ABSTRACT

Title of Document: WHOLE STREAM NITROGEN UPTAKE AND DENITRIFICATION IN A RESTORED STREAM OF THE CHESAPEAKE BAY

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Little is currently known about the effects of stream restoration practices on in-stream processing and nitrogen removal. This study quantified nitrate retention in a survey of two restored and two unrestored streams in Baltimore, MD using unenriched nitrate additions, denitrification enzyme assays, and a ^{15}N isotope tracer addition in one of the urban restored streams, Minebank Run. Denitrification potential in sediments was variable across streams, whereas nitrate uptake length was significantly correlated to surface water velocity, which was lowest in restored streams. *In situ* denitrification rates in Minbank Run were 153 mg NO_3^-- N m^{-2} d^{-1}, and approximately 40% of the daily load of nitrate could be removed over a distance of 220.5 m. Stream restoration projects that decrease water velocity and increase residence time may lead to considerable rates of nitrate removal through denitrification.

WHOLE STREAM NITROGEN UPTAKE AND DENITRIFICATION IN A RESTORED STREAM OF THE CHESAPEAKE BAY

By

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Chapter 1: Introduction

Humans have greatly altered the global nitrogen cycle through the application of agricultural fertilizers, the combustion of fossil fuels, and widespread changes in land use (Vitousek 1997). In particular, rapidly urbanizing areas may represent a major source of nitrogen to many streams, rivers, and estuaries (Castro et al. 2003). Both nitrogen concentration and export to rivers has been strongly correlated to human population density and wastewater inputs (Peierls et al. 1991, Howarth et al. 1996, Valiela et al. 1997, Castro et al. 2003). Population growth and urbanization is expected to continue increasing in coastal regions of the U.S. in the future (NOAA 2004). In the Chesapeake Bay region, increased nitrogen export from land use change has contributed to eutrophication and hypoxia, decreased plant diversity, and formation of harmful algal blooms and fish kills (e.g. Jaworski et al. 1992, Boesch et al. 2001, D'Elia et al. 2003, Glibert et al. 2001, Kemp et al. 2005, Paerl 2006).

Although human activities have greatly increased nitrogen inputs to watersheds, in-stream processing of nitrogen by headwater streams may be an important sink for anthropogenic nitrogen (Alexander et al. 2000, Bernhardt et al. 2005a). Previous studies have reported substantial amounts of nitrogen retention in headwater streams (Alexander et al. 2000, Peterson et al. 2001) and in larger rivers (Seitzinger et al. 2002, Wollheim et al. 2006). In-stream retention of nitrogen can occur by several mechanisms such as uptake by autotrophic algae and plants, heterotrophic uptake by microbes, storage in sediments, and microbial denitrification (e.g. Böhlke et al. 2004, Grimm et al. 2005). Biotic uptake, the conversion to plant and microbial biomass, and sediment storage, however, only provide a temporary

means of reducing nitrogen in stream water, whereas the process of denitrification results in permanent removal of nitrogen from the ecosystem through the production of dinitrogen gas (N_2) and nitrous oxide (N_2O). *In situ* measurements of denitrification in streams and rivers have shown that denitrification can account for approximately 15% of nitrate uptake in a forest stream with very low concentrations of nitrate (Mulholland et al. 2004), and approximately 50% of nitrate uptake in an agricultural stream with higher concentrations of nitrate (Böhlke et al. 2004).

Recent studies have shown that stream degradation due to urbanization can decrease the ability of streams to process and remove nitrogen by increasing channel incision and decreasing the hydrologic connectivity between the stream channel and riparian areas (e.g. Groffman et al. 2002, Walsh et al. 2005). Additionally inputs of nitrogen from human land use often exceed biotic demand in urban streams and can saturate the ability of headwater streams to attenuate increased nitrogen (Gücker et al. 2006, Kaushal et al. 2006). Many researchers have found increased uptake lengths for nitrogen in urban streams (e.g. Grimm et al. 2005, Meyer et al. 2005, Gücker et al. 2006). For example, Grimm et al. (2005) found uptake lengths to significantly and positively correlate with both stream discharge and nitrate inputs in urban desert streams and Gücker et al. (2006) observed decreased ammonium uptake efficiency with increasing nitrogen concentration. Therefore, urbanization may not only increase the amount of nitrogen entering streams, but it may also simultaneously reduce a stream's ability to efficiently process and remove it.

In an attempt to offset the negative effects of urbanization and other forms of land use change on streams and riparian zones, over 37,000 river restoration projects

have occurred or are currently underway within the United States (Bernhardt et al. 2005b). The goals of many of these projects are to restore water quality, riparian zones, improve in-stream habitat, reduce channel erosion and promote bank stability (e.g. Bernhardt et al. 2005b, Hassett et al. 2005, Palmer et al. 2005, Wohl et al. 2005). A recent study investigating nitrate removal in the hyporheic zone of Minebank Run, a restored stream Maryland, USA found significantly higher *in situ* denitrification rates in a restored stream reach than an unrestored degraded stream reach (Kaushal et al. in review). In particular, restoration practices increasing hydrologic connectivity and residence time in stream subsurface zones may be important in stimulating denitrification and N uptake (Kasahara and Hill 2006, Kaushal et al. in review). A recent study at the whole channel scale, also observed relationships between decreased flow velocities in response to restoration with reduced downstream transport of nitrogen in an agricultural stream (Bukaveckas 2007). Similarly, another recent study found that stream restoration can increase transient storage with potential effects on N retention at the reach scale (Roberts et al. 2007). Little is currently known about the effects of restoration practices that foster increased hydrologic connectivity between stream channels and floodplains on whole-stream rates of nitrogen removal. Theoretically, restoration techniques that decrease channel incision and increase hydrologic "connectivity" between streams and floodplains should lead to higher retention and removal of nitrogen through promoting biotic uptake and microbial denitrification (e.g. Tockner et al. 1999, Boulton 2007). Although the management implications of stream restoration are great, there has been little work

actually documenting changes in denitrification and nitrate retention as a result of stream restoration (Bernhardt et al. 2005 b, Hassett et al. 2005).

The objectives of this study were to (1) conduct preliminary measurements of nitrate uptake and sediment denitrification potential in a survey of two restored and two unrestored streams in Baltimore, Maryland, U.S.A. and (2) to quantify rates of *in situ* denitrification in a restored urban stream using ^{15}N stable isotope techniques. The restored stream that was the primary focus of the present study was Minebank Run. The study reach of this stream was restored with low, hydrologically connected banks to promote flooding and dissipate erosive force for stormwater management (Kaushal et al. in review). The work described here was part of two larger research programs; the Baltimore Ecosystem Study (BES, http://beslter.org), an urban long-term ecological research (LTER) project funded by the U.S. National Science Foundation, and an intensive hydrological and biogeochemical study of a restored stream, Minebank Run, funded by the U.S. Environmental Protection Agency (Mayer et al. 2003). The BES includes studies of forested reference and urban/suburban streams (e.g. Groffman et al. 2004, Kaushal et al. 2005) and the EPA study focuses on studying the effects of restoration at Minebank Run on hydrological, geomorphological, and biogeochemical changes related to nutrient retention and removal (e.g. Mayer et al. 2003, Groffman et al. 2005, Kaushal et al. in review).

Chapter 2: Methods

Study Design and Background on Different Techniques

Preliminary measurements of sediment denitrification (similar to Groffman et al. 2005) and nitrate uptake using reach scale nutrient additions of nitrate (similar to Stream Solute Workshop 1990) were conducted in a survey of two restored streams and two degraded urban streams in the Baltimore metropolitan area from June - August of 2006. These types of measurements are relatively commonplace and were conducted to provide a context for the detailed measurements of water chemistry and *in situ* denitrification rates at Minebank Run, also conducted during the summer of 2006, using ^{15}N isotopic tracer techniques (similar to Mulholland et al. 2004). All study sites were located in Baltimore County, MD in the Gwynns Falls and Gunpowder Falls watersheds (Figure 1).

The most commonly used method to measure denitrification in stream sediments is the acetylene inhibition method (Seitzinger 1993, Greene 2005, Groffman et al. 2005). This method uses acetylene to block the final step of denitrification, thereby allowing the accumulation of N_2O instead of N_2, which alleviates the problem of distinguishing between N_2 produced by denitrification from that which already exists in the atmosphere (Seitzinger 1993, Groffman et al. 2006). The acetylene inhibition method has led to a large body of denitrification estimates and has increased our understanding of the process. A problem of this technique, however, is that acetylene not only blocks the reduction of N_2O to N_2, but also inhibits nitrification, thus potentially underestimating denitrification coupled with

nitrification (Seitzinger 1993, Groffman et al. 2006). Another potential problem is that this technique does not provide removal rates at the whole stream reach scale.

Nutrient additions are the most frequently used method of determining nitrate uptake lengths at the reach scale in many streams largely due to their low cost. Nutrient additions use metrics of nutrient spiraling theory that describe the path of a nutrient molecule within the stream, incorporating both biogeochemical cycling and downstream transport (e.g Newbold 1981, Stream Solute Workshop 1990, Mulholland 2002, Doyle 2005, Grimm 2005). Briefly, nutrient additions involve artificially elevating concentrations of the nutrient of interest in the stream (nitrate in the present study), above ambient concentrations through additions of known quantities of the nutrient coupled with additions of a conservative tracer to compare uptake of the reactive nutrient (Newbold 1981, Stream Solute Workshop 1990). The nutrient/conservative tracer solutions are dripped into the stream reach and monitored until the system has reached plateau concentrations, indicating a conservative tracer steady state (Stream Solute Workshop 1990, Webster and Ehrman 1996). The conservative tracer is used to correct downstream values of the nutrient for dilution (Stream Solute Workshop 1990, Webster and Ehrman 1996). Using the corrected average concentration of the nutrient, uptake lengths, uptake rate, and uptake velocity can be calculated using information from the slope of the regression of the ln corrected concentration of the nutrient plotted against the downstream distance at which the nutrient was sampled and equations describing its decay over distance (e.g. Stream Solute Workshop 1990, Grimm et al. 2005, Ensign and Doyle 2005). Three of the most commonly measured metrics associated with nutrient spiraling theory are:

uptake length, $S_{w,}$ (the mean distance traveled by a particular nutrient atom or ion dissolved in water from its release until its removal from the water column); uptake rate, U, (the rate at which a particular nutrient is removed from the water column); and uptake velocity, V_f (the vertical velocity at which a particular nutrient moves throughout the water column until it is taken up in the benthic zone; Newbold 1991, Stream Solute Workshop 1990, Mulholland et al. 2002, Grimm et al. 2005). Using these metrics a stream with high nitrogen retention would have a low uptake length, high uptake rate and high uptake velocity (Grimm et al. 2005).

A potential drawback of nutrient additions of nitrate is that they raise the background concentration of nitrate and may artificially influence processes (Mulholland et al. 2002). In addition, these nutrient additions of nitrate do not measure permanent removal through denitrification, and previous work has shown that increased nitrate may be converted to bioavailable forms of organic N in streams by heterotrophic microbes when labile carbon is abundant (Kaushal and Lewis 2005). The conversion of nitrate to bioavailable dissolved/particulate organic N may need to be considered in estimates of retention under some conditions and can have detrimental and underestimated effects on receiving waters and contribute to eutrophication (e.g. Seitzinger and Sanders 1997).

Recently there has been development of methods using isotope tracers to quantify denitrification rates at the reach scale in streams with high nitrate levels (Böhlke et al. 2004). Currently, two studies have used this technique to quantify *in situ* denitrification rates in a forested stream (Mulholland et al. 2004) and an agricultural stream (Böhlke et al. 2004). Isotope tracer additions require smaller

additions of nutrients, thus ambient nutrient concentrations can often be maintained during isotope additions. Comparative studies suggest that the nutrient addition approach produces longer uptake lengths for PO_4^-, NH_4^+ and NO_3^- than the isotope approach (Mulholland 2002, Grimm 2005), with the increase in uptake length positively related to the increase in nutrient concentration used for the nutrient addition (Mulholland 2002).

With the addition of $^{15}NO_3^-$ to aquatic systems, N_2 can be collected and analyzed for $^{14}N^{14}N$, $^{14}N^{15}N$, and $^{15}N^{15}N$ ratios, allowing for denitrification rates of a reach to be determined (e.g. Middleburg et al. 1996, Böhlke et al. 2004, Mulholland et al. 2004). The advantage of this method is that both direct denitrification of $^{15}NO_3^-$ and coupled nitrification-denitrification can be measured (Seitzinger 1993), with a relatively negligible increase in ambient nitrogen levels. Some assumptions must be made with this method, however, such as: a) complete mixing of $^{15}NO_3^-$ with the $^{14}NO_3^-$ pool occurs, b) inputs of $^{15}NO_3^-$ do not cause overestimation by increasing the pool of available N to denitrify, and c) that the two isotopes diffuse similarly (Middleburg et al. 1996, Groffman et al. 2006). Drawbacks of using ^{15}N tracer additions are that dilution of gas samples by ambient atmospheric N_2 can reduce the sensitivity of this method, and isotope additions can be expensive, especially where ambient nitrate concentrations are high.

Land Use Classifications for Study Watersheds

In order to present land use data using uniform methods, land use characteristics were determined for the 12 digit watersheds of each study site using a 2002 GIS layer of Land Use and Land Cover data of Baltimore County, MD, created

by the Maryland Department of Planning. A series of layers were also created from a digital elevation model (DEM) of the Baltimore County area, obtained from the National Elevation Dataset, to determine the area of each watershed that was upstream of and "contributed" directly to the stream reach sampled. Land use classification of the "contributing" portion of the watershed above the study reaches was determined by the GIS layers and Land Use/Land Cover data described above.

The Land Use/Land Cover data obtained was classified using a modified Anderson Level 2 classification that was much more detailed than necessary for the purpose of this simple analysis. Therefore a more general classification was applied that grouped low density residential and open urban land into a suburban land use category. Medium-density and high-density residential were grouped along with commercial, industrial, institutional, extractive and transportation land uses into an urban land use category. All agriculture land uses were grouped into one category, as were all forested land covers into another category. Finally, all other land covers, water, wetlands, and bare ground, were classified as other.

Site Descriptions for Restored Sites

Minebank Run

Minebank Run (MNBK) is a 2nd order stream located in the Gunpowder Falls watershed, a predominantly suburban watershed within Baltimore County, Maryland (Figure 1). The 12 digit Lower Gunpowder watershed is approximately 11,828 ha with 30% agricultural, 32% forested, 18% urban, 19% suburban land cover, and 1% other land use. Land use for the 113 ha of the contributing portion of the watershed to the study stream reach was 13% forested, 83% urban, and 4% suburban. The

section of Minebank Run chosen for this study was restored in 1998 and 1999 (Mayer et al. 2003). The goal of the restoration was to improve the geomorphic stability of the streambed and reduce channel incision (Mayer et al. 2003). The restoration included techniques such as installing step-pool structures designed to reduce erosion, reshaping the stream banks to reconnect the stream channel to the flood plain, armoring stream banks against erosion with large boulders, reconstructing stream meander features and riffle zones, and re-establishing riparian vegetation (Mayer et al. 2003, Groffman et al. 2005, Kaushal et al. in review). In particular, previous work has shown that this study reach has low, hydrologically connected banks with high *in situ* denitrification rates and substantial hydrologic residence times (Kaushal et al. in review).

Spring Branch

Spring Branch (SPBR), a restored 1st order stream in Baltimore County, MD, drains the suburban Loch Raven watershed eventually emptying into the Loch Raven Reservoir, a major drinking supply for the Baltimore Metropolitan area (Figure 1). Land use for the 12 digit Loch Raven, 9437 ha, watershed was 12% agriculture, 36% forested, 14% urban, 29% suburban and 9% other. Land use of the 188 ha contributing to the study stream reach was 2% forested, 77 % urban, and 20% suburban. The Spring Branch Stream Restoration project began in 1994 and was completed in 1997 (US EPA River Corridor and Wetland Restoration 2002). The goal of this restoration was to manage the flow of the stream to control for erosion and floods (US EPA River Corridor and Wetland Restoration 2002). Restoration features

used included step pools at the outfall channel, plunge pools below pipe outfalls, rip rap in outfall channels and downstream of culverts, catch basins to attenuate flow, and floodplain access for bankfull discharges (US EPA River Corridor and Wetland Restoration 2002). Stabilization of stream banks and enhancement of aquatic habitats were also attempted through the construction of features such as vortex rock weirs, root wad revetments, gravel riffles, step pools, meander bend pools, live brush mattresses, live fascines, live branch layering, as well as live joint planting (US EPA River Corridor and Wetland Restoration 2002).

Site Descriptions for Degraded Sites

Gwynns Falls at Glyndon

Glyndon (GLYN) is the 1st order headwater sub-watershed of the 19,000 ha Gwynns Falls watershed that is monitored routinely as part of the National Science Foundation funded Baltimore Ecosystem Study Long-term Ecological Research (LTER) project (Groffman et al. 2004, Kaushal et al. 2005) (Figure 1). The 4607 ha, 12 digit, Upper Gwynn Falls watershed that Glyndon is within consists of 7% agriculture, 24% forested, 50% urban, 17% suburban, and 1% other land cover. Land cover for the 79 ha of the contributing portion of the watershed was 6% forested, 70% urban, and 24% suburban. The particular reach of the Glyndon stream studied here had visible channel incision and riparian zones consisted largely of mowed lawns extending to the edge of the stream bank. Further details and descriptions of this site and its characteristics can be found in Groffman et al. (2004), Kaushal et al. (2005) and at the BES LTER website, www.beslter.org.

Tributary of Dead Run

DR 5 is a headwater tributary of the larger 3rd order Dead Run stream located in the Gwynns Falls watershed of Baltimore County, MD (Figure 1). Land use for the 12,233 ha of the Lower Gwynns Falls watershed, in which DR5 is located, is 2% agriculture, 14% forested, 75% urban, 8% suburban, and 1% other. Land use for the 189 ha of the contributing portion of the watershed was 6% forested, 85% urban, and 8% suburban. DR5 was similar to Glyndon as there was visible channel incision and little remaining of the riparian buffer. Further details and descriptions of Dead Run and its tributaries can be found in Groffman et al. (2004), Kaushal et al. (2005), and the BES LTER website, www.beslter.org.

Streamwater Chemistry at Minebank Run

Surface water samples from Minebank Run were collected approximately every two weeks for the 2006 water year in the hydrologically connected, low bank, reach studied. Time-series samples for nitrate concentrations were collected at USGS gauged stations 0158397925, Minebank Run at Intervale Court, Towson, Maryland, U.S.A., since June of 2004 (Saffer et al. In Press) (further information on this site location, description, and data from the USGS gauged station can be found at www.usgs.gov). Samples were stored, filtered and analyzed for water chemistry using analytical methods similar to those described below.

Denitrification Enzyme Assays (DEA)

Sediment samples were collected during from each of the four study reaches in June 2006 to measure their potential for denitrification. The upper 10 cm of

sediment was collected using a random sampling design from the measured center of the stream every 25 m (along the 200 m designated reach) and were refrigerated until analysis within a week of collection. Sediment moisture content was determined gravimetrically by drying 5 grams of sediment at 70°C and subtracting the dry weight of the sediment from the wet weight. Denitrification enzyme activity was measured using a short term assay according to protocols described by Groffman et al. (1999). Briefly, 10 grams of a homogenized sediment sample were amended in Erlenmeyer flasks with 10 mL of a media solution containing KNO_3^-, glucose, and chloramphenicol. Acetylene (10% of the headspace volume) was then added to the flasks and samples were incubated under anaerobic conditions for 16 hrs. Gas samples taken at 1 hr, 4 hr, and 16 hr time periods were then analyzed for N_2O using electron capture gas chromatography (Groffman and Crawford 2003, Groffman et al. 2005).

Stream Nitrate Additions

Nitrate injections were conducted at all four sites during July through August of 2006. A solution of KNO_3^- and NaBr was dripped at the upstream location of all four study reaches for 6-10 hours, allowing enough time for all stations to plateau. Concentrations of NO_3^-N are typically between 1 - 2 mg L^{-1} in the urban streams of Baltimore (Groffman et al. 2004) therefore the goal of the injection was to raise ambient stream concentrations of nitrate by at least 500 μg –N L^{-1} so that differences could be detected.

Water samples were collected at six stations just before the start of the nitrate/conservative tracer addition (Pre) and throughout the addition every 30 minutes. One station was located just above the injection location, which was used to monitor background concentration of nitrate and bromide throughout the addition, the second station was located at the end of a mixing riffle, and the four remaining stations were spread out over approximately equidistant intervals throughout the remainder of the study reaches. Stations were located in areas of the stream where the channel was constricted to ensure well-mixed samples at each station (LINX II 2004). Stream study reaches ranged from 74 – 212 m in length. Samples collected before each addition were used to determine ambient concentrations of nitrate and bromide in the stream at each station. Samples taken throughout the addition at each site were used to estimate the time of arrival of the nitrate and bromide at each station as well as to verify and determine when plateau had been reached at each site (Stream Solute Workshop 1990, Webster and Ehrman 1996, LINX II 2004). Samples of water were collected and transported to the University of Maryland Center for Environmental Science Appalachian Laboratory, in Frostburg, MD for filtration and storage. Bromide and nitrate analyses were performed using a Dionex 500 ion chromatograph. Analyses of nitrate, nitrite, ammonium and total nitrogen were performed on all pre and plateau samples using a Lachat Quick Chem 8000 autoanalyzer (Lachat Instruments, Milwaukee, WI).

Reach travel time, stream discharge, and average stream surface water velocity were estimated using channel measurements and analysis of the conservative tracer data. Channel measurements of wetted width and reach length were collected

within the study reaches using a meter tape (Webster and Ehrman 1996). Reach travel time was calculated as the difference between the times at which the [Br-] breakthrough curves for the upstream and downstream stations reached the maximum rate of increase (Houser et al. 2005, Roberts et al. 2007). Average stream surface water velocity (u) was then calculated as reach length divided by reach travel time. Discharge (Q) was calculated as:

$$Q = \frac{Q_{pump} \text{ X } [Br_{inj}]}{(Br_p - Br_b)} \quad (1)$$

where Q_{pump} = the injection rate of the pump, Br_{inj} = the concentration of bromide in the injection solution, Br_p = the concentration of bromide at the station during plateau and Br_b = the background concentration of bromide at the station (Webster and Ehrman 1996, Houser et al. 2005, Roberts et al. 2007). Field measurements of surface water velocity were not used as it was difficult to obtain accurate measurements at such low flows.

Nitrate uptake length in all four reaches was estimated using the nutrient spiraling metrics equations described by Newbold et al. (1981), Stream Solute Workshop (1990), Webster and Ehrman (1996), and others. Plateau concentrations of both nitrate and bromide were corrected for background concentrations. Nitrate was also corrected for dilution using the ratio of nitrate to bromide (Stream Solute Workshop 1990, Webster and Ehrman 1996). Uptake length (S_w) was calculated as the negative inverse slope of the regression line of the natural log of the dilution-corrected concentration of nitrate versus distance downstream (Stream Solute Workshop 1990, Grimm et al. 2005, Gücker and Pusch 2006). The slope of this line is also referred to as the fractional rate of decline of the nitrate, k. Once uptake length

is determined, the two other metrics, uptake rate and uptake velocity, can be calculated using the spiraling metric equations:

$$S_w = -\frac{1}{k} = \frac{Q\,C}{U\,w} \qquad U = V_f\,C \qquad V_f = \frac{Q}{S_w\,w} \qquad (2, 3, \& 4)$$

where S_w = uptake length, U = uptake rate, V_f = uptake velocity, Q = discharge, C = concentration of the nutrient, and w = stream wetted width.

Isotope Addition and Denitrification in Minebank Run

^{15}N-NO_3^- Addition and In situ Denitrification

An isotope addition was conducted at Minebank Run from August 16 – 17, 2006, to provide a more intensive measurement of the three nutrient spiraling metrics as well as actual denitrification rates without raising the ambient concentration of nitrate in the stream. The addition lasted approximately 27 hours. The objective of the longer addition was to allow each station to remain at plateau for several hours such that ^{15}N would be available to microbes at a constant rate and detectable in the dissolved N_2 pool. A solution of 99% ^{15}N labeled KNO_3^- along with the conservative tracer Br^- was dripped in the stream to increase the $\delta\ ^{15}N$ of nitrate in the stream by approximately 20,000 per mil (LINX II 2004). Methods were similar to those used for the nutrient injections except that water samples for Br^- and NO_3^- analysis were collected every hour, instead of every 30 minutes. Two additional sampling stations were also added downstream of those used in the nutrient addition studies to extend the reach to 220.5 m in length.

In addition to taking water samples, as was performed during the unenriched nitrate addition studies, 1 liter water samples were collected at each station before and at plateau for $^{15}NO_3^-$ analysis. Samples were put on ice and then refrigerated upon arrival at the lab. Within 2-3 days, samples were filtered and frozen until analysis could be conducted. Nitrate in the 1 liter samples was reduced to ammonium, using Devarda's alloy, and an ammonium alkaline headspace diffusion procedure was used to remove ^{15}N from the sample (Sigman et al. 1997, LINX II 2004). Filters infused with ^{15}N were then sent to the UC Davis Stable Isotope Laboratory for analysis.

At the same time that samples were collected for analysis of $^{15}NO_3^-$, two 120 mL water samples were collected at each station in 140 mL plastic syringes, affixed with a one-way luer lock valve, for extraction of ^{15}N labeled gases (Mulholland et al. 2004). Samples were carefully checked and all air bubbles removed. Upon collection, syringes were stored under stream water (LINX II 2004). Once all the samples had been collected, a 20 mL helium headspace was added to each syringe via a 60 mL injection syringe, while under stream water to further guard against contamination of N_2 from the atmosphere (Mulholland 2004, LINX II 2004, Hamilton and Ostrom 2007). Sample syringes were then vigorously shaken for 5 minutes to allow the dissolved gases within the stream water to diffuse into the helium headspace (Mulholland 2004, LINX II 2004, Hamilton and Ostrom 2007). Approximately 13 mL of headspace was then injected into 12 mL Labco evacuated exetainers (Labco, Buckinghamshire, England), which were stored in centrifuge tubes filled with water, to prevent diffusion of dinitrogen from the atmosphere into the exetainer (Mulholland 2004, LINX II 2004, Hamilton and Ostrom 2007). Gas

samples were sent to the UC Davis Stable Isotope Laboratory to be analyzed for $^{15}N/^{14}N$ isotopic ratios using isotope ratio mass spectrometry.

Reaeration Rates

The dissolved N_2 concentration in a stream is a function of both N_2 production and atmospheric exchange (Laursen and Seitzinger 2002). In order to account for any $^{15}N_2$ lost to the atmosphere, the reaeration flux of N_2 was determined using a volatile non-reactive gas (propane) and conservative tracer addition during the isotope addition. Whole stream metabolism and the reaeration rate of O_2 were determined with a propane/bromide addition using the two station approach according to the methods described by Marzolf et al. (1994) and adapted by Young and Huryn (1998). Briefly, propane was injected at a constant rate into the stream through two 1.5 m long bubblers attached to a tank through a series of tubes. Dissolved oxygen was measured at each station every 30 min throughout the isotope additions. In order to quantify propane dissolved in the water, three 40 mL water samples were collected at each station before the addition and at plateau in 60 mL syringes. A 20 mL headspace of air, taken away from the stream so as to not have any propane from the injection, was then added to each syringe. The syringes were shaken vigorously for 5 minutes to allow the dissolved propane in the water to diffuse into the headspace of the syringe. Approximately 10 mL of gas sample was injected into a 9 mL vial and analyzed for propane by gas chromatography using standard methods developed by the U.S. EPA (EPA, Ground Water and Ecosystems Restoration Division, Ada, OK). Propane concentration was normalized for dilution using the conservative tracer concentrations. The gas exchange rate of propane was determined by the slope of a

regression of the natural log of the dilution-corrected concentration of propane versus distance downstream. From this value, a reaeration rate (k_2) for dissolved O_2, N_2 and N_2O was determined (Marzolf 1994, Young and Huryn 1998, Mulholland 2004).

^{15}N Mass Flux Calculations

Tracer ^{15}N flux was calculated from measured $\delta^{15}N$ using a series of equations developed by Mulholland et al. (2004). Briefly, $\delta^{15}N$ values were converted to mole fraction ratios using the equation:

$$\frac{^{15}N}{(^{15}N+^{14}N)} = \frac{[(\delta^{15}N/1000) + 1] * 0.0036765}{1 + [(\delta^{15}N/1000) + 1] * 0.0036765} \quad (5)$$

The $^{15}NO_3^-$ mass flux ($^{15}N\ flux_i$) was then calculated as

$$^{15}N\ flux_i = \{MF_i * [NO_3^- - N] * Q_i\} - \{MF_{bi} * [NO_3^- - N] * Q_i\} \quad (6)$$

where MF_i= the plateau mole fraction at the station, MF_{bi} = is the background (or Pre) mole fraction at the station, $[NO_3^- - N]$ is the concentration of nitrate at the station and Q_i = stream discharge at each station. Q_i was calculated from the same equation used for the nutrient additions (Eq. 1). The ln ^{15}N flux was plotted against distance downstream to calculate fractional rate of decline of the nitrate (k_{total}) and uptake length (Sw). Uptake rate (U) and uptake velocity (V_f) were then calculated similar to the nitrate additions using equations 3 and 4.

In order to determine the concentrations of $^{15}N_2$ and $^{15}N_2O$, the measured headspace $\delta^{15}N$ values were first corrected for isotopic fractionation (Mulholland 2004). N mass values were corrected for incomplete gas transfer into the headspace using the volumes of headspace and water for each sample along with the Bunsen

coefficients for N_2 and N_2O at the same pressure and temperature the headspace equilibration was performed (Mulholland et al. 2004). Mole fraction values were calculated from the fractionation corrected $\delta^{15}N$ using equation 5. Fluxes of $^{15}N_2$ and $^{15}N_2O$ were then calculated using equation 6.

Production rates of N_2 and N_2O from denitrification were estimated by fitting a denitrification model, created by Mulholland et al. (2004), to the average tracer ^{15}N flux data for N_2 and N_2O at each station. A least squares fitting technique was used with the model to estimate values for fractional rate of decline of the nitrate due to denitrification (k_{den}) from the ^{15}N mass flux data for N_2 and N_2O separately. Denitrification rates were also calculated as a nitrate mass removal rate per unit area for N_2 and N_2O using the uptake rate equation (Eq. 3) and the model predicted k_{den} values for each (Mulholland et al. 2004).

To adequately quantify uncertainty in these measurements due to variation in gas exchange rates and scatter in our data points, different simulation runs of the model were also run under varying scenarios. Similar to Mulholland et al. (2004), simulations were run varying the gas exchange rate of N_2 and N_2O (k_2) by 0.5 k_2 and 2 k_2. Simulations were also run varying k_{den} so that the model bound all of the data points (Mulholland et al 2004).

Statistical Analysis

All statistical analyses were performed using SAS Analyst (version 9.1, SAS Institute, Cary, North Carolina). Significance for all of the reported data was determined at $\alpha = 0.05$. Regression analyses were used to examine the longitudinal pattern of $N\text{-}NO_3^-$ uptake within each stream and the produced slope (k) was used to

calculate the uptake length (S_w) for each stream. To evaluate the differences in uptake length, and the related uptake rate and uptake velocity, an analysis of covariance (ANCOVA) was used on the pooled regression data. Analysis of variance (ANOVA) followed by the Tukey-Kramer adjusted least square means test, was used to examine statistically significant differences in denitrification potential and background [$N\text{-}NO_3^-$] between sites. For comparisons in which there was not already a mathematical or assumed relationship (i.e. denitrification potential and uptake length), pairwise Pearson correlations were used on pooled data across all streams. A significance level of $\alpha = 0.05$ was used for all analyses.

Chapter 3: Results

Streamwater Chemistry at Minebank Run

Nitrate concentrations in the streamwater at Minebank Run ranged between 0.76 – 1.36 mg L^{-1} for the months of June – August, with a mean concentration of 1.03 mg L^{-1} (0.12 S.E.) for the month of June, 1.04 mg L^{-1} (0.08 S.E.) for July, and 0.87 mg L^{-1} (0.04 S.E.) for August (Figure 2).

Small Survey of Denitrification Enzyme Activity and Nitrate Injections

Nitrate concentrations varied significantly in the four streams. SPBR had the highest nitrate-N concentrations, DR5 had the lowest nitrate-N concentrations, and MNBK and GLYN were intermediate (Table 1).

Sediment denitrification rates were variable and showed no predictable pattern across sites or with streamwater nitrate concentrations. MNBK, SPBR, and DR5 had significantly higher mean denitrification potential than GLYN (Table 2). DR5 had the highest mean denitrification potential of all the four streams and was significantly higher than GLYN and MNBK, but not SPBR.

Surface water velocity measurements were low in all four streams particularly the two restored sites MNBK and SPBR (2.1 cm s^{-1} and 2.0 cm s^{-1}, Table 1). Travel times for the four reaches ranged from 1-3 hours over the range in distances of 74 - 212 m, with plateau at the final stations not occurring at streams until several hours after additions began. Travel time for Minebank was especially slow, as it took 5.5 hours to reach plateau at the furthest station, only 74 m from the injection (Figure 3).

MNBK had the shortest uptake length, 357 m while DR5 had the longest, 1341 m (Table 2), but there was no significant differences between uptake slopes (k, the fractional rate of decline of nitrate) among the four sites. DR5 and GLYN showed a significant longitudinal pattern of the ln corrected concentration of $N\text{-}NO_3^-$ versus distance downstream, the pattern used to determine uptake length, at $p < 0.05$ ($R^2 = 0.84$ and 0.98, respectively).

Although the number of sites was small ($n = 4$), there was a significant relationship between uptake length, (S_w) and mean surface velocity (u) in the four streams (Figure 4). There was no significant correlation between S_w and Q from the small survey. There was also no correlation between S_w and either background [$N\text{-}NO_3^-$] or experimental [$N\text{-}NO_3^-$]. There were significant correlations between background [$N\text{-}NO_3^-$] and both U and V_f.

Isotope Addition and In situ Measurements of Denitrification

Background physical and chemical properties of Minebank Run for the day of the isotope addition are presented in Table 3. A significant longitudinal linear decrease was observed for the tracer $^{15}NO_3^-$ flux versus distance downstream, $R^2 = 0.89$, $p < 0.05$ (Figure 5). An uptake length (S_w) of 556 m was calculated from the slope of this line (k_{tot}). Uptake rate (U) was calculated to be 1.75 μg m^{-2} s^{-1} and uptake velocity (V_f) was 1.80 x 10^{-3} mm s^{-1}(Table 4). When examining patterns at the first three stations, the portion of the reach also used for the nitrate additions, uptake length (S_w) was shorter at 204 m ($R^2 = 0.99$ and $k_{tot} = 0.0049$). The corresponding uptake rate (U) and uptake velocity (V_f) were 5.0 μg m^{-2} s^{-1} and 4.7 mm s^{-1} respectively.

The air water exchange rate of propane for the entire stream reach was 0.0223 m^{-1}. This rate was then used to calculate an exchange rate (k_2) for N_2, 0.0292 m^{-1}, and N_2O, 0.0286 m^{-1}. A hump shaped curve, similar to those reported by other researchers (Mulholland et al. 2004), was found for the values of ^{15}N Mole Fraction for N_2 versus distance downstream (Figure 6).

Denitrification rates were determined using the Mulholland et al. (2004) denitrification model, which is separately fitted to the ^{15}N flux data for both N_2 and N_2O. Due to detection problems with N_2O, the model was fit to data from only the first 50 meters of the reach for N_2O. The best fit k_{den} for N_2 production was 0.0016, 89% of the k_{total} (0.0018). N_2O production was considerably less and the best fit k_{den} was 1.96 x 10^{-4}, approximately 11% of k_{den}. The mass flux rates of N_2 and N_2O production per unit area (U_{den}- N_2 and U_{den}- N_2O) were 136.6 and 16.5 mg-N m^{-2} d^{-1} respectively, totaling 153 mg-N m^{-2} d^{-1} removed by denitrification (Table 4). When adjusting values of the gas exchange rates (k_2) and k_{den} for uncertainty analysis k_{den} for N_2 production ranged from 0.0010 to 0.0025 m^{-1}, and ranged from between 1.10 x 10^{-4} to 2.95 X 10^{-4} m^{-1} for N_2O.

Chapter 4: Discussion

Sediment denitrification potential showed no obvious pattern in the survey of four streams and was highly variable. Nonetheless, results showed that substantial denitrification could be occurring in the sediments of Minebank Run, Spring Branch and the tributary of Dead Run, and these rates were similar to ranges reported for urban streams in Baltimore by Groffman et al. (2005). Denitrifying microbes are facilitative anaerobes, thus highly influenced by oxygen availability. Urban streams may have sediment microzones with low oxygen that function as "hot spots" of denitrification (Gold et al. 2001, McClain et al. 2003, Groffman et al. 2005). These microzones should be related to factors that lead to low oxygen levels, e.g., low flow areas, or accumulations of organic carbon that consumes oxygen. Denitrification studies in other streams have found rates to be highly variable and "patchy" both within and among stream sediments, similar to the present study (Royer et al. 2004).

Nitrate uptake at the reach scale in the survey of the four streams suggested that travel times can be long in the restored and degraded urban streams as evidenced by the long time for conservative tracers to reach a stable plateau after beginning continuous additions. Surface water velocity was particularly low at both restored sites and the travel time for water at Minebank Run was estimated to be five hours for the original 116 m reach. Within 9.5 hours, surface water velocity for the entire 116 m was so slow (0.64 cm s^{-1}) that solute concentrations at the final downstream station did not reach plateau and therefore had to be eliminated from analysis. The slow surface water velocities and length of time it took to reach plateau conditions in Minebank (5 hrs for the 74m reach) and the tributary of Dead Run (7 hrs for the 180

m reach) suggest that both restored and degraded urban streams have the potential to have long travel times. An increase in travel time may increase a nutrient molecules opportunity for removal via assimilation and/or denitrification and therefore decrease uptake length. Smaller streams and rivers have been shown to have a higher capacity for N uptake than larger streams because of their higher benthic sediment to surface water ratios which increases contact time between nutrients and potential removal sites (Alexander et al. 2000, Peterson et al. 2001).

A strong relationship between uptake length, S_w, and discharge, Q, where S_w increases with Q is often found in stream nutrient addition studies (Peterson et al. 2001, Grimm et al. 2005). In most streams as Q increases, the average surface water velocity (u) also increases, which results in a decreased travel time for the reach. This relationship was not found for S_w and Q in this study but we did observe an increase in uptake length (S_w) with increasing surface water velocity (u). Minebank Run and Spring Branch, the two restored streams, had the smallest surface water velocities as well as the shortest uptake lengths of the four sites. Previous work has shown that decreased flow velocity and meandering of the stream channel due to restoration resulted in reduced downstream transport of both nitrogen and phosphorus in an agricultural Kentucky stream (Bukaveckas 2007). In addition, pool and riffle sequences used in stream restoration may also slow the flow velocity of water and have effects on N retention (Kasahara and Hill 2006). This reach of Minebank Run studied has low, hydrologically connected banks and pools, which may be related to N retention at the reach scale (Kaushal et al. in review). Previous work in this reach has shown very high *in situ* denitrification rates and long hydrologic residence times

in hyporheic ground water response to stream restoration (Kaushal et al. in review). This slow velocity and interaction with benthic sediments may have contributed to the increased uptake of nitrate in Minebank Run and Spring Branch.

Although most studies in the past have focused on reporting S_w there can be a high amount of variability in S_w among streams due to both biogeochemical and hydrogeomorphic effects (Doyle et al. 2003). Therefore, recent research has taken more of an interest in uptake velocity, V_f , the rate at which a nutrient travels vertically before being taken up by the stream bed, when making comparisons because it normalizes for the effects of stream hydrogeomorphology so that differences in nutrient retention between streams are then based on biogeochemical changes (Davis and Minshall 1999, Doyle et al. 2003). Our uptake velocity values were on the lower end of those reported (4.6 – 32.4 mm hr^{-1}, Table 5) and were lower than the average V_f value for first order streams (168 mm hr^{-1}) determined in a comprehensive review of 52 reported literature values by Ensign and Doyle (2006). Uptake rate and length, when compared to other streams, would indicate that Minebank Run is highly retentive yet uptake velocity indicated otherwise. Uptake velocity is calculated as the discharge of the stream divided by uptake length and channel width (eq. 4) therefore streams with smaller discharges may have smaller uptake velocities than streams with larger discharges yet similar uptake lengths. Discharge for Minebank Run, as well as the other three streams of the survey, was much smaller than most other streams were these types of nutrient and isotope additions have been conducted therefore this may have led to the low uptake velocity in the four sites studied when compared to other sites.

The two restored streams of this survey had the shortest uptake lengths but despite this pattern, there was no significant difference between restored and degraded streams. This may have been due to the small sample size of this study. Uptake rate and uptake velocities also did not follow the same pattern as uptake length. This variability between the three metrics may have occurred due to the variability of environmental factors at the sites that would affect nitrogen retention and these metrics (i.e. nitrate concentration, discharge, biotic uptake, denitrification, etc…). Future work should be conducted to clarify these relationships and truly examine if restoration can significantly affect nitrate retention in urban streams. Studies looking at the flow effects on nutrient retention as well as the relationship between discharge and surface water velocity may further inform us on the importance of restorations that slow and dissipate the high flows typically associated with urban streams. Although there may be considerable variability in the effectiveness of different restoration designs on N removal (Kaushal et al. in review), the present study suggests that slowing hydrologic flow and increasing travel time of water may increase the potential for N removal in restored streams.

Nitrate uptake length measured from the ^{15}N isotope addition was 556 m. When comparing our isotope spiraling metric results to other studies that used the isotope tracer technique, uptake length for Minebank Run was longer than the forested Walker Branch (35.7 m) studied by Mulholland et al. (2004) and the arid desert Agua Fria River (36 m) studied by Grimm et al. (2005) but shorter than the agricultural streams (800-9600 m) studied by Bernot et al. (2006). Minebank Run was similar to and within the mid range for the three urban arid streams also studied

by Grimm et al. (2005). Nitrate uptake rate (U) was high in Minebank Run (1.75 μg-N m^{-2} s^{-1}) compared to other streams as only two other streams, both of which were urban streams, had higher rates (Table 6). Uptake velocity (V_f) was lower in Minebank Run than all of the other streams except Sugar Creek (Table 6).

According to the Mulholland et al. (2004) model the best fit fractional NO_3^- removal rate due to denitrification (k_{den}) was 0.0018 m^{-1}, with an uncertainty of approximately ± 0.001 m^{-1}. Thus, the model suggests 100% of uptake was explained by denitrification, with the uncertainty being ± 40%. Production of N_2 made up approximately 89% of denitrification while N_2O production made up the remaining 11%. The uncertainty was relatively high and may have been due to analytical factors such as incomplete gas transfer (although these samples were adjusted based on equations by Mulholland et al. 2004).

Despite the large uncertainty, denitrification still appeared to comprise the majority of NO_3^- uptake within Minebank Run during the isotope addition. Even at our lowest uncertainty estimate, denitrification in Minebank Run comprised up to 60% of the NO_3^- uptake within the reach. This result for Minebank Run was higher than those reported in the two isotope tracer experiments where denitrification was also measured using similar methodology (Table 7). Of the three streams, the forested Walker Branch had the shortest S_w (36 m) and largest fractional rate of nitrate decline due to denitrification, k_{den} (0.0046 m^{-1}, Mulholland et al. 2004), followed by the urban Minebank Run (356 m and 0.0018 m^{-1} respectively, this study), while the agricultural Sugar Creek had the largest S_w (9600m) and smallest k_{den} (5.4 x 10^{-5} m^{-1}, Böhlke et al. 2004). Denitrification made up only 16% of uptake within

Walker Branch and 52% of uptake within Sugar Creek. Interestingly, Sugar Creek and Minebank Run had similar nitrate concentrations (990 μg-N L^{-1} and 973 μg-N L^{-1} respectively) but Sugar Creek had a larger discharge and average surface water velocity (45 L s^{-1} and 0.19 m s^{-1}) than Minebank Run (2.16 L s^{-1} and 0.0061 m s^{-1}). Theoretically, the slower discharge and velocity in Minebank Run may have allowed NO_3^- molecules to be in contact with potential denitrification sites longer, therefore the larger measurement of uptake explained by denitrification in Minebank Run verse Sugar Creek may be possible.

Denitrification rate for Minebank Run from the isotope addition, expressed as removal per unit of area, was 153 mg N m^{-2} d^{-1}. For the 220.5 m reach studied, denitrification could potentially remove 40 % of the daily nitrate load assuming a nitrate concentration and discharge similar to those during the isotope addition. Although these rates are high, they are not the highest reported in the literature. For example, denitrification ranged between 0.24 – 360 mg N m^{-2} d^{-1} for Illinois agricultural streams that ranged in NO_3^- concentration of 0.12 -9.99 mg/L (Royer et al. 2004). Mean rates of nitrate-N removal of 3.63 mg NO_3^--N per liter of groundwater flow were found in piezometers located within the reach of Minebank Run used for the present study, and there was a strong relationship between hydrologic residence time and denitrification rates (Kaushal et al. in review). This rate was much higher than those found for an unrestored reach of Minebank Run from the same study (0.20 – 1.74 mg NO_3^- - N per L of groundwater through 1 m^3 of hyporheic sediments, Kaushal et al. in press). The high rates of in-stream denitrification and implications for N load reductions in the present study, and results

from previous work at Minebank Run in hyporheic ground water by Kaushal et al. (in review) and benthic sediments (Groffman et al. 2005) suggest that denitrification may represent an important component of the N budget of this restored stream.

Our results, showing that denitrification was the dominant fate for the $^{15}NO_3^-$ that we added to Minebank Run, are further supported from the propane addition and dissolved oxygen measurements. These measurements allow us to estimate a gross primary production (GPP) of 1.12 g O_2 m^{-2} d^{-1}during the time of this study and community respiration (CR_{24}) of 9.26 g O_2 m^{-2} d^{-1} (calculated the same as Marzolf et al. 1994, Young and Huryn 1998, Mulholland 2006). This estimate of GPP, although not extremely low, was not overly high (Mulholland 2006) and suggests that GPP could only account for a fraction of the N uptake in this study. Furthermore, net ecosystem metabolism (NEM) of a stream is calculated as community respiration (CR_{24}) subtracted from gross primary productivity (GPP), NEM = GPP - CR_{24} (Marzolf 1994, Young and Huryn 1998, Meyer et al. 2005) and was estimated to be approximately -8.14 g O_2 m^{-2} d^{-1} indicating that the metabolism of Minebank Run during the time of the isotope addition was dominated by heterotrophic activity and not autotrophic primary producers.

Minebank Run was shaded with increased hydrologic connectivity between the channel and ground water. These environmental conditions associated with stream restoration may have fostered N transformations through heterotrophic microbial pathways. Restorations of this type may lead to decreases in nitrogen loading of estuaries and bays by promoting heterotrophic microbial processing of N and denitrification. Despite this potentially positive implication, N loading in urban

streams is often larger than biotic demand (Gücker and Pusch 2006), and hydrologic disturbance from uplands (e.g. increased runoff from impervious surfaces) may compromise restoration effectiveness during high flows (Booth 2005, Walsh 2005). For example, urban streams in Michigan were found to have higher rates of denitrification than forested streams but nitrate loads were high enough so that a smaller proportion of loads were removed in urban streams compared to forested (Inwood et al. 2005). This isotope study was conducted in the summer under very low flow and high temperature which are both optimal for maximum denitrification. On the other hand, nitrogen loads to many urban streams are often highest in the winter when flows are high and temperatures are low (Shields et al. in review). Therefore, it is unclear how restoration of urban streams can influence nitrate load reductions under different hydrologic flow conditions. Future studies of this nature should look at effects of varying loads, different flows, and seasonality on this type of data. Studies should also include the use of models, such as the OTIS and OTIS-P models (Runkel 1998), that are transport-based, time-series approaches that provide estimates of both U and V_f and are completely independent of hydrologic effects, allowing for separation of hydrologic and non-hydrologic processes (Runkel 2007). In addition, more studies are needed in restored streams to compare with forest and degraded urban references to determine the efficacy of various restoration designs and approaches (Bernhardt et al. 2005b, Palmer and Bernhardt 2006).

Chapter 5: Conclusion

Nitrate retention from a survey of urban degraded and restored streams in Baltimore County, MD showed variability among nitrate retention metric and denitrification. The restored streams, Minebank Run and Spring Branch, had the shortest uptake lengths, but were not significantly different from the degraded urban streams, Glyndon and the tributary of Dead Run. Surface water velocities (u) were slowest in the restored streams and uptake length was significantly correlated to surface water velocity among the study streams. This, along with results from a growing body of research, supports the finding that stream restorations that decrease water velocity and increase residence time may lead to higher retention of nutrients, via shorter uptake lengths (Kasahara and Hill 2006, Bukaveckas 2007, Roberts et al. 2007, Kaushal et al. in review). More detailed research examining the relationships between discharge, velocity, "hydrologic connectivity", and N uptake can provide us with important management information that would not only increase or understanding of these processes but also how restorations may play an important role in reducing N loads in our urban streams.

Results from the isotope tracer technique for the urban restored stream, Minebank Run, demonstrated that denitrification appeared to encompass the majority of nitrate uptake in the stream. This study suggested that denitrification contributed to a larger portion of nitrate uptake than in a forested stream and an agriculture stream for two other studies using the same technique. More work is necessary to determine whether restoration contributed to the high amount of uptake explained by denitrification seen in Minebank Run. Research should also look at the effects of

restoration, urbanization, and varying flow regimes, on N removal and the efficiency of different restoration designs.

Tables

Table 1. Stream chemistry and channel characteristics for study sites in the survey of restored and unrestored streams. w = wetted width, u = surface water velocity, and Q = discharge. Values in parenthesis are the standard error of the mean for the measurement above. Letters after a value represent significant differences in values of the same characteristic with a different letter.

Stream	Type	Reach length (m)	Background $[NO_3^-]$ (mg L^{-1})	$[NH_4^+]$ (µg L^{-1})	Average w (m)	u (cm s^{-1})	Q (L s^{-1})
MNBK	Restored	74	1.0[c] (0.04)	2.7 (1.02)	2.0 (0.10)	2.1	2.2 (0.25)
SPBR	Restored	212	2.7[d] (0.03)	8.4 (1.84)	2.9 (0.27)	2.0	16.2 (0.48)
DR5	Degraded	180	0.5[a] (0.02)	14.8 (2.48)	1.5 (0.03)	5.0	2.6 (0.21)
GLYN	Degraded	142.5	1.8[b] (0.06)	20.2 (5.13)	1.3 (0.13)	2.6	4.9 (0.18)

Table 2. Nitrate retention metrics and denitrification potential results of the nitrate additions for the small survey study sites. k = fractional rate of NO_3^- lost, S_w = uptake length, V_f = uptake velocity, and U = uptake rate. Values in parenthesis are the standard error of the mean for the measurement above. Letters after a value represent significant differences in values of the same characteristic with a different letter.

Stream	Experimental $[NO_3^-]$ (mg L^{-1})	k_{tot} (m^{-1})	S_w (m)	U (µg m^{-2} s^{-1})	V_f (mm hr^{-1})	Mean DEA (ng N/ g^{-1} soil hr^{-1})
MNBK	2.1 (0.21)	2.8×10^{-3}	356	6.7	11.0	19.7^{b} (3.9)
SPBR	2.9 (0.03)	1.6×10^{-3}	621	26.3	32.4	$53.1^{a,b}$ (21.2)
DR5	2.0 (0.20)	7.5×10^{-4}	1341	2.5	4.6	73.1^{a} (9.9)
GLYN	3.1 (0.04)	1.5×10^{-3}	671	17.5	20.4	4.2^{c} (2.0)

Table 3. Stream chemistry and channel characteristics of Minebank Run for the ^{15}N isotope addition. w = wetted width, u = surface water velocity, and Q = discharge. Values in parenthesis are the standard error of the mean for the measurement to the right.

Stream characteristics	Value	S.E.
Reach length (m)	220.5	
$[NO_3^-]$ (mg L^{-1})	0.85	(0.02)
$[NH_4^+]$ (µg L^{-1})	3.0	(1.03)
Avg. w (m)	2.2	(0.40)
u (cm s^{-1})	0.61	
Q (L s^{-1})	2.2	(0.30)

Table 4. Results of NO_3^- uptake from the ^{15}N isotope addition at Minebank Run. Metrics labeled "tot" represent total NO_3^- lost due to all forms of uptake and metrics labeled "den" represent NO_3^- lost due to denitrification alone. k = fractional rate of NO_3^- lost, S_w = uptake length, V_f = uptake velocity, and U = uptake rate.

Total uptake rates	
k_{tot} (m^{-1})	0.0018
$S_{w,\ tot}$ (m)	556
U_{tot} (mg m^{-2} s^{-1})	151
$V_{f,\ tot}$ (m h^{-1})	0.0065
Denitrification uptake rates	
k_{den} N_2+N_2O (m^{-1})	0.0018
$S_{w,den}$ (m)	549
U_{den} (mg m^{-2} s^{-1})	153
$V_{f,den}$ (m h^{-1})	0.0066

Table 5. Nitrate spiraling metric results for other studies using the unenriched nitrate addition technique. Q = discharge, S_w = uptake length, U = uptake rate, and V_f = uptake velocity.

Site	Land Use	NO_3^- ($\mu g\ L^{-1}$)	Q ($L\ s^{-1}$)	S_w (m)	U ($\mu g\ N\ m^{-2}\ s^{-1}$)	V_f ($mm\ hr^{-1}$)	Citation
Minebank Run	Restored Urban	1010	2.2	356	6.7	11.0	This study
Spring Branch	Restored Urban	2740	16.2	621	26.3	32.4	
Dead Run trib.	Urban	540	2.6	1341	2.5	4.6	
Glyndon	Urban	1770	4.9	671	17.5	20.4	
Agua Fria River	Desert	5	15	67	0.38	277	
Sycamore Creek	Desert	21	55	90	4.3	720	Grimm et al. 2005
Rio Rancho Drain	Urban	18	27	294	0.38	76	
Indian Bend Wash	Urban	100	49	555	2.2	79	
Gila Drain	Urban	1220	113	526	87	259	
Price Road Drain	Urban	5241	187	833	294	202	
Highline Canal	Urban	6111	306	1245	734	432	
DMB-D	Agriculture	800 – 16400		23	10304	4.4	Gücker and Pusch 2006
DMB-P	Agriculture	800 – 16400		22	27534	2.1	
Erpe-D	Agriculture	800 – 16400		164	11529	4.4	
Erpe-P	Agriculture	800 – 16400		511	6977	92	
Pioneer Creek	Forested	52	88	549	4.25	0.30	Davis and Minshall 1999
Cliff Creek	Forested	330	83.3	1839	7.5	0.083	

Kings Creek	Prairie		55	168	7.4	446	Dodds et al. 2002
Kings Creek	Prairie	57.4	4	300	1.8	31	
Kings Creek	Prairie	57.4	4	311	3.5	30	
Kings Creek	Prairie	57.4	4	402	5.6	24	
Kings Creek	Prairie	57.4	4	225	17	42	

Table 6. Nitrate spiraling metric results for other studies using the ^{15}N isotope addition technique. Q = discharge, S_w = uptake length, U = uptake rate, and V_f = uptake velocity.

Site	Land Use	NO_3^- ($\mu g\ L^{-1}$)	Q ($L\ s^{-1}$)	S_w (m)	U ($\mu g\ N\ m^{-2}\ s^{-1}$)	V_f ($m\ h^{-1}$)	Citation
Minebank Run	Restored Urban	973	2.2	555	1.75	0.0065	This study
Agua Fria River	Desert	0.4	10	36	0.04	0.34	Grimm et al. 2005
Rio Rancho Drain	Urban	7	14	84	0.43	0.14	
Indian Bend Wash	Urban	202	69	609	3.0	0.094	
Highline Canal	Urban	4747	502	1245	1231	0.97	
Sand Creek trib.	Agriculture	500	7	800	0.02	0.29	Bernot et al. 2006
Red Run Drain	Agriculture	600	17	1900	0.0002	0.036	
Little Rabbit River	Agriculture	3200	63	1400	0.0003	0.18	
Cobb Ditch	Agriculture	2900	575	2200	0.007	0.14	
Walker Branch	Forested	26	0.4	36	0.32	0.044	Mulholland et al. 2004
Sugar Creek	Agriculture	990	45	9600	0.86	0.0031	Böhlke et al. 2004

Table 7. Denitrification results and nitrate uptake metrics using the ^{15}N isotope addition technique compared with results from other studies using the same technique. Metrics labeled "tot" represent total NO_3^- lost due to all forms of uptake and metrics labeled "den" represent NO_3^- lost due to denitrification alone. k = fractional rate of NO_3^- lost, S_w = uptake length, V_f = uptake velocity, and U = uptake rate.

	Minebank Run (present study)	**Sugar Creek** (Bölke et al. 2004)	**Walker Branch** (Mulholland et al. 2004)
Stream characteristics			
Discharge, Q, (L s^{-1})	2.2	45	0.4
Average surface water velocity, u, (m s^{-1})	0.0061	0.19	0.029
NO_3^- concentration (µg-NO_3^- - N L^{-1})	973	990	26
NO_3^- flux (µg- NO_3^- - N s^{-1})	2100	44550	10.4
NO_3^- uptake rates			
k_{tot} (m^{-1})	0.0018	0.00010	0.028
k_{den} (m^{-1})	0.0018	0.000054	0.0046
$S_{w, tot}$ (m)	556	9600	36
$S_{w, den}$ (m)	556	18500	217
$V_{f, tot}$ (m h^{-1})	0.0065	0.0031	0.044
$V_{f, den}$ (m h^{-1})	0.0066	0.0061	0.0062
U_{tot} (mg- NO_3^- - N m^{-2} d^{-1})	151	73.9	27.7
U_{den} (mg- NO_3^- - N m^{-2} d^{-1})	151	38.6	3.9

Figures

Figure 1. Map of stream sites for the survey study, located within Baltimore County, MD. All sites are pictured within their 12 digit watershed. Minebank Run (MNBK) and Spring Branch (SPBR) are both part of the larger Gunpowder Falls watershed while Glyndon (GLYN) and the tributary of Dead Run (DR5) are both part of the larger Gwynns Falls watershed. Baltimore City is represented by the area highlighted in grey within the Baltimore County map.

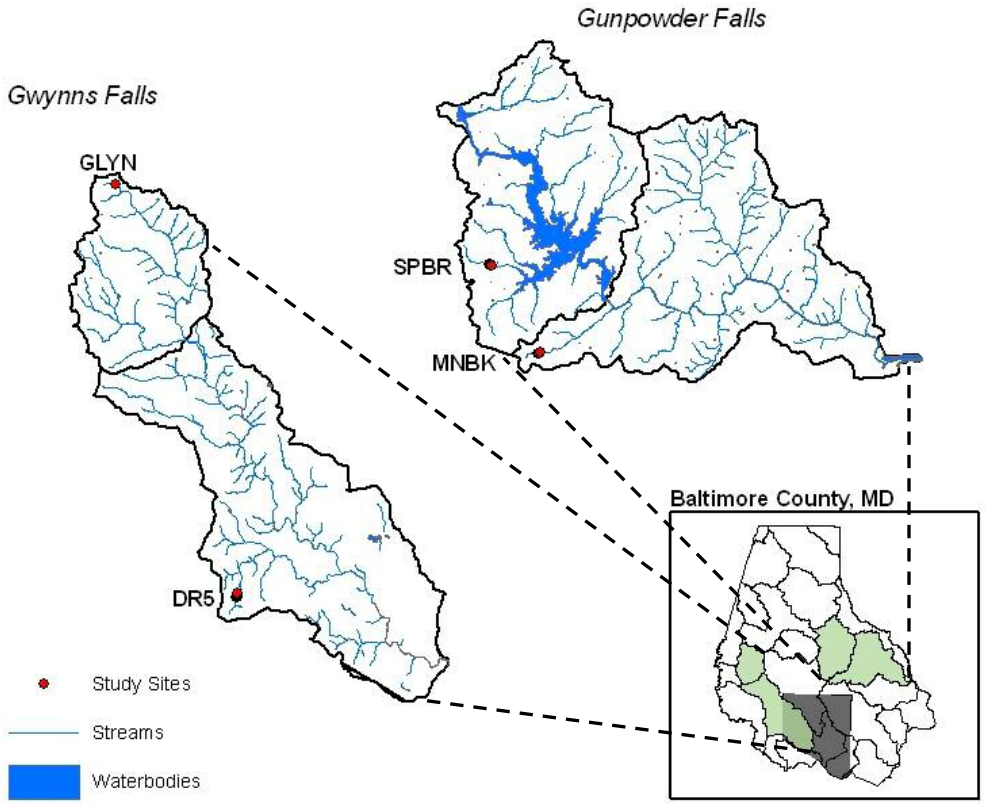


Figure 2. Streamwater nitrate concentrations at Minebank Run for the 2006 water year. Arrows indicate times at which particular experimental methods were conducted. DEA = sediment collection for the denitrification enzyme assays, NT = the unenriched nitrate addition at Minebank, and ISO = the ^{15}N isotope addition at Minebank.

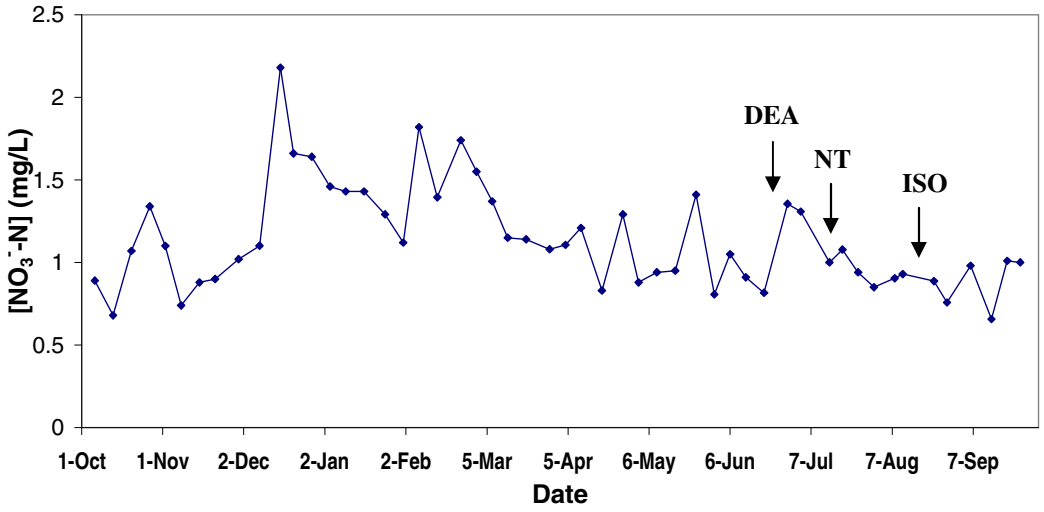


Figure 3. Bromide concentrations and travel times for the nitrate addition at Minebank Run on 7-17-06.

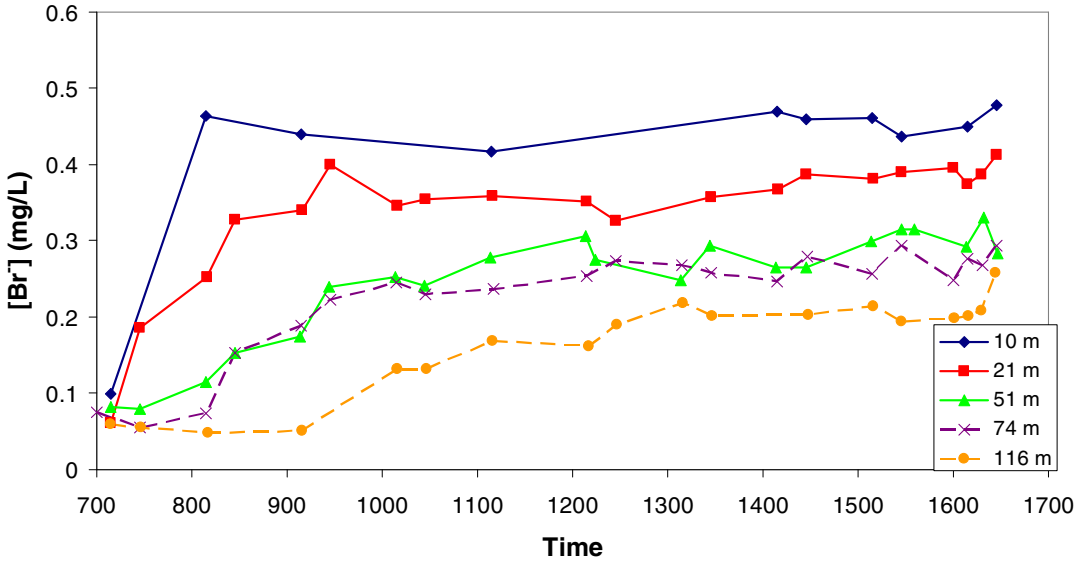


Figure 4. Relationship between stream surface water velocity, u, and nitrate uptake length, S_w, for the four urban streams of the small survey study; ($R^2 = 0.92$, $p < 0.05$, $n = 4$). Restored streams (MNBK and SPBR) had the slowest stream water velocities.

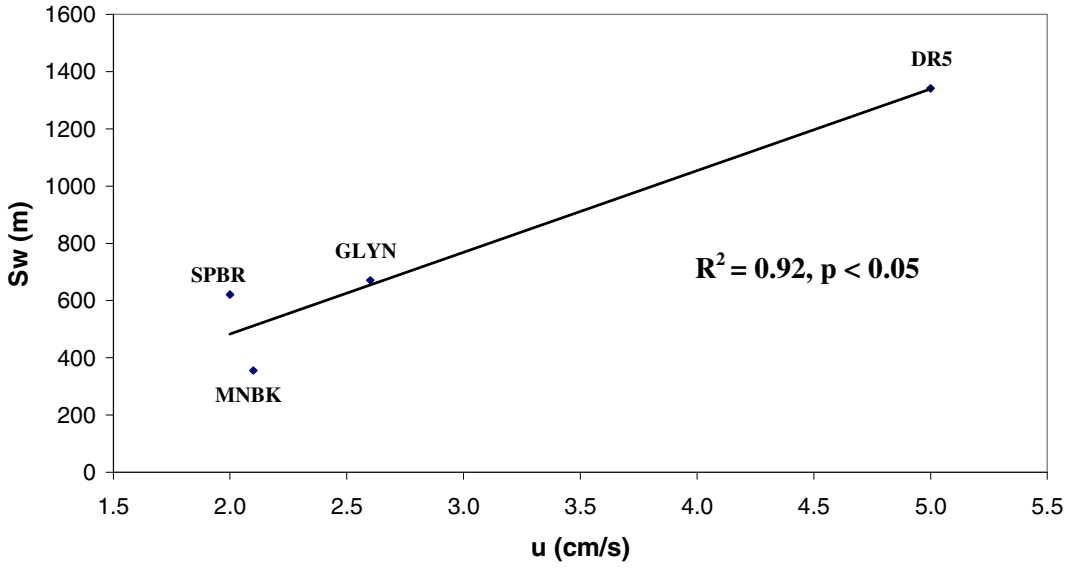


Figure 5. The natural log of the tracer $^{15}NO_3^-$ flux versus distance downstream for the ^{15}N isotope addition at Minebank Run; ($R^2 = 0.89$, $p < 0.05$, $n = 7$).

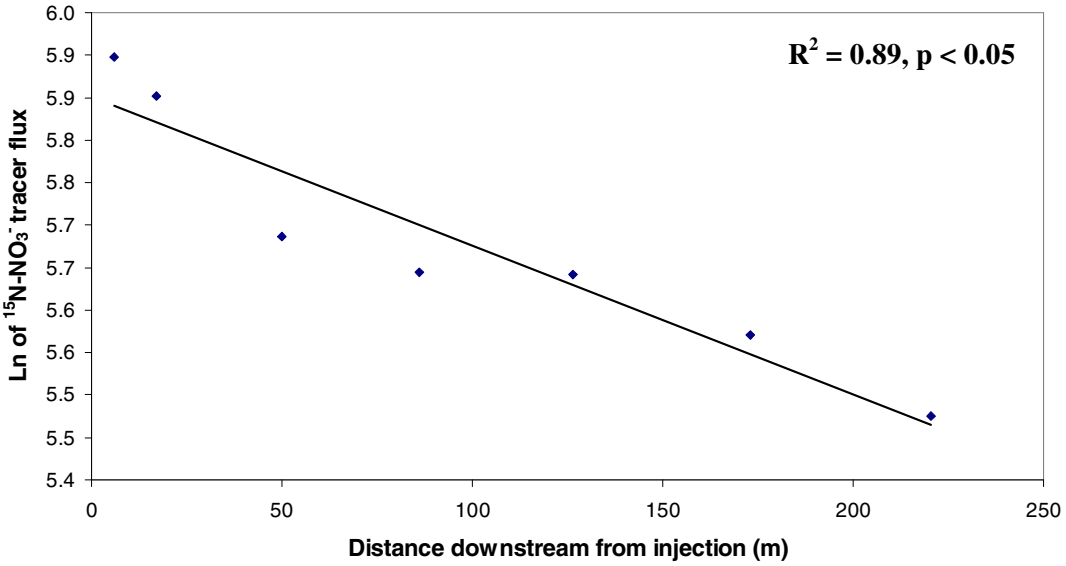
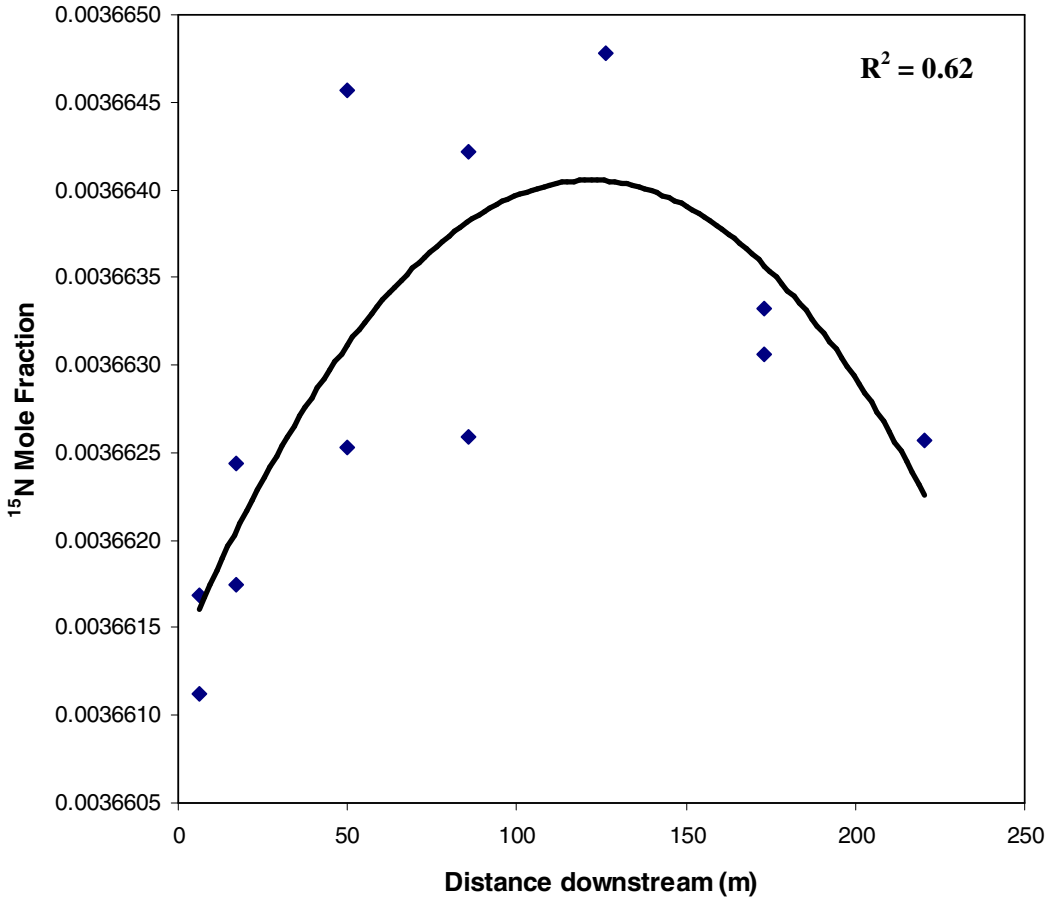


Figure 6. ^{15}N Mole fraction values versus distance downstream for N_2 at Minebank Run.



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