Title: **Identifying Hot Spots and Hot Moments of Metabolic Activity in Salt Marsh Sediments Through BONCAT-FISH Microscale Mapping**

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Project Abstract: Complex microbial communities are essential constituents of soil and sediment ecosystems: they modulate nutrient and metabolite flux in ways that filter runoff, determine greenhouse gas emissions, and support higher trophic levels. However, our ability to derive net biogeochemical fluxes from knowledge of a community's constituents is limited by a lack of suitable methods connecting metabolic activity on a single-cell level to bulk processes. In salt marsh sediments, where redox zones are compressed due to substantial organic loading, this challenge is particularly pronounced: connections between dominant electron-donating (carbon) and electron-accepting (sulfur) metabolic cycles lack spatial and temporal specificity.

Our work to identify the "hot spots" and "hot moments" of metabolic activity in salt marsh sediment follows up on recent efforts conducting *in situ* substrate analog probing incubations in Little Sippewissett Salt Marsh in Falmouth, MA. Through the development of customized incubation chambers, we exposed intact sediment columns to the substrate analog L-homopropargylglycine (HPG), which is incorporated into growing biomass and subsequently visualized through click chemistry and fluorescence microscopy. The approach is known as bio-orthogonal non-canonical amino acid tagging (BONCAT); initial work revealed growing microbes in precise spatial arrangements with respect to specific mineral grains (Marlow et al., *Environmental Microbiology*, 2021).

The next steps are to gain additional temporal and phylogenetic resolution on which organisms are active under distinct conditions. Here, we share preliminary results of a two-phase BONCAT technique, as well as background spectral profiling to enable optimized fluorescence microscopy analysis. Using both HPG and a different substrate analog (L-azidohomoalanine, AHA), we have developed a novel method to visualize anabolically active organisms under two different environmental conditions, doubling BONCAT's temporal resolution and revealing which community members are growing during the day or night. In preparation for multiplexed fluorescence *in situ* hybridization (FISH), we have developed microscale maps of salt marsh sediment spectral profiles under UV/vis excitation. These spectral maps, developed with sub-micron spatial resolution, will indicate which spectral windows are most promising for FISH. We have also incorporated fluorescence lifetime data into this map, offering an additional "dimension" of differentiation to both characterize the substrate and guide fluorescence analysis.

These efforts have set the stage for a late summer field deployment to further resolve the "hot spots" and "hot moments" of microbial activity, offering a promising opportunity to link single-cell activity with broader environmental biogeochemical processes.