Denitrification in a Coastal Plain Riparian Zone Contiguous to a Heavily Loaded Swine Wastewater Spray Field

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ABSTRACT

Riparian zones are recognized as landscape features that buffer streams from pollutants, particularly nitrogen. The objectives of this experiment were to (i) assess denitrification activity within a riparian zone and (ii) determine the influence of physical, chemical, and landscape features on denitrification. This experiment was conducted from 1994 to 1997 in North Carolina on a riparian zone contiguous to a spray field that was heavily loaded with swine lagoon wastewater. Denitrification enzyme activity (DEA) was measured on soils collected from (i) the soil surface, (ii) midway between the soil surface and water table, and (iii) above the water table. The DEA ranged from 3 to 1660 μg N_2O-N kg^{-1} soil h^{-1}. The DEA was highest next to the stream and lowest next to the spray field. Nitrate was found to be the limiting factor for denitrification. The DEA generally decreased with soil depth; means for the surface, middle, and bottom depths were 147, 83, and 67 μg N_2O-N kg^{-1} soil h^{-1}, respectively. These DEA values are higher than those reported for riparian zones adjoining cropland of the southeastern United States, but are lower than those reported for a constructed wetland used for treatment of swine wastewater. Regression analysis indicated that soil total nitrogen was the highest single factor correlated to DEA (r^2 = 0.65). The inclusion of water table depth, soil depth, and distance from the spray field improved the R^2 to 0.86. This riparian zone possessed sufficient soil area with high denitrifying conditions to be a significant factor in the removal of excess nitrogen in the ground water.

The importance of riparian zones to water quality has become widely recognized over the last two decades (Peterjohn and Correll, 1984; Hill, 1996; Lowrance et al., 2002). This importance is directly related to their strategic landscape position next to both large and small streams. In this location, their physical, chemical, and biological processes can function in assimilation and transformation of contaminants before they can be transported into stream waters. Moreover, their importance has been documented throughout the world in various ecosystems to include deciduous forest in the Atlantic Coast of the USA (Peterjohn and Correll, 1984; Lowrance et al., 1984; Jacobs and Gilliam, 1985; Jordan et al., 1993; Hanson et al., 1994; Stone et al., 1998a; Novak et al., 2002), fens of Scandinavia (Brusch and Nilsson, 1993), grass areas of New Zealand (Cooper, 1990), forest and grass areas of Canada (Hill et al., 2000), and forest and grass areas of England (Haycock and Pinay, 1993).

Riparian zones can be particularly effective in the removal of nitrogen. Although there is variability, especially in the hydrological and biological conditions that promote nitrogen removal, effective riparian zones generally contain shallow ground water, an active plant community, massive and dynamic soil microbial populations, and hydric soils (Lowrance et al., 1984; Ambus and Lowrance, 1991; Hill et al., 2000). Often the removal of nitrogen from the point of entry into the riparian zone to the point of stream entry is more than 90% (Peterjohn and Correll, 1984; Jacobs and Gilliam, 1985; Jordan et al., 1993; Lowrance et al., 1995). What is more, the actual mass removal of nitrogen can be large; for instance, Brusch and Nilsson (1993) reported 390 kg N ha^{-1} yr^{-1} removed by a stream valley fen. While denitrification is considered a major nitrogen removal process for the entire riparian zone, there is considerable variation from one area to another with significant hot spots and zones of high and low activity (Hill et al., 2000; Flite et al., 2001). Thus, the interconnected cycling of nutrients from the ground water into plant biomass with the subsequent soil deposit and decomposition of the leaf litter is important for both the translocation of nitrogen and microbial energy from carbon (Ambus and Lowrance, 1991).

While the importance and function of riparian zones are documented for natural and agricultural systems with modest nitrogen loads, they are less well documented for riparian zones that are contiguous to heavily loaded animal waste application fields. Lowrance and Hubbard (2001) documented the nitrogen removal in various combinations of grass and forested wetland zones when swine wastewater was applied via overland flow. Although they found effective removal of nitrogen at high rates, they also noted the accumulation of ammonia in soil profiles along the entire length of the overland flow slope. Vellidis et al. (2003) also reported the importance of a restored riparian zone in the mitigation of nitrogen (>50%) from a manure application area. Sloan et al. (1999) reported that stream nitrogen concentration was elevated as a stream passed by a riparian zone contiguous to a swine wastewater spray field despite high levels of denitrification in the riparian zone. The importance of effective riparian zones in a watershed with significant livestock was also reported by Hunt et al. (1995) and Stone et al. (1995, 1998a). The need for riparian buffering of nitrogen inputs is especially important when there is a history of very high waste loading and elevated nitrogen in the shallow ground water (Stone et al., 1998a, 1998b). The objectives of this investigation, which used the acetylene blockage method, were to (i) assess the denitrification activity within this riparian zone that was heavily affected by swine wastewater application

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Abbreviations: DEA, denitrification enzyme activity.

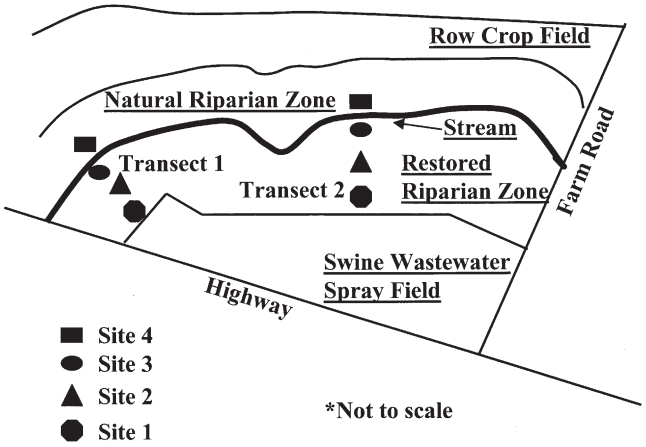

Fig. 1. A schematic of the experimental site including spray field, riparian zone, stream, and soil sampling sites.

and (ii) determine the influence of various physical, chemical, and landscape features on denitrification.

MATERIALS AND METHODS

Site Description

A field study was conducted from 1994 to 1997 to assess the denitrification enzyme activity of soil from a newly planted forested riparian zone. The soil was an Autryville loamy sand soil (loamy, siliceous, subactive, thermic Arenic Paleudults). This riparian zone varied from five to 30 m wide and was 200 m long. It was located on an unnamed tributary of the Herrings Marsh Run watershed in Duplin County, North Carolina. It was downslope from a swine lagoon effluent spray field (Fig. 1) (Stone et al., 1998b).

This riparian landscape feature was used to minimize the effect of surface and ground water outflows from a coastal bermudagrass [*Cynodon dactylon* (L.) Pers.] pasture irrigated with swine lagoon effluent. The riparian zone was divided into (i) a swine wastewater-affected portion (the newly planted forested riparian zone) on the spray field side of the stream and (ii) a control portion (the natural riparian zone) on the opposite side of the stream. The natural riparian zone did not receive any drainage from wastewater application. In April 1993, the affected portion was planted to trees (1–1.5 m in height) on 2-m spacings. Starting at the spray field edge and moving toward the stream, species planted were green ash (*Fraxinus pennsylvanica* Marshall), red maple (*Acer rubrum* L.), sycamore (*Platanus occidentalis* L.), water oak (*Quercus nigra* L.), and bald cypress [*Taxodium distichum* (L.) Rich.]. Native vegetation remained on the control portion of the riparian zone.

Ground water monitoring wells were installed in the riparian zone adjacent to the edge of the spray field and on both sides of the stream along with those installed in the spray field. A description of the installation procedure was presented by Stone et al. (1998a).

Table 2. Riparian site soil characteristics at Transects 1 and 2.

Site†	Soil depth	Total N‡	Total C‡
	mm	g kg^{-1}	
		Transect 1	
1	0–150	0.41 ± 0.13	9.54 ± 2.54
1	600–750	0.18 ± 0.10	4.98 ± 2.56
1	1220–1370	0.16 ± 0.06	3.67 ± 2.09
2	0–150	1.20 ± 0.76	49.21 ± 22.06
2	300–450	0.71 ± 0.48	40.30 ± 19.07
2	600–750	0.40 ± 0.30	24.73 ± 13.50
3	0–150	3.76 ± 2.63	91.68 ± 31.93
3	300–450	3.77 ± 2.40	102.03 ± 34.39
3	600–750	3.77 ± 2.05	119.56 ± 35.82
4	0–150	6.59 ± 3.43	179.10 ± 50.10
4	150–300	5.41 ± 3.35	187.70 ± 62.40
4	300–450	4.88 ± 3.48	208.37 ± 76.16
		Transect 2	
1	0–150	0.50 ± 0.19	11.70 ± 4.09
1	750–900	0.20 ± 0.09	5.96 ± 5.59
1	1520–1670	0.12 ± 0.05	4.98 ± 2.64
2	0–150	3.97 ± 2.99	127.37 ± 46.80
2	150–300	5.12 ± 2.68	156.68 ± 64.56
2	300–450	4.41 ± 2.02	155.52 ± 50.38
3	0–150	4.52 ± 2.86	153.38 ± 78.01
3	150–300	4.44 ± 3.28	171.84 ± 96.59
3	300–450	4.62 ± 2.38	163.40 ± 107.9
4	0–150	1.07 ± 0.49	38.39 ± 12.16
4	300–450	0.67 ± 0.67	27.27 ± 23.69
4	750–900	0.43 ± 0.88	7.76 ± 4.94
$LSD_{(0.05)}$		1.05	21.58

† 1, Field edge; 2, middle; 3, edge of the stream contiguous to the wastewater spray field; 4, edge of the stream opposite the wastewater spray field.
‡ Values are means and standard deviations.

Sample Collection and Analysis

Soil samples were collected from four sites on two transects (three sites in the newly planted forested riparian zone and one site in the natural riparian zone) on 13 sampling dates between September 1994 and April 1997 (Table 1). Samples (50-mm diameter by 152-mm length) were collected from three depths: (i) from the soil surface, (ii) midway between the soil surface and water table, and (iii) above the water table. The depth samples were obtained from the same core-hole with a vertical penetration to each respective sample depth. Three cores were taken and composited at each site. Locations and depths of soil samples are presented in Table 2. Soil samples were placed in plastic bags, stored on ice, transported to the laboratory, and stored overnight at 4°C. Subsamples of soil were digested with sulfuric acid and analyzed for TKN with a TRAACS 800 (Bran + Luebbe, Buffalo Grove, IL). Total carbon was determined by analysis with a Model CN2000 analyzer (LECO, St. Joseph, MI).

Denitrification enzyme activity (DEA) was measured by the acetylene blockage method (Tiedje, 1994). All analyses were performed in triplicate. Field moist soil (10–15 g) was placed in 60-mL serum bottles (four bottles per sample). Each bottle received one of the following amendments: (i) 5 mL of

Table 1. Denitrification enzyme activity (DEA) sampling dates and mean monthly temperature (MMT).

1994		1995		1996		1997	
Date	MMT	Date	MMT	Date	MMT	Date	MMT
	°C		°C		°C		°C
6 Sept.	21.3	7 Mar.	12.3	29 Feb.	6.2	21 Jan.	6.7
5 Oct.	15.5	5 Apr.	17.2	21 May	21.3	7 Apr.	14.1
1 Nov.	14.3	12 June	23.9	27 Aug.	24.8		
		7 Dec.	4.4	24 Oct.	16.7		

chloramphenicol (1 g L^{-1}) to inhibit protein synthesis, (ii) 5 mL of chloramphenicol with nitrate N (200 mg NO_3–N L^{-1}), (iii) 5 mL of chloramphenicol with glucose (2 g glucose C L^{-1}), or (iv) 5 mL of chloramphenicol with nitrate N (200 mg NO_3–N L^{-1}) and glucose (2 g glucose C L^{-1}). Bottles were capped with rubber septa, evacuated, and purged with nitrogen gas three times. They were then injected using a syringe with 15 mL of acetylene. The bottles were incubated on a horizon shaker at 90 rpm. Samples of the headspace gases were removed after 1, 5, and 24 h with a Plastipak syringe with eccentric tip (Becton Dickinson, Franklin Lakes, NJ) and placed in vials (borosilicate glass, crimp top with butyl septum).

A Varian (Palo Alto, CA) Model 3600 CX gas chromatograph with a 15-m Ci^{63} Ni electron capture detector operating at 350°C was used for measuring N_2O in the samples. A 1.8-m-long by 2-mm-i.d. stainless steel column packed with Poropak Q (80–100 mesh) was used to separate CO_2, N_2O, and C_2H_2. The column and injector temperature was 70°C. Samples were injected onto the column by a Varian 8200 autosampler.

Water Samples

Water samples from the monitoring wells were collected with a manual pump every month (Stone et al., 1998a). Samples were acidified to pH < 2 with sulfuric acid, packed in ice, and transported to the laboratory. Nitrate N and ammonia N were determined on a TRAACS 800 autoanalyzer using Methods 353.2 and 350.1 (USEPA, 1983).

Data Analysis

The data were pooled for dates to allow an analysis and discussion of the mean denitrification function over the study period rather than a focus on denitrification on any one date. Data were analyzed by analysis of variance (ANOVA), stepwise regression, and least significant difference (LSD) with SAS (SAS Institute, 1999).

RESULTS AND DISCUSSION

Ground Water Nitrogen

Ground water under the spray field had excessive N. The mean nitrate N and ammonia N were 78 and 11 mg L^{-1}, respectively (Table 3). While the N in the ground water at the edge of the riparian zone dropped to <15 and 2 mg L^{-1} nitrate N and ammonia N, respectively; they rose to >30 and 12 mg L^{-1} nitrate N and ammonia N, respectively, at the stream edge. However, as the water moved the remaining short distance into the stream, the N content dropped substantially. Nonetheless, the stream had nitrate N and ammonia N means of 12.2 ± 4.6 and 2.5 ± 1.1 mg L^{-1}, respectively; these concentrations show that the riparian zone was unable to totally buffer the stream from the heavily loaded wastewater spray field. This effect is similar to that reported by Sloan et al. (1999). Nitrate concentrations in the ground water across the stream were similar to those found in the stream, but the ammonia N concentrations were much lower, <0.12 mg L^{-1}. These data indicate that biogeochemical processes in the stream and in the stream-edge riparian areas were very active. Furthermore, the variation in ground water nitrogen content would suggest the presence of considerable and varied denitrification enzyme activity throughout this wastewater-affected riparian zone.

Table 3. Nitrogen in the stream and in the ground water of both the spray field and the riparian zone.

Transect	Site†	Nitrate N‡	Ammonia N‡
		mg N kg^{-1}	
Spray field	spray field	77.99 ± 71.38	10.97 ± 11.37
1	1	9.58 ± 6.74	1.17 ± 0.82
1	2	–	–
1	3	68.99 ± 29.93	12.67 ± 8.26
Stream	stream	12.2 ± 4.62	2.50 ± 1.07
1	4	13.91 ± 3.39	0.08 ± 0.04
2	1	14.20 ± 1.15	0.59 ± 0.43
2	2	–	–
2	3	32.01 ± 14.95	12.14 ± 6.96
Stream	stream	12.2 ± 4.62	2.50 ± 1.07
2	4	16.48 ± 1.60	0.12 ± 0.10
$LSD_{0.05}$		8.15	3.59

† 1, Field edge; 2, middle; 3, edge of the stream contiguous to the wastewater spray field; 4, edge of the stream opposite the wastewater spray field.
‡ Values are means and standard deviations.

Denitrification Enzyme Activity in the Control Treatment

The four-way interaction of transect by site by soil depth by amendment for DEA was highly significant ($F \geq 0.001$); DEA values ranged from 3 to 1660 μg N_2O-N kg^{-1} soil h^{-1} (Table 4). This indicates that the DEA at the specific sites responded differently to nitrate and glucose amendments, and the responses were likely related to the fact that transects, sites, and soil depths were quite different in their soil N and C contents as well as water table depths (Table 2). Whereas the control treatment had only the existing soil N and C, it provided information on the extent of denitrification in the unamended soil at different positions in the riparian landscape. The DEA range of the control treatment was smaller, 4 to 372 μg N_2O-N kg^{-1} soil h^{-1}. The relative

Table 4. Denitrification enzyme activity (DEA) values for riparian transect, site location, soil depth, and amendments.

Transect	Site†	Soil layer	None	NO_3–N added	Glucose C added	NO_3–N + C added
			μg N_2O-N kg^{-1} soil h^{-1}			
1	1	top	38	35	42	48
		middle	12	13	10	17
		bottom	16	18	8	15
1	2	top	19	109	33	183
		middle	76	54	38	76
		bottom	39	41	21	32
1	3	top	154	324	208	469
		middle	97	320	103	389
		bottom	74	195	92	242
1	4	top	142	1050	126	1146
		middle	96	574	51	943
		bottom	46	528	36	640
2	1	top	47	56	66	73
		middle	18	20	5	6
		bottom	12	41	13	5
2	2	top	314	1335	337	1660
		middle	148	673	127	930
		bottom	219	592	161	728
2	3	top	372	920	277	1507
		middle	205	632	339	1004
		bottom	125	414	109	620
2	4	top	86	157	95	196
		middle	13	21	10	21
		bottom	4	6	3	8
$LSD_{0.05}$ = 192						

† 1, Field edge; 2, middle; 3, edge of the stream contiguous to the wastewater spray field; 4, edge of the stream opposite the wastewater spray field.

magnitude of these DEA values can be seen by comparison with other studies in the southeastern Coastal Plain. While DEA values in this riparian zone were more than 100 times greater than the values reported by Ambus and Lowrance (1991) for riparian soil contiguous to a row-crop field in Georgia, they were much lower than the 210 to 516 mg N_2O-N kg^{-1} soil h^{-1} for constructed wetlands used for treatment of swine wastewater in Duplin County, North Carolina, reported by Hunt et al. (2003).

At five of the eight sites, DEA decreased with depth. The means of the three depths for both transects and all sites were 147, 83, and 67 μg N_2O-N kg^{-1} soil h^{-1}, respectively, for the top, middle, and bottom soil depths. There was a slight variation from the typical decrease with depth at the mid-riparian sites of both transects; the top depth was the lowest on Transect 1 (19 μg N_2O-N kg^{-1} soil h^{-1}), and the middle depth was the lowest on Transect 2 (148 μg N_2O-N kg^{-1} soil h^{-1}).

As with soil depth, the sites along the transects varied greatly in their denitrification characteristics. At the field–riparian edge sites, soil and water conditions were more similar to an agronomic field. The water tables were deeper than at the sites farther into the riparian zone. The water table at this site on Transect 2 was deeper than on Transect 1 (1.67 vs. 1.37 m). Both transects had relatively low mean soil N with values ranging from 0.12 to 0.50 g kg^{-1}. Similarly, carbon at the edge of the riparian zone ranged from 3.7 to 11.7 g kg^{-1}, which is similar to that found in the surface layer of a sandy Coastal Plain soil under long-term conservation tillage (Hunt et al., 1996). The DEA at these sites for the control treatment was low even at the surface depth, which had values of 38 and 47 μg N_2O-N kg^{-1} soil h^{-1} for Transects 1 and 2, respectively. Values for the lower two depths only ranged from 12 to 18 μg N_2O-N kg^{-1} soil h^{-1}.

Farther into the riparian zone, both the soil composition and hydric conditions became somewhat more typical of riparian zones. Specifically, the water table depths rose to 0.75 m at the middle section of Transect 1; however, the soil C and N concentrations remained relatively low (24.7–49.2 g C kg^{-1} and 0.4–1.2 g N kg^{-1}). The DEA rates were also low, 19 to 76 μg N_2O-N kg^{-1} soil h^{-1}, with the middle depth being the highest. The middle section of Transect 2 was more typical of a high denitrifying soil. The water table was at 0.45 m, and the soil C and N were higher (127–157 g C kg^{-1} and 4.0–5.1 g N kg^{-1}). Accordingly, the mean DEA rates were much higher; values ranged from 148 to 314 μg N_2O-N kg^{-1} soil h^{-1} with the top depth being the highest.

At the stream edge closest to the spray field, both transects were characteristic of high denitrifying wetland soils; the water table depths were 0.75 and 0.45 m for Transects 1 and 2, respectively. The soil N on Transects 1 and 2 was 3.76 to 4.62 g kg^{-1}, respectively; and the soil C mean was very high, ranging from 92 to 172 g kg^{-1}. The DEA rates were highest at the surface and lowest at the bottom depths, and Transect 1 was lower than Transect 2 at all depths; values ranged from 74 to 372 μg N_2O-N kg^{-1} soil h^{-1}.

Just across the stream, conditions were different for the two transects. On Transect 1 the water table rose to 0.45 m while it dropped to 0.90 m on Transect 2. The soil N on Transect 1 was high (4.9–6.6 g kg^{-1}) while the soil N on Transect 2 dropped dramatically (0.43–1.1 g kg^{-1}). The DEA was lower across the stream on both transects; and similar to C and N, it dropped more on Transect 2. On Transect 1 the surface, middle, and bottom depths had DEA values of 142, 96, and 46 μg N_2O-N kg^{-1} soil h^{-1}, respectively. On Transect 2 the values were 86, 13, and 4 μg N_2O-N kg^{-1} soil h^{-1} for the surface, middle, and bottom depths, respectively. In addition to the soil and water table characteristics, the DEA across the stream was probably less affected by the spray field because of the high denitrification on the spray field side of the stream and dilution from the stream.

Denitrification Enzyme Activity in Samples Amended with Nitrate and/or Glucose

Amendments significantly affected DEA ($F \geq 0.01$); and the four-way interaction of amendments with sites, transects, and soil depths was also significant ($F \geq 0.01$). The response to amendments was least in both transects at the location next to the field edge. At this location, even in the surface layer, DEA values only ranged from 38 μg N_2O-N kg^{-1} soil h^{-1} without amendments to 73 μg N_2O-N kg^{-1} soil h^{-1} with amendments of both nitrate and glucose. Similarly, DEA at the lower depths was only increased from a high of 18 μg N_2O-N kg^{-1} soil h^{-1} in the control to a high of 41 μg N_2O-N kg^{-1} soil h^{-1} by the addition of nitrate and/or glucose. This lack of denitrifying response was probably related to the relatively deeper water table and oxidative conditions in the soils at these locations.

In the middle riparian sites where more hydric soil conditions existed, the response to amendments was very large. Nitrate was clearly the limiting factor for denitrification in the surface depth of both transects, and denitrification was nearly 10 times greater in the middle of Transect 2 than in Transect 1. On Transect 1, DEA increased from 19 to 109 μg N_2O-N kg^{-1} soil h^{-1} on the addition of nitrate. Such an increase was not found by amending the soil with additional glucose, which indicated that denitrification was not limited by carbon. However, addition of both nitrate and glucose increased DEA nearly 10-fold to 183 μg N_2O-N kg^{-1} soil h^{-1}. This increase in DEA was limited to the surface depth on Transect 1; there was no significant response to amendments at the lower depths. The increase in DEA with nitrate and glucose amendments was more consistent with depth in the middle section of Transect 2. As in Transect 1, addition of nitrate to the surface-depth soil increased DEA dramatically (314–1335 μg N_2O-N kg^{-1} soil h^{-1}). Addition of glucose alone caused no increase in DEA, but addition of both nitrate and glucose increased DEA to 1660 μg N_2O-N kg^{-1} soil h^{-1}. This pattern of response was the same for the middle-depth and the water-table-depth soils where DEA was 930 and 728 μg N_2O-N kg^{-1} soil h^{-1}, respectively, when both nitrate and glucose were added.

At the stream edge next to the spray field on Transect

1, the increase in DEA on addition of nitrate was two- to threefold, and the increase occurred at all depths. Addition of both nitrate and glucose caused even larger increases: surface depth (154–469 μg N_2O-N kg^{-1} soil h^{-1}), middle depth (97–389 μg N_2O-N kg^{-1} soil h^{-1}), and water-table depth (74–242 μg N_2O-N kg^{-1} soil h^{-1}). The pattern of response to addition of nitrate and glucose on Transect 2 was similar but of greater magnitude than Transect 1. The magnitude of the increase was similar to that found in the middle section of Transect 2, and again nitrate was shown to be limiting while carbon was sufficient for control levels of nitrate. Addition of both nitrate and carbon resulted in very large increases in denitrification: surface depth (372–1507 μg N_2O-N kg^{-1} soil h^{-1}), middle depth (205–1004 μg N_2O-N kg^{-1} soil h^{-1}), and water-table depth (125–620 μg N_2O-N kg^{-1} soil h^{-1}). These DEA values were in the range of those reported by Hunt et al. (2003) for waste treatment constructed wetlands. Furthermore, the DEA of the lower depths increased greatly on the amendment with nitrate or nitrate and glucose, but it did not increase with the addition of only glucose. Thus, this riparian area had established a very active denitrifying population, and it had the potential for removing significant nitrate that was formed or passed through this area of the riparian zone. Varying levels of nitrate probably came into this area via seasonally fluctuating water tables and significant autumn leaf drop (Stone et al., 1998b; Ambus and Lowrance, 1991).

The differences in DEA on the two transects on the side of the stream opposite the spray field were magnified on the addition of nitrate and glucose. On Transect 1, DEA values on both sides of the stream were very similar for the control treatment, but the response to addition of nitrate and glucose was much greater for the site on the opposite side of the stream. This response was probably related to the fact that the soil on the opposite side of the stream had much higher total N and C concentrations. At this site, the increases in DEA on addition of both nitrate and glucose were: surface depth (142–1146 μg N_2O-N kg^{-1} soil h^{-1}), middle depth (96–943 μg N_2O-N kg^{-1} soil h^{-1}), and water-table depth (46–640 μg N_2O-N kg^{-1} soil h^{-1}). On Transect 2, where the total N and C were relatively low on the side of the stream opposite the spray field, the responses to addition of nitrate and glucose were minimal in the surface depth, and even smaller in the lower depths. Specific DEA increases from the control rate on the addition of both nitrate and glucose were: surface depth (86–196 μg N_2O-N kg^{-1} soil h^{-1}), middle depth (13–21 μg N_2O-N kg^{-1} soil h^{-1}), and water-table depth (4–8 μg N_2O-N kg^{-1} soil h^{-1}).

Correlation of Denitrification Enzyme Activity With Site Conditions—Stepwise Analyses

The previous discussion was based on an analysis of variance to distinguish among the various landscape positions and their interactions with nitrogen and carbon amendments. Regression analyses provided additional insight about the effect of site conditions on the capacity of this riparian zone to denitrify excess nitrogen. In stepwise regression analyses of the log of DEA, soil total N was the most highly correlated site parameter (Table 5). The partial r^2 for the control treatment and total soil N was 0.65, and the p value was <0.01. Water table depth, distance from the field, and depth from the soil surface were also significant parameters ($p \leq 0.11$). Stepwise regression of these parameters yielded a model r^2 of 0.86 and a $C(p)$ value that was nearly equal to the number of variables (4.1 vs. 4.0). The predictive equation was log DEA (μg N_2O-N kg^{-1} soil h^{-1}) = 0.0003 total soil N (μg g^{-1}) − 0.17 soil depth (m) + 0.0004 water depth (m) + 1.08 distance from field (m) − 10.20.

Considering denitrification activity was generally limited by the soil nitrate concentration, addition of nitrate alone improved and simplified the stepwise regression. Only three parameters were significant (total soil N, soil depth, and total soil C). The partial r^2 for total N was 0.82, and the p value was <0.01. Stepwise regression with the addition of soil depth and soil C concentration produced a model r^2 of 0.91 and again a $C(p)$ value that

Table 5. Stepwise regression values for denitrification enzyme activity (DEA) (log 10 transformation) correlation to site characteristics of Transects 1 and 2.†

Variable	Number of variables	Intercept	Partial r^2	Model R^2	$C(p)$	F	$P > F$
			No amendment				
Total N	1	6.81	0.65	0.65	25.4	40.06	<0.01
Water table depth	2	6.45	0.14	0.78	9.9	13.27	<0.01
Distance from field	3	−8.97	0.05	0.84	4.9	6.73	0.02
Depth from soil surface	4	−10.20	0.02	0.86	4.1	2.84	0.11
			Nitrate N added				
Total N	1	6.89	0.82	0.82	20.8	97.90	<0.01
Depth from soil surface	2	7.50	0.08	0.89	5.4	15.59	<0.01
Carbon	3	7.61	0.02	0.91	3.1	4.48	0.05
			Glucose C added				
Total N	1	6.79	0.51	0.51	16.6	23.32	<0.01
Depth from soil surface	2	7.53	0.22	0.73	2.1	17.26	<0.01
			Nitrate N and Glucose C added				
Total N	1	6.97	0.78	0.78	27.0	77.76	<0.01
Depth from soil surface	2	7.72	0.11	0.89	6.4	19.50	<0.01
Carbon	3	7.85	0.03	0.91	3.0	5.64	0.03

† Two transects, eight sites, three depths, and DEA mean for 13 sampling dates.

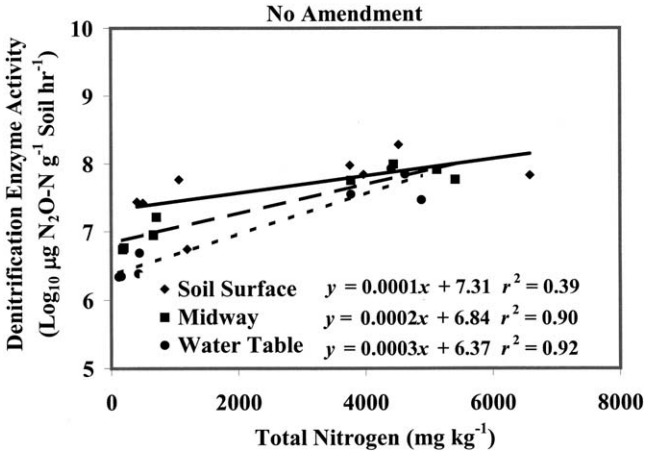

Fig. 2. Linear regression of denitrification enzyme activity vs. total soil nitrogen in the soil of a riparian zone contiguous to a swine wastewater spray field.

was nearly equal to the number of variables (3.1 vs. 3.0). The predictive equation was log DEA (μg N_2O-N kg^{-1} soil h^{-1}) = 0.0001 total soil N (μg g^{-1}) + 0.08 soil C (μg g^{-1}) − 0.36 soil depth (m) + 7.61.

When glucose was added alone, the partial r^2 for total N was 0.51 with a $P < 0.01$. Using stepwise regression, the correlation was improved by the inclusion of soil to a model r^2 of 0.73, and the $C(p)$ was very close to the number of variables (2.1 vs. 2.0). The predictive equation was log DEA (μg N_2O-N kg^{-1} soil h^{-1}) = 0.0002 total soil N (μg g^{-1}) − 0.36 soil depth + 7.53.

When both glucose and nitrate were added, the best correlated single factor was again total N, with a partial r^2 of 0.78. With stepwise regression analysis, a model r^2 of 0.91 and a $P < 0.03$ were obtained when total C and soil depth were added. The predictive equation was log DEA (μg N_2O-N kg^{-1} soil h^{-1}) = 0.000064 soil total N (μg g^{-1}) + 0.09 total soil C (μg g^{-1}) − 0.43 soil depth (m) + 7.85.

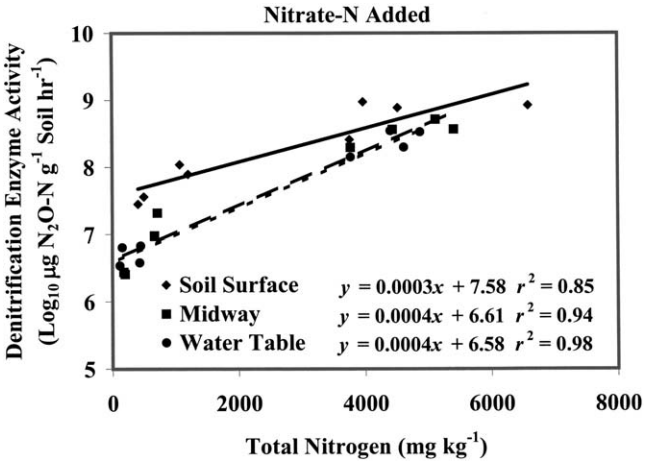

Fig. 3. Linear regression of denitrification enzyme activity vs. total soil nitrogen in the soil of riparian zone contiguous to a swine wastewater spray field when nitrate was added to the soil before incubation.

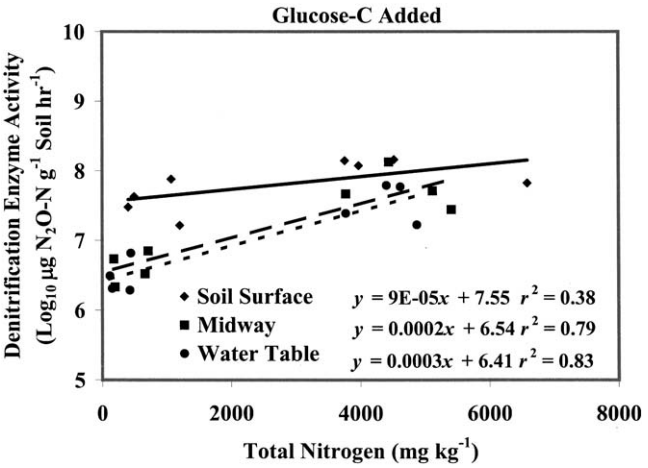

Fig. 4. Linear regression of denitrification enzyme activity vs. total soil nitrogen in the soil of riparian zone contiguous to a swine wastewater spray field when glucose was added to the soil before incubation.

Linear Regression of Total Soil Nitrogen and Denitrification Enzyme Activity

Results of the analysis of variance and stepwise regression suggested that linear regression of DEA with total soil N would be informative, particularly for soil depth layers. This expectation was found to be true for the middle and bottom soil depths. At these soil depths, simple linear regression of DEA vs. total soil N was very highly correlated with an $r^2 \geq 0.90$ in the control treatment (Fig. 2). At the bottom (water table) depth, the r^2 was 0.92 (log DEA = 0.0003 soil total N + 6.37), and the middle depth had an r^2 of 0.90 (log DEA = 0.0002 soil N + 6.84). However, DEA in the soil surface layer was not well as correlated to soil total N (r^2 = 0.39). This may be related to greater variations in soil oxidative–reductive conditions as well a total soil N in the surface, and this explanation is substantiated by the improvement in the correlation on the addition of nitrate.

If nitrate was added to the soil, the correlation of DEA to total soil N improved for all depths, and it continued to be more highly correlated at the lower soil depths (Fig. 3). In the surface soil depth, the r^2 was 0.85 (log DEA = 0.0003 soil N + 7.58). Although the bottom soil depth had a slightly higher r^2 than the middle depth, 0.98 and 0.94, respectively, their slope and intercepts were almost identical. Additionally, at the higher soil N concentrations, the log DEA rates were very similar for all depths.

When only glucose was added, the r^2 values and regression equations were similar to the control treatment, and the r^2 for the surface was 0.38 (Fig. 4). In the middle and bottom depths, the r^2 values were lower, 0.79 and 0.83, respectively; but the slopes and intercepts were nearly identical to the control. When both nitrate and glucose were added, there was little difference from the correlation or regression equation obtained when only nitrate was added in the lower two soil depths (Fig. 5). This indicated that there was little carbon limitation of denitrification even when larger amounts of nitrate

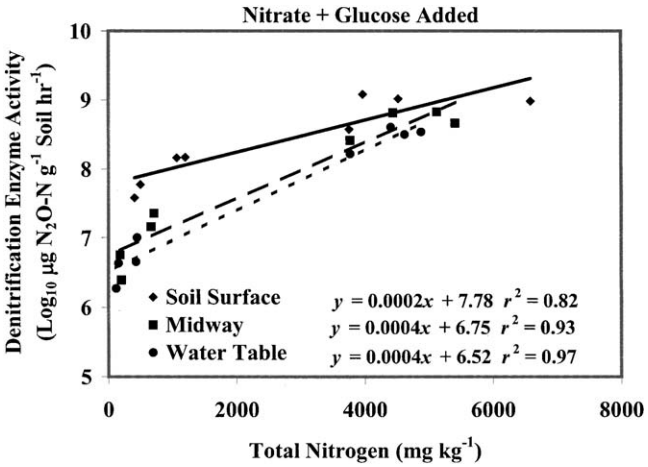

Fig. 5. Linear regression of denitrification enzyme activity vs. total soil nitrogen in the soil of riparian zone contiguous to a swine wastewater spray field when nitrate and glucose was added to the soil before incubation.

became available. In the surface layer, addition of both nitrate and glucose increased the DEA values more where the total soil N was low, and this resulted in a regression equation that was slightly less sloped than when only nitrate was added.

These data indicate that riparian soils with high total soil N had the capacity to denitrify considerable amounts of N, while those that did not have this soil characteristic were not effective in establishing high denitrifying capabilities even though they were probably exposed to periodically high nitrates from high water table or autumn leaf fall. Furthermore, this relationship was much stronger in the middle and water-table depths of the soil. Whereas the riparian zone had substantial areas with high total soil N, its capacity to develop zones for high denitrification was considerable and important in the buffering of excessive N transport. These finding are in agreement with the fact that the riparian zone was able to greatly reduce the concentration of N in the water table as it moved through the riparian zone to the stream.

CONCLUSIONS

- Denitrification enzyme activity generally decreased with soil depth. Mean values for the surface, middle, and bottom depths were 147, 83, and 67 μg N_2O-N kg^{-1} soil h^{-1}, respectively.
- The DEA values were lowest next to the spray field most likely because of their relatively low soil carbon and nitrogen concentrations. The DEA was also lower on the stream side opposite the spray field relative to the stream side closest to the spray field.
- Nitrate was generally found to be the limiting factor for denitrification.
- In a stepwise regression, soil total nitrogen was found to be the most highly correlated factor to DEA. However, inclusion of soil depth and total soil carbon further improved the predictive capability.
- Linear regression of total soil N vs. DEA was highly correlated in the middle and bottom layers in the control as well as the glucose- and nitrate-amended treatments. However, the surface layer was not well correlated unless nitrate was added. This was probably related to the greater variability of oxidative–reductive soil conditions as well as total soil N in the surface layer.
- This riparian zone possessed sufficient soil areas with high denitrifying conditions to be a significant factor in the removal of excess nitrogen in the ground water, and this high denitrifying capacity is consistent with the substantial measured reduction in ground water nitrogen as it passed through the riparian zone into the stream.

REFERENCES

Ambus, P., and R.R. Lowrance. 1991. Comparison of denitrification in two riparian soils. Soil Sci. Soc. Am. J. 55:994–997.

Brusch, W., and B. Nilsson. 1993. Nitrate transformations and water movement in a wetland area. Hydrobiologia 251:103–111.

Cooper, A.B. 1990. Nitrate depletion in the riparian zone and stream channel of a small headwater catchment. Hydrobiologia 202:13–26.

Flite, O.P., III, R.D. Shannon, R.R. Schnabel, and R.R. Parizek. 2001. Nitrate removal in a riparian wetland of the Appalachian Valley and Ridge Physiographic Province. J. Environ. Qual. 30:254–261.

Hanson, G.C., P.M. Groffman, and A.J. Gold. 1994. Symptoms of nitrogen saturation in a riparian wetland. Ecol. Appl. 4:750–756.

Haycock, N.E., and G. Pinay. 1993. Groundwater nitrate dynamics in grass and poplar vegetated riparian buffer strips during the winter. J. Environ. Qual. 22:273–278.

Hill, A.R. 1996. Nitrate removal in stream riparian zones. J. Environ. Qual. 25:743–755.

Hill, A.R., K.J. Devito, S. Campagnolo, and K. Sanmugadas. 2000. Subsurface denitrification in a forest riparian zone: Interactions between hydrology and supplies of nitrate and organic carbon. Biogeochemistry 51:193–223.

Hunt, P.G., D.L. Karlen, T.A. Matheny, and V.L. Quisenberry. 1996. Changes in carbon content of a Norfolk loamy sand after 14 years of conservation or conventional tillage. J. Soil Water Conserv. 51: 255–258.

Hunt, P.G., T.A. Matheny, and A.A. Szogi. 2003. Denitrification in constructed wetlands used for treatment of swine wastewater. J. Environ. Qual. 32:727–735.

Hunt, P.G., K.C. Stone, F.J. Humenik, and J.M. Rice. 1995. Impact of animal waste on water quality in an eastern Coastal Plain watershed. p. 257–264. *In* K. Steele (ed.) Animal waste and the landwater interface. Lewis Publ., Boca Raton, FL.

Jacobs, T.C., and J.W. Gilliam. 1985. Riparian losses of nitrate from agricultural drainage waters. J. Environ. Qual. 14:472–478.

Jordan, T.E., D.L. Correll, and D.E. Weller. 1993. Nutrient interception by a riparian forest receiving inputs from adjacent cropland. J. Environ. Qual. 22:467–473.

Lowrance, R.R., S. Dabney, and R. Schultz. 2002. Improving water and soil quality with conservation buffers. J. Soil Water Conserv. 57:36–43.

Lowrance, R.R., and R.K. Hubbard. 2001. Denitrification from a swine lagoon overland flow treatment system at a pasture riparian zone interface. J. Environ. Qual. 30:617–624.

Lowrance, R.R., R.L. Todd, and L.E. Asmussen. 1984. Nutrient cycling in an agricultural watershed. II. Stream flow and artificial drainage. J. Environ. Qual. 13:27–32.

Lowrance, R.R., G.R. Vellidis, and R.K. Hubbard. 1995. Denitrification in a restored riparian forested wetland. J. Environ. Qual. 24: 808–815.

Novak, J.M., P.G. Hunt, K.C. Stone, D.W. Watts, and M.H. Johnson. 2002. Riparian zone impact on phosphorus movement to a Coastal Plain black water stream. J. Soil Water Conserv. 57:127–133.

Peterjohn, W.T., and D.L. Correll. 1984. Nutrient dynamics in an

agricultural watershed: Observations on the role of a riparian forest. Ecology 65:1466–1475.
SAS Institute. 1999. The SAS system for Windows. Release 8.02. SAS Inst., Cary, NC.
Sloan, A.J., J.W. Gilliam, J.E. Parsons, R.L. Mikkelsen, and R.C. Riley. 1999. Groundwater nitrate depletion in a swine lagoon effluent-irrigated pasture and adjacent riparian zone. J. Soil Water Conserv. 54:651–656.
Stone, K.C., P.G. Hunt, S.W. Coffey, and T.A. Matheny. 1995. Water quality status of a USDA water quality demonstration project in the eastern Coastal Plain. J. Soil Water Conserv. 59:567–571.
Stone, K.C., P.G. Hunt, F.J. Humenik, and M.H. Johnson. 1998b. Impact of swine waste application on ground and stream water quality in an eastern Coastal Plain watershed. Trans. ASAE 41:1665–1670.
Stone, K.C., P.G. Hunt, M.H. Johnson, and S.W. Coffey. 1998a. GLEAMS simulation of groundwater nitrate-N from row crop and swine wastewater spray fields in the eastern Coastal Plain. Trans. ASAE 41:51–57.
Tiedje, J.M. 1994. Denitrifier enzyme activity (DEA). p. 256–257. *In* R.W. Weaver, J.S. Angle, and P.S. Bottomley (ed.) Methods of soil analysis. Part 2. SSSA Book Ser. 5. SSSA, Madison, WI.
USEPA. 1983. Methods for chemical analysis of water and wastes. Rep. 600/4-79-020. U.S. Gov. Print. Office, Washington, DC.
Vellidis, G.R., R.R. Lowrance, P. Gay, and R.K. Hubbard. 2003. Nutrient transport in a restored riparian wetland. J. Environ. Qual. 32: 711–726.