Independent Research Proposal

Background

Modern large-scale agricultural practices elevate the nitrogen levels in watershed discharges. The increased nitrogen availability accelerates eutrophication of estuaries and coastal marine systems where primary production is nitrogen limited. Readily available nitrogen in the form of ammonium (NH₄) and nitrate (NO₃⁻) augment algal and microbial biomass. Ensuing phytoplankton and periphyton algal blooms and microbial productivity remove oxygen to create “dead zones” devoid of other aquatic life, such as are found in the Gulf of Mexico, Chesapeake Bay, and other places in the world (Fisher, et al., 2006; National Academy of Sciences, 2000; Jordan, et al., 1997).

Nitrogen exports from watersheds are usually less than net anthropogenic inputs creating an unbalanced nitrogen budget. Inputs include fertilizer, food for human consumption, feed for animals, atmospheric nitrogen deposition and nitrogen fixation whereas outputs are riverine export. Areas where agriculture dominates the land use have additional nitrogen inputs from agricultural practices. Net anthropogenic nitrogen input (NANI) in excess of riverine export has been termed the “missing nitrogen” (Van Breemen, 2002).

There are two possible fates of the missing nitrogen: 1) Nitrogen is stored and accumulates in the ecosystem. 2) Nitrogen is denitrified and escapes to the atmosphere as N₂ gas. Nitrogen gas offers a potentially large sink for nitrogen and can account for the missing nitrogen in the nitrogen budget. I propose to test the hypothesis that the majority of the missing nitrogen is denitrified, and the resulting N₂ emerges in streams and escapes to the atmosphere as gas.

If the excess nitrogen is stored in the watershed, the storage capacity may be limited. Therefore, as NANI increases, the nitrogen may reach carrying capacity. Nitrogen saturation would result in an increase in the rate of export with future inputs. Denitrification removes nitrogen from the ecosystem. Denitrification to nitrous oxide and gaseous nitrogen is especially important because N₂ and N₂O are nitrogen forms that are not available to primary producers. Therefore,
Denitrification offers the only permanent removal of nitrogen from the watershed (Laursen and Seitzinger, 2002).

Denitrification is the bacterial conversion of nitrate ($\text{NO}_3^-$) to nitrogen gas ($\text{N}_2$). Under anaerobic reducing conditions, nitrate acts as the electron acceptor for nitrate reductive metabolism. Nitrate is denitrified to nitrite, nitric oxide, nitrous oxide, and finally nitrogen gas. The reaction proceeds along the following pathway.

$$\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$$

Nitrate is the main form of nitrogen discharged from watersheds. Nitrate comes from the oxidation of ammonium ($\text{NH}_4^+$). Fertilizers contribute large amounts of ammonium ($\text{NH}_4^+$) to cropland, which crops utilize during the growing season. Some ammonium is oxidized to nitrate ($\text{NO}_3^-$). Nitrate remaining in the soil post-harvest provides elevated nitrate concentrations to groundwater within the watershed. Denitrification of nitrate occurs under anaerobic conditions when the consumption of oxygen creates reducing conditions favorable to denitrification. Some nitrate leaves the watershed through riverine export, but a large portion is denitrified. The resulting nitrogen ($\text{N}_2$) gas and small amounts of nitrous oxide ($\text{N}_2\text{O}$) emerges in streams and escapes to the atmosphere in unmeasured amounts.

Measuring denitrification within an entire watershed is difficult due to heterogeneity and the large spatial scale. An entire watershed presents challenges, but by focusing on a small stream the measure of denitrification is simplified. Study of a single small stream reach can reveal function of a small watershed. Determination of the fate of the missing nitrogen on a small scale can then eventually be extrapolated to the watershed as a whole.

Denitrification is hard to measure because of inaccurate field methods and variation of denitrification rates (Weller, et al., 1994). Previous studies have used various experimental techniques for determining denitrification rates such as acetylene inhibition analysis of sediment cores, mass balance calculations, and volatile gas tracers (Herrman, Bouchard, & Moore, 2008; Van Breemen, et al., 2002; Laursen and Seitzinger, 2002). Large discrepancy in the rate of denitrification as
well as the percentage of nitrogen export due to denitrification show further research is needed.

Goals and Rationale

I propose to assess the nitrogen in a stream reach with large amounts of denitrification. I chose the site because it is in an area with large amounts of NANI and therefore potential for denitrification. The Choptank watershed on Maryland’s eastern shore is an area dominated by agriculture. Watersheds in this area have strong correlation between increased NANI and elevated nitrogen, in particular nitrate, riverine export (Jordan et al., 1997). Yet riverine discharge from watersheds accounts for at most one-third of the NANI as nitrate. The Choptank is not unusual because larger watersheds in the Upper Mississippi region, Western Europe, and West Africa similarly discharge at most a third of the NANI (Jordan and Weller, 1996). Therefore, the remaining two-thirds is unaccounted for, the missing nitrogen. It is important to know how much is lost via denitrification versus how much is stored in the system.

It is possible that denitrification can occur and N₂ can escape to the atmosphere before reaching the stream. N₂ can emerge through vadose zone in the soil and escape to the atmosphere. Calculation of dissolved nitrogen in excess of equilibrium relative nitrate will yield the percent of denitrified N₂ emerging through the stream. I propose that a relatively small percentage of the total denitrification emerges through the soil and that the majority emerges through streams.

Denitrification in transit to the stream can be calculated from dissolved N₂ concentrations. Dissolved N₂ concentrations are determined from the N:Ar ratio in solution (Laursen and Seitzinger, 2002). The amount of argon in solution depends on temperature and atmospheric concentration. The concentration of dissolved Ar is inversely proportional to temperature, and the atmospheric concentration is known. Therefore, the concentration of argon is used to calculate the temperature of recharge water. Then, the temperature is used to calculate the amount of dissolved N₂ in the recharge water when N₂ in the water and atmosphere was at
equilibrium. Denitrification produces $N_2$ in excess of $N_2$ at equilibrium. The excess $N_2$ is the difference between the measured $N_2$ and the equilibrium $N_2$.

The site selected is uniquely suited to conduct the study because of the large portion of missing nitrogen and thus potential for denitrification. The proposed stream is a drainage ditch in an area with predominantly agricultural land, and preliminary data from fall sampling shows approximately 34% emerging groundwater as well as evidence of gas evasion. The landowner has given permission for conducting the proposed experiments on his property. The study will focus on a small stream to develop a method for measuring the fate of nitrogen with a single watershed as a prototype. Goals of the study include the following.

- Estimate denitrification in a watershed of a small stream reach.
- Measure $N_2$ flux in stream reach and compare it to nitrate flux.
- Infer how much denitrification occurred in the watershed of the reach.
- Characterize the incoming groundwater.
- Compare methodological approaches of measuring emerging groundwater with piezometer data to surface water flow calculations using a conservative tracer.

Understanding groundwater and riverine processes is difficult because of heterogeneity, multiple flow pathways, and variations due to precipitation. A single sampling method does not allow for total understanding, but a combination of methods offers a more complete picture. Sampling of the whole reach and piezometer sampling allow both integration along the stream and a snapshot of what is happening in the groundwater at one specific location. Neither method alone generates a definitive outline of the aquatic processes. Combination of the two approaches provides a more comprehensive view into what is happening in the stream system.

I hypothesize the difference between watershed nitrogen input and output, the missing nitrogen, is attributed to denitrification. Nitrogen inputs include
atmospheric deposition and fixation as well as fertilizers and food inputs (i.e. crop and animal production of fruits, vegetables, eggs, etc.). Watershed nitrogen losses are through riverine export. Research agrees that denitrification is a likely fate of the missing nitrogen, but there is uncertainty about the proportion of denitrification. Estimations vary from 20 to 51 percent of export nitrogen is denitrified (Kudeyarov and Bashkin, 1980; Van Breemen, et al., 2002).

Main goal of this project is to measure the $N_2$ flux through the stream as a component of total denitrification in the watershed. It would be difficult to distinguish denitrification occurring in transit to the stream and occurring within the stream. However, inferences can be made based on the amount of emerging groundwater and stream temperature. Complete determination of whether the denitrification happens in the stream, hyperheic zone, or en route to the stream is beyond the scope of this proposal.

Study of a single reach of a small stream allows for completion of the nitrogen budget on a small scale, understanding of the composition of the emerging groundwater, and comparison of data from piezometer and whole reach sampling methods. I propose that streams with large amounts of emerging groundwater will allow a window into the groundwater processes and that the nitrogen flux into the atmosphere accounts for the missing nitrogen.

Method

I will measure the change in dissolved $N_2$ and nitrate ($NO_3^-$) concentrations in two ways 1) looking at a single unit of water traveling downstream and 2) measuring the groundwater at point locations. The instream dissolved nitrogen concentration is measured from stream water sampling, the groundwater dissolved nitrogen concentration is measured from piezometer sampling, and $N_2$ gas evasion is calculated from comparison to a volatile gas tracer.

Experimental procedure and data analysis will follow previously tested methods (Bott, 2006; Laursen and Seitzinger, 2002), and follow the protocol a previous intern at the Smithsonian Environmental Research Center (SERC) established for gas evasion experiments during the summer 2010. A solution of
bromide and rhodamine act as a conservative tracer to calculate emerging groundwater, steady state, and travel time. Propane acts as a volatile tracer and allows for calculation of nitrogen (N$_2$) gas evasion to the atmosphere based upon a first-order transfer rate of N$_2$ (Laursen and Seitzinger, 2002). Piezometers allow for measure of hydraulic head, hydraulic conductivity, and groundwater velocity as well as collection of groundwater samples (Praamsma, 2009; Fetter, 2001; Novotny and Olem, 1994).

The study will focus on a small reach, approximately 400 m, of a stream within the Choptank watershed on Maryland’s eastern shore. The stream is a drainage ditch in an agricultural area and preliminary measurements found the stream gains groundwater. 34% of the water leaving comes from groundwater emerging in the reach. Experimental methodology involves the use of piezometers in conjunction with whole stream sampling.

A solution of rhodamine and sodium bromide will be pumped into the stream at a constant rate to establish a steady state. Slightly upstream of the tracer solution injection site, propane gas is pumped into the stream also at a constant rate. The bubbling of the propane should facilitate mixing of the bromide/rhodamine tracer solution across the stream width. Background samples, stream morphology, and environmental conditions including air and water temperature and relative humidity will be recorded, and upstream and downstream dissolved nitrogen samples will be collected.

Upstream and downstream water samples are collected at short intervals during the beginning and ending of the experiment in order to precisely capture the halfway point to steady state. The halfway point is needed at upstream and downstream locations to determine time travel of a unit of water. Samples are collected less frequently during steady state. The upstream sampling location is far enough downstream of the tracer solution and propane injection sites to ensure proper mixing across the stream width. Propane samples are collected during steady state.

Piezometers provide another way to look at groundwater and offer a check for what is happening in the stream reach. Furthermore, they offer comparison
between denitrification in the reach compared to what comes in through groundwater. Piezometers will be installed, three upstream and three downstream, within the experimental stream reach flow pathway (Winter et al., 1988). Previous study indicates three depths of 1 m, 0.5 m, and 0.25 m stepwise down the length of the stream are appropriate. Measurement of hydraulic conductivity will be taken with a pressure transducer. Subsequently Darcy’s equation can calculate groundwater velocity (Novotny and Olem, 1994).

Both instream water samples and piezometer groundwater samples will be analyzed with ion chromatograph. Propane samples will be given headspace in the laboratory and analyzed with gas chromatography with a flame ionization detector and nitrogen carrier (Marzof et al., 1994). Dissolved nitrogen samples are collected for membrane inlet mass spectroscopy (MIMS) system analysis at University Maryland’s Horn Point laboratory (Kana et al., 1994). Due to logistics of the upstream and downstream sampling, I will work with a team to conduct the actual experiment. The team’s lab technicians are familiar with the sampling techniques and have extensive experience with the instrumentation I will use.

My tenure at SERC during the fall 2010 provided the foundation knowledge for this proposal. I studied the experimental procedure and the instrumental methods I propose to use, and I created a framework for data analysis calculations. I sampled once a week in the fall with a total of four experiments. Therefore, I propose a similar schedule conducting three to four experiments between February and March, depending on field conditions. Field conditions are important because experiments cannot be conducted if the stream reach is frozen or dry. Field and laboratory work will be conducted through the SERC under grant funding from the National Science Foundation.

Expected Results

I hypothesize the output of N$_2$ will be high compared to the output of nitrate indicating the missing nitrogen is denitrified and N$_2$ generated flows out of the groundwater and into the stream. Denitrification produces N$_2$ in excess of the equilibrium between water and atmosphere. Therefore, measure of excess
dissolved nitrogen equates to denitrification. I expect instream denitrification to be small compared to overall denitrification. Assuming the stream reach is a black box, the difference between N\textsubscript{2} inputs from upstream and groundwater and N\textsubscript{2} outputs downstream and atmospherically is the N\textsubscript{2} generated from instream denitrification.

Furthermore, the measure of the whole stream reach provides a more complete look at stream processes than piezometers alone because gas evasion cannot be calculated from piezometric data. Thus, piezometers cannot determine N\textsubscript{2} lost to the atmosphere. The piezometers will reveal the hydraulic conductivity, hydraulic gradient, and the samples from piezometers will show composition of groundwater. Piezometers will also provide the volume of groundwater emerging in the stream reach. The source of emerging groundwater would require isotopic data analysis, but the potential area the emerging groundwater covers can be calculated.

NO\textsubscript{3} and N\textsubscript{2} concentrations will reveal nitrate and dinitrogen fluxes within the stream reach. Assuming the stream reach drains a small watershed, the input and export of nitrate and dinitrogen should create a balanced nitrogen budget for that watershed. Comparing upstream and downstream N\textsubscript{2} and NO\textsubscript{3} concentrations allows inference into the fate of the missing nitrogen. An N\textsubscript{2} flux larger than the nitrate flux will support the hypothesis that the majority of nitrogen is leaving the watershed as N\textsubscript{2} gas via the stream. Furthermore, this would account for the missing nitrogen, which was denitrified.
References


