

# Report as of FY2009 for 2009AL78B: "Understanding the Role of Denitrification as a Mechanism for Nitrogen (N) Removal Along a River Continuum in Central Alabama"

## Publications

- Other Publications:

- ◆ Chapman, E.L., C. Tatariw, J.W. Edmonds, B. Mortazavi, and R.A. Sponseller. 2009. Understanding Denitrification as a Mechanism of Nitrogen Removal in the Cahaba River, AL. Alabama Water Resources Conference, Orange Beach, AL, September 9-11, 2009. Poster Presentation
- ◆ Chapman, E.L., R.A. Sponseller, J.W. Edmonds. Relationships Between Geomorphology and Ecosystem Metabolism in the Cahaba River, AL. Joint meeting of the American Society of Limnology and Oceanography (ASLO) and the North American Benthological Society (NABS). Santa Fe, New Mexico. Poster Presentation 2010.
- ◆ Riggs, A.A., A.J. Vedral, T.B. Reeves, J.L. Turner, and B. Mortazavi. Denitrification in Weeks Bay, AL. Frontiers in Biology, Research Colloquium, University of Alabama, Department of Biological Sciences, Tuscaloosa, AL. October 2009. Poster Presentation
- ◆ Riggs, A.A., K.M. Beebe, A.J. Vedral, T.B. Reeves, J.L. Turner, and B. Mortazavi. Benthic Nitrogen Fluxes and Denitrification in Weeks Bay, AL. Alabama Water Resources Conference, Orange Beach, AL. September 9-11, 2009. Poster Presentation
- ◆ Tatariw, C., E. Chapman, R.A. Sponseller, J.W. Edmonds. Exploring Community Dynamics of Denitrifying and Anaerobic Ammonia Oxidizing Bacteria in a Large, Unregulated River in Alabama, USA. International Society for Microbial Ecology, August 2010. Poster Presentation
- ◆ Tatariw, C., E. Chapman, B. Mortazavi, R.A. Sponseller, J.M. Edmonds. Exploring Community Dynamics of Denitrifiers in the Cahaba River, Alabama. University of Alabama Frontiers in Biology Symposium, Tuscaloosa, AL. October 2009. Poster Presentation

## Report Follows

**Title: Understanding the role of denitrification as a mechanism for nitrogen (N) removal along a river continuum in central Alabama**

**Participants**

PI: Ryan Sponseller

Co-PI: Jennifer Edmonds

Co-PI: Behzad Mortzavi

Department of Biological Sciences

University of Alabama

Tuscaloosa, AL 35487

**Statement of the problem and research objectives**

*Globalization of the nitrogen cycle*

In the last century, humans have significantly altered the global nitrogen (N) cycle, resulting in a dramatic increase in the rate at which ‘fixed’ (or biologically active) N is created globally. The magnitude and scale of this increase has been remarkable: since 1970 alone, the rate at which N<sub>2</sub> has been fixed has gone up by 120% worldwide. Excessive N inputs to ecological systems are linked to several environmental concerns, including the eutrophication of inland freshwater and marine ecosystems, and the loss of species diversity. Increasingly, elevated N inputs are also being linked to ecological changes that have negative consequence for human health, including the distribution and abundance of key disease vectors, as well as the occurrence of harmful algal blooms (HABs). **For these reasons, understanding controls on the cycling and fate of reactive nitrogen in regional landscape remain an important research priority.**

*The role of denitrification in the nitrogen cycle*

Given concern over this escalation in N inputs to ecosystems, much current research focuses on understanding how bioavailable forms of N are transformed, transported, and retained within landscapes. Large-scale budgets indicate that only 20-25% of the N added to the biosphere is exported to oceans. This suggests that a significant N sink must exist within landscapes, and **denitrification** within riparian and aquatic habitats has been identified as the process most likely responsible for this N removal. Denitrification is a heterotrophic process in which organic matter oxidation to CO<sub>2</sub> is coupled to the reduction of dissolved nitrate (NO<sub>3</sub><sup>-</sup>) and nitrite (NO<sub>2</sub><sup>-</sup>). This is carried out by bacteria in anoxic, or low-oxygen environments (where O<sub>2</sub> is < ~0.2 mg/L), with an end product of nitrogen gas (N<sub>2</sub> or N<sub>2</sub>O). Bacteria capable of denitrification are widespread in soils and sediments, and this process can occur throughout terrestrial, freshwater, and marine environments. **Importantly, denitrification is thought to be the primary mechanism by which bioavailable N can be permanently removed from ecosystems.**

*Eutrophication in the Mobile River Basin*

Widespread nutrient enrichment within the Mobile River Basin of the southeastern US has the potential to increase the delivery of nitrogen (N) and phosphorus (P) to ecosystem of the Gulf Coast. This notion is supported by research showing that variation phytoplankton abundance in several Mobile Bay estuaries is directly linked to differences in N delivery from the upstream catchment, and is presaged by well-documented eutrophication in the northern Gulf of Mexico associated with nutrient delivery from the Mississippi River. The extent to which nutrient enrichment and associated anoxic conditions persist in the Mobile Bay is largely unknown; however, reducing N transport to Alabama’s coastal waterways will likely ameliorate possible loss of commercial and recreational value due to hypoxic events similar to the Dead Zones widely observed in the Gulf of Mexico. **The goal of this proposed research is to evaluate the patterns and controls**

## on sediment denitrification within the Cahaba River, a key drainage system within the Mobile basin in central Alabama.

One key observation motivating this research comes from a long-term survey of stream chemistry from multiple stations located along the Cahaba River (Fig 1; data from UA, *Center for Freshwater Studies*). These show sharp increases in N concentration associated with Birmingham, AL, followed by a clear decline downstream of the urban environment. **Even correcting for the effects of dilution downstream, these data indicate a strong potential for N removal in this river, and the goal of our research is to help explain this observation.**

### Brief explanation of the methods

Research is organized into three elements (RE I-III), and includes both laboratory and field activities. For RE I and II, we have collected sediments from multiple locations in the Cahaba River and elsewhere in the Mobile drainage. With these, we have carried out experiments designed to quantify rates of denitrification, and to assess the relative importance of dissolved  $\text{NO}_3^-$  and labile carbon as drivers of this process. We have also used these sediments to characterize changes in the structure of microbial communities associated with denitrification. For RE III, we have initiated field studies aimed at making estimates of ecosystem metabolism (gross primary production; GPP and ecosystem respiration; R) along a channel continuum in the Cahaba River. In conjunction with this, we are working to develop methods for making whole-system estimates of net denitrification via membrane inlet mass spectrometry (MIMS). The long-term goal of RE III is to get field estimates of denitrification, contrast these with laboratory results, and evaluate how these vary with  $\text{NO}_3^-$  concentration, channel size, and rates of metabolism.

Two approaches have been used to characterize the activity and status of sediment denitrifiers along the Cahaba River. The first, denitrification potential assays (DNPs), involve a direct measure of respiration in bottle experiments that are amended with varying levels of nitrate and an organic carbon source (glucose). To complete DNP assays, sediments are homogenized in the field from several subsamples at a site, brought into the laboratory, and a known mass placed into gas-tight sampling bottles. Water from each site is then amended with N and/or C and added to sampling bottles, bottles are then flushed with  $\text{N}_2$  gas to create an anaerobic environment, and acetylene is added. Acetylene blocks the conversion of  $\text{N}_2\text{O}$  to  $\text{N}_2(\text{g})$  during denitrification, so accumulation of  $\text{N}_2\text{O}$  over time in bottle headspace represents net denitrification. Because resource limitation and oxygen inhibition are alleviated through nutrient additions and purging bottles with  $\text{N}_2$ , these measurements represent rates under ideal conditions (hence the name ‘potentials’). Our second approach for evaluating the denitrifier community uses genetic tools to measure gene expression (mRNA) in un-manipulated sediment communities. For this analysis, samples are immediately preserved upon collection in the field to capture in situ activity. Gene expression measurements in these preserved samples targets the genes coding for a critical denitrification enzyme, nitrite reductase (gene abbreviation, *nir*). Using quantitative PCR (qPCR), we measure the number of copies of the *nir* genes found in the samples after DNA and RNA extraction. The more copies of the genes, the more active those enzymes are in the sample. We are also using the variation in different *nir* sequences found in sediments along the Cahaba River to “fingerprint” the denitrifier community. Our fingerprinting method, called terminal restriction length polymorphisms (T-RFLP),

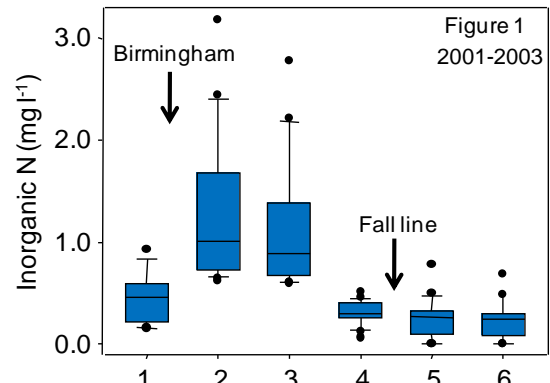


Fig. 1: Dissolved inorganic nitrogen (N) concentration from six sampling stations located along the Cahaba River, AL. Sites correspond to USGS monitoring stations and include: Liberty (1), Acton (2), Helena (3), Centerville (4), Suttle (5), and Marion Junction (6).

can be used to distinguish changes in denitrifier community structure that might be indicative of shifts in N cycling along the Cahaba River.

Finally, field research for this project has focused on ecosystem metabolism at six sites identified in Figure 1. We are currently using diel measurements of dissolved oxygen to make estimates of GPP, and R (and GPP/R) following standard single-station mass balance approaches. These metrics provide an estimate of the assimilative N demand in different sections of the river (e.g., above and below the fall line), and at different seasons. Further, this effort will allow us to explore potential indirect links between river productivity and denitrification. We made initial estimates of metabolism in summer 2009 from two locations; since March of 2010, we have started a field campaign in which oxygen data are collected at all six stations at monthly intervals, with greater frequency (e.g., biweekly) during important seasonal transitions (e.g., spring-summer). Our plan is to continue this effort through May of 2011. In addition, in the summer of 2009, we collected water samples from two stations (Helena and Sprott) to make estimates of dissolved N<sub>2</sub> concentration, with the goal of using these to calculate whole-system, net denitrification.

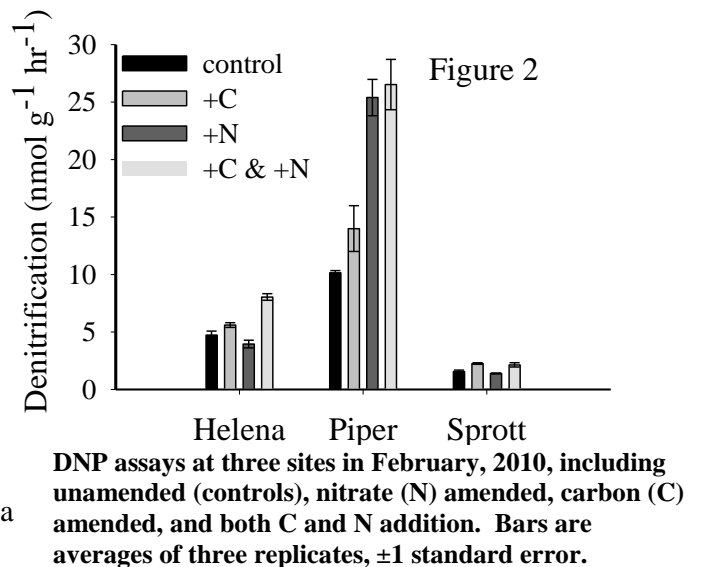
### Principal findings and their significance

**Lab experiments:** results to date provide evidence for carbon (C) limitation of denitrification, as well as secondary limitation by N (nitrate) as indicated by a larger effect of C and N amendment versus C amendment alone (Figure 2). Results from these limitation also assays suggest variable controls on denitrification among sites. Specifically, C amendment stimulated rates of denitrification at Helena and Sprott, where N addition had no effect. In contrast, the shoals at Piper had only a small response to C additions, but responded strongly to N addition, with no additive effect of C and N together (Figure 2). This suggests that C limitation of denitrifying bacteria was alleviated by the presence of organic matter rich sediments associated with shoal macrophytes.

Furthermore, denitrification potentials measured at coastal sites by the Mortazavi lab as part of this same project also showed evidence of strong N limitation, but no effects of adding C, most likely due to high primary production (i.e., organic matter production) coupled with low water nitrate concentrations (data not shown).

**Genetic analyses:** Sediment samples were collected in the summer of 2009 and winter of 2010, and are currently being processed in the Edmonds lab. This genetic work involves amplification of *nirS* and *nirK* genes using 3 primer sets for each gene, a combination of 3 different fluorescent tags, and 3 different restriction enzymes. DNA and RNA extractions have been started, and gene products have been found for all samples for all primer sets. Once completed, both longitudinal and seasonal patterns will be evaluated.

**Field results:** To date, estimates of metabolism suggest significant differences in productivity with longitudinal position in the river and strong seasonal variability. Specifically, rates of productivity (and P/R) were greater below (at Sprott) than above (at Helena) the fall line in the summer of 2009. Furthermore, sites below the fall line appear to show greater seasonal fluctuation in metabolism when compared to upstream reaches. This metabolism research is ongoing, and understanding these



patterns will improve as we continue this effort, which represents a major component of Elise Chapman's dissertation. Finally, we are still working to develop and improve the field and analytical methods required to get accurate and repeatable estimates of N<sub>2</sub> gas concentration via MIMS.