NSF EAR 1024662

Anammox in a shallow coastal aquifer - combining in situ stable isotope and molecular approaches to examine controls on rates and communities

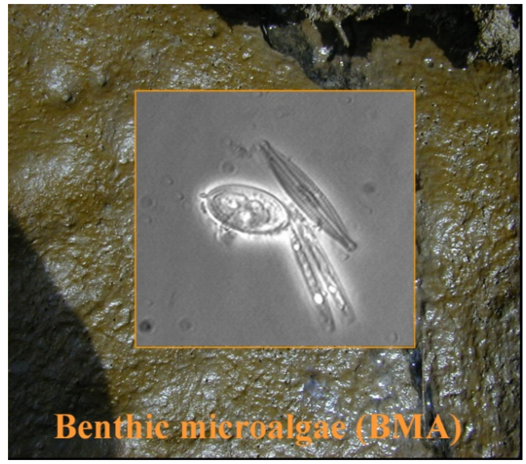
PROJECT SUMMARY

This proposal addresses a fundamental question that spans the geological and microbiological sciences. How do geochemistry and microbial community interaction regulate rates of biogeochemical reactions? We focus our efforts on reactive dissolved inorganic nitrogen (DIN) removal in groundwater because of the increasing DIN saturation of the hydro and biospheres. The work specifically targets a newly discovered DIN reaction pathway, anaerobic ammonium oxidation (anammox) that combines NH_4^+ with NO_2^- to produce N_2. Although anammox has proven to be an important route of DIN loss in many marine systems, it is almost completely uncharacterized in groundwater environments despite geochemical conditions that should promote its activity. We propose to investigate anammox, and its connection(s) to denitrification and nitrification at the geochemical and microbial levels through a series of laboratory and newly developed *in situ* molecular and isotopic investigations. The overall goal of this study is to address the following questions: **1)** Does anammox occur at sufficient *in situ* rates to warrant its consideration as an important mechanism of N loss during groundwater transport? **2)** Are there indicative chemical or isotopic signatures for anammox in groundwater that can aid in its detection and quantification? **3)** What are the subsurface geochemical conditions that promote anammox? **4)** To what extent is anammox dependent on, or competes with, other N-cycling reactions (denitrification and nitrification) for substrate(s)? Three highly novel aspects of work are proposed: First-of-its kind *in situ* natural gradient isotope tracer experiments targeting anammox in groundwater; Application of new molecular tools for gene expression and quantification of *in situ* per cell activity; Generation of fractionation factors for anammox and detailed interpretation of how anammox influences natural abundance $\delta^{15}N$ distribution among DIN species.

Intellectual Merit and Transformative Science - Groundwater holds a large reserve of DIN with extended residence times that permit slow removal rates to impact total in-aquifer loads and the magnitude of DIN subsequently discharged to surface waters. Current analysis suggests that 20% of global DIN conversion to N_2 (via denitrification) occurs in aquifers. Our preliminary evidence suggests that anammox may exceed denitrification in N-rich aquifers. Global groundwater DIN loss estimates cannot be complete without considering anammox. The proposed work will yield the first reported *in situ* anammox rates for groundwater and define how microbial nitrogen cycling communities (anammox, denitrification, nitrification) interact in a mutualistic or competitive fashion to regulate nitrogen fate in aquifers. Through collaboration among a hydrologist, isotope geochemist(s), and microbial ecologist(s), the work crosses traditional disciplinary boundaries. The results provide a heretofore missing component for the assessment of anammox importance across ecosystems. The work is specifically made transferable to other systems by supplementing anammox rate information with quantifying the general geochemical and/or microbial controls on anammox. Further, by establishing the isotopic fractionation effects for this reaction we can potentially provide an anammox diagnostic tool for researchers in other disciplines (e.g. soil scientists, oceanographers).

CLICK HERE FOR PROJECT DATA AND ASSORTED DETAILS

Benthic Microalgal Regulation of Carbon and Nitrogen Turnover in Land-Margin Systems: A Dual Isotope Tracer Approach is a four-year project is funded by the National Science Foundation (NSF-DEB) in collaboration with an ecosystem ecologist and organic geochemist at the Virginia Institute of Marine Science (Drs. Iris Anderson and Elizabeth Canuel), and an ecosystem modeler at the Marine Biological Laboratory (Dr. Joe Vallino). The research investigates the importance of small algae that live in estuarine sediments (benthic microalgae). Excess nutrients (nitrogen) fuel water column algal blooms which sink to the sediments, decompose, and recycle nutrients back to the water column where they enhance further algal blooms. Continued recycling of nutrients between sediments and overlying water supports chronic eutrophication. Benthic microalgae might attenuate this recycling by intercepting ('capping') sediment-water nutrient exchange. The research investigates how the 'cap' functions, its efficiency, what causes the 'cap' to fail in eutrophied systems, and how it can be restored. The project uses a variety of chemical, isotopic, ecological, and modeling tools to track algal carbon and nitrogen through the sediments. *Key words: benthic algae, benthic diatoms, nitrogen, carbon, sediments, coastal bays, isotope tracer*



Linking Hydrogeomorphology and Denitrification in the Tidal Freshwater Region of Coastal Streams is a three-year project is funded by the National Science Foundation (NSF-EAR). This project is a collaboration between Tobias' lab and hydrologists / geochemists (Drs. Jud Harvey and J.K. Bohlke) at the US Geological Survey and a marine scientist (Dr. Mike Piehler) at UNC-Chapel Hill. This proposal addresses a fundamental question in the hydrologic sciences: *How does hydrology regulate chemical reactivity at the reaction-site and reach scales?*. We focus our efforts on nitrogen (N) reactivity because of its increasing trajectory of N loading to the hydro- and biospheres, and resultant eutrophication of aquatic habitats. Denitrification, the only N reaction that completely removes N from a water mass, has been estimated at numerous points along the aquatic continuum (e.g. streams, lakes, rivers, and estuaries). Tidal stream networks, however have largely escaped much inquiry with respect to hydrology and nitrogen reactivity. We hypothesize that tidal freshwater streams possess hydrogeomorphic properties that optimize for N removal through direct and coupled denitrification. Four factors (low volume:surface area, enhanced

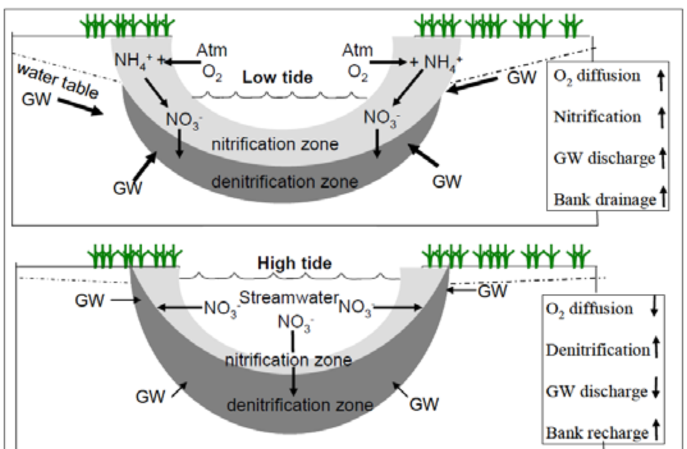
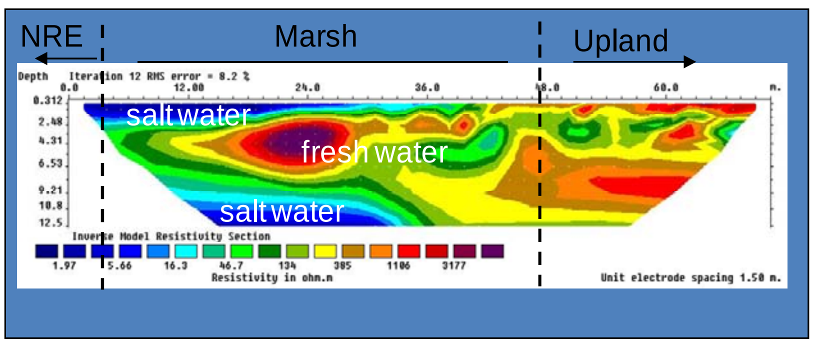
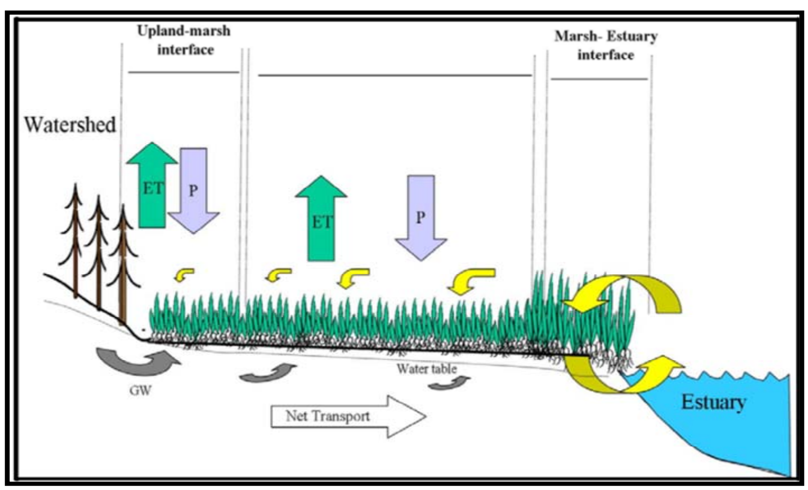


Figure 1. Conceptual illustration of how tidal hydrology initiates: Porewater / stream water / groundwater pumping; redox cycling; and enhances coupled nitrification-denitrification. Not to scale.

transient storage and throughput, redox pumping, and extended water residence time per linear distance of stream bed) contribute to enhanced N removal via denitrification, and all are optimized in tidal freshwater streams. These extensively distributed networks may be an exceptionally denitrification-reactive component of the aquatic continuum yet currently lack a synthesized reach-scale characterization with respect to hydrology and N reactivity. Stream hydrologists and biogeochemists have rarely considered the hydrologic and biogeochemical differences between tidally-influenced freshwater streams and upland streams. Few estuarine scientists extend their investigations upstream to include tidal freshwater streams. We seek to quantify the extent of tidal forcing on the size, travel time, and flushing rate of the bed and bank hyporheic zones, measure the N reactivity of these hyporheic zones, and assess the impact of the flowpath scale hydrology and reactivity on N fate and transport at the reach scale. The proposed research couples field-scale measurements of stream hydrology, characterization of the geomorphology and stream physical / chemical characteristics, and in situ measurements of N processing rates (denitrification). The work relies on a series of diel / tidal hydro-chemical monitoring efforts, conservative (Br^-) tracer studies, ^{15}N isotope tracer experiments, and stream tracer modeling conducted under natural and hydrologically manipulated conditions. *Key words: hydrology, geomorphology, streams, tidal, denitrification, isotope tracer , stream manipulations.*

<u>Hydraulic Exchange and Nutrient Reactivity in the Wetlands of the New River</u> is a five-year project funded by the US Department of Defense through the Strategic Environmental Research and Development Program (SERDP). The project is designed to quantify the source of sink strength for reactive nitrogen with respect to the adjacent New River Estuary, NC. The efforts focus on multiple pathways by which the marsh receives nutrients (groundwater discharge from the watershed, tidal infiltration, particle trapping), modifies those nutrient loads (plant uptake, mineralization, denitrification), and releases those modified loads back to the estuary via drainage. Hydrologic, chemical, and isotopic techniques are employed to construct a marsh-scale nitrogen balance and infer how changes in marsh area and geomorphology in response to sea level rise will impact how marshes modify the water quality of proximal waters. *Key words: groundwater, marsh hydrology, porewater chemistry, denitrification, isotope tracers, nutrient burial.*





Implication of ANAMMOX community structure and microbial interactions in estuarine N removal processes is a 3-year project in collaboration with a microbial ecologist (Dr. Bongkuen Song, UNC-Wilmington). Estuaries are the dynamic meeting points between the freshwater and marine environment, including some of the most productive marine ecosystems that act as breeding and feeding grounds for many species of birds, fish and crustaceans. Nutrient loading, particularly nitrogen loading, in estuarine environments causes nuisance phytoplankton and macroalgal blooms, shifts in food web structure, and expanded areas of severe hypoxia as symptoms of eutrophication. The intensity and duration of estuarine eutrophication in response to high N loading, and the rate of estuarine recovery by N reduction are primarily controlled by biogeochemical N removal processes. While numerous processes transform N, only denitrification (nitrate conversion to N_2) and anaerobic ammonium oxidation (ANAMMOX; ammonium plus nitrite conversion to N_2) remove fixed N from the environment. In addition, nitrification becomes a part of N removal processes as a supplier of nitrite and nitrate to ANAMMOX and denitrifying bacteria. Denitrification and nitrification have been intensively studied in various estuaries to reveal their significance in the natural attenuation of excess N. However, ANAMMOX, as a recently discovered N removal pathway, has only begun to be studied in estuarine ecosystems. We are investigating ANAMMOX communities along the estuarine gradients. Our goals in this study are to determine 1) the correlation between ANAMMOX activities and community structures (composition and abundance), 2) the environmental parameters influencing the community structure and activity of ANAMMOX bacteria, and 3) the interaction of ANAMMOX bacteria with denitrifiers and nitrifiers in the estuarine ecosystem. Tobias' lab is specifically looking at how the differences in geochemical gradients, microbial community structure translate, if at all, into altered rates of N processing via ANAMMOX, nitrification, and denitrification. This study will provide better understandings of the significance of ANAMMOX in the estuarine N cycle and clarify the interactions between microbial functional groups involved in N-loss from both microbial and ecosystem perspectives.

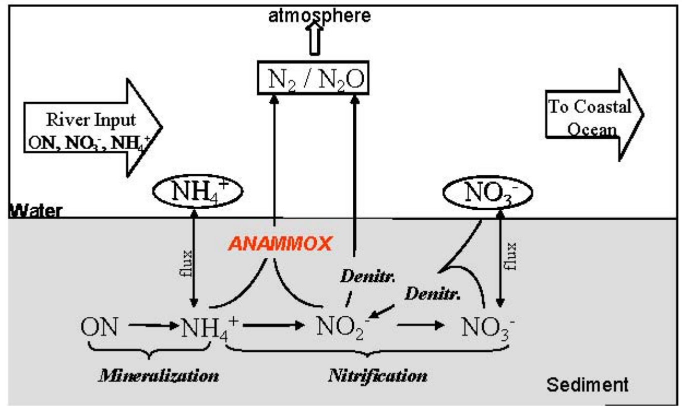


Figure 1. Estuarine N removal pathway. ANAMMOX is a new pathway removing DIN from water/ sediment to the atmosphere.

Keywords: Anammox, denitrification, nitrification, salinity, geochemistry, estuary, microbial communities.