Patchiness in Microbial Nitrogen Transformations in Groundwater in a Riparian Forest

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ABSTRACT

We measured microbial N transformations in 15 cm diam. by 40 cm intact horizontal sections of aquifer material (mesocosms), taken from a riparian forest in Rhode Island, USA, incubated under ambient conditions. The mesocosms allowed us to measure these transformations on the same scale as hydrologic tracer methods (Br^-/NO_3^- ratios) that measure net NO_3^- removal. Our objective was to reconcile discrepancies between hydrologic tracer and microbial measurements in previous studies where laboratory-based microbial NO_3^- consumption measurements were much lower than in situ hydrologic measurements of net NO_3^- removal. We hypothesized that small "patches" of organic matter in the aquifer matrix, which are easily missed when sampling for microbial measurements, are "hotspots" of NO_3^- removal and are responsible for these discrepancies. Mesocosms were subjected to three treatments [Br^- only, $Br^- + {}^{15}NO_3^-$, $Br^- + {}^{15}NO_3^-$ + dissolved organic carbon (DOC)]. Solution (NH_4^+, NO_3^-, dissolved organic N) and gaseous (N_2O, $^{15}N_2O$, and $^{15}N_2$) inputs and outputs to the mesocosms were measured over a 132-d incubation, followed by destructive sampling for the presence of patches and residual ^{15}N in aquifer matrix and patch material. Total (gross) NO_3^- consumption by denitrification and immobilization was greater than net removal of NO_3^- measured by Br^-/NO_3^- ratios. Net NO_3^- consumption was only observed in mesocosms that contained "patches" of organic matter and was not increased by addition of DOC, suggesting that these patches, which represent <1% of aquifer weight, are critical to groundwater NO_3^- removal in riparian forests.

MICROBIAL transformations in groundwater are a topic of great interest and uncertainty in environmental science. While there is great interest in the fate of a variety of pollutants in groundwater, it has proven difficult to sample and characterize the microbial communities capable of transforming these pollutants (Madsen, 1995). This problem is particularly acute for NO_3^-, a pollutant that is exceptionally widespread, highly amenable to biological removal, and of great concern as a drinking water contaminant and agent of coastal ecosystem eutrophication (Keeney, 1986; Howarth et al., 1996).

The fate and transport of NO_3^- in groundwater has been particularly well studied in riparian forests. Many studies have shown that these ecosystems have the ability to prevent the movement of groundwater NO_3^- from agricultural or residential upland areas into streams (Hill, 1996). However, there is considerable uncertainty as to the mechanism of NO_3^- removal from groundwater in these studies. Several studies have found that laboratory-based measurements of microbial processes that can remove NO_3^- (e.g., denitrification and immobilization) were too low to account for the amount of NO_3^- removal observed in situ in groundwater monitoring well networks (Cooper, 1990; Ambus and Lowrance, 1991; Lowrance, 1992; Groffman et al., 1992, 1996; Correll 1997; Gilliam et al., 1997; Verchot et al. 1997). These results are particularly intriguing because NO_3^- removal from riparian groundwater appears to go on throughout the year, including periods when plant uptake is not likely to be an important mechanism of NO_3^- removal (Simmons et al., 1992; Nelson et al., 1995).

The discrepancy between laboratory and field-based studies of groundwater NO_3^- dynamics is exacerbated by differences in the approach and temporal and spatial scale of the laboratory-based microbiological and field-based hydrology measurements (Smith et al., 1996). In situ groundwater monitoring well networks measure NO_3^- removal over relatively large spatial (meters of riparian zone width, kg of soil) and temporal (days, weeks, and months) scales relative to laboratory-based microbiology measurements (g of soil, hours). We suggest that these methodological differences create two problems. First, sampling for laboratory microbial measurements that require only small amounts of soil may miss "patches" or "hotspots" of organic matter and microbial activity in the subsurface. Such patches have been shown to be important for surface soil denitrification (Parkin, 1987; Christensen et al., 1990) and we hypothesize here that they are critical for groundwater denitrification as well. Second, it is important to recognize that very low rates of denitrification can remove large amounts of NO_3^- if groundwater is moving very slowly through a large volume of material, as is frequently the case in riparian forests. Such low denitrification rates are often not detectable in short-term incubations of small amounts of groundwater material (which often has very low levels of organic matter and microbial biomass and activity) in the laboratory.

In this study, we attempted to reconcile the differences between field and laboratory-based studies of groundwater NO_3^- removal by carrying out microbial and hydrologic measurements at one, intermediate (mesocosm) scale. Large (15 cm diam. by 40 cm long) intact horizontal sections of aquifer material were used as groundwater "mesocosms". Rates of NO_3^- removal were measured using standard hydrologic tracer techniques (i.e., NO_3^-/Br^- ratios) and denitrification and immobilization were measured using highly sensitive stable N isotope techniques. After 130 d of incubation, the mesocosms were dissected to examine the importance of "patches" as "hotspots" of microbial activity. Construction of, and net groundwater NO_3^- removal in, the mesocosms is described in a companion paper (Gold et al., 1998), while this paper describes the microbial

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Abbreviations: MWD, moderately well drained; PD, poorly drained; DO, dissolved oxygen; DOC, dissolved organic carbon; DON, dissolved organic nitrogen.

analysis. The specific objectives of the work described in this paper were: (i) to quantify rates of denitrification and immobilization activity in groundwater mesocosms and (ii) to determine the importance of subsurface patches of organic matter to this activity.

MATERIALS AND METHODS

Collection of Cores and Instrumentation

Undisturbed cores (40 cm long, 15 cm diam.) of aquifer material were obtained from two locations within a riparian forest near the campus of the University of Rhode Island (Kingston, RI). Cores were taken from beneath a moderately well drained (MWD) soil at the upland/wetland transition zone end of the riparian forest and in a poorly drained (PD) soil in a wetland section of the riparian zone. Soils in this riparian zone are coarse-textured Endoaquepts derived from glaciofluvial deposits of structureless, granitic sands and loamy sands with an average slope of 3%. Water table depth during the dormant season (November–April) ranged from 20 to 40 cm in the PD soil and from 90 to 100 cm in the MWD soil. Vegetation in the PD section of the riparian zone was dominated by 60- to 80-yr-old red maple (*Acer rubrum*) while the MWD section was dominated by a mixture of red maple and oak (*Quercus*) sp.

Core extraction procedures are described in detail in the companion paper (Gold et al., 1998). Briefly, three pits were excavated in July 1996 to depths corresponding to the lowest water table elevation that occurs each year in each soil type in the riparian zone. To obtain undisturbed horizontal sections of the aquifer material, PVC pipes were pushed horizontally along an exposed side of the pit using a hydraulic jack. The mean sampling depth was 61 cm for the PD cores and 155 cm for the MWD cores. Once collected, cores were stored at 4°C in a cold room. To maintain the soil material in place, and to ensure the structural integrity of the cores, a clean, perforated fiberglass disc was placed directly in contact with the soil inside the PVC pipe. At both ends of the cores, PVC endcaps were installed and fitted with plastic connectors to serve as inlet and outlet ports. The top endcap was fitted with a threaded male connector that allowed insertion of a 1.2 cm diam. probe used for dissolved oxygen (DO) measurement (Fig. 1).

Description of Treatments

A noncirculating continuous flow-through system (Fig. 1) was designed to measure rates of NO_3^- removal under conditions that closely simulated the ambient groundwater environment (temperature, natural groundwater, and oxygen). Groundwater collected from the aquifer at the PD and MWD locations within the riparian forest was transferred into 10-L polypropylene "carboys", and purged with Ar and Ar–O_2 mixtures to yield DO concentrations of 2 and 5 mg L^{-1}, respectively. These values represent DO concentrations at the PD and MWD locations in the aquifer measured in a previous field study (Nelson et al., 1995). Water in the carboys was amended with either: bromide (Br^-) only (control), Br^- + NO_3^- or Br^- + NO_3^- + DOC. Bromide and NO_3^- concentrations were 10 mg L^{-1} each (Br^-/NO_3^- ratio, 1:1). The NO_3^- source (KNO_3, 99 atom %^{15}N) was purchased from Isotech, Inc. (Miamisburg, OH). The final ^{15}N content of the solution entering the cores was between 94 and 98 atom% due to dilution with NO_3^- originally present in the groundwater. Dissolved organic carbon was obtained by water extraction of forest floor (0–15 cm depth) material. A suspension was prepared and allowed to decant overnight (4°C). The supernatant

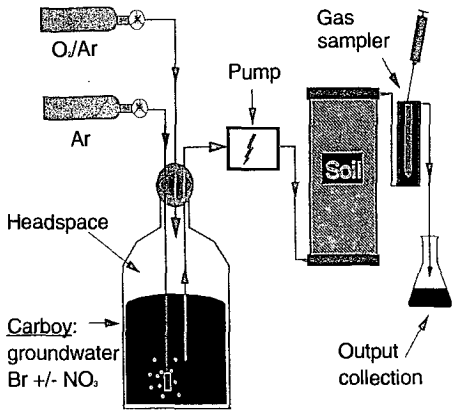

Fig. 1. Schematic of mesocosm dosing system showing oxygen-regulating system for the groundwater input solutions, carboy dosing reservoir and samplers for gas and solution outputs.

was filtered by successive passage through Whatman GF/A (1.6 μm) and GF/F (0.7 μm) filters. An aliquot of the filtrate was analyzed for DOC content, and an appropriate volume of DOC extract was added to the carboys to achieve a target concentration of 20 mg C L^{-1}. This concentration was based on data on forest floor DOC concentrations in the literature (Qualls and Haines, 1991; Guggenberger and Zech, 1993). There were three replicates of each treatment for a total of 18 cores.

After addition of amendments to the carboys, the DO of the solution was adjusted by bubbling with high-purity Ar. When the desired DO levels were attained, the carboys were capped and their headspace was flushed for 3 min with an Ar–O_2 mixture at a rate of ≈350 mL min^{-1}. The experiment was carried out in a temperature-controlled room set at 11°C, which is the groundwater temperature at the field site in November. At this temperature, to maintain the targeted DO levels of 5 and 2 mg O_2 L^{-1} in the MWD and PD cores, O_2 partial pressure in the carboys headspace was set at 12.9 and 4.7 kPa, respectively (Stumm and Morgan, 1981). To avoid creation of a vacuum in the carboys as solution was pumped into the cores, and to maintain a constant dissolved gas concentration in the carboy headspace, an Ar–O_2 mixture was continuously supplied (Fig. 1). Delivery rate of the gas mixture to the carboys was ≈0.5 mL min^{-1}, and was controlled by a flowmeter (Aalborg Instruments, Monsey, NY). Dissolved O_2 level in the carboys was measured every 1 to 2 d and adjusted when needed. Overall, DO concentrations remained fairly stable in the carboys, averaging 5.4 and 2.2 mg L^{-1} in the carboys feeding the MWD and PD cores, respectively. Once or twice a week, carboy headspace was sampled to measure the concentrations of CO_2 and N_2O originally present in solution and to determine the extent of microbial activity in the carboys.

Solutions from the carboys were pumped into the cores with an Ismatec multichannel peristaltic pump (Cole Parmer, IL) at a rate of 170 mL^{-1} d^{-1} to simulate actual rates of groundwater flow at the field site (Nelson et al., 1995; Gold et al., 1998). Solution entered the bottom of the cores and effluent was collected in acid-washed mason jars. Three times a week, effluent volume was recorded and liquid samples were collected. Samples were stored at 4°C until analyzed.

Solutions were analyzed for NO_3^-, NO_2^-, and Br^- on a Dionex 500 ion chromatograph (Dionex, Sunnyville, CA) equipped with an AS4A column. The mobile phase (flow rate, 2 mL min^{-1}) was made of Na_2CO_3 (1.8 m*M*) and $NaHCO_3$ (1.7 m*M*). Effluent NH_4^+ concentrations were analyzed on a Perstorp (Perstorp Analytical, Silver Spring, MD) autoana-

lyzer using the salicylate–hypochlorite method. The concentration of NO_3^- + NO_2^- is reported as NO_3^- because NO_2^- concentration was always <0.1 mg N L^{-1}. Liquid samples were also analyzed for dissolved organic N (DON) using the persulfate digestion procedure described by Cabrera and Beare (1993).

Isotopic composition of the inorganic N (NO_3^- + NO_2^-–N, NH_4^+–N) present in liquid samples was obtained using the microdiffusion method described by Saghir et al. (1993) and Mulvaney et al. (1997). Isotope analysis was done by the N-15 Service of the University of Illinois on a Nuclide mass spectrometer.

Dissolved Gas Measurement

A sampling port located on top of the cores (Fig. 1) allowed direct measurement of DO content in the core effluent with a DO-meter (Cole Parmer, Niles, IL). During sampling, the screw cap was removed and the probe was immediately inserted. The concentration of DO was recorded when the reading became stable.

A sampler made of silicone rubber was used to collect dissolved N_2O and N_2. Previous studies have established the permeability of silicone rubber to these gases (Jacinthe and Dick, 1996). Core effluent was conducted into a PVC (15 cm long, 5 cm diam.) enclosure at the center of which was inserted a sampling cell of 2.4 mm thick silicone tubing (Cole-Parmer, IL). The cell was closed at one end with silicone caulking and was fitted, on the outer end, with a plastic reducer and a butyl rubber stopper firmly secured with a screw cap. Nominal volume of the silicone cell thus formed was 20 mL. Core effluent flowed into the PVC enclosure and the gases present in solution diffused through the silicone membrane. The silicone tubing headspace was sampled by syringe, concentrations of N_2O were quantified by electron capture gas chromatography, and dissolved gas concentrations were calculated using constants and equations provided by Stumm and Morgan (1981). A sampling frequency of 2 to 4 d was more than adequate to allow equilibrium between the liquid and the gas phases in the PVC enclosures (Jacinthe and Dick, 1996).

Gas samples were analyzed for $^{15}N_2$ and $^{15}N_2O$ on a dual inlet isotope ratio mass spectrometer designed for isotopic composition determination of both N_2 and N_2O as described by Mosier and Schimel (1993). Each sample was analyzed concurrently with unenriched ultra-high purity N_2 gas used as a standard against which sample mass ratio measurements were compared. The ^{15}N enrichment of the substrate (NO_3^-) undergoing denitrification and of the N gases produced was computed using the equations provided by Mulvaney and Kurtz (1982) and Siegel et al. (1982).

Destructive Sampling

After 132 d of incubation, each core was dissected into 5-cm-long sections. Patches of organic matter were identified and isolated based on color, signs of illuviation and mineral accretion, and the presence of decomposed roots. When present, patch samples were collected with care being taken to minimize mixing with the aquifer matrix. Patch as well as matrix samples were stored in plastic bags at 4°C until used. Within 2 d of their collection, samples were extracted with 2 *M* KCl and analyzed for NO_3^- and NH_4^+. For all extracts, both NO_3^- and NH_4^+ were analyzed on a Perstorp autoanalyzer using the Cd-reduction method for NO_3^- and the salicylate–hypochlorite method for NH_4^+.

Between 30 and 50 mg of finely ground patch or aquifer matrix material was used for total N and ^{15}N content analysis by coupled gas chromatography–isotope ratio mass spectrometery (Isotope Ratio Mass Spectrometry Laboratory, Michigan State University). For some of the soil matrix samples, however, the amount of N present in the 30- to 50-mg samples was too low to yield reliable results. In these cases, total N was determined using a salicylic acid–thiosulfate modification of the Kjeldahl method. For these analyses, aquifer matrix samples (5 g) were digested in H_2SO_4 (18 *M*) and salicylic acid (25 g L^{-1}) at 360°C for 4 h. The digest was distilled and the distillate was collected into dilute acid (0.025 *M* H_2SO_4). A subsample of the distillate was used to determine NH_4^+ content as described above, and another distillate aliquot, containing 50 to 150 μg N, was processed for ^{15}N analysis as described above.

Both patch and aquifer matrix samples were assayed for denitrification potential in a medium of (per liter): 0.2 g KNO_3–N, 0.4 glucose-C, and 0.25 mg chloramphenicol (Smith and Tiedje, 1979). Slurries were made using a soil/medium ratio (v/v) of 1:1 in crimp-sealed serum bottles fitted with butyl rubber septa. Bottles were evacuated and flushed with N_2 gas three times and amended with acetylene (C_2H_2) to obtain an C_2H_2 partial pressure of 10 kPa. Bottles were incubated at room temperature (22°C) on a rotary shaker (110 rpm) for a 24-h period during which gas samples were taken from the bottle headspace for N_2O analysis. Denitrification potential was determined from the rate of N_2O accumulation in the bottle headspace.

Statistical Analysis

Data on N gas production were analyzed using repeated measures analysis of variance with soil type, treatment, and sampling date as main effects using the SAS statistical software (SAS Institute, 1992). Data on soil variables (residual ^{15}N, denitrification potential) were evaluated by analysis of variance with soil type and treatment as main effects. Data were log-transformed before analysis where appropriate. Separate analyses were run for each soil and treatment when interactions were significant. Fisher's protected least significant difference test was used a posteriori to determine differences between specific treatments.

RESULTS

Background Levels of Nutrients in Groundwater

Groundwater used in the experiment was collected as needed during the period October 1995 to March 1996, and variations in background NO_3^- were noted. Concentrations of NO_3^- in groundwater collected in late October averaged 0.3 in the PD and 0.5 mg NO_3^-–N L^{-1} in MWD wells. In subsequent collections (December–March), background NO_3^- levels dropped slightly (<0.2 mg NO_3^-–N L^{-1}) in the PD groundwater, whereas background NO_3^- concentrations in the MWD groundwater remained between 0.1 and 0.4 mg NO_3–N L^{-1}. Isotopic composition of groundwater NO_3^- was slightly higher than natural abundance, averaging 0.379 ± 0.005 atom% in the PD and 0.377 ± 0.005 atom% in the MWD groundwater, respectively.

Dissolved Oxygen Concentrations

During the first 56 d of the experiment, DO concentrations in core effluents were between 4 and 10 mg L^{-1} which was twice the DO levels maintained in the dosing solution reservoirs (i.e., the carboys). Illumination of

the incubation-room occurred only during sampling operations; thus the growth of O_2-producing algae was excluded as an explanation for these high DO levels in core effluents. Examination of the O_2-diffusion characteristics of components of the incubation system showed that the Tygon tubing (Cole Parmer H-06409-16, wall thickness 1.4 mm) used to transport solution from the carboys to the cores was O_2-permeable. On Day 56, the Tygon tubing was replaced with stainless steel tubing. Thereafter, DO concentrations in the core output gradually decreased to closely reflect DO levels in the influent.

Denitrification: Nitrogen Gases Production

Rates of N gas production ($\mu g\ N\ kg^{-1}\ d^{-1}$) were computed using dissolved gas concentrations ($\mu g\ N\ L^{-1}$ water), groundwater flow rates ($L\ d^{-1}$) and the mass of the cores (kg). During the first 10 d of the experiment, N_2O production in the PD cores submitted to the three treatments were of similar magnitude (<2 $\mu g\ N_2O$–N $kg^{-1}\ d^{-1}$) (Fig. 2). After the breakthrough of Br^- and NO_3^- (Day 10; Gold et al., 1998), N_2O production in the NO_3^--amended PD cores increased significantly ($P < 0.05$). Between Days 20 and 30, the highest production rates (up to 24 $\mu g\ N_2O$–N $kg^{-1}\ d^{-1}$) were recorded. These production rates corresponded with relatively high rates of NO_3^- removal as reported by Gold et al. (1998). It is interesting to note that O_2 levels were relatively high during this period. Between Days 20 and 50, production of N_2O was lower ($P < 0.05$) in PD cores receiving NO_3^- + DOC (mean: 5 $\mu g\ N_2O$–N $kg^{-1}\ d^{-1}$) than in cores treated with Br + NO_3^- (12 $\mu g\ N_2O$–N $kg^{-1}\ d^{-1}$). In the MWD cores, N gas production remained very low throughout the experiment and never exceeded 0.4 $\mu g\ N_2O$–N $kg^{-1}\ d^{-1}$. The average rate of N gas production was 0.05 $\mu g\ N_2O$–N $kg^{-1}\ d^{-1}$ in MWD cores receiving DOC and 0.03 $\mu g\ N_2O$–N $kg^{-1}\ d^{-1}$ in those treated with NO_3^- only.

In all soil and treatment combinations, production of N_2 was almost nil during the first 40 d of the experiment (Fig. 3). After better control of DO levels was achieved (Day 56), N_2 production increased in the PD cores (Fig. 3). Production of N_2 in the MWD cores was extremely low and reliable data could be obtained for only a few sampling dates. Due to limited data, statistical analysis of N_2 production was not performed. Maximum rates of N_2 production were recorded during Days 100 to 130 in the PD cores treated with NO_3^- + DOC. However, the average rate of N_2 production (0.3 $\mu g\ N\ kg^{-1}\ d^{-1}$) was only 10% of the total N gas production (3 $\mu g\ N\ kg^{-1}\ d^{-1}$) (Fig. 2 and 3) during that period. Because N_2

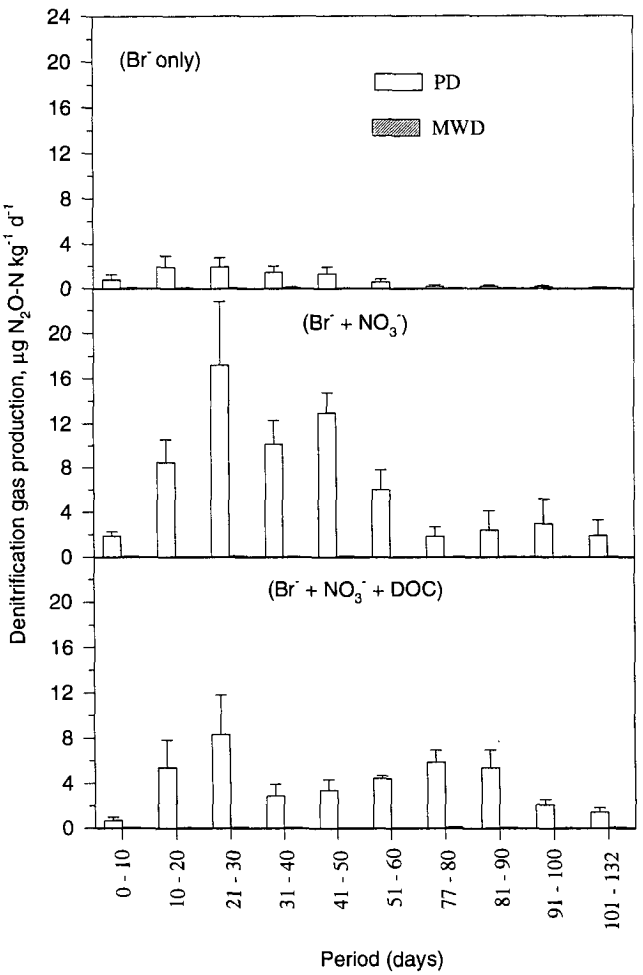

Fig. 2. Denitrification N gas production (N_2O only) from aquifer mesocosms taken from beneath MWD and PD soils in a riparian forest subjected to Br^- only, Br^- + NO_3^-, and Br^- + NO_3^- + DOC treatments. Values are mean (standard error) of three to five samples of three replicate mesocosms per soil per treatment during 10-d periods over a 132-d experiment. Production of N_2O is taken as the rate of denitrification because N_2 production was very low.

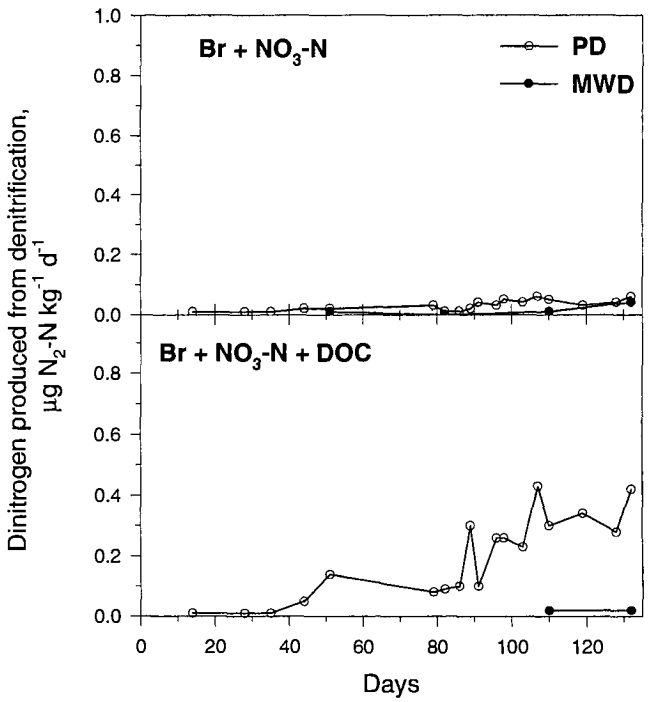

Fig. 3. Dinitrogen production from aquifer mesocosms taken from beneath MWD and PD soils in a riparian forest subjected to Br^- + NO_3^- and Br^- + NO_3^- + DOC treatments. Values represent selected single samples from three replicate mesocosms per soil per treatment sampled at selected dates over a 132-d experiment. The limited number of samples is due to the low ^{15}N content of the gas causing most samples to be below the limit of detection.

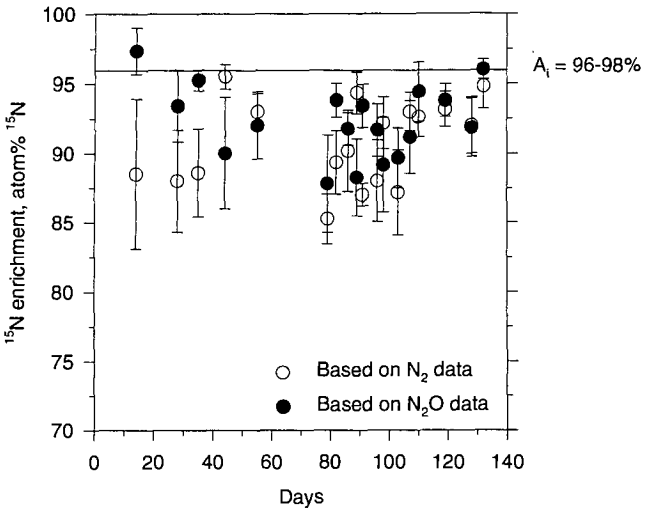

Fig. 4. Nitrogen-15 content of the NO_3^- that was denitrified, derived from isotopic ratios of evolved N_2 and N_2O, in aquifer mesocosms taken from beneath PD soils in a riparian forest subjected to Br^- + NO_3^- and Br^- + NO_3^- + DOC treatments. Values are mean (standard error) of samples from three replicate mesocosms taken at selected dates over a 132-d experiment. A_i is the ^{15}N content of the NO_3^- in the input dosing solution.

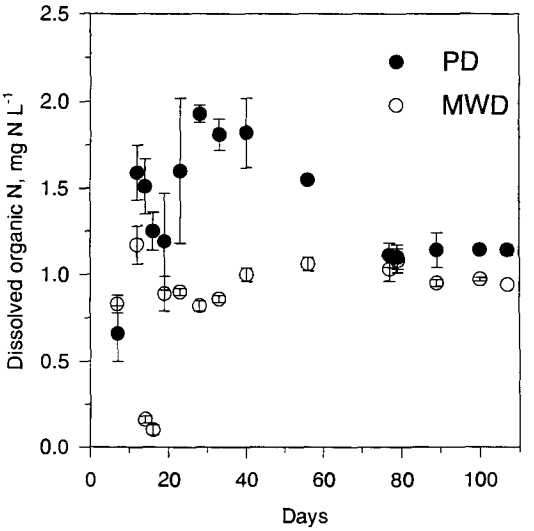

Fig. 5. Dissolved organic N production over a 132-d incubation of aquifer mesocosms taken from beneath MWD and PD soils in a riparian forest subjected to the Br^- only treatment. Values are mean (standard error) of three replicate cores per soil per treatment.

production rates were so low, and because we had much higher temporal resolution in our analysis of N_2O production, we used N_2O production as an estimate of total denitrification N gas production.

Isotopic Composition of Substrate

After effluent Br^- concentrations equaled influent concentrations, complete distribution of the ^{15}N-labeled NO_3^- within the cores could, in theory, be expected. However, production of unlabeled NO_3^- within the cores during incubation, and subsequent mixing of this NO_3^- with the ^{15}N-labeled NO_3^-, was likely. Therefore, the isotopic composition of the NO_3^- actually present at the site of denitrification cannot be inferred directly from that of the input solution. Using procedures outlined in Siegel et al. (1982) and Mulvaney and Kurtz (1982), the isotopic composition of the substrate being denitrified was obtained from the mass ratios of the evolved denitrification gases.

The ^{15}N content of the substrate obtained using isotopic ratios of evolved N_2O was, in general, slightly greater than that derived from data of evolved N_2, however the difference was not statistically significant (Fig. 4). The ^{15}N content of the substrate (NO_3^-) undergoing denitrification was almost always lower than that of the NO_3^- in the inflow, a clear indication of internal NO_3^- production.

Dissolved Organic Nitrogen and Ammonium Release

Both DON (Fig. 5) and NH_4^+ (Fig. 6) release were higher ($P < 0.05$) in the PD cores than in the MWD cores. There were no treatment differences for either DON or NH_4^+. In the cores receiving NO_3^-, DON measurement was limited to only the first 30 d of the experiment because the large background of inorganic N made determination of low levels of DON technically difficult. Therefore, treatment comparisons were possible only for days <30. Overall mean DON concentration in effluents from the PD cores (1.3 mg N L^{-1}) was significantly ($P < 0.05$) higher than in the MWD cores (0.8 mg N L^{-1}). However, DON reached a maximum concentration in the PD cores of 1.9 mg N L^{-1} on Day 28, and then gradually decreased to values comparable (1 mg N L^{-1}) to those recorded in the MWD core effluent.

In the PD core effluents, NH_4^+–N concentrations followed a similar temporal pattern as DON (Fig. 6, top). Ammonium-N concentrations reached a peak of 2.8 mg NH_4^+–N L^{-1} on Day 30 and then dropped sharply. In the MWD cores, NH_4^+–N concentrations showed less variability and were always <0.5 mg NH_4^+–N L^{-1} during that same period. Concentrations of NH_4^+–N in the groundwater added to the columns were always <0.3 mg NH_4^+–N L^{-1} (Fig. 6, bottom) suggesting that NH_4^+ was internally produced in the PD cores. Internal production via mineralization (and not dissimilatory NO_3^- reduction to NH_4^+) is also suggested by the fact that addition of NO_3^- and NO_3^- + DOC had no effect on NH_4^+ concentrations in core effluents.

Immobilization: Residual Nitrogen-15 in Soil

During destructive sampling at the end of the experiment, materials found inside the cores were divided into: soil matrix, illuvial deposits, patches of decomposed roots, and patches of mixed composition. Illuvial patches had no visible root material and appeared to be material that had been transported from elsewhere in the soil profile. Patches were found in all the PD cores and in none of the MWD cores. All patch-related discussion, therefore, pertains to the PD soil. Patch mass in the PD cores ranged from 5 to 170 g (0.04–1.41% of core weight).

Total organic N (TON) was higher ($P < 0.05$) in the PD matrix than in the MWD matrix in all three treatments (Table 1). In both soil types, the TON content of matrix material was lower ($P < 0.05$) in cores

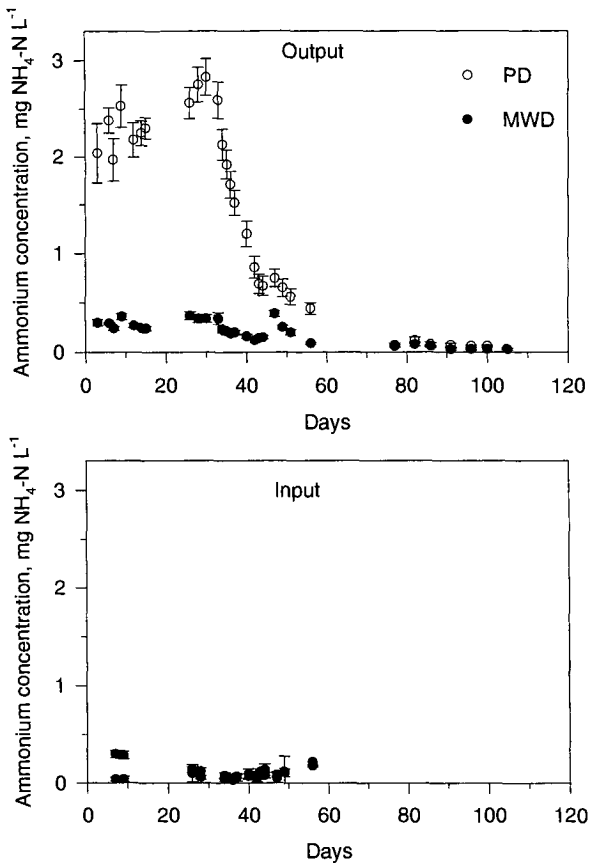

Fig. 6. (*Top*) Production of NH_4^+ over a 132-d incubation of aquifer mesocosms taken from beneath MWD and PD soils in a riparian forest subjected to the Br^- only treatment. Values are mean (standard error) of three replicate cores per soil per treatment. (*Bottom*) NH_4^+ concentrations in the groundwater dosing solution reservoirs (carboys).

that received either NO_3^- or NO_3^- + DOC than in those treated with Br only. Among the patches found in the PD cores, those consisting of decomposed root fragments had the highest ($P < 0.05$) TON content.

Residual organic ^{15}N in the soil matrix was significantly ($P < 0.05$) affected by soil, treatment, and their interactions. Data from the Br^- only treatment provide a measure of background ^{15}N in organic N, which was higher ($P < 0.05$) in the PD matrix than in the MWD matrix. As expected, residual organic ^{15}N above background levels was recorded in cores treated with ^{15}N-labeled NO_3^-. In the PD cores, similar amounts of residual organic ^{15}N were found in the soil matrix of the Br + NO_3^- and Br + NO_3^- + DOC treatments. However, in the MWD cores, DOC treatment caused a significant ($P < 0.05$) increase in residual organic ^{15}N. Residual organic ^{15}N in the patches consisting of decomposed roots was several-fold greater than in the matrix. In the illuvial deposits and the patches of mixed composition, residual organic ^{15}N contents were in the same range as in the soil matrix.

Denitrification Potential

Irrespective of soil type and previous treatment, denitrification activity was low in the soil matrix, averaging 1.9 μg N_2O–N kg^{-1} d^{-1}. Overall, mean denitrification potential in patches was 88 μg N_2O–N kg^{-1} d^{-1} (range 9.8–175 μg N_2O–N kg^{-1} d^{-1}).

Process Summary

The different processes affecting NO_3^- in the cores are summarized in Table 2. This summary is restricted to the Br^- + NO_3^- treatment because this is the only treatment where it was possible to directly compare estimates of NO_3^- removal computed by hydrologic tracer methods (Br^-/NO_3^- ratios) from our companion study (Gold et al., 1998) with the N gas production and immobilization rates computed for this study. Methodological errors ruined the NO_3^-/Br^- ratio data for the Br^- + NO_3^- + DOC treatment.

Total N gas production and total NO_3^- removal from the Br^-/NO_3^- ratio data were calculated by summing

Table 1. Total N, isotopic composition, and residual organic N in soil matrix from moderately well-drained (MWD) and poorly drained (PD) cores and from patch samples from PD cores at the end of the study.

Material	Treatment	N	Total N		^{15}N in total N		Residual organic ^{15}N†	
			Mean	SE	Mean	SE	Mean	SE
			mg N kg^{-1}		atom% ^{15}N		mg ^{15}N kg^{-1}	
Matrix (MWD)	Br^-	24	49a+*	5.2	0.39c+	0.01	0.25c+	0.03
	Br^- + NO_3^-	24	27b+	2.5	2.87b+	0.22	0.74b+	0.12
	Br^- + NO_3^- + DOC	24	26b+	1.8	6.32a+	0.18	1.62a	0.10
Matrix (PD)	Br^-	24	70a	5.8	0.41b	0.00	0.35b	0.02
	Br^- + NO_3^-	24	46b	4.6	4.09a	0.19	1.52a	0.13
	Br^- + NO_3^- + DOC	24	37b	3.3	4.35a	0.13	1.66a	0.16
Illuvial	Br^-	NA‡	NA	NA	NA	NA	NA	NA
	Br^- + NO_3^-	3	86	9.6	2.63	0.02	2.26	0.24
	Br^- + NO_3^- + DOC	6	34	4.8	4.71	0.07	1.59	0.23
Decomposed roots	Br^-	6	3296	331	0.41	0.00	13.62	1.36
	Br^- + NO_3^-	5	5312	797	1.93	0.47	104.5	25.64
	Br^- + NO_3^- + DOC	4	3574	456	1.07	0.04	37.54	4.38
Mixed	Br^-	8	103	16	0.41	0.00	0.43	0.06
	Br^- + NO_3^-	9	118	23	2.67	0.28	2.64	0.15
	Br^- + NO_3^- + DOC	9	112	23	2.75	0.26	2.62	0.26

* Different letters following values (a,b,c) indicate significant ($P < 0.05$) differences among treatments for a given material. A + indicates a significant difference between soil types for a given treatment.

† Residual organic $^{15}N = (N_t)(A_t/100) - (N_i)(A_i/100)$ where N_t and N_i are total N and inorganic N; A_t and A_i represent atom% ^{15}N in total N and in the inorganic pool, respectively.

‡ NA = not available.

Table 2. Total net nitrate (NO_3^-) removal and internal NO_3^- production estimated by Br^-/NO_3^- ratios (from Gold et al., 1998) and total NO_3^- consumption by denitrification and immobilization over the entire 132-d study in the $Br^- + NO_3^-$ treatment of the poorly drained (PD) and moderately well-drained (MWD) aquifer material. Values are mean and standard error (SE) of three replicate cores per soil.

Process	PD		MWD	
	Mean	SE	Mean	SE
	μg N core^{-1} 132 d^{-1}			
Net removal (from Br^-/NO_3^- ratios)	5 924	2 656	−7 351	545
Internal NO_3^- production	7 350	210	2 890	190
Denitrification	8 350	272	59	8
Immobilization-matrix	14 040	2 859	5 600	2 086
Immobilization-POM†	6 370	4 240	0	0

† POM = patches of organic matter.

the 10 d period gas production rates from Fig. 2 in this paper and the 10 d NO_3^- removal rates from our companion paper (Gold et al., 1998). Total immobilization was calculated from the residual organic ^{15}N content of the matrix and patch materials from Table 1. The ^{15}N content of matrix and patch material in the Br^- only treatment were subtracted from the ^{15}N content of material from the $Br^- + NO_3^-$ treatment to account for background levels of ^{15}N.

Total (gross) NO_3^- consumption by denitrification and immobilization was greater than the "net" removal of NO_3^- measured by Br^-/NO_3^- ratios and reported in Gold et al. (1998). The excess consumption was ~23 000 μg in the PD cores and ~13 000 μg in the MWD cores. Internal NO_3^- production averaged 7350 μg N in the PD and 2890 μg^{-1} N in the MWD cores over the course of the study (Gold et al., 1998), which accounts for some of the discrepancy between gross and net consumption. The rest of the difference likely can be accounted for by substitution of ^{15}N for ^{14}N in the organic matter of the matrix and patch materials, that is, gross vs. net immobilization.

DISCUSSION

The use of relatively large, intact horizontal sections of aquifer material as "mesocosms" allowed us to measure net NO_3^- removal using hydrologic tracer (Br^-/NO_3^- ratios) methods and microbial processes that consume NO_3^- on the same scale, at the same time. As a result, we were able to measure more than enough microbial NO_3^- consumption to account for the net NO_3^- removal measured with hydrologic techniques. In previous studies (Simmons et al., 1992; Groffman et al., 1996) we had never been able to reconcile rates of NO_3^- removal measured in groundwater monitoring well networks with rates of microbial NO_3^- consumption measured in laboratory microcosms. The results of this study support our hypothesis that small "patches" of organic matter, which are easily missed when sampling for microcosm construction, are critical "hotspots" of denitrification in subsurface ecosystems. These patches support rates of microbial activity high enough to consume enough O_2 to allow anaerobic processes such as denitrification to occur. The use of mesocosms, which are big enough to include these patches, thus provides a much more accurate depiction of subsurface microbial NO_3^- dynamics than microcosm techniques. Mesocosms also allow for the use of ^{15}N gas flux and flow-through incubation to measure denitrification which are better than static, C_2H_2-based denitrification methods.

The importance of patches as hotspots of microbial activity in the subsurface is shown by two lines of evidence. First, we observed no net NO_3^- removal and very low rates of denitrification and immobilization in the MWD cores, which had no patches. Second, the patch material had very high rates of denitrification potential relative to the aquifer matrix material. It is quite likely that there was no actual denitrification in the matrix material at all. While we were able to measure rates of denitrification potential in the matrix material, they were very low, and it is likely that the anaerobic, C-rich conditions present in our denitrification potential assay are never present in the matrix material. It is quite possible that the N gas that was produced in the MWD cores came from nitrification, an aerobic process. The lack of patches and roots in the MWD cores suggests that NO_3^- may be conservative in groundwater beneath these soils.

While previous studies have shown that patches and hotspots are important to surface soil denitrification (Parkin, 1987; Christensen et al., 1990), they may be especially important in the subsurface due to the very low C content of aquifer matrix material. A lack of organic C to provide energy to denitrifiers is often identified as the major factor limiting NO_3^- removal in subsurface soils and contaminated aquifers (Parkin and Meisinger, 1989; Obenhuber and Lowrance, 1991; Groffman et al., 1992; Bradley et al., 1992; McCarty and Bremner, 1992; Yeomans et al., 1992; Starr and Gillham, 1993; Groffman et al., 1996; Starr et al., 1996). Our data suggest that there is a strong need to determine the factors that influence the production and maintenance of patches in the subsurface, for example, upland vs. wetland vegetation. Patches comprised of decomposing roots were the most biologically active in this study, suggesting that there is a need to know which plants are capable of colonizing the subsurface, and the biogeochemical factors that influence this colonization. That fact that patches were common in the subsurface of PD soils located <30 m downslope from MWD soils that had no patches suggests that the factors regulating subsurface patch occurrence are likely to be dynamic within riparian zones. Understanding these dynamics is important to understanding the function, and managing the long-term NO_3^- removal capacity, of these zones.

Data from this study suggest that patches are a much more important source of C to subsurface denitrification than surface-derived DOC. It is often speculated (Trudell et al., 1986; Simmons et al., 1992) that during leaching events, water-soluble organic compounds can be translocated to lower soil depths with percolating water and contribute to denitrification. McCarty and Bremner (1992) questioned the significance of such a mechanism as most soluble C would be oxidized in surface soils and would not reach subsoils. Results from this study, where

forest floor extract was directly injected into the cores as a DOC source, suggest that even if surface-derived DOC reaches the subsurface it will not stimulate NO_3^- removal. Our DOC treatment had no significant effect on N gas production and stimulated immobilization only in the matrix of the MWD cores. The "extra" immobilization induced by the DOC in the MWD cores was small (4.8 $\mu g\ ^{15}N\ kg^{-1}\ d^{-1}$) relative to N gas production and immobilization in the patches. Moreover, our direct injection DOC treatment should strongly overestimate the role of surface-derived DOC in subsurface processes.

One disadvantage of the mesocosm technique used in this study is that it creates disturbance of several types. Cutting of roots to create mesocosms could create a pool of labile organic matter that could stimulate both the production and consumption of inorganic N by microbes. The pulse of NH_4^+ production and the high rates of denitrification that we observed early in our incubation of the mesocosms may have been artificially stimulated by this disturbance. In contrast, extended incubation of the mesocosms may have inhibited microbial activity, especially denitrification, because the subsurface patches were isolated from any new source of C that would be supplied by actively growing roots. Mesocosm techniques cannot provide estimates of in situ activity. However, they were extremely useful in this study because they allowed us to study microbial processes in a more realistic physical context than is possible with laboratory microcosms, but with more control than is possible in field studies.

In addition to showing the importance of patches, results from this study suggest that the aquifer matrix also has a capacity to process NO_3^-. The use of highly enriched $^{15}NO_3^-$ allowed us to quantify rates of NO_3^- immobilization and production in the subsurface matrix material. The matrix immobilization data suggest that the subsurface matrix organic matter has the capacity to take up NO_3^-. However, the matrix does not appear to function as a net NO_3^- sink. We observed several lines of evidence that suggest that there was net N production via mineralization and nitrification in aquifer matrix material. First, in the MWD cores, which contained only matrix material, we observed only net NO_3^- production (Gold et al., 1998). Second, we observed significant amounts of NH_4^+ production in the Br^- only treatment of both the MWD and PD cores. This production went on for several flushing volumes, suggesting that this was not just "wash out" of NH_4^+ produced during core sampling and set up. In the PD cores, much of the NH_4^+ likely came from mineralization of patch material. However, in the MWD cores, the NH_4^+ production suggests that the matrix material can be a source of inorganic N. Finally, we observed a significant decrease in the TON content of the matrix material of both the MWD and PD cores in response to the $Br^- + NO_3^-$ and $Br^- + NO_3^- +$ DOC treatments. This decrease suggests that NO_3^- additions stimulated mineralization of organic N in the matrix material. These results suggest that microbial NO_3^- processing in the matrix material of shallow aquifers is more dynamic than previously thought. In addition to the need for research on subsurface patches, there is a need for further work on internal N dynamics in the subsurface matrix, and a particular need to understand the effects of NO_3^- additions on these dynamics.

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