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Louisiana State University and Agricultural & Mechanical College

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COMPARISON OF BIOGEOCHEMICAL FUNCTIONS BETWEEN RESTORED AND NATURAL BOTTOMLAND HARDWOOD WETLANDS

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in

The Department of Oceanography and Coastal Studies

by

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B.S., University of Tennessee, 1989
M.S., Tennessee Technological University, 1996
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ABSTRACT

The purpose of this research was to compare biogeochemical functions of restored and natural bottomland hardwood (BLH) wetlands to determine if re-establishment of hydrology and vegetation is equivalent to restoration of ecosystem function. Three wetland types were studied: natural BLH (NAT), restored with hydrology re-established (RWH), and restored without hydrology re-established (RWOH). Denitrification potential (DEA), soluble organic carbon (SOC), soil moisture, readily mineralizable carbon (RMC), and heterotrophic microbial activity were measured seasonally from 1997-99. Leaf litter mass, total carbon and nitrogen concentrations, moisture, and microbial activity were also measured at three soil depths in study wetlands. The effect of organic matter amendments on microbial activity and denitrification rates was determined during a one-month incubation. Mean μg nitrous oxide evolved/g soil/day for NAT, RWH, and RWOH were 17,112, 8,694, and 4,179, respectively, with a significant difference among wetland types measured in fall. During the two-year period, average soil saturation in the upper 15 cm was 4, 4.25, and 2 months, for NAT, RWH, and RWOH, respectively. Carbon amendments added to restored wetland soils significantly increased denitrification rates and microbial activity but there were no differences in measured parameters among wetland types. Although no significant differences were detected among wetland types for RMC, SOC, and three of the four seasons for heterotrophic microbial activity, values were
consistently highest in NAT and lowest in RWOH. Litter mass was significantly higher on NAT soils than on restored, corresponding to higher microbial activities in NAT soils than in restored. Because parameters measured in the RWH wetland soils were much higher than those in RWOH soils, these results illustrate that changes in hydrology will effect changes in soil characteristics and microbial dynamics. Therefore, a BLH wetland restored without re-establishing hydrology will not have biogeochemical functions comparable to those of a natural BLH wetland. Although RWOH soils have the capability to denitrify, because these wetlands are not connected to the surface hydrology of the watershed they will not be in contact with nitrate and thus will not be important in removing nutrients from agricultural runoff water.
CHAPTER ONE
INTRODUCTION

When North America was first colonized by the Europeans, there were approximately 221 million acres of wetlands in the conterminous United States (Wiebe et al., 1995). Since the late 1700’s, over 53% of these wetlands have been lost, primarily due to clearing and draining for agriculture and urban development. Between 1950 and 1970 over 185,000 hectares disappeared annually, with 87% of the losses attributable to agricultural conversion (Mitsch and Gosselink, 1993; Wiebe et al., 1995). Since the 1970’s, however, as perceptions of wetland values and functions changed, the rate of wetland conversion slowed and the number of wetland creation and restoration projects increased.

Interest in protection of wetlands grew significantly in the early 1970’s when the scientific community began to identify and quantify wetland values (Mitsch and Gosselink, 1993). As the amount of research increased and a scientific foundation was laid, attitudes towards wetlands changed and the federal government began to implement legislature designed to protect wetlands. The first of such policies was Executive Order 11990, Protection of Wetlands, issued by President Jimmy Carter in 1977. This order required all federal agencies to take action to minimize destruction of wetlands by ending federal assistance for wetland conversion. A similar order, Executive Order 11988, Floodplain Management, issued at the same time protected floodplains (Mitsch and Gosselink, 1993). These executive orders forced federal agencies to
rethink their activities in wetlands and floodplains and focused their sights on conservation of these areas.

The next significant event in wetland protection was the "no net loss" policy developed in 1987 by the National Wetlands Policy Forum. George Bush adopted this policy as a national goal and it became an important stepping stone in the United States' attitude towards wetland conservation (Mitsch and Gosselink, 1993). However, the most important vehicle in wetland conservation has been Section 404 of the Federal Water Pollution Control Act, also known as the Clean Water Act. Because of this act, the U.S. Army Corps of Engineers established a permit system to regulate the dredging and filling of materials in waters of the U.S. and the definition was designed to include wetlands, although normal agricultural practices were exempt (Mitsch and Gosselink, 1993; Wiebe et al., 1995).

A fairly new program in restoration, the Wetland Reserve Program (WRP), was authorized by the Food, Agricultural, Conservation, and Trade Act of 1990 to restore and permanently protect prior-converted wetlands and functionally dependent land adjacent to farmland (Wiebe et al., 1995). This program allows farmers to enroll land for restoration and receive conservation easements and retain the rights to hunting, fishing, and timber management. The goal of the WRP program, implemented by the USDA-NRCS, is to enroll 405,000 hectares by the year 2000. One important benefit of this restoration program is the improvement of water quality. By restoring wetlands in the lower landscape adjacent to agricultural cropland, runoff water flows from cropland through the wetland, carrying with it sediment and nutrients. These biological buffer zones reduce sediment
and nutrient concentrations by deposition, plant and microbial uptake, adsorption/fixation reactions, and denitrification, resulting in an improvement of the quality of runoff water before it reaches a nearby river or groundwater (Hill, 1996).

The WRP program focuses primarily on restoration of forested wetlands such as bottomland hardwood (BLH) wetlands. Bottomland hardwood wetlands are riparian wetlands that occur in river floodplains of the southeastern United States (Mitsch and Gosselink, 1993). The largest expanse of BLH wetlands in the southeast occurs in the Mississippi River floodplain. Historically, about 21 million hectares existed, however, as of 1991, only about 4.9 million hectares had not been cleared for agricultural cropland and other uses (Mitsch and Gosselink, 1993). Between 1883 and 1991, 77% of southern bottomland hardwood forests were lost, primarily due to conversion to agriculture (Mitsch and Gosselink, 1993). In the 1970's, conservationists began to work towards preservation of these forests, when rates of land clearing exceeded 120,000 hectares per year in the Mississippi River Alluvial Valley (Loesch et al. 1995). Despite their efforts, by 1986 only 20% of forested wetlands remained in this area (Llewellyn et al., 1996). Loss of BLH wetlands results in water quality problems, decline in wildlife habitat, and problems with flood control (Walbridge, 1993).

The location of BLH wetlands in river floodplains results in seasonal flooding by surface water or as near-surface groundwater and causes soils to be saturated or inundated during the late winter and early spring (Messina and Conner, 1998). This annual flooding causes anoxic
conditions in soils of these ecosystems, which influences growth and composition of vegetation and soil microbial populations (Conner, 1994). Many researchers believe hydrology to be the dominant factor which controls ecosystem dynamics in forested wetlands (Day and Megonigal, 1993; Wharton et al., 1982).

Wetland hydrology affects soil and vegetation characteristics and biogeochemical functions. Soils of BLH forests are the product of river deposition and are generally fine-grained alluvial soils. The flood and drain cycle of these riparian wetlands affects properties such as oxygen concentration, organic matter concentration, and nutrient content. Percent clay, silt, and sand content in a soil will affect hydrologic properties such as hydraulic conductivity and water-holding capacity. Mitsch and Gosselink (1993) characterized physiochemical characteristics of soils in floodplain wetlands as primarily dominated by clays, anoxic for part of the year, and about 3 to 4% organic matter.

Changes in hydrology which occur due to man-made alterations or which happen naturally over time will affect soil characteristics such as moisture content and oxygen concentrations. Elevation and rate of sediment deposition are also affected by hydrology and will alter species occurrence and natural patterns of ecological succession (Hodges, 1997). As soil characteristics change, over time vegetation dynamics are altered as allogenic forces primarily determine which plants will grow in more flooded or drier conditions. Differences in vegetation composition will lead to changes in litter quality which will affect heterotrophic microbial populations (Messina and Conner, 1998). If soils remain saturated or
inundated, facultative anaerobes and obligate anaerobes will dominate the microbial community. A shift in microbial dynamics affects primary productivity, decomposition, and biogeochemical functions.

Biogeochemical functions of BLH wetlands describe soil chemical reactions and nutrient uptake and transformations by soil microbial populations, invertebrates, and plant communities (Walbridge, 1993). Because riparian areas are seasonally flooded, this hydrologic regime creates a unique environment for nutrient transformations, especially denitrification, an important biogeochemical function of BLH wetlands. When soils are flooded during late winter and early spring, O₂ is consumed and anoxic conditions are created in soils, promoting growth of facultative and obligate anaerobes. As redox potential falls below +300 mV, denitrifying microorganisms utilize nitrate (NO₃⁻) as an electron acceptor to create a gaseous end product, N₂ (Groffman and Tiedje, 1989). Annual flooding dictates organic matter cycling as well, with decomposition much greater when soils are drained than when they are flooded (Patrick et al., 1985).

There is a great deal of research measuring the denitrification ability of riparian wetlands (Haycock and Pinay, 1993; Hanson et al., 1994; Seitzinger, 1994; Nelson et al., 1995; Hill, 1996; Groffman et al., 1998; Addy et al., 1999; Martin et al., 1999). Riparian ecosystems also lower NO₃⁻ concentrations through plant uptake and microbial immobilization, but denitrification and plant uptake are generally considered the main mechanisms of NO₃⁻ removal, while microbial immobilization is considered to be of minor importance (Hill, 1996; Martin et al., 1999). Regardless of
the mechanism through which $\text{NO}_3^-$ is removed, riparian wetlands have been shown to remove 80 to 99% of $\text{NO}_3^-$ from subsurface water inputs from agricultural and unsewered residential land uses (Hill, 1996).

While plant uptake and denitrification are considered the primary mechanisms of $\text{NO}_3^-$ removal, there is disagreement on the relative importance of each (Gilliam, 1994). For denitrification to occur, oxygen must be depleted so that facultative and obligate anaerobes use $\text{NO}_3^-$ rather than oxygen as an electron acceptor. In addition, sufficient concentrations of available organic matter must be present to provide an energy source for denitrifiers, almost all of which are heterotrophs. Several researchers working in organic riparian wetlands have identified denitrification as the main mechanism for $\text{NO}_3^-$ removal, probably because the energy source available to denitrifiers was not limiting (Cooper, 1990; Ambus and Christensen, 1993; Schipper et al., 1993).

The importance of vegetation uptake in $\text{NO}_3^-$ removal varies with season (Simmons et al., 1992; Haycock and Pinay, 1993). Most $\text{NO}_3^-$, especially that originating from agricultural fields, flows through neighboring wetlands when vegetation is dormant in winter and early spring, a time when plant uptake would not occur (Gilliam, 1994). The type of vegetation present may also affect $\text{NO}_3^-$ removal either directly through the amount of uptake or indirectly through quality and quantity of organic matter added to the soil (Seitzinger, 1994; Hill, 1996; Martin, 1999). Schnabel et al. (1996) measured denitrification in a grassed and a wooded riparian ecotone and found denitrification rates were greater in the grassed section of the riparian zone than the forested section. Addition of carbon
amendments (glucose) increased rates of denitrification in the forest but did not affect denitrification rates in the grass ecosystem, suggesting that carbon in the latter system was more available to microorganisms than in the former. Haycock and Pinay (1993) measured greater NO$_3^-$ removal in a poplar vegetated riparian buffer than in a grass vegetated one during winter in the River Leach catchment in the UK. They hypothesized that, although vegetation was not actively uptaking NO$_3^-$, the poplar was providing a better carbon source to the microbial populations responsible for denitrification than the grass, accounting for higher NO$_3^-$ removal.

Because soil organic matter concentrations and redox status greatly influence denitrification rates in riparian wetlands, disturbances such as conversion to cropland and subsequent restoration or clearing for timber harvest may affect denitrification ability. Lowrance et al. (1995) measured denitrification in a restored riparian forest wetland and found that denitrification rates were comparable in the recently restored forest and a mature riparian forest. The restored forest studied, however, had not been hydrologically modified, a factor which significantly affects biogeochemical functioning. Addy et al. (1999) found no significant differences in removal rates within the subsoils of hydrologically intact forested and mowed riparian wetlands, suggesting that vegetation composition may not be critical if the hydrologic regime has not been disturbed. Unfortunately, until now there has been no research conducted comparing denitrification rates in restored wetlands with and without hydrologic modifications.

The ability of an ecosystem to denitrify also changes within the system due to microsite differences in soil moisture, organic matter, and
substrate concentrations. There has been significant variability in measured denitrification rates and potentials within the same wetland (Christensen et al., 1990; Ambus and Lowrance, 1991; Groffman et al., 1992; Hill, 1996; Ashby et al., 1998). Much of this variation has been attributed to “hot spots” of particulate organic matter in soils (Parkin, 1987; Christensen et al., 1990).

The purpose of this research was to compare biogeochemical functions of restored and natural BLH wetlands to gain an understanding of the effectiveness of current restoration methods. This research was conducted in the Tensas River Basin, part of the Mississippi River Alluvial Plain which consists of 1,000,000 hectares in northeastern Louisiana. Between 1959 and 1987, over 200,000 hectares of these forested wetlands were converted to other uses, primarily agricultural cropland (Llewellyn et al., 1995). Recently the Tensas River Basin has received a lot of attention from conservationists and, as a result, the Tensas River Basin Steering Committee was formed. The focus of this committee is to implement a plan that will emphasize reforestation and compatible farming practices (Llewellyn et al., 1995).

The Tensas River Basin leads the nation in WRP enrollment, with over 20,250 hectares restored to BLH forests. Because of the emphasis on restoration projects, the Tensas River Basin was selected for this research. Six BLH wetlands were chosen for study. Two of the sites were natural, undisturbed BLH wetlands, while the remaining four were restored. All of the wetlands were restored by replanting with typical BLH hardwood trees. Only two of the four restored wetlands had hydrology re-established by
placing.flashboard.risers.in.the.drainage.ditches.Using.this.experimental
design.it.is.possible.to.relate.structure.(vegetation.and.hydrology.or.the
lack.thereof)to.biogeochemical.functioning.

In.addition.to.biogeochemical.functions,we.also.wanted.to.study
specific.soil.characteristics.which.would.affect.ecosystem.function. It.is.well
documented.that.the.primary.factors.affecting.denitrification.are.$\text{NO}_3^-$
concentration,.organic.matter.quality.and.quantity,.and.soil.moisture
(Burford.and.Bremner,.1975;.Groffman.and.Hanson,.1997;.Martin.et.al.,
1999),.therefore,.these.soil.characteristics.were.measured.along.with
denitrification. These.factors.will.fluctuate.with.time.and.with.changes.in
vegetation.and.hydrology.as.succession.occurs.in.an.ecosystem. Soil
characteristics.will.also.affect.microbial.populations,.which.affect.nutrient
cycling.and.decomposition.

The.specific.objectives.of.this.research.were.to.i) quantify
denitrification.potentials;.ii) measure.soluble.organic.carbon.and.readily
mineralizable.carbon.concentrations.and.soil.moisture;.iii) measure.soil
heterotrophic.microbial.activity;.iv) measure.$\text{NO}_3^-$.concentrations.in.soils;.v)
quantify.litter.layer.accumulation;.vi) measure.heterotrophic.microbial
activity,.and.carbon.and.nitrogen.concentrations.in.different.depths.of.soils;
and.vii) measure.heterotrophic.microbial.activity.and.denitrification.rates.in
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moisture. Chapter.three.describes.the.measurements.of.soluble.organic
carbon, readily mineralizable carbon, heterotrophic microbial activity, and soil moisture in the upper soil surface of the study wetlands. Chapter four presents a nutrient analysis of the upper soil surface, along with a comparison of nutrient and carbon concentrations between the litter layer and the upper 15 cm of soil. Chapter five describes the results of a study to determine the effects of organic matter amendments on microbial activity and denitrification rates in restored wetland soils. Chapters six presents a synthesis of the data and overall conclusions of this research.
CHAPTER TWO

COMPARISON OF DENITRIFICATION POTENTIALS IN RESTORED AND NATURAL BOTTOMLAND HARDWOOD WETLANDS

INTRODUCTION

Overview

Wetland restoration involves replanting appropriate vegetation and restoring the hydrologic regime. However, because ecosystem characteristics have changed from the original wetland, biogeochemical functions may be different as well. Biogeochemical functions are not as easily evaluated as structural features and, thus, they are not always measured when determining the success of a restoration project. One biogeochemical function, denitrification, occurs in flooded soils and may be a useful indicator of functioning wetlands. Denitrification potential, soluble organic carbon concentrations, and soil moisture were measured seasonally over a one-year period in bottomland hardwood (BLH) wetlands in northeastern Louisiana. Three types of wetlands were studied: natural bottomland hardwood wetlands (NAT), restored wetlands with hydrology restored (RWH), and restored wetlands without hydrology restored (RWOH). Although no significant differences were measured in denitrification potentials among wetland types in winter, spring and summer, in fall denitrification was highest in the NAT wetlands and lowest in the RWOH wetlands. Although differences in denitrification enzyme activity were not detected for most of the year, mean nitrous oxide-nitrogen (N₂O-N) evolved/g dry soil/hr for NAT, RWH, and RWOH were 657, 372, and 162, respectively, when nitrate and energy source were not
limiting. Soil moisture was highest in NAT wetlands and lowest in RWOH, but no differences were measured in soluble organic carbon concentrations among wetland type. These results suggest that changes in hydrology will effect changes in soil characteristics and microbial dynamics. Because RWOH wetlands exhibited denitrification potentials which were much lower than the other wetland sites, these results indicate that restoring a BLH wetland without restoring hydrology will affect biogeochemical functioning.

**Background**

Ecosystem restoration is a difficult endeavor due to the inherently dynamic nature of ecosystem processes. Complexity in both time and space creates a significant challenge when restoring an altered ecosystem and evaluating the results. Site-specific goals should be established at the outset so that the appropriate methods may be applied and the results evaluated according to desired measures of success. The selection criteria for determining success, i.e., performance standards, are particularly important if we are to truly restore an altered system back to its previous condition or, at the very least, restore the most important aspects of the system. Specifically, these performance standards should include both structural and functional attributes of the ecosystem at both the site and landscape scales.

These concepts are particularly important in the area of wetland restoration where wetlands are often created or restored in order to mitigate the loss of wetland functions caused by conversion to some other land use. Compensatory mitigation is required under Section 404 of the
Clean Water Act and may occur on-site or through the purchase of acreage "credits" in a mitigation bank. Millions of dollars are also being spent through governmental programs such as the Wetland Reserve Program (WRP) to restore wetlands on marginal croplands (USDA-NRCS, 1995). Although structure (hydrology and vegetation) is restored in these wetlands, very few studies have been conducted to determine if functions are being restored as well.

In the Mississippi River alluvial valley, less than 25% of the forested wetlands remain (Abernathy and Turner, 1987) and loss of these wetlands affects water quality in the watershed and wildlife populations, and reduces flood control. One type of forested wetland, bottomland hardwood (BLH) wetlands, performs many valuable functions including nutrient uptake and transformations, sediment retention, floodwater storage, and organic carbon export to downstream ecosystems (Mitsch and Gosselink, 1993).

Because of the unique hydrological conditions under which these ecosystems evolved, restoration is a complex process. While mature BLH wetlands may be excellent transformers of nutrients and may be able to provide flood control, the ability of newly created or restored wetlands to perform these same functions has not been studied to any extent (Vellidis et al., 1993). In order to determine causal relationships between ecosystem structure and function, restored wetland functions should be evaluated and compared to those of natural wetlands.

One important water quality function of natural riparian wetlands is denitrification, the microbial reduction of nitrate ($\text{NO}_3^-$) to nitrogen ($\text{N}_2$) gas (Hanson et al., 1994). Because denitrification occurs in soils when oxygen
is not available, it is indicative of soils that are flooded. Therefore, potential for denitrification may be used as a measurement of wetland function. Denitrification may also be a good indicator of how well a restored BLH wetland is functioning because this process is affected by several conditions which have evolved in wetland ecosystems. Amount and type of organic matter accumulated in soils, soil moisture, vegetation, and hydrologic regime all either directly or indirectly affect microbial populations which are capable of denitrifying (Groffman et al., 1996; Groffman and Hanson, 1997; Ashby et al., 1998).

Comparison of denitrification potentials between restored and natural wetlands may be used to determine if restored wetlands have biogeochemical functions similar to natural wetlands. The denitrification enzyme activity (DEA) assay measures the potential of a soil's microbial populations to produce enzymes able to reduce $\text{NO}_3^-$ to $\text{N}_2$ gas. The DEA assay optimizes conditions for denitrification by providing unlimited amounts of substrate ($\text{NO}_3^-$) and energy source (glucose) while blocking further reduction of nitrous oxide ($\text{N}_2\text{O}$) to $\text{N}_2$ gas. When comparing the ability of two soils to reduce $\text{NO}_3^-$, it is useful to use the DEA assay rather than measuring actual denitrification rates because the former test will produce maximum amounts of $\text{N}_2\text{O}$, if other conditions are not limiting.

Our study examines how restoration affects biogeochemical functions in bottomland hardwood wetlands. The primary objectives of this research were (i) to compare biogeochemical functions of natural wetlands with those of wetlands restored with and without hydrologic modifications; and (ii) to relate measured denitrification potentials to specific soil
characteristics. It was hypothesized that denitrification would be higher in natural wetlands than in restored due to a longer hydroperiod and higher organic matter concentrations in the natural wetland soils than in restored wetland soils.

MATERIALS AND METHODS

Site Description

Study wetlands were located in the Tensas River Basin in northeastern Louisiana (see Fig. 2.1), an area in which agriculture is the primary land use (USDA-NRCS, 1995). The 291,500 hectare Tensas River Basin was once over 90% bottomland hardwood forests. Conversion of about 85% of these forests to cropland resulted in a decrease in water quality of the Tensas River, caused by runoff of sediments and nutrients from adjacent agricultural fields (USDA-NRCS, 1995). To try to improve water quality of this river, and to provide wildlife habitat and flood storage, agricultural croplands are being restored back to bottomland hardwood wetlands.

Three types of BLH wetlands were chosen for study: natural mature (NAT) wetlands, wetlands restored with hydrology re-established (RWH), and wetlands restored without hydrology re-established (RWOH). Restored wetlands were once natural BLH wetlands which were converted to agricultural fields by clearing vegetation and digging drainage ditches around the wetlands. Both RWH and RWOH sites were replanted with appropriate bottomland hardwood species (*Quercus lyrata*, *Q. nigra*, *Q. phellos*, *Acer rubrum*). Hydrologic restoration of the RWH sites consisted of installing flashboards risers in drainage ditches adjacent to sites. The water
2.1. Location of bottomland hardwood wetland study sites in the Tensas River Basin in northeastern Louisiana (Adapted from Heggem et al., 1999).
height in these ditches can be controlled by the risers to allow surface runoff from the adjacent agricultural fields to inundate the sites. This simulates the hydrologic regime of the natural BLH wetlands in agricultural watersheds. The RWOH sites did not have flashboards risers installed in drainage ditches and agricultural surface runoff simply bypassed these sites via the drainage ditches. Study wetlands were chosen for their proximity to agricultural fields as well as for their similarities in soil type, slope, and topography. Two replicates of each type of wetland were chosen. The RWH wetlands were both restored in 1990 and the RWOH wetlands were restored in 1993 and 1994. Textural classes were clay for NAT and RWH soils and silty clay for RWOH wetland soils. Soils in the Tensas Basin are alluvial clays and fine silts. Approximately 60% of these soils are classified as hydric, including the following series: Alligator, Sharkey, and Tunica. The study wetlands chosen for this research contain primarily Sharkey and Sharkey-Tunica soil associations. Sharkey map unit consists of poorly drained clayey soils with slopes of generally 0-3%. Sharkey-Tunica map unit consists of poorly drained and somewhat poorly drained clayey soils with slopes of 0-3%. Sharkey soils, occurring in swales, and Tunica soils, occurring on low ridges, make up 58 and 26% of this map unit, respectively (USDA-NRCS, 1995).

Experimental Design

A 20- x 30-m grid system was established in each of the study wetlands. The grid consisted of three columns 10 m apart and four rows 10 m apart (Fig. 2.2). The exception to the this grid system was one of the
2.2. Layout of plot and location of sampling equipment in natural and restored bottomland hardwood study wetlands. 30, 60, and 90 designate the depth of piezometers in cm. Well designates the position of 90-cm groundwater sampling wells. Arrows beside the flow path indicate the direction of agricultural runoff water.
mature wetlands (Little Fork). This wetland contained a 15- x 40-m grid system where the rows were 10 m apart and the columns were five meters apart, due to the shape of the wetland. In all wetlands, the grid system was set up so that rows were horizontal to the water source. In restored wetlands, the water source was overflow from drainage ditches. All wetlands were chosen for their proximity to cropland so that surface flow to the wetlands included runoff from adjacent fields. Natural wetlands have not had trees harvested in over seventy years so a mature stand is present on these wetlands. The RWH sites were both restored in 1990 and the RWOH sites were restored in 1993 and 1994.

Rainfall and groundwater levels were monitored monthly in each study wetland. For groundwater depth measurements, one 90-cm groundwater well was installed every 10 meters in the middle of each site. Water levels were determined by measuring the depth to water in the well using a flashlight and tape measure. Rainfall was measured using tipping bucket rain gauges installed in Dorsey (RWH), Brown (RWOH), Windham (RWOH), and Greenlea (RWH) sites. Because Windham and Canon (NAT) are located beside each other, as are Dorsey and Little Fork (NAT), only one rain gauge was installed for each of these pairs of study wetlands.

**Statistical Analysis**

Data were analyzed using the Proc Mixed procedure of SAS® Institute (1994). Wetland type, distance from the water source, season, and, for the denitrification study, soil treatment, were analyzed as main effects. Main effects were considered significant if they had a $P \leq 0.05$. Differences among means within each main effect and within interaction
effects were evaluated using Tukey’s Studentized Range (HSD) test \((\alpha = 0.05)\). Relationships between dependent and independent variables were examined using a linear regression analysis and correlations among variables were conducted using Pearson’s correlation test.

**Collection of Soil Samples**

Twelve soil samples were collected from the upper 15 cm at each study site using a 5-cm diameter soil auger. Samples were collected in July and October, 1997 and January and April, 1998. One soil sample was collected near each piezometer nest. After collection, the soils were stored on ice until they were brought to the Wetland Biogeochemistry Institute and refrigerated \((4^\circ C)\). Each soil sample was homogenized by passing through a mesh screen with 1.25 cm\(^2\) openings and then analyzed for percent moisture, soluble organic carbon concentration, and denitrification potential.

**Soluble Organic Carbon**

Ten grams of field moist soil from each sample was shaken in 100 ml deionized water at high speed for 30 minutes and allowed to stand for approximately 18 hours (overnight) (Kaiser and Zech, 1996). The solution was then shaken by hand and 40 ml was poured into a centrifuge tube and centrifuged at 6500 rpm using a DuPont Instruments Sorvall\textsuperscript{®} SA-600 rotor for 10 minutes at 25°C. Twenty milliliters of the supernatant was filtered through a 0.45 \(\mu\)m polysulfone membrane filter into a scintillation vial and refrigerated at 4°C until analysis could be completed. Samples were analyzed for nonpurgable organic carbon using a Shimadzu TOC-5000A Total Organic Carbon (TOC) analyzer. Nonpurgable organic carbon in
each sample was measured by acidifying the sample with 40 µl of HCl and then purging for eight minutes with TOC grade compressed air. Acidification reduces inorganic carbon to primarily carbon dioxide (CO$_2$) in these samples and purging volatilizes CO$_2$ out of solution. Samples were then analyzed for organic carbon concentrations. Results were given as the mean of three replicates per sample. Results were also corrected for soil moisture so that final results were expressed as mg SOC/g soil on a dry weight basis.

**Denitrification Enzyme Activity (DEA) Assay**

Potential denitrification rates were determined using the DEA procedure of Tiedje (1982). Four 25-gram subsamples were taken from soil samples and placed in four 125-ml incubation flasks. The four subsamples were treated with 25 ml of one of the following solutions:

- Treatment A = 1 g/L solution of chloramphenicol (chl); Treatment B = 1 mM KNO$_3$ (14 mg NO$_3$-N/L) and 1 g/L chl; Treatment C = 1 mM glucose and 1 g/L chl; and Treatment D = 1 mM KNO$_3$, 1 mM glucose, and 1 g/L chl.

Chloramphenicol inhibits protein synthesis so that the microbial population size is the same as at the time of field sampling. By applying these different treatments, it can be determined if denitrification is limited by substrate (NO$_3^-$), energy source (glucose), or both. The mixtures were shaken vigorously to obtain a slurry. The flasks were capped with gas-impermeable stoppers and made anaerobic by flushing with argon for one minute. Ten milliliters of purified acetylene was added to each flask to achieve a final concentrations of 10% (10 kPa) in the gas phase. The soil slurries were placed on a rotary shaker for 1 1/2 hours and headspace gas...
was sampled by syringe at 30 and 90 minutes. Gas samples were stored in evacuated 10 ml vacutainer vials until N₂O could be measured by gas chromatography. Nitrous oxide concentrations were measured using a Tremetrics 9001 gas chromatograph with an electron capture detector. Nitrous oxide dissolved in sample water was corrected with the Bunsen relationship, \( M = C_g (V_g + V_l D) \), where \( M \) = total amount of N₂O in water plus gas phase, \( C_g \) = concentration of N₂O in gas phase, \( V_g \) = volume of gas phase, \( V_l \) = volume of liquid phase, and \( D \) = Bunsen absorption coefficient (at 25°C). Denitrification rates are expressed as μg N₂O evolved per g dry soil per day. Because this assay measures denitrification enzyme activity, these values give an indication of denitrifier population size in soils and they also provide an index of denitrification capacity which is strongly related to annual soil denitrification rates (Ambus, 1993; Hanson et al., 1994; Groffman et al., 1996).

**RESULTS**

**Soil Moisture**

Significantly higher percent soil moisture was found in NAT wetlands than in restored (\( P = 0.0097 \))(Fig. 2.3). Soil moisture was also significantly different among seasons, with highest moisture measured in winter and lowest measured in fall (\( P = 0.0001 \)). Soil moisture did not change as a function of distance from the water source (\( P = 0.8845 \)). Results of monthly water table and rainfall measurements are shown for each of the study sites in Figures 2.4 to 2.9. Length of saturation in the upper 15 cm of soils from October 1997 to April 1998 averaged 4, 4, and 3 months for NAT, RWH, and RWOH wetlands, respectively.
2.3. Percent moisture in bottomland hardwood (BLH) wetland soils. NAT = Natural BLH wetland. RWH = Restored BLH wetland with hydrology re-established. RWOH = Restored BLH wetland without hydrology re-established. Standard deviation is denoted by error bars. Treatments with different letters are significantly different at $\alpha = 0.05$. 
2.4. Rainfall and water table levels measured during a two-year period in Brown study wetland (a restored wetland without hydrology re-established).
2.5. Rainfall and water table levels measured during a two-year period in Windham study wetland (a restored wetland without hydrology re-established).
2.6. Rainfall and water table levels measured during a two-year period in Dorsey study wetland (a restored wetland with hydrology re-established).
2.7. Rainfall and water table levels measured during a two-year period in Greenlea study wetland (a restored wetland with hydrology re-established).
2.8. Rainfall and water table levels measured during a two-year period in Canon study wetland.
2.9. Rainfall and water table levels measured during a two-year period in Little Fork study wetland.
Soluble Organic Carbon (SOC)

Mean SOC concentrations were not significantly different among wetland types ($P = 0.4310$), however, they were significantly affected by season ($P = 0.0001$) (Fig. 2.10). Distance from sampling row to water source also significantly affected mean SOC concentrations ($P = 0.0023$) (Table 2.1). Tukey's HSD test revealed that no differences existed 10, 20, 30, or 40 m from the water source, but carbon concentrations in soils sampled 50 m from water source were lower than concentrations in soils in the other four distances ($\alpha = 0.05$).

Table 2.1. Soluble organic carbon concentrations (mg/g soil) in bottomland hardwood (BLH) wetland soils. Distance away from the surface water source is shown in meters (m). NAT = natural BLH wetland. RWH = restored BLH with hydrology re-established. RWOH = restored BLH without hydrology re-established. Concentrations with different letters are significantly different at $\alpha = 0.05$. nd = not determined.

<table>
<thead>
<tr>
<th>Distance</th>
<th>NAT</th>
<th>RWH</th>
<th>RWOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 m</td>
<td>0.24 ± 0.07$^a$</td>
<td>0.20 ± 0.08$^a$</td>
<td>0.18 ± 0.07$^a$</td>
</tr>
<tr>
<td>20 m</td>
<td>0.23 ± 0.10$^a$</td>
<td>0.19 ± 0.06$^a$</td>
<td>0.18 ± 0.06$^a$</td>
</tr>
<tr>
<td>30 m</td>
<td>0.22 ± 0.08$^a$</td>
<td>0.19 ± 0.06$^a$</td>
<td>0.18 ± 0.05$^a$</td>
</tr>
<tr>
<td>40 m</td>
<td>0.22 ± 0.09$^a$</td>
<td>0.19 ± 0.05$^a$</td>
<td>0.19 ± 0.06$^a$</td>
</tr>
<tr>
<td>50 m</td>
<td>0.16 ± 0.07$^b$</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

Denitrification Enzyme Activity (DEA) Assay

Mean DEA was significantly enhanced by addition of substrate and/or energy source to soil sample ($P = 0.0001$)(Fig. 2.11). Treatments D (addition of glucose and $\text{KNO}_3$) and C (addition of glucose) produced the highest soil denitrification while treatment A (no additions) produced the lowest. There were no differences found between treatment D ($\text{NO}_3^-$ + glucose) and treatment C (glucose) using Tukey’s HSD test. Season significantly affected denitrification enzyme activity, with the highest
2.10. Soluble organic carbon concentrations in wetland soils. NAT = natural bottomland hardwood (BLH) wetland. RWH = restored BLH wetland with hydrology re-established. RWOH = restored BLH wetland without hydrology re-established. Standard deviation is denoted by error bars.
2.11. Denitrification enzyme activity measured in wetland soils. NAT = natural bottomland hardwood (BLH) wetland. RWH = restored BLH wetland with hydrology re-established. RWOH = restored BLH wetland without hydrology re-established. Standard deviation is denoted by error bars. Treatments with different letters are significantly different at $\alpha = 0.05$. 

**Diagram Description:**
- The y-axis represents ng N$_2$O-N evolved/g soil/hr.
- The x-axis represents different treatments: No addition-c, KNO3-b, Glucose-a, and KNO3+Glucose-a.
- The graph shows bar plots with error bars for each treatment in different wetland conditions (NAT, RWH, RWOH).
evolution measured in fall for all wetland types and the lowest measured in spring and summer (P = 0.0001) (Fig. 2.12). Denitrification potential was not significantly different among wetland types in summer (P = 0.0649), winter (P = 0.3798), or spring (P = 0.3776), but in fall denitrification was highest in the NAT wetlands and lowest in the RWOH (P = 0.0218). Although NAT soils consistently exhibited the greatest N₂O evolution, regardless of season, and RWOH soils exhibited the lowest N₂O evolution, significant differences were probably not found because of the high sample variability. Distance from the water source did not significantly affect denitrification potential (P = 0.4572).

Stepwise regression conducted between denitrification potential (using denitrification enzyme activity from Treatment D), moisture, and SOC concentrations revealed no linear relationship between variables (α ≤ 0.05). Treatment D was used in this analysis because it is not limiting in amount of available substrate or energy source to denitrifiers. Pearson correlation coefficients for these variables are shown in Table 2.2. There was a significant correlation between SOC and moisture (r = 0.47) and lesser correlations between moisture and N₂O evolution (r = 0.16) and SOC and N₂O evolution (r = 0.06).

Table 2.2. Pearson correlation coefficients for N₂O production from the denitrification enzyme activity (DEA) assay, soluble organic carbon (SOC) concentrations, and soil moisture content. *denotes significance at α = 0.05.

<table>
<thead>
<tr>
<th></th>
<th>N₂O</th>
<th>SOC</th>
<th>Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₂O</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOC</td>
<td>0.06*</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>0.16*</td>
<td>0.47*</td>
<td>1.00</td>
</tr>
</tbody>
</table>

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2.12. Seasonal denitrification enzyme activity measured in wetland soils. Results shown are from treatment D (addition of nitrate and glucose). NAT = natural bottomland hardwood (BLH) wetland. RWH = restored BLH wetland with hydrology re-established. RWOH = restored BLH wetland without hydrology re-established. Standard deviation is denoted by error bars. Treatments with different letters are significantly different at $\alpha = 0.05$. 
DISCUSSION

Soil Moisture

Soil moisture was higher in NAT wetlands than in the other wetland types most likely because these wetlands have not had disturbances in their hydrology as have the restored wetlands. RWH wetlands always had higher soil moisture than the RWOH wetlands, probably because the RWOH wetlands have drainage ditches which prevent the degree of soil moisture present in the RWH wetlands. Drainage ditches in the RWH wetlands have had water control structures placed in them so that these wetlands are flooded for portions of the year to imitate the natural flooding regime. During 1997-98, the NAT and RWH wetland soils were saturated in the upper 15 cm for one month longer than RWOH wetlands soils. Because the only difference in hydrology between the restored wetlands is the establishment of flashboards risers in drainage ditches to allow surface flooding in the RWH wetlands, it appears that surface flooding may prolong length of soil saturation. In addition, soils in NAT and RWH wetlands were classified as clay while soils in RWOH wetlands were classified as silty clay. Because clay soils have an overall smaller pore size than silty clay soils, this may prevent clay soils from drying out as quickly as silty clay soils after a rainfall event.

Soil moisture for all sites was lower in the fall than in any other season, which is typically the period of greatest moisture deficit. Rainfall was measured monthly and, for the 1997-98 sampling period, the highest rainfalls were recorded in January (exceeding a thirty year average rainfall, data not shown). Lowest rainfalls of the sampling year were recorded in
August and September, 1997 and this caused soils to be very dry by October, 1997, when fall samples were collected. Soil moisture was lower in NAT soils than in the RWH soils in fall probably because transpiration by mature trees growing in these study sites caused a drop in the water table.

**Soluble Organic Carbon (SOC) Concentrations**

No differences were seen in SOC concentrations among wetland types and this was not expected. It was assumed that SOC would be greater in the NAT wetlands than in restored because the former wetlands have a thick leaf litter layer on soil surfaces. Restored wetlands were once cultivated and organic matter that accumulated while the area was wetland may have been oxidized when soils were aerated upon drainage for agriculture and during tillage. Cultivation over a long period of time will reduce concentrations of soluble sugars in soils (Boyer and Groffman, 1996). In addition, soluble carbon is mobile and can leach out of the upper soil surface.

Soluble organic carbon concentrations were higher in winter than in any other season in all wetland types. Litterfall, dead herbaceous vegetation, and root biomass may be decomposed in late fall and early winter, thus creating higher concentrations of mineralized carbon when these soils were sampled in January. In addition, as stated previously, the highest rainfall of the year was recorded in January and in the NAT wetlands excessive rainfall may have leached carbon out of the litter layer and into the upper soil surface (Moore, 1997). DeLuca and Keeney (1994) consistently found the greatest SOC concentrations in cultivated soils in Iowa occurred in summer and winter while Dosskey and Bertsch (1997)
found no seasonal patterns in SOC concentrations of forest soils in the southeastern U.S. coastal plain.

Although significant differences were found among SOC concentrations due to distance from water source, no apparent trend was seen. Because there was no difference in soil moisture as soils were sampled further away from the water source, detected differences in SOC concentrations were probably not due to waterlogged conditions.

**Denitrification Enzyme Activity (DEA) Assay**

The highest denitrification activity was measured in soils receiving treatment D probably because this treatment supplied both a substrate \( \text{NO}_3^- \) and an energy source (glucose) for denitrifiers, while the other treatments are limited by substrate (Treatment B), energy source (Treatment C), or both (Treatment A). If wetland soils were limited by either substrate and/or energy source, we would expect denitrification to be highest in treatment D. Because no significant differences were found between treatments C and D, soils of these wetlands may be carbon limited relative to the available soil \( \text{NO}_3^- \) (Beauchamp et al., 1989; Hill, 1996; Luo et al., 1998). Schnabel et al. (1996) measured denitrification in wooded riparian ecosystems and found that it was carbon limited. They hypothesized that the amount or composition of organic matter available to microbes caused the limitation. Verchot et al. (1998) found that carbon additions increased denitrification potentials in forested filter zones. They also measured denitrification rates in these systems and found them to be limited by carbon availability.
There were no significant differences in $\text{N}_2\text{O}$ evolution found among wetland types most likely because of the high sample variation for $\text{N}_2\text{O}$ evolution within each wetland type. Many studies measuring both denitrification rates and potentials have shown high variation in measured $\text{N}_2\text{O}$ evolution (Groffman et al., 1992; Groffman et al., 1996; Parkin, 1987; Verchot et al., 1998). Study wetland soils have a high clay content (NAT = 59%, RWH = 68%, and RWOH = 47%) and the small particle size of clay can create microsites within soil that exhibit extreme variation in oxygen and organic matter concentrations and water content, leading to differences in microbial populations (Christensen et al., 1990; Hill, 1996). In addition, mean $\mu\text{g} \text{N}_2\text{O}/\text{g soil/day}$ shown in Figs. 2.11 & 2.12 are averages of two different wetlands for each type (NAT, RWH, RWOH) and this would also account for some of the variability from the overall mean for each wetland type.

Although results were not significantly different, RWOH wetlands consistently had denitrification potentials which were much lower than the other two wetland types, indicating that these wetlands did not have denitrifying microbial populations as numerous as NAT and RWH soils. Lower values for denitrification potential, and thus smaller numbers of denitrifiers, were probably measured in the RWOH wetlands because these sites, on average, had lower soil moisture throughout the year than NAT and RWH sites. Flooding soil will create conditions conducive to denitrification as oxygen is depleted and redox potentials fall below +300 mV. According to Groffman and Tiedje (1989), soil oxygen concentration is the primary factor controlling denitrification. As mentioned previously, the
NAT and RWH soils are saturated in the upper 15 cm for longer than the RWOH soils. Length of saturation will affect oxygen concentrations and thus microbial activity and composition. Therefore, surface flooding may indirectly affect numbers of denitrifiers because it directly affects length of soil saturation. Soils in NAT and RWH wetlands have a higher clay content than RWOH wetlands and, because clay particles hold water more tightly than other soil particles, soils of NAT and RWH wetlands may have become anoxic, increasing populations of microbes which were able to denitrify.

Another probable reason that denitrification potential was lower in the RWOH wetlands than in the other wetlands is related to the source of surface floodwater. The NAT and RWH wetlands receive direct runoff from agricultural fields. This runoff contains nitrogen, phosphorous, and organic matter which may cause an increase in soil microbial populations. Verchot et al. (1998) found that microbial populations in subsoils of vegetated filter zones were significantly impacted by exposure to agricultural runoff, increasing the number of denitrifiers. Drury et al. (1998) studied the long-term effects of fertilization on clay loam soil and found that it resulted in 35% higher denitrification capacity and 65% higher CO₂ production than in unfertilized soils, indicating that microbial populations were higher with fertilization.

Higher denitrification potentials were measured in fall than in other seasons probably because microbial populations were highest at this time due to an influx of organic matter when trees drop their leaves and herbaceous vegetation dies (Dalva and Moore, 1991). Increases in available carbon will lead to increases in heterotrophic populations in the
upper 15 cm of soils (Tate, 1987). Because fall soil moisture was lowest of all seasons measured, oxidation of organic matter may have been higher than in portions of the year when soils were saturated or flooded. Because denitrifiers are primarily heterotrophs, elevated carbon concentrations may increase numbers of these microbes. Although this hypothesis is not supported by SOC data, it must be emphasized that SOC includes water soluble sugars, amino acids, and fulvic and humic acids (Dalva and Moore, 1991), but does not include other carbon sources that are not water soluble such as cellulose, which is readily utilized by heterotrophs once it has been hydrolyzed into smaller subunits (i.e., cellobiose and glucose) by soil fungi (Wagner and Wolf, 1998). Concentrations of SOC were higher in winter than in other seasons, but lower temperature in January may have inhibited microbial growth. Microbial activity is inhibited by soil moisture lower than 45% and, in fall, percent moisture in all wetland soils was lower than this. However, because soils in the DEA assay are made into a slurry, moisture limitations are relieved.

Correlation between soil moisture and DEA potential was lower than that found by Ambus (1993) \( (r = 0.767) \) and Groffman et al. (1991) \( (r^2 = 0.56) \), however we are looking at a much narrower soil moisture range than Groffman et al. Drury et al. (1991) found a strong correlation between background \( \text{N}_2\text{O} \) production and organic carbon concentration \( (r = 0.901) \), and moisture content \( (r = 0.916) \), however these correlations were not as strong when comparing denitrification potentials and organic carbon concentrations \( (r = 0.154) \) and moisture content \( (r = 0.155) \). Drury et al. attributed these discrepancies to carbon limitations when additional NO\textsubscript{3}^{-}
was added as a soil treatment. Because the DEA assay corrects for soil moisture limitations by adding 25 ml of deionized water solution, this may cause there to be no correlation between moisture and other variables measured.

There has been extensive research investigating denitrification capacity of riparian areas, demonstrating that these areas are capable of providing a buffer zone for removal of NO$_3^-$ from agricultural runoff water (Ambus and Lowrance, 1991; Groffman et al., 1992; Lowrance, 1992; Hanson et al., 1994; Schipper et al., 1994; Maag et al., 1997; Jordan et al., 1998; Verchot et al., 1998). On an annual basis, the NAT, RWH, and RWOH wetlands would remove 8.2, 5.7, and 1.4 g NO$_3^-$N/kg soil/year, respectively. These figures were calculated based on mean results for treatment B in the DEA assay. Treatment B was used for this annual calculation because it measured denitrification activity with added NO$_3^-$, a condition which would occur in late winter and early spring when NO$_3^-$ is present in surface runoff from adjacent cropland. Thus, even though denitrification enzyme activity was lower in the RWOH wetlands than in the other two types, this type of wetland does have the capacity to remove NO$_3^-$ through denitrification. Surface runoff does not flood these restored wetlands, however, it bypasses them because it is confined to drainage ditches. Therefore, because these wetlands are removed from the surface hydrology of the watershed, RWOH wetlands will not provide improvements in water quality in adjacent rivers.
CONCLUSIONS

Our results indicate that restoration of wetland biogeochemical functions is connected to on-site hydrology because denitrification potentials were lower in the RWOH wetlands than in the wetlands which are flooded or saturated in the upper 15 cm of soils for part of the year. The denitrification enzyme assay is a reliable indicator of long-term reducing conditions since expression of the nitrate reductase enzyme is strongly correlated with the need for that enzyme, i.e., anoxic conditions. Since wetlands restored with hydrologic modifications had lower denitrification potentials than the natural wetlands, restoration of this important water quality function is dependent upon more than just hydrology. Further work is necessary to identify the role of land-use practices and carbon quality (among others) as additional factors controlling denitrification in these restored wetlands.

Like many agricultural watersheds in the southeastern U.S., the natural hydrologic regime of the Tensas River Basin has been forever altered by the combination of flood-control levees and drainage ditches. Modification of these drainage ditches is necessary if the goal is to restore water quality functions since sediment- and nutrient-laden runoff simply bypasses the restored wetlands via these ditches. This restoration approach not only emulates the natural cycle of winter and spring flooding with dry fall periods (which maximizes the nitrification/denitrification cycle), but also reconnects the restored wetland to agricultural runoff in the watershed. This linkage is critical if we are to truly restore water quality functions on a watershed scale.
CHAPTER THREE

COMPARISON OF MICROBIAL ACTIVITY AND ORGANIC
MATTER CONCENTRATIONS IN SOILS OF RESTORED AND
NATURAL BOTTOMLAND HARDWOOD WETLANDS

INTRODUCTION

Overview

As the number of wetland mitigation and restoration projects increases, it is important to understand how ecosystem structure affects biogeochemical functions of wetlands. To provide data on how restoration affects biogeochemical functioning, soil characteristics of restored and natural bottomland hardwood (BLH) wetlands were measured and compared. Three types of wetlands were studied: natural BLH wetlands, restored BLH wetlands with hydrology re-established, and restored BLH wetlands without hydrology re-established. Two study wetlands were chosen per wetland type for a total of six study sites. Soil moisture, heterotrophic microbial activity, readily mineralizable carbon, and soluble organic carbon were measured seasonally in the upper 15 cm of soils. There was no difference in soil moisture, readily mineralizable carbon concentrations, or soluble organic carbon concentrations among different wetland types. No significant differences were detected in heterotrophic microbial activity in summer, fall, or winter, but in spring microbial activity was highest in NAT wetlands and lowest in RWOH. Although no significant differences existed in most measured parameters, it is noteworthy that these parameters were all higher in the natural wetlands and those restored with hydrology re-established than in those restored without hydrology re-established, regardless of season. These results suggest that
restoration of hydrology is important when the goal of a restoration project is to restore a BLH wetland with biogeochemical functions similar to those of a natural BLH wetland.

**Background**

Biogeochemical processes describe the transformation of nutrients or energy between biotic and abiotic compartments of an ecosystem (Schlesinger, 1991). Biogeochemical functions of wetlands include decomposition and export of organic carbon to downstream ecosystems, phosphorous uptake and sorption, nitrification, and denitrification (Kellison and Young, 1997). The majority of biogeochemical functions are microbially mediated and, therefore, specific rates will be governed by factors affecting microbial growth. The primary factors which affect growth, activity, and speciation of heterotrophic soil microorganisms are quality and quantity of organic matter, oxygen concentrations, moisture, pH, temperature, and nutrient concentrations (Killham, 1994; Mesquita et al., 1998).

Heterotrophic microorganisms are critical to soil nutrient cycling and decomposition of organic matter. Quality of organic matter (i.e., how readily utilizable it is by microbes) and quantity available will affect microbial population size and activity in a soil (Gorres et al., 1998). Organic carbon comes in many forms depending on the source (e.g., plant species composition) and age of the organic matter (Wagner and Wolf, 1998). Thus, vegetative growth stage and species present in a bottomland hardwood wetland will affect microbial activity because these factors affect amount and type of organic matter that accumulates on soil surfaces.
Because quality and quantity of organic matter in and on top of soil will affect microbial populations, these factors will also influence biogeochemical functions (Killham, 1994; Groffman et al., 1996).

Ecosystem disturbances such as deforestation and conversion to cropland will also affect soil organic matter concentrations and there have been numerous studies documenting these findings (DeLuca and Keeney, 1994; Boyer and Groffman, 1996; Gorres et al., 1998). For example, converting a forest to agricultural land will generally result in a marked decline in total and soluble organic carbon concentrations (Boyer and Groffman, 1996). Tate (1987) reported a 57% loss of soil organic matter in a variety of forest soils in Georgia upon conversion to cropland. Cultivation has also been demonstrated to reduce microbial biomass carbon present in soils (DeLuca and Keeney, 1994). In addition, draining a wetland for agricultural purposes will reduce organic matter concentrations because aerobic degradation of organic substrates is faster than anaerobic degradation (Patrick et al., 1985). When an ecosystem is converted to cropland, fundamental characteristics of that system are removed. As a result of vegetation clearance and soil tillage, soil characteristics will undergo changes in carbon and microbial dynamics, which affect biogeochemical functions of that ecosystem. Wetland restoration and creation projects involve major changes in ecosystem structure so it is reasonable to assume that biogeochemical functions will be altered or different from those of the original wetland.

One important biogeochemical function of wetlands is denitrification. This microbially mediated process is important for removing $\text{NO}_3^-$ from
surface and subsurface waters, thereby maintaining water quality of nearby rivers, streams, or estuaries. Factors which affect denitrification are the same as those which affect microbial activity (e.g., organic matter type and quality, oxygen concentrations, and nutrient availability). There are numerous studies measuring denitrification in riparian wetlands (Lowrance, 1992; Merrill and Zak, 1992; Groffman et al., 1996; Hill 1996; Maag et al., 1997; Cey et al., 1999), however no studies have compared organic matter concentrations and soil heterotrophic microbial activity in restored and natural BLH wetlands. Because denitrification is important in maintaining water quality of rivers and streams, it is essential to understand how to maximize soil characteristics which affect this process when restoring or creating a wetland.

In an attempt to improve water quality and restore wildlife habitat, marginal agricultural croplands are being restored back to BLH wetlands in the lower Mississippi River Alluvial Valley (Llewellyn et al., 1995). Of the original 10 million hectares of bottomland forests in this region, 80% were cleared by 1978 (Sifneos et al., 1992). Loss of these wetlands creates water quality problems because buffer zones between agricultural lands and receiving water bodies are eliminated. Bottomland hardwood wetlands remove sediments and nutrients from runoff water through deposition, denitrification, nutrient uptake, and adsorption/fixation reactions (Messina and Conner, 1998). At each restoration site, hardwood trees are replanted and, in some, hydrology is re-established as well. What needs to be examined is if biogeochemical functions are being restored when these structural features are restored because it is essential to system
sustainability to determine how ecosystems are affected by restoration
methods.

To determine how restoration of vegetation and hydrology affected
soil characteristics of wetlands, we measured specific features of restored
and natural wetland soils which affect biogeochemical functions. The
objectives of this research were (i) to measure heterotrophic microbial
activity, readily mineralizable carbon, and soluble organic carbon
concentrations in soils of natural and restored BLH wetlands; and (ii) to
determine if relationships exist between these soil variables and previously
measured denitrification potentials.

MATERIALS AND METHODS

Site Description

Study wetlands were located in the Tensas River Basin in
northeastern Louisiana, an area in which agriculture is the primary land
use (USDA-NRCS, 1995). The 291,500 hectare Tensas River Basin was
once over 90% bottomland hardwood forests. Conversion of about 85% of
these forests to cropland resulted in a decrease in water quality of the
Tensas River, caused by runoff of sediments and nutrients from adjacent
agricultural fields (USDA-NRCS, 1995). To try to improve water quality of
this river, and to provide wildlife habitat and flood storage, agricultural
croplands are being restored back to bottomland hardwood wetlands.

Three types of BLH wetlands were chosen for study: natural mature
(NAT) wetlands, wetlands restored with hydrology (RWH), and wetlands
restored without hydrology (RWOH). Restored wetlands were once natural
BLH wetlands which were converted to agricultural fields by clearing
vegetation and digging drainage ditches around the wetlands. Both RWH and RWOH sites were replanted with appropriate bottomland hardwood species (Quercus lyrata, Q. nigra, Q. phellos, Acer rubrum). Hydrologic restoration of the RWH sites consisted of a flashboard riser in the drainage ditch adjacent to the site. The height of water in the ditch can be controlled by the riser to allow surface runoff from the adjacent agricultural fields to inundate the sites. This simulates the hydrologic regime of the natural BLH wetlands in agricultural watersheds. The RWOH sites did not have a flashboard riser system and agricultural surface runoff simply bypassed the site via existing drainage ditches. Study wetlands were chosen for their proximity to agricultural fields as well as for their similarities in soil type, slope, and topography. Two replicates of each type of wetland were chosen. The RWH wetlands were restored in 1990 and the RWOH wetlands were restored in 1993 and 1994. Textural classes were clay for NAT and RWH soils and silty clay for RWOH wetland soils.

Soils in the Tensas Basin are alluvial soils of clay and fine silt. Approximately 60% of these soils are classified as hydric, including the following series: Alligator, Sharkey, and Tunica. The study wetlands chosen for this research contain primarily Sharkey and Sharkey-Tunica soil associations. Sharkey map unit consists of poorly drained clayey soils with slopes of generally 0-3%. Sharkey-Tunica map unit consists of poorly drained and somewhat poorly drained clayey soils with slopes of 0-3%. Sharkey soils, occurring in swales, and Tunica soils, occurring on low ridges, make up 58 and 26% of this map unit, respectively (USDA-NRCS, 1995).
Experimental Design

A 20- x 30-m grid system was established in each of the study wetlands (Fig. 2.2). The grid consisted of three columns 10 m apart and four rows 10 m apart. The exception to this grid system was one of the natural wetlands (Little Fork). This wetland contained a 15- x 40-m grid system in which rows were 10 m apart and columns were five meters apart, due to the shape of the wetland. The system was designed so that rows were horizontal to the water source. In restored wetlands, the water source is overflow from drainage ditches. All wetlands were chosen for their proximity to cropland so that surface flow to the wetlands included runoff from adjacent fields. One 90-cm groundwater well was installed every 10 meters in the middle column.

Statistical Analysis

Data were analyzed using the Proc Mixed procedure of SAS® Institute (1994). Wetland type, distance from the water source, and season were analyzed as main effects. Main effects were considered significant if they had a P < 0.05. Differences among means within each main effect and within interaction effects were evaluated using Tukey's Studentized Range (HSD) test (α = 0.05). Relationships between dependent and independent variables were examined using a linear regression analysis and correlations among variables were conducted using Pearson's correlation analysis.

Collection of Soil Samples

Twelve soil samples were collected from the upper 15 cm at each study site using a 5-cm diameter soil auger. Soils were collected in August.
and November, 1998 and February and April, 1999. After collection, the soils were stored on ice until they were brought to the Wetland Biogeochemistry Institute and refrigerated (4°C). Each soil sample was homogenized by passing through a mesh screen with 1.25 cm² holes. Percent soil moisture of each sample was measured gravimetrically by drying for 24 hours at 105°C.

**Fluorescein Diacetate Hydrolysis (FDA)**

The FDA assay (Reed et al., 1989) was used to measure heterotrophic microbial activity. Most studies measuring microbial populations in soils measure microbial biomass carbon or utilize direct counts or plate counts. These are not adequate methods when describing microbial community structure because they do not provide information about physiological differences of these microbes (Borga et al., 1994). The theory behind the FDA method is that heterotrophic microorganisms hydrolyze fluorescein diacetate, producing fluorescein. Upon death of the cells with the addition of acetate, fluorescein can be quantified spectrophotometrically at 490 nm. The amount of absorbance of fluorescein is indicative of the hydrolytic activity of the soil's heterotrophic microbial population (Reed et al., 1989). The FDA assay does not quantify microbial biomass, but it is a simple and sensitive method to be used for comparing microbial activity in similar soil ecosystems (Schnurer and Rosswall, 1982).

An FDA stock solution was made by dissolving 0.200 g fluorescein diacetate in acetone and bringing the volume to 100 ml with deionized water. Ten grams of soil from each sample was weighed and placed in a
250 ml plastic Nalgene® bottle. Fifty milliliters phosphate buffer (pH 7.6) and 0.5 ml FDA stock solution was added to each bottle. The bottles were capped and placed on a rotary shaker (200 rpm) for one hour. After exactly one hour, 50 ml acetone was added. Each solution was swirled by hand and 40 ml decanted into a centrifuge tube. The solutions were centrifuged at 6000 rpm using a DuPont Instruments Sorvall® SA-600 rotor for 10 minutes. Absorbance values were read at 490 nm with a Hach DR/2000 direct reading spectrophotometer. Absorbance values were converted to μg fluorescein produced/g soil/hour by using a standard absorbance curve created for each set of soils.

An FDA standard solution was made by dissolving 0.0399 g fluorescein in acetone and bringing the volume to 100 ml. Standards were made by adding 50 ml phosphate buffer, 50 ml acetone, and 10 g of soil from the wetland site being analyzed to each of six flasks, and then adding 0, 0.1, 0.2, 0.3, 0.5, 1.0, and 1.5 ml of fluorescein standard to the flasks. The resulting solutions contained the equivalent of 0, 50, 100, 150, 250, 500, and 750 μg FDA converted to fluorescein/flask. Standards were incubated on a rotary shaker (200 rpm) for one hour and then absorbance values were read at 490 nm. The absorbance values were plotted to obtain a calibration curve and a 1st-order regression equation was obtained for converting samples to μg fluorescein produced/g sample/hour.

**Readily Mineralizable Carbon (RMC)**

Five grams of field-moist soil was weighed into a 40-ml incubation flask and stoppered with a serum stopper. The samples were incubated in
the dark for seven days at 25°C and then 10 ml gas samples were collected by syringe and stored in vacutainer vials until analysis. Gas samples were analyzed for CO₂ using a Tremetrics 9001 gas chromatograph with a flame ionization detector and a methanizer to convert CO₂ to CH₄ by catalytic reduction (Davidson et al., 1987). The production of CO₂ using this method has been shown to be linear up to seven days (Burford and Bremner, 1975; Davidson et al., 1987)

**Soluble Organic Carbon**

One hundred milliliters of deionized water was added to 10 g field moist soil from each sample and the solution was shaken at high speed for 30 minutes and allowed to stand for approximately 18 hours (overnight) (Kaiser and Zech, 1996). The solution was then shaken by hand and 40 ml was poured into a centrifuge tube and centrifuged at 6500 rpm using a DuPont Instruments Sorvall® SA-600 rotor for 10 minutes at 25°C. Twenty milliliters of the supernatant was filtered through a 0.45 μm polysulfone membrane filter into a scintillation vial and refrigerated at 4°C prior to analysis. Samples were analyzed for nonpurgable organic carbon using a Shimadzu TOC-5000A Total Organic Carbon (TOC) analyzer. Nonpurgable organic carbon concentration in each sample was measured by acidifying the sample with 40 μl of HCl and then purging for eight minutes with TOC grade compressed air. Acidification reduces inorganic carbon to primarily CO₂ in these samples and purging volatilizes CO₂ out of solution. Samples were then analyzed for organic carbon concentrations. Results were given as the mean of three replicates per sample. Results
were also corrected for soil moisture so that final results were expressed as mg SOC/g soil on a dry weight basis.

RESULTS AND DISCUSSION

Soil Moisture

Percent soil moisture was not significantly different among the three wetland types ($P = 0.1061$) (Fig. 3.1), but was affected by season, with the highest moisture content measured in winter and the lowest measured in summer ($P = 0.0001$). This data corresponds to rainfall data in Figures 2.4 to 2.9 where, during a two-year period, the highest rainfalls occurred in winter months and the lowest rainfalls occurred in summer. Soil moisture was not significantly affected by distance from the surface water source ($P = 0.1223$).

There were no differences in soil moisture due to wetland type and this was not expected because it was assumed that those wetlands with winter flooding regimes would have higher soil moistures than those that do not flood. In summer and fall, the two seasons when rainfall is lower and soils are drier than winter and spring, soil moisture was similar among the different wetlands. Water table data shows that in all wetlands the water table was 90 cm below soil surfaces from May to late October, causing soil moistures to be low in all wetlands (Figures 2.4 to 2.9). However, during winter and spring when there were much higher rainfalls than in summer and fall, exceeding the 30-year average rainfall in December, January, and February, there was a much greater difference.
3.1. Percent soil moisture measured in study wetlands during 1998-99. NAT = natural bottomland hardwood (BLH) wetland. RWH = restored BLH wetland with hydrology re-established. RWOH = restored BLH wetland without hydrology re-established. Standard deviation is denoted by error bars.
among wetland soil moistures. Because a normal hydrologic regime of winter flooding is present in NAT and RWH wetlands, these soils are inundated in the upper 15 cm from approximately December to March or April. The RWOH wetlands are never flooded with surface water because of the drainage ditches, although soils in the upper 15 cm are saturated for an average of two months out of the year because the water table is at or right below the soil surface. Average period of soil saturation within the upper 15-cm of soil surfaces was two, 4.25, and four months in RWOH, RWH, and NAT wetlands, respectively. Because the only difference in hydrology between the RWOH wetlands and the others is surface flooding, it appears that surface flooding leads to prolonged soil saturation in RWH and NAT wetlands. Longer periods of soil saturation may influence other soil characteristics such as carbon and oxygen concentrations and microbial activity.

**Fluorescein Diacetate Hydrolysis (FDA) Assay**

Heterotrophic microbial activity was not significantly different among wetland types during summer (P = 0.2310), fall (P = 0.0991), or winter (P = 0.1557), but in spring microbial activity was highest in NAT wetlands and lowest in RWOH (P = 0.0141)(Fig. 3.2). Season in which samples were collected significantly affected microbial activity in wetland soils (P = 0.0001), with activity increasing from summer throughout the year to spring. Distance from the water source also significantly affected microbial activity (P = 0.0196), with microbial activity higher near the surface water source in NAT and RWH wetlands than 40 m away while the opposite pattern was observed in RWOH wetlands (Table 3.1).
3.2. Heterotrophic microbial activity (measured as μg fluorescein produced/g soil/hour) in wetland soils. NAT = natural bottomland hardwood (BLH) wetland. RWH = restored BLH wetland with hydrology re-established. RWOH = restored BLH wetland without hydrology re-established. Standard deviation is denoted by error bars. Treatments with different letters are significantly different at $\alpha = 0.05$. 
Table 3.1. Heterotrophic microbial activity (measured as μg fluorescein produced/g soil/hour) in bottomland hardwood (BLH) wetland soils. NAT = natural BLH wetland. RWH = BLH wetland with hydrology re-established. RWOH = BLH wetland without hydrology re-established. Distance from the water source is shown in meters (m). nd = not determined. Activities with different letters are significantly different at α = 0.05.

<table>
<thead>
<tr>
<th>Distance (m)</th>
<th>NAT</th>
<th>RWH</th>
<th>RWOH</th>
</tr>
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<tbody>
<tr>
<td>10</td>
<td>80.7 ± 29.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.0 ± 22.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.4 ± 16.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>88.3 ± 35.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.6 ± 15.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.8 ± 11.7&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>77.0 ± 27.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.7 ± 13.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.7 ± 13.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>40</td>
<td>68.5 ± 23.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.2 ± 20.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>46.9 ± 13.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>72.4 ± 20.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>nd</td>
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</table>

Although no statistical differences existed between microbial activity among wetland types except in the spring, it is ecologically significant that the highest microbial activity was consistently measured in NAT soils and the lowest in RWOH soils, regardless of season. Microbial activity was probably higher in NAT wetlands than restored because these wetlands have a thick leaf litter layer on top of the soil. Continual renewal and decomposition of this organic material provides an energy source for heterotrophic microbes and therefore presence of a litter layer will affect population size and activity (Schnurer and Rosswall, 1982; Davidson and Swank, 1987; Smolander et al., 1994). Microbial activity may also have been higher in NAT and RWH wetlands because, as discussed previously, soils were inundated in the upper 15 cm for twice as long as RWOH soils and microbes in RWOH soils may have been limited by soil moisture. Less than 45% moisture is limiting to microbial growth (Stratton et al., 1995) and percent moisture in the RWOH wetlands was less than this in every season sampled.
Microbial activity was lower in summer and fall than in spring and winter, corresponding to times when soil moisture was also lower. In summer and fall, soil moisture was less than 45% in all wetland types. Reduction in soil moisture will affect microbes by limiting movement through pore water and increasing both matric tension and osmotic tension which stresses the cell by limiting water availability and affecting internal water potential (Metting, Jr., 1993; Killham, 1994). When microorganisms are stressed, less energy can be applied to metabolism and must be reappropriated to cell maintenance (Killham, 1994).

Microbial activity declined in the NAT and RWH soils as the distance from the water source increased and this would suggest that moisture limitations may have decreased microbial activity. However, because no differences were seen in soil moisture, differences in microbial activity may be attributable to RMC concentrations. As will be discussed, in RWH soils RMC concentrations were higher near the surface water source that further away (30 - 40 m distance). Because anaerobic decomposition of organic matter is much slower than aerobic, soils nearer the surface water source may remain flooded longer thereby having higher organic matter concentrations and supporting larger populations of microbes.

From July 1997 to May 1998 we studied seasonal denitrification potentials in these wetland soils using the DEA assay of Tiedje (1982)(Hunter and Faulkner, In review). Because denitrification is an important function of wetland soils, quantification of its potential may provide a measure of functional restoration of these ecosystems. Although not significantly different, the highest denitrification potentials were
measured in natural wetlands while the lowest were measured in those restored without hydrology re-established, regardless of season. Because the primary factors which affect denitrification are substrate (NO$_3^-$), amount and type of organic matter available for heterotrophic denitrifiers, soil moisture, and oxygen concentrations (Drury et al., 1991; Ambus, 1993; Drury et al., 1998), the most probable reason for differences in denitrification is due to changes in soil characteristics upon conversion to cropland and subsequent restoration. Changes in these ecosystems due to clearing, draining, cropping and restoration probably altered factors which affect microbial soil ecology and, thus, altered biogeochemical functions from their original capacity. Measurements of microbial activity follow the same pattern as measurements of denitrification potentials in these soils. Patterns of microbial activity corresponds to DEA potentials, which would be expected because the predominate number of denitrifiers are heterotrophic (Myrold, 1998).

**Readily Mineralizable Carbon (RMC)**

Soil RMC concentrations were not significantly different among wetland types (P = 0.1449), however concentrations in soils of NAT and RWH wetlands were higher than RWOH wetlands in every season (Fig. 3.3). Readily mineralizable carbon concentrations were highest in spring and lowest in summer of all four seasons sampled (P = 0.0001). Mineralizable carbon concentrations in soil samples were also significantly affected by distance from the water source in RWH wetlands (P = 0.0087) (Table 3.2). Soil RMC concentrations in RWH wetlands declined as distance from the surface water source increased, however values
3.3. Readily mineralizable carbon concentrations in wetland soils. NAT = natural bottomland hardwood (BLH) wetland. RWH = restored BLH wetland with hydrology re-established. RWOH = restored BLH wetland without hydrology re-established. Standard deviation is denoted by error bars.
remained fairly constant in the NAT and RWOH soils, regardless of distance from the water source.

Table 3.2. Readily mineralizable carbon concentrations (measured as ug CO₂ evolved/g soil/day) in bottomland hardwood (BLH) wetland soils. NAT = natural BLH wetland. RWH = BLH wetland with hydrology re-established. RWOH = BLH wetland without hydrology re-established. Distance from the water source is shown in meters (m). nd = not determined. Values with different letters are significantly different at α = 0.05.

<table>
<thead>
<tr>
<th>Distance (m)</th>
<th>NAT</th>
<th>RWH</th>
<th>RWOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 m</td>
<td>50.0 ± 30.9a</td>
<td>57.6 ± 22.5a</td>
<td>27.7 ± 10.7a</td>
</tr>
<tr>
<td>20 m</td>
<td>62.2 ± 39.7a</td>
<td>50.2 ± 25.7ab</td>
<td>31.9 ± 13.1a</td>
</tr>
<tr>
<td>30 m</td>
<td>56.8 ± 30.6a</td>
<td>42.3 ± 16.8b</td>
<td>32.8 ± 18.7a</td>
</tr>
<tr>
<td>40 m</td>
<td>43.6 ± 22.3a</td>
<td>43.2 ± 19.3b</td>
<td>35.0 ± 15.8a</td>
</tr>
<tr>
<td>50 m</td>
<td>41.8 ± 19.9a</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

Although these results were not statistically significant, higher RMC concentrations were consistently measured in NAT wetland soils than in RWOH wetland soils. Soil RMC concentrations were lower in restored wetlands than in the NAT wetlands most likely because the former wetlands were drained and used for agriculture (Tate, 1987; DeLuca and Keeney, 1994; Boyer and Groffman, 1996). Readily mineralizable carbon concentrations in the RWOH wetlands were lower than those in the RWH soils probably because the RWOH wetlands have remained drained while the RWH flood seasonally and organic matter decomposition is slower in flooded soils than in drained soils (Lockaby et al., 1996). Surface flooding from upstream ecosystems will also bring in organic matter, sediments, and nutrients which will settle onto soil surfaces if water remains standing for a sufficient period of time. In addition, as mentioned previously, soils of RWH wetlands are saturated for 4.25 months in the upper 15 cm, while RWOH
soils are only saturated in this area for two months of the year and length of saturated will influence organic matter decomposition.

RMC concentrations were highest in spring and lowest in summer, following the same trend as microbial activity and soil moisture. Concentrations may be higher in spring and winter than in summer and fall because leaf litter that was deposited in fall was being broken down. In summer and fall, moisture limitations may reduce microbial activity and decomposition of organic matter.

**Soluble Organic Carbon (SOC)**

Wetland type did not affect soil SOC concentrations (P = 0.0630), however season was significant (P 0.0001), with the highest concentrations measured in winter and the lowest measured in summer (Fig. 3.4). Concentrations of SOC in soil samples were not significantly different when analyzed by distance from the water source (P = 0.5389). Although no trend was seen in SOC seasonal measurements, concentrations were generally higher in NAT wetlands than in the restored. This is most likely due to the same reasons mentioned for differences in RMC, namely oxidation of organic matter during cropping, absence of surface flooding in the RWOH wetlands, and length of soil saturation.

Soluble organic carbon is that portion of soil organic matter that is water soluble and is comprised of sugars, amino acids, and fulvic and humic acids (Dalva and Moore, 1991). Soluble organic carbon usually consists of the highest percentage of organic matter that is readily mineralized by microbes (DeLuca and Keeney, 1994) and production of
3.4. Soluble organic carbon concentrations in wetland soils. NAT = natural bottomland hardwood (BLH) wetland. RWH = restored BLH wetland with hydrology re-established. RWOH = restored BLH wetland without hydrology re-established. Standard deviation is denoted by error bars.
SOC is dependent upon microbial populations in the soil (Christ and David, 1996). Christ and David (1996) therefore hypothesized that production of SOC from organic matter is controlled by the same factors that control biological activity, namely soil oxygen concentrations, soil moisture, and temperature. Concentrations of SOC can also increase as precipitation passes through canopy (throughfall) and down tree trunks and herbaceous vegetation (stemflow) (Dalva and Moore, 1991). Soluble organic carbon concentrations may not directly correlate to RMC concentrations because not all carbon classified as RMC, such as cellulose, is water soluble.

Soluble organic carbon concentrations in natural wetlands could increase in the upper soil surface as soluble carbon is leached out of the litter layer. Along with SOC dissolved in throughfall and stemflow water, this is most likely why there was a peak in SOC concentrations in NAT wetlands in the winter sampling period, when rainfall was the highest of the year. No peaks were seen in the restored wetlands and this is probably because they do not have a leaf litter layer on soil surfaces.

**Relationships Between Soil Characteristics**

Table 3.3 shows correlation relationships between measured soil variables. There was a significant correlation between microbial activity and RMC and between microbial activity and SOC. There was also a strong correlation between soil moisture and FDA, RMC, and SOC. Weak correlations were detected between denitrification potentials and soil characteristics.
Table 3.3. Pearson correlation coefficients for denitrification potential (DEA), heterotrophic microbial activity (FDA), readily mineralizable carbon (RMC), soil moisture, and soluble organic carbon (SOC) of restored and natural bottomland hardwood wetland soils. *denotes significant at $\alpha = 0.05$

<table>
<thead>
<tr>
<th></th>
<th>FDA</th>
<th>RMC</th>
<th>Moisture</th>
<th>SOC</th>
<th>DEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDA</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMC</td>
<td>0.73*</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>0.67*</td>
<td>0.67*</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOC</td>
<td>0.48*</td>
<td>0.41*</td>
<td>0.68*</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>DEA</td>
<td>0.41*</td>
<td>0.22*</td>
<td>0.33*</td>
<td>0.21*</td>
<td>1.00</td>
</tr>
</tbody>
</table>

The strong correlation between FDA and RMC illustrates that higher microbial activity is, in part, due to higher soil carbon concentrations. The correlation between SOC and FDA was not as high and this may be because SOC was leached out of the upper 15 cm or because not all of the SOC measured is available to microorganisms. Some SOC may be bound to clay particles and not available in pore water, but during the assay to measure concentrations it becomes solubilized (Jardine et al., 1989). Soil moisture was strongly correlated with FDA, RMC, and SOC and these results are important when considering the effects of hydrology and saturation on carbon pools and microbial activity in soils. The variables measured had a low correlation with denitrification potential. This is most likely because the high standard deviation in $N_2O$ samples prohibited any type of relationship from being detected.

**IMPLICATIONS FOR BOTTOMLAND HARDWOOD WETLAND RESTORATION**

Of the restored wetlands studied, those with hydrologic modifications displayed soil characteristics most similar to natural wetlands and these results suggest that the method of restoration will affect biogeochemical
functioning of these wetlands. Clearly, there are differences in microbial populations and organic matter concentrations in soils of our study wetlands. It is reasonable that changes in hydrology, the driving force of any wetland, are creating these differences.

Relationships between hydrology, biotic, and abiotic characteristics are closely tied together because soil moisture affects oxygen concentrations which, in turn, affects numbers of aerobes, facultative anaerobes, and anaerobes present in the soil. Size of heterotrophic microbial populations determines degradation of organic matter and, because decomposition of organic matter will release nutrients needed by plants, vegetation growth and composition will be affected as well. Because these processes are closely tied together, further studies need to be conducted to clearly identify what is happening in agricultural areas restored to forested wetlands. Studies over time should also be conducted to learn how restored wetlands change as a function of ecological succession.

Although the restored wetlands without hydrology re-established do not flood with surface water, groundwater continues to create saturated soil conditions in the winter months. Because soils are saturated in the upper 15 cm longer in NAT and RWH wetlands than in RWOH, it is clear that the hydrology of these wetlands is an interaction between groundwater and surface flooding. This is also an important consideration when restoring BLH wetlands, or any wetland. Because the wetlands will recharge groundwater during rainfall events, they are valuable in maintaining groundwater quality as well as surface water quality.
When feasible, attempts should be made to re-establish the natural hydrologic regime of winter flooding to areas being restored back to BLH wetlands. Restoring surface flooding will extend the period of time that soils are saturated, possibly creating a soil environment where facultative anaerobic microorganisms (such as those responsible for denitrification) will thrive. When \( \text{NO}_3^- \) runoff occurs in late winter and early spring, wetlands with higher soil populations of denitrifying microbes may remove more \( \text{NO}_3^- \) than those with soils that are not saturated as long. Wetlands are valuable ecosystems within watersheds which can provide buffer zones between uplands and receiving water bodies. Therefore, during restoration, every attempt should be made to create a functioning, self-sustaining ecosystem.
CHAPTER FOUR
MEASUREMENT OF CARBON, NITROGEN, AND HETEROTROPHIC MICROBIAL ACTIVITY AT DIFFERENT DEPTHS IN WETLAND SOILS

INTRODUCTION

Overview

Microorganisms are primarily responsible for plant nutrient recycling within ecosystems through decomposition and nutrient transformations. Thus, factors which affect microbial growth and activity will affect biogeochemical functions of an ecosystem. One important factor affecting microbial growth is type and quality of soil organic matter. We measured litter mass on soil surfaces, total carbon and nitrogen, nitrate, heterotrophic microbial activity, and moisture at three depths (0 - 5, 5 - 10, and 10 - 15 cm) in soils of six riparian wetlands located adjacent to agricultural cropland. Two of the wetlands were natural, mature bottomland hardwood forests, two were restored by replanting vegetation and re-establishing hydrology, and two were restored only by replanting vegetation. The amount of litter on soil surfaces in natural wetlands was much greater than on restored soils. Total carbon and nitrogen concentrations changed with depth, with higher concentrations in the 0- to 5-cm depth than in deeper soils. There was no change in soil nitrate concentrations with increasing distance from the source, i.e., agricultural runoff water. Percent soil moisture was greater in the 10 - 15 cm depth than in the 0 - 5 cm depth, but was not different due to wetland type. Although microbial activity and carbon, nitrogen, and nitrate concentrations were not significantly different among wetland types, these parameters were consistently higher in the
natural wetlands than in restored, suggesting that presence of a litter layer is affecting soil nutrient dynamics and microbial populations.

**Background**

Riparian wetlands, such as bottomland hardwood (BLH) forests, play an important role in maintaining water quality of streams and rivers by intercepting nutrients and sediments from surface water runoff. In particular, research has shown the usefulness of wetlands located between agricultural fields and receiving water bodies in removing nitrate (NO$_3^-$), phosphorous, and suspended sediments which contribute to eutrophication and declines in water quality (Jordan et al., 1993; Walbridge, 1993, Gilliam, 1994). One way NO$_3^-$ is removed from surface and subsurface waters is through denitrification, a microbial process which occurs in anoxic wetlands soils. The ability of riparian wetlands to denitrify influent NO$_3^-$ in surface and subsurface water has been extensively researched (Ambus and Lowrance, 1991; Hill, 1996; Groffman et al., 1998; Martin et al., 1999). In particular, the use of riparian wetlands for removal of NO$_3^-$ from agricultural runoff has been studied because these wetlands usually occupy a lower landscape position in watersheds than adjacent cropland and therefore surface water runs off cropland and through these wetlands before reaching a receiving water body (Verchot et al., 1997; Ashby et al., 1998; Verchot et al., 1998).

In the southern United States BLH wetlands are the primary riparian wetland type. Historically, over 21 million hectares existed but over 75% of these have been lost, with the main reason being conversion to agricultural cropland (Mitsch and Gosselink, 1993). This is particularly disturbing
because it results in the loss of an ecosystem with beneficial functions, both in water quality and wildlife habitat, and replaces it with a land use that is the primary contributor to impaired water quality. Water quality has declined particularly in the Tensas River Basin in northeastern Louisiana, the area where this research was undertaken and in which agriculture is the primary land use (USDA-NRCS, 1995). This 291,500 hectare river basin was once over 90% BLH forests. Conversion of about 85% of these forests to cropland increased sediment and nutrient runoff from adjacent agricultural fields into the Tensas River (USDA-NRCS, 1995). To try to improve water quality of this river, and to provide wildlife habitat and flood storage, agricultural croplands are being converted back to BLH wetlands.

Because over 20,250 hectares of BLH wetlands have been restored in the Tensas River Basin, there is a need to evaluate restoration techniques to determine if functions are being restored when structure is re-established. One important area to be studied is how restoration affects soil microbial population size and activity and, subsequently, biogeochemical functions (Lowrance et al., 1995; McLatchey and Reddy, 1998). If wetlands are restored to improve water quality and biogeochemical functions are not restored, then the wetland won’t be improving water quality. In many projects it is assumed that once vegetation and hydrology are restored specific functions will follow, while in reality these functions may never be restored or they evolve slowly as the ecosystem matures (Simenstad and Thom, 1996).

The wetlands in this study included two mature BLH wetlands and two types of restored BLH wetlands. Of the four restored wetlands, all have
been replanted with BLH vegetation and two have had hydrology restored as well, while two have not. In previous research, denitrification, microbial activity, soluble organic carbon, readily mineralizable carbon, and moisture were measured in soils of these wetlands. Although all parameters were not significantly different among wetland types, the natural wetlands had the highest values while those wetlands restored without hydrology re-established had the lowest in all parameters measured. It was assumed that hydrologic regime in these systems was affecting biogeochemical functions because the restored wetlands which are flooded annually were most similar in soil characteristics measured to the natural wetlands.

An obvious difference in soil structure among these study wetlands, and one affected by hydrology, is that the natural BLH wetlands have a large amount of accumulated organic matter on soil surfaces while the restored wetlands soils do not. We hypothesized that this litter layer was supplying organic matter to microbes, causing microbial growth and activity in the NAT soils to be higher than those of restored soils. This research was therefore initiated to determine if soil characteristics which would affect microbial activity were higher in NAT soils than in restored and also higher in the 0- to 5-cm soils than in 5- to 15-cm soils. The primary objectives of this experiment were i) to measure organic matter on soil surfaces in six wetland ecosystems; ii) to measure carbon and nitrogen concentrations and microbial activity in soil samples collected at different depths; and iii) to determine how NO$_3^-$ concentrations changed as soils were sampled further away from the influent NO$_3^-$ source.
MATERIALS AND METHODS

Site Description

Three types of BLH wetlands in the Tensas River Basin were chosen for study: natural mature (NAT) wetlands, wetlands restored with hydrology re-established (RWH), and wetlands restored without hydrology re-established (RWOH). Restored wetlands were once natural BLH wetlands that were converted to agricultural fields by clearing vegetation and digging drainage ditches around the wetlands. Upon restoration, RWH and RWOH sites were replanted with appropriate BLH species (*Quercus lyrata, Q. nigra, Q. phellos, Acer rubrum*). Vegetation on the natural wetlands are primarily 70+ year-old trees, along with herbaceous understory. The RWH sites have many trees approximately four meters tall, along with herbaceous vegetation that is primarily *Sesbania* sp. The RWOH sites have thick growths of goldenrod and several trees less than 2.5 m tall. Hydrologic restoration of the RWH sites consisted of installing a flashboard riser in drainage ditches adjacent to the sites. The height of the water in these ditches can be controlled by the risers to allow surface runoff from the adjacent agricultural fields to inundate the sites, simulating the natural winter flooding hydrologic regime. The RWOH sites did not have a flashboard riser system installed and agricultural surface runoff simply bypassed the sites via drainage ditches, eventually reaching the Tensas River and, therefore, these restored areas were never flooded. The RWH sites were restored in 1990 and the two RWOH sites were restored in 1993 and 1994. Study wetlands were chosen for their proximity to agricultural fields as well as for their similarities in soil type, slope, and topography.
Two replicates sites for each type of wetland were chosen. Textural classes were clay for NAT and RWH soils and silty clay for RWOH wetland soils.

**Collection of Soil Samples**

Twelve samples were randomly collected from the upper 15 cm of soils within each study site using a 5-cm diameter hammer probe. Soils were collected on June 19, 1999 and each sample was immediately divided into three subsamples according to depth: 0 - 5 cm, 5 - 10 cm, and 10 - 15 cm. The top of the soil with the litter layer removed was considered the soil surface and was called the 0-cm depth for this study. Samples were then stored in separate bags on ice until they were brought to the Wetland Biogeochemistry Institute and refrigerated (4°C). Each soil sample was homogenized by passing through a mesh screen with 1.25 cm² openings. Percent soil moisture of each sample was measured gravimetrically by drying for 24 hours in an oven at 105°C. Bulk density of these soils was determined in a previous study using the method of Patrick (1958).

The FDA assay (Reed et al., 1989) was used to measure heterotrophic microbial activity. The theory behind the FDA method is that most, if not all, heterotrophic microorganisms hydrolyze fluorescein diacetate, producing fluorescein. Upon death and lysis of the cells by the addition of acetone, the fluorescein can be quantified colorimetrically. The amount of absorbance of fluorescein is indicative of the hydrolytic activity of the soil's heterotrophic microbial population (Reed et al., 1989). The FDA assay does not quantify microbial biomass, but it is a simple and sensitive
method to be used for comparing microbial activity in similar soil ecosystems (Schnurer and Rosswall, 1982).

Twelve litter samples were collected from each study site by placing a 12 cm² frame on the soil surface and collecting all of the organic matter which lay on top of the soil within the frame. Locations for litter collection were randomly selected within each wetland.

Samples of soil and litter were dried at 70°C overnight and then ground using a Thomas Wiley® plant tissue grinder (40 mesh). Total carbon and nitrogen concentrations in soils and litter were measured by direct combustion using a Perkin-Elmer CHN Model #2400 Series 2 analyzer. Soluble, inorganic NO₃⁻ and ammonium (NH₄⁺) were extracted by shaking five grams of soil with 50 ml of 2M KCl solution for 30 minutes. The samples were filtered through Whatman no. 42 (.45 µm) filter paper after extraction and the filtrate was analyzed using a Lachat automated flow analyzer (Lachat QuickChem Method 12-107-04-1-13).

**Statistical Analysis**

Data were analyzed using the Proc Mixed procedure of SAS® Institute (1994). Wetland type, distance from the water source, and soil depth were analyzed as main effects. Main effects were considered significant if they had a $P \leq 0.05$. Differences among means within each main effect and within interaction effects were evaluated using Tukey's Studentized Range (HSD) test ($\alpha = 0.05$). Relationships between dependent and independent variables were examined using a linear regression analysis and correlations among variables were conducted using Pearson's correlation analysis.
Results

The amount of leaf litter collected on soil surfaces was much greater in NAT wetlands than in restored (P = 0.0036) (Table 4.1). Litter collected in NAT wetlands was primarily decomposing tree leaves and woody debris. Although quantitative data was not collected, this litter layer appeared about 2.5-cm or more thick and had sediment trapped in between leaves. The litter collected on soil surfaces of restored wetlands was primarily herbaceous plant material that had not accumulated to any degree. While the ages of these restored wetlands differed by a few years, the amount of organic matter accumulated on soil surfaces did not. Total carbon concentrations and C/N ratios were higher in restored wetland litter than in NAT litter (P = 0.0202 and 0.0348, respectively) (Table 4.1). No differences were seen in litter nitrogen concentrations among wetland types (P = 0.0596). The distance that litter was sampled from the water source did not affect carbon and nitrogen concentrations (P = 0.8975 and 0.1841, respectively).

Table 4.1. Litter layer weights and litter carbon (C) and nitrogen (N) concentrations. NAT = natural bottomland hardwood (BLH) wetlands. RWH = restored BLH wetlands with hydrology re-established. RWOH = restored BLH wetlands without hydrology re-established. Values with different letters are significantly different at α = 0.05.

<table>
<thead>
<tr>
<th>Type</th>
<th>Litter (kg/m²)</th>
<th>mg C/g litter</th>
<th>mg N/g litter</th>
<th>C/N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAT</td>
<td>25.1 ± 13.5a</td>
<td>113.6 ± 43.2b</td>
<td>6.5 ± 1.9a</td>
<td>17.2b</td>
</tr>
<tr>
<td>RWH</td>
<td>1.2 ± 2.3b</td>
<td>330.2 ± 121.6a</td>
<td>11.5 ± 3.4a</td>
<td>30.8a</td>
</tr>
<tr>
<td>RWOH</td>
<td>1.2 ± 0.6b</td>
<td>368.6 ± 81.4a</td>
<td>11.9 ± 2.1a</td>
<td>31.4a</td>
</tr>
</tbody>
</table>

Soil carbon and nitrogen concentrations and microbial activity were not significantly different among wetland types (P = 0.1560, 0.1289, 0.1404, 0.1006, respectively).
respectively). Although not significantly different, it is noteworthy that carbon and nitrogen concentrations and microbial activity in NAT wetland soils were higher than in restored, at every depth. Soil carbon and nitrogen concentrations were significantly affected by soil sample depth (P = 0.0001 and 0.0001, respectively) (Table 4.2). In the natural wetlands mean heterotrophic microbial activity was greater in the litter layer than in the soil, however there was a large amount of variability and no significant differences were detected (P = 0.1721). Microbial activity was not measured in litter layers of restored wetlands because there was not enough sample available to perform this analysis. Nitrate concentrations were not significantly different due to wetland type (P = 0.1152), distance soil was sampled from the surface water source (P = 0.2578), or soil depth (P = 0.0972).

Soil moisture was highest in the 10- to 15-cm depth and lowest in the 0- to 5-cm depth in restored wetlands (P = 0.0003) but was not different in NAT wetlands (Table 4.2). Soil moisture was not different due to wetland type (P = 0.1244). Percent soil moisture at saturation was determined in homogenized soil samples by addition of water to dry soils until the sticky point was reached. Soil moisture at saturation was 70, 85, and 84% for RWOH, RWH, and NAT, respectively. Percent soil moisture measured at the time of sample collection was 26, 38, and 39% of the saturation moisture content for RWOH, RWH, and NAT wetlands, respectively. Soils were collected on June 19, 1999 and it had rained the day prior to sample collection. Rainfall reported by the Southern Regional Climate Center for Tallulah, Louisiana was 6.25 cm in May, 1999 and 6.80 cm in June, 1999.
Both monthly totals were lower than May and June monthly averages for
the past 38 years, 7.9 cm and 3.75 cm lower than average, respectively.

Table 4.2. Carbon (C), nitrogen (N), nitrate N (NO₃-N), and ammonium N
(NH₄-N) concentrations, moisture, and heterotrophic microbial activity in
study wetlands soils. NAT = natural bottomland hardwood (BLH) wetlands.
RWH = restored BLH wetlands with hydrology re-established. RWOH =
restored BLH wetlands without hydrology re-established. Values with
different letters are significantly different at α = 0.05. nd = not determined.
Microbial activity is reported as μg fluorescein produced/g soil/hour.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NAT</th>
<th>RWH</th>
<th>RWOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg C/g soil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0- to 5-cm</td>
<td>38.7 ± 10.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.7 ± 6.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.9 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5- to 10-cm</td>
<td>25.6 ± 6.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.0 ± 2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.0 ± 2.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10- to 15-cm</td>
<td>21.8 ± 8.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.6 ± 4.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.0 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>mg N/g soil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0- to 5-cm</td>
<td>3.3 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5- to 10-cm</td>
<td>2.7 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5 ± 0.3&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>10- to 15-cm</td>
<td>2.4 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C/N ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0- to 5-cm</td>
<td>11.6 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.4 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.0 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5- to 10-cm</td>
<td>9.5 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.3 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.8 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10- to 15-cm</td>
<td>9.3 ± 3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.5 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.7 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>μg NO₃-N/g soil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0- to 5-cm</td>
<td>8.1 ± 5.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5- to 10-cm</td>
<td>6.4 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10- to 15-cm</td>
<td>4.8 ± 4.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.9 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>microbial activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>litter layer</td>
<td>73.8 ± 46.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>0- to 5-cm</td>
<td>55.0 ± 6.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.2 ± 4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.7 ± 6.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5- to 10-cm</td>
<td>52.9 ± 12.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.8 ± 9.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.2 ± 8.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10- to 15-cm</td>
<td>44.0 ± 15.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.8 ± 15.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.9 ± 5.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>% soil moisture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0- to 5-cm</td>
<td>31.7 ± 6.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.9 ± 3.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.2 ± 1.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5- to 10-cm</td>
<td>33.8 ± 8.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.8 ± 3.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>18.8 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10- to 15-cm</td>
<td>32.8 ± 7.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.5 ± 3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.6 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

A model for predicting microbial activity using forward stepwise
regression included carbon, nitrogen, C/N ratio, and soil moisture (adjusted
r² = 0.73). The model for predicting microbial activity in these soils is:

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Microbial activity = \(-56.86 - 22.34(C \text{ concentration}) + 322.42(N \text{ concentration}) + 5.74(C/N \text{ ratio}) + 0.78(\text{soil moisture})\). Individual regressions between microbial activity and carbon concentration, percent soil moisture, and nitrogen concentration provided adjusted $r^2$'s of 0.48, 0.52, and 0.53, respectively. From these analyses, nitrogen concentration was seen to be the greatest predictor of microbial activity in these soils.

Results of Pearson's correlation analysis performed on carbon, nitrogen, C/N ratio, soil sample depth, microbial activity, and soil moisture are shown in Table 4.3. Carbon concentrations were highly correlated with nitrogen concentrations, microbial activity, and soil moisture and inversely correlated with depth. Thus, as depth increased, carbon concentrations decreased. Nitrogen concentrations were also highly correlated with microbial activity and soil moisture and inversely correlated with depth. Microbial activity was highly correlated with soil moisture as well.

Tables 4.4, 4.5, and 4.6 show results of Pearson's Correlation analysis performed on carbon, nitrogen, C/N ratio, microbial activity, and soil moisture at 0 - 5, 5 - 10, and 10 - 15 cm depths, respectively. The strength of relationships between carbon and nitrogen concentrations, carbon concentrations and soil moisture, and nitrogen concentrations and soil moisture decreased with depth. The relationships between microbial activity and carbon concentrations, nitrogen concentrations, and soil moisture were also strongest in the 5 - 10 cm depths and weakest in the 10 - 15 cm depths.
Table 4.3. Pearson correlation coefficients for parameters measured in study wetland soils. FDA = heterotrophic microbial activity measured as µg fluorescein produced/g soil/hour. * denotes significance at $\alpha < 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>Carbon</th>
<th>Nitrogen</th>
<th>C/N ratio</th>
<th>Depth</th>
<th>FDA</th>
<th>Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.90*</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/N ratio</td>
<td>0.51*</td>
<td>0.12</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth</td>
<td>-0.49*</td>
<td>-0.36*</td>
<td>-0.43*</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FDA</td>
<td>0.70*</td>
<td>0.73*</td>
<td>0.24*</td>
<td>-0.21</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>0.54*</td>
<td>0.61*</td>
<td>0.03</td>
<td>0.17</td>
<td>0.72*</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 4.4. Pearson correlation coefficients for parameters measured in 0 - 5 cm of study wetland soils. FDA=heterotrophic microbial activity µg fluorescein produced/g soil/hour. * denotes significance at $\alpha < 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>Carbon</th>
<th>Nitrogen</th>
<th>C/N ratio</th>
<th>FDA</th>
<th>Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.97*</td>
<td>1.00</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>C/N ratio</td>
<td>0.42*</td>
<td>0.20</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FDA</td>
<td>0.77*</td>
<td>0.75*</td>
<td>0.39</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>0.82*</td>
<td>0.82*</td>
<td>0.26</td>
<td>0.76*</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 4.5. Pearson correlation coefficients for parameters measured in 5 - 10 cm of study wetland soils. FDA=heterotrophic microbial activity µg fluorescein produced/g soil/hour. * denotes significance at $\alpha < 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>Carbon</th>
<th>Nitrogen</th>
<th>C/N ratio</th>
<th>FDA</th>
<th>Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.92*</td>
<td>1.00</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>C/N ratio</td>
<td>0.08</td>
<td>-0.29</td>
<td>1.00</td>
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<td></td>
</tr>
<tr>
<td>FDA</td>
<td>0.86*</td>
<td>0.85*</td>
<td>-0.08</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>0.77*</td>
<td>0.76*</td>
<td>-0.10</td>
<td>0.84*</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 4.6. Pearson correlation coefficients for parameters measured in 10-15 cm of study wetland soils. FDA=heterotrophic microbial activity µg fluorescein hydrolyzed/g soil/hour. * denotes significance at $\alpha < 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>Carbon</th>
<th>Nitrogen</th>
<th>C/N ratio</th>
<th>FDA</th>
<th>Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.73*</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/N ratio</td>
<td>0.53*</td>
<td>-0.17</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FDA</td>
<td>0.68*</td>
<td>0.63*</td>
<td>0.24</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>0.69*</td>
<td>0.62*</td>
<td>0.21</td>
<td>0.77*</td>
<td>1.00</td>
</tr>
</tbody>
</table>
DISCUSSION

The amount of litter accumulated on soil surfaces of NAT wetlands was much greater than that on RWH or RWOH soils probably because the NAT wetlands are mature, undisturbed forests whereas the restored wetlands were cultivated for agriculture. Conversion to agricultural cropland will cause losses of organic material due to increased oxidation upon cultivation when previously occluded organic matter is uncovered and made available to microbes (DeLuca and Keeney, 1994; Boyer and Groffman, 1996). In addition, the restored wetlands have had disturbances in their natural hydrology, including being drained for more than 20 years while in cropland, while the NAT wetlands have an annual flood/drain cycle. During surface flooding, anoxic conditions may cause buildup of organic matter because decomposition rates are slower than in oxic conditions (Gale et al., 1992). While the RWH wetlands have had annual flooding cycles re-established, organic matter accumulates on soil surfaces over time and most likely sufficient time has not passed for accumulation to occur (Mason, 1976; Bishel-Machung et al., 1996).

The litter layer in NAT wetlands was composed of a range of freshly deposited to highly decomposed litter and older leaf litter may be lower in carbon because it has been leached, used as a substrate for extracellular enzymes, or utilized by aerobic and anaerobic microbes (McLatchey and Reddy, 1998). Thus, the stage of litter decomposition was most likely the cause of differences in carbon concentrations in litter layers and soils of these wetlands. In addition, vegetative leaf litter and organic matter may be more quickly decomposed in restored wetlands than in mature forests due
to vegetation type. Herbaceous plants and young trees dominate the restored sites while 70+ year old trees dominate the natural sites. Decomposition of litter from pioneer species is faster than older trees because pioneer plants invest less energy in chemical defenses (tannins and phenolic compounds) and structural carbohydrates (lignin) (Mesquita et al., 1998).

The high C/N ratio of litter collected on soil surfaces of restored wetlands indicated that the litter was relatively fresh and it did appear to have been fairly recently deposited. The C/N ratio of the litter collected in the NAT wetlands was much lower than that of restored and showed less variability, indicating that it was, as a whole, in a more advanced state of decomposition. This was apparent when collecting the litter because much of it was very decomposed, especially litter nearest the soil surface. At times, it was hard to distinguish where the soil surface ended and the leaf layer began, however for the most part the leaf litter was easily separated as a distinct layer from the soil. Generally, fresh organic matter has a higher C/N ratio than decomposed organic matter because in the latter organic matter has been oxidized by microbes for energy and/or soluble carbon has been leached out. Ping et al. (1998) reported that the C/N ratio narrows after humification from greater than 20 in fresh organic matter to about 8 - 20 in humus.

Leaf litter on soil surfaces will contribute nutrients to underlying soil as it decomposes. Bishel-Machung et al. (1996) found significant differences in soil organic matter in natural wetlands between 5- and 20-cm depths. They attributed this disparity to a surface accumulation of organic
matter. Because the NAT wetlands had a much greater litter mass than restored, it would be expected that soil carbon and nitrogen concentrations would be higher, especially in the upper five centimeters of soil. Although no significant differences were seen in total soil carbon and nitrogen concentrations among wetland types, mean concentrations were higher for every depth in NAT wetlands than in restored. Because the litter layer will continually provide nutrients to soil microbes as it decomposes, it may support higher microbial populations than in soils without a surface litter layer such as the restored wetlands. Again, although no significant differences were seen in microbial activity among wetland types, activity in the natural wetland soils was higher than in restored soils at every depth. The key difference in soils between these wetlands is the presence of leaf litter and it is highly probable that this is affecting microbial populations and, consequently, biogeochemical functions. Results of the regression analysis also demonstrate a strong relationship between microbial activity and carbon and nitrogen concentrations and moisture in these soils.

Carbon and nitrogen concentrations were higher in RWH soils than in RWOH soils, although the same amount of litter was collected on soil surfaces. This apparent discrepancy creates a problem in our hypothesis that the litter layer is supplying nutrients to soils. While it is well researched that decomposing leaves will release nutrients, in these ecosystems there must be an additional source of nutrients as well. To further investigate, carbon and nitrogen concentrations in RWH soils were analyzed by site. The reason for doing this is that there were differences in the flooding regime measured in Dorsey and Greenlea. Data collected in 1997-98

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showed that Greenlea was flooded with up to 50 cm of surface water from late November until early March while Dorsey had only a few cm of surface water briefly in February. The following sampling year, 1998-99, Greenlea was flooded with surface water for three months while Dorsey had surface water for only one month. This floodwater, which is runoff from agricultural cropland, contains dissolved nutrients, fine organic matter, and sediments will contribute to soil nutrient concentrations. When analyzed by individual site, carbon concentrations were significantly higher in Greenlea (P = 0.0262)(mean = 22.02 mg C/g soil) than in Dorsey (mean = 17.49 mg C/g soil) but no differences were measured in nitrogen concentrations (P = 0.0592)(mean = 2.02 and 1.80 mg N/g soil in Greenlea and Dorsey, respectively). Heterotrophic microbial activity was also much higher in Greenlea (P = 0.0001)(mean = 47.04 µg fluorescein produced/g soil/hour) than in Dorsey (mean = 32.75 µg fluorescein produced/g soil/hour). In addition to increasing soil nutrient content, standing water may lead to anoxic conditions in soils, slowing breakdown of organic matter that is present in soils and leading to higher carbon concentrations. These results indicate that both leaf litter and surface water flooding may contribute to higher nutrient concentrations in surface soils of these wetlands.

Soil microbial activities in this study were strongly correlated with total carbon and nitrogen concentrations. Growth of heterotrophs in soils is carbon limited and presence of organic matter has been shown to have the greatest influence on microbial populations (Grayston et al., 1998). Groffman et al. (1996) found a strong correlation (r = 0.90, α = 0.01, n = 36) between microbial biomass carbon and total carbon in wetland soils. In
addition, they also found a strong correlation \( r = 0.89, \alpha = 0.01, n = 36 \) between microbial biomass carbon and total nitrogen.

Microbial activity was also significantly correlated with percent moisture in soils. Not only was percent moisture higher in NAT and RWH soils, but soil water content was closer to saturation in the former soils than in the latter. Soil moisture can regulate microbial activity because not only are most microbes 70% or more water but because water must be available to maintain turgor pressure, act as a medium for microbial movement, dissolve solutes and organic substrates, act as a transport mechanism for gases, and facilitate removal of microbial metabolic by-products (Killham, 1994; Scott et al., 1996). The NAT and RWH wetlands are periodically flooded and this may cause soil moisture to be greater than in RWOH wetlands, especially because the NAT and RWH soils are classified as clay soils which will hold water more tightly than the silty clay soils of the RWOH wetlands. The presence of a litter layer on soil surfaces may also help to retain moisture by lowering evaporation from the upper soil surface. It is probable that evaporation from soil surfaces is lowering soil moisture in the upper five centimeters, especially when one notes that moisture increased as sample depth increased to 15 cm in the restored wetlands but not in the NAT wetlands, where the litter layer may have prevented water evaporation from soil surfaces.

Soil bulk density will also indirectly affect microbial activity because it directly affects soil aeration. Bulk density of the RWOH soils was higher (mean = 1.34 g/cc) than in NAT (mean = 0.96 g/cc) and RWH (mean = 1.17 g/cc) soils. Soils with higher bulk densities have less pore space, less
room for gas concentrations such as oxygen, and reduced gas diffusion from the atmosphere (Dragun, 1988; Wagner and Wolf, 1998). Because oxygen concentrations will affect activity of aerobic microorganisms, higher soil bulk density in RWOH may have contributed to lower heterotrophic microbial activity. Organic matter in soil will reduce bulk density (Hartel, 1998) and these results are consistent with higher carbon concentrations in NAT soils than in restored.

As sample depth increased, the relationship between carbon and nitrogen concentrations decreased because as organic matter decomposes nutrients are released and not closely associated with litter anymore (Mason, 1976). Heterotrophic microbial activity and carbon and nitrogen concentrations were not as strongly correlated in the 10 - 15 cm depth as in the 0 - 10 cm of soil probably because nutrient concentrations decreased with depth, causing a reduction in microbial activity. This latter result supports the hypothesis that the presence of an organic layer on soil surfaces is affecting microbial activity, causing increases in activity where carbon and nitrogen concentrations are higher. Therefore, one would expect that higher soil and litter carbon concentrations in the NAT wetlands would support a greater number of heterotrophs.
CONCLUSIONS

The results of this study indicate that the presence of leaf litter is providing nutrients necessary to support higher populations of soil microorganisms in NAT wetlands than in restored wetlands. Results of this study also indicate that surface flooding, i.e., the normal winter and early spring flood regime of BLH wetlands, may contribute to nutrient status of these soils, leading to increases in microbial populations as well. Because microorganisms are the primary organisms responsible for biogeochemical functions it is beneficial to have higher soil microbial populations if an ecosystem is being managed for specific functions which are microbially mediated, for example denitrification. One way to increase heterotrophs may be to increase soil carbon concentrations. In riparian wetlands restored for the purpose of NO$_3^-$ removal, increasing heterotroph populations (of which denitrifiers are included) may lead to higher populations of denitrifiers. Because these microbes are primarily facultative anaerobes, reduced soil conditions upon flooding may yield higher denitrification rates in wetlands with higher microbial populations.

Litter layer accumulation is a product of time and ecological succession and, thus, cannot be immediately established when a wetland is restored or created. Some wetland creation projects import soils high in organic matter so that these newly created ecosystems will have higher microbial populations and nutrients than those without imported soils. This approach may be wise in restoration projects as well, especially if wetlands are being restored for water quality improvement purposes such as removal of NO$_3^-$ from non-point source pollution. Long-term studies are
needed to determine the amount of time required for restored wetlands to have biogeochemical functions equivalent to natural wetlands, especially in ecosystems such as BLH wetlands which take many years to reach maturity.
CHAPTER FIVE
EFFECT OF SOIL CARBON AMENDMENTS ON HETEROTROPHIC MICROBIAL ACTIVITY AND DENITRIFICATION RATES

INTRODUCTION

Overview

Organic matter concentrations affect population size and activity of heterotrophic microbes in soils and previous research has indicated that bottomland hardwood wetlands with higher soil organic matter concentrations will have higher heterotrophic microbial activities and denitrification rates. To determine how heterotrophic microbes would respond to carbon additions, the effect of organic matter amendments on soil heterotrophic microbial activities and denitrification rates were measured during a one-month study. Composted green waste, paper mill sludge, cotton gin trash, and composted municipal sewage sludge were added to homogenized soils of restored wetlands at a concentration of 1 - 1.5\% of soil dry weight. Controls consisted of natural and restored wetland soils with no amendments added. Heterotrophic microbial activity was measured weekly using the fluorescein diacetate assay (FDA) and denitrification rates were measured at the end of the month using the acetylene-block technique. Total nitrogen, ammonium (NH$_4^+$), and nitrate (NO$_3^-$) were measured in soils at the end of the incubation using the ammonium diffusion technique. No significant differences in heterotrophic microbial activity were measured among wetland type, however cotton gin trash produced higher microbial activity in restored soils than most of the other amendments. Denitrification rates were also not significantly different

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among wetland types, but again cotton gin trash produced the highest denitrification rates of all amendments. No differences were measured in total nitrogen, $\text{NH}_4^+$, and $\text{NO}_3^-$ concentrations among wetland types at the end of the four-week incubation period. This study indicates that organic matter amendments will increase heterotrophic microbial activity and denitrification rates in restored bottomland hardwood wetlands, and these results have important implications for future restoration projects. In particular, amendments may increase capacity of restored wetlands to remove $\text{NO}_3^-$ from agricultural runoff water. Specifically, this research demonstrates that cotton gin trash, when added to wetland soils, will increase microbial activity and denitrification rates, while not promoting $\text{NO}_3^-$ leaching into ground and surface waters.

**Background**

Bottomland hardwood (BLH) wetlands in the Tensas River Basin are being restored in an effort to re-establish ecosystems with beneficial water quality and habitat functions. The Tensas River Basin is an area of intense agricultural activity and nutrient and sediment runoff from agricultural fields into the Tensas River has caused a decline in water quality (USDA-NRCS, 1995). Bottomland hardwood wetlands may help improve water quality because they may act as buffer zones to remove nutrients and sediments as runoff water flows through them (Hill, 1996; Groffman et al., 1998; Martin et al., 1999). In particular, nitrate ($\text{NO}_3^-$), a mobile nutrient which promotes eutrophication, is denitrified in BLH wetland soils by primarily heterotrophic denitrifying microorganisms (Jordan et al., 1993). Because 85% of the original BLH forests have been converted to agriculture in the Tensas River
Basin, restoration efforts are important to improve water quality and to provide new wildlife habitat and floodwater storage.

We have studied biogeochemical functions and soil characteristics of restored and natural BLH wetlands in the Tensas River Basin for the past three years. Although not significantly different, most likely due to the large amount of variation, denitrification potentials were much higher in natural BLH wetland soils than in restored wetland soils for every season measured during a one-year sampling period (Hunter and Faulkner, In review). One reason denitrification may be lower in restored wetlands than in natural wetlands is because organic matter concentrations are lower in the former than in the latter and restored soils may not be able to support a population of heterotrophs as large as those in natural wetland soils. Organic carbon content is 3% in the upper 15 cm of natural BLH soils, 1 to 1.5% higher than in restored wetland soils (Table 4.2). Seasonal heterotrophic microbial activities were also measured in the upper 15 cm of soil for one year, and activities were higher in natural BLH wetland soils than in restored in every season measured (Fig. 3.2).

Because of measured differences in denitrification potentials, soil carbon concentrations, and microbial activity, we wanted to determine how different organic matter amendments applied to soils would affect heterotrophic microbial activity and denitrification rates. By using these amendments, which are waste products that are hard to dispose of in a useful manner, two problems may be solved at one time. First, industrial, agricultural, and/or municipal wastes may be disposed of in a way that is beneficial to the environment, as opposed to addition to landfills or burning.
Louisiana annually produces more than two million tons of organic wastes through manufacturing and municipalities and these wastes are typically disposed of through storage in lagoons or landfills or by incineration (Boquet et al., 1999). Second, during a restoration project organic matter added to soils may not only result in an increase in heterotrophic microbial activity but may also improve soil structure as well (Chantigny et al., 1999). Because heterotrophic microbial activity is directly correlated with carbon concentrations in wetland soils, an increase in organic matter may result in an increase in microbial activity, possibly leading to increases in denitrification.

Many studies have been conducted demonstrating the beneficial use of industrial, municipal, and agricultural wastes in agriculture and forestry (Brockway, 1983; Bellamy et al., 1995; Boquet and Breitenbeck, 1999). In particular, research on land application of organic wastes has shown increased plant growth and yield, higher organic carbon concentrations, and increased water holding capacity in soils with amendments over those without amendments added (Mays et al., 1973; Khaleel et al., 1981; Bellamy et al., 1995; Fierro et al., 1997; Boquet et al., 1999). To our knowledge, no research has been conducted to determine the effects of organic matter amendments on soil biogeochemical functions and microbial activity of restored wetlands. To be an appropriate soil amendment to wetlands restored for the purpose of improving water quality, the amendment must increase microbial activity, result in higher denitrification rates, and not contribute $\text{NO}_3^-$ to downstream ecosystems.
The objective of this study was to determine effects of composted municipal sewage sludge, cotton gin trash, paper mill sludge, and composted green waste on heterotrophic microbial activity and denitrification rates in soils of restored bottomland hardwood wetlands. It was hypothesized that soils amended with organic matter would have higher heterotrophic microbial activity than soils with no amendments. Higher heterotrophic microbial activity may also result in higher denitrification rates in amended soils than in unamended soils. It was also hypothesized that soils amended with organic matter would have heterotrophic microbial activity and denitrification rates comparable to or greater than soils of natural BLH wetlands with no added amendments.

MATERIALS AND METHODS

Site Description

Soils from three types of BLH wetlands were chosen for study: natural mature (NAT) wetlands, wetlands restored with hydrology re-established (RWH), and wetlands restored without hydrology re-established (RWOH). Restored wetlands were once natural BLH wetlands which were converted to agricultural fields by clearing vegetation and digging drainage ditches around the wetlands. Both RWH and RWOH sites were replanted with appropriate BLH species (*Quercus lyrata, Q. nigra, Q. phellos, Acer rubrum*). Hydrologic restoration of the RWH sites consisted of installing flashboard risers in the drainage ditches adjacent to the sites. The water height in these ditches can be controlled by the risers to allow surface runoff from adjacent agricultural fields to inundate the sites. This simulates the winter and early spring flooding hydrologic regime of natural...
BLH wetlands in this watershed. The RWOH sites did not have flashboards installed in drainage ditches and agricultural surface water runoff simply bypassed sites via the drainage ditches. Study wetlands were chosen for their proximity to agricultural fields as well as for their similarities in soil type, slope, and topography. Two replicates of each type of wetland were chosen. Textural classes were clay for NAT and RWH soils and silty clay for RWOH wetland soils.

**Experimental Design**

The experimental design was a split plot design with wetland type as the whole plot treatment and type of amendment added as the split plot treatment. Each of the restored sites had soil subsamples with each of the four amendments added and also a subsample with no amendment added for a control. Three replicates of each subsample were established. No amendments were added to natural wetland soils.

**Soil Collection, Preparation, and Analyses**

Soil samples were gathered from six study wetlands using a 12.5-cm diameter bucket auger on October 21, 1999. Samples were collected to a depth of 15 cm. Soils were randomly collected from four points in a 30-x 40-m established plot within each wetland. Soils from each site were homogenized by passing through a mesh screen with 1.25 cm² holes. The soils were then air dried for at least 72 hours and pulverized to approximately two millimeters using a soil pulverizer. On October 28, 1999 moisture content at saturation was determined. One hundred grams of soil was weighed and water was added until saturation was reached and then the soil was reweighed and water content was determined. Moisture
content at saturation was determined for each soil with each amendment added and without any amendment added. Thus a total of 22 determinations were made (five per restored site and one for each natural site with no amendment added). After this, 100 g of each soil was weighed into 8-oz plastic containers and water was added to bring the moisture content to 70% of saturation. Seventy percent of saturation was chosen because between 55 and 65% moisture is effective for maximum microbial growth and less than 45% moisture is limiting to growth (Stratton et al., 1995). In addition, when water-holding capacity of soils drops below 60-70%, oxygen partial pressures will negatively affect denitrification (Tisdale et al., 1985). The appropriate amendment was added at a rate of 1% dry weight for RWH soils and 1.5% dry weight for RWOH soils. After water and amendments were added to each of the containers, the containers were reweighed and the weight was recorded. Every two days soils were reweighed and water was added to bring the soil to its original weight. By doing so, 70% of moisture content of saturation was maintained in each container.

The four amendments used in this study were paper mill sludge, composted green waste (i.e., yard clipping, leaves, etc.), composted municipal sewage sludge, and cotton gin trash. The amendments were dried for 48 hours at 60°C and then ground in a Thomas Wiley® plant tissue grinder (40 mesh). Total carbon and nitrogen concentrations of amendments were measured at the end of the month-long incubation by direct combustion using a Perkin-Elmer CHN analyzer (#2400 Series 2). Percent carbon and nitrogen content and C/N ratios of each amendment
are shown in Table 5.1. One gram and 1.5 g of amendment was added for every 100 g of soil to the RWH and RWOH wetland soils, respectively.

Table 5.1. Carbon (C) and nitrogen (N) contents of soil amendments determined by direct combustion.

<table>
<thead>
<tr>
<th>Amendment</th>
<th>N  (% dry wt.)</th>
<th>C  (% dry wt.)</th>
<th>C/N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton gin</td>
<td>2.2</td>
<td>41.3</td>
<td>19.2</td>
</tr>
<tr>
<td>Sewage sludge</td>
<td>1.3</td>
<td>31.4</td>
<td>24.4</td>
</tr>
<tr>
<td>Paper mill</td>
<td>0.1</td>
<td>41.5</td>
<td>328.7</td>
</tr>
<tr>
<td>Green waste</td>
<td>2.6</td>
<td>34.4</td>
<td>13.4</td>
</tr>
</tbody>
</table>

Heterotrophic microbial activity was measured weekly for one month using the fluorescein diacetate hydrolysis (FDA) assay (Reed et al., 1989). The theory behind the FDA method is that heterotrophic microorganisms hydrolyze fluorescein diacetate, producing fluorescein. Upon death of cells with the addition of acetate, fluorescein can be quantified spectrophotometrically at 490 nm. The amount of absorbance of fluorescein is indicative of the hydrolytic activity of the soil's heterotrophic microbial population (Reed et al., 1989). The FDA assay does not quantify microbial biomass, but it is a simple and sensitive method to be used for comparing microbial activity in similar soil ecosystems (Schnurer and Rosswall, 1982).

Denitrification rates were determined using the denitrification assay of Myrold and Tiedje (1985). At the end of the month-long incubation, one 10-gram subsample was taken from each soil and placed in a 125-ml incubation flask with 25 ml of a solution containing 20 mg NO₃-N/L as KNO₃. The mixture was shaken vigorously to obtain a slurry. Each flask was capped with gas impermeable stoppers and made anoxic by flushing with argon for 30 seconds. Ten milliliters of purified acetylene was added.
to each flask to achieve a final concentrations of 10% (10 kPa) in the gas phase. The soil slurries were placed on a rotary shaker for one hour. Headspace gas was sampled by syringe after 24 hours. Gas samples were stored in evacuated 10 ml vacutainer vials until nitrous oxide (N₂O) could be measured by gas chromatography. Nitrous oxide concentrations were measured using a Tremetrics 9001 gas chromatograph with an electron capture detector. Nitrous oxide dissolved in sample water was corrected for using the Bunsen relationship, \( M = C_g(V_g + V_lD) \), where \( M \) = total amount of \( N_2O \) in water plus gas phase, \( C_g \) = concentration of \( N_2O \) in gas phase, \( V_g \) = volume of gas phase, \( V_l \) = volume of liquid phase, and \( D \) = Bunsen absorption coefficient (at 25°C). Denitrification rates are expressed as \( \mu g \ N_2O \) evolved/g dry soil/day.

Total soil nitrogen, \( NH_4^+ \), and \( NO_3^- \) concentrations were determined at the end of the month-long incubation by shaking four grams of soil with 40 ml of 2M KCl solution for one hour. The samples were then centrifuged at 6500 rpm for 10 minutes and filtered using Whatman no. 42 (.45 μm) filter paper and refrigerated. The samples were analyzed for total nitrogen, \( NH_4^+ \), and \( NO_3^- \) using a modification of the ammonium diffusion technique of Carlson et al. (1990).

**Statistical Analysis**

Data were analyzed using the Proc Mixed procedure of SAS® Institute (1994). Wetland type and amendment were analyzed as main effects. Effect of sampling time on microbial activity was analyzed using repeated measures. Main effects were considered significant if they had a \( P \leq 0.05 \). Differences among means within each main effect and within
interaction effects were evaluated using Tukey's Studentized Range (HSD) test ($\alpha = 0.05$).

**RESULTS AND DISCUSSION**

At the end of the month-long incubation, there were no significant differences in denitrification rates among wetland types ($P = 0.6550$). This result is important because our goal was to increase denitrification rates in restored wetlands to be equal to or greater than those of natural BLH wetlands. Although not significantly different among wetland types, it is noteworthy that denitrification rates in restored wetland soils containing carbon amendments were higher than those measured in unamended NAT soils (Fig. 5.1). The one exception to this was RWOH soils amended with composted sewage sludge. Thus, in this bench-scale study adding organic wastes to soils increased denitrification rates in restored wetlands, with a dramatic increase in soils amended with cotton gin trash. Type of amendment significantly affected denitrification rates in soil ($P = 0.0001$) (Fig. 5.1). Cotton gin trash increased denitrification rates 23.7 and 45.2%, in RWH and RWOH soils respectively, over those same soils with no amendments added (Table 5.2). The other amendments resulted in a much smaller increase over unamended soils from the same wetlands. In some amended soils denitrification rates were lower than in unamended soils from the same wetland type.

It is unclear why denitrification rates were highest in soils amended with cotton gin trash. Cotton gin trash has not been widely used as a soil amendment and very little data exists. As will be discussed, heterotrophic
5.1. Denitrification rates in wetland soils with different organic carbon amendments. NAT = natural bottomland hardwood (BLH) wetland. RWH = restored BLH wetland with hydrology re-established. RWOH = restored BLH wetland without hydrology re-established. None = no amendment added. CG = cotton gin trash. GW = composted green waste. PM = paper mill sludge. SS = composted sewage sludge. Standard deviation is denoted by error bars. Treatments with different letters are significantly different at $\alpha = 0.05$. 
Table 5.2. Percent increase in denitrification rates measured in amended soils over rates in soils from the same wetland type with no amendment added. RWH = restored bottomland hardwood (BLH) wetlands with hydrology re-established. RWOH = restored BLH wetlands without hydrology re-established.

<table>
<thead>
<tr>
<th>Amendment</th>
<th>RWH</th>
<th>RWOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton gin</td>
<td>23.7</td>
<td>45.2</td>
</tr>
<tr>
<td>Green waste</td>
<td>-2.4</td>
<td>9.0</td>
</tr>
<tr>
<td>Paper mill</td>
<td>8.3</td>
<td>14.1</td>
</tr>
<tr>
<td>Sewage sludge</td>
<td>-6.8</td>
<td>-11.9</td>
</tr>
</tbody>
</table>

Microbial activity was also higher in soils amended with cotton gin trash than in most other soils and an increase in microbial activity may lead to an increase in denitrification. Certainly, our previous research demonstrates that facultative anaerobes capable of denitrification exist in these wetland clay soils (Hunter and Faulkner, In review). If these soils are carbon limited, when organic matter amendments are added facultative anaerobes may outcompete other microbes and denitrify influent NO₃⁻. The soil solutions in which denitrification rates were measured were made anoxic by flushing with argon and incubating for 24 hours. During this incubation period, facultative anaerobes may outcompete aerobic microbes because they can utilize NO₃⁻ as an electron acceptor while the aerobes can only use oxygen, which will quickly be depleted.

In a previous study, denitrification potentials were measured using the denitrification enzyme activity (DEA) assay (Hunter and Faulkner, in review). This assay gives an indication of denitrifier population size and research has shown that denitrification potential, measured as N₂O evolution, is strongly correlated to annual denitrification rates (Ambus, 1993; Hanson et al., 1994; Groffman et al., 1996). In this year-long study
conducted seasonally from July 1997 to April 1998, denitrification potentials were higher in NAT soils than in restored soils, although significant differences among type were not detected most likely due to the high sample variation. Because of these results, one of the goals of adding organic matter amendments was to increase denitrification rates of restored wetlands to equal to or greater than rates measured in NAT wetland soils. That goal was achieved in all restored soils with the exception of RWOH soils amended with sewage sludge.

When the results of this study are compared to those of the DEA study, it may be noted that trends seen in denitrification rates for NAT, RWH, and RWOH soils are not the same as those measured in soils in this present study. In the DEA assay, denitrification potentials were highest in the NAT soils and lowest in the RWOH soils, regardless of treatment (see Chapter 2 for treatment description). In the amendment study, denitrification rates in unamended NAT, RWH, and RWOH soils were very similar to each other, with slightly lower rates measured in the NAT soils than in restored. Several explanations for this discrepancy are possible. In the DEA assay, chloramphenicol is added to the soil slurry to prevent any new microbial growth, providing a “picture” of the soil microbial population at the time of sample collection. In addition, N₂O evolution was measured after 30 and 90 minutes and rates were extrapolated to a 24-hour time period. In this amendment study, chloramphenicol was not added to soil slurries and N₂O evolution was actually measured after 24 hours. Thus, in the latter study, microorganisms could grow and reproduce while chloramphenicol prevented reproduction during the DEA assay. In
addition, the month-long incubation previous to denitrification measurements allowed growth of microbes in a controlled setting and also promoted decomposition of soil organic matter. Finally, the concentration of $\text{NO}_3^-$ in the amendment study was chosen because between 10 - 20 mg/L is the average concentration measured in agricultural runoff (Hill, 1996).

No differences existed in heterotrophic microbial activity among wetland types ($P = 0.4304$), but amendments did affect activity ($P = 0.0001$), with microbial activities higher in amended soils than in soils from the same wetland types with no amendments added (Table 5.3). It is also noteworthy that, although not significantly different from the other soils in every week measured, soils amended with cotton gin trash consistently had the highest microbial activity. This was especially true in RWOH soils amended with cotton gin trash where microbial activity was 27 - 46% higher than unamended soils (Table 5.4). Soils with no amendments added and those with composted green waste added typically had lower microbial activity than the other soils.

Although it contained 34% carbon, the green waste was composted and the organic matter was in a more stable form than if it was freshly deposited. In addition, the green waste contained some twigs and tree limbs and this would lower the microbial degradability of the amendment on a weight basis when compared to only leaves and grass. Therefore, it may take longer for microbes to break down this organic matter and utilize the carbon than if the organic matter contained only readily mineralizable
Table 5.3. Heterotrophic microbial activity measured weekly during the month-long incubation of soils with different amendments. Heterotrophic microbial activity was measured as μg fluorescein hydrolyzed/g soil/hour. NAT = natural bottomland hardwood (BLH) wetlands. RWH = restored BLH wetlands with hydrology re-established. RWOH = restored BLH wetlands without hydrology re-established. None = soils with no amendment added. CG = cotton gin trash. GW = composted green waste. PM = paper mill sludge. SS = composted municipal sewage sludge. Amendments with different letters are significantly different from one another within a particular wetland type at α = 0.05.

<table>
<thead>
<tr>
<th></th>
<th>NAT</th>
<th>RWH</th>
<th>RWOH</th>
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<tbody>
<tr>
<td>Week 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>44.9 ± 4.4</td>
<td>42.7 ± 5.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.4 ± 7.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CG</td>
<td>51.8 ± 13.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.0 ± 9.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>GW</td>
<td>43.1 ± 6.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.9 ± 6.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>PM</td>
<td>51.4 ± 7.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.7 ± 14.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>47.5 ± 6.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.0 ± 5.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
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<td>Week 2</td>
<td></td>
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<tr>
<td>none</td>
<td>51.1 ± 5.0</td>
<td>51.8 ± 2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.0 ± 3.3&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>CG</td>
<td>68.0 ± 8.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.1 ± 5.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>GW</td>
<td>55.5 ± 4.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>41.1 ± 2.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>PM</td>
<td>61.4 ± 7.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.2 ± 3.3&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>SS</td>
<td>57.4 ± 5.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>43.9 ± 4.0&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Week 3</td>
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<tr>
<td>none</td>
<td>54.3 ± 5.3</td>
<td>46.9 ± 3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.5 ± 4.8&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>CG</td>
<td>55.0 ± 5.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.3 ± 15.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>GW</td>
<td>48.9 ± 6.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.1 ± 2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>PM</td>
<td>54.8 ± 5.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.1 ± 7.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>SS</td>
<td>55.4 ± 4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.2 ± 9.3&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Week 4</td>
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<tr>
<td>none</td>
<td>54.4 ± 4.3</td>
<td>56.9 ± 7.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.7 ± 10.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CG</td>
<td>74.2 ± 10.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.0 ± 9.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>GW</td>
<td>57.6 ± 7.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>49.3 ± 8.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>PM</td>
<td>67.2 ± 6.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>51.9 ± 9.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>SS</td>
<td>63.4 ± 7.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>54.0 ± 8.6&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>
Table 5.4. Percent increase in heterotrophic microbial activity (reported as μg fluorescein produced/g soil/hour) in amended soils over non-amended soils of the same type. CG = cotton gin trash. GW = composted green waste. PM = paper mill sludge. SS = composted municipal sewage sludge. RWH = restored bottomland hardwood (BLH) wetlands with hydrology re-established. RWOH = restored BLH wetlands without hydrology re-established.

<table>
<thead>
<tr>
<th></th>
<th>CG</th>
<th>GW</th>
<th>PM</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RWH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>week 1</td>
<td>17.5</td>
<td>0.9</td>
<td>17.0</td>
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<td>week 2</td>
<td>23.9</td>
<td>6.7</td>
<td>15.8</td>
<td>9.8</td>
</tr>
<tr>
<td>week 3</td>
<td>14.8</td>
<td>4.1</td>
<td>14.4</td>
<td>15.3</td>
</tr>
<tr>
<td>week 4</td>
<td>23.3</td>
<td>1.2</td>
<td>15.2</td>
<td>10.3</td>
</tr>
<tr>
<td>RWOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>week 1</td>
<td>34.0</td>
<td>1.5</td>
<td>16.2</td>
<td>14.8</td>
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<tr>
<td>week 2</td>
<td>39.3</td>
<td>5.3</td>
<td>7.6</td>
<td>11.2</td>
</tr>
<tr>
<td>week 3</td>
<td>46.5</td>
<td>4.3</td>
<td>13.6</td>
<td>17.9</td>
</tr>
<tr>
<td>week 4</td>
<td>26.7</td>
<td>3.2</td>
<td>8.1</td>
<td>11.6</td>
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</table>

carbon. Lignin and cellulose are more resistant to degradation by microbes than sugars, starches, and proteins in plants (Stratton et al., 1995). Cotton gin trash and paper mill sludge were not composted and contained essentially the same carbon concentrations, 41.3 and 41.5%, respectively. However carbon in the paper mill sludge may be harder to break down because it is primarily wood fiber with a low nitrogen concentration and a high lignin content, whereas the gin trash contained parts of the cotton plant which are more easily broken down by soil microorganisms.

One important factor when adding any amendment to soil is availability of nitrogen. Nitrate leaching is of special concern because it is a primary contributor to water quality problems. Total nitrogen (P = 0.2766), NH₄⁺ (P = 0.1330), and NO₃⁻ (P = 0.2835) concentrations were not significantly different among wetland types, but concentrations were
significantly different due to amendment added ($P = 0.0001, 0.0001$, and $0.0205$, respectively). In this study, total nitrogen, $\text{NH}_4^+$, and $\text{NO}_3^-$ concentrations were consistently lower in soils amended with cotton gin trash and paper mill sludge than in unamended soils and those amended with green waste (Table 5.5). Papermill sludge has an extremely high C/N ratio so in those soils amended with it any available nitrogen was most likely immobilized by microbes, making nitrogen concentrations very low in soil KCl extracts. Soils amended with cotton gin trash had very low nitrogen concentrations and this may be because it was being denitrified. In the uncomposted cotton gin trash, nitrogen and carbon are readily available to soil microorganisms. Because results of this study have shown that microbial activity and denitrification were increased by addition of this amendment, perhaps any $\text{NO}_3^-$ was readily denitrified, leading to low soil nitrogen concentrations.

**CONCLUSIONS**

The results of this research indicate that if a wetland is being restored for water quality functions, amending the soil with cotton gin trash will stimulate microbial activity and denitrification, while not leaching $\text{NO}_3^-$ into nearby water bodies. Clearly, because this was a bench-scale study, the results may not translate to field settings. Additional research should be conducted in restored wetlands to determine how cotton gin trash will affect denitrification rates and soil nitrogen concentrations and also how long the amendment will continue to provide organic matter to soil microorganisms.
Table 5.5. Potassium chloride extractable soil nitrogen concentrations (µg/g dry soil) sampled at the end of the month-long incubation. NAT = natural bottomland hardwood (BLH) wetland soils. RWH = restored BLH wetlands with hydrology re-established. RWOH = restored BLH wetlands without hydrology re-established. None = no amendment added. CG = cotton gin trash. GW = composted green waste. PM = paper mill sludge. SS = composted municipal sewage sludge. Amendments with different letters are significantly different from one another within a wetland type at α = 0.05.

<table>
<thead>
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<th></th>
<th>NAT</th>
<th>RWH</th>
<th>RWOH</th>
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<tbody>
<tr>
<td><strong>Total N</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>44.5 ± 30.1</td>
<td>19.5 ± 7.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.1 ± 3.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CG</td>
<td>4.9 ± 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>GW</td>
<td>15.4 ± 4.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.1 ± 2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>PM</td>
<td>4.7 ± 2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>SS</td>
<td>13.0 ± 8.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.6 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><strong>NH₄⁺</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>none</td>
<td>4.1 ± 0.6</td>
<td>3.9 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CG</td>
<td>1.8 ± 0.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.7 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>GW</td>
<td>4.2 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2 ± 1.1&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>PM</td>
<td>1.4 ± 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.6 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>SS</td>
<td>3.5 ± 1.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.5 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><strong>NO₃⁻</strong></td>
<td></td>
<td></td>
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<tr>
<td>none</td>
<td>40.47 ± 29.77</td>
<td>15.7 ± 7.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5 ± 3.6&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>CG</td>
<td>3.2 ± 1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>GW</td>
<td>11.3 ± 4.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.9 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>PM</td>
<td>3.4 ± 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.4 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>SS</td>
<td>9.4 ± 8.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.2 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
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The weight of one acre of Sharkey clay soil six inches deep is about three million pounds. In order to add 1% of soil weight as amendment, 30,000 pounds of cotton gin trash, or about 15 tons, would be added per acre. Cotton gin trash is a readily available waste product in Louisiana, especially in the Tensas River Basin where many BLH wetlands are being restored. Use of this agricultural by-product may result in its beneficial disposal and in increased soil biogeochemical functions of restored wetlands, resulting in greater reduction of NO$_3^-$ from agricultural run-off water and improved water quality of the Tensas River.
CHAPTER SIX
SYNTHESIS AND CONCLUSIONS

Overall results of this research indicate that bottomland hardwood (BLH) wetlands restored without hydrology re-established (RWOH) will have biogeochemical functions that are compromised when compared to those of natural (NAT) wetlands or restored wetlands with hydrology re-established (RWH). The typical hydrologic regime of BLH wetlands is a seasonal flooding cycle which begins in late November or early December and lasts, on average, about three to four months. Surface flooding is important because it affects soil moisture content, nutrient dynamics, microorganism composition, and microbial processes such as denitrification. During the two years in which hydrology was monitored in this study, NAT and RWH wetlands were saturated in the upper 15 cm, the biologically active soil zone, for twice as long as RWOH wetlands. This was most likely because the former wetlands were flooded with surface water for part of the year while the latter were never flooded because surface water was confined to drainage ditches. Soil saturation will lead to depletion of oxygen by aerobic microorganisms because gas diffusion into saturated soils is much slower than in dry soils. Saturation will also reduce rates of organic matter decomposition and effect shifts in microbial communities, thus affecting biogeochemical functions of these wetlands (Mitsch and Gosselink, 1993).

It was hypothesized that denitrification potential would be lower in RWOH wetlands than in those wetlands with winter surface flooding because denitrification occurs in anoxic soils and sediments (Groffman and
Hanson, 1997). Denitrifiers are facultative anaerobic microorganisms that do not use NO$_3^-$ as a terminal electron acceptor unless oxygen is absent or concentrations are very low. Therefore, soils which are inundated or saturated should have higher populations of denitrifiers than soils which are not inundated or are inundated for shorter periods of time. No significant differences in denitrification potentials were detected among wetland types in any season but fall, most likely due to the high sample variation. Despite this, N$_2$O evolution was consistently highest in NAT soils and lowest in RWOH soils. Although the RWOH wetlands were saturated in the upper 15 cm of soils, the NAT and RWH wetland soils were saturated for twice as long during the year. Length of saturation will affect microbial composition (i.e., numbers of aerobes vs. anaerobes) and oxygen concentrations in soils (Mitsch and Gosselink, 1993). The denitrification enzyme activity (DEA) assay is an indicator of reducing conditions because expression of the denitrification enzyme, nitrate reductase, is strongly correlated with the need for that enzyme (Ambus 1993; Hanson et al., 1994; Groffman et al., 1996). Because denitrification potential, a measure of nitrate reductase, was much higher in NAT and RWH soils than in RWOH soils, the results of the year-long DEA measurements clearly indicate moisture conditions of these soils and demonstrate that restoration of surface flooding will lead to greater denitrification capacity because it affects soil characteristics which influence heterotrophic microbial activity.

Other factors which affect denitrification in soils are substrate concentrations and organic matter availability to denitrifiers, the majority of which are heterotrophs (Drury et al., 1991; Ambus, 1993; Drury et al., 1996).
1998). Our year-long measurements of soluble organic carbon (SOC) did not indicate any differences among soil SOC concentrations in the different wetlands during most of the year. In January, however, when rainfall was frequent, a large peak was measured in SOC concentrations in surface soils of NAT wetlands. This peak is most likely due to SOC leaching out of litter present on soil surfaces in the NAT wetlands and into the soil. Average litter mass accumulated on soils of NAT wetlands was about twenty times greater than litter accumulated on soils of restored wetlands. Because organic carbon concentrations will affect the numbers of heterotrophs that soils can support, presence of a litter layer is an important component of forested ecosystems (Davidson and Swank, 1987).

It was also hypothesized that presence of a leaf litter layer on soil surfaces was affecting nutrient dynamics and microbial activity in the upper soil surface. Although not statistically significant, the NAT wetlands had higher heterotrophic microbial activity and readily mineralizable carbon (RMC) concentrations in the upper 15 cm of soils than restored wetlands. Results of this research indicate that litter present in NAT wetlands is a significant contributor to soil organic matter concentrations. Litter layer accumulation is a product of time and ecological succession and therefore a newly restored wetland will not have leaf litter accumulated on soil surfaces. As time passes however, natural processes of vegetation succession, deposition, and decomposition will add organic matter to soils. Therefore, it may take longer for a newly restored BLH wetland to have soil characteristics comparable to those of one that has remained undisturbed.
Along with presence of a litter layer, this research indicates that surface flooding may be bringing in dissolved and particulate organic matter which will contribute to soil nutrient dynamics. Although the RWH wetlands did not have any more litter accumulated on soil surfaces than RWOH wetlands, carbon concentrations and microbial activity were higher in the former than in the latter. Because RWH wetlands had hydrology re-established, they have standing water present out of which organic matter may settle, thus influencing microbial activity and microbial community composition. In addition, flooding influences length of saturation which will directly influence microbial activity and indirectly affect decomposition and accumulation of organic matter. It has long been understood that hydrology is the driving force of any wetland and results of this research confirm this belief. The hydrology of a wetland includes length and duration of flooding and these factors will affect soil saturation and, thus, ecosystem biogeochemistry.

One way to improve biogeochemical functions of a restored wetland may be to increase microbial activity. Numbers of heterotrophs will rapidly increase upon addition of an organic carbon substrate (Gaudy and Gaudy, 1983). It was hypothesized that introduction of organic matter to RWH and RWOH soils would increase heterotrophic microbial activity and denitrification rates. The results of a one-month amendment study demonstrated that after addition of cotton gin trash to soils both microbial activity and denitrification rates were much greater than in the same soils without amendments added. This effect was especially predominant in RWOH soils amended with cotton gin trash. These results indicate that
RWOH soils may be carbon limited relative to microbial growth and relative to carbon concentrations in soils of the other wetlands studied. Cotton gin trash is a readily available amendment in Louisiana and its use in wetland restoration could provide a means for its beneficial disposal, in addition to increasing biogeochemical functions of restored wetlands. Field studies should be conducted to determine how cotton gin trash will affect biogeochemistry in a natural setting, the length of time carbon will remain in soils, and the optimum time for amendment addition.

Although denitrification potentials, microbial activity, soil moisture, and carbon concentrations were lower in the RWOH wetlands than in the other two wetland types, it is important to note that denitrification did occur in soils of these wetlands. Therefore, RWOH wetlands have the capability to denitrify influent NO$_3^-$ from runoff water. However, runoff water from agricultural fields does not flood RWOH wetlands because it flows through drainage ditches which have been dug around these areas. Because they are not connected to the surface hydrology of the watershed, RWOH wetlands would not be effective in improving water quality.

Hydrology is relatively easy to restore, through the installation of flashboards or the filling in of drainage ditches. However, because these wetlands are part of an agricultural watershed, care must be taken to prevent flooding of upstream agricultural areas. Thus, although it is relatively simple to install a flashboard riser or fill in a drainage ditch, it is much more difficult to establish land and water elevational relationships. The goals of hydrologic re-establishment in these BLH wetlands are to restore the seasonal flooding regime and to flood agricultural runoff water.
onto these wetlands so that nutrients and sediments may be removed. To accomplish these goals, it is essential to understand the annual flood cycle and hydrologic relationships of these watersheds to provide water delivery at appropriate flood times and to control water depth across the wetland, while not affecting cropland upstream.

After four years of research in restored and natural BLH wetlands in the Tensas River Basin, it is the overall conclusion of this study that re-establishment of hydrology is essential to creating a functional BLH wetland and to maintaining the integrity of receiving water bodies within the watershed. Because these wetlands are important for maintaining water quality in areas where agriculture predominates, it is important to fill in or establish flashboard risers in drainage ditches so that runoff water will flood these wetlands. As this research has demonstrated, surface flooding and prolonged soil saturation in the upper 15 cm of soil is essential to organic matter dynamics and soil microbial populations. It is important to optimize these soil characteristics so that water quality functions of these wetlands, primarily denitrification, will be enhanced. Although the natural hydrology of the Tensas Basin has been permanently altered by a combination of flood control levees and drainage ditches, biogeochemical functions can be restored through planned manipulation of these ditches. Wetlands which have drainage ditches around them have essentially been removed from the surface hydrology of the watershed and, in order to receive benefits such as denitrification and organic matter export from these valuable ecosystems, they must be connected back to the watershed.
REFERENCES


VITA

Rachael Grimsley Hunter graduated from the University of Tennessee, Knoxville, in May 1989. In August 1992 she began attending Tennessee Technological University in Cookeville, Tennessee, taking biology and chemistry classes. She entered the graduate program in biology during the spring semester, 1994. Rachael received her master of science degree in biology from Tennessee Technological University in May 1996. For her master's research Rachael investigated factors affecting softstem bulrush growth and removal of nitrogen, phosphorous, and organic carbon from municipal wastewater in wetland microcosms. Rachael is a candidate for the degree of Doctor of Philosophy in the Department of Oceanography and Coastal Sciences.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Rachael Grimsley Hunter

Major Field: Oceanography and Coastal Sciences

Title of Dissertation: Comparison of Biogeochemical Functions Between Restored and Natural Bottomland Hardwood Wetlands

Approved:

[Signatures]

EXAMINING COMMITTEE:

[Signatures]

Date of Examination: March 3, 2000