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Denitrification and N_2O emission from forested and cultivated alluvial clay soil

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Abstract. Restored forested wetlands reduce N loads in surface discharge through plant uptake and denitrification. While removal of reactive N reduces impact on receiving waters, it is unclear whether enhanced denitrification also enhances emissions of the 'greenhouse' gas N_2O, thus compromising the water-quality benefits of restoration. This study compares denitrification rates and N_2O:N_2 emission ratios from Sharkey clay soil in a mature bottomland forest to those from an adjacent cultivated site in the Lower Mississippi Alluvial Valley. Potential denitrification of forested soil was 2.4 times of cultivated soil. Using intact soil cores, denitrification rates of forested soil were 5.2, 6.6 and 2.0 times those of cultivated soil at 70, 85 and 100% water-filled pore space (WFPS), respectively. When NO_3 was added, N_2O emissions from forested soil were 2.2 times those of cultivated soil at 70% WFPS. At 85 and 100% WFPS, N_2O emissions were not significantly different despite much greater denitrification rates in the forested soil because N_2O:N_2 emission ratios declined more rapidly in forested soil as WFPS increased. These findings suggest that restoration of forested wetlands to reduce NO_3 in surface discharge will not contribute significantly to the atmospheric burden of N_2O.

Abbreviations: LMV – Lower Mississippi Alluvial Valley; PDA – potential denitrification assay; WFPS – water-filled pore space

Introduction

Seasonally flooded lower elevation clay alluvium lands in agricultural watersheds within the Lower Mississippi River Valley (LMV) are typically bottomland hardwood forests. The Sharky soil series (very-fine, smectitic, thermic, Vertic Haplaquepts) is one of the major soil types in the LMV, an area historically developed under bottomland hardwood forests. This very poorly drained, very slowly permeable soil formed in clayey alluvium of the Mississippi River (Fisk 1951). About 78% of native bottomland hardwood forest has been cleared in the LMV since European colonization (MacDonald et al. 1979) primarily for agricultural purposes. Because of the exceptionally poor natural drainage of Sharky and similar clay alluvium soils, it is generally necessary to ditch and drain these areas after clearing in preparation for crop production. These fields are used mainly for soybean, rice, corn, wheat, cotton, grain sorghum and pecan orchards (NRCS 1959).

Conversion of forested wetlands to agricultural lands in the LMV altered the natural role of this ecosystem from potential sinks for NO_3 and sediments to potential sources leading to water-quality problems (Mitsch et al. 2001). Drainage and cropping of Sharky and similar soils over decades is likely to have altered their potential for denitrification compared to Sharky soil maintained under bottomland hardwood forests (Hunter and Faulkner 2001). Moreover, N fertilizer use in agricultural watersheds with reduced denitrification potential may partly enhance their contribution of biologically available N to receiving water bodies (Breitenbeck et al. 1980; Baggs et al. 2002). Application of N fertilizer results in accumulation of NO_3 in surface soil that is subject to runoff during rainfall, storm events and irrigation. NO_3 runoff from croplands, if not denitrified, can reach water bodies and subsequently contribute to eutrophication (Mitsch et al. 2001).

Restoration of marginally productive, low-lying agricultural fields to bottomland forests has become a highly recommended practice in the LMV (Mitsch et al. 2001). A number of conservation programs such as the Conservation Reserve program, Wetland Reserve program and conservation buffer programs sponsored by the Natural Resource Conservation Service (NRCS) and other federal and state programs have been established to offset the costs of restoration to landowners (Allen et al. 2001). Restoration of bottomland forests, especially the restoration of riparian zones along streams and rivers, can reduce the impact of nearby agricultural production on surface water quality by entrapping eroded sediments and plant nutrients (Sanders and Kalff 2001). Forested bottomlands can also intercept flow and accelerate denitrification of NO_3 in agricultural runoff prior to discharge into receiving water bodies. An additional advantage of bottomland restoration is the ability of these systems to sequester atmospheric CO_2 into stable organic forms (Turner et al. 1995). There is growing interest in subsidizing the restoration of bottomlands in the LMV by marketing carbon credits to power generators and other facilities releasing CO_2 from fossil fuel consumption. Because restored bottomland forests enhance denitrification, the possibility that they will also enhance the atmospheric burden of N_2O, a greenhouse gas, remains a principal uncertainty in establishing their carbon credit value.

The end products of denitrification are N_2O and N_2 gas, which are emitted to the atmosphere (Sexstone et al. 1985; Weier et al. 1993; Dalal et al. 2003). The emission ratio of N_2O:N_2 is affected by soil moisture, NO_3 concentration, pH, available carbon (Sahrawat and Kenney 1986; Groffman and Tiedje 1988; Weitz et al. 2001) and soil management (Khalil et al. 2002; Simek et al. 2004). While restoration of forested wetlands is very likely to enhance the extent of denitrification, the impact on N_2O:N_2 emission ratio is more difficult to predict on the basis of soil management, different hydrologic regimes and NO_3 loading. An increased N_2O:N_2 emission ratio can compromise the benefits of restoration because N_2O is a potent greenhouse gas that has an atmospheric radiative forcing 320 times more than that of CO_2 (Granli and Bockman 1994; Tilsner et al. 2003). Increasing the atmospheric burden of N_2O poses an additional threat because of the capacity of this gas to catalyze stratospheric ozone depletion. Mitsch et al. (1999) speculated that restoration projects will not significantly affect the atmospheric burden of N_2O. The validity of this observation is dependent not only on the extent of land restored, but changes in the rates of denitrification and N_2O:N_2 emission ratio as a result of different restoration techniques. Even so, the N_2O emission remains important when assessing the overall value of riparian restoration in the current efforts to market greenhouse gas emission credits.

The primary objective of this study was to compare N_2O:N_2 emission ratios from soils under row crop cultivation and under mature bottomland hardwood forest collected from adjacent sites occupying similar positions in the landscape. Soil water content and NO_3 concentration can influence N_2O:N_2 emission ratio as well as denitrification rate, and therefore emissions were compared under NO_3 amendment and a range of water contents ranging from 70% water-filled pore space (WFPS) to saturation. Because of extensive area of Sharky soil within the LMV and its extensive use for crop production, sites containing Sharky soil were selected for this study.

Materials and methods

Field sites

Two adjacent sites occupying similar positions on the landscape were located in the Beasley Agricultural Watershed in the Yazoo Delta region of northwestern Mississippi. One site consisted of a mature riparian zone of bottomland hardwood forest dominated by American elm (*Ulmus Americana*), oaks (*Quercus* spp.), red maple (*Acer ruburum*), hackberry (*Celtis leavigata*) and green ash (*Fraxinus pensylvanica*). The adjacent site had been cleared and ditched more than 20 yr previously for cultivation of soybeans (*Glycine max* (L.) Merr.) and occasional other crops. These near-level sites are within the floodplain of Sunflower River, a tributary of Mississippi River, and drain into Beasley Lake, an oxbow occluded from the Sunflower River. Sharkey is the predominant soil series throughout the lower elevations of this region. This fine-textured soil is high in montmorillonite clay, very poorly drained and very slowly permeable. Selected physiochemical properties of the soil within the sampling sites are shown in Table 1.

Soil sampling

In July 2002, soil samples (0–10 cm) were collected from eight sampling sites located within the forested zone and the adjacent cultivated area for the

Table 1. Physiochemical properties of forested and cultivated Sharky soil (± standard error of the mean).

Soil property	Units	Forested soil	Cultivated soil
Bulk density	g cm^{-3}	0.86 ± 0.03	1.20 ± 0.01
Total pore space	cm^3 cm^{-3}	0.68 ± 0.01	0.54 ± 0.00
Clay	g/100 g	53	51
Silt	g/100 g	45	46
pH		5.4	6.1
Organic carbon	g kg^{-1}	37.7 ± 3.2	16.4 ± 0.8
Total N	g kg^{-1}	3.3 ± 0.2	1.6 ± 0.1
C:N		11.4	10.3
Soluble organic C	mg kg^{-1}	152 ± 14	137 ± 11
Mineralizable organic C	mg CO_2 m^{-2} h^{-1}	122 ± 34	29 ± 2.1
Organic C (0–15 cm)	Mt ha^{-1}	324	197
Total N (0–15 cm)	Mt ha^{-1}	28	19
NO_3	mg kg^{-1}	3.30 ± 0.25	3.5 ± 0.27

determination of potential denitrification assay (PDA). In July 2003, 15 sampling sites were randomly selected within each of the ecosystems. At each sampling site, two intact soil cores of (5 cm diameter × 10 cm height) were collected in plastic liners (5 cm diameter × 15 cm height) using a slide hammer core sampler (AMS Inc., American Falls, ID). Liners were capped on each end to create columns with an average 101 cm^{-2} of headspace above the soil core surface. The columns were put on ice for transport to the laboratory. Bulk soil samples (0–10 cm) were also collected from each site for destructive determination of soil moisture, pH, particle size distribution, and concentrations of soluble N (NO_3^-), soluble organic C, total organic C, and total N. Additional intact soil cores (5 cm diameter × 10 cm) were collected at each site using rings fitted in the AMS core sampler for determination of bulk density, total porosity and WFPS. Bulk density of the moist cores was calculated by dividing initial volume by the mass determined gravimetrically after oven-drying for 48 h (105 °C). Total porosity was determined by the displacement caused by dispersal of field-moist soil cores in water and adjusted for initial soil moisture content. Water-filled porosity was calculated as the difference between total porosity and moisture content, assuming a water density of 1 g cm^{-3}.

Potential denitrification assay (PDA)

PDA analyses (Tiedje 1982) were performed to compare denitrification potential in a similar soil type from adjacent forested and cultivated sites. Soil samples were homogenized by hand and 10 g (field-moist) weighed into each of six 150-ml glass serum bottles. Three were amended with 15 ml NO_3 solution (10 mg NO_3 l^{-1}) and 5 ml deionized water. Twenty milliliter of deionized water was added to the other three bottles. All bottles were fitted with airtight septum caps and purged with oxygen-free N_2 gas for 20 min to induce anaerobic conditions. About 15% of the headspace was replaced with acetylene (C_2H_2) to block the enzymatic reduction of N_2O to N_2 gas during denitrification. Prior to use, C_2H_2 was purified by bubbling through 1 M $CuCl_3$ solution. The bottles were wrapped in aluminum foil and placed on a reciprocating Eberbach shaker for continuous shaking at low speed and room temperature (22–25 °C). Headspace samples were collected after 2, 4 and 6 h incubation using a syringe and transferred to Vacutainers for analysis of N_2O concentration using a Tremetric 9001 gas chromatograph fitted with a Porapak Q column and equipped with an electron capture detector (ECD). N_2O concentration of the headspace samples was determined using a standard calibration curve and total N_2O production rate (μg N_2O-N g^{-1} h^{-1}) was calculated after making adjustment for dissolved N_2O using the Bunsen absorption coefficient.

Determination of denitrification and N_2O emission rates

One column from each sample pair was used to measure total denitrification by the acetylene-blockage technique (Tiedje 1982). Percent WFPS of the soil cores were determined (Linn and Daron 1984) and paired cores were randomly assigned to various treatments, by addition of dilute NO_3 solution to deliver 15 μg NO_3-N g^{-1} soil. To ensure even distribution of water and NO_3 solution, they were added by multiple injections using a syringe fitted with a 16 gauge × 10 cm needle. Initial WFPS were 70, 85 and 100%. Emissions from cores adjusted to 85 and 100% with NO_3 solution were compared to those adjusted to similar WFPS with deionized water (all treatments were performed in triplicates).

After addition of NO_3 or water and WFPS adjustments, the cores were capped tight at both ends. One column from each sample pair was injected with 10 ml of purified C_2H_2 in small aliquots at the interface between the soil core and liner using a syringe fitted with a 16 gauge × 10 cm needle. An additional 10 ml of C_2H_2 was injected into the headspace after the tubes were sealed with airtight caps fitted with septum to allow periodic sampling of the headspace atmosphere. Addition of C_2H_2 to one column from each pair was used to determine total denitrification and N_2O:N_2 emission based on the difference of N_2O emitted by the paired cores. The columns were incubated for 72 h (22–25 °C) and gas samples were collected daily from the headspaces for N_2O and CO_2 determination. Gas samples were stored in Vacutainers® until GC analysis described above.

Soluble organic carbon

Soluble organic carbon (SOC) of the soil samples was determined using the technique described by Mahdun et al. (1986). Ten gram field-moist soil was weighed into duplicate 250 ml acid washed clean flasks. To each flask 100 ml deionized water was added and the slurry was shaken at reciprocating shaker at high speed for 30 min. After shaking, the suspension was allowed to stand for 20 h (overnight) in a refrigerator. The solution was shaken by hand and about 40 ml of the solution was poured into centrifuge tubes for centrifugation at 6500 rpm for 20 min at room temperature. Thirty milliliter of the supernatant was filtered into cleaned scintillation vial through a Whattman filter paper (0.45 μM size) and stored in refrigerator at 4 °C. The samples were analyzed using a Shimadzu TOC Analyzer for SOC concentration. The SOC values for the duplicate samples were averaged and reported as SOC in mg g^{-1} of oven-dried soil.

Total soil carbon, total nitrogen and nitrate

Total soil carbon (C) and nitrogen (N) were determined using a Shimadzu CNS Analyzer. Soil samples were oven dried, pulverized and thoroughly homogenized. A sub-sample of about 35 mg was weighed into a tin capsule for automated analysis. Total soil carbon and nitrogen are reported in Mt ha^{-1} oven-dried soil. Soil NO_3 was determined using 2 M KCl soil extracts and a Lachat Flow Injection Analysis instrument. These values are reported as mg NO_3-N kg^{-1} soil.

Statistical analysis

Denitrification rates and N_2O:N_2 emission ratio of forested and cultivated soil was tested for significant differences (Fisher's LSD at alpha of 0.05) at different WFPS using the pooled error from ANOVA (SAS, Inc. 1998). Effects of ecotype and NO_3 additions on PDA and denitrification rates were also compared by ANOVA (SAS, Inc. 1998).

Results

PDA of forested soil averaged 1.42 μg N g^{-1} h^{-1} dry soil, whereas the PDA of cultivated soil was significantly less (0.62 μg N g^{-1} h^{-1} dry soil). When no NO_3 was added, total denitrification rates were similar and averaged 0.67 and 0.84 μg N g^{-1} h^{-1} in forested and cultivated soils, respectively (Figure 1). Addition of NO_3 caused a 129% increase in denitrification rate in forested soil, but had no significant impact on denitrification in cultivated soil. The response to added NO_3 is reflected in the amounts of total and mineralizable C found in soil of the two ecosystems (Table 1). Mineralizable carbon averaged 122 and 29 μg CO_2 g^{-1} h^{-1} dry soil in the forested and cultivated soils, respectively.

Denitrification rates of the soil cores collected from both forest and cultivated sites showed an increase with increase in soil WFPS from 70 to 100%

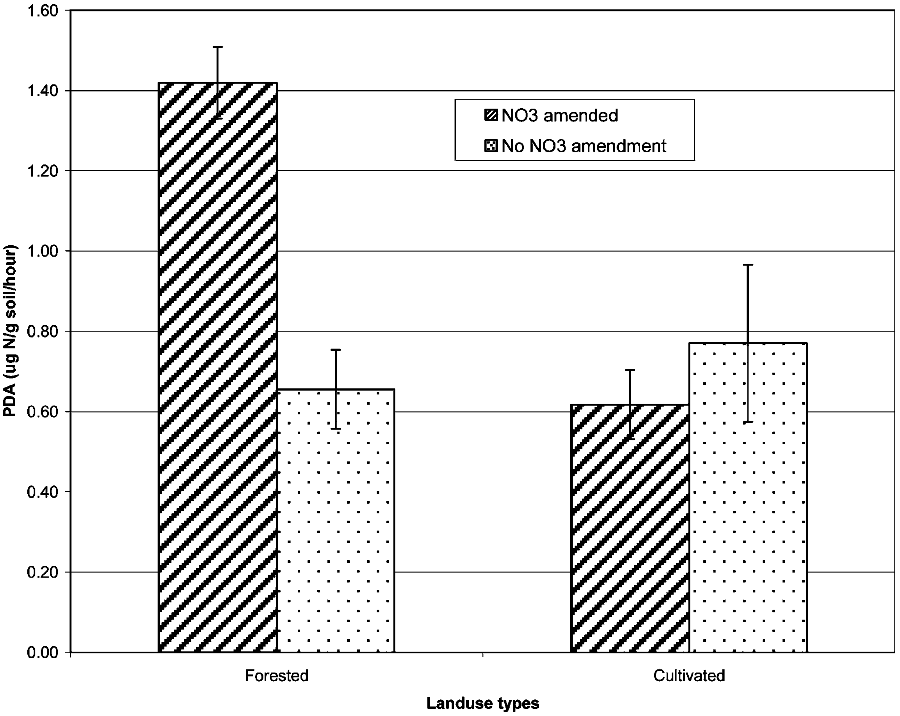

Figure 1. Denitrification potential of forested wetland and cultivated soil. Error bars represent standard error of the mean ($p = 0.05$).

(Table 2). Mean denitrification rates in NO_3 amended forest soil increased from 0.89 mg N m^{-2} h^{-1} at 70% WFPS to 2.04 mg N m^{-2} h^{-1} at 100% WFPS. The corresponding increases in cultivated soil were from 0.17 to 1.02 mg N m^{-2} h^{-1}. Denitrification rates of forested soil amended with NO_3 were significantly higher than that of cultivated soil at 70 and 85% WFPS

Table 2. Denitrification and net N_2O emission rates of forested and cultivated Sharky soil.

WFPS[a]	NO_3^- added 15 μg N g^{-1}	Denitrification rate mg N m^{-2} h^{-1}			N_2O emission rate mg N m^{-2} h^{-1}		
		Forested	Cultivated	Diff.[b]	Forested	Cultivated	Diff.[b]
70%	+	0.89 ± 0.28	0.17 ± 0.06	*	0.25 ± 0.09	0.11 ± 0.03	*
85%	+	3.28 ± 0.29	0.49 ± 0.17	*	0.40 ± 0.13	0.32 ± 0.13	ns
85%	–	0.77 ± 0.29	0.27 ± 0.23	ns	0.09 ± 0.03	0.15 ± 0.12	ns
100%	+	2.04 ± 0.62	1.02 ± 0.40	ns	0.22 ± 0.05	0.40 ± 0.28	ns
100%	–	1.35 ± 1.0	1.49 ± 1.01	ns	0.15 ± 0.02	0.29 ± 0.20	ns

Data show the mean and standard errors of three replicate analyses.

[a] WFPS, water-filled pore space.

[b] *Significant difference ($p < 0.05$) between ecotypes using pooled variance *t*-test; ns, differences not significant.

($p < 0.05$). At 100% WFPS, the average denitrification rate of the forested soil with NO_3 addition was 2.04 mg N m^{-2} h^{-1}, much higher than the corresponding rate of cultivated soil (1.02 mg N m^{-2} h^{-1}).

Addition of NO_3 led to a marked increase in denitrification rate in forested soil, but had a lesser effect on denitrification rate in cultivated soil. NO_3 amended forest soil at 85 and 100% WFPS resulted in average denitrification rates of 3.28 and 2.04 mg N m^{-2} h^{-1}, respectively (Table 2). These rates were 4.3 and 1.5 times those observed in cores at 85 and 100% WFPS without added NO_3. In contrast, NO_3 amendment of cultivated soil did not lead to significantly greater denitrification at 85 and 100% WFPS. These findings are similar to those found in PDA analysis.

Overall mean N_2O:N_2 emission ratios decreased with increasing WFPS and were greater in cultivated soil than in forested soil (Figure 2). In forested soil, the N_2O:N_2 ratio decreased from 0.28 to 0.11 as WFPS increased from 70 to 100% WFPS. WFPS greater than 70% led to a marked decrease ($p < 0.05$) in the N_2O:N_2 emission ratio of forested soil both with and without NO_3 additions. In cultivated soil, the N_2O:N_2 emission ratio decreased from 0.64 to 0.19 as WFPS increased from 70 to 100%. At 70 and 85% WFPS, the N_2O:N_2 emission ratio of cultivated soil was similar with and without NO_3 amendment,

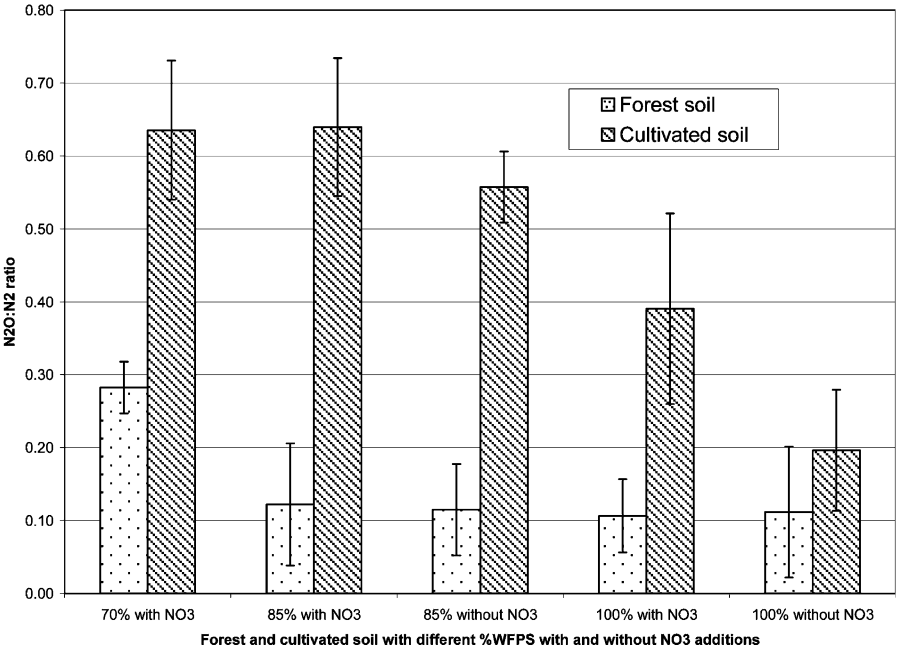

Figure 2. Ratio of N_2O to N_2 evolved from forested and cultivated soil adjusted to various WFPS with or without the addition of added NO_3^-. Error bars represent standard error of the mean ($p = 0.05$).

while at 100% WFPS ratio of the NO_3 amended cores was double the corresponding ratio of unamended cores.

To compare the potential contribution of these ecotypes to the atmospheric burden of N_2O, the net N_2O emission rate was calculated on an area basis assuming a microbially active soil depth of 10 cm. In forested soil, N_2O emission rates were 0.25, 0.40 and 0.22 mg N m^{-2} h^{-1} at 70% WFPS, 85% WFPS, and 100% WFPS, respectively, when amended with NO_3 (Table 2). The corresponding emissions from cultivated soil were 0.11, 0.32 and 40 mg N m^{-2} h^{-1} at 70, 85 and 100% WFPS, respectively, from NO_3 amended soil (Table 2). These findings indicate that the contribution of N_2O from forested and cultivated soil were similar at higher moisture contents, but that the forest soil emitted significantly greater amount of N_2O at 70% WFPS ($p < 0.05$).

The distribution of clay and silt was similar in forested and cultivated soils (Table 1), though significant differences in a number of soil properties were observed. These differences in adjacent soils were likely due to differences in land use over the past decades. The forested soil was more acidic (pH 5.4) than the cultivated soil (pH 6.1). Perhaps the most notable differences between the soils of these two ecosystems were the substantially greater organic matter and porosity of soils under forest. Soil organic C in forested soil was 37 g kg^{-1} dry soil, more than twice that of the cultivated soil (16 g kg^{-1} soil). Higher soil organic matter contributed to improved soil structure and greater porosity. Soil bulk densities in forested soil averaged 0.84 g cm^{-3}, nearly 40% less than those of cultivated soils (1.20 g cm^{-3}). Total porosities in the forested and cultivated soils averaged 0.68 and 0.54 cm^3 cm^{-3}, respectively.

The amounts of NO_3 available to support denitrification were similar in both ecosystems (Table 1), although the amount of total soil N in the forested soil (3.3 g kg^{-1}) was more than twice that of the cultivated soil (1.6 g kg^{-1}). The ratio of C to N was slightly greater in the forested soil, suggesting a somewhat greater pool of readily degradable organic carbon. Aerobic incubation showed that the amounts of mineralizable C in the forested soil averaged 4.2 times those in the cultivated soil and this substrate undoubtedly contributed to greater denitrification activity observed in the forested soil (Burford and Bremner 1975; Singh et al. 1988; Weier et al. 1993). It is noteworthy that despite differences in total and mineralizable C, the amounts of water–SOC in the forested and cultivated soil were similar (152 and 137 μg SOC g^{-1}, respectively). The ratio of mineralizable carbon to SOC in forest soil was 3.8 times that of cultivated soils, suggesting that the quality of SOC in the forested soil was more suitable for use by denitrifying microorganisms.

Moisture contents of soil samples collected through out the year showed that the percentage of WFPS in the forested soil averaged 79% whereas the WFPS in the cultivated soil averaged only 50% (Table 3). Moisture contents and WFPS % were lower in summer than at other seasons. Moisture contents, expressed as WFPS %, were similar in samples collected in spring, fall and winter and averaged 84 and 55%, respectively, in forested and cultivated soils.

Table 3. Seasonal percent water-filled pores spaces of forested and cultivated soil.

Ecotype	Spring	Summer	Fall	Winter
	WFPS %			
Forest soil	86	64	81	84
Cultivated soil	56	34	55	54

Discussion

Land use resulted in differences in the physiochemical attributes of a heavy, alluvial clay soil (Sharkey) that influenced its capacity to denitrify. Wetter soil conditions, increased soil porosity, and higher amounts soil organic C reserves in the forested ecosystem lead to a greater capacity to retain and denitrify NO_3^- in runoff from nearby cultivated fields. This conclusion is supported by PDA analyses (Figure 1) and incubations of intact soil cores (Table 2), which showed that soil from mature bottomland forests possesses a greater potential to denitrify than a similar cultivated soil occupying a similar position on the landscape.

When soils from each ecosystem were amended with NO_3^- and incubated as slurries under anaerobic conditions, the potential denitrification rates in forested samples averaged nearly $2\frac{1}{2}$ times those in samples from adjacent cultivated areas (Figure 1). When no NO_3^- was added, denitrification rates were similar for the two ecosystems. Addition of NO_3^- caused a marked increase in denitrification rate in forested soil but did not cause a similar response in cultivated soil. These findings indicate that potential denitrification rate in the forested ecosystem is limited by NO_3^-, and by other factors, most probably C substrate availability, in the cultivated system.

Experiments using intact soil cores showed that in addition to land use and NO_3 concentration, WFPS % (Table 3) influenced not only the rate of denitrification but also the ratio of N_2O to N_2 emitted (Figure 2). Denitrification rates in cores of forested soil averaged 2.66 mg N m^{-2} h^{-1} when amended with 15 mg N kg^{-1} as NO_3 and were 3.5 times greater than the corresponding rates in cultivated soil. It is noteworthy that even though unamended cores from both ecosystems contained low amounts of NO_3 (average 3.4 mg N kg^{-1}), denitrification rates at moisture contents of 85 and 100% WFPS averaged 1.06 and 0.88 mg N m^{-2} h^{-1} in forested and cultivated soils, respectively. In the cultivated system, water content typically remains below that where denitrification rates can be maintained (Table 3). The moisture content of the forested soil, however, remains above 80% WFPS throughout the year except in summer. At the denitrification rates observed, the amount of soil NO_3 present in upper 15 cm of forested soil represents less than a 161 h supply, and therefore soil NO_3^- must be continually replenished through runoff or mineralization and nitrification to maintain the denitrification rates and soil NO_3

levels observed in unamended cores. Comparison of denitrification rates determined after 24 h (data not shown) were more variable but not significantly different from those determined after 72 h incubation for any treatment, suggesting that NO_3 or substrate depletion during incubation did not significantly influence denitrifying activity.

In the forested systems, denitrification rates at 85 and 100% WFPS were 3.7 and 2.3 times, respectively, those observed at 70%. Addition of NO_3 increased denitrication rates in forested soils by 4.2 and 1.5 times at 85 and 100% WFPS, respectively, and supports the conclusion that denitrification rates in that system are limited by NO_3 rather than C availability. In the cultivated systems, denitrification rates at 85 and 100% WFPS were 2.8 and 6.0 times greater, respectively, than at 70%. Unlike the forested system, addition of NO_3 did not lead to a significant increase in denitrification rates at higher moisture contents.

Whereas denitrification rates increased with increasing WFPS, the ratios of N_2O to N_2 decreased from 0.28 to 0.11 in forested soils and from 0.64 to 0.39 in cultivated soils as WFPS increased from 70 to 100%. At lower WFPS %, the higher ratios of N_2O to N_2 observed may have been augmented by the production of N_2O by nitrifying rather than denitrifying populations (Blackmer et al. 1980). At high WFPS %, the diffusion of O_2 into the soil is reduced, promoting conditions favorable for denitrification. Increased WFPS also reduces the diffusion of N_2O from the soil, increasing the probability that this gas will be subsequently reduced to N_2 by active denitrifier populations. It is not surprising, then, that numerous studies have observed an increase in denitrification rate and decrease in $N_2O:N_2$ as WFPS % increases (Mosier and Hutchison 1981; Sexstone et al. 1988; Weier et al. 1993; Klein De and Van Logtestijn 1996; Hunter and Faulkner 2001; Weitz et al. 2001).

Few studies have assessed the effects of cultivation on the extent or products of denitrification. Linn and Doran (1984) compared the effects of tillage systems on emissions of N_2O and CO_2 from agricultural soils at various % WFPS. While those studies did not measure the extent of denitrification, they showed that N_2O emissions from soils collected from no-till systems averaged 9.4 times those of 'plowed' soils. These differences were attributed largely to differences in % WFPS that averaged 62% under no till and 44% with tillage. In the current study, soil water contents of samples collected at various times in 2002–2003 averaged 79% WFPS in forested soils and 50% in cultivated soils, suggesting that denitrification in bottomland hardwoods remains active throughout most of the year whereas drained, cultivated soil remain aerated and denitrification is likely to occur only after periods of intense rainfall.

The significantly greater ratios of N_2O to N_2 evolved from cultivated soil were undoubtedly due to several factors and their interactions. A number of marked differences in physicochemical properties were evident between cultivated and forested soil, including soil pH, bulk density, porosity and organic matter content. Soil pH in the forested soil (pH 5.4) was significantly less than in the cultivated soil (pH 6.1). Gaskell et al. (1981) reported that the ratio of N_2O to N_2 evolved during denitrification increases sharply as soil pH decreases, but this relationship is not supported by the data in Figure 1 showing higher $N_2O:N_2$ evolution from the soil with higher pH. The greater amounts of available organic substrate in the forested soil may have been the overriding factor influencing $N_2O:N_2$ ratios by supporting more complete reduction during denitrification (Vinther 1984; Weier et al. 1993; Tilsner et al. 2003).

Despite lower ratios of $N_2O:N_2$, higher denitrification rates in the forested soil resulted in somewhat larger emissions of N_2O at the lower moisture content (70% WFPS). Differences in emissions of N_2O from intact soil cores at 85 and 100% WFPS from forested areas were not statistically different than those from cultivated areas. While the denitrification rates observed indicate that at least some flooded pores were sufficiently reduced at 70% WFPS in both soils to support denitrifying activity, the forested soil contained a greater volume of air-filled pores because of its higher total porosity. At 70% WFPS, for example, the forested soil cores contained 0.20 $cm^3 \ cm^{-3}$ air-filled pores, whereas the cultivated soil contained only 0.16 $cm^3 \ cm^{-3}$. This difference in air volume may have facilitated more rapid gaseous diffusion at lower WFPS, reducing the possibility of subsequent reduction of N_2O to N_2. It is also possible that the greater volume of air-filled pores at low WFPS and the substantially larger amounts of mineralizable organic matter in the forested soil led to more extensive N mineralization and release of N_2O during nitrification. At saturation (100% WFPS), N_2O emissions tended to be greater from cultivated soil, though these differences were below statistical significance ($p > 0.05$).

When no NO_3^- was added, N_2O emissions from the forested soil averaged 10.5 kg N_2O-N $ha^{-1} \ yr^{-1}$. Addition of 15 mg NO_3^--N kg^{-1} resulted in average emissions of 25.4 kg N_2O-N $ha^{-1} \ yr^{-1}$, and were within the range of 24–30 kg N_2O-N $ha^{-1} \ yr^{-1}$ reported for other riparian wetlands (Walker et al. 2002; Hefting et al. 2003). N_2O emissions from the cultivated area averaged 24.2 and 19.3 kg N_2O-N $ha^{-1} \ yr^{-1}$ with and without the addition of NO_3^-, respectively. The finding that addition of NO_3^- invariably led to an increase in total denitrification as well as N_2O production at all water contents but did not significantly alter the ratio of N_2O to N_2 evolved suggests denitrification rather than nitrification plays a principal role in N_2O production in these soils.

The higher amounts of soil organic matter observed in forested areas support the general conclusion that restoration of bottomland hardwoods not only results in an increased capacity to denitrify, but also increases sequestration of atmospheric C both as stable soil humus as well as standing biomass. The organic C content of the forested soil (37.7 g C kg^{-1}) was more than twice that of the cultivated soil (16.4 g C kg^{-1}). While the bulk density of the surface soil in the forested soil was substantially less than that of the cultivated soil, the forested system nevertheless contained 1.6 times more soil organic carbon in the upper 10 cm. Extrapolating to a hectare basis, the forested soil contained 32,400 kg C ha^{-1} in the surface 10 cm or 12,700 kg ha^{-1} more C sequestered as organic matter than in the cultivated system. The sequestration of organic C invariably results in the sequestration of N in organic forms. Despite the higher denitrification rates in the forested system, the surface 10 cm of soil contained 2838 kg N ha^{-1}, or 918 kg N ha^{-1} more than in the cultivated soil.

In summary, the results of these experiments indicate that denitrifying activity is more persistent and rapid in forested bottomland soil than in similar cultivated soil. Even so, N_2O emissions resulting from the restoration of bottomland forests are likely to be similar to that of similar cultivated soil due to a reduced ratio of N_2O to N_2 evolved from forested soil at water contents that support high rates of denitrification.

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References

Allen J.A., Keeland B.D., Stauturf J.A., Clewell A.F. and Kennedy H.E. Jr. 2001. A guide to bottomland hardwood restoration. Technical Report, USGS/BRD/ITR-2000-0011, 132 pp.

Baggs E.M., Rees R.M., Castle K., Scott A., Smith K.A. and Vinten A.J.A. 2002. Nitrous oxide release from soils receiving N-rich crop residues and paper mill sludge in eastern Scotland. Agric. Ecosyst. Environ. 90: 109–123.

Blackmer A.M., Bremner J.M. and Schmidt E.L. 1980. Production of nitrous oxide by ammonia-oxidizing chemiautotrophic microorganisms in soil. Appl. Environ. Microbiol. 40: 1060–1066.

Breitenbeck G.A., Blackmer A.M. and Bremner J.M. 1980. Effects of different nitrogen fertilizers on emissions of nitrous oxide from soils. Geophys. Res. Lett. 7: 85–88.

Burford J.R. and Bremner J.M. 1975. Relationship between the denitrification capacities of soils and total, water-soluble and readily decomposable soil organic matter. Soil Biol. Biochem. 7: 389–394.

Dalal R.C., Wang W., Robertson G.P. and Parton W.J. 2003. Nitrous oxide emission from Australian agricultural lands and mitigation. Aust. J. Soil Res. 41: 165–195.

Fisk N.H. 1951. Mississippi River geology relation to river regime. American Society of Civil Engineers Transactions. Paper no: 2511, pp. 667–682.

Gaskell J.F., Blackmer A.M. and Bremner J.M. 1980. Comparison of the effects of nitrate, nitrite and nitric oxide on the reduction of nitrous oxide to dinitrogen by soil microorganisms. Soil Sci. Soc. Am. J. 45: 1124–1127.

Granli T. and Bockman O.C. 1994. Nitrous oxide emissions from agriculture Norway. J. Agric. Sci. Suppl. 12 .

Groffman P.M. and Tiedje J.M. 1988. Denitrification hysteresis during wetting and drying cycles in soil. Soil Sci. Soc. Am. J. 52: 1626–1629.

Hefting M.M., Bobbink R. and de Caluwe H. 2003. Nitrous oxide emissions and denitrification in chronically nitrated-loaded riparian buffer zones. J. Environ. Qual. 32: 1194–1203.

Hunter R.G. and Faulkner S.P. 2001. Denitrification potential in restored and natural hardwood wetlands. Soil Sci. Soc. Am. J. 65: 1865–1872.

Khalil M.I., Rosenani A.B., Van Cleemput O., Fauziah C.I. and Shamshuddin J. 2002. Nitrous oxide emissions from an ultisol of the humid tropics under Maize-Groundnut rotation. J. Environ. Qual. 31: 1071–1078.

Klein De C.A.M. and Van Logtestijn R.S.P. 1996. Denitrification in grassland soils in the Netherlands in relation to irrigation, N-application rate, soil water content and soil temperature. Soil Biol. Biochem. 28: 231–237.

Linn D.M. and Doran J.W. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and non-tilled soils. Soil Sci. Soc. Am. J. 48: 1267–1272.

MacDonald P.O., Frayer W.E. and Clauser J.K. 1979. Documentation, chronology and future projections of bottomland hardwood habitat losses in the lower Mississippi alluvial plain. USFWS, Washington, DC, 427 pp.

Mahdun B.Y., Young J.L. and Freed V.H. 1986. Binding of herbicides by water soluble organic materials from soil. J. Environ. Qual. 15: 64–68.

Mitsch W.J., Day J.W. Jr., Gilliam J.W., Groffman P.M., Hey D.L., Randall G.W. and Wang N. 1999. Reducing nutrient loads, especially nitrate-nitrogen, so surface water, groundwater and the Gulf of Mexico: Topic 5 Report for the Integrated Assessment on Hypoxia in the Gulf of Mexico. NOAA Coastal Ocean Office, Silver Spring, MD, Decision Analysis Series No. 19.

Mitsch W.J., Day Jr. J.W, Gilliam J.W., Groffman P.M., Hey D.L., Randall G.W. and Wang N. 2001. Reducing nitrogen loading to the Gulf of Mexico from the Mississippi River basin: strategies to counter a persistent ecological problem. BioScience 51: 373–388.

Mosier A.R. and Hutchison G.L. 1981. Nitrous oxide emissions from cropped fields. J. Environ. Qual. 10: 169–173.

NRCS 1959. Soil Survey-Sunflower County, Mississippi, Series No. 5. Natural Resource Conservation Service and Mississippi Agricultural Experiment Station.

Sahrawat K.L. and Keeney D.R. 1986. Nitrous oxide emission from soils, Advances in Soil Science, Vol. 4. Springer-Verlag, Inc., New York, pp. 103–143

Sanders D.L. and Kalff J. 2001. Nitrogen retention in wetlands, lakes and rivers. Hydrobiologia 443: 205–212.

SAS, Inc. 1998. SAS User Manual and Software. SAS Inc., North Carolina, US.

Sexstone A.J., Parkin T.B. and Tiedje J.M. 1985. Temporal response of soil denitrification rates to rainfall and irrigation. Soil Sci. Soc. Am. J. 49: 99–103.

Sexstone A.J., Parkin T.B. and Tiedje J.M. 1988. Denitrification response to soil wetting in aggregated and unaggregated soil. Soil Biol. Biochem. 20: 767–769.

Simek M., Elhottova D., Klimes F. and Hopkins D.W. 2004. Emissions of N_2O and CO_2, denitrification measurements and soil properties in red clover and ryegrass stands. Soil Biol. Biochem. 36: 9–21.

Singh B., Ryden J.C. and Whitehead D.C. 1988. Some relationships between denitrification potential and fractions of organic carbon in air-dried and field moist soils. Soil Biol. Biochem. 20: 737–741.

Tiedje J.M. 1982. Denitrification. In: Page A.L. (ed.), Methods of Soil Analysis, 2nd ed., Agronomy Monograph, Vol. 9. American Society of Agronomy, Madison, WI.

Tilsner J., Wrage N., Lauf J. and Gebauer G. 2003. Emission of gaseous nitrogen oxides from extensively managed grassland in NE Bavaria, Germany. 1. Annual budget of N_2O and NO_x emissions. Biogeochemistry 63: 229–247.

Turner D.P., Koerper G.J., Harmon M.E. and Lee J.J. 1995. A carbon budget for forests of the conterminous United States. Ecol. Appl. 5: 421–436.

Turner R.E. et al. 1998. Fluctuating silicate: nitrate ratios and coastal plankton food webs. Proc. Natl. Acad. Sci. USA 95: 13048–13051.

Vinther P.F. 1984. Total denitrification and the ratio between N_2O and N_2 during the growth of spring barley. Plant Soil 76: 227–232.

Walker J.T., Geron C.D., Vose J.M. and Swank W.T. 2002. Nitrogen trace gas emissions from a riparian ecosystem in southern Appalachia. Chemosphere 49: 1389–1398.

Weier K.L., Doran J.W., Power J.F. and Walters D.T. 1993. Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon and nitrate. Soil Sci. Soc. Am. J. 57: 66–72.

Weitz A.M., Linder E., Frolking S., Crill P.M. and Keller M. 2001. N_2O emissions from humid tropical agricultural soils: effects of soil moisture, texture and nitrogen availability. Soil Biol. Biochem. 33: 1077–1093.