

Assessment of Nitrification Potential in Ground Water Using Short Term, Single-Well Injection Experiments

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Received: 27 July 2004 / Accepted: 3 October 2004 / Online publication: 31 December 2005

Abstract

Nitrification was measured within a sand and gravel aquifer on Cape Cod, MA, using a series of single-well injection tests. The aquifer contained a wastewater-derived contaminant plume, the core of which was anoxic and contained ammonium. The study was conducted near the downgradient end of the ammonium zone, which was characterized by inversely trending vertical gradients of oxygen (270 to 0 μM) and ammonium (19 to 625 μM) and appeared to be a potentially active zone for nitrification. The tests were conducted by injecting a tracer solution (ambient ground water + added constituents) into selected locations within the gradients using multilevel samplers. After injection, the tracers moved by natural ground water flow and were sampled with time from the injection port. Rates of nitrification were determined from changes in nitrate and nitrite concentration relative to bromide. Initial tests were conducted with ^{15}N -enriched ammonium; subsequent tests examined the effect of adding ammonium, nitrite, or oxygen above background concentrations and of adding difluoromethane, a nitrification inhibitor. *In situ* net nitrate production exceeded net nitrite production by 3- to 6- fold and production rates of both decreased in the presence of difluoromethane. Nitrification rates were 0.02–0.28 $\mu\text{mol (L aquifer)}^{-1} \text{ h}^{-1}$ with *in situ* oxygen concentrations and up to 0.81 $\mu\text{mol (L aquifer)}^{-1} \text{ h}^{-1}$ with non-limiting substrate concentrations. Geochemical considerations indicate that the rates derived from single-well injection tests yielded overestimates of *in situ* rates, possibly because the injections promoted small-scale mixing within a transport-limited reaction zone.

Nonetheless, these tests were useful for characterizing ground water nitrification *in situ* and for comparing potential rates of activity when the tracer cloud included non-limiting ammonium and oxygen concentrations.

Introduction

The presence of inorganic nitrogen compounds in ground water can result from both natural sources and a variety of human activities. In most cases, nitrate is the predominant nitrogen species found in ground water and in the US has been the focal point of regional and national surveys to document aquifer susceptibility to nonpoint source contamination [43, 66]. However, ammonium also is found in ground water in many situations. It can occur naturally in association with recalcitrant organic materials such as shale, coal, or peat [15, 39, 51], or as a contaminant associated with disposal of wastewater [48, 49, 61], landfills [5, 11, 12], feedlots, and manure applications [38], or other types of point sources [4]. In large regions of the former Soviet Union, mean ammonium concentrations in unconfined ground water exceed 28 μM [75], the drinking water guideline set by the European Union. Transport and distribution of ammonium in an aquifer is primarily controlled by cation exchange with aquifer solids, even within sand and gravel formations [8, 9, 16], as well as by nitrification, the microbial oxidation of ammonium to nitrite and then nitrate.

Compared to other environments, such as surface soils and the open ocean, almost nothing is known about nitrification in the terrestrial subsurface below the water table. Distributions of inorganic nitrogen species at different study sites have suggested that nitrification does occur in ground water [5, 8, 9, 16]. In the limestone Chalk aquifer in England, high concentrations of nitrous oxide were attributed to nitrification [27] and significant populations of nitrifying bacteria were detected with

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culture-based enumerations [73]. Core material collected from a sand and gravel aquifer on Cape Cod contained a higher abundance of nitrite oxidizers than ammonium oxidizers, whereas aerobic flask incubations oxidized ammonium to both nitrite and nitrate [41]. In general, however, detailed studies on ground water nitrification as a process are lacking, and how the process interacts with the physical and chemical environment and other biogeochemical processes present in an aquifer to control the transport and attenuation of ammonium is largely open to speculation.

There are many barriers to accurate assessment of biogeochemical processes in ground water; most are related to the inaccessible nature of the subsurface. Activity assays conducted in the laboratory allow bench-scale control, but representative cores can be difficult and expensive to obtain and may yield overestimates of *in situ* rates [10, 46, 59]. On the other hand, tracer tests conducted in the field allow *in situ* measurement. Long-term tracer tests, which follow a tracer cloud through a well field, have been used to measure processes such as denitrification [56, 59], methane oxidation [62], and organic contaminant degradation [2, 36]. In a much shorter-term field approach, push-pull tracer tests have been used to introduce a tracer cloud into a single well and then soon thereafter remove it by continuous pumping. Push-pull tests have been used to measure aerobic respiration and denitrification [29, 30, 39, 52], sulfate reduction [29, 35, 53], methanogenesis [29], uranium and technetium reduction [30, 44, 54], and cometabolism of aliphatic chlorinated hydrocarbons [33]. The field methods can also have shortcomings: in general, subsurface microbial processes are slow, particularly in pristine environments [19], which can make rates of activity difficult to measure, while some tracers, such as ammonium, are not transported conservatively. Additional, less complicated techniques are clearly needed to assist in quantifying the effect of microbial processes in aquifers.

The purpose of this study was to examine nitrification as a process within ground water and to explore the use of single-well injection experiments to assess the process within *in situ* conditions. The study was conducted within a sand and gravel aquifer on Cape Cod that had been contaminated with treated wastewater and within which an earlier study had suggested that nitrification was actively occurring [37]. The short-term tracer tests provided the opportunity to quantify nitrification *in situ* within existing geochemical conditions and to assess the response of the process to altered concentrations of ammonium, nitrite, and oxygen.

Materials and Methods

Site Description and Ground Water Sampling. This study was conducted in a regional, unconfined freshwater

aquifer located on Cape Cod, MA, that has been the subject of several previous investigations regarding contaminant transport [3, 18, 26, 31, 32, 36, 37, 70], characterization of microbial communities [21, 23, 25, 34, 40, 45], and nitrogen cycling [9, 56, 58, 59, 61, 63] in the subsurface. At this site, disposal of treated wastewater for more than 60 years resulted in the formation of a large ground water contaminant plume that is >5 km long, >1 km wide, and 30 m thick.

Biogeochemical processes control the concentrations and speciation of dissolved oxygen and nitrogen within the contaminant plume [57–59, 61]. In general, the central core along the long axis of the plume is anoxic and contains ammonium, but little or no methane, whereas the contaminated ground water surrounding the central core contains nitrate [61]. Due to restricted vertical mixing [18], the plume consists of steep, overlapping, vertical gradients that persist even after several kilometers of downgradient transport [60]. Differential transport rates of nitrate and ammonium affect their relative distributions. Nitrate, like sodium and chloride, is hydrologically conservative, and travels at the rate of ground water flow. In contrast, ammonium transport is significantly slowed by cation exchange, which creates a large pool of residual ammonium that is reversibly sorbed [9]. Denitrification is a predominant electron accepting reaction within the contaminant plume and has been examined in several studies [56, 58, 59, 61, 63].

This study was conducted in a well field (F593) located at a distance of 2.4 km (2.6 km via ground water flow path) from the downgradient edge of the disposal location. The well field consisted of a cluster of 16 multilevel samplers (MLSs) located in rows perpendicular to ground water flow (Fig. 1). Each MLS contains 15 separately screened ports, providing discrete sample collection within fairly narrow vertical intervals. For this study, multiple injection tests were conducted simultaneously under similar conditions by using ports with similar elevations in different MLSs.

Ground water was collected from the MLS ports using a peristaltic pump fitted with Norprene[®] tubing. Specific conductance was measured with an Orion 128 conductivity meter. For dissolved oxygen analysis, when O₂ exceeded 30 μM, water samples were collected in 0.3-L BOD bottles and assayed with an oxygen-specific electrode. When O₂ was less than 30 μM, water samples were analyzed using a colorimetric reagent in ampoules (Chemetrics, Inc., Calverton, VA, USA) and a battery-powered spectrophotometer [32]. Water samples for analysis of nitrite, nitrate, chloride, and bromide concentrations were filtered with an in-line capsule filter (0.45 μm pore size) and frozen. Samples for ammonium analysis were filtered and preserved with concentrated H₂SO₄ (final pH < 2.0). Samples for dissolved organic carbon (DOC) analysis were filtered with a silver mem-

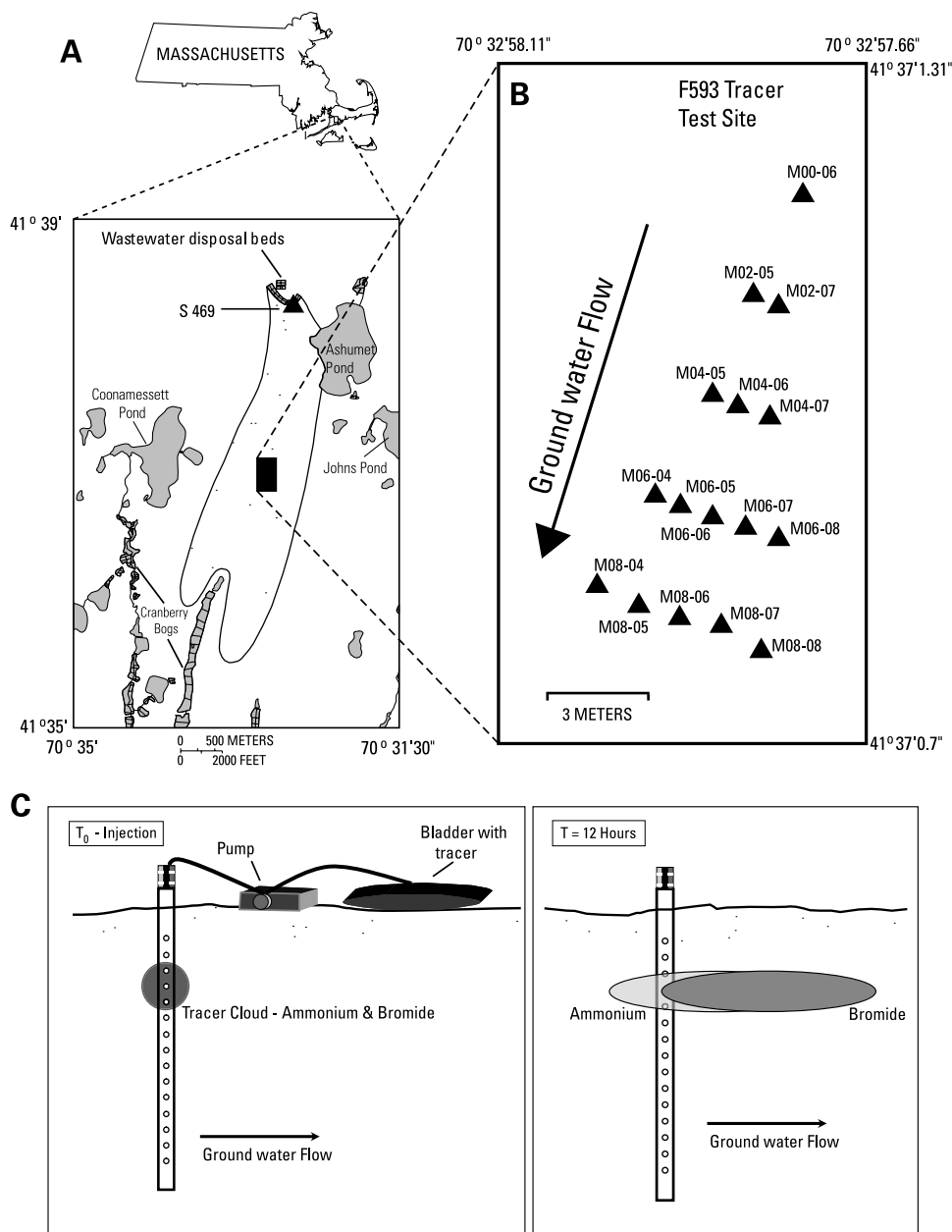


Figure 1. (A) Map of study site and contaminant plume on Cape Cod. (B) Map of the F593 MLS array used for the *in situ* injection tests. (C) Diagram of a single-well injection experiment with bromide and ammonium demonstrating the separation of the tracers with time due to ground water flow and retardation of ammonium by cation exchange.

brane filter (0.45 μm pore size) into baked (250 $^{\circ}\text{C}$) amber 40-mL VOA vials, which were filled completely (no headspace), capped with septa, and stored at 4 $^{\circ}\text{C}$. For nitrous oxide determination, 15-mL water samples were collected in a syringe and injected into stoppered 30-mL serum bottles that contained 0.2 mL of 12.5 N NaOH in a He headspace.

^{15}N Tracer Tests. In 1997 and 1998, two natural gradient tracer tests were conducted at well site F593 using ^{15}N -enriched ammonium to assess *in situ* rates of nitrification within the Cape Cod contaminant plume. The tests were similar to previously described tests to quantify methane oxidation and denitrification [56, 59,

62] using an injection MLS and a target grid of MLSs to intercept the path of the tracer cloud as it was transported downgradient. Briefly, a tracer solution was prepared by pumping 200 L of ground water (1997 from port 4 of M0207 and M0205; 1998 from port 5 of M0207 and port 4 of M0407; port selection based on target ammonium and oxygen concentrations) into a gas-impermeable bladder. The bladder had been previously prepared by flushing with argon gas to remove air, and then loaded with an anoxic solution (~ 3 L) containing 25.7 g of sodium bromide and 0.46 g (1997) or 0.20 g (1998) of ($^{15}\text{NH}_4$) $_2\text{SO}_4$ (98+ atom %). After adding the ground water, the bladder was mixed vigorously, any gas bubbles present were vented, and the tracer solution

pumped back into the aquifer via port 4 of M0207. Samples were collected from the bladder during the injection process; the tracer cloud was periodically sampled from the injection MLS for 240 h after completion of the injection. Samples were collected and preserved as described above; separate 1-L samples were collected and preserved for ammonium and nitrate + nitrite isotope analysis. Subsequent samples were also collected from the downgradient grid of MLSs (Fig. 1); the data from these analyses are presented elsewhere [6A].

Single-Well Injection Experiments. A series of single-well injection tests were conducted at site F593 from 1997 to 2000 to examine the effect of added nitrite or ammonium on *in situ* nitrification, as well as the *in situ* nitrification potential across the vertical geochemical gradient. These experiments were conducted by preparing the tracer solution using one of two methods. The first method was used to maintain the *in situ* dissolved oxygen concentration or for adding a dissolved gas as a tracer. In this case, a 40-L Tedlar[®] gas sampling bag was first filled and evacuated six times with nitrogen gas. Then, 0.25 L of an anoxic solution containing sodium bromide (1200 μM) and ammonium chloride or potassium nitrite (at varied concentrations) was added to the bag with a peristaltic pump. The bag was prepared the day before an experiment and stored overnight under water to minimize gas exchange. At the field site, the Tedlar[®] bag was placed in a water-filled, plastic wading pool. For one set of experiments, 0.05 kPa of difluoromethane, a compound known to inhibit nitrification [42], was added as headspace gas to the Tedlar[®] bag. The bag was then filled with 39.5 L of ground water from the appropriate MLS port, with frequent agitation to mix the tracer solution. After filling, any headspace gas in the bag was vented, a water sample was collected from the bag, then the pump was reversed and the contents of the bag pumped into the ground through the injection port at $\sim 1 \text{ L min}^{-1}$. A second injection sample was collected when the bag was half empty. The second tracer preparation method was used to assess potential nitrification rates. In this case, the tracer solution was prepared in open 120-L containers and the solution was bubbled with air to raise the oxygen concentration to near atmospheric equilibrium. All other aspects of the two methods were the same.

Immediately after injection, tracer cloud samples were collected from the injection port and the adjacent ports and at periodic intervals thereafter. Samples were collected as described above for nitrate, nitrite, ammonium, bromide, and dissolved oxygen. Difluoromethane samples were collected using the same procedure as for nitrous oxide. Details for all the tracer tests, including specific MLSs and ports, the tracers used, and the tracer concentrations, are listed in Table 1.

Analyses. Nitrite was reduced to nitric oxide in the presence of sodium iodide and glacial acetic acid and analyzed by chemiluminescent detection on a Sievers NOA 280. Anions (chloride, bromide, nitrate, and sulfate) were analyzed with a Dionex 300 Ion Chromatograph using carbonate buffered eluent (1.8 mM $\text{Na}_2\text{HCO}_3/1.7 \text{ mM NaHCO}_3$, pH 7.5) at a flow rate of 2.0 mL min^{-1} through an IonPac AS4A analytical column and an AG4A guard column. Ammonium samples were analyzed with a Dionex 100 Ion Chromatograph with 5 or 15 mN sulfuric acid eluent at 0.6 mL min^{-1} through a CS12A analytical column and CG12A guard column. Dissolved organic carbon (DOC) was oxidized with ammonium persulfate and analyzed by membrane-based conductometric analysis on a Sievers TOC 800. Inorganic carbon was first removed as carbon dioxide with the addition of 6 M phosphoric acid.

Nitrous oxide was assayed using a headspace equilibration technique as described by Brooks *et al.* [7]. Difluoromethane was analyzed using a similar headspace equilibration method and a Packard Chrompac gas chromatograph equipped with a flame ionization detector and a 2-m packed column of Poropak N operating at 100°C with nitrogen carrier gas at 15 mL min^{-1} . Detection limits for nitrate, nitrite, nitrous oxide, and ammonium were 5, 0.01, 0.01, and 5 μM , respectively.

Nitrate samples were prepared for N isotopic analysis by freeze drying at high pH (>11), then sealed into evacuated glass tubes with Cu + Cu_2O and baked at 850°C to produce N_2 gas [6, 56]. Ammonium samples were prepared for N isotopic analysis by sorption onto a zeolite cation exchanger (Union Carbide IONSIEV W-85; modified from [72]), which was filtered, dried, and baked to produce N_2 gas. N_2 was analyzed by dual-inlet isotope-ratio mass spectrometry and calibrated by analyses of ammonium and nitrate isotope reference materials that were prepared the same way. Analyses of artificial solutions containing nontracer nitrate and ^{15}N -enriched ammonium indicate that these species were separated without significant cross-contamination [6A]. The $\delta^{15}\text{N}$ values are reported relative to atmospheric N_2 and were normalized to reference values of +0.4‰ for IAEA-N1 +4.7‰ for IAEA-N3, +180‰ for USGS32, and +4730‰ for IAEA-311 [6A].

Results

Ground Water Geochemistry. The location of the sampling well array used in this study was 2.6 km downgradient from the contaminant plume source (Fig. 1). As is characteristic throughout the Cape Cod contaminant plume, a steep vertical gradient of dissolved oxygen was evident at the top of the contaminant plume at the study site (Fig. 2). The core of the plume was suboxic or anoxic and contained high concentrations of dissolved ammonium, up to 600 μM , and other contaminant

Table 1. List of single-well injection experiments conducted for this study at well site F593

Test	MLS # (Port #)	Injection addition				Explanation
		Ammonium ^a (μM)	Nitrite ^b (μM)	Diffuoromethane ^b (μM)	Oxygen ^c (μM)	
1	0207 (4)	75/106	–	–	17	¹⁵ N-NH ₄ ⁺
2	0806 (4)	50/74	–	–	44	
3	0804 (5)	150/156	–	–	72	
4	0407 (4)	0/5	–	–	44	No bromide control ^d
5	0405 (4)	0/20	–	–	45	Bromide-only control
6	0804 (4)	300/281	–	4.3	19	
7	0604 (5)	300/402	–	7.0	44	
8	0605 (5)	0/19	1.3	–	68	
9	0805 (4)	0/19	4.5	–	62	
10	0807 (4)	0/62	9.4	–	80	
11	0807 (4)	0/63	10.7	–	33	
12	0604 (4)	0/NA	12.5	–	87	
13	0606 (4)	0/16	20.1	–	58	
14	0606 (2)	300/325	–	–	241	
15	0805 (3)	300/305	–	–	212	
16	0605 (4)	300/347	–	–	197	
17	0806 (5)	300/496	–	–	237	
18	0605 (7)	300/558	–	–	175	
19	0805 (9)	300/805	–	–	222	
20	0606 (11)	300/843	–	–	193	
21	0806 (13)	300/870	–	–	232	

^aAdded concentration/final concentration (added + background) at t_0 . NA = no data.

^bAdded concentration. Background concentrations were all less than 1 μM .

^cFinal concentration at t_0 .

^dSodium bromide (1200 μM) was added for all tests, except the no bromide control.

constituents, such as chloride, sulfate, and dissolved organic carbon (Fig. 2). In 1996, the F593 site was located near the downgradient end of the ammonium zone; ammonium concentrations were undetectable 0.5 km downgradient from this site (data not shown). Typically, the contaminant plume in this region contains nitrate throughout the ammonium zone (Fig. 2) as well as downgradient from the ammonium toe (data not shown). This is unlike the contaminant plume within ~ 1.5 km of the source, where there is little or no overlap in nitrate and ammonium concentrations (e.g., see [61]). The co-occurrence of nitrate and ammonium at well site F593 and the differential rate of transport of the two nitrogen species suggested that nitrification might have been occurring within the aquifer at this location. Nitrite concentrations at this location were somewhat higher in the shallow portion of the contaminant plume, where the ammonium and oxygen gradients overlapped and there was a small decrease in pH (Fig. 2). On the other hand, nitrous oxide was undetectable in the shallow zone, but did have a concentration peak deeper, at the same altitude as the peak in ammonium concentration (Fig. 2).

¹⁵N Tracer Test. To assess ground water nitrification *in situ*, two natural gradient tracer tests were conducted at site F593 using ¹⁵NH₄⁺ and bromide as tracers. These tests were intended to quantify the prod-

uction and transport of ¹⁵N-enriched nitrate and nitrite from ammonium within the tracer cloud after several meters of transport and consisted of sample collection for a period of several weeks from a downgradient target grid as the tracer cloud passed by. However, as part of the protocol for these tests multiple post-injection samples were collected from the injection well to ensure that the tracer cloud did not significantly alter the geochemistry of the zone in which the test was being conducted (particularly with respect to oxygen concentrations). A fortuitous result from both of these tests was a significant post-injection increase in nitrite and nitrate concentrations with time (Fig. 3; results from 1997 not shown). During the first 12 h after the second test injection, when tracer bromide concentrations were relatively constant, nitrite concentrations increased 38-fold, from a background level of 27 nM to 1.03 μM , whereas nitrate concentrations increased about 1.5 μM against a relatively high background concentration. Both nitrite and nitrate concentrations continued to increase, even after the bromide was being diluted by dispersion. Some of the secondary nitrate increase (12–48 h) may have been due to concentration differences between the tracer solution (which was prepared by pooling water from 2 wells) and the background (gray vs black squares on day 0, Fig. 3B), but the ¹⁵N results clearly indicated that the tracer ammonium was being oxidized. The ¹⁵N content of the ammonium oxidation products (nitrate + nitrite)

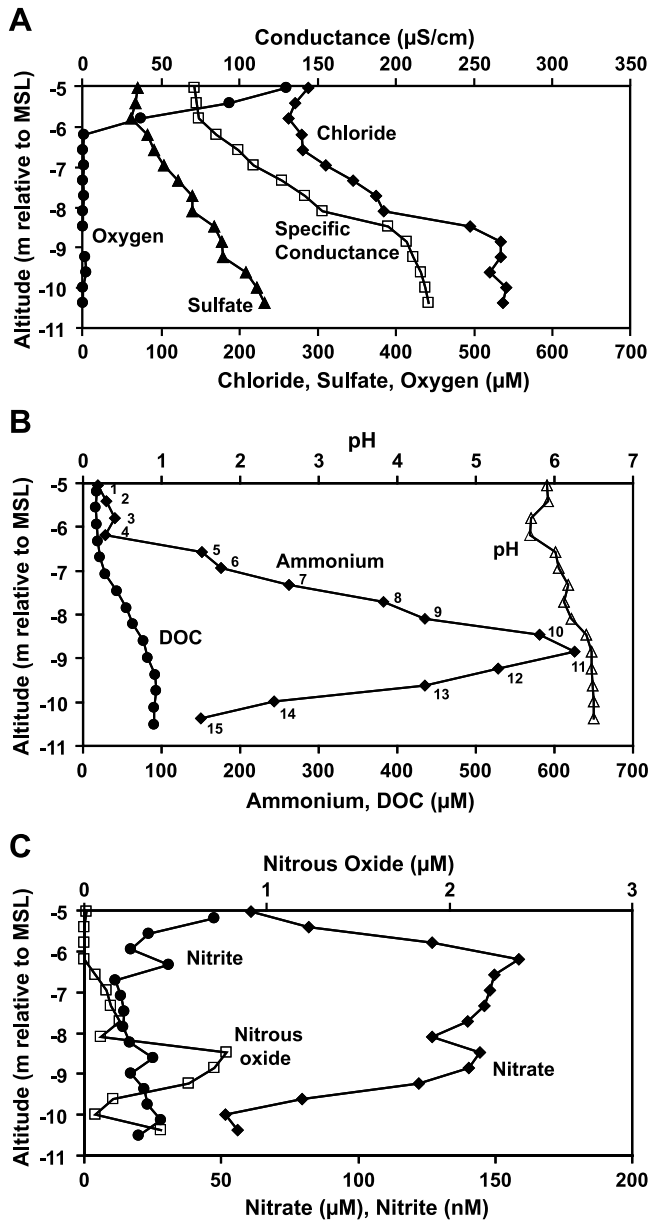


Figure 2. Vertical profiles of ground water constituents relative to mean sea level (MSL) in June 1999 from MLS F593-M0806 (DOC is from F593-M0804). (A) Oxygen, sulfate, specific conductance, and chloride. (B) Dissolved organic carbon (DOC), ammonium and pH. (C) Nitrous oxide, nitrite, and nitrate. Open symbols pertain to upper axes; MLS port numbers are labeled on the ammonium profile.

increased substantially in only 18 h and remained elevated for more than 9 days (Fig. 3C). Injection well time courses of nitrate, nitrite, and ^{15}N after day 1 reflect an early separation of the bromide and ammonium tracers (as depicted in Fig. 1) as well as continued oxidation of the retarded ammonium cloud throughout most of the nearly 10-day sampling interval (Fig. 3). The *in situ* rate of nitrification for the first 18 h (when there was no dispersive loss of bromide), as determined from the ^{15}N

content of the nitrate + nitrite pool, was $0.02 \mu\text{mol} (\text{L aquifer})^{-1} \text{h}^{-1}$.

Single-Well Injection Experiments with Ammonium. In response to the ^{15}N tracer results, a series of single-well injection experiments was conducted to examine the potential for using these as short-term tests to assess ground water nitrification *in situ*. The first set of experiments tested different concentrations of added ammonium under varying conditions. When the ambient ammonium concentration was increased by 50 or $150 \mu\text{M}$ within the same altitude as for the ^{15}N test, an immediate increase was evident for both nitrate and nitrite concentrations (Fig. 4). Again, the total increase was greatest for nitrate. A smaller injection volume (40 vs 200 L for the ^{15}N tests) was used for these tests, which

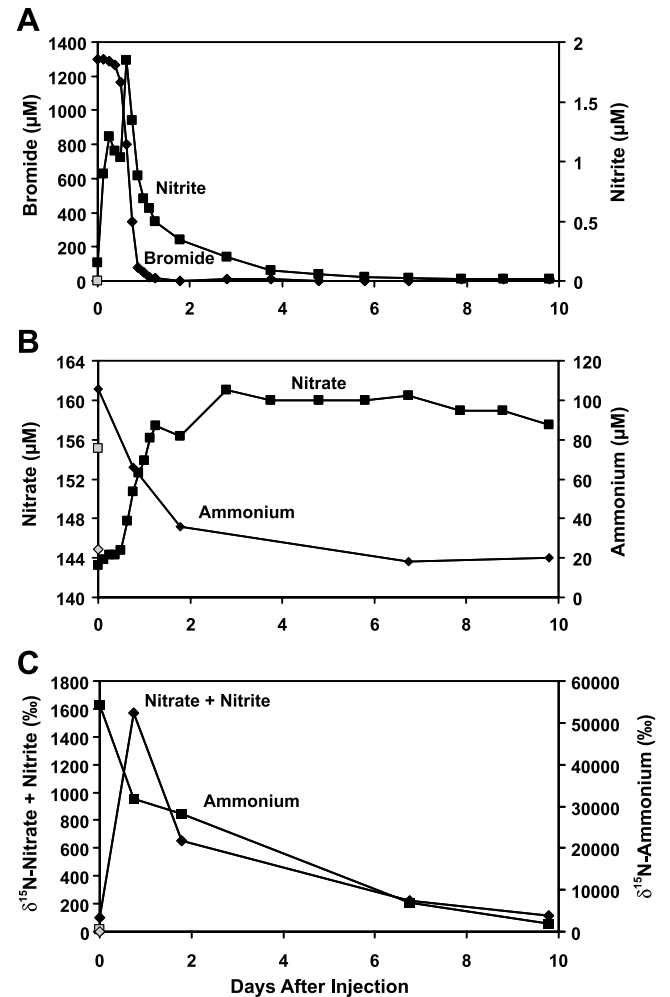


Figure 3. Time course of concentrations and $\delta^{15}\text{N}$ values at well F593-M0207 for the 1998 tracer experiment to assess subsurface nitrification using $^{15}\text{NH}_4^+$. (A) Bromide and nitrite concentrations. (B) Nitrate and ammonium concentrations. (C) $\delta^{15}\text{N}$ for ammonium and nitrate + nitrite. Gray symbols represent pre-injection values.

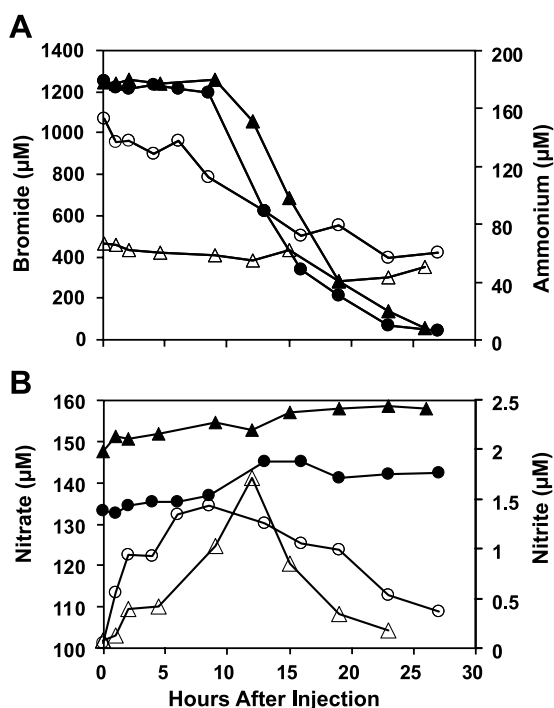


Figure 4. Time course of two single-well injection experiments with added ammonium, 50 μM (triangles) and 150 μM (circles), near the top of the *in situ* ammonium gradient (ports 4 and 5) at well site F593. These are Tracer Tests 2 and 3, respectively (listed in Table 1). Solid symbols are (A) bromide and (B) nitrate concentrations. Open symbols are (A) ammonium and (B) nitrite concentrations.

resulted in a smaller tracer cloud and a shorter time period until the bromide concentrations returned to background. Control injections of unamended ground water or ground water amended only with bromide resulted in a minimal change in nitrate, nitrite, or ammonium concentrations from background (data not shown).

In situ rates of nitrate and nitrite production were calculated from the concentration increases during the

time interval when the bromide concentration was constant (Table 2). Generally, this interval was from 0 to 8–10 h. Rates of nitrate accumulation were 3- to 6-fold higher than for nitrite. Rates for total ammonium oxidation (nitrate + nitrite) were very similar [$0.24\text{--}0.28 \mu\text{mol (L aquifer)}^{-1} \text{h}^{-1}$] for the two different ammonium concentrations tested (Table 2). On the other hand, the rate of the ammonium concentration decrease was dissimilar for the two ammonium concentrations tested and greater than the amount oxidized to nitrite and nitrate (Fig. 4A). Dissolved ammonium loss was 0.35 and 1.80 $\mu\text{mol (L aquifer)}^{-1} \text{h}^{-1}$ for the 50 and 150 μM addition, respectively. The additional ammonium loss was due, at least in part, to sorption onto aquifer solids. Additional tests were conducted using ammonium and difluoromethane, a nitrification inhibitor, as injectate constituents. In those tests some nitrate and nitrite production did occur (Fig. 5), but the rates of accumulation, and therefore total ammonium oxidation, were decreased (Table 2). For one test, inhibition was nearly complete, but for a second test that was conducted one port deeper into the contaminant plume, the rates were 50% of the rates without difluoromethane. There was some apparent loss (or consumption) of the difluoromethane in these tests. Difluoromethane concentrations normalized to the t_0 time point showed a decrease relative to bromide and ammonium (Fig. 5).

Single-Well Injection Experiments with Nitrite.

The injection experiments with ammonium indicated that nitrite oxidation to nitrate could be independently assessed with similar injections using nitrite additions. Consequently, six injections were conducted within the apparent zone of nitrification using a set of nitrite concentrations ranging from 1.3 to 20.1 μM (Table 1). The time course for the 4.5 μM injection is shown in Fig. 6. As described by Snodgrass and Kitanidis [65], first-order rate constants for nitrite consumption were determined

Table 2. Effect of ammonium concentration and addition of difluoromethane (DFM) on net nitrate and nitrite production rates for single-well injection experiments at *in situ* oxygen concentrations

Test ^a	Ammonium concentration ^b (μM)	Time points regressed (hours)	Production rate ^c $\mu\text{mol (L aquifer)}^{-1} (\text{h})^{-1}$		
			Nitrate	Nitrite	Total
4 and 5 ^d	12	0–10	0.04	0.01	0.05
2	74	0–9	0.24 (0.07)	0.04 (0.01)	0.28
3	156	0–8.5	0.18 (0.03)	0.06 (0.01)	0.24
Injection with DFM					
6	281	0–8	0.04 (0.06)	0.01 (<0.001)	0.05
7	402	0–10	0.07 (0.03)	0.06 (0.006)	0.13

^a See Table 1.

^b Total concentration (added + background) at t_0 .

^c Parentheses enclose standard error.

^d Mean result of two control injections; one with NaBr, one without.

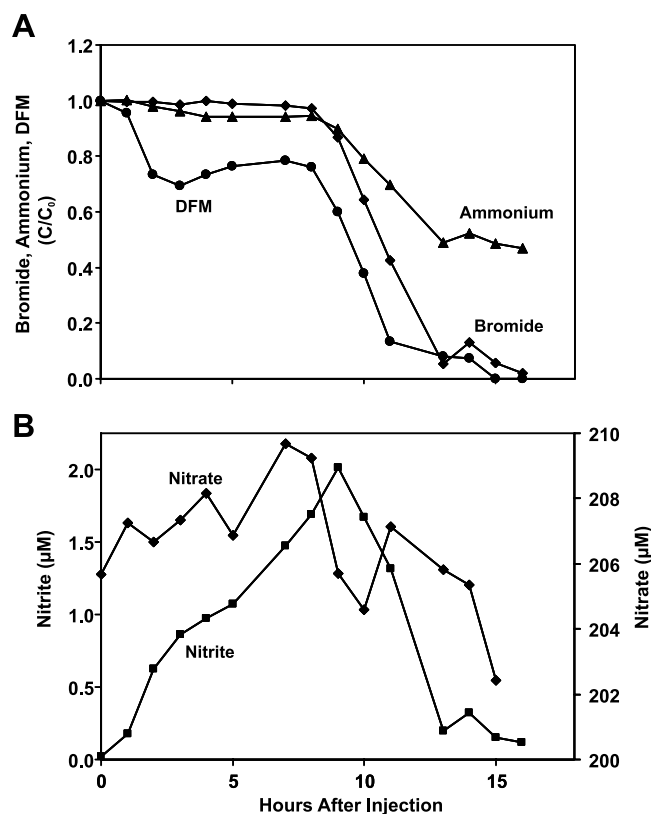


Figure 5. Effect of difluoromethane on *in situ* nitrate and nitrite production for a single-well injection experiment (Test 7, Table 1). (A) Concentration change (normalized to the injection concentration, C/C_0) of bromide, ammonium, and difluoromethane (DFM). (B) Nitrate and nitrite concentrations.

from a natural log plot of the nitrite concentration at each point in time normalized to the bromide concentration (Fig. 6B). In the case of the 4.5- μM nitrite injection, there was no quantifiable nitrite consumption; that is, the slope of the line in Fig. 6B was essentially zero. The same was true for all nitrite concentrations less than about 12 μM (Fig. 7). Measurable nitrite consumption was only evident for the 12.5- and 20.1- μM nitrite injections. The amount of nitrite consumed was small (2–4 μM in 8 h) and was not detectable as a concentration increase in the much larger nitrate pool.

In Situ Determination of Nitrification Potential. The *in situ* capacity for nitrification by the microbial communities within the various geochemical horizons of the aquifer was assessed using single-well injection tests that included relatively high concentrations of oxygen and ammonium as substrates. The results from such a test conducted at the same altitude (port 4) as the ^{15}N and ^{14}N ammonium injection experiments and the nitrite injection tests are shown in Fig. 8. As before, the concentrations of nitrate and nitrite increased with time during the period when the bromide concentration was

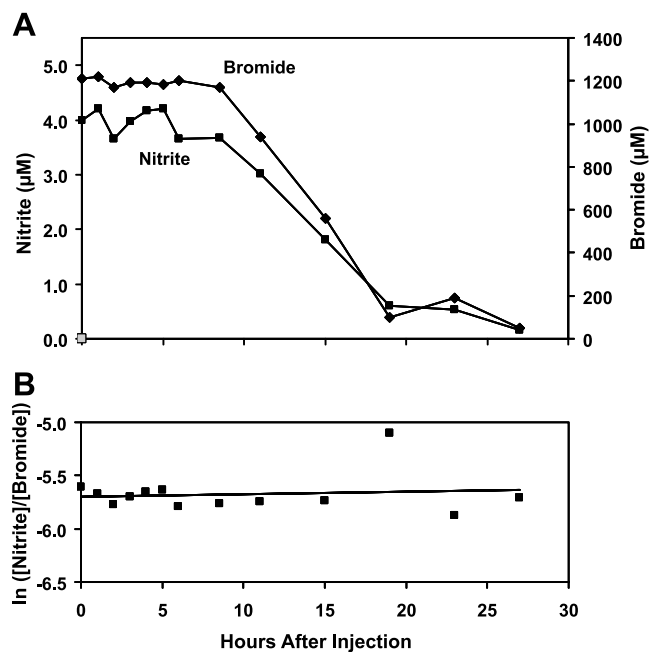


Figure 6. Fate of added nitrite for a single-well injection experiment (Test 9, Table 1) conducted at the top of the *in situ* ammonium gradient (port 4) at well site F593. (A) Nitrite and bromide concentrations. (B) Log-transformed change in nitrite concentration relative to bromide concentration. The line is a best-fit linear regression; negative slope of the line is the first-order rate constant for nitrite consumption. Gray symbol represents nitrite pre-injection value.

relatively constant; those concentration increases were used to calculate potential nitrification rates. Oxygen concentrations during this time interval were consistently greater than 100 μM (Fig. 8B), even in zones that were

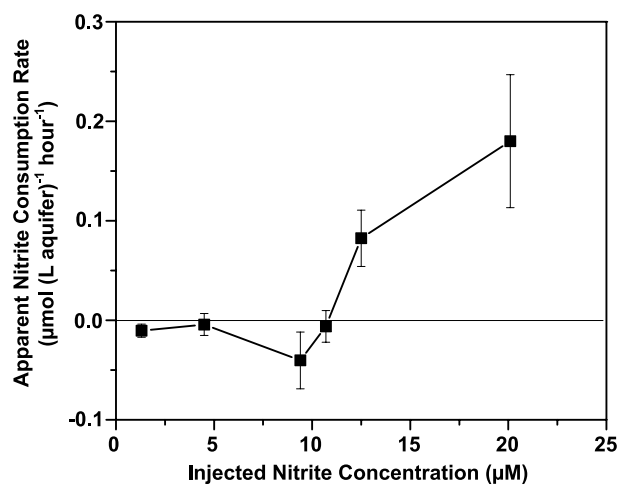


Figure 7. Effect of nitrite concentration on the apparent rate of nitrite consumption for single-well injection experiments conducted at the top of the *in situ* ammonium gradient (ports 4 and 5) at well site F593. Results correspond to Tests 7–13 in Table 1. Error bars represent ± 1 standard error.

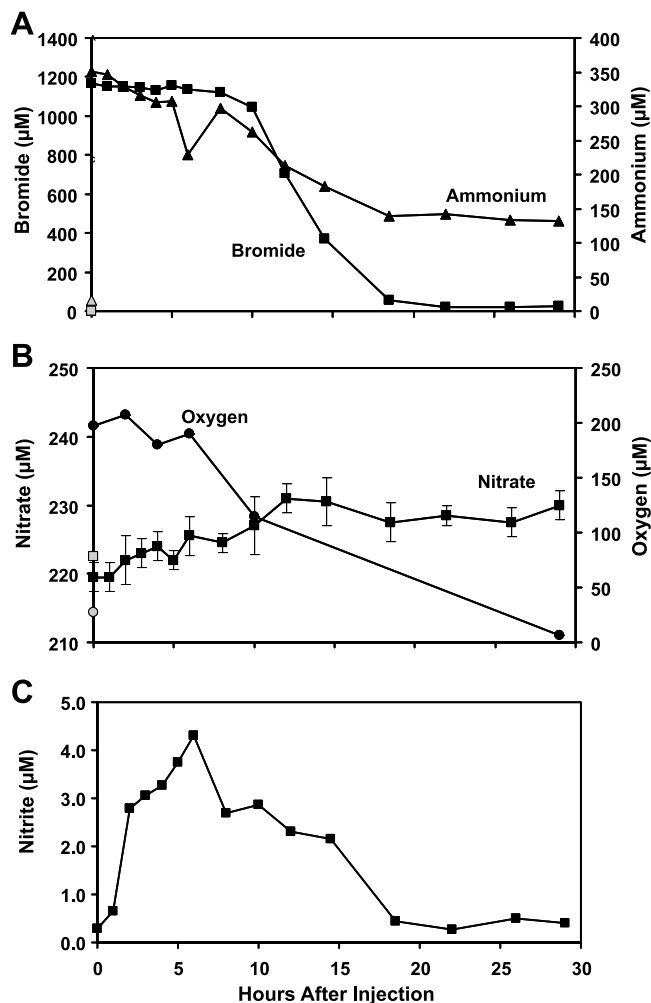


Figure 8. Time course of a single-well injection experiment with added ammonium and oxygen (Tracer Test 16, Table 1) to measure *in situ* nitrification potential at well site F593, port 4. (A) Bromide and ammonium concentrations. (B) Nitrate and oxygen concentrations. (C) Nitrite concentration. Gray symbols represent pre-injection values; error bars are ± 1 standard deviation.

normally anoxic. Rates of nitrification potential were greatest in the shallower regions of the contaminant plume (Fig. 9) near the overlap of the oxygen and ammonium gradients (Fig. 2). The highest rate of total (nitrate + nitrite) net nitrification potential was $0.81 \mu\text{mol} (\text{L aquifer})^{-1} \text{h}^{-1}$ at port 3. Unlike the ports above and below it, the primary ammonium oxidation product at port 3 was nitrate; apparent nitrite accumulation was negligible and quickly leveled off at $\sim 0.15 \mu\text{M}$ for most of the quantified time interval. Rates of total net nitrification potential for ports 2 and 4 were 0.54 and $0.61 \mu\text{mol} (\text{L aquifer})^{-1} \text{h}^{-1}$, respectively. There also was clear evidence for aerobic nitrification potential at port 7 (Fig. 9), a lower, anoxic depth that presumably has not seen oxygen for perhaps as long as several decades.

Discussion

Geochemical profiles, laboratory incubations with aquifer sediments, and DNA extractions from sediments have all suggested that nitrification was active within the Cape Cod contaminant plume [41]. Near the toe of the ammonium zone, ammonium and oxygen gradients overlapped vertically, providing a suitable redox gradient for nitrification (Fig. 2). Nitrate concentrations downgradient suggested a net N oxidation coupled to a change in the rate of transport, with the product (nitrate) moving faster than the substrate (ammonium). Even the dip in the pH gradient suggested active nitrification, a process that is known to produce protons [14, 48]. Initially, our approach for assessing nitrification *in situ* was to conduct natural gradient tracer tests using ^{15}N -enriched ammonium and 3–10 m transport intervals. Similar tests have been used in this aquifer to study denitrification [56, 59], methane oxidation [62], surfactant degradation [36], and bacterial [1, 22, 24], viral [1, 47, 50], protozoan [22, 24, 25], and heavy metal transport [32]. Somewhat unexpectedly, nitrification rates proved to be sufficient to detect nitrite + nitrate production within the tracer cloud before it moved beyond the injection MLS. The injection well results showed a large increase in nitrite concentration and a smaller increase in nitrate (relative to background; Fig. 3). This was associated with a strong ^{15}N signal in the nitrate + nitrite pool, clearly indicating that the effect was due to nitrification.

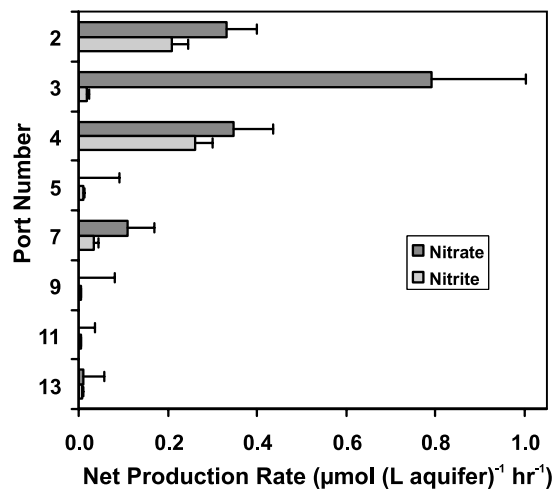


Figure 9. Vertical profile of net production rates of nitrate and nitrite during single-well injection experiments with added oxygen and ammonium to measure *in situ* nitrification potential at well site F593. Production rates were calculated as the slope of the concentration increase during the time interval when the bromide tracer concentration was relatively unchanged (usually 0 to 6–8 h after injection). Error bars are the standard error of the calculated slope. MLS port numbers can be correlated with concentration profiles shown in Fig. 2.

For the ^{15}N tests, both the concentration increases and the isotope enrichment in nitrate + nitrite persisted well after the conservative bromide tracer was no longer detectable (Fig. 3). Ground water velocities in the aquifer are $\sim 0.5 \text{ m day}^{-1}$ [18]. Thus, after 24 h only the ammonium tracer would have remained in the immediate vicinity of the injection well because it was retarded by cation exchange. This chromatographic effect on the tracer cloud is depicted in Fig. 1C. Other potential effects of the injection process, such as unintended changes in the dissolved oxygen or dissolved inorganic carbon concentrations or pH changes, would no longer be in direct contact with the aquifer immediately around the injection port or with the small volumes of water collected from the injection port. With time, the nitrification products decreased as the added ammonium decreased (both concentration and ^{15}N content for each; Fig. 3). The ^{15}N experiments clearly indicated that single-well injection tests could be used as a tool within this aquifer to assess *in situ* nitrification.

The single-well injection tests used in this study are similar to push-pull tests developed at other sites to assess microbial activity in ground water [20, 28, 29, 52, 53]. The difference is that for the single-well tests at Cape Cod the tracer cloud was subjected to the natural hydraulic gradient. Thus, ground water flow velocities within the interstitial pores represented *in situ* conditions during the “incubation” portion of the test. For push-pull tests, ground water is continuously pumped, first into and then out of the injection well, with an optional resting (or non-pumping) phase in between [20, 29]. During pumping, pore flow velocities are directly related to the pumping rate and inversely related to the radial distance from the injection port [20], but always exceed the natural hydraulic gradient. The “pull” portion of push-pull tests is designed to recover a high percentage of the added tracers by the end of the test [20, 29], whereas the tests for this study only remove a small fraction of the tracer cloud during the entire “incubation” and sample collection process. For either approach, first-order rate constants can be derived from the relative differences in concentration between reactive, but non-retarded and non-reactive tracers [20, 65], as was done for the nitrite injections (Fig. 6). However, when a tracer addition was not transported conservatively, as with ammonium, then nitrification rates were derived during the first 6–10 h of a test, when the bromide concentration was essentially constant and differential dispersion between the products and substrate of nitrification was less of a factor (e.g., Fig. 4).

A series of single-well injection experiments were conducted to examine some of the factors affecting nitrification at the interface of the ammonium and oxygen gradients within the Cape Cod aquifer. For comparison purposes, similar tests were conducted

concurrently at separate MLSs but within the same vertical horizon, and as much as possible, without altering the *in situ* geochemistry. It should be noted that there is some spatial variability in the location of the gradients and that the tracer injection process homogenizes the gradient within the vertical interval affected by the tracer cloud. Tracer tests with added ammonium, similar to the ^{15}N test, indicated that the net rate of nitrate production usually exceeded the net rate of nitrite production by 3- to 6-fold, whereas control tests did not produce significant amounts of nitrite or nitrate (Table 2). The addition of difluoromethane, which inhibits nitrification and methane oxidation [42], nearly completely inhibited nitrification in one test and partially inhibited it in a second test. Interestingly, the second test was somewhat deeper into the ammonium gradient than the first test (ammonium concentration was $\sim 100 \mu\text{M}$ higher), but had nearly double the initial concentration of difluoromethane. The latter was slightly less than the recommended concentration for complete inhibition ($\sim 10 \mu\text{M}$), which was determined in closed flask incubations with soils and added ammonium [42]. As in the flask incubations, there was a small amount of difluoromethane consumed within the aquifer during the *in situ* tests. The mechanism of difluoromethane loss is not known.

The rapid accumulation of nitrite during the first few hours of these nitrification experiments suggested that the initial rate of nitrite production exceeded the rate of nitrite consumption. It seemed likely that the consumption rate would be related to the bulk water nitrite concentration, unless the ammonium and nitrite oxidizers were very closely juxtaposed on the aquifer sediments. Thus, nitrite consumption was quantified using a series of tests that titrated the amount of nitrite in contact with the aquifer. Somewhat unexpectedly, nitrite behaved conservatively at concentrations less than about $12 \mu\text{M}$; there was no measurable nitrite consumption except for the two highest concentrations tested, 12.5 and $20.1 \mu\text{M}$ (Fig. 7). Clearly nitrite oxidation must have occurred in the nitrification tracer tests that produced nitrate (the ammonium injection tests). However, in all of those tests, the bulk ground water nitrite concentration did not exceed $12 \mu\text{M}$. It appears, therefore, that the net nitrite produced under those conditions was not turning over at a rate commensurate with nitrate production. Although it is possible that a process other than nitrification produced the nitrite, we note that its production occurred at both high and low oxygen concentrations (Figs. 4 and 8), which would tend to rule out denitrification and dissimilatory nitrate reduction to ammonium, and it did not occur in the absence of ammonium. It is also unlikely that assimilatory nitrate reduction would occur to any significant extent given the presence of the ammonium. It appears that the two steps of nitrification must be tightly coupled

within the aquifer, to the extent that the bulk water nitrite (which is $<1 \mu\text{M}$ at the study site; Fig. 2) is a separate distinct pool from the nitrite that is being oxidized to nitrate. This could be related to intragranular diffusion and sorption of ammonium into quartz and feldspar grains resulting in localized microsites for nitrification. Wood *et al.* [74] demonstrated a similar effect with Li^+ sorption in the Cape Cod aquifer resulting in a time-dependent chemical disequilibrium between the interstitial pores and the interior of sand grains. Within these microsites, the nitrite concentration may be significantly greater than the bulk water nitrite concentration and more closely coupled to the overall rates of nitrification than is the bulk water nitrite concentration.

The apparent rates of nitrification determined in this study at *in situ* oxygen concentrations were 0.02 and $0.24\text{--}0.28 \mu\text{mol (L aquifer)}^{-1} \text{h}^{-1}$ for the ^{15}N and ammonium addition tests, respectively. Even the lowest value is probably significantly higher than the actual *in situ* rate of nitrification. For example, at an ammonium concentration of $100 \mu\text{M}$, the lowest rate would correspond to a turnover time of 353 days for the total ammonium pool (sorbed + dissolved), calculated using an average K_d of $0.7 \text{ mL (g dry sediment)}^{-1}$ and a porosity of 0.39 for this aquifer [9, 37]. Yet on a plume-wide basis, the time for ammonium to travel from the source to this location within the contaminant plume is approximately 40 years (using a velocity of 0.5 m day^{-1} and an ammonium retardation factor of 3 [9, 18]). Within-plume production of ammonium is not likely a significant source, particularly beyond about 0.5 km downgradient from the wastewater disposal beds [58, 64]. Therefore, the measured rates would have exhausted the ammonium pool well before the ammonium reached the study site location. On a site-specific basis, nitrification rates derived from downgradient transport of the ^{15}N ammonium tracer shown in Fig. 3 were more than 10-fold lower after up to 150 days of travel time than the rates derived from samples collected from the injection well [6A].

At present, there are very few reported rates for subsurface nitrification; rates for comparable field sites are lower than the rates found here. Erskine [17] computed ammonium consumption based on concentration changes with time in two landfill plumes. Half-life estimates were 3.5 years in an unsaturated sandstone and 4–10 years in a sand and gravel aquifer. Buss *et al.* [8] speculate that the general range of half-lives for aerobic subsurface nitrification is from 1 to 10 years.

It is not known for certain why the single-well injection experiments would overestimate *in situ* rates of nitrification. We suspect that the discrepancy may be related to the difficulty in maintaining the opposing *in situ* vertical gradients of oxygen and ammonium when injecting the tracer cloud (Fig. 2). For example, tracer

cloud concentrations of both species were somewhat elevated above background for several of the tests conducted during this study (Table 1). Potential disturbance and mixing at the redox interface could be sufficient to stimulate *in situ* nitrification by increasing available oxygen or ammonium. This is an important point because nitrification (and redox reactions in general) commonly may be limited in aquifers by rates of transport of electron acceptors and donors toward each other across redox boundaries or sharp gradients. In the current situation, this must be accomplished by transverse (vertical) dispersion along the plume boundary, which is severely limited. Therefore, the injection of a tracer cloud with dimensions of 1–2 m could cause significant mixing of gradients in which oxygen and ammonium only seem to overlap in a similar or smaller vertical dimension. Nonetheless, the single-well injection tests were useful for assessing nitrification potential within given aquifer zones on a comparative basis. Tests that were similar to nitrification potential assays conducted in laboratory flask incubations were conducted *in situ* in the aquifer by adding non-limiting amounts of ammonium and oxygen. These tests clearly showed that nitrifying activity could commence immediately within many zones of the aquifer. Nitrification potential was highest where background oxygen concentrations were highest and the ammonium concentration lowest, but also was present at locations that had probably been anoxic for several decades. The highest rate of nitrification potential was $0.81 \mu\text{mol (L aquifer)}^{-1} \text{h}^{-1}$ or $0.15 \mu\text{g N (g dry wt.)}^{-1} \text{day}^{-1}$. This is similar to nitrification potentials determined in microcosms using shallow, saturated zone sediments collected under croplands [$0.16\text{--}0.81 \mu\text{g N (g dry wt.)}^{-1} \text{day}^{-1}$] and grasslands [$0\text{--}0.13 \mu\text{g N (g dry wt.)}^{-1} \text{day}^{-1}$] [67], but up to 20-fold lower than nitrification potentials in stream and lake sediments [55].

Recent studies have demonstrated the presence of anaerobic ammonium oxidation in marine and estuarine sediments [13, 68, 71]. This process utilizes nitrate or manganese oxides as an electron acceptor, oxidizing ammonium to nitrogen gas via nitrite. Although it has not been documented in freshwater aquifers, some evidence in a landfill leachate plume suggested that anaerobic nitrification might be occurring at that site [5]. A similar situation occurs within the Cape Cod contaminant plume, in that nitrate, nitrite, and ammonium coexist in an oxygen-depleted portion of the plume (Fig. 2). The nitrate source at site F593 has long suggested aerobic nitrification (see [37]), but there also is the potential that anaerobic nitrification might be occurring. Although the results leave some room for uncertainty, it seems unlikely that the single-well tracer tests within the anoxic and suboxic zones were assessing anaerobic nitrifying activity. Nitrite production could result from such activity, but

not nitrate production. And, for each of these tests, some dissolved oxygen was present, although in some cases the concentrations were very low (Table 1). Likewise, there was no detectable increase above background in $^{29}\text{N}_2$ (a product of anaerobic nitrification from $^{15}\text{NH}_4^+$ [69]) in the small number of samples collected for this analysis during the ^{15}N tests [6A].

In conclusion, this study demonstrated that short-term, single-well injection tests could be used to assess ground water nitrification. A detectable level of *in situ* activity was evident within a 24-h time period for a process for which the primary substrate is subject to reversible sorption onto aquifer sediments and to differential rates of transport relative to the reaction products. These tests were particularly appropriate for examining the *in situ* response to experimental manipulations and for assessing potential rates of nitrifying activity by adding non-limiting substrate concentrations. The latter could have utility as a tool for assessing bioremediation potential. Tests conducted with *in situ* geochemistry appeared to overestimate actual *in situ* rates. This overestimation serves as a reminder that although *in situ* tracer tests within the natural hydraulic gradient represent the least intrusive approximation to the physical environment of the subsurface, all tracer tests, both push-pull and natural gradient, still represent a perturbation that is imposed upon the system.

Acknowledgments

We thank Denis LeBlanc, coordinator of the Cape Cod site, and Kathy Hess, Tim McCobb, and Seanne Buckwalter for field and technical assistance. Thanks also to Eric Strauss and Steven Harris for manuscript reviews. This study was supported by U.S. Dept. of Agriculture (grant no. 95-37101-1713) and by the U.S. Geological Survey Toxic Substances Hydrology Program and the U.S. Geological Survey National Research Program. The use of trade or product names in this report is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.

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