Direct measurement of denitrification activity in a Gulf coast freshwater marsh receiving diverted Mississippi River water

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Abstract

Wetland loss along the Louisiana Gulf coast and excessive nitrate loading into the Gulf of Mexico are interrelated environmental problems. Nitrate removal by soil denitrification activity was studied in a ponded freshwater marsh receiving diverted Mississippi River water for the purpose of reversing or slowing wetland loss. Labeled ^{15}N-nitrate was applied at 3.8 g N m^{-2} into four replicate study plots after removing above ground vegetation. Nitrogen gas (N_2) and nitrous oxide (N_2O) emissions from the plots were determined by isotope ratio mass spectrometry (IRMS). Nitrous oxide emissions were also compared with the results determined by gas chromatograph (GC). Results showed that it took 2 weeks to remove the added nitrate with N_2O emission occurring over a period of 4 d. The apparent denitrification dynamics were assumed to follow the Michaelis–Menten equation. The maximum denitrification rate and K_m value were determined as 12.6 mg N m^{-2} h^{-1}, and 6.5 mg N l^{-1}, respectively. Therefore the maximum capacity for nitrate removal by the marsh soil would be equivalent to 110 g N m^{-2} yr^{-1}, with more than 30% of nitrogen gas evolved as N_2O. For typical nitrate concentrations in Mississippi River water of about 1 mg N l^{-1}, nitrate would be removed at a rate of 14.7 g N m^{-2} yr^{-1} with N_2O emission about 1.5%. A denitrification dynamic model showed that the efficiency of nitrate removal would largely depend on the water discharge rate into the ponded wetland. Higher discharge rate will result in less retention time for the water in the marsh where nitrate is denitrified.
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Keywords: Coastal restoration; Denitrification; Freshwater marsh; Labeled ^{15}N-nitrate; Mississippi River; Stable isotope

1. Introduction

The Mississippi River watershed is the largest in the Unites States comprising mainly of the Ohio, Missouri, and Mississippi Rivers. Anthropogenic activities, primarily agricultural practices, have been introducing large amounts of nutrients into the watershed, which ultimately reach water bodies through the drainage basin. The Mississippi River accounts for about 90% of the freshwater input into the northern Gulf of Mexico (Mitsch et al., 2001). The Mississippi River discharge, containing elevated nutrient levels, is the major cause of coastal eutrophication and hypoxia (water O_2 level <2 mg l^{-1}) documented over the last several decades (Rabalais et al., 1994; Turner and Rabalais, 1994). The Mississippi River levee built for flood control has prevented major course changes, allowing nutrients entering the river to discharge into the Gulf without the buffering effect of coastal wetlands. Levee construction also prevents coastal wetlands from receiving sufficient fluvial sediment to counteract rapid subsidence (Swanson and Thurlow, 1973), nutrients for wetland vegetation growth, and freshwater to prevent saltwater intrusion (Nyman et al., 1990; Turner, 1997). This has ultimately caused Louisiana to experience a significant wetland loss, a pressing environmental problem for the State.

Wetlands are an important sink for nitrogen (N), which can be immobilized by soil microorganisms and wetland vegetation, and consumed by soil denitrification activities (DeLaune et al., 1996; Brodrick et al., 1988). Nitrification

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in soils and coupled nitrification–denitrification on interfaces of soil/water and soil/plant roots are also possible mechanisms of N removal in wetlands. Nitrification activity is probably limited due to anaerobic nature of the inundated soils. Denitrification is the most important mechanism of the N removal by which fixed N in the biosphere, especially in wetland soils, returns to the atmosphere. Nitrogen gas is the major product of denitrification, with N_2O as an intermediate product (Firestone et al., 1980). Nitrous oxide production is of concern because it is an important greenhouse gas contributing to global climate change (IPCC, 2001).

Nitrate concentration in the Mississippi River varies, but is generally in the magnitude of 1 mg N l^{-1} at New Orleans (Battaglin et al., 2001). Diversion of the Mississippi River water into coastal wetlands before discharging to the Gulf of Mexico is a feasible approach currently used for reducing wetland loss in coastal Louisiana. The diversion can also reduce N loading to offshore areas experiencing annually hypoxic conditions associated with excess nutrients. Freshwater diversion can imitate seasonal flooding of the wetlands, supplying nutrients and sediments needed for marsh vegetative growth. In consideration of the related environmental issues associated with greenhouse gases and global warming, it is desirable for such a wetland system to efficiently remove nitrate with minimum N_2O production.

In the design and operation of the Mississippi River diversion project, it is important to know the amount of nitrate that can be removed by wetlands receiving the diverted river water. Nitrogen can be immobilized temporarily by wetland vegetation and soil microbial communities. All major N transformation process can take place in wetland ecosystem, including mineralization, nitrification, denitrification, and possible coupled nitrification–denitrification. Because of the anaerobic environment under the flooded conditions, denitrification is believed the major process that is ultimately responsible for N removal from such an ecosystem. In this study, we have conducted an in situ measurement of soil denitrification activity in a freshwater marsh. The information is needed for quantifying the area of similar wetlands needed to remove specific quantities of nitrate, and the potential amount of N_2O produced from the Mississippi River water diverted into Louisiana coastal wetlands.

2. Materials and methods

2.1. Description of the study site

The study was conducted at a freshwater marsh (located in the northern portion of the Barataria Basin Estuary) receiving water from the Davis Pond Mississippi River Freshwater Diversion Structure, Louisiana USA (N 29°52.16′, W90°14.83′). This is the world's largest freshwater diversion structure and consists of 4 iron gated $4.3 \times 4.3 \text{ m}$ box culverts built into the Mississippi River levee. An inflow channel 163 m long and 26 m wide connects the Mississippi River to the structure. An outflow channel 3400 m long and 37 m wide discharges the diverted water into a 3800 ha ponding area. The structure has a capacity to divert up to $302 \text{ m}^3 \text{ s}^{-1}$ of water (Addison, 1999), and became operational in March 2002. The ponding area is bounded by constructed levees, and is connected to Lake Cataouatche, then Lake Salvador and ultimately to lower portion of Barataria Bay estuary (Fig. 1). The freshwater marsh site was dominated by *Panicum hemitomon schult.*, *Sagittaria lancifolia* L., and *Polygonum* spp. L. Soil samples (top 15 cm) at the study site were collected at the beginning of the experiment, and major soil characteristics were analyzed and are presented in Table 1.

2.2. Experiment setup

The study was conducted in September 2004 during which the air temperature was $27.2 \pm 1.3 \text{ °C}$ (annual temperature range 1.1–30.6 °C, mean 21.1 °C). Four treatment and three control plots that are slightly larger than chamber basal area were established in the freshwater marsh after removal of above-ground vegetation. A static cham-
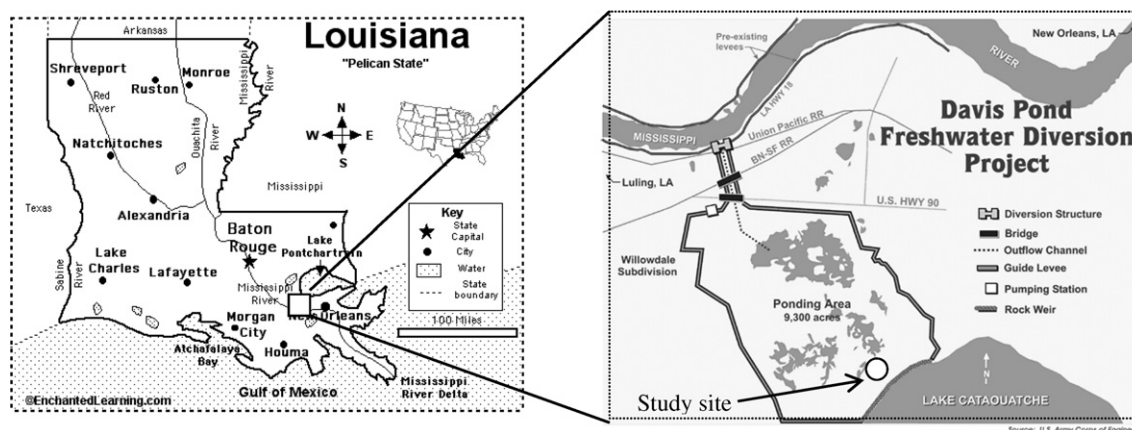

Fig. 1. Study site and sketch map of the Davis Pond Freshwater Diversion Structure.

Table 1
Major characteristics of the soil at the study site

	Total OM%	Total N%	pH	Extractable nutrients (mg kg^{-1})				
				Ca	Mg	P	K	Na
Mean	23.48	2.14	5.45	5719	1734	23.4	291.5	1270
SD ($n = 3$)	1.53	0.03	0.13	281	94	0.5	1.4	157

OM: organic matter.

ber technique was used for measuring N_2O and N_2 emission from the soil surface due to denitrification. The chamber consisted of a base and a top unit (Fig. 2). Both were constructed of Plexiglas with dimensions of $30 \times 30 \times 30$ cm. The base unit of the chamber was inserted 15 cm into the marsh soil. Labeled ^{15}N-potassium nitrate (60 atom% in total abundance) was applied by adding 1 l solution containing 2.5 g N (equivalent to 3.8 g N m^{-2}) into each treatment plot. During each gas emission measurement, the base unit was covered with the top, and sealed with water. For $^{15}N_2O$ and $^{15}N_2$ analysis, 300 ml gas samples were collected 60 min after chamber closure
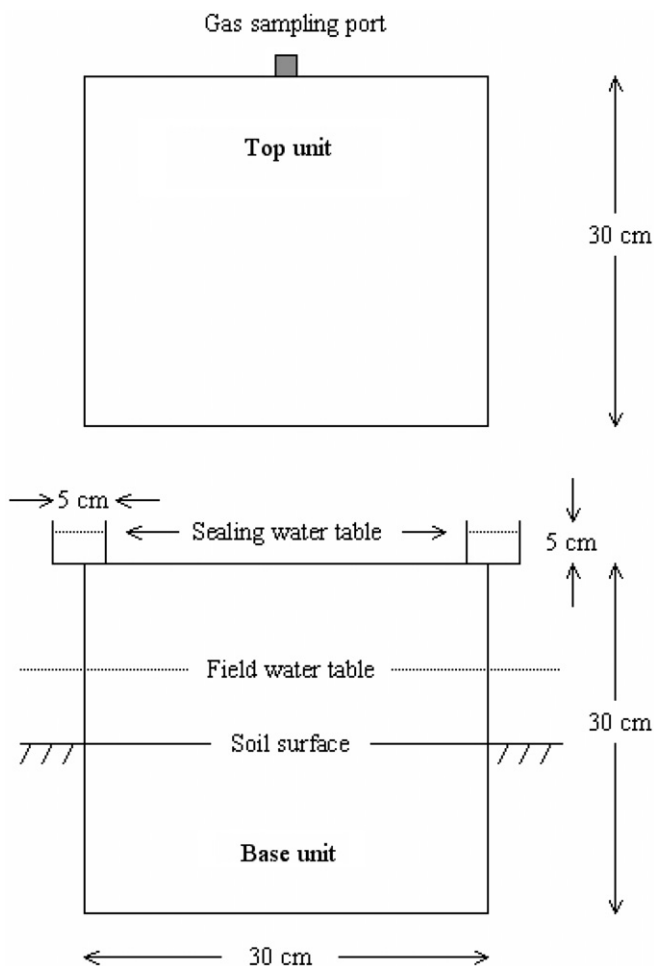

Fig. 2. Side view of the static chamber. The base unit remained in the field during the study period, and the top unit was manually removed after each measurement. During the 1-h measurement, the two units were sealed for gas accumulation by the added water in the collar of the base unit.

and immediately transferred into a 250-ml amber glass vial for over-pressured storage. Gas samples for N_2O-GC analysis were collected from the headspace of the chamber at 0, 30, and 60 min after chamber closure using a 20-ml plastic syringe sealed with a gas tight valve. After the gas samplings, top unit of the chamber was removed and water table inside the chamber base unit was recorded. Then a 50 ml water sample was taken using a plastic bottle from each study plot to monitor changes in nitrate concentration.

2.3. Sample analysis

Total organic matter (OM) was measured colorimetrically after oxidizing with $K_2Cr_2O_7$ and concentrated sulfuric acid. Total N was analyzed in dry combustion by a Leco N analyzer (Leco Corp. St. Joseph, MI). Soil pH was measured in soil–water (1:1) slurry. Extractable nutrients were analyzed by inductively coupled plasma (ICP) after extracting with neutral ammonium acetate solution (for Calcium, Magnesium, Potassium and Sodium), and with Bray 2 solution (for Phosphorus). Nitrate concentration in the water samples were analyzed using a Lachat auto-analyzer (Hach Company, Loveland, CO, USA). Reliable detection limit of the instrument is 0.01 mg N l^{-1}.

Both N_2 and N_2O gas emissions were determined by ^{15}N analysis using a trace gas unit (TGII, PDZ Europa, UK) coupled to an Isotope Ratio Mass Spectrometer (IRMS) (20-20, Sercon, UK). For $^{15}N_2O$ analysis, after cryofocusing and cryotraping, N_2O was further purified on a capillary GC column and then entered into the IRMS that was tuned for mass 44, 45 and 46. Similar procedure was used for $^{15}N_2$ analysis, but cryofocusing and cryotraping was not required, and the IRMS was tuned for mass 28, 29 and 30. Atmospheric N_2 ($\delta^{15}N_2 = 0‰$) and N_2O (atom% $^{15}N_2O = 0.366\%$) were used as a working standard, respectively. Both N_2 and N_2O flux rates were calculated from the enrichment of samples' isotope ratio (^{15}N/^{14}N) that can be derived from δ value (for N_2) and atom% of ^{15}N (for N_2O) of the samples:

$$^{15}\text{N}/^{14}\text{N(sample)} = 0.003675 \times \left(\frac{\delta(\text{sample})}{1000} + 1 \right)$$

$$^{15}\text{N}/^{14}\text{N(sample)} = \frac{\text{atom}\%^{15}\text{N(sample)}}{100 - \text{atom}\%^{15}\text{N(sample)}}$$

Nitrous oxide concentration was also analyzed using a Tremetrics 9001 gas chromatograph (GC) with an electron capture detector (ECD), and calibrated with a certified N_2O standard (Scott Specialty Gases, Inc. Plumsteadville, PA). Detail information on the GC specifications and operation conditions can be found in a recent publication (Yu et al., 2006). Nitrous oxide flux rate was calculated by linear regression of its concentration in the chamber against time. All water and gas analyses were subject to conventional quality control with a standard spike in every three samples, and the standard curve $R^2 > 0.999$.

2.4. Data analysis

Simple linear regression using PROC REG (SAS 9.1, SAS Institute Inc. Cary, NC, USA) was conducted to test if the slope of a regression was significantly different from a theoretical model ($\alpha = 0.05$). A system dynamic model was developed to simulate nitrate removal by soil denitrification activities (STELLA 8, High Performance Systems, Inc.). The model assumed the apparent denitrification process was a single step reaction from nitrate to $N_2O + N_2$, and followed the Michaelis–Menten equation:

$$\text{Denitrification rate} = \frac{V_{max} \times [\text{Nitrate}]}{(K_m + [\text{Nitrate}])}$$

where the denitrification rate was represented by an emission rate of $N_2O + N_2$, and K_m was the nitrate concentration when the reaction was at half maximum rate. Model input parameters, maximum denitrification rate (V_{max}) and K_m value, were determined from this study. Nitrate concentrations between 0.2 and 1.2 mg N l^{-1} were used, representing the typical range in the Mississippi River water. Water level was assumed to be a constant 10 cm above the soil surface. Thus, total nitrate quantity can be calculated by multiplying the nitrate concentration with water volume. Simulation model was run for periods of up to 5 d with 1-h steps using Euler's integration method. The model was run at each selected nitrate concentration, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mg N l^{-1} (inlet nitrate concentration), and N removal rate was determined by calculated $N_2O + N_2$ emissions. The outlet nitrate concentration was calculated by subtracting inlet nitrate concentration with N removal by denitrification over a period of time (1–5 d).

3. Results and discussion

The study site was flooded during the two-week measurement period, except for the last measurement when there was almost no standing water. When flooded, the water table was approximately 10 cm above the soil surface and fluctuated less than 1.5 cm. The nitrate concentrations in the treatment plots declined rapidly during the course of the study.

Table 2 summarizes the analysis results. It is assumed that soil denitrification activities were mainly responsible

for the observed nitrate disappearance in this study since the marsh plants in the plots were removed. At day 0, nitrate concentration was equivalent to 32.8 mg N l^{-1} in the treatment plots after applying labeled ^{15}N-nitrate. Water samples were not available at day 13, because there was no standing water in the study plots. All denitrification products are assumed to come from added ^{15}N-nitrate, because of neglectable amount of background nitrate as seen in the control plots. Enrichment of both ^{15}N–N_2O and ^{15}N–N_2 was detected upon chamber enclosure, indicating immediate denitrification activity following addition of labeled nitrate. Nitrous oxide and N_2 emissions from the treatment plots are presented in Fig. 3. Nitrogen gas emissions, the major products of denitrification, continued for two weeks. Nitrous oxide emission lasted for 4 d, subsequently it was undetectable by the GC method, and no significant ^{15}N enrichment could be found by the IRMS method (Table 2). The results indicate there was no significant difference in N_2O emission rates determined by IRMS and GC methods as seen in Fig. 3 ($P = 0.89$, $n = 5$). The N_2/N_2O ratio ranged from 2.5 on day 1 to 80.5 on day 6. A linear regression showed a significant ($n = 4$, $P < 0.01$) negative relationship between the N_2/N_2O ratio and nitrate concentration in the treatment plots (N_2/N_2O ratio = $-2.9 \times [\text{Nitrate}] + 71.3$, $R^2 = 0.94$). The cumulative emission rate of N_2O and N_2 decreased rapidly as did the nitrate concentration in the treatment plots.

Based on the emission rates of $N_2O + N_2$, and observed nitrate concentrations in the treatment plots, the V_{max} and K_m value were determined, using the best fit approach, as 12.6 mg N m^{-2} h^{-1}, and 6.5 mg N l^{-1}, respectively. At the beginning of the study, the nitrate concentration in the treatment plots was much higher than the K_m, thus the initial denitrification rate was close to its maximum rate (Fig. 3). The calculated denitrification rates based on the

Table 2
Results of nitrate measurement in the plots and isotope analysis of the gas samples

Days after nitrate addition	Nitrate (mg N l^{-1})		$\delta^{15}N_2$	atom% $^{15}N_2O$
	Control	Treatment		
Day 1	0.04 ± 0.08	25.62 ± 1.91	2.70 ± 0.84	1.45 ± 1.15
Day 2	<0.01	27.56 ± 1.87	3.18 ± 0.95	1.02 ± 0.11
Day 4	<0.01	4.37 ± 3.77	2.36 ± 0.29	0.41 ± 0.01
Day 6	0.05 ± 0.08	0.05 ± 0.08	0.50 ± 0.81	0.38 ± 0.01
Day 13		0.02 ± 0.03	0.38 ± 0.03	

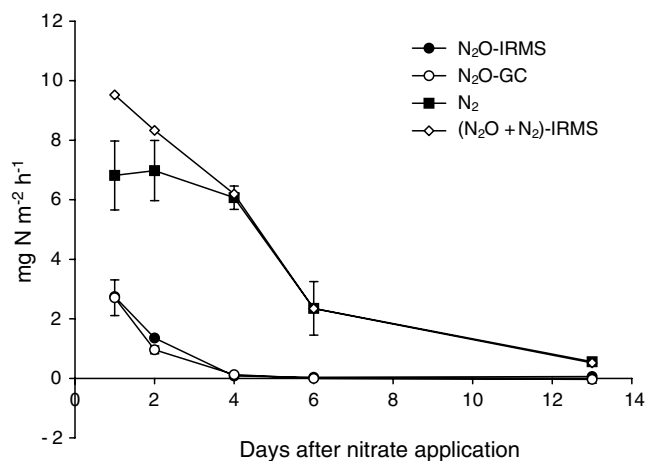All data are expressed as mean ± SD.

Fig. 3. Denitrification activities following ^{15}N-nitrate addition. The error bars denote the standard deviations of the replicate measurements. The experiment was designed to measure a large range of denitrification rate in order to better estimate the two inherent denitrification parameters (V_{max} and K_m).
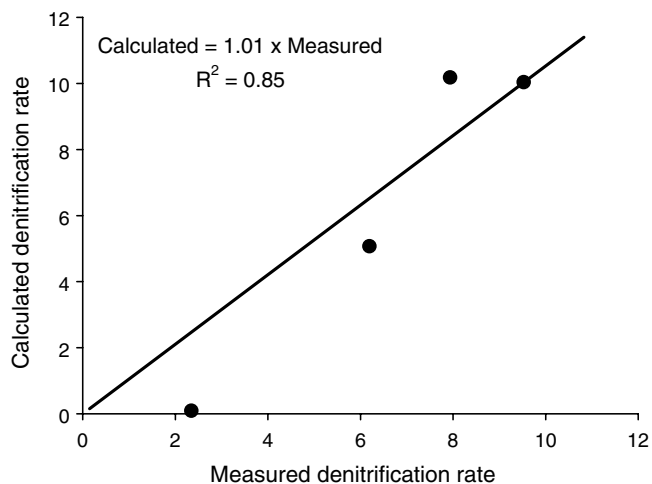
Fig. 4. Comparison of measured and calculated denitrification rates (based on the estimated V_{max} and K_m, as well as the measured in situ nitrate concentrations). The regression showed no significant difference ($P = 0.36$, $n = 4$) between the calculated denitrification rate and the measured denitrification rate.

obtained V_{max} and K_m values were compared with the actual measured rates (Fig. 4). The results showed that the calculated denitrification rates were not statistically different from the measured rates ($P = 0.36$).

If the marsh received water containing elevated concentrations of nitrate, the maximum capacity of nitrate removal by the soils would be about 110 g N m^{-2} yr^{-1}. Maximum denitrification rate reported in a greenhouse study using a freshwater marsh soil was 100 and 165 g N m^{-2} yr^{-1}, when nitrate was added at 10 and 30 g N m^{-2}, respectively (Lindau et al., 1991). In a sediment core study using a saltmarsh soil, maximum denitrification rate was found to be 50 g N m^{-2} yr^{-1} with nitrate addition of 10 g N m^{-2} (Lindau and DeLaune, 1991). Nitrate removal by the system would be more significant under elevated nitrate conditions, because of higher denitrification rate. However, higher levels of nitrate introduce more competition for electrons from soil organic matter, favoring smaller N_2/N_2O ratio in the end products of denitrification (Blackmer and Bremner, 1978). The first measurement (day 1) after nitrate addition showed a N_2/N_2O ratio of 2.5. If denitrification took place under even higher nitrate conditions, the N_2/N_2O ratio might be smaller than 2.5 due to negative relationship between the N_2/N_2O ratio and nitrate concentration. It can be expected that the higher the nitrate concentrations, the larger the percentage of N_2O in denitrification products. It was estimated that N_2O emission would account for at least 30% of the denitrified N when the marsh soil reached its maximum denitrification rate (as happened in the first day after adding nitrate in this study). The results suggest the importance of management practices to balance the N removal capacity of wetlands and other environmental effects (such as N_2O emission).

The Davis Pond Freshwater Diversion Structure enables a portion of the Mississippi River water to enter Barataria Basin of Louisiana. The diverted Mississippi River water contains elevated concentrations of nitrate and other nutrients from the upper reaches of the Mississippi River drainage basin (Battaglin et al., 2001). The elevated nitrate concentrations raise concerns regarding possible eutrophication in the downstream estuaries (Rabalais et al., 1996; Donner, 2003). A monitoring study showed that nitrate concentration in the diverted Mississippi River water passing through the ponded wetland ranged from 1.2 to 0.8 mg N l^{-1} (Johnson, 2004). As shown by this study, for a typical nitrate concentration of 1 mg N l^{-1}, the removal rate by the marsh would be 14.7 g N m^{-2} yr^{-1}. Nitrous oxide emission would account for less than 1.5% of the total denitrified N (based on the N_2/N_2O ratio 68.4 calculated from the regression equation). Under low nitrate conditions, soil N_2O emissions are low or even negative (consumption of atmospheric N_2O, the final step of denitrification process where N_2O is used as an electron acceptor). In this study, the control plots actually functioned as a minor sink of atmospheric N_2O with an average N_2O flux rate of -0.01 mg N m^{-2} h^{-1} (data for each measurement were not shown). Therefore, the small amount of N_2O emission from the wetland receiving diverted Mississippi River water could be partially compensated by the consumption of atmospheric N_2O during the period without diverted water flow to the wetlands.

Temperature plays a critical role in denitrification rate. The Q_{10} value (the increase factor of a biological reaction rate when temperature increases by 10 °C) for denitrification varies for different soils and in different temperature ranges. Peterjohn (1991) reported a Q_{10} value of 1.74 in a desert soil with optimal conditions for denitrification being pH 7.0 and temperature 40 °C. Ambus (1993) reported the Q_{10} value for denitrification was 2.71 and 2.53 for temperature range of 2–12 °C and 12 to 22 °C, respectively. Assume the Q_{10} value was 2.0 for denitrification activity in the studied marsh soil, the effect of temperature on denitrification can be characterized as: Ln (denitrification rate in g N m^{-2} yr^{-1}) = $0.07 \times T$ (°C) + 0.80. Thus, the denitrification capacity of this study site can seasonally vary between 2.4 and 18.9 with a mean value of 9.8 g N m^{-2} yr^{-1} (at 21.1 °C). The results will provide an important guidance for operating the diversion project to maximize its performance.

Based on the two basic parameters of denitrification (the V_{max} and K_m) determined in this study, a simple denitrification model was developed that simulated the dynamics of nitrate removal by the marsh soil. The results indicated that the retention time of water in the ponded freshwater marsh is critical to nitrate removal efficiency (Fig. 5). In order to achieve a specific nitrate reduction objective, the diverted water must be discharged at rates capable of providing sufficient time for the marsh soils to denitrify the nitrate. There was only a slight difference in nitrate removal efficiency with different water retention time among the simulated six concentrations of nitrate. In the order of water retention time in the marsh from 1 d to 5 d, nitrate
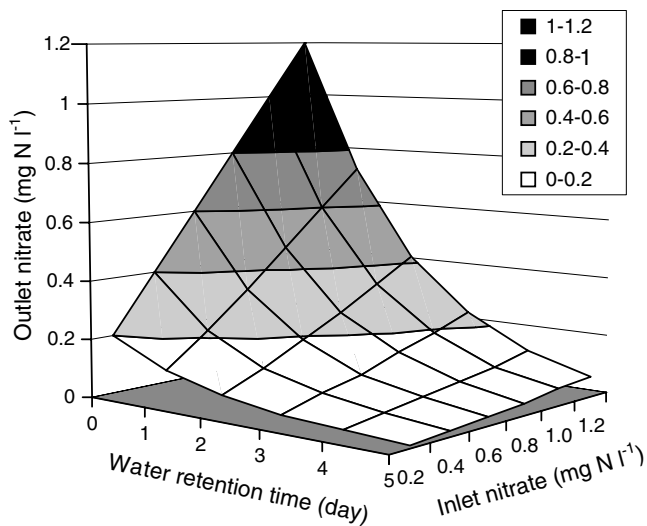
Fig. 5. Simulation results of nitrate reduction with time when the Mississippi River water is diverted into the marsh. Only soil denitrification activity was included in the simulation model. Outlet nitrate concentration = Inlet nitrate concentration − denitrified nitrate in the same volume of water.

removal efficiency would be $42 \pm 2.5\%$, $68 \pm 2.1\%$, $82 \pm 1.8\%$, $90 \pm 0.3\%$, and $95 \pm 0.5\%$, respectively (the standard deviation represented the variations using the six simulated nitrate concentrations). In other word, nitrate removal efficiency (percentage of nitrate removal) was independent of nitrate concentration in the water, but on water retention time in the wetland that is controlled by the discharge rate of the diverted river. On site monitoring studies showed that a low pulse of $25\ m^3\ s^{-1}$ in April 2003 resulted in 96% reduction in nitrate load when the water passed through the marsh, and 48% reduction with a higher pulse discharge rate of $78\ m^3\ s^{-1}$ in December 2003 (Johnson, 2004). The relative poor nitrate removal performance in December 2003 was partially caused by higher discharge rate that resulted in less time for the water to be retained in the wetland. In addition, seasonal temperature effects on denitrification rates as well as nitrate immobilization by marsh vegetation and soil microorganisms also contributed to the observed difference in nitrate removal rates. As a result the actual nitrate removal may be somewhat different than that reported in this study, since plants can compete with denitrification for inorganic nitrogen (Hoagland et al., 2001).

This is the first labeled denitrification study trial in this area. Uncertainty in operational time of the structure and the high cost of stable isotope analysis limited the frequency of measurement. Seasonal measurements may provide parameters to cover seasonal effects (e.g. temperature variation) on soil denitrification activity, which will help to construct a more accurate model for better estimating annual N removal by the marsh soil. Additional research is needed to address the long-term efficacy of such a wetland for nitrate removal by soil denitrification activities. With accumulation of soil organic carbon, denitrification

activities may increase. Higher denitrification rates supported by higher electron availability is generally associated with higher N_2/N_2O ratio (Betlach and Tiedje, 1981; Masscheleyn et al., 1993), which is beneficial in reducing N_2O emission to the atmosphere. Carbon sequestration and methane (another greenhouse gas) production and emission in the wetlands also deserve careful evaluation since N loading will affect the system's carbon cycle. This study represented a step in the process of developing an integrated ecological and environmental protocol for managing a river system for coastal restoration. Integrated research provides information necessary for developing realistic models that serve as a tool for the management of coastal ecosystems.

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