Integrating single-cell wetland microbiome structure, function, and activity to ecosystem-scale biogeochemical fluxes

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Project Goals: Short statement of goals. (Limit to 1000 characters)

We propose to enhance understanding of the impact of climate change stressors on microbial wetland communities by reverse engineering natural wetland sediments, using *hydrogel* particles (concept A in Fig. 1) of varying sizes to trap sediment microbiota in stratified *bioreactor* setups (B in Fig. 1). These experimental systems are fully controlled and monitored analogs of the wetland soil column, and their microbial populations will be investigated with molecular tools such as *proteomic-SIP* (C in Fig. 1) and *NanoSIMS coupled with FISH* (D in Fig. 1). The results will be incorporated into mathematical wetland models (E in Fig. 1) to eventually run simulations that can predict how climate change stressors impact carbon and nitrogen fluxes across spatial and temporal scale. In the text below, we further explain concepts A-E which are also summarized visually in Figure 1. This proposal bridges different biological scales, linking single-cell scale measurements (with NanoSIMS and FISH) to population scale (with metagenomics and Proteomic SIP), to the ecosystem scale (with biogeochemical flux measurements) to inform global scale mathematical models.

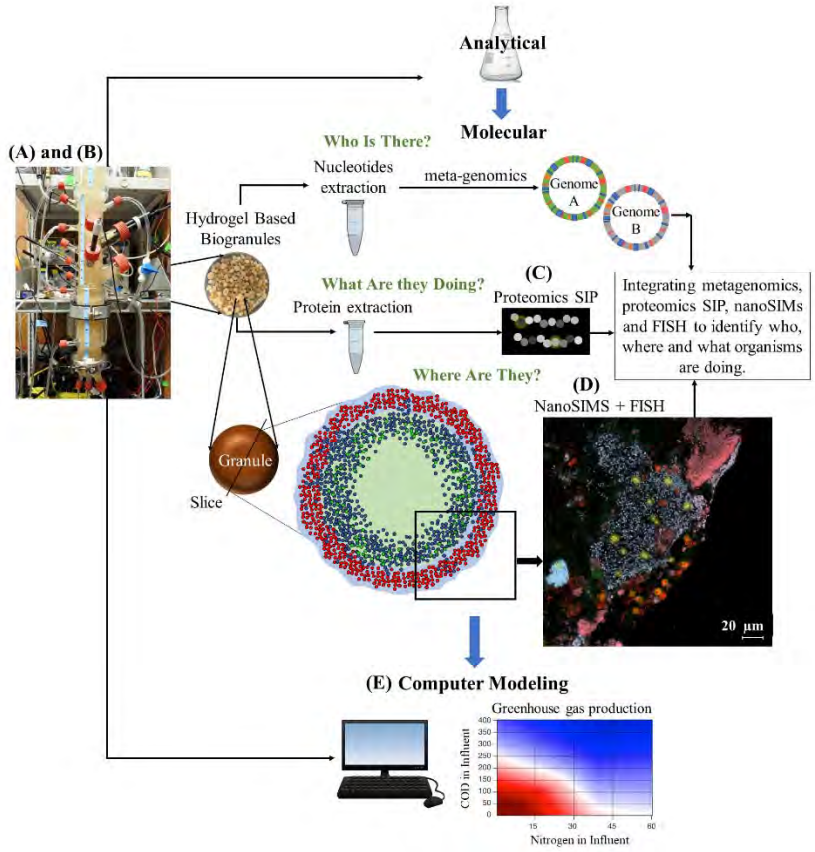

Figure 1: *Overview of the hydrogel based bioreactor setup (A, B), and analysis of the microbial populations with proteomic-SIP (C), NanoSIMS and FISH (D) and mathematical modeling (E).*

Abstract:
Wetland habitats serve essential functions in an ecosystem, including acting as water filters, providing flood and erosion control, and furnishing food and habitats for fish and wildlife. Wetlands cover 6% of the world's land surface but contain about 12% of the global carbon pool, playing an important role in the global carbon cycle (*1, 2*). Wetlands are responsible for roughly one third of global methane (CH_4) emissions. They help regulate atmospheric composition by absorbing and releasing greenhouse gases (GHG), such as CH_4 and

carbon dioxide (CO_2). However, these critical ecosystems are under increasing stress due to anthropogenic activity and climate change, and it is not clear how the accompanying environmental disturbances will impact the underlying microbial communities that drive these processes. The overall effects on microbially mediated functions that produce GHG emissions will likely unfold in distinct ways, depending on the type of wetland and the specific environmental impact. For this research proposal, we have identified two wetland habitats expected to be impacted differently by climate change.

- **Estuarine wetlands** are expected to be altered by changes in temperature, the intensity and duration of precipitation, and by sea level rise (*4*). The resulting intrusion of seawater into coastal wetlands will substantially alter the sediment chemistry, introducing abundant sulfate (SO_4^-) ions that may promote sulfate reduction over methanogenesis for the decomposition of organic matter and also facilitate the oxidation of methane (Fig. 2 left).
- **Lacustrine wetlands** are expected to be impacted by reduced snowpack, earlier snowmelt, and drought events, which will result in increased exposure of soil organic matter to oxygen. This may promote the aerobic microbial respiration of C stocks that have accumulated over centuries to millennia, with CO_2 as the primary product (*3*) (Fig. 2 right).

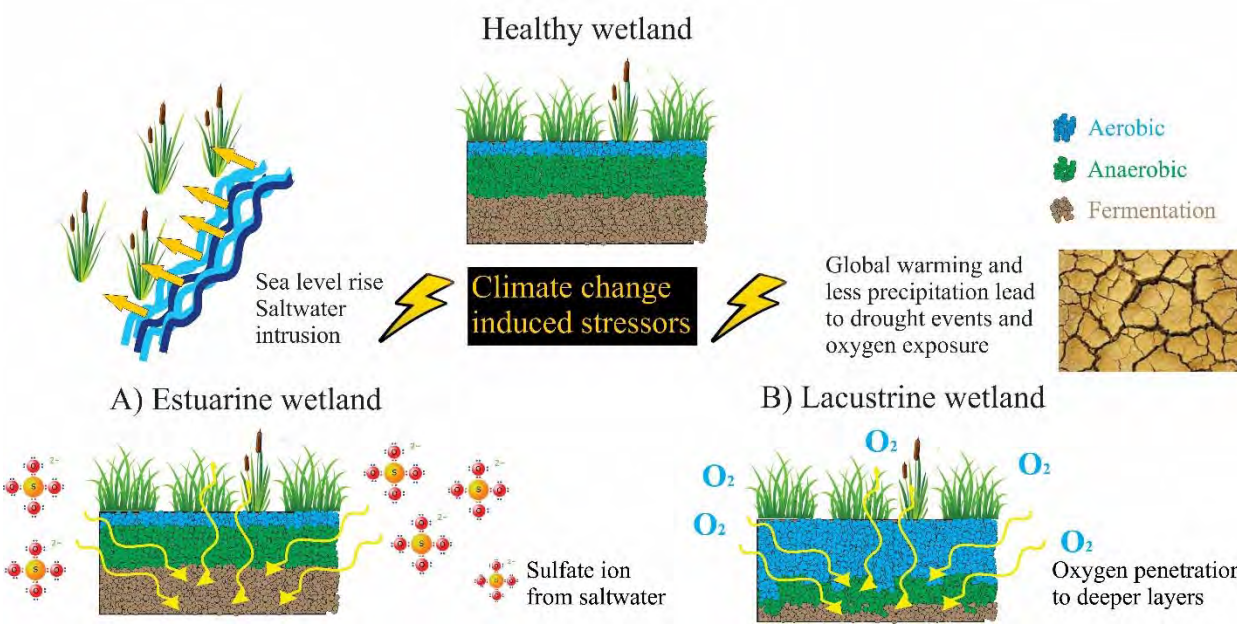

Figure 2: *Schematic overview of the scope of work. Environmental samples will be obtained from A) an estuarine and B) a lacustrine wetland to assess the impact of climate change stressors (sea-level rise for A and drought events for B) on changes in microbial community composition and the herewith associated C, N, P, S cycling including the release and capture of greenhouse gases (N_2O, NO, CO_2, and CH_4).*

This collaborative project takes advantage of expertise in four disciplines (biological process engineering, mathematical modeling, sediment microbiology, and omics driven science) to implement a game-changing technological approach to link the physiology of individual wetland microbial cells to larger mesoscale ecosystem processes. The **encapsulation of naturally resident microbiota in hydrogel particles** (called biogranules) of different sizes provides a format that closely replicates natural diffusion-limited processes, and the entrapment allows the system to experience extremes of imposed environmental conditions without community washout. Native communities entrapped in a novel biogranule format (i.e., biogranule column mesocosms) will be used to examine how anticipated climate-related changes in wetland habitats will impact biogeochemical activities in different redox zones at different scales of resolution, from the functional roles of individual cells, to interacting populations, and to system-level processes. Specifically, the proposed research will **quantify the impact of climate change stressors on model wetland microbial communities and the ecosystem processes** they sustain through the integration of mathematical modeling

and highly controlled hydrogel column experiments. This experimental design provides for both a system-level analysis (by continuous sampling of net fixation and release of gaseous microbial excretion products) and a coincident fine scale analysis of the supporting microbiology using **NanoSIMS paired with FISH, metagenomics, and Proteomic Stable Isotope Probing**. This research will use highly automated column mesocosms to mimic natural wetland conditions by closely simulating their chemical, thermal and physical gradients.

We will specifically investigate how predicted climate change-associated stressors impact the microbial populations of two wetland systems. ***Lacustrine wetlands*** are known to be impacted by lower rainfall and warmer temperature leading to more frequent draught events and therefore to lower above-ground productivity and higher below-ground oxygen exposure. In our experiments we will mimic this scenario by changing the availability of oxygen to test the impact on anaerobic respiratory processes, alterations of the microbial community structure, net metabolic rates, and transformations / partitioning of carbon and nitrogen including the stepwise conversion of intermediates (NO_2-NO-N_2O-N_2) in the denitrification pathway. ***Estuarine wetlands*** are expected to be impacted by saltwater intrusion due to sea level rise, which will shift the microbial community from methanogenesis towards sulfate reduction at a specific salt concentration (which we will experimentally alter). In both wetlands we will trace how carbon mineralization rates will vary as a function of the different redox zones, local microbial functional guild structures, and the forms of available organic carbon. We will infuse the hydrogel biogranule community with **a mixture of organic carbon polymers designed to approximate plant fiber composition** (cellulose, xylan, lignin, and pectin), providing for the controlled and stable microbial contact required for hydrolysis and metabolism of these complex carbon sources. Stable isotope labeling with ^{15}N-labeled ammonium in combination with NanoSIMS will be used to assess changing activities with depth in the individual hydrogel grains, and in relation to varying redox zones of the mesocosm system. Organism-specific activity profiles will be inferred by combined metagenomic and proteomic SIP analyses, and validated by more selective NanoSIMS analysis of carbon mineralization using ^{13}C-labeled intermediates of polymer degradation (coniferyl alcohol, glucose, acetate, CH_4, and CO_2) to resolve patterns of activity distribution and possible metabolic interactions among individual organisms and populations. This experimental format offers a unique way to **bridge different biological scales,** linking **single-cell scale** measurements (with NanoSIMS and FISH) to **population scale** (with metagenomics and Proteomic SIP), to the **ecosystem scale** (with biogeochemical flux measurements) to inform **global scale** mathematical models. The system response to imposed perturbations will be used for iterative modeling. Particle-level transformations will serve to both model and predict net C and N conversions, which should be consistent with measured net capture and release of CO_2/CH_4 from the column system and activities identified by proteomic SIP. These microscale models may contribute to more robust large-scale models and to project future climate-related changes in wetland processes.

Funding statement:
This work is funded by the Department of Energy (grant ##DE-SC0020356).

References:
1. T. R. Christensen *et al.*, *Geophysical Research Letters* **30**, (2003).
2. M. Cao, K. Gregson, S. Marshall, *Atmospheric Environment* **32**, 3293-3299 (1998).
3. C. Potter *et al.*, *Scientific Reports* **7**, 11314 (2017).
4. B. Blankespoor, S. Dasgupta, B. Laplante, *Ambio* **43**, 996-1005 (2014).