Experimental models bridging single cell-to-ecosystem scales to evaluate climate-wetland feedback mechanisms

Dongyu Wang[2*], Zach Flinkstrom[1*], Pieter Candry[1*], Bruce Godfrey[1], James Britt Abrahamson[2], Juliet Johnston[3], Sam Bryson[1], Ludmila Chistoserdova[1], David Stahl[1], Chongle Pan[2], Xavier Mayali[3], and Mari Winkler[1*] (mwinkler@uw.edu)
*Denote co-first authors
[1] University of Washington, Seattle, Washington, USA
[2] University of Oklahoma, Norman, Oklahoma, USA
[3] Lawrence Livermore National Laboratory, Livermore, California, USA

Project Goals: Wetlands capture and release large amounts of greenhouse gases (CO_2, CH_4 and N_2O) and it is of prime importance to predict their response to climate change induced stressors such as drought and sea level rise associated saltwater intrusion. This project aims to link wetland microbial activities 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 and plant-like carbon, 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.

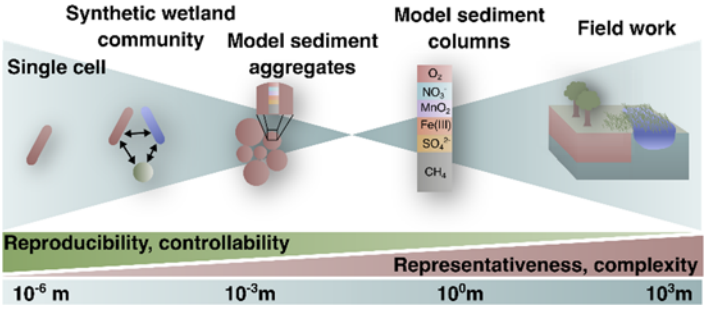

Figure 1 Bottom-up and top-down approaches to begin bridging the microbe-climate scale divide.

Abstract Text: This work aims to construct reproducible experimental model systems to test the relationships between macro-scale climate stressors and processes at the microbial scale to understand future trajectories of wetlands as carbon cycle nodes (Figure 1).

Estuarine wetlands are expected to experience significant alterations of greenhouse gas fluxes due to the introduction of sulfate (SO_4^-) ions that may promote sulfate reduction over methanogenesis for the decomposition of organic matter. We have developed a four-member synthetic community (Figure 2) converting cellulose to CH_4 and CO_2 representative of estuaries at the edge of transition towards saline systems. Using *Desulfovibrio vulgaris* as key node in the community enables investigating the role of sulfate on community dynamics in a simplified system. Proteomics of the four-member community grown with and without sulfate revealed the impact of sulfate on the population size and functional behaviors. The increased population size of *Desulfovibrio vulgaris* and the enhanced abundance of key functional proteins in the sulfate reduction pathway indicated that the addition of sulfate promoted sulfate reduction. Reduced population size of *Clostridium cellulolytiucm* and the abundance of identified cellulolases suggested a reduced cellulose degradation after being treated by sulfate. There were no significant changes on the population sizes of two methanogens between two conditions, but the sulfate addition significantly reduced the protein abundance of enzymes in the acetoclastic methanogenesis pathway in *Methanosaeta concilii* and the hydrogenotrophic methanogenesis pathway in *Methanospirilum hungatei.* This was consistent with the observed reduction in the methane production in the sulfate addition condition. Moving towards higher-scale processes, diffusion limitations become more important. We have developed an *in silico* biofilm model of *D. vulgaris* and *Methanospirillum hungatei* to

investigate the role of electron donor (lactate) and electron acceptor (sulfate) concentration gradients on the spatial organization of methanogenic syntrophy by these partners. In parallel, we have entrapped a cellulose degrader (*Clostridium cellulolyticum*) and the four-member synthetic community in hydrogel-based synthetic sediment aggregates to investigate how organisms and communities access solid carbon substrates in diffusion limited environments. Connecting these experiments with nanoSIMS and SIP-proteomics provides a bottom-up experimental model connecting single cells to millimeter-scale processes.

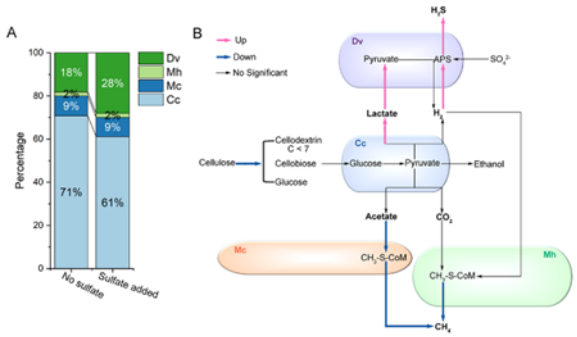

Figure 2 Metaproteomics analysis of the synthetic community. Dv, *Desulfovibrio vulgaris,* Mh, *Methanospirilum hungatei* Mc, *Methanosaeta concilii* Cc, *Clostridium cellulolytiucm*

Freshwater wetlands are expected to experience longer droughts and more extreme floods in the future. To investigate the consequences of these impacts on GHG fluxes from freshwater wetlands, we are using a top-down approach from field sites to spatially explicit lab-scale model systems. In a wetland field site on Lake Washington (a managed, freshwater lake), we measured *in situ* surface GHG fluxes as well as depth-resolved metabolite (e.g., ammonia, sulfate, sulfide, organic acids, etc.) and microbial community profiles. Field campaigns across seasons and associated water table changes will show the extent to which the GHG fluxes are controlled by temperature and varying level of submergence. Our results suggest that disturbance of anaerobic conditions, can increase decomposition of organic matter in soils and continue to promote the release of GHGs even if the wetland's condition is restored. In parallel, we are investigating the effect of water table changes on GHG emissions in a more reproducible, on-line monitored bioreactor systems, enabling long-term non-destructive measurements of metabolite, gas, and microbial community depth profiles throughout a 20-centimeter soil column. Preliminary results show that lowering water tables triggers a massive transient CH_4 efflux (100-fold higher than before disturbance), observations that may be due to both physical and biological responses to water table drops. Conversely, increasing water tables drastically lowered CH_4 fluxes in the system. Such lab-scale experimental systems provide powerful templates to design simple experiments answering questions on centimeter-scale community organization, carbon conversion and meter-scale process outcomes. These columns will be sampled at different column depth for transcriptomic and/or proteomic analyses to better understand the key players contributing to carbon release.

Towards the future, these bottom-up and top-down experimental models will converge in a hydrogel-based sediment column bioreactor. This will enable leveraging the single-cell to millimeter-scale strengths of the hydrogel model system, while providing the centimeter-to-meter-scale process heterogeneity needed to appropriately investigate the responses of wetland ecosystems to climate disturbances.

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