

Denitrification in a Grassed and a Wooded, Valley and Ridge, Riparian Ecotone

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ABSTRACT

Denitrification is one of the major mechanisms responsible for changes in NO_3^- -N concentrations in shallow groundwater as subsurface flow passes from agricultural fields to the stream. In this study, denitrification is examined in a grassed and wooded riparian ecotone common to the Valley and Ridge physiographic province in Pennsylvania. Denitrification rates of 102 and 10 $\mu\text{g N kg}^{-1} \text{ soil d}^{-1}$ were measured using soil slurry and intact core incubation techniques, respectively. The grassed riparian site exhibited greater denitrification rates than the wooded site. Carbon amendments resulted in an increase in denitrification at the wooded site and suggest that denitrification may be C limited at this site. Denitrification generally decreased with distance away from the stream and also decreased with depth in the soil. Patterns of denitrification measured with the soil slurries corresponded closely to patterns of soil NO_3^- content.

ELEVATED LEVELS of N impair the beneficial uses of ground and surface waters in agricultural and mixed-use watersheds. Groundwater transports much of the N contained in stream flow to channel systems as NO_3^- -N. This subsurface flow normally passes through a band of riparian zone soil before discharging to the stream. A body of research has documented the effectiveness of riparian zone processes in removing NO_3^- -N from agricultural drainage (Lowrance et al., 1984; Peterjohn and Correll, 1984; Schnabel, 1986; Cooper, 1990; Groffman et al., 1992; Lowrance, 1992; Hanson et al., 1994; Haycock and Pinay, 1993). Thus, the management or establishment of riparian buffer systems between agricultural fields and streams is a focus of nonpoint source pollution control efforts.

Nitrate losses within riparian forests result primarily from denitrification in the saturated zone (Jacobs and Gilliam, 1985; Peterjohn and Correll, 1984; Cooper, 1990; Lowrance, 1992) and vegetative uptake from the unsaturated soil and capillary fringe (Fail et al., 1986). Peterjohn and Correll (1984) measured plant uptake rates that accounted for 33% of observed groundwater NO_3^- -N removal in a riparian forest. Groffman and Tiedje (1989) associate seasonal patterns of denitrification with the activity of forest trees. They observed that the end of the spring pulse of denitrification coincided with tree leaf breakout, and the onset of the fall pulse of denitrification was coincident with leaf die-off and litterfall. These authors suggest that water and mineral N uptake by trees during the growing season may be substantial, and thus may hamper denitrification.

The width of riparian zone necessary to remove NO_3^- from shallow groundwater, and what type of vegetation significantly affects NO_3^- removal remain major

questions in riparian zone research. Researchers on the Atlantic Coastal Plain (Lowrance 1992; Jordan et al., 1993) and in France (Pinay et al., 1993) report nearly complete NO_3^- removal, from groundwater, in 30 m of riparian forest. Most of the NO_3^- reduction measured by Lowrance (1992); and Pinay et al., (1993) occurred in the first 10 m of the riparian buffer. Pinay et al., (1993) also measured denitrification at several positions within the riparian forest. Denitrification enzyme assays were not different throughout the riparian forest; however, when they measured denitrification on unamended cores, rates were highest at positions most distant from the stream.

Forested sites were the focus of most riparian zone research in recent years. A few studies compared NO_3^- removal from groundwater in forested and grassed riparian zones. Forested and grassed vegetated buffer strips along the Embarras River in Illinois both reduced the NO_3^- concentration of shallow groundwater by up to 90% (Osborne and Kovacic, 1993); however, on an annual basis the forested buffer was more effective at retaining nitrate. A poplar riparian buffer along the River Leach in southern England retained 99% of N entering it (Haycock and Pinay, 1993). A similarly situated ryegrass riparian buffer retained only 84% of entering N. Groffman et al., (1991) measured denitrification rates in two forested and two grassed vegetated filter strips in Rhode Island. Denitrification rates measured on cores amended with NO_3^- or NO_2^- in addition to glucose were an order of magnitude greater for the grassed strips. Each of the above authors suggested that either the content or nature of organic C accounted for the observed differences.

Incubations of soil slurries (Tiedje, 1982), and intact soil cores (Ryden et al., 1987) are commonly used to measure denitrification rates of riparian zone soils. Several authors have used both techniques (Ambus and Lowrance, 1991; Pinay et al. 1993; among others). The slurry techniques, which provide an index of denitrification, are frequently used to examine the effects of C and NO_3^- additions on denitrification, while the unamended core techniques more closely represent denitrification under ambient conditions.

While studies of the N removal efficiency of riparian zone soils have received considerable attention in the past two decades, the vast majority of these studies have been conducted in coastal plain regions of the USA. A review of the literature reveals limited research performed on the NO_3^- -N removal efficiency of riparian areas common to the Ridge and Valley physiographic province that encompasses much of the Chesapeake Bay drainage.

The objectives of this study were (i) to determine denitrification rates and their distribution in a grassed and wooded riparian ecotone in the Ridge and Valley

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physiographic province of Pennsylvania, (ii) to determine if denitrification rates were NO_3^- -N or C limited, and (iii) to compare results from two commonly used tests of denitrification; an anaerobic, soil slurry incubation technique and an aerobic, intact core incubation technique. Both methods provide better indices of denitrification than representations of in-situ rates. If the distribution of denitrification within an area and ranks among different areas are similar with either method, then perhaps wider inferences can be drawn about denitrification in riparian ecotones by combining studies using either method.

MATERIALS AND METHODS

Site and Soils Description

This research was conducted at a wooded and a grassed riparian buffer along the East Mahantango Creek, approximately 40 km north of Harrisburg in central Pennsylvania ($76^\circ 34'$ W. long, $40^\circ 41'$ N. lat., 210 m msl). The site is in the Valley and Ridge physiographic province on soils derived from sandstone and shale. Climate in the Mahantango Valley is humid and temperate. Annual precipitation and stream flow average approximately 1000 and 580 mm, respectively. Sixty to eighty percent of stream flow is predominantly groundwater runoff (Gburek et al., 1986). East Mahantango Creek is a second order stream at the study site. The soils at the site are alluvial, medium-textured, and well to somewhat poorly drained (Soil Survey Staff, 1982). The parent material is a transported, yellowish brown, medium to fine grained sand, typical of alluvial deposits in the area. Mahantango Valley is underlain by two geologic formations, Trimmers Rock (Late Devonian) and Catskill (Late Devonian-Early Mississippian). The Trimmers rock formation is primarily shale in the study area. The overlying Catskill formation consists of interbedded sandstones, siltstones, and shales, and becomes increasingly coarse-grained to the North. Previous analysis of well yields (Cline, 1968) indicated that rock fracture patterns are as important to formation permeability as rock type, and based on specific capacity data (Urban, 1977) the two formations are hydrologically similar. The site was characterized with a seismic refraction survey, and contours of piezometric head.

Most of the time-distance plots from the seismic survey of the site showed three or four layers with different velocities (Schnabel and Gburek, 1995). The upper layer with a seismic velocity of approximately 300 m/s is typical of dry soil. Seismic velocities increased to approximately 1500 m/s indicating the position of the water table 1 to 1.5 m below land surface. The next recognizable layer had a seismic velocity of approximately 3000 m/s, typical of highly fractured bedrock in the area (Urban and Pasquarell, 1992). The deepest layer began about 4.5–12 m below land surface, and had seismic velocity typical of unfractured bedrock (4800–7500 m/s). Geologic materials with seismic velocities in the range of layer four have very low permeabilities and are considered flow restrictive. The contours of bedrock depth provide no evidence of buried stream channels that might divert flow. Piezometric water potentials measured once a week for a month during site characterization showed water potential gradients towards the stream (Schnabel and Gburek, 1995). The pattern of piezometric water potentials is indicative of flow from the field to the stream rather laterally along it.

The wooded riparian buffer is 40 m wide and contains an assortment of woody vegetation. This site is on a gently rolling landscape located down gradient of an agricultural field planted

to a corn and small grain rotation. Basher silt loam (coarse-loamy, mixed mesic Fluventic Dystrochrepts) is the soil mapped within this site. The 18 m wide grass riparian buffer is on an alluvial flood plain immediately down-gradient of the same agricultural field as the wooded site. The riparian vegetation of this site is tall fescue (*Festuca arundinacea* L.) grass and Linden silt loam (coarse-loamy, mixed, mesic, Fluventic Dystrochrepts) is the soil mapped at this site. The water table near the stream at both these sites ranges from 10 cm above to 60 cm below the land surface. The water table at the field–riparian zone interface ranges from 15 to 90 cm below the land surface.

Field Sampling

Soil sampling for this study was conducted along four transects at each site. The transects, spaced 15-m apart, extended from the stream edge, through the riparian area, toward the riparian zone–agricultural field interface. Soil samples were collected at four positions along each transect: at the stream edge, and 3, 9, and 18 m up-gradient of the stream. The transects in the wooded stream section also were sampled at the riparian zone–agricultural field interface, 40 m from the stream.

Intact soil cores measuring 5.8-cm diam. were collected in December 1993, and in April and May 1994 with a metal soil sampling probe fitted with a removable plastic sleeve. The soil sampling probe was attached to a hydraulic hammer (Geoprop; Salina, Kansas) and driven to the desired depth (~ 1 m) or until fractured rock limited further penetration. Fractured rock was frequently encountered at depths shallower than 1 m, resulting in shorter cores. The plastic sleeves containing the soil core were sealed with rubber caps to prevent evaporation. Each core was then returned to the laboratory and stored in a walk-in cooler at 4°C prior to analysis.

Each plastic sleeve containing an intact soil core was split down its length and the core sectioned into several 7.5-cm long increments. Due to differences in length of the intact cores, the number of depth increments obtained from each core varied from three to four. Generally, four depth increments were sectioned from each core sample: 7.5–15 cm, 37.5–45 cm, 60–67.5 cm, and 87.5–95 cm below the soil surface. When we were unable to get a full core, the first section was 7.5–15.0 cm below the soil surface and the other depth increments were collected from the bottom of each intact core and two intermediate sections along the core.

Soil moisture was determined on each core segment by air drying 10 g at 90°C . Soil NO_3^- was determined by ion-exchange chromatography of a 2M KCl extract.

Denitrification Experiments

Denitrification rates were measured using the anaerobic, soil slurry incubation method described by Tiedje (1982), and the intact soil core incubation method described by Ryden et al. (1987).

Soil Slurry Incubations

Denitrification rates were determined by measuring N_2O production at ambient NO_3^- and C concentrations for samples collected in December 1993. Soil slurries were created by treating 10 g of soil with 10 mL of a 1 g chloramphenicol (chl) L^{-1} solution to inhibit the synthesis of enzymes during incubation. These soil slurries were made anaerobic by repeated evacuation and flushing with N_2 gas containing acetylene (C_2H_2) at a concentration of 0.1 L L^{-1} . We incubated the slurries anaerobically at 22°C on a reciprocating shaker and sampled

headspace gas 24 h after incubation began. We stored headspace samples in evacuated crimp top vials until analysis. All gas samples were analyzed for N_2O concentrations by gas chromatography. Nitrous oxide concentrations for each gas sample were determined by comparing the area under the peak to a curve created with 0.5, 1.16, 5, and 10 $\mu L L^{-1}$ prepared standards. All N_2O concentrations were corrected for the amount of N_2O dissolved in water.

Factors limiting denitrification were examined by measuring the separate effects of NO_3^- -N and C additions and the effect of NO_3^- plus C additions. The effects of NO_3^- -N additions on denitrification were determined with soil slurries created by treating 10 g soil samples with 10 mL of a 200 mg NO_3^- -N L^{-1} and 1 g chl L^{-1} solution. Soil slurries used to measure C effects were created by adding 10 mL of a 2 g glucose-C L^{-1} and 1 g chl L^{-1} solution to the soil samples. Denitrification Enzyme Assays (DEA) were determined by adding 10 mL of a 200 mg NO_3^- -N L^{-1} , 2 g glucose-C L^{-1} and 1 g chl L^{-1} solution to 10 g of field moist soil. The soil slurries were then treated as described above.

Intact Core Incubations

Denitrification rates for each riparian site were also determined using the acetylene (C_2H_2) inhibition soil core method described by Ryden et al. (1987) on samples collected in April and May 1994. Intact cores measuring 2.5-cm diam. by 7.5-cm were removed from the center of each depth increment, collected during April and May 1994, and placed into disposable 60 mL syringes. The head space volume of each syringe was adjusted to approximately 30 mL and the syringes were then sealed with rubber serum stoppers. Three milliliters of head space gas were removed from each syringe and replaced with 3 mL of C_2H_2 . The syringes containing the soil samples were incubated aerobically at 16°C and 5 mL of head space gas were removed for N_2O analysis after 2 and 6 h. Head space gases were treated as described above. Denitrification rates were calculated from differences in headspace concentration at 6 and 2 h.

Statistical Methods

All data were examined using standard statistical procedures contained in the Statistical Analysis System software package (SAS Institute, 1985). Simple descriptive statistics and details on the distribution of collected data were obtained with the Univariate procedure. The data were not normally distributed and, therefore, transformed using a natural log transformation to stabilize error variance. All subsequent statistical analyses of denitrification rates were performed using the log transformed data. Although the statistical analyses were performed on transformed data, all denitrification rates given in the following tables were untransformed to give rates in meaningful units. Backtransforming means of log transformed data gives geometric means of the initial data rather than arithmetic means. Consequently, the means in the tables will be smaller than the values reported in the text.

A two-step approach to data analysis was used in this study. Denitrification, soil moisture, and soil NO_3^- interact in a complex and dynamic manner. Consequently, correlations among these variables may mask true differences resulting from the independent variables in a simple univariate procedure. Thus, a multivariate analysis of variance was first run with these three variables simultaneously used as the dependent variables in the model. The independent variables used were: type of vegetation (type), solution used to create the slurry (amendment), soil depth (depth), and distance from the stream (dis-

tance). The second step was a separate univariate analysis of N_2O , soil moisture, and soil nitrate, using the same independent variables as the multivariate analysis.

For both analyses, transect was considered a random effect and the transects within (nested) each type were used as replication. All other variables were considered fixed effects. Higher order interactions that were not significant were dropped from the model and pooled with the error term. The overall model with the class effect of type and the continuous effects of 'depth' and 'distance' results in a homogeneity of slopes model (Freund and Littell, 1981).

RESULTS

Denitrification Rates from Soil Slurries

Data Description

Denitrification rates for all soil slurry incubations ranged from 0.01 to 2821.28 $\mu g N kg^{-1}$ dry soil d^{-1} . The mean denitrification rate was 101.81 $\mu g N kg^{-1}$ dry soil d^{-1} with a standard deviation of 245.91. Soil nitrate and soil moisture in the original soil samples were normally distributed with means equal to 2.81 mg kg^{-1} , and 23.4% on a weight basis, respectively.

Relationships between denitrification and soil nitrate or soil moisture have been shown by many researchers. If these dependent variables are correlated, then the assumptions of a univariate analysis of variance are violated and inferences drawn about denitrification are in doubt. Under these circumstances, multivariate analysis of variance is a more appropriate beginning point for data analysis. Denitrification rate, soil nitrate and soil moisture were all correlated ($P > 0.05$).

Type of vegetation ($P > 0.0001$), distance from the stream and depth in the soil ($P > 0.03$) affected the combination of denitrification rate, soil moisture and soil nitrate by the Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Greatest Root statistics. Two interactions also significantly affected the combination of dependent variables. They were interactions between the type of vegetation and soil depth, and between the type of vegetation and distance from the stream. The interaction between type of vegetation and amendment significantly affected the combination of denitrification rate, soil moisture and soil NO_3^- ($P > 0.0433$) only by Roy's Greatest Root.

The multivariate analysis showed that the independent variables affected the data as a whole. It is now appropriate to examine the univariate analyses of denitrification rate. Distance from the stream, depth in the soil and type of riparian vegetation affected denitrification rates (Table 1). Interactions between: type of vegetation and amendments added during incubation, type of vegetation and distance from the stream, and added amendments and distance from the stream also affected denitrification rates (Table 1).

The Effect of Vegetative Type, and Nitrate and Carbon Amendments

Primary objectives of this research were to determine if the type of vegetation, woods or grass, and amendments

Table 1. Analysis of variance for log(denitrification) from soil slurries using univariate analysis.

Source	df	Type I SS	F Value	P > F
Distance	1	82.56	23.48	0.0001
Depth	1	353.99	100.68	0.0001
Type	1	91.38	25.99	0.0001
Amendment	3	9.30	0.88	0.4511
Type × Amendment	3	51.37	4.87	0.0026
Distance × type	1	57.52	16.36	0.0001
Depth × type	1	9.18	2.61	0.1075
Distance × depth	1	27.91	7.94	0.0053
Distance × Amendment	3	43.24	4.10	0.0073
Depth × Amendment	3	3.53	0.33	0.8005
Distance × Depth × Type	1	7.44	2.12	0.1470

added to the soil during incubation have an effect on denitrification rates measured in riparian ecotones.

Denitrification rates were greater in the grassed section of the riparian zone than the forested section (17.3 vs. 5.8 $\mu\text{g N kg}^{-1}$ dry soil d^{-1} , respectively). Rates were greater in the grassed section of the riparian zone for each amendment except for the carbon amendment (Table 2), where they were not different.

Adding organic C to samples from the wooded section of riparian zone increased denitrification rates (Table 2). The addition of C and NO_3^- did not increase denitrification over that for either C or N additions alone. Denitrification rates for the grassed riparian zone were not different for any of the amendments. Neither soluble C nor NO_3^- limited denitrification in the grassed section.

We can infer from Table 2 that the availability of soluble organic C limits denitrification in the wooded riparian ecotone. Prior to slurrying, NO_3^- contents of soil collected from the grassed section of riparian zone were greater than that of soil from the wooded section. There was no difference in moisture contents. Differences in denitrification rates corresponded more closely to differences in soil nitrate content than soil moisture, for the dataset as a whole.

The Effect of Depth and Position

Denitrification rates frequently exhibit systematic variations with soil depth (Lowrance, 1992) and distance from streams (Pinay et al., 1993). These systematic variations result from the distribution of variables such as soil NO_3^- and soil moisture within near-stream soil, and their relationships with denitrification. The distribution of denitrification rates in near-stream soils may be complex since controlling variables can be distributed in ways that either reinforce their effect on denitrification, or counteract their effect.

Denitrification rates and soil NO_3^- decreased with depth, while soil moisture increased with depth. Although the slopes were small, the trend in denitrification rates with depth is in agreement with that expected from soil nitrate concentrations, and in opposition to the trend expected from soil moisture alone.

Denitrification rates in both the grassed and wooded riparian zones were lowest in samples collected nearest the stream (Fig. 1a). Under both types of vegetation, denitrification rates increased, to a point, then decreased with distances from the stream. There is an interaction

Table 2. Least-squares means of denitrification rate ($\mu\text{g N kg}^{-1}$ dry soil d^{-1}) in the wooded and grassed section of the riparian zone.

Amendment	Wooded	Grassed
Chloramphenicol	2.6c†	14.1a
Chloramphenicol + NO_3^-	2.1c	14.4a
Chloramphenicol + organic C	8.8a,b	9.5a,b
Chloramphenicol + NO_3^- + organic C	4.4b,c	15.2a

† Values followed by the same letter are not different at the 0.05 level.

between distance and vegetation type because near the stream denitrification is greater in the grassed riparian ecotone than the wooded riparian ecotone. Further from the stream, denitrification is greater in the wooded riparian ecotone. Both soil NO_3^- and soil moisture were significantly affected by distance from the stream. While both are significant, the pattern in soil moisture (Fig. 1c) is not as useful as the NO_3^- pattern (Fig. 1b) for explaining the distribution of denitrification rates with

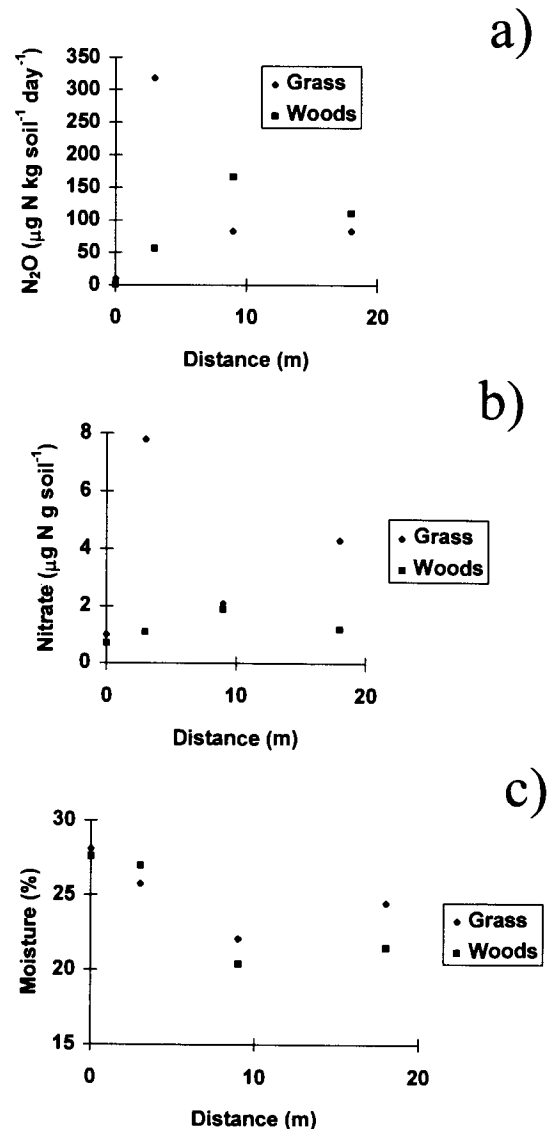


Fig. 1. Patterns of (a) denitrification (mean of all treatments), (b) NO_3^- , and (c) moisture content with distance from the stream for soil slurry incubations.

distance from the stream. The general pattern of nitrate concentration within the riparian ecotones mimics the pattern of denitrification rate (Fig. 1), unlike the pattern of moisture content. It appears that, at this site, soil NO_3^- concentrations were a greater influence on denitrification rates than moisture content, at least for rates measured with the soil slurry technique.

The fact that the depth increments were not the same for all cores caused the interactions between type and depth, and depth and distance. An assessment of the significance of interactions involving depth would require deleting much of the data in order to balance the dataset. Much of the power to detect differences in other factors would be lost by balancing to run a traditional analysis of variance; however, the sequential consideration of factors in the homogeneity of slopes allows us to sequentially examine effects entered into the model even when numerous interactions are significant.

Denitrification Rates from Intact Cores

Data Description

Denitrification rates for all intact core incubations ranged from 0.01 to 735.43 $\mu\text{g N kg}^{-1} \text{ soil d}^{-1}$. The mean denitrification rate was 10.26 $\mu\text{g N kg}^{-1} \text{ soil d}^{-1}$ with a standard deviation of 63.59. As with the slurry data, all statistical analyses of denitrification rates were performed using the log transformed data. Soil NO_3^- and soil moisture content data were normally distributed with means equal to 2.29 mg kg^{-1} , and 23.1% on a weight basis, respectively.

A multivariate analysis of variance with denitrification rate, soil nitrate and soil moisture as dependent variables was performed because the dependent variables, denitrification rate and soil moisture, were correlated ($P = 0.0002$).

Type of vegetation ($P > 0.0431$), distance from the stream ($P > 0.0060$), and depth in the soil ($P > 0.0001$) all significantly affected the combination of denitrification rate, soil moisture and soil NO_3^- by each of Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Greatest Root statistics. No interactions were significant and were therefore removed from the model.

Only the type of vegetation and distance from the stream affected log(denitrification rate) determined on intact cores (Table 3).

The Effect of Vegetative Type, Depth, and Position

The geometric mean of denitrification rates in the grassed riparian zone was 1.4 $\mu\text{g N kg}^{-1} \text{ dry soil d}^{-1}$ compared with 0.7 $\mu\text{g N kg}^{-1} \text{ dry soil d}^{-1}$ for the wooded riparian zone. There were no differences in soil moisture or soil NO_3^- between the sites.

The denitrification rates and soil moisture were greatest near the stream and generally decreased with distance from the stream. Soil nitrate concentration was constant along the transect unlike the December sampling used in the slurry incubations. Hence, denitrification appears to be controlled by moisture contents. The higher moisture content near the stream causes a greater resistance to O_2 diffusion resulting in a larger volume of anaerobic microsites. While soil moisture increased and soil nitrate decreased with soil depth, denitrification rates were not different for the depths tested. Superimposing the expected effect of NO_3^- and moisture content on denitrification, negates the effect of either variable individually.

DISCUSSION AND SUMMARY

We measured denitrification rates from intact cores that average 10 $\mu\text{g N/kg soil}^{-1} \text{ d}^{-1}$ at our riparian zone sites within the Valley and Ridge physiographic province of Pennsylvania. Although denitrification rates for other riparian soil of the Valley and Ridge are not available, these rates are within the wide range of values reported by researchers on the Atlantic Coastal Plain, in Rhode Island and southern England for incubations of intact soil cores. Rates determined by incubating soil slurries (102 $\mu\text{g N/kg soil}^{-1} \text{ d}^{-1}$) were substantially higher. Differences in denitrification rates measured by these two methods have also been reported (Ambus and Lowrance, 1991; Lowrance, 1992; Pinay et al., 1993). The slurry method provides an anaerobic atmosphere that is more favorable for denitrification, and reduces the resistance for reactants to diffuse to the bacteria and for products to diffuse away. Three of the four solutions used to produce the slurry also supply one or more reactant that otherwise may limit denitrification. This research was not conducted to provide reliable, long-term estimates of field N loss. Consequently, the magnitude of measured rates is not very important. The measured rates are useful as indicators for comparing denitrification among locations and its distribution at a location.

Denitrification rates were generally greater in the grassed section of riparian zone for both measurement methods. There was also a significant linear trend, with both of the methods, in which denitrification rates decreased with distance from the stream. The pattern of denitrification in the slurried samples was consistent with that expected from the original soil NO_3^- content. In contrast, denitrification in the intact cores was more consistent with the soil moisture content distribution. This pattern of denitrification with distance from the stream is the reverse of that reported by other researchers (Lowrance, 1992; Pinay et al., 1993). A reversal of flow with nitrate enriched stream water flowing into the riparian ecotone could cause the pattern we observed; however, piezometric head data collected as part of the overall project has always shown the direction of flow to be from the field to the stream. The difference in denitrification patterns more likely results from the impact that depth to flow restrictive layers has on riparian zone hydrology. The greater depth to an impermeable layer at our site results in a greater vertical component

Table 3. Analysis of variance for log(denitrification) from intact cores using univariate analysis.

Source	df	Type I SS	F Value	P > F
Distance	1	15.96	6.35	0.0127
Depth	1	4.41	1.75	0.1872
Type	1	17.20	6.84	0.0097

to groundwater discharge at the stream. The combination of properties favoring denitrification (elevated levels of NO_3^- -N, moisture and organic C) is more likely near the field-riparian zone interface where impermeable layers are shallow, and near the stream where impermeable layers are deeper.

A trend in denitrification with depth below the soil surface was only apparent for the soil slurries, and it was consistent with the nitrate distribution. The soil slurries are made artificially anaerobic minimizing the impact of moisture content in providing an environment favoring denitrification. In the soil cores, the reduction in denitrification rate expected from decreasing NO_3^- concentration with depth is probably more effectively balanced by an increase in denitrification anticipated from the greater moisture content at depth. The net effect for the soil cores was no significant trend with depth. The differences in relative patterns of dependent variables between the methods also could be effected by moisture and nitrate differences when the samples were collected.

Denitrification rates were limited by organic C in the wooded riparian ecotone. Adding organic C to soil from the wooded riparian ecotone brought denitrification rates up to levels measured in the grassed riparian ecotone. This suggests that differences in the content or composition of organic C are the cause of denitrification rate differences between the riparian ecotones.

Unsurprisingly denitrification rates measured with the two methods (soil slurries and intact cores) were significantly different; however, the inferences drawn from the data, regarding the effect of vegetation, distance from the stream and depth in the soil, were similar. Consequently, either of the methods could be used to compare the denitrification activity within a site or among a group of sites.

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