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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 N₂O, thus compromising the water-quality benefits of restoration. This study compares denitrification rates and N₂O:N₂ 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% WFPS, respectively. When NO₃ was added, N₂O emissions from forested soil were 2.2 times those of cultivated soil at 70% WFPS. At 85% and 100% WFPS, N₂O emissions were not significantly different despite much greater denitrification rates in the forested soil because N₂O:N₂ emission ratios declined more rapidly in forested soil as WFPS increased. These findings suggest that restoration of forested wetlands to reduce NO₃ in surface discharge will not contribute significantly to the atmospheric burden of N₂O.

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 $\text{N}_2\text{O}:\text{N}_2$ 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% 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 $\text{N}_2\text{O}:\text{N}_2$ 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 .

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2 **ABSTRACT**

3 Restored forested wetlands reduce N loads in surface discharge through plant uptake and
4 denitrification. While removal of reactive N reduces impact on receiving waters, it is
5 unclear whether enhanced denitrification also enhances emissions of the “greenhouse”
6 gas N₂O, thus compromising the water-quality benefits of restoration. This study
7 compares denitrification rates and N₂O:N₂ emission ratios from Sharkey clay soil in a
8 mature bottomland forest to those from an adjacent cultivated site in the lower
9 Mississippi Alluvial Valley. Potential denitrification of forested soil was 2.4 times of
10 cultivated soil. Using intact soil cores, denitrification rates of forested soil were 5.2, 6.6
11 and 2.0 times those of cultivated soil at 70%, 85% and 100% WFPS, respectively. When
12 NO₃ was added, N₂O emissions from forested soil were 2.2 times those of cultivated soil
13 at 70% WFPS. At 85% and 100% WFPS, N₂O emissions were not significantly different
14 despite much greater denitrification rates in the forested soil because N₂O:N₂ emission
15 ratios declined more rapidly in forested soil as WFPS increased. These findings suggest
16 that restoration of forested wetlands to reduce NO₃ in surface discharge will not
17 contribute significantly to the atmospheric burden of N₂O.

18

19 **Abbreviations:** LMV, Lower Mississippi Alluvial Valley; PDA, potential denitrification
20 assay; WFPS, water-filled pore space

21

1 **Introduction**

2
3 Seasonally flooded lower elevation clay alluvium lands in agricultural watersheds
4 within the Lower Mississippi River Valley (LMV) are typically bottomland hardwood
5 forests. The Sharky soil series (very-fine, smectitic, thermic, Vertic Haplaquepts) is one
6 of the major soil types in the LMV, an area historically developed under bottomland
7 hardwood forests. This very poorly drained, very slowly permeable soil formed in clayey
8 alluvium of the Mississippi River (Fisk, 1951). About 78% of native bottomland
9 hardwood forest has been cleared in the LMV since European colonization (MacDonald
10 et al. 1979) primarily for agricultural purposes. Because of the exceptionally poor natural
11 drainage of Sharky and similar clay alluvium soils, it is generally necessary to ditch and
12 drain these areas after clearing in preparation for crop production. These fields are used
13 mainly for soybean, rice, corn, wheat, cotton, grain sorghum and pecan orchards (NRCS,
14 1959, 2003).

15 Conversion of forested wetlands to agricultural lands in the LMV altered the
16 natural role of this ecosystem from potential sinks for NO_3 and sediments to potential
17 sources leading to water quality problems (Mitsch et al. 2001). Drainage and cropping of
18 Sharky and similar soils over decades is likely to have altered their potential for
19 denitrification compared to Sharky soil maintained under bottomland hardwood forests
20 (Hunter et al. 2001). Moreover, N fertilizer use in agricultural watersheds with reduced
21 denitrification potential may partly enhance their contribution of biologically available N
22 to receiving water bodies (Breitenbeck et al. 1980, Baggs et al. 2002). Application of N
23 fertilizer results in accumulation of NO_3 in surface soil that is subject to run-off during

1 rainfall, storm events and irrigation. NO_3 run-off from croplands, if not denitrified, can
2 reach water bodies and subsequently contribute to eutrophication (Mitsch et al. 2001).

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

21 The end products of denitrification are N_2O and N_2 gas, which are emitted to the
22 atmosphere (Weier et al. 1993, Dalal et al. 2003, and Sexstone et al. 1985). The emission
23 ratio of $\text{N}_2\text{O}:\text{N}_2$ is affected by soil moisture, NO_3 concentration, pH, available carbon

1 (Groffman et al. 1988, Sahrawat and Kenney 1986, Weitz et al. 2001) and soil
2 management (Khalil et al. 2002, Simek et al. 2004). While restoration of forested
3 wetlands is very likely to enhance the extent of denitrification, the impact on $N_2O:N_2$
4 emission ratio is more difficult to predict on the basis of soil management, different
5 hydrologic regimes and NO_3 loading. An increased $N_2O:N_2$ emission ratio can
6 compromise the benefits of restoration because N_2O is a potent greenhouse gas that has
7 an atmospheric radiative forcing 320 times more than that of CO_2 (Granli and Bockman,
8 1994, Tilsner et al. 2003). Increasing the atmospheric burden of N_2O poses an additional
9 threat because of the capacity of this gas to catalyze stratospheric ozone depletion.
10 Mitsch et al. (1999) speculated that restoration projects will not significantly affect the
11 atmospheric burden of N_2O . The validity of this observation is dependent not only on the
12 extent of land restored, but changes in the rates of denitrification and $N_2O:N_2$ emission
13 ratio as a result of different restoration techniques. Even so, the N_2O emission remains
14 important when assessing the overall value of riparian restoration in the current efforts to
15 market greenhouse gas emission credits.

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

1 **Materials and methods**

2 *Field sites*

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

16 *Soil sampling*

17 In July 2002, soil samples (0-10 cm) were collected from eight sampling sites
18 located within the forested zone and the adjacent cultivated area for the determination of
19 potential denitrification assay (PDA). In July 2003, 15 sampling sites were randomly
20 selected within each of the ecosystems. At each sampling site, two intact soil cores of (5
21 cm dia. x 10 cm height) were collected in plastic liners (5 cm dia. x 15 cm height) using a
22 slide hammer core sampler (AMS Inc., American Falls, ID). Liners were capped on each
23 end to create columns with an average 101 cm² of headspace above the soil core surface.

1 The columns were put on ice for transport to the laboratory. Bulk soil samples (0-10 cm)
2 were also collected from each site for destructive determination of soil moisture, pH,
3 particle size distribution, and concentrations of soluble N (NO_3^-), soluble organic C, total
4 organic C, and total N. Additional intact soil cores (5 cm dia. X 10 cm) were collected at
5 each site using rings fitted in the AMS core sampler for determination of bulk density,
6 total porosity and WFPS. Bulk density of the moist cores was calculated by dividing
7 initial volume by the mass determined gravimetrically after oven-drying for 48 h (105
8 °C). Total porosity was determined by the displacement caused by dispersal of field-
9 moist soil cores in water and adjusted for initial soil moisture content. Water-filled
10 porosity was calculated as the difference between total porosity and moisture content,
11 assuming a water density of 1 g cm^{-3} .

12 *Potential Denitrification Assay (PDA)*

13 PDA analyses (Tiedje, 1982) were performed to compare denitrification potential
14 in a similar soil type from adjacent forested and cultivated sites. Soil samples were
15 homogenized by hand and 10 g (field-moist) weighed into each of six 150-mL glass
16 serum bottles. Three were amended with 15 mL NO_3^- solution ($10 \text{ mg NO}_3^- \text{ L}^{-1}$) and five
17 mL deionized water. Twenty mL of deionized water was added to the other three bottles.
18 All bottles were fitted with airtight septum caps and purged with oxygen-free N_2 gas for
19 20 minutes to induce anaerobic conditions. About 15% of the headspace was replaced
20 with acetylene (C_2H_2) to block the enzymatic reduction of N_2O to N_2 gas during
21 denitrification. Prior to use, C_2H_2 was purified by bubbling through 1 M CuCl_2 solution.
22 The bottles were wrapped in aluminum foil and placed on a reciprocating Eberbach
23 shaker for continuous shaking at low speed and room temperature (22-25 °C). Headspace

1 samples were collected after 2, 4 and 6 h incubation using a syringe and transferred to
2 Vacutainers for analysis of N₂O concentration using a Tremetric 9001 gas chromatograph
3 fitted with a Porapak Q column and equipped with an electron capture detector (ECD).
4 N₂O concentration of the headspace samples was determined using a standard calibration
5 curve and total N₂O production rate (ug N₂O-N g⁻¹ h⁻¹) was calculated after making
6 adjustment for dissolved N₂O using the Bunsen absorption coefficient.

7 *Determination of denitrification and N₂O emission rates*

8 One column from each sample pair was used to measure total denitrification by
9 the acetylene-blockage technique (Tiedje, 1982). Percent WFPS of the soil cores were
10 determined (Linn et al. 1984) and paired cores were randomly assigned to various
11 treatments, by addition of dilute NO₃ solution to deliver 15 μg NO₃-N g⁻¹ soil. To ensure
12 even distribution of water and NO₃ solution, they were added by multiple injections using
13 a syringe fitted with a 16 gauge x 10 cm needle. Initial WFPS were 70%, 85% and
14 100%. Emissions from cores adjusted to 85% and 100% with NO₃ solution were
15 compared to those adjusted to similar WFPS with deionized water (all treatments were
16 performed in triplicates).

17 After addition of NO₃ or water and WFPS adjustments, the cores were capped
18 tight at both ends. One column from each sample pair was injected with 10 mL of
19 purified C₂H₂ in small aliquots at the interface between the soil core and liner using a
20 syringe fitted with a 16 gauge x 10 cm needle. An additional 10 mL of C₂H₂ was injected
21 into the headspace after the tubes were sealed with airtight caps fitted with septum to
22 allow periodic sampling of the headspace atmosphere. Addition of C₂H₂ to one column
23 from each pair was used to determine total denitrification and N₂O:N₂ emission based on

1 the difference of N₂O emitted by the paired cores. The columns were incubated for 72
2 hours and gas samples were collected daily from the headspaces for N₂O and CO₂
3 determination. Gas samples were stored in Vacutainers® until GC analysis described
4 above.

5 *Soluble organic carbon*

6 Soluble organic carbon (SOC) of the soil samples was determined using the
7 technique described by Mahdun (1986). The samples were analyzed using a Shimadzu
8 TOC Analyzer for SOC concentration. The SOC values for the duplicate samples were
9 averaged and reported as SOC in mg g⁻¹ of oven-dried soil.

10 *Total soil carbon, total nitrogen and nitrate*

11 Total soil carbon (C) and nitrogen (N) were determined using a Shimadzu CNS
12 Analyzer. Soil samples were oven dried, pulverized and thoroughly homogenized. A
13 sub-sample of about 35 mg was weighed into a tin capsule for automated analysis. Total
14 soil carbon and nitrogen are reported in Mt ha⁻¹ oven-dried soil. Soil NO₃ was determined
15 using 2M KCl soil extracts and a Lachat Flow Injection Analysis instrument. These
16 values are reported as mg NO₃-N kg⁻¹ soil.

17 *Statistical analysis*

18 Denitrification rates and N₂O:N₂ emission ratio of forested and cultivated soil was
19 tested for significant differences (Fisher's LSD at alpha of 0.05) at different WFPS using
20 the pooled error from ANOVA (SAS Inc. 1998). Effects of ecotype and NO₃ additions on
21 PDA and denitrification rates were also compared by ANOVA (SAS Inc. 1998).

22 **Results**

1 Potential denitrification assay (PDA) of forested soil averaged $1.42 \text{ ug N g}^{-1} \text{ h}^{-1}$
2 dry soil, whereas the PDA of cultivated soil was significantly less ($0.62 \text{ ug N g}^{-1} \text{ h}^{-1}$ dry
3 soil). When no NO_3 was added, total denitrification rates were similar and averaged 0.67
4 and $0.84 \text{ ug N g}^{-1} \text{ h}^{-1}$ in forested and cultivated soils respectively (Figure 1). Addition of
5 NO_3 caused a 129% increase in denitrification rate in forested soil, but had no significant
6 impact on denitrification in cultivated soil. The response to added NO_3 is reflected in the
7 amounts of total and mineralizable C found in soil of the two ecosystems (Table 1).
8 Mineralizable carbon averaged 122 and $29 \text{ ug CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ dry soil in the forested and
9 cultivated soils respectively.

10 Denitrification rates of the soil cores collected from both forest and cultivated
11 sites showed an increase with increase in soil WFPS from 70% to 100% (Table 2). Mean
12 denitrification rates in NO_3 amended forest soil increased from $0.89 \text{ mg N m}^{-2} \text{ h}^{-1}$ at 70%
13 WFPS to $2.04 \text{ mg N m}^{-2} \text{ h}^{-1}$ at 100% WFPS. The corresponding increases in cultivated
14 soil were from 0.17 to $1.02 \text{ mg N m}^{-2} \cdot \text{h}^{-1}$. Denitrification rates of forested soil amended
15 with NO_3 were significantly higher than that of cultivated soil at 70% and 85% WFPS (p
16 <0.05). At 100% WFPS, the average denitrification rate of the forested soil with NO_3
17 addition was $2.04 \text{ mg N m}^{-2} \text{ h}^{-1}$, much higher than the corresponding rate of cultivated
18 soil ($1.02 \text{ mg N m}^{-2} \text{ h}^{-1}$).

19 Addition of NO_3 led to a marked increase in denitrification rate in forested soil,
20 but had a lesser effect on denitrification rate in cultivated soil. NO_3 amended forest soil at
21 85% and 100 % WFPS resulted in average denitrification rates of 3.28 and 2.04 mg N m^{-2}
22 h^{-1} respectively (Table 2). These rates were 4.3 and 1.5 times those observed in cores at
23 85% and 100% WFPS without added NO_3 . In contrast, NO_3 amendment of cultivated

1 soil did not lead to significantly greater denitrification at 85% and 100% WFPS. These
2 findings are similar to those found in PDA analysis.

3 Overall mean $N_2O:N_2$ emission ratios decreased with increasing WFPS and were
4 greater in cultivated soil than in forested soil (Figure 2). In forested soil, the $N_2O:N_2$
5 ratio decreased from 0.28 to 0.11 as WFPS increased from 70% to 100% WFPS. WFPS
6 greater than 70% led to a marked decrease ($p < 0.05$) in the $N_2O:N_2$ emission ratio of
7 forested soil both with and without NO_3 additions. In cultivated soil, the $N_2O:N_2$
8 emission ratio decreased from 0.64 to 0.39 as WFPS increased from 70% to 100%. At
9 70% and 85% WFPS, the $N_2O:N_2$ emission ratio of cultivated soil was similar with and
10 without NO_3 amendment, while at 100% WFPS ratio of the NO_3 amended cores was
11 double the corresponding ratio of unamended cores.

12 To compare the potential contribution of these ecotypes to the atmospheric burden
13 of N_2O , the net N_2O emission rate was calculated on an area basis assuming a microbially
14 active soil depth of 10 cm. In forested soil, N_2O emission rates were 0.25, 0.40 and 0.22
15 $mg\ N\ m^{-2}\ h^{-1}$ at 70% WFPS, 85% WFPS, and 100% WFPS, respectively, when amended
16 with NO_3 (Table 2). The corresponding emissions from cultivated soil were 0.11, 0.32
17 and 40 $mg\ N\ m^{-2}\ h^{-1}$ at 70%, 85% and 100% WFPS, respectively, from NO_3 amended
18 soil (Table 2). These findings indicate that the contribution of N_2O from forested and
19 cultivated soil were similar at higher moisture contents, but that the forest soil emitted
20 significantly greater amount of N_2O at 70% WFPS ($p < 0.05$).

21 The distribution of clay and silt was similar in forested and cultivated soils (Table
22 1), though significant differences in a number of soil properties were observed. These

1 differences in adjacent soils were likely due to differences in land use over the past
2 decades. The forested soil was more acidic (pH 5.4) than the cultivated soil (pH 6.1).
3 Perhaps the most notable differences between the soils of these two ecosystems were the
4 substantially greater organic matter and porosity of soils under forest. Soil organic C in
5 forested soil was 37 g kg^{-1} dry soil, more than twice that of the cultivated soil (16 g kg^{-1}
6 soil). Higher soil organic matter contributed to improved soil structure and greater
7 porosity. Soil bulk densities in forested soil averaged 0.84 g cm^{-3} , nearly 40% less than
8 those of cultivated soils (1.20 g cm^{-3}). Total porosities in the forested and cultivated soils
9 averaged 0.68 and $0.54 \text{ cm}^3 \text{ cm}^{-3}$, respectively.

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

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

7

8

9 **Discussion**

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

18 When soils from each ecosystem were amended with NO_3^- and incubated as
19 slurries under anaerobic conditions, the potential denitrification rates in forested samples
20 averaged nearly 2 ½ times those in samples from adjacent cultivated areas (Fig. 1).
21 When no NO_3^- was added, denitrification rates were similar for the two ecosystems.
22 Addition of NO_3^- caused a marked increase in denitrification rate in forested soil but did
23 not cause a similar response in cultivated soil. These findings indicate that potential

1 denitrification rate in the forested ecosystem is limited by NO_3^- , and by other factors,
2 most probably C substrate availability, in the cultivated system.

3 Experiments using intact soil cores showed that in addition to land use and NO_3
4 concentration, WFPS % (Table 3) influenced not only the rate of denitrification but also
5 the ratio of N_2O to N_2 emitted (Figure 2). Denitrification rates in cores of forested soil
6 averaged $2.66 \text{ mg N m}^{-2} \text{ h}^{-1}$ when amended with 15 mg N kg^{-1} as NO_3 and were 3.5 times
7 greater than the corresponding rates in cultivated soil. It is noteworthy that even though
8 unamended cores from both ecosystems contained low amounts of NO_3 (average 3.4 mg
9 N kg^{-1}), denitrification rates at moisture contents of 85% and 100% WFPS averaged 1.06
10 and $0.88 \text{ mg N m}^{-2} \text{ h}^{-1}$ in forested and cultivated soils, respectively. In the cultivated
11 system, water content typically remains below that where denitrification rates can be
12 maintained (Table 3). The moisture content of the forested soil, however, remains above
13 80% WFPS throughout the year except in summer. At the denitrification rates observed,
14 the amount of soil NO_3 present in upper 15 cm of forested soil represents less than a 161
15 hour supply, and therefore soil NO_3^- must be continually replenished through runoff or
16 mineralization and nitrification to maintain the denitrification rates and soil NO_3 levels
17 observed in unamended cores.

18 In the forested systems, denitrification rates at 85% and 100% WFPS were 3.7
19 and 2.3 times, respectively, those observed at 70%. Addition of NO_3 increased
20 denitrification rates in forested soils by 4.2 and 1.5 times at 85% and 100% WFPS,
21 respectively, and supports the conclusion that denitrification rates in that system are
22 limited by NO_3 rather than C availability. In the cultivated systems, denitrification rates
23 at 85% and 100% WFPS were 2.8 and 6.0 times greater, respectively, than at 70%.

1 Unlike the forested system, addition of NO_3 did not lead to a significant increase in
2 denitrification rates at higher moisture contents.

3 Whereas denitrification rates increased with increasing WFPS, the ratios of N_2O
4 to N_2 decreased from 0.28 to 0.11 in forested soils and from 0.64 to 0.39 in cultivated
5 soils as WFPS increased from 70% to 100%. At lower WFPS%, the higher ratios of
6 N_2O to N_2 observed may have been augmented by the production of N_2O by nitrifying
7 rather than denitrifying populations (Blackmer et al, 1980). At high WFPS %, the
8 diffusion of O_2 into the soil is reduced, promoting conditions favorable for denitrification.
9 Increased WFPS also reduces the diffusion of N_2O from the soil, increasing the
10 probability that this gas will be subsequently reduced to N_2 by active denitrifier
11 populations. It is not surprising, then, that numerous studies have observed an increase in
12 denitrification rate and decrease in $\text{N}_2\text{O}:\text{N}_2$ as WFPS % increases (Weitz et al. 2001,
13 Klein et al. 1996, Hunter et al. 2001, Weier et al. 1993, Sexstone et al. 1988, and Mosier
14 et al. 1981).

15 Few studies have assessed the effects of cultivation on the extent or products of
16 denitrification. Linn and Doran (1984) compared the effects of tillage systems on
17 emissions of N_2O and CO_2 from agricultural soils at various %WFPS. While those
18 studies did not measure the extent of denitrification, they showed that N_2O emissions
19 from soils collected from no-till systems averaged 9.4 times those of 'plowed' soils.
20 These differences were attributed largely to differences in %WFPS that averaged 62%
21 under no till and 44% with tillage. In the current study, soil water contents of samples
22 collected at various times in 2002-2003 averaged 79% WFPS in forested soils and 50% in
23 cultivated soils, suggesting that denitrification in bottomland hardwoods remains active

1 throughout most of the year whereas drained, cultivated soil remain aerated and
2 denitrification is likely to occur only after periods of intense rainfall.

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

14 Despite lower ratios of $N_2O:N_2$, higher denitrification rates in the forested soil
15 resulted in somewhat larger emissions of N_2O at the lower moisture content (70%
16 WFPS). Differences in emissions of N_2O from intact soil cores at 85% and 100% WFPS
17 from forested areas were not statistically different than those from cultivated areas. While
18 the denitrification rates observed indicate that at least some flooded pores were
19 sufficiently reduced at 70% WFPS in both soils to support denitrifying activity, the
20 forested soil contained a greater volume of air-filled pores because of its higher total
21 porosity. At 70% WFPS, for example, the forested soil cores contained $0.20 \text{ cm}^3 \text{ cm}^{-3}$
22 air-filled pores, whereas the cultivated soil contained only $0.16 \text{ cm}^3 \text{ cm}^{-3}$. This difference
23 in air volume may have facilitated more rapid gaseous diffusion at lower WFPS, reducing

1 the possibility of subsequent reduction of N_2O to N_2 . It is also possible that the greater
2 volume of air-filled pores at low WFPS and the substantially larger amounts of
3 mineralizable organic matter in the forested soil led to more extensive N mineralization
4 and release of N_2O during nitrification. At saturation (100% WFPS), N_2O emissions
5 tended to be greater from cultivated soil, though these differences were below statistical
6 significance ($p > 0.05$).

7 When no NO_3^- was added, N_2O emissions from the forested soil averaged 10.5 kg
8 $\text{N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$. Addition of 15 mg $\text{NO}_3^- \text{-N kg}^{-1}$ resulted in average emissions of 25.4 kg
9 $\text{N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$, and were within the range of 24-30 kg $\text{N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$ reported for other
10 riparian wetlands (Walker et al. 2002; Hefting et al., 2003). N_2O emissions from the
11 cultivated area averaged 24.2 and 19.3 kg $\text{N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$ with and without the addition
12 of NO_3^- , respectively. The finding that addition of NO_3^- invariably led to an increase in
13 total denitrification as well as N_2O production at all water contents but did not
14 significantly alter the ratio of N_2O to N_2 evolved suggests denitrification rather than
15 nitrification plays a principal role in N_2O production in these soils.

16 The higher amounts of soil organic matter observed in forested areas support the
17 general conclusion that restoration of bottomland hardwoods not only results in an
18 increased capacity to denitrify, but also increases sequestration of atmospheric C both as
19 stable soil humus as well as standing biomass. The organic C content of the forested soil
20 (37.7 g C kg^{-1}) was more than twice that of the cultivated soil (16.4 g C kg^{-1}). While the
21 bulk density of the surface soil in the forested soil was substantially less than that of the
22 cultivated soil, the forested system nevertheless contained 1.6 times more soil organic
23 carbon in the upper 10 cm. Extrapolating to a hectare basis, the forested soil contained

1 32,400 kg C ha⁻¹ in the surface 10 cm or 12,700 kg ha⁻¹ more C sequestered as organic
2 matter than in the cultivated system. The sequestration of organic C invariably results in
3 the sequestration of N in organic forms. Despite the higher denitrification rates in the
4 forested system, the surface 10 cm of soil contained 2,838 kg N ha⁻¹, or 918 kg N ha⁻¹
5 more than in the cultivated soil.

6 In summary, the results of these experiments indicate that denitrifying activity is
7 more persistent and rapid in forested bottomland soil than in similar cultivated soil. Even
8 so, N₂O emissions resulting from the restoration of bottomland forests are likely to be
9 similar to that of similar cultivated soil due to a reduced ratio of N₂O to N₂ evolved from
10 forested soil at water contents that support high rates of denitrification.

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Figure 2. Ratio of N₂O to N₂ evolved from forested and cultivated soil adjusted to various WFPS with or without the addition of added NO₃⁻. Error bars represent standard error of the mean ($p=0.05$).

Table 1. Physiochemical properties of forested and cultivated Sharky soil (\pm 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	$\text{cm}^3 \text{cm}^{-3}$	0.68 ± 0.01	0.54 ± 0.00
Clay	$\text{g}/100 \text{g}$	53	51
Silt	$\text{g}/100\text{g}$	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	$\text{mg CO}_2 \text{m}^{-2} \text{h}^{-1}$	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}	$3.30 \pm .25$	$3.5 \pm .27$

+

Table 2. Denitrification and net N₂O emission rates of forested and cultivated Sharky soil

WFPS [†]	NO ₃ ⁻ added 15 μg N g ⁻¹	Denitrification rate (mg N m ⁻² h ⁻¹)			N ₂ O emission rate (mg N m ⁻² h ⁻¹)		
		Forested	Cultivated	Diff. [‡]	Forested	Cultivated	Diff. [‡]
70%	+	0.89	0.17	*	0.25	0.11	*
85 %	+	3.28	0.49	*	0.40	0.32	ns
85 %	-	0.77	0.27	ns	0.09	0.15	ns
100 %	+	2.04	1.02	ns	0.22	0.40	ns
100 %	-	1.35	1.49	ns	0.15	0.29	ns

[†]WFPS, water-filled pore space.

[‡]*, significant difference (p<0.05) between ecotypes using pooled variance T-test; ns, differences not significant

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

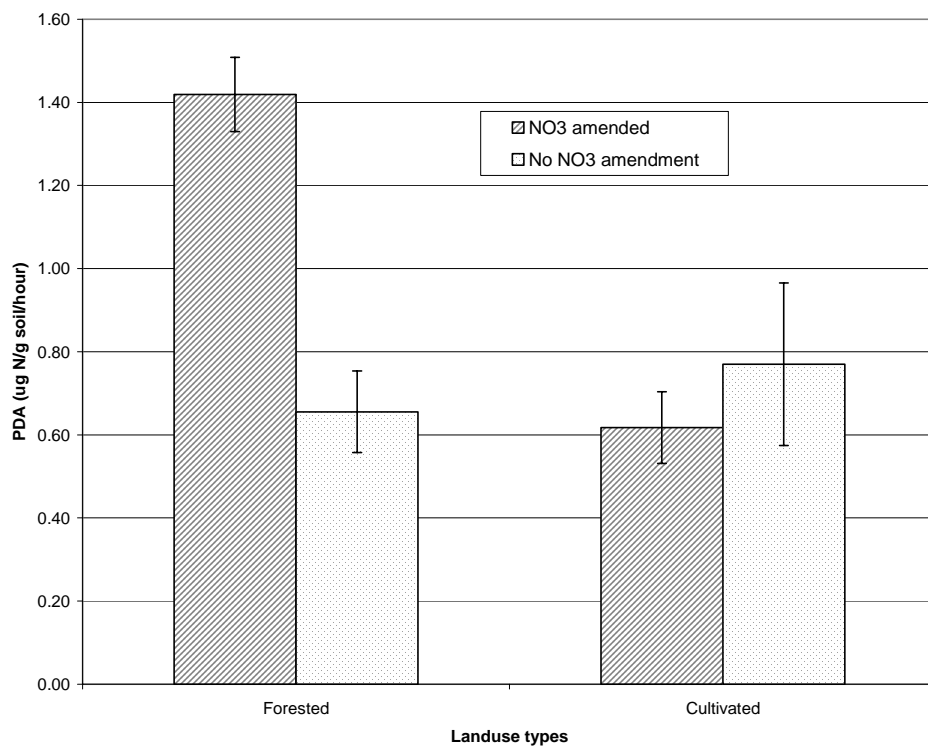


Figure 1. Denitrification potential of forested wetland and cultivated soil Error bars represent standard error of the mean ($p=0.05$).

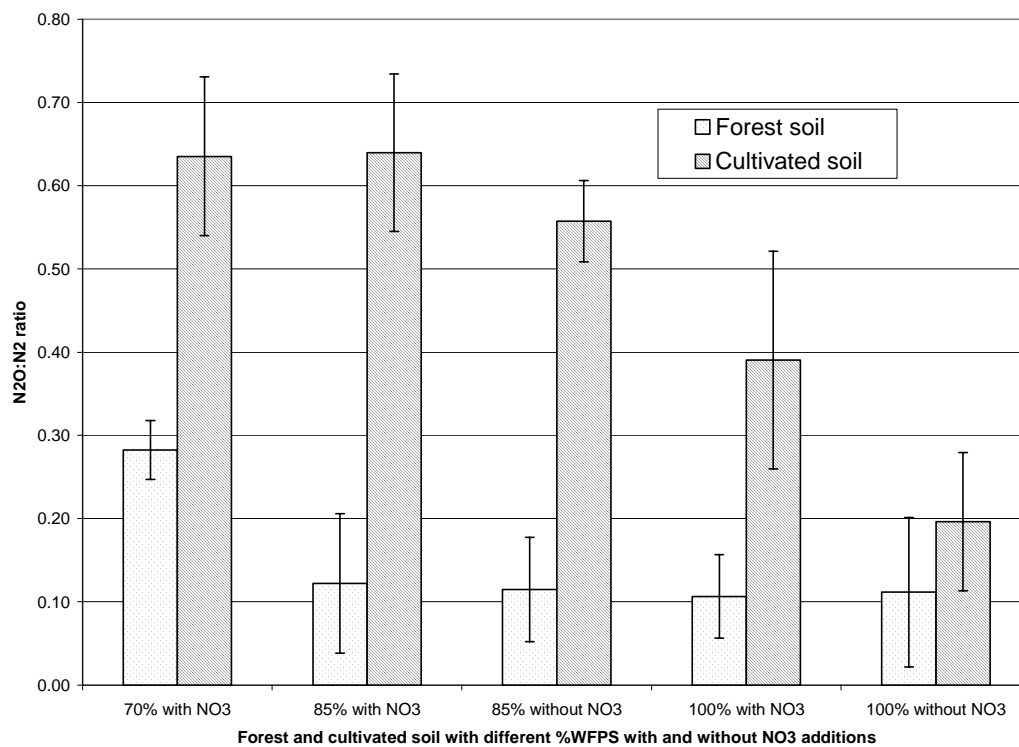


Figure 2. Ratio of N_2O to N_2 evolved from forested and cultivated soil adjusted to various WFPS with or without the addition of added NO_3^- . Error bars represent standard error of the mean ($p=0.05$).