Potential soil denitrification under riparian cropland, forests and pastures

in northeast Missouri

by

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Signatures have been redacted for privacy.
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**POTENTIAL SOIL DENITRIFICATION UNDER RIPARIAN CROPLAND, FORESTS AND PASTURES IN NORTHEAST MISSOURI**

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ABSTRACT

Riparian zones in agricultural watersheds can serve as important intercepts of non-point source (NPS) pollution exported from upland crop fields via overland flow and subsurface flow. Denitrification, the microbial reduction of nitrate (NO$_3^-$) to nitrogen (N) gases, is an important process that can reduce NPS pollution reaching receiving surface and subsurface waters. In this study, we measured potential denitrification of riparian soils under crops, forests and pastures in three sub-watersheds of the Mark Twain Lake watershed in northeast Missouri. Soils cores (110 cm deep) were taken in spring, summer and fall in 2000 and 2001. Denitrification enzyme activity (DEA) was determined using the acetylene block technique. In surface soils, there was one difference in DEA between forests and pastures, and few differences in DEA between crops and forest and/or pastures. Below 15 cm, denitrification potential decreased sharply. DEA was generally higher in soils under perennial vegetation, and lower in sandier soils. Soil texture, soil moisture and microbial biomass (measured in a companion study) greatly influenced potential denitrification in this study.
GENERAL INTRODUCTION

Thesis organization

This thesis is composed of three chapters. The first chapter is the General Introduction, within which the literature review is contained. The second chapter is a paper evaluating potential denitrification in riparian soils under crops, forests, and pastures in northeast Missouri. The third chapter is a general conclusion.

Introduction

Since Euro-American settlement, the landscape of North America has been highly altered. One such major alteration has been the conversion of tall-grass prairie to intensive row-crop agriculture in the Corn Belt eco-region of the Central USA (Burkart et al., 1994). The common practices of annual tillage and fertilization contribute non-point source (NPS) pollutants to receiving waters both in the immediate region and downstream, dramatically impacting the ecologic, sociologic and economic functions of these waters (Qiu and Prato, 1998; Colletti et al., 1994). The riparian areas that interface the crop fields with the water (surface or subsurface) bodies can serve as important intercepts for NPS pollution. However, land use practices in the riparian zone have a major impact on the efficacy of a site to reduce NPS pollution, and without proper management of these sensitive areas, that potential may not be realized.

The federal government has recognized the importance of these riparian areas. In the 1996 Farm Bill, funds were allocated to the National Conservation Buffer Initiative with the goal of buffering two million miles (3.2 million km) of riparian zones with perennial woody and herbaceous vegetation (www.nrcs.usda.gov). Also, the Environmental Quality Incentive Program (EQIP) allocates funds to private landowners to implement best management
practices (BMPs) in field, to reduce NPS pollution exported from agricultural lands (www.nrcs.usda.gov).

Many researchers have attempted to quantify the attenuation of sediment and agrichemicals in natural or re-established riparian zones, (Addy, Gilliam, Gold, Groffman, Jacinthe, Lowrance, Parkin, Schultz, and others). For example, Lee et al. (2000) found a 16.3 m wide switchgrass-woody riparian buffer trapped more than 92% of sediment and over 60% inorganic-N in run-off. O’Neill and Gordon (1994) found Carolina poplar (Populus x Canadensis Moench), approximately 1 m in height, filtered up to 14% more of the groundwater NO₃-N exported from a constructed riparian zone than a zone with no trees and a zone with a lower planting density. This implies that as the trees age, their capacity to reduce groundwater NO₃⁻ would increase.

**Denitrification**

Much of the research done to date shows riparian zones can be effective nitrate sinks (Peterjohn and Correll, 1984; Jacobs and Gilliam, 1984; Schipper et al., 1993; Lowrance et al., 1995). It is commonly believed that the two primary pathways for this N sink are plant uptake or denitrification; however, denitrification is the only process that converts the NO₃⁻ to a gas (N₂ or N₂O), removing it entirely from the site (Hanson, et al., 1994). However, there are many factors that influence the efficacy of a soil matrix to denitrify.

The three limiting/driving factors for denitrification are carbon, nitrate and oxygen (Tiedje, 1994). Carbon serves as the electron donor in denitrification, and is essential in contributing to microbial growth. Continuous inputs of carbon to a site through plant litter or root exudates or decomposition provide this carbon source, and therefore site-specific vegetation plays a major role in denitrification. For example, pine needle litter has been
shown to provide a less usable form of carbon than watercress leaves, and potential
denitrification with the pine carbon source was lower (Schipper et al., 1994). In addition,
there is much demand for labile carbon forms in soil from various microbes, so new inputs of
organic matter (OM) are quickly decomposed, leaving the recalcitrant fraction (Schipper et
al., 1994). Several amendment studies, in concert with denitrification studies, have shown
that adding a solution form of labile carbon (usually glucose or sucrose) to the matrix
increases denitrification (Jordan et al., 1998; Groffman et al., 1996;), especially at depth
(Yeomans et al., 1992). Frequently, in areas where dissolved organic carbon (DOC) does not
reach subsurface soil, denitrification is limited (White and Reddy, 1999; Yeomans et al.,

The second primary driving factor for denitrification is nitrate (NO₃⁻). As the electron
acceptor, nitrate in solution is reduced to N₂ or N₂O, and released to the atmosphere. When
oxygen is limited for respiration, nitrate, if present, becomes the alternate electron acceptor
for facultative anaerobes. This means that if conditions turn anoxic, nitrate is used by the
organisms already in place, and the enzymes needed for denitrification may be rapidly
produced. Like carbon, many enrichment studies have shown that NO₃⁻ is often the limiting
factor of denitrification (Jordan et al., 1998; Hanson et al., 1994; Strong and Fillery, 2002).

The third primary driving factor for denitrification is oxygen, as denitrification occurs
in the absence of oxygen. With rapid decomposition, oxygen is quickly consumed, leaving
conditions favorable for denitrification (Schipper et al., 1994). Water plays an important role
in creating microsites for denitrification. Not only can water encourage anoxic conditions, it
also serves to disperse substrate by redistributing C and N sources in the soil (Sexstone et al.,
1988). Jordan et al. (1998) showed that just the addition of water increased denitrification,
inferring that periods of frequent wetting could produce high rates of denitrification and maintain denitrifying populations.

Soil structure and texture also play important roles in creating conditions ideal for denitrification. Aggregated soils tend to have more carbon (C) (Marquez et al., 1998; Cambardella and Elliott, 1993), which can serve as the C source for denitrification. Additionally, aggregated soils generally hold water better than unaggregated soils, creating microsites for microbial activity. However, soils with low moisture and high aggregation have been shown to have diffusional constraints, thus limiting denitrification (Myrold and Tiedje, 1985). Particle size distribution of a soil is one control of aggregation, and sandier soils tend to have less aggregation than finer-textured soils. Furthermore, sandy soils are generally well-drained, quickly moving moisture off site and creating aerated conditions for rapid decomposition and consumption of the essential C sources. Sexstone et al. (1988) concluded that a sandy unaggregated soil had lower denitrification rates than a finer-textured unaggregated soil due to oxygen inhibition, but denitrification in the finer-textured unaggregated soil was also low, limited by “spatial discontinuity of available carbon, nitrate and denitrifying bacteria”. Conversely, finer textured soils tend to have more aggregation and slower OM decomposition due to more anoxic conditions, generally facilitating higher denitrification. Poorly drained soils have been shown to have higher rates of denitrification as well (Hanson et al., 1994).

Because of all the constraints on denitrification, rates are highly spatially and temporally variable. Parkin (1987) found that in one case, the variability between neighboring samples could be as much as an order of magnitude. Parkin (1987) also found that small patches of OM accounting for < 0.1 % of the mass of the sample, accounted for 85
% of the denitrification. Other researchers have found similar patchiness in NO$_3$-N removal in surface and subsurface denitrification (Groffman et al., 1992b; Jacinthe et al., 1998). Denitrification has also been shown to vary seasonally in surface soils (White and Reddy, 1999; Groffman and Tiedje, 1989). In humid regions, spring and fall tend to have higher rates (Hanson et al., 1994), likely due to the increased moisture contents and organic inputs from litterfall and root decomposition and exudation. This seasonality may also be due to the pulse of litter in the fall and the exhaustion of that C source by the following summer.

**Denitrification Method**

Denitrification can be measured several ways, but the two dominant methods are the acetylene inhibition/block method and N-isotope method, or $^{15}$N, both of which have limitations (Tiedje et al., 1989). For the N-isotope method, $^{15}$N is injected into a site and all $^{15}$N exported quantified. The missing fraction from the mass balance is considered to be lost via denitrification. This works well in mesocosms where N uptake by vegetation can be accounted for; however, in a field study it is difficult to account for all the mass lost. This method is typically used to create a mass balance using mathematical modeling of annual loss for a large area or landscape based on the subsample rates (Tiedje et al., 1989). The acetylene inhibition method uses C$_2$H$_2$ additions to inhibit the conversion of N$_2$O to N$_2$. N$_2$O is found in only trace concentrations in the atmosphere and is therefore more easily measured than N$_2$. The N$_2$O produced is simply converted to a rate of N loss. A disadvantage of the acetylene block method is that acetylene also inhibits nitrification, a process tightly coupled to denitrification that can provide the NO$_3^-$ source for denitrification. However, in a setting where off-site contributions of NO$_3^-$ are the primary input to denitrification, and where the
study seeks to identify the capacity of a site to ameliorate excess NO$_3^-$, this is not a limitation (Hill, 1996).

Denitrification enzyme activity (DEA) is commonly measured by the acetylene block technique. DEA is a measure of potential denitrification, reflecting the denitrification history of a site (Tiedje et al., 1989). The DEA method removes limiting factors by incubating the soil in a solution containing a labile C source and NO$_3^-$ in an anaerobic atmosphere, allowing expression of soil denitrification enzyme activity (Tiedje et al., 1989). Enzymes essential to denitrification are left as a residual in soil, so if the microbial population is induced by ideal denitrification conditions, the relative magnitude can be indicative of the recent past enzyme activity (Tiedje et al., 1989).

It has been shown that intact denitrification and DEA are correlated if many measurements are made over time (Groffman and Tiedje, 1989, Groffman et al., 1992a). DEA can be a good indicator of potential denitrification and is less variable than intact measurements (Parkin, 1987), and is therefore often used to make comparisons between sites with different vegetation and management aspects. Hunter and Faulkner (2001) used DEA to compare the denitrification potentials of a restored v. natural bottomland hardwood wetland, and Groffman and Crawford (2003) used the method to compare urban v. rural denitrification potentials.

Land Use

Land use has a major role in denitrification potential. Forests provide OM input in the form of litter fall (Groffman and Tiedje, 1989) and root decomposition (Tufekcioglu et al., 1999), which can become the C substrate for denitrification. The diversity of species in a forest also provides different forms of C, enhancing microbial populations by providing a
dynamic substrate. Trees have complex rooting systems, and the below-ground biomass can be a major source of carbon for denitrifiers (Tufekcioglu et al., 1999). This increased OM fraction contributes to greater aggregate stability and water holding capacity. In addition, forests have higher infiltration capacity (Bharati et al., 2002), allowing the necessary water to penetrate the soil surface and create microsites for denitrification. Groffman et al. (1992b) measured rates of DEA up to 732 µg N kg⁻¹ h⁻¹ in the top 15 cm in a riparian forest soil, and Groffman and Crawford (2003) recorded DEA rates of up to 4160 µg N kg⁻¹ h⁻¹ in the top 10 cm in a riparian forest soil. Although less research has focused on grass systems, they have been shown to function much like forests. Groffman and Crawford (2003) found no difference in potential denitrification in surface soils between herbaceous and forested sites, with DEA rates in the top 10 cm of herbaceous riparian soil up to 7600 µg N kg⁻¹ h⁻¹. Likewise, Addy et al. (1999) also found no differences in groundwater NO₃⁻ removal between herbaceous and forested sites. (Rates have been converted from those published for sake of comparison).

In contrast, surface soils under cultivated row crops have been shown to have lower rates of potential denitrification (Sotomayor and Rice, 1996). Annual tillage destroys macroaggregates that hold water and provide sites for denitrification (Cambardella and Elliott, 1993; Marquez et al., 1998), as well as induce rapid decomposition of OM (McCarty and Bremner, 1992) and leaching of dissolved C (Brye et al., 2001). Depending on the cropping system, the soil surface can be left bare for up to six months at a time, depriving the microbes of much needed C inputs. The shallow rooting depths and relatively small root biomass of corn (*Zea mays* L.) and soybeans (*Glycine max* (L.) Merr.) also deprive the subsurface of C inputs from decomposed roots (Tufekcioglu et al., 1999). Tillage can also
decrease infiltration and increase compaction (Bharati et al., 2002), impeding flow of substrate (C and NO$_3^-$) to the subsurface. Contrary to the higher rates found in forest or herbaceous systems, Sotomayer and Rice (1996) found average DEA in the top 20 cm of a cultivated field up to 5.38 µg N kg$^{-1}$ h$^{-1}$, and Parkin (1987) reported mean DEA in the surface 16 cm of a no-till corn field of 237 µg N kg$^{-1}$ h$^{-1}$.

**Study Description**

This research contributes to the program of the Agroecology Issue Team (AIT) of Iowa State University. The overall goal of the AIT is to develop locally-acceptable, economically viable, watershed management systems that increase the sustainability of agriculture in the Midwest with respect to surface and ground water quality, while improving the integrity of the aquatic and terrestrial ecosystems.

Specifically, the group has been working since 1990 to quantify the benefits of multi-species riparian buffers. Some of these projects have included: evaluating overland flow and sediment and nutrient transport (Lee et al., 2000); infiltration and bulk density (Bharati et al., 2002); soil organic matter and soil aggregate dynamics (Marquez et al., 1998); root biomass and dynamics (Tufekcioglu et al., 1999); soil microbial biomass and nitrogen immobilization (Pickle, 1999); intact and potential denitrification (Isenhart et al., unpublished); and hydrogeological constraints on riparian buffers (Simpkins et al., 2002).

Much of this work has been conducted in the Bear Creek watershed in Central Iowa, where over 11 km of riparian buffers have been established. However, it is important to understand these processes in different geologic and ecological regions to design buffers for maximum NPS pollution reduction.
Therefore, in 1998 the AIT began a collaborative project with the University of Missouri Center for Agroforestry (UMCA), Columbia, to expand the work from Iowa to Missouri. This suite of projects is evaluating riparian soil quality parameters and function for NPS pollution reduction in the Mark Twain Lake Watershed in northeast Missouri, a source of water to 16 rural water districts and communities in the region. The larger project evaluated dynamics such as nitrogen mineralization and denitrification, microbial biomass (Haake, 2003), overland flow, soil aggregate stability and the organic matter composition of those aggregates (Zhang, 2003).

Specifically, this component of the project evaluated the denitrification potential of riparian soils under crops, forests and pastures. We hypothesized that the forested sites would have the highest denitrification potential, followed by pastures and then crop fields. This was based on previous studies that have shown bottomland forests have a high potential to remove NO$_3^-$ (Hunter and Faulkner, 2001; Lowrance et al., 1995). We hypothesized that the pasture sites would have lower denitrification potential than forests due to reduced soil quality. Marquez et al. (1998) showed pastures to have fewer stable aggregates and less soil C and N, and Bharati et al. (2002) showed that infiltration rates in pastures were significantly lower than in woody plantations. Lastly, we hypothesized that the crop sites would have the lowest rates of denitrification potential because of low infiltration (Bharati et al., 2002), low C and N (Marquez et al., 1998) and frequent disturbance of the soil structure.
References


POTENTIAL SOIL DENITRIFICATION UNDER RIPARIAN CROPLAND,
FORESTS AND PASTURES IN NORTHEAST MISSOURI

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Abstract

Riparian zones in agricultural watersheds can serve as important intercepts of non-point source (NPS) pollution exported from upland crop fields via overland flow and subsurface flow. Denitrification, the microbial reduction of nitrate (NO$_3^-$) to nitrogen (N) gases, is an important process that can reduce NPS pollution reaching receiving surface and subsurface waters. In this study, we measured potential denitrification of riparian soils under crops, forests and pastures in three sub-watersheds of the Mark Twain Lake watershed in northeast Missouri. Soils cores (110 cm deep) were taken in spring, summer and fall in 2000 and 2001. Denitrification enzyme activity (DEA) was determined using the acetylene block technique. In surface soils, there was one difference in DEA between forests and pastures, and few differences in DEA between crops and forest and/or pastures. Below 15 cm, denitrification potential decreased rapidly. DEA was generally higher in soils under perennial vegetation, and lower in sandier soils. Soil texture, soil moisture and microbial biomass (measured in a companion study) greatly influenced potential denitrification in this study.

Introduction

Since Euro-American settlement, the landscape of North America has been highly altered. One such major alteration has been the conversion of tall-grass prairie to intensive row-crop agriculture in the Corn Belt eco-region of the Central USA (Burkart et al., 1994).
The common practices of annual tillage and fertilization contribute non-point source (NPS) pollutants to receiving waters both in the immediate region and downstream, dramatically impacting the ecologic, sociologic and economic functions of these waters (Qiu and Prato, 1998; Colletti et al., 1994). Reducing NPS pollution dramatically will require more than in-field best management practices (BMP's), but the riparian areas that interface the crop fields with the water (surface or subsurface) bodies can serve as important intercepts for this off-site NPS pollution. However, land use practices in the riparian zone have a major impact on the efficacy of a site to reduce NPS pollution, and without proper management of these sensitive areas, that potential may not be realized.

One major constituent of NPS pollution is nitrogen, primarily in the form of nitrate (NO$_3^-$). Much of the research done to date shows riparian zones can be effective nitrate sinks (Peterjohn and Correll, 1984; Jacobs and Gilliam, 1984; Schipper et al., 1993; Lowrance et al., 1995). It is commonly believed that the two primary pathways for this N sink are plant uptake or denitrification; however, denitrification is the only process that converts the NO$_3^-$ to a gas (N$_2$ or N$_2$O), removing it entirely from the site (Hanson, et al., 1994). However, there are many factors that influence the efficacy of a soil matrix to denitrify.

Because the primary driving factors for denitrification are carbon, nitrate and oxygen (Tiedje, 1994), how the landscape is vegetated and managed greatly influences loss of NO$_3^-$ via denitrification. Continuous inputs of carbon to a site through plant litter or root exudates or decomposition provide a carbon source for denitrification. Frequently, in areas where dissolved organic carbon (DOC) does not reach subsurface soil, denitrification is limited (White and Reddy, 1999; Yeomans et al., 1992). While NO$_3^-$ can be the limiting factor of
denitrification (Jordan et al., 1998; Hanson et al., 1994; Strong and Fillery, 2002), nitrate limitations are less common in agricultural watersheds where nitrogen is applied as fertilizer (Hill, 1996). When oxygen is limited for respiration, nitrate, if present, becomes the alternate electron acceptor for facultative anaerobes. Water plays an important role in encouraging these anoxic microsites for denitrification, and it also serves to disperse substrate by redistributing C and N sources in the soil (Sexstone et al., 1988).

Land use has a major impact on the controls of denitrification. Forests provide OM input in the form of litter fall (Groffman and Tiedje, 1989) and root decomposition (Tufekcioglu et al., 1999), which can become the C substrate for denitrification. Trees have complex rooting systems, and the below-ground biomass can a major source of carbon for denitrifiers (Tufekcioglu et al., 1999). In addition, forests have higher infiltration capacity (Bharati et al., 2002), allowing the necessary water to penetrate the soil surface and create microsites for denitrification. Although less research has focused on grass systems, they have been shown to function much like forests in terms of litter fall, root exudation and decomposition. Groffman and Crawford (2003) found no difference in potential denitrification in surface soils between herbaceous and forested sites. Likewise, Addy et al. (1999) also found no differences in groundwater NO₃⁻ removal between herbaceous and forested sites.

In contrast, soils under cultivated row crops can have lower denitrification potential due to disturbance and lack of C. Annual tillage destroys macroaggregates that hold water and provide sites for denitrification (Cambardella and Elliott, 1993; Marquez et al., 1998), as well as induce rapid decomposition of OM (McCarty and Bremner, 1992) and leaching of dissolved C (Brye et al., 2001). The shallow rooting depths and relatively small root
biomass of corn (*Zea mays* L.) and soybeans (*Glycine max* (L.) Merr.) also deprive the subsurface of C inputs from decomposed roots (Tufekcioglu et al., 1999). Tillage can also decrease infiltration and increase compaction (Bharati et al., 2002), impeding flow of substrate (C and NO$_3^-$) to the subsurface.

Therefore, the need exists to evaluate the denitrification potential of riparian areas in agricultural landscapes under different land use practices. In this study we examined denitrification potential of riparian soils under crops, forests and pastures in three sub-watersheds of the Mark Twain Lake watershed in northeast Missouri. Denitrification enzyme activity (DEA) and several other soil parameters were measured in spring, summer and fall of 2000 and 2001. The objective was to determine if forests had higher potential soil denitrification than pastures and crops, as was hypothesized, and to investigate the factors influencing potential denitrification.

**Materials & Methods**

**Study Site**

The study was conducted in three sub-watersheds of the Mark Twain Lake reservoir, a major drinking water source for a 12 county region of northeast Missouri. Crooked Creek, Long Branch Creek and Otter Creek are all third-order streams with watersheds of approximately 100 to 110 square miles (260 to 285 km$^2$). All sampling locations were located in Monroe County (39° 27′N, 92° 03′W).

Samples were collected in spring, summer and fall in 2000 and 2001 from riparian soils under corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) rotational crop fields; mixed riparian hardwood forests; and riparian cool-season grass pastures in each of the three watersheds. The Crooked Creek crop site was planted to soybeans both years; the Otter
Creek crop site was planted to soybeans in 2000 and corn in 2001; the Long Branch crop site was planted to soybeans in 2000 and was left fallow in 2001. The forests were multi-aged, composed primarily of walnut (*Juglans nigra* L.), hickory (*Carya ovata* Mill.), green ash (*Fraxinus pennsylvanica* Marsh.), elm (*Ulmus americana* L.), white oak (*Quercus alba* L.), hackberry (*Celtis occidentalis* L.) and basswood (*Tilia americana* L.). Cattle stocking densities in the pastures during the study period were 0.5 animal units/acre, 0.6 animal units/acre, and 0.7 animal units/acre for Crooked, Long Branch and Otter Creeks, respectively. The cattle were allowed to graze the pastures for about six months, from mid-spring to early autumn.

**Field Methods**

At each site, samples were collected from an area 100 meters long by 25 meters wide, which was oriented lengthwise along the creek and approximately 10 meters from the stream channel. Samples were collected from the four corners and from the middle of the 100 m sides, for a total of six sampling points per site. The sites were located on exclusively one vegetation type, for a total of nine independent sites.

Soils sampled were Moniteau silt loam (fine-silty mixed mesic Typic Ochraqualf), Piopolis silty clay loam (fine-silty, mixed, acid, mesic Typic Fluvaquent), Blackoar silt loam (fine-silty mixed, mesic Fluvaquentic Haplaquoll), and Arbela silt loam (fine, montmorillonitic, mesic Argiaquic Argialboll). Piopolis-Blackoar-Arbela is the dominant association characterized by poorly drained soils in bottomlands (Watson, 1979; Young and Geller, 1995).

Intact soil cores were collected using a 120 cm metal soil tube with a 110 cm long by 6 cm diameter zero-contamination liner (Giddings Company, Ft. Collins, CO). The tube 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.

**Lab Methods**

The cores were divided into five depths: 0-15 cm (Depth 1), 15-35 cm (Depth 2), 35-60 cm (Depth 3), 60-85 cm (Depth 4) and 85-110 cm (Depth 5). Depths 1, 3 and 5 were used for this study, while all depths were used for companion studies that evaluated microbial biomass and nitrogen mineralization (Haake, 2003). Soil was sieved through a 4 mm screen to remove rocks and roots, and a subsample for gravimetric moisture was weighed, dried at 105°C to constant weight (Gardner, 1986). The wet sample was stored at 4°C to be used for DEA analysis. The remaining soil was air-dried and sieved to 2 mm for particle size analysis, pH determination and total carbon and nitrogen content.

**Denitrification Measurements**

Denitrification enzyme activity (DEA) was determined using the acetylene inhibition method (Tiedje, 1994). Sieved wet soil (50g) was placed in a 250 ml Erlenmeyer flask with 50 ml nutrient broth containing NO₃⁻, glucose and chloramphenicol, which inhibits potentially interfering protein synthesis. The flasks were evacuated three times using a vacuum-helium flush cycle. The overpressure of helium was released so the pressure inside the flask was approximately equal to that of 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 minutes and stored in evacuated glass vials until analysis. The gas samples were analyzed for nitrous oxide using electron capture gas chromatography with a fraction collector autosampler (Parkin, 1985). The
concentration of nitrous oxide was converted to a loss rate of N using calculations from Tiedje (1994) with slight modifications.

**Other Soil Parameters**

Particle size was determined using a modified pipet method as described by Gee and Bauder (1986). Ten grams of soil were combined with 10 ml of a 5% sodium hexa-metaphosphate (HMP) solution and approximately 250 ml water. The samples were shaken for approximately 15 h and then filled with water to a mass of 410 g before stirring and pipetting. Using Stokes’ Law, the clay fraction was extracted with a pipet at a depth of 5 cm (Gee and Bauder, 1986) and dried for 24 h at 105°C. After extracting the clay subsample, the slurry was wet sieved through a 53 µm sieve (270 mesh size) for sand determination. The difference of the total weight (10g) minus (clay + sand) was assumed to be the silt fraction. Soil pH was determined with a glass electrode using a 1:1 (w/v) soil : water ratio (Thomas, 1996).

Total soil C and N were determined by high-temperature dry combustion using a Carlo Erba NA 1500 C/N/S elemental analyzer (Nelson and Sommers, 1996).

In a companion study (Haake, 2003), initial NO₃⁻ concentration, dissolved organic carbon (DOC) and microbial biomass were measured. These data were included in the multiple regression analysis, as N and C conditions can be controlling factors of denitrification.

**Statistical Analysis**

Differences in potential denitrification between vegetation types and depth were determined using a mixed model procedure (SAS Institute, 2001). Crooked Creek data were analyzed separately from Long Branch and Otter Creeks due to an unexpected texture difference, a consequence of working in areas of alluvial deposition. Multiple regression
analysis was performed to determine the factors that most influenced potential soil denitrification. Because the DEA data were log-transformed for analysis, estimates of error can not be reported.

Results

Texture

Soil texture varied among sites, despite similar soil mapping units. Crooked Creek (CC) sites had an average of 40% sand in the surface 15 cm, whereas Long Branch (LB) and Otter Creek (OC) sites had 13 and 5% sand, respectively. At 35-60 cm, CC had at least 35% more sand than LB and OC, and at 85-110 cm, CC had at least 26% more sand than LB and OC (Fig. 1). These differences were significant throughout the soil profile (p < 0.01). Because of these differences, land use, depth and seasonal effects on DEA for CC were evaluated separately from LB and OC (pooled). Differences in texture between LB and OC were not significant (α = 0.05), and therefore data for LB and OC were pooled for all analyses.

Moisture

Seasonal wetness varied among creeks, but CC had consistently less soil moisture than the other creeks. Figures 2 and 3 illustrate seasonal differences in soil moisture with vegetation types pooled. Figures 4 and 5 illustrate seasonal differences in soil moisture in the surface 15 cm across vegetation types. In LB and OC, fall 2000 was the wettest season in 2000 (p < 0.05) for the surface 15 cm, though fall 2000 was not statistically different from other seasons at Crooked Creek (p > 0.05) (Fig. 2). Fall 2001 was, in general, wetter than spring and summer 2001, but not statistically different (p > 0.05) for LB and OC (Fig. 3).
However, fall 2001 in the surface 15 cm was wetter than summer (p < 0.05) but not spring (p > 0.05) at CC. In general, CC had 3 to 5 % less soil water than LB and OC.

**DEA**

DEA decreased sharply with depth across all vegetation types and seasons (Figs. 6 & 7). In general, DEA at 35-60 cm and 85-110 cm was five to 150 times less than the surface 15 cm. With one exception, DEA in the surface 15 cm was significantly greater than DEA in subsurface soil across all vegetation types and seasons (p < 0.10). DEA in the surface 15 cm reached 33,068 µg N kg⁻¹ d⁻¹, whereas the maximum rates in the 35-60 and 85-110 cm depths were 563 µg N kg⁻¹ d⁻¹ and 277 µg N kg⁻¹ d⁻¹, respectively.

Over the course of the study, DEA ranged from 1 to 33,068 µg N kg⁻¹ d⁻¹, with the maximum occurring in the top 15 cm of a forest soil. However, DEA between forest and pasture sites was seldom different, regardless of how creeks were pooled. Surface DEA under crops was significantly lower than forest and/or pasture in all sampling periods except fall 2000 for CC (α = 0.10) (Table 1). Surface DEA under crops was significantly lower than forest and/or pasture only in fall 2000 and summer 2001 at LB and OC (Table 1), though in general, DEA was greater in forest and pasture (Figs. 8 & 9). Vegetation effects in subsurface soils were also more apparent within CC. Forest and/or pasture subsurface (35-60 and/or 85-110 cm) DEA was significantly greater than crops in all of 2000 at CC in (p < 0.10). However, at LB and OC, forest and/or pasture subsurface (35-60 and/or 85-110 cm) DEA was significantly greater than DEA under crops only in spring 2000 and 2001 (p < 0.10) (Table 1). Additionally, DEA was generally lower in CC soils than in LB and OC soils (Figs. 8 & 9).
Seasonal variation in DEA in forests was consistent throughout the study, increasing from spring to summer to fall, with fall the greatest in both years at all creeks (Figs. 10 & 11). However, seasonal variation in DEA was inconsistent from 2000 to 2001 for pastures and crop fields. Like forests, DEA in pastures was greatest in the fall for both years at LB and OC and in 2000 at CC (Fig. 11); however, in 2001, DEA was lowest in the fall at CC pasture (Fig. 8). DEA in LB and OC crops was highest in spring and lowest in fall in 2000, opposite the pattern in 2001 (Fig. 11).

From the multiple regression analysis, there was a significant relationship between DEA and soil moisture at LB and OC (p = 0.01). On the other hand, there was no significant relationship between DEA and soil moisture at CC (p = 0.26). DEA also had a significant relationship with microbial biomass C at CC and at LB and OC (p = 0.01 and p = 0.04, respectively). There were no significant relationships between DEA and initial dissolved organic carbon (DOC) and initial nitrate (α = 0.10).
Figure 1. Percent sand, silt and clay of soils for Crooked, Long Branch and Otter Creeks pooled across vegetation types for A) 0-15 cm, B) 35-60 cm, and C) 85-110 cm.
Figure 2. Percent soil water at different depths pooled among vegetation types for Crooked Creek in 2000 and 2001.

Figure 3. Percent soil water at different depths pooled among vegetation types and pooled for Long Branch and Otter Creeks in 2000 and 2001.
Figure 4. Percent soil water, 0-15 cm, under crops, forests and pastures along Crooked Creek seasonally for 2000 and 2001.

Figure 5. Percent soil water, 0-15 cm, under crops, forests and pastures pooled for Long Branch and Crooked Creeks seasonally for 2000 and 2001.
Figure 6. Potential denitrification through the soil profile for soils under crop, forest and pasture at Crooked Creek in fall 2000.

Figure 7. Potential denitrification through the soil profile for soils under crop, forest and pasture pooled for Long Branch and Otter Creeks in fall 2000.
Figure 8. Potential denitrification in soils, 0-15 cm, under crops, forests and pastures along Crooked Creek seasonally for 2000 and 2001. Land uses with the same letter within a season are not different ($\alpha = 0.10$).

Figure 9. Potential denitrification in soils, 0-15 cm, under crops, forests and pastures pooled for Long Branch and Otter Creeks seasonally for 2000 and 2001. Land uses with the same letter within a season are not different ($\alpha = 0.10$).
Table 1. Potential denitrification (µg N kg soil$^{-1}$ day$^{-1}$) under crops, forests and pastures for Crooked Creek and pooled for Long Branch and Otter Creeks in 2000 & 2001.

<table>
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</tr>
<tr>
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* Different letters within a season for creeks at each depth denote significant difference (p < 0.10). (n = 6 for Crooked Creek; n = 12 for Long Branch and Otter Creeks pooled)
Figure 10. Seasonal variation in potential denitrification in soils, 0-15 cm, under crops, forests and pastures at Crooked Creek.

Figure 11. Seasonal variation in potential denitrification in soils, 0-15 cm, under crops, forests and pastures pooled for Long Branch and Otter Creeks.
Discussion

Previous work has shown that perennial plant systems provide more suitable conditions for many biological and physical processes important to reducing NPS pollution than annually harvested crop systems. (Lee et al., 2000; Marquez et al., 1998; Tufekcioglu et al., 1999). Consistent with these works, results from this study show few differences in potential denitrification within riparian soils under forests and pastures, but show more differences between riparian crop soils and riparian perennial plant system soils (forest and/or pasture). Also, the differences in rates between Crooked Creek and Long Branch and Otter Creeks suggest that soils with less sand, in general, have higher potential for denitrification.

Differences in NO₃⁻ removal via denitrification between forest and herbaceous plant cover has been previously discounted (Groffman and Crawford, 2003; Lowrance et al., 1995). Groffman and Crawford (2003) found potential denitrification in highly disturbed riparian cool-season grass sites no different from natural riparian forests in surface soils. Additionally, Lowrance et al. (1995) found similar denitrification in a wet grassed area and a newly restored forest. While it is possible that denitrification in the newly restored forest was not different from the grassed area due to lack of establishment of the trees, rates reported were very similar to those of the mature natural riparian forest. In both cases, researchers commented on the high relative wetness of the grass sites. Therefore, the lack of differences is likely due to several controlling factors, including OM inputs, soil moisture, and available C and NO₃⁻.

Potential denitrification rates in the forests and pastures were similar to rates found in other studies. In Long Branch and Otter Creek soils, potential denitrification averaged
between 1270 µg N kg⁻¹ d⁻¹ and 5030 µg N kg⁻¹ d⁻¹ in the top 15 cm of the forest and pasture soils over all seasons. Flite et al. (2001), Hunter and Faulker (2001), and Groffman and Crawford (2003) all recorded DEA of similar magnitude for surface soils under riparian forests and cool-season grasses.

This suggests that perennial vegetation facilitates high denitrification in moist silty loam soils. Lower DEA in the sandy Crooked Creek soils, relative to Long Branch and Otter soils, also suggests moisture strongly influenced denitrification. While the vegetation and precipitation dynamics of Crooked v. Otter and Long Branch Creek soils were nearly identical, the percent water in Crooked Creek soils was lower throughout the study by approximately 3 to 5 % in the surface 15 cm.

It is important to note that the grass sites in this study were actively grazed pastures, not undisturbed natural areas. However, the pastures were not heavily used, and therefore the biological integrity of the soil was relatively uncompromised. The low cattle stocking rates on the pastures allowed a substantial grass cover to accumulate throughout the growing season. Anecdotally, by the summer sampling season, the grass cover was thick and 0.5 to 1 m tall, suggesting ample recovery of the grass root structures.

Low potential denitrification in the crop fields relative to forests and pastures suggests land use has a major impact on the ability of a site to reduce NO₃⁻ via denitrification. This coincides with results from companion studies that found lower soil microbial biomass (Haake, 2003) and lower large and small macroaggregates and higher silt+clay fractions (e.g. higher unstable aggregates) (Zhang, 2003) in the same crop sites. The continual input of OM to the forest and pasture soils seemingly provides the needed C
for microbial processes. Annual cultivation and harvest of crops encourages rapid
decomposition at the surface and reduces potential organic input from the site.

Lower DEA at depth was expected. Yeoman and Bremner (1992) noted decreased
denitrification at depth in Iowa soils, and attributed the low rates to lack of organic C.
Sotomayor and Rice (1996) reported rates of approximately $2 \mu g \text{ N kg}^{-1} \text{ d}^{-1}$ to $72 \mu g \text{ N kg}^{-1} \text{ d}^{-1}$ for soils 20-80 cm deep, and undetectable below 80 cm. Vegetation with deep or large
amounts of fine root biomass may be especially important in our study soils. Throughout the
three watersheds, there is a claypan layer between approximately 55 and 100 cm (Watson,
1979), potentially eliminating transport of OC to the subsurface via percolation. Therefore,
the only way to get OC to the unsaturated subsurface zone is root exudation and
decomposition, which would be reduced under an annual crop system.

Seasonality of denitrification is well documented. Lowrance et al. (1995) and White
and Reddy (1999) found rates generally higher in periods of higher moisture, specific to the
climatological region of the study. For this study, potential denitrification was generally
greater in the fall, coupled with greater soil moisture content. However, spring was generally
wetter, but this higher moisture did not result in higher observed rates of DEA. This suggests
that while moisture can be a control of denitrification, it is not the only driving factor for the
process. Low DEA in spring may be reflective of depressed microbial activity over the
winter and lag time before the population is fully active.

The differences in DEA between Crooked Creek and Long Branch and Otter Creeks
indicate soil texture strongly influences DEA. In Crooked Creek soils, there were more
significant differences in DEA among vegetation types than in Long Branch and Otter Creek
soils. Higher DEA under forests and pastures in Crooked Creek soils may suggest that
perennial vegetation is especially important in sandy soils. The more rapid drying of the sandy soils in the Crooked Creek watershed suggest that OM inputs and not water may be the controlling factor of denitrification within that watershed.

**Conclusions**

Perennial vegetation increases potential soil denitrification, inferring that perennial vegetation may also increase *in situ* denitrification (Groffman and Tiedje, 1989). This is very important to consider when managing riparian zones in agricultural landscapes. If overland flow is intercepted and infiltrated into riparian soils under perennial vegetation, it is more likely to have reduced NO$_3^-$ concentrations due to high denitrification activity in the surface soils. Additionally, while DEA decreased sharply below 15 cm, DEA was still present, and denitrification is a possible sink for NO$_3^-$ in the vadose and saturated zones. While in this study potential denitrification was similar between forest and pasture soils, this is likely the result of low pasture utilization.

Additionally, groundwater monitoring has been initiated along a transect from the crop field through a newly-established grass filter to the stream at Crooked Creek. Preliminary results show shallow groundwater NO$_3$-N concentrations up to 17 mg/L at crop edge and up to 10 mg/L at stream edge (Simpkins, personal communication), indicating a general trend of NO$_3^-$ reduction through the riparian zone. It is possible this reduction is a result of subsurface denitrification, indicating future research evaluating subsurface NO$_3^-$ removal pathways is critical to understanding buffer function.
References


CONCLUSIONS

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Additionally, groundwater monitoring has been initiated along a transect from the crop field through a newly-established grass filter to the stream at Crooked Creek. Preliminary results show shallow groundwater \( \text{NO}_3^- \)-N concentrations up to 17 mg/L at crop edge and up to 10 mg/L at stream edge (Simpkins, personal communication), indicating a general trend of \( \text{NO}_3^- \) reduction through the riparian zone. It is possible this reduction is a result of subsurface denitrification, indicating future research evaluating subsurface \( \text{NO}_3^- \) removal pathways is critical to understanding buffer function.
References

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I am also grateful to Tim Parkin for use of his lab facilities and equipment, and his expertise and insightfulness regarding denitrification and data analysis.

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