SEASONAL NITRATE DYNAMICS IN AN AGRICULTURALLY INFLUENCED NEW HAMPSHIRE HEADWATER STREAM

BY

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THESIS

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Nitrate (NO$_3^-$) uptake and denitrification in small headwater streams can have significant implications for downstream water quality. I measured seasonal NO$_3^-$ uptake and longitudinal patterns in potential sediment denitrification in a New Hampshire agriculturally influenced stream during 2009. NO$_3^-$ uptake was measured through short-term NO$_3^-$ enrichments and potential denitrification was measured using the Chloramphenicol-amended acetylene-inhibition method. NO$_3^-$ was taken up in autumn but not in spring or summer. In autumn, on average 87% of the daily NO$_3^-$ load was taken up and a maximum of 4% was removed via denitrification. Results suggest that, in this stream, carbon availability from leaf fall drives NO$_3^-$ uptake primarily via heterotrophic assimilation. This study helps to better characterize controls on NO$_3^-$ uptake and denitrification in New England, where few such studies have been conducted on agricultural streams, and where seasons are pronounced.
INTRODUCTION

Human activities have nearly doubled the amount of reactive nitrogen, including nitrate ($\text{NO}_3^-$) and ammonium ($\text{NH}_4^+$), in ecosystems since the industrial revolution (Galloway et al. 1995, Smil 2001). Nitrogen (N) is an essential nutrient for biological growth. However, chronic N loading to aquatic ecosystems from sources such as manure, fertilizer applications and sewage effluent can alter species composition and lead to water quality impairments such as eutrophication (Paul and Meyer 2001). Characterized by increased productivity, algal blooms, and anoxia, eutrophication leads to decreased biodiversity and potential fish kills (Carpenter et al. 1998, Diaz and Rosenberg 2008). Additionally, $\text{NO}_3^-$ is a federally listed drinking water contaminant and can cause methemoglobinemia (blue baby syndrome) in infants (Knobeloch et al. 2000). Currently, N is one of the leading causes of water quality impairment in the U.S., with agriculturally-influenced waters making up a significant portion of cases (Birgand et al. 2005).

Headwater streams are important N cycling pathways, linking terrestrial ecosystems to larger rivers, lakes and estuaries. Once N enters a stream it can cycle between organic (e.g. amines, proteins) and inorganic (e.g. $\text{NO}_3^-$, $\text{NH}_4^+$) forms as it moves downstream; a process known as nutrient spiraling (Webster and Patten 1979, Newbold et al. 1981- Figure 1). $\text{NO}_3^-$ can be taken up via autotrophic (e.g. algal and plant) and heterotrophic (microbial) assimilation, reduced to $\text{NH}_4^+$ via dissimilatory nitrate reduction to ammonium (DNRA), or emitted into the atmosphere in gaseous forms through denitrification (Webster and Valett 2006). Because $\text{NO}_3^-$ does not sorb well to sediments (Hedin et al. 1998), if it is not taken up it is typically transported farther downstream.
Figure 1. Conceptual diagram illustrating the nitrogen cycle and spiraling. Nitrogen gas (N₂) is converted to organic N and ammonia (NH₃⁺) through nitrogen fixation. During decomposition organic N is converted to NH₃⁺ and then NH₄⁺. Nitrification is the conversion of NH₄⁺ to nitrite (NO₂⁻) and NO₃⁻. NO₃⁻ can then be converted to gaseous forms of N (nitric oxide (NO), nitrous oxide (N₂O) and nitrogen gas (N₂)) and emitted into the atmosphere.

The pathway NO₃⁻ takes in headwater streams can determine its impact on larger downstream waterbodies. Assimilatory uptake is considered a temporary NO₃⁻ removal pathway because it will eventually return to the water column through mineralization. DNRA is also only a temporary removal pathway and actually converts the NO₃⁻ back into a more biologically available form of N. In contrast, denitrification permanently removes N from the water column, which minimizes downstream export and potential eutrophication in larger waterbodies.

Denitrification, the reduction of NO₃⁻ to gaseous forms including nitric oxide- NO, nitrous oxide- N₂O, and nitrogen gas- N₂, occurs when denitrifying microbes use NO₃⁻ as an electron acceptor in the absence of oxygen. In order for denitrification to occur there must be denitrifying microbes present, low oxygen conditions (<0.2 mg O₂ L⁻¹), and available NO₃⁻ and carbon (Seitzinger et al. 2006). Sediment composition and depth


\[
\text{Reaction: } \text{CH}_2\text{O} + (0.8)\text{NO}_3^- + (0.8)\text{H}^+ \rightarrow \text{CO}_2 + (0.4)\text{N}_2 + (1.4)\text{H}_2\text{O} \quad (\text{Hedin et al. 1998}).
\]
affect denitrification rates due to these factors. For example, sediment with higher organic matter content may exhibit higher rates of denitrification due to enhanced carbon availability (Seitzinger et al. 2006). In surface waters, denitrification is greatest in the top 5 cm of underlying anoxic sediment, which receives NO$_3^-$ from the water column (Inwood et al. 2007). Overall rates of denitrification show high spatial and temporal variability, sometimes differing by orders of magnitude in adjacent locales or within a few hours (Inwood et al. 2005, Groffman et al. 2006). Despite this variability, quantifying rates of denitrification is key to minimizing the impacts of NO$_3^-$ on downstream waterbodies since it is the only pathway that permanently removes N.

Stream NO$_3^-$ uptake is often estimated by conducting $^{15}$N- isotope additions (Hall et al. 2009), or non-isotopic NO$_3^-$ additions (Dodds et al. 2002, Payn et al. 2005) and measuring downstream NO$_3^-$ attenuation. NO$_3^-$ additions are significantly less expensive than $^{15}$N- isotope additions, and are a common approach to measuring uptake. However, increasing ambient stream NO$_3^-$ can lead to a fertilization effect, potentially overestimating NO$_3^-$ uptake (Dodds et al. 2002).

NO$_3^-$ uptake can be described with several parameters including uptake length, uptake rate and uptake velocity (Stream Solute Workshop 1990). Uptake length is the average distance NO$_3^-$ travels downstream before being removed from the water column. Stream discharge can influence uptake length so it is not often used in cross-site comparisons. More commonly, uptake rate is used, which adjusts for stream size. Uptake velocity accounts for stream NO$_3^-$ concentration and is defined as the speed at which NO$_3^-$ moves toward the benthos. Similar metrics are used to characterize stream denitrification (see Methods for further description).

NO$_3^-$ uptake and rates of denitrification vary and are influenced by physiochemical (e.g. temperature, oxygen availability), hydrological (e.g. stream size and discharge), and watershed characteristics (e.g. land use). Smaller, headwater
streams with high width to depth ratios often have greater uptake rates because the N has more contact with the benthos (Peterson et al. 2001, Wollheim et al. 2001, Ensign and Doyle 2006). Additionally, higher discharge may lower rates of denitrification due to increased oxygen availability (Hill and Lymburner 1998). Warmer temperatures, greater light availability, higher rates of primary production and longer growing seasons may increase uptake (Fenn et al. 1998, Alexander et al. 2000, Dodds et al. 2002, Ensign and Doyle 2006).

Previous NO$_3^-$ uptake and denitrification studies have found that streams overwhelmed with NO$_3^-$ inputs can reach saturation, when inputs exceed the amount of NO$_3^-$ removed from the water column (Dodds et al. 2002, Mulholland et al. 2008). N-saturation is indicated by a negative or asymptotic (e.g. Michaelis-Menten) relationship between stream NO$_3^-$ and uptake rate and uptake velocity (Stream Solute Workshop 1990, Mulholland et al. 2004). The point at which NO$_3^-$ uptake no longer increases proportionately with ambient NO$_3^-$ is referred to as the point of saturation (Dodds et al. 2002). Results from the LINX II project, a series of NO$_3^-$ uptake experiments conducted on 72 streams in eight biomes, indicate that as NO$_3^-$ concentrations increase, uptake velocity and denitrification decrease (Hall et al. 2009, Mulholland et al. 2009). This phenomenon leads to greater downstream NO$_3^-$ export and potential water quality impairments (Mulholland et al. 2008).

Despite evidence that NO$_3^-$ uptake and denitrification are dependent on factors that are temporally and spatially variant, the majority of NO$_3^-$ uptake studies have been based on results from NO$_3^-$ enrichments typically performed once, during peak growing seasons in spring and summer (Mulholland 2004, Hall et al. 2009). Few studies have examined seasonal differences in NO$_3^-$ uptake (Simon et al. 2005, Hoellein et al. 2007, Arango et al. 2008). Additionally, only a few NO$_3^-$ uptake and denitrification studies have been carried out in agriculturally influenced streams in New England (Hall et al. 2009).
This study attempts to help fill these gaps and better understand the importance of seasonality on NO₃⁻ uptake in New Hampshire, where seasons are pronounced.

The objectives of this study were to measure seasonal differences in NO₃⁻ uptake and longitudinal patterns in potential sediment denitrification at Burley-Demeritt Creek, an agriculturally influenced headwater stream at the University of New Hampshire Organic Dairy Research Farm in Lee, New Hampshire. The study was conducted during a one year period in 2009. NO₃⁻ uptake was measured using standard short-term NO₃⁻ additions. Potential sediment denitrification was measured through Denitrification Enzyme Assays (DEA) using the Chloramphenicol-amended acetylene-inhibition method (Groffman et al. 1999). I hypothesized that NO₃⁻ uptake would be greatest in spring coinciding with low canopy cover and peak biological activity, and in autumn, when heterotrophic metabolism increases with leaf litter inputs and greater light availability. I also hypothesized that potential sediment denitrification rates would be low, with higher rates occurring upstream where organic matter is the dominant substrate.

Burley-Demeritt Creek receives N-rich runoff from pastures and from a small, intermittent tributary draining the pastures. The creek flows into the Lamprey River, a municipal drinking water supply, which discharges into Great Bay Estuary, an N-impaired estuarine ecosystem (Piscataqua Region Estuaries Partnership 2009). Estuaries are typically N-limited (Vitousek and Howarth 1991) and high N-loading to these ecosystems can lead to eutrophication and loss of eel grass and other aquatic plant and animal species. In Great Bay Estuary, N concentrations have increased nearly 60% since the late 1980s, threatening the estuarine ecosystem (Castro and Driscoll 2002, Piscataqua Region Estuaries Partnership 2009). Understanding seasonal differences in NO₃⁻ uptake and denitrification at Burley-Demeritt Creek will assist agricultural planners as they work to minimize their environmental impact on the Lamprey River and Great Bay Estuary.
CHAPTER I

METHODS

Site Description

Burley-Demeritt Creek (BDC) is at the University of New Hampshire Organic Dairy Research Farm in Lee, New Hampshire (Figure 2). The first-order stream is approximately 30 m above sea level, with a slope of 0.76% and drains a wetland. A forested riparian buffer between the creek and pastures ranges from approximately 50 m upstream to 1000 m downstream. Riparian vegetation is dominated by eastern hemlock (Tsuga canadensis), eastern white pine (Pinus strobus), red maple (Acer rubrum), and northern red oak (Quercus rubra). Specific stream and watershed characteristics are in Table 1. Soil within BDC is predominantly clay, however the top 5-10 cm of underlying sediment is silt (near the Lamprey River junction), sandy/cobble (between BDC-100 and BDC-350), and predominantly organic matter (upstream from BDC-350). Station names indicate the distance (m) upstream from the Lamprey River junction. There are very few macrophytes in the stream and minimal algal growth was only observed on two sample collection dates in early spring. A small intermittent tributary draining cow pastures enters BDC from the west approximately 380 m upstream from the Lamprey River junction. The tributary has no riparian forested buffer.
Figure 2. Burley-Demeritt Creek site map. Sample stations indicate the distance (m) upstream from the Lamprey River Junction.

Table 1. Watershed and stream reach characteristics.

<table>
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<th>Value</th>
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<tbody>
<tr>
<td>Latitude (N)</td>
<td>43.09247</td>
</tr>
<tr>
<td>Longitude (W)</td>
<td>-70.99199</td>
</tr>
<tr>
<td>Reach Length (m)</td>
<td>516</td>
</tr>
<tr>
<td>Watershed Area (acres)</td>
<td>317</td>
</tr>
<tr>
<td>% Agricultural</td>
<td>60.4</td>
</tr>
<tr>
<td>% Forested</td>
<td>30.3</td>
</tr>
<tr>
<td>% Wetland</td>
<td>8.8</td>
</tr>
<tr>
<td>% Water</td>
<td>0.6</td>
</tr>
<tr>
<td>% Developed</td>
<td>0.003</td>
</tr>
</tbody>
</table>
Mean air temperature ranges from -6.6 °C in January to 21.1 °C in July and mean annual precipitation is 95.5 cm (NRCC 2009). June and July of the 2009 study period were exceptionally wet, with precipitation amounts that were nearly 50% greater than the thirty year average (NWS 2008). When the Lamprey River rises after large rain events, it often floods up to BDC-150, taking several days to recover to base flow.

**Monthly Sampling and NO$_3^-$ Additions**

**Stream Water Sampling**
Water samples were collected monthly (January, February, and December) and bi-monthly (March-November) during 2009. Samples were taken approximately every 50 m of stream reach starting at the Lamprey River junction (BDC-0) to 516 m upstream (BDC-516). The tributary was also sampled if there was measureable flow, as well as any groundwater seepage along BDC at the surface water interface. Stream water was collected with 60 ml plastic syringes and filtered in the field through pre-combusted (450 °C for 4 hr) glass fiber filters (Whatman GF/F) into acid-washed high density polyethylene (HDPE) bottles. Prior to collection, the syringe and bottle were rinsed three times with sample water. Temperature, conductivity, specific conductivity, dissolved oxygen (DO- % and mg L$^{-1}$), and pH were measured at each site using a multiparameter water quality sensor (YSI 556). All samples were frozen until analysis.

**Stream Discharge**
Stream discharge was measured by an on-site removable six inch v-notch aluminum weir downstream from BDC-300, installed in August, 2009. The average of hourly discharge measurements recorded by the weir was used to estimate stream

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ii Based on 30 year average (1971-2000) from data collected in Concord, NH.

iii Based on 30 year average (1971-2000).
discharge on sample days. On all addition dates, discharge was quantified using NaBr (see calculations below).

**NO₃⁻ Additions**

To measure NO₃⁻ uptake, short-term (≤ 5 hr depending on stream discharge) NaNO₃, NaBr, and NaCl additions were conducted on April 16th (before leaf out), July 13th (full canopy cover), October 6th (before leaf fall), October 17th and 26th (during leaf fall), and November 22nd (after leaf fall) in 2009. To understand NO₃⁻ uptake at ambient NO₃⁻ concentrations, a tracer only (NaBr and NaCl) addition was conducted on August 16th. NaCl was used as a tracer to readily evaluate solute mixing and transit time as indicated by a rise and plateau in specific conductivity at the sampling point farthest downstream (Figure 3). Because of the low ambient Br⁻ concentrations, NaBr was used as a tracer to quantify stream discharge and hydrologic dilution. NO₃⁻ uptake was measured through the downstream attenuation of NO₃⁻. Target enrichments for NO₃⁻, Br⁻ and Cl⁻ were 1 mg N L⁻¹, 2.5 mg Br⁻ L⁻¹, and 20 mg Cl⁻ L⁻¹, respectively. The amount of solute added to reach these target enrichments was dependent on stream discharge (measured the day before using a Marsh-McBirney flow meter or the weir) and background stream solute concentration (estimated based on average values from most recent sampling).

Figure 3. Stream solute mixing diagram. Specific conductivity measurements demonstrating stream water and solute mixing during the August 6th addition.
Using a peristaltic pump (Masterflex E/S portable sampler), the NO$_3^-$ and tracer solution was pumped at a constant rate into a turbulent area of the stream upstream from the tributary. Multiparameter YSI water quality meters recorded specific conductivity at various stations and at the bottom of the reach (BDC-60 unless stream flow was low in which case BDC-125 was used as the downstream station). BDC-60 was chosen because it was upstream from a small organic dam. Stream samples were collected at six to 12 stations downstream from where thorough mixing was reached. Specific conductivity measurements indicated that tracers were thoroughly mixed in the stream by BDC-350. For each addition, samples were collected before the addition to determine background NO$_3^-$ concentrations, at plateau (to measure decay rate), and 24 hours after the addition (to measure recovery). The pump was turned off after the plateau samples were collected. Stream wetted width was measured approximately every 50 m.

**Water Chemistry Analysis**

All stream water samples were analyzed at the University of New Hampshire Water Quality Analysis Laboratory. NH$_4^+$ and PO$_4^{3-}$ were analyzed by robotic automated colorimetry (Westco Smartchem), cations (Na$^+$, K$^+$, Mg$^+$, Ca$^+$) and anions (NO$_3^-$, Cl$^-$, Br$^-$, SO$_4^{2-}$) by ion chromatography (Dionex Model), and dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) by high temperature catalytic oxidation (Shimadzu TOC-V).

**Potential Sediment Denitrification**

**Sediment Sample Collection**

Sediment samples were collected at BDC-60, BDC-300 and BDC-500 to characterize potential denitrification rates in the stream reach. Samples were collected on May 13th, July 2nd and October 18th, 2009. 15 cm$^3$ of sediment was collected from the top ~5 cm of sediment at six locations (in a zigzag pattern) at each station, using a cut-
Samples were placed into pre-weighed glass Mason jars and covered with 10 ml stream water filtered through Whatman GF/F to prevent the sediment from drying out. All jars were sealed with tops fitted with butyl septa. At the same time sediments were collected, 3 L stream water was also collected in acid-washed and stream water rinsed high density polyethylene bottles for use in the Denitrification Enzyme Assay (DEA) method.

**Denitrification Enzyme Assays**

Sediment denitrification potential was measured through Chloramphenicol-amended acetylene-inhibition DEA (Tiedje et al. 1989, Groffman et al. 1999). DEA’s were conducted on May 11th, July 2nd, and October 18th, 2009. After sample collection, stream water (3 L) was filtered through Whatman GF/F and amended with NO$_3^-$ and dextrose (to ensure NO$_3^-$ and carbon would not be limiting), and Chloramphenicol. Chloramphenicol limits further microbial growth by preventing production of the nitrate reductase enzyme (Tiedje et al. 1989). Target concentrations for NO$_3^-$, dextrose and Chloramphenicol were 100 mg N L$^{-1}$, 40 mg L$^{-1}$, and 10 mg L$^{-1}$, respectively. The uncapped sample jars, solution, and equipment were placed overnight in an anaerobic chamber.

The following morning, after anoxia had been reached, 75 ml of the NO$_3^-$, dextrose and Chloramphenicol solution were added to each jar. The jars were then quickly capped and removed from the anaerobic chamber. Using a gas-tight plastic syringe, 10 ml acetylene purified with sulfuric acid was injected into each jar through the butyl septa. The purpose of the acetylene was to inhibit the conversion of N$_2$O to N$_2$ gas, which is difficult to measure due to its ubiquity in the atmosphere. Five ml gas was sampled from each jar with a syringe, 30 and 90 minutes after the acetylene had been injected. To replace the removed gas from the headspace, 5 ml helium was immediately
injected into each jar. Gas samples were analyzed for N\textsubscript{2}O with a HP 5890 Series II gas chromatograph, using N\textsubscript{2}O standards of 0.1, 1, and 10 parts per million by volume (ppmv) N\textsubscript{2}O.

To determine sediment dry weight and headspace volume, the jars with sediment and solution were weighed, filled to the top with DI water, re-weighed, and placed in a drying oven for several days. The samples were then re-weighed, placed in an oven at 550 °C for four hours, and again re-weighed to determine ash-free dry mass (AFDM).

**Calculations and Statistical Analyses**

All statistical analyses were performed using SPSS 17.0, with a significance level of α=0.05. Seasons were designated as: winter (Jan-Feb, Dec), spring (Mar-May), summer (Jun-Aug), and autumn (Sep-Nov).

**Stream Chemistry**

Monthly and seasonal differences in stream water chemistry were determined through ANOVA and post hoc Tukey tests. To more accurately represent average stream chemistry, samples from stations BDC-50 to BDC-500 were used in analyses and included bi-monthly samples and pre-addition samples only. Pearson correlations were run between NO\textsubscript{3}\textsuperscript{-} and parameters that were determined to potentially influence NO\textsubscript{3}\textsuperscript{-}, including stream temperature, DO, DOC, NH\textsubscript{4}\textsuperscript{+}, PO\textsubscript{4}\textsuperscript{3-}, and stream discharge. Stream discharge measurements were taken from the weir unless the samples were taken on an addition date, in which case NaBr was used to measure discharge (see “NO\textsubscript{3}\textsuperscript{-} Uptake” section for calculations).

**NO\textsubscript{3}\textsuperscript{-} Uptake**

Natural hydrologic dilution and temporary storage of NaBr was calculated by dividing the plateau NaBr concentration at each station by the plateau NaBr at BDC-350.
(first station downstream from injection where solute was thoroughly mixed). Stream discharge \( (Q) \) was calculated for each station using the formula (Stream Solute Workshop, 1990):

\[
Q = \frac{(C_1 - C_b) \cdot Q_1}{(C_p - C_b)}
\]

where \( C_1 \) is the NaBr drip concentration, \( C_b \) is the pre-injection stream NaBr concentration, \( Q_1 \) is the pump rate, and \( C_p \) is the plateau NaBr concentration. Specific discharge was calculated by dividing stream discharge by the average wetted width of the stream.

A linear regression of concentration versus distance was performed using log-transformed \( \text{NO}_3^- \) concentration corrected for dilution, dispersion and station-specific background \( \text{NO}_3^- \) concentrations. The negative slope of the regression line is the uptake rate coefficient \( (k) \), the inverse of which is uptake length \( (S_w) \). Areal \( \text{NO}_3^- \) uptake rate \( (U) \), was calculated using the equation (Newbold et al. 1981, Stream Solute Workshop 1990):

\[
U = \frac{F}{S_w \cdot w}
\]

where \( F \) is the \( \text{NO}_3^- \) flux calculated using average discharge and average pre-injection \( \text{NO}_3^- \), and \( w \) is calculated as the average stream wetted width. Areal uptake rate was compared with the \( \text{NO}_3^- \) flux to determine the percent \( \text{NO}_3^- \) removal. If the areal uptake rate was higher than the actual \( \text{NO}_3^- \) flux at the time of the addition, it was assumed that 100% of the \( \text{NO}_3^- \) was taken up. \( \text{NO}_3^- \) uptake velocity \( (V_f) \) was calculated with the equation (Stream Solute Workshop 1990):

\[
V_f = \frac{Q \cdot k}{w}
\]

Pearson correlations were run between uptake length, uptake rate and uptake velocity, and water characteristics and chemistry data that were thought to potentially...
influence uptake, including discharge, specific discharge, DO (mg L\(^{-1}\)), stream temperature, % N-enrichment (the amount of N added to the stream relative to background concentration), DOC, NH\(_4^+\), and PO\(_4^{3-}\). For additions where NO\(_3^-\) uptake was not statistically significant, uptake rate and uptake velocity were assumed to be zero and uptake length was omitted from statistical analyses. ANOVA and post-hoc Tukey tests were used to determine significant differences between downstream NO\(_3^-\) decline slopes.

A power analysis was conducted for additions where a decline in NO\(_3^-\) was not statistically significant. The purpose of the analysis was to test the ability of the methods to reject the null hypothesis of no NO\(_3^-\) uptake, and determine the minimum uptake length at which a decline in NO\(_3^-\) would be statistically detectable. Regressions were plotted using the log-transformed NO\(_3^-\) concentration at each station. The residuals were assumed to be normally distributed. Simulations were conducted on 1,000 hypothetical data sets with similar residual variance but different slopes from the actual NO\(_3^-\) values collected in the field. These simulations were used to determine the slope, and therefore uptake length, at which the variance would be too high to detect NO\(_3^-\) uptake.

**Denitrification Enzyme Assays**

The concentration of N\(_2\)O in the headspace of each sample at 30 and 90 minutes was calculated with the equation (Holland et al. 1999):

\[
C_m = \frac{C_y \times M \times P}{R \times T}
\]

where \(C_m\) is the concentration of N\(_2\)O in \(\mu g\) N\(_2\)O-N L\(^{-1}\), \(C_y\) is the concentration of N\(_2\)O in ppmv, \(M\) is the molecular weight of N\(_2\)O (28 \(\mu g\) N\(_2\)O-N L\(^{-1}\)), \(P\) is the barometric pressure within the anaerobic chamber (atm), \(R\) is the universal gas constant (0.0821 L atm K mole\(^{-1}\)), and \(T\) is the temperature within the anaerobic chamber (\(^\circ\)C).
Potential sediment denitrification rates were calculated for both the dry mass and
ash free dry mass of sediments with the equation (Groffman et al. 1999):

\[
DR = \frac{(C_{90} \times H) - (C_{30} \times H)}{D \times T}
\]

where DR is the rate of sediment denitrification (μg N g soil hr\(^{-1}\)), C\(_{90}\) is the concentration
of N\(_2\)O at 90 minutes, C\(_{30}\) is the N\(_2\)O concentration at 30 minutes, H is headspace
volume in the Mason jar, D is either the sediment dry mass or ash free dry mass
(organic matter weight) depending on the desired calculation, and T is the duration of the
incubation. The percent organic content for each sample was determined by dividing
DM by AFDM and multiplying by 100.

Areal denitrification rates (U\(_{den}\)) were calculated by correcting DR for average
sediment AFDM for each sample station and average stream area (length=516 m,
width=1.1 m) based on measurements from each NO\(_3^-\) addition. The total mass of
sediment at BDC was calculated assuming denitrification was occurring in the top 5 cm
of underlying sediment only. U\(_{den}\) was divided by stream NO\(_3^-\) flux to produce V\(_{f-den}\), or
the velocity at which NO\(_3^-\) enters the benthos via denitrification (Royer et al. 2004,
Arango et al. 2008).

ANOVA and post-hoc Tukey tests were used to determine significant differences
among stations and seasons. If no there were no significant differences, denitrification
results were pooled for all analyses.
CHAPTER II

RESULTS

Stream Chemistry

Stream temperature and DO followed seasonal patterns, with higher stream temperatures in summer and autumn coinciding with low DO. The creek was covered in ice January through March, and in December, 2009 (Table 2). Mean stream temperature ranged from 0-16 °C, and mean DO ranged from 4.3 to 15.1 mg O₂ L⁻¹.

Table 2. BDC seasonal stream chemistry (mean ± standard error) in 2009. Values calculated using samples BDC-50 to BDC-500 only.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Winter (Jan-Feb, Dec)</th>
<th>Spring (Mar-May)</th>
<th>Summer (Jun-Aug)</th>
<th>Autumn (Sep-Nov)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stream Temp.</td>
<td>°C</td>
<td>0.10 ± 0.15</td>
<td>5.62 ± 0.63</td>
<td>15.2 ± 0.14</td>
<td>7.76 ± 0.47</td>
</tr>
<tr>
<td>DO</td>
<td>mg L⁻¹</td>
<td>14.46 ± 0.37</td>
<td>9.75 ± 0.34</td>
<td>9.18 ± 0.27</td>
<td>8.84 ± 0.35</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>mg N L⁻¹</td>
<td>1.34 ± 0.11</td>
<td>0.85 ± 0.06</td>
<td>0.78 ± 0.05</td>
<td>0.99 ± 0.06</td>
</tr>
<tr>
<td>DOC</td>
<td>mg C L⁻¹</td>
<td>5.33 ± 0.20</td>
<td>6.79 ± 0.33</td>
<td>10.78 ± 0.40</td>
<td>10.97 ± 0.27</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>mg N L⁻¹</td>
<td>0.02 ± 0.001</td>
<td>0.06 ± 0.008</td>
<td>0.07 ± 0.005</td>
<td>0.02 ± 0.002</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>mg P L⁻¹</td>
<td>0.03 ± 0.001</td>
<td>0.08 ± 0.006</td>
<td>0.16 ± 0.006</td>
<td>0.12 ± 0.004</td>
</tr>
</tbody>
</table>

Daily mean NO₃⁻ concentrations for sites BDC-50 to BDC-500 were variable throughout the year ranging from 0.3 to 2.4 mg N L⁻¹ (Figure 4). The highest concentrations were in September, though three-month seasonal averages indicated higher overall NO₃⁻ in winter (Table 2). DOC in autumn was higher than winter and spring concentrations (p< 0.001), with mean DOC ranging from 4.2 to 15.6 mg C L⁻¹ (Figure 4 and Table 2). NO₃⁻ and DOC were negatively correlated (p< 0.001, r²=0.31-Figure 5). There was no correlation between NO₃⁻ and NH₄⁺. Concentrations of NH₄⁺ and
PO$_4^{3-}$ were low, with daily means ranging from <0.01 to 0.15 mg N L$^{-1}$, and 0.02 to 0.22 mg P L$^{-1}$, respectively. Concentrations for both NH$_4^+$ and PO$_4^{3-}$ were lowest in winter and highest in summer.

**Figure 4.** Mean monthly stream NO$_3^-$ and DOC (+ standard error) for stations BDC-50 to BDC-500. Error bars are on all points but may not be visible if too small. Sampling was conducted once during Jan, Feb, and Dec, and at least twice for all other months.

**Figure 5.** NO$_3^-$ and DOC concentrations from bi-monthly and pre-addition samples at BDC. n=278 representing sample stations BDC-50 through BDC-500.
Stream $\text{NO}_3^-$ was inversely related to stream discharge ($p<0.001$, $r^2=0.61$).

Figure 6. Discharge ranged from 0.2 to 4.6 L sec$^{-1}$ with lowest flows in September and early October, coinciding with high $\text{NO}_3^-$. 

Figure 6. BDC mean daily $\text{NO}_3^-$ (BDC-50 to BDC-500) and stream discharge from bi-monthly and pre-addition samples. Discharge measurements were from the weir unless on an addition date, in which case NaBr was used to measure flow. Measurements from the December sampling were not included due to the influence of ice on the weir.

Stream $\text{NO}_3^-$ was positively correlated with tributary $\text{NO}_3^-$ ($p<0.001$, $r^2=0.81$).

Figure 7. BDC $\text{NO}_3^-$ was typically highest at the tributary junction and declined downstream towards the Lamprey River. Tributary $\text{NO}_3^-$ was temporally variable, ranging from $<0.01$ to 10.1 mg N L$^{-1}$ within a five-hour time frame on the August 6th sampling, for example.
There were no statistical differences between the slope of the ambient NO$_3^-$ decline downstream and season. There was no relationship between ambient NO$_3^-$ and DOC concentrations, though ambient NO$_3^-$ slope was strongly correlated with stream discharge ($p = 0.006$, $r^2 = 0.54$).

**NO$_3^-$ Uptake**

NO$_3^-$ uptake was statistically significant for all autumn additions, but not during the spring or summer additions (Figure 8). For the autumn additions mean uptake length ($S_w$) was 509 m, uptake velocity ($V_f$) was 0.36 mm min$^{-1}$, and uptake rate ($U$) was 317 ug N m$^{-2}$ min$^{-1}$ (Table 3). Measured uptake was greatest during the October 17$^{th}$ addition,

---

$^iv$ NO$_3$ concentrations declined downstream from the tributary on all dates except for 5/27/09 when there was a slight increase in concentration and the slope was positive.
when discharge, specific discharge, and N-enrichment were lower and background stream NO$_3^-$ concentrations were higher than the other autumn additions.

Figure 8. Downstream patterns in NO$_3^-$ during all additions. NO$_3^-$ attenuation was only statistically significant during the autumn additions (p<0.001).
Table 3. NO$_3^-$ uptake and denitrification measurements as well as stream characteristics for all addition dates. -- signifies non-detectable uptake length because the p value > 0.05. N-enrichment represents the percent increase in N from each addition relative to ambient concentration. N-enrichment was not consistent due to changes in stream discharge. Note that August 6$^{th}$ was a conservative tracer only addition and no NO$_3^-$ was added.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>4/16/09</th>
<th>7/13/09</th>
<th>8/6/09</th>
<th>10/6/09</th>
<th>10/17/09</th>
<th>10/26/09</th>
<th>11/22/09</th>
</tr>
</thead>
<tbody>
<tr>
<td>p value</td>
<td></td>
<td>0.667</td>
<td>0.091</td>
<td>0.722</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$S_w$ (m)</td>
<td></td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>534</td>
<td>111</td>
<td>752</td>
<td>640</td>
</tr>
<tr>
<td>U (ug N m$^{-2}$ min$^{-1}$)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>208</td>
<td>712</td>
<td>114</td>
<td>233</td>
</tr>
<tr>
<td>$V_f$ (mm min$^{-1}$)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.25</td>
<td>0.60</td>
<td>0.29</td>
<td>0.31</td>
</tr>
<tr>
<td>$V_{f-den}$ (mm min$^{-1}$)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.009</td>
<td>0.006</td>
<td>0.019</td>
<td>0.010</td>
</tr>
<tr>
<td>Total NO$_3^-$ Uptake (%)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>96.6</td>
<td>100.0</td>
<td>68.6</td>
<td>80.6</td>
</tr>
<tr>
<td>NO$_3^-$ Removed via</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.4</td>
<td>1.0</td>
<td>6.3</td>
<td>3.1</td>
</tr>
<tr>
<td>Denitrification (%)</td>
<td></td>
<td>103</td>
<td>31</td>
<td>0</td>
<td>138</td>
<td>55</td>
<td>281</td>
<td>104</td>
</tr>
<tr>
<td>N-Enrichment (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discharge (L s$^{-1}$)</td>
<td></td>
<td>4.6</td>
<td>2.3</td>
<td>1.4</td>
<td>2.4</td>
<td>1.3</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Spec. Discharge (L sec$^{-1}$ m$^{-1}$)</td>
<td></td>
<td>4.2</td>
<td>2.2</td>
<td>1.6</td>
<td>2.2</td>
<td>1.1</td>
<td>3.7</td>
<td>3.4</td>
</tr>
</tbody>
</table>
Simulations were conducted to test the statistical power of the methods to detect a downstream decline in NO₃⁻ on the dates when uptake was not detected (Figure 9). For the April addition, there was a 95% chance of detecting uptake if the uptake length was less than 1,430 m, indicating that actual uptake length on that date was greater than 1,430 m. The point of rejection was 500 m and 110 m for the July and August additions, respectively.

Figure 9. Results from the power analysis conducted to determine the minimum uptake length detectable based on the variance of data from the spring and summer additions.

NO₃⁻ uptake length and discharge were positively related, though the trend was not statistically significant ($p=0.067$). Specific discharge was positively correlated with uptake length ($p=0.042$, $r^2=0.92$- Figure 10a) and negatively correlated with background stream NO₃⁻ ($p=0.027$, $r^2=0.87$- Figure 10b). Wetted width was similar for all addition dates, ranging from 0.9 to 1.2 m, and specific discharge ranged from 1.1 to 4.2 L sec⁻¹ m⁻¹. NO₃⁻ uptake length had a weak negative correlation with ambient stream NO₃⁻ ($p=0.051$, $r^2=0.90$- Figure 10c).
There was no correlation between N-enrichment and uptake length ($p=0.224$), uptake rate ($p=0.940$), or uptake velocity ($p=0.540$). N-enrichment varied considerably between additions due to changes in stream flow and background NO$_3^-$ concentration (Table 3).
Other chemical characteristics that could potentially influence uptake were DOC, stream temperature, DO, NH₄⁺, and PO₄³⁻ (Table 4). There was no correlation between DOC and any of the uptake measurements, though DOC was significantly higher \((p<0.001)\) on the October 6th and 26th addition dates. When uptake was compared to the other chemical characteristics there were no statistically significant correlations.

### Table 4. Mean stream chemistry data ± standard error for each addition. Values are from pre-addition samples.

<table>
<thead>
<tr>
<th>Date</th>
<th>DO (mg O₂ L⁻¹)</th>
<th>Stream Temp. (°C)</th>
<th>DOC (mg C L⁻¹)</th>
<th>NO₃⁻ (mg N L⁻¹)</th>
<th>NH₄⁺ (mg N L⁻¹)</th>
<th>PO₄³⁻ (mg P L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/16/09</td>
<td>12.3 ± 0.29</td>
<td>3.0 ± 0.06</td>
<td>5.2 ± 0.10</td>
<td>0.8 ± 0.02</td>
<td>0.02 ± 0.002</td>
<td>0.04 ± 0.002</td>
</tr>
<tr>
<td>7/13/09</td>
<td>8.8 ± 0.33</td>
<td>14.7 ± 0.06</td>
<td>7.4 ± 0.55</td>
<td>1.0 ± 0.09</td>
<td>0.06 ± 0.009</td>
<td>0.15 ± 0.011</td>
</tr>
<tr>
<td>8/6/09</td>
<td>8.7 ± 0.20</td>
<td>16.3 ± 0.11</td>
<td>9.3 ± 0.24</td>
<td>1.0 ± 0.09</td>
<td>0.11 ± 0.017</td>
<td>0.19 ± 0.010</td>
</tr>
<tr>
<td>10/6/09</td>
<td>6.0 ± 0.25</td>
<td>9.8 ± 0.03</td>
<td>12.8 ± 0.23</td>
<td>0.8 ± 0.05</td>
<td>0.02 ± 0.009</td>
<td>0.19 ± 0.005</td>
</tr>
<tr>
<td>10/17/09</td>
<td>10.0 ± 0.47</td>
<td>3.2 ± 0.05</td>
<td>9.5 ± 0.32</td>
<td>1.2 ± 0.05</td>
<td>0.004 ± 0.001</td>
<td>0.10 ± 0.005</td>
</tr>
<tr>
<td>10/26/09</td>
<td>9.6 ± 0.37</td>
<td>6.2 ± 0.03</td>
<td>15.6 ± 0.17</td>
<td>0.4 ± 0.02</td>
<td>0.004 ± 0.000</td>
<td>0.13 ± 0.004</td>
</tr>
<tr>
<td>11/22/09</td>
<td>13.4 ± 0.54</td>
<td>3.0 ± 0.02</td>
<td>9.9 ± 0.17</td>
<td>0.7 ± 0.01</td>
<td>0.03 ± 0.002</td>
<td>0.06 ± 0.002</td>
</tr>
</tbody>
</table>

**Potential Sediment Denitrification**

Potential sediment denitrification rates ranged from <0.001 to 0.048 ug N g DM⁻¹ hr⁻¹ or 0.01 to 1.93 ug N g AFDM⁻¹ hr⁻¹ (Figure 11). Overall, rates were variable even within the same site, differing by an order of magnitude. There were no statistically significant differences in potential denitrification rates between seasons or stations. The percent organic content at each site was 7.2%, 3.0%, and 7.4% at BDC-60, BDC-300 and BDC-500, respectively.
Areal denitrification rates ($U_{den}$) were calculated using pooled data, and assuming a sediment depth of 5 cm, where denitrification was most likely to occur. Areal rates were: 4.9, 12.2, and 14.9 mg N m$^{-2}$ day$^{-1}$ for BDC-60, BDC-300 and BDC-500, respectively. Denitrification velocity ($V_{den}$) ranged from 0.006 to 0.01 mm min$^{-1}$ (Table 3).

**Fate of NO$_3^-$ in BDC**

Mean NO$_3^-$ uptake rate at BDC in autumn was 316.7 mg N m$^{-2}$ day$^{-1}$. When compared to average NO$_3^-$ flux this accounted for approximately 87% $^{v}$ removal of NO$_3^-$ N from BDC. When comparing areal denitrification with areal NO$_3^-$ uptake, denitrification accounted for 3.6% of stream NO$_3^-$ removal over the 516 m reach (Table 3, Figure 12a-d). Because there was no significant NO$_3^-$ uptake in spring or summer, it was assumed that there was no uptake or denitrification occurring and therefore 100% of the NO$_3^-$ flux was discharged to the Lamprey River.

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$^{v}$ When comparing areal uptake rate with actual NO$_3^-$ flux, on 10/17/09 the percent NO$_3^-$ removal was over 100%. For that date it was assumed that 100% of the NO$_3^-$ was removed. See Methods.

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Figure 12a-d. Fate of NO$_3^-$ during the autumn additions. Assumes 3.6% of NO$_3^-$ load removed via denitrification.
CHAPTER III

DISCUSSION

Patterns in Ambient Stream NO$_3$-

Stream NO$_3$- concentration is influenced by watershed land use, with generally higher concentrations in agriculturally influenced streams compared to forested streams. NO$_3$- in BDC ranged from 0.3 to 2.4 mg N L$^{-1}$, which, when compared to other published values, was higher than streams classified as forested (Mulholland et al. 2004), but significantly lower than agricultural streams, where NO$_3$- concentrations as high as 12.6 mg N L$^{-1}$ have been reported (Royer et al. 2004). Unlike most agricultural streams which are often ditches flowing through pastures, BDC has a large forested riparian area that likely reduces N-loading. Based on longitudinal patterns in NO$_3$- concentrations, the intermittent, non-buffered tributary draining the cow pastures seems to be the primary source of NO$_3$- in BDC. The temporal variability in tributary NO$_3$- was likely linked to groundwater mixing, dilution during high discharge, and proximity of the dairy cows to the tributary.

Background NO$_3$- concentration declined downstream from the tributary junction on all sampling dates except for one. Most of the measurable decline in background NO$_3$- can be attributed to natural hydrologic dilution from surface runoff or groundwater, or from uptake, which, based on the NO$_3$- additions, was not occurring in spring or summer. Because NO$_3$- was only taken up in autumn, and because of the strong negative correlation between NO$_3$- and DOC, I would have expected steeper NO$_3$- slopes in autumn when DOC was high. However, there were no seasonal differences in slope and there was no correlation between NO$_3$- slope and DOC. The data suggest that
stream discharge had the greatest overall impact on the slope of the NO$_3^-$ decline, as well as other NO$_3^-$ dynamics including background NO$_3^-$ concentration, and NO$_3^-$ uptake length.

**Spring and Summer NO$_3^-$ Additions**

The lack of measurable NO$_3^-$ uptake in the spring and summer at BDC was unusual when compared to other studies. Other published seasonal NO$_3^-$ uptake studies have found that, not only is NO$_3^-$ taken up throughout the year, but rates are highest in spring and summer (Simon et al. 2005, Hoellein et al. 2007). Out of all the published studies on NO$_3^-$ uptake, few found no significant NO$_3^-$ uptake when only NO$_3^-$ was added (Richey et al. 1985, Hall and Tank 2003). Based on the power analysis, NO$_3^-$ uptake could not be detected if uptake length was greater than approximately 1400 m for the spring addition. Despite this limitation, BDC is only 516 m in length so even if longer uptake lengths could be detected, this would still indicate that, on average, NO$_3^-$ is not being taken up prior to discharging into the Lamprey River. The power of detecting NO$_3^-$ uptake during the summer and spring additions was limited by shallow NO$_3^-$ decline slopes and high variance.

Research conducted on floodplain NO$_3^-$ dynamics at BDC in 2009 supports the finding of no NO$_3^-$ uptake in spring or summer (Galvin 2010). Galvin (2010) measured ground and surface water in BDC during flooding events in the spring (March, April) and summer (May, July) and found that, during flooding events, NH$_4^+$ and DOC were taken up, but that NO$_3^-$ removal was minimal and only occurred after prolonged periods of inundation in the spring.

NO$_3^-$ values were variable during the spring and summer additions and appeared to slightly increase downstream. This increase is unlikely due to groundwater input,
which contains little NO$_3^-$ (Galvin 2010), but may be explained by nitrification (the conversion of NH$_4^+$ to NO$_3^-$). BDC drains a wetland, where low DO concentrations would inhibit nitrification. As DO concentrations increase downstream, as evidenced by field measurements (see Appendix), a portion of the NH$_4^+$ is likely nitrified. Additionally, downstream from BDC-300 the stream has more riffles which introduce oxygen into the system, therefore further oxidizing NH$_4^+$. The occurrence of nitrification in BDC may be supported by the downstream decline in NH$_4^+$ observed on all sample dates except for four, one of which was the spring addition. However, further experiments such as NH$_4^+$ additions are necessary to quantify nitrification rates in BDC.

While NH$_4^+$ concentrations were low, the availability of NH$_4^+$ in BDC may have inhibited NO$_3^-$ uptake. NH$_4^+$ is more easily assimilated than NO$_3^-$ and sorbs well to sediments (Hedin et al. 1998, Peterson et al. 2001). Therefore, if sufficient NH$_4^+$ is available, it would likely be taken up prior to NO$_3^-$. In LINX I (similar to LINX II though NH$_4^+$ additions were conducted in 12 streams), despite higher NO$_3^-$ than NH$_4^+$ concentrations, NO$_3^-$ uptake lengths were ten times greater than those for NH$_4^+$ (Peterson et al. 2001).

Precipitation, light availability and stream DOC concentrations may have also influenced NO$_3^-$ uptake in spring and summer. In summer 2009, there was an unusually large amount of precipitation, leading to higher stream flows. These higher flows may have scoured autotrophs and heterotrophs from the surface, inhibiting NO$_3^-$ uptake. During spring, DOC was lower and light availability was higher than during both summer additions. In the summer months, the increase in DOC may be explained by increased microbial activity in the upstream wetland, organic matter breakdown, and thus DOC export to BDC. It is possible that NO$_3^-$ uptake in spring was limited by the lack of DOC, and in summer, uptake was limited by reduced light availability.
Influence of NO$_3^-$ Concentration on Uptake

Stream NO$_3^-$ concentration can significantly impact NO$_3^-$ uptake. Because of the link between stream NO$_3^-$ concentrations and uptake, there are inherent short-falls in bulk additions, which can have a fertilization effect leading to an overestimation of uptake length and underestimation of uptake rate (Dodds et al. 2002). When using bulk additions, uptake can best be estimated by conducting multiple enrichments within a short time frame (Payn et al. 2005). In October three additions were performed with varying N-enrichments (range= 55% to 280% increase in N), though stream temperature, ambient NO$_3^-$ and discharge varied considerably between each addition. While there was an apparent positive relationship (though not statistically significant) between N-enrichment and uptake length, because of the differences in stream chemistry it is not possible to attribute any differences to N-enrichment alone.

At BDC, stream NO$_3^-$ was not a strong predictor of uptake rate or uptake velocity. If BDC were N-saturated I would have expected to see a negative or Michaelis-Menten relationship between stream NO$_3^-$ and uptake rate and uptake velocity (Stream Solute Workshop 1990, O'Brien et al. 2007). It is possible that the range of NO$_3^-$ values in BDC (0.4 to 1.2 mg N L$^{-1}$) was not great enough to detect a relationship. For example, Arango et al. (2008) found a Michaelis-Menten relationship between uptake rate and ambient NO$_3^-$, and their stream NO$_3^-$ values ranged from 0.01 to 17.4 mg N L$^{-1}$. Simon et al. (2005) detected a strong negative relationship between uptake velocity and ambient NO$_3^-$, which ranged from 0.001 to 0.013 mg N L$^{-1}$, though they conducted 12 additions. With the relatively small range of NO$_3^-$ concentrations at BDC I may have needed to conduct more than seven additions to detect a relationship.

Background stream NO$_3^-$ appeared to predict uptake length, though the negative relationship was contrary to what has been found in other studies (Peterson et al. 2001, Grimm et al. 2005, O'Brien et al. 2007, Mulholland et al. 2008). The negative relationship
was either another indication that BDC is not N-saturated, or was simply an indirect
effect of the influence of discharge on both stream ambient NO$_3^-$ and uptake length.
Higher specific discharge was correlated with lower background NO$_3^-$ and longer uptake
lengths, thus explaining the apparent correlation between long uptake lengths and low
background NO$_3^-$ concentrations. Therefore, it appears that specific discharge more
strongly predicts uptake length than background stream NO$_3^-$ concentrations.

**Autumn NO$_3^-$ Additions**

When comparing the autumn NO$_3^-$ uptake velocity, or the demand for NO$_3^-$, to
published literature values, it is apparent that NO$_3^-$ removal at BDC is less efficient than
some forested, headwater streams (Hoellein et al. 2007), though significantly more
efficient than most agricultural reaches (Birgand et al. 2005- Table 5). Uptake efficiency
at BDC was within the range of values found in the LINX II project (Mulholland et al.
2008).

The discrepancy between NO$_3^-$ uptake at BDC and other agricultural streams can
be attributed to basic stream characteristics. Unlike BDC, typical agricultural reaches are
un-buffered ditches draining pastures and crop fields. The lack of canopy cover, and
therefore higher light availability and stream temperatures often leads to significantly
higher ambient NO$_3^-$ concentrations and rates of primary production (Bernot et al. 2006).
Additionally, because these ditches are designed to quickly divert water away from
fields, stream flow is often higher, resulting in reduced NO$_3^-$ contact with the benthos. At
BDC, the large forested riparian buffer significantly reduces NO$_3^-$ loading and provides
shade, reducing stream temperature and light availability. At BDC there is little aquatic
plant or algal growth, which is an indication of low primary productivity. Because
agricultural streams often receive high loads of NO$_3^-$, these systems can become N-
saturated, resulting in reduced NO$_3^-$ uptake efficiency (Birgand et al. 2005, Bernot et al. 2006, Mulholland et al. 2008).
Table 5. Comparison of NO$_3^-$ uptake measurements from BDC and from other published studies. Where possible, the range of values was given, otherwise the mean value was provided. * is the inter-quartile range rather than the full range. -- signifies that the data was not reported. Methods: 1=NO$_3^-$ addition; 2=$^{15}$N addition.

<table>
<thead>
<tr>
<th>Stream Type</th>
<th>Location</th>
<th>$S_w$ (m)</th>
<th>$U$ (ug N m$^{-2}$ min$^{-1}$)</th>
<th>$V_t$ (mm min$^{-1}$)</th>
<th>Method</th>
<th>NO$_3^-$ (mg N L$^{-1}$)</th>
<th>Q (L sec$^{-1}$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural, 1st order</td>
<td>Lee, NH</td>
<td>111-752</td>
<td>114-712</td>
<td>0.25-0.60</td>
<td>1</td>
<td>0.4-1.2</td>
<td>1-4</td>
<td>This study-autumn only</td>
</tr>
<tr>
<td>Mixed, 1st order</td>
<td>North America, Puerto Rico</td>
<td>20-18,332</td>
<td>0.3-7,594</td>
<td>0.02-17.9</td>
<td>2</td>
<td>&lt;0.01-21.2</td>
<td>0.2-267.9</td>
<td>Hall et al. 2009</td>
</tr>
<tr>
<td>Mixed, 1st order</td>
<td>Various</td>
<td>101-478</td>
<td>5.8-19.1*</td>
<td>0.8-4.2</td>
<td>1, 2</td>
<td>--</td>
<td>--</td>
<td>Ensign and Doyle 2006</td>
</tr>
<tr>
<td>Urban, 1st order</td>
<td>Baltimore, MD</td>
<td>356-1341</td>
<td>150-1050</td>
<td>0.08-0.34</td>
<td>1</td>
<td>0.5-1.8</td>
<td>2-5</td>
<td>Klocker et al. 2009</td>
</tr>
<tr>
<td>Grassland, 1st order</td>
<td>South Island, New Zealand</td>
<td>57-704</td>
<td>2-39</td>
<td>1.10-12.40</td>
<td>1, 2</td>
<td>0.003-0.005</td>
<td>15-23</td>
<td>Simon et al. 2005</td>
</tr>
<tr>
<td>Agricultural</td>
<td>Various</td>
<td>--</td>
<td>243-868</td>
<td>0.05-0.17</td>
<td>Various</td>
<td>--</td>
<td>--</td>
<td>Birgand et al. 2005</td>
</tr>
<tr>
<td>Forested, 1st order</td>
<td>Untonagon River basin, MI</td>
<td>348-556</td>
<td>472-1213</td>
<td>2.70-7.56</td>
<td>1</td>
<td>0.3</td>
<td>40-68</td>
<td>Hoellein et al. 2007</td>
</tr>
</tbody>
</table>
One of the primary differences between the autumn additions and the April and July additions at BDC was carbon availability, potentially indicating carbon-limitation. Carbon inputs to streams from leaf-fall in autumn are a significant energy source and can be responsible for as much as 11% of the total annual respiration in headwater streams in New England (McDowell and Fisher 1976). For two of the autumn additions DOC was twice as high as in the April and July additions. Of the autumn additions the October 17th and November 22nd additions had the highest uptake velocity and uptake rate, shortest uptake length, and lowest DOC. The lower DOC may be an indication of higher heterotrophic carbon uptake and autotrophic carbon-fixation, resulting in higher NO\textsubscript{3}\textsuperscript{-} uptake due to C:N stoichiometry (Hall and Tank 2003). Tank and Webster (1998) note that heterotrophic activity is often carbon-limited and is therefore higher in autumn during leaf fall.

Carbon-limitation of NO\textsubscript{3}\textsuperscript{-} uptake at BDC is further suggested by the inverse relationship between stream DOC and NO\textsubscript{3}\textsuperscript{-} throughout 2009. This relationship has also been documented in other New England streams (Goodale et al. 2005), and in experimental additions of leaf litter and labile DOC (Hedin et al. 1998, Bernhardt and Likens 2002). This inverse relationship not only highlights the importance of available carbon in NO\textsubscript{3}\textsuperscript{-} uptake, but also demonstrates that changes in DOC availability can impact NO\textsubscript{3}\textsuperscript{-} uptake (Goodale et al. 2005).

While leaf-litter provides a carbon source to the stream, leaf-fall also corresponds to increased light availability to the stream. Autotrophic demand plays a key role in NO\textsubscript{3}\textsuperscript{-} uptake and is often light-limited (Mulholland et al. 2008). The dense canopy cover at BDC during the summer may have inhibited autotrophic activity. It is unlikely that light-limitation influenced biological activity in April because the addition was conducted prior to leaf-out.
**Potential Sediment Denitrification Rates**

Sediment denitrification rates in this study were likely overestimates of actual rates in the field because they were measured under non-limiting conditions (i.e. anoxia, sufficient NO$_3^-$ and carbon). As predicted, DM denitrification rates were highest at BDC-500 where the underlying stream sediment was predominantly organic matter, and the percent organic content was highest (7.4%). When expressed as AFDM, however, denitrification rates were highest at BDC-300. AFDM at BDC-300 likely primarily represents microbial organic content since the sediment is predominantly inorganic material (Inwood et al. 2007). In contrast, the organic content at BDC-500 and BDC-60 is high and therefore AFDM most likely includes both microbial organic content and organic matter. Despite the apparent differences in denitrification rates, there were no statistical differences between sites, likely a result of high variance which is typical in denitrification studies (Groffman et al. 2006, Seitzinger et al. 2006).

Overall, potential denitrification rates for both DM and AFDM sediment and areal denitrification were within the range of published values (Table 6). Denitrification velocity was also within the range of values found in headwater agricultural streams (Royer et al. 2004).
Table 6. Comparison of sediment denitrification rates and areal sediment denitrification at BDC and in published studies. DM= dry mass sediment and AFDM= ash free dry mass sediment. U\text{den} is areal denitrification. ** signifies that the data were extrapolated from figures. -- signifies that the data was not provided. All U\text{den} values are for DM sediment only. Methods: 1= Denitrification Enzyme Assay (DEA- NO\textsubscript{3}, dextrose, Chloramphenicol, acetylene (C\textsubscript{2}H\textsubscript{2})); 2= DEA (Chloramphenicol, C\textsubscript{2}H\textsubscript{2}); 3=\textsuperscript{15}N addition; 4= DEA (Chloramphenicol, C\textsubscript{2}H\textsubscript{2}, glucose).

<table>
<thead>
<tr>
<th>Stream Type</th>
<th>Location</th>
<th>Sediment</th>
<th>Denitrification Rate (ug N g soil\textsuperscript{-1} hr\textsuperscript{-1})</th>
<th>U\text{den} (mg N m\textsuperscript{-2} day\textsuperscript{-1})</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural, 1st order</td>
<td>Lee, NH</td>
<td>DM</td>
<td>&lt;0.01-0.05</td>
<td>--</td>
<td>1</td>
<td>This study</td>
</tr>
<tr>
<td>Urban, 1st order</td>
<td>Baltimore, MD</td>
<td>DM</td>
<td>&lt;0.01-0.07</td>
<td>--</td>
<td>1</td>
<td>Klocker et al. 2009</td>
</tr>
<tr>
<td>Forested, agricultural, urban, 1st order</td>
<td>Kalamazoo River basin, MI</td>
<td>DM</td>
<td>0.1-1.0*</td>
<td>--</td>
<td>2</td>
<td>Inwood et al. 2005</td>
</tr>
<tr>
<td>Forested, 1st order</td>
<td>Walker Branch, TN</td>
<td>AFDM</td>
<td>--</td>
<td>3.9</td>
<td>3</td>
<td>Mulholland et al. 2004</td>
</tr>
<tr>
<td>Agricultural, 1st order</td>
<td>Lee, NH</td>
<td>AFDM</td>
<td>0.01-1.93</td>
<td>4.9 - 4.9</td>
<td>1</td>
<td>This study</td>
</tr>
<tr>
<td>Agricultural 1st order</td>
<td>Sangamon, Embarras, and Kaskaskia Rivers, IL</td>
<td>AFDM</td>
<td>0.20-24.82</td>
<td>0.2 - 360.0</td>
<td>2</td>
<td>Royer et al. 2004</td>
</tr>
<tr>
<td>Forested, agricultural, urban, 1st order</td>
<td>Kalamazoo River basin, MI</td>
<td>AFDM</td>
<td>3-18*</td>
<td>0.7 - 15.6</td>
<td>2</td>
<td>Inwood et al. 2005</td>
</tr>
<tr>
<td>Forested, 1st order</td>
<td></td>
<td>AFDM</td>
<td>11.8</td>
<td>2</td>
<td>4</td>
<td>Findlay et al. In press</td>
</tr>
<tr>
<td>Agricultural, 1st order</td>
<td>North America, Puerto Rico</td>
<td>AFDM</td>
<td>107.0</td>
<td>5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban, 1st order</td>
<td></td>
<td>AFDM</td>
<td>94.2</td>
<td>6.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
At BDC a maximum of 4% of the daily NO$_3^-$ load over the 516 m reach was removed via denitrification. Actual removal is likely lower, where the sediments may not be completely anoxic and carbon may be limiting, which is often the case with denitrification (Seitzinger et al. 2006). As reviewed by Bernot and Dodds (2005), denitrification typically accounts for 1-20% of NO$_3^-$ removal in streams. More recently the LINX II experiments demonstrated that on average, denitrification is responsible for 16% of NO$_3^-$ removal in small streams (Mulholland et al. 2009).

**Conclusions**

The results of this study suggest that NO$_3^-$ is only taken up in autumn during leaf fall, and denitrification is responsible for a small portion of NO$_3^-$ removal. Overall the stream appears to be co-limited by light and carbon availability, since uptake was only detected in autumn, when both were highest. There are several pathways in which NO$_3^-$ can be removed from the water column (burial, DNRA, denitrification, autotrophic and heterotrophic assimilation). Based on the NO$_3^-$ additions and denitrification assays as well as chemistry data collected throughout the year it is apparent that heterotrophic assimilation most likely dominates NO$_3^-$ uptake at BDC, while the other pathways are negligible.

First, NO$_3^-$ does not sorb well to sediments so burial is likely not responsible for a significant portion of NO$_3^-$ removal. Second, DNRA, the conversion of NO$_3^-$ back to NH$_4^+$, is likely minimal if occurring at all because it is most common in systems with low NO$_3^-$ and high carbon availability (Tiedje 1988), which is not characteristic of BDC. Third, based on my measurements of potential denitrification, this pathway is responsible for a very small portion of NO$_3^-$ removal. Fourth, autotrophic assimilation is likely low because the creek supports very few rooted aquatic plants, and algae were only observed in
small sections of the creek on two occasions in spring. Additionally, the lack of measurable NO$_3^-$ uptake during the spring addition, when light availability was high and autotrophic activity would presumably also have been high, suggests little influence of autotrophic assimilation on NO$_3^-$ uptake. Finally, heterotrophic activity is often carbon limited (Tank and Webster 1998) and given that NO$_3^-$ was only taken up in autumn during leaf fall, this is most likely the dominant form of NO$_3^-$ removal in BDC.

This study highlights the importance of further research on seasonal differences in NO$_3^-$ uptake in headwater streams. NO$_3^-$ dynamics at BDC are influenced by factors that vary temporally (e.g. stream discharge, carbon availability) and therefore uptake and denitrification exhibit seasonal variation. On average, 87% of the daily NO$_3^-$ load was taken up in autumn whereas NO$_3^-$ uptake was not detectable in spring or summer. More research in this area will enable scientists to further understand what drives NO$_3^-$ uptake, especially in areas like New England where seasons are pronounced.

Because NO$_3^-$ uptake is minimal in BDC for the majority of the year, it is imperative that agricultural planners work to minimize NO$_3^-$ loading into BDC. Since a primary source of NO$_3^-$ to BDC appears to be the tributary, N-loading may be minimized by either creating a vegetative buffer or minimizing contact of cows with the tributary. The results of this study have implications for Great Bay Estuary, an estuary that has recently been listed as N-impaired, and the Lamprey River, the local drinking water supply.
LIST OF REFERENCES


