Hydrogel Beads to Encapsulate Sediment Microbes as a Strategy to Quantify Climate Impacts on Microscale Biogeochemical Activity

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Project Goals: Wetlands capture and release large amounts of greenhouse gases (CO_2, CH_4 and N_2O) and predicting their response to climate change induced stressors such as drought and saltwater intrusion is of prime importance. This project aims to link wetland microbial activity to ecosystem-scale processes by developing a reproducible experimental model for lacustrine and estuarine wetland ecosystems to quantify responses to controlled manipulations representing climate impacts. Hydrogel beads, controllable in size, with entrapped wetland microbes and soil plat-like, act as models for sediment aggregates. Bioreactors with real-time gas and liquid metabolite flux monitoring, integrated multi-omics analyses, and stable isotope tracing will be conceptually incorporated into mathematical models to predict how climate change stressors impact C and N fluxes across different wetland spatial and temporal scales.

Abstract: Microbial communities are the driving force for carbon degradation and nutrient cycling throughout wetland ecosystems and are expected to experience significant disruption due to climate change. For instance, estuarine wetlands are expected to suffer from increased saltwater intrusion while freshwater lacustrine wetlands may experience more frequent and more severe drought events over the coming decades. Accurately predicting the impacts of climate change on these microbial communities necessitates a mechanistic understanding of micro-scale processes and how these scale-up to ecosystem-scale fluxes. To develop such a multi-scale approach, this project aims to connect microbial processes occurring at the µm-scale to community organization in a single sediment aggregate (0.5-1mm in size) and to the nutrient flux within the soil column (meter scale). These results could then inform the quantification of greenhouse gas release and capture from wetlands (km scale) and their impact on global climates (Mm scale) by mathematical modelling. This abstract focuses on building an experimental framework to develop a sediment model system that mimics diffusion limiting conditions under controlled and hence, experimentally manipulable conditions. We will work with novel polymer based synthetic sediment-like aggregates that encapsulate native microbial wetland communities. These PEGDMA (polyethylene glycol dimethacrylate) based hydrogel beads maintain the complex spatial organization of sediment microbial communities while facilitating downstream molecular analysis due to simplifications in both matrix and community complexity. PEGDMA beads are not biodegradable, allowing entrapment of plant like material into the bead interior and hence the assembly of a sediment model system that can mirror carbon availability of real wetland systems. A special feature of these hydrogel beads is that they can be produced in various sizes to match the soil compositions from coarse granules of several mm down to fine sand smaller than 1 mm. This provides us with the opportunity to design the proper beads to match each individual study's requirements, as well as investigate the role bead size, or sediment aggregate size, plays in microbial community structure and nutrient cycling.

An initial step towards the application of hydrogel entrapment to study complex soil microbial communities is the validation of the experimental methodology and the subsequent challenges in data analysis. Important milestones for developing this experimental approach include assessing

the activity of microbes entrapped in the hydrogel and evaluating the compatibility of hydrogel encapsulated microbes with downstream 'omics'-based and stable isotope probing (SIP) analyses such as NanoSIMS and proteomic-SIP. A particular challenge for the latter will be identification of cross-feeding between potential synergistic partnerships that occur in complex communities such as wetland soils. In order to validate the technology prior to encapsulating complex wetland communities we tested the approach with two simplified biofilm communities. In the first hydrogel experiment, we paired a complete ammonium oxidizing bacterium (Comammox, *Nitrospira inopinata*) with an enriched anaerobic ammonium oxidizing (Anammox, *Candidatus* Brocadia anammoxidans) community. *Nitrospira inopinata* localized on the aerobic bead periphery to provide *Ca.* Brocadia anammoxidans with nitrite in the inner anaerobic core to form N_2. An unexpected result was the complete removal of nitrogen as Anammox produces some nitrate in its anabolic pathway. We hypothesize that Anammox and Comammox form a mutualistic relationship under anaerobic conditions in which Comammox utilizes formate and nitrate (supplied by anammox) to perform anaerobic nitrate reduction to nitrite, which is then removed by anammox to N_2, leading to complete nitrogen removal. This hypothesis was also supported with *N. inopinata* batch testing and observations of spatial organization using FISH (fluorescent in-situ hybridization) (Figure 1). The second synthetic community under investigation is an Anammox community that has been shown to support a significant heterotrophic community. This partnership has exhibited stability when maintained within bioreactors and without the addition of organic carbon sources for more than one year. This suggests that the autotrophic Anammox bacteria are the primary producers of the system, supplying carbon to the diverse heterotrophic organisms for secondary production. By supplying ^{13}C-bicarbonate over an extended incubation period (~1 month) and collecting samples at multiple time-points for proteomic SIP analysis, we will identify how autotrophs fuel the heterotrophic community. We are scaling up the successes from simple communities entrapped in hydrogels towards more complex microbial cross-feeding dynamics and relationships for this study on climate change. Therefore, we have developed protocols to extract and immobilize live cells from freshwater and saltwater wetland sediments at high cell densities while drastically reducing humic matter that potentially inhibits molecular analysis. Overall, our hydrogel beads show promise for downstream analytical applications such as meta-omics for cellular functions and activities as well as NanoSIMS for spatial-analysis and nutrient uptake in complex wetland environments.

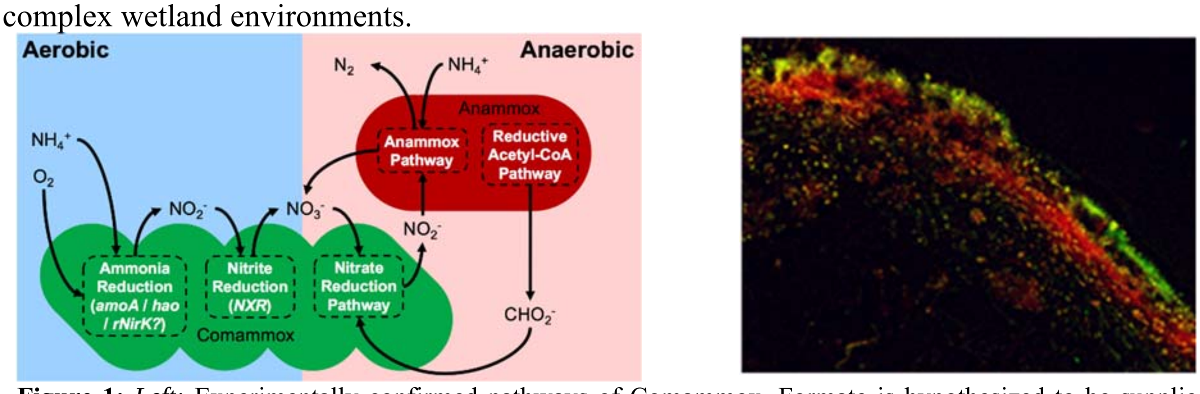

Figure 1: *Left:* Experimentally confirmed pathways of Comammox. Formate is hypothesized to be supplied by Anammox. *Right:* Hydrogel bead confirms partnership of Comammox (green) and Anammox (red).

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