

Autotroph-Heterotroph Communities Increase Interactions and Metabolite Exchange as They Undergo Ecological Succession

William C. Nelson* (William.Nelson@pnnl.gov), Stephen R. Lindemann, Jessica K. Cole, Eric Huang, Jason Xu, Yukari Maezato, Jennifer Mobberley, Beau Morton, Ronald A. Taylor, Stephen Wiley, Mary S. Lipton, Thomas Metz, Margaret F. Romine and **Janet K. Jansson**

Pacific Northwest National Laboratory, Richland, WA

<http://www.pnl.gov/biology/programs/fsfa>

Project Goals: The PNNL FSFA goal is to identify the fundamental mechanisms by which microbial interactions and spatial organization impact rates and pathways of carbon and energy flow in microbial communities. The strategy involves the study of highly interactive and tractable model autotroph-heterotroph consortia whose member genome sequences have been defined. Our project leverages unique capabilities including multi-omics measurements, advanced functional imaging, taxonomic profiling and metabolic and regulatory network modeling to elucidate underlying reaction mechanisms within complex microbial communities. Our research plan supports DOE goals to achieve a predictive understanding of microbially-mediated carbon and energy transformation.

A major barrier to investigating microbial community function and dynamics is the high organismal diversity found in most environments. To address this challenge, we have derived stable consortia of reduced complexity from benthic microbial mats, by sequential enrichment on defined media under constant light¹. Two of the resulting consortia, referred to as unicyanobacterial consortia (UCCs), each include a single cyanobacterium and up to 18 heterotrophs. Complete or near-complete genome sequences from these consortia were generated using a combination of metagenome and isolate genome sequencing². Comparison of the metagenomic sequences to the assembled genome sequences uncovered microdiversity within the community, both in the form of distinct species conflated within OTUs and genomic microheterogeneity within species.

These consortia are a unique and powerful model system for the study of microbial interactions because of: 1) their tractable richness, 2) stable community structure and reproducible ecological succession, 3) a defined growth medium enabling detailed analyses of metabolic interdependencies, 4) axenic isolates of most members, 5) available genetic systems for most of the isolates, 6) the presence of microdiversity and 6) complete genomic information. We have used these consortia to examine microbial community dynamics and mechanisms associated with division of labor among the community members.

To test the hypothesis that *microbial interactions increase over community ecological succession*, we applied a multi-omics approach to 28-day succession cycles of two different UCC consortia, each with a different cyanobacterium, and nearly identical heterotrophic membership. Reproducible changes in the relative abundances of populations over time were observed, with distinct succession patterns between the consortia, suggesting that the two consortia display different photoautotroph-driven carbon and energy fluxes. Analysis of metatranscriptomics data for nitrogen metabolism genes suggested that nitrogen acquisition shifted from an initial phase dominated by incorporation of inorganic nitrogen (nitrate in the

growth medium) to a recycling phase in which organic resources were exchanged. Metaproteomics data supported this observation, showing a decrease in expression of the assimilatory nitrite reduction protein NirA and a concomitant increase in the expression of the ammonium transporter Amt, the urea transporter UreT and urease UreC. Conversely, sulfur metabolism genes had stable expression across succession, suggesting that sulfur metabolites were either retained or remineralized, leading to a consistent requirement for acquisition of inorganic sulfur.

We also quantified extracellular amino acid levels during the succession experiments. This metabolite analysis required the development of a novel suite of methods for the isolation of polar metabolites from hypersaline media. The workflow, called MetFish, is based on chemoselective enrichment in which dansyl or related probes are used to derivatize metabolites *in situ*, followed by their isolation and analysis. In this study, dansyl chloride was used to isolate amine-containing metabolites. Using MetFish, we found that the concentrations of extracellular amino acids peaked early in succession, declining thereafter. These data suggest that the efficiency of recycling of nitrogen-containing metabolites increases as the community matures.

Together our observations suggest that early in community formation, microorganisms must act more independently and acquire nutrients from inorganic sources. As succession progresses, specific interactions are established, likely in concert with the development of spatial structure, and metabolite exchange dominates nutrient and energy fluxes. Moving forward, we plan to continue to investigate mechanisms of interaction, explore the relationship between spatial arrangement and cooperative metabolism, and test the hypothesis that microdiversity stabilizes community function to environmental change through the presence of subpopulations with similar functional profiles but differing optimal growth conditions.

References

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