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DENITRIFICATION POTENTIAL OF NONTIDAL RIPARIAN WETLAND SOILS IN THE VIRGINIA COASTAL PLAIN

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Abstract—In the Atlantic Coastal Plain, riparian wetlands are thought to play an important role in the removal of NO_3^- from groundwater. The denitrification potential of the Bibb (coarse-loamy, siliceous, acid, thermic Typic Fluvaquents) series, located in the Nomini Creek Watershed, Virginia, was evaluated in the laboratory using soil columns under saturated flow conditions. Soil columns were infiltrated with synthetic groundwater containing 3.2 ± 0.1 mmol NO_3^--N (44 ± 2.0 mg NO_3^--N l^{-1}). Soil samples were collected from two surface horizons and one subsurface horizon in May (soil temperature 16.4°C), September (19.9°C), and November (13.5°C) of 1993 at 12 sites along the stream. Denitrification rates (N_2O-N evolution in the presence of acetylene) were significantly higher for soils incubated at 19.9°C than at 16.4 or 13.5°C. The highest mean denitrification rates were measured in the ponded surface horizon (0.40 ± 0.17 μmol N_2O-N (g DW)$^{-1}$ d^{-1} at 16.4°C, 0.65 ± 0.27 at 19.9°C, 0.40 ± 0.25 at 13.5°C) in comparison to the terrestrial surface (0.17 ± 0.11, 0.28 ± 0.13, 0.16 ± 0.11) and subsurface (0.04 ± 0.02, 0.11 ± 0.06, 0.04 ± 0.01) horizons. Rates of denitrification were significantly correlated with organic carbon for the ponded surface horizon across all three temperatures. For the terrestrial surface and subsurface horizons, organic carbon was related to denitrification rates for only those soils incubated at 13.5°C. Relations between NO_3^- loss and denitrification rates were present for the terrestrial surface horizon across all temperatures, and at 13.5 and 16.4°C for the ponded surface and subsurface horizons. Spatial variability accounted for less than 10% of the variation in denitrification rates, while soil horizon amounted to about 50%. Within the soil columns, the relative NO_3^- concentration decreased rapidly with increasing temperature in the surface horizons but much less so in the subsurface horizon. For the surface horizons, incubated at the higher temperatures, NO_3^- concentration in the effluent was very low, suggesting that denitrification may have been limited by NO_3^- availability. The denitrification potential for the surface horizons was very high, related in part to the high levels of organic carbon present in those horizons. Copyright © 1996 Elsevier Science Ltd

Key words—denitrification, riparian wetlands, NO_3^- loss, spatial variability

INTRODUCTION

Within the last decade, water quality preservation in the Chesapeake Bay and the surrounding watersheds has become a high priority issue. As a result of intense agricultural activity in the Atlantic Coastal Plain region, large amounts of nutrients and pesticides enter groundwater and surface waters on an annual basis, leading to a widespread contamination of the Bay (Mostaghimi *et al.*, 1993). Thus, a significant portion of the water quality degradation in the Bay is caused by nonpoint source pollution due to agricultural activities (Hamilton, 1992). In many areas of the Atlantic Coastal Plain, riparian wetlands border intensively managed agricultural fields and may act as important biological filters with the potential to remove nutrients, such as NO_3^-, as they move with groundwater through this zone.

Several studies, conducted in the Coastal Plain of Eastern and Southern U.S.A., have indicated that riparian wetlands effectively remove NO_3^- from groundwater flowing from beneath agricultural fields before it can enter into nearby streams. Reductions in groundwater NO_3^- concentrations of up to 90% have been measured by Jacobs and Gilliam (1985) in North Carolina, by Lowrance *et al.* (1984) in Georgia, and by Peterjohn and Correll (1984) in Maryland. In Virginia, Snyder (1995) observed that elevated NO_3^- concentrations in groundwater moving from beneath an upland agricultural field (located in the Nomini Creek watershed) and then under a riparian forest was reduced by 48% before entering a stream bordering the field. The topography of the Nomini Creek site differs from the North Carolina, Georgia, and Maryland study sites. The depth to the water-table is deeper at Nomini Creek and the

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Table 1. Physicochemical properties of the three different soil horizons from the Bibb series ($n = 48$)

	Soil horizons		
Parameter	Ponded surface	Terrestrial surface	Subsurface
pH (±SD)	5.2 (±0.7)	4.1 (±0.9)	4.2 (±0.5)
Texture	Silty loam, sandy loam, loamy sand	Sandy loam, loamy sand	Loamy sand, sand
Organic carbon (% ± SD)	9.3 (±4.8)	7.8 (±4.6)	0.8 (±0.7)

distance between the agricultural and riparian wetland is greater. The Nomini Creek watershed site is also unique, since the water-table (and stream) is atop of a restrictive layer. Processes responsible for removing NO_3^- from groundwater as it moves through the riparian buffer zones include denitrification, plant uptake, and assimilative NO_3^- reduction by microbes (Simmons *et al*., 1992).

Information regarding the potential of riparian wetlands to act as decontamination buffer zones is important in the continued development of best management practices (BMPs), designed to reduce nonpoint source pollution problems. The objective of this study was to evaluate temporal and spatial variability of riparian wetland soils in terms of their denitrification potential in the laboratory using soil columns under saturated-flow conditions. Relationships between soil organic carbon, NO_3^- loss, and denitrification rates were also investigated.

MATERIALS AND METHODS

Soil and site description

The Nomini Creek watershed, located 80 km northeast of Richmond, Virginia, was selected for this study because it is typical of the Coastal Plain of Virginia, with a distribution of land use activities of approximately 43% agricultural, 54% woodland, and 3% homestead. Also, there are nonpoint source pollution discharges in the watershed to impact water quality. Riparian wetland soils were collected down-gradient from an agricultural field, where corn, wheat, and soybeans are grown in rotation. The stream draining this area is approximately 12 m lower in elevation than the field edge at a distance of 60 to 150 m. The seepage face formed at the outflow boundary of the aquifer generally contains large quantities of organic matter and low dissolved O_2 contents (1.5 mg l^{-1} ± 0.5), conditions suitable for supporting denitrifying populations. The riparian wetland soils within the watershed are mapped as Bibb (coarse-loamy, siliceous, acid, thermic Typic Fluvaquents) and Levy (fine, mixed, acid, thermic Typic Hydraquents) soils (Nicholson, 1981). The soils used in this study were deep, poorly drained, associated with freshwater marshes, frequently flooded, and belonged to the Bibb series. Texture, pH, and organic carbon content for the three soil horizons are shown in Table 1. The existence of forested riparian zones in this region is common due primarily to extreme slopes and incessant wetness.

Soil samples were collected in May (soil temperature 16.4°C), September (19.9°C), and November (13.5°C) of 1993 at 12 equidistant locations distributed along a 250 m section of the stream. At each location, samples were taken from a surface horizon (1–15 cm depth within 1 m of the stream) that was almost continuously ponded, designated as ponded surface horizon, and from a terrestrial surface (1–15 cm) and a subsurface (24–45 cm) horizon 1–3 m distant from the stream. Terrestrial surface and subsurface horizons were collected from the same profile at each location that ranged from 15 to 30 cm higher in elevation than the ponded surface horizon. Groundwater is frequently flowing through the terrestrial surface horizon during periods of high seasonal water-tables, and the subsurface horizon experiences groundwater flow much of the time. Soil samples were placed into 1 l. Qorpak polypropylene wide-mouth bottles, sealed, and then transported back to the laboratory on ice in coolers. The soils were stored at 4°C.

Preparation of synthetic groundwater and soil columns

Synthetic groundwater was prepared by adding 44 ± 2.0 mg NO_3^--N l^{-1} (3.2 ± 0.1 mmol NO_3^--N), 1.30 mg NH_4^+-N l^{-1}, 0.14 mg *ortho*-P l^{-1}, and 1.11 g $CaCl_2$ l^{-1} to water that had been passed through a VHP-3 Vaponics ultrapure water system (Vaponics, Inc., MA, U.S.A.). Based on field measurements, the pH of the synthetic groundwater was adjusted to 5.5 with HCl.

Soil subsamples for each horizon were wet-packed into Plexiglas columns (8 cm length, 2.2 cm diameter). The soils were sealed in the columns with glass-wool plugs and rubber stoppers, pierced by a 5 cm long and 3.18 mm o.d. stainless steel tube fitted with Teflon tubing. Teflon tubing from the influent end of the vertically positioned column was connected to either 1.02 or 0.38 mm i.d. manifold pump tubing, depending on the desired flow rate. Two (four channel) peristaltic pumps (MCP 2500, Haake Buchler Instruments, NJ, U.S.A.) were used to deliver groundwater to the bottom of vertically positioned soil columns (Fig. 1). The groundwater reservoir consisted of a 5 l. Knotes solvent delivery system with three on/off CTFE valves. The entire column set-up, including the reservoir and pumps, was placed in an incubator for temperature control.

Prior to each column run, the synthetic groundwater was sparged with N_2 for 15 min (while stirring) to reduce the dissolved oxygen concentration to about 0.75 mg l^{-1}, as measured by an oxygen meter (model 57, YSI Corp., OH, U.S.A.). Afterwards, the synthetic groundwater was sparged with acetylene that was passed through water to remove

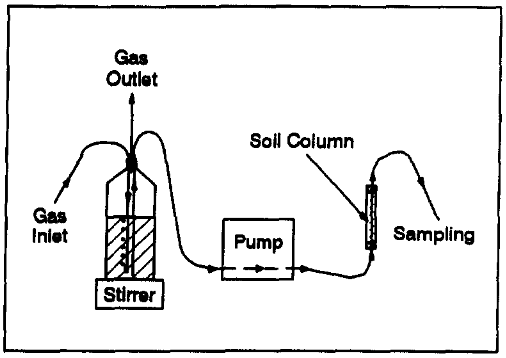

Fig. 1. Schematic showing the soil column set-up and direction of fluid flow.

impurities for 3 min. The oxygen concentration measured 1.3 mg l^{-1} following the acetylene strip and was well within the range of the field measurements (i.e. 1.5 mg l^{-1} ± 0.5). Soil columns were acclimated for 45 h at either 13.5, 16.4, or 19.9 ± 0.3°C. Acetylene containing synthetic groundwater was delivered to the soil columns for 1 h at a flow rate of 0.74 ± 0.2 ml min^{-1}, amounting to about twice the column volume and initiating acetylene inhibition (Yoshinari *et al.*, 1977). The volume flow was then adjusted to 0.01 ± 0.001 ml min^{-1} to accurately reflect field measurements of hydraulic conductivity. The determination of hydraulic conductivity (below the water-table) in the field was conducted according to the method of Boersma (1965). After the 45 h equilibration period, at the time when both N_2O production and NO_3^- consumption were constant (Fig. 2), aqueous effluent samples were collected by inserting the needle of a syringe into the outlet tubing (Fig. 1). Then 50 μl aliquots (effluent) were injected into each of five gas-tight vacutainer tubes (4 ml) equilibrated with laboratory air (minus 50 μl).

The sample-containing vacutainer tubes were vigorously shaken to facilitate establishment of equilibrium of N_2O between the aqueous phase and the headspace of the vacutainer tubes. Following equilibration to room temperature (10 min), 0.5 ml gas samples were withdrawn from the headspace and injected into a gas chromatograph (GC; injector temperature 60°C) (model 3700, Varian Assoc., CA, U.S.A.), equipped with a ^{63}Ni electron capture detector (340°C) and integrator (HP 3390A, Hewlett Packard, PA, U.S.A.), for N_2O analysis. The gases were separated with a Poropak Q column (80/100 mesh; 183 × 0.2 cm) (Alltech Assoc., IL, U.S.A.) at 40°C with argon–methane (9:1) (Airco, NJ, U.S.A.) as carrier gases flowing at 30 ml min^{-1}. N_2O-N standard curves, in the range of 1–5 nmol, were prepared using a Scott certified gas mixture (4.5% N_2O with the balance of He). The amount of N_2O dissolved in the effluent was determined using the Bunsen absorption coefficient and added to the headspace N_2O as described by Tiedje (1982). After each column run, soils were dried at 75°C, and their dry weight determined. NO_3^--N concentrations of the influent and effluent solutions were determined colorimetrically with an autoanalyzer (AP 200, Orion Research, NY, U.S.A.) using the cadmium reduction method (APHA, 1992). The loss of NO_3^- was calculated by difference of NO_3^- concentrations between influent and effluent solutions.

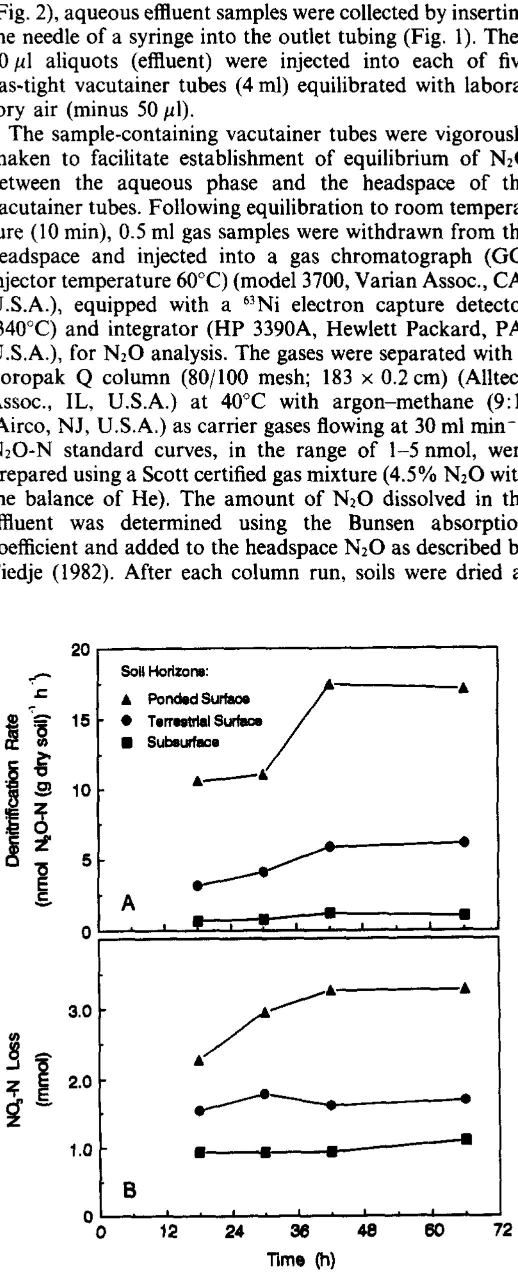

Fig. 2. Relationship between denitrification rate (N_2O production) (a), and NO_3^- loss (b) as a function of column operation time. Equilibrium conditions were established within 45 h. Soils, collected in May of 1993, were incubated at 16.4°C.

For each season of sampling, the combinations of soil type and sampling location within the site generated a large number of soil samples which could not be analyzed in the laboratory at the same time. Two pumps with four channels were used in the study. To reduce the possibility of confounding from laboratory factors, the soil column study was designed as a replicated Latin rectangle with two pumps acting as replicates. The blocking factors were the date the trial was run and the soil horizon. To generate the design, two Latin squares were generated and stacked to form a rectangle with six rows (representing the date the samples were analyzed) and three columns (representing the pump channel). The dates were then randomly assigned to the rows, separately for each pump. Each channel within the pump received one of the soil samples from the three horizons. The fourth channel was used as a repetition of one of the soil samples. The repetitions were selected so that each soil horizon appeared on exactly two randomly selected dates within each pump. This spare sample was only used to evaluate between channel variability. Finally, each of the 12 (field) sampling sites was randomly assigned to one of the pumps by date combinations.

Analysis of variance was used to describe differences between denitrification rates due to soil horizon and season. The analysis used a split plot model, with pump acting as the whole plot factor and season and soil horizon being subplot factors. As part of the analysis, the residuals were investigated and a log transformation was used to improve heterogeneity of variance and normality (see also Parkin and Robinson, 1993). Correlation coefficients were calculated to assess the strength of linear relationships between denitrification rates, soil organic carbon, and NO_3^- loss.

Soil horizon samples were characterized by particle size analysis (Gee and Bauder, 1986). Organic carbon of each soil sample was determined by dry combustion (1371°C) as described by Nelson and Sommers (1982) using a carbon analyzer (CR 12, Leco, IL, U.S.A.) equipped with an infrared detector. The pH of the soil samples was measured with an Orion combination pH electrode. Water had to be added to the terrestrial surface horizon (1:1) for pH measurements.

The relative NO_3^- distribution within soil columns was determined at 13.5, 16.4, or 19.9°C by using samples of the three soil horizons collected at two locations in November of 1993. Following the 45 h equilibration period, soil cores were divided into 2 cm sections. The water from each section was extracted and analyzed for NO_3^-. The mean influent NO_3^--N concentrations for these experiments was 2.99 ± 0.32 mmol.

RESULTS AND DISCUSSION

Mean denitrification rates were greatest for the ponded surface horizon (0.40 ± 0.17 μmol N_2O-N (g DW)$^{-1}$ d^{-1} at 16.4°C, 0.65 ± 0.27 at 19.9°C, 0.40 ± 0.25 at 13.5°C) followed by the terrestrial surface horizon (0.17 ± 0.11, 0.28 ± 0.13, 0.16 ± 0.11) across all variables (Fig. 3A). Only minimal mean rates of denitrification were observed for the subsurface horizon (0.04 ± 0.02 μmol N_2O-N (g DW)$^{-1}$ d^{-1} at 16.4°C, 0.11 ± 0.06 at 19.9°C, 0.04 ± 0.01 at 13.5°C). Rates of denitrification were correlated to loss of NO_3^- for the terrestrial surface

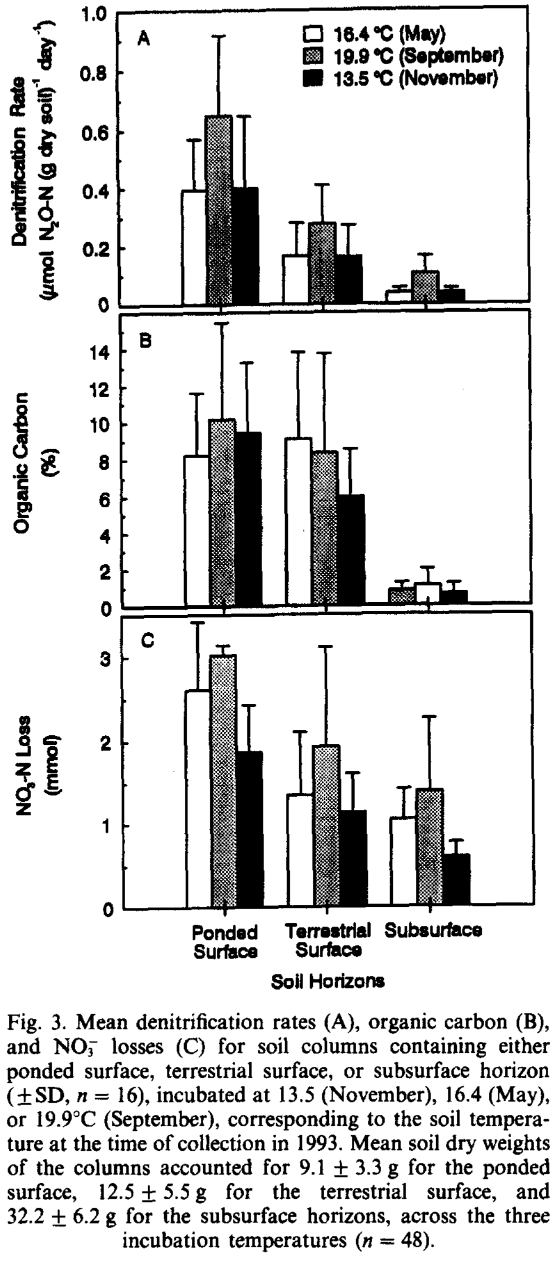

Fig. 3. Mean denitrification rates (A), organic carbon (B), and NO_3^- losses (C) for soil columns containing either ponded surface, terrestrial surface, or subsurface horizon (±SD, $n = 16$), incubated at 13.5 (November), 16.4 (May), or 19.9°C (September), corresponding to the soil temperature at the time of collection in 1993. Mean soil dry weights of the columns accounted for 9.1 ± 3.3 g for the ponded surface, 12.5 ± 5.5 g for the terrestrial surface, and 32.2 ± 6.2 g for the subsurface horizons, across the three incubation temperatures ($n = 48$).

horizon across the three incubation temperatures and for the ponded surface and subsurface horizons, incubated at 13.5 and 16.4°C (Table 2, Fig. 3C).

Although the surface horizons of both the ponded and adjacent terrestrial sites exhibited similar organic carbon contents (Fig. 3B) and soil textures (Table 1), a substantial difference in denitrification rates was observed between these two soils. While the two surface horizons shared some similarities, there were also some major differences, including the composition of organic matter and pH. The terrestrial surface horizon consisted primarily of humus with living root tissue, while the ponded surface horizon was primarily composed of humus. The compositional nature of the organic carbon source can affect denitrification (Parkin, 1987). We observed a strong correlation between organic carbon and denitrification in the ponded surface horizon across all incubation temperatures, and for the terrestrial surface and subsurface horizons incubated at 13.5°C (Table 2). Burford and Bremmer (1975) and Stanford *et al.* (1975a) reported a stronger correlation between denitrification and soil organic matter that was readily decomposable than for total organic carbon. However, denitrification rates were not correlated with organic carbon for the terrestrial surface and subsurface horizons collected in May and August, suggesting that other factors, such as carbon availability, may have played a major role in influencing denitrification. The subsurface horizon contained very little organic matter and was practically devoid of root mass.

Differences in organic matter composition, pH, and degree of water saturation probably impacted the diversity of microbial metabolic processes in the surface horizons (Table 1). The terrestrial surface horizon was about 10 times more acidic than the ponded surface horizon. Although denitrifying populations can adapt to low soil pH, an overall reduction in denitrification is expected (Parkin *et al.*, 1985). High denitrification potentials have been, however, observed in acid soils (Gilliam and Gambrell, 1978; Müller *et al.*, 1980; Koskinen and Keeney, 1982; Parkin *et al.*, 1985).

The low organic carbon content coupled with low pH in the subsurface horizon (Fig. 3B) represents less than ideal conditions for denitrifying activity. Direct comparisons of mean denitrification rates between the surface horizons and the subsurface horizons,

Table 2. Correlations between denitrification rate and organic carbon and NO_3^- loss, respectively, and *P*-values for the different soil horizons of the Bibb series ($n = 16$)

Soil horizons	Temperature (°C) (season)	NO_3^--N loss (mmol)		Organic carbon (%)	
		r	*P*	*r*	*P*
Ponded surface	16.4 (May)	0.47	0.0635	0.70	0.0024
	19.9 (September)	−0.13	0.6405	0.90	0.0001
	13.5 (November)	0.63	0.0087	0.84	0.0001
Terrestrial surface	16.4 (May)	0.77	0.0004	0.23	0.3914
	19.9 (September)	0.76	0.0007	−0.09	0.7341
	13.5 (November)	0.79	0.0030	0.79	0.0003
Subsurface	16.4 (May)	0.78	0.0004	0.37	0.1527
	19.9 (September)	0.19	0.4831	0.28	0.2293
	13.5 (November)	0.48	0.0599	0.90	0.0001

however, need to be approached with the knowledge that the (experimentally imposed) average pore water velocity of the two surface horizons was approximately 3 mm min^{-1}, while the subsurface horizon experienced a velocity of approximately 5 mm min^{-1}. Thus, the residence time for a given volume of water infiltrating through the subsurface horizon was less than the residence time of that same volume of water passing through either of the two surface horizons. This difference in average pore water velocities between the surface and subsurface horizons certainly accounted for some of the large differences observed in denitrification rates between these soil horizons. Substrate (organic carbon) availability and thus denitrifying activity may have been influenced by the differences in flow rate. Ambus and Lowrance (1991) also observed higher denitrification rates in surface compared to subsurface horizons for two riparian forest soils, including a Kinston fine-loamy sand (fine-loamy, siliceous, acid, thermic Typic Fluvaquent) and an Alapaha loamy sand (loamy, siliceous, thermic Arenic Plinthic Paleaquult).

Mean denitrification rates were significantly greater for soils incubated at 19.9°C compared to either 16.4 or 13.5°C. There was little difference in rates of denitrification when soils were incubated at the lower temperatures (Fig. 3A). Similar seasonal patterns for denitrification have been described by Struwe and Kjøller (1990) in water-logged alder stands, and in riparian wetlands by Hanson *et al.* (1994). Effects of temperature on denitrification rates have been reported by several authors (Stanford *et al.*, 1975b; Smid and Beauchamp, 1976; Jacobsen and Alexander, 1980).

Of the total variability accounted for by the analysis of the variance model, the greatest portion was due to soil horizon (about 50%), while variation between sampling sites (i.e. spatial variability) accounted for about 10% (Fig. 4). The remainder of the variability was due to season (or incubation temperature). The pump effect was not significant. Hence, differences between the two pumps were minor relative to differences between soil horizons. High spatial variability in soil denitrification rates has been reported by several authors (Folorunso and Rolston, 1984; Parkin, 1987; Groffman and Tiedje, 1989; Christensen *et al.*, 1990; Parsons *et al.*, 1991). Soil microsites exhibiting high denitrification activity were associated with particulate organic carbon material, such as leaves, in the soil. The patchy dispersion of organic material in soil is apparently a major factor affecting the variability of denitrification rates between sites (Parkin, 1987). Since the two surface horizons of the riparian wetland soils contained such high organic carbon contents (Table 1), spatial variability would be expected to be minimal.

The NO_3^- concentration of the infiltrating aqueous solution decreased within the soil columns as a function of distance (Fig. 5), displaying a similar pattern as that reported by Doner *et al.* (1974) and Misra *et al.* (1974). Disappearance of NO_3^- was curvilinear for the two surface horizons (Fig. 5A and B) and linear within the subsurface horizon (Fig. 5C). Incubation temperature had much less of an effect on NO_3^- disappearance within the subsurface horizon columns as compared to the surface horizon columns. NO_3^- concentration within the columns containing the surface horizon soils was strongly affected by incubation temperature. Surface horizons incubated at 19.9°C showed the steepest decline in NO_3^- over the first 2 cm distance. Misra *et al.* (1974) reported lower relative NO_3^- concentrations within soil columns incubated at 34.5 than at 19.9°C. For the two surface horizon soils, incubated at 16.4 and 19.9°C, the NO_3^- concentration at the effluent end of the columns had decreased to nominal levels, indicating that denitrification was apparently NO_3^- limited. In the subsurface horizon, however, NO_3^- was not limiting, and the denitrification potential appeared to be controlled primarily by organic carbon availability or possibly by the size of denitrifying populations. Ambus and

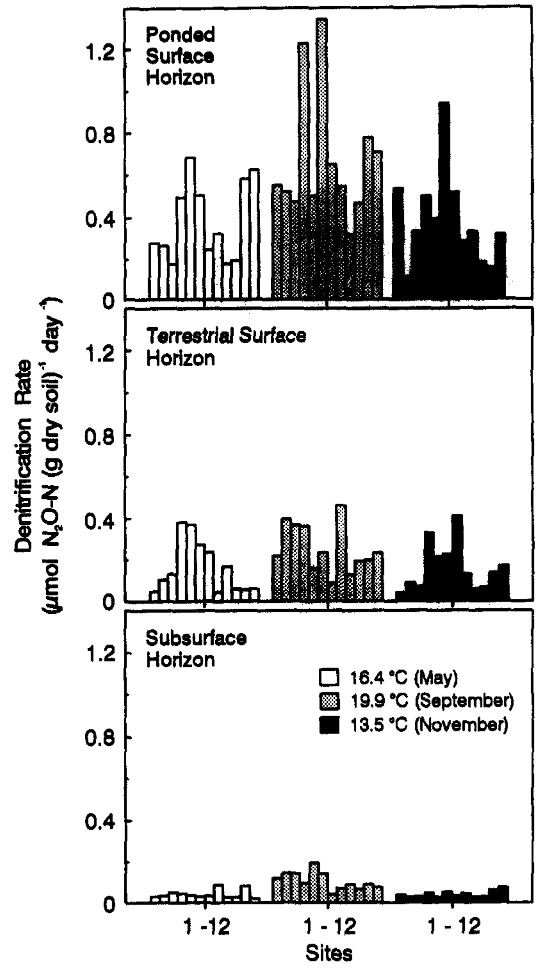

Fig. 4. Comparison of denitrification rates for the three soil horizons collected from the 12 sampling sites in May (16.4°C), September (19.9°C), and November (13.5°C) of 1993.

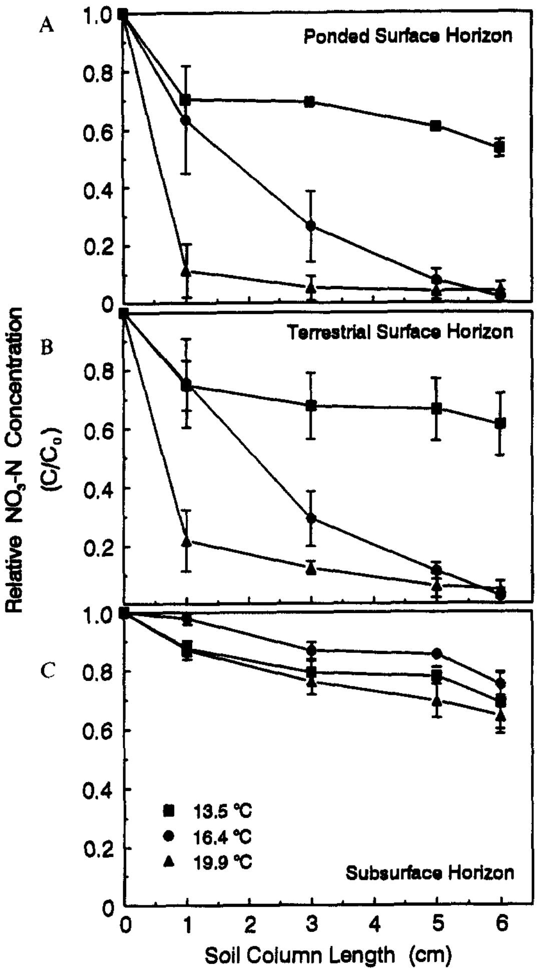

Fig. 5. NO_3^- concentrations within the columns representing the three soil horizons following equilibration ($\pm$SD, $n = 3$). Soil samples were collected in November and incubated at 13.5, 16.4, and 19.9°C, corresponding to season temperatures of 1993.

Lowrance (1991) observed that additions of NO_3^- or a readily usable carbon source did not increase the rates of denitrification of two subsurface riparian soils.

In those areas within the study site where the seepage face is separated from the stream by the ponded surface horizon, one could expect that denitrification would be effective in removing NO_3^- from contaminated groundwater, especially during spring, summer, and fall seasons when the soil temperature is above 13°C. Groundwater temperatures near the seepage face are rarely lower than 7°C even during the coldest winter months (Snyder, 1995). Denitrification in the terrestrial surface horizon is much less effective in removing NO_3^- from groundwater, primarily because groundwater does not move *en masse* through this horizon, except during a wet spring season when the water-table is high. For those areas of the site where the groundwater enters the stream directly from the subsurface horizon, denitrification is probably only minimally effective in removing NO_3^-.

CONCLUSIONS

Riparian wetlands are believed to contribute substantially to the preservation of groundwater quality by removal of NO_3^- through denitrification (Gilliam, 1994) and plant uptake (Peterjohn and Correll, 1984; Ehrenfeld, 1987; Cooper, 1990). Our studies show that, despite their acidity, the surface horizons of the riparian Bibb soil possess a high denitrification potential, while the subsurface horizon does not. Thus in the Nomini Creek watershed, the potential of riparian wetland soils to remediate groundwater depends largely upon the percentage of groundwater passing through the highly active surface horizons.

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