High-Resolution Spatial Analysis Reveals How Nitrogen Source Governs Carbon Partitioning Between Members in a Phototrophic Consortium

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Project Goals: The goal of the Metabolic and Spatial Interactions in Communities (MOSAIC) Foundational Scientific Focus Area is to understand the fundamental mechanisms by which microbial metabolic interactions and spatial organization impact carbon, nitrogen, and energy dynamics in microbial communities. Our studies focus on the coupling of carbon and nitrogen cycles in microbial communities, the role of environmental variables in governing the rates of these cycles, and the impact of environmental perturbations on microbial community dynamics. We employ tractable model consortia whose member genome sequences have been defined, advanced omics measurements, functional imaging, taxonomic profiling, and modeling to elucidate interaction mechanisms within complex microbial communities. Our research supports the DOE goals to achieve a predictive understanding of Earth's integrated biogeochemical processes.

Interactions between microbial photoautotrophs and associated heterotrophic organisms are ubiquitous in nature and exert significant impacts on global biogeochemical cycling. Hence, elucidating the molecular mechanisms by which environmental conditions impact community interactions in phototrophic-heterotrophic consortia is critical to predicting how they will respond to environmental change.[1,2] Here, we used a model consortium containing one photoautotrophic cyanobacterium (*Phormidium* sp. OSCR) and 18 associated heterotrophic species, for which a species-resolved metagenome reconstruction is available,[3] to understand fundamental carbon and nitrogen coupling in microbial communities. We hypothesized that the genome-predicted inability of most heterotrophic consortium members to directly acquire NO_3^- from the medium would result in widespread nitrogen limitation for these species. Altering the available nitrogen source (NO_3^- vs. $NO_3^- + NH_4^+$) drastically altered community composition and dynamics, though not in ways easily predictable from members' ability to assimilate NO_3^-. Examination of the consortia, using a multimodal mass spectrometry-based stable isotope probing approach, revealed that although NH_4^+ was acquired approximately twice as rapidly as NO_3^-, there was no significant difference in community $H^{13}CO_3^-$ incorporation. To resolve whether C/N partitioning among members was altered, we used high-lateral resolution mass spectrometry imaging (NanoSIMS), in conjunction with our previously developed image processing pipeline[4]. These data showed significant differences in the rate at which heterotrophs

acquired cyanobacterially-fixed carbon, where carbon was much more rapidly transferred to heterotrophs when NO_3^- was the sole nitrogen source than when NH_4^+ was also available. Relating these results to that from proteomic analysis of the cyanobacteria indicates altered iron acquisition and pyruvate metabolism based on the available nitrogen source, suggesting that carbon transfer rate may be increased when NO_3^- is the sole nitrogen source as the cyanobacteria are discarding excess reductant. Fluorescence *in situ* hybridization (FISH) imaging with species-specific probes provided insight into the potential role of select heterotrophs within the community. We observed that the most abundant heterotroph (*Aliidiomarina calidilacus*), independent of nitrogen source, was always proximal to the cyanobacterial filaments, whereas the heterotroph (*Algoriphagus marincola*) whose abundance was significantly reduced upon NH_4^+ amendment co-localized with lysed cyanobacterial cells. These results suggest that *A. calidilacus* cells form an epibiotic or parasitic relationship with the cyanobacteria, and that *A. marincola* cells are detritivores that recycle proteinaceous biomass.

References

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