2002

Denitrification potential of sediment from a future Mississippi River diversion site in Louisiana

Roy Ryuta Iwai
Louisiana State University and Agricultural and Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_theses
Part of the Oceanography and Atmospheric Sciences and Meteorology Commons

Recommended Citation
Iwai, Roy Ryuta, 'Denitrification potential of sediment from a future Mississippi River diversion site in Louisiana' (2002). LSU Master's Theses. 3872.
https://digitalcommons.lsu.edu/gradschool_theses/3872

This Thesis is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Master's Theses by an authorized graduate school editor of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.
DENITRIFICATION POTENTIAL OF SEDIMENT FROM A FUTURE MISSISSIPPI RIVER DIVERSION SITE IN LOUISIANA

A Thesis

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

in

The Department of Oceanography and Coastal Sciences

by
Roy Ryuta Iwai
B. Arch., University of Oregon, 1992
May, 2002
ACKNOWLEDGEMENTS

I would like to thank my co-major professors, Dr. Robert P. Gambrell and Dr. Charles W. Lindau, for their guidance and reviews of my work. I would also like to thank Dr. Ronald D. DeLaune for serving as a member on my thesis committee and for providing the financial support to conduct this research. I am grateful for the support from Dr. R. Eugene Turner who also served as a member of my thesis committee. Particular acknowledgement must be made to Dr. W. James Catallo for not only taking such a strong interest in my development as a scientist, but also for helping me to understand the complexity and depth of the human spirit through our long and excited discussions.

This research was funded by a grant through the Louisiana Water Resources Research Institute.
# TABLE OF CONTENTS

Acknowledgements ................................................................. ii

List of Tables ................................................................. iv

List of Figures ................................................................. v

Abstract ................................................................. vi

Chapter

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Introduction .................................................................. 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Review of Literature ............................................. 1</td>
</tr>
<tr>
<td></td>
<td>Objectives .................................................................. 18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Materials and Methods ............................................. 20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Site Description ..................................................... 20</td>
</tr>
<tr>
<td></td>
<td>Sediment Sampling ................................................... 22</td>
</tr>
<tr>
<td></td>
<td>Microcosm Preparation .............................................. 24</td>
</tr>
<tr>
<td></td>
<td>Nitrate Removal Analysis ......................................... 24</td>
</tr>
<tr>
<td></td>
<td>Acetylene Inhibition Technique ................................... 26</td>
</tr>
<tr>
<td></td>
<td>$^{15}$N Isotope Technique .......................................... 29</td>
</tr>
<tr>
<td></td>
<td>Statistical Analysis ................................................ 29</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Results .................................................................. 31</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sediment Characteristics ....................................... 31</td>
</tr>
<tr>
<td></td>
<td>Nitrate Removal .................................................... 31</td>
</tr>
<tr>
<td></td>
<td>Acetylene Inhibition Technique .................................. 41</td>
</tr>
<tr>
<td></td>
<td>Nitrous Oxide Emission ............................................ 48</td>
</tr>
<tr>
<td></td>
<td>$^{15}$N Isotope Technique ........................................ 50</td>
</tr>
<tr>
<td></td>
<td>Entrapped $^{15}$N gases in Sediment .......................... 54</td>
</tr>
<tr>
<td></td>
<td>Thickness of the Oxidized Layer ................................ 55</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Discussion ............................................................. 57</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Conclusions ................................................................ 68</th>
</tr>
</thead>
</table>

References ........................................................................ 70

Appendix

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Mean Nitrate Concentrations for Sites A, B, C, D and E .......... 77</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Mean Nitrous Oxide Flux from Site A with Acetylene Treatment ...... 78</td>
</tr>
<tr>
<td>B</td>
<td>Mean Nitrous Oxide Flux from Site A without Acetylene Treatment .... 79</td>
</tr>
<tr>
<td>C</td>
<td>Sample Calculations .................................................................. 80</td>
</tr>
</tbody>
</table>

Vita ............................................................................. 81
LIST OF TABLES

1. Macronutrient concentrations in the Mississippi River and upper Barataria Basin ................................................................. 4

2. Sampling site coordinates .................................................................................................................. 22

3. Sediment characteristics .................................................................................................................. 31

4. Mean nitrate removal rates from sediment floodwater in low and high nitrate treatment microcosms ................................................ 37

5. Mean nitrate removal rates for Sites A, B, C, D, and E ................................................................ 41

6. Mean nitrous oxide flux from high nitrate treatment microcosms .................................................. 47

7. Mean nitrous oxide fluxes for day 5 for Sites A, B, C, D, and E ................................................. 48
LIST OF FIGURES

1. A simplified diagram of nitrogen transformations in a wetland environment .............................................. 7

2. Map of Barataria Basin showing major water bodies................................. 21

3. Sampling sites within Lake Cataouatche....................................................... 23

4. Diagram of a static microcosm................................................................. 25

5. Changes in nitrate levels versus time in the overlying floodwater of control and low nitrate treatment sediment microcosms .......................... 33

6. Changes in nitrate levels versus time in the overlying floodwater of high nitrate treatment sediment microcosms................................. 34

7. Linear regressions of selected intervals on the mean nitrate removal curves......................................................... 36

8. Mean denitrification rates using the acetylene inhibition technique in control microcosms......................................................... 42

9. Mean denitrification rates using the acetylene inhibition technique in low nitrate treatment microcosms................................. 44

10. Mean denitrification rates using the acetylene inhibition technique in high nitrate treatment microcosms ........................ 45

11. Mean nitrous oxide emission rates in control microcosms without acetylene ......................................................... 49

12. Mean nitrous oxide emission rates in low nitrate treatment microcosms without acetylene ......................................................... 51

13. Mean nitrous oxide emission rates in high nitrate treatment microcosms without acetylene ......................................................... 52

14. Comparison between denitrification potential using the acetylene inhibition technique and $^{15}$N isotope technique ......................... 53

15. Mean thickness of the oxidized layer in sediment microcosms with and without the acetylene treatment................................. 55
ABSTRACT

Denitrification potential was determined in surface sediment from Lake Cataouatche, the receiving basin for a future Mississippi River diversion located in the northern portion of the Barataria Basin estuary. Nitrate removal and denitrification was measured in the laboratory using static sediment microcosms flooded with lake water. Dissolved potassium nitrate (KNO₃) was added to the microcosms to achieve: 1) an initial nitrate concentration similar to the mean Mississippi River concentration (~1.4 mg NO₃-N l⁻¹), and, 2) a high initial nitrate concentration to elicit a denitrification potential (~50 mg NO₃-N l⁻¹). Denitrification was determined by the acetylene inhibition technique. The denitrification potential during the most active period (day 3-10) of nitrate removal in September, December, and March studies ranged from 37 to 55, 29 to 60, and 34 to 111 mg N m⁻² d⁻¹, respectively. A mean denitrification potential of 49±17 mg N m⁻² d⁻¹ was estimated from the three sampling periods. The denitrification potential was 16 times greater than estimates of denitrification under nitrate concentrations similar to the Mississippi River (3.0±1.2 mg N m⁻² d⁻¹), which ranged from 1.7 to 4.1, 0.89 to 3.8, and 2.3 to 4.5 mg N m⁻² d⁻¹ in September, December, and March studies, respectively. Mean nitrate removal from the 50 mg NO₃-N l⁻¹ addition to the microcosm floodwater was estimated at 177±25 mg N m⁻² d⁻¹ (with 6.3-18.5% error using an assigned estimate of floodwater volume). However the quality of data was poor resulting from not measuring initial floodwater volumes and evaporation. The nitrate removal rate estimate is qualitative and may vary as much as 100% at the high nitrate addition rate (see Table 4). At the low nitrate addition (1.4 mg NO₃-N l⁻¹) it was difficult to assign a removal rate since many measurements were
below the analytical method detection limit used. Results demonstrated that Lake Cataouatche sediment has a large capacity to remove nitrate from the water column, and also suggest that denitrification could remove a significant portion of the nitrate inputs from the Davis Pond diversion.
CHAPTER 1. INTRODUCTION

Review of Literature

The Mississippi River receives high levels of nutrients over its entire course. During the past century, nutrient levels have increased steadily primarily due to agricultural practices in the Corn Belt region of the northern United States (Goolsby et al., 2000; Turner and Rabalais, 1991). The heavy use of fertilizers along with current crop management practices contributes to large amounts of nutrient runoff and leaching directly into the Mississippi River and its tributaries. With additional inputs from urbanization, industry, and other land use practices, the current load of nitrogen represents 2 to 5 times the nutrient concentration in the Mississippi River in 1900. Most of the increase occurred within the past thirty years (Goolsby et al., 2000). As a result of human influences, historic oligotrophic flows have been replaced by eutrophic conditions that threaten to permanently alter the ecology and economy of the Mississippi River delta and coastal zone (Turner and Rabalais, 1994). Similarly, such impacts have been noted for other major rivers of the North Atlantic (Howarth et al., 1996).

Like many estuarine systems, the coastal zone of the Mississippi River delta is nutrient limited, particularly in nitrogen, and thus large inputs of nutrients can have major ecological effects on the receiving waters (Turner and Rabalais, 1991). The development of a large seasonal hypoxic zone in the Gulf of Mexico has been a concern in recent years (Rabalais et al., 1994). Hypoxia of the offshore bottom waters occurs each summer because of blooms of phytoplankton from river borne nutrients. Their subsequent death and decomposition by oxygen-consuming bacteria in the water
column and at the water-sediment interface give rise to anoxic or reducing conditions in the normally oxygenated benthic zone. The disturbance of trophodynamics and changes in water quality affects important Gulf coast fisheries and may have long-term impacts that remain unknown (Turner and Rabalais, 1994).

There also has been a reduction in sediment load of the Mississippi River by some 50% as a result of land use changes and the construction of dams and levees along the Mississippi River (Kesel, 1988). The resultant changes in sediment and water distribution are considered major causes of wetland loss along the Louisiana Gulf Coast (Salinas et al., 1986). In the absence of historic seasonal flooding across large expanses of coastal plains, the lack of sediment and nutrient inputs may be a factor that contributes to the 12,500 ha yr⁻¹ of wetland loss in Louisiana (Turner, 1990).

Recent intervention by state and federal agencies has provided an opportunity to restore wetlands in some areas by constructing large river diversions in the coastal zone. Six diversions are currently in operation and several others are being planned or are under construction. The diversions at Bonne Carre Spillway (in planning), Caernarvon (1991), and Davis Pond (under construction) are the largest of these projects, capable of diverting a total of 1376 cms at maximum discharge or 1% of mean Mississippi River flow (Rabalais et al., 1995). During episodic flooding, however, up to 16% of the Mississippi flow has been diverted through the Bonne Carre Spillway into Lake Pontchartrain (Day et al., 1999). The Caernarvon and Davis Pond diversions have the potential to release a maximum discharge of 230 and 300 cms into Breton Sound and northern Barataria Basin estuaries, respectively.
Nutrient-rich discharge from diversions can affect the ecology of receiving basins in both beneficial and harmful ways. By mimicking historical overbank flows into a basin isolated from the river, added sediment and nutrients can stimulate macrophyte production and contribute to soil stability and marsh accretion (Mendelssohn and Kuhn, 1999). Recent studies at Caernarvon reported 164 ha of new marsh (Villarubia, unpublished data), and increased marsh accretion as a result of diversion inputs (DeLaune, unpublished data). Diversions also may stimulate freshwater fisheries and enhance wildlife in the receiving basins. Nutrient levels in the discharge water, however, may be significantly higher than that of the receiving basin (Table 1), thereby increasing the potential for eutrophication and its consequences (e.g. anoxia, changes in soil chemistry, changes in trophic structure). The nitrate concentration, for example, was found to be 20-50 times higher in the Mississippi River during spring peak discharge than the average in the Barataria Basin estuary, though concentrations in the river vary considerably with differences in seasonal rainfall and river flows (Rabalais et al., 1995).

Eutrophication of the receiving waters can lead to deleterious consequences both ecologically and economically. The impacts may be short-term, such as the algal blooms in Lake Pontchartrain at the Bonne Carre Spillway that have resulted from singular pulsed events (Day et al., 1999). Chronic effects such as the displacement of natural vegetation by invasive species in freshwater marsh environments may also occur (Otto et al., 1999). For some lakes in the northern Barataria Basin already considered eutrophic, the introduction of diversion water may worsen algal blooms and low
Table 1. Macronutrient concentrations in the Mississippi River and Upper Barataria Basin. Number of samples (n) are in parenthesis. (Adapted from USACOE, 1982)

<table>
<thead>
<tr>
<th></th>
<th>Mississippi River</th>
<th></th>
<th></th>
<th>Upper Barataria Basin</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum</td>
<td>Minimum</td>
<td>Mean</td>
<td>(n)</td>
<td>Maximum</td>
<td>Minimum</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>63</td>
<td>0</td>
<td>6.9</td>
<td>(929)</td>
<td>9.6</td>
<td>1</td>
</tr>
<tr>
<td>Bicarbonate ion</td>
<td>182</td>
<td>67</td>
<td>124</td>
<td>(953)</td>
<td>115</td>
<td>32</td>
</tr>
<tr>
<td>Calcium, dissolved</td>
<td>59</td>
<td>26</td>
<td>40</td>
<td>(876)</td>
<td>50</td>
<td>6.1</td>
</tr>
<tr>
<td>Magnesium, dissolved</td>
<td>21</td>
<td>3.2</td>
<td>11</td>
<td>(877)</td>
<td>63</td>
<td>3.1</td>
</tr>
<tr>
<td>Sodium, dissolved</td>
<td>690</td>
<td>0</td>
<td>24</td>
<td>(1555)</td>
<td>590</td>
<td>0</td>
</tr>
<tr>
<td>Potassium, dissolved</td>
<td>8.1</td>
<td>1</td>
<td>3</td>
<td>(677)</td>
<td>77</td>
<td>34</td>
</tr>
<tr>
<td>Unionized + Ionized Ammonia, total</td>
<td>1.26</td>
<td>0</td>
<td>0.33</td>
<td>(504)</td>
<td>2.06</td>
<td>0.08</td>
</tr>
<tr>
<td>Unionized Ammonia, NH₃</td>
<td>0.08</td>
<td>0</td>
<td>0.01</td>
<td>(410)</td>
<td>0.08</td>
<td>0.00</td>
</tr>
<tr>
<td>Nitrite + Nitrate as N, total</td>
<td>5.10</td>
<td>0.01</td>
<td>1.18</td>
<td>(409)</td>
<td>2.18</td>
<td>0</td>
</tr>
<tr>
<td>Phosphate as P, soluble</td>
<td>0.20</td>
<td>0</td>
<td>0.07</td>
<td>(417)</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>Phosphorus as P, total</td>
<td>1.40</td>
<td>0</td>
<td>0.32</td>
<td>(537)</td>
<td>0.70</td>
<td>0.04</td>
</tr>
</tbody>
</table>
dissolved oxygen conditions and increase the frequency of fish kills (USACOE, 1982). Eutrophication on a larger spatial scale can lead to the decline of general estuary health that may threaten coastal Louisiana fisheries, since approximately 90% of commercial fisheries are estuary dependent (Mitsch and Gosselink, 1993). In the worst cases, severe loss of biodiversity or other permanently irreversible trends may result (McComb and Davis, 1993).

Although eutrophication may be result from the relatively high influx of nitrogen and phosphorus in forms readily available to primary producers, this discussion will focus on nitrogen processes relating to the fate of nitrate in the Mississippi River. There are significant differences in nitrate uptake and transformation in fresh, brackish, and salt marsh biota and sediments. Studies in Barataria Basin show that N:P ratios decline along the salinity gradient (Rabalais et al., 1995). The limitation of nitrogen depends largely on the biogeochemical processes that can alter N:P ratios within an estuary, such as ammonification, nitrification, and denitrification. The increase in the availability of ammonium in higher salinity zones, for instance, is largely due to the inhibitory effect of increased sulfate from seawater on nitrification (Joye and Hollibaugh, 1995). The partitioning of nitrogen between specific pathways in the upper estuary strongly influences its availability in the coastal zone.

It is evident that the spatial and temporal distribution of nitrogen is important in determining the level of eutrophication in aquatic environments. The turnover and transport of nitrogen in an estuary is accordingly related to initial loading and removal rates (Middelburg and Nieuwenhuizee, 2000). It is extremely difficult, however, to assess the biogeochemical cycling of nitrogen and its impacts on a large scale. As
multiple pathways generate and remove nitrogen species simultaneously, coastal wetlands can be sinks, transformers, and sources of nutrients (Mitsch and Gosselink, 1993). The flow of nutrients from adjacent land and waterways intersect with coastal transport mechanisms, making it nearly impossible to quantify with current methods.

Wetlands and shallow open water bodies attenuate nitrogen transformation and transport through physical and biogeochemical mechanisms. Studies at the Caernarvon Diversion showed that total suspended sediment and nitrate concentrations rapidly decreased within a few kilometers of the input (Lane, 1999). Similarly, 95% of nitrate from treated wastewater inputs to a Louisiana forested wetland was removed from the water column over a 1 km transect (Boustany et al., 1997). Several factors, including residence time, hydraulic loading rate, and nutrient concentration affect the rates under which nutrient transformation and nutrient removal occur (Ingersoll and Baker, 1998; Blahnik, 1997). For freshwater marshes, flood duration and frequency also influence the fate of nutrients in the marsh (Mitsch and Gosselink, 1993).

The biogeochemical processes occurring in the water column and sediment, however, are pathways with several endpoints. The cycling of nitrogen in wetland and aquatic ecosystems is perhaps the most complex among the major macronutrients (Figure 1). Nitrogen may undergo several transformations, and is likely turned over several times as it passes through the estuary (Day et al., 1989). In estuarine systems, macrophytes, benthic algae and phytoplankton can assimilate a significant fraction of available inorganic nitrogen (Day et al., 1989). Wetland macrophytes can assimilate inorganic nitrogen up to 3.5 g N m\(^{-2}\) d\(^{-1}\) in treatment wetlands (Kaldec and Knight,
Figure 1. A simplified diagram of nitrogen transformations in wetland and aquatic environments with the dissimilatory reduction of nitrate to N gases (denitrification) in bold. Adapted from Day et al, 1989.
1996). Bacterial immobilization of inorganic and organic nitrogen can also be significant (0.167 umol l⁻¹ h⁻¹), particularly where the contact time with nitrogen is very short (Wheeler and Kirchman, 1986). Microbially-mediated processes play a major role in nitrogen transformations within the sediment and at the water-sediment interface.

The cycling of nitrogen begins with ammonification, which is the production of ammonium (NH₄⁺) from the microbial decomposition of organic material in the water column, soil and sediment. Ammonium may also be formed by the dissimilatory reduction of nitrate in freshwater, estuarine, and marine aquatic sediment (Jorgensen, 1989; Koike and Hattori, 1978; Priscu and Downes, 1987). Ammonium is the preferred form of nitrogen under certain conditions that is assimilated by primary producers and immobilized by microbes. Unconsumed ammonium can be adsorbed to sediment by cation exchange, or may diffuse into the water column, undergo deprotonation, and be released into the atmosphere through volatilization.

In submerged freshwater systems, essentially all (80-100%) of net ammonium produced in sediment may undergo nitrification, the transformation of ammonium to nitrate (NO₃⁻), while in marine sediments, the salinity limits nitrification activity to 40 to 60% (Seitzinger, 1990). Nitrification activity within the surface sediment may be a significant source of denitrified nitrogen in sediment with relatively low nitrate in the overlying water (Nishio et al., 1983). The close coupling of nitrification with denitrification is responsible for the removal of internally cycled nitrogen to the atmosphere (Van Luijn et al., 1999).

Denitrification is the microbially-mediated reduction of nitrate to gaseous end products, and represents a major pathway for the removal of nitrogen in wetland and
aquatic ecosystems. Nitrate may be reduced to the gaseous products, nitric oxide (NO), nitrous oxide (N₂O), or dinitrogen gas (N₂), by facultative denitrifying bacteria. Nitrate also may be reduced by ferrous iron in a chemical reaction to form the same products as microbial denitrification in the subsoil (Mosier and Schimel, 1993). Seitzinger (1990) reported that denitrification in aquatic sediments can remove up to 75% of nitrogen loading.

Denitrification is very significant in aquatic environments where external inputs of nitrate are proportionally greater than internal inputs. The high mobility and solubility of nitrate allows the rapid diffusion of nitrate from the water column down into anaerobic sediment layers where active denitrifying organisms interact with the substrate. Heterotrophic bacteria such as Psuedomonas spp. (most numerous) use nitrate as an electron acceptor in the absence of oxygen to carry out the oxidation of organic compounds to carbon dioxide gas, CO₂ (Germon, 1985). The reaction is described by the stoichiometric equation:

\[ 4 \text{HNO}_3 + 5 \text{CH}_2\text{O} \rightarrow 5 \text{CO}_2 + 7 \text{H}_2\text{O} + 2 \text{N}_2 \]

The reduction of nitrate follows a sequence that reduces the oxidation status of nitrogen species from +5 (NO₃⁻) to 0 (N₂) in the order:

\[ \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow (\text{NO}) \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2 \]

The key irreversible step in denitrification is the conversion of nitrite (NO₂⁻) to nitric oxide (NO) and nitrous oxide (N₂O) that is mediated by dissimilatory NO₂⁻ reductase (Coyne and Tiedje, 1990).

The biological process comprises several steps catalyzed by metal-containing enzymes (e.g. Fe, Mo, Cu). NO₂⁻ reductase has two major structures, one containing a
copper center and the other a heme group. Although denitrification involves several steps, many bacteria can carry out only one or two (Germon, 1985). Complete denitrification with N₂O and N₂ as the only end products is the result of denitrifying biocommunities with several different species; the imbalanced activity of nitrate and nitrite reductases may result in the accumulation of intermediate species (Martienssen and Schops, 1999). The efficiency of reducing bacteria with respect to these steps is variable for different populations (Martienssen and Schops, 1999).

Of the intermediates produced, NO is evolved in minor quantities because of its highly reactive nature, while the evolution of N₂O may be substantial. The evolution of N₂, however, exceeds that of the other species being the final reduced species in denitrification. Nitrification may also be a primary N₂O producing process in organic carbon limited soils, however, in most circumstances, N₂O evolution by nitrification is not quantitatively significant (Mosier and Heinemeyer, 1985). The N₂:N₂O ratio observed in several denitrification studies ranges from less than 10:1 to greater than 100:1 with large variation among and within sites (Reddy and Patrick, 1984). The relative proportion of the gases evolved depends on nitrate, carbon, and oxygen availability (Weier et al., 1993). Notable differences in end products and differences in denitrification rates in general are indicative of the spatial heterogeneity of sediment.

Denitrification occurs beneath the aerobic/anaerobic sediment boundary where the redox potential is below approximately +220 mV as measured between the platinum and SHE reference electrodes (Patrick and Mahapatra, 1968). Denitrification increases with decreasing redox potential (Van Cleemput and Patrick, 1974). The aerobic-anaerobic boundary zone facilitates simultaneous nitrification and denitrification, as
nitrate produced in or that diffuses into the aerobic layers of surface sediment can be transported in close proximity to the anaerobic sediment where it is subsequently reduced to N₂O and N₂ by facultative heterotrophic bacteria. The aerobic layer is limited in depth by the slow diffusive property of dissolved oxygen in submerged sediment and rapid reduction of the small amounts of diffused oxygen by benthic organisms. Increases in the concentration of oxygen in the water column can increase the thickness of the aerobic layer (Patrick and Gotoh, 1974), and thereby stimulate the coupled nitrification-denitrification effect. The diffusion of nitrate from the overlying water, however, is hindered by the longer path through the aerobic sediment layer.

Oremland et al. (1984) found that denitrification occurred endogenously up to 3 cm in depth from the sediment surface, but potential activity existed at all depths (the deepest was 15 cm) when nitrate was added to sediment. This concurs with the observation by Dunigan and DeLaune (1978) that although nitrifiers were found to be concentrated in the upper few centimeters in flooded soil, the numbers of denitrifiers actually increased with depth. In the presence of oxygen, denitrifiers may be present but may be repressed, as facultative anaerobes would use oxygen rather than nitrate in respiration. Enzyme repression may also be due to a limited nitrate supply. Smith and Tiedje (1979), however, observed that a full de-repression of enzymes occurred only 4 hours after a drained soil was wetted and anaerobic conditions were re-established.

Denitrification may also occur in the water column to some extent as well as in the sediment. Anoxic layers in stratified lakes support denitrification in the water column when the oxygen concentration is less than 0.2 mg l⁻¹ (Knowles, 1982). A limited amount of denitrification, however, was found to occur in turbid oxygenated
estuarine water with a high nitrate concentration, where organic matter provided microniches of anaerobic activity (Omnes et al., 1996).

Denitrification rates for aquatic sediments span a wide range, varying by ecosystem type (e.g. marine and freshwater, emergent marsh and forested swamp) and denitrification measurement technique. A review by Seitzinger (1990) revealed low denitrification rates for deep-sea sediments (0.3 –2.4 µmol N m⁻² h⁻¹, or 0.1 – 0.8 mg N m⁻² d⁻¹), higher rates for estuarine sediments (5 – 250 µmol N m⁻² h⁻¹, or 1.7 – 84 mg N m⁻² d⁻¹), and highest rates for polluted estuarine sediments (> 500 µmol N m⁻² h⁻¹, or > 168 mg N m⁻² d⁻¹).

Since denitrification is a microbially mediated process, denitrification rates are dependent on the activity of denitrifiers and environmental factors influencing denitrification. Several studies have reported increased denitrification rates with additions of nitrate in nitrogen-limited sediment systems (Nowicki, 1984; Smith et al., 1985). In fact, long-term addition of nitrate can raise the maximum denitrification capacity in wetland soils, but the proportion of nitrate load that is not reduced increases with increasing nitrate concentration (Maag et al., 1997). Denitrification rate is not correlated to nitrate concentrations at high concentrations (Nowicki, 1994). Beyond the linear relationship of denitrification rates with nitrate concentration, a denitrification maximum is reached. Drury et al. (1991) found that although microbial biomass was correlated with background denitrification rates, no correlation was found for potential denitrification. Studies of English sloughs by Livingstone et al. (2000) showed that the denitrification potential matched present external loading or else exceeded it several-
fold, suggesting that some sediments had the potential to remove greater anthropogenic inputs over others.

Denitrification rates may be limited or influenced by the composition of sediment (Gordon et al., 1986). Clay particles in sediment provide attachment points for denitrifying bacteria (Chalamet, 1985). Soil texture also influences organic matter content, which affects denitrification rates (Van Luijn et al., 1999). Water soluble carbon, mineralizeable carbon, and to a lesser degree, total carbon in sediment were found to be good indices of denitrification potential for a broad range of soils incubated under anaerobic conditions (Burford and Bremner, 1975; Stanford et al., 1975). Ingersoll and Baker (1998) found that the carbon-nitrogen ratio was highly predictive of nitrate removal efficiency. Pfenning and McMahon (1996) also found that in carbon limited riverbed sediment, the type of organic carbon (i.e., acetate, fulvic acid) added to the sediment greatly influenced denitrification rates.

Although denitrification rates are limited primarily by nitrate concentration, the presence of oxygen, and organic matter availability, several external factors are associated with these major factors. Aquatic plants alter dissolved nutrient concentration through root uptake, and some develop oxidized rhizomes around their roots. Root rhizomes create sites for coupled nitrification-denitrification by increasing the aerobic-anaerobic sediment interface within the sediment (Knowles, 1982). Root exudates also can provide denitrifiers with organic substrate. Otto et al. (1999) reported that the absence or presence of vegetation in a tidal freshwater marsh influenced denitrification, but the type of plants did not. In contrast, studies of riparian wetlands showed that differences in organic carbon supply from leaf litter and root exudates
between woody and grassy sites produced different denitrification rates (Martin et al., 1999). The influence of primary producers on denitrification in aquatic environments, however, is complex, and further investigation using models may be required (Seitzinger, 1990).

The presence of benthic macrofauna and meiofauna was also shown to stimulate denitrification in several studies (Binnerup et al., 1992; Svensson and Leonardson, 1996; Tuominen et al., 1999). Binnerup et al. (1992) found that 38 to 66% of the denitrification rate was due to the increased diffusion of nitrate into sediment via infauna burrows combined with the venting of burrow water, which released nitrous oxide into the water column. Animal burrows also create oxidized zones around their burrows, which allow increased nitrification-denitrification activity.

The seasonal effects on denitrification are the result of several factors. Temperature raises the overall metabolic activity in the sediment and therefore may stimulate benthic activity, particularly the microbial decomposition of organic matter and influence of benthos on the oxidized layer. Temperature was found to increase denitrification rates from 25 umol N₂ m⁻² h⁻¹ (17 mg N m⁻² d⁻¹) at 5°C to 200 umol N₂ m⁻² h⁻¹ (130 mg N m⁻² d⁻¹) at 20°C in enriched cores of estuarine sediment (Nowicki, 1994). Denitrification in estuarine sediments at higher temperatures (20°C), however, may be limited by competition for nitrate with fermentative microorganisms that reduce nitrate to ammonia (Ogilivie et al., 1997). A review by Herbert and Newdell (1990) reported the development of denitrifiers in low winter temperatures in estuarine sediment, whereas summer high temperatures favored the dominance of nitrate-ammonification.
Microbial activity, conversely, can affect the pH of their environment. The release of CO$_2$ during denitrification can form OH$^-$ (hydroxide) and HCO$_3^-$ (bicarbonate), which can increase pH of the sediment pore water beyond the range preferred by most heterotrophic soil microbes (Rust et al., 2000). A review by Knowles (1982) reports the optimum pH range for denitrifiers as 7.0 to 8.0. Nitrate reduction also decreases with decreasing pH from 8 to 4.5 in slurries maintained at a redox potential of 0 mV, however, substantial nitrogen loss occurred in the most acidic slurry (Van Cleemput and Patrick, 1974).

The measurement of denitrification has been historically limited by the development of analytical techniques for laboratory and field studies. Studies of denitrification by mass balances were common, though results often have been influenced by endogenous nitrogen. This is because nitrogen losses were typically estimated as the difference of total inputs and recovered nitrogen in the water column, sediment, sediment pore water, and plant tissue. Direct measurement of gases with acetylene inhibition and $^{15}$N isotope techniques are now common and provide better results than estimates using mass balance techniques. Spatial variability in sediments, however, limits the reproducibility of field studies. Even small “homogeneous” areas are not uniform so that the coefficient of spatial variability can vary from 40% to 80% (Mosier and Heinemeyer, 1985). Their research showed, however, that a four-fold replication in “homogeneous” sites gave general estimates of the variability. Mosier and Heinemeyer (1985) also discuss the issue of scaling effects on overall experimental error: i.e., extrapolating denitrification results from small plots to large scales can cause errors to be multiplicative. Denitrification measurements may also be underestimated.
by large amounts of reduced gases that may be trapped in the sediment column (Lindau and DeLaune, 1991).

Laboratory experiments may provide better reproducibility because many potentially important input variables can be controlled, or measured easily. Denitrification potential studies often employ the use of soil and sediment cores or mixed sediment slurries. Denitrification rates in slurries usually are higher than in situ conditions, mainly because of decreased heterogeneity in factors such as oxygen and substrate concentrations (Koike, 1990). The laboratory conditions, therefore, are only approximately representative of real environmental situations, and, as a result, lab findings cannot be used to predict the extent of denitrification in the field (Ryden and Rolston, 1983).

The acetylene inhibition technique has recently come into wide usage for its simplicity and economy. Acetylene (C₂H₂) inhibits the dissimilatory N₂O reductase by noncompetitive inhibition and prevents the formation of N₂ from N₂O. The emission of N₂O from the sediment can be readily detected with gas chromatography with an electron capture detector (Mosier and Heinmeyer, 1985). As ambient N₂O concentrations are very low compared to N₂, the technique can also be used in non-fertilized systems. Acetylene also does not interfere with other denitrifying reductases nor is it reduced in the process. Not many details of the mechanism of acetylene blockage are known, however (Knowles, 1990).

Mosier and Heinmeyer (1985) discusses several disadvantages of the acetylene inhibition technique: 1) the acceleration of nitrate reduction, 2) the disappearance of acetylene with prolonged incubation (>3-5 d), 3) the inhibition of soil carbon
mineralization at low nitrate concentration and the acceleration of carbon mineralization when sufficient nitrate is supplied, and, 4) the anaerobic oxidation of acetylene by estuarine sediments. Knowles (1990) reported that acetylene inhibits nitrification when low nitrate concentrations are present in the floodwater or sediment. Ryden and Dawson (1982) found, however, that acetylene inhibition of nitrification was not important when the method was applied in field settings, and that their repeated short-term acetylene treatment did not lead to acetylene decomposition or enhancement of denitrification. Incomplete blockage of N₂O reductases may also occur at low concentrations of acetylene (or nitrate), and may result in the loss of N₂O in laboratory experiments (Oremland et al., 1984). Knowles (1990) also acknowledged a possible lack of an inhibitory effect of acetylene at low nitrate concentrations in the presence of sulfide in highly reduced marine sediments.

Direct measurement of denitrification using the ¹⁵N method is also commonly used and often conducted in support of the acetylene blockage technique. The method involves applying highly enriched KNO₃⁻¹⁵N (>20 atom %) to a plot of soil or sediment core, and finding the ¹⁵N mole fraction of the NO₃⁻ that serves as the N₂ source (Mosier and Heinemeyer, 1985). Since N₂ is 78% of the atmosphere, an isotope ratio mass spectrometer must be used for analysis. ¹⁵N-labelled fertilizers are frequently used in mass balance studies in which the inputs, outputs, and pools of N are measured.

Difficulty with analytical errors and sensitivity often results in an overestimated value according to Mosier and Heinemeyer (1985), though they report this technique to be sensitive to 5 g N d⁻¹ ha⁻¹. Variations in ¹⁵N natural abundance and differences in reaction and mixing of ¹⁴N and ¹⁵N can also pose problems with isotope work.
Specifically with denitrification measurements, as both N₂O and N₂ are evolved, the problem of differential diffusion must also be faced in waterlogged soils. A practical disadvantage of ¹⁵N isotope technique is the relative expense of ¹⁵N that can be prohibitive in field studies or extensive laboratory studies.

Compared with ¹⁵N isotope direct measurement, the acetylene inhibition technique gives similar denitrification values. Similar rates are obtained from these two methods that measure gaseous products because both methods are subject to similar physical processes (Myrold, 1990). Rolston et al (1982) found that in a direct comparison of the ¹⁵N method to the acetylene inhibition technique, the amount of N evolved was similar in both, but the timing of the N evolution was not the same for both methods. This is thought to be due to differences in solubility of both gases in water and their respective diffusion rates. Both of these techniques often underestimate the amount of denitrification measured by mass balance, but current research suggests these as the preferred methods that provide the best estimate for denitrification. Use of these methods also allows for comparison to the growing body of data on processes affecting nitrogen in soils and sediment-water systems.

**Objectives**

This research sought to quantify the nitrate processing capacity of sediment from Lake Cataouatche, the receiving water body of the Davis Pond Mississippi River Diversion. The objectives of this study included: 1) the quantification of the emission of dinitrogen and nitrous oxide from static sediment microcosms using the acetylene inhibition and ¹⁵N isotope techniques, 2) the quantification of the nitrate removal capacity of the sediment from the overlying floodwater, and, 3) the identification of the
denitrification potential and its significance relative to total nitrate removal from the water column.

The interpretation of these results will provide part of a preliminary assessment of the impact of the Davis Pond Diversion on upper Barataria Basin estuary. These data on nitrate removal capacity of Lake Cataouatche sediment are part of the groundwork for further, more inclusive discussions on the impact of freshwater diversion impacts where eutrophication and nitrogen loading may be of concern.
CHAPTER 2. MATERIALS AND METHODS

Site Description

The Davis Pond Diversion is located near Luling, Louisiana in St. Charles Parish on the west bank of the Mississippi River. The freshwater diversion structure is capable of delivering a maximum discharge of 300 cms through four 4.3 m x 4.3 m gated box culverts. The diversion will potentially benefit 13,400 ha of marsh in Barataria Basin estuary, a 314,400 ha wetland complex hydrologically bound on the east by the Mississippi River levee and on the west by Bayou LaFourche (Figure 2). The diversion discharge will follow a course south through a 3,400 m long channel under the Highway 90 underpass and into a 3,700 ha ponding area bound by constructed levees before entering Lake Cataouatche. The description of the Davis Pond Diversion is reported on the US Army Corps of Engineers website:

(http://www.mvn.usace.army.mil/pao/dpond/davispond.htm)

Lake Cataouatche is a shallow (mean depth ~ 2 m) freshwater lake in the northern portion of the Barataria Basin estuary with a mean tidal range of 0.03 m (Swenson and Turner, 1998). Lake Cataouatche has open connections with Lake Salvador to the south, through which discharge water will reach the brackish and salt marshes in the lower reaches of the Barataria Basin estuary and then enter the Gulf of Mexico.

Since the initial ponding area likely will become channelized by diversion flows, Lake Cataouatche is considered the first major receiving body for this discharge where retention time will be significant for considerable nutrient transformations to occur. Lake Cataouatche is a 3,700 ha freshwater lake that receives freshwater inputs primarily
Figure 2. Map of Barataria Basin showing major water bodies and highways.
by rainfall and nutrients by runoff from adjacent uplands. Water quality in the area has been characterized as poor by the US Army Corps of Engineers as a result of low dissolved oxygen, frequent algal blooms and fish kills (USACOE, 1982). Results of preliminary studies suggest that although freshwater inputs could alleviate low dissolved oxygen conditions in some areas, additional nutrients in the influent may foster more severe eutrophic conditions in the lake (USACOE, 1982).

Five sites within the lake were selected for sample collection and were located using a global positioning system (Table 2). The primary sampling site location (Site A) was selected because it is near the proposed outflow from the ponding area, while an additional four sites (B-E) were selected to determine homogeneity of bottom sediment (Figure 3). Sediment samples were collected on September 15, 2000 and December 14, 2000 at Site A only and at all sites (A-E) on March 27, 2001.

Table 2. Sampling site coordinates.

<table>
<thead>
<tr>
<th>Location</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latitude</td>
<td>29.8548</td>
<td>29.8500</td>
<td>29.8400</td>
<td>29.8337</td>
<td>29.8264</td>
</tr>
<tr>
<td>Longitude</td>
<td>90.2521</td>
<td>90.2240</td>
<td>90.2650</td>
<td>90.2404</td>
<td>90.2136</td>
</tr>
</tbody>
</table>

Sediment Sampling

At each site, 3-5 samples of surface sediment (upper 15 cm) were collected from a boat using a Petersen dredge. Sediment was composited on the boat, and transported in plastic-lined containers to the laboratory. Lake water was also collected in plastic
Figure 3. Sampling sites (✪) within Lake Cataouatche.
carboys from Site A. The sediment was immediately homogenized upon arrival at the laboratory using a trowel, and all large debris (including mollusks) were removed by hand. The prepared sediment was transferred immediately to two sets of incubation jars.

**Microcosm Preparation**

All experiments were conducted in static microcosms, constructed of glass jars (8.9 cm x 16.9 cm) with gas-tight lids with rubber septa (Figure 4). Wet sediment (299-314 g) was added to each incubation jar to obtain a depth of approximately 6 cm. Lake water then was added to achieve a floodwater depth of approximately 5 cm, with water volumes varying to accommodate an initial headspace height of 6 cm. A headspace volume of 373 ml remained above the floodwater when lids were fitted. The jars were pre-incubated without the lids at ambient laboratory temperature (approximately 22°C) for about two weeks prior to headspace gas and floodwater analysis to allow the sediment to form an oxidized surface layer above the anaerobic sediment. During the incubation, the jars were wrapped with aluminum foil to prevent the proliferation of algae in the water column and sediment surface. Small needle holes in the aluminum foil at the tops of jars were made to allow natural gas exchange from the sediment and floodwater, and to limit the loss of water through evaporation. The foil covers were used throughout each experiment except during headspace gas sampling times.

**Nitrate Removal Analysis**

Samples of floodwater (5 ml) were removed from each jar for nitrate analysis on days when headspace gas sampling was performed (see below). Water samples were collected in glass vials, sealed and stored in a refrigerator (4°C) until analysis. Water
Figure 4. Diagram of a static microcosm.
samples were analyzed with a Spectronic Genesys 5 UV single beam spectrophotometer at a wavelength of 220 nm and the absorbance was corrected for dissolved organic matter interference by subtracting twice the absorption at 275 nm (APHA, 1992). This method is considered a screening method with limitations based on organic matter interference, which should not exceed 10% of the absorbance value for nitrate. The detection limit of nitrate was 0.24 mg l⁻¹, as determined by multiplying the standard deviation of ten replicate blanks by 2.82 (APHA, 1992). Nitrate removal rates were expressed in the units of mg N m⁻² d⁻¹, using an estimate of floodwater volume (311 ml) in the conversion of units. Accurate measurements of initial floodwater volume were difficult to obtain as a result of differences in the sediment settling in each microcosm during preparation. Evaporative losses were also not quantified during the experiment. The nitrate removal rates were corrected for the volume and concentration of nitrate removed from the system during floodwater sampling, however, the final estimates have an error of approximately 30% and are therefore only qualitative measures.

**Acetylene Inhibition Technique**

Denitrification in Lake Cataouatche sediment from Site A was measured in the fall, winter and spring (September 15, 2000; December 14, 2000; March 27, 2001) using the acetylene inhibition technique. A truncated study comparing all five sites with the acetylene inhibition technique also was conducted simultaneously with the March experiment. In the truncated study, microcosms from Sites B-E were sampled only on the fifth day (during peak N₂O emission) of the investigation.

The experiments using Site A sediment was conducted with two sets of incubation jars each with three levels of nitrate treatment: a control, a low nitrate
treatment, and a high nitrate treatment. Set 1 was treated with acetylene gas to
determine denitrification rates and potentials, and Set 2 was used to determine only the
N$_2$O emission (without acetylene). Each nitrate treatment (including controls) had three
replicates in each set, except the control and low nitrate treatment without acetylene in
the September study (two replicates each). The low and high nitrate treatments were
prepared by adding dissolved potassium nitrate (KNO$_3$) to the floodwater to achieve
initial floodwater nitrate concentrations of approximately 1.4 and 50 mg NO$_3$-N l$^{-1}$,
respectively. The nitrate concentrations represented a low nitrate concentration
representative of the nitrate level in the Mississippi River, and a high nitrate
concentration to elicit the denitrification potential. A preliminary investigation showed
that an initial concentration of 50 mg N l$^{-1}$ as nitrate produced N$_2$O fluxes similar to that
using an initial concentration of 100 mg N l$^{-1}$ and therefore it was concluded that 50 mg
N l$^{-1}$ was a sufficient concentration to produce a maximum denitrification potential
(data not shown). Headspace gas sampling was initiated on the first day after the
addition of nitrate to the incubation jars.

The experiments using sediment from Sites B, C, D, and E in the March study
were conducted using two replicates for controls and three replicates for the high nitrate
treatment. The high nitrate treatment was prepared with dissolved KNO$_3$ to achieve an
initial floodwater concentration of approximately 50 mg NO$_3$-N l$^{-1}$. All microcosms in
the study with Sites B, C, D, and E were treated with acetylene.

At the start of each sampling period, the incubation jars were fitted with gas
tight caps with rubber septa. Prior to acetylene injection, headspace air was removed
from jars in Set 1 to create a slight vacuum, which allowed the injection of an equal
volume of acetylene without overpressure in the headspace. Forty-five ml of acetylene gas, injected at approximately 12% of the headspace volume, was injected slowly into the floodwater at the start of each sampling period. Emission measurements of nitrous oxide were taken from the closed jars using a 2-ml syringe at 0, 100, 160, 220, and 280 min after the incubation jars were sealed. Sampling occurred over the course of 16 days for controls and low nitrate treatment jars on days 1, 3, 5, 7, 10, 13, and 16 following the addition of KNO₃. For high nitrate treatment jars, sampling occurred for 24 days on days 1, 3, 5, 7, 10, 13, 16, 20, and 24. The jars were open to the atmosphere and covered with foil after the 280 min sampling periods to allow natural gas exchange within the floodwater and sediment.

The gas samples were immediately analyzed using a Shimadzu GC-14A Gas Chromatograph fitted with a 2-ml sampling loop, Poropak Q 1.8 m column, and an electron capture detector. The instrument used a carrier gas of ultra high pure nitrogen and operated at temperatures of 40, 100, and 290°C for the oven, injector, and detector, respectively. Rates of denitrification were determined by the linear regression of gas flux, corrected with the Bunsen absorption coefficient (Tiedje, 1982) to estimate total N₂O. Nitrous oxide flux was estimated using the closed chamber equation by Rolston (1986):

\[ F = \frac{(V/A)(273/T)}{(\Delta C/\Delta T)}, \]

where \( V \) is the headspace volume in the jar, \( A \) is the sediment surface area of the jar, \( T \) is the absolute temperature of the headspace gases, and \( \Delta C/\Delta T \) is the change in concentration of N₂O per unit of time. Changes in headspace volume during the
experiment were estimated from initial and final measurements of headspace volume in each microcosm. Nitrous oxide flux was reported in the units: mg N m\(^{-2}\) d\(^{-1}\).

\textbf{15N isotope Technique}

The \(^{15}\)N isotope technique allows for the direct measurement of \(^{15}\)N flux from sediments (Koike and Hattori, 1978). Denitrification was studied once using the \(^{15}\)N isotope method (November 22, 2000). Two replicate pre-incubated jars were amended with 56.6 atom \% labeled \(^{15}\)N potassium nitrate to achieve an initial concentration of approximately 50 mg NO\(_3\)-N l\(^{-1}\). Nitrogen gases were allowed to collect in the sealed headspace of the incubation jars for 24 h periods prior to gas sampling. Headspace gas samples (13 ml) were collected using a 20 ml syringe and transferred into 10 ml Vacuutainers. Gas samples were stored at ambient laboratory temperature until analysis was performed. Sampling occurred on days 2, 4, 6, 8, 10, 13, 15, 17, 19, 21, 23, 25, 27, and 29 from the initial amendment of nitrate. Lids were removed during the days when the headspace gas sampling was not performed. After a run of 29 days, the regular sampling was discontinued and the sediment was analyzed for entrapped gases. Both incubation jars were sealed and shaken vigorously by hand to release entrapped gases. Headspace gas was sampled after the sediment was allowed to settle for 15 min.

Gas samples containing labeled \(^{15}\)N were analyzed with a Finnigan Mat Delta Plus gas isotope ratio mass spectrometer. The recovery of \(^{15}\)N from gas samples was calculated using the equations of Mulvaney and Boast (1986).

\textbf{Statistical Analysis}

The statistical analysis of all data was performed using the SAS statistical software (SAS, 1998). The statistical assessment of nitrate removal from the overlying
floodwater was performed with an analysis of variance (ANOVA) to determine differences between seasons and sites for different treatments. A logarithmic transformation was first made on the data to convert the exponential decay form to a linear equation. The slopes determined from a linear regression were used in the ANOVA.

Comparisons of the two intervals of nitrate removal rates in high nitrate treatment microcosms from Sites A, B, C, D, and E were made using least square means with the Tukey adjustment. The ANOVA assumption of normality was tested for the data using the Shapiro-Wilk statistic, normal probability plot, and stem and leaf plot. The values used in this comparison were estimates made through a conversion of units from the nitrate concentration data. These estimates have a broad range of error (see Table 4) and the validity of the statistical analysis of these values is of some question. An ANOVA with repeated measures (days) was applied to N₂O emission data to determine differences in sampling periods and sites. This analysis was conducted with the Tukey adjustment to the least square means comparisons. The ANOVA assumption of homogeneity of variance was tested with a plot of variances and means and the assumption of normality was tested using the Shapiro-Wilk statistic. To assess the differences in N₂O flux between all sites, the results from day 5 in the March study for Sites A, B, C, D, and E were compared using an ANOVA with contrast statements. An alpha value of 0.05 was used in all statistical analysis.
CHAPTER 3. RESULTS

Sediment Characteristics

The sediment collected from Lake Cataouatche was a very dark gray-brown sediment containing no visible live or decomposing plant material. Sediment characteristics for samples collected at Sites A-E are given in Table 3. Sediment from all five sites was visually and texturally similar. Site B, however, contained lower concentrations of Na, K, Ca, and Mg compared to Sites A, C, D, and E. The sediment pH at all sites was neutral to slightly acidic, and within range of ideal conditions for denitrifying organisms in all sites.

Table 3. Sediment characteristics for Sites A-E.

<table>
<thead>
<tr>
<th>Location</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>P (mg/kg)</td>
<td>188</td>
<td>161</td>
<td>152</td>
<td>158</td>
<td>118</td>
</tr>
<tr>
<td>Na (mg/kg)</td>
<td>4982</td>
<td>780</td>
<td>4470</td>
<td>4788</td>
<td>3677</td>
</tr>
<tr>
<td>K (mg/kg)</td>
<td>695</td>
<td>320</td>
<td>649</td>
<td>658</td>
<td>576</td>
</tr>
<tr>
<td>Ca (mg/kg)</td>
<td>3047</td>
<td>1329</td>
<td>2535</td>
<td>3217</td>
<td>2099</td>
</tr>
<tr>
<td>Mg (mg/kg)</td>
<td>2351</td>
<td>1002</td>
<td>2394</td>
<td>2275</td>
<td>1775</td>
</tr>
<tr>
<td>pH</td>
<td>6.7</td>
<td>7.0</td>
<td>6.6</td>
<td>6.8</td>
<td>6.4</td>
</tr>
<tr>
<td>OM (%)</td>
<td>6.77</td>
<td>4.81</td>
<td>5.33</td>
<td>5.66</td>
<td>6.27</td>
</tr>
<tr>
<td>Bases (meq/100g)</td>
<td>57.9</td>
<td>19.1</td>
<td>53.4</td>
<td>57.2</td>
<td>42.5</td>
</tr>
<tr>
<td>Nitrogen (%)</td>
<td>0.62</td>
<td>0.28</td>
<td>0.75</td>
<td>0.65</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Nitrate Removal

The nitrate removal from the overlying floodwater in Site A microcosms following KNO₃ amendments was similar between replicates in each nitrate treatment.
The mean values for the three sampling periods (September 15, 2000; December 14, 2000; March 27, 2001) at Site A are plotted in Figures 5 and 6. A statistical comparison of nitrate removal curves for all treatments showed no significant difference between microcosms treated with and without acetylene in each treatment level (P=0.45-0.95, a=0.05).

The removal of nitrate followed an exponential decrease for the low and high nitrate treatments (initial concentrations of approximately 1.4 mg l⁻¹ and 50 mg l⁻¹, respectively) and no significant changes occurred for controls without acetylene treatment. In control and low nitrate treatment microcosms treated with acetylene, lower final nitrate concentrations were observed than in those microcosms without acetylene. As many of the nitrate concentrations in the control microcosms with the acetylene treatment were below the detection limit (0.24 mg l⁻¹) for the screening method used in the analysis, it should be noted that these values may not reflect a true quantitative measure of nitrate. Also, the correction factor for dissolved organic matter in the control and low nitrate treatment floodwater exceeded the recommended 10% of nitrate concentration, and so these values may not necessarily be representative of actual nitrate concentrations in these microcosms.

The mean nitrate concentration for controls without acetylene treatment over the sampling period of 16 days was 0.42±0.08 mg N l⁻¹ (mean±SD) with a range of values from below the detection limit to 0.71 mg N l⁻¹. For acetylene treated controls, a mean nitrate concentration of 0.25±0.07 mg N l⁻¹ was found with a range of values from below detection the limit to 0.52 mg N l⁻¹. The lower nitrate concentration in acetylene treated controls may be due to the decreased net production of nitrate as a result of the
Figure 5. Changes in nitrate levels versus time in the overlying floodwater of control and low nitrate treatment sediment microcosms of Site A. Data points represent a mean value for September, December, and March studies. Error bars represent one standard deviation (n=8 for both control and low nitrate treatments(no acetylene); n=9 for both control and low nitrate treatments with acetylene). Note: several data points are below the detection limit.
Figure 6. Changes in nitrate levels versus time in the overlying floodwater of high nitrate treatment sediment microcosms from Site A. Data points represent a mean value for September, December, and March studies. Errors bars represent one standard deviation (n=9).
Nitrate removal rates for low and high nitrate treatments were estimated from a linear regression of two specific segments of the removal curve where major rate changes were observed graphically (Figure 7). The time intervals used for low nitrate treatments were between days 1 to 7 and from day 7 until sampling ended for N\textsubscript{2}O at day 16. For high nitrate treatments, time intervals between days 1 to 10 and day 10 through 24 were selected.

These rates, however, have a range of error as actual initial floodwater was not measured and a conservative estimate of the initial floodwater volume (311 ml) was used in converting the nitrate removal units from mg N l\textsuperscript{-1} d\textsuperscript{-1} to mg N m\textsuperscript{2} d\textsuperscript{-1} (Table 4). The error range was estimated in the high nitrate treatments at 6.3-18.5\% for the interval day 1-10 and 9.8-23.3\% for day 10-24. In the low nitrate treatments the error range was 2.6-19.2\% for day 1-7 and 16.0-106\% for day 7-16. The nitrate removal rates for the low nitrate treatment also may not be accurate estimates since the organic matter interference in this treatment obscured the nitrate concentration analysis.

The error was estimated by two separate methods to produce the ranges of error. First, the error was estimated from the difference in removal rates using the initial floodwater volumes of 311 ml or 373 ml in the conversion of units, corresponding to the smallest (5 cm) and largest (6 cm) estimated floodwater depths, respectively. Changes in floodwater volumes, resulting from evaporation and floodwater sampling for the
Figure 7. Linear regressions of selected intervals on the mean nitrate removal curves for high nitrate and low nitrate treatment used to determine nitrate removal rates. Regression equations and $R^2$ values are shown beside each regressed interval.
Table 4. Mean nitrate removal rate from sediment floodwater in low nitrate treatment microcosms and high nitrate treatment microcosms for selected time intervals. Nitrate removal rates with different letters within the same interval and treatment type is significantly different at P < 0.05. (n=17 for low nitrate treatment; n=18 for high nitrate treatment). Error ranges for each treatment and interval is also given. Note: as a result of large error ranges, the statistical evaluation may not be valid.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Interval</th>
<th>Sampling Period</th>
<th>Mean</th>
<th>Overall R²</th>
<th>Error Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>September</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low nitrate*</td>
<td>day 1-7</td>
<td>9.8±.36a</td>
<td>12±.72a</td>
<td>10±1.7</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>day7-16</td>
<td>0.93±.29a</td>
<td>0.83±.69a</td>
<td>0.70±.53</td>
<td>0.79</td>
</tr>
<tr>
<td>High nitrate</td>
<td>day 1-10</td>
<td>173±19ab</td>
<td>198±25a</td>
<td>177±25</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>day 10-24</td>
<td>52±3.9a</td>
<td>39±7.6b</td>
<td>42±10</td>
<td>0.91</td>
</tr>
</tbody>
</table>

*Nitrate removal rates in the low nitrate treatment are qualitative as a result of the high organic matter interference in the nitrate analysis.
nitrate analysis during the course of the experiment, were taken into account by using the linear increase of headspace volumes to estimate floodwater decrease. The removal of small amounts of nitrate in the samples was also taken into account. The conversion of units from mg N L\(^{-1}\) to mg N m\(^{-2}\) was made with the following formula:

\[
\left( \frac{x \text{ mg N}}{L} \right) \left( \frac{d}{1000 \text{ cm}^3} \right) - \left( \frac{x \text{ mg N}}{L} \right) \left( \frac{5 \text{ ml}}{1000 \text{ ml}} \right) \left( \frac{1 \text{ L}}{10000 \text{ cm}^2} \right) = x' \text{ mg N m}^2
\]

where \(x\) is the concentration of nitrate, \(A\) is the area of the jar, and \(d\) is the depth of the floodwater. By calculating the linear regression of the converted nitrate data, the method produced two sets of nitrate removal rates corresponding to the initial floodwater volumes of 311 ml and 373 ml. A comparison of these rates produced errors in the high nitrate treatment of 18.5±0.4% for the interval day 1-10 and 23.3±3.8% for day 10-24. In the low nitrate treatments, error ranges of 19.2±0.3% for day 1-7 and 16.0±2.2% were determined.

In the second method, nitrate concentration data were converted using a one initial volume of 342 ml (the mean of 311 and 373 ml) and two different final volumes. The final volumes were 200 ml, which estimates the lowest final volume, and 300 ml, an estimate of final volume if only the floodwater removal from sampling was considered. Linear regressions were made on the converted nitrate concentration data and a comparison of the two removal rates provided another set of error estimates. In the high nitrate treatments, error ranges of 6.3±1.8% and 9.8±3.1% were estimated for the intervals day 1-10 and day 10-24, respectively. Error ranges for the low nitrate
treatments were 1.8±1.4% for day 1-7 and 106±196% for day 7-16. The error ranges are summarized in Table 4.

The nitrate removal rates were calculated using a linear regression of each of the two intervals in the high and low nitrate treatment data. The R values (0.89 - 0.98) for these segments suggest a good fit (R_{0.05} > 0.632, d.f.=8; R_{0.05} > 0.666, d.f.=7) for each regressed interval in both low and high nitrate treatments. Although there is considerable error in these rates, the results are presented here if only to represent general qualitative values and trends.

The rapid initial decrease of nitrate in the low nitrate treatment floodwater occurred until day 7 with a mean nitrate removal of 10±1.7 mg N m\(^{-2}\) d\(^{-1}\) (with 2.6-19.2% error), from values ranging between 9.5 to 10, 6.8 to 13, and 11 to 13 mg N m\(^{-2}\) d\(^{-1}\) in September, December, and March studies, respectively. The organic matter interference, however, exceeded the recommended value for the nitrate screening method, and the nitrate removal rates in the low nitrate treatments are considered as qualitative values. A summary of mean nitrate removal for each interval is given in Table 4.

Nearly all of the nitrate from the water column was removed after the first seven days and subsequent rates decreased to a mean of 0.70±0.53 mg N m\(^{-2}\) d\(^{-1}\) (with 16.0-106% error) until sampling was terminated after 16 days. The nitrate removal rates ranged from 0.48 to 1.3, -0.18 to 1.6, and 0.36 to 1.4 mg N m\(^{-2}\) d\(^{-1}\) in September, December, and March studies, respectively. The final nitrate concentration for the low nitrate treatments approached that of the controls at approximately 0.3 mg N l\(^{-1}\). The
nitrate removal rates between sampling periods in low nitrate treatment microcosms were not statistically different in either interval (P=0.12-0.99, a=0.05).

The removal of nitrate in high nitrate treatments achieved a mean nitrate removal rate of $177 \pm 25$ mg N m$^{-2}$ d$^{-1}$ (with 6.3-18.5% error) from day 1 until day 10, with values ranging from 151 to 194, 133 to 178, and 164 to 235 mg N m$^{-2}$ d$^{-1}$ in September, December, and March studies, respectively (Table 4). The highest nitrate removal rates were observed in the March study, and these values were statistically different from results in December (P=0.01, a=0.05), but not significantly different in the September study (P=0.11, a=0.05). The samples at day 24 for the high nitrate treatment had a final concentration of less than 5 mg NO$_3$-N l$^{-1}$ with a nitrate removal rate from day 10 to 24 of $42 \pm 10$ mg N m$^{-2}$ d$^{-1}$ (with 9.8-23.3% error) with a range of 51 to 57, 28 to 47, and 23 to 44 mg N m$^{-2}$ d$^{-1}$ in September, December, and March studies, respectively. The nitrate removal in this interval was highest in September, and was significantly different from the December (P=0.01, a=0.05) and March results (P=0.003, a=0.05).

Nitrate removal from Sites B, C, D, and E with high nitrate treatment in the March experiment was compared with data from Site A over the entire sampling period (Table 5). The raw data are given in Appendix A. The differences between nitrate removal in Site A microcosms and those of site B, C, D and E were not statistically significant during days 1-10 (P=0.18-0.96, a=0.05). The nitrate removal from Site D had the lowest rate of removal during days 1-10, but had the highest removal rate during days 10-24. Site A removal rates in this second interval were not statistically different
Table 5. Mean nitrate removal rates (± one standard deviation) for Sites A, B, C, D, and E in the March study. Nitrate removal within an interval with different letters is significantly different at P < 0.05. (n=3 for all sites and intervals). Note: all nitrate removal rates have an error of 6.3-18.5%, since an estimate of floodwater volume was used in the conversion of units.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Interval</th>
<th>Site A</th>
<th>Site B</th>
<th>Site C</th>
<th>Site D</th>
<th>Site E</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>day 1-10</td>
<td>188±21a</td>
<td>177±21a</td>
<td>179±8.3a</td>
<td>154±21a</td>
<td>178±3.2a</td>
</tr>
<tr>
<td></td>
<td>day 10-24</td>
<td>39±7.9a</td>
<td>44±3.5a</td>
<td>50±2.4ab</td>
<td>59±4.3b</td>
<td>42±2.2a</td>
</tr>
</tbody>
</table>

from all other sites (P=0.08-0.95, a=0.05), except with Site D (P=0.003, a=0.05). As there were a small number of replicates for each site (n=3), the statistical comparison was limited. In subsequent work, it is recommended that the replicates be increased to give a better statistical evaluation. These results suggest that the capacity of sediment to remove nitrate from overlying floodwater in static microcosms was generally similar between sites.

**Acetylene Inhibition Technique**

The rates of denitrification in control microcosms determined by the acetylene inhibition technique ranged between –0.03 and 1.4 mg N m⁻² d⁻¹ for the three sampling periods (Figure 8). A small peak in N₂O emission occurred between days 3-7, coincident with a small decrease in nitrate concentration in the overlying floodwater. This is probably due to a stimulatory effect of acetylene on denitrifiers. The denitrification rates in controls averaged 0.19±0.24 mg N m⁻² d⁻¹, with no significant differences between sampling periods (P=0.99, a=0.05).
Figure 8. Mean denitrification rates using the acetylene inhibition technique in control microcosms for September, 2000, December, 2000, and March, 2001 experiments. Data are shown in Appendix B.
The denitrification rate in sediment treated with acetylene showed similar patterns among replicates for the low nitrate treatment, where N$_2$O emissions increased rapidly to a peak coinciding with the rapid removal of nitrate from the floodwater, then fell with a decline in emission rates until sampling was terminated. The apparent lag in N$_2$O flux on day 1 may be the result of enzyme induction following nitrate addition. N$_2$O flux achieved a mean maximum flux of 3.7, 3.7, and 4.2 mg N m$^{-2}$ d$^{-1}$ for low nitrate treatments at day 3 in the September, December, and March studies (Figure 9). The data are shown in Appendix B. The mean N$_2$O flux for the period corresponding to the most active period of nitrate removal in low nitrate treatment microcosms (day 1-7) was calculated from a mean of days 3-5, omitting values from day 1, where an initial lag occurred, and day 7, where nitrate concentrations returned to control levels. The mean N$_2$O flux was estimated at 3.0±1.1 mg N m$^{-2}$ d$^{-1}$ with values ranging from 1.7 to 4.1, 0.89 to 3.8, and 2.3 to 4.5 mg N m$^{-2}$ d$^{-1}$ in the September, December, and March studies, respectively. The denitrification rates fell rapidly to values similar to that in the controls after day 7. The denitrification rates of sediment with low nitrate treatments were not statistically different between September, December, and March studies (P=0.90, a=0.05). This apparent similarity in the N$_2$O flux for the low nitrate treatment between sampling periods may indicate that carbon sources were not limiting denitrification under the experimental conditions in the laboratory.

For high nitrate treatments, the peak mean denitrification rates of 53, 54, and 94 mg N m$^{-2}$ d$^{-1}$ was observed during the most active period of nitrate removal (day 1-10) of the September, December, and March studies, respectively (Figure 10). The mean peak values were recorded on day 10 in September, day 3 for December and on day 5
Figure 9. Mean denitrification rates using the acetylene inhibition technique in low nitrate treatment microcosms for September, 2000, December, 2000, and March, 2001 experiments. Data are in Appendix B.
Figure 10. Mean denitrification rates using the acetylene inhibition technique in high nitrate treatment microcosms for September, 2000, December, 2000, and March, 2001 experiments. Data are in Appendix B.
for the March samples. A consistent pattern of denitrification was observed in high nitrate treatments, which was characterized by a rapid increase in N\textsubscript{2}O emission from day 1 and a sustained period of denitrification activity until day 10, corresponding to the most active portion of the nitrate removal curves. Denitrification rates tended to decrease following peak activity through day 24 when the headspace gas sampling ended. The ranges of N\textsubscript{2}O flux values during the most active period (not including day 1, for reasons stated above) of nitrate removal in September, December, and March were 37 to 55, 29 to 60, and 34 to 111 mg N m\textsuperscript{-2} d\textsuperscript{-1}, respectively. The mean denitrification potential rate was 49±17 mg N m\textsuperscript{-2} d\textsuperscript{-1}. The denitrification potentials for the September, December, and March studies were statistically different (P<0.0001, a = 0.05). A summary of denitrification results for Site A microcosms treated with and without acetylene is in Table 6.

A comparison of the mean N\textsubscript{2}O flux from day 5 for Site A with the mean N\textsubscript{2}O flux observed on day 5 from sites B, C, D, and E was made in the March study. The N\textsubscript{2}O flux values ranged from 34 to 111, 52 to 61, 35 to 42, 25 to 36, and 32 to 41 mg N m\textsuperscript{-2} d\textsuperscript{-1} for Sites A, B, C, D, and E, respectively. On day 5 in the March study, the highest N\textsubscript{2}O fluxes over all sampling periods were observed for Site A microcosms. Statistical differences between Site A and Sites B, C, D, and E (P<0.0002 for all sites, a=0.05) were found, as well as statistical difference between Site B and Site C (P<0.0002, a=0.05)(Table 7). The N\textsubscript{2}O flux from day 5 from controls for Site B, C, D, and E also was significantly different from mean control N\textsubscript{2}O flux from Site A (P<0.0002, a=0.05). The N\textsubscript{2}O flux from controls was not statistically different among Sites B, C, D, and E. The apparent lack of similarity between the five sites in N\textsubscript{2}O flux
Table 6. Mean nitrous oxide fluxes (± one standard deviation) for Site A microcosms treated with and without acetylene (C$_2$H$_2$). Means for control microcosms with and without acetylene are determined from all sampling data in the period. Means for low and high nitrate treatments were determined from day 3-5 and day 3-10, respectively. N$_2$O flux within a treatment type with different letters is significantly different at P < 0.05 for the entire sampling period. The number of observations (n) is in parentheses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>September</th>
<th>Sampling Period</th>
<th>March</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_2$H$_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.24±.29a</td>
<td>0.13±.15a</td>
<td>0.22±.25a</td>
<td>0.19±.24</td>
</tr>
<tr>
<td>Low Nitrate</td>
<td>2.86±1.0a</td>
<td>2.34±1.5a</td>
<td>3.52±.84a</td>
<td>2.96±1.2</td>
</tr>
<tr>
<td>High Nitrate</td>
<td>46.6±5.2a</td>
<td>38.9±10b</td>
<td>62.3±22c</td>
<td>49.2±17</td>
</tr>
<tr>
<td>No C$_2$H$_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.03±.04a</td>
<td>0.12±.04a</td>
<td>0.02±.04a</td>
<td>0.05±.06</td>
</tr>
<tr>
<td>Low Nitrate</td>
<td>0.17±.14a</td>
<td>0.23±.12a</td>
<td>0.07±.04a</td>
<td>0.16±.12</td>
</tr>
<tr>
<td>High Nitrate</td>
<td>14.7±9.8a</td>
<td>9.29±11b</td>
<td>23.4±14c</td>
<td>15.8±13</td>
</tr>
</tbody>
</table>
Table 7. Mean nitrous oxide fluxes (± one standard deviation) for day 5 for Sites A, B, C, D and E in the March study with acetylene. (n=3 for Site A control; n=2 for Site B, C, D, and E controls; n=3 for Site A, B, C, D, and E high nitrate treatments). N₂O flux within a treatment type with different letters is significantly different at P < 0.05.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>0.5±0.05a</td>
</tr>
<tr>
<td>High Nitrate</td>
<td>94.1±15a</td>
</tr>
</tbody>
</table>

Nitrous Oxide Emission

Sediment microcosms without the acetylene treatment allowed for the estimation of N₂O production. The theoretical difference in N₂O emission between the controls and the acetylene treated microcosms is the amount of N₂ presumably produced by the sediment. The results show significant differences between the timing and quantity of N₂O produced in these microcosms compared to those treated with acetylene. The N₂O flux data are in Appendix C. The control microcosms without acetylene treatment ranged between –0.05 and 0.15 mg N m⁻² d⁻¹ with a mean N₂O flux of 0.05±0.06 mg N m⁻² d⁻¹ (Figure 11). The N₂O flux in these microcosms was not statistically different between sampling periods (P=0.99, a=0.05).
Figure 11. Mean nitrous oxide emission rates in control microcosms without acetylene for September, 2000, December, 2000, and March, 2001 experiments. Data are in Appendix C.
For low and high nitrate treatments without acetylene, N₂O flux followed a similar pattern to N₂O flux in acetylene treated microcosms (Figures 12 and 13). The mean N₂O flux of the low nitrate treatments between days 3-5 was 0.16±0.12 mg N m⁻² d⁻¹ with a range of 0.08 to 0.31, 0.06 to 0.36, and 0.03 to 0.12 mg N m⁻² d⁻¹ for the September, December, and March studies, respectively. No statistical difference in N₂O flux was found between the sampling periods in the low nitrate treatment microcosms (P=0.99, a=0.05). For the high nitrate treatments, a mean flux of 16±13 mg N m⁻² d⁻¹ was estimated from a range of 1.1 to 28, 0.67 to 25, and 4.7 to 43 mg N m⁻² d⁻¹ for the September, December, and March studies, respectively. The N₂O flux was statistically different between sampling periods (P<0.0001, a=0.05). The N₂O flux without acetylene was always lower than the in acetylene treated microcosms except on day 1 for the high nitrate treated microcosms.

¹⁵N Isotope Technique

The total N flux (¹⁴N and ¹⁵N) from sediment cores was determined by multiplying direct measurements of N₂+N₂O·¹⁵N emission from the cores by 3.1541, which was determined from a ratio of estimates of total N·¹⁵N flux in two samples. A rapid increase of N flux that reached a mean peak of 16±0.39 mg N m⁻² d⁻¹ on day 6 was followed by a slow decline until the end of the experiment (Figure 14). A range from 3.3 to 16 mg N m⁻² d⁻¹ during the time corresponding to the most active period of nitrate removal in acetylene treated microcosms with a mean value of 12±5.1 mg N m⁻² d⁻¹. In the results, however, two anomalous values, 2.5 and 1.8 mg N m⁻² d⁻¹ for day 13 and day 21 for the ¹⁵N isotope technique, respectively, were found that may have been caused by sampling or instrument error. A comparison between the mean
Figure 12. Mean nitrous oxide emission rates in low nitrate treatment microcosms without acetylene for September, 2000, December, 2000, and March, 2001 experiments. Data are in Appendix C.
Figure 13. Mean nitrous oxide emission rates in high nitrate treatment microcosms without acetylene for September, 2000, December, 2000, and March, 2001 experiments. Data are in Appendix C.
Figure 14. Comparison between denitrification potential using the acetylene inhibition technique and $^{15}$N isotope technique. Error bars represent one standard deviation (n=9 for acetylene inhibition technique; n=2 for $^{15}$N isotope technique).
denitrification potential determined by the acetylene inhibition technique and $^{15}$N isotope technique showed a 3-4 fold difference in mean peak values.

**Entrapped $^{15}$N Gases in Sediment**

The gas entrapment study at the end of the $^{15}$N isotope experiment revealed that a significant fraction of $\text{N}_2 + \text{N}_2\text{O}$ produced in the sediment over the 29 days remained trapped in the sediment. The entrapped $\text{N}_2 + \text{N}_2\text{O}$-$^{15}$N determined from two replicates at the end of the experiment was 0.83 and 1.0 mg $^{15}$N. Adding the entrapped $\text{N}_2 + \text{N}_2\text{O}$-$^{15}$N (5.2 mg $^{15}$N m$^{-2}$ d$^{-1}$) to estimates of $^{15}$N flux resulted in a 30-100% increase in $^{15}$N flux values. The maximum $^{15}$N flux increased from approximately 16 mg $^{15}$N m$^{-2}$ d$^{-1}$ to 21 mg $^{15}$N m$^{-2}$ d$^{-1}$. It might be incorrect to assume that these gases became trapped in the sediment uniformly over time, and further study to determine the amount of entrapped gases as they are produced over time would be necessary to apply an accurate correction factor to $\text{N}_2\text{O}$ flux estimates.

Although the entrapped gas study was conducted only with the NO$_3^{-15}$N amended microcosms, small pockets of entrapped gases were also visible in microcosms treated with and without acetylene. The $\text{N}_2\text{O}$ flux results from the acetylene inhibition technique have not included this entrapped gas and, therefore, are acknowledged to be low estimates relative to the actual production of $\text{N}_2\text{O}$ in the sediment. The estimate of the denitrification potential with added entrapped gases, however, would likely fall in the range of one standard deviation from the mean of previously stated results from the acetylene technique.
**Thickness of the Oxidized Layer**

A thin light-brown aerobic layer was apparent in all microcosms after the two-week pre-incubation for September, 2000, December, 2000 and March, 2001 experiments. The bottom boundary of this layer, however, was not clearly visible as it advanced deeper in the sediment profile in many of the microcosms during the September and December experiments, and thus only the measurements in the March study were analyzed (Figure 15).

The thickness of the oxidized layer ranged from 0.4 to 0.6 cm (mean = 0.5 cm) in all replicate microcosms prior to the addition of nitrate. In controls with and without the acetylene treatment, the increases were minimal (mean thickness = 0.6 cm) at the end of 16 days. Similarly, in the low nitrate treatments, the microcosms with and without acetylene treatment had a final thickness of 0.8 and 0.7 cm, respectively. The addition of nitrate in the high nitrate treatment resulted in a rapid increase in oxidized layer thickness until day 10, achieving 1.8 and 2.2 cm in microcosms treated with and without acetylene, respectively. The increases after this initial period were negligible.
Figure 15. Mean thickness of the oxidized layer in sediment microcosms with and without the acetylene treatment. Error bars represent one standard deviation (n=3).
CHAPTER 4. DISCUSSION

The results of the denitrification enzyme assay conducted in static microcosms with the acetylene inhibition technique indicated that sediment from Lake Cataouatche has the potential to denitrify nitrate in the floodwater at nitrate concentrations far greater than typically found in the Mississippi River (~1.4 mg NO3-N l⁻¹). The mean denitrification potential (49±17 mg N m⁻² d⁻¹) estimated from values ranging from 29 to 111 mg N m⁻² d⁻¹ was approximately 16 times greater than the mean denitrification rate in the low nitrate treatment microcosms amended with an initial nitrate concentration similar to that of the Mississippi River (3.0±1.2 mg N m⁻² d⁻¹). A range of 1.7 to 4.5 mg N m⁻² d⁻¹ was found in the low nitrate treatment. Also, compared with the N₂O flux from control microcosms treated with acetylene (0.19±0.24 mg N m⁻² d⁻¹), the denitrification potential was approximately 250 times greater, indicating that denitrification in Lake Cataouatche sediment is primarily limited by the availability of nitrate. The addition of nitrate always stimulated denitrification activity in the replicate microcosms.

Although the acetylene-based methods have been used widely in enzyme activity assays, under certain conditions the acetylene inhibition technique has several limitations (Knowles, 1990). With low concentrations of nitrate, the inhibitory effect of acetylene on denitrifiers may be incomplete and would thus result in a loss of N₂O over a period of a few hours (Oremland et al., 1984). As a result, the estimates of N₂O flux in our controls using the acetylene inhibition technique may only be qualitative. Also, the internal cycling of nitrogen may be interrupted by the inhibition of nitrifying enzymes by acetylene, reducing the flow of nitrate ions to denitrifiers in the anaerobic
Facies  

sediment layer. As nitrification and denitrification may be closely coupled, denitrification rates may be underestimated (Knowles, 1990).

Under prolonged anaerobic incubation (3-5 d), acetylene may be used as a carbon source by denitrifiers and, conversely, stimulate activity to give an overestimate of denitrification (Mosier and Heinemeyer, 1985). The incubation time with acetylene in sealed microcosms in this experiment was approximately five hours, and so it is unlikely that acetylene metabolism had significant effects on N2O flux estimates.

The results of field measurements of denitrification in Lake Cataouatche bottom sediments would be useful data to compare with our laboratory experiments, however, due to resource limitations, no such study was undertaken. Previous field determinations of denitrification rates measured by the production of N2O and N2 in freshwater marshes and adjacent open water bodies in northern Barataria basin measured 0.15 and 0.09 mg N m⁻² d⁻¹, respectively (Smith and DeLaune, 1983b). A laboratory study by Smith and DeLaune (1983a) also reported N₂O+N₂ emission of 0.09 mg N m⁻² d⁻¹ from bottom sediment of a eutrophic freshwater lake in the Barataria basin (Lac Des Allemands). The denitrification estimates observed in the controls of this study are within the range of these values, but the denitrification estimates in our low nitrate treatment microcosms are several-fold higher. Differences in field and laboratory techniques, and natural variation in carbon and nitrate availability are probable factors contributing to the range of these denitrification estimates.

The addition of nitrate to our sediment-water microcosm systems was followed by a rapid removal of nitrate in the floodwater and increases in N₂O emission as a result of denitrification from sediment. Several studies have observed a relationship between
nitrate concentrations and denitrification rates, but the denitrification rates may not keep pace with high nitrate concentrations (Maag et al., 1997). The preliminary investigation for this study showed that the maximum denitrification rate observed with an initial nitrate concentration of 50 mg NO$_3$-N l$^{-1}$ in the floodwater of the microcosm did not significantly increase when the nitrate concentration was doubled. The denitrification potential elicited from the microcosms treated with nitrate to achieve 50 mg NO$_3$-N l$^{-1}$ likely represents the capacity of denitrification in the sediment microcosms. Denitrification potential rates were probably limited by microbial activity and growth, available carbon, or oxygen concentration in the sediment profile.

The denitrification potential estimates, however, are subject to variability depending largely on the method. Rudolph et al. (1991) reported 100% recovery of added nitrate in anaerobic sediment slurries, while recovery was less than 50% in intact cores. This might suggest that incomplete inhibition of the N$_2$O reductase occurred in deeper portions of intact cores using the acetylene inhibition technique as a result of the limited diffusion of acetylene. In this study, the acetylene inhibition technique produced somewhat higher values than for the $^{15}$N isotope technique, and thus it was not apparent that losses of N$_2$O occurred because of incomplete inhibition.

Denitrification in anaerobic sediment slurries also occurs in the entire soil volume, whereas in pre-incubated static microcosms, denitrification activity is typically the greatest at the boundary of the aerobic and anaerobic zone. This is a result of nitrification in the aerobic sediment layer and nitrate diffusion into the sediment column. In these experiments, the static microcosms allowed the advantage of identifying denitrification potential in the context of several other sediment processes,
so that the fate of excess nitrate may resemble processes occurring in the natural environment.

Denitrification potential estimates from the acetylene inhibition technique ranging from 29 to 111 mg N m⁻² d⁻¹ with a mean of 49±17 mg N m⁻² d⁻¹ were 3-4 fold higher than the results from direct measurement of N flux using the ¹⁵N isotope method, which ranged from 11 to 16 mg N m⁻² d⁻¹ with a mean of 12±5.1 mg N m⁻² d⁻¹. Although there was some agreement in the range of these values, the difference between them may be reduced with the use of more replicates. Also, because we used only two measurements of total ¹⁵N flux to estimate total ¹⁴N+¹⁵N measurements for the experiment, the results of the ¹⁵N isotope work may not reflect a true direct measurement of nitrogen gas emission. The calculated maximum thus may be somewhat qualitative in nature. These results, however, are similar to those of Livingstone et al. (2000), where denitrification potentials observed in estuarine sediment slurries with the acetylene inhibition technique and ¹⁵N isotope were in good agreement, but that the acetylene block method gave somewhat higher rates than was observed for the ¹⁵N gas-flux method. However, because the denitrification potentials using the ¹⁵N isotope technique may not have given quantitative results, the comparison between the two techniques was not conclusive.

The investigation of the entrapped ¹⁵N gases in sediment at the end of the ¹⁵N gas sampling showed that over the period of 29 days, approximately one-third of the gases produced in the sediment were not released into the floodwater and atmosphere. This result may imply that the denitrification potential estimated in this study are seriously underestimated. However, because we did not investigate the development of
sediment gas entrapment over time, it is unclear whether a significant portion of the N gases were produced and became trapped during the most active period (day 3-10) over which the denitrification potential was determined. It is possible and likely that the depth of the oxidized layer may have influenced N gas entrapment, since the production of the gases occurred deeper in the sediment core over time, and that the gases produced later became entrapped. The entrapped N gases may have also been formed later in the experiment as a result of coupled nitrification-denitrification from existing ammonium pools deeper in the sediment or from new pools created through the dissimilatory reduction of nitrate to ammonia.

The denitrification potential of Lake Cataouatche sediment was within the range of values reported for other wetlands and open water bodies in the region. Lindau et al. (1988) estimated a peak N₂ flux of 194 µg N₂ m⁻² min⁻¹ (140 mg N m⁻² d⁻¹) from soil spiked with ¹⁵N-enriched NO₃ from a hardwood swamp in northern Barataria basin. Boustany et al. (1997) observed denitrification potentials of 30 to 450 mg N m⁻² d⁻¹ from a hardwood swamp in Terrebonne Parish, Louisiana. Denitrification studies of Big Mar (Caernarvon Diversion) sediment resulted in values as high as 87 mg N m⁻² d⁻¹ (DeLaune, unpublished data). Denitrification potential estimates from open waters, however, are typically lower than in adjacent wetlands in Barataria basin (Smith and DeLaune, 1983b), thus it is not surprising that the denitrification potential of Lake Cataouatche is lower than the reports for wetland areas.

Although the effect of seasonal temperature differences on denitrification potential has been reported in several studies (Gilliam and Gambrell, 1978; Livingstone et al., 2000; Van Luijn et al. 1999), the effects of temperature on the denitrification rates
of Lake Cataouatche sediment may have been partially masked by controlled laboratory temperature during sediment incubation. The ranges of N₂O flux observed in the low and high nitrate microcosms both with and without acetylene, however, are highest for the March study and lowest for the December study, indicating that some seasonal effect contributed to this variation in N₂O emission. The statistical difference in nitrate removal and N₂O flux observed between the March, September, and December studies, therefore, may be due to differences in sediment composition that affected the microbial activity in the surface sediment of Lake Cataouatche. The sampling of the top 15 cm of bottom sediment and subsequent homogenization may not have masked seasonal differences in the accumulation of organic and mineral matter in the surface sediment.

The nitrate removal from the overlying floodwater of the low and high nitrate treatments was rapid, following an exponentially decreasing curve over time. The estimated nitrate removal rates, however, were confounded by the fact that initial floodwater volumes and the evaporative losses were not measured, producing an error of approximately 30% when units were converted from mg N l⁻¹ to mg N m⁻² d⁻¹. The mean nitrate removal rate of 177±25 mg N m⁻² d⁻¹ with a range of 133 to 235 mg N m⁻² d⁻¹ is therefore qualitative. The estimates of nitrate removal in the low nitrate treatment were also qualitative as a result of the limitations of the screening method for nitrate analysis. The primary limitation was high organic matter interference (beyond the recommended 10% for the technique) that produced questionable results after corrections for the interference were made to the nitrate concentration (APHA, 1992). In addition, the screening method used in the nitrate analysis had an experimentally derived detection limit of 0.24 mg l⁻¹, which invalidated much of the data for the
controls and low nitrate treatment microcosms. As a result of the qualitative nature of
the nitrate removal analysis, the denitrification potential measurements using the
acetylene inhibition technique were considered the best estimates for denitrification in
this study.

A portion of added nitrate that was removed from the water column was not
recovered as N₂O in the headspace of the low and high nitrate treatment microcosms.
This fraction of nitrate may have been channeled into several pathways following its
addition into nitrate-limited systems. The initial lag in nitrous oxide production may be
partially attributed to the induction of denitrifying enzymes (Smith and Tiedje, 1979).
Following this phase, the change in nitrate availability probably increased dentrifier
activity, and also may have stimulated growth (i.e., increased number of cells)(Twilley
et al., 1986). Wheeler and Kirchman (1986) found that most heterotrophic bacteria in
marine systems obtain nitrogen from free amino acids and ammonia, but not much from
nitrate. Cooke (1994) found that microbial assimilation accounted for only 5-10% of
added ¹⁵N-labeled nitrate in sediment microcosms in a treatment wetland. Hence, it is
likely that only a small fraction of nitrate was assimilated into the microbial pool.
Rapid diffusion and temporary storage of nitrate into the sediment may also partially
account for the removal of nitrate from the overlying floodwater. Increases in the
oxidized layer also provide greater accumulation of nitrate in the aerobic surface layer
of the sediment.

Nitrate ions also may have been channeled into the dissimilatory pathway to
ammonium. Cooke (1994) found that 35 % of ¹⁵N-labeled nitrate was reduced to
ammonia in unvegetated microcosms in a treatment wetland. Priscu and Downes
(1987) reported a partitioning of nitrate into the dissimilatory reduction pathway to ammonium of 30 to 50% in lake sediment with high carbon to nitrate ratios. It has been suggested that this new ammonium ion pool may be recovered as dissolved ammonia, or in some cases, the primary conversion of nitrate may be to sediment-bound ammonium (Oremland et al., 1984). Under reducing conditions, ammonium ions tend to be in solution, rather than being adsorbed on the cation exchange complex (DeLaune et al. 1981), thus it is conceivable that a new ammonium pool exists as either dissolved ammonium in the anaerobic sediment layer, or adsorbed to mineral matter in the aerobic zone. The subsequent nitrification of the available ammonium in the sediment, coupled with denitrification, may remove this nitrogen fraction from the anaerobic sediment layer.

It is possible to interpret the differences between N$_2$O flux in acetylene treated microcosms and controls (no acetylene) as the quantity of N$_2$ ascribed to sediment production. The timing of nitrous oxide emission peaks, however, were different between microcosms with and without acetylene, and obtaining an estimate of N$_2$ from the ratio of N$_2$:N$_2$O is somewhat questionable. Lindau et al. (1988) also observed that the emission of N$_2$ lagged several days after the detection of N$_2$O fluxes in denitrification chambers in the field. The lag time may be due to differences in the solubility of each gas produced and also to a microbial response to abrupt nitrate additions. N$_2$:N$_2$O ratios during the most active period (days 3-10) of denitrification increased from <1:1 to 35:1. The increase in this ratio has also been noted in other studies (Lindau and DeLaune, 1991). The range of N$_2$:N$_2$O ratio in this study was low compared to those reported for a Barataria Basin swamp (10:1 to 100:1), salt marsh (3:1
and 250:1), and eutrophic lake (Lac Des Allmands)(19:1 to 164:1)(Lindau et al., 1988; Lindau and DeLaune, 1991; Smith and DeLaune, 1983b). The range of N₂:N₂O ratio for Big Mar at the Caernarvon diversion was also considerably larger (1:1 to 245:1)(DeLaune, unpublished data).

It is difficult to isolate the mechanism for changes in the net N₂O flux because nitrous oxide is produced by denitrification, nitrification, and dissimilatory reduction of nitrate to ammonia (Seitzinger, 1990). Net N₂O flux, representing the total production and consumption of nitrous oxide in sediment, is highly variable depending on the concentration of nitrate, carbon supply and the presence of inhibitors. The proportion of nitrous oxide produced by denitrification also is influenced by the pH and temperature of the sediment, as well as by the presence of oxygen and sulfide (Knowles, 1982). Seitzinger and Nixon (1985) also demonstrated that the addition of nutrients (N, P and Si) to flooded sediment systems increases the flux of N₂O. The relationship between eutrophication and N₂O flux, however, remains unknown. Although N₂:N₂O ratios may be important in identifying the major species evolved from denitrification rates, using the N₂:N₂O ratio to estimate denitrification from field measurements of N₂O may not be prudent because of large variations of the ratio produced under various environmental conditions (Weier et al., 1993).

A major difficulty in extrapolating denitrification rates obtained from small cores in the laboratory to larger spatial scales is the extent of spatial heterogeneity that exists in the natural environment. Tiedje (1982) reports that the coefficient of variation (CV) for extrapolation may be in the range of 100%. The CV values were lower for nitrate-amended cores than in unamended cores in a study of artificially flooded
sediment (Davidsson et al., 1996). The permanent uniform flooding of Lake Cataouatche bottom sediment and the uniform addition of nitrate concentrations in our microcosms also may contribute to less variation in denitrification estimates.

The investigation to assess the heterogeneity of Lake Cataouatche bottom sediment was not firmly conclusive, although we found that nitrate removal from the floodwater was similar among the five selected sites. The N2O flux for the five sites measured on day 5 showed that Site A values were significantly higher compared to Sites B, C, D, and E. Some similarity in the mean N2O flux, however, existed among some sites. The high variability of N2O emission over time did not allow good comparison of data of the one day in which headspace gases from microcosms of all sites occurred. More sampling is required over the most active period of nitrate removal to assess the range of denitrification potentials and spatial variability of Lake Cataouatche.

The study of denitrification potential of Lake Cataouatche sediment in its current state (pre-diversion) will be useful for future studies to investigate effects of diversions on denitrification potential over time (years). Similarly, it may be possible to find good reference sediments near the Caernarvon diversion to estimate the effects from the past seven years of water diversion at that site. Determining or predicting the influence of denitrification on diversion impacts to Lake Cataouatche, or Barataria Basin estuary, for that matter, is extraordinarily difficult for the reasons stated throughout the discussion. Without an understanding of competing mechanisms for nitrate removal (i.e., plant and phytoplankton uptake, dissimilatory reduction to ammonia), not only in Lake Cataouatche, but also for brackish and saline environments
down-estuary, we cannot begin to accurately predict the fate of nitrate in the system and associated risks of eutrophication.

The Davis Pond Diversion is capable of replacing the entire volume of Lake Cataouatche in three days at maximum discharge (Swensen and Turner, 1998), and at that volume it is probable that turbidity will reduce nitrate consumption by phytoplankton and transport nitrate down estuary, shifting the principal site for denitrification to Lake Salvador. Recent lawsuits and management practices concerning the effects of the diversion on oyster reefs have forced the diversion discharge at Caernarvon to lower volumes. At a low discharge rate for the Davis Pond diversion, the retention time of water may support greater nitrate removal through denitrification in Lake Cataouatche. While the denitrification potential is estimated to be 24 times greater than estimated denitrification rates using Mississippi River concentrations, the likelihood of reaching the denitrification potential is low considering that the maximum recorded nitrate concentration in the Mississippi near the diversion is approximately 5 mg NO$_3$-N l$^{-1}$ (USACOE, 1982). During spring flooding, however, the sediment of Lake Cataouatche has the capacity to remove increased nitrate inputs through higher denitrification, if the nitrate concentration in the diversion water is also elevated. The transport of increased nitrate load may therefore be reduced, while also lowering the risks of eutrophication through increased nitrate removal. Future studies using a continuous flow apparatus in sediment microcosms to estimate denitrification rates and to determine the partitioning of nitrate into specific pathways under varying environmental conditions are suggested to more accurately estimate the effects of the diversion on Lake Cataouatche.
CHAPTER 5. CONCLUSIONS

Denitrification potential was determined for sediments of Lake Cataouatche with the acetylene inhibition technique and compared with the $^{15}$N isotope technique. An average denitrification potential flux of $49\pm17 \text{ mg N m}^{-2} \text{ d}^{-1}$ with mean peaks of 53, 54, and 94 $\text{ mg N m}^{-2} \text{ d}^{-1}$ and a range from 37 to 55, 29 to 60, and 34 to 111 $\text{ mg N m}^{-2} \text{ d}^{-1}$ occurred in the September, December, and March experiments, respectively. The results from the $^{15}$N isotope technique were 3-4 fold lower than the results with the acetylene technique. The N flux using the $^{15}$N isotope technique had a mean of $12\pm5.1 \text{ mg N m}^{-2} \text{ d}^{-1}$ with a range of 3.3 to 16 $\text{ mg N m}^{-2} \text{ d}^{-1}$. As a result of the limited data obtained from the $^{15}$N isotope technique, the comparison between these two techniques was not conclusive. From the results of the denitrification enzyme assays, we concluded that the Lake Cataouatche sediment has a large potential for denitrification similar to other water bodies in Barataria Basin.

The sediment in Lake Cataouatche also has a large potential for nitrate removal from the water column. The nitrate removal rate estimates, however, are qualitative and may vary as much as 100% at the high nitrate addition rate. This is because a screening method of nitrate analysis was used to measure nitrate concentration and the quality of data was poor from not measuring initial floodwater volumes and evaporation. The estimated nitrate removal rates ranged from 151 to 194, 133 to 178, and 164 to 235 $\text{ mg N m}^{-2} \text{ d}^{-1}$ in September, December, and March studies, respectively, with a mean of $177\pm25 \text{ mg N m}^{-2} \text{ d}^{-1}$ (with 6.3-18.5% error using an assigned estimate of floodwater volume). At the low nitrate addition (1.4 mg NO$_3$-N l$^{-1}$) it was difficult to assign a removal rate since many measurements were below the analytical method detection
limit used. Because these values have a large error, the acetylene inhibition technique is thought to produce the best estimate of denitrification potential in this study. The sediment processes may significantly decrease the movement of nitrate into the lower estuary, with a significant portion of the nitrogen load from diversion water removed from the system. The level of eutrophication, therefore, may be mitigated temporally and spatially. However, nitrate removal in Lake Cataouatche will depend largely on retention time and nitrogen turnover.
REFERENCES


US Army Corps of Engineers. 1982. Louisiana Coastal Area, LA: Feasibility Report on Freshwater Diversion to Barataria and Breton Sound Basins. USACOE NOD.


Appendix A. Mean Nitrate Concentrations (± one standard deviation) in the Floodwater of High Nitrate Treatment Microcosms and Controls for Sites A, B, C, D and E in the March study. (n=2 for controls in Site B, C, D, and E; n=3 in all other treatments and sites). BDL = below detection limits.

Nitrate concentrations (mg NO₃-N l⁻¹)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Site</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>10</th>
<th>13</th>
<th>16</th>
<th>20</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A</td>
<td>0.44±0.08</td>
<td>0.28±0.05</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.34±0.04</td>
<td>0.26±0.04</td>
<td>BDL</td>
<td>BDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.32±0.04</td>
<td>0.24±0.00</td>
<td>BDL</td>
<td>BDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.76±0.08</td>
<td>0.60±0.09</td>
<td>0.42±0.09</td>
<td>0.32±0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.44±0.00</td>
<td>0.29±0.03</td>
<td>BDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.27±0.09</td>
</tr>
<tr>
<td>High nitrate</td>
<td>A</td>
<td>44±3.4</td>
<td>37±3.5</td>
<td>25±2.9</td>
<td>19±3.2</td>
<td>14±3.5</td>
<td>8.8±1.6</td>
<td>4.9±2.0</td>
<td>2.3±1.4</td>
<td>0.6±4.1</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>47±2.2</td>
<td>39±4.1</td>
<td>39±9.1</td>
<td>27±3.8</td>
<td>16±1.9</td>
<td>12±2.4</td>
<td>7.2±.43</td>
<td>4.9±.76</td>
<td>2.5±.48</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>50±1.8</td>
<td>38±.66</td>
<td>31±2.7</td>
<td>26±1.4</td>
<td>18±1.4</td>
<td>13±.41</td>
<td>9.5±1.7</td>
<td>5.4±.55</td>
<td>2.8±.37</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>49±1.9</td>
<td>41±1.6</td>
<td>34±3.4</td>
<td>29±2.0</td>
<td>23±2.5</td>
<td>17±1.6</td>
<td>12±.40</td>
<td>6.8±.15</td>
<td>5.2±.71</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>46±1.2</td>
<td>34±1.1</td>
<td>25±1.4</td>
<td>21±.51</td>
<td>14±.68</td>
<td>8.1±.89</td>
<td>4.6±1.0</td>
<td>2.3±.64</td>
<td>0.6±2.1</td>
</tr>
</tbody>
</table>
Appendix B. Mean Nitrous Oxide Flux (± one standard deviation) from Sediment Microcosms from Site A with Acetylene Treatment. (n=3 for all other treatments).

<table>
<thead>
<tr>
<th>Nitrous Oxide Flux (mg N m⁻² d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Sept</td>
</tr>
<tr>
<td>Dec</td>
</tr>
<tr>
<td>Mar</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Low Nitrate</td>
</tr>
<tr>
<td>Sept</td>
</tr>
<tr>
<td>Dec</td>
</tr>
<tr>
<td>Mar</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>High Nitrate</td>
</tr>
<tr>
<td>Sept</td>
</tr>
<tr>
<td>Dec</td>
</tr>
<tr>
<td>Mar</td>
</tr>
<tr>
<td>Mean</td>
</tr>
</tbody>
</table>
Appendix C. Mean Nitrous Oxide Flux (± one standard deviation) from Sediment Microcosms from Site A without Acetylene Treatment. (n=2 for control and low nitrate treatments in September; n=3 for all other treatments).

**Nitrous Oxide Flux (mg N m\(^{-2}\) d\(^{-1}\))

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>10</th>
<th>13</th>
<th>16</th>
<th>20</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sept</td>
<td>0.04±0.04</td>
<td>0.03±0.00</td>
<td>0.07±0.08</td>
<td>0.05±0.06</td>
<td>0.01±0.03</td>
<td>-0.01±0.04</td>
<td>0.00±0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dec</td>
<td>0.08±0.04</td>
<td>0.14±0.02</td>
<td>0.10±0.03</td>
<td>0.08±0.00</td>
<td>0.10±0.00</td>
<td>0.14±0.08</td>
<td>0.10±0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mar</td>
<td>0.05±0.02</td>
<td>0.01±0.10</td>
<td>0.02±0.01</td>
<td>0.02±0.02</td>
<td>0.01±0.02</td>
<td>0.00±0.02</td>
<td>0.03±0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.06±0.04</td>
<td>0.06±0.08</td>
<td>0.06±0.05</td>
<td>0.05±0.03</td>
<td>0.04±0.05</td>
<td>0.05±0.09</td>
<td>0.05±0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Nitrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sept</td>
<td>0.21±0.07</td>
<td>0.28±0.04</td>
<td>0.05±0.03</td>
<td>0.00±0.02</td>
<td>-0.02±0.00</td>
<td>-0.03±0.01</td>
<td>-0.03±0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dec</td>
<td>0.29±0.04</td>
<td>0.33±0.05</td>
<td>0.14±0.08</td>
<td>0.12±0.08</td>
<td>0.09±0.07</td>
<td>0.09±0.02</td>
<td>0.06±0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mar</td>
<td>0.09±0.03</td>
<td>0.10±0.02</td>
<td>0.04±0.01</td>
<td>0.06±0.01</td>
<td>0.01±0.02</td>
<td>-0.01±0.02</td>
<td>0.04±0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.20±0.10</td>
<td>0.23±0.11</td>
<td>0.08±0.07</td>
<td>0.07±0.07</td>
<td>0.04±0.06</td>
<td>0.02±0.06</td>
<td>0.03±0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Nitrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sept</td>
<td>9.56±1.8</td>
<td>20.6±3.3</td>
<td>21.0±6.7</td>
<td>2.77±1.5</td>
<td>0.71±1.6</td>
<td>0.75±1.11</td>
<td>0.82±0.08</td>
<td>1.48±0.28</td>
<td>0.73±.28</td>
<td></td>
</tr>
<tr>
<td>Dec</td>
<td>10.6±1.9</td>
<td>23.6±2.4</td>
<td>3.40±1.4</td>
<td>0.91±.41</td>
<td>0.95±.08</td>
<td>0.97±.37</td>
<td>0.83±.44</td>
<td>0.75±.37</td>
<td>0.38±.17</td>
<td></td>
</tr>
<tr>
<td>Mar</td>
<td>4.80±1.3</td>
<td>39.5±2.9</td>
<td>24.0±1.7</td>
<td>6.67±1.9</td>
<td>2.68±.91</td>
<td>0.95±.44</td>
<td>0.44±.21</td>
<td>0.08±.06</td>
<td>0.00±.01</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.33±3.1</td>
<td>27.9±9.2</td>
<td>16.1±10</td>
<td>3.45±2.8</td>
<td>1.45±1.0</td>
<td>0.89±.31</td>
<td>0.70±.31</td>
<td>0.77±.65</td>
<td>0.37±.36</td>
<td></td>
</tr>
</tbody>
</table>
Appendix D. Sample Calculations.

**N₂O Flux Calculations**

The headspace gas analysis using the Shimadzu Gas Chromatograph gives N₂O concentration in the units: ppm by volume.

\[
\text{ppm by volume} = \frac{x}{10^6}
\]

\[
\left( \frac{x}{10^6} \right) \left( \frac{1 \text{ L}}{10^3 \text{ ml}} \right) \left( \frac{1 \text{ mol N}_2\text{O}}{22.4 \text{ L}} \right) \left( \frac{44 \text{ g N}_2\text{O}}{1 \text{ mol N}_2\text{O}} \right) \left( \frac{28 \text{ g N}}{44 \text{ g N}_2\text{O}} \right) \left( \frac{10^3 \text{ mg}}{1 \text{ g}} \right) = x' \frac{\text{mg N}}{\text{ml}}
\]

\[
F = \left( \frac{V}{A} \right) \left( \frac{273}{T} \right) \left( \frac{\text{Conc}}{\text{Time}} \right) = \left( \frac{\text{ml}}{\text{cm}^2} \right) \left( \frac{C}{C} \right) \left( \frac{x' \text{ mg N/ml}}{d} \right) \left( \frac{10^4 \text{ cm}^2}{1 \text{ m}^2} \right) = x'' \text{ mg N m}^{-2} \text{ d}^{-1}
\]

**NO₃ Removal Calculations**

Estimated initial floodwater volume = 3.11 x 10⁻⁴ m³
Diameter of microcosm = 7.238 x 10⁻³ m²

\[
\left( \frac{x \text{ mg N}}{L} \right) \left( \frac{1 \text{ L}}{1000 \text{ cm}^3} \right) \left( \frac{10^6 \text{ cm}^3}{1 \text{ m}^3} \right) \left( \frac{3.11 \times 10^{-4} \text{ m}^3}{1} \right) \left( \frac{1}{7.238 \times 10^{-3} \text{ m}^2} \right)
\]

\[= x' \text{ mg m}^{-2} \text{ d}^{-1}\]
VITA

Roy Ryuta Iwai was born in Tokyo, Japan, in 1969. He immigrated to the United States at the age of three and attended elementary and secondary school in Orange County, California. He earned his bachelor’s degree in architecture from University of Oregon in 1992. Upon graduation, he pursued a career in culinary arts, primarily as a sushi chef in Portland, Oregon. Post-baccalaureate education in science at Portland Community College and Portland State University and service in the AmeriCorps with Oregon Trout, a non-profit conservation organization, led to an interest in water quality and wetland ecology. Roy was accepted to the graduate school at Louisiana State University in the fall of 1999, and is a candidate for a Master’s of Science in Oceanography and Coastal Sciences at Louisiana State University.