

# In Situ Push–Pull Method to Determine Ground Water Denitrification in Riparian Zones

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## ABSTRACT

To quantify ground water denitrification in discrete locations of riparian aquifers, we modified and evaluated an in situ method based on conservative tracers and  $^{15}\text{N}$ -enriched nitrate. Ground water was “pushed” (i.e., injected) into a mini-piezometer and then “pulled” (i.e., extracted) from the same mini-piezometer after an incubation period. This push–pull method was applied in replicate mini-piezometers at two Rhode Island riparian sites, one fresh water and one brackish water. Conservative tracer pretests were conducted to determine incubation periods, ranging from 5 to 120 h, to optimize recovery of introduced plumes. For nitrate push–pull tests, we used two conservative tracers, sulfur hexafluoride and bromide, to provide insight into plume recovery. The two conservative tracers behaved similarly. The dosing solutions were amended with  $^{15}\text{N}$ -enriched nitrate that enabled us to quantify the mass of denitrification gases generated during the incubation period. The in situ push–pull method detected substantial denitrification rates at a site where we had previously observed high denitrification rates. At our brackish site, we found high rates of ground water denitrification in marsh locations and minimal denitrification in soils fringing the marsh. The push–pull method can provide useful insights into spatial and temporal patterns of denitrification in riparian zones. The method is robust and results are not seriously affected by dilution or degassing from ground water to soil air. In conjunction with measurements of ground water flow-paths, this method holds promise for evaluating the influence of site and management factors on the ground water nitrate removal capacity of riparian zones.

ALTHOUGH RIPARIAN ZONES can markedly decrease the flux of nitrogen from watersheds, major questions surround the influence of site and management factors on the ground water nitrogen (N) removal capacity of riparian zones (Hill, 1996). In particular, we are still developing our understanding of the response of riparian ground water nitrate removal to water table dynamics (Correll, 1997), soil drainage class (Simmons et al., 1992; Nelson et al., 1995), surficial geology (Lowrance et al., 1997), and vegetation (Haycock and Pinay, 1993; Osborne and Kovacic, 1993; Addy et al., 1999). Within a riparian zone, we need to resolve the factors that influence the depth of the biologically active zone (i.e., the portion of the saturated zone that is altered by the riparian ecosystem and generates substantial nitrogen transformations). In addition, there is a great need to evaluate the effectiveness of various riparian zone restoration and management approaches on riparian zone function (Lowrance et al., 1995; Schultz et al., 1995;

Clausen et al., 2000). The extent of questions surrounding riparian ground water nitrate removal argues for timely and affordable in situ methods of assessment.

Hill (1996) summarized the types of field and laboratory studies that have contributed to our understanding of ground water nitrate N ( $\text{NO}_3^-$ -N) dynamics in riparian zones. Field studies often rely on intensive well networks that track changes in nitrate concentrations as ground water moves through a riparian zone. Many of these studies (Peterjohn and Correll, 1984; Jacobs and Gilliam, 1985; Lowrance, 1992; Haycock and Pinay, 1993; DeVito et al., 2000; Hill et al., 2000) are situated on riparian sites downgradient from a source of nitrate-enriched ground water (e.g., cropland). Other field studies introduce an enriched plume of nitrate into the ground water and observe transformations following an incubation period or travel path (Trudell et al., 1986; Simmons et al., 1992; Nelson et al., 1995; Starr et al., 1996; Verchot et al., 1997). These in situ studies are well suited to evaluate the ground water nitrate removal capacity of riparian zones, but they require extensive time and effort and often cannot directly explore the removal mechanisms (i.e., plant uptake vs. microbial immobilization vs. microbial denitrification).

A major challenge in field studies is that reductions in nitrate concentrations can occur as a result of both biological removal processes as well as physical processes (i.e., dispersion or dilution with other ground water of low nitrate concentration). Many field studies compare changes in nitrate concentrations along a flow-path to changes in “conservative” tracer concentrations along the flowpath to account for physical processes. Changes in ambient chloride to nitrate ratios are often used in studies downgradient of agricultural lands (Jacobs and Gilliam, 1985; Lowrance, 1992; Verchot et al., 1997; Devito et al., 2000; Hill et al., 2000). Bromide to nitrate ratios are commonly used where enriched plumes are introduced within the ground water, either through natural gradient tests where transformations are observed in downgradient wells (Simmons et al., 1992; Nelson et al., 1995; Smith et al., 1996) or within the injection well over a series of different time periods (Trudell et al., 1986). Recent studies suggest that these anion tracers are susceptible to plant uptake, potentially confounding the reliability of tracers in certain situations (Kung, 1990; Schnabel et al., 1996; Whitmer et al., 2000). In addition, these anion tracers are of limited value in coastal riparian zones where brackish ground water has high ambient concentrations of chloride ( $\text{Cl}^-$ ) and bromide ( $\text{Br}^-$ ).

Another approach to examining ground water nitrate

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**Abbreviations:** C/C<sub>0</sub>, concentration of pulled sample/concentration of pushed sample; DO, dissolved oxygen; DOC, dissolved organic carbon.

removal is to conduct laboratory microcosm studies with aquifer sediments; however, such studies do not always corroborate observations from the field (Groffman et al., 1996). Samples of media from different depths below the water table are difficult to obtain, microcosm rates are often lower than in situ-derived rates, and, most importantly, the small sample size used in microcosm assays can generate extremely high variability. Several studies suggest that ground water nitrate removal might occur in small patches or "hotspots" that might be missed using microcosm techniques (Parkin, 1987; Christensen et al., 1990b; Jacinthe et al., 1998). Mesocosm studies (Gold et al., 1998; Jacinthe et al., 1998; Addy et al., 1999) with >10 kg undisturbed aquifer sediments can provide insights into riparian ground water denitrification; however, obtaining mesocosms from below the water table is highly labor intensive.

Here, we present a rapid, in situ method based on conservative tracers and  $^{15}\text{N}$ -enriched nitrate to quantify ground water denitrification in discrete locations of riparian aquifers. Our method was adapted from the push-pull method (Trudell et al., 1986; Istok et al., 1997) where a single piezometer was used for both dosing and sampling of ground water. Application of this method at fresh water and brackish water sites with different hydrologic properties is also considered.

## METHODS

### Approach

We adapted the push-pull method (Trudell et al., 1986; Istok et al., 1997) to estimate in situ rates of denitrification in the shallow aquifer of riparian zones rapidly and at scales relevant to riparian research. We "pushed" (i.e., injected) 10 L of previously collected ground water into a mini-piezometer and then "pulled" (i.e., extracted) ground water from the same mini-piezometer after an incubation period (Fig. 1). Prior to injection, the ground water was amended with  $^{15}\text{N}$ -enriched nitrate and  $\text{Br}^-$ . Then, this amended solution was adjusted to ambient dissolved oxygen (DO) concentrations to mimic aquifer conditions by bubbling a sulfur hexafluoride ( $\text{SF}_6$ ) gas mixture through the dosing solution. We used relatively brief incubation periods (i.e., 5 to 72 h) to optimize recovery of the

introduced plume. Two conservative tracers, gaseous tracer  $\text{SF}_6$  and soluble anion  $\text{Br}^-$ , provided insight into the recovery of the introduced plume. To minimize the effects of confounding factors such as dilution and dispersion, denitrification rates were estimated from only the "core" of the plume (i.e., the first 2 L of the plume pulled from the mini-piezometer after the incubation period). This portion of the plume consistently exhibited the highest conservative tracer recovery rate. In sandy media (bulk density =  $1.65 \text{ g cm}^{-3}$ , porosity = 0.38) the 2-L plume core interacts with 8.7 kg (dry wt.) of soil.

### Site Descriptions

We field-tested the in situ mini-piezometer method at two riparian sites in Rhode Island. Site A was a streamside riparian area where Addy et al. (1999) previously found high rates of ground water nitrate removal. By using Site A, we could compare denitrification rates generated by the in situ push-pull mini-piezometer method to rates obtained using the mesocosm method (Addy et al., 1999). Site B was a coastal riparian area with brackish ground water where we explored the ability of this method to discern differences in denitrification rates at two discrete locations located in different ground water environments separated by less than 15 m.

Site A was located along Tanyard Brook, a first-order tributary of Watchaug Pond, Charlestown, RI ( $41^\circ 22' \text{ N}$ ,  $71^\circ 42' \text{ W}$ ). Soils at the site were poorly drained sands and loamy sands derived from glaciofluvial deposits (average slope of 3%) and classified as sandy, mixed, mesic Typic Humaquepts. Vegetation included a mix of emergent vegetation, sedges, bluegrass (*Poa* spp.) and brome grass (*Bromus inermis* Leyss.) with an overstory dominated by speckled alder [*Alnus incana* (L.) Moench subsp. *rugosa* (Du Roi) R.T. Clausen]. Further site characterization can be found in Table 1.

Site B was located along Brushneck Cove, a tidally influenced cove of Narragansett Bay, Warwick, RI ( $41^\circ 41' \text{ N}$ ,  $71^\circ 24' \text{ W}$ ). We explored two discrete locations below the water table at Site B within (i) the salt "marsh" and (ii) the transition area between the salt marsh and the upland, the area referred hereafter as the *fringe*. At both locations, we assessed ground water denitrification in fine sands derived from glaciofluvial deposits. Mineral soils in the marsh were below a 30- to 90-cm-thick organic horizon, whereas no organic horizon was present in the fringe location. Marsh soils were very poorly drained, classified as sandy, mixed, eudic, mesic Terric Sulfihe-mists and tidally inundated twice daily (average slope of 3%). Fringe soils were somewhat poorly drained, classified as mixed, mesic Typic Psammaquents and rarely tidally inundated (average slope of 10%). Vegetation was dominated by smooth cordgrass (*Spartina alterniflora* Loisel.) in the marsh location and by marsh elder [*Iva frutescens* Pursh var. *oraria* (Bartlett) Fernald & Griscom], sea lavender [*Limonium carolinianum* (Walter) Britton] and seaside goldenrod (*Solidago sempervirens* L.) in the fringe location. Further site characterization can be found in Table 1.

### Mini-Piezometer Instrumentation

The mini-piezometers, similar to the sampling system described by Winter et al. (1988), are small steel well points (1.8-cm o.d., 2-cm screen length; AMS, American Falls, ID) attached to gas-impermeable Teflon tubing (0.7-cm o.d.) that extend into the soil. We used the AMS gas vapor probe system to install the mini-piezometers. After installation, the narrow hole surrounding the mini-piezometer and tubing was back-filled with sand and bentonite to prevent water flow along the side of the tubing. In sandy media, we were able to install and develop at least three mini-piezometers in one day.

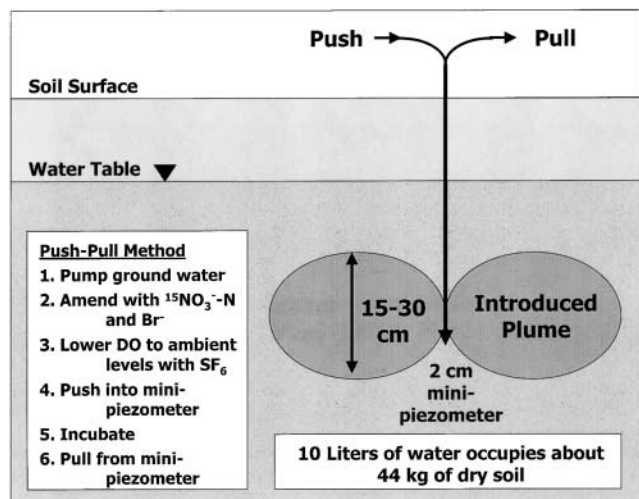


Fig. 1. Schematic of the in situ push-pull mini-piezometer method.

**Table 1.** Site characteristics.

Characteristic	Site A mini-piezometers	Site B marsh mini-piezometers	Site B fringe mini-piezometers
Number of replicate mini-piezometers	3	4	3
Depth of mini-piezometers, † cm	65	125	125
Water table depth at dosing time, † cm	44	19	92
Ground water temperature, ‡ °C	14.1	15.7	13.3
Dissolved oxygen, ‡ mg L <sup>-1</sup>	2.9	0.9	2.3
pH, ‡	6.5	4.2	4.9
Dissolved organic C, ‡ mg L <sup>-1</sup>	39.6	7.2	5.5
NO <sub>3</sub> <sup>-</sup> -N, ‡ mg L <sup>-1</sup>	0.4	0.0	0.0
Br <sup>-</sup> , ‡ mg L <sup>-1</sup>	0.0	NA§	NA§
Salinity, ‡ g L <sup>-1</sup>	0.0	13.4	0.1
Soil texture class¶	loamy sand	fine sand	fine sand

† Depth below soil surface.

‡ Mean value of replicate mini-piezometers.

§ Not applicable.

¶ Based on analysis of sample collected from soil pits dug in the vicinity of mini-piezometers.

At Site A, we installed three replicate mini-piezometers in the mineral soil at 65 cm below the soil surface. At Site B, four marsh and three fringe replicate mini-piezometers were installed in mineral soil at 125 cm below the soil surface (total of seven mini-piezometers at Site B). Replicate mini-piezometers were at least 2.5 m apart. To develop the mini-piezometers we pumped at least one liter of water from each. Water was sampled with a Masterflex L/S portable peristaltic pump (Cole Parmer, Vernon Hills, IL). From each mini-piezometer, we measured ground water temperature and ambient concentrations of DO, NO<sub>3</sub><sup>-</sup>-N, Br<sup>-</sup>, dissolved organic carbon (DOC), and salinity prior to the tracer push-pull pretests and nitrate push-pull tests. At all mini-piezometer locations, soil samples were collected from nearby soil pits for analysis of soil textural class. During the study at Site A, the water table was 44 cm below the soil surface. During the study at Site B, the water table at low tide was 19 and 92 cm below the soil surface in the marsh and fringe, respectively.

### Hydrologic Characterization: Push-Pull Pretest

Prior to the in situ nitrate study, we conducted an in situ conservative tracer push-pull pretest at both sites. This tracer pretest provided insight into the relationship between the length of incubation period and plume recovery. The recovery rate of the tracer reflected the extent of ground water advection, dispersion, and diffusion that occurred during the push phase and incubation period. We then adjusted the length of the incubation period for the in situ nitrate push-pull test to obtain high rates of tracer recovery in the plume core (i.e., the first 2 L extracted in the pull phase).

We used SF<sub>6</sub> as the pretest conservative tracer at both sites. Prior to the pretest, we collected 10 L of ground water from one mini-piezometer at Site A, the Site B marsh, and the Site B fringe. The three ground water solutions were each bubbled with a mixture of SF<sub>6</sub>-O<sub>2</sub>-N<sub>2</sub> (100 mg L<sup>-1</sup> SF<sub>6</sub>, 2 mg L<sup>-1</sup> O<sub>2</sub>, balanced in N<sub>2</sub>; unanalyzed mixture in portable cylinder; Matheson Trigas, Gloucester, MA) to saturate the solutions with SF<sub>6</sub> (approximately 20 min per solution). These amended ground water solutions were pushed into the same mini-piezometer via a peristaltic pump. The amended dosing solution was sampled during the push phase to obtain the undiluted concentration of SF<sub>6</sub> (C<sub>0</sub>). The plume was left in the ground for at least the same incubation period we expected to use in our in situ nitrate push-pull test. After the incubation period, we pulled two to three times the dosing volume, taking samples at 1- to 6-L intervals. We analyzed gas extracted from ground water (method described below) for SF<sub>6</sub> and determined the recovery of this tracer at each sampled interval.

We selected 10 L as our injected ground water volume for

experimental and logistical reasons. Experimentally, 10 L of ground water interacts with a large volume of aquifer material, around 44 kg of soil (bulk density = 1.65 g cm<sup>-3</sup>, porosity = 0.38). This injection volume also helps to minimize dilution in the plume core. Logistically, 10 L of ground water solution is relatively easy to transport into and out of remote sites and can be pushed into the wells in a reasonable time period even with low pumping rates.

After at least 2 wk, we resampled the pretested mini-piezometers and analyzed for SF<sub>6</sub> to ensure that tracer concentrations were at ambient levels before conducting another pretest with a shorter incubation period if the original pretest recovery was poor or before conducting the in situ nitrate push-pull test. If SF<sub>6</sub> concentrations were still above ambient levels, we extracted additional volumes of water or waited additional time until ambient concentrations were found. At Site A, we conducted 120-h and 72-h incubated SF<sub>6</sub> pretests in May and November of 1999, respectively. At Site B, we conducted a 24-h and 5-h incubated SF<sub>6</sub> pretest in June and July of 2000, respectively.

### In Situ Nitrate Push-Pull Test

We conducted in situ nitrate push-pull tests at Site A in November 1999 and Site B in October 2000. To prepare for the in situ nitrate push-pull tests, we collected bulk quantities of ground water from one mini-piezometer at Site A, the Site B marsh, and the Site B fringe. Ground water was stored at 4°C (maximum of 2-wk storage) until the push-pull test. Each dosing solution (10 L per mini-piezometer) at Site A consisted of ambient ground water enriched with 32 mg L<sup>-1</sup> Br<sup>-</sup> (as KBr) and 32 mg L<sup>-1</sup> isotopically enriched (20 atom % <sup>15</sup>N) NO<sub>3</sub><sup>-</sup>-N (as KNO<sub>3</sub>-N). Site B dosing solutions were similar, except they did not contain Br<sup>-</sup> because high ambient Br<sup>-</sup> concentrations in the brackish ground water limited its usefulness as a tracer here.

Prior to injection, we bubbled the SF<sub>6</sub> mixture into the dosing solution to saturate the solution with SF<sub>6</sub> and lower the DO to ambient levels (approximately 20 min per solution). We then capped the carboy, filled its headspace with the SF<sub>6</sub> gas mixture, and sealed its vents for transport to the study site. Alternatively, a gas-impermeable bag could have been used to collect ground water, receive the enriched solution, and reinject the ground water solution without exposing it to the atmosphere (Smith et al., 1991, 1996). We found that the carboy setup facilitated the use of the SF<sub>6</sub> gas tracer.

The 10-L dosing solutions were pushed into mini-piezometers over the course of an hour with the peristaltic pump at very low rates (10 to 12 L h<sup>-1</sup>) to minimize changes in the hydraulic potential surrounding the mini-piezometer. The dos-

ing solution carboy was maintained under constant pressure through connection to the SF<sub>6</sub> cylinder. A small quantity of the dosing solution (targeted 500 mL and measured later in the lab) was left at the bottom of the carboy to measure DO and ensure that the DO content remained stable. Based on the pretest results, the incubation period was set at 72 h at Site A and 5 h at Site B. At Site B, the incubation period occurred in the period approximately 2 h before low tide to 3 h after low tide, when Site B was not inundated with tidal water. After the incubation period, we pulled 18 L of ground water from each mini-piezometer. We pumped ground water from the mini-piezometers slowly (9 to 13 L h<sup>-1</sup>) to avoid generating gas bubbles within the tubing. We collected ground water samples at periodic intervals throughout the pull and push phases. Dissolved gases were extracted from ground water samples as described below. All ground water samples were stored at 4°C until analysis.

### Conservative Tracer Recovery Estimates

For each mini-piezometer, we calculated the recovery or  $C/C_o$  of the conservative tracers where  $C$  was the pulled ground water concentration following incubation and  $C_o$  was the original pushed ground water concentration (Freeze and Cherry, 1979). Relative concentration profiles were created by plotting the  $C/C_o$  versus the normalized plume volume (cumulative pulled volume when the sample was collected/total pushed volume).

### Gas Extraction from Ground Water

To sample for N<sub>2</sub>, N<sub>2</sub>O, and SF<sub>6</sub> gases in ambient, pushed, and pulled samples, we used the phase equilibration headspace extraction technique (Lemon, 1981; Davidson and Firestone, 1988). We collected ground water samples with a syringe attached to an air-tight sampling apparatus made of stainless steel tubing connected to the peristaltic pump. These ground water samples were injected into an evacuated serum bottle and the headspace was filled with high-purity argon gas. After incubating overnight at 4°C and shaking, we sampled the bottle headspace to extract SF<sub>6</sub> and gases produced by denitrifying microbes (N<sub>2</sub> and N<sub>2</sub>O).

### Denitrification Rate Calculations

Only samples taken from the plume core (i.e., first 2 L extracted in the pull phase with tracer recovery > 80%) were used in denitrification rate calculations. To calculate the masses of N<sub>2</sub>O-N and N<sub>2</sub> gases (μg) in our headspace extraction samples, we used equations and constants provided by Tiedje (1982) and Mosier and Klemetsson (1994). The mass of N<sub>2</sub>O-N or N<sub>2</sub> was transformed to the mass of <sup>15</sup>N<sub>2</sub>O-N or <sup>15</sup>N<sub>2</sub> by multiplying it by the respective <sup>15</sup>N sample enrichment proportion (ratio of pulled atom % of the dissolved N<sub>2</sub> and N<sub>2</sub>O-N to pushed NO<sub>3</sub><sup>-</sup>-N atom %, both corrected for ambient atom %). Sample <sup>15</sup>N<sub>2</sub>O-N and <sup>15</sup>N<sub>2</sub> gas production rates were expressed as μg N kg<sup>-1</sup> d<sup>-1</sup> (total mass of <sup>15</sup>N<sub>2</sub>O-N or <sup>15</sup>N<sub>2</sub> per volume of water pulled/[dry mass of soil per volume of water pulled × incubation period]).

Each pulled sample represented 1 L of ground water that occupied 4.37 kg of soil (bulk density = 1.65 g cm<sup>-3</sup>, porosity = 0.38). The incubation period was defined as the length of time between the end of the push phase and the start of the pull phase since the plume core would consist mostly of the later injected ground water. Denitrification rates were the sum of <sup>15</sup>N<sub>2</sub>O-N and <sup>15</sup>N<sub>2</sub> generation rates. Denitrification rates may be underestimated since we did not measure NO<sub>2</sub><sup>-</sup> and NO, other intermediates of the denitrification process.

All samples used in denitrification calculations contained at least 2 mg L<sup>-1</sup> NO<sub>3</sub><sup>-</sup>-N to ensure that our denitrification rate estimates were not limited by the amount of nitrate available (Schipper and Vojvodic-Vukovic, 1998).

Another option to quantify ground water nitrate transformations was to generate ground water nitrate removal estimates based on differences between Br<sup>-</sup> and NO<sub>3</sub><sup>-</sup>-N concentrations. However, at the relatively short incubation periods used in the push-pull design, notable rates of nitrate removal (i.e., 5–15 μg N kg<sup>-1</sup> d<sup>-1</sup>) require Br<sup>-</sup> and NO<sub>3</sub><sup>-</sup>-N concentrations at resolutions at the 0.1 mg L<sup>-1</sup> level. Therefore, we chose to estimate only denitrification rates from our samples. Denitrification rates are derived from the total concentration of <sup>15</sup>N<sub>2</sub>O-N and <sup>15</sup>N<sub>2</sub> gases obtained through mass spectrometer analysis and were of finer resolution (at the μg L<sup>-1</sup> level) than Br<sup>-</sup> and NO<sub>3</sub><sup>-</sup>-N data (at the 0.5 mg L<sup>-1</sup> level) obtained from ion chromatography.

### Analytical Methods

Ground water DO and temperature were measured with a YSI Model 55 DO/temperature meter (YSI, Yellow Springs, OH). Ground water samples were analyzed for NO<sub>3</sub><sup>-</sup>-N and Br<sup>-</sup> (detection limit: 0.2 mg L<sup>-1</sup>) on a DX-120 ion chromatograph (Dionex, Sunnyvale, CA), for dissolved organic carbon by infrared analysis using an O.I. Corporation (College Station, TX) Model 1010 carbon analyzer, for pH on an Accumet Model 925 pH meter (Fisher Scientific, Pittsburgh, PA), and for salinity on a YSI Model 30 salinity/conductivity/temperature meter. Concentrations and isotopic composition of N<sub>2</sub> and N<sub>2</sub>O gases were determined on a dual inlet isotope ratio mass spectrometer (Stable Isotope Facility, UC Davis, Davis, CA) as described by Mosier and Schimel (1993). Concentrations of N<sub>2</sub>O and SF<sub>6</sub> gases were analyzed by electron-capture gas chromatography (Tracor [Houston, TX] 540). Soil texture was determined by dry sieve analysis (Troeh and Thompson, 1993).

### Statistical Analyses

Paired  $t$  tests (Ott, 1993) were performed to determine significant differences in (i) recovery ( $C/C_o$ ) of SF<sub>6</sub> within the plume core between different incubation periods in each mini-piezometer and (ii) recovery ( $C/C_o$ ) between Br<sup>-</sup> and SF<sub>6</sub> in Site A mini-piezometers. Mann-Whitney  $U$  tests (Ott, 1993) were performed to determine significant differences in (i) denitrification rates observed at Site A and those determined in the Addy et al. (1999) mesocosm study and (ii) denitrification rates at the marsh and fringe locations at Site B. All statistical analyses were performed on Statistica for Windows (StatSoft, 1999).

## RESULTS AND DISCUSSION

### Recovery of Conservative Tracer: Pretest

Selecting an appropriate incubation period based on hydrologic properties of specific sites was a critical component of the push-pull mini-piezometer method. At both sites, we found significantly higher recovery ( $p < 0.01$ ) of the pretest tracer SF<sub>6</sub> in the plume core using the shorter incubation period. With the 120-h incubated pretest at Site A, the highest recovery of SF<sub>6</sub> was only 28% (Fig. 2). With the 72-h incubated pretest at Site A, recovery of SF<sub>6</sub> improved to >80% in the plume core (Fig. 2). At Site B, we recovered no SF<sub>6</sub> after the 24-h incubation, but SF<sub>6</sub> recovery in the 5-h incubation

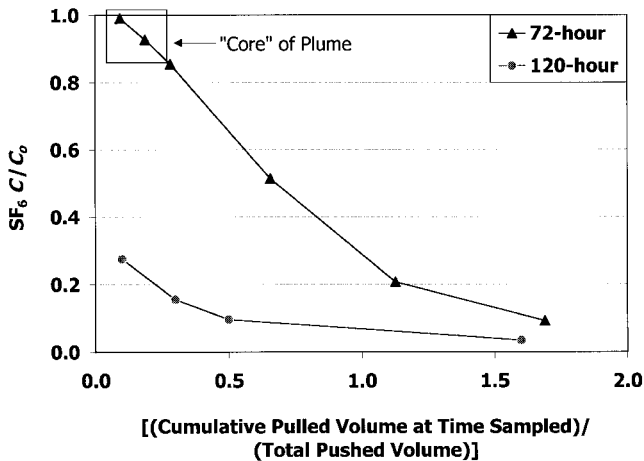


Fig. 2. Relative concentration profiles of  $\text{SF}_6$  from the 72- and 120-h conservative tracer push-pull pretests at Site A. The term  $C$  represents the concentration of the sample pulled from the mini-piezometer. The term  $C_0$  represents the concentration of the dosing solution originally pushed into the mini-piezometer.

(data not shown) was comparable with the 72-h incubated pretest results at Site A. Clearly, the 72-h incubation at Site A and the 5-h incubation at Site B were well suited to determine denitrification rates with minimal dilution influences on the introduced plume.

A variety of site factors, including hydraulic conductivity and hydraulic gradient, can affect the extent of plume displacement and dilution via advection and dispersion. These site factors can require rigorous field effort to elucidate and incorporate into sampling designs. Conducting a push-pull tracer pretest was an efficient approach to estimate the effects of advection and dispersion on an introduced plume. By modifying incubation period length in a series of  $\text{SF}_6$  pretests, we obtained high recovery of the tracer in the plume core at both sites. The range in incubation periods required to generate similar plume recoveries suggests substantial variation in hydrologic factors between sites; however, the push-pull mini-piezometer method should be equally effective in determining denitrification rates at both sites as long as we have high plume recovery. Since Site B was tidally influenced, there was unusually high hydraulic gradient around low tide, indicating the need for shorter incubation periods.

With the appropriate incubation period, the in situ push-pull mini-piezometer method should be effective at characterizing ground water nitrate dynamics at a range of sites. Further exploration of this method is needed at heterogeneous sites. When we pretested Sites A and B, we only used one mini-piezometer per location for characterization since the soil was fairly uniform within sites. However, at sites with less homogeneous soils, multiple mini-piezometers may need to be pretested with conservative tracers to determine the appropriate incubation period for each specific location.

#### Recovery of Conservative Tracers: Nitrate Push-Pull Tests

In the nitrate push-pull tests, tracer recovery in the plume core of all mini-piezometers at both Sites A and

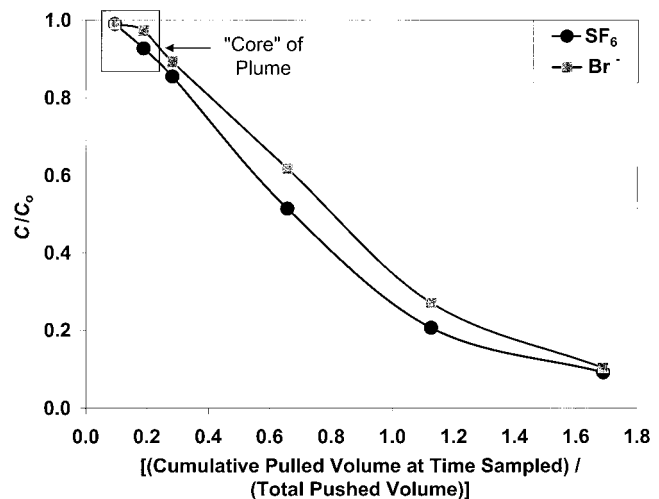


Fig. 3. Relative concentration profiles of conservative tracers ( $\text{SF}_6$  and  $\text{Br}^-$ ) from the 72-h in situ nitrate push-pull test in one Site A mini-piezometer. The term  $C$  represents the concentration of the sample pulled from the mini-piezometer. The term  $C_0$  represents the concentration of the dosing solution originally pushed into the mini-piezometer.

B exceeded 80%, indicating minimal loss due to physical processes. Tracer concentration in the pulled samples dropped steadily after the first 2 L extracted. Concentrations approached ambient levels after we extracted close to two dosing volumes from mini-piezometers. Within each mini-piezometer at Site A, the relative concentration profiles of  $\text{Br}^-$  and  $\text{SF}_6$  were very similar (Fig. 3). In each mini-piezometer at Site A,  $\text{Br}^-$  recovery was not significantly different from  $\text{SF}_6$  recovery. The difference in  $\text{Br}^-$  and  $\text{SF}_6$  recovery at each point of measurement within the plume never exceeded 10% in any mini-piezometer.

Bromide has been used as the conservative tracer in many riparian ground water nitrate studies (Simmons et al., 1992; Nelson et al., 1995; Starr et al., 1996), but its value as a tracer may be compromised if it is subjected to plant uptake. Sulfur hexafluoride is a gaseous tracer that has been found to behave conservatively in sandy aquifers (Wilson and Mackay, 1993, 1996). Sulfur hexafluoride is not suspected of plant uptake and can be used in situations where high ambient ion concentrations confound the use of  $\text{Cl}^-$  and  $\text{Br}^-$ , as in tidal riparian areas; however, its value as a conservative ground water tracer could potentially be compromised by degassing. The similarities in the high recoveries of both  $\text{Br}^-$  and  $\text{SF}_6$  in the plume core demonstrated that this portion of the plume was not substantially altered by physical or biological processes, enhancing our confidence in denitrification estimates based on  $^{15}\text{N}$ -enriched gas in samples from this portion of the plume.

#### Denitrification Rates: Nitrate Push-Pull Tests

The in situ nitrate push-pull test detected substantial denitrification rates at Site A, where we had previously observed high denitrification rates (Addy et al., 1999). However, denitrification rates obtained from the in situ nitrate push-pull test (mean =  $96.7 \mu\text{g N kg}^{-1} \text{d}^{-1}$ , SE =

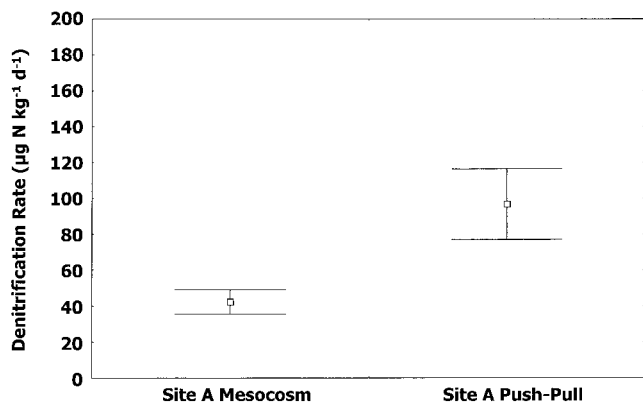


Fig. 4. Ground water denitrification rates from the in situ nitrate push-pull test conducted at Site A. Values for mesocosm data are the mean (SE) of three replicate mesocosms (Addy et al., 1999) and values for push-pull data are the mean (SE) of three replicate mini-piezometers.

19.7) were significantly greater ( $p < 0.05$  level) than those found by Addy et al. (1999) using mesocosms from the same depth (Fig. 4). The variation in denitrification rates between replicates was comparable with results obtained from other mesocosm and in situ dosing-well studies (Nelson et al., 1995; Gold et al., 1998; Addy et al., 1999).

The higher denitrification rates detected with this in situ method compared with mesocosms taken from the same location (Addy et al., 1999) may have resulted from seasonality and labile carbon limitations within the Addy et al. (1999) study. In a long-term in situ study at a similar riparian site in Rhode Island, Nelson et al. (1995) found significantly higher rates of ground water nitrate removal in November, the time of our in situ nitrate push-pull test, than in June, when Addy et al. (1999) collected mesocosms. Nelson et al. (1995) speculated that root turnover in the autumn might enhance denitrification rates at that period. In addition, the mesocosms may have underestimated daily denitrification rates since the results were generated from a closed system over a 50-d period and the labile carbon supply may have dwindled over the course of the study. Hot-spots of microbial activity may persist for only days to weeks (Christensen et al., 1990b) but are constantly replenished in natural systems (Christensen et al., 1990a). In our in situ nitrate push-pull test, incubation periods were relatively brief and capable of exploiting recurring pools of labile carbon that result from an intact plant-soil-hydrologic system.

In situations with low ground water nitrate removal rates and relatively brief incubation periods (i.e., less than 24 h), the resolution of ion chromatograph methods may obscure direct comparison of nitrate removal estimates based on changes in  $\text{Br}^-$  to  $\text{NO}_3^-$ -N ratios with denitrification rates derived from  $^{15}\text{N}$ -enriched  $\text{N}_2$  and  $\text{N}_2\text{O}$ , as mentioned earlier in the Methods. However, based on our 72-h incubations at Site A, we found that in situ push-pull estimates of denitrification rates agreed closely with mass balance estimates of nitrate removal corrected for dilution. The mean denitrification rate at Site A was  $96.7 \mu\text{g N kg}^{-1} \text{d}^{-1}$ , equivalent to a change

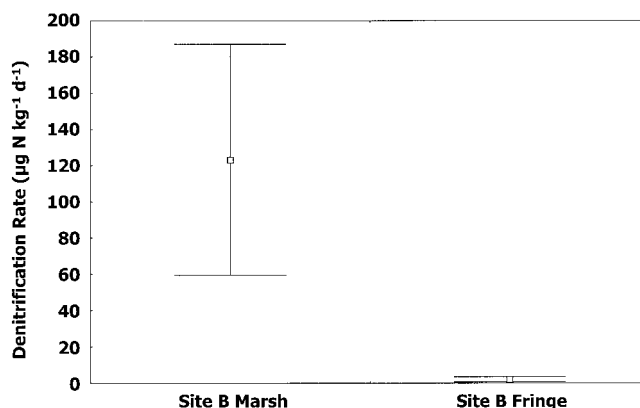


Fig. 5. Ground water denitrification rates from the in situ nitrate push-pull test conducted at Site B. Values are the mean (SE) of four marsh and three fringe mini-piezometers.

in concentration of  $1.3 \text{ mg NO}_3^- \text{N L}^{-1}$  over the 72-h incubation. This value is near the observed changes in mean  $\text{NO}_3^- \text{N}$  concentration within the plume core of those Site A mini-piezometers, ranging from 1.4 to 1.9  $\text{mg N L}^{-1}$ . The discrepancy could result from losses due to other removal processes, such as immobilization, dissimilatory nitrate reduction to ammonium, or plant uptake, and from differences in the precision of the different analytical procedures.

At Site B, we found significantly higher denitrification rates (Fig. 5;  $p < 0.05$ ) in marsh mini-piezometers (mean =  $123.2 \mu\text{g N kg}^{-1} \text{d}^{-1}$ , SE = 63.8) than in fringe mini-piezometers (mean =  $2.1 \mu\text{g N kg}^{-1} \text{d}^{-1}$ , SE = 1.4). These results are in accordance with the difference in ground water denitrification rates expected for these types of ecosystems.

### Potential Confounding Factors

Several factors could confound the denitrification rate estimates from  $^{15}\text{N}$  gas generation in the push-pull mini-piezometer method: (i) dilution of denitrification gases, (ii) degassing from ground water to soil air, and (iii) a lag time between dosing and microbial response. However, the specific set of conditions associated with the push-pull mini-piezometer method suggests that the method is quite robust and likely to yield useful results over a range of conditions.

During incubation, ground water velocity contributes to displacement and dilution through advection and dispersion, while concentration gradients contribute through molecular diffusion (Freeze and Cherry, 1979). Because it is extremely difficult to directly measure these processes and the physical factors governing them, we rely on conservative tracers to characterize their effects. At the beginning of the incubation period, we assume no concentration gradient of the denitrification gases,  $\text{N}_2$  and  $\text{N}_2\text{O}$ , within the injected plume. As incubation and denitrification progress, these gases increase within the plume and are subject to the same processes governing tracer dilution.

The second factor that could contribute to the loss of denitrification gases before sampling is the movement of dissolved gases from the ground water to the air (i.e.,

degassing). While minor amounts of degassing may have occurred, the excellent agreement between SF<sub>6</sub> and Br<sup>-</sup> recoveries suggests that degassing is not a major process affecting our results (Fig. 3). For degassing to occur, gases must first move vertically upward from the introduced plume to the air–water interface. Assuming no vertical ground water velocity component at the mini-piezometer tip, degassing would require that transverse dispersion and molecular diffusion account for the flux of gases to the air–water interface—a highly unlikely occurrence given the combination of brief incubation periods, low transverse dispersivities, and low rate of molecular diffusion in most soils. In addition, the movement of denitrification gases into the soil air is impeded by the partial saturation of the capillary fringe and the slow air exchange through the porous media, thus reducing the concentration difference at the interface that drives degassing. Although the likelihood of degassing is minor, we now use He rather than N<sub>2</sub> to make up the balance of the SF<sub>6</sub> mixture, minimizing N<sub>2</sub> concentration gradients between the plume and the soil air at the start of the incubation.

The third factor that should be considered when interpreting denitrification rates is the possibility of a time lag between dosing a mini-piezometer and the response of the microbial community (Aelion and Shaw, 2000), particularly over short incubation periods and at pristine sites where there is very low ambient nitrate. In these cases, it may be important to conduct multiple in situ nitrate push–pull tests over several weeks at a site to allow the microbial community the opportunity to respond.

### Advantages of the Push–Pull Mini-Piezometer Method

The push–pull mini-piezometer method has many advantages for use in determining rates of in situ ground water nitrate removal in riparian zones:

- (i) Site instrumentation with multiple replicates was relatively easy. We were able to characterize hydrologic properties and quantify ground water denitrification rates at a site within several weeks.
- (ii) This in situ design provided only minimal soil and hydrological disturbance.
- (iii) Our push–pull tests encompassed 8.7 kg (dry weight) of soil, which aggregates microsites of denitrification, providing great advantages over microcosm-based estimates of denitrification.
- (iv) We were able to isolate both <sup>15</sup>N<sub>2</sub>O and <sup>15</sup>N<sub>2</sub> to measure directly in situ denitrification, thus avoiding the use of acetylene. Acetylene is used to quantify denitrification by causing the reaction to terminate at N<sub>2</sub>O (Balderston et al., 1976; Yoshinari and Knowles, 1976; Groffman et al., 1999). However, there can be a number of complications in the use of acetylene, including the inhibition of nitrification, incomplete diffusion of acetylene into active denitrification sites in soil, provision of energy to denitrifiers, and the failure to terminate denitrification at N<sub>2</sub>O under low nitrate conditions (Groffman et al., 1999). All of these potential problems were avoided with the use of <sup>15</sup>N-enriched nitrate.
- (v) We found little evidence of SF<sub>6</sub> degassing even when our introduced plume was within 16 cm of the water table, indicating the usefulness of SF<sub>6</sub> as a ground water conservative tracer in estuarine and fresh water settings.
- (vi) In addition, our gas analysis provided N<sub>2</sub> to N<sub>2</sub>O ratios generated by denitrification, a potentially important finding for researchers interested in greenhouse gases (Groffman et al., 2000).

The push–pull mini-piezometer method can provide useful insights into spatial and temporal patterns of denitrification in riparian zones. In conjunction with measurements of ground water flowpaths (Devito et al., 2000; Hill et al., 2000), this method holds promise for establishing the role of riparian zones in the flux of nitrate within watersheds.

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