

# Nitrate Dynamics in Riparian Forests: Microbial Studies

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## ABSTRACT

While riparian forests have a demonstrated ability to remove nitrate ( $\text{NO}_3^-$ ) moving from uplands before it enters streams, there is considerable uncertainty as to the mechanisms of  $\text{NO}_3^-$  removal in these areas. We characterized spatial and temporal variation in denitrification enzyme activity (DEA), microbial biomass C and N content, soil respiration and potential net N mineralization and nitrification in three riparian forest sites consisting of soil catenas containing moderately well, somewhat poorly, poorly, and very poorly drained soils (inceptisols and entisols). These measurements were made in conjunction with studies of  $\text{NO}_3^-$  removal from groundwater during growing and dormant seasons that are reported in a companion paper. Two of the sites were on stratified glacial drift, one with an undeveloped upland and one with an upland with high density unsewered residential development that produced groundwater at the edge of the riparian zone with  $\text{NO}_3^-$ -N concentrations between 8 and 12 mg/L. The third site was on unstratified glacial drift with an undeveloped upland. Hydric surface (0–15 cm) soils (poorly and very poorly drained) consistently had higher DEA than upland-wetland transition zone (moderately well and somewhat poorly drained) surface soils. Spatial patterns of microbial biomass C and N content were more variable but showed the same general pattern as DEA. Levels of DEA and microbial biomass were consistently low or undetectable at and below the seasonal high water table. Surface soil DEA and microbial biomass were correlated with  $\text{NO}_3^-$  removal from groundwater during the growing season. Low levels of DEA and microbial biomass in the subsurface however, suggested that plant uptake was the dominant groundwater  $\text{NO}_3^-$  sink during the growing season. During the dormant season, water table levels were higher and groundwater-borne  $\text{NO}_3^-$  was able to interact with near surface soil and be removed by denitrification and/or microbial immobilization. Potential net N mineralization was quite variable both within and between sites, while potential net nitrification was very low at most sites. A notable exception was in the transition-zone soils at the stratified drift site with a densely developed upland, which had relatively high rates of net  $\text{NO}_3^-$  production. The high nitrification rates observed at this site may suggest that the long-term buffering potential of this site is limited.

WHILE RIPARIAN FORESTS have a demonstrated ability to remove  $\text{NO}_3^-$  moving from uplands before it enters streams (Lowrance et al., 1984; Peterjohn and Correll, 1984; Jacobs and Gilliam, 1985; Pinay and Decamps, 1988; Cooper, 1990), there is considerable uncertainty as to the mechanisms of  $\text{NO}_3^-$  removal in these areas. The strong affinity of plants and microorganisms for N creates a high potential for  $\text{NO}_3^-$  removal in riparian zones, but there is considerable spatial and temporal variability in plant and microbial activity and consequently, in riparian zone N dynamics. Understanding this variability is necessary for the development of broadly applicable models and management strategies for the use of riparian zones for water quality maintenance and improvement (Lowrance and Shiromhamadi, 1985).

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Possible  $\text{NO}_3^-$  fates in riparian zones include uptake by plants, denitrification (conversion of  $\text{NO}_3^-$  into N gases by facultative anaerobic microorganisms), and microbial immobilization. Within riparian zones, marked changes in vegetation, soils and hydrology occur over small spatial scales (Warwick and Hill, 1988; Cooper, 1990). These changes cause marked spatial variation in  $\text{NO}_3^-$  attenuation processes. In addition to spatial variation, seasonal variation, especially in vegetation uptake, is likely to be significant. Over longer time scales, the nature and extent of N input into riparian zones will likely affect attenuation processes. Riparian zones that have been subject to long-term  $\text{NO}_3^-$  inputs may have different attenuation capacities than non- $\text{NO}_3^-$  enriched areas.

Understanding the relative magnitude of different  $\text{NO}_3^-$  attenuation mechanisms, or the partitioning of  $\text{NO}_3^-$  to different fates in riparian zones, is important for evaluating the long-term effectiveness of these zones as  $\text{NO}_3^-$  sinks. While denitrification results in removal of  $\text{NO}_3^-$  from the system as N gas,  $\text{NO}_3^-$  removed by plant uptake and microbial immobilization is subject to remineralization and release back to the soil solution. Over time, plant and microbial pools can become enriched, or “saturated” with N, resulting in a decline in their N absorbing capacity (Aber et al., 1989). Long-term N inputs to riparian zones may lead to increases in N mineralization and nitrification induced by N enrichment of soil organic matter and microbial populations. Increases in mineralization and nitrification could increase within-site  $\text{NO}_3^-$  production and export from the riparian zone (Hill and Shackleton, 1989). Sites where denitrification is the dominant  $\text{NO}_3^-$  sink should be more effective as long-term buffer zones than areas where plant uptake and immobilization are the dominant sinks.

In this study, we characterized microbial processes in three forested riparian zones with catenas consisting of moderately well, somewhat poorly, poorly, and very poorly drained soils. The study presented here was carried out in conjunction with direct measurements of  $\text{NO}_3^-$  removal from groundwater at these sites (see the companion paper by Simmons et al., 1992). We measured microbial biomass C and N content as indices of the size of the active pool of microorganisms (Smith and Paul, 1990), and several indices of microbial activity including DEA, soil respiration, and potential net N mineralization and nitrification. Our objectives were to: (i) characterize spatial variation in microbial processes in riparian zones and (ii) elucidate the mechanisms responsible for the patterns of  $\text{NO}_3^-$  attenuation observed in the companion groundwater study.

## MATERIALS AND METHODS

### Sites

The sites used in this study are described in detail in the companion paper by Simmons et al. (1992). Briefly, there

**Abbreviations:** DEA, denitrification enzyme activity.

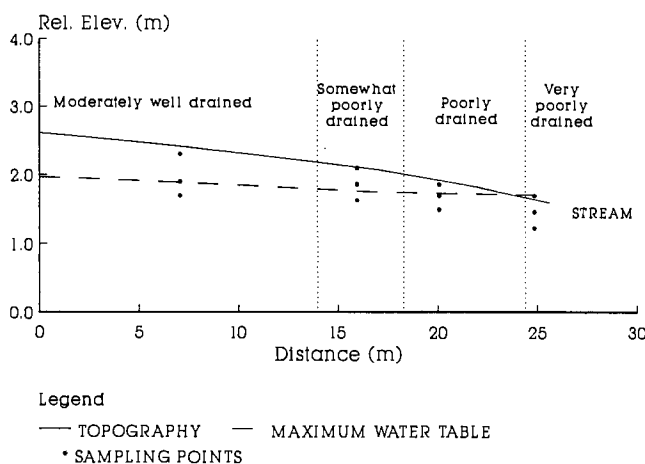
were three sites located within 35 km of Kingston, RI. Each site consisted of a soil catena containing moderately well, somewhat poorly, poorly, and very poorly drained soils (Fig. 1). Soils were coarse-textured inceptisols and entisols and the poorly and very poorly drained soils were classified as hydric. Two of the sites were located on stratified glaciofluvial deposits (outwash) and one was on unstratified glacial drift. The unstratified drift site and one of the outwash sites had undeveloped uplands characterized by oak (*Quercus* spp.), beech (*Fagus* spp.) and maple (*Acer* spp.), while the other outwash site had an upland with high density unsewered residential development. The experiment was not designed to explicitly examine the effects of geologic setting and upland land use. Groundwater entering the riparian zone at the undeveloped sites consistently had less than 0.1 mg/L  $\text{NO}_3^-$ -N, while  $\text{NO}_3^-$ -N concentrations in groundwater entering the riparian zone at the outwash site with the developed upland ranged from 8 to 12 mg/L. Vegetation in the riparian zones at all the sites was dominated by oak in upland/wetland transition zones and red maple (*A. rubrum*) in wetlands.

### Sampling

In summer 1989 (July and August), samples were collected from each soil type within each site at three depths: (i) the surface (0–15 cm), (ii) at the top of seasonal high water table, and (iii) 0.5 m below this depth, within the permanently saturated zone (Fig. 1). These depths corresponded to the depths of well screening in the companion groundwater study (Simmons et al., 1992). To collect the subsurface samples, overlying soil was carefully removed to avoid “contamination” of the deep samples with surface material. In 1990, samples of surface soil only were collected at two dates, during April and August. All samples were stored at 4 °C between the time of sampling and all analyses. Percentage moisture was determined gravimetrically.

### Microbial Assays

Dentrification enzyme activity was measured using the procedure described by Smith and Tiedje (1979). Field moist soils (3 replicates per site for the summer 1989 samples, 5 replicates for the dates in 1990) were amended with  $\text{NO}_3^-$  (200 mg/kg), dextrose (40 mg/kg) chloramphenicol (10 mg/kg) and acetylene ( $\text{C}_2\text{H}_2$ , 10 kPa) and incubated under shaken, anaerobic conditions for 90 min. Gas samples were taken



**Fig. 1.** Cross-section view of riparian forest experimental layout in the glacial outwash site with an undeveloped upland showing sampling locations for microbial studies.

at 30 and 90 min, stored in evacuated glass tubes, and analyzed for nitrous oxide ( $\text{N}_2\text{O}$ ) by electron capture gas chromatography. In the DEA procedure, soils were made anaerobic by repeated evacuation and flushing with  $\text{O}_2$ -free gas.

Microbial biomass C and N content were measured using the chloroform fumigation–incubation method (Jenkinson and Powlson, 1976). Field-moist soils (3 replicates per site for the summer 1989 samples, 5 replicates for the dates in 1990) were fumigated to kill and lyse microbial cells in soil samples. The fumigated samples were then inoculated with fresh soil, and microorganisms from the fresh soil grew vigorously using the killed cells as substrate. The flushes of carbon dioxide ( $\text{CO}_2$ ) and ammonium ( $\text{NH}_4^+$ ) released by the actively growing cells during a 10-d incubation were directly proportional to the amount of C and N in the microbial biomass of the original sample (Jenkinson and Powlson, 1976). A proportionality constant (0.45) was used to calculate biomass C from the  $\text{CO}_2$  flush. No constant was used for biomass N and data reported are just the “flush” of  $\text{NH}_4^+$ -N released following fumigation. Carbon dioxide was measured on a SRI 8610 gas chromatograph (SRI, Redondo Beach, CA) equipped with a methanizer (to convert  $\text{CO}_2$  to methane) and a flame ionization detector. Ammonium was quantified colorimetrically using an Alpkem RFA 300 rapid flow analyzer (Alpkem Corp., 1986).

For samples collected in 1990, soil respiration was quantified as the accumulation of  $\text{CO}_2$  over a 10-d incubation of field moist soil held at 25 °C. Potential net N mineralization and nitrification were quantified as the accumulation of inorganic N ( $\text{NH}_4^+ + \text{NO}_3^-$ ) and  $\text{NO}_3^-$  alone during the same incubations. Nitrate was quantified colorimetrically (Alpkem Corp., 1986).

### RESULTS

Surface soil DEA was consistently higher in wetland (poorly and very poorly drained) soils than in upland–wetland transition zone (moderately well and somewhat poorly drained) soils (Table 1, Fig 2) at all sites.

Dentrification enzyme activity declined sharply from surface to subsurface soils at all sites and was undetectable at the seasonal high water table and in the permanently saturated zone at several locations (Fig. 2). There were no consistent seasonal patterns in DEA, although variation from date to date was high in some cases (Table 1). Soil  $\text{NO}_3^-$  levels were highly variable and were not correlated with DEA.

Microbial biomass C showed more spatial variation than DEA, but was generally higher in wetlands than transition zones at all sites (Table 2). Microbial biomass C was not detectable in the subsurface. As for DEA, temporal variation was high in many cases, but showed no consistent pattern (Table 2).

Soil respiration was significantly correlated with soil moisture (Fig. 3a,  $r = 0.63$ ,  $P < 0.01$ ), microbial biomass C (Fig. 3b,  $r = 0.43$ ,  $P < 0.01$ ), and microbial biomass N (Fig. 3c,  $r = 0.72$ ,  $P < 0.01$ ). Respiration and DEA were weakly correlated ( $r = 0.14$ ,  $P < 0.10$ ). Potential net N mineralization was quite variable both within and between sites, while potential net nitrification was very low at most sites (Table 3). The transition zone soils at the outwash site with a densely developed upland had relatively high rates of net  $\text{NO}_3^-$  production. Soil  $\text{NO}_3^-$  levels were also relatively high at this site (Table 3).

There were significant linear correlations between

**Table 1. Denitrification enzyme activity in surface soil (0–15 cm) along catenas in three riparian forest sites. Values are mean (standard error).**

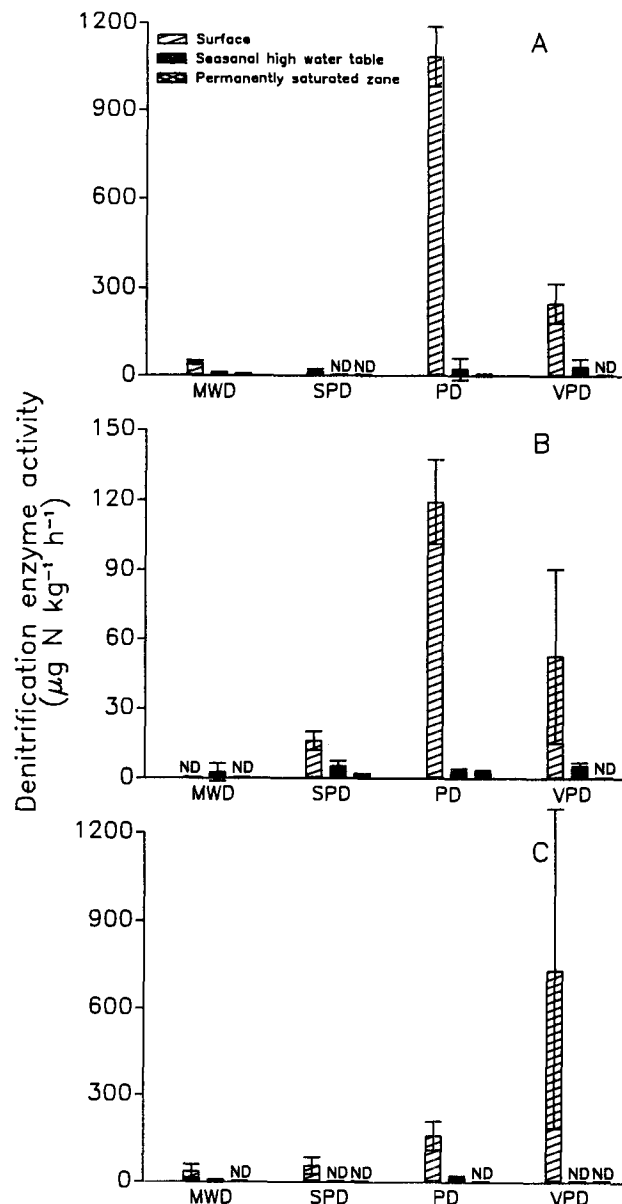
Site	Summer 1989	Spring 1990	Summer 1990
	$\mu\text{g kg}^{-1} \text{h}^{-1}$		
<b>Site A—Unstratified drift, undeveloped upland</b>			
Moderately well-drained soil	43a (8)*	29a (11)	33a (9)
Somewhat poorly drained soil	11b (10)	68a (30)	22a (9)
Poorly drained soil	1084c (104)	106a (38)	207b (75)
Very poorly drained soil	247b (68)	73a (26)	124ab (21)
<b>Site B—Glacial outwash, undeveloped upland</b>			
Moderately well-drained soil	0a (7)	39a (6)	3a (4)
Somewhat poorly drained soil	16a (4)	75a (18)	15a (8)
Poorly drained soil	119b (18)	240b (53)	98a (24)
Very poorly drained soil	52ab (38)	350b (78)	289b (114)
<b>Site C—Glacial outwash, developed upland</b>			
Moderately well-drained soil	35a (24)	91a (11)	0a (7)
Somewhat poorly drained soil	54a (30)	97a (28)	0a (13)
Poorly drained soil	157a (51)	252a (44)	295b (95)
Very poorly drained soil	732a (606)	1064b (328)	682c (99)

\* Values followed by different superscripts within each site are significantly different at  $P < 0.05$  in a one-way analysis of variance with a Duncan's multiple comparisons test.

$\text{NO}_3^-$  removal from groundwater (measured in the companion study reported by Simmons et al., 1992, Table 4) and surface soil DEA ( $r = 0.73$ ,  $P < 0.01$ ), and microbial biomass C ( $r = 0.63$ ,  $P < 0.07$ ) in the growing season sampling, but not in the dormant season sampling. The range of attenuation values was very low in the dormant season studies, making it difficult to explore relationships between attenuation and environmental factors. A nonlinear model accounted for more than 85% of the variance in growing season attenuation with DEA (Fig. 4).

## DISCUSSION

We observed significant spatial variation in microbial processes within riparian zones. Denitrification, as indicated by DEA, was higher in wetland portions of the riparian zone than in upland-wetland transition zones likely due to the presence of anaerobic conditions and higher levels of organic C in the wetland soils (Groffman and Tiedje, 1989a; Groffman et al., 1991). While DEA does not provide information on the actual rate of denitrification at any particular point in time (Smith and Parsons, 1985; Groffman, 1987; Martin et al., 1988; Parsons et al., 1991), it has been shown to be highly correlated with annual rates of denitrification in north temperate forest soils (Groffman and Tiedje, 1989b) and is useful for site comparison studies. Microbial biomass and activity (as indicated by soil respiration) were also high in wetland areas relative to transition zones, likely due to more favorable moisture conditions and higher levels of organic



**Fig. 2. Denitrification enzyme activity in four soils of three riparian forest sites at three depths, summer 1989. (A) Unstratified drift site with an undeveloped upland; (B) Glacial outwash site with an undeveloped upland; (C) Outwash site with a developed upland. MWD = moderately well-drained soil, SPD = somewhat poorly drained soil, PD = poorly drained soil, VPD = very poorly drained soil, ND = not detectable activity.**

C in the wetlands. These patterns of denitrification and microbial biomass and activity are consistent with the high rates of  $\text{NO}_3^-$  attenuation observed in wetland soils relative to transition zones. The DEA and microbial biomass and activity data suggest that wetlands have a greater potential for microbial  $\text{NO}_3^-$  attenuation than transition zones or uplands.

Although surface soil DEA and microbial biomass C were highly correlated with  $\text{NO}_3^-$  attenuation during the growing season, this does not prove that denitrification or microbial immobilization were responsible for the observed  $\text{NO}_3^-$  removal. In contrast, the low levels of these parameters in the subsurface suggest

**Table 2. Soil microbial biomass C in surface soil (0–15 cm) along catenas in three riparian forest sites. Values are mean (standard error).**

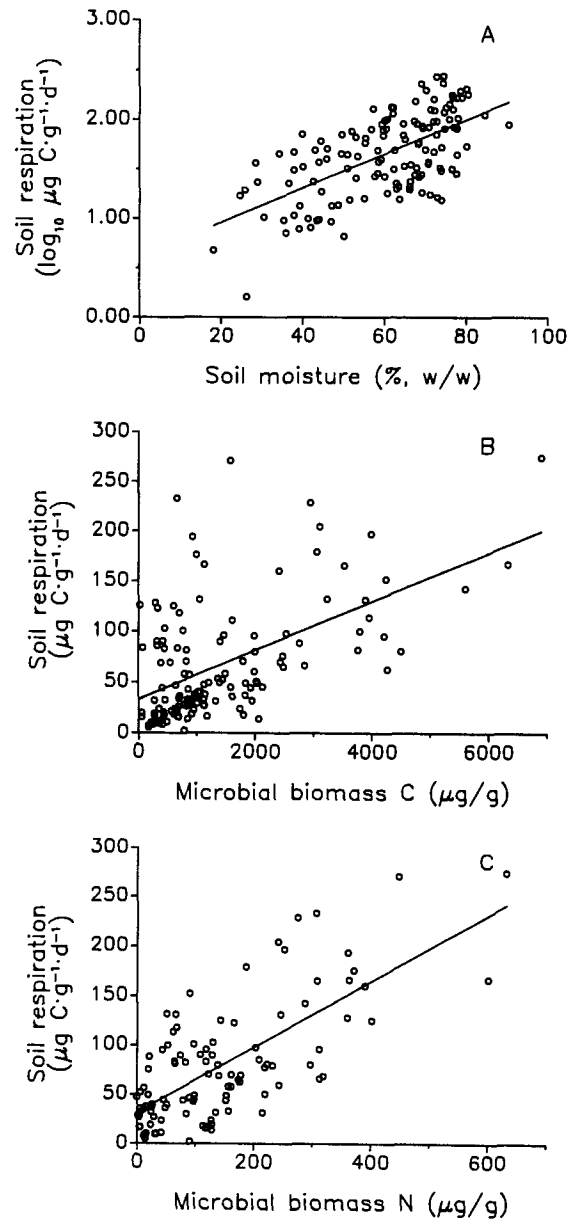
Site	Summer 1989	Spring 1990	Summer 1990
	mg kg <sup>-1</sup>		
<b>Site A—Unstratified drift, undeveloped upland</b>			
Moderately well-drained soil	340a (11)	2565a (1108)	943b (292)
Somewhat poorly drained soil	282a (79)	709a (132)	385a (84)
Poorly drained soil	1122b (124)	803a (77)	755ab (51)
Very poorly drained soil	375a (144)	1386a (434)	630ab (9)
<b>Site B—Glacial outwash, undeveloped upland</b>			
Moderately well-drained soil	161a (68)	NA†	840ab (304)
Somewhat poorly drained soil	227a (104)	NA	1427b (232)
Poorly drained soil	342a (107)	NA	270a (60)
Very poorly drained soil	127a (31)	NA	275a (95)
<b>Site C—Glacial outwash, developed upland</b>			
Moderately well-drained soil	156a (45)	1499a (503)	918a (229)
Somewhat poorly drained soil	437a (146)	2138ab (340)	1709a (156)
Poorly drained soil	326a (140)	3656b (690)	4166b (503)
Very poorly drained soil	416a (156)	2735ab (651)	3665b (308)

\* Values followed by different superscripts within each site are significantly different at  $P < 0.05$  in a one-way analysis of variance with a Duncan's multiple comparisons test.

† NA = data not available.

that plant uptake was the dominant  $\text{NO}_3^-$  sink during the growing season. This apparent importance of plants was especially marked in several transition zone sites where significant  $\text{NO}_3^-$  attenuation was observed during the growing season and DEA and microbial biomass in the subsurface were undetectable. While DEA does not provide information on the actual rate of denitrification occurring at any point in time, a lack of detectable DEA is a strong indicator that denitrification is *not* occurring. In wetlands, and during the dormant season in transition zone sites, water table levels were higher, allowing groundwater  $\text{NO}_3^-$  to interact with near surface soil and be removed by denitrification and immobilization. Thus while we observed little seasonal variation in  $\text{NO}_3^-$  removal from groundwater, it appears that different processes were responsible for  $\text{NO}_3^-$  attenuation in different seasons; plants (and wetland denitrification) during the growing season, and microbial processes during the dormant season.

The lack of DEA at depth that we observed is consistent with results obtained by Parkin and Meisinger (1989) but is in contrast to studies that have found significant potential for denitrification in groundwater (Trudell et al., 1986; Slater and Capone, 1987; Smith and Duff, 1988; Obenhuber and Lowrance, 1991). Our results suggest that groundwater denitrification may exhibit significant spatial and temporal variation. Although DEA was low or nondetectable at the top



**Fig. 3. Soil respiration vs. soil moisture: (A) Microbial biomass C; (B) and microbial biomass N; (C) over all sites and soils, spring and summer 1990 with linear regression lines. See text for regression statistics.**

of the water table during the growing season, the observed  $\text{NO}_3^-$  disappearance during the dormant season suggests that denitrification and/or microbial immobilization was occurring, assuming that plant uptake of  $\text{NO}_3^-$  was negligible during the dormant season. Since water tables were higher during the dormant season, groundwater-borne  $\text{NO}_3^-$  was moving through strata with higher DEA levels and higher organic C than during the growing season. Parkin and Meisinger (1989) observed an exponential decrease in DEA with depth that suggests that relatively small changes in water table elevation could yield large changes in groundwater denitrification activity.

Even though plant uptake was likely the dominant groundwater  $\text{NO}_3^-$  sink during the growing season, surface soil DEA was highly correlated with ground-

**Table 3. Potential net N mineralization and nitrification and soil NO<sub>3</sub>-N concentrations in surface soil (0–15 cm) along catenas in three riparian forest sites, summer 1990. Values are mean (standard error).**

Site	N mineralization mg N kg <sup>-1</sup> d <sup>-1</sup>	Nitrification mg N kg <sup>-1</sup> d <sup>-1</sup>	Soil nitrate mg kg <sup>-1</sup>
<b>Site A—Unstratified drift, undeveloped upland</b>			
Moderately well-drained soil	-0.08 (0.13)	-0.01 (0)	0.06 (0)
Somewhat poorly drained soil	-0.14 (0.06)	-0.01 (0)	0.06 (0)
Poorly drained soil	0.99 (1.03)	-0.02 (0)	0.20 (0.11)
Very poorly drained soil	2.99 (0.19)	0.18 (0.08)	0.81 (0.32)
<b>Site B—Glacial outwash, undeveloped upland</b>			
Moderately well-drained soil	1.84 (0.40)	-0.04 (0.01)	0.44 (0.07)
Somewhat poorly drained soil	1.87 (0.67)	-0.04 (0.01)	0.62 (0.20)
Poorly drained soil	1.69 (0.77)	-0.03 (0.02)	0.55 (0.22)
Very poorly drained soil	4.51 (1.31)	2.66 (0.45)	22.04 (2.33)
<b>Site C—Glacial outwash, developed upland</b>			
Moderately well-drained soil	0.70 (0.18)	0.89 (0.17)	11.03 (1.80)
Somewhat poorly drained soil	1.74 (0.47)	1.55 (0.11)	35.72 (1.75)
Poorly drained soil	0.94 (1.20)	0.66 (0.75)	30.37 (8.76)
Very poorly drained soil	0.03 (0.87)	0.06 (0.97)	19.72 (6.89)

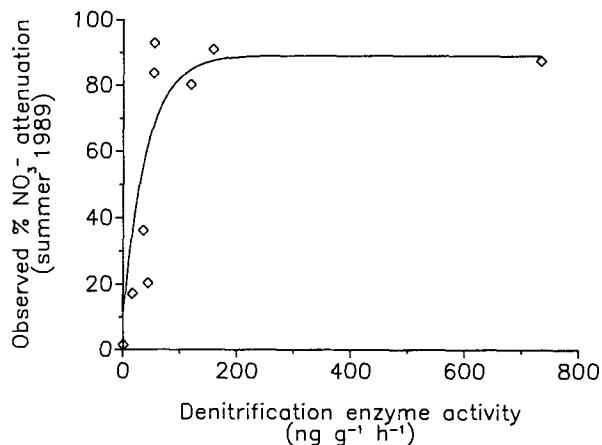
**Table 4. Observed percentage groundwater NO<sub>3</sub> attenuation in three riparian forest sites, summer 1989 (growing season) and winter 1990 (dormant season). Data from Simmons et al. (1992). Values are mean (standard deviation).**

Site	Growing season	Dormant season
	% removal	
<b>Site A—Unstratified drift, undeveloped upland</b>		
Moderately well-drained soil	20 (28)	62 (18)
Somewhat poorly drained soil	ND†	78 (38)
Poorly drained soil	ND	89 (17)
Very poorly drained soil	ND	97 (8)
<b>Site B—Glacial outwash, undeveloped upland</b>		
Moderately well-drained soil	1 (3)	NA‡
Somewhat poorly drained soil	17 (15)	NA
Poorly drained soil	80 (21)	NA
Very poorly drained soil	84 (24)	NA
<b>Site C—Glacial outwash, developed upland</b>		
Moderately well-drained soil	36 (28)	50 (25)
Somewhat poorly drained soil	91	59 (31)
Poorly drained soil	93 (17)	61 (27)
Very poorly drained soil	87 (28)	90 (16)

† ND = plume not detected.

‡ NA = contaminant and tracer not applied.

water NO<sub>3</sub> removal because this parameter was a sensitive indicator of subsurface moisture conditions and of the interaction of groundwater with aboveground ecosystems. Much of the variation in riparian zone soil, plant and microbial properties arises from variation in groundwater levels that influences the nature and extent of plant production and microbial activity. In our study, surface soil DEA and observed groundwater NO<sub>3</sub> attenuation both increased from transition zone to wetland soils due to changes in the water table along the transition zone to wetland gradient. As the water table approaches the surface, soils become more anaerobic which leads to increases in soil organic matter and denitrifier populations (Groffman and Tiedje, 1989b). Coincidentally, NO<sub>3</sub> in groundwater is able to interact with the biologically active zone of the soil. We therefore expect to observe strong relationships between DEA and NO<sub>3</sub> attenuation wherever changes in subsurface moisture influence surface soil and mi-

**Fig. 4. Observed NO<sub>3</sub> attenuation (1989 growing season data from Simmons et al., 1992) vs. surface soil denitrification enzyme activity (samples from summer 1989). Regression line is drawn from a nonlinear model of the function  $y = B_1 \times \exp(B_2 \times X) + B_3$ , where  $B_1 = -77.4$ ,  $B_2 = -0.24$ ,  $B_3 = 89.0$ ; where  $y$  = observed NO<sub>3</sub> attenuation, and  $x$  = surface soil denitrification enzyme activity.**

crobial properties. The DEA will not be a sensitive indicator of differences in subsurface moisture when there are other factors that control surface soil microbial biomass and DEA, such as differences in surface soil texture or organic matter input, such as manure or sludge application.

A major question when evaluating the water quality maintenance value of riparian zones relates to long-term effectiveness. If a site receives high levels of subsurface NO<sub>3</sub> inputs for many years, the ability of that site to attenuate NO<sub>3</sub> may increase or decrease over time. It is possible that plant and microbial sinks can become "saturated" with N (Aber et al., 1989; Kadlec and Bevis, 1990). On the other hand, groundwater nutrient inputs have been shown to stimulate both plant (Ehrenfeld, 1987) and microbial (Smith and Duff, 1988) populations, increasing their potential to act as sinks.

One of our sites (outwash with a developed upland) had been receiving high levels of subsurface NO<sub>3</sub> inputs for many years (at least 25 yr). Although our

experimental design was not adequate to explicitly address the effects of upland land use, data from this site raises some interesting questions. The fact that this site showed high  $\text{NO}_3^-$  attenuation suggests that high  $\text{NO}_3^-$  inputs have not decreased the ability of this site to serve as an effective  $\text{NO}_3^-$  sink. On the other hand, the high nitrification observed at this site suggests that this is an N-rich site, susceptible to high  $\text{NO}_3^-$  losses, especially if the site is disturbed by logging, fire or blowdowns (Vitousek et al., 1979; Aber et al., 1989; Hill and Shackleton, 1989). This symptom of N enrichment was especially marked in the transition zone soils at the outwash site with a developed upland. The high nitrification rates may indicate that the long-term buffering potential of this site may be limited. Monitoring of this and/or other sites with a long history of exposure to subsurface  $\text{NO}_3^-$  inputs is warranted.

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