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Nitrification in a Shallow, Nitrogen - Contaminated Aquifer, Cape Cod, Massachusetts

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ABSTRACT

Little is known about nitrification in ground-water environments when compared to marine systems and surface soils. Ground-water geochemistry near the upper boundary of a shallow, sewage-contaminated ground-water plume on Cape Cod, Mass., indicated a transition zone where O_2 [24 micromolar (μM)] and NH_4^+ (37 μM) coexist. The occurrence and rate of nitrification in this zone were investigated by a combination of isotopic, biogeochemical, microbial, and molecular techniques. $\delta^{15}N$ values of the NH_4^+ increased from +13 per mil (‰) within the NH_4^+ plume to +31‰ in the transition zone consistent with partial nitrification of the NH_4^+. Core incubations under nitrifying conditions demonstrated that nitrifying organisms were present and indicated a low, but measurable potential activity. Molecular analysis of core DNA also specifically detected *Nitrosomonas eutropha* DNA in sediment extracts. A small-scale, natural-gradient tracer test was conducted with ^{15}N-enriched NH_4^+ and Br^- as tracers. Transport of NH_4^+ was at least four times slower than transport of the conservative Br^- tracer. A low nitrification rate (13 to 96 nanomole per liter aquifer per day) was calculated from the natural-gradient tracer test data. From this study, we conclude that nitrification can occur in ground-water environments and can play a significant role in the speciation and transport of nitrogen.

INTRODUCTION

Nitrate and NH_4^+ contamination of freshwater aquifers and soils is a serious problem and affects watersheds throughout the United States. Ground-water nitrogen contamination is commonly associated with septic wastes, landfill leachates, and agricultural activities (Barcelona and Naymik, 1984; Bjerg and Christensen, 1993; Pederson and others, 1991). Ultimately, the movement of NO_3^- and NH_4^+ is controlled largely by aquifer geochemistry, the rate of ground-water flow, and by microbial nitrogen transformations within the aquifer.

Microbial transformation of NH_4^+ to NO_3^- (nitrification) plays an important role in surface soils and affects the speciation, transport, and fate of NO_3^- and NH_4^+ in watersheds (Fisk and Fahey, 1990; Hill and Shackleton, 1989). Ammonium transport is normally retarded in sediments and soils, but its conversion in microaerobic environments to NO_3^-, a highly mobile compound, can lead to widespread nitrogen contamination. Nitrification in soils is well documented, but this process is poorly characterized in ground-water ecosystems. The purpose of this study was to use a variety of microbial, molecular, biogeochemical, and isotopic techniques to detect, characterize, and measure the *in situ* activity of nitrifying organisms in an NH_4^+ and NO_3^- contaminated aquifer in Cape Cod, Mass.

METHODS

The study site is a shallow sand and gravel aquifer characterized by an extensive NO_3^- and NH_4^+ contamination plume that developed over sixty years as a result of continuous discharge of dilute, treated sewage (LeBlanc, 1984). An on-going research project at this field site has documented nitrogen cycling (Smith and Duff 1988), and studies have indicated that there are overlapping vertical NO_3^-, O_2, and NH_4^+ gradients within the contamination plume, suggesting that there are zones where nitrification may be occurring (Ceazan, 1987; Ceazan and others, 1989). Well site F168 (fig. 1) was selected for

this study because of its pronounced O_2, NO_3^-, and NH_4^+ gradients (see below) and its close proximity to tracer-test well array F593. This array consists of 16 multilevel sample (MLS) wells, each having 15 evenly spaced [vertical spacing = 31.75 centimeters (cm)] sampling ports at altitudes from –5.18 to –10.52 meters (m) relative to mean sea level (MSL). One MLS well (F593M02-07) served as the injection well. Thirteen downgradient MLS wells were arranged in three rows 3.05, 6.10, and 9.15 m from the injection sampler, each row roughly perpendicular to the direction of ground-water flow.

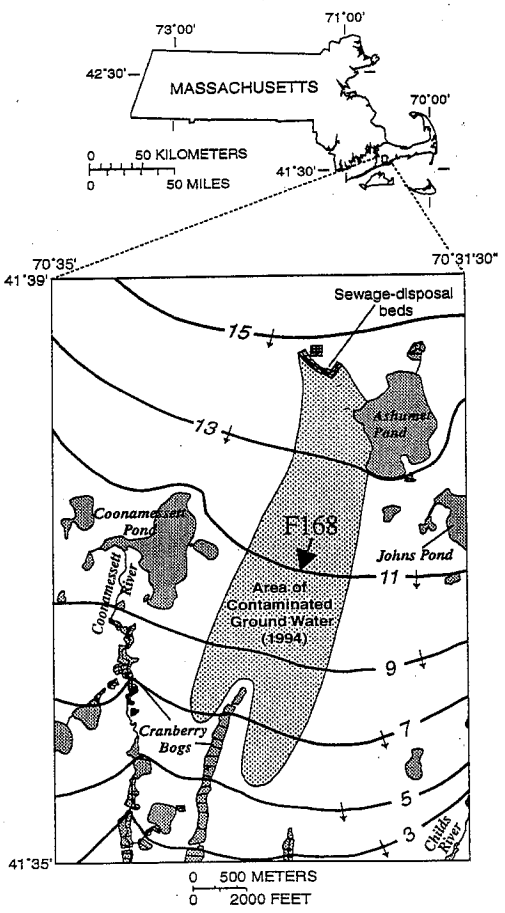

Figure 1. Ground-water study site on Cape Cod, Mass., showing the location of the contaminant plume and multilevel sampler site F168 used in this study.

Sample Collection and Incubations

Ground-water samples were collected, preserved, and analyzed as detailed by Savoie and LeBlanc, (1998). Six core samples from an elevation of –5.85 to –9.25 m relative to MSL from well site F168 were each sub-divided into three portions. One portion was used to measure nitrification potential. This portion was slurried and amended with NH_4Cl [final conc. 0.14 millimolar (mM)] and ground water. Nitrifying organisms were enumerated in the second portion of core by most probable number (MPN) technique (Schmidt and Belser, 1994). The third core portion was frozen for deoxyribonucleic acid (DNA) extraction.

Molecular Analysis of Extracted DNA

DNA was extracted from 40 grams (g) of core in 10 milliliters (ml) of TE buffer [10 mM Tris[hydroxymethyl]aminomethane (Tris), 1 mM ethylenediaminetetraacetic acid (EDTA), pH 8] containing 0.9% sodium dodecyl sulfate (SDS) during a 70 °C, 1 hour incubation with occasional mixing. Extract was separated from the core, and the DNA was precipitated by adding 1/10 volume of 3 molar (M) sodium acetate and 2 volumes ethanol. The DNA pellet was resuspended in TE buffer and purified on a Sephadex G-200 spin column (Boccuzzi and others, 1995). Two primer sequences, NITB (5'-ACCCATCCCGAAGTGTGCATT) (Voytek and Ward, 1995) and Nso190R (5'-GGAGAAAAGCAGGGGATCG) (Mobarry and others, 1996) were used to amplify ammonium-oxidizer 16S ribosomal ribonucleic acid (rRNA) genes. Polymerase Chain Reaction (PCR) amplifications were in 25 microliters (µl) and contained 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris (pH 8.8), 0.1 milligrams per milliliter bovine serum albumin, 0.05% Tween 20, 0.5 µM each primer, 50 µM deoxynucleotidetriphosphates, and 1 unit Taq polymerase. The following PCR temperature cycle was used to amplify nitrifying bacterial 16s rRNA genes: 80 °C hot start followed by 35 cycles of 95 °C (1 min), 60 °C (1 min), and 72 °C (1.5 min). After cycling, the reactions were subjected to a final 5 min extension at 72 °C.

A qualitative estimate of the diversity of nitrifying organisms in core material was made by comparing the restriction fragment (RFLP) patterns of the PCR-amplified 16s rRNA genes. PCR amplifications (100 µL) were purified using a BioRad DNA purification kit and resuspended in 50 µl TE buffer. The restriction endonuclease *Mbo I* was used to digest the PCR product according to the manufacturer's protocol (Promega, Milwaukee, WI). Restriction fragments were separated on a 3% agarose gel at four volts per centimeter. The gel was stained with ethidium bromide and visualized under short-wave, ultraviolet illumination. Banding patterns were compared to the banding patterns from control strains.

Tracer Injection and Sampling

A small-scale, natural-gradient tracer test was performed from June to September 1997. Approximately 200 liters (L) of ground water was withdrawn from two sampling ports with a peristaltic pump outfitted with gas impermeable tubing. The ground water was withdrawn from a zone of putative nitrification -6.3 m relative to MSL and pumped into a gas impermeable bladder that had been previously flushed with argon and contained 25.76 g NaBr and 0.46 g $(^{15}NH_4)_2SO_4$ dissolved in 1 L distilled water. After mixing the ground water and tracer in the bladder, the liquid was injected back into the aquifer (approx. 1.5 liters per min) at a single sample port -6.3 m relative to MSL. Samples (60 ml and 1 L) were collected and analyzed for Br^-, NH_4^+, NO_3^-, NO_2^-, $^{15}NH_4^+$ and $^{15}NO_3^-$ during injection from the bladder, post injection from the injection well, and during the next 10 weeks from downgradient wells. All samples were filtered (0.45 micron) and preserved in the field by freezing (60 ml NO_3^-/NO_2^-), acidification (60 ml and 1 L NH_4^+), or by adding approximately 100 mg KOH (1 L NO_3^-). Bromide was analyzed on the day of collection with an ion-specific electrode. Temperature and O_2 measurements (CHEMetrics, Calverton, VA) made during injection and post injection showed that there was only a slight increase in either of these factors during tracer injection.

RESULTS AND DISCUSSION

Groundwater Chemistry

A series of narrow vertical chemical gradients consistent with nitrification was observed in ground-water samples from F168 (fig. 2). A zone where O_2 and NH_4^+ coexist is a prerequisite for nitrification, and both O_2 and NH_4^+ are present –6.3 meters relative to MSL in ground-water samples. High concentrations of NO_3^-, a product of nitrification, were also measured in this zone. $\delta^{15}N$ values as high as +21‰ and +31‰ were measured in the transition zone in nearby wells. In contrast, $\delta^{15}N$ values below the transition zone averaged less than +13‰.

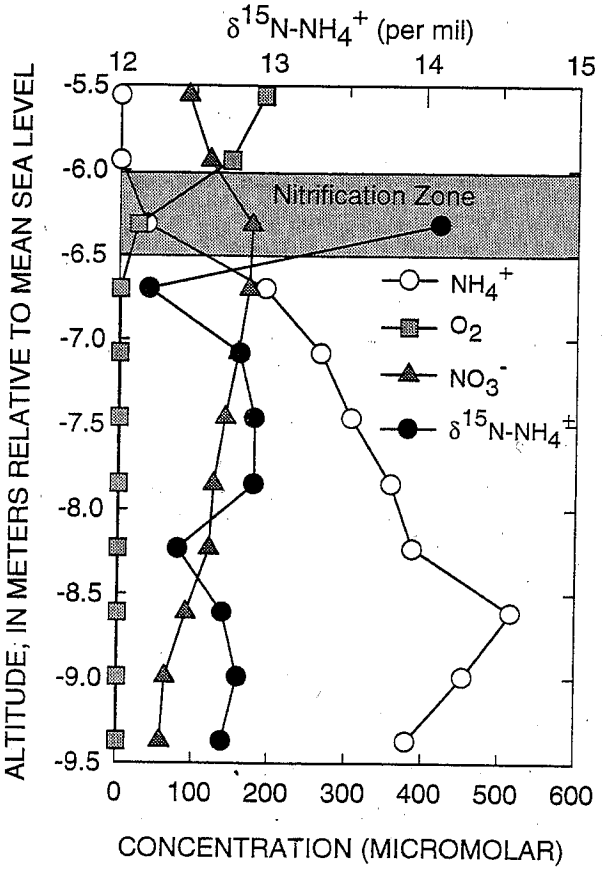

Figure 2. Concentration of dissolved nitrogen species and O_2 at tracer injection well in June 1997. Delta (δ) values are given in units of parts per thousand (‰, or per mil), as defined by $\delta = 1000*[R/R_{air} - 1]$, where R and R_{air} are the ratios $^{15}N/^{14}N$ of the sample and of atmospheric nitrogen, respectively.

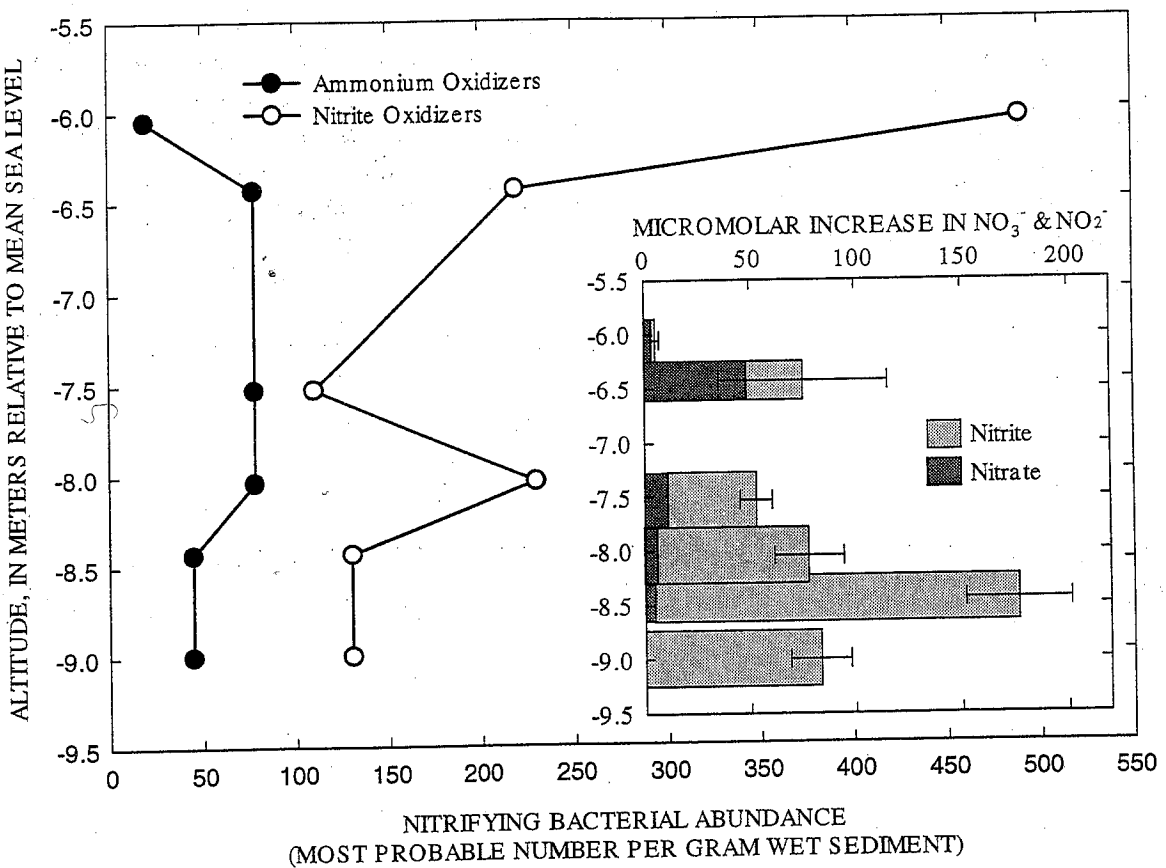

Figure 3. Abundance of nitrifying organisms and nitrification potential (inset figure) in sediment cores collected from well site F168.

Sediment Core Experiments

Nitrification is a biologically mediated, two step process where NH_4^+ is first oxidized to NO_2^- by one group of bacteria (ammonium oxidizers), and then a second group of bacteria (nitrite oxidizers) complete the transformation by oxidizing NO_2^- to NO_3^-. Ammonium- and nitrite-oxidizing bacteria were found throughout the core profile (fig. 3). The abundance of nitrite-oxidizers was consistently higher than the abundance of ammonium-oxidizers. Although nitrifier abundance was lower than expected, their presence accounts for nitrification potential observed in aerobic sediment slurries spiked with NH_4^+ (fig 3., inset). Very little NO_2^- or NO_3^- accumulated in sediment slurries above –6.25 m even though nitrifying organisms present in this core material. This is due in part to the very low numbers of ammonium oxidizers. Surprisingly, even cores taken from anaerobic zones could produce NO_2^- and NO_3^- from NH_4^+.

DNA was successfully extracted and purified from the -6.5 m core section. PCR amplification with ammonium-oxidizer specific primers produced a PCR product of the expected size (1 kilobase). Restriction analysis of the PCR product was used to (i) verify that the PCR product generated from core DNA was from nitrifying organisms and not from a closely related, non-nitrifier like *Spirillum volutans*, and (ii) determine the nitrifier species responsible for generating the PCR product. When the PCR product was restricted with *Mbo I*, a 4-cutter endonuclease, several smaller fragments were produced (fig. 4). The resultant bands corresponded exactly to the bands generated from the nitrifier strain *Nitrosomonas eutropha*.

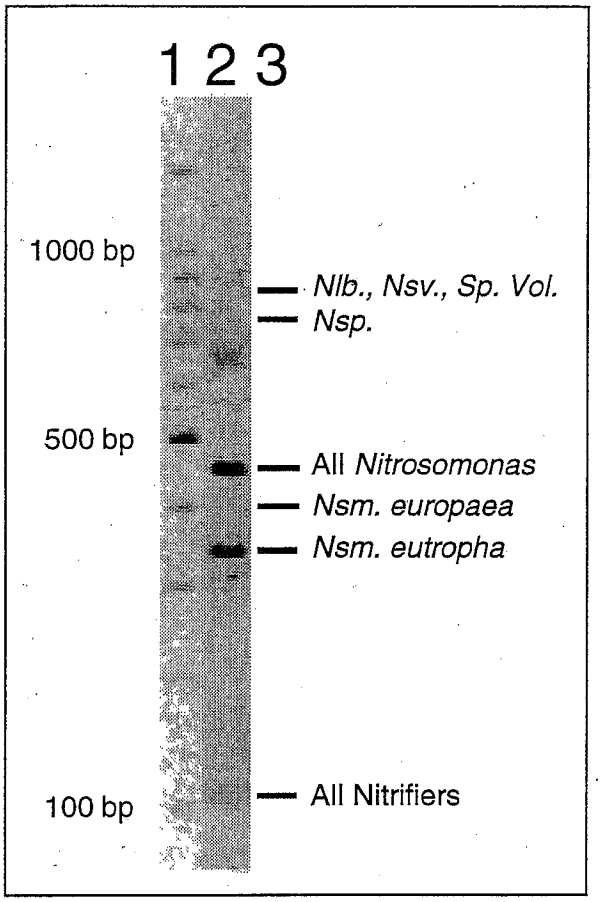

Figure 4. Endonuclease restriction analysis of the ammonia oxidizer polymerase chain reaction product with *Mbo I*. DNA fragments were separated on a 3% agarose gel, stained with ethidium bromide, and viewed under ultraviolet light. Lane 1: 100 base pair DNA ladder. Lane 2: Sediment core PCR product restricted with *Mbo I*. Lane 3: Potential restriction pattern from available nitrifying organisms and *Spirillum volutans*, a closely related non-nitrifier. Abbreviations: *Nsm* = genera *Nitrosomonas*, *Nlb* = general *Nitrosolobus*, *Nsv* = genera *Nitrosovibrio*, *Nsp* = genera *Nitrosospira, and Sp. vol* = *Spirillum volutans.*

Natural-Gradient Tracer Test

A conceptualization of the natural-gradient tracer test is presented in fig. 5. A mixture of ground water, Br^-, and $^{15}NH_4^+$ is injected in the aquifer. Because the movement of $^{15}NH_4^+$ is retarded by sorption to aquifer sediments, its movement is much slower than Br^-, which moves with ground-water flow. As $^{15}NH_4^+$ is nitrified, a cloud of $^{15}NO_3^-$ is formed. Since NO_3^- moves much faster than the NH_4^+, $^{15}NO_3^-$ will move out of the $^{15}NH_4^+$ cloud trailing behind the Br^- cloud.

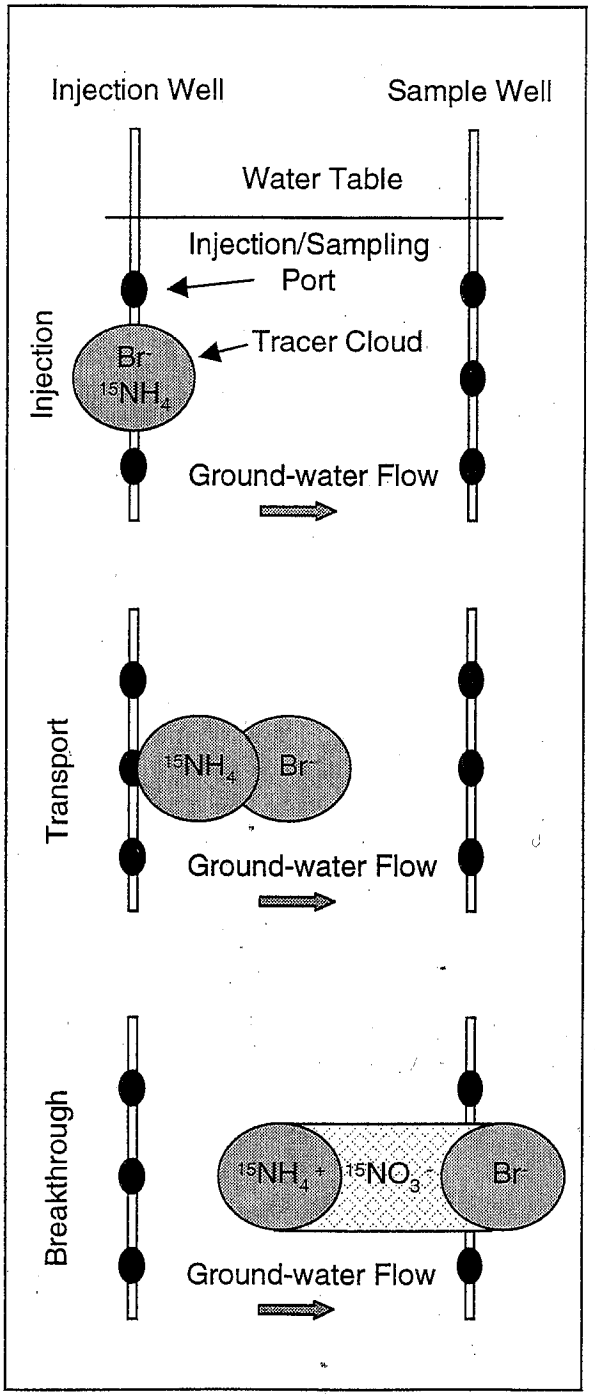

Figure 5. Idealized model of $^{15}NH_4^+$ and Br^- natural-gradient tracer test. Tracer clouds overlap immediately after injection. Clouds separate during transport owing to the slower transport of NH_4^+ compared to Br^-. Tracer clouds completely separated during breakthrough with $^{15}NO_3^-$ (formed in the $^{15}NH_4^+$ cloud) transported faster than the $^{15}NH_4^+$.

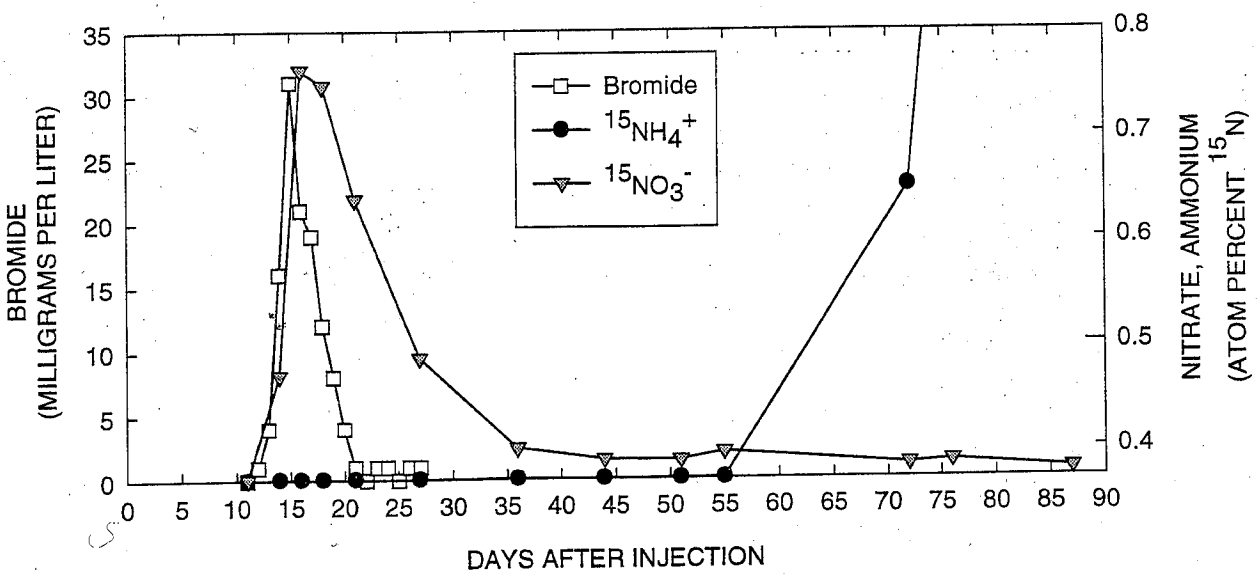

Figure 6. Breakthrough curves for Br^-, $^{15}NH_4^+$, and $^{15}NO_3^-$ at a sampling well 9.15 meters downgradient of the injection well

The initial concentration of tracer constituents was 103 milligrams (mg) $Br^- L^{-1}$ and 53.6 Atom percent (At%) $^{15}NH_4^+$. Peak Br^- breakthrough (31 mg $Br^- L^{-1}$) occurred 15 days after injection at well F593 M08-06 (9.15 m from the injection well) at a depth of –6.2 m (fig. 6). Initial $^{15}NH_4^+$ breakthrough began more than 55 days after injection. A large peak of $^{15}NO_3^-$ is seen immediately after Br^- at the sampling well. We interpret this large peak as an injection artifact, possibly because nitrification was stimulated by the two-fold increase in NH_4^+ concentration and/or by additional O_2 (concentrations increased from 24 to 27 µM). However, $^{15}NO_3^-$ did not return to background levels. It is this low concentration of $^{15}NO_3^-$ that we attribute to *in situ* nitrification.

A rough estimate of the *in situ* nitrification rate in the transition zone can be calculated based upon ground-water flow rate, the mass of $^{15}NO_3^-$ produced immediately before the $^{15}NH_4^+$ breakthrough, assumed $^{15}NH_4^+$ peak width and shape, the porosity of the aquifer, and a constant nitrification rate. A minimum and maximum nitrification rate of 13 and 96 nanomoles of NO_3 per L aquifer per day was calculated (table 1). Given these rates, it would take from 2 to 16 years to consume the NH_4^+ in the nitrification zone. In that amount of time, NO_3^- would have traveled from 500 to 3800 m while the NH_4^+ would have traveled 63 to 480 m. Our interpretation of these results is that nitrification plays a significant role in nitrogen speciation and transport in the transition zone between the sewage plume and uncontaminated ground water. A second tracer test, with complete Br^-, $^{15}NO_3^-$, and $^{15}NH_4^+$ breakthrough curves, is currently under way. This new data set will allow us to accurately measure *in situ* nitrification rates and predict the fate and transport of nitrogen species at this site.

Table 1. Nitrification natural-gradient tracer test results.

Nitrogen Species	Transport Rate (centimeters/day)	Retardation Factor	Oxidation Rate (nanomoles per liter aquifer per day)
NH_4	8 - 16	3.8	13 – 96
NO_3	32 - 64	1.0	NA

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