Microbial Nitrate Processing in Shallow Groundwater in a Riparian Forest

Peter M. Groffman,* Galen Howard, Arthur J. Gold, and William M. Nelson

ABSTRACT

We measured denitrification, immobilization, and respiration in microcosms that simulated groundwater conditions in a riparian forest in Rhode Island. Rates were higher in summer than in winter and were nearly always limited by a lack of organic C. Limited evidence suggested that immobilization was not a significant sink for NO$^\text{3}$ in aquifer material exposed to the 10 mo of NO$^\text{3}$ dosing and unexposed control material. While there was significant variation in water table levels, dissolved oxygen, dissolved organic C, and total C within the aquifer in the riparian forest, there was little spatial variation in denitrification or respiration rates. Rates were higher in summer than in winter and were nearly always limited by a lack of organic C. Limited evidence suggested that immobilization was not a significant sink for NO$^\text{3}$. Measured groundwater denitrification rates were high enough to eliminate 27% of an incoming NO$^\text{3}$ concentration of 10 mg N L$^{-1}$ over a riparian zone width of 60 m, with a travel time of 1600 d, and equaled 6.0 kg N ha$^{-1}$ yr$^{-1}$. The measured denitrification rates were much lower than rates of groundwater NO$^\text{3}$ removal directly measured in the companion hydrologic study at the same time (120 kg N ha$^{-1}$ yr$^{-1}$), suggesting that there is still considerable uncertainty about the mechanisms of NO$^\text{3}$ removal from groundwater in riparian forests.

A problem that has emerged in riparian zone research is that when denitrification and immobilization have been directly measured in laboratory microcosms of shallow aquifer material, the measured rates are frequently too low to account for the amount of NO$^\text{3}$ removal observed in companion field studies based on groundwater monitoring well networks (Lowrance, 1992; Groffman et al., 1992). Laboratory-based measurements of low groundwater denitrification rates are especially puzzling in situations when high in situ NO$^\text{3}$ removal rates are observed during the dormant season, when subsurface microbial activity is the most likely mechanism of removal (Lowrance, 1992; Haycock and Pinay, 1993). This puzzle suggests that our understanding of subsurface microbial activity, in general, and of subsurface denitrification, in particular, is incomplete.

A major question in microbial ecology is the source of energy to support groundwater microbial activity (Korom, 1992; Starr and Gillham, 1993). High rates of groundwater denitrification have been observed in several studies (Trudell et al., 1986; Slater and Capone, 1987; Smith and Duff, 1988; Francis et al., 1989; Obenhuber and Lowrance, 1991); however, other studies have found the potential for denitrification beneath the water table to be low or nonexistent (Parkin and Meisinger, 1989; Groffman et al., 1992; Bradley et al., 1992; Yeomans et al., 1992; Starr and Gillham, 1993). Groundwater microbial activity is usually limited by organic C availability since the total and dissolved organic C contents of the solid and liquid phases of aquifers are low and are generally poor quality substrates for microbial growth (Lind and Eiland, 1989; Hiscock et al., 1991; Johnson and Wood, 1992; McCarty and Bremer, 1992).

Rates of groundwater denitrification in riparian forests are likely to vary substantially from upland to streamside areas due to changes in soil C content, water table heights, vegetation, and oxygen levels (Starr and Gillham, 1993). In riparian areas, there can be marked changes in these factors over small spatial scales (Warwick and Hill, 1988; Cooper, 1990; Nelson et al., 1995). At the upland edge of a typical riparian forest, the water table is beneath the biologically active zone (surface soil horizons), aerobic conditions are likely, and there is little C in the subsurface to support denitrification. At the wetland edge of a typical riparian zone, the water table is closer to the soil surface, anaerobic conditions are common, and the groundwater is likely within the biologically active zone of the soil (Simmons et al., 1992). While denitrification should be relatively low at the upland edge and high in the wetlands, rates in those areas in between (transition zones) are less easy to predict.

This study was performed in conjunction with a study of in situ NO$^\text{3}$ removal from groundwater in a riparian zone of Rhode Island, Kingston, RI 02881; and W.M. Nelson, Wehran EMCOM NE, Wallingford, CT 06492. Received 23 Oct. 1995. *Corresponding author (capg@vm.marist.edu).


Riparian ecosystems have been found to be important sinks for NO$^\text{3}$ in groundwater from upland areas toward streams in many studies (Lowrance et al., 1984; Peterjohn and Correll, 1984; Jacobs and Gilliam, 1985; Pinay and Decamps, 1988; Simmons et al., 1992; Jordan et al., 1993; Haycock and Pinay, 1993). However, in several of these studies, the mechanism of NO$^\text{3}$ removal within the riparian zone has not been clear. While both plants and microbes can take up NO$^\text{3}$ from groundwater, the ability of plants to scavenge NO$^\text{3}$ from below the water table throughout the year, and the capacity of microbes to be active in the commonly low C subsurface environment are uncertain and poorly studied.

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Abbreviations: MWD, moderately well drained; SPD, somewhat poorly drained; PD, poorly drained; VPD, very poorly drained; DO, dissolved oxygen; DOC, dissolved organic carbon.
near Kingston, RI (41°30' N, 71°30' W). This area is located within the Pawcatuck watershed and includes 500 m of stream bordered by a forested riparian zone. The upland is composed of agricultural fields and forest. Soils at the site comprise a toposequence derived from glaciofluvial deposits of structureless, granitic, sands, and loamy sands with an average slope of 3%. The soils are classified as sandy, mixed mesic Haplaquepts and include moderately well drained (MWD), somewhat poorly drained (SPD), poorly drained (PD), and very poorly drained (VPD) series (Fig. 1). The PD and VPD soils meet hydric soil criteria. Mean water table depths over the course of the study were 104 cm at the upland edge of the SPD soil, 75 cm at the boundary between the SPD and PD soils, and 50 cm at the wetland edge of the PD soil (Table 1). Vegetation at the site varies in response to water table depth with the more upland soils dominated by white oak (Quercus alba L.) and the wetland soils dominated by red maple (Acer rubrum L.).

Groundwater Dosing and Monitoring
As described in Nelson et al. (1995), a groundwater dosing and monitoring well network was established for the companion hydrology study. Dosing wells (piezometers) were established at two groundwater depths: at the top of the permanently saturated zone (shallow) and 1.5 m below this depth (deep). Only the lower portion of each dosing well was screened. Three shallow dosing wells were placed at the MWD/SPD interface, three shallow and three deep dosing wells at the SPD/PD interface, and three shallow and three deep dosing wells at the PD/VPD interface (Fig. 1). A network of monitoring wells was established in a circular pattern at a distance of 0.6 m from each of the dosing wells. Nitrate and Br were continuously added to the dosing wells from February to December 1992. Rates of NO₃ removal were quantified by coupling changes in NO₃/Br⁻ ratios between the dosing wells and the monitoring wells with estimates of groundwater flow. The detection of Br⁻ in the monitoring wells was used to determine the location of the NO₃ plume produced by the dosing well. Throughout the study, regular measurements of water table heights, dissolved oxygen (DO), and temperature were made in the wells at the site. Measurements of DO and temperature in the groundwater were made using a YSI DO meter. Water table heights were measured by the steel tape and chalk method or with an electric tape.

METHODS

Site Description
The study site was located at the University of Rhode Island Peckham Farm Soil and Water Conservation Field Laboratory between Kingston, RI, and the Pawcatuck River near the New York State border. This area is in the Pawcatuck watershed and includes 1 km of stream bordered by a forested riparian zone. The upland is composed of agricultural fields and forested riparian zone. The upland is composed of agricultural fields and forest. Soils at the site comprise a toposequence derived from glaciofluvial deposits of structureless, granitic, sands, and loamy sands with an average slope of 3%. The soils are classified as sandy, mixed mesic Haplaquepts and include moderately well drained (MWD), somewhat poorly drained (SPD), poorly drained (PD), and very poorly drained (VPD) series (Fig. 1). The PD and VPD soils meet hydric soil criteria. Mean water table depths over the course of the study were 104 cm at the upland edge of the SPD soil, 75 cm at the boundary between the SPD and PD soils, and 50 cm at the wetland edge of the PD soil (Table 1). Vegetation at the site varies in response to water table depth with the more upland soils dominated by white oak (Quercus alba L.) and the wetland soils dominated by red maple (Acer rubrum L.).

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Microcosm Studies
To create microcosms for measurement of denitrification, respiration, and NO₃ immobilization within the NO₃ plume...
created by the dosing wells, samples of aquifer material were collected at the screen depth of each of the dosing wells. Samples were taken from immediately outside the ring of monitoring wells around each dosing well, but within the NO\textsubscript{3}~* plume produced by the dosing well (one sample per dosing well = 15 samples total) in March, June, and September 1992 and in February 1993. Samples were obtained by hand auguring down to the desired depth within a casing to prevent contamination of samples by surface soil. All samples were taken within the saturated zone of the aquifer. The shallow sampling depth varied seasonally, depending on the height of the water table.

The microcosms consisted of 50 g of aquifer material in Erlenmeyer flasks amended with 45 mL of groundwater saturated with acetylene (C\textsubscript{2}H\textsubscript{2}) gas and 5 mL of 1000 mg L\textsuperscript{-1} NO\textsubscript{3}~*-N solution. The groundwater in the individual microcosms was taken from the same well location and soil class as the aquifer material.

To initiate the incubation, flasks were evacuated and flushed three times with N\textsubscript{2} gas. After the final evacuation, the headspace was filled with N\textsubscript{2}-air mixtures and C\textsubscript{2}H\textsubscript{2} to produce DO levels similar to those in the field. The composition of these mixtures was determined by calculating the headspace air concentration that would produce ambient groundwater O\textsubscript{2} concentrations in the microcosm water at ambient groundwater temperatures. Oxygen concentrations in the microcosms were generally within 2 to 3 mg L\textsuperscript{-1} of ambient concentrations in the different treatments. The flasks were incubated at in-field temperatures for 5 d (15 dosing piezometers × 5 d + 5 blanks = 80 flasks). Incubations were limited to 5 d because after 1 wk C\textsubscript{2}H\textsubscript{2} can stimulate microbial activity by acting as a C source (Terry and Duxbury, 1985). Flasks were not shaken during the incubation.

Incubation temperatures were 5, 13, 15, and 5°C for the March, June, and September 1992, and February 1993 incubations, respectively. There were no significant differences in temperature with soil depth or drainage class in the field, so all flasks were incubated at the same temperature.

Fifteen flasks were destructively sampled on each of the 5 d of the incubation. Before destruction, flasks were shaken by hand for 30 s and gas samples were extracted from the headspace for analysis of N\textsubscript{2}O, CO\textsubscript{2}, and C\textsubscript{2}H\textsubscript{2}. A Tracor 540 gas chromatograph equipped with an electron capture detector was used to measure N\textsubscript{2}O, a Shimadzu GC8A gas chromatograph equipped with a thermal conductivity detector was used to quantify CO\textsubscript{2}, and another Shimadzu GC8A with a flame ionization detector was used to measure C\textsubscript{2}H\textsubscript{2}. Levels of C\textsubscript{2}H\textsubscript{2} were monitored to check for utilization of C\textsubscript{2}H\textsubscript{2} as a C source by microbes. No decreases in C\textsubscript{2}H\textsubscript{2} were observed.

Rates of denitrification and respiration were calculated from changes in the concentrations of N\textsubscript{2}O and CO\textsubscript{2} over the 5-d incubation. High variation and low sampling frequency (daily) made it impossible to determine if rates were stable over the course of the incubation.

Following gas sampling, the overlying water in the flasks was poured off for immediate analysis of DO using a YSI DO meter. After DO analysis, the water was filtered and concentrations of NH\textsubscript{4}*, NO\textsubscript{3}~*, and dissolved organic carbon (DOC) were measured using an Alpkem TFS autoanalyzer and an Astro 2001 TOC Analyzer. Following filtration of the overlying water, 50 mL of KCl was added to the remaining aquifer material and flasks were shaken for 30 min to extract NH\textsubscript{4}* and NO\textsubscript{3}~* from the pore spaces of the aquifer material.

**Amendment Studies**

To determine the factors limiting denitrification, aquifer samples from each sampling station and date were incubated at 25°C in stoppered flasks under four different conditions: (i) aerobic (atmospheric), (ii) anaerobic, (iii) aerobic with 1000 mg kg\textsuperscript{-1} glucose, and (iv) anaerobic with 1000 mg kg\textsuperscript{-1} glucose. All treatments were amended with 100 mg kg\textsuperscript{-1} NO\textsubscript{3}~*-N and C\textsubscript{2}H\textsubscript{2} (10 kPa). Samples were made anaerobic where necessary by repeated evacuation and flushing with N\textsubscript{2} gas. Samples were incubated for 3 d and gas samples were taken daily from the headspace to quantify N\textsubscript{2}O production as described above.

**Other Analyses**

Aquifer total C and N content, microbial biomass C and N content, root biomass, and potential net N mineralization and nitrification were measured in the same aquifer samples used to construct the microcosms and in control samples taken at each dosing station outside the NO\textsubscript{3}~* plume. The control samples were taken within 1 m and at the same depth as the dosed samples, allowing for evaluation of the effects of dosing on subsurface ecosystem properties. The dosing stations produced well defined, relatively stable NO\textsubscript{3}~* plumes; therefore, it was possible to choose locations for control sampling based on Br\textsuperscript{-1} analysis of samples from the monitoring well networks at each dosing station.

Total C and N were measured with a Carlo Erba 1500 C-N-S analyzer. Microbial biomass C and N content were measured using the chloroform fumigation–incubation method (Jenkinson and Powlson, 1976). An unfumigated control was not subtracted from the fumigated samples (Voroney and Paul, 1984). A k\textsubscript{c} value of 0.41 was used to convert the flush of C produced in the fumigated and inoculated samples to values for microbial biomass C (Jenkinson and Powlson, 1976). No k\textsubscript{c} value was used to compute microbial biomass N, and the values presented are just the flush of N produced in the fumigated and reincubated controls.

Potential net N mineralization and nitrification were quantified from the accumulation of NH\textsubscript{4}~* plus NO\textsubscript{3}~* and NO\textsubscript{2}~* alone during a 10-d aerobic incubation at 25°C. Readily mineralizable C was quantified from the accumulation of CO\textsubscript{2} during these same 10-d incubations. Ammonium, NO\textsubscript{3}~*, and CO\textsubscript{2} were measured as described above.

Root biomass was measured using a hydrodynamic root washer (Smucker et al., 1982) for the March sampling and by an original flotation method for the other three samplings. In the flotation method, soil samples were placed in a container and flushed with water. After agitation, roots were removed from the water, dried, and weighed.

**Statistical Analysis**

Each of the five soil–depth combinations was classified as a separate treatment and comparisons were made by analysis of variance using treatment, date, and treatment × date as main effects. Separate analyses were done for each date when the treatment × date interaction was significant. Fisher's protected least significant difference test was used a posteriori to determine specific treatment and date differences. Samples taken from within the NO\textsubscript{3}~* plume (dosed) were compared with control samples by analysis of variance with dosing treatment, soil/depth treatment, and date as main effects (with interactions). Separate analyses were run for each soil/depth treatment and date when interactions were significant. Data were log-transformed before analysis where appropriate.
Table 2. Denitrification rates in microcosms of aquifer solid phase material from beneath three soil drainage classes in a riparian forest in Rhode Island at four sample dates between March 1992 and February 1993. Values are the mean (standard error) of three replicate microcosms.

<table>
<thead>
<tr>
<th>Soil drainage class†</th>
<th>Relative depth‡</th>
<th>Denitrification rate</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>March b§</td>
<td>June a</td>
<td>September a</td>
<td>February b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>µg N kg⁻¹ d⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPD</td>
<td>Shallow</td>
<td>0.00 (0.00)a†</td>
<td>1.79 (1.16)a</td>
<td>4.16 (1.84)a</td>
<td>0.00 (0.00)a</td>
</tr>
<tr>
<td>SPD/PD</td>
<td>Shallow</td>
<td>0.00 (0.00)a</td>
<td>1.68 (1.10)a</td>
<td>1.54 (0.40)a</td>
<td>0.04 (0.04)a</td>
</tr>
<tr>
<td></td>
<td>Deep</td>
<td>0.00 (0.00)a</td>
<td>1.35 (0.88)a</td>
<td>0.26 (0.26)a</td>
<td>0.07 (0.07)a</td>
</tr>
<tr>
<td>PD</td>
<td>Shallow</td>
<td>0.05 (0.04)a</td>
<td>3.50 (2.06)a</td>
<td>0.40 (0.26)a</td>
<td>0.70 (0.70)a</td>
</tr>
<tr>
<td></td>
<td>Deep</td>
<td>0.23 (0.19)a</td>
<td>0.91 (0.91)a</td>
<td>4.10 (3.84)a</td>
<td>0.00 (0.00)a</td>
</tr>
</tbody>
</table>

† SPD = samples taken from along the upland edge of a somewhat poorly drained soil; SPD/PD = samples taken along the boundary between somewhat poorly and poorly drained soils; PD = samples taken from along the wetland edge of a poorly drained soil.
‡ Shallow samples were taken from the top of the permanently saturated zone. Deep samples were taken from 1.5 m below this depth.
§ Dates followed by different superscripts are significantly different in a one-way analysis of variance with a Fisher's least significant difference test comparing activity over all soils and depths.
¶ Values followed by different superscripts within each column are significantly different in a one-way analysis of variance with a Fisher's least significant difference test comparing activity among different soils and depths at each date.

RESULTS

Denitrification and respiration showed much less spatial variation than other site properties. While there was significant variation in water table levels, DO, DOC, and total C with depth and soil drainage class (Table 1), there was little or no significant spatial variation in denitrification (Table 2) or respiration rates (Table 3). Rates were higher (p < 0.05) in summer (June, September) than in winter (March, February). There were no correlations between either denitrification or respiration and DO, DOC, total C, or total N (data not presented).

Denitrification activity was limited by C availability in nearly all cases. In the amendment studies, in four of five soil–depth treatments, denitrification did not occur unless C was added (Fig. 2). In the PD–shallow treatment, denitrification was stimulated (p < 0.05) by the anaerobic treatment, suggesting that C was available to support denitrification in this treatment.

Although we theoretically could quantify immobilization rates by measuring changes in NO₃⁻ over the course of our microcosm incubations, because the NO₃⁻ concentrations in our microcosms were high (100 mg N L⁻¹), our ability to detect immobilization was limited to rates greater than 20 µg N kg⁻¹ d⁻¹. Therefore, while we did not measure any significant decreases in NO₃⁻ during the microcosm incubations, this is not strong evidence for a lack of immobilization. Stronger evidence for a lack of immobilization comes from a lack of any increase in microbial biomass N in the dosed relative to the control samples, even after 10 mo of continuous dosing (Table 4). Microbial biomass and readily mineralizable C were also not affected by dosing (Table 5). Root biomass was negatively affected (p < 0.10) by dosing (Table 5).

DISCUSSION

Groundwater denitrification rates must be evaluated relative to fluxes of NO₃⁻, oxygen, and C in the aquifer. While the rates measured here appear to be quite low relative to surface soil denitrification rates measured in other studies (Groffman, 1994), they may be high enough to represent a significant sink for NO₃⁻ in this riparian forest because groundwater flow rates are slow, even in sandy material (Robertson et al., 1991).

Our study was designed to quantify the ability of the riparian zone to denitrify a load of NO₃⁻-enriched groundwater (i.e., all our samples came from within an experimentally induced plume of NO₃⁻-rich groundwater, and were incubated with high NO₃⁻ concentrations). To evaluate the potential importance of our measured denitrification rates, consider a unit of the riparian zone aquifer consisting of a rectangle 0.5 m deep by 0.037 m long by 1 m wide. Groundwater movement in this aquifer averages 0.037 m d⁻¹ (Nelson et al., 1995). The volume of soil contained in this rectangle that will hold water for 1 d is therefore 0.0185 m³. Assuming a bulk density of 1.6 × 10³ kg m⁻³, the mass of soil in the rectangular cube is 29.6 kg. The pore space within this cube is 0.0074 m³ [porosity = 1 – (bulk density/particle density) (2.65)]. If the groundwater NO₃⁻ concentration was 10 mg N L⁻¹, loading of NO₃⁻ into the cube would be 0.073 g d⁻¹ (assuming the pore space is saturated). We can estimate % removal of this 10 mg L⁻¹ NO₃⁻ solution using the microcosm rates of denitrification and...
the lengths of the soil drainage classes within the riparian zone at our site. For this analysis, we used the mean of the denitrification rates in Table 1 (1.03 µg N kg⁻¹ d⁻¹) and soil drainage class widths of 10, 10, and 40 m for the MWD/SPD, SPD/PD, and PD/VPD soil classes. These widths produce travel times of 270, 270, and 1081 d from the MWD/SPD, SPD/PD, and PD/VPD soil classes, respectively (travel time = width ÷ 0.037 m d⁻¹). Using the mean actual rate and these actual soil class lengths, denitrification would remove 27% of an incoming 10 mg L⁻¹ NO₃⁻-N solution over a 1600-d travel time through the riparian zone.

While the spatial and temporal variation of our measured denitrification rates was not large relative to surface soil studies (where rates often vary over two or three orders of magnitude), it is quite significant in a groundwater context. For example, our highest measured actual denitrification rate (4.0 µg N kg⁻¹ d⁻¹) would remove 0.118 x 10⁻³ g of NO₃⁻-N d⁻¹ from the cube of aquifer described above. If we had a riparian zone 20 m in length, the expected travel time would be 540 d and the soil would therefore denitrify 0.064 g NO₃⁻-N or 84% of an initial 10 mg L⁻¹ NO₃⁻-N loading.

We can also express our groundwater denitrification rates on a kg ha⁻¹ of riparian zone basis. Assuming an active depth of 1 m and using the average of all summer (June, September) rates (1.96 µg N kg⁻¹ d⁻¹) for 182 d, and the average of all winter (March, February) rates (0.109 µg N kg⁻¹ d⁻¹) for 183 d, groundwater NO₃⁻ removal by denitrification equals 6.0 kg N ha⁻¹ yr⁻¹. Our maximum denitrification rate (4.0 µg N kg⁻¹ d⁻¹) would represent 23.4 kg N ha⁻¹ yr⁻¹ of groundwater NO₃⁻ removal. Lowrance et al. (1995) summarized existing data on groundwater NO₃⁻ removal in riparian forests and found a range of 20 to 39 kg N ha⁻¹ yr⁻¹. Their analysis included removal by all mechanisms, not just denitrification, and included mostly riparian forests dominated by wetland soils, which generally have higher NO₃⁻ removal rates than the upland–wetland transition zone soils in this study (Simmons et al., 1992).

The groundwater denitrification rates we measured are similar to those measured in other studies that have taken care to use realistic levels of O₂ and C. Lowrance (1992) and Beare et al. (1994) measured unamended (no NO₃⁻ or C additions) denitrification rates of less than 5 µg N kg⁻¹ d⁻¹ in groundwater in a riparian forest in Georgia. Most groundwater denitrification measurements have been made under anaerobic conditions, often with added C, and are thus much higher than the rates measured in this study. In situ studies using either C₂H₂ block or geochemical methods have found that groundwater denitrification rates are very sensitive to O₂ and are strongly limited by available C (Gambrell et al., 1975; Starr and Gillham, 1993; Spalding and Parrott, 1994). Our study is in agreement with the many previous studies (see citations in the introduction) that have found that subsurface denitrification of exogenous NO₃⁻ is limited by available C.

Although the groundwater denitrification rates that we measured could represent a significant sink for NO₃⁻ in this riparian zone, they were much lower than the rates

![Fig. 2. Denitrification in subsurface material from different soil/depth treatments incubated under different conditions. Values are the mean (standard error) of three replicate samples taken from each soil/depth combination depth at three sampling dates between March 1992 and February 1993 (n = 9).](image-url)
Table 4. Microbial biomass nitrogen flush and potential net N mineralization and nitrification in nitrate-dosed and control aquifer solid phase material from beneath three soil drainage classes in a riparian forest in Rhode Island. Values are the mean (standard error) of three replicate samples taken from each soil/depth combination at four sampling dates between March 1992 and February 1993 (n = 12).

<table>
<thead>
<tr>
<th>Soil drainage class†</th>
<th>Relative depth‡</th>
<th>Microbial biomass N</th>
<th>Mineralization</th>
<th>Nitrification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Dosed</td>
<td>Control</td>
<td>Dosed</td>
</tr>
<tr>
<td>SPD Shallow</td>
<td>2.7 (0.2)a§</td>
<td>2.7 (0.3)a§</td>
<td>-1.2 (0.5)a</td>
<td>-0.7 (1.0)a</td>
</tr>
<tr>
<td>SPD/PD Shallow</td>
<td>2.9 (0.4)a</td>
<td>3.0 (0.5)a</td>
<td>0.0 (0.8)a</td>
<td>-1.1 (0.9)a</td>
</tr>
<tr>
<td>PD Shallow Deep</td>
<td>3.1 (0.4)a</td>
<td>2.7 (0.4)a</td>
<td>0.3 (0.6)a</td>
<td>-1.1 (0.7)a</td>
</tr>
</tbody>
</table>

† SPD = samples taken from along the upland edge of a somewhat poorly drained soil; SPD/PD = samples taken along the boundary between somewhat poorly and poorly drained soils; PD = samples taken from along the wetland edge of a poorly drained soil.
‡ Shallow samples were taken from the top of the permanantly saturated zone. Deep samples were taken from 1.5 m below this depth.
§ Values followed by different superscripts within each column are significantly different in a one-way analysis of variance comparing different soil and depths over all sampling dates. There were no significant differences between control and dosed treatments.

Table 5. Microbial biomass and readily mineralizable C levels and root biomass in nitrate-dosed and control aquifer solid phase material from beneath three soil drainage classes in a riparian forest in Rhode Island. Values are the mean (standard error) of three replicate samples taken from each soil/depth combination at four sampling dates between March 1992 and February 1993 (n = 12).

<table>
<thead>
<tr>
<th>Soil drainage class†</th>
<th>Relative depth‡</th>
<th>Microbial biomass C</th>
<th>Readily mineralizable C</th>
<th>Root biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Dosed</td>
<td>Control</td>
<td>Dosed</td>
</tr>
<tr>
<td>SPD Shallow</td>
<td>62 (12)a§</td>
<td>84 (21)a</td>
<td>20 (8)a</td>
<td>13 (5)a</td>
</tr>
<tr>
<td>SPD/PD Shallow</td>
<td>62 (10)§</td>
<td>57 (9)a</td>
<td>11 (4)a</td>
<td>11 (2)a</td>
</tr>
<tr>
<td>PD Shallow Deep</td>
<td>60 (10)a</td>
<td>59 (16)a</td>
<td>9 (2)a</td>
<td>11 (3)§</td>
</tr>
</tbody>
</table>

† SPD = samples taken from along the upland edge of a somewhat poorly drained soil; SPD/PD = samples taken along the boundary between somewhat poorly and poorly drained soils; PD = samples taken from along the wetland edge of a poorly drained soil.
‡ Shallow samples were taken from the top of the permanantly saturated zone. Deep samples were taken from 1.5 m below this depth.
§ Values followed by different superscripts within each column are significantly different in a one-way analysis of variance comparing different soil and depths over all sampling dates. There were no significant differences between control and dosed treatments, except for root biomass, which was significantly higher (p < 0.10) in control than in dosed treatments in a one-way analysis of variance comparing treatments over all soils, depths, and sampling dates.
works with denitrification rates measured in laboratory microcosms is differences in scale. In our study, the monitoring well networks measured NO$_3^-$ removal rates over a mass of soil of 20 to 30 kg, whereas the microcosms were constructed from 50 g of material removed from one point within each of the well fields. One possible explanation for the discrepancy between the field and laboratory results is that there were patches of high denitrification activity within the well fields that were not hit when samples were taken for microcosm construction (i.e., there is spatial variation that our sampling did not characterize). Small patches or hotspots of high activity are thought to account for a very high percentage of the denitrification activity in surface soils (Parkin, 1987; Christensen et al., 1990). These hotspots are centered around patches of labile organic matter that support high rates of microbial activity. We suggest that hotspots may be important to denitrification in subsurface riparian ecosystems, just as they are in surface soils (Fujikawa and Hendry, 1991). Given the long travel path and extremely long retention time of groundwater, a small volume of hotspots (e.g., 5%) could have a large impact on a parcel of water passing through a riparian zone.

A second phenomenon that may account for the lack of measured denitrification in groundwater is the importance of non-C-based energy sources. Although most denitrification research has focused on heterotrophic organisms capable of carrying out this process, a variety of chemoautotrophic organisms are capable of denitrifying while using reduced S and Fe compounds or CH$_4$ as energy sources (Pedersen et al., 1991; Postma et al., 1991; Korom, 1992; Parkin and Simpkins, 1993). While these compounds are abundant in many subsurface environments, their role in driving denitrification may have been overlooked due to methodological problems. Most denitrification studies have employed the C$_2$H$_2$ inhibition method to measure denitrification. However, C$_2$H$_2$ is also a potent inhibitor of most chemoautotrophic energy-yielding reactions including CH$_4$, S, and NH$_3$ oxidation (Payne, 1984; Juliette et al., 1993). It is quite possible that these reactions are responsible for observed NO$_3^-$ disappearance in the field but are not present in laboratory incubations with C$_2$H$_2$. Preliminary analysis of CH$_4$, S, and NH$_3$ levels in groundwater at our site suggest that these are not important substrates for subsurface denitrification at our site, but we are conducting more detailed studies to verify this.

A final phenomenon that may contribute to microbial removal of NO$_3^-$ from groundwater is abiotic fixation via chemical and physical condensation reactions between old organic matter, e.g., humus and inorganic N (Haider et al., 1975; Davidson et al., 1991; Johnson, 1992). Controlled studies with $^{15}$NO$_3^-$ can be done to test for this mechanism.

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