

# Patchiness in Groundwater Nitrate Removal in a Riparian Forest

A. J. Gold,\* P. A. Jacinthe, P. M. Groffman, W. R. Wright, and R. H. Puffer

## ABSTRACT

Our ability to identify and manage riparian sites for groundwater nitrate ( $\text{NO}_3^-$ ) removal is limited by uncertainty surrounding the relative importance of plant uptake vs. microbially mediated removal processes. Microcosm studies often demonstrate negligible transformation rates in the subsoil of riparian forests, even in situations where groundwater well networks showed substantial groundwater  $\text{NO}_3^-$  removal during the winter and a decline in dissolved oxygen (DO) in ambient groundwater moving through the site. We hypothesize that microcosm studies may miss groundwater transformations that occur within microsites, that is, "hotspots" of riparian subsoils. We created mesocosms of large (15 cm diam.  $\times$  40 cm length), undisturbed cores from the seasonally saturated zone of poorly drained (PD) and moderately well drained (MWD) sandy soils from a forested riparian area in southern New England. We dosed the mesocosms for 130 d with ambient groundwater amended with  $\text{NO}_3^-$ -N and  $\text{Br}^-$ . Changes in the  $\text{NO}_3^-$ -N/ $\text{Br}^-$  ratios were used to calculate groundwater  $\text{NO}_3^-$ -N removal rates. The PD treatment demonstrated substantial groundwater  $\text{NO}_3^-$ -N removal rates. The PD mesocosms contained patches of dark-stained material that often surrounded roots in various stages of decay. The dry mass of patches in the PD treatment ranged from 0.07 to 1.4% of the mesocosms. The MWD treatment contained no patches and exhibited no groundwater  $\text{NO}_3^-$ -N removal. Further investigations on the relationships between the extent of subsurface patchiness, water table dynamics and plant characteristics might yield fruitful insights into the management of vegetated riparian zones for groundwater  $\text{NO}_3^-$ -N removal.

CONSIDERABLE controversy surrounds the fate of nitrate-nitrogen ( $\text{NO}_3^-$ -N) in groundwater. Several studies suggest that  $\text{NO}_3^-$  is relatively conservative in groundwater (Keeney, 1986; Bradley et al., 1992; Starr and Gillham, 1993), but several studies indicate that  $\text{NO}_3^-$  may undergo marked transformations in portions of aquifers. In particular, groundwater  $\text{NO}_3^-$  removal has been noted in the shallow groundwater below vegetated riparian zones (Lowrance et al., 1984; Jacobs and Gilliam, 1985; Lowrance, 1992; Nelson et al., 1995; Hill, 1996; Verchot et al., 1997). Riparian zones are defined functionally as transition areas that have the capacity to affect the exchange of energy and matter between uplands and surface waters (Gregory et al., 1991).

Because groundwater contributes substantially to the base flow of many lower order streams, the fate of groundwater  $\text{NO}_3^-$  in riparian zones can have a major effect on the export of  $\text{NO}_3^-$  from watersheds. Our ability to identify and manage riparian sites for groundwater  $\text{NO}_3^-$  removal is limited by uncertainty surrounding the relative importance of plant uptake vs. microbially mediated removal processes (Hill, 1996).

Riparian settings with permeable surface soils, shal-

low groundwater, and extended groundwater retention times (i.e., days vs. hours) are the most likely locations for groundwater  $\text{NO}_3^-$  removal (Hill, 1996). But these settings have been found to exhibit substantial intra-site variation in shallow groundwater  $\text{NO}_3^-$  removal rates (Cooper, 1990; Simmons et al., 1992; Schipper et al., 1993; Nelson et al., 1995). Starr and Gillham (1993) suggested that denitrification rates in groundwater may be inversely related to the depth of the water table. Soil characteristics, particularly the depth to redoximorphic features can be a useful indicator of water depth and groundwater  $\text{NO}_3^-$  removal within riparian zones. At the upland edge, a riparian zone may contain well-drained (WD) and moderately well-drained (MWD) soil drainage classes with water table depths  $>1$  m from the surface. Near the stream, the water table depths of PD and very poorly drained (VPD) soils may be within 0.4 m from the surface. We have found that PD riparian soils consistently demonstrate more groundwater  $\text{NO}_3^-$  removal capacity than MWD riparian soils (Simmons et al., 1992; Nelson et al., 1995).

Much of our knowledge of groundwater  $\text{NO}_3^-$  removal capacity of riparian locations has been obtained through field studies based on groundwater monitoring well networks (Hill, 1996). In situ studies are cumbersome for isolating the magnitude and dynamics of individual removal processes such as plant uptake, microbial immobilization, and denitrification. In addition to the need for process-level research, there is a role for techniques that can provide a more rapid assessment of the variation in removal capacities that results from different hydrogeomorphic settings, plant communities, groundwater depth, and seasonal conditions.

Our research group (Groffman et al., 1992, 1996) and others (Lowrance, 1992) have compared the shallow groundwater  $\text{NO}_3^-$  removal estimated from monitoring well networks with microcosm studies of microbial  $\text{NO}_3^-$  transformations, that is, denitrification and/or immobilization, in soil derived from the same depths and locations. In all cases the microcosms demonstrated negligible transformation rates, even in situations where groundwater well network studies showed substantial groundwater  $\text{NO}_3^-$  removal during the winter and a decline of DO in ambient groundwater moving through the site. The microcosm results suggest that either plant uptake is the predominant process involved in  $\text{NO}_3^-$  removal or that microcosm results may not capture a sufficient sample volume to characterize microbial processes in the groundwater of riparian zones (Groffman et al., 1996; Smith et al., 1996).

Microcosm studies often rely on  $<50$  g of sample to represent field conditions. Even with intensive sampling, this sample size may miss "hot spots" of microbial activity (Parkin et al., 1987; Christensen et al., 1990a).

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Parkin (1987) found that 25 to 85% of the denitrification generated from 98 g soil cores originated from <1% of the sample. Alternately, microcosms often involve extensive soil disturbance, which can artificially increase rates of microbial activity (Smith et al., 1996). We hypothesize that hot spots may play an important role in groundwater  $\text{NO}_3^-$  removal in riparian soils. In soil morphological studies on our riparian sites we have often seen small (<2 cm), intermittent patches of roots and dark-stained media located well below the B horizon. These patches occur at depths that are inundated by groundwater during the dormant season.

To alleviate the scale incongruence between field monitoring and microcosm studies, we began a series of mesocosm studies to evaluate the rates and processes involved in groundwater  $\text{NO}_3^-$  removal in riparian soils. We created mesocosms of relatively large (15 cm diam.  $\times$  40 cm length) intact horizontal sections, from the seasonally saturated zone of PD and MWD soils located within a riparian forest that we had used for previous studies (Nelson et al., 1995; Groffman et al., 1996). To examine groundwater  $\text{NO}_3^-$  dynamics we dosed the mesocosms with ambient groundwater amended with  $^{15}\text{N}$  labeled  $\text{NO}_3^-$  and a bromide ( $\text{Br}^-$ ) tracer. Bromide was used as a conservative, nonreactive tracer to differentiate between changes in ionic concentrations due to biological removal mechanisms vs. reductions that result from physical processes such as mixing, diffusion, and dispersion.

In this paper we focus on mesocosm estimates of groundwater  $\text{NO}_3^-$  removal from seasonally saturated PD and MWD soils of a riparian forest. Groundwater  $\text{NO}_3^-$  in the mesocosms was not subject to any direct plant uptake, hence we examined the nature and extent of microbial removal of  $\text{NO}_3^-$  in the mesocosms. The mesocosms permit us to further our examinations of the effect of sampling scale on estimates of microbially mediated groundwater  $\text{NO}_3^-$  transformations. In particular, we explore the relationship of hotspots, that is, patches of elevated organic matter, to the groundwater  $\text{NO}_3^-$  removal rates. In a companion paper (Jacinthe et al., 1998), we use the enriched  $^{15}\text{N}$  to examine the microbial processes responsible for  $\text{NO}_3^-$  transformations within the mesocosms.

## METHODS

### Site Description and Field Sampling

The study area was located on a forested riparian site along White Horn Brook at the Peckham Farm of the University of Rhode Island, Kingston, RI (41°30' N, 71°30' W). Soils and groundwater at the site were described by Nelson et al. (1995) and Groffman et al. (1996). The forested riparian area contains a toposequence of soils and includes MWD, somewhat poorly, PD and VPD soils. Drainage class boundaries were established from the depth to redoximorphic features based on visual inspection of the soil profile. The soils are classified as Endoaquepts and are derived from glaciofluvial deposits of structureless, granitic sands and loamy sands with an average slope of 3%. The forested riparian area is approximately 60 m wide and is bordered on its upland edge by well-drained forest and old fields. High water table depths during the dormant season

(1 November–1 April) ranged from 0.9 m in the MWD soils to 0.2 m in the PD soils, while summer water tables fell to 1.6 and 0.7 m in the MWD and PD soils, respectively (Table 1). The forest is uniform throughout the site. The overstory is dominated by a mixture of white oak (*Quercus alba* L.) and red maple (*Acer rubrum* L.) with the frequency of maples increasing in the wetter soils. The overstory trees are approximately 40 to 50 yr old. The trunk diameter of the overstory trees at 1.35 m elevation (dbh) ranges from 17 to 40 cm.

We obtained horizontal, undisturbed soil cores during July 1995 from the bottom of pits located in the PD and MWD soils. The center of the cores were 0.61 and 1.55 m below ground surface for the PD and the MWD soils, respectively. The cores consisted of Schedule 35 PVC, 15 cm diam. and 40 cm long (volume: 7070  $\text{cm}^3$ ). We beveled the outer diameter of the cutting edge of the cores to ease the insertion of the cores into the soil and minimize compaction and disturbance during sampling. The cores were laid horizontally in the direction of groundwater flow (Nelson et al., 1995) and pushed into the soil with an 8 ton hydraulic jack. The cores were then dug out and stored at 4°C until dosing ensued. The cores were obtained from partially saturated media, thus we can assume that the cores may have contained some oxygen in the air-filled pores at the inception of storage.

Based on a measured dry bulk density of 1.65  $\text{g cm}^{-3}$ , we calculated the dry soil mass of the cores to be 11.7 kg, with a pore volume of 2670  $\text{cm}^3$ . Sampling occurred during the annual water table minimum, thus we were able to extract cores from media that was at least 20 to 40 cm below the water table depth during the dormant season. All of the cores were obtained from "C" horizon material composed of acidic (pH 4.8–5.3), medium to fine sands. This horizon is uniform throughout all the drainage classes of the riparian site. The media was moist during the time of sampling, which allowed us to push the cores into sand. We had very fine control over the extension rate on our hydraulic jack, thus we were able to avoid compaction and compression within the core during sampling.

The two drainage class locations were separated by 25 to 30 m. In each drainage class location there were three separate pits. Sampling depth and characteristics of the sampling locations are given in Table 1. At each location the three replicate pits were randomly located along a line of equal elevation approximately 30 m long. Three cores were extracted from each pit. During the mesocosm experiment one core per pit was dosed with groundwater amended with  $\text{NO}_3^-$  and  $\text{Br}^-$ , another core was dosed with groundwater amended with  $\text{NO}_3^-$  and  $\text{Br}^-$  and DOC, and the third core was used as a control and dosed with groundwater amended only with  $\text{Br}^-$ . Due to an experimental error associated with input ratios of the amended constituents, we do not report on the results of the DOC amended mesocosms in this paper. However,

**Table 1. Selected characteristics of sampling locations.**

Drainage class	Core sampling† depth	Dormant season‡ water table depth	Depth to C horizon	Soil classification
Poorly drained	61	20–40	25	Sandy, mixed mesic, Typic Endoaquepts
Moderately well drained	155	90–110	85	Sandy, mixed mesic, Aeric Endoaquepts

† All depths are measured from ground surface.

‡ Dormant season: 1 November to 1 April.

microbial transformation data is presented on this treatment in our companion paper (Jacinthe et al., 1998).

### Mesocosm Setup

We created an experimental mesocosm setup to simulate closely the ambient groundwater conditions found at the site (Fig. 1). We used 12 mesocosms to examine saturated groundwater  $\text{NO}_3^-$  dynamics in the following treatments and controls (three replicated mesocosms per experimental condition):

- Poorly drained soil amended with  $\text{NO}_3^-$ -N and  $\text{Br}^-$
- Poorly drained soil amended only with  $\text{Br}^-$  (control)
- Moderately well-drained soil amended with  $\text{NO}_3^-$ -N and  $\text{Br}^-$
- Moderately well-drained soil amended only with  $\text{Br}^-$  (control).

The ends of each core were covered by a plexiglass plate perforated with 1/8 in. holes, then capped with a PVC end cap. A 1/16 in. plastic adapter plug was inserted into the center of the end caps to permit connection to dosing and sampling tubing. The cores were positioned vertically and dosed from the bottom to avoid flow impedance from entrapped air. The outflow from each core was first directed into a PVC enclosure with a silicone cell inside to collect gases (Jacinthe and Dick, 1996) and then discharged into an acid washed mason jar. The results of the gas analyses are reported in our companion paper (Jacinthe et al., 1998).

The mesocosms were dosed with groundwater that was obtained every 4 to 6 wk from shallow wells at the two sampling locations. Ambient levels of groundwater  $\text{NO}_3^-$ -N were always  $<0.5 \text{ mg L}^{-1}$ . Groundwater was stored in a controlled environment chamber at  $11^\circ\text{C}$  until it was transferred to the dosing carboys. Initially, we dosed the mesocosms for several days with unamended groundwater to saturate the mesocosms and assure that the output volume equaled the input volume. Thereafter, the groundwater was amended with either  $\text{Br}^-$  at  $10 \text{ mg L}^{-1}$  or with both  $\text{Br}^-$  and  $^{15}\text{N}$ -labeled  $\text{NO}_3^-$ -N at  $10 \text{ mg L}^{-1}$ .

All carboys were sampled two to three times per week to assess the stability of the dosing solution. The concentrations of  $\text{NO}_3^-$ -N and  $\text{Br}^-$  in the carboys remained constant throughout each batch of amended groundwater, suggesting negligible transformations within the carboys.

The amended groundwater was pumped continuously into

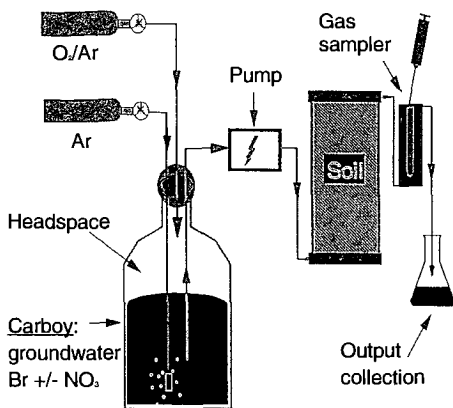


Fig. 1. Schematic of mesocosm dosing system showing oxygen-regulating system for the groundwater input solution, carboy dosing reservoir and samplers for gas and solution outputs. A separate carboy served as the input to each set of treatments or controls. Mesocosm is labeled *Soil*.

the bottom of each mesocosm with an Ismatec multi-channel peristaltic pump (Cole Palmer, IL) at a rate of  $170 \text{ mL/d}$ . The amended groundwater for each of the four sets of mesocosms (two types of amendments  $\times$  two drainage classes) was stored in separate 10 L dosing carboys. The same dosing solution was pumped into all three of the replicates. Assuming a simple breakthrough model with no preferential flow, the pumping rate would generate an average retention of about 16 d and an average pore velocity of  $2.5 \text{ cm d}^{-1}$ , which is comparable to flow rates measured in our previous well network study of groundwater  $\text{NO}_3^-$  removal (Nelson et al., 1995). Once dosing began, the mesocosms were placed in a controlled climate chamber and maintained at a constant temperature of  $11^\circ\text{C}$ , the groundwater temperature observed at the study site during November. Dosing began in October, 1995 and continued for the next 130 d.

We intended to maintain DO concentrations in the mesocosms at levels similar to those observed in our field studies. Our goal was to generate a DO concentration of  $2 \text{ mg L}^{-1}$  in the PD mesocosms and  $5 \text{ mg L}^{-1}$  in the MWD mesocosms. We obtained the desired DO concentration in our storage carboys by bubbling high-purity mixtures of  $\text{O}_2/\text{Ar}$  gas through the solution (Jacinthe et al., 1998). However, during the first 56 d of the study, the DO concentration in the output of the mesocosms ranged from  $4.0$  to  $10 \text{ mg L}^{-1}$ , which was twice the DO levels maintained in the dosing carboys. Examination of the  $\text{O}_2$ -diffusion characteristics of the components of the incubation system showed that the Tygon tubing (Cole Parmer H-06409-16, wall thickness  $1.4 \text{ mm}$ ) used to transport solution from the carboys to the mesocosms was  $\text{O}_2$  permeable. Thus, on Day 56 of dosing, the tygon tubing was replaced with stainless steel tubing. Thereafter, the DO concentrations in the mesocosms gradually approached the levels in the dosing carboy.

### Calculation of Nitrogen Removal

We obtained  $\text{NO}_3^-$ -N removal rates by coupling the daily output flow volume ( $Q$ ) with estimates of the change between the input and output  $\text{NO}_3^-$ -N concentrations (Nelson et al., 1995). Because we calculated removal rates based on differences between the input and output concentrations of amended ions, any  $\text{NO}_3^-$ -N added to the outflow through internal transformations reduces our estimates of groundwater  $\text{NO}_3^-$ -N removal within the mesocosms. Thus, our estimates constitute net removal rates rather than gross removal rates.

We amended the mesocosms with equal concentrations of  $\text{NO}_3^-$ -N and  $\text{Br}^-$ . Changes in  $\text{NO}_3^-$ -N/ $\text{Br}^-$  or  $\text{NO}_3^-$ -N/ $\text{Cl}^-$  ratios are frequently used to document groundwater  $\text{NO}_3^-$  removal in situations where  $\text{NO}_3^-$  concentrations may be changing due to physical processes, such as dispersion and dilution or where the input concentrations may be changing over time, as occurred during the start-up period before the output concentration of the conservative tracer equaled the input concentration (Jacobs and Gilliam, 1985; Trudell et al., 1986; Warwick and Hill, 1988; Nelson et al., 1995; Starr et al., 1996; Verchot et al., 1997). When  $\text{NO}_3^-$ -N concentrations in the output decreased below output concentrations of  $\text{Br}^-$ , the decrease was attributed to biological removal processes.

The daily flux ( $\text{mg d}^{-1}$ ) of  $\text{Br}^-$  and  $\text{NO}_3^-$ -N was determined by multiplying the concentration of each ion by the daily outflow per mesocosm ( $0.170 \text{ L d}^{-1}$ ). The mass of  $\text{NO}_3^-$ -N removed or retained in the mesocosms is the difference between the mass of  $\text{Br}^-$  and  $\text{NO}_3^-$ -N leaving the mesocosms:

$$\text{MR}_{\text{NO}_3^-} = [(M_{\text{Br}^-} - M_{\text{NO}_3^-}) \times 10^3 \mu\text{g mg}^{-1}]/M_s \quad [1]$$

where

$MR_{NO_3^-N}$  = mass of  $NO_3^-N$  retained or removed per kg of soil per day ( $\mu\text{g kg}^{-1} \text{d}^{-1}$ )

$M_{Br^-}$  = mass of  $Br^-$  leaving the mesocosm ( $\text{mg d}^{-1}$ )

$M_{NO_3^-N}$  = mass of  $NO_3^-N$  leaving the mesocosm ( $\text{mg d}^{-1}$ )

$M_s$  = Mass of soil in the mesocosm (11.7 kg)

For a situation where the daily output concentrations were 9 and 8 for  $Br^-$  and  $NO_3^-N$ , respectively, the groundwater removal would be  $14 \mu\text{g kg}^{-1} \text{d}^{-1}$ . Given our analytical resolution levels ( $0.1 \text{ mg L}^{-1}$  for  $Br^-$  and  $NO_3^-N$ ) the minimum removal rate we could detect was  $1.4 \mu\text{g NO}_3^-N \text{ kg}^{-1} \text{d}^{-1}$ .

We chose to remix the amended solution of  $NO_3^-N$  and  $Br^-$  each time we refilled the dosing carboys (refilling occurred approximately every 15 d). Unfortunately, due to weighing errors, in the first 100 d of the experiment the different amendment solutions differed slightly in the concentrations of  $NO_3^-N$  and  $Br^-$ . To account for these differences, we used a correction factor that may result in a maximum underestimation of groundwater  $NO_3^-N$  removal of  $7.0 \mu\text{g kg}^{-1} \text{d}^{-1}$  in the PD and  $4 \mu\text{g kg}^{-1} \text{d}^{-1}$  in MWD mesocosms. From 100 to 130 d the relative concentrations of  $Br^-$  and  $NO_3^-N$  varied more widely. Accordingly, we have limited our analyses to the first 100 d.

### Analytical Methods

The outflow from each mesocosm was collected in acid washed containers and the volume measured two to three times per week. At that time samples were obtained for chemical analyses from the outflow of each mesocosm and dosing carboys. Samples were stored at  $4^\circ\text{C}$  until analysis. Samples were analyzed for  $NO_3^-N$ ,  $NO_2^-N$ , and  $Br^-$  on a Dionex 500 ion chromatograph (Dionex, Sunnyville, CA) and for  $NH_4^+N$  with a Perstorp autoanalyzer (Perstorp Analytical, Silver Spring, MD) using the salicylate-hypochlorite method. Nitrite- $N$  concentrations were consistently  $<0.1 \text{ mg L}^{-1}$  and are not reported in the data. Dissolved oxygen was measured with a 1.2 cm diam. probe and Cole-Palmer meter (Cole-Palmer, Niles, IL). A sampling port located on top of the mesocosms allowed direct measurement of DO in the mesocosm effluent.

### Characterization of Mesocosms

At the conclusion of dosing, each mesocosm was cut into 5-cm sections (eight sections per mesocosm). The soil in each individual section was then extracted and any patches of darker material were removed. For each mesocosm the patch material was aggregated and analyzed for dry mass and %C using a Carlo-Erba CNS analyzer. Microbial and chemical characterization of the patches and other process level investigations on the fate of the  $^{15}\text{N}$ -labeled  $NO_3^-N$  are reported in our companion paper (Jacinthé et al., 1998).

### Statistical Analysis

We used the nonparametric Mann-Whitney U Test (Bhattacharyya and Johnson, 1977) to determine differences in groundwater  $NO_3^-N$  removal rates between the  $NO_3^-N$  amended treatments for the whole period between Days 11 and 100 and within each 10-d interval during that period. The 10-d interval was selected as a unit for analysis based on the daily patterns of removal in the PD mesocosms and the fact that we had at least three separate samples per mesocosm within each 10-d period. For comparisons between treatments,

daily groundwater removal rates were pooled by mesocosm for the period of interest, because each mesocosm represented a distinct spatial unit. We used a Fisher's least significant difference test to compare mean groundwater  $NO_3^-N$  removal rates between mesocosms of the amended PD treatment. Differences were considered significant at the  $P < 0.05$  level.

## RESULTS

### Flow Characteristics

Based on the breakthrough curves of the conservative tracer, none of mesocosms showed pronounced evidence of rapid bypass or macropore flow. Measurable outflow  $Br^-$  concentrations were not observed until at least 0.6 pore volumes of outflow had occurred (approximately 10 d after the enriched solution was added to the mesocosms). Within each treatment the breakthrough curves were generally similar and relatively symmetrical (Fig. 2). The 0.5 relative concentration (output concentration/input concentration, that is,  $C/C_0$ ) in these mesocosms corresponded to outflow pore volumes between 0.88 to 1.13, suggesting minimal retardance or preferential flow (Li and Ghodrati, 1994). Within treatments, only mesocosm 5 of the  $NO_3^-$ -amended PD mesocosms displayed a notable difference—a comparatively greater retardance—in the timing of the breakthrough curve.

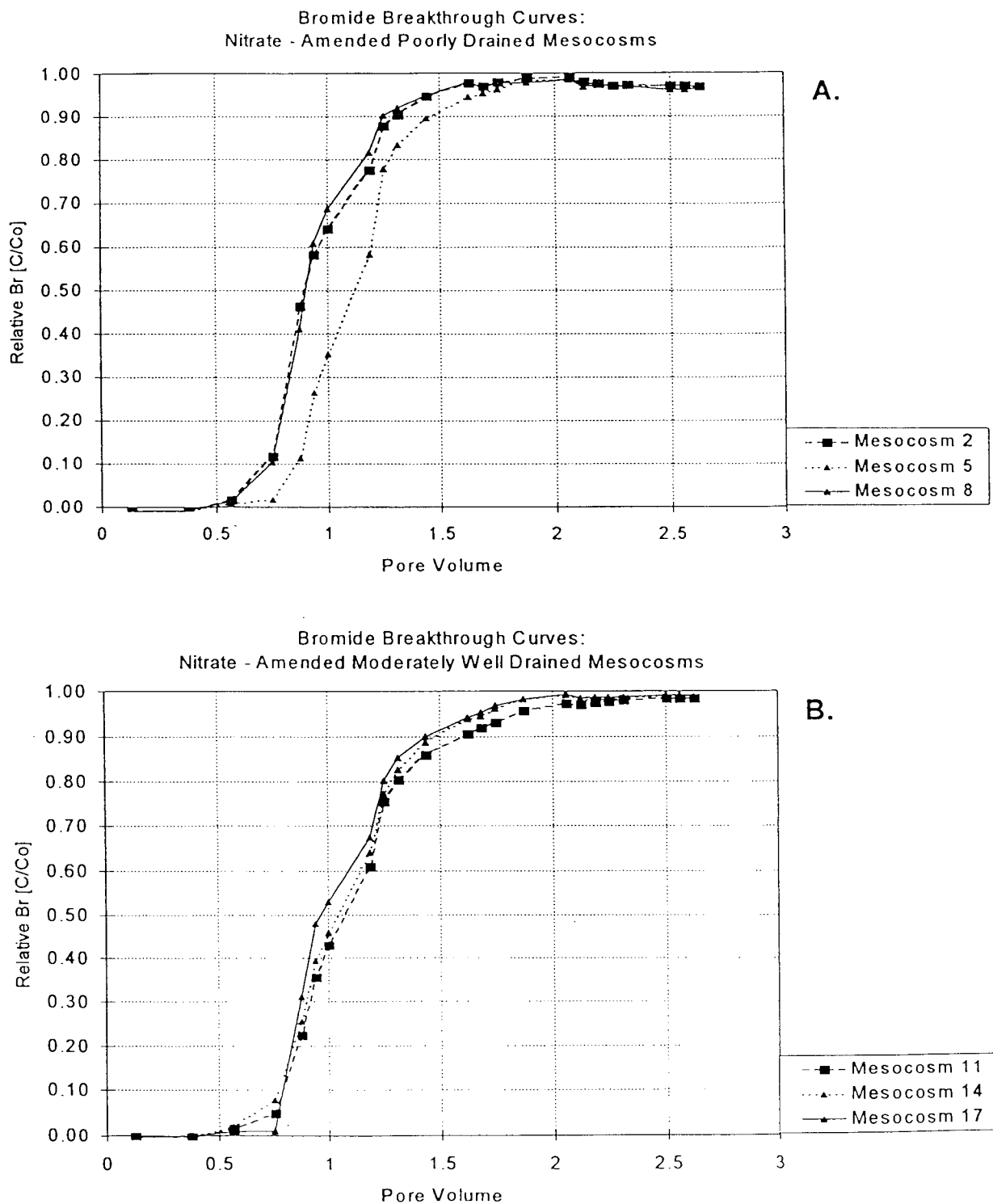
Our approach for estimating groundwater  $NO_3^-N$  removal assumes that the difference between the  $Br^-$  concentrations and  $NO_3^-N$  concentrations in the output results from differences in the fate of the introduced  $NO_3^-N$  and  $Br^-$  within the mesocosm. Until the introduced  $Br^-$  has emerged, we cannot estimate groundwater  $NO_3^-N$  removal using this approach. Accordingly, given the lack of elevated  $Br^-$  concentrations in output until Day 11, we have not included groundwater  $NO_3^-N$  removal estimates for the first 10 d of the study.

### Control Mesocosm Results

The pattern and magnitude of the outflow  $NO_3^-N$  concentrations in the control (no  $NO_3^-N$  addition) mesocosms differed between drainage classes (Fig. 3). Outflow  $NO_3^-N$  concentrations from the MWD control mesocosms were similar to input concentrations. Outflow concentrations never exceeded input concentrations by more than  $0.5 \text{ mg L}^{-1}$  and remained relatively stable over time.

In contrast, the PD control mesocosms outflow concentrations of  $NO_3^-N$  consistently exceeded the input concentrations. Initial outflow concentrations from the PD control mesocosms were almost  $2 \text{ mg L}^{-1}$ , and the concentrations diminished in an exponential pattern throughout the 100-d dosing period. After 20 d of dosing, the outflow concentrations were never more than  $0.4 \text{ mg NO}_3^-N \text{ L}^{-1}$  above the input concentrations.

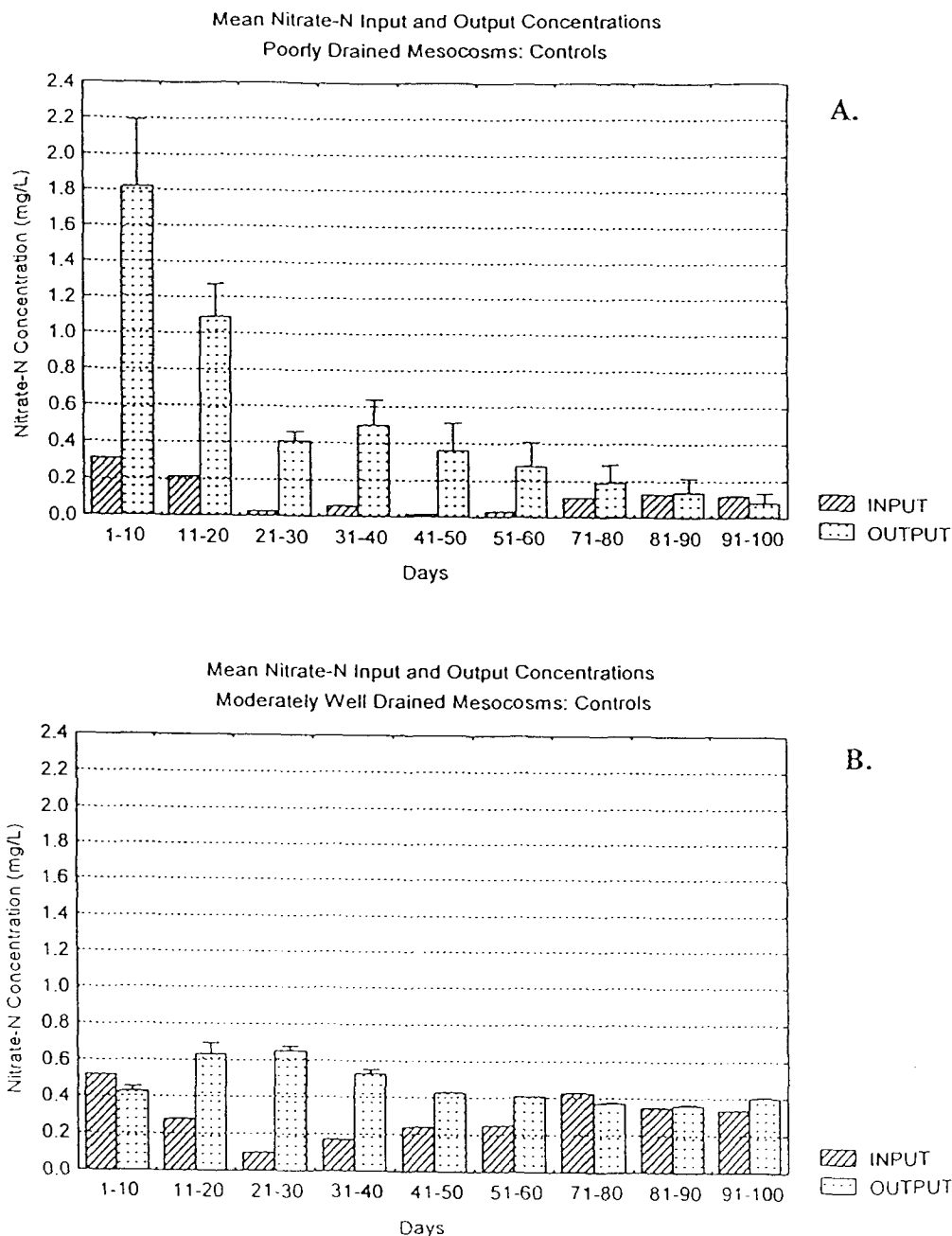
The high initial outflow concentrations of the PD mesocosms occurred before bromide was measured in the outflow, suggesting that we observed a flush of residual  $NO_3^-N$  that had accumulated during the 60 d of storage before dosing began. Given the low ambient groundwater  $NO_3^-N$  concentration ( $<0.5 \text{ mg L}^{-1}$ ), we



**Fig. 2.** Experimental bromide breakthrough curves of nitrate ( $\text{NO}_3^-$ )-amended aquifer mesocosms taken from beneath: (A) poorly drained; and (B) moderately well-drained soils in a riparian forest.

suggest that during the storage period mineralization and nitrification may have occurred, even though the cores were held at  $4^\circ\text{C}$ . Our method for characterizing groundwater  $\text{NO}_3^-$  removal is limited to net removal, thus we cannot quantify the gross nitrification and extent of denitrification that may have occurred during storage. The low initial output concentrations ( $0.3 \text{ mg L}^{-1}$ ) of the MWD control mesocosms suggest that less

$\text{NO}_3^-$ -N had accumulated within these mesocosms during storage. Assuming that internal  $\text{NO}_3^-$ -N production in the  $\text{NO}_3^-$ -N amended mesocosms was the same as in the control mesocosms, the internally generated  $\text{NO}_3^-$ -N would raise gross estimates of groundwater  $\text{NO}_3^-$ -N removal by an average (Day 11–100) of  $6.3$  and  $2.5 \mu\text{g N kg}^{-1} \text{ d}^{-1}$  for the PD and MWD treatments, respectively, as compared to the net rates that we report.



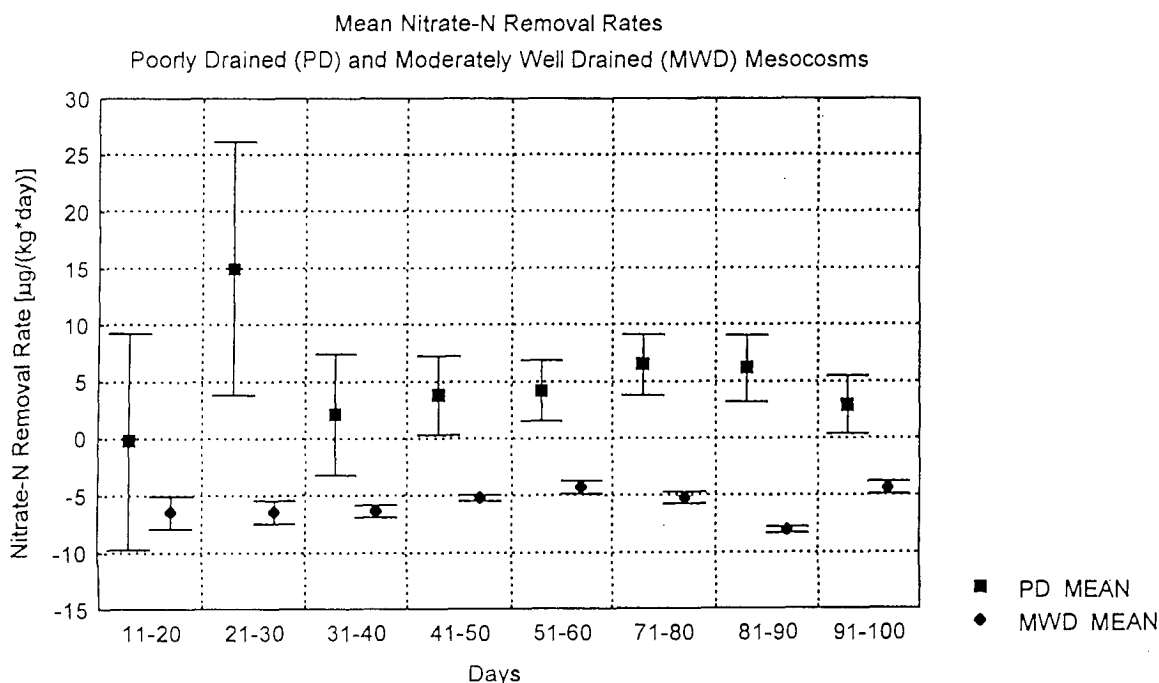
**Fig. 3.** Groundwater nitrate–nitrogen ( $\text{NO}_3^-$ -N) concentrations from control (bromide-amended) aquifer mesocosms taken from beneath: (A) poorly drained and (B) moderately well-drained soils in a riparian forest. Input values are the mean (three to five samples per period) from a single carboy; output values are the mean (SE) of three replicate mesocosms per soil during 10-d periods over a 100-d experiment.

Our companion study (Jacinthe et al., 1998) demonstrated that dissimilatory reduction of  $\text{NO}_3^-$  to  $\text{NH}_4^+$  was not the process responsible for the observed decline in  $\text{NO}_3^-$ -N concentrations. Jacinthe et al. (1998) also noted an initial elevation in output  $\text{NH}_4^+$  concentrations from the PD mesocosms and suggested that this increase may have resulted from mineralization during the storage period.

### Groundwater Nitrate Removal

The pattern and magnitude of groundwater  $\text{NO}_3^-$  removal varied significantly between drainage class (Fig.

4). The study-wide mean daily removal rate (Day 11–100) was significantly higher in the PD mesocosms (mean:  $5.4 \mu\text{g kg}^{-1} \text{d}^{-1}$ ; SE: 4.8) than in the MWD mesocosms (mean:  $-6.0 \mu\text{g kg}^{-1} \text{d}^{-1}$ ; SE: 0.65). The mean removal rates of the PD treatment were significantly higher than the MWD treatments during all but one of the 10-d periods from Day 21 to the end of the study. Based on our observations of the PD control mesocosms, we believe that the initial removal rates (Day 10–15) observed in the  $\text{NO}_3^-$ -amended PD mesocosms substantially underestimate actual removal of the introduced plume and reflect internally generated  $\text{NO}_3^-$ -N.



**Fig. 4.** Groundwater nitrate-nitrogen ( $\text{NO}_3^-$ -N) removal rates from  $\text{NO}_3^-$ -amended aquifer mesocosms taken from beneath poorly drained and moderately well-drained soils in a riparian forest. Values are mean (SE) of three replicate mesocosms per soil during 10-d periods (three to five samples per period) over a 100-d experiment.

The PD treatment showed considerable temporal and spatial variation (Fig. 5). Within the PD treatment two of the mesocosms generated groundwater  $\text{NO}_3^-$ -N removal in excess of  $15 \mu\text{g kg}^{-1} \text{d}^{-1}$  between Days 15 and 30, while the third mesocosm (mesocosm 2) generated negative removal rates during the first 56 d of the experiment. After Day 40 the removal rates did not exceed  $11 \mu\text{g kg}^{-1} \text{d}^{-1}$  in any of the PD mesocosms. Although the mesocosm with the greatest groundwater  $\text{NO}_3^-$ -N removal (mesocosm 5) exhibited somewhat greater flow retardance than the other PD replicates (Fig. 2), the intra-treatment difference in PD groundwater  $\text{NO}_3^-$ -N removal cannot be attributed solely to flow characteristics. The breakthrough curves of mesocosms 2 and 8 were almost identical, yet we observed substantial removal in mesocosm 8 and negligible removal in mesocosm 2.

The MWD treatment generated little removal throughout the study and displayed very minor spatial and temporal variation. No significant difference was observed between time periods for the MWD treatment. The removal rates from the MWD mesocosms were always negative, suggesting either that mineralization and nitrification were occurring within the mesocosms, or that we used too large a correction factor to account for the occasional differences that we observed between the input concentrations of  $\text{NO}_3^-$ -N and  $\text{Br}^-$ .

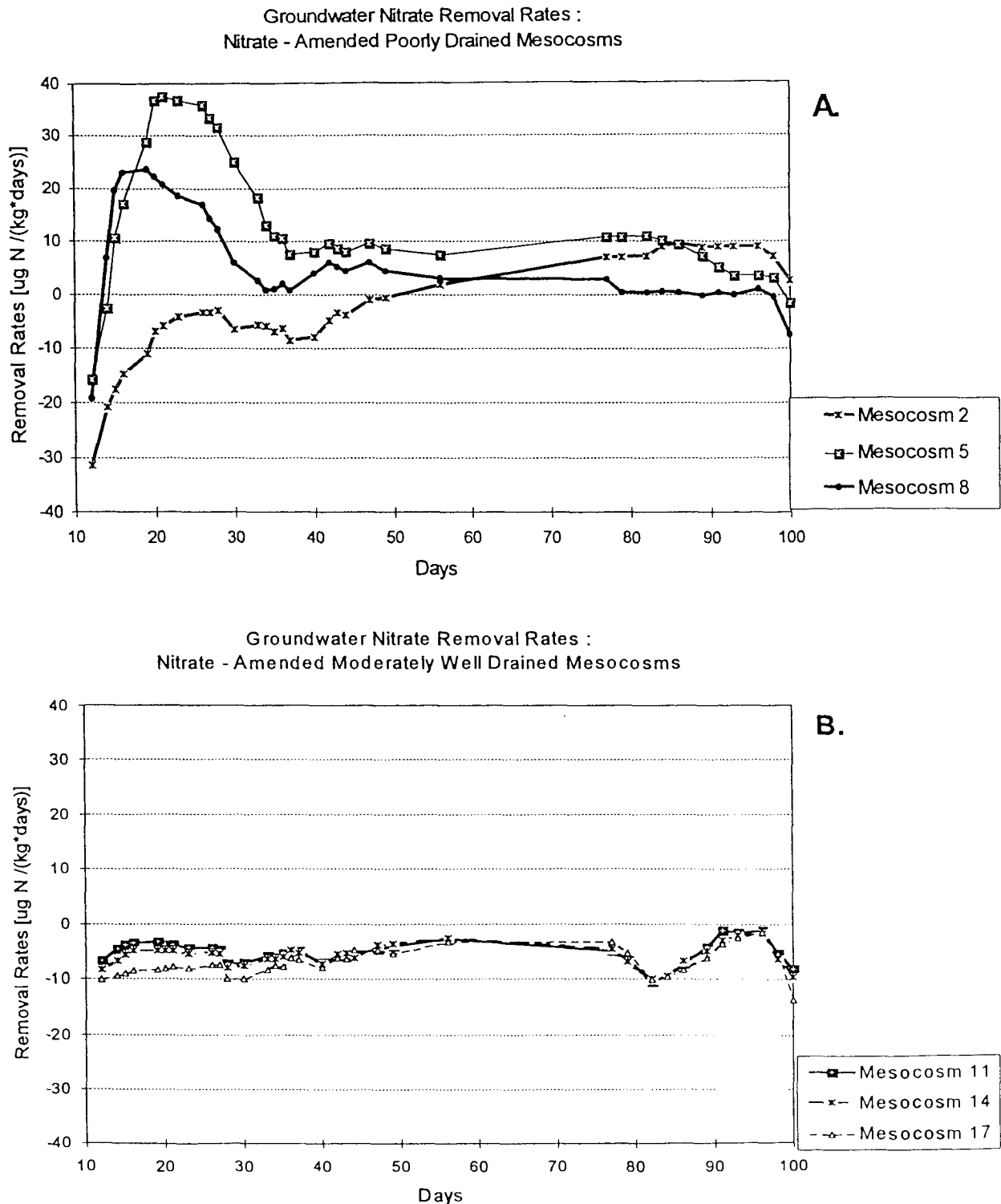
#### Patch Characteristics

Patches of dark-stained media and roots were only found in the PD mesocosms. The PD mesocosms contained patches of dark-stained material that often surrounded roots in various stages of decay. For all six of the PD mesocosms the dry mass of the patches ranged

from 0.07 to 1.41% of the entire mesocosm mass. The organic C content of the patches ranged from 7 to 9  $\text{g kg}^{-1}$ . This is several fold higher than the organic C content of the matrix material in mesocosms from both drainage classes, which averaged about 2  $\text{g kg}^{-1}$ . The patch content of each of the  $\text{NO}_3^-$ -N amended PD mesocosms is compared to groundwater  $\text{NO}_3^-$ -N removal rates in Table 2. Within the amended PD treatment there was no significant relationship between the mass of patches within a mesocosm and the groundwater  $\text{NO}_3^-$ -N removal rates.

#### DISCUSSION

In our previous studies of groundwater  $\text{NO}_3^-$  removal in riparian forests we have observed serious discrepancies between direct measurements of  $\text{NO}_3^-$  removal in groundwater monitoring well networks and measurements of microbial  $\text{NO}_3^-$  removal processes in laboratory microcosms. In a previous study at the same research site as the study reported here, groundwater  $\text{NO}_3^-$  removal rates in aquifer material beneath PD soils averaged  $46 \mu\text{g N kg}^{-1} \text{d}^{-1}$  while denitrification rates in laboratory microcosms averaged only  $2 \mu\text{g N kg}^{-1} \text{d}^{-1}$  (Nelson et al., 1995; Groffman et al., 1996). This mesocosm study was initiated to eliminate scale incongruencies between the field and laboratory studies and to test the hypothesis that small patches of organic matter function as "hotspots" of  $\text{NO}_3^-$  removal in the subsurface. Results from this and the companion study described by Jacinthe et al. (1998) strongly suggest that patches are hotspots of  $\text{NO}_3^-$  removal and that microcosm-based studies that do not include these patches are likely to underestimate microbial  $\text{NO}_3^-$  removal pro-



**Fig. 5.** Groundwater nitrate–nitrogen ( $\text{NO}_3^-$ -N) removal rates from  $\text{NO}_3^-$ -amended aquifer mesocosms taken from beneath: (A) poorly drained; and (B) moderately well-drained soils in a riparian forest. Values represent single samples from individual mesocosms sampled at selected dates over a 100-d experiment.

cesses in the subsoil of riparian zones. We did not observe any  $\text{NO}_3^-$  removal in mesocosms that did not have patches or roots. Moreover, our companion work (Jacinthe et al., 1998) showed that the patches had rates of denitrification and immobilization high enough to account for the observed rates of  $\text{NO}_3^-$  removal.

Our results suggest that the scale of our mesocosms is an improvement over microcosm studies for process level studies. The mesocosms appear to have been sufficiently large to facilitate denitrification within anaerobic microsites surrounding the patches. The highest rates of groundwater removal occurred under conditions gen-

**Table 2. Groundwater nitrate-N removal rates and patch characteristics in nitrate-amended PD mesocosms.**

Mesocosm	Removal rate		Patch characteristics	
	Studywide† mean (SE)	Peak period‡ mean	Total mass	Relative mass
				% of core
μg kg <sup>-1</sup> d <sup>-1</sup>		g		
2	-3.1a§ (1.6)	-4.8a	12	0.10
3	13.5b (2.2)	33.9b	30	0.25
8	5.4c (1.6)	15.8c	115	0.96

† Studywide: Days 11 to 100; *n* = 35.

‡ Peak period: Days 21 to 30; *n* = 7.

§ 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.

erally considered too aerobic for denitrification. Several studies have found that the rhizosphere can create anaerobic conditions and generate markedly higher denitrification activity than the surrounding soil (Woldendorp, 1962; Bailey, 1976; Smith and Tiedje, 1979).

Although these mesocosms were a great improvement over our previous microcosms, the variation between replicates suggests that even larger sample volumes may be needed to quantify mean groundwater NO<sub>3</sub><sup>-</sup> removal rates within a riparian zone. The PD mesocosms were large enough to contain numerous patches, however the relative mass of patches varied markedly between mesocosms. Moreover, there appears to be significant variation in the activity of patches. For example, the mesocosm with the greatest mass of patches did not have the highest rates of NO<sub>3</sub><sup>-</sup> removal.

There was a brief period when several of the PD mesocosms generated removal rates comparable to our previous field studies at the same location, but after the first 30 to 40 d we observed substantially lower removal rates from the mesocosms than from our field studies. These results are not surprising if patches are the source of the removal, because hotspots of denitrification have been found to persist for up to several weeks, and the distribution of hotspots can vary seasonally (Parkin, 1987; Christensen et al., 1990b). For a variety of reasons we expect field studies to generate higher removal rates than those observed in our mesocosm study. In the field, root turnover and root exudates may serve as a routine source of C replenishment to the shallow groundwater of certain riparian locations (Haycock and Pinay, 1993; O'Neill and Gordon, 1994; Hill, 1996). In addition, groundwater NO<sub>3</sub><sup>-</sup> is subject to plant uptake as well as microbial processes.

An inherent source of uncertainty in our study was the possibility that the coring severed living roots within the cores. During the measurement period, these roots could have served as a source of mineralized C and N. Thus a portion of our results might be attributed to artifacts surrounding root senescence, rather than patch dynamics.

Odum et al. (1995) have suggested that forested swamps may be "pulsating" ecosystems, similar to tidally flushed estuaries. In these environments, a portion of the required materials for a given process are transported to a site during one period, then transformations

occur during a later period when other materials and conditions prevail. Whereas the periodicity in estuaries is the hours between tidal cycles, in riparian forests, seasonal changes in water table elevation and plant growth generate the periodicity.

Because of seasonal fluctuations in water tables, at some riparian locations roots may grow into subsoil that is unsaturated only in the summer. Root turnover and exudates may stimulate microbial transformations at a later time when the subsoil becomes inundated during the dormant season. The water table at our riparian study sites, and at many other riparian sites, rises in the dormant season and falls in the growing season with vertical fluctuations in the water table often ranging from 0.3 to 1 m. Denitrification in the portion of the soil profile that is saturated only during the dormant season may be quite important to the annual flux of NO<sub>3</sub><sup>-</sup> from a watershed, because much of the annual movement of groundwater through riparian zones occurs as baseflow to streams during the dormant season.

Starr and Gillham (1993) have observed that groundwater NO<sub>3</sub><sup>-</sup> removal rates are relatively high in sites with shallow water tables. Although the mesocosms at both locations in this study were taken from media more than 20 cm below the dormant season water table, the water table of the PD location was much closer to the surface than at the MWD site. The shallow groundwater of the PD soil, although below the B horizon, was still within the rootzone. It is also important to consider that the PD location supports more obligate wetland species than the MWD location. These plants may have different water and nutrient requirements and root dynamics that may also contribute to the differences in patch occurrence in the PD soils.

We suggest that several lines of investigations are warranted if groundwater NO<sub>3</sub><sup>-</sup> removal in riparian zones is sensitive to the interaction of root dynamics and the depth and fluctuations of the water table. First, we need to encourage the use of soil classification schemes that recognize soil morphological features that relate to the depth of the rootzone, the dormant season water table depth and the extent of water table fluctuations within riparian soils. Second, further investigations on the relationships between site characteristics, plant species, plant management, root development, and the nature and extent of subsurface patchiness might yield fruitful insights into management of vegetated riparian zones for groundwater NO<sub>3</sub><sup>-</sup> removal.

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