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Denitrification enzyme activity as an indicator of nitrate loading in a wetland receiving diverted Mississippi River water

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DENITRIFICATION ENZYME ACTIVITY AS AN INDICATOR OF NITRATE LOADING IN A WETLAND RECEIVING DIVERTED MISSISSIPPI RIVER WATER

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ABSTRACT

The Davis Pond freshwater diversion discharges nutrient-rich Mississippi River water to a 3,760 ha receiving marsh in upper Barataria Basin, LA. Excess nitrate in the Mississippi River has been linked to algal blooms and hypoxia in the Gulf of Mexico with potential to negatively impact Barataria Basin. We hypothesized that 1) soil denitrification enzyme activity (DEA) will increase with higher surface water nitrate concentrations, and 2) the spatial distribution of DEA in Davis Pond marsh will provide information about the extent nitrate loading at a specific discharge rate. Intact soil cores collected from the marsh received a continuous flow of nitrate solution (0.0, 0.5, 1.0, or 2.0 mg NO_3-N l^{-1}) for a period of 7, 20, or 45 days. Overall, DEA for the 1.0 mg NO_3-N l^{-1} was significantly higher than the control treatment ($P < 0.05$). A strong positive correlation between DEA and surface water nitrate in the 0-5 cm ($P < 0.05$) and 5-10 cm ($P < 0.001$) soil horizons was observed on day 20. However, the correlation between DEA and nitrate was not significant on days 7 and 45. Measureable DEA was observed in the 0.0 mg NO_3-N l^{-1} on all days, indicating the contribution of internal biochemical N cycling to DEA in organic wetland soils. Approximately 92% of all DEA was observed in the top 5 cm of soil, 7% occurred at 5-10 cm, and <1% below 10 cm. DEA was also quantified for 88 randomly distributed soil cores in Davis Pond marsh collected May - July, 2007. At a mean discharge rate of 39 m^3 s^{-1}, high rates of DEA (0.41 to 2.10 mg N_2O-N kg^{-1} h^{-1}) occurred in a 715 ha area proximal to the diversion inflow, while background rates (0 to 0.30 mg N_2O-N kg^{-1} h^{-1}) were observed outside this area. The 715 ha area contained > 80% of all the DEA observed in Davis Pond marsh, yet encompassed only 19% of the total marsh area. The area of elevated DEA included the

highest observed surface water nitrate concentrations, suggesting DEA is a potential indicator of nitrate loading.

CHAPTER 1: REVIEW OF LITERATURE

1.1 LOUSIANA'S COASTAL WETLANDS

The Louisiana coastal zone is experiencing the highest rate of land loss in the United States (Barras et al., 1994). Land loss is defined as the conversion of coastal wetlands to open water. Several factors, both natural and anthropogenic, have contributed to the high rate of land loss in Louisiana. Southern Louisiana includes approximately 40 percent of all coastal wetlands in the US and was formed over several thousand years by the deposition of nutrients and sediments carried with the Mississippi River (Penland and Ramsey, 1990). The alluvium deposited by the river composed of sand, silt, and clay, is highly susceptible to dewatering, compaction, and consolidation. Additionally, down warping of older geological deposits, tectonic activity, and eustatic sea level rise have all culminated in the natural subsidence or "sinking" of the delta region (Evers et al., 1992; National Research Council, 2006) .

Anthropogenic forces have accelerated natural subsidence. For example, the course of the lower Mississippi River to the Gulf of Mexico has shifted numerous times throughout geologic history in an ongoing sequence of land building (accretion) and abandonment (subsidence) (Roberts, 1997). Humans eliminated the natural process of delta switching by constructing extensive flood control levees along the river, thus preventing freshwater, nutrients, and sediments from reaching the coastal wetlands. Although much of the delta region would remain in the degradation phase of the delta cycle regardless of the levee construction, the loss of land from subsidence would be compensated for with the construction of a new delta elsewhere along the coast (Nyman et al., 1990). The current river delta, known as the Balize or Birdfoot Delta, has been maintained by human intervention despite having entered the degradation phase. The delta front continues to prograde into deep

water where sediments are deposited on the continental slope and are unavailable for land building (Coleman et al., 1998).

Canal dredging in the coastal zone for navigation and access to oil deposits causes direct marsh loss, as well as indirect impacts via hydrology alterations and accelerated salt water intrusion. For several decades, dredge spoils were deposited adjacent to the canal, creating a slightly elevated berm along the marsh edge. These spoil banks can prevent tidal exchange. Studies have found a strong correlation between canal density and marsh loss (Scaife et al., 1983; Turner and Rao, 1990). Canals connecting inland freshwater areas with the Gulf create a conduit for saltwater intrusion that is further enhanced by a lack of freshwater flowing seaward and rising sea level (Wang, 1988). Wetland vegetation adapted to freshwater will die when salinity increases, resulting in a feedback loop in which increasing salinity causes plant mortality, which in turn reduces peat accumulation, increases subsidence, and results in higher water levels and greater saltwater intrusion (Flynn et al., 1995; Gough and Grace, 1998). According to Turner (1999), 88% of Louisiana's wetland loss is a result of 'indirect' impacts, including hydraulic alterations, altered sediment supply, and subsurface fluid (i.e., oil and gas) withdraw. Other factors attributed to coastal wetland loss include herbivory (Gough and Grace, 1998) and coastal population growth (Adams et al., 2004).

The overall result of these natural and human induced changes to Louisiana's coastal zone is land accretion occurring at a slower rate than sea level rise, leading to vast areas of wetlands reverting to open water (Day et al., 1995) According to Barras et al. (1994) the average annual rate of land loss in coastal Louisiana between 1956 and 1990 was 34.9 $mi^2\ y^{-1}$.

1.1.1 Coastal Restoration

The preservation of coastal wetlands is vital to both the environment and the economy of Louisiana. Wetland values include storm surge abatement, water quality improvement, and wildlife habitat (Mitsch and Gosselink, 2000). Commercial fisheries in Louisiana account for 25% of all US seafood landings and are the nation's top provider of shrimp, oysters, crab, and menhaden (Adams et al., 2004; LA Coast, 2006). Ninety percent (90%) of the species that make up the multi-million dollar Louisiana commercial fishing industry utilize coastal wetlands for at least some portion of their life cycles (National Research Council, 2006). Louisiana's coastal wetlands offer vital habitat for migratory waterfowl and support the largest concentration of overwintering waterfowl in the US. Coastal wetlands also protect billions of dollars worth or oil and gas infrastructure, and reduce storm surge impacts to millions of residents (Turner and Cahoon, 1987).

Coastal restoration efforts are occurring on private, state, and federal levels. Most are small-scale, isolated projects that involve water level control structures, the deposition of dredge material on subsiding marshes, or marsh vegetation plantings. Some of the largest and most costly projects to date have been initiated by the US Army Corps of Engineers (ACOE) and state agencies in an effort to reintroduce Mississippi River water to the coastal wetlands. The primary goals of these freshwater diversion projects are to reduce salinity in the receiving basin and provide nutrients and sediments to help offset marsh subsidence (Green, 2006). Two diversion structures are now in operation (Caernarvon and Davis Pond) and 10 more future projects are in the feasibility/planning stages (Villarrubia, 2006).

1.2 FRESHWATER DIVERSION PROJECTS

The Caernarvon Freshwater Diversion began operation in 1991 with a maximum discharge capacity of 226 $m^3 s^{-1}$ (8,000 cfs). It is located on the east bank of the river, south of New Orleans, and discharges into Benton Sound estuary. The Davis Pond diversion was completed in 2002, but began regular operation in 2007 following design modifications. The Davis Pond diversion can divert up to 302 $m^3 s^{-1}$ (10,650 cfs) into Barataria Basin and is located on the west bank of the Mississippi River, approximately 19 km upstream (west) of New Orleans (Figure 1.1) (US ACOE, 2006; Villarrubia, 2006).

Freshwater diversions are designed to utilize gravity flow and are managed in response to 1) salinities in the receiving basin and 2) the height of the Mississippi River. This is unlike a sediment diversion, which actively siphons part of the bedload of the Mississippi River and deposits the material outside the river channel (Villarrubia, 2006).

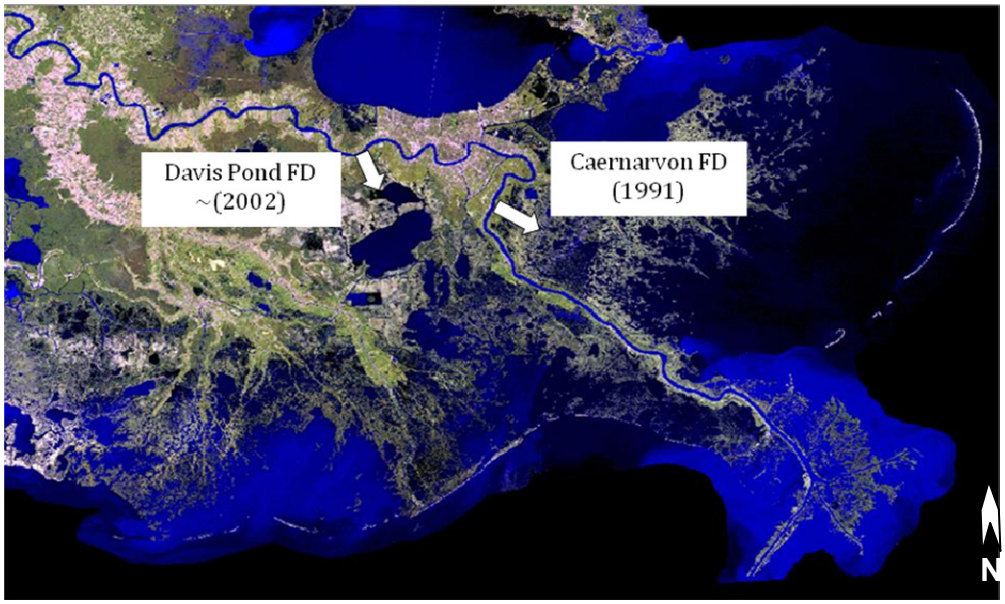

Figure 1.1 Aerial photograph of southeast Louisiana. The approximate locations of the two largest freshwater diversions and their dates of completion are indicated.

1.2.1 Soil Formation

Freshwater diversions operate on the principle that organic matter accumulation is the primary driving factor in marsh accretion. Hatton et al. (1983) and Nyman et al. (1993) both found that autochthonous organic matter accumulation was the main mechanism for vertical accretion in coastal Louisiana marshes without a freshwater input other than precipitation. Organic soils are composed of living and dead root and plant material. Therefore, factors affecting plant productivity also directly affect organic soil formation and strength. Greater plant growth not only increases peat formation, but higher stem densities can trap more sediment and increase the stability of soil against erosion (DeLaune et al., 1990). Similarly, marsh plant mortality can lead to peat collapse and significant elevation loss (DeLaune et al., 1994). In general, organic soil formation is believed to account for 70-80% of accretion in the Mississippi River delta plain between 1933 and 1990 (Day et al., 2000).

The second constituent of wetland soils, mineral sediments, can play an important role in plant productivity. The addition of mineral sediment to the inland portions of a *Spartina alterniflora* marsh in Louisiana resulted in a significant increase in above-ground biomass and an increase in nutrient and mineral content of the plant tissue (DeLaune et al., 1990). Mineral sediment promotes plant growth by providing nutrients (Ca, K, and P), cation exchange, and sorption sites to reduce leaching of phosphate (Patrick and Khalid, 1974). Furthermore, predominately organic soils (bulk density less than 0.08 g cm^{-3}) have been correlated with low above-ground biomass in a *Spartina patens* dominated marsh (Nyman et al., 1994). Salt marshes appear to require the greatest amount of mineral sediments, followed by brackish marshes and freshwater marshes. The high mineral sediment demand of salt marshes may be a product of the need for inorganic elements in sulfide precipitation (Nyman et al., 1990).

For example, iron (a substantial component of mineral sediments) has a high affinity to precipitate sulfide, thereby neutralizing the stressor and allowing for increased plant growth (King et al., 1982). Therefore, lowering the salinity with freshwater diversions can reduce the mineral sediment requirements of the marsh (DeLaune et al., 2003).

Measuring the spatial gradient of bulk density, accretion, and mineral content in the receiving basin of the Caernarvon diversion revealed a significant increase in mineral sediment deposition, organic matter accumulation, and nutrient input at the sites nearest the diversion structure (DeLaune et al., 2003). However, more mineral sediment needs to be diverted into the coastal marshes to have a significant impact on slowing relative sea level rise (RSLR) (Nyman et al., 1990).

1.2.2 Salinity

Wetland plants have different levels of tolerance to salinity, with freshwater adapted species being most sensitive to salinity changes (Batzer and Shartiz, 2006). Salt can negatively impact plant productivity by three mean: 1) osmotic stress, 2) accumulation of hydrogen sulfide, and 3) inhibition of nutrient uptake. Osmotic stress occurs when the water potential (ψ) of the soil becomes less than the water potential of the plant, preventing water from flowing into the roots, and subsequently inducing physiological drought (Batzer and Shartiz, 2006). Sulfate is the second most abundant anion in salt water. When sulfate is exposed to reducing conditions typically found in wetlands, it can be reduced to H_2S, which is considered a phytotoxin. Sulfide concentrations greater than 10μg S g^{-1} will inhibit root production in *S. alterniflora* (DeLaune et al., 1983). Some of the mechanisms suggested for the growth inhibition caused by H_2S include the blocking of a key enzyme involved in alcohol dehydrogenase (ADH), a form of anaerobic respiration, and the inhibition of N uptake (Koch

and Mendelssohn, 1990). Finally, the abundant Na^+ ions in salt water can compete with NH_4^+, thus inhibiting the plants ability to assimilate N (Bradley and Morris, 1991).

Due to the high rate of RSLR in Louisiana, salt water from the Gulf is moving farther inland, converting brackish marshes into salt marshes, and freshwater marshes into brackish. Under natural conditions, salt water would encroach on an abandoned delta lobe over the course of thousands of years, allowing time for species composition to evolve. However, in Barataria Basin, Louisiana, this process is occurring over decades and freshwater plants are lost before they can be replaced by more salt tolerant species (Sasser and Dozier, 1986). As previously mentioned, plant mortality can lead to peat collapse and increased erosion.

Increasing salinity in the coastal zone also has economic implications because historic fishing and oyster grounds are being pushed further inland. The low salinity areas are vital for juvenile fish, shrimp, and crab development (Schexnayder and Caffey, 2006).

The Caernarvon diversion has been managed in an effort to move the 15 ppt and 5 ppt isohalines seaward approximately 24 km, near to their historic location in the early 1900s (Villarrubia, 2006). Davis Pond managers have established similar target salinities and are using a hydrodynamic model to determine width and duration the diversion needs to be opened to reach these goals (Mashriqui et al., 2002).

1.2.3 Nutrients

High concentrations of N and P in the Mississippi River are directly related to land use changes, fertilizer application, and population growth in the drainage basin (Mitsch et al., 2001; Turner and Rabalais, 1991; 2003). Nitrogen and P increase primary productivity, which increases organic matter formation. Nitrate concentration in the Mississippi River averages 1.0 to 1.2 mg N L^{-1} (Antweiler at l., 1995). Mississippi River water nitrate

concentrations can be 50 times higher than that of Barataria Basin in the spring, the receiving estuary for the Davis Pond diversion (Battaglin et al., 2001). Excess nutrients can cause harmful algae blooms (HABs) that can ultimately lead to hypoxia during their decline, as well as introduce toxins into the food web (Anderson et al., 2002). Hypoxia caused by the high nutrient load of the Mississippi River is well documented on the continental shelf (Rabalais et al., 2002). Several studies addressing the fate of river nutrients downstream of the diversions have already been completed in Caernarvon (DeLaune and Jugsujinda, 2003; Lane et al., 1999; Lane et al., 2002; Wissel and Fry, 2005), and fewer in Davis Pond (DeLaune et al., 2005; Johnson, 2004). Nitrate/nitrite is rapidly assimilated or transformed downstream of the Caernarvon diversion, with 88 to 97% removal efficiency at low discharge. However, removal efficiency decreased as discharge rate increased (Lane et al., 1999). The increased nutrient supply from the Caernarvon diversion has enhanced plant productivity along the major flow path in Breton Sound and is being incorporated into the food web (Wissel and Fry, 2005).

Although these studies seem promising for the success of the recently completed Davis Pond diversion, there are significant differences in the diversion capacity and the morphology of the receiving basins of the two projects. First, Davis Pond has a greater discharge capacity than the Caernarvon diversion (302 $m^3 s^{-1}$ and 226 $m^3 s^{-1}$, respectively) (U.S. ACOE, 2006). The Breton Sound estuary has experienced continuous inputs of Mississippi River water throughout this century via intermittent crevasses and at least four smaller diversion projects that flow either continuously or with high river stages (Lane et al., 1999). In contrast, Barataria Basin has been completely isolated from the Mississippi River and other freshwater sources since 1904, when Bayou Lafourche was damned (Evers et al.,

1992). Finally, Caernarvon diversion is geographically closer to the Gulf of Mexico and discharges into an open estuary, whereas the Davis Pond diversion discharges into a 3,700 ha "ponding area" confined by earthen levees, then flows into Lake Cataouatche, Lake Salvador, and eventually the Gulf of Mexico.

The Davis Pond marsh (the ponding area) has demonstrated near complete removal of Mississippi River nitrate at low discharge rates (35 $m^3 s^{-1}$), but approximately 0.75 mg N l^{-1} was discharged into Lake Cataouatche at moderate flows (100 $m^3 s^{-1}$) (DeLaune et al., 2005). Lake Cataouatche was found unable to remove all N exported downstream from Davis Pond at a 100 $m^3 s^{-1}$ discharge rate (Miao et al., 2006).

The opening of the Bonnet Carré Spillway in 1997 introduced Mississippi River water into Lake Pontchartrain to reduce the risks associated with high water levels in the river. Following the opening of the spillway in 1997, an extensive blue-green algae bloom persisted in Lake Pontchartrain for a period of two months. Additionally, fish kills were reported, which were attributed to the high nutrients levels from the Mississippi River (Day et al., 1999).

1.3 THE NITROGEN CYCLE

Nitrogen is of particular concern at Davis Pond because it is the limiting nutrient in Barataria Basin (Patrick and DeLaune, 1976). The majority of N entering Davis Pond is in the form of nitrate, and denitrification has been identified as the major process of N removal in the system (DeLaune et al., 2005; Goolsby et al., 2001).

Nitrogen, one of the most abundant elements on earth, is also one of the most bioenergetic, having at least five stable oxidation states (Table 1.1). The existence of multiple oxidation states creates a high potential for N to undergo oxidation-reduction (redox)

reactions. Despite its abundance, N is often the major limiting nutrient in flooded or drained soils, as well as aquatic systems (Mitsch and Gosselink, 2000).

The nitrogen cycle is almost completely controlled by soil micro fauna, most notably, bacteria. The major processes of the N cycle are 1) Mineralization/ Ammonification: the conversion of organic nitrogen to ammonium (NH_4^+), 2) Volatilization: the conversion of ammonium to ammonia gas (NH_3), a process that only occurs at pH $\geq$ 7, 3) Nitrification: the oxidation of ammonium to nitrate (NO_3^-), 4) Denitrification: the reduction of nitrogen oxides to nitrous oxide (N_2O) or elemental nitrogen (N_2), and 5) Nitrogen Fixation: the conversion of elemental nitrogen to organic nitrogen (Figure 1.2).

Since N is a major limiting nutrient, adding N fertilizers can be an effective way to increase agricultural production. Nitrogen is normally applied to agricultural fields as NH_4^+ or urea. Soil naturally has a net negative charge, so the ammonium ion adheres to the soil and promotes plant growth. However, if oxygen is present, it is thermodynamically favorable for specific bacteria (e.g. *Nitrosomonas spp.* and *Nitrobacter spp.*) to use NH_4^+ as an electron donor, resulting in the oxidation of ammonium. The product of this nitrification is an anion (NO_3^-, NO_2^- or NO^-), which is subsequently repelled by the soil, highly soluble, and easily

Table 1.1 Naturally occurring oxidation states of nitrogen and their most common molecular form.

Oxidation State	Common Forms
+5	NO_3^-
+3	NO_2^-
+1	N_2O
0	N_2
-3	NH_3, NH_4^+

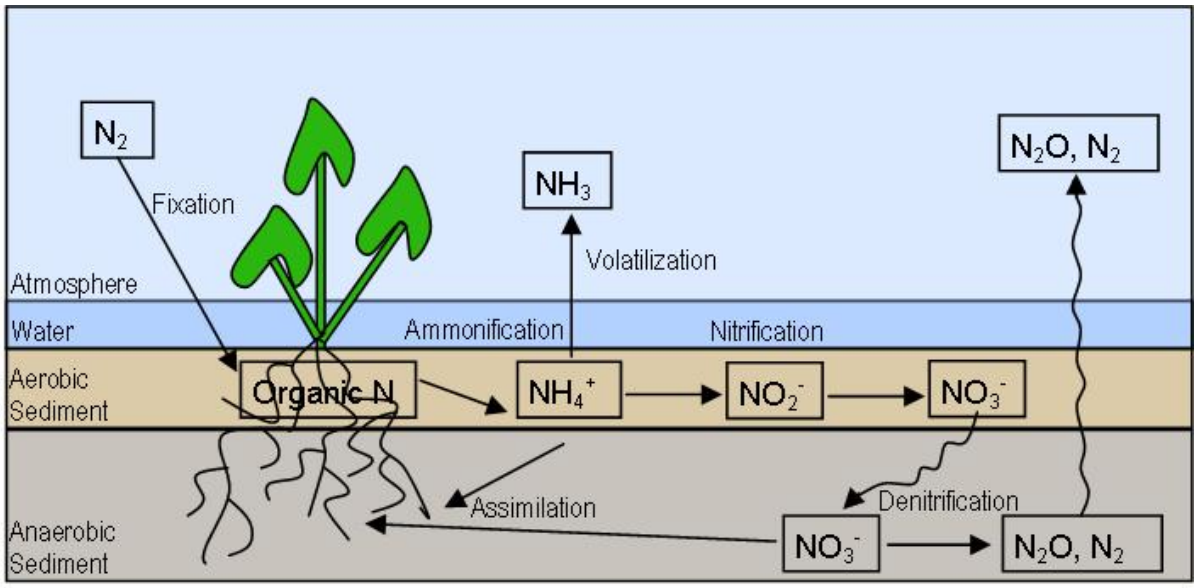

Figure 1.2 Major pathways for nitrogen transformation and the location/redox condition in which they normally occur.

leached into nearby water bodies or groundwater. Once in aquatic systems, N oxides are very mobile and have a similar effect as in agricultural systems- they stimulate the growth of aquatic plants. Phytoplankton can exploit excess nutrients and proliferate into an algal bloom. Not only can some species of alga produce toxins, but when the bloom dies, the phytoplankton sink and accumulate on the sediment surface. Detritivores (fauna that obtain energy from breaking down dead material) soon proliferate in response to the food source. Detritivores can respire most (or all) of the oxygen in the water column, creating a condition known as hypoxia (or anoxia), in which few macro fauna can exist due to the lack of oxygen.

A local and frequently cited case of excess N contributing to harmful algae blooms (HABs) occurs annually in the northern Gulf of Mexico. First recorded in the 1970s, an area of the continental shelf up to 20,000 km^2 in size has been dubbed the "dead zone" because bottom waters have a dissolved oxygen concentration < 2 mg l^{-1} (Rabalais et al., 2001;

Rabalais, 2000). Increases in N fertilizer use in the Mississippi River watershed has been correlated with increased concentrations of river nitrate, increased primary productivity on the shelf, hypoxia, and fish kills (Antweiler et al., 1995; Mitsch et al., 2001; Rabalais et al., 2000; Turner, 1991).

1.3.1 Denitrification

Denitrification is unique as a major pathway of nitrogen removal from the environment through gaseous diffusion into the atmosphere. Phosphorus, however, does not have a significant stable gas phase, and can be more difficult to remove from aquatic systems. Ideal conditions which promote denitrification involve both biotic and abiotic factors. First, the microbes capable of nitrate reduction must be present and active in the soil. Second, two of the regulating factors (nitrate and carbon) must be present and there must be a paucity of O_2.

Denitrifiers are free-living, facultative, aerobic bacteria. These organisms can be classified as either true denitrifiers, which are capable of catalyzing the entire denitrification pathway ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$), or partial denitrifiers, which can only catalyze one or a few steps in the pathway. Denitrifiers are diverse, including species from every major physiological and taxonomic group of prokaryotes, and both gram-positive and gram-negative bacteria (Ingraham, 1981). Denitrifiers are thought to be ubiquitous in the soil, suggesting that denitrification is not limited by presence of denitrifiers, but limited by ideal conditions. The most numerous genera of denitrifiers are *Pseudomonas*, followed by *Alcaligenes* and *Flavobacterium* (Germon, 1985).

Specialized reductase enzymes regulate each step of the denitrification process. *Nitrate reductase* is the most well studied denitrifying enzyme, and is synthesized via similar

mechanisms by a variety of species (Bryan, 1981). The *nitrate reductase* enzyme is a highly soluble, membrane-bound molybdoprotein, which is synthesized only under anaerobic conditions (Payne, 1985). The presence of nitrate is required for the production of *nitrate reductase*, and the concentration synthesized is directly proportional to the concentration of nitrate in the medium (Downey, 1966).

The second step in the denitrification pathway, the reduction of nitrite to nitric oxide (NO), is regulated by *Nitrite reductase.* One of two distinct types of enzymes can be involved in *nitrite reductase*, a copper containing non-heme protein, or a non-copper containing *cd*-cytochrome (Bryan, 1981). Since NO does not appreciably accumulate in nature, there is speculation as to whether NO is a true intermediate of denitrification. Additionally, the *nitric oxide reductase* enzyme has not yet been purified (Payne, 1985).

The final step of denitrification is catalyzed by *nitrous oxide reductase*, a non-heme copper-containing enzyme (Payne, 1985). *Nitrous oxide reductase* is at least partially repressed by oxygen and is the only denitrification pathway inhibited by acetylene (Bryan, 1981).

1.3.2 Oxygen

In order for denitrification to occur, oxygen must be limiting. In both freshwater and marine systems, an oxygen concentration of ≤ 0.2 mg l^{-1} is needed for denitrification to commence (Seitzinger, 1988). Denitrifiers are facultative aerobes, meaning they are capable of utilizing O_2 as the terminal electron acceptor. In accordance with the first and second laws of thermodynamics, the Gibbs free energy (ΔG) for O_2 is lower than the ΔG for NO_3^-, making it energetically favorable for microbes to use O_2 as a terminal electron acceptor when available (Fenchel et al., 1998). However, when O_2 is depleted, NO_3^- has the next lowest ΔG.

Therefore, nitrate is only respired by facultative aerobes under anaerobic or moderately reduced conditions (Bryan, 1981). Oxygen affects both the activity and synthesis of denitrifying enzymes (Knowles, 1982). Although denitrifying enzyme synthesis is repressed in the presence of oxygen, the stability of the enzyme does not appear to be affected if O_2 concentration increases following synthesis (Bryan, 1981).

The relationship between oxygen and denitrification can become complicated when nitrate is limiting. Ammonium is the dominate form of inorganic nitrogen in anaerobic systems. In order for ammonium to be converted to nitrate, it must first be oxidized, after which it can undergo denitrification. The coupling of nitrification and denitrification has been demonstrated in several flooded systems (Patrick and Reddy, 1976; Reddy and Patrick, 1975). The occurrence of aerobic zones adjacent to anaerobic zones in shallow surface water systems and wetlands make these environments ideal for promoting coupled nitrification-denitrification (Jones, 1985). Maio et al. (2006a) found that 59% of denitrification in a shallow lake was a result of sequential nitrification-denitrification. The coupling of these

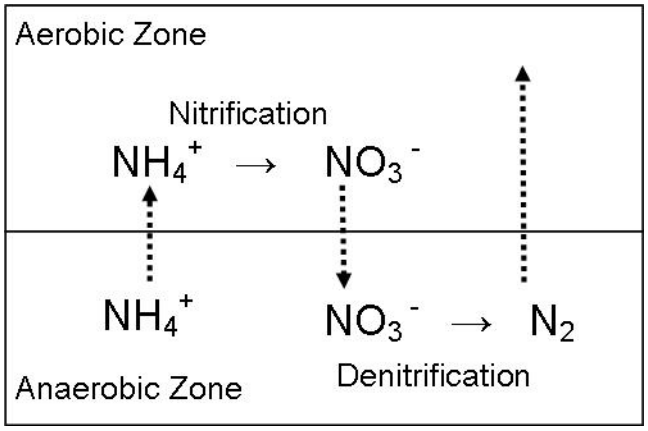

Figure 1.3 Diffusion gradients associated with the coupling of nitrification and denitrification in sediments of different oxygen content.

redox reactions can be strongly regulated by diffusion. Ammonium must diffuse upward into the aerobic zone to be nitrified; nitrate must diffuse downward to the anaerobic zone to be denitrified (Figure 1.3) (Patrick and Reddy, 1976). Diffusion is driven in response to the concentration gradient, in accordance with Fick's laws of diffusion. At times the distance a molecule must travel is very small because soils are highly heterogeneous, developing micro zones of aerobic and anaerobic processes on a single soil particle or within the rhizosphere (Parkin, 1990).

In summary, denitrification requires anaerobic conditions. However, adjacent zones of aerobic and anaerobic soils can promote coupled nitrification-denitrification, which can substantially increase the net rate of denitrification as N species diffuse between the two layers in response to the concentration gradient.

1.3.3 Nitrate

Nitrate is not only required for denitrification enzyme synthesis, but the concentration of nitrate determines the amount of *nitrate reductase* enzymes produced (Bryan, 1981). Both nitrate (NO_3^-) and nitrite (NO_2^-) can be denitrified, however, the instability of nitrite makes it a temporary intermediate. Therefore, nitrate concentration is considered the controlling factor for denitrification in wetland systems.

There are three general sources of nitrate for soil microbes, 1) nitrate present in the water column that diffuses into the sediment, 2) organic nitrogen in the sediment that is mineralized to ammonium and subsequently nitrified, and 3) nitrate advected from groundwater. Factors such as vegetation and bioturbation can increase nitrate concentrations in the sediment by providing oxygen for nitrification. For example, the activity of a benthic polychaete has been shown to significantly enhance potential denitrification in the top 0.5 cm

of sediment (Sayama and Kurihara, 1983). In wetlands, where anaerobic conditions normally occur along with the accumulation of organic matter (a carbon source), nitrate is typically the factor limiting potential denitrification rates (Cooper, 1990; White and Reddy, 1999). A study conducted in a wastewater treatment wetland found a direct relationship between nitrate load and nitrate removal efficiency (Blahnik and Day, 2000; Gale et al., 1993).

1.3.4 Carbon

Most denitrifiers are chemo-organotrophs, meaning they obtain energy from the chemical reduction of organic carbon compounds. Carbon supply influences denitrification directly by providing a substrate for microbial growth, and indirectly through the consumption of O_2 by microbes which creates anaerobic conditions (Chalamet, 1985). The rate of denitrification can be correlated with total C (TC), water-soluble C, as well as the rate of C mineralization (Reddy et al., 1982). When nitrate is added to an anaerobic soil, the most easily degradable C is quickly utilized as an electron acceptor. As the remaining C store becomes more recalcitrant, the rate of denitrification is proportional to the rate of C mineralization (Focht and Verstraete, 1977). Several studies have added glucose (a simple, easily degradable C source) and observed a substantial increase in denitrification rate. As the recalcitrance of the remaining C increases, the effect of added glucose becomes more pronounced (Payne, 1981).

1.3.5 Other Factors Affecting Denitrification

Several additional factors have been cited as secondary controls of denitrification. These include pH, temperature, and soil properties. Although pH does not appear to be a significant factor limiting denitrification, rates are most rapid at a pH of 7 to 8 (Chalamet, 1985). However, pH may influence the completion of the denitrification pathway. Nitrous

oxide production is more common at a pH of 7 or less. It may also be formed at a pH above 7, but it is typically diffuses back into the anaerobic zone and is reduced to N_2 (Bryan, 1981). Temperature can have several indirect effects on denitrification because it influences microbial activity, oxygen solubility, and oxygen diffusion. The Q_{10} for denitrification has been studied in several systems. Results indicate Q_{10} values typically range from 1.5 to 3.5 (Chalamet, 1985; Seitzinger, 1988). Soil texture can be important as it relates to surface to volume ratios (S:V). Larger S:V provides a greater number of sites for microbial colonization and aerobic/anaerobic micro zones (Chalamet, 1985). Denitrification rate is normally greatest in organic and fine-textured mineral soils. However, this may be attributed to the typically higher carbon content of these soil types (Dury et al., 1991).

1.3.6 Additional Pathways for Nitrate Reduction

There are three alternate pathways that may compete with denitrification for nitrate; assimilatory nitrate reduction to ammonium (ANRA), dissimilatory nitrate reduction to ammonium (DNRA), and anaerobic ammonium oxidation (anammox). Assimilatory nitrate reduction to ammonium has been recognized for many years and involves the assimilation of nitrate proteins and amino acids, followed by reduction to ammonium (Reddy and White, 1997). ANRA is suppressed when ammonium is present and is unaffected by oxygen concentration (Germon, 1985).

In contrast, DNRA was discovered recently. It also reduces nitrate to ammonium, but does so as a means of energy generation by using nitrate as an electron acceptor. DNRA has the potential to compete with denitrification because it occurs in anaerobic conditions and is not suppressed by ammonium (Germon, 1985). The DNRA pathway is controlled by fermentative and obligate anaerobic bacteria that exist under nitrate-limiting conditions where

C availability is high. This circumstance is consistent with deep-water, continuously anoxic sediments where the redox potential may drop below 0 mV (Kelso et al., 1997). Evidence of DNRA includes the accumulation of nitrite, which may be a result of the repression of nitrite reductase, and an increase in ammonium, especially at depth within the soil profile (Kelso et al., 1997). Understanding the mechanisms that control whether nitrate is reduced to ammonium (DNRA) or N_2 gas (denitrification) is important because DNRA retains N in the system, rather then releasing it into the atmosphere as does denitrification. Current literature suggests denitrification is the dominate pathway in high nutrient conditions and in systems that undergo periods of oxygenation. However, DNRA may predominate in marine and estuarine systems that are continuously anoxic and develop strongly reduced conditions. Under these circumstances, the high electron availability can favor DNRA (which requires 8 electrons) over denitrification (which requires 5 electrons). The high sulfide concentrations in seawater may also enhance DNRA by serving as an electron donor (An and Gardner, 2002).

The anammox pathway was discovered very recently and is defined as the oxidation of ammonium with nitrite to produce N_2 and H_2O. Anammox has been documented in wastewater systems (Mulder et al., 1995) and estuarine sediments (Rich et al., 2008) that are characterized by continuously high inputs of NO_3^- and NH_4^+.

1.3.7 Nitrous Oxide Emissions

Nitrous oxide (N_2O) is a greenhouse gas 300 times more potent than CO_2 on a per mole basis; it is also a free obligate intermediate of denitrification. The majority of denitrified nitrate completes the entire pathway to N_2 gas. However, a small portion can be released as N_2O, especially in upland soils where the absence of water allows for quicker diffusion. The

ratio of N_2O/N_2 produced by denitrification varies significantly (Schlesinger, 1997). The factors affecting this ratio are poorly understood, but vitally important in relation to global climate change. In addition to nitrification and denitrification, nitrous oxide is emitted from industrial processes, fossil fuel combustion, soil disturbance (such as tillage), fertilizer application, human waste disposal, and is naturally produced in the ocean. Since the industrial revolution, atmospheric concentrations have increased from 280 ppb to 311 ppb (Schlesinger, 1997).

Research suggests that higher concentrations of NO_3^- and NO_2^- released into the environment will increase the proportion of N_2O relative to N_2, and that the larger the component of NO_2^- present, the more N_2O produced (Firestone et al., 1979; Hefting et al., 2003). In constructed wetlands for wastewater treatment, a gradient within the wetland has been observed, with higher N_2O emissions nearest the inflow where loading is highest and significantly lower emissions near the outflow (Sovik et al., 2006). The oxygen status of soil is also important to N_2O production. The presence of O_2 inhibits each step denitrification, thus increasing the mole fraction of N_2O that is not completely reduced to N_2. Oxygen may even specifically inhibit *nitrous oxide reductase* synthesis and activity (Yoshinari, 1990). The presence of vegetation can increase the portion of N_2O, possibly as a result of oxygen released from the rhizosphere and gas transport through the plant (Sovik et al., 2006). Temperature appears to be inversely correlated with the product ratio, with the mole fraction of N_2O increasing as temperatures decrease (Keeney et al., 1979). Finally, a low pH may increase the proportion of N_2O relative to N_2 (Yoshinari, 1990). Managing wetlands to reduce the emissions of N_2O will likely become an important subject in the near future as global warming concerns increase.

1.4 DENITRIFICATION ENZYME ACTIVITY (DEA)

Denitrification enzyme activity (DEA) is an assay used to quantify the initial rate, or Phase I, of denitrification using the acetylene block technique to prevent the reduction of N_2O to N_2. DEA represents the activity of denitrifying enzymes *in situ* (i.e., enzymes active at the time of sampling), and therefore is indicative of the historic site conditions (Tiedje et al., 1989). The rate represents not only enzyme activity, but also the environmental factors that control enzyme expression (oxygen content, C availability, and nitrate concentration). In anaerobic environments without carbon limitations, the amount of enzyme produced is proportional to the concentration of nitrate available, and the rate of N_2O production is proportional the enzyme content (Bryan, 1981; Tiedje et al., 1989).

Although DEA is normally considered a quantification of potential denitrification because all limiting factors are removed, studies have also indicated it may be useful in estimating field denitrification rates. Groffman and Tiedje (1989) found a strong correlation between annual denitrification N loss and DEA in a temperate forest. DEA was also correlated with field denitrification rates in an organic riparian soil (Schipper et al., 1993). However, other attempts to establish such a relationship have not succeeded, and the lack of correlation is often attributed to high spatial and temporal variability of denitrification in the landscape (Parkin, 1990). The problem of extrapolating DEA to landscape scale denitrification rates is most prevalent in upland soils. Denitrifying enzymes are known to be repressed or deactivated in the presence of oxygen, even trace amounts of O_2 can repress N_2O production during DEA assays (Martin et al., 1988; Murray and Knowles, 2004). Upland soils are better drained and more likely to be exposed to oxygen. In addition, they tend to exhibit limitations in organic C, which can restrict denitrification by reducing microbial

activity and decreasing the water holding capacity of coarse-textured soils (Groffman and Tiedje, 1989; Parsons et al., 1991). This explains why denitrification rates in upland soils are most strongly correlated with C availability and soil respiration (Myrold, 1988; Parsons et al., 1991). DEA rates can vary significantly depending on soil and landscape variables (Table 1.2).

Table 1.2 Example DEA rates from the published literature.

Location	Soil Type/Use	DEA *(g N kg^{-1} h^{-1})*	Reference
Devon, UK	Upland silty clay/Agriculture	1.25 – 1.87	Dendooven and Anderson, 1995
Everglades, Florida USA	Wetland Histosol	0.04 - 7.75	White and Reddy, 1999
Kentucky, USA	Upland Mollisol/Sod	0.93 ± 0.05	Martin et al., 1988
Kentucky, USA	Upland Alfisol/Pasture	0.102 - 5.4	Smith and Parsons, 1985
North Island, New Zealand	Riparian Inceptisol	0.81 ± 0.4	Schipper et al., 1993

Conversely, wetland soils are anaerobic for part or most of the year and accumulate large quantities of organic carbon. Water logging reduces oxygen diffusion into the soil, which is at least partly responsible for poorly-drained soils having higher denitrification activity. For example, a well drained clay loam in Michigan exhibited half the annual N loss from denitrification of a poorly drained clay loam (18 kg N $ha^{-1}yr^{-1}$ and 40 kg N $ha^{-1}yr^{-1}$, respectively) (Groffman and Tiedje, 1989). With two of the three factors controlling denitrification satisfied in wetland soils (i.e. anoxic conditions and ample carbon), nitrate concentration becomes the limiting factor for denitrification (Cooper, 1990). The correlation

between nitrate and DEA in flooded soils was first observed by Reddy et al. (1978) when high nitrate concentrations in flood water enhanced the diffusion gradient delivering nitrate into the soil, thus increasing the rate of denitrification. Schipper et al. (1993) applied this concept to a riparian soil and found a strong correlation between NO_3^- concentration and DEA. In the northern Everglades, DEA was measured in the surface soils of a wetland receiving point source of nitrate-rich surface water. A soil transect extending from the nutrient source to the wetland interior revealed an exponential decrease in DEA, which was strongly correlated with a similar decrease in surface water NO_3^- concentration (White and Reddy, 1999). DEA has also proven to be an effective indicator of anthropogenic N loading in fringe salt marshes (Wigand et al., 2004). These studies suggest DEA may be an effective tracer for the spatial distribution of NO_3^- loading in wetlands.

1.4.1 Methods for Quantifying DEA

In performing a DEA assay, all factors that may limit denitrification are removed so the functioning enzymes can be fully expressed. Field moist soil and oxygen-free deionized (DI) water are incubated under anaerobic conditions with 10 kPa pure acetylene to prevent the reduction of N_2O to N_2. Non-limiting quantities of nitrate and available C are added and the slurry is continuously shaken to eliminate diffusion constraints. Finally, chloramphenicol is added to inhibit the synthesis of new enzymes, ensuring the observed N_2O production is exclusively a result of pre-existing enzymes (Smith and Tiedje, 1979; Tiedje, 1982). Chloramphenicol can also inhibit the expression of existing enzymes, especially when sampling occurs over several hours (Brooks et al., 1992; Smith and Tiedje, 1979). For this reason, the assay is limited to a maximum of 2 hours, during which at least four gas samples should be analyzed to confirm a linear 'rate' relationship between mg N_2O kg $soil^{-1}$ hr^{-1} and

time (Tiedje, 1982; Tiedje et al., 1989). Several studies have investigated the implications of adding acetylene to the incubation. In addition to inhibiting N_2O reduction, acetylene (and anaerobic conditions) can also inhibit nitrification, a processes often tightly coupled with denitrification, resulting in an underestimation of the denitrification rate (Berg et al., 1982; Knowles, 1990). Other disadvantages of using the acetylene inhibition technique include possible degradation of C_2H_2 by soil microbes for energy if the added C becomes depleted, potentially stimulating denitrification (Klemedtsson et al., 1990; Knowles, 1990; Tiedje et al., 1989). Most of these problems are nearly eliminated by limiting the incubation time to 2 hours and the advantages of this method (cost effective, simplistic, and fast) seem to outweigh any possible disadvantages.

1.5 SITE DESCRIPTION

The Davis Pond diversion is located in the upper portion of Barataria Basin, a 190 km long estuary located between the west bank of the Mississippi River and Bayou LaFourche. Barataria basin was formed during the progradation phases of the LaFourche Delta Lobe (60-3,500 years before present) and the Plaquemines Delta Lobe (200-1,000 years before present) (Turner and Cahoon, 1987). Presently, all sources of freshwater to Barataria Basin have been removed as a result of the construction of flood control levees along the Mississippi River and the damming of Bayou Lafourche in 1902. Land loss within Barataria Basin occurs at an average rate of 28.7 $km^2\ y^{-1}$, the highest rate among the 10 major coastal basins in Louisiana (Figure 1.4) (Barras et al., 1994). Commonly cited problems leading to land loss in the Basin include erosion, subsidence, sea level rise, herbivory, dredging, levee construction, and development. Approximately 40 percent of the area is wetland (swamp, fresh, intermediate, brackish, or saline marsh) and 22 percent is leveed or developed (LACoast, 2006).

The Davis Pond freshwater diversion is located at 29°55'01"N; 90°19'04" W (NAD83) in St. Charles Parish, Louisiana (hydrologic unit code 08090301). The inflow structure is positioned on the west bank of the Mississippi River near Luling, approximately 19 km upstream from New Orleans. Four 4.3 m^2 box culverts, operated according to salinities with the Basin and the stage of the Mississippi River, allow for a maximum discharge capacity of 302 $m^3 s^{-1}$ (10,650 cfs). River water flows through the diversion by gravity, down a 3 km inflow channel, and into Davis Pond marsh, a 3,760 ha ponding area (Figure 1.5) (US ACOE, 2003). Davis Pond marsh is confined by earthen levees on three sides and an outflow weir along the downstream boundary. The design was intended to temporarily retain water in the marsh until the wetland reached its maximum hydraulic holding capacity, at which point water would sheet-flow over the outflow weir into Lake Cataouatche, Lake Salvador, and eventually Barataria Bay (Figure 1.6). Construction was completed in 2002 by the U.S. Army Corps of Engineers. However, several design modifications have been required along the outflow weir structure to permit greater water transport and to reduce the water depth of the marsh (Letter, 2005; LDWF 2005). Therefore, full-scale operation of the structure did not commence until 2006/2007 (LDNR, 2004; Villarrubia, 2006).

Davis Pond marsh soils consists of moderately decomposed histosols several meters thick, overlying historic fluvial sediments deposited by Mississippi River overflow events. A crevasse in the Mississippi River levee in 1884 assisted in the formation of the current marsh, which has been further modified by logging operations, oil and gas exploration, the installation of two mineral pipelines, and continued hunting and trapping operations (Ensminger and Simon, 1993). The western portion of the marsh contains a series of ridges running east-west that support declining stands of bald cypress (*Taxodium distichum*), tupelo

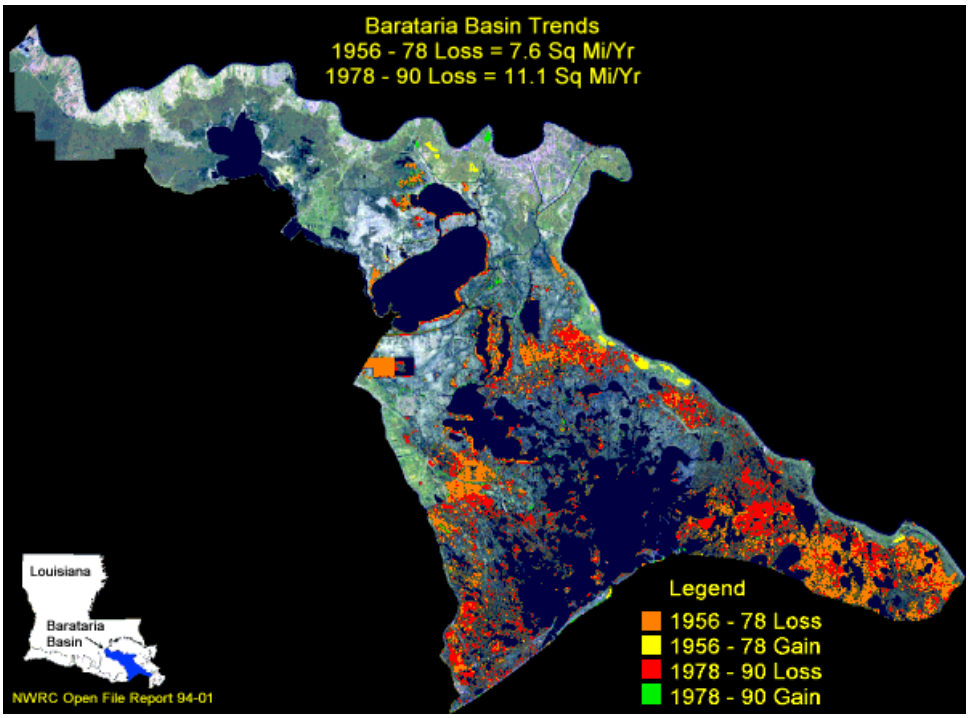

Figure 1.4 Map of Barataria Basin depicting land changes (loss and gain) between 1956 and 1990 (Barras et al., 1994).

Figure 1.5 Aerial photograph of the Davis Pond diversion structure and inflow canal (US ACOE, 2003).

gum (*Nyssa aquatica*), and green ash (*Acer rubrum*) (Figure 1.7). The majority of the area is characterized by emergent herbaceous plants, predominately bulltongue (*Sagittaria lancifolia*), water hyacinth (*Eichhornia crassipes*), alligator weed (*Althernathera philoxeroides*), *Biden spp*. and *Typha spp.* (Ensminger and Simon, 1993), with pockets of open water and naturally developing channels (Figure 1.8).

Figure 1.6 Aerial photograph depicting Davis Pond and surrounding area.

Figure 1.7 Photograph of Bald cypress (*Taxodium distichum*) ridges in Davis Pond (Jeremy Conkle, 10/20/07).

Figure 1.8 Photograph of herbaceous plant community (*Sagittaria lancifolia*) and open water areas. (Brett Marks, 5/16/07).

CHAPTER 2: LABORATORY STUDY

NITRATE LOADING EFFECTS ON RATE AND DISTRIBUTION OF DENITRIFICATION ENZYME ACTIVITY IN AN ORGANIC WETLAND SOIL

2.1 INTRODUCTION

Nutrient concentrations of the world's surface waters have increased dramatically over the past several decades. This increase has been linked to anthropogenic causes, including land-use changes, increased synthetic fertilizer use, sewage disposal, and fossil fuel consumption (Mitsch et al., 2001; National Research Council, 2006). Nitrogen (N) is normally the limiting nutrient in aquatic systems (Mitsch and Gosselink, 2000), and consequently, excess N is often cited as the main cause of eutrophication, harmful algal blooms, and hypoxia (Anderson et al., 2002; Rabalais et al., 2001).

Denitrification provides a major pathway for N removal from terrestrial and aquatic systems through gaseous diffusion to the atmosphere. Denitrifying microbes synthesize specialized *reductase* enzymes which allow the organism to utilize N oxides, in the place of O_2, as the terminal electron acceptor in the generation of ATP. Organisms capable of denitrification are thought to be ubiquitous in the soil, suggesting denitrification is not limited by presence of denitrifiers, but the appropriate environment for their expression. This environment must include anaerobic conditions, a high concentration of available carbon (C), and the presence of nitrate (Germon, 1985). The most studied of these enzymes, *nitrate reductase*, is only synthesized and/or activated under anaerobic conditions where nitrate is present (Bryan, 1981; Tiedje, 1982).

Denitrification assays include two distinct phases. The initial rate, or Phase I, is a zero order reaction which occurs over 1-3 h of anaerobic soil incubation and represents potential denitrification enzyme activity (DEA). Phase II is a first order reaction which begins after ~4 h and reflects the synthesis of new enzymes, as seen by the dramatic increase following the addition of C and/or N (Smith and Tiedje, 1979). Both DEA and denitrification have

inherently high spatial variability (Parkin, 1990), but are important to distinguish and quantify due to their ecological importance. DEA corresponds with *in-situ* denitrification- the amount of enzymes active during sampling indicates the favorability of the site to support denitrification and the system's current capacity for N removal (Tiedje et al., 1989). DEA is therefore considered to be more directly related to field denitrification rates than Phase II or potential denitrification rates (Smith and Tiedje, 1979). However, several studies have found no correlation between DEA and actual field denitrification rates, possibly due to the stability of denitrifying enzymes (Groffman, 1987; Martin et al., 1988; Parsons et al., 1991). When DEA is viewed as a time-averaged rate, the relationship with field denitrification rates strengthens. DEA was found to explain 86% of the variation in annual denitrification in a temperate forest soil (Groffman and Tiedje, 1989). Schipper et al. (1993) also found a strong correlation between DEA, NO_3^- concentration, and field denitrification rates ($r^2 = 0.77$, $p < 0.05$) in a riparian wetland soil.

The amount of denitrifying enzymes synthesized is directly proportional to the concentration of nitrate in the medium (Downey, 1966), suggesting DEA reflects the nitrate concentration in aquatic systems. Theoretically, the relationship between denitrification rate and surface water nitrate concentration is first-order. High nitrate in overlying floodwater enhances the diffusion gradient at the soil-water interface, increasing the rate of nitrate transmission to the soil, and therefore increasing the rate of denitrification (Reddy et al., 1978). There are several conditions which can create challenges in correlating DEA with nitrate concentration. For example, if soils have a high mineral content or experience aerobic periods, C may be the limiting factor for denitrifying enzyme synthesis (Dhondt et al., 2004; Parsons et al., 1991). DEA may exhibit no relationship with nitrate concentration in some

systems because assimilation and immobilization may be the dominate N removal mechanisms (Groffman et al., 1992).

Organic wetland soils provide an ideal environment for the promotion of denitrification since they are characterized by anaerobic conditions and C accumulation. With two of the three major conditions for denitrification satisfied, nitrate becomes the limiting factor (Cooper, 1990). DEA showed a strong correlation ($p < 0.01$) with surface water nitrate in a south Florida wetland receiving a point-source input of agricultural drainage water, represented by an exponential decrease of DEA in the upper 10 cm of soil with increasing distance from the loading source (White and Reddy, 1999).

We conducted a laboratory experiment to test the hypothesis that DEA can be used as an indicator of surface water nitrate concentration in an organic wetland soil. We also investigated 1) DEA with depth, 2) the effect of time of nitrate loading on DEA, and 3) the correlation between DEA and wetland soil properties.

2.2 MATERIALS AND METHODS

2.2.1 Experimental Design

Forty intact replicate soil cores were collected within a 20 x 20 m^2 area in the southwest quadrant of Davis Pond marsh (St. Charles Parish, Louisiana; 29°52'44" N, 90°15'47" W) on June 25, 2007 (Figure 2.1). On the sampling date, the mean discharge rate of the Davis Pond Freshwater Diversion was 32.8 $m^3 s^{-1}$ (Figure 2.2). Davis Pond marsh began receiving diverted Mississippi River water in 2002 at discharge rates ranging from 0 to 320 $m^{-3} s^{-1}$ (US ACOE, 2003). The area of the marsh where the cores were collected was characterized as emergent freshwater and was colonized almost exclusively by *Sagitaria lancifolia* (common name, bull tongue). A minimum of 20 cm of the soil profile was

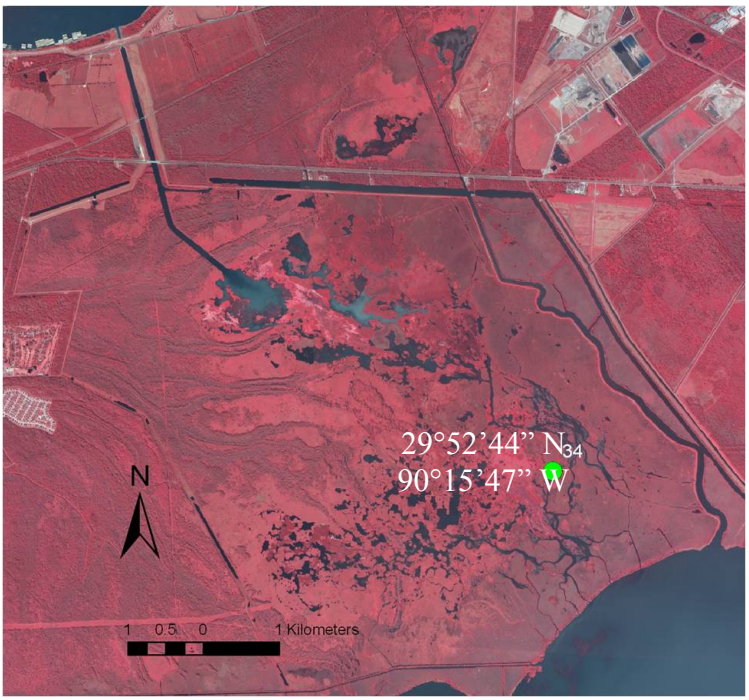

Figure 2.1 Location map where intact soil cores were collected on July 25, 2007.

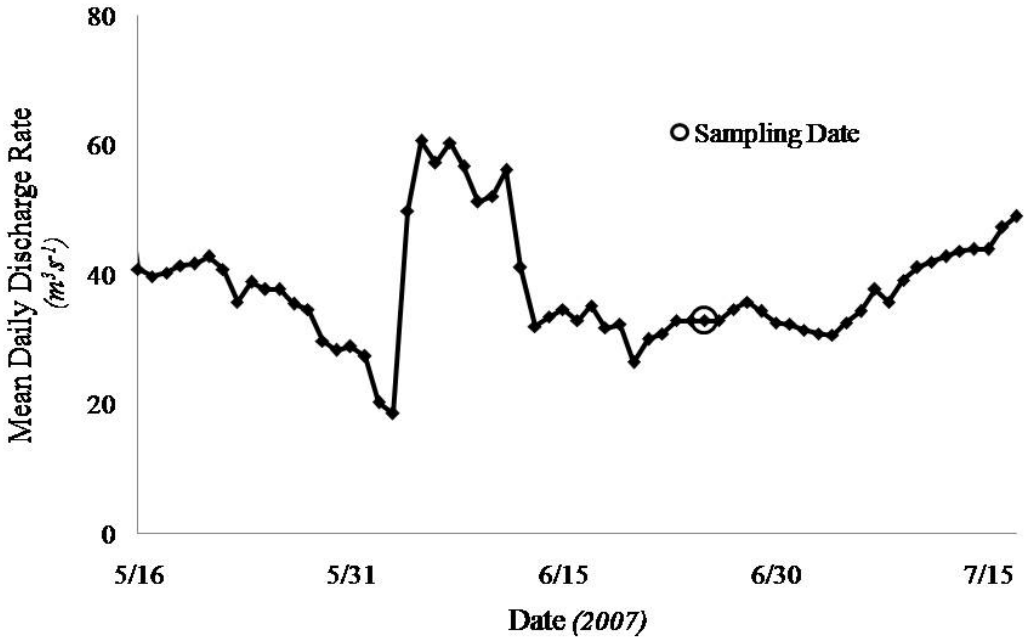

Figure 2.2 Mean daily discharge rate of the Davis Pond diversion when the intact soil cores were collected.

collected in 7-cm diameter clear Plexiglas tubes using care to minimize compaction (< 25%) during coring. Cores were removed from the ground, fully flooded with site water, sealed with rubber stoppers, and transported back to the lab. Upon visual inspection, four cores with unusually large root masses or rhizomes were discarded to minimize variability. All surface water was removed and cores were wrapped in aluminum foil to the soil-water interface for light exclusion. A 1-cm diameter hole was drilled in each core tube exactly 10 cm above the soil surface. This hole served as a drain to ensure identical water column depth among cores.

Four groups of nine (36 total replicate cores) were randomly assigned to one of four NO_3^--N concentration treatment groups: 0.0, 0.5, 1.0, or 2.0 mg NO_3^--N l^{-1}. Concentrations were chosen to simulated levels commonly observed in the Mississippi River (Antweiler et al., 1995). The lower half of each core was submerged in a water bath to minimize temperature fluctuations and cores were incubated in the dark. Nitrate solution was continuously pumped into each core using peristaltic pumps that turned-over the water

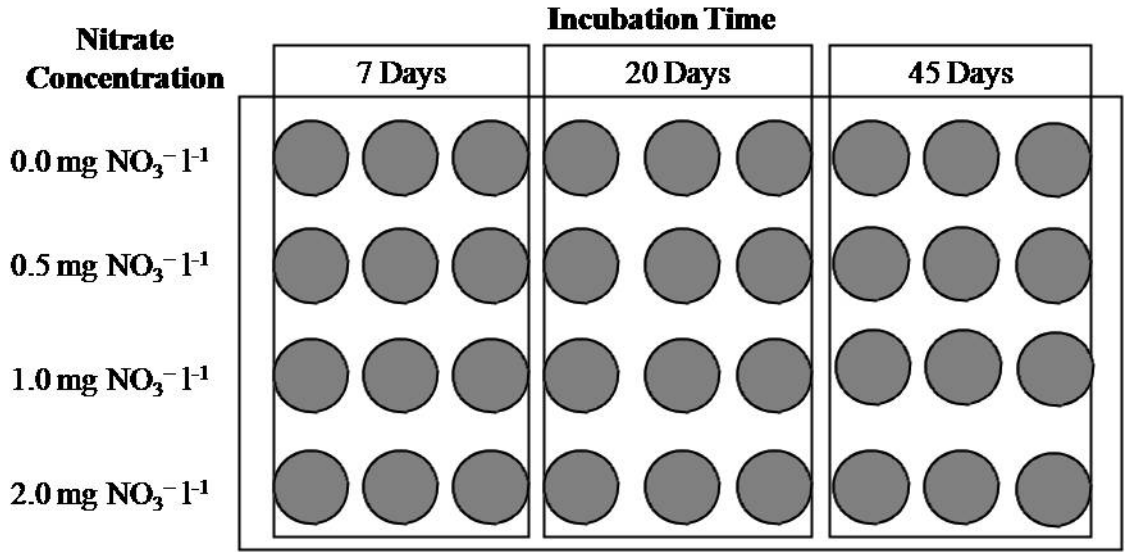

Figure 2.3 Schematic of the experimental design where each field replicate intact soil core (represented as a circle) was randomly assigned to 1 of 4 nitrate treatments and 1 of 3 incubation times.

column approximately 4 times daily. Three cores from each nitrate treatment (3 cores x 4 treatments = 12 total cores) were randomly chosen and sacrificed by separating into 3 depth segments (0-5 cm, 5-10 cm, and 10-20 cm) after either 7, 20, or 45 days of nitrate loading (Figure 2.3). Surface water within the cores was collected approximately every 9 days and analyzed for NO_3^--N (EPA Method 353.2; US EPA, 1983) using a SEAL AQ2 Automated Discrete Analyzer (SEAL Analytical, West Sussex, England). Dissolved oxygen, temperature, and redox potential (Eh) were also monitored over the course of the incubation. Redox measurements were obtained using a platinum working electrode and a saturated calomel (SCE) reference electrode. A correction factor of 245 mV was applied to all readings. Soil samples were homogenized and stored in the dark at 4°C until analyzed for soil properties and DEA.

2.2.2 Soil Characterization

The following soil characteristics were analyzed on the sectioned soils at the end of the incubation period: moisture content, bulk density, total C (TC), total N (TN), extractable NO_3^-, extractable NH_4^+, microbial biomass C (MBC), and microbial biomass N (MBN). Moisture content and bulk density were determined after drying a subsample at 70°C until constant weight and calculating percent water and soil g per volume (cm^{-3}). TC and TN were measured on the dried, ground subsample 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^- and extractable NH_4^+ were measured using soil extractants (25 ml of 0.5 *M* K_2SO_4) and were analyzed on 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 limits for NO_3^- and NH_4^+ were 0.006 and 0.007 mg l^{-1}, respectively.

Microbial biomass C and N were determined using the fumigation-extraction method (Vance et al., 1987). Duplicate 5 g wet weight samples were prepared in 25 ml centrifuge tubes. One set was designated as the fumigate and the other as the non-fumigate. One-half ml of pure chloroform was added to each fumigate sample, then the samples were placed in a dessicator with a beaker containing ~50 ml of chloroform and 5-10 boiling stones. All air within the desiccators was removed and re-filled with room air 3 consecutive times, and then the desiccator was sealed under a vacuum and placed in a fume hood for 24 h. After 1 day, the chloroform was completely evacuated from the desiccator with 7 cycles of removing the headspace, and then refilling it with room air. Following the chloroform treatment, fumigate and non-fumigate samples underwent the same treatment. Twenty-five milliliters of 0.5 *M* K_2SO_4 was added to each sample, samples were shaken for 30 min, and then centrifuged. The supernatent was vacuum filtered using 47 mm Whatman filter paper and stored at 4°C until analyzed for total organic carbon (TOC) (Shimadzu Scientific Instrument TOC-VCSN, Columbia, MD). MBC was determined by subtracting the TOC of the non-fumigate from the corresponding fumigate sample. An extraction efficiency coefficient of $k_{EC} = 0.37$ was applied (Sparling et al., 1990)

MBN was measured using the chloroform fumigation method developed by (Brookes et al., 1985b), followed by a general total kjeldahl nitrogen (TKN) digestion (Bremner and Mulvaney, 1982) . TKN was quantified on a SEAL AQ2 Automated Discrete Analyzer (SEAL Analytical, West Sussex, England) using US EPA Method 351.2 with a detection limit of 0.035 mg l^{-1} (US EPA, 1983). An extraction efficiency coefficient of $k_{EN}= 0.54$ was applied (Brookes et al., 1985a).

2.2.3 Denitrification Enzyme Activity (DEA)

DEA was measured in accordance with the methods outlined in Tiedje (1982), with adaptations by White and Reddy (1999). Five grams of wet weight soil were added to a glass serum bottle. The bottle was sealed with a rubber septa and aluminum crimp cap. The headspace was evacuated from the bottle to -75 kPa, then purged with O_2-free N_2 gas for one minute. Eight milliliters of N_2 purged DI water was added to create a slurry and approximately 15% of the headspace was replaced with acetylene gas (C_2H_2) while maintaining atmospheric pressure within the bottle (Yoshinari and Knowles, 1976). Bottles were shaken on a longitudinal shaker for 30 min to distribute the acetylene. Eight milliliters of a solution of 56 mg KNO_3^--N l^{-1}, 288 mg dextrose-C l^{-1}, and 2 mg chloramphenicol l^{-1} was added, creating a slight overpressure. Chloramphenicol is an enzyme inhibitor used to prevent *de novo* enzymes from synthesizing during incubation (Smith and Tiedje, 1979). Samples were continuously agitated on a longitudinal shaker in the dark at 25°C and the headspace was sampled at approximately 30, 60, 90, and 120 min. Gas samples were analyzed on a Shimadzu GC-8A ECD (Shimadzu Scientific Instruments, Columbia, MD, detection limit 0.006 mg N_2O-N kg^{-1} h^{-1}) and N_2O production was calculated with consideration for product in a aqueous phase using the Bunsen absorption coefficient (0.544) (Tiedje, 1982). The DEA was calculated as the slope of the line when mg N_2O-N kg $soil^{-1}$ was plotted against time (See sample calculation A.9). Ninety-percent of 0-5 cm samples were measured in duplicate or triplicate, as well as several randomly selected samples from 5-10 cm and 10-20 cm.

2.2.4 Data Analysis

The effect of nitrate treatment, incubation time, and soil depth were determined using a three-way ANOVA model ($P < 0.05$) and the Tukey's Studentize (HSD) post-hoc test. Homogeneity and normality were verified with Levene's Test and Spario-Wilk Test, respectively, both at $P < 0.05$. Log transformations were performed as appropriate. Linear regressions and correlations were performed to determine the relationship between soil properties (bulk density, TC, TN, extractable NO_3^--N, extractable NH_4^+-N, MBC, and MBN) and DEA. All analyses were conducted using SAS 9.1 (SAS Institute Inc., Cary, NC).

2.3 RESULTS

2.3.1 Soil Properties

Bulk density decreased significantly ($r = -0.60$) with depth, from 0.095 ± 0.005 g cm^{-3} at 0-5 cm to 0.059 ± 0.004 g cm^{-3} at 10-20 cm (Tables 2.1 and 2.2). Total C and total N increased significantly with depth ($r = 0.91$ and $r = 0.92$, respectively) and were directly correlated with one another ($r = 0.99$) and indirectly correlated with bulk density (TC $r = -0.67$ and TN $r = -0.68$; Table 2.2). Soil pH was fairly constant, with an overall average of 6.9 ± 0.2. Extractable NO_3^- data was highly skewed due to the abundance of values below the detection limit (0.006 mg l^{-1}). Values for extractable NO_3^- ranged from <0.006 to 0.69 mg l^{-1}, with a mean of 0.12 ± 0.16 mg l^{-1} for all depths. Extractable NH_4^+ ranged from below detection (0.007 mg l^{-1}) to 5.26 mg l^{-1}. Extractable NH_4^+ data was also strongly skewed and increased significantly with incubation time ($r = 0.26$), whereas extractable NO_3^- was not related to time ($r = 0.12$). Microbial Biomass C increased significantly with depth ($r = 0.46$), from 23.8 ± 6.0 g kg^{-1} at 0-5 cm to 30.9 ± 6.6 g kg^{-1} at 10-20 cm. MBN was not correlated with depth and averaged 0.28 ± 0.18 g kg^{-1}. The significant decrease in bulk density and

increase in TC, TN, and MBC with depth is contrary to most studies. This can be explained by the growth patterns of *Sagitaria lancifolia*, the dominate vegetation in Davis Pond marsh, which develops large rhizomes and root mats below 5 cm. Collecting the vertical soil profile required shearing the belowground biomass, which would release soluble C in the lower horizons and increase MBC. When the percent of total C and N represented by microbial biomass was considered (e.g. MBC / TC * 100), both show a significant decrease with depth ($P < 0.01$). The proportion of C as MBC ranged from 12.6 ± 0.4% in the surface 0-5 cm of soil, to 8.0 ± 0.3% at 5-10 cm, and 6.9 ± 0.2% at 10-20 cm. The percent of total N as MBN decreases from 1.9 ± 0.2% to 1.6 ± 0.2%, and 1.1 ± 0.1% for the three depth segments, respectively (Figure 2.4). This represents a mean C:N for microbial biomass of 6:1.

Table 2.1 Select soil properties of intact cores measured after incubation. Data are mean values (n=36 for each depth) ± standard deviation.

Soil Parameter	Soil Depth *(cm)*		
	0-5	5-10	10-20
Total C *(g kg^{-1})*	119 ± 37	323 ± 67	453 ± 27
Total N *(g kg^{-1})*	15.7 ± 2.7	25.2 ± 4.9	33.8 ± 2.0
Bulk Density *(g cm^{-3})*	0.095 ± 0.005	0.073 ± 0.003	0.059 ± 0.004
pH	6.9 ± 0.2	6.9 ± 0.3	7.0 ± 0.2
MBC *(g kg^{-1})*	23.8 ± 6.0	25.4 ± 5.0	30.9 ± 6.6
MBN *(g kg^{-1})*	0.29 ± 0.18	0.39 ± 0.22	0.37 ± 0.22
Extractable NO_3^- *(mg kg^{-1})*	0.03 ± 0.06	0.24 ± 0.18	0.11 ± 0.14
Extractable NH_4^+ *(mg kg^{-1})*	1.73 ± 0.54	1.81 ± 0.97	1.13 ± 0.59

Table 2.2 Product-moment correlation coefficients for soil properties. Bold indicates significance at P < 0.05 (for n = 106, at P = 0.05, r = 0.20, at P = 0.01, r = 0.25).

	Depth	Time	Treat-ment	MBC	MBN	NO_3^-	NH_3^+	DEA	TN	TC	Bulk Dens.
Time	0.00										
Treat-ment	0.01	0.00									
MBC	**0.46**	-0.14	0.00								
MBN	0.18	0.17	-0.11	**0.22**							
NO_3^-	**0.20**	0.12	-0.08	0.04	0.18						
NH_3^+	**-0.31**	**0.26**	-0.11	**-0.40**	0.03	0.13					
DEA	**-0.65**	-0.11	0.14	-0.12	**-0.23**	**-0.26**	0.03				
TN	**0.91**	0.12	0.03	**0.56**	**0.23**	0.18	**-0.35**	**-0.61**			
TC	**0.92**	0.11	0.02	**0.55**	**0.22**	0.16	**-0.37**	**-0.62**	**0.99**		
Bulk Dens.	**-0.60**	-0.09	0.15	**-0.50**	**-0.21**	**-0.22**	0.18	**0.43**	**-0.68**	**-0.67**	
pH	0.05	0.12	0.05	0.17	**0.20**	-0.01	**0.22**	-0.02	0.10	0.07	-0.08

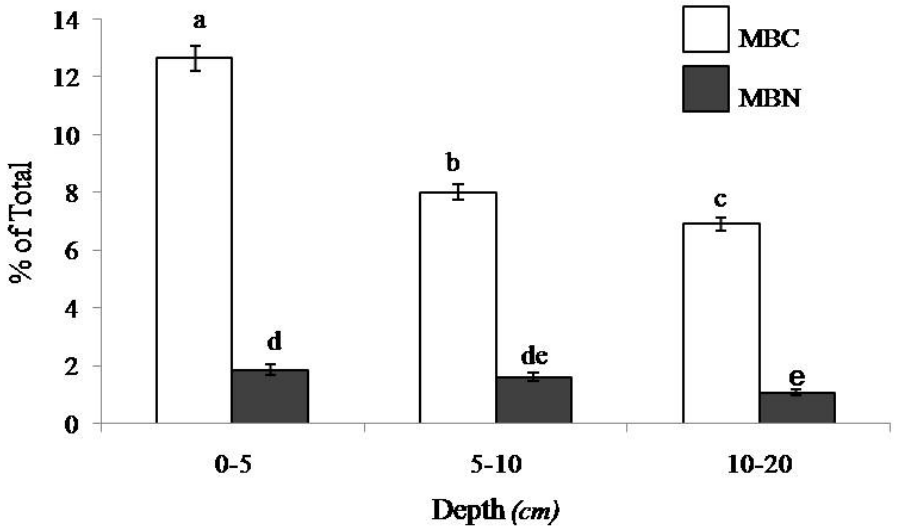

Figure 2.4 Percent of total C and N as microbial biomass (MB) C and N, respectively (i.e. MBC / TC *100 and MBN / TN * 100) with standard error bars, n = 36. Different letters indicate different Tukey's groupings.

2.3.2 Experimental Variables

The average flow rate of nitrate treatment into each core was 55.4 ml h^{-1}, causing the 10-cm water column to turning-over approximately 4 times daily (Table 2.3). The inflow concentration of nitrate added to each core was within 0.01 mg l^{-1} of the specified treatment. Floodwater in the cores had nitrate concentrations up to 27% less than the added treatment concentration, indicating nitrate removal was occurring faster than nitrate loading. Assuming all nitrate in the water column was removed through denitrification, the rate of denitrification for the 2.0 mg l^{-1} treatment (137 ± 24 mg m^{-2} h^{-1}) was significantly higher than for the 1.0 mg l^{-1} treatment (95 ± 29 mg m^{-2} h^{-1}), and both were significantly higher than the 0.5 mg l^{-1} treatment (44 ± 11 mg m^{-2} h^{-1}) at $P < 0.001$ (Table 2.3; See sample calculations A.10). Dissolved oxygen (D.O.) in the surface water ranged from 2.3 to 3.2 mg l^{-1} and temperature averaged 23 ± 1.1°C. Redox potential varied significantly between and within treatments, but was always below 0 mV. The average soil Eh at 3 cm depth was -100 mV, and -107 mV at 7 cm depth (Table 2.3).

2.3.3 DEA

Results from a three-way ANOVA indicated DEA differed significantly with nitrate concentration ($P<0.01$; Table 2.4). Overall, the 1.0 mg NO_3^--N l^{-1} treatment was significantly higher than the control treatment. However, when each incubation time was viewed independently, day 20 was the only day that showed significant differences between nitrate treatment was significantly lower than that of the 2.0 mg NO_3^--N l^{-1} treatment. This trend was significant at both the 0-5 cm depth ($P < 0.05$) and the 5-10 cm depth ($P < 0.01$) and was also observed when correlating treatment and DEA on day 20 (Table 2.5). For all incubation times, measurable DEA was observed in the 0.0 mg NO_3^--N l^{-1} treatment (Figure 2.5).

Table 2.3 Characteristics of intact cores by treatment presented as mean ± standard deviation, n = 12 for each treatment. 'Surface water' refers to samples collected from the water column within individual cores. ND = not determined (e.g. since no nitrate was added, denitrification could not be calculated).

Treatment *(mg NO_3-N l^{-1})*	0.0	0.5	1.0	2.0
Flow Rate *(ml h^{-1})*	55.4 ± 2.3	55.4 ± 2.3	55.4 ± 2.3	55.4 ± 2.3
Surface Water NO_3^- *(mg l^{-1})*	0.003 ± 0.001	0.37 ± 0.03	0.73 ± 0.08	1.60 ± 0.07
NO_3^- Denitrified *(%)*	ND	26 ± 6	27 ± 8	20 ± 3
Denitrification Rate *(mg N m^{-2} d^{-1})*	ND	44 ± 11	95 ± 29	137 ± 24
Surface Water D.O. *(mg l^{-1})*	2.3 ± 0.3	2.8 ± 0.7	2.7 ± 0.6	3.2 ± 0.7
Surface Water Temp *(°C)*	23.0 ± 1.1	23.0 ± 1.1	23.0 ± 1.1	23.0 ± 1.1
Redox (3 cm) *(mV)*	-148 ± 47	-57 ± 22	-115 ± 93	-81 ± 57
Redox (7 cm) *(mV)*	-89 ± 97	-115 ± 74	-103 ± 80	-120 ± 80

Table 2.4 Results of a three-way ANOVA indicating the significance value (P) for the main effects (nitrate, time, and depth), and the interaction effects. DF = degrees of freedom (n-1).

Effects	DF	P
Nitrate Concentration	3	0.008
Incubation Time	2	0.018
Soil Depth	2	<0.001
Nitrate*Time	6	0.680
Nitrate*Depth	6	0.020
Time*Depth	4	<0.001

Table 2.5 Product-moment correlation coefficients for DEA and treatment at each day and soil depth. Bold indicates significance at $P < 0.01$(for $n = 12$, at $P = 0.01$, $r = 0.71$).

Day, Depth	DEA and Treatment
Day 7, 0-5 cm	0.34
Day 7, 5-10 cm	-0.14
Day 20, 0-5 cm	**0.74**
Day 20, 5-10 cm	**0.78**
Day 45, 0-5 cm	0.11
Day 45, 5-10 cm	0.15

Table 2.6 Product-moment correlation coefficients for DEA and depth at each treatment level. Bold indicates significance at $P < 0.01$(for $n = 36$, at $P = 0.01$, $r = -0.42$).

Treatment	DEA and Depth
0.0 mg NO_3^--N l^{-1}	**-0.52**
0.5 mg NO_3^--N l^{-1}	**-0.64**
1.0 mg NO_3^--N l^{-1}	**-0.76**
2.0 mg NO_3^--N l^{-1}	**-0.80**

treatments (Figure 2.5). On day 20, DEA for the 0.0 mg NO_3^--N l^{-1} treatment was significantly lower than that of the 1.0 and 2.0 mg NO_3^--N l^{-1} treatments, and DEA for the 0.5 mg l^{-1}

DEA showed high variation among replicates. The greatest variability and the highest rates of DEA were observed on day 7 (Figure 2.6). DEA on days 20 and 45 were significantly lower than on day 7 ($P < 0.05$), but did not differ from one another, suggesting the establishment of steady-state conditions. Time showed significant interaction with nitrate treatment concentration ($P = 0.68$), while nitrate and depth, and time and depth, showed no interaction ($P < 0.05$ and $P < 0.001$, respectively; Table 2.4).

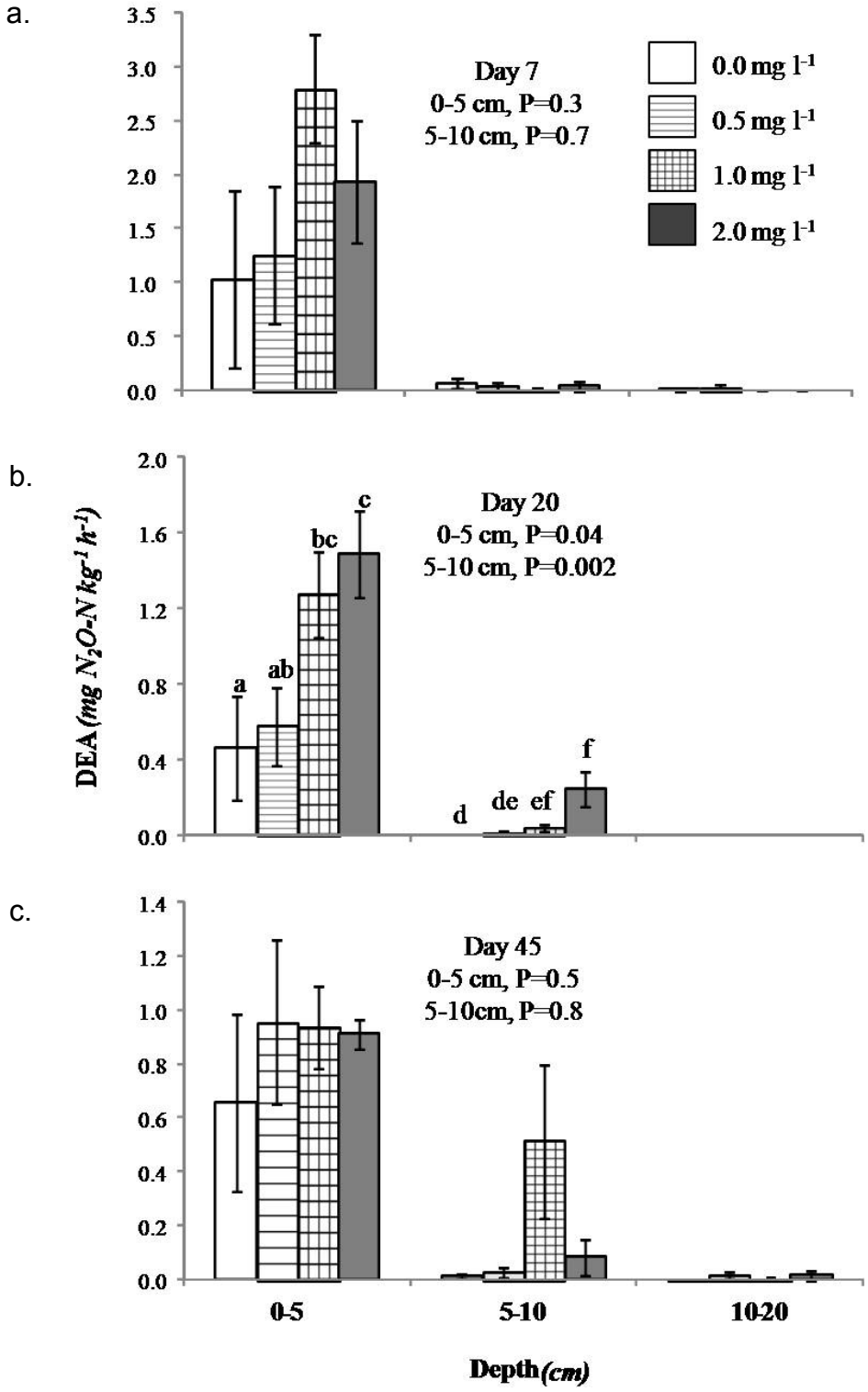

Figure 2.5 Mean DEA and standard error. P values for each day and depth were determined with one-way ANOVAs. Factors with different letters are significantly different Tukey's groupings, n= 3, $P < 0.05$.

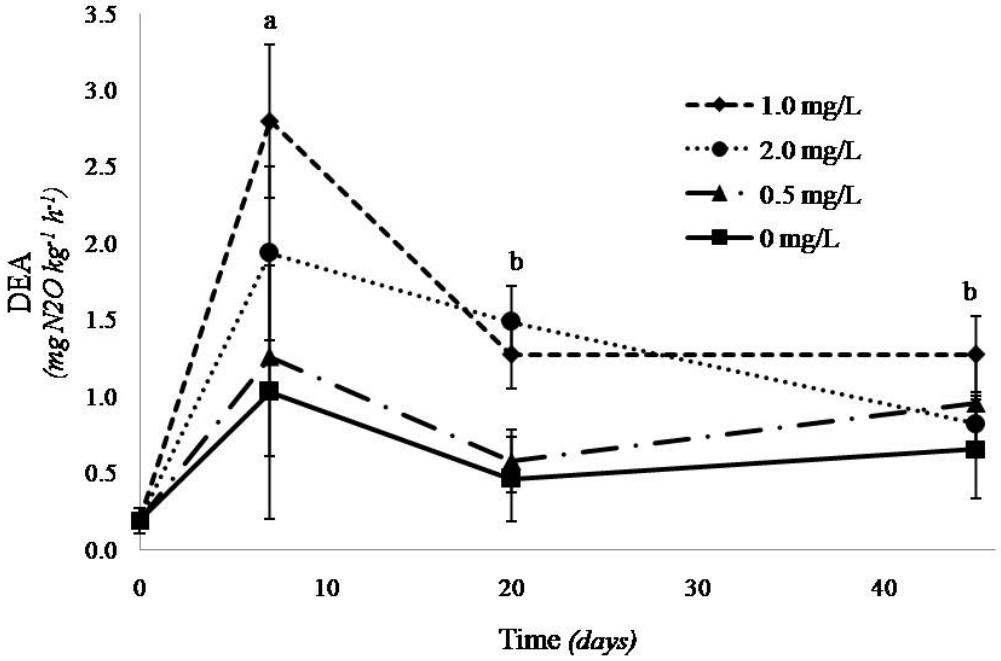

Figure 2.6 Changes in mean DEA (with standard error bars) over the course of the incubation. Factors with different letters have different Tukey's groupings. n= 3, $P < 0.05$.

DEA decreased significantly with depth on all days ($P < 0.001$; Table 2.4). For the 0.0, 0.5, and 1.0 mg NO_3^--N l^{-1} treatments, the 0-5 cm soil depth had significantly higher DEA than both the 5-10 and 10-20 cm soil depths. The 2.0 mg NO_3^--N l^{-1} treatment showed significant differences in DEA for all three depth segments (Figure 2.5). The correlation between DEA and depth strengthened as the nitrate loading concentration increased (Table 2.5).

The percent of DEA occurring below 5 cm increased significantly over the course of the incubation in all treatments ($P < 0.001$), from representing 3% of the total DEA on day 7, to 7% on day 20, and 16% on day 45 (Figure 2.7). Averaged over all days, approximately

92% of all enzyme activity was observed in the top 0-5 cm of soil, 7% at the 5-10 cm depth, and <1% at 10-20 cm.

DEA showed significant correlations with the following soil properties: MBN (r = -0.23), extractable NO_3^- (r = -0.26), TC (r = -0.62), TN (r = -0.61), and soil pH (r = 0.22) (Table 2.2). However, the strongest correlation was between DEA and depth (r = -0.65). Many of these soil properties were also correlated with depth (i.e., extractable NO_3^-, TC, and TN), indicating their correlation with DEA may be indirect.

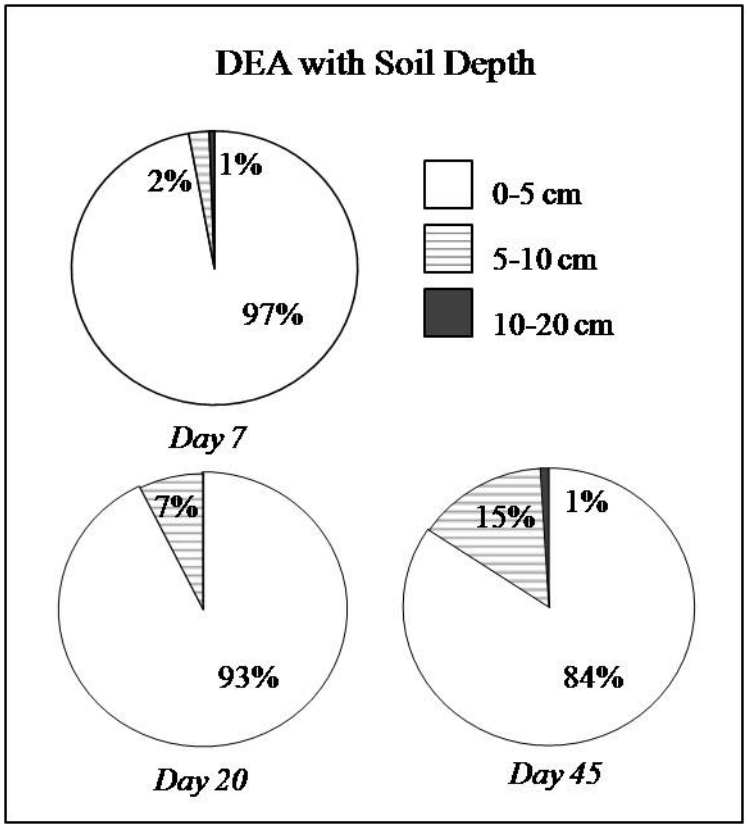

Figure 2.7 The percent of overall DEA occurring at each soil depth over the course of the incubation.

2.4 DISCUSSION

All soil cores had similar properties consistent with an organic wetland soil, including low bulk density (<0.1 g cm^{-3}) and high TC content (overall mean 320 ± 117 g C kg^{-1}). Extractable NO_3^- in the soil ranged from below detection (0.006) to 0.69 mg l^{-1}, with an overall mean value of 0.12 ± 0.16 mg l^{-1}. Between 20 and 27% of the loaded nitrate was not represented in the surface water of the cores, indicating quick diffusion into the surface soil. The low redox conditions of the soils (-103 ± 27 mV) also indicate the environmental conditions were poised for denitrification (Patrick et al., 1996).

The proportion of total C as microbial biomass decreased from 12.6% in the surface 0-5 cm to 6.9% at 10-20 cm. This is slightly higher than found for a peat soil in the northern Everglades, FL, where 4.3% of C is MBC in the 0-10 cm horizon of this phosphorus (P) limited system (DeBusk and Reddy, 1987). The high proportion of TC as microbial biomass is indicative of high substrate quality (DeBusk et al., 2001). The percent of N as MBN also decreased significantly with depth, from 1.9% to 1.1%, and represented a proportion of TN similar to that found in northern Everglades soil (White and Reddy, 2000).

The rate of denitrification (calculated as N loss in core surface water) increased significantly as the nitrate concentration increased, which is consistent with previous work indicating denitrification is a first-order rate reaction (Reddy et al., 1978). The role of diffusion as a controlling factor for DEA was also apparent in the interaction between soil depth and incubation time- the longer the soil was exposed to nitrate loading, the greater the proportion of DEA at depth. Averaging the denitrification rate among the 0.5, 1.0, and 2.0 mg NO_3^--N l^{-1} treatments, the mean rate was 92 ± 44 mg N m^{-2} d^{-1}. This is similar to the

average rate of 81 ± 37 mg N m^{-2} d^{-1}, which was estimated using the mass-balance approach to determine the nitrate removal capacity of Davis Pond marsh (DeLaune et al., 2005).

Groffman (1987) suggested DEA is an effective indicator of treatment differences because it represents an integrated product of all treatment effects. Overall, our results indicate nitrate concentration did significantly influence DEA rate ($P < 0.05$). However, when days were viewed individually, the effect of nitrate concentration on DEA was only significant on day 20, at which time DEA increased significantly with nitrate concentration at both the 0-5 cm and 5-10 cm soil depths. Statistical significance was observed when the differences in nitrate concentration were $\geq$1 mg NO_3^--N l^{-1}. The trend of higher DEA with increased nitrate loading continued on day 45 (with the exception of the 2.0 mg NO_3^--N l^{-1} treatment), but the differences between treatments were not significant ($P = 0.5$ for 0-5 cm). Long term incubation studies are often complicated by other biological processes (e.g. photosynthesis and respiration) (Groffman, 1987), which may have accounted for the weakened relationship on day 45. Differences in DEA among treatments was also not significant on day 7 ($P=0.3$ for 0-5 cm), which could be a result of insufficient time for the microbial communities adjusted to the new conditions.

The present study utilized intact soil cores in attempt to replicate field conditions in a laboratory setting where nitrate load and temperature could be controlled. However, the inevitable heterogeneity of organic soils and the artificial conditions created when the cores were removed from the field probably contributed to weakened relationship between nitrate and DEA on days 7 and 45. Future studies may be able to decrease variability by using homogenized soil slurries, or by investigating the relationship between DEA and nitrate concentration on a mesocosm scale, were soil structure would remain intact.

The existence of measurable DEA in the cores receiving the 0.0 mg NO_3^--N l^{-1} treatment was observed on all days, suggesting the significance of internal biochemical N cycling in this system. A study conducted directly downstream of Davis Pond marsh in lake sediments found an average NH_4^+-N flux of 1.42 mg m^{-2} d^{-1} in anaerobic conditions, which contributed substantially to the total N load and the denitrification rate of that system (Miao et al., 2006b). In the lab, these lake sediments exhibited denitrification rates of 3.3 mg m^{-2} d^{-1} with no added N (Miao et al., 2006b). Similarly, the present study found a denitrification rate of 0.46 mg kg^{-1} h^{-1}, or 5.2 mg m^{-2} d^{-1}, for the 0-5 cm soil horizon in 0.0 mg NO_3^--N l^{-1} treatment on day 20. The influence of internal nutrient cycling has already been quantified in riverine (Malecki et al., 2004), estuarine (Burdige and Zheng, 1998), and lake (D'Angelo and Reddy, 1993) sediments, and appears to be significant in organic wetland soils. Future studies should consider the contribution of internal N cycling when establishing target loading rates in wetlands and other aquatic systems.

Incubation time significantly influenced DEA, as seen by a peak in rates for all treatments on day 7, followed by a decrease to steady-state conditions on days 20 and 45. A lag time between nitrate addition and the peak in denitrification rate has been observed in other lab studies. At temperatures comparable to this experiment (22°C), sediments incubated under anaerobic conditions exhibited a peak in denitrification on day 3, followed by a rate decrease (Lindau et al., 2008). It is difficult to compare the results of the present study with others because previous workers often add NO_3^- once and then observe the removal rate over time. Conversely, the present study provided a continuous flow of nitrate to the incubating soils in attempt to more closely simulate field conditions. One of the only studies to incorporate a continuous flow of nitrate into the experimental design involved leaching of an

upland soil in a packed column. The study found the nitrate concentration in the leachate reached a steady-state after nine days of continuous loading (Doner, 1975), similar to the equilibrium state reached between days 7 and 20 in the present study. Since nitrate was non-limiting in our cores, it follows that the peak in activity at day 7 may be an artifact of the soluble C released during the shearing of belowground roots and rhizomes when the cores where collected from the field. A decline in enzyme activity over time is often attributed to C limitations (Burns and Ryder, 2001).

DEA decreased significantly as soil depth increased. Previous work has shown DEA is significantly higher at the 0-10 cm depth, as compared to 10-30 cm depth. The current study refines the point of decline in DEA further, to within the 0-5 cm soil depth for organic wetland soils. It is therefore reasonable to suggest that DEA in the surface soils be measured in the smallest possible increment to obtain a clearer picture of the variability with depth. However, it is also important to note that the relationship between DEA and depth relies upon the assumption of uniform soil composition. Riparian soils containing pockets of high C alluvial deposits at depth can weaken this relationship (Dhondt et al., 2004). A significant increase in DEA was observed at lower depths as the time soils were exposed to nitrate loading increased. On day 7, only 3% of the total enzyme activity occurred below 5 cm, while 16% of total activity occurred below 5 cm on day 45.

2.5 CONCLUSION

Our results indicate DEA is strongly influenced by the length of time soils are exposed to nitrate loading and the depth of the soil sample. The diffusion of nitrate below 5 cm required several days. Even after 45 days of continuous nitrate loading, 84% of DEA was observed in the surface 5 cm. This study also indicates a significant contribution of

biochemical cycling of soil N to the overall rate of DEA, an important consideration when managing nutrient inputs to aquatic systems.

In the surface soils (0-5 cm), DEA responded quickly (< 7 days) to nitrate loading and exhibited a general trend of higher rates of DEA when exposed to higher nitrate concentrations in the surface water. Field studies have also found DEA will increase in response to increased N loads (Wigand et al., 2004). An oscillation in DEA from higher rates in the summer when N inputs were high, to lower rates in the winter when N loading decreased, has also been observed in the field (White and Reddy, 1999). This suggests that measuring surface soil DEA will indicate if a wetland has been exposed to external N loading over the previous weeks to months. However, further study is needed to determine if the rate of DEA can serve as a proxy for surface water nitrate concentration.

CHAPTER 3: FIELD STUDY

DENITRIFICATION ENZYME ACTIVITY AS A POTENTIAL SPATIAL INDICATOR OF NITRATE LOADING IN A WETLAND RECEIVING MISSISSIPPI RIVER WATER

3.1 INTRODUCTION

The Louisiana coastal zone is experiencing the highest rate of land loss (Barras et al., 1994) and relative sea level rise (RSLR) (Penland and Ramsey, 1990) in the United States. Several factors, both natural and anthropogenic, have coalesced to cause the rapid deterioration of the Mississippi River delta plain. These factors include natural subsidence (Penland and Ramsey, 1990), eustatic sea level rise (Day et al., 1995), the construction of artificial levees along the Mississippi River (Turner and Cahoon, 1987), and hydrologic modifications associated with canal dredging (Evers et al., 1992; Turner and Rao, 1990).

Large-scale freshwater diversion projects along the lower Mississippi River are intended to restore coastal wetlands by reintroducing freshwater, nutrients, and sediments to the historic floodplain (Green, 2006). The Caernarvon diversion, completed in 1991, has successfully decreased salinity (Lane et al., 2007) and increased productivity and marsh accretion near the freshwater inflow to Breton Sound estuary (DeLaune et al., 2003). The recently completed Davis Pond diversion has a greater discharge capacity than the Caernarvon diversion. The Davis Pond diversion discharges into Barataria Basin, a 190 km long estuary located between the west bank of the Mississippi River and Bayou LaFourche. Land loss within Barataria Basin was estimated at 28.7 $km^2 y^{-1}$ between 1978 and 1990, representing the highest rate of loss among the 10 major coastal basins in Louisiana (Barras et al., 1994).

Barataria Basin is a nitrogen limited system (Patrick and DeLaune, 1976). Nitrate concentrations in the Mississippi River peak at 50 times higher than the estuary in the spring (Battaglin et al., 2001) and annually average 1.0 - 1.2 mg NO_3^--N l^{-1} (Antweiler et al., 1995). The high nutrient concentrations in the Mississippi River have been implicated as a major

cause of algal blooms, hypoxia, and fish kills in the northern Gulf of Mexico (Anderson et al., 2002; Rabalais et al., 2002), raising concern that freshwater diversions may negatively impact downstream habitats (Day et al., 1999).

Downstream of the Caernarvon diversion, NO_3^-/ NO_2 was rapidly transformed and/or removed upon entering the Breton Sound estuary, demonstrating an 88-97% removal efficiency (Lane et al., 1999). Similarly, studies in Davis Pond marsh (the 3,760 ha receiving wetland for the diversion) indicate Mississippi River nitrate was nearly completely removed when the diversion discharge rate was very low (35 $m^3 s^{-1}$). However, approximately 0.75 mg NO_3^--N l^{-1} was transported out of the marsh and into Barataria Basin during moderate discharge events (100 $m^3 s^{-1}$) (DeLaune et al., 2005). This data provides information on the overall NO_3^- removal efficiency of Davis Pond marsh, but it does not indicate the area of the marsh exposed to Mississippi River nitrate or the contribution of other N pools to the denitrification rate.

Denitrification is the major mechanism for NO_3^- removal in Davis Pond marsh (DeLaune et al., 2005). Organic wetland soils, such as those found in Davis Pond marsh, are characterized by low redox potential (-103 ± 27 mV) and high C content (244 ± 141 mg kg^{-1}). With two of the factors regulating denitrification met in wetland soils (a paucity of oxygen and abundance of available C), nitrate becomes the limiting factor for denitrification (Cooper, 1990).

Denitrification enzyme activity (DEA) is an assay used to quantify the amount of denitrifying enzymes present in the soil (Smith and Tiedje, 1979). Therefore, DEA is directly related to *in-situ* denitrification rates (Groffman, 1987). The enzymes synthesized by denitrifiers to catalyze nitrate reduction are produced in direct proportion to the concentration

of nitrate available in the environment (Downey, 1966). The DEA assay is limited to two hours, ensuring no *de novo* synthesis of enzymes occurs, and provides non-limiting conditions for enzyme expression (Smith and Tiedje, 1979; Teidje, 1982).

A study of riparian wetland organic soils found a strong correlation between *in-situ* denitrification rate, DEA, and NO_3^- concentration ($r^2 = 0.77$) (Schipper et al. 1993). A significant correlation between DEA and surface water NO_3^- concentration ($P < 0.01$) was also found in organic wetland soils from the northern Everglades, FL (White and Reddy, 1999). Furthermore, the laboratory study present in Chapter 2 found a significant correlation between NO_3^- concentration and DEA in the surface horizon of Davis Pond soils (0-5 cm; $P < 0.05$) and the 5-10 cm soil horizon ($P < 0.01$) after 20 days of continuous nitrate loading.

We hypothesized that the correlation between soil DEA and surface water NO_3^- would make DEA an effective spatial indicator of Mississippi River nitrate loading in Davis Pond marsh. Our objectives were to quantify DEA in surface soils throughout the marsh and use this data to estimate the area of the marsh receiving exogenous nitrate at a given discharge rate. We also compared *in-situ* NO_3^- concentrations to the area of elevated DEA and made inferences about the flow path of river water through the marsh using the spatial distribution of DEA.

3.2 MATERIALS AND METHODS

3.2.1 Site Description

The Davis Pond freshwater diversion was constructed by the US Army Corps of Engineers (ACOE) and is operated by the Louisiana Department of Natural Resources (LDNR) (Villarrubia, 2006). Operation began in July 2002 and the structure is capable of diverting up to 302 $m^3 s^{-1}$ (10,650 cfs) of Mississippi River water, making it one of the largest

surface water diversions in the country. Four 4.3 m^2 box culverts were constructed in the west bank levee of the Mississippi River in St. Charles Parish, approximately 19 km upstream of New Orleans (LDNR, 2004). Before entering the Barataria Basin estuary, river water flows down a 3 km inflow channel to Davis Pond marsh, a 3,760 ha ponding area (US ACOE, 2003). When Davis Pond marsh reaches capacity, the design intention was for water to sheet-flow over the outflow weir, into Lake Cataouatche, Lake Salvador, and eventually Barataria Bay. Since construction, several design modifications have been required along the weir structure to permit greater water transport and reduce the water depth of the marsh (Letter, 2005; LDWF 2005). As a result, full-scale operation of the structure did not commence until 2006/2007 (LDNR, 2004).

Davis Pond marsh is a freshwater wetland overlying fluvial sediments deposited when the LaFourche (60 - 3,500 years before present (Y.B.P.)) and Plaquemines (200 - 1,000 Y.B.P.) delta lobes were active (Turner and Cahoon, 1987). A crevasse in the Mississippi River levee in 1884 assisted in the formation of the current marsh, which has been further modified by logging operations, oil and gas exploration, the installation of two mineral pipelines, and continued hunting and trapping operations (Ensminger and Simon, 1993). The western portion of the marsh contains a series of ridges running east-west that support declining stands of baldcypress (*Taxodium distichum*), tupelo gum (*Nyssa aquatica*), and green ash (*Acer rubrum*). The majority of the marsh is characterized by emergent herbaceous plants, predominately bulltongue (*Sagittaria lancifolia*), water hyacinth (*Eichhornia crassipes*), alligator weed (*Althernathera philoxeroides*), *Biden spp*. and *Typha spp.* (Ensminger and Simon, 1993), with pockets of open water and naturally developing channels.

3.2.2 Field Sampling

Soil samples were collected at 88 randomly distributed sites spanning the entire area of the marsh on eight sampling trips in 2007 (May 13, 16, 24, 31; June 7, 12, 25; and July 10). GPS coordinates for each site were recorded and field triplicates were collected at 5 of the 88 sampling sites. A minimum of 20 cm of the soil profile was collected in a 7 cm diameter clear Plexiglas tube. Since the soils consisted of moderately decomposed organic matter, coring involved using a serrated knife to cut through the plant matter as the core tube was pushed down. While some surface compaction was unavoidable, if the soil surface in the coring tube was compacted more than ~5 cm (25%), the sample was discarded and re-collected. Soils were extruded in the field and divided into 0-10 cm and 10-20 cm increments, placed on ice, and transported back to the laboratory for storage at 4°C. All sites had either standing water or saturated soils at the time of sampling.

Vegetation community was categorized at the 88 sampling site where soils were collected. At each sampling station, dominate vegetation was recorded as one of three categories, 1) open water/submerged aquatic vegetation (SAV), 2) emergent macrophytes, or 3) woody species. General soil characteristics were also categorized as either 1) organic (attached), 2) organic (floating mat), or 3) organic with mineral sub-horizons at sites.

Eleven water samples were collected in shallow channels along a transect from the inflow structure on the Mississippi River to Lake Cataouatche. The water samples were collected on the final day of sampling (July 10, 2007) when the discharge rate had been 38.0 ± 11.3 $m^3 s^{-1}$ for 57 continuous days (Figure 3.1). Samples were field filtered, placed on ice, and transported back to the laboratory for storage at 4°C. Water samples were analyzed

within 2 weeks of collection for NO_3^--N. Water samples were not collected at every soil sampling site because approximately half of the sites did not having standing water.

3.2.3 Laboratory Analysis

Soils were analyzed for moisture content, bulk density, total C (TC), total N (TN), and percent organic matter (% OM). Moisture content and bulk density were determined after drying a subsample at 70°C until constant weight. TC and TN were measured on the dried, ground subsample using an Elemental Combustion System with a method detection limit of 0.005 g kg^{-1} (Costech Analytical Technologies, Inc., Valencia, CA). Percent OM was estimated by mass loss on ignition (LOI) where dry soils were combusted at 550°C for 5 h and final weight was subtracted from initial weight.

Surface water samples were field filtered through a 0.45 µ membrane filter and analyzed for NO_3-N on a SEAL AQ2 Automated Discrete Analyzer (SEAL Analytical, West Sussex, England), using US EPA Method 353.2 (US EPA, 1983). The method detection for NO_3^--N was 0.006 mg l^{-1}.

DEA was determined in accordance with the methods outlined in Tiedje (1982) with adaptations by White and Reddy (1999). The 0-10 cm soil sample was homogenized and a 5 g wet weight sub-sample was placed in a glass serum bottle. In addition to the field triplicates, 5 individual core samples were also measured in triplicate for DEA. The bottle was sealed with a rubber septa and aluminum crimp cap and headspace was evacuated from the bottle to -75 kPa, then purged with O_2-free N_2 gas for one minute. Eight milliliters of N_2 purged DI water was added to create a slurry and approximately 15% of the headspace was replaced with acetylene gas (C_2H_2) while maintaining atmospheric pressure within the bottle (Yoshinari and Knowles, 1976). Bottles were agitated on a longitudinal shaker for 30 min to

distribute the acetylene. Eight milliliters of a solution of 56 mg KNO_3^--N l^{-1}, 288 mg dextrose-C l^{-1}, and 2 mg chloramphenicol l^{-1} was added, creating a slight overpressure. Chloramphenicol is an enzyme inhibitor used to prevent *de novo* enzymes from synthesizing during incubation (Smith and Tiedje, 1979). Samples were continuously agitated in the dark at 23°C and the headspace was sampled at approximately 30, 60, 90, and 120 min. Gas samples were analyzed on a Shimadzu GC-8A ECD (Shimadzu Scientific Instruments, Columbia, MD) and N_2O production was calculated with consideration for product in a aqueous phase using the Bunsen absorption coefficient (0.544) (Tiedje, 1982). The rate was calculated as the slope of the line when mg N_2O-N kg $soil^{-1}$ was plotted against time (See sample calculation, A.9).

Denitrification potential was measured over a 24-h period on eight duplicate surface soil (0-10 cm) samples. Four samples were chosen from sites near the diversion inflow, representing soils with elevated DEA, and four were chosen far from the inflow, representing soils with background DEA (Figure 3.2). All 8 soils had % OM between 47 and 62. Incubations were prepared in glass serum bottles as described above, with the addition of 1.0 mg NO_3^--N l^{-1}. This nitrate concentration was chosen to simulate levels found in Mississippi River water. Samples were continuously agitated on a longitudinal shaker in the dark at 25°C. Headspace was sampled for N_2O-N every 2 to 6 h, analyzed on a Shimadzu GC-8A ECD (Shimadzu Scientific Instruments, Columbia, MD), and plotted against time.

3.2.4 Data Analysis

DEA rates and surface water nitrate concentrations were mapped using ArcGIS 9.0 (ESRI Software, Redlands, CA). Differences between data sets were determined using a one-way ANOVA model ($P < 0.05$) and the Fisher's least significant difference (LSD) post-hoc

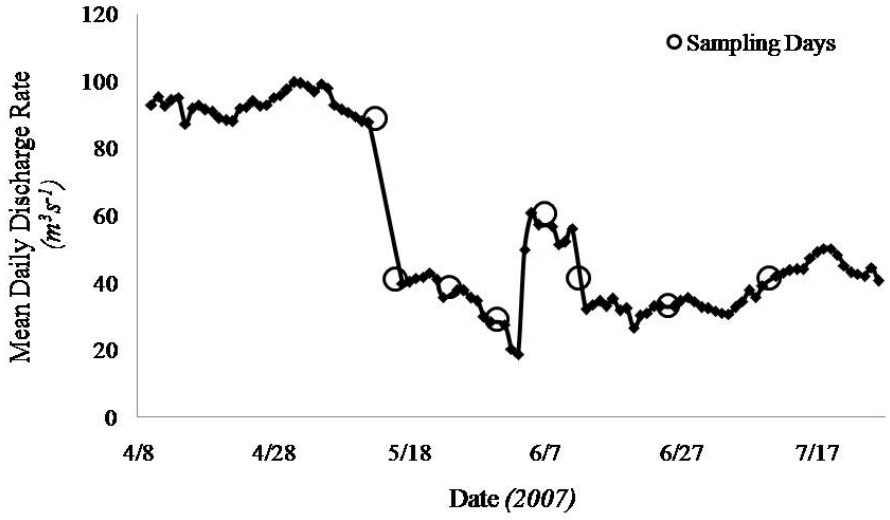

Figure 3.1 Mean daily discharge rate of the Davis Pond diversion between May 8 and July 25, 2007.

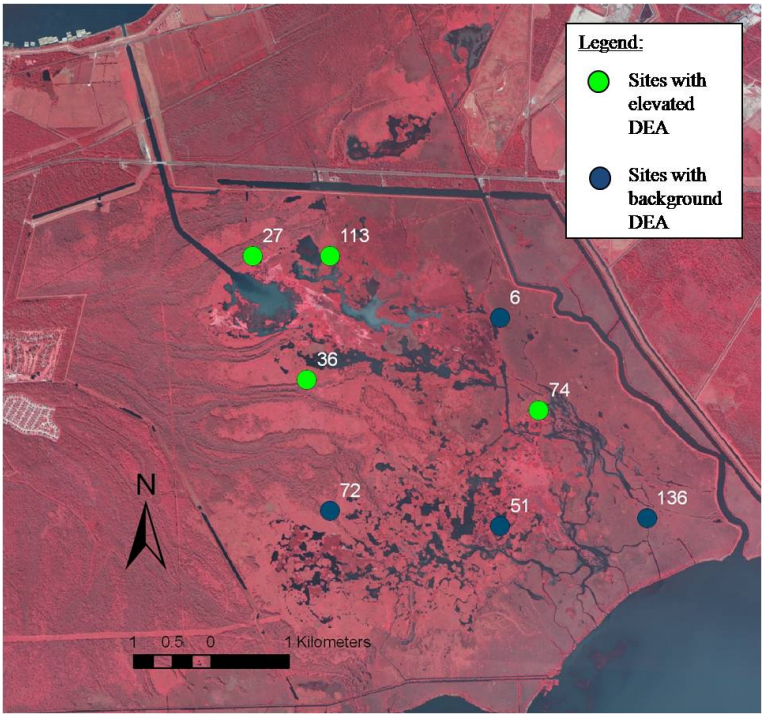

Figure 3.2 Site locations for the 8 soil samples (0-10 cm) measured for denitrification potential.

test. Homogeneity and normality were verified with Levene's Test and Spario-Wilk Test, respectively, and log transformations were performed as appropriate. Pearson's Product correlations were performed to determine the relationship between soil parameters, site characteristics and DEA. All analyses were conducted using SAS 9.1 (SAS Institute Inc., Cary, NC).

3.3 RESULTS

3.3.1 Study Area Characteristics

The discharge rate of the Davis Pond diversion remained steady at 93.1 ± 3.6 $m^3 s^{-1}$ for one month prior to field sampling. Soil collection began on May 13, 2007 when the discharge rate was 88 $m^3 s^{-1}$. The following day, the discharge rate dropped to 39.5 ± 10.4 $m^3 s^{-1}$, and remained at a similar rate from May 14 to July 10, 2007 (Figure 3.1). On sample collection days, the minimum air temperature ranged from 19 to 26°C and the maximum air temperature was between 27 and 33°C (Table 3.2). Water temperature was influenced by many variables (e.g. proximity to the diversion inflow, the discharge rate, river water temperature, air temperature, etc), but tended to be lower closer to the inflow due to the mixing of cold river water. Since standing water was not present at all sites (despite all sites being saturated at/near field capacity), water temperature, NO_3^- concentration, and other surface water parameters were not collected.

The soils of the Davis Pond marsh can be generalized as hemist histosols. Approximately 50% of the sampling sites consisted of peat soils (>50% organic matter). Mean (± standard deviation) bulk density was 0.14 ± 0.12 g cm^{-3}, % moisture was 88 ± 9, and % organic matter (LOI) was 50 ± 25 (Table 3.1). The soil pH was neutral (7.0 ± 0.3) and total C and total N averaged 244 ± 141 and 16 ± 8 mg kg^{-1}, respectively (Table 3.1). Strong

Table 3.1 General soil parameters for the 88 sites where DEA was measured.

	Mean ± Std. Dev.	Min.	Max.
Soil Moisture *(%)*	88 ± 9	59	98
Bulk Density *(g cm^3)*	0.14 ± 0.12	0.03	0.55
Soil pH	7.1 ± 0.3	6.1	7.7
Organic Matter *(%)*	50 ± 25	8	91
Soil TC *(mg kg^{-1})*	244 ± 131	26	499
Soil TN *(mg kg^{-1})*	16 ± 8	2	31

Table 3.2 Discharge rate and weather conditions on each of the 8 field sampling days, plus monthly averages.

Sampling Date	Discharge Rate *(m^3s^{-1})*	Min Temp *(°C)*	Mean Temp *(°C)*	Max Temp *(°C)*	Precipitation *(cm)*
5/13/2007	88.6	22	27	32	0.0
5/15/2007	40.8	20	24	28	0.4
5/24/2007	38.8	20	23	27	0.0
5/31/2007	28.9	21	24	27	2.1
May Average	*62.6*	*19*	*24*	*28*	*0.7*
6/7/2007	60.3	25	28	31	0.2
6/12/2007	41.1	22	27	33	0.0
6/25/2007	32.9	22	27	33	2.9
June Average	*38.0*	*22*	*27*	*31*	*0.7*
7/10/2007	41.1	26	30	32	0.5
July Average	*40.9*	*23*	*27*	*31*	*0.4*

correlations were found between several of the soil parameters and site characteristics (Table 3.3). Soil nutrients (TC, and TN) were correlated with one another and with the general soil properties (e.g. % soil moisture, bulk density, soil pH, and % organic matter). DEA was correlated with both soil nutrients (r = -0.39 for TC and r = -0.42 for TN), soil pH (r = 0.37), and % OM (r = -0.42). However, DEA was not correlated with sampling date, soil type, % moisture, bulk density, or vegetation type (Table 3.3).

Eight-two percent of the sites sampled in the Davis Pond marsh had attached organic soils, while 12% consisted of an organic floating mat (flotant), and 6% contained some mineral sediment in the surface 0-20 cm (Figures 3.3 and 3.4). Soils containing mineral sediments were concentrated along the western edge of the marsh, coinciding with the location of the historic splay ridge deposits, and also in the northeast corner. Mineral sediments not associated with splay deposits are likely an artifact of past construction activities. Sixty-four percent of the marsh was dominated by emergent macrophytes. An area of open water existed at the end of the inflow canal and in small pockets further downstream. Woody vegetation predominated along the outer edges of the marsh (Figure 3.5).

3.3.2 Spatial Distribution of DEA

Rates of DEA ranged from below detection (0.006 g N_2O-N kg^{-1} h^{-1}) to 2.10 mg N_2O-N kg^{-1} h^{-1} (0.08 to 92.4 g N_2O-N m^{-2} d^{-1}) in the upper 0-10 cm of soils. The highest rates were concentrated proximal to the inflow channel where river water first enters the marsh. An area of approximately 715 ha contained over 80% of all observed DEA and represented 19% of the total marsh area (Figure 3.6). Outside the 715 ha area, DEA ranged from below detection to

0.30 mg N_2O-N kg^{-1} h^{-1} (3.90 mg N_2O-N m^{-2} d^{-1}) while elevated DEA within the 715 ha area ranged from 0.41 to 2.10 mg N_2O-N kg^{-1} h^{-1} (21.9 to 92.4 mg N_2O-N m^{-2} d^{-1}). The natural break in the data set between 0.30 and 0.41 mg N_2O-N kg^{-1} h^{-1} defined the difference between sites with elevated DEA and sites with background DEA. This delineation was also based on 1) the location where the difference in DEA between two sampling sites was greatest, 2) the area that encompassed at least 80% of all DEA measured, and 3) the results of previous work that demonstrated internal N cycling processes can produce DEA rates between 0.006 to 0.46 mg N_2O-N kg^{-1} h^{-1} with no added nitrate (see Chapter 2). The mean DEA in the 715 ha area

Table 3.3 Product-moment correlation coefficients for soil parameters and site characteristics of Davis Pond marsh. Bold indicates significance at $P < 0.01$ (for $n = 88$, at $P = 0.05$, $r = 0.22$, at $P = 0.01$, $r = 0.28$).

	Sampling Date	% Moisture	Bulk Density	Soil pH	% OM	Soil TN	Soil TC	DEA	Vegetation Type
% Moisture	0.06								
Bulk Density	-0.05	**-0.99**							
Soil pH	0.10	**-0.36**	**0.37**						
% OM	0.01	**0.80**	**-0.78**	**-0.53**					
Soil TN	0.02	**0.81**	**-0.80**	**-0.51**	**0.98**				
Soil TC	0.01	**0.79**	**-0.77**	**-0.51**	**1.00**	**0.98**			
DEA	0.04	-0.06	0.06	**0.37**	**-0.42**	**-0.39**	**-0.42**		
Vegetation Type	-0.19	**0.32**	**-0.32**	**-0.29**	**0.33**	0.26	**0.31**	-0.20	
Soil Type	0.14	-0.05	0.05	0.11	-0.04	-0.05	-0.02	0.05	0.00

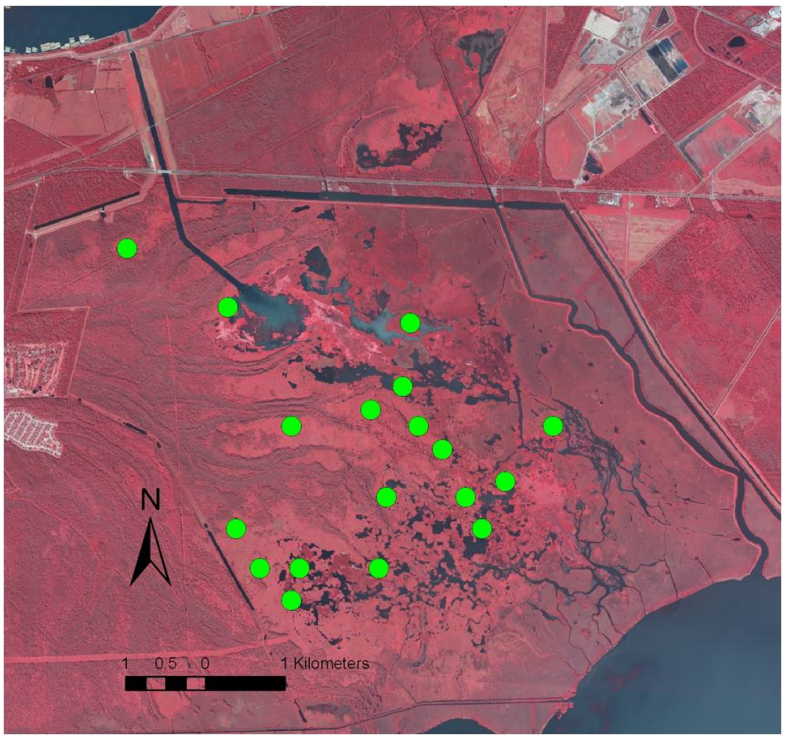

Figure 3.3 Sites with floating mat (flotant) organic soils.

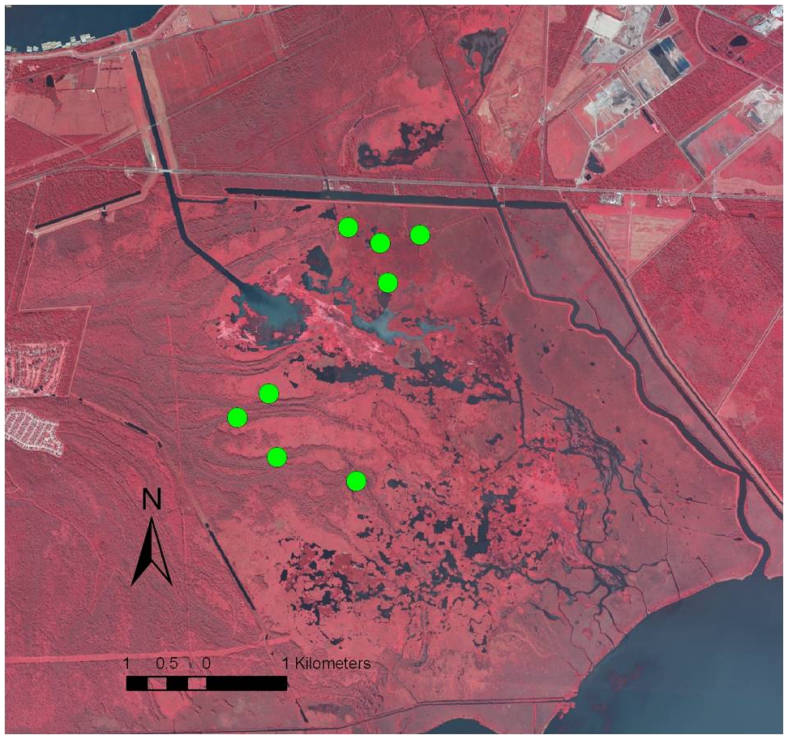

Figure 3.4 Sites with mineral sediment components in the top 20 cm of organic soil.

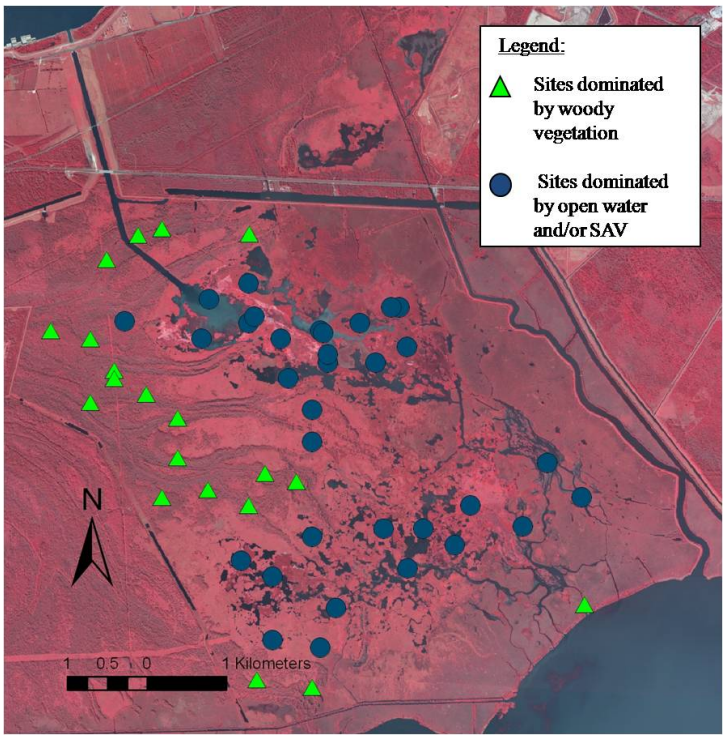

Figure 3.5 Sites characterized by woody vegetation or open water/SAV.

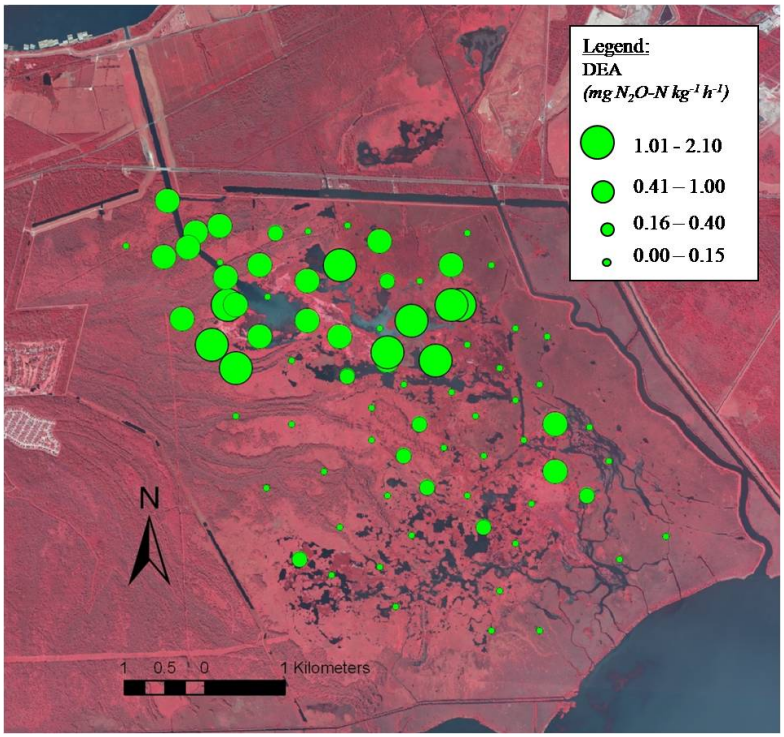

Figure 3.6 Spatial distribution of DEA rates at 88 field sites in Davis Pond marsh.

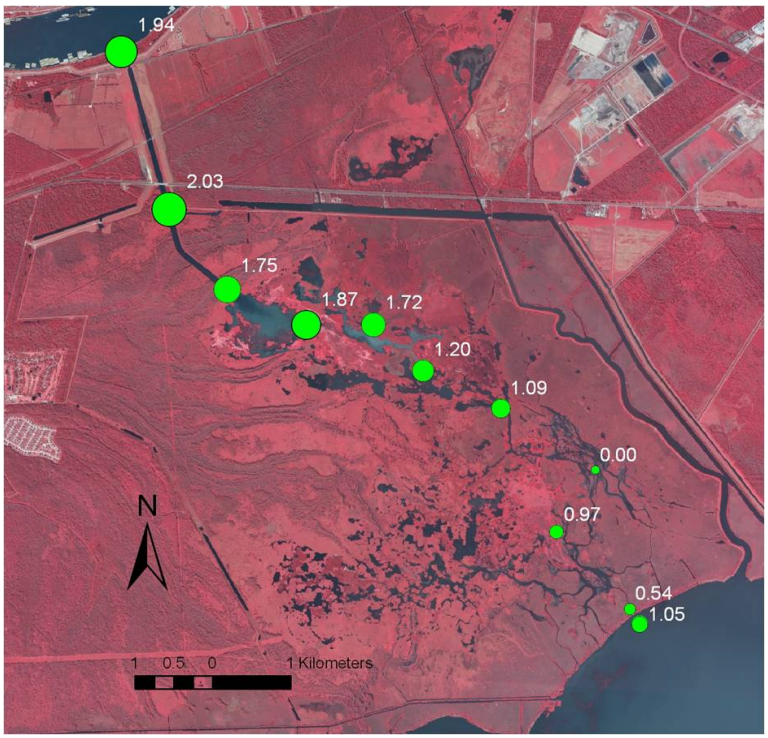

Figure 3.7 Surface water nitrate concentrations (mg NO_3^--N l^{-1}) from samples collected on July 10, 2007.

proximal to the inflow was 15 times higher than the mean DEA of sites outside this area ($O < 0.001$). The area of elevated DEA was oriented in a south-easterly direction from the inflow, possibly a result of channelization created by the historic splay ridges located along the western side of the marsh.

Nitrate concentrations at the Mississippi River inflow were 2.0 mg NO_3-N l^{-1}. No detectable nitrate removal occurred in the 3 km inflow channel. However, nitrate concentrations steadily decreased as water flowed through the marsh area, to a low of 0.5 mg NO_3^--N l^{-1} at the outflow weir (Figure 3.7). A direct comparison cannot be made between DEA and surface water nitrate concentration because NO_3^- was not quantified at all sites where DEA was measured. However, the highest nitrate concentrations were observed within

the area of elevated DEA. The spike in NO_3^--N l^{-1} in Lake Cataouatche (downstream of the outflow) may be a product of turbulence created as water flows over the outflow weir.

3.3.3 Potential Denitrification Capacity

Denitrification potential was measured at 8 sites in the marsh with similar % OM, 4 located within the area of elevated DEA and 4 located in the area exhibiting background rates of DEA (Figure 3.2). As expected, the first 2 hours of N_2O-N production was significantly higher in the soils close to the inflow (0.83 mg N_2O-N kg^{-1} h^{-1}), compared to the soils located farther from the inflow (0.13 mg N_2O-N kg^{-1} h^{-1}). The soils with elevated rates of DEA reached their maximum potential denitrification rate (2.3 mg N_2O-N kg^{-1} h^{-1} or 44.2 mg N_2O-N m^{-2} d^{-1}) within 4 hours of incubation. The soils with low rates of DEA exhibited a lag time of approximately 10 h before reaching the same maximum potential denitrification rate (Figure 3.8). The ability of all soils to reach equivalent rates of denitrification, regardless of proximity to the inflow, suggests denitrifiers are ubiquitous in the marsh soils (Germon, 1985). Soils from the area of elevated DEA contained active denitrifying enzymes from *in situ* nitrate exposure (i.e., Mississippi River water), and therefore reached their maximum potential quickly. The soils with background rates of DEA required additional time (as seen by the lag) to synthesize denitrifying enzymes before attaining their maximum potential, a result of nitrate limitations *in situ* preventing the initiation of enzyme synthesis prior to the laboratory addition.

3.4 DISCUSSION

The spatial distribution of DEA in Davis Pond marsh showed the highest rates were concentrated in a 715 ha area proximal to the Mississippi River inflow and the lowest rates occurred farthest from the inflow at an average discharge rate of 39.5 ± 10.4 m^3 s^{-1}. DEA

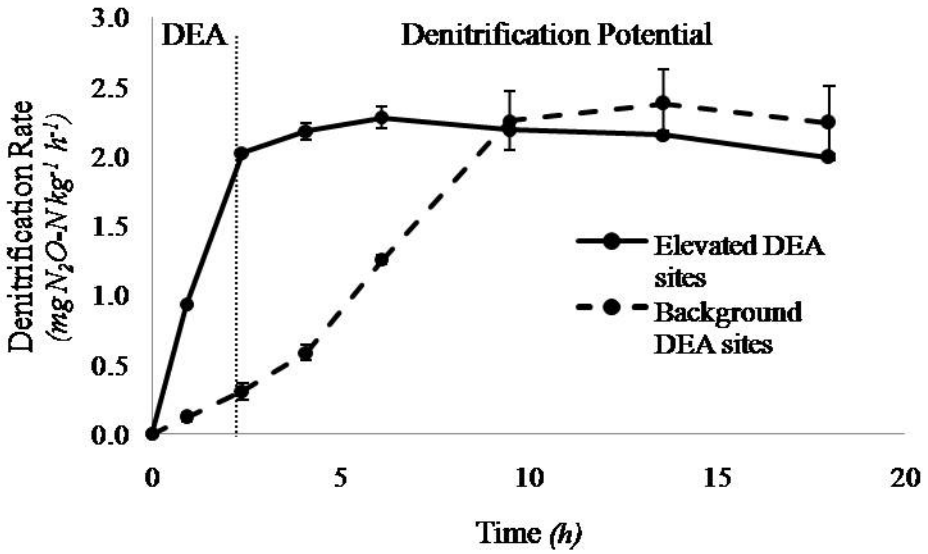

Figure 3.8 Denitrification potential of sites with elevated DEA and background DEA rates when exposed to the same concentration of nitrate (1.0 mg N l^{-1}).

ranged from 0.41 to 2.10 mg N_2O-N kg^{-1} h^{-1} (21.9 to 92.4 mg N_2O-N m^{-2} d^{-1}) within the 715 ha area. Outside this area, DEA ranged from below detection to 0.30 mg N_2O-N kg^{-1} h^{-1} (3.90 mg N_2O-N m^{-2} d^{-1}). Yu et al. (2007) found a maximum denitrification rate of 302 mg N_2O-N m^{-2} d^{-1} for Davis Pond soils (0-15 cm) and an overall nitrate removal capacity of 110 g N_2O-N m^{-2} y^{-1}

Organic wetland soils (such as those of Davis Pond marsh) generally exhibit reduced conditions and an accumulation of carbon. When these two regulating conditions for denitrification are satisfied, nitrate availability becomes the limiting factor (Cooper, 1990; Gale et al., 1993; Schipper et al., 1993). Since nitrate must be present for denitrifying enzymes to synthesize (Bryan, 1981; Germon, 1985) and the amount of enzymes synthesized is directly proportional to the nitrate concentration (Downey, 1966), it follows that areas with higher DEA represent areas of higher NO_3^- concentrations. Previous studies have found significant correlations in the spatial distributions of denitrification and NO_3^- concentration,

and in the spatial distributions of DEA and NO_3^- concentration in organic soils (Schipper et al., 1993; White and Reddy, 1999).

Surface water NO_3^- could not be quantified at every sampling site due to differences in flood status. However, several sites characterized as open water/SAV showed background rates of DEA, suggesting the presence of floodwater alone was not controlling the rate of DEA. On the contrary, the distribution of sites with high surface water NO_3^- coincided with the area of elevated DEA.

In this study, it was assumed that denitrification was the major pathway for surface water NO_3^- disappearance. Several studies have shown denitrification is the major pathway of nitrate removal in flooded soils (DeLaune et al., 2005; DeBusk et al., 2001: Seitzinger, 1988). However, other processes such as assimilation to plant tissue, immobilization to microbial organisms, or dissimilatory reduction to ammonium (DRNA), will also result in nitrate loss. A riparian wetland microcosm experiment indicated that in macrophyte dominated soils, the percent of NO_3^- loss attributed to denitrification was 61-63%, while 24-26% was lost to immobilization, 11-15% to assimilation, and <1% to DNRA (Matheson et al., 2002). Processes such as dilution and mixing are also contribute to the NO_3^- concentration. DEA only reflects enzyme activity associated with the denitrification pathway and does not provide insight on the importance of other pathways in this system.

The nitrate removal efficiency of Davis Pond can be calculated as the difference in NO_3^- from inflow (2.0 mg NO_3^--N l^{-1}) to outflow (0.5 mg NO_3^--N l^-), a 75% removal efficiency. Measuring soil DEA can provide more detailed information than surface water NO_3^- concentrations alone because it allows you to distinguish between the denitrification of background nitrate and exogenous nitrate. In this study, DEA data suggests all *exogenous*

nitrate was removed within the 715 ha area proximal to the inflow. Low levels of DEA are observable in wetland soils and sediments receiving no nitrate, a product of internal N cycling and coupled nitrification-denitrification (Miao et al., 2006a; Miao et al., 2006b). A nitrate loading lab study using Davis Pond soils also found DEA rates from 0 to 0.46 mg N_2O-N kg^{-1} h^{-1} in soils receiving no exogenous nitrate (see Chapter 2). This work suggests the contribution of internal (soil) biogeochemical N cycling to denitrification can be significant and should be considered when determining target effluent concentrations.

The benefits of quantifying DEA rather than only surface water NO_3^- concentration include the ability to measure DEA when standing water is not present, and the ability of this quick, one-time collection method to indicate a time-averaged rate of nitrate exposure. Denitrifying enzymes can be synthesized and/or activated quickly (White and Reddy, 1999) and can remain stable during temporarily unfavorable conditions (Smith and Parsons, 1985). These unique characteristics make DEA a good indicator of the prevailing nitrate concentration at a specific location over the previous weeks to months, compared to a single surface water sampling event, which reflects only instantaneous conditions.

DEA may also reflect small-scale spatial variations in nitrate loading that traditional tracers cannot, as well as provide information about the flow path of nitrate-rich water. Traditional hydrologic studies use tracer additions (dyes, anions, cations, or isotopes) which are released, collected, and quantified at specified locations downstream. Such studies are often compromised by the conservation, sorption, and reactivity of the added tracer, resulting in < 100% recovery. In addition, these methods are also labor intensive and only feasible in smaller (e.g. < 500 ha) systems (Dierberg et al., 2005; Martinez and Wise, 2003; Wang et al., 2006). DEA measurements from this study not only demonstrated the aerial extent of

elevated DEA, but also provided insight about the flow path of introduced river water through the marsh. The south-eastern orientation of the high DEA soils suggests river water is not flowing directly toward the outflow, but is being deflected eastward, away from the historic splay ridges on the western side of the marsh. The elevation differences within Davis Pond appear to be contributing to the channelization and short-circuiting of river water, especially during low discharge events.

Future work should investigate how the area of elevated DEA in Davis Pond changes at higher discharge rates. Due to the strong influence of hydraulic retention time on denitrification rate, the area of marsh exhibiting elevated DEA at higher discharges is unlikely to show a linear relationship (Blahnik and Day, 2000; Kjellin et al., 2007)

3.5 CONCLUSION

The highest DEA rates (0.41 to 2.10 mg N_2O-N kg^{-1} h^{-1}) were found within a 715 ha area proximal to the diversion inflow at a discharge rate of 39.5 ± 10.4 m^3 s^{-1}. This area of elevated DEA contained over 80% of all observed DEA in the marsh, but represented only 19% of the total marsh area (Figure 3.6). Outside the 715 ha area, DEA ranged from below detection to 0.30 mg N_2O-N kg^{-1} h^{-1}. The paucity of O_2 and accumulation of C in this organic wetland soil dictates that NO_3^- concentration would control the synthesis of denitrifying enzymes. Therefore, areas of elevated DEA represent soils exposed to higher concentrations of NO_3^- from the re-introduced Mississippi River water. Surface water NO_3^- concentrations at a limited number of sites support this statement.

Quantifying the DEA of organic wetland soils collected within an area of suspected nitrate loading provides more detailed spatial information then traditional methods, such as using tracers or measuring surface water NO_3^- concentrations alone. Due to the high levels of

nitrate in the Mississippi River, DEA serves as an effective indicator for the spatial area of the marsh impacted by nitrate loading and provides insight on the flow path of river water. Future work should test the applications of DEA mapping in other organic wetland soils, such as coastal, urban, or agricultural wetlands receiving anthropogenic nutrients.

CHAPTER 4: SUMMARY AND CONCLUSIONS

4.1 SUMMARY

Coastal land loss in Louisiana is a natural phenomenon that has been accelerated by anthropogenic forces. As attention focuses on the ecological, economic, and cultural resources at risk of being lost, federal and state agencies are attempting to develop restoration plans that provide the greatest benefit to the landscape with the least impacts to property and commercial interests. As a result, much of the costal restoration funds have been allocated to the construction of freshwater diversion projects. Freshwater diversions are intended to re-introduce Mississippi River water to what would be the natural floodplain if the river were not restricted by levees. Providing freshwater to the subsiding estuaries bordering the Mississippi River is expected to combat salt water intrusion, promote wetland productivity by providing nutrients, and locally supply mineral sediment. Large-scale diversion projects have been met with equal amounts of hope and skepticism. A major concern among scientists is the potential for nutrient-rich river water to negatively impact habitats downstream of a diversion. Specifically, questions regarding how the river water will alter the biochemistry of the receiving estuary, and over what spatial scale the effects will be seen, remain unanswered.

Excess nitrogen is of greatest consequence downstream of these diversions because it is the limiting nutrient in flooded soils and estuarine environments. Research interests include the fate of nitrate in the estuary, its affect on primary production, and the ability of the estuary to serve as a substrate for denitrification. The main goal of this research was to determine if denitrification enzyme activity (DEA) can be used as a spatial indicator of nitrate loading in the Davis Pond marsh, a 3,760 ha wetland directly downstream of the Davis Pond freshwater diversion. The objectives were to, 1) determining the relationship between DEA and surface

water nitrate concentration in a controlled laboratory setting, and 2) identify the area of the marsh with elevated rates of DEA at a given discharge rate.

First, nitrate was loaded to intact soil cores in a flow-though design to establish the correlation between nitrate concentration and DEA. Thirty-six soil cores (0-20 cm of soil) were collected from a small area in the SW portion of Davis Pond marsh and brought back to the lab. Each core was connected to a peristaltic pump that conveyed a nitrate solution containing 0.0, 0.5, 1.0, or 2.0 mg NO_3^--N l^{-1}, mimicking concentrations normally observed in Mississippi river water. The 10 cm water column turned-over approximately 4 times daily. Cores were incubated in a water bath at room temperature in the dark and environmental conditions (e.g. temperature, soil redox, pH, DO) were regularly monitored throughout the experiment. Triplicates from each nitrate treatment were analyzed after 7, 20, or 45 days of nitrate loading. The soils were divided into 3 depth segments (0-5, 5-10, and 10-20 cm) and DEA, microbial biomass, TC, TN, and general soil properties were measured.

Overall, results from the laboratory core study indicated a significant difference in DEA with nitrate treatment ($P < 0.05$). After 20 days of nitrate loading, both the 0-5 cm and 5-10 cm soil horizons showed a strong positive relationship with surface water nitrate; the relationship was not significant on days 7 and 45. Denitrification rate, calculated by the loss of NO_3^--N from the core surface water, increased significantly with treatment and averaged 92 ± 44 mg N m^{-2} d^{-1}. DEA rates in all treatments peaked at day 7, and then decreased to a steady-state on days 20 and 45. The control treatment (0.0 mg NO_3^--N l^{-1}) had measureable DEA on all days, indicating the contribution of internal biochemical N cycling to DEA in organic wetland soils. On average, 92% of all enzyme activity was observed in the upper 0-5 cm of soil, 7% occurred at 5-10 cm, and <1% below 10 cm.

DEA was then measured on 88 soil samples randomly distributed in Davis Pond marsh to determine the spatial distribution of DEA in the field. Each core was divided into 0-10 cm and 10-20 cm, and DEA and general soil properties were quantified. The location of each sample and rate of DEA in the surface soil (0-10 cm) was then mapped using ArcGIS software. The resulting map revealed an aggregation of the highest rates of DEA nearest to the diversion inflow. Within this 715 ha area proximal to the inflow, DEA rates were between 0.41 and 2.10 mg N_2O-N kg^{-1} h^{-1}, while outside this area, DEA ranged from below detection (0.006) to 0.30 mg N_2O-N kg^{-1} h^{-1}. For comparison, the lab study found DEA rates of 0.46 mg N_2O-N kg^{-1} h^{-1} in the 0-5 cm horizon of soils receiving no external nitrate.

The highest surface water nitrate concentrations were observed within the area of elevated DEA. Additionally, by measuring potential denitrification on soils with high DEA and soils with low DEA, we confirmed that all soils had the same capacity to denitrify. This suggests DEA rates were limited by nitrate exposure *in situ*. At the low discharge rate (39 m^3 s^{-1}) during which the soil samples were collected, all excess nitrate was removed within a 715 ha area, or 19% of the total marsh area. Soils with high DEA were oriented in a south-easterly direction upon entering the marsh, suggesting the splay ridges on the western side of the marsh may be contributing to channelization and/or short-circuiting of surface water at low discharge rates. Future work should focus on measuring DEA after high discharge events.

4.2 CONCLUSIONS

- A laboratory study found significantly higher DEA in surface soils receiving 1.0 mg NO_3^--N l^{-1} compared to soils receiving no nitrate.

- After 20 days of continuous nitrate loading in the lab, a significant positive correlation was observed between DEA and nitrate concentration in the 0-5 cm and 5-10 cm soil horizons of intact cores. This correlation was not significant on days 7 and 45.
- Internal biochemical N cycling and coupled nitrification-denitrification produced DEA rates of 0.46 mg N_2O-N kg^{-1} h^{-1} (5.2 mg N_2O-N m^{-2} d^{-1}) in the 0-5 cm soil horizon of the intact cores (day 20).
- Approximately 92% of all DEA occurred in the surface 0-5 cm of soil, 7% occurs at a depth of 5-10 cm, and <1% occurs below 10 cm in the lab study.
- More than 80% of DEA observed in the marsh occurred in a 715 ha area proximal to the diversion inflow at a discharge rate of 39 m^3 s^-. This area of elevated DEA represented only 19% of the total Davis Pond marsh area.
- The distribution of soils with high DEA suggests river water is flowing in a south-easterly direction upon entering the marsh, possible to circumvent the elevated splay ridges in the western portion of the marsh.

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APPENDIX: SUPPLEMENTAL DATA

Table A. Intact soil core properties
a. 0.0 mg NO_3^--N l^{-1}treatment

Core No.	Soil Depth *(cm)*	Incubation Length *(days)*	Bulk Density *(g cm^{-3})*	pH	TC *(g kg^{-1})*	TN *(g kg^{-1})*	DEA *(g kg^{-1} h^{-1})*	MBC *(g kg^{-1})*	MBN *(g kg^{-1})*	Extrac. NO_3^- *(mg kg^{-1})*	Extrac. NH_4^+ *(mg kg^{-1})*
1	0	20	0.055	6.5	207.7	17.0	0.002	27.38	0.75	0.00	2.95
1	5	20	0.069	6.5	418.9	31.1	0.000	21.21	0.38	0.10	0.99
1	10	20	0.057	6.6	459.5	33.0	0.000	30.16	0.70	0.30	0.34
2	0	7	0.094	6.8	133.4	11.1	0.060	17.69	0.11	0.00	1.85
2	5	7	0.081	6.5	315.3	24.5	0.000	27.28	0.40	0.35	2.08
2	10	7	0.060	7.0	459.8	33.8	0.000	39.17	0.32	0.13	1.61
3	0	20	0.113	7.0	185.6	14.9	0.959	19.99	0.07	0.00	2.22
3	5	20	0.074	7.0	413.5	28.1	0.000	30.10	0.47	0.00	0.00
3	10	20	0.059	6.8	454.8	34.0	0.000	35.54	0.00	0.13	1.26
4	0	45	0.001	7.0	206.9	16.6	0.816	24.00	0.00	0.02	2.26
4	5	45	0.061	7.1	336.3	27.8	0.016	23.83	0.00	0.24	1.72
4	10	45	0.062	7.1	474.0	36.5	0.004	30.66	0.62	0.19	1.53
5	0	45	0.132	7.1	142.0	12.3	1.124	14.15	0.43	0.11	3.46
5	5	45	0.076	7.2	294.0	23.0	0.030	22.82	0.66	0.24	3.45
5	10	45	0.058	7.2	481.4	35.0	0.000	27.97	0.46	0.13	1.56
6	0	7	0.062	6.8	171.3	14.8	2.676	24.69	0.12	0.00	1.17
6	5	7	0.106	7.1	183.7	14.9	0.075	10.96	0.67	0.14	2.75
6	10	7	0.066	6.8	333.0	26.0	0.033	31.38	0.49	0.05	0.73
7	0	45	0.068	7.0	212.9	18.3	0.027	26.86	0.58	0.00	2.20
7	5	45	0.061	7.7	288.9	23.5	0.000	25.07	0.19	0.00	5.26
7	10	45	0.047	7.5	442.6	35.1	0.000	28.85	0.58	0.09	1.83
8	0	7	0.105	7.0	174.9	15.1	0.350	22.66	0.32	0.00	1.30
8	5	7	0.052	6.9	304.3	25.0	0.138	25.77	0.47	0.57	1.64
8	10	7	0.060	6.8	440.9	33.6	0.000	34.11	0.72	0.14	1.36
9	0	20	0.086	7.0	194.5	16.4	0.424	27.00	0.48	0.10	1.66
9	10	20	0.060	7.0	452.0	33.9	0.000	25.74	0.29	0.00	0.57

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b. 0.5 mg NO_3^--N l^{-1} treatment

Core No.	Soil Depth *(cm)*	Incubation Length *(days)*	Bulk Density *(g cm^{-3})*	pH	TC *(g kg^{-1})*	TN *(g kg^{-1})*	DEA *(g $kg^{-1}h^{-1}$)*	MBC *(g kg^{-1})*	MBN *(g kg^{-1})*	Extrac. NO_3^- *(mg kg^{-1})*	Extrac. NH_4^+ *(mg kg^{-1})*
1	0	45	0.095	6.6	207.0	17.3	1.515	21.06	0.29	0.00	1.06
1	5	45	0.068	7.1	363.6	28.6	0.000	27.87	0.74	0.15	1.64
1	10	45	0.060	7.0	472.3	34.4	0.000	30.16	0.45	0.00	1.92
2	0	7	0.102	6.7	184.3	14.8	0.541	17.93	0.19	0.01	1.37
2	5	7	0.055	6.6	268.4	21.1	0.101	28.83	0.31	0.44	1.68
2	10	7	0.069	6.3	441.5	32.1	0.067	27.92	0.00	0.26	0.96
3	0	45	0.097	6.8	185.8	15.3	0.892	25.26	0.17	0.03	1.42
3	5	45	0.068	7.1	310.2	24.0	0.065	24.43	0.50	0.46	1.45
3	10	45	0.048	6.9	454.9	35.1	0.014	34.00	0.49	0.04	0.80
4	0	20	0.117	6.9	155.6	12.7	0.424	15.86	0.28	0.00	1.27
4	5	20	0.068	7.1	315.2	23.9	0.000	27.38	0.13	0.00	0.16
4	10	20	0.063	7.1	474.9	34.2	0.000	34.89	0.18	0.69	1.04
5	0	7	0.084	7.2	196.1	17.3	0.686	23.56	0.35	0.00	1.61
5	5	7	0.068	7.1	318.6	25.0	0.000	33.14	0.11	0.05	0.59
5	10	7	0.061	6.9	434.3	30.5	0.027	29.04	0.03	0.03	0.00
6	0	20	0.088	6.8	205.1	15.8	0.320	19.39	0.30	0.04	2.33
6	5	20	0.074	6.9	378.1	28.1	0.003	32.43	0.62	0.42	2.27
6	10	20	0.054	7.2	452.0	33.9	0.000	31.40	0.57	0.46	2.02
7	0	7	0.100	7.1	161.9	13.6	2.545	33.95	0.32	0.02	1.17
7	5	7	0.098	6.9	231.8	18.9	0.000	26.30	0.39	0.26	2.53
7	10	7	0.052	7.0	449.0	34.5	0.000	25.70	0.43	0.00	0.29
8	0	45	0.088	6.7	185.9	15.7	0.456	23.87	0.38	0.00	1.89
8	5	45	0.071	6.9	399.2	29.3	0.026	26.02	0.76	0.48	1.99
8	10	45	0.064	6.8	467.1	32.7	0.043	26.07	0.31	0.00	1.83
9	0	20	0.073	7.2	256.6	20.5	0.986	29.59	0.54	0.10	2.34
9	5	20	0.067	7.3	465.9	35.4	0.035	24.19	0.48	0.06	1.68

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c. 1.0 mg NO_3^--N l^{-1} treatment

Core No.	Soil Depth *(cm)*	Incubation Length *(days)*	Bulk Density *(g cm^{-3})*	pH	TC *(g kg^{-1})*	TN *(g kg^{-1})*	DEA *(g$kg^{-1}h^{-1}$)*	MBC *(g kg^{-1})*	MBN *(g kg^{-1})*	Extrac. NO_3^- *(mg kg^{-1})*	Extrac. NH_4^+ *(mg kg^{-1})*
1	0	7	0.099	7.0	168.9	14.7	3.115	26.81	0.05	0.01	0.90
1	5	7	0.066	6.8	342.8	28.7	0.019	36.32	0.11	0.28	1.64
1	10	7	0.049	6.9	462.9	36.1	0.000	40.77	0.08	0.09	0.87
2	0	45	0.118	6.5	140.9	12.1	0.870	12.11	0.12	0.02	1.73
2	5	45	0.075	6.6	285.7	23.4	0.983	21.27	0.20	0.54	2.18
2	10	45	0.052	6.8	478.2	34.8	0.000	24.34	0.44	0.13	0.69
3	0	7	0.062	6.8	212.4	17.3	3.456	31.38	0.39	0.02	1.50
3	5	7	0.104	7.0	202.0	16.4	0.029	18.20	0.21	0.43	1.96
3	10	7	0.063	6.9	419.3	30.5	0.000	33.12	0.34	0.06	0.06
4	0	45	0.101	7.0	176.1	15.4	1.726	25.80	0.35	0.10	1.80
4	5	45	0.048	7.6	288.4	24.3	0.559	30.80	0.59	0.47	1.72
4	10	45	0.041	7.1	438.7	33.0	0.012	44.01	0.47	0.07	1.15
5	0	20	0.134	7.1	193.5	16.5	1.723	31.03	0.39	0.00	1.35
5	5	20	0.064	6.8	357.2	27.6	0.045	31.20	0.71	0.08	1.15
5	10	20	0.055	7.1	462.9	34.9	0.000	40.54	0.65	0.00	0.68
6	0	45	0.088	6.8	219.7	17.8	1.224	20.51	0.31	0.26	2.10
6	5	45	0.055	7.0	360.2	27.3	0.000	27.35	0.36	0.19	2.25
6	10	45	0.067	6.7	469.2	34.6	0.000	27.32	0.52	0.22	0.97
7	0	7	0.095	6.9	155.8	12.7	1.816	18.80	0.13	0.00	1.77
7	5	7	0.076	6.5	276.7	21.7	0.000	23.65	0.00	0.35	1.08
7	10	7	0.061	6.8	460.2	33.4	0.000	36.60	0.31	0.05	1.49
8	0	20	0.141	6.8	141.1	12.1	1.029	12.24	0.19	0.00	2.04
8	5	20	0.063	6.6	286.4	21.3	0.071	17.64	0.35	0.07	2.24
8	10	20	0.069	7.0	444.6	32.6	0.000	23.41	0.08	0.07	2.32
9	0	20	0.078	6.8	223.5	17.5	1.066	22.89	0.66	0.01	1.27
9	5	20	0.075	6.9	437.3	34.7	0.008	30.68	0.07	0.00	1.91
9	10	20	0.060	6.6	464.7	33.5	0.000	37.89	0.29	0.00	0.26

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d. 2.0 mg NO_3^--N l^{-1} treatment

Core No.	Soil Depth *(cm)*	Incubation Length *(days)*	Bulk Density *(g cm^{-3})*	pH	TC *(g kg^{-1})*	TN *(g kg^{-1})*	DEA *(g$kg^{-1}h^{-1}$)*	MBC *(g kg^{-1})*	MBN *(g kg^{-1})*	Extrac. NO_3^- *(mg kg^{-1})*	Extrac. NH_4^+ *(mg kg^{-1})*
1	0	20	0.085	6.8	223.9	18.0	1.887	31.58	0.06	0.02	1.50
1	5	20	0.112	6.9	212.8	16.6	0.188	18.13	0.37	0.00	3.32
1	10	20	0.066	6.8	420.9	31.5	0.000	26.15	0.35	0.01	1.71
2	0	45	0.108	6.9	163.8	13.3	0.871	19.07	0.22	0.01	2.12
2	5	45	0.060	7.0	318.2	25.7	0.035	23.23	0.42	0.52	2.26
2	10	45	0.062	6.7	444.4	35.5	0.001	22.84	0.47	0.12	1.68
3	0	45	0.078	7.0	334.7	26.0	0.478	33.22	0.42	0.02	1.19
3	5	45	0.071	7.1	465.8	37.0	0.000	24.60	0.31	0.24	1.37
3	10	45	0.089	6.7	461.5	34.2	0.000	22.36	0.00	0.02	0.99
4	0	7	0.095	6.9	155.3	13.3	0.931	19.98	0.09	0.00	2.02
4	5	7	0.062	6.9	294.5	23.0	0.000	23.36	0.22	0.08	1.83
4	10	7	0.058	7.0	441.2	32.6	0.000	20.99	0.18	0.02	1.71
5	0	20	0.137	7.0	180.9	15.6	1.087	25.41	0.36	0.00	1.24
5	5	20	0.070	6.9	334.6	26.3	0.123	28.80	0.70	0.35	0.88
5	10	20	0.063	7.1	488.8	35.7	0.000	41.09	0.62	0.00	0.56
6	0	20	0.162	7.2	170.6	14.1	1.485	27.98	0.16	0.00	1.31
6	5	20	0.062	7.2	341.5	26.4	0.423	20.80	0.37	0.08	1.07
6	10	20	0.048	7.1	467.2	36.3	0.000	42.06	0.62	0.00	0.80
7	0	7	0.094	7.2	194.8	16.1	1.976	35.96	0.23	0.02	1.65
7	5	7	0.063	7.2	302.8	24.0	0.137	27.44	0.45	0.37	2.22
7	10	7	0.050	7.3	451.8	34.3	0.000	35.37	0.33	0.04	1.03
8	0	45	0.097	6.8	180.7	14.4	1.104	18.09	0.14	0.16	1.65
8	5	45	0.087	7.1	303.2	23.9	0.215	19.97	0.07	0.24	1.88
8	10	45	0.059	6.9	458.8	33.8	0.051	29.25	0.59	0.15	1.41
9	0	7	0.089	7.0	197.2	17.1	2.898	27.25	0.41	0.01	1.47
9	5	7	0.088	7.1	279.1	21.1	0.000	21.63	0.55	0.03	0.63
9	10	7	0.072	7.0	433.7	34.6	0.000	20.22	0.19	0.00	1.38

Table B. Core water nitrate concentration

Date	Treatment *(mg NO_3-N l^{-1})*	NO_3-N *(mg NO_3-N l^{-1})*	Denitrification Rate *(mg $d^{-1}m^{-2}$)*
7/2/2007	0.0	0.004	N/A
	0.0	0.003	N/A
7/7/2008	0.0	0.001	N/A
	0.0	0.001	N/A
7/12/2008	0.0	0.004	N/A
	0.0	0.002	N/A
7/18/2008	0.0	0.004	N/A
	0.0	0.002	N/A
7/2/2007	0.5	0.366	46.2
	0.5	0.313	64.6
7/7/2008	0.5	0.357	49.5
	0.5	0.422	26.9
7/12/2008	0.5	0.375	43.4
	0.5	0.396	35.8
7/18/2008	0.5	0.380	41.5
	0.5	0.368	45.7
7/2/2007	1.0	0.591	141.4
	1.0	0.662	116.8
7/7/2008	1.0	0.768	80.3
	1.0	0.729	93.6
7/12/2008	1.0	0.852	51.2
	1.0	0.680	110.6
7/18/2008	1.0	0.811	65.5
	1.0	0.714	99.0
7/2/2007	2.0	1.597	139.3
	2.0	1.583	144.3
7/7/2008	2.0	1.584	143.7
	2.0	1.581	144.9
7/12/2008	2.0	1.562	151.5
	2.0	1.612	134.3
7/18/2008	2.0	1.542	158.2
	2.0	1.767	80.7

Table C. Core water dissolved oxygen (DO)

Date	Treatment *(mg NO_3-N l^{-1})*	DO *(mg l^{-1})*
7/3/2008	0.0	2.7
	0.0	2.6
7/9/2008	0.0	2.1
	0.0	1.8
7/13/2008	0.0	2.1
7/31/2008	0.0	2.5
	0.0	2.5
8/4/2008	0.0	2.2
7/3/2008	0.5	3.2
	0.5	4.6
7/9/2008	0.5	2.6
	0.5	2.4
7/13/2008	0.5	2.5
	0.5	2.2
7/31/2008	0.5	2.3
	0.5	2.7
	0.5	3.0
8/4/2008	0.5	2.1
7/3/2008	1.0	3.7
	1.0	3.3
7/9/2008	1.0	2.9
	1.0	3.2
7/13/2008	1.0	2.0
	1.0	1.9
7/31/2008	1.0	2.5
	1.0	2.7
	1.0	2.4
8/4/2008	1.0	2.4
7/3/2008	2.0	3.9
	2.0	4.0
7/9/2008	2.0	4.1
	2.0	3.9
7/13/2008	2.0	3.1
	2.0	2.0
7/31/2008	2.0	2.7
	2.0	2.8
	2.0	2.6
8/4/2008	2.0	3.0

Table D. Oxidation-reduction potential at soil depth during core incubation.

Date	Treatment	3 cm*	7 cm*
	(mg NO_3-N l^{-1})	*mV*	*mV*
7/17/2007	0.0	-188	-201
7/31/2007	0.0	-96	-31
8/7/2007	0.0	-160	-34
7/17/2007	0.5	-128	-203
	0.5	-30	-144
7/31/2007	0.5	-58	-31
8/7/2007	0.5	-35	-139
7/17/2007	1.0	-206	-193
7/31/2007	1.0	-55	-49
	1.0	-50	-29
	1.0	-53	-39
8/7/2007	1.0	-122	-62
	1.0	-66	2
	1.0	-168	-168
7/17/2007	2.0	-27	-210
	2.0	-41	-216
	2.0	-85	-186
7/31/2007	2.0	-31	-10
	2.0	-60	ND
8/7/2007	2.0	-198	-4
	2.0	-95	-33

* Redox has been corrected to SCE by adding 245mV to each reading

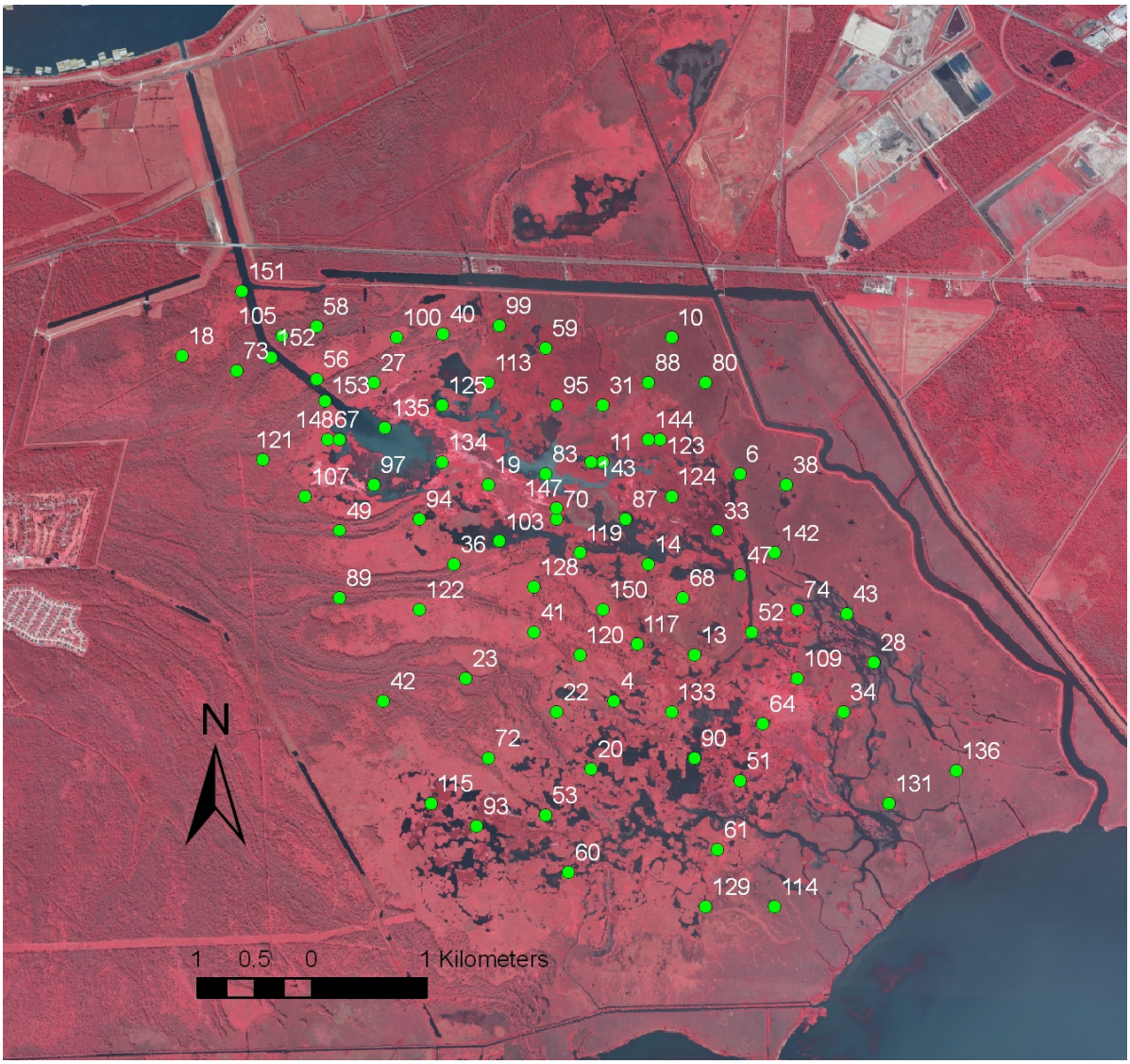

Figure A. Location map of the 88 field sampling sites.

Table E. Field study soil properties

Site Number	Long. *(UTM)*	Lat. *(UTM)*	Moisture *wt %*	Bulk Dens. *g cm^{-3}*	pH	OM *%*	Total N *mg kg^{-1}*	Total C *mg kg^{-1}*
1	765171	3306901	94.5	0.057	7.1	89.8	30.3	499.2
4	762300	3308600	94.4	0.055	6.9	63.2	23.9	317.5
6	763400	3310600	91.0	0.087	6.9	47.0	15.0	211.6
8	758500	3310100	92.5	0.080	7.0	68.4	21.6	354.0
10	762800	3311800	94.8	0.055	6.8	77.1	26.6	392.3
11	762200	3310700	79.4	0.241	7.3	18.7	5.6	71.3
13	763000	3309000	91.6	0.082	6.6	68.4	23.8	332.1
14	762600	3309800	91.4	0.082	6.6	38.8	14.3	175.0
18	758528	3311636	96.0	0.053	6.4	88.8	27.3	470.1
19	761200	3310500	77.5	0.242	7.6	15.3	5.1	56.2
20	762100	3308000	94.6	0.061	7.0	87.2	30.5	456.6
22	761800	3308500	96.3	0.035	7.4	71.1	26.9	359.7
23	761000	3308800	91.8	0.092	6.9	70.3	22.9	337.7
27	760200	3311400	90.8	0.096	7.4	51.7	18.9	255.2
28	764571	3308935	93.1	0.072	6.7	73.6	24.1	345.4
31	762200	3311200	79.3	0.242	7.3	12.0	3.5	44.5
33	763200	3310100	88.1	0.137	6.8	38.7	13.5	174.8
34	764300	3308500	89.7	0.118	6.9	56.4	19.6	272.7
36	760900	3309800	92.5	0.080	7.0	53.8	18.2	267.4
38	763800	3310500	84.0	0.169	6.8	44.6	14.4	197.3
39	759500	3309800	91.6	0.072	7.3	59.3	18.9	287.8
40	760801	3311825	95.6	0.044	7.1	68.0	23.3	329.5
41	761600	3309200	94.0	0.058	7.1	80.0	27.0	409.8
42	760279	3308597	92.5	0.071	7.2	85.5	23.3	444.9
43	764329	3309362	90.8	0.099	6.8	56.9	21.6	288.4
47	763400	3309700	90.8	0.083	7.1	59.4	21.4	304.4
49	759900	3310100	92.2	0.088	7.4	31.9	12.2	154.2
51	763400	3307900	93.4	0.066	7.2	53.8	20.2	270.7
52	763500	3309200	58.5	0.498	7.0	12.1	2.9	38.2
53	761700	3307600	67.3	0.377	6.8	19.2	6.1	87.8
56	759700	3311432	67.8	0.410	7.3	16.4	4.3	68.9
58	759701	3311889	90.3	0.129	7.0	35.2	11.6	179.9
59	761700	3311700	94.6	0.056	7.3	39.9	13.7	185.2
60	761900	3307100	92.4	0.077	6.7	76.6	24.4	400.1
61	763200	3307300	92.3	0.081	7.2	67.2	25.9	332.7
64	763600	3308400	78.9	0.279	6.8	27.7	8.2	155.1
67	759800	3310900	90.2	0.111	7.7	28.7	9.8	132.7
68	762900	3309500	92.9	0.079	6.9	82.8	27.4	417.4
69	759200	3309400	85.5	0.162	7.5	31.8	10.9	170.0

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Site Number	Long. *(UTM)*	Lat. *(UTM)*	Moisture *wt %*	Bulk Dens. *g cm^{-3}*	pH	OM *%*	Total N *mg kg^{-1}*	Total C *mg kg^{-1}*
70	761800	3310200	80.1	0.220	7.4	15.2	5.5	59.3
71	759365	3308950	87.9	0.137	7.2	41.9	12.0	199.6
72	761200	3308100	97.6	0.030	7.2	85.5	25.0	419.8
73	759000	3311500	77.4	0.279	7.6	15.8	4.9	72.5
74	763900	3309400	93.2	0.101	6.6	46.3	17.2	232.8
80	763100	3311400	92.0	0.068	6.1	91.4	26.1	426.7
83	761700	3310600	62.1	0.511	7.5	9.6	3.0	56.4
87	762400	3310200	89.0	0.115	7.1	34.5	12.0	169.6
88	762600	3311400	95.4	0.047	6.5	64.5	23.2	319.5
89	759900	3309500	91.9	0.089	7.4	58.6	14.8	281.5
90	763000	3308100	93.5	0.071	7.3	64.5	22.6	313.9
93	761100	3307500	94.8	0.058	7.2	70.1	21.1	342.2
94	760600	3310200	92.5	0.095	7.1	48.9	16.0	249.6
95	761800	3311200	61.1	0.514	7.3	9.5	2.3	31.0
97	760200	3310500	68.0	0.371	7.5	9.6	2.6	26.4
99	761300	3311900	75.4	0.294	6.7	15.8	3.4	44.9
100	760400	3311800	92.1	0.081	6.3	77.1	25.0	376.5
103	761300	3310000	92.0	0.089	7.4	40.9	14.1	212.3
105	759398	3311809	90.8	0.109	7.2	42.4	11.1	215.3
106	758300	3310600	88.9	0.123	6.9	68.2	22.3	334.0
107	759600	3310400	88.3	0.139	7.2	21.6	7.0	82.2
109	763900	3308800	94.2	0.072	7.2	56.2	20.0	270.9
113	761200	3311400	89.0	0.123	7.1	23.2	7.2	91.7
114	763700	3306800	92.6	0.086	6.7	81.5	27.2	406.5
115	760700	3307700	94.7	0.055	6.7	68.1	25.2	349.0
117	762500	3309100	93.0	0.070	7.0	82.3	25.7	417.8
119	762000	3309900	88.4	0.089	6.6	41.0	14.7	195.4
120	762000	3309000	96.3	0.034	7.1	70.9	23.9	338.9
121	759231	3310720	89.7	0.122	6.9	64.7	15.1	318.9
122	760600	3309400	93.6	0.068	7.4	69.1	22.0	343.0
123	762700	3310900	79.4	0.234	7.2	16.9	6.0	75.8
124	762800	3310400	89.1	0.111	7.5	37.3	12.3	184.6
125	760800	3311200	77.1	0.296	7.2	14.0	5.0	53.6
128	761600	3309600	93.0	0.071	7.0	64.5	21.1	327.8
129	763100	3306800	90.5	0.097	6.7	69.6	23.2	368.4
130	758743	3311089	84.5	0.180	7.2	26.4	7.7	128.6
131	764700	3307700	93.1	0.077	7.1	76.2	26.0	382.2
133	762800	3308500	79.3	0.221	6.7	28.3	10.2	165.6
134	760800	3310700	71.0	0.372	7.7	9.8	2.9	32.6
135	760300	3311000	59.3	0.552	7.6	8.0	2.5	30.2
136	765285	3307988	89.7	0.102	6.7	55.6	19.2	275.2

Continued on page 101

Site Number	Longitude *(UTM)*	Latitude *(UTM)*	Moisture *wt %*	Bulk Density *g cm^{-3}*	pH	OM *%*	Total N *mg kg^{-1}*	Total C *mg kg^{-1}*
137	758800	3309700	96.3	0.039	7.2	71.8	21.8	364.3
140	758800	3310500	90.1	0.118	7.2	56.8	17.7	296.7
142	763700	3309900	91.2	0.087	6.5	57.1	17.9	271.1
143	762100	3310700	83.1	0.192	7.4	14.2	6.3	86.6
144	762600	3310900	76.7	0.267	7.4	14.4	5.0	67.5
147	761800	3310300	76.2	0.303	7.5	12.4	4.9	58.3
148	759900	3310900	88.1	0.124	7.4	31.7	10.7	151.8
150	762200	3309400	92.9	0.073	7.2	65.3	23.2	329.4

Table F. Field study DEA and site characteristics

Station Number	Longitude *(UTM)*	Latitude *(UTM)*	DEA *(mg kg^{-1} h^{-1})*	Vegetation Category§	Soil Category†
1	765171	3306901	0.000	2	2
4	762300	3308600	0.295	1	1
6	763400	3310600	0.122	3	1
8	758500	3310100	0.032	4	1
10	762800	3311800	0.000	3	1
11	762200	3310700	0.058	1	1
13	763000	3309000	0.046	3	1
14	762600	3309800	0.054	2	1
18	758528	3311636	0.000	2	2
19	761200	3310500	0.634	1	1
20	762100	3308000	0.019	2	1
22	761800	3308500	0.062	2	2
23	761000	3308800	0.063	4	1
27	760200	3311400	0.772	2	1
28	764571	3308935	0.045	2	1
31	762200	3311200	0.008	2	3
33	763200	3310100	0.118	3	1
34	764300	3308500	0.188	3	1
36	760900	3309800	2.085	2	2
38	763800	3310500	0.053	3	1
39	759500	3309800	0.053	4	1
40	760801	3311825	0.022	4	1
41	761600	3309200	0.021	1	1
42	760279	3308597	0.140	4	1
43	764329	3309362	0.000	2	1
47	763400	3309700	0.020	3	1
49	759900	3310100	1.725	2	1
51	763400	3307900	0.084	1	0
52	763500	3309200	0.000	2	1
53	761700	3307600	0.002	2	2
56	759700	3311432	0.000	2	1
58	759701	3311889	0.457	4	1
59	761700	3311700	0.804	2	3
60	761900	3307100	0.000	1	1
61	763200	3307300	0.000	2	1
64	763600	3308400	0.002	2	1
67	759800	3310900	1.973	2	2
68	762900	3309500	0.003	2	1
69	759200	3309400	0.064	2	1
70	761800	3310200	0.414	1	1
71	759365	3308950	0.685	2	1
72	761200	3308100	0.000	2	1

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Station Number	Longitude *(UTM)*	Latitude *(UTM)*	DEA *(mg kg^{-1} h^{-1})*	Vegetation Category§	Soil Category†
73	759000	3311500	0.644		
74	763900	3309400	0.837	3	1
80	763100	3311400	0.000	3	1
83	761700	3310600	0.067	1	1
87	762400	3310200	1.281	1	1
88	762600	3311400	0.563	3	1
89	759900	3309500	0.024	4	3
90	763000	3308100	0.201	1	2
93	761100	3307500	0.032	1	1
94	760600	3310200	0.119	2	1
95	761800	3311200	0.263	2	3
97	760200	3310500	0.532	1	1
100	760400	3311800	0.054	2	1
101	760600	3307200	0.183	2	2
103	761300	3310000	0.239	1	1
105	759398	3311809	0.619	4	1
106	758300	3310600	0.030	4	1
107	759600	3310400	1.160	2	1
109	763900	3308800	0.567	2	1
113	761200	3311400	1.673	2	1
114	763700	3306800	0.006	2	1
115	760700	3307700	0.206	1	1
117	762500	3309100	0.000	2	2
119	762000	3309900	0.051	2	2
120	762000	3309000	0.223	3	1
121	759231	3310720	0.491	2	1
122	760600	3309400	0.145	2	2
123	762700	3310900	1.220	1	1
124	762800	3310400	0.017	1	1
125	760800	3311200	0.797	1	1
128	761600	3309600	0.108	2	2
129	763100	3306800	0.001	2	1
130	758743	3311089	0.087	4	1
131	764700	3307700	0.000	2	1
133	762800	3308500	0.000	2	2
134	760800	3310700	0.492	1	1
135	760300	3311000	0.074	1	1
136	765285	3307988	0.046	3	1
137	758800	3309700	0.267	4	1
140	758800	3310500	0.124	4	1
142	763700	3309900	0.009	3	1
143	762100	3310700	1.262	2	2
144	762600	3310900	1.065	1	1

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Station Number	Longitude *(UTM)*	Latitude *(UTM)*	DEA *(mg kg^{-1} h^{-1})*	Vegetation Category§	Soil Category†
147	761800	3310300	1.271	1	1
148	759900	3310900	0.739	2	1
150	762200	3309400	0.242	2	2
151	759047	3312202	0.561	2	3
152	759303	3311619	0.626	2	3
153	759777	3311239	0.576	2	3

§ 1 = open water/SAV, 2&3 = emergent macrophytes, 4 = woody vegetation

† 1 = organic attached, 2 = flotant, 3 = organic with mineral components

Table G. Field surface water properties, 7/10/2007

Field ID	Longitude *(UTM)*	Latitude *(UTM)*	Temp *(ºC)*	Salinity *(ppt)*	DO *(mg l^{-1})*	pH	NH_4^+ *(mg l^{-1})*	NO_3^- *(mg l^{-1})*	SRP *(mg l^{-1})*
1	765171	3306901	31.2	0.28	8.4	7.6	0.024	1.051	0.077
2	765038	3307093	31.2	0.28	9.1	7.7	0.071	0.539	0.070
3	764600	3308900	31.3	0.27	7.9	7.3	0.055	0.001	0.124
4	764100	3308100	30.8	0.28	8.5	7.7	0.058	0.968	0.074
5	763375	3309704	30.2	0.28	7.1	7.5	0.011	1.094	0.070
6	762377	3310190	30.2	0.28	8.0	7.6	0.059	1.198	0.078
7	761735	3310782	29.3	0.28	7.7	7.7	0.062	1.724	0.101
8	760870	3310782	29.7	0.29	7.2	7.7	0.006	1.870	0.093
9	759847	3311235	30.3	0.29	7.6	7.9	0.033	1.752	0.116
10	759098	3312275	29.9	0.29	7.4	7.9	0.039	2.028	0.125
11	758479	3314314	29.5	0.29	7.4	ND	0.031	1.938	0.114

Table H. Denitrification potential of field soils

Site Number	Time	DEA (Rep.1)	DEA (Rep.2)
	(h)	*(mg kg^{-1} h^{-1})*	*(mg kg^{-1} h^{-1})*
36	0.0	0.000	0.000
	0.9	0.926	0.937
	2.4	2.031	2.016
	4.1	2.239	2.115
	6.1	2.195	2.359
	9.5	2.211	2.161
	13.6	2.177	2.125
	18.0	1.965	2.012
51	0.0	0.000	0.000
	0.9	0.149	0.084
	2.4	0.364	0.248
	4.1	0.638	0.533
	6.1	1.284	1.224
	9.5	2.046	2.463
	13.6	2.131	2.623
	18.0	1.976	2.500

Table I. DEA Sample Calculations

CALIBRATION CURVE:

Standard Gas Conc. *(N_2O-N ppm)*	GC Peak Area	Injection Vol. *(µL)*	*(N_2O-N ppm)*	x-coefficient	y-intercept
			(A*C/1,000,000)	(LINEST)	(LINEST)
100	22908	1000	0.1	4.38675E-06	-0.001441687
100	12116	500	0.05	6.76918E-08	0.000800181
100	4984	200	0.02	0.999286166	0.001259156
10	1374	500	0.005	4199.657357	3
10	610	200	0.002	0.006658444	4.75642E-06

N_2O-N ppm = Standard Gas Conc. (N_2O-N ppm) * Injection Vol (µL) / 1,000,000

x and y = LINEST (N_2O-N ppm, Peak Area, true, true)

KNOWN VALUES: (Example: Site 109)

Soil % moisture: 94.22

Wet sub-sample (g): 10.99

Dry sub-sample (g): 0.68

Temperature (K): 298

Bunsen Absorption Coefficient (25 C): 0.544

Gas Constant (mL atm/K µmol): 0.0821

Injection Volume *(µL)*	Time *(h)*	Pressure *(Kpa)*	Corrected Pressure *(Kpa)*	Pressure *(atm)*	GC Peak Area
1000	0.48	12.6	10	1.1	8127
500	0.97	11.2	8.6	1.086	8684
400	1.50	8.2	5.6	1.056	12326
200	2.03	7.7	5.1	1.051	9602

Corrected Pressure = Pressure (Kpa) – 2.6

Pressure (atm) = Corrected pressure / 101

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Vg *(μL)*	Vl *(μL)*	Cg *(μL N_2O/ μL gas)*	M *(μL N_2O)*	n *(μ moles N_2O)*	*(mg N_2O-N/kg soil)*	DEA Rate *(mg N_2O-N/kg soil/h)*	*(mg N_2O-N/kg soil/h)*
6085.13	1044.86	3.42E-05	0.23	0.0102	0.4247	1.2825	-0.2718
6085.13	1044.86	7.33E-05	0.49	0.0216	0.8985	0.0937	0.1287
6085.13	1044.86	1.32E-04	0.88	0.03773	1.5681	0.9894	0.1086
6085.13	1044.86	2.03E-04	1.35	0.05813	2.4127	187.28	2
6085.13	1044.86	3.42E-05	0.23	0.0102	0.4247	1.2825	-0.2718
6085.13	1044.86	7.33E-05	0.49	0.0216	0.8985	0.0937	0.1287

Vg ($Volu_{me}$ in gas phase) = Total bottle volune (μL) – Vl

Vl (Volume in liquid phase) = 8 mL [vol. enzyme soln.] + 5 mL [vol. H2O] – Soil dry weight * 100 [conversion factor from mL to μL]

Cg = (x-coefficient of calibration curve * Peak area + y-intercept) / Injection Volume

M = Cg * (Vg + (Vl * Bunsen coeff)

n = (M * Pressure (atm)) / Temp (K) * Gas Constant (mL atm/K μmol)

mg N2O-N/Kg soil = n (umoles N2O) * 28 [N_2 formula weight]/ dry soil weight (g)

DEA Rate = LINEST (mg N2O-N/Kg soil, Peak area, true, true)

Table J. Denitrification Rate Sample Calculation

Nitrate Treatment *(mg NO_3-N l^{-1})*	Measured in Surface Water *(mg NO_3-N l^{-1})*	Mass Loss *(mg NO_3-N)*	Percent Loss *(%)*	Loading Rate *(mg h^{-1})*	Mass Loss with Time *(mg NO_3-N h^{-1})*	Area of Core *(m^2)*	Denitrification Rate *(mg NO_3-N m^{-2} d^{-1})*
0.50	0.38	0.12	24.0	55.4	0.007	.00385	41.5

Mass Loss = Treatment NO_3-N – Surface Water NO_3-N

Percent Loss = (Mass Loss / Treatment NO_3-N) * 100

Mass Loss with Time = (Mass Loss / 1000) * Loading Rate

Denitrification Rate = (Mass Loss with Time / Area of Core) * 24 h

VITA

Lisa Gardner grew up in North Canton, Ohio, with her parents, Bruce and Marcia, and older brother, Chris. Her family instilled in her a great appreciation for nature through weekend trips to the lake and family vacations to national parks in the western U.S.

Lisa attended Ohio State University in Columbus, Ohio and graduated magna cum laude with a Bachelor of Science degree in the Department of Natural Resources, in 2003. At Ohio State, her desire to study wildlife veterinary medicine encouraged her to enroll in an introductory environmental science course. This course permanently changed her focus toward understanding, restoring, and conserving ecological resources. Her undergraduate mentor, Dr. Virginie Bouchard, was influential in refining Lisa's interests to wetland ecology.

After graduation, Lisa spent two years working as a Wetland Consultant in Dayton, Ohio, and one year as the Watershed Director of a non-profit organization. However, her strong desires to continue her education and be part of the historic wetland restoration effort in southern Louisiana lead her to accept a fellowship at Louisiana State University in Baton Rouge, Louisiana, with Dr. John White. Her master's education has succeeded in intensifying her interest and curiosity in wetlands and she plans to pursue a Doctor of Philosophy degree with K. Ramesh Reddy at the University of Florida beginning in the fall of 2008.