

# Denitrification in Riparian Wetlands Receiving High and Low Groundwater Nitrate Inputs

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## ABSTRACT

Wetlands potentially remove a high percentage of the groundwater-borne nitrate ( $\text{NO}_3^-$ ) that moves from upland environments before it reaches streams. It is important to determine how much of the  $\text{NO}_3^-$  that enters wetlands is actually removed from the ecosystem by denitrification (conversion of  $\text{NO}_3^-$  into  $\text{N}_2$  gas) rather than cycled between plants and soil. We measured denitrification in riparian forests with upland to wetland transition zones (moderately well drained and somewhat poorly drained soils) and red maple (*Acer rubrum* L.) swamps (poorly and very poorly drained soils) on two sides of a stream. Soils on the two sides were similar, but the upland land use on one side was a high density, unsewered residential development (enriched site), while the upland on the other side was undeveloped (control site). Denitrification was measured using an acetylene-based intact core (0–15 cm) technique under unamended, water amended, and water plus nitrate-amended conditions. Denitrification (both unamended and amended rates) and soil and groundwater  $\text{NO}_3^-$  levels were consistently higher in soils on the enriched site. Estimates of annual denitrification ranged from  $<5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  on the moderately well drained control site soil to nearly  $40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  on the very poorly drained enriched site soil. Stimulation of surface soil denitrification by subsurface  $\text{NO}_3^-$  enrichment requires a complex interaction between hydrology, plant uptake of  $\text{NO}_3^-$ , and movement of plant N into soil  $\text{NO}_3^-$  pools through litterfall, mineralization, and nitrification. Comparison of measured denitrification rates with estimates of groundwater  $\text{NO}_3^-$  loading suggested that denitrification may have removed up to 50% of the groundwater  $\text{NO}_3^-$  that entered the enriched site.

**N**ITRATE ( $\text{NO}_3^-$ ) is a federally regulated drinking water pollutant that may contribute to the eutrophication of marine and fresh water bodies (Keeney, 1987). Wetlands have been found to remove  $\text{NO}_3^-$  moving from upland areas, preventing its movement into surface water bodies (Nixon and Lee, 1986; Johnston, 1991). Riparian wetlands, which exist at the interface between upland and aquatic systems, have a particularly high potential to affect groundwater and surface water quality through filtering and attenuation of pollutants (Lowrance et al., 1984; Peterjohn and Correl, 1984; Jacobs and Gilliam, 1985).

Denitrification, the anaerobic process in which microbes convert  $\text{NO}_3^-$  to N gases in the absence of oxygen, is one of the major processes consuming  $\text{NO}_3^-$  in wetlands. There are other possible fates for  $\text{NO}_3^-$  in wetlands, such as plant uptake and microbial immobilization, but only denitrification removes  $\text{NO}_3^-$  from the system as a gas (Bowden, 1987). Nitrate immobilized by plants or microbes can be rereleased to soil following death and decomposition of these organisms (Paul and Clark,

1989). Moreover, over time, chronic N inputs can cause plant and microbial  $\text{NO}_3^-$  sinks to become saturated and thus less effective (Aber et al., 1989).

Soil denitrification is affected by many factors, including  $\text{NO}_3^-$  availability, soil moisture, temperature, and C availability (Tiedje, 1988). Denitrification in wetland ecosystems has been found to vary in response to water levels, sediment C content and quality, and the nature of ecosystem N cycling (Bowden, 1987; Groffman, 1994). Many studies have found that  $\text{NO}_3^-$  limits denitrification (Robertson and Tiedje, 1984; Bowden, 1987; Broderick et al., 1988; Groffman and Tiedje, 1989; Morris, 1991; Lowrance, 1992; Merrill and Zak, 1992), which implies that wetland denitrification should increase with inputs of  $\text{NO}_3^-$  from upland areas. The amount of upland-derived  $\text{NO}_3^-$  that is denitrified rather than immobilized by plants or microbes likely varies with season, hydrology, vegetation and soil type, and other factors. The partitioning of  $\text{NO}_3^-$  between denitrification and other sinks is likely important to the long-term water quality maintenance value and biotic integrity of wetland ecosystems.

In this study, we compared denitrification rates in two riparian forest sites with similar soils, vegetation, and hydrology. The sites were located on the east and west sides of a small stream. Each site contained an upland to wetland transition zone with moderately well drained (MWD) and somewhat poorly drained (SPD) soils above a red maple (*Acer rubrum* L.) wetland with poorly drained (PD) and very poorly drained (VPD) soils. The sites differed in that the eastern side of the stream was below an intensive residential development with on-site septic systems, while the upland above the western side was not developed. Previous studies on the eastern site found that groundwater entering this riparian forest was highly enriched in  $\text{NO}_3^-$  (Simmons et al., 1992). The objectives of this study were (i) to quantify surface soil denitrification in riparian wetlands, (ii) to assess the effects of groundwater  $\text{NO}_3^-$  enrichment on denitrification in these wetlands, and (iii) to determine the factors limiting denitrification in  $\text{NO}_3^-$ -enriched and nonenriched riparian wetlands.

## METHODS

### Site Selection

The two sites selected for this study were similar in all physical features except for upland land use (Fig. 1, Table 1). The sites were located within 25 km of Kingston, RI, on the eastern and western sides of a small stream (Sandhill Brook) that is tributary to Narragansett Bay. The dominant vegetation at both sites consisted of oak (*Quercus* sp.) and maple (*Acer* sp.) dominated forest in the upland to wetland transition zones (MWD and SPD soils) with red maple dominated wetlands

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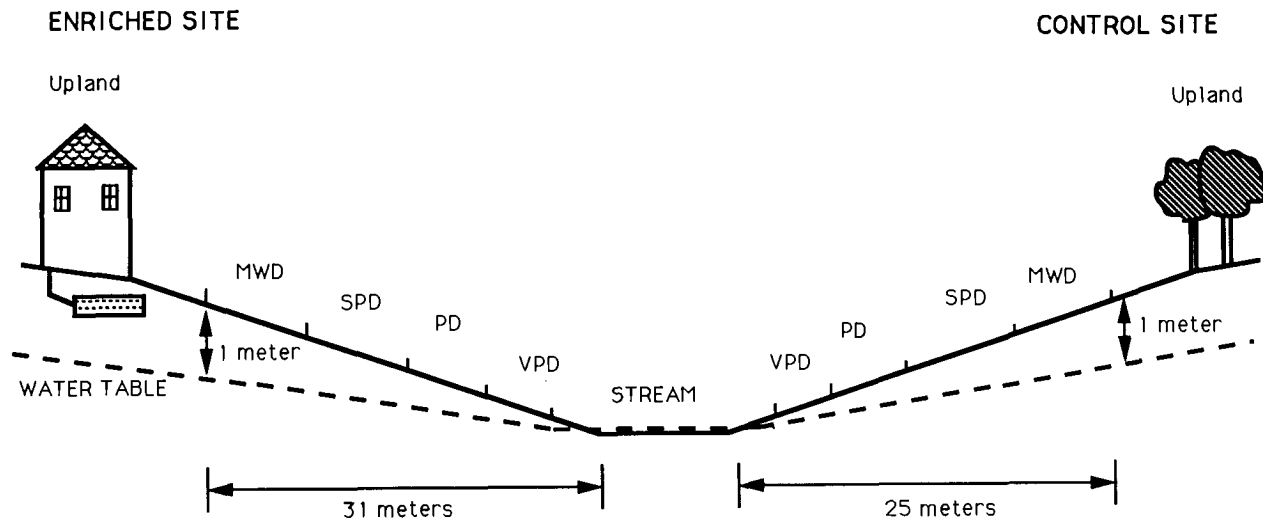


Fig. 1. Site diagram showing enriched and control sites located on eastern and western sides of Sandhill Brook, Rhode Island.

(PD and VPD soils). Soils at both sites were derived from stratified glaciofluvial deposits with variable amounts of alluvial material and were coarse-textured inceptisols and entisols. The poorly drained and very poorly drained soils at each site were classified as hydric. Soil drainage classes were mapped based on soil morphological characteristics (Wright and Sautter, 1988).

The eastern or *enriched* site was directly downgradient of a dense unsewered residential development that was built in the 1950s. In our previous groundwater study, soils and groundwater flow on this site were well characterized. Groundwater  $\text{NO}_3^-$ -N concentrations ranged from an average of  $8 \text{ mg L}^{-1}$  at the upland edge of the riparian zone to an average of  $0.5 \text{ mg L}^{-1}$  beneath the VPD soils in the wetland (Simmons et al., 1992).

In order to quantify the effects of  $\text{NO}_3^-$  loading on the riparian wetland, a *control* site was located on the western side of the stream, which has an undeveloped upland. The direction of groundwater flow at the control site was determined by analysis of water table elevations and triangulation (Driscoll, 1986). Groundwater monitoring wells of 5-cm diam. slotted PVC pipe were already in place on the enriched site. There were 30 wells in place at this site, six in each of the four soil drainage classes, and six just above the upland edge of the riparian zone. Three of the wells at each location in the enriched site sampled the top meter of the permanently saturated zone, while the other three were placed at the top of the maximum seasonal high water table. For this study, nine new wells were installed at the control site (three at the upland edge, three in the SPD soil, three in the VPD soil) to sample within the top meter of the permanently saturated zone. Wells were installed

by hand augering to the desired depth, inserting the well casing, backfilling with clean sand to the top of the slots, then backfilling to within 15 cm of the surface with native fill, and filling the remaining hole with bentonite pellets. The well was then capped.

Groundwater  $\text{NO}_3^-$  levels were measured in water samples taken from the wells after purging three times the well volume. These water samples were refrigerated for later analysis using an Alpkem RFA 300 Rapid Flow Analyzer. Groundwater  $\text{NO}_3^-$  concentrations were measured three times between March 1991 and March 1992.

### Soil Analysis

Sampling plots of 4 by 8 m were located within each soil type on each of the two sites for a total of eight plots. Replicate samples of the upper 15 cm of soil on each plot were taken with a soil tube auger weekly or biweekly during the spring and fall, and monthly during the summer for a total of 15 sampling dates between March 1991 and March 1992. Soil moisture was measured gravimetrically. Soil  $\text{NO}_3^-$ -N was extracted with 2 M KCl and quantified with an Alpkem RFA Rapid Flow Analyzer. Soil organic matter content was measured by loss on ignition at  $450^\circ\text{C}$  on soil samples taken from test pits dug in each drainage class in August 1991. Soil pH was measured on seven of the 15 sampling dates in a 2:1 water/soil solution using an electrometric method (US EPA, 1979) with a glass electrode (Corning Glass Works, Corning, NY).

### Denitrification Method

Denitrification rates were measured using the acetylene-based intact core technique described by Tiedje et al. (1989). This technique has been found to produce similar denitrification rates to  $^{15}\text{N}$  balance (Parkin et al., 1985), in-field chamber (Ryden et al., 1987), and direct  $^{15}\text{N}_2$  flux (Christensen et al., 1991; Groffman et al., 1993) methods.

Intact soil cores (0–15 cm depth) were taken using a 2-cm diam. punch auger fitted with plexiglass inserts. A total of five or six intact core samples were taken from each soil type on both sites, for 40 to 48 cores every sampling date.

The core samples were stored overnight at in-field soil temperatures in a BOD type incubator. On the following day, the cores were removed from the incubation unit and sealed with rubber stoppers. Acetylene (to at least 10 kPa final conc.) was added to the headspace of each core and mixed into the soil pores by repeated pumping with a 60-mL syringe. The

Table 1. Soil (A horizon) properties at the enriched and control sites.

Site	Soil drainage class†	% Sand	% Silt + clay	% Soil organic matter	pH
Enriched	MWD	99.3	0.7	5.9‡	3.95‡
	SPD	99.3	0.7	6.3‡	3.90‡
	PD	99.5	0.5	8.6‡	4.05‡
	VPD	91.8	8.2	12.5	5.05
Control	MWD	99.4	0.6	3.6	4.74
	SPD	99.2	0.8	4.9	4.79
	PD	96.9	3.1	3.5	4.63
	VPD	98.0	2.0	3.5	4.97

† MWD = moderately well drained, SPD = somewhat poorly drained, PD = poorly drained, VPD = very poorly drained.

‡ Indicates significant difference between enriched and control site soils.

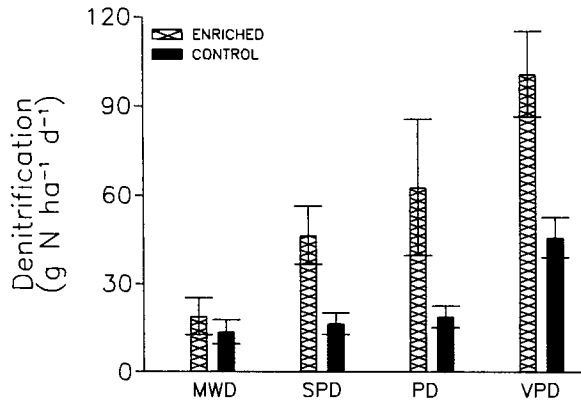


Fig. 2. Mean denitrification rates over 15 sampling dates between March 1991 and March 1992 in four soil types in enriched and control sites. Values are mean (standard error).

cores were then incubated at in-field soil temperatures for 6 h. Gas samples (3 mL) were removed from the headspace after 2 and 6 h. Gas samples and blanks were stored in 3-mL rubber stoppered glass vials (Venoject, Terumo Scientific, NJ) and were analyzed for  $N_2O$  by electron-capture gas chromatography. The rate of  $N_2O$  production between 2 and 6 h was taken as the rate of denitrification.

Following incubation, cores were weighed and measured for area and bulk density calculations. The internal headspace volume of each core was measured with a pressure transducer (Parkin et al., 1984). Annual denitrification N flux was calculated by extrapolating measured rates over the intervals between sampling dates. Denitrification rate was assumed to be zero for 88 d during the coldest part of the year. This assumption may cause a slight underestimate of annual denitrification flux since some microbial activity likely goes on during winter.

### Amendment Studies

Amendment studies were begun in May of 1991 to determine the factors limiting denitrification rates. After the initial incubation of the unamended cores, the cores were unstoppered and amended with either 8 mL of distilled  $H_2O$  to increase the anaerobic volume in the cores (three cores per drainage class) or 8 mL of a  $100 \text{ mg L}^{-1} \text{ NO}_3^- \text{-N}$  solution to remove any  $\text{NO}_3^-$  limitation of denitrification (three cores per drainage class). The amended cores were stored overnight at in-field temperatures. On the following day, the cores were incubated again as described above. Amendment studies were done on eight sampling dates throughout the year; 21 May, 7 June, 8 July, 5 August, 23 September, 24 October, and 18 November of 1991, and 8 March of 1992.

### Data Analysis

Both parametric (one-way analysis of variance) and nonparametric (Wilcoxon, Kruskal-Wallis) statistical comparisons were performed using the Statistical Analysis System (SAS Inst., 1985). Results of nonparametric tests were used when data did not fit a normal distribution (all variables other than soil moisture, pH, and organic matter content). Both overall site as well as soil by soil comparisons of the enriched and control sites were performed.

## RESULTS

In an analysis over all soil types and sampling dates, denitrification rates were higher ( $p < 0.01$ ) on the en-

Table 2. Estimates of annual denitrification N loss in soils at enriched and control sites, March 1991 to February 1992.

Site	Soil drainage class†	Denitrification ( $\text{kg N ha}^{-1} \text{ yr}^{-1}$ )
Enriched	MWD	7.1
	SPD	15.9
	PD	21.4
	VPD	38.5
Control	MWD	4.8
	SPD	5.7
	PD	6.3
	VPD	16.3

† MWD = moderately well drained, SPD = somewhat poorly drained, PD = poorly drained, VPD = very poorly drained.

riched site than the control site (Fig. 2). On a soil-by-soil basis, the difference between sites was significant ( $p < 0.05$ ) for all soils other than MWD. Estimates of annual denitrification N flux for soils on the sites ranged from less than 5 to nearly  $40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  (Table 2). Statistical comparison of annual denitrification flux estimates was not possible with only 1 yr of data.

Denitrification rates were generally higher at the wetland end of the riparian zone (PD and VPD soils) than at the upland end (MWD and SPD), but the differences were not dramatic. Over all sampling dates, there were no significant differences among the soils within the enriched site. In the control site, denitrification was higher ( $p < 0.05$ ) in the VPD soil than in the MWD, SPD, and PD soils.

Denitrification was generally highest in spring and fall and lowest in summer, but the seasonal pattern was not dramatic, especially in the enriched site soils (Fig. 3).

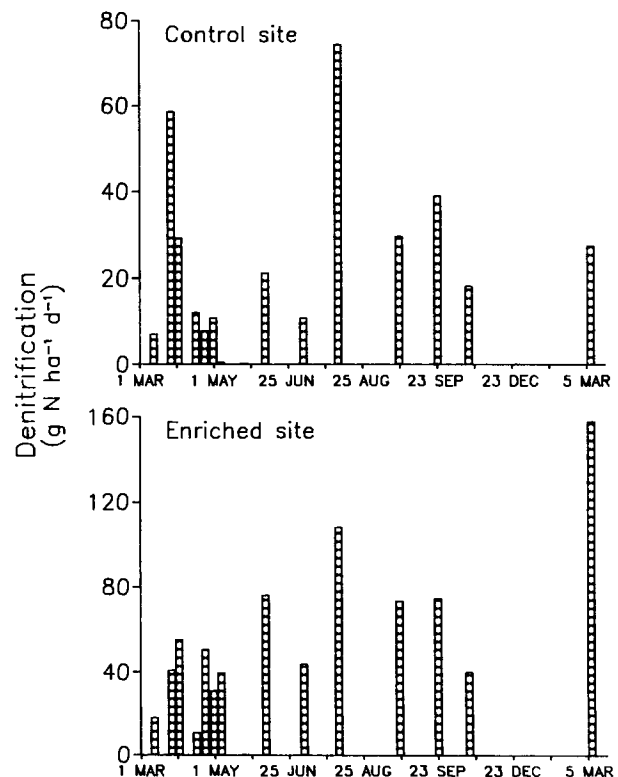


Fig. 3. Mean daily denitrification rates for 15 sampling dates between March 1991 and March 1992 over all soil types in control and enriched sites.

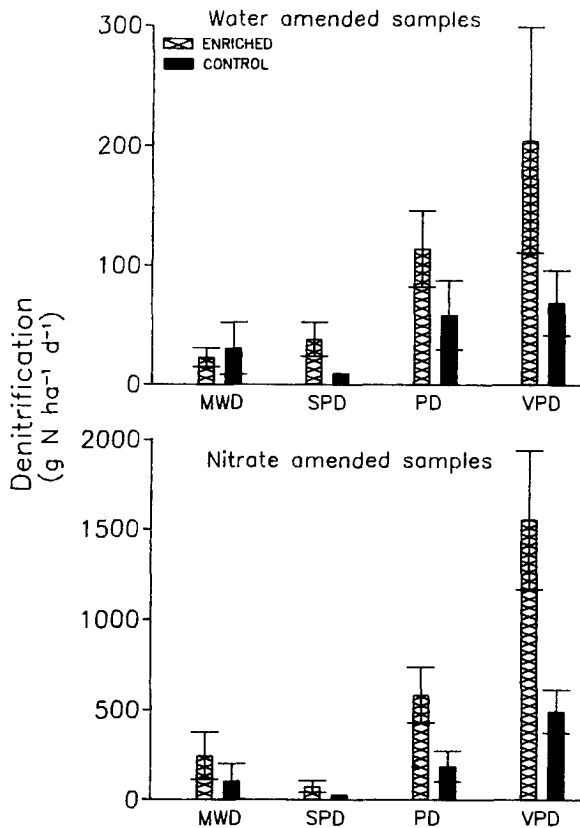


Fig. 4. Water-amended and water plus nitrate-amended mean denitrification rates over eight sampling dates between March 1991 and March 1992 in four soil types in enriched and control sites. Values are mean (standard error).

A marked exception to the general pattern was the August sample date, which followed a 5-cm rainfall event.

Water and water plus  $\text{NO}_3^-$ -amended denitrification rates showed similar patterns as unamended denitrification rates (Fig. 4). Both amended activities were higher ( $p < 0.05$ ) in the enriched site overall, and in all soils other than MWD on a soil-by-soil basis. Activity in water-amended cores was not significantly higher than activity in unamended cores in any soil type. Water plus  $\text{NO}_3^-$  amendments stimulated ( $p < 0.05$ ) activity in both sites in an analysis over all soil types. On a soil-by-soil basis, water plus  $\text{NO}_3^-$ -amended rates were higher than unamended or water amended rates only in the VPD soils on the enriched site and in the PD and VPD soils on the control site.

Soil moisture and  $\text{NO}_3^-$  levels (Fig. 5) were higher ( $p < 0.01$ ) in the enriched site than the control site in all soil types (Fig. 5). Groundwater  $\text{NO}_3^-$  concentrations were higher ( $p < 0.01$ ) on the enriched site at all sampling locations at all sample dates (Table 3).

## DISCUSSION

Although we have no direct evidence of  $\text{NO}_3^-$  transport from groundwater to surface soils, our results suggest that subsurface  $\text{NO}_3^-$  enrichment increased denitrification in surface soils in the enriched site. Soil and groundwater  $\text{NO}_3^-$  levels, and unamended, water-amended, and water plus  $\text{NO}_3^-$ -amended denitrification rates were all higher in the surface soils on the enriched site than the control

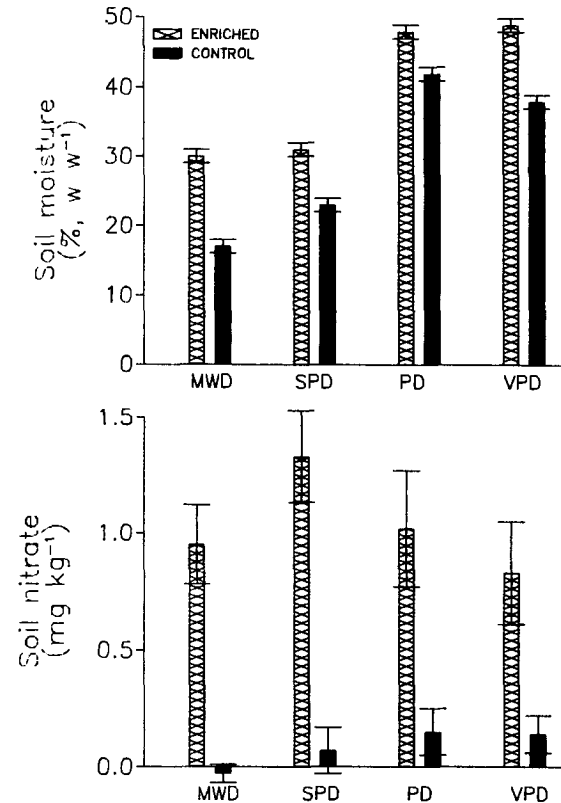


Fig. 5. Mean soil moisture and nitrate levels over 15 sampling dates between March 1991 and March 1992 in four soil types in enriched and control sites. Values are mean (standard error).

site. Stimulation of surface soil denitrification by subsurface enrichment requires a complex interaction between hydrologic, plant, and soil processes. Plants must take up  $\text{NO}_3^-$  from groundwater which results in increased levels of N in plant litter and root detritus. Nitrogen enrichment of litter and root detritus likely leads to high rates of decomposition, mineralization, and nitrification, producing elevated levels of  $\text{NO}_3^-$  in the surface soil. Where conditions are anaerobic and there is a sufficient C source (as in these riparian wetlands), unamended denitrification rates rise in response to the increase in available  $\text{NO}_3^-$ . The increase in water-amended and water plus  $\text{NO}_3^-$ -amended denitrification rates suggests that the population of denitrifying organisms has increased at this site as well. We also measured increases in denitrification enzyme activity (an index of denitrifier population size) at the enriched site (Hanson, 1993).

Lowrance (1992) suggested that surface soil denitrification may be a major route of removal of groundwater  $\text{NO}_3^-$  in riparian forests. Several studies have found that the potential for  $\text{NO}_3^-$  removal in the subsurface itself is low due to low levels of available C (Parkin and Meisinger, 1989; Obenhuber and Lowrance, 1991; Groffman et al., 1992; Lowrance, 1992). In areas where groundwater flow is the dominant mode of  $\text{NO}_3^-$  movement from uplands into riparian zones, the water quality maintenance value of these zones may be dependent on the complex interaction of hydrologic, plant, and soil processes that facilitates surface soil denitrification of groundwater  $\text{NO}_3^-$ .

In addition to the elevated soil  $\text{NO}_3^-$  concentrations,

**Table 3. Groundwater nitrate concentrations in enriched and control sites.**

Date	Soil drainage class†	Groundwater nitrate	
		Enriched	Control
— NO <sub>3</sub> <sup>-</sup> -N (mg L <sup>-1</sup> ) —			
24 Oct. 1991	MWD	3.5	0.2
	SPD	4.1	<0.1
	VPD	0.2	<0.1
18 Nov. 1991	MWD	2.1	<0.1
	SPD	2.2	<0.1
	VPD	2.4	<0.1
8 Mar. 1992	MWD	15.3	<0.1
	SPD	2.2	<0.1
	VPD	2.1	0.3

† MWD = moderately well drained, SPD = somewhat poorly drained, VPD = very poorly drained.

high levels of soil moisture on the enriched site relative to the control site also contributed to the high denitrification on the enriched site. Higher soil moisture on the enriched site is likely a product of the relatively high levels of soil organic matter on this site. High soil organic matter levels may be a product of the chronic N inputs to this site. Long-term N inputs have likely increased the productivity of vegetation at this site, which has led to higher levels of soil organic matter.

As expected, the rate of denitrification was higher at the wetland end (PD and VPD soils) of the riparian zone than at the upland end (MWD and SPD soils) but the difference was not dramatic. The PD and VPD soils had higher moisture contents and soil organic matter levels, which favored denitrification in these soils. However, while the PD and VPD soils generally had the highest denitrification rates, the MWD and SPD soils were also removing a significant amount of NO<sub>3</sub><sup>-</sup>. These soils are transitional between wetlands and uplands, and are characterized as having fluctuating water tables (Wright and Sautter, 1988). Fluctuating water tables are favorable for the development of vigorous coupled nitrification-denitrification activity (Reddy and Patrick, 1984). Transitional soils may thus be important to the water quality maintenance value of riparian zones and may need to be considered in riparian area protection and management plans.

Denitrification has been shown to have a seasonal pattern in temperate climates (Goodroad and Keeney, 1984; Myrold, 1988; Schmidt et al., 1988; Groffman and Tiedje, 1989; Groffman et al., 1993). Pulses of activity have been found to occur in the spring and fall, when soil moisture levels are at their highest and plant uptake of water and NO<sub>3</sub><sup>-</sup> is minimal. Denitrification in natural ecosystem soils frequently does not increase in response to rainfall or water inputs during the growing season because plant and heterotrophic microbes keep soil NO<sub>3</sub><sup>-</sup> levels low. In this study, we observed peaks of activity in the spring and fall, but activity during summer was occasionally significant, especially in response to a large rainfall event. In highly N-enriched sites, seasonal patterns of activity may not be as marked as has been observed in other studies. In enriched sites, soil NO<sub>3</sub><sup>-</sup> levels may be high enough in summer to allow for the development of vigorous denitrification activity following rainfall events that increase the anaerobic volume in soil.

The annual denitrification rates observed on the sites in this study ranged from <5 kg N ha<sup>-1</sup> yr<sup>-1</sup> in the MWD soil on the control site to <40 kg N ha<sup>-1</sup> yr<sup>-1</sup> in the PD soil on the enriched site. These rates are similar to rates measured by Groffman and Tiedje (1989) using the same intact core technique in a range of soils in Michigan. In that study annual rates of N loss to denitrification ranged from less than 1.0 kg N ha<sup>-1</sup> yr<sup>-1</sup> in a well-drained sandy soil to nearly 40 kg N ha<sup>-1</sup> yr<sup>-1</sup> in a poorly drained clay loam soil adjacent to an agricultural field. Hendrickson (1981) used a similar intact core method to ours and measured denitrification rates of 31.5 kg N ha<sup>-1</sup> yr<sup>-1</sup> (range from 1.4 to 295 kg N ha<sup>-1</sup> yr<sup>-1</sup>) in riparian forest soils adjacent to agricultural uplands in Georgia. Zak and Grigal (1991) also used a similar technique to ours and measured 10 kg N ha<sup>-1</sup> yr<sup>-1</sup> removal by denitrification in red maple swamps on sandy, N-poor soils in Minnesota.

It is important to evaluate the amount of NO<sub>3</sub><sup>-</sup> removed by denitrification relative to the amount of NO<sub>3</sub><sup>-</sup> entering the riparian zone. Using the groundwater flow and groundwater NO<sub>3</sub><sup>-</sup>-N data obtained by Simmons et al. (1992) directly upgradient of the MWD soil, we estimated that 62.7 g N m<sup>-1</sup> yr<sup>-1</sup> enter the riparian area at the upland boundary of the MWD soil of the enriched site. This estimate of loading does not account for the possibility of upwelling bringing NO<sub>3</sub><sup>-</sup>-laden groundwater into the interior of the riparian zone and therefore may be an underestimate.

We evaluated our denitrification results relative to this loading estimate by calculating how much *extra* denitrification occurred between the upland edge and the streamside of the enriched riparian zone. To make this calculation, we subtracted denitrification rates in the control site soils from the rates in the enriched site soils to produce an estimate of the extra denitrification induced by NO<sub>3</sub><sup>-</sup> loading in each soil type. We then multiplied this extra denitrification rate by the width (in the direction of groundwater flow) of each soil type within the enriched site riparian zone and summed the results. This analysis produced an estimate of how much extra denitrification occurred in response to groundwater NO<sub>3</sub><sup>-</sup> enrichment between the upland edge of the riparian zone and the stream.

Total extra denitrification induced by groundwater NO<sub>3</sub><sup>-</sup> input was calculated as 36.8 g m<sup>-1</sup> width (orthogonal to groundwater flow) of riparian zone per year. This indicates that denitrification removed an amount of N equal to approximately 59% of the NO<sub>3</sub><sup>-</sup> that entered the enriched site. If upwelling of NO<sub>3</sub><sup>-</sup>-laden groundwater in the interior of the riparian zone was significant, then the percent removal was lower. This analysis suggests that denitrification is removing a significant amount of the upland-derived NO<sub>3</sub><sup>-</sup> that enters the site, and that this process is moderating NO<sub>3</sub><sup>-</sup> delivery to surface water.

The relative contribution of denitrification, plant uptake and other processes to NO<sub>3</sub><sup>-</sup> removal has been a major question in riparian zone research. Peterjohn and Correll (1984) measured plant uptake rates that accounted for 33% of observed groundwater NO<sub>3</sub><sup>-</sup> removal in a riparian forest in Maryland and assumed that denitrification was responsible for the remaining 67% of removal.

Lowrance et al. (1984) measured both denitrification and plant uptake rates that were greater than groundwater  $\text{NO}_3^-$  inputs to a riparian forest in Georgia and drew no conclusions about which process was responsible for actual removal of  $\text{NO}_3^-$  from the subsurface. The relative contribution of denitrification to  $\text{NO}_3^-$  removal may be important to the long-term water quality maintenance value of riparian zones, because while denitrification results in removal of N from the ecosystem as gas, plant and microbial uptake allow for recycling and possible hydrologic loss of  $\text{NO}_3^-$ .

Our amendment studies showed that denitrification was still  $\text{NO}_3^-$  limited in the soils of the enriched site. These results suggest that the capacity of this site to denitrify upland-derived  $\text{NO}_3^-$  is not saturated. The amount of  $\text{NO}_3^-$  that reaches denitrifiers is affected by complex interactions between hydrology, vegetation, and soil processes, however, and it is not clear how denitrification has or will continue to respond to increases in  $\text{NO}_3^-$  inputs to this site.

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