

Nitrogen Dynamics Control Carbon Partitioning in Model Complex Microbial Consortia

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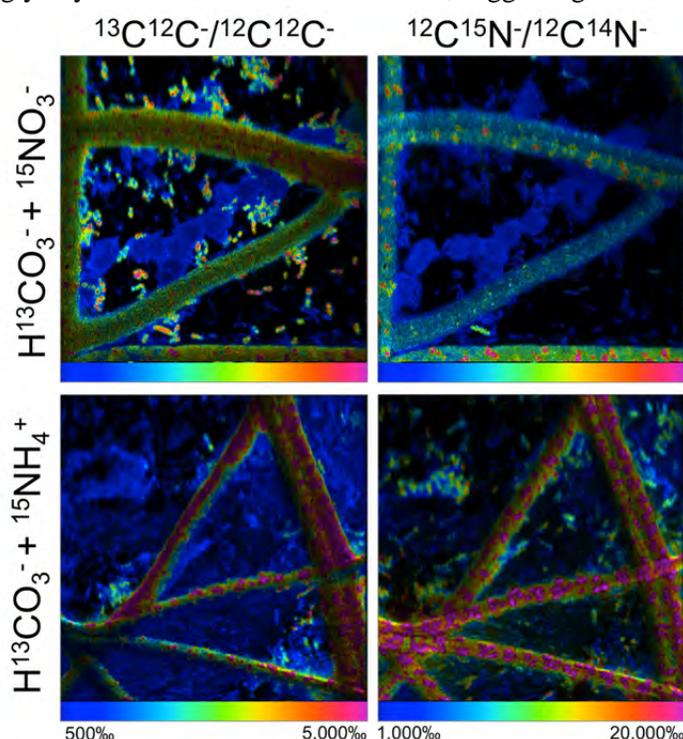
Project Goals: The PNNL FSFA goal is to identify the fundamental mechanisms by which microbial interactions and spatial organization impact rates and pathways of carbon and energy flow in microbial communities. The strategy involves the study of highly interactive and tractable model autotroph-heterotroph 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.

Interactions between microbial autotrophs and heterotrophs exert globally significant impacts on the cycling of carbon and energy, as well as other nutrients (e.g. nitrogen, phosphorus, sulfur). In order to predict the consequences of changing environmental conditions on autotroph-heterotroph interactions, it is first necessary to understand the fundamental principles driving their community dynamics. Here we investigated the principles governing interactions between phototrophs and heterotrophs in a model unicyanobacterial consortium (UCC), which contains a single cyanobacterium and co-isolated heterotrophs^{1,2}. Species-resolved metagenome reconstruction³ of two consortia revealed that most member species within the consortia were unable to directly assimilate nitrate as a nitrogen source, despite the fact that nitrate was the sole nitrogen source. Our aim was to elucidate the key metabolic interactions within the consortium that enabled relatively few member organisms (those that can consume nitrate) to supply the nitrogen requirements of the other members.

Because of the high energy cost of nitrate reduction to ammonium, *we hypothesized that the cyanobacteria in the consortia served as the major primary assimilators and providers of community nitrogen*. To test this hypothesis, we investigated member macronutrient acquisition over a 28-day succession cycle of the consortium UCC-O, which contains the cyanobacterium *Phormidium* sp. OSCR and 19 associated heterotrophs, using an integrated transcriptomic and proteomic approach. These analyses revealed that cyanobacterial nitrate assimilation was initially high, but was supplanted late in succession by apparent recycling of reduced nitrogen species (e.g. ammonium, urea). However, cyanobacterial expression of proteins involved in ammonium incorporation into amino acids (i.e., glutamine synthetase, GlnA) was constant. Notably, heterotrophic species responded divergently in their expression of glutamine synthetase and ammonium transporters, revealing species-specific strategies to acquire reduced N within the community.

Subsequently, we tested the hypothesis *that reliance upon the cyanobacterium for access to bioavailable N resulted in heterotrophic N limitation* by amending UCC-O with reduced N (NH_4^+) at inoculation. This amendment drastically altered the community dynamics compared to NO_3^- -only cultures, but in ways that were not readily explainable from the heterotrophs' predicted ability to reduce NO_3^- . Although there was no change in total carbon uptake rates between the two conditions, spatially-resolved analysis of ^{13}C and ^{15}N flow through the biofilm via nanoSIMS suggested that the cyanobacterium shared carbon with its heterotrophic partners much more rapidly when NO_3^- was the sole

nitrogen source than when amended with NH_4^+ (Fig. 1). Proteomic investigation revealed significant differences in proteins involved in cyanobacterial glycolysis between the two conditions, suggesting that kinetic limitation in NO_3^- uptake resulted in release of fixed C in the photosynthate to maintain redox balance. The protein data also revealed that lactate dehydrogenase and pyruvate-formate lyase were more highly expressed when NH_4^+ was absent, presumably as routes for shunting fixed C away from the TCA cycle via export of formate and/or lactate. Interestingly, these pathways for organic acid production are typically anaerobic processes. Together these observations suggest two new candidate principles governing autotroph-heterotroph interactions in communities: (1) N availability to microbial photoautotrophs governs community C partitioning, and (2) Spatial organization around hypoxic pockets in phototrophic biofilms enables anaerobic autotroph-heterotroph interactions that balance community redox and increase total productivity. Future research will test the generality of these principles using a broader array of consortia.



References

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