TITLE

USE OF COTTON GIN TRASH TO ENHANCE DENITRIFICATION IN RESTORED FORESTED WETLANDS

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Authors:

Sami Ullah[a *] and Stephen P. Faulkner[b]

a. Louisiana State University, Wetland Biogeochemistry Institute, Baton Rouge, Louisiana 70803, USA.
b. USGS National Wetlands Research Center, 700 Cajundome Blvd., Lafayette, LA 70506, USA.

* Author for correspondence: Global Environmental and Climate Change Center, Department of Geography, McGill University, 610 Burnside Hall, 805 Sherbrooke St. West. Montreal, Quebec H3A 2K6, Canada
Email: sami.ullah@mcgill.ca phone: +1-514-398-4957, Fax: +1-514-398-7437

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Abbreviations: CGT: Cotton gin trash, LMV: Lower Mississippi Alluvial Valley

Abstract

Lower Mississippi Valley (LMV) has lost about 80% bottomland hardwood forests, mainly to agriculture. This landscape scale alteration of the LMV resulted in the loss of nitrate (NO_3) removal capacity of the valley, contributing to nitrogen (N)-enhanced eutrophication and potentially hypoxia in the northern Gulf of Mexico. Restoration of hardwood forests in the LMV is a highly recommended practice to reduce NO_3 load of the Mississippi River. However, restored bottomland forests take decades to develop characteristic ecological functions including denitrifier activity. One way to enhance denitrifier activity in restored wetland forests is to amend the soils with an available carbon (C) source. This research investigated the effects of cotton gin trash (CGT) amendment on denitrification rate and N_2O:N_2 emission ratio from a restored bottomland forest soils and compared it to those from an adjacent unamended natural forest soils. CGT amendment increased denitrification rates in the restored forest soils to the level of the natural forest soils. N_2O:N_2 emission ratios from the restored and natural forest soils were highly variable and were not significantly different from each other. These findings suggest that restoration of bottomland hardwood forests in the LMV will require organic carbon amendment to achieve enhanced denitrifier activity for NO_3 removal while the restored forest is developing into a mature state over time.

Introduction

There is growing global concern about the increasing mineral nitrogen (N) levels in the environment and its subsequent impacts on aquatic ecosystems (Howarth et al. 2002; Galloway et al. 2002). Increasing discharge of reactive N from terrestrial landscapes to estuaries and coastal ecosystems results in algal blooms and high primary productivity, which leads to oxygen depletion and anoxia (Thompson et al. 2000). Run-off from cultivated lands is the major cause of increased reactive N in rivers and lakes, which affect more than 50% of surface water in the southeastern US (Neary et al. 1989). Intensive agricultural practices in the Mississippi River basin have resulted in an increase of NO_3 concentration in the Mississippi River (Mitsch et al. 2005). Up to 70% of the current total NO_3 load of the Mississippi River has been attributed to agricultural runoff (Goolsby 2000; Turner and Rabalais 2003). Widespread eutrophication and hypoxia in the northern Gulf of Mexico has been linked to the increased NO_3 and sediment loading of the Mississippi River (Mitsch et al. 2001).

The Lower Mississippi Alluvial Valley (LMV) has lost more than 80% of its native bottomland hardwood forests mainly from its conversion to agriculture (MacDonald et al. 1979). This large-scale alteration has changed these landscapes from a net NO_3 sink to a net NO_3 source. Natural forested wetlands have a tightly coupled N cycle and additional NO_3 input from agricultural run-off into these ecosystems is either used by vegetation, denitrified by heterotrophic microbes, or immobilized by bacterial cells (Silvan et al. 2003: Ullah et al. 2005). Compared to upland forests, forested wetlands are recognized for their high denitrification rates, which are a function of their anaerobic soil conditions, high denitrifier populations, and readily available organic carbon (C) substrates (Lowrance et al. 1984; Delaune et al. 1996; Ingrid-Brettar and Hofle 2002; Ullah et al. 2005).

Restoration of formerly forested wetlands as a method to improve water quality in watersheds dominated by agriculture has received increased attention. Among others, one goal of wetland restoration in agricultural watersheds is to enhance denitrification capacity of the restored wetland for NO_3 removal (Lowrance et al. 1984; Hunter and Faulkner 2001; Mitsch et al. 2001; Ullah and Faulkner 2006). However, forested wetland restoration is a long-term endeavor as restored forests take decades to reach maturity and fully develop characteristic ecological functions including biogeochemical (Niswander and Mitsch 1995; Shear et al. 1996; Battaglia et al. 2002; Ruiz-Jaen and Aide 2005). Organic C is a key substrate for important microbiological processes including denitrification in soils (DeLaune et al. 1996) and newly restored wetland soils often have lower soil C than natural wetland soils (Craft and Reader 1999; Hunter and Faulkner 2001). Hunter (2000) reported that denitrification potential in a 10-year old restored forested wetland was limited by available C substrate. Addition of cotton gin trash (CGT) to soils collected from a 10-year old restored forest increased its denitrification rate by 45%, suggesting that denitrification potential of restored forested wetlands can be enhanced by amending soils with organic C such as CGT. **CGT is produced at ginning industries as flower residues while separating cotton fibbers from the rest of the cotton flower. The southeastern states of the US (east of the Mississippi River) produce about 500,000 to 700,000 tons of CGT from about 4.5 million acres of cotton growing area annually (Rossi 2006). CGT is available at ginning industries**

free of cost and can be used as a C source for enhancing microbial activities in restored wetlands in the region.

Organic C substrate in soils supports greater N_2O reductase activity during denitrification, leading to lower N_2O emissions under low to moderate levels of soil NO_3 (Sahrawat and Keeney 1986: Arah et al. 1990; Skiba et al. 1998). However, high NO_3 loading into soils lead to higher N_2O emissions (Bowden et al. 1991; Llyod 1995) raising the issue of whether newly restored forested wetlands will increase the atmospheric burden of N_2O emissions when exposed to NO_3 run-off from agricultural lands. Given the significance of N_2O as a potent greenhouse gas (IPCC 1996), it is important to account for N_2O emissions from all of its potential sources (Groffman et al. 2000b), including newly restored forested wetlands.

We measured the effects of CGT amendment on denitrification rates and $N_2O:N_2$ emission ratios from restored forested wetlands and compared it those from an adjacent natural forested wetlands in the LMV. We hypothesized that amending restored forested wetland soils with CGT would increase denitrification rates and reduce $N_2O:N_2$ emission ratio from the restored forested wetlands

Material and Methods

Description of the Research Sites

The research sites were located on the Panther Swamp National Wildlife Refuge in the Yazoo delta region of Northwestern Mississippi (Figure 1). A 13-year-old restored forested wetland (~ 5 acres) and adjacent natural forested wetlands (~ 10 acres) were selected for this study. We selected sites containing Sharkey clay soils (non-acidic montmorilinitic, Vertic Haplaquept), because this soil series is common in the lowelevation areas of the LMV, covering about 12,150 km^2. The natural forested wetland was dominated by a mature stand of American elm (*Ulmus americana)*, water oak (*Quercus nigra.),* laurel oak (*Q. laurifolia*), red maple *(Acer ruburum, L.*), bitter pecan (*Carya x lecontei),* hackberry (*Celtis leavigata*) and dogwood (*Cornus spp*.). The soil surface in the natural forest site was interspersed with dead logs and snags. The restored site was dominated by young tree species of water oak (*Quercus nigra*), green ash (*Fraxinus pennsylvanica),* honeylocust *(Gleditsia triacanthos L.),* dogwood (*Cornus spp*.), and red maple *(Acer ruburum* L.). This site was re-planted in 1990 after being abandoned as an agricultural land.

Eight replicate sampling sites (pseudo-replicates) were randomly selected in both the restored and natural forested wetlands. In the natural forested wetland, eight 1 m^2 area plots were marked at each sampling site. In the restored forested wetland, two plots each of 1 m^2 area were placed and marked at each sampling site. **Two kilograms of CGT was spread manually on the soil surface of one plot of the two plots of the restored forested wetland, 15 days before the start of denitrification studies.** The amendment was left on the soil surface of the selected plots to avoid altering soil porosity and gas flux. Cotton gin trash amendment represented 20 Mt ha^{-1} or about 1.5% of the total soil dry weight in the upper 10 cm. Cotton gin trash is 40% organic C and has a C:N ratio of 18:1 (determined on CNS Finnigan analyzer), which can provide a readily mineralizable organic C substrate to microbes in soils. The mean NO_3-N and NH_4-N contents of the CGT were 15.4 ± 3.6 and 788 ± 40 mg kg^{-1} cotton gin trash, respectively.

Denitrification, N_2O:N_2 Emission Ratio and CO_2 Production Rates

Duplicate intact soil cores (5cm dia. x 10 cm length) were collected from each plot using a slide hammer (AMS-samplers, American Falls, Idaho) fitted with plastic liners (5 cm dia. x 15 cm length) for the determination of denitrification rates, N_2O:N_2 emission ratios and CO_2 production rates at 6-week intervals between October 2003 and April 2004 (5 times). Each core was amended with 3.3 mL of 1g NO_3 L^{-1} solution to deliver 15 µg NO_3 g^{-1} dry soil to allow zero-order kinetics during denitrification with reference to NO_3 availability, and thus be able to assess CGT amendment effects. The soil core liners were capped at the base and put back in the holes from which the cores were collected to maintain field soil temperature conditions during incubation. To measure denitrification rates, 10 ml of purified C_2H_2 gas was injected in small aliquots into one of the duplicate cores at the interface of soil and plastic liner to ensure diffusion of C_2H_2 throughout the soil column (Ullah et al. 2005). After injection of C_2H_2 gas, the cores were capped and fitted with a gas-tight rubber stopper for gas sampling. The final headspace of each core after capping was 101 cm^3. After capping, about 10 ml additional C_2H_2 was replaced in the headspace of C_2H_2 injected cores using a syringe. To measure net N_2O emissions, the other core was incubated without C_2H_2 addition.

Gas samples were collected from the headspaces of cores with a hypodermic needle attached to a syringe at 0, 30 and 60 minutes duration for N_2O and CO_2 concentration determination. The samples were stored in 5-ml crimp-topped evacuated vials and transferred to the laboratory for analysis within one week of collection on a Varian CP38001 gas chromatorgraph (GC) equipped with an electron capture and flame ionization detectors (ECD and FID). The GC was fitted with a methanizer, which reduced CO_2 in the samples to CH_4 for detection by the FID. The rates of N_2O and CO_2

production were determined in µg N_2O-N m^{-2} h^{-1} and mg CO_2 m^{-2} h^{-1}, respectively. Corrections were made for dissolved N_2O and CO_2 by using the Bunsen's absorption coefficients of 0.54 and 0.75 respectively. N_2O:N_2 emission ratio was calculated from the difference of N_2O emitted from soil cores with and without C_2H_2 addition.

Soil Sampling

Bulk soil samples (0-10 cm deep) were collected from all 24 plots at six-week intervals between October 2003 and April 2004 using a mud-auger. The soil samples were transported on ice to the laboratory and refrigerated until use under their field-moisture conditions. Intact soil cores (5 cm dia. x 10 cm length) were collected from each plot using a slide hammer fitted with bronze liners for the determination of soil moisture, bulk density, total porosity and percent water-filled pore spaces (WFPS).

Soil Chemical Properties

Field-moist soils (5 gram oven-dry soil weight equivalents) were weighed into duplicate 250 ml glass bottles and 50 ml of 2 molar KCl solution was added to each bottle. The bottles were shaken continuously for 1 hour on a reciprocating shaker, centrifuged at a force of 50 Hertz/minute for 5 minutes, and were then filtered into 20 ml scintillation vials through a No.42 Whatman filter. The filtered samples were frozen until analyzed for NO_3 and NH_4 with an automated Lachat flow injection analyzer. Average NO_3 and NH_4 values for each soil sample were determined and reported in mg Kg^{-1} oven-dried soil. Soil pH was determined in the laboratory using 1:1 soil to de-ionized water mixing ratio.

The bulk soil samples collected from each plot were oven dried, homogenized thoroughly and pulverized. A subsample of about 35 mg was weighed into a tin capsule prior to their injection into a Thermo Finnigan CNS analyzer for total soil C and N contents at the USGS National Wetland Research Center, Lafayette, Louisiana. Total N and organic C concentrations and bulk density measurements were used to calculate the amounts of N and C present in the upper 10 cm on an area basis (Mt ha^{-1}).

Soil Physical Properties

Intact soil cores (5 cm dia. x 10 cm length) were collected at each sampling date and dried at 105 ^{o}C for 72 hours for the determination of soil moisture, bulk density and porosity. These values were used to determine the percent water-filled pore spaces (WFPS) for each core (Ullah et al. 2005) for the five sampling dates. Soil texture was determined by the modified pipette method (Sheldrick and Wang 1993). Soil temperature was measured with a soil temperature probe (inserted up to 10 cm depth) during the field denitrification studies. Some of the soil physico-chemical characteristics of the selected sites are given in Table 1.

Statistical Analysis

Differences in denitrification rates among the natural, CGT amended and unamended restored forests were analyzed by two-way ANOVA analysis using the general linear model. In the GLM model forest type and the 5 sampling dates were treated as the categorical variables to assess the significance of differences among denitrification rates of the restored and natural forested wetlands for each sampling date. Significant differences in selected physico-chemical properties, N_2O:N_2 emission ratio and mineralizable organic carbon production rates among the forest types were analyzed by one-way ANOVA. Fisher's protected LSD was used for comparison purposes at α = 0.05 for all the ANOVA analysis. Pearson's correlation coefficients among denitrification rates, mineralizable organic C, total soil C and N, NO_3 and NH_4 concentrations were determined. All statistical analyses were performed using SAS (SAS 1998). The proc univariate procedure in SAS was applied to the data to check if the data met the normal distribution and homogeneity of variance assumptions.

Results

Addition of CGT led to significant increases in denitrification rates in restored forested wetland plots. **In October, December, February, and March sampling, denitrification rates in the CGT-amended plots were 5.7, 1.4, 2.6, and 1.3 times greater than the unamended plots respectively, and were not significantly different from the natural forested wetland soils (Figure 2).** On average denitrification rates were lower in February than in December despite high %WFPS in all plots (93%) (Table 2). Decrease in soil temperature from 8 ^{o}C in December to 5.8 ^{o}C in February (Table 2) may have decreased denitrifier activity. In March, soil temperatures rose to 14 ^{o}C (Table 2) and denitrification rates increased significantly in the CGT-amended, unamended and natural forested wetland plots (Figure 3) compared to their rates in October, December, February and April. After 6 months of CGT addition (April sampling) denitrification rates of the CGT-amended plots remained higher than the unamended restored forest plots (1.4 times higher), although statistically not significant. When compared within each forest type, denitrification rates in the CGT-amended, unamended and natural forested plots were highest in March and lowest in October. Higher denitrification rates in March are attributed to higher %WFPS and mineralizable organic C contents relative to other sampling dates (Figure 2 and Tables 2 and 3).

Denitrification rates in all the plots correlated significantly with mineralizable organic C contents except in October and February sampling dates (Table 3). On average mineralizable organic C of the natural and CGT amended restored forest plots were 2.3 and 1.5 times greater than those of the restored forest plots without CGT addition. An exception occurred in October, shortly after the addition of CGT, when CGT amended plots contained more mineralizable organic C than the natural forest plots. No significant relationship between total soil C and N with denitrification rate was observed. Higher denitrification rates observed in CGT amended plots resulted in 1.2 times lower soil NO_3-N concentration compared to the NO_3 levels of the unamended plots (Table 1), even though CGT amendment added an estimated 31 mg NO_3-N m^{-2} area initially. Soil NO_3-N concentration of the natural forested wetland was also 1.3 times lower than those of the unamended restored forested soil, although statistically non-significant (Table 1).

Amending restored forest soil with CGT lowered N_2O:N_2 emission ratio by 33% compared to the unamended restored forest plots. However, due to the highly variable N_2O:N_2 emission ratio those differences were statistically non-significant, except in March (Figure 3). CGT amended and unamended restored forest plots had an average N_2O:N_2 emission ratio of 0.40 and 0.53, respectively, while natural forest plots had an average emission ratio of 0.35 (across all sampling dates).

Discussion

Significant differences in a number of soil properties (Table 1) among the selected forest types influenced denitrification rates. Lower bulk density, greater amount of total soil C, higher C:N ratio (Table 1) and wetter soil conditions (Table 2) in the soils of the natural forest ecosystem may have been the overriding factors supporting greater denitrifier activity than those observed in the unamended restored forest plots (Figure 2). These findings indicate that restored forested wetland maintained significantly lower denitrification rates than natural forested wetland, even though these measurements were performed 13 years after restoration and both sites possessed similar soil type and landscape position. Unlike vegetation structure and diversity which recover rapidly in restored forests (Ruiz-Jean and Aide 2005), biogeochemical scale functions in restored forested wetlands seem to be recovering at slower rates. Similar evidence is reported by Ruiz-Jean and Aide (2005), who concluded that nutrient cycling, litter turnover and bulk density in restored forests will take longer to recover to the level of mature forests.

Mineralizable organic C is an index of the amount of C substrate available to denitrifiers (Blackmer et a. 1980; Singh-Bijay et al. 1988). The 42% higher denitrification rates observed in the CGT amended plots compared to the unamended restored forest plots (Figure 2) were due to the availability of higher amounts of mineralizable organic C measured in the CGT amended plots (Table 3). These findings suggest that addition of readily decomposable organic C substrate like CGT can enhance denitrification rates in restored forested wetland soils to a level comparable to a more mature forest system. This result is consistent with the findings of Hunter (2000), who found 45% increase in denitrification rates in response to CGT amendment of soils collected from similar ecosystems in the LMV. These observations support our hypothesis that CGT addition enhances NO_3 removal through denitrification by providing a relatively higher and sustained organic C source to denitrifiers in restored forest soils in the LMV. **Mississippi, Louisiana and Arkansas lead the nation in the number of acres restored under the wetland reserve program (WRP) of the US Department of**

Agriculture (USDA). By year 2001, about 346,994 acres were enrolled by private landowners with USDA under the WRP program in the three states. These restored acres provide ample opportunities for CGT re-use. Thus the practice of CGT addition to restored wetland soils as a restoration technique to enhance denitrifier activity in soils can also help recycle tons of CGT waste produced in the LMV, besides water quality improvement.

In addition to forest age and organic C availability, WFPS also affected denitrification rates in both the restored and natural forest soils. Denitrification rates increased significantly with an increase in soil WFPS from an average 41% in October to 77% in December (Table 2). Similarly, denitrification rates were highest in March when the soils were saturated and the rates lowered again in April most likely due to drying of the soils as WFPS had declined to an average of 63% by the time of April sampling. It appears that lower soil temperature in February (Table 2) in spite of high WFPS resulted in the observed decrease in denitrification rates in all the plots, which is in agreement with the findings of Magg et al. (1997) who reported a decrease in denitrification potential in riparian buffer soils with a decrease in soil temperature. Our findings clearly show the importance of WFPS in regulating denitrifier activity in soils and are in agreement with similar studies (Linn and Doran 1984; de Klein and van Logtestijn 1996; Hefting et al. 2003; Ullah and Faulkner 2006). Given the importance of WFPS in regulating denitrifier activity (Ullah et al. 2005; Ullah and Faulkner 2006), denitrification rates in restored forested wetlands will depend on the extent of hydrologic modification of the restored sites in addition to available organic C substrates.

CGT-amended forest plots had relatively lower soil NO_3 concentration than the unamended plots. This observation support our finding that denitrifier activity in CGT amended plots was sustained at higher rates, which resulted in lowering soil NO_3 levels compared to the unamended plots. NO_3 immobilization by microbes in the CGT amended and natural forest plots with higher C:N ratios may have also contributed to lower soil NO_3 concentration in these plots compared to the uamended restored plots. Soils with higher C:N ratios have been reported to immobilize relatively more NO_3 than soils with lower C:N ratios (Silvan et al. 2003). At the observed rates of denitrification (averaged over the study period), the amount of soil NO_3 present per m^2 (in the upper 10 cm of soils) of natural, CGT-amended and unamended restored forests represent 29, 38 and 61 days supply in a year. Thus to maintain the observed denitrification rates and soil NO_3 levels in these forests, NO_3 must be continuously replenished either through nitrification or run-off from agricultural lands and river diversions. Being N limited, forested wetlands in the LMV can retain and reduce loss of external source NO_3 through denitrification besides plant uptake and microbial immobilization (Silvan et al. 2003). At the average observed rates of denitrification, mature forested wetlands in the LMV can denitrify 28 kg $N\text{-}NO_3$ ha^{-1} y^{-1}, which is in the range of denitrification rates (20 to 60 kg $N\text{-}NO_3$ ha^{-1} y^{-1}) in riparian forests reported by Mitsch et al. (2001) and Ullah et al. (2005).

Although CGT amendment significantly increased denitrification rates, it resulted in an averaged 33% lower $N_2O:N_2$ emission ratio than those observed from the restored site receiving no CGT (Figure 3). Being highly variable, the $N_2O:N_2$ emission ratios from the CGT and unamended restored forests sites were not significantly different from each other. Similar high scatter in N_2O relative to N_2 emissions during denitrification from forest soils is encountered by other researchers (Tilsner et al. 2003; Groffman et al. 2000a and 2000b). Higher scatter in the N_2O:N_2 emission data does not support our hypothesis that CGT addition significantly reduces net N_2O emissions during denitrification in forested wetlands. The effect of CGT on reducing N_2O:N_2 emission ratio may have been obscured by the additional NO_3 added to soil columns before incubation as higher NO_3 levels in soil leads to higher net N_2O emissions (Llyod 1995). **Since this research had no experimental replicates for both the restored and natural forest sites, therefore, temporally intensive (at daily cycle at least) N_2O emission monitoring from multiple restored and natural forested wetlands in the LMV is recommended to accurately quantify the effects of organic C substrates and NO_3 addition on net N_2O emission rates.**

In summary, these results demonstrate that even after 13 years, the restored forested wetland had not yet achieved the same denitrifying activity observed in the more mature but otherwise similar naturally forested site. Addition of CGT to restored forested wetlands in the LMV can enhance denitrification rates to the level of natural forested wetlands provided denitrification is not limited by lower WFPS and soil temperature. Restoration of forested wetlands in the LMV will require organic C amendments in order to provide the same level of NO_3 removal as natural forested wetlands before the restored forest succeed into a mature state.

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List of Figures:

Figure 1. Location map of the Panther Swamp National Wildlife Refuge

Figure 2. Denitrification rates of natural, CGT amended and unamended restored forested wetland soils in Panther Swamp National Wildlife Refuge, Mississippi. Similar small-scale letters on top of each bar shows no significant difference in emission ratio among the three forest types within each sampling date ($p > 0.05$). Error bars are standard error of the means.

Figure 3. Mean $N_2O:N_2$ emission ratio of natural, CGT-amended and unamended restored forested wetlands in Panther Swamp National Wildlife refuge, Mississippi. Similar small-case letters on top of each bar shows no significant difference in emission ratio among the three forest types within each sampling date ($p > 0.05$). Error bars are standard error of the means.

Table 1. Physio-chemical properties of soil (0-10 cm depth) with standard errors from restored and natural forested wetland sites samples collected in October 2003.

Variables	---------------------------Forest Types	----------------------------------	
	Natural	Restored + CGT[§]	Restored
Texture Class	Clay	Clay	Clay
% Clay	63 (1.1) a†	62 (2.9) a	60 (1.9) a
% Silt	22 (1.6) a	24 (3.5) a	25 (1.3) a
Bulk Density (g cm^{3})	0.85 (0.03) a	0.93 (0.02) b	0.93 (0.02) b
Porosity (cm^{3} cm^{-3})	0.68 a	0.65 a	0.65 a
pH	4.7	5.6	5.5
NO_3-N mg Kg^{-1}(5 months average)	2.7 (0.7) a	2.9 (0.6) a	3.4 (0.7) a
NH_4-N mg Kg^{-1} (5 months average)	7.2 (1.3) a	4.9 (1.0) ab	3.7 (1.0) b
Total soil C (%)	3.5 (0.2) a**	3.1 (0.1) ab	2.7 (0.1) b
Total soil N (%)	0.22 (0.01) a	0.22 (0.01)a	0.22 (0.01) a
C:N	15	14	12.3

[§] CGT: Cotton gin trash

† Means followed by different letters shows significant difference at $p < 0.05$ and ** $p < 0.10$ between forest types for different soil properties (ANOVA).

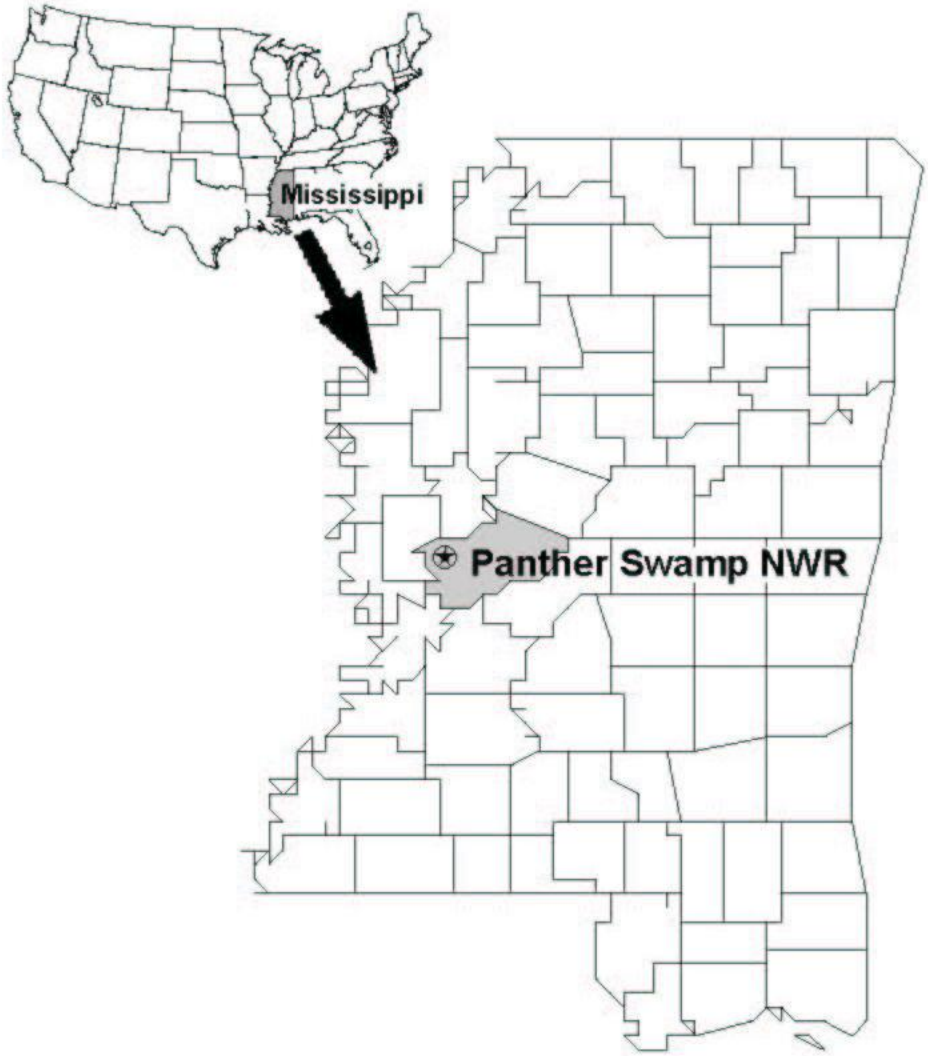

Figure 1. Location map of the Panther Swamp National Wildlife Refuge

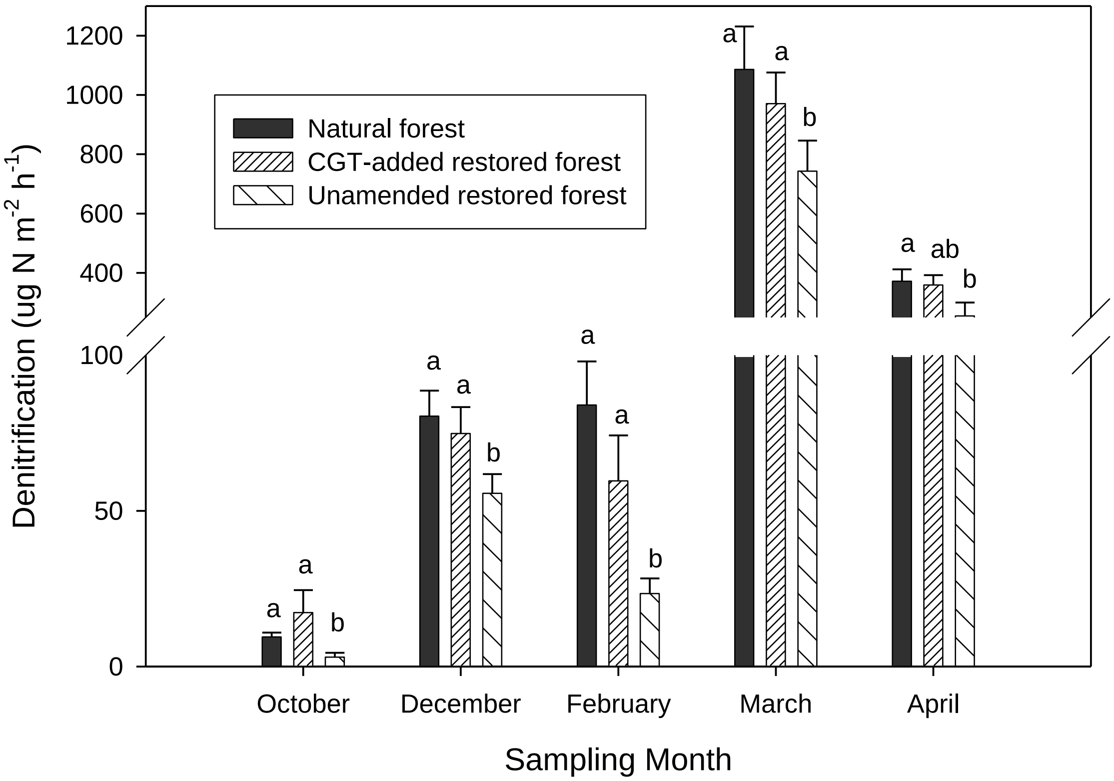

Figure 2. Denitrification rates of natural, CGT amended and unamended restored forested wetland soils in Panther Swamp National Wildlife Refuge, Mississippi. Similar small-scale letters on top of each bar shows no significant difference in emission ratio among the three forest types within each sampling date (p> 0.05). Error bars are standard error of the means.

Table 2. Percent water-filled pore space and soil temperature (0-10 cm) of restored and natural forest soils determined during denitrification studies. Each value in the table is an average of 8 data points. Values in brackets are standard error of the means.

Forest Type	% WFPS				
	October 2003	December 2003	February 2004	March 2004	April 2004
Natural Forest	37 (0.01)	71 (0.03)	92 (0.04)	100 (0.03)	70 (0.02)
Restored Forest	44 (0.02)	82 (0.01)	94 (0.02)	88 (0.02)	56 (0.02)
Soil Temp (^{o}C)	19	8	5.8	14	19

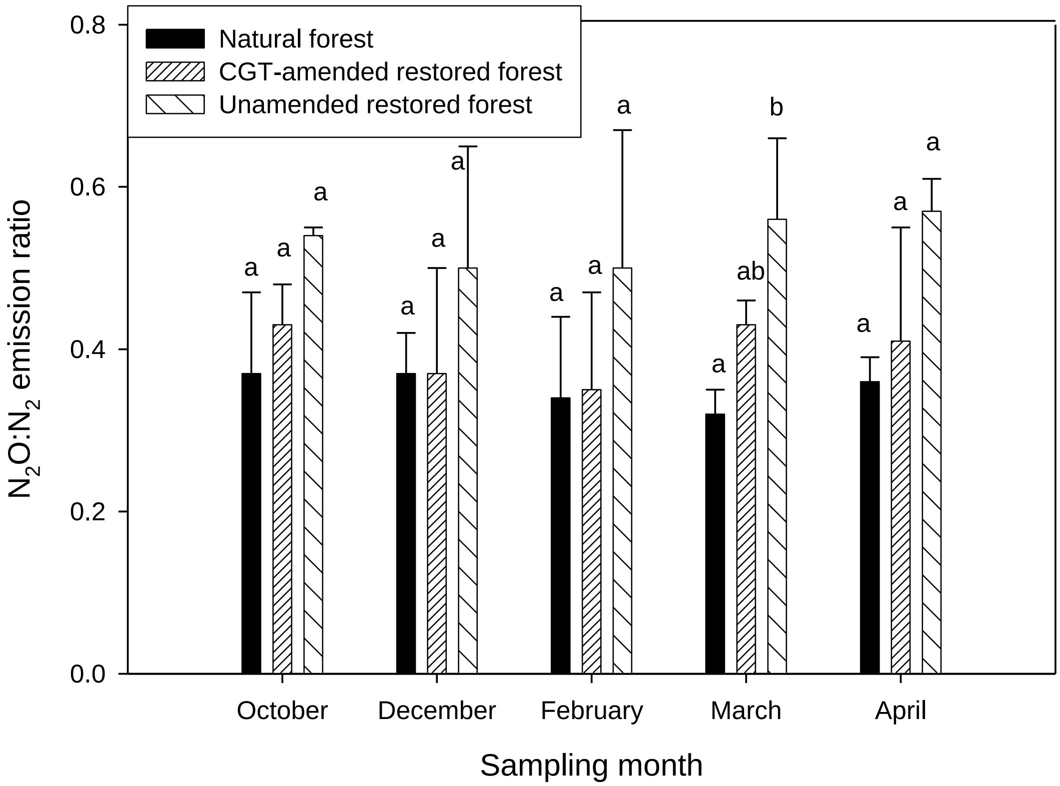

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Table 3. Organic carbon mineralization rate and its correlation with denitrification rates of restored and natural forested wetland soils. Each value in the table is an average of 8 data points. Values in brackets are standard error of the means.

Sampling times	------------Forest Types------------			Correlation of Organic C with denitrification
	Natural	Restored + CGT	Restored	
	--------mg CO_2 emitted $m^{-2}h^{-1}$------------			
October	955 (89) b[†]	1661 (212) a	1267 (196) ab	0.28 ns
December	109 (15) a	51 (9) b	44 (4) b	0.57*
February	37 (1.4) a	13 (3) b	10 (2) b	0.22 ns
March	249 (56) ab	379 (64) a	194 (30) b	0.56*
April	326 (26) a	307 (18) a	194 (29) b	0.70*

[†] Means followed by different letter indicate significant ($p < 0.05$) difference between forest types within each sampling time (ANOVA).

ns: non-significant, * significant correlation at $p < 0.05$ and $n = 24$