ABSTRACT

KNIES, SARA VICTORIA. Riparian Buffer Effectiveness at Removal of $NO_3$-N from Groundwater in the Middle Coastal Plain of North Carolina. (Under the direction of Dr. Deanna L. Osmond).

Non-point source pollution from agriculture is one of the causes of surface water quality degradation in the Coastal Plain of North Carolina. Riparian buffers are an important best management practice for reducing $NO_3$ concentrations in natural waters, predominantly by vegetation uptake and denitrification. However, there continues to be debate over the optimal design of buffers, specifically buffer width, and vegetation type. This project was designed to investigate the effects of vegetation type, groundwater depth, and buffer width on $NO_3$ removal from groundwater. Four buffers have been established at a research farm in the Middle Coastal Plain of North Carolina to investigate these factors; individual buffers are comprised of five vegetation types, two buffer widths, and two well depths.

The influence of vegetation type on $NO_3$-N groundwater decreases were as follows: revegetation had a decrease of 14% (5.75 mg N/L to 4.97 mg N/L); switchgrass had a decrease of 40% (9.19 mg N/L to 5.48 mg N/L); trees had a decrease of 32% (9.18 mg N/L to 6.20 mg N/L); native vegetation had a decrease of 35% (8.36 mg N/L to 5.41 mg N/L); fescue had a decrease of 23% (7.34 mg N/L to 5.67 mg N/L); the control had a decrease of 0% (5.85 mg N/L to 5.86 mg N/L).

Influence of width and depth on $NO_3$-N decreases were as follows: deep wells in 15 m buffers had a $NO_3$-N decrease of 77% (5.76 mg N/L to 1.34 mg N/L), deep wells in 8 m buffers had a decrease of 53% (4.55 mg N/L to 2.13 mg N/L), intermediate wells in 15 m buffers had a decrease of 47% (7.51 mg N/L to 4.00 mg N/L), and intermediate wells in 8 m buffers had a decrease of 14% (8.38 mg N/L to 7.19 mg N/L)

There was a significant three-way interaction ($p = 0.001$) between vegetation type, buffer width, and well depth. This interaction was desegregated by depth: at the deep depth, the effect of switchgrass was significant ($p=0.0120$) in removal of $NO_3$-N in both the narrow and wide buffer widths. The effect of the revegetation treatment was significant ($p=0.0093$) at removal of $NO_3$-N in the narrow width.

The ratio of $NO_3$-N/Cl was evaluated to determine if dilution of groundwater was responsible for observed $NO_3$-N concentration decreases. Dilution was slight and did not significantly account for any observed $NO_3$-N decreases. Reduction potential (Eh) values indicated reducing conditions at the deep well depth in three of the four buffers, suggesting denitrification was most likely responsible for observed $NO_3$-N decreases in groundwater. Inhibition of denitrification rates could be occurring in buffers due to low levels of organic C ($\approx 3.4 \pm 0.6$ mg C/L). To test this hypothesis, a laboratory study was designed to complement the field study. Flow-thru soil columns were constructed to determine the effect of dissolved organic carbon (DOC) concentration on denitrification rates and products in buffer soils. Four DOC concentrations (2.0 mg DOC/L, 4.0 mg DOC/L, 8.0 mg DOC/L, and 16.0 mg DOC/L) and a control (0.0 mg DOC/L) were utilized to study this relationship between DOC and denitrification.

There was no trend between DOC concentration and rate of $NO_3$-N loss. DOC concentrations > 4.0 mg DOC/L increased up until *12.0 mg DOC/L*, after which rates leveled off. There was a linear relationship between DOC concentration and rate of $N_2O$-N production with the exception of *12.0 mg DOC/L*, with the rate of $N_2O$-N production increased with increasing concentrations of DOC.

# Riparian Buffer Effectiveness at Removal of $NO_3$-N from Groundwater in the Middle Coastal Plain of North Carolina

by
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# DEDICATION

This thesis is dedicated to my parents, Leonard Andrew Knies Jr., and Laurie Ann Knies. Thank you for raising us to be different from everyone else, and always encouraging us to pursue our interests in life.

# BIOGRAPHY

Sara Victoria Knies was born on January 10, 1982 in Hackensack, New Jersey to Leonard and Laurie Knies. She is the second of six children, with four sisters, and one brother. Her father, Leonard Knies, is a life-long employee of the utility company PSE&G. Laurie Knies, her mother, is a registered nurse working for a county hospital. When she was five, her parents moved to Sussex, located in northeast New Jersey. She graduated from High Point Regional High School in 2000.

Sara attended Washington College, located on the Eastern Shore of Maryland, where she earned a Bachelor of Arts in Environmental Studies in 2004. From January to December 2006, Sara was an AmeriCorps VISTA at the U.S. Environmental Protection Agency in Philadelphia, PA. During the year she spent at the EPA in the non-point source program, Sara became interested in agriculture, and non-point source pollution. Wishing to pursue a Master's degree, she came to NC State University in January, 2007 to study under Dr. Deanna Osmond in the Soil Science program, focusing on riparian buffers and water quality.

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## Chapter 1: Literature Review of Riparian buffers

Nonpoint source pollution has caused increasingly serious water quality issues throughout the world. In the United States, agriculture is the leading contributor to non-point source pollution, with associated pollution responsible for 64% of impaired rivers, and 57% of impaired lakes (U.S. EPA, 2008, Marquez-Cuyno, 1995). Agricultural activities, such as confined animal feeding operations (CAFOs), overgrazing of land, excessive and ill-timed application of wastes and fertilizers, and tillage of crop land all contribute to non-point source pollution (Monaghan et al, 2008; Mayer et al, 2005) in the form of nutrients, pesticides, metals, and sediment. Effects of this pollution on surface waters can be severe, resulting in algal blooms; decaying algae has a high biological oxygen demand, which in turn can lead to hypoxic conditions, and fish kills (Sloan et al, 1999).

During the 1990's, the Neuse River experienced algal blooms, fish kills, and a Pfesteria outbreak, which called attention to water quality issues and agricultural practices. The Neuse River basin encompasses 23 counties, and makes up approximately 8.8% of landmass in the state of North Carolina. Agriculture makes up 35% of land use in the basin, and is the primary contributor of sediment, organics, and nutrients, specifically nitrogen (N), to the river (Neuse River Education Team). To work towards protection of water resources, the NC Department of Environment, Health, and Natural Resources (NC DENR) mandated that N loading be decreased by 30% by the year 2003 (NC Division of Water Quality, 2008). Strategies employed to meet these reductions have focused on implementation of best management practices (BMPs), which include nutrient management plans, controlled

drainage, and riparian buffers; in fact, riparian buffers have now been made mandatory in the Neuse River Basin (NC Division of Water Quality, 2008).

Riparian buffers are transitional vegetated strips of land between uplands and adjacent water bodies that provide wildlife habitat, stream bank stabilization, and water quality benefits such as nutrient filtering and sediment retention (Davis et al, 2007a; Osmond et al, 2002). Establishment and maintenance of riparian buffers in the correct landscape position can have major positive implications for water quality (Mayer et al, 2005). Hydrology and dissolved organic carbon (DOC) are thought to be the most critical factors influencing the effectiveness of buffers; however, interaction between these environmental parameters and design factors such as buffer width and vegetation type are not fully understood (Young and Briggs, 2007; Naiman and Decamps, 1997). Landowners, scientists, agricultural producers, and policy makers all have a vested interest in land-use and buffer establishment on land. To promote buffers that are the most environmentally and economically effective, a greater understanding of the physical and biological factors that promote the effective function of riparian buffers is needed.

**Loss of nutrients and sediment**

Fertilizers and animals waste contains N that is in the form of, or readily converted, to nitrate ($NO_3$), a highly soluble nutrient that moves with meteoric or irrigation water through the soil profile into shallow groundwater. The Coastal Plain is characterized by sandy soils with clay lenses at varying subsoil depths; the sandy texture encourages movement of nutrients with precipitation. When infiltrating $NO_3$ encounters clay lenses, water and

contaminants are forced in a lateral direction, eventually delivering nutrients to surface waters (Osmond et al, 2002).

In addition to N, sediment and attached nutrients can also be a water quality issue in agricultural landscapes. Surface run-off from agricultural fields commonly carries sediment, as well as associated nutrients and pesticides, to surface waters (Smith et al, 2008).

## Methods of nutrient and sediment removal

Riparian buffers can remove sediment and nutrients from both surface run-off, and groundwater that infiltrates buffers or moves laterally through rooting zones (Yamada et al, 2007).

Removal of $NO_3$ as it moves with shallow groundwater through buffer rooting zones can occur through plant root absorption (vegetation uptake), or through the microbial mediated conversion of $NO_3$ and $NO_2$ into a gas ($N_2O$ or $N_2$), with root and organic matter serving as the microbial energy source (Gilliam et al, 1997). Other modes of removal from groundwater include microbial immobilization, and Dissimilatory Nitrate Reduction to Ammonium (DNRA)(Groffman et al, 1992, Revsbech et al., 2005). Vegetation uptake and denitrification are believed to be the primary processes of $NO_3$ removal in riparian buffers (Jacobs and Gilliam, 1985; Pinay et al 1993; Lowrance et al 1984).

Sediment and attached contaminants are carried in surface run-off to surface waters. Run-off that encounters buffer vegetation will slow in velocity, allowing sediment and nutrients to settle out, and thus be retained in buffers; up to 90% of sediment load can be removed in this manner (Daniels and Gilliam, 1996). In addition to retaining sediment, this

slowing of run-off allows for infiltration of nutrients and pesticides into the soil profile, where microbial breakdown, plant uptake, and sorption can occur (Smith et al, 2008).

## Denitrification

In anaerobic conditions, $NO_3$ can be used as a terminal electron acceptor for specific microbes (denitrifiers), resulting in its conversion to nitrogen oxides (NO, $N_2O$) and dinitrogen gas ($N_2$). These nitrogen oxides are then volatized from the soil system into the atmosphere (Starr and Gillham, 1993, Coyne, 1999). The optimal environmental conditions for denitrifiers are (1) anaerobic conditions, (2) available substrate ($NO_3$), and (3) available organic carbon (C), which serves as the electron donor to denitrifiers (Starr and Gillham, 1993; Altman and Parizek, 1995; Hunt et al, 2004).

Control of denitrification rates and products is difficult to attribute to one particular factor; instead, interaction of multiple physical and chemical factors is more likely, as illustrated below (Coyne, 1999).

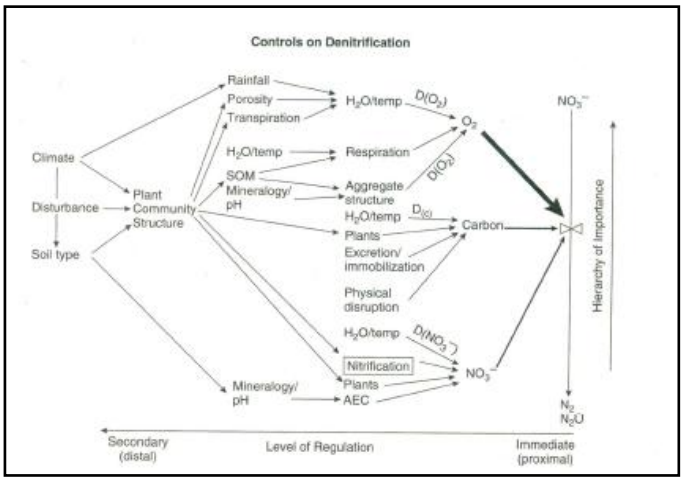


Figure 1. Environmental factors controlling denitrification (Adapted from Tiedje 1988).

Although it is difficult to isolate effects of specific factors when measuring denitrification, general relationships between factors and products have been identified by researchers.

## *Factors Effecting Denitrification*

### *Acidity*

The ideal pH range for soil denitrification is neutral (6 to 8) (Wijler and Delwiche, 1954). Acidic conditions ($pH \leq 4.0$) inhibit denitrification, as denitrifying microbe populations must adapt to low soil pH (Parkin et al, 1985). In acidic conditions, reduction of $N_2O$ to $N_2$ is inhibited; as a result, $N_2O$ dominates gaseous products (Wijler and Delwiche, 1954).

### *Temperature*

Biological activity of microbes is regulated by temperature. The rates of biological activity typically increase by a factor of 2 or 3 for every 10-degree rise in temperature (Stanford, 1975). Denitrifiers can function in a temperature range of 5ºC - 75ºC; however, the optimal temperature is believed to be 30ºC (Hebraud et al, 1994; Keeney et al, 1979). Researchers have argued that temperature effects on denitrification rates are dependent on the concentration of C available to microbes; where C is adequate in quantity and quality, denitrification can occur below 5º (Novak, 1974). In general, as temperature increases, production of $N_2O$ and $N_2$ increases, with $N_2$ dominating gaseous products (Kenney et al, 1979; Gilliam and Gambrell, 1978).

### *Soil Oxygen Status*

Activity of denitrifiers is strongly correlated to soil moisture (Davis et al, 2007a;

Luke et al, 2007); the presence of oxygen in soils has a significant effect on denitrifiers, and as a result denitrification products and rates. Oxygen will inhibit electron flow to denitrifying enzymes, and synthesis of denitrifying enzymes (Coyne, 1999). Researchers have found that the "later" reductase in the denitrification sequence are more sensitive than reductase earlier in the denitrification sequence. Due to reductase sensitivity, as oxygen increases overall denitrification rates decrease, with $N_2O$ dominating the proportion of gaseous products (Focht, 1974).

*Carbon Concentration*

Carbon (C) concentration is considered to be one of the primary factors regulating denitrification rates; as the electron donor for denitrifying bacteria, C is essential to the denitrification process (Gambrell et al, 1975). The exact concentration of DOC (dissolved organic carbon) that is adequate as an energy source for denitrifiers is unknown; concentrations less than 4.0 mg C/L are thought to inhibit denitrification, while concentrations greater than 8.0 mg C/L enable elevated denitrification rates (Lowrance and Smittle, 1988; Obenhuber and Lowrance, 1991; Sloan et al, 1999; Gilliam, personal communication, 2007). Composition of organic matter will also have an effect on availability of carbon to denitrifiers (Pavel et al, 1996). Labile organic matter, such as plant litter, is more readily available to microbes as compared to recalcitrant compounds (e.g., lignin) (Melillo et al, 1989); composition will thus have an effect on microbial activity, and denitrification rates.

Denitrification is believed to be the dominant pathway of $NO_3$ reduction in riparian areas where shallow groundwater flows laterally through biologically active zones, and there

is a sufficient C supply (McCarty et al, 2006; Davis et al, 2007b; Fennessy and Cronk, 1997; Jacobs and Gilliam, 1985; Peterjohn and Correll, 1984; Hanson et al, 1994). In conditions where neither C nor $NO_3$ is limiting, denitrification rates of over 1,278 kg N $ha^{-1}$ $yr^{-1}$ have been recorded (Pinay and Decamps, 1988). The highest rate of denitrification in soils has been found where C is concentrated (Robertson and Schiff, 2008; McCarty et al, 2006; Pavel et al, 1996). Higher denitrification rates are found in surface layers (due to the presence of living and dead root mass and decomposed leaf litter), at the depth of roots (due to C from root senesce and exudates), or in carbon-rich buried sediments (Haycock and Burt, 1992). Hill et al (2004) found denitrification activity concentrated in the upper 0-15 cm; additional studies by Lowrance (1992) found denitrification activity to be more than two orders of magnitude higher in the upper 10 cm of soil. Both of these studies found higher concentrations of C at the depth where denitrification rates were greatest. Studies have also found elevated denitrification rates in buried sediments; elevated denitrification rates were due to carbon-rich sediments and anaerobic conditions (Haycock and Burt, 1992). These studies and others support the idea of hotspots, or areas of concentrated organic matter and associated microbial activity, that support locally elevated denitrification rates (McCarty et al, 2006; Addy et al, 1999).

## Vegetation Uptake

Vegetation uptake is the second primary method by which $NO_3$ is removed from groundwater. Plant roots absorb $NH_4^+$ (ammonium) and $NO_3$ as a means of acquiring N, an essential nutrient for growth (Brady and Weil, 2004). Vegetation removal occurs as $NO_3$

moves through the soil profile and is intercepted and absorbed by roots, or through absorption of $NO_3$ in shallow groundwater flowing through the buffer rooting zone (Gilliam et al, 1997). Through absorption and sequestration, vegetation serves as a sink by storing N in woody mass and leafy vegetation. Vegetation root interception and adsorption (uptake) can removal significant N. Researchers have suggested that these two methods of removal will fluctuate seasonally, with denitrification dominating in the dormant seasons, and vegetation uptake dominating in the growing season (Simmons et al, 1992; Groffman et al, 1992; Lowrance, 1992; Schoonover and Willard, 2003). In addition, evapotranspiration needs and rainfall patterns typically reduces the groundwater table. Lack of plant growth during the dormant season may lead to a higher water table, and greater denitrification rates (Schoonover and Willard, 2003). The interaction between these two processes is very important to $NO_3$ removal in riparian buffers (Naiman and Decamps, 1997).

**Dilution**

Dilution of groundwater can occur as a result of underlying unconfined aquifers or spring upwelling, or by groundwater recharge by precipitation (Davis et al, 2007b). This dilution can reduce $NO_3$ concentrations in groundwater systems, which may lead to incorrect assumptions about $NO_3$ removal by vegetation uptake and/or denitrification. To monitor and detect dilution, the ratio of $NO_3$ to chloride (Cl) is compared in groundwater samples prior to and after flowing through buffers. Cl is a conservative tracer ion that is fairly resistant to biological and chemical soil transformations (Altman and Parizek, 1995). Any decreases in $NO_3$ should be mirrored by a decrease in the $NO_3$/Cl ratio. If the ratio were to stay the same

even though a $NO_3$ decrease was observed, dilution may be occurring in the buffers. Analysis for this study assumes that any dilution is from uncontaminated groundwater (King, 2005).

## Vegetation Type

Buffer vegetation type (trees versus grass) can have an effect on denitrification, vegetation uptake of $NO_3$, and sediment and nutrient removal from surface run-off (Lui et al, 2008; Smith et al, 2008). Studies are inconclusive relative to effect of vegetation type on $NO_3$ removal. Some researchers have recorded high $NO_3$ removal in grass buffers due to denitrification (Davis et al, 2007a; Groffman et al, 1991; Hubbard et al; 1998; Lowrance et al; 1995; Schnabel et al, 1996). These higher rates of denitrification are attributed to greater amounts of C in the soil profile due to density of grass rooting systems, which contribute C through root senesces and exudates (Gilliam et al, 1997). Other researchers, however, have identified sites where forest buffers had higher denitrification rates (Osbourne and Kovacic, 1993; Haycock and Pinay, 1993; Hefting and Klein, 1998). Reasons for these findings range from site specific factors, such as longer residence time of groundwater at buffer sites and deeper tap roots of trees that may provide C to microbes at a greater range of soil depth (Haycock and Pinay, 1993; Hefting and Klein, 1998).

Forest buffers provide additional ecosystem services such as stream bank stabilization and shading (Ghermandi et al, 2008; Machtans et al, 1996). Shading can reduce algal blooms by reducing phytoplankton productivity in the adjacent waterbodies (Ghermadi et al, 2008). Shading is also imperative for maintaining in-stream water temperature for sensitive aquatic species, specifically fish (Barton et al, 1985).

Both grass and forest buffers can provide habitat and corridors for bird movement (Machtans et al, 1996; Smith et al, 2008). In a study specific to North Carolina, researchers compared number of bird species in a three-zone buffer (mainly shrubs and grasses) versus both a primarily shrub and a primarily woodland buffer. Researchers observed the highest number of, and richness of, bird species in the three-zone buffer. They concluded that the vegetation present in each respective buffer dictated the bird community found to inhabit the buffer (Smith et al, 2008). This study highlights the habitat benefits provided by both grass and forest buffers.

When the water quality goal is retention of sediment (and attached nutrients and/or pesticides), grasses have proven to be more effective than trees (Lui et al, 2008, Smith et al, 2008). Effectiveness of grasses at sediment removal is due to density of grass vegetation, which slows velocity of water-borne sediment; this slowing allows for infiltration of water through the soil profile, where nutrient and pesticide attenuation can occur (Lui et al, 2008).

Overall, researchers caution against attributing variation in rates of $NO_3$-N reduction solely to differences in vegetation type; instead, site specific factors such as land-use legacy, soil type, adjacent vegetation, landscape position, and water table interactions need to also be considered (Addy et al, 1999).

## Buffer Width

Researchers have attempted to determine the optimal buffer width that will maximize $NO_3^-$ decreases from groundwater. Residence time of groundwater in narrow buffers may not allow sufficient time for denitrification to occur; however, wider buffers may not increase

water quality benefits sufficiently to offset the economic loss of land to producers (Lui et al, 2008).

Narrow buffers (10-20 m) have shown a range of $NO_3$ concentration decreases, with a maximum of 82% (Osbourne and Kovacic, 1993; Jordan et al, 1993); wider buffers (20-60 m) have demonstrated concentration decreases of 81-100% (Lowrance et al, 1995; Hubbard et al, 1998; Schoonover and Willard, 2003, Peterjohn and Correll, 1984). Although wider buffers showed greater decrease in [$NO_3$], widening a buffer does not necessarily guarantee greater $NO_3$ removal. In studies where narrow and wide buffer were studied alongside one another, rates of $NO_3$ removal were nearly ideal due to shallow water tables and high C concentrations (Osbourne and Kovacic, 1993; Mander et al, 1997).

Research studying the effect of buffer width on the removal of sediment and attached nutrients from surface run-off is also inconclusive; research suggests that a buffer should be wide enough for finer particles such as clay to be retained, which requires low flow velocities through buffer vegetation. Finer sediments are known to carry higher concentrations of pollutants and nutrients (Naiman and Decamps, 1997). However, other factors, such as buffer vegetation and height of vegetation, also have an effect on an appropriate buffer width (Lui et al, 2008).

Wenger (1999) theorized that slope of land adjacent to buffers may be as critical a factor in buffer width as groundwater flow and C concentration. Steep slopes will increase velocity of over-land flow, minimizing infiltration of nutrients, and settling out of sediment and attached contaminants from run-off. Specifically, researchers have hypothesized that for

every degree slope increases, buffer width should be increased by two feet (Lui et al, 2008). When all factors are ideal, researchers predict a trapping efficiency of over 95% with a 10 m buffer is possible (Lui et al, 2008).

## Anammox and DNRA

Besides denitrification, an alternative pathway for the reduction of $NO_3$ is Dissimilatory Nitrate Reduction to Ammonium (DNRA). In highly reduced, carbon-rich conditions, $NO_3$ is used as a terminal electron acceptor to drive the oxidation of organic compounds with the end product being $NH_4^+$ (Coyne, 1999; Maier et al, 2000). Due to the need for continuously anaerobic conditions, the DNRA pathway typically occurs in specific environmental niches, such estuaries and sediments, where the C/N ratio is $\geq 4.0$ (Coyne, 1999, Fazzolari et al, 1998).

Our field study conditions [low C concentration ($\approx$3.36 mg C/L), negligible $NH_4^+$, anaerobic and semi-aerobic soil profile) suggest that DNRA is not a pathway utilized in reduction of $NO_3$ in our study, and is thus not considered in our analysis.

Anaerobic ammonium oxidizing (anammox) bacteria are a relatively recent discovery; their existence was discovered in the sludge of waste water in the 1990's (Kuenen, 2008). Anammox bacteria are responsible for the anaerobic oxidation of ammonium ($NH_4^+$) coupled with $NO_2^-$ reduction under anoxic conditions to $N_2$ (Penton, 2009). Anammox bacteria are "metabolically flexible", meaning they are capable of alternative metabolic pathways. Because anammox operate in anoxic zones, these microorganisms may compete

with denitrifiers. In contrast to denitrifiers, however, anammox growth is slow, and their reductive activity is inhibited by even small amounts of $O_2$ (Penton, 2009).

The majority of research on anammox bacteria is associated with deep ocean sediments (Penton, 2009), where researchers believe these bacteria are responsible for 24-67% of N loss in marine environments (Francis, 2007). Although the possibility exists that anammox are active in riparian buffers, anammox activity has yet to be measured in soils. In addition, groundwater samples from our field study were analyzed for $NH_4^+$ for approximately eight years; concentrations were always below detection levels, so NH4+ sampling ceased in 2004. Based on these observations, anammox is excluded from further analysis of nitrate loss in our system.

## Need for Additional Research

Due to the wide range of research conclusions on removal of $NO_3$ from groundwater in riparian buffers, there is need for additional research on types of vegetation, buffer width, and $NO_3$ removal as effected by DOC concentration. Greater understanding of factors effecting $NO_3$ removal, in addition to greater understanding of factor interaction, will have a positive impact on water quality policy regarding buffer design and establishment.

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Chapter 2: Riparian Buffer Effectiveness at Removal of $NO_3$-N from Groundwater in the Middle Coastal Plain of North Carolina


Abstract

KNIES, SARA VICTORIA. Riparian Buffer Effectiveness at Removal of $NO_3$-N from Groundwater in the Middle Coastal Plain of North Carolina. (Under the direction of Dr. Deanna L.Osmond).

Non-point source pollution from agriculture is one of the causes of surface water quality degradation in the Coastal Plain of North Carolina. Riparian buffers are an important best management practice for reducing $NO_3^-$ concentrations in natural waters, predominately by vegetation uptake and denitrification. A temporal study of riparian buffer effectiveness has been underway at the Center for Environmental Farming Systems (CEFS). The study has investigated the effects of vegetation type, groundwater depth, and buffer width on $NO_3^-$ removal from groundwater. Five vegetation types (switchgrass [Panicum virgatum], fescue [festuca elatior], native vegetation [vines, weeds, grass], tree trees [Pinus taeda], and a control) have been established. In addition, two buffer widths (8 and 15 m), and two well depths (1.5 m and 2.1 m) have been established, and monitored for $NO_3^-$ removal efficiency. On a monthly basis, water table depth, reduction potential measurements, and groundwater samples were taken. Groundwater samples were analyzed for $NO_3$-N, $PO_4$-P, Cl, and DOC.

There was a significant three-way interaction ($p < 0.05$) between vegetation type, buffer width, and well depth. This interaction was desegretated by depth, resulting in a significant two-way interaction ($p < 0.05$) between vegetation type and buffer width at the deep depth. The effect of vegetation at the narrow width was significant ($p = 0.0412$) at the deep well depth, meaning there was a positive reduction in groundwater $NO_3$-N by

vegetation at this interaction of depth and width. The effect of switchgrass was significant ($p=0.0120$) in removal of groundwater $NO_3$-N in both the narrow and wide buffer widths. The effect of the revegetation treatment was significant ($p=0.0093$) at removal of groundwater $NO_3$-N in the narrow width.

Reduction potential (Eh) values indicated that deep wells were predominantly anaerobic; this suggests denitrification was responsible for $NO_3^-$ removal in buffers.

## Introduction

During the 1990's, the Neuse River experienced algal blooms, fish kills, and an outbreak of Pfesteria. These water quality problems were believed to be the result of excessive nutrients in surface waters, originating from multiple sources, including agricultural practices such as over application of fertilizers and animal waste (Mayer et al, 2005). In response to water quality problems, rules mandating implementation of best management practices were introduced; establishment of riparian buffers became mandatory in the Neuse River Basin as a result of these rules. Riparian buffers are transitional vegetated strips of land established between agricultural lands and adjacent waterbodies. Riparian buffers are capable of filtering nutrients, sediments, and attached contaminants from surface run-off and groundwater flow (Osmond et al, 2002).

Research has demonstrated that riparian buffers are capable of removing nitrate ($NO_3$) from groundwater (Lowrance et al, 1995; Hubbard et al, 1998; Schoonover and Willard, 2003, Peterjohn and Correll, 1984). Removal rates up greater than 90% have been recorded in buffer studies. However, there is a wide range of $NO_3$-N decreases in the literature, with varying explanations for buffer effectiveness In addition the debate about the processes responsible for $NO_3$ removal, there is debate among researchers about the most effect buffer vegetation and widths. Studies of buffer vegetation have evaluated the effectiveness of forest versus grass buffers at removal of $NO_3$, often with conflicting findings. Certain studies have found forest buffers to be most effective at $NO_3$ removal (Osbourne and Kovacic, 1993; Haycock and Pinay, 1993; Hefting and Klein, 1998), while

others have identified grass buffers as being more effective at removal (Groffman and Axelrod, 1991; Schnabel et al, 1996; Lowrance et al; 1995; Hubbard et al; 1998). Similar differences have been found with regards to buffer width. Studies have identified wider buffers as being more effective at $NO_3$ removal (Hubbard et al, 1998; Schoonover and Willard, 2003, Peterjohn and Correll, 1984), while other studies have identified narrow buffers as having nearly identical rates of removal (Osbourne and Kovacic, 1993; Mander et al, 1997). The issue of buffer width is a critical one, as wider buffers require more land to be taken out of agricultural production.

Besides buffer vegetation and width, there are several other factors which have a significant effect on buffer effectiveness. These factors include soil carbon (C) concentration, and groundwater hydrology. Carbon concentration is an extremely important factor, as it is believed that low C concentrations will inhibit denitrification, and higher concentrations will elevate denitrification rates (Lowrance and Smittle, 1988; Obenhuber and Lowrance, 1991; Sloan et al, 1999; Gilliam, personal communication, 2007).

As demonstrated by contrasting data, additional research is needed to elucidate ideal buffer widths and vegetation type. Thus, this field study was designed to accomplish to following objectives:

- Determine if denitrification is responsible for groundwater $NO_3$-N decreases in buffers CEFS.
- Determine the influence of vegetation type, buffer width, and groundwater depth on $NO_3$-N removal from buffer groundwater.

## Methods and Materials

### Site Description

Four vegetated buffers were established twelve years ago (1997) at the Center for Environmental Farming Systems (CEFS) Cherry Farm research farm located in Goldsboro, North Carolina (Appendix B, Figure 1). Goldsboro is located in Wayne County, which is within the Neuse River Basin and the Middle Coastal Plain physiographic province. The buffers at CEFS are adjacent to deeply incised drainage ditches, which drain into the Neuse River.

The Middle Coastal Plain, in the thermic soil temperature regime, is characterized by smooth, gently rolling uplands. These uplands give way to river valleys that can range from gentle to steep in gradient. The Middle Coastal Plains elevation upper and lower boundaries are the Coasts Scarp at 94 m and the Surry Scarp at 29 m, respectively (Daniels et al, 1999). Uplands have a seaward sloping gradient of 0.1 to 0.3 m/km. Maximum elevation differences between upland areas and valley floors are rarely greater than 30 m (98 feet), with most valley floors being flat (Daniels et al, 1999).

Upland soil series of the Middle Coastal Plain include Lynchburg, Pantego, Goldsboro, and Rains. Series delineated on valley slopes include Bibb, Johnston, Kinston, Gritney, Wagram, Orangeburg and Norfolk (Daniels et al, 1999).

### Buffer Design

The four buffers that are being monitored for this study are designated as R1, R2N, R4W, and R5N and are located along four drainage ditches (approximately 10” deep and

20”wide), some of which are incised. Land-use adjacent to buffers has historically included wheat, livestock pasturing, soybeans, sudangrass, corn, millet, ryegrass, peanuts, clover, and fescue grass (King, 2005). Each buffer is divided into two widths, 7.6 m (narrow) and 15.2 m (wide); each width is further subdivided into five strips, 25 m in length, in which five vegetation types have been established.

Vegetation types are fescue grass (*Festuca elatior*), switchgrass (*Panicum virgatum*), trees [tree trees (*Pinus taeda*),water oak (*Quercus nigra*), cherry bark oak (*Quercus pagoda*), green ash (*Fraxinus pennsylvanica*), sweetgum *(Liquidambar styraciflua)* cedar (*Cedrus*)], native vegetation (vines, weeds, grass) and a control. Each vegetation type and control is represented twice per buffer; once in the narrow buffer width, and once in the wide buffer width. The fescue treatments are mowed once a year, and the fescue is left on the treatment plot. Established vegetation (fescue, switchgrass, tress, and native vegetation) is twelve years old. During previous monitoring, the control treatment varied among buffers, and consisted of livestock, pasture, (R4W and R5N) or rotations of soybeans, wheat, ryegrass, corn and other crops (R1 and R2N). Since 2005, previously cropped controls have not been managed, and have been allowed to reestablish native vegetation. This native vegetation has never been cut or fertilized, and consists of a mixture of vines, weeds, and grass. The controls in R4W and R5N remain in beef and dairy pasture, and are managed in the same manner as adjacent fields. For purposes of discussion, controls in R1N and R2 are hereafter referred to “revegetation”, and controls in R4W and R5N are referred to as “controls”.

## Groundwater Monitoring

Within each vegetation treatment, well nests were installed at the ditch edge and field edge. Well nests have also been installed in the fields adjacent to each buffer, approximately 15 m from the buffer edge (Figure1). Wells were constructed using 5.1 cm diameter polyvinyl chloride (PVC) pipe, with 0.6 m of slotted screen at the bottom. Each well nest consists of three wells, installed to depths of 0.6-1.0 m (shallow), 1.5-2.1m (intermediate), and 2.1-3.5 m (deep) as measured from the ground surface to the top of the well screen (Appendix B, Figure 6).

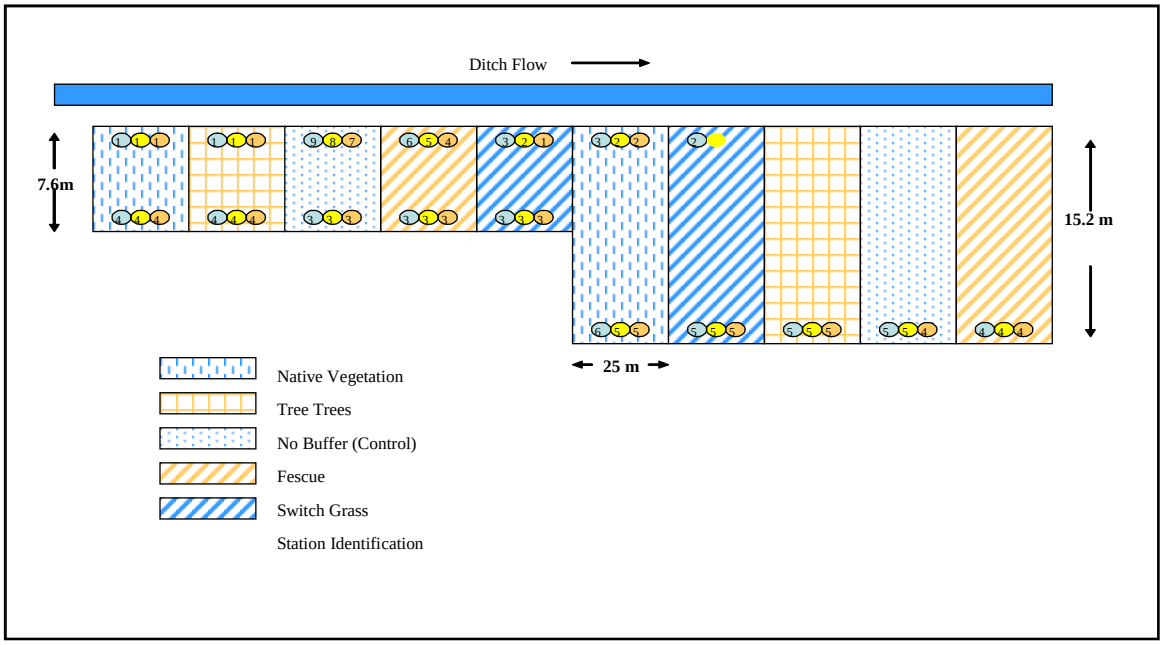


Figure 1. Example of buffer at CEFS illustrating well nest location, and vegetation treatment establishment. Not to scale.

The purpose of these three depths was to actively monitor the water table profile.

The deep well was installed to 2.1-3.5 m so that it would provide a sample of groundwater just above the impermeable layer of the existing aquifer (Dukes et al, 2002). The intermediate well was placed to sample the intermittently saturated zone at the top of the aquifer; redoximorphic features were used to determine this zone. The shallow well was installed as to sample the upper surface beneath the root zone (Dukes, 2000). All wells were surveyed to a local benchmark. Only intermediate and deep wells were monitored as the shallow wells were dry for significant periods of time. Wells were capped between samplings. Standard 3-point surveying was previously completed on buffers to ensure groundwater flow was perpendicular to drainage ditches (King, 2005).

Groundwater sampling for this study began in January 2007, with wells being sampled approximately every 30 days for the duration of the study. Using a handheld Solinst water level meter, respective water levels were measured in all wells. Water table depth was used to calculate the time needed to purge wells three well volumes before a sample was collected using a portable Teledyne Isco peristaltic pump. Once purged, water samples were pumped into acid-washed 40 ml glass bottles. Duplicate samples of random wells, spikes, and blanks were taken to meet QA/QC protocol. Samples were placed on ice, and transported back to NC State University where they were stored at 4ºC until analyzed.

Samples were acidified to a pH of 2.0 using 5% $H_2SO_4$ and filtered through 0.45um HV Millipore syringe filters into acid-washed 40 ml glass bottles using 10 ml syringes. Samples were submitted to the Analytical Services Laboratory in the Soil Science

Department at NC State University for analysis (see Laboratory Analysis of Groundwater Samples).

## Reduction Potential (Eh) Monitoring

Prior to this study, platinum tipped redox probes were installed in buffers R1, R2N, and R4W by Kunickis (2000) and Ricks (2002). Probes were installed at three depths, designated as shallow (76 cm), intermediate (152 cm), and deep (300 cm). Probes were installed around a salt bridge constructed using an open ended 3.81 cm diameter PVC pipe (Appendix B, Figure 7). Salt bridges were filled with a gelatinous potassium chloride agar solution and then capped. Once depth of probe was identified, appropriately labeled redox wires were attached to a wooden platform.

Eh was measured in mV using an Accumet AP62 Portable pH/mV meter manufactured by Fisher Scientific. The reference electrode was inserted into the salt bridge enabling an electric circuit to be created between soil and redox probe; readings were taken from each individual probe wire. Eh data was collected at each scheduled monthly sampling event. A standard correction factor of +240 mV was added to all values recorded from the field in order to compensate for the potential of the reference electrode.

The pH of respective buffer soil was determined so that the predicted Eh at which denitrification is expected to occur could be determined. A hole was augured in order to remove either an intermediate or deep redox probe at each buffer. A soil sample was than collected from the bottom of the augured hole. The pH was analyzed using a 1:1 soil to water

mix. The hole was then backfilled using a slurry of water pumped from an adjacent well and the removed soil. The pH at both probe depths was determined to be 5.2.

### Laboratory Analysis of Groundwater Samples

Approximately 200 samples were collected during each sampling event. Each month, approximately 192 groundwater samples were pumped directly from wells, with two duplicate samples per buffer, one blank, and one spike. Upstream and downstream surface water samples were also collected. During the summer months, wells were frequently dry, preventing groundwater samples from being collected. Groundwater samples were analyzed for $NO_3$-N, phosphate ($PO_4$-P), chloride (Cl), and dissolved organic carbon (DOC). Prior sampling determined that ammonium concentrations were too low to be detected. The Analytical Services Lab in the Soil Science Department analyzed samples using the following equipment:

- $NO_3$-N, $PO_4$-P and Cl were analyzed using a Lachat Instruments QuikChem brand 8000 Automated Ion Analyzer Continuum Series (Lachat Instruments, Milwakee, WI).
- DOC was analyzed using a Shimadzu Total Organic Carbon Analyzer 5050 (SN30623181) (Shimadzu Scientific Instruments, Inc., Columbia, MD)

## Results

### Water Table Data

Water table measurements to monitor groundwater depth were taken on a monthly basis from the intermediate and deep wells in all well nests. As noted in Methods and

Materials, shallow wells were not monitored.

Monthly water table measurements from intermediate and deep wells were averaged across all buffers for a monthly water table level (Figure 2):

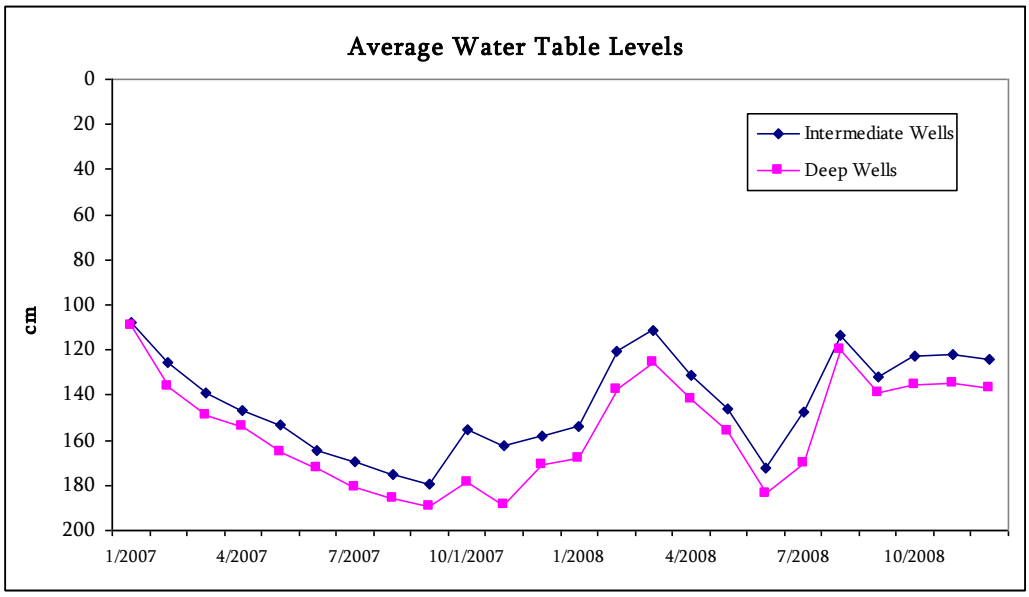


Figure 2. Average monthly water table levels for intermediate and deep depth wells at CEFS.

Although there was not a large difference between intermediate and deep water table depth, during the summer and prolonged periods of low rainfall, intermediate wells were frequently dry.

At both the intermediate and deep well depth, the water table was shallower to the ground surface at the ditch edge versus the field edge. This resulted in establishment of reducing and oxidizing field conditions at respective field locations as discussed below.

## Overall Reduction Potential (Eh) Data

Monthly Eh values (mV) for three buffers (R1, R2, R4) were calculated by averaging

values from the three probes at each of the two respective depths (deep probes, 300 cm; intermediate probes, 152 cm). No redox probes were installed in the fourth buffer (R5N). Electron activity and pH are considered master variables in reduction potential, as both have a significant influence on the chemistry of compounds found in soil (Essington, 2004). The pH of buffer soil was used as an indicator of the value where denitrification is expected to occur in CEFS buffers. It is generally accepted for soils pH ≈ 5.2 (average pH of buffer soil at the deep and intermediate probe depths) that $NO_3$-N reduction will begin to occur at approximately 350 mV. This number is not exact however; the accepted range where $NO_3$-N reduction can occur at pH ≈ 5.2 is 325 mV-375 mV (Essington, 2004).

*Eh Measurements: Deep Wells*

At the deep well depth, the water table was shallower to the ground surface at the ditch edge (versus the field edge). Shallower water table depth and long periods of anaerobic conditions at the ditch edge resulted in average Eh measurements that were always reducing (<350 mV). The water table was further from the ground surface at the field edge, and wells were occasionally dry. With the exception of R4W, Eh values were on average oxidizing (> 350 mV) at the field edge. The soil aeration gradient in buffers at the deep well depth suggests that the denitrification process would occur most readily at the position of the ditch wells.

*Eh Measurements: Intermediate Wells*

Intermediate depth wells were frequently dry during the summer or periods of reduced rainfall. Although the water table was shallower to the ground surface in ditch edge

wells versus field edge wells, reduction potential measurements in intermediate wells were on average > 350 mV, indicating oxidizing conditions (Figure 3):

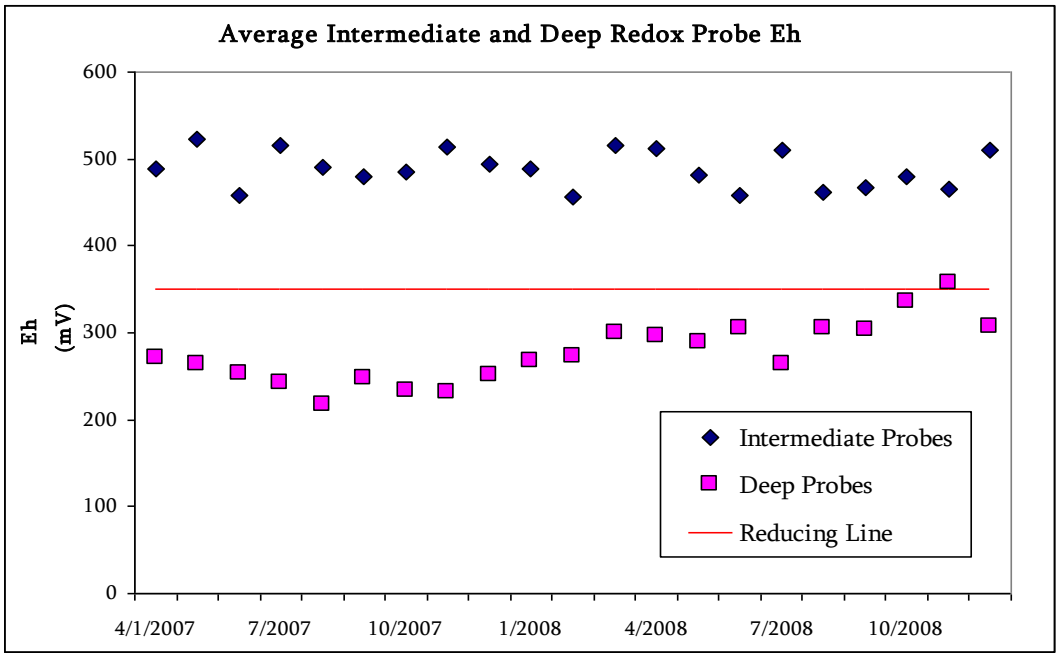


Figure 3. Average Eh in intermediate and deep probes corresponding to intermediate and deep well depth.

## Dilution

The ratio of $NO_3$-N to Cl)was calculated to ensure that any decreases in $NO_3$-N were due to either vegetation uptake or denitrification. Chloride is a conservative ion in groundwater, so decrease in concentration in not expected (Altman and Parizek, 1995). If the ratio $NO_3$-N/Cl were to stay the same even though a $NO_3$-N decrease was observed, an upwelling, and thus dilution could be occurring in the buffers. Any decreases in $NO_3$-N concentration should be mirrored by a decrease in the $NO_3$-N/Cl ratio.

Comparison of the $NO_3$-N/Cl ratio was made between field-edge wells and ditch-edge wells for both buffer widths. The ratio for the 15 m buffer width decreased 71% (from 0.52 mg to 0.15 mg) in the deep wells, and 44% in the intermediate wells (from 0.84 to 0.47). Ratio reductions generally correspond to $NO_3$-N decreases of 73% and 44%. The $NO_3$-N/Cl ratio for the 8 m buffer width decreased 51% (from 0.35 to 0.17) in the deep wells, and 29% (from 0.83 to 0.59) in intermediate wells. These generally corresponded to $NO_3$-N decrease of 47% for the deep wells. There was a $NO_3$-N decrease of 19% in the intermediate wells, which is 10% different from the $NO_3$-N/Cl ratio reduction.

Comparison of ratios was also made between field-edge wells and ditch-edge wells for vegetation treatments. The $NO_3$-N/Cl ratio for trees decreased 52% (from 0.73 to 0.35), fescue decreased 40% (from 0.45 to 0.27), switchgrass decreased 62% (from 0.60 to 0.23), native vegetation decreased 46% (from 0.61 to 0.33), and the control decreased 46% (from 0.45 to 0.22). Ratio decreases all corresponded to $NO_3$-N decreases, which respectively were 48% (trees), 43% (fescue), 64% (switchgrass), 43% (native vegetation), and 50% (no-buffer control).

There appeared to be slight dilution in the vegetation treatments of switchgrass and fescue. In these treatments, the $NO_3$-N decreases are slightly greater than the $NO_3$-N/Cl ratios, indicating dilution may be occurring (see Appendix F for complete data).

## Statistical Analysis: $NO_3$-N & DOC

*$NO_3$-N and DOC concentrations*

Incoming $NO_3$-N concentrations varied greatly both between, and within replicates as a function of buffer width and well depth (Table 1).

Table 1. Average incoming $NO_3$-N concentrations

| | | $NO_3$-N (mg N/L) |
|---|---|---|
| R1 | 15 m width | 14.96 |
| | 8 m width | 5.42 |
| | Deep depth well | 8.93 |
| | Interm. depth well | 12.27 |
| R2 | 15 m width | 3.83 |
| | 8 m width | 2.53 |
| | Deep depth well | 2.81 |
| | Interm. depth well | 3.47 |
| R4 | 15 m width | 6.34 |
| | 8 m width | 15.06 |
| | Deep depth well | 7.9 |
| | Interm. depth well | 13.16 |
| R5 | 15 m width | 1.84 |
| | 8 m width | 2.87 |
| | Deep depth well | 1.08 |
| | Interm. depth well | 3.58 |

Approximately 7% of incoming $NO_3$-N concentrations were below machine minimum detection limits (MDL = 0.1 mg N/L); these values were concentrated in replicate R5. To address concentrations below the MDL, all concentrations <0.1 mg N/L were set to half of the MDL (0.05 mg N/L).

No DOC concentrations were below machine minimum detection limits (MDL= 0.5 mg DOC/L).

### *Model Development*

Analyses were run for the response variable of $NO_3$-N and DOC percent reduction difference, or "change" as:

$NO_3$-N Change = $NO_3$-N(mg N/L) before - $NO_3$-N(mg N/L) after/ $NO_3$-N(mg N/L) before

DOC Change = DOC (mg C/L) before – DOC (mg C/L) after/ DOC (mg C/L) before

where before is pretreatment concentrations, and after is post-treatment concentrations. Analyses were run in SAS 9.2, using a PROC MIXED model:

log (change) is a function of vegetation type, buffer width, and groundwater depth

### *$NO_3$-N Change*

Computation of $NO_3$-N change resulted in left skewed data, with a range in values from 0.99 to -279. Change was multiplied by (-1), resulting in a data range of -0.99 to 279, shifting skewness to the right. The value of 1.9986911 was then added to all negative values so that the minimum value in the data set was equal to 1, which would allow natural log transformation of data. After natural log transformation, the resulting data range (1.0086911 to 280.99869) was normal, and thus analyzed for significance. Owing to complexity of data issues, there was no commonly accepted method of dealing with data results such as ours.

Data transformations were thus unique to our data set, with the main purpose being achievement of data normalcy for purposes of analysis.

### *DOC Change*

Computation of DOC change resulted in left skewed data (-9.588235 to 0.925764). Values were multiplied by -1, which resulted in moving the tail to the right (-0.925764 to 9.588235). The value of 1.925764 was then added to negative values so that all data was greater than or equal to1. This allowed data to be natural log transformed, which resulted in normalcy of data. Data was then analyzed for significance. As with $NO_3$-N, transformations of DOC data were unique to our study due to data complexity, with the main purpose being achievement of data normalcy for purposes of analysis.

### *Model Results*

#### *$NO_3$-N*

The main effects of vegetation, width, and depth were all significant ($p < 0.05$) as listed in Table 2. In addition, there were significant two-way interactions (veg*width, veg*depth), and a significant three-way interaction (veg*width*depth).

| Table 2. Type 3 Tests of Fixed Effects for $NO_3$-N | | | |
|---|---|---|---|
| Effect | Numerator Degrees of Freedom | Denominator Degrees of Freedom | Pr > F |
| Veg | 5 | 909 | <.0001* |
| Width | 1 | 923 | 0.0012* |
| Veg*width | 5 | 909 | <.0001* |
| Depth | 1 | 901 | <.0001* |
| Veg*depth | 5 | 881 | <.0001* |
| Width*depth | 1 | 901 | 0.0825 |
| Veg*width*depth | 5 | 881 | <.0001* |

*Indicates value is significant ($p < 0.05$)

Reduction potential (Eh) values were different at the two depths. For most samples, Eh suggested a saturated system for the deeper well depths. The Eh values of the medium well depth fluctuated between anoxic and oxic. We determined that depth represented two biologically different systems and we separated their analysis, (Table 3):

| Table 3. Type 3 Tests of Fixed Effects of $NO_3$-N desegregated by depth | | | |
|---|---|---|---|
| Effect | Numerator Degrees of Freedom | Denominator Degrees of Freedom | Pr > F |
| Deep well depth | | | |
| Veg | 5 | 39 | 0.3256 |
| Width | 1 | 39 | 0.2829 |
| Veg*width | 5 | 39 | 0.0046* |
| Intermediate well depth | | | |
| Veg | 5 | 39.6 | 0.6646 |
| Width | 1 | 39.9 | 0.5777 |
| Veg*width | 5 | 39.6 | 0.6852 |

*Indicates value is significant ($p < 0.05$)

At the deep depth, there was a significant two-way interaction between veg*width. This interaction was desegregated to investigate the effect of *vegetation at each width*, and the effect of *width for each vegetation type* (Table 4):

| Table 4. Type 3 Deep well effects for vegetation type at each width and width for each vegetation type | | | | |
|---|---|---|---|---|
| | Effect | Numerator Degrees of Freedom | Denominator Degrees of Freedom | Pr > F |
| **Effect of vegetation at each width** | | | | |
| Narrow | Width | 5 | 19 | 0.0412* |
| Wide | Width | 5 | 20.1 | 0.0699 |
| **Effect of width at each vegetation type** | | | | |
| Switchgrass | | 1 | 6.95 | 0.0120* |
| Control | | 1 | 3.98 | 0.3596 |
| Fescue | | 1 | 8 | 0.0715 |
| Native | | 1 | 8.02 | 0.4908 |
| Revegetation | | 1 | 3.98 | 0.0093* |
| Pine | | 1 | 8 | 0.5315 |

*Indicates value is significant ($p < 0.05$)

The effect of vegetation at the narrow width was significant ($p = 0.0412$) at the deep well depth, meaning there was a positive reduction in groundwater $NO_3$-N by vegetation at this interaction of depth and width. The effect of switchgrass was significant ($p=0.0120$) in removal of groundwater $NO_3$-N in both the narrow and wide buffer widths. The effect of the revegetation treatment was significant ($p=0.0093$) at removal of groundwater $NO_3$-N in the narrow width.

### *DOC*

The main effects of width, and depth were all significant ($p < 0.05$) for DOC as listed in Table 5. The two-way interactions of veg*width, veg*depth, and width*depth were all significant ($p < 0.05$); however, the three way interaction was not significant.

Table 5. Type 3 Tests of Fixed Effects for DOC

| Effect | Numerator Degrees of Freedom | Denominator Degrees of Freedom | Pr > F |
|---|---|---|---|
| Veg | 5 | 979 | 0.0782 |
| Width | 1 | 989 | 0.0003* |
| Veg*width | 5 | 979 | 0.0084* |
| Depth | 1 | 970 | 0.0001* |
| Veg*depth | 5 | 960 | 0.0320* |
| Width*depth | 1 | 970 | 0.0129* |
| Veg*width*depth | 5 | 960 | 0.2697 |

*Indicates value is significant ($p < 0.05$)

### *Non-modeled Data: $NO_3$-N*

### *Influence of Vegetation on $NO_3$-N Removal*

Influence of vegetation type on groundwater $NO_3$-N decreases was calculated by averaging concentrations for field-edge wells and ditch-edge wells for both well depths and buffer widths, in all four buffer replications (Figure 4).

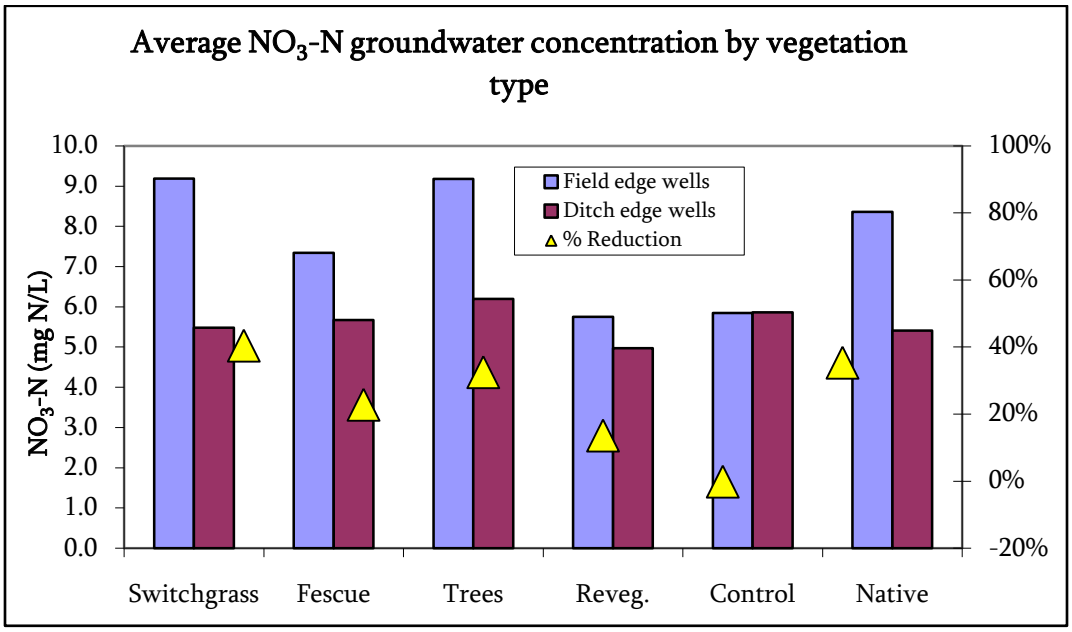


Figure 4. Average $NO_3$-N groundwater concentration by vegetation type

The influence of vegetation type on $NO_3$-N groundwater decreases were as follows: revegetation had a decrease of 14% (5.75 mg N/L to 4.97 mg N/L); switchgrass had a decrease of 40% (9.19 mg N/L to 5.48 mg N/L); trees had a decrease of 32% (9.18 mg N/L to 6.20 mg N/L); native vegetation had a decrease of 35% (8.36 mg N/L to 5.41 mg N/L); fescue had a decrease of 23% (7.34 mg N/L to 5.67 mg N/L); the control had a decrease of 0% (5.85 mg N/L to 5.86 mg N/L).

*Influence of width and depth on $NO_3$-N Removal*

Influence of buffer width and groundwater depth on $NO_3$-N decreases was calculated by averaging concentrations for field-edge wells and ditch-edge wells for well depths and buffer widths, in all four buffer replications (Figure 5).

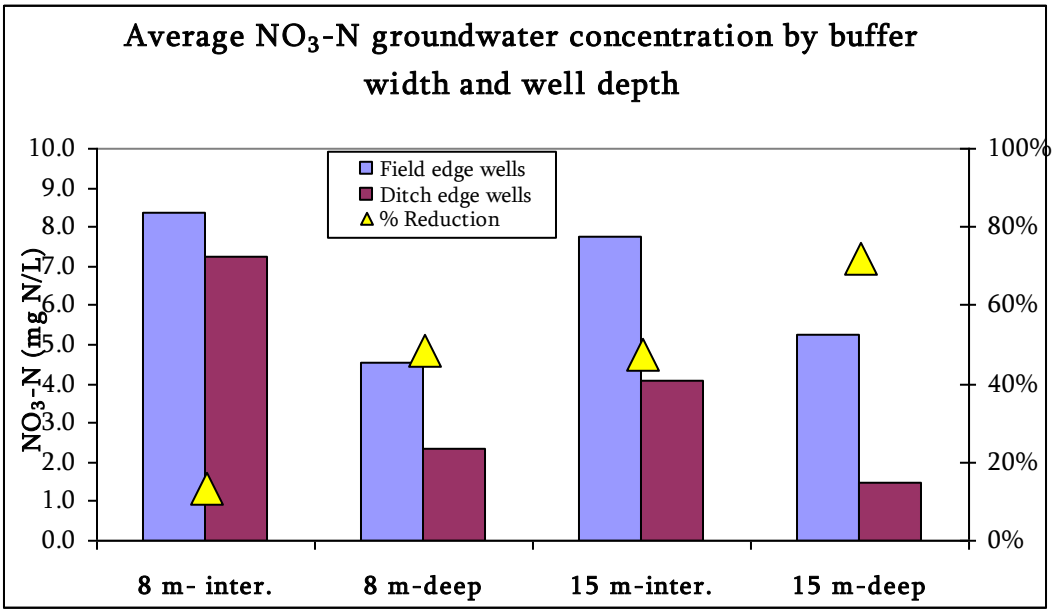


Figure 5. Average $NO_3$-N groundwater concentration by buffer width and well depth

Influence of width and depth on $NO_3$-N decreases were as follows: deep wells in 15 m buffers had a $NO_3$-N decrease of 77% (5.76 mg N/L to 1.34 mg N/L), deep wells in 8 m buffers had a decrease of 53% (4.55 mg N/L to 2.13 mg N/L), intermediate wells in 15 m buffers had a decrease of 47% (7.51 mg N/L to 4.00 mg N/L), and intermediate wells in 8 m buffers had a decrease of 14% (8.38 mg N/L to 7.19 mg N/L).

*Non-modeled results: DOC concentration*

Average DOC concentration was calculated by averaging non-transformed concentrations across all widths, depths, and vegetation types (Figures 6 & 7). Average concentration was 3.4 ± 0.6 mg C/L.

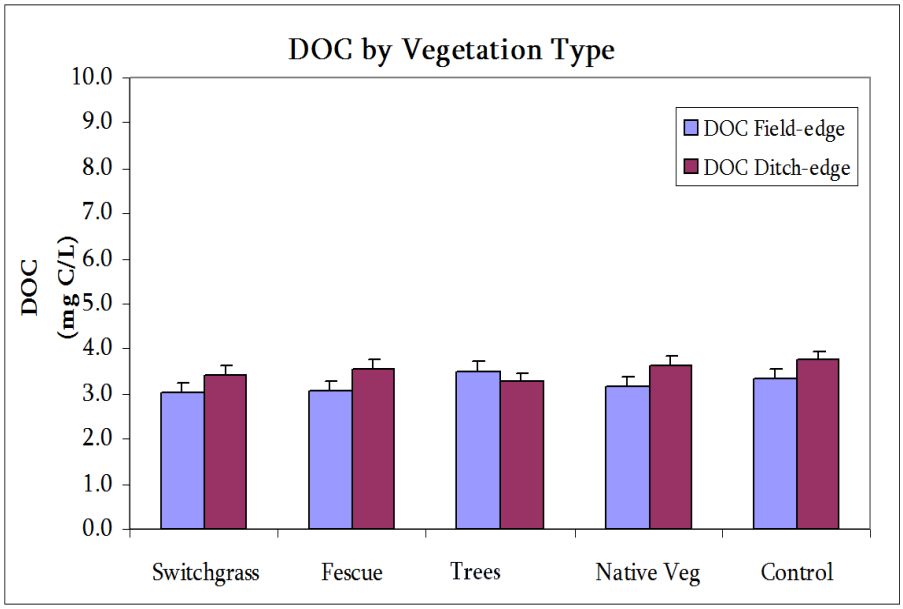


Figure 6. Average DOC groundwater concentration by vegetation type.

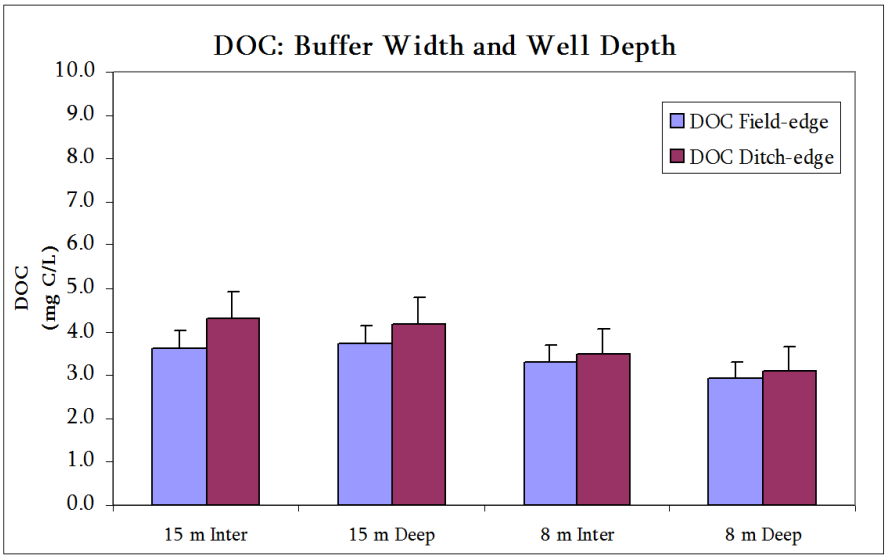


Figure 7. Average DOC groundwater concentration by buffer width and well depth

Due to the concentration of DOC being so low across all four replicates, there were no discernable trends in DOC data, even though there were significant ($p < 0.05$) main effects, and significant two way interactions. With an average DOC concentration of 3.4 ± 0.6 mg C/L mg for all buffers at CEFS, $NO_3$-N removal by the denitrification process would be expected to be minimal (Obenhuber and Lowrance, 1991; Gilliam, personal communication, 2007). However, even though removal did not approach those demonstrated by other buffer studies, high percentages of $NO_3$-N removal were found in specific treatments. It is important to note that quality of organic C, versus quantity of C, may be more important in terms of the denitrification process. "High quality" (labile) C has a lack of recalcitrant lignin, and a higher C/N ratio, and is utilized quicker by the microbial community (Hill and Cardaci, 2004). Although quantity of C at CEFS is low, quality may be sufficient for some measure of denitrification to occur (see Appendix E for supplementary C data).

*Riparian buffer replicates as Case Studies*

Replicates differed not only with regards to incoming $NO_3$-N concentrations, but also with regards to land practices adjacent to buffers, intensity of incised ditches adjacent to buffers, and most importantly, soils within buffers. Varying depths of deeply incised drainage ditches resulted in deeper water table conditions in some buffers versus others, while land practices influenced incoming $NO_3$-N concentrations both between, and within buffer replicates. Due to soils and other replicate properties changing so considerably within buffers, it is difficult, if not misleading, to evaluate overall buffer effectiveness of $NO_3$-N removal of all replicates together. Having four replicates whose properties differ allows us to

instead evaluate buffer effectiveness as individual case scenarios, allowing the application of results to a range of environmental conditions that are encountered where buffers may be established.

### *Case studies of riparian buffer $NO_3$-N removal*

#### R1

Land use adjacent to R1 was organic hay in 2007 receiving 3.5 tons/ac turkey litter, and piper sudan grass in 2008, which did not receive any fertilization. Average incoming $NO_3$-N concentrations are listed below in Table 6:

Table 6. Average incoming $NO_3$-N concentrations in R1

| | | $NO_3$-N (mg N/L) |
|---|---|---|
| R1 | 15 m width | 14.96 |
| | 8 m width | 5.42 |
| | Deep depth well | 8.93 |
| | Interm. depth well | 12.27 |

The drainage ditches adjacent to R1 were the most deeply incised drainage ditches (in comparison to other rep's buffers (approx. 15 ft deep).

#### *Soils*

Soils in buffer R1 were Lumbee sandy loam, with Wickham sandy loam in the adjacent field. Soil profile data is only available for the narrow width in R1. At the field

edge, there were clay lenses of varying depths in the approximate depth range of 40 cm – 140 cm. Clay lens were present in all vegetative treatments. At the ditch edge, clayey lends were only present at a depth of 60 cm to 80 cm in the tree and native vegetation treatment.

*Reduction Potential (Eh) Values*

Eh conditions in deep wells were oxidizing at the field edge, and reducing at the ditch edge (Figure 8):

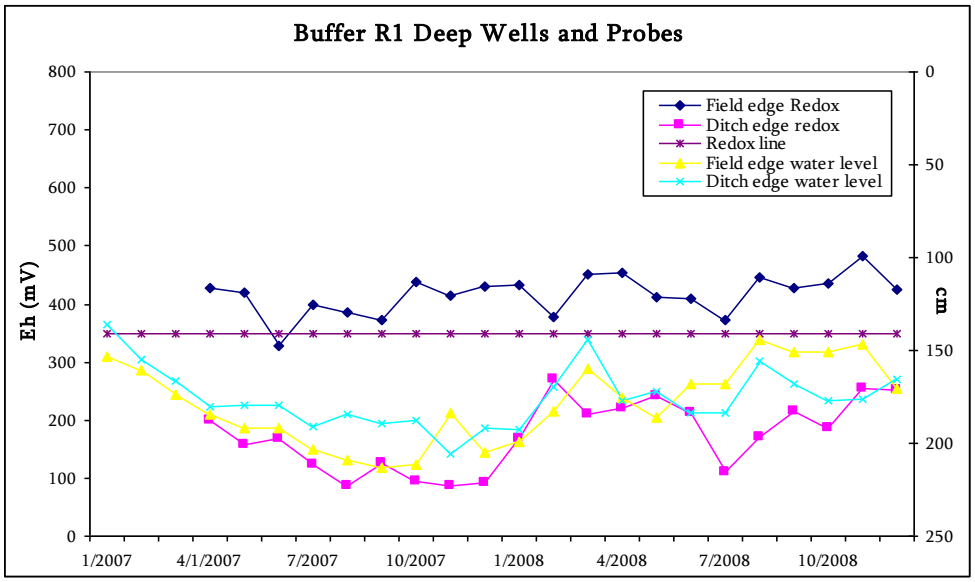


Figure 8. Average groundwater depth and redox measurements for deep wells.

Eh conditions in intermediate wells were always oxidizing (Figure 9):

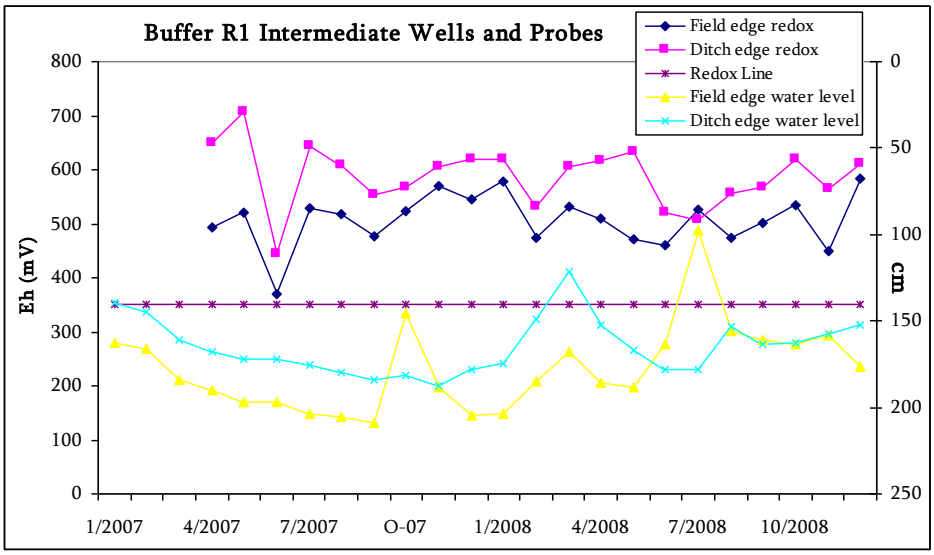


Figure 9. Average groundwater depth and redox measurements for intermediate wells in R1.

Nitrate reduction in R1 from greatest to least was as follows (Table 7):

| Table 7. $NO_3$-N Percent Reduction in R1 | |
|---|---|
| Wide, deep, Pine | 99.20% |
| Wide, deep, Switchgrass | 95.16% |
| Wide, deep, Native | 93.47% |
| Narrow, deep, Pine | 86.95% |
| Narrow, deep, Fescue | 78.65% |
| Wide, inter, Switchgrass | 60.50% |
| Wide, deep, Revegetation | 59.80% |
| Wide, inter, Native | 55.45% |
| Narrow, inter, Pine | 43.55% |
| Wide, inter, Revegetation | 32.63% |
| Wide, inter, Pine | 29.74% |
| Narrow, inter, Native | 28.50% |
| Narrow, inter, Fescue | 19.79% |
| Wide, inter, Fescue | 9.07% |
| Wide, deep, Fescue | 7.42% |
| Narrow, deep, Revegetation | 0.00% |
| Narrow, deep,Native | -2.55% |
| Narrow, inter, Revegetation | -22.16% |

There were notable trends with regards to $NO_3$-N removal in R1. Higher percentages of $NO_3$-N reduction were observed in the wide (vs. narrow) buffer width. Within the wide width, greater $NO_3$-N reduction was observed in the deep depth wells versus intermediate depth wells. There was no obvious pattern of $NO_3$-N reduction in the narrow width. In terms of vegetation, the greatest reduction at the narrow width was observed in the pine treatment, while in the wide width it was switchgrass. Clayey lens present in all treatments at the field edge, and in two treatments at the ditch edge did not appear to have a direct effect on Eh values, as the lens were shallower then the intermediate (150 cm) and deep (300 cm) probe depths. Instead, clay lens most likely actively retard the movement of $NO_3$-N through the

profile, increasing residence time of $NO_3$-N, and thus increasing $NO_3$-N reduction rates. Overall, 43% of $NO_3$-N entering from the adjacent field was removed in R1.

R2

Land use adjacent to the 15 m width was a wheat and sorghum rotation in 2007 which received 117 lbs/ac N, and grain sorghum in 2008 which received 104 lbs/ac N in 2008. Land use adjacent to the 8 m width was a successional meadow. The drainage ditch adjacent to R2 was approximately 4 ft deep. Averaging incoming nitrate concentrations are listed in Table 8:

Table 8. Average incoming $NO_3$-N concentrations in R2

| | | $NO_3$-N (mg N/L) |
|---|---|---|
| R2 | 15 m width | 3.83 |
| | 8 m width | 2.53 |
| | Deep depth well | 2.81 |
| | Interm. depth well | 3.47 |

*Soils*

Soils in R2 were Nahunta very fine sandy loam, with Wickham loamy sand in the adjacent field. At the field edge, moderately coarse-textured and medium textured soil material dominated the soil profile, with a narrow band of moderately fine-textured material present in four of the 6 vegetative treatments at a depth of 60 cm to 80 cm. At the ditch edge, clayey soil material was present in all vegetative treatments in both the wide and narrow widths with the exception of the narrow switchgrass and wide native treatments. Clayey material was found at a depth of 50 cm, and ranged from 20 cm to 60 cm in profile thickness.

*Reduction Potential (Eh values)*

Conditions in deep wells were predominantly oxidizing at the field edge, and reducing at the ditch edge (Figures 10).

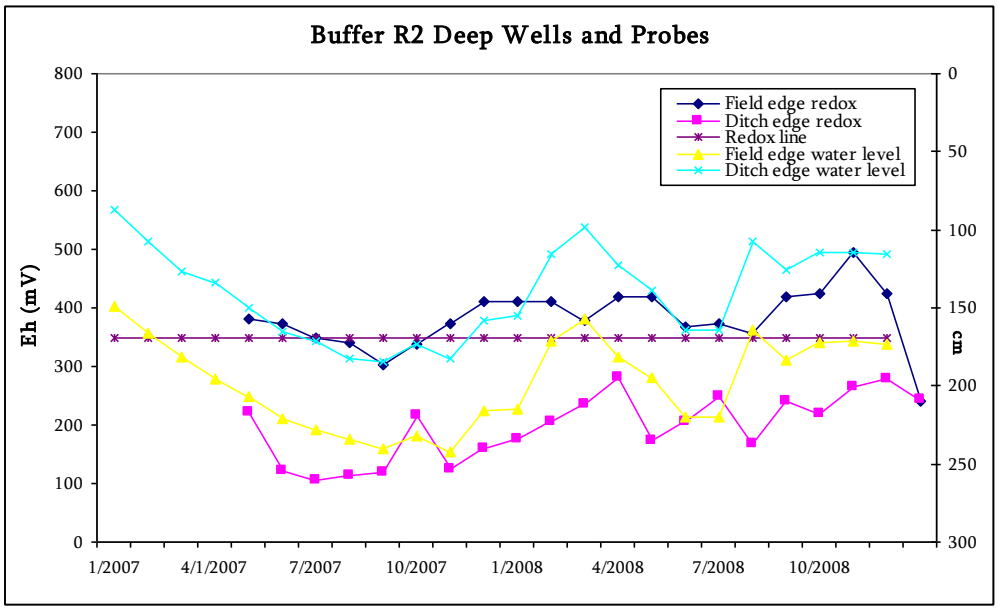


Figure 10. Average groundwater depth and redox measurements for deep wells in R2.

Conditions in intermediate wells were oxidizing at the field edge, and were borderline reducing (fluctuating around 350 mV) at the ditch edge (Figure 11):

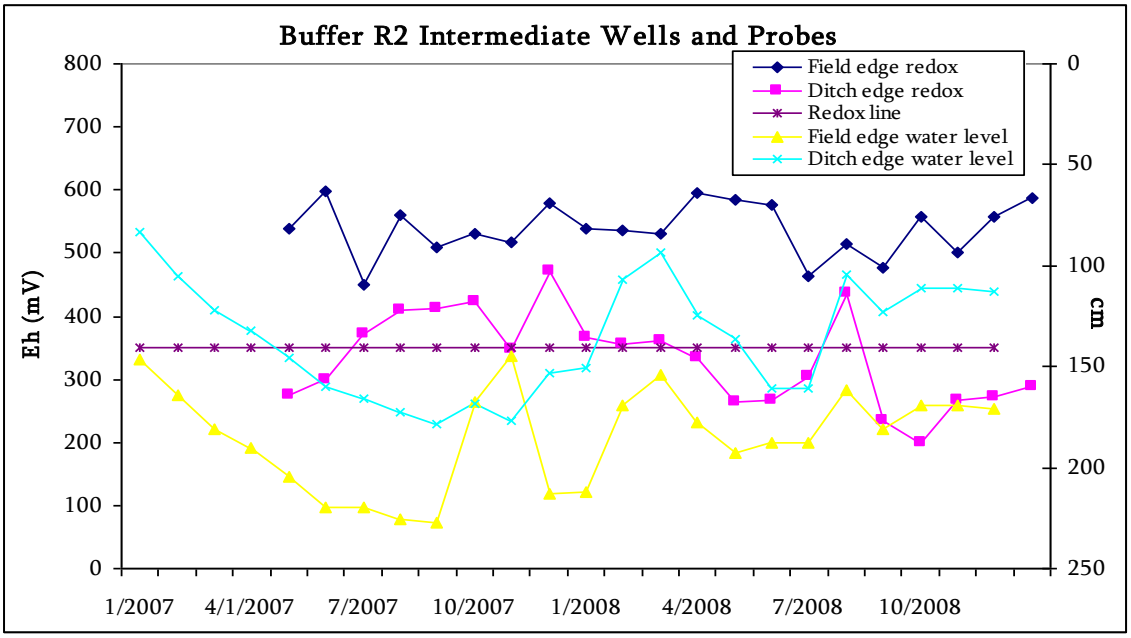


Figure 11. Average groundwater depth and redox measurements for intermediate wells in R2.

Nitrate reduction in R2 from greatest to least was as follows (Table 9):

| Table 9. $NO_3$-N Percent Reduction in R2 | |
|---|---|
| Wide, inter, Fescue | 96.32% |
| Wide, deep, Switchgrass | 92.98% |
| Wide, deep, Native | 88.28% |
| Wide, deep, Fescue | 85.81% |
| Wide, deep, Revegetation | 83.69% |
| Wide, deep, Pine | 75.17% |
| Wide, inter, Pine | 69.69% |
| Wide, inter, Switchgrass | 35.28% |
| Wide, inter, Revegetation | 32.78% |
| Narrow, inter, Fescue | 26.98% |
| Narrow, deep, Pine | 17.11% |
| Narrow, inter, Pine | 13.69% |
| Narrow, deep, Fescue | 12.76% |
| Narrow, inter, Switchgrass | 12.47% |
| Narrow, deep,Native | 11.42% |
| Narrow, deep, Revegetation | 5.72% |
| Narrow, inter, Revegetation | -17.11% |
| Wide, inter, Native | -32.05% |
| Narrow, inter, Native | -32.05% |
| Narrow, deep, Switchgrass | -48.25% |

As with R1, the greatest $NO_3$-N removal was observed in the 15 m width, deep wells versus intermediate depth wells in the 15 m width. With the exception of the 8 m width deep wells, the greatest reduction was observed in fescue treatments. Overall, 33% of $NO_3$-N that entered R2 was removed before reaching the adjacent ditch.

Similar to R1, $NO_3$-N movement through the soil profile into the groundwater would have been promoted at the field edge due to the coarse texture of soil material. $NO_3$-N movement would have been retarded at the ditch edge due to the presence of clay lens.

## R4

Land use adjacent to R4 was beef cattle pasture which was planted in a sorghum sudan and ryegrass in both 2007 and 2008. R4 received 130 lbs/ac N, and 40 lbs/ac N in 2007 and 2008 respectively. The drainage ditch adjacent to the buffer was the least incised of all the ditches (approx. 3 ft). Averaging incoming $NO_3$-N concentrations were highly variable. Averaging incoming nitrate concentrations were as follows:

Table 10. Average incoming $NO_3$-N concentrations in R4

| | | $NO_3$-N (mg N/L) |
|---|---|---|
| R4 | 15 m width | 6.34 |
| | 8 m width | 15.06 |
| | Deep depth well | 7.9 |
| | Interm. depth well | 13.16 |

### *Soils*

The entirety of R4 has been mapped as Lumbee sandy loam, with Wagram loamy sand in the adjacent field. There were no clay lens present in R4.

### *Reduction Potential (Eh) Values*

Conditions in deep wells were always reducing at the field and ditch positions (Figure 12):

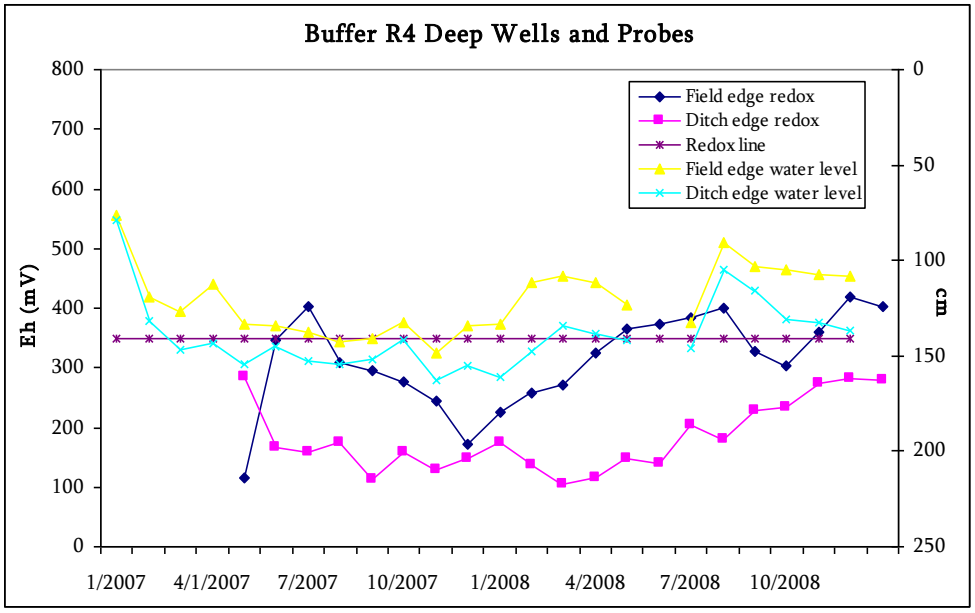


Figure 12. Average groundwater depth and redox measurements for deep wells in R4.

Conditions in intermediate depth wells were always oxidizing at the field edge and ditch edge, with the exception of 9/2007 and 3/2008, when conditions were reducing at the ditch edge.

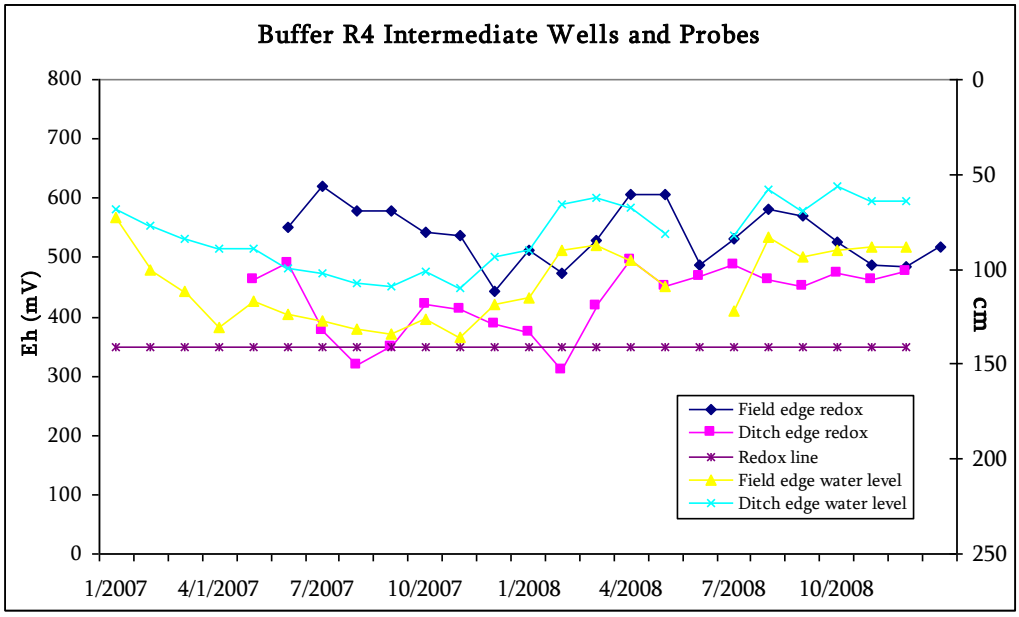


Figure 13. Average groundwater depth and redox measurements for intermediate wells in R4.

Nitrate reduction in R4 from greatest to least was as follows (Table 11):

| Table 11. $NO_3$-N Reduction in R4 | |
|---|---|
| Wide, deep, Pine | 98.39% |
| Narrow, deep, Fescue | 95.40% |
| Narrow, deep, Pine | 93.93% |
| Wide, deep, Switchgrass | 93.29% |
| Wide, inter, Switchgrass | 76.25% |
| Narrow, deep, Control | 54.42% |
| Wide, inter, Pine | 52.38% |
| Wide, deep, Native | 48.41% |
| Wide, inter, Native | 40.50% |
| Wide, deep, Control | 37.50% |
| Wide, inter, Fescue | 35.15% |
| Narrow, deep,Native | 29.80% |
| Narrow, inter, Native | 18.50% |
| Narrow, inter, Pine | 10.03% |
| Wide, inter, Control | 9.91% |
| Narrow, inter, Switchgrass | 9.18% |
| Narrow, inter, Fescue | 9.06% |
| Narrow, deep, Switchgrass | 8.49% |
| Narrow, inter, Control | 3.06% |
| Wide, deep, Fescue | -36.80% |

There was greater reduction in the deep wells versus the intermediate wells at the 8 m width. Overall, R4 was 39% effective at $NO_3$-N removal. The lack of stronger trends was most likely due to the effect of cattle. Although cattle had favorite loafing places in summer, random movement throughout the two years of monitoring created random $NO_3$-N hotspots, and therefore lack of strong $NO_3$-N reduction trends and their inputs into incoming $NO_3$-N concentrations.

R5

Land use adjacent to R5 was dairy pasture planted in Durana clover with received 1 ton/ac lime in both 2007 and 2008, but no N from fertilizer. Cattle had access to controls in the wide and narrow width buffer. The average drainage ditch depth was approximately 4-5 ft. There is no reduction potential data for this replication. Average incoming concentrations were very low as shown in Table 12:

Table 12. Average incoming $NO_3$-N concentrations in R5

| | | $NO_3$-N (mg N/L) |
|---|---|---|
| R5 | 15 m width | 1.84 |
| | 8 m width | 2.87 |
| | Deep depth well | 1.08 |
| | Interm. depth well | 3.58 |

Soils in R5 were Weston loamy sand in the 15 m width, and predominantly Kalmia sandy loam in the 8 m width, with Rains sandy loam in the control of the 8 m width. Rains soils are considered the wettest of its catena. No clayey lens were present in soil profiles.

Nitrate reduction in R5 from greatest to least was as follows (Table 13):

| Table 13. $NO_3$-N Reduction in R5 | |
|---|---|
| Wide, inter, Fescue | 78.96% |
| Wide, inter, Pine | 62.75% |
| Wide, inter, Native | 60.20% |
| Narrow, deep, Pine | 57.05% |
| Narrow, inter, Pine | 45.70% |
| Wide, deep, Switchgrass | 41.44% |
| Narrow, inter, Switchgrass | 35.29% |
| Wide, inter, Control | 30.87% |
| Narrow, deep, Control | 27.10% |
| Narrow, deep,Native | 22.14% |
| Narrow, inter, Native | 21.19% |
| Narrow, inter, Control | 13.08% |
| Wide, deep, Native | 11.01% |
| Narrow, deep, Fescue | 9.95% |
| Wide, deep, Control | 0.00% |
| Wide, deep, Pine | -1.67% |
| Narrow, inter, Fescue | -6.25% |
| Narrow, deep, Switchgrass | -27.75% |
| Wide, deep, Fescue | -44.57% |
| Wide, inter, Switchgrass | -48.30% |

In contrast to R1, R2, and R4, there was greater $NO_3$-N removal in the intermediate deep wells versus the deep wells in the 15 m width buffer. Intermediate depth wells in the 15 m width had greater reduction then identical depth wells at the narrow width. There was no difference in $NO_3$-N reduction between deep and intermediate depth wells in the narrow width.

As incoming $NO_3$-N were low in R5, slight increases or decreases in concentration convert into seemly significant percent reductions or increases. In reality, incoming $NO_3$-N concentrations were so low that little to no biological changes were occurring in the system. In addition to low incoming $NO_3$-N concentrations, random cattle movement and $NO_3$-N hotspots, as with R4, could be confounding results.

*Cumulative CEFS groundwater monitoring data*

In terms of cumulative data from this site, reductions by vegetation type are shown in Table 2:

Table 14. Cumulative CEFS $NO_3$-N (mg/L) reduction by vegetation type (Adapted from King, 2005)

| Study | Trees | Fescue | Switchgrass | Native Vegetation | Control |
|---|---|---|---|---|---|
| Dukes (2000) | 49% | 37% | 33% | 32% | 35% |
| Ricks (2002)* | 48% | 48% | 41% | 33% | 32% |
| King (2005) | 57% | 40% | 44% | 37% | 27% |
| Current (2008) | 32% | 23% | 40% | 35% | Reveg: 14%** Pasture: 0%** |

*Excludes R5N

** Control was different in current monitoring versus previous monitoring periods.

As discussed in *Methods and Materials: Buffer Design*, controls during current monitoring were different than controls during previous monitoring. Comparison between monitoring periods for the control is thus not possible. During the most current two years of monitoring, North Carolina was affected by a drought, which may further confound comparison between current and past data (see Appendix G, Figure 16 for precipitation data). Longitudinal $NO_3$-N removal is not increasing with time at CEFS; instead, the most recent $NO_3$-N data suggests the established trend of $NO_3$-N reduction at CEFS are being maintained.

*General CEFS Discussion*

The 15 m deep wells had the highest decrease in $NO_3$-N concentration, which is expected due to increased buffer width (as compared to the 8 m buffer), and the positioning and depth of the wells. The deep wells (2.1-3.5 m) were placed to sample the lower portion of the existing aquifer. This position in the soil profile is always saturated, leading to anaerobic conditions, which are optimal for the denitrification process. The intermediate depth wells (1.5-2.1 m) were placed to sample the upper limit of the aquifer. During summer months, or times of low precipitation, these wells were dry, meaning the soil surrounding the wells was either completely aerobic, or "semiaerobic". As with deep wells, the water table was shallower to the ground surface in ditch edge wells versus field edge wells. Reduction potential measurements in intermediate wells were only rarely <350 mV. Elevated Eh values, and thus oxidizing conditions, support water data that showed minimal rates of $NO_3$-N reduction in intermediate depth wells. These minimal rates of denitrification may be due to the fact that denitrification can occur in anaerobic microsites of aerobic soils (Smith and Tiedje, 1978). Depending upon soil properties, intra-aggregate water-filled pores may be surrounded by inter-aggregate air-filled pores. Diffusion of $NO_3^-$ to water-filled pores can lead to solute reduction (Knowles, 1982), which can explain $NO_3$-N reduction in intermediate wells where Eh measurements were not reducing.

### *Effect of In-situ Properties on Study Results*

Due to differences between replicate soils, land management practices, and groundwater table dynamics, isolation of factors effecting $NO_3$-N removal is difficult to elucidate.

Fertilization, hotspots as a result of cattle loafing, soil compaction by cattle, and variation in incoming $NO_3$-N concentrations make interpretation of study results difficult. In addition, a severe drought in 2007 would have influenced groundwater flow paths. In non-drought conditions, groundwater flow paths were found to be perpendicular to buffers; however, drought conditions may have disrupted flow paths. Disrupted flow paths could result in groundwater from field edge wells flowing to non-partner ditch edge wells, meaning comparison of partner wells would not be an accurate evaluation of $NO_3$-N concentration reduction.

### *Implications of Research*

Instead of an overall analysis of buffer effectiveness at $NO_3$-N removal, the four replications instead serve as case studies showing how variability in soil properties and land management practices, in addition to buffer width and groundwater depth, will result in a range of efficiencies of $NO_3$-N removal by riparian buffers.

Based on water table and Eh measurements, there were two distinct biological systems operating in the soil profile. With the exception of one buffer (R4W), buffers were located alongside deeply incised drainage ditches, which influenced water table depth, and thus existence of anaerobic and aerobic conditions in wells. This site was a good example of

$NO_3$-N removal in both anaerobic and aerobic conditions, meaning rates can be compared to buffers that have either condition.

It is possible that low levels of C are inhibiting the denitrification process at CEFS. However, other site conditions, such as incised drainage ditches besides buffers, could also be contributing to inhibition of denitrification. Incised drainage ditches could result in flow of $NO_3$-N laden groundwater beneath the zone of vegetation roots, and associated C. Although controlled drainage structures with flashboard risers are installed along ditches upstream of all four buffers, flashboard risers were never lowered during our two years of sampling, meaning drainage ditch water table level was never artificially raised. Even with site limitations, based on water table data and Eh values, it is a reasonable assumption that denitrification is the dominant process responsible for observed $NO_3$-N removal from groundwater.

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# APPENDICES-CHAPTER 2

**Appendix A: Site location**

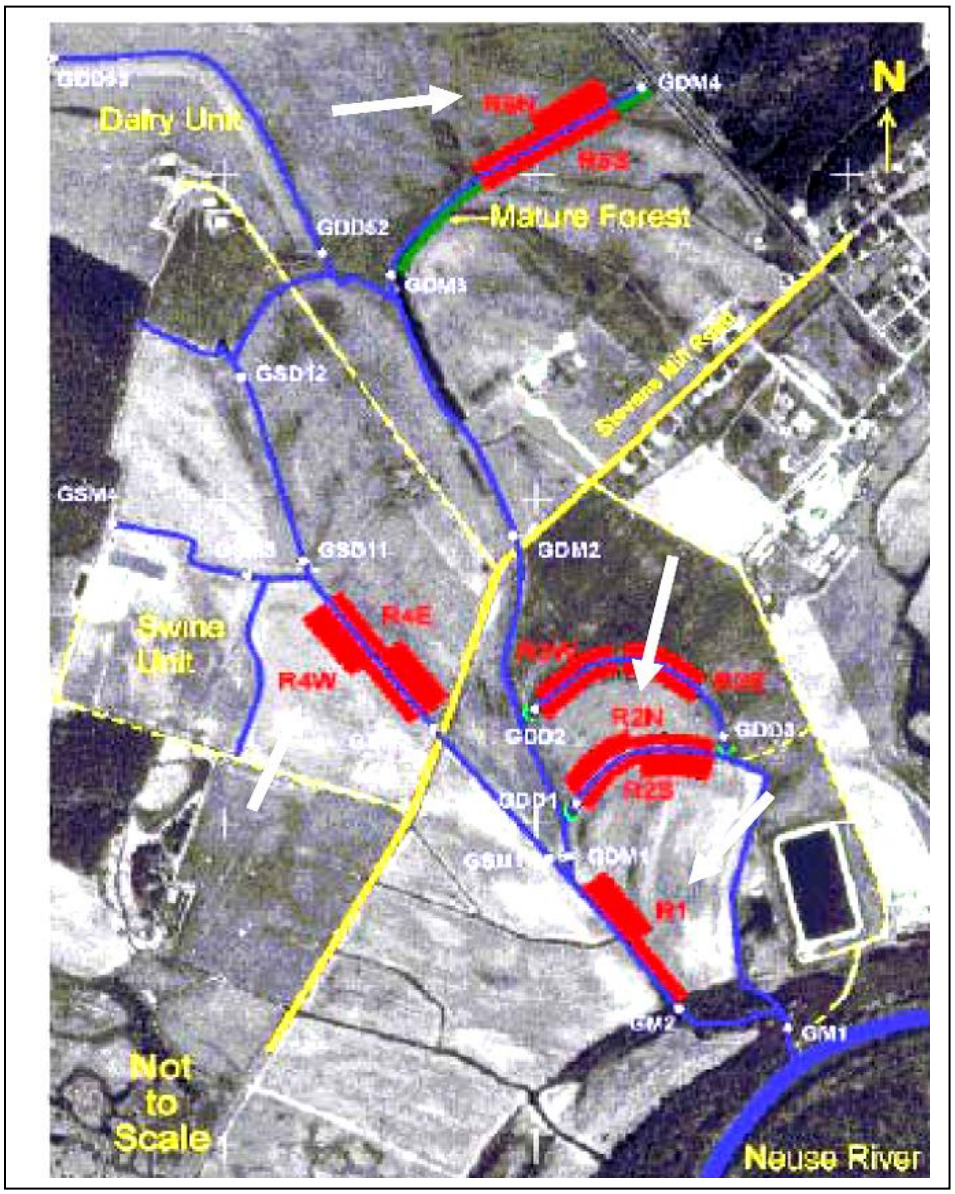


Figure 1. Location of buffer at CEFS. Arrows indicate buffers (R1, R2N, R4W, R5N). Figure adapted from Dukes (2000). Not to scale.

**Appendix B. Buffer design**

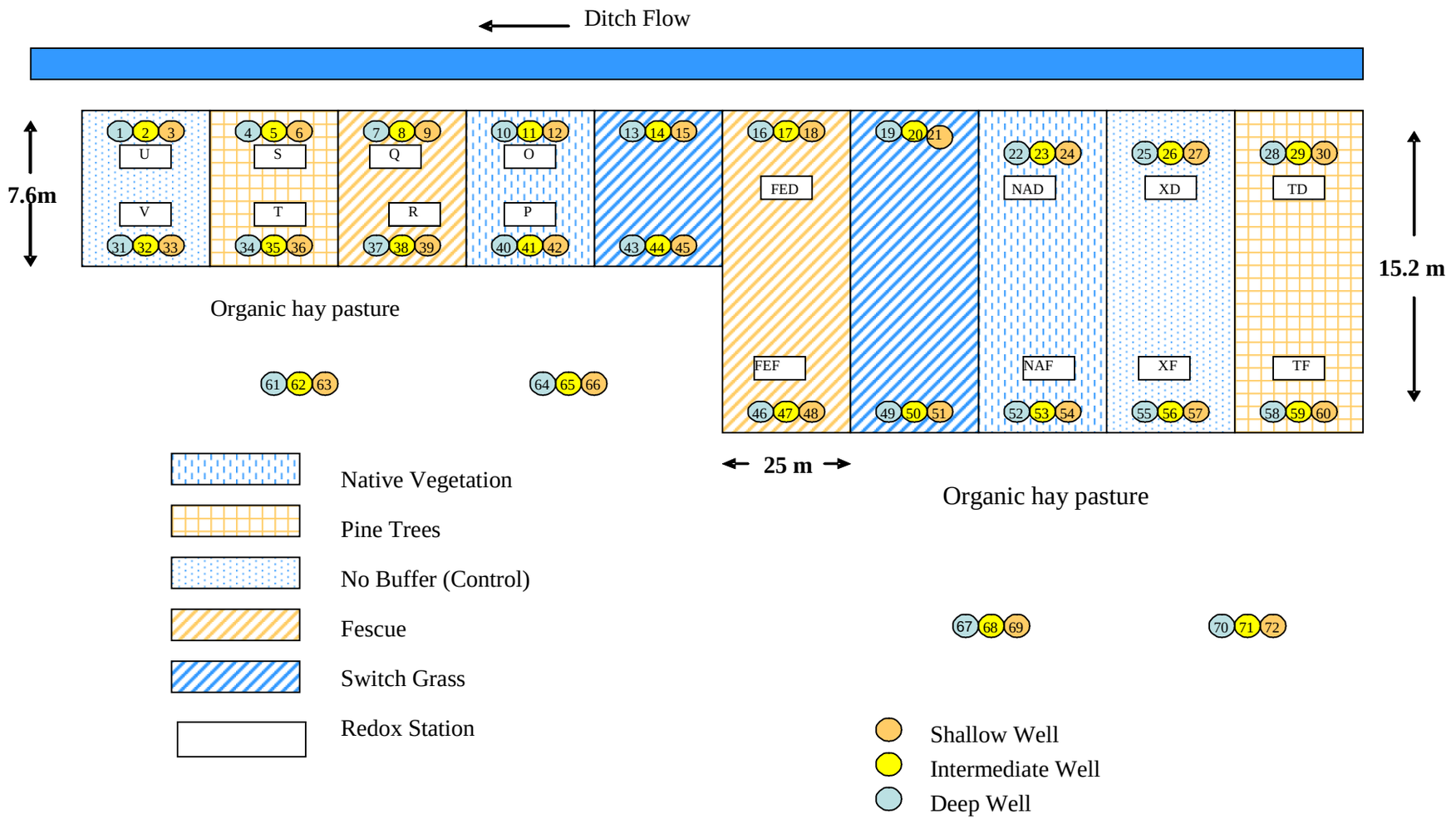


Figure 2. Well numbers and vegetation treatments in R1. Figure not to scale.

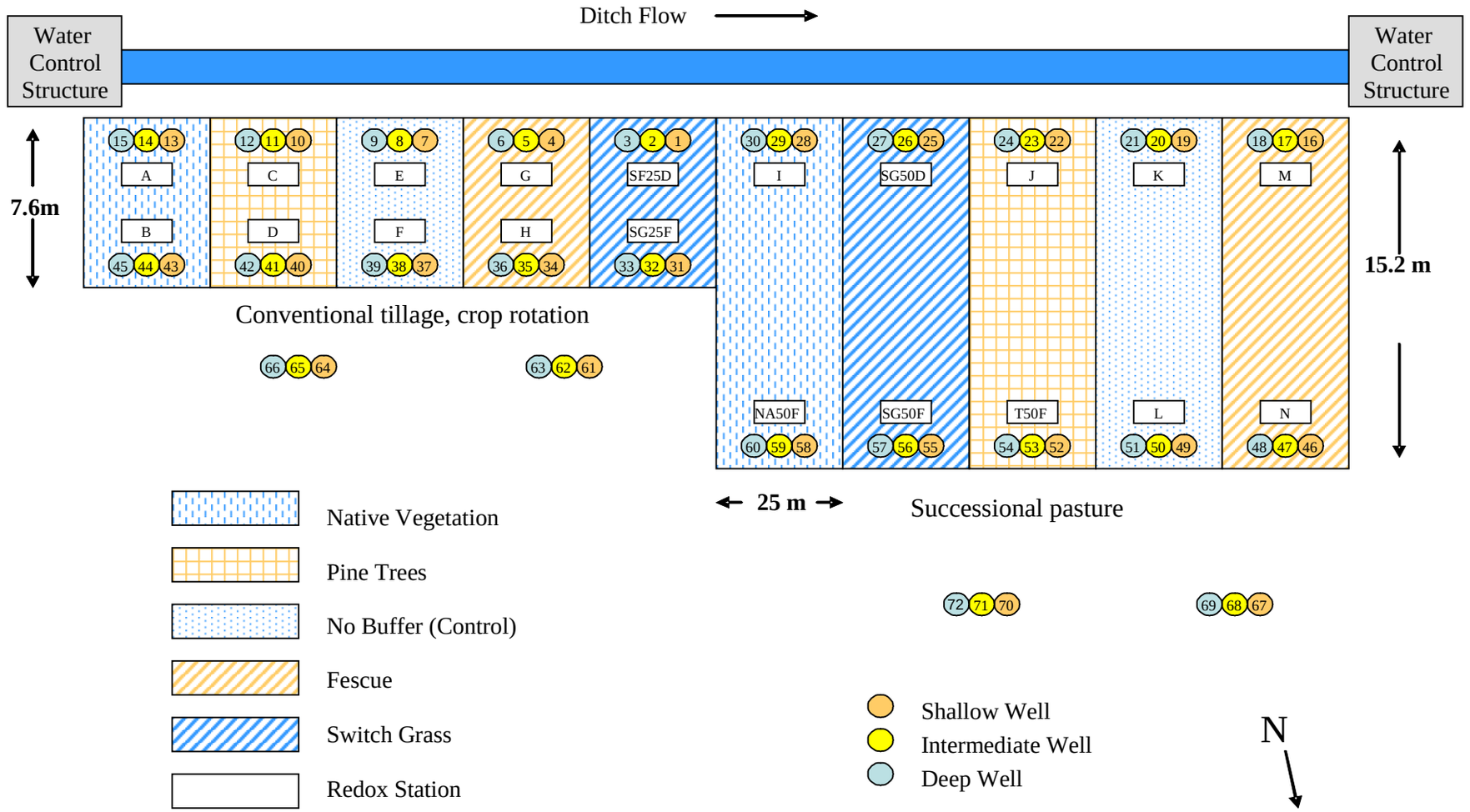


Figure 3. Well numbers and vegetation treatments in R2N. Figure not to scale.

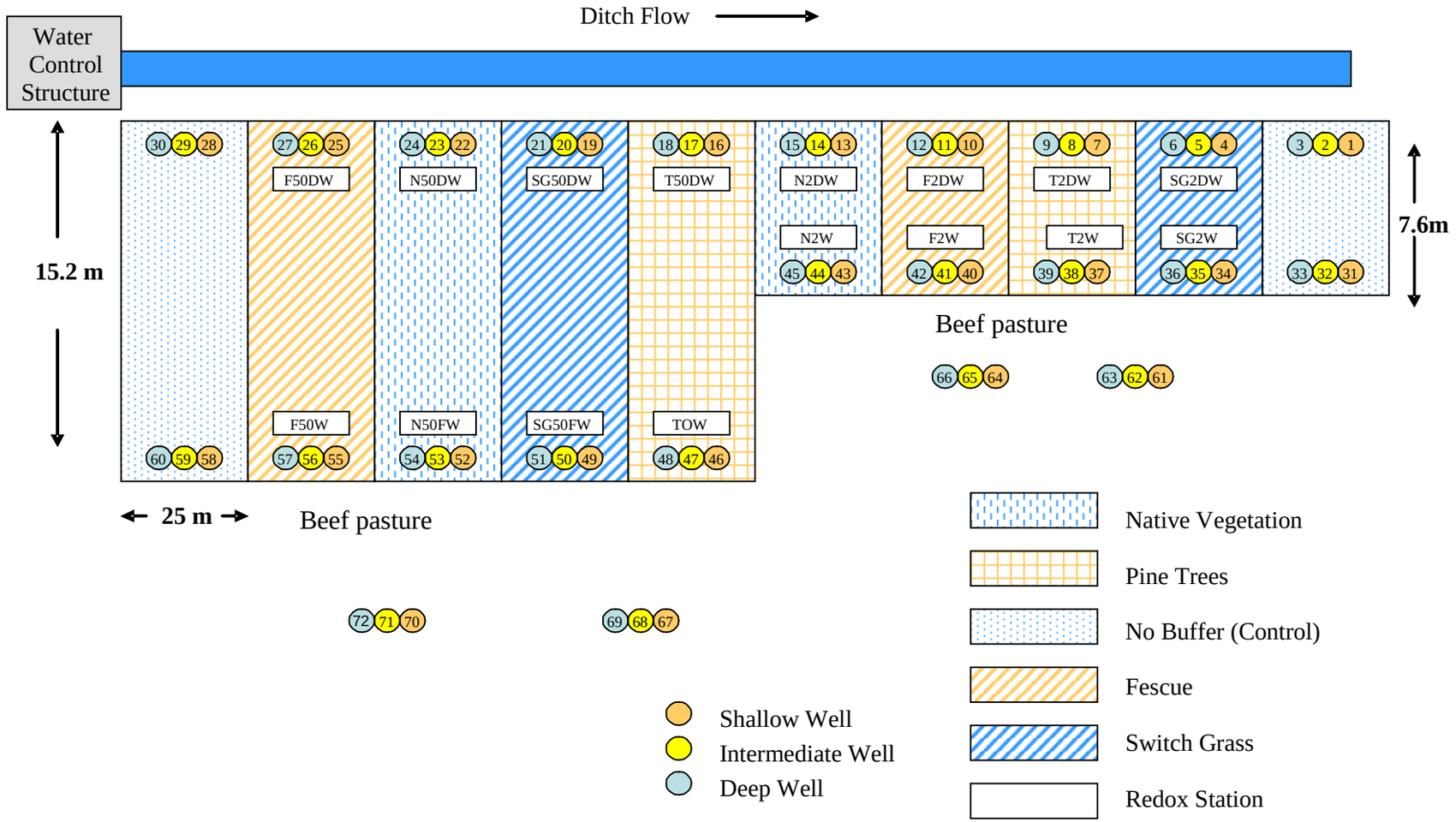


Figure 4. Well numbers and vegetation treatments in R4W. Figure not to scale.

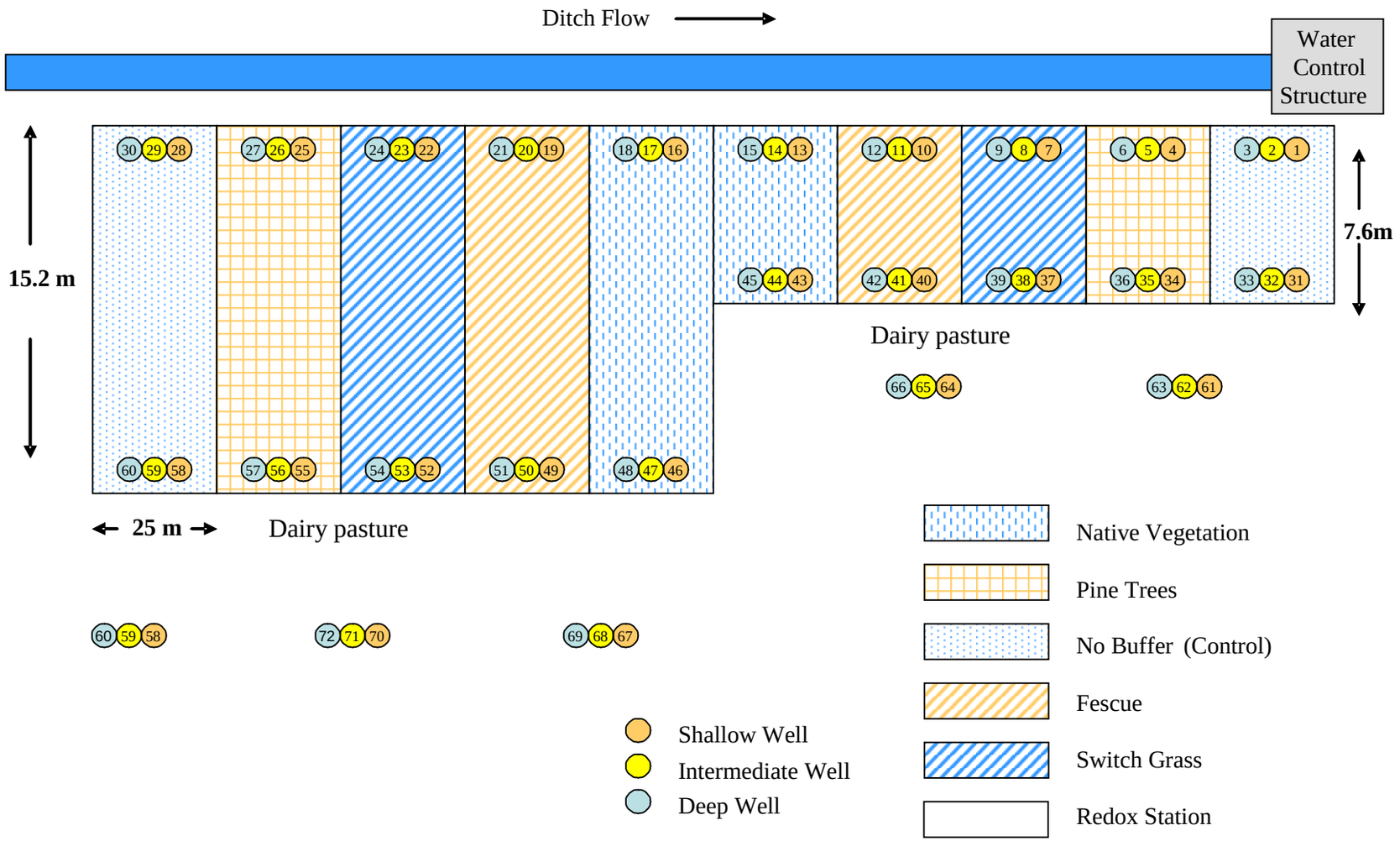


Figure 5. Well numbers and vegetation treatments in R5N. Figure not to scale.

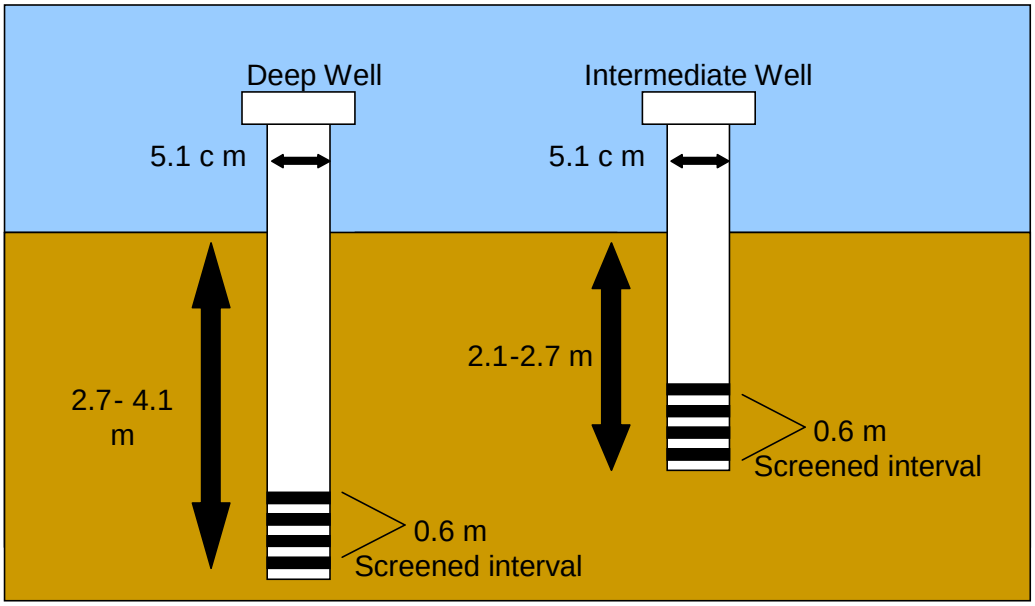


Figure 6. Well depth in R1, R2N, R4W, and R5N.

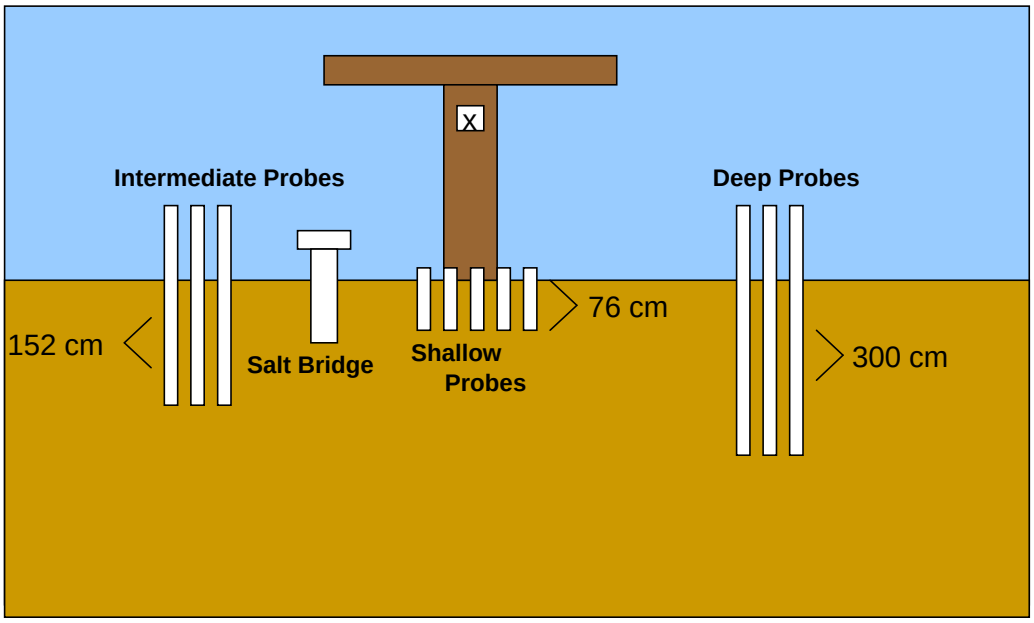


Figure 7. Redox probe depths and establishment around salt bridges for R1, R2N, and R4W.

## Appendix C: R5 water table data

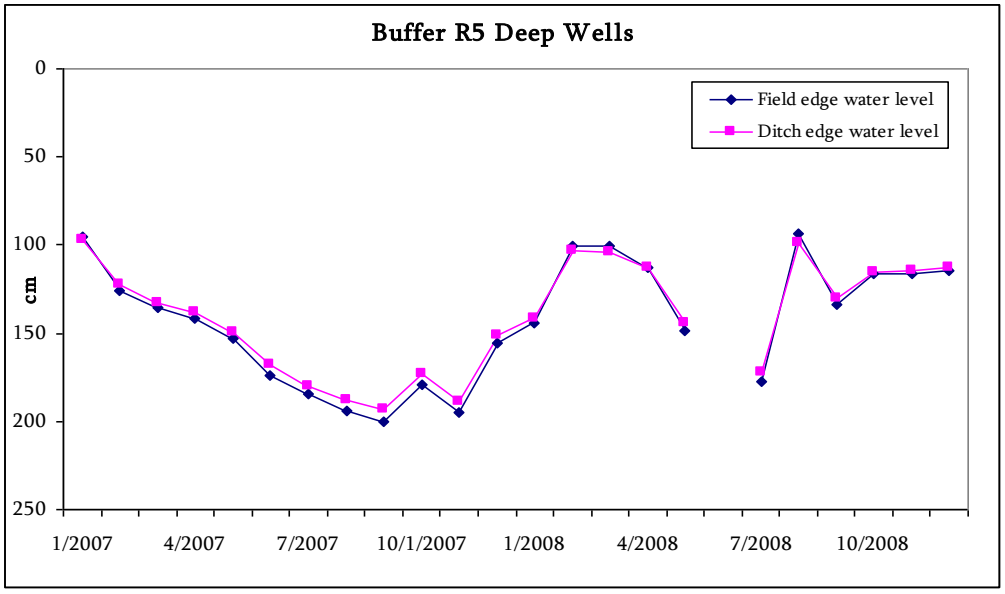


Figure 8. Water table data for deep wells in R5N. Water table depth is recorded as cm below soil surface.

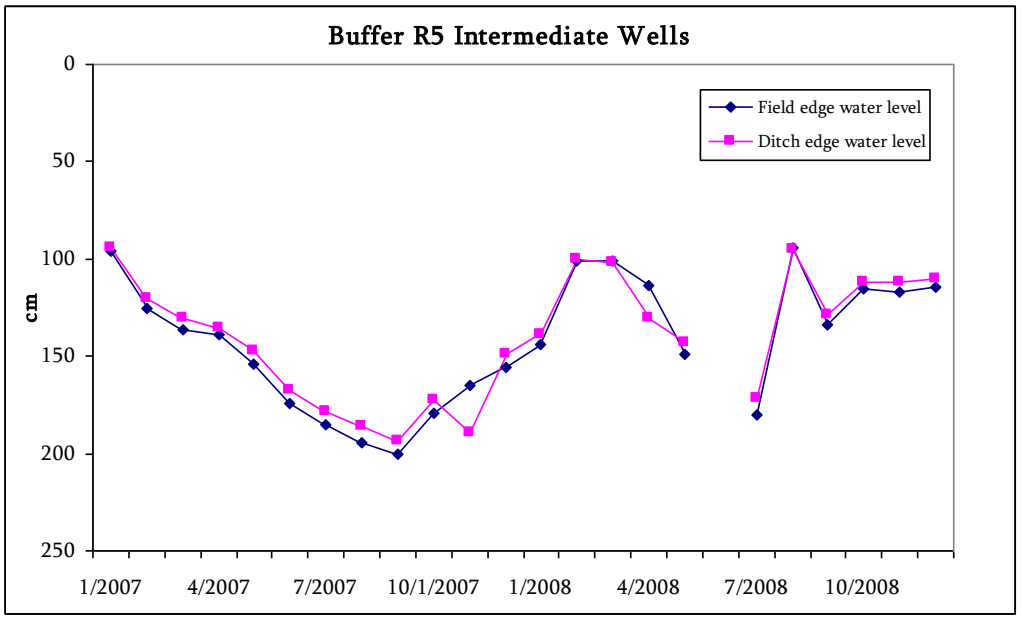


Figure 9. Eh values and water table data for intermediate wells in R5N. Water table depth is recorded as cm below soil surface.

**Appendix D.** $NO_3$-N (mg N/L) concentration data.

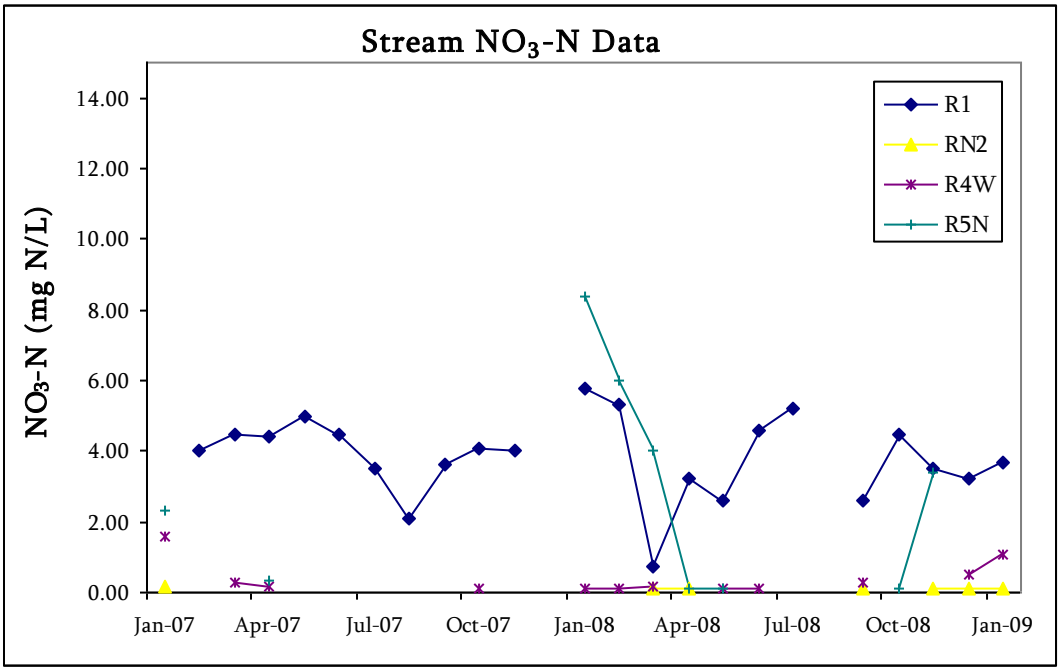


Figure 10. Stream data for drainage ditches adjacent to buffers. Ditches were frequently dry due to drought conditions.

| Table 1. Incoming $NO_3$-N (mg N/L) concentrations by vegetation type. | | | | | | |
|---|---|---|---|---|---|---|
| Buffer | Trees | Fescue | Switchgrass | Native Vegetation | Control | Revegetation |
| R1 | 9.10 | 8.16 | 12.75 | 12.34 | N/A | 11.02 |
| R2N | 3.05 | 3.67 | 3.72 | 2.69 | N/A | 3.97 |
| R4W | 15.29 | 10.51 | 9.92 | 10.90 | 6.00 | N/A |
| R5N | 2.22 | 2.37 | 3.24 | 3.77 | 1.30 | N/A |

| Table 2. Average incoming $NO_3$-N (mg N/L) concentrations by buffer width and well depth. | | | | |
|---|---|---|---|---|
| Buffer | 8 m Intermediate | 8 m Deep | 15 m Intermediate | 15 m Deep |
| R1 | 6.87 | 4.02 | 17.92 | 12.82 |
| R2 | 2.54 | 2.55 | 5.52 | 3.37 |
| R4 | 18.92 | 9.91 | 7.35 | 5.70 |
| R5 | 4.63 | 1.72 | 2.95 | 1.01 |

**Appendix E.** Supplementary DOC data.

| Table 3. Average DOC (mg C/L) concentration by buffer width and well depth. | | | | |
|---|---|---|---|---|
| Width | Depth | Field-edge wells (mg C/L) | Ditch-edge wells (mg C/L) | % Reduction |
| 8 m | Intermediate | 3.53 | 3.80 | -8% |
| | Deep | 2.87 | 2.97 | -4% |
| 15 m | Intermediate | 3.52 | 4.39 | -25% |
| | Deep | 3.02 | 3.21 | -6% |

| Table 4. Average DOC (mg C/L) concentration by vegetation type. | | | |
|---|---|---|---|
| Vegetation Type | Field-edge wells (mg C/L) | Ditch-edge wells (mg C/L) | % Reduction |
| Trees | 3.24 | 3.32 | -21% |
| Fescue | 3.21 | 3.65 | -14% |
| Switchgrass | 3.15 | 3.53 | -12% |
| Native Vegetation | 3.24 | 3.71 | -15% |
| Control | 3.83 | 4.30 | 12% |
| Revegetation | 2.72 | 3.01 | -11% |

**Appendix F.** Dilution ratio data

| Table 5. Dilution analysis: Average $NO_3$-N/Cl ratios by buffer width and well depth. | | | | | |
|---|---|---|---|---|---|
| Width | Depth | Ratio: Field-edge wells | Ratio: Ditch-edge wells | Ratio reduction | $NO_3$-N reduction |
| 8 m | Intermediate | 0.83 | 0.59 | 29% | 19% |
| | Deep | 0.35 | 0.17 | 51% | 47% |
| 15 m | Intermediate | 0.84 | 0.47 | 44% | 40% |
| | Deep | 0.52 | 0.15 | 71% | 73% |

| Table 6. Dilution analysis: $NO_3$-N/Cl ratios by vegetation type. | | | | |
|---|---|---|---|---|
| Vegetation Type | Field-edge wells | Ditch-edge wells | Ratio reduction | $NO_3$-N reduction |
| Trees | 0.73 | 0.35 | 52% | 48% |
| Fescue | 0.45 | 0.27 | 40% | 43% |
| Switchgrass | 0.60 | 0.23 | 62% | 64% |
| Native Vegetation | 0.61 | 0.33 | 46% | 43% |
| No-buffer control | 0.45 | 0.22 | 51% | 50% |

**Appendix G:** Climate Data for CEFS (Monthly sum of daily precipitation)

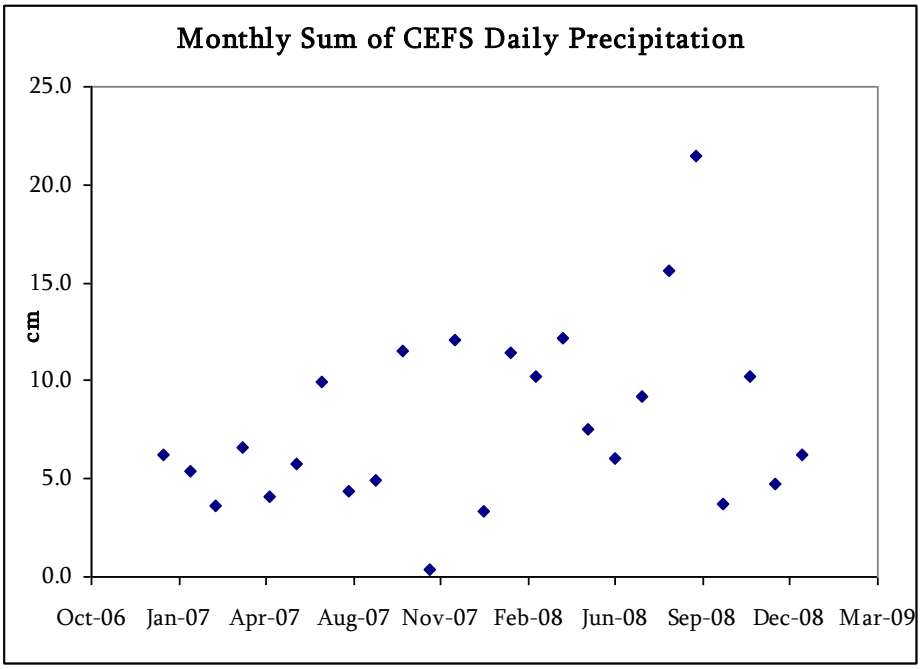


Figure 12. Daily mean of soil temperature at a depth of 10 cm.

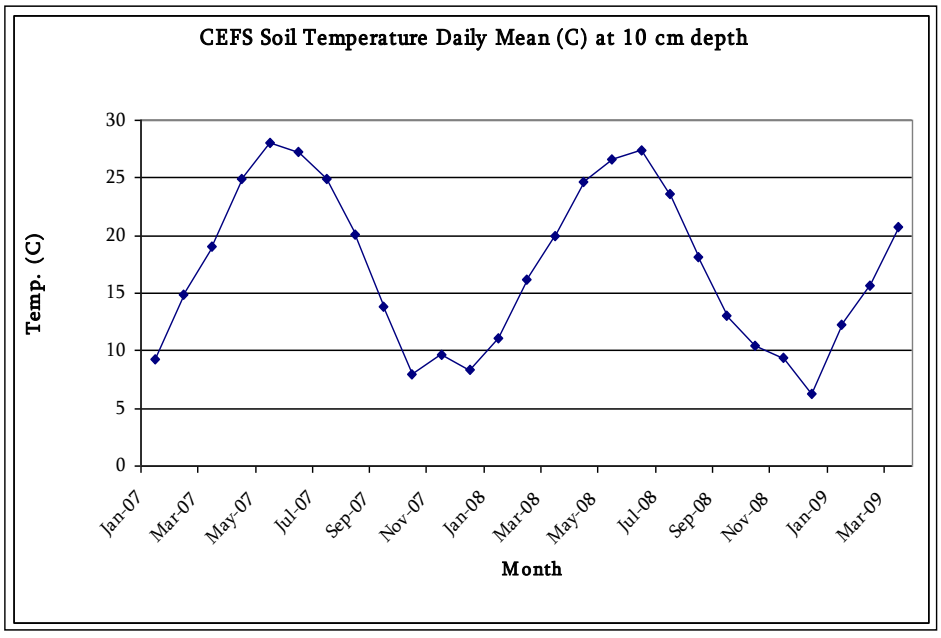


Figure 11. Monthly sum of daily precipitation at CEFS (cm).

Chapter 3: Effect of carbon concentration on denitrification rate and products in riparian soil materials


## Abstract

Non-point source pollution from agriculture is one of the causes of surface water quality degradation in the Coastal Plain of North Carolina. Riparian buffers are an important best management practice for reducing $NO_3^-N$ concentrations in natural waters, predominately by vegetation uptake and denitrification. Inhibition of denitrification is possible at CEFS due to low levels of organic C (≈ 3.36 mg C/L). To test this hypothesis, a parallel laboratory study was designed to complement current riparian buffer field studies. Flow-thru soil columns were utilized to determine the effect of dissolved organic carbon (DOC) concentration on denitrification rates and products in riparian buffer soils. Four DOC concentrations (*2.0 mg DOC/L*, *4.0 mg DOC/L*, *8.0 mg DOC/L*, *12.0 mg DOC/L* and *16.0 mg DOC/L*) and a control (*0.0 mg DOC/L*) were utilized to study this relationship between DOC and denitrification.

There was no clear trend between DOC concentration and rate of $NO_3$-N loss. However, DOC concentrations > 4.0 mg DOC/L increased up until the experiment containing *12.0 mg DOC/L*, after which rates leveled off. There was a linear relationship between DOC concentration and rate of $N_2O$-N production with the exception of *12.0 mg DOC/L*, with the rate of $N_2O$-N production increased with increasing concentrations of DOC. However, the interaction of factors effecting denitrification requires more study to be fully understood, specifically data on resident microbe populations, as this will provide greater insight into the metabolic processes occurring.

## Introduction

Nitrogen (N) contamination of groundwater and the resulting water quality issues are a concern in the Coastal Plain of North Carolina due to intensive agriculture, and native soil and hydrologic properties. Field-applied fertilizers and animal waste often contain N that is readily converted to nitrate ($NO_3^-$), a highly soluble nutrient that moves with precipitation or irrigation through the soil profile into shallow groundwater (Osmond et al, 2002). The Coastal Plain is characterized by sandy soils with clay lenses at varying depths of the subsoil, which forces the lateral flow of groundwater and contaminants to surface waters (Osmond et al, 2002).

Riparian buffers, areas of uncultivated land established between agricultural fields and surface waters, are established to decrease nutrient concentration, specifically $NO_3^-$, from groundwater drainage. Elimination of excess $NO_3^-$ prevents its movement into surface waters, reducing the potential for environmental impacts from eutrophication, hypoxia, and for health impacts from $NO_3^-$ in drinking water [(EPA standard = 10 mg $NO_3$-N/L; EPA, 2006)]. Reductions of $NO_3^-$ concentrations greater than 90% have been documented, highlighting the importance of riparian buffers to water quality (Jacobs and Gilliam, 1985; Lowrance et al, 1984). These reductions in $NO_3^-$ concentration from groundwater can occur through vegetation uptake, dilution, or biogeochemical transformation (e.g., denitrification). Denitrification is the reduction of nitrate and nitrite ($NO_3^-$ and $NO_2^-$) to N gases ($N_2O$ and $N_2$); the efficiency of this process is contingent on existing environmental conditions in buffers. The four existing environmental factors needed for denitrification to proceed are (1) reducing conditions, (2) substrate availability ($NO_3^-$), (3) availability of organic carbon (OC),

and (4) presence of denitrifying bacteria (Starr and Gilliam, 1983; Altman and Parizek, 1995; Hunt et al, 2004). Although denitrification rates vary as a function of all these factors, if reducing conditions are present, OC availability is most likely to be the critical factor regulating denitrification rates. Low concentrations of OC limit the activity of denitrifying microbes, inhibiting denitrification (Lowrance and Smittle, 1988).

Riparian buffers at the Center for Environmental Farming Systems (CEFS) in Goldsboro, NC were monitored for groundwater concentrations of $NO_3^-$ in an experimental design including 2 buffer widths and 5 vegetation types as treatments (Chapter 2). To date, over 12 years of water quality data has been collected and analyzed. Riparian buffers at the Center for Environmental Farming Systems have low levels of dissolved organic C (DOC) ($3.4 \pm 0.6$ mg/L) that may inhibit denitrification.

Further research is needed to quantitatively understand the influence of DOC on denitrification rates, and to elucidate the effects of DOC on the nature of end-products (e.g., $N_2$ vs. $N_2O$). A laboratory experiment controlling other factors effecting denitrification while varying DOC has allowed focus on the role of DOC, and end-product production. The objectives of this study were:

- Determine the effect of DOC concentration on denitrification rates in riparian buffers through controlled lab experiments that complement current field studies.
- Determine the effects of DOC concentration on the relative production of $N_2O$ and $N_2$.

## Denitrification

Denitrification is the microbial mediated reduction of $NO_3^-$ to N gases. In anaerobic conditions, $NO_3^-$ is used as a terminal electron acceptor for denitrifiers, and reduced to nitrogen oxides (i.e., NO and $N_2O$) and dinitrogen gas ($N_2$). Denitrification rates and products are the result of interrelated environmental factors and soil properties. It is difficult to attribute rates and products to one particular environmental factor; instead, a complicated web of interactions between factors is more likely. These factors can be better controlled and isolated in laboratory settings in order to understand specific variable effects of soils.

## Soil Column Experiments

The soil ecosystem is multifaceted and complex, which makes it difficult to isolate and understand the factors controlling physical, chemical, and biological processes, and end products of these processes. Soil columns, which are microcosms designed to simulate the soil ecosystems, can be employed to simplify and explore specific processes, specifically biological transformations of chemical contaminants (Obenhuber and Lowrance, 1991). Soil columns are constructed using cylindrical pipes that are packed with soil material, and have influent pumped through to allow collection and analysis of effluent. Column dimensions, flow rate, and influent properties are all predetermined, and reflect specific objectives of the study. Columns are typically either flow-through or saturated in design. A flow-through design allows constant movement of solution through columns, whereas solution is only collected from saturated columns at specific sampling points. Soil column experiments have been successfully used by researchers to explore functions of riparian buffers and their

continued use as best management practices to mitigate non-point source pollution (Pavel et al, 1996; Willems et al, 1997; Fazzolari et al., 1998).

Successful experiments utilizing continuous flow columns have provided a greater understanding of the relationship between $NO_3^-$ loss and denitrification rates, and can attempt to replicate groundwater flowing through riparian buffers. These experiments have explored denitrification rates as influenced by variables including flow rate, temperature, soil depth, and DOC and $NO_3^-$ concentration (Pavel et al, 1996, Obenhuber and Lowrance, 1991, Willems et al, 1997).

## Methods and Materials

### Soil Collection

Soil material was collected from the 8 m wide switchgrass plot in the R4W buffer at the CEFS (Chapter 2, Appendix B, Figure 1). Collection occurred approximately 3 ft. from the edge of the field-buffer interface. Soil material was collected from a depth of 2.1-3.5 m using an auger with a bucket head attached. Soil samples were placed in polypropylene bottles, sealed, and kept on ice while transported back to NCSU. Soil was stored at 4°C until packed into soil columns. A soil sample was submitted to the Analytical Service Lab at NCSU yielded organic N and C concentrations of 0.02% and 0.42% respectively; a 1:1 ratio of soil to distilled water was used to measure pH, which was found to be pH = 5.2.

### Soil Columns and Solution Preparation

Continuous flow column experiments were conducted to measure nitrogen transformation rates (Pavel et. al., 1996) Columns were constructed using PVC pipe (13.2 cm length, 3.0 cm diameter) and wet-packed with a homogenous mixture of collected soil

material to an approximate density of 1.60 g/cm$^3$, a bulk density representative of sandy coastal plain soil (Lowrance and Smittle, 1998). Rubber stoppers equipped with 6 cm long and 3 mm plastic tubing outlets were inserted into the end of the column that were screened with PVC fabric. Tubing from the influent end of the vertically positioned column was a passed through a six channel peristaltic pump (Manostat Cassette), which was used to transfer solution from a light-shielded 25 L carboy. Fluorinated ethylene propylene (FEP) tubing wrapped in aluminum foil was used to minimize contamination from atmospheric gases. Tubing that passed though the pump cartridge was not wrapped in foil in order to facilitate more accurate control of flow rate and observation of condition of tubing.

Figure 1. Laboratory set-up; solution (in foil covered carboys). Solution was pumped to soil columns, with flow-rate regulated by a peristaltic pump. Effluent was collected from exit tubing of each column. Conditions: Argon-purged solution containing 5.0 mg N/L $NO_3^-$ at 25ºC.

Solutions with known concentrations of potassium nitrate ($KNO_3$), dextrose ($C_6H_{12}O_6$), and Type I dionized (DI) water were boiled and then poured into respective

carboys; the solution was purged with humified argon gas immediately after transfer. Initially, approximately 3 days worth of solution was prepared, and carboys were resupplied with solution every 3 days with new solution by opening and pouring the new solution into the carboy. However, due to concerns of oxygen contamination loss of anoxic conditions resulting to transfer, in subsequent experiments sufficient solution was prepared for the experiment duration and heavily purged with humified argon gas before solution began passing through columns. The $KNO_3$ concentration was set at 5.0 mg N/L for all experiments. The $C_6H_{12}O_6$ concentrations were set at 0.0 mg DOC/L, 2.0 mg DOC/L, 4.0 mg DOC/L, 8.0 mg DOC/L, 12.0 mg DOC/L, and 16.0 mg DOC/L, and were thereafter labeled as, and will be referred to, as *0.0 mg DOC/L, 2.0 mg DOC/L, 4.0 mg DOC/L, 8.0 mg DOC/L, 12.0 DOC/L* and *16.0 mg DOC/L*. Solution was continuously purged with humified argon gas throughout the duration of experiments in order to keep solution anoxic.

Solution was delivered to columns at a flow rate of approximately 0.3 mL/min. Denitrification rates in columns have been shown to depend on flow rate (Pavel et al, 1996). No attempt was made to quantify these effects. However, columns were designed and flow rate optimized to result in a 10-35% decrease in $NO_3^-$, thus insuring limited depletion of reactants. The rate was kept constant between experiments to allow for comparison between experiments. Triplicates of experiments were run for approximately 25 days for the control, and 7 days for the remainder of the experiments. On a daily basis, approximately 40 mL of effluent was collected in 50 mL disposable plastic beakers. Immediately after effluent collection, column exit tubing was connected to a universal flow-through adaptor (Cole-Palmer 00652-85) with a combination redox electrode (Orion 9778BNWP) to measure Eh.

As indicated by the manual, +220mV was added to measured values to correct the electrode potential to standard hydrogen electrode reference. The pH of collected samples was measured using an Accument Excel pH/conductivity meter (XL20). Samples were than filtered through 0.45 um HV Millipore syringe filters and frozen until analysis, at which point they were thawed, and vigorously shaken. For $NO_3$-N and $NO_2$-N determination, samples were analyzed with a sulfanilamide color reagent using an Automated Ion Analyzer (QuikChem Method 10-107-01-1-A, Lachat Instruments QuickChem brand 8000). For determination of $N_2O$-N, a hypodermic needle (23 gauge) was attached to the exit tubing on each respective column. The needle was than inserted into a vacutainer tube that had been exposed to air; approximately 30 mL of solution was collected. For analysis, ten mL of gas samples were withdrawn from the headspace of the vacutainer tubes, and injected into a gas chromatograph for $N_2O$-N analysis (Hewlett Packard 5890 GC-ECD). Sample concentration was determined by subtracting the background concentration of $N_2O$-N from the measured sample concentration.

**Data Analysis**

The sum of the N bearing species expected in our column is:

$$N_{TOTAL} = NO_3\text{-}N + NO_2\text{-}N + N_2O\text{-}N + N_2\text{-}N \quad (1)$$

Because of the high concentration of atmospheric $N_2$ and the resulting difficulty in measuring production against background concentration, no attempt was made to measure $N_2$. Instead, we calculated $N_2$ –N production as the difference between N total and measured N species

($NO_3$-N+ $NO_2$-N + $N_2O$-N). This calculation implicitly assumes no other nitrogen species, other than those enumerated in the equation, are present.

Analysis focused on quantifying rates of N loss ($NO_3$-N+ $NO_2$-N), and denitrification production ($N_2O$-N); as no $NO_2$-N was found in any experiments, N loss represented loss of $NO_3$-N.

Rate of N loss (mg $hour^{-1}$ $g^{-1}$)was calculated using the following equation (Willems et al, 1997):

$$R = \frac{q(\Delta C)}{m} \qquad (2)$$

where q is flow rate (L $hour^{-1}$), $\Delta C$ is the change in $NO_3^-$ + $NO_2^-$ (mg $L^{-1}$) concentration, and m is mass of soil in the column (g).

Rate of $N_2O$-N production (ng $hour^{-1}$ $g^{-1}$) was calculated in a similar manner (Willems et al, 1997):

$$R = -\frac{q\ (N_2O\text{-}N)}{m} \qquad (3)$$

where q is flow rate (L $hour^{-1}$), $N_2O$-N is the concentration dissolved in column effluent (ppbv), and m is mass of soil in the column (g). Rate of N loss and rate of $N_2O$-N production were separately plotted as a function of time for individual experiments. Average rates of $NO_3$-N loss and $N_2O$-N production were calculated for respective replications in experiments.

## Results and Discussion

### pH in Column Experiments

The pH of column replicates remained fairly constant throughout the duration of all four experiments, as demonstrated in the example data plotted in Figure 2 (see Appendix A, Tables A5-A10 for complete pH data):

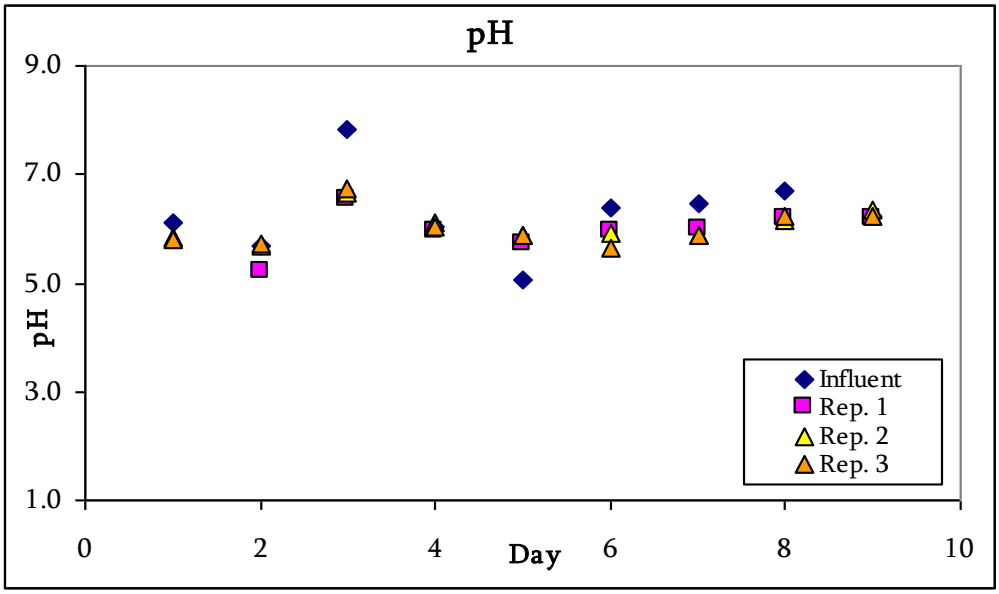


Figure 2. Example pH values as a function of time for a column experiment. Conditions: Argon-purged solution containing 5.0 mg N-$NO_3$/L at 25ºC.

As discussed previously, the denitrification process is inhibited by acidic conditions (Wijler and Delwiche, 1954). The average pH of the effluent solution used in experiments was 5.2. Although the pH in our system is below optimal for denitrification (pH range of 6 to 8), previous studies have shown denitrification occurring at pH $\leq$ 4.9 (Waring and Gilliam, 1983; Wijler and Delwiche, 1954, Parkin et al, 1985). It is possible, however, that denitrification was slowed due to the less than optimal soil pH.

**Reduction Potential (Eh) in Column Experiments**

Reduction potential (Eh) fluctuated slightly during experiments, but stayed in the general range of 450 mV to 500 mV, as illustrated by Figure 3 (see Appendix A, Tables A11-A15 for complete data):

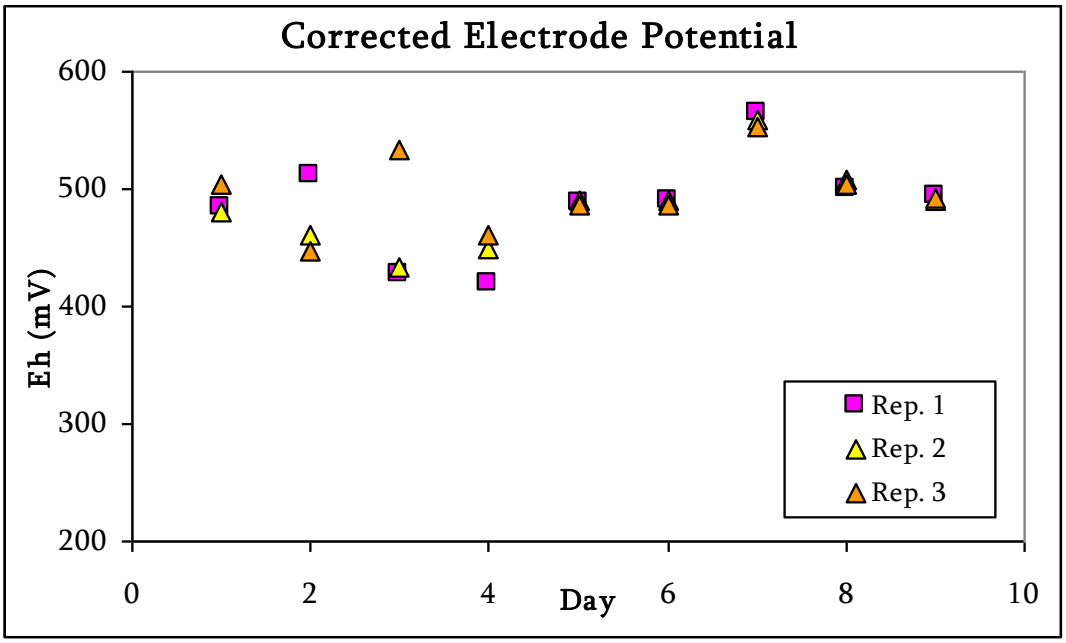


Figure 3. Example of time course of Eh (mV) measured on the outlet of columns. Conditions: Argon-purged solution containing 5.0 mg N/L $NO_3$ at 25ºC.

The Eh at which $NO_3^-$ reduction occurs will vary according to soil pH; it is generally accepted for soils pH ≈ 5.2 that $NO_3^-$ reduction will occur at approximately 350 mV, with an accepted range where $NO_3$ reduction can occur between 325 mV to 375 mV (Essington, 2004). Eh values never dropped into this range in any of the experiments; equipment associated error may have artificially raised measured Eh values. A flow-through adapter equipped with combination redox electrode was utilized for taking Eh measurements.

However, due to the mechanics of the flow-through adapter, there was a possibility of contamination by atmospheric oxygen. Alternatively, poising of Eh could have occurred. Poising of Eh is similar to pH buffering in soils; a soil that is poised resists changes in Eh, and elevated Eh values are observed (Sposito, 1989). The poising of Eh by $NO_3^-$ is possible, and would have falsely indicated suboxic conditions (Sposito, 1989), explaining Eh values in this experiment. Measured Eh values did not support evidence of $NO_3^-$ reduction observed in columns, although values were consistent across all four experiments (Appendix D, Table D1). We thus conclude that measurements of Eh from column outlets did not have accurately reflect the actual redox state in our system.

## Rate of Nitrate Loss

Concentration of $NO_3$-N in both influent and effluent (mg N/L) for replications of individual experiments were plotted as a function of time. Examples of these plots are shown in Figures 4 and 5.

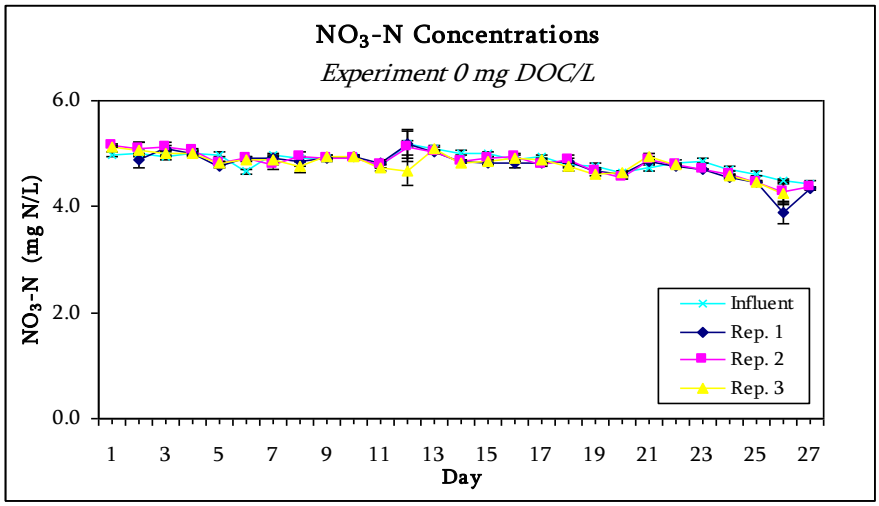


Figure 4. Example of concentration data for a column experiment. Conditions: Argon-purged solution containing 5.0 mg N-$NO_3$/L at 25ºC.

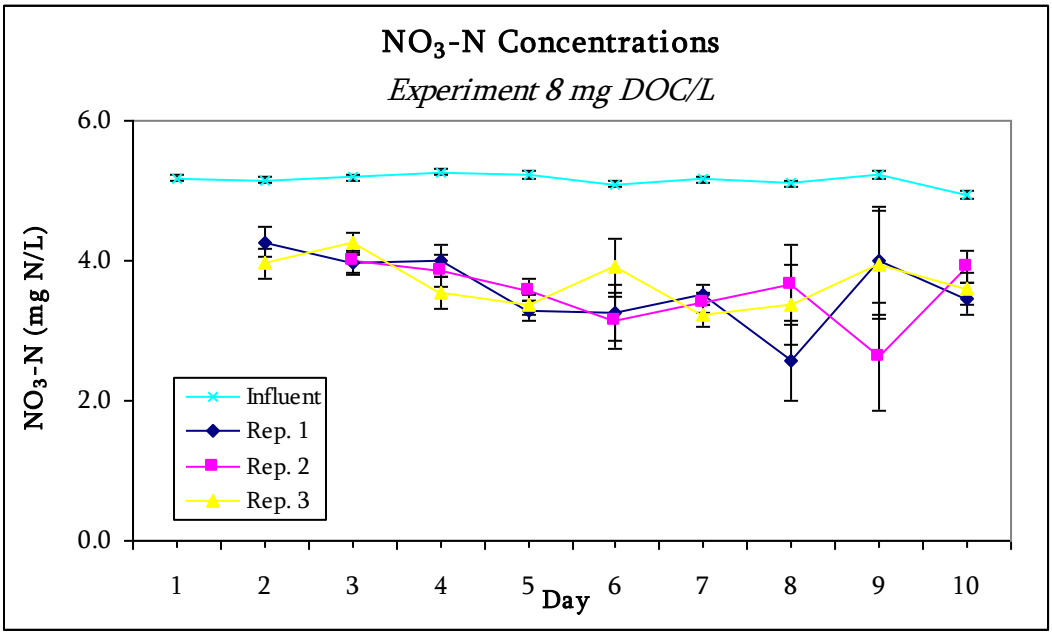


Figure 5. Example of concentration data for a column experiment. Conditions: Argon purged solution containing 5.0 mg N-$NO_3$/L at 25ºC.

For all experiments (excluding the *0 mg DOC/L* control, which showed no measurable change in $NO_3^-$ concentration across the column), effluent $NO_3$-N decreased from days 1 to 3. At approximately day 3, effluent $NO_3$-N stabilized at a roughly steady-state $NO_3$-N concentration for the duration of the experiment. Data points that showed extreme reduction in concentration as a result a known experimental artifact (such as a pump shut-down or column failure) were removed from the data set (see Appendix A for complete data set). Concentration changes were used to calculate steady-state rate of $NO_3$-N loss (mg N/h/g), as described in methods section of this chapter (see Appendix B for complete data set). Replicate measurements showed good agreement (standard deviation $\leq$ 0.00004 mg N/h/g). In our control, without the addition of glucose, minimal loss of $NO_3$-N occurred. Measurable loss of $NO_3$-N occurred in all other trials, with rate of $NO_3$-N loss in the order of

*2.0 mg DOC/L > 12.0 mg DOC/L > 8.0 mg DOC/L > 16.0 mg DOC/L > 4.0 mg DOC/L* (Figure 6). However, error estimates overlap for all treatments, suggesting all treatments were not statistically different.

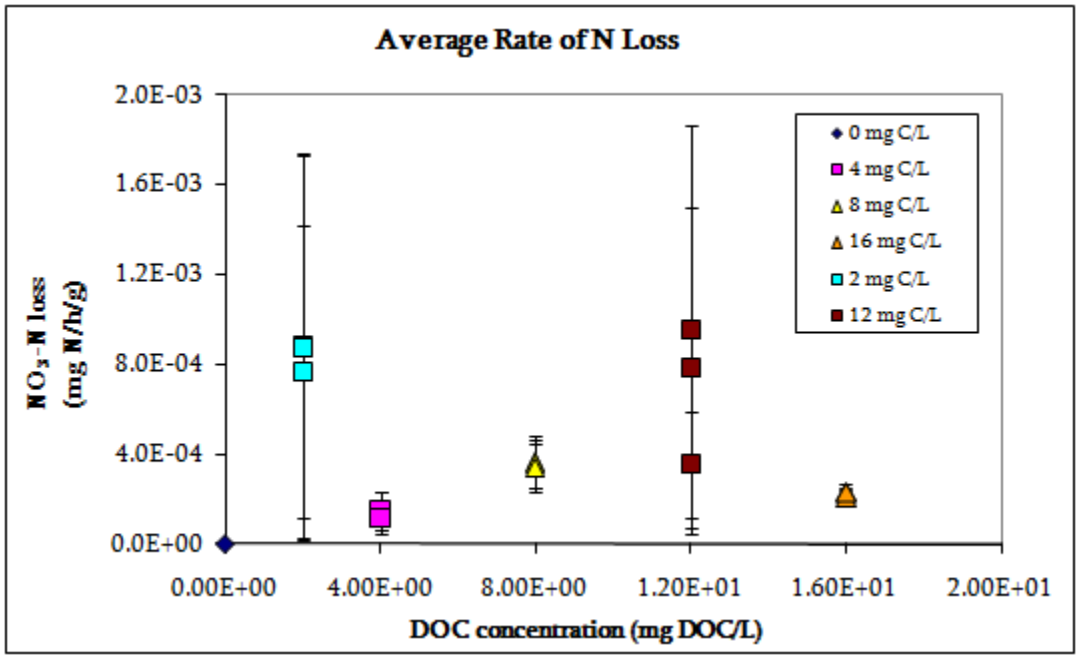


Figure 6. Average rate of $NO_3$-N loss (mg N/h/g) as a function of DOC concentration. Conditions: Argon-purged solution containing.0 mg N-$NO_3$/L at 25ºC.

Results were not consistent with the expected trend of increasing $NO_3$-N loss with increasing DOC. In fact, the highest rate of $NO_3$-N loss was measured in the *2.0 mg DOC/L* experiment, a carbon concentration which is not considered to be adequate for denitrification (Obenhuber and Lowrance, 1991, Lowrance and Smittle, 1998). The lack of increasing rates of N loss with increasing C is difficult to explain. It is possible that 2.0 mg DOC/L, if DOC is in a labile form of glucose, is sufficient to promote a maximum rate of denitrification. We suspect that inconsistent oxygen contamination may reduce the effective concentration of C in columns, leading to large fluctuations in the data between experiments.

The rates of $NO_3$-N loss were compared to those in similar column studies and were found to generally be in good agreement, although trends were dissimilar (Pavel et al, 1996, Obenhuber and Lowrance, 1991). Pavel et al (1996) employed continuous flow-through soil columns, using soil material from three horizons (ponded surface, terrestrial surface, subsurface) with varying OC concentrations (9.3%, 7.8%, and 0.8% respectively). In contrast, we utilized a carbon-poor (0.42%) subsurface soil but added labile DOC in varying concentrations. Despite this difference in experimental approach, the observed range of rates of $NO_3$-N loss from 4.06 $\times 10^{-5}$ mg N/h/g to 6.59 $10^{-4}$ mg N/h/g in their study is similar to the rates obtained in our experiments (1.16 $\times 10^{-4}$ mg N/h/g to 8.43 x $10^{-4}$ ). In a partner study to Pavel et al. (1996), Willems (1997) observed rates of $NO_3$-N loss from 7.24 $\times 10^{-5}$ mg N/h/g to 8.15 $\times 10^{-4}$ mg N/h/g, again, showing good agreement with our rates of $NO_3$-N loss. Good agreement in study rates suggests different sources of C [total organic carbon (TOC) in soil versus DOC] produced similar rates of $NO_3$-N loss in studies.

Other processes may also result in $NO_3$- loss. One such class of processes is $NO_3$-reduction via biological pathways other than denitrification. However, conditions in our column are not expected to promote the activity of DNRA or ANAMMOX bacteria. Another possible source of $NO_3$- loss other then denitrification is microbial assimilation of N. However, microbial biomass N per soil column was approximately 0.25 mg N, as calculated in the following equation:

$$\frac{100\text{ g soil}}{\text{column}} \times \frac{10^8\text{ bacteria}}{1\text{ g soil}} \times \frac{1.0\times10^{-12}\text{ g bacteria}}{1\text{ bacteria}} \times \frac{0.025\text{ g bacteria N}}{1\text{ g bacteria}} \times \frac{1000\text{ mg bacteria N}}{1\text{ g bacteria N}} = \frac{0.25\text{ mg bacteria N}}{\text{column}}$$

Over the complete time course of experiments, microbial biomass N would account for <1% of total influent N. Using microbial data, similar column studies have found immobilization to be < 2%, supporting our findings (Obenhuber and Lowrance, 1991).

**Rate of $N_2O$-N Production**

Production of $N_2O$-N (ng N/h/g) was calculated for replications in experiments and plotted as a function of time (see Appendix C, Figures C1-C7 for complete $N_2O$-N data). Despite a high range of uncertainty in measurements, a notable increase in $N_2O$-N production was observed at the beginning of the experiment containing *8.0 mg DOC/L* and the experiment containing *16.0 mg DOC/L*. After initial higher $N_2O$-N production at the first sampling point, production then decreased over the duration of the experiment (Figure 7 and 8).

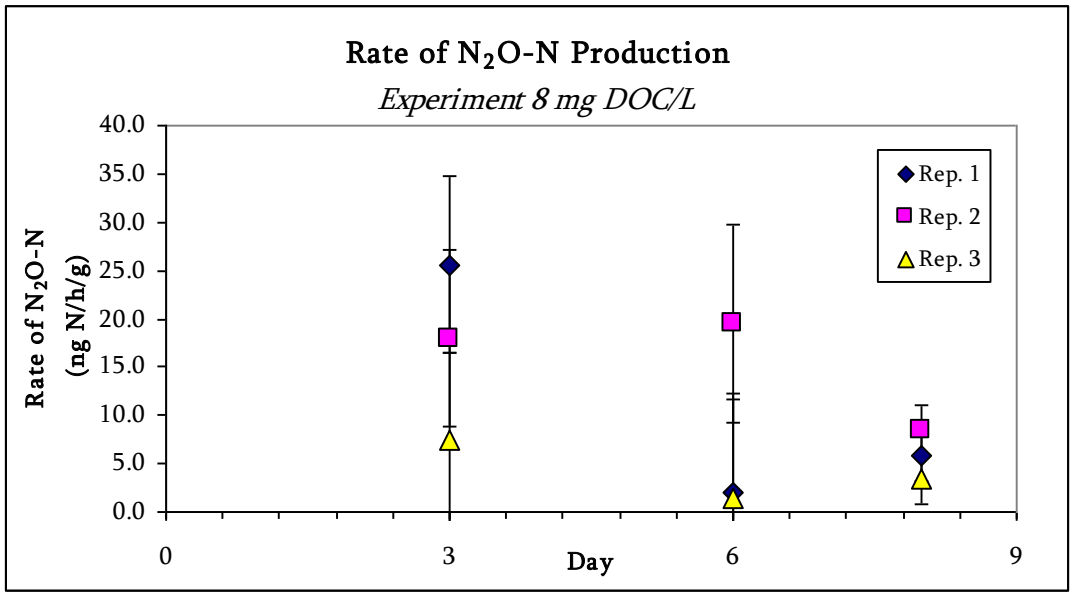


Figure 7. Rate of $N_2O$-N production (ng N/h/g) as a function of time for experiment 8 mg DOC/L. Conditions: Argon-purged solution containing 5.0 mg $N\text{-}NO_3$/L at 25ºC.

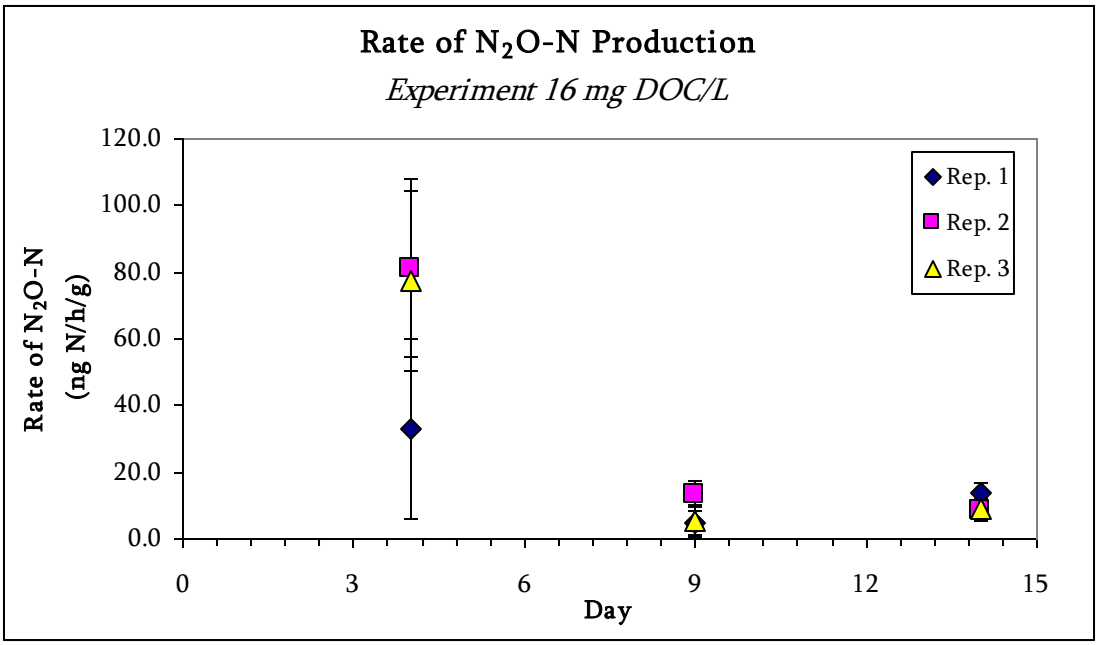


Figure 8. Rate of $N_2O$-N production (ng N/h/g) as a function of time for experiment 16 mg DOC/L. Conditions: Argon-purged solution containing ≈ 5.0 mg N-$NO_3$/L at 25ºC.

Although it is difficult to interpret because of the small size of the dataset, this trend may be reflective of the onset of anaerobic conditions, and the initial introduction of DOC and $NO_3^-$ to columns. Significant increases in available DOC and $NO_3^-$, in addition to the onset of anaerobic conditions may produce a transient period with greater net production of $N_2O$ (Firestone and Davidson, 1989). With microbe acclimation to conditions, a decreased, steady-state of $N_2O$ production will be reached for the duration of the experiment. A similar trend has been observed by researchers study the onset of anaerobic conditions in soils (Clayton et al, 1997). This observed trend was not present in the experiments containing *0.0 mg DOC/L* and *4.0 mg DOC/L* because no denitrification occurred (*0 mg DOC/L*) or slower rates of denitrification occurred (*4 mg DOC/L*).

Rates of $N_2O$-N production (ng N/h/g) increased as DOC concentration increased with the exception of *12.0 mg DOC/L*, as illustrated in Figure 9:

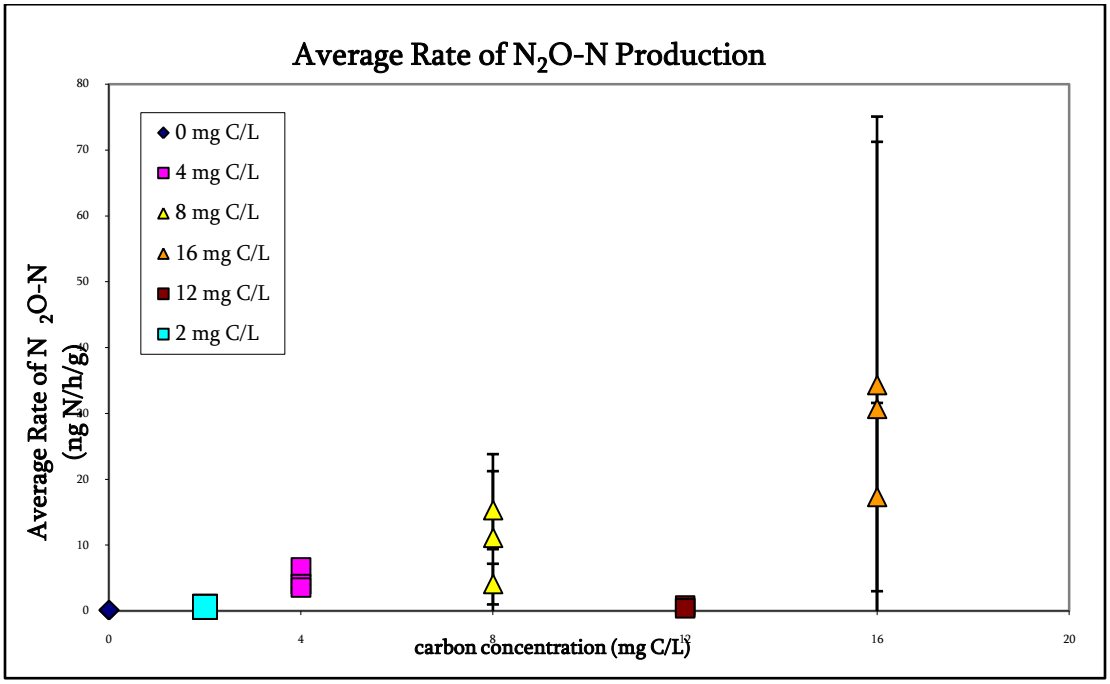


Figure 9. Rate of $N_2O$-N production (ng N/h/g) as a function of time DOC concentration. Conditions: Argon-purged solution containing ≈ 5.0 mg N/L $NO_3$ at 25ºC.

With the exception of *12.0 mg DOC/L*, there was a linear relationship between carbon concentration and rate of $N_2O$-N. As DOC increases, electron donor's increase, leading to higher rates of $NO_3^-$ reduction and $N_2O$-N production. These findings are consistent with findings of other researchers (Pavel et al, 1996; Obenhuber and Lowrance, 1991).Results in the *12.0 mg DOC/L* are difficult to explain; we suspect that experimental artifacts in $N_20$ measurements may have lead to the dataset for this experiment to be unrealiable.

## Production of $N_2$-N

As shown in equation 1, we employed a mass balance approach to describe the species of N produced in the column experiments. The difference between N total ($N_T$) and measured N species ($NO_3$-N + $NO_2$-N + $N_2O$-N) was used to calculate production of $N_2$-N.

$N_2$-N production constituted the majority of denitrification products produced (Figure 10). There was no $NO_2$-N found in any experiments, and NO-N (nitric oxide-nitrogen) was assumed to be negligible. Microbial immobilization could have incorporated minimal amounts of $NO_3$-N; however, previous work and our estimated calculation suggest that immobilization was negligible. Therefore, rates of $NO_3$-N loss, $NO_2$-N production, and $N_2$-N production were the only N species rates calculated in our mass N balance (Table 1).

Table 1. $NO_3$-N loss and $N_2$-N production

| [DOC] (mg C/L) | Average Rate of $NO_3$-N Loss (mg/d/g) | Average Rate of $N_2O$-N Production (mg/d/g) | Average Rate of $N_2$-N Production (mg/d/g) | Ratio $N_2O$-N/$N_2$-N |
|---|---|---|---|---|
| 2.0 mg | $2.0 \times 10^{-2}$ | $2.9 \times 10^{-6}$ | $2.0 \times 10^{-2}$ | $1.4 \times 10^{-4}$ |
| 4.0 mg | $3.3 \times 10^{-3}$ | $1.5 \times 10^{-5}$ | $3.3 \times 10^{-3}$ | $4.7 \times 10^{-3}$ |
| 8.0 mg | $8.5 \times 10^{-3}$ | $1.1 \times 10^{-4}$ | $8.4 \times 10^{-3}$ | $1.4 \times 10^{-2}$ |
| 12.0 mg | $1.7 \times 10^{-2}$ | $2.4 \times 10^{-4}$ | $1.7 \times 10^{-2}$ | $1.5 \times 10^{-2}$ |
| 16.0 mg | $5.3 \times 10^{-3}$ | $1.5 \times 10^{-5}$ | $5.3 \times 10^{-3}$ | $2.8 \times 10^{-3}$ |

The ratio of $N_2O$-N/$N_2$-N was calculated to understand the effect of DOC concentration on relative proportions of $N_2O$-N and $N_2$-N produced during denitrification. On a percentage basis, $N_2$-N constituted nearly all (> 99%) of gaseous products in all experiments containing . In all experiments, $N_2O$-N production was highly variable, resulting

in both large with-in experimental error, and overlapping error bars between experiments. Although variation can be partially explained by onset of column conditions (resulting in higher initial $N_2O$-N production rates as discussed previously), results suggest that production of $N_2O$-N is extremely variable and sensitive to environmental factors. This supposition is fits into the conceptual framework of  by the "hole-in-the-pipe" model, which attempts to explain both process rates, and the factors effecting the portioning of gases between $N_2O$-N and $N_2$-N (Firestone and Davidson, 1989). Firestone and Davidson (1989) suggest that in addition to biological production/consumption of trace N-gases, water-air transfer rates and gaseous diffusion in soil could also effect fluxes, with highly variable fluxes in response to environmental controls.

As with rate of $NO_3$-N loss, results of rates of $N_2O$-N and $N_2$-N production would benefit from data on resident column microbe communities; this information would give us direct insight into the metabolic processes occurring at set DOC concentrations.

## Future Research

Improvements in column design in future flow-through studies would decrease experimental error and create a more stable system. First, our attempts to measure Eh were unsuccessful due to probable oxygen contamination in our flow-thru adaptor. I suggest a column design that would allow in-situ Eh measurements, thereby eliminating exposure of effluent to atmospheric oxygen. Second, collection of $N_2O$ samples may have increased the chances of column clogging. There was a pressure difference between column and vacutainer that occurred when syringes were inserted into vacutainers. This resulted in increased pressure pulling effluent and sediment through screening fabric and exit tubing of the

column. Sampling for $N_2O$ with minimal disturbance to columns could prevent future clogging issues. Third, data on column microbial population density and species would be beneficial in experimental findings, specifically $N_2O$-N and $N_2$-N production.

## Conclusions

There was no clear trend between DOC concentration and rate of $NO_3$-N loss. However, DOC concentrations > 4.0 mg DOC/L increased up until the experiment containing *12.0 mg DOC/L*, after which rates leveled off. There was a linear relationship between DOC concentration and rate of $N_2O$-N production with the exception of *12.0 mg DOC/L*, with the rate of $N_2O$-N production increased with increasing concentrations of DOC.

Reduction of $NO_3^-$ by denitrification is thought to be inhibited in riparian buffers at CEFS due to low levels of organic DOC (3.4 ± 0.6 mg DOC/L). To elucidate the relationship between C and denitrification and to understand better how buffers function, our laboratory study measured denitrification rates and products as a function of C concentrations in a design that replicated field conditions as closely as possible. Although it is difficult to relate laboratory studies to field studies, we attempted to design a laboratory experiment with properties similar to our field study (Table 2):

| Table 2. Parameters of field and laboratory study | | |
|---|---|---|
| **Field** | **Parameter** | **Laboratory** |
| 12 cm/hr | Hydraulic Conductivity | 12 cm/hr |
| 17ºC | Average Groundwater Temperature | 25ºC |
| 5.2 | Soil pH | 5.2 |
| Highly variable: 5.0 average | $NO_3$-N (mg N/L) | 5.2 |
| ≈4.0 mg DOC/L | DOC (mg DOC/L) | 0.0, 2.0, 4.0, 8.0, 12.0, 16.0 mg DOC/L |

The interaction of factors affecting denitrification requires more study to be fully understood. While our laboratory study was beneficial in helping to elucidate certain aspects of the relationship between C and microbial activity, our system was too unstable to accurately capture trends in $NO_3$ loss and $N_2O$ production. Improvements in column design, and data on resident microbe populations would provide greater insight into metabolic processes occurring both in soil columns, and in riparian buffers.

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# APPENDICES-CHAPTER 3

**Appendix A**: Concentration changes between influent $NO_3$-N and effluent $NO_3$-N (mg N/L) as a function of time for all experiments with supplementary pH and redox data.

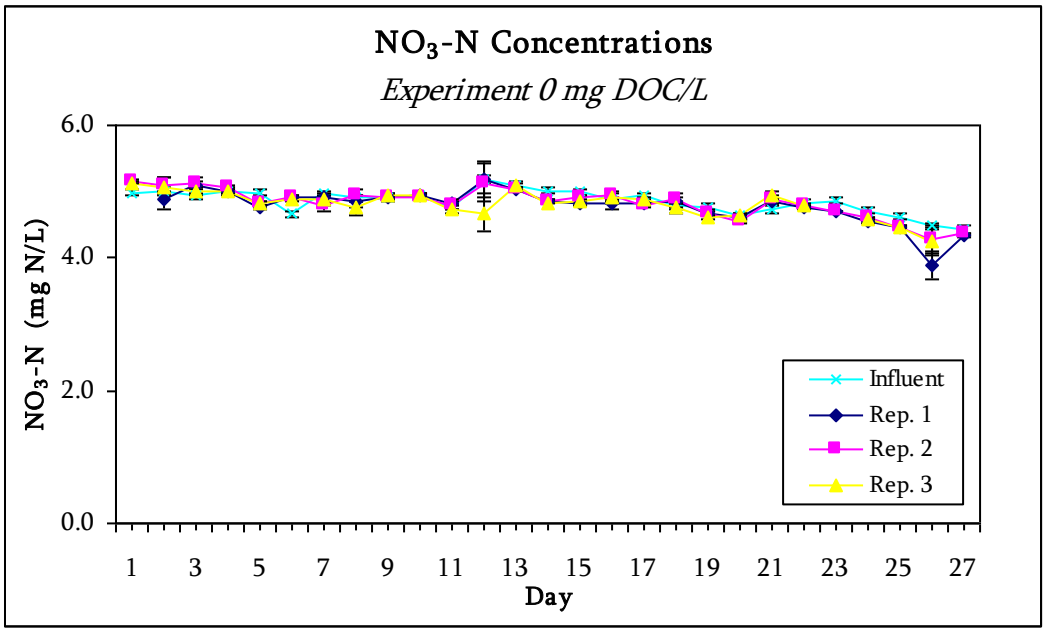


Figure A1. Concentration changes between influent $NO_3$-N and effluent $NO_3$-N (mg N/L) as a function of time for *0 mg C/L*. Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

Table A5. Daily pH of influent and effluent for *0 mg C/L**

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Influent | 6.13 | 5.70 | 7.81 | 6.03 | 5.07 | 6.38 | 6.46 | 6.71 | 6.23 |
| Rep. 1 | ---** | 5.23 | 6.54 | 5.94 | 5.72 | 5.95 | 6.01 | 6.20 | 6.18 |
| Rep. 2 | 5.84 | 5.69 | 6.64 | 6.11 | 5.6 | 5.90 | 5.89 | 6.14 | 6.33 |
| Rep. 3 | 5.80 | 5.71 | 6.73 | 6.02 | 5.89 | 5.66 | 5.87 | 6.22 | 6.23 |

**pH is shown for first nine days of experiment; values did not significantly fluctuate from displayed data for the remainder of the experiment.*
***No data*

Table A11. Daily corrected redox values of effluent for *0 mg C/L**

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Rep. 1 | 484 | 511 | 428 | 420 | 488 | 490 | 565 | 500 | 494 |
| Rep. 2 | 480 | 460 | 433 | 450 | 491 | 490 | 559 | 507 | 491 |
| Rep. 3 | 504 | 448 | 533 | 460 | 486 | 486 | 552 | 503 | 492 |

**Redox values are shown for first nine days of experiment; values did not significantly fluctuate from displayed data for the remainder of the experiment.*

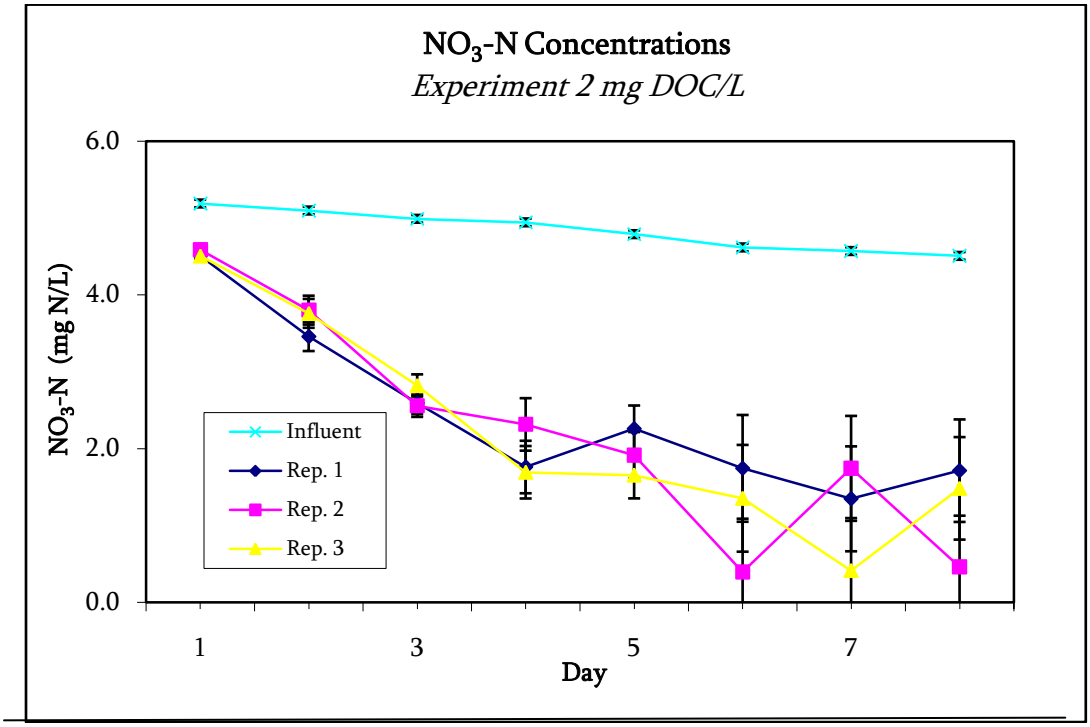


Figure A2. Concentration changes between influent $NO_3$-N and effluent $NO_3$-N (mg N/L) as a function of time for *2 mg C/L*. Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC

Table A6. Daily pH of influent and effluent for *2 mg C/L*.

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Influent | 5.25 | 5.37 | 5.52 | 5.44 | 5.56 | 5.55 | 5.63 |
| Rep. 1 | 6.1 | 5.66 | 5.29 | 5.53 | 5.68 | 5.31 | 5.87 |
| Rep. 2 | 5.98 | 5.04 | 5.8 | 5.66 | 5.46 | 5.72 | 6.08 |
| Rep. 3 | 5.63 | 5.43 | 5.54 | 5.49 | 5.41 | 5.83 | 5.83 |

**Appendix A continued**

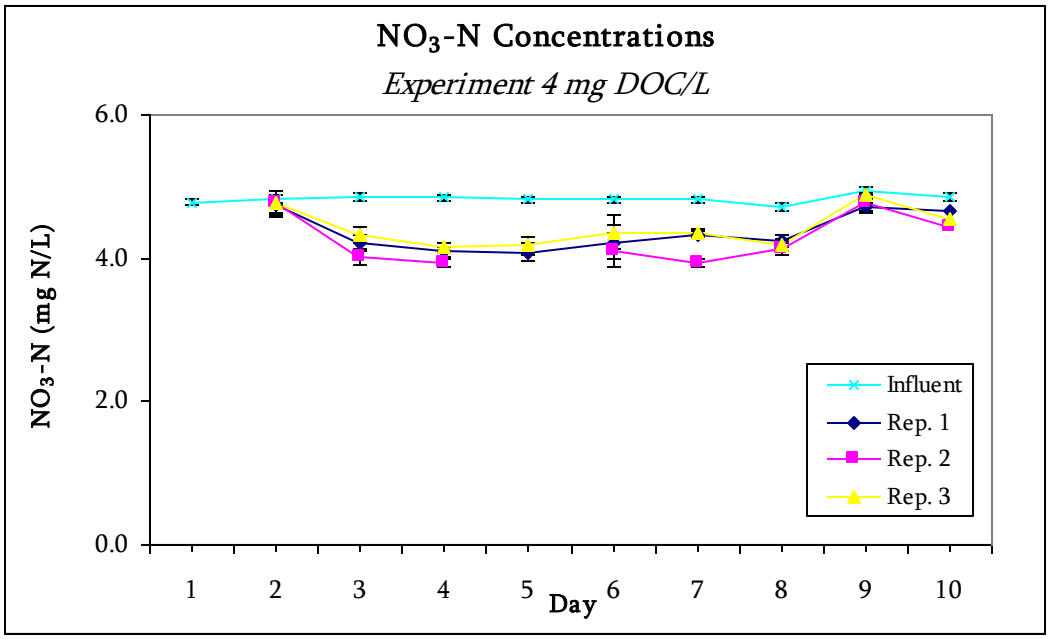


Figure A3. Concentration changes between influent $NO_3$-N and effluent $NO_3$-N (mg N/L) as a function of time for *4 mg C/L*. Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

Table A7. Daily pH of influent and effluent for *4 mg C/L*.

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Influent | 5.54 | 5.51 | 5.54 | 5.39 | 5.22 | 6.00 | 6.77 | 6.60 | 6.08 |
| Rep. 1 | 5.63 | 5.79 | 6.29 | 6.08 | 6.13 | 5.96 | 5.69 | 6.07 | 6.08 |
| Rep. 2 | 5.7 | 5.96 | 6.39 | 6.24 | 6.22 | 6.27 | 6.39 | 6.17 | 5.59 |
| Rep. 3 | 5.62 | 5.99 | 6.3 | 6.22 | 6.12 | 6.18 | 6.24 | 6.29 | 6.04 |

Table A12. Daily corrected redox values of effluent for *4 mg C/L*.

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Rep. 1 | 475 | 464 | 475 | 475 | 480 | 489 | 497 | 486 | 484 |
| Rep. 2 | 473 | 465 | 475 | 469 | 474 | 487 | 494 | 477 | 486 |
| Rep. 3 | 470 | 462 | 471 | 466 | 480 | 485 | 490 | 476 | 486 |

**Appendix A continued**

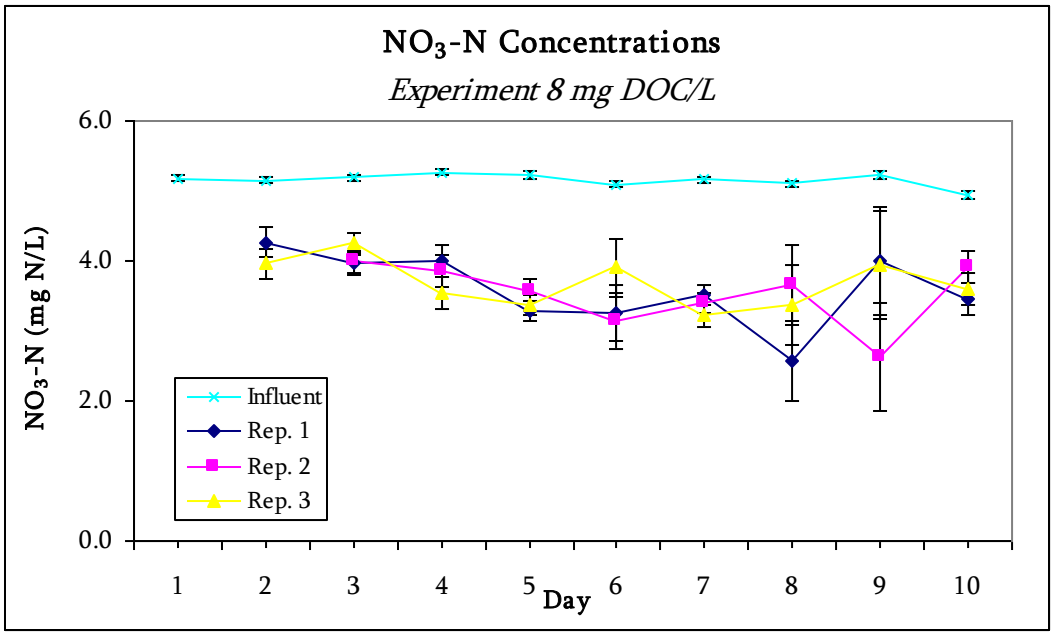


Figure A4. Concentration changes between influent $NO_3$-N and effluent $NO_3$-N (mg N/L) as a function of time for *8 mg C/L* Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

Table A8. Daily pH of influent and effluent for *8 mg C/L.*

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Influent | 5.54 | 5.45 | 5.45 | 5.89 | 5.94 | 6.30 | 6.76 | 6.78 | 6.24 |
| Rep. 1 | 5.70 | 6.22 | 6.48 | 6.48 | 6.43 | 6.44 | 6.59 | 6.74 | 6.19 |
| Rep. 2 | 5.83 | 6.18 | 6.50 | 6.46 | 6.27 | 6.44 | 6.52 | 6.57 | 6.31 |
| Rep. 3 | 5.66 | 6.06 | 6.61 | 6.42 | 6.04 | 6.44 | 6.61 | 6.75 | 6.31 |

Table A13. Daily corrected redox values of effluent for *8 mg C/L.*

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Rep. 1 | 475 | 464 | 470 | 469 | 482 | 487 | 495 | 480 | 482 |
| Rep. 2 | 475 | 465 | 468 | 473 | 480 | 485 | 496 | 477 | 483 |
| Rep. 3 | 474 | 464 | 467 | -----* | 481 | 488 | 492 | 476 | 483 |

**No data*

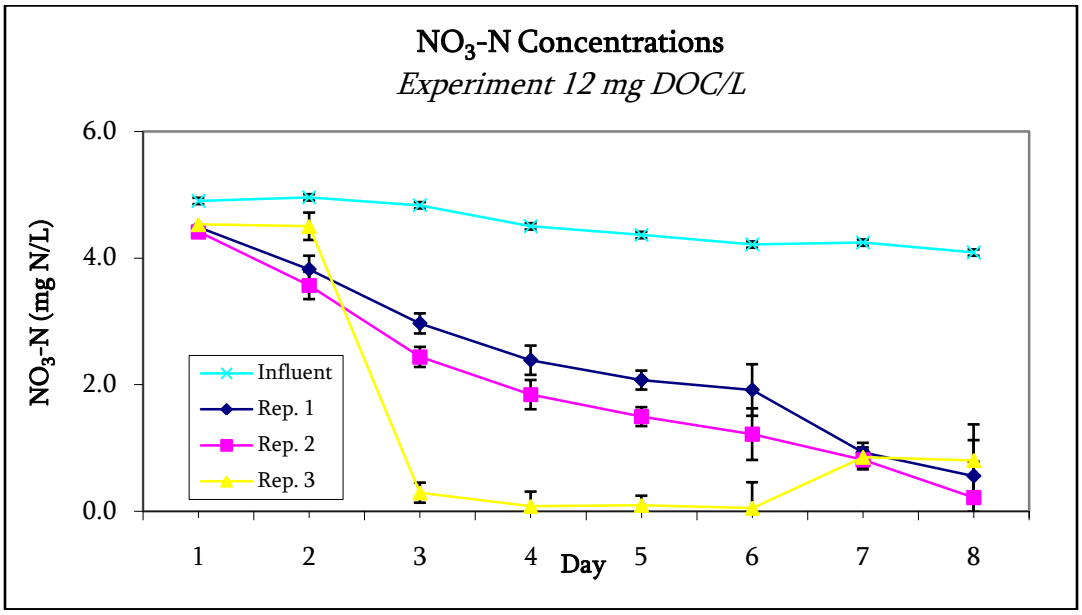


Figure A5. Concentration changes between influent $NO_3$-N and effluent $NO_3$-N (mg N/L) as a function of time for *12 mg C/L* Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

| Table A9. Daily pH of influent and effluent for *12 mg C/L.* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Influent | 5.84 | 5.17 | 5.43 | 6.23 | 6.48 | 6.73 | 6.63 | 6.56 |
| Rep. 1 | 6.07 | 6.04 | 6.37 | 6.75 | 6.71 | 6.68 | 7.04 | 7.2 |
| Rep. 2 | 6.05 | 6.21 | 6.58 | 7 | 6.7 | 6.92 | 7.28 | 6.88 |
| Rep. 3 | 5.9 | 6.8 | 6.45 | 6.67 | 6.7 | 6.71 | 6.97 | 7.05 |

| Table A14. Daily corrected redox values of effluent for *12 mg C/L.* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Rep. 1 | 461 | 490 | 468 | 464 | 481 | 474 | 452 | 461 |
| Rep. 2 | 468 | 492 | 470 | 468 | 485 | 488 | 460 | 468 |
| Rep. 3 | ---- | 497 | 467 | 459 | 467 | 489 | 467 | ------ |

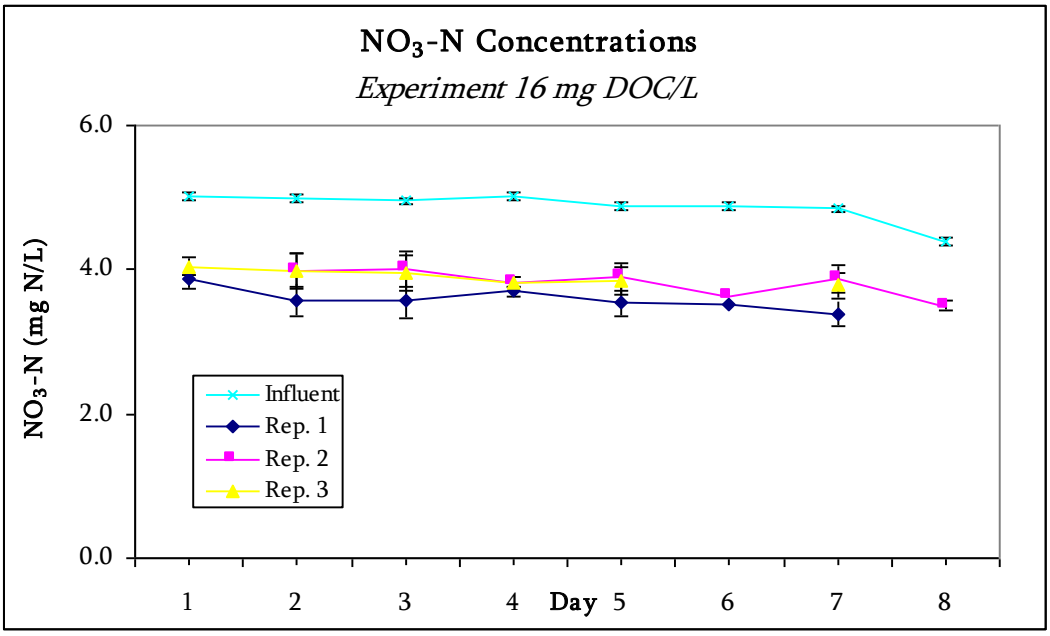


Figure A6. Concentration changes between influent $NO_3$-N and effluent $NO_3$-N (mg N/L) as a function of time for *16 mg C/L* Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

Table A10. Daily pH of influent and effluent for *16 mg C/L.*

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Influent | 5.58 | 5.47 | 5.29 | 5.41 | 5.21 | 6.42 | 6.67 | 6.84 |
| Rep. 1 | 6.12 | 6.54 | 6.66 | 6.68 | 6.66 | 6.34 | 6.68 | 6.67 |
| Rep. 2 | 6.01 | 6.50 | 6.58 | 6.70 | 6.49 | 6.27 | 6.47 | 6.55 |
| Rep. 3 | 5.95 | 6.50 | 6.58 | 6.83 | 6.62 | 5.89 | 6.49 | 6.69 |

Table A15. Daily corrected redox values of effluent for *16 mg C/L.*

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Rep. 1 | 460 | 472 | 480 | 478 | 481 | 482 | 475 | 477 |
| Rep. 2 | 461 | 471 | 480 | 486 | 482 | 484 | 481 | 477 |
| Rep. 3 | 462 | 468 | 479 | 485 | 481 | 473 | 482 | 474 |

**Appendix B:** Rate of N loss (mg N/h/g)

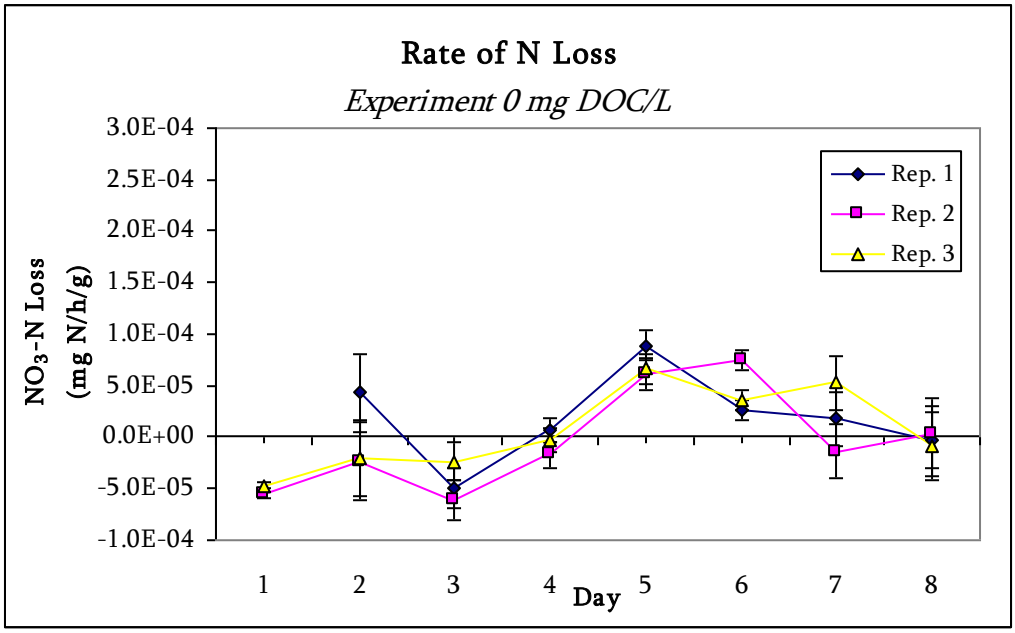


Figure B1. Rate of $NO_3$-N loss (mg N/h/g) as a function of time for *0 mg C/L*. Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

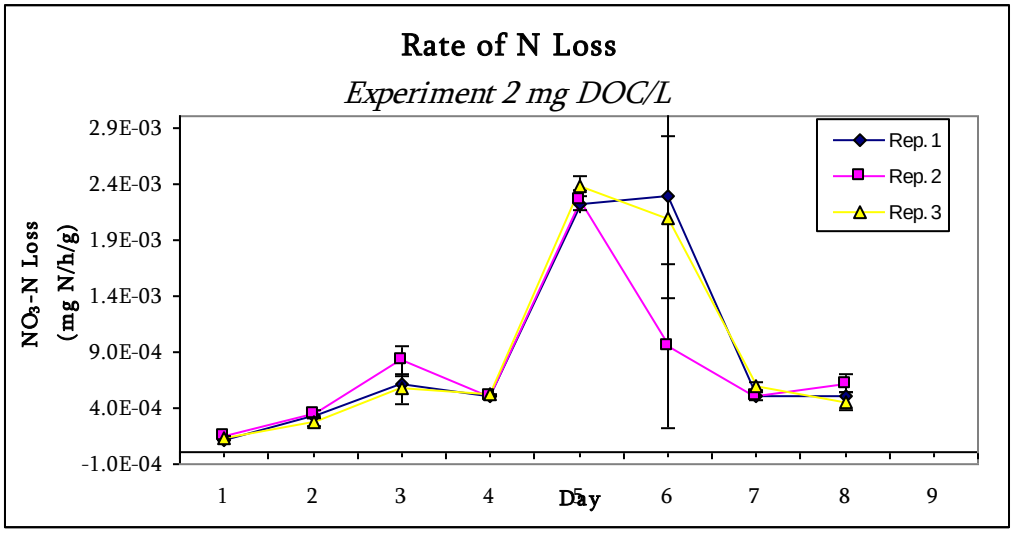


Figure B2. Rate of $NO_3$-N loss (mg N/h/g) as a function of time for*20 mg C/L*. Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

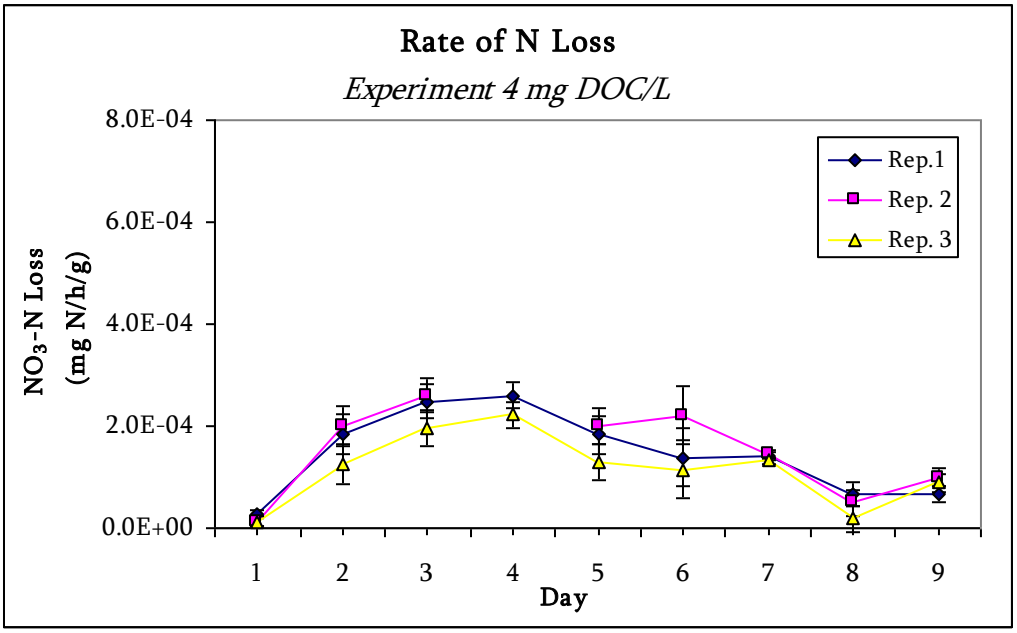


Figure B3. Rate of $NO_3$-N loss (mg N/h/g) as a function of time for *4 mg C/L*. Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

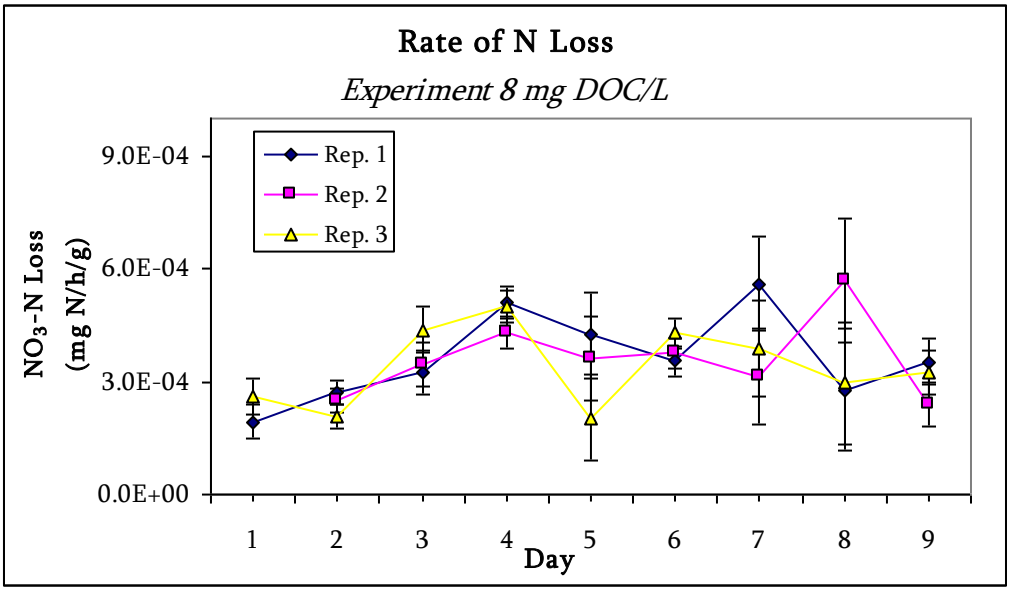


Figure B4. Rate of $NO_3$-N loss (mg N/h/g) as a function of time for *8 mg C/L*. Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

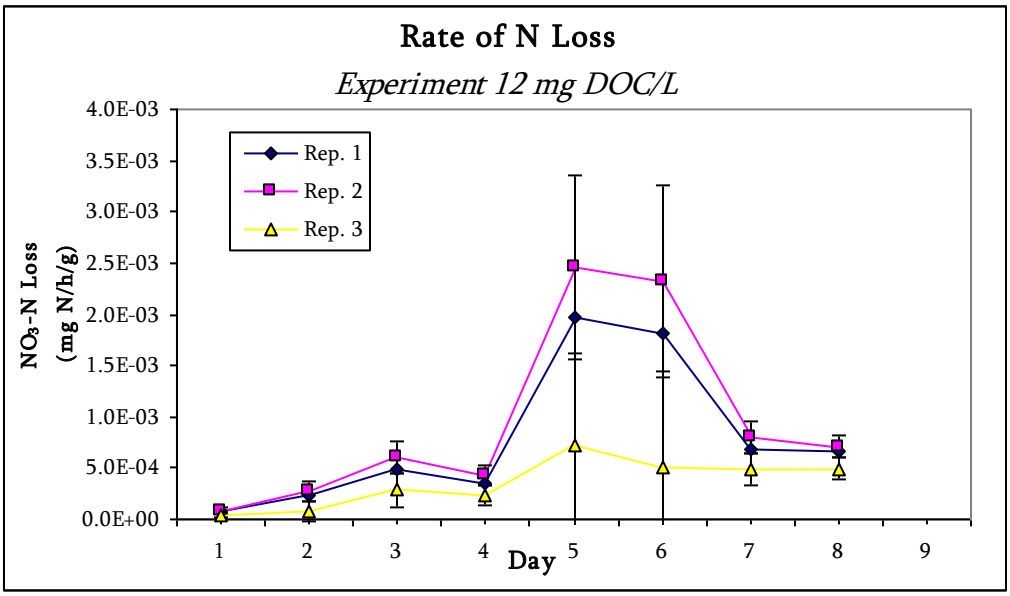


Figure B5. Rate of $NO_3$-N loss (mg N/h/g) as a function of time for *16 mg C/L*. Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

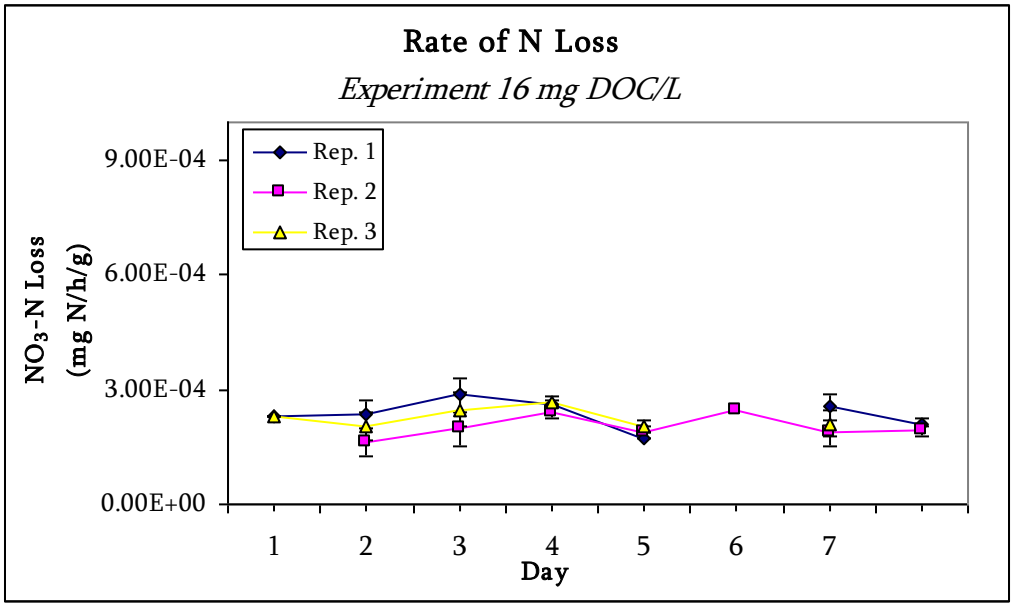


Figure B6. Rate of $NO_3$-N loss (mg N/h/g) as a function of time for *16 mg C/L*. Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

**Appendix C:** Rate of $N_2O$-N Production (ng N/h/g)

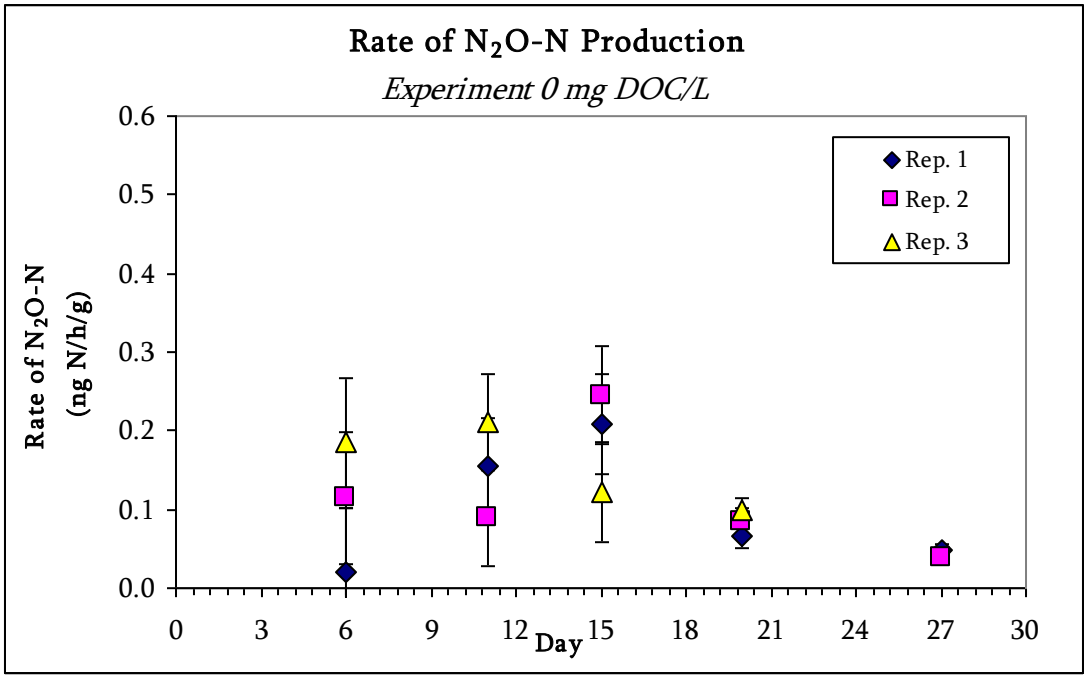


Figure C1. Rate of $N_2O$-N production (ng N/h/g) as a function of time for *0 mg C/L*. Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

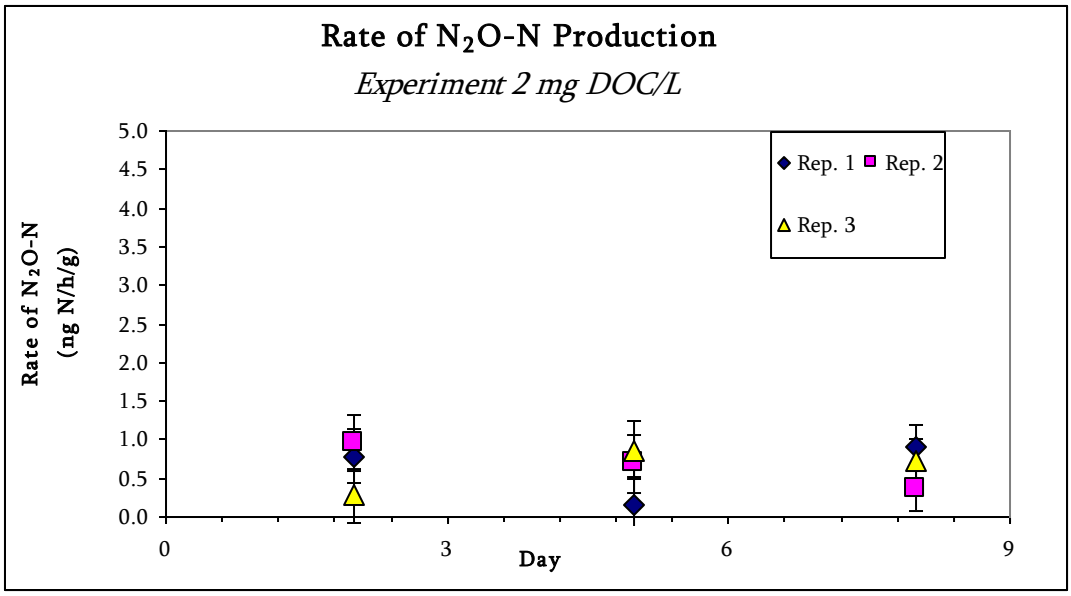


Figure C2. Rate of $N_2O$-N production (ng N/h/g) as a function of time for *2 mg C/L*. Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC

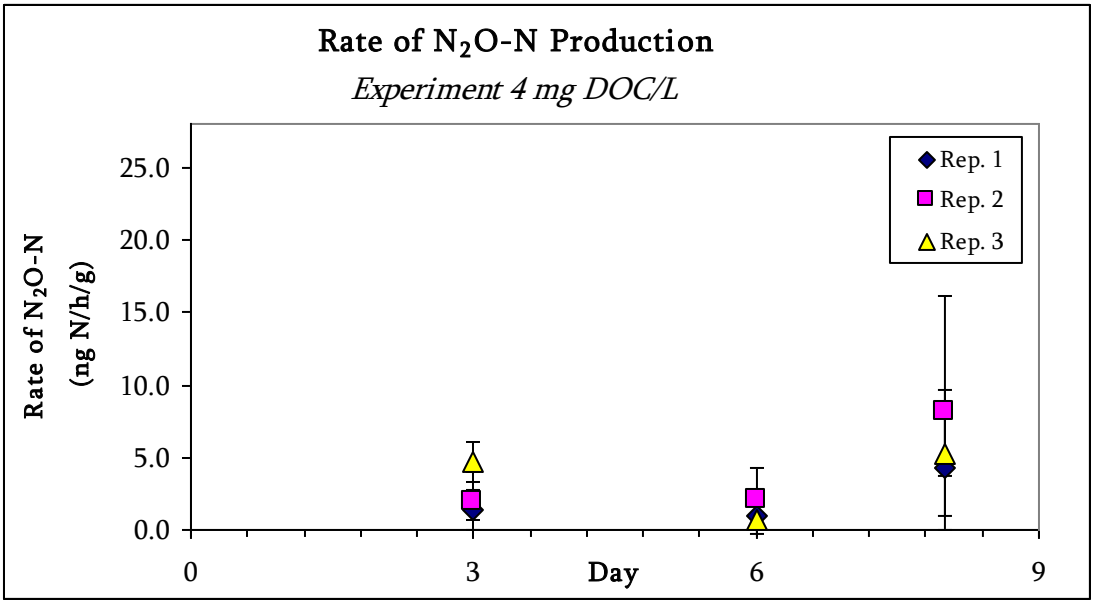


Figure C3. Rate of $N_2O$-N production (ng N/h/g) as a function of time for *4 mg C/L*. Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

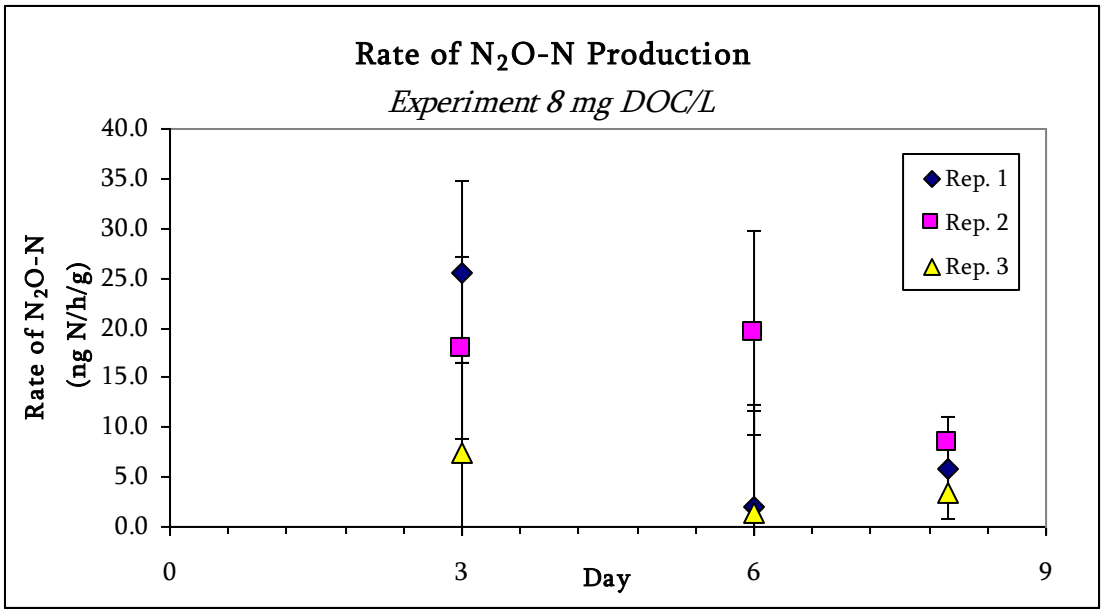


Figure C4. Rate of $N_2O$-N production (ng N/h/g) as a function of time for *8 mg C/L*. Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

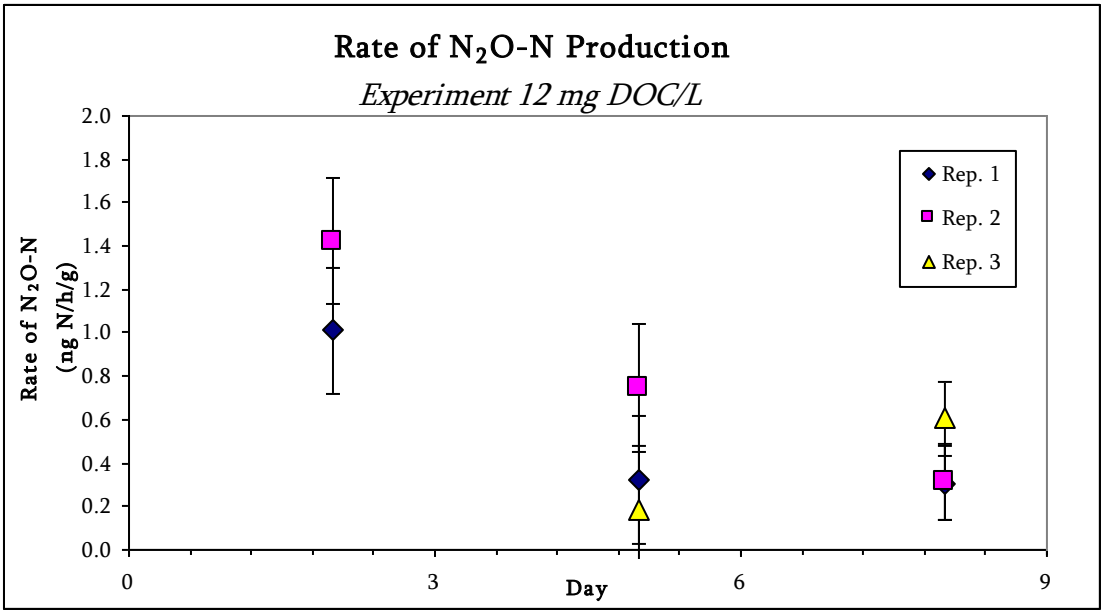


Figure C5. Rate of $N_2O$-N production (ng N/h/g) as a function of time for *8 mg C/L*. Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

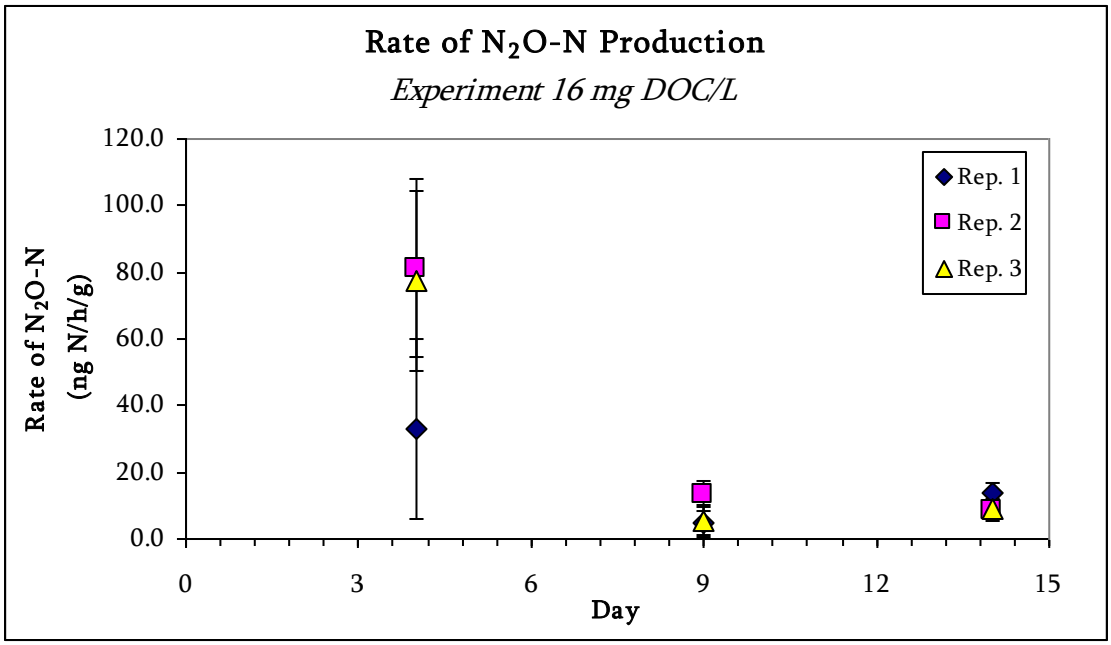


Figure C6. Rate of $N_2O$-N production (ng N/h/g) as a function of time for16 *mg C/L*. Conditions: Argon-purged solution containing ≈ 5.00 mg N/L $NO_3^-$ at 25ºC.

## Appendix D: N Immobilization Calculation

Assumptions:

1. 20% of microbe is dry-weight.
2. Microbe C/N: 8

$$\frac{100\text{ g soil}}{\text{column}} \times \frac{10^{8}\text{ bacteria}}{\text{g soil}} \times \frac{1.0\times10^{-12}\text{ g bacteria}}{1\text{ bacteria}} \times \frac{0.025\text{ g bacteria N}}{\text{g bacteria}} \times \frac{1000\text{ mg bacteria N}}{1\text{ g bacteria N}} = \frac{0.25\text{ mg bacteria N}}{\text{column}}$$