Wetlands
DOI 10.1007/s13157-014-0521-6

ARTICLE

Nitrous Oxide Emissions from Soils in Louisiana's Atchafalaya River Basin

Amy E. Scaroni · Siyuan Ye · C. W. Lindau · J. A. Nyman

Received: 20 September 2013 / Accepted: 6 February 2014

Abstract The Atchafalaya River Basin functions as a net sink for nitrogen delivered by the Mississippi and Red Rivers. Continued influx of river-borne sediment drives rapid habitat change on the floodplain, and lakes are transitioning to seasonally flooded baldcypress swamps and bottomland hardwood forests. As flooding regimes change, leading to more intermittent wet and dry cycles, soil conditions become more conducive to incomplete denitrification resulting in increased N_2O emissions. The objective of this laboratory study was to determine if sediments in the Basin are potential sources of N_2O emissions, and if emission rates differ among habitats types. Results indicate that potential N_2O emission rates are low, and differ by habitat type. Lake sediments had the highest potential denitrification rates (53.68 g N_2-N ha^{-1}d^{-1}), while baldcypress sediments had the highest potential N_2O emissions (2.31 g N_2O-N ha^{-1}d^{-1}). Unflooded bottomland hardwood sediments had very low denitrification potential and were not a source of N_2O emissions, but emissions of both N_2 and N_2O increased after bottomland hardwood sediments were flooded. These results suggest that overall N_2O emissions are low, but management strategies aiming to improve water quality and reduce greenhouse gas emissions should consider each habitat separately when modeling nitrogen removal within large floodplains.

Keywords Denitrification · Floodplain · Nutrients · Nitrogen · Nitrous oxide · Wetland · Habitat

A. E. Scaroni (✉)
University of Maryland Sea Grant Extension, Wye Research and Education Center, 124 Wye Narrows Drive, PO Box 169, Queenstown, MD 21658, USA
e-mail: ascaroni@umd.edu

S. Ye
Key Laboratory of Coastal Wetland Biogeosciences, China Geologic Survey, Qingdao Institute of Marine Geology, QingDao 266071, China

C. W. Lindau
School of the Coast and Environment, Louisiana State University, Baton Rouge, LA, USA

J. A. Nyman
School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA, USA

Introduction

The literature contains many studies of denitrification in various habitats, but there are few studies specifically comparing differences due to habitat or ecosystem type. Those that do often depend on an extensive literature review and use the results of separate studies to make comparisons (Pina-Ochoa and Alvarez-Cobelas 2006; Seitzinger 1988). While this has advanced our understanding of factors leading to spatial variability in denitrification rates, it can be difficult to compare across habitat and ecosystem types when there are differing methodologies, analysis techniques, and seasons and frequency of sampling.

Saunders and Kalff (2001) compared differences in nitrogen retention in wetlands, lakes and rivers. Their intent was to determine which removal process (denitrification, sedimentation, or plant uptake) was the primary pathway for nitrogen retention/removal within each of these ecosystem types. Data were collected from previously published mass-balance studies, and denitrification was identified as primary pathway for nitrogen removal. The authors' ultimately attributed the differences in nitrogen retention among wetlands, lakes and rivers almost entirely to differences in water discharge (Saunders and Kalff 2001).

A study of landscape scale denitrification in forest soils identified a strong relationship between denitrification rates and soil texture and drainage properties (Groffman and Tiedje

Published online: 25 February 2014

1989). Pinay et al. (2000) reported a significant relationship between denitrification rates and soil texture in floodplain soils; fine textured soils had higher rates of denitrification. Harms et al. (2009) detected greater rates of denitrification potential at a wet floodplain site than a dry one.

Denitrifying microorganisms can permanently remove nitrogen by mediating the reduction of nitrate to nitrogen gas (Seitzinger 1988; Pina-Ochoa and Alvarez-Cobelas 2006; Rivera-Monroy et al. 2010). The denitrification reaction occurs under anaerobic conditions in the presence of a carbon source and a supply of nitrate. When nitrate diffuses into the anaerobic zone in the sediment, it can be incrementally reduced to nitrogen gas via the denitrification pathway ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$). However, environmental conditions are not always conducive to completion of the pathway. Incomplete denitrification releases nitrous oxide (N_2O), which is favorably produced in well-drained, aerated sites with course textured soils (Groffman 1991). Low temperature, low pH, and the presence of O_2 all favor the production of N_2O relative to N_2 (Knowles 1982; Granli and Bockman 1994; Stevens et al. 1998). As soil water content increases, thereby decreasing oxygen content and lowering the redox potential (Eh) of the soil, the ratio of N_2: N_2O emissions increases. It follows that water filled pore space may be the best predictor of the production and emission of N_2O from soils (Granli and Bockman 1994). Alternating wet and dry cycles, commonly seen on floodplains, tend to produce higher N_2O emissions (Granli and Bockman 1994), because the soil may be less waterlogged. Because flooding affects these soil characteristics, soil moisture and soil management schemes will affect emission ratios (Ullah et al. 2005). At the ecosystem scale, the emission ratio of N_2:N_2O is of concern because riparian wetlands and freshwater marshes can produce substantial quantities of N_2O (Burt et al. 1999; Kroeze et al. 2005; Swarzenski et al. 2008). N_2O is a gas with a high global warming potential (IPCC 2007), so understanding the factors controlling N_2 vs. N_2O emissions is essential to minimizing greenhouse gas emissions from natural and restored freshwater wetlands.

Past research has advanced our understanding of factors leading to spatial variability in denitrification rates, and provided estimates for actual and potential denitrification in treatment wetlands, freshwater ecosystems, estuaries, groundwater, and even the ocean. Denitrification is not the sole process mitigating excess nitrogen in a floodplain; sedimentation and biomass assimilation also remove nitrogen from water and soil. However, denitrification is the only permanent removal process and is likely the dominant process for removal of river-borne nitrogen (Howarth et al. 1996). The authors have previously characterized rates and spatial variability of denitrification in floodplain habitats of the Atchafalaya River Basin (ARB) in South Louisiana (Lindau et al. 2008; Scaroni et al. 2010, Lindau et al. 2011; Scaroni et al. 2011). Scaroni et al. (2011) showed that the three major habitats differ in their effectiveness at nitrogen removal via denitrification. Notably, intermittently flooded habitats had lower rates of denitrification potential than permanently flooded habitats. Because habitat change in the Basin is being driven by sedimentation, these results have implications for Basin management. Originally managed for flood control and navigation, water management projects now aim to also improve water quality and internal circulation, and address sediment reduction, removal, and diversion (FY2011 Atchafalaya Annual Basin Plan).

Attempts to model the possible outcomes of different water management regimes have been limited by lack of site-specific data. Combined with the data from previous work in the Basin, the baseline data from this study can generally indicate whether future management strategies for the ARB that result in shifts in habitat type could also result in enhanced N_2O emissions. Because differences in the degree of inundation leads to differences in the potential of sediments to complete the denitrification pathways, we hypothesize that intermittently flooded habitats will release more N_2O relative to N_2, i.e. have lower N_2:N_2O ratios. We designed an experiment to determine (1) if incomplete nitrate reduction is a substantial source of N_2O emissions in the ARB, and (2) if N_2O emission rates differ among habitat types.

Study Site

The Atchafalaya River is regulated to be 30 % of the combined flow of the Mississippi and Red Rivers. Not only does this make the Atchafalaya River the largest distributary of the Mississippi River, at nearly 5,000 km^2, the ARB is the largest deepwater swamp in North America. As the Atchafalaya River enters the ARB, the distance between levees increases to at least 24 km, which widens the floodplain and provides increased opportunity for nutrient transformation and removal. Recent work indicates that the ARB retains up to 14 % of the total nitrogen entering the system from the Mississippi and Red Rivers (Xu 2006).

The hydroperiod varies greatly across the floodplain; some areas rarely flood, while others are constantly inundated. Rapid sedimentation has transformed the ARB from a lake-dominated floodplain into a system that is nearly 70 % forested (bottomland hardwood habitat) (Hupp et al. 2008). Estimates by Faulkner et al. (2009) of total baldcypress swamp area in the ARB translates to approximately 19 %, which leaves 11 % as lake habitat, and sedimentation continues to convert lakes into swamps and forested bottomlands (Hupp et al. 2008). As surface elevations increase, connectivity between the river and floodplain is reduced. At the same time, continued influx of nutrients derived from upstream activities fuel a seasonal area of hypoxia downstream in the Gulf of Mexico.

Methods

Sample Collection

To estimate variability in N_2:N_2O emission ratios within and among habitats we used the same nine sites that were randomly selected for a previous study (Scaroni et al. 2011): three bottomland hardwood forests (BLHW) sites, three baldcypress swamp sites, and three lake sites (Fig. 1). The BLHW, baldcypress, and lake sites were 75, 40, and 25 km upstream from the mouth of the Atchafalaya River, respectively. Sites within habitats were separated by at least 2 km but not more than 15 km.

Sediment samples were collected from these nine sites during June and July of 2009. For the BLHW sites, which were dry during sampling, we used a hand shovel to sample the top 15 cm. Sediment samples from the rest of the sites, which were flooded during sampling, were collected with an Eckman Dredge. Immediately upon collection, samples were sealed in 4-L wide-mouth heavy duty polyethylene (HDPE) bottles, put on ice, and transported to the laboratory where they were stored at 2 °C. Subsamples were removed and analyzed for total nitrogen (N) and total carbon (C) using a Leco C-N Analyzer (Leco Corp., St. Joseph, MI, USA), total phosphorus (P) using an Inductively Coupled Plasma (ICP) spectrophotometer after ashing and HCl digestion, particle size using the Hydrometer Method (Gee and Bauder 1986), salts using a conductivity meter, and pH using a pH meter (Table 1). Soil moisture was determined on a dry weight basis, and soil organic matter was determined by loss on ignition at 550 degrees for 2 h.

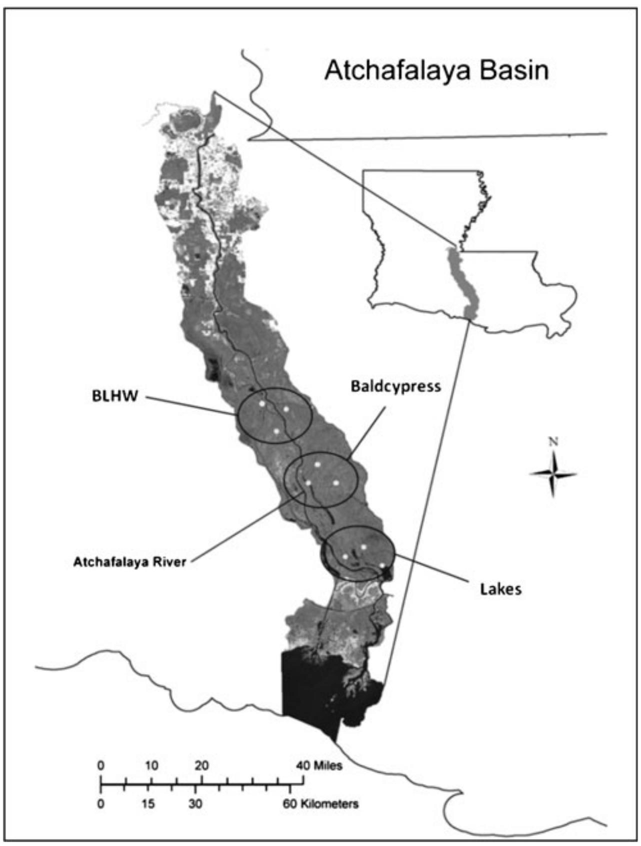

Fig. 1 Atchafalaya Basin and location of sampling sites

Laboratory Experimental Set-up

For our experimental set-up, we established two full sets of microcosms (for each set, n=24) (Fig. 2). Within each set we established duplicate microcosms for baldcypress samples (3 sites×2 microcosms per site) and lake samples (3 sites×2 microcosms per site), and we established four microcosms for each BLHW sample (3 sites×4 microcosms per site). Our intention in doubling the number of BLHW microcosms was to flood two microcosms per BLHW site, and to leave two microcosms per BLHW site unflooded (to simulate field conditions upon collection and to account for the pulse of denitrification generally seen upon rewetting). Each microcosm (glass incubation jar with diameter=9 cm) received approximately 576 g (5 cm) of sediment, which was compacted to vent entrapped gas. Next, all baldcypress and lake microcosms, and half of the BLHW microcosms (two microcosms per site) were flooded with approximately 240 ml (4 cm) of nitrate-free deionized water, while the remaining BLHW microcosms (two microcosms per site) remained unflooded.

Jars were wrapped in foil and covered with tissue paper to discourage growth of plants and microbes. The microcosms sat for approximately 2 weeks under these conditions to allow for equilibration. During this time flooded sediments developed an observable oxidized layer at the sediment-water interface as was seen in the field. Initial soil moisture content (for the unflooded BLHW cores) was roughly maintained throughout the experiment. Despite unavoidable disturbance to cores in the lab, prior research has shown good agreement between results from in situ and laboratory denitrification studies (Well et al. 2003).

Denitrification Measurements

For the first round of measurements, in order to measure background N_2O emission rates, neither set #1 nor set #2 received added nitrate (NO_3-N) or acetylene. For the second round of measurements, all microcosms in set #1 and set #2 were amended with 3 mg NO_3-N L^{-1} (low treatment) to simulate slightly elevated nitrate loading rates. For the third round of measurements, we established new microcosms in the same manner as above, and then all microcosms in set #1 and set #2 were amended with 5 mg NO_3-N L^{-1} (high treatment) to simulate elevated nitrate loading rates.

Table 1 Sediment characteristics (total carbon, total nitrogen, % organic matter, phosphorus, salts, pH, % sand, % silt, % clay, and % moisture) from nine sites sampled in the Atchafalaya River Basin. Subsamples from each site were taken prior to the experiment and sent to a laboratory for analysis

Site	Habitat	Total C (%)	Total N (%)	OM %	P (ppm)	Salts (ppm)	pH	Sand %	Silt %	Clay %	% Moisture
1	BLHW	1.8	0.1	3.4	14.5	330.2	6.7	39.4	50	10.6	16.7
2	BLHW	3.5	0.2	4.9	19.2	632.3	7.4	31.5	52.9	15.6	27.5
3	BLHW	3.1	0.2	4.4	44.4	468.5	6.6	26.5	60.7	12.8	24.7
4	CYP	8.9	0.6	7.6	15.7	673.3	6	13.3	29.5	57.2	68.9
5	CYP	9.3	0.6	7.5	14.3	963.8	5.7	14.8	23.1	62.1	76.9
6	CYP	8.7	0.5	7.4	19.8	585	6.4	12.6	32.2	55.2	68.5
7	LAKE	2	0.1	3.2	91	410.9	7.3	6.8	70.4	22.8	46.1
8	LAKE	4.2	0.3	6.3	39.9	839.7	7.3	8.9	48.8	42.3	58.5
9	LAKE	8.8	0.4	7.5	55.6	960	6.5	13.8	37.1	49.1	71.6

Background (no NO_3-N added) and potential (3 and 5 mg NO_3-N L^{-1} additions) rates of denitrification were characterized using the indirect acetylene block technique (Groffman 1994). We acknowledge several drawbacks to this technique, including underestimation of denitrification rates, and the possibility of acetylene serving as an energy source for microbes (Hynes and Knowles 1978). However, research shows that these are less of a concern over the short term (Ryden and Dawson 1982), and the technique remains popular due to convenience and low cost.

Acetylene was added directly to the headspace and floodwater of microcosms (~10 % v/v) in set # 2 only, which were then capped to prevent gaseous exchange with the atmosphere. No acetylene was added to the microcosms in set #1, which were also capped to prevent gaseous exchange. N_2O emissions were measured from set #1 (no acetylene added, thus evolved N_2O results from incomplete denitrification only), and N_2+N_2O emissions were measured from set #2 (with acetylene added, thus evolved N_2O includes both emissions of N_2O resulting from incomplete denitrification, as well as N_2 that would have been produced if not blocked by acetylene).

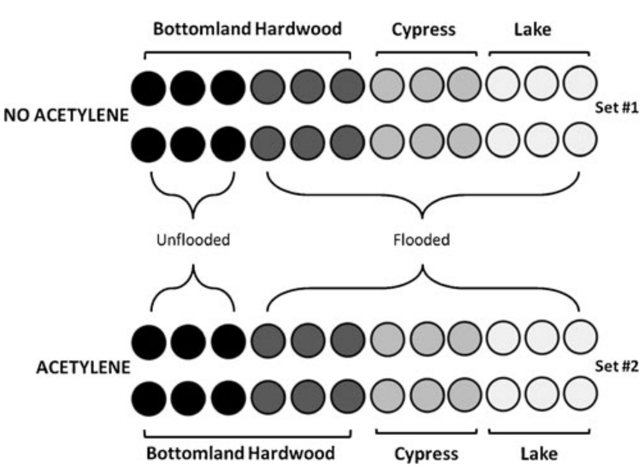

Fig. 2 Laboratory experimental setup, repeated with new microcosms for each level of nitrate added (3, and 5 mg NO_3-N L^{-1})

Gas samples were collected from the microcosms, via a rubber septum sealed in the lid, with a 2-ml gas tight syringe at 0 and 24 h (set #1) and 2 and 6 h (set #2) after nitrate addition, to determine the linear rate of N_2O buildup in the headspace. Samples were injected into a Shimadzu GC-14A gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) fitted with a 1-ml sampling loop, Porpak Q 1.8 m ss column, electron capture detector (ECD) and calibrated with certified N_2O gas standards (Scott Specialty Gases, Inc., Plumsteadville, PA, USA). Ultra high purity nitrogen was the carrier gas and the instrument operated at temperatures of 40, 100 and 290 °C for the oven, injector, and ECD detector, respectively (Lindau et al. 1988). Calculations were performed using the Bunsen absorption coefficient (which corrects for solubility of N_2O in water) to determine recovery of N_2O-N in both the headspace and floodwater (Tiedje 1982). The closed chamber equation of Rolston (1986) was used to calculate final N_2O flux, which we reported as g N ha^{-1} d^{-1}.

Background emissions (no NO_3-N addition) were measured on day 0. Potential emissions (low and high NO_3-N additions) rates were measured at 0, 1, 3, and 7 days after NO_3-N additions. Previous work in the ARB indicates that these habitats reach peak emission rates at different times (Scaroni et al. 2011) so sampling continued until day 7, when emissions from all sites at all NO_3-N concentrations returned to background levels. All tests were run at room temperature (22 °C). For temperature effects on denitrification at these same baldcypress sites, see Lindau et al. (2008).

Statistical Analysis

Background (0 mg NO_3-N L^{-1} addition) and potential (3, 5 mg NO_3-N L^{-1} additions) N_2O emissions, and potential N_2O+N_2 emissions were measured to characterize denitrification. Total N_2 emissions were then estimated by calculating the difference between these direct measurements from each set (Ryden et al. 1979). N_2:N_2O ratios were also calculated using this approach. Data were tested for normality and analyzed using PROC MIXED for analysis of variance and PROC REG for regression analysis in SAS (SAS Institute 2006). Correlations between emissions and soil components (C, N, P, organic matter content, pH, sand, silt and clay content) were tested using the PROC CORR function in SAS (SAS Institute 2006). Regression coefficients relating N_2 and N_2O emissions to NO_3-N concentrations were estimated using PROC REG. Results are reported for both maximum emissions and mean emissions averaged over the entire sampling period.

Results

N_2O Emission Rates

N_2O emissions were directly measured from set #1, which did not have acetylene added, thus evolved N_2O results from incomplete denitrification only (Fig. 3).

Unflooded BLHW Sediments

N_2O emissions remained below detection levels throughout the course of the experiment for all NO_3-N levels (0, 3, and 5 mg NO_3-N L^{-1}).

Flooded BLHW Sediments

N_2O emissions peaked on day 3 (0.14 g N_2O-N ha^{-1} d^{-1}) with the low treatment, and averaged 0.11 g N_2O-N ha^{-1} d^{-1} (SE=0.02, n=18) on days 1 and 3; on day 7 emissions were below the detection limit.

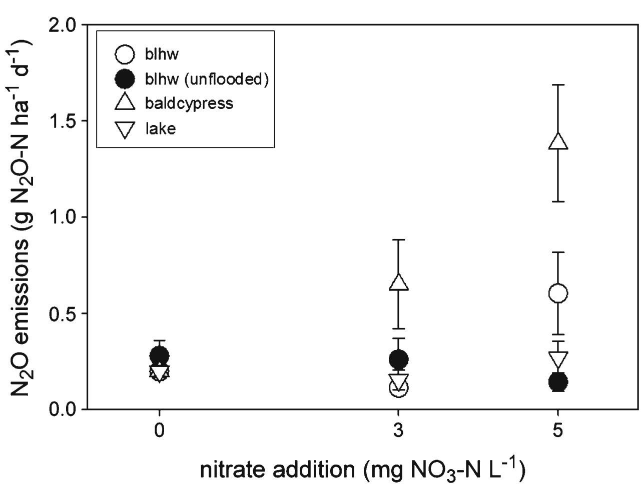

Fig. 3 Mean N_2O emission rates averaged over three sampling dates for three habitat types in the Atchafalaya River Basin, Louisiana; background, 3, and 5 mg NO_3-N L^{-1} addition. Graph shows Least Squares Means and Least Squares Standard Error bars

N_2O emissions peaked on day 1 (1.46 g N_2O-N ha^{-1} d^{-1}) with the high treatment, and averaged approximately 0.60 g N_2O-N ha^{-1} d^{-1} (SE=0.21, n=18) over the three sampling days.

Baldcypress Sediments

N_2O emissions peaked on day 1 (1.35 g N_2O-N ha^{-1} d^{-1}) with the low treatment, and averaged 0.65 g N_2O-N ha^{-1} d^{-1} (SE=0.23, n=18) over the three sampling days. N_2O emissions peaked on day 1 (2.31 g N_2O-N ha^{-1} d^{-1}) with the high treatment, and averaged 1.39 g N_2O-N ha^{-1} d^{-1} (SE=0.30, n=18).

Lake Sediments

N_2O emissions peaked on day 1 (0.29 g N_2O-N ha^{-1} d^{-1}) with the low treatment, and averaged 0.15 g N_2O-N ha^{-1} d^{-1} (SE=0.05, n=18) over the three sampling days. N_2O emissions peaked on day 1 (0.61 g N_2O-N ha^{-1} d^{-1}) with the high treatment, and averaged 0.27 g N_2O-N ha^{-1} d^{-1} (SE=0.09, n=18).

There was a significant three-way interaction between NO_3-N, day, and habitat (p=0.02). Baldcypress sites showed the greatest spike in N_2O emissions after NO_3-N addition, but all habitats peaked after 24 h. N_2O was positively correlated with C, N, organic matter content, clay content, and moisture content, and negatively correlated with pH, P, and silt content (Table 2).

N_2 Emission Rates

N_2+N_2O emissions were measured from set #2, which had acetylene added, thus evolved N_2O includes both emissions of N_2O resulting from incomplete denitrification, as well as N_2 that would have been produced if not blocked by acetylene. Total N_2 emissions were then estimated by calculating the difference between the measurements from set #1 (N_2) and set #2 (N_2+N_2O) (Fig. 4).

Unflooded BLHW Sediments

N_2 emissions peaked on day 7 (1.44 g N_2-N ha^{-1} d^{-1}) with the low treatment, and averaged 1.35 g N_2-N ha^{-1} d^{-1} (SE=0.43, n=18) over the three sampling days. N_2 emissions peaked on day 1 (2.12 g N_2-N ha^{-1} d^{-1}) with the high treatment, and averaged 2.08 g N_2-N ha^{-1} d^{-1} (SE=0.64, n=18) over the three sampling days.

Flooded BLHW Sediments

N_2 emissions peaked on day 3 (11.94 g N_2-N ha^{-1} d^{-1}) with the low treatment, and averaged 13.07 g N_2-N ha^{-1} d^{-1} (SE=2.35, n=18) over the three sampling days. N_2 emissions

Table 2 Correlation table showing correlations between emissions (N2O, N2O+N2, N2, and the ratio of N2:N2O) and soil components (total carbon, total nitrogen, phosphorus, % organic matter, pH, % sand, % silt, % clay, and moisture content)

	N_2O	N_2O+N_2	N_2	N_2:N_2O	C	N	P	OM	pH	Sand	Silt	Clay	Moisture Content
N_2O	1	**0.41925** **0.0002**	**0.37743** **0.0011**	**−0.25062** **0.0337**	**0.40133** **0.0005**	**0.4527** **<.0001**	**−0.23185** **0.05**	**0.33897** **0.0036**	**−0.5227** **<.0001**	−0.15024 0.2078	**−0.4406** **0.0001**	**0.38596** **0.0008**	**0.32537** **0.0053**
N_2O+N_2		1	**0.99896** **<.0001**	**0.47184** **<.0001**	**0.31854** **0.0064**	**0.26876** **0.0224**	**0.31727** **0.0066**	**0.31625** **0.0068**	−0.1619 0.1742	**−0.51417** **<.0001**	−0.17305 0.146	**0.40899** **0.0004**	**0.4702** **<0.0001**
N_2			1	**0.49388** **<.0001**	**0.30474** **0.0092**	**0.25138** **0.0332**	**0.33528** **0.004**	**0.30552** **0.0091**	−0.13888 0.2446	**−0.51691** **<.0001**	−0.15437 0.1954	**0.39777** **0.0005**	**0.46326** **<0.001**
Ratio				1	−0.18115 0.1278	−0.18348 0.1229	**0.52131** **<.0001**	−0.14097 0.2376	**0.30773** **0.0085**	**−0.34464** **0.003**	**0.3387** **0.0036**	−0.03648 0.761	0.06436 0.5912

The top number is the correlation coefficient and the bottom number is the *p*-value

Bold text indicates significance at $\alpha=0.05$

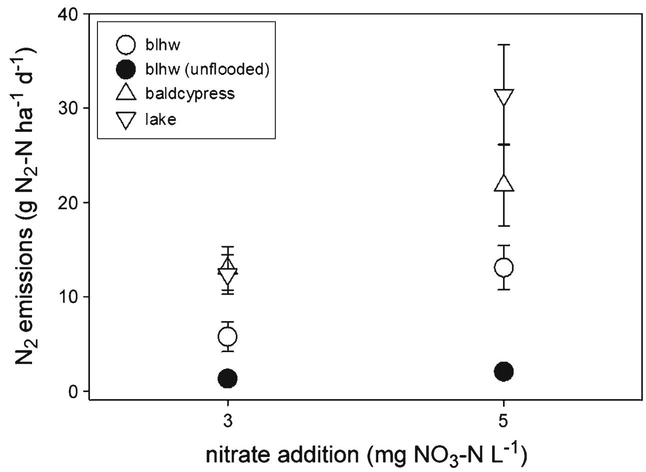

Fig. 4 Calculated mean N_2 emission rates averaged over three sampling dates for three habitat types in the Atchafalaya River Basin, Louisiana; 3 and 5 mg NO_3-N L^{-1} addition. Graph shows Least Squares Means and Least Squares Standard Error bars

peaked on day 1 (22.23 g N_2-N ha^{-1} d^{-1}) with the high treatment, and averaged 5.75 g N_2-N ha^{-1} d^{-1} (SE=1.58, n=18) over the three sampling days.

Baldcypress Sediments

N_2 emissions peaked on day 3 (20.4 g N_2-N ha^{-1} d^{-1}) with the low treatment, and averaged 13.03 g N_2-N ha^{-1} d^{-1} (SE=2.31, n=18) over the three sampling days. N_2 emissions peaked on day 1 (41.9 g N_2-N ha^{-1} d^{-1}) with the high treatment, and averaged 21.97 g N_2-N ha^{-1} d^{-1} (SE=4.32, n=18) over the three sampling days.

Lake Sediments

N_2 emissions peaked on day 3 with a 3 mg NO_3-N L^{-1} nitrate addition (20.48 g N_2-N ha^{-1} d^{-1}), and on day 1 with a 5 mg NO_3-N L^{-1} addition (53.68 g N_2-N ha^{-1} d^{-1}), and averaged 12.34 (SE=2.10, n=18) and 31.41 (SE=5.31, n=18) g N_2-N ha^{-1} d^{-1}, respectively over the entire incubation period.

There was a significant three way interaction between NO_3-N, day and habitat ($p<0.0001$). At the low concentration of nitrate (3 mg NO_3-N L^{-1}) emissions didn't peak until day 3 (for all but unflooded BLHW, which stayed at background levels despite nitrate addition), whereas N_2 emissions were highest on day 1 following 5 mg NO_3-N L^{-1} nitrate addition. N_2 was positively correlated with C, N, P, organic matter content, clay content, and moisture content, and negatively correlated with pH, silt, and sand content (Table 2).

Ratio of N_2:N_2O

Ratios of N_2:N_2O were highly variable across habitat type and between replicates (Fig. 5).

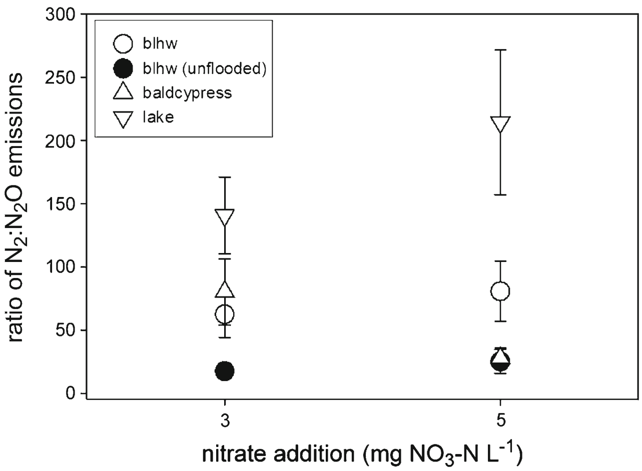

Fig. 5 Ratio of N_2:N_2O emission rates averaged over three sampling dates for three habitat types in the Atchafalaya River Basin, Louisiana; 3 and 5 mg NO_3-N L^{-1} addition. Graph shows Least Squares Means and Least Squares Standard Error bars

Unflooded BLHW Sediments

N_2:N_2O ratios peaked on day 3 (19.2) with the low treatment, and averaged 17.4 (SE=6.19, n=18) over the three sampling dates. One sediment core collected from the unflooded BLHW sites displayed N_2:N_2O ratios less than one (N_2:N_2O=0.5), indicating N_2O emissions were greater than N_2 fluxes over the 7 day incubation. N_2:N_2O ratios peaked on day 3 (24.8) with the high treatment, and averaged 25.2 (SE=9.42, n=18) over the three sampling dates.

Flooded BLHW Sediments

N_2:N_2O ratios peaked on day 3 (123.5) with the low treatment, and averaged 62.4 (SE=18.01, n=18) over the three sampling dates. This indicates that N_2 emissions were 3.6 times higher than N_2O emissions from the flooded sites. N_2:N_2O ratios peaked on day 3 (119.8) with the high treatment, and averaged 80.7 (SE=23.95, n=18) over the three sampling dates.

Baldcypress Sediments

N_2:N_2O ratios peaked on day 3 (135.9) with the low treatment, and averaged 80.2 over the three sampling dates. N_2:N_2O ratios peaked on day 1 (141.8) with the high treatment, and averaged 27.7 over the three sampling dates. Ratios dropped sharply on day 7 (averaging 8.7 across both NO_3-N treatments).

Lake Sediments

Highest N_2:N_2O ratios were observed from the lake sediment cores. N_2:N_2O ratios peaked on day 3 (239.2) with the low treatment and averaged 140.9 (SE=30.36, n=18) across the three sampling dates. N_2:N_2O ratios peaked on day 3 (373.8) with the high treatment and averaged 214.4 (SE=57.19, n=18) across the three sampling dates. N_2:N_2O ratios dropped sharply on day 7 (18.7 with the low treatment, and 43.9 with the high treatment).

The ratio of N_2:N_2O differed among the habitats, but in different ways, as indicated by the two way interaction between NO_3-N and habitat (p=0.02). The ratio increased with increasing NO_3-N concentration for lake habitats, decreased with increasing NO_3-N concentration for baldcypress habitats, and remained the same for both flooded and unflooded BLHW habitats when NO_3-N increased. The ratio of N_2:N_2O was positively correlated with P, pH, and silt content, and negatively correlated with sand content (Table 2).

Relationship Between Nitrate and Denitrification

N_2O emissions from BLHW and baldcypress soils were positively related to NO_3-N addition level (p=0.07 and p=0.002, respectively), but NO_3-N level only explained 7 % and 18 % of the variation in N_2O emissions, respectively. The relationship between NO_3-N and N_2O was not significant for unflooded BLHW habitats (p=0.28) or lake habitats (p=0.48), thus it was appropriate to use the overall means to predict emission rates (Table 3).

N_2 emissions from BLHW and lake soils were positively related to NO_3-N addition level (p=0.01 and p=0.002, respectively), but NO_3-N level only explained 16 % and 25 % of the variation in N_2 emissions, respectively. The relationship between NO_3-N and N_2 was not significant for unflooded BLHW habitats (p=0.33) or baldcypress habitats (p=0.08), thus it was appropriate to use the overall means to predict emissions rates (Table 3).

Discussion

Background levels of denitrification were below detection limits in nearly all jars. This was surprising considering that sediments collected from the same locations for a previous study measured background levels of denitrification ranging from 1.4 g N_2-N ha^{-1} d^{-1} to 5.4 g N_2-N ha^{-1} d^{-1} (Scaroni et al. 2011). We attribute this to the high degree of spatial and temporal variability in denitrification rates within habitats and to differences in soil organic nitrogen associated with seasonal differences in plant growth and senescence. Lindau et al. (1988) also measured levels below detection limits of background N_2O emissions from a Louisiana baldcypress swamp, and then similarly saw an increase upon amendment with NO_3-N. Our northern BLHW sites have either indirect or no contact with the Atchafalaya River, thus external NO_3-N loading to these sites may be lower than expected, resulting in lower background denitrification rates. Our southern lake sites

Table 3 Regression coefficients (for habitats with a significant linear relationship between nitrate and N_2O or N_2) or overall means with standard deviations of emissions from all levels of nitrate additions (for habitats without a significant linear relationship between nitrate and N_2O or N_2)

Habitat	N_2O (g N_2O-N ha^{-1} d^{-1})	N_2 (g N_2-N ha^{-1} d^{-1})
BLHW	N_2O=0.0767+0.081 (NO_3-N)	N_2=−5.2519+3.6653 (NO_3-N)
BLHW unflooded	<0.4 g N_2O-N ha^{-1} d^{-1}±0.3	1.66 g N_2O-N ha^{-1} d^{-1}±2.3
BCS	N_2O=0.1048+0.2365 (NO_3-N)	17.42 g N_2O-N ha^{-1} d^{-1}±15.1
LAKE	0.21 g N_2O-N ha^{-1} d^{-1}±0.3	N_2=−16.218+9.5256 (NO_3-N)

are directly connected to the Atchafalaya River; however, they receive river water after it has travelled a considerable distance through the ARB with ample opportunities for NO_3-N removal via sedimentation, biomass uptake, and denitrification.

N_2O emissions responded to nitrate additions, but our potential N_2O rates were similar to background rates reported by DeLaune et al. (1989) from a Louisiana freshwater marsh (1.5 g N_2O-N ha^{-1} d^{-1}), Lindau and DeLaune (1991) from a Louisiana salt marsh (2–3 g N_2O-N ha^{-1} d^{-1}), and Smith et al. (1983) from a Louisiana freshwater marsh (1.5 g N_2O-N ha^{-1}). Morse et al. (2012) saw slightly higher emissions from restored and forested wetlands in North Carolina (scaled up to 7.2–15.6 g N_2O-N ha^{-1} d^{-1}).

A review of N_2O emissions from treatment wetlands reported an average rate of emission of 40 g N_2O-N ha^{-1} d^{-1} across 15 wetlands (Kaldec and Wallace 2008). This amounted to a removal of approximately 2.2 % of the nitrogen load in the wetlands. These elevated rates were in response to a higher external NO_3-N loading. The elevated NO_3-N loads we applied to our microcosms in the laboratory (3 and 5 mg NO_3-N L^{-1}) were more than double the concentration introduced by the Atchafalaya River, and still saw N_2O emissions of less than 6 % of the average from the treatment wetlands mentioned above (Kaldec and Wallace 2008). Because the majority of the ARB is BLHW, we do not expect increasing river access to the lake and baldcypress habitats to produce a large spike in N_2O emissions.

Increasing the concentration of NO_3-N from 0 to 3 to 5 mg NO_3-N L^{-1} stimulated denitrification rates, as expected. The significant interaction between NO_3-N and habitat with denitrification rates indicates the importance of considering habitat type when estimating denitrification rates across a spatially variable system. We also recommend consideration of habitat type when modeling ecosystem-scale N_2O emissions, as not all the habitats in this study responded with linear increases to increasing nitrate additions.

While we accept the limitations of scaling up from microcosms in the laboratory to large scale Basin-wide estimates, it is still a useful approach for comparing differences among habitat types. Based on area estimates of habitat coverage in the ARB, we used our data from this experiment (with samples collected during the summer season) to estimate habitat-scale emissions of N_2O resulting from increases in NO_3-N loading to the ARB (Table 4).

It appears that differences in soil moisture and texture were driving differences in gas emissions among habitat types. Water filled pore space is one of the best predictors of N_2O emissions, relative to N_2 emissions (Granli and Bockman 1994), so it follows that soils with a high clay content and a high percentage of water filled pore space would realize higher emissions up to a certain threshold. N_2O emissions generally increase with increasing soil water content, as this reduces O_2 availability and increases redox potential (Eh) (Granli and Bockman 1994). However, at a certain point the soil becomes saturated, and highly anaerobic, causing N_2O emissions to decrease in favor of N_2 emissions. Alternating wet and dry cycles, commonly seen in the BLHW habitats of the ARB, tend to produce higher N_2O emissions (Granli and Bockman 1994). Although our flooded BLHW sediments exhibited higher potential N_2O emission rates than the unflooded BLHW sediments, these rates were still less than emissions from the lake sediments. BLHW sediments in the ARB have a higher sand content, whereas the texture of lake sediments is predominately silt and clay. These lake soils have higher water content, lower Eh, and exhibit higher denitrification rates. The flooded BLHW sediments had higher potential denitrification rates than the unflooded BLHW sediments; it appears that the moisture content of unflooded sediments was too low to denitrify. This was corroborated by the positive correlation between soil moisture and N_2O emissions (p= 0.005), and between soil moisture and N_2 emissions (p<0.0001). Without flooding, there was adequate gas exchange between the sediment and the atmosphere, prohibiting

Table 4 Estimate of habitat-scale (based on area estimates of habitat coverage) emissions of N_2O resulting from increases in NO_3-N loading to the ARB

	Area (ha)	N_2O emissions (t yr^{-1}) Low treatment	High treatment
BLHW	396,900	20	211
Baldcypress	106,227	52	89
Lake	63,873	7	14

the formation of an anaerobic layer necessary for the denitrification reaction. Weitz et al. (2001) reported an increase in N_2O production following precipitation, i.e. with an increase in soil moisture. We found a similar pattern; when the BLHW sediments were flooded and spiked with nitrate in the laboratory N_2O emissions increased compared to the unflooded, spiked BLHW cores.

We speculate that the negative correlation between P and N_2O could indirectly result from the negative correlation between P and organic matter content. The P in the system is primarily associated with mineral sediment, whereas the C in the system is associated with organic sediment. A carbon source is required for denitrification; more organic matter and bioavailable C stimulates denitrification (Dolda et al. 2008), whereas more P could indicate there is less C available to stimulate dentrifiers. This is corroborated by the positive correlation between N_2 (and N_2O) with C, N, and organic matter.

A longer duration of flooding increases pH in wetland sediments and reduces Eh. Therefore, as pH increases, the ratio of N_2:N_2O emissions should increase. It follows that we also saw a positive correlation between pH and N_2:N_2O. The negative correlation between pH and N_2O emissions agrees with previous studies. Low pH tends to favor a higher proportion of N_2O emissions relative to N_2 (Knowles 1982).

Conclusion

We found that background N_2O emissions were low compared to N_2, with a slight increase in response to NO_3-N additions when anaerobic conditions were maintained. This suggests that increases in external nitrate loading to isolated areas in the ARB will not produce emissions on par with those from treatment wetlands, or from areas directly in the path of agricultural runoff. Diverting water across the floodplain in the ARB is being considered to combat anoxic conditions in backwater swamps. Our results suggest that introducing river water to seasonally dry areas will not stimulate drastic increases in N_2O emissions. Many BLHW areas in the ARB will remain dry, in spite of diversions, due to their higher elevation. Thus, diverting water will not result in uniform flooding, but will instead allow a higher percentage of river water to access areas with a higher potential for nitrogen retention and removal (such as baldcypress swamps). Because field conditions in these areas are generally anaerobic, we expect that N_2O emissions will remain low.

Our results indicate that when attempting to quantify nitrogen retention and removal in a floodplain, regardless of location, it is necessary to sample each habitat separately to account for differences in spatial variability across the floodplain. In this study we classified habitats based on the dominant vegetation, because vegetation, among other factors, controls soil organic matter. While the data we obtained will not be applicable to all floodplains, the procedure we used can apply elsewhere. We recommend that all large scale denitrification studies account for habitat type in the experimental design, maintain in the laboratory similar soil moisture regimes as observed in the field, and continue sampling gas emissions from microcosms until they return to background levels. The tradeoffs between water pollution (high NO_3-N loading) and greenhouse gas (N_2O) emissions should be considered when making management decisions that will redirect the flow of water, impact water retention time, or alter the rate of habitat change resulting from sedimentation. Creating gaps in spoil banks and natural levees appears to be a viable option for removing nutrients and minimizing nitrogen loading to receiving waters, as demonstrated by this work in the ARB.

Acknowledgments We are grateful to two anonymous reviewers who provided constructive feedback for improving the manuscript. Also, thanks to Robert Ourso for providing the Atchafalaya Basin map adapted here.

References

Burt TP, Matchett LS, Goulding WT, Webster CP, Haycock NE (1999) Denitrification in riparian buffer zones: the role of floodplain hydrology. Hydrol Process 13:1451–1463

DeLaune RD, Feijtel TC, Patrick WH (1989) Nitrogen flows in Louisiana Gulf Coast salt marsh: spatial considerations. Biogeochemistry 8: 25–37

Dolda SK, Wang JJ, DeLaune RD, Cook RL (2008) Denitrification potential and its relation to organic carbon quality in three coastal wetland soils. Sci Total Environ 407:471–480

Faulkner SP, Bhattarai P, Allen Y, Barras J, Constant G (2009) Identifying baldcypress–water tupelo regeneration classes in forested wetlands of the Atchafalaya Basin, Louisiana. Wetlands 29:809–817

Gee GW, Bauder JW (1986) Particle-size analysis. In: Klute A (ed) Methods of soil analysis. Part 1. 2nd ed. Agronomy monograph 9. ASA and SSSA, Madison, pp 383–411

Granli T, Bockman OC (1994) Nitrogen oxide from agriculture. Nor J Agric Sci 12:7–127

Groffman PM (1991) Ecology of nitrification and denitrification in soil evaluated at scales relevant to atmospheric chemistry. In: Rogers JE, Whitmas WB (eds) Microbial production and consumption of greenhouse gases: methane, nitrous oxides and halomethanes. American Society for Microbiology, Washington DC, pp 201–217

Groffman PM (1994) Denitrification in freshwater wetlands. Curr Top Wetl Biogeochem 1:15–35

Groffman PM, Tiedje JM (1989) Denitrification in north temperate forest soils: spatial and temporal patterns at the landscape and seasonal scales. Soil Biol Biogeochem 21(5):613–620

Harms TK, Wentz EA, Grimm NB (2009) Spatial Heterogeneity of Denitrification in Semi-Arid Floodplains. Ecosystems 12(1):129–143

Howarth RW, Billen G, Swaney D, Townsend A, Jaworski N, Lajtha K, Downing JA, Elmgren R, Caraco N, Jordan T, Berendse F, Freney J, Kudeyarov V, Murdoch P, Zhao-Liang Z (1996) Regional nitrogen budgets and riverine N, P fluxes for the drainage to the North Atlantic Ocean: Natural and human influences. Biogeochemistry 35: 75–139

Hupp CR, Demas CR, Kroes DE, Day RH, Doyle TW (2008) Recent sedimentation patterns within the central Atchafalaya Basin, Louisiana. Wetlands 28:125–140

Hynes RK, Knowles R (1978) Inhibition by acetylene of ammonia oxidation in *Nitrosomonas europaea*. FEMS Microbiol Lett 4: 319–321

IPCC (2007) Climate Change 2007: the physical science basis. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, p 996

Kaldec RH, Wallace SD (2008) Treatment wetlands, 2nd edn. CRC Press, Boca Raton

Knowles R (1982) Denitrification. Microbiol Mol Biol Rev 46:43–70

Kroeze C, Dumont E, Seitzinger SP (2005) New estimates of global emissions of N_2O from rivers and estuaries. J Integr Environ Sci 2:159–165

Lindau CW, DeLaune RD (1991) Dinitrogen and nitrous oxide emission and entrapment in *Spartina alterniflora* saltmarsh soils following addition of N-15 labelled ammonium and nitrate. Estuar Coast Shelf Sci 32:161–172

Lindau CW, DeLaune RD, Jones GL (1988) Fate of added nitrate and ammonium-nitrogen entering a Lousiaina Gulf Coast swamp forest. J Water Pollut Control Fed 60:386–390

Lindau CW, DeLaune RD, Scaroni AE, Nyman JA (2008) Denitrification in cypress swamp within the Atchafalaya River Basin, Louisiana. Chemosphere 70:886–894

Lindau CW, Scaroni AE, Rivera-Monroy VH, Nyman JA (2011) Comparison of $^{15}N_2$ flux and acetylene inhibition denitrification methods in Atchafalaya River Basin sediments. J Freshw Ecol 26: 337–334

Morse JL, Ardon M, Bernhardt ES (2012) Greenhouse gas fluxes in southeastern U.S. coastal plain wetlands. Ecological Applications 22(1): 264–280

Pina-Ochoa E, Alvarez-Cobelas M (2006) Denitrification in aquatic environments: a cross-system analysis. Biogeochemistry 81:111–130

Pinay G, Black VJ, Planty-Tabacchi AM, Gumiero B, Decamps H (2000) Geomorphic control of denitrification in large river floodplain soils. Biogeochemistry 50:163–182

Rivera-Monroy VH, Lenaker P, Twilley RR, DeLaune RD, Lindau CW, Nuttle W, Habib E, Fulweiler RW, Castaneda-Moya E (2010) Denitrification in coastal Louisiana: a spatial assessment and research needs. J Sea Res 63:157–172

Rolston DE (1986) Gas flux. In: Klute A (ed) Methods of soil analysis, Part 1, physical and mineralogical methods. American Society of Agronomy, Madison, pp 1103–1109

Ryden JC, Dawson KP (1982) Evaluation of the acetylene-inhibition technique for the measurement of denitrification in grassland soils. J Sci Food Agric 33:1197–1206

Ryden JC, Lund LJ, Focht DD (1979) Direct measurement of denitrification loss from soils: laboratory evaluation of acetylene inhibition of nitrous oxide reduction. Soil Sci Soc Am J 43:104–110

SAS Institute (2006) Version 9.1.3. SAS Institute, Cary

Saunders DL, Kalff J (2001) Nitrogen retention in wetlands, lakes and rivers. Hydrobiologia 443:205–212

Scaroni AE, Lindau CW, Nyman JA (2010) Spatial Variability of Sediment Denitrification Across the Atchafalaya River Basin, Louisiana, USA. Wetlands 30:949–955

Scaroni AE, Lindau CW, Nyman JA (2011) Comparison of denitrification characteristics among three habitat types of a large river floodplain; Atchafalaya River Basin, Louisiana, USA. Hydrobiologia 658:17–25

Seitzinger SP (1988) Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. Limnol Oceanogr 33:702–724

Smith CJ, DeLaune RD, Patrick WH (1983) Nitrous oxide emission from Gulf Coast Wetlands. Geochim Cosmochim Acta 47:1805–1814

Stevens RJ, Laughlin RJ, Malone JP (1998) Soil pH affects the processes reducing nitrate to nitrous oxide and di-nitrogen. Soil Biol Biogeochem 30:1119–1126

Swarzenski CM, Doyle TW, Fry B, Hargis TG (2008) Biogeochemical response of organic-rich freshwater marshes in the Louisiana delta plain to chronic river water influx. Biogeochemistry 90:49–63

Tiedje JM (1982) Denitrification. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis, Part 2. Chemical and microbiological properties, agronomy monograph no. 9, 2nd edn. American Society of Agronomy, Madison, pp 1011–1026

Ullah S, Breitenbeck GA, Faulkner SP (2005) Denitrification and N_2O emission from forested and cultivated alluvial clay soil. Biogeochemistry 73:499–513

Weitz AM, Linder E, Frolking S, Crill PM, Keller M (2001) N_2O emissions from humid subtropical agricultural soils: effects of soil moisture, texture, and nitrogen availability. Soil Biol Biogeochem 33:1077–1093

Well R, Augustin J, Meyer K, Myrold DD (2003) Comparison of field and laboratory measurement of denitrification and N_2O production in the saturated zone of hydromorphic soils. Soil Biol Biogeochem 25:783–799

Xu YJ (2006) Total nitrogen inflow and outflow from a large river swamp basin to the Gulf of Mexico. Hydrol Sci J 51:531–542