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Denitrification and greenhouse gas emissions from cultivated and wetland alluvial soils

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`DENITRIFICATION AND GREENHOUSE GAS EMISSIONS FROM CULTIVATED AND WETLAND ALLUVIAL SOILS

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ABSTRACT

Agricultural development in the Mississippi River Basin has contributed to an 3-fold increase in NO_3 loading of the river. Increased NO_3 loading is a primary cause of eutrophication in the northern Gulf of Mexico. Identification of best management practices (BMPs) to reduce NO_3 loss and wetlands restoration to remove NO_3 through denitrification are critically needed. The objectives of this research were to determine factors controlling denitrification potential of different landscape units in an agricultural watershed and quantify the effects of BMPs and organic C amendments on denitrification rates of cultivated lands and restored forested wetlands. N_2O, CH_4 and CO_2 emissions from restored and natural forested wetlands were measured to determine if restoration for NO_3 removal will increase greenhouse gas emissions, thereby contributing to global warming and compromising the water quality benefits of restoration.

Low-elevation, wetland clay soils exhibited 6.3 and 2.5 times greater denitrification potential than the high-elevation silt-loam and low-elevation clay soils under cultivation, respectively. Denitrification potentials of vegetated ditches were 1.3 to 4.2 times greater than the unvegetated ditches and cultivated soils, respectively. Soil cores collected from forested wetlands displayed 2.0 - 6.6 times greater denitrification rates than cultivated soils when incubated at 70 to 100% water-filled pore space (WFPS). Significantly lower N_2O:N_2 emission ratios were observed from wetlands than from cultivated soils. Denitrification rates in the cultivated and restored forested wetland soils increased 200% and 42%, respectively, when amended with cotton gin trash (CGT). BMPs increased denitrification rates of restored wetlands. Nitrate addition to forested wetlands led to a 48% increase in N_2O emissions. Forested wetlands exhibited net CH_4

sink of 438-1050 g CH_4 ha^{-1} y^{-1}. CO_2 emission decreased as WFPS increased from 40 to100% in forested wetlands.

Low-elevation clay soils in agricultural watersheds are the best candidates for wetland restoration for water quality improvement. CGT amendment of cultivated and restored soils in conjunction with BMPs can help reduce on-site NO_3 loss. Wetland restoration in the Lower Mississippi valley will not significantly affect the global greenhouse gas emissions budget; however, increased N_2O emissions due to NO_3 additions merit consideration when establishing CO_2 storage credits on restored wetlands.

CHAPTER 1

INTRODUCTION

Overview

To meet the needs of human dietary requirements, the use of synthetic nitrogen (N) fertilizers in an expanding agriculture has increased worldwide (Howarth, et al. 2002). The high demand for N fertilizers has resulted in a10-fold increase in production worldwide since the1940s (Galloway, et al. 1995). Synthetic N fertilizers helped increase food production to accommodate increasing human population and improve human health and living standards (Galloway, 2002). N fertilizer use increased from 3.1 to 11.2 T g N y^{-1} from 1961 to 1999 in the US, making it consume about 13% of the synthetic N used globally (Howarth, et al. 2002). Since 1950, N fertilizer application to croplands has increased 20-fold in the US (Battaglin and Goolsby, 1996). However, the increased input of the available N into agricultural lands has adverse effects on the environment (Jordan and Weller, 1996). Excessive loading of the natural ecosystems with available N leads to changes in the productivity of terrestrial and aquatic ecosystems, invasion by weeds, changes in the soil microbial community composition, and eutrophication and hypoxia in lakes and coastal waters (Galloway, 2002, Rabalias, et al. 2002).

Agricultural expansion in the Mississippi River Basin of the USA during the past 200 years has resulted in the loss of many natural ecosystems including forested wetlands. Drainage and cultivation of the converted lands, expanded use of N fertilizers (Galloway, 2003) and the loss of wetlands in the Mississippi River Basin (Mitsch et al. 2001; Lowrance et al. 1984) have contributed to increased NO_3 concentration in the Mississippi River (Donner 2004). About 74% of the NO_3 load of the Mississippi River is currently derived from agricultural run-off (Rabalais, et al. 2002). The subsequent increase in dissolved and particulate NO_3 levels in the Mississippi

River is one of the major causes of extensive eutrophication and hypoxia in the northern Gulf of Mexico (Rabalais, et al. 2002; Howarth et al. 2000).

The lower Mississippi River alluvial valley (LMV) has lost about 80% of its bottomland hardwood forests, mainly to agriculture (Allen, et al. 2001, McDonald, et al. 1978). The conversion of bottomland forests to cultivation changed these natural ecosystems from a net NO_3 sinks to net NO_3 sources. Currently there is growing interest in controlling or reversing NO_3 pollution of surface and ground waters due to land use changes in the Mississippi River watershed. Critical research needs to sustain agricultural productivity while protecting the environment in this region include identification of soil management practices to enhance N use efficiency (Inarappong, et al. 2003) through reduced N loss from croplands, and restoration of wetlands and riparian ecosystems at suitable locations between croplands and water bodies to trap and remove NO_3 from agricultural run-off (Mitsch, et al. 2001; Galloway, et al. 2002). This dissertation research investigates factors controlling denitrification in cultivated and wetland soils in an agricultural watershed in the LMV in an effort to identify measures for the reduction of run-off NO_3. The impacts of forested wetlands restoration on N_2O, CH_4 and CO_2 flux are also evaluated.

Lower Mississippi River Valley: Ecological and Agricultural Significance

The LMV is the largest floodplain in the US covering about 23, 300 km^2 (Figure 1.1). It extends from southern Missouri and Illinois to the northern Gulf of Mexico, encompassing parts of Tennessee, Kentucky, Arkansas, Mississippi and Louisiana (Allen et al. 2001). This floodplain was formed by river alluvium deposited by the over-bank flooding events of the Mississippi River and its tributaries in the region after the recession of the Pleistocene era

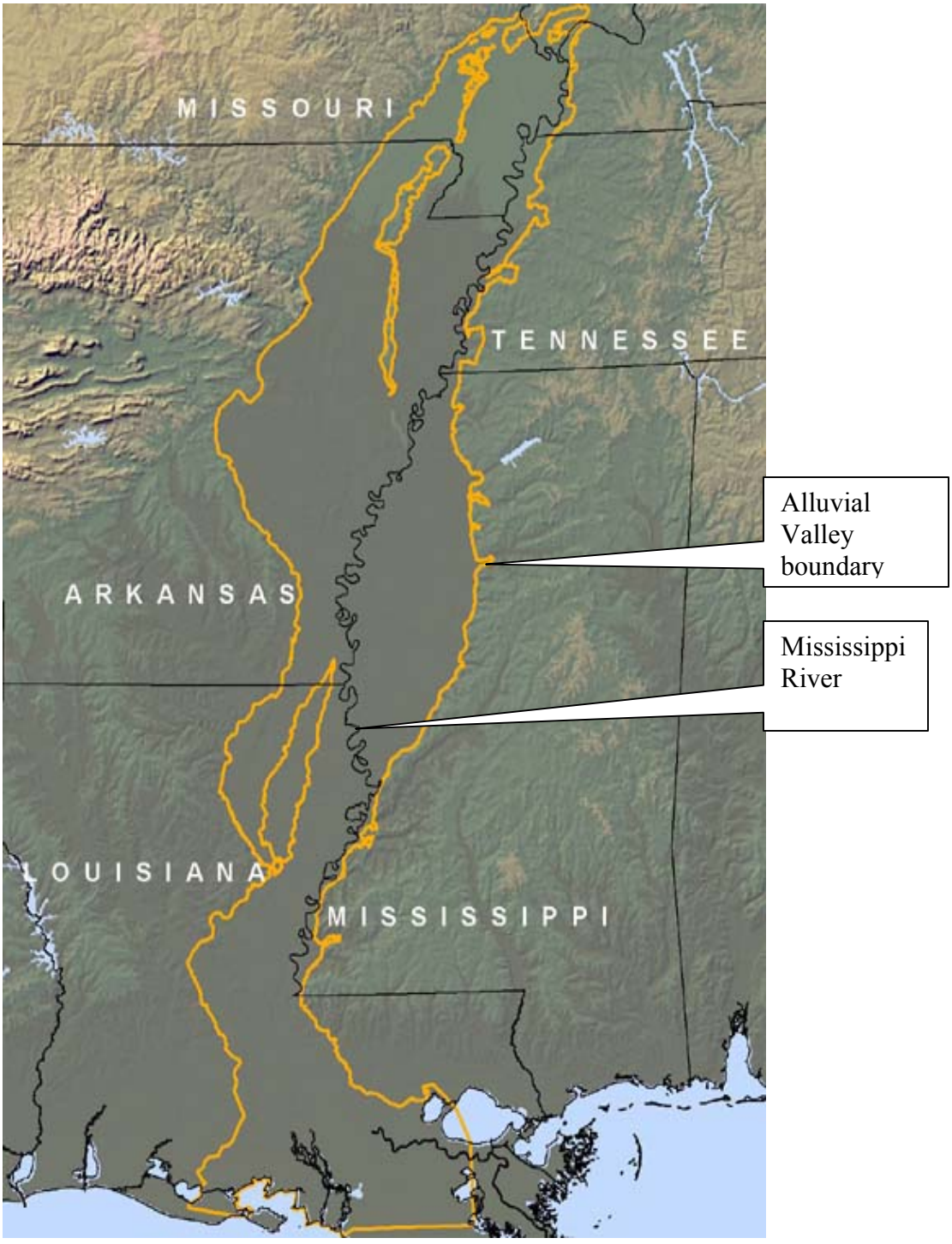

Figure 1.1. Boundary map of the Lower Mississippi River Alluvial Valley (Adopted from Miller and Nasser, 2000)

(Fisk, 1951). The distribution of alluvium and the development of a mosaic of topographic features including ridges, swales, meander belts and backswamp areas were predominately determined by the frequency, depth and duration of over-bank flooding by the Mississippi River (Wharton et al. 1982).

Historically, bottomland hardwood forest were developed on the floodplain and their growth was subsidized by the sediments and nutrients carried by the over-bank flooding water, mainly in the spring (Gosselink et al. 1990). These forests covered an estimated 10 million hectares of land in the LMV at the time of the European colonization of America (McDonald, et al. 1979). With an increasing market for soybeans and cotton during the last half of the 20th century, about 120,000 ha of forested wetlands were cleared annually during 1950s to 1970s (McDonald et al. 1979). By 1979, only 2.1 million hectare of bottomland hardwoods remained (Allen et al. 2001).

Natural forested wetlands in the LMV perform various vital ecological functions. These functions include hydrologic (flood attenuation and ground water recharge), biogeochemical (nutrient retention and cycling, soil C and N storage, denitrification, etc.) and biotic functions (maintenance of characteristic plant community and provision of wildlife habitat) (Ainslie, et al. 1999; Ullah, 2001). Flood control structures and levees were built to secure agricultural land, residential and commercial areas. Once cleared and drained these fertile alluvial soils were brought under row crop cultivation including cotton, corn and soybeans. Agricultural production is the backbone economic activity of the region sustaining local and regional agro-based industries and food production. However, agricultural development in the LMV resulted in the loss of the ecological functions rendered by forested wetlands (Scott et al. 1990).

Agriculture and Water Quality Issues in the LMV

Fertilizer and pesticide use and conventional cultivation in the LMV accelerated soil erosion and loss of nutrients and pesticides from agricultural lands. Soil erosion is one of the serious issues in the LMV (Locke et al. 2001). Sediments, nutrients and pesticides washed from the cultivated lands by run-off are discharged into ox-bow lakes, streams and rivers. The chances of the sediments, pesticides and run-off NO_3 being retained, sequestered or recycled by the remnant fragmented patches of wetlands (scattered in agricultural watersheds) before their discharge into surface waters are minor, largely because direct drainage of agricultural run-off into surface water bodies bypasses natural flow through these wetlands. Increased sediment, nutrient, and pesticide concentrations in the receiving lakes and rivers are threatening the ecological integrity of water bodies in and well beyond the boundaries of agricultural watersheds in the LMV.

Among the water pollutants of concern arising agricultural activities, NO_3 is the most mobile pollutant (Myrold, 1998). NO_3 is both negatively charged (like most soil particles) and highly water soluble, which limits its adsorption to soil particles or retention through precipitation (Myrold, 1998). This mobility allows it to travel long distances from its origin through hydrologic pathways (Galloway, et al. 2003). For example N fertilizer use in the upper and middle Mississippi River basins are linked to the increased NO_3 loading of the northern Gulf of Mexico (Donner, 2004; Rabalais, et al. 2002; McIssac et al. 2001; Battaglin and Goolsby, 1996). Not only are increased agricultural activities in the LMV contributing to the higher NO_3 loading of the Mississippi River (McIssac et al. 2001, Mitsch, et al. 2001), but the role of former wetlands as a net NO_3 sink for the over-bank flooding water of the river is also diminished due to its hydrologic isolation from the rivers (Hunter and Faulkner, 2001; DeLaune et al. 1996).

Current Programs of Water Quality Improvement in the LMV

Growing public concern regarding the loss of wetland functions in the US led to the recognition of the importance of these ecosystems and the need for their protection and restoration. The Clean Water Act of 1972 was enacted to protect waters from pollution. Under its definition of the waters of the US, wetlands were identified part of this definition. Section 404 of the Act empowered the US Army Corps of Engineers to ensure 'no net loss in wetlands' through regulatory measures of avoidance, mitigation and restoration of any wetland lost due to development. Under this act, forested wetlands in LMV came under the protection custody of the US Army Corps of Engineers to ensure wetlands mitigation. Wetlands restored under mitigation program at suitable locations in LMV can substantially help improve water quality. At the same time, in the 1970s and 1980s, the US Fish and Wildlife Service initiated re-planting lands in the LMV with an impetus on the rehabilitation of wildlife habitat (Allen et al. 2001). However, these restoration programs were envisioned to improve water quality also. The US Department of Agriculture (USDA) initiated the Conservation Reserve Program (CRP) in 1985 authorized by the US Congress under the Food Security Act. This program subsidizes farm owners with an annual per-acre rental payment and half the cost of establishing a permanent land cover, in exchange for removing ecologically critical croplands from production for 10-15 years (USDA 2002). Nearly 500, 0000 ha of grass buffer and forest CRP has been established in Arkansas, Mississippi and Louisiana by 2002 (USDA, 2002). CRP sites are designed to reduce soil erosion, improve water quality and provide habitat to wildlife. The USDA also started the Wetlands Reserve Program (WRP) through the 1990 Farm Bill. This program provides incentives to land owners to restore lands which were historically wetlands. About 62,500 ha of bottomlands were restored under this program in LMV by 2000 (USDA, 2002). Both CRP and WRP will have their

impact on improving water quality in LMV including NO_3 removal, however, no scientifically valid restoration techniques or guidelines are established to ensure CRP and WRP sites are restored in ways that results in substantial removal of run-off NO_3. For example, a restored CRP or WRP site with no consideration given to their location in the landscape and hydrologic connection to croplands in agricultural watersheds may not serve a run-off NO_3 removal function, although the restored site may have a high wildlife value.

Certain best management practices (BMP) are also implemented and evaluated for their role in improving water quality in agricultural watersheds in LMV by the USDA. These BMPs are under evaluation for their significance in slowing down soil erosion and retaining and processing pesticides and nutrients. Establishment of grassed filter strips and vegetated ditches in agricultural watersheds is recommended by the USDA for water quality improvement purposes. No detailed assessments of the importance of these BMPs in removing NO_3 through denitrification has been undertaken (Rebich and Knight, 2001). To ensure optimal environmental benefits, all of these water quality improvement programs including restoration of wetlands merit evaluation regarding their effect on the rate and extent of NO_3 removal through denitrification.

Fate of NO_3 in Agricultural Watersheds

NO_3-N in cultivated lands is derived from several major sources. These sources are synthetic N fertilizer added to cultivated lands, atmospheric deposition of nitrogen oxides, release of NO_3 from the soil organic N pool through ammonification and nitrification, and biological N_2 fixation into soil by nitrogen-fixing bacteria (Galloway et al. 2002 and 2003; Jordon and Weller, 1996; Howarth et al. 2002). Nitrogen in the form of NO_3 in soils is taken up by plants, immobilized by bacteria, reduced back to NH_4, denitrified or washed away by irrigation water or rainfall (Bowden 1986). If during its transport from croplands to receiving

waters, NO_3 encounters organic-rich, poorly aerated wetlands, it is most likely denitrified, taken up by plants, or immobilized by bacteria. Wetlands in general and forested wetlands in the LMV in particular are N-limited systems with great potential to retain and recycle run-off NO_3. Among the NO_3 retention and cycling processes in wetlands, denitrification is the major pathway known to reduce the reactive NO_3 to N_2O and N_2 gases.

Denitrification is a microbial process in which heterotrophic facultative bacteria reduce NO_3 to N_2O and N_2 gases under anaerobic conditions (Tiedje, 1982). Whenever soils become anoxic, denitrifier bacteria shift from O_2 to NO_3 to use it as their terminal electron acceptor during respiration. At a biogeochemical scale, denitrification is mainly regulated by soil moisture, availability of organic C substrate and soil NO_3 concentration (Figure 1.2) (Tiedje, 1982). Other factors which have bearing on this process are soil pH, temperature, and soil particle size distribution. These factors in turn are influenced by soil topography, climate, vegetation type, geology and the pattern of organic C production and decomposition. Moreover, in agro-ecosystems, soil management affects one or more of the controlling factors of denitrification (Khalil, et al. 2002), which can either enhance or retard denitrification rates.

The end products of denitrification are N_2O and N_2 gases (Skiba et al. 1998, Weier, et al. 1993), although there are two other intermediate products as shown below:

$$NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

The relative amount of N_2O and N_2 gases (N_2O:N_2 ratio) produced during denitrification is influenced by soil moisture, NO_3 concentration, pH, available carbon (Groffman et al. 1988; Sahrawat and Kenney 1986; Weitz et al. 2001; Linn and Doran, 1984) and soil management (Khalil et al. 2002). High soil moisture (Linn and Doran, 1984; Groffman and Tiedje 1988) and greater quantities of labile organic C reduced the N_2O:N_2 emission ratio, while higher NO_3

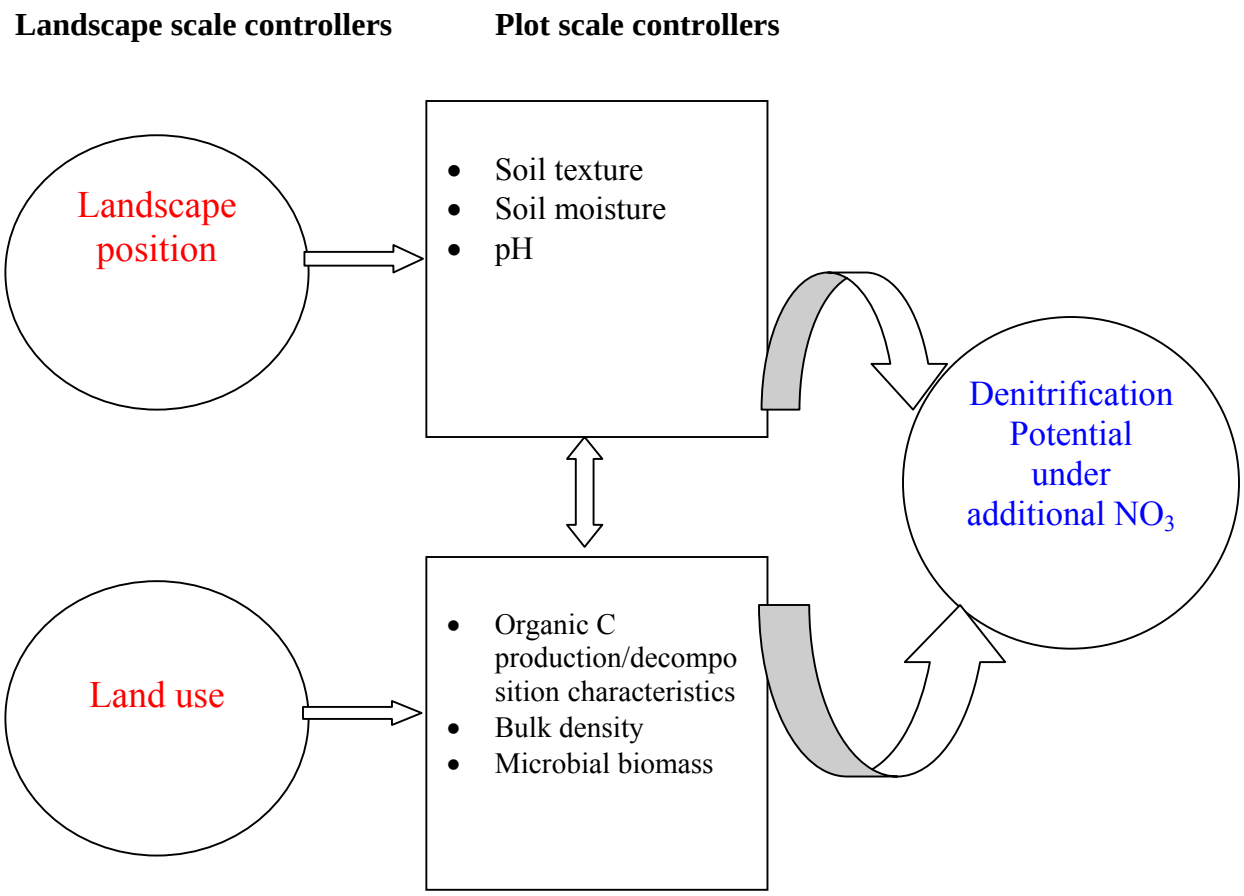

Figure 1.2 Landscape scale and biogeochemical scale controllers of denitrification potential

concentration increases the ratio (Weier et al. 1993; Dalal et al. 2003; and Sexstone et al. 1985). There is increasing concern about N_2O emissions during denitrification since it is a potent greenhouse gas (Hefting et al. 2003), having 300 times greater global warming potential than CO_2 (Granli and Bockman, 1994).

Needs for Further Research

Agricultural watersheds in the LMV are not homogenous croplands, but are a mosaic of land uses including aerated and poorly drained soils under row crop cultivation, a network of drainage ditches and access roads, and sometimes patches of bottomland hardwood forests and

depressional wetlands. The topography, hydrology and land use of these different landscape units may either enhance or reduce both soil denitrification rates and N_2O:N_2 emission ratios by affecting the various biogeochemical factors controlling denitrification and N_2O:N_2 emission ratio (Weitz et al. 2001). The specific impacts of land use changes, however, are not well understood and there is a need to investigate landscape and other environmental factors which regulate denitrification in different land use types at an agricultural watershed scale. To our knowledge, no such studies at a watershed scale on landscape and land use controls of denitrification have been undertaken in LMV. A more complete understanding of the factors and land uses which enhance denitrification is essential for optimal design of programs to reduce non-point source NO_3 pollution at a watershed scale.

Restored wetland buffers between croplands and rivers have been found to be effective in the removal of NO_3 from run-off (Hefting et al. 2003; Groffman et al. 1991; Lowrance et al. 1984), hence restoration of wetlands in agricultural watersheds is recommended (Mitsch et al. 2001; Lowrance et al. 1997; Hunter and Faulkner 2001; Lindau, et al. 1994). LMV has a great potential for forested wetlands restoration for improving water quality including NO_3 removal. While restoration of forested wetlands in the LMV is very likely to enhance denitrification rates, the impact on N_2O: N_2 emission ratio is more difficult to predict on the basis of land use, different soil hydrologic regimes and NO_3 loading. An increased N_2O:N_2 emission ratio from restored wetlands can compromise the benefits of restoration because N_2O is 300 times more potent as a greenhouse gas than CO_2 in inducing global warming (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 deplete stratospheric ozone. Not only N_2O, but CH_4 flux from restored wetlands in the LMV may be affected by the way we restore wetlands. CH_4 has 21 times

more global warming potential than the atmospheric CO_2 gas (Robertson et al. 2000). There are no published reports of the CH_4 flux from bottomland hardwood wetlands in the LMV. Because of the potential impact of restoration on the atmospheric burden of greenhouse gases, an investigation is merited regarding the effects of restoration on the fluxes of N_2O and CH_4.

Some studies have found that newly restored sites are not functionally comparable to natural wetlands regarding denitrification rates. Hunter and Faulkner (2001) reported significantly lower denitrification rates in a restored wetland compared to a natural forested wetland in LMV. A newly restored wetland site having low available organic C when loaded with addition NO_3 may lead to high N_2O emissions (Hefting et al. 2003).This raises the question of how to increase the rate of denitrification and at the same time reduce the N_2O:N_2 emission ratio from restored wetlands receiving NO_3.

Other research needs include evaluations of the impact of BMPs (structural and grassed ditches) on rates of denitrification in cultivated soils, and what additional measures are needed to ensure that the BMPs remove run-off NO_3. This question is rooted in the general observation that structural BMPs increase soil moisture-a major controlling factor of denitrification (Myrold, 1998) and thus may enhance denitrification rates in cultivated soils under their influence. But cultivated soils are often carbon limited for optimum denitrification, thus questions arise concerning identification of an economically feasible way of amending existing soil organic C to increase denitrification and the effects of these amendments on greenhouse gas emissions.

Research Objectives and Hypotheses

The objectives of this research were:

1. Determine landscape and land use controls over denitrification potential in an agricultural watershed.

The following hypotheses were tested in the Beasley Lake agricultural watershed in the LMV (Chapter 2).

- Coarse-textured soils have lower denitrification potential than fine-textured soils in agricultural watersheds.
- Landscape units under natural wetlands have higher denitrification potential than similar units under cultivation.
- Most of the cultivated lands are limited by microbially available organic C to denitrify.
- Denitrification potential is influenced by season and soil moisture contents.

2. Determine the impacts of BMPs and organic C amendments on denitrification rate of active croplands and restored wetlands in an agricultural watershed.

The following hypotheses were tested under this objective:

- Addition of organic C to cultivated and newly restored wetland sites increase denitrification rates
- Structural BMPs applied at cultivated and restored wetland sites increas soil moisture content, which results in higher denitrification rates

To accomplish this objective, cultivated and restored sites under the influence of structural BMPs were selected and amended with cotton gin trash to assess their effectiveness in enhancing denitrification rates in an agricultural watershed (Chapter 3).

3. Quantify the effects of the primary controls- saturation, microbially available carbon, and NO_3 concentration- on denitrification rates and greenhouse gas emissions from restored and natural forested wetlands.

The following hypotheses were tested under this objective:

- An increase in percent water-filled pore space (WFPS) increases denitrification rates and reduces N_2O:N_2 emission ratio from soils
- Newly restored forested wetlands are not functionally equivalent to natural forested wetlands with reference to denitrification capacity
- Organic C amendment and NO_3 additions to restored forested wetlands increase denitrification rates; however, it also results in higher N_2O and CH_4 emissions into the atmosphere

Several experiments were run to accomplish this objective. Denitrification rates and N_2O:N_2 emission ratio from Sharkey soil under row crop cultivation and adjacent forested wetlands with and without the additions of NO_3 were determined in an agricultural watershed. Chapter 4 presents the results of this study. Denitrification rates and N_2O:N_2 emission ratio from a 13-year old restored and an adjacent forested wetland were determined under the influence of cotton gin trash amendment and NO_3 additions (Chapter 5). The impacts of cotton gin trash and NO_3 additions on N_2O, CH_4 and CO_2 emissions from restored and natural forested wetlands were investigated (Chapter 6). Chapter 7 synthesizes the overall findings of all the experiments in the context of forested wetlands restoration for NO_3 pollution control in agricultural watersheds in the LMV.

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CHAPTER 2

DENITRIFICATION POTENTIAL OF AGRICULTURAL AND WETLAND SOILS IN THE LOWER MISSISSIPPI ALLUVIAL VALLEY

Introduction

The primary source of increased NO_3 in surface waters is nitrogen (N) fertilizer applied to croplands (USEPA, 1996). Increases in the NO_3 concentration of water bodies is correlated with increased agricultural activity in river watersheds (Smith et al., 1987, Galloway et al. 2003). Nitrogen fertilizer use in the US increased by 300% from 1961 to 1999 and current usage consumes 13% of the inorganic N fertilizer used globally (Howarth et al. 2002). Agricultural lands often contribute more NO_3 to surface waters than forests even if croplands are not fertilized, due to lower evapotranspiration, greater water run-off (Seyfried, et al. 1991) and higher soil erosion (Rebich, 2001). Thus, expansion of agricultural activities coupled with an increased use of synthetic N fertilizer in the US has resulted in excessive accumulations of reactive N in environments external to croplands (Galloway, 2002; Howarth et al. 2002).

Extensive agricultural development and N fertilizer use over the past 200 years in the Mississippi River basin has increased NO_3 loading into the river and the northern Gulf of Mexico (Turner and Rabalais, 2003). Natural ecosystems (forests, grasslands, and wetlands) were cleared, ditched and drained to reclaim agricultural lands throughout the watershed. Ditching and draining (surface and tile drains) agricultural lands accelerated the transport of N to the Mississippi River (Donner, 2004). Since the 1950's, N fertilizer use has increased 20-fold in the basin (Battaglin and Goolsby 1996), which has contributed to a 3-fold increase in the NO_3 load of the Mississippi River (Turner and Rabalais, 1994; Donner, 2004). Agricultural run-off contributes about 74% of the current NO_3 loading carried by the Mississippi River (Rabalais, et

al. 2002) and the increased NO_3 loading is cited one of the major causes of the extensive hypoxia in the northern Gulf of Mexico (Rabalais, et al. 2002). A 30% reduction of the N load delivered by the Mississippi River has been recommended to reduce the hypoxia (EPA 2001; Mitsch et al. 2001).

The Lower Mississippi Alluvial Valley (LMV) has lost about 80% of its bottomland hardwood forests to other land uses primarily agriculture (Allen, et al. 2001). The LMV was the largest floodplain ecosystem in the US covering about 23,300-km^2 area. Bottomland hardwood forests covered this floodplain and were flooded seasonally as a result of over-bank flooding by the Mississippi River. Due to growth in agriculture, the bottomland hardwoods were cleared, drained, ditched and cultivated for decades for different row crops cultivation. This practice not only led to the loss of the NO_3 sinks in the form of greater denitrification rates of bottomland wetlands (Hunter and Faulkner, 2001), but enhanced its potential of loading additional NO_3 into surface waters including the Mississippi River through N fertilizer use, soil erosion (Rebich, 2001; ARS, 2001), mineralization of organic nitrogen, and direct drainage of the cultivated lands.

Measures and research work recommended by Mitsch et al. (2001) for reducing the NO_3 loading of the Gulf of Mexico from the Mississippi River basin includes on-farm soil and N fertilizer management to enhance N use efficiency, alternative cropping and management systems for reducing N loss from croplands and creation or restoration of wetlands and riparian ecosystems at suitable locations between croplands and water bodies to remove run-off NO_3 before its outfall into the river. Like elsewhere in the basin, restoration of forested and riparian wetlands to filter agricultural run-off to reduce NO_3 through plant uptake and denitrification (Lowrance et al. 1984, Comin et al. 1997) in LMV is recommended (Lindau et al. 1994).

Moreover, re-connecting the forested and riparian wetlands with the rivers for over-bank flooding is another measure suggested for NO_3 removal from river water through denitrification in the LMV (Lindau et al. 1994, Lowrance et al. 1997). Denitrification is one of the major biological processes for NO_3 removal from soil and water. Soil organic carbon and NO_3 contents, water regime, temperature and soil texture affect the rate and extent of denitrification (Galloway, et al.2003). These physiochemical soil characteristics are the result of interactions of topography, soil hydrology and soil management at basin and sub-watershed scales in the landscape (Lowrance et al. 1997, Peterjohn and Correll, 1984).

Agricultural watersheds in the LMV are not homogenous croplands, but are a mosaic of land uses including aerated and poorly drained soils under row crop cultivation, a network of drainage ditches and access roads, and patches of bottomland hardwood forests and depressional wetlands. Based on the current land use, hydrology, and topography, these different land use types can either enhance or retard denitrification. Maintaining environmentally sound crop production in the LMV and reducing NO_3 loading into aquatic ecosystems warrants investigation of landscape and environmental factors regulating denitrification potential in agricultural watersheds. To our knowledge, there are no scientific studies available on this topic in the LMV. Our objectives were to 1) determine the denitrification potential of different land use types of an agricultural watershed in the LMV and 2) assess some of the environmental and land use management factors regulating the denitrification capacities.

Material and Methods

Study Area

The study area was the 8.5 km^2 Beasley Lake watershed in Sunflower County, MS (Figure 2.1). The watershed is part of the Yazoo delta region of Northwestern Mississippi formed

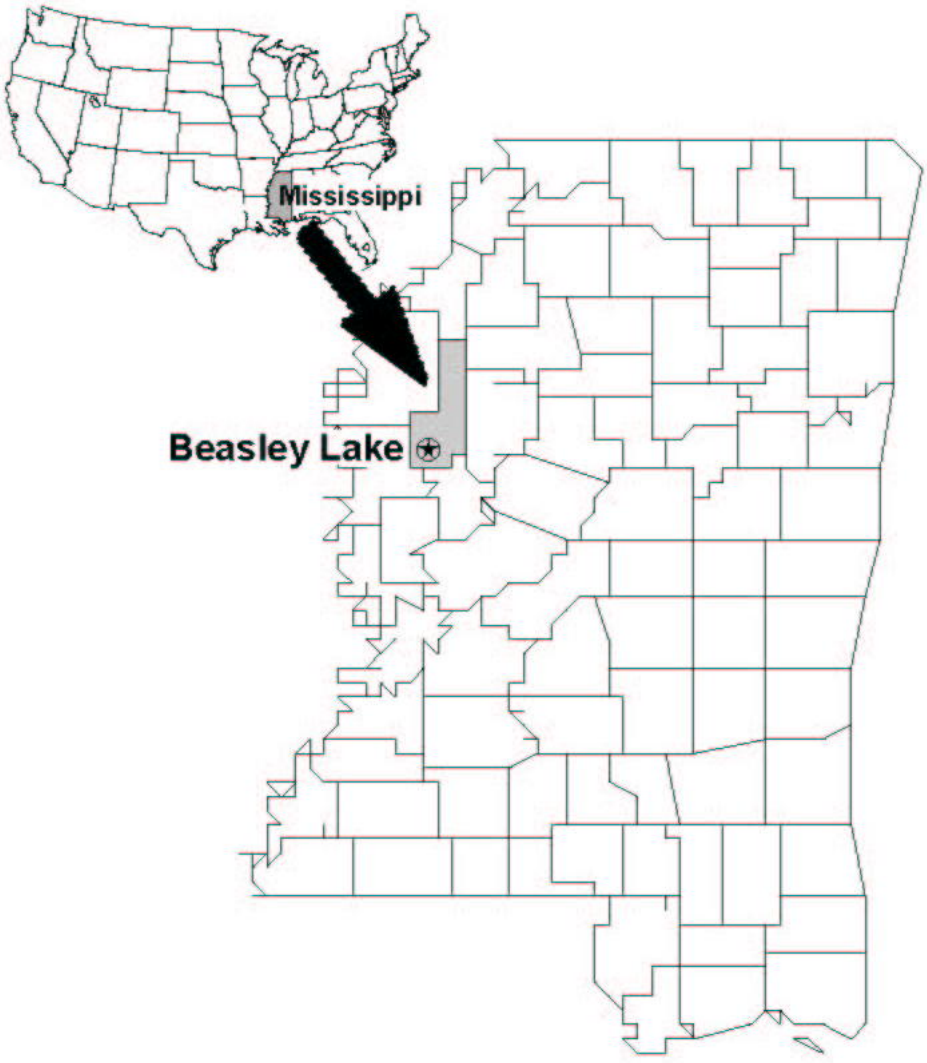

Figure 2.1. Location of the Beasley Lake watershed, Mississippi

by the alluvial deposits of the Mississippi River and its tributaries in the region (Fisk, 1951). Soils of the watershed range from coarse-textured silty-loam and loam to fine-textured clay alluvium. Dominant soil series of the watershed are Sharkey clay (non-acidic montmorrilinitic, Vertic Haplaquept), Dowling (Very-fine, smectitic, nonacid, thermic Vertic Endoaquept), Alligator (Very-fine, smectitic, thermic Chromic Dystraquert), Dundee (Fine-silty, mixed, active,

thermic Typic Endoaqualf), Dubbs silt loam (Fine-silty, mixed, active, thermic Typic Hapludalf), and Forestdale (Fine, smectitic, thermic Typic Endoaqualf), (NRCS, 1959).

The elevation gradient between the highest and lowest points in the watershed is 5.5 meters. Current land uses consist of high (Ag-high) and low elevation croplands (Ag-low), vegetated ditches (veg-ditches), unvegetated ditches (unveg-ditches), natural forested wetland and depressional wetlands (Figure 2.2). Forested wetlands are dominated by bottomland hardwood tree species. Depressional wetlands are small depressions next to the Bealey Lake, which remain ponded during winter and spring and are dominated by submerged and emergent wetland vegetation. These depressions are the remnants of the swale and ridge topographic features of an ox-bow lake watershed where the swales developed into depressional wetlands. Ag-high covers mainly well-drained soils while Ag-low covers poorly drained soils next to depressional wetlands and forested wetlands. A low elevation natural ditch next to the forested wetlands was developed into a constructed wetland in Spring 2002, through excavation and installation of a water control structure to increase the aerial extent of the flooded soil. The heavy clay soil of the constructed wetland was similar to that of the nearby natural wetlands. Some of the physio-chemical properties of the soils of different land use types of the watershed are shown in Table 2.1.

The major management activity in the watershed is crop cultivation. Recreation land uses include fishing and hunting in the lake and in forested wetlands. Overhead irrigation is applied as needed for crop cultivation. Irrigation run-off and rain water are drained through the ditches into Beasley Lake. Due to cultivation and the shunting of agricultural runoff directly to the lake, sedimentation in the lake has increased to a degree where it now threatens its ecology (ARS,

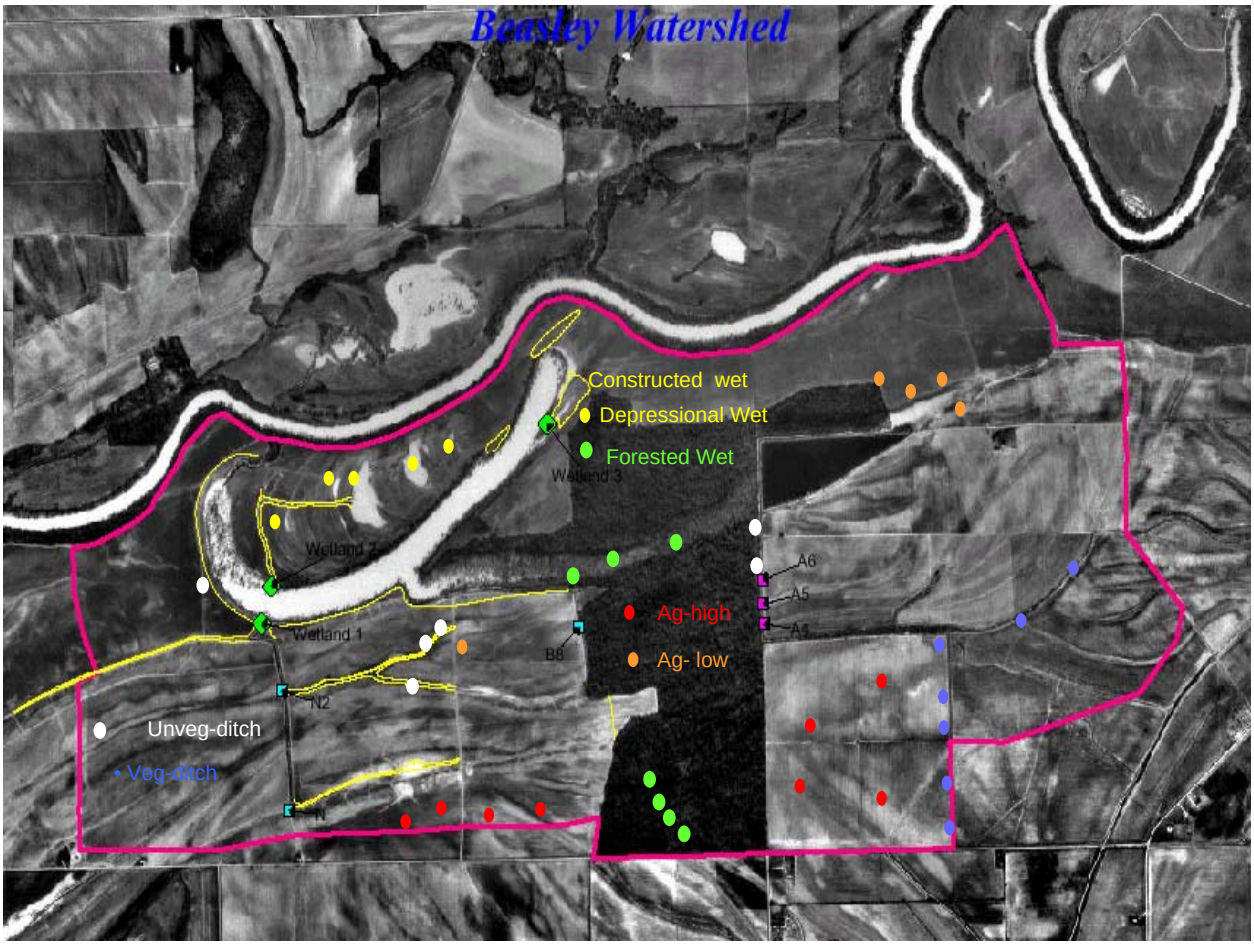

Figure 2.2. Sampling locations within seven landuse types of Beasley watershed

2001). Maintaining ditches under grass cover is one of the best management practices (BMP) to help reduce sedimentation in Beasley Lake promoted by USDA (Rebich, 2001).

Seven land use types of the watershed were selected for this research (Ag-high, Ag-low, veg-ditches, unveg-ditches, forested wetlands, depressional wetlands and the constructed wetland). Eight sampling points were selected randomly (n =8) in each land use type. Four soil cores (0-10 cm deep; 3 cm dia.) were collected from each sampling point of the seven land-use types of the watershed using a hand auger in March 2002, July 2002, October 2002 and January 2003. The four cores from each sampling point were composited and were transferred to the laboratory on ice and refrigerated at 4 ^{o}C for further analysis.

Denitrification Potential (DP)

The inherent capacity of a soil to undergo denitrification under an unlimited supply of NO_3 and organic carbon under anaerobic conditions is called denitrification enzyme assay (Beauchamp and Bergstrom, 1993, Groffman, et al.1999). This assay measures the amount of denitrification enzymes available at the time of soil sampling. We modified this procedure by amending the soil slurries only with NO_3 as we were interested in the denitrification potential (DP) of the different land use types under their existing soil carbon contents using the C_2H_2 block technique (Hill and Cardaci, 2004; Knowels, 1982). It is well established that adding carbon to NO_3 amended slurries will increase denitrification rates (Hunter and Faulkner, 2001; Groffman and Crawford, 2003), however there is no practical way of increasing soil carbon at the landscape scale. Therefore, this approach is the more realistic evaluation of the existing ability of the different land use units to remove NO_3 from surface or ground water. For the purpose of this study DP is defined as the capacity of soil slurries to denitrify NO_3 under anoxic conditions with and without additional NO_3. Field moist soils were thoroughly homogenized by hand and brought to room temperature overnight before incubation. The next morning, six subsamples (10 g) of the homogenized soil from each of the eight soil samples from each of the seven land use types were weighed into serum bottles (150 mL). Fifteen mL of 10 mg NO_3 L^{-1} solution and 5 ml of de-ionized water were added to triplicate bottles out of the six bottles to deliver 15 µg of NO_3 g^{-1} dry soil, while 20 mL of de-ionized water was added to the rest of the triplicate bottles as un-amended control. The bottles were then capped airtight and purged with O_2-free N_2 gas for 20 minutes to induce anaerobic conditions. Ten percent of the serum bottle

Table 2.1. Soil physio-chemical properties of soils (mean (SE)) of the7 landscape units in Beasley watershed

Soil parameters	Forested wetland	Dep.wetland	Constructed wetland	Veg-ditches	Unveg-ditches	Ag.low	Ag.high
Bulk Density (g cm^{-3})	0.90 (0.03)	0.96 (0.01)	1.20 (0.2)	1.17 (0.3)	1.24 (0.4)	1.24 (0.9)	1.31 (0.4)
Porosity cm^3 cm^{-3}	0.66	0.64	0.55	0.56	0.53	0.53	0.51
Clay (%)	51 (0.5)	47 (1.2)	45 (1.2)	51 (2.5)	45 (5.4)	48 (2.1)	23 (0.0)
Silt (%)	47 (0.7)	49 (3)	45 (1.6)	44 (3.5)	33 (2.8)	46 (1.3)	65 (0.8)
pH	5.4	5.3	5.5	5.6	5.6	6.1	6.3
Total C 0-10 cm(Mt ha^{-1})	297 (30)	167 (18)	218 (9)	189 (11)	156 (44)	168 (13)	85 (26)
Total N 0-10 cm (Mt ha^{-1})	26 (2)	17 (1.4)	23 (0.9)	24 (2.2)	13 (2.6)	11 (1.0)	5 (3.5)
NO_3 (mg kg $soil^{-1}$)	5.7 (1.7)	6.5 (2.5)	6.1 (2.8)	7.6 (3.2)	7.4 (1.9)	8.0 (1.6)	7.7 (3.2)

headspace was replaced with cleaned C_2H_2 gas to block the bacterial conversion of N_2O to N_2 gas. The bottles were then wrapped in Al foil and put on a reciprocating shaker for continuous shaking at room temperature (22 to 25 ^oC). Headspace gas samples were collected at 2, 4 and 6 hours with a syringe and stored in Beckton Dikinson vacutainers®. The gas samples wereanalyzed on a Tremetrics 9001GC having a porapack Q column with ECD detector for N_2O concentration determination. The rate of N_2O production was calculated in mg N_2O-N Kg^{-1} h^{-1} using the 3 gas sample readings during the 6 hour incubation. Adjustments were made for soluble N_2O in the bottles using a Bunsen absorption coefficient of 0.54 for N_2O at 25 ^oC.

Anaerobically Mineralizable Organic Carbon (AMOC)

Denitrification depends directly on the amount of mineralizable organic C available to the denitrifier population under anaerobic conditions (Singh, et al. 1988, Gale, et al. 1992). Since denitrification is an anaerobic process, the amount of mineralizable organic C available under anaerobic conditions would help explain any trend in the DP among different land use types. Field moist soil equivalents of 5 g oven-dried soil from each soil sample were weighed into 150 mL duplicate serum bottles. Twenty ml of 50 mg NO_3 L^{-1} solution was added into each bottle, which delivered 200 μg NO_3 g^{-1} soil. The bottles were capped airtight and purged with oxygen-free N_2 gas for 20 minutes to induce anaerobic conditions. After purging, the bottles were wrapped in aluminum foil and were shaken for 15 minutes on a reciprocating shaker. After shaking, the bottles were stored at room temperature (22-25 ^oC). The headspaces of the bottles were sampled with a syringe at 7, 14, 21 and 28 days of incubation and stored in 5-mL Beckton Dikinson vacutainers®. The gas samples were analyzed on a Tremetric 9001 GC

equipped with an FID detector for CO_2 concentration. The amount of CO_2 produced was calculated in μg CO_2 g^{-1} per 4 weeks. Corrections were made for soluble CO_2 in the incubation bottles by using the Bunsen Adsorption coefficient of 0.752 at 25 ^oC.

Total Soil Carbon and Nitrogen

Total soil carbon (C) and nitrogen (N) were determined using a Perkins Elmer 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 to determine concentrations of organic C and total N. These values and bulk density measurements were used to calculate Mt of C and N ha^{-1}.

Soil NO_3

The denitrifying bacteria use soil NO_3 as the terminal electron acceptor during their anaerobic respiration and the amount of NO_3 available determines the rate of denitrification (Patrick and Reddy, 1976). Moist soil equivalents of 5 g oven-dried soil were weighed into 250 mL duplicate bottles. Fifty mL of 2M KCL solution was added to each bottle. The bottles were put on a reciprocating shaker for continuous shaking for 1 hour. After shaking, the bottles were centrifuged at 3000 rpm for 5 min and were then filtered into 20 mL scintillation vials through a N0. 42 Whatman filter paper. The samples were stored in a freezer until analysis for NO_3 on a Lachat automated flow injection analyzer (Wendt, 2000). Average values for each soil sample were determined and reported in mg N Kg^{-1} oven-dried soil.

Soil Bulk Density, Porosity and Particle Size Distribution

At each land use type, eight intact soil cores (2.5 cm dia. x 10 cm long) were taken and transferred to the lab for the determination of soil moisture and bulk density

(Blake, 1965). Soil porosity was determined using the equation of 1- (bulk density/particle density) (Vomocil, 1965). Soil particle size distribution was determined by the filtration method according to Sheldrick and Wang (1993).

Statistical Analysis

Differences in DP among the landscape units within each season were analyzed by a two-way analysis of variance using the General Linear Model (GLM) in SAS (SAS Institute, 1998). Landscape was treated as main effect, NO_3 amendment was treated as a sub-plot effect and season was treated as repeated measures in the ANOVA model. Post ANOVA tests were conducted with Fisher's protected LSD at 5% significance level. Pearson correlation coefficients among DP, AMOC and soil moisture were calculated for each season. Linear regression of the yearly averaged DP on total soil C, N, clay contents and bulk density of the 7 land use types was done using SAS. The data were analyzed for homogeneity of variance of the residuals and when necessary log transformed.

Results

Landscape effects on Denitrification Potential

Landuse, season, NO_3 amendment and their interactions significantly affected the DP (Table 2.2) The natural and constructed wetlands had significantly greater ($p < 0.05$) DP than the Ag-high and Ag-low sites in all seasons (Figures 2.3-2.6). Forested wetlands showed the highest DP while Ag-high showed the lowest during the four seasons when dditional NO_3 was added during the assay. The DP of forested wetlands was 4.5 to 11 times greater than the Ag-high during the year. Similarly, the forested wetlands showed 2.4 to 4.3 times greater DP than the Ag-low sites in all the seasons. Except in summer,

Ag-high and Ag-low had similar DP values ($p > 0.05$). On average, forested wetlands exhibited 3.0 and 2.7 times greater DP than those of high-ditches and low-ditches.

Depressional and constructed wetlands showed similar DPs, except in summer when the depressional wetland had 1.5 times greater DP than the constructed wetland. On average both depressional and constructed wetlands had 3.7 to 7.4 and 1.2 to 3.7 times greater ($p < 0.05$) DP than that of the Ag-high and Ag-low respectively during the four seasons. Depressional and constructed wetlands also showed 1.2 and 1.6 times greater DP than veg.ditches and unveg-ditches, respectively , but were less than the DP of forested wetlands.

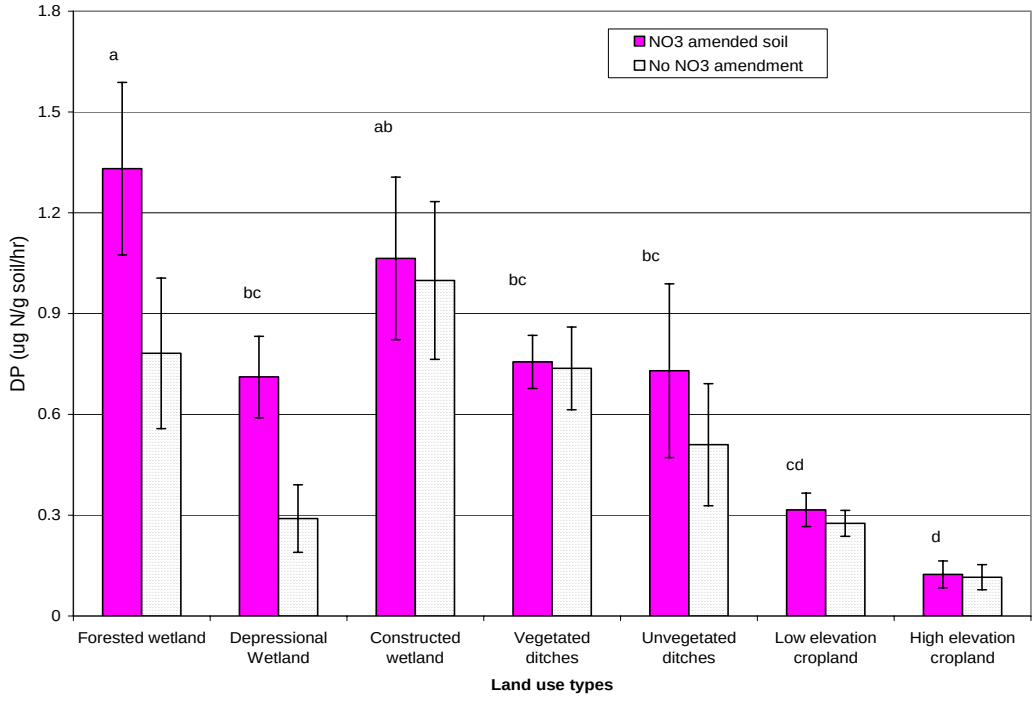

Figure 2.3. Spring denitrification potential of different land use types with standard error of the means (same letters on top of NO_3 amended bars show significant differences among land uses at $p < 0.05$ for the NO_3 amended bars only)

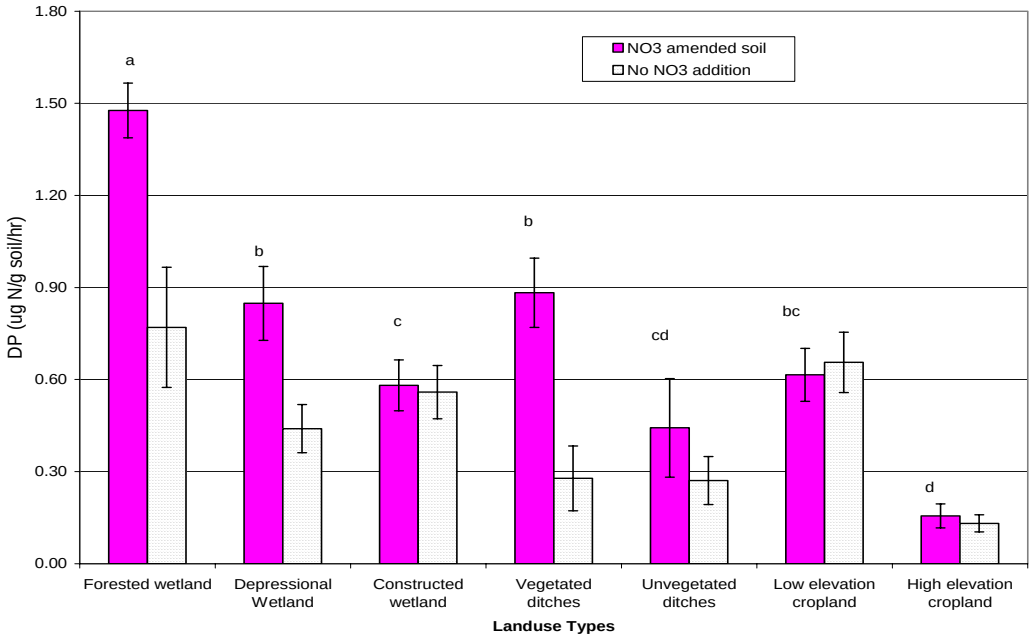

Figure 2.4. Summer denitrification potential of different land use types with standard error of the means (same letters on top of NO_3 amended bars show significant differences among land uses at $p < 0.05$ for the NO_3 amended bars only)

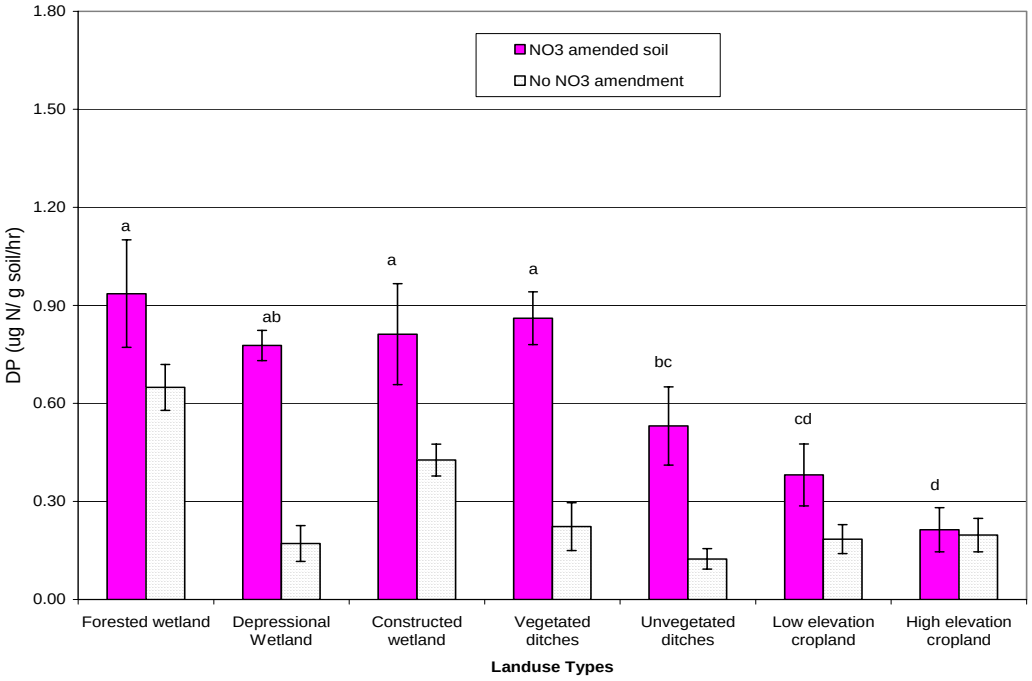

Figure 2.5. Fall denitrification potential of different land use types with standard error of the means (same letters on top of NO_3 amended bars show significant differences among land uses at $p < 0.05$ for the NO_3 amended bars only)

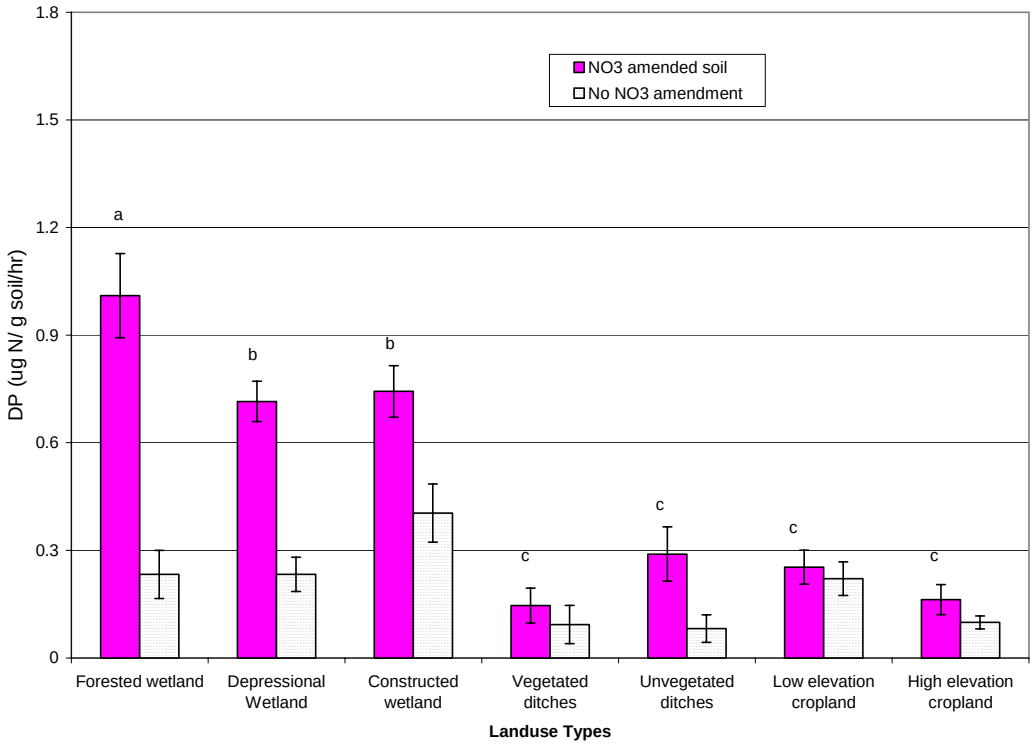

Figure 2.6. Winter denitrification potential of different land use types with standard error of the means (same letters on top of NO_3 amended bars show significant differences among land uses at $p < 0.05$ for the NO_3 amended bars only)

Table 2.2. Showing type 3 test of fixed effects results of the split-plot model

Effects	Numerator degrees of freedom	Denominator degrees of freedom	F value	P value
Landuse type	6	399	38	<0.0001
Season	3	399	19	<0.0001
NO_3 amendment	1	14	47	<0.0001
Land*Season	6	399	7	<0.0001
Land*NO_3 amendment	18	399	7	<0.0001

When the DP of individual land use types were averaged for the four seasons (Table 2.3), the DP of veg-ditches was 4.1 times of the Ag-high ($p < 0.05$) and 1.7 times of Ag-low, although statistically not significant ($p > 0.05$). Moreover, veg-ditches had 1.3

times greater DP than unveg-ditches. On the whole forested wetlands exhibited significantly greater DP ($p < 0.05$) than the rest of the land use types. Similarly, depressional and constructed wetlands had higher DP than unveg-ditches, Ag-high and Ag-low sites (Table 2.3), but had lower DP than the forested wetlands. The DP of veg-ditches was more variable and thus was not significantly different than Ag-low, unveg-ditches, constructed and depressional wetlands ($p > 0.05$).

Environmental Variables and DP

Among the environmental variables measured, NO_3 concentration, organic C availability, bulk density and soil moisture contents significantly influenced DP of all the land use types. NO_3 availability is a key factor controlling denitrification. When amended with additional NO_3 forested and depressional wetlands soils showed 55% and 67% greater DP compared to the DP of soil without NO_3 additions. Constructed wetland and unveg-ditches showed an increase in DP with NO_3 enrichment during fall and winter only while veg-ditches responded in summer and fall. Ag-high and Ag-low soil did not respond to NO_3 amendment during the four seasons (Table 2.3), which shows that their DPs were limited by factors other than NO_3, probably by the availability of organic C and lack of anaerobosis.

Season significantly affected the DPs of all the land uses except Ag-high and unveg-ditches (Table 2.3). When the DPs of all the land use types were averaged together and tested for a seasonal effect, the winter DP was found the lower than the rest of the seasons. Lower winter soil temperatures are speculated to have limited the denitrifier activity. Similar seasonal effect on denitrification potential of forested wetland soils in the LMV was observed by Hunter and Faulkner (2001).

Significant differences in a number of soil properties of the different land uses were observed (Table 2.1). Wetlands had 1.8 times greater total soil organic C than the cultivated soils. Higher soil organic C in wetlands contributed to improved soil structure resulting in low bulk densities and high soil porosities. Wetlands had lower soil bulk densities and greater soil pores spaces than the Ag-high and Ag-low sites. Linear

Table 2.3. Yearly average denitrification potential (DP) with post-ANOVA comparisons, and NO_3 addition and seasonal effects on denitrification potential of different land use types at $p < 0.05$

Land use types	DP of NO_3 amended soils (ug N g^{-1} h^{-1})	NO_3 addition effects on DP	Seasonal effects on DP
Forested wetland	1.18 a	*	*
Depressional wetland	0.77 b	*	*
Constructed wetland	0.82 b	*	*
Veg-ditches	0.66 bc	*	*
Unveg-ditches	0.50 c	*	ns
Low-elevation Agric.	0.40 cd	Ns	*
High-elevation Agric.	0.16 d	Ns	ns

*: significant effect, ns: non-significant effect

regression identified a significant relationship between bulk density and DP with an R^2 value of 0.80 (Figure 2.7). Anaerobic incubation of soils from the 7 land use types showed that the amount of anaerobically mineralizable organic carbon (AMOC) of wetland soil was 1.4 times those of the cultivated soils. Similarly, veg-ditch soil had a relatively lower soil bulk density, higher porosity and 1.3 time greater AMOC values

compared to those of the cultivated soils (Table 2.4). Unveg-ditches had similar soil bulk density, porosity and AMOC values to those of the cultivated soils. High AMOC values observed in the wetland and veg-ditch soils supported significantly greater DP compared to those in the cultivated soils (Singh et al. 1988). AMOC showed a significant correlation with DP of NO_3 amended soils during the four seasons ($p<0.05$). AMOC values of the spring significantly correlated with DP ($p <0.05$) with an r value of 0.51 (Table 2.4). In summer the AMOC values for each landscape position increased compared to the spring AMOC values. A significant correlation of 0.57 between summer AMOC and DP values was observed ($p <0.05$). Similarly, significant correlations of 0.75 and 0.81 between AMOC and DP values of fall and winter seasons were observed ($p <0.05$). Like the AMOC, total soil N and C significantly correlated with the DP of the NO_3 amended soils with R^2 values of 0.80 and 0.78, respectively (Figures 2.8 and 2.9). Other studies have also reported a close correlation between available soil carbon and the denitrification potential of wetland and agricultural soils (Ingrid-Brettar, et al. 2002, DeLaune, et al. 1996, Singh, et al. 1988, Davidsson, and Stahl, 2000).

Soil moisture content regulates oxygen diffusion into soil and hence denitrifying activity (Patrick and Reddy, 1976). Potential denitrification was significantly correlated with soil moisture in each of the four seasons. The Pearson's correlation coefficients of spring, summer, fall and winter soil moisture with DP were 0.46, 0.44, 0.69 and 0.57 respectively at $p <0.05$ (Table 2.5). This result is in agreement with other studies reporting a significant effect of soil moisture on denitrification rates (Weier, et al. 1993; Groffman and Crawford, 2003; Jordan et al. 1998; Sexstone, et al. 1985).

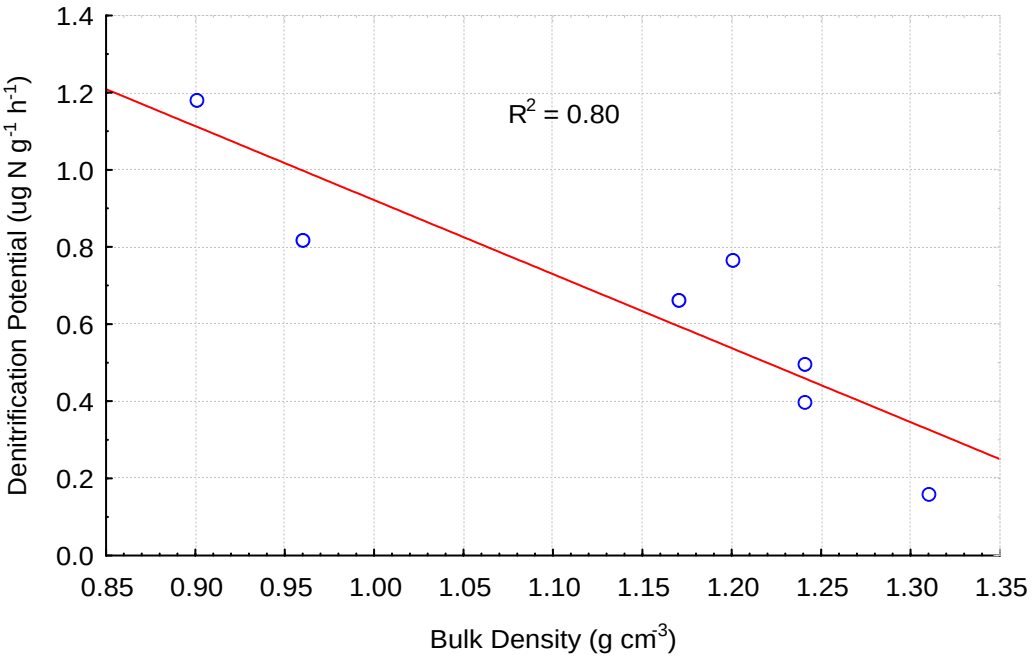

Figure 2.7. Linear regression of average potential denitrification on soil bulk density

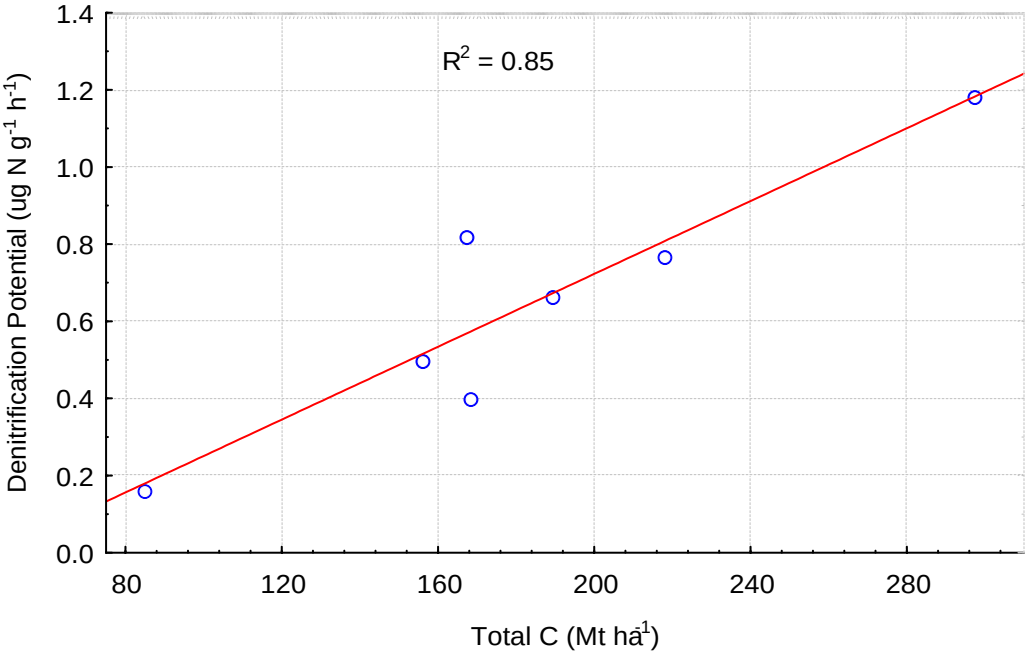

Figure 2.8. Linear regression of average potential denitrification on total soil C

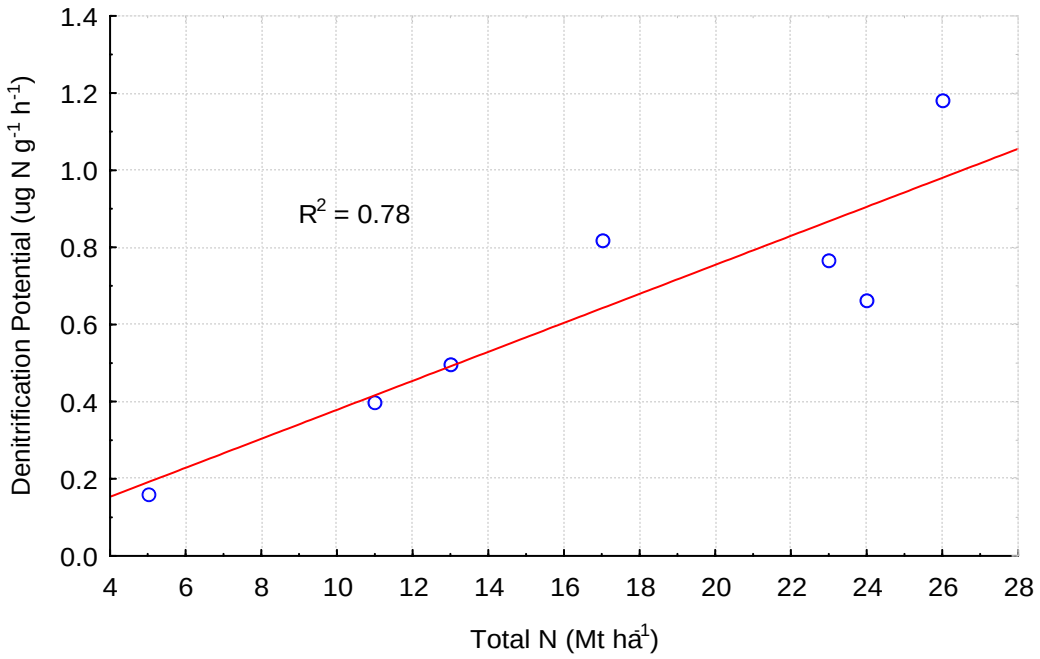

Figure 2.9. Linear regression of average potential denitrification on total soil N

Discussion

Higher total soil carbon, mineralizable organic C, wetter soil conditions, greater soil porosity and the fine clay texture of natural and constructed wetlands (Table 2.1) supported greater denitrifier activity than those observed in the Ag-high and the pedogenically similar Ag-low and unveg-ditch soils (Hill and Cardaci 2002; Davidsson and Stahl, 2000). At the biogeochemical scale, soil moisture content, soil texture, available C and NO_3 are the major factors controlling denitrification (Myrold, et al.1998; Parkin, et al. 1984) and the same factors were found significantly affecting the DPs in the Beasley watershed. The status of these factors is a result of the interaction of soil O_2 diffusion rate, landscape position, plant community structure, climate, physical disruption (landuse), ecosystem type, microbial biomass, and organic matter production characteristics of a given landscape unit (Pastor and Post, 1986). Some of these

interacting controlling factors of denitrification were found different for the seven landscape units, which resulted in significant differences in the observed DPs among the land use types (Table 2.1).

Significant differences in the DPs of different land use types (Figures 2.3 to 2.6) are attributed to differences in the position and management of different land uses in the watershed. Low-lying heavy clay soil (Sharkey, Dowling and Alligator soil series) under forested and depressional wetlands led to 6.3 greater DPs than the Ag-high silt-loam soils. Groffman and Tiedje (1989) also found significant relationship between soil texture and drainage class in forest soils with higher denitrification rates in fine-textured, poorly drained soils compared to coarse-textured, well-drained soils. Mohn et al. (2000) also found low denitrification rates in drained mounds compared to wetland soils. These finding shows that poorly-drained, fine-textured soils in natural wetlands support higher denitrifier activity than the coarse textured Ag-high soils.

Forested, depressional and constructed wetlands showed 195%, 92% and 104% greater DP than the pedogenically similar (Table 2.1) Ag-low soils, respectively. Drainage and cultivation of the Ag-low soils over the years led to lower available organic C contents (Table 2.1), which resulted in lower denitrifier activity compared to similar soils under natural and constructed wetlands in the watershed. This finding suggests that land use of a landscape unit exerts major control over soils denitrifying activity, which is in agreement with the findings of Clement, et al (2002). In another study by the authors, using intact soil cores, higher denitrification rates were observed from the forested wetland soil compared to the Ag-low soils (Ullah et al., in press), showing that

Table 2.4. Anaerobically mineralizable organic carbon content of different land use types (mean ± SE) and its correlation with DP

Season	----------AMOC (mg CO_2 produced/ g soil /4 weeks)----------							
	Forested wetland	Dep. wetland	Constructed wetland	Veg-ditches	Unveg-ditches	Ag-low	Ag-high	Pearson's correlation with DP (n=57)
Spring	1.92 ± 0.24	1.55 ± 0.18	2.01 ± 0.26	1.89 ± 0.17	0.35 ± 0.06	1.13 ± .01	0.87 ± 0.17	0.51*
Summer	3.11 ± 0.33	3.17 ± .08	2.97 ± 0.12	2.24 ± 0.12	2.09 ± 0.09	2.18 ± 0.07	2.20 ± 0.10	0.57*
Fall	2.55 ± 0.06	2.56 ± .08	2.43 ± 0.10	2.42 ± 0.13	2.19 ± 0.08	2.25 ± 0.06	1.62 ± 0.02	0.75*
Winter	2.12 ± 0.10	1.71 ± 0.09	1.72 ± 0.10	1.29 ± 0.07	1.54 ± 0.07	1.46 ± 0.16	1.77 ± 0.06	0.81*

* Significant difference at $p < 0.05$

Table 2.5. Seasonal average soil moisture and temperature of the different land uses

Land use Types	----------Soil moisture (g g^{-1} oven-dried soil)--------			
	Spring	Summer	Fall	Winter
Forested wetland	0.41	0.32	0.39	0.35
Depressional wetland	0.40	0.18	0.33	0.28
Constructed wetland	0.28	0.16	0.30	0.26
Veg-ditches	0.26	0.34	0.38	0.28
Unveg-ditches	0.26	0.32	0.31	0.27
Low-elevation croplands	0.30	0.18	0.30	0.24
High-elevation croplands	0.17	0.14	0.23	0.16
Moisture correlation with DP	0.46*	0.44*	0.69*	0.57*
Soil Temp. ^{o}C	22	28	21	9

Significant at $p < 0.05$, and n= 56

cultivation diminished the capacity of these soils to denitrify compared to forested wetland soils. The natural vegetation cover and the resulting soil litter of forested and depressional wetland soils provide ample organic C for supporting greater heterotrophic denitrifier activity than similar textured Ag-low soils or coarse-textured Ag-high soils.

Veg-ditches had 1.3 to 4.2 times greater DP than that of the unve-ditches, Ag-low and Ag-high soils. The high denitrifier activity in the veg-ditches is attributed to its maintenance as a grassed waterway which resulted in maintaining high soil moisture and producing greater AMOC contents compared to the croplands and unveg-ditches. This finding supports the current practices of maintaining grassed-ditches as a BMP for

erosion control and water quality improvement as recommended by the USDA (ARS, 2001; Shreiber, et al. 2001).

The DPs observed in spring, summer and fall of all the land use types were significantly greater ($p < 0.05$) than their DPs of winter except Ag-high and unveg-ditch soils (Figures 2.3 to 2.6), respectively. This observation suggests that the lower average winter soil (0-10 cm) temperature (6 to 9 ^{o}C) (Table 2.5) substantially reduced denitrifier activity. Lower winter temperatures may have suppressed microbial activity (Magg, et al. 1997) and/or reduced the supply of mineralizable organic C to denitrifiers (Mohn, et al. 2000; Mogge, et al. 1998; Klein and Logtestijn, 1996). The winter AMOC contents were significantly lower than the summer and fall values of all the land use types (Table 2.4), showing temperature control on mineralizable C availability to denitrifiers.

NO_3 availability was found limiting DPs of forested and depressional wetlands, because NO_3 additions to these soils led to increases in DPs during the four seasons compared to the Ag.high and Ag.low soils (Figures 2.3 to 2.6). When averaged over the four seasons, forested and depressional wetlands showed 3 and 4-fold increase in DP under additional NO_3 compared to the unamended soils. Higher denitrification rates under additional NO_3 can happen, if the process is not limited by the availability of organic C (Weier, et al. 1993). No response of Ag-high and Ag-low soils to NO_3 amendment shows that DP in these land uses was limited by the availability of organic C (Table 2.3). Constructed wetland responded with increased DP to NO_3 additions in fall and winter only, about 6-9 months after its construction. Veg-ditches also responded with increased DPs to NO_3 additions. These results demonstrates that wetlands and veg-ditches can denitrify additional NO_3 coming from cultivated soils. This finding is in

agreement with the findings of similar studies conducted regarding NO_3 removal potential of riparian wetlands and grassed buffer-strips in agricultural watersheds (Lowrance, et al. 1995; Groffman and Crawford, 2003; Groffman et al. 2002; Ingrid Brettar et al. 2002; Clement et al. 2002; Gregory, et al. 1991; Lindau, et al. 1994).

High soil organic C and AMOC contents, lower bulk densities, higher porosities and fine soil textures of the wetland soils retained more water, resulting in greater anoxic microsites in soils (Weitz et al. 2001), and hence supported higher denitrifier activity (Hill and Cardaci 2002; Davidsson and Stahl; 2000, Skiba et al. 1998; Bradley, et al. 1992) than the croplands and unveg-ditches. Soil bulk density, total soil C, N, AMOC and soil moisture correlated significantly with DPs of the NO_3 amended soils, showing that these variables are exerting major controls over denitrification potential of different land use types in the watershed. Based on our results, the status or condition of these biogeochemical scale factors was significantly influenced by the landscape position and land uses in the watershed. Soil particle size distribution and soil moisture holding capacity are determined by the soil topographic position of a land use in these watersheds. Similarly, land use influences the organic C production and decomposition characteristics and bulk density of soils which in combination with the soil texture and moisture contents regulates denitrification potential under additional NO_3. These findings clearly shows that denitrification potential is regulated by more than one biogeochemical scale factors which are significantly influenced by landscape position and land uses in the watershed. This finding is similar to that of Marshal (1999) and Groffman et al. (1999), who found that denitrification cannot be predicted on the basis of one factor, rather a combination of factors are involved in the regulation of the denitrification process.

Therefore, it is very important to consider landscape scale variables (landscape position and land use) in addition to biogeochemical scale variables in assessing denitrification potential of soils and identification of sites for wetland restoration for water quality improvement.

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CHAPTER 3

USE OF WATER CONTROL STRUCTURES AND COTTON GIN TRASH AMENDMENTS TO ENHANCE DENITRIFICATION IN CULTIVATED SOILS

Introduction

Cultivation of land often diminishes the denitrification capacity of soils to reduce NO_3 to N_2O and N_2 gases. Consequently, soil NO_3 not taken by plants, immobilized by microbes or denitrified may be lost in humid areas by leaching below the rhizosphere or to receiving waters through agricultural run-off (Randal et al. 1997). An increase in the NO_3 level of water bodies is correlated with increased agricultural activity in the watershed (Smith et al., 1987). Humans have doubled the NO_3 load into terrestrial ecosystems and much of this increase is estimated to be from the use of N fertilizers and cultivation of leguminous crops (Vitousek, et al., 1997). This enrichment of soils and waters with additional reactive nitrogen (N) is of global concern (Galloway and Cowling, 2002).

Cultivation leads to better soil aeration, which results in greater aerobic decomposition of soil organic carbon (C) and lower soil moisture holding capacity (Breitenbeck, et al. 1980). Subsequently, the amount of labile organic C available to microbes in the cultivated soils including the denitrifiers is reduced compared to similar soils under natural ecosystems including wetlands. The amount of soil organic C substrate, NO_3 and soil O_2 concentration are all important biogeochemical regulators of denitrification (Tiedje, 1982). Greater quantities of labile organic C (Hill and Cardaci, 2004) and NO_3 under anaerobic conditions enhance denitrification rates, while higher soil aeration reduces it (Myrold, 1998).

Agricultural expansion in the Lower Mississippi River valley (LMV) during the past 200 hundred years resulted in the loss of about 80% of its bottomland hardwood forests. This practice not only led to the loss of the NO_3 sinks in the form of higher NO_3 removal through plants uptake and denitrification, but enhanced its potential of delivering additional NO_3 into receiving waters through N fertilizer use, soil erosion and direct drainage of the cultivated lands (ARS, 2001). Excessive NO_3 discharged into lakes, rivers and coastal waters can cause eutrophication. A 3-fold increase in the NO_3 loading of the Mississippi River, due mainly to agricultural activities, is cited as the major cause of eutrophication and the resulting hypoxia in the northern Gulf of Mexico (Rabalais, et al. 2002).

One means to reduce NO_3 run-off from agricultural watersheds is to identify agricultural best management practices (BMPs) which can reduce NO_3 through denitrification. BMPs are being implemented to reduce soil erosion and nutrient loss (including NO_3) from cultivated soils in the LMV (Locke, et al. 2001). Structural BMPs that control the rate of water discharge, such as slotted board risers (SBR) and slotted pipes coupled with sub-surface tile drains have been implemented on an experimental basis to assess their effectiveness in controlling soil erosion and nutrient loss. In the case of SBR wooden boards are put in slots in front of a drainage pipe, which covers half of the pipe's opening, in order to hold back drainage water from cultivated lands (Figure 3.1). This results in water ponding behind the SBR in the cropland up to the height of the board riser. This BMP is mainly designed to reduce suspended sediments in surface run-off from croplands, which have steep gradients and are more prone to erosion (Parkman, 2001). Anaerobic conditions in the inundated cropland behind the SBR may have higher

denitrification rates. Moreover, holding back the drainage water may provide sufficient time for NO_3 to be denitrified in the inundated soil before it is carried by the drainage. The SBR BMP has also been applied at newly restored Wetlands Reserve Program (WRP) and Conservation Reserve Program (CRP) sites for soil erosion and water quality improvement purposes in agricultural watersheds. As with agricultural systems, the use of SBR to retard discharge may enhance denitrification rates in the restored sites. The slotted pipe (SP) BMP is a slotted pipe placed at the main surface drainage end of a steep to gently sloping cropland to hold back water before its outfall into the main ditch (Figure 3.2). The SP BMP functions on the same concept like SBR, however, it has smaller dimension leading to shallower water ponding than the SBR. Moreover, the SP BMP is applied in conjunction with sub-surface tile drains in the cultivated land. The purpose is to reduce surface runoff by promoting sub-surface drainage and thereby reduce suspended sediment discharged from the field. The SP BMP may have an also impact on NO_3 removal through denitrification.

Denitrification in cultivated lands in the LMV is often limited by available C (Ullah et al. in press). Thus even if the structural BMPs are implemented in cultivated sites in the LMV, it may not be sufficient to promote denitrification due to limitation of the process by available C. A bench scale research study by Hunter (2000) showed a 45% increase in denitrification rates of restored wetland soils in the LMV amended with cotton gin trash (CGT). The author recommended CGT application to newly restored wetland soils for enhancing denitrifier activity. Tons of CGT are produced in the LMV and can be applied to soils as a BMP to provide C for enhancing denitrifier activity in cultivated and restored CRP and WRP sites.

Figure 3.1. Slotted board riser BMP installed in Beasley watershed

Figure 3.2. Slotted pipe BMP installed in Beasley watershed

No information is available on the impact of these water control structures and CGT amendment on denitrification rates of cultivated and restored soils in agricultural watersheds in the LMV. Field studies were conducted to determine whether water control structures and amending cultivated and newly restored soils with CGT can be used to better manage diffuse NO_3 pollution at watershed scale.

Material and Methods

Study Area

A study area was established in the 8.5 km^2 Beasley Lake watershed located in Sunflower County, MS (Figure 3.3). The watershed is part of the Yazoo delta region of Northwestern Mississippi formed by the alluvial deposits of the Mississippi River and its tributaries in the region (Fisk, 1951). Soils of the watershed range from coarse-textured silty-loam and loam to fine-textured clay alluvium. Dominant soil series of the watershed are Sharkey clay (non-acidic montmorrilinitic, Vertic Haplaquept), Dowling clay (Very-fine, smectitic, nonacid, thermic Vertic Endoaquept), Alligator clay (Very-fine, smectitic, thermic Chromic Dystraquert), Dundee silt loam (Fine-silty, mixed, active, thermic Typic Endoaqualf), Dubbs silt loam (Fine-silty, mixed, active, thermic Typic Hapludalf), and Forestdale silt loam (Fine, smectitic, thermic Typic Endoaqualf), (NRCS, 1959). The elevation gradient between the highest and lowest points in the watershed is 5.5 meters. Forestdale, Dubbs and Dundee soil series occur in the high elevation areas and ridges of the watershed, while Sharkey, Alligator and Dowling soil series occur in the low elevation areas of the watershed.

Cultivated lands, drainage ditches, riparian forests and depressional wetlands are the major land uses in the watershed. Some of the well-aerated high elevation and poorly

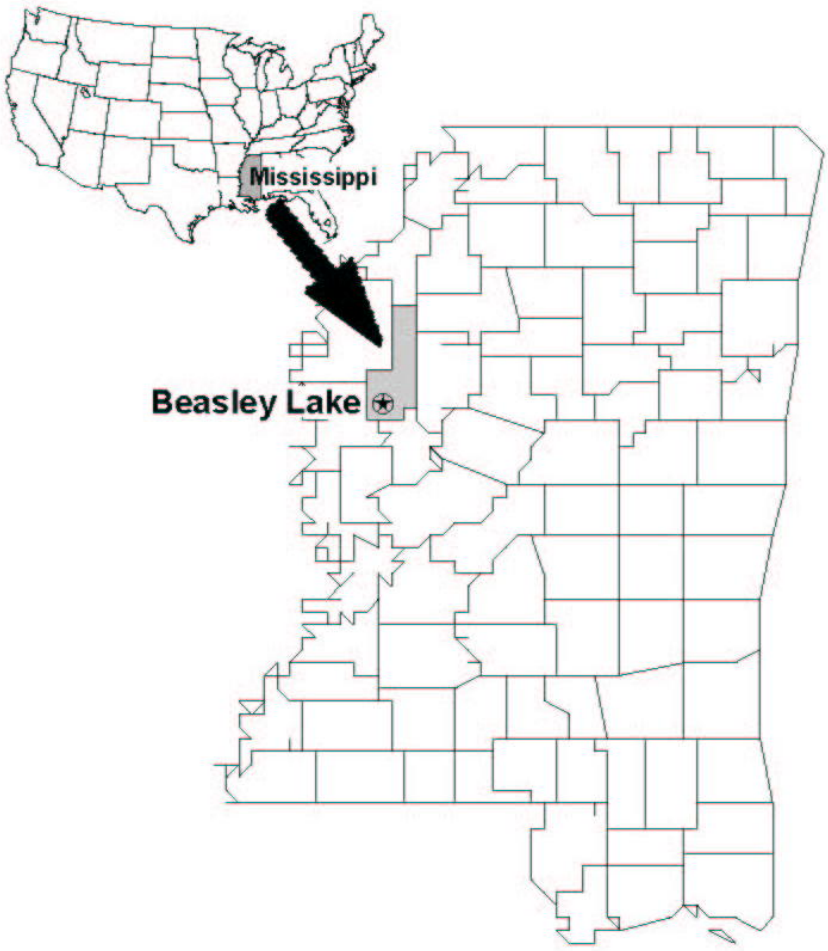

Figure 2.3. Beasley Lake watershed, Mississippi

drained low-elevation areas to the north of the watershed were restored under the CRP and WRP programs in the spring of 2002. Steep to gently sloping croplands and two CRP and WRP sites each in the watershed have SBR placed at their drainage end points to reduce soil erosion and possibly remove nutrients. These sites are named as SBR-cropland, SBR-grass and SBR-wet for the cropland, CRP and WRP sites respectively.

Two high elevation croplands have SP BMPs (SP-cropland) installed. The SP BMP sites have sub-surface tile drains. SBR BMP is operated from October to March (non-crop growing period). At the start of growing season in spring, the board riser is removed and the land is drained in preparation for planting. SBR causes more waterponding and inundation of the land compared to soils where discharge is controlled by SP.

We selected sites under row crop cultivation and one-year-old WRP and CRP sites for investigation of the SBR and SP effects on denitrification with and without CGT amendment. Two row crop sites (Control-cropland) with free surface drainage and without BMPs were also selected for this study. Selected physio-chemical characteristics of the soils of all the sites are given in Table 3.1.

Research Design and Soil Sampling

Six replicate sampling plots were randomly selected in the SP-cropland, Control-cropland and SBR-cropland sites (n=6), while 3 sites were selected in the SBR-wetland (n=3) and 2 in the SBR-grass site (n=2). The SBR-grass and SBR-wet sites were smaller in area, thus we were not able to establish 6 sampling plots for a balanced design like that of the SP-cropland and Control-cropland sites. The design is a split-plot with repeated measures, where BMPs are the main effect with CGT amendment as a split. The aerial extent of inundation behind the SBR and SP was demarcated with a laser level and the selected plots were placed with the BMPs hydrological influence. At each sampling site 2 plots of 1 m^2 area were placed next to each other. One plot at each selected sites was amended with CGT in October 2003. Two kilograms of CGT was spread manually on the soil surface of the selected plots. 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, and provides a readily mineralizable organic C substrate to microbes. The NO_3-N and NH_4-N contents of CGT are 15.4 ± 3.6 and 788 ± 40 mg kg^{-1} gin trash, respectively. Soil samples were collected from all 46 plots at 6-week intervals between October 2003 and April 2004. Bulk soil samples (10 cm deep) were collected and transported on ice to the laboratory for further analysis. Intact soil cores (5 cm x 10 cm) 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).

Denitrification and CO_2 Production Rates

Intact soil cores (5cm dia. x 10 cm ht.) were collected in plastic liners (5 cm x 15 cm dimensions) for the determination of denitrification rates 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 soils to allow zero-order kinetics during denitrification with reference to NO_3 and thus be able to assess CGT amendment effects. The cylinders were capped at the base and put back in the holes from which the cores were collected to maintain field temperatures. To measure denitrification rates, 10 ml of purified C_2H_2 gas was injected in small aliquots into the cores at the interface of soil and plastic liner to ensure diffusion of C_2H_2 throughout the soil column. After injection of C_2H_2 gas, the cores were capped tight and fitted with a 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.

Table 3.1. Physio-chemical properties of soils (0-10 cm) of the research sites

Sites	Texture Class	% Clay	% Silt	Bulk Density (g cm^3)	Porosity (cm^3 cm^{-3})	pH	Total C Mt C ha^{-1} (pre-CGT addition)	Total C Mt C ha^{-1} (post-CGT addition)	Total N Mt N ha^{-1} (pre-CGT addition)	Total N Mt N ha^{-1} (post-CGT addition)	C:N (pre-CGT addition)	C:N (Post-CGT addition
SBR-Wet	Clay	48	46	1.29 (0.05)	0.51	5.2	147	212	14	16	11:1	13:1
SBR-Crop	Loam	14	50	1.30 (0.03)	0.50	5.3	120	160	12	14	10:1	11:1
SBR-Grass	Silt Loam	13	58	1.44 (0.08)	0.45	5.5	115	134	9	10	13:1	13:1
SP-cropland	Silt Loam	22	66	1.21 (0.04)	0.54	6.4	140	200	15	17	9:1	12:1
Control-cropland	Silt Loam	23	65	1.21 (0.04)	0.54	6.5	144	171	14	15	10:1	11:1

(*CGT= Cotton gin trash)

Gas samples were taken from the headspaces of cores with 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 lab for analysis on a Varian 38001GC equipped with ECD and FID detectors. The rates of N_2O and CO_2 production were determined in μg N_2O-N m^{-2} h^{-1}a 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 coefficient.

Total Soil Carbon and Nitrogen

Total soil carbon (C) and nitrogen (N) of both the sites were determined using a Thermo Finnigan CNS analyzer during each sampling at the USGS National Wetland Research Center, Louisiana. The bulk soil samples collected from each plot were oven dried, homogenized thoroughly and pulverized. A sub-sample of about 35 mg was weighed into a tin capsule prior to their injection into the CNS analyzer. 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 NO_3, NH_4, pH and Temperature

Moist soils (5 gram oven-dry soil weight equivalent) were weighed into duplicate 250 mL bottles. Fifty mL of 2M KCl solution was added to each bottle. The bottles were put on an Eberbach shaker for continuous shaking for 1 hour. After shaking, the bottles were centrifuged at 300 rpm for 5 minutes and were then filtered into 20 mL scintillation vials through a No. 42 Whatman filter paper. The samples were frozen until analyzed for NO_3 and NH_4 with an automated Lachat FIA analyzer (Wendt, 2000). Average values for each soil sample were determined and reported in mg Kg^{-1} oven-dried soil. Soil pH was determined in the lab using 1:1 soil to de-ionized water mixing ratio. Soil temperature

was measured with a soil temperature probe (inserted up to 10 cm depth) during the field denitrification studies.

Soil Bulk Density, Water-filled Pore Spaces, and Particle Size

One intact core each was (5cm dia. x 10 cm ht.) collected at each sampling date and was dried in an oven 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 (Watts and Seitzinger, 2001) for the five sampling dates. Soil texture was determined by the modified pipette method (Sheldrick and Wang, 1993).

Statistical Analysis

Differences among denitrification rates of the different BMP sites were analyzed by two-way ANOVA using BMPs as the main effect and CGT amendment as a split. The 5 sampling times were treated as repeated measures in the ANOVA model. Fisher's protected LSD at p= 0.05 was used for post-hoc comparisons. Simple linear regression analysis of denitrification rates on mineralizable organic C was performed to identify any relationship between the two variables for each sampling date. All statistical analyses were performed using SAS (SAS Inc. 1998).

Results

The effect of BMPs on denitrification rates was significant in the SBR-wet and SBR-grass sites ($p < 0.05$), while non-significant in the SBR-cropland and SP-cropland sites. The SBR-wet site had 1.5 times greater denitrification rates than the rest of the sites over the study period (Figures 3.4-3.8). However, the differences in denitrification rates of the SBR-wet site were significant ($p < 0.05$) from the rest of the sites in October and December only ($p < 0.05$). SBR-grass site exhibited significantly greater ($p < 0.05$)

denitrification rates than the control-cropland and SP-cropland sites in March (Figure 3.7) and than all the sites in April (Figure 3.8). Denitrification rates of the SBR-cropland and SP-cropland sites were not different from the control-cropland site, although both the sites under the influence of the BMPs had higher average %WFPS (76%) than the control-cropland site (64%) (Table 3.3).

Denitrification rates were significantly increased by CGT amendment in all the sites. CGT amended plots of the SBR-wet site had 1.4 to 10.4 times greater denitrification rates than the unamended plots (Figures 3.4-3.8). The difference between CGT and un-amended plots of the SBR-wet site were not significant in February and April only. Lower soil temperatures (Table 3.2) observed in February (5.5 ^{o}C) and lower %WFPS (63%) observed in April may have led to non-significant differences in denitrification rates.

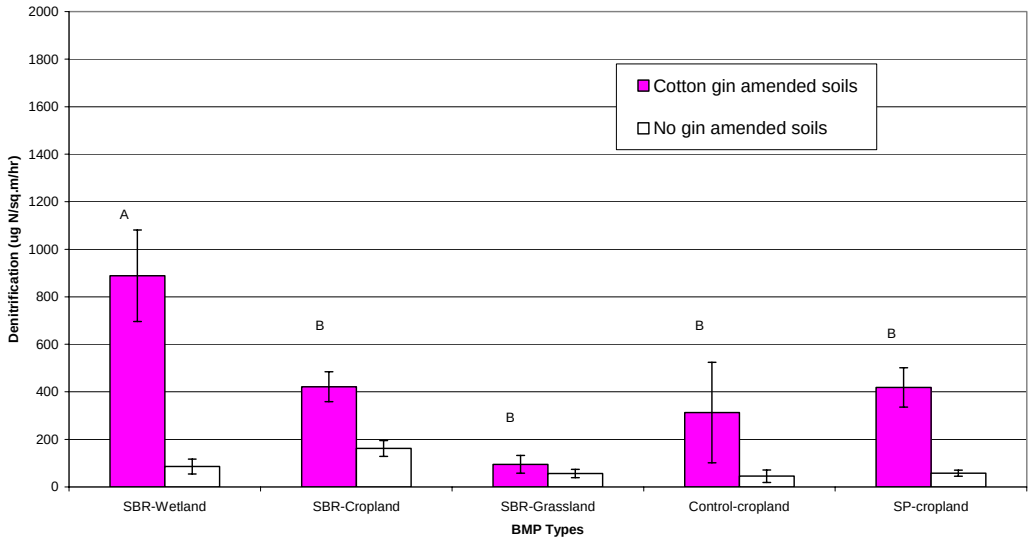

Figure 3.4. Denitrification rates of different land uses under different BMPs with and without cotton gin trash amendments (Different letters at the top of CGT amended bars shows significant differences between BMP types at $p < 0.05$) (October 2003).

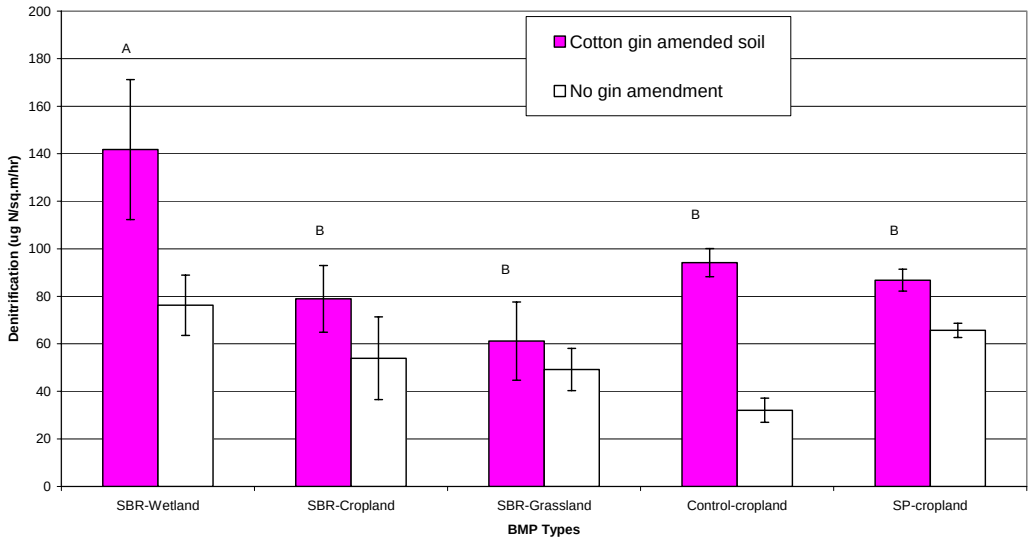

Figure 3.5. Denitrification rates of different land uses under different BMPs with and without cotton gin trash amendments (Different letters at the top of CGT amended bars shows significant differences between BMP types at $p < 0.05$) (December, 2003).

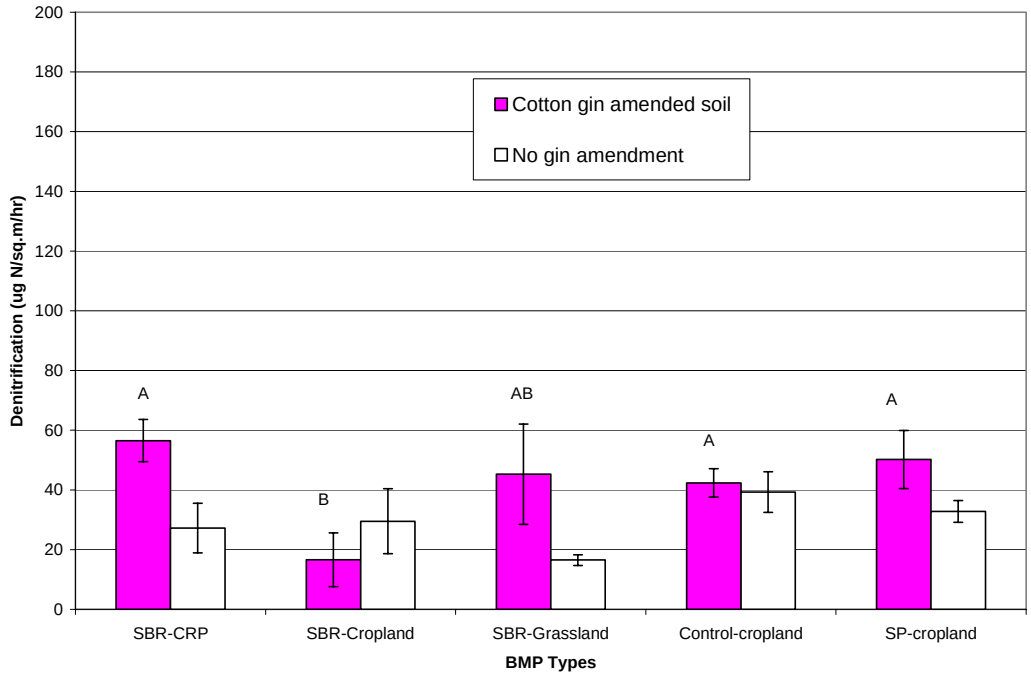

Figure 3.6. Denitrification rates of different land uses under different BMPs with and without cotton gin trash amendments (Different letters at the top of CGT amended bars shows significant differences between BMP types at $p < 0.05$) (February, 2004).

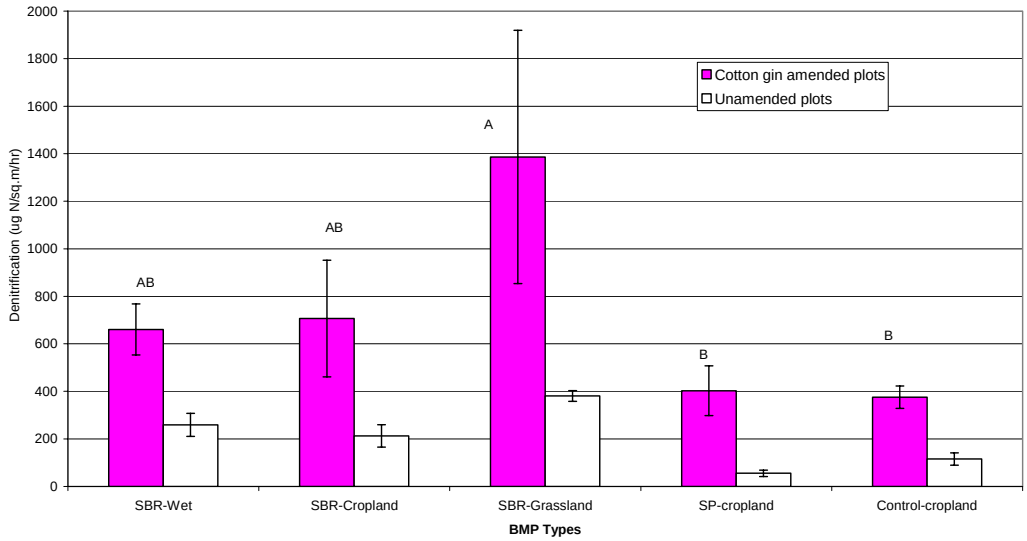

Figure 3.7. Denitrification rates of different land uses under different BMPs with and without cotton gin trash amendments (Different letters at the top of CGT amended bars shows significant differences between BMP types at p <0.05) (March, 2004).

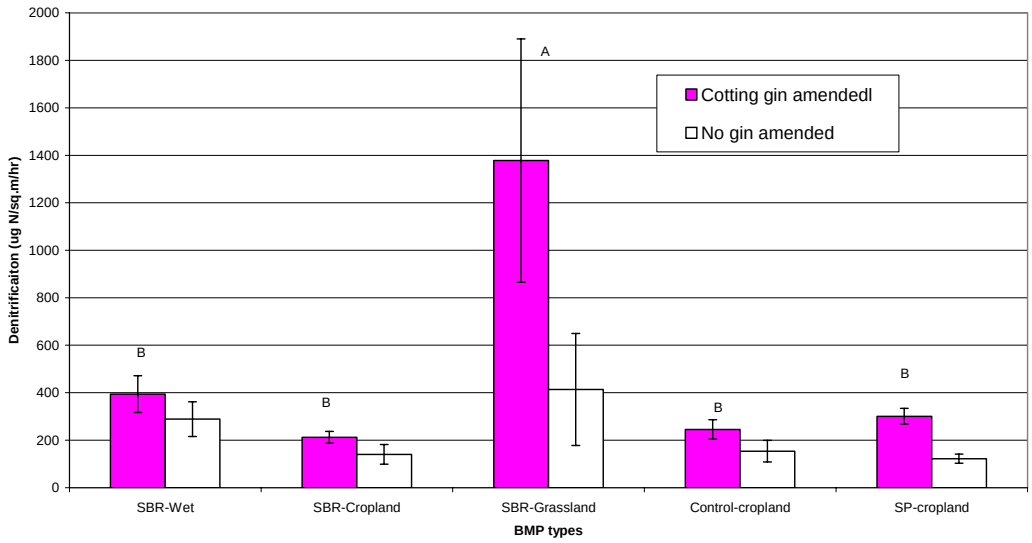

Figure 3.8. Denitrification rates of different land uses under different BMPs with and without cotton gin trash amendments (Different letters at the top of CGT amended bars shows significant differences between BMP types at p <0.05) (April, 2004).

Table 3.2. Mineralizable organic C contents (mean ± SE) of the different land uses/BMPs

Sites	CGT additions	----------Mineralizable Organic Carbon (mg CO_2 m^{-2} h^{-1})---------- October 2003	December 2003	February 2004	March 2004	April 2004
SBR-Wet	+	2047 (315)	52 (14)	10 (1)	119 (15)	410 (48)
SBR-Wet	-	440 (59)	27 (10)	7 (2)	102 (12)	291 (63)
SBR-Crop	+	609 (99)	32 (5)	10 (5)	98 (35)	207 (16)
SBR-Crop	-	469 (57)	33 (5)	8 (3)	56 (23)	16 (5)
SBR-Grass	+	232 (53)	27 (3)	16 (1)	453 (155)	669 (320)
SBR-Grass	-	115 (5)	12 (0.3)	3 (1)	267 (74)	289 (137)
Control-cropland	+	996 (73)	54 (10)	14 (3)	68 (16)	246 (39)
Control-cropland	-	458 (191)	19 (6)	15 (4)	21 (5)	153 (29)
SP-cropland	+	1967 (317)	39 (7)	18 (8)	85 (31)	87 (42)
SP-cropland	-	551 (114)	26 (4)	8 (3)	20 (8)	149 (29)
Soil Temp. (0-10 cm, ^{o}C)		19.4	9.1	5.5	17.6	22.0

SBR-grass plots amended with CGT showed an overall 3.2 fold increase in denitrification rates compared to the unamended plots. Increased denitrification response to CGT amendment was not significant during October, December and February incubations ($p > 0.05$). However in March and April, this site showed much greater

denitrification rates ($p < 0.01$) than the unamended plots. SBR-grass site had the highest denitrification rate and %WFPS (66%) in April of all sites. The denitrification rate of CGT amended plots of SBR-crop site was 2.4 times those of the unamended plots over the study period. Denitrification rates of the CGT amended SBR-crop plots were significantly greater than the unamended plots in October and March only ($p < 0.05$). The CGT amended SP-cropland and control-cropland plots showed an average 3.8 and 2.8 fold increases in denitrification rates compared to their respective unamended plots.

The production of mineralizable organic C (Table 3.2) was greater in the CGT amended plots compared with unamended plots and was 1.7 (SBR-cropland) to 3.1 times (SBR-wet) those of the unamended plots . It is not surprising then that the CGT amended

Table 3.3. Average (SE) Percent Water-filled pore spaces of different land use types, Beasley Watershed

Sites	----------Water-filled pore spaces (%)----------				
	October 2003	December 2003	February 2004	March 2004	April 2004
SBR-wet	100 (0.07)	100 (0.0)	97 (0.04)	100 (0.04)	63 (0.03)
SBR-cropland	88 (0.04)	100 (0.0)	84 (0.02)	100 (0.02)	35 (0.06)
SBR-grassland	65 (0.01)	100 (0.0)	72 (0.03)	87 (0.06)	66 (0.2)
Control-cropland	65 (0.03)	74 (0.01)	64 (0.02)	82 (0.02)	37 (0.03)
SP-cropland	68 (0.04)	80 (0.03)	71 (0.02)	100 (0.02)	36 (0.03)

Plots of all the sites had greater denitrification rates than the unamended plots. Seasonal variation in mineralizable organic C was observed with lowest values in December and February when soil temperatures were low (9.1 and 5.5 ^{o}C, respectively) (Table 3.2). Denitrification rates of all the sites were also lower in December and February (Figures 3.5 and 3.6), which shows that the lower soil temperatures affected both denitrifier activity and microbial respiration/decomposition.

Linear regression analysis identified a significant relationship between denitrification and mineralizable organic C production rates ($p < 0.05$). Mineralizable organic C production rates accounted for 0.35%, 0.34%, 0.40%, 0.58% and 0.71% of the variability in denitrification rates (Figures 3.9-3.13). CGT amendment led to an average 32% increase in total soil C and 13% increase in total soil N of the plots (Table 3.1), which increased the C:N ratios providing relatively more organic C for denitrifier activity than the unamended plots. This effect is evidenced by the high denitrification rates in CGT amended plots. No significant relationship between total soil C and N with denitrification rates was detected ($p > 0.05$).

Interestingly, NO_3-N concentration of the CGT amended plots was lower than the unamended plots at all sites (Table 3.4) although addition of CGT to soils added 31 mg NO_3-N m^{-2} area. Average lowest NO_3 concentration was observed in the CGT amended plots of SBR-wet site (2.27 mg NO_3-N kg^{-1} soil) while the highest average concentration was found in the unamended plots of the SBR-crop site (7.36 NO_3-N mg kg^{-1}). No significant correlation between NO_3 concentration and denitrification rate was detected ($p >0.05$).

Discussion

Water control BMPs and CGT amendment affected denitrification rates. BMPs affected denitrification rates in the SBR-wet and SBR-grass sites only. The effect of these BMPs on denitrification rates were observed under CGT amendment in both the sites (Figures 3.4-3.8). BMPs maintained higher %WFPS in the SBR-wet and SBR- grass sites (92% and 78%, respectively) compared to the WPFS of the control-cropland site (64%) (Table 3.3), which led to higher denitrification rates in these sites under CGT amendment. These results are consistent with other studies showing an increase in denitrification rates with increase in soil %WFPS (Linn and Doran, 1984; Groffman and Tiedje, 1988), provided the process in not limited by the availability of organic C

Table 3.4. Mean NO_3-N concentration (± SE) of the different land use types/BMPs

Sites	CGT additions	----------NO_3-N concentration (mg kg^{-1} dry soil)---------- October 2003	December 2003	February 2004	March 2004	April 2004
SBR-Wet	+	0.72 (0 .03)	0.10 (00)	3.30 (0.92)	0.57 (0.06)	6.7 (1.6)
SBR-Wet	-	2.57 (0.31)	0.62 (0.15)	5.38 (0.97)	3.75 (0.80)	8.5 (2.0)
SBR-Crop	+	1.32 (0.48)	0.79 (0.35)	2.64 (0.90)	5.25 (1.98)	14.3 (0.5)
SBR-Crop	-	6.05 (1.18)	5.08 (1.33)	7.37 (2.0)	6.2 (2.2)	12.1 (1.9)
SBR-Grass	+	1.54 (0.61)	0.90 (0.5)	2.20 (0.0)	2.40 (1.9)	9.3 (2.1)
SBR-Grass	-	1.68 (0.09)	1.2 (0.10)	10.8 (1.3)	6.18 (2.4)	6.2 (0.3)
Control-cropland	+	2.60 (0.57)	2.91 (1.1)	2.80 (0.66)	0.23 (0.07)	4.4 (1.2)
Control-cropland	-	4.15 (0.61)	4.24 (0.78)	4.35 (0.68)	0.93 (0.39)	5.9 (1.2)
SP-cropland	+	2.07 (0.40)	1.08 (0.34)	nd	0.66 (0.19)	6.6 (1.4)
SP-cropland	-	3.39 (0.59)	2.28 (0.77)	nd	1.38 (0.38)	5.0 (1.2)

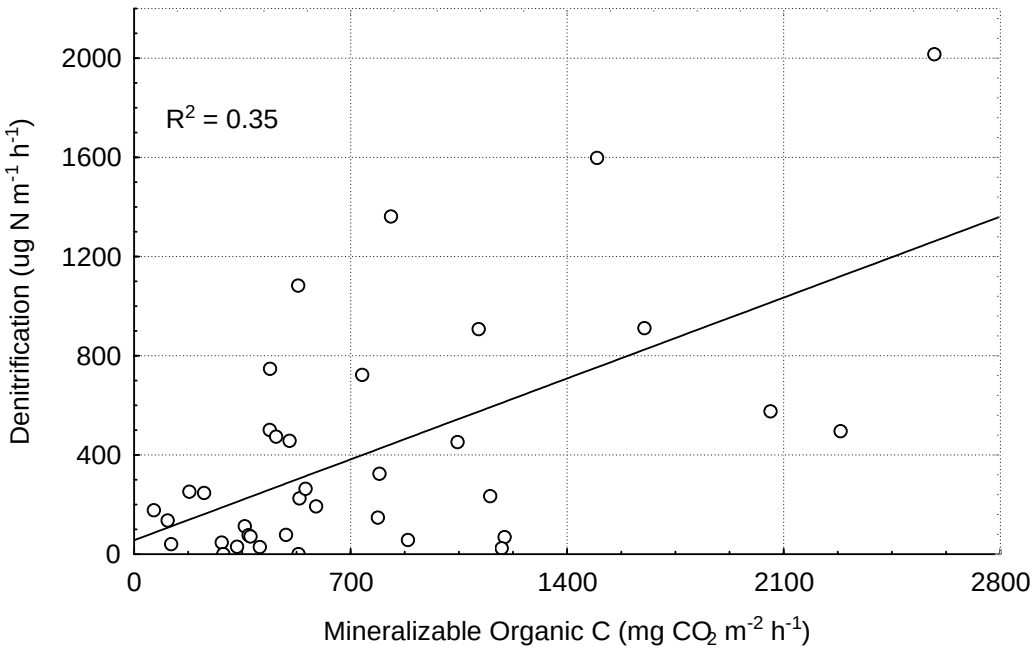

Figure 3.9. Regression of denitrification rates on mineralizable C (October 2003)

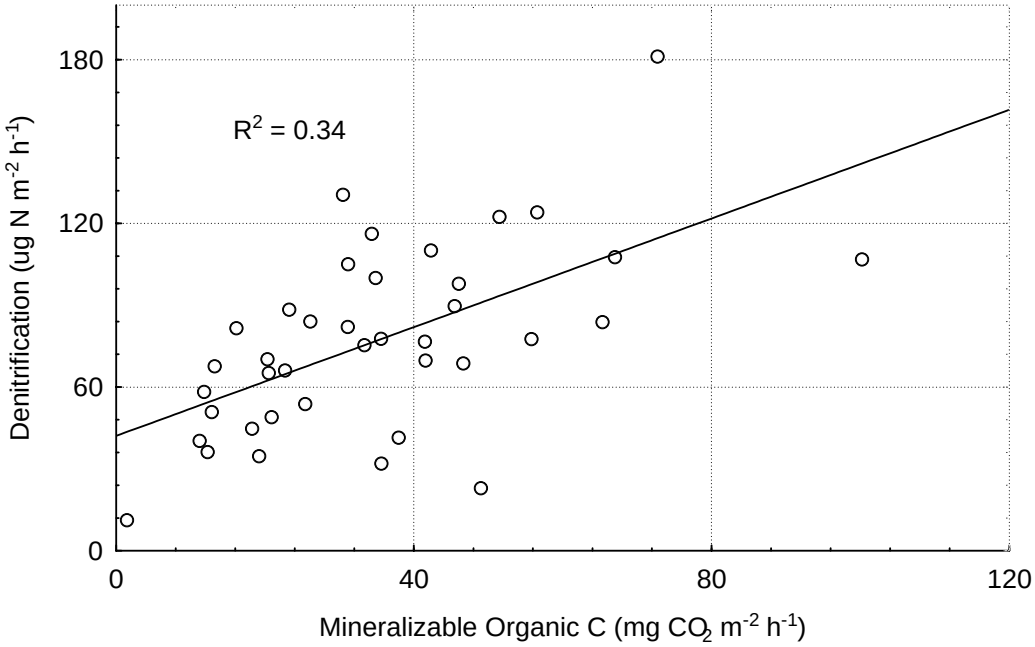

Figure 3.10. Linear regression of denitrification rates on mineralizable organic C (December 2003)

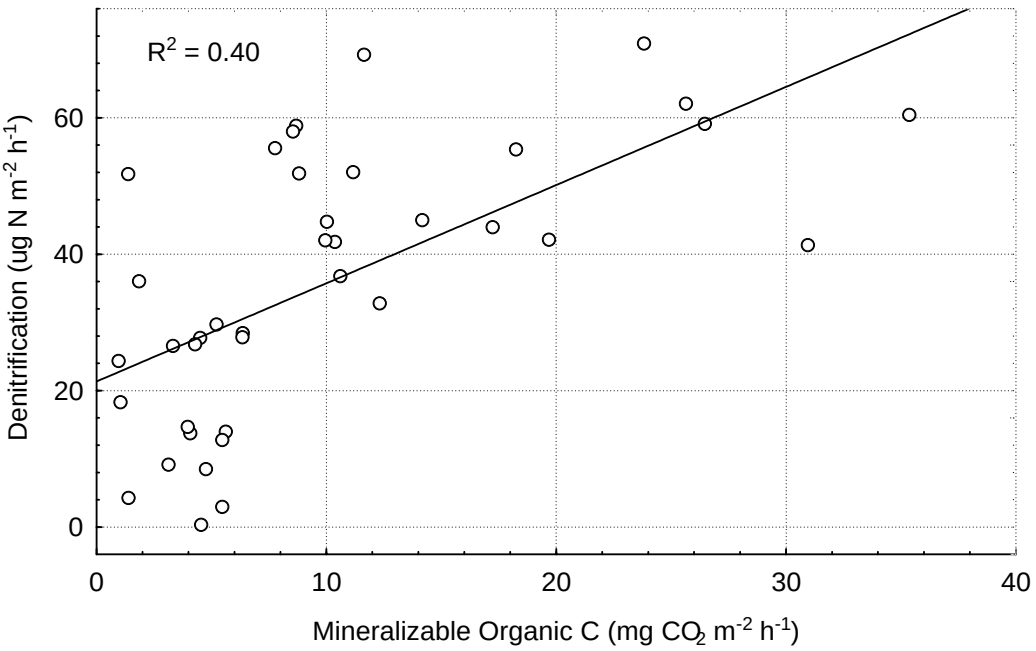

Figure 3.11. Linear regression of denitrification rates on mineralizable organic C (February 2004)

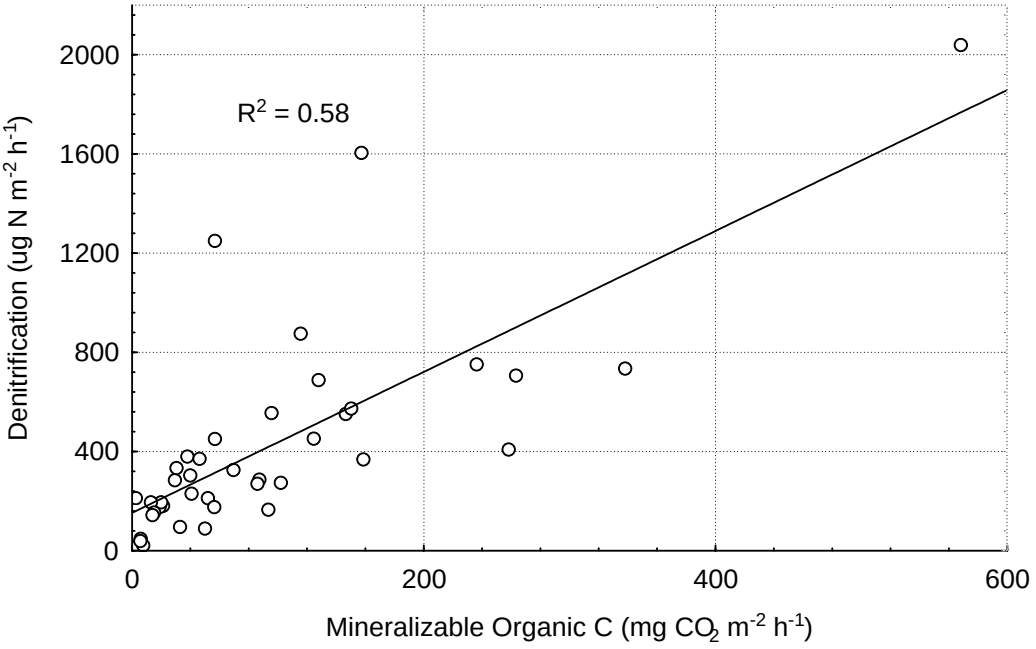

Figure 3.12. Linear regression of denitrification rates on mineralizable organic C (March 2004)

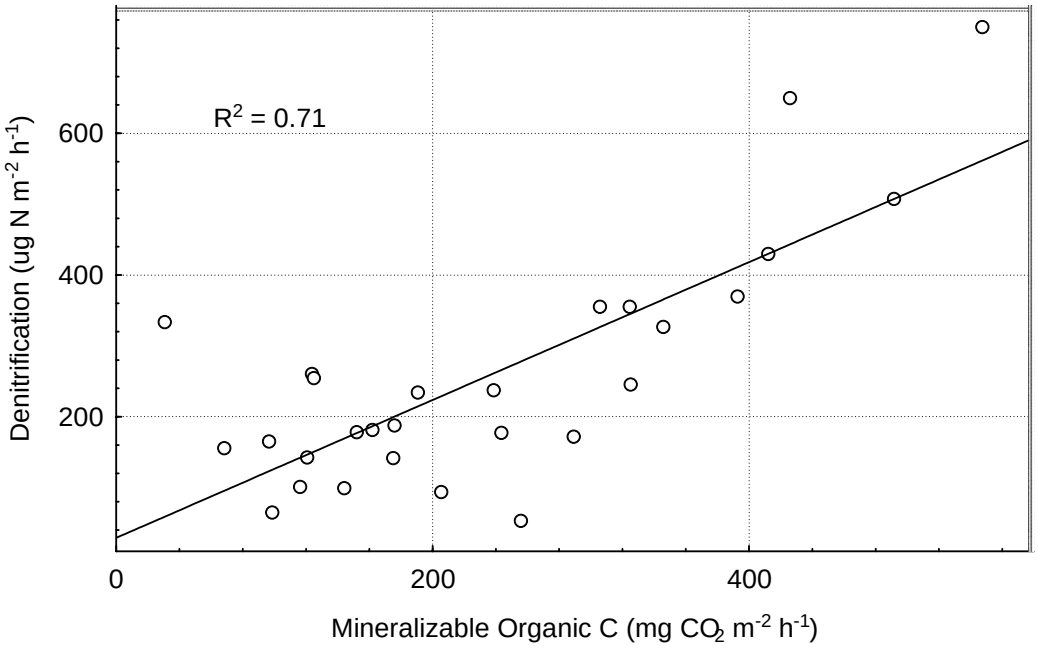

Figure 3.13. Linear regression of denitrification rates on mineralizable organic C (April 2004)

substrate and NO_3. Although %WFPS of SBR-cropland and SP-cropland sites (81% and 71%, respectively) maintained by these BMPs were also higher like that of the SBR-wet and SBR-grass sites, their denitrification rates were lower than the rates of the restored sites, suggesting that cultivation of these gently sloping soils diminished their capacity to have comparable denitrifier populations and activity relative to soils of the restored soils.

Greater available organic C contents due to CGT additions increased the denitrification rates of all the sites under additional NO_3 supply (Figures 3.4-3.8). These results are in agreement with other studies showing a close correlation between denitrification rates and available organic C (Bradley et al., 1992, Linn and Doran, 1984, Baggs, et al. 2002, Brettar, and Hoefle 2002, DeLaune et al. 1996, Seitzinger, 1994, Burford and Bremner, 1975). CGT amendment led to an average 200% increase in denitrification rates of all the land use types compared to the unamended plots. Our result

of 200% increase denitrification rate is higher than that reported by Hunter (2000), where CGT amendment led to 45% increase in denitrification rates of restored wetland soils in the LMV. This finding suggests that cultivated soils are more C limited to denitrify than restored forested wetlands and hence addition of CGT to cultivated soils in the LMV can enhance denitrifier activity. Mineralizable organic C accounted for 48% of the variability in denitrification rates of all the sites over the study period (Figures 3.9-3.13). CGT amended SBR-wet site produced 1.5 times greater mineralizable organic C than SBR-cropland, SP-cropland and control-cropland sites, which led to 1.5 times greater denitrification rates than those observed in the SBR-cropland, SP-cropland and control-cropland soils. Conversion from cultivation to wetland, and high %WFPS due to retention of field discharge of SBR-wet soil supported greater denitrifier activity than the rest of the sites. On the whole all the sites showed 3 fold increases in mineralizable organic C production rates and 3.1 fold increases in denitrification rates for plots with added CGT. Vinten et al. (1998) also reported similar impact of mineralizable organic C amendment sources on denitrification rates. CGT amended plots from all the sites denitrified 16 kg N ha^{-1} from October to April (6 months period), while the unamended plots denitrified 5.2 kg N ha^{-1} over the same period. Like other organic carbon amendment sources such as paper mill sludge, domestic wastes, and crop residue (Baggs, et al. 2002; Vinten e tal. 1998; de Catanzaro and Beasuchamp, 1985), CGT can be used as a cultural BMP in combination with structural BMPs to promote NO_3 removal through denitrification in cultivated and restored wetland soils.

Plots with added CGT had an average 1.6 times lower soil NO_3 concentration than those of the unamended plots. Although CGT amendment led to 13% increase in total

soil N (Table 3.1), the greater denitrification rates triggered by CGT addition subsequently reduced NO_3 at significantly greater rates than the unamended plots. The high denitrification rates observed in the CGT amended plots resulted in lower NO_3 levels in soils compared to the un-amended plots. The difference in NO_3 concentration between CGT and unamended plots of all the sites was higher during October (10 days after application of CGT) and declined over time. During the last sampling time in April (140 days after CGT application) some of the CGT amended plots had greater NO_3 contents than the unamended plots (Table 3.4). Denitrification rates of CGT amended and unamended plots in April were also not statistically different from each other for all the sites except the SP-cropland site (Figure 3.8). This finding suggests that the amount of remaining mineralizable organic C coming from CGT was to low to support greater denitrification rates six months after the amendment.

Denitrification rates observed varied through time with high rates observed in October, March and April, while lower rates were found in December and February. Lower soil temperatures in December and February (9.1 and 5.5 ^{o}C respectively) seem to have reduced denitrifier activity, in spite of soil WFPS in a range (78 to 91%), where optimum denitrification activity can be supported (Meixner and Eugster, 1999; Mohn et al. 2000).

In summary, addition of CGT to cultivated and CRP and WRP sites coupled with the operation of BMPs in agricultural watersheds in the LMV can substantially enhance denitrification rates and thus can help reduce NO_3 loss from agricultural lands. Denitrification rates observed in the cultivated soils were lower than the restored CRP and WRP sites in spite of similar WFPS and organic C addition rates. We speculate that

cultivation may have led to lower denitrifier population and activity in these soils. Quantification of the denitrifier population in soils of the cultivated and restored sites is recommended to verify this speculation.

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CHAPTER 4

DENITRIFICATION AND N_2O EMISSION FROM FORESTED AND CULTIVATED ALLUVIAL CLAY SOIL[1]

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Introduction

Seasonally flooded lower elevation clay alluvium lands in agricultural watersheds within the Lower Mississippi River Valley (LMV) are typically bottomland hardwood forests. The Sharkey 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 Sharkey 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, 2003).

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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 Sharkey and similar soils over decades is likely to have altered their potential for denitrification compared to Sharkey soil maintained under bottomland hardwood forests (Hunter et al. 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 run-off during rainfall, storm events and irrigation. NO_3 run-off 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 et al. 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 (Weier et al. 1993, Dalal et al. 2003, and Sexstone et al. 1985). The emission ratio of N_2O:N_2 is affected by soil moisture, NO_3 concentration, pH, available carbon (Groffman et al. 1988, Sahrawat and Kenney 1986, 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 Sharkey soil within the LMV and its extensive use for crop production, sites containing Sharkey 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 years 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 physio-chemical properties of the soil within the sampling sites are shown in Table 4.1.

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 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 dia. x 10 cm height) were collected in plastic liners (5 cm dia. x 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.

Table 4.1. Physiochemical properties of forested and cultivated Sharkey 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/100g	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 ± .25	3.5 ±.27

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 dia. X 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 five mL deionized water. Twenty mL 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 minutes 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 ^oC). 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 (ug 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 et al. 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 x 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 x 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 hours (22-25 ^oC) 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 (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 minutes. After shaking, the suspension was allowed to stand for 20 hours (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 minutes at room temperature. Thirty mL of the supernatant was filtered into cleaned scintillation vial through a Whatman filter paper (0.45 uM size) and stored in refrigerator at 4 ^{o}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 2M 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

Potential denitrification assay (PDA) of forested soil averaged 1.42 ug N g^{-1} h^{-1} dry soil, whereas the PDA of cultivated soil was significantly less (0.62 ug 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 ug N g^{-1} h^{-1} in forested and cultivated soils respectively (Figure 4.1). 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 4.1). Mineralizable carbon averaged 122 and 29 ug 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% (Table 4.2). 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} $\cdot h^{-1}$. Denitrification rates of forested soil amended with NO_3 were significantly higher than that of cultivated soil at 70% and 85% WFPS ($p < 0.05$). 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 4.2). 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 4.2). 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, 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 4.2). 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 4.2). 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 ($p < 0.05$).

Table 4.2. Denitrification and net N_2O emission rates of forested and cultivated Sharkey soil. Data show the mean and standard errors of three replicate analyses

WFPS[†]	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.[‡]	Forested	Cultivated	Diff. ‡
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

[†]WFPS, water-filled pore space.

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

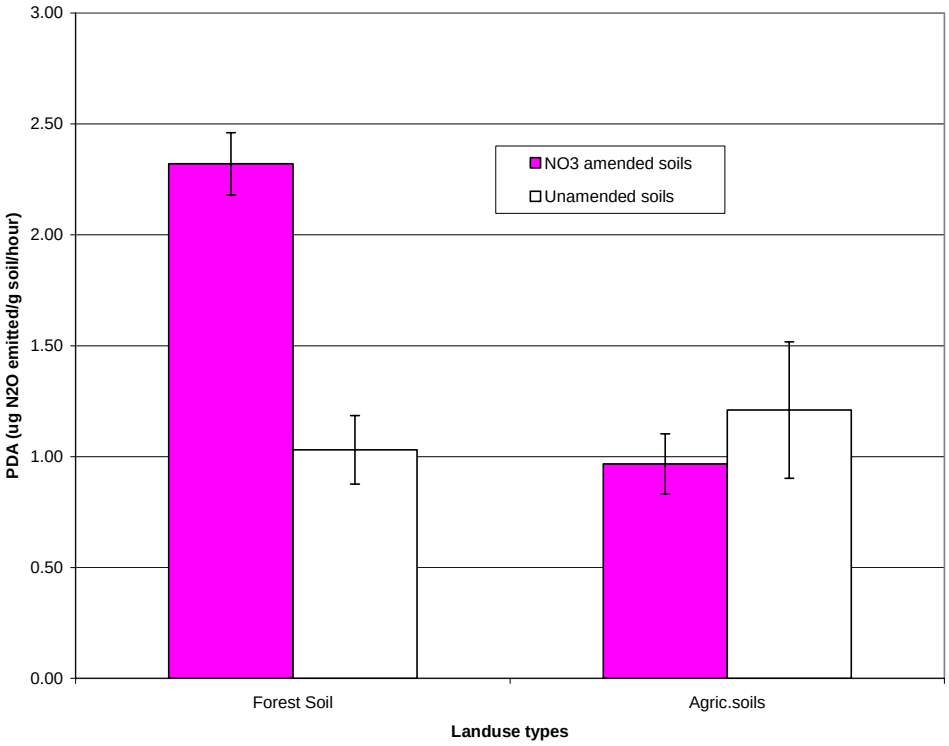

Figure 4.1. Denitrification potential of forested wetland and cultivated soil Error bars represent standard error of the mean (p=0.05).

The distribution of clay and silt was similar in forested and cultivated soils (Table 4.1), 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 4.1), 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; and Weier, et al. 1993). It is noteworthy that despite differences in total and mineralizable C, the amounts of water-soluble organic carbon (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 4.3). 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.

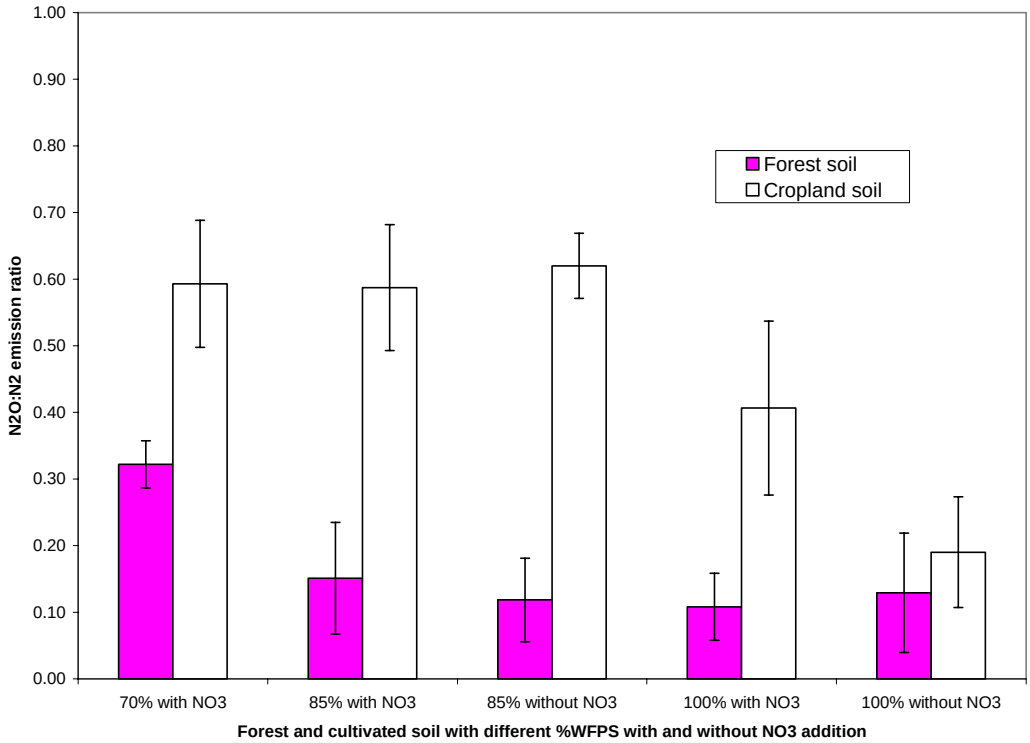

Figure 4.2**.** Ratio of N_2O to N_2 evolved from forested and cultivated soil adjusted to various WFPS with or without the addition of added NO_3^-. Error bars represent standard error of the mean (p=0.05).

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 (Fig.4.1) and incubations of intact soil cores (Table 4.2), 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 ½ times those in samples from adjacent cultivated areas (Fig. 4.1). 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 4.3) influenced not only the rate of denitrification but also the ratio of N_2O to N_2 emitted (Figure 4.2). 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 4.3). 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 hour 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. Observed denitrification rates at 24 and 72 h incubation were linear in both ecotypes with and without NO_3 amendment, which indicated zero-order kinetics with reference to NO_3 availability to denitrifiers.

Table 4.3. 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

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 denitrification 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. 19 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 (Weitz et al. 2001, Klein et al. 1996, Hunter et al. 2001, Weier et al. 1993, Sexstone et al. 1988, and Mosier et al. 1981).

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 Fig. 4.1 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 (Weier et al. 1993, Tilsner et al. 2003, and Vinther, 1984).

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 2,838 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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CHAPTER 5

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

Introduction

There is growing global concern about increasing biologically available nitrogen (N) in the environment due to N fertilizer use and its subsequent impacts on aquatic ecosystems (Howarth et al. 2002, Galloway, et al. 2002). Increase in reactive N load of estuaries and coastal waters results in algal blooms and high primary productivity, which leads to oxygen depletion and anoxia (Thompson, et al. 2000). Agricultural run-off is the major cause of increased reactive N in rivers and lakes, which affects more than 50% of surface waters in the southeastern US (Neary, et al. 1989). Intensified agricultural practices in the Mississippi River Basin over the past 200 years have resulted in increased NO_3 loading of the Mississippi River (Goolsby 2000; Turner and Rabalias, 2003) and 70% of the current total NO_3 load of the river has been attributed to agricultural runoff. Widespread eutrophication in the northern Gulf of Mexico has been linked to the increased NO_3 and sediment loading of the Mississippi River due to agricultural expansion and N fertilizers use in the river watershed (Rabalais, et al. 2002, Mitsch et al. 2001)

The Lower Mississippi River alluvial valley (LMV) has lost more than 80% of its native bottomland hardwood forests mainly from conversion to agriculture (MacDonald et al. 1979). Forested wetlands are recognized for their high denitrification rates (Lowrance et al. 1984, Delaune et al. 1996) and the conversion to agriculture changed these landscapes from a net NO_3 sink to a net NO_3 source. Natural forested wetlands have a tightly coupled N cycle with any additional NO_3 coming from nitrification or

agricultural run-off, is taken either by plants, denitrified or immobilized by bacteria. The high denitrification rates observed in forested wetlands are ascribed to their high soil moisture holding capacity, high denitrifier population and availability of high organic C substrate to denitrifiers (Ingrid-Brettar and Hofle 2002; Ullah et al. in press; Lindau, 1994; Lowrance et al. 1984).

With deteriorating water quality issues including NO_3 pollution of rivers, coastal waters and oceans (Mitsch, et al. 2001), interest in restoring wetlands for water quality improvement has received increased attention (Lowrance et al. 1984, Hunter and Faulkner, 2001). However, forested wetland restoration is a long-term endeavor compared to herbaceous wetlands or grassland ecosystems restoration, because it takes 30-40 years at least for the forest to reach maturity and fully develop characteristic ecological functions (Niswander and Mitsch, 1995). A newly restored site may have lower organic C available to microbes than in a natural wetland, a key substrate for important microbiological processes including denitrification (Groffman and Tiedje, 1989). Hunter (2000) found that denitrification in 10-years old restored forested wetlands were limited by available C when compared to natural forested wetlands in the LMV. Preliminary studies by the authors showed that addition of cotton gin trash (CGT) to soils from a restored forest increased denitrification rate by 45%, suggesting that the capacity of restored forested wetlands can be enhanced by amending soils with organic C.

Forested wetlands restoration is likely to enhance denitrification rates, however, it is uncertain if restoration will result in significant increases in the atmospheric burden of N_2O emissions. High available organic C substrate in soils have been found to support greater N_2O reductase activity during denitrification, leading to lower N_2O emissions

(Arah, 1990, Skiba et al.1998, Sahrawat and Keeney, 1986). Therefore, amending restored forested wetland soils with labile organic C sources is most likely to reduce N_2O:N_2 emission ratio during denitrification. On the other hand, high NO_3 loading of soils lead to higher N_2O emissions (Bowden et al. 1991, Lloyd, 1995) raising the question if newly restored forested wetlands will increase the atmospheric burden of N_2O emissions. Because of the significance of N_2O as a potent greenhouse gas (IPCC, 1996) and as a destroyer of the stratospheric ozone (Hahn and Crutzen, 1982), it is important to account for its emissions from all of its potential sources (Skiba, et al. 1998), including newly restored forested wetlands.

The objectives of this research were to assess the effect of CGT amendment on denitrification rates and N_2O:N_2 emission ratio from restored forested wetland by comparison to corresponding emissions from an adjacent natural forested wetland.

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 5.1). A 13-year-old restored forested wetland and an adjacent natural forested wetland were selected for this study. We selected sites containing Sharkey clay soils (non-acidic montmorilinitic, Vertic Haplaquept) for this study, because this soil series is very common in the low elevation areas of the LMV, covering about 12,150 km^2. Some of soil the physio-chemical characteristics are given in Table 5.1. Dominant tree species of the natural forested wetland are American elm (*Ulmus americana)*, water oak (*Quercus nigra, L.), r*ed maple *(Acer ruburum, L.*), bitter pecan (*Carya x lecontei Little),* dwarf palmetto (*Sabal minor*),

laurel oak (*Quercus laurifolia Michx*), dogwood species (*Cornus spp.*) and sugarberry (*Celtis leavigata, Willd*). The restored sites have dominant tree species of oak species (*Quercus sp*), ash (*Fraxinus pennsylvanica),* honey-locust *(Gleditsia triacanthos L.),* dogwood species (*Cornus spp.*) and red maple *(Acer ruburum* L.).

Eight replicate sampling sites 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 the one plot of the two plots of the restored forested wetland. 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 17.5:1, and provides a readily mineralizable organic C substrate to microbes. The NO_3-N and NH_4-N contents of CGT are 15.4 ± 3.6 and 788 ± 40 mg kg^{-1} soil, respectively.

Soil Sampling

Soil samples were collected from all 24 plots at six-week intervals between October 2003 and April 2004. Bulk soil samples (10 cm deep) were collected and transported on ice to the laboratory for further analysis. Intact soil cores (5 cm x 10 cm) 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).

Table 5.1. Soil (0-10 cm) variables with standard errors from restored and natural forested wetlands sites sampled in October 2003

Variables	----------Forest Types----------		
	Natural	Restored + CGT*	Restored
Texture Class	Clay	Clay	Clay
% Clay	63 (1.1)	62 (2.9)	60 (1.9)
% Silt	22 (1.6)	24 (3.5)	25 (1.3)
Bulk Density (g cm^3)	0.85 (0.03)	0.93 (0.02)	0.93 (0.02)
Porosity (cm^3 cm^{-3})	0.68	0.65	0.65
pH	4.7	5.6	5.5
NO_3-N mg Kg^{-1}	2.7 (0.7)	2.9 (0.6)	3.4 (0.7)
NH_4-N mg Kg^{-1}	7.2 (1.3)	4.9 (1.0)	3.7 (1.0)
Total C	3.3 (0.2)	3.1 (0.1)	3.1 (0.1)
Kg C m^{-2}			
Total N	0.22	0.22	0.25
Kg N m^{-2}			
C:N	15	14	12.4

Denitrification, N_2O:N_2 Ratio and CO_2 Production Rates

Duplicate intact soil cores (5cm x 10 cm) were collected in plastic liners (5 cm x 15 cm dimensions) 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 and thus be able to assess CGT amendment effects. The cylinders were capped at the base and put back in the holes from which the cores were collected to maintain field temperatures. To measure denitrification rates, 10 ml of purified C_2H_2 gas was injected in small aliquotes

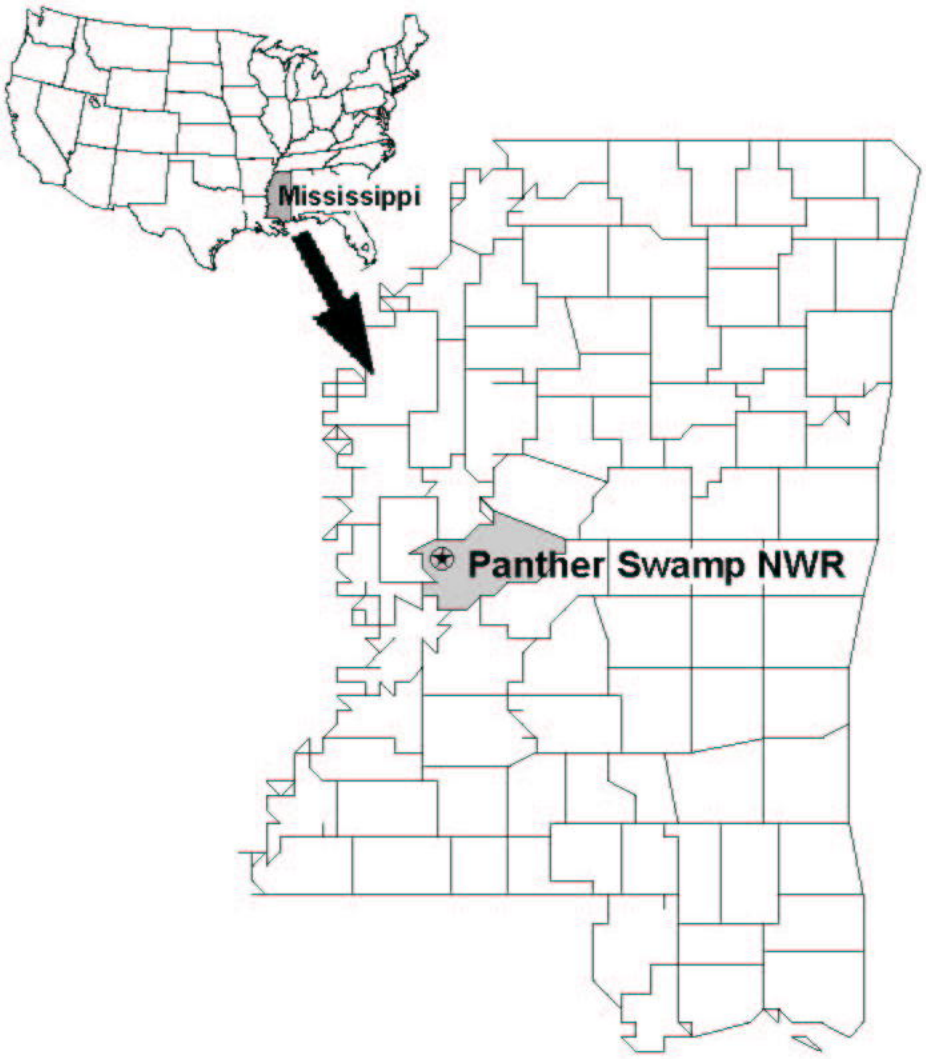

Figure 5.1. Location map of the Panther Swamp National Wildlife Refuge

into one of the duplicates cores at the interface of soil and plastic liner to ensure diffusion of C_2H_2 throughout the soil column. After injection of C_2H_2 gas, the cores were capped tight, fitted with a 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 natural N_2O emissions, the other core was incubated without C_2H_2 addition.

Gas samples were taken from the headspaces of cores with 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 lab for analysis on a Varian 38001GC equipped with ECD and FID detectors. The rates of N_2O and CO_2 production were determined in μg N_2O-N m^{-2} h^{-1}a 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 coefficient. N_2O:N_2 emission ratio was calculated from the difference of N_2O emitted with and without C_2H_2 addition.

Total Soil Carbon and Nitrogen

Total soil carbon (C) and nitrogen (N) of both the sites were determined using a Thermo Finnigan CNS analyzer during each sampling at the USGS National Wetland Research Center, Louisiana. The bulk soil samples collected from each plot were oven dried, homogenized thoroughly and pulverized. A sub-sample of about 35 mg was weighed into a tin capsule prior to their injection into CNS analyzer. 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 NO_3, NH_4, pH and Temperature

Moist soils (5 gram over-dry soil weight equivalent) were weighed into 250 mL duplicate bottles. Fifty mL of 2M KCl solution was added to each bottle. The bottles were put on Eberbach shaker for continuous shaking for 1 hour. After shaking, the bottles were centrifuged at 300 rpm for 5 minutes and were then filtered into 20 mL scintillation vials through a No.42 Whatman filter paper. The samples were frozen until analyzed for NO_3 and NH_4 with an automated Lachat FIA analyzer (Wendt, 2000). Average values for

each soil sample were determined and reported in mg Kg^{-1} oven-dried soil. Soil pH was determined in the lab using 1:1 soil to de-ionized water mixing ratio. Soil temperature was measured with a soil temperature probe (inserted up to 10 cm depth) during the field denitrification studies.

Soil Bulk Density, Water-filled Pore Spaces, and Particle Size

Intact 5 x 10 cm soil cores were collected at each sampling date were dried in oven at 105 degrees Celsius 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 (Watts and Seitzinger, 2001) for the five sampling dated. Soil texture was determined by the modified pipette method (Sheldrick and Wang, 1993).

Statistical Analysis

One-way ANOVA analysis was performed to assess the significance of differences among denitrification rates of the restored and natural forested wetlands for each sampling date. Fisher's protected LSD was used for comparison purposes at α = 0.05. Multiple linear regression analysis of denitrification rates on CO_2 production rates, total C, total N, NO_3, and NH_4 was performed to identify factors significantly related to denitrification rates. Pearson's correlation coefficients among the variables were also determined. All statistical analyses were performed using SAS (SAS Inc. 1998).

Results

Addition of CGT led to significant increases in denitrification rates of restored forested wetlands. In October, denitrification rates of the CGT-amended plots were 5.7 times greater than the unamended plots (Table 5.2). Moreover, denitrification rates of the CGT-amended plots were not different from that of the natural forested wetland soils (p >

0.05). Six weeks after addition of the CGT (December), denitrification rates remained 1.4 times greater than those in unamended plots ($p < 0.05$). No significant difference was found between CGT-amended restored site and natural forested wetland site. In February, denitrification rates in the CGT-amended plots were significantly ($p < 0.05$) higher than the unamended plots of the restored wetlands. Denitrification rates in CGT amended plots were again similar to those in the natural forested wetland. Denitrification rates were lower in February than in December despite %WFPS (93%) (Table 5.3). Decrease in soil temperature from 8^{o}C in December to 5.8 ^{o}C in February may have accounted for the observed decrease in denitrifier activity.

In March, soil temperatures rose to 14 ^{o}C (Table 5.3) and denitrification rates increased significantly ($p < 0.05$) in the CGT-amended, unamended and natural forested wetland plots. Denitrification in CGT amendment plots was 1.3 times those of unamended plots and similar to the natural forested wetland plots. In April, natural wetland plots again had significantly greater denitrification rates ($p <0.05$) than the unamended restored plots. The denitrification rates of the CGT-amended plots were 1.4 times those of unamended restored wetland plots, although statistically not significant ($p > 0.05$).

Amending restored forest soil with CGT lowered N_2O:N_2 emission ratios (Table 5.4). The restored site with CGT amendment was found with an average N_2O:N_2 emission ratio of 0.40 (across all dates), while unamended plots had an average emission ratio of 0.53. The natural forested wetland plots had an average N_2O:N_2 emission ratio of0.35, which is significantly different than the unamended plots ($p < 0.05$), except in February (Table 5.4).

Table 5.2. Denitrification rates (± SE) of the restored and natural forested wetland soils. Means followed by the same letter indicate no significant ($p > 0.05$) difference between forest types within each sampling time

Sampling times	Forest Types		
	Natural	Restored + CGT*	Restored
	µg N m^{-2} h^{-1}		
October	9.5 (1.4) a	17.3 (7.2) a	3.0 (1.4) b
December	80.4 (8.2) a	74.8 (8.5) a	55.6 (6.2) b
February	84.0 (13.9) a	59.6 (14.6) a	23.4 (4.9) b
March	1086 (145) a	971 (105) ab	743 (103) b
April	372 (40) a	359 (33) ab	255 (45) b

CGT: Cotton gin trash

Denitrification rates in all the plots significantly correlated with mineralizable organic C (Table 5.5). The Pearson's correlation coefficients of denitrification with mineralizable organic C were 0.57, 0.56 and 0.70 during December, March and April respectively ($p < 0.05$). Mineralizable soil organic C of the natural and CGT amended restored forest sites were 2.3 and 1.5 times greater than those of the restored sites without CGT. An exception occurred in October, shortly after addition of CGT, when the amended sites contained more mineralizable soil C than the natural forest site. No significant relationship between total soil C and N with denitrification rate was observed ($p > 0.05$). Owing to the high denitrification rates, CGT amended plots had 1.2 times lower NO_3-N compared to the unamended plots even though CGT amendment added 31 mg NO_3-N m^{-2} area. Soil NO_3-N concentration of the natural forested wetland was also 1.3 times lower than those of the unamended restored forested soil (Table 1). No significant relationship between NO_3 concentration and denitrification was detected ($p>0.05$).

Table 5.3. Percent water-filled pore space (± SE) and soil temperature (0-10 cm) of restored and natural forest soil determined during denitrification studies

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

Table 5. 4. Seasonal N_2O:N_2 emissions ratio (± SE) of restored and natural forest soils. Means followed by the same letter indicate no significant ($p > 0.05$) difference between forest types within each sampling time

Sampling times	----------Forest Types----------		
	Natural	Restored + CGT*	Restored
	----------N_2O:N_2 emissions ratio----------		
October	0.37 (0.17) a**	0.43 (0.05) ab	0.54 (0.01) b**
December	0.37 (0.05) a	0.37 (0.10) ab	0.50 (0.11) b
February	0.34 (0.10) a	0.35 (0.11) a	0.50 (0.17) a
March	0.32 (0.03) a	0.43 (0.02) b	0.56 (0.09) c
April	0.36 (0.02) a	0.41 (0.08) ab	0.57 (0.11) b

CGT: Cotton gin trash, ** Significant at $p < 0.10$

Table 5.5. Organic carbon mineralization rate (± SE) and its correlation with denitrification rates of restored and natural forested wetland soils

Sampling times	----------Forest Types----------			
	Natural	Restored + CGT†	Restored	Correlation of Organic C with denitrification (n=24, $p < 0.05$)
	--------mg CO_2 emitted $m^{-2}h^{-1}$----------			
October	955 (89)	1661 (212)	1267 (196)	0.28 ns
December	109 (15)	51 (9)	44 (4)	0.57*
February	37 (1.4)	13 (3)	10 (2)	0.22 ns
March	249 (56)	379 (64)	194 (30)	0.56*
April	326 (26)	307 (18)	194 (29)	0.70*

ns: non-significant, * significant difference, †CGT: Cotton gin trash

Discussion

Forest type, CGT amendment, soil WFPS, and soil temperature influenced denitrification and N_2O:N_2 emission ratio from the selected sites. Lower soil bulk density, wetter soil conditions (Table 5.1) and greater mineralizable organic C contents observed in the natural forested wetland supported persistent and higher denitrifier activity with lower N_2O:N_2 emission ratios (Table 5.4) than the restored forested wetland site. These findings indicate that the restored forested wetland had 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, landscape position and hydrology. The differences in denitrification observed can only be attributed to differences in the maturity of the forests.

Mineralizable organic C is an index of the amount of C substrate available to denitrifiers (Singh, et al. 1988, Blackmer and Bremner, 1975). It is likely that the 42% higher rates of denitrification found when restored forest was amended with CGT was due to the e higher amounts of mineralizable organic C measured. 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 finding is consistent with the findings of Hunter (2000), who found 45% increase in denitrification rates in response to CGT amendment of soils from similar ecosystems.

Although the CGT amendment significantly increased the denitrification rates, it resulted in 33% lower N_2O:N_2 emission ratio than observed from the restored site receiving no CGT (Table 5.4). N_2O:N_2 emission ratio indicates the relative activity of

N_2O reductase enzyme activity during denitrification and can be used as an index to identify restoration techniques which can lead to higher N_2O reductase activity (Bergsma et al. 2002). This ratio can also be used to predict net N_2O emissions out of the total denitrification in soils under different restoration scenarios. Out of the overall mean denitrification rate of 296 μg N m^{-2} h^{-1} observed in the CGT-amended restored forest, 119 μg N m^{-2} h^{-1} were emitted as net N_2O into the air, while out of the averaged 216 μg N m^{-2} h^{-1} denitrification rate observed in the unamended plots, 114 μg N m^{-2} h^{-1} were emitted as net N_2O. Natural forested soils emitted 114 μg N m^{-2} h^{-1} as net N_2O gas out of its averaged denitrification rate of 326 μg N m^{-2} h^{-1}. This result suggests that restored forested wetlands emit net N_2O at the same rate as natural forested wetlands. However, for the same amount of N_2O emitted, natural forested wetlands denitrify more than twice NO_3 than the restored forested wetlands. Forested wetlands seem to support greater nitrous oxide reductase activity compared to restore forested wetlands. However, the additional available C supplied by CGT amendment (Table 5.5) increased the nitrous oxide reductase activity in the restored forest. Addition of readily mineralizable organic C substrate such as CGT to restored forested wetlands can enhance their denitrification rates and at the same time will lead to a lower N_2O:N_2 emission ratio. CGT amendment of restored forested wetland soils results in lower net N_2O emissions compared to restored soils without CGT additions for the same amount of NO_3 reduced. Similar effects of organic C amendment on denitrification rates of restored forested wetlands (Hunter, 2000) and N_2O:N_2 emission ratios and net N_2O emissions (Weier, et al. 1993, Skiba et al. 1998, Vinther, 1984, Tilsner, et al. 2003) are reported in the literature. The

practice of CGT addition to restored wetland soils will also help recycle tons of CGT waste produced in the LMV.

In addition to forest age, organic C availability and soil temperature, WFPS also affected denitrification rates in both the ecosystems. It is not surprising then that denitrification rates increased with an increase in soil WFPS from an average 41% in October to 77% in December (Table 5.3). 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 and improved soil aeration as WFPS had declined to an average of 63% by the time of April sampling. Lower denitrification rates observed in February in spite of high %WFPS were attributed to the lower soil temperature in February (Table 5.3), which limited the denitrification rates. Our findings clearly show the importance of WFPS in regulating denitrifier activity in soils and are in agreement with similar studies reported in the literature (Linn and Doran, 1998; Groffman and Tiedje, 1988; Hefting et al. 2003; Klein De and Logtestijn, 1996). Given the importance of WFPS in regulating denitrifier activity, denitrification rates in restored forested wetlands will depend on the extent of hydrologic modification of the restored sites in addition to available C.

In summary, these results demonstrate that even after 13 years restored bottomland forest wetlands have not yet achieved the same denitrifying activity observed in more mature but otherwise similar naturally forested sites. Addition of CGT to restored forested wetlands in the LMV can enhance denitrification rates to the level of natural forested wetlands. CGT addition also reduces the N_2O:N_2 emission ratio during denitrification, which results in N_2O emissions similar to those from natural forested wetlands. Restoration of forested wetlands in the LMV will require appropriate

hydrologic modification and organic C amendments in order to provide the same level of NO_3 removal as natural forested wetlands.

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CHAPTER 6

GREEN HOUSE GAS EMISSIONS FROM RESTORED AND NATURAL FORESTED WETLANDS IN LOWER MISSISSIPPI ALLUVIAL VALLEY

Introduction

Approximately 80% of the bottomland hardwood forests of the lower Mississippi River alluvial valley (LMV) have been converted to other land uses, primarily agriculture (McDonald, et al. 1979). This conversion not only led to a reduction in the NO_3 sink activity in the form of higher denitrification rates (Hunter and Faulkner, 2001, Lindau et al. 1994), but increased its potential for loading additional NO_3 into surface waters though N fertilizer use, soil erosion (Rebich, 2001) and direct drainage of agricultural lands. Interests in restoring forested wetlands for water quality improvement in the LMV has received increased attention (Hunter and Faulkner, 2001; Lowrance et al. 1984 ;Trettin and Jurgensen, 2003) due to the deteriorating water quality issues including NO_3 pollution in the Mississippi River (Mitsch, et al. 2001) and the resulting hypoxia in the northern Gulf of Mexico (Rabalias, et al. 2002).

Hunter and Faulkner (2001) reported that newly restored forested wetlands in the LMV have lower denitrification potentials than natural forested wetlands due to limitation by available organic carbon (C) and lower soil moisture due to lack of appropriate hydrologic restoration (Hunter and Faulkner, 2001). To enhance denitrification rates in the restored forested wetlands, they recommended addition of a readily decomposable organic C source to the restored sites and the establishment of an appropriate soil hydrologic regime. Restoration of forested wetlands in the LMV according to the above recommendations is likely to enhance denitrification rates,

however, it is uncertain how forested wetlands restoration under organic C amendments and hydrologic modifications will influence N_2O, CH_4 and CO_2 emissions. Since both N_2O and CH_4 have greater global warming potential (300 and 21 times, respectively) than CO_2 (IPCC, 1994), any forested wetland restoration designed to offset CO_2 emissions through C sequestration must factor in emissions of these greenhouse gases.

Soil nitrogen (N) availability and cycling is closely linked to C cycling and greenhouse gas emissions from soils. Productive forest ecosystems with high rates of N cycling have high soil respiration potential and higher CO_2 production rates (Pastor et al. 1984, Bowden et al. 2000). Soil CH_4 uptake is also influenced by soil N. High soil NH_4 competitively inhibits CH_4 oxidation by methanotrophs (Nesbit and Breitenbeck, 1992, Wang and Ineson, 2002) and some studies have found NO_3 as a potential inhibitor of CH_4 oxidation in soils (Dunfield and Knowles, 1995, Wang and Ineson, 2002). High NO_3 loading of restored wetlands designed for non-point source pollution control increases N_2O emissions into the air ((Dobbie and Smith, 2003, Hefting et al. 2003, Lloyd, 1995), which may offset the C sequestration benefits of restoration. Like N, soil C also influences greenhouse gas emissions from soils. High organic C substrate available to denitrifiers reduces N_2O emission from soils into the air. Thus the emissions of N_2O, CH_4 and CO_2 from soils are affected by the amount of available soil N and C, besides soil moisture, temperature, pH, redox potential, soil particle size distribution and soil management (which influences one or more of these controlling factors). These factors can be affected differently the way we restore forested wetlands for water quality improvement in the LMV.

There is paucity of research work on the impacts of forested wetlands restoration on greenhouse gas emissions in the LMV; hence it is imperative to clearly understand how forested wetlands restoration for water quality improvement will affect greenhouse gas emissions from soils. Our objective was to evaluate the effects of soil organic C amendments and NO_3 additions on greenhouse gas emissions (N_2O, CH_4 and CO_2) from restored and natural forested wetlands.

Material and Methods

Sites Description

The research sites were located on the Panther Swamp National Wildlife Refuge in Yazoo delta region of Northwestern Mississippi (Figure 6.1). A 13-year-old restored forested wetland and an adjacent natural forested wetland was selected for this study. We selected sites containing Sharkey clay soils (non-acidic montmorilinitic, Vertic Haplaquept) for this study, because this soil series is very common in the low elevation areas of the LMV, covering about 12,150 km^2. Some of the physio-chemical properties of the soils are given in Table 6.1. Dominant tree species of the natural forested wetland are American elm (*Ulmus americana)*, water oak (*Quercus nigra, L.),* red maple *(Acer ruburum, L.*), bitter pecan (*Carya x lecontei Little),* dwarf palmetto (*Sabal minor*), laurel oak (*Quercus laurifolia Michx*), dogwood (*Cornus sp.*) and sugarberry (*Celtis leavigata, Willd*). The restored sites have dominant tree species of oak (*Quercus sp*), ash (*Fraxinus pennsylvanica),* honeylocust *(Gleditsia triacanthos L.),* dogwood (*Cornus sp.*) and red maple *(Acer ruburum* L.).

Eight replicate sampling sites 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 cotton gin trash (CGT) was spread manually on the soil surface of the one plot of the two plots of the restored forested wetland. 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, and provides a readily mineralizable organic C substrate to microbes. The NO_3-N and NH_4-N contents of CGT are 15.4 ± 3.6 and 788 ± 40 mg kg^{-1} soil, respectively. Static chambers (15 cm diameter) were inserted into the soil at each plot up to a depth of 10 cm, 10 days before the start of the gas sampling in October 2003, resulting in eight chambers in each treatment (natural forest, restored forest, and restored forest amended CGT). The chambers were standing 15 cm above the soil surface with a total volume of 2735 cm^3 and a volume to area ratio of 15:1.

Soil samples were collected from all 24 plots at 6-week intervals between October 2003 and April 2004. Bulk soil samples (10 cm deep) were collected and transported on ice to the laboratory for further analysis. Intact soil core (5 cm dia.x 10 cm ht.) 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).

Greenhouse Gas Emissions Measurement

Methane, N_2O, and CO_2 fluxes from each site were measured at 6-week intervals between October 2003 and April 2004 (5 times). Soils in each chamber were amended with 15 μg NO_3 g^{-1} soil just before the closure of chambers for the gas flux measurement.

The chambers were sealed with an airtight cover fitted with a sampling port and gas samples were from the headspaces at 0, 30 and 60 minutes. The samples were collected by filling a 10-ml polypropylene disposable syringe and injecting the full volume into pre-evacuated 5-ml crimp-topped vials. The gas samples were transferred to the

Table 6.1. Selected soil (0-10 cm) properties from restored and natural forested wetlands sites sampled in October 2003 before CGT amendment[mean (SE)]

Property	----------Forest Types----------		
	Natural	Restored + CGT*	Restored
Texture Class	Clay	Clay	Clay
% Clay	63 (1.1)	62 (2.9)	60 (1.9)
% Silt	22 (1.6)	24 (3.5)	25 (1.3)
Bulk Density (g cm^3)	0.85 (0.03)	0.93 (0.02)	0.93 (0.02)
Porosity (cm^3 cm^{-3})	0.68	0.65	0.65
pH	4.7	5.6	5.5
NO_3-N (mg kg^{-1})	2.7 (0.7)	2.9 (0.6)	3.4 (0.7)
NH_4-N (mg kg^{-1})	7.2 (1.3)	4.9 (1.0)	3.7 (1.0)
Total C kg C m^{-2}	3.3 (0.2)	3.1 (0.1)	3.1 (0.1)
Total N kg N m^{-2}	0.22	0.22	0.25
C:N	15	14	12.4

laboratory for analysis on a Varian CP 3800 GC equipped with an FID and ECD detectors for CH_4, CO_2 and N_2O concentration determination using packed columns. The fluxes of the three gases were calculated from the linear regression of the concentration

versus time. Positive fluxes show net emission by soils while negative fluxes show a net uptake by soils. The calculated fluxes are reported as μg CH_4 flux m^{-2} h^{-1}, μg N_2O-N flux m^{-2} h^{-1}, and mg CO_2 flux m^{-2} h^{-1}.

In order to evaluate the effects of NO_3 additions on N_2O, CO_2 and CH_4 emissions, we added 15 mg NO_3 Kg-1 soil (what is this in kg/ha? 30-40 kg N/ha?) to only four of the chambers in each treatment in May 2004.

We used the same gas sampling and analytical techniques mentioned above and compared emissions from NO_3 amended and unamended plots.

Total Soil Carbon and Nitrogen

Total soil carbon (C) and nitrogen (N) of both the sites were determined using a Thermo Finnigan CNS analyzer during each sampling at the USGS National Wetland Research Center, Louisiana. The bulk soil samples collected from each plot were oven dried, homogenized thoroughly and pulverized. A sub-sample of about 35 mg was weighed into a tin capsule prior to their injection into CNS analyzer. 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 NO_3, NH_4, pH and Temperature

Moist soils (5 gram over-dry soil weight equivalent) were weighed into 250 mL duplicate bottles. Fifty mL of 2M KCl solution was added to each bottle. The bottles were put on Eberbach shaker for continuous shaking for 1 hour. After shaking, the bottles were centrifuged at 300 rpm for 5 minutes and were then filtered into 20 mL scintillation vials through a No. 42 Whatman filter paper. The samples were frozen until analyzed for NO_3 and NH_4 with an automated Lachat FIA analyzer (Wendt, 2000). Average values for each

soil sample were determined and reported in mg Kg^{-1} oven-dried soil. Soil pH was determined in the lab using 1:1 soil to de-ionized water mixing ratio. Soil temperature was measured with a soil temperature probe (inserted up to 10 cm depth) during the field denitrification studies.

Soil Bulk Density, Water-filled Pore Spaces, and Particle Size

Intact 5 x 10 cm soil cores were collected at each sampling date were dried in oven at 105 ^{o}C for 72 h 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 (Watts and Seitzinger, 2001) for the five sampling dates. Soil texture was determined by the modified pipette method (Sheldrick and Wang, 1993).

Statistical Analysis

Differences in greenhouse gas flux among the different treatments for each sampling occasion were analyzed by one-way analysis of variance using the general linear model in SAS (SAS Inc. 1998). Similarly, differences in gas fluxes among sampling dates for each treatment were also analyzed by ANOVA. Post-ANOVA comparisons were conducted using the Fisher's protected LSD at $p < 0.05$.

Results

Methane

Methane fluxes from restored and natural forested wetlands were not significantly different from each other ($p > 0.05$), except in March, where CGT amended plots of the restored site had higher emission rates than the unamended plots (Table 6.2). In October, both restored and natural forested wetlands displayed strong sink activity with net CH_4 fluxes ranging from -64 to -119 μg CH_4 m^{-2} h^{-1}. In December, when soil %WFPS were

82% and 71% in the restored and natural forest plots respectively, net CH_4 flux was positive for both the sites (Table 6.3). High soil moisture and lower soil temperature (8 ^oC) in December may have led to either higher methanogenic activity or lower methanotrophic activity or reduction in the oxidized zones supporting CH_4 production. In February, the natural forested wetland plots had an average small negative flux of -1.9 CH_4 m^{-2} h^{-1}, while CGT and unamended restored forested wetland plots exhibited small positive fluxes of 6.7 and 7.6 μg CH_4 m^{-2} h^{-1}, respectively.. However due to high variability in flux rates all the sites were not statistically different ($p > 0.05$).

With a rise in soil temperature from 5.8^oC in February to 14 ^oC in March (Table 6.3), natural and CGT-amended plots exhibited higher net CH_4 emissions ($p < 0.05$) compared to the emissions observed in February and December (Table 6.2). The CGT amended plots had significantly greater emissions ($p < 0.05$) than the unamended plots. Nearly saturated soils conditions (94% WFPS) in March seem to have increased methanogenic activity on one hand and reduced methanotrophic activity on the other hand, by reducing the oxidized soil zone where methanotrophs occurs (Nesbitt and Breitenbeck, 1992). With a decrease in soil %WFPS from 94% in March to an average 63% in April led to a more variable but smaller CH_4 flux compared to the fluxes observed in March. All these sites had small net positive CH_4 fluxes of 1.6 to 2.2 μg CH_4 m^{-2} h^{-1} in April which was significantly lower than those observe in March (p , 0.05).

NO_3 additions did not significantly affect CH_4 flux from either the restored or natural forested wetlands. The variability of CH_4 flux was high (Table 6.4) and the flux rates ranged from -2.9 ± 5.7 μg CH_4 m^{-2} h^{-1} from CGT amended restored forest plots to

Table. 6.2. Methane emissions (± SE) from restored and natural forested wetland soils. Means followed by the same letter indicate no significant ($p > 0.05$) difference between forest types within each sampling date.

Sampling times	Forest Types		
	Natural	Restored + CGT*	Restored
	CH_4 flux (μg CH_4 m^{-2} h^{-1})		
October	-64 (19) a	-119 (46) a	-71 (53) a
December	11 (6) a	16 (3) a	15 (3) a
February	-1.9 (6) a	6.7 (3) a	7.6 (3) a
March	47 (7) ab	83 (24) a	28 (5) b
April	2.2 (5) a	1.6 (2) a	1.6 (5) a

Table 6.3. Percent water-filled pore space (± SE) and soil temperature (0-10 cm) of restored and natural forested wetlands

Forest Type	% WFPS					
	October 2003	December 2003	February 2004	March 2004	April 2004	May 2004
Natural Forest	37 (0.01)	71 (0.03)	92 (0.04)	100 (0.03)	70 (0.02)	79 (0.03)
Restored Forest	44 (0.02)	82 (0.01)	94 (0.02)	88 (0.02)	56 (0.02)	71 (0.05)
Soil Temp (^oC)	19	8	5.8	14	19	22

27 ± 26 μg CH_4 m^{-2} h^{-1} from the natural forested wetlands. The average WFPS at the time of incubation was 75%, which may have likely led to slightly greater methanogenic activity than the methanotrophic activity by developing higher soil anaerobosis. Soil temperature of 22 degrees Celsius in April was in a range that would support both CH_4 production and oxidation activity.

Table. 6. 4. NO_3 amendment effects on greenhouse gas flux (± SE) from restored and natural forested wetland soils. Means followed by the same letters indicate no significant ($p > 0.05$) difference within each forest type (with and without NO_3 additions)

Wetland Types	NO_3 additions	CH_4 flux $\mu g\ CH_4\ m^{-2}\ h^{-1}$	N_2O flux $\mu g\ N\ m^{-2}\ h^{-1}$	CO_2 flux $mg\ CO_2\ m^{-2}\ h^{-1}$
Natural Forest site	+	1.5 (3) a	258 (9) a	315 (34) a
Natural Forest site	-	9.8 (10) a	176 (12) b	204 (46) b
CGT amended site	+	-1.1 (2) a**	216 (36) a	240 (37) a**
CGT amended site	-	7.0 (3) b	123 (19) b	161 (55) b
No CGT amended site	+	2.3 (1) a	109 (33) a	91 (77) a
No CGT amended site	-	4.0 (2) a	94 (39) b	113 (38) a

** Significant at $p < 0.10$

Nitrous Oxide

Nitrous oxide emissions varied seasonally and were significantly lower in October, December and February compared to March and April (Table 6.5). In October, WFPS averaged 41%, which did not support high denitrifier activity and thus the overall N_2O emissions were significantly lower ($p < 0.05$) than those observed in March and April where WFPS were 94 % and 63% respectively (Table 6.5). N_2O emissions in October likely originated through nitrification rather than denitrification. Linn and Doran (1984) reported that N_2O is produced through nitrification in soils having WFPS below 60%. The N_2O emissions in December and February were also significantly lower ($P < 0.05$) compared to the emissions in March and April. The lower soil temperatures of 8 and 5.8 ^oC in December and February seem to have reduced denitrifier activity, resulting in lower N_2O emissions.

Table 6.5 . N_2O emissions (± SE) from restored and natural forested wetlands. Means followed by the same letter indicate no significant ($p > 0.05$) difference between forest type within each sampling date.

Sampling times	Forest Types		
	Natural	Restored + CGT*	Restored
	Net N_2O emissions ($\mu g\ N\ m^{-2}\ h^{-1}$)		
October	3.51 (2) b	7.43 (1) a	1.63 (0.03) b
December	30 (4) a	28 (7) a	28 (6) a
February	29 (8) a	21 (7) ab	12 (4) b
March	348 (33) a	418 (19) a	416 (67) a
April	134 (7) a	147 (29) a	145 (28) a

CGT*= Cotton gin trash amended soils

N_2O emissions from natural forested wetlands were similar to those from the CGT amended restored wetlands ($p > 0.05$), except in October, when the CGT-amended plots emitted 2.1 times greater N_2O than the natural forested wetland (Table 6.5). Similarly, unamended restored plots had similar N_2O emissions rates to those from natural forested wetland soil except in February (Table 6.5), when the unamended plots had lower rates ($p < 0.05$). According to the results presented in Chapter 5 of this report, CGT amendment of the restored forested wetland soils, reduced N_2O:N_2 emission ratio from 0.53 to 0.40 and increased denitrifier activity by 40%. As a result, CGT amended and unamended restored plots emitted N_2O at similar rates, although CGT amended plots reduced much more NO_3 than the un-amended plots. Similarly, for the same amount of N_2O emitted by the natural forested wetlands, it reduced more than twice NO_3 than those in the un-amended restored forested wetlands

Addition of NO_3 increased N_2O emissions from both the restored and natural forested wetlands by 1.6 to 1.8 times the unamended treatments in May 2004 (Table 6.4). The %WFPS at the time of incubation averaged 75% at all the sites, which is sufficient to support high rates of denitrification.

Carbon Dioxide

The flux of CO_2 from all the sties varied seasonally and was closely related to the variation in soil temperature and %WFPS (Table 6.6). Carbon dioxide emissions from the three treatments were significantly higher in October compared to those observed in the rest of the sampling dates. Lower soil temperatures in December and February (8 and 5.8 ^oC respectively) resulted in significantly lower CO_2 emissions ($p < 0.05$) than those observed in March and April (Table 6.6). The averaged emission rates increased 5 fold in March and April from all the sites when soil temperatures increased to 14 and 19 ^oC respectively, compared to the emission rates of December and February. Lower WFPS (41%) in October led to significantly greater CO_2 emission rates than the other sampling dates (Table 3). The addition of CGT resulted in 156% increase in CO_2 emissions from the restored forested wetland soils compared to the unamended restored forest plots. The natural forested wetland had 20% and 160% times greater CO_2 emission rates compared to the CGT amended and unamended restored forested wetlands, except in October.

Table 6.6. CO_2 emissions (± SE) from restored and natural forested wetlands. Means followed by the same letter indicate no significant ($p > 0.05$) difference between forest types within each sampling date.

Sampling times	----------Forest Types----------		
	Natural	Restored + CGT*	Restored
	----------CO_2 flux (mg CO_2 m^{-2} h^{-1})----------		
October	866 (108) b	3453 (437) a	1306 (218) b
December	53 (5) a	66 (9) a	37 (6) a
February	38 (1) a	13 (3) b	11 (2) b
March	248 (56) a	220 (23) a	79 (21) b
April	254 (27) a	193 (25) ab	101 (20) b

NO_3 amendment led to 1.6 times higher CO_2 production rates from CGT amended and natural forested wetland soils compared to soils without NO_3 additions (Table 6.4).

The unamended restored forest did not show any significant response to NO_3 additions in terms of CO_2 evolution rates. The amount of CO_2 produced from the unamended plots were lower than those produced by the CGT amended restored and natural forested wetlands.

Discussion

Methane flux from the restored and natural forested wetland soils was not influenced by CGT and NO_3 amendments. Nesbitt and Breitenbeck (1992) also observed no NO_3 addition effect on CH_4 uptake of soils collected from different ecosystems in the LMV. The CH_4 flux was affected by soil WFPS and temperature. Lower WFPS led to CH_4 uptake while high WFPS led to net emissions (Table 6.2). The impact of increased soil saturation on CH_4 flux is two-fold: it reduces the oxic zones in the soil where methanotrophs can oxidize CH_4 (Davidson et al. 2004) and the anaerobic soil conditions increase methanogenic activity resulting in higher CH_4 emissions. Nesbitt and Breitenbeck (1992) found 56% lower CH_4 uptake by soils at WFPS above 75%. They reported WFPS between 25 to 75% as the optimum conditions for CH_4 oxidation in soils. Similarly, Bowden et al. (1998) reported that moisture contents (field capacity) can lead to maximum CH_4 uptake by forest soil and further increase in WFPS reduces CH_4 oxidation.

A decrease in soil temperature led to lower CH_4 flux rates in the three sites. This result is similar to the findings of Smith et al. (2003). These results indicate that soil WFPS and temperature are the major regulators of CH_4 flux from forested wetlands in the LMV, similar to results reported by Castro et al. (1995), Rask et al. (2002) Nesbitt and Breitenbeck (1992), and Bowden et al. (1998). As forested wetlands in LMV have higher

WFPS (above 80%) in winter and part of the spring season (Table 6.3) when soil temperatures are low, not much CH_4 is expected to be emitted into the atmosphere. These minor CH_4 emission in winter and spring are offset by the far higher CH_4 consumption rates under lower WFPS (less than 60%) during most of the year in bottomland hardwood forests in LMV.

N_2O emissions from the restored and natural forested wetland soils did not differ significantly. Soil WFPS was found closely related to N_2O emissions from both the sites, where an increase in WFPS led to higher N_2O emissions, which is similar to results reported by Linn and Doran (1986), Smith et al. (2003), and Dobbie and Smith (2003).

Not surprisingly, adding NO_3 to both restored and natural forested wetlands increased N_2O emissions into the atmosphere. Restored and natural forested wetland soils showed 48% increase in response to NO_3 amendment at a rate of 15 μg NO_3-N g^{-1} soil compared to N_2O emissions from similar soils without NO_3 additions (Table 6.4), a result in agreement with research studies reported by Magill et al. (1997) and Bowden et al. (1991), where increase in N_2O emission was observed from temperate forests under additional NO_3 in Northeastern USA. When extrapolated to per hectare per year basis, average N_2O emissions rates from the both natural and restored forested wetlands observed at 75% WFPS ranged from 10 to 22 kg N_2O-N ha^{-1} y^{-1} and 8 to 15 kg N_2O-N ha^{-1} y^{-1} with and without NO_3 additions respectively. Similar, N_2O emission rates ranging from 20-30 N_2O-N ha^{-1} y^{-1} are reported for riparian forest soils loaded with high levels of NO_3 by Hefting et al. (2003) and Walker et al. (2002). Dobbie and Smith (2003) also reported a similar range of N_2O emissions from grassland soils ranging from <1 to 44 N_2O-N ha^{-1} y^{-1}). The range of N_2O emissions observed from restored wetlands can

significantly alter the global N_2O budget and can offset the C sequestration benefits of restored wetlands. The 48% increase in N_2O emissions observed from the CGT amended restored and natural wetlands due to NO_3 amendment should be taken into consideration when establishing CO_2 emission credits on restored forested wetlands in the LMV.

Nitrous oxide emissions aside, run-off NO_3 loaded into forested wetlands can have beneficial on soil organic C storage, by increasing plant growth, litter fall and belowground biomass, as these ecosystems are reported to be N limited (Delaune, et al. 1996). Moreover, Berg and Matzner (1997), Waldrop et al.(2004) and Hagedorn et al. (2003) reported that addition of N to soils retards decomposition of soil humus leading to more C storage in soils. Further mechanistic research on NO_3 loading on soil C storage potential in forested wetlands in the LMV would help reduce uncertainties in estimates of C sequestration in these ecosystems.

Emissions of CO_2 were affected by wetland age, soil temperature, moisture, and CGT and NO_3 amendments. Lower soil temperatures reduced CO_2 emissions in the three sites, a fact already established by other studies (Lomandar, et al. 1998). Soil WFPS regulated the CO_2 emission rates whenever not limited by soil temperature. Under drier soil conditions, CO_2 emissions were significantly higher ($p < 0.05$) than those observed at WFPS above 80% (Table 6.6). Bowden et al. (1998) reported lower CO_2 emissions under higher soil moisture conditions, which is similar to our finding. This result shows that wetlands restored under saturated soil conditions can reduce CO_2 emissions and thus can increase soil C storage as well.

NO_3 amendment led to greater CO_2 evolution rates from natural and CGT amended restored forest soil compared to soils without NO_3 additions. This result of

increased CO_2 evolution with N fertilization is consistent with the research findings reported by Brumme and Beese (1992) and West and Marland (2003). Bottomland hardwood forest in the LMV are N limited, hence, it is likely that addition of NO_3 to these forests enhances both autotrophic and heterotrophic soil respiration leading to high CO_2 evolution rates. The net amount of CO_2 evolved from natural forested wetland soil was 7.5 Mg C ha^{-1} y^{-1} and 4.9 Mg C ha^{-1} y^{-1} with and without NO_3 additions respectively. The net amount of CO_2 evolved from CGT-amended and unamended restored forest soil was 5.7 and 2.2 Mg C ha^{-1} y^{-1} after NO_3 addition and 3.9 and 2.7 Mg C ha^{-1} y^{-1} without NO_3 amendments, respectively. These emission rates are within the range of 1.10 to 8.0 Mg C ha^{-1} y^{-1} reported for temperate forests (Bowden, et al. 2000, Bowden et al. 1993) and Florida slash pine plantations under N fertilization (Castro et al. 1994), suggesting that restoration will not contribute substantially to atmospheric CO_2. Our data also suggests that restored wetlands maintained under saturated soil conditions are likely to lead to a reduce CO_2 emissions. However, restoration of forested wetlands in the LMV for water quality improvement should take into account the effect of NO_3 addition on CO_2 emission rates, while modeling C storage potential of soils.

In summary, NO_3 additions, soil temperature, WFPS and CGT amendment were found to be key environmental variables influencing greenhouse gas flux from both the restored and natural forested wetlands in the LMV. CH_4 flux from forested wetlands in LMV is relatively small and is regulated by soil WFPS and temperature. Lower WFPS and higher soil temperature led to net CH_4 uptake by soils while higher WFPS and lower soil temperatures led to either high CH_4 production or suppressed CH_4 consumption resulting in small, but highly variable average flux rates. CGT and NO_3 additions had no

effect on CH_4 flux and both restored and natural forested wetlands were net minor sinks for CH_4 during the study, indicating that restoration of forested wetlands in the LMV will not result in increased CH_4 emissions. Addition of NO_3 to these ecosystems increased net N_2O emissions, which should be taken into consideration during any C sequestration program. NO_3 additions also increased CO_2 emissions from wetland soils. However, based on our limited data, the overall emissions of CO_2 from restored wetlands will be reduced substantially under saturated soil conditions benefiting C storage in soils. Restoration of forested wetlands in the LMV will affect the cycling and interaction of soil C and N differently based on the differences in the degree of hydrologic modifications and loading of run-off NO_3 into the restored ecosystems. The impacts of nutrient loading, organic C amendment and hydrologic modifications on multiple ecological functions including water quality, C sequestration, habitat provision and flood protection are all important in the planning of forested wetlands restoration programs in the LMV to obtain optimal environmental benefits.

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CHAPTER 7

SYNTHESES AND CONCLUSIONS

Overview

Water quality degradation due to diffuse NO_3 pollution is a significant problem worldwide. The major causes of elevated levels of NO_3 in surface and ground waters are the loss of natural ecosystems (including wetlands), N fertilizer use in the agricultural systems, and increased artificial drainage. Remedial measures recommended for reducing and controlling NO_3 pollution involve increasing denitrification and reducing NO_3 loss from croplands by implementing BMPs on agricultural lands and restoring wetlands and riparian buffers at suitable locations between croplands and receiving waters. This research was designed to answer questions concerning A) landscape and land use effects on soil denitrification potential in an agricultural watershed; B) impacts of structural BMPs and soil organic carbon (C) amendments on denitrification rates and in cultivated, and a year-old restored CRP and WRP sites; C) effects of water-filled pore space and NO_3 amendments on denitrification rates and N_2O:N_2 emission ratio from low elevation heavy clay soils (Sharkey series), in cultivated and an adjacent forested wetland; D) impact of organic C amendment on denitrification rates of Sharkey soils under a 13 year-old restored bottomland hardwood wetland; and E) influence of NO_3 and organic C additions on greenhouse gas flux from alluvial Sharkey clay soils under restored forested and adjacent natural forested wetlands.

Landscape Position and Land use Impacts on Denitrification Potential

Much research work has been done on biogeochemical scale factors controlling denitrification potential and rates in soils (Groffman, et al. 1988; Gambrell, et al. 1975).

However, there is lack of research studies on landscape controls over denitrification potential in agricultural watersheds, especially in the LMV. It is imperative to identify landscape units and land uses which can promote denitrification so as to be able to plan programs for diffuse NO_3 pollution control in agricultural watersheds while maintaining environmentally sound crop production.

Both landscape position and land use significantly affected soil denitrification potential in the Beasley Lake watershed (Chapter 2). Natural and constructed wetlands occupying low-elevation landscape positions with clays soils had significantly greater denitrification potential than those of the low and high elevation cultivated lands. Wetlands exhibited 2.5-6.3 times greater denitrification than the cultivated soils. Veg-ditches had 1.3 to 4.2 times greater denitrification potentials than that of the high and low elevation cultivated soils. Differences in denitrification potentials of the different land-use types are the result of the interaction of landscape position and land uses in the watershed, both of which influenced soil moisture contents and organic C availability to denitrifiers. Gambrell et al. (1975) also reported soil texture and drainage as key factors controlling soil anaerobosis, which has a direct bearing on denitrification rates of agricultural soils.

High denitrification potential in wetlands was supported by the availability of 1.4 and 1.6 times greater amounts of anaerobically mineralizable organic C to denitrifiers compared to the cultivated and unvegetated ditch soils respectively. Similar results of the importance of available organic C on denitrification have been widely reported in the literature (Blackmer and Bremner, 1975; Hill and Cardaci, 2004; Khalil et al. 2002). NO_3 addition to wetlands and vegetated-ditches led to significantly greater denitrification

potentials compared to the cultivated soils. This finding shows that denitrification in the wetlands and vegetated-ditches were limited by available NO_3 to denitrify while the cultivated soils were limited by factors other than NO_3, most probably microbially available C and anaerobic conditions. Season also significantly affected denitrification potential. Denitrification potential of all the land use types was high in spring, summer, and fall compared to winter. Lower winter denitrification potential is attributed to the observed lower soil temperatures (Klein and Logtestijn. 1996). High amounts of available organic C, greater soil moisture contents, fine soil texture and low permeability observed in the wetland soils maintained conditions favorable for higher denitrifier activity (Hill and Cardaci 2002; Davidsson et al. 2000) compared to the well aerated high and low elevation croplands and unvegetated ditches. Groffman and Tiedje (1989) also observed similar effects of soil texture and drainage classes on denitrification rates of temperate forests at landscape scale.

It is evident from these results that low-elevation, fine-textured soils of natural wetlands are the best locations for mediating diffuse NO_3 pollution in agricultural watersheds in the LMV. These results support the recommendations of Mitsch et al. (2001). Agricultural run-off drained through vegetated-ditches and wetlands before entering receiving waters will help reduce significant quantities of run-off NO_3. Landscape position and land use types can be used as useful indices for assessment of the denitrification potential of soils in agricultural watersheds and identification of sites for wetlands restoration for water quality improvement in the LMV.

BMPs and Cotton Gin Trash Amendment Effects on Denitrification Rates Of Alluvial Soils

Best management practices (BMPs) implemented for soil erosion control in agricultural watersheds can provide opportunities for NO_3 pollution control (Schreiber et al. 2001). Structural BMPs hold back agricultural run-off from cultivated lands to help increase sedimentation in erosion prone landscapes.

Best management practices and CGT amendment affected denitrification rates (Chapter 3). CGT amendment led to an average 200% increase in denitrification rates of all the sites. The increased rate of denitrification observed in the CGT amended plots was supported by the 141% higher rates of mineralizable organic C sources due to CGT additions. CGT amended plots denitrified 16 kg of NO_3-N ha^{-1} during the 6 months study period, while the unamended plots denitrified 5.2 kg NO_3-N ha^{-1}, in spite of similar NO_3 loading rates of 15 μg NO_3-N g^{-1} soil. Structural BMPs affected denitrification rates in different land use types differently. The grass buffer CRP and herbaceous WRP sites under the influence of slotted board riser BMPs, maintained high average WFPS compared to cultivated soils without BMPs. The high WFPS in the WRP and CRP sites resulted in significantly greater denitrification rates than the rates of cultivated site without BMPs. Cultivated soils under the slotted board riser and slotted pipes BMPs also maintained high average WFPS than the cultivated soils without BMPs, however, their denitrification rates were not significantly different from each other ($p > 0.05$). This result suggests that cultivation may have led to lower denitrifier population in these soils.

These findings clearly show that addition of CGT to cultivated soils and newly restored CRP and WRP sites in conjunction with BMPs application can help reduce run-off NO_3 in agricultural watersheds. As tons of CGT are produced in the LMV, its

application to soils for the enhancement of denitrifier activity can help recycle this waste in an environmentally beneficial way.

Land Use, Water-filled Pore Space, and NO_3 Amendment Effects on Denitrification Rates and N_2O:N_2 Emission Ratio

Restoration of forested wetlands on low-elevation clay soils will clearly increase NO_3 removal through denitrification. However, it is uncertain if increased denitrification in the restored soils also increases N_2O emissions into the atmosphere. Land use, WFPS and NO_3 addition affected both denitrification rates and N_2O:N_2 emission ratios (Chapter 4). Forested wetland soils had 5.2, 6.6 and 2 times greater denitrification rates than those observed in cultivated soils, incubated at 70%, 85% and 100% WFPS respectively. Denitrification rates observed in the forested soils under NO_3 loading ranged from 41 to 78 mg N m^{-2} day^{-1}. These rates are comparable to the denitrification rates of riparian wetlands reported by several similar studies (Lowrance et al. 1995; Hefting et al. 2003; Cooper, 1990) The N_2O:N_2 emission ratio decreased markedly from 0.28 to 0.11 in forested wetland soils and from 0.69 to 0.19 in the cultivated soils as WFPS increased from 70% to 100%. NO_3 additions led to an average 2.6 times greater denitrification rates in forested wetland soils incubated at 85 and 100% WFPS, while cultivated soils exhibited no response to NO_3 additions. Cultivated soils were limited by the availability of organic C to denitrify, as these soils had 4.2 times lower mineralizable organic C contents than that of the forested wetland soil. N_2O emissions increased in both the forested and cultivated soils when amended with NO_3. N_2O emissions from forested wetlands ranged from 10 to 25 kg N ha^{-1} y^{-1} without and with the additions of NO_3 respectively. These rates are within the range of emissions reported in the literature from forested wetlands (Lindau et al. 1994; Hefting, et al. 2003; Walker et al. 2002). In the

cultivated soils, N_2O emission ranged from 19 to 24 kg N ha^{-1} y^{-1} without and with additional NO_3 loading.

These findings demonstrate that restoration of forested wetlands on low elevation clay soils can remove significant quantities of run-off NO_3 and will not contribute significantly to the atmospheric burden of N_2O. However, any restoration program designed to sequester C to offset atmospheric CO_2 emissions needs to account for the increased N_2O emission under additional NO_3 loading. Moreover, restoration of wetlands should make sure to restore hydrology of these ecosystems to maintain high WFPS (above 80%), so that high denitrifier activity can be supported by the restored wetland soils.

Use of Cotton Gin Trash to Enhance Denitrification in Restored Forested Wetlands

The success of forested wetlands restoration efforts for water quality improvement depends on how long the restored system will take to achieve the same level of functioning performed by similar natural forested wetlands. There is paucity of information on the rate of functional restoration of forested wetlands in the LMV, including water quality improvement function. One reason for low microbial activity in restored wetlands is the lower amounts of microbially available C compared to natural wetlands. Denitrification rates observed in the natural forested wetlands were on average 52% greater than those in the restored forested wetlands (Chapter 5). Similarly, average N_2O:N_2 emission ratio from the natural forested wetland was 0.35, significantly lower than the ratio of 0.53 observed from the restored forested wetlands. When averaged and extrapolated to the 6 months study period, the natural forested wetlands denitrified 14 kg of N ha^{-1}, a rate which is 2.2 times of the restored forested wetlands. These results clearly

demonstrate that even after 13 years of restoration, the restored wetland ecosystem did not achieve the same level of denitrifier activity exhibited by the natural forested wetlands. When amended with CGT, restored forested wetlands showed an average 40% increase in denitrification rates and 33% decrease in $N_2O:N_2$ emission ratios compared to unamended restored wetland. This result of increased denitrification rate due to organic C amendment is in agreement with the findings of Hunter (2000) and Davidson and Stahl (2000). As CGT additions led to enhanced denitrifier activity in the restored forested wetlands, it is recommended that 15-20 metric tons of CGT ha^{-1} applied to the soil surface in restored forested wetland soils will significantly enhance denitrifier activity.

Among the environmental variables influencing denitrification, soil WFPS regulated denitrification rates in both the restored and natural forested wetland soils. High WFPS in soils led to higher denitrification rates, which is in agreement with the research findings in Chapter 4, Arah, et al. (1990), and Weier, et al. (1993). Thus it is important to restore forested wetlands in a way that ensure more flooding and saturation of soils (Hunter and Faulkner, 2001). Filling up drainage ditches to control quick draw down of rain water from wetlands and its surrounding land uses and re-routing run-off from uplands and agricultural lands to restored wetlands is recommended.

Nitrate and Cotton Gin Trash Amendment Effects on N_2O, CH_4 and CO_2 Emissions from Forested Wetlands

The overall averaged flux of CH_4 from the restored and natural forested wetlands was negative and the soils consumed 12 μg CH_4 m^{-2} h^{-1}(Chapter 6). Merino et al (2004) reported a net CH_4 uptake in southern European forest soils ranging from 3 to 116 μg CH_4 m^{-2} h-1. The lower net CH_4 uptake rates observed during my study from the forested wetlands coincided with an average high soil WFPS of 73% which seem to have reduced

methanotrophic activity. Reduced CH_4 uptake by forest soils at WFPS > 60% have also been reported by Yavitt et al (1995), Merino et al (2004) and Nesbitt and Breitenbeck (1992). No significant difference in CH_4 flux from the restored and natural forested wetlands was observed. Higher CH_4 consumption in forested wetlands in the region is expected during summer and early fall when soil water contents are low. CH_4 flux from CGT amended restored forested wetland soils was similar to that from the unamended soils, showing that organic C amendment of restored wetland soils will not significantly influence soil CH_4 flux. Similarly, NO_3 amendment did not influence CH_4 flux from both the restored and natural forested wetland soils. This result of no NO_3 amendment effect on soil CH_4 uptake is consistent with the findings of Nesbitt and Breitenbeck (1992). Among the environmental variables, soil WFPS and soil temperature were found regulating CH_4 flux from these sites. High WFPS and low soil temperatures led to significantly low but highly variable CH_4 flux, which shows that both methanogenic and methanotrophic activity in these soils were influenced by these two variables. Lower WFPS and higher soil temperatures led to CH_4 consumption by soils. This finding is commensurate with the findings of Castro et al (1995), Bowden et al (1998) and Nesbitt and Breitenbeck (1992). In summary, these finding shows that forested wetlands restoration will not result in increased CH_4 emissions.

NO_3 additions resulted in an average 48% increase in N_2O emissions from both the restored and natural forested wetlands soils. Similar increases in N_2O emissions from forest soils with NO_3 additions were observed by Magill et al. (1997) and Bowden et el. (1991). When extrapolated to per hectare per year basis, average N_2O emissions rates from both the natural and restored forested wetlands observed at 75% WFPS ranged from

10 to 23 kg N_2O-N ha^{-1} y^{-1} and 8 to 15 kg N_2O-N ha^{-1} y^{-1} with and without NO_3 additions respectively. These emission rates are in the range of N_2O emissions reported from riparian forests (Hefting et al. 2003, Walker et al. 2002, Lindau et al. 1994) and cultivated soils elsewhere (Simek, et al. 2004; Ullah et al. in press). The range of N_2O emissions observed from restored wetlands can offset the C sequestration benefits of restored wetlands. The 48% increase in N_2O emissions observed from the CGT amended restored and natural wetlands due to NO_3 amendment needs to be accounted for while establishing CO_2 emission credits on restored forested wetlands in the LMV.

CO_2 flux from the restored and natural forested wetland soils was influenced by soil moisture, temperature, organic C amendment and NO_3 loading. The flux of CO_2 from natural and CGT amended restored wetland soils was 2.5 times of the no CGT amended restored wetland soil. This result shows that CGT amendment led to higher CO_2 emissions, which were statistically not different than those observed from the natural forested wetlands. As far as the effects of environmental variables are concerned, CO_2 emissions were significantly reduced by increasing soil WFPS and decreasing soil temperatures. Similar effects of soil WFPS and temperature have been reported in the literature (Lomander, et al. 1998, Merino et al. 2004). This result suggests that forested wetlands restored under higher WFPS can reduce CO_2 emissions from soils, which will benefit the sequestration of atmospheric CO_2 into the soil organic matter. NO_3 additions to the restored and natural forested wetland soils led to a marked 35% increase in CO_2 emissions compared to soils without NO_3 amendment. Brumme and Beese (1992) and West and Marland (2003) also reported an increase in CO_2 emission with N fertilization of soils. Our findings show that restoration of forested wetlands in the LMV will lead to

reduced CO_2 emissions into the air. However increased CO_2 emission due to NO_3 loading needs to be accounted for while calculating the C storage credits for restored forested wetlands in the LMV, to minimize uncertainties associated with C storage budgeting.

Further Research

Quantification of denitrifier population in different land uses of an agricultural watershed in the LMV is recommended to elucidate the effects of landscape and land uses on denitrification population size and activity. Mechanistic research on tracing the pathways of run-off NO_3 in wetlands is recommended using the ^{15}N tracer techniques, to get insight into the relative contribution of plant uptake and microbial immobilization to NO_3 removal in wetlands, beside denitrification. Denitrification and greenhouse gas emissions from soils are highly variable temporally, more frequent measurements of these processes is recommended to establish more accurate estimates of NO_3 removal and greenhouse gas emissions from cultivated and wetland soils in the LMV. The effect of NO_3 addition on soil organic C storage potential is recommended in the LMV to help reduce uncertainties in estimates of C sequestration in forested wetlands.

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APPENDIX. LETTER OF PERMISSION TO REPRINT

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