Determining Uptake Rates of Nitrate-Nitrogen in Low-Order Streams in a Semi-Arid Agricultural Watershed

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Honors Thesis
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Précis:

According to the U.S. EPA’s 2000 National Water Quality Inventory, agricultural pollution is the leading cause of declining water quality in America’s rivers and lakes and a major factor in the impairment of wetlands and groundwater resources. In particular, nitrogen contamination of surface waters can cause a surfeit of adverse and varied impacts, ranging from losses of biodiversity to fatal ailments in humans and livestock. Application of inorganic fertilizers to crops, one of the principal anthropogenic actions that lead to nitrogen contamination in rivers, is likely to intensify as the rapidly increasing global population demands more cereal grains. Therefore understanding the in-stream processes that affect and potentially mitigate nitrogen inputs in agricultural watersheds is critical to maintaining water quality for human and ecological needs.

Large amounts of nitrogen fertilizer are added to the Palouse wheat farms, and a substantial portion of the fertilizer percolates into the local streams. This research project arose from previous work to monitor the high nitrate concentrations coming from the WSU Cook Agronomy Farm (CAF) and discharging into the Missouri Flat Creek (MFC). Ten years of data from the tile drain of the CAF and several sites downstream along the MFC show that the nitrate concentrations are consistently highest at the tile drain but decrease in the downstream direction (Keller et al. 2008, Keller unpublished data). These data suggest the possibility of natural attenuation of the nitrate inputs via biological uptake and metabolism, although the decline could also be caused by groundwater dilution. My research topic was therefore to distinguish between several in-stream processes that can alter nitrate concentrations and determine the fate of the nitrate fertilizers.
In order to differentiate between dilution and biological nitrate uptake along the MFC continuum, short-term conservative tracer additions were performed along two separate study reaches. Reach A was chosen near the CAF tile drain along a shallow MFC tributary, whereas the Reach B on the MFC had greater depth and discharged approximately ten times as much water. Conservative tracers are not used by stream biota and therefore reflect the physical processes occurring on a study reach, in this case measuring dilution. During the additions, tracer concentrations were logged at upstream and downstream sites on each reach and compared to corresponding nitrate measurements.

The results confirmed the longitudinal trend in nitrate concentrations observed by Keller et al. (2008), with nitrate concentrations approximately one order of magnitude higher along the upstream Reach A than the downstream Reach B. Further, nitrate values steadily declined along Reach A while concentrations along Reach B were steady or possibly increased slightly. The conservative tracer additions along both study reaches of the MFC revealed that dilution was negligible during summer baseflow. Knowing that dilution was not responsible for the decrease in nitrate concentrations along Reach A, uptake of nitrate was calculated at 3,053 mg m$^{-2}$ d$^{-1}$. This uptake calculation is reasonable based on published values and suggests a high capacity for natural mitigation of fertilizers. Changes in nitrate along Reach B, however, were negligible. The lack of nitrate removal along Reach B compared to reach A is consistent with literature results in demonstrating the inability of larger streams to effectively process high nitrate loads. This study indicates that the smallest streams play a disproportionately large role in the natural attenuation of fertilizer runoff in the Palouse region. These modest streams likely mitigate the water quality impacts of local intensive agriculture and may be the key to protecting valuable water resources.
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I. Introduction:

Nitrogen contamination of surface waters is a global problem due to widespread human activities such as crop fertilizer application, combustion of fossil fuels, and concentration of animal waste, which have increased reactive nitrogen fluxes into freshwater systems by up to 50 times natural rates (Vitousek et al. 1997, Carpenter et al. 1998). Nitrogen is often a limiting nutrient for biological growth, and the input of high nitrate concentrations into aquatic systems has been shown to cause algal blooms, hypoxia, eutrophication, alteration of food chains, and loss of biodiversity (Vitousek et al. 1997, Rabalais 2002). In addition, nitrate is readily reduced to the toxic form of nitrite in the digestive tracts of humans and livestock, increasing the risk of methemoglobinemia, or “blue baby syndrome,” as well as reproductive issues and cancers (Swann 1975, Townsend et al. 2003). An estimated 2 million American families drink from water supplies that exceed nitrate concentration standards, but remediation can be expensive, making natural attenuation extremely valuable (Nolan et al. 1998).

Nitrogen loading is a serious concern within the Palouse region in Eastern Washington, where 60% (486,000 ha) of the land area is devoted to crop agriculture and the average farmer adds 80-200 kg N ha\(^{-1}\) for fertilizer in forms such as ammonium nitrate and manure (Hall et al. 1999, Keller et al. 2008). A study of nitrogen cycling at the Washington State University experimental Cook Agronomy Farm (CAF), demonstrated that at least one tenth of the nitrogen fertilizer applied to the crops at the site percolates through the soil and enters the Missouri Flat Creek (MFC) Watershed (Keller et al. 2008).

Despite the influence that Palouse agriculture exerts on the local surface waters, to my knowledge there have been no studies in the region that have sought to quantify aquatic nitrate spiraling dynamics. Many stream nitrogen studies in the United States have focused on relatively
pristine, forested sites with lower nutrient levels, less direct sunlight, and undisturbed riparian vegetation (Bernot et al. 2006). Research sites in Midwestern and Northeastern North American agricultural watersheds have differences in sediment types and hydrologic regimes that preclude their use as models for the Palouse region (e.g. Bernot et al. 2006, Kellman 2004).

Previous and ongoing experiments at the CAF have sought to complete a nitrogen budget, noting the amount of fertilizer added to the fields and tracking it into the crop biomass, atmosphere, and streams. These projects measured surface water parameters from the CAF tile drain outflow and several sites up to five kilometers downstream on the MFC continuum (Keller et al. 2008). Ten years of data show that the nitrate concentration in the MFC surface waters is consistently highest at the farm's tile drain discharge and decreases in the downstream direction, but it is not known whether this decrease is due to dilution from groundwater inputs or caused by in-stream biological retention or removal (Keller unpublished data). Explaining the observed nitrate trend by differentiating between the effects of groundwater influx and biological activity is critical to understanding the CAF nitrogen budget. Quantifying MFC nitrogen export also depends on this experiment because biological processes may permanently remove the nitrogen from the stream whereas dilution simply waters it down.

Conservative tracer additions have been shown to effectively distinguish between dilution and nutrient retention/removal in surface waters (e.g. Triska et al. 1989, Stream Solute Workshop 1990, Mulholland et al. 2004). The principle of this method is to compare the behavior of a nonconservative nutrient such as nitrate that can be uptaken or transformed by in-stream processes to that of a conservative tracer, which is biogeochemically inactive (i.e. biological modification and sorption to sediments are assumed to be negligible). Any change in the concentration of a conservative tracer between a point upstream and a point downstream can
therefore be attributed to groundwater fluxes. For example, a decrease in the concentration of a conservative tracer indicates that dilution has occurred, and the nonconservative tracer concentrations can then be corrected for this. Any decrease in the non-conservative nutrient beyond that explained by dilution is attributed to removal and retention. The relative influence of each process can vary along the stream continuum as a function of local geology as well as stream parameters such as discharge rate and mean depth (Alexander et al. 2000, Peterson et al. 2001), thus one single study reach may not be representative of the processes occurring at a different point on the same river continuum. If the nitrate trend is due to biological processes rather than dilution, it would indicate that the MFC has a high natural capacity to mitigate the impacts of the local inorganic fertilizer applications.

II. Research Question

The proposed project will evaluate the influences of dilution versus nutrient retention and removal along two reaches of dissimilar discharge along the MFC in order to explain the observed nitrate concentration trend of the stream.

III. Methodology

Site Description

The Washington State University Cook Agronomy Farm (CAF) is located ten kilometers northeast of Pullman, Washington, at 46°46'44" N, 117°05'19" W. The study site lies within the semi-arid Palouse region and is characterized by annual precipitation rates between 31-58 cm yr⁻¹ and mean high temperatures ranging from 27°C to -7°C seasonally (Donaldson 1980, Geyer 1992). Wheat is the principal crop grown on the Palouse, but cultivation of peas, lentils, barley
and canola is also significant (Hall et al. 1999). A tile drain underlays approximately 12 hectares of the CAF (Moravec et al. 2010) and delivers the subsurface flow into a first-order tributary of the MFC. This tributary collects other ditch drainage and joins the still modest Missouri Flat Creek approximately 800m downstream of the CAF tile drain. The total drainage area of the MFC is 72 km² (Farnsworth 1991).

The first study reach (Reach A) begins approximately 400m downstream from the Cook Agronomy Farm and extends for 46.6m. The steep-sided channel of the tributary divides the WSU Plant Pathology Farm to the West and a private farm growing wheat to the East. This site was selected because of its low-discharge of approximately 1 L s⁻¹ and rapid observed change in nitrate concentrations during preliminary sampling.

The second study reach (Reach B) is located on the MFC proper, approximately 3km downstream from Reach A. Land-use along the 58m Reach B is predominantly undeveloped but cleared space that also receives drainage from farms and office buildings located uphill. The Reach B discharge was approximately one order of magnitude higher than that of Reach A.

Five sites, referred to as S1 through S5, were marked at rough intervals along each reach (see Figure 1). A surface drain that steadily discharged relatively high nitrate (9 mg L⁻¹) water

![Figure 1 - Generic Site layout](image-url)
between S1 and S2 at Reach A proved problematic for calculations, so the reach was shortened to the 38.2m section between S2-S5. No other surface inputs existed on the reaches, so any dilution is attributable to subsurface inputs from soil water and groundwater.

**Stream chemistry**

Water samples for nitrate were taken from the stream thalweg, filtered (Whatman GF/F filters, nominal pore size 0.711µm), and stored in acid-washed Nalgene bottles that were triple-rinsed with filtered stream water. Four replicates were taken for each sampling point along the reaches. Water sampling occurred immediately before the tracer addition began at each site and was conducted from downstream to upstream to prevent interference from disturbed sediments entering the water column. The samples were transported on ice to the laboratory where they were frozen until analysis. Nitrate concentration was determined by cadmium reduction and subsequent colorimetric spectrophotometry following a procedure adapted from standard methods (Standard Methods: Eaton 2005).

Stream temperature and dissolved oxygen were also monitored using YSI Model 85 probes (YSI, Yellow Springs, Ohio). Dissolved oxygen was measured at the beginning of the experiment and temperatures were taken throughout each addition along with the corresponding specific conductance readings.

**Stream physiology**

Stream width, depth, and velocity were taken at each site in order to calculate mean depth and discharge. Wetted stream perimeter was also measured to establish the mean water-sediment contact area. Velocity was determined with a flow meter (Marsh Mc Birney Flowmate Model
2000), however it was not designed for the shallow depths observed at Site A in particular (<10 cm), so discharge was instead calculated using the following equation from the Stream Solute Workshop (1990):

\[(1) \quad C_r(Q_w + Q_a) = (C_w*Q_w) + (C_a*Q_a)\]

Where \(Q_w\) is stream discharge, \(Q_a\) is the discharge of the tracer injection, \(C_w\) is the concentration of NaCl in the stream (specific conductance), \(C_a\) is the NaCl concentration of the tracer injection, and \(C_r\) is the resultant NaCl concentration.

**Tracer addition**

The conservative tracer for this experiment was 99.6% pure NaCl. Bromide or chloride anions are typically used for conservative tracers, however sensing of specific ions requires expensive equipment. Sodium is virtually as conservative as chloride when reach length is small (<100 m), and the total ion content of water can be measured indirectly via electrical conductivity (Bencala and Walters 1983, Pellerin et al. 2008). Therefore sodium chloride salt offers a low-cost alternative to sensing anion concentrations directly (Pellerin et al. 2008). MFC stream temperature exhibits a strong diurnal pattern during summer months, so electrical conductivity was measured as specific conductance (\(\mu S \text{ cm}^{-1}\)) which corrects for temperature changes during the addition.

No injection of nitrate was necessary during this experiment due to high ambient concentrations. Nitrogen amendments are necessary in streams with low background nitrogen concentrations, but using background concentrations when possible provides more accurate data about the actual ambient conditions (Bernot and Dodds 2005).
A 120 liter reservoir was filled with streamwater and mixed with concentrated salt tracer solution. The tracer solution was continuously injected into the reach with a QB pump (Fluid Metering Inc. Syosset, NY) powered by a 6V battery. At each site the addition occurred just upstream from a turbulent area of mixing. Site S1 of each reach began below the turbulence at a point where steady specific conductance readings indicated even mixing. One of two co-calibrated YSI Model 85 probes was placed at S1 and the other at S5, the downstream end of the reach. Background readings were taken and the probes were consistently within 0.6 μS cm⁻¹ of each other. The probes provided specific conductance breakthrough curves at the beginning and end of the reach. Each addition continued until specific conductance reached plateau, steady-state conditions at S5 (~2 hours) so the breakthrough curves at S1 and S5 could be compared. After correcting the specific conductance values by subtracting background concentrations, the downstream NaCl concentrations were normalized by dividing the S5 specific conductance by the S1 specific conductance. This yields the downstream NaCl concentration as a proportion of that which was observed upstream and indicates the level of dilution occurring along the reach.

The tracer addition and nitrate sampling at Reach A were performed on 22-June 2010. There was a disruption of the tracer injection that prevented the completion of the addition, so a second addition was performed on 25-June. The Reach B experiment took place on 12-July 2010.

Additional calculations and metrics

The nitrate uptake length ($S_w$) is a common comparison metric defined as the average distance traveled by a nitrate molecule along a reach before being taken out of solution. $S_w$ is determined by first plotting the natural log of nitrate concentration divided by corrected NaCl
concentration versus distance. A linear regression of these points produces a line with the equation $Kx+b$, with $K$ being negative if any nitrate removal or retention has occurred (Stream Solute Workshop 1990). Taking $(1/-K)$ yields $S_w$.

The mean nutrient uptake velocity is a useful parameter for inter-stream comparisons because it is not discharge-dependent, and it is related to the $S_w$ by the following equation from the Stream Solute Workshop (1990):

$$V_r = (d*u) / S_w$$

Where $V_r$ is the uptake velocity in m s$^{-1}$, $d$ is average stream depth (m), $u$ is water flow velocity (m s$^{-1}$), and $S_w$ is uptake length (m).

Nitrate loss and retention are referred to as uptake, $U$, which is the mass of nitrate removed from the streamwater per unit area per unit time. The Stream Solute Workshop (1990), Newbold (1981), and most work since (e.g. Earl et al. 2006, Mulholland et al. 2004) has used the following equation for uptake:

$$U = V_r * C$$

With uptake in mg NO$_3^-$ m$^{-2}$ s$^{-1}$, $V_r$ in m s$^{-1}$, and $C$ being background nutrient concentration in mg m$^{-3}$. This equation is necessary to correct for atypical behavior measured on the stream during the addition due to the temporary increase in nutrient concentrations. For experiments done at ambient nitrate concentrations however, equation 3 is unnecessarily complicated and also problematic, as background nutrient concentration, $C$, varies along the reach. Therefore uptake in this study was calculated using the following equation:

$$U = (\Delta [NO_3^-] Q_r) / SA$$

Where $U$ is in mg m$^{-2}$ s$^{-1}$, $\Delta [NO_3^-]$ is the change in nitrate concentration along the reach, corrected for dilution, in mg L$^{-1}$, $Q_r$ is resultant discharge after injection in L s$^{-1}$, and $SA$ is the
stream benthic surface area in m² found by multiplying average wetted perimeter times reach length. A constant multiple of 86,400 can be added to convert seconds to days.

Statistics

Resulting values from the injection experiments were analyzed using a one-way Analysis of Variance test to determine whether changes in nitrate concentration along each reach were significant (α = 0.05).

Results

Nitrate

Nitrate data matched the trend observed by Keller et al. (2008) with concentrations generally one order of magnitude lower on Reach B than upstream on Reach A. Nitrate levels on Reach A steadily declined from 5.31 to 4.81 mg L⁻¹ from S2 to S5, a significant (α = 0.05)

![Figure 2 - Observed Nitrate Trends](image)

Note: Nitrate trends are plotted using the same scale, but concentrations are approximately one order of magnitude higher at Reach A than Reach B.
Figure 3 – Nitrate Concentration Data Table

<table>
<thead>
<tr>
<th>Site</th>
<th>Distance (m)</th>
<th>$[\text{NO}_3^-]$ (mg L$^{-1}$)</th>
<th>Sig. ($\alpha$)</th>
<th>$\sigma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S2</td>
<td>0</td>
<td>5.31</td>
<td>-</td>
<td>0.04</td>
</tr>
<tr>
<td>S3</td>
<td>12.1</td>
<td>5.25</td>
<td>0.692</td>
<td>0.07</td>
</tr>
<tr>
<td>S4</td>
<td>26.8</td>
<td>5.01</td>
<td>0.11</td>
<td>0.16</td>
</tr>
<tr>
<td>S5</td>
<td>38.2</td>
<td>4.81</td>
<td>0.142</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Total: $\Delta[\text{NO}_3^-] = -0.50$ 0.002*

Note: $\alpha$ values represent the statistical difference from one site to the next adjacent site in the downstream direction. S2 on Reach A and S1 on Reach B were the first sites on their respective reaches and therefore have no upstream site to be compared to. * designates significance at $\alpha = 0.05$ level.

decrease of 0.5 mg L$^{-1}$ over a distance of 38.2m. Along Reach B, however, the nitrate levels demonstrated a significant ($\alpha = 0.05$) increase from upstream to downstream. The positive nitrate trend on Reach B was much less consistent between sites than the negative trend on Reach A. For this and other reasons, the results will not focus on Reach B.

On Reach A, the S1 nitrate concentration was 5.14 mg L$^{-1}$, and the adjacent drain steadily inputted water at 9.31 mg L$^{-1}$ nitrate, elevating the stream concentration to 5.31 mg L$^{-1}$ at S2. By S4, however, uptake along the reach had already negated the effect of the input, decreasing the stream nitrate level to 5.01 mg L$^{-1}$.

Specific Conductance

Technical difficulties during the Reach A experiment on 22-June necessitated a repeat of the salt injection on 25-June in which S5 reached 100% of upstream specific conductance. Reach B took much longer to approach steady-state conditions, but came to 99% of the averaged...
S1 plateau value. Dilution on Reach B was therefore assumed to be negligible although no nitrate uptake calculations were made for Reach B due to lack of clear nitrate trend.

![Figure 4 - Conservative Tracer Breakthrough Curves (BTC) for Reaches A and B](image)

**Nitrate Metrics**

Reach A $S_w$ and $V_f$ were calculated to be 384.6 m and $6.29 \times 10^{-6}$ m s$^{-1}$, respectively. Uptake on the reach was determined to be 3,053 mg m$^{-2}$ d$^{-1}$ using equation 4. For comparison, nitrate uptake was also approximated using equation 3 and the nitrate concentration from S2, which yielded a relatively similar value of 2,884 mg m$^{-2}$ d$^{-1}$. 

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Discussion

Nitrate data from the two sites match the documented long-term nitrate spatial pattern observed by Keller et al. (2008). Reach A demonstrated a substantial decline in nitrate concentration. Although no two adjacent sites were significantly different from each other, there was a steady decreasing trend that was strongly significant between S2-S4, and S3-S5, and S2-S5 ($\alpha = 0.04, 0.008, \text{ and } 0.002$, respectively). The decline observed along Reach A was sufficient to cancel out the effects of a 9.31 mg L$^{-1}$ input along the reach in only 27 m, which proves the mitigation potential of the reach and other small streams in the area.

While the trend of NO$_3^-$ increase on Reach B was statistically significant, this was driven almost exclusively by the uniqueness of S1 compared to all the other sites. For example, the trend between S2 and S5 is not significant, and all sites between S2-S5 had at least one overlapping nitrate concentration value. The magnitudes of the variations between sites on Reach B were slight compared to the imprecisions of the analytical method. For these reasons I am cautious to conclude that nitrate concentrations were in fact increasing along Reach B.

Specific conductance data generated high quality breakthrough curves that demonstrated a lack of groundwater inputs along either reach during the additions, which signifies that the nitrate concentrations were not affected by dilution. The conservative tracer method effectively addressed the research question but required one assumption to be made. The upstream breakthrough curves plateaued relatively quickly, as expected, and maintained steady-state conditions for the majority of the experiment. Late in the addition, however, the concentrations drifted higher at S1, by approximately 7% of the previous plateau. The drift may have been due to changes in the salinity of the streamwater coming from upstream beyond the start of the addition, as it was not possible to monitor the incoming salt concentrations. Another potential
explanation is that density gradients developed in the saltwater reservoir, increasing the concentration of NaCl being injected late in the addition. The reservoir was frequently stirred to maintain a well-mixed fluid, but this may not have been fully effective. Although the drift is a potential problem in longer additions, steady-state conditions were achieved at downstream sites of both reaches before the higher concentrations arrived. I decided to exclude the drift data as a limitation of the method. The conclusion that groundwater inputs were absent or negligible along the reach is reasonable based on the data and also given the decrease in groundwater levels during the dry summer months.

This study demonstrates that the observed decrease in nitrate concentrations along Reach A was due to retention or removal rather than dilution. There are many processes that can affect nitrate transport, some of which temporarily retain the ion while others permanently remove it. For this reason it is important to determine or predict, where possible, which processes were responsible for the decline in nitrate concentrations along Reach A. Retention routes include adsorption to sediment and burial (Dodds et al. 2002, Bernot and Dodds 2005). Biotic assimilation is another temporary storage of nitrate although plant biomass can retain nitrate for long periods of time. Permanent removal mechanisms for nitrate are bacterial metabolism of \( \text{NO}_3^- \) to dinitrogen or nitrous oxide gases, called denitrification, and export downstream (Dodds et al. 2002, Bernot and Dodds 2005). Nitrate concentrations can also be increased by nitrification, in which bacteria convert ammonium to nitrate. This yields the following mass balance equation:

\[
(5) \quad -\Delta\text{NO}_3^- = \text{Adsorption} + \text{Burial} + \text{Denitrification} + \text{Assimilation} - \text{Nitrification}
\]

Studies that use nutrient additions to quantify nitrate retention and removal parameters cannot distinguish between these processes without additional testing. At ambient concentrations...
however it is assumed that physical storage processes of nitrate are at equilibrium. This study takes advantage of the steady-state physical conditions, which signify that there is no net adsorption or burial. Without data on ammonium concentrations I cannot predict a relative rate of nitrification. If the process was occurring on Reach A, it would mean that the calculated uptake rate underestimated the true value because it would replace nitrate that was removed via other processes. Nitrification generally occurs at rates similar to denitrification although there are variations and one process can temporarily be much more active than the other (Arango et al. 2008). For these reasons nitrification is ignored with the understanding that uptake rates would only be higher than calculated if it was occurring. The following uptake equation results from these assumptions:

\[
(6) -\Delta \text{NO}_3^- = \text{Denitrification} + \text{Assimilation}
\]

Thus the observed decline in nitrate concentrations was caused by the sum of denitrification and biological assimilation.

The calculated nitrate removal metrics support the widely accepted theory that as nitrate levels increase, so does uptake but with less efficiency as the system becomes saturated (e.g. Dodds et al. 2002). In terms of metrics, saturation is demonstrated by high nitrate uptake, long \( S_w \), and low \( V_f \), although \( S_w \) in particular is heavily influenced by other factors such as stream discharge. The calculated \( V_f \) of \( 6.29 \times 10^{-6} \text{ m s}^{-1} \) is much lower than values reported for relatively pristine streams, such as \( 1.5 \times 10^{-4} \text{ m s}^{-1} \) in Spread Creek of the Grand Tetons (Hall and Tank 2003). The nitrate uptake rate of 3,053 mg m\(^{-2}\) d\(^{-1}\) is pointedly higher than the values of 28-143 mg m\(^{-2}\) d\(^{-1}\) observed along the forested East Fork of Walker Branch (Mulholland 2004). Compared to nitrate uptake rates in an agricultural watershed in Michigan, however, the MFC value is average to low (Arango et al. 2008).
A study very similar to my own was conducted by Kellman (2004) on a first-order agricultural stream in Nova Scotia that is very useful for comparison. Like the MFC, the studied watershed was primarily devoted to cropland with many farmers growing wheat in tile-drained fields. The discharge was not provided, but mean stream width and depth were comparable to Reach A. Additionally, the site was located at 45°N and sampling occurred during the summer months, suggesting that solar inputs were similar to those of the MFC study. To complement the field experiments in Nova Scotia, the conditions were also simulated in a laboratory setting using transported sediment cores and stream water. In the laboratory tests, total nitrogen removal averaged between 93-353 mg m$^{-2}$ d$^{-1}$ (Kellman 2004). In situ experiments, however, demonstrated no significant nitrogen loss beyond that which was explained by dilution. The author noted that the high hydraulic conductivity of the sandy benthic sediment would have allowed fast rates of water flow between the hyporheic zone and the stream channel. The resultant mixing of the boundary layer water would have prevented anoxia from developing, which is a necessary condition for denitrification. Other conditions necessary in order for

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Figure 5 – Stream Cross Section
bacteria to metabolize nitrates via denitrification are sources of nitrate and carbon in the water, pH between 4-8, and warm temperatures above 5°C (Farnsworth 1991).

In many ways the hydrologic conditions in the first-order tributaries of the MFC during summer months are ideal for denitrification. The clayey soils of the MFC watershed and Palouse region as a whole suggest very low fluxes of water from the hyporheic zone. This means that mixing probably occurs much more slowly and anoxic conditions that develop in the boundary layer at the soil-water interface are likely to persist much longer than in the sandy, permeable sediments in the Kellman study. This relatively impermeable barrier at the sediment-water interface would also prevent nitrates and dissolved organic carbon (DOC) in the groundwater from reaching the bacteria, but other dynamics help to offset this effect.

The first such factor is that the low discharge and shallow depths equate to high average contact time between nitrate and DOC molecules in the water column and biofilms at the sediment-water interface. These parameters have a strong influence on nitrogen retention and removal (Alexander et al. 2000, Mulholland et al. 2004, and Peterson et al. 2001). Another factor is that the MFC has high concentrations of nitrate and DOC levels are likely elevated as well. Nitrate concentrations measured along Reach A were around 5 mg L⁻¹, which match typical summer values (Keller et al. 2008). DOC was measured in the groundwater below the CAF during previous experiments at the study site (Farnsworth 1991). Upslope field positions had an extremely high average DOC level of 8.6 mg L⁻¹, which decreased as the groundwater flowed downhill toward the MFC. The decline was caused by denitrifying bacteria in the soil which were found to be nitrate-limited (Farnsworth 1991).

While groundwater DOC near the MFC was generally less than 1 mg L⁻¹, several wells registered average concentrations higher than 2 mg L⁻¹ (Farnsworth 1991). Another important
consideration is that tile drains like that of the CAF act as conduits for groundwater flow. Rapid draining of the subsurface water bypasses natural filtration (Peterjohn and Correl 1984) and in this case, denitrification. Thus the tile drain allows for higher concentrations of DOC to reach the streams than the 1-2 mg L\(^{-1}\) observed in the wells near the MFC, which are still relatively elevated. High DOC is common in agricultural watersheds, and often peaks during storm events and summer baseflow (Royer and David 2005). Therefore it is likely that high levels of nitrate and DOC were consistently available for bacterial metabolism at the sediment-water interface.

As for pH, values along the MFC were consistently measured between 6.5-7.5 through the summer of 2010. Finally, the warm water typical of the MFC due to unobstructed solar radiation during daytime promotes biotic activity. Stream temperatures on Reach A during the addition were as high as 22\(^{\circ}\)C.

The theoretical maximum nitrate removal via denitrification using these parameters was calculated to be nearly 500 mg m\(^{-2}\) d\(^{-1}\) (Bernot and Dodds 2005). A recent study done on nitrogen cycling in Michigan streams of varying land-use concludes that denitrification accounted for only a small proportion of the observed nitrate losses and that biological assimilation dominated overall nitrate reduction (Arango et al. 2008). Close examination of their data suggests that this may be a result of extraordinary biological assimilation rather than insignificant denitrification. While average values were lower, denitrification rates as high as 384 and 336 mg m\(^{-2}\) d\(^{-1}\) were observed along two agricultural streams but were simply eclipsed by total nitrate uptake, which was up to 4.8-8 times MFC values at two sites. It is important to note that the sediments in these reaches were up to 80% sand and fine organic matter so anoxia may have been limiting factor as it was for Kellman (2004).

With nitrate uptake lower on the MFC than that observed in the Arango study, possibly
due to less vegetation in the stream channel, and very favorable conditions for bacterial metabolism of nitrate, denitrification could make up a much higher proportion of total removal on the Palouse than that observed in Michigan. With greater chance of anoxia and similarly high nitrate concentrations and stream temperatures, it would be reasonable to predict denitrification uptake on the MFC to meet or exceed those values from Arango et al. (2008) so long as DOC is in fact high. Thus denitrification along MFC Reach A could reasonably occur at rates of 300-400 mg m$^{-2}$ d$^{-1}$ or even greater, which could represent up to 10-15% of the nitrate removal.

Denitrification is a particularly important process because it permanently removes nitrate from the aquatic system. Mulholland et al. (2004) found that gas produced via denitrification was over 99% inert dinitrogen gas and less than 1% of the greenhouse gas nitrous oxide, although this percentage can vary. Based on equation 6 the remaining uptake not explained by denitrification can be attributed to biotic assimilation. This process is ultimately represents only temporary storage, as the accumulated biomass is seasonal. When the bacteria and plants die and decay, the nitrogen is released back into the stream (Bernot and Dodds 2005).

**Conclusions**

In this study, nitrate uptake along the shallow Reach A was found to be 3,053 mg m$^{-2}$ d$^{-1}$, whereas Reach B had no substantial uptake. I was also able to predict relative rates of denitrification and biotic assimilation based on available data and comparison to literature on other agricultural stream systems, with denitrification potentially representing 10-15% of the observed nitrate removal along the upstream reach of the MFC. It must be restated that denitrification rates were not measured by the tracer additions and this is only a prediction.
The results of this study will be very useful to ongoing work on the MFC, which is closely tied to the experimental agriculture conducted at the CAF. The data from this experiment build on previous efforts to understand the processes that govern fertilizer fate and transport after their entry to this reach of the MFC. By differentiating between nitrate retention and dilution, as well as discussing potential influences of denitrification and biotic assimilation, this project helps to explain the in-stream portion of the nitrogen budget. In particular, the large nitrate uptake value is important given the high potential for denitrification, which offers permanent removal of the ion from the watershed. The conclusions from this study will promote future research to quantify summer denitrification rates as well as winter nitrogen dynamics, which play a dominant role in overall nitrogen export due to higher discharge rates.

In addition, the MFC watershed is representative of streams in much of the Palouse region as well as other dryland agricultural areas. The demonstrated ability of Reach A to substantially mitigate the impacts of the CAF tile drain and other high nitrate discharges suggests that small streams and ditch drainages are critical to protecting the local surface waters. Without the disproportionately large uptake provided by these modest streams, the nitrate concentration of the MFC would rapidly rise, causing potential problems for downstream environments. It is my hope that the results from this research could help to inform better fertilizer application practices and have far-reaching implications beyond this project site.

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V. References


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