2012

Linking nitrogen biogeochemistry to different stages of wetland soil development in the Mississippi River Delta, Louisiana

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LINKING NITROGEN BIOGEOCHEMISTRY TO DIFFERENT STAGES OF WETLAND SOIL DEVELOPMENT IN THE MISSISSIPPI RIVER DELTA, LOUISIANA

A Dissertation

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
Doctor of Philosophy

in

The Department of Oceanography and Coastal Sciences

by
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August 2012

To Scott W. Nixon, my mentor and friend

ACKNOWLEDGEMENTS

This dissertation would not have been possible without the support of my family, guidance of my committee, and encouragement of my friends. I am most grateful to my parents, Joe and Christine Palmer. Without their emotional, and occasionally financial support, I would not be here today. I would like to extend my sincerest thanks to my Aunt JeanMarie, Grandma Komyathy, and Grandma Henry. My family has always encouraged me to strive for new, more challenging goals and believed with all their hearts that I will achieve them.

I would like to express my gratitude to my major professor, Dr. Robert Twilley. My success at Louisiana State University would not have been possible without his guidance. I would also like to thank the members of my committee, Dr. David Blouin, Dr. Brian Fry, Dr. Robinson Fulweiler, and Dr. Irving Mendelssohn, for their interest and insight into my research. This dissertation has greatly benefited from all of their involvement. Much appreciation goes to Thomas Blanchard. Without his analytical expertise and laboratory assistance, I would still be looking at a freezer full of dissolved inorganic nutrient samples yet to be analyzed. I greatly appreciate the generous help Benjamin Branoff, Azure Bevington, Edward Castañeda, Jeremy Conkle, Mark Robinson, and Jonathan West provided in the field. I would also like to acknowledge all the student workers who assisted in the field and with sample preparation: Jason Bordelon, Lindsay Bordelon, Ramsey Coronado, Theresa Crupi, Ross Fossum, Emma-Leah Hale, Matthew Heels, Jennifer Ladner, Leander Lavergne, William Ohlenforst, Dustin Reid, Sarah Simmons, and Havalend Steinmuller. Many thanks to Gregg Snedden for generously providing the hydroperiod analysis included in Chapter 2 and Yvonne Allen for helping to process the Landsat images presented in Chapter 2.

I would also like to thank Edward for sharing his wisdom and love of mangroves with me. Much appreciation goes to Scott for his continued interest and encouragement, even after I left Rhode Island for warmer climates and better funding. I would like to express my sincerest thanks to Wally for her invaluable advice, unwavering support, patience, and friendship. Her passion for science is contagious and was a true inspiration through the tougher parts of this process. I would like to extend a special thanks to Hilary and Gregg, Whitney and Adam, Bryan and Sarai, and Kathy for welcoming me into their homes and treating me like family. To everyone I swam, biked, and ran through Louisiana with, thank you for helping me stretch my legs and enjoy the sunshine every now and again. And to Jefe… Thank you for not just tolerating, but loving the crazy.

This project was funded by a fellowship from the State of Louisiana Board of Regents, U.S. Shell, the Northern Gulf Institute, and the National Science Foundation via the National Center for Earth-Surface Dynamics (EAR-0120914).

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ABSTRACT

Extensive wetland loss and nutrient-enhanced eutrophication occur across the Mississippi River delta and include newly emergent landscapes, in the early stages of ecological succession, and older landscape formations, with fully developed ecological communities. Here I tested how the anthropogenic effects of a climate-induced vegetation shift, an oil spill, and nitrate-enrichment regulate the principal environmental factors controlling nutrient biogeochemistry in wetland soils at different stages of development throughout the Mississippi River delta. In the older, transgressing Barataria basin, there was no clear effect of the climate-induced species shift from *Spartina alterniflora* Loisel to *Avicennia germinans* L. on soil nutrient chemistry. Observed soil development patterns were attributed to allochthonous sediment deposition from disturbances rather than autochthonous soil development. Throughout the salt marsh-mangrove ecotone, gross denitrification (mean net N_2 flux 81.4 μmol N m^{-2} h^{-1}) was the dominant N_2 pathway and low nitrate concentrations (< 10 μM) likely limited direct denitrification. The oiling of *Avicennia* and *Spartina* habitats, during the Deepwater Horizon oil spill, doubled soil organic matter stimulating net N_2 production and nitrate/nitrite uptake. In the actively regressing Wax Lake delta (WLD), soil nutrient chemistry exhibited patterns characteristic of primary substrate development; total nitrogen and organic matter increased, while total phosphorus remained relatively constant. Under ambient nitrate concentrations (> 60 μM), gross denitrification dominated the mean net N_2 flux (163.2 μmol N m^{-2} h^{-1}). However, under low nitrate concentrations (< 2 μM), soils switched from net denitrification to net nitrogen fixation. As soils in the WLD aged, the subsequent increase in organic matter stimulated fluxes of N_2 and nitrate/nitrite in more mature soils.

In conclusion, patterns of soil nitrogen biogeochemistry were linked to the distinct stages of delta formation. Low nitrate availability in the older, transgressive regions limited direct denitrification yielding a net N_2 flux dominated by coupled nitrification-denitrification fueled by organic matter mineralization. In contrast, young, regressive regions demonstrated a high capacity for direct denitrification of riverine nitrate that was regulated by substrate age and organic matter accumulation. Throughout the delta cycle, nitrate availability and soil organic matter were the principal factors regulating nitrogen biogeochemistry, and thus the anthropogenic impact of nitrate-enrichment had a marked influence on the observed patterns.

CHAPTER 1
INTRODUCTION

Coastal Louisiana, like all river-dominated deltaic earth surfaces, is a highly dynamic environment (Paola et al. 2011). Throughout the Holocene, interactions between sea level and sediment deposition from the Mississippi River formed the 30,000 km^2 of wetlands, shallow inshore water bodies, and low elevation uplands referred to as the Mississippi River delta (Boesch et al. 1994; Roberts 1997; Day et al. 2005). The modern Mississippi River plain is composed of six delta lobes, which formed in succession as the river changed its path to the Gulf of Mexico (Roberts 1997; Coleman et al. 1998). This succession of lobe formation follows the well-established cyclic nature of delta development (Roberts 1997; Coleman et al. 1998). Briefly, after a distributary captures the river, there is a period of rapid subaerial growth (regressive phase) of newly emerged landforms followed by relative stability. These soils are dominated by mineral sediment, and much like the classic Walker and Syers (1976) model of soil development, are rich in phosphorus and poor in nitrogen content. Eventually, the river abandons its course in favor of a more hydraulically efficient path and the delta lobe begins to deteriorate (transgressive phase). By the time this phase of the delta cycle has been achieved, the soils should have developed an equilibrium in nitrogen and phosphorus as found in more mature ecosystems on developing landscapes.

Historically, shifts in the course of the river resulted in a cycle of land-building and land loss, with an overall net increase in wetland area over the last 7,000 years (Roberts 1997; Coleman et al. 1998). During this time, the delta was sustained by river-pulsing events that delivered sediment, nutrients, and freshwater to the wetlands within the floodplain (Day et al. 2000; Twilley and Rivera-Monroy 2009). However, since the 1930s, the delta has experienced dramatic land loss, with approximately 65 km^2 of wetlands converted to open water each year

(Boesch et al 1994; Barras et al. 2008). Research shows that wetland loss in the delta is a complex process resulting from reduced sediment delivery due to flood control levees constructed along the river, a decrease in the sediment load within the river, altered wetland hydrology from canal construction, saltwater intrusion, wave erosion, subsidence, and eustatic sea level rise (Boesch et al. 1994, Turner 1997; Day et al. 2000, 2007; Blum and Roberts 2009).

The need for protection and restoration of wetlands within the Mississippi River delta is evident given the numerous economic and ecological goods and services they provide (Costanza et al. 1989; Mitsch et al., 2001, 2005; Twilley and Rivera-Monroy 2009). In an attempt to stimulate wetland development and mitigate further loss, large-scale restoration efforts have been proposed to reintroduce Mississippi River water into the state's coastal wetlands (Louisiana Coastal Master Plan 2012). These freshwater diversion projects are designed to reduce salinity and increase sediment delivery to the receiving basin by mimicking the historical overbank flooding of the Mississippi River (Twilley and Rivera-Monroy 2009; Paola et al. 2011). Complicating the widespread use of freshwater diversions in Mississippi River delta restoration is how the chemistry of the river has changed during the 20th century, particularly in the decades following the 1950s (Mitch et al. 2001; Twilley and Rivera-Monroy 2009). The Mississippi River connects the deltaic coast to 40% of the land in the contiguous United States; and because of this river network, the delta integrates processes occurring throughout the entire catchment basin (Twilley and Rivera-Monroy 2009). A prime example is the widespread use of industrial fertilizers throughout the basin, which has tripled the concentrations of reactive nitrogen in the Mississippi River over the last half-century (Goolsby and Battaglin 2001; Broussard and Turner 2009). Considerable evidence links this increase in reactive nitrogen to enhanced levels of

primary productivity and depletion of bottom water dissolved oxygen concentrations over the Louisiana-Texas continental shelf (Rabalais et al. 2002; Scavia et al. 2003; Turner et al. 2008).

In addition to accelerated rates of wetland loss and nitrogen enrichment, coastal Louisiana is positioned to experience many of the effects associated with global climate change. Climate change is expected to alter temperatures and increase rates of sea level rise (IPCC 2007). Scientists predict climate change to affect atmospheric and oceanic circulation and thus the timing, frequency, and magnitude of precipitation and hurricane activity (IPCC 2007). In Louisiana, recent evidence suggests that temperatures have increased (Ning et al. 2003) and the frequency of extreme freezes has decreased over the last several decades (Figure 1.1). These changes are facilitating expansions of the tropical black mangrove (*Avicennia germinans* L.) into smooth cordgrass (*Spartina alterniflora* Loisel) marshes throughout the delta (Day et al. 2005; Perry and Mendelssohn 2009). The survival of mangroves and their replacement of salt marsh are directly related to freeze frequency (Chen and Twilley 1998). The occurrence of greater than one freeze every eight years inhibits the survival of mangroves, while a freeze frequency of 12 years allows for mangroves to replace salt marsh (Chen and Twilley 1998; Day et al. 2005). The last frost-induced dieback of Louisiana *Avicennia* occurred in 1989, over 20 years ago, and since then the northward expansion of *Avicennia* into *Spartina* marshes has been unhampered by sustained periods of below freezing winter temperatures.

The main goal of this research was to characterize the principal environmental factors controlling nutrient biogeochemistry in wetland soils at different stages of delta development. I specifically sought to determine patterns of soil nutrient biogeochemistry in the Barataria and Atchafalaya basins, which represent distinct stages of soil development within the Mississippi River delta. Barataria basin formed approximately 2,500 years ago as an inter-distributary of the

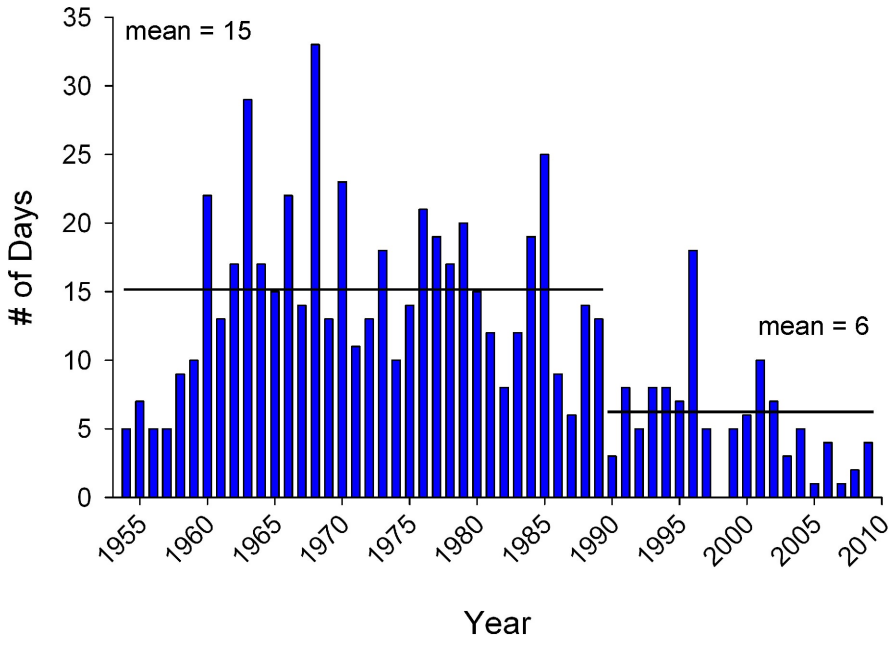

Figure 1.1. Number of days per year with a mean temperature below freezing (0 ºC) in New Orleans, Louisiana (National Climactic Data Center 2012). Horizontal black lines represent mean number of days prior to- and post-1989, which marks the most recent frost-induced *Avicennia* dieback in southern Louisiana.

Mississippi River and Bayou Lafourche (Roberts 1997). Presently, the basin is isolated from riverine influences and marine processes control hydrology, sediment load, and salinity in the salt marshes at the seaward edge of the system. Contrasting the transgressing Barataria basin is the regressing Atchafalaya basin, which is the only prograding delta lobe along the coast and affords a model of nutrient cycling in an emerging landscape. Still in the early stages of delta lobe formation, hydrology in the Atchafalaya basin is largely controlled by the Atchafalaya River, mineral sediment load is high, and salinity is low. Similar to the well-established relationship between the ecological features of wetland succession and delta formation (Gosselink 1984, Gosselink et al. 1998), my primary hypothesis anticipated that distinct patterns of soil development and nutrient biogeochemistry are linked to the different phases of the delta cycle.

The Mississippi River delta today represents an anthropogenically impacted landscape that may require adjustment to any concept of natural biogeochemical patterns linked to delta development. Therefore, the secondary goal of this research was to understand how anthropogenic impacts regulate the principal environmental factors controlling nutrient biogeochemistry, thereby modifying the observed patterns within these distinct coastal basins. In the transgressing region of Barataria basin, I focused on how shifts in vegetation from salt marsh to mangrove alter soil development and impact nutrient biogeochemistry. This represents a climate change effect on soil development in a mature wetland system of the deltaic coast. During this study, the Deepwater Horizon oil spill provided the additional opportunity to explore the immediate impacts of an oil spill on nutrient biogeochemistry in a mature wetland system. In the regressing region of the Atchafalaya basin, my secondary goal sought to understand how short-term patterns of soil development influence nutrient biogeochemistry and the importance of nitrate-enrichment in regulating these processes. My secondary hypothesis anticipated the anthropogenic effects of a climate-induced species shift, an oil spill, and nitrate-enrichment to exhibit a marked influence on patterns of soil development and nutrient biogeochemistry within the Mississippi River delta.

Chapters 2 through 4 of this dissertation examined a climate change effect on soil development and nutrient biogeochemistry within Barataria basin, Louisiana. In Chapter 2, I evaluated how the climate-induced vegetation shift from *Spartina* marshes to *Avicennia* scrub mangroves influenced the nutrient chemistry of soil development in the mature wetlands of the Fourchon region, located within the greater Barataria basin system. Additionally, I explored whether the proximity and exposure of the salt-marsh mangrove ecotone to storm events from the Gulf of Mexico altered the soil response to this species shift.

In Chapter 3, I investigated the effect *Avicennia* scrub mangrove expansions have on soil-water column net N_2, oxygen, and dissolved inorganic nutrient fluxes within mature *Spartina* marsh habitats of the Fourchon region. I determined if the shift from *Spartina* to *Avicennia* altered the balance between nitrogen fixation and denitrification. Then, I evaluated whether preexisting differences in marsh elevation, hydroperiod, and soil nutrient content modified the effect of this shift on inorganic nutrient cycling.

In Chapter 4, I captured the immediate impacts of the Deepwater Horizon oil spill on inorganic nutrient cycling within the salt marsh-mangrove ecotone of Barataria Bay, also located within the greater Barataria basin system. The unforeseen occurrence of this oil spill provided the opportunity to determine how organic matter enrichment of mature wetland soils affected soil-water column net N_2, oxygen, and dissolved inorganic nutrient fluxes. However, the oil spill and subsequent property restrictions forced me to terminate my sampling in the salt marsh-mangrove ecotone and continue my research in a different region of coastal Louisiana. To facilitate comparison of soil development and nutrient biogeochemistry during the different phases of the delta cycle, I chose to continue exploring patterns of soil nutrient biogeochemistry in the newly emerged Wax Lake delta (WLD) located within the rapidly prograding Atchafalaya basin, Louisiana.

In Chapter 5, I applied the Walker and Syers (1976) conceptual model of soil development to describe patterns of soil nutrient biogeochemistry on newly emerging landscapes of a river-dominated deltaic coast. I used a 35-year chronosequence in the WLD to determine if short-term patterns of soil chemistry and net N_2, oxygen, and dissolved inorganic nutrient fluxes in an emerging delta were consistent with the Walker and Syers (1976) model of long-term nutrient availability during ecosystem development. I contrasted the current anthropogenically

enriched high-nitrate scenario, with a low-nitrate scenario more typical of primary substrate development, in order to evaluate the role of nitrate-enrichment in determining the net N_2 flux across the WLD chronosequence.

Finally, Chapter 6 summarizes patterns of soil development and nutrient, specifically nitrogen, biogeochemistry observed across the different phases of the delta cycle examined in this study. I contrast soil nitrogen biogeochemistry in the mature wetlands of Barataria basin to the young, newly emergent wetlands of the Atchafalaya basin. Then, I discuss how the principal factors of nitrate availability and soil organic matter content determined the observed patterns of net N_2, oxygen, and dissolved inorganic nitrogen fluxes and the relative importance of a climate-induced species shift, an oil spill, and nitrate-enrichment in regulating these factors.

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CHAPTER 2
SOIL DEVELOPMENT IN A COASTAL LOUISIANA WETLAND DURING A CLIMATE-INDUCED VEGETATION SHIFT FROM SALT MARSH TO MANGROVE

INTRODUCTION

Located at the northernmost extent of mangroves in the Gulf of Mexico, coastal Louisiana provides an excellent opportunity to study the effects of a climate-induced vegetation shift on soil development within a wetland ecosystem. Low energy coastlines throughout temperate latitudes are dominated by salt marshes, while mangrove forests occupy a similar niche in the coastal tropics. Over the last several decades, climate throughout the Gulf Coast region has experienced a general warming trend and scientists are predicting hotter summers (+1.5 to 4 °C) and warmer winters (+1.5 to 5.5 °C) by 2100 (Twilley et al. 2001; Ning et al. 2003; IPCC 2007). Mild winter temperatures associated with this warming have facilitated the northward expansion of black mangroves (*Avicennia germinans* L.) into the smooth cordgrass (*Spartina alterniflora* Loisel) marshes of Florida, Louisiana, and Texas (Sherrod and McMillan 1985; Stevens et al. 2006; Perry and Mendelssohn 2009). In Louisiana, *Avicennia* were historically restricted to the barrier islands and southernmost beaches (Penfound and Hathaway 1938); however, in recent years they have become a common occurrence in mainland *Spartina* marshes. Louisiana *Avicennia* are typically found in scrub form (< 2.5 m in height) due to the occasional frost and reduced number of degree days (Lugo and Zucca 1977; Chen and Twilley 1998).

Previous research in the Louisiana salt marsh-mangrove ecotone has examined controls on plant zonation (Patterson et al. 1993, 1997) as well as differences in soil physicochemical variables (Patterson and Mendelssohn 1991), and ecosystem function (Perry and Mendelssohn 2009) between the two habitats. The results pertinent to soil organic matter and nutrient content

are highly variable. Bulk density, elevation, and oxidation-reduction potential are frequently greater in soils containing *Avicennia* (Patterson and Mendelssohn 1991; Perry and Mendelssohn 2009), while soils in *Spartina* habitats are more reducing, with higher concentrations of porewater sulfides, ammonium, and phosphate (Patterson and Mendelssohn 1991). In contrast to these disparities, no significant differences have been detected in sediment accretion, belowground production, decomposition, and carbon assimilation (Perry and Mendelssohn 2009).

Additional studies from *Spartina* marshes and neotropical scrub mangrove stands draw attention to potential differences between these species in productivity and tissue chemistry. Salt marsh productivity exhibits a latitudinal gradient with the most productive salt marshes in North America occurring along the Gulf of Mexico (Turner 1976). Published rates of aboveground primary productivity (APP) in *Spartina* marshes throughout Louisiana range from 700 to 3,570 g m^{-2} yr^{-1} (Kirby and Gosselink 1976; Kaswadji et al. 1990; Edwards and Mills 2005; Darby and Turner 2008). *Spartina* below ground primary productivity (BPP) appears more variable, with rates falling between 1,670 and 11,680 g m^{-2} yr^{-1} (Edwards and Mills 2005; Darby and Turner 2008; Perry and Mendelssohn 2009). Rates of mangrove (aboveground) productivity also show a distinct trend with latitude; however, the most productive systems are located near the equator (Twilley et al. 1992; Saenger and Snedaker 1993). Studies measuring the APP of scrub mangrove stands throughout the neotropics found rates to vary from 170 to 490 g m^{-2} yr^{-1} (Twilley et al. 1986; Day et al. 1996; Ewe et al. 2006; Castañeda-Moya 2010), while published rates of BPP in this same region fall between 20 and 1,630 g m^{-2} yr^{-1} (McKee and Faulkner 2000; McKee et al. 2007; Perry and Mendelssohn 2009; Castañeda-Moya et al. 2011). These studies

suggest that the APP and BPP of coastal Louisiana *Avicennia* is only half the APP and BPP of *Spartina*, which may ultimately affect the quantity of organic matter deposited in marsh soils.

The subtle difference in foliar carbon (C) and nitrogen (N) content between *Spartina* and *Avicennia* may also affect soil development, more specifically the soil C:N ratio. Reported molar C:N ratios in the green leaves of *Spartina* and *Avicennia* range from 23 to 37 and 26 to 29, respectively (Twilley et al. 1986; Lawton-Thomas 1997; Osgood and Zieman 1993). When comparing green versus senescent leaves, it is clear that both species retranslocate nitrogen before shedding leaves. *Spartina* retranslocates nitrogen to underground rhizomes and roots, whereas nitrogen from mangroves leaves is stored in aboveground stems. Although, it appears as though *Spartina* retranslocates more nitrogen when shedding leaves resulting in higher a C:N ratio in *Spartina* litter (65 to 92) than *Avicennia* (47 to 55) litter (Breteler et al. 1981; Twilley et al. 1986; Lawton-Thomas 1997). As *Spartina* marshes change to *Avicennia* stands, the lower C:N ratio in the litter of *Avicennia* may increase the nitrogen content of the soil.

To better understand how the shift from *Spartina* marshes to *Avicennia* scrub mangrove stands impacts nutrient chemistry during wetland soil development, I measured changes in soil bulk density as well as organic matter, nitrogen, and phosphorus content within the salt marsh-mangrove ecotone of Fourchon, Louisiana. Soils in low energy coastal systems preserve historical conditions of plant community dynamics and maintain a record of natural and anthropogenic disturbances. Soil stratigraphy is frequently used as a tool in paleoecological studies to reconstruct past ecosystem dynamics and elucidate patterns, causes, and rates of change (Willard and Cronin 2007). Rates of sediment, nutrient, and heavy metal accumulation (DeLaune et al. 1981; Reddy et al. 1993), the timing and frequency of episodic events (e.g. fires and hurricanes) (Liu and Fearn 2000; Liu et al. 2008), and shifts in hydrology and vegetation

(Brenner et al. 2001; Kim and Rejemánková 2002) can all be determined from soil records. In this study, shallow soil stratigraphy from the two different habitats were compared to evaluate whether expanding populations of *Avicennia* will affect organic matter and nutrient accumulation during soil development. I specifically sought to answer the following questions: (1) Does the shift from *Spartina* to *Avicenni*a influence the chemistry of soil development? (2) Does the proximity and exposure of the salt marsh-mangrove ecotone to storm events from the Gulf of Mexico alter the soil response to this species shift?

Wetland soil formation is the result of both organic matter accumulation and mineral sediment deposition (Baumann et al. 1984; Hatton et al. 1983; Nyman et al. 1990, 2006). In Louisiana's coastal wetlands, mineral sediment deposition during episodic storm events can account for a significant portion of vertical marsh accretion (Turner et al. 2006, 2007). I anticipated that increased exposure to the Gulf of Mexico and high mineral sediment input during storm events would mask any differences in soil development between these species. However, in regions more protected from the Gulf of Mexico, I anticipated that wetland soils would exhibit a decrease in carbon content and a subtle increase in nitrogen content as they shifted from *Spartina* marshes to *Avicennia* scrub mangrove stands.

MATERIALS AND METHODS

Study Area and Sampling Locations

The study area was located in salt marsh-mangrove ecotone of Fourchon, Louisiana, just east of Bayou Lafourche, less than 2 km from the Gulf of Mexico (Figure 2.1). The Fourchon region is located in the Barataria basin system of the Mississippi River delta plain and formed as part of the Lafourche lobe 2500 to 800 years BP (Roberts 1997; Coleman et al. 1998). Prior to the completion of the Donaldsonville dam in 1904, Bayou Lafourche served as an outlet for the

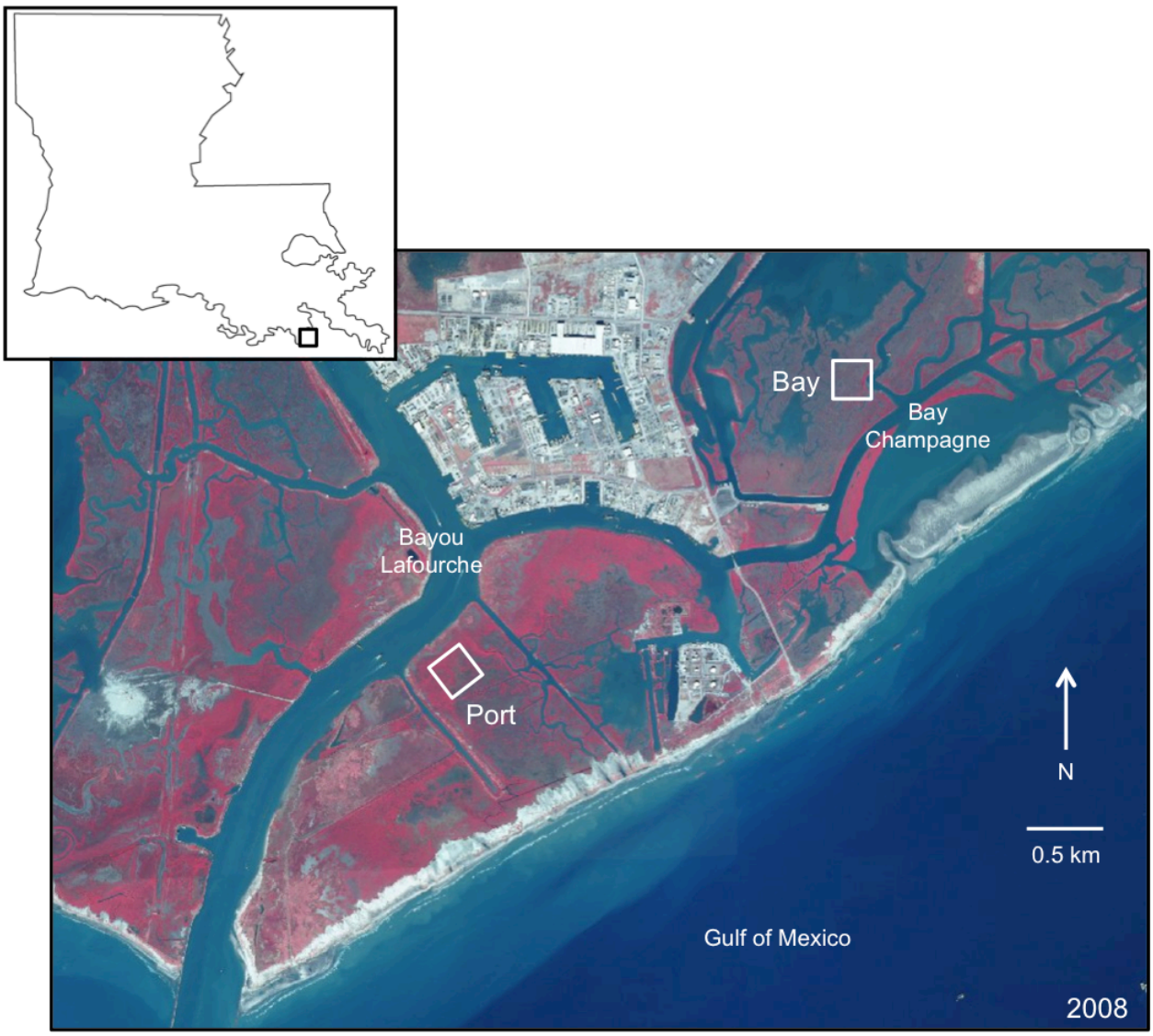

Figure 2.1. Sampling locations in Fourchon, Louisiana. Image courtesy of the Louisiana Department of Natural Resources. Wetlands in light pink were dominated by *Spartina*, wetlands in bright pink were dominated by *Avicennia*. White areas were non-vegetated sediment.

Mississippi River. A pumping station constructed in 1955 now allows approximately 6 m^3 s^{-1} of Mississippi River flow into Bayou Lafourche (CWPPRA 2011). Hydroperiod in the region is predominately controlled by diurnal microtides averaging 0.3 m in range, with strong meteorological influences during winter cold fronts and summer tropical storms and hurricanes (Zetler and Hansen 1972). Precipitation (160 cm yr^{-1}) comprises the majority of freshwater inputs to the bayou and as a result, salinities are comparable to the Gulf of Mexico (~30 ‰)

(Louisiana Department of Natural Resources 2011). The climate of southern Louisiana is humid subtropical (Peel et al. 2007), with mean monthly temperatures between 6 and 30 °C (National Climatic Data Center 2011). The Port of Fourchon serves as a major support center for petroleum exploration and production in the central Gulf of Mexico and is considered a highly industrialized area.

Natural tidal creeks as well as dredged channels intersect the wetlands of Fourchon. When selecting sites, an effort was made to select sites near natural creeks and avoid sites adjacent to dredged canals and their associated spoil banks. Two sites were established in Fourchon (Figure 2.1). The Bay site (29°07'14" N 090°10'54"W) was located less than 0.5 km northwest of Bay Champagne in a wetland that is highly exposed to winds and waves from the Gulf of Mexico. The Port site (29°06'21" N 090°12'30"W) was located south of Port Fourchon and east of Bayou Lafourche. A levee prevents direct overbank flooding from Bayou Lafourche into the Port site and approximately 1 km of wetland and barrier beach protect this site from the Gulf of Mexico. Each site contained monospecific habitats of *Spartina* and *Avicennia*.

Elevation Survey and Water Level Measurements

In July 2009, a 0.01 km^2 grid of approximately 35 sampling points and a permanent benchmark were established at the Bay and Port sites. Elevation of each sampling point relative to the benchmark was measured with a Sokkia LP30A Class 1 laser level and the habitat type (*Avicennia* or *Spartina*) was recorded. The benchmark elevations were corrected to North American Vertical Datum of 1988 (NAVD88) using a Trimble R8 GNSS dual frequency receiver and elevation control points within the Continuously Operating Reference Stations (CORS) of the Louisiana State University (LSU) GULFNet real-time network (error ± 2 cm; Center for GeoInformatics 2011).

After completing the elevation survey, an Onset HOBO U20 titanium water level logger was installed at the lowest point in each site. Each water level recorder was suspended inside a well casing and placed approximately 1.5 m below the soil surface. Water levels relative to the soil surface were recorded hourly from August 2009 to August 2010.

Soil Core Collection and Analysis

In July 2009, six cores (15 cm internal diameter by 30 cm length) were collected from the Bay site, three cores each from the *Spartina* and *Avicennia* habitats. Soil cores were collected using a PVC suction-coring device (Meriwether et al. 1996). I was only able to retrieve five cores from the Port site, two from the *Spartina* habitat and three from the *Avicennia* habitat. Cores were extruded and sectioned into 2 cm intervals that were oven-dried at 60 °C to a constant weight and then ground to 250 μm with a Wiley Mill. Prior to grinding, bulk density was calculated for each section as the total dry weight divided by the section volume (365 cm^3). Total organic matter was determined by loss on ignition at 550 °C for 2 hours (Davies 1974). Total carbon and nitrogen concentrations were determined with an ECS 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, California). Total phosphorus was extracted from soil samples with 1 M HCl after combustion in a furnace for 2 hours at 550 °C (Aspila et al. 1976) and determined by colormetric analysis using a Flow Solution IV autoanalyzer (OI Analytical, College Station, Texas). Soil nutrient data are expressed on a volume basis using bulk densities.

Statistical Analysis

Elevation data were analyzed with a two-way analysis of variance (ANOVA) to test for differences between sites (Bay and Port) and habitat types (*Spartina* and *Avicennia*). A three-way ANOVA with repeated measures was used to determine if statistical differences existed

between sites or habitat types with depth for all soil variables (bulk density, organic matter content, nitrogen density, phosphorus density, molar carbon to nitrogen ratio (C:N), and nitrogen to phosphorus ratio (N:P)). The three-way ANOVA was run twice; the first run incorporated each individual section (0-2 to 28-30 cm) and will be referred to as the section analysis. In the second run, the mean of 0-10 cm was compared to the mean of 20-30 cm and will be referred to as the mean analysis. Interaction effects were considered for all analyses and pairwise comparisons among treatments were described with a Tukey's honestly significant difference (HSD) test. All statistical analyses were performed with SAS PROC Mixed and significance was assessed at the 0.05 level (SAS Institute 2011).

RESULTS

On average, elevation in the Bay site was 13.3 ± 1.2 cm significantly greater than in the Port site (Table 2.1). Within both sites, *Spartina* occurred at significantly lower elevations than *Avicennia*. Hydroperiod varied between the two sites (Table 2.1). The Bay site was flooded 42% of the year and experienced 246 flood events yr^{-1}, while the Port site was flooded 66% of the year, but only experienced 183 flood events yr^{-1}. It appears as though the lower elevation

Table 2.1. Elevation and hydroperiod data for *Spartina* and *Avicennia* habitats in the Bay and Port sites of Fourchon, Louisiana.

	Bay		Port	
	Spartina	*Avicennia*	*Spartina*	*Avicennia*
Elevation (cm)[†]	28.1 ± 0.9[b‡]	38.7 ± 1.1[a]	18.2 ± 0.3[d]	21.4 ± 0.4[c]
(*n*)	(16)	(16)	(15)	(21)
Hydroperiod				
Duration (h yr^{-1})	3707		5780	
Duration (%)	42		66	
Frequency (events yr^{-1})	246		183	

[†]Mean ± 1 standard error presented for elevation data

[‡]Within row, means followed by the same letter are not significantly different according to Tukey's HSD ($p > 0.01$)

Port site does not drain as frequently and as a result, a longer flood at the Port site registers as two or more flood events at the higher elevation Bay site.

The full depth profile for all variables from the Bay site showed marked variability in the top 0 to 10 cm relative to the lower 20 to 30 cm, with a transition zone from 10 to 20 cm (Table 2.2, Figure 2.2). Soils in the top 10 cm exhibited a mineral rich signature, with high bulk density and phosphorus density, and low organic matter content and nitrogen density. Below 20 cm, soil bulk density and phosphorus density decreased, while organic matter content and nitrogen density increased. There were no notable differences between the *Spartina* and *Avicennia* profiles (Figure 2.2). The profiles for all soil variables from the five Port cores strongly contrasted those from the Bay cores exhibiting the opposite trends with depth (Table 2.2, Figure 2.2). Soils in the top 10 cm were enriched in organic matter, with low bulk density and phosphorus density. From 20 to 30 cm, soil bulk density and phosphorus density increased, while organic matter content and nitrogen density decreased.

The section analysis demonstrated a significant effect of depth and a significant interaction between site and depth for all soil variables except C:N (Table 2.3). The only significant difference between habitat types was in total phosphorus density, with lower phosphorus content in *Spartina* soils (mean: 0.28 ± 0.01 mg cm^{-3}) than *Avicennia* soils (mean: 0.32 ± 0.01 mg cm^{-3}) (Table 2.3). The means of each soil variable from 0-10 cm and from 20-30 cm further supported the results of the section analysis (Figure 2.3). There was a significant interaction between site and depth for all variables measured except C:N and the only significant difference between habitat types was in phosphorus density (Table 2.3).

Table 2.2. Vertical distribution of bulk density, organic matter content, and nitrogen and phosphorus density for each site in the salt marsh-mangrove ecotone of Fourchon, Louisiana.

Depth (cm)	Bulk Density ($g\ cm^{-3}$)				Organic Matter Content (%)				Nitrogen Density ($mg\ cm^{-3}$)				Phosphorus Density ($mg\ cm^{-3}$)			
	Bay		Port		Bay		Port		Bay		Port		Bay		Port	
	X	± SE	X	± SE	X	± SE	X	± SE	X	± SE	X	± SE	X	± SE	X	± SE
0-2	0.79	± 0.10	0.33	± 0.04	3.74	± 0.24	8.88	± 0.89	0.54	± 0.13	0.79	± 0.05	0.40	± 0.05	0.17	± 0.01
2-4	1.08	± 0.05	0.53	± 0.06	3.59	± 0.37	8.77	± 0.57	0.52	± 0.33	1.11	± 0.09	0.53	± 0.03	0.27	± 0.03
4-6	1.05	± 0.07	0.44	± 0.04	3.54	± 0.74	14.12	± 0.16	0.47	± 0.30	1.61	± 0.18	0.47	± 0.02	0.21	± 0.02
6-8	0.90	± 0.04	0.38	± 0.02	5.05	± 0.55	14.99	± 1.09	1.27	± 0.27	1.39	± 0.12	0.45	± 0.02	0.19	± 0.01
8-10	0.87	± 0.10	0.46	± 0.02	6.18	± 1.08	12.87	± 0.53	1.13	± 0.24	1.39	± 0.10	0.40	± 0.03	0.21	± 0.02
10-12	0.69	± 0.04	0.54	± 0.01	8.33	± 0.63	11.21	± 0.95	1.38	± 0.09	1.55	± 0.09	0.30	± 0.02	0.24	± 0.01
12-14	0.72	± 0.08	0.61	± 0.03	7.84	± 0.89	9.40	± 0.94	1.32	± 0.13	1.41	± 0.07	0.30	± 0.03	0.27	± 0.02
14-16	0.59	± 0.07	0.70	± 0.03	9.65	± 0.95	7.51	± 0.50	1.30	± 0.12	1.34	± 0.09	0.25	± 0.03	0.31	± 0.02
16-18	0.52	± 0.04	0.78	± 0.04	10.99	± 1.00	5.66	± 0.31	1.38	± 0.09	1.22	± 0.07	0.22	± 0.02	0.35	± 0.02
18-20	0.53	± 0.07	0.85	± 0.02	11.59	± 1.12	4.66	± 0.37	1.33	± 0.04	1.11	± 0.12	0.22	± 0.03	0.36	± 0.01
20-22	0.48	± 0.03	0.82	± 0.03	12.62	± 0.62	5.44	± 0.34	1.42	± 0.08	0.79	± 0.04	0.19	± 0.01	0.35	± 0.01
22-24	0.49	± 0.02	0.92	± 0.05	11.95	± 0.48	4.60	± 0.46	1.39	± 0.03	0.82	± 0.03	0.20	± 0.01	0.40	± 0.01
24-26	0.50	± 0.02	0.93	± 0.05	11.54	± 0.46	4.44	± 0.58	1.48	± 0.09	0.78	± 0.05	0.19	± 0.01	0.39	± 0.02
26-28	0.49	± 0.02	0.96	± 0.06	11.78	± 0.41	4.51	± 0.59	1.42	± 0.07	0.77	± 0.04	0.20	± 0.01	0.41	± 0.01
28-30	0.54	± 0.02	0.90	± 0.06	9.96	± 0.42	4.27	± 0.68	1.35	± 0.05	0.79	± 0.03	0.22	± 0.01	0.39	± 0.02

[†]Bay site $n = 6$, Port site $n = 5$

[‡]Mean (X) ± 1 standard error (SE) presented for all data

DISCUSSION

A Note on the Chronosequence Analyzed in this Study

Extensive documentation exists of daily (sediment traps), annual (feldspar marker horizons), and decadal-scale (^{137}Cs and ^{210}Pb) accretion rates throughout coastal Louisiana wetlands (Cahoon and Turner 1989; DeLaune et al. 1989; Reed 1989; Perry and Mendelssohn 2009 among others). Most accretion estimates for the region fall between 0.6 and 1.5 cm yr^{-1}, with higher rates of deposition occurring along natural creeks and during episodic events such as winter cold fronts and summer storms (Hatton et al. 1983; Baumann et al. 1984; Reed 1989; Childers and Day 1990). In 2006, Perry and Mendelssohn (2009) measured accretion rates in the salt marsh-mangrove ecotone of Fourchon, Louisiana and observed constancy between daily,

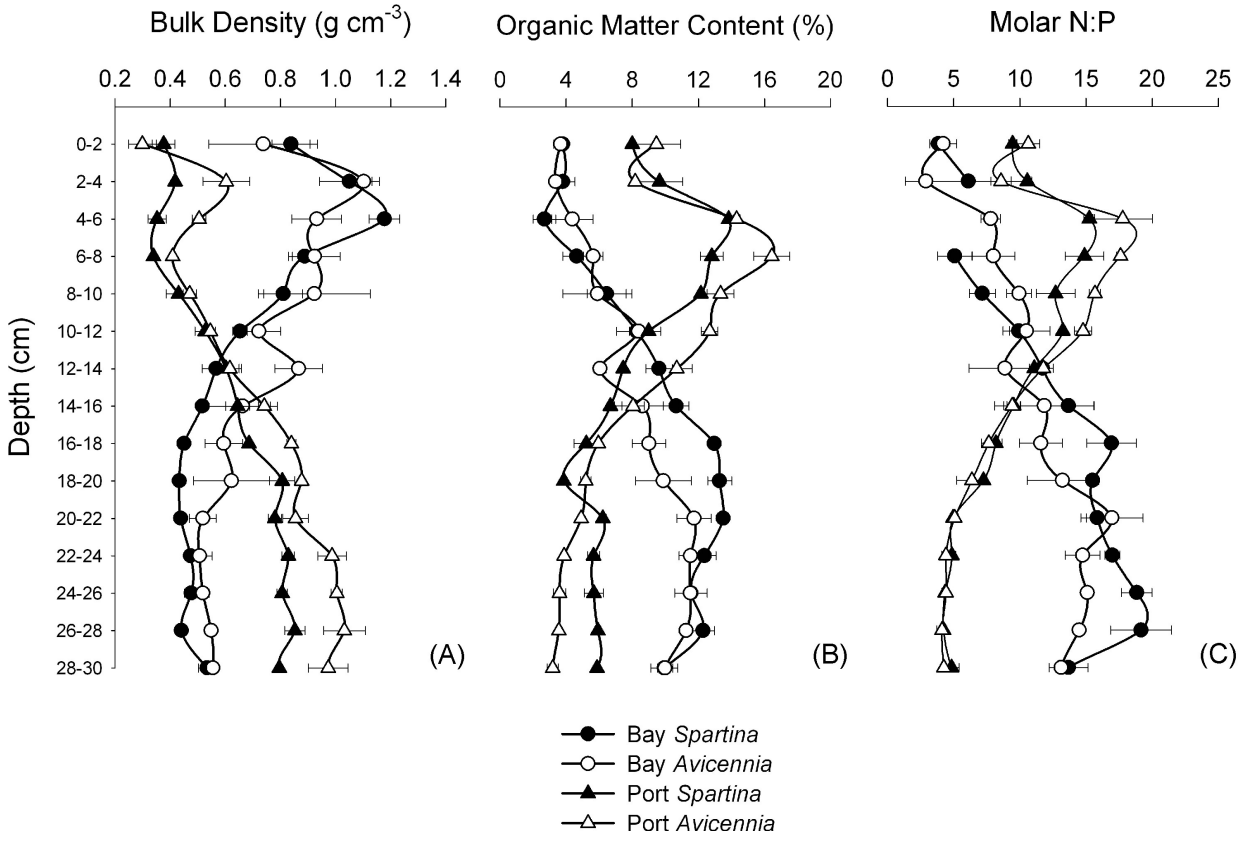

Figure 2.2. (A) Bulk density, (B) organic matter content, and (C) molar N:P profiles for *Spartina* and *Avicennia* soils in Bay and Port sites. Each data point is mean (± 1 SE) of three cores, except *Spartina* at the Port site, which is the mean of two cores.

Table 2.3. ANOVA results from section analysis and mean analysis testing for effects of site, habitat, and depth on soil properties in the salt marsh-mangrove ecotone of Fourchon, Louisiana.

	Section Analysis[†]					
	Bulk density ($g\ cm^{-3}$)	Organic matter (%)	Nitrogen ($mg\ cm^{-3}$)	Phosphorus ($mg\ cm^{-3}$)	Molar C:N[‡]	Molar N:P[‡]
Site	ns	ns	ns	ns	ns	0.04
Habitat	ns	ns	ns	0.05	ns	ns
Site x Habitat	ns	ns	ns	ns	ns	ns
Depth	<0.0001	<0.0001	<0.0001	<0.0001	0.05	<0.0001
Site x Depth	<0.0001	<0.0001	<0.0001	<0.0001	ns	<0.0001
Habitat x Depth	ns	0.0003	0.01	ns	ns	0.02
S x H x D[§]	ns	<0.0001	ns	ns	ns	ns
	Mean Analysis[¶]					
	Bulk density ($g\ cm^{-3}$)	Organic matter (%)	Nitrogen ($mg\ cm^{-3}$)	Phosphorus ($mg\ cm^{-3}$)	Molar C:N[‡]	Molar N:P[‡]
Site	ns	ns	ns	0.02	ns	ns
Habitat	ns	ns	ns	0.02	ns	ns
Site x Habitat	ns	ns	ns	ns	ns	ns
Depth	ns	ns	ns	0.004	ns	ns
Site x Depth	<0.0001	<0.0001	0.0005	<0.0001	ns	<0.0001
Habitat x Depth	ns	0.03	ns	ns	ns	0.04
S x H x D[§]	ns	ns	ns	ns	ns	ns

[†]Section analysis incorporated each individual depth section from 0 to 30 cm for a total of 15 depths
[‡]Molar carbon to nitrogen ratio (C:N), molar nitrogen to phosphorus ratio (N:P)
[§]S x H x D = three-way interaction effect between site, habitat, and depth
[¶]Mean analysis compared mean of 0 to 10 cm to the mean of 20 to 30 cm
[#]ANOVA p values from SAS PROC Mixed, ns = not significant ($p > 0.05$)

annual, and decadal-scale rates. Furthermore, their results showed no significant difference in accretion between *Spartina* and *Avicennia* habitats. To facilitate interpretation of my data, I divided soil profiles from the Bay and Port sites into two periods, recent soil development (0-10 cm) and early soil development (20-30 cm), with a transitional period in between. I assumed similar accretion rates between the habitats of *Spartina* and *Avicennia*. Based on annual (1 cm yr^{-1}) and decadal-scale (0.6 cm yr^{-1}) accretion rates for the Fourchon region, early soil development likely covers the 1960s to the 1980s (hereafter referred to as soil development prior to 1990), while recent soil development spans the 1990s to 2009 (hereafter referred to as soil development post 1990) (Castañeda-Moya et al. 2005; Perry and Mendelssohn 2009).

Soil Development Prior to 1990

At the Bay site, soil development prior to 1990 (20-30 cm) exhibited a signature characteristic of a coastal Louisiana wetland, with low bulk density and high organic matter content (Figure 2.3). Both bulk density and organic matter content in these soils were within the range of values previously published for salt marshes (DeLaune et al. 1981, 1989; Patterson and Mendelssohn 1991; Perry and Mendelssohn 2009). While few studies specific to the region have measured nitrogen and phosphorus content, my values are on the same order of magnitude as those reported by DeLaune et al. (1981) (mean N: 2.8 ± 0.075 mg g^{-1}, mean P: 0.4 ± 0.003 mg g^{-1}). The relatively high bulk density and low organic matter and nitrogen content in these soils in comparison to previous studies may be attributed to the close proximity of the Bay site to the Gulf of Mexico and a disproportionate contribution of marine sediment to the site (Hatton et al. 1983).

Vegetation surveys as early as 1932 document the presence of *Avicennia* in Louisiana *Spartina* marshes; however these populations were sparse in their occurrence and primarily

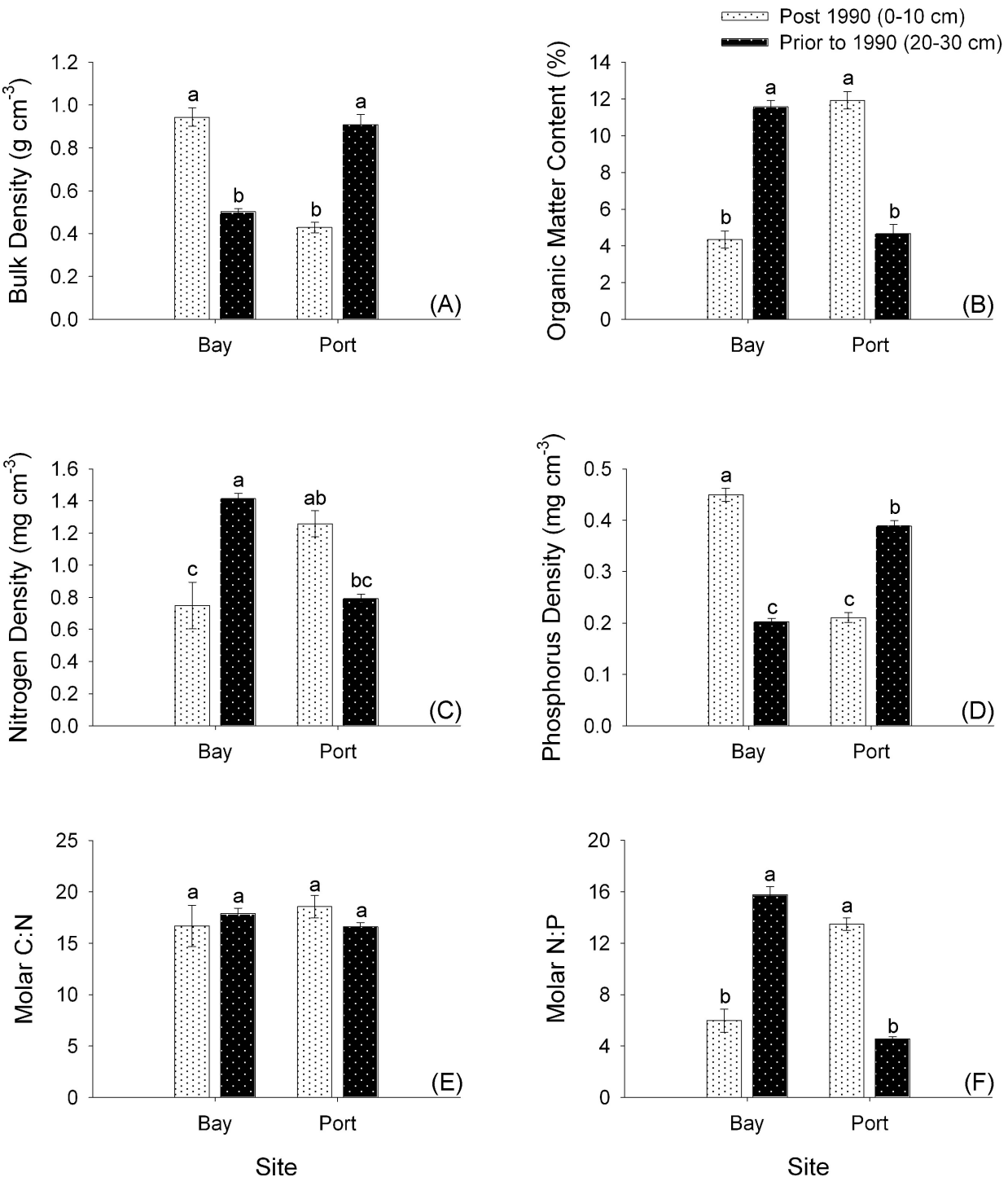

Figure 2.3. Mean (± 1 SE) (A) bulk density, (B) organic matter content, (C) nitrogen density, (D) phosphorus density, (E) molar C:N, and (F) molar N:P in top 10 cm (post 1990) and 20 to 30 cm (prior to 1990) of Bay and Port sites. Lowercase letters correspond to ANOVA results from SAS PROC Mixed. Within figures, means (Bay $n = 6$; Port $n = 5$) followed by the same letter are not significantly different according to Tukey's HSD ($p > 0.05$).

restricted to the southernmost portion of the state and the barrier islands (Penfound and Hathaway 1938). After analyzing aerial photographs and Landsat images of Lafourche Parish from 1952, 1957, and 1972, I determined that *Avicennia* was not present in the Bay site during these years (Figure 2.4 and 2.5). And while there may have been a few individuals of *Avicennia* at this site in 1983, the dominant vegetation type in both the *Spartina* and *Avicennia* habitats was likely *Spartina* (Figure 2.5). The absence of *Avicennia* from the Bay site would explain the similarities in soil development between the two habitat types prior to 1990.

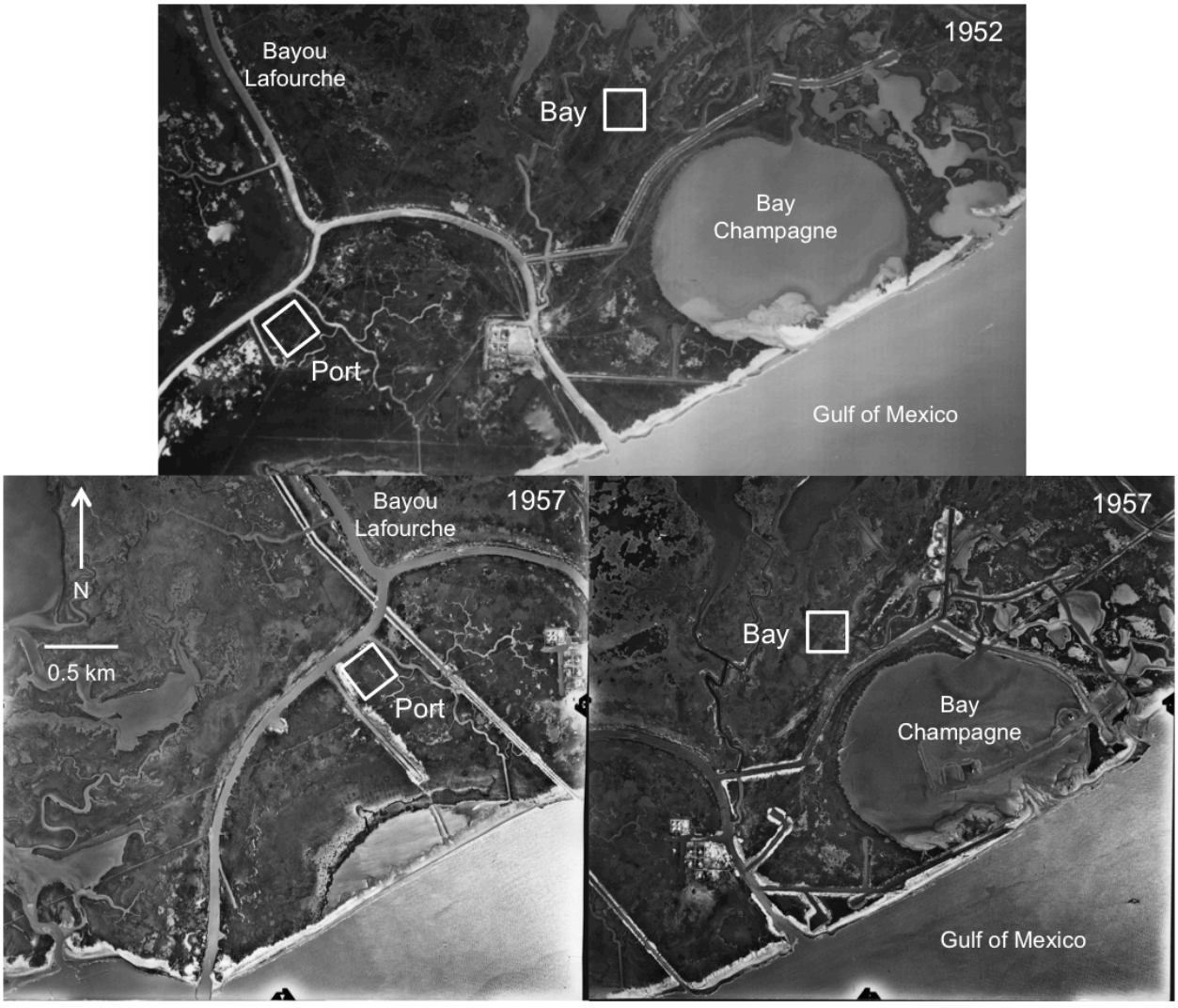

Figure 2.4. Sampling locations in 1952 and 1957. Images courtesy of the Louisiana State University Map Library. Wetlands in gray were dominated by *Spartina*, wetlands black were dominated by *Avicennia*. White areas were non-vegetated sediment.

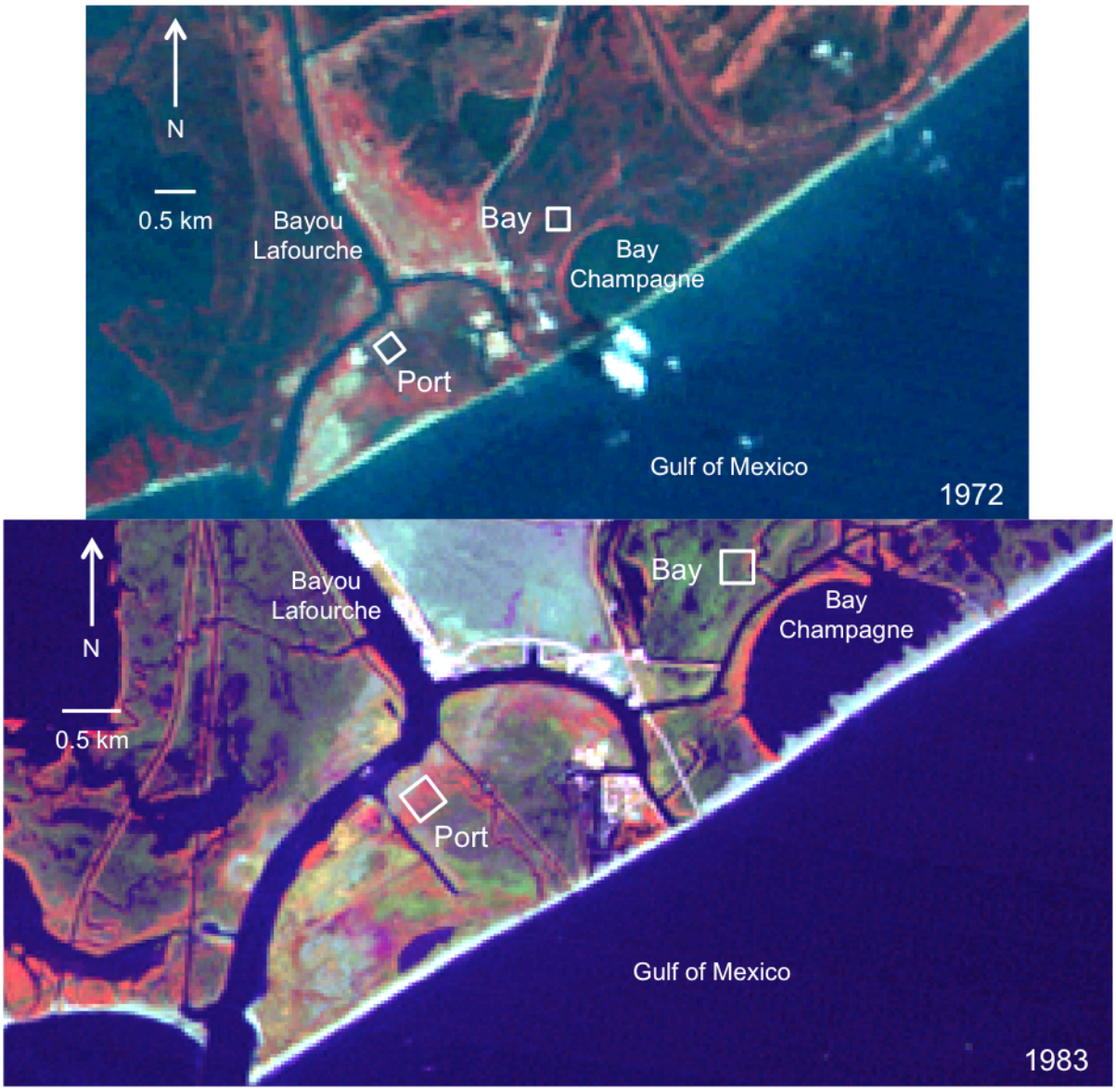

Figure 2.5. Sampling locations in 1972 and 1983. Images courtesy of the United States Geological Survey. Note difference in scale between 1972 and 1983. Wetlands in light gray-pink were dominated by *Spartina*, wetlands bright pink were dominated by *Avicennia*. White areas were non-vegetated sediment.

Soil development prior to 1990 (20-30 cm) in the Port site drastically contrast that of the Bay site discussed above (Figure 2.3). The Port soil profile from this time period was enriched in mineral sediment exhibiting a signature more typical of dredge spoil or storm surge deposits. While the composition of storm deposits (and dredge spoil) can vary depending on sediment origin, they are typically dominated by mineral material with high bulk densities (> 0.8 g cm^{-3})

and low organic matter content (< 10%) (Cahoon et al. 1995; Nyman et al. 1995; Ford et al. 1999; Turner et al. 2006).

The Port site is located just south of Bayou Lafourche, which is a major transportation channel for vessels servicing the Gulf of Mexico petroleum industry from the Port of Fourchon. Frequent hydraulic dredging from the Flotation Canal to Belle Pass maintains these lower reaches at 24 to 30 feet; and up until the late 1990s, the spoil was pumped into disposal areas along the channel banks (Ted M. Falgout, Ted M. Falgout & Associates, written communication, October 5, 2011). Aerial photographs of the Port site from 1952 and 1957 show the construction of at least two additional canals with distinct spoil banks less than a half kilometer from the Port site (Figure 2.4). The natural creeks bisected by these canals may have facilitated transport and redistribution of dredge spoil into the Port site. The 1972 and 1983 Landsat images provide further evidence of dredge spoil in the wetlands neighboring the Port site during this time period (Figure 2.5). While a conscious effort was made to avoid locating my sites on visible levees and spoil banks, it appears as though the Port cores were sampled on remnant dredge spoil. The aerial photographs and Landsat images from 1972 and 1983 show the presence of *Avicennia* in the Port site during this time period (Figure 2.5). However, the high input of mineral sediment seems to have concealed any potential differences between *Spartina* and *Avicenna* soil development prior to 1990.

Soil Development Post 1990

From 1990 to 2009 (0-10 cm), mineral sediment deposition dominated the Bay site soil profile. Bulk density and phosphorus content were significantly higher, and organic matter and nitrogen content were significantly lower than soils deposited prior to 1990 (Figures 2.3). All the soil variables measured were outside the range reported for Louisiana salt marsh soils and

more representative of dredge spoil or storm surge deposits (DeLaune et al. 1981; Nyman et al. 1995; Ford et al. 1999; Perry and Mendelssohn 2009 among others).

In the absence of fluvial inputs, episodic events such as winter cold fronts and summer tropical storms and hurricanes dominate allochthonous sediment deposition in the Mississippi River delta (Turner et al. 2006, 2007). From 1991 to 2009, approximately 18 tropical storms or hurricanes made landfall in coastal Louisiana, 14 of which occurred between 1999 and 2009 (National Hurricane Center 2011). Significant sediment deposition in wetlands throughout the delta plain was documented with the passage of Hurricane Andrew in 1992 (Cahoon et al. 1995; Nyman et al. 1995). Directly impacting the Fourchon region were Hurricanes Lili (2002), Katrina and Rita (2005), and Gustav and Ike (2008) (Liu et al. 2011). The storm surge during these events breached the sand barrier between Bay Champagne and the Gulf of Mexico depositing prominent overwash fans behind the barrier beach (Liu et al. 2011). A soil core collected from a wetland directly adjacent to the Bay site after the passage of Katrina and Rita showed approximately 5 cm of storm-derived sediment deposition (Castañeda-Moya et al. 2005). More recently, the 2008 combination of Gustav and Ike completely inundated the coastal plain and caused extensive flooding in the Fourchon region (Liu et al. 2011). These results support my hypothesis that the mineral rich composition observed in soils at the Bay site from 1990 to 2009 corresponds to a period of enhanced storm activity in coastal Louisiana.

It is important to note that the distance today from the Bay site across Bay Champagne to the Gulf of Mexico is approximately half the distance that it was in 1952 (Figures 2.1 and 2.4). Furthermore, marsh deterioration during the last 10 years opened a direct path from Bay Champagne to the Bay site (Figures 2.1 and 2.6). The Bay site is situated to receive large amounts of reworked Bay Champagne and near-shore sediment during winter cold fronts and

Figure 2.6. Sampling locations in 1991 and 1998. Images courtesy of the United States Geological Survey and the Louisiana Department of Natural Resources. Wetlands in brown (1991) and green (1998) were dominated by *Spartina*, wetlands in orange were dominated by *Avicennia*. White areas were non-vegetated sediment.

summer storm events due to its unprotected position. Images from 1998 and 2008 show the presence of *Avicennia* in the Bay site; however, physical storm events appear to be the dominant signal following 1990 obscuring any subtle differences in soil development between *Spartina* and *Avicennia* (Figures 2.1 and 2.6).

Post 1990 soil development (0-10 cm) at the Port site exhibited a signature more typical of a Louisiana salt marsh. Bulk density and phosphorus content were significantly lower, and organic matter and nitrogen content were significantly higher than in soils deposited prior to 1990 (Figure 2.3). The significantly lower contribution of mineral sediment from 1990 to 2009 in soil from the Port site (relative to the Bay site) during a period of high storm activity can be attributed to the more protected location of this site from the Gulf of Mexico. Despite the loss of nearly 1 km of shoreline between 1952 and 2008, there is still > 1 km of wetland between the Port site and the Gulf of Mexico (Figures 2.1 and 2.4). Thus, it appears as though the influence of storm events on soil development was not significant in the Port site as it was in the Bay site.

Several severe winters in the 1980s killed most of the *Avicennia* populations in the Fourchon region (Patterson and Mendelssohn 1991; Perry and Mendelssohn 2009). Since 1989, mild winters have allowed populations of *Avicennia* to expand back into areas of *Spartina* marsh. At the Port site, *Avicennia* began recolonizing the *Spartina* marshes in the 1990s and was firmly established by 2008 (Figures 2.1 and 2.6). However, even after nearly two decades of *Avicennia* presence at this site, I found no significant difference between soil stratigraphy from the *Spartina* and *Avicennia* habitats.

Throughout the entire study, the only significant differences observed between *Spartina* and *Avicennia* were in elevation and phosphorus density; *Avicennia* occurred at higher elevations in soils with greater phosphorus content. Previous research in the salt marsh-mangrove ecotone observed similar differences in elevation between *Avicennia* and *Spartina* that coincided with significantly lower soil moisture and higher oxidation-reduction (redox) potential in *Avicennia* habitats (Patterson and Mendelssohn 1991; Perry and Mendelssohn 2009). Redox potential as well as the availability of sulfate and iron strongly influence the geochemical cycling of

phosphorus in both salt marsh and mangrove wetlands (see Tobias and Neubauer 2009 and Twilley and Rivera-Monroy 2009 for comprehensive reviews). As redox potential increases and oxidizing conditions become more prominent, dissolved phosphate adsorbs onto iron oxyhydroxides decreasing concentrations of soluble reactive phosphate in the porewater and increasing total phosphorus in the soil. Therefore, the significantly greater phosphorus density observed in the *Avicennia* soils was likely caused by the more oxidizing conditions in this habitat.

The shift from *Spartina* to *Avicennia* had no effect on soil bulk density, organic matter content, or nitrogen content. My data did show significantly higher phosphorus densities in *Avicennia* habitats, which were likely caused by the occurrence of *Avicennia* at higher elevations in more oxidizing soils. The significant differences between the Bay and Port sites observed throughout the chronology can be attributed to a combination of autochthonous soil development and allochthonous deposition from both natural and anthropogenic disturbances. In conclusion, the initial expansion of *Avicennia* does not appear to be affecting soil development in the highly disturbed region of Fourchon, Louisiana. However, if the warming trend of the last several decades continues and populations of *Avicennia* increase in structural complexity (e.g. basal area, tree height, and density) or areal coverage, there may be the potential for changes in wetland soil chemistry during soil development provided a low occurrence of physical disturbance from storm events and human activities.

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CHAPTER 3
INORGANIC NUTRIENT CYCLING IN A COASTAL LOUISIANA WETLAND DURING A CLIMATE-INDUCED VEGETATION SHIFT FROM SALT MARSH TO MANGROVE

INTRODUCTION

Southern Louisiana is a highly dynamic and heavily impacted river-dominated deltaic coast. Over the last 7,000 years, sediment delivery from the Mississippi River formed the deltaic and chenier plains creating the largest area of coastal wetlands in the contiguous United States (Fisk et al. 1954; Gould and McFarlan 1959). Historically, wetlands in the delta plain were sustained by the delivery of sediment, nutrients, and freshwater during river-pulsing events (Day et al. 2000; Twilley and Rivera-Monroy 2009a). However today, an extensive network of water control structures, oil and gas infrastructure, and nutrient enrichment have led to dramatic rates of wetland loss and water quality deterioration throughout the region (Boesch et al. 1994; Day et al. 2000; Ko and Day 2004). Coastal Louisiana is also positioned to experience many of the effects associated with global climate change, including changes in temperature, ocean and atmospheric circulation, and sea-level rise (IPCC 2007). In addition, the interaction between climate change and the highly engineered landscape of the Mississippi River delta will likely result in very complex outcomes yet to be predicted (Twilley 2007).

Enhanced eutrophication, harmful algal blooms, and oxygen depletion resulting from excessive nutrient loading are among the most pressing environmental issues affecting many coastal ecosystems (Rabalais 2002; Howarth and Marino 2006). In the northern Gulf of Mexico, nitrate-enhanced primary productivity has been linked to extensive summer hypoxia (dissolved oxygen < 2 mg L^{-1}) (Rabalais et al. 2002; Scavia et al. 2003; Turner et al. 2008). The annual recurrence of these hypoxic events highlights the importance of determining the fate of reactive nitrogen within the Mississippi River basin. Located at the interface between agricultural

uplands and estuarine systems, wetlands can remove a significant portion of reactive nitrogen from the waters of the Mississippi River (Mitsch et al. 2001, 2005). Within wetlands, nitrogen can be retained by macrophyte and microbial assimilation or removed via denitrification (canonical as well as anaerobic ammonium oxidation (anammox)).

In the absence or near absence of oxygen, select heterotrophic prokaryotes use nitrate or nitrite as the terminal electron acceptor during the oxidation of organic matter converting it to nitrous oxide or dinitrogen gas (N_2). This process, referred to as canonical denitrification, is a sink for reactive nitrogen transforming it to a biologically unavailable form thereby removing the nutrient from a system. Denitrification in wetland soils and estuarine sediments can be fueled by nitrate diffusing from the water column (direct denitrification), nitrate formed within the soil through the nitrification of ammonium (coupled nitrification-denitrification), or nitrate advected from the groundwater (Seitzinger 1988). The primary factors influencing denitrification include oxygen concentration, nitrate availability, and organic matter quantity and quality; however, it is the interaction of these and several secondary factors that determine denitrification rates within a system (Seitzinger 1988; Cornwell et al. 1999).

Numerous studies specific to saline wetlands show a positive relationship between denitrification and concentrations of dissolved inorganic nitrogen (Smith et al. 1985; Corredor and Morell 1994; Eriksson et al. 2003 among others) and organic matter quality and quantity (Sherr and Payne 1978; Lee and Joye 2006; Dodla et al. 2008 among others). The relationship between oxygen concentration and denitrification depends on whether the dominate nitrate source is from the water column or from within soil nitrification of ammonium. In a system with high concentrations of water column nitrate, increasing oxygen concentration may inhibit denitrification. Conversely, when soil nitrification is the major source of nitrate, increasing

oxygen concentrations frequently stimulates coupled nitrification-denitrification rates by stimulating nitrification (Rysgaard et al. 1994). Although after nitrification, the nitrate must still diffuse to a nearby hypoxic zone in order for denitrification to occur.

Studies measuring denitrification in vegetated wetland soils versus non-vegetated sediments highlight the important, yet complex role emergent macrophytes play in denitrification. Oxygen diffusion from the roots of aquatic macrophytes can stimulate coupled nitrification-denitrification (Reddy et al. 1989; Risgaard-Petersen and Jensen 1997). Higher rates of direct denitrification in vegetated soils have also been attributed to higher nitrate concentrations (Koch et al. 1992) and root exudates (Sherr and Payne 1978). However, in some systems with higher nitrate concentrations in creeks relative to the wetland, higher rates of denitrification were measured in non-vegetated creek sediments (Kaplan et al. 1979; Eriksson et al. 2003). Furthermore, competition for a nitrogen source between denitrifiers and macrophytes can limit denitrification rates in wetland soils, especially in highly productive systems with low nitrogen availability (Hamersley and Howes 2005).

Perhaps more important than quantifying rates of gross denitrification is understanding the balance between denitrification and the opposing process of nitrogen fixation. Biological nitrogen fixation is the reduction of atmospheric N_2 into a biologically available form, which can then be incorporated into cellular material. This process is performed by specialized prokaryotes containing the nitrogenase enzyme. The formation and maintenance of the nitrogenase enzyme and the reduction of N_2 are energetically expensive processes and as a result nitrogen fixation typically occurs when there is a competitive advantage over assimilation of fixed forms of nitrogen (Howarth et al. 1988a). Biological nitrogen fixation is considered a “new” source of

nitrogen into a system and together with denitrification determines the net flux of nitrogen across the soil-water interface (Howarth et al. 1988b).

Capone (1983) and Howarth et al. (1988b) reviewed rates of benthic nitrogen fixation and they found notably higher rates in salt marsh and mangrove soils relative to non-vegetated sediments. They suggest this activity may constitute a major fraction of the nitrogen demand to these wetland ecosystems. Furthermore, experimental work in both salt marsh and mangrove wetlands has found nitrogen fixation rates may balance or even exceed losses due to denitrification (Nedwell et al. 1994; Kristensen et al. 1998; Davis et al. 2004; Lee and Joye 2006). With the development of new techniques for measuring N_2 fluxes, scientists are now finding ecologically significant rates of nitrogen fixation in systems where it was previously considered unimportant (Capone et al. 2005; Gardner et al. 2006; Fulweiler et al. 2007). Taking all of this into consideration, the contribution of fixation to the nitrogen budget of a wetland system should not be ignored.

Coastal Louisiana has a rich history of denitrification research (see Rivera-Monroy et al. 2010 for a comprehensive review). Studies measuring denitrification rates cover a diverse range of ecological settings including agricultural soils, bottomland hardwood forests, bald cypress swamps, and fresh, brackish, and saltwater marshes and benthic sediments (Rivera-Monroy et al. 2010). Techniques used to directly measure or indirectly estimate denitrification include the acetylene block technique, a variety of ^{15}N isotopic tracer techniques, nitrous oxide emission, nitrate uptake, mass balance, and stoichiometry (Rivera-Monroy et al. 2010). The majority of this research has focused on the potential of wetland soils and sediments to denitrify under experimentally enriched nitrate and ammonium concentrations (Rivera-Monroy et al. 2010). Soils and sediments enriched with nitrate have a high potential for denitrification (> 1000 μmol

N m^{-2} h^{-1}); however, ambient rates (< 100 μmol N m^{-2} h^{-1}) are at the lower end of the range reported for other aquatic systems (Seitzinger 1988; Steingruber et al. 2001; Hopkinson and Giblin 2008; Twilley and Rivera-Monroy 2009b). In contrast to the rich history of denitrification research in coastal Louisiana, only three studies have measured rates of nitrogen fixation (Casselman et al. 1981; DeLaune et al. 1986; DeLaune and Patrick 1990). These studies report low nitrogen fixation rates that are comparable to ambient rates of denitrification in coastal Louisiana (< 100 μmol N m^{-2} h^{-1}; Casselman et al. 1981; DeLaune et al. 1986; DeLaune and Patrick 1990).

A Climate-Induced Vegetation Shift

The recent climate-induced expansion of black mangroves (*Avicennia germinans* L.) into smooth cordgrass (*Spartina alterniflora* Loisel) marshes along the northern Gulf of Mexico is well documented (Sherrod and McMillan 1985; Day et al. 2005; Stevens et al. 2006; Perry and Mendelssohn 2009; Chapter 2). Studies have examined controls on plant zonation (Patterson et al. 1993, 1997) as well as differences in soil physicochemical variables (Patterson and Mendelssohn 1991), ecosystem function (Perry and Mendelssohn 2009), and soil development (Chapter 2). To date, no research has measured nutrient cycling in the salt marsh-mangrove ecotone. However, important differences exist between these habitat types that are likely to affect inorganic nutrient retention and removal in the Mississippi River delta.

Previous studies measured more oxidized conditions in *Avicennia* soils relative to *Spartina* soils and suggested a link between higher elevations, better drainage, lower soil moisture, and higher oxidation-reduction (redox) potential (Patterson and Mendelssohn 1991; Perry and Mendelssohn 2009). In addition to differences in elevation, root morphology may contribute to the observed disparities in redox potential. Root architecture differs considerably

between these two species. *Avicennia* has an extensive underground cable root system that is linked to aerial roots (pneumatophores). The pneumatophores are covered in specialized cells (lenticels) that facilitate gas exchange and increase oxygen transport to the roots that can then be released to the surrounding soils (Scholander et al. 1955; McKee et al. 1988). In contrast, the roots of *Spartina* lack pneumatophores and oxygen transport must occur through the leaves and stem to get to the rhizome and fine roots, where oxygen can also be released to the soil (Teal and Kanwisher 1966). This differentiation in root morphology may result in species-specific gas exchange capacities and soil redox potentials as well as differences in labile root exudates. Shifts in soil redox conditions as well as the labile organic matter released from the roots have the potential to alter net N_2 and inorganic nutrient fluxes.

Here I present the first net N_2 flux measurements for coastal Louisiana, determining the balance between nitrogen fixation and denitrification. I investigate the effect of *Avicennia* expansions on inorganic nutrient cycling within *Spartina* marsh habitats of southern Louisiana. By comparing net N_2, oxygen, and dissolved inorganic nutrient (NO_2^-, NO_3^-, NH_4^+, PO_4^{-3}) fluxes in *Spartina* and *Avicennia* habitats, I address the following questions: (1) Does the shift from *Spartina* to *Avicennia* alter the balance between nitrogen fixation and denitrification? (2) Do preexisting differences in marsh elevation, hydroperiod, and soil nutrient content modify the effect of this shift on inorganic nutrient cycling? In the nitrate-nitrogen enriched region of the Mississippi River delta, I anticipated that net N_2 fluxes throughout the salt marsh-ecotone would indicate greater rates of denitrification than nitrogen fixation. Based on differences in root morphology, I hypothesized that the shift from *Spartina* to *Avicennia* would increase soil redox potential and subsequently decrease nitrate uptake and denitrification. I expected high soil redox

potential and low organic matter content to inhibit denitrification in the higher elevation regions of the salt marsh-mangrove ecotone that are more exposed to the Gulf of Mexico.

MATERIALS AND METHODS

Study Area and Sampling Locations

The study was conducted in Fourchon, Louisiana in the wetlands directly east of Bayou Lafourche and north of the Gulf of Mexico (Figure 3.1). The Fourchon region is located in the Barataria basin system and formed as part of the Lafourche lobe of the Mississippi River delta plain 2500 to 800 years BP (Roberts 1997; Coleman et al. 1998). Climate in the region is humid subtropical (Peel et al. 2007), with mean monthly temperatures between 6 and 30 °C and mean annual precipitation of 160 cm (National Climatic Data Center 2012). Flow from the Mississippi River into Bayou Lafourche is highly restricted and as a result diurnal microtides in conjunction with meteorological events control hydroperiod throughout the year (Zetler and Hansen 1972).

Spartina and *Avicennia* grow sympatrically in Louisiana's coastal wetlands. *Spartina* dominates during periods of harsh winters with below freezing temperatures, while mild winters favor the expansion of *Avicennia*. The last hard freeze in the region occurred in December 1989 and killed most of the *Avicennia* in southern Louisiana (Day et al. 2005; Perry and Mendelssohn 2009; Chapter 2). Since then, two decades of freeze-free winters have allowed populations of *Avicennia* to expand back into areas of *Spartina* marsh. *Avicennia* are now common throughout the *Spartina* marshes of southern Louisiana and can be found up to 15 km north of the Gulf of Mexico. In Louisiana, *Avicennia* tree heights are generally less than 2.5 m due to the occasional frost and reduced number of degree days (Lugo and Zucca 1977; Chen and Twilley 1998).

Two sites were selected in Fourchon, each containing monospecific stands of *Spartina* and *Avicennia* (Figure 3.1). The Bay site (29°07'14" N 090°10'54" W) was located northwest of

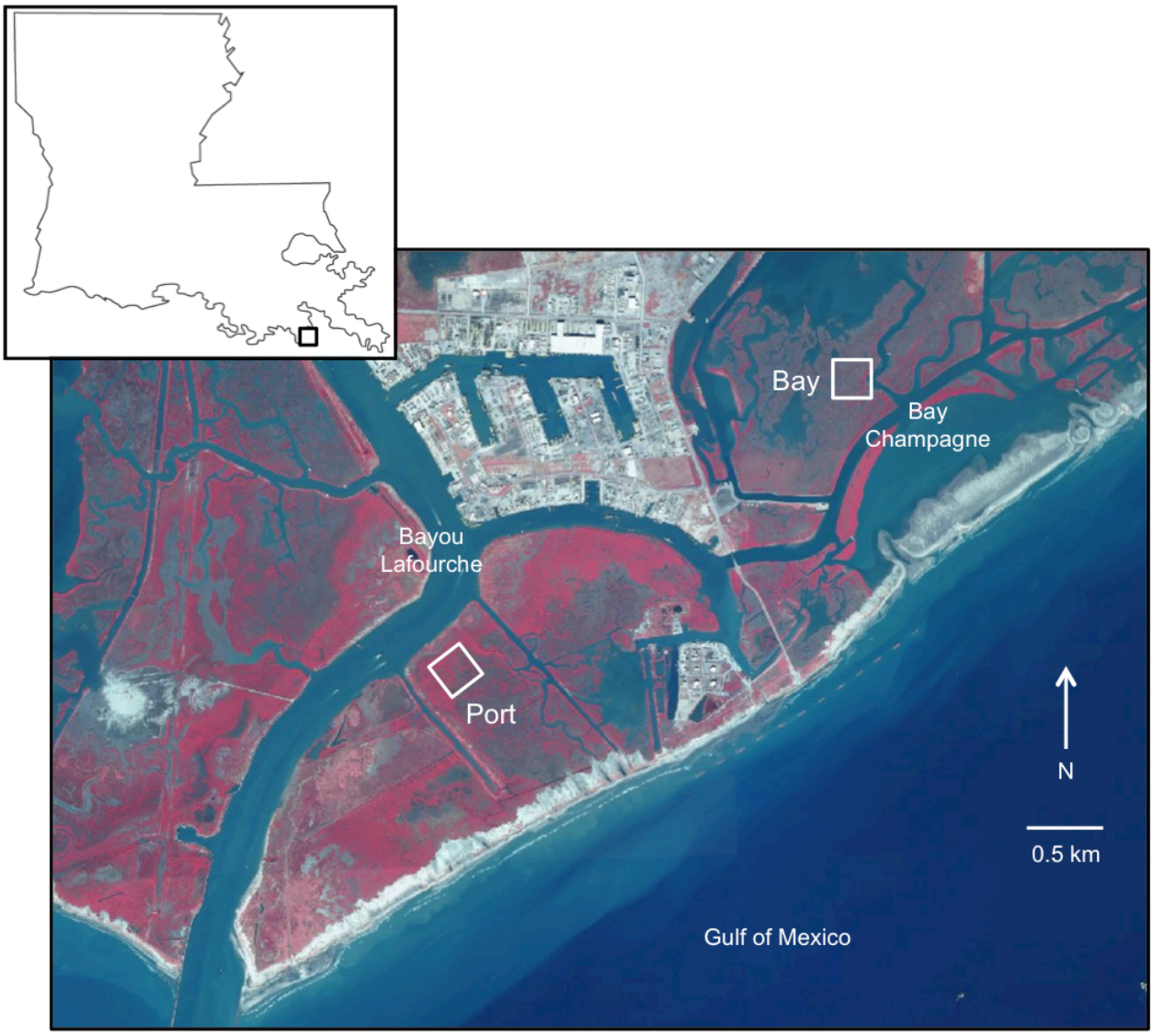

Figure 3.1. Sampling locations in Fourchon, Louisiana. Image courtesy of the Louisiana Department of Natural Resources. Wetlands in light pink are dominated by *Spartina* and wetlands in dark pink are dominated by *Avicennia.*

Bay Champagne and the Port site (29°06'21" N 090°12'30" W) was located south of Port Fourchon and east of Bayou Lafourche. These sites differ in their exposure to winds and waves from the Gulf of Mexico as well as their elevation, hydroperiod, and soil chemistry (Chapter 2). The higher elevation Bay site experiences a lower duration of inundation, but a higher flood frequency than the Port site (Chapter 2). Surface (0 to 10 cm) soil in the more exposed Bay site is dominated by mineral material, high in bulk density and phosphorus content, and low in

organic matter and nitrogen content (Chapter 2). Soils in the top 10 cm of the more protected Port site are enriched in organic matter, with low bulk density and phosphorus content (Chapter 2).

Field Sampling

In the winter (December 2009) and summer (August 2010), I collected porewater samples and soil cores from *Spartina* and *Avicennia* habitats within each site. Triplicate 60 mL porewater samples were collected at a depth of 10 cm from each habitat. I measured porewater temperature, salinity, and pH in the field, with a Hach HQ Series portable meter. Five mL of porewater were then added to an equal volume of antioxidant buffer and analyzed for hydrogen sulfide concentration (H_2S) within 24 hours using a micro mono ion sulfide electrode (McKee et al. 1988). The remaining 55 mL of porewater were filtered with a Whatman GF/F glass fiber filter (0.7 μm) and frozen for later analysis of dissolved inorganic nitrogen (nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+)) and dissolved inorganic phosphate (PO_4^{-3}) (analytical techniques described below). I measured soil oxidation-reduction (redox) potential at a depth of 10 cm, with a platinum electrode. Redox potential was measured using a portable volt meter, connected to a saturated calomel reference electrode, and pushed into the surface soil approximately 2 to 3 cm. The multi-depth platinum electrodes were standardized in a quinhydrone solution buffered to pH 4 (Hargis and Twilley 1994).

Triplicate, intact soil cores (10 cm internal diameter by 10 cm depth) were collected from each habitat, placed in a cooler, and transported to an environmental growth chamber at Louisiana State University. Care was taken to avoid aboveground vegetation; yet roots, rhizomes, and an occasional pneumatophore were captured in the cores. Once in the chamber, cores were placed in a water bath and pre-incubated for 24 to 48 hours with air gently bubbling

through the overlying water. Chamber conditions were maintained at ambient field temperature (winter: 15 °C, summer: 29 °C) and in continuous darkness during the pre-incubation period. Surface water was collected from Bayou Lafourche at the Louisiana Universities Marine Consortium's (LUMCON) Fourchon research facility. This water was filtered immediately to 0.5 μm (winter) or 0.2 μm (summer) and stored at ambient field temperature, in continuous darkness.

Soil Core Incubations

Before beginning the dark portion of the core incubation to measure dissolved gas fluxes (hereafter referred to as the gas incubation), the overlying water from each soil core was carefully replaced with the filtered water from Bayou Lafourche. Cores were sealed with gas-tight lids, which contained magnetic stirrers to gently mix (~55 rpm) the overlying water. I collected replicate water samples for N_2:Ar dissolved gas analysis into 12 mL exetainers (© Labco Limited) at five sampling intervals evenly spaced throughout the incubation. These samples were immediately preserved with 250 μL $ZnCl_2$ (50% w/v; Nielsen and Glud 1996) and stored underwater, in the dark, at incubation temperature until analysis. I measured initial and final dissolved oxygen concentrations in the overlying water with a Hach LDO101-01 dissolved oxygen probe. These measurements were used to determine the duration of core incubations using the time required for oxygen concentrations to decrease by approximately 2 ppm (Giblin et al. 1997; Fulweiler et al. 2008). At the end of the dark portion of the gas incubation, the light portion of the gas incubation commenced by providing light levels of approximately 300 μmol m^{-2} s^{-1} (LI-COR 1400). Cores remained sealed during the transition from dark to light conditions and overlying water was not replaced. Cores were incubated in a dark to light cycle without unsealing in order to lower oxygen gas pressure in the water column and avoid bubble formation

(Eyre and Ferguson 2002). I measured initial and final dissolved oxygen concentrations and collected N_2:Ar samples at approximately the same five time intervals during the light portion of the gas incubation.

After completing the dark and light portions of the gas incubation, cores were left to re-equilibrate in the dark for approximately 12 hours with air gently bubbling into the overlying water. The following day, I replaced the overlying water, resealed the cores, and began measurements of dissolved inorganic nutrient fluxes (hereafter referred to as the nutrient incubation). Temperature (winter: 15 °C, summer: 29 °C) and light (dark/light cycle) conditions during the nutrient incubation were identical to those of the gas incubation. During the nutrient incubation, water samples were collected at six (three dark and three light, winter) or ten (five dark and five light, summer) sampling intervals evenly spaced throughout the incubation. Inorganic nutrient samples were filtered either with a Whatman GF/F glass fiber filter (0.7 μm, winter) or a Cole-Parmer RC-membrane filter (0.45 μm, summer) and then frozen for later analysis of NO_2^-, NO_3^-, NH_4^+, and PO_4^{-3}.

Analytical Techniques

Overlying water samples from the cores were assayed for N_2:Ar dissolved gas concentrations with a Pfeiffer Prisma QME 200 quadrupole membrane inlet mass spectrometer (Bay Instruments, Easton, Maryland) using the technique of Kana et al. (1994) modified with a copper reduction column and furnace heated to 600 °C (Eyre et al. 2002). This modification removes oxygen from the dissolved gas samples, thereby eliminating the potential for an oxygen effect on N_2 measurements and subsequently increasing sample precision to ± 0.01% (Eyre et al. 2002). Dinitrogen concentrations were determined for each sample by multiplying the N_2:Ar ratio by the Ar concentration at air saturation (Colt 1984). Filtered porewater and nutrient

incubation water samples were analyzed colorimetrically for NO_2^-, NO_3^-, NH_4^+, and PO_4^{-3} using a Flow IV OI Analytical Autoanalyzer (Strickland and Parsons 1972; Parsons et al. 1984).

I calculated N_2 and dissolved inorganic nutrient fluxes across the soil-water interface from the slope of a 3-point (winter incubations of dissolved inorganic nutrients) or 5-point linear regression of concentration as a function of time. To calculate the flux, the slope was multiplied by the headspace water volume and then divided by core surface area. If the relationship between analyte concentration and time was nonlinear ($r^2 < 0.65$), the slope and subsequently the flux were considered zero (Prairie 1996). Negative fluxes denote soil consumption or uptake, while positive fluxes denote soil production or release. Since the N_2:Ar ratio is a measure of the net N_2 flux (gross denitrification – gross nitrogen fixation), a positive flux equals net denitrification and a negative flux equals net nitrogen fixation. A major limitation of the N_2:Ar technique is the inability to differentiate between N_2 production pathways (e.g. direct denitrification, coupled nitrification-denitrification, and anammox) (Fulweiler et al. 2008).

Statistical Analysis

Porewater data were analyzed with a two-way analysis of variance (ANOVA) to test for differences between sites (Bay and Port) and habitat types (*Spartina* and *Avicennia*). A three-way ANOVA with repeated measures was used to determine if differences existed between sites, habitat types, or light conditions (dark and light) for all dissolved gas and inorganic nutrient fluxes. For both the porewater and the flux analyses, I considered site, habitat type, and light condition fixed effects. Interaction effects were considered for all analyses and pairwise comparisons among treatments were described with a Tukey's honestly significant difference (HSD) test. I was unable to test for differences between my winter and summer data due to insufficient replication of my site by habitat interaction effect. Therefore, I present and discuss

these results separately. All statistical analyses were performed with SAS PROC Mixed and significance was assessed at the 0.05 level (SAS Institute 2012).

RESULTS

Winter 2009

During the winter sampling event, mean porewater temperature was 15.4 ± 0.8 °C. Porewater salinities ranged from 21.2 to 34.9 ‰, with significantly higher values in the *Avicennia* habitats (Table 3.1). There was no significant effect of site or habitat type on soil redox potential; however, some general trends were observed (Table 3.1). Soils at the Bay site

Table 3.1. Winter 2009 means and the effects of site and habitat type on porewater chemistry in the salt marsh-mangrove ecotone of Fourchon, Louisiana.

		Means[†]				ANOVA Results[‡]		
		Site		Habitat		Site	Habitat	S x H[§]
		Bay	Port	*Spartina*	*Avicennia*			
Salinity	X	29.90	28.38	27.08	31.20	ns	0.03	0.05
‰	(SE)	(0.59)	(2.20)	(1.41)	(1.32)			
pH	X	7.10	7.78	7.45	7.42	0.0001	ns	ns
	(SE)	(0.04)	(0.08)	(0.15)	(0.18)			
Redox	X	50.33	-87.75	-53.67	16.25	ns	ns	ns
mV	(SE)	(61.09)	(53.86)	(51.07)	(73.78)			
H_2S	X	1.56	3.42	3.69	0.86	0.0006	<0.0001	0.009
mM	(SE)	(0.46)	(0.90)	(0.55)	(0.24)			
NO_2^-	X	0.40	0.43	0.42	0.41	ns	ns	ns
µM	(SE)	(0.01)	(0.05)	(0.04)	(0.02)			
NO_3^-	X	0.00	0.63	0.37	0.07	0.03	ns	ns
µM	(SE)	(0.00)	(0.22)	(0.20)	(0.07)			
NH_4^+	X	117.61	139.48	229.18	5.60	ns	0.06	ns
µM	(SE)	(86.89)	(68.10)	(78.36)	(0.99)			
PO_4^{-3}	X	23.50	36.42	36.83	20.42	ns	ns	0.01
µM	(SE)	(10.72)	(10.25)	(6.48)	(14.16)			

[†]Mean at 10 cm, standard error in parenthesis (n = 6)
[‡]ANOVA p values from SAS PROC Mixed, ns = not significant ($p > 0.05$)
[§]S x H = two-way interaction effect between site and habitat

were more oxidized, as were soils in the *Avicennia* habitats. Both site and habitat type had a significant effect on porewater H_2S concentrations (Table 3.1). Mean H_2S concentrations were significantly lower at the Bay site than the Port site and significantly lower in the *Avicennia* habitats relative to the *Spartina* habitats. Porewater NH_4^+ concentrations were highly variable ranging from 2.7 to 546.5 μM, with significantly lower concentrations in the *Avicennia* habitats (Table 3.1). Porewater PO_4^{-3} concentrations were high in all site by habitat combinations (range: 15.8 to 73.4 μM) except the *Avicennia* habitat at the Bay site (mean < 1.0 μM). Porewater NO_2^- and NO_3^- concentrations were low (< 1.0 μM) in both sites and habitat types.

During the winter incubations, temperature and salinity in the water overlying the soil cores was 15 °C and 23 ‰, respectively. Mean net N_2 fluxes across the soil-water interface for all sites and habitat types were positive, indicating net denitrification (Table 3.2, Figure 3.2). Mean net denitrification rates at the Bay site (19.8 ± 5.7 μmol N m^{-2} h^{-1}) were significantly lower than at the Port site (49.5 ± 9.4 μmol N m^{-2} h^{-1}). In addition, only the Port site exhibited diurnal variability in net denitrification, with slightly lower rates during the light incubation. Nitrate fluxes were dominated by uptake into the soil (Table 3.2, Figure 3.2). Similar to net denitrification, NO_3^- uptake was significantly lower in soil cores from the Bay site relative to the Port site (means: -5.2 ± 1.5 and -69.5 ± 7.5 μmol m^{-2} h^{-1}, respectively). Mean oxygen fluxes varied over the dark/light cycle, with oxygen consumption in the dark (-47.7 ± 7.0 mg m^{-2} h^{-1}) and production in the light (8.9 ± 3.9 mg m^{-2} h^{-1}; Table 3.2). Ammonium fluxes exhibited diurnal variability (Table 3.2). During the dark incubation, most soil cores showed a release of NH_4^+, which then switched to uptake during the light incubation. In the winter, mean NO_2^- and PO_4^{-3} fluxes were low (< 1.0 μmol m^{-2} hr^{-1}) across all site, habitat, and light treatments (Table

3.2). I found no significant difference between *Spartina* and *Avicennia* habitats in any of the dissolved gas or inorganic nutrient fluxes measured (Table 3.2).

Table 3.2. Winter 2009 means and the effects of site, habitat, and light on dissolved gas and inorganic nutrient fluxes in the salt marsh-mangrove ecotone of Fourchon, Louisiana.

Means[†]							
		Site		Habitat		Light	
		Bay	Port	*Spartina*	*Avicennia*	Dark	Light
N_2-N	X	19.82	49.54	33.08	36.28	43.66	25.70
μmol m^{-2} h^{-1}	(SE)	(5.74)	(9.41)	(7.68)	(10.11)	(10.77)	(5.56)
O_2	X	-14.80	-23.99	-22.79	-16.00	-47.72	8.93
mg m^{-2} h^{-1}	(SE)	(9.44)	(14.05)	(11.20)	(12.80)	(6.96)	(3.86)
NO_2^-	X	0.10	0.32	0.58	-0.16	0.47	-0.05
μmol m^{-2} h^{-1}	(SE)	(0.09)	(0.43)	(0.27)	(0.31)	(0.40)	(0.16)
NO_3^-	X	-5.15	-69.50	-38.13	-36.53	-40.47	-34.19
μmol m^{-2} h^{-1}	(SE)	(1.51)	(7.49)	(10.81)	(11.39)	(12.66)	(9.20)
NH_4^+	X	-3.44	26.28	10.01	11.61	27.24	-4.31
μmol m^{-2} h^{-1}	(SE)	(11.61)	(13.23)	(16.90)	(6.31)	(13.72)	(10.90)
PO_4^{-3}	X	-0.65	-0.22	-0.67	-0.19	-0.05	-0.86
μmol m^{-2} h^{-1}	(SE)	(0.35)	(0.23)	(0.35)	(0.23)	(0.24)	(0.33)

ANOVA Results[‡]						
	N_2-N	O_2	NO_2^-	NO_3^-	NH_4^+	PO_4^{-3}
Site	0.01	ns	ns	<0.0001	ns	ns
Habitat	ns	ns	ns	ns	ns	ns
Site x Habitat	ns	ns	ns	ns	ns	ns
Light	ns	ns	ns	ns	ns	ns
Site x Light	0.05	ns	ns	ns	ns	ns
Habitat x Light	ns	ns	ns	ns	ns	ns
S x H x L[§]	ns	ns	ns	ns	ns	ns

[†]Means from winter sampling event, standard error in parenthesis (n = 12)
[‡]ANOVA p values from SAS PROC Mixed, ns = not significant ($p > 0.05$)
[§]S x H x L = three-way interaction effect between site, habitat, and light

Summer 2010

In the summer, mean porewater temperature was 30.0 ± 0.4 °C and porewater salinities ranged from 25.1 to 40.4 ‰. Site and habitat type had a significant effect on porewater

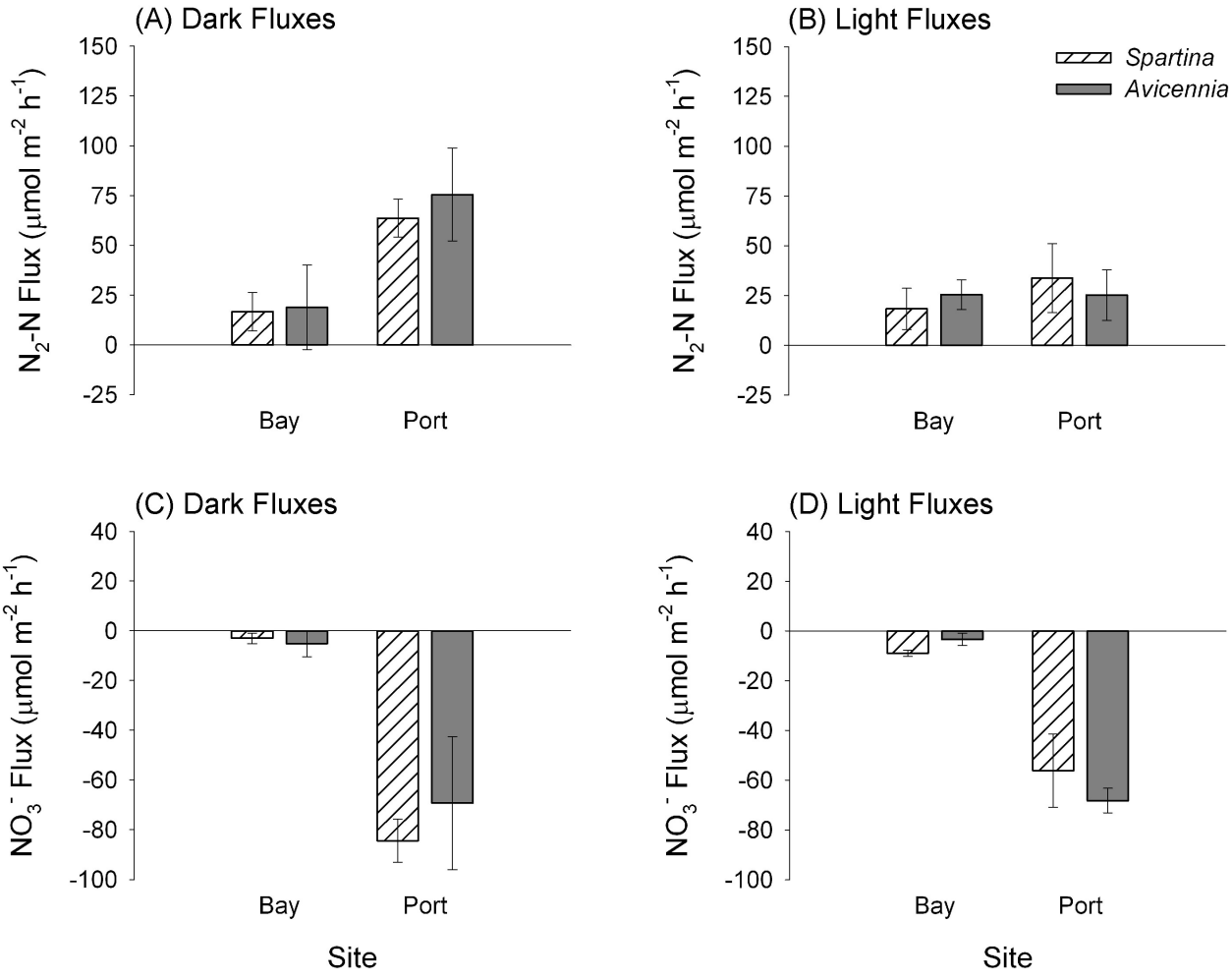

Figure 3.2. Winter 2009 mean (±1 SE) (A) dark N_2-N fluxes, (B) light N_2-N fluxes, (C) dark NO_3^- fluxes, and (D) light NO_3^- fluxes for *Spartina* and *Avicennia* habitats in the Bay and Port sites (n = 3).

salinities, with higher values at the Bay site and in the *Avicennia* habitats (Table 3.3). Soil redox potential exhibited trends across sites and habitat types similar to those observed in the winter. Soils at the Bay site were significantly more oxidized than at the Port site and *Avicennia* soils were slightly more oxidized than *Spartina* soils (Table 3.3). Hydrogen sulfide concentrations were slightly lower at the Bay site and significantly lower in the *Avicennia* habitats (Table 3.3). Porewater NO_2^- concentrations were low (< 1.0 μM) and NO_3^- concentrations were below the detection limit in all sites and habitat types. There was no significant effect of site or habitat type on porewater NH_4^+ or PO_4^{-3} concentrations during the summer sampling event (Table 3.3).

Table 3.3. Summer 2010 means and the effects of site and habitat type on porewater chemistry in the salt marsh-mangrove ecotone of Fourchon, Louisiana.

		Means[†]				ANOVA Results[‡]		
		Site		Habitat		Site	Habitat	S x H[§]
		Bay	Port	*Spartina*	*Avicennia*			
Salinity	X	35.28	30.02	28.52	36.78	0.01	0.001	ns
‰	(SE)	(1.77)	(2.46)	(1.61)	(1.60)			
pH	X	6.55	6.70	6.63	6.61	0.02	ns	ns
	(SE)	(0.03)	(0.03)	(0.05)	(0.04)			
Redox	X	77.25	-65.67	-5.67	17.25	0.0009	ns	ns
mV	(SE)	(22.42)	(16.09)	(39.75)	(34.22)			
H_2S	X	0.55	1.70	1.82	0.18	ns	0.009	ns
mM	(SE)	(0.30)	(0.68)	(0.53)	(0.07)			
NO_2^-	X	0.44	0.39	0.35	0.48	ns	0.0003	ns
µM	(SE)	(0.03)	(0.04)	(0.02)	(0.02)			
NO_3^-	X	0.00	0.00	0.00	0.00	-	-	-
µM	(SE)	(0.00)	(0.00)	(0.00)	(0.00)			
NH_4^+	X	5.22	145.83	114.53	36.51	ns	ns	ns
µM	(SE)	(1.09)	(76.78)	(78.95)	(32.13)			
PO_4^{-3}	X	9.08	25.75	18.70	16.13	ns	ns	ns
µM	(SE)	(5.00)	(9.09)	(5.08)	(10.43)			

[†]Mean at 10 cm, standard error in parenthesis (n = 6)
[‡]ANOVA p values from SAS PROC Mixed, ns = not significant ($p > 0.05$)
[§]S x H = two-way interaction effect between site and habitat

During the summer incubations, temperature and salinity in the water overlying the soil cores was 29 °C and 29 ‰, respectively. Similar to winter, mean net N_2 fluxes were positive indicating net denitrification (62.3 ± 12.1 µmol N m^{-2} h^{-1}); however, there was no significant difference between sites (Table 3.4, Figure 3.3). In summer, net N_2 fluxes exhibited diurnal variability, with significantly lower fluxes occurring during the light incubation. Nitrate fluxes were variable (range: -40.5 to 70.1 µmol m^{-2} h^{-1}) and showed no consistent trends across sites, habitat types, or light conditions (Table 3.4, Figure 3.3). In the summer, all 12 soil cores consumed oxygen in both the dark and light incubations. Rates of oxygen consumption were

Table 3.4. Summer 2010 means and the effects of site, habitat, and light on dissolved gas and inorganic nutrient fluxes in the salt marsh-mangrove ecotone of Fourchon, Louisiana.

Means[†]							
		Site		Habitat		Light	
		Bay	Port	*Spartina*	*Avicennia*	Dark	Light
N_2-N	X	65.80	58.72	63.14	61.38	77.83	46.70
μmol m^{-2} h^{-1}	(SE)	(17.44)	(17.41)	(16.20)	(18.63)	(17.82)	(15.75)
O_2	X	-69.71	-135.23	-97.60	-107.34	-135.98	-68.97
mg m^{-2} h^{-1}	(SE)	(8.06)	(17.77)	(15.39)	(18.29)	(17.37)	(8.35)
NO_2^-	X	0.31	-3.01	-1.85	-0.85	-2.43	-0.27
μmol m^{-2} h^{-1}	(SE)	(1.95)	(2.90)	(2.03)	(2.92)	(2.17)	(2.79)
NO_3^-	X	-8.99	-6.63	-11.89	-3.72	-2.83	-12.78
μmol m^{-2} h^{-1}	(SE)	(4.29)	(10.36)	(8.22)	(7.45)	(7.16)	(8.38)
NH_4^+	X	NA[‡]	NA	NA	NA	NA	NA
μmol m^{-2} h^{-1}	(SE)						
PO_4^{-3}	X	0.70	1.80	-1.02	3.51	1.75	0.75
μmol m^{-2} h^{-1}	(SE)	(0.33)	(1.85)	(0.60)	(1.52)	(1.49)	(1.15)

ANOVA Results[§]						
	N_2-N	O_2	NO_2^-	NO_3^-	NH_4^+	PO_4^{-3}
Site	ns	<0.0001	ns	ns	-	ns
Habitat	ns	ns	ns	ns	-	0.01
Site x Habitat	ns	ns	ns	ns	-	0.05
Light	0.05	<0.0001	ns	ns	-	ns
Site x Light	ns	0.01	0.003	ns	-	ns
Habitat x Light	ns	ns	ns	ns	-	ns
S x H x L[¶]	ns	ns	ns	ns	-	ns

[†]Means from summer sampling event, standard error in parenthesis (n = 12)
[‡]NA = no sample available
[§]ANOVA p values from SAS PROC Mixed, ns = not significant ($p > 0.05$)
[¶]S x H x L = three-way interaction effect between site, habitat, and light

significantly lower in soils from the Bay site and significantly decreased during the light incubation (Table 3.4). I was unable to calculate NH_4^+ fluxes for the summer dark and light incubations due to a sample contamination issue. Although statistically significant, the site by light differences in NO_2^- fluxes were small and unlikely to have an ecologically meaningful effect (Table 3.4). Phosphate was the only flux to show a significant difference between habitat

types during the summer incubations (Table 3.4). Mean *Spartina* fluxes were negative (-1.0 ± 0.6 μmol m^{-2} h^{-1}), while mean *Avicennia* fluxes were positive (3.5 ± 1.5 μmol m^{-2} h^{-1}).

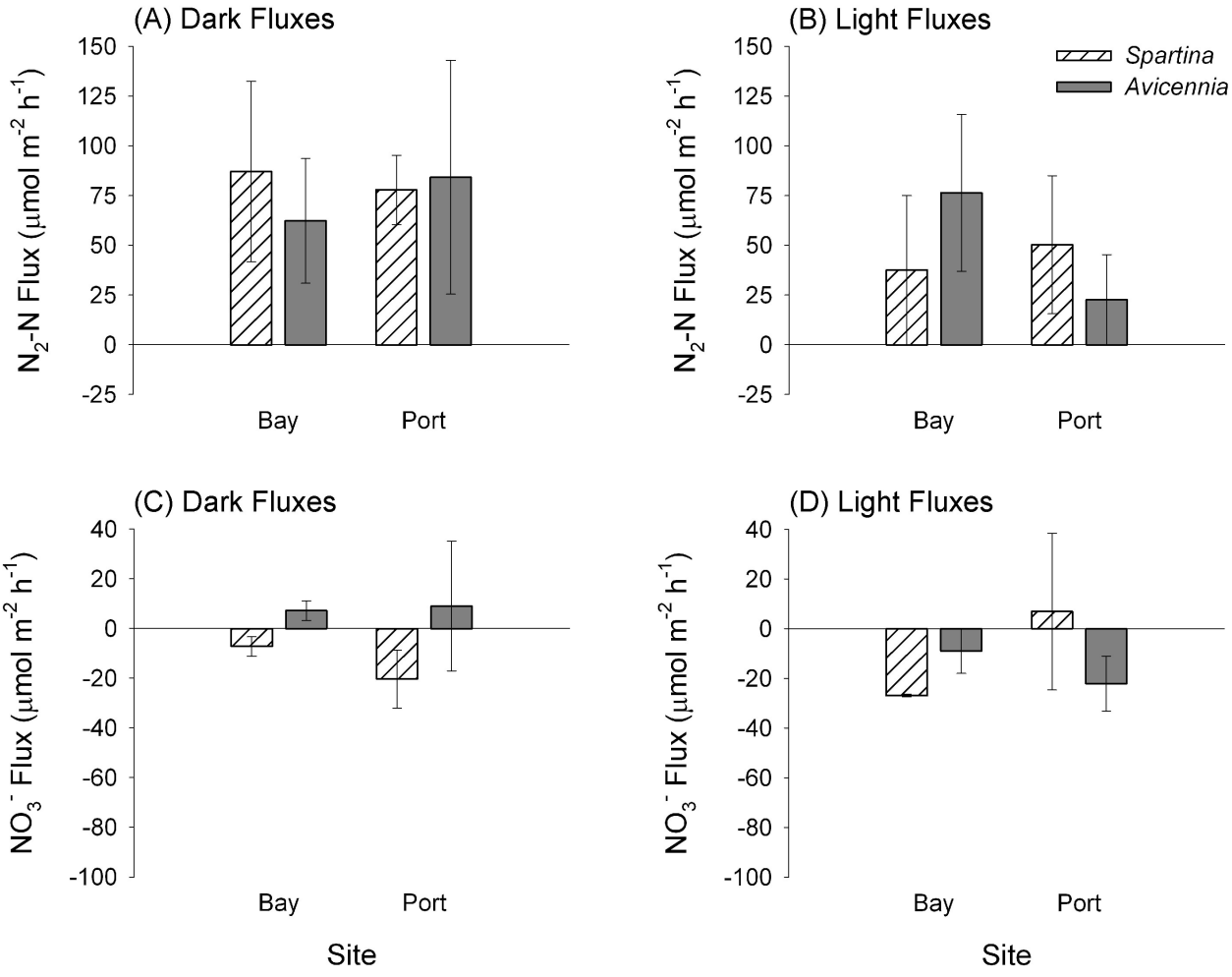

Figure 3.3. Summer 2010 mean (±1 SE) (A) dark N_2-N fluxes, (B) light N_2-N fluxes, (C) dark NO_3^- fluxes, and (D) light NO_3^- fluxes for *Spartina* and *Avicennia* habitats in the Bay and Port sites (n = 3).

DISCUSSION

The Net N_2 Balance in a Coastal Louisiana Wetland

The net exchange of N_2 gas in cores from the salt marsh-mangrove ecotone of Fourchon, Louisiana was positive suggesting greater rates of gross denitrification than gross nitrogen fixation. Whereas the large number of methodological approaches employed by researchers hampers direct comparison of denitrification and nitrogen fixation in salt marsh and mangrove

ecosystems, some generalizations can be made. A summary of rates for salt marshes throughout North America and Europe indicates that nitrogen inputs via nitrogen fixation may balance losses from denitrification (Table 3.5). However, in both new and old world mangrove wetlands, rates of denitrification are at least twice those of nitrogen fixation (Table 3.6). Net denitrification rates measured during my study are similar to the lower range of rates reported in salt marsh and mangrove literature (Tables 3.5 and 3.6). My rates are also in the lower range previously published for coastal Louisiana (0 to 2,850 μmol N m^{-2} h^{-1}; Rivera-Monroy et al. 2010).

In the winter, a significant difference was detected in both nitrate uptake and net denitrification rates between the Bay and the Port sites. Here I explore two possible explanations for the observed differences: (1) soil organic matter content and (2) nitrate availability. Organic matter can have both a direct and indirect effect on rates of denitrification in marine systems (Seitzinger and Giblin 1996; Davis et al. 2004; Fulweiler et al. 2007; Dodla et al. 2008). Denitrifiers are heterotrophs and increasing labile organic matter can directly stimulate denitrification rates (Fulweiler et al. 2007, 2008). High organic matter loading can increase soil oxygen demand, decrease oxygen penetration into the soil, and create conditions more favorable for nitrate reduction (Cornwell et al. 1999). This more indirect influence of organic matter loading tends to decrease the contribution of coupled nitrification-denitrification to the total denitrification rate (Caffrey et al. 1993). In the current study, winter net denitrification rates appear to be positively correlated to soil organic matter content. Soil organic matter content is significantly greater in the top 10 cm of the Port site relative to the Bay site (12% and 4%, respectively), which corresponds to the significant trend observed in nitrate uptake and net

Table 3.5. Denitrification and nitrogen fixation estimates for salt marshes.

Location	Range (μmol N m^{-2} h^{-1})	Technique	Reference
Denitrification Estimates for Salt Marshes			
Canada	11 to 84	isotope pairing	Poulin et al. 2007
MA	25 to 182	*in situ* N_2 gas partitioning	Kaplan et al. 1979
MA	ND to 94	direct N_2 flux	Nowicki et al. 1999
MA	17 to 496	short-term ^{15}N budget	Hamersley and Howes 2005
MA	18 to 21	tracer ^{15}N and isotope pairing	Koop-Jakobsen and Giblin 2010
RI	-750 to 850	porewater N_2 accumulation	Davis et al. 2004
RI	50	N_2:Ar	Caffrey et al. 2007
NC	< 2 to 25	acetylene block	Thompson et al. 1995
LA	30 to 164	N_2O:N2	Smith and DeLaune 1983
LA	14 to 17	acetylene block	Smith et al. 1985
LA	-2 to 153	N_2:Ar	Present study
England	1 to 118	acetylene block	Koch et al. 1992
Italy	7 to 286	isotope pairing	Ericksson et al. 2003
Portugal	51 to 676	isotope pairing	Sousa et al. 2012
Nitrogen Fixation Estimates for Salt Marshes			
MA	2 to 19	acetylene reduction	Carpenter et al. 1978
MA	0 to 51	acetylene reduction	Teal et al. 1979
VA	12 to 448	acetylene reduction	Tyler et al. 2003
NC	UD to 23	acetylene reduction	Currin et al. 1996
NC	4 to 93	acetylene reduction	Currin and Paerl 1998
GA	129 to 805	acetylene reduction	Hanson 1977
LA	3 to 122	acetylene reduction	Casselman et al. 1981
LA	55	acetylene reduction	DeLaune and Patrick 1990
CA	< 1	acetylene reduction	Langis et al. 1991
CA	18 to 235	acetylene reduction	Joye and Paerl 1994
CA	3 to 41	acetylene reduction	Moseman 2007
England	0 to 24	acetylene reduction	Abd Aziz and Nedwell 1986

Table 3.6. Denitrification and nitrogen fixation estimates for mangrove wetlands.

Location	Range (μmol N m^{-2} h^{-1})	Technique	Reference
Denitrification Estimates for Mangrove Wetlands			
LA	-21 to 197	N_2:Ar	Present study
Mexico	1 to 250	tracer ^{15}N enrichment	Rivera-Monroy and Twilley 1996
Puerto Rico	1 to 161	acetylene block	Morell and Corredor 1993
Puerto Rico	10 to 183	acetylene block	Corredor and Morell 1994
Puerto Rico	50 to 90	acetylene block	Corredor et al. 1999
Belize	0 to 46	acetylene block	Lee and Joye 2006
Jamaica	8 to 83	acetylene block	Nedwell et al. 1994
China	92 to 315	direct N_2 flux	Alongi et al. 2005
India	8 to 1320	isotope pairing	Fernandes et al. 2012
Thailand	1 to 2	isotope pairing	Kristensen et al. 1998
Thailand	0 to 160	direct N_2 flux	Alongi et al. 2002
Vietnam	0 to 183	direct N_2 flux	Alongi et al. 2000
Malaysia	33 to 917	direct N_2 flux	Alongi et al. 2004
Australia	242 to 572	direct N_2 flux	Alongi et al. 1999
Nitrogen Fixation Estimates for Mangrove Wetlands			
Puerto Rico	13 to 32	acetylene reduction	Morell and Corredor 1993
Belize	0 to 50	acetylene reduction	Lee and Joye 2006
Jamaica	0 to 100	acetylene reduction	Nedwell et al. 1994
China	0 to 4	acetylene reduction	Alongi et al. 2005
Thailand	12 to 16	acetylene reduction	Kristensen et al. 1998
Thailand	0 to 24	acetylene reduction	Alongi et al. 2002
Vietnam	20 to 119	acetylene reduction	Alongi et al. 2000
Malaysia	0 to 250	acetylene reduction	Alongi et al. 2004
Tanzania	9 to 112	acetylene reduction	Lugomela and Bergman 2002
Tanzania	1 to 41	acetylene reduction	Sjöling et al. 2005
Australia	ND to 13	acetylene reduction	Boto and Robertson 1990
New Zealand	2 to 15	acetylene reduction	Hicks and Silvester 1985

denitrification (Chapter 2). In addition, soil oxygen demand exhibited a positive relationship with organic matter content in the Port site during both the winter and summer incubations.

The significant difference between sites in net denitrification observed during the winter incubations may be the result of a disparity in water column nitrate concentrations. Initial nitrate concentrations in the overlying water were an order of magnitude higher in cores from the Port site relative to the Bay site (13 μM and 2 μM, respectively). Soil cores and water for the Bay and Port incubations were collected approximately one week apart and it appears as though nitrate concentrations in Bayou Lafourche exhibited a marked decrease during this time. Since direct denitrification is proportional to water column nitrate concentration, the significant difference observed could be the result of initial water column nitrate concentrations (Cornwell et al. 1999 and references within).

In contrast, nitrate uptake and net denitrification rates in the summer were not significantly different between sites and show no correlation with soil organic matter content. Summer nitrate concentrations in the overlying water were uniformly low (2 μM) in cores from both sites. Thus, direct denitrification was likely limited by nitrate availability and the majority of my observed net N_2 production was probably the result of coupled nitrification-denitrification. Nitrate limitation of direct denitrification is common in salt marsh and mangrove wetlands; and as a result, coupled nitrification-denitrification is often the dominant N_2 production pathway (Kristensen et al. 1998; Hamersley and Howes 2003; Poulin et al. 2007). Assuming all nitrate consumed was denitrified, direct denitrification could only be responsible for 14% and 11% of the summer net N_2 production in the Bay and Port sites, respectively. This suggests that over 85% percent of the N_2 produced likely came from porewater ammonium nitrification-denitrification. It is important to mention that anaerobic ammonium oxidation, or anammox, is

another possible source of N_2 production in wetland soils and estuarine sediments. However, the contribution of anammox to the net N_2 flux in salt marsh and mangrove wetlands is often less important than denitrification (Koop-Jakobsen and Giblin 2009a; Fernandes et al. 2012).

During the winter incubation, the contribution of coupled nitrification-denitrification to net N_2 production appears less significant, particularly at the Port site where nitrate consumption exceeded net N_2 production. The excess nitrate consumed at the Port site was likely immobilized by microbes or reduced to ammonium via dissimilatory nitrate reduction to ammonium (DNRA). DNRA is an important nitrate reduction pathway in many coastal environments (An and Gardner 2002; Koop-Jakobsen and Giblin 2010; Dong et al. 2011). A recent study found DNRA accounted for more than 30% of the total dissimilatory nitrate reduction (denitrification + DNRA) occurring in a Massachusetts salt marsh (Koop-Jakobsen and Giblin 2010). In summary, it appears as though coupled nitrification-denitrification was more important to the net N_2 flux in the summer than in the winter. Further research is needed to determine if this is directly related to lower water column nitrate concentrations or a temperature dependence of nitrification. In the absence of substrate (oxygen and ammonium) limitation, nitrifying communities frequently exhibit higher activity in the warmer summer months (Canfield et al. 2005). In addition, Poulin et al. (2007) observed that the coupling of nitrification and denitrification was temperature dependent and suggested that coupled nitrification-denitrification might be more important under summer conditions.

The diel variations in net N_2 fluxes observed during this study likely reflect a direct light effect on nitrogen fixation in combination with a more indirect effect on denitrification. Prior work in coastal systems found light to increase the activity of microbial mats and microphytobenthos stimulating nitrogen fixation, while inhibiting denitrification (Joye and Paerl

1994; Sundbäck and Miles 2000; Sundbäck et al. 2000; Lee and Joye 2006). The decrease from dark to light conditions observed in net N_2 production during both the winter and summer incubations may be the result of enhanced nitrogen fixation. In the winter, the significant decrease in net N_2 production at the Port site could be explained by an increase in oxygen diffusion into the soil. High rates of primary productivity, as indicated by the positive oxygen flux, may have increased the depth that oxygen penetrated into the soil. Since winter net denitrification was primarily driven by direct denitrification (see discussion above), an increase in oxygen penetration into the soil would likely decrease net N_2 production (Risgaard-Petersen et al. 1994; Rysgaard et al. 1994). In the summer, the significant decrease in net N_2 production could be attributed to a decrease in denitrification; however, the underlying mechanism was likely oxygen limitation of nitrification. Dissolved oxygen concentrations decreased throughout both the dark and the light incubations; and before the light incubation was terminated, hypoxic (< 2 mg L^{-1}) conditions developed in several of the cores. Since coupled nitrification-denitrification dominated the summer net N_2 flux, low concentrations of dissolved oxygen in the overlying water may have limited rates of nitrification and subsequently decreased net N_2 production (Patrick and Reddy 1976; Rysgaard et al. 1994). Furthermore, microphytobenthos are a major nitrogen sink in some shallow-water systems and competition between the microphytobenthos and denitrifiers for available nitrogen could have decreased denitrification rates during both the winter and summer incubations (Sundbäck and Miles 2000; Sundbäck et al. 2000).

Overall Effect of *Avicennia* Expansions on Inorganic Nutrient Cycling

In the present study, *Avicennia* soils were more oxidized, with higher porewater salinities. I observed more reducing conditions in *Spartina* habitats that corresponded to higher

porewater concentrations of hydrogen sulfide and ammonium. These difference may be the result of higher elevations, better drainage, and higher redox potential in *Avicennia* soils (Patterson and Mendelssohn 1991; Perry and Mendelssohn 2009). However, when soil redox potential and porewater measurements were made in high and low elevation plots across *Spartina* and *Avicenna* habitats, I only observed a significant elevation effect on soil redox potential and porewater nitrite concentration (Appendix A). During this same experiment, habitat type significantly altered soil redox potential as well as porewater salinity and concentrations of hydrogen sulfide, nitrite, nitrate, and ammonium (Appendix A). This suggests that some of the observed differences in soil and porewater properties are inherent to vegetation type and not a direct result of the elevation difference between *Spartina* and *Avicennia* habitats.

Despite the significant differences measured in soil and porewater properties, I observed no significant difference in net N_2, oxygen, or inorganic nitrogen fluxes between *Spartina* and *Avicennia* habitats. The similarities between habitats observed in these measurements may be directly related to the *in vitro* core technique employed. The available methods for measuring both denitrification and nitrogen fixation, particularly in wetland environments, are fraught with problems (Seitzinger and Garber 1987; Hamersley and Howes 2005; Groffman et al. 2006; Koop-Jakobsen and Giblin 2009b among others). Quantifying the relatively small changes in N_2 associated with denitrification and nitrogen fixation against high background nitrogen gas concentrations is extremely difficult. As a result, most methods alter substrate concentrations or isolate soils from *in situ* physical and biological factors (Hamersley and Howes 2005; Groffman et al. 2006; Koop-Jakobsen and Giblin 2009b). I selected the N_2:Ar technique because it allows for the direct measurement of net N_2 fluxes in intact, un-amended soil cores. In addition to its widespread use in non-vegetated subtidal sediments, it has been successfully applied to intertidal

and subtidal soils with emergent macrophytes (Eyre and Ferguson 2002; Caffrey et al. 2007). Unfortunately, it does require that soils be removed from the field and incubated under flooded conditions. By separating soils from *in situ* hydrology and the photosynthesizing parts of *Avicennia* and *Spartina*, the *in vitro* technique I chose may have masked subtle differences in fluxes between the habitats of these two species.

In contrast to the similar nitrogen and oxygen fluxes observed between habitat types, I measured a significantly greater release of phosphate in *Avicennia* soils during the summer incubation. Phosphorus solubility in both salt marsh and mangrove wetlands is driven by soil redox potential (Tobias and Neubauer 2009; Twilley and Rivera-Monroy 2009b); and after exposure to anoxic conditions, benthic sediments typically exhibit a rapid release of phosphorus (Sundby et al. 1986; Jensen et al. 1995; Fulweiler et al. 2010). Within the study area, total phosphorus content was significantly greater in the higher elevation, more oxidized *Avicennia* soils (Chapter 2). Thus, the marked decrease in dissolved oxygen concentration during the summer incubation likely had a disproportionate effect on phosphorus release from the *Avicennia* soils.

In summary, *Avicennia* expansions do not appear to be affecting net N_2, oxygen, or inorganic nitrogen fluxes within the *Spartina* marshes of Fourchon, Louisiana. Rates of gross denitrification were greater than gross nitrogen fixation, yielding a positive net N_2 flux throughout the salt marsh-mangrove ecotone. Results from this study indicate the potential for nitrate limitation of direct denitrification and temperature regulation of coupled nitrification-denitrification. However, further investigation is necessary to clarify the contribution of direct versus coupled nitrification-denitrification to the net N_2 production in the region. *In situ* differences between habitat types in soil redox potential as well as porewater salinity, hydrogen

sulfide, and ammonium concentrations were not realized in the net N_2, oxygen, or inorganic nutrient fluxes. Furthermore, preexisting differences between sites in marsh elevation, hydroperiod, and soil nutrient content had no clear impact on inorganic nutrient cycling. Some of these negative results may be attributed to the *in vitro* core technique employed in this study and I suggest an *in situ* approach that does not isolate soils from hydrology and plant growth for future research.

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CHAPTER 4
IMMEDIATE IMPACTS OF THE DEEPWATER HORIZON OIL SPILL ON INORGANIC NUTRIENT CYCLING WITHIN A COASTAL LOUISIANA WETLAND

INTRODUCTION

The wetlands of coastal Louisiana provide numerous economic and ecological goods and services, including flood mitigation, shoreline protection and stabilization, water quality improvement, and wildlife habitat (Costanza et al. 1989; Twilley and Rivera-Monroy 2009). These wetlands are important in the cycling, retention, and removal of nutrients, especially nitrogen and phosphorus (Mitsch et al. 2001, 2005). In Louisiana, the quantity of river-born reactive nitrogen entering the Gulf of Mexico has increased markedly during the last century (Turner and Rabalais 1991; Goolsby and Battaglin 2001). Considerable evidence links this increase to enhanced levels of primary productivity and depletion of bottom water dissolved oxygen concentrations over the Louisiana-Texas continental shelf (Rabalais et al. 2002). Previous research in Louisiana has focused on the potential of wetlands to remove nutrients from the Mississippi River, with particular attention given to the removal of reactive nitrogen (Mitsch et al. 2005; Twilley and Rivera-Monroy 2009). Pathways for reactive nitrogen removal include denitrification (canonical as well as anaerobic ammonium oxidation or anammox), assimilation by macrophytes and microbes, sedimentation and burial; however denitrification is frequently herald as the most important mechanism for removing reactive nitrogen (Galloway et al. 2003; Seitzinger 2008).

Despite their economic and ecologic importance, Louisiana's coastal marshes are constantly threatened by both natural and anthropogenic causes of degradation. Navigation and flood control structures as well as petroleum exploration and production accelerate natural rates of land loss by directly converting wetlands to open water and by indirectly altering remaining

marsh elevation and increasing saltwater intrusion (Boesch et al. 1994; Ko and Day 2004). The negative impacts of extensive oil and gas exploration, production, and transportation are not limited to canal dredging and well drilling, but include oil spills (Ko and Day 2004). Each year the Louisiana Oil Spill Coordinator's Office (2011) receives nearly 4,000 spill alerts. On April 20, 2010, an explosion on the Deepwater Horizon offshore oil platform initiated the largest marine oil spill in the history of the United States. Lasting for nearly three months, the Deepwater Horizon oil spill released approximately four million barrels of crude oil into the Gulf of Mexico. By mid-May, the surface slick reached the shores of Louisiana and began depositing weathered crude oil into wetlands of this deltaic coast.

The ability of hydrocarbon-utilizing microbial populations to effectively biodegrade crude oil as well as the negative impacts of crude oil on the abundance of less tolerant species are well documented (Atlas et al. 1991; Atlas 1995; Margesin et al. 2000; Labud et al. 2007). In contrast, there exists very little information on crude oil impacts to community-level microbial functioning, particularly on key biogeochemical processes occurring in wetland soils (DeLaune and Wright 2011). Of the available research, low level hydrocarbon amendments appear to increase carbon dioxide production, methanogenesis, nitrogen fixation, and denitrification, while more extreme applications inhibit these processes in coastal soils and sediments (DeLaune et al. 1979; Li et al. 1990; Gilbert et al. 1997; Nyman 1999). By providing additional substrate and removing micronutrient limitation as well as increasing soil oxygen demand and decreasing oxygen diffusion, crude oil amendments stimulate both aerobic and anaerobic microbial respiration (DeLaune et al. 1979; Li et al. 1990; Nyman 1999; Shin et al. 2000).

In an effort to understand how the Deepwater Horizon oil spill may have altered some of the key inorganic nutrient cycling pathways in coastal wetlands, I measured net dinitrogen (N_2),

oxygen, and dissolved inorganic nutrient fluxes in both reference and impacted soils. The purpose of this paper is to present a "snapshot" of the immediate impact weathered crude oil had on wetland nutrient fluxes. The oil released during the spill was south Louisiana sweet crude oil, which is a relatively non-toxic oil enriched in light aromatic hydrocarbons, paraffins, and olefins, and low in nitrogen, oxygen, and sulfur containing heterocyclic compounds (Jackson and Pardue 1999; DeLaune et al. 2003). Approximately one month elapsed between commencement of the oil spill and its landfall on Louisiana's shores allowing the crude oil to weather before its application to the marsh, further decreasing its toxicity (Proffitt 1996). In a nutrient rich region such as the Mississippi River delta, I hypothesized that the application of Louisiana sweet crude would stimulate microbial activity ultimately enhancing net N_2, oxygen, and dissolved inorganic nutrient fluxes across the soil-water interface.

MATERIALS AND METHODS

Study Area and Sampling Locations

Barataria Bay is located within the Barataria basin system between two major distributaries of the Mississippi River delta, the Plaquemines-Balize deltaic headland to the east and the Lafourche deltaic headland to the west (Figure 4.1). Construction of artificial flood control levees along the Mississippi River in the early 1900s and the damming of Bayou Lafourche in 1904 isolated river floods from the basin and prevented any major riverine sediment inputs over the last century. Direct rainfall and runoff from the surrounding drainage basin comprise the majority of freshwater inputs to Barataria Bay with three Mississippi River diversions contributing less than 25% of the total freshwater entering the system (Louisiana Department of Natural Resources 2011). Wetland hydroperiod in the region is controlled by a combination of diurnal microtides averaging 0.3 m in range and meteorological events such as

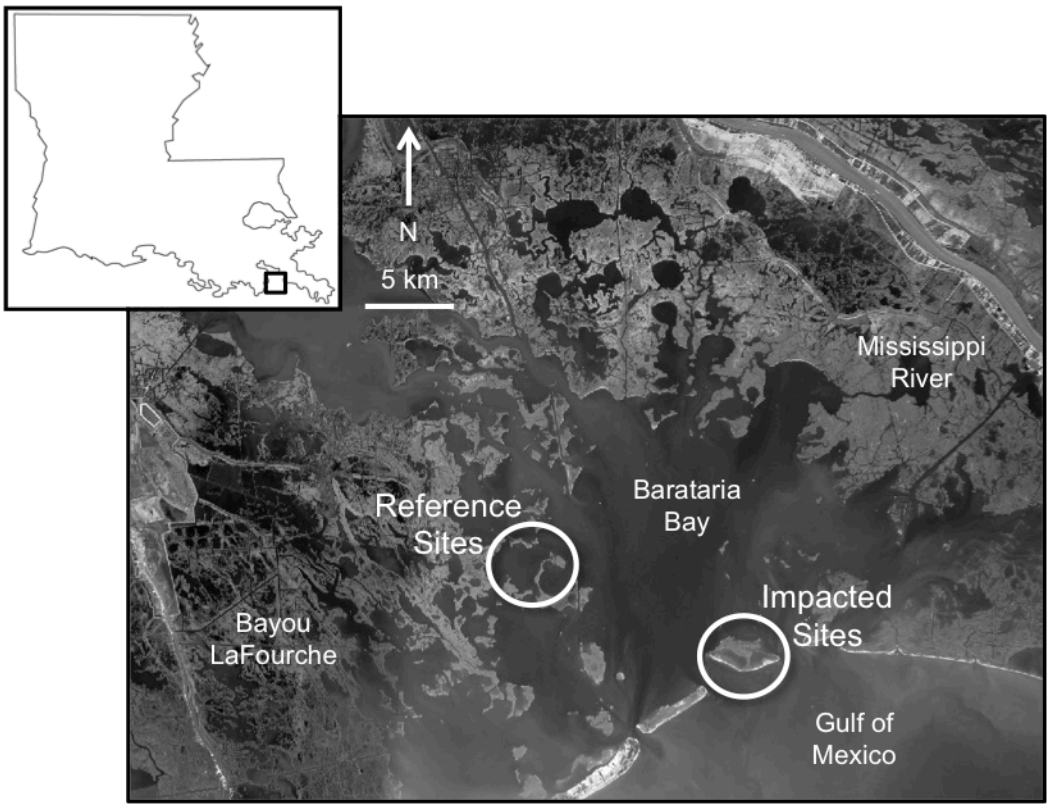

Figure 4.1. Sampling locations in Barataria Bay, Louisiana. Image courtesy of Louisiana Department of Natural Resources.

winter cold fronts and summer tropical storms and hurricanes (Zetler and Hansen 1972). The climate of southern Louisiana is humid subtropical (Peel et al. 2007), with monthly mean temperatures between 6 and 30 °C and mean annual precipitation of 160 cm (National Climatic Data Center 2011).

On June 7, 2010, I collected porewater samples and non-vegetated soil cores from four sites within Barataria Bay, two sites each in the oil impacted location and the reference location (Figure 4.1). The reference location included habitats of *Spartina alterniflora* Loisel (29°22'48" N 090°00'36" W) and *Avicennia germinans* L. (29°20'57" N 090°00'41" W) surrounding Creole Bay as the oil had not reached this inland region. The impacted location included habitats of *Spartina* (29°19'22" N 089°53'30" W) and *Avicennia* (29°19'8" N 089°53'53" W) on the heavily oiled East Grand Terre Island at the mouth of Barataria Bay.

Porewater Collection

At each site, triplicate 60 mL samples of porewater were collected from a depth of 10 cm using rigid aquarium tubing affixed to a 60 mL syringe. Porewater temperature, salinity, and pH were analyzed in the field using a Hach HQ Series portable meter. Five mL of porewater were added to an equal volume of antioxidant buffer and transported to the laboratory to be analyzed for hydrogen sulfide (H_2S) concentration with a micro mono ion sulfide electrode (McKee et al. 1988). The remaining 55 mL of porewater were filtered with a Whatman GF/F glass fiber filter (0.7 μm) for nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), and phosphate (PO_4^{-3}) analysis. Soil oxidation-reduction (redox) potential was measured in the field at 0, 10, and 45 cm using a multi-depth platinum electrode (Hargis and Twilley 1994).

Core Collection and Incubation

Triplicate intact soil cores (10 cm internal diameter by 10 cm depth) were collected from each site and transported to an environmental growth chamber located at Louisiana State University. Growth chamber conditions were maintained at ambient field temperature (25 °C) and in continuous darkness for the duration of the experiment. Cores were placed in a water bath and pre-incubated for 24 hours with air gently bubbling through the overlying water.

To insure comparable experimental conditions between the reference and impacted soil cores, filtered (0.2 μm) freshwater (0.4 ‰) from Bayou Des Allemands was mixed with filtered seawater (35.0 ‰) and used as the overlying water source during both the gas and nutrient incubations. Bayou Des Allemands is located approximately 75 km upstream of my sampling locations and was selected as a non-impacted freshwater end member. To achieve the ambient porewater salinity of my four sites (mean: 12.5 ‰), the water from Bayou Des Allemands was mixed in a 2:1 ratio with seawater obtained from the Louisiana Marine Consortium's (LUMCON) facility in Cocodrie, LA. The seawater obtained from LUMCON was collected

from the Gulf of Mexico prior to the Deepwater Horizon oil spill. Inorganic nutrient concentrations of the filtered water mixture were comparable to concentrations in Barataria Bay on the day of sampling (NO_2^- < 1 μM, NO_3^- < 1 μM, and NH_4^+ = 6 μM, PO_4^{-3} < 1 μM).

Before beginning the soil core incubation to measure dissolved gas fluxes (hereafter referred to as the gas incubation), the overlying water in each soil core was replaced with the filtered water mixture. All cores were then sealed with a gas-tight lid and two water samples for N_2:Ar analysis were collected into 12 mL exetainers (© Labco Limited) and preserved with 250 μL $ZnCl_2$ (50% w/v; Nielsen and Glud 1996). Water samples were collected every hour for a total of five sampling intervals. Initial and final dissolved oxygen concentrations were measured with a Hach LDO101-01 dissolved oxygen probe. Throughout the incubation, the overlying water was gently mixed (~55 rpm) with a magnetic stir bar suspended from the lid. After completing the gas incubation, I replaced the overlying water and began measurements of dissolved inorganic nutrient fluxes (hereafter referred to as the nutrient incubation). All soil and water column conditions during the nutrient incubation were identical to those of the gas incubation. Water samples were collected and filtered with a Cole-Parmer RC-membrane filter (0.45 μm) for dissolved inorganic nutrients (NO_2^-, NO_3^-, NH_4^+, and PO_4^{-3}) every hour at approximately the same five time intervals. Both incubations lasted until a 2 mg L^{-1} decrease in dissolved oxygen occurred (5 to 6 hours) (Fulweiler et al. 2008). After completing both incubations, all cores were sectioned in 2 cm increments from the soil surface to 8 cm depth. Soil samples were oven-dried at 60 ºC to a constant weight and then ground. Organic matter content was determined by loss on ignition at 550 ºC for 2 hours (Davies 1974).

Dissolved Gas and Inorganic Nutrient Analysis

Water samples from the gas incubation were assayed for N_2:Ar dissolved gas concentrations with a Pfeiffer Prisma QME 200 quadrupole membrane inlet mass spectrometer (Bay Instruments, Easton, Maryland) modified with a copper reduction column and furnace heated to 600 °C (Kana et al. 1994; Eyre et al. 2002). The N_2:Ar ratio is actually a measure of net N_2 flux (gross denitrification – gross nitrogen fixation); a positive flux indicates net denitrification, while a negative flux indicates net nitrogen fixation. The change in N_2 concentration was determined for each core by taking the change in the N_2:Ar and multiplying by the Ar concentration at air saturation (Colt 1984). Water samples were analyzed colorimetrically for NO_2^-, NO_3^-, NH_4^+, and PO_4^{-3} using a Flow IV OI Analytical Autoanalyzer (Strickland and Parsons 1972; Parsons et al. 1984). Net N_2 and inorganic nutrient production were determined from the slope of a 5-point linear regression. Rates were then prorated for the volume of water overlying the core and area of the core.

Statistical Analysis

A two-way analysis of variance (ANOVA) was used to determine if statistical differences existed between oil treatments (reference or impacted) or habitat types (*Spartina* or *Avicennia*) for porewater variables. I used a three-way ANOVA with repeated measures to determine if statistical differences existed in soil redox potential and organic matter content between oil treatments, habitat types, or depths. A two-way ANOVA was used to determine if statistical differences existed between oil treatments or habitat types for all dissolved gas and inorganic nutrient fluxes. Interaction effects were considered for all analyses and pairwise comparisons among treatments were described with a Tukey's honestly significant difference (HSD) test. All

tests were run with SAS PROC Mixed and significance was assessed at the 0.05 level (SAS Institute 2011).

RESULTS

Porewater and Soil Properties

Porewater salinity ranged from 5.8 ‰ in the impacted *Avicennia* soil to 29.0 ‰ in the reference *Avicennia* soil. Mean porewater salinity was significantly different between the oil treatment locations, but not between the habitat types at either location (Table 4.1). Mean porewater pH was significantly lower in the reference location than the impacted location, with no significant difference between habitat types (Table 4.1). Mean porewater NH_4^+ concentration

Table 4.1. Means and the effects of oil treatment and habitat type on porewater and soil chemistry in the salt marsh-mangrove ecotone of Barataria Bay, Louisiana.

		Means[†]				ANOVA Results[‡]		
		Oil Treatment		Habitat Type		Oil	Habitat	O x H[§]
		Reference	Impacted	*Spartina*	*Avicennia*			
Salinity	X	17.14	10.30	11.67	16.87	0.04	ns	ns
‰	(SE)	(3.10)	(1.37)	(0.79)	(4.25)			
pH	X	6.15	6.96	6.41	6.71	0.0007	ns	ns
	(SE)	(0.06)	(0.16)	(0.18)	(0.23)			
Redox[¶]	X	39.50	78.67	71.92	46.25	0.03	ns	0.02
mV	(SE)	(24.67)	(2.00)	(14.06)	(22.41)			
Sulfide	X	0.07	0.09	0.14	0.01	ns	ns	ns
mM	(SE)	(0.03)	(0.07)	(0.06)	(0.01)			
NO_{2+3}^-	X	2.40	0.24	1.86	0.78	ns	ns	ns
µM	(SE)	(1.20)	(0.07)	(1.28)	(0.38)			
NH_4^+	X	15.11	1.99	8.20	8.90	0.01	ns	ns
µM	(SE)	(3.72)	(0.23)	(4.34)	(3.49)			
PO_4^{-3}	X	1.07	2.95	3.47	0.55	ns	ns	ns
µM	(SE)	(0.31)	(2.53)	(2.45)	(0.14)			

[†]Mean from a depth of 10 cm presented, with standard error in parentheses ($n = 3$)
[‡]ANOVA p values from SAS Proc Mixed, ns = not significant, ($p > 0.05$)
[§]O x H = two-way interaction effect between oil treatment and habitat type
[¶]ANOVA results for redox are from three-way model; effect of depth was significant ($p < 0.0001$)

was significantly higher in the reference location than the impacted location, with no difference between habitat types (Table 4.1). There was no significant difference between oil treatment locations or habitat types in the concentrations of porewater H_2S, NO_x, or PO_4^{-3} (Table 4.1). Concentrations of NO_2^- and NO_3^- were near the detection limit, yielding a negative NO_3^- concentration in many instances. As an alternative, I present the concentration of $NO_2^- + NO_3^-$ as NO_x throughout the results and discussion.

The top 10 cm of soil had a redox potential ranging from -21 mV to 145 mV, with more reduced conditions by 45 cm. Redox potential was significantly lower in soils from the reference location, with no difference between habitat types (Table 4.1). In the lower soil layers, the impacted location contained significantly less organic matter than the reference location (Figure 4.2). However, in the surface layer (0 to 2 cm), soils at the impacted location had similar organic matter content to the reference reflecting the presence of oil at this location (Figure 4.2). Habitat type had no significant effect on soil organic matter content.

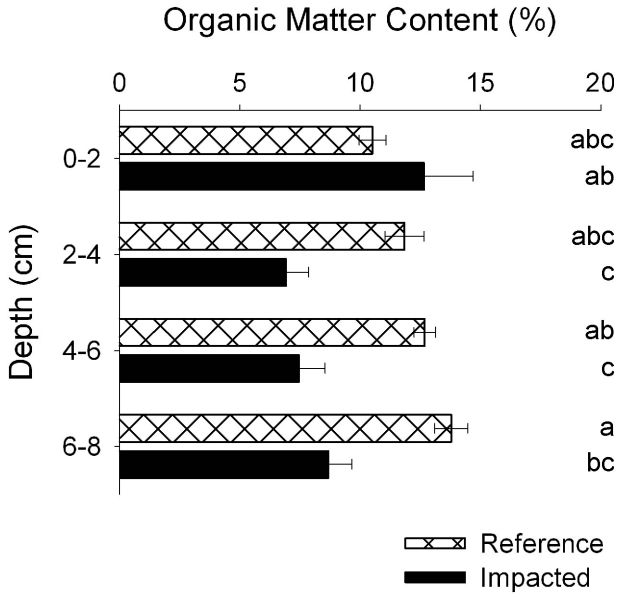

Figure 4.2. Mean (± 1 SE) organic matter content in reference and impacted soil. Lowercase letters correspond to ANOVA results. Means ($n = 6$) followed by the same letter are not significantly different according to Tukey HSD ($p > 0.05$).

Dissolved Gas and Inorganic Nutrient Fluxes

Net N_2 fluxes across the soil-water interface in all 12 cores were positive, indicating net denitrification (Figure 4.3). Mean net N_2 fluxes were higher for the impacted oil treatments; however, differences were not significant (Table 4.2). All soil cores exhibited oxygen consumption, with no significant differences between oil treatment locations or habitat types (Table 4.2, Figure 4.3). NO_x fluxes ranged from -134.9 to 0 μmol m^{-2} h^{-1}, indicating uptake into the soil (Figure 4.4). NO_x uptake was approximately the same order of magnitude as net

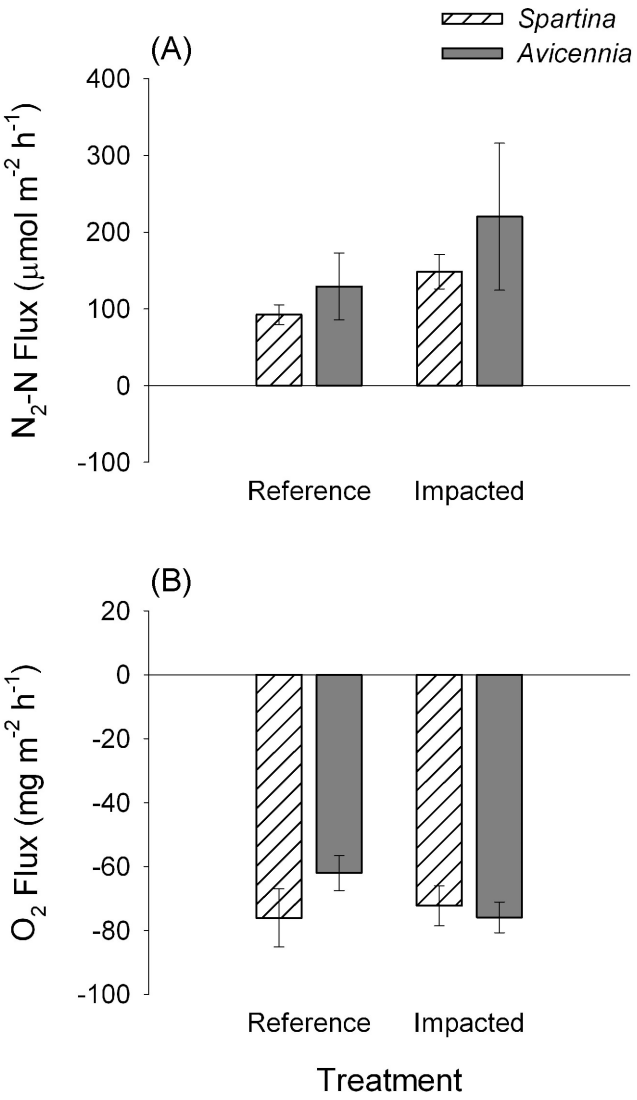

Figure 4.3. Mean (± 1 SE) (A) N_2-N and (B) O_2 fluxes for reference and impacted, *Spartina* and *Avicennia* soil cores (n = 3).

denitrification (as moles of N; Figures 4.3 and 4.4). There was a significant difference in mean NO_x uptake between oil treatments, but not between habitat types (Table 4.2). Ammonium fluxes ranged from 0 to 222.8 µmol m^{-2} h^{-1}, and there was no significant difference in NH_4^+ release between the oil treatment locations or habitat types (Table 4.2, Figure 4.3). Phosphate fluxes were the only variable to exhibit a significant difference between habitat types (Tables 4.1 and 4.2). Mean *Spartina* fluxes were positive, while mean *Avicenna* fluxes were negative (Figure 4.3).

Table 4.2. Means and the effects of oil treatment and habitat type on dissolved gas and inorganic nutrient fluxes in the salt marsh-mangrove ecotone of Barataria Bay, Louisiana.

		Means[†]				ANOVA Results[‡]		
		Oil Treatment		Habitat Type		Oil	Habitat	O x H[§]
		Reference	Impacted	*Spartina*	*Avicennia*			
N_2-N	X	110.65	184.08	120.21	174.52	ns	ns	ns
µmol m^{-2} h^{-1}	(SE)	(21.90)	(46.77)	(17.07)	(51.18)			
O_2	X	-69.04	-74.07	-74.14	-68.98	ns	ns	ns
mg m^{-2} h^{-1}	(SE)	(5.67)	(3.64)	(5.01)	(4.50)			
NO_{2+3}^-	X	-24.83	-95.42	-53.60	-66.65	0.01	ns	ns
µmol m^{-2} h^{-1}	(SE)	(10.29)	(20.14)	(18.84)	(25.25)			
NH_4^+	X	54.02	75.75	50.25	79.53	ns	ns	ns
µmol m^{-2} h^{-1}	(SE)	(37.60)	(34.17)	(32.09)	(38.89)			
PO_4^{-3}	X	-1.52	0.50	5.63	-6.65	ns	0.05	ns
µmol m^{-2} h^{-1}	(SE)	(1.73)	(6.43)	(3.41)	(4.25)			

[†]Means are presented with standard error in parentheses (n = 3)
[‡]ANOVA p values from SAS Proc Mixed, ns = not significant ($p > 0.05$)
[§]O x H = two-way interaction effect between oil treatment and habitat type

DISCUSSION

Previous experimental work in wetland soils and sediments found nitrogen fixation rates may balance or even exceed losses due to denitrification (Nedwell et al. 1994; Kristensen et al. 1998; Davis et al. 2004; Lee and Joye 2006). In the present study, all net N_2 fluxes across the soil-water interface were positive, indicating the dominance of gross denitrification over gross

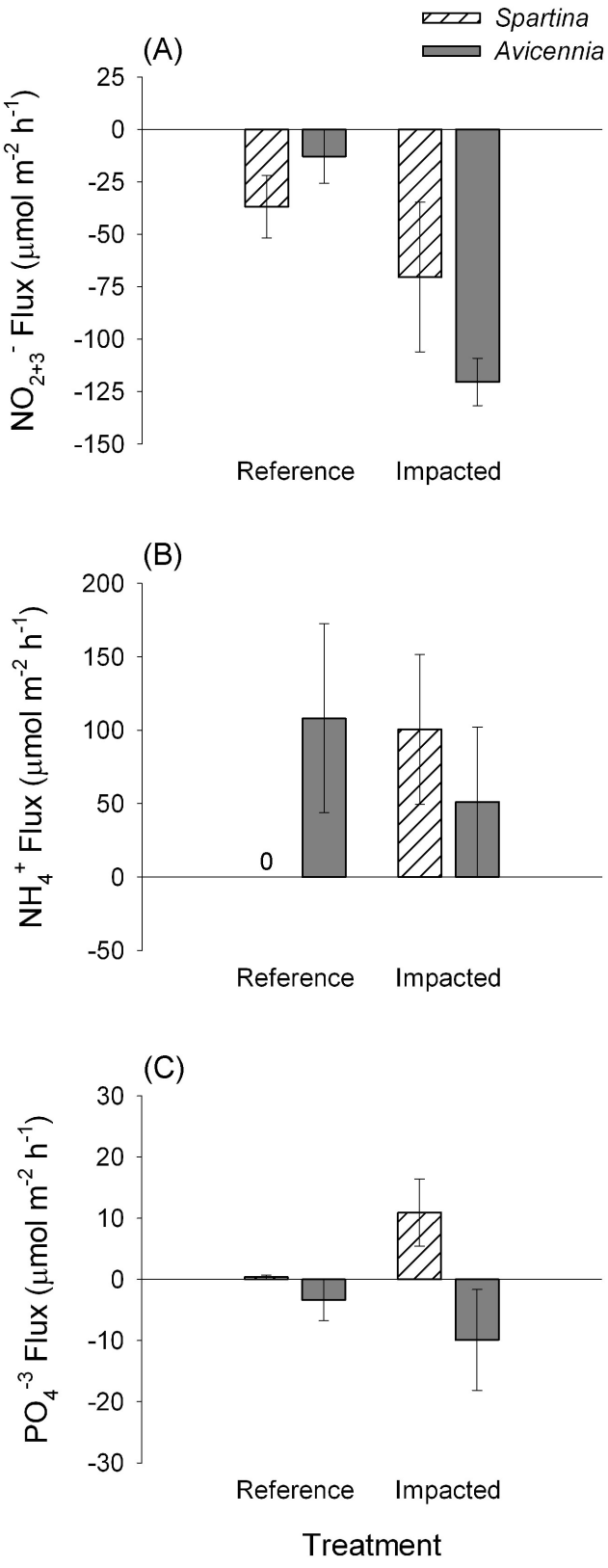

Figure 4.4. Mean (± 1 SE) (A) NO_{2+3}^-, (B) NH_4^+, and (C) PO_4^{-3} fluxes for reference and impacted, *Spartina* and *Avicennia* soil cores (n = 3).

nitrogen fixation in these wetland sites of Barataria Bay. Net denitrification rates for all oil treatments and habitat types measured during this experiment are at the lower end of the range previously reported for coastal Louisiana (0 to 2,853 μmol N m^{-2} h^{-1}; Rivera-Monroy et al. 2010). Whereas surface water nitrate concentrations in the Mississippi River frequently exceed 100 μM, nitrate concentrations in Barataria Bay are considerably lower due to restricted flow from the river into this region (Goolsby and Battaglin 2001). This generates an inverse-type estuary where the limiting nutrient (e.g. nitrate) is supplied by the coastal waters of the Gulf of Mexico instead of the Mississippi River watershed (Patrick and DeLaune 1976; Das et al. 2011). The ambient nitrate concentrations during my sampling event (which were comparable to the NO_x concentrations in the cores) were less than 2 μM, indicating nitrate limitation of direct denitrification in this system. Throughout the experiment, the moles of NO_x taken up by the soil were slightly less than the moles of N_2-N released suggesting the occurrence of coupled nitrification-denitrification as well as direct denitrification and further supporting the conclusion that nitrogen fixation rates were negligible.

The effects of petroleum hydrocarbons on porewater chemical properties, dissolved gas, and inorganic nutrient fluxes are complex and appear to depend on pre-existing soil conditions as well as the type and quantity of oil applied. Prior research found oil applications lowered dissolved oxygen concentration and soil redox potential (DeLaune et al. 1979; Nyman 1999; Suprayogi and Murray 1999; LaRiviere et al. 2003). However, in the present study, more oxidizing conditions as well as lower porewater salinities were measured in the impacted soils. This suggests that the close proximity of the impacted location to mouth of Barataria Bay and to a freshwater eddy that passes by the Bay increased flushing relative to the reference location (Walker et al. 2005; Das et al. 2010).

Soils and sediments amended with low levels of hydrocarbons frequently exhibit enhanced rates of nitrogen fixation and denitrification, while more extreme applications primarily inhibit these processes (Li et al. 1990; Gilbert et al. 1997). In the current study, the presence of weathered crude oil on *Spartina* and *Avicennia* soils significantly increased NO_x uptake and slightly enhanced net denitrification rates, though the latter was not statistically significant. These increases may be attributed to a decrease in oxygen diffusion into the soil and a subsequent reduction in redox potential (DeLaune et al. 1979; Nyman 1999; Shin et al. 2000). Decreasing diffusion and soil redox potential would steepen the oxic-anoxic gradient making conditions more favorable for denitrification (Nyman 1999). However, the reference soils were actually more reduced and rates of oxygen consumption were very similar between the reference and impacted soils, so this is an unlikely explanation for the observed differences in net denitrification. Another more probable explanation is an increase in denitrifier activity with the application of oil. Louisiana's coastal wetlands show a high capacity for the degradation of crude oil and in the absence of nutrient limitation, oil applications have been shown to stimulate microbial activity (Jackson and Pardue 1999; Nyman 1999; Shin et al. 2000).

If we take into account the difference in soil type between the reference and impacted locations, the magnitude of net denitrification measured in cores from the impacted location becomes even more striking. The impacted *Spartina* and *Avicennia* sites were located on the barrier island of eastern Grand Terre; soils were very coarse, dominated by shell hash, and low in organic matter. In contrast, the reference location was in a more protected marsh of Barataria Bay with finer soils, more enriched in organic matter. In the absence of pre-impacted denitrification rates for the barrier island soils, I summarized the relationship between denitrification and soil (or sediment) organic matter for a selection of coastal wetlands (Figure

4.5). The positive relationship between denitrification and soil organic matter can be described by the following linear regression ($r^2 = 0.53$, $p < 0.0001$):

$$\text{Denitrification} = 15.17\ (\text{Organic Matter}) + 11.40 \quad (1)$$

where denitrification rate is in μmol N m^{-2} h^{-1} and organic matter is in % dry weight soil. This regression shows that soil organic matter explains at least 50% of the variability in coastal denitrification rates. If we assume the soil below 2 cm at the impacted location was not yet affected by the oil spill, the mean organic matter content of 8% would yield a denitrification rate approximately 56 μmol N m^{-2} h^{-1} lower than was measured in the impacted soil cores. Thus, it appears as though the oiling of the impacted location increased the organic matter content of these soils. This not only stimulated rates of NO_x uptake, but likely enhanced denitrification as well.

There were no consistent trends between *Spartina* and *Avicennia* habitats in porewater properties, dissolved gas, or inorganic nutrient fluxes. Results from previous studies in the Louisiana salt marsh-mangrove ecotone are variable (Patterson and Mendelssohn 1991; Perry and Mendelssohn 2009). In the mid 1980's Patterson and Mendelssohn (1991) compared several physicochemical variables in the salt marsh-mangrove ecotone of southern Louisiana and found significant differences between *Spartina* and *Avicennia* zones in elevation, percent soil moisture, redox potential, and porewater sulfide, ammonium, and phosphorus concentrations (Patterson and Mendelssohn 1991). A more recent study addressing ecosystem effects of expanding *Avicennia* populations in southern Louisiana observed similar variability in elevation, soil moisture, and redox potential; however, they were unable to detect significant differences between habitats in the majority of variables measured, including sediment accretion, primary productivity, decomposition, and many of the soil and porewater physicochemical variables

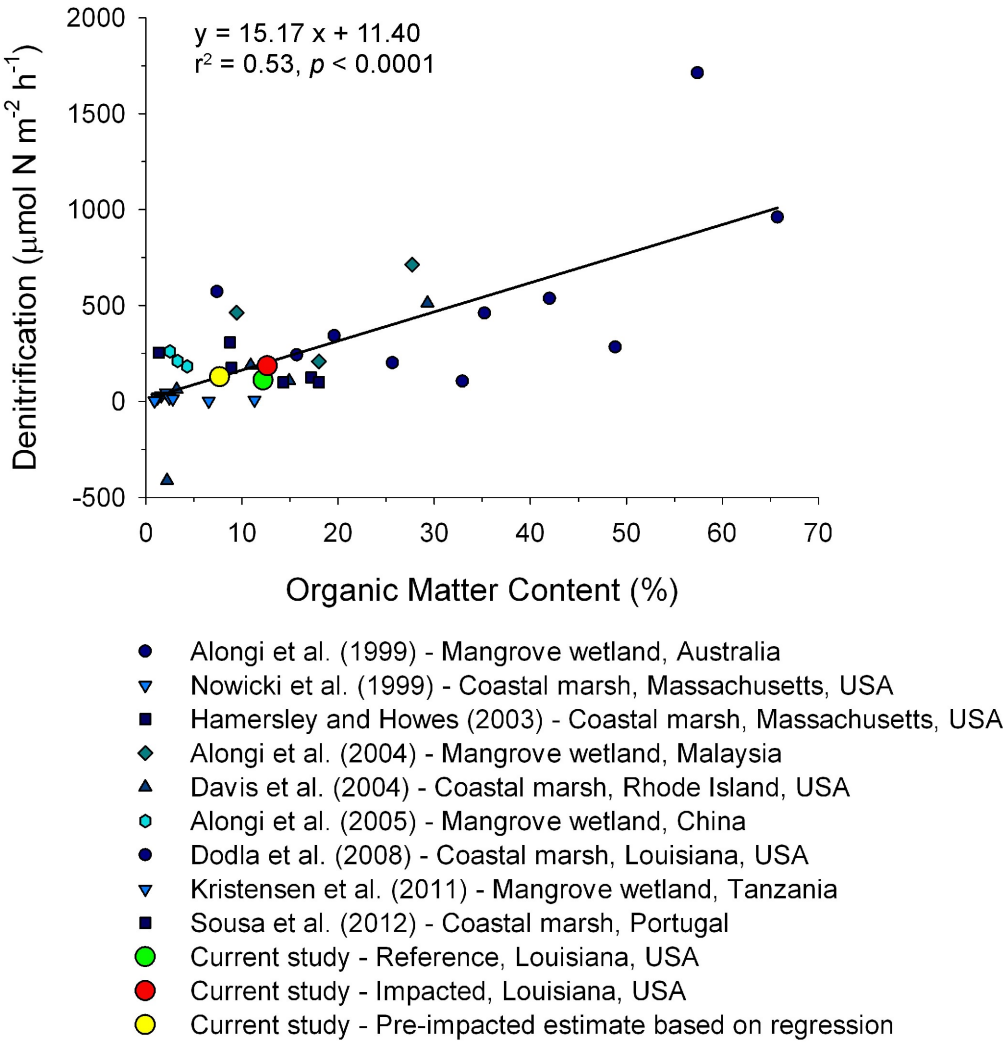

Figure 4.5. Linear regression between denitrification and soil organic matter content. Denitrification estimates presented were summarized from a variety of temperate and tropical coastal wetlands throughout the world. When possible, seasonal denitrification rates measured at temperatures comparable to the present study (> 20 °C) were used as apposed to annual integrated rates. When necessary, the relationship between soil organic carbon and organic matter described by Craft et al. (1991) was used to convert from organic carbon (% carbon) to organic matter (% loss on ignition).

analyzed (Perry and Mendelssohn 2009). The different phosphate fluxes measured between *Spartina* and *Avicenna* in this study may be the result of differing concentration gradients. Initial water column phosphate concentrations were less then 0.5 µM in all 12 of the cores; however mean porewater phosphate concentrations in the *Spartina* soils were six times the mean concentrations in the *Avicenni*a soils.

The presence of weathered crude oil from the Deepwater Horizon oil spill had no clear effect on soil oxygen demand, ammonium production, or phosphate fluxes. While there were differences between reference and impacted locations in several of the porewater and soil properties measured (e.g. salinity, pH, ammonium concentration, and redox potential), these differences could not be attributed to the application of weathered crude oil. Of the immediate impacts, the most pronounced was uptake rates of NO_x in the impacted soils that were nearly four times higher than rates in the reference soils. The application of weathered crude oil appears to have stimulated net denitrification by 30 to 40% over pre-oiled conditions. These results highlight several of the immediate impacts of the Deepwater Horizon oil spill on inorganic nutrient cycling within coastal Louisiana's wetland soils. However, the question remains as to how wetland biogeochemical cycling and important ecosystem functions like denitrification will be altered by this oil in the long-term.

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CHAPTER 5
A CONCEPTUAL MODEL OF BIOGEOCHEMICAL CYCLING DURING DELTA DEVELOPMENT IN THE ANTHROPOCENE

INTRODUCTION

A Model of Primary Substrates

Erosion of sediment from hillslopes and floodplains within the Mississippi River basin produces mineral particles that are transported through the basin at highly variable temporal and spatial patterns (Mulder and Syvitski 1996; Papanicolaou and Abaci 2008). Environmental factors within the watershed such as rainfall distribution and intensity, land cover, and terrain characteristics control the quantity and composition of mineral sediment delivered to the coast where it is deposited to form new land (Mulder and Syvitski 1996). Through natural ecological succession, land created by this process supports the development of diverse wetlands habitats that are associated with the early transgressional sequences of the delta cycle (Neill and Deegan 1986). The ecological stages of plant succession linked to the land formation are closely related to hydroperiod and salinity patterns during the early states of delta development (Gosselink et al. 1998).

The biogeochemical patterns linked to the formation of new land in depositional environments (hereafter referred to as a primary substrate) have not been clearly defined for the early stages of delta evolution. Such a model should follow the biogeochemical patterns of sediment-derived nutrients and minerals that are high in phosphorus and very low in carbon and nitrogen according to the classic model of Walker and Syers (1976). When a primary substrate is formed, it is composed largely of rock-derived nutrients and minerals. With time, nitrogen fixation and atmospheric deposition increase nitrogen towards a theoretical equilibrium nitrogen to phosphorus ratio. Eventually, thousands to millions of years after formation, most phosphorus

having been lost to leaching, erosion, or occlusion becomes biologically unavailable and henceforth the limiting nutrient.

Patterns of soil development and nutrient accumulation after mud flows (Dickson and Crocker 1953), glacial recession (Crocker and Major 1955; Bormann and Sidle 1990), volcanic eruption (Vitousek et al. 1983; Vitousek and Farrington 1997), and sand dune stabilization (Lichter 1998; Tackett and Craft 2010) largely follow this model. Developmentally young systems tend to exhibit nitrogen limited plant growth (Vitousek and Howarth 1991; Vitousek and Farrington 1997) and support symbiotic nitrogen fixation (Hobbie et al. 1998; Pearson and Vitousek 2002). As soils develop, they generally show a marked decrease in phosphorus coupled to an increase in nitrogen (Bormann and Sidle 1990; Chapin et al. 1994; Crews et al. 1995; Hedin et al. 2003). Coinciding with increased nitrogen availability are increased rates of nitrogen mineralization, nitrification, and nitrous oxide production (Crews et al. 1995; Riley and Vitousek 1995; Hedin et al. 2003). With time, the increase in nitrogen shifts plant growth to phosphorus limitation (Chapin et al. 1994; Vitousek and Farrington 1997). Whereas the Walker and Syers (1976) conceptual model has been extensively applied to describe long-term patterns of soil development, nutrient availability, and plant succession in terrestrial ecosystems, no work to date applies their model to short-term evolution of biogeochemical processes during delta formation.

Nitrate Enrichment of Primary Substrates

The application of the Walker and Syers (1976) model to explain biogeochemical patterns in recently exposed primary substrates is complicated by humankind's growing influence on the environment. In 2002, Crutzen coined the term "Anthropocene" to describe the human-dominated, geological epoch following the Holocene. Amongst other things, the

Anthropocene is characterized by rapid population expansion and exploitation of the earth's resources, increasing concentrations of greenhouse gases, and an exponential increase in biologically available nitrogen (Crutzen 2002; Steffen et al. 2007). By doubling the rate at which reactive nitrogen is added to the biosphere, human activity has greatly altered the global nitrogen cycle (Vitousek et al. 1997). This increase in reactive nitrogen may accelerate the long-term development of nitrogen enrichment in primary substrates and alter the eventual equilibrium of nitrogen and phosphorus.

Perhaps nowhere is the increase in reactive nitrogen more prevalent than in the Mississippi River basin, thus a significant impact on the development of primary substrates during delta formation. Since the advent of the Haber-Bosch process and the widespread use of industrial fertilizers in modern agriculture, concentrations of reactive nitrogen (specifically nitrate) in watersheds of the Mississippi River basin have tripled (Goolsby and Battaglin 2001; Broussard and Turner 2009). The elevated nitrate levels in the Mississippi River serve as an additional source of nitrogen in the early depositional substrates of the deltaic coast and present an interesting perspective on the biogeochemical model of primary substrates.

The Walker and Syers (1976) model assumes low nitrogen availability during primary substrate development, while soils are slowly enriched with nitrogen via nitrogen fixation. However, the presence of high nitrate concentrations in the waters of the Mississippi River may reduce rates of nitrogen fixation relative to denitrification during delta formation. The microbially mediated process of denitrification converts reactive nitrate (or nitrite) to biologically unavailable dinitrogen gas (N_2). Denitrification is considered a permanent sink for reactive nitrogen and together with nitrogen fixation determines the net N_2 flux across the soil-water interface. Rates of denitrification are dependent on the organic matter content of wetland

soils (Davis et al. 2004; Dodla et al. 2008; Wolf et al. 2011 among others); and as a result, denitrification may be limited in early depositional substrates that lack organic matter to fuel the dissimilative processes of denitrification. Consequently, the biogeochemical model of Walker and Syers (1976) may require some modification from observations made during primary substrate formation in other types of ecosystems.

Finally, the biogeochemical model associated with deltaic wetland succession is important to understanding how these wetlands reduce nitrogen loading to coastal and offshore environments. Present management of the lower Mississippi River basin is based on the mid-1800s decision to control floods and protect people using a continuous network of levees rather than a system of major outlets (Criss and Shock 2001). Today, the Mississippi River delta is losing extensive areas of wetland because the delta is cut off from the sediment supply of the river (Day et al. 2000; Fischetti 2001). To stimulate wetland development and mitigate further loss, large-scale restoration efforts have been proposed to divert freshwater and sediment from the Mississippi River back into coastal bay environments (Louisiana Coastal Master Plan 2012). These freshwater diversion projects are designed to reduce salinity and increase sediment delivery to the receiving basin by mimicking the historical overbank flooding of the Mississippi River (Twilley and Rivera-Monroy 2009; Paola et al. 2011). There is a particular interest in the capacity of deltaic wetlands to remove the reactive nitrogen introduced by these diversions via the process of denitrification (Lindau et al. 1988, 1994; DeLaune and Jugsujinda 2003; DeLaune et al. 2005; Lindau et al. 2008). Ultimately, the ability of emerging wetlands to reduce the nitrate load in the Mississippi River depends on the development of biogeochemical cycles as sediment deposition from diversions forms primary substrates.

The present study applies the Walker and Syers (1976) conceptual model to describe patterns of nutrient biogeochemistry during primary substrate development of a river-dominated coast. Primary substrate formation (delta islands or lobes) in this region is the result of sediment deposition delivered by the Mississippi River from upland watersheds. Here I use a "space-for-time substitution" (Pickett 1989) to measure changes in soil chemistry and soil-water column fluxes of net N_2, oxygen, and dissolved inorganic nutrients (NO_2^-, NO_3^-, NH_4^+, PO_4^{-3}) since emergence of the Wax Lake delta (WLD) in 1973. In this paper, I determine if short-term patterns of soil chemistry and dissolved inorganic nutrient fluxes along an emerging delta chronosequence are consistent with the Walker and Syers (1976) model of long-term nutrient availability during ecosystem development. Then, by contrasting the current anthropogenically enriched high-nitrate scenario with a low-nitrate scenario more typical of primary substrate development, I evaluate the role of nitrate enrichment in determining the net N_2 flux during the evolution of the WLD. Finally, I assess the capacity of primary deltaic substrates to reduce nitrogen load by denitrification.

Wax Lake Delta as a Model Primary Substrate

Located within the Atchafalaya basin of the Mississippi River delta plain, the Wax Lake delta (WLD) provides an ideal ecosystem for the application of Walker and Syers (1976) biogeochemical model of primary substrate formation in the Anthropocene. The Atchafalaya River begins at the convergence of the Mississippi and the Red Rivers and flows approximately 275 km through the wooded lowland and cypress-tupelo swamps of the Atchafalaya basin before reaching the Gulf of Mexico. At the convergence, the Old River Control Structure stabilizes the Atchafalaya River at 30% of the combined flows of the Mississippi and Red Rivers (mean annual discharge 6,371 $m^3\ s^{-1}$; Ford and Nyman 2011). In 1942, the Wax Lake outlet was

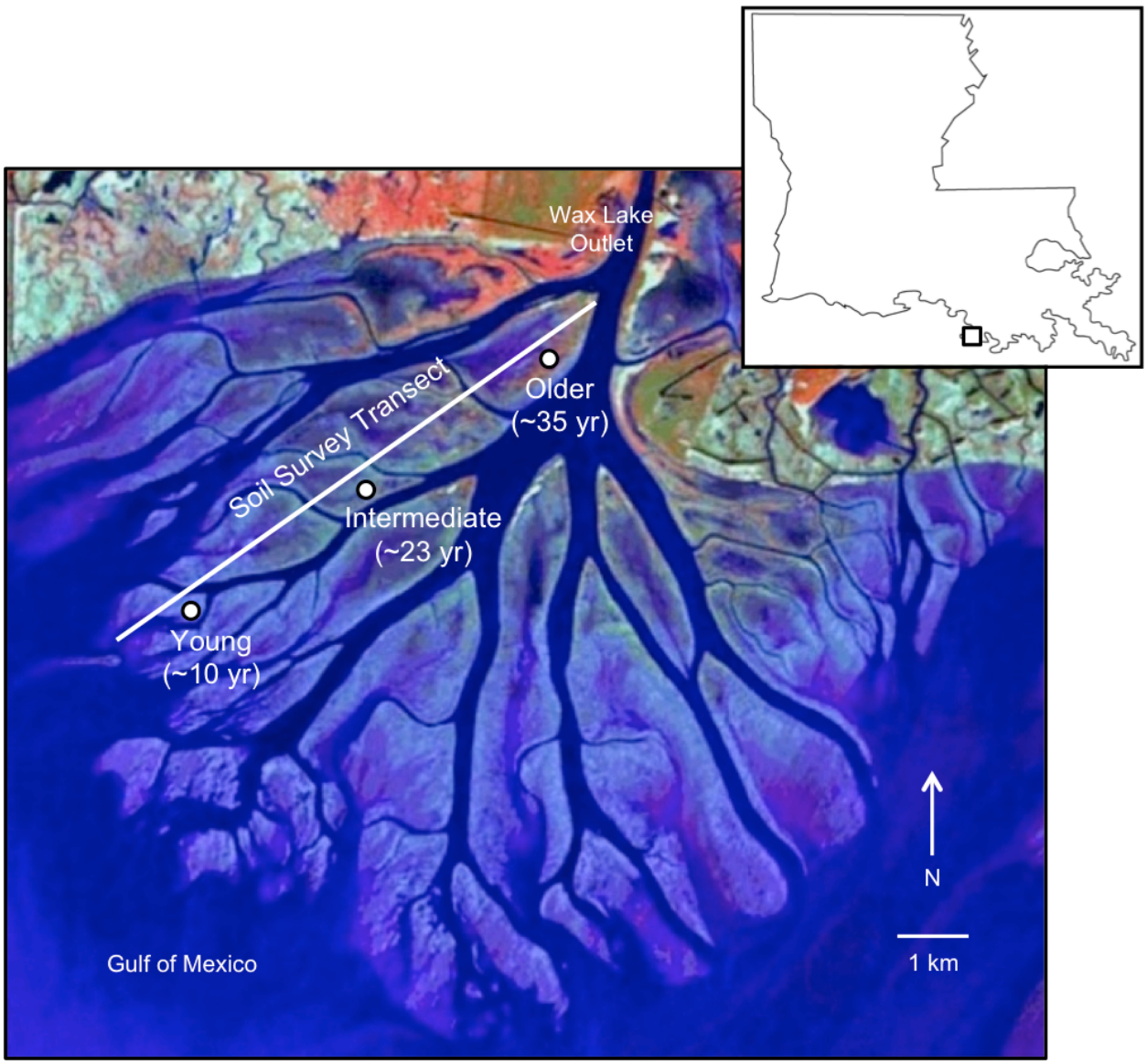

Figure 5.1. Location of the age-gradient of study sites across the Wax Lake delta, Louisiana. The 35-year old site is closest to the head of the delta and the Wax Lake outlet. The 10-year-old site is closest to the Gulf of Mexico. Image courtesy of United States Geological Survey.

constructed to provide flood relief for the lower Atchafalaya (Figure 5.1). Thirty percent of flow from the Atchafalaya River is diverted through the outlet, approximately 10% of the Mississippi River discharge.

Two deltas are currently forming in Atchafalaya Bay, the Atchafalaya and the Wax Lake deltas; however, only the WLD has been allowed to form naturally without any major

morphological manipulation (Roberts et al. 1997; Allen et al. 2012). The WLD first emerged above mean sea level after the unusually high spring flood of 1973 and has experienced rapid subaerial growth throughout the last 35 years (1 km^2 yr^{-1}; Allen et al. 2012). Because it is such a young delta, aerial photographs and satellite images allow for approximate aging of the delta lobes (Wellner et al. 2006). Using distance from head of the WLD delta as a proxy for substrate age, three sites were established on lobes 1.0, 4.5, and 7.5 km from the head of the delta corresponding to primary substrates that are approximately 35, 23, and 10 years old, respectively (Figure 5.1). These lobes differ markedly in substrate age and plant community, but share similar parent material, hydrology, and climate.

At the Young (~10 yr) site (29°30'06" N 091°28'46" W), *Sagitarria platyphylla* is the dominant vegetation in the summer and fall; however, during the winter and spring, vegetation is completely absent from this site. Vegetation at the Intermediate (~23 yr) site (29°30'45" N 091°27'56" W) is a mixed community composed of *Colocasia esculenta*, *Phragmites australis*, *Polygonum punctatum*, *Typha spp.*, *Schoenoplectus spp.*, and *Zizaniopsis miliacea*. *Salix nigra* is the dominant vegetation present at the Older (~35 yr) site (29°31'56" N 091°26'10" W), with an understory of *C. esculenta* and *P. punctatum.* Sedimentary facies of the WLD display the typical coarsening upward trend, with a high percentage of medium to fine sand and coarse silt throughout the sedimentary framework of the delta (Roberts et al. 1997). Water level in the WLD is influenced by diurnal microtides (0.3 m), seasonal wind-driven events, and the annual flood cycle of the Atchafalaya River (Swenson and Sasser 1992; Holm and Sasser 2001). The combination of these hydrological forcing factors causes water levels to rise through the winter, peak in the spring, and decrease to a minimum in the fall (Swenson and Sasser 1992; NOAA Tides and Currents 2012). Climate in the region is humid subtropical (Peel et al. 2007) and

characterized by mild winters (6 °C), hot summers (30 °C), and ample rainfall (160 cm yr^{-1}; National Climatic Data Center 2012). From this perspective, these sites define a sequence of delta lobes assembled from the same parent material differing in substrate age and stage of ecological succession. My approach was to measure soil chemistry and net N_2, oxygen, and dissolved inorganic fluxes in order to draw relationships between primary substrate development and nutrient biogeochemistry during the evolution of the WLD.

MATERIALS AND METHODS

Initial Soil Survey

In August 2010, I established an 8 km transect from the head of the Wax Lake delta (WLD) to the Gulf of Mexico (Figure 5.1). Triplicate soil cores (10 cm internal diameter by 10 cm depth) were collected at 1.0, 3.0, 4.5, 6.0, and 7.5 km to capture soil conditions across the substrate age gradient of the WLD. Soil from the top 4 cm of each core was oven-dried at 60 °C to a constant weight and bulk density was determined by dividing the total dry weight by the soil volume (81 cm^3). Prior to soil organic matter and nutrient analysis, each sample was ground to 250 μm in a Wiley Mill. Total organic matter was determined by loss on ignition at 550 °C for 2 hours (Davies 1974). Total carbon and nitrogen concentrations were determined with an ECS 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, California). After combustion in a furnace for 2 hours at 550 °C, total phosphorus was extracted from soil samples with 1 M HCl (Aspila et al. 1976) and determined by colorimetric analysis using a Flow Solution IV autoanalyzer (OI Analytical, College Station, Texas). To express soil organic matter and nutrient content per unit volume, I multiplied by the bulk density of each sample.

Seasonal Field Sampling

In late summer/early fall (September 2010), winter (January 2011), and spring (May 2011), triplicate 90 mL samples of porewater were collected from a depth of 10 cm within the Young, Intermediate, and Older sites. Temperature, salinity, and pH were measured in the field with a Hach HQ Series portable meter. Five mL of porewater were added to an equal volume of antioxidant buffer and analyzed for hydrogen sulfide concentration (H_2S) with a micro mono ion sulfide electrode (McKee et al. 1988). Thirty mL of porewater were filtered with a Whatman 42 filter paper, acidified with 1 M HCl, and analyzed for dissolved organic carbon using a TOC-V series carbon analyzer (Shimadzu Scientific Instruments, Columbia, Maryland). The remaining 55 mL of porewater were filtered with a Whatman GF/F glass fiber filter (0.7 μm), acidified with 6 M H_2SO_4, and frozen for analysis of dissolved inorganic nitrogen (nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+)), and dissolved inorganic phosphate (PO_4^{-3}) (dissolved inorganic nutrient analysis described below). Soil oxidation-reduction (redox) potential was measured at 10 cm with a platinum electrode (Chapter 3; Hargis and Twilley 1994).

Triplicate, intact soil cores (10 cm internal diameter by 10 cm depth) were collected from each site. Care was taken to avoid aboveground vegetation; yet roots, rhizomes, and an occasional small plant were captured within the cores. After collection, soil cores were placed in a cooler and transported to an environmental growth chamber at Louisiana State University. Cores were pre-incubated in a water bath for 24 to 48 hours with air gently bubbling through the overlying water. During the pre-incubation period, chamber conditions were maintained at ambient field temperature (25 °C in summer/fall and spring, and 12 °C in winter) and in continuous darkness. Surface water was collected from the Wax Lake outlet (hereafter referred

to as site water) and filtered immediately to 0.2 µm. Site water was stored in the dark, at ambient field temperature.

In winter (February 2011) and spring (June 2011), three additional soil cores (10 cm internal diameter by 10 cm depth) were collected from each site for microbial biomass carbon and chlorophyll *a* analysis. The chloroform fumigation extraction method (Vance et al. 1987 as modified by White 2006) was used to determine microbial biomass carbon in the soil. Replicate (fumigate and non-fumigate) soil subsamples from the top 4 cm of the core were weighed (equivalent to 1 g dry weight) into 50 mL centrifuge tubes. Each replicate for fumigation received 0.5 mL of chloroform. Fumigation replicates were placed in a glass vacuum desiccator, with an additional beaker of chloroform, and fumigated for approximately 24 hours. The following day, both fumigated and non-fumigated samples were extracted with K_2SO_4, shaken on a reciprocating shaker (lowest setting, 30 minutes), and centrifuged (800 to 1,000 gravities, 10 minutes). The supernatant was filtered through a Whatman 42 filter paper, acidified with 1 M HCl, and analyzed for total carbon on a Shimadzu TOC-V series carbon analyzer. Using the following equations, I determined the extractable total organic carbon (TOC mg kg^{-1} dry soil):

TOC (mg L^{-1}) * volume extracted (L) * dilution factor / soil dry weight (kg) (1)

and the microbial biomass carbon (MBC mg kg-1):

k_{ec} x ($TOC_{fumigated} - TOC_{nonfumigated}$) (mg kg^{-1}) (2)

where k_{ec} is a conversion factor of 2.70 (Sparling et al. 1990).

Soil chlorophyll *a* was measured using a modification of U.S. Environmental Protection Agency Method 445.0 (1997). A known volume (1 to 2 cm^3) of soil was subsampled from the top 4 cm of each core and placed into a 50 mL centrifuge tube. Pigments were extracted by adding 25 mL of 90% acetone to each centrifuge tube and sonicating with a probe sonicator (700

W, 30 seconds). Samples were stored in the freezer overnight (12 to 16 hours) and then centrifuged (800 to 1,000 gravities, 10 minutes). Fluorescence of the supernatant was measured before and after acidification with 1 M HCl.

Soil Core Experiments

A total of five experiments were completed throughout this study to capture seasonal variability in net N_2, oxygen, dissolved inorganic nutrient fluxes and evaluate the importance of nitrate enrichment in determining the net N_2 flux (Table 5.1). During each season (summer/fall, winter, and spring), soil cores from each of the three sites were incubated under ambient nitrate (> 60 μM) concentrations. These three experiments are hereafter referred to as the seasonal experiments. Then in winter and spring, two additional experiments were conducted on the soil cores with reduced nitrate (< 10 μM), hereafter referred to as the low nitrate experiments. Using a nitrate specific resin (© ResinTech SIR-100-HP), I decreased the nitrate concentration in a subsample of the site water to 10 μM in the winter and 2 μM in the spring. After the seasonal experiment, the same cores were pre-incubated for 24 to 48 hours with the low nitrate site water. Once the pre-incubation was complete, the soil cores were incubated again under low nitrate conditions. During each of the five experiments (three seasonal and two low nitrate), two separate incubations were conducted. The first incubation collected dissolved gas samples (N_2:Ar and O_2; gas incubation), and the second incubation collected dissolved inorganic nutrient samples (NO_2^-, NO_3^-, NH_4^+, and PO_4^{-3}; nutrient incubation). Both the gas and the nutrient incubations included a sub-incubation in the dark followed by another sub-incubation in the light. Temperature, salinity, nitrate concentrations, and light conditions were identical between the gas and nutrient incubations (Table 5.1).

Table 5.1. Incubation conditions for the seasonal and low nitrate experiments in the Wax Lake delta, Louisiana.

Experiment	Season	Temperature (°C)	Salinity (‰)	Nitrate Conc. (µM)	Incubation	Light Conditions
1[†]	Summer/Fall	25	0.2	ambient (75)	gas	dark/light
					nutrient	dark/light
2[†‡]	Winter	12	0.3	ambient (94)	gas	dark/light
					nutrient	dark/light
3[‡]		12	0.3	low (10)	gas	dark/light
					nutrient	dark/light
4[†‡]	Spring	25	0.1	ambient (62)	gas	dark/light
					nutrient	dark/light
5[‡]		25	0.1	low (2)	gas	dark/light
					nutrient	dark/light

[†]Data from experiments 1, 2, and 4 were used in the season analysis
[‡]Data from experiments 2, 3, 4, and 5 were used in the low nitrate analysis

Before beginning the dark portion of the gas incubation, I carefully replaced the overlying water in each core with the filtered site water and then sealed the cores with a gas-tight lid. Magnetic stir bars affixed to each lid gently mixed (~ 55 rpm) the overlying water throughout the incubation. Replicate water samples were collected into 12 mL exetainers (© Labco Limited, High Wycombe, Buckinghamshire, England) and preserved with 250 μL $ZnCl_2$ (50% w/v; Nielsen and Glud 1996). Water samples for N_2:Ar analysis were collected five times evenly spaced throughout the dark sub-incubation. Initial and final dissolved oxygen concentrations were measured with a Hach LDO101-01 dissolved oxygen probe. The dark sub-incubation lasted until a 2 ppm decrease in dissolved oxygen occurred (Giblin et al. 1997; Fulweiler et al. 2008). At the end of the dark sub-incubation, light was provided in the chamber environment (~ 300 μmol m^{-2} s^{-1}) and the light sub-incubation commenced. Cores remained sealed between the dark and light sub-incubations and overlying water was not replaced (Eyre and Ferguson 2002). I measured initial and final dissolved oxygen concentrations and collected N_2:Ar samples at approximately equal sampling times over the course of the light sub-incubation.

After completing the gas incubation, the overlying water was replaced and the nutrient incubation commenced. Water samples were collected and filtered with a Cole-Parmer RC-membrane filter (0.45 μm) for dissolved inorganic nutrients (NO_2^-, NO_3^-, NH_4^+, and PO_4^{-3}) at approximately the same sampling intervals. Once both the gas and nutrient incubations were complete, the top 4 cm of soil was collected from each core, oven-dried at 60 °C, and analyzed for organic matter, carbon, nitrogen, and phosphorus content (soil organic matter and nutrient analysis described above).

Dissolved Gas and Inorganic Nutrient Analysis

N_2:Ar dissolved gas samples were analyzed using a Pfeiffer Prisma QME 200 quadrupole mass spectrometer (Bay Instruments, Easton, Maryland), with a flow-through silicone capillary membrane inlet (MIMS; Kana et al. 1994). The MIMS at Louisiana State University is modified with a copper reduction column and furnace heated to 600 °C (Eyre et al. 2002). This modification removes oxygen from the dissolved gas samples, thereby eliminating the potential for an oxygen effect on N_2 measurements and subsequently increasing sample precision to ± 0.01% (Eyre et al. 2002). Dinitrogen concentrations were determined for each sample by multiplying the N_2:Ar ratio by the Ar concentration at air saturation (Colt 1984). Filtered porewater and nutrient incubation water samples were analyzed colorimetrically for NO_2^-, NO_3^-, NH_4^+, and PO_4^{-3} using a Flow IV OI analytical autoanalyzer (Strickland and Parsons 1972; Parsons et al. 1984).

Dinitrogen and dissolved inorganic nutrient fluxes across the soil-water interface were calculated from the slope of a 5-point linear regression of concentration as a function of time. To calculate the flux, the slope was multiplied by the headspace water volume and then divided by the core surface area. If the relationship between analyte concentration and time was nonlinear ($p > 0.1$), the slope and subsequently the flux were considered zero. Negative fluxes denote soil consumption or uptake, while positive fluxes denote soil production or release. The N_2:Ar ratio is a measure of the net N_2 flux (gross denitrification – gross nitrogen fixation); therefore, a positive flux equals net denitrification, while a negative flux equals net nitrogen fixation. A major limitation of the N_2:Ar technique is the inability to differentiate between N_2 production pathways (e.g. direct denitrification, coupled denitrification, and anammox) (Fulweiler et al. 2008).

Statistical Analysis

The relationship between distance from the head of the WLD (used as a proxy for substrate age) and soil organic matter and nutrient content were described with a series of simple linear regressions. Soil microbial biomass carbon and chlorophyll *a* data were analyzed using a two-way analysis of variance (ANOVA) with repeated measures to test for differences among sites (Young, Intermediate, and Older) and between seasons (winter and spring). Porewater and soil redox data were analyzed using a two-way ANOVA with repeated measures to test for differences among sites (Young, Intermediate, and Older) and seasons (summer/fall, winter, and spring). The dissolved gas and inorganic nutrient flux data were divided into two separate analyses. The first analysis only included data from the seasonal experiments and will be referred to as the season analysis. This analysis tested for significant differences among sites (Young, Intermediate, and Older) and seasons (summer/fall, winter, and spring), and between light conditions (dark and light) using a three-way ANOVA with repeated measures. The second analysis only included the two seasons I conducted experiments under ambient and low nitrate conditions and will be referred to as the low nitrate analysis. The low nitrate analysis tested for significant differences among sites (Young, Intermediate, and Older), and between seasons (winter and spring), nitrate concentrations (ambient and low), and light conditions (dark and light) using a four-way ANOVA with repeated measures. For all analyses, site, season, nitrate concentration, and light condition were considered fixed effects. Interaction effects were considered for all analyses and pairwise comparisons among treatments were described with a Tukey's honestly significant difference (HSD) test. All statistical analyses were performed with SAS PROC Mixed and significance was assessed at the 0.05 level (SAS Institute 2012).

RESULTS

Soil Properties

Organic matter content in the top 4 cm of the soil ranged from a mean of 2.8 ± 0.4% at the Young site (~10 years) to 18.2 ± 1.7% at the Older site (~35 years) and exhibited a significant, positive relationship with substrate age (Figure 5.2). Total nitrogen demonstrated a similar relationship with substrate age increasing from a mean of 0.06 ± 0.01% to 0.56 ± 0.05% from the Young to the Older site (Figure 5.2). While the relationship between total phosphorus and substrate age was significant, only 42% of the variability in the data was explained by linear regression (Figure 5.2). There was no relationship between the molar carbon to nitrogen ratio (C:N) and substrate age (overall mean: 13.2 ± 0.3; Figure 5.2). Mean molar nitrogen to phosphorus ratios (N:P) at the Young, Intermediate, and Older sites were 3.6 ± 0.4, 9.1 ± 0.7, and 15.6 ± 1.5 respectively, and N:P was positively correlated with substrate age (Figure 5.2).

Microbial biomass carbon in the top 4 cm of the soil was significantly different among sites and between seasons, and there was a significant interaction between site and season (Table 5.2). Overall, microbial biomass increased with substrate age across the Wax Lake delta (WLD) (Table 5.2). Between the winter and spring sampling events, microbial biomass decreased by 45% at the Intermediate site and 34% Older site, with no significant seasonal effect at the Young site (Table 5.2). Site, season, and their interaction significantly affected soil chlorophyll *a* (Table 5.2). However, it appears as though the majority of the significant differences observed in chlorophyll *a* were driven by the winter mean at the Intermediate site (12,773.3 ± 3,325.8 μg cm^{-3}), which was an order of magnitude greater than the remainder of the site by season combinations (mean: 1,605.6 ± 296.3 μg cm^{-3}; Table 5.2).

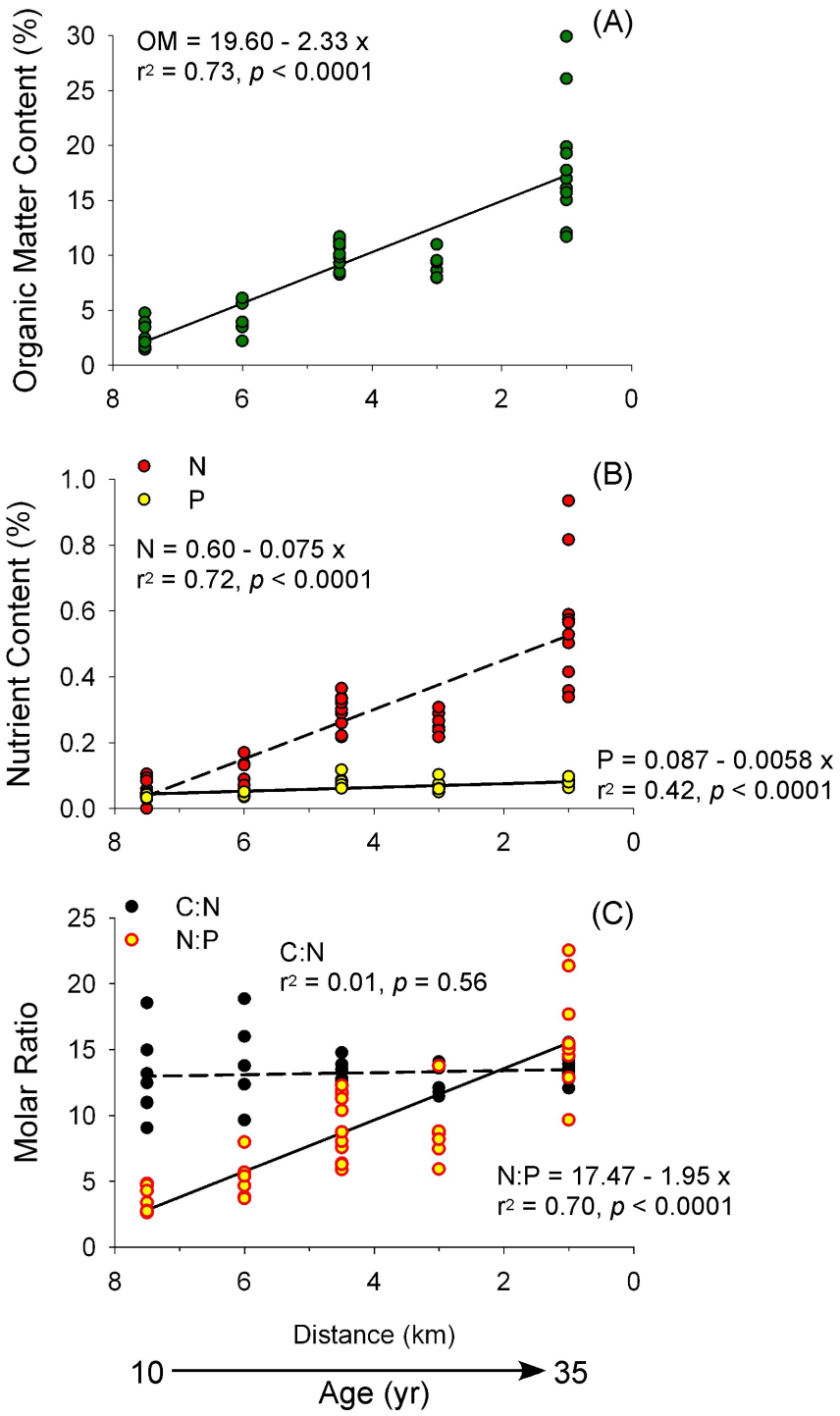

Figure 5.2. Soil (A) organic matter content, (B) nutrient content, and (C) molar ratios as a function of substrate age. Distance from the head of the Wax Lake delta used as a proxy for age. Note that values on the x-axis were inversed to clarify the positive relationship between each soil variable (y-axis) and substrate age.

Table 5.2. Means and the effects of site and season on soil microbial biomass and chlorophyll *a* in the Wax Lake delta, Louisiana.

		Means[†]						ANOVA Results[‡]	
		Winter			Spring				
		Young	*Intermediate*	*Older*	*Young*	*Intermediate*	*Older*		
Microbial Biomass	X	1.04[§d]	9.10[b]	15.90[a]	0.88[d]	5.06[c]	10.51[b]	Site	<0.0001
g C g^{-1} soil	(SE)	(0.02)	(0.89)	(0.69)	(0.08)	(1.19)	(0.19)	Season	<0.0001
								Site x Season	0.006
Chlorophyll *a*	X	1156.8[b]	12773.3[a]	2953.9[b]	351.6[b]	1332.7[b]	2232.9[b]	Site	0.002
µg cm^{-3}	(SE)	(603.8)	(3325.8)	(532.7)	(87.4)	(105.1)	(613.4)	Season	0.003
								Site x Season	0.004

[†]Mean from top 4 cm, standard error in parenthesis (n = 3)
[‡]ANOVA p values from SAS PROC Mixed
[§]Superscript letters represent significant ($p < 0.05$) differences for two-way interactions between site and season using Tukey's HSD test

Porewater Properties

Mean porewater temperature at 10 cm was 24.8 ± 0.2, 10.8 ± 0.9, and 26.2 ± 0.7 °C in the summer/fall, winter, and spring, respectively. Porewater salinity throughout the study was low with values ranging from 0.2 to 1.2 ‰. The significant differences observed in porewater salinity appear to be the result of the spring mean at the Young site, which contained the only values greater than 1.0 ‰ (Table 5.3). Soil redox potential[1] was highly variable (range: 116 to 522 mV), indicating moderately reducing to oxidizing conditions at 10 cm throughout the study period. Site, season, and their interaction effect had a significant affect on soil redox potential (Table 5.3); however, I observed no consistent trends among the sites and seasons. Hydrogen sulfide concentrations were low (overall mean: 0.01 ± 0.002 mM) and showed no significant difference among sites (Table 5.3). As a result, I only measured H_2S concentrations during the summer/fall sampling event. Porewater DOC concentrations were consistently higher at the Young site and significantly greater in the spring (Table 5.3). There was no significant effect of site or season on porewater NO_2^- (overall mean < 1.0 μM) or NO_3^- (overall mean < 1.0 μM) concentrations (Table 5.3). Mean porewater NH_4^+ was low (< 12 μM) for all site by season combinations except the winter mean at the Young site (37.1 ± 12.1 μM; Table 5.3). Porewater phosphate concentrations were only measured in the spring, during which there was no significant difference among sites (Table 5.3).

Seasonal Experiment

Initial nitrate concentrations in the water overlying the soil cores were 75, 94, and 62 μM for the summer/fall, winter, and spring incubations, respectively (Table 5.1). Mean net N_2 fluxes (181.8 ± 15.9 μmol N m^{-2} h^{-1}) across the soil-water interface were positive (net denitrification) for all site by season combinations except the winter mean at the Young site (-25.0 ± 45.5 μmol

[1]Water was too deep to measure soil redox potential at the Young site during the spring sampling event.

Table 5.3. Means and the effects of site and season on porewater and soil chemistry in the Wax Lake delta, Louisiana.

Means[†]		Summer/Fall			Winter			Spring		
		Young	*Intermediate*	*Older*	*Young*	*Intermediate*	*Older*	*Young*	*Intermediate*	*Older*
Salinity	X	0.47[‡b]	0.27[b]	0.65[b]	0.51[b]	0.37[b]	0.56[b]	1.08[a]	0.28[b]	0.64[b]
‰	(SE)	(0.03)	(0.01)	(0.20)	(0.03)	(0.03)	(0.12)	(0.09)	(0.02)	(0.06)
pH	X	6.92[b]	6.75[bc]	6.42[c]	7.38[a]	6.79[bc]	6.69[bc]	6.96[ab]	6.61[bc]	6.76[bc]
	(SE)	(0.10)	(0.03)	(0.06)	(0.09)	(0.04)	(0.21)	(0.04)	(0.01)	(0.02)
Redox	X	168.67[bc]	121.00[c]	285.17[abc]	257.50[abc]	291.50[abc]	430.67[a]	NA[§]	340.68[ab]	219.43[bc]
mV	(SE)	(10.55)	(4.51)	(20.73)	(14.74)	(24.88)	(44.04)		(90.45)	(20.33)
H_2S	X	0.01[a]	0.01[a]	0.01[a]	NA	NA	NA	NA	NA	NA
mM	(SE)	(0.005)	(0.003)	(0.003)						
DOC	X	NA	NA	NA	777.32[b]	465.85[b]	669.93[b]	2348.89[a]	1017.44[b]	1925.69[a]
µM	(SE)				(71.76)	(101.89)	(99.50)	(176.65)	(170.64)	(73.03)
NO_2^-	X	0.10[a]	0.00[a]	0.05[a]	0.08[a]	0.12[a]	0.13[a]	0.02[a]	0.06[a]	0.47[a]
µM	(SE)	(0.04)	(0.00)	(0.02)	(0.01)	(0.01)	(0.03)	(0.003)	(0.003)	(0.37)
NO_3^-	X	1.46[a]	0.00[a]	0.00[a]	0.32[a]	0.15[a]	1.61[a]	0.37[a]	1.08[a]	0.24[a]
µM	(SE)	(1.46)	(0.00)	(0.00)	(0.13)	(0.005)	(1.20)	(0.08)	(0.82)	(0.04)
NH_4^+	X	4.93[b]	9.29[b]	4.77[b]	37.14[a]	6.85[b]	11.28[b]	9.82[b]	5.75[b]	7.95[b]
µM	(SE)	(0.27)	(2.85)	(1.59)	(12.08)	(0.64)	(6.66)	(2.03)	(0.39)	(0.41)
PO_4^{-3}	X	NA	NA	NA	NA	NA	NA	1.87[a]	4.42[a]	5.12[a]
µM	(SE)							(0.26)	(1.31)	(1.96)

ANOVA Results[¶]	Salinity	pH	Redox	H_2S	DOC	NO_2^-	NO_3^-	NH_4^+	PO_4^{-3}
Site	<0.0001	<0.0001	0.04	ns	<0.0001	ns	ns	0.03	ns
Season	0.02	0.006	0.002	-	<0.0001	ns	ns	0.01	-
Site x Season	0.004	0.03	0.006	-	0.004	ns	ns	0.02	-

[†]Mean at 10 cm, standard error in parenthesis (n = 6)
[‡]Superscript letters represent significant ($p < 0.05$) differences for two-way interactions between site and season using Tukey's HSD test
[§]NA = no sample available
[¶]ANOVA p values from SAS PROC Mixed, ns = not significant ($p > 0.05$)

N m^{-2} h^{-1}; Figure 5.3). In the summer/fall, there were no significant differences among sites; however, in both the winter and the spring, net N_2 fluxes at the Young site were significantly lower than fluxes at the Older site. Only winter net N_2 fluxes demonstrated diurnal variability, with significantly lower fluxes occurring in the light (Table 5.4). Mean oxygen fluxes were dominated by soil consumption and exhibited significant differences by season with winter (-17.9 ± 6.5 mg O_2 m^{-2} h^{-1}) < summer/fall (-76.5 ± 6.5 mg O_2 m^{-2} h^{-1}) < spring (-118.3 ± 15.6 mg O_2 m^{-2} h^{-1}; Table 5.5, Figure 5.3). Within each season, soil oxygen demand was 36 to 83% lower at the Young site. Oxygen fluxes exhibited variability between dark and light sub-incubations with significantly lower rates of consumption (summer/fall) or production (winter) occurring in the light (Table 5.4).

Spatial and temporal patterns of NO_3^- uptake and release were similar to those of net N_2 and oxygen (Figure 5.3). Mean NO_3^- fluxes were dominated by uptake into the soil (-367.3 ± 52.1 μmol m^{-2} h^{-1}), except during the winter when the mean at the Young site exhibited a release (131.4 ± 61.7 μmol m^{-2} h^{-1}). Season had a significant effect on NO_3^- uptake such that the winter mean (-18.6 ± 48.7 μmol m^{-2} h^{-1}) was significantly lower than the summer/fall mean (-369.8 ± 71.1 μmol m^{-2} h^{-1}), which was significantly lower than the spring mean (-526.2 ± 97.4 μmol m^{-2} h^{-1}; Table 5.5). Within each season, rates of NO_3^- uptake were lower at the Young site than the Intermediate or Older sites. In the winter, NO_3^- uptake switched to release from the dark to the light sub-incubation (Table 5.4). Soil fluxes of NO_2^- typically showed uptake at the Young site (mean: -1.2 ± 1.3 μmol m^{-2} h^{-1}) and release at the Intermediate and Older sites (mean: 15.5 ± 2.9 μmol m^{-2} h^{-1}; Figure 5.3). Nitrite flux exhibited diurnal variability in the summer/fall and spring,

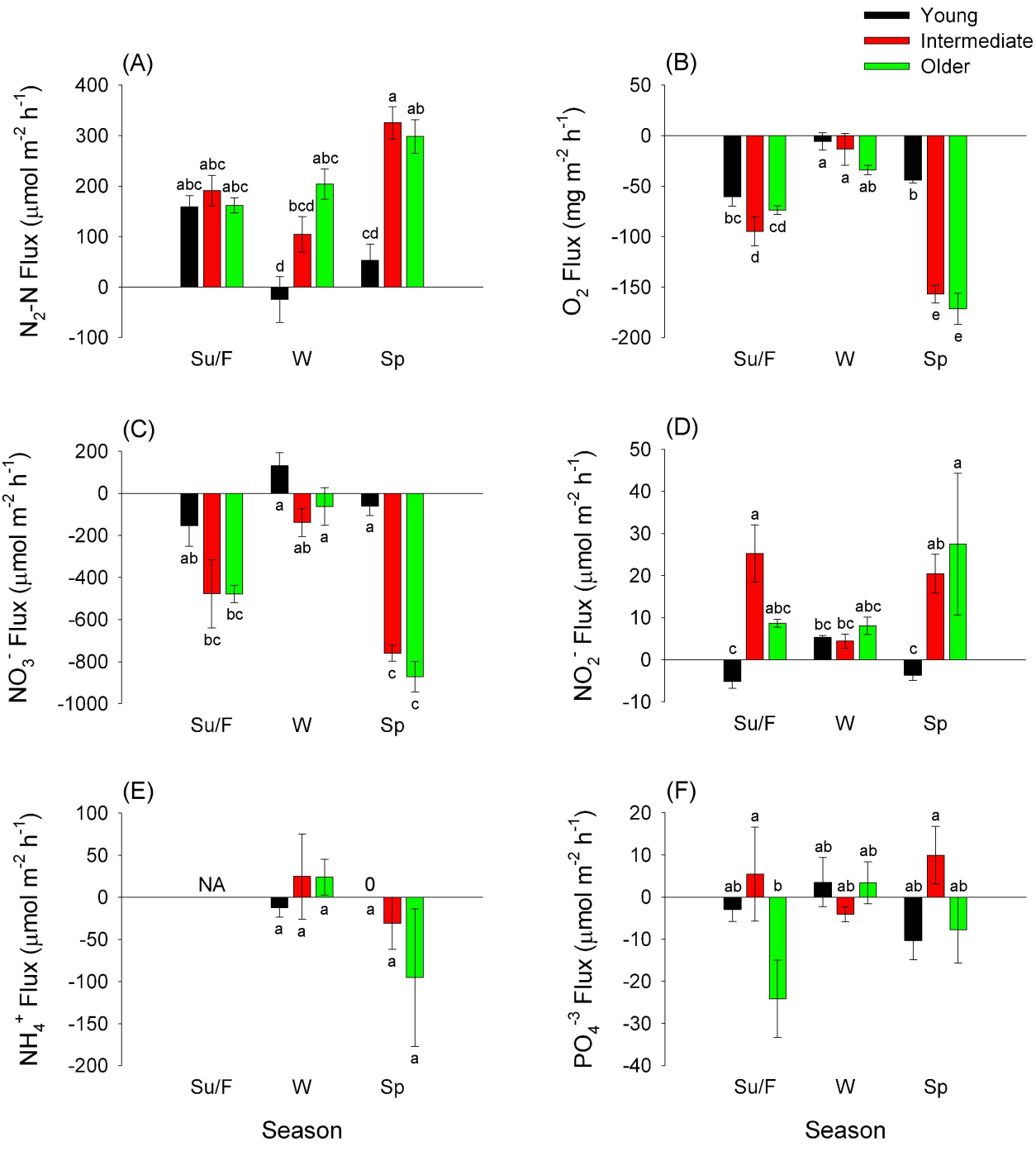

Figure 5.3. Mean (± 1 SE) (A) N_2-N, (B) O_2, (C) NO_3^-, (D) NO_2^-, (E) NH_4^+, and (F) PO_4^{-3} fluxes from the season analysis under ambient nitrate concentrations (n = 6). Within each figure, superscript lowercase letters represent significant ($p < 0.05$) two-way interactions between site and season using Tukey's HSD test. NA = no sample available.

Table 5.4. Means and ANOVA results from season analysis exploring the effects of season and light on dissolved gas and inorganic nutrient fluxes in the Wax Lake delta, Louisiana.

Means[†]		Summer/Fall		Winter		Spring	
		Dark	*Light*	*Dark*	*Light*	*Dark*	*Light*
N_2-N	X	181.35[‡a]	158.38[a]	151.16[a]	37.57[b]	207.09[a]	225.15[a]
μmol m^{-2} h^{-1}	(SE)	(19.81)	(16.04)	(32.69)	(45.40)	(49.76)	(58.68)
O_2	X	-91.90[d]	-61.05[c]	-38.21[b]	2.43[a]	-118.17[e]	-118.52[e]
mg m^{-2} h^{-1}	(SE)	(9.51)	(5.53)	(3.89)	(7.89)	(22.43)	(23.37)
NO_2^-	X	8.42[ab]	10.77[ab]	7.36[b]	3.93[b]	6.03[b]	20.23[a]
μmol m^{-2} h^{-1}	(SE)	(4.13)	(6.47)	(1.07)	(1.05)	(2.86)	(10.04)
NO_3^-	X	-305.80[bc]	-433.74[bc]	-155.25[ab]	118.06[a]	-529.41[c]	-523.06[c]
μmol m^{-2} h^{-1}	(SE)	(106.60)	(95.46)	(50.11)	(48.30)	(157.90)	(125.27)
NH_4^+	X	NA[§]	NA	60.50[a]	-39.61[ab]	-65.46[b]	-5.39[ab]
μmol m^{-2} h^{-1}	(SE)			(24.25)	(18.07)	(45.25)	(5.39)
PO_4^{-3}	X	-10.10[a]	-4.37[a]	10.01[a]	-8.17[a]	-2.59[a]	-1.61[a]
μmol m^{-2} h^{-1}	(SE)	(10.91)	(2.91)	(2.68)	(1.08)	(8.58)	(0.85)

[†]Mean presented with standard error in parenthesis (n = 9)
[‡]Superscript letters represent significant ($p < 0.05$) two-way interactions between season and light using Tukey's HSD test
[§]NA = no sample available

with increased release during the light sub-incubations (Table 5.4). Ammonium fluxes[2] (mean: -12.5 ± 15.5 μmol m^{-2} h^{-1}) were variable, and I did not observe a significant effect of site or season (Table 5.5, Figure 5.3). Phosphate fluxes (mean: -2.8 ± 2.5 μmol m^{-2} h^{-1}) were variable, with no consistent trends among sites and seasons (Table 5.5, Figure 5.3).

Low Nitrate Experiment

In the winter, initial nitrate concentrations in the overlying water were 94 and 10 μM for the ambient and low nitrate experiments, respectively (Table 5.1). Patterns of net N_2 and NO_3^- fluxes from the ambient to low nitrate experiments were not consistent among the three sites (Figure 5.4). At the Young site, mean net N_2 flux reversed from negative (net nitrogen fixation)

[2]Due to a sample contamination issue, I do not have NH_4^+ fluxes for the summer/fall nutrient incubation.

Table 5.5. ANOVA results from season analysis testing for effects of site, season, and light on dissolved gas and inorganic nutrient fluxes in the Wax Lake delta, Louisiana.

ANOVA Results[†]	N_2-N	O_2	NO_2^-	NO_3^-	NH_4^+	PO_4^{-3}
Site	<0.0001	<0.0001	<0.0001	<0.0001	ns	0.05
Season	0.002	<0.0001	ns	<0.0001	ns	ns
Site x Season	0.009	<0.0001	0.002	0.008	ns	0.03
Light	0.001	<0.0001	ns	ns	ns	ns
Site x Light	ns	ns	ns	ns	ns	ns
Season x Light	0.0006	0.004	0.02	0.02	0.009	0.03
Site x Season x Light	ns	0.001	ns	ns	ns	ns

[†]ANOVA *p* values from SAS PROC Mixed, ns = not significant ($p > 0.05$)

under ambient nitrate conditions to positive (net denitrification) under low nitrate conditions. Soils from the Young site exhibited a significant shift in NO_3^- flux from release to uptake between the ambient and the low nitrate experiments. At the Intermediate site, mean net N_2 production and NO_3^- uptake decreased in magnitude from the ambient to the low nitrate experiments, whereas net N_2 production and NO_3^- uptake at the Older site exhibited no change. Nitrite release under ambient nitrate concentrations changed to uptake under low nitrate concentrations at both the Young and Older sites (Figure 5.4). There were no notable trends in O_2, NH_4^+, or PO_4^{-3} fluxes between the ambient and low nitrate experiments during winter (Table 5.6).

In the spring, initial water column nitrate concentrations were lower than in the winter for both the ambient (62 μM) and low nitrate (2 μM) experiments (Table 5.1). At all sites, mean net N_2 flux shifted from positive (net denitrification) to negative (net nitrogen fixation) and NO_3^- uptake decreased between the ambient and low nitrate experiments (Figure 5.4). Nitrite flux decreased in magnitude under low nitrate conditions; though, the direction and magnitude varied among the different sites (Figure 5.4). At the Young site, NO_2^- uptake exhibited a decrease of 92%, while at the Intermediate site, NO_2^- release significantly decreased by 80%. Soils from the

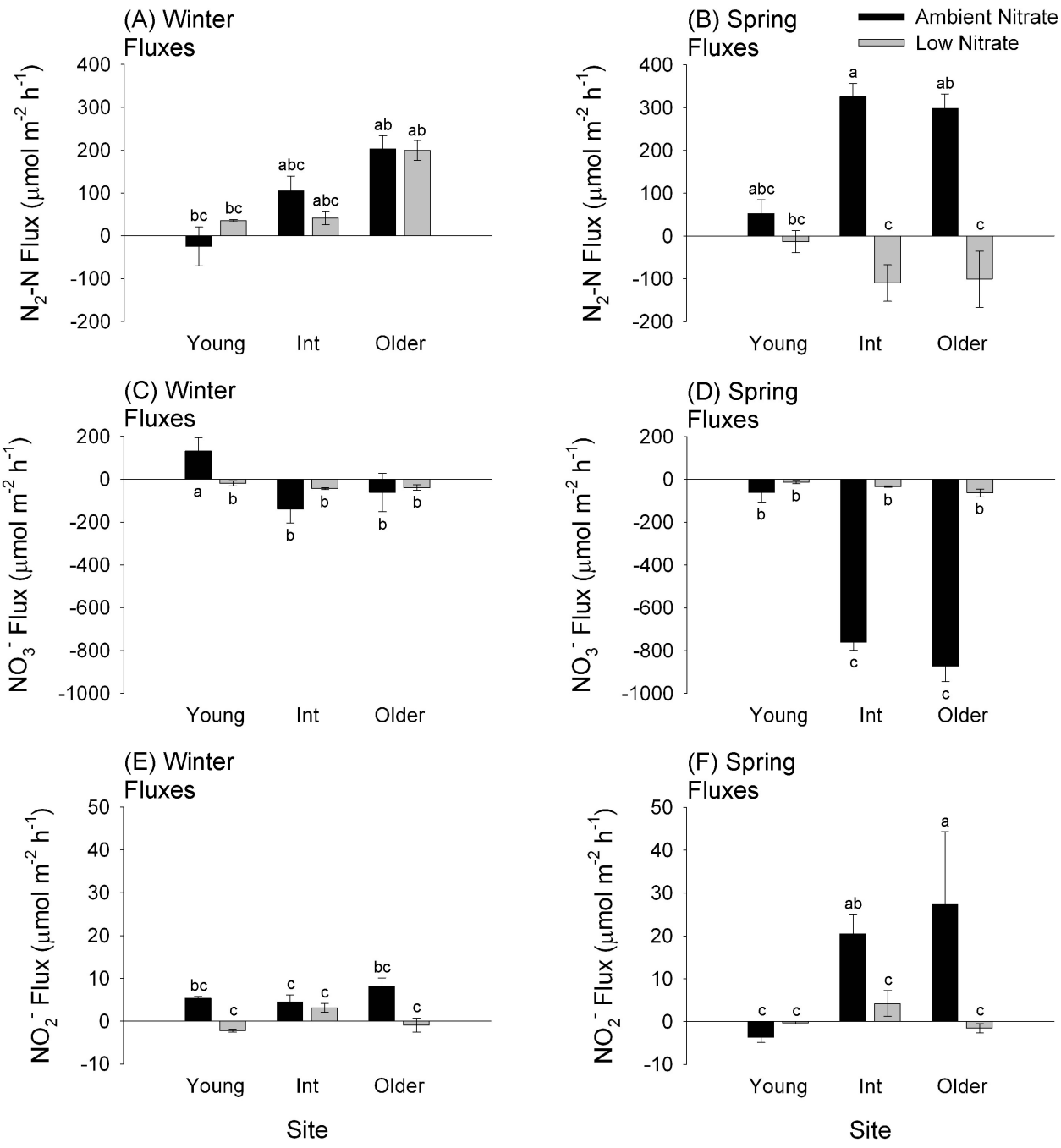

Figure 5.4. Mean (± 1 SE) (A) winter N_2-N, (B) spring N_2-N, (C) winter NO_3^-, (D) spring NO_3^-, (E) winter NO_2^-, and (F) spring NO_2^- fluxes from the low nitrate analysis (n = 6). By analyte, superscript lowercase letters represent significant ($p < 0.05$) three-way interactions among site, season, and nitrate concentration using Tukey's HSD test.

Table 5.6. Means and ANOVA results from low nitrate analysis exploring the effects of site, season, and nitrate concentration on dissolved gas and inorganic nutrient fluxes in the Wax Lake delta, Louisiana.

Means[†]		Winter						Spring					
		Young		Intermediate		Older		Young		Intermediate		Older	
		Ambient	*Low*	*Ambient*	*Low*	*Ambient*	*Low*	*Ambient*	*Low*	*Ambient*	*Low*	*Ambient*	*Low*
N_2-N	X	-24.96[‡bc]	35.42[bc]	104.53[abc]	41.10[abc]	203.52[ab]	199.28[ab]	52.69[abc]	-12.87[bc]	324.93[a]	-109.68[c]	298.05[ab]	-100.85[c]
µmol m^{-2} h^{-1}	(SE)	(45.48)	(2.77)	(35.02)	(14.82)	(30.00)	(23.15)	(32.19)	(25.52)	(31.70)	(42.68)	(33.43)	(65.73)
O_2	X	-5.95[a]	-18.38[abc]	-13.54[ab]	-39.07[cd]	-34.18[bcd]	-38.60[cd]	-44.15[d]	-32.66[bcd]	-157.04[ef]	-136.61[e]	-171.60[f]	-167.68[f]
mg m^{-2} h^{-1}	(SE)	(8.70)	(4.07)	(15.79)	(5.73)	(4.82)	(5.08)	(2.79)	(3.82)	(8.88)	(6.40)	(15.21)	(19.38)
NO_2^-	X	5.27[bc]	-2.25[c]	4.41[c]	3.12[c]	8.06[bc]	-0.96[c]	-3.71[c]	-0.30[c]	20.41[ab]	4.18[c]	27.47[a]	-1.57[c]
µmol m^{-2} h^{-1}	(SE)	(0.48)	(0.32)	(1.67)	(1.04)	(2.01)	(1.62)	(1.18)	(0.30)	(4.61)	(3.01)	(16.79)	(1.07)
NO_3^-	X	131.37[a]	-19.24[b]	-139.27[b]	-43.32[b]	-62.52[b]	-39.33[b]	-61.00[b]	-12.95[b]	-760.65[c]	-34.66[b]	-872.45[c]	-63.76[b]
µmol m^{-2} h^{-1}	(SE)	(61.69)	(11.47)	(65.91)	(4.58)	(89.23)	(12.43)	(44.70)	(8.43)	(38.22)	(3.19)	(72.83)	(17.53)
NH_4^+	X	-12.62[a]	6.77[a]	24.58[a]	102.89[a]	23.84[a]	59.67[a]	0.00[a]	-19.98[a]	-30.82[a]	56.48[a]	-95.47[a]	-65.74[a]
µmol m^{-2} h^{-1}	(SE)	(11.13)	(10.50)	(50.69)	(7.57)	(21.18)	(29.17)	(0.00)	(23.91)	(30.82)	(24.91)	(81.74)	(9.56)
PO_4^{-3}	X	3.50[a]	-0.52[a]	-4.09[a]	-0.17[a]	3.36[a]	-1.00[a]	-10.33[a]	-0.69[a]	9.95[a]	26.87[a]	-7.83[a]	-2.90[a]
µmol m^{-2} h^{-1}	(SE)	(5.84)	(0.52)	(1.83)	(0.17)	(4.95)	(0.74)	(4.57)	(0.48)	(6.83)	(11.38)	(7.83)	(3.47)

[†]Mean presented with standard error in parenthesis ($n = 6$)

[‡]Superscript letters represent significant ($p < 0.05$) three-way interactions among site, season, and nitrate concentration using Tukey's HSD test

Older site exhibited a significant shift in NO_2^- flux from release to uptake. Again in the spring, there were no notable trends in O_2, NH_4^+, or PO_4^{-3} fluxes between the ambient and low nitrate experiments (Table 5.6).

The overall main effect of lowering the initial water column nitrate concentration significantly decreased net N_2 production from 151.7 ± 25.7 to 8.7 ± 22.1 μmol N m^{-2} h^{-1}, NO_3^- uptake from -272.4 ± 70.3 to -35.5 ± 4.9 μmol m^{-2} h^{-1}, and NO_2^- release from 9.4 ± 2.8 to 0.4 ± 0.7 μmol m^{-2} h^{-1} (Table 5.7). And while the season by nitrate concentration by light condition interaction had a significant affect on oxygen and all dissolved inorganic nutrient fluxes, I observed no consistent trends among the experimental combinations (Tables 5.7; Appendix B).

Table 5.7. ANOVA results from low nitrate analysis testing for effects of site, season, nitrate concentration and light on dissolved gas and inorganic nutrient fluxes in the Wax Lake delta, Louisiana.

ANOVA Results[†]	N_2-N	O_2	NO_2^-	NO_3^-	NH_4^+	PO_4^{-3}
Site	0.05	<0.0001	0.001	<0.0001	0.03	0.05
Season	ns	<0.0001	0.02	<0.0001	0.003	ns
Site x Season	ns	<0.0001	0.01	<0.0001	0.04	0.009
Nitrate	<0.0001	ns	<0.0001	<0.0001	0.03	ns
Site x Nitrate	0.0009	ns	0.005	<0.0001	ns	ns
Season x Nitrate	<0.0001	<0.0001	0.04	<0.0001	ns	0.03
Site x Season x Nitrate	0.04	0.04	0.005	<0.0001	ns	ns
Light	ns	<0.0001	0.05	<0.0001	ns	0.01
Site x Light	ns	0.001	0.02	ns	ns	0.01
Season x Light	<0.0001	0.01	0.009	<0.0001	0.001	ns
Site x Season x Light	ns	<0.0001	0.02	ns	ns	0.0002
Nitrate x Light	0.0004	ns	ns	0.0001	0.04	ns
Site x Nitrate x Light	ns	0.02	ns	ns	ns	ns
Season x Nitrate x Light	ns	0.0005	0.004	0.0005	0.02	0.001
Site x Season x Nitrate x Light	0.02	ns	0.04	ns	ns	ns

[†]ANOVA *p* values from SAS PROC Mixed, ns = not significant ($p > 0.05$)

DISCUSSION

Soil Development

Short-term patterns of nutrient accumulation observed along the 35-year Wax Lake delta (WLD) chronosequence were consistent with the Walker and Syers (1976) conceptual model of soil development. Total nitrogen in the soil significantly increased with substrate age, while total phosphorus remained relatively constant. Corresponding to the significant increase in soil nitrogen was a significant increase in organic matter content. Increasing concentrations of nitrogen and organic matter during soil development have been documented in both natural (Tyler and Zieman 1999; Krull and Craft 2009) and created wetlands (Craft et al. 1999, 2002; Anderson et al. 2005; Hernandez and Mitsch 2007; Wolf et al. 2011). Tight coupling between organic matter (carbon) and nitrogen is expected during wetland ecosystem succession as biological rather than geochemical processes control their accumulation (Craft 1997). Low salinity and freshwater (non-floating) marshes throughout Louisiana exhibit high variability in organic matter content (25 to 70%), with a median value of 45% (Hatton et al. 1983; Smith et al. 1983; Nyman et al. 1990; Howard and Mendelssohn 2000; Yu et al. 2006; Gardner and White 2010). Assuming the rate of organic matter accumulation in the WLD remains constant (~0.6% yr^{-1}), percent organic matter will achieve equivalence to these more mature marshes within the next 40 years.

Nutrient Biogeochemistry during the Anthropocene

The Walker and Syers (1976) model predicts nitrogen fixation and atmospheric deposition to increase soil nitrogen content during early ecosystem development. Research in terrestrial ecosystems supports this prediction and shows the relative importance of nitrogen fixation to decrease with substrate age (Hobbie et al. 1998; Pearson and Vitousek 2002).

Similarly, rates of nitrogen fixation tend to be greater in newly created or restored marshes relative to more mature marsh systems (Currin et al. 1996; Piehler et al. 1998; Tyler et al. 2003). However, there is no evidence as to how these relationships may change under nitrate-enriched conditions.

In the current study, net N_2 fluxes (mean: 157.5 ± 17.6 μmol N m^{-2} h^{-1}) under ambient nitrate concentrations (> 60 μM) were largely positive. A positive net N_2 flux does not preclude the possibility of nitrogen fixation; it simply indicates greater rates of gross denitrification than gross nitrogen fixation. In contrast to the negative relationship between nitrogen fixation and substrate age, others have found rates of denitrification generally increase as created wetlands mature (Thompson et al. 1995; Currin et al. 1996; Hernandez and Mitsch 2007; Wolf et al. 2011). The low denitrification rates measured in the coarse soils of newly created wetlands have been attributed to a lack of organic matter and low moisture (Hernandez and Mitsch 2007; Wolf et al. 2011) as well as oxygen inhibition and increased tidal flushing of porewater nutrients (Thompson et al. 1995).

Various studies have reported a positive relationship between denitrification and soil organic matter content in wetland ecosystems (Davis et al. 2004; Arango et al. 2007; Dodla et al. 2008; Hopfensperger et al. 2009; Wolf et al. 2011). Organic matter can directly control the size or activity of the heterotrophic denitrifying population (Groffman 1994); or more indirectly influence denitrification rates through regulation of soil metabolism, oxygen demand, and nitrogen remineralization (Seitzinger 1988; Cornwell et al. 1999). In the WLD, soil organic matter content explained 51 and 35% of the variability in net N_2 and oxygen fluxes, respectively (Figure 5.5). During the winter and spring sampling events, net N_2 fluxes largely paralleled changes in organic matter; with significantly lower (or negative) fluxes occurring at the Young

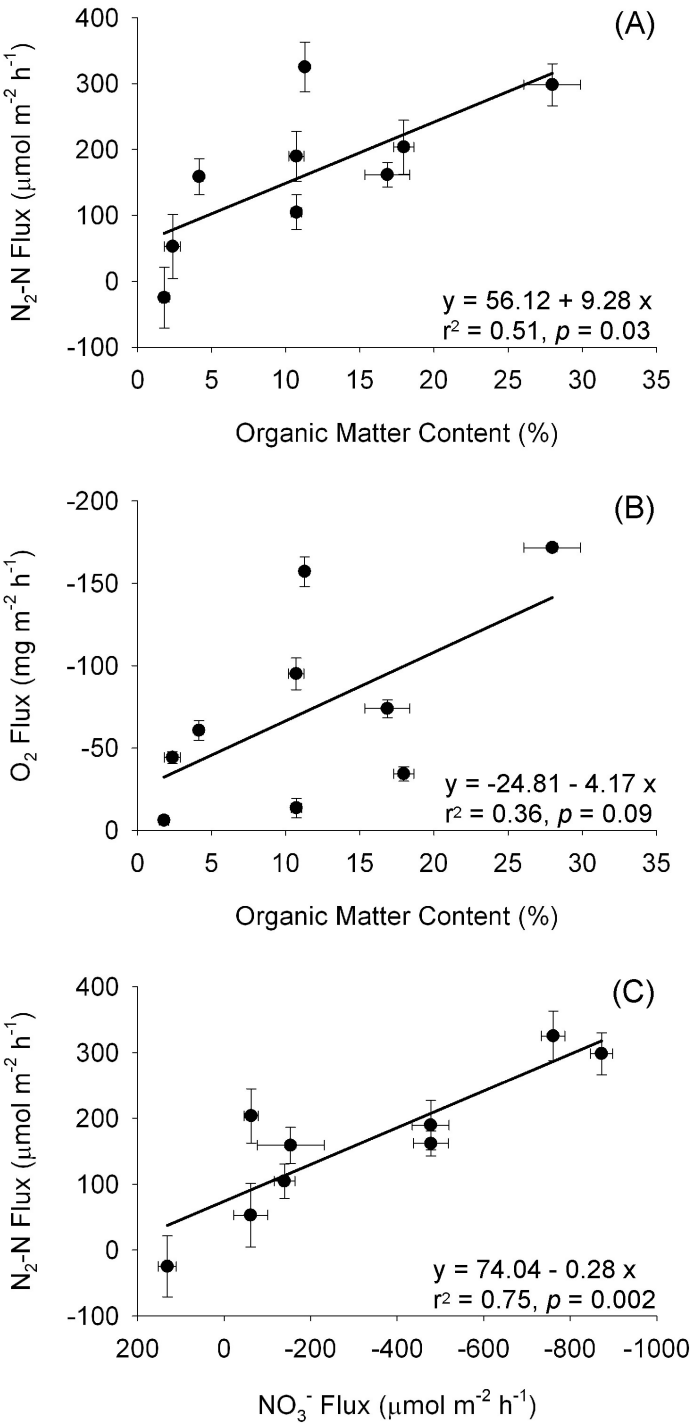

Figure 5.5. (A) Net N_2-N flux as a function of soil organic matter content, (B) O_2 flux as a function of soil organic matter content, and (C) net N_2-N flux as a function of NO_3^- flux. All data points represent mean (± 1 SE) from season analysis under ambient nitrate concentrations (flux n = 6; organic matter content n = 3). Note that values on the y-axis of figure (B) were inversed to present the relationship between the magnitude of oxygen uptake and organic matter content. Values on the x-axis of figure (C) were also inversed to present the relationship between N_2-N flux and the magnitude of nitrate uptake.

site. However, net N_2 fluxes in the summer/fall were similar across the delta and showed no correlation with organic matter.

Temporal variability of denitrification is well established; however, rates are seldom a direct function of temperature (Sherr and Payne 1978; Canfield et al. 2005; Fulweiler and Nixon 2011). To describe the seasonal differences observed in net N_2, oxygen, and nitrate fluxes among sites in the WLD, organic matter content must be interpreted together with the presence of vegetation. During the summer/fall sampling event, vegetation was present at all sites and there were no significant differences between fluxes. In the winter, vegetation was completely absent from the Young site, coverage was largely reduced at the Intermediate site, and vegetation remained present at the Older site. At this time, the gradient in fluxes across the chronosequence was the most pronounced. Finally, the spring sampling event occurred before vegetation re-established at the Young site and the magnitude of soil-water column fluxes were significantly lower than at the Intermediate or Older sites. Thus, it appears as though the presence of vegetation significantly affects net N_2, oxygen, and nitrate fluxes, particularly at the younger sites with low soil organic matter content.

Possible mechanisms for enhanced denitrification in vegetated soils include increase oxygen diffusion and leaching of labile organic carbon from the roots of macrophytes (Sherr and Payne 1978; Reddy et al. 1989; Caffrey and Kemp 1992; Risgaard-Petersen and Jensen 1997). Since an increase in oxygen diffusion into the soil is unlikely to have stimulated nitrate uptake, an increase in labile organic carbon seems more probable. Concentrations of porewater dissolved organic carbon (DOC) did not vary consistently with seasonal vegetation patterns; however, these measurements were made in the field before my soil cores were collected. When vegetation was present, the process of coring may have damaged roots and rhizomes providing

additional labile carbon and stimulating net N_2, oxygen, and nitrate fluxes (Howes et al. 1985; Eriksson et al. 2003).

During the winter sampling event, I observed a significant diurnal variability in net N_2 and oxygen fluxes. This likely reflects the influence of benthic primary production on nitrogen cycling. Light tends to increase the activity of microbial mats and microphytobenthos, which can stimulate nitrogen fixation as well as inhibit denitrification (Joye and Paerl 1994; Currin et al. 1996; Sundbäck and Miles 2000; Sundbäck et al. 2000). Soil chlorophyll *a* concentrations were notably higher in the winter, particularly at the Young and Intermediate sites. The significant decrease in net N_2 production during the light sub-incubation may be the result of enhanced nitrogen fixation. The observed decrease in net N_2 production could have been driven by a decrease in denitrification. The reduction of nitrate to N_2 gas is an oxygen sensitive process. The high rates of primary productivity, as indicated by the positive oxygen flux, may have increased oxygen penetration into the soil and decreased denitrification (Risgaard-Peterson et al. 1994). In addition, microphytobenthos are a nitrogen sink in some shallow-water systems, outcompeting denitrifiers for inorganic forms of nitrogen (Sundbäck and Miles 2000; Sundbäck et al. 2000). With winter water-column nitrate concentrations exceeding 90 μM, it is more plausible that either an increase in nitrogen fixation or oxygen inhibition of denitrification resulted in the diel pattern observed in net N_2 fluxes.

The Role of Nitrate Enrichment

Canonical denitrification in wetland soils and sediments can be fueled by nitrate diffusing from the water column (direct denitrification), nitrate formed within the soil during the oxidation of ammonium (coupled nitrification-denitrification), or nitrate advected from groundwater (Seitzinger 1988; Groffman 1994; Hopkinson and Giblin 2008). In coastal environments, nitrate

availability often limits rates of direct denitrification; and as a result, coupled nitrification-denitrification is the dominant N_2 production pathway (Giblin et al. 2010; Hamersley and Howes 2003; Poulin et al. 2007). Under ambient nitrate concentrations, net N_2 production in the WLD was highly correlated to nitrate uptake ($r^2 = 0.75$; Figure 5.5). This suggests that the production of N_2 gas was primarily via direct denitrification and the contribution of coupled nitrification-denitrification to the net N_2 flux was relatively insignificant.

A closer look at the relationship between nitrate uptake and net N_2 production reveals that for every 1 mole of NO_3-N entering the soil only 0.28 moles of N_2-N were released. This begs the question, where did the excess nitrate go? There are three known pathways for the dissimilatory reduction of nitrate: (1) canonical denitrification to nitrous oxide (N_2O) or N_2, (2) dissimilatory nitrate reduction to ammonium (DNRA), and (3) anammox, which couples the reduction of nitrite (an intermediate) to the oxidation of ammonium (Canfield et al. 2005). Of the few studies quantifying anammox in wetlands, most find it to be of minor importance to the total N_2 production (Koop-Jakobsen and Giblin 2009; Fernandes et al. 2012). Furthermore, since the end product is also N_2 gas, the presence of anammox does not help clarify the additional nitrate uptake.

A number of studies provide evidence that DNRA is a significant nitrate reduction pathway in freshwater and marine ecosystems (An and Gardner 2002; Gardner et al. 2006; Scott et al. 2008; Giblin et al. 2010). Increasing salinities and corresponding sulfide concentrations can enhance rates of DNRA by inhibiting denitrification and providing an electron donor for DNRA (An and Gardner 2002; Giblin et al. 2010). Organic matter loading can increase rates of DNRA, particularly in systems with limited nitrate supply (McCarthy et al. 2007; Scott et al. 2008). In addition to DNRA, assimilation by the microbial community could account for the

surplus of nitrate entering the soil. Research shows that benthic microphytes are capable of high rates of nitrate uptake, even under dark conditions (Rysgaard et al. 1993). Throughout my study, porewater salinities as well as hydrogen sulfide and ammonium concentrations were low and ammonium fluxes highly variable; therefore, the more probable nitrate sink was microbial assimilation.

It is plausible that what appears to be excess nitrate taken up by the soil was an underestimate of denitrification due to N_2O production or nitrogen fixation. Canonical denitrification can end at either N_2O or N_2, with the relative proportion of N_2:N_2O depending on conditions within the soil (Firestone and Tiedje 1979; Knowles 1982). In coastal Louisiana, the N_2:N_2O ratio ranges from 3 to 250 and is inversely correlated to nitrate concentration (Smith and DeLaune 1983; Lindau and DeLaune 1991; Yu et al. 2006). Throughout the current study, ambient nitrate concentrations were relatively high and it is possible that N_2O was significant end product of nitrate reduction. Unfortunately, N_2O fluxes were not measured; and as a result, the denitrification rates presented here may be underestimates.

Since the N_2:Ar technique only provides information on the net N_2 flux (gross denitrification – gross nitrogen fixation), the presence of nitrogen fixation could offset the 1:1 balance of nitrate (NO_3-N) reduced to N_2 (N_2-N) produced. In the spring, when nearly all the nitrate was removed from the overlying water (nitrate concentration: 2 μM), soils in the WLD switched from net denitrification (positive N_2 flux) to net nitrogen fixation (negative N_2 flux). While it is possible that nitrogen fixation simply "turned on" in the absence of a reactive nitrogen source, the winter net N_2 flux at the Young site was also negative (net nitrogen fixation) under ambient nitrate concentrations of 94 μM. Thus, the disproportional amount of nitrate uptake to N_2 produced may not be the result of an additional nitrate sink, but evidence of nitrogen fixation.

Nitrogen fixation is an energetically expensive process and the common paradigm is that fixation only occurs when the availability of reactive nitrogen is low (Howarth et al. 1988; Howarth and Marino 2006). Recent research challenges this model showing appreciable rates of nitrogen fixation can occur even when reactive nitrogen is available (Gardner et al. 2006; Fulweiler et al. 2007; Scott et al. 2008; Bertics et al. 2010). Furthermore, studies measuring both denitrification and nitrogen fixation provide evidence that these processes take place simultaneously in many systems (Gardner et al. 2006; Lee and Joye 2006; McCarthy et al. 2007; Scott et al. 2008).

Surprisingly, the winter reduction in water column nitrate concentration from 94 to 10 μM had no significant effect on net N_2 fluxes. It is unclear whether a water column nitrate concentration of 10 μM was low enough to reduce denitrification or a temperature of 12 °C was limiting nitrogen fixation. Nitrogen fixation is highly correlated to temperature, with significantly lower rates occurring in the winter (< 15 °C) (Carpenter et al. 1978; Marcarelli and Wurtsbaugh 2006; Fulweiler and Nixon 2011). In addition to temperature, substrate age seems to influence nitrogen fixation in the WLD. Spring net fixation was significantly lower at the Young site, which likely reflects the low organic matter content and absence of vegetation (see discussion above). Nitrogen fixing organisms have a diverse range of metabolic and ecologic strategies, including autotrophic and heterotrophic metabolisms as well as free-living and symbiotic associations (Capone 1983). When heterotrophic organisms dominate the nitrogen fixing community, low organic matter content frequently limits rates of nitrogen fixation (Langis et al. 1991; Cole and McGlathery 2012).

Overall, this study suggests that the Walker and Syers (1976) conceptual model applies to short-term patterns of nutrient biogeochemistry during primary substrate development of a river-dominated deltaic coast. Throughout the 35-year Wax Lake delta (WLD) chronosequence, total

soil nitrogen and organic matter content exhibited a marked increase, while total phosphorus remained relatively constant. Nitrate availability as well as substrate age appears to regulate the net N_2 flux during delta development. Under ambient nitrate concentrations (> 60 μM), the net exchange of N_2 gas was largely positive, indicating greater rates of gross denitrification than gross nitrogen fixation. In the absence of nitrate, the net N_2 flux reversed and soils exhibited net fixation. Soil organic matter content in conjunction with the presence of vegetation significantly affected net N_2, oxygen, nitrate, and nitrite fluxes yielding lower fluxes at the Young site (~10 years old). Consequently, as soils in the WLD aged, their capacity for nitrate uptake and net denitrification increased. Results from this study indicate the possibility of dissimilatory nitrate reduction to ammonium (DNRA) as well as potentially high rates of N_2O production. However, further investigation is necessary to clarify the contribution of DNRA to the total nitrate uptake and the relative proportion of N_2O to N_2 produced.

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CHAPTER 6
SUMMARY AND SYNTHESIS

The main focus of this research was to explore how selected environmental factors regulate nutrient biogeochemistry in wetlands soils across distinct developmental stages of the Mississippi River delta. The primary hypothesis was that patterns of soil development and nutrient biogeochemistry are linked to the different phases of the delta cycle, much like the ecological features of a delta have been described (Gosselink 1984, Gosselink et al. 1998). To test this hypothesis, I determined patterns of nutrient biogeochemistry associated with soil development in two distinct coastal basins of the Mississippi River delta, the transgressive Barataria basin and the regressive Atchafalaya basin. Soil development was predicted to follow the classic model of Walker and Syers (1976); however, the Mississippi River delta today represents an anthropogenically impacted landscape that may require adjustment to any concept of biogeochemical patterns. Therefore, the secondary goal of this research was to understand how anthropogenic impacts regulate the principle environmental factors controlling nutrient biogeochemistry, thereby altering the predicted patterns within these distinct coastal basins.

During the transgressive phase of the delta cycle, soil chemistry was strongly influenced by episodic disturbances. Soil stratigraphy from the Fourchon region showed that the climate-induced vegetation shift from *Spartina alterniflora* Loisel to *Avicennia germinans* L. had no clear effect on soil nutrient chemistry. However, both natural (e.g. hurricanes) and anthropogenic (e.g. dredging) disturbances significantly modified patterns of soil development. These events increased soil bulk density and total phosphorus content, while decreasing organic matter and total nitrogen content. In Barataria Bay, the anthropogenic disturbance of the Deepwater Horizon oil spill impacted wetland soil chemistry by significantly increasing soil organic matter content following the application of weathered crude oil. Flux measurements

indicated that initial *Avicennia* expansions had no significant effect on net N_2, oxygen, or dissolved inorganic nitrogen fluxes within the *Spartina* marshes of Fourchon or Barataria Bay. Nitrate availability and soil organic matter content appeared to influence net N_2 fluxes (Figure 6.1). When water column nitrate concentrations were < 2 μM, direct denitrification was likely limited in both Fourchon and Barataria Bay; and as a result, coupled nitrification-denitrification was likey the dominant N_2 production pathway. In Barataria Bay, the application of weathered crude oil from the Deepwater Horizon oil spill significantly increased soil organic matter content, which appeared to stimulate rates of nitrate uptake and net denitrification.

In the early regressive phase of rapid subaerial delta growth, soil nutrient chemistry exhibited patterns characteristic of primary substrate development. Specifically, total nitrogen and organic matter content increased, while total phosphorus remained relatively constant. Both nitrate availability and substrate age appeared to regulate net N_2 fluxes throughout the 35-year WLD chronosequence (Figure 6.1). In the presence of ambient nitrate concentrations (60 to 100 μM), mean net N_2 fluxes were largely positive indicating greater rates of gross denitrification than gross nitrogen fixation. Net denitrification was highly correlated with nitrate uptake, suggesting direct denitrification was the primary N_2 production pathway (versus coupled nitrification-denitrification). Rates of nitrate uptake into the soil were more than three times the net denitrification rates, which may be indicative of nitrous oxide (N_2O) production, dissimilatory nitrate reduction to ammonium (DNRA), or microbial assimilation. In the absence of an appreciable nitrate source (< 2 μM), soils switched from net denitrification to net nitrogen fixation, highlighting the potential importance of nitrogen fixation in these newly developing soils. As soils in the WLD aged, the subsequent increase in organic matter content and the presence of vegetation stimulated net N_2, oxygen, nitrate, and nitrite fluxes yielding greater

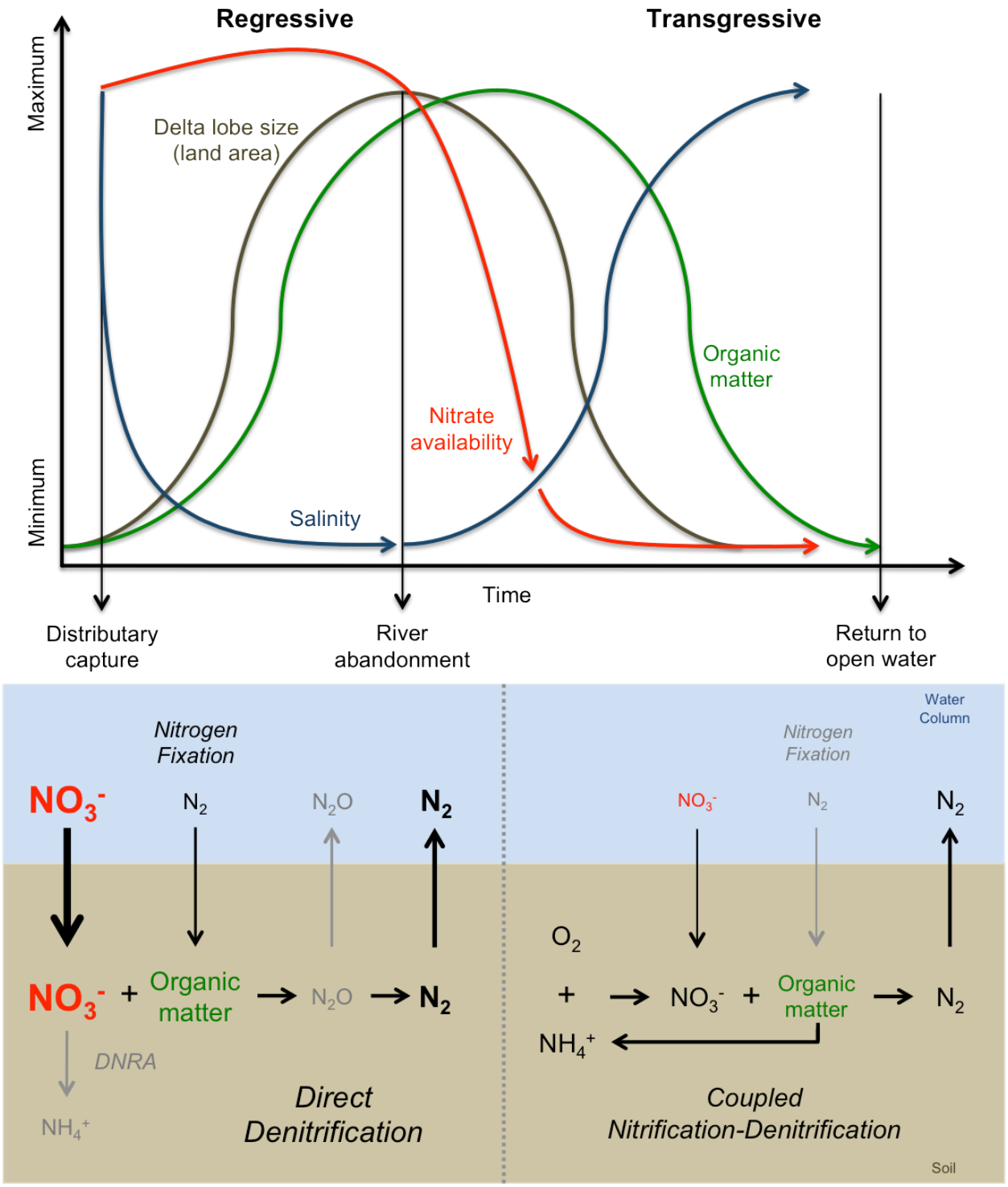

Figure 6.1. Conceptual model of nitrogen biogeochemistry during the regressive (growth) and transgressive (decay) phases of the delta cycle. Size of the font reflects relative importance of a specific analyte or transformation. Processes in gray require further research to confirm their relative importance. (Figure adapted from Gosselink 1984 and Gosselink et al. 1998).

fluxes at the older sites (23 to 35 years old), which suggests organic matter regulation of dissolved inorganic nitrogen cycling.

Overall, the presence of water column nitrate and soil organic matter content played an important role in determining soil nutrient biogeochemistry, more specifically inorganic nitrogen cycling, in wetland soils throughout the Mississippi River delta. Under ambient conditions, net N_2 fluxes were positively correlated with rates of nitrate uptake ($r^2 = 0.73$, $p < 0.0001$; Figure 6.2). The majority of measurements from the WLD fall below the 1:1 ratio of net N_2-N to NO_3-N flux, while measurements from Fourchon and Barataria Bay are much closer to or just above this ratio. Points below the line represent soils with lower rates of net denitrification than nitrate uptake (N_2-N < NO_3^-) in which net N_2 fluxes were likely dominated by direct denitrification. A low net denitrification to nitrate uptake ratio is suggestive of additional nitrate utilization processes such as DNRA and microbial assimilation, and could be indicative of denitrification

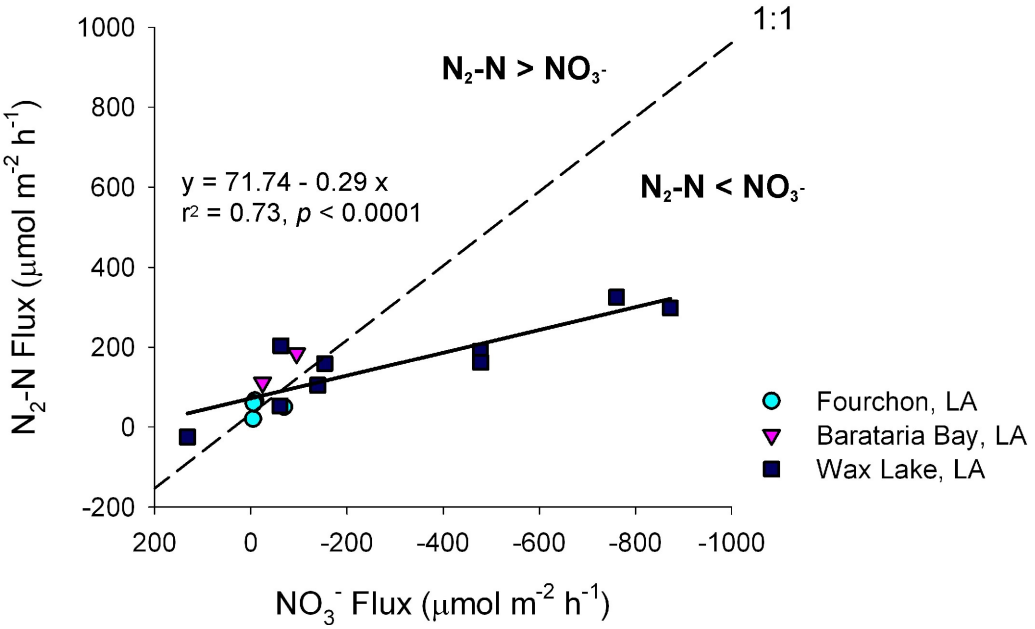

Figure 6.2. Net N_2-N flux as a function of NO_3^- flux for all sites sampled in the wetlands of the Mississippi River delta. Data from cores incubated under ambient nitrate concentrations. Note that values on the x-axis were inversed to present the relationship between the magnitude of N_2-N flux and the magnitude of NO_3^- flux. Line is the 1:1 ratio between denitrification and nitrate uptake.

that terminates in N_2O production as well as the presence of nitrogen fixation. Points above the line represent soils in which net denitrification was greater than nitrate uptake (N_2-N > NO_3-N) where net N_2 fluxes were likely dominated by coupled nitrification-denitrification.

In the absence of nitrate (< 2 μM), there was a marked difference in net N_2 fluxes between the WLD, which exhibited net fixation, and Fourchon and Barataria Bay, which exhibited net denitrification (Figure 6.3). Furthermore, there was no significant relationship between net N_2 fluxes and initial water column nitrate concentrations. The lack of a relationship between these factors was likely due to the occurrence of coupled nitrification-denitrification in Fourchon and Barataria Bay and the confounding effects of soil organic matter, temperature, and salinity throughout the different regions included in my study. The positive relationship between net N_2 flux and soil organic matter content was significant throughout the wetlands of the Mississippi River delta examined in this study ($r^2 = 0.53$, $p = 0.002$; Figure 6.4).

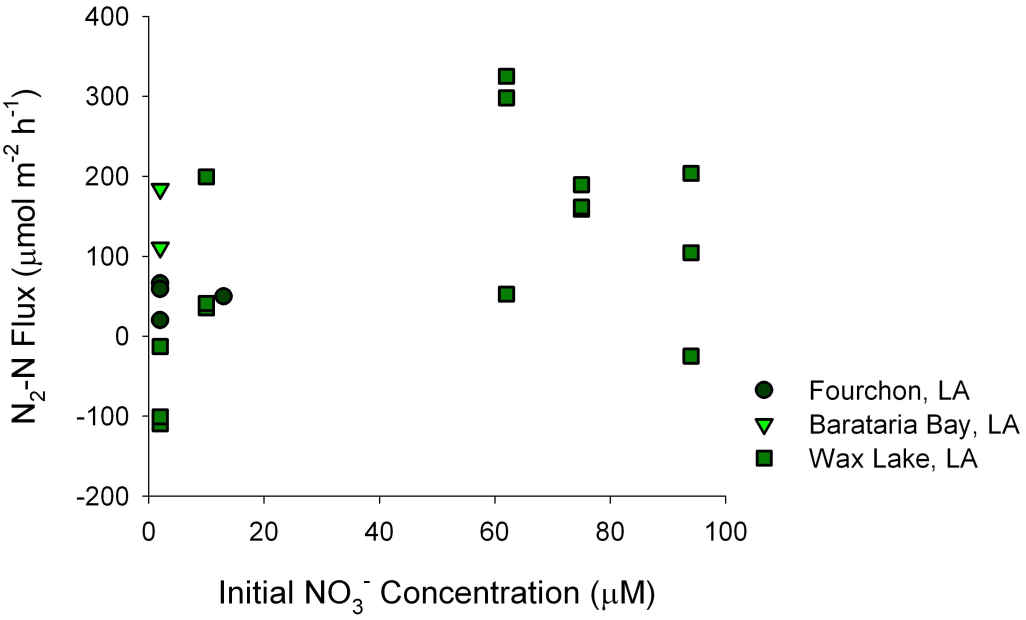

Figure 6.3. The relationship between net N_2-N flux and initial water column NO_3^- concentration for all sites sampled in wetlands of the Mississippi River delta. Data from Wax Lake include N_2-N fluxes under ambient and reduced nitrate concentrations.

This research documents patterns of nitrogen biogeochemistry coupled to different stages (early versus late) of delta formation in the Mississippi River delta. Net N_2 flux across the soil-water interface was dominated by denitrification during both early and late stages. Young regressive regions of the Mississippi River delta reflected a high capacity for direct denitrification of riverine nitrate. This capacity was largely regulated by substrate age and the accumulation of organic matter. After the river switches its course and abandons a delta lobe, nitrate availability and subsequently direct denitrification decreased. The net N_2 flux was dominated by coupled nitrification-denitrification likely fueled by the remineralization of organic matter. Of the anthropogenic disturbances captured in this study, the nitrate enrichment of the Mississippi River appears to exert the largest influence on patterns of nitrogen biogeochemistry.

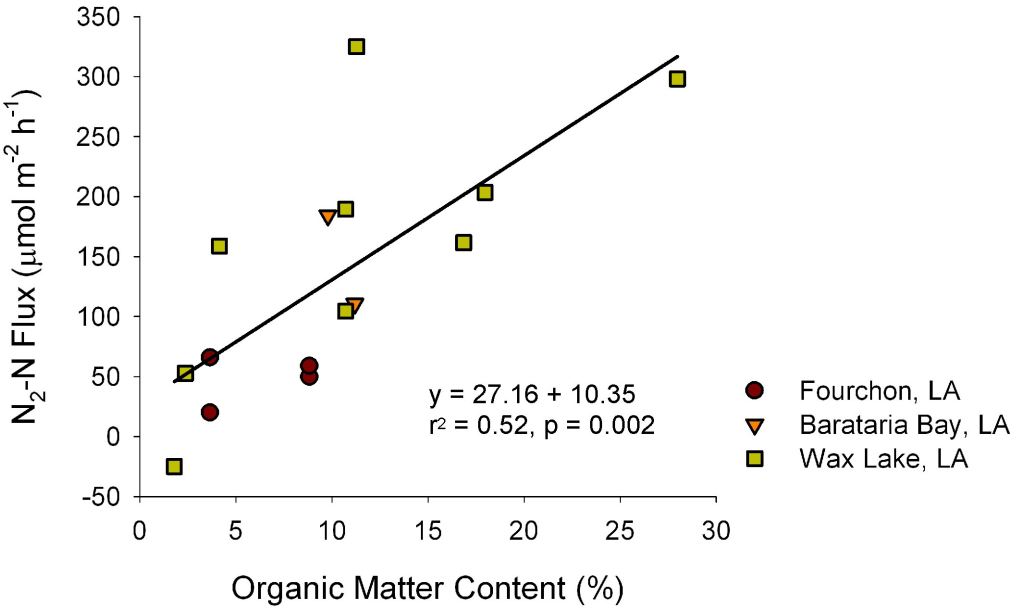

Figure 6.4. The relationship between net N_2-N flux and soil organic matter content for all sites sampled in wetlands of the Mississippi River delta. Data from cores incubated under ambient nitrate concentrations.

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APPENDIX A
EFFECT OF MARSH ELEVATION ON INORGANIC NUTRIENT CYCLING IN THE SALT MARSH-MANGROVE ECOTONE OF SOUTHERN LOUISIANA

Table A.1. Elevation and hydroperiod data for High and Low elevation plots in the Bay and Port sites located in Fourchon, Louisiana.

	Bay		Port	
	High	Low	High	Low
Elevation (cm)[†]	33.4 ± 1.2[a‡]	20.3 ± 0.6[b]	32.9 ± 0.7[a]	20.1 ± 0.4[c]
(*n*)	(32)	(39)	(41)	(36)
Hydroperiod				
Duration (h yr^{-1})	3707	3739	4301	5780
Duration (%)	42	43	49	66
Frequency (events yr^{-1})	246	240	221	183

[†]Least squares means ± 1 standard error presented for elevation data

[‡]Within row, means followed by the same letter are not significantly different according to Tukey's HSD test ($p > 0.005$)

Table A.2. Means and the effects of site, elevation, and habitat type on porewater chemistry in the salt marsh-mangrove ecotone of Fourchon, Louisiana.

Means[†]							
		Site		Elevation		Habitat	
		Bay	Port	High	Low	*Spartina*	*Avicennia*
Salinity	X	28.53	27.30	28.06	27.76	25.60	30.23
‰	(SE)	(0.55)	(1.57)	(1.28)	(1.09)	(1.00)	(0.92)
pH	X	6.89	7.60	7.26	7.24	7.26	7.23
	(SE)	(0.07)	(0.09)	(0.07)	(0.17)	(0.13)	(0.14)
Redox	X	63.96	37.50	106.54	-5.08	8.79	92.67
mV	(SE)	(35.41)	(46.56)	(34.90)	(40.86)	(36.74)	(42.24)
H_2S	X	1.55	2.57	1.71	2.40	3.15	0.82
mM	(SE)	(0.24)	(0.65)	(0.48)	(0.50)	(0.43)	(0.18)
NO_2^-	X	0.40	0.32	0.32	0.41	0.35	0.37
µM	(SE)	(0.01)	(0.05)	(0.04)	(0.02)	(0.05)	(0.02)
NO_3^-	X	0.01	0.53	0.24	0.26	0.43	0.03
µM	(SE)	(0.01)	(0.17)	(0.14)	(0.13)	(0.16)	(0.03)
NH_4^+	X	90.05	114.21	105.38	97.49	187.57	7.83
µM	(SE)	(44.90)	(37.68)	(46.54)	(35.61)	(42.65)	(1.41)
PO_4^{-3}	X	32.65	44.45	37.32	39.35	34.60	42.32
µM	(SE)	(7.72)	(8.20)	(8.90)	(7.13)	(5.15)	(10.54)

ANOVA Results[‡]								
	Salinity	pH	Redox	H_2S	NO_2^-	NO_3^-	NH_4^+	PO_4^{-3}
Site	ns	<0.0001	ns	0.02	ns	0.002	ns	ns
Elevation	ns	ns	0.008	ns	0.04	ns	ns	ns
Site x Elevation	0.03	0.0006	0.002	ns	0.02	ns	ns	ns
Habitat	0.0004	ns	0.04	<0.0001	ns	0.01	0.003	ns
Site x Habitat	0.001	ns	0.007	0.003	ns	0.01	ns	0.01
Elevation x Habitat	ns	ns	ns	ns	ns	ns	ns	ns
S x E x H[§]	ns	ns	ns	ns	ns	ns	ns	0.04

[†]Mean at 10 cm from winter 2009 sampling event, standard error in parenthesis (n = 12)
[‡]ANOVA p values from SAS PROC Mixed, ns = not significant ($p > 0.05$)
[§]S x E x H = three-way interaction effect between site, elevation, and habitat

Table A.3. Means and the effects of site, elevation, habitat, and light on dissolved gas and inorganic nutrient fluxes in the salt marsh-mangrove ecotone of Fourchon, Louisiana.

Means[†]

		Site		Elevation		Habitat		Light	
		Bay	Port	High	Low	*Spartina*	*Avicennia*	Dark	Light
N_2-N	X	23.02	46.65	31.79	37.88	40.17	29.50	45.74	23.94
μmol m^{-2} h^{-1}	(SE)	(8.48)	(7.57)	(7.06)	(9.52)	(9.27)	(7.26)	(10.01)	(5.54)
O_2	X	-14.03	-28.00	-23.40	-18.63	-21.24	-20.80	-45.01	2.97
mg m^{-2} h^{-1}	(SE)	(5.14)	(9.01)	(7.73)	(7.32)	(6.89)	(8.16)	(5.16)	(3.28)
NO_2^-	X	-0.07	0.47	0.35	0.04	0.33	0.07	0.46	-0.06
μmol m^{-2} h^{-1}	(SE)	(0.12)	(0.26)	(0.16)	(0.24)	(0.21)	(0.21)	(0.24)	(0.16)
NO_3^-	X	-5.18	-75.64	-43.47	-37.36	-43.83	-37.00	-41.66	-39.16
μmol m^{-2} h^{-1}	(SE)	(0.94)	(6.63)	(9.66)	(7.66)	(9.07)	(8.34)	(9.60)	(7.78)
NH_4^+	X	0.66	31.54	15.80	15.05	19.14	11.37	32.29	-0.03
μmol m^{-2} h^{-1}	(SE)	(7.26)	(10.46)	(10.69)	(8.05)	(11.18)	(6.73)	(9.32)	(8.35)
PO_4^{-3}	X	-0.56	1.43	1.08	-0.34	0.51	0.31	0.83	0.02
μmol m^{-2} h^{-1}	(SE)	(0.24)	(0.53)	(0.54)	(0.21)	(0.52)	(0.34)	(0.44)	(0.45)

ANOVA Results[‡]

	N_2-N	O_2	NO_2^-	NO_3^-	NH_4^+	PO_4^{-3}
Site	0.04	0.02	ns	<0.0001	0.02	0.007
Elevation	ns	ns	ns	ns	ns	0.04
Site x Elevation	ns	ns	ns	ns	ns	0.01
Habitat	ns	ns	ns	ns	ns	ns
Site x Habitat	ns	ns	ns	ns	ns	ns
Elevation x Habitat	ns	ns	ns	ns	ns	ns
Site x Elevation x Habitat	ns	ns	ns	ns	ns	ns
Light	0.05	ns	ns	ns	0.02	0.02
Site x Light	ns	ns	ns	ns	ns	ns
Elevation x Light	ns	ns	ns	ns	ns	ns
Site x Elevation x Light	ns	ns	ns	ns	ns	ns
Habitat x Light	ns	ns	ns	ns	ns	ns
Site x Habitat x Light	ns	ns	ns	ns	ns	ns
Elevation x Habitat x Light	ns	ns	ns	ns	ns	0.01
Site x Elevation x Habitat x Light	0.04	ns	ns	ns	ns	ns

[†]Means from winter 2009 sampling event, standard error in parenthesis (n = 24)
[‡]ANOVA p values from SAS PROC Mixed, ns = not significant ($p > 0.05$)

APPENDIX B
SUPPLEMENTAL DATA FROM THE CONCEPTUAL MODEL OF BIOGEOCHEMICAL CYCLING DURING DELTA DEVELOPMENT IN THE ANTHROPOCENE

Table B.1. Means and the effects of site, season, and depth on soil microbial biomass and chlorophyll *a* in the Wax Lake delta, Louisiana.

			Means[†]						ANOVA Results[‡]	
			Winter			Spring				
	Depth (cm)		*Young*	*Intermediate*	*Older*	*Young*	*Intermediate*	*Older*		
Microbial	0-1	X	1.10	12.02	20.91	0.76	5.66	12.81	Site	<0.0001
Biomass		(SE)	(0.33)	(1.71)	(2.41)	(0.11)	(1.10)	(1.42)	Season	<0.0001
g C g^{-1} soil	1-2	X	0.94	8.36	15.19	0.93	4.66	11.14	Site x Season	0.007
		(SE)	(0.09)	(0.34)	(0.15)	(0.15)	(1.31)	(0.48)	Depth	<0.0001
	2-4	X	1.10	6.92	11.60	0.93	4.88	7.57	Site x Depth	0.0004
		(SE)	(0.06)	(0.82)	(0.25)	(0.22)	(1.43)	(1.11)	Season x Depth	0.04
									Site x Season x Depth	ns
Chlorophyll *a*	0-1	X	2520.6	34180.0	5496.5	406.3	1647.6	3568.5	Site	0.002
µg cm^{-3}		(SE)	(1507.9)	(9607.6)	(913.6)	(105.9)	(156.5)	(1687.8)	Season	0.003
	1-2	X	641.3	2964.9	2272.5	535.4	1691.1	2411.2	Site x Season	0.004
		(SE)	(307.0)	(500.3)	(693.3)	(317.8)	(198.1)	(91.9)	Depth	<0.0001
	2-4	X	308.5	1174.9	1092.7	113.2	659.3	719.0	Site x Depth	0.0002
		(SE)	(16.3)	(12.3)	(290.0)	(45.2)	(21.0)	(163.0)	Season x Depth	0.0001
									Site x Season x Depth	0.0001

[†]Mean from top 4 cm, standard error in parenthesis ($n = 3$)

[‡]ANOVA p values from SAS PROC Mixed, ns = not significant ($p > 0.05$)

Table B.2. Means and ANOVA results from season analysis exploring the effects of site and season on dissolved gas and inorganic nutrient fluxes in the Wax Lake delta, Louisiana.

Means[†]		Summer/Fall			Winter			Spring		
		Young	*Intermediate*	*Older*	*Young*	*Intermediate*	*Older*	*Young*	*Intermediate*	*Older*
N_2-N	X	158.64[‡abc]	190.90[abc]	161.64[abc]	-24.96[d]	104.53[bcd]	203.52[abc]	52.69[cd]	324.93[a]	298.05[ab]
µmol m^{-2} h^{-1}	(SE)	(22.03)	(30.06)	(14.98)	(45.48)	(35.02)	(30.00)	(32.19)	(31.70)	(33.43)
O_2	X	-60.60[bc]	-94.98[d]	-73.84[cd]	-5.95[a]	-13.54[a]	-34.18[ab]	-44.15[b]	-157.04[e]	-171.60[e]
mg m^{-2} h^{-1}	(SE)	(9.33)	(14.35)	(4.48)	(8.70)	(15.79)	(4.82)	(2.79)	(8.88)	(15.21)
NO_2^-	X	-5.14[c]	25.25[a]	8.68[abc]	5.27[bc]	4.41[bc]	8.06[abc]	-3.71[c]	20.41[ab]	27.47[a]
µmol m^{-2} h^{-1}	(SE)	(1.64)	(6.80)	(0.90)	(0.48)	(1.67)	(2.01)	(1.18)	(4.61)	(16.79)
NO_3^-	X	-153.85[ab]	-477.39[bc]	-478.06[bc]	131.37[a]	-139.27[ab]	-62.52[a]	-61.00[a]	-760.65[c]	-872.45[c]
µmol m^{-2} h^{-1}	(SE)	(97.75)	(162.32)	(41.32)	(61.69)	(65.91)	(89.23)	(44.70)	(38.22)	(72.83)
NH_4^+	X	NA[§]	NA	NA	-12.62[a]	24.58[a]	23.84[a]	0.00[a]	-30.82[a]	-95.47[a]
µmol m^{-2} h^{-1}	(SE)				(11.13)	(50.69)	(21.18)	(0.00)	(30.82)	(81.74)
PO_4^{-3}	X	-2.91[ab]	5.40[a]	-24.18[b]	3.50[ab]	-4.09[ab]	3.36[ab]	-10.33[ab]	9.95[a]	-7.83[ab]
µmol m^{-2} h^{-1}	(SE)	(2.91)	(11.16)	(9.20)	(5.84)	(1.83)	(4.95)	(4.57)	(6.83)	(7.83)

[†]Mean presented with standard error in parenthesis (n = 6)
[‡]Superscript letters represent significant ($p < 0.05$) two-way interactions between site and season using Tukey's HSD test
[§]NA = no sample available

Table B.3. Means and ANOVA results from low nitrate analysis exploring the effects of season, nitrate concentration, and light on dissolved gas and inorganic nutrient fluxes in the Wax Lake delta, Louisiana.

Means[†]		Winter				Spring			
		Ambient		Low		Ambient		Low	
		Dark	*Light*	*Dark*	*Light*	*Dark*	*Light*	*Dark*	*Light*
N_2-N	X	151.16[‡a]	37.57[a]	110.00[a]	73.86[a]	207.09[a]	225.15[a]	-116.47[a]	-32.46[a]
µmol m^{-2} h^{-1}	(SE)	(32.69)	(45.40)	(31.67)	(25.99)	(49.76)	(58.68)	(43.26)	(31.55)
O_2	X	-38.21[b]	2.43[a]	-39.74[b]	-24.29[b]	-118.17[d]	-118.52[d]	-123.78[d]	-100.86[c]
mg m^{-2} h^{-1}	(SE)	(3.89)	(7.89)	(4.59)	(4.32)	(22.43)	(23.37)	(25.07)	(18.76)
NO_2^-	X	7.36[b]	3.93[b]	-0.38[b]	0.31[b]	6.03[b]	20.23[a]	0.95[b]	0.60[b]
µmol m^{-2} h^{-1}	(SE)	(1.07)	(1.05)	(1.39)	(0.97)	(2.86)	(10.04)	(2.33)	(0.60)
NO_3^-	X	-155.25[c]	118.06[a]	-43.68[b]	-24.25[b]	-529.41[d]	-523.06[d]	-36.56[b]	-37.68[b]
µmol m^{-2} h^{-1}	(SE)	(50.11)	(48.30)	(9.35)	(6.68)	(157.90)	(125.27)	(12.13)	(11.09)
NH_4^+	X	60.50[a]	-39.61[bc]	42.87[ab]	70.02[a]	-65.46[c]	-5.39[abc]	-39.47[bc]	19.98[ab]
µmol m^{-2} h^{-1}	(SE)	(24.25)	(18.07)	(23.45)	(14.65)	(45.25)	(5.39)	(18.47)	(24.66)
PO_4^{-3}	X	10.01[ab]	-8.17[c]	-0.87[abc]	-0.26[abc]	-2.59[bc]	-1.61[abc]	10.86[a]	4.65[abc]
µmol m^{-2} h^{-1}	(SE)	(2.68)	(1.08)	(0.54)	(0.26)	(8.58)	(0.85)	(9.00)	(4.65)

[†]Mean presented with standard error in parenthesis (n = 9)

[‡]Superscript letters represent significant ($p < 0.05$) three-way interactions among season, nitrate concentration, and light using Tukey's HSD test

VITA

Kelly Marie Henry was born in Norfolk, Virginia, and raised in Richmond, Rhode Island. She attended Chariho High School and graduated in May 1999. She enrolled at Franklin Pierce College (FPC) in the fall of 1999 to pursue studies in environmental science and chemistry. During her undergraduate program, Ms. Henry was a member of the varsity field hockey team. She received Verizon Academic All-America, Verizon District I Academic All-America, and National Field Hockey Coaches Association Division II All-Academic accolades as well as the Richard Burns Female Scholar-Athlete of the Year award. While attending FPC, she participated in a Sea Education Association Semester program that took her through the Caribbean Sea aboard the schooner *Westward* giving her a taste of oceanographic research. In May 2003, she graduated *summa cum laude* as the class valedictorian. After graduation, she worked as a deckhand and education coordinator aboard the sloop *Providence* in Narragansett Bay, Rhode Island, a kayak guide in the San Juan Islands, Washington, and a tour guide in a butterfly garden located in Monte Verde, Costa Rica. In the fall of 2004, Ms. Henry returned to Rhode Island and began her Master of Science program at the University of Rhode Island's (URI) Graduate School of Oceanography. Under the guidance of Dr. Scott Nixon she explored environmental influences on the growth pattern and growth rate of the hard clam, *Mercenaria mercenaria*, in Narragansett Bay, Rhode Island. While at URI, she was awarded a Nature Conservancy Global Marine Initiative Student Research Award, the Rhode Island Surfrider Foundation – Robert Lloyd Scholarship, and the Henry S. Farmer Award in Biological Oceanography. After completing her Master of Science Degree in the summer of 2007, Ms. Henry began her doctoral studies at Louisiana State University. She was awarded a Louisiana Board of Regents Fellowship to pursue a doctoral degree in the Department of Oceanography

and Coastal Sciences. Under the supervision of Dr. Robert Twilley, she determined patterns of nutrient biogeochemistry associated with wetland soil development in the Mississippi River delta, Louisiana. In the summer of 2009, she received a National Oceanic and Atmospheric Administration – Northern Gulf Institute Minority Summer Internship. She will be receiving her Doctor of Philosophy degree from the Department of Oceanography and Coastal Sciences with an unofficial minor in applied statistics on August 3, 2012, with a 4.0 GPA.