NITRATE-NITROGEN DYNAMICS IN TRIBUTARIES OF THE SANTA FE RIVER WATERSHED, NORTH-CENTRAL FLORIDA

By

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Abstract of Thesis Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Master of Science

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Nitrate runoff from agricultural systems is an increasing concern because of its potential effect on the health of both humans and ecosystems. Riparian systems have been shown to reduce nitrate concentrations in soil and water as a result of denitrification processes that occur under anaerobic conditions. The Santa Fe River Basin in north central Florida contains many tributaries that drain adjacent agricultural systems and in the eastern part of the watershed that discharge to the Santa Fe or New Rivers. In central areas of the Santa Fe River, however, these tributaries on occasion discharge directly to the Floridian aquifer due to the karst and partially confined geology of the region. Increasing evidence suggest that nitrate concentrations in surface and groundwater are increasing, and in some instances have exceeded EPA safe drinking water standards. In an effort to better understand nitrate dynamics and denitrification potential of channel bed and riparian wetlands along tributaries of the Santa Fe River, a two year research investigation was established at Boston Farm-UF/IFAS Santa Fe River Beef Research Unit (SFRBRU).

Fundamental questions addressed by this research include 1) what are the seasonal dynamics of nitrate concentrations within two tributaries of the Santa Fe River, 2) are there differences in stream reach or stream fluvial morphology that influence nitrate assimilative

capacity, 3) what effect does distance from stream have on soil denitrification potential and 4) what effect does nitrate concentration have on denitrification potential within stream reaches. To answer these questions two streams on the SFBRU were monitored.

Results show little variation in nitrate concentration along a low nitrate concentration tributary. Along a high nitrate tributary, however, concentrations were reduced an average of 31% from headwaters to discharge during the study. Decreases in nitrate concentration were not uniform along the length of the stream, but instead indicate that several types of stream reaches have significantly greater nitrate assimilative capacities than others.

Soil characterization and denitrification studies indicate that nitrate, carbon and anaerobic conditions are limiting denitrification in these tributaries.

CHAPTER 1
BACKGROUND AND SITE DESCRIPTION

Introduction

Nitrogen is the most abundant element on earth, yet it is often the most limiting nutrient for plants and microbes in marine and terrestrial ecosystems (Goldman 1999, Casblanq 1999, Burns 1992). Nitrogen is limiting because it is predominantly present in the atmosphere as dinitrogen gas (N_2), a nitrogen form unavailable to most organisms. Humans, however, have dramatically increased the amount of available N on earth by a factor of 10 through anthropogenic and industrial N fixation (Galloway et al. 2004).

In 1913, the Haber-Bosch process was developed to convert N_2 to NH_3 for fertilizer to improve food production. Combustion of fossil fuels along with the cultivation of rice, legumes, and other N-fixing crops has also increased biologically available forms of N, commonly in the form of ammonium (NH_4^+) or nitrate (NO_3^-) (Galloway et al. 2004). Inputs of available nitrogen dramatically increase plant productivity; however, with extensive nitrogen loading to an ecosystem, more N may be available than plants and microbes can use (Aber et al. 1989). As excess nitrogen accumulates over time, it can have significant effects within an ecosystem. The Nitrogen Cascade refers to changes that occur as an ecosystem becomes saturated with nitrogen. There is an initial increase in productivity; however, over time, nitrogen loading has been shown to decrease biodiversity in forests, grasslands, lakes, and streams (Aber et al. 1995, Vitousek et al. 1997). Soil acidicification and a decrease in soil fertility may also occur because leaching of nitrate ions from the soil facilitates the release of base cations such as calcium.

There are a number of other detrimental effects that nitrogen accumulation can have on the growth and health of plants in natural and agroecosystems. For instance, with an oversupply of nitrogen, excessive vegetative growth and plant cell enlargement can cause a plant to become

weak and top heavy. Other effects include delayed plant maturity and reduced resistance to disease and pests (Brady et al. 2002).

Excess nitrogen loading also has profound effects on waterways. As streams, creeks, or rivers with elevated levels of dissolved organic nitrogen (DON), ammonium (NH_4^+) or nitrate (NO_3^-) drain into ponds, lakes, and oceans, eutrophication and degradation of water quality can occur (Seitzinger 1988). This can lead to algal blooms, fish kills, change in species composition, and hypoxic conditions (Rabalais et al. 1996, van der Hoek 2004). Many zones of severe hypoxia occur where freshwater rivers high in nutrients enter coastal waters such as those near Louisiana, New York, New Jersey, Alabama, Texas, and Florida leading to mass mortality of benthic communities and stressed fisheries (Diaz 2001).

Nitrate, an inorganic form of nitrogen, is unique because it is a negatively charged ion, making it more susceptible to leaching than other positively charged nitrogen species that adhere to negatively charged soil particles. As nitrate moves in water through the soil and enters ground and surface waters, it can have detrimental effects on humans, animals, and ecosystems. Concentrations of nitrate in drinking water greater than 10 mg L^{-1} are considered a health hazard to humans and animals. Excess NO_3^- can cause methemoglobinemia or blue baby's syndrome and has also been linked to brain tumors in children and to forms of stomach cancer (Forman 2004).

Respiratory infections and problems related to thyroid metabolism are also effects associated with high nitrate levels in drinking water (Follett and Follett 2001). Nitrate concentrations above 1 mg L^{-1} have also been shown to be toxic to amphibians and insects (Rouse et al. 1999).

Nitrogen Removal in Riparian Wetlands

Intact riparian ecosystems have been found to reduce nitrogen concentrations in surface and groundwater. The ability of these buffer areas to transform nutrients is important in streams adjacent to agriculture areas that drain to freshwater and marine systems subject to eutrophication (Lowrance 1992). Although these wetlands can be relatively small in area, they can be a major zone for nitrogen retention in plants (Schaede and Lewis 2006) or nitrogen transformation through denitrification (Fennessy and Cronk 1997).

If ground or surface water comes into contact with plant roots, riparian plants can take up nutrients from the water column or soil porewater, thus providing a temporary sink for nitrogen. Schaede and Lewis (2006) found that increased N loading in a nitrogen limited system caused an increase in plant tissue %N and changes in the root to shoot ratio in plants due to increases in nutrient use efficiency and productivity. Yet, as plants senesce, most of the nitrogen will leach from the plant or be mineralized by microbes, releasing it to the ecosystem. Plants can remove a significant amount of nitrogen from soil and water; thus, unless plants are harvested or a portion of the biomass accumulates as peat, they do not provide a long term sink for nitrogen.

Denitrification, another process that removes nitrogen in riparian areas, takes place in soils and sediments under anaerobic conditions. This reaction occurs when facultative heterotrophic bacteria must use alternate electron acceptors during respiration under low oxygen conditions. Nitrate is reduced to dinitrogen, nitric and nitrous oxide gases that are lost to the atmosphere and thus nitrogen is removed from the water column.

This is a long-term sink for nitrogen since these gases are only available to a few micro-organisms during nitrogen fixation. As a result of flooded conditions, at least half of the denitrification on land has been found in wetlands, lake sediments, and riparian ecosystems (Bowden 1986).

Restored wetlands in agricultural landscapes can be self-sustaining and effective at removing excess N if properly managed. For the management of animal waste, denitrification in riparian areas can be a valuable process to remove N from liquid manure and other non-point source pollutants that are land applied (Lowrance et al. 1998). Cleaner water, however, may come at a cost to air quality. Denitrification plays a role in global climate change because it generates greenhouse gases. If NO_3^- is not reduced completely to N_2, microbial by-products N_2O and NOx will be the end products of denitrification. Production of N_2O rather than N_2 is favored at low pH (Johns et al. 2004), low temperature, and high oxygen and nitrate concentrations (Chapin et al. 2002). N_2O has a long residence time in the atmosphere due to its low reactivity. This gas contributes to global warming since it can absorb infrared radiation and has the capacity to contribute about 300 times the greenhouse effect as one molecule of CO_2 (Schlesinger 1997). Also, in reactions in the stratosphere, this produces NO, a gas that contributes to the destruction of good ozone.

Another intermediate product of denitrification is NOx. This is a very reactive gas that is involved in the production of stratospheric ozone, or the photochemical smog that is common in highly populated urban areas. Smog is known to cause lung problems in humans. NOx is also a component of acid rain in the form of nitric acid. Not only is this a strong acid that decreases the pH of soils, it also deposits available N in ecosystems.

Regulators of Denitrification

Several factors influence where and at what rate denitrification occurs. Denitrification requires the presence of a labile carbon source, anaerobic conditions, a nitrate source, and an active microbial community. Other abiotic factors such as temperature and soil texture can affect rates of denitrification.

A number of studies have found the presence of a readily available carbon source to be the primary factor affecting rates of denitrification in ecosystems (Marienssen and Schops 1999). Under waterlogged conditions, the breakdown of organic matter is slow because, in the absence of O_2, microbes must use an alternate electron acceptor such as CH_4, NO_3^-, Fe^{3+}, Mn^{4+}, or SO_4^{2-} during respiration. These electron acceptors are not as energetically efficient as O_2, which leads to a slower decomposition rate and the accumulation of organic matter and, thus, electron donors for the denitrification process (D'Angelo and Reddy 1999). Dissolved organic carbon (DOC), another source of carbon in riparian ecosystems, has been shown to be highly correlated with rates of denitrification (Desimone and Howes 1996).

Moisture content also affects denitrification since anaerobic conditions must be present for denitrification to occur. Studies show that denitrification rates have a significant relationship with moisture content (Schnabel et al. 1997, Schipper et al. 1993). Schnabel et al. (1997) found that moisture content decreased with distance from streams in riparian areas and increased with soil depth; however, where moisture conditions are optimal, other factors such as carbon may be limited. Moisture content in soils is also affected by water table fluctuations, therefore seasonal or event driven changes in water table can strongly influence the nitrogen cycle in the processes of nitrification, mineralization, and denitrification (Reddy et al. 1989). In areas subject to high loads of nitrogen, a fluctuating water table will increase the nitrogen removal efficiency in riparian zones (Hefting et al. 2004).

The process of denitrification is also controlled by the presence of a nitrate source. Systems that are flooded year-round must rely on the diffusion of nitrate from aerobic to anaerobic layers for denitrification to take place. If a system does not have a nitrate source, then denitrification may be controlled by nitrification rates. Nitrification is the process where NH_4^+ is

oxidized to NO_3^- by autotrophic bacteria. This occurs in aerobic portions of the soil, and nitrate can diffuse to anaerobic soils along a concentration gradient. Even if microsites within the soil are anaerobic, seasonal water table fluctuations stimulate nitrification (Schipper et al. 1993). If O_2 concentrations are too low and nitrification cannot occur, however, NO_3^- production can be a rate-limiting step of denitrification.

Denitrification is indirectly affected by soil texture. Higher rates of denitrification have been found in fine-textured soils rather than sandy soils (Hefting et al. 2004). For instance, during storm events, flooded conditions ideal for denitrification can be short-lived because of the rapid drainage that occurs in coarse, sandy soils. This is related to water filled pore space (WFPS); as WFPS increases so do rates of denitrification. Aulakh et al. (1992) found that denitrification only occurs at a WFPS of 60% and higher.

All microbial processes are regulated by temperature. Q_{10} is the rule of thumb that with every 10 degree increase in temperature, biological activity will double. Denitrification is a mechanism carried out by microbes, and it has also been shown to be highly affected by temperature in lab studies (Fischer and Whalen 2005, Maag et al. 1997).

The limiting factor for denitrification varies among ecosystems, as well as in microsites within an ecosystem. For instance, denitrification can take place in microsites of the soil profile if a soil is well-drained with seasonal wetting periods. This creates anaerobic "hotspots" within the soil profile where denitrification can take place. High carbon microsites are also hypothesized to be a major source of error for rates of denitrification measured. Knowledge of soil properties, hydrology, climate, and the biotic community can help predict how effective a system will be at removing nitrate through denitrification. This may be especially important near agricultural areas with connections to groundwater.

Nitrogen and Florida's Waters

Waterways impacted with nitrates are especially problematic in areas where direct connections between ground and surface waters occur. These connections can lead to the contamination of aquifers, and, subsequently to sources of drinking water, especially near industrial and agricultural landscapes. In many parts of Florida, connections can be numerous as a result of geology.

Limestone from the state's marine origins lies beneath Florida soils. A geological formation known as karst forms when limestone comes into contact with carbonic acid. When CO_2 is dissolved in water from the break-down of organic matter, it forms carbonic acid (H_2CO_3). As carbonic acid comes into contact with limestone, calcium carbonate is easily dissolved. Over time, holes in the limestone develop from this erosion process forming karst. Some examples of karst formations in Florida are caves, springs, and sinkholes, all of which provide a conduit between surface and ground waters.

In most of Florida, this direct connectivity is not a concern because an impermeable layer of silt and clay, called the Hawthorne layer, underlays the soil. The Hawthorne formation was formed by the deposition of phosphorus-rich clay and sand from ancient rivers and can be as deep as 800 ft in parts of western Florida. When the Hawthorne layer is intact, there is no direct connection to the Floridan aquifer. In the north–central portion of the state, however, along the Ocala Uplift, the Hawthorne layer has thinned so limestone is within 0–50 ft of the ground surface (Figure 1-1). The interface zone between intact and eroded Hawthorne layer is called the Cody Scarp. Along this interface, thinning of the Hawthorne layer allows increased infiltration of surface water to underlying limestone leading to dissolution and occasional collapse forming sinkholes. Once the Hawthorne layer is completely eroded, direct leaching of surface waters and rainfall through the soils to the aquifer is possible. Interaction between surface water and

groundwater along this zone is significantly increased and can lead to water quality degradation within the aquifer.

Agriculture management practices in areas where karst formations are present can have significant ecological impacts on ecosystems and watersheds from applied fertilizers or animal waste. These non-point sources are subject to runoff and leaching into ground and surface waters, introducing nitrogen to waterways that otherwise might be nutrient limited. One area that illustrates the change in hydrologic connectivity along the Cody Scarp and potential impacts of agricultural activities due to these connections is the Santa Fe River Watershed.

The Santa Fe River Watershed

The Santa Fe River watershed covers 3,585 square kilometers in north central Florida and drains into the 121 km long Santa Fe River. This watershed lies within the Suwannee River Basin that drains to the Gulf of Mexico (Figure 1-2). This area of Florida typically receives a mean annual precipitation of 1.3 meters and has a mean annual temperature of 24^{o}C.

Dominant land use types in this area of Florida are silviculture, row crop and pasture agriculture, and undeveloped natural areas (Figure 1-3). In the upper and middle watershed, agricultural and timber production areas are of concern because fertilizer and animal waste may be susceptible to runoff and leaching into tributaries, creeks, and springs. Along the Santa Fe River, numerous tributaries drain agriculture areas that contribute water and nutrients to the river (Figure 1-4). Major tributaries include the Ichetucknee, Olustee, New, and Sampson Rivers.

Because of the geology of this region, these surface waters can come into contact with karst formations through sinkholes. The river actually enters a major sinkhole near the Cody Scarp and goes completely underground for 5 km and re-emerges before entering the Gulf of Mexico (Figure 1-4). Therefore surface waters in the upper and middle Santa Fe watershed will eventually enter groundwater and then eventually drain to freshwater and marine systems

possibly leading to eutrophication, aquifer contamination, algal blooms, and other degradations in water quality. Possible nitrate sources in this watershed include septic tanks, atmospheric deposition, fertilizers, and animal waste.

Objectives and Hypotheses

The main goal of this study was to characterize water quality in several tributaries of the middle Santa Fe River watershed and to determine the extent to which riparian soils can effectively reduce nitrate concentrations in waters impacted by agriculture. Specific objectives were to

- evaluate spatial nitrate-nitrogen dynamics in tributaries and riparian wetlands at the Boston Farm – Santa Fe River Ranch Beef Unit;
- identify reaches within the tributaries that may have a greater capacity to remove nitrate;
- determine if carbon or nitrogen is limiting denitrification in these riparian areas;
- determine the denitrification potential in a riparian wetland zone characteristic of tributaries in the Santa Fe River basin;

Findings from studies that addressed these objectives are outlined in the following chapters of this thesis. In Chapter 2, nitrate concentrations as well as other nutrients in surface waters of the two tributaries are discussed.

Soil characteristics and denitrification potential of soils along the tributary and adjacent wetlands are addressed in Chapter 3. Chapter 4 is a summary chapter to discuss implications of these finding and suggestions for future research.

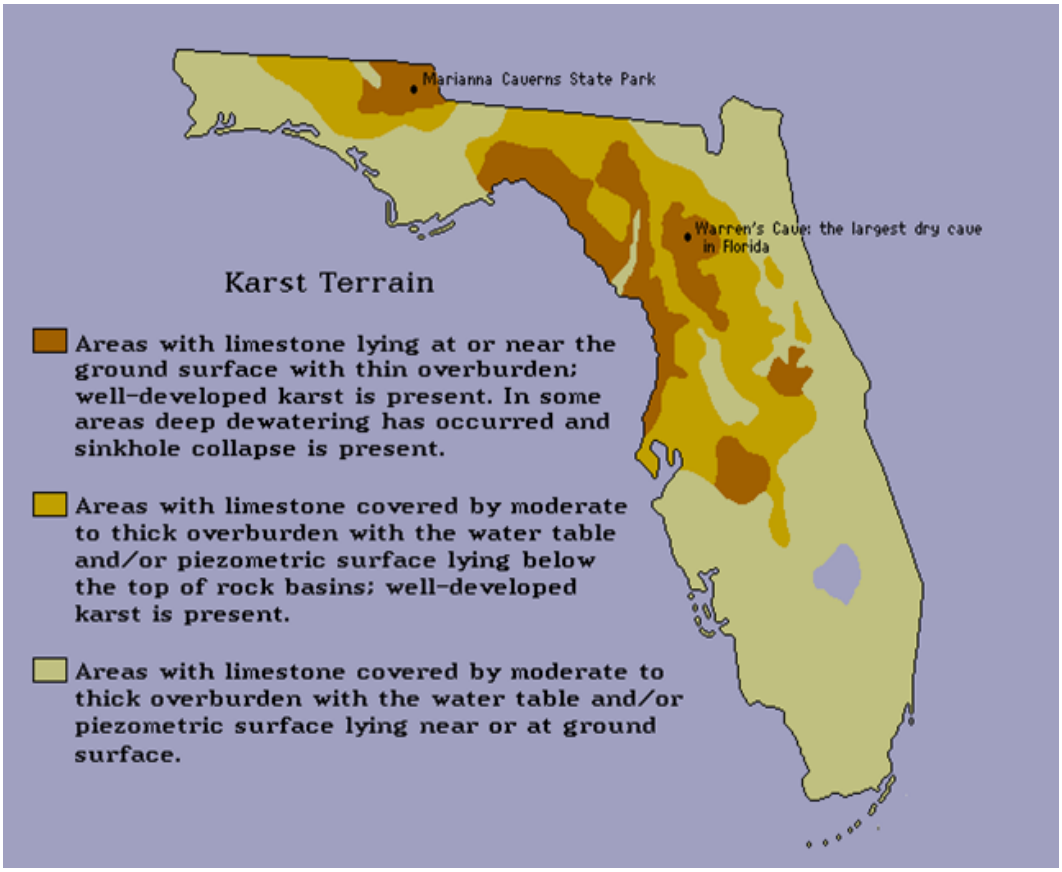

Figure 1-1. Along the Ocala Uplift in eastern Florida, the Hawthorne layer becomes discontinuous, allowing surface and groundwater connections to occur. (Reprinted with permission from The Florida Speological Society, Gainesville, Florida, http://www.caves.com/fss/pages/misc/geology.htm.)

Figure 1-2. Location of the Santa Fe River and Suwannee River, relative to the Cody Scarp (Reprinted with permission from Martin, J, Screaton, E., and Moore, P.2004. Surface and ground water mixing along the Cody Scarp: An example from the Santa Fe River Sink-Rise system. USGS Suwannee River Basin and Estuary Integrated Science Workshop Proceedings.

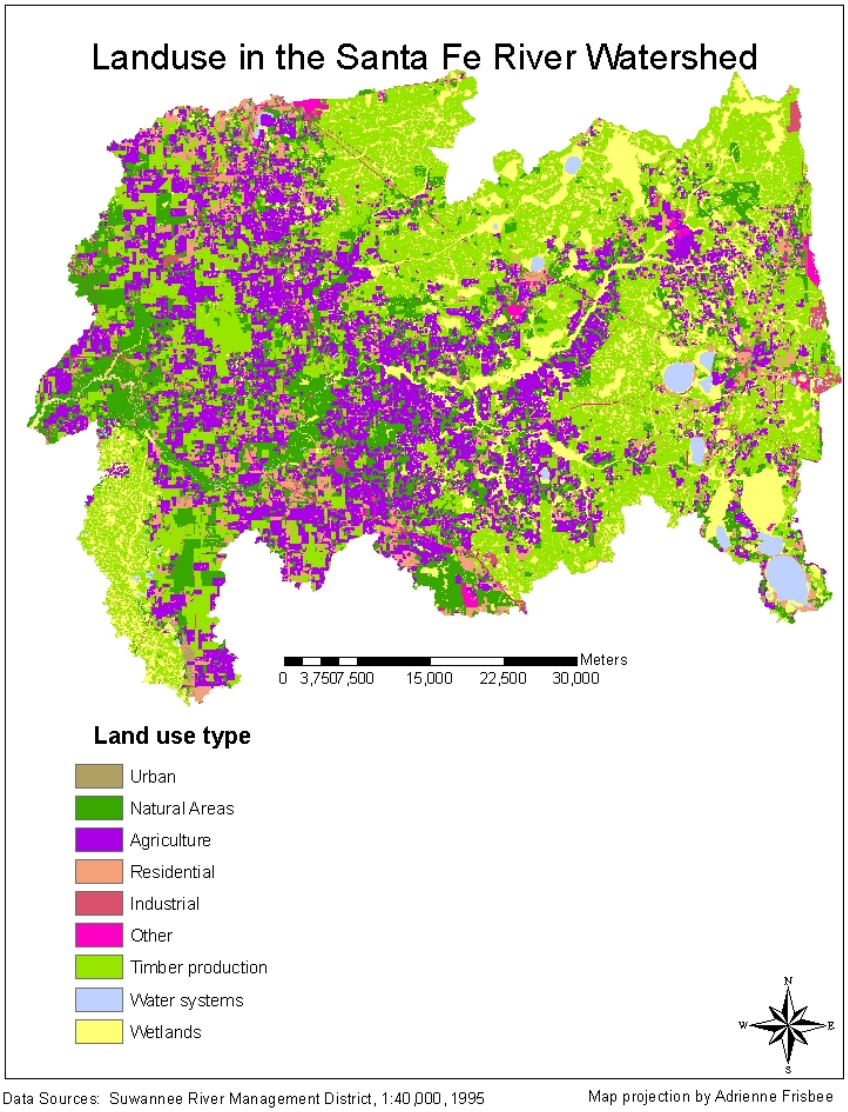

Figure 1-3. Dominant land uses common in the Santa Fe River Watershed

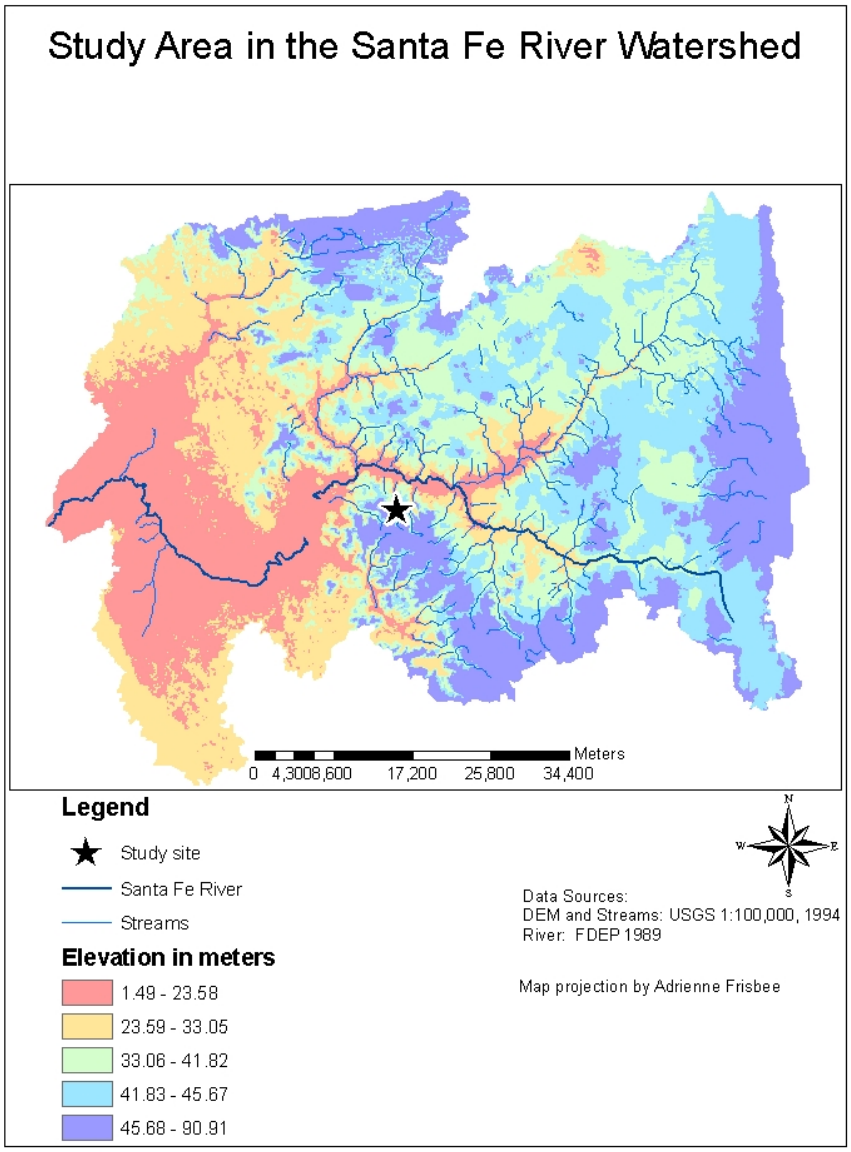

Figure 1-4. A digital elevation map of the Santa Fe River Watershed that shows the repeating pattern of tributaries and where the river goes underground in the western portion of the watershed

CHAPTER 2
WATER QUALITY MONITORING

Introduction

Numerous tributaries drain agriculture areas leading to the Santa Fe River that eventually drains to the Gulf of Mexico. High concentrations of nitrate in water can be detrimental to humans, and to marine or freshwater ecosystems. The research and development of methods to decrease nitrate in water is of interest especially where groundwater will be impacted. If a sufficient riparian buffer exists along these tributaries, there is the potential for nitrates and other nutrients to be reduced in the water column through denitrification or plant uptake. Water column nitrate concentrations may also decrease when freshwater systems are diluted by surface runoff or groundwater intrusion.

Tributaries of the Santa Fe River may have spatial differences in water quality as a result of biotic and abiotic factors. For instance, some reaches of a tributary may have plants that are able to immobilize nitrate from the water column. Other areas may be anaerobic with a labile carbon source, conditions ideal for denitrification. Other tributary reaches may be too channelized or sandy for significant nitrogen removal to occur. As a result, nitrate removal efficiencies in tributaries impacted by agriculture may vary along the length of each tributary.

Nitrate concentrations in tributaries draining agricultural areas may also have seasonal variations in water quality. For example, irrigation and fertilization practices are maximized at different times of the year according to plant needs, which can cause nitrate concentrations to vary in tributaries.

Precipitation and evapotranspiration can also alter tributary nitrate concentrations by diluting or concentrating nitrates. While long-term studies are necessary to thoroughly understand seasonal changes, inferences can be made on what may be driving measured fluctuations over time.

In order to better understand nitrate-nitrogen dynamics in tributaries of the Santa Fe River Watershed, a one year study was conducted on two tributaries that drain to the Santa Fe River at the Boston Farm- Santa Fe Beef Research Unit (SFRBU).

Objectives and Hypotheses

The major goal of the water quality study was to understand the fate of nitrate in two tributaries that drain to the Santa Fe River.

Specific objectives were to

1. make observations of how nitrate concentrations vary from month to month;
2. determine if different stream reaches remove more nitrate than others.

Specific Hypotheses:

1. Nitrate concentrations will vary over the course of the study as a result of season changes, fertilization, or irrigation. It is expected that highest fertilization rates will be in spring and summer, and therefore highest nitrate concentrations will occur during these months.
2. Some reaches in the tributaries will be more effective at removing nitrate from the water column than others. Reaches with plants in the water column or an available carbon source are hypothesized to remove more nitrate than sandy channel reaches without plants.

Materials and Methods

Site Description

The Boston Farm –Santa Fe River Ranch Beef Unit (SFBRU), a University of Florida property, provides an excellent representative site of typical landuse and topography along the middle third of the Santa Fe River. The research site is located about 30 miles northeast of Gainesville, Florida in Alachua County (Figure 2-1).

Soils in this watershed are sandy and are predominantly Ultisols, Spodosols, and Entisols. Specific soils in sampling areas are Sparr fine sand, Pelham, Plummer, and Masotte soils, and Chipley sand (SSURGO). The site has a number of features characteristic of north central

Florida's geologic and biotic communities. These include groundwater seeps, sinkholes, tributaries, ponds, and wetland communities.

Land use on the site consists of low intensity pastures bordered by forests and riparian areas associated with the tributaries. The research unit supports a low density cattle operation with about 300 heifers on 1,600 acres. Adjacent to the property is a plant nursery that is potentially responsible for elevated nitrate concentrations measured in water sampled from the site in 2004.

Two tributaries run the length of the property and drain to a floodplain leading to the Santa Fe River (Figure 2-2). Tributary 1 (T1) drains cattle pastures on the Santa Fe Beef Research Unit SFBRU as well as The Holly Factory, an ornamental plant nursery adjacent to the research site. During this study, cattle were only observed in the pasture bordering this tributary during the month of October. Tributary 2 (T2) had less flow than T1 and at times went underground or had low water levels during the sampling period. Cattle are kept out of this tributary by barbwire fencing, although runoff can still enter the stream from nearby pasture and from upstream during larger rainfall events

Field Methods

To address the hypotheses posed in this study, two tributaries in the Santa Fe watershed were selected on the Santa Fe River Beef Research Unit –Boston Farm (Figure 2-2). Along these tributaries, we designated transitional zones between morphologically different stream reaches. Eight morphologically discrete stream segments were designated using dominant vegetation type, degree of bank incision, and whether depositional or erosional processes were the principal drivers along the reach (Table 2-1, Figures 2-2 to 2-9). These classifications can be compared to the widely used Rosgen stream classification system which uses shape, slope and

pattern to classify streams and rivers (Rosgen and Silvey 1996). The Rosgen classification, however, does not take into account dominant vegetative community.

Once stream reaches were classified according to the above criteria, monthly water samples were taken at the beginning and end of each reach. This sampling method led to a total of 20 sampling stations along T1 and 10 along T2. For each sample station, an acid washed 250 mL bottle was rinsed three times with site water before collecting a sample. Care was taken to collect samples from an undisturbed portion of the water column in the middle of the channel at mid-water column depth. Water samples were then acidified to a pH of 2 with ultra pure concentrated sulfuric acid and put on ice for transport to the lab. In the lab, samples were transferred to scintillation vials. Samples to be analyzed for NO_3^-, NH_4^+, and DOC were filtered with a Whatman 0.45µm filter. Samples analyzed for total Kjeldahl nitrogen (TKN) were not filtered.

In May 2005 and March 2006, water samples were collected in T1 from the end of the tributary, through the floodplain and improved pasture up to the Santa Fe River, this resulted in an additional four samples. These samples were analyzed for NO_3^-, SRP and Cl^-.

Analytical Methods

All water samples were refrigerated and analyzed for nutrients within 28 days as recommended by the EPA. Nitrate, ammonium, and TKN were analyzed to determine the dominant nitrogen forms present in the water column. Nitrate was analyzed colorimetrically using the cadmium reduction method on a rapid flow or a discrete analyzer (EPA method 353.2). Ammonium was analyzed colorimetrically on a Technicon AAIII autoanalyzer (EPA. 350.1). TKN was determined by digesting the water samples with sulfuric acid and a copper sulfate mixture to convert organic forms of nitrogen to ammonium. Ammonium was then analyzed colorimetrically on a Technicon AAII (EPA. 351.2).

Dissolved organic carbon (DOC) and soluble reactive phosphorus (SRP) were analyzed because of their potential roles in nutrient limitation to plants and microbes, which can in turn affect the nitrogen cycle. DOC was analyzed on a Shimadzu TOC 5050a (EPA 415.1). SRP was measured colorimetrically on a spectrophotometer (EPA 365.1).

Chloride concentrations were measured to determine if nitrate concentrations in the tributaries were being diluted by surface runoff or groundwater intrusion. Chloride concentrations were analyzed on a Dionex Ion Chromatograph.

All above methods used the QA/QC requirements set by the Wetland Biogeochemistry laboratory which require a spike, repeat, standard, and blank to be run for every 20 samples analyzed.

Statistical Methods

All statistics were analyzed with JMP IN 5.1. T-tests were used to compare two means, and ANOVAs followed by Tukey-Kramer tests were used when comparing more than one mean. All data were tested for a normal distribution and transformed if necessary before performing analyses.

Results

Nitrate

For eleven months of sampling, nitrate concentrations in T1 had an average nitrate concentration of 4.73 ± 1.01 mg L^{-1} (mean ± SD). Nitrate concentrations in T2 were significantly lower than nitrate concentrations in T1 ($p < 0.001$) for all months, with an average of 0.03 ± 0.03 mg L^{-1} (Figure 2-10).

Nitrate concentrations in T2 were consistently low and showed no significant spatial or temporal variability. Therefore, the remainder of this chapter will focus on T1 for further

analyses of tributary nitrate-nitrogen dynamics. Nitrate concentration decreased by 13–81% from headwaters to discharge in T1 with an average reduction of 31% (Figure 2-11).

The Open Water (OW), Depositional Herbaceous (DH), Moderately Incised Herbaceous (MIH), and Slightly Incised Woody (SIW) reaches all removed significantly more nitrate per meter than the other six reach designations (Table 2-2).

Because of the relatively short sampling period, statistics were not performed to determine if differences existed between months or seasons. There were, however, considerable differences in mean tributary nitrate concentration between months and seasons that may be related to fertilization, irrigation, cattle grazing, or seasonal climate differences (Figures 2-12 and 2-13).

Ammonium and Organic Nitrogen

TKN and NH_4^+ were not significantly different in the two tributaries (Table 2-3). T1 had NH_4^+ concentrations of 0.14 ± 0.11 mg L^{-1} and TKN concentrations of 0.51 ± 0.32 mg L^{-1}. T2 had NH_4^+ concentrations of 0.22 ± 0.23 mg L^{-1} and TKN concentrations of 0.43 ± 0.29 mg L^{-1}.

Dissolved Organic Carbon and Soluble Reactive Phosphorus

T2 DOC concentrations were significantly higher than T1 concentrations ($p= 0.005$). T1 had an average DOC concentration of 5.79 mg L^{-1} ± 1.59, whereas T2 had an average of 12.94 mg L^{-1} ± 8.69.

SRP concentrations were analyzed for the March 2006 sample event. SRP concentrations were not significantly different in the two tributaries ($p= 0.5$). In T1, SRP concentrations decreased 72% along the length of the tributary (Figure 2-14). In T2, SRP was reduced by 60%, but increased when the tributary reached the floodplain (Figure 2-15).

Chloride

Chloride concentrations measured in T1 remained similar along the tributary until the sampling station just before the floodplain where it increases from 6.5 to 13.0 mg L^{-1} (Figure 2-16). Chloride concentrations were more variable in T2, with a maximum concentration of 8.2 mg L^{-1} (Figure 2-17).

Floodplain

Nitrate

Nitrate in T1 was reduced another 81% in May 20, 2005 and 86% in March 29, 2006 from the last station in T1 through the floodplain and improved pasture to the Santa Fe River (Figure 2-18).

Phosphate

March 29, 2006, SRP was reduced 10% as T1 went through the floodplain to the river (Figure 2-19).

Discussion

T1 and T2 were not significantly different in NH_4^+ or TKN concentrations. NO_3^- concentrations, however, were significantly higher in T1 than in T2. Because both tributaries are bordered by cattle pastures, T1 is believed to be significantly higher in nitrate as a result of runoff from landuse practices in the upper watershed which in Figure 2-2 can be identified as a horticultural nursery. T1 receives irrigation and storm water from the nursery, which is fertilized year round with NH_4NO_3, urea, and KNO_3 (T. Stevens personal communication 2006). Nolan and Stone (2000) sampled over 50 sites across the United States and found that the major source of nitrogen to groundwater was found to be from fertilizers rather than manure or atmospheric deposition. Average nitrate concentrations in groundwater were shown to be highest near agriculture areas (3.4 mg L^{-1}) when compared to urban areas (1.6 mg L^{-1}) and major aquifers

(0.48mg L^{-1}) (Nolan and Stone 2000). Although our study did not address nutrients in groundwater, it did find T1 surface waters to be impacted certain types of agricultural runoff.

Over a year of monitoring, T1 consistently showed a reduction of nitrate in the water column as water moved from headwaters to discharge. Many studies have shown that riparian areas can reduce nitrates in runoff before it reaches freshwater systems (Fennessy and Cronk 1997). This study, however, shows a reduction in nitrates in the stream channel. This may be a result of plant uptake, denitrification, or dilution by ground or surface waters. Chloride concentrations measured in this tributary showed no major change along the length of T1 suggesting that the decrease in nitrate concentrations is not due to a dilution by groundwater or surface runoff, but rather from plant uptake or denitrification.

Studies have shown phytoplankton and plants remove substantial amounts of nitrogen from water systems (Schaede and Lewis 2006, Bledsoe et al. 2004). Phlips et al. (2002) found phytoplankton in the Indian River Lagoon, Florida were most often limited by nitrogen. Phytoplankton populations were frequently observed at the SFBRU in the Open Water reach and in the floodplain (in winter), thus phytoplankton may provide a sink for nitrate in T1. Phytoplankton has also been shown to increase rates of denitrification by providing a labile carbon source to microbes (Sirivedhin and Gray 2006) Reaches with a closed tree canopy, however, are likely light limited, which would inhibit phytoplankton growth. Indeed, reaches with woody species showed little to no nitrate removal in the water column.

Smialek et al. (2006) showed higher rates of denitrification in soils with herbaceous species (*Juncus sp*) when compared to soils with woody species (*Salix sp*) present. Both woody and herbaceous plant species occur along the tributaries, and aquatic plant species grow in some

stream reaches. Both plant and algal species in these tributaries may have a role in the decrease in nitrate concentrations of the water column.

The decrease in nitrate was not uniform along the length of the tributary; some reaches removed more nitrate than others, while some reaches released nitrate into the water column. The Open Water, Depositional Herbaceous, and Moderately Incised Herbaceous reaches removed the most nitrate from T1 during the year of monitoring. Numerous characteristics may contribute to high nitrate removal in the Open Water. For instance, water has a long residence time in this reach, which increases contact time with soils, plants and phytoplankton. The long residence time also leads to deposition and build up of organic matter. The sediments in the stream channel are constantly flooded and likely anaerobic, making this an ideal location for denitrification. Finally, this reach has a number of species of aquatic plants along the edges of the tributary that may be assimilating nitrate.

The Depositional Herbaceous reach is a portion of the tributary that braids through organic and mineral deposits. These depositional areas have built up over time, and a number of plant species are present. As water braids through these zones, it may come into contact with plant roots that take up nitrate. The plants can also provide a carbon source for denitrification.

The Moderately Incised Herbaceous reaches have riparian and aquatic plants present that can take up nitrate. These reaches may also be receiving DOC as it leaches from the upland. It was, however, unexpected that this reach would remove a significant quantity of nitrate.

Nitrate concentrations in T1 did appear to vary over the course of the year. Highest mean concentrations were observed in October 2005, the only month that cattle were observed in the pasture directly adjacent to the tributary. As a result, nitrate concentrations did not decrease much along the length of the tributary. Spring was found to have the highest initial nitrate

concentrations in the water column. This likely corresponds with the higher fertilizer application rates at the beginning of the growing season at the nursery upstream (T. Stevens, nursery owner, personal communication 2006). The most nitrate removed along the length of T1, however, occurred in April. This is likely the result of warming soil temperatures and plant growth, which occur in the spring. On the other hand, the least amount of nitrate was removed along the length of the T1 in the fall. This may correspond with the release of nitrogen that occurs as plants senesce at the end of the growing season.

Carbon quality and quantity is important to nitrogen cycling in aquatic environments because of its effects on denitrification, nitrification, and mineralization. Strauss and Lamberti (2000) found that glucose and leaf leachates inhibited nitrification because heterotrophic bacteria outcompeted the chemoautotrophic bacteria responsible for nitrification. DOC was found to be significantly higher in T2 than T1, and this may inhibit nitrification in this tributary. On the other hand, DOC may be providing a carbon source for denitrification in T1. Finally, if a stream has high carbon and low nitrogen, most inputs of nitrogen will be rapidly assimilated into plant and microbial biomass (Schlesinger 1997). All of these processes could explain in part why nitrate concentrations were significantly lower in T2 than in T1.

Available phosphorus concentrations in the water column in T1 decreased from headwaters to discharge. Microbial and plant uptake may both have a part in SRP removal from T1. Phosphorus may also be adsorbing on to the surface of stream sediments.

Nitrate and SRP concentrations were both dramatically reduced in May 20, 2005 and March 29, 2006 in the floodplain. This may be due to a combination of denitrification, dilution, and plant uptake. Although it is unknown what process is reducing nutrient concentrations, it is

clear that the floodplain is important in removing nitrate and SRP from agriculture impacted waters in T1.

Overall, unlike T2, T1 waters were impacted by agricultural runoff. Nitrate concentrations, however, were reduced as T1 moved from headwaters to discharge in the floodplain. No change in chloride concentration along T1 suggests this reduction in nitrate is from denitrification or plant uptake rather than dilution from groundwater intrusion. SRP concentrations were also reduced along the length of the tributary.

Some nitrate reaches were more effective at nitrate removal than others, likely due to differences in carbon availability, retention time, and plant community. Nitrate was also significantly reduced as T1 passed through the floodplain.

Figure 2-1. Santa Fe River Beef Research Unit relative to Gainesville, Florida and the Santa Fe River watershed.

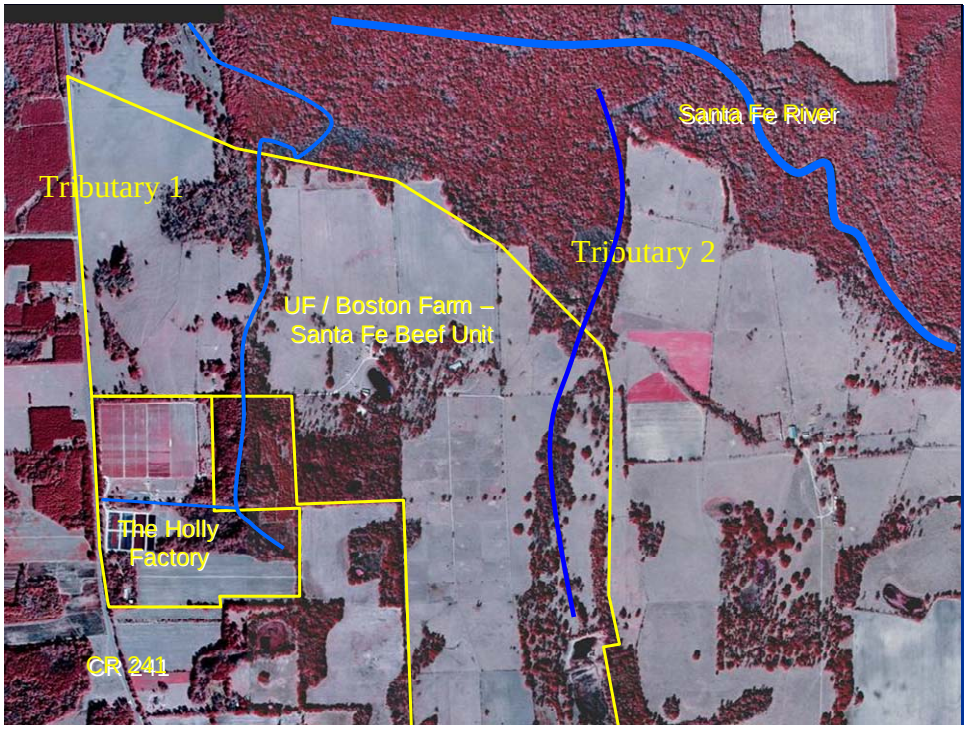

Figure 2-2. The SFBRU cattle pastures with an ornamental plant nursery south of the property and tributaries that drain to the Santa Fe River floodplain.

Table 2-1. Summary of stream reach characteristics.

Reach type	Acronym	Dominant vegetation	Depositional or Erosional	Degree of Bank Incision	Rosgen equivalent	Figure
Depositional woody	DW	tree species specificly *Carya sp., Pinus sp., Quercus sp., Magnolia grandiflora*	depositional	none	D or DA	2-2
Depositional herbaceous	DH	herbaceous plant species such as *Saururus cernuus, Juncus sp., Cephalanthus occidentalis, Hydrocotle umbellata, and Polygonum sp.*	depositional	none	D or DA	2-3
Slightly incised woody	SIW	tree species specificly *Carya sp., Pinus sp., Quercus sp., Magnolia grandiflora*	erosional	<30cm	B	2-4
Slightly incised herbaceous	SIH	herbaceous plant species such as *Saururus cernuus, Juncus sp., Cephalanthus occidentalis, Hydrocotle umbellata, and Polygonum sp.*	erosional	<30cm	B	2-5
Moderately incised woody	MIW	tree species specificly *Carya sp., Pinus sp., Quercus sp., Magnolia grandiflora*	erosional	<50cm	A	2-6
Deeply incised woody	DIW	tree species specificly *Carya sp., Pinus sp., Quercus sp., Magnolia grandiflora*	erosional	>50cm	Aa+	2-7
Open water	OW	aquatic emergent and floating plants	depositional	none	F	2-8
Floodplain	FP	tree species such as *Taxonium distichum and Nyssa sylvatica*	depositional	none	F	2-9

Figure 2-3. An example of Depositional Woody stream reach.

Figure 2-4. A Depositional Herbaceous reach.

Figure 2-5. An example of a Slightly Incised Woody reach.

Figure 2-6. Slightly Incised Herbaceous

Figure 2-7. Deeply Incised Woody.

Figure 2-8. An example of an Open Water reach

Figure 2-9. A Moderately Incised Woody reach.

Figure 2-10. The Santa Fe River floodplain.

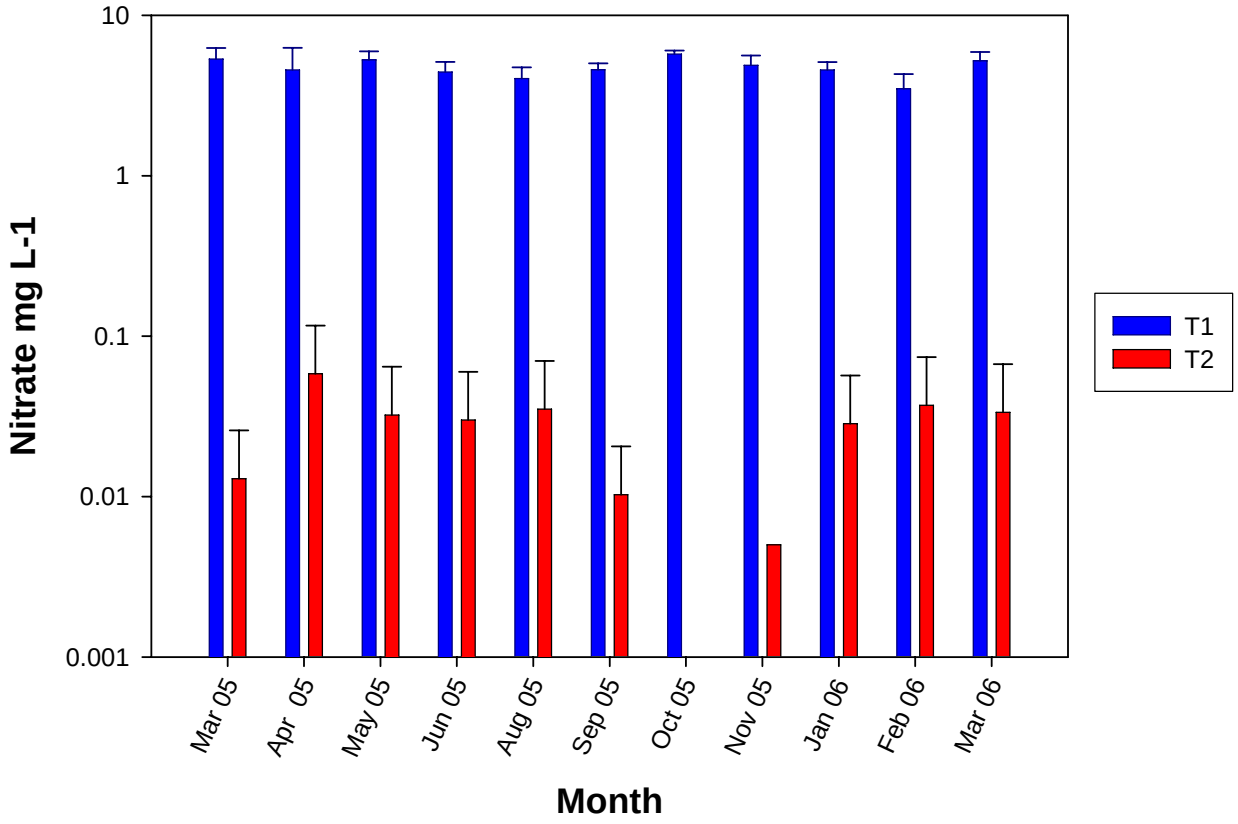

Figure 2-11. Log of mean nitrate concentrations of tributary 1 (T1) compared to tributary 2 (T2). Bars represent the standard deviation. T2 was not sampled in October due to the absence of surface water.

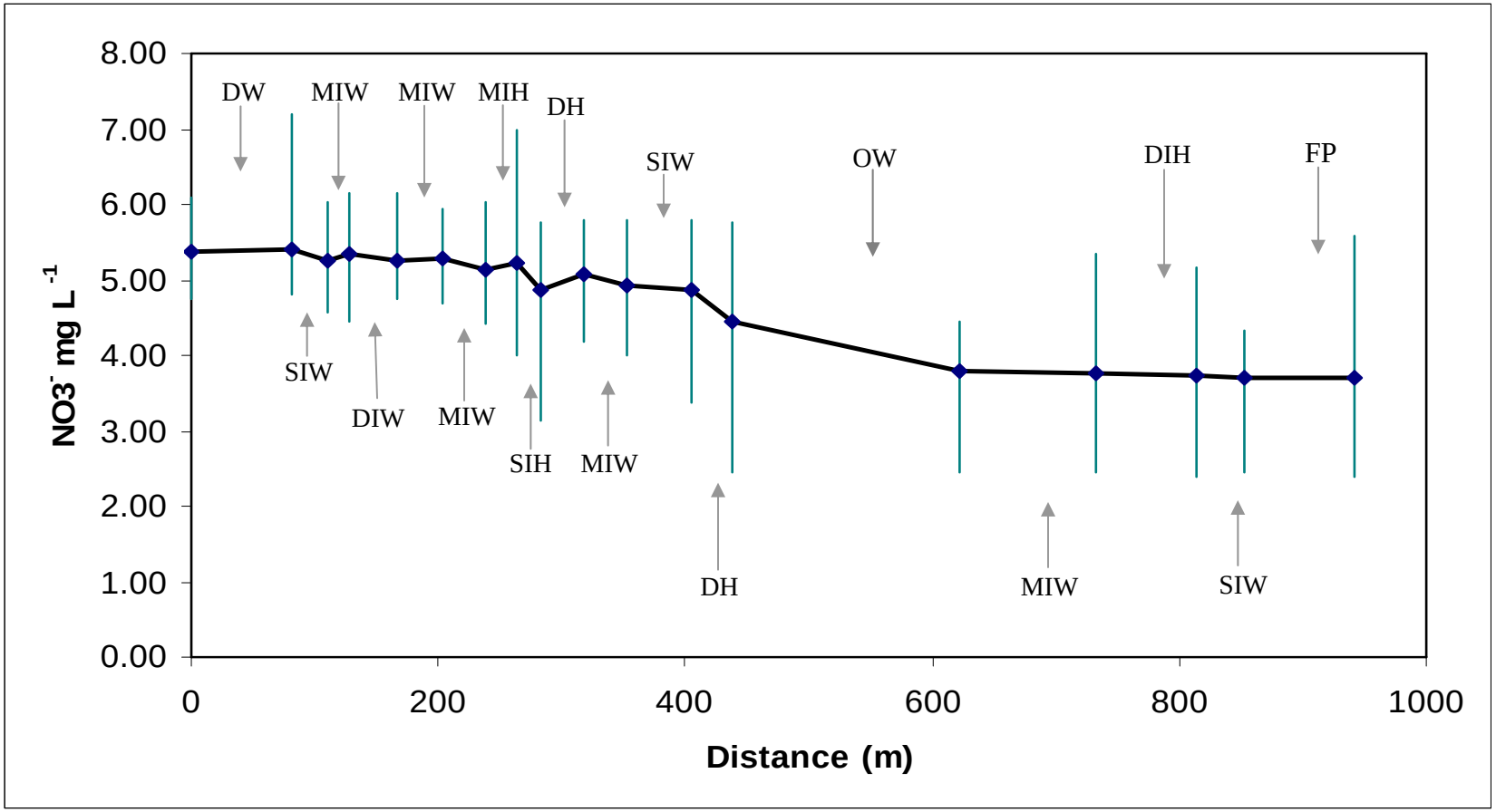

Figure 2-12. Average and range of nitrate concentration in tributary 1 (T1) from headwaters to discharge for all months sampled. Samples were collected at transitional point between classified stream reaches. The reach classifications are as follows: DW= Depositional Woody, SIW= Slightly Incised Woody, MIW= Moderately Incised Woody, MIH= Moderately Incised Herbaceous, SIH= Slightly Incised Herbaceous, DH= Depositional Herbaceous, OW= Open Water, DIH= Deeply Incised Herbaceous, and FP= Floodplain.

Table 2-2. Average percent change in nitrate per meter in tributary 1 (T1) for 11 months. Values with the same letter for significance level (SL) are not significantly different.

Reach Type	Mean	SD	SL
	% m^{-1}		
Open water	0.27	0.21	a
Depositional Herbaceous	0.13	0.65	ab
Moderately incised herbaceous	0.08	0.09	abc
Slightly incised herbaceous	0.04	0.10	abc
Deeply incised herbaceous	0.01	0.02	c
Floodplain	0.00	0.14	c
Moderately incised woody	-0.01	0.36	bc
Deeply incised woody	-0.04	0.20	bc
Slightly incised herbaceous	-0.04	0.42	abc

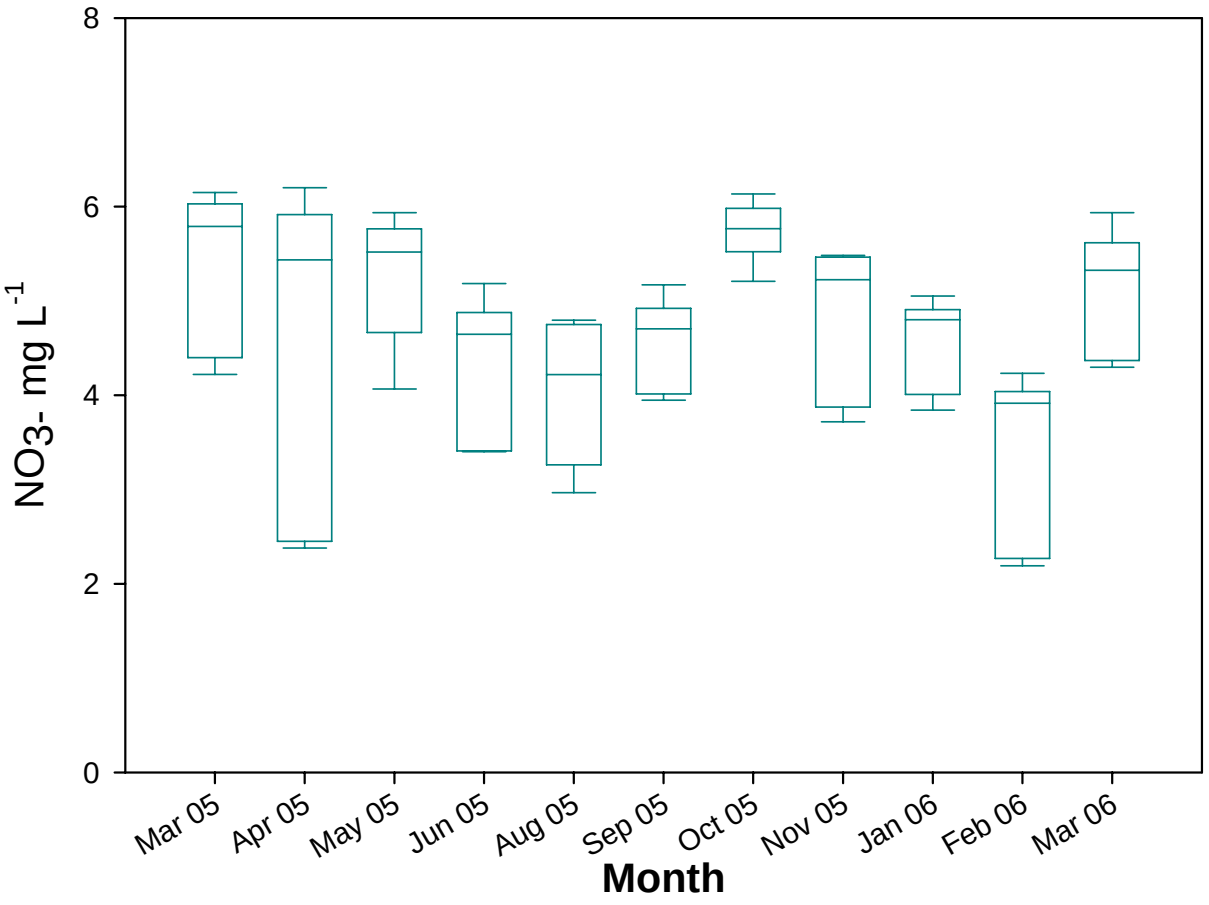

Figure 2-13. Quantiles of monthly nitrate concentrations measured in T1.

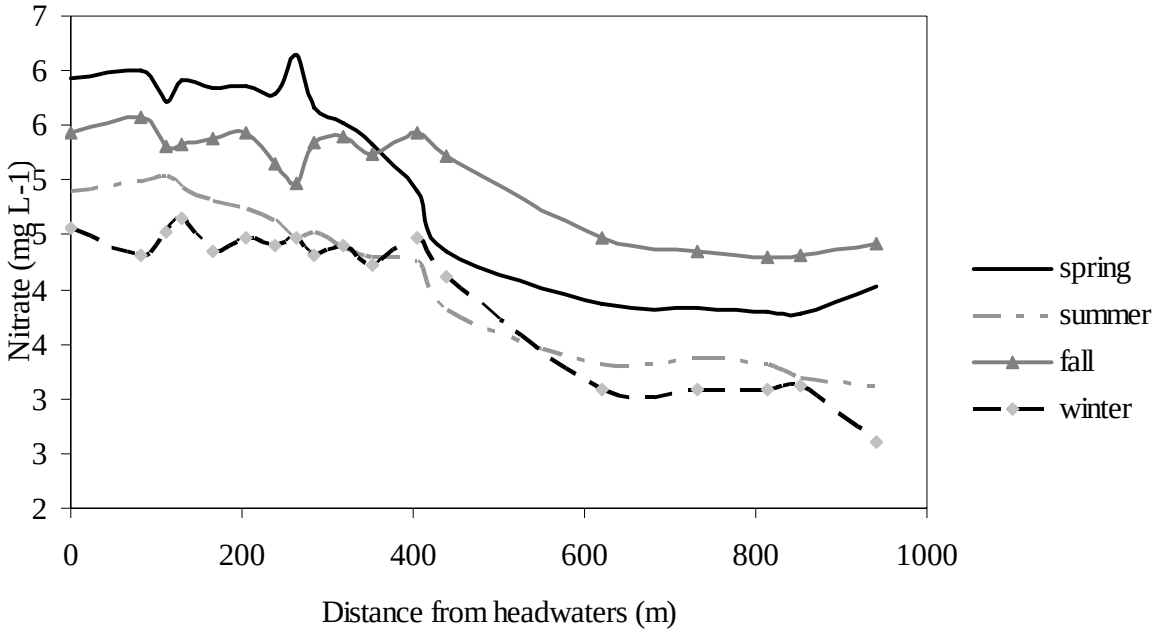

Figure 2-14. Nitrate concentrations by season in tributary 1 (T1). Spring = March, April, May; summer = June, August; fall = September, October, November; and winter = January, February.

Table 2-3. Summary of NH_4^+ and TKN measured in tributary (T1) and tributary 2 (T2) with one standard deviation in parentheses. Dashes indicate months that were not analyzed for NH_4^+ or TKN.

Month	Mean [NH4+]	Mean [TKN]
	mg L^{-1}	
	T1	
Mar-05	0.15 (0.08)	0.32 (0.07)
Apr-05	0.22 (0.08)	—
May-05	0.22 (0.24)	—
Jun-05	0.20 (0.35)	0.50 (0.32)
Aug-05	0.26 (0.31)	—
Feb-06	—	0.86 (0.25)
Mar-06	0.06 (0.02)	0.26 (0.16)
Average	0.14 (0.11)	0.51 (0.32)
	T2	
Mar-05	0.25 (0.11)	0.41 (0.16)
Apr-05	0.13 (0.03)	—
May-05	0.27 (0.04)	—
Jun-05	0.09 (0.20)	0.30 (0.05)
Aug-05	0.05 (0.03)	—
Feb-06	—	0.63 (0.35)
Mar-06	0.04 (0.01)	0.22 (0.04)
Average	0.22 (0.23)	0.43 (0.29)

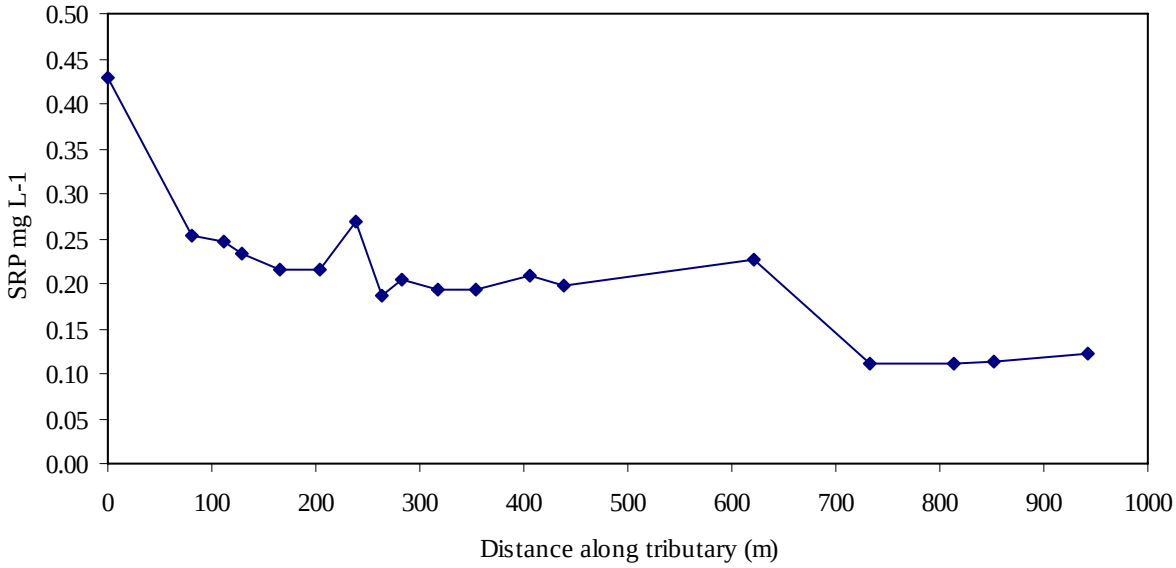

Figure 2-15. SRP concentrations in tributary 1 (T1), March 29, 2006.

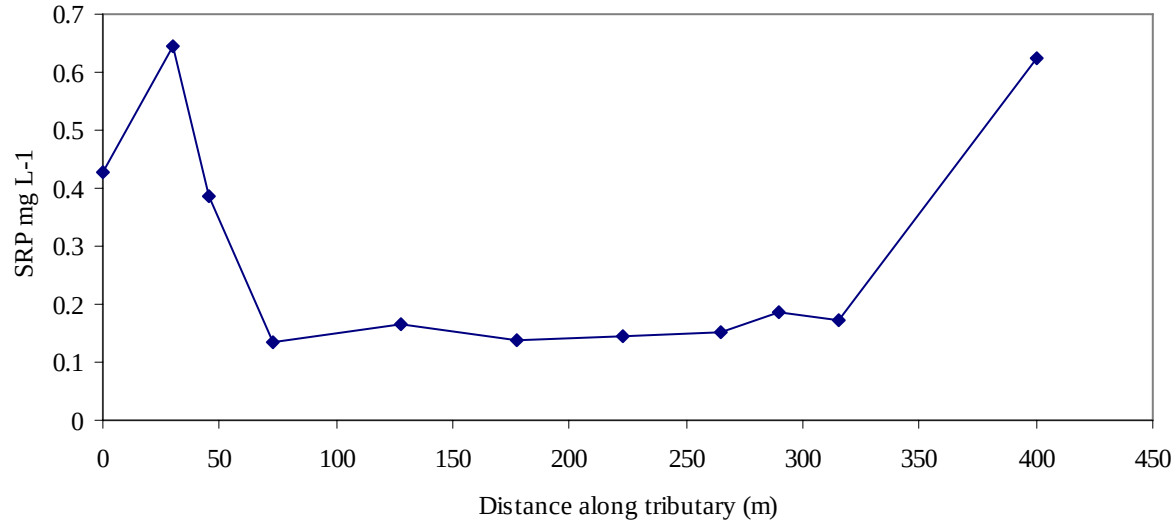

Figure 2-16. SRP concentrations for tributary 2 (T2), March 29, 2006.

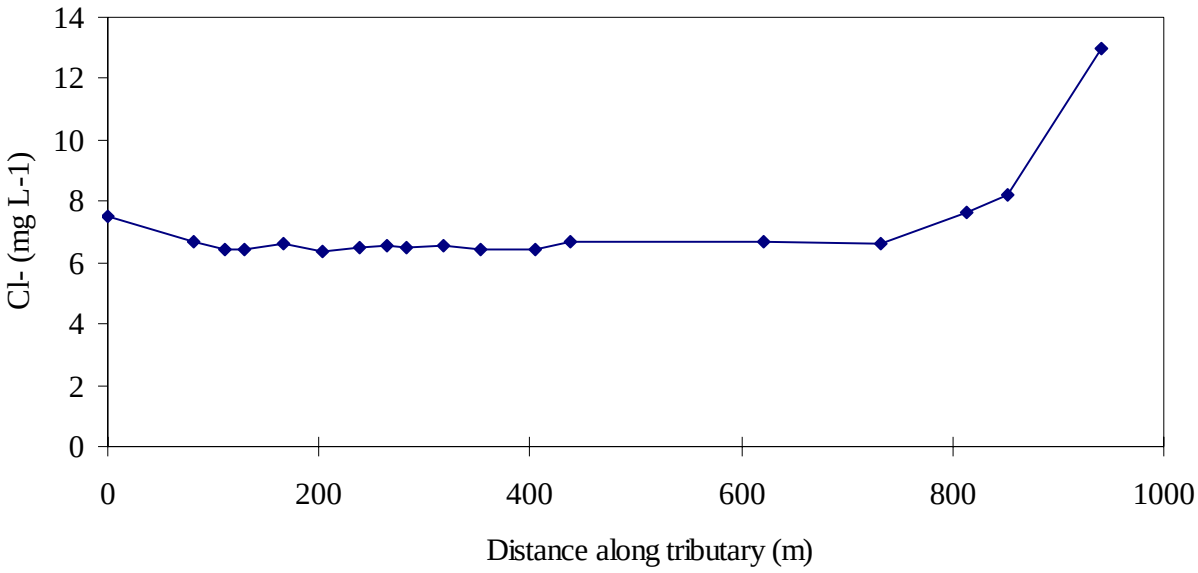

Figure 2-17. Chloride concentrations for May 20, 2005 along the length of tributary 1 (T1).

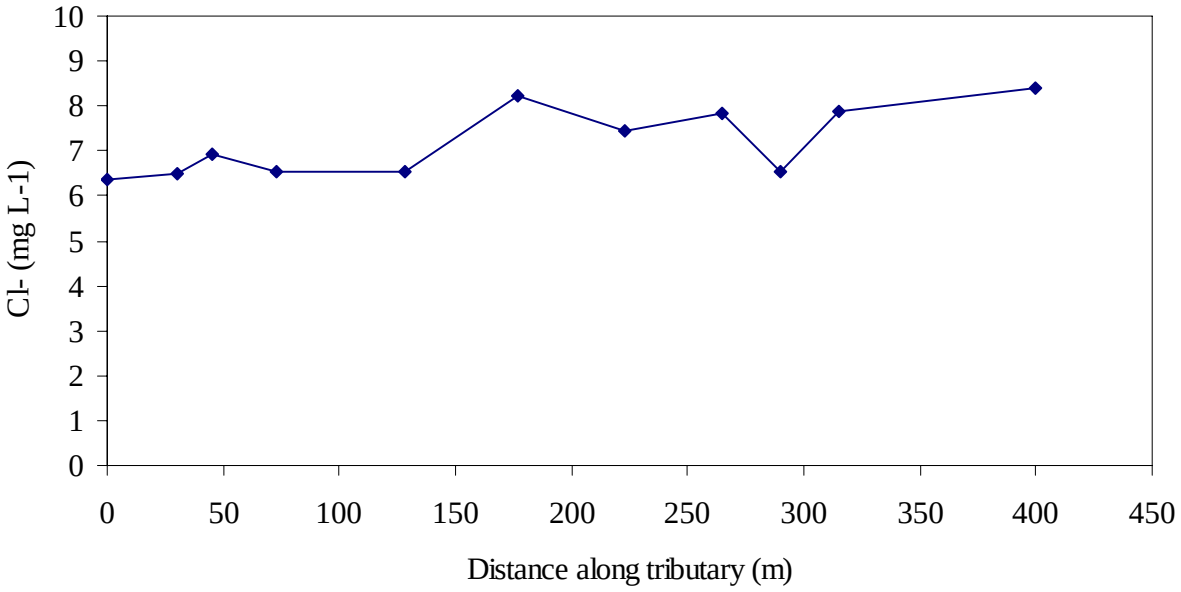

Figure 2-18. Chloride concentrations for May 20, 2005 along the length of tributary (T2).

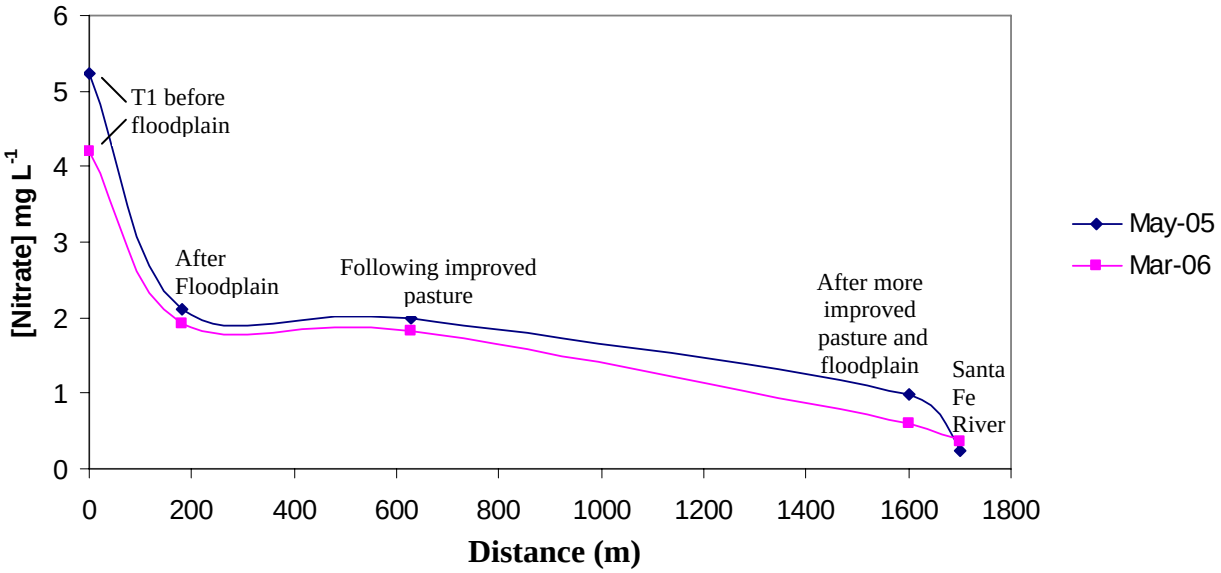

Figure 2-19. Change in nitrate concentrations as tributary 1 (T1) flows through the floodplain and improved pasture to the Santa Fe River.

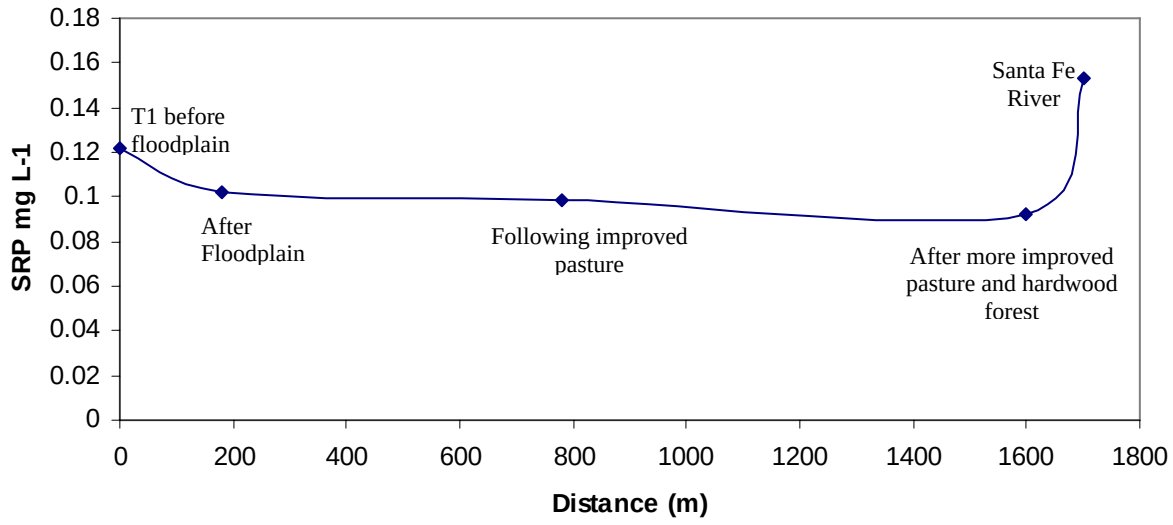

Figure 2-20. Change in soluble reactive phosphorus (SRP) concentrations from the last tributary 1 (T1) sample station, through the floodplain and improved pasture to the Santa Fe River.

CHAPTER 3
SOIL CHARACTERIZATION AND DENITRIFICATION

Introduction

Denitrification, a major removal pathway for nitrogen from ecosystems, requires low oxygen, a labile carbon source, a nitrate source, and an active microbial community. Because wetlands are often anaerobic with an accumulation of carbon, these ecosystems can provide ideal conditions for denitrification.

Nitrate-nitrogen concentrations in a tributary impacted by agricultural runoff are being reduced by some mechanism as water moves along the length of the tributary (Chapter 2). To determine the possible role of denitrification in reducing nitrate in this system, three studies were conducted on two tributaries at the Santa Fe Beef Research Unit (SFBRU). A soil characterization study, a nutrient limitation study, and an intact core study were conducted on soils of the stream, bank, and upland of tributary 1 (T1) and tributary 2 (T2).

These studies were conducted to investigate soil characteristics that have been shown to directly or indirectly influence denitrification in soils such as carbon and nitrogen (Fischer and Whalen 2005, Lowrance 1992, Aber et al. 1991).

Objectives

Specific objectives were to

1. determine if denitrification is a major removal pathway for nitrogen from the soils of the SFBRU;
2. determine what nutrients, if any, are limiting denitrification in this system;
3. determine if there are differences in nitrate removal rates among upland, bank, and stream channel soils in intact cores;
4. determine if nitrate removal rates in intact soil cores correspond to denitrification rates measured by the soil slurry method.

Hypotheses

Specific hyptheses were that

1. denitrification is a dominant pathway for nitrogen loss under anaerobic conditions;
2. because soils are mostly sandy in this region of Florida, carbon limits denitrification;
3. the bank and upland will have higher rates of nitrate removal from the water column than the channel due to more available soil carbon;
4. denitrification measurements using intact soil cores will be more variable than the soil slurry method because the experiment is conducted in a less controlled environment.

Materials and Methods

Sampling Locations

Soil sampling was conducted along three transects established on a high nitrate (T1) and a low nitrate (T2) tributary at the SFBRU. All transects run perpendicular to the tributary and are located at the upper (headwater), middle, and lower portion (near floodplain) of each tributary. Five sampling stations along each transect were established at the center of the stream channel, on either bank of the main channel and 25 meters upland from each bank sampling point (Figure 3-1). Bank sampling stations were representative of riparian areas, and the site 25 meters from the bank was representative of upland areas. These sample sites and transects were used for all soil sampling events.

Soil Characterization Study

Field methods

On June 20, 2005, triplicate soil samples were randomly taken within a 1 meter radius of each sampling location along each transect. A 7 cm diameter soil corer with sharpened metal head was used to extract a soil sample to a depth of 5 cm, making sure to minimize compaction of the soil. A knife was used to cut any roots and to ensure the sample obtained was flush with the end of

the soil corer. Each sample was extruded into a plastic storage bag and excess air was removed before sealing the bag. A total of 90 samples were put on ice for transport to the lab.

In addition to soil sampling, redox potential of the soil was measured four times between November 2005 and March 2006. Redox potential is a measure of how reduced a soil is and therefore what dominant electron acceptor is being used by microbes during respiration. To measure redox, platinum electrodes were set up in duplicate at sample sites along the middle transect of each tributary. Each electrode was inserted to a depth of 5 cm, the depth at which all soil cores were taken. An Accumeter redox probe was connected to a pH meter and a platinum electrode to measure soil redox potential. All values were adjusted to the standard hydrogen electrode by adding 207mV to the measured value.

Laboratory methods

Once in the lab, soils were weighed for bulk density. Soils were then processed by homogenizing samples and removing any large live and dead plant material, roots, or rocks. Samples were stored in sealed plastic tubs at 4^{o}C until analysis.

Soil moisture content was measured gravimetrically by drying 10–20g of soil at 70^{o}C for at least 72 hours. Samples were then reweighed and soil moisture content was calculated.

Soil organic matter content was determined by the loss on ignition method. Oven dried soil samples were ground and passed through a #60 sieve (0.25mm). Any soil remaining in the sieve was ground with a mortar and pestle until it could pass through the sieve. Approximately 2g of dry soil was placed in an aluminum tin, weighed, and combusted in a muffle furnace for 30 minutes at 250^{o}C then for 3–4 hours at 550^{o}C. Ashed samples were reweighed to determine total loss of organic matter (Jackson 1993).

Soil pH was measured by placing 10 grams of soil and 10 grams of deionized water in a beaker and allowing the mixture to equilibrate for 30 minutes. The soil-water solution was then measured with a calibrated pH meter (Thomas 1996 and Hanlon 1984).

To measure water extractable carbon and nitrate**,** 2.5g of soil and 25mL of distilled deionized (DDI) water were added to plastic extraction tubes. A rubber stopper was placed in each tube and the soils were shaken on an end to end shaker at an intermediate speed for one hour. Samples were then centrifuged for 10 minutes at 6000 rpm. Extractions were filtered with a Whatman # 41 filter (0.45µm). Water extractable nitrate (WEN) was analyzed by the cadmium reduction method discussed in chapter 2. Water extractable carbon (WEC) was measured on a Shimadzu TOC 5050A.

Denitrification potential

To measure denitrification potential of these soils, denitrification enzyme activity (DEA) was measured within 2 weeks of soil collection. This process measures the activity of microbes, specifically denitrifying bacteria under anaerobic conditions.

For the DEA procedure, 8–10 g of soil was weighed into a 120mL glass serum bottle. Bottles were capped, crimped, and evacuated with N_2 gas to establish anaerobic conditions. Five milliliters of purged H_2O were added to create a soil slurry.

The acetylene block method was used because acetylene gas (C_2H_2) blocks the final step in denitrification when N_2O is reduced to N_2. Acetylene was generated by adding water to calcium carbide rocks which immediately produces high grade acetylene gas. Twenty milliliters of acetylene gas were injected into each serum bottle. Samples were put on a shaker for 30 minutes to ensure complete mixing of acetylene throughout the soil. Eight milliliters of DEA solution (288 mg L^{-1} glucose, 56 mg L^{-1} KNO_3, and 100mg L^{-1} chloroamphenicol) were added to each sample and soils were put on an end to end shaker to incubate in the dark at a constant temperature of

25°C. The volume of chloroamphenicol used in this study was selected based on experiments conducted by Murray and Knowles (1999). Gas samples were collected every 30 minutes for organic soils and every hour for sandy soils for up to 3 hours. Samples were stored in 4 milliliters evacuated, crimp-top, glass, serum bottles until analysis. N_2O gas samples were measured on a Shimadzu gas chromatograph 14A with a ^{63}Ni electron capture detector. Column temperature was 30°C, detector temperature was 240°C, and injector temperature was 120°C. The carrier gas was Argon and 5% methane. Denitrification rates were obtained by calculating the slope of the line obtained when gas concentrations were plotted over time.

Nutrient Limitation Study

Field methods

Using the same soil transects discussed above, in January 2006, triplicate soil samples were taken to a depth of 5 cm in the stream channel, at the east bank, and at the east upland soil sampling locations. Triplicate samples at each location were combined into one sample. Samples were homogenized and stored in a sealed plastic bag on ice for transport to the lab.

Laboratory methods

Soil samples were processed and moisture content and loss on ignition determined according to the methods described above. To determine what may be limiting denitrification rates in this system, 8-10g of soil from each sample were added to 3 serum bottles to represent each treatment: ambient, + nitrogen (+N), and + nitrogen + carbon (+N+C). Each serum bottle was capped, crimped, and flushed with N_2. Five milliliters of N_2 purged water and 20 milliliters of acetylene were added to each sample as described above.

For ambient samples, 8 milliliters of DDI water were added to the serum bottles, and samples were set to incubate in the dark on an end to end shaker at 25°C. Based on previous sampling, the ambient soils presumably had low nitrate concentrations, so nitrate consumption was

expected to occur quickly. Gas samples were taken at approximately 20 min, 40 min, 2 hours, and 4 hours.

For the +N treatment, 8mL of a 5mg L^{-1} nitrate solution were added to each serum bottle. This concentration was chosen because it is similar to the average nitrate concentration of the water sampled in T1. Samples were set to incubate in the dark on an end to end shaker at 25^{o}C. Gas samples were taken at 1, 2, 4, 16 and 48 hours. These sample times were selected to try to catch the linear portion of the denitrification reaction.

For the +N+C treatment, 8 mL of 5 mg L^{-1} nitrate solution and 4 grams of ground litter as a carbon source were added to each sample. Litter was collected from the sample site near the stream channel and was composed of a mix of woody (pine and oak) and herbaceous (knotweed, *Juncus sp*, and grass) species. Samples were incubated in the dark on a shaker at 25^{o}C. Based on the analysis of the +N gas samples, samples for the +N+C reaction were sampled at: 1.5, 3, 10, 13, and 28 hours.

All gas samples were stored in evacuated 4mL glass serum bottles. The N_2O gas concentration of each sample was measured on a Shimadzu 14A gas chromatograph.

Intact Core Study

Field methods

To measure nitrate removal capacity of SFBRU soils, an intact core study was carried out in April 2006. Triplicate intact soil samples were taken along three transects in the T1 tributary (described in Chapter 3) within the stream channel, east bank, and east upland of the impacted tributary. Soil cores were also taken in the floodplain to determine the nitrate removal rate of floodplain soils. Each soil core was taken to a depth of 5 cm with a sharpened steel head placed on a 35 cm long, clear polycarbonate tube. Care was taken to minimize compaction when the

apparatus was either pushed or hammered into the soil. The steel head was removed and both ends of the tube capped for transport. All collected cores were transported upright, on ice to the lab.

Laboratory methods

Site water from T1 with an initial nitrate concentration of 6.21 mg L^{-1} was added until each soil core was saturated and covered with 20cm of water. All flooded cores were placed in an aquarium filled with water to moderate ambient temperature changes and maintain a neutral hydraulic head difference between the inside and the outside of the core . The water column of each core was mixed by continuous bubbling with ambient air pumped through tubes fixed with a 1.5 gauge hypodermic needle. Bubbling rate was sufficient to keep the water column mixed and under aerobic conditions, but not to the level that sediments became suspended. Black plastic was placed over the entire experiment to minimize light and, therefore, deter algal growth in the cores.

Water samples and temperature readings were collected from the water column 14 times over 8 days (time sampled = 0, 4, 8, 12, 24, 36, 48, 60, 72, 96, 120, 144, 168, and 192 hours).

Samples were analyzed with the Cadmium reduction method on an AQ2, a discrete autoanalyzer, to measure nitrate concentrations in the water over time.

Following completion of the experiment, soils from the intact cores were analyzed for organic matter, moisture content, and denitrification enzyme activity rate (DEA).

Statistics

All statistical analyses were performed in JMP IN 5.1, Sigma Plot 8.0, or Statistica. To test differences between tributaries, a t-test was performed. To compare differences between denitrification rates by stream location, when comparing more than two means, ANOVAs followed by a Tukey–Kramer test were used. ANOVAs and Tukey–Kramer tests were also used to compare differences between treatments in the nutrient limitation study.

DEA rates were correlated with soil characteristics to see what factors if any had an affect on denitrification. Correlations were also analyzed for soil properties and denitrification rates for the treatments. All Pearson product moment correlations were performed in JMP IN 5.1 and Statistica.

A factor analysis was performed by the Principle Component extraction method to get an overview of how soil characteristics affect variation in soils samples by location. Factor analyses were run with Statistica.

Differences between soil cores were analyzed with ANOVAs followed by a Tukey–Kramer test in JMP 5.1. Correlations were run in JMP 5.1.

Results

Soil Characterization Study

Soil bulk density, pH, % moisture content, % organic matter, water extractable carbon (WEC), and water extractable nitrate (WEN) measurements were used for initial soil characterization (Table 3-1). When combining sites along each tributary, bulk density was significantly higher in T1 than in T2 ($p= 0.04$). WEN and WEC were not significantly different ($p= 0.13$ and 0.10, respectively). Finally, % organic matter, % moisture content, and pH were not significantly different for T1 compared to T2 ($p= 0.22$, 0.57, and 0.12, respectively).

Each tributary had a number of differences in soil properties between upland, bank and stream soils. Differences between locations were observed for all soil properties except pH in T1 and moisture in T2 (Table 3-1).

Overall, the mean DEA rate was 5.89 ± 9.83 mg N_2O kg $soil^{-1}$ d^{-1}. T1 had an average DEA rate of 8.73 ± 12.78 mg N_2O kg $soil^{-1}$ d^{-1}which was significantly higher than T2, with an average DEA rate of 2.50 ± 2.68 mg N_2O kg $soil^{-1}$ d^{-1} ($p= 0.04$).

For both tributaries, the upland and bank soils had significantly higher DEA rates than the stream channel soils ($p<0.001$; Figure 3-2). There were no differences in DEA rates between transects on either tributary.

Percent organic matter had the strongest correlation with T1 and T2 DEA rates. DEA rate was also correlated with bulk density and WEC in both tributaries. There was not a strong relationship in either tributary between DEA rate and pH, WEN, or % moisture content (Table 3-2).

A factor analysis was performed by the Principle component extraction method to get an overview of how these soil characteristics affected variation in soils samples by location (Figure 3-3). Factor 1 describes 49% of the variability in soil properties, and the parameters selected were % organic matter, DOC, DEA rate, and bulk density. Percent organic matter, DOC, and DEA varied together, whereas bulk density was inversely related to these soil properties. Factor 2 describes 24% of the variability in soil characteristics, and the parameters selected by the Factor analysis were moisture content and soil NO_3^-. These parameters were inversely related.

Upland soils were most strongly influenced by organic matter and soil nitrate concentration. Bank soils overlapped with all soil characteristics, but clusters existed near soil nitrate and moisture content. Finally, stream soils were inversely related to DOC, and organic matter, but positively related to bulk density (Figure 3-3).

Redox potentials were not significantly different between T1 and T2 ($p= 0.21$, $n= 53$). For both tributaries, however, stream redox potentials were significantly lower than those measured at the bank and upland ($p<0.001$, Figure 3-4).

Nutrient Limitation Study

Denitrification rates in the ambient and +N samples were significantly lower than the +N+C treatment ($p= 0.013$). Mean denitrification rates were 0.54 ± 0.64 mg kg $soil^{-1}d^{-1}$ for ambient soils, 1.56 ± 2.68 mg kg $soil^{-1}d^{-1}$for + N soils, and 7.17 mg kg $soil^{-1}d^{-1}$ for +N+C (Figure 3-5).

In this experiment, there were significant relationships between denitrification rate and organic matter, moisture content, WEC, and WEN (Table 3-3).

When soil samples were compared by location or transect there were no differences in denitrification rates among treatments. Denitrification rates were compared between tributaries, however, and rates in T2 were significantly higher than T1 for all treatments ($p<0.001$; Table 3-4). T1 had an average denitrification rate of 1.51 mg kg $soil^{-1}d^{-1} \pm 3.64$ whereas T2 had an average of 5.18 ± 9.18 mg kg $soil^{-1}d^{-1}$.

Intact Core Study

For all 30 cores, average NO_3^- removed from the water column was 0.67 ± 0.40 mg $L^{-1}d^{-1}$. There were no significant differences in nitrate removed per day between the floodplain, upland, bank or stream channel (Figure 3-6). One set of cores from the stream channel in the middle transect were outliers and had high rates of denitrification likely due to the presence of worms. Worms can affect denitrification either via gut denitrification or increased sediment water mixing. When cores were analyzed without these soils, the channel soils had significantly lower rates of denitrification than the bank soils ($p=0.04$, Table 3-5, Figure 3-6).

There were also differences in nitrate removal rate by transect. The middle transect had an average NO_3^- removal rate of 1.02 ± 0.42 mg L^{-1} d^{-1} and was significantly different from the upper transect (0.49 ± 0.39 mg $L^{-1}d^{-1}$; $p= 0.19$) but not the lower transect (0.66 ± 0.10 mg $L^{-1}d^{-1}$). The lower and upper transects were not significantly different.

Nitrate removal rate in the core water column was significantly correlated with DEA rate, moisture content and organic matter, but not with other soil parameters (Table 3-6).

The soil cores had an average DEA rate of 29.62 ± 42.84 mg kg soil^{-1} d^{-1}, however, this rate is much higher because it includes the floodplain DEA. Without floodplain rates, the mean DEA rate was 16.49 ± 17.94 mg kg soil^{-1} d^{-1}. DEA rates for the core soils were highly correlated with organic matter and moisture (Figure 3-7 and 3-8). DEA was also significantly correlated with WEC (r = 0.57), but not WEN (r= 0).

Discussion

Soil Characterization Study

For all soil characteristics measured in T1 and T2, only bulk density and denitrification enzyme activity (DEA) rates were significantly different. DEA rates were quite variable in both T1 and T2, but this is likely the result of microsites with high carbon or high moisture (Parkin 1987 and Tiedje et al. 1984).

DEA rates measured in this system were an order of magnitude lower than those measured by White and Reddy in the Everglades, Florida (2003). Everglades soils, however, are peat soils that accumulate carbon and receive waters high in nitrogen and phosphorus. Lowrance (1992) measured a mean DEA rate of 0.191 mg kg soil^{-1}d^{-1} on soils in the Gulf Atlantic coastal plain in Georgia, compared to a mean of 5.89 mg kg soil^{-1}d^{-1} found in the SFBRU soils.

Because DEA is a measure of denitrification potential, the results suggest that under ideal conditions, higher rates of denitrification will occur in T1 compared to T2. This is likely because T1 soils have a steady nitrate source to utilize in the water column, whereas T2 has low nitrate concentrations and thus, denitrification is limited by nitrate.

Redox potentials measured in the stream channel also show T1 redox to be in the optimum range for nitrate reduction, whereas in T2, redox potentials are too low for nitrate to be the

dominant electron acceptor. Rates of denitrification in T1 suggest the reduction of nitrate observed along the length of the tributary is likely in part due to denitrification.

DEA rates were significantly higher in the bank and upland soils of both tributaries compared to soils in the stream channel. Because denitrification rates were highly correlated with organic matter content and water extractable carbon, it is likely that the observed differences in denitrification by stream location are related to carbon availability. T1 upland and bank soils were significantly higher in carbon than stream channel soils and T2 upland soils were higher in carbon than bank and stream channel soils.

Although denitrification potentials were higher in the upland and bank soils, redox measurements show these soils were using O_2 as the dominant electron acceptor and were, therefore, aerobic. These zones would be ideal for denitrification, but only when flooded will the soils become anaerobic enough to carry out denitrification.

Nutrient Limitation Study

The nutrient limitation study showed that denitrification rates were limited by both carbon and nitrogen, but most strongly by carbon. Nitrogen and carbon, however, might be co-limiting. Fischer and Whalen (2005) measured the effect of the addition of nitrate, glucose, and nitrate + glucose on DEA rates. Highest rates were obtained in the nitrate + glucose treatment, similar to our findings. In their experiment, however, there were no significant differences between the nitrate and glucose treatments.

Unlike the DEA experiments, soils in T2 had significantly higher denitrification rates than in T1. This is likely due to the presence of chloroamphenicol in the DEA solution, which blocks the microbial production of new enzymes for denitrification, allowing only enzymes already present in the soil to be used for denitrification. T2 has low nitrate availability so it is less likely that microbes are using NO_3^- as an electron acceptor for respiration. Depending on how reduced these

soils are, microbes would be using other electron acceptors such as O_2, Fe^{3+} or CH_4 during respiration. The nutrient limitation study, however, does not use chloroamphenicol, so microbes can produce new enzymes to carry out denitrification. There are likely differences between the tributaries in microbial activity, micronutrients, or soil texture that are driving differences in denitrification rates with and without chloroamphenicol. Studies have shown that soil texture can affect denitrification (Godde and Conrad 2000; Groffman and Tiejde 1991). D'Haene et al. (2003) found highest denitrification rates in clay soils (low bulk densities) and lowest rates in sandy soils (high bulk densities). This may help explain differences in our findings for the nutrient limitation study since higher rates of denitrification were found in T2 soils with lower bulk densities than those in T1.

Intact Core Study

Intact soil core nitrate removal rates were highly variable, ranging from 0.01 to 1.94 mg $L^{-1}d^{-1}$. When soils were initially compared by sample location, no significant differences were found. Tubificid worms were observed in a set of cores from the stream channel of the middle transect. Some tubificid worms are able to tolerate low oxygen conditions and often occur in low nutrient conditions (Howmiller 1975). Although cores with worms had extremely low organic matter content, they had the highest net nitrate removed from the water column during the experiment. Studies have shown that tubificid worms significantly increase rates of microbial processes because bioturbation allows surface particles and chemical species to infiltrate to lower depths in the soil (Mermillod-Blondin et al. 2004). This could have increased the NO_3^- transport rate from the aerobic water column to anaerobic sites in the soil where denitrification takes place.

When these cores were excluded from analysis, mean nitrate removal rates from channel cores were lower than upland soils and significantly lower than bank soils. This is similar to findings in previous experiments, and is likely due to carbon availability.

Intact core nitrate removal rates can be quite different from denitrification potentials measured in the lab. The presence of plants and bioturbators can influence the process of denitrification. Studies have shown that the presence of plant roots can increase denitrification rates because nitrification can take place in the oxygenated zone surrounding roots (Hernandez and Mitsch 2006). Nitrate is then available to diffuse to surrounding anaerobic zones in saturated soils. This experiment did not remove small plants or plant roots from soil cores due to the soil disturbance it would have caused in the intact cores. All cores with plants present removed more nitrate than those without plants, but it was unclear if this was a result of plant uptake or denitrification. In the future, it would be interesting to compare N_2O emission from cores with and without plant roots.

The DEA in intact core soils were higher than in previous experiments. These findings are likely the result of soils in the intact cores being saturated throughout the experiment, allowing them to become anaerobic for a longer period of time. Under anaerobic conditions, more enzymes would be produced by denitrifying bacteria to carry out denitrification in the presence of a nitrate source. The microbial community would be utilizing nitrate as an electron acceptor in the process of denitrification, and nitrate concentrations in the water column would decrease.

DEA rates were also highly correlated with porosity in these soils. This may be because larger porosities allow more nitrate to diffuse into the anaerobic portion of the soil profile.

In summary, DEA rates were higher in T1 than in T2 for the soil characterization study likely due to the lack of denitrification occurring in the low nitrate T2. Denitrification rates were highest in upland and bank soils compared to stream channel soils, likely due to carbon availability. The nutrient limitation study showed that denitrification in both tributaries was limited by nitrate and carbon. T2 denitrification rates were higher when soils were incubated for

longer time periods in the absence of chloroamphenicol possibly due to differences in microbial activity and soils texture. Finally, in the intact core study, intact soil core nitrate removal rates were lowest in the stream channel in the absence of tubificid worms.

Highest nitrate removal rates were found in bank soils indicating that, when flooded, these zones would be optimal for denitrification. DEA rates were also higher in these soils than in previous experiments, likely due to the fact that soils were flooded previous to denitrification potential measurements.

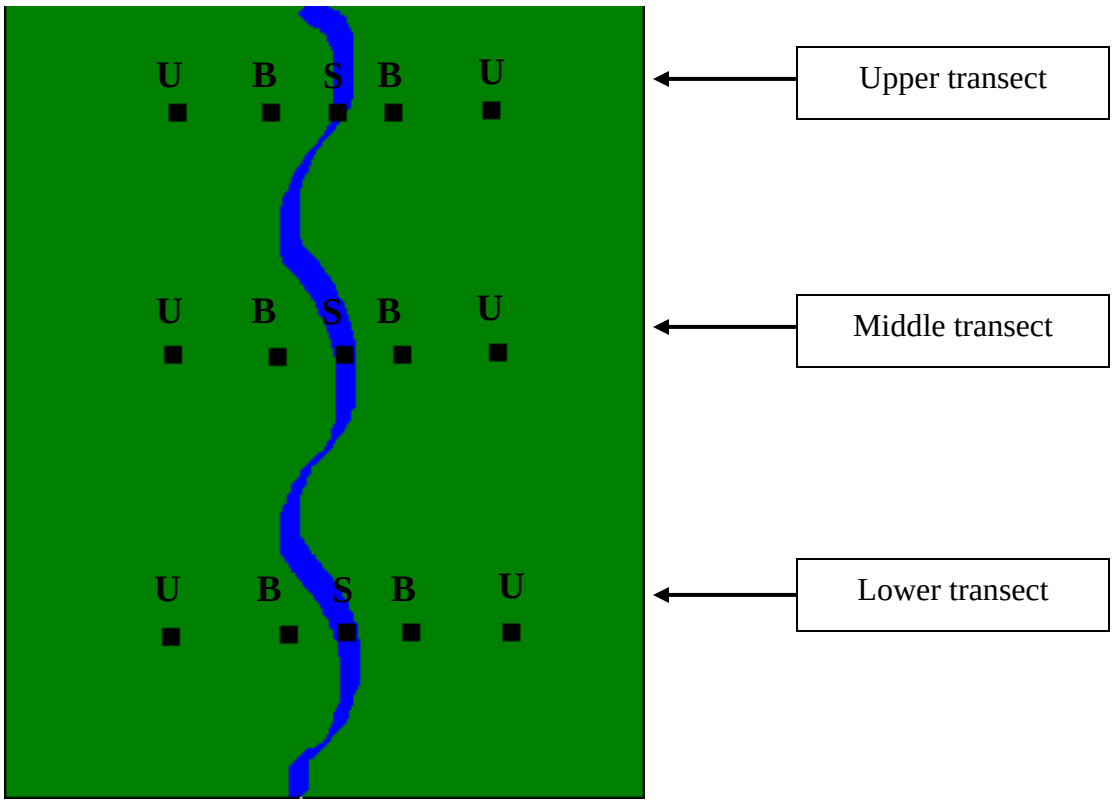

Figure 3-1. Sampling sites on each tributary. Each transect had sample stations at the upland (U), bank (B), and stream channel (S).

Table 3-1. Soil characteristics in tributary 1 (T1) and tributary 2 (T2) for the upland, bank, and stream. Values (n=15) represent mean and ± one standard deviation. Values with different letters indicate that upland, bank, and stream characteristics are significantly different within each tributary (α=0.05).

	Tributary 1					
	BD (g cm^{-3})	pH	WEN (mg kg^{-1})	WEC (mg kg^{-1})	%OM	%Moisture
Mean	1.23 (0.32)	5.82 (0.98)	1.25 (2.45)	9.33 (5.03)	5.21 (3.67)	18.85 (12.16)
Upland mean	1.05 (0.19) a	5.43 (1.14) a	2.70 (3.41) a	10.62 (3.41) a	7.22 (1.52) a	8.36 (2.72) a
Bank mean	1.24 (0.35) a	6.00 (0.75) a	0.33 (0.40) b	10.54 (6.06) a	5.19 (4.41) a	27.50 (12.96) b
Stream mean	1.58 (0.16) b	6.22 (0.87) a	0.22 (0.33) b	4.32 (1.14) b	1.23 (0.93) b	22.56 (3.09) b

	Tributary 2					
	BD (g cm^{-3})	pH	WEN (mg kg^{-1})	WEC (mg kg^{-1})	%OM	%Moisture
Mean	1.08 (0.34)	5.55 (0.64)	2.45 (4.20)	7.76 (4.78)	6.43 (5.82)	17.30 (12.35)
Upland mean	0.89 (0.24) a	5.13 (0.54) a	4.05 (4.79) a	11.16 (5.21) a	10.17 (6.26) a	18.70 (16.41) a
Bank mean	1.06 (0.19) a	5.59 (0.40) b	0.97 (0.74) b	6.38 (2.98) b	4.91 (3.14) b	12.94 (8.49) a
Stream mean	1.58 (0.16) b	6.31 (0.50) c	0.27 (0.28) b	3.72 (0.66) b	1.00 (0.66) b	20.29 (2.27) a

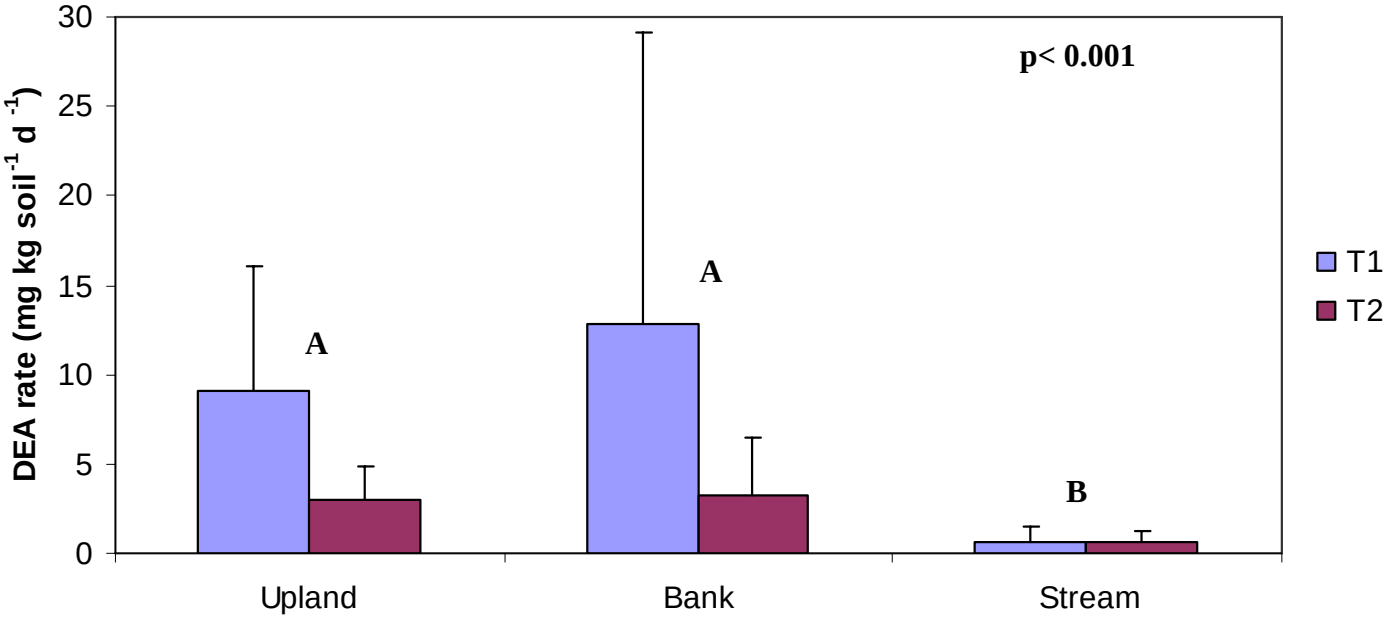

Figure 3-2. Mean denitrification enzyme activity (DEA) rates in tributary 1 (T1) and tributary 2 (T2) in the upland, bank, and stream. Error bars represent one standard deviation.

Table 3-2. Pearson product moment correlations (r value) between denitrification enzyme activity (DEA) rates and soil characteristics for tributary 1 and 2.

	Soil Parameters					
Tributary	Bulk Density	pH	WEN	WEC	%OM	%Moisture
1 DEA rate	0.73	0.35	0.33	0.69	0.80	0.08
2 DEA rate	0.60	0.07	0.32	0.50	0.70	0.12

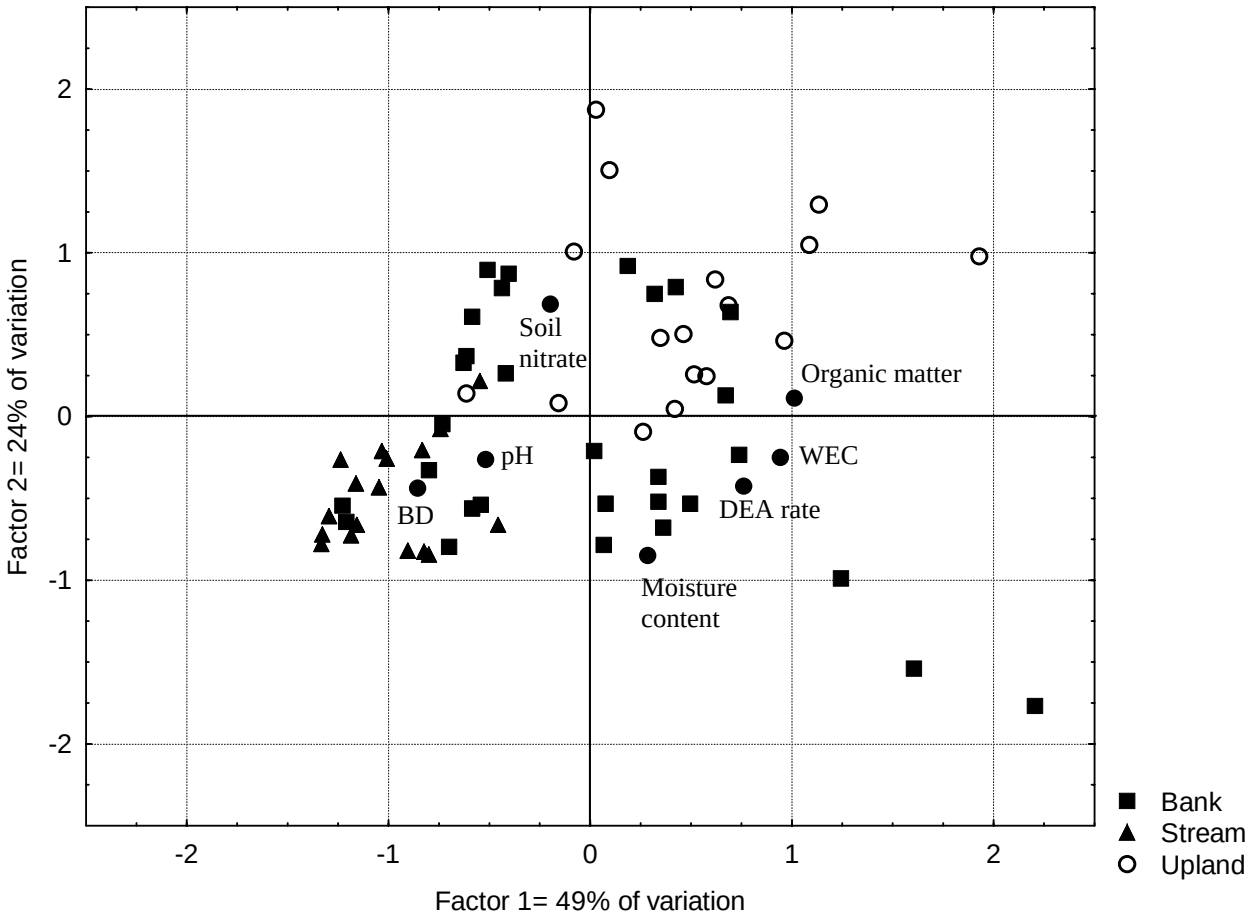

Figure 3-3. Factor analysis of soil characteristics and stream location.

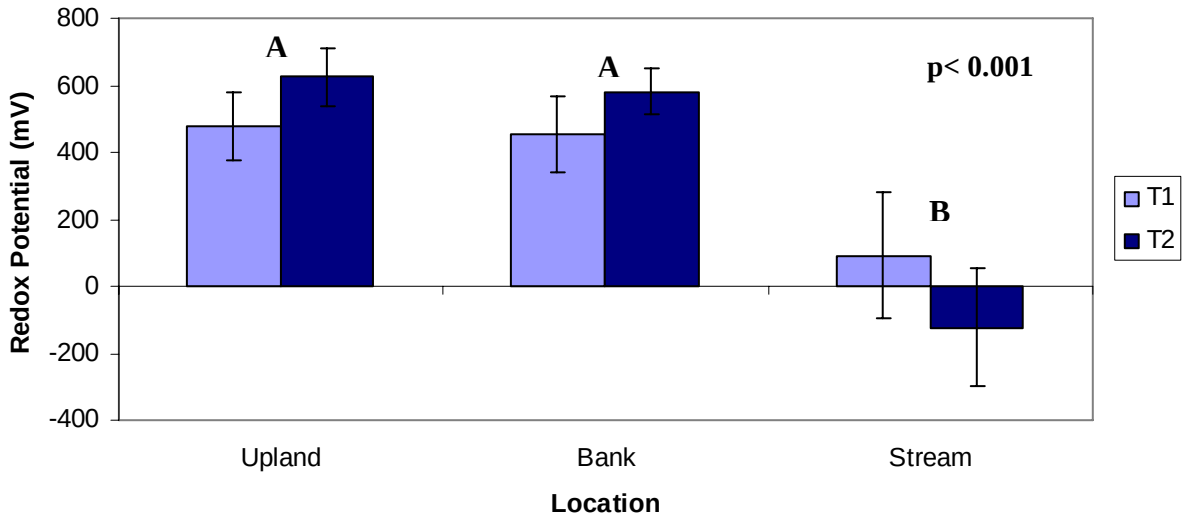

Figure 3-4. Mean redox potentials in tributary 1 (T1) and tributary 2 (T2). Nitrate is the dominant electron acceptor for redox potentials from 200-250mV. Error bars represent one standard deviation.

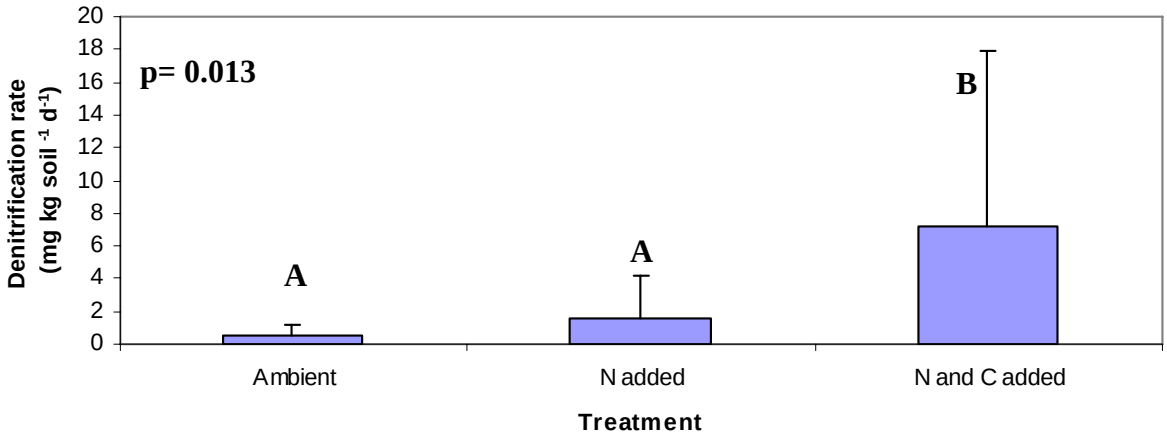

Figure 3-5. Mean + one standard deviation of denitrification rates for each treatment of a nutrient limitation experiment (N= nitrogen, N and C= nitrogen and carbon).

Table 3-3. Pearson product moment correlations (r value) between denitrification rates of each treatment and soil characteristics.

Soil parameter	Ambient	N added	C and N added
% Organic matter	0.50	0.73	0.66
% moisture	0.26	0.39	0.16
WEC	0.40	0.74	0.55
WEN	0.24	0.56	0.43

Table 3-4. Mean ± one standard deviation of denitrification rates for each treatment in tributary 1 (T1) and tributary 2 (T2).

Treatment	T1	T2
	mg kg soil^{-1} d^{-1}	
Ambient	0.19 ± 0.13	0.73 ± 0.71
(+) N	0.38 ± 0.33	2.74 ± 3.46
(+) N (+) C	3.18 ± 5.34	11.17 ± 13.28

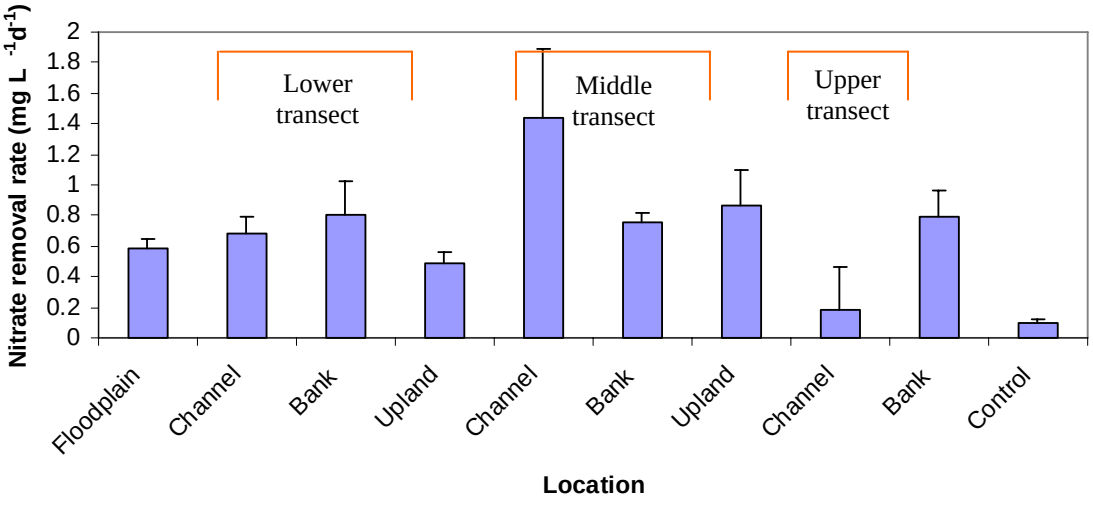

Figure 3-6. Mean nitrate removal rates + one standard deviation of each set of intact soil cores. The upland of the upper transect was not sampled because of equipment problems. Tubificid worms were found in the set of three cores taken from the channel of the middle transect.

Table 3-5. Mean nitrate removal rate ± one standard deviation by sampling site. Mean values followed by the same value are not significantly different. This analysis excludes a set of cores that were outliers.

Location	Nitrate removal rate	SD
	$mg\ L^{-1}d^{-1}$	
Upland	0.67 ab	0.26
Bank	0.79 a	0.14
Stream	0.44 b	0.33

Table 3-6. Pearson product moment correlations between nitrate removal rate per day and soil characteristics (DEA is denitrification enzyme activity, WEC and WEN are water extractable carbon and water extractable nitrogen, respectively).

Soil Parameter	r
Organic matter (%)	0.38
Moisture content (%)	0.36
DEA rate ($mg\ kg^{-1}\ d^{-1}$)	0.47
WEC ($mg\ kg\ soil^{-1}$)	0.22
WEN ($mg\ kg\ soil^{-1}$)	0.16

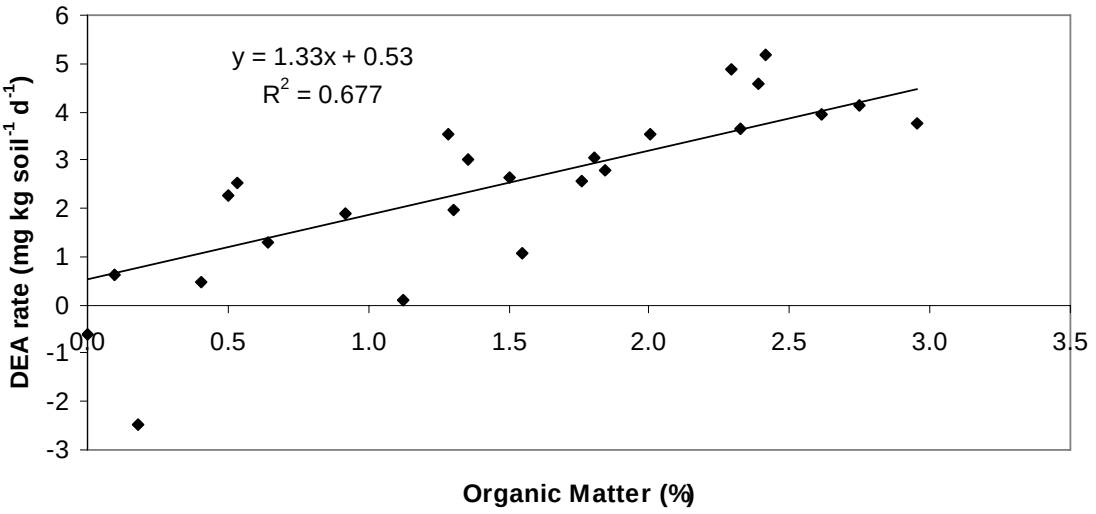

Figure 3-7. Linear relationship between organic matter and denitrification enzyme activity (DEA) for the core soils.

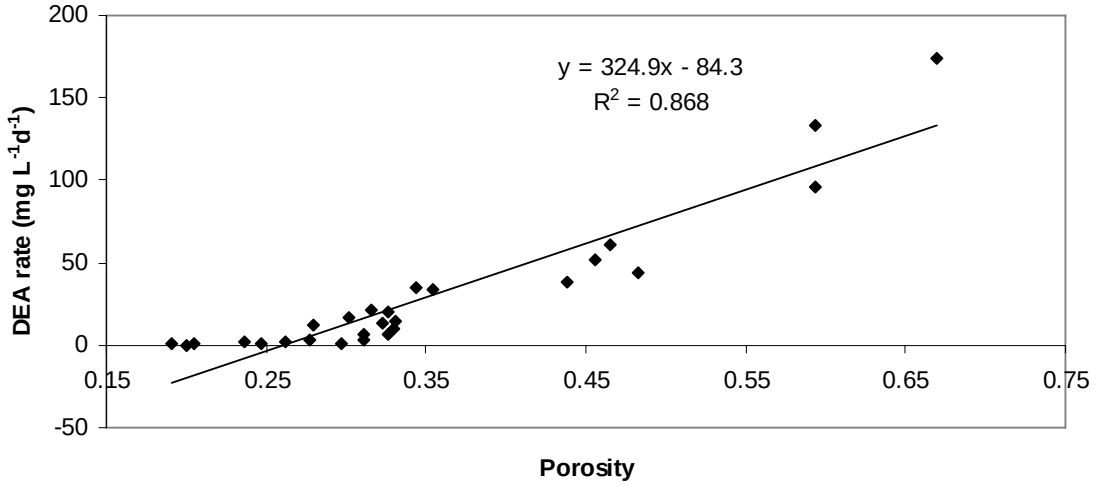

Figure 3-8. Linear relationship between porosity and denitrification enzyme activity (DEA) rate for core soils.

CHAPTER 4
SUMMARY, IMPLICATIONS AND FUTURE RESEARCH

Water Quality Monitoring

Many tributaries of the Santa Fe River drain agricultural land in north-central Florida. There are direct connections between ground and surface waters in this region as a result of a discontinuous clay layer (the Hawthorne layer) that overlays limestone bedrock. When water runs off agricultural areas, waters high in nitrogen, phosphorus, and other nutrients can impact fresh and marine waters. Nitrate-nitrogen can affect the health of humans, animals, and ecosystems, so improvements in management of agricultural runoff are important to maintain Florida's drinking water quality and ecosystem health.

Two tributaries of the Santa Fe River were studied for one year. One tributary had consistently low nitrate concentrations, while the other was high in nitrate from runoff of an ornamental plant nursery. Our study found that nitrate is being at least partially reduced in the impacted tributary as a result of denitrification processes prior to water reaching the Santa Fe River. Nitrate concentrations varied over the year of monitoring likely due to season, fertilizer application rates, and irrigation rates.

Our results indicate that open water reaches and reaches with herbaceous vegetation of this tributary are more efficient at nitrate removal than others. To decrease nitrogen loading to Florida's waters, a number of Best Management Practices (BMPs) could be implemented that create or enhance these open water and herbaceous vegetation reaches in tributaries of the Santa Fe River. For instance, small dams or weirs could be installed to decrease flow or pool water to create open water reaches. To create reaches with herbaceous vegetation, organic and mineral material could be deposited in a tributary. After stabilization, these depositional areas could be planted with native hydrophilic herbaceous plants.

Soil Characterization and Denitrification

Our denitrification experiments showed that bank and upland soils had the highest denitrification potentials. Unless the stream channel overflows, however, these aerobic soils are not active sites for nitrate removal from the water column. BMPs could be implemented that decrease bank incision to encourage water to overflow banks or increase contact with riparian and upland soils. Otherwise, greatest denitrification will only occur in these soils during storm events.

To increase denitrification occurring in the stream channel, where our findings suggest carbon is limiting, adding a carbon substrate of some sort may be feasible. One such BMP employs a "denitrification wall" that is constructed in water systems to improve nitrate removal. These walls have been shown to greatly improve nitrate removal capacity by providing a carbon source for the process of denitrification (Greenan et al 2006). These walls are long-lasting, inexpensive, and easy to install. Common carbon sources are sawdust, peanut shells, wood chips and plant residues. In fact, a denitrification wall is planned for installation upstream of T1 in conjunction with the plant nursery. Schipper and Vojvodic-Vukovic (2001) found that after five years, a denitrification wall had the same performance, and only when the water table dropped below the wall did nitrate concentrations downstream increase.

The nutrient limitation study indicated that nitrate and carbon are both limiting denitrification in these soils. The intact core study also indicated that denitrification is limited by carbon, and low oxygen, anaerobic conditions. Saturation of upland and bank soils could significantly increase denitrification in this system and improve nitrate removal from tributaries in the Santa Fe River. Intact soil core nitrate removal rates were more variable than DEA rates measured in the soils likely because intact soil cores are more representative of field conditions.

Future Research

This research focused on two tributaries in the Santa Fe River Watershed. A study that monitors multiple tributaries across the watershed would be helpful to address if processes across tributaries are similar. A number of BMPs could then be implemented and tested for success to decrease the impact that agricultural areas have on the Santa Fe River and other freshwater systems in the Santa Fe River watershed.

More research is also needed to understand what role plants have in removing nitrate from reaches of these tributaries. Plants do not provide a long-term sink for nitrogen and, thus, if they are removing nitrogen from the system, it may be helpful to harvest the plants to prevent the release of stored nitrogen back into the tributaries.

Soil samples from different tributary reaches could also be tested for denitrification potentials. This would increase understanding of the role of denitrification in individual reaches within the stream channel. To increase nitrate removal, construction of similar reaches along tributaries, or increasing the residence time of water in these tributaries could greatly increase nitrate removal efficiencies. For instance, in this study, Open Water and Depositional Herbaceous reaches removed the most nitrate from the water column. Open Water reaches can be created in a tributary by narrowing the tributary channel downstream from a reach. Depositional Herbaceous reaches can be constructed by depositing materials within the stream that will recruit plants and force a shallow water column to flow over the reach.

The floodplain was shown to reduce both nitrate and SRP concentrations in T1, and floodplain soils had high DEA rates. More research would be useful to study the importance of

the floodplain in reducing nutrients in agriculture runoff. It may be beneficial to restore abandoned pastures and other land to floodplain to reduce N and P loading to the Santa Fe River.

Conclusion

This study provided evidence that a tributary of the Santa Fe River reduces nitrate concentrations in agricultural runoff. Denitrification is believed to be a major process reducing nitrate concentrations, though a combination of carbon, nitrogen, and saturated anaerobic soils are limiting denitrification. BMPs such as denitrification walls and morphological stream reach enhancements are suggested to increase nitrate removal from tributaries near agriculture areas. These BMPs, however, could alter stream ecosystem function, so, it would be ideal to manage agriculture runoff before it enters water systems. This involves reducing fertilizer applications, intercepting runoff with buffer strips, or controlling on-site drainage (Hey 2002). Reducing or optimizing fertilizer applications would also decrease greenhouse gases produced as by-products of denitrification. There is currently little incentive for agriculture, industry or municipalities to regulate nitrates.

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BIOGRAPHICAL SKETCH

Adrienne Elizabeth Frisbee was born September 23, 1979, in Dallas, Texas. She grew up in Tulsa, Oklahoma and discovered her love of science early in biology class at Bishop Kelley High School. When she graduated in 1998, she moved to New Orleans, Louisiana to attend Loyola University. There, she majored in biology with a minor in environmental studies. She graduated cum laude in 2002.

For the next two years, Adrienne was a biologist in New York, California, and Oklahoma studying vegetation restoration and coastal and grassland bird species. After deciding to continue her education, she moved to Florida in 2004 to get her Masters of Science degree in soil and water science. There she studied wetlands and water quality and was also involved in research projects in Alaska.

After graduating in May 2007, Adrienne will be working in San Francisco, CA with NASA on nitrogen cycling in microbial mats.