Denitrification and organic carbon in a series of riparian buffers in the Bear Creek National Demonstration Watershed

by

Amber Denton Johnson

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Ecology and Evolutionary Biology

Program of Study Committee:
Richard C. Schultz, Major Professor
Thomas M. Isenhart
Timothy B. Parkin
James W. Raich

Iowa State University
Ames, Iowa
2003

Graduate College
Iowa State University

This is to certify that the Master's thesis of

Amber Denton Johnson

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy

Special thanks to all those who have made it possible
for me to complete this work
Especially
Jason Johnson
And all those who have helped care for
Phoebe Rose Johnson
The very best thing from the past three years

TABLE OF CONTENTS

ABSTRACT

Riparian buffers have been shown to be effective at improving surface water quality. Vegetative uptake and denitrification are interrelated nitrogen removal services of riparian buffers. Surface denitrification rates are much higher than subsurface rates, however, subsurface denitrification is also essential to overall nutrient removal services. Soil quality can vary under different vegetation types in surface and subsurface soils. This study was conducted to determine if denitrification potential and its controlling factors were different under warm season grasses and introduced cool season grasses at different depths. Denitrification potential measured by the Denitrifier Enzyme Activity Assay (DEA), Total Organic Carbon (TOC), Total Nitrogen (TN), Dissolved Organic Carbon (DOC), Bioavailable DOC (%BDOC) and Inorganic Nitrogen (IN) were measured during three sampling periods (Summer 2001, Fall 2001, and June 2002) at five depths up to the non-permeable aquitard (3-4 m). Sampling was done following soil morphological features, from the surface, throughout the rest of the vadose zone, at the border with fluctuating groundwater conditions evidenced by mottling, throughout the mottled zone, and at the border with impermeable till. Surface samples were higher than all other samples. Vadose zone samples were significantly different from other subsurface depths during active plant growth, but were not significantly different from other subsurface depths when grasses were dormant. Multivariate analysis and amendment studies showed that after accounting for depth in the soil profile, soil water was the most important factor controlling denitrification followed by organic matter (% C and N) and C availability (%BDOC). The results were confounded by age of the buffers, i.e. all the warm season grasses were relatively recently established, and all cool season grasses had been established for more than 50 years. The lack of startling differences between vegetation types indicates that warm season grasses may rapidly restore organic C and soil processes after establishment. The results suggest that different plant communities affect denitrification potential in the surface and vadose zones, and imply that a diverse mixture of plants should be used in buffer establishment to maximize year-long denitrification potential.

GENERAL INTRODUCTION

By the 1880's an almost complete transformation of the native prairie-pothole ecosystem of Iowa was underway. The complex native ecosystem was converted from a perennial vegetation system to an annual today almost exclusively devoted to corn and soybeans. Soil development was halted and erosion greatly increased. Erosion caused by the conversion from a native perennial vegetation system has increased from 220 - 440 kg of soil per ha per year to more than 13.3 metric tons per ha per year (Burkart et al., 1994). Mechanical cultivation replaced soil mixing by burrowing insects and mammals. The loss of native vegetation and wetlands has also greatly reduced wildlife species diversity. Natural depressions that collected water were drained by field tiles converting wetlands into cultivated fields. Field tiles greatly decrease the residence time of surface water by providing a direct conduit to streams and bypassing the remaining natural soil and vegetation buffers. Excess nitrogen from field runoff is often in the form of nitrate (Schilling and Libra, 2000), which can enter the drinking water system and have serious health consequences (Weyer et al., 2001). Field tile water is a major source of nitrate that has been measured at 80 to 90 ppm during the summer months in Iowa (Kelly, 1990). This is an especially important consideration in North-Central Iowa where during much of the year stream base flow is dependent upon tile drainage, and where surface waters and shallow groundwater wells often exceed the US EPA limit of 10 ppm (Schilling and Libra, 2000).

Watershed contamination by NPS pollutants is not only a local problem. Besides contaminating local water sources, these pollutants can be carried downstream to other rivers and eventually to the ocean, where they can cause further environmental and social problems. An example of this type of situation is found in the "dead zones" in the Gulf of Mexico. Areas of hypoxia, created by algal blooms, are suspected to be caused by nutrient loading to the Gulf from agriculture in the Midwest. These dead zones kills organisms in a large area and persist seasonally from May to September after algae has died. They potentially have a large economic impact on fisheries as well as obvious environmental problems (Rabalais et al., 2002).

Riparian management systems along with in-field management and other off-site technologies, have been identified as part of a possible solution to NPS pollution (Schultz et al., 1995). The riparian management system (RiMS) designed by the Agroecology Issue Team of the Leopold Center for Sustainable Agriculture at Iowa State University (AIT) may consist of a grass filter or forest buffer, constructed wetlands that intercept field drainage tiles and reduce nutrient loads before they enter the channel, streambank bioengineering practices that reduce bank erosion and/or controlled riparian grazing to reduce the impact of livestock on the stream channel. The perennial grasses, native trees and shrubs used in RiMS physically reduce overland and some subsurface flow

from fields and trap sediment, nutrients, herbicides and pesticides to prevent their introduction to streams (Schultz et al., 2000). Riparian buffers also add organic matter and associated soil carbon to the riparian zone (Marquez, 2001). The result is an improvement in soil microbial activity (Pickle, 1999), soil aggregation (Marquez, 2001) and infiltration (Bharati et al., 2002) that allows surface runoff from adjacent fields to enter the plant-soil system. Riparian buffer systems may also provide habitat for aquatic, mammal and bird species, alternative products for landowners and improve the aesthetic diversity of the landscape (Schultz et al., 2000). Organic carbon, especially dissolved organic carbon is very important in providing a carbon source for denitrification and forming strong complexes with chemical pollutants which facilitates their degradation. These ecological processes restore the "living filter" of the riparian area and prevent NPS pollution from field runoff to surface waters (Schultz et al., 2000).

This research was conducted as part of the overall efforts of the AIT. The mission of the AIT is to do basic and applied research of riparian buffer function and to improve agricultural sustainability by restoring perennial plant communities in riparian landscapes that have been severely degraded by years of cropping and grazing down to the edge of the stream channel. In Central Iowa, the AIT has completed years of research in riparian buffer system function and has restored perennial vegetation to an 11 km stretch of the upper reaches of the Bear Creek Watershed. Buffers have been planted over a course of 12 years, creating a chronosequence of riparian buffer systems that mimic the historical function of the native vegetation at these sites. These buffers were all established on the same soil series that were previously under row crop cultivation or livestock grazing and were established in 1990 (12 year buffer), 1994 (7 year buffer), 1997 (5 year buffer), 1999 (3 year buffer), and 2001 (0 year buffer). The sites were located roughly 2.5 km N of Roland, IA. All sampling locations were on Coland soils (fine-loamy, mixed, mesic Cumulic Endoaquoll) which have been described as a poorly drained, moderately permeable soil. The Coland solum ranges from 0.9 to 1.2 meters in thickness and forms in loamy alluvium. The A horizon is generally a clay loam to a silty clay loam and the C horizon is generally a sandy loam to a clay loam, but includes silty clay to loamy sand layers (DeWitt, 1984). Deeper horizons are oxidized and unoxidized till, depending on groundwater flow paths, with gravel and sand layers interspersed with silty clay.

In the oldest buffer, sediment and nutrient trapping, soil organic matter and particulate organic matter fractions (Marquez, 2001), above and belowground biomass and respiration rates (Tufekcioglu, 1999), soil microbial biomass and microbial nitrogen immobilization (Pickle, 1999), as well as in situ and potential denitrification have been evaluated to 1 m depths (Isenhart, unpublished data). In addition, the hydrogeology of the buffer zone has been described through the use of piezometers, lysimeters and tensiometers (Johnston, 1998; Wineland, 2002; Simpkins et al., 2002).

A potential problem with riparian buffer systems is that although they have been shown to be very effective at preventing pollution from water moving through the buffer, hydrogeology of the area may cause the water to bypass the buffer rooting zone completely. In areas with an aquitard near the surface, subsurface water is forced through the rooting zone of the buffer before entering the stream. In areas where the aquitard is well below the rooting zone or does not exist at all, water may move by deep subsurface flow underneath the buffer directly into the stream. At the Bear Creek National Demonstration and Research Site, as recognized by the Clean Water Act program, RiMS there is an aquitard at a depth of 4-5 m that prevents deep groundwater from reaching the stream, which effective forces shallow groundwater originating in the crop field through the buffer system (Wineland, 2002). In these study sites the riparian vegetation has the opportunity to filter subsurface water.

With increasing depth in the soil profile the influence of the surface vegetation is reduced and less nutrient immobilization occurs. Preliminary data suggest that at depths greater than about 30 cm the major form of carbon available in the soil is dissolved organic carbon (DOC) (Marquez 2001). Therefore, deep rooted species such as warm season grasses and trees may help alleviate carbon limitations at depth by providing direct carbon input from roots, and by immobilizing nitrate and other pollutants.

Nitrate is immobilized by plant and microbial uptake, but this does not remove N from the system. Denitrification removes nitrate from the system by converting it to nitrogen gasses (N_2, N_20). Denitrification is primarily controlled by six main factors: oxygen supply, nitrate availability, carbon supply, pH, temperature and soil moisture (Tiedje, 1994). Denitrification generally will not occur in aerobic environments. Carbon supply directly and indirectly affects denitrification rates. Carbon supplies the major substrate for the growth of denitrifiers and through aerobic respiration reduces the amount of oxygen in the soil (Tiedje, 1994). Soil temperature and moisture are also very important limiting factors of denitrification. Denitrification is slowed considerably at cold temperatures and in dry soils and increased with warmer temperatures (more denitrifying bacteria) and wetter soils (less 0_2 diffusion)(Paul and Clark, 1989). Water table fluctuations, and corresponding soil moisture in riparian buffers can vary dramatically over the season. In the Bear Creak research sites the upper limit of the water table can vary from just below the soil surface to depths of over 4 meters in one season. Wet/dry cycles in soil can spur soil aerobic respiration and denitrification due to the accumulation of labile organic matter during dry periods then the subsequent flush of activity following redistribution of substrate and onset of anaerobic conditions during wet periods (Kalbitz et al., 2000). Multilevel piezometers were used at Bear Creek (Andress, 1999) to show that denitrification decreases with depth in the aquifer, possibly suggesting that other factors being equal, denitrification is limited by carbon supply at depth. To make reasonable predictions about the amount of NPS pollution reduction

through quantification of soil physical and biological parameters, an understanding of physiological processes and the hydrology of the buffer area is necessary.

Study Description and Objectives

This study from the Bear Creek Watershed compared potential denitrification, and many of its controlling factors in surface and in subsurface soils, within the chronosequence of warm season grass riparian buffers, and within long established cool season grass buffers. Understanding differences between carbon inputs and carbon quality provided by various vegetation types, and the relationship between carbon and nutrient removal processes such as denitrification, will give us important strategic information for designing effective RiMS. The project investigated changes with depth of denitrification potential, organic carbon, dissolved organic carbon, and bioavailable dissolved organic carbon as well as other controlling factors of denitrification including total soil N, and inorganic N. Specifically, we hypothesized:

1) that denitrification potential would differ between warm and cool season grasses,

2) that denitrification potential would vary seasonally, and

3) that organic carbon quantity and quality would mirror denitrification patterns and that bioavailable dissolved organic carbon would be more important at depth than total organic carbon or dissolved organic carbon to denitrification potential.

Results from this study will help fulfill an overall goal of the AIT to determine the ability of riparian buffers to attenuate nitrogen concentrations over time and under different vegetation types.

Thesis organization

This thesis is organized into five chapters: 1) General introduction, 2) Denitrification potential in surface and subsurface soils in a riparian buffer, 3) Factors controlling nitrate removal capability in a riparian buffer, and 4) General conclusions. The general introduction is a broad overview of the importance of the thesis research and reviews the pertinent body of literature. Chapter 2 is a paper based on this research to be submitted to the Journal of Environmental Quality. It contains an abstract, a materials and methods sections, a results and discussion section, a conclusions section and a references cited section as required by the journal. This paper lists three coauthors. Amber Denton Johnson, Graduate Student, primary researcher and author, Thomas M. Isenhart, Associate Scientist and Adjunct Assistant Professor, and Richard C. Schultz, Professor, all in the Department of Natural Resource Ecology and Management, Iowa State University Ames, IA. Timothy B. Parkin, Research Microbiologist, U.S.D.A. A.R.S. National Soil Tilth Laboratory, Ames IA. Chapter 3 is a thesis chapter reporting the results of field and laboratory testing of dissolved organic C and percent bioavailable dissolved organic C as well as other measured soil parameters. This chapter also

discusses results of statistical analysis to determine the relative importance of different forms of organic C to denitrification potential. It includes a materials and methods section, a results and discussion section and a summary and conclusions section. The general conclusion chapter (4) summarizes the major results and management implications of the research as a whole.

References

Andress, R.J. 1999. Fate and transport of nitrate in groundwater within a riparian buffer in the Bear Creek Watershed. M.S. Thesis. Iowa State University. Ames, Iowa.

Bharati, L., K-H. Lee, T.M. Isenhart, and R.C. Schultz. 2002. Soil-water infiltration under crops, pasture, and established riparian buffer in Midwestern USA. Agroforestry Systems. 56:249-257.

Bowman, R.A., J.D.,Reeder, R.W. Lober. 1990. Changes in soil properties in a central plains rangeland soil after 3, 20 and 60 years of cultivation. Soil Science. 150 (6)851-857.

Burkart, M.R., S.L. Oberle, M.J. Hewitt, and J. Pickus. 1994. A framework for regional agroecosystems characterization using the National Resources Inventory. Journal of Environmental Quality 23:866-874.

DeWitt, T. A. 1984. Soil survey of Story County, Iowa. United States Department of Agriculture, Soil Conservation Service.

Johnston, D. A. 1998. Hydrogeology and geology of three restored riparian buffers near Roland, Iowa. M.S. Thesis. Iowa State University, Ames, Iowa.

Kalbitz, K., S. Solinger, J. H. Park, B. Michalzik, and E. Matzner, 2000. Controls on the dynamics of dissolved organic matter in soils: A review. Soil Science. 165:(4)277-304.

Kelly, R. D. (1990) Iowa's surface water quality. Iowa groundwater association newsletter 10.

Lee, K-H., T.M. Isenhart, R.C. Schultz, and S.K. Mickelson. 2000. Multispecies riparian buffers trap sediment and nutrients during rainfall simulations. Journal of Environmental Quality. 29:1200-1205.

Marquez, C. O. 2001. Soil aggregate dynamics and aggregate-associated carbon under different vegetation types in riparian soils. Ph.D. Dissertation. Iowa State University. Ames, IA.

Paul, E. A. and F.E. Clark. 1989. Soil microbiology and biochemistry. Academic Press, New York.

Pickle, J. E. 1999. Microbial biomass and nitrate immobilization in a multi-species riparian buffer. M.S. Thesis. Iowa State University. Ames, Iowa.

Rabalais, N.N., R.E.Turner, and D. Scavia. 2002. Beyond science into policy: Gulf of Mexico hypoxia and the Mississippi River. BioScience 52(2):129-142.

Rolston, D. E. 1981. Nitrous oxide and nitrogen gas production in fertilizer loss. In: Denitrification, nitrification and atmospheric nitrous oxide. Ed. C. Delwiche. Wiley and Sons, New York, NY.

Schultz, R. C., T. M. Isenhart, W.W. Simpkins, C.W. Mize, and M.L Thompson. 1995. Design and placement of a multi-species riparian buffer strip system. Agroforestry Systems 29:201-226.

Schultz, R.C., J.P. Colletti, T.M. Isenhart, C.O. Marquez, W.W. Simpkins, C.J. Ball, 2000. Riparian Forest Buffer Practices. pp. 189-281. In: North American Agroforestry: An Integrated Science and Practice. ASA, Madison, WI.

Simpkins, W.W., T. R. Wineland, R. J. Andress, D. A. Johnston, T. M. Isenhart, and R. C. Schultz 2002. Hydrogeological constraints on riparian buffers for reduction of diffuse pollution; Examples from the Bear Creek Watershed in Iowa. Journal of Water Science and Technology 45:61-68.

Tiedje, J.M. 1994. Denitrifiers. pp. 245-267. In: R.W. Weaver, J.S. Angle, and P.S. Bottomley (ed). Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties. SSSA Book Series no. 5. SSSA, Madison, WI.

Tufekcioglu, A., J.W. Raich, T.M. Isenhart, and R.C. Schultz. 1999. Fine root dynamics, coarse root biomass, root distribution, and soil respiration in a multispecies riparian buffer in Central Iowa, USA. Agroforestry Systems. 44:163-174.

Weyer, P.J., J.R. Cerhan, B.C. Cross, G.R. Hallberg, , J. Kantamneni, G. Breuer, M.P. Jones, W. Zheng, and C.F. Lynch, 2001. Municipal drinking water nitrate level and cancer risk in older women: The Iowa Women's Health Study. Epidemiology 11:322-338.

Wineland, T.R. 2002. Assesing the role of geology for nitrate fate and transport in groundwater beneath riparian buffers. M.S. Thesis, Iowa State University, Ames, IA.

Literature Review

Denitrification is the reduction of nitrate (NO_3^-) and nitrite (NO_2^-) to dinitrogen gases (N_2, N_20). Biological denitrification in soils is carried out by facultative anaerobic bacteria that use NO_3^- as an alternate electron acceptor in respiration. This reduction of nitrogen from a mobile dissolved form to a gaseous form provides an important removal pathway in systems that may be negatively impacted by excess nitrate. A great deal of research has been carried out in agricultural, wetland and forested systems to study denitrification and other related microbial processes. When these processes and their interactions are well understood, we will be able to design riparian buffers and wetlands to maximize natural nutrient removal processes, and therefore mitigate some of the harmful effects of industrialized agriculture.

Along with in-field management and other off-site technologies, riparian areas have been identified as important in attenuating nitrogen contamination of surface and ground waters. Their

position in the landscape between streams and rivers and agricultural land use areas makes them uniquely capable of providing nutrient capture and removal services (Gold et al., 2001; Hill, 1996; Lowrance, 2001). Riparian areas generally have sufficient water and nutrient levels to support high biomass and biological activity (Swanson et al., 1982), and riparian vegetation is effective at filtering excess nutrients and pesticides from soil water (Naiman and Decamps, 1997). However, not all types of vegetation are equally effective at removing pollutants. For example, C_3 and C_4 grasses are active at different times of the year and their growth periods may or may not coincide with nitrate availability in soils. Trees, grasses and shrubs have different rooting depths which may affect their ability to remove pollutants from groundwater depending on groundwater levels; and different vegetation may be better at taking up pollutants from groundwater than others. Greater understanding of the biological processes that capture and remove pollutants is needed in order to design buffer systems that maximize these functions.

Factors controlling denitrification in terrestrial soils: proximal and distal

While soil pH, texture and temperature may affect denitrification, the three most important controls on denitrification in soils are O_2, NO_3^- and C. (Groffman et al., 1988; Merrill and Zak, 1992; Tiedje, 1988). At the cellular level, a lack of NO_3^- or C or the presence of O_2 will preclude denitrification. Researching denitrification in the field is difficult because it is hard to isolate any one of these controlling factors. O_2, NO_3^- and C can be referred to as proximal factors and they are each controlled by a multitude of interrelated distal factors including rainfall, soil texture, vegetation type etc (Groffman et al., 1988).

For denitrification to occur at the organismal level there must be anaerobic conditions. This does not mean, however, that the entire soil matrix must be anaerobic. Many researchers have found that denitrification occurs almost entirely in anaerobic microsites that are often related to soil aggregates and decomposing plant material (Groffman et al., 1988; Parkin, 1987; Sextone et al., 1985). Aggregate formation is tightly linked to soil organic matter content which is influenced by many factors including vegetation type, land use and tillage and climate (freeze thaw cycles, radiation intensity) (Marquez, 2000; Six et al., 1998). Therefore, at the field level, anaerobic microsites are affected by many other, distal, factors and O_2 may be present at some level in the soil matrix without precluding denitrification.

Figure 1.1. Factors controlling denitrification at different scales of investigation (Groffman et al., 1988).

Organism – oxygen, nitrate, carbon
Microsite – organic matter, physical disruptions
Field – water, nitrification, decomposition
Landscape – soil type, land use
Regional – soil type, land use, community structure, geography
Global – biome type, climate

Groffman et al., (1988) describes a simplified hierarchy of importance in controlling factors for denitrification. It places O_2 at the highest level of importance with NO_3^- second and C third. This hierarchy of importance may shift as the overall scale of interests shifts. At the organismal level, O_2 is clearly the most important factor. However, as you shift to the field level, the distal factors of water content, nitrification and decomposition rates are more important in controlling overall denitrification rates.

Carbon, nitrogen and oxygen limitations

Decomposition of organic matter can greatly affect the other main controlling factors of denitrification. Organic matter affects the number of anaerobic sites even in the unsaturated surface horizons. For example, it serves as the energy source during aerobic respiration, which then reduces O_2 concentrations, and thus can create anaerobic microsites within aggregates and behind water films where diffusion of new O_2 is limited. Additionally, OM can provide a NO_3^- source from organic N mineralization as it decomposes (Tiedje, 1988). In surface soils that are not continually saturated, O_2 is an important controlling factor, but, soil organic matter affects the number of anaerobic microsites available for denitrification. Parkin (1987) found that most of the denitrification that occurred in surface soils was related to small "hotspots" associated with plant litter. Hence, at the organismal level denitrification is limited by the presence of oxygen, lack of C and N, but at the field level, the more important controlling factor is OM content because of its role in creating anaerobic microsites and providing substrate.

OM is important in controlling the oxygen status in both surface soils and in subsurface soil. Denitrification may be limited in shallow groundwater systems with constant percolation of dissolved oxygen (DO) rich waters if there is not sufficient DOC. If there is abundant DOC and limited diffusion of new O_2, anaerobic conditions can quickly be created by microbial aerobic respiration. Then when all the O_2 is used up, if there is still enough C available, denitrification occurs (Tiedje,

1988). Likewise, if aerobic respiration does not take up all the O_2 in unsaturated subsoils, denitrification will be limited; especially in subsoils without C-associated microsites where much of the denitrification takes place (Gold et al., 1998). Therefore, in order for denitrification to occur at depth, oxygen inputs must be low from inflowing groundwater and C sources must be sufficient to both use up the available pool of O_2 and have enough left for denitrification. In some groundwater sites, anaerobic conditions conducive to denitrification occur primarily during spring and fall following inputs of DOC leaching from surface soils stemming from litter input, freeze-thaw cycles, and microbial turnover (Clay, 1996).

Ambus and Christensen (1993) found that in a riparian fen irrigated with agricultural drainage water, water-filled pore space as an indicator of the oxygen status of the soils was the only significant variable controlling denitrification rates. NO_3^- availability and water soluble C were either poorly or not at all correlated with denitrification. They believed that this was a result of water-flow patterns and microtopography through the fen. The small ponds on the edges of the fen were generally much lower in NO_3^- concentrations due to rapid denitrification and low irrigation water inputs as compared to central portions of the fen. The authors determined from amendment studies on slurries that NO_3^- was indeed limiting to denitrification and determined that this was related to poor diffusion of NO_3^- during the flooded periods. They found that denitrification rates were much higher in surface soils and the areas that received higher drainage water inputs. Water-soluble C was not at all related to denitrification. The authors felt that this was because C was not needed to control anaerobisis as in other, drier, sites and it was present in high enough concentrations that it was not limiting as an electron donor. Therefore, in this riparian fen, anaerobisis as controlled by water-filled pore space was the most important variable governing denitrification because not only was O_2 limited when pore space was filled with water, but the water redistributed N and made it available for denitrification.

In systems without anthropogenic N inputs, denitrification is often limited by nitrate supply rates (Ashby et al., 1998). Nitrate can have a very rapid turnover rate (0.7 d) and small pool sizes are not necessarily indicative of lack of importance in site N cycling (Corre et al., 2002). Tiedje (p. 219, 1988) said that

"In habitats that are exposed to the atmosphere, oxygen is the principal factor limiting denitrification. . . (in) habitats that are primarily anaerobic . . . the lack of nitrate limits denitrification because nitrate is quickly denitrified and its re-supply (nitrification) is blocked by the absence of O_2. Carbon as the electron donor almost never prevents denitrification, although it is often not present in amounts to saturate the reaction."

The more important role of C is in creating anaerobic environments. However, denitrification is often carbon as opposed to nitrate limited in systems that receive large N inputs. In systems with anthropogenic N-inputs, e.g. atmospheric, agriculture, water treatment etc, there are abundant NO_3^-

sources and denitrification rates are then limited by organic carbon availability (Starr and Gillham, 1993; Bradley et al., 1992; Yeomans et al., 1992). Therefore, we hypothesized that in riparian buffers associated with agricultural activity, carbon will possibly be more closely correlated with denitrification potential than NO_3^- or water content.

Because denitrification is limited by C, one question of interest is whether or not different vegetation treatments produce more total and/or more available organic C which not only spurs denitrification in surface soils, but also could be transported as DOC to shallow groundwater and stimulate denitrification in groundwater systems. DOC is mineralized in surface soils and in the vadose zone and the DOC that percolates into shallow and subsequently deeper groundwater systems is progressively less available to microbes. Starr and Gillham (1993) found that denitrification was lower in a deep groundwater system (>4 m) and was more limited by carbon availability than denitrification in a shallow groundwater system (>1m). They concluded that this was due to DOC oxidation in the vadose zone above the deep groundwater system, which prevented available C from leaching into the deep groundwater. Lack of organic carbon substrate severely limited denitrification in this location.

Measurements of denitrifier populations, and denitrification capacity and potentials in 5 Iowa soils (0-3 m) found that denitrifier populations and denitrification capacity decreased with depth but denitrification potential did not (Yeomans et al., 1992). The authors measured denitrification capacity by anaerobic slurry methods without additions of N or C. Denitrification potential included N and C additions. In subsoils, denitrification potentials were much higher than denitrification capacities which indicates that low denitrification rates in these subsoils was not a result of low denitrifier populations, but instead a result of low available C. Follow-up to this research showed that adding aqueous extracts of surface soils to subsurface soil resulted in a quick denitrification, indicating that subsoils do have substantial denitrification potential, however due to the lack of water soluble, available carbon they do not denitrify to the extent that surface soils do (McCarty and Bremner, 1992).

In a bottomland forested wetland in Arkansas, denitrification was shown to be very limited by C as opposed to nitrate availability (DeLaune et al., 1996). This study demonstrated the importance of C limitations to denitrification as a result of slow decomposition rates and the quality of OC substrate in influencing N-transformation rates. Amendment studies clearly showed that denitrification was limited by C availability (glucose additions more than doubled denitrification rates). Other differences in denitrification rates between sites could easily be explained by litter and thereby leachate DOC quality (cypress needles as opposed to deciduous leaves). Litter quality was not only correlated with denitrification rates, but also with nitrification rates. In natural systems without additional N fertilization, denitrification is often limited by NO_3^- availability, because the main supplier of soil

NO_3^- is from mineralization of organic materials and nitrification, which is an aerobic process. More easily broken down litter provides more organic N for nitrification. Alternatively, DeLaune et al. (1996) found that only 5-12% of the total nitrate reduced came from local nitrification, and the rest came from agricultural run-off. They believed that denitrification in this site was limited by the amount of biologically available C rather than nitrate influx.

In another study in a riparian fen irrigated with agricultural drainage water, water-soluble C was not found to be significantly related to denitrification, whereas NO_3^- availability was very important (Ambus and Christensen, 1993).This riparian fen had much higher inputs of organic-C than did the Arkansas bottomland forest described above.

Wet/dry cycles

Wet/dry cycles and air-filled porosity affect denitrification rates by controlling anaerobicity of soils and affecting substrate availability through stimulation of mineralization, microbial death, transport of substrate etc. (Groffman and Tiedje, 1991; Sextone et al., 1988). In a discussion of research in this area, Groffman and Tiedje (1991) found that wet/dry cycles significantly affected C availability for both denitrification and aerobic respiration especially in the late spring and summer. They found there was a significant relationship between CO_2 production and air-filled porosity.

Similar results were found from a study of soils in permanent pasture in New Zealand (Luo et al., 1999). Increasing soil water also significantly increased denitrification rate, but the effect was most significant during the warm dry period. Soil wetting and subsequent transport of available C and N to denitrification sites is particularly important during summer.

It has been assumed that rewetting physically releases soil organic matter and redistributes it, resulting in the C source for significant soil respiration. However, Fierer and Schimel (2003) found that while re-wetting did increase the amount of extractable SOM-C by 200%, neither it nor cell lyis was the energy source for the following pulse in soil respiration. Instead, they propose that microbes release stored cytoplasmic solutes which are then readily available for respiration. It may be possible that microbes can conserve readily available C-sources, which are then made available in the presence of good conditions for respiration including sufficient water and nutrient levels. This phenomenon may also help to explain why in some sites C is limiting and in others N appears to be more limiting.

Aggregates

Soil aggregates are held together by sticky polysaccharides that are produced by fungal and especially bacterial breakdown. These polysaccharides are usually unavailable to soil microbes because they are in clay interstitial spaces that are too small for microbes to create colonies (Foster, 1981). However, destruction of aggregates by freeze/thaw cycles, tillage or heavy rainfall on bare

soil can potentially release labile C. Aggregate structure, size and strength potentially affect denitrification.

The presence of aggregates can improve conditions for denitrification, by providing structural habitat for denitrifiers, improving water infiltration and water storage capacity of soil. Anaerobic microsites behind water films on aggregates or in aggregate centers can provide for denitrification in otherwise aerated soils. Sextone et al. (1988) found that aggregated soils had higher denitrification rates than unaggregated soils. Their results indicated that solute redistribution from the breakup of soil structure in the anaerobic slurry was important in providing substrate to denitrifiers.

Besides the simple presence of aggregates, aggregate size can potentially affect denitrification and varies by vegetation type as well. Marquez (2001) discussed aggregate dynamics in a riparian buffer system over different vegetation types and seasons. She found that smaller, more stable aggregates with more available C were found under cool-season (C3) grass plots and riparian forest plots as compared to switchgrass (C4) plots and crop fields. Additionally, she found that stability varied seasonally and hypothesized that temporal changes in particulate organic matter may be synchronized with temporal changes in denitrification. Seech and Beauchamp (1988) found that N_2O production was up to 12 times higher in small aggregates than in larger aggregates and that the rate generally decreased with aggregate size. When aggregates were crushed this relationship was less obvious, and rates increased in all size classes. This result plus amendment study results suggested that: C limitation was stronger in larger aggregates; and that physical breakdown of aggregate structure lessened this limitation. C limitation was probably stronger in larger aggregates because of transport problems with labile C.

Beauchamp and Seech (1991) hypothesized that wet-sieving soils would remove more water-soluble C than dry-sieving, but instead concluded that water-soluble C and denitrification were related to aggregate size, not to sieving technique. In wet-sieved soils, denitrification rates increased with increasing aggregate size. The opposite was found in dry-sieved soil. They concluded that this may be related to increased dominance of bacterial derived polysaccharides in large aggregates, which become more available after wetting, but do not necessarily readily leach.

Effects of texture and drainage class

Groffman and Tiedje (1989) studied the effects of texture and drainage class on denitrification rates. Overall they found that finer textured soils have higher rates of denitrification than coarser soils. They found that drainage class alone could not predict denitrification rates well. In a poorly drained sandy soil with low O_2 concentrations, they found insignificant denitrification rates. This soil supported vegetation with low litter quality. The authors concluded that lack of available NO_3^- limited

denitrification and therefore low net N mineralization in these poorly drained sandy soils was probably more important than aeration.

Denitrification is affected by both texture and drainage class and they are interrelated, i.e., fine-textured soils easily become anaerobic at lower water contents than coarse-textured soils because of their improved ability to hold water, inorganic N, mineralize C and therefore, improve conditions for denitrification, whereas a poorly drained coarse-textured soil may never have high oxygen, but still may never denitrify due to other factors.

Spatial and Temporal Variability

Spatial and temporal variability in soils and nutrient parameters are interrelated. Undestanding their relationship and considering its implications is important in designing riparian buffers with optimum nutrient removal capability. Corre et al., (2000) found that nitrogen transformation processes were significantly influenced by both spatial and temporal variability. Spatial variability was controlled by topography and vegetation type which in turn influenced water and nutrient distribution. Temporal variability was controlled mainly by seasonal changes in groundwater movement that provided flushes of organic matter.

There is great variability in measured rates of denitrification within sites, especially in surface soils (CV>100%) (Ambus and Christensen, 1993; Parkin, 1987). This variability seems to be lessened and overall denitrification rates increased during the wetter spring and fall seasons and shortly after rainfall events when soils are saturated (Groffman and Tiedje, 1989). This is probably a result of a complex set of interacting factors including: reduced O_2 diffusion within soils and soil aggregates due to water saturation, increased N mineralization and transport of available C and N to anaerobic microsites, and corresponding stimulation of respiratory O_2 uptake by microbes within the aerobic volume of the soil (Groffman and Tiedje, 1989).

Spatial Variability

Spatial variability in field denitrification rates has been found to be highly dependent on "activity centers in the soil environment" (Christensen et al., 1990). The majority of denitrification was tied to microsites or "hotspots" within the soil matrix, and the denitrification rate found in these sites approximated the potential rate of the entire soil (Parkin, 1987; Christensen et al., 1990). Water plays an important role in reducing spatial variability of denitrification. Christensen et al. (1990) found that when they added enough water to simulate flooding conditions and brought the cores to above field capacity, spatial variability was lessened and denitrification activity was more uniform throughout the soil. This also occurred to a lesser extent after glucose was added to field moist soils and after fall

litter additions (Christensen et al., 1990). Several other studies have corroborated these results (Sextone et al., 1988; Groffman and Tiedje, 1989; Parkin and Robinson, 1989).

Contrary to the above results, Ambus and Christensen (1993) found that in a riparian fen that was flood irrigated with agricultural drainage water there was no change in variability related to seasonal changes or flooding events. They concluded that spatial variability in their site was not controlled by anaerobic patches as in other, drier soils that have been studied, but related to water flow patterns through the microtopography of the fen and resulting high variability was controlled by patchiness in NO_3^- and C substrate availability, or possibly by distribution of denitrifying organisms.

Patchiness or high spatial variability related to hotspots of denitrification does not only occur in surface soils. In a mesocosm study of sandy mostly well-drained (1.55 m deep) and poorly-drained (0.61 m deep) subsoils in New England, denitrification rates were much higher in the poorly drained soils. This higher denitrification rate and the high variability was found to be related to dark patches surrounding decaying roots (Gold et al., 1998). In an additional mesocosm study in the same area (Addy and Gold, 1999), researchers confirmed these results and further found that there was no difference between denitrification rates in mesocosms formed from soil from mowed riparian areas and from forested riparian areas. They discovered that even though all mowed riparian areas were >5 meters from any trees, substantial tree roots were found throughout the subsurface soil in the mowed areas. This illustrates the importance of assigning potential denitrification rates to particular aboveground vegetation types without also considering the effects of nearby vegetation, and further demonstrates the importance of deep rooted species in contributing to overall site denitrification.

Deep rooted species can have other effects on the NO_3^- removal capabilities in a site besides direct C input to subsoils. Lowrance (1992) found that denitrification potential (DEA) was two orders of magnitude lower in the shallow groundwater than in the surface soils of a Coastal Plain riparian forest. He also found that while denitrification potential was much lower in the shallow groundwater, NO_3^- was being removed at a significant rate by other processes. He proposed that tree uptake of NO_3^- from the shallow groundwater was the removal mechanism. This resulted in increased litter quality and improved conditions for surface denitrification. While plant uptake is not a direct NO_3^- removal pathway, higher quality litter inputs to surface soils as a result of this uptake from shallow groundwater may result in stimulation of surface soil denitrification, and overall increase NO_3^- removal for the site.

Spatial variability of denitrification in soils can be related to SOM content, patchiness in substrate availability and due to the influence of nearby vegetation.

Temporal Variability

Considering temporal aspects of denitrification and NO_3^- removal is important because soil N fluxes vary seasonally. In temperate climates, soil inorganic N content and losses are highest in winter when plant uptake does not occur (Martin et al., 1999), and in some temperate systems, denitrification is also highest during winter months (Davidsson and Leonardson, 1998). In other systems, denitrification is limited by temperature during cool wet winters, but does continue at a reduced rate depending on the extent of the temperature limitation (Davidsson and Leonardson, 1998; Teepe et al., 2001).

Spring and fall freeze/thaw cycles and fall litter input may control much seasonal denitrification. Studies of temporal variability in denitrification rates show that denitrification rates are much greater in the spring and fall than during the rest of the year (Christensen S. and Tiedje, 1990; Cates and Keeney, 1987). This corresponds with documented higher DOC concentrations after spring thaw (Clay et al., 1996; DeLuca and Keeney, 1994). Freezing and thawing breaks up soil aggregates and lyses microbial cells making available an easily mineralizable C source for respiratory denitrification (Groffman and Tiedje, 1989). Additionally, soil moisture content is generally higher in many soils during the spring and fall, further improving potential conditions for denitrification. This can have two effects; first, it creates a more homogenous, less variable environment for surface denitrification, and second, it can provide more DOC that can then percolate down to subsoils.

Groffman and Tiedje (1989) found that spring and fall pulses of denitrification are often correlated with vegetative activity. They found that spring denitrification ended at about the time that trees broke bud and that fall denitrification activity began about the time of litter fall. Their explanation for this phenomenon is that vegetation uptake and consequent competition for water and inorganic N decreases denitrification rates substantially in the spring, and that fall litter inputs and initial decomposition provided an available C source.

Winter Denitrification-- In a laboratory experiment undisturbed soil columns were repeatedly frozen and thawed, and N_2O fluxes were measured (Teepe, et al. 2001). This experiment showed that N_2O was not only produced during thawing, but was also during freezing and while the soils were frozen. N_2O was produced even at very low temperatures (-8°C and -16°C). CO_2 fluxes were also measured and indicated that microbial activity continued throughout the frozen periods. In their discussion the authors postulated that part of the soil water was not frozen solid, and in fact, isolated liquid water pockets provided very favorable conditions for denitrification. These pockets of liquid soil water had high available C from recently killed microbial cells, low O_2 from reduced diffusion and rapid microbial uptake, high inorganic nutrients because they are frozen after the water in the ice grid, all which favor denitrification. The N_2O loss seen during freezing could be from soluble N_2O

escaping from cracks formed by frost heaving, and part of the dramatic spring flux of N_2O seen in many systems could be built up N_2O escaping as the ice diffusion barrier melts. This quick pulse of N_2O loss during thaw that is probably stored N_2O from winter denitrification that is different from the stronger spring denitrification pulse. These N_2O pulses are separated by a matter of two weeks during which the soil can warm substantially, and thus increase denitrification rates (Groffman and Tiedje, 1989).

Temporal variability of denitrification in subsurface soils

Several studies have shown that subsoil and shallow aquifers often do not have enough organic carbon to produce continuously anaerobic conditions (Parkin and Meisinger, 1989; McCarty and Bremner, 1992; Obenhuber and Lowrance, 1991), however, there are seasonal fluxes of DOC that stimulate spring and fall pulses of denitrification. Clay et al. (1996) reported that DOC concentrations peaked in the spring and fall in South Dakota, and that nitrate concentration peaks lagged somewhat behind. They concluded that this would be expected if those spring and fall DOC peaks corresponded with high dissolved oxygen (DO) concentrations. This would result in aerobic oxidation of the DOC at first, and then if DOC concentrations were still high enough to keep up with influx of O_2 from percolating water a shift to denitrification would occur. They assumed that if nitrate concentration declines were due solely to denitrification then they could approximate that nitrate loss rate with laboratory methods. This may or may not be a correct assumption to perpetuate based on results from Lowrance (1992) who demonstrated that groundwater N removal was due to vegetative uptake. Clay et al. (1996) did not report if roots were present in their subsoils. Clay et al (1996) commented that their DOC peaks corresponded with freeze-thaw cycles and supposed that "freezing and thawing produces conditions where organic C can be transported from the soil surface to the aquifer." They also reported that DOC concentrations in the aquifer only reached a level that could create anaerobic conditions necessary for denitrification directly following freeze/thaw cycles (~3-~10 μg C/l) (McCarty and Bremner, 1992; Obenhuber and Lowrance, 1991)).

On the contrary, Dosskey and Bertsch (1997) did not see any seasonal changes in DOC concentrations at depth. They supposed however, that this was due to lack of seasonality (and lack of corresponding spring and fall organic C influxes) in their southern coastal plain study sites.

Denitrification Rates

Denitrification rates can vary by a factor of a thousand between surface and subsurface soils. This is related to lower levels of carbon (C) and nitrogen (N) with depth in the soil profile. Changes in carbon content and quality by depth can be due to many factors including: microbial mineralization, different soil textures and associated carbon sorption to mineral soil particles,

inorganic C neutralization of organic acids, as well as the influence of incoming shallow groundwater on the nutrient budget of a site. Surface soils are richer in organic C than subsurface soils and a larger portion of that organic C is available to microbes. Furthermore, deeper in the soil profile organic C is less abundant and the major source of C for denitrification is dissolved organic carbon (DOC) (Dosskey and Bertsch, 1997; Zsolnay and Steindl, 1991). Changes in carbon content and quality could potentially change the denitrification potential of a site.

Methods

Denitrification rate measurements are highly variable depending on the type of system, measurement technique, soils and the time of year sampling takes place. Care must be taken when comparing rates to assure that similar measurement procedures were used in the rate determinations. For example, Sextone et al (1988) measured denitrification rates using three different methods on the same soil types and received very different rates for each procedure. They measured the anaerobic intact core rate (1-3 h of N_2O accumulation in an anaerobic 80% Ar-20% C2H2 environment), the aerobic intact core rate (20% C_2H_2, 18% O_2) and an anaerobic rate from a slurry of soil and distilled water (80% Ar-20% C_2H_2, shaken during incubation). The intact aerobic rate gives a rate theoretically most similar to field conditions since it maintains soil structure and gas exchange capabilities. The anaerobic intact core rate gives a potential field rate, since soil structure is maintained, but the entire core is made anaerobic, removing O_2 limitations. Finally, the anaerobic slurry rate should be a maximum potential rate from the soil since there is no limitation from O_2 and N and C are readily redistributed to microorganisms. There were significant differences between all three methods. Typical rates over 3 h at 40% air-filled porosity were 765 μg N_2O-N kg-1 h-1 in the anaerobic slurry, 85 μg N_2O-N kg-1 h-1 in the anaerobic core and 15μg N_2O-N kg-1 h-1 in the aerobic core. Another study comparing techniques was done using aquifer materials that receive wastewater inputs with similar results (DeSimone and Howes, 1996). The anaerobic slurry method described above is widely done and is described as the denitrification enzyme activity assay (DEA). DEA is a measure of the maximum denitrification potential of a soil, based on the potential of the enzyme present in the soil to reduce NO_3^- to N_2O. Chloramphenicol is generally added to prevent de novo protein synthesis, and acetylene is added to halt denitrification at N_2O rather than N_2 and to block production of N_2O by nitrification that would confound results.

Surface soil rates

Barton et al. (1999) reviewed denitrification rates in agricultural and forest soils and compared reported rates from undisturbed forests, disturbed forests and agricultural systems (See Table 1.1). Denitrification rates in the undisturbed forests were generally less than the disturbed forest soils and

the agricultural soils. Disturbance tended to increase denitrification rates for a short time (months to 4 yrs) because of the increased NO_3^- availability following disturbance. Furthermore in forest soils, denitrification rates changed according to stand age, i.e., recent disturbance>mature forest>aggrading forest following NO_3^- availability to soil denitrifiers (Robertson and Tiedje, 1984) and possibly other controlling factors such as soil moisture, pH, SOC and vegetation cover (Davidson et al., 1990).

Davidson et al. (1990) found that deciduous forest soils generally have higher denitrification rates than coniferous forest soils. Conifer forest litter is generally harder to break down than deciduous forest litter. Deciduous forests also have more regular pulses of organic matter input. Lennon and Pfaff (2003) found that denitrifying microbial communities not only have higher productivity under beech-hemlock-oak derived dissolved organic matter than under pine-maple-birch which was explained by differences in the chemical make-up of DOM, but also that the denitrifier communities are favored under pulses of OM rather than continuous inputs. Furthermore, soil texture and structure can affect what vegetation type is found on a site and have secondary effects on denitrification. Deciduous forests in the northern US often have finer textured soils and higher soil pH than coniferous forests. These factors combined provide a better overall environment for denitrification in deciduous forest soils (Davidson et al., 1990).

Subsurface soil rates

Denitrification rates in subsurface soils are generally much lower than in surface soils (See Table 1.2). Denitrification in most subsurface soils is limited by carbon availability rather than nitrate availability as in unamended surface soils. In addition, surface vegetation or agricultural surface treatments often do not affect increased DOC or denitrification below the rooting zone because DOC is quickly mineralized as it travels downward in the soil profile (McCarty and Bremner 1992; Parkin and Meisinger 1989; Zsolsnay and Steindl 1991). DOC import to rivers has been shown to be related to soil C:N however (Aitkenhead and McDowell, 2000). Therefore in soils with appropriate conditions, DOC may be leached from surface soils. Denitrification rates in subsoils may potentially be affected by direct C inputs of deep rooted species by either DOC following preferential flow paths (Zsolsnay and Steindl 1991) or by rhizodeposition (Lynch and Whipps 1990).

Table 1.1. Summary of annual denitrification rates (aerobic intact cores) in forest and agricultural soils. Adapted from Barton, et al. 1999 individual references included in original table.

System	Observations	Geometric mean (kg N/ha/yr)	Range (kg N/ha/yr)
Forest	25	2.2	0.1-40
Undisturbed coniferous	8	0.3	0.1-2.4
Disturbed coniferous	6	2.4	0.1-40
Undisturbed deciduous	7	3.0	0.3-28
Disturbed deciduous	1	5.4	1.4-5.4
Wastewater irrigated	2	6.6	2.4-16
Agricultural	70	13	0-239
Unfertilized, not irrigated	14	3.2	0-17.4
N-fertilized, not irrigated	49	13.4	0.5-110
N-fertilized, irrigated	7	113	49-239

Table 1.2. Selected research examining denitrification in riparian soils (Martin et al., 1999) Adapted and added to.

Reference	Location of study	Depth of soil samples	Tests performed	Results and conclusions
Richards and Webster (1998)	Harpenden, Herts, UK	(i) 0-23 cm (ii) 23-40 cm (iii) 20 cm increments thereafter to 2 m	DEA and DEA with amendments, TOC and DOC	DEA declined with depth, no relationship between surface practices and DOC at depth, denitrification is C limited
Schnabel et al. (1997)	Pennsylvania, USA	(i) 7.5-15 cm, (ii) 37.5-45 cm, (iii) 60-67.5 cm, (iv) 87.5-95 cm	Denitrification rate	Rates were greatest nearest the stream and in the surface samples
Pavel et al. (1996)	Virginia, USA	Ponded surface horizon (1-15 cm); terrestrial surface horizon (1-15 cm); terrestrial subsurface horizon (24-45 cm)	Denitrification rate	Rates were greatest in ponded surface horizon and minimal in the terrestrial subsurface
Hanson et al. (1994)	Rhode Island, USA	1-15 cm from enriched and control sites	Denitrification rate, amendment denitrification rates	Rates higher in enriched site than in control site
Ambus et al. (1993)	Copenhagen, Denmark	(i) surface 5 cm (ii) top 5 cm of saturated soil layer, to a max depth of 20 cm	DEA	DEA decreased markedly with depth
Starr and Gillham (1993)	Ontario, Canada	At water table depth in two watersheds (i) 1 m (ii) 4 m	DEA, DOC	DEA decreased with depth. DOC was much lower in deeper system. Denitrification limited by available C.
Bradley et al. (1992)	Florida, USA	In the aquifer at (i) 1-2 m (ii) 3-4 m	Denitrification rates, total SOC	Denitrification and total SOC highly correlated, C-limited
Groffman et al. (1992)	Rhode Island, USA	(i) 0-15 cm (ii) top of high water table, (iii) 0.5 cm below water table	DEA microbial biomass	DEA decreased markedly with depth No detectable microbial biomass at depth
Lowrance (1992)	Georgia Coastal Plain, USA	(i)0-6 cm (ii)7-12 cm (iii) 13-18 cm (iv) 19-24 cm	Denitrification rate	Rate was positively related to depth. 19-24 cm depth higher than surface.
Ambus and Lowrance (1991)	Georgia Coastal Plain, USA	(i) 0-10 cm (ii) top 10 cm of aquifer at disturbed site	DEA	DEA higher in 0-10 cm layer than top of aquifer. DEA stratified within the top 10 cm
Parkin (1987)	Maryland, USA	1-16 cm	Denitrification rate DEA	Most activity associated within the top 5 cm within the soil core. Obvious hotspots of denitrification associated with organic matter.

Dissolved Organic Carbon and Bioavailable Dissolved Organic Carbon

Soil Organic Carbon Quality derived from different vegetation sources

The possibility exists that differences in SOC quantity and quality derived from different vegetation types can result in different denitrification potentials under the varying vegetation types. There is so far insufficient evidence to quantify differences but some studies have shown that they exist and that total SOC is additionally influenced by age of vegetation.

Burford and Bremner (1975) discussed results that showed that the effect of different types of organic additions to soil on denitrification rates varied according to the decomposability of those additions. More easily decomposable organic materials had a larger, positive effect on denitrification. They measured denitrification capacities and total, water-soluble and readily decomposable organic matter in 17 different soil types and explored the relationship between denitrification rates and available C. They found that TOC and denitrification were significantly correlated ($r=0.77$) and water-soluble OC was very highly correlated ($r=0.99$) with denitrification. They concluded that measurements of water-soluble OC could be a good predictor of the capacity of a soil to denitrify. In addition, when C inputs to a site are more readily broken down, denitrification rates are increased.

Denitrification rates and CO_2 production rates in organic riparian soils with additions of fresh and senescent pine needles and watercress leaves were measured in order to investigate the importance of C lability in soil microbial processes (Schipper et al., 1994). They found that when the same amount of total C was added to all treatments, denitrification and CO_2 production was 5 times higher in the watercress and fresh pine needle treatments as opposed to the senescent pine needle treatments. This suggests that the quality or lability of the OC added to the soil was more important in controlling soil microbial processes than simply the quantity.

DOC concentrations in litter leachate were found to differ depending on the initial chemistry of the litter. Litter with low lignin and high extractatives was found to provide the highest DOC concentrations in leachate. Samples treated with additional N also had higher DOC concentrations in leachate (Magill and Aber, 1999). This suggests that different vegetation types can provide varying levels of DOC, and that DOC from different vegetation types will have different chemistries and availability.

C:N ratio is considered a good indicator of litter quality, and litter quality affects soil C:N ratios. Soil C:N ratios in different biomes with vegetation types ranging from forests (tropical, temperate coniferous, deciduous, and mixed) to peatlands and grasslands, have been successfully used to predict annual DOC flux in rivers (Aitkenhead and McDowell 2000). In fact, 99.2% of variance in riverine

DOC flux was explained by the soil C:N. This concept is important when considering that DOC export from surface soils to subsurface soils may be controlled by litter quality of surface vegetation.

Vegetation type and resulting differences in litter quality as well as the length of time vegetion has been established can potentially affect denitrification rates. In a study conducted in riparian buffer systems in the north-eastern US, researchers evaluated water-extractable organic carbon (WEOC), bioavailable DOC (portion taken up by microbes from WEOC after 30 day incubation) and total SOC under forests, C3 and C4 grasses. Though they could not determine differences in WEOC, BDOC or total SOC between vegetation types when they pooled data from all sampling locations. They believed that their data was confounded by the age of the riparian buffer systems. Analysis of individual sampling locations showed that total SOC is less under C4 grass than under C3 grass or under forest vegetation shortly after the establishment of the C4 grass plots. However, by the time that the plots had been established for 16 to 18 years the total SOC was approximately the same as in other vegetation types. They determined that conversion from forest or C3 grass to C4 grass resulted in an early loss of SOC, but that it eventually caught up to levels similar to forest and C3 grasses (Corre, Schnabel, and Shaffer, 1999).

Marquez et al (2001) examined C dynamics in riparian buffers in central Iowa. They found that vegetation type affected aggregate size and stability, with larger, more unstable aggregates associated with C4 grasses and crop fields, and smaller, more stable aggregates associated with C3 grasses and riparian forests. She found that C4 grass plots after 7 years did not have more aggregates or more available C than the crop fields. Additionally she found that soils under C3 grasses and forests are initially higher in available soil C. This is important when we consider that aggregate size and stability affect denitrification rates and would suggest that C3 grasses provide a better environment for denitrification to occur in surface soils.

DOC fractions and sorption dynamics

DOC concentrations vary by depth in soil. Higher concentrations are found in surface soils while subsoils generally contain much lower concentrations (about 1/5th of surface soils) (Dosskey and Bertsch, 1997). Some of this decrease in concentration can be explained by microbial uptake as soil water percolates downward. Microbial uptake also leaves a remaining DOC fraction which is less suitable for microbial respiration (Zsolnay and Steindl, 1991). DOC is also adsorbed to mineral soil and may be removed from the soil water in this manner. Therefore, the amount of DOC that is bioavailable (%BDOC) might be a more important controlling factor of denitrification than total DOC especially at depth.

Jandl and Sollins (1997) characterized water-extractable soil carbon from below a forest floor. They separated the DOC into hydrophobic and hydrophilic acid groups and hydrophilic neutral groups. Jandl and Sollins (1997) assumed that the hydrophobic acids (HoAs) were plant litter derived and more recalcitrant (ligno-cellulose and complex aliphatic and aromatic acids) than: hydrophilic acids (HiAs) which were microbially derived and more labile (simpler aliphatic acids, polysaccharides, and more carboxyls per C atom); and more recalcitrant than hydrophilic neutral groups (HiNs) which were even more labile byproducts from both plant and microbial breakdown (simple sugars, carbohydrates, alcohols and ketones). From incubation experiments they were able to determine that HoAs were indeed the most recalcitrant followed by HiAs and HiNs. They also corroborated results by Zsolnay and Steindl (1991) that showed that the labile portion of DOC is rapidly (<2-3 d) taken up by microbial activity, leaving behind a more recalcitrant total DOC. For all of their samples, the largest initial fraction of DOC was HoAs, although the actual percentage of the total DOC depended upon the substrate from which it was leached. Litter leachate was dominated by HoAs and HiNs which generally came from microbial sources (Jandl and Sollins, 1997). In their concluding discussion, the authors reviewed the current understanding of the relationship between DOC and its role as an energy source for soil respiration. They concluded that although it is difficult to determine the exact nature of the relationship because of measurement difficulties, the positive relationship between available, labile or soluble DOC and respiration does seem to hold out and merits further investigation.

DOC Sorption

In addition to microbial uptake which can change the composition and availability of DOC in soil water, preferential adsorption to soil particles can also change the relative availability of DOC to microbes. Several studies have shown (Dunnivant et al., 1992; Mclaughlin et al., 1994; Jardine et al., 1989) that hydrophobic molecules in DOC are preferentially adsorbed to aquifer and subsoil sediments. This may or may not result in total DOC that is more labile – depending on microbial activity as above, and initial DOC concentrations. DOC sorption studies have shown that the ability of soil particles to adsorb DOC decreases with increasing DOC concentration and that sorption potential increases with depth (because concentrations generally decrease with depth) (Dosskey and Bertsch, 1997; Mclaughlin et al., 1994).

Soil texture may also play a role in DOC retention through sorption. Dosskey and Bertsch (1997) reported that in many forest soils DOM is retained through podzolization in finely textured Spodosols and through sorption to soil clays in medium textured Ultisols and some Inceptisols. Based on these results, one might suppose that sandy soils would not limit DOC transport as much as in finer-

textured soils, because fine-textured soils have greater surface area and therefore greater sorption potential. However, contrary to this, DOC transport was not greater through sandy soils in the Coastal Plain. Dosskey and Bertsch (1997) found that DOC was strongly sorbed to sandy E horizons, and that external transport of DOC was also limited. This low DOC export may be a result of effective microbial immobilization of DOC, or a result of preferential sorption to Fe and Al oxide coatings found on sand particles in this study. DOC has been shown to be retained by Fe and Al oxides in other systems (Mclaughlin et al., 1994). More research needs to be conducted in this area.

Summary and Discussion

Riparian soils are uniquely positioned in the landscape to capture non-point source pollutants such as NO_3^- and remove them through biological processes such as denitrification (Hill, 1996; Gilliam, 1994). Understanding the controlling factors of denitrification is essential if riparian buffers are to be managed to maximize the soil denitrification potential. Denitrification in soils is primarily controlled by O_2 concentrations, NO_3^- supply rates and organic C availability. These controls are in turn controlled by distal factors such as precipitation, soil texture and aggregation, OM, aerobic respiration, plants, and community structure (Groffman et al., 1988). Each of these distal factors is highly interrelated and hard to study in isolation. C availability plays a large role in controlling the other factors, e.g. O_2 concentrations in soil matrix lowered as a result of aerobic respiration (Tiedje, 1988).

Very high variability (CV>100%) in denitrification rates is common in surface soils and much of this variation can be tied to favorable anaerobic microsites within the soil matrix (Parkin, 1987). Soil texture, variable water content and biological factors such as microbial distribution also affect high variability. In many studies this effect seems to be lessened in spring and fall and following high precipitation events as increased soil water lowers O_2 diffusion, transports available C and N and creates a more homogenous, more favorable environment for denitrification (Parkin, 1987; Sextone et al, 1988; Groffman and Tiedje, 1989; Parkin and Robinson, 1989). In other studies from places without strong seasonal changes, this was not a noticeable occurrence (Dosskey and Bertsch, 1997). We can draw the conclusion that increasing soil water will increase denitrification rates and reduce spatial variability because it serves as a homogenizing mechanism through substrate transport and lower soil O_2 concentrations.

Soil aggregates are very important in denitrification processes. They provide anaerobic microsites in surface soils; although due to low denitrifer populations or substrate limitations they may not support denitrification (Sextone, et al., 1988). Soil aggregates are held together by polysaccharides that are generally unavailable to microbes because of physical size limitations

(Foster, 1981). However, polysaccharides are easily taken up as energy sources for microbes when aggregates are broken up by freeze/thaw cycles or tillage. Newly available C is then transported by soil water to anaerobic sites where denitrification can occur. Denitrification also is correlated with aggregate size and stability. Smaller aggregates have higher denitrification rates than larger aggregates (Beauchamp and Seech, 1990; Seech and Beauchamp, 1988) and aggregate size and stability changes seasonally and with different vegetation types (Marquez, 2001).

Soil texture and drainage class are important factors in controlling denitrification by controlling anaerobisis. However, neither texture nor drainage class is an ideal predictor of the denitrification capacity of a soil. More important are interrelated factors such as the ability of a fine-textured soil to retain substrate and water essential for denitrification (Groffman and Tiedje, 1989).

Denitrification in surface and subsurface soils is controlled by organic carbon. However, denitrification is subject to slightly different processes in surface soils as compared to subsurface soils. In surface soils, most denitrification is associated with decomposing vegetation, particulate organic matter and aggregates. In addition, the main C-limitation is not as a direct denitrification substrate, but rather because of its ability to create anaerobic microsites. In deep soils denitrification generally is controlled by DOC (Bradley et al., 1992), rather than OC that is adsorbed to soil particles. Adsorbed OC is generally more recalcitrant than DOC because of preferential adsorption (Dosskey and Bertsch, 1997) and is not physically available to microbial populations. Therefore, adsorbed OC does not generally affect denitrification rates. If deep roots exist in the subsoil, then plant associated C in the rhizosphere could potentially provide available C for denitrification (Gold et al., 1998; Addy et al., 1999). DOC is limiting in subsurface soils because of its ability to create anaerobic conditions by serving as an O_2 sink, and is also limiting as a substrate. DOC can be particularly limiting in sites with continual influxes of DO. If DOC inputs cannot keep pace with DO inputs, denitrification will be limited because anaerobic conditions will never occur.

OM quality, as measured by the amount of total OM that is available for microbial respiration, not just quantity may be an important control of denitrification. The ability of vegetation to provide quality DOC to subsurface aquifer materials can be very important in maximizing the denitrification of a particular site. Surface management, i.e. fertilization, straw and manure additions has been found to have no effect on subsoil DOC (Parkin and Meisinger, 1989; Richards and Webster, 1999), therefore other methods of introducing DOC deep within the soil profile must be considered. Deep rooted species such as alfalfa, switchgrass and trees have been shown to take up NO_3^- from shallow groundwater (Lowrance, 1992; Huang et al., 1996). Deep rooted species may vary in their abilities to take up NO_3^- from shallow ground water and in the quality of C derived from their litter and roots.

Different species also promote different soil aggregate structures that may affect denitrification (Marquez, 2000).

Depth of water table has a large effect as well. If DOC has to travel through a large unsaturated vadose zone, it will be mostly oxidized before it reaches the aquifer. Substantial NO_3^- could then just keep moving through, unaffected by the buffer. Water table levels vary seasonally, which in many areas does not correspond with plant growth. Therefore if water table levels are only high during winter and early spring before vegetation becomes active (Bormann and Likens 1979), direct NO_3^- removal through plant uptake may not occur. However, during this time, water table level movement redistributes accumulated substrate for denitrification and may create anaerobic conditions which promote denitrification as a removal mechanism.

References

Addy, K.L., A.J. Gold, P.M. Groffman, and P.A. Jacinthe. 1999. Groundwater nitrate removal in subsoil of forested and mowed riparian buffer zones. Journal of Environmental Quality 28:962-970.

Ambus, P. and S. Christensen. 1993. Denitrification variability and control in a riparian fen irrigated with agricultural drainage water. Soil Biology and Biochemistry 25:915-923.

Ashby, J.A., W.B. Bowden, and P.S. Murdoch. 1998. Controls on denitrification in riparian soils in headwater catchments of a hardwood forest in the Catskill mountains, U.S.A. Soil Biology and Biochemistry 30:853-864.

Aitkenhead, J.A., W.H. McDowell. 2000. Soil C:N ratio as a predictor of annual riverine DOC fluxes at local and global scales. Global Biogeochemical Cycles. 14(1):127-138.

Beauchamp, E.G. and A.G. Seech. 1990. Denitrification with different sizes of soil aggregates obtained from dry-sieving and from sieving with water. Biology and Fertility of Soils 10:188-193.

Bradley, P.M., M. Fernandez Jr., and F.H. Chapelle. 1992. Carbon limitation of denitrification rates in an anaerobic groundwater system. Environmental Science and Technology 26:2377-2391.

Cates, R.L.Jr. and D.R. Keeney. 1987. Nitrous oxide production throughout the year from fertilized and manured maize fields. Journal of Environmental Quality 16:443-447.

Christensen S. and J.M. Tiedje. 1990. Brief and vigorous N_20 production by soil at spring thaw. Journal of Soil Science 41:1-4.

Christensen, S., S. Simkins, and J.M. Tiedje. 1990. Spatial variation in denitrification: Dependency of activity centers on the soil environment. Soil Science Society of America Journal

Clay, D.E., S.A. Clay, T.B. Moorman, K. Brix-Davis, K.A. Scholes, and A.R. Bender. 1996. Temporal variability of organic C and nitrate in a shallow aquifer. Water Resources 30:559-568.

Corre, M.D., R.R. Schnabel, and J.A. Shaffer. 1999. Evaluation of soil organic carbon under forests, cool-season and warm-season grasses in the northeastern US. Soil Biology and Biochemistry 31:1531-1539.

Corre, M.D., R.R. Schnabel, and W.L. Stout. 2002. Spatial and seasonal variation of gross nitrogen transformations and microbial biomass in a Northeastern US grassland. Soil Biology and Biochemistry 34(4):445-457.

Davidson, E.A., D.D. Myrold, and P.M. Groffman. 1990. Denitrification in temperate forest ecosystems. Pages 196-220. in Gessel, S. P., Lacate, D. S., Weetman, G. F., and Powers, R. F., editors. Proceedings of the 7th North America forest soils conference. University of British Columbia, Faculty of Forestry Publications, Vancouver, British Columbia.

Davidsson, T.E. and L. Leonardson. 1998. Seasonal dynamics of denitrification activity in two water meadows. Hydrobiologia 364:189-198.

DeLaune, R.D., R.R. Boar, C.W. Lindau, and B.A. Kleiss. 1996. Denitrification in bottomland hardwood wetland soils of the Cache River. Wetlands 16:309-320.

DeLuca, T.H. and D.R. Keeney. 1994. Availability of organic carbon for denitrification of nitrate in subsoils. Soil Science Society of America Journal 58:835-840.

DeSimone, L.A. and B.L. Howes. 1996. Denitrification and nitrogen transport in a coastal aquifer recieving wastewater discharge . Environmental Science and Technology 30:1152-1162.

Dosskey, M.G. and P.M. Bertsch. 1997. Transport of dissolved organic matter through a sandy forest soil. Soil Science Society of America Journal 61:920-927.

Dunnivant, F.M., P.M. Jardine, D.L. Taylor, and J.F. McCarthy. 1992. Transport of naturally occurring dissolved organic carbon in laboratory columns containing aquifer material. Soil Science Society of America Journal 56: 437-444.

Fierer, N., J.P. Schimel. 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal. 67(3):798-805.

Foster, R.C.. 1981. Polysaccharides in soil fabrics. Science 214 :665-667.

Gilliam, J.W.. 1994. Riparian wetlands and water quality. Journal of Environmental Quality 23:896-900.

Gold, A.J., P.A. Jacinthe, P.M. Groffman, W.R. Wright, and R.H. Puffer. 1998. Patchiness in groundwater nitrate removal in a riparian forest. Journal of Environmental Quality 27:146-155.

Gold, A. J., P.M. Groffman, K. Addy, D.Q. Kellogg, M. Stolt, A.E. Rosenblatt, 2001. Landscape Attributes as Controls on Ground Water Nitrate Removal Capacity of Riparian Zones. Journal of the American Water Resources Association. 37(6): 1457-1464.

Groffman, P.M., Tiedje, J.M., Robertson, G.P., and Christensen S. 1988. Denitrification at different temporal and geographical scales: Proximal and distal controls. Pages 174-192. in J.R. Wilson editor., CAB International, Wallingford.

Groffman, P.M. and J.M. Tiedje. 1989. Denitrification in north temperate forest soils: Spatial and temporal patterns at the landscape and seasonal scales. Soil Biology and Biochemistry 21:613-620.

Groffman, P.M. and J.M. Tiedje. 1991. Short Communication: Relationship between denitrification, $C O_2$ production and air-filled porosity in soils of different texture and drainage. Soil Biology and Biochemistry 23:299-302.

Hill, A.R.. 1996. Nitrate removal in stream riparian zones. Journal of Environmental Quality 25:743-755.

Huang, Y., D.H. Rickerl, and K.D. Kephart. 1996. Recovery of deep-point injected soil Nitrogen-15 by switchgrass, alfalfa, ineffective alfalfa, and corn. Journal of Environmental Quality 25:1394-1400.

Jandl, R. and P. Sollins. 1997. Water-extractable soil carbon in relation to the belowground carbon cycle. Biology and Fertility of Soils 25:196-201.

Jardine, P.M., N.L. Weber, and J.F. McCarthy. 1989. Mechanisms of dissolved organic carbon adsorption on soil. Soil Science Society of America Journal 53:1378-1385.

Lennon J.T., Pfaff, L.E. Terrestrial subsidies in lake ecosystems: Importance of detrital souce and supply on microbial metabolism. Poster presentation in: Cary Conference 2003: Ecosystem Function in Heterogeneous Landscapes. April 29-May 1. 2003. Institute of Ecosystem Studies. Millbrook, NY. USA.

Lowrance, R.. 1992. Groundwater nitrate and denitrification in a coastal plain riparian forest. Journal of Environmental Quality 21:401-405.

Lowrance, R. R.G. Williams, S.R. Inamdar, D.D. Bosch, J\.M Sheridan, 2001. Evaluation of Coastal Plain Conservation Buffers Using the Riparian Ecosystem Management Model. Journal of the American Water Resources Association. 37(6): 1445-1455

Luo, J., R.W. Tillmann, and P.R. Ball. 1999. Factors regulating denitrification in a soil under pasture. Soil Biology and Biochemistry 31:913-927.

Magill, A.H. and J.D. Aber. 1999. Dissolved organic carbon and nitrogen relationships in forest litter as affected by nitrogen deposition. Soil Biology and Biochemistry 32:603-613.

Marquez, C.O..2001. Soil aggregate dynamics and aggregate-associated carbon under different vegetation types in riparian soils. Ph.D. Dissertation. Iowa State University. Ames, IA.

Martin, T.L., N.K. Kaushik, J.T. Trevors, and H.R. Whiteley. 1999. Review: Denitrification in temperate climate riparian zones. Water, air and soil pollution 111:171-186.

McCarty, G.W. and J.M. Bremner. 1992. Availability of organic carbon for denitrification of nitrate in subsoils. Biology and Fertility of Soils. 14:219-222.

Mclaughlin, J.W., J.C. Lewin, D.D. Reed, C.C. Trettin, M.F. Jurgensen, and M.R. Gale. 1994. Soil factors related to dissolved organic carbon concentrations in a black spruce swamp, Michigan. Soil Science 158:454-464.

Merrill, A.G. and D.R. Zak. 1992. Factors controlling denitrification rates in upland and swamp forests. Canadian Journal of Forest Resources. 22:1597-1604.

Naiman R.J. and Decamps H. 1997. The ecology of interfaces: riparian zones. Annual Reviews of Ecology and Systematics 28:621-658.

Obenhuber, D.C. and R. Lowrance. 1991. Reduction of nitrate in aquifer microcosms by carbon additions. Journal of Environmental Quality. 20:

Parkin, T.B. and Meisinger J. J. 1989. Denitrification below the crop rooting zone as influenced by surface tillage. Journal of Environmental Quality. 18:12-16.

Parkin, T.B.. 1987. Soil microsites as a source of denitrification variability. Soil Science Society of America Journal 51:1194-1199.

Parkin, T.B. and J.A. Robinson. 1989. Stochastic models of soil denitrification. Applied and environmental microbiology 55:72-77.

Richards, J.E. and C.P. Webster. 1999. Denitrification in the subsoil of the Broadbalk Continuous Wheat Experiment. Soil Biology and Biochemistry 31:747-755.

Robertson, G.P. and J.M. Tiedje. 1984. Denitrification and nitrous oxide production in sucessional and old-growth Michigan forests. Soil Science Society of America Journal 48:383-389.

Schipper, L.A., C.G. Harfoot, P.N. McFarlane, and A.B. Cooper. 1994. Anaerobic decomposition and denitrification during plant decomposition in an organic soil. Journal of Environmental Quality 23:923-928.

Seech, A.G. and E.G. Beauchamp. 1988. Denitrification in soil aggregates of different sizes. Soil Science Society of America Journal 52:1616-1621.

Sextone, A.J., T.B. Parkin, and J.M. Tiedje. 1988. Short communication: Denitrification response to soil wetting in aggregated and unaggregated soil. Soil Biology and Biochemistry 20:767-769.

Sextone, A.J., N.P. Revsbech, T.B. Parkin, and J.M. Tiedje. 1985. Direct measurement of oxygen profiles and denitrification rates in soil aggregates. Soil Science Society of America Journal 49:645-651.

Six, J. E.T. Elliot, K. Paustian, and J.W. Doran. 1998. Soil Science Society of America Journal. 62:1367-1377.

Starr, R.C. and R.W. Gillham. 1993. Denitrification and organic carbon availability in two aquifers. Ground Water. 31(6):934-947.

Swanson F.J. S.V Gregory, J.R. Sedell and A.G. Campbell. 1982. Land-water interactions: The riparian zone. pp. 267-289, IN: Analysis of Coniferous Forest Ecosystems in the Western United States. R.L. Edmonds (ed.). Hutchison Ross Publishing Company, Pennsylvania, USA.

Teepe, R., R. Brumme, and F. Beese. 2001. Nitrous oxide emissions from soil during freezing and thawing periods. Soil Biology and Biochemistry 33:1269-1275.

Tiedje, J.M. 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium. Pages 179-244. in A. J. B. Zehnder editor., Biology of anaerobic organisms. John & Sons, New York..

Yeomans, J.C., J.M. Bremner, and G.W. McCarty. 1992. Commun. Soil Sci. Plant Anal., 23(9&10):919-927.

Zsolnay, A. and H. Steindl. 1991. Geovariability and biodegradability of the water-extractable organic material in an agricultural soil. Soil Biology and Biochemistry 23:1077-1082.

CHAPTER 2. DENITRIFICATION POTENTIAL IN SURFACE AND SUBSURFACE SOILS IN THE BEAR CREEK RIPARIAN BUFFER SYSTEM

A paper to be submitted to Journal of Environmental Quality

Amber Denton Johnson, Thomas M. Isenhart, Timothy B. Parkin, Richard C. Schultz

Abstract

Riparian buffers have been shown to be effective at improving surface water quality. Vegetative uptake and denitrification are interrelated nitrogen removal services of riparian buffers. While surface denitrification rates are much higher than subsurface rates, subsurface denitrification is also essential to overall nutrient removal services. Carbon and nitrogen supply can vary under different vegetation types in surface and subsurface soils. This study was conducted to determine if denitrification potential and its controlling factors were different under warm season grasses and introduced cool season grasses at different depths. Denitrification potential measured by the Denitrifier Enzyme Activity Assay (DEA), Total Organic Carbon (TOC), Total Nitrogen (TN), and Inorganic Nitrogen (IN) were measured during three sampling periods (Summer 2001, Fall 2001, and June 2002) at five depths up to the non-permeable aquitard (3-4 m). Sampling was done following soil morphological features, from the surface, throughout the rest of the vadose zone, at the border with fluctuating groundwater conditions evidenced by mottling, throughout the mottled zone, and at the border with impermeable till. Mean DEA, TOC and TN were generally higher in cool season grasses than warm season grasses. Vadose zone samples were significantly different from other subsurface depths when the respective vegetation type was actively growing, but were not significantly different from other subsurface depths when grasses were not active. Deep in the soil profile TOC and TN were higher in warm season grass than cool season grasses, although not significantly. The results suggest that different plant communities affect denitrification potential in the surface and vadose zones, and imply that a diverse mixture of plants should be used in buffer establishment to maximize year-long denitrification potential.

Introduction

Riparian buffers have been identified as an integral part of a suite of land management practices that potentially reduce non-point source pollution of surface and ground waters and eutrophication of coastal waters (Hill, 1996). Denitrification and immobilization are important N-removal processes in riparian buffers. Denitrification is the anaerobic, microbially mediated process whereby NO_3^- is converted to N gases (N_2, N_2O, NO) and returned to the atmosphere. Oxygen, organic carbon and nitrate control denitrification (Tiedje, 1982). Vegetation type can directly affect site denitrification potential by C-input quality and rooting depths. For example, it has been shown that different vegetation types affect C-related processes such as aggregation (Marquez 2001), soil respiration and below ground biomass production (Tufekcioglu et al., 1999, 2003), decomposition of organic matter and dissolved organic matter lability (Schipper et al., 1994), microbial biomass (Haake 2003; Pickle 1999), as well as direct C-inputs through rhizodeposition (Lynch and Whipps, 1990). Increasing C and improving soil structure provides better conditions for denitrification.

Riparian zone denitrification has been shown to be highly variable both vertically and horizontally, and is affected by site hydrogeology, plant impacts on soil properties and by land-use history (Corre et al., 1999, 2002; Hill, 1996). Groffman et al. (1992) and other studies (Hanson et al., 1994; Lowrance, 1992; Peterjohn and Correl, 1984) make an important linkage between surface and subsurface denitrification and plant uptake. These studies generally found that surface soil denitrification was related to plant uptake of groundwater nitrate during the growing season; that denitrification rarely took place in groundwater during the growing season; and that DEA and microbial biomass were low or undetectable below the seasonal water table. Groffman et al. (1992) found that during the dormant season, when groundwater levels rose to the surface, denitrification and microbial immobilization occurred. Hanson et al., (1994) found that surface soil denitrification, as well as soil and groundwater nitrate levels, were higher in a site with nitrater enriched groundwater than in a control site, and suggested that this was due to plant uptake from groundwater and subsequent surface enrichment. Peterjohn and Correl (1984) and Lowrance (1992) found that below the seasonal high water table, plant uptake accounted for the majority of subsurface N-removal. Additionally, Lowrance (1992) found that at depth there was very little DEA, and that activity did increase with C and N additions because of lack of available enzyme.

Some studies which have indicated that plant uptake of nitrate from groundwater occurred have been criticized for relying on mass balance approaches to nitrate removal that do not take into account other, unmeasured N sources (Hill, 1996). It is also possible that root growth and direct plant uptake from groundwater could be suppressed due to lack of available oxygen. However, many studies have shown that lack of available C in shallow groundwater prevents anaerobic conditions from forming

(McCarty and Bremner, 1992; Parkin and Meisinger, 1989; Starr and Gillham, 1993), or may only reach high enough levels following fall litter inputs and freezing and thawing (Clay et al., 1996). Therefore, saturation may not equal anaerobicity. However, there were not roots present in the shallow groundwater of these studies. In addition, deeply rooted plants even in unsaturated soils may need to provide extra oxygen to the rooting zone in order to facilitate root growth and nutrient uptake (Van Noordwijk and Brouwer, 1988). Finally, a variety of upland plants have been shown to respond to lack of oxygen by producing aerenchyma (Aschismiti et al., 2003; Baruch, 1994; Baruch and Merida, 1995; McDonald et al., 2002; Shimamura et al., 2003; Subbaiah Chalivendra and Sachs Martin, 2003). It is reasonable to assume that the grass species planted at the Bear Creek National Demonstration and Research Watershed, which were selected for reasonable tolerance to flooded conditions, would be able to adapt to seasonally flooded conditions, particularly if the greatest depth of flooding did not occur during the growing season. Plant uptake of nitrate may be important in the overall loss of nitrate from a system by removing it from groundwater systems where denitrification is limited and stimulating surface denitrification through enriched litter inputs. In addition, plant and microbial immobilization of nitrate during the growing season may prevent its loss to waterways and conserve it in the system until better conditions for denitrification occur in the fall and spring (Fennesy and Cronk, 1997). However, few studies have been done which directly measure plant uptake of nitrate from groundwater (Huang et al., 1996) and this area needs further attention.

Results from the study reported here, among others, have shown that subsurface or groundwater denitrification does occur, albeit to a lesser degree than surface soils, and other studies have shown that it is probably the greatest N removal process below the rooting zone (Addy et al., 1999; Castle et al., 1998; Gold et al., 1998; Hill, 2000; Schipper et al., 1993). Localized subsurface denitrification can be very high where conditions are right. Subsurface variability is controlled by vegetation type and organic matter depositions. For example, buried A-horizons are common in alluviual soils and may provide carbon for microbial processes deep in the soil profile and explain some of the high spatial variability of denitrification (Gurwick et al., 2003). Patches of OM in subsoils associated with vegetation have much higher denitrification potential than the soil matrix without OM patches (Addy et al., 1999; Gold et al., 1998). Additionally, Hill et al. (2000) found that denitrification in groundwater occurred primarily in areas where flow paths intersected areas with high organic matter. The potential for high denitrification has not been realized in many subsoils. If organic C and other substrate increase through increased root biomass, the ability of the soil to remove N will be improved (Fennesy and Cronk, 1997). Conditions that promote subsurface denitrification combined with plant uptake and subsequent stimulation of surface denitrification are ideal for promoting on-site N-removal.

This research was conducted to examine the functional effects of vegetation on surface and subsurface soil denitrification in a series of multi-species riparian buffers re-established on previously cropped soils. We hypothesized that surface soils would have higher denitrification potentials than subsurface soils and that vegetation type and possibly the age of the riparian buffer would impact potential denitrification. We compared DEA, TOC, TN, and other soil parameters at 5 different soil depths (up to 3-4 m) under planted riparian vegetation. While DEA does not reflect instantaneous denitrification rates, it provides a longer term picture of the denitrification history of a soil since denitrifying conditions (e.g. anaerobicity, and available C and N) spur the microbial production of denitrifying enzyme. DEA can be used to compare the potential of a soil to denitrify across sites and depths.

Materials And Methods

Site description and soil sampling

The study area consisted of one warm season grass plot in each of five riparian buffers that ranged in age from 0 to 12 years since establishment, and five long-established introduced cool season grass plots in two riparian buffers. Plots were all located on the same soil mapping unit in the Bear Creek Watershed of Hamilton and Story counties in central Iowa (Coland, fine-loamy, mixed, mesic Cumulic Endoaquoll) and had similar topography (DeWitt, 1984). At each of three sampling times (Summer 2001, Fall 2001, June 2002) five cores were taken in each plot. Cores were randomly located using GIS, and sampling locations were flagged and returned to each season. Soil cores for the Summer 2001 and Fall 2001 sampling were extracted using a tractor mounted Giddings hydraulic soil sampler to the depth of the consolidated glacial till parent material (generally 3-4 m) using a 120 cm metal soil tube, 6 cm in diameter which was fitted with a carbide toothed bit (Giddings Company, Ft. Collins, CO).

Five samples were taken from each core based upon soil morphological features. An 8 cm long sample was taken from each end, surface and bottom. Next, the area with redoximorphic features such as mottling was identified. Where mottles first appeared, an 8 cm long sample was taken from the core and referred to as the top of the mottling sample. Composited subsamples were taken from throughout the rest of the vadose zone, i.e. between the surface sample and the top of the mottling, to approximately the same sample mass and volume as the surface, top mottling and bottom samples. The final sample was taken from throughout the entire mottled section, between the top mottling and bottom samples. surface, vadose and top mottling samples were generally in the A, B and BC horizons, respectively. Mottled and bottom samples were taken from the C horizon which was a mostly oxidized till (diamicton) with interbedded sand. Samples were mixed in a bag, and a

subsample was taken for in-field inorganic N extraction (Van Miegroet 1995), placed on ice, transported to the laboratory and stored at 4° C until analysis.

Sampling in Summer 2001 took place over a seven week period in June and July, with warm season grass plots sampled first and cool season grass plots sampled later. In Fall 2001, sampling again took place over an extended period, with warm season grass plots and three cool season grass plots sampled in a two week period in the middle of October, then due to equipment failure and logistical problems, continued sampling of cool season grass plots was not finished until early December. One of the plots that was sampled in October was resampled in December to check for possible differences in DEA due to the extended time since the original sampling. Differences were undetectable, so samples from October and December were considered part of the same sampling period for analysis. In June of 2002 cores were taken only to a depth of 110 cm. The metal sampling tube, with a zero-contamination liner, was pounded into the ground with a gas-powered jackhammer and extracted from the ground using a 48" (120 cm) hi-lift jack. The liner containing the intact soil core was then pulled from the tube and capped for transport. We were able to complete the sampling in one day, but only had the surface, vadose and top mottling depths in most cases, and a few mottled depths in the warm season grass plots.

Laboratory Analysis

In the laboratory, each sample was sieved through a 4-5 mm sieve to remove large rocks and roots. Field moist soil was subsampled for DEA and gravimetric water content analysis and the remainder air dried for storage and further analysis. Each sample was analyzed for denitrification potential using the Denitrifier Enzyme Activity (DEA) acetylene block technique (Tiedje, 1994). Sieved wet soil (50g) was placed in a 250 ml Erlenmeyer flask with 50 ml nutrient broth containing NO_3^-, glucose and chloramphenicol, which inhibits potentially interfering protein synthesis. The flasks were evacuated three times and flushed with helium four times in a continuous cycle. The overpressure of helium was released to bring the flask pressure to ambient air pressure. Twenty-five ml of acetylene were added and the samples were shaken on a reciprocal shaker for two hours. Nine ml of the headspace gas was sampled at 30, 60 and 120 min and stored in evacuated glass vials until analysis. The gas samples were analyzed for nitrous oxide using electron capture gas chromatography (Shimadzu Gas Chromatograph 17A) with a fraction collector autosampler (Parkin, 1985). The concentration of nitrous oxide was converted to an N loss rate using calculations from Tiedje (1994). Moisture content was determined by oven drying a subsample at 105°C for 24 hours.

In June 2002, a DEA amendment study was conducted to determine relative limitations of C and N. Replicate samples from each depth were treated with chloramphenicol and either water without

added nutrients (DEA+0), water and potassium nitrate (DEA+N), water and dextrose (DEA+C), or water and both potassium nitrate and dextrose (DEA+C&N).

For TOC and TN analysis, soils were air dried at room temperature and sieved (2 mm). Roots and recognizable organic debris were removed; soils were ground with mortar and pestle, then sieved through a 40 μm sieve. Subsamples of each ground and air-dried sample were oven dried at 105°C for 24 h and the % total C (TC) and % inorganic C (IC) data were corrected for water content. Inorganic carbon content was measured using the modified pressure calcimeter method described by Sherrod et al. (2002). Total carbon and nitrogen were measured using a Flash EA 2000 (ThermoFinnigan, Italy) direct combustion instrument. TOC and TN were calculated by subtracting IC and inorganic N (IN) from TC and total N (TN) values. In the two deepest depths, which had high IC content, about 30% of the samples were negative when IC was subtracted from TC. A subset of these samples was independently tested (Iowa State University Soil and Plant Testing Laboratory) for OC using the Walkley-Black procedure to verify the lack of OC. In addition, a subset of samples was rerun with a lower mass on the Flash EA 2000, and IC content was verified by repeat analysis in another laboratory. All samples tested were at or below the detection limit for Walkley-Black organic carbon determination, decreasing the sample mass for the direct combustion method did not increase total C measurements, and IC quality control checks run independently verified our initial IC measurements. We believe that the negative TOC values reflect very low TOC numbers and result from the compound error involved with using two procedures to measure one variable.

Soil inorganic N was extracted in-field with potassium chloride, placed on ice, transported to the laboratory, stored at 4° C until filtration (within 24 h of sampling) (Van Miegroet 1995). Filtrates were frozen and stored until further analysis. NO_3^- -N and NH_4^+ -N contents were analyzed with a Lachat (Lachat Quickchem method IDs: Ammonia 12-107-06-2-A, Nitrate 12-107-04-1-B).

Statistical Analysis

DEA summary statistics, including means, standard deviation and 90% confidence intervals (CI) of the means were calculated using Unbiased Minimum Variance Estimators (UMVUE) as outlined by Parkin (1994). UMVUE estimation is an appropriate method of calculating mean and variance when the data are log-normally distributed and highly skewed. Differences in DEA by sampling period, vegetation type and depth were observed when the 90% CI did not overlap. In addition, non-parametric Wilcoxon or Kruskal-Wallis tests based on the rank sums were performed, depending on the number of comparisons (JMP, SAS institute 2002). P-values reported for differences in DEA by vegetation type and depth at each sampling period were generated by these non-parametric tests. There was general agreement between the significance based on overlapping 90% CI of the UMVUE mean and the non-parametric rank-sum testing. However, occasionally one test returned a significant

difference when the other did not. These disagreements were the result of extremely high variance, and are noted in the results.

Summary statistics of other variables and differences by vegetation type and depth at each sampling period were calculated using ANOVA and pairwise comparisons for differences between depths were calculated with Tukey-Kramer Honest Significant Differences in the JMP statistical package. Multivariate comparisons with all sampling periods included were made using the PROC MIXED command of SAS (SAS Institute 2002). PROC MIXED was used when the design was not balanced due to missing data in June 2001, as this procedure compensates for missing numbers. Differences were considered significant at the 0.1 alpha level.

Results and Discussion

Vegetation effects

We expected that DEA would differ by vegetation type due to differences in factors such as carbon and nitrogen levels. In the first sampling period, Summer 2001, warm season grass DEA was higher than cool season grass DEA at every depth except the bottom (Table 2.1) (Non-parametric test results, surface $p = 0.044$, vadose $p = 0.057$, top mottling $p = 0.095$, mottled $p = 0.04$, bottom $p = 0.384$). This is probably due to the fact that cool season grass plots were not sampled until July, when they had become dormant. In the Fall of 2001, this pattern reversed and DEA was significantly higher in cool season grass than warm season grass DEA at the two upper depths, but differences disappeared in deeper subsoil (surface $p = 0.0007$, vadose $p = 0.002$, top mottling $p = 0.309$, mottled $p = 0.479$, bottom $p = 0.808$). In June of 2002, cool season grass DEA continued to be higher than warm season grass DEA in the surface and vadose zones (surface $p < 0.0001$, vadose $p = 0.002$). If individual 90% CIs generated by UMVUE are compared, in Summer 2001 warm season grass plots are almost always higher than cool season grass plots. In Fall 2001 and June 2002 cool season grass means are higher than warm season grass means although the CIs overlap indicating lack of significance (Table 2.1). Cool season grasses tended to have higher variability and larger 90% confidence intervals than warm season grasses.

In the top three depths, mean TOC was generally higher in cool season grasses than warm season grasses, although by the top mottling depth the differences were no longer significant ($p < 0.0001$, $p = 0.0002$, $p \geq 0.261$, respectively, by depth) (Table 2.2). Similarly, in the top two depths TN was higher in cool season grasses than in warm season grasses, but by the third depth the difference was no longer significant ($p < 0.0001$, $p = 0.0022$, $p \geq 0.466$, respectively, by depth) (Table 2.2).

Overall differences in mean DEA between cool season grasses and warm season grasses are confounded with differences in variability between the vegetation types and the effect of buffer age

on soil properties, i.e. as buffers age, beneficial soil properties such as TOC, TN and denitrification potential increase. We saw that TOC and TN were higher in cool season grasses at the surface, vadose and top mottling depths, however, deeper in the soil profile at the mottled and bottom depths mean TOC and TN were higher in the warm season grass plots, although not significantly (Table 2.2). Overall, differences between vegetation types were difficult to see. Recently established warm season grasses have relatively high denitrification potentials, and perhaps with increased time and development of the buffer warm season grasses may surpass cool season grasses in denitrification capacity due to higher live root biomass.

Additionally, the species composition within warm season grass plots may have affected the results. The five year buffer, which had much higher means and variance than any other warm season grass buffer, excepting the 12 y buffer (Figure 2.2), (switchgrass, *Panicum virgatum*, monoculture) contains more species than the other warm season grass plots (personal observation).

Rooting depth

Cool season grasses have a shallower rooting depth compared to warm season grasses or riparian trees and shrubs (Kramer and Boyer, 1995), but it has been suggested that they contribute more available C to soil, which can then be leached downward to provide C at depth (Corre et al., 1999). Our results showed slightly higher mean TOC and TN in cool season grass plots as compared to warm season grass plots in the upper horizons (Table 2.2), which appears to support this conclusion. However, our cool season grass plots have been relatively undisturbed for at least the past 13 years, and were in continuous cool season grass vegetation for many years before that. When land is converted from cool season grasses to warm season grasses, TOC is reduced and does not recover for as much as 18 years (Corre et al., 1999). Land conversion to agricultural uses, such as cropping and pasture, also tend to have an homogenizing impact on soil properties, greatly reducing the variability, which can take 60 years or longer to recover once native vegetation is restored (Fraterrigo et al. 2003, submitted). Our results showed much higher variability in the long established cool season grasses than in warm season grasses, which may be an artifact of previous cropping of the warm season grass buffers.

Riparian buffers may not effectively reduce nitrogen in groundwater if the site does not constrict shallow groundwater movement to the rooting zone of the buffer (Burt et al., 1999; Simpkins, 2002). This problem can be alleviated by placing buffers in areas with favorable hydrology, and by maximizing the effective depth of the buffer by species with deeper roots (Burt et al., 1999; Groffman and Crawford, 2003; Schultz et al., 2000). All of the riparian buffers in this study were in areas where an aquitard prevented further percolation of surface waters beyond our sampling depth and funneled it through the rooting zone of the buffer before entering the stream as base flow (Simpkins, 2002). We

also consistently found live roots as deep as 2.5 - 3 m under the warm season grass plots, and dead woody roots surrounded by organic mottles in a recently established warm season grass plot (3 y buffer) (personal observation). Another study in the 12 year old buffer used in this research showed differences in root densities and below-ground biomass between vegetation types (Tufekcioglu, 2003).

Sampling period and depth effects

Differences in DEA by season and by depth in the soil profile were expected. High denitrification rates have been recorded in all seasons, but spring and fall in the temperate zones seem to provide the best overall conditions for denitrification (Groffman and Tiedje, 1989). Fall denitrification is spurred by relatively warm soils, a pulse of litter inputs, lack of active plant N-uptake and the beginning of freeze-thaw cycles that break up soil aggregates and release C from surface soils. Spring sees large jumps in N_2 production rates, with generally high water content from precipitation and high groundwater levels, a buildup of C and mineral N from the winter months and warming soil temperatures which spurs OM decomposition and N mineralization. In our statistical analysis we did not see an overall difference in DEA between sampling periods ($p = 0.7615$) when depth, vegetation type and other related factors were included in a multivariate analysis (proc mixed, SAS Institute, 2002). However, the three way interaction term: vegetation type by depth by sampling date was highly significant ($p=0.0001$), which indicates that patterns in DEA changed under each vegetation type by depth and by sampling date.

Consequently, we examined seasonal differences in DEA by separating the data by vegetation type. Based on overlapping 90% CIs of the mean, there appear to be large differences in DEA seasonally in the cool season grasses, but not in the warm season grasses (Table 2.1), indicated by much larger confidence intervals in the cool season grasses. During the summer of 2001, sampling was carried out over a seven week period from June to July, with the warm season grass samples taken in June and the cool season grass samples taken in July. This may have affected the surface soil cool season grass results somewhat as the mean DEA of surface soils in summer 2001 was lower than samples taken the following June. In fact, cool season grass samples taken the following June were more similar to fall samples than the previous summers samples.

Denitrification generally tends to decrease in summer months as plant uptake competes for mineral N and soil moisture decreases (Groffman and Tiedje, 1989). We saw however, that active plant growth, even in the summer months, may have been correlated with increased DEA. This might be related to active rhizodeposition, and corresponding denitrification activity in anaerobic microsites.

There appears to be a trend where DEA is highest under grasses that are active at the time of year of sampling. During the first summer, DEA in cool season grasses was lower than warm season

grasses when the cool season grasses were dormant and the warm season grasses were actively growing (late June and early July). In the second summer all sampling was completed by mid June and cool season grasses were still actively growing during the sampling period. Even though there were no statistically significant differences in mean DEA between sampling periods, the sampling date by vegetation interaction term was significant ($p=0.0008$). Therefore, we were able to determine that DEA behaved differently under different vegetation types at different times of the year.

The difference in time of sampling between summers may explain the extremely high mean DEA in June 2002 in the cool season grass surface soils. The relatively wet spring weather, and active growth of the cool season grasses (the warm season grasses were just starting to grow) combined to favor DEA in the cool season grass surface soils in the weeks previous to sampling. During the first Summer 2001 sampling period, cool season grass plots were sampled almost a month after warm season grasses and after active growth had stopped.

The similarity between Fall 2001 and June 2002 DEA is related to patterns seen in gravimetric water content. This also may help explain why sampling period was not found to be significant in the multivariate model, since the mixed model takes into account error variation explained by other variables. Gravimetric soil water content in June 2002 was roughly 0.24, which was not significantly different from gravimetric soil water content in the partial sampling in December 2001 (0.23), and was significantly different from water content in October 2001 and Summer 2001 of around 0.19. It seems that seasonal differences in DEA can be adequately explained by differences in vegetation type and in differences in gravimetric soil moisture, since when these variables are included in the model, differences by sampling period are no longer significant.

Surface soils exhibited much higher DEA than any of the other subsurface depths ($p<0.0001$) (Table 2.1). Interestingly, although the subsurface depths were not generally different from one another, in a particular season, the vadose zone samples occasionally were different from the other subsurface depths and the surface. In the summer seasons, the vadose zone samples in warm season grass buffers were lower than the surface, but higher than the rest of the subsurface samples. In the fall, warm season grass vadose zone samples were not significantly different from other subsurface samples. This phenomenon was the opposite in cool season grass buffers. In the summer, cool season grass vadose zone samples were not different from other depths, but in the fall they were. This may be related to differences in rooting zone activity in the different vegetation types. In the fall, cool season grasses are more active, which could explain increases in denitrification in the vadose zone because of increased rhizodeposition, C-inputs from litter and break up of aggregates from freezing and thawing, and subsequent percolation of DOC to the vadose zone. To check to see if this pattern was significant, the vegetation by depth interaction was tested. This relationship was significant ($p =$

0.007), indicating that denitrification in the vadose zone was significantly different from other subsurface depths under warm season grass in the summer and under cool season grass in the fall.

Soil water effects

Gravimetric water content was significantly related to DEA and both TOC and TN. This relationship with DEA disappeared with depth in the soil, indicating that soil water was not limiting deep in the profile, but that it was in the surface. A contributing factor to these patterns may be the fluctuating water table. Higher water tables in spring and fall than in early-mid summer may influence aeration, nitrate concentrations and available carbon. Groundwater fluctuates seasonally in response to snowmelt, precipitation events and vegetative uptake. The rise and fall of the water table in riparian buffers affects spatial heterogeneity of denitrification and improves local conditions for denitrification. Clément et al. (2002) found that surface soil denitrification was highest when the seasonally fluctuating groundwater was at the surface and that subsurface denitrification, while low, also contributed to an overall NO_3^- reduction in a riparian wetland. When water tables are high, oxygen diffusion is limited, substrate (labile C and NO_3^-) is redistributed and microbes shift to denitrification from aerobic respiration to meet their energy needs (Tiedje, 1988). Clément et al. (2002) found that the microbial community was relatively stable despite the fluctuating water table, indicating that there was no shift in microbial populations seasonally, rather a shift in respiration pathway.

There is some evidence as well that the fluctuating water table promotes microbial communities that are adapted to fluctuating anaerobicity and other stresses caused by drying and re-wetting. Pett-Ridge and Firestone (2003) found that microbial biomass was higher under fluctuating aerobic and anaerobic conditions than under static conditions, and those microbial communities under fluctuating anaerobicity had different N-transformation capacities than those from static conditions. Other research has also shown that that drying and re-wetting results in microbial communities that are adapted to changing conditions and are different from the communities that would have existed without fluctuating water potentials (Fierer et al., 2003a). Fierer et al., (2002) also showed that microbial community structures change by depth in the soil, which may be in response to soil moisture variability or different C and N availability by depth.

If the water table remains high, nitrification will be limited by lack of oxygen and unless incoming groundwater continually resupplies N, denitrification will also be limited. Some nitrification can also occur in the relatively oxygenated rhizosphere of deep-rooted plants, but plants are effective competitors for this NO_3^- supply (Tiedje, 1988). Drawdown of the water table allows nitrification to resupply NO_3^- to the soil matrix. The area near the top of the variable water table may provide ideal conditions for denitrification to occur. Simpkins et al. (2002) found that denitrification

rates in the groundwater were higher near the water table than lower in the aquifer because of slower groundwater velocities providing longer residence times and higher quantities of organic carbon from the overlying buffer. While we did not see a direct relationship between inorganic N levels and DEA, amendment studies showed that nitrate was limiting in these soils, although C was more important (See Chapter 3).

Seasonal groundwater fluctuations in this buffer may have had a significant effect on denitrification. We saw that including water content and vegetation type to the multivariate regression effectively explained the lack of significance in seasonal fluctuations of DEA. It may be that improved conditions for denitrification provided by the fluctuating water table affected denitrification in the vadose zone, which may partially explain increased denitrification in the vadose zone during seasonal vegetative activity.

Fierer and Schimel, (2003) found that even though microbial respiration increased up to 475% after rewetting soil, the observed carbon dioxide pulse did not originate in soil organic matter that was released during rewetting, nor did they observe significant cell lysis, rather they believe that release of cytoplasmic solutes from existing microbes resulted in the large jump in soil respiration. Perhaps microbial communities are able to conserve readily available carbon sources in their living biomass and release it for use when nutrient or water conditions become favorable. The ability to do this would be maximized in areas of low nutrient availability, or when available C resources were not necessarily timed with nutrient availability. Even though groundwater levels are not necessarily in the vadose zone during the growing season, the ability to store resources opportunistically would result in microbial activity during times of active rhizodeposition. If maximizing denitrification over the entire soil profile is the goal, locating riparian zones in areas with fluctuating water tables and planting water tolerant, deep-rooted species will be effective.

Nutrient effects

A DEA amendment study showed that while adding only nitrate increased DEA substantially more than just adding water, adding C alone increased the rate by an even greater amount in the surface soils (Figure 2.1). In the vadose zone, adding both C and N increased DEA, but by a proportionally lesser amount than in the surface soils, and in the deeper depths adding C and N together did not increase DEA more than adding either substrate singly. The DEA test prevents the production of new enzyme, so it may be that adding any extra substrate effectively saturates the reaction for the small amount of enzyme available at depth, while at the surface, more enzyme is present and the reaction is not saturated as quickly. The microbial community at depth given a short period of time may have responded similarly to the surface if new cell growth had not been prevented. These results indicate that soils are both N and C limited at all depths, but in the surface

and vadose zone, a larger enzyme pool allows for increased N_2 production with unlimited substrate. Organic carbon is essential to microbial growth, whether it is used in aerobic respiration or in anaerobic respiration using nitrate as the alternate electron acceptor. In this riparian buffer it may be that at depth, organic C is first limiting to microbial growth, whether that growth is mediated by either aerobic or anaerobic means, then secondarily to the denitrification reaction.

Significant relationships were shown between DEA and both TOC and TN using multivariate analysis, although TN was the stronger predictor (See Figure 2.1). TOC:TN ratio was not significantly related to DEA, but TOC and TN were highly significantly related to each other. The mean TOC:TN dropped significantly from the surface soils where it was about 11:1, to about 8:1 at the deepest depths, depending on the location. This may indicate that at depth the microbial biomass-C and N pools are the largest contributors to the TOC and TN pools, while at the surface inorganic-N and plant associated C and N provide relatively greater contributions to the total N pool and raise the soil C:N.

General discussion

One of the objectives of this study was to determine if denitrification was favored under certain vegetation types, however, vegetation plays a complicated role in the N-removal services of a riparian buffer. Not only does vegetation affect denitrification through changing soil properties, but it also plays an important role in N-immobilization. Although plant uptake of shallow groundwater N can significantly reduce the amount of N exported from the buffer, its result is only temporary storage of N in plant biomass. Much of this N is eventually returned to surface soils through litterfall, fine-root turnover and rhizodeposition, unless it is removed by harvest. For example, Janzen (1990) found that 18-33% of mineral N taken up by plants was returned to the soil by rhizodeposition and Lynch and Whipps in a 1990 review found that 30-60% of net photosynthetic C in annual plant systems is allocated belowground for roots, and of that C allocated to roots, 4-70% is lost through rhizodeposition. Additionally, Fogel (1988) found that 5-30% of net photosynthates are allocated belowground for roots, mycorrhizas and respiration in a coniferous system. If site conditions are suitable for denitrification to occur, enriched plant material can potentially stimulate surface denitrification, and facilitate greater overall site denitrification potential (Hanson et al., 1994).

We cannot discount the potential importance of rooting depth and rhizodeposition as it relates to subsurface denitrification. If a great deal of previously immobilized substrate is returned to the subsoil by deep roots, it may provide positive feedback to increased soil respiration and denitrification at depth. Haycock and Pinay (1993) studied winter denitrification and found that in the non-growing season roots provided as substantial C-source to denitrifying bacteria. Root growth during the dry season may result in N-removal through denitrification when water tables rise.

It is notable that although mean denitrifier enzyme activity was very low in our deepest samples, it was often detectable and sometimes relatively high. One recent study showed that a third of the microbial biomass found in soil 2 m deep was below 25 cm, and concluded not only that there were effectively large microbial populations in subsoils, but also that there was no reason to believe that individual subsoil microbes were not as active as surface microbes (Fierer et al., 2002). In another study, Fierer et al. (2003b), found that microbial communities at depth responded differently to changes in temperature, soil moisture and nutrient additions than surface communities did. In fact subsurface communities dramatically responded to increased nutrient (N and P) additions, while surface microbial communities did not (subsurface CO_2 production increased by 450%). We may find that stimulating denitrifying activity of surface soils with enriched plant materials is not as effective as stimulating subsurface soils, especially if subsurface microbial communities indeed have the capability to conserve labile C sources in their cytoplasm for later use (Fierer and Schimel, 2003).

Finally, it has been shown that chronic N additions can eventually saturate plant and microbial pools and that the end result may be an increase in net N export (Aber et al., 1998; Hill and Shackleton, 1989). If site conditions are regularly suitable for denitrification, N-saturation may not occur, as excess nitrogen will be permanently removed from the site. At the landscape level, sites such as riparian zones where denitrification is relatively more important as a fate of nitrate than plant uptake, will be better long-term N-sinks than sites where denitrification is limited (Groffman et al., 1992).

Conclusions

Our research clearly shows that in order to maximize nitrate removal from shallow groundwater in riparian zones through denitrification a wide variety of vegetation types needs to exist. Riparian zones that include both warm season and cool season grasses will have denitrification activity throughout the year not only at the surface, but also at depth. During spring and fall when warm season grasses are dormant, cool season grasses will be active, and stimulate microbial activity in the surface and the vadose zone. Deeper rooting depths provided by warm season grasses, and potentially by other deeply rooted species such as trees and shrubs, may stimulate denitrification by providing carbon and nitrogen directly to deep soils. Trees and other deep rooted species such as warm season grasses will actively pump nitrate from groundwater, and stimulate further denitrification throughout the soil profile through enriched C and N litterfall, fine-root turnover and rhizodeposition. Planting diverse mixtures of riparian vegetation provides added horizontal and vertical spatial variability to the buffer, which can increase denitrification potential by providing more denitrification sites.

Acknowledgements

This research has been funded in part by the U.S. Department of Agriculture National Research Initiative Competitive Grants Program; the Iowa Department of Natural Resources through a grant from the U.S. Environmental Protection Agency under the Federal Nonpoint Source Management Program (Section 319 of the Clean Water Act); and the Leopold Center for Sustainable Agriculture. Special thanks to Jennifer Nelson, Alicia Shaffer and the great crews of undergraduate laboratory and field assistants.

References

Aber, J.D., W. McDowell, K. Nadelhoffer, A. Magill, G. Berntson, Mark K. S.G. McNulty, W. Currie, L. Rustad, and I. Fernandez. 1998. Nitrogen saturation in temperate forest ecosystems: hypotheses revisited. BioScience 48(11):921-934.

Addy, K. L., A. J. Gold, Peter M. Groffman, and P. A. Jacinthe. 1999. Groundwater nitrate removal in subsoil of forested and mowed riparian buffer zones. Journal of Environmental Quality 28:962-970.

Aschismiti, S., W. Chaïbi, B. Renaud, R. Bérénice, S. Pierre. 2003. Assesment of enzyme induction and aerenchyma formation as mechanisms for flooding tolerance in *Trifolium suberraneum* 'Park'. Annals of Botany. 91(2):195-204.

Baruch, Z. 1994. Responses to drought and flooding in tropical forage grasses .2. Leaf water potential, photosynthesis rate and alcohol-dehydrogenase activity. Plant and Soil. 164(1):97-105.

Baruch Z. and T. Merida, 1995. Effects of drought and flooding on root anatomy in four tropical forage grasses. International Journal of Plant Sciences. 156(4):514-521.

Burt, T.P., L.S. Matchett, K.W.T. Goulding, C.P. Webster, and N.E. Haycock. 1999. Denitrification in riparian buffer zones: the role of floodplain hydrology. Hydrological Processes 13:1451-1463.

Castle K., J.R.M. Arah, A.J.A. Vinten. 1998. Denitrification in intact subsoil cores. Biology and Fertility of Soils. 28:12-18.

Clay, D.E., S.A. Clay, T.B. Moorman, K. Brix-Davis, K.A. Scholes, and A.R. Bender. 1996. Temporal variability of organic C and nitrate in a shallow aquifer. Water Resources. 30:559-568.

Corre, M. D., R. R. Schnabel, and J. A. Shaffer. 1999. Evaluation of soil organic carbon under forests, cool-season and warm-season grasses in the northeastern US. Soil Biology and Biochemistry 31:1531-1539.

Corre, M.D., R.R. Schnabel, and W.L. Stout. 2002. Spatial and seasonal variation of gross nitrogen transformations and microbial biomass in a Northeastern US grassland. Soil Biology and Biochemistry 34(4):445-457.

DeWitt, T.A., 1984. Soil Survey of Story County, Iowa. USDA Soil Conservation Service, Washington D.C.

Fennesey, M.S., J.K. Cronk. 1997. The effectiveness and restoration potential of riparian ecotones for the management of nonpoint source pollution, particularly nitrate. Critical Reviews In Environmental Science And Technology. 27(4):285-317.

Fierer, N., J.P. Schimel. 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal. 67(3):798-805.

Fierer, N., J.P. Schimel, P.A. Holden. 2002. Variations in microbial community composition through two soil depth profiles. Soil Biology and Biochemistry. 35:167-176.

Fierer, N., J.P. Schimel, P.A. Holden. 2003a. Influence of drying-rewetting frequency on soil bacterial community structure. Microbial Ecology. 45(1):63-71.

Fierer, N., A.S. Allen, J.P. Schimel, P.A. Holden. 2003b. Controls on microbial CO_2 production: a comparison of surface and subsurface soil horizons. Global Change Biology. 9:1322-1332.

Fraterrigo, J.M., M.G. Turner, S.M. Pearson, and P. Dixon. 2003. Effects of prior land use on spatial heterogeneity of soil nutrient in southern Appalachian forests. Ecology. Submitted July 2003.

Fogel, R. 1988. Interactions among soil biota in coniferous ecosystems. Agriculture Ecosystem Environment 24:69-85.

Gold, A.J., P.A. Jacinthe, P.M. Groffman, W.R. Wright, R.H. Puffer. 1998. Patchiness in groundwater nitrate removal in a riparian forest. Journal of Environmental Quality 27:146-155.

Groffman, P.M. and M.K. Crawford. 2003. Denitrification potential in urban riparian zones. Journal of Environmental Quality 32(3):1144-1149.

Groffman, P.M., A.J. Gold, and R.C. Simmons. 1992.Nitrate dynamics in riparian forests: Microbial studies. Journal of Environmental Quality 21:666-671.

Groffman, P.M. and J.M. Tiedje. 1989. Denitrification in north temperate forest soils: Spatial and temporal patterns at the landscape and seasonal scales. Soil Biology and Biochemistry. 21(5):613-620.

Gurwick, N.P., P.M. Groffman, G. Blazejewski, and M. Stolt. 2003. Carbon availability in riparian zones: The role of buried soils. Poster presented at Cary Conference 2003: Ecosystem function in heterogeneous landscapes. Institute of Ecosystem Studies. Millbrook, NY USA. April 29-May 1 2003.

Janzen, H.H. 1990. Deposition of nitrogen in to the rhizosphere by wheat roots.Soil Biology and

Biochemistry. 22:1155-1160.

Hanson, G.C., P.M. Groffman, A.J. Gold. 1994. Denitrification in riparian wetlands receiving high and low groundwater nitrate inputs. Journal of Environmental Quality. 23:917-922.

Haycock, N.E., and G. Pinay. 1993. Nitrate retention in gras and poplar vegetated riparian buffer strips during winter. Journal of Environmental Quality. 22:273.

Hill, Alan R. 1996. Nitrate removal in stream riparian zones. Journal of Environmental Quality 25:743-755.

Huang, Y., D.H. Rickerl, K.D. Kepert. 1996. Recovery of deep-point injected soil Nitrogen-15 by switchgrass, alfalfa, ineffective alfalfa and corn. Journal of Environmental Quality. 25:1394-1400.

Kramer, P.J., J.S. Boyer. 1995. Water relations of plants and soils. Academic Press, Harcourt Brace and Company. San Diego, California, USA.

Lennon, J.T. and L.E. Pfaff. 2003. Terrestrial subsidies in lake ecosystems: Importance of detrital source and supply on microbial metabolism. Poster presented at Cary Conference 2003: Ecosystem Function in Heterogeneous Landscapes. Institute of Ecosystem Studies. Millbrook, NY USA. April 29-May 1 2003.

Lynch, J.M. and Whipps, J.M., 1990. Substrate flow in the rhizosphere. Plant Soil 129:1-10.

Marquez, C. O., C. A. Cambardella, T. M. Isenhart, and R. C. Schultz. 1999. Assessing soil quality in a riparian buffer by testing organic matter fractions in central Iowa, USA. Agroforestry Systems 44, 141-149.

Marquez, C. O. 2001. Soil aggregate dynamics and aggregate-associated carbon under different vegetation types in riparian soils. Ph.D. Dissertation. Iowa State University. Ames, IA.

McCarty, G.W. and J.M. Bremner. 1992. Availability of organic carbon for denitrification of nitrate in subsoils. Biology and Fertility of Soils. 14:219-222.

McDonald, M.P., N.W. Galwey., T.D. Colmer. 2002. Similarity and diversity in adventitious root anatomy as related to root aeration among a range of wetland and dryland grass species. Plant Cell and Environment. 25:441-451.

Parkin, T.B. and Meisinger J. J. 1989. Denitrification below the crop rooting zone as influenced by surface tillage. Journal of Environmental Quality. 18:12-16.

Peterjohn, W.T. and D.L. Correll. 1984. Nutrient dynamics in an agricultural watershed: observations on the role of a riparian forest. Ecology 65[5], 1466-1475.

Pickle, J. E. 1999. Microbial biomass and nitrate immobilization in a multi-species riparian buffer. M.S. Thesis. Iowa State University. Ames, Iowa.

SAS Institute. 2003. SAS user's guide. SAS Inst., Cary, NC.

Shimamura S., T. Mokchizuki, Y. Nada and M. Fukuyama. 2003. Formation and function of

secondary aerenchyma in hypocotyl, roots and nodules of soybean (*Glycine max*) under flooded conditions. Plant and Soil. 251(2):351-359.

Schipper, L.A., A.B. Cooper, C.G. Harfoot and W.J. Dyck. 1993. Regulatorrs of denitrification in an organic riparian soil. Soil Biology and Biochemistry. 25(7):925-933.

Schipper, L. A., C. G. Harfoot, P. N. McFarlane, and A. B. Cooper. 1994. Anaerobic decomposition and denitrification during plant decomposition in an organic soil. Journal of Environmental Quality 23:923-928.

Simpkins, W. W., T. R. Wineland, R. J. Andress, D. A. Johnston, G. C. Caron, T. M. Isenhart, and R. C. Schultz. 2002. Hydrogeological Constraints on Riparian Buffers for Reduction of Diffuse Pollution: Examples From the Bear Creek Watershed in Iowa, Usa. Water Science and Technology 45(9):61-68.

Schultz, R.C., J.P. Colletti, T.M. Isenhart, C.O. Marquez, W.W. Simpkins, C.J. Ball, 2000. Riparian Forest Buffer Practices. In: North American Agroforestry: An Integrated Science and Practice. ASA, Madison, WI. 189-281.

Sherrod, L.A., G. Dunn, G.A. Peterson, R.L. Kolberg, 2002. Inorganic carbon analysis by modified pressure-calcimeter method. Soil Science Society of America Journal. 66(1):299-305.

Starr, R.C. and R.W. Gillham. 1993. Denitrification and organic carbon availability in two aquifers. Ground Water. 31(6):934-947.

Subbaiah Chalivendra, C., and M. Sachs Martin. 2003. Molecular and cellular adaptations of Maize to flooding stress. Plant and Soil. 91(2):119-127.

Tiedje, J.M. 1982. Denitrification. Chapter 47, pp. 1011-1026 In: Methods of soil analysis. Soil Science Society of America.

Tiedje, J.M. Denitrifiers. 1994. Chapter 14, pp. 245-267 In: Methods of soil analysis. Soil Science Society of America.

Tiedje, J.M. 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium. pp. 179-244 In: Zehnder, A.J.B. (ed.) Biology of anaerobic organisms. John & Sons. New York. New York. USA.

Tufekcioglu, A., J.W. Raich, T.M. Isenhart, and R.C. Schultz. 1999. Fine root dynamics, coarse root biomass, root distribution, and soil respiration in a multispecies riparian buffer in Central Iowa, USA. Agroforestry Systems. 44:163-174.

Tufekcioglu, A., J.W. Raich, T.M. Isenhart, and R.C. Schultz. 2003. Biomass, carbon and nitrogen dynamics of multi-species riparian buffers within an agricultural watershed in Iowa, USA. Agroforestry Systems. In press.

Van Miegroet, H. 1995. Inorganic nitrogen determined by laboratory and field extractions of two forest soils. Soil Science Society of America Journal. 59(2): 549-553.

Van Noordwijk, M. and G. Brouwer. 1988. Quantification of air-filled root porosity: A comparison of two methds. Plant Soil 111:255-258. As cited in: pp.151-152. Kramer, P.J., J.S. Boyer. 1995. Water relations of plants and soils. Academic Press, Harcourt Brace and Company. San Diego, California, USA.

FIGURES AND TABLES

Table 2.1. Denitrifier Enzyme Activity in warm and cool season grass plots in Coland soils in the Bear Creek Riparian Buffer System, near Roland, Iowa from the soil surface to up to 4 m in depth.

	Cool Season Grasses[†]			Warm Season Grasses		
	Mean[‡§]	STE[¶]	90% CI[#]	Mean	STE	90% CI
Soil depth designation	ng-N g-soil^{-1} day^{-1}					
	Summer 2001					
Surface	4023*	1373	2848,7617	9334*	3447	6439,19083*
Vadose	124	23	101,170	821*	471	509,2459
Top Mottling	70	17	54,106	234	90	144,474
Mottling	67	16	52,102	150	42	112,247
Bottom	85	16	69,115	342	182	216,948
	Fall 2001					
Surface	23747*	7342	17146,42483	9151*	3896	6133,20529
Vadose	509*	225	333,1239	53	103	126,534
Top Mottling	388*	297	218,2036	211	157	123,858
Mottling	193	126	113,828	80	28	55,159
Bottom	62	24	42,142	87	48	54,257
	June 2002					
Surface	75835*	61362	43238,346160	8669*	4243	5605,22085
Vadose	555*	172	402,986	443*	347	239,2044
Top Mottling	6	6	-12,24	62	32	38,241
Mottling	---	---	---	34	12	22,97
Bottom	---	---	---	---	---	---

* Significant at the 0.10 probability level, signifies differences between depths at each sampling period.

[†]Long established (>50 y) cool season grass plots; recently reestablished (≤12 y) warm season grass plots.

[‡]calculated using Unbiased Minimum Variance Estimators.

[§] $n \leq 25$

[¶] Standard Error of the Mean.

[#] 90% Confidence Interval of the Mean; overlapping CIs indicate no significant difference between means.

--- no samples

Table 2.2. Percent Total Organic Carbon and Percent Total Nitrogen in warm and cool season grass plots in Coland soils in the Bear Creek Riparian National Demonstration Watershed, near Roland, Iowa from the soil surface to up to 4 m in depth.

	% TOC[†]				%TN			
	Cool Season Grass		Warm Season Grass		Cool Season Grass		Warm Season Grass	
Soil depth designation	Mean[‡]	STE [§]	Mean	STE	Mean	STE	Mean	STE
	Summer 2001							
Surface	2.92*	0.128	2.11*	0.155	0.251*	0.0091	0.181*	0.0099
Vadose	2.15*	0.131	1.66*	0.155	0.176*	0.0093	0.139*	0.0099
Top Mottling	0.992*	0.128	0.917*	0.158	0.078*	0.0091	0.076	0.0101
Mottling	0.543	0.134	0.486	0.155	0.049	0.0095	0.050	0.0101
Bottom	0.087	0.131	0.192	0.155	0.036	0.0093	0.029	0.0099
	Fall 2001							
Surface	2.79*	0.161	2.16*	0.122	0.198*	0.014	0.189*	0.0095
Vadose	2.10*	0.161	1.83*	0.122	0.155*	0.015	0.161*	0.0095
Top Mottling	0.575	0.164	0.413	0.124	0.065	0.015	0.052	0.0097
Mottling	0.132	0.169	0.247	0.130	0.026	0.015	0.041	0.0099
Bottom	0.049	0.190	0.108	0.127	0.023	0.017	0.035	0.0099
	June 2002							
Surface	2.87*	0.170	2.12*	0.159	0.250*	0.130	0.179*	0.015
Vadose	2.41*	0.167	1.61*	0.163	0.196*	0.127	0.140	0.015
Top Mottling	0.365	0.578	0.589	0.240	0.042	0.044	0.074	0.022
Mottling	---	---	0.14	0.325	---	---	0.032*	0.030
Bottom	---	---	---	---	---	---	---	---

* Significant at the 0.10 probability level, signifies differences between depths at each sampling period.

[†]Long established (>50 y) cool season grass plots; recently reestablished (≤12 y) warm season grass plots.

[‡] $n \leq 25$

[§] Standard Error of the Mean.

--- no samples

Figure 2.1. Denitrifier Enzyme Activity amendment study. Four treatments were applied to fifty replicates at each depth. DEA where both added C (dextrose) and N (Potassium Nitrate) were added to the incubation flask, DEA with only added C, DEA with only added N and DEA without added substrate.

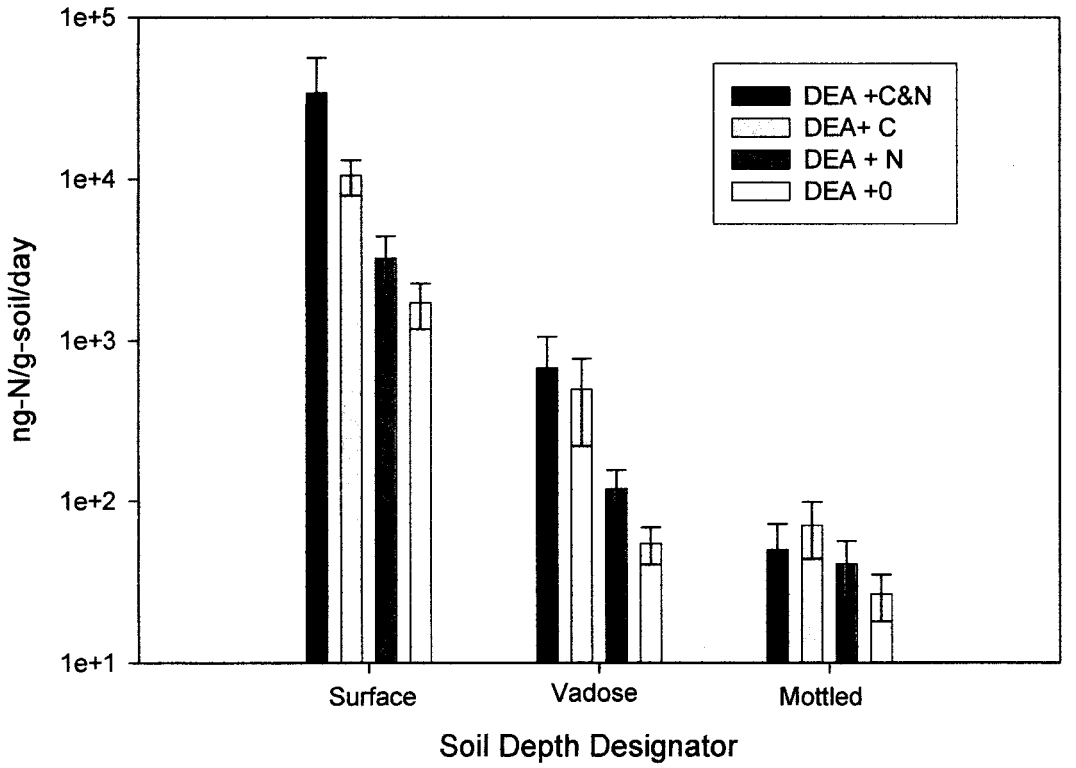


Figure 2.2. Relationships between ln transformed Denitrifier Enzyme Activity, Total Organic C and Total Organic N in Coland soils up to 4 m in depth at Bear Creek, Roland IA.

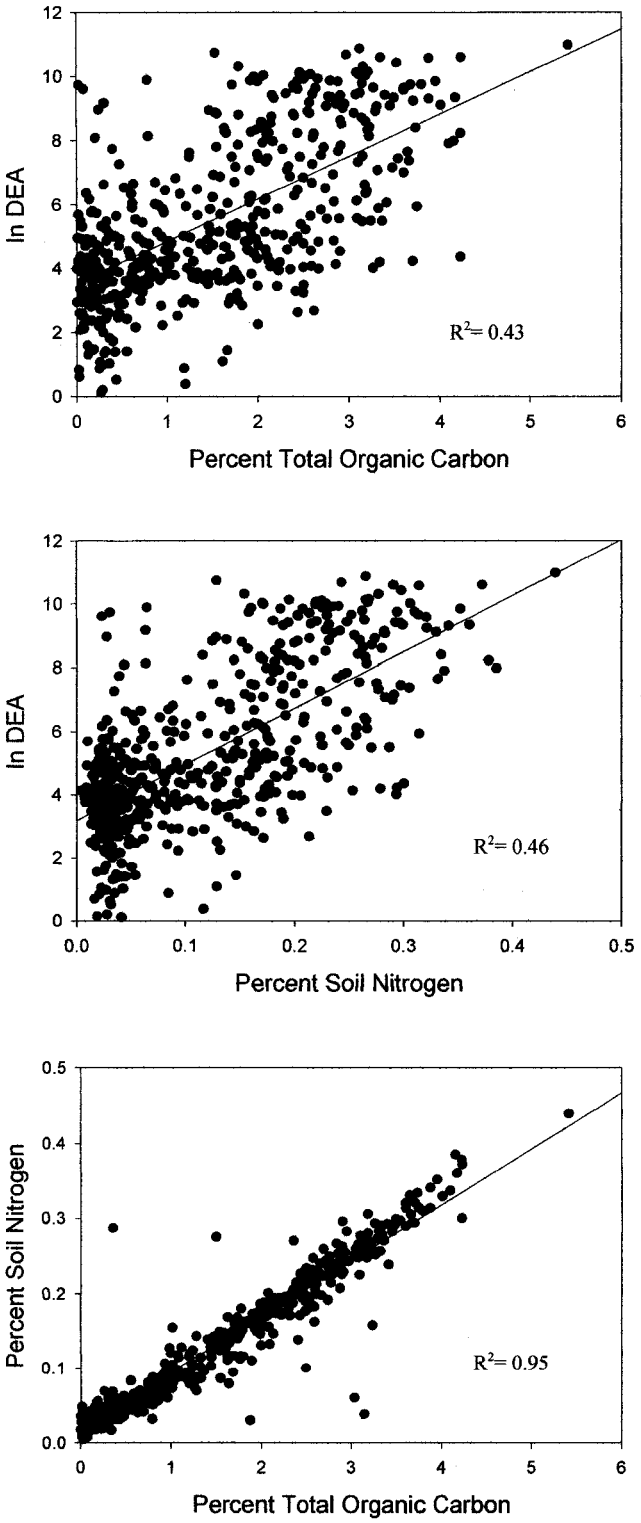
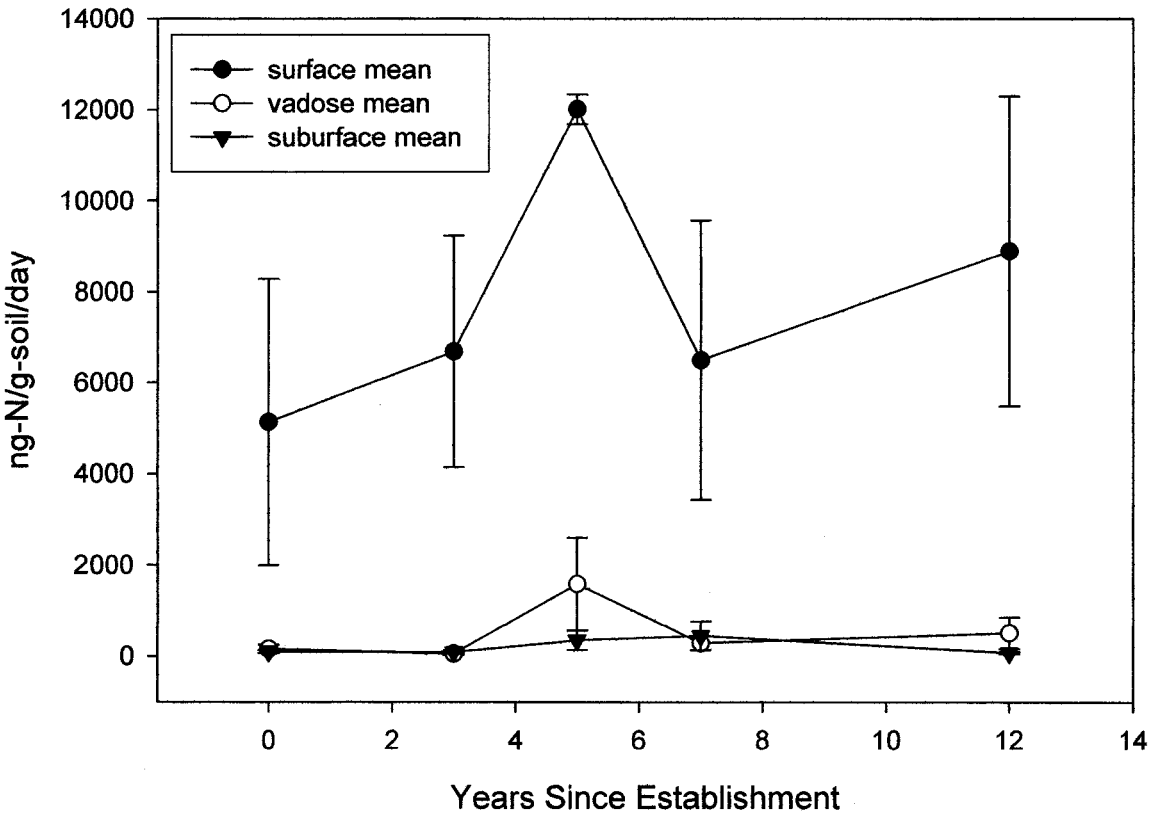


Figure 2.3. Denitrifier Enzyme Activity at different depths in the Warm Season Grass Chronosequence of Riparian buffers in the Bear Creek National Restoration and Demonstration Watershed, near Roland, IA.



CHAPTER 3. FACTORS CONTROLLING DENITRIFICATION IN A RIPARIAN BUFFER SYSTEM

Introduction

While carbon and nitrogen have long been known to control denitrification, relatively little is known about the relative contributions of different forms of carbon to denitrification. It may be possible that different forms of carbon are better able to predict denitrification than others. If this is true, then understanding the various roles of carbon forms in soil, and their input and transport will help us in designing more effective buffers. We measured total organic carbon (TOC), dissolved organic carbon (DOC), % bioavailable dissolved organic carbon (% BDOC), and hypothesized that they would affect denitrification in different ways, depending upon the depth in the soil profile. For example, we hypothesized that in the surface soils, TOC would be the most important controller of denitrification because of the role of humus in forming good soil structure for water infiltration and storage, and particulate organic matter in providing both substrate and anaerobic microsites. Deeper in the soil profile, the dominant form of organic carbon is DOC. We hypothesized that we would see a shift in relative importance to denitrification from TOC to DOC, and that %BDOC, as a measure of what portion of the DOC is actually used by the microbes, would be even more important to denitrification. Total organic N and inorganic N were also measured and reported on.

The other major idea that was explored in this research was that different vegetation types can affect the form and quantity of organic C, nitrogen status and denitrification potential in a soil. The structure and function of different vegetation types may affect these parameters at varying depths in the soil profile related to their rooting depths and the amount of substrate that is leached from the surface to deeper depths. Statistical tests were designed with these factors in mind. The guiding hypotheses to the overall research are reviewed below.

Hypotheses

1) Denitrification potential would be greatest in the surface soil, and would decrease by depth, but that near the top of the fluctuating water table we would see a spike in enzyme activity, corresponding to previous work in the buffer that showed C and N removal concomitantly with low oxygen levels and stable chloride levels.

2) Denitrification potential would follow observed patterns in Organic C and N, which would limit enzyme activity.

3) Vegetation would have a significant effect on denitrification by controlling C and N distribution both between vegetation types and throughout the soil profile, i.e., DEA in surface soils would be higher under introduced cool season grasses and in deep soils DEA would be higher under native warm season grasses, following observed patterns in root distribution, and growing season activity.

4) Denitrification potential would be affected by the amount of available carbon and that DOC and %BDOC would be more closely related to denitrification potential than TOC. We also hypothesized that the relative importance of each of these C parameters would change by depth, i.e in the surface, TOC would be best related to DEA, but deeper in the soil profile, as C became more limiting first DOC then %BDOC would be the better predictor of DEA.

Materials and Methods

Total C, Total N and Inorganic N

The results from these data sets will not be discussed in detail in this chapter, however, relevant points were included in the discussion. For methods description and tables of the means by depth and vegetation type, please see chapter 2.

DOC and %BDOC

DOC was extracted with water by adding 300 ml ultrapure, C-free, distilled, deionized water to 100 g of soil, shaking for 2 hours, centrifuging and filtering with Whatman 40 (8 μm pore size) to remove particulate C and sediment. Approximately ½ of the filtrate was filtered again through Whatman GF/F (0.7μg pore size) glass fiber filters to remove large microbial biomass. This aliquot was frozen, until analysis on a Phoenix 8000 organic carbon autoanalyzer (Tekmar Dohrmann, Cincinnati, Ohio) using a persulfate oxidation method and pretreatment with phosphoric acid to remove carbonates. The second half of the filtrate was poured into 250 ml Ehrlenmeyer flasks that had been acid washed and thoroughly rinsed with ultrapure water, covered with parafilm, perforated 6-8 times with a hypodermic needle, and incubated in the dark in a closed cabinet. After 30 days, the liquid in the flasks was filtered through Whatman GF/F filters, and frozen until analysis. %BDOC was the percentage of the initial DOC that was used up during the 30 day incubation period (((Initial DOC-Final DOC)/(Initial DOC))*100).

Two smaller incubation studies were also conducted to verify the appropriateness of the 30 d incubation time: one in September of 2002 and one in December 2002. In each case, three composited surface soil samples from the 12 y buffer were taken in both warm season and cool

season grass plots. These samples were returned to the lab, sieved through a 5 mm screen to remove rocks and roots, a subsample for moisture content was removed, and the rest of the sample was treated as above for %BDOC determination. Each sample was extracted in triplicate to result in 9 incubating flasks for both warm season grasses and cool season grasses, and a 50 ml aliquot was filtered every 7 days for one month and then at 60 d and 90 d to follow the DOC loss process. The results are shown in Figure 3.1.

Methodological problems

During the first two sampling periods the Whatman GF/F filter papers were folded into filter funnels. We did not realize that folding the glass-fiber filter papers would break down the structure of the filter and allow particles greater than 0.7μg pore size to pass through. During summer of 2002, these filters were left flat and were filtered using a vacuum pump attached to a Buchner funnel. A randomly selected subset of 20 samples were analyzed twice (two separate aliquots from the same soil extraction), one through folded papers and one through flat papers. This experiment showed that there was more DOC was in the folded paper aliquot than in the aliquot filtered through flat filter papers. However, the error introduced by this mistake was randomly distributed among the samples. It should not have biased the samples, which would lead us to inflate the Type I error (i.e. find differences that are actually not there). Instead, random error should make it harder to see differences between means, and tends to inflate the Type II error (failing to detect differences in means that are actually there).

Statistical analysis

ANOVA and pair-wise comparisons were used to determine differences between depths and vegetation types within a sampling period and were calculated with Tukey-Kramer Honest Significant Differences using the JMP statistical package (SAS Institute 2003). Multivariate comparisons with all sampling periods included were made using the Proc Mixed command of SAS (SAS Institute 2002). Differences were considered significant at the 0.1 alpha level.

Proc Mixed was also used to determine significant relationships between DEA and other variables. The multivariate prediction model included vegetation type (warm or cool season grass), depth, sampling period, gravimetric moisture content, soil N-NO_3^- and NH_4^+, %Total N, %TOC, %BDOC, interaction terms of vegetation type by sampling period and vegetation type by depth, with plots in each farm as a random effect, and with repeated measure of plots over time. The mixed model used natural log transformed data for DEA to correct for log-normal distribution.

Results and Discussion

Dissolved Organic Carbon

We hypothesized that DOC would be higher at the surface under cool season grasses and higher at depth under warm season grasses, and that DOC would be significantly related to DEA. In this study there was no apparent relationship between DOC and DEA ($p=0.3829$).The methodological problems during the laboratory analysis of DOC may have introduced enough error to prevent us from seeing a relationship between DOC and DEA and possibly clear-cut differences between warm and cool season grasses. However, it is very likely that the nature of the laboratory tests makes clear statistical relationships between these parameters difficult.

Our original assumption was that higher DOC would result in higher denitrifying capacity which is supported in the literature (Clay et al., 1996; Sobczak et al., 2002; Cosandey et. al., 2003). However, in soils, diffusional constraints of substrate can result in serious limitations even if total quantity of substrate is relatively high (Myrold and Tiedje, 1985). DEA is a measure of the activity of the enzyme available in the soil, i.e. what has been built up by the microbial community in response to positive denitrifying conditions over a period of time. DOC is highly variable in time and is quickly used up by respiration. The resupply of organic C to microbes is facilitated by water movement through the soil interface, which is dependant upon many other factors. It is possible to have relatively high C concentration in soil water (DOC), but also dry, aerated soil. These conditions will ultimately limit denitrification, resulting in low DEA. Alternatively, DOC may be low in saturated soils because soil respiration used up most of the available C, but if denitrifying conditions had been good for the past while, the result would be a build-up of denitrifying enzyme. It is probable that although we were unable to see a relationship between our 'moment in time' DOC measurement and DEA, that functionally denitrification is limited by DOC.

Alternatively, Brugger et al., (2001) found that in riparian soils (up to 1 m in depth) particulate organic C and not DOC was the direct C source for microbial biomass and its quality determined microbial distribution. This fits well with our data, TOC or TON were more strongly related to DEA than DOC, especially in the surface soils. It may be that within the top of the soil profile, where TOC is plentiful, DOC is simply less important in creating ideal conditions for denitrification.

Differences by depth and vegetation

As expected in DOC was higher in the surface than in the subsurface soil depths, and there were no detectable differences between the subsurface depths (Table 3.1). Overall, DOC differed by depth ($p<0.0001$, $R^2=0.068$), by sampling period ($p=0.0602$, $R^2=0.013$), and marginally by vegetation type ($p=0.100$, $R^2=0.005$). However, even though these were significant effects, the R^2s suggest that none

of these models explained a high proportion of the variability found in DOC. In addition the CV% of each of these variables was about 100%.

As stated above, we hypothesized that DOC would be higher at the surface under the cool season grasses and higher at depth under warm season grasses. This did not turn out to be the case. Generally, DOC was higher in warm season grasses than in cool season grasses regardless of depth in the profile, although differences by vegetation type were hard to see, especially in the surface soils.

To see if the differences in DOC by depth were preventing us from seeing potential differences by vegetation type, surface samples were analyzed separately from the combined subsurface depths. In the surface samples, the mean DOC of warm season grass was 139 μg-C/L-soil water and cool season grass was 129 μg-C/L-soil water, however, they were not significantly different from each other ($p=0.6099$). At depth, warm season grass DOC levels (90 μg-C/L-soil water) were significantly higher than cool season grass (73 μg-C/L-soil water) ($p=0.0485$). This was especially surprising since the TOC pattern was almost the opposite. TOC was higher under the cool season grasses at the first two depths and marginally higher in deeper depths (not significantly higher after depth 2) than warm season grasses (See Chapter 2). DOC and TOC were significantly related to one another, although the relationship was relatively weak ($p<0.0001$, $R^2=0.036$, CV= 67 %). It may be that DOC makes up a relatively small amount of the TOC pool. DOC was related to total nitrogen ($p=0.01$, $R^2 = 0.033$, CV=78.3%), but the relationship was not as strong as that of TOC to TON ($p=0.0000$ $R^2 = 0.962$ CV=25%).

The sampling period of June 2002 was free from methodological problems and when statistical analyses were done on that data set alone, there was still no significant relationship between DOC and DEA ($p=0.290$), nor were there differences between warm and cool season grasses ($p=0.2111$). Again, comparisons were made between warm season grass and cool season grass at only the surface depth ($p=0.1551$) and at the combined subsurface depth ($p=0.1090$). There was no difference in mean DOC between warm season grasses and cool season grasses although again, mean DOC tended to be higher in warm season grasses. This lack of significance may be because the model lacked power due to low sample size and high variability, as there were differences in mean DOC by vegetation type in the other sampling periods, albeit close to the significance level.

Since patterns in mean DOC were similar between June 2002 and the other two sampling periods, it was assumed that even though DOC numbers from Summer 2001 and Fall 2001 may not be entirely accurate, they were probably unbiased, and any differences that were detectable could be accepted with some degree of certainty. A more appropriately designed and managed study may be able to pick out more clearly differences in DOC quality, composition and its relationship to denitrification.

In addition, the age and species diversity of the warm season grass buffers may have confounded the results somewhat. The warm season grass samples were combined for analysis. We were unable

to see differences between warm season grass buffers of different ages probably due to lack of power in the study. There was a slight trend toward increasing DEA with age of the buffer, but the confidence limit of the 0 y buffer and the 12 y buffer did overlap slightly. Additionally, the 5 y buffer has much higher species diversity than the other buffers, and consistently has higher means and lower variance than the other warm season grass buffers (See Figure 3 from chapter 2). However, we expected that the confounding effect of age would result in artificially lowered DOC means, perhaps preventing us from seeing a difference between vegetation types, or leading us to believe that cool season grasses provide more DOC than warm season grasses, when in fact the opposite was true. The fact that our results showed consistently higher DOC in warm season grasses, both at the surface and at depth, added strength to the assumption that the vegetation effect was stronger than the confounding effect of age of the buffer.

Bioavailable Carbon

% BDOC (% Bioavailable Dissolved Organic Carbon) is the percentage of the initial DOC that was used by microbes during a 30 d incubation, and is a measure of the availability of the carbon source to the microbial population. The methodological problem that affected DOC measurements was probably not as important to the quality of % BDOC data since it is a percentage of an initial quantity rather than a direct measurement, i.e. the initial measurement doesn't matter as much as how much of it was used up.

We hypothesized that carbon availability would be the best predictor of denitrification capability since it was a measure of the portion of the C pool that was actually available to denitrifiers. It was clearly significantly related to DEA ($p=0.0344$) when it was included in a multivariate model that included all organic C parameters. %BDOC varied by sampling period ($p<0.0001$), by vegetation type ($p=0.0308$), but not by depth ($p=0.2554$) (Table 3.1). Since our original hypothesis was that %BDOC would decrease with depth along with denitrification this was a surprising result. However, it makes sense in light of recent research by Fierer, (2002) who showed that microbial communities at depth were specially adapted to make use of recalcitrant carbon sources since that was the carbon that was available in the soil-water interface. In this case the percentage of the initial DOC that was used up by microbes would not change by depth, because the microbial community would shift by depth according to what C-sources are available to them. Possibly, if we had used a microbial community innoculum from surface soils and added it to DOC extracts from deep soils that had been biologically sterilized, we might have seen differences by depth of %BDOC since the microbial community at the surface is probably not adapted to make use of the highly recalcitrant DOC found at depth. Our results correspond to other studies. Corre et al., (1999) found that %BDOC did not decline by depth (only to 1 m) and assumed that resource availability was similar throughout the soil profile. These

results could also be explained by changing microbial communities by depth. Much is unknown about the ability of microbial communities to adapt in response to changing food sources. More research into microbial community structure by depth and into composition of DOC (fractionation) at different depths would be enlightening. Additionally, testing %BDOC using the methods we employed (based on Corre et al., 1999) did not give us really useful information for determining resource availability by depth. However there were differences in % BDOC by sampling period and vegetation type. There is no standard method for testing % BDOC and more research needs to be done in this area to determine more suitable methods (Chantigny, 2003).

Seasonal variation

Our hypothesis was that % BDOC would differ seasonally and would be generally higher in the fall and spring than in the summer due to vegetation inputs and reduced respiration in response to cooler temperatures. This hypothesis was held to be true, although we did have some surprising results. Differences in % BDOC by sampling period were driven by a large number of negative values in the Summer 2001 sampling period. A negative value for % BDOC is obtained when the final DOC value is greater than the initial value for any one sample. This could be due to microbial death and cell lysis in the incubation flask. The negative numbers were more frequent in the cool season grass plots, during the summer months, and at depth indicating that this was not random error since we could see a pattern in the distribution of negative numbers. However, negative numbers were seen at all sampling periods, including June 2002 using flat, unfolded filter papers, although the greatest percentage of them occurred during Summer 2001.

Summer 2001 %BDOC was lower than Fall 2001 and June 2002 when all depths were compared. June 2002 did not include the deepest depths and sampling occurred during just one day as opposed to sampling in Summer of 2001 which took place over a 7 week period, with cool season samples being taken near the end of July. To try and get a more realistic picture of true differences between the two summer sampling periods, Summer 2001 and June 2002 were compared using only the depths that they both had in common (surface, vadose, top mottling). Summer 2001 was still lower than June 2002 ($p<0.0001$) using this reduced data set. This may be a result of when the samples were taken (cool season 2001 samples taken in July instead of in June). June 2002 was probably closer to spring than mid-summer conditions, with corresponding differences in soil moisture, temperature and vegetational activity. Although we have no direct evidence, the negative numbers could also potentially be the result of some sort of pathogenic outbreak in the incubation flasks that was more severe in the Summer 2001 incubation.

Vegetation effects

When compared across all sampling dates and depths, cool season grass samples had much lower mean % BDOC than warm season grass samples ($p=0.0308$). These means were pushed down by the large number of negative % BDOC values. The lowest % BDOC numbers and the greatest quantity of negative numbers were in cool season grass samples, but a good number of warm season grass samples were negative as well. The most severely negative values tended to occur in the deeper depths, but the absolute number of negative values was relatively well spaced between the depths. This pattern of negative numbers suggests that there was something about the cool season grass samples at depth that made them more likely to be negative, but was not exclusive to them.

The difference between % BDOC means by vegetation type over all sampling periods and depths ($p=0.0308$) was driven by differences between vegetation types in June 2002. When differences in % BDOC by vegetation type were examined for each sampling period individually, only June 2002 showed differences between warm and cool season grasses ($p=0.0236$), Summer 2001 ($p=0.6489$) Fall 2001 ($p=0.3165$). It is unknown whether or not this is because we folded the filter papers in Summer and Fall 2001, but didn't in June 2002, or if it is a seasonal effect that we didn't capture the first time because of the long sampling period. We can say that the differences in June 2002 were so strong that they were able to overcome the lack of difference at the other two sampling periods.

Excluding negative numbers

The same comparisons between mean %BDOC by vegetation type and sampling period were run on the data set excluding all negative numbers. Interestingly, there was no difference between mean %BDOC in the two summer sampling periods, and fall %BDOC was higher than either summer. Additionally, during both summer periods, warm season grass %BDOC was higher than cool season grass, but during the fall this pattern disappeared with %BDOC in both vegetation types increasing dramatically and generally equalizing. In my opinion, something unexplainable by this experiment happened during the incubation process that caused the negative %BDOC numbers. This resulted in means that were depressed, especially during Summer 2001, which resulted in lower means than in June 2002 when negative numbers were included. When negative numbers were excluded from the data set, the patterns were more obvious because Summer 2001 and June 2002 were similar. We were able to see that generally, warm season grass had higher %BDOC in the summer when vegetation was actively growing, but that during the fall, %BDOC increased in both vegetation types, but especially in cool season grasses and differences between vegetation types disappeared. This fits with trends we saw in denitrification activity and with the idea that DOC inputs are greater with plant senescence.

C:N

The organic C:N ratio should give additional insight into C limitations due to availability. C:N differed by soil depth ($p<0.0001$), by sampling period ($p=0.0063$), but did not differ between warm and cool season grasses ($p=0.3431$). Organic C:N ratio dropped by depth as follows: surface and vadose > top mottling > mottled and bottom. Surface and vadose depths were not significantly different from each other and neither were mottled and bottom depths. Mean C:N ratio differences between sampling periods roughly followed the pattern of no difference between summer sampling periods (although mean of summer 2002 was slightly higher than the mean C:N of June 2002), no difference between December and October periods in the fall sampling with fall sampling only being different from June 2002 sampling period. An interesting trend with C:N ratio is that surface samples have a C:N of 11.4, vadose, 11.9, top mottling 10.26, mottled 4, 7.3 and bottom, 7.5. Soil humus C:N ratio is usually from 10:1 to 12:1 and soil microbial biomass generally is around 5:1 to 7:1. These soils, regardless of vegetation type, have relatively low C:N ratios that reflect mineralizing conditions. C:N ratios in these soils may indicate that the percentage of total C and N pools that are made up of microbial C and N increases with depth. This may help explain the relationship between total C and N and DEA. In the surface soils, higher TOC may result in better conditions for denitrification because particulate C makes good structure, aggregates, anaerobic microsites etc., but doesn't leave much in the way of available C to percolate downward through the profile. At depth where the only C inputs are from percolating DOC, or possibly from deep root inputs, most of the C and N are conserved in microbial biomass. The microbes would need C inputs from percolating DOC or direct root input in order to maintain populations over time, but the co*nservation* of C and N through quick uptake of lysed cells during periods of the year when inputs are slower may result in small adapted populations that quickly increase following pulses of DOC. Indeed we saw that DOC and TOC were incredibly low in many of our deep soils, and notwithstanding low substrate levels and high variability, we did see denitrifying enzyme activity at up to 4 m in depth. We know from other research that microbial populations are specially adapted to the food source they are most likely to find available to them, (Fierer, 2002); community structure is altered to optimize the redox conditions in which they are found (ability to switch quickly from aerobic to anaerobic respiration) (Pett-Ridge and Firestone, 2003); and do best under pulses of DOC rather than continual input (Lennon and Pfaff, 2003). These are exactly the conditions found at depth in the Bear Creek riparian buffer system. It is likely that at depth in these soils there is a small microbial population that is specially adapted to make use of occasional pulses of DOC and respond by increasing microbial biomass, and in anaerobic conditions, making use of nitrate as an alternate electron receptor during respiration.

Organic and Inorganic N

Total organic N was the only nitrogen factor that was significantly related to DEA (%TON p=0.0220, NO_3^- p=0.1419, NH_4^+ p=0.1576). TON was higher under cool season grasses than under warm season grasses (p=<0.0001), was higher in June of 2002 than any other sampling period (p=<0.0001), and varied by depth in descending order (surface>vadose>top mottling>mottled>bottom, p=<0.0001) (Table 2.2). As discussed in chapter 2 in greater detail, TON was highly correlated with TOC which may indicate that microbial biomass is limited by C, which in turn limits denitrification.

Total inorganic N (NO_3^- and NH_4^+) generally decreased by depth in the soil profile, with surface soil levels about twice what subsurface levels were (p<0.0001, surface = 4.3 mg-N kg-soil^{-1},subsurface (pooled) = 2.3 mg-N kg-soil^{-1}) (Table 3.2). There were no significant differences between any of the subsurface depths. Warm season grass samples had higher total inorganic N than cool season grass samples. Total inorganic N was significantly higher in June 2002 than the other sampling dates. This may be a result of the difference in sampling regime, or it may be a result of longer storage time of frozen KCl extracts from Summer 2001 and Fall 2001, especially since there was no seasonal difference found for nitrate, but there was for ammonium, which is more easily volatilized.

During Summer 2001 and Fall 2001, mean soil NH_4^+ content dropped with depth, although the only significant difference found was between the surface and everything else. In June 2002, there were no differences by depth, however, there were only three depths as the deeper depths were not sampled. All of the cool season grass samples were taken earlier in the year than the previous summer as well. June 2002 had higher ammonium levels than other sampling periods (p=<0.0001). Mean soil NH_4^+ levels were equal between vegetation types at all seasons and at all depths (p=0.9338).

Overall, mean soil nitrate levels did not appear to vary seasonally (p=0.1001), although warm season grass mean soil nitrate levels were higher than cool season grass levels in Summer 2001 and Fall 2001, but were not different in June 2002. High nitrate levels in June 2002, plus high ammonium levels resulted in an overall increase in total inorganic N levels during the last sampling season. The highest DEA numbers were also found in June 2002. It was surprising that inorganic N did not correlate with DEA, however, extremely high variation in DEA numbers may have prevented us from seeing the relationship. The same argument made to explain lack of correlation between DOC and DEA applies here as well since inorganic N is extremely variable within a short period of time and is used quickly. It may be that the absolute pool size is not as important to denitrification as the rate of nitrate supply to the pool, i.e. when nitrate is being supplied at a fast rate, more denitrification occurs,

but the absolute pool size may not be large. We do not have the type of data that could verify this statement.

The most interesting trend found in the inorganic N data was declining nitrate levels in the vadose and top mottling depths, with subsequent rises to near surface levels at the mottled and bottom depths. This suggests that nitrate is being used up in these depths, where there is relatively more C than deeper in the soil profile and corresponds with multilevel piezometer data taken in previous years from some of these sites (Andress 1999).

Multivariate Regression

A major hypothesis of this research was that denitrification potentials were affected by the amount of available carbon. To this end, we tested each soil sample for %TOC, DOC and %BDOC to determine which carbon indicator was most related to denitrification after accounting for the other controlling factors of denitrification that we had data for. Using the Proc Mixed procedure of SAS, several models were developed using Schwarz's BIC as a determinant of best fit (lower BIC is better). However, to determine which of the carbon variables was the most important in predicting denitrification the model was run with each of them separately, and BIC was compared to see which individual variable improved the model more. %BDOC seemed to be the best predictor (BIC 1759), followed by DOC (1925) and finally %TOC (1963) after the effects of all the other variables were considered. These results give some credence to the hypothesis that since DOC is the direct supplier of C to denitrifiers, especially at depth, it should be a better predictor of denitrification than total organic C alone, even though this data did not show a direct relationship between DOC and DEA. In addition, %BDOC is considered to be a measure of what proportion of the DOC is actually available to microbes for respiration, therefore it is a better predictor of denitrification potential than DOC. Of the other variables, only soil depth ($p<0.0001$), gravimetric water content ($p=0.0007$) and percent total organic N (TON) ($p=0.022$) significantly affected DEA.

In the above model, TOC was not significantly related to DEA ($p=0.9112$), but TON was. However, there was strong collinearity of the model between TOC and TON, which prevents us from having a robust understanding of their individual contributions to DEA. TOC and TON were highly significantly related to each other, with an r^2 of 0.95. The model was run twice more, once including TOC but not TON, and once including TON but not TOC to determine which was the best predictor of DEA. When the BICs were compared, TON by itself returned a BIC of 1731 and was highly significantly related to DEA ($p<0.0001$), and TOC by itself returned a BIC of 1740 and was also highly significantly related to DEA ($p<0.0001$). We can say that TON is probably a better predictor of DEA than TOC because the BIC is lower, although they both have some effect on DEA.

Additionally, when TON was removed from the model, the BIC of the model including TOC dropped from 1963 to 1740 which was lower than the %BDOC BIC of 1759. It may be that TOC is a better predictor of DEA than either %BDOC or DOC, but it is hard to determine if this is truly the case from these data.

We hypothesized that the relative importance of each C-parameter to DEA would change by depth in the soil profile. We checked to see if the above relationships would stay the same if we ran the model on only surface soils or only the soils at depth. For the surface samples only, a mixed multivariate regression model was run for DEA with sampling date, vegetation, a vegetation*sampling date interaction term, gravimetric water content NO_3^-, NH_4^+, TON, DOC, and %BDOC. At the surface, gravimetric water content ($p<0.0001$) and TON ($p<0.0001$) were the only two variables that were significantly related to DEA. TON probably reflects the relationship of TOC to DEA if it were included, since they tended to cancel out each other's effects in the model. The p-value for %BDOC went up from 0.0344 when all the depths were included to 0.6859 with just the surface depth, indicating that it was less important to DEA in the surface than at depth. The p-value for DOC dropped from 0.3829 with all the depths to 0.2933 with only surface. These trends may indicate that at the surface, carbon quantity rather than carbon availability per se may be more important to DEA. This is probably related to organic C in humus and greater aggregation in surface soils, which is related to more anaerobic microsites (Seech and Beachamp 1998, Sextone et al., 1988). This fits with patterns in DEA and %BDOC. DEA declined significantly with depth but %BDOC did not (see above discussion and Tables 2.1, 3.1). However, TOC (Table 2.2) and DOC amounts were much higher in the surface than at depth. While the percentage of OC that was bioavailable did not change by depth, the overall quantity of OC at the surface supported much higher denitrification potential.

To determine the relative importance of the parameters to DEA at depth, the same mixed model as outlined above for surface soils was run on all of the subsurface depths combined, excluding the surface depth. In this iteration, TON ($p<.0001$), vegetation type ($p=0.0367$) and %BDOC ($p=0.0432$) were significantly related to DEA. Gravimetric water was no longer significant at alpha 0.05 ($p=0.0591$), nor was DOC ($p=0.8721$). Deeper in the soil profile, when carbon is limiting to microbial growth, carbon availability is more important overall to DEA than other C parameters. In the surface soils OC is found in aggregates and particulate organic matter which is greatly reduced or non-existent in subsurface soils. Gravimetric water was less variable at depth and subsurface horizons (M and B) tended to be saturated or close to it.

Summary and Conclusions

After accounting for depth in the soil profile, TOC and TON, together with gravimetric moisture were the most important factors regulating denitrification capability in soils. DEA, TOC and TON were higher in cool season grasses as was gravimetric water content at all seasons. DOC and % BDOC were higher in warm season grasses, but this did not influence DEA until deep in the soil profile, i.e. below the rooting zone of cool season grasses. In the surface, high TOC and TON created conditions that supported high levels of denitrification. TOC and TON contained in humus are instrumental in creating well developed soil structure, and providing direct substrate to microbial respiration. In addition, soils with high organic matter tend to hold moisture better. Overall these conditions are favorable for creating anaerobic microsites within an aerated soil.

Deeper in the soil profile, TOC and TON were very low, and higher DOC and % BDOC in warm season grass soils, especially in Fall 2001, which may explain higher DEA at depth in warm season grasses. This may be because warm season grasses could provide more DOC which then could then percolate downward to subsoils. Warm season grasses also have greater rooting depths which results in a shorter distance for DOC to travel and correspondingly less microbial mineralization on its way down. Tufekcioglu, (2000) found that warm season grasses had significantly higher live fine and small root biomass than cool season grasses, which may help explain higher DOC levels in warm season grasses if we assume that much of the DOC comes from rhizodeposition. Additionally, modeling of the relative contributions of DOC and roots to total soil profile C, showed that in a temperate forest, dissolved organic C provided only 25% of the C, and roots provided the rest (Neff and Asner, 2001). If this is the case, rhizodeposition rather than percolation from surface soils may provide the more important C-source at depth.

Another explanation for low DEA in surface warm season grasses notwithstanding high DOC measurements might be related to lower gravimetric water in the warm season grasses. Greater plant uptake of water in warm season grasses may also result in lower DEA, even if substrate (such as DOC and inorganic N) is higher in warm season grasses, because water facilitates substrate transport and anaerobic conditions (Myrold, 1985). High DOC might simply be a reflection of concentrated DOC, because the soils are fairly dry, rather than an increase in C production. We saw a significant negative relationship between DOC and gravimetric water content that supports this conclusion (Figure 3.2). In this case, lower DEA may be more of a reflection of conditions that are not as good for denitrification such as smaller water holding capacity as a result of less aggregation (Sextone et al., 1988; Marquez, 2001), and less dead fine and small root biomass biomass, under warm season grasses as compared to cool season grasses (Tufekcioglu, 2000). Conditions that are less suitable for microbial respiration and transformations in surface soils may result in a "leaky" system where more, unmineralized DOC

and other substrates percolate deeper in the soil profile and may contribute to the slightly higher rates of DEA found at depth under warm season grasses. Finally, deeper rooting depths provided by warm season grasses (Kramer and Boyer, 1995) and greater above ground biomass in warm season grasses (Tufeckioglu 2000), can provide both nitrate removal from groundwater that provides 22% of the total N requirement for switchgrass (Huang et al. 1996).

The lack of significance of C-variables to DEA is probably more an artifact of the type of testing than a true lack of significant effect between OC and potential denitrification. Because of strong colinearity of the model, the effects of organic carbon and organic nitrogen tend to cancel the other out when they are both included in the model. Including either one separately results in a significant relationship. DEA provides an estimate of the activity of the enzyme present in the soil at the time of sampling which is a function of the recent microbial activity and site conditions (i.e. past anaerobicity, and C and N availability). Our measured OC parameters are instantaneous measures, which vary both temporally and spatially. Seasonal variation in OC and N may be related to inputs and exports due to grass and root growth, activity and decomposition, while spatial variation is related to depth in soil profile, aggregate presence and stability and moisture regime of the soil which redistributes OC and N and prevents O_2 diffusion.

References

Andress, R.J. 1999. Fate and transport of nitrate in groundwater within a riparian buffer in the Bear Creek Watershed. M.S. Thesis. Iowa State University. Ames, IA.

Brugger, A., B. Reitner, I. Kolar, N. Quéric, G.J. Herndl. 2001. Seasonal and spatial distribution of dissolved and particulate organic carbon and bacteria in the bank of an impounding reservoir on the Enns River, Austria. Freshwater Biology. 46: 997-1016.

Chantigny, M.H. 2003. Dissolved and water-extractable organic matter in soils: a review on the influence of land use and management practices. Geoderma. 113:3-4.

Clay, D.E., S.A. Clay, T.B. Moorman, K. Brix-Davis, K.A. Scholes, and A.R. Bender. 1996. Temporal variability of organic C and nitrate in a shallow aquifer. Water Resources. 30:559-568.

Corre, M.D., R.R. Schnabel, and J.A. Shaffer. 1999. Evaluation of soil organic carbon under forests, cool-season and warm-season grasses in the northeastern US. Soil Biology and Biochemistry 31:1531-1539.

Cosandey, A.C., V. Maitre, C. Guenat. 2003. Temporal denitrification patterns in different horizons of two riparian soils. European Journal of Soil Science. 54:25-37.

Fierer, 2002. Abstract from General Meetings of the Soil Science Society of America, Indianapolis, Indiana. ASA. Madison Wisconsin.

Huang, Y., D.H. Rickerl, and K.D. Kephart. 1996. Recovery of deep-point injected soil Nitrogen-15 by switchgrass, alfalfa, ineffective alfalfa, and corn. Journal of Environmental Quality 25:1394-1400.

Lee, K-H., T.M. Isenhart, R.C. Schultz, and S.K. Mickelson. 2000. Multispecies riparian buffers trap sediment and nutrients during rainfall simulations. J. Environ. Qual. 29:1200-1205.

Lennon, J.T. and L.E. Pfaff. 2003. Terrestrial subsidies in lake ecosystems: Importance of detrital source and supply on microbial metabolism. Poster presented at Cary Conference 2003: Ecosystem Function in Heterogeneous Landscapes. Institute of Ecosystem Studies. Millbrook, NY USA. April 29-May 1 2003.

Marquez, C. O. 2001. Soil aggregate dynamics and aggregate-associated carbon under different vegetation types in riparian soils. Ph.D. Dissertation. Iowa State University. Ames, IA.

Myrold, D.D., J.M. Tiedje, 1985. Diffusional constraints on denitrification in soil. Soil Science Society of America Journal . 49(3):651-657.

Neff, J.C., G.P. Asner. 2001. Dissolved organic carbon in terrestrial ecosystems: Synthesis and model. Ecosystems. 2001:29-48.

Pett-Ridge, J., M. Firestone. 2003. Temporal Heterogeneity is a determinant of ecosystem N-Cycling. Poster presented at Cary Conference 2003: Ecosystem Function in Heterogeneous Landscapes. Institute of Ecosystem Studies. Millbrook, NY USA. April 29-May 1 2003.

Seech, A.G. and E.G. Beauchamp. 1988. Denitrification in soil aggregates of different sizes. Soil Science Society of America Journal 52:1616-1621

Sextone, A.J., T.B. Parkin, and J.M. Tiedje. 1988. Short communication: Denitrification response to soil wetting in aggregated and unaggregated soil. Soil Biology and Biochemistry 20:767-769.

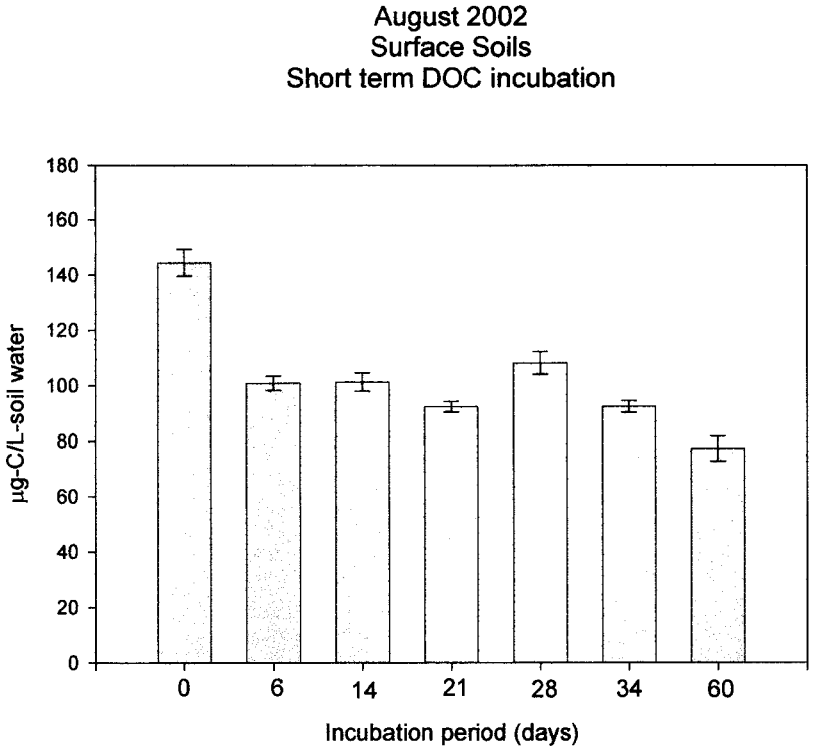
Sobczak, W.V., S. Findlay, S. Dye. 2002. Relationships between DOC bioavailability and nitrate removal in an upland stream: An experimental approach. Biogeochemistry. 00:0-19.

Sherrod, L.A., G. Dunn, G.A. Peterson, R.L. Kolberg, 2002. Inorganic carbon analysis by modified pressure-calcimeter method. Soil Science Society of America Journal. 66(1):299-305.

Tufekcioglu, A., J.W. Raich, T.M. Isenhart, and R.C. Schultz. 1999. Fine root dynamics, coarse root biomass, root distribution, and soil respiration in a multispecies riparian buffer in Central Iowa, USA. Agroforestry Systems. 44:163-174.

Tufekcioglu, A., J.W. Raich, T.M. Isenhart, and R.C. Schultz. 2003. Biomass, carbon and nitrogen dynamics of multi-species riparian buffers within an agricultural watershed in Iowa, USA. Agroforestry Systems. In press.

Figure 3.1. Dissolved Organic Carbon incubation experiment using surface soils from both warm and cool season grasses in August and December 2002.



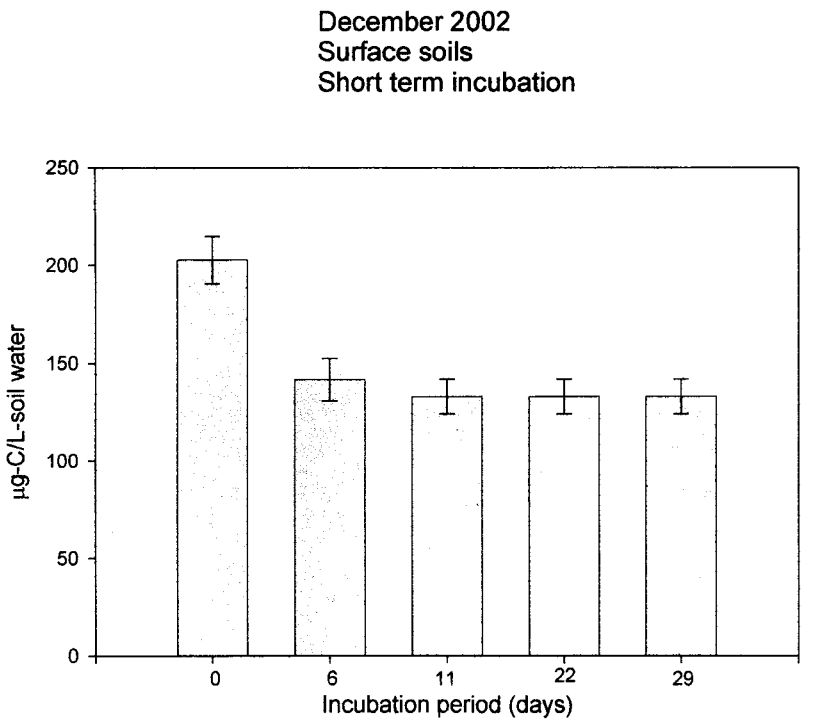


Figure 3.2 Relationship between gravimetric water content and mean dissolved organic carbon (µg-C l-soil water $^{-1}$) from the surface of Coland soils in the Bear Creek National Demonstration Watershed.

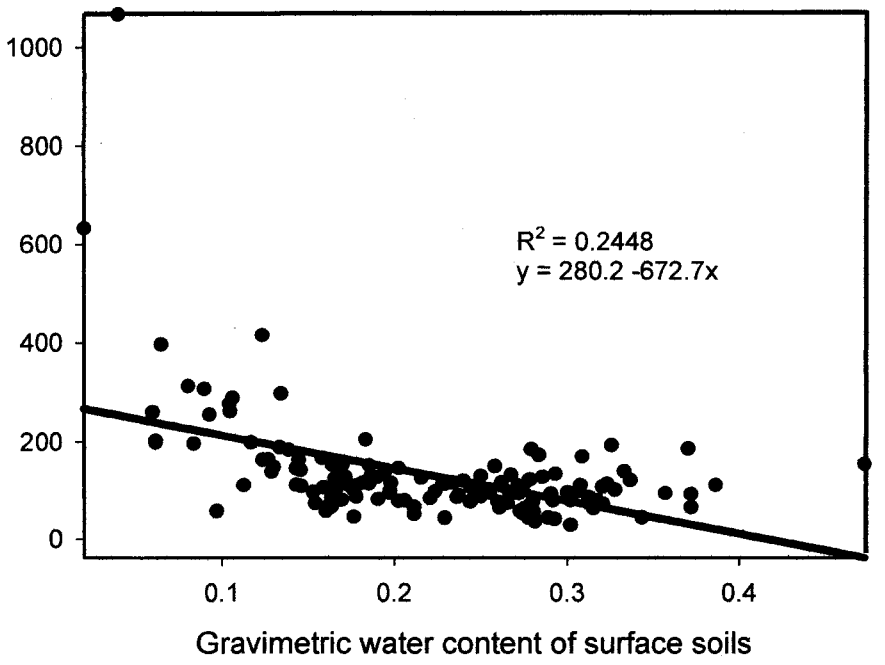


Table 3.1. Dissolved Organic Carbon and Percent Bioavailable Dissolved Organic Carbon in warm and cool season grass plots in Coland soils in the Bear Creek Riparian National Demonstration Watershed, near Roland, Iowa from the soil surface to up to 4 m in depth.

	DOC[†]				%BDOC			
	μg-C L-soil water^{-1}				% of initial DOC used in 30d			
	Cool Season Grass		Warm Season Grass		Cool Season Grass		Warm Season Grass	
Soil depth designation	Mean[‡]	STE[§]	Mean	STE	Mean	STE	Mean	STE
	Summer 2001							
Surface	161.9	12.6	150.8	32.0	14.6	27.8	-11.2[¶]	16.4
Vadose	80.3	12.4	143.1	32.0	-25.0	27.2	-4.87	16.0
Top Mottling	58.9	13.2	60.0	32.0	-7.6	29.1	-36.2	15.7
Mottling	43.8	12.9	52.2	31.3	-20.3	28.4	-31.9	15.7
Bottom	54.2	12.7	50.3	31.3	-91.8	27.8	-13.3	157
	Fall 2001							
Surface	125.7	9.7	149.6	20.4	51.2	4.5	51.0*	4.0
Vadose	115.8	9.7	116.9	20.4	50.3	4.8	59.8	4.0
Top Mottling	88.1	10.0	101.1	20.4	65.2	5.0	56.9	4.0
Mottling	86.4	10.2	101.3	21.3	68.1	4.8	73.4	4.2
Bottom	70.4*	11.2	110.9	21.8	72.6*	5.8	66.4	4.3
	June 2002							
Surface	99.7*	5.1	112.2*	7.1	9.8	3.9	23.2	8.1
Vadose	70.0	5.1	80.7	6.9	15.1	3.9	32.1	7.7
Top Mottling	47.9	18.0	81.2	9.4	32.3	13.52	35.3	10.4
Mottling	---	---	77.1	13.3	---	---	-1.4	14.7
Bottom	---	---	---	---	---	---	---	---

* Significant at the 0.10 probability level, signifies differences between depths at each sampling period.

[†] Long established (>50 y) cool season grass plots; recently reestablished (≤12 y) warm season grass plots.

[‡] $n \leq 25$

[§] Standard Error of the Mean.

[¶] Negative numbers are a result of final incubation numbers that were higher than initial incubation numbers. Reasons for this trend were unexplained, however, were more frequent in the cool season grass plots, during the summer months, and at depth. The means are generally depressed as a result of this phenomenon.

--- no samples

Table 3.2. Soil Nitrate and Ammonium in warm and cool season grass plots in Coland soils in the Bear Creek Riparian National Demonstration Watershed, near Roland, Iowa from the soil surface to up to 4 m in depth.

	Soil Nitrate[†]				Soil Ammonium			
	Cool Season Grass		Warm Season Grass		Cool Season Grass		Warm Season Grass	
Soil depth designation	Mean[‡]	STE[§]	Mean	STE	Mean	STE	Mean	STE
	Summer 2001							
Surface	0.57	0.23	1.33	0.44	2.9*	0.34	2.20	0.396
Vadose	0.49	0.23	0.87	0.44	1.33	0.34	1.42	0.396
Top Mottling	0.91	0.24	1.15	0.45	0.99	0.35	1.76	0.394
Mottling	0.63	0.24	1.93	0.45	1.10	0.35	0.71	0.394
Bottom	0.78	0.24	1.37	0.44	1.17	0.35	1.17	0.394
	Fall 2001							
Surface	0.78	0.20	2.4*	0.37	3.4*	0.35	2.77	0.65
Vadose	0.18	0.20	1.68	0.37	1.72	0.35	1.1	0.65
Top Mottling	0.29	0.21	0.69	0.37	0.94	0.36	0.78	0.65
Mottling	0.43	0.21	0.88	0.38	0.65	0.36	2.03	0.67
Bottom	0.52	0.23	0.88	0.38	0.797	0.41	0.93	0.67
	June 2002							
Surface	4.43	1.90	1.62	0.19	3.04	0.398	2.9	0.48
Vadose	0.77	1.90	1.09	0.19	2.99	0.390	3.2	0.47
Top Mottling	0.22	6.71	0.88	0.269	2.83	1.37	3.1	0.64
Mottling	---	---	0.68	0.396	---	---	2.7	0.94
Bottom	---	---	---	---	---	---	---	---

* Significant at the 0.10 probability level, signifies differences between depths at each sampling period.

[†]Long established (>50 y) cool season grass plots; recently reestablished (≤12 y) warm season grass plots.

[‡] $n \leq 25$

[§] Standard Error of the Mean.

--- no samples

Table 3.3 Gravimetric moisture in warm and cool season grass plots in Coland soils in the Bear Creek Riparian Buffer System, near Roland, Iowa from the soil surface to up to 4 m in depth. Subsurface soils are likely underestimates as water drained from sampling tube after reaching the water table.

	Cool Season Grasses[†]		Warm Season Grasses	
Soil Depth Designator	Mean[‡§]	STE[§]	Mean	STE
	Summer 2001			
Surface	0.17	0.013	0.16	0.014
Vadose	0.21	0.013	0.20	0.014
Top Mottling	0.24	0.014	0.20	0.014
Mottling	0.22	0.014	0.19	0.014
Bottom	0.19	0.014	0.18	0.013
	Fall 2001			
Surface	0.30	0.010	0.20	0.013
Vadose	0.24	0.010	0.19	0.012
Top Mottling	0.21	0.011	0.16	0.012
Mottling	0.19	0.011	0.17	0.013
Bottom	0.22	0.012	0.16	0.013
	June 2002			
Surface	0.28	0.010	0.21	0.012
Vadose	0.26	0.010	0.22	0.013
Top Mottling	0.18	0.040	0.18	0.017
Mottling	---	---	0.18	0.025
Bottom	---	---	---	---

* Significant at the 0.10 probability level, signifies differences between depths at each sampling period.

[†]Long established (>50 y) cool season grass plots; recently reestablished (≤12 y) warm season grass plots.

[‡] $n \leq 25$

[§] Standard Error of the Mean.

--- no samples

CHAPTER 4. GENERAL CONCLUSIONS

Assuming that soil organic C in riparian areas is limiting, in order to increase year-round denitrification potentials of soils in riparian areas, an increase in SOC in surface and especially subsurface soils is essential. Planting vegetation that has short term benefits to surface SOC such as cool season grasses will increase denitrification rates in surface soils, but may not have any effect on subsurface denitrification rates. In this research, we saw increased denitrification potential in surface soils under cool season grasses and under warm season grasses, while at depth, warm season grass soils had slightly higher denitrification potential than cool season grass soils. Depending on the location of the buffer, plant species on site, water flow paths, geology, and physical substrate, an increase in surface denitrification alone may not make substantial changes in overall field level NO_3^- removal rates, especially since high levels of NO_3^- may be leaving the system through groundwater export.

The fluctuating water table common in riparian buffers tends to increase the ability of buffer soils to denitrify. Water redistributes built up C and N substrate, limits O_2 diffusion and creates suitable conditions to denitrify. Plant uptake of groundwater N and direct inputs from agricultural runoff and atmospheric deposition may result in surface enrichment of N through litter fall and fine root turn-over, which may eventually saturate plant and microbial pools resulting in a net N export from the site. If the water table reaches the surface or close to the surface during part of the year, the potential exists to permanently remove much of the excess nitrogen. Drawdown of the water table redistributes available C and N downward in the soil profile, bringing fresh supplies of substrate to deeper microbial communities. In addition, fluctuating water selects for microbial communities that are specifically adapted to be good denitrifiers (Pett-Ridge and Firestone 2003; Tiedje 1988). This research clearly showed that soil water content controlled denitrification potential, it was the second most important controlling factor after accounting for depth in the soil profile.

Planting deep-rooted species may be the best way to get available C into shallow groundwater. Deep-rooted species such as trees and many warm season grasses and native prairie forbs put C directly into the phreatic zone in some cases, and at the very least provides C farther down in the soil profile, so that it has less distance to travel before it gets to the saturated zone. Even if denitrification potential in shallow groundwater is not directly increased by the presence of roots (in the case where high DO inputs prevent denitrification) the potential for direct plant uptake of NO_3^- from deep in the soil profile will stimulate denitrification in the surface indirectly through N-enriched plant litter (e.g. Lowrance 1992). During field sampling, we observed clear differences in root density and biomass between warm and cool season grasses. We often saw warm season grass roots as deep as 3 m below

the surface, while cool season grass roots were rarely found deeper than 50 cm. DOC was higher in warm season grasses than in cool season grasses. This may be accounted for by differences in live fine and small root biomass and soil respiration (Tufeckioglu, 2000) and also in microbial biomass (Pickle 1999). Pickle (1999), found that warm season grasses consistently had lower microbial biomass than cool season grasses. The combination of more DOC due to higher root biomass, plus lower microbial biomass and lower soil soil respiration may result in more DOC percolating to deep in the soil profile than in cool season grasses. Consequently, denitrification may be favored deep under warm season grasses.

Care should be taken when providing recommendations for creation of riparian buffers to increase N-removal functions. The proposed buffer design must integrate knowledge of the location's physical substrate and hydrology as well as current understanding of soil C sources and dynamics. One should not recommend only cool season grasses be planted because they have a quick effect on surface SOC and denitrification rates, nor should one recommend that warm season grasses be exclusively planted because over the long-term they might provide higher N-removal capacities.

Knowing that SOC might initially drop after conversion to warm season grass plots (Corre et al., 1999), that cool season grass plots have aggregate structure that is more conducive to denitrification (Marquez, 2001; Seech and Beauchamp 1988), different vegetation types have different litter qualities which can lead to differences in DOC lability (Burford and Bremner, 1975; Schipper et al., 1994), and that rooting depth can have potential impacts on denitrification (Gold et al. 1998; Lowrance 1992) lead me to believe that a mixed planting of various vegetation types would be most effective at maximizing denitrification potential of a site.

An integrated system including various vegetation types is the most effective way to increase a sites N-removal capacity over the long-term. Especially in sites with significant NO_3^- concentrations in groundwater, trees and warm season grasses planted for direct nitrate uptake from groundwater, as well as long-term deep additions of SOC to the subsurface soils, and cool season grasses for immediate surface improvements in soil quality and denitrification potential, should be planted. We found that the one warm season buffer with significantly higher species diversity than the other warm season grass buffers had higher DEA and variance than was expected for its relatively young age. The 5 year buffer was roughly equivalent to the 12 year buffer, which was a switchgrass monoculture.

Riparian buffer zones have been shown to be an extremely effective way to reduce the impact of non-point source pollutants such as NO_3^- in water. With increased knowledge about the biological processes that remove pollutants and their controls, we can improve buffer design to maximize nutrient removal capacities. Finally, correct placement of riparian buffers where groundwater levels fluctuate seasonally and ground water flow paths are constricted to near the rooting zone of the buffer will maximize the effective N removal capabilities of buffers.

Suggestions for further research

Current research at the Bear Creek buffer system, as well as others has clearly demonstrated that riparian buffers can effectively remove non-point source pollutants from surface and shallow ground water. However, further understanding of functional relationships will make the placement and design of such buffers more effective. Some suggestions for further research include:

1) A better understanding of the relationship between SOC and DOC with denitrification is essential. It would be greatly helpful to have better information about the relative make-up of DOC in both warm and cool season grasses and differences between them, and their temporal relationships. Documenting differences in native DOC by depth in the soil profile would also be useful for understanding how microbial mineralization of percolating DOC affects denitrification.
2) Study of the microbial community composition by depth would help to elucidate the relationships between vegetation, DOC and denitrification
3) Plant uptake of nitrate from groundwater by the different vegetation types at the buffer would be extremely useful information. This information could help us determine if subsurface nitrate uptake stimulates surface denitrification, and subsequent permanent removal from the site.

References

Burford, J.R., J.M. Bremner. 1975. Relationships between the denitrification capacities of soils and total, water-soluble and readily decomposable soil organic matter. Soil Biology and Biochemistry. 7: 389-394.

Corre, M.D., R.R. Schnabel, and J.A. Shaffer. 1999. Evaluation of soil organic carbon under forests, cool-season and warm-season grasses in the northeastern US. Soil Biology and Biochemistry 31:1531-1539.

Gold, A.J., P.A. Jacinthe, P.M. Groffman, W.R. Wright, and R.H. Puffer. 1998. Patchiness in groundwater nitrate removal in a riparian forest. Journal of Environmental Quality 27:146-155.

Marquez, C.O..2001. Soil aggregate dynamics and aggregate-associated carbon under different vegetation types in riparian soils. Ph.D. Dissertation. Iowa State University. Ames, IA.

Lowrance, R.. 1992. Groundwater nitrate and denitrification in a coastal plain riparian forest. Journal of Environmental Quality 21:401-405.

Pickle, J. E. 1999. Microbial biomass and nitrate immobilization in a multi-species riparian buffer. M.S. Thesis. Iowa State University. Ames, Iowa.

Pett-Ridge, J., M. Firestone. 2003. Temporal Heterogeneity is a determinant of ecosystem N-Cycling. Poster presented at Cary Conference 2003: Ecosystem Function in Heterogeneous Landscapes. Institute of Ecosystem Studies. Millbrook, NY USA. April 29-May 1 2003.

Seech, A.G. and E.G. Beauchamp. 1988. Denitrification in soil aggregates of different sizes. Soil Science Society of America Journal 52:1616-1621.

Schipper, L.A., C.G. Harfoot, P.N. McFarlane, and A.B. Cooper. 1994. Anaerobic decomposition and denitrification during plant decomposition in an organic soil. Journal of Environmental Quality 23:923-928.

Tiedje, J.M. 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium. Pages 179-244. in A. J. B. Zehnder editor., Biology of anaerobic organisms. John & Sons, New York.

Tufekcioglu, A., J.W. Raich, T.M. Isenhart, and R.C. Schultz. 1999. Fine root dynamics, coarse root biomass, root distribution, and soil respiration in a multispecies riparian buffer in Central Iowa, USA. Agroforestry Systems. 44:163-174.

Tufekcioglu, A., J.W. Raich, T.M. Isenhart, and R.C. Schultz. 2003. Biomass, carbon and nitrogen dynamics of multi-species riparian buffers within an agricultural watershed in Iowa, USA. Agroforestry Systems. In press.