

## **Methane and nutrient cycling by sediment-hosted archaeal-bacterial syntrophic consortia and their viral predators**

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**Project Goals: Our overarching goal is to develop a systems-level understanding of the interactions and fundamental activities by syntrophic methane-oxidizing archaeal-bacterial consortia and viral assemblages involved in cycling of C and nutrients in anoxic sedimentary environments. As part of this work, we are developing single cell and virus-targeted analytical and metagenomics techniques in tandem with high resolution imaging methods, stable isotope analysis, and modeling for application both in the laboratory and field settings.**

Microbially-mediated anaerobic oxidation of methane (AOM) represents a significant methane sink in anoxic sediments. This process is catalyzed primarily by consortia of methanotrophic ‘ANME’ archaea and syntrophic bacteria coupled with the reduction of sulfur, nitrogen, iron, and manganese. These methane-consuming consortia are also involved in the transformation of essential nutrients, through processes including nitrogen fixation and intracellular storage of iron, carbon, and phosphate. The nutritional requirements and interdependencies of different methanotrophic archaeal-bacterial partnerships and their collective impact on nutrient cycling within the sedimentary ecosystem is poorly understood and represents a major focus of this work. Viruses are also important mediators of nutrient cycling in diverse environments. As selective agents of microbial mortality, viruses have been shown to enhance the bioavailability of essential nutrients such as N and P and stimulate microbial growth. The degree to which viruses influence syntrophic AOM consortia and their potential role in transforming methane-derived carbon and nutrients within sediment communities has not yet been investigated. Here we are developing a multi-modal analytical imaging pipeline and comparative ‘omics datasets for studying the ecophysiology of diverse uncultured ANME-bacterial consortia and associated viral predators in sediment ecosystems.

Comparative ‘omics analysis of metagenome assembled genomes and BONCAT-based single consortia sequencing show notable differences in gene content and predicted physiologies of different ANME and associated syntrophic bacterial lineages, including differences in the predicted ability to fix nitrogen among the syntrophic bacterial partners of ANME. Application of mRNA-FISH and FISH-nanoSIMS analyses with <sup>15</sup>N<sub>2</sub> support the involvement of syntrophic sulfate-reducing bacteria and ANME archaea in nitrogen fixation, but active diazotrophy appears to be unevenly distributed among archaeal and bacterial partners within individual consortia. Initial analyses of viral activity and morphological diversity within methane saturated sediments using viral-BONCAT and TEM surveys revealed active viral-like particle production and distinct morphotypes in sediments with active ANME-SRB consortia compared to sediments where only

the ANME were active, with an increase in ‘lemon-shaped’ morphologies reminiscent of viruses infecting archaea.

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