

Denitrification in cypress swamp within the Atchafalaya River Basin, Louisiana

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Abstract

Nitrogen has been implicated as a major cause of hypoxia in shallow water along the Louisiana/Texas, USA coasts. Excess nitrogen (mainly nitrate) from Mississippi and Atchafalaya River drainage basins may drive the onset and duration of hypoxia in the northern Gulf of Mexico. Restoring and enhancing denitrification have been proposed to reduce and control coastal hypoxia and improve water quality in the Mississippi River Basin.

Sediments were collected from six baldcypress restoration sites within the Atchafalaya River Basin, Louisiana, USA. The acetylene blockage technique was used to measure background and potential sediment denitrification rates. Denitrification fluxes were measured before nitrate addition (background rates) and after nitrate addition of 100 mg N l⁻¹ (potential denitrification) at three seasonal temperatures. Background denitrification was low across all cypress swamp sites ranging from 0.9 to 8.8, 0.6 to 28.5 and 8.8 to 47.5 g N evolved ha⁻¹ d⁻¹ at water/sediment column temperatures of 8, 22 and 30 °C, respectively. After nitrate addition, temperature had a significant effect on sediment denitrification potential. Maximum rates measured at 8, 22 and 30 °C were approximately 250–260, 550 and 970 g N ha⁻¹ d⁻¹, respectively. Most of the added nitrate in water columns, incubated at 8 °C, was removed after 65 d compared to 32 d and 17 d at 22 and 30 °C, respectively. These results indicate cypress swamps have the potential to assimilate and process elevated levels of floodwater nitrate with denitrification being a major removal mechanism.

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1. Introduction

The Mississippi–Atchafalaya River Basin (henceforth, the Basin) is the largest North American drainage basin, draining an area of approximately 3208700 km² or 41% of the conterminous United States. The Basin contains productive farming regions and the majority of fertilizers and pesticides used in the United States are applied to Basin cropland. Fertilizer input to the Basin from 1951 to 1996 increased from less than 1 to about 7 million metric tons of nitrogen per year (Goolsby et al., 2001).

The Mississippi and Atchafalaya Rivers account for about 80% of the freshwater and 90% of the total nitrogen discharged annually to the Gulf of Mexico (Dunn, 1996). Mean annual nitrogen flux (1980–1996) from the Basin to the Gulf of Mexico was approximately 1568000 metric tons. About 70–75% of the nitrogen enters the Gulf via the Mississippi River and the remainder, about 390000 metric tons through the Atchafalaya River. Total nitrogen discharge is about 61% nitrate and 37% organic nitrogen. From 1955 to 1970 nitrate discharged averaged 328000 metric tons per year but from 1980 to 1999 averaged about 969000 metric tons per year (Goolsby et al., 2001). Over 90% of the nitrate entering the Gulf emanates from non-point sources (mainly agriculture) and highest discharges are usually in the spring and early summer.

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nitrogen loads from the Mississippi and Atchafalaya Rivers to the Gulf of Mexico (USEPA, 2001). Approaches to increase denitrification include: creating and restoring wetlands, creating and restoring riparian buffers and diverting river water in coastal Louisiana. Denitrification is the microbially-mediated reduction of nitrate (NO_3^-) to gaseous end products (N_2O , N_2) and represents a major pathway for nitrogen removal from wetland and aquatic ecosystems (Kadlec and Knight, 1996; Mitsch and Gosslink, 2000). Numerous studies have shown denitrification rates are affected by nitrate concentration, temperature, organic matter, microbial populations, soil texture and moisture, pH and sediment O_2 (Reddy and Patrick, 1984; Groffman, 1994; Watts and Seitzinger, 2000; Clement et al., 2002; Ullah and Faulkner, 2006; Yu et al., 2006; Hernandez and Mitsch, 2007).

The Atchafalaya River Basin (ARB) is the largest swamp wilderness (4662 km²) in the United States, containing vast expanses of bottomland hardwoods, bayous, swamps and backwater lakes. Approximately 50% of migratory bird species in the North American Flyway use the ARB each year. The primary environmental concern is water with low dissolved oxygen and the resulting stress and mortality on fish and crustaceans. Such waters originate in backwater areas where river flow is hindered by spoil banks within the ARB. Pilot projects to restore river flow through backwater areas by gapping spoil banks are underway and restoration efforts on dissolved oxygen and fish abundance are currently being assessed (ABP, 2003). A likely byproduct of restoring river flow to backwater areas may be reducing nitrogen discharges from the ARB to the Gulf of Mexico through denitrification, soil burial and biological assimilation.

An online search was conducted to identify denitrification research within the ARB but most results addressed the Atchafalaya Bay not the cypress swamp restoration sites where our research was conducted.

The purpose of this study was to quantify background denitrification rates and estimate denitrification potential at three seasonal water/sediment column temperatures at two habitat areas at each of three cypress swamp restoration sites within the ARB. Studies conducted provide

researchers and restoration managers an opportunity to assess denitrification as a water management strategy to improve water and habitat quality within the ARB and adjacent coastal areas.

2. Materials and methods

2.1. Description of sites and sample collection

Bayou Cowan (BC) – Iberia Parish, Bee Bayou (BB) – St. Martin Parish and Jones Bayou (JB) – Iberville Parish, LA were selected as the baldcypress swamp restoration sites (Fig. 1). At each site, denitrification was measured at locations where river water directly floods swamps (high flow/energy – HE) and backwater swamps where spoil banks and natural levees cause cypress swamps to flood indirectly (low flow/energy – LE). Soils at the six sites are in the Fausse Association formed in clayey alluvium inside the Atchafalaya Basin Floodway (Soil Surveys of Iberia, St. Martin and Iberville Parishes, LA, 1977–1978). Fausse soils typically have a slightly acidic dark grayish brown mucky clay surface layer and subsoils are neutral dark gray clay which are very poorly drained and slowly permeable. Soils are wet throughout the year and generally flooded (up to 4 m) for 6–10 months of the year. Fausse soils are high in organic matter, and high in available phosphorus, potassium and calcium (Soil Surveys). The UTM site coordinates and major sediment characteristics of the samples collected from the six baldcypress locations are shown in Table 1. Bulk sediment samples were collected (0–15 cm depth) manually in February (8–10 °C sediment temperature) and August (30–32 °C sediment temperature) 2006 and sealed in 4-l wide mouth heavy duty polyethylene (HDPE) bottles and iced down. Four sediment samples were collected from each site, composited and mixed. Composite samples were not homogeneous due to incorporated plant material at various stages of decomposition. Overlying floodwater (where available) was collected from four sites in 500 ml narrow mouth HDPE plastic bottles, sealed and immediately iced down. Sediment and water samples were transported back to the laboratory and stored at 1–2 °C.

Table 1
UTM coordinates and initial sediment characteristics

Site	UTM east	UTM north	OM (%)	Total-N (%)	Clay (%)	pH	Extractable nutrients (mg kg ⁻¹)						
							P	Ca	Mg	K	Na	S	Fe
BC-LE ^a	15R0652424	3323317	32.3 ^c	0.83	65.8	4.6	24	4902	866	371	193	564	451
BC-HE ^a			27.9	0.80	65.2	5.2	37	5577	1058	378	301	192	328
BB-LE ^b	15R0665505	3329455	16.8	0.46	57.9	5.0	24	4179	871	392	135	185	339
BB-HE ^a			18.9	0.51	51.9	5.5	49	5488	1000	379	130	589	270
JB-LE ^b	15R0655397	3332966	23.7	0.70	64.3	4.4	17	3862	894	642	129	318	421
JB-HE ^a			26.2	0.71	52.4	5.2	35	4964	1078	538	164	173	422

^a Floodwater nitrate <0.02 mg N l⁻¹.

^b Insufficient floodwater for nitrate analysis.

^c All sediment characteristics determined on composite samples ($n = 1$).

2.2. Denitrification experiments

Aliquots of equal sediment weight (dry weight basis) were transferred into 1 l glass jars (9 cm diameter) and packed to field bulk densities. A 4 cm layer of nitrate free distilled/deionized water was added and cores were allowed to incubate for approximately one week to reestablish the thin oxidized surface layer (observed in the field) overlaying the reduced sediment layer. After aerobic surface layer development cores were equilibrated for two days at selected temperatures (8, 22 and 30 °C) prior to nitrate addition. Denitrification potential was measured after 100 mg NO₃-N l⁻¹ was added to the floodwater of each treatment replication (*n* = 2). A high concentration of NO₃-N was required to assure the system was not nitrate limited. Prior to nitrate addition, background denitrification fluxes were measured.

The indirect acetylene blockage method was used to estimate denitrification (Groffman, 1994). Before and after nitrate addition, at each selected temperature, glass jars were sealed (Teflon lined plastic screw caps fitted with rubber septums), purified acetylene was added to the headspace volume (10% v/v) and additional acetylene was injected at the floodwater/sediment interface. Cores were only incubated with acetylene for short period of time to minimize acetylene effects on microorganisms (Watts and Seitzinger, 2000). Gas samples were collected (at 2 h and up to 6 h after acetylene addition) over time with a 2 ml gas tight syringe to monitor the linear buildup of nitrous oxide (N₂O) in the core headspace. After incubation, tops were removed and cores exposed back to the atmosphere. Acetylene incubation and gas sampling occurred every few days until the floodwater nitrate and/or N₂O gas headspace concentrations approached or decreased to background levels. Depending upon temperature, incubation conditions and gas sampling, laboratory experiments took up to two months to complete.

2.3. Analysis method

Sediment characteristics were determined on composite samples collected from the six sampling sites. Total organic matter was measured by loss on ignition at 550 °C (for 2 h) and total N analyzed by combustion in a Leco C and N analyzer (Leco Corp., St. Joseph, MI, USA). Percent clay was determined by the pipette method and sediment pH measured in a 1:1 soil to water slurry. Phosphorus, calcium, magnesium, potassium, sodium and sulfur were extracted with Mehlich 3 solution and iron with a weak DPTA solution. Extractable nutrients were analyzed by an inductively coupled plasma (ICP) instrument. Floodwater nitrate concentrations were analyzed on a Dionex ion chromatograph (Dionex Corp., Sunnyvale, CA, USA) according to EPA method 300.0 (USEPA, 1999).

Collected headspace gas samples were analyzed for N₂O concentration on a Shimadzu GC-14A gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD,

USA) fitted with a 1-ml sampling loop, Poropak Q 1.8 m ss column, electron capture detector (ECD) and calibrated with certified N₂O 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., 1998).

2.4. Data analysis

Denitrification rates were determined by linear regression of N₂O buildup, corrected with the Bunsen absorption coefficient (Tiedje, 1982) to estimate total N₂O-N evolved into the headspace and dissolved in the floodwater. Nitrous oxide flux was calculated using the closed chamber equation of Rolston (1986)

$$F = (V/A)(273/T)(\Delta C/\Delta T),$$

where *V* in the core headspace volume, *A* is the core sediment area, *T* is the absolute temperature of the headspace gases and $\Delta C/\Delta T$ is the change in N₂O concentration per unit of time. Nitrous oxide flux is reported as: g N₂O-N evolved ha⁻¹ d⁻¹ which represents total denitrification (N₂O + N₂) with a detection limit of 0.6 g N ha⁻¹ d⁻¹.

Denitrification data was analyzed by the analysis of variance (ANOVA) test procedure and significant differences between treatment means were calculated by Duncan's Multiple Range Test (SAS Institute, 2006). An alpha level of 0.05 was used for all statistical analysis. Error bars were not added to line graphs due to close proximity and overlapping of many denitrification mean values.

3. Results and discussion

3.1. Background denitrification rates

By using the acetylene blockage technique, denitrification in cypress swamp and other natural wetland systems can be measured without the addition of nitrogen fertilizer (Duxbury, 1986).

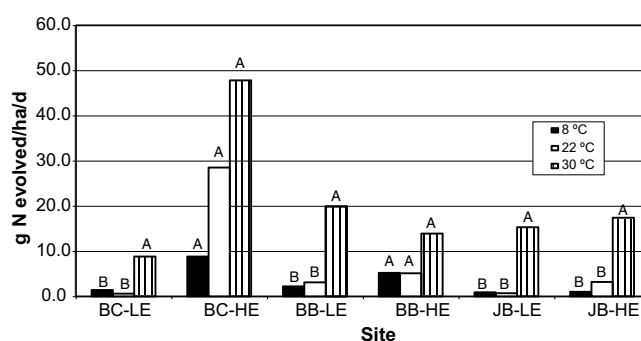


Fig. 2. Background sediment denitrification rates as affected by temperature prior to nitrate addition. Means (*n* = 2) with same letter at the same site are not significantly different (α = 0.05).

Background denitrification rates are presented in Fig. 2. At 8 °C, sediment denitrification rates ranged from 0.9 (JB-LE) to a high of 8.8 (BC-HE) g N evolved ha⁻¹ d⁻¹. Fluxes at 22 °C were generally higher and varied from a low mean of 0.6 (BC-LE) to 28.5 (BC-HE) g N ha⁻¹ d⁻¹. Highest background denitrification rates were measured in floodwater/sediment cores incubated at 30 °C. Values ranged from 8.8 (BC-LE) to 47.8 (BC-HE) g N ha⁻¹ d⁻¹ (Fig. 2). Significant (*p* ≤ 0.05) temperature affects were measured at four (BC-LE, BB-LE, JB-LE and JB-HE) of the six sampling sites. At these sites, background sediment denitrification mean rates measured at 30 °C were significantly higher compared to 8 and 22 °C mean rates. At the three incubation temperatures, no significant treatment differences were observed for the BC-HE and BB-HE sediment denitrification fluxes (Fig. 2).

3.2. Sediment denitrification potential at 8 °C

Denitrification potential was measured in floodwater/sediment cores incubated at 8 °C (Fig. 3). Rates were measured for approximately 65 days after NO₃-N addition (100 mg l⁻¹) to the core floodwater. Denitrification rates measured at time zero reflect background fluxes. Denitrification measured over the six treatments were highly variable but several general trends were observed. After nitrate addition, rates for all sediment treatments rapidly increased over the first 8 days (Fig. 3). Maximum flux rates for five of the six treatments sites (147.6–259.3 g N ha⁻¹ d⁻¹) occurred at 8 or 12 d of incubation. The highest denitrification rate was recorded on day 8 from the BC-HE site. Mean maximum flux (165.6 g N ha⁻¹ d⁻¹) from the BC-LE site occurred at 30 d after nitrate addition. For many of the treatments, mean denitrification rates decreased after day 12 until the end of sampling (65 d) but rates were highly variable as shown in Fig. 3. At 65 d, denitrification rates still ranged from 4.7 (BC-HE) to a high of 93.8 (JB-LE) g N evolved ha⁻¹ d⁻¹.

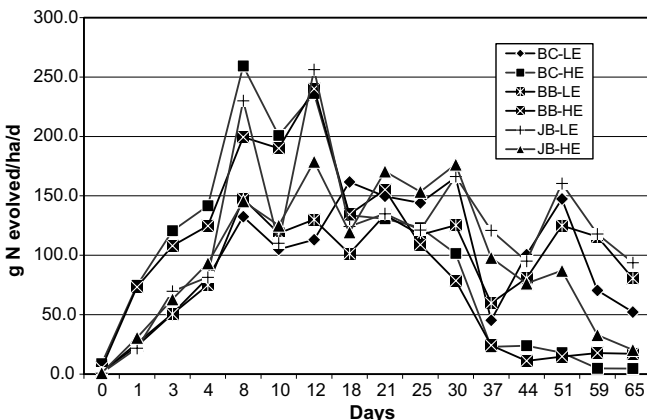


Fig. 3. Denitrification potential mean (*n* = 2) rates measured at 8 °C sediment temperature after nitrate addition.

Significant treatment differences were observed in 11 out of 15 sampling intervals. No significant treatment differences were observed on days 18, 21, 25 and 37. On days 1, 3, 4 and 10, denitrification rates measured from the BC-HE and BB-HE sites were significantly higher compared to the remaining four experimental sites. On these days, no significant differences were observed between the BC-HE and BB-HE denitrification means and no significant differences were calculated among the remaining four treatment site means (BC-LE, BB-LE, JB-LE and JB-HE). On days 12, 51, 59 and 65 the highest denitrification flux was measured from the JB-LE site which was significantly higher than at least 2 other site treatment means.

3.3. Sediment denitrification potential at 22 °C

Denitrification results measured in sediments incubated at 22 °C are shown in Fig. 4. For most treatments, rates increased sharply over the first three days after nitrate addition followed by a sharp and steady decline for days 4–32. Denitrification potential rates from the Bayou Cowan LE and HE sediments ranged from 0.6 (day 32) to 549.7 (day 3) and from 0.6 (days 25 and 32) to 443.6 (day 3) g N emitted ha⁻¹ d⁻¹, respectively. Bee Bayou-LE sediment displayed lower fluxes ranging from 12.8 (day 32) to a maximum of 349.2 (day 3) g N ha⁻¹ d⁻¹. For the BB-HE site, denitrification potential varied from a low of 2.9 (day 32) to a maximum value of 318.1 g N ha⁻¹ d⁻¹ measured on day 3 after nitrate addition (Fig. 4). Measured denitrification from sediment collected at the JB-LE and JB-HE sites ranged from a low mean of 18.5 (day 32) to a maximum mean of 238.1 (day 6) and from 1.3 (day 32) to 420.5 (day 3) g N emitted ha⁻¹ d⁻¹, respectively. At the end of incubation (32 d), very little sediment denitrification was measured from any of the six sites. Four of the six baldcypress sites had sediment denitrification rates less than 3 g N ha⁻¹ d⁻¹ which corresponded with floodwater

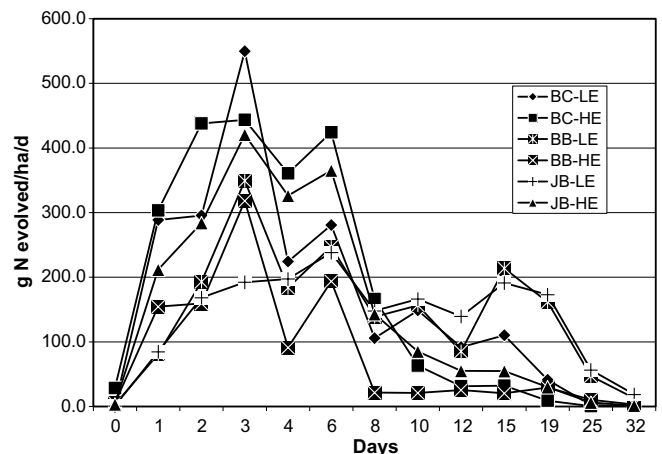


Fig. 4. Denitrification potential mean (*n* = 2) fluxes measured at 22 °C sediment temperature after nitrate addition.

nitrate-N concentrations of $<0.02 \text{ mg N l}^{-1}$ (below detection limit).

Significant treatment differences were calculated for 10 of 12 sampling times after nitrate addition. Only on days 1 and 3 were no significant treatment differences observed. At days 2, 4 and 6, the mean BC-HE denitrification rate was the highest and was significantly higher than at least four of the remaining sediment denitrification treatment means. On day 8, the BC-HE mean was only significantly higher than the BB-HE denitrification mean. For days 10, 12, 19 and 32, Jones Bayou-LE sediment denitrification was significantly higher compared to at least 3 of the remaining treatment means. On sampling day 15, the BB-LE sediment denitrification mean ($213.7 \text{ g N ha}^{-1} \text{ d}^{-1}$) was significantly higher compared to the BC-LE, BC-HE, BB-HE and JB-HE denitrification means.

3.4. Sediment denitrification potential at 30°C

Denitrification potential results at a floodwater/sediment temperature of 30°C are graphed in Fig. 5. Denitrification potential was measured over 17 days after floodwater nitrate addition. Sediment denitrification rates for all six treatments increased rapidly over the first 2 days. A maximum rate of $973.0 \text{ g N evolved ha}^{-1} \text{ d}^{-1}$ was measured from the BC-HE site at day 2. After day 2, denitrification fluxes steadily declined over the remainder of the incubation period for 5 of the 6 treatment sites. At the end of the 17 days, denitrification rates were low and ranged from <0.6 to $4.5 \text{ g N evolved ha}^{-1} \text{ d}^{-1}$ (Fig. 5) which corresponds with low floodwater nitrate concentrations ($<0.02\text{--}0.93 \text{ mg N l}^{-1}$). Sediment denitrification potential at Bayou Cowan ranged from 0.6 (day 17) to 688.5 (day 3) for the LE site and from 0.6 (day 17) to a maximum mean of $973.0 \text{ g N emitted ha}^{-1} \text{ d}^{-1}$ measured at HE cypress site. Bee Bayou denitrification fluxes were lower, LE site rates ranged from 4.1 (day 17) to a high of $465.7 \text{ g N ha}^{-1} \text{ d}^{-1}$ measured on day 2 and HE site denitri-

fication rates varied from 0.9 to $731.8 \text{ g N evolved ha}^{-1} \text{ d}^{-1}$ (Fig. 5). Denitrification nitrogen emitted from the JB-LE and -HE sediment cores ranged from 4.5 to 596.4 and from 1.5 to $636.4 \text{ g N ha}^{-1} \text{ d}^{-1}$, respectively.

Significant differences in denitrification rates were observed for 7 of 9 sampling intervals after nitrate addition. Only on days 4 and 17 were no significant treatment differences calculated. On days 1, 2 and 3, BC-HE mean sediment denitrification fluxes were significantly higher compared to at least 2 of the remaining treatment values. For days 6, 8 and 10, JB-LE mean denitrification rates were the highest measured but only significantly higher compared to the Bayou Cowan-HE mean.

3.5. Temperature affect on sediment denitrification potential

Fig. 6 displays the effect of temperature on denitrification potential at days 1, 3, 4, 8, 10 and 12 (common sampling times across incubation temperatures). Each observation point for each temperature represents a mean of 12 values (BC, BB and JB sites). Means were combined because no significant treatment differences were consistently observed between and within the cypress sites over the entire incubation periods. Mean sediment denitrification measured at 8°C ranged from 3.3 (day 10) to a maximum of $192.4 \text{ g N evolved ha}^{-1} \text{ d}^{-1}$ recorded on day 12. Denitrification measured at 22 and 30°C varied from 6.9 (day 0) to 378.9 (day 3) and from 20.5 (day 0) to a mean maximum of $529.8 \text{ g N evolved ha}^{-1} \text{ d}^{-1}$ (day 3), respectively. At day 3, the sediment denitrification potential at 30°C was 1.4 times higher compared to the 22°C mean and 6.9 times greater than the average mean denitrification mean recorded at 8°C .

Statistical analysis revealed significant treatment differences at all days after nitrate addition. On days 1 and 3 all temperature treatments were significantly different with

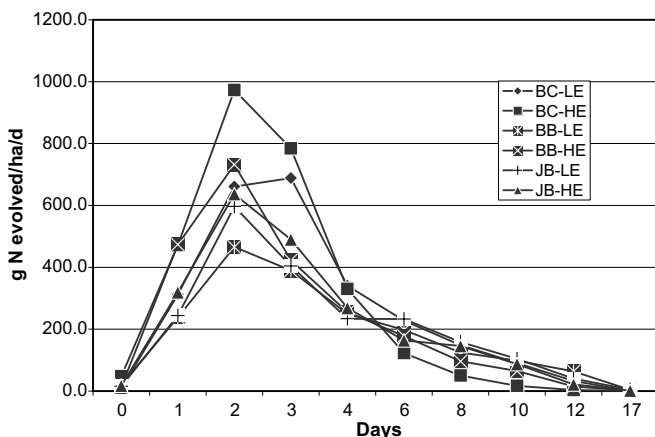


Fig. 5. Denitrification potential mean ($n = 2$) rates measured at 30°C sediment temperature after nitrate addition.

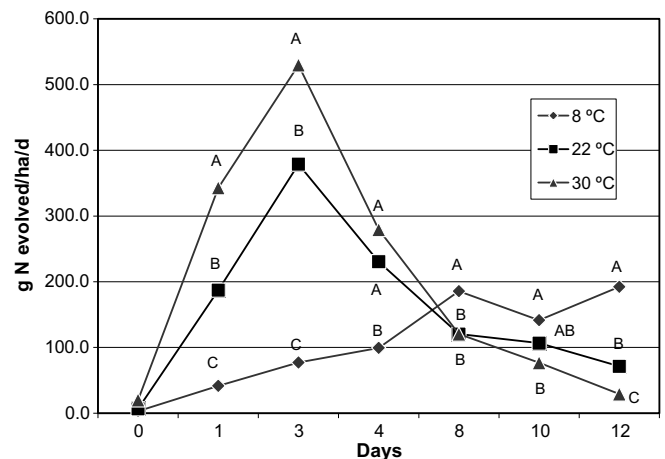


Fig. 6. Influence of temperature on denitrification potential rates ($n = 12$). Time zero represents background rates. Means with the same letter on the same day are not significantly different.

the 30 °C sediment denitrification mean the highest and 8 °C mean the lowest. At day 4, the 30 °C mean was only significantly higher than the 8 °C mean. For days 8, 10 and 12 the 8 °C sediment denitrification was significantly higher than at least one of the two remaining temperature treatments. Data plotted in Fig. 6 shows nitrate concentration may be the limiting factor affecting denitrification rates at 22 and 30 °C for days 4, 8, 10 and 12 after nitrate addition (100 mg N l⁻¹). At 8 °C, denitrification rates showed a gradual and steady increase over the 12 d sampling period. This trend may indicate denitrification rates are limited by core sediment temperature rather than nitrate concentration and availability.

Averaged over the six sites, the mean sediment background denitrification rate (20.6 g N ha⁻¹ d⁻¹) at 30 °C was 6.2 and 3.0 times greater compared to rates measured at 8 °C (3.3 g N ha⁻¹ d⁻¹) and 22 °C (6.8 g N ha⁻¹ d⁻¹). By using the acetylene blockage procedure, these denitrification rates may be underestimated. Numerous studies have shown prolonged acetylene exposure may inhibit nitrification and may not capture coupled nitrification/denitrification (Watts and Seitzinger, 2000; Clement et al., 2002). The baldcypress floodwater/sediment cores were exposed to acetylene only for short periods of time to minimize acetylene problems inhibiting nitrification and/or depletion of the sediment nitrate pool due to mineralization of organic N. Using acetylene blockage, Delaune and Jugsujinda (2003) measured background denitrification rates in a Louisiana wetland receiving diverted Mississippi River water. Over the 11 d incubation, background rates were low and ranged from 0.3 to 9.5 g N evolved ha⁻¹ d⁻¹ at 22 °C. This compares to the six baldcypress sediment sites where background rates ranged from 0.6 to 28.5 g N ha⁻¹ d⁻¹ (22 °C).

Sediment denitrification data (after nitrate addition) plotted in Figs. 3–5 were highly variable, strongly influenced by sediment temperature and consistent significant differences between and within the three baldcypress sites were not observed over the entire incubation periods. Maximum sediment denitrification potential means measured at 8, 22 and 30 °C averaged 207.9 (147.6–259.3), 386.6 (238.1–549.7) and 682.0 (465.7–973.0) g N emitted ha⁻¹ d⁻¹, respectively. The 30 °C means average was approximately 3.3 and 1.8 times greater compared to the 8 and 22 °C means average, respectively. Denitrification or disappearance of added nitrate N (100 mg N l⁻¹) at 30 °C was much more rapid. Rates measured at a 30 °C sediment temperature approached background levels after 17 d or about one-half the time required at 22 °C (32 d) and about one-fourth the time for 8 °C (about 65 d). The lag time between addition of nitrate to the water column and peak denitrification decreased as temperature increased. At a sediment temperature of 8 °C, the lag time was from 8 to 12 d compared to 3 d at 22 °C and 2 d at 30 °C. This data shows denitrifying communities can respond quickly once nitrate is added to the system and season or temperature may influence the population and distribution of sediment denitrifiers.

In an earlier study, Nowicki (1994) demonstrated temperature increases increased denitrification in control and nitrate enriched estuarine sediment cores. The denitrification rate in control cores increased from approximately 80 g N ha⁻¹ d⁻¹ at about 4 °C to about 170 g N ha⁻¹ d⁻¹ at 22 °C. Rates in nitrate enriched cores increased exponentially from about 170 g N ha⁻¹ d⁻¹ (4 °C) to approximately 1275 g N ha⁻¹ d⁻¹ at 18 °C. Poe et al. (2003) also demonstrated a significant positive correlation between denitrification rates and temperature in a constructed wetland receiving agricultural runoff. In June, denitrification averaged about 1570 g N ha⁻¹ d⁻¹ and November about 170 g N ha⁻¹ d⁻¹. A review by Seitzinger (1990) showed a large range in denitrification rates for estuarine sediments (approximately 20–840 g N ha⁻¹ d⁻¹) and higher rates for polluted sediments (>1600 g N emitted ha⁻¹ d⁻¹). The review also showed highest rates in eutrophic streams or rivers. Using the acetylene blockage method, Xue et al. (1999) measured denitrification in-situ in a constructed wetland after addition of 9–20 mg NO₃-N l⁻¹ to the water column. Denitrification rates range from 20 to about 120 g N ha⁻¹ d⁻¹.

In a Louisiana coastal wetland receiving diverted Mississippi River water, Delaune and Jugsujinda (2003) measured denitrification in water-sediment columns (22 °C) amended with nitrate-N at 1750 and 3500 mg m⁻². Using the acetylene technique the average denitrification rate over the most active period (days 1–5) was 570 and 870 g N ha⁻¹ d⁻¹, respectively. Using labeled ¹⁵N – nitrate, Yu et al. (2006) measured denitrification in a freshwater marsh after nitrate addition (32.8 mg N l⁻¹) to field plots. The maximum denitrification potential was estimated to be approximately 3000 g N emitted ha⁻¹ d⁻¹.

Measured denitrification rates are strongly influenced by experimental methods and protocol including temporal and spatial patterns (Dhondt et al., 2004). Comparison of reported denitrification rates among ecosystems is difficult due to differences in sediment environmental conditions, nitrate application rates, analysis method (indirect or direct), incubation periods, seasonal frequency of sampling, field collection procedures (slurry vs. intact cores vs. bulk sediment) and denitrification flux units (sediment weight vs. sediment area).

3.6. Summary

In sediments collected from six baldcypress restoration sites within the Atchafalaya Basin, LA, USA background and potential denitrification rates were highly variable between and within sites. Sediment incubation temperature significantly influenced denitrification fluxes as expected. Denitrification within the ARB should be highest during the summer and reduced during the winter months. Consistent significant denitrification differences were not observed between sediments collected where river water directly floods swamp habitats (HE sites) compared to backwater swamps where spoil banks and natural levees cause cypress

swamps to flood indirectly (LE sites). This may have been partially due to limited replication ($n = 2$) and/or heterogeneity of composite sediment samples. Sediments from all habitats showed potential to remove significant quantities of added nitrate (100 mg N l^{-1}) through the denitrification process. Due to limited sampling sites, sample analysis and replication further studies are needed to assess the overall spatial variability of denitrification within the vast Atchafalaya Basin. Detailed studies are also needed to determine correlation of sediment properties to denitrification rates. Once additional studies have been completed the significance of denitrification on a landscape scale (ARB) can be assessed. In addition, the generated acetylene blockage denitrification data needs to be compared to direct denitrification measurement techniques (isotope pairing, N_2/Ar ratios). Ultimately, to use denitrification as a restoration tool, denitrification must be assessed and quantified over all habitats and environmental conditions at nitrate levels ($1\text{--}3 \text{ mg N l}^{-1}$) typically found in river water passing through the ARB. Even a small increase ($0.2\text{--}0.3 \text{ mg N l}^{-1}$) in the Mississippi River nitrate concentration could significantly increase the amount of nitrate-N entering and exiting the ARB on a yearly basis. Gapping spoil banks to restore flow to backwater swamps of the ARB should increase removal of riverine nitrate. Allowing redirected river water to spread out and slow down over reduced backwater sediment should help optimize the denitrification process. However, results clearly demonstrate denitrification has the potential to remove a significant amount of riverine nitrate entering the ARB during yearly flood events. With less nitrate entering the Gulf of Mexico, Louisiana and Texas coastal hypoxic regions should decrease in size resulting in less stress and mortality of aquatic species due to low dissolved oxygen.

Acknowledgements

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