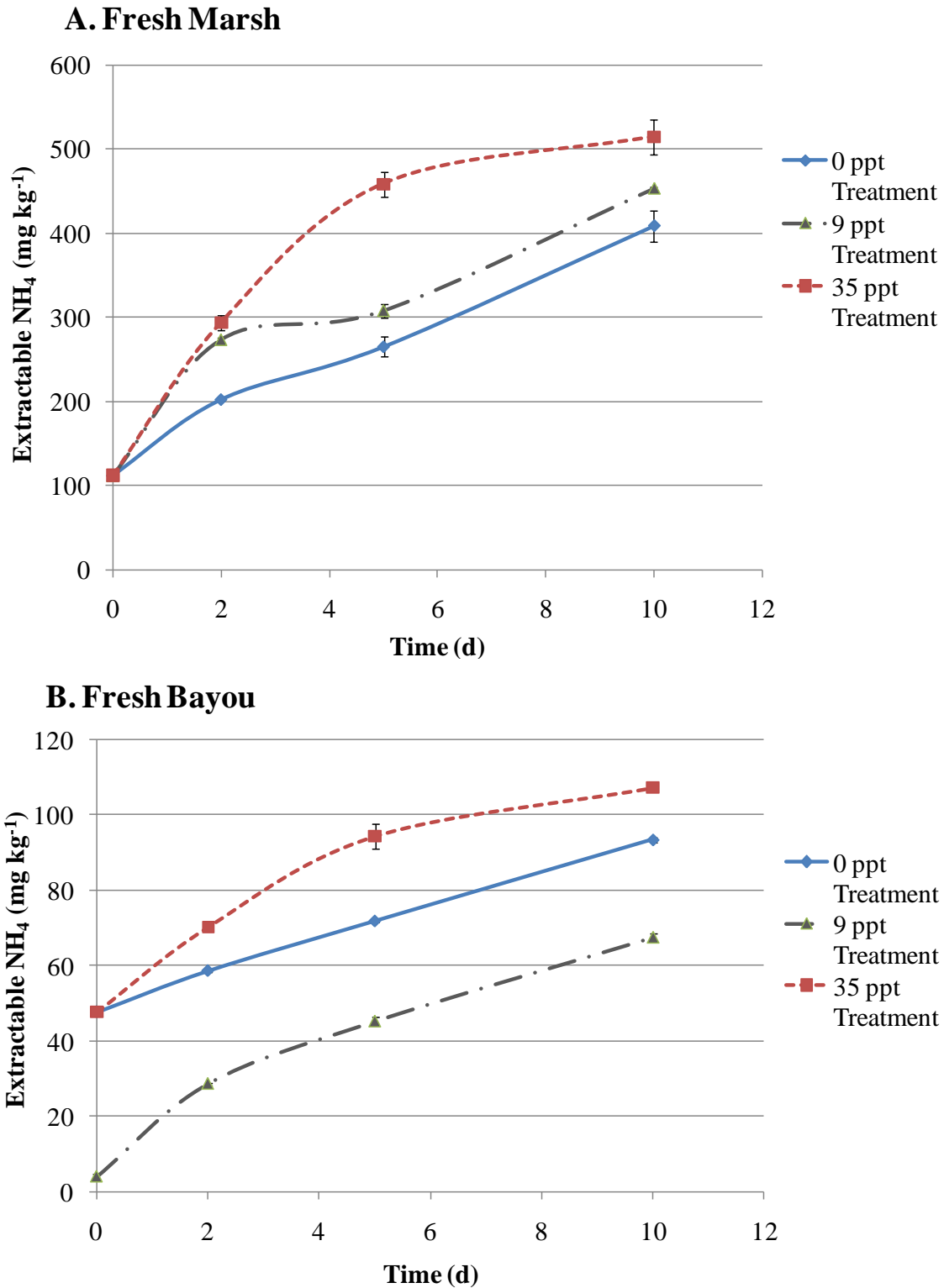


Figure 3.6 Net N mineralization in soils from the fresh marsh site.

Freshwater discharge from the Caernarvon Diversion exerts the strongest hydrological influence on wetlands found in the upper Breton Sound estuary. These systems rarely experience salinity regimes similar to those in areas regularly flushed with seawater. Strong storm systems, such as hurricanes do occasionally push high salinity seawater far inland (Edmiston et al., 2008). This surge of saltwater can alter porewater chemistry in surface soils instantly and have long lasting effects (Blood et al., 1991; Fritz et al., 2007; Gong et al., 2007). Net N mineralization rates witnessed in fresh bayou sediments under control (0 ppt), 9 ppt and 35 ppt were significantly lower than those produced by the adjacent marsh soils (Table 3.3). Ammonification in fresh marsh samples constantly increased during incubation (Fig. 3.6A). The rates of N mineralization in these soils under all salinization treatments were not significantly different from one another (Table 3.3). The trend of net ammonification rates in fresh marsh soil incubated at 9 ppt water was depressed between days 2 and 5 (Fig. 3.6A). This short-lived reduction in mineralization potential may represent the salinity induced interference to microbial efficiency. In fresh bayou sediments, a similar short-term decrease in ammonification rate was found during this period (Fig. 3.6B). The porewater salinity of soils and sediments from the freshwater region were between 0.23 and 0.26 ppt (Table 3.2). The salinity treatments applied to these soils were significantly higher than what the indigenous microbial assemblages are likely acclimated to, albeit short lived. This enhanced salinization of wetland soils has been shown to decrease the efficiency of microbial processes (Jackson and Vallaire, 2009).

Storm surge into fresh marshes can cause considerable disruption to the plant community, not only from the physical force of the water and flooding, but also by the delivery of salt. Numerous studies have shown that fresh marsh plants are affected by salt (Piazza and La Peyre, 2009; Rodgers et al., 2009; Baldwin and Mendelssohn, 1998). While we expect the changes in

salinity to not affect the PMN rate of the salt marsh site due to the diversion and tidal wide range of salinity experiences over the year. Due to the diversion and tidal exchange, the fresh marsh site also showed a resilience to changes in salinity. There was only a short-lived (3 day) depression of PMN and the rates were not significantly different from the control thereafter. These results suggest that post storm surge, the fresh marsh vegetation will still have N provided by mineralization despite the salinity shift and may provide a mechanism for vegetative recovery.

The PMN rates in salt marsh, salt bayou, and fresh bayou sediments at 9 ppt showed lower mean extractable NH_4^+ concentrations than soils under 0 and 35 ppt (Fig. 3.5, Fig. 3.6). This difference was likely due to the fact that this portion of the experiment was conducted about 5 months following the 0 and 35 ppt incubations. Though the soil samples were continuously stored in refrigeration, microbes were most likely able to continuously function even in a cold system, though most likely inhibited by the low temperature. This extended period of time allowed before the soil was used for the 9 ppt treatment would decrease the amount of measurable ammonium to be extracted in the PMN assay. Though this may occur, the PMN rates calculated from this experiment would still represent the present microbial activity under the salinity treatment.

3.5 Conclusions

N mineralization rates in each fresh and saline marsh soil/sediment at the 3 salinity treatments did not significantly differ from one another. Though each rate remained positive throughout the PMN assay, there were short-term reductions in ammonification in marsh and bayou soils that possibly represented effects of salinity (Fig. 3.5A, B; Fig. 3.6A).

The stable N mineralization rates observed in both salt marsh and bayou soils at 0 ppt suggests that freshwater introduced to these saline wetlands via diversions may not stimulate the release of bio-available N from soils into coastal waters. This would illustrate a properly functioning system which will not add to eutrophication in adjacent marine systems. In fresh marsh and bayou soils there was no significant change in ammonification efficiency due to experimental salinization. The salinity effects on fresh marsh soils (9 ppt treatment) and fresh bayou sediments (35 ppt) were short-term and resumed healthy N mineralization rates once soil microbes acclimated to the treatments. In this soil type high seawater introduction, such as those caused by storm surge in the Breton Sound, did not hinder the ammonification process for long periods of time. The increase in available N, released by ammonification may facilitate marsh vegetation recover from physical damage caused by storm surge and temporarily enhance marsh productivity within these estuarine systems.

CHAPTER 4: SUMMARY AND CONCLUSIONS

4.1 Summary

The coastal wetlands of Louisiana are subject to processes that cause salinity conditions to be in constant flux. Estuarine circulation due to wind, tides, and upland runoff influences can lead to widely fluctuating salinities in the coastal basin. Hurricanes moving along the Louisiana coast or making land-fall are capable of flooding the coastal zone with seawater. Storm surge is capable of rapidly increasing the salinity regimes of the brackish and fresh marsh soils and surface waters. Following the near instantaneous flooding, the retreat of high salinity water can take many weeks. Elevated salinity in marsh and bayou soils has been shown to affect microbial assemblages in the soils and also alter the biogeochemical processes that are occurring in these systems.

The rapid losses of land along the Louisiana coast due to natural processes and anthropogenic forces have become a major concern. Man-made river diversion structures are now being utilized in southeast Louisiana to slow the processes leading to coastal land loss. The Caernarvon diversion is a large-scale project that can deliver fresh water from the Mississippi River into the Breton Sound estuary. These diverted waters are resupplying the wetlands with sediments and nutrients dissolved in the river waters. The distribution of freshwater to wetlands near the Mississippi River is expected to increase productivity and soil accretion by mitigating saltwater intrusion. When these diversions are diverting water into the coastal basins at high flow, the high salinity regimes in the salt marsh region at the outer edges of these systems are altered due to constant flushing with fresh water. If changes in salinity cause negative effects in biogeochemical processes occurring in these coastal wetlands, then the associated nutrients will be delivered to the coastal waters. The introduction of high concentrations of nitrate into the coastal waters can contribute to eutrophication in the Northern Gulf of Mexico. The hypoxic

zone occurring off of Louisiana's coast is just one product of nutrients being discharged into the ocean without first being naturally treated by coastal wetlands.

Nitrogen is one of these valuable nutrients that is limiting in marine and terrestrial environments alike. The fate of nitrogen in coastal wetlands is of great concern due to the great value of coastal wetlands and the ocean. Nitrate is one form of bio-available N that is dissolved in high concentrations within Mississippi River waters diverted into wetlands and discharged directly out into the ocean. Denitrification is one biogeochemical process occurring in wetlands soils that is very important in the fate of nitrate in these systems. Mississippi river waters directed through coastal systems are expected to act as filters to reduce excess nitrogen.

Wetlands possess the natural ability to retain surface and subsurface waters that enter the system. These flooded conditions allows for the accumulation of organic matter and suppression of decomposition occurring in the inundated soils and sediments. Most nitrogen is sequestered in these large deposits of organic matter in wetlands. Ammonification is a biogeochemical process that allows this unavailable N in the organic matter to be released in the form of ammonium. Due to the high biological demand for available N by primary producers in the wetlands, N mineralization is another important function of wetlands.

The main goal of this study was to determine any salinity effects on potential denitrification and potentially mineralizable N in fresh and salt marsh soils from the Breton Sound estuary. The specific objectives concerning potential denitrification were, to 1) determine the effects of high salinity water on fresh marsh and bayou soils, 2) identify the effects of fresh water treatments on salt marsh and bayou soils, and 3) verify whether marsh soils or bayou sediments possess higher denitrifying potential under varying salinities in a controlled laboratory setting. The objectives concerning N mineralization were, to 1) determine the effects of high

salinity water on fresh marsh and bayou soils and 2) identify the effects of fresh water on salt marsh and saline bayou soils using PMN assays.

The effects of salinity on denitrification were studied using fresh and salt marsh/bayou soils collected from the Breton Sound estuary. Potential denitrification rates were determined in homogenized subsamples incubated and sampled in a short-term experiment (2 days) and a long-term experiment (11 days). Denitrification activity was analyzed under constant anaerobic conditions, with the addition of non-limiting C and N solutions. Salinity effects on potential denitrification in soils/sediments from the fresh and salt marshes were determined at 0, 15, 35 ppt salinity. The results from the long-term and short-term potential denitrification rates indicated a significant difference ($P < 0.05$) between rates of denitrification in salt and fresh marsh soils/sediments under varying salinity regimes. The fresh marsh and bayou control was 0 ppt water and the salt marsh and bayou soils control was 15 ppt treatment. The maximum rate of denitrification in salt marsh soils and saline bayou sediments at 15 ppt were 507 ± 27.0 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ and 149 ± 51.2 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$, respectively. At 35 ppt, the rates of denitrification in salt marsh and bayou soils were 615 ± 182 and 373 ± 35.9 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$. Potential denitrification in salt marsh soil (9.18 ± 3.27 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$) and saline bayou sediments (94.3 ± 9.55 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$) at 0 ppt was significantly lower than the 15 ppt and 35 ppt treatments. In fresh marsh and bayou soils the rates of potential denitrification averaged 373 ± 22.2 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ and 330 ± 23.0 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$ at 0 ppt. The rates of denitrification in the fresh marsh soils (99.7 ± 21.1 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$) and bayou sediments (131 ± 86.4 mg $\text{N}_2\text{O-N kg}^{-1} \text{ d}^{-1}$) in 35 ppt were significantly lower than the control (0 ppt).

PMN assays were performed on homogenized subsamples from the four soils collected in the Breton Sound estuary: salt marsh, salt bayou, fresh marsh, and fresh bayou. Net N

mineralization was measured in the soils incubated in 0, 9, and 35 ppt salinity solutions at 0, 2, 5, and 10 days. The 9 ppt salinity was utilized as a control treatment for saline soils and 0 ppt was used in fresh marsh soils. The results of this experiment indicated that rates of PMN produced by the soils in each salinity regime did not significantly differ ($P < 0.05$). Fresh marsh soils in 35 ppt did have a short-term reduction in net N mineralization between days 2-5. Salt marsh and saline bayou soils also showed a temporary decline in ammonification rates from 2-5 d in the 35 ppt treatment.

4.2 Major Findings in the Research

- Potential denitrification in fresh marsh soils and bayou sediments were 3.7 times and 2.5 times, respectively, lower than the control (0 ppt). Denitrification in salt marsh soil at 0 ppt was 55 times lower than the control (15 ppt).
- There was no significant differences ($P < 0.05$) between potential denitrification rates in Breton Sound marsh and bayou sediments under most salinity treatments.
- PMN rates over the 10 day incubations in marsh and bayou soils were not significantly affected by salinity treatments, however some short-term trends of reduced N mineralization were observed in some soils under salinity treatments.
- The high N mineralization observed in soils and sediments under all salinity treatments suggests that microbial assemblages were more susceptible to salinity fluxes in the environment.
- Rapid salinity changes due to freshwater diversions and storm surge events may not significantly alter the N mineralization occurring in soils within the Breton Sound estuary; however, denitrification seems to be more sensitive to rapid salinity shifts in wetland soils.

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VITA

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