FATE OF MISSISSIPPI RIVER DIVERTED NITRATE ON VEGETATED AND NON-VEGETATED COASTAL MARSHES OF BRETON SOUND ESTUARY

A Thesis

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ABSTRACT

The Caernarvon Diversion meters Mississippi River water into coastal marshes of Breton Sound. Elevated levels of nitrogen in river water have sparked concerns that nutrient loading may affect marsh resilience and belowground biomass, as evidence from several marsh fertilization studies. These concerns resulted from observation that fresh and brackish Breton Sound marshes suffered extensive damage from Hurricane Katrina. The goal of this study is to determine the fate of nitrate (the dominant inorganic nitrogen form in the Mississippi River) in Breton Sound Estuary marshes. We hypothesized that most nitrate would be removed by denitrification and that nitrate loading would not affect belowground biomass. To test this hypothesis, a mass balance study was conducted using ^{15}N-labeled nitrate. Twelve plant-sediment cores were collected from a brackish marsh located proximal to Delacroix, Louisiana. Six cores received dionized water (control), while another six (treatment) received 2 mg L^{-1} of ^{15}N-labeled potassium nitrate twice a week for three months. A set of three control and treatment cores were destructively sampled after three months and analyzed for ^{15}N in the above and below ground biomass, as well as the soil. The remaining three treatment cores received 20 mg L^{-1} of ^{15}N-labeled potassium nitrate twice a week for one month, and a similar mass balance was determined to distinguish N removal, including denitrification, surface algae and microbial uptake and incorporation into aboveground and belowground biomass. Twelve hrs after the addition of 2 mg N L^{-1} water for each flooding event, nitrate levels were below detection (0.014 mg NO_3^- L^{-1}. In comparison, after 24 hrs, 20 N mg L^{-1} water column nitrate levels were approximately zero. The ^{15}N analyses determined 68, 65, and 74% of added labeled nitrate as unaccounted for, which represents gaseous losses. The remaining ^{15}N was incorporated in plant and soil compartments. Labeled N data from the 2 mg N L^{-1} treatment and 20 mg N L^{-1} treatment suggests denitrification as the major removal pathway for nitrate in Caernarvon

Diversion. Comparison of nitrate loss in bayou sediment and marsh soil suggests nitrate removal would be enhanced if diverted Mississippi River water flooded the marshes.

CHAPTER 1:

REVIEW OF LITERATURE

1.1 COASTAL WETLANDS OF LOUISIANA

Coastal Louisiana is experiencing wetland loss at rates as high as 100 $km^2\ yr^{-1}$ (Gagliano, 1981; Penland, 1990) with total land losses approximately 4900 km^2 since 1900 (Day et al., 2007). Coastal wetlands in Louisiana make up 40 % of the total number of wetlands in the United States (Boesch et al., 1994). This loss constitutes 80 % of coastal wetlands in the United States (Boesch et al., 1994; Penland, 1990). Wetland loss in Louisiana is the result of a combination of natural and anthropogenic factors. Wetland loss factors include lack of sediment and freshwater sources from levees along the Mississippi River (DeLaune et al., 2005b), salt water intrusion, subsidence, eustatic sea level rise, dredged canals for the oil industry (Turner, 1997; Turner, 2009) and catastrophic events (i.e. hurricanes) (Farris, 2007).

Coastal wetlands are important for ecological and economic reasons. Wetlands provide prime habitat for coastal fish and migratory bird populations (Boesch et al., 1994). Ecosystem values include flood protection, storm surge abatement, aquifer recharge, and improved water quality (Boyer, 1997; Mitsch and Gosselink, 2007). Also, wetlands offer recreational and economic benefits for human population located near coastal wetlands (Boyer, 1997).

Non-point sources of nutrients in the Mississippi River, particularly nitrate resulting from agricultural runoff of fertilizers, are reported to be the cause of the seasonal hypoxic zone off the coast of Louisiana. Since the 1960's, nitrate concentrations in the Mississippi River have been increasing with the rise of nitrogen fertilization application to agricultural fields in the Mississippi River basin during the same time period (Rabalais et al., 2002; Rabalais et al., 1996). The steady use of nitrogen fertilizers has resulted in a 300 % increase in nitrogen in the Mississippi River (Rabalais, 2002; Rabalais et al., 1996). High concentrations of nitrate cause a zone of low oxygen that adversely affects aquatic life in the northern part of the Gulf of Mexico. The seasonal hypoxic zone in the Gulf of Mexico near the Louisiana coast is present during the

late spring and early summer when spring flooding of the Mississippi River occurs. The hypoxic zone was relatively constant in size from 1985 to 1992. However, after the 1993 flood, the hypoxic zone increased from 10,000 km^2 to 17,000 km^2 (Battaglin et al., 2001). Since 1993, the hypoxic zone has increased to 22,000 km^2 (Liu et al., 2010).

The Caernarvon and Davis Pond Diversions off the lower Mississippi River are restoration projects designed for salinity control in the receiving estuary. An added benefit of diversions, are the ability of wetlands to remove nutrients by discharging river flow through coastal wetlands. This would decrease high nutrient concentrations reaching the Gulf of Mexico by moving some Mississippi River flow through coastal wetlands. The nutrient load can then be removed by coastal wetlands before discharged water reaches the Gulf of Mexico. The nitrate load, in particular, can be removed by burial, assimilation into plant or algal biomass, returned to the atmosphere by denitrification, or reduced to ammonia (Reddy and DeLaune, 2008).

There are a number of sources of nutrients in the Mississippi River. Nitrogen derives from a combination of natural and anthropogenic sources. Natural sources of nitrogen include mineralization of soil nitrogen, fixation of atmospheric nitrogen by legumes, and atmospheric deposition of nitrogen (Battaglin et al., 2001). Nitrogen fertilizer from agricultural runoff, animal wastes, and discharge of municipal and industrial waste are the main anthropogenic sources of nitrogen in the Mississippi River (Battaglin et al., 2001). Industrial fixation of N_2 by the Haber-Bosch process for fertilizer production has more than doubled the amount of bioavailable nitrogen entering ecosystems (Boesch, 2002; Reddy and DeLaune, 2008). The sources and type of nitrogen that are entering the Mississippi River are dependent on land use in each sub basin in the Mississippi River Basin, as well as physical and chemical processes that control nutrient cycling in these systems (Battaglin et al., 2001).

The Mississippi Delta is made up of several overlapping delta lobes formed over time by changes in river direction. Annual spring flooding of the delta have brought large amounts of freshwater, nutrients, and sediments into the surrounding wetlands. Freshwater inputs help decrease the effect of salt water intrusion as many plants and wildlife in wetlands have limited salt tolerance. Mississippi River water historically delivered nutrients that spurred plant growth and biomass accumulation in the wetlands. With each spring flood, the Mississippi River replenished nutrients and increased elevation by sediment accumulation. Mineral sediments found in the Mississippi River are important for plant growth and maintaining adequate elevation in low lying wetland areas (DeLaune et al., 2005a).

1.1.1 Coastal Restoration

The Mississippi Delta Plain is made up of 6 different aged delta lobes, the first that began forming 6,000 to 7,000 years ago when the rise in sea level rise began to slow (Figure 1.1). Each delta lobe was formed in successive progradating stages, lasting approximately 1,000 to 1,500 years. A new delta lobe was formed when the course of the Mississippi River switched, finding the shortest route to the Gulf of Mexico (Blum and Roberts, 2009). New active deltas began land building as deposition of Mississippi sediment occured. Sediments from the river were either deposited at the mouth of the river forming the delta or deposited in wetlands when overbank flooding occurred. Older abandoned delta lobes began a transgressive, or erosional stage, and were susceptible to loss of elevation by compaction and dewatering of fine grain sediments. Sediment supply was maintained in transgressive lobes each time the Mississippi River over flowed it’s banks bringing in new sources of sediment (Blum and Roberts, 2009). Sediments from overbank flooding maintained marsh elevation needed to keep pace with local eustatic and

sea level rise. Only the Plaquemines-Balize and Atchafalaya-Wax Lake deltas are currently experiencing land building.

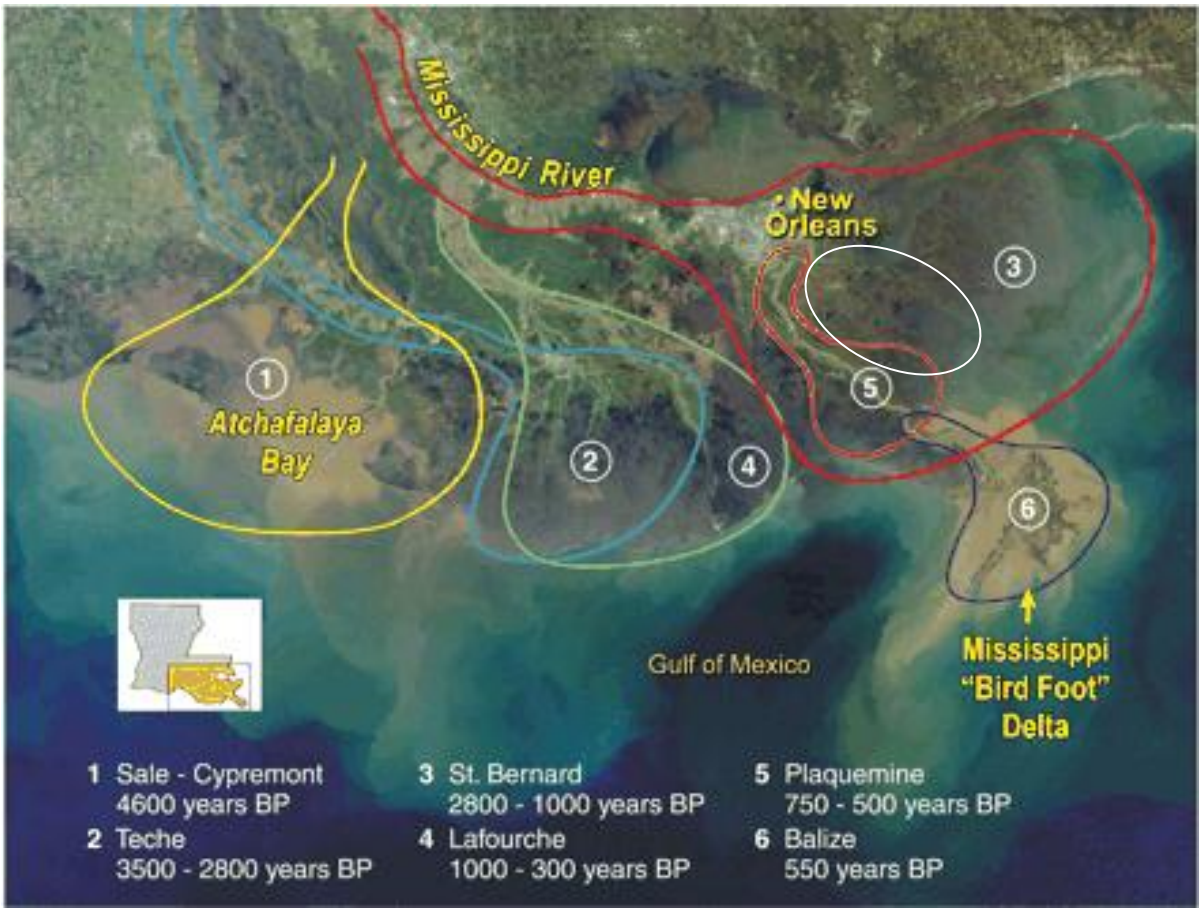

Figure 1.1 Historic map of Mississippi River delta complexes (Day et al., 2007). The white circle indicates the location of Breton Sound Estuary.

Sediment inputs from Mississippi River overbank flooding are especially important to the health and maintenance of Louisiana's wetlands. Sediment inputs from the Mississippi River add minerals to the soil, which supply micronutrients for plants and increase elevation directly (Day et al., 2007; Lane et al., 2003). Plant growth is also important in maintaining marsh elevation. Plant growth increases the organic content of the soil while sediments from the Mississippi River increase the mineral content of the soil. Plant growth also reduces water velocities allowing

mineral matter deposition to occur (Reddy and DeLaune, 2008). Loss of riverine sediment inputs from overbank flooding when the Mississippi River levees where built has had huge implications on wetland elevation change in the Breton Sound estuary.

Wetland loss in Louisiana is directly or indirectly a result of the decoupling of the Mississippi River from the surrounding wetlands by the building of levees. Levees prevent over bank flooding of the Mississippi River and therefore the addition of mineral sediment to the surrounding wetlands. Mineral sediment from the Mississippi River directly increase vertical elevation and indirectly by additions of nutrients that stimulate plant production and increase organic content that increases elevation (Day et al., 2007). Wetland loss from subsidence and dewatering naturally occurs; however, lack of new sediment from spring flooding of the Mississippi River no longer counteracts elevation loss by subsidence (Baumann and Turner, 1990). Subsidence increases flooding and erosion rates by increased wave action that consequently result in land loss and change in dominate vegetation from increasing salt water intrusion (Boesch et al., 1994).

Marsh accretion is the result of a combination of mineral and organic matter accumulation (DeLaune et al., 2003). Soil mineral matter was received yearly when overbank flooding of the Mississippi River occurred. Inputs of soil allowed wetlands to maintain vertical elevation with respect to local and eustatic sea level rise. Restoration projects such as diversion projects aim to couple river sediment and wetlands together again. However, the Mississippi River has seen a decrease in sediment load by 50% on account of dam construction in the upper drainage basin. The resulting decrease in sediment load in the Mississippi River is not enough sediment to counteract subsidence, local and eustatic sea level rise, and build new land, even if overbank flooding of the river was permitted (Blum and Roberts, 2009). Finally, major

diversions off the Mississippi River, such as the Caernarvon Diversion, were not designed to introduce sediments into wetlands since they redirect surface water, but rather to decrease salinity with the introduction of surface water column.

Freshwater supplied by yearly flooding buffered wetlands against salt water intrusion in the Mississippi River Delta. As levees around the Mississippi River prevented spring flooding, salinity increased in the Breton Sound. A study by Merino et.al. (2010) found that increasing salinity in coastal wetlands decreased plant productivity, specifically in *Spartina patens*. However, the introduction of freshwater as a restoration tool to decrease plant stress by decreasing salinity can potentially lead to problems associated with nutrients and contaminants in the diverted freshwater. Separating the effects of decreasing salinity levels and introduction of high nutrient concentrations as well as water level stress then becomes an issue. A study using *Spartina patens* found that even with an introduction of nutrients, high salinity resulted in a decrease in plant biomass (Merino et al., 2010). Results from a study by DeLaune et al. (2005a) also support lower salinities stimulate *Spartina patens* growth regardless of nutrient concentrations. These studies suggest that the effects of salinity are much greater than the effects of high nutrient additions in limiting the growth of *Spartina patens*.

Lack of freshwater from flooding of the Mississippi River has increased salt water intrusion causing indirect loss of wetlands (Baumann and Turner, 1990). Other wetland loss is from the dredging of canals through wetlands, oil withdraw, and reduction of sediment supply in the Mississippi River (Day et al., 2007) and land reclamation projects (Craig et al., 1979). Anthropogenic impacts from canal widening, oil extraction, and spoil bank creation also increased subsidence rates, further increased salt water intrusion (Baumann and Turner, 1990), and caused changes in hydrological regimes (Boesch et al., 1994).

Wetlands in the Breton Sound Estuary can no longer maintain adequate elevation with respect to local sea level rise, eustatic sea level rise, and compaction of sediments (Lane et al., 2003). In an effort to restore the Breton Sound Estuary, the Caernarvon Freshwater Diversion was proposed to link the Mississippi River and the Breton Sound Estuary back together by to mimicking yearly spring flooding events. This project was designed to increase freshwater inputs to the upper estuary in order decrease salt water intrusion to enhance oyster bed production (2003). The design of this project limits sediment input only to the upper estuary by using only the surface water of the Mississippi River (Reddy and DeLaune, 2008).

1.2 Freshwater Diversion Projects

The Mississippi River delta complex consists of several basins. These basins are the Pontchartrain, Breton Sound, Terrebonne, and Teche/Vermilion, which lie between major deltas. The Breton Sound Estuary is located on the eastern side of the Mississippi River bird's foot delta. Breton Sound Estuary functions as a hydrologic component of the delta allowing tidal exchange and freshwater drainage (Boesch et al., 1994). The Breton Sound Estuary is the location of one of several freshwater diversions off the lower Mississippi River.

There are several diversions off the lower Mississippi River south of New Orleans, LA. The Caernarvon Freshwater Diversion is one of the largest diversions and is located south of New Orleans on the east bank of the Mississippi River near mile marker 81.5. The maximum discharge rate of the Caernarvon Diversion is 226 m^3s^{-1} (8000 ft^3s^{-1}) and had been in operation since 1991. This diversion meters Mississippi River water into the Breton Sound Estuary, which contains 1,100 km^2 of fresh, brackish, and salt marshes. The Breton Sound Estuary is unique in geometry, with the Mississippi River levee located to the west, Bayou La Loutre natural levees to the north, and Mississippi River Gulf Outlet spoil banks to the east (Lane et al., 1999).

The Caernarvon diversion was designed to reduce salt water intrusion in the Breton Sound Estuary by mimicking yearly overbank flooding of the Mississippi River. Yearly flooding brought high nutrients and freshwater into the Breton Sound wetlands (2003). The Caernarvon Diversion was also designed to enhance vegetation growth, reduce marsh loss, and improve productivity of recreational and commercial fisheries (2003). The main commercial fishery is the oyster. The Breton Sound Estuary suffered 100 km^3 loss of wetlands after Hurricanes Katrina and Rita hit the Louisiana coast in 2005 (Day et al., 2007).

The construction of the Caernarvon Diversion began in 1988 and was completed in 1991. Diverted Mississippi River water began flowing in August 1991 from the Caernarvon Diversion. There were three years of preconstruction monitoring and four years of post construction monitoring. A forty six year long monitoring phase in the Breton Sound Estuary is also in place to evaluate the effectiveness of the Caernarvon Diversion (2003).

Loss of historic fresh and brackish marshes in the Breton Sound Estuary is the result of salt water intrusion resulting from levees along the Mississippi River preventing overbank flooding of freshwater. Levee construction on the lower Mississippi River prevented overbank flooding that otherwise would have increased marsh elevation by sedimentation, added nutrients needed for plant growth, and increased freshwater preventing marsh intrusion by salt water (Yu et al., 2006). The main goal of the Caernarvon Diversion is to return the Breton Sound estuary to the historic salinity regime in the estuary. Wetland habitat in the Breton Sound estuary consisted of only brackish and saline wetlands, with no freshwater wetlands, before discharge from the Caernarvon Diversion began. Low habitat diversity also leads to low wildlife diversity in the estuary. Since the introduction of freshwater into the Breton Sound Estuary, salinities are less than 5 ppt in the upper estuary, freshwater and intermediate marshes have been established and

diversity of wildlife increased (2003). In the upper basin, *Schoenoplectus americanus* (Chairmaker's bulrush) and *Spartina patens* (saltmeadow cordgrass) are dominant (Piazza and La Peyre, 2007). Dominant species in the brackish and salt marshes are *Spartina patens* (saltmeadow cordgrass) and *Spartina alterniflora* (smooth cordgrass) (Visser et al., 1998).

1.3 THE NITROGEN CYCLE

Nitrogen is an important nutrient in biologically active processes and is often the limiting nutrient in coastal system for primary production (Figure 1.2). The nitrogen cycle is energetically important in biological processes because of its five naturally occurring oxidation states, ranging from +5 to -3 (Reddy and DeLaune, 2008). N_2 gas completes several conversions from inorganic to organic and back to inorganic nitrogen with the release of N_2 gas into the atmosphere. Nitrogen fixation occurs only in few bacterial species where elemental nitrogen (N_2) is converted to ammonium (NH_4^+) (Reddy and DeLaune, 2008). This conversion makes N_2 gas available for use by assimilation into plant biomass. Mineralization is the breakdown of organic nitrogen to inorganic nitrogen by the conversion of organic nitrogen to NH_4^+. Nitrification is the microbial mediated conversion of NH_4^+ to nitrate (NO_3^-), with an intermediate conversion to nitrite (NO_2^-), to break down organic matter. Denitrification is the conversion of NO_3^- to NO_2^- to N_2O and finally to N_2 gas, where it is released back to the atmosphere.

Nitrogen is an essential nutrient in plant growth and is generally the limiting nutrient in a system. Since nitrogen is usually limiting, nitrogen also limits productivity. Plants and algae utilize either ammonium or nitrate as the source of nitrogen for growth. Ammonium is assimilated directly, while nitrate is reduced to ammonium in plant cells before use. Nitrate is reduced to ammonia via the nitrate reductase enzyme. Ammonium is then incorporated into plant and algae as amino acids and proteins (Reddy and DeLaune, 2008).

Nitrogen cycling in wetlands includes inputs, storage, and outputs. Inputs of nitrogen are from both natural and anthropogenic sources. Natural inputs include biologically fixed nitrogen, precipitation, and particulate matter, which are ubiquitous in the environment (Antweiler et al., 1995; Reddy and DeLaune, 2008). Natural sources of nitrogen are usually low because the environment is at equilibrium between production and use of nitrogen (Antweiler et al., 1995).

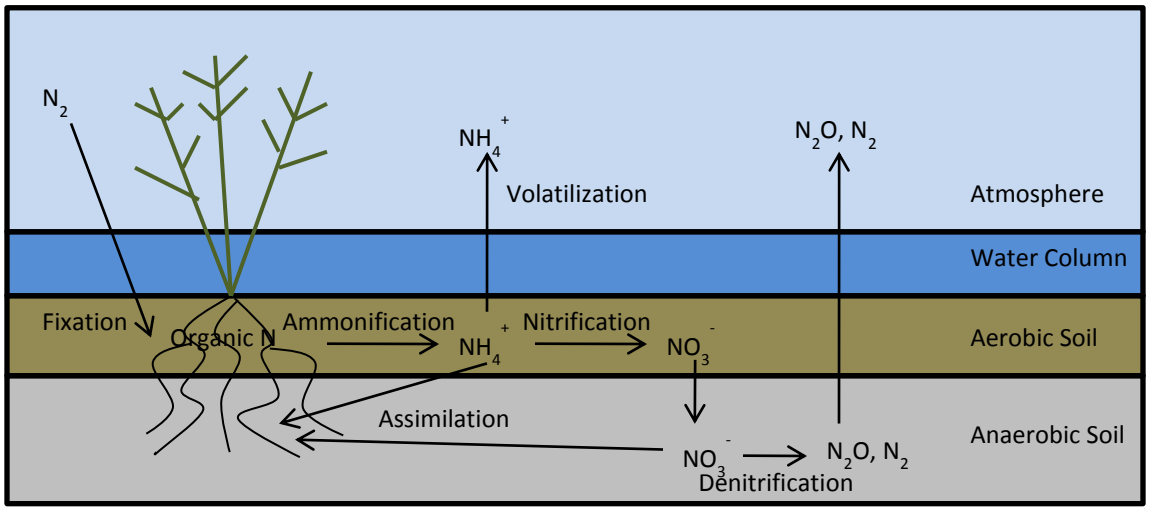

Figure 1.2 Major pathways in the nitrogen cycle for N transformation and the location of redox conditions where each transformation occurs.

Anthropogenic nitrogen inputs are point and non point sources to wetlands. Point sources of nitrogen are waste water discharge and industrial wastes (Antweiler et al., 1995). Non point sources of nitrogen are from runoff from urban areas (Reddy and DeLaune, 2008), agricultural fields (Antweiler et al., 1995; Reddy and DeLaune, 2008), and animal wastes (Antweiler et al., 1995). Storage in wetlands is a combination of plant and microbial biomass, soil organic nitrogen, pore water nitrogen, and exchangeable nitrogen. Outputs are the loss of nitrogen from storage in the wetland and are lost from the system from outflow of water, loss of dissolved gasses, and loss of plant biomass (Reddy and DeLaune, 2008).

1.3.1 Dinitrogen (N_2)

Dinitrogen is the most common form of nitrogen, making up 78% of the atmosphere. Dinitrogen has a very stable triple bond that is energetically expensive to break, such that few organisms are able to use dinitrogen as the source of nitrogen needed in biological processes. Nitrogen fixation is the process of converting dinitrogen to ammonia that is readily used in biological processes. The Haber-Bosch industrial process has increased nitrogen fixation and its product, ammonia, for use in agricultural fields. The Haber-Bosch process has increased the bioavailable nitrogen pool by 50%, changing the global nitrogen balance. The process of denitrification returns other inorganic forms of nitrogen back to dinitrogen (Reddy and DeLaune, 2008).

1.3.2 Ammonia/ Ammonium (NH_3/NH_4^+)

Sources of ammonia are two-fold. First, mineralization of organic matter is the process of converting bound nitrogen to ammonium. Second, nitrogen fixation is the process of converting dinitrogen gas to ammonium. Nitrogen fixation only occurs through specific microbes that possess the nitrogonase enzyme (Mitsch and Gosselink, 2007). Ammonium is an important inorganic nitrogen form used in biological processes. Under acidic conditions, ammonium is present and under basic conditions, ammonia is present. Since the Haber-Bosch process began, ammonia fertilizers are commonly applied to agricultural fields. Excess ammonia is oxidized to nitrate in the process of nitrification. Ammonium is used as a nutrient in plant, microbes, and algal growth (Reddy and DeLaune, 2008).

1.3.3 Nitrate (NO_3^-)

The main source of nitrate in wetland soils is the oxidation of ammonia to nitrate by nitrification. Other sources of nitrate are from the direct use of nitrate fertilizers rather than ammonia fertilizers, non point source discharge from land, and point source discharge from

urban areas (Reddy and DeLaune, 2008). Nitrate is used in several processes in wetlands. One process is the use of nitrate as a bioavailable nutrient by assimilation into plant and microbial biomass by assimilatory nitrate reduction. Assimilatory nitrate reduction requires aerobic conditions, where aerobes reduce nitrate to ammonia during cell synthesis (Reddy and Delaune, 2008). Nitrate can also be used as an alternate electron acceptor for the oxidation of organic matter under anaerobic conditions. This is the catabolic process of denitrification. Another process of catabolic nitrate reduction is dissimilatory nitrate reduction to ammonia (DNRA). DNRA is mediated by obligate anaerobes that use nitrate as an alternate electron acceptor in cellular respiration (Reddy and DeLaune, 2008).

Nitrate is the predominate form of nitrogen present in the Mississippi River. Mississippi River water diverted into the Breton Sound estuary is high in nitrate concentration. Excess ammonia from agricultural fields within the drainage basin is oxidized to nitrate under aerobic conditions in the Mississippi River before being diverted into the Breton Sound Estuary. As water from the diversion flows through the estuary, nitrate concentration is decreased. Day et. al. (2003) found that nitrate concentrations ranging from 2.5-17.7 mg L^{-1} were reduced to 0.062-4.6 mg L^{-1} by mid-estuary. Possible reductions in nitrate are from denitrification, dilution by exchange with the Gulf of Mexico, assimilation by plants, algae, or bacteria, and burial (Day et al., 2003). This suggests that excess nitrate received in the Breton Sound Estuary from the Caernarvon Diversion does not reach the mouth of the estuary and is not released to the Gulf of Mexico.

Nitrate removal depends on several factors. Residence time (Reddy and DeLaune, 2008) and loading rate (Lane et al., 2003) are the main factors in the effectiveness of nitrate removal in wetlands. Residence time is related to discharge rate, where maximum removal rate occur at low

flow from the Caernarvon Diversion that results in long residence times. A study on residence time in the Davis Pond Diversion, found high discharge rates into Barataria Bay marshes resulted in nitrate reaching the Gulf of Mexico (DeLaune et al., 2005b). Also, low nitrate loading results in maximum removal efficiency. The soil water interface is important for nitrate removal. The more surface area of wetlands diverted water comes into contact with, the greater nitrate removal occurs. Removal of nitrate occurs by assimilation into plant or algal biomass, denitrification, or burial (Lane et al., 2003).

Over the past 80 years, nitrate concentration in the Mississippi River has steadily increased. Nitrate concentrations before 1940 were between 0.2 to 0.4 mg N L^{-1}. Since 1940, the nitrate concentration has increased to between 1.0 and 2 mg L^{-1} (Antweiler et al., 1995; Lane et al., 1999). It is estimated that seventy five percent of the nitrate entering the Gulf of Mexico from the Mississippi River since 1940 is from anthropogenic sources (Antweiler et al., 1995). Nitrate concentrations in the Mississippi River vary seasonally following seasonal rainfall patterns in the drainage basin. Nitrate concentrations are high during the late winter, spring, and early summer, while nitrate concentrations are lower during the mid to late summer, fall, and early winter (Antweiler et al., 1995).

1.3.4 Denitrification

Denitrification is an important component of the nitrogen cycle by removing excess nitrogen from the system and releasing N_2 gas back to the atmosphere. Because nitrogen is lost from the system by denitrification, denitrification is a primary controlling factor in primary productivity by removing excess nitrate that would otherwise be available for assimilation by plant or algal growth. Denitrification is mediated by microbes and occurs only in anaerobic conditions, where nitrate is used as the alternative electron donor in place of oxygen to oxidize

organic matter (Lane et al., 2003; Reddy and DeLaune, 2008). Nitrate is converted in a process of steps from nitrate to elemental nitrogen following this pathway:

$$NO_3^- \longrightarrow NO_2^- \longrightarrow N_2O \longrightarrow N_2 \text{ gas}$$

N_2O is an obligate intermediate in the process of reducing NO_3^- to N_2 gas by facultative microbes (Blackmer and Bremner, 1978). A ratio of N_2O to N_2 gas indicates that about 94% of N_2O is reduced to N_2 during denitrification before being released to the atmosphere (Blackmer and Bremner, 1978).

Denitrification needs a source of electron acceptors, a carbon source, and anaerobic conditions. Under anaerobic conditions, nitrate can be used as an alternate electron acceptor for the oxidation of organic carbon. The rate of denitrification is limited by the nitrate concentration and presence of organic carbon (Dodla et al., 2008). As a result, denitrification may play a role in the removal of nitrate since diverted Mississippi River water entering the Breton Sound estuary contains nitrate (Dodla et al., 2008).

1.4 FERTILIZATION STUDIES

Coastal land loss is wide spread in the Mississippi River deltaic region, occurring at rates as high as 100 km^2 yr^{-1} (Gagliano, 1981) with total land losses approximately 4900 km^2 since 1900 (Day et al., 2007). Coastal land loss in Louisiana is a result of physical, chemical, and biological processes in the Mississippi River delta. Catastrophic events such as hurricanes are regular forces that affect the Mississippi River delta and have been the cause of erosion of wetlands since the formation of the delta 6,000 to 7,000 yrs ago (Day et al., 2007; Walker et al., 1987). In 2005, Hurricanes Katrina made landfall on the Louisiana coast. The storm path of Hurricane Katrina was over the Breton Sound Estuary eroding approximately 100 km^2 of wetlands in the estuary (Day et al., 2007). Wetland loss estimates as high as 527 km^2 in the Breton Sound Estuary have also been reported (Howes et al., 2010).

The Breton Sound Estuary has been receiving Mississippi River water since 1991 from the Caernarvon Diversion as part of a marsh restoration project. The upper Breton Sound Estuary has also been receiving nitrate concentration as high as 2 mg NO_3 -N L^{-1} from the Mississippi River since the 1960's (Lane et al., 1999). Since the operation of the Caernarvon Diversion fresh marshes have increased from 0 to 628 acres and intermediate marshes have increased by 10,582 acres (2003). Increases in fresh and intermediate marshes are closer to historic vegetation types in the Breton Sound since the addition of Mississippi River freshwater by Carnarvon Diversion. Storm surge in the Breton Sound from Hurricane Katrina preferentially eroded the upper fresh and brackish marshes in the upper basin. It has been hypothesized that eutrophication of wetlands wetland receiving nitrate laden Mississippi River water contributed to high wetland loss in the Breton Sound Estuary following Hurricane Katrina (Howes et al., 2010; Turner, 2010; Turner et al., 2009).

There are several hypotheses suggested for why high wetland loss occurred following Hurricane Katrina in the Breton Sound Estuary. One hypothesis is increased nitrate availability from Mississippi River water to Breton Sound wetlands decreased belowground biomass, resulting in shallow rooting as N limitation was eased (Darby and Turner, 2008a; Darby and Turner, 2008c). A second hypothesis suggests a decrease in soil strength results from decreases in soil redox conditions and higher decomposition rates with the addition of Mississippi River water (Swarzenski et al., 2008). Finally, a third hypothesis states that the tight coupling of denitrification and carbon uses carbon reserves as excess nitrate is removed by denitrification, thus decreasing soil strength (Turner, 2010; Turner et al., 2009).

The role of excess nitrogen has been fairly well studied in coastal environments using plot fertilization studies. Fertilization studies up to this point use granular fertilizers. Past studies

were designed to distinguish effects of elevated nutrients on plant aboveground biomass. More recently, interest in the effects of nutrient loading on belowground biomass after wetland loss in Breton Sound following Hurricanes Katrina have increased (Day et al., 2007). Consequently, fertilization studies began including changes in belowground biomass to address hypotheses about wetland loss in Breton Sound.

Several studies addressed the first hypothesis, that nitrate loading resulted in shallow rooting. For example, Darby and Turner (2008a) used granular ammonium sulfate with loading rates of 744, 22, and 60 kg ha^{-1} $month^{-1}$ (Table 1.1). Results show that the addition of nitrogen to these marshes resulted in an increase in aboveground biomass, but no detectable change in belowground biomass. A second nutrient addition field experiment using N, P, and Fe was designed to resolve changes in either below or above ground biomass to various combinations of these three nutrients. These results suggest that P rather than N resulted in a decrease in belowground biomass. Laursen (2004) also found similar results, where the addition of N and P increased decomposition of labile root components. Darby and Turner (2008b) suggest that the addition of P reduced the root standing biomass because of relaxed P competition by soil microbes, not competition for N.

A second study by Darby and Turner (2008c) supports results from Darby and Turner (2008b), where aboveground biomass increased in response to N and P additions. Analysis of belowground biomass found that the addition of N alone did not change total biomass of roots and rhizomes. However, the addition of N + P or P alone resulted in the decrease of belowground biomass. Darby and Turner (2008b) suggest that a decrease in belowground biomass decreases carbon production because of increased soil microbial response to the addition of P. Conclusions

of this study imply that coastal wetland restoration that includes the use of nutrient rich waters may decrease coastal marsh ability to resist erosion.

Darby and Turner (2008b) studied the variations of below and aboveground biomass over one year, sampling once a month in a *Spartina alternifora* salt marsh. Aboveground biomass was lowest in March and increased during the growing season until September. Belowground biomass had large amounts of roots and rhizomes immediately before aboveground spring growth and was then followed by a decrease in belowground biomass as aboveground biomass increased during the growing season. This study found that the seasonal differences in belowground biomass were more distinct than the seasonal changes in the aboveground biomass, suggesting translocation of nutrients from the belowground biomass to the aboveground biomass during spring growth. Also, belowground biomass was concentrated in the 0-10 cm soil section. Darby and Turner (2008c) suggest the shallow rooting depth is the result of nitrogen fixation, surface water nutrient source, or soil oxidation zone.

Changes in redox conditions can affect soil strength, as supported by the second hypothesis. The effect of chronic river influx to a freshwater marsh was evaluated by comparing pore water, soil redox, and soil strength in a marsh receiving Mississippi River water for more than 30 years and a marsh that only receives rain water (Swarzenski et al., 2008). This study found that marshes receiving Mississippi River water were more reducing, had a higher concentration of sulfide, and had higher alkalinity. This resulted in a higher rate of decomposition and loss of soil strength in marsh receiving river water in comparison to the marsh receiving only precipitation. Swarzenski et. al. (2008) suggests that the chronic inflow of nutrient in the Mississippi River has not increased accretion rates in comparison to the marsh that

did not receive Mississippi River water. However, these marshes are not historically precipitation driven systems.

Organic matter accumulation in coastal marshes ranging from 219-301 g C m^{-2} for fresh marshes and 132-334 g C m^{-2} for saline marshes in Louisiana (DeLaune and White, in press). This accumulation of organic matter helps maintain coastal marsh elevation in relation to relative sea level rise, preventing plant stress by flooding. Coastal nutrient enrichment increase aboveground biomass; however, studies reported by Turner et. al. (2009) found that nutrient enrichment (N + P, Urea + P) decreased root and rhizome biomass as well as carbon accumulation, supporting the third hypothesis. They suggest that nutrient additions result in a loss of marsh elevation by the tight coupling of denitrification and carbon reserves. A series of 14 multiyear fertilization field experiments compared soil strength to various combinations of N and P. Turner (2010) found that soil strength decreased 35% at the 60 to 100 cm soil layer with the highest loading rates (N, P, and N + P). This study concluded that nutrient loading into coastal marshes increases organic matter decomposition and at the same time reduces belowground root depth, therefore decreasing soil strength.

Lastly, storm surge and wave action from Hurricane Katrina in 2005 resulted in the loss of 527 km^2 of coastal wetlands in the Breton Sound (Howes et al., 2010). Fresh and brackish marshes were preferentially eroded in the Breton Sound, with little damage to salt marshes in the Breton Sound. Damage in the low salinity marshes occurred at the base of the rooting zone, at 30 cm in depth. Howes et. al . (2010) speculated that low salinity wetlands were preferentially eroded as a result of introduced high nutrient Mississippi River water into the Breton Sound. This conclusion agrees with Swarzenski et. al (2008), Darby and Turner (2008b), and Turner (2010) in that the introduction of Mississippi River water results in more reduced soil conditions

and decreasing belowground root biomass creating less resistant low salinity marshes and more destruction during hurricanes as observed by loss of marshes in the Breton Sound.

Studies presented here suggest eutrophication of marshes receiving excess nutrients including changes in aboveground and belowground biomass and changes in soil properties. However, we feel that there are problems with the use of granular fertilizer to determine the

Table 1.1 Examples of form of N and loading rates of N used in fertilization studies from published literature.

Reference	Form N	Loading Rate N
Darby and Turner (2008a)	Ammonium sulfate	2246 kg ha^{-1} N
	Osmocote slow release fertilizer	174 kg ha^{-1} N
Darby and Turner (2008b)	Ammonium sulfate	744 kg ha^{-1} $month^{-1}$ N
DeLaune et. al (1986)	1 M $(NH_4^+)_2SO_4$ solution	0, 30, 100 kg ha^{-1}
Laursen (2004)	46-0-0 urea, enclosed gelatin capsule	0, 50, 200, 1200 kg ha^{-1} yr^{-1}
Turner et. al. (2009)	Urea 10%	7.56 kg N km^{-2} yr^{-1}
	Milorganite fertilizer 10%	2.46, 7.56, 22.68 kg N km^{-2} yr^{-1}

effects of wetland loss in the Breton Sound. The first problem is the use of granular fertilizers themselves as granular fertilizers create a concentration of high nutrients at the soil surface, where application of fertilizers generally occurs. This could result in the reduction of roots as an artifact of the fertilization process itself, as root biomass will decrease if available N is concentrated at the surface. A second problem is the use of ammonium or urea as the N source. Ammonium and urea are not removed by the same process (denitrification) as nitrate. The difference in removal mechanism will change what effect excess nutrients have in wetlands. This is particularly true if soil metabolism is important in answering hypotheses about soil strength in the Breton Sound. Finally, loading rates of N in several fertilization studies are orders of magnitude higher than marshes receive from diverted Mississippi River water (Table 1.1). Also,

some granular fertilizers used contained SO_4^{2-} and under anaerobic conditions form H_2S which is toxic to plants (Koch et al., 1990).

Finally, Darby and Turner (2008a) and Darby and Turner (2008b) both suggest that belowground biomass decreased only when the addition of P occurred resulting from relaxed competition for P between belowground biomass and microbial communities. Although competition between belowground biomass and microbial communities is known to occur (Sundareshwar et.al. 2003), there is no data presented to suggest changes in root and microbial competition resulted in a reduction in belowground biomass in these marshes. Secondly, Darby and Turner (2008c) did not report nutrient data in their study to support the conclusion that nutrient concentration resulted in lower root biomass over the growing season.

The geometry in the Breton Sound Estuary is particularly important in looking at historical hurricane damages. Howes et. al (2010) did not take into account the geometry of the Breton Sound, which funnels storm surge into the upper basin as a result of levees located on the western, eastern, and northern edges of the Breton Sound Estuary (Wamsley et al., 2010). This funneling results in high energy in the upper basin and heavy destruction of the fresh and brackish marshes that are also located in the upper basin. Similar marsh destruction by hurricanes in upper Breton Sound Estuary observed in aerial photographs previous to the building of the Caernarvon Diversion suggest that damage of the marshes following Hurricane Katrina was not the result of elevated nutrients from diverting part of the Mississippi River into the Breton Sound (Farris, 2007). This is contrary to the study by Turner (2010) where loss of wetlands in the Breton Sound by hurricanes before the operation of the Caernarvon Diversion began was not taken into consideration.

Experiments presented here aim to 1) mimic the nutrient addition of diversions into coastal wetlands and 2) use the same N source (nitrate) as found in the Mississippi River. The delivery mechanism of Mississippi River water into coastal wetlands is flooding over the surface of the wetlands. This creates a nitrate concentration gradient from the water column into wetland soil. Nitrate was used as the source of excess nitrogen because nitrate is the main nitrogen source present in Mississippi River. Nitrate was delivered in solution much like the nitrate received by wetlands when flooded by river water. Elevated water column nitrate concentrations mimic the delivery mechanism of the diversion and type of nitrogen that is delivered via the Caernarvon Diversion. This series of experiments are unlike previous fertilization studies that use granular fertilizers and N sources other than nitrate that may contain sulfate.

1.5 SITE DESCRIPTION

The Caernarvon Diversion is located in Caernarvon, LA south of New Orleans, LA on the east bank of the Mississippi River at mile marker 81.5. Built in 1991, the purpose of the Caernarvon diversion is to reroute Mississippi River water into the Breton Sound Estuary. Freshwater diversions like Caernarvon are built with the intension of decreasing salt water intrusion unlike sediment diversions that move sediment from the Mississippi River into the catchment area. The design of Caernarvon was twofold, one using the height of the Mississippi River and one using gravitational forces to move water over the structure and into the Breton Sound Estuary. Maximum discharge into the Breton Sound Estuary is 8000 $ft^3\ s^{-1}$ (226 $m^3\ s^{-1}$). Five 4.6 m wide box culvert control structures with vertical lift gates meter Mississippi River water into the Breton Sound Estuary. The Breton Sound Estuary is hydrologically cut off any Mississippi River freshwater exchange by three levees, the Mississippi River levee to the west,

the natural levee of Bayou La Loutre to the north, and the Gulf of Mexico Outlet spoil banks to the east (Lane et al., 1999). The estuary has open exchange with the Gulf of Mexico to the south.

Discharge rate into the Breton Sound Estuary depends on 1) height of the Mississippi River and 2) salinity in the estuary. Since Caernarvon Diversion was designed using gravitational forces to move water in the estuary, the flood stage of the Mississippi River regulates how much water is metered into Breton Sound Estuary. During high flood years, such as 2008, the Caernarvon Diversion discharged at the maximum rate for approximately 2 months (Figure 1.3). The following year, the Mississippi River experienced low flow so that the discharge rate was

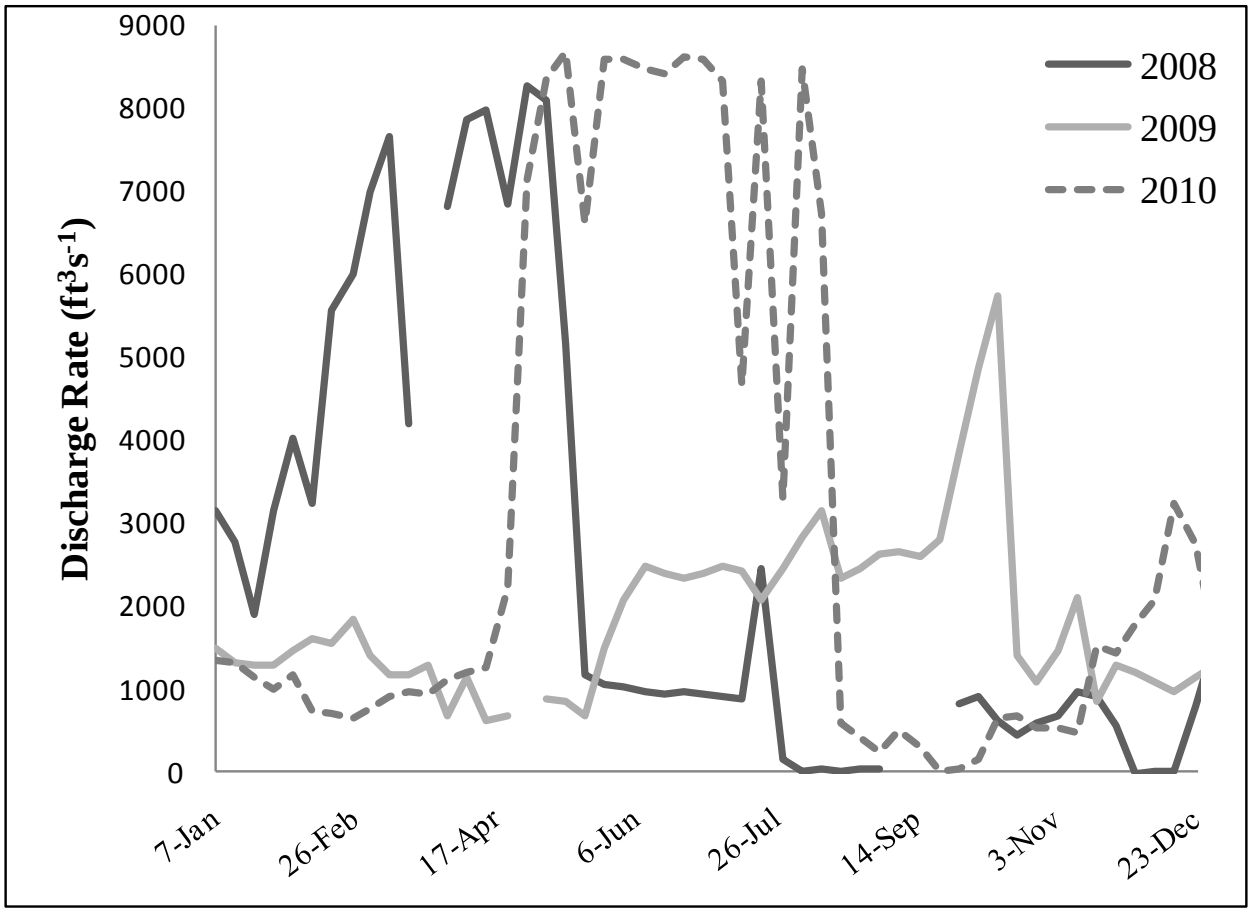

Figure 1.3 Caernarvon Diversion mean weekly discharge rates from 2008, 2009, and 2010 (USGS, 2010).

approximately 1000 ft^3 s^{-1}, one eighth the discharge in 2008. In 2010, discharge into the Breton Sound was unusually high during the summer. Salinity in the estuary also regulates the discharge rate off the Mississippi River. Years where salinity is higher in the Breton Sound results in higher river water discharge. Year to year changes in timing and discharge rate also change the timing and amount of nitrate received by marshes in the Breton Sound Estuary.

The Breton Sound estuary was formed during the Plaquemines-St. Bernard delta complex several thousand years ago. The estuary consists of 1,100 km^2 of wetlands from freshwater to saline marshes. Once metered water off the Mississippi River enters the Breton Sound estuary, the water flows directly into Big Mar, a failed reclaimed agricultural field (Lane et al., 2006). Two major pathways of water flow occur after diverted water enters Big Mar. One flow path is through Big Mar into Lake Lery and then east through Bayou Terra aux Boeufs. This pathway moves approximately two thirds of diverted water through Breton Sound Estuary towards the Gulf of Mexico. The second flow pathway is through Big Mar, then water flows to the west through Manuel's Canal and River aux Chene to the Gulf of Mexico (Lane et al., 2004). This flow path moves the remaining one third of diverted Mississippi River water that enters the Breton Sound estuary via Caernarvon Diversion.

The Breton Sound Estuary is a 70 to 80 km long section of wetlands that extends southeast towards the Gulf of Mexico. The upper 40 km of wetlands includes most of the 1,100 km^2 of wetlands while the lower 30 to 40 km^2 of the estuary is mostly open water of the Breton Sound Estuary and where exchange with the Gulf of Mexico occurs (Day et al., 2003). Tidal mixing is minimal since the tidal amplitude in the Gulf of Mexico is 35 cm. Strong winds and discharge rate of Caernarvon Diversion have the most effect in water level variation in the Breton Sound Estuary.

The Breton Sound Estuary consists of freshwater, brackish, and saline marshes. The Caernarvon Diversion has significantly reduced salt water intrusion in the estuary and salinity now ranges from less than 5 ppt in the upper estuary year round to 35 ppt at the mouth of the estuary (Lane et al., 2004). Since operation of the diversion began freshwater marshes have increased from 0 in 1978 to 628 acres in 2000 (2003). Intermediate marshes have increased by an additional 10,582 acres and brackish and salt marshes have declined with introductions of fresh water.

CHAPTER 2: MASS BALANCE

FATE OF DIVERTED MISSISSIPPI RIVER NITRATE IN VEGETATED BRACKISH COASTAL MARSH

2.1 INTRODUCTION

It is well recognized that wetlands within the coastal environment are important for providing nutrient removal and accumulation of organic matter due to anaerobic conditions in the soil. In coastal wetlands, nitrogen is generally the limiting nutrient. The importance of wetlands as nutrient sinks and coastal buffers is countered by possible adverse effects of eutrophication from intense agricultural practices in the Mississippi River Basin (Lane et al., 1999). The Mississippi River drains 41% of the United States, and as a result of agricultural practices within the Mississippi River drainage basin, the Mississippi River water has an elevated concentration of nitrate, between 1 and 2 mg N L^{-1} (Lane et al., 1999). Agricultural practices in the Mississippi River Basin increase the nutrient load in the river from runoff of ammonium (NH_4^+) and nitrate (NO_3^-). Under aerobic water column conditions in the Mississippi River, some of the NH_4-N is nitrified producing nitrate (NO_3^-). This inorganic form of nitrogen is in high demand for use in biological processes such as plant assimilation, microbial immobilization, and denitrification.

In conjunction with possible eutrophication of wetlands from elevated nutrients in the Mississippi River, lack of new sediment and freshwater have resulted in subsidence of wetlands in Louisiana. The Mississippi River levee system was built in the early 1900s to prevent spring flooding of the delta each year. As a result of the levee system, the wetlands around the Mississippi River delta have also been removed from yearly sources of sediments, freshwater, and nutrients. The lack of nutrients and sediments in the Breton Sound estuary, located southeast of New Orleans, LA, has resulted in deterioration of these wetlands (Delaune et al., 1983).

A proposed restoration tool in the Mississippi River delta is the use of diversions to redirect Mississippi River water into wetlands in Louisiana to simulate yearly spring flooding of the Mississippi River. In 1991, the Caernarvon Diversion was completed to restore annual

freshwater, directing up to 226 m^3s^{-1} (8000 ft^3s^{-1}) of Mississippi River water into the Breton Sound Estuary, LA (Lane et al., 2006). Recent concerns over eutrophication were highlighted after large scale disturbance of fresh and brackish marshes, approximately 100 km^2, in the Breton Sound Estuary was observed after Hurricanes Katrina and Rita (Day et al., 2007). It has been suggested that possible eutrophication of the marshes in Breton Sound from elevated nitrate from the Mississippi River was the underlying cause of the marsh destruction. In particular, Darby and Turner (2008a,b) suggest that elevated nitrate in the Mississippi River caused lower belowground biomass that was more easily damaged from high energy events like hurricane storm surge. Turner and Darby (2008a,b) suggest that nutrient loading lessens rooting depth resulting in shallow roots and less root biomass.

Wetlands are effective at removing excess nutrients, especially nitrogen, by assimilation into organic material or by gaseous loss by denitrification. Under anaerobic conditions, nitrate is used as an alternate electron donor by facultative anaerobic bacteria to oxidize organic matter. Denitrification in coastal wetlands is particularly important because conditions are ideal. A source of carbon, high primary productivity, and a lack of oxygen are all present as ideal conditions for denitrification. The end product of denitrification is nitrogen gas. Denitrification is an important component of the nitrogen cycle because nitrate is removed from the biosphere. Denitrification occurs at high rates in wetland soils and therefore regulates primary productivity and possible adverse effects of eutrophication (Lane et al., 1999).

The use of the stable isotopic techniques can help clarify the effects of eutrophication from high nitrate concentrations in the Breton Sound Estuary, LA. Nitrogen isotope tracer experiments allow us to identify nutrient allocation, specifically, mineralization, immobilization, nitrification, assimilation, and denitrification processes (Barraclough, 1991). The major

pathways for removal of nitrate in the Breton Sound are denitrification and assimilation by plant biomass. Other possible pathways for reduction of nitrate in the Breton Sound are dilution by the Gulf of Mexico or rainwater, phytoplankton uptake, and burial (Day et al., 2003). The addition of labeled nitrate can also be used to detect possible effects of excess nitrate on belowground root biomass. Experiments focused on natural abundance to determine nitrate removal rates suggest the main removal mechanism for nitrate in wetlands is denitrification at 94% and 89-95%, respectively (Lund et al., 1999; Reinhardt et al., 2006). Possible removal mechanisms in this experiment are denitrification, plant uptake, immobilization by the microbial pool, assimilations by algal biomass, and soil adsorption.

This study examines the effects of elevated nitrate levels in surface water for *Spartina patens*. A greenhouse core study planted with *Spartina patens* investigated changes in belowground biomass. Removal pathways of nitrate in the Breton Sound wetlands using labeled nitrogen and mass balance calculations was also investigated. We hypothesize that belowground biomass will not be significantly different under elevated water column nitrate concentrations. Furthermore, we hypothesize that the majority of added nitrate is removed by denitrification.

2.2 MATERIALS AND METHODS

2.2.1 Experimental Design

Five vegetated soil plugs were collected from a brackish marsh located proximal to Delacroix (St. Bernard Parish, Louisiana; 29º44’21.3”N, 89º41’45.6”W) on April 7, 2010. The Caernarvon Diversion began discharging Mississippi River Water into Breton Sound marshes in 1991 and the mean discharge rate of the Caernarvon Diversion on the day of sampling was 1090 $ft^3\ s^{-1}$. The Caernarvon Diversion discharge rate ranged from 0 to 8940 $ft^{-3}\ s^{-1}$ during 2010. Vegetated soil plugs were collected from an emergent brackish marsh approximately 16 km from

the diversion and were colonized almost entirely by *Spartina patens*. The vegetated soil plugs were removed from the site and transported back to the Wetland and Aquatic Biogeochemistry Laboratory (WABL) at Louisiana State University (LSU). The following day, the collected vegetated soil plugs were fitted into 12-15.2 cm diameter PVC tubes and placed in a greenhouse on LSU property.

A mass balance study was initiated using added labeled NO_3-N. Two groups of six (12 total replicate cores) were randomly assigned to one of two nitrate concentration treatment groups, 0.0 (control) or 2.0 mg L^{-1} (treatment). The treatment level of nitrate was chosen based on observed concentrations within the Mississippi River (Lane et al., 1999). The nitrate added was 99% atom ^{15}N (Cambridge Isotope Laboratory, Andover, MA). A 10 cm water column was maintained within each core for the duration of the experiment. Nitrate solution was replaced approximately twice a week for three months. Twelve flood events occurred during the first six week experimental time and 11 flood events occurred during the second six week experimental time, for a total of 23 flood events. The solution was changed in the water column by manually pouring the water column out of each core at the end of each flooding event. The water column was replaced by first filling each core with dionized water up to the soil surface. Then each core was filled 10 cm above the soil surface with the either dionized water (control) or 2 mg L^{-1} KNO_3-N solution (treatment).

Water column sampling was performed at roughly two week intervals. At each time, two consecutive flooding events, for a total of 4 flood events during each 6 week time period, were sampled during the 12 week study period. At 6 weeks, aboveground biomass was harvested by clipping all stems approximately 2 inches above the soil surface. The removal of the aboveground biomass was designed to simulate a disturbance event. At the end of 12 weeks, 6

cores (3 each from control and treatment) were destructively harvested by clipping aboveground biomass and sectioned belowground biomass into 0-10 cm and 10-20 cm sections. Harvest of cores at 6 weeks did not occur for two reasons 1) expense of ^{15}N analysis and 2) root separation is very time consuming. Assimilation of ^{15}N into plant biomass was assumed to be constant over time and therefore was divided in half for use in analysis for weeks 1-6 (this chapter) and weeks 7-12 (Chapter 4). Aboveground biomass was separated into live and dead biomass. Half of each soil section was used to determine belowground biomass by separating root biomass into dead, live, and stem biomass. The other half of each soil section was used for soil samples by removing large roots and blending to a homogenous soil sample. Total weights of each component were recorded before separation occurred. All samples were stored refrigerated in the dark at 4ºC until analyses were completed. Temperature in the greenhouse, redox potential (Eh), pH, and conductivity were monitored over the course of the experiment.

The pH was measured using an Accumet® Research AR25 Dual Channel pH/Ion Meter. Redox potential was taken at 5 and 10 cm soil depth in six randomly selected cores, 3 control and 3 treatment cores. Redox potential was measured using a platinum working electrode and saturated calomel (SCE) reference electrode. A correction factor of +242 was applied to each redox potential measurement to correct for the potential of the calomel reference electrode (Twilley and Nyman, 2005). Conductivity was monitored using an Accument® Basic AB30 Conductivity Meter and converted to salinity using 0.67 as a conversion factor.

2.2.2. Water Column, Plant, and Soil Characterization

Water column subsamples were taken at 0, 4, 8, 12, and 24 hours after flooding for each of the 8 flood events. Each 20 mL sample was filtered using an Acrodisc® Premium 25mm Syringe Filter with 0.45 µm GHP Membrane. Water samples were stored in the dark at 4ºC until

analysis of NO_3^-, NH_4^+, and SRP completed. NO_3^-, NH_4^+, and SRP were analyzed using a SEAL AQ2 Automated Discrete Analyzer (SEAL Analytical, West Sussex, England; US EPA Methods 353.2 and 350.2 respectively (US EPA, 1983)). Method detective limits for NO_3^-, NH_4^+, and SRP were 0.014, 0.012, and 0.005 mg L^{-1}, respectively. Aboveground biomass was analyzed for total C (TC), total N (TN), total P (TP) and $\delta^{15}N$ by drying separated dead and live biomass at 70ºC until constant weight. Belowground biomass was separated into live, dead, and stems, dried at 70ºC until constant weight and analyzed for TC, TN, TP, and $\delta^{15}N$. Root separation was completed by the same person for the duration of the experiment to maintain consistency in determining live versus dead root biomass. Roots that were categorized as live were gold in color, turgid, floated when placed in water, and had the presence of fine root hairs. Roots that were partially decomposed were considered dead.

Each soil section was analyzed for moisture content, bulk density, TC, TN, TP, extractable NO_3-N, extractable NH_4-N, potentially mineralizable nitrogen (PMN), microbial biomass C (MBC), and microbial biomass N (MBN), and $\delta^{15}N$. Moisture content was calculated by drying a soil subsample at 70ºC until constant weight. Percent moisture and soil g per volume (cm^{-3}) were calculated. TC and TN were measured using dried, ground subsamples of soil sections 0-10 and 10-20 cm using an Elemental Combustion System with a method detection limit of 0.005 g kg^{-1} (Costech Analytical Technologies, Inc., Valencia, CA).

Extractable NO_3-N and NH_4-N soil samples were measured using a soil extractant (25 ml of 2 M Potassium chloride (KCl)) then analyzed using the same EPA methods on the SEAL AQ2 Automated Discrete Analyzer for determination of water column NO_3^- and NH_4^+. PMN was measured using 2.0 M KCl soil extractant for the time zero control soil samples. PMN soil samples were incubated for 3 and 10 days with 5 mL of dionized water and agitated each day.

Incubate PMN soil samples were extracted with 20 mL of 2.0 M KCl. The PMN subsamples were subjected to the same EPA methods on the SEAL AQ2 Automated Discrete Analyzer for determination of water column NO_3^- and NH_4^+. The PMN rate was calculated as the increase in NH_4-N over time by regression.

Microbial biomass C and N were calculated using the fumigation-extraction method (Brookes et al., 1985; Sparling et al., 1990) . Two sets of triplicate 5 g wet weight samples were prepared in 25 ml centrifuge tubes. One set was used for non-fumigate samples and the other set was used for fumigate samples. Non-fumigate samples were measured using soil extractant (25 ml of 2 M HCl), shaken for 30 minutes then centrifuged. The supernatant was filtered through 47 mm Supor®-450 membrane filter and stored in the dark at 4ºC until analysis was completed. Analysis of the supernatant included total organic carbon (TOC) and total organic nitrogen (TON) using a Shimadzu Scientific Instrument TOC-VCSN, Columbia, MD. Fumigated triplicates were placed in a desiccator with 0.5 ml chloroform added to each centrifuge tube as well as a beaker with approximately 50 ml of chloroform with 5-10 boiling stones. The air within the desiccator was removed and refilled three times. The fourth time, the desiccator was sealed by evacuatation and placed in the fume hood for 24 hours. After 24 hours, the chloroform was removed by evacuating the head space at least seven times. After fumigation, this set of triplicates was treated with the same process as the non-fumigate triplicates. MBC and MBN was calculated by subtracting the non-fumigate samples from the fumigate samples.

TP was determined using a TP ashing-digestion method (Andersen, 1976) for aboveground plant biomass, belowground plant biomass, soil scrapings, and soil. Samples were prepared in a 50 mL beaker using 0.5 g dried soil weight and between 0.2 and 0.3 dried plant biomass weight. Triplicate samples occurred 10% of the time, with an external peach leaf

standard and a blank for each set. Samples were burned over night using a muffle furnace (Barnstead Thermolyne 62700 Furnace) at 550°C for 4 hours. Samples were reweighed after burning and prepared for acid extraction. The ash was moistened using dionized water to prevent any loss of sample before the addition of 20 mL of 6 M HCl. Each sample was placed on a 120°C hot plate until dry. Additional 2.25 mL of 6 M HCl was added and brought to a near boil. Samples were filtered through Whatman #41 filter paper into 50 mL volumetric flasks. Samples were stored at room temperature until analysis was completed. TP was analyzed using a SEAL AQ2 Automated Discrete Analyzer (SEAL Analytical, West Sussex, England; US EPA Methods 353.2 and 350.2 respectively (US EPA, 1983)). The method detection limit for TP was 0.05 mg P L^{-1}.

2.2.3 ^{15}N Analysis

Live aboveground plant biomass and dead aboveground plant biomass for harvest at 6 and 12 weeks and live root biomass, dead root biomass, stem root biomass, soil scrapings, and soil samples for harvest at 12 weeks were sent to the Stable Isotope Laboratory at Woods Hole Oceanographic Institute for ^{15}N analysis. Analysis was done using a Europa 20-20 CF-IRMS interfaced with the Europa ANCA-SL elemental analyzer. Stable isotope values were used in the mass balance calculation of the nitrogen cycle for this study. Isotope values for the 12 week belowground root biomass and soil of the harvested cores where divided in half to assess isotope values at 6 weeks, before aboveground biomass clipping occurred, and used for the mass balance calculation at 6 weeks. Aboveground biomass cut at 6 weeks was used for the isotope content for this mass balance calculation at 6 weeks.

Percentage of recovered ^{15}N for each component of a core was calculated by first converting the δ^{15}N value to the atom % using the following formula:

$$\% \ ^{15}N = 0.000365 * \delta^{15}N + 0.0155726 \text{ (Fry, 2006)} \quad \textbf{[Equation 1]}$$

Recovered ^{15}N percentage was calculated by first multiplying % ^{15}N/100, % nitrogen/100, and total dry weight (mg) together. This value was then divided by total added ^{15}N (mg) for each time period and then multiplied by 100 to give the percentage of ^{15}N recovered for each component such that:

$$\% \text{ Recovered } ^{15}N = [((\% \ ^{15}N/100)*(\% \text{ nitrogen}/100)*(\text{total dry weight}))/^{15}N \text{ added}]*100 \quad \textbf{[Equation 2]}$$

Percentage of recovered ^{15}N was calculated for live aboveground biomass, dead aboveground biomass, soil scraping, live root biomass, dead root biomass, stem biomass, and soil for each treatment core. Live roots, dead roots, stems and soil % ^{15}N were calculated for 0-10 cm and 10-20 cm sections. Each value for all components of a core were added together to determine the total percent loss of added nitrate to each core. A mass balance calculation for each core was performed as well as averages of each component for each time period.

2.2.4 Data Analysis

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

The effect of nitrate addition on aboveground and belowground biomass between control and treatment cores was also determined using a student t-test ($P < 0.05$). Data normality was

determined using the Kolmogorov-Smirnov test ($\alpha = 0.01$). Data was log-transformed to fit a normal distribution when necessary.

2.3 RESULTS

2.3.1 Soil Properties

In the 0-10 cm soil section, the mean TC (n = 6) from the control and treatment cores were 67 ± 3.4 g kg^{-1} and 62 ± 25 g kg^{-1}, respectively (Table 2.1). The mean TN (n = 6) was similar between the control core at 4.87 ± 0.23 g kg^{-1} and the treatment cores at 4.56 ± 1.33 g kg^{-1}. MBC and MBN were similar in the control and treatment cores at 3.35 ± 0.22 g $kg^{-}1$, 10.3 ± 12.3 mg kg^{-1}, 3.71 ± 0.61 g kg^{-1}, and 11.7 ± 9.07 mg kg^{-1}, respectively. Extractable NO_3-N was also alike at 2.40 ± 0.32 mg kg^{-1} and 2.37 ± 0.76 mg kg^{-1} for the control and treatment cores. In the 0-10 cm soil interval, only TP was significantly different in the control and treatment cores (638 ± 33.4 g kg^{-1} and 502 ± 14.0 g kg^{-1}, respectively; Table 2.1).

Similar results were seen in the 10-20 cm soil section as in the 0-10 cm soil section when comparing control and treatment cores for the same soil section. Mean TC (n = 6) was 57 ± 3.8 g kg^{-1} for the control cores and 50 ± 7.0 g kg^{-1} for the treatment cores (Table 2.2). There was no difference for TN between the control and treatment cores at 4.32 ± 0.22 g kg^{-1} and 3.89 ± 0.50 g kg^{-1}, respectively. MBC and MBN were alike in treatment and control cores for both soil parameters. The mean (n = 6) MBC was 2.81 ± 0.09 g kg^{-1} for the control cores and 2.66 ± 0.24 g kg^{-1} and the mean (n = 6) for MBN was 7.5 ± 8.82 mg kg^{-1} for the control cores and 4.64 ± 5.54 mg kg^{-1} for the treatment cores. Extractable NO_3-N was similar in control and treatment cores at 2.80 ± 0.44 mg kg^{-1} and 1.31 ± 0.61 mg kg^{-1}, respectively. In the 10-20 cm soil interval, only % moisture was significantly difference in the control and treatment cores (63 ± 1.1 % and 60 ± 3.3 %, respectively; Table 2.2)

Table 2.1 Soil characteristics for harvest at 6 weeks for soil section 0-10 cm. Data are mean values (n = 6) ± sd. Difference letters indicate significant differences between columns at p = 0.05. *Indicates extraction by 2 M KCl

Soil Parameter	Units	Controls	Treatments
Bulk Density	**g cm^{-3}**	0.24 ± 0.05	0.25 ± 0.03
% Moisture	**%**	67 ± 1.8	67 ± 2.9
TC	**g kg^{-1}**	67 ± 3.4	62 ± 25
TN	**g kg^{-1}**	4.87 ± 0.23	4.56 ± 1.33
TP	**mg kg^{-1}**	638 ± 33.4[a]	502 ± 14.0[b]
MBC	**g kg^{-1}**	3.35 ± 0.22	3.71 ± 0.61
MBN	**mg kg^{-1}**	10.3 ± 12.3	11.7 ± 9.07
NO_3-N*	**mg kg^{-1}**	2.40 ± 0.32	2.37 ± 0.76
NH_4-N*	**mg kg^{-1}**	41 ± 21	39 ± 11
PMN	**mg kg^{-1} day^{-1}**	2.17 ± 2.19	3.33 ± 0.88
TC:TN		14	14

Table 2.2 Soil characteristics for harvest at 6 weeks for soil section 10-20 cm. Data are mean values (n = 6) ± sd. Difference letters indicate significant differences between columns at p = 0.05. *Indicates extraction by 2 M KCl

Soil Parameter	Units	Controls	Treatments
Bulk Density	**g cm^{-3}**	0.33 ± 0.03	0.35 ± 0.08
% Moisture	**%**	63 ± 1.1[a]	60 ± 3.3[b]
TC	**g kg^{-1}**	57 ± 3.8	50 ± 7.0
TN	**g kg^{-1}**	4.32 ± 0.22	3.89 ± 0.50
TP	**mg kg^{-1}**	622 ± 104	510 ± 35.6
MBC	**g kg^{-1}**	2.81 ± 0.09	2.66 ± 0.24
MBN	**mg kg^{-1}**	7.50 ± 8.82	4.64 ± 5.54
NO_3-N*	**mg kg^{-1}**	2.80 ± 0.44	1.31 ± 0.61
NH_4-N*	**mg kg^{-1}**	24 ± 7.6	44 ± 18
PMN	**mg kg^{-1} day^{-1}**	1.25 ± 0.54	2.24 ± 4.00
TC:TN		13	13

2.3.2 Soil Properties Relationships

In the 0-10 cm soil interval, TC and TN were significantly positively correlated with % moisture (r = 0.84 and r = 0.83, respectively) and were strongly correlated with each other (r = 1.00; Table 2.3). Extractable NO_3-N was negatively correlated with MBC (r = -0.88). Finally, PMN was positively correlated with MBN (r = 0.90). In the 10 to 20 cm soil section, TC was positively correlated with % moisture (r = 0.82) and was directly correlated with TN (r = 0.90; Table 2.4). MBC was positively correlated with TC (r = 0.92). Extractable NO_3-N was positively correlated with TP in the 10-20 cm soil section (r = 0.81).

Table 2.3 Product-moment correlation coefficients for 0-10 cm soil section properties at 6 weeks. Bold indicates significance at P < 0.05 (for n = 24, r = 0.40)

	Bulk Density	% Moisture	TC	TN	TP	MBC	MBN	NO_3^-	NH_4^+
% Moisture	**-0.75**								
TC	**-0.49**	**0.84**							
TN	**-0.47**	**0.83**	**1.00**						
TP	-0.14	-0.04	0.03	0.05					
MBC	**-0.48**	**0.74**	**0.48**	0.41	-0.38				
MBN	0.13	-0.26	-0.12	-0.19	-0.37	0.25			
NO_3^-	**0.61**	**-0.66**	-0.28	-0.22	0.00	**-0.88**	-0.11		
NH_4^+	-0.14	0.41	0.06	0.09	-0.16	0.27	**-0.76**	-0.29	
PMN	0.39	-0.33	-0.13	-0.19	-0.16	0.21	**0.90**	-0.10	**-0.70**

Table 2.4 Product-moment correlation coefficients for 10-20 cm soil section properties at 6 weeks. Bold indicates significance at P < 0.05 (for n = 6, r = 0.81)

	Bulk Density	% Moisture	TC	TN	TP	MBC	MBN	NO_3^-	NH_4^+
% Moisture	**-0.63**								
TC	-0.17	**0.82**							
TN	0.19	**0.59**	**0.90**						
TP	-0.35	**0.46**	**0.42**	0.15					
MBC	-0.11	**0.76**	**0.92**	**0.76**	**0.53**				
MBN	0.03	**0.50**	**0.42**	0.37	0.17	**0.64**			
NO_3^-	**-0.41**	**0.65**	**0.47**	0.18	**0.81**	**0.69**	**0.68**		
NH_4^+	**0.54**	-0.12	-0.06	0.31	**-0.57**	-0.13	0.39	-0.28	
PMN	-0.13	0.25	-0.08	-0.13	0.25	0.14	**0.76**	**0.66**	0.35

2.3.3 Plant Biomass

There was no significant differences in live aboveground biomass when control and treatment cores where compared (Figure 2.1). Similarly, there was no response in the dead aboveground biomass when comparing control and treatment cores. Belowground biomass did not have any response to nitrate addition when comparing live and dead biomass for control and treatment cores for 0-10 cm soil section (Figure 2.2, Table 2.5). Stem biomass in the 0-10 cm soil section was significantly different ($p = 0.05$) in the control and treatment cores, where stems where higher in the treatment cores.

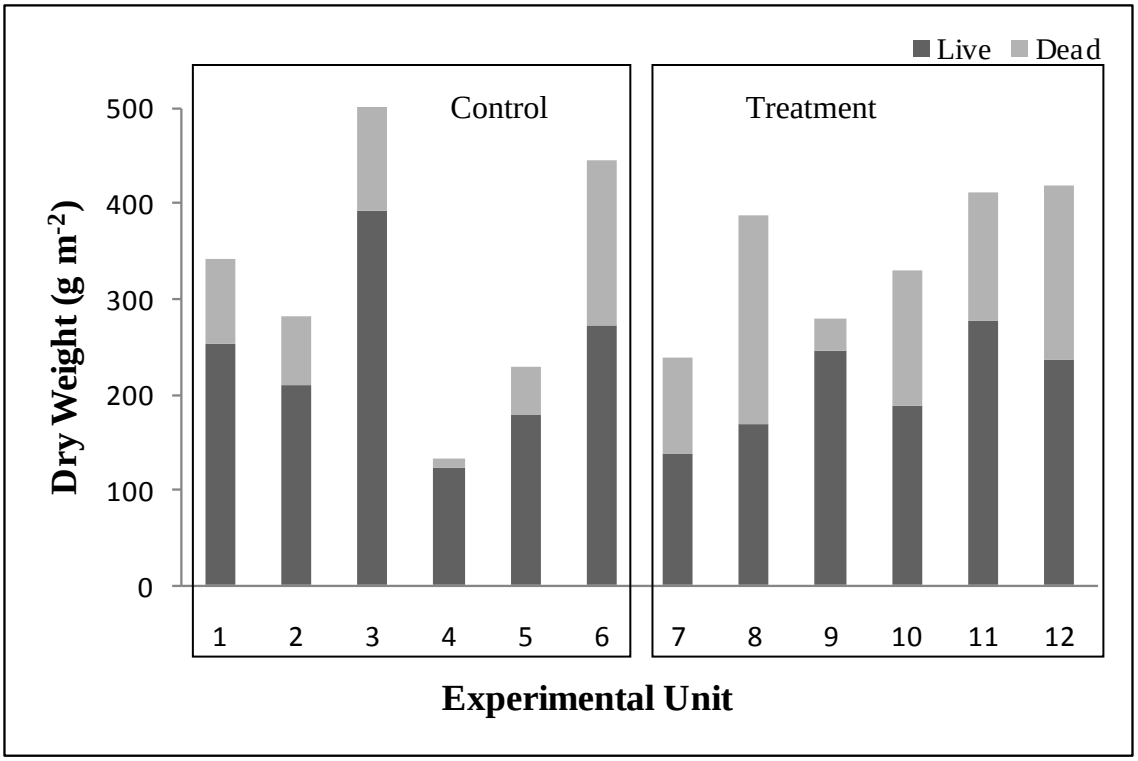

Figure 2.1 Total dry weight of live and dead aboveground biomass for control cores and treatment cores during 6 weeks of growth.

Similarly to soil section 0-10 cm, there was no significant difference in live belowground biomass in the 10-20 cm soil section when comparing control and treatment cores (Figure 2.3).

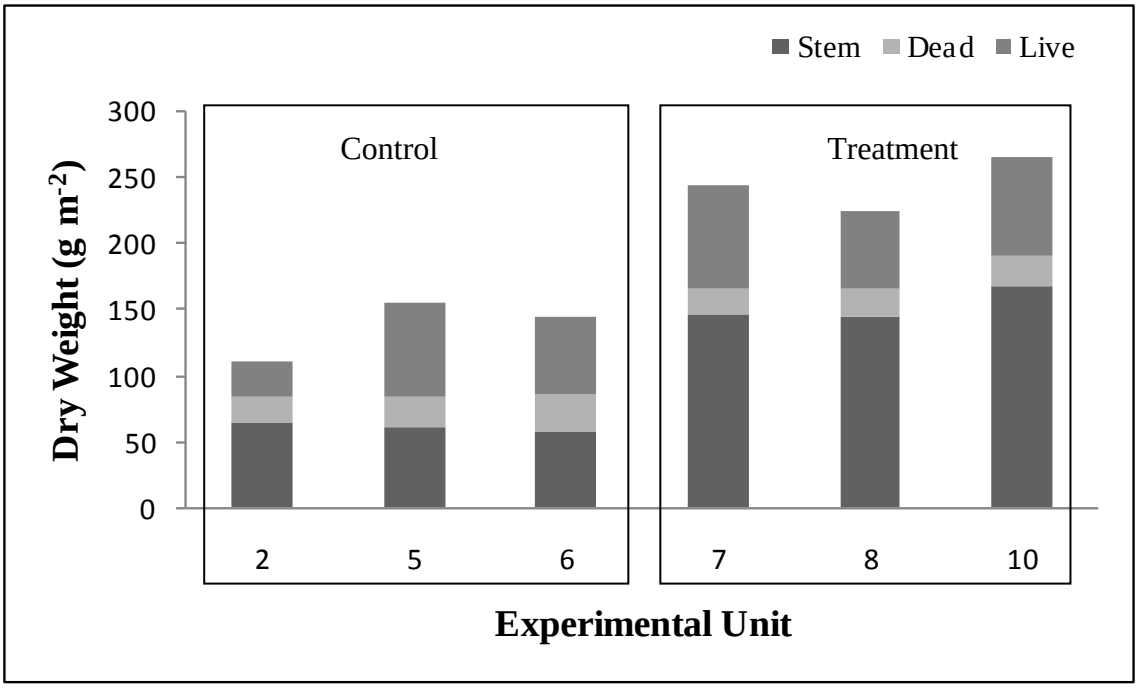

Figure 2.2 Total dry weight of live, dead, and stem belowground biomass in the 0-10 cm soil section for control cores and treatment cores during 6 weeks of growth.

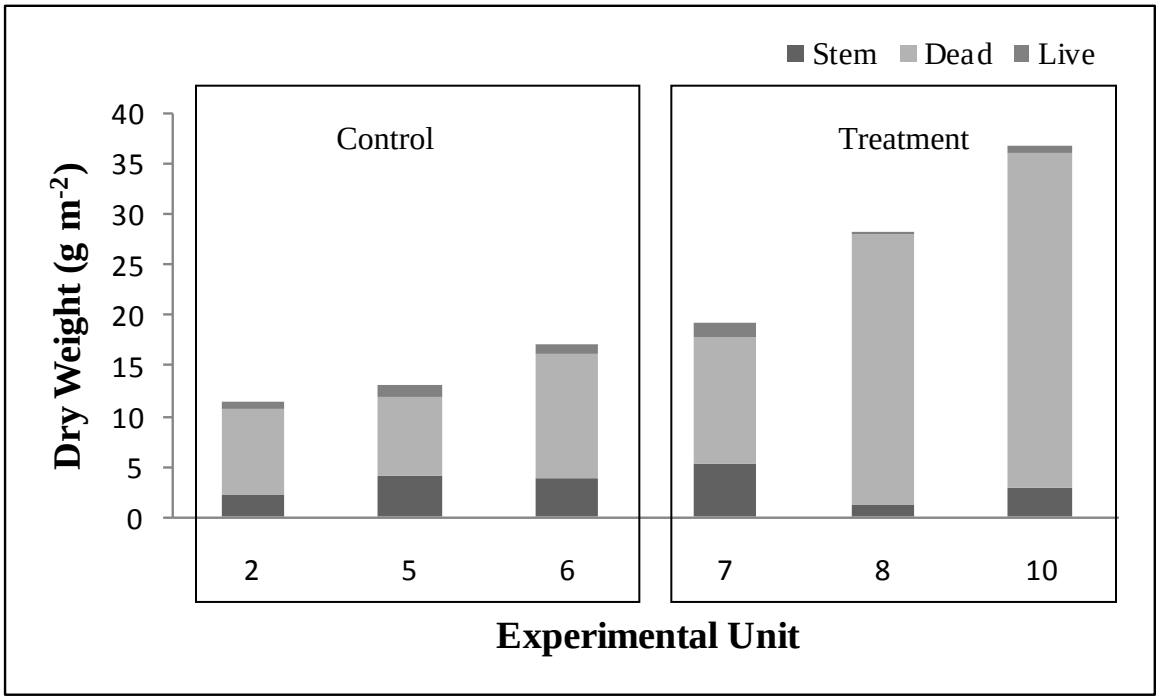

Figure 2.3 Total dry weight of live, dead, and stem belowground biomass in the 10-20 cm soil section for control cores and treatment cores during 6 weeks of growth.

There was no response to stem belowground biomass in the 10-20 cm soil section when comparing control and treatment cores. However, there was a significant difference ($p = 0.05$) in dead belowground biomass when comparing control and treatment cores in the 10-20 cm soil section (Table 2.5).

Table 2.5 Dry weight in g of live and dead above and below ground components, and soil. Data are mean ± sd (*n = 6 for aboveground biomass, soils scraping, all other components n = 3). Difference letters indicate significant differences between columns at $p = 0.05$.

Experimental Component	Control	Treatment
Live Aboveground*	20.0 ± 7.79	17.5 ± 4.44
Dead Aboveground*	7.16 ± 4.68	11.4 ± 5.38
Live Roots 0-10 cm	2.05 ± 0.34	1.82 ± 0.14
Dead Roots 0-10 cm	5.09 ± 0.24	12.9 ± 1.09
Stem Roots 0-10 cm	4.33 ± 1.89[a]	5.85 ± 0.83[b]
Soil 0-10 cm	202 ± 42	202 ± 24
Live Roots 10-20 cm	0.78 ± 0.21	0.79 ± 0.67
Dead Roots 10-20 cm	9.70 ± 2.50	24.3 ± 10.6
Stem Roots 10-20 cm	3.44 ± 1.02[a]	3.22 ± 2.01[b]
Soil 10-20 cm	286 ± 30	286 ± 68
Soil Scraping*	3.81 ± 1.57	3.11 ± 1.44

2.3.4 Experimental Variables

The nitrate treatment addition was 2 mg $K^{15}NO_3$-N L^{-1} of 99 % atom ^{15}N; however, dilution by pore water occurred such that nitrate concentrations at time zero averaged 1.52 mg NO_3-N L^{-1} (Figure 2.4). Complete loss of nitrate from the water column occurred in 12 hrs over 4 different flood cycles during the 6 weeks of 2 mg NO_3-N L^{-1} additions. Denitrification rate during the 4 flood events sampled over the 6 weeks remained relatively constant, ranging from 929 to 1182 mg N m^{-2} day^{-1} (Table 2.6). Mean temperature in the greenhouse was 32.5 ± 4.8 ºC during the experimental time period. Redox potential was similar in each core measured and at each soil depth (5 and 10 cm) averaging -149.27 ± 27.04 mV. The average pH was 6.82 ± 0.12 and the average salinity was 0.553 ± 0.18 ppt.

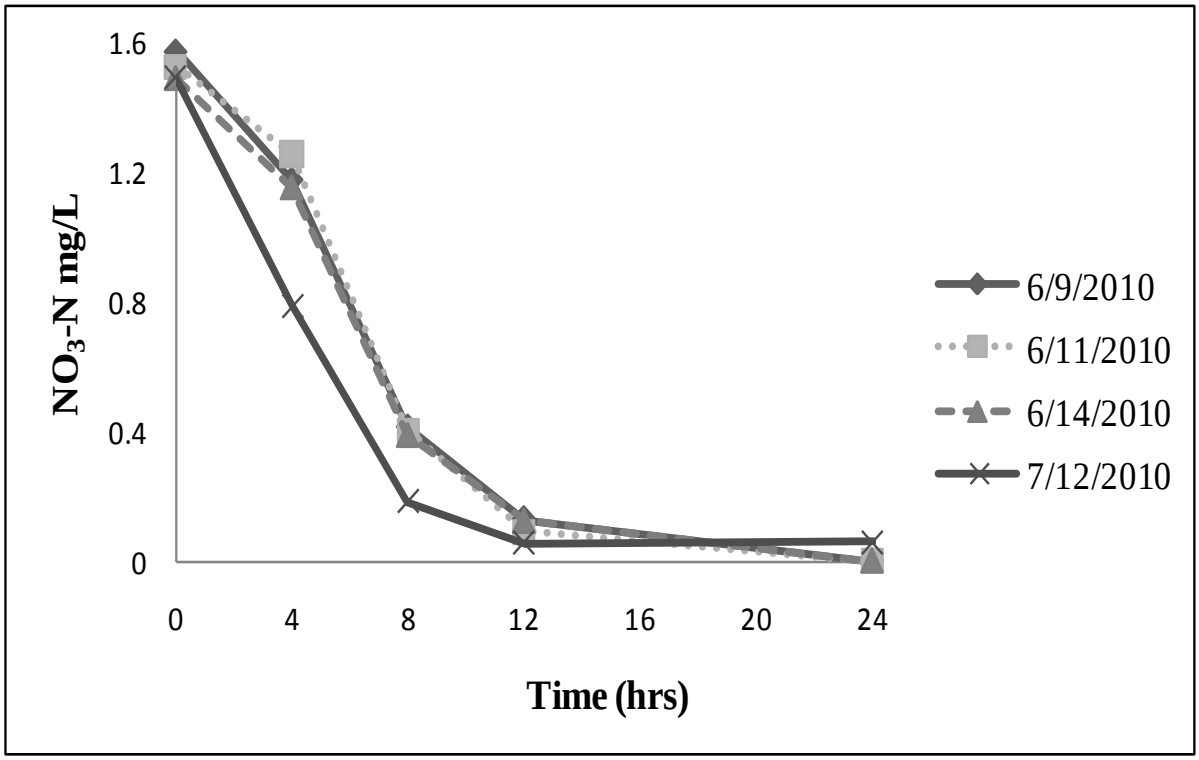

Figure 2.4 Mean water column nitrate concentration for 4 flood dates at 2 mg N L^{-1} over 6 weeks (n = 6).

2.3.5 Percent ^{15}N Recovery

A total of 43.44 mg $^{15}NO_3$-N was added in solution during the 6 week time period. The average percent recovery of added ^{15}N for each component is shown in Figure 2.5. The 10-20 cm belowground biomass (live roots, dead roots, and stems combined) and the 10-20 cm soil section both recovered less than 1 % of ^{15}N added to the treatment cores. Soil scrapings, dead roots, and stems each recovered approximately 1 % of the added ^{15}N. Live roots in the 0-10 cm soil section accounted for about 2 % of the added labeled nitrate. Soil from the 0-10 cm soil section retained about 4 % of the added ^{15}N. Aboveground biomass (live + dead biomass) accounted for 21 % of recovered ^{15}N. The largest component was unaccounted gaseous losses at 70 % of added ^{15}N.

External and internal N sources were calculated for each core component to compare main N sources in the Breton Sound Estuary. Added labeled nitrate represented external N

sources and N mineralization represented internal N sources. The plant biomass, both above and below ground biomass, only recovered 30% of the total added labeled N (Figure 2.5). The remaining 70% of N in all of the plant components then has to come from internal N source. External N accounted for 19 mg N and internal N accounted for 2737 mg N for this time period in the 0-20 cm soil section (Table 2.7). External N from added labeled nitrate was only 0.7 % if the total N recovered N over 6 weeks. Internal N was 99.3 % of the total N over the 6 weeks experimental time.

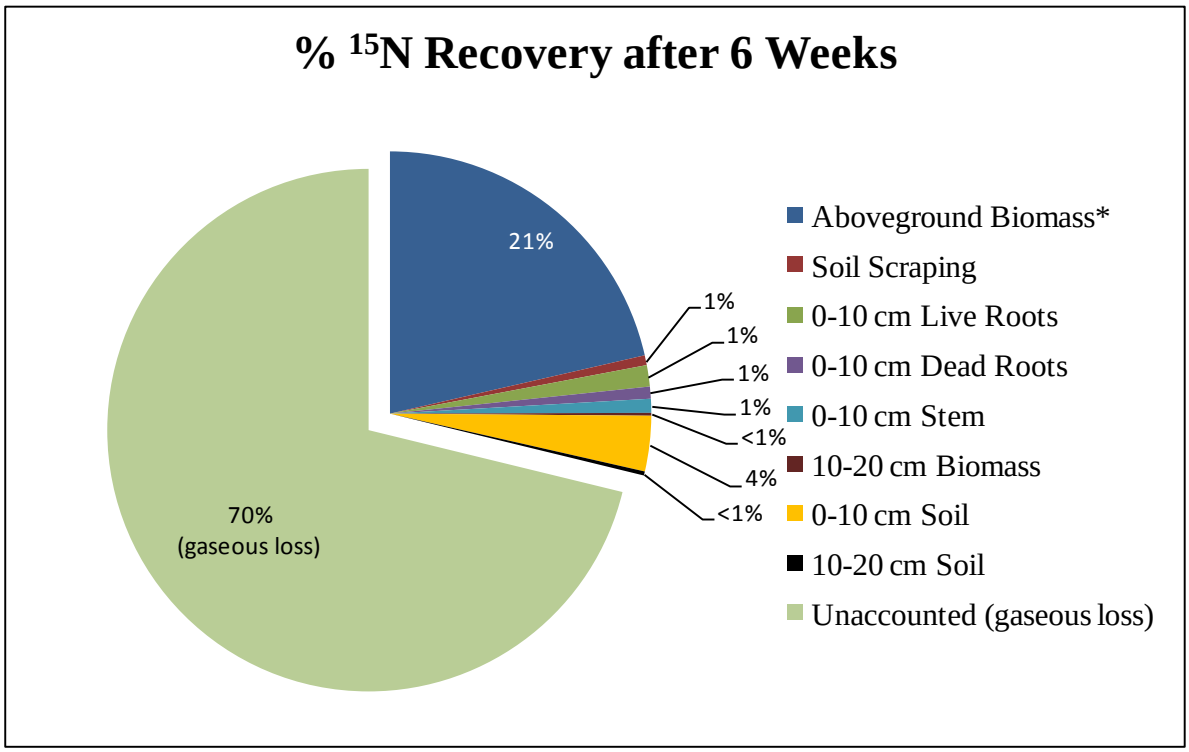

Figure 2.5 Mass balance of labeled nitrate addition over 6 weeks for above and below ground components, represented as % of recovered ^{15}N in each component (*n = 6 for aboveground biomass, all other components n = 3).

Table 2.6 Maximum daily denitrification rate for 4 flood events over 6 weeks with the addition of 2 mg N L^{-1}. Denitrification rate is maximum rate of nitrate loss corrected for ^{15}N loss by assimilation into plant biomass (maximum rate of nitrate loss x gaseous loss from Figure 2.5).

Core Number	Denitrification Rate (mg N m^{-2} day^{-1})			
	6/9/2010	6/11/2010	6/14/2010	7/12/2010
1	673	777	862	688
2	1131	1047	950	1460
3	820	864	786	1306
4	846	1231	918	1409
5	1196	1206	1074	1084
6	934	1006	986	1145
Mean ± stdev	933 ± 198	1022 ± 181	929 ± 100	1182 ± 283

Table 2.7 External and Internal N sources over 6 weeks for above and below ground components in the 0-20 cm soil section. Data are mean values ± sd. (*n = 6 for aboveground biomass and soils scraping, all other components n = 3).

Experimental Component	mg ^{15}N	mg ^{14}N
Live Aboveground*	7.7 ± 2.2	163 ± 37
Dead Aboveground*	1.1 ± 1.7	83 ± 37
Live Roots 0-10 cm	2.2 ± 0.21	20 ± 1.5
Dead Roots 0-10 cm	1.2 ± 0.70	128 ± 6.8
Stem Roots 0-10 cm	1.3 ± 0.75	44 ± 6.9
Soil 0-10 cm	4.6 ± 1.4	901 ± 175
Live Roots 10-20 cm	0.01 ± 0.02	5.8 ± 5.1
Dead Roots 10-20 cm	0.25 ± 0.26	227 ± 101
Stem Roots 10-20 cm	0.05 ± 0.04	21 ± 16
Soil 10-20 cm	0.45 ± 0.14	1123± 369
Soil Scraping*	0.25 ± 0.20	20 ± 10
Total N	19	2737
% of Total N	0.7	99.3

2.4 DISCUSSION

All cores had similar soil properties when comparing control and treatment cores in the 0-10 cm and 10-20 cm soil sections. Soil properties that were expected to change with the addition

of nitrate were TC, TN, MBC, MBN, and extractable NO_3-N. These properties were expected to change because of increased microbial biomass (higher MBC and MBN) in response to additions of nitrate, which is usually a limiting nutrient. This suggests that immobilization of added nitrate into microbial biomass did not take place. Changes in TC and TN were expected if carbon and nitrogen reserves were used as nitrate was used during denitrification. These properties, however, did not significantly change after the addition of excess nitrate for 6 weeks. Similar values in the five soil properties that we expected to see changes in indicate that the addition of nitrate did not affect soil properties in the treatment cores when compared with control cores.

Table 2.8 Examples denitrification rates from published literature.

System	**Denitrification Rate (mg N m^{-2} day^{-1})**	**Reference**
Chesapeake Bay, *Z. marina*	225-702	Caffrey and Kemp (1990)
Colne Estuary	1-154	Ogilvie at al. (1997)
Great Ouse Estuary	7-32	Trimmer at al. (1998)
Guadalupe Estuary	15-116	Yoon and Benner (1992)
Patuxent River Estuary	259-299	Jenkins and Kemp (1984)
Barataria Bay marsh	44-137	Gardner and White (2010)
Breton Sound Estuary marsh	199-253	This study
Colne Point salt marsh	13-44	Aziz and Nedwell (1986)
Torridge River marsh	8-198	Koch et al. (1992)

* Modified from Herbert (1999)

The rate of nitrate loss in the water column was consistent throughout the 6 week experiment at approximately 12 hours, suggesting that gaseous losses (denitrification or ammonia volatilization) was occurring in response to nitrate additions. Low ammonium concentrations in the water column over each flood event were at or below detection limits and mean pH was 6.82 ± 0.12, suggesting ammonia volatilization was not a significant process for removing added nitrate. Redox conditions (-149 ± 27 mV) indicated environmental conditions were available for denitrification to occur (Patrick et.al, 1996). The presence of anaerobic

conditions, nitrate, and high soil carbon advocate that the excess nitrate was removed within 12 hrs by denitrification. Furthermore, denitrification rates in this study ranging from 929 to 1182 mg N m^{-2} day^{-1}. When corrected for temperature in the greenhouse, by dividing in half for Q_{10}, denitrification rate are on the high end of published denitrification rates (Table 2.8).

Percentage of recovered ^{15}N in the above and belowground biomass support the conclusion that excess nitrate was removed by denitrification. If assimilation by plants was an important process in removing excess nitrate, then above and belowground biomass should have a large percentage of ^{15}N incorporated in the biomass. Live roots in the 0-10 cm soil section assimilated only 2% of added labeled nitrate. In total, belowground biomass in the 0-10 cm soil sections assimilated only 4% of the added labeled nitrate into biomass. Also, there was no significant difference in live roots in either soil section when comparing control and treatment cores. The hypothesis that eutrophication causes lower live belowground biomass in coastal marshes receiving high nitrate Mississippi River water was not substantiated in this study.

Above and below ground biomass percentage of recovered labeled nitrate does show that assimilation of excess nitrate to above and below ground biomass occurs in Breton Sound Estuary. However, only 30 % of labeled nitrate was recovered in all the above and below ground plant biomass (Figure 2.5). Therefore, the other 70 % of N in the plant biomass had to be available through internal N sources by N mineralization. Calculations of internal and external N sources support the conclusion that N mineralization is the main source of N in the Breton Sound. This is further supported as there were no significant changes in belowground biomass in response to nitrate additions.

No significant differences in TC or MBC when control and treatment cores were compared indicate that an increase in soil metabolism did not occur. This is contrary to other

studies that suggest the addition of nitrate laden Mississippi River water is increasing soil metabolism and decreasing marsh stability (Howes et al., 2010; Turner, 2010; Turner et al., 2009). Using an accumulation rate of 301 g C m^{-2} (DeLaune and White, 2011) for 20 years, a 0-20 cm soil section has 6020 g C m^{-2}. The denitrification rate during the 6 weeks was found by multiplying the total N added during the experiment, then dividing by meter square equivalent of the core and the number of experimental days. Finally, this value was multiplied by the gaseous loss component of the mass balance (Figure 2.5), so that the time integrated denitrification rate was 39.07 mg N m^{-2} day^{-1}. We can assume that 99 % nitrate loss was to N_2 (Smith et al., 1981), so that the total N needed for 30, 60 and 90 days are 1.17, 2.34, and 3.52 g N m^{-2}, respectively. Solving simultaneous stoichiometric equations for the conversion of nitrate to N_2 gas and glucose to CO_2 shows that for every 4 g of N, 5 g of C are needed.

$$2(12H^+ + 10e^- + 2NO_3^- \longrightarrow N_2 + 6H_20)$$

$$\underline{5(H_2O + CH_2O \longrightarrow CO_2 + 4H^+ + 4e^-)}$$

$$4\ NO_3^- + 5CH_2O \longrightarrow 2N_2 + 5CO_2$$

Accordingly, for 30, 60, and 90 days the amount of C needed for denitrification to take place is 1.46, 2.93, and 4.40 g C m^{-2}, respectively. This is equivalent to 0.02, 0.05, and 0.07 % of the total C (6020 g C m^{-2}) calculated for this experiment over 30, 60, and 90 days, respectively. These carbon calculations do not take into account carbon accumulation that would also occur. DeLaune and White (2011) report carbon accumulation rates for fresh marshes in LA between 219-301 g C m^{-2} $year^{-1}$.

2.5 CONCLUSION

Our results indicate that gaseous losses are the main removal mechanism for diverted Mississippi River nitrate as loss of added nitrate occurred in 12 hours. Gaseous losses include denitrification and ammonia volatilization. Denitrification is the most likely removal pathway

given low redox conditions observed in cores, the presence of excess nitrate, and concentrations of NH_4^+ at or below detection limits. This study also confirms that assimilation of nitrate into live root from the Mississippi River does occur; however, after 6 weeks of labeled nitrate addition, only 2% of added labeled nitrate was assimilated into live root biomass. Also, no significant differences in total live root biomass at either the 0-10 cm or 10-20 cm soil sections indicate Mississippi River nitrate diverted into coastal marshes is not affecting the total amount of belowground biomass or rooting depth by the addition of excess nitrate. Calculations of external and internal N sources in plant biomass, at 68.61 mg N (2.5 %) and 2686.08 mg N (97.5 %), respectively, support the conclusion that N mineralization is the main source of N for plant assimilation in the Breton Sound Estuary. As a result, the hypothesis that excess nitrate is affecting belowground root biomass in Breton Sound Estuary was not confirmed in this experiment.

Furthermore, results indicate that even though denitrification was the main removal mechanism for excess nitrate, carbon reserves were not significantly affected. This was indicated by the lack of significant differences in TC or MBC when comparing control and treatment cores. Stoichoimetric calculations of grams of C needed for the maximum denitrification rate is equivalent to 0.26, 0.53, and 0.70 % for 30, 60, and 90 days, respectively, of the total grams of C in the 0-20 cm soil profile. Therefore, the hypothesis that tight coupling of denitrification and C use C reserves potentially decreasing soil strength was not validated in this experiment.

CHAPTER 3: MASS BALANCE

FATE OF DIVERTED MISSISSIPPI RIVER NITRATE IN VEGETATED BRACKISH COASTAL MARSH AFTER A SIMULATED DISTURBANCE

3.1 INTRODUCTION

It is well recognized that wetlands within the coastal environment are important for providing nutrient abatement and accumulation of organic matter due to anaerobic conditions in the soil. In coastal wetlands, nitrogen is generally a limiting nutrient. The importance of wetlands as nutrient sinks and coastal buffers is countered by possible adverse effects of eutrophication from intense agricultural practices in the Mississippi River Basin (Lane et al., 1999). The Mississippi River drains 41% of the United States, and as a result of agricultural practices within the Mississippi River drainage basin, the Mississippi River water has an elevated concentration of nitrate, between 1 and 2 mg N L^{-1} (Lane et al., 1999). Agricultural practices in the Mississippi River Basin increase the nutrient load in the river from runoff of ammonium (NH_4^+) and nitrate (NO_3^-). Under aerobic condition in the Mississippi River NH_4^+ undergoes nitrification producing nitrate (NO_3^-). These inorganic forms of nitrogen are in high demand for use in biological processes. Nitrate is used in biological processes such as plant assimilation, microbial immobilization, and denitrification.

In conjunction with possible eutrophication of wetlands from elevated nutrients in the Mississippi River, lack of new sediment and freshwater has resulted in subsidence of wetlands in Louisiana. The Mississippi River levee system was built in the early 1900s to prevent spring flooding of the delta each year. As a result of the levee system, the wetlands around the Mississippi River delta have also been removed from yearly sources of sediments, freshwater, and nutrients. The lack of nutrients and sediments in the Breton Sound Estuary, located southeast of New Orleans, LA, has resulted in deterioration of these wetlands (Delaune et al., 1983).

A proposed restoration tool in the Mississippi River delta is the use of diversions to redirect Mississippi River water into wetlands in Louisiana to simulate yearly spring flooding of

the Mississippi River. In 1991, the Caernarvon diversion was completed to restore annual freshwater, directing up to 226 $m^3 s^{-1}$ (8000 $ft^3 s^{-1}$) of Mississippi River water into the Breton Sound Estuary, LA (Lane et al., 2006). Recent concerns over eutrophication were highlighted after large scale disturbance of fresh and brackish marshes, approximately 100 km^2, in the Breton Sound Estuary was observed after Hurricanes Katrina and Rita (Day et al., 2007). It has been suggested that possible eutrophication of the marshes in Breton Sound Estuary from elevated nitrate from the Mississippi River was the underlying cause of the marsh destruction. In particular, Darby and Tuner (2008a,b) suggest that elevated nitrate in the Mississippi River is causing lower belowground biomass that is more easily damaged from high energy events. Turner and Darby (2008a,b) argue that nutrient loading lessens rooting depth resulting in shallow roots and less root biomass.

Wetlands are effective at removing excess nutrients, especially nitrogen, by assimilation into organic material or by gaseous loss by denitrification. Under anaerobic conditions, nitrate is used as an alternate electron donor by facultative anaerobic bacteria to oxidize organic matter. Denitrification in coastal wetlands is particularly important because conditions are ideal for denitrification to occur. A source of carbon, high primary productivity, and a lack of oxygen are ideal conditions for denitrification. The end product of denitrification is nitrogen gas. Denitrification is an important component of the nitrogen cycle because nitrate is removed from the biosphere. Denitrification occurs at high rates in wetland soils and therefore regulates primary productivity and possible adverse effects of eutrophication (Lane et al., 1999).

The use of the stable isotopic techniques can help clarify the effects of eutrophication from high nitrate concentrations in the Breton Sound Estuary, LA. Nitrogen isotope tracer experiments identify nutrient allocation, specifically, mineralization, immobilization,

nitrification, assimilation, and denitrification processes (Barraclough, 1991). The major pathways for removal of nitrate in the Breton Sound are denitrification and assimilatory nitrate reduction (Reddy and DeLaune, 2008). Other possible pathways for reduction of nitrate in the Breton Sound are dissimilatory nitrate reduction to ammonium (DRNA) (Reddy and DeLaune, 2008), dilution by the Gulf of Mexico or rainwater, phytoplankton uptake, and burial (Day et al., 2003). The addition of labeled nitrate can also be used to detect possible effects of excess nitrate on belowground root biomass. Experiments focused on natural abundance of ^{15}N to determine nitrate removal rates suggest the main removal mechanism for nitrate in wetlands is denitrification at 94% and 89-95%, respectively (Lund et al., 1999; Reinhardt et al., 2006). Possible removal mechanisms in this experiment are denitrification, plant assimilation, immobilization by the microbial pool, assimilation by algal biomass, and soil adsorption.

This study examines the effects of elevated nitrate levels in surface water for *Spartina patens* after a simulated disturbance event occurred. A greenhouse core study planted with *Spartina patens* investigated changes in above and below ground biomass. Removal pathways of nitrate, including denitrification, in the Breton Sound wetlands using labeled nitrogen and mass balance calculations were investigated. We hypothesize that belowground biomass will not be significantly different under elevated water column nitrate concentrations after a disturbance event. Furthermore, we hypothesize the majority of added nitrate is removed by denitrification.

3.2 MATERIALS AND METHODS

3.2.1 Experimental Design

Vegetated soil sections were collected from a brackish marsh located proximal to Delacroix (St. Bernard Parish, Louisiana; 29º44’21.3”N, 89º41’45.6”W) on April 7, 2010. On the day of sampling, the mean daily discharge rate of the Caernarvon Diversion was 1090 ft3/s.

The Caernarvon Diversion began discharging Mississippi River Water into Breton Sound marshes in 1999. The Caernarvon Diversion discharge ranged from 0 to 8940 ft^{-3} s^{-1} during 2010. The area of marsh where soil sections were collected from was approximately 10 miles from the diversion outfall and was characterized as emergent brackish marsh, colonized almost entirely by *Spartina patens*. The vegetated soil sections were removed from the site and transported back to the Wetland and Aquatic Biogeochemistry Laboratory (WABL) at Louisiana State University (LSU) until processing occurred. The following day, the collected vegetated soil sections of marsh were partitioned into 12 equal sizes and fitted into 15.2 cm diameter PVC tubes and placed in a greenhouse on LSU property.

Two groups of 6 (12 total replicate cores) were randomly assigned to one of two nitrate concentration treatment groups, 0.0 (control) or 2.0 mg L^{-1} (treatment). The treatment level of nitrate was chosen based on observed concentrations within the Mississippi River (Lane et al., 1999). The nitrate added was 99% atom ^{15}N (Cambridge Isotope Laboratory, Andover, MA). Twelve flood events occurred during the first six week experimental time and 11 flood events occurred during the second 6 week time period for a total of 23 flood events. Aboveground biomass was clipped at 6 weeks to simulate a non lethal disturbance event similar to other studies (Slocum and Mendelssohn, 2008). Three control and treatment cores each were destructively harvested at the end of 12 weeks. Destructive harvest was clipping aboveground biomass and separating soil into 0-10 cm and 10-20 cm soil sections. Belowground biomass was used for ^{15}N assessment. Harvest of cores at 6 weeks did not occur for two reasons 1) expense of ^{15}N analysis and 2) root separation is very time consuming. Assimilation of ^{15}N into plant biomass was assumed to be constant over time and therefore was divided in half for use in analysis for weeks 1-6 (Chapter 2) and weeks 7-12 (this chapter).

The remaining 6 replicate cores (3 control and 3 treatment cores) received a tenfold increase in nitrate solution (20.0 mg L^{-1}) and aboveground biomass was similarly clipped at 16 weeks to assess plant uptake rates during a disturbance event with very high nitrate concentrations. A 10 cm water column was maintained within each core for the duration of the experiment. Nitrate solution was replaced approximately twice a week for 12 weeks (2 mg N L^{-1}) and 4 weeks (20 mg N L^{-1}) for a total of 23 and 9 flood events, respectively. The nitrate solution was replaced in the water column by manually pouring the water column out of each core. The water column was replaced by first filling each core with dionized water up to the soil surface. Then each core was filled 10 cm above the soil surface with the either dionized water (control) or $^{15}KNO_3$-N solution (treatment).

Water column subsamples were taken at roughly two week intervals sampling two consecutive flooding events for a total of 4 flood events subsampled for each of the experiments. Aboveground biomass was harvested at 6, 12, and 16 week experimental times and then separated into live and dead biomass. Also, at 6, 12, and 16 weeks, scrapings of the top 1 cm of soil from each core were taken to analyze possible use of added nitrate by a thin layer of algae. The remaining three cores from the control and nitrate treatment were harvested destructively at 16 weeks and separated into 0-10 cm and 10-20 cm soil sections. Cores receiving the 10 fold increase in nitrate level were harvested in similar fashion as harvest from the 2 mg L^{-1} study and used for ^{15}N analysis. All samples were stored in the dark at 4ºC until analysis was completed. Temperature in the greenhouse, redox potential (Eh), pH, and conductivity were monitored over the course of the experiment.

The pH was measured using an Accumet® Research AR25 Dual Channel pH/Ion Meter. Redox potential was taken at 5 and 10 cm soil depth in six randomly selected cores, 3 control

and 3 treatment cores. Redox potential was measured using a platinum working electrode and saturated calomel (SCE) reference electrode. A correction factor of +242 was applied to each redox potential measurement to correct for the potential of the calomel reference electrode (Twilley and Nyman, 2005). Conductivity was monitored using an Accument® Basic AB30 Conductivity Meter and converted to salinity using the 0.67 conversion factor.

3.2.2. Water Column, Plant, and Soil Characterization

Water column sub samples were taken at 0, 4, 8, 12, and 24 hours after flooding. Each 20 mL sample was filtered using an Acrodisc® Premium 25mm Syringe Filter with 0.45 µm GHP Membrane. Water samples were stored in the dark at 4ºC until analysis of NO_3^-, NH_4^+, and SRP was completed. NO_3^-, NH_4^+, and SRP were analyzed using a SEAL AQ2 Automated Discrete Analyzer (SEAL Analytical, West Sussex, England; US EPA Methods 353.2 and 350.2 respectively (US EPA, 1983)). Method detective limits for NO_3^-, NH_4^+, and SRP were 0.014, 0.012, and 0.005 mg L^{-1}, respectively. Aboveground biomass was analyzed for total C (TC), total N (TN), total P (TP) and $\delta^{15}N$ by drying separated dead and live biomass at 70ºC until constant weight. Belowground biomass was separated into live, dead, and stems, and were dried at 70ºC until constant weight and analyzed $\delta^{15}N$. Root separation was completed by one person for the duration of the experiment for consistency in determining live versus dead root biomass. Roots that were categorized as live were gold in color, turgid, floated when placed in water, and had the presence of fine root hairs. Roots that were partially decomposed were considered dead. Also, each soil section was analyzed $\delta^{15}N$.

3.2.3 ^{15}N Analysis

Live aboveground plant biomass and dead aboveground plant biomass for harvest at 12 and 16 weeks were sent to the Stable Isotope Laboratory at Woods Hole Oceanographic Institute

for ^{15}N analysis. Live root biomass, dead root biomass, stem root biomass, soil scrapings, and soil samples at 12 and 16 week harvests were also sent to the Stable Isotope Laboratory at Woods Hole Oceanographic Institute for ^{15}N analysis. Analysis was done using a Europa 20-20 CF-IRMS interfaced with the Europa ANCA-SL elemental analyzer. Stable isotope values were used in the mass balance calculation of the nitrogen cycle for this study. Aboveground biomass growth from 7- 12 weeks was used for the isotope content for the mass balance calculation at 12 weeks. Isotope values for the 12 week belowground root biomass and soil were divided in half to assess isotope values during the weeks 7-12, after the disturbance event occurred, and used for the mass balance calculation during this period. Aboveground biomass growth from week 13 to week 16 was used for isotope content or the mass balance calculation at 16 weeks. Isotope values of belowground root biomass and soil for harvest at 16 week were used for the mass balance calculation at 16 weeks. Mass balance for weeks 13-16 represent isotope content with 20 mg N L^{-1} additions for 4 weeks.

Percentage of recovered ^{15}N for each component of a core was calculated by first converting the $\delta^{15}N$ value to the atom % using the following formula:

$$\%\ ^{15}N = 0.000365*\delta^{15}N + 0.0155726 \text{ (Fry, 2006)} \quad \textbf{[Equation 1]}$$

Recovered ^{15}N percentage was calculated by first multiplying % $^{15}N/100$, % nitrogen/100, and total dry weight (mg) together. This value was then divided by total added ^{15}N (mg) for each time period and then multiplied by 100 for the percentage of ^{15}N recovered for each component such that:

$$\%\ \text{Recovered}\ ^{15}N = [((\%\ ^{15}N/100)*(\%\ \text{nitrogen}/100)*(\text{total dry weight}))/^{15}N\ \text{added}]*100 \quad \textbf{[Equation 2]}$$

Percentage of recovered ^{15}N was calculated for live aboveground biomass, dead aboveground biomass, soil scraping, live root biomass, dead root biomass, stem biomass, and soil for each treatment core. Live roots, dead roots, stems and soil % ^{15}N recovered were calculated for 0-10 cm and 10-20 cm sections. The % ^{15}N recovered mean of each component was used for the mass balance calculation as 12 weeks and at 16 weeks.

3.2.4 Data Analysis

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

3.3 RESULTS

3.3.1 Plant Biomass

There was no significant differences in live aboveground biomass when comparing control and treatment cores after a disturbance event occurred and the addition of 2 mg N L^{-1} for 12 weeks (Figure 3.1). Similarly, there was no response in dead aboveground biomass when comparing control and treatment cores at 12 weeks. Live aboveground biomass was significantly different ($p = 0.05$) at 16 weeks, with 20 mg N L^{-1} addition for the last 4 weeks, when comparing control and treatment cores (Figure 3.2). There was no significant difference in the dead aboveground biomass for control and treatment cores at 16 weeks.

3.3.2 Experimental Variables

The nitrate treatment addition to each core was approximately 2 mg $K^{15}NO_3$-N L^{-1} for 12 weeks, however, dilution by pore water occurred. Nitrate concentrations at time zero averaged 1.39 mg KNO_3-N L^{-1} (Figure 3.3). Loss of nitrate within the water column took place in

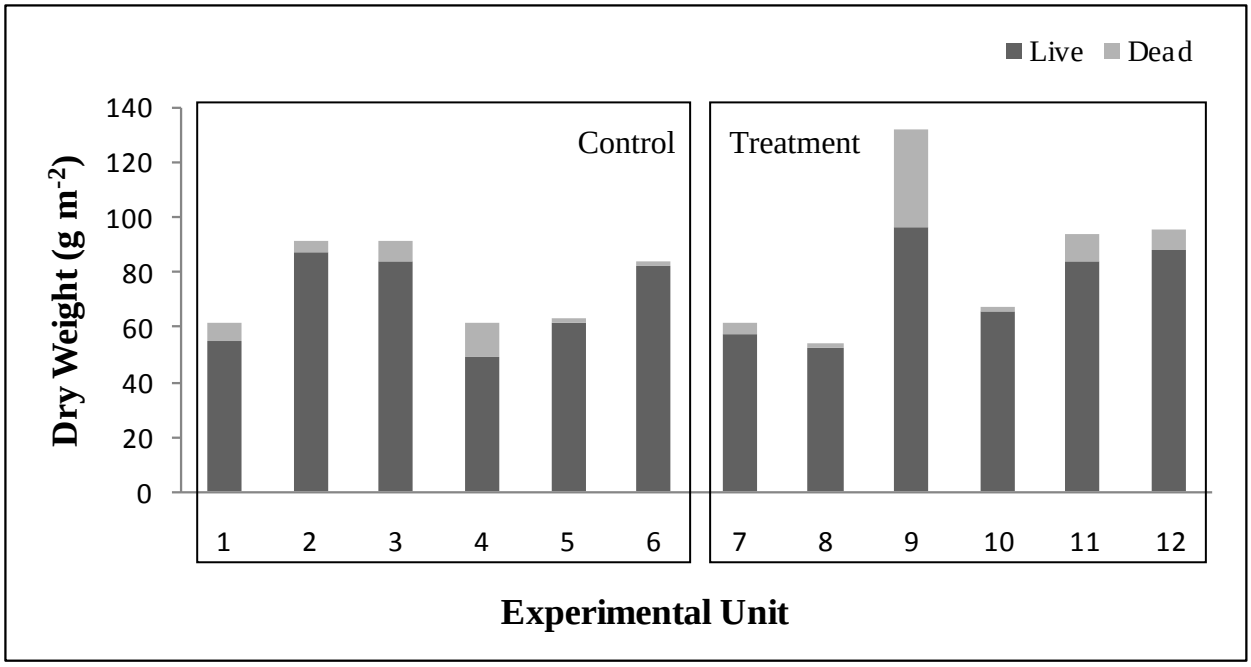

Figure 3.1 Total dry weight of live and dead aboveground biomass for control cores and treatment cores at 12 weeks.

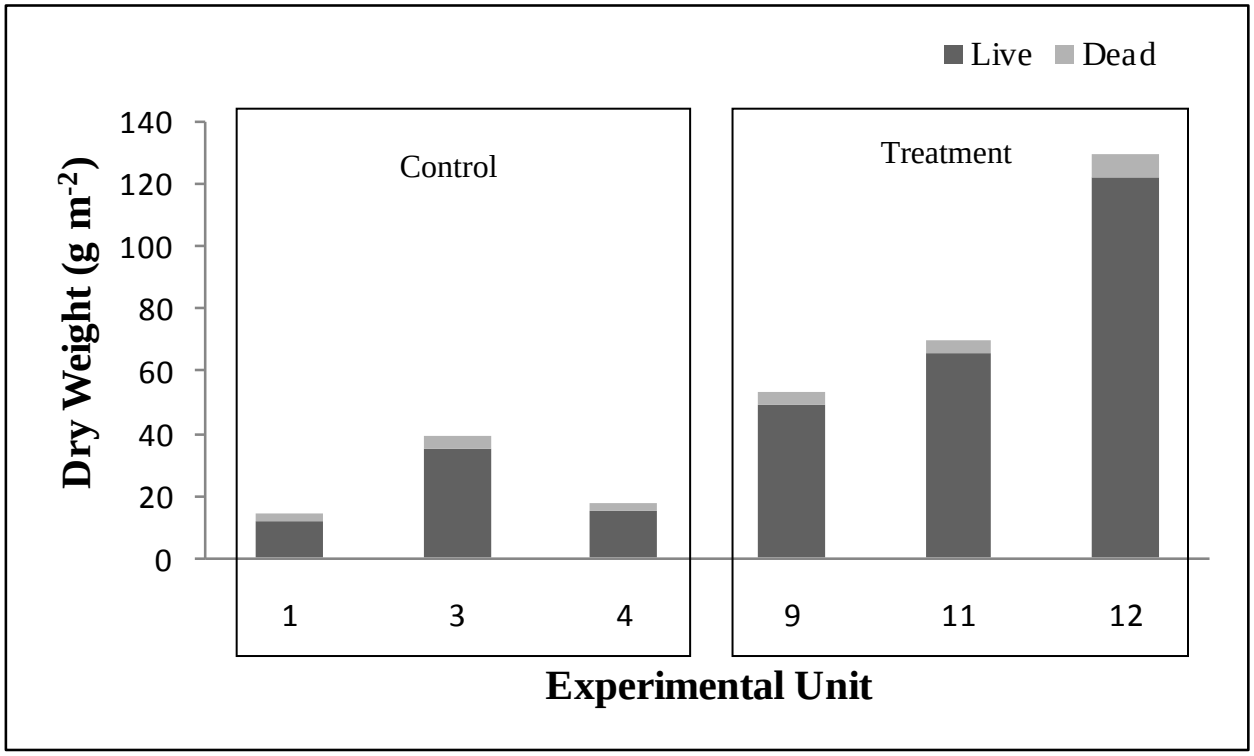

Figure 3.2 Total dry weight of live and dead aboveground biomass for control cores and treatment cores at 16 weeks.

approximately 12 hrs over 4 different flood cycles during the 12 weeks. Denitrification rate remained relatively constant for the 4 flood events sampled over the 12 weeks, ranging from 901 to 1126 mg N m^{-2} day^{-1} (Table 3.1). Temperature in the greenhouse ranged from 32.6 ± 4.8 °C during the experimental time period. Redox potential was similar in each core measured and at each soil depth (5 and 10 cm) averaging -149.27 mV ± 27.04. The average pH was 6.82 ± 0.12 and the average salinity was 0.553 ± 0.18 ppt.

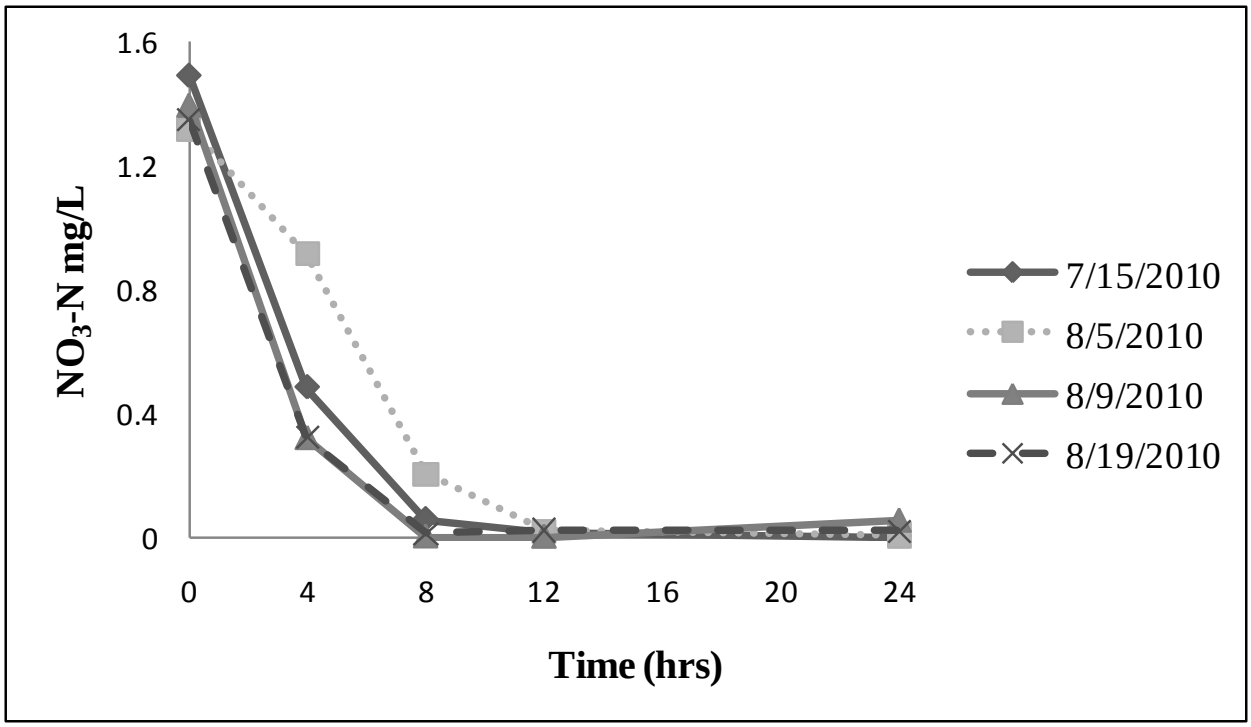

Figure 3.3 Mean water column nitrate concentration for 4 flood events at 12 weeks (n = 6).

Nitrate treatment addition of 20 mg $K^{15}NO_3$-N L^{-1} for 4 weeks was also diluted by pore water. At time zero nitrate concentration averaged 15.41 mg KNO_3-N L^{-1} (Figure 3.4). Loss of nitrate in the water column took place in approximately 24 hours over 4 different flood cycles with 20 mg KNO_3-N L^{-1} additions. Denitrification rate remained relatively constant over the 4 flood events samples at 16 weeks, ranging from 7226 to 8155 mg N m^{-2} day^{-1} (Table 3.2). Temperature in the

greenhouse ranged from 19 to 43°C during the experimental time period. Redox potential was similar in each core measured and at each soil depth (5 and 10 cm) averaging -149.27 mV ± 27.04. The average pH was 6.82 ± 0.12 and the average salinity was 0.553 ± 0.18 ppt.

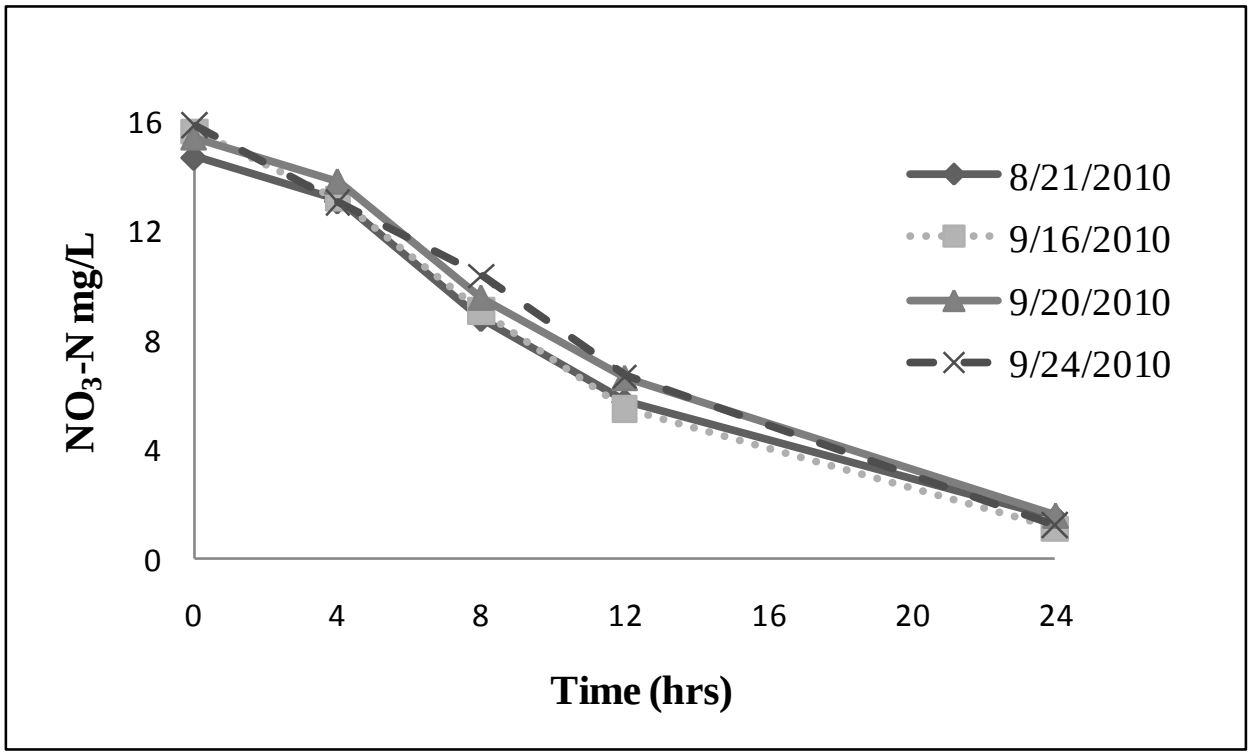

Figure 3.4 Average water column nitrate concentration of 4 flood events with 20 mg N L^{-1} additions (n = 6).

Table 3.1 Denitrification rate over 4 flood events at 12 weeks.

Core Number	Denitrification Rate (mg N m^{-2} day^{-1})			
	7/15/2010	8/5/2010	8/9/2010	8/19/2010
1	1076	545	791	729
2	1209	1097	1049	1130
3	1139	1085	1122	1270
4	1250	988	1344	1235
5	1077	762	1163	1031
6	1006	929	1098	889
Mean ± stdev	1126 ± 91	901 ± 213	1095 ± 180	1047 ± 209

Table 3.2 Denitrification rate over 4 flood events at 16 weeks.

Core Number	Denitrification Rate (mg N m^{-2} day^{-1})			
	8/21/2010	9/16/2010	9/20/2010	9/24/2010
1	7381	7383	8552	8345
2	7947	8173	6988	8677
3	6354	8638	7711	7443
Mean ± stdev	7227 ± 808	8065 ± 635	7750 ± 782	8155 ± 638

3.3.3 Percent ^{15}N Recovery

A total of 39.82 mg $^{15}NO_3$-N was added to each core 6 weeks after the disturbance event took place. The average % ^{15}N recovery for each component is shown in Figure 3.5. The soil scraping, 10-20 cm belowground biomass, and 10-20 cm soil section accounted each for less than 1 % of added ^{15}N. The 0-10 cm live roots, 0-10 cm dead roots and, 0-10 cm stem each accounted for approximately 1 % of the added ^{15}N. Aboveground biomass (live + dead) represents 27 % of the added ^{15}N. The largest component of the mass balance was 65 % that was unaccounted for by gaseous losses.

External and internal N sources were calculated for each core component to compare the main N sources in the Breton Sound with 2 mg N L^{-1} treatment for 6 weeks. Added labeled nitrate represented external N sources and N mineralization represented internal N sources. The soil and plant biomass, both above and below ground biomass, only recovered 35 % of the total added labeled N (Figure 3.5). The remaining 65 % of N in all of the plant and soils components then has to come from internal N source by N mineralization. External N accounted for 21 mg N and internal N accounted for 2578 mg N for this time period (Table 3.3). External N from added labeled nitrate was only 0.79 % if the total N recovered N over 12 weeks. Internal N was 99.2 % of the total N over the 6 weeks experimental time with 2 mg N L^{-1} addition.

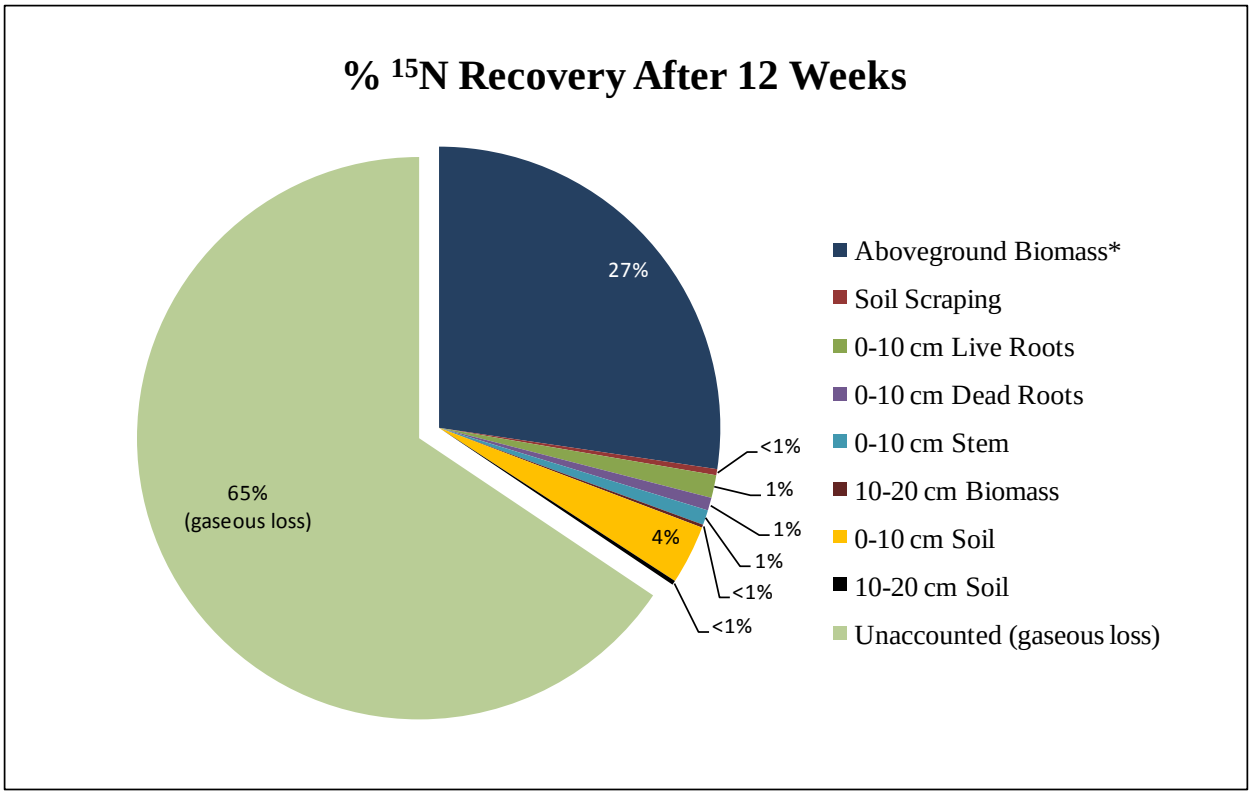

Figure 3.5 Mass balance of labeled nitrate addition after 12 weeks of 2 mg N L^{-1} for above and belowground components, represented as % of recovered ^{15}N in each component (*n = 6 for aboveground biomass, all other components n = 3).

A total of 325.80 mg $^{15}NO_3$-N was added to the remaining three control and treatment cores during the 4 weeks of 20 mg N L^{-1}. The 0-10 cm live roots and 10-20 cm belowground biomass both recovered less than 1 % of added ^{15}N (Figure 3.9). Soil scraping, 0-10 cm stems, and 10-20 cm soil accounted for 1 % each of the added ^{15}N. After 4 weeks at a 10 fold increase in nitrate concentration, 16 % of ^{15}N was recovered in the aboveground (live + dead) biomass. Unaccounted for gaseous losses were 74 % of the added ^{15}N during this experimental time period.

External and internal N sources were calculated for each core component to compare main N sources in the Breton Sound under the 20 mg N L^{-1} treatment for 4 weeks. Similarly to the 2 mg N L^{-1} treatment, labeled nitrate represented external N sources and N mineralization

Table 3.3 External and Internal N sources over 12 weeks for above and below ground components receiving 2 mg N L^{-1} for the 0-20 cm soil section. Data are mean values ± standard deviation. (*n = 6 for aboveground biomass and soils scraping, all other components n = 3).

Experimental Component	mg ^{15}N	mg ^{14}N
Live Aboveground*	9.7 ± 2.1	84 ± 16
Dead Aboveground*	0.66 ± 0.89	6.1 ± 6.8
Live Roots 0-10 cm	2.2 ± 0.21	20 ± 1.5
Dead Roots 0-10 cm	2.1 ± 0.70	128 ± 6.8
Stem Roots 0-10 cm	1.3 ± 0.75	44 ± 6.9
Soil 0-10 cm	4.6 ± 1.4	901 ± 175
Live Roots 10-20 cm	0.01 ± 0.02	5.8 ± 5.1
Dead Roots 10-20 cm	0.25 ± 0.26	227 ± 101
Stem Roots 10-20 cm	0.05 ± 0.04	21 ± 16
Soil 10-20 cm	0.45 ± 0.14	1123 ± 369
Soil Scraping*	0.30 ± 0.07	19 ± 9.7
Total N	21	2578
% of Total N	0.79	99.2

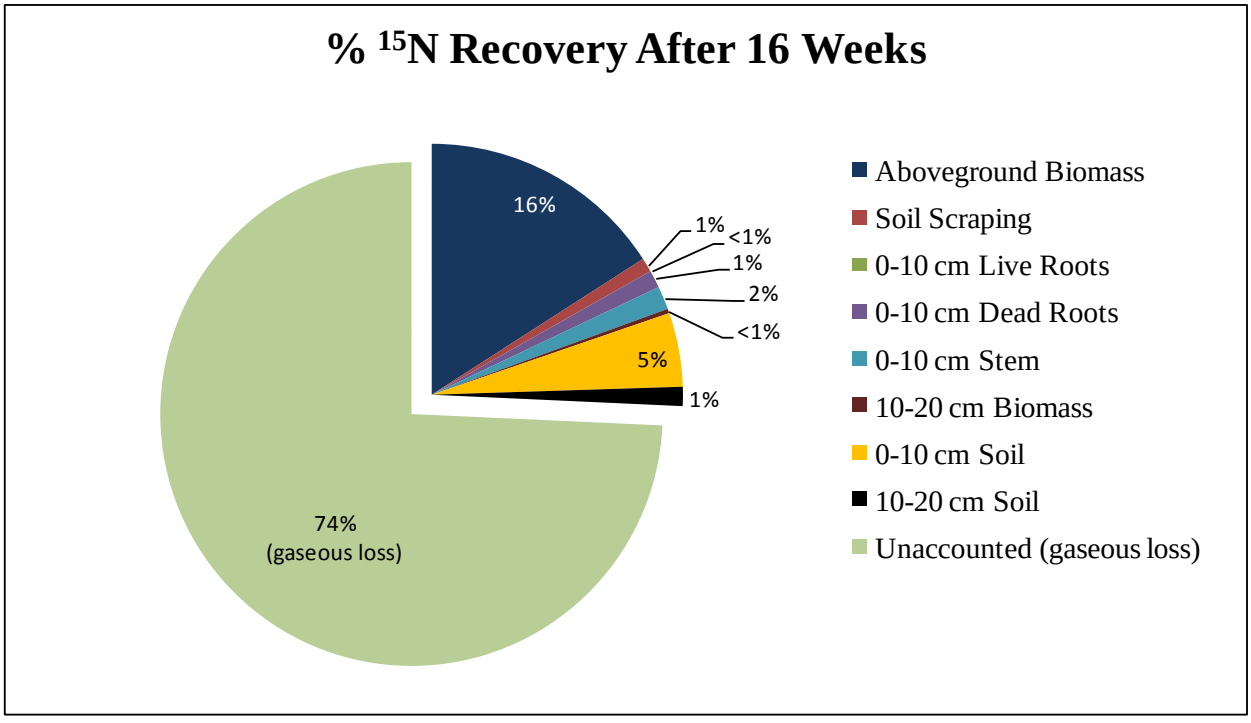

Figure 3.6 Mass balance of labeled nitrate addition after 16 weeks (4 weeks of 20 mg N L^{-1}) for above and belowground components, represented as % of recovered ^{15}N (n = 3).

represented internal N sources. The soil and plant biomass, both above and below ground biomass, only recovered 26 % of the total added labeled N (Figure 3.6). The remaining 74 % of N in all of the plant and soils components then has to come from internal N source. External N accounted for 169 mg N and internal N accounted for 3266 mg N for this time period (Table 3.4). External N from added labeled nitrate was only 4.9 % if the total N recovered N over 4 weeks. Internal N was 95.1 % of the total N over the 4 weeks experimental time with 20 mg N L^{-1} additions

Table 3.4 External and Internal N sources over 16 weeks for above and below ground components receiving 20 mg N L^{-1} for the 0-20 cm soil section. Data are mean values ± standard deviation (*n = 1 for aboveground biomass and soils scraping, all other components n = 3).

Experimental Component	mg ^{15}N	mg ^{14}N
Live Aboveground	29 ± 10	90 ± 27
Dead Aboveground*	0.41	2.5
Live Roots 0-10 cm	0.19 ± 0.02	36 ± 0.33
Dead Roots 0-10 cm	0.68 ± 0.06	147 ± 8.1
Stem Roots 0-10 cm	0.37 ± 0.30	49 ± 23
Soil 0-10 cm	7.1 ± 1.1	1564 ± 270
Live Roots 10-20 cm	0.05 ± 0.02	1.8 ± 0.63
Dead Roots 10-20 cm	25 ± 4.9	188 ± 22
Stem Roots 10-20 cm	0.12 ± 0.02	11 ± 5.2
Soil 10-20 cm	85 ± 87	1144 ± 107
Soil Scraping	2 1± 9.6	32 ± 6.3
Total N	169	3266
% of Total N	4.9	95.1

3.4 DISCUSSION

There were no significant differences in the aboveground biomass for with 2 mg N L^{-1} additions for 12 weeks. There was a significant difference in live aboveground biomass with 20

mg N L^{-1} additions. The difference in live aboveground biomass was not unexpected given a tenfold increase in nitrate additions occurred during this time period.

The denitrification rates with 2 mg NO_3-N L^{-1} and 20 mg NO_3 -N $L^{-}1$ additions were consistent throughout the 6 week and 4 weeks experiment time at approximately 12 hrs and 24 hrs, respectively, suggesting that gaseous losses (denitrification or ammonia volatilization) was occurring in response to nitrate additions. Ammonium concentrations in the water column in both nitrate treatment concentration over the flood event were at or below detection limits suggesting that ammonia volatilization was a minimal process for removing added nitrate. Also, mean pH of 6.82 ± 0.12 suggests high pH needed for ammonia volatilization was not present. Redox conditions (-149 ± 27 mV) indicated environmental conditions were available for denitrification to occur (Patrick et al., 1996) for both 2 mg NO_3-N L^{-1} and 20 mg NO_3-N L^{-1} treatments. The presence of anaerobic conditions, nitrate, and carbon sources advocate that denitrification was removing excess nitrate 12 hrs after the addition of 2 mg NO_3- N L^{-1} and 24 hrs after the addition of 20 mg NO_3-N L^{-1}.

A tenfold increase in nitrate concentration doubled the removal time from 12 to 24 hrs, even after a disturbance event took place. The rate of 20 mg NO_3-N L^{-1} was consistent in each of the 4 flood events where subsamples were taken. This suggests that added nitrate was removed by denitrification. Ideal conditions for denitrification to occur were present during the 4 weeks that 20 mg NO_3-N L^{-1} suggesting denitrification was the main removal mechanism for excess nitrate in this experiment. Live aboveground biomass was significantly different comparing control and treatment core with 20 mg N L^{-1} additions. This was not unexpected as the nitrate concentration was 10 times greater during this experimental time period.

Percentage of recovered ^{15}N in the above and belowground biomass in both the 2 mg NO_3-N L^{-1} and 20 mg NO_3-N L^{-1} experiments support the conclusion that excess nitrate was removed by denitrification or other gaseous losses. If assimilation by plants was an important process in removing excess nitrate, then above and belowground biomass should have a large percentage of ^{15}N incorporated in the biomass. Live roots in the 0-10 cm soil section assimilated only 1 % of added labeled nitrate after 12 weeks of 2 mg NO_3-N L^{-1} additions. In total, for the same time period and nitrate concentration, belowground biomass in the 0-10 cm soil sections assimilated only 3 % of the added labeled nitrate into biomass. Aboveground biomass for the 2 mg NO_3^- -N L^{-1} treatment assimilated 27 % of the added labeled nitrate after the disturbance event took place. However, 65 % of the added labeled nitrate was unaccounted for by gaseous losses. Similar results occurred in the cores receiving 20 mg NO_3 -N L^{-1} for 4 weeks. Live belowground biomass accounted for less than 1% and total belowground biomass for soil section 0-10 cm was 4 %. Live aboveground biomass was 16 % after 4 weeks of 20 mg NO_3 -N L^{-1} and unaccounted losses of added nitrate was 74 %. The hypothesis that eutrophication of live belowground biomass in coastal marshes receiving high nitrate Mississippi River water appears to be unsubstantiated based on the small percentage of recovered ^{15}N in the live belowground biomass.

Calculations of external and internal N sources for both the 2 mg N L^{-1} and 20 mg N L^{-1} verify that external labeled nitrate was not the main source of N assimilated into plant biomass. Internal N sources from N mineralization, it turns out, was the main N source assimilated into plant biomass. External N in the 2 mg N L^{-1} treatment was 3.5 % of the total N for plant and soil components and internal N sources was 96.5 % for the plant and soil components. Remarkably, external and internal N percentages were very similar in the 2 mg N L^{-1} and 20 mg N L^{-1}

treatments. In the 20 mg N L^{-1} treatment, the external N was 3.4 % and the internal N was 96.6 % of the total N for plant and soil components. External and internal sources of N further confirm that excess nitrate is not negatively impacting root biomass.

No significant differences in TC or MBC between control and treatment cores indicating that an increase in the soil microbial pool did not occur. This is contrary to other studies that suggest the addition of nitrate laden Mississippi River water is increasing soil metabolism and decreasing marsh stability (Howes et al., 2010; Turner, 2010; Turner et al., 2009). Using an accumulation rate of 301 g C m^{-2} (DeLaune and White, in press) for 20 years, a 0-20 cm soil section has 6020 g C m^{-2}. The denitrification rate during the 12 weeks was found by multiplying the total N added during the experiment, then dividing by meter square equivalent of the core and the number of experimental days. Finally, this value was multiplied by the gaseous loss component of the mass balance (Figure 3.5), so that the denitrification rate was 34.24 mg N m^{-2} day^{-1}. The total N needed for 30, 60 and 90 days is 1.03, 2.05, and 3.08 g N m^{-2}, respectively. Solving simultaneous stoichiometric equations for the conversion of nitrate to N_2 gas and glucose to CO_2 shows that for every 4 g of N, 5 g of C are needed.

$$2(12H^+ + 10e^- + 2NO_3^- \longrightarrow N_2 + 6H_20)$$

$$\underline{5(H_2O + CH_2O \longrightarrow CO_2 + 4H^+ + 4e^-)}$$

$$4\ NO_3^- + 5CH_2O \longrightarrow 2N_2 + 5CO_2$$

Accordingly, for 30, 60, and 90 days the amount of C needed for denitrification to take place is 1.29, 2.56, and 3.85 g C m^{-2}, respectively. This is equivalent to 0.02, 0.04, and 0.06 % of the total C (6020 g C m^{-2}) calculated for this experiment over 30, 60, and 90 days, respectively. These carbon calculations do not take into account carbon accumulation that would also occur.

DeLaune and White (2011) report carbon accumulation rates for fresh marshes in LA between 219-301 g C m^{-2} $year^{-1}$.

This same calculation can be done with the 20 mg N L^{-1} additions to compare the carbon use with a 10 fold increase in nitrate addition. Using the denitrification rate of 0.48 g N m^{-2} day^{-1} with 20 mg N L^{-1} additions for 4 weeks, total N needed for 30, 60 and 90 days is 14.4, 28.8, and 43.2 g N m^{-2}, respectively. Using the same stoichiometry, for every 4 g of N, 5 g of C is needed. Therefore, for 30, 60, and 90 days the amount of C needed for denitrification to take place in this time period is 18.0, 36.0, and 54.0 g C m^{-2}, respectively. This is equivalent to 0.3, 0.6, and 0.9 % of the mean total C (6020 g C m^{-2}) over 30, 60, and 90 days, respectively.

3.5 CONCLUSION

Our results indicate that gaseous losses are the main removal mechanism for diverted Mississippi River nitrate, not plant assimilation, as loss of added nitrate occurred in 12 hours for the 2 mg NO_3-N L^{-1} treatment and 24 hours in the 20 mg NO_3-N L^{-1} treatment. Gaseous losses include denitrification and ammonia volatilization. Denitrification is the most likely removal pathway given low redox conditions observed in cores, the presence of excess nitrate, and concentrations of NH_4^+ at or below detection limits. This study also confirms that assimilation of excess nitrate into live root from the Mississippi River does occur; however, 6 weeks after a disturbance event took place, only 1% of added labeled nitrate was assimilated into live root biomass with 2 mg NO_3-N L^{-1} addition. Four weeks after a disturbance event took place, less than 1% of added labeled nitrate was recovered in the live roots for 0-10 cm soil section with 20 mg NO_3-N L^{-1} addition. This suggests that diverted Mississippi River water nitrate that is received by coastal marshes in the Breton Sound Estuary is not affecting belowground biomass by releasing root foraging in the soil profile by N limitation.

Also, no significant differences in total live root biomass at either the 0-10 cm or 10-20 cm soil sections indicate there was no effect seen in total live root amounts or rooting depth by the addition of excess nitrate in this experiment. Calculations of external and internal N sources in plant biomass at 2 mg N L^{-1}, at 21 mg N (0.79 %) and 2578 mg N (99.2 %), respectively, support the conclusion that N mineralization is the main source of N for plant assimilation in the Breton Sound. Similar external and internal N calculations for the 20 mg N L^{-1} treatment further support N mineralization as the main N source for plant assimilation. External N accounted for 169 mg N (4.9 %) and internal N accounted for 3266 mg N (95.1 %) with 20 mg N L^{-1}. As a result, the hypothesis that excess nitrate is affecting belowground root biomass in Breton Sound Estuary was not confirmed in this experiment.

Furthermore, results indicate that even though denitrification was the main removal mechanism for excess nitrate, carbon reserves were not significantly affected as indicated by the lack of significant differences in TC or MBC when comparing control and treatment cores. Stoichoimetric calculations of grams of C needed for the maximum denitrification rate for 2 mg N L^{-1} is equivalent to 0.27, 0.54, and 0.80 % for 30, 60, and 90 days, respectively, of the total C in the 0-20 cm soil profile. Therefore, the hypothesis that tight coupling of denitrification and C use C reserves and potentially decrease soil strength was not validated in this experiment. This conclusion is further substantiated by stoichoimetric calculations of grams of C needed for maximum denitrification rate for 20 mg N L^{-1} equivalent to 1.56, 3.13, and 4.69 % for 30, 60, and 90 days, respectively, of the total C in the 0-20 cm soil profile.

CHAPTER 4: CORE STUDY

DENITRIFICATION RATE COMPARISON BETWEEN A BAYOU SEDIMENT AND MARSH SOIL

4.1 INTRODUCTION

Coastal wetlands play an important role in the coastal environment by providing nutrient abatement and organic matter accumulation as anaerobic conditions in the soil are present. Nitrogen is generally the limiting nutrient in coastal environments. The importance of coastal wetlands as nutrient sinks is countered by possible eutrophication by intense agriculture as the Mississippi River basin drains 41 % of the United States (Lane et al., 1999). Elevated nitrate from agricultural practices in the drainage basin has resulted in hypoxic conditions in the northern Gulf of Mexico (GOM) (Rabalais et al., 2002). Proposed restoration tools in the lower Mississippi River are diversions that direct Mississippi River water high in nitrate in to coastal wetlands. However, recent concerns with possible eutrophication of coastal marshes receiving nitrate laden Mississippi River water (Howes et al., 2010; Turner, 2010) have been suggested following large scale disturbance of fresh and brackish marshes (100 km^2) that were observed in the Breton Sound Estuary, LA following Hurricanes Katrina and Rita (Lane et al., 2006).

The Caernarvon Diversion is one of several large scale restoration projects located southeast of New Orleans, LA, which meters Mississippi River water into the Breton Sound Estuary. The wetlands in this estuary are deteriorating as a result of salt water intrusion, lack of new sediment, subsidence, and sea level rise. The goal of this restoration project is to decrease salt water intrusion as a way to improve oyster harvest in the Breton Sound and at the same time improve deteriorating marshes in the sound (2003).

The Caernarvon Diversion began distributing Mississippi River water into the Breton Sound in 1991. The maximum discharge rate of this diversion is 226 $m^3 s^{-1}$ (8,000 $ft^3 s^{-1}$). If the discharge rate of the diversion is less than 4,000 $ft^3 s^{-1}$, most of the metered water remains in channels and bayous as the water moves through the estuary and flows into the Gulf of Mexico.

If this occurs, residence time decreases and marsh soil water interface is lost. This loss results in a decrease in nitrate removal efficiency. If the discharge rate of the diversion is greater than 4,000 $ft^3 s^{-1}$, flow rerouted off the Mississippi River is sufficiently high to flood wetlands in the Breton Sound Estuary.

Nitrate removal depends on several factors. Residence time (Reddy and DeLaune, 2008) and loading rate (Lane et al., 2003) are the main factors in the effectiveness of nitrate removal in wetlands. Maximum removal rate occur at low flow from the Caernarvon Diversion that results in long residence times. Also, low nitrate loading results in maximum removal efficiency. The sediment water interface is important for nitrate removal. The more surface area of wetlands diverted water comes into contact with, the greater nitrate removal occurs. Removal of nitrate occurs by assimilation into plant or algal biomass, denitrification, or burial (Lane et al., 2003).

This experiment was designed to investigate changes in net nitrate removal under different discharge rates. As second question investigated was the importance of plants in nitrate removal. Discharge rates investigated were less than 4000 $ft^3 s^{-1}$ and greater than 4000 $ft^3 s^{-1}$. Discharge rates less than 4000 $ft^3 s^{-1}$ are sufficiently low for Mississippi River water to remain in the channel. Discharge rates greater than 4000 $ft^3 s^{-1}$ are great enough to allow flooding of the marshes. This is an important nutrient removal question as marshes in the Breton Sound Estuary receive Mississippi River water with elevated nitrate concentrations only when discharge rates exceed 4000 $ft^3 s^{-1}$.

This study examines the possible differences in nitrate removal capacity of bayou sediments and marsh soils. A laboratory study using bayou sediment and marsh soil was used to investigate the rate of net nitrate loss when excess nitrate is added. Removal of nitrate in the Breton Sound Estuary was investigated using nitrate addition and monitoring concentration over

time. We hypothesize that a decrease in nitrate concentration over time will occur faster in the marsh soil than in bayou sediment. Furthermore, we hypothesize that this decrease in excess nitrate from the Mississippi River is being removed by denitrification and lost from wetlands in the Breton Sound estuary.

4.2 MATERIALS AND METHODS

4.2.1 Experimental Design

Two five gallon buckets, one bucket each of marsh soil and bayou sediment, were collected from Delacroix (St. Bernard Parish, Louisiana; 29°44.932'N, 89°47.861') on January 28, 2011. This site is approximately 16 km from the diversion outfall. Soil was collected proximal to the bayou and is classified as marsh soil in this study. Sediment was collected in the canal adjacent to the marsh soil site. Sediment was collected on the canal bottom at equal distance from either vegetated side and is classified at bayou sediment in this study. Sediment in the canal bottom was collected using a hand dredge, while marsh soil was collected using a shovel. Water depth in the canal was approximately 8 feet deep with no flood water apparent in the adjacent marsh. On the day of sample collection, the mean daily discharge rate of the Caernarvon Diversion was 1270 ft^3 s^{-1} The Caernarvon Diversion began discharging Mississippi River water into the Breton Sound in 1991. The discharge range for January – April 2011 was 464 – 4750 cfs. The marsh are where soil and sediment collection took place was characterized as emergent brackish marsh and colonized as *Spartina patens*. Soil and sediment were transported back to the Wetland and Aquatic Biogeochemistry Laboratory (WABL) at Louisiana State University (LSU) and refrigerated at 4 ºC until processing occurred.

Bayou sediment was prepared for use in cores by removing large roots and blending to produce a homogenous sample. Blended soil was then placed into 8-10.2 cm diameter PVC pipe

to a depth of 10 cm. Two groups of 4 cores (8 total replicate cores) were randomly assigned to one of two nitrate treatment concentration groups, either 0.0 or 2.0 mg NO_3-N L^{-1}. The 2 mg NO_3-N L^{-1} concentration was chosen based on nitrate level in the Mississippi River (Lane et al., 1999). Each core was flooded with either 0.0 or 2.0 mg NO_3-N L^{-1} and the change in nitrate and ammonium concentrations were monitored over 9 days. Marsh soil was prepared in similar fashion as the bayou sediment, for a total of 2 sets of 8 replicate cores (8 marsh soil and 8 bayou sediment). Cores were placed in a water bath to maintain a consistent temperature and kept in the dark to prevent the growth of algae. Water column subsamples were taken approximately once a day for the duration of the flood event. Cores were destructively harvested at the end of experiment by removal of the entire sediment core. All samples were stored in the dark at 4°C until analysis was completed. Conductivity, redox potential, pH, and dissolved oxygen were monitored once at the beginning and once at the end of the experiment. Conductivity was converted to salinity using the 0.67 conversion factor.

The pH was measured using an Accumet® Research AR25 Dual Channel pH/Ion Meter. Redox potential was taken at approximately 5 cm soil depth in 8 selected cores, 2 control and 2 treatment cores for each of the bayou sediments and marsh soils. Redox potential was measured using a platinum working electrode and saturated calomel (SCE) reference electrode. A correction factor of +242 was applied to each redox potential measurement (Twilley and Nyman, 2005). Conductivity was monitored using an Accumet® Basic AB30 Conductivity Meter and converted to salinity using the 0.67 conversion factor. Dissolved oxygen (DO) was measured using an Accumet® Research AR40 Dissolved Oxygen Meter.

4.2.2 Soil Characterization

Five subsamples of each the bayou sediment and marsh soil were used for soil/sediment characterization. The following characteristics were measure for all subsamples, moisture content, total C (TC), total N (TN), KCl extractable NH_4-N, KCl extractable NO_3^-, total P (TP), potentially mineralizable nitrogen (PMN), microbial biomass C (MBC), and microbial biomass N (MBN). Soil moisture was determined by drying a wet subsample at 70°C to constant weight. Dried ground soil subsamples were analyzed for TC and TN using an Elemental Combustion System with a detection limit of 0.005 g kg^{-1} (Costech Analytical Technologies. Inc., Valencia, CA). Extractable NH_4-N was measured using on 25 mL 2M KCl soil extractant. Extractable NH_4-N was analyzed on a SEAL AQ2 Automated Discrete Analyzer (SEAL Analyzer, West Sussex, England; US EPA Methods 353.2 (US EPA, 1983). Method detection limits for NH_4-N was 0.012 mg L^{-1}. PMN was determined on 25 mL 2M KCl soil extracts after incubation of 0, 2, 8, and 10 days at 40°C. PMN subsamples were subjected to the same EPA methods on the SEAL AQ2 Automated Discrete Analyzer for determination of the extractable NH_4-N. The PMN rate was calculated as the increase in NH_4-N over time by regression.

Microbial biomass C and N were calculated using the chloroform fumigation-extraction method (Brookes et al., 1985; Sparling et al., 1990) . Two sets of triplicate 5 g wet weight samples were prepared in 25 ml centrifuge tubes. One set was used for non-fumigate samples and the other set was used for fumigate samples. Non-fumigate samples were measured using soil extractant (25 ml of 2 M HCl), shaken for 30 minutes then centrifuged. The supernatant was filtered through 47 mm Whatman filter paper and stored in the dark at 4ºC until analysis was completed. Analysis of the supernatant included total organic carbon (TOC) and total organic nitrogen (TON) using a Shimadzu Scientific Instrument TOC-VCSN, Columbia, MD. Fumigated

triplicates were placed in a desiccator with 0.5 ml chloroform added to each centrifuge tube as well as a beaker with approximately 50 ml of chloroform with 5-10 boiling stones. The air within the desiccator was removed and refilled three times. The fourth time, the desiccator was sealed by evacuatation and placed in the fume hood for 24 hours. After 24 hours, the chloroform was removed by evacuating the head space at least seven times. After fumigation, this set of triplicates was extracted using the same procedure as the non-fumigate triplicates. MBC and MBN was calculated by subtracting the non-fumigate samples from the fumigate samples.

TP was calculated using the TP ashing method after Andersen (1976) for sediment and soil samples. Dried ground sediment and soil samples were prepared in a 50 mL beaker using 0.5 g dried soil weight at between 0.2g and 0.3g dried material weight. Triplicate samples occurred 10% of the time, with an external peach leaf standard and a blank for each set. Samples were ashed using a muffle furnace (Barnstead Thermolyne 62700 Furnace) at 550°C for 4 hours. Samples were reweighed after burning to determine loss on ignition (LOI). The ashed samples were then moistened using ~ 2 mL of dionized water before the addition of 20 mL of 6 M HCl. Each sample was placed on a 100 °C hot plate until dry. Additional 2.25 mL of 6 M HCl was added and brought to a near boil. Samples were filtered through Whatman #41 filter paper into 50 mL volumetric flasks. Samples were stored at room temperature until analysis was completed. TP was analyzed using a SEAL AQ2 Automated Discrete Analyzer (SEAL Analytical, West Sussex, England; US EPA Methods 353.2 and 350.2 respectively (US EPA, 1983)). The method detection limit for TP was 0.05 mg P L^{-1}.

4.2.3 Data Analysis

The effect of nitrate addition between control and treatment cores for the bayou sediment and marsh soil was determined using a student t-test ($P < 0.05$). Data normality was determined

using the Kolmogorov-Smirnov test ($\alpha = 0.01$). Data was log-transformed to fit a normal distribution when necessary. Soil properties analyzed include bulk density, % moisture, TC, TN, TP, loss on ignition (LOI), MBC, MBN, and extractable NH_4^+ for each soil section.

4.3 RESULTS

4.3.1 Soil Properties

Mean % moisture was 80 ± 0.25 % for the bayou and 81 ± 0.13 % for the marsh (Table 4.1). TC, TN, TP, and LOI were all significantly higher in the marsh soil at 165 ± 1.79 g C kg^{-1}, 11 ± 0.10 g N kg^{-1}, 676 ± 6.85 mg P kg^{-1}, 32 ± 0.31 %, respectively. While the bayou sediment, TC, TN, TP, LOI were 105 ± g C kg^{-1}, 7.0 ± 0.66 g N kg^{-1} and 589 ± 12.2 mg P kg^{-1}, respectively. MCB and MBN were significantly different in the bayou sediment and marsh soil. However, MBC was higher and MBN was lower in the marsh soil. MBC was 6.94 ± 0.30 g C kg^{-1} in the bayou sediment and 8.04 ± 0.40 g C kg^{-1} in the marsh soil. MBN was 35 ± 14 mg N kg^{-1} in the bayou sediment. 20 ± 5.8 mg N kg^{-1} in the marsh soil. Extractable NH_4-N was about 2.3 times higher in the marsh soil than the bayou sediment at 330 ± 7.17 mg kg^{-1} and 145 ± 8.58 mg kg^{-1}, respectively. The PMN rate was not significantly different at 9.63 mg kg^{-1} day^{-1} for the bayou sediment and 9.42 ± 1.62 mg kg^{-1} day^{-1} for the marsh soil.

4.3.2 Soil Properties Relationships

PMN in the bayou sediment was positively correlated with % moisture and TC (r = 0.96, r = 0.95, respectively; Table 4.2) and negatively correlated with TN and TP (r = -1.00, r = -0.89, respectively). No correlation between TC and % moisture may be an artifact of homogenizing the sediment and repacking of bayou sediment into cores. In the marsh soil, TC was negatively correlated with % moisture (r = -0.98, n = 5; Table 4.3). Extractable NH_4^+ increased as TP increased (r = 0.96). PMN was positively correlated with TP, MBC, and extractable NH_4^+ (r =

1.00, r = 0.95, and r = 0.98, respectively). The MBC:MBN ratio for the bayou sediment was lower than the marsh soil, at 0.2 and 0.4, respectively, suggesting the bayou sediment is more N limited than the marsh soil.

Table 4.1 Soil characteristics for bayou sediment and marsh soil. Data are mean values (˜ n = 2 for bayou PMN and n = 3 for marsh PMN, all other components n = 5) ± standard deviation. Difference letters indicate significant differences between columns at p = 0.5. *Indicates extraction by 2 M KCl

Soil Parameter	Units	Bayou	Marsh
% Moisture	**%**	80 ± 0.25^{a}	81 ± 0.13^{b}
TC	**g kg^{-1}**	105 ± 8.69^{a}	165 ± 1.79^{b}
TN	**g kg^{-1}**	7.0 ± 0.66^{a}	11 ± 0.10^{b}
TP	**mg kg^{-1}**	589 ± 12.2^{a}	676 ± 6.85^{b}
LOI	**%**	22 ± 0.53^{a}	32 ± 0.31^{b}
MBC	**g kg^{-1}**	6.94 ± 0.30^{a}	8.05 ± 0.399^{b}
MBN	**mg kg^{-1}**	35 ± 14^{a}	20 ± 5.8^{b}
NH_4-N*	**mg kg^{-1}**	145 ± 8.58^{a}	330 ± 7.17^{b}
PMN˜	**mg kg^{-1} day^{-1}**	9.63	9.42 ± 1.62
TC:TN		15	14

Table 4.2 Product-moment correlation coefficients for bayou sediment characteristics. Bold indicates significance at P < 0.05 (*n = 3 for PMN, all other components n = 8, r = 0.63).

	% Moisture	TC	TN	TP	LOI	MBC	MBN	NH4
TC	0.00							
TN	-0.54	**0.79**						
TP	-0.62	-0.27	0.01					
LOI	**0.65**	-0.41	**-0.70**	**-0.65**				
MBC	-0.36	0.57	**0.83**	-0.04	**-0.71**			
MBN	-0.25	0.13	0.30	-0.54	0.37	0.03		
NH4	-0.01	**0.63**	0.43	-0.49	0.22	-0.04	**0.71**	
PMN	**0.96**	**0.95**	**-1.00**	**-0.89**	**0.72**	**-0.69**	-0.04	0.58

Table 4.3 Product-moment correlation coefficients for marsh soil characteristics. Bold indicates significance at $P < 0.05$ (*n = 3 for PMN, all other components n = 8, r = 0.63).

	% Moisture	TC	TN	TP	LOI	MBC	MBN	NH_4^+
TC	**-0.98**							
TN	0.23	-0.35						
TP	**0.64**	-0.48	-0.06					
LOI	**-0.64**	**0.76**	-0.24	0.17				
MBC	0.40	-0.43	0.54	0.43	-0.08			
MBN	-0.13	0.28	0.01	0.58	**0.80**	0.03		
NH_4^+	**0.65**	-0.53	0.22	**0.96**	0.15	0.53	**0.63**	
PMN*	0.39	0.02	**-0.63**	**1.00**	**0.67**	**0.95**	**0.74**	**0.98**

4.3.3 Nitrate Concentration in Bayou Sediment and Marsh Soil

The nitrate treatment addition was 2 mg NO_3-N L^{-1}; however, dilution by pore water occurred such that nitrate concentrations at time zero averaged 1.92 mg NO_3-N L^{-1} in the bayou sediment cores (Figure 4.1). After 9 days, the mean nitrate concentration decreased to 0.92 mg NO_3-N L^{-1}. Two mg NO_3-N per L was also added to the marsh soil as the bayou sediment. Dilution also occurred in the marsh soils but less than in the bayou sediment cores, so that the nitrate concentration 1.98 mg NO_3-N L^{-1} at time zero. After 9 days, the mean nitrate concentration in the marsh soil cores was 1.22 mg NO_3-N L^{-1}. Redox potential was similar in each core measured at approximately 5 cm soil depth, averaging -215 ± 34 mV. The mean pH, salinity, and DO were 7.32 ± 0.11, 70 ± 94 ppt and 2.97 ± 0.59 mg L^{-1}, respectively.

4.3.4 Internal and External Nitrogen Sources

Over the duration of the experiment, in both the bayou sediment and marsh soil cores, nitrate concentration decreased and ammonium concentration increased. For the bayou sediment nitrate concentration at time zero was 1.92 mg NO_3-N L^{-1} and after 9 days of incubation, the

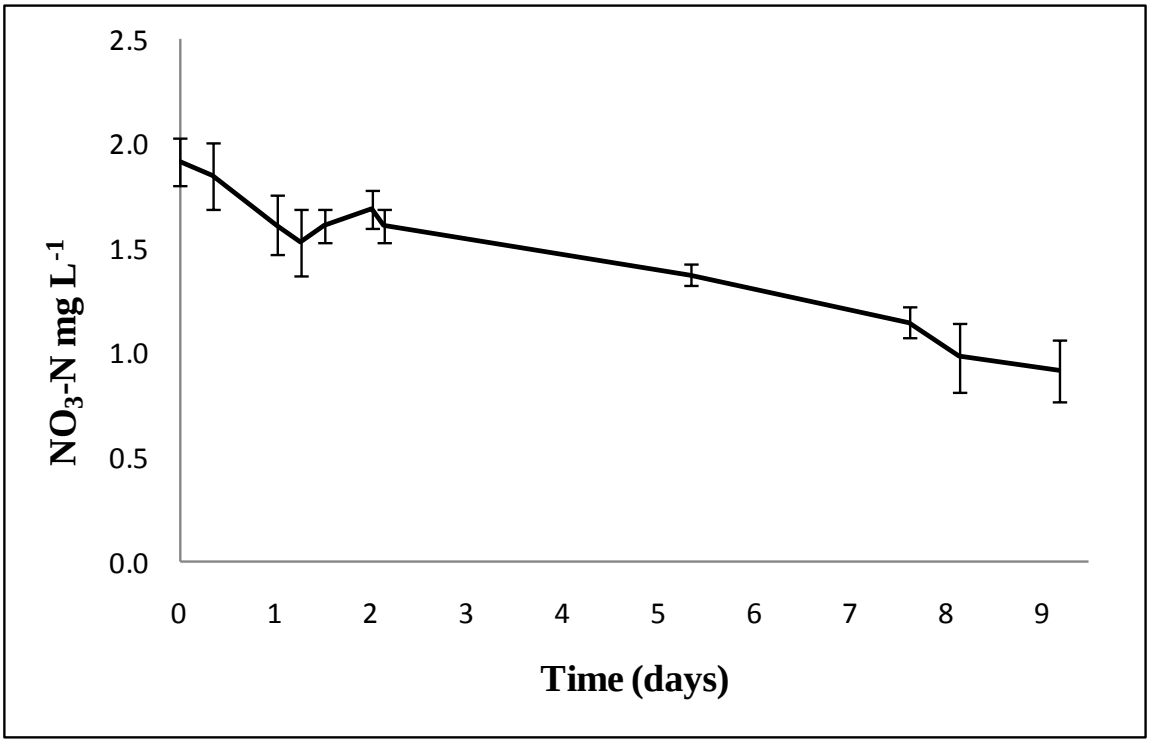

Figure 4.1 Mean water column nitrate concentration over 9 days in a bayou sediment, presented as mean ± one standard deviation (n = 4).

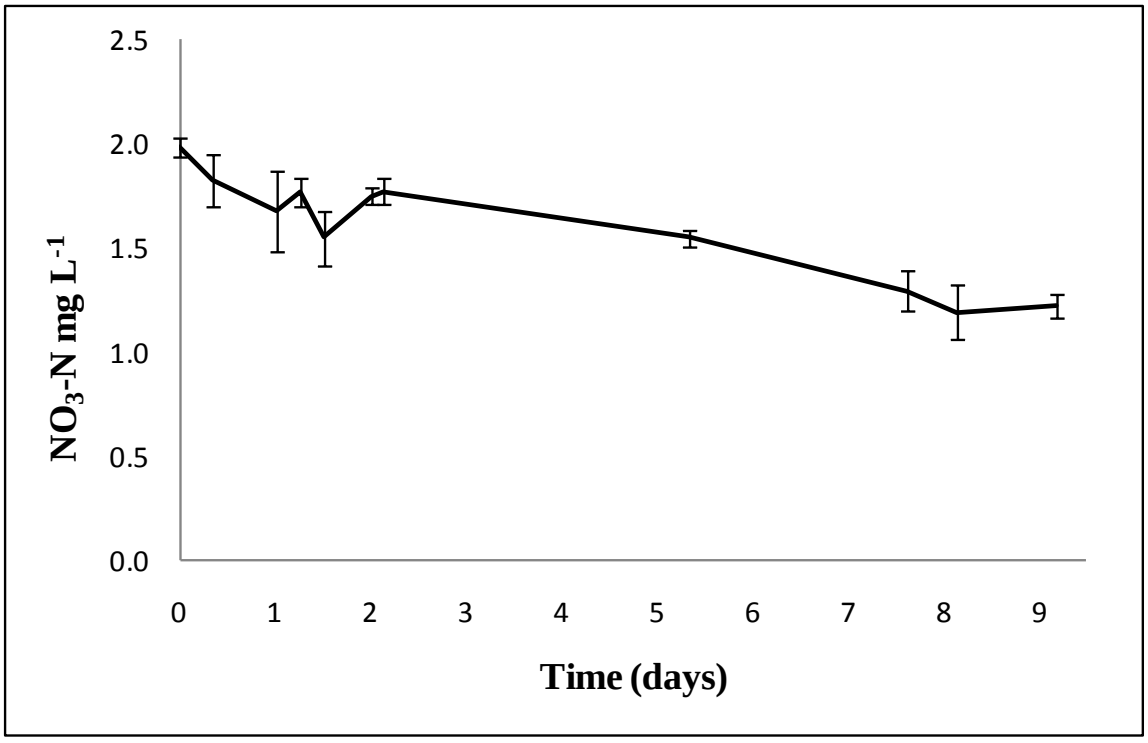

Figure 4.2 Mean water column nitrate concentration over 9 days in a marsh soil, presented as mean ± one standard deviation (n = 4).

nitrate concentration decreased to 0.92 mg NO_3-N L^{-1} (Figure 4.3). At the same time ammonium concentrations at time zero were 0.09 mg NH_4-N L^{-1} at time zero and increased over the experimental time to 1.51 mg NH_4-N L^{-1}. In the bayou sediment cores, over 9 days a net loss of nitrate occurred.

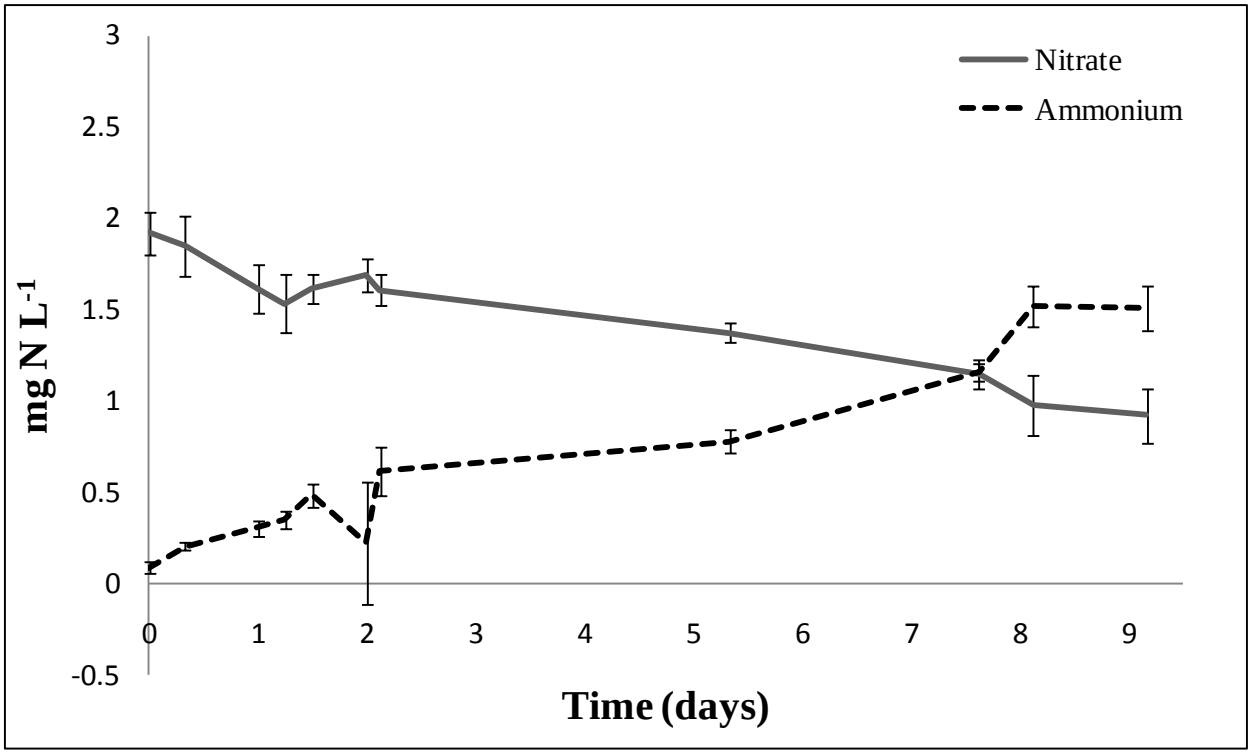

Figure 4.3 Mean water column N concentration over 9 days in a bayou sediment, presented as mean ± one standard deviation (n = 4).

Similar results occurred in the marsh soil core experiment. Nitrate concentration at time zero was 1.98 mg NO_3-N L^{-1} and decreased over 9 days to 1.22 mg NO_3-N L^{-1} (Figure 4.4). Ammonium concentration increased over time in the marsh soils, beginning at 0.20 mg NH_4-N L^{-1} at time zero and increasing to 2.22 mg NH_4-N L^{-1} at the highest concentration (day 8) during the experimental time period. Over the experimental time period, net nitrate loss occurred in the marsh soil, but less than in the bayou sediment.

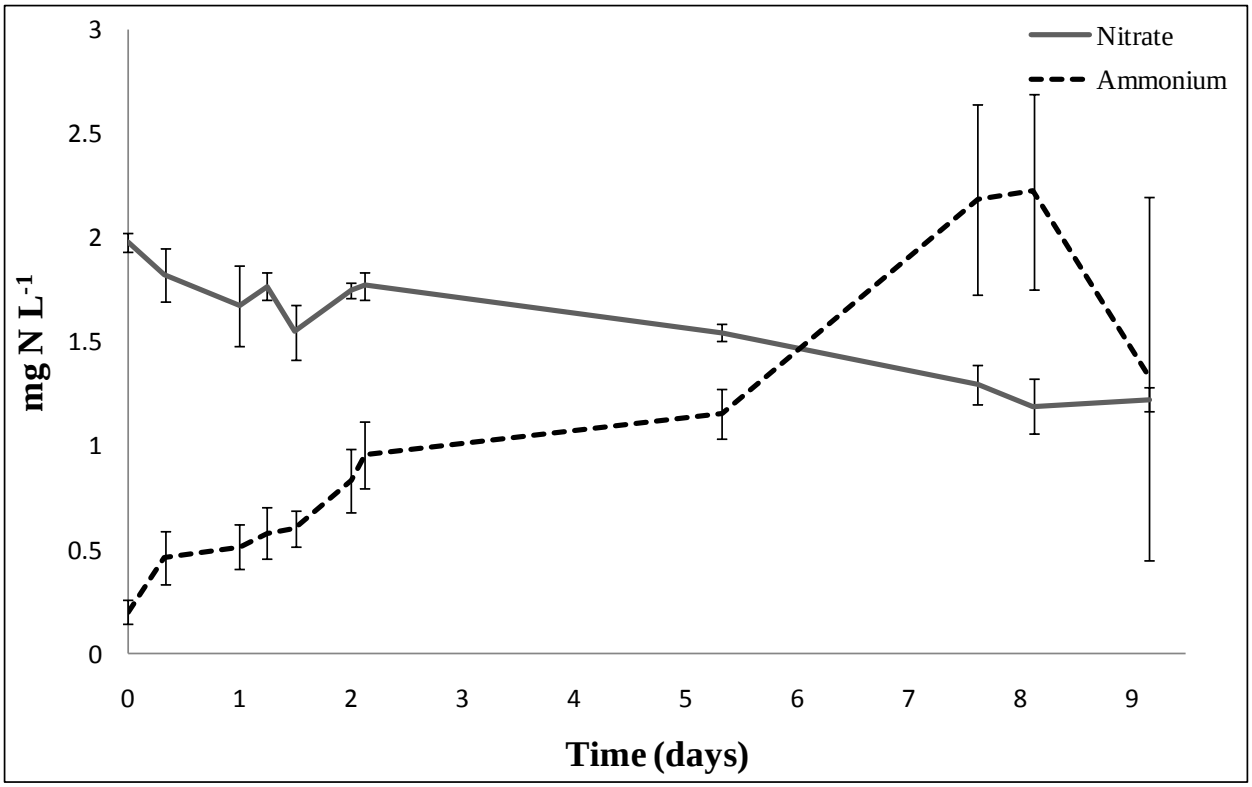

Figure 4.4 Average water column N concentration over 9 days in a marsh soil, presented as mean ± one standard deviation (n = 4).

4.4 DISCUSSION

Soil properties in the bayou sediment and the marsh soil were significantly different for most soil characteristics tested. This difference was expected as marsh soils are high in organic matter and bayou sediments are lower in organic matter. Extractable NH_4-N was also expected to be higher in marsh soil as organic matter is needed for N mineralization. Higher organic matter in the marsh soils will have higher N mineralization and higher extractable NH_4^+.

Both the bayou sediment cores and marsh soil cores had a net loss of nitrate over 9 days. Nitrate concentration in bayou sediment cores went from 1.92 to 0.93 mg NO_3-N L^{-1}, a loss of 1.00 mg NO_3-N L^{-1}. Marsh soil core nitrate concentrations went from 1.98 mg NO_3-N L^{-1} to 1.22 mg NO_3-N L^{-1} over 9 days. Loss of nitrate was only 0.76 mg NO_3-N L^{-1}, less than nitrate loss

from the bayou sediment cores. However, tight coupling of nitrification and denitrification explains the lower rate of nitrate loss in the marsh soil cores.

Marsh soil had significantly higher extractable NH_4-N, indicating that N mineralization was taking place faster than in bayou sediment. Significantly higher TC and extractable NH_4-N in marsh soils supports occurrence of N mineralization in the marsh soils. Increasing water column ammonium concentrations also support higher N mineralization in marsh soil than the bayou sediment. Water column ammonium increased from 0.09 mg NH_4-N L^{-1} to 1.51 mg NH_4-N L^{-1} over 9 days in the bayou sediment cores, an increase of 1.42 mg NH_4-N L^{-1}. Water column ammonium increased from 0.20 mg NH_4-N L^{-1} to 2.22 mg NH_4-N L^{-1}, an increase of 2.02 mg NH_4-N L^{-1} over 9 days in the marsh soil cores.

Zones of aerobic and anaerobic conditions need to exist for coupling of nitrification and denitrification to occur. Nitrification of ammonium to nitrate occurs only in aerobic conditions. Water column mean DO level of 2.97 ± 0.59 mg L^{-1} suggest conditions for nitrification to occur existed in this experiment. On the other hand, denitrification only occurs in anaerobic conditions. Mean redox measurements of -215 ± 34 mV at approximately 5 cm below the sediment/ soil surface suggest conditions existed for denitrification to occur (Patrick et al., 1996). This supports tight coupling of nitrification-denitrification.

Conditions for the coupling of nitrification and denitrification to occur are present in wetland soils and sediments. Aerobic and anaerobic conditions were both present during this study as well as high ammonium and nitrate concentrations that can be limiting factors. Diffusion of ammonium from N mineralization in sediment/soil into the oxygenated water column increased nitrate concentration in the water column. However, net loss of nitrate in both the bayou sediment and marsh soil suggests that removal of excess nitrate is possible.

Concentrations of nitrate and ammonium over this 9 day experiment show that a net loss of excess nitrate is possible when the discharge rate less than 4000 ft^3s^{-1} and Mississippi River water remains in bayous. Also, marsh soil still has the potential to remove excess nitrate, but high release of ammonium by N mineralization decreased net removal of excess nitrate. This suggests that these marshes are not N limited, as N mineralization converts organic N to inorganic N (NH_4^+). Since ammonium is a biologically active form of N, ammonium generally is assimilated into plant biomass before reaching the water column.

4.5 CONCLUSION

Our results indicate that net nitrate removal occurs when the discharge rate of Caernarvon Diversion is low and river water remains in the bayou. However, net nitrate loss over 9 days was only 1 mg N L^{-1}. Ammonium concentration, monitored over the 9 days, suggest bayou sediment can be a source of nitrate thus decreasing the potential nitrate removal capacity. A possible solution would be to increase the discharge rate of Caernarvon Diversion so that flow is greater than 4000 ft^3 s^{-1}. Higher discharge rates from Caernarvon Diversion would result in flooding the marsh. Net nitrate loss in the marsh soil further supports the conclusion that higher discharge rates that result in flooding marshes will increase net nitrate loss. Over 9 days, nitrate removal was less in the marsh soil then in the bayou sediment because of N mineralization and subsequent nitrification. If plants were present, ammonium that would otherwise be a potential source of nitrate will be intercepted and assimilated into plant biomass. This is supported by previous experiments (Chapters 2 and 3) where nitrate removal occurred in 12 hrs and there was no increase in ammonium concentration with the presence of plants.

CHAPTER 5:

SUMMARY AND CONCLUSIONS

5.1 SUMMARY

Diversions of the lower Mississippi River meter river water into coastal Louisiana marshes. The Caernarvon Diversion is one of several diversions in coastal LA. The Caernarvon Diversion can deliver up to 8000 ft^3 s^{-1} of Mississippi River water into Breton Sound Estuary and has been in operation since 1991. Excess nitrate in the Mississippi River, approximately 2 mg NO_3-N L^{-1}, also enters the Breton Sound Estuary via the Caernarvon Diversion. One potential benefit of having diversions is decreasing the amount of high nutrient water reaching the Gulf of Mexico by moving some Mississippi River flow through coastal wetlands for removal by denitrification. However, concerns have been raised with possible eutrophication of Breton Sound Estuary marshes, especially with potential negative impacts to belowground biomass. Concerns with belowground biomass resulted from the preferential damage of fresh and brackish marshes in the upper Breton Sound Estuary after Hurricane Katrina in 2005. However, marshes in the Breton Sound Estuary only receive nitrate laded Mississippi River water when flow from Caernarvon Diversion is greater than 4000 ft^3 s^{-1}.

Nitrate from the Mississippi River is of important for the Breton Sound Estuary, as nitrogen is generally the limiting nutrient for plant growth in coastal marshes. The fate of this nitrate is of great consequence as diversions were built to restore coastal wetlands by mimicking spring flooding of the Mississippi River and returning freshwater to these marshes. However, the possible negative effect of nitrate on plant resilience in the Breton Sound Estuary is of concern. Therefore, the main goal of this research was to determine response of belowground biomass with nitrate addition delivered in surface water. The specific objectives of this research was to 1) determine how much nitrate was assimilated into above and below ground plant biomass and to determine affects on belowground biomass, 2) establish rate of nitrate loss by denitrification, 3)

calculate carbon usage by denitrification, and 4) determine rate of denitrification in bayou sediment in the Breton Sound Estuary.

To determine nitrate assimilation into plant biomass, ^{15}N-labeled nitrate was used as a tracer to study the movement of nitrate in the nitrogen cycle. Nitrate concentration of 2 mg N L^{-1} was added in solution to 12 *Spartina patens* cores over three time periods, 6, 12, and 16 weeks. After 12 weeks six cores were destructively harvested. Aboveground biomass was clipped and belowground biomass was separated into 0-10 cm and 10-20 cm soil sections. Biomass was separated into live and dead components for ^{15}N analysis. At 12 weeks, the remaining 6 cores received a 10 fold increase in nitrate concentration to simulate high nitrate loading. After 4 weeks of 20 mg NO_3-N L^{-1} additions, the remaining 6 cores were destructively harvested in the same fashion as the earlier harvest.

Overall, results indicate that denitrification is the main removal mechanism for excess nitrate in the Breton Sound Estuary. At 6 and 12 weeks, gaseous losses accounted for 70 and 65 % of added labeled nitrate at 2 mg N L^{-1}. After a tenfold increase in nitrate additions, gaseous loss accounted for 74 % of added labeled nitrate. Belowground biomass was not significantly different comparing control and treatment cores indicating belowground biomass was not impacted by nitrate addition. Furthermore, carbon calculations indicate that less than 5 % of carbon in the 0-20 cm soil profile in these marshes was used during denitrification at 20 mg N L^{-1}. Carbon calculations do not include any new carbon accumulation by plants, at about 1 cm a year (DeLaune and White, 2011).

Marsh soil and bayou sediment was used to compare denitrification rates under different discharge rates. Discharge rates less than 4000 ft^3 s^{-1} result in Mississippi River water remaining in bayous, decreasing residence time and possibly reducing nitrate removal. Discharge rates

greater than 4000 ft^3 s^{-1} result in the flooding of marshes in Breton Sound Estuary, increasing residence time and increasing nitrate removal. The goal of this experiment was to determine net nitrate removal under these different discharge scenarios. Large roots were removed from the bayou sediment and marsh soils and blended to form a homogenous sample. Nitrate additions occurred at 2 mg N L^{-1} in a 10 cm water column and nitrate concentrations was monitored over 9 days. Also, ammonium concentration was monitored over the 9 days. Overall, results indicate that net loss of nitrate occurred by denitrification in both the bayou sediment and marsh soil. However, bayou sediments can actually be a source of nitrate by N mineralization and subsequent nitrification, with ammonium concentrations increasing from 0.09 mg NH_4-N L^{-1} to 1.51 mg NH_4-N L^{-1}. Increase in ammonium concentrations occurred faster in the marsh soil, where ammonium increased from 0.20 mg NH_4-N L^{-1} to 2.22 mg NH_4-N L^{-1}. Results from the mass balance indicate the presence of plants assimilate ammonium before nitrification can occur.

We confirmed that belowground biomass in the Breton Sound Estuary is not negatively impacted by nitrate with the use of labeled nitrate in the *Spartina patens* core study. Carbon calculations suggest that tight coupling of carbon and denitrification does not appear to affect Breton Sound Estuary marshes by removing carbon stores. The bayou sediment and marsh soil study confirm excess nitrate is removed by denitrification in the Breton Sound Estuary, however the unvegetated bayou sediment can actually be a source of N, decreasing the net removal of N. These results further indicate that nitrate removal is enhanced if discharge rates from Caernarvon Diversion are sufficiently high to flood marshes in Breton Sound Estuary.

5.2 CONCLUSIONS

- A greenhouse *Spartina patens* core study found that loss of 2 mg NO_3-N L^{-1} occurred in 12 hours compared to cores receiving no nitrate.

- After 6 weeks of labeled nitrate additions, 21% was recovered in aboveground biomass (live + dead) and 2% was recovered in the live belowground biomass in the 0-10 cm soil section.
- Seventy percent of added labeled nitrate after 6 weeks was unaccounted for gaseous losses. Sixty five percent of labeled nitrate was unaccounted for after an additional 6 weeks. With 20 mg NO_3-N L^{-1}, 74% of labeled nitrate was unaccounted for after 4 weeks.
- Lack of labeled N in plant biomass, low ammonium concentrations, and anaerobic conditions in the soil indicate denitrification was the main removal mechanism for excess nitrate.
- A 10 fold increase in nitrate concentration also supports nitrate removal by denitrification, where loss of nitrate occurred in 24 hrs.
- Carbon calculations needed for denitrification indicate less than 5% of the total g of C in the 0-20 cm soil profile was needed at 20 mg N L^{-1} additions. Loss of carbon by denitrification was not substantiated in this study.
- Net loss of nitrate over 9 days in bayou sediment indicate removal of nitrate can occur if Mississippi River water remains in the bayou.
- Bayou sediments and marsh soils are a source of nitrate by coupled nitrification-denitrification indicating the importance of flooding vegetated marshes for higher nitrate removal.

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APPENDIX: SUPPLEMENTAL DATA

Table A. *Spartina patens* Core Properties

Experimental Unit	Harvest Date	Soil Section (cm)	Total Weight (g)	Bulk Dens. g cm^{-3}	Moisture (%)	TC g kg^{-1}	TN g kg^{-1}	TP mg kg^{-1}	MBC g kg^{-1}	MBN mg kg^{-1}	NO_3^- mg kg^{-1}	NO_4^+ mg kg^{-1}	PMN mg kg^{-1} day^{-1}
1	10/4/2010	0-10	1759.50	0.32	66.54	61.61	4.55	426.91	9.25	21.39	2.54	17.11	0.87
1	10/4/2010	10-20	1475.47	0.33	59.82	38.22	3.01	454.12	7.40	6.31	1.45	28.62	-0.83
2	8/20/2010	0-10	1516.13	0.29	65.70	64.50	4.75	641.55	3.13	13.03	2.77	36.68	3.74
2	8/20/2010	10-20	1534.68	0.30	64.54	60.93	4.56	634.34	2.88	12.88	2.16	68.13	0.57
3	10/4/2010	0-10	2272.70	0.46	63.29	61.69	4.50	446.87	8.98	6.02	2.56	32.24	1.87
3	10/4/2010	10-20	1666.81	0.34	62.93	60.03	4.14	592.38	8.52	3.68	2.04	99.27	0.54
4	10/4/2010	0-10	1568.08	0.29	66.79	66.63	4.80	424.17	9.68	8.60	3.32	23.46	1.02
4	10/4/2010	10-20	1654.63	0.38	58.26	37.23	3.14	577.45	8.96	6.69	1.67	76.39	-2.67
5	8/20/2010	0-10	1244.85	0.21	69.15	70.42	5.13	603.38	3.61	12.06	2.16	64.27	-0.33
5	8/20/2010	10-20	1763.46	0.37	62.29	53.39	4.27	513.12	2.73	16.77	2.23	166.58	3.84
6	8/20/2010	0-10	1137.85	0.21	66.86	64.75	4.72	670.03	3.29	23.14	2.28	22.60	3.11
6	8/20/2010	10-20	1606.44	0.33	63.15	57.61	4.13	719.69	2.83	12.02	2.62	35.18	2.83
7	8/20/2010	0-10	1373.54	0.27	64.70	53.84	4.24	496.62	2.97	15.57	3.25	34.72	2.32
7	8/20/2010	10-20	1242.80	0.25	62.95	51.63	3.76	511.91	2.79	6.66	2.13	50.23	1.53
8	8/20/2010	0-10	1330.08	0.25	65.64	41.74	3.42	517.79	3.80	14.31	1.93	44.14	3.96
8	8/20/2010	10-20	1535.39	0.37	56.42	42.26	3.46	544.01	2.42	1.77	1.91	72.17	1.30
9	10/4/2010	0-10	2214.90	0.46	62.53	60.24	4.51	478.19	9.83	16.32	1.72	60.12	3.12
9	10/4/2010	10-20	1641.06	0.40	55.53	35.88	3.07	548.65	5.82	15.67	1.35	81.78	-2.24
10	8/20/2010	0-10	1321.45	0.22	70.03	90.07	6.03	491.26	4.35	19.77	1.94	38.50	3.71
10	8/20/2010	10-20	1876.45	0.42	59.54	55.94	4.44	472.95	2.78	5.99	1.88	100.46	-0.13
11	10/4/2010	0-10	2174.40	0.44	62.91	60.25	4.59	589.15	5.07	12.64	1.61	47.36	5.72
11	10/4/2010	10-20	1906.16	0.43	59.04	47.28	3.89	567.00	3.96	32.42	1.66	108.91	3.18
12	10/4/2010	0-10	1777.19	0.37	62.35	52.08	4.08	472.94	3.98	11.12	0.62	24.59	-2.13
12	10/4/2010	10-20	1761.62	0.42	56.45	42.52	3.44	456.68	3.10	12.61	0.68	53.16	7.27

Table B. *Spartina patens* above and below ground plant properties
a. Experimental units 1-3 (* indicates no data)

Experimental Unit	Harvest Date	Treatment (mg N/L)	Core Section	Plant Type	Total Weight (g)	TC g kg^{-1}	TN g kg^{-1}	TP mg kg^{-1}
1	7/14/2010	0	Above	Live	21.30	427.64	8.86	1261.35
1	7/14/2010	0	Above	Dead	7.40	419.23	6.43	751.47
1	8/20/2010	0	Above	Live	4.60	433.45	15.55	2396.17
1	8/20/2010	0	Above	Dead	0.58	401.27	8.54	1639.34
1	10/4/2010	0	Above	Live	1.01	444.52	12.27	941.85
1	10/4/2010	0	Above	Dead	0.18	411.82	9.74	ND*
1	10/4/2010	0	0-10	Live	1.25	424.06	9.25	467.48
1	10/4/2010	0	0-10	Dead	18.42	422.13	7.96	350.75
1	10/4/2010	0	0-10	Stem	4.36	467.88	4.75	192.65
1	10/4/2010	0	10-20	Live	0.56	429.75	8.09	3163.91
1	10/4/2010	0	10-20	Dead	17.90	428.58	8.48	325.27
1	10/4/2010	0	10-20	Stem	2.87	465.74	6.88	277.81
2	7/14/2010	0	Above	Live	17.60	428.80	11.43	1001.91
2	7/14/2010	0	Above	Dead	6.07	407.34	11.91	907.49
2	8/20/2010	0	Above	Live	7.32	433.97	14.93	1328.21
2	8/20/2010	0	Above	Dead	0.37	274.26	6.69	879.60
2	8/20/2010	0	0-10	Live	1.72	406.60	9.49	3135.88
2	8/20/2010	0	0-10	Dead	5.35	370.63	11.64	1213.18
2	8/20/2010	0	0-10	Stem	2.25	442.93	9.75	1489.19
2	8/20/2010	0	10-20	Live	0.56	420.80	10.37	3048.06
2	8/20/2010	0	10-20	Dead	8.64	434.12	12.22	980.16
2	8/20/2010	0	10-20	Stem	2.28	446.47	11.53	684.07
3	7/14/2010	0	Above	Live	32.92	430.24	7.35	817.95
3	7/14/2010	0	Above	Dead	9.83	413.65	5.14	488.19
3	8/20/2010	0	Above	Live	7.03	428.42	14.35	1758.75
3	8/20/2010	0	Above	Dead	0.68	429.75	6.77	751.09
3	10/4/2010	0	Above	Live	2.93	443.64	13.65	1212.08
3	10/4/2010	0	Above	Dead	0.35	415.66	9.51	ND*
3	10/4/2010	0	0-10	Live	3.14	442.49	8.38	510.34
3	10/4/2010	0	0-10	Dead	26.14	409.72	8.35	383.71
3	10/4/2010	0	0-10	Stem	9.13	463.02	5.48	486.17
3	10/4/2010	0	10-20	Live	0.33	440.96	7.70	ND*
3	10/4/2010	0	10-20	Dead	31.90	429.62	9.30	391.19
3	10/4/2010	0	10-20	Stem	1.86	460.50	7.22	241.35

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b. Experimental units 4-6 (* indicates no data)

Experimental Unit	Harvest Date	Treatment (mg N/L)	Core Section	Plant Type	Total Weight (g)	TC g kg^{-1}	TN g kg^{-1}	TP mg kg^{-1}
4	7/14/2010	0	Above	Live	10.29	428.46	11.10	701.42
4	7/14/2010	0	Above	Dead	0.80	410.57	9.07	944.90
4	8/20/2010	0	Above	Live	4.10	431.45	17.16	2298.36
4	8/20/2010	0	Above	Dead	1.06	407.50	10.28	1710.46
4	10/4/2010	0	Above	Live	1.25	439.74	15.88	1414.49
4	10/4/2010	0	Above	Dead	0.21	373.21	9.45	ND*
4	10/4/2010	0	0-10	Live	1.03	423.58	10.96	1116.69
4	10/4/2010	0	0-10	Dead	10.89	387.74	10.51	512.89
4	10/4/2010	0	0-10	Stem	1.63	465.39	7.89	658.96
4	10/4/2010	0	10-20	Live	0.11	425.57	8.57	ND*
4	10/4/2010	0	10-20	Dead	24.64	406.43	9.16	397.26
4	10/4/2010	0	10-20	Stem	1.41	454.79	5.90	317.93
5	7/14/2010	0	Above	Live	14.93	422.91	7.47	1588.15
5	7/14/2010	0	Above	Dead	4.39	413.73	7.15	974.98
5	8/20/2010	0	Above	Live	5.15	416.92	13.56	2441.59
5	8/20/2010	0	Above	Dead	0.19	357.04	8.41	ND*
5	8/20/2010	0	0-10	Live	2.04	398.48	9.15	2534.30
5	8/20/2010	0	0-10	Dead	5.05	399.79	11.33	692.34
5	8/20/2010	0	0-10	Stem	5.95	444.09	6.87	845.90
5	8/20/2010	0	10-20	Live	0.98	437.23	8.01	406.35
5	8/20/2010	0	10-20	Dead	7.90	391.04	10.94	466.33
5	8/20/2010	0	10-20	Stem	4.22	454.64	8.54	423.88
6	7/14/2010	0	Above	Live	22.95	479.67	12.56	937.59
6	7/14/2010	0	Above	Dead	14.45	407.89	10.07	797.60
6	8/20/2010	0	Above	Live	6.93	415.57	17.93	1564.96
6	8/20/2010	0	Above	Dead	0.12	393.17	8.30	ND*
6	8/20/2010	0	0-10	Live	2.40	372.71	9.44	3290.05
6	8/20/2010	0	0-10	Dead	4.88	405.43	12.11	924.51
6	8/20/2010	0	0-10	Stem	4.78	445.97	7.73	1176.29
6	8/20/2010	0	10-20	Live	0.79	371.77	10.03	7950.60
6	8/20/2010	0	10-20	Dead	12.56	417.80	11.36	878.55
6	8/20/2010	0	10-20	Stem	3.82	448.39	6.30	877.08

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c. Experimental units 7-9 (* indicates no data)

Experimental Unit	Harvest Date	Treatment (mg N/L)	Core Section	Plant Type	Total Weight (g)	TC g kg^{-1}	TN g kg^{-1}	TP mg kg^{-1}
7	7/14/2010	2	Above	Live	11.48	429.24	9.86	603.60
7	7/14/2010	2	Above	Dead	8.55	413.08	9.66	614.45
7	8/20/2010	2	Above	Live	4.82	409.73	16.03	2550.45
7	8/20/2010	2	Above	Dead	0.35	413.85	8.90	1984.24
7	8/20/2010	2	0-10	Live	1.68	405.03	11.99	1697.81
7	8/20/2010	2	0-10	Dead	12.29	417.61	10.50	546.71
7	8/20/2010	2	0-10	Stem	6.49	426.05	8.15	742.57
7	8/20/2010	2	10-20	Live	1.50	439.97	7.59	262.23
7	8/20/2010	2	10-20	Dead	12.62	419.62	9.48	324.58
7	8/20/2010	2	10-20	Stem	5.33	457.33	7.14	282.89
8	7/14/2010	2	Above	Live	14.13	424.67	10.62	1070.66
8	7/14/2010	2	Above	Dead	18.39	421.73	6.50	764.73
8	8/20/2010	2	Above	Live	4.43	412.13	16.22	1824.81
8	8/20/2010	2	Above	Dead	0.15	378.95	16.31	ND*
8	8/20/2010	2	0-10	Live	1.82	467.52	12.77	1583.40
8	8/20/2010	2	0-10	Dead	12.15	358.37	11.23	816.66
8	8/20/2010	2	0-10	Stem	4.91	459.27	7.67	884.57
8	8/20/2010	2	10-20	Live	0.18	422.80	8.02	ND*
8	8/20/2010	2	10-20	Dead	27.00	429.28	8.95	689.66
8	8/20/2010	2	10-20	Stem	0.99	468.90	5.77	172.76
9	7/14/2010	2	Above	Live	20.66	425.49	10.06	1097.99
9	7/14/2010	2	Above	Dead	2.93	407.08	6.12	563.02
9	8/20/2010	2	Above	Live	8.13	413.49	13.33	1368.81
9	8/20/2010	2	Above	Dead	2.98	416.34	7.40	668.74
9	10/4/2010	20	Above	Live	4.10	439.46	15.04	1116.48
9	10/4/2010	20	Above	Dead	0.35	430.65	10.79	ND*
9	10/4/2010	20	0-10	Live	2.80	414.76	13.05	822.55
9	10/4/2010	20	0-10	Dead	16.89	407.93	8.73	398.46
9	10/4/2010	20	0-10	Stem	6.77	466.97	4.96	528.92
9	10/4/2010	20	10-20	Live	0.19	433.78	8.59	ND*
9	10/4/2010	20	10-20	Dead	23.37	409.62	10.22	385.54
9	10/4/2010	20	10-20	Stem	1.46	454.48	5.81	172.48

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d. Experimental units 10-12 (* indicates no data)

Experimental Unit	Harvest Date	Treatment (mg N/L)	Core Section	Plant Type	Total Weight (g)	TC g kg^{-1}	TN g kg^{-1}	TP mg kg^{-1}
10	7/14/2010	2	Above	Live	15.82	423.38	9.89	1045.51
10	7/14/2010	2	Above	Dead	11.83	415.79	9.01	880.83
10	8/20/2010	2	Above	Live	5.54	407.08	15.36	2132.51
10	8/20/2010	2	Above	Dead	0.13	360.39	13.24	ND*
10	8/20/2010	2	0-10	Live	1.96	425.68	11.55	1370.39
10	8/20/2010	2	0-10	Dead	14.11	406.99	8.71	359.22
10	8/20/2010	2	0-10	Stem	6.16	467.89	7.31	771.27
10	8/20/2010	2	10-20	Live	0.68	443.34	6.84	284.19
10	8/20/2010	2	10-20	Dead	33.37	420.09	9.56	355.11
10	8/20/2010	2	10-20	Stem	3.02	460.87	6.21	293.01
11	7/14/2010	2	Above	Live	23.20	425.00	8.37	952.34
11	7/14/2010	2	Above	Dead	11.41	410.71	5.97	432.74
11	8/20/2010	2	Above	Live	7.07	411.15	15.03	1381.61
11	8/20/2010	2	Above	Dead	0.81	394.79	8.69	1221.37
11	10/4/2010	20	Above	Live	5.53	432.32	16.79	834.37
11	10/4/2010	20	Above	Dead	0.33	400.47	8.72	ND*
11	10/4/2010	20	0-10	Live	3.09	418.85	11.82	1448.13
11	10/4/2010	20	0-10	Dead	16.79	440.38	8.34	594.59
11	10/4/2010	20	0-10	Stem	12.88	468.60	5.92	503.81
11	10/4/2010	20	10-20	Live	0.26	443.96	10.11	ND*
11	10/4/2010	20	10-20	Dead	22.67	406.52	8.37	448.68
11	10/4/2010	20	10-20	Stem	2.06	460.25	8.39	726.52
12	7/14/2010	2	Above	Live	19.91	428.06	10.26	953.74
12	7/14/2010	2	Above	Dead	15.35	422.72	7.21	581.79
12	8/20/2010	2	Above	Live	7.43	410.98	15.05	1670.94
12	8/20/2010	2	Above	Dead	0.59	362.97	7.47	1370.31
12	10/4/2010	20	Above	Live	10.23	435.10	14.24	1211.12
12	10/4/2010	20	Above	Dead	0.68	391.49	8.14	911.48
12	10/4/2010	20	0-10	Live	2.78	414.94	12.96	1377.67
12	10/4/2010	20	0-10	Dead	15.85	441.02	9.86	448.46
12	10/4/2010	20	0-10	Stem	6.99	466.67	5.49	608.27
12	10/4/2010	20	10-20	Live	0.16	430.95	9.05	ND*
12	10/4/2010	20	10-20	Dead	25.25	388.17	8.39	405.90
12	10/4/2010	20	10-20	Stem	1.30	452.80	6.09	153.43

Table C. Bayou/Marsh sediment and soil characterization

Experimental Unit	Type	Moisture (%)	TC g kg^{-1}	TN g kg^{-1}	TP mg kg^{-1}	MBC g kg^{-1}	MBN mg kg^{-1}	NO_4^+ mg kg^{-1}	PMN mg kg^{-1} day^{-1}
1	Bayou	79.53	105.44	7.73	599.73	7.22	41.52	144.09	5.88
2	Bayou	80.03	113.19	7.28	576.03	6.83	51.34	159.96	10.06
3	Bayou	80.07	114.41	7.40	591.06	7.20	17.76	142.10	9.21
4	Bayou	79.98	95.02	6.13	600.98	6.50	23.82	139.47	ND*
5	Bayou	80.14	98.13	6.55	576.22	6.96	38.53	139.46	ND*
6	Marsh	81.67	163.79	11.43	688.12	8.22	24.13	341.02	11.17
7	Marsh	81.50	165.50	11.63	676.60	8.14	25.01	333.88	9.11
8	Marsh	81.63	163.37	11.53	672.00	8.00	10.72	325.98	7.97
9	Marsh	81.44	166.38	11.49	674.32	8.48	17.78	327.86	ND*
10	Marsh	81.37	167.66	11.36	671.24	7.41	20.54	323.00	ND*

VITA

Christine M. VanZomeren was brought up in Charlotte, North Carolina, with her parents Annmarie and Anthony, and older sister Catherine. She grew up spending many weekends backpacking and canoeing in western North Carolina, where her love of the outdoors grew.

Christine attended University of North Carolina Wilmington in Wilmington, North Carolina, where she graduated summa cum laude in 2009 with two Bachelor of Science degrees, one in the Department of Biology and Marine Biology and one in the Department of Environmental Science. During her time at University of North Carolina Wilmington, she worked for a year and half at the Wetland Ecology Laboratory. The experience of working in wetlands on the Cape Fear River, North Carolina, for the Wetland Ecology Laboratory influenced and refined her interest in wetland ecology.

After graduation, Christine accepted a fellowship to Louisiana State University in Baton Rouge, Louisiana with Drs. John R. White and Ronald D. DeLaune to continue with wetland research. Her master's research has strengthened her interest in wetland biogeochemistry and she plans to pursue her Doctor of Philosophy in the Soil and Water Science Department at University of Florida with K. Ramesh Reddy in the fall of 2011.