

**NITROGEN REMOVAL FROM WETLANDS VIA DENITRIFICATION:
LITERATURE REVIEW**

GOCA FINAL REPORT 2005

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Nitrogen Removal from Wetlands via Denitrification: Literature Review

Abstract

Nitrogen cycling in wetland and aquatic ecosystems may be the most complex interactions among major wetland nutrients. Measurement of rates of denitrification in marsh and estuarine water and sediment has gained national attention due to agricultural nitrogen loading in surface waters and eutrophication of coastal waters. Approximately 970,000 metric tons of nitrate-N are annually discharged into the Gulf of Mexico via the Mississippi–Atchafalaya River basin and may be partially responsible for the large area of coastal hypoxic water observed off the Louisiana and Texas coasts. Denitrification is the microbially mediated reduction of nitrate-N (NO_3^- -N) to gaseous end products such as elemental nitrogen (N_2) and nitrous oxide (N_2O), which escape to the atmosphere. Denitrification represents a major pathway for nitrogen removal from wetland and aquatic ecosystems and could lead to improved water and habitat quality within a nitrogen polluted drainage basin or ecosystem. This report discusses in detail nitrogen cycling and transformations in wetland environments. Both indirect and direct methods have been used to measure denitrification in freshwater, coastal, and marine environments. These methods are also discussed. Commonly used denitrification measurement procedures include nitrogen mass-balance techniques, the acetylene inhibition method, measurement of nitrate disappearance, and N-15 labeled procedures.

1.0 Introduction

The Mississippi—Atchafalaya River Basin is the largest North American drainage basin, draining an area of approximately 3,208,700 km² or 41% of the coterminous United States. The basin stretches from Pennsylvania to Idaho and from Minnesota to Louisiana (Goolsby et al. 2001). Land use in the basin includes: cropland (58%), range and barren land (21%), woodland (18%), wetland and water (2.4%), and urban land (0.6%). The basin contains the most productive farming regions in the world, and the majority of all pesticides and fertilizers used in the United States are applied to basin cropland. From 1951 to 1996, fertilizer input to the basin increased from less than 1 to about 7 million metric tons of nitrogen per year (Goolsby et al. 2001).

The Mississippi and Atchafalaya Rivers are the primary sources of freshwater and nutrients discharged into the Gulf of Mexico. The combined mean annual stream flow since 1980 is about 22,000 m³ s⁻¹, which represents about 80% of the freshwater discharge to the Gulf of Mexico. The two rivers account for 90% of total nitrogen flux annually discharged to the Gulf of Mexico (Dunn 1996). Mean annual nitrogen flux (1980-1996) from the Mississippi-Atchafalaya River basin to the Gulf of Mexico is about 1,568,000 metric tons. About 70-75% of the nitrogen flux enters the Gulf via the Mississippi River, and the remainder, about 390,000 metric tons, discharges through the Atchafalaya River (Goolsby et al. 2001). Total nitrogen flux is about 61% nitrate and dissolved/particulate organic nitrogen (37%). The annual flux of nitrate N to the Gulf has increased dramatically from 1955 through 1999. From 1955 to 1970, nitrate flux averaged 328,000 metric tons per year, but from 1980 to 1999, nitrate flux averaged about 969,000 metric tons per year—approximately a three fold increase (Goolsby et al. 2001). Over 90% of the nitrate flux to the Gulf of Mexico emanates from nonpoint sources (mainly agriculture), and the remaining 10% from municipal and industrial point sources. The highest nitrate flux is usually in the spring and early summer.

Nitrogen and other nutrients have been implicated as possible causes of hypoxia in a large area (“Dead Zone”) of coastal water along the Louisiana-Texas coast (Rabalais et al. 2002). Hypoxia is a deficiency in the amount of oxygen reaching living tissues. In coastal water, hypoxia is characterized by low dissolved oxygen levels (< 2 mg L⁻¹); meaning that there is not enough oxygen available to support fish and other aquatic species. Nutrients such as nitrogen and phosphorus are essential for healthy freshwater, coastal, and marine environments. An overabundance of nutrients can trigger excessive algal growth (eutrophication), which results in reduced sunlight, loss of aquatic habitat, and a decrease in dissolved oxygen in coastal waters. About 40% of U.S. fisheries are located in the waters off Louisiana’s coasts. Parts of this area are affected by hypoxia during the summer months, which can lead to loss of shrimp, crabs, zooplankton, and other important fish. Scientific evidence indicates that excess nitrogen (mainly nitrate) from the Mississippi and Atchafalaya River drainage basins drives the onset and duration of hypoxia in the Gulf of Mexico (EPA 2001). The hypoxic areas off the Louisiana and Texas coasts have increased in area over the past two decades. From 1985 to 1992 the hypoxic regions averaged 8,300 km². The same regions increased to 16,000 km² from 1993 through 2001 (Rabalais et al. 2002).

A major goal of the Mississippi River/Gulf of Mexico Watershed Nutrient Task Force Action Plan is to reduce the size of the Gulf's hypoxic zone to less than 5,000 km² by 2015. This will protect valuable resources and improve water quality throughout the vast Mississippi River Basin. To obtain this goal, the Task Force suggested that a 30% reduction from the 1980–1996 average nitrogen load to the Gulf of Mexico will be needed (EPA 2001). Modeling efforts by other researchers (Scavia et al. 2003) suggest that the proposed 30% reduction might not be sufficient to obtain that goal.

Approaches to reduce, mitigate, and control coastal hypoxia include:

- reducing nitrogen inputs from agriculture, point sources, atmospheric deposition, and other sources to streams and rivers in the basin; and
- restoring and enhancing natural denitrification and nitrogen retention processes to reduce nitrogen loads from the Mississippi and Atchafalaya Rivers to the Gulf of Mexico.

Potential approaches to increasing denitrification include: (1) creating and restoring wetlands, (2) creating and restoring riparian buffers, and (3) diverting rivers in coastal Louisiana.

2.0 Narrative

Nitrogen cycling in wetlands may be the most complex interactions among major aquatic ecosystem nutrients. Organic and inorganic nitrogen forms undergo various transformations and may be recycled several times as they pass through wetlands (Day et al. 1989). Wetland macrophytes, algae, and phytoplankton can assimilate significant inorganic nitrogen found in the water column and sediment. In treatment wetlands, macrophytes can assimilate inorganic nitrogen up to 3 to 4 g N m⁻² d⁻¹ (Kaldec and Knight 1996). Bacteria have also been shown to immobilize inorganic and organic nitrogen (about 0.2 μmol L⁻¹ h⁻¹) when the contact time with inorganic nitrogen is short (Wheeler and Kirchman 1986). At the water column-sediment interface, microbially mediated reactions play a major role in nitrogen transformations.

2.1 Ammonification and Ammonia Volatilization

Nitrogen cycling in the water column and/or sediment begins with the microbial decomposition of organic matter (ammonification) producing inorganic ammonium (NH₄⁺). Ammonium can also be formed by dissimilatory reduction of inorganic nitrate (NO₃⁻) in freshwater and estuarine sediments (Priscu and Downes 1987). Under specific environmental conditions, ammonium is the preferred N form that is assimilated and/or immobilized by primary producers and microbes. Ammonium-N can also be adsorbed by sediments or released to the water column. If the water column or sediment pH is high (above 8), ammonium-N can be transformed to ammonia (NH₃) and released into the

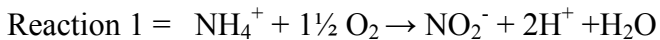
atmosphere via volatilization. Ammonia volatilization is the process whereby ammonium-N is in equilibrium with the gaseous form (NH₃) and hydroxyl ion:



2.2 Nitrification

If sufficient oxygen is available in the wetland water column, sediment ammonium-N will be transformed into nitrate -N (NO₃⁻-N) through biological oxidation (nitrification). Nitrification is a two-step process initiated by the activity of chemoautotrophic bacteria (obtain energy for growth and biosynthetic reactions from oxidation of inorganic compounds) of the genera *Nitrosomonas* (NH₄⁺-N → NO₂⁻) and *Nitrobacter* (NO₂⁻ → NO₃⁻). These organisms require oxygen (O₂) during ammonium-N oxidation to nitrite (NO₂⁻) and nitrite-N oxidation to nitrate (NO₃⁻). Oxygen is used as a terminal electron acceptor or directly incorporated into the substrate of the organism (Focht and Verstraete 1977). The two-step conversion of ammonium-N to nitrate-N is as follows:

Ammonium oxidation to nitrite (*Nitrosomonas* bacteria)



Nitrite oxidation to nitrate (*Nitrobacter* bacteria)



Combining the two reactions:



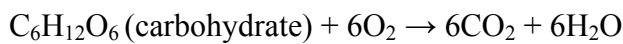
Oxygen is essential for these reactions, but nitrification can occur readily down to about 0.3 ppm dissolved oxygen. The actual limit is more dependent on oxygen flux into the system (water or sediment) rather than the actual O₂ level at a specific time. The transformation of ammonium-N to nitrate-N can potentially use large quantities of oxygen and at times produce oxygen depleted waters in wetlands and estuaries receiving large amounts of high nitrogen content wastes.

In a wetland ecosystem, nitrification can occur in: (1) the water column above submerged sediments, and (2) the surface oxidized sediment layer of wetlands (Reddy and Patrick 1984). In an aquatic ecosystem, nitrification flux rate is influenced by pH, temperature, alkalinity (water), microbial population, carbon source, and ammonium-N substrate concentration. Nitrification depends on the metabolism of nitrifying organisms, so adequate bacterial populations must be present to achieve oxidation of ammonium-N to nitrate-N. Large numbers of nitrifying organisms occur in most flooded soils and sediments and usually decrease with soil depth. In submerged freshwater ecosystems, approximately 80 to 100% of net ammonium production in sediments may undergo

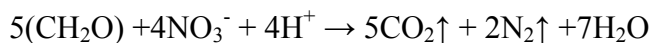
nitrification, compared to only 40 to 60% in marine sediments affected by salinity (Seitzinger 1990).

2.3 Denitrification

Denitrification is the microbially mediated reduction of nitrate-N (NO_3^- -N) to gaseous end products such as elemental nitrogen (N_2) and nitrous oxide (N_2O) and represents a major pathway for nitrogen removal in wetlands and aquatic ecosystems. Denitrification in wetlands is carried out by facultative bacteria (developed in the presence or absence of O_2). In the presence of oxygen, these organisms oxidize carbohydrates to carbon dioxide (CO_2) and water (H_2O) and use O_2 as an electron acceptor:



Under anaerobic or oxygen-free conditions and in the presence of available organic substrate, these organisms (denitrifying bacteria) use nitrate-N as the electron acceptor during respiration. Nitrate-N is converted to dinitrogen gas (N_2) according to the overall reaction:



This reaction is irreversible in wetlands, and nitrogen is lost from the system permanently. Reduction of nitrate follows a sequence where the oxidation state of the nitrogen species is reduced from +5 (NO_3^-) to 0 (N_2) in the order:



The irreversible step in the denitrification sequence is nitrite (NO_2^-) conversion to nitric oxide (NO) and nitrous oxide (N_2O) gases (Coyne and Tiedje 1990). Denitrification of NO_3^- -N to N_2O and N_2 as the only end products is the result of several different bacterial communities (Martienssen and Schops 1999). Nitric oxide (NO) gas (intermediate product) is only evolved in minor quantities due to its highly reactive nature. The evolution of N_2O may be substantial, but N_2 evolution is much more significant under flooded wetland conditions (Mosier and Heinemeyer 1985). The ratio of N_2 : N_2O observed in several studies ranged from less than 10:1 to greater than 100:1, depending upon concentrations of carbon, oxygen, and nitrate (Weier et al. 1993).

Figure 1 (from Iwai 2002) depicts a simplified diagram of nitrogen transformations and cycling in wetland ecosystem with denitrification occurring in the anaerobic soil layer resulting in loss of N_2O and N_2 to the atmosphere. In wetland and aquatic systems, under reducing sediment conditions, denitrification is a major removal mechanism of large inputs of inorganic nitrogen. Additional nitrogen transformations and nitrogen species cycling, as well as ammonification, nitrification, ammonia volatilization, and assimilation (microbial, plankton, and plant uptake), are also shown in Figure 1.

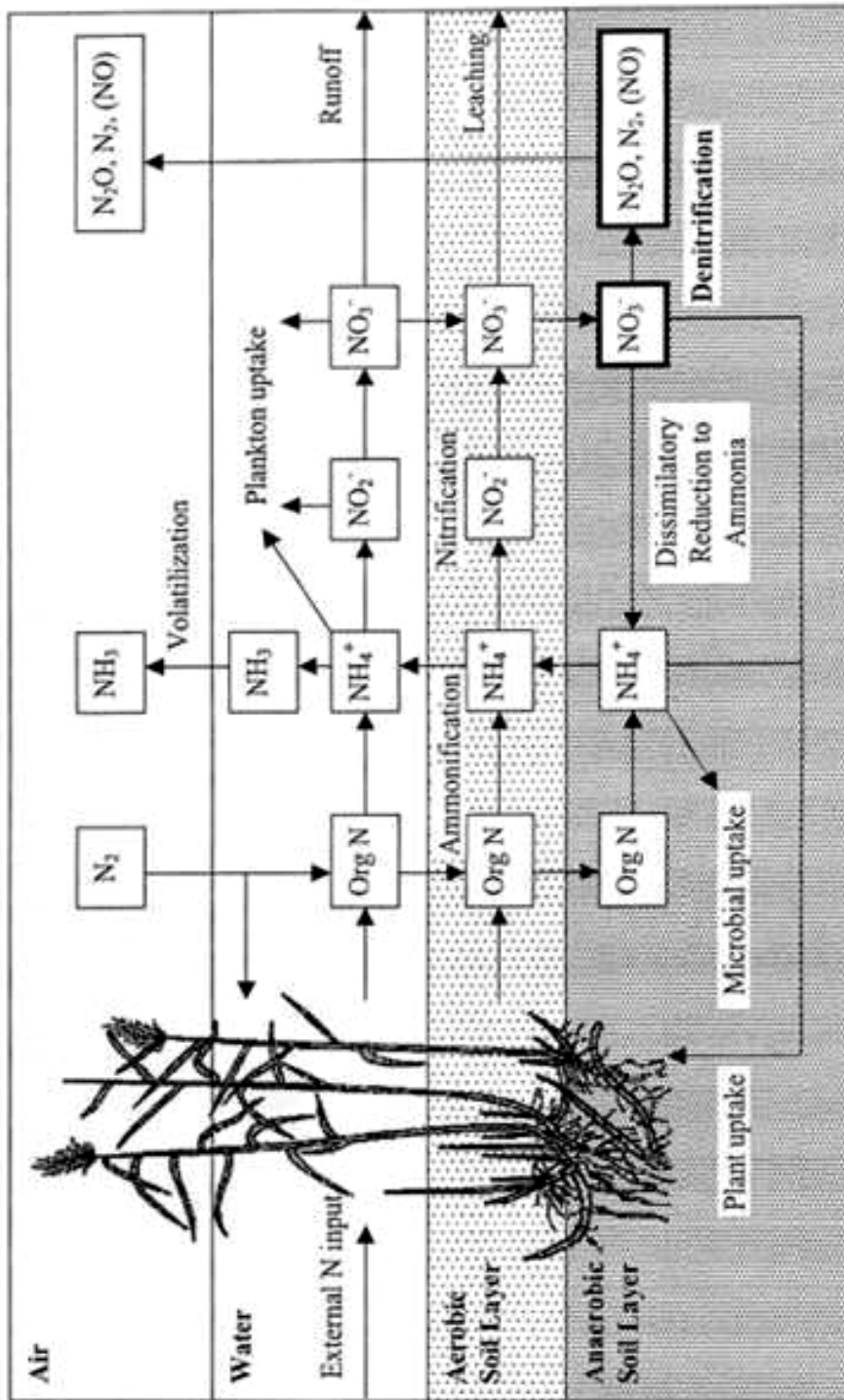


Figure 2.1. Nitrogen transformations and cycling in aquatic and wetland ecosystems (Iwai 2002).

2.4 Sediment Redox Potential

Wetland and aquatic ecosystem sediments are characterized by the absence of molecular oxygen and activity of facultative and anaerobic (grow only in absence of O₂) bacteria. Within six to 10 hours after submergence or flooding, the sediment is virtually O₂ free except at the floodwater-sediment and rhizosphere (environment under the influence of plant roots)-sediment interfaces (Mikkelsen 1987).

Oxygen movement through the overlying floodwater to the sediment surface layer is usually much slower than the rate of oxygen consumption in the sediment. Depletion of sediment oxygen forces the facultative and obligate (only one life condition) anaerobic microorganisms to use NO₃⁻, Mn⁺⁴, Fe⁺³, SO₄⁻², and CO₂ as electron acceptors for respiration and decomposition of sediment organic compounds (Van Breeman and Feijtel 1990). Oxidized inorganic species (listed above) that undergo oxidation-reduction reactions contribute to the redox potential of sediment. Redox potential is the most diagnostic and easiest to measure (platinum electrode) parameter that indicates whether a soil/sediment is oxidized or reduced. A well-oxidized soil has a redox potential of +400 to +700 mV, but flooded or submerged wetland sediments may exhibit potentials down to as low as -250 to -300 mV (Patrick and Delaune 1977). As illustrated in Figure 2, oxygen (O₂) is the first inorganic compound reduced after submergence at a redox potential of about +350 mV. Once oxygen is depleted in the sediment, microorganisms are forced to use

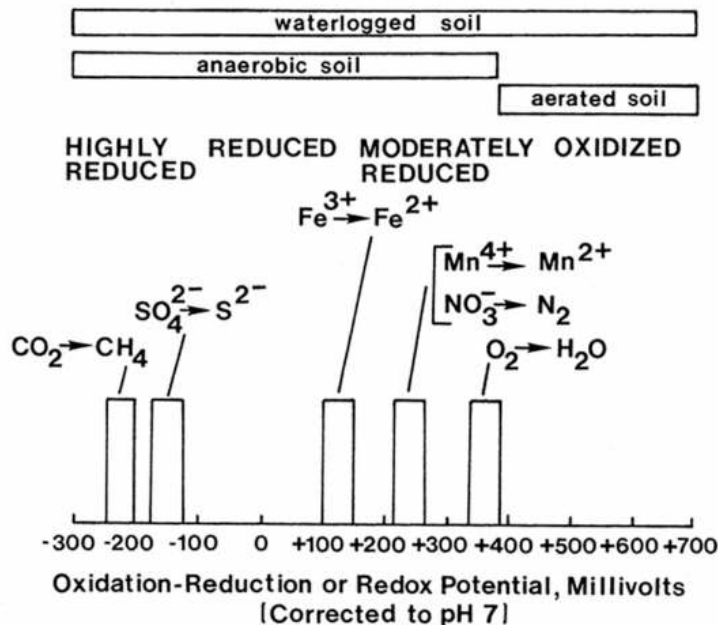
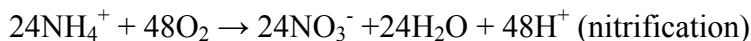


Figure 2.2. Critical sediment redox potential at which oxidized inorganic substances begin to undergo reduction (Patrick and Delaune 1977).

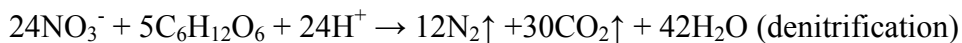
other inorganic compounds (NO_3^- , Mn^{+4} , Fe^{+3} , SO_4^{-2} , and CO_2) as terminal electron acceptors for respiration. Once oxygen is removed (reduced) from the sediment, nitrate is the next inorganic compound to be reduced at about +250 mV. At or below this redox potential, inorganic nitrate undergoes denitrification to N_2O and N_2 gaseous end products, which escape to the atmosphere. As the sediment redox potential continues to drop, remaining inorganic species are sequentially reduced by bacteria, provided carbon or energy sources are available (see Figure 2). Denitrification increases with decreasing redox potential (Van Cleemput and Patrick 1974).

2.5 Nitrification-Denitrification Coupling

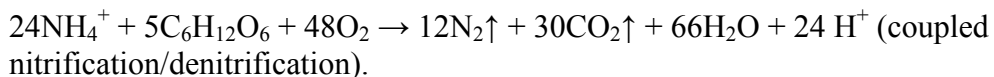
Due to diffusion of oxygen through the water column to the sediment surface, a thin oxidized or aerobic layer will develop. Below the aerobic zone, microorganisms will consume more oxygen than can be supplied by diffusion, resulting in a reduced or anaerobic zone directly beneath the thin oxidized layer (Reddy and Patrick 1984). The aerobic-anaerobic boundary zone facilitates simultaneous nitrification and denitrification. Nitrification occurs in the aerobic zone, and denitrification occurs in the anaerobic layer. The two reactions can be combined, producing a balanced equation in the oxidized and reduced layers:



+



equals



The main supply of ammonium to the aerobic soil layer and floodwater comes from: (1) mineralization of organic nitrogen (organic N \rightarrow NH_4^+ -N) in the oxidized soil layer and water column, and (2) diffusion of ammonium-N from the underlying anaerobic soil layer. Nitrate-N in waterlogged sediments is mainly derived from the nitrification of ammonium-N in the surface aerobic layer and/or can be supplied by river water containing elevated levels of nitrate (Atchafalaya River). Nitrate formed in or added to the aerobic layer is constantly moving downward to the anaerobic layer by diffusion due to a concentration gradient and its negative charge, and is removed by the denitrification reaction ($\text{NO}_3^- \rightarrow \text{N}_2\text{O}$ and N_2). The thickness of the oxidized sediment layer is limited by the slow diffusive property of dissolved oxygen from the water column to the sediment surface and the consumption rate on the diffused oxygen by benthic microorganisms. Increasing the concentration of oxygen in the water column can increase the thickness of the aerobic sediment layer, thereby stimulating the coupled nitrification-denitrification reaction.

Oremland et al. (1984) observed that denitrification occurred up to about 3 cm in depth from the sediment surface, but potential activity existed at depths to 15 cm when nitrate was added to the sediment. Dunigan and Delaune (1978) found nitrifying microorganisms to be concentrated in the upper few centimeters in flooded sediments; the denitrifier numbers actually increased with depth. In the presence of oxygen, denitrifiers may be repressed since facultative bacteria prefer to use oxygen rather than nitrate for respiration and decomposition of sediment organic matter. Denitrification may also occur in the water column if dissolved oxygen concentrations are less than 0.2 mg L^{-1} (Knowles 1982). Limited denitrification was found in turbid oxygenated estuarine water containing a high nitrate level. In this case, denitrification was considered to be confined to organic matter, which provided microniches for anaerobic activity (Omnes et al. 1996).

A review by Seitzinger (1990) showed that the lowest denitrification rates were found for deep sea sediments (0.03 to $2.4 \mu\text{m N m}^{-2} \text{ h}^{-1}$), higher rates were found for estuarine sediments (5 to $250 \mu\text{m N m}^{-2} \text{ h}^{-1}$), and the highest rates for polluted estuarine sediments ($>500 \mu\text{m N m}^{-2} \text{ h}^{-1}$). The review also revealed denitrification in eutrophic streams or rivers ranging from 40 to $2,121 \mu\text{m N m}^{-2} \text{ h}^{-1}$.

Denitrification rates are dependent upon activity and populations of denitrifiers and environmental factors (nitrate load, organic carbon, temperature, soil redox potential, soil texture, soil pH, salinity, and water/sediment oxygen content). Temperature was found to increase denitrification rates from $25 \mu\text{m N m}^{-2} \text{ h}^{-1}$ at 5°C to $200 \mu\text{m N m}^{-2} \text{ h}^{-1}$ at 20°C in nitrate enriched estuarine sediment (Nowicki 1994). Additional studies have reported increased denitrification rates as nitrate additions increased in nitrogen-limited sediments (Smith et al. 1985; Nowicki 1994). Long-term addition of nitrate to wetland sediments can increase the maximum denitrification capacity, but the proportion of nitrate load that is not denitrified increases as nitrate concentration increases (Maag et al. 1977). Denitrification rates are not linear at high rates of nitrate input compared to lower nitrate addition rates. Research has also pointed out that some sediments have the potential to remove greater nitrate inputs compared to other wetland sediments (Livingstone et al. 2000).

Denitrification is also affected by sediment composition. Clay particles provide attachment sites for denitrifying bacteria, and soil texture influences organic matter content, all of which affects rates of denitrification (Chamalet 1985; Van Luijn et al. 1999). Labile and water soluble carbon, mineralizable carbon, and total carbon in sediment have been found to be good indicators of denitrification potential over a broad range of soils incubated under anaerobic conditions (Bunford and Bremner 1975). Sediment/carbon ratios were found to be highly predictive of denitrification efficiency (Ingersoll and Baker 1998).

Aquatic plants can also affect denitrification rates in wetland ecosystems. Nitrate is removed through root uptake, and some plants develop an oxidized rhizosphere around the root system. The rhizosphere creates additional sites for coupled nitrification-denitrification reactions within the sediment column (Knowles 1982). Organic matter given off by roots as root exudates provides an additional energy source for denitrifiers,

which may increase denitrification. The absence or presence of vegetation in marshes can influence denitrification (Otto et al. 1999).

Microbial activity can affect denitrification by altering the pH of the sediment environment. The release of carbon dioxide (CO₂) by microorganisms during denitrification can form hydroxide (OH⁻) and bicarbonate (HCO₃⁻), raising the sediment pore water pH above the optimum range preferred by heterotrophic soil microbes (Rust et al. 2000). Knowles (1982) reported the optimum pH range for denitrifiers to be from 7.0 to 8.0.

3.0 National Interest

Measurement of denitrification rates in lake, river, and estuarine water and sediment columns has gained national attention and importance due to increasing nitrogen loading in surface waters and eutrophication of coastal waters (Tyrrell 1999; Steingruber et al. 2001). National interest and research has focused on the Mississippi-Atchafalaya River basins discharging into the Gulf of Mexico. Approximately 969,000 metric tons of nitrate-N are discharged into the Gulf of Mexico annually, which represents a three fold increase compared to 1955-1970 nitrate input rates (328,000 metric tons). Approximately 70% of the nitrate-N enters the Gulf via the Mississippi River, and the remaining 30% discharges through the Atchafalaya River (Goolsby et al. 2001).

This large nitrate-N input has been implicated as a possible cause of hypoxia in a large area (“Dead Zone”) of coastal water along the Louisiana-Texas coasts (Rabalais et al. 2002). In the mid 1980s to the early 1990s, the hypoxic regions averaged 8,300 km². From 1993 through 2001, this region increased to about 16,000 km² (Rabalais et al. 2002). To reduce the size of the Gulf of Mexico’s hypoxic zone, the Mississippi River/Gulf of Mexico Watershed Nutrient Task Force Action Plan has proposed restoring and enhancing denitrification to reduce nitrogen loads from the Mississippi and Atchafalaya Rivers to the Gulf of Mexico (EPA 2001).

4.0 Measurement of Denitrification

Over the years, many different methods have been developed to quantify denitrification rates in freshwater, coastal, and marine waters and sediments. Both indirect and direct methods have been used to measure the denitrification products of nitrous oxide (N₂O) and dinitrogen (N₂) fluxes from sediments and anoxic waters. Current denitrification methods include: (1) nitrogen mass-balance techniques (Legg and Meisinger 1982), (2) measurement of NO₃⁻-N disappearance (Anderson 1977), (3) nitrate flux calculations into sediment from pore water distribution data (Mengis et al. 1997), (4) N-15 nitrate dilution method (Koike and Hattori 1978), (5) ¹⁵N₂ flux methods (Devol 1991), (6) N-15 isotope pairing technique (Nielsen 1992), (7) acetylene inhibition technique (Sorensen 1978), and (8) unlabeled N₂ flux procedures (Seitzinger et al. 1993). All of these methods have advantages as well as potential problems and assumptions that

must be assessed prior to denitrification measurement (Seitzinger et al. 1993; Steingruber et al. 2001).

4.1 Nitrogen Mass-Balance Techniques

The nitrogen-balance technique is an indirect method commonly used in the past to measure nitrogen losses via denitrification. The method essentially involves measuring the difference in unlabeled N content of a soil and plant at the beginning and end of a laboratory or field experiment. The difference (portion of N unaccounted for) is assumed to be the nitrogen loss mainly through the denitrification pathway (Hauck 1986). The technique's main advantages are convenience and the fact that the net result of many opposing nitrogen transformations is quantified without requiring extrapolation over time and space. Complications arise when all the components of the nitrogen balance cannot be measured with comparable accuracy where the net value for nitrogen loss includes an accumulation of systematic errors (analytical procedures). For example, problems arise if a significant fraction of the total nitrogen flow is retained in plant roots. There is an immobilization of N during bacterial decomposition of organic material, or the immobilization is enhanced by mineralization of soil N (Ryden and Rolston 1983).

N-15 can also be used for nitrogen mass balance studies. The basic approach is to add enriched N-15 labeled fertilizers or compounds to the soil and subsequently sample the sediment, water and plant N pools after a desired time interval. To determine denitrification, inorganic N-15 fertilizer is added to the soil/sediment; all other inputs and outputs of nitrogen and changes in water, soil and plant N are measured; and denitrification (N-15 unaccounted for) is determined from the total N-15 recovered subtracted from total N-15 input (fertilizer N-15). A major advantage of the N-15 mass balance approach for denitrification measurement is that it integrates biological activity over time and space. But it is poorly adapted to resolve temporal dynamics and spatial variability of denitrification because the method requires destructive sampling of sediments and plants (Myrold 1990).

4.2 Nitrate Disappearance Method

One of the simplest methods for estimating denitrification is to follow the disappearance of nitrate-N from the system. This method is acceptable provided that measurable amounts of nitrate are not assimilated or reduced to ammonia through dissimilatory reduction. If significant amounts of nitrate are assimilated or reduced by microorganisms, then the disappearance of nitrate can overestimate denitrification. But the method may also underestimate denitrification because it does not take into consideration coupled nitrification-denitrification (Rysgaard et al. 1993; Steingruber et al. 2001).

4.3 Nitrate Pore Water Distribution Technique

Calculations of nitrate fluxes from pore water column profiles into the sediment to estimate denitrification have the same problems described above for the nitrate-N disappearance method. Nitrate fluxes across the sediment-water interface and diffusive boundary zone can underestimate denitrification because of insufficient vertical

resolution of the profiles. Also, turbulent transport is not considered when nitrate-N fluxes are calculated (Mengis et al. 1996).

4.4 N-15 Nitrate Dilution Method

Studies by Koike and Hattori (1978) demonstrated that nitrification and denitrification rates could be determined in coastal sediment systems and flooded rice soils using $^{15}\text{NO}_3^-$ as a tracer by measuring the temporal changes in the atom % N-15 and nitrate pool concentration. This technique is known as pool dilution or isotope dilution because the rate of nitrification is determined from the dilution on the N-15 tracer labeled product pool with the natural abundance NO_3^- pool. Myrold and Tiedje (1986) showed that the nitrate N-15 dilution technique could be used to measure denitrification in soils. This method has the advantages of being integrative in time and space and can estimate rates of other nitrogen cycle processes. The sensitivity for determining denitrification rates is mainly a function of the sensitivity of measuring soil nitrate concentration changes (Myrold 1990). The N-15 nitrate dilution technique with subsequent quantification of N-15 nitrate disappearance and N-15 production of ammonia measures both denitrification and nitrification but neglects coupled nitrification-denitrification and assimilation under field conditions (Steingruber et al. 2001).

4.5 $^{15}\text{N}_2$ Flux Methods

Denitrification rates can be measured most directly by determining the main products on this process: N_2O and N_2 in a closed chamber system. It is relatively easy to measure nitrous oxide flux rates from soil/sediment systems because the ambient concentration N_2O in air is low (about 300 ppbv), and simple gas chromatography techniques (electron capture detector) can measure N_2O with great sensitivity (Myrold 1990). However, quantitatively assaying increases in dinitrogen (N_2) is difficult because the atmosphere is 78% N_2 . This inherent insensitivity in measuring N_2 evolution due to denitrification can be overcome using isotope ratio mass spectrometer (IRMS) techniques to measure the emission of $^{15}\text{N}_2$ following application of $^{15}\text{NO}_3^-$ to the sediment system.

N-15 can be used to directly measure N_2 evolution from sediments and soils. The theory behind this method goes back over 45 years (Hauck et al. 1958; Hauck and Bouldin 1961) where $^{15}\text{N}_2$ evolution could be measured after highly labeled N-15 fertilizer is added to the soil or sediment. It took almost another 25 years in instrument advances before its applicability for field experiments became evident. Siegel et al. (1982), Mulvaney (1984), and Mulvaney and Boast (1986) revitalized the mass spectrometer techniques and modified the calculations originally proposed by Hauck et al. (1958). The method requires applying highly ^{15}N -labeled (>20 atom %) fertilizer to the sediment/soil surface and then placing a chamber over the ^{15}N -fertilized plot to isolate the atmosphere above the soil for a specified time (Mosier and Schimel 1993). Isolation of the chamber headspace permits determining the rate of increase of ^{15}N atoms ($^{15}\text{N}_2\text{O}$ and $^{15}\text{N}_2$ denitrification products) over time. The revised calculations take advantage of the fact that the isotopic distribution of the evolved soil denitrification gases (mainly $^{15}\text{N}_2$) is different from that already present in the confined chamber headspace. When $^{15}\text{N}_2$ formed from soil denitrification of ^{15}N -labeled nitrate is evolved into this confined

atmosphere (already containing 78% N₂ composed of 0.3667 atom % ¹⁵N and 99.6337 atom % ¹⁴N), the ¹⁴N and ¹⁵N atoms in the entire chamber headspace mixture are not distributed randomly among the N₂ molecules (¹⁴N¹⁴N, ¹⁴N¹⁵N, ¹⁵N¹⁵N – masses 28, 29 and 30). Using this nonrandom ¹⁵N distribution, the equations permit calculation of the amount of N gas evolved not only from the added ¹⁵N-labeled fertilizer but also from soil nitrogen when denitrification occurs. It is not necessary to disturb the soil in the plot during the experiment because the ¹⁵N mole fraction of the NO₃⁻-N in the soil that serves as the denitrification N-gas source is calculated directly from headspace gas mass spectral data. This is done by measuring the m/e 29/28 and 30/28 ratios using a triple collector isotope ratio mass spectrometer (Mosier and Schimel 1993). To use the equations, a number of assumptions are made. The first is that the total amount of ²⁸N₂ inside the chamber headspace does not change over the sample collection period. However, later refinements in the equations (Mulvaney and Boast 1986) do not rely on this assumption. The major assumption is that after adding ¹⁵N-labeled fertilizer to the soil, the ¹⁵N-nitrate formed mixes uniformly with the unlabeled soil nitrate or unlabeled NO₃⁻ from organic N mineralization. The detection limit for ¹⁵N-enriched N₂ using the closed chamber technique is dependent on the applied fertilizer N-15 enrichment, headspace volume of chamber, amount of time chambers are left in place over the soil, and precision of the mass spectrometer used to measure the nitrogen 28, 29, and 30 peaks. Using a high N-15 enrichment (64 atom % N-15) and a triple collector mass spectrometer, Siegel et al. (1982) were able to measure N₂ denitrification fluxes down to about 2.5 g N ha⁻¹d⁻¹. Recent advances in direct combustion mass spectrometer techniques now permit detection of ¹⁵N at ambient atmospheric concentrations, which allows for tracer level additions of ¹⁵NO₃⁻ (Brooks et al. 1991).

4.6 Acetylene Inhibition Technique

The acetylene (C₂H₂) inhibition technique is a relatively easy and rapid method for measuring denitrification in aquatic sediments. Acetylene blocks the reduction of N₂O to N₂ during denitrification, and buildup of N₂O in samples exposed to acetylene is equated to denitrification rates (Yoshinari and Knowles 1976). Adding C₂H₂, it is possible to measure denitrification rates by end product formation without measuring N₂ production, since N₂O is the terminal denitrification product (Groffman 1994).

If two sets of soil or sediment samples are used, the amount of N₂ and N₂O produced can be estimated using acetylene. Acetylene is added to one set of identical sediment samples, and no acetylene is added to the second set. All sediment samples are incubated for a few hours under identical conditions, and N₂O produced from each set is determined by gas chromatography (electron capture detector). The difference in N₂O measured between the acetylene treated soil and the untreated (no acetylene) soil estimates the amount of N₂ produced from denitrification. Major advantages of the acetylene inhibition method include: (1) it is relatively simple, rapid, and straightforward; (2) inexpensive sample collection and use of inexpensive gas chromatographic equipment (compared to expensive isotope ratio mass spectrometer instruments) are needed for gas collection of N₂O and N₂O analysis; (3) it can be used on nonfertilized ecosystems to measure *in-situ* denitrification rates; and (4) it has an N₂O detection limit of about 1 g N ha⁻¹ d⁻¹ (Mosier and Heinemeyer 1985). Parkin et al. (1985)

and Mosier et al. (1986) compared acetylene methods with N-15 measurement methods and showed that acetylene methods can accurately measure soil denitrification rates if precautions are taken to avoid several problems caused by acetylene additions.

The acetylene inhibition technique is not without its problems, and research has documented that the method systematically underestimates denitrification (Mengis et al. 1997). The most important problem is that acetylene inhibits nitrification. At the same time, the coupled nitrification-denitrification reaction is extremely important in flooded or wetland systems (Watts and Seitzinger 2000). If long-term laboratory or field incubations are conducted with acetylene or when soil nitrate concentrations are low, the denitrification can be underestimated because nitrate levels can become depleted (Groffman 1994). In addition, acetylene does not always effectively block N_2O reduction to N_2 , and denitrification rates may again be underestimated (Seitzinger et al. 1993). Further, N_2O may diffuse toward deeper sediment layers where it is reduced to N_2 (Steingruber et al. 2001).

A second major problem involves diffusion of added acetylene into pore spaces. If acetylene within the soil or sediment is not uniformly distributed throughout the sediment layers containing the denitrifying microorganisms, then rates of denitrification will be underestimated. Acetylene diffusion rates vary widely with soil type and soil properties. Fine-textured and/or submerged sediments significantly inhibit acetylene diffusion rates. Diffusion of acetylene is a much larger concern for flooded or wetland systems than for upland aerobic soils (Rudolph et al. 1991). It has also been pointed out that acetylene affects soil carbon metabolism. Under certain soil conditions, soil microorganisms can consume acetylene and use it as an energy yielding substrate, making it difficult to maintain the optimum acetylene soil concentrations necessary for N_2O reductase inhibition (Terry and Duxbury 1985). In addition, Topp and Germon (1986) showed that acetylene consuming microorganisms oxidize acetylene to ethanol and other compounds, which can stimulate denitrification by supplying an energy source to denitrifiers.

Long-term soil/sediment exposure to acetylene appears to be the main cause of many of the acetylene inhibition method problems described above. Ryden and Dawson (1982) showed in a field study that short-term exposure to acetylene did not significantly inhibit nitrification, lead to acetylene decomposition, or lead to denitrification enhancement.

4.7 Direct N_2 (Unlabeled) Flux Methods

Direct flux methods have also been developed that do not use highly N-15 labeled nitrate (Groffman 1994). Quantifying production of the main gaseous denitrification product (N_2) is difficult against its high atmospheric background (78% N_2). Using this N_2 flux method, the headspace volumes of intact sediment cores are flushed with a mixture of 21% O_2 and 79% He (He substituted for N_2) to reduce background N_2 concentrations from 78% nitrogen to about 1% N_2 (Seitzinger et al. 1993; Watts and Seitzinger 2000). In aquatic sediment cores, a 10 day preincubation is usually required to decrease the background N_2 concentration in the pore water. The resulting low background N_2 then

makes it possible to detect and measure increases in N_2 in the gas phase and overlying water column due to denitrification. Method refinement had allowed the preincubation and flushing period to be reduced to two to three days (Nowicki 1994; Watts and Seitzinger 2000). A major assumption is that the long preincubation of the sediment cores does not significantly change the ambient field denitrification rates. In addition, the operator must be able to distinguish between N_2 created due to degassing of atmospheric N_2 from soil pore space and the portion of N_2 flux due to denitrification. Making this distinction requires detailed calculations (Watts and Seitzinger 2000). In addition, isotope pairing (^{15}N with ^{14}N) and $N_2:Ar$ ratio methods have been used to measure denitrification in sediment systems (Steingruber et al. 2001; Eyre et al. 2002; Dalsgaard and Thamdrup 2002).

4.8 Denitrification Methods Comparisons

A few studies have directly compared denitrification methods and critically evaluated the results. In upland field sites, Rolston et al. (1982) and Mosier et al. (1986) compared N_2O production in acetylene amended soils to $^{15}N_2$ production in the same soils. Both studies concluded that only minor differences were observed between the two methods, with N losses from the acetylene treatment being somewhat higher compared to the $^{15}N_2$ method. This may have been caused when multiple treatments of acetylene on the field plots increased available C to denitrifying organisms. In another study, the acetylene method was compared to the ^{15}N difference method (Parkin et al. 1985). No significant N loss differences were calculated between the two methods. Seitzinger et al. (1993) measured denitrification in aquatic sediment cores using $^{15}N_2$ isotope tracer, N_2 flux, and acetylene inhibition methods. The $^{15}N_2$ tracer and N_2 (unlabeled) laboratory flux experiments showed higher rates of coupled nitrification-denitrification in the cores compared to the acetylene inhibition method. When nitrate was added ($100 \mu M$) to the overlying water of the sediment cores, the denitrification rate after acetylene was added was about $100 \mu mol N m^{-2} h^{-1}$, and denitrification calculated by the $^{15}N_2$ tracer method was approximately $120 - 290 \mu mol N m^{-2} h^{-1}$. The highest denitrification rate was observed in the sediment cores using the N_2 flux method (about $400 \mu mol N m^{-2} h^{-1}$). In a later study, Watts and Seitzinger (2000) compared denitrification rates from riparian sites using the N_2 flux and acetylene techniques. Denitrification rates determined by the N_2 flux method ranged from $35 - 245 \mu mol N m^{-2} h^{-1}$ across two soil types. Rates calculated by acetylene inhibition were much less and ranged from $0.1 - 10 \mu mol N m^{-2} h^{-1}$. Underestimation of denitrification by the acetylene method was attributed to inhibition of nitrification by acetylene and the poor penetration of acetylene into the sediment microsites (Watts and Seitzinger 2000).

4.9 Electronic Literature Search

An electronic search of peer-reviewed documents was conducted to identify published research on denitrification in the Atchafalaya Basin. Most of the search results addressed the Atchafalaya Bay, not the basin where our research is being conducted. The search failed to produce any published articles comparing denitrification between back water swamp sites and well flushed sites within the basin (our proposed research sites). The computer search was broadened to include denitrification studies in Louisiana, the

United States, and other countries. The additional case studies briefly described below include previous denitrification laboratory and field experiments conducted in wetlands and bottomland hardwood forests similar to the ecosystems found in the Atchafalaya Basin, Louisiana.

5.0 Relevant Denitrification Studies

1. In a laboratory study, Smith and Delaune (1983) estimated denitrification in bottom sediments collected from freshwater and saline lakes in Barataria Basin, Louisiana after nitrate-N and ammonium-N additions. Using the acetylene inhibition technique, maximum gaseous N_2O from the nitrate amended ($4.5 \mu\text{g}$ nitrate-N g^{-1} sediment) freshwater and saline sediments was 1.6 and $1.4 \mu\text{g N g}^{-1}$. Following addition of $50 \mu\text{g}$ ammonium-N g^{-1} dry sediment, denitrification rates estimated by the acetylene technique ranged from 3 to $659 \mu\text{g N m}^{-2} \text{h}^{-1}$ from the saline sediment samples and 14 to $5,149 \mu\text{g N m}^{-2} \text{h}^{-1}$ from the freshwater sediment.

2. The fate of Atchafalaya River nitrate entering a Louisiana estuary was investigated by Smith et al. (1985). The study was conducted on sediment samples collected from the bay and marshes surrounding Four League Bay, Louisiana. The bay is connected to the Gulf of Mexico on its southern end, and its northern end opens into the northeastern section of the Atchafalaya Bay. Mixed wet sediment samples were incubated with 60 cm^3 of $70 \mu\text{M}$ or $35 \mu\text{M}$ KNO_3 solution. In the presence of 10 kPa acetylene, total denitrification was measured by the acetylene inhibition technique. Annual total N emission ($\text{N}_2\text{O} + \text{N}_2$) was equal to 142 and $120 \mu\text{g N g}^{-1}$ sediment from the five bay and five marsh stations, respectively. The authors estimated that approximately 50% of the Atchafalaya riverine nitrate entering the estuary was removed and returned to the atmosphere via denitrification.

3. Lindau et al. (1988) directly measured denitrification fluxes of N_2O and N_2 from a cypress tupelo gum swamp located near Lac Des Allemands in Barataria Basin, Louisiana. Labeled NH_4^+ -N and NO_3^- -N (99 atom % ^{15}N) at a rate of 10 g m^{-2} was applied to the swamp forest substrate, and over a 27 day period gas samples were collected from the field site and analyzed for N_2O and $^{15}\text{N}_2$. N_2O was analyzed by gas chromatographic techniques and $^{15}\text{N}_2$ was determined on an isotope ratio mass spectrometer. Maximum N_2O and N_2 fluxes were estimated at $24 \text{ g N m}^{-2} \text{y}^{-1}$ and $110 \text{ g N m}^{-2} \text{y}^{-1}$, respectively. This field study showed that large inputs of nitrogen could be removed via the denitrification process.

4. Nitrous oxide and dinitrogen denitrification emissions were measured from Louisiana *Panicum hemitomon* S. freshwater marsh soils after addition of labeled ammonium- and nitrate-N (Lindau et al. 1991). High rates (100, 200 and 300 kg N ha^{-1}) of N-15 labeled NH_4^+ -N and NO_3^- -N were applied to the marsh cores to simulate nutrient loading from anthropogenic sources. Nitrous oxide (gas chromatography) and $^{15}\text{N}_2$ (mass spectrometry) were measured over 46 days after N addition. Maximum $^{15}\text{N}_2$ emissions from the cores treated with 100, 200, and 300 kg N ha^{-1} were <15 , 650 and $1,080 \text{ g ha}^{-1} \text{ d}^{-1}$ for NH_4^+ -N addition and 2,700, 3,430 and $4,490 \text{ g ha}^{-1} \text{ d}^{-1}$ for the NO_3^- -N additions. Nitrous oxide flux ranged from nondetectable concentrations to $1,980 \text{ g ha}^{-1} \text{ d}^{-1}$ for the

300 kg N ha⁻¹ nitrate treatment. This study demonstrated that *Panicum hemitomon* marsh soils have significant capacity to transform and remove large inputs of inorganic N by denitrification.

5. N-15 isotope dilution and direct ¹⁵N₂ flux techniques were used to measure nitrification and denitrification in a Louisiana bald cypress/tupelo gum swamp forest following addition of ¹⁵N labeled NO₃⁻-N and NH₄⁺-N (Delaune et al. 1988). Swamp forest sediment cores were amended (100 kg N ha⁻¹) with highly labeled KNO₃ (61.3 atom % ¹⁵N) and NH₄Cl (99.9 atom % ¹⁵N). Using the N-15 isotope dilution technique, rates of NO₃⁻ production (nitrification) and reduction in the ¹⁵NO₃⁻ treated sediment cores were about 240 and 2,320 g N ha⁻¹ d⁻¹, whereas NH₄⁺ production (mineralization) and removal rates in ¹⁵NH₄⁺ treated cores were 270 and 2,160 g N ha⁻¹ d⁻¹, respectively. Calculations based on labeled ¹⁵N₂ emissions showed that denitrification was about three times greater for the NO₃⁻-N treatment than for the NH₄⁺-N treatment, with rates of 818 and 266 g N emitted ha⁻¹ d⁻¹, respectively. The study demonstrates that swamp forest soils have the potential to process large inputs of NH₄⁺-N and NO₃⁻-N, with a significant amount of added N being returned to the atmosphere via denitrification.

6. Using acetylene blockage and ¹⁵N-tracer methods, denitrification was measured *in-situ* in constructed wetlands in Illinois (Xue et al. 1999). Nitrate pulse input (about 15 mg N L⁻¹, 70 atom % ¹⁵N) was applied to field mesocosms in the constructed wetlands, and N₂O and N₂ were measured over time. Acetylene and ¹⁵N methods yielded comparable denitrification rates. With 9 to 20 mg NO₃⁻-N L⁻¹ initially in the mesocosm water columns, denitrification rates measured by the acetylene method ranged from 2 to about 12 mg N m⁻² h⁻¹. In the N-15 experiment, the maximum denitrification rate of 9.3 mg N m⁻² h⁻¹ was measured about five days after a nitrate input of 15 mg N L⁻¹.

7. Hunter and Faulkner (2001) measured the denitrification potential in natural and restored bottomland hardwood wetlands located in the Tensas River Basin in northeastern Louisiana. Soil samples were collected from the field and the acetylene inhibition technique was used to measure and compare denitrification enzyme activity rates after addition of 1 mM KNO₃. Denitrification potential in the natural bottomland hardwood wetland site was 657 ng N₂O-N g⁻¹ soil h⁻¹ and was significantly higher compared to the restored wetland site without hydrology reestablished (167 ng N₂O-N g⁻¹ soil h⁻¹). Results showed that a restored bottomland hardwood wetland without a reestablished natural hydrologic regime did not denitrify added nitrogen as efficiently as natural wetland sites.

8. Iwai (2002) measured the denitrification potential of sediments collected from a future Mississippi River freshwater diversion site (Davis Pond). Samples were collected (three sites) from Lake Cataouatche located in the northern portion of the Barataria Basin estuary. Denitrification was determined using the acetylene inhibition technique after addition of two rates of nitrate: (1) 1.4 mg NO₃⁻-N L⁻¹ concentration similar to mean Mississippi River nitrate content, and (2) 50 NO₃⁻-N L⁻¹ to estimate maximum sediment denitrification potential. Denitrification rates measured after the 1.4 mg NO₃⁻-N L⁻¹ addition were low and averaged 3.0 ± 1.2 mg N m⁻² d⁻¹, which was about 16 times lower compared to the high nitrate addition where denitrification averaged 49 ± 17 mg N m⁻² d⁻¹. Results demonstrated that Lake Cataouatche bottom sediments have the capacity to

remove large quantities of nitrate through the denitrification process. This suggested that future nitrate inputs from the Davis Pond diversion could be effectively removed by sediment denitrification processes.

9. Using the acetylene blockage technique, Ullah et al. (2005) measured the denitrification potential of intact soil cores collected from: (1) a mature riparian zone of bottomland hardwood forest (elm, oak, red maple, green ash), and (2) an adjacent cultivated site cleared about 20 years earlier and planted with soybeans in the Yahoo Delta region of northwestern Mississippi. Denitrification rates were determined after the addition of a dilute nitrate solution ($15 \mu\text{g NO}_3^- \text{-N g}^{-1}$ soil) or deionized water to cores at 70, 85, and 100% water-filled pore space and the addition of 10 ml of acetylene. Denitrification rates of forested intact soil cores were 5.2, 6.6 and 2.0 times those of cultivated soil cores at 70, 85, and 100% water-filled pore space, respectively.

10. Eyre et al. (2002) used the $\text{N}_2:\text{Ar}$ and isotope pairing methods to measure sediment denitrification. Undisturbed sediment cores were collected from Aarhus Bay, Denmark in October and November. $\text{N}_2:\text{Ar}$ gas ratios were determined with a quadruple mass spectrometer, and abundance and concentration of $^{14}\text{N}^{15}\text{N}$ and $^{15}\text{N}^{15}\text{N}$ were analyzed on a gas chromatograph coupled to a triple collector isotope ratio mass spectrometer. Using the $\text{N}_2:\text{Ar}$ method, denitrification rates ranged from 92 to 140 $\mu\text{mol N m}^{-2} \text{ h}^{-1}$ and were three to four times the rates obtained by the isotopic pairing technique (32 to 34 $\mu\text{mol N m}^{-2} \text{ h}^{-1}$).

11. Direct measurement of denitrification in mangrove sediments in Terminos Lagoon, Mexico was conducted using ^{15}N isotope techniques. Intact sediment cores were collected, different nitrate concentrations were injected, and $^{15}\text{N}_2$ was measured in the core headspace over time to calculate denitrification rates. Additional $^{15}\text{N-KNO}_3$ (99 atom % ^{15}N) concentrations ranged from 25 to 200 $\mu\text{mol core}^{-1}$. Headspace gas samples were analyzed for nitrogen isotope masses 28, 29, and 30 on a Nuclide model 3-60-RMS isotope ratio mass spectrometer. The highest denitrification rate of 9.4 $\mu\text{mol m}^{-2} \text{ h}^{-1}$ (after 200 $\mu\text{mol core}^{-1} \text{ }^{15}\text{N-KNO}_3$ addition) was measured in the fringe mangrove after three days of incubation. Basin mangrove denitrification rates ($^{15}\text{N}_2$) ranged from 1.9 to 4.5 $\mu\text{mol m}^{-2} \text{ h}^{-1}$. Fluxes of $^{15}\text{N}_2$ from the fringe mangrove sediment cores ranged from 4.5 to 7.7 $\mu\text{mol m}^{-2} \text{ h}^{-1}$ and were significantly higher compared to cores enriched with 25 and 100 $\mu\text{mol core}^{-1} \text{ }^{15}\text{N-KNO}_3$ ($<1 \mu\text{mol m}^{-2} \text{ h}^{-1}$). Study results showed that less than 10% of the applied $^{15}\text{N-KNO}_3$ was lost via denitrification.

12. The denitrifying capacity of a riparian wetland was estimated using the denitrification enzyme activity technique (Maitre et al. 2005). Soil samples were collected at different soil horizons from a riparian area at the foot of the Jura Mountains in Switzerland. The area contained hydric soils containing accumulated organic matter, which has been used as an indicator of denitrification. Soil samples (30 g) collected at different sampling depths were amended with 30 mL of a solution containing nitrate ($10 \mu\text{g KNO}_3\text{-N g}^{-1}$ soil) and 4 mg glucose carbon. Denitrification was measured over time using the acetylene inhibition method. Denitrification mean rates ranged from 24 to 604 $\text{ng N g}^{-1} \text{ h}^{-1}$; the rates corresponded to which soil denitrifying class the soil samples were grouped under. The highest denitrifying rates were associated with the soil horizons containing

higher organic carbon. The denitrifying removal capacity for the riparian wetland was estimated to be about $1.8 \text{ kg N m}^{-2} \text{ yr}^{-1}$. The calculated removal capacity was less than expected and was attributed to the fact that not all groundwater interacts with soil horizons having the highest denitrifying capacity.

13. Kellogg et al. (2005) measured *in-situ* groundwater denitrification rates at three hydric soil depths at four riparian sites located within the Pawcatuck River watershed of Rhode Island, USA using a ^{15}N -enriched nitrate “push-pull” method. Mini-piezometers were installed within the hydric soils at each site at 65, 150, and 300 cm below the soil surface. The mini-piezometers were dosed (pushed) with 10 L of groundwater containing $32 \text{ mg L}^{-1} \text{ NO}_3^- \text{-N}$ as enriched KNO_3 (20 atom % ^{15}N). After 24 hours, groundwater was extracted (pulled) from the same mini-piezometers and analyzed for dissolved gases (labeled and unlabeled N_2 and N_2O). At three sites no significant differences in denitrification rates were observed among depths. Mean denitrification rates ranged from 30 to $120 \text{ } \mu\text{g N kg}^{-1} \text{ d}^{-1}$ within 10 m versus <1 to $40 \text{ } \mu\text{g N kg}^{-1} \text{ d}^{-1}$ at greater than 30 m from the stream. Variations in measured denitrification rates were affected by temperature and dissolved organic carbon.

14. Using the acetylene based intact core method, Hanson et al. (1994) measured denitrification in Rhode Island, USA riparian wetlands receiving high and low groundwater nitrate inputs. Denitrification was measured in riparian forests with upland to wetland transition zones with red maple swamps on both sides of a stream. The upland area on one side of the stream housed an unsewered residential development (enriched side), and the upland site on the other side was undeveloped (control site). Denitrification was measured on enriched and control site samples. Estimates of annual denitrification N loss from the enriched site ranged from 7.1 to $38.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and from 4.8 to $16.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for the control site. The highest rates were observed for the poorly drained and very poorly drained sample locations compared to the moderately well drained sampling sites (lowest denitrification values).

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