Measuring Nitrogen Attenuation in Septic-Contaminated Coastal Groundwater

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Abstract

Anthropogenic inputs of nitrogen (N) to septic systems result in the release of excess N to groundwater, which reduces water quality and causes eutrophic conditions in bodies of water. 52% of Buzzards Bay uses Title V septic systems, which remove little N from effluent. We measured the efficiency of N-removal in an Innovative Alternative (IA) system and found that IA systems were 2.7 times more efficient at removing N from wastewater than Title V. By injecting nitrate into the wastewater plume in the unsaturated zone, we determined that 0%-9% N in the effluent was naturally attenuated in the groundwater. We attempted to increase denitrification rates in groundwater by injecting necessary electron acceptors and donors, nitrate (NO$_3^-$) and an ethanol carbon source, respectively. Upon adding carbon, N-attenuation did not increase because the groundwater remained oxic. Measuring N attenuation in groundwater is complicated by complex hydrology and variable measurement strategies. Septic management solutions are best targeted towards processes that occur within, and not downstream, of the septic system.

Introduction

Septic wastewater is the main source of nitrogen to coastal watersheds (Valiela et al. 1997). Sewage carries nitrogen (N) to groundwater, which contaminates water systems with excess N. A limiting nutrient for many freshwater systems, the presence of nitrogen increases primary productivity. A common side effect is eutrophication, the process by which algal decomposition creates anoxic conditions in which fish and other organisms cannot survive. The loss in biodiversity disrupts the food web, can change ecological function, and lead to a decline in water quality. The extent of N-pollution in West Falmouth Harbor (WFH) put it on the Federal “Dirty Water List.”
In Falmouth, MA, the town of study, about 50% of the drinking water comes from N-polluted sources – namely, groundwater (Table 1). Consuming nitrate can result in methemoglobinemia, or blue baby disease. Nitrite present in the body converts hemoglobin to methemoglobin, which cannot carry oxygen to the body. In most cases, minor digestive or respiratory problems occur, but in severe cases the disease causes brain damage or even death (Shearer et al., 1972). For most adults, nitrate contamination is less of a threat, but has been documented to form with amines in the body, which form nitrosamines, a cancer-causing substance (Shuval and Gruener, 1972).

Most of the pollution in WFH comes from anthropogenic sources: sewage and fertilizer. Sewage, which includes both wastewater treatment facilities (WWTF) and septic system, accounts for 80% of the total N load in West Falmouth Harbor (Buzz. Bay Co., 2017). Of this, WWTFs make up 30% and septic systems make up the remaining 70% of the N load (Fig. 1). Septic systems are critical yet solvable point sources of pollution.

About 50% of the Falmouth population is on septic, and most of these systems are called Title V. The most standard form of septic in Massachusetts, the systems capture solid waste in a tank, and allow liquid waste to leach out into soil. Wastewater eventually percolates to the groundwater, which moves into the Harbor and contaminates the ecosystem with N-species nitrate, NO$_3^-$, and ammonium, NH$_4^+$.

Through the septic process, environmental conditions will alter which species of N are present. In a standard Title V system, urea, the main form of N in human waste, ammonifies to NH$_4^+$, which is released to the groundwater. One solution to septic pollution is through Innovative Alternative (IA) systems, which are modifications to current septic systems which remove N before the wastewater reaches the leach field. They vary in efficiency and may remove very little
N or up to 90% of the N present, (Heufelder et al. 2007). A second approach is to enhance N removal in the wastewater plume downstream of the leach field by creating conditions that foster denitrification.

In this study in Falmouth, MA, we examined a Hoot IA system, which undergoes a two-step process before N reaches the leach field (Fig. 2). First, an aerobic tank nitrifies wastewater from ammonium to nitrate. Next, an anaerobic tank denitrifies nitrate to harmless N\textsubscript{2} gas. Denitrogen gas (N\textsubscript{2}) is biologically unavailable and returns to the atmosphere, thus reducing sewage N input to the water. Denitrification is the key N-removal process.

Denitrification is a microbe-mediated metabolic reduction that uses nitrate as an electron acceptor to respire in the absence of dissolved oxygen. Various forms of labile carbon serve as the electron donor (Eqn. 1, Schlesinger and Bernhardt, 2013).

\[ 5\text{CH}_2\text{O} + 4\text{NO}_3^- + 4\text{H}^+ \rightarrow 2\text{N}_2 + 5\text{CO}_2 + 7\text{H}_2\text{O} \]

Nitrate reduction is limited by available carbon and nitrate compounds. The availability of both compounds will vary based on the chemical conditions of the soil (Pabich et al., 2001). Past research has shown evidence of denitrification in groundwater when injected NO\textsubscript{3} does not disappear faster than a tracer (Korom, 1991).

In order to better understand septic-water dynamics and to consider management solutions for the future, we pose three questions:

1) How efficiently does an IA system remove nitrogen?
2) When effluent leaves an IA system, is N attenuated in the groundwater?
3) Can denitrification be increased by adding carbon to the effluent leaving the leaching field?
We measured N attenuation in the groundwater leaving the leachfield of an IA system and quantified the effect of carbon on N attenuation rates by installing a series of wells. To measure attenuation, we injected a solution containing a conservative tracer and source of nitrate to measure how much nitrate was taken up relative to the tracer. To test for denitrification, we also injected ethanol to compare N attenuation rates in the groundwater with and without a carbon source.

Methods

We installed wells in a backyard with a Hoot IA system. Over 25 wells were installed to attempt to map the groundwater plume, but only seven indicated presence of water from the leachfield. To create a horizontal gradient, (Fig. 3a), the site had four wells (A, B, C, 1) in the leachfield, and three wells downstream of the leachfield in the direction of groundwater flow (2, 3, and 4) each 1.5 m apart. The leach field contained lysimeters, which are porous clay wells designed to extract soil water in the unsaturated (vadose) zone via a pressurized vacuum. Lysimeters were 1.5 m and 2.5 m below ground surface. Downstream wells were installed at three depths characterized as shallow (s), shallow-intermediate (si), or intermediate (i) (Table 2). Multiple depths create a vertical gradient to measure water flow (Fig 3b).

We conducted three injections, in which groundwater was pumped up from below the leachfield, mixed with a tracer-containing solution, and pumped back down into the aquifer. Over the following 70 hours, water samples were taken at wells in the downstream plume of the injection site. We ran three injections total: one to determine the timing of water flows in the well field, one to quantify NO$_3^-$ attenuation in groundwater, under existing conditions, and one to test the effect of NO$_3^-$ uptake when carbon is added to the effluent leaving the leaching field.
We took samples by pulling up water with a 60 mL syringe, filtering it through a 20 mm non-ashed GF/F Swinex filter, and into a 20 mL scint vial. Samples were brought to the lab on ice. All nitrate samples were analyzed by absorbance on a Lachat XYZ Autoanalyzer 260 series. All ammonium samples were colorimetrically analyzed via the phenol hypochlorite method on a Cary 5000 spectrophotometer (Solarzano, 1969).

**Injection 1: Determine timing of water flow**

We pumped up 36 liters of groundwater from Well 1s, to which we added a pre-mixed solution of 500uM NaNO₃, 20mL rhodamine, and 1L deionized water (DI) to the groundwater and mixed thoroughly. The water was pumped back down into Well 1s using a geopump. We took samples from each well in Fig. 3b over 95 hours. The "clock" for this experiment started 15 minutes after all the water was pumped back down. Injecting a large volume of water (37L) into a tightly porous reservoir creates a bulge of liquid in the system, so some water flows backward against the normal flow direction. By waiting 15 minutes, the ‘bulge’ has mostly equilibrated and water again flows in the normal direction.

A total of 195 samples were taken over 95 hours. The rhodamine dye acted as a tracer. It has a deep red color, and dilutes as it moves away from the injection sites, so as we pulled samples we could check for pink water and optically determine if the injected fluid had reached a downstream well.

We analyzed for nitrate and rhodamine (Rh) concentration in water samples. Pigmented nitrate samples were diluted before Lachat analysis and then run with the cadmium column on and off in order to subtract the color interference from Rh. Rhodamine was measured by fluorescence on a Spectra Max M2 Microplate Reader. Light was emitted at 530 nm and measured at 555 nm.
Injection 2: Nitrate addition and $\text{NO}_3^-$ attenuation

We pumped up 36 L of groundwater from Well 1s, to which we added a pre-mixed 1000 uM $\text{NO}_3^-$ and 1000 uM KBr solution. Sampling methods are the same as injection 1. Some samples were taken using a geopump to pump water into a 60 mL biological oxygen demand (BOD) bottle. We measured oxygen content of these water samples using a Hoch LTR oxygen probe. The second injection lasted 48 hours and included 12 total sampling times. We collected 77 total samples. Ammonium samples were preserved with 10 μL of 6M HCl and later analyzed colorimetrically. We measured the conservative tracer, bromide, on a Dionex ion gas chromatograph. The amount of $\text{NO}_3^-$ or Br present is represented as a percent remaining of the original injected concentration (Eqn 2). N attenuation is calculated as the difference between the expected and actual nitrate concentration. Expected $\text{NO}_3^-$ was determined by calculating how much bromide had been diluted by water from behind (Eqn. 3).

Injection 3: Carbon addition and enhanced denitrification

Using a geopump, we pumped up 36 L of groundwater from Well 1s, to which we added a solution containing 1000 μM $\text{NO}_3^-$, 1000 μM KBr, and 0.1%v/v ethanol. Ethanol was chosen as a carbon source because of its low cost, its availability to a diverse microbial community (Cherchi et al, 2009), and its high rates of denitrification (EPA, 2013). We injected 0.9 g COD/L $\text{H}_2\text{O}$, assuming that there are 907 g COD/L ethanol, which results in 1mL ethanol/L $\text{H}_2\text{O}$. We filled BOD bottles to determine dissolved oxygen (DO) content. We preserved NH$_4$ samples with 5N HCl. Nitrate, ammonium, bromide, and oxygen were determined following previous methods. The third injection lasted 43 hours and included 12 sampling intervals. We collected 55 total samples.

Results
Initial baseline data for water chemistry showed that in the leachfield where the contaminant plume is releasing nutrients, NO$_3^-$ ranged between 2000 uM at 1m below ground surface and 500 uM 2.5 m below ground surface (Fig. 4). Nitrate decreased with depth in the unsaturated zone. In Wells 1-3 downstream of the leachfield, nitrate concentration increased with depth, ranging from about 50 μM at 0.5 meters to 250 μM at 1.5 meters below water table (Fig. 5). Ammonium concentrations were very small.

Injection 1:

Water appeared in wells 1-4 sequentially. The rhodamine concentration indicated that the bulk of the water mass passed through Well 1 in twelve hours, arrived at Well 2 after 20 hours elapsed, and passed Well 3 after 32 hours elapsed (Fig. 6). The water was most densely concentrated at the shallow-intermediate depth, 1.5 m below the water table. At Well 1, the max amount of nitrate was 100% of original, well 2 peaked at 61% of injected concentration, and Well 3 peaked at 54% of the injected concentration. About 50% of water leaving the leachfield stayed fairly concentrated in the dominant direction of groundwater flow, and the remaining water disperses to mechanical mixing.

Injection 2: Nitrate addition

Nitrate concentrations closely followed the KBr tracer, indicating that measured NO$_3^-$ came from the injected mix. Bromide cannot be uptaken biologically and its decrease in each well was caused only by dilution from incoming water. Nitrate did not decrease significantly compared to the tracer, indicating that decreases in NO$_3^-$ concentrations were caused by dilution and not attenuation (Fig. 7, Table 3). Wells 1-3 captured the most direct plume of groundwater from the leach field. In each well the peak of NO$_3^-$ and Br represented the arrival of the
contaminant plume as water flowed downstream. Each peak was subsequently lower than the previous because water also diffused laterally post-release from the leach field.

The injected mixture was 990 μM NO$_3^-$ and 968 μM Br. The initial concentration of Well 1s was 44.8 μM NO$_3^-$ and 116 μM Br. At 0.25 hours post-injection, concentrations peaked at 100% of their initial value. The plume declined, and left Well 1 after 12 hours. Decline in bromide indicated dilution. Nitrate concentration was slightly higher than Bromide.

In Well 2si (1m below water table) the initial NO$_3^-$ concentration was 70 μM. Water began entering the well 4 hours post-injection, peaked at 14 hours, and left after 20 hours. At its maximum, NO$_3^-$ and Br were 68% and 69% of their original concentration, respectively. Nitrate patterns mimic those of bromide, indicating that most N remained in the water. In Well 2, 9% NO$_3^-$ was attenuated in the groundwater (Table 3).

Well 3si (1m below) intercepted water downstream of 2si. The initial nitrate concentration of well 3si was 51.2 μM. Water began entering the well at 20 hours since injection, peaked at 27 hours, and left by 37 hours elapsed. At its maximum, NO$_3^-$ and Br were 47% and 53% of their original concentration, respectively. In Well 3, 0% N was attenuated in the groundwater. In all wells, ammonium made up less than 1% of the total N output. Ammonium levels increased slightly, but were insignificant when considered in relation to total N output.

**Injection 3: Carbon addition**

With a carbon addition, N attenuation did not increase (Fig. 8, Table 3). Water was injected at 951 μM NO$_3^-$ and 1000μM Br. Water moved through Well 1 for the first ten hours, spiking at 0.25 hours elapsed, and 5% of the present N was attenuated (Eqn 2, Table 3). The highly concentrated injected NO$_3^-$ also corresponded with an immediate 400% decrease in NH$_4$. 

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From the time of injection to hour 19, oxygen levels decreased from 6.9 mg/L to 5.1 mg/L O2. At hour 23, O2 increased to 6.8 mg/L and decreased again to 4.7 mg/L at Tfinal, 45 hours (Fig. 9a).

In Well 2, no nitrogen was attenuated. Nitrate and bromide concentrations reached maximum at 12 hours, reaching 60% and 50% of their original injected values, respectively. Ammonium fluctuated greatly, decreasing in concentration as water approached and departed Well 2, but peaking at 12 hours, when the NO3− and Br peak occurred. Oxygen followed a similar pattern as the previous well. It declined steadily from the time of injection until 23 hours elapsed, dropping from 7.3 mg/L to 4.5 mg/L. After this O2 levels increased and again decreased until the final sampling time (Fig. 9b).

In Well 3, no N was attenuated with the addition of carbon. Nitrate and bromide concentrations reached maximum at 32 hours, reaching 64% and 52% of their original injected values, respectively. Ammonium had insignificant fluctuations and largely remained stable. Oxygen levels steadily decreased from 6.6 mg/L to 4.1 mg/L over the first 25 hours, after which they spiked to 7.05 right before the injected mix approached Well 3. At the peak NO3− concentration, O2 levels decline to 4.6 mg/L once more (Fig. 9c).

**Discussion:**

**Initial chemistry**

Nitrate levels decreased throughout the unsaturated zone likely because denitrification was still occurring. As NO3− increased below the water table, one explanation may be that dissolved organic carbon (DOC) was decreasing (Pabich et al, 2001), resulting in fewer electron donors present for NO3− removal. While we did not measure DOC values, the pattern found by Pabich (2001) may explain rising nitrate.

**Innovative Alternative Systems**
Innovative Alternative septic systems are more efficient at nitrogen removal than a standard Title V. Compared to a Title V, which releases 35.8 mg/L N-DIN, we measured that the IA system is 30% more efficient at removing N (Table 4). Of the total N released, over 90% is NH₄, and only a small portion is NO₃⁻. Both NH₄ and NO₃⁻ can cause eutrophication in coastal water. NH₄ must convert to NO₃⁻ before being reduced to N₂, so more NO₃⁻ is preferable to NH₄ in order to increase likelihood of denitrification. Of the 11.0 mg/L N-DIN released by the innovative alternative system, less than 1% is in ammonium form, and the remaining 99% is nitrate. Because of its smaller output and preferable N-composition, our IA site is preferable to Title V for reducing N pollution.

While the studied Hoot IA system is efficient, it is not the most efficient. Other IA solutions include the Layer Cake, which creates an anoxic zone underneath the leachfield through layers of sand, silt, and woodchips that act as a carbon source to foster denitrification. The surface aquifer water coming out of a Layer Cake contains 0.4 mg/L N-DIN, which is more efficient than the 0.6 mg/L N-DIN in the surface of the Hoot aquifer. Efficiencies within systems vary.

**Attenuation post-release from System**

Processes inside a septic system are the most critical to N attenuation, because little uptake occurs in the groundwater once effluent is released from the leachfield. Using a rhodamine tracer to measure attenuation created error because the tracer sorbed to some particles in the aquifer, which made the water appear to have a longer residence time than reality. The clear KBr tracer provided more accurate results.
Injecting 1000uM nitrate into the groundwater showed that on average, 3% of the N was attenuated. Little biological uptake occurs likely due to oxic conditions because organic matter is not available to denitrifiers (Postma et al, 1991; Starr and Gillham, 1993). With the presence of dissolved oxygen (DO), heterotrophic microbes do not require NO$_3^-$ as an electron acceptor.

Natural attenuation in the groundwater is minimal, indicating that any N leaving the leachfield will remain in the groundwater.

When carbon was added, an average of 1.6% N was attenuated. We expected to see lower nitrate concentrations because carbon additions have been shown to create anoxic conditions that foster denitrification (Pabich, 2001). Instead, nitrate patterns were almost exactly the same in each injection (Fig. 10) External carbon did not increase denitrification because we did not add enough C to drive the system anoxic. Microbes in the system responded to the carbon addition and began to consume the oxygen present, but not enough C was present to sustain increased respiration rates, so oxygen levels rose as respiration declined. Had the system already been anoxic, 0.1% v/v ethanol would likely have provided sufficient carbon, but creating and maintaining anoxia would require more.

Experimentally, increasing the carbon addition is possible. We added 1 mL ethanol/1 L H$_2$O, determined from past research (Nyberg, 1996) and the chemical oxygen demand (COD) of ethanol (EPA, 2013). While possible, increasing the external carbon source in an individual septic system may not be a practical management solution. Large scale wastewater treatment plants use a maximum of 1.5 g COD/L H$_2$O to enhance nutrient removal (EPA, 2013). We injected 0.9 g COD/L H$_2$O, which is on the high end of what is currently used. If external carbon additions were used on a network of septic systems or a watershed, the cost and quantity of necessary carbon would be high. Additionally, external carbon would require high maintenance
to inject each septic system continually as waterflushes through. External carbon additions in WWTFs are centralized and are an efficient use of time and carbon source, but may not be as practical for individual septic systems.

Challenges to study

Difficulties in measuring attenuation also occur from the complex hydrology on Cape Cod, which is characterized by unconsolidated coarse-grain sandy sediment of glacial origin (Valiela et al, 1997). In each of the three injections, we lost the tracer at Well 4, which was 4.3 m downstream of the injection well. One of two options may have occurred: first, the tortuosity of the aquifer sediment could have altered the flow direction. Our site has complex hydrology.

More than 20 wells were set up in the expected groundwater plume, but effluent moved in multiple directions. It is possible that underground complexities resulted in more unexpected flow patterns. Second, the sediment may have changed to a more poorly-sorted, porous material that results in increased flow rate. In this case, the water would have left Well 3, and moved through Well 4 very quickly. Examinations of the aquifer material, water table head and gradient, and hydraulic conductivity are necessary to further determine water flow, but with present data, we can determine that water flows approximately 1.3 m laterally over a ~12 hour period.

Nitrogen attenuation was lower during the carbon injection than during the nitrate-only injection, which shows a trend opposite to what we expected. The variability in these measurements supports a historic difficulty in measuring groundwater attenuation (Keeny, 1986; Korom, 1992). Because of these difficulties, N attenuation may be underrepresented. Evidence of measurable denitrification (20%-60%) in sandy aquifers occurred in Ontario and Lake Michigan (Gillham, 1991; Cherkauer et al., 1992). The complex nature of groundwater has resulted in
models that predict N-transfer through Cape Cod groundwater to be ~35% (Valiela et al, 1997).

Our measurements fall far below this value but our measurements occurred over short time (~50 hours) and distance (3m) scales.

Our three major findings lead several conclusions: First, attenuation rates are low, so most nitrogen leaving the septic system and entering groundwater remains in groundwater. Second, external carbon additions to the effluent of individual septic systems prove timely and care-intensive. Third, hydrology of groundwater flow is complex and challenging to measure precisely. These three findings show that improving septic design is an ever-more critical task because it is one aspect of nitrogen pollution that can be controlled. Continuing research on wastewater management and N-flow through aquifers is necessary to reducing nitrogen pollution and improving water quality of coastal ecosystems.
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Works Cited


