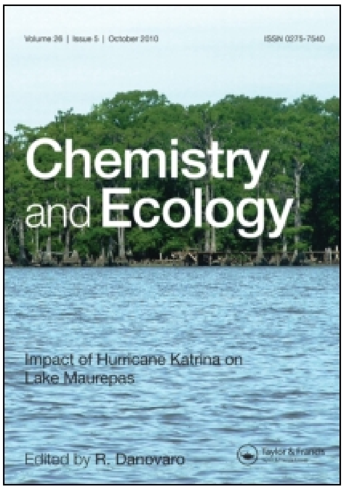

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

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To cite this Article Delaune, R. D. and Jugsujinda, A.(2003) 'Denitrification potential in a Louisiana wetland receiving diverted Mississippi River water', Chemistry and Ecology, 19: 6, 411 — 418
To link to this Article: DOI: 10.1080/02757540310001618820
URL: http://dx.doi.org/10.1080/02757540310001618820

Chemistry and Ecology, Vol. 19(6), December 2003, pp. 411–418

DENITRIFICATION POTENTIAL IN A LOUISIANA WETLAND RECEIVING DIVERTED MISSISSIPPI RIVER WATER

R. D. DELAUNE* and A. JUGSUJINDA

Wetland Biogeochemistry Institute, School of the Coast and Environment,
Louisiana State University, Baton Rouge, Louisiana 70803-7511, USA

(Received 12 May 2003; In final form 27 August 2003)

Excess nitrate in Mississippi River water entering offshore areas is reported to contribute to low oxygen (hypoxia) conditions in the Gulf of Mexico. Excessive algal growth driven by the excess nitrogen results in a decrease in dissolved oxygen in bottom water. Reintroduction of Mississippi River waters into a Louisiana coastal wetland has the potential to reduce the amount of nitrate reaching offshore waters. In this study, reduction in the concentration of added NO_3^- was determined in sediment–water-columns collected from a wetland site in Breton Sound estuary receiving nutrient inputs from the Mississippi River. The capacity of a wetland to process nitrate in floodwater was determined in the laboratory. The rates of NO_3^- removal (determined from change in nitrate concentration in the floodwater) averaged 97 mg $N\,m^{-2}\,d^{-1}$ over 16 d for a 1750-mg NO_3-$N\,m^{-2}$ addition, and 170 mg $N\,m^{-2}\,d^{-1}$ over 16 d for a 3500-mg NO_3-$N\,m^{-2}$ addition. The total N_2O-N emissions from the 1750- and 3500-mg NO_3-$N\,m^{-2}$ additions were 19 and 54 mg $N\,m^{-2}$ accounting for 1.1% and 1.5% of the applied NO_3-N, respectively. Using the acetylene-inhibition technique, the average denitrification rate was determined to be 57 and 87 mg $N\,m^{-2}\,d^{-1}$ (21 and 32 g $N\,m^{-2}\,yr^{-1}$) during the most active denitrification period of 5 d after incubation for 1750 and 3500 mg NO_3^--$N\,m^{-2}$ of added nitrate in floodwater, respectively. The total N evolved over 11 d as $N_2O + N_2$ was equivalent to 436 and 921 mg $N\,m^{-2}$ (24.9% and 26.3%, respectively, of added N). Increasing the amount of NO_3^- applied to the overlying water increased the rate of NO_3^- loss and N_2O emission significantly. The thickness of the oxidized surface sediment layer was also influenced by the NO_3^- application to the floodwater with a significant linear correlation between nitrate addition and thickness of the oxidized layer ($r = 0.9998$, $p = 0.01$). This study indicates that wetlands receiving diverted Mississippi River water have the potential to process and remove NO_3^- in the river water, reducing the amount of NO_3^- reaching to offshore areas.

Keywords: NO_3^- loading; Denitrification; Acetylene blockage technique; NO_3^- reduction; N_2O emission, Mississippi River; Wetland

1 INTRODUCTION

The excess nitrate in Mississippi River waters reportedly contributes to hypoxic conditions, creating dead zones in the north central Gulf of Mexico. It is estimated that several million hectares of wetlands and riparian ecosystems are needed to remove sufficient nitrogen to alleviate the hypoxic zones in the Gulf of Mexico (Mitsch *et al.*, 2001). Annual estimated loads, based on water-quality measurements, indicate that a 40% reduction in total nitrogen

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* Corresponding author. E-mail: rdelaune@aol.com

ISSN 0275-7540 print; ISSN 1029-0370 online
DOI: 10.1080/02757540310001618820

flow to the Gulf is necessary to return to loads comparable to 1955–1970 levels (Mississippi River/Gulf of Mexico Nutrient Task Force, 2001). Proposed approaches to reduce hypoxia in the Gulf of Mexico are to: (1) reduce nitrogen loads from watersheds to streams and rivers in the Mississippi River drainage basin, and (2) restore and enhance denitrification in the Basin and on the coastal plain of Louisiana (Mississippi River/Gulf of Mexico watershed Task Force, 2001).

As a result of restoration efforts to counteract the extensive wetland loss in coastal Louisiana, the state developed a plan for freshwater diversion of Mississippi River water to mimic flood events of the Mississippi River. There have been concerns about the effects of such diversions on water quality in Louisiana coastal wetlands and estuaries. By siphoning, pumping, or cutting through lower levees, diversion projects are moving millions of gallons of water from the Mississippi River into wetlands and estuaries. The infusion of freshwater and sediment supplies essential nutrients, reduces salinity allowing for enhanced vegetation growth, and increases vertical marsh accretion. The Caernarvon structure (see Fig. 1) constructed in 1991, on the east bank of the Mississippi River downstream from New Orleans, has the capacity of passing 226 $m^3 s^{-1}$ to adjacent wetlands in Breton Sound estuary, a 1.100 km^2 area of fresh, brackish, and saline wetlands (Lane *et al.*, 1999). An average of 212 $m^3 s^{-1}$ flowed into the estuary from December 1993 to February 1994. Since then, due to concerns about the effect of decreasing salinity on oyster culture in lower portions of the estuary, flow has generally been less than 50 $m^3 s^{-1}$.

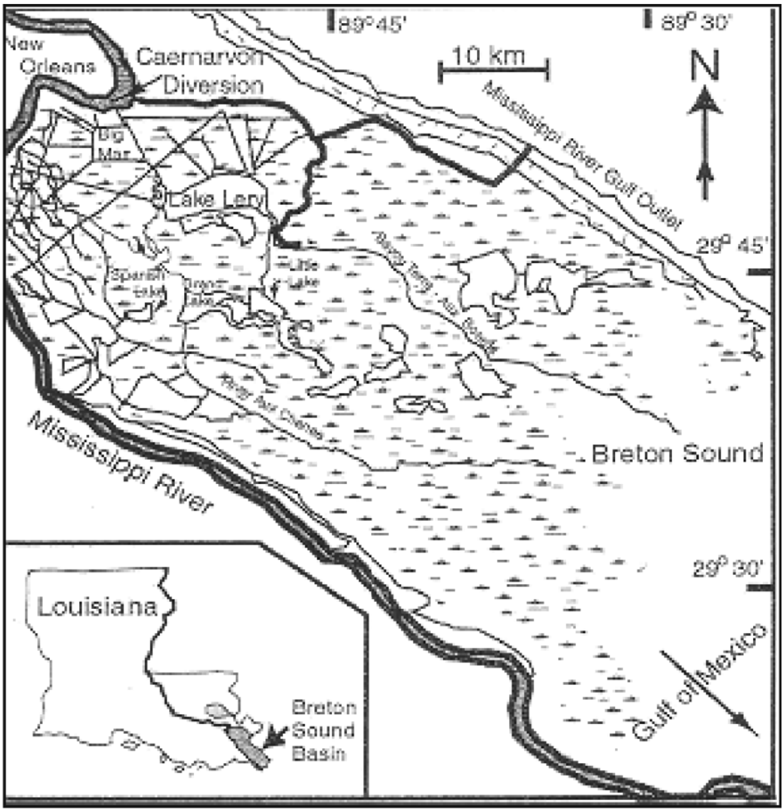

FIGURE 1 Caernarvon Diversion showing Big Mar study site (modified from Lane, 1999).

The amount of nitrate in the Mississippi River has tripled during the past several decades (Turner and Rabalais, 1994). Nitrate-N in the Mississippi River entering the wetland at Caernarvon was between 1 and 1.5 ppm (Lane *et al.*, 1999). Between April 1991 and April 1992, the Mississippi River delivered 900,000 metric tons of nitrates to the Gulf of Mexico (Antweiler *et al.*, 1995). Loading rates of nitrite + nitrate N ($5.6–13.4\,\mathrm{g\,N\,m^{-2}\,yr^{-1}}$) and total nitrogen ($8.9–23.4\,\mathrm{g\,N\,m^{-2}\,yr^{-1}}$) entering the northern portion of the estuary have been documented (Lane *et al.*, 1999). In this study, we quantified denitrification potential at a wetland site (Caernarvon freshwater diversion) receiving nitrogen input from the diverted Mississippi River water.

2 MATERIALS AND METHODS

2.1 Study Site

Caernarvon, about 100 miles south of Baton Rouge, Louisiana (Latitude 29.8227 N, Longitude 89.92175 W), was selected as the location for this study. Surface sediment was collected from Big Mar, an abandoned agricultural site (see Fig. 1). Due to failure of the levee and pumping system, the area has reverted to open water. Freshwater from the Mississippi is currently diverted directly into Big Mar, which drains through a series of channels into marshes located in upper Breton Sound estuary.

2.2 Field and Laboratory Procedures

Fresh sediments collected in June 2000 at six sites from the top 30 cm of the Big Mar Lake (see Fig. 1) were mixed homogeneously, and added into the incubation jars (8.9 cm width × 16.0 cm height) to obtain a 5.0-cm sediment depth. The sediment had a pH of 6.8, organic matter content of 0.43%, P (10 N HCl), Na, K, Ca, and Mg (1 N NH_4OAc, pH 7.0) of 11.84, 11.8, 10, 235, and $30\,\mathrm{mg\,kg^{-1}}$, respectively, and sum of bases of 1.5 meq/100 g dry sediment. The sediment placed in the jars was flooded with lake water collected from Big Mar on the same day as the sediment to establish a floodwater column of 5.0 cm. The water was not stirred but maintained oxygenated. The jars were then covered with aluminum foil (with needle holes) to prevent algal growth and reduce water loss due to evaporation. The holes ensuring an ambient atmosphere was maintained in the jars. The sediment–water-columns were incubated for 12 d to allow for equilibrium at 22 °C.

Following development of a 2–3 mm soil surface oxidized layer at the end of 12 d, KNO_3 was dissolved and applied to the overlying floodwater at an amount equivalent to 1750 or 3500 mg NO_3^--N $\mathrm{m^{-2}}$ of added surface water, respectively. The rate of added NO_3^--N was only a portion of the yearly loading rate of nitrite + nitrate ($5.6–13.4\,\mathrm{gm^{-2}\,yr^{-1}}$) for the upper portion of Breton Sound Estuary (Lane *et al.*, 1999). Each N treatment (including control) had six replicates. Three of the jars were used to study N_2O flux from sediment–water-column and the rate of NO_3^- loss from the overlying water over time. Another set ($n = 3$) was used to estimate denitrification rate using acetylene-inhibition technique. The jars were incubated at laboratory temperature (22 °C). Water lost through evaporation and sampling was replaced by adding deionized water containing no nitrate to maintain 5.0-cm depth.

To determine nitrous oxide emission and nitrate removal, headspace gas and floodwater were sampled for N_2O and NO_3^-, respectively, at 0, 1, 2, 3, 5, 7, 9, 11, 13, and 16 d following the initial application of NO_3^-. To determine denitrification rate, jars with added nitrate were treated with acetylene to block reduction of N_2O to N_2 (Sorensen, 1978). The jar was sealed with a lid containing a rubber septum and 20% C_2H_2 (based on the volume of headspace gas) was injected into the water in the sediment–water columns without shaking to ensure that acetylene came into contact with the active zone of denitrification. Shaking to obtain true equilibrium between water and air phase in N_2O measurements would have impacted measurements of NO_3 disappearance from water column. Therefore, the thin oxidized layer (a zone where both denitrification and nitrification occur) was not disturbed. After sealing the jar with a lid containing a septum for sampling, headspace gas samples were collected at 0, 2, 4, and 6 h. After the acetylene-inhibition measurements were finished, the jars were left open until the next flux measurement. Headspace gas samples were transferred into evacuated glass Vacutainers (Vol. 15 mL) for N_2O analysis. The concentration of N_2O was determined using a Tremitrics 9001 (Austin, TX) gas chromatograph equipped with an electron capture detector, using a column packed with Porapak Q and a 5% methane, 95% Argon carrier gas. Total N_2O content in the water plus gas phases was calculated as described by Tiedje (1982) using the Bunsen absorption coefficient. Denitrification rate was defined as the total N_2O concentration in the water plus gas phases and was expressed as mg $N\,m^{-2}\,d^{-1}$. Following collection of the last gas sampling, 3 mL of water was removed from floodwater for nitrate analysis. Collected floodwater samples were stored in the refrigerator at 4 °C until the NO_3^- measurement the following day. NO_3^- was measured using a Dionex Model 2010I Ion Chromatography System with a detection limit of 0.01 mg L^{-1}. The nitrate removal rate was expressed as mg $N\,m^{-2}\,d^{-1}$.

3 RESULTS

3.1 Denitrification Rate (Acetylene Blockage)

Denitrification rates determined using the C_2H_2 blockage technique are shown in Table I. Rates of denitrification in the control treatment (no added nitrate) were low, remaining relatively stable through the experiment, averaging 0.3 mg $N\,m^{-2}\,d^{-1}$. For 11 d, significantly higher denitrification rates were measured for treatments receiving added NO_3^-. The rates increased with time reaching a maximum of 67 mg $N\,m^{-2}\,d^{-1}$ on day 3 for the 1750-mg NO_3-N m^{-2} treatments and 118 mg $N\,m^{-2}\,d^{-1}$ on day 5 for the 3500-mg NO_3-N m^{-2} treatments. The rates decreased to non-detectable at the end of 16 d incubation (data not shown) in both the low and high N treatments, indicating denitrification or immobilization of the added nitrate. Statistical analysis using Duncan's Multiple Range Test procedure (SAS Institute, 1988) showed that denitrification rates were significantly higher ($p = 0.01$)

TABLE I Acetylene Inhibition Denitrification Rate for Treatments with Low and High Rates of NO_3-N with C_2H_2.

Treatment (with C_2H_2)		*Days of incubation*						
	0	*1*	*2*	*3*	*5*	*7*	*9*	*11*
	Denitrification rate (mg N $m^{-2}\,d^{-1}$)							
Control	0.44 (0.10)	0.00 (0.00)	0.95 (0.17)	0.56 (0.07)	0.28 (0.03)	0.02 (0.08)	0.38 (0.04)	0.03 (0.00)
1750 mg NO_3-N m^{-2}	9.9 (1.31)	53.49 (5.34)	60.71 (7.37)	67.08 (17.93)	48.22 (7.59)	37.90 (7.68)	16.07 (5.54)	4.60 (2.31)
3500 mg NO_3-N m^{-2}	14.1 (0.75)	53.60 (2.32)	67.04 (7.51)	110.43 (14.79)	117.59 (2.20)	83.54 (10.93)	48.19 (0.78)	18.54 (6.51)

Note: Data are mean with standard error in parenthesis, $n = 3$.

in the treatment receiving 3500 mg NO_3-N m^{-2} as compared to the treatment receiving 1750 mg NO_3-N m^{-2}. These results are consistent with the results of Lindau *et al.* (1991), who found in a forested wetland that the fluxes of labeled have significantly higher N_2 from the 300-kg NO_3-N ha^{-1} treatment than the fluxes from the 100-kg NO_3-N ha^{-1} treatment.

Rates of denitrification averaged 57 mg N m^{-2} d^{-1} (approximately 20.8 g N m^{-2} yr^{-1}) for the 1750-mg NO_3-N m^{-2} treatments and 87 mg N m^{-2} d^{-1} (approximately 31.7 g N m^{-2} yr^{-1}) for the 3500-mg NO_3-N m^{-2} treatments over the most active period (3 d). The total N evolved as $N_2O + N_2$ over the sampling periods where significant denitrification occurred was about 436 and 921 mg N m^{-2} (data not shown), which represents 24.9% and 26.3% of the applied NO_3^--N. Estimates of denitrification obtained by measuring evolution of N_2O may be underestimated since several studies suggest that N_2O can be trapped in the soil (Lindau *et al.*, 1988; Katyal *et al.*, 1989) and thus the acetylene-inhibition assay tends to underestimate denitrification.

3.2 NO_3^--Concentration and Removal Rate

NO_3^- concentrations in the control treatments (receiving no added nitrate) remained low and changed little throughout the incubation period, concentrations ranging from 48 to 22 mg NO_3-N m^{-2}. Measured NO_3^- concentrations in floodwater in the 1750- and 3500-mg NO_3-N m^{-2} treatments were 1716 and 3135 mg NO_3-N m^{-2} (see Fig. 2). Concentration of nitrate in the floodwater decreased over time reaching background after 16 d incubation. Regression analysis of the change in NO_3 concentrations over 16 d period indicated NO_3^- reduction rates were 97 and 170 mg NO_3-N m^{-2} d^{-1} for 1750- and 3500-mg NO_3-N m^{-2} treatments, respectively (see Fig. 2). The NO_3^- removal rates are shown in Figure 3. The rates of NO_3^- removal from the water column ranged from 46 to 427 mg N m^{-2} d^{-1} and averaged 169 mg N m^{-2} d^{-1} for the 1750 mg NO_3-N m^{-2} application rate over 16 d. For the 3500-mg NO_3-N m^{-2} treatment, the reduction rates ranged from 131 to 467 mg N m^{-2} d^{-1} with an average of 258 mg N m^{-2} d^{-1} over 16 d. A faster removal rate occurred shortly following nitrate addition, and gradually decreased toward the end of the incubation period. Since maximum denitrification rates were measured on day 3 and day 5 for the low and high N treatments (see Tab. I), this indicates that the rapid decrease in NO_3^- concentration served at the beginning of

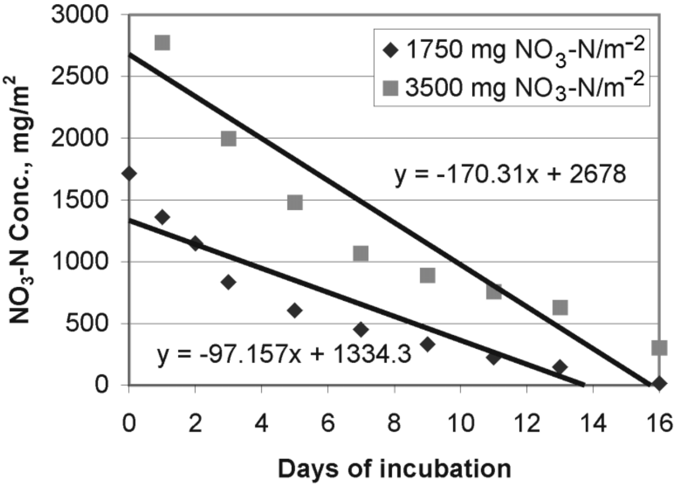

FIGURE 2 Regression analysis of NO_3 reduction in water column following addition of low and high rates of NO_3-N.

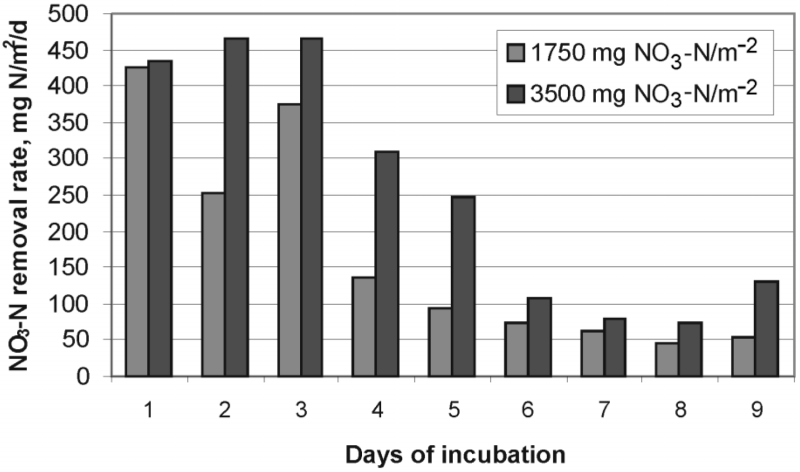

FIGURE 3 NO_3 removal rate from water column after addition of low and high rates of NO_3-N.

the experiment could in part be attributed to diffusion of NO_3^- from water into the soil column following nitrate addition. The removal rates in the high N application were significantly greater than that in the lower N application. It has been shown previously that the higher NO_3^- concentration favors a greater NO_3^- reduction rate (DeLaune *et al.*, 1998). The denitrification rate decreased with time of reduction of nitrate concentration in the flood water. The rates shown for day 9 and 11 (Tab. I) were considerably lowered and may best represent rates for nitrate concentration found in diverted river water.

3.3 N_2O Emission

N_2O emission was also measured in jars receiving no acetylene, and the measured rates of N_2O emission over time following the NO_3^- application are shown in Figure 4. Emission of N_2O from the control treatment was very low or even negative (ranged from 0 to $-0.77\,\mathrm{mg\ N\,m^{-2}\,d^{-1}}$) at sampling points. The low N_2O flux is expected since most of the nitrate is reduced to N_2 which was not measured. The negative flux measurement would suggest that open-water wetland ecosystems consume ambient N_2O and that nitrification–denitrification at the sediment water interface (without added nitrate to the flow water) is low. The average daily N_2O emission–production in this study was estimated to be 0.3 mg $\mathrm{N\,m^{-2}\,d^{-1}}$. N_2O emission from the sediment–water columns increased in both treatments

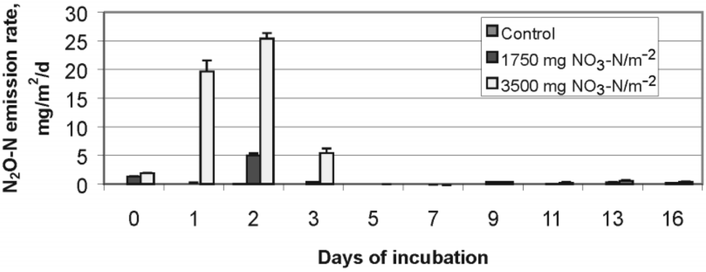

FIGURE 4 N_2O emission rate following addition of low and high rates of NO_3-N (without C_2H_2).

TABLE II Thickness of Oxidized Soil Layer after 16 d of Incubation Following Addition of Low and High Rate of NO_3-N After 16 d of Incubation.

Treatments	*Rep 1 (mm)*	*Rep 2 (mm)*	*Rep 3 (mm)*	*Average (mm)*	*Standard deviation*
Control	3	2.5	3	2.8	0.29
1750 mg NO_3-N m^{-2}	8	8.5	8	8.2	0.29
3500 mg NO_3-N m^{-2}	13.5	13	13	13.2	0.29

receiving added nitrate. N_2O emission rates declined rapidly, reaching undetectable levels after 3 d for both the 1750- and 3500-mg NO_3-N m^{-2} treatments (see Fig. 4). Maximum N_2O emission rate was recorded on day 2 at 20 mg N m^{-2} d^{-1} for the 1750-mg NO_3-N m^{-2} application rate and at 25 mg N m^{-2} d^{-1} for the 3500-mg NO_3-N m^{-2} rate. The total evolved N_2O-N without C_2H_2 added was calculated to be 19 and 54 mg N m^{-2} and accounts for 1.1% and 1.5% of the applied NO_3^--N for the 1750- and 3500-mg NO_3-N m^{-2} treatments, respectively. This demonstrated that the sediment removes significant amount of NO_3^- from water column by denitrification processes but only a relatively small amount of added NO_3^- is reduced to N_2O, an important greenhouse gas emitted to the atmosphere. A portion of the applied NO_3-N was likely immobilized by soil microorganisms.

3.4 Thickness of Oxidized Soil Layer

The application of NO_3^- increased the thickness of the surface oxidized sediment layer (see Tab. II). After the 16 d incubation period, the thickness of the light-brown aerobic layer ranged from 2.8 mm in the control to 8.2 and 13.2 mm for the low and high NO_3^- treatments, respectively. A significant correlation between the application rate of NO_3^- and the thickness of oxidized layer was observed ($r = 0.9998$, $n = 3$, 1% confidence level). Oxidation of organic matter would, with time, allow oxygen to penetrate deeper into the surface layer, oxidizing the reduced iron, creating a lighter color in the top layer and the consumed oxygen leads to an anaerobic zone creating a darker color in the bottom layer.

The effect of NO_3^- application on the thickness of aerobic zone was attributed to the applied NO_3^- diffusing downward into the sediment. The microorganisms utilize the NO_3 as electron acceptors in decomposing the organic matter when O_2 is not available. Generally, the faster uptake of oxygen in the bottom sediments, compared to supply rate from the water column, results in the development of an oxidized surface layer and an underlying reduced layer. Thickness of the oxidized layer is determined by the net oxygen consumption rate. It has been observed that NO_3^- is present in the surface oxidized layer, but cannot be detected in the underlying reduced layer (Reddy *et al.*, 1976; Reddy and Patrick, Jr., 1977). Nitrate diffusing through the oxidized surface layer down into the reduced zone is quickly denitrified.

4 DISCUSSION

There is no simple solution for reducing the nitrogen loads that will cause hypoxia in the Gulf of Mexico. Management strategies such as Mississippi River Diversion into Louisiana coastal zones can contribute to reduction of N loading. Such diversions, in addition to slowing or reversing marsh deterioration, will process and remove nitrate from river water, which would normally be transported to offshore areas. There are thousands of hectares of fresh, brackish, and salt marsh or flood plain wetlands in the Mississippi River Delta Plain, which could be used to processes or remove nitrogen in diverted Mississippi River or Atchafalaya River water. The total area of swamps and marshes and shallow coastal lakes in association with the Mississippi Delta Plain in Southeastern Louisiana is 36,000 km^2 (Mitsch and Gosselink, 1993). Due to leveeing of the Mississippi River, much of Louisiana coastal wetlands do not currently receive Mississippi River water and thus could potentially serve as areas for processing nitrogen in diverted river water.

This study shows that anaerobic conditions existing in Big Mar sediment may provide an ideal environment for reduction of nitrate in Mississippi River water diverted through the wetland. Denitrification accounted for a significant part of the rapid rate of nitrate N loss from the water column. The results suggest that diversions can play an important role in the overall strategy for nitrate removal. The maximum short-term potential denitrification rate found in this laboratory experiment ranged from 21 to 32 $g\ N\,m^{-2}\,yr^{-1}$. Lane *et al.* (1999) found that Mississippi River water entering Brenton Sound estuary had a loading rate of nitrite + nitrate of 5.6–13.4 $g\ N\,m^{-2}\,yr^{-1}$. Our study suggests that the estuary has the capacity to process significant quantities of nitrate, helping to reduce nitrate levels entering the lower portion of the estuary and its offshore areas. Further studies are needed to determine seasonal removal rates at nitrate levels typically found in diverted river water.

Acknowledgements

Funding for this study was provided in part by the Louisiana Water Resource Research Institute and the Louisiana Department of Natural Resources, Coastal Restoration Division.

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