

217. Using Genome-Resolved Metagenomics to Uncover Trophic Structure in Microbial Communities: Its Influence on Community Dynamics

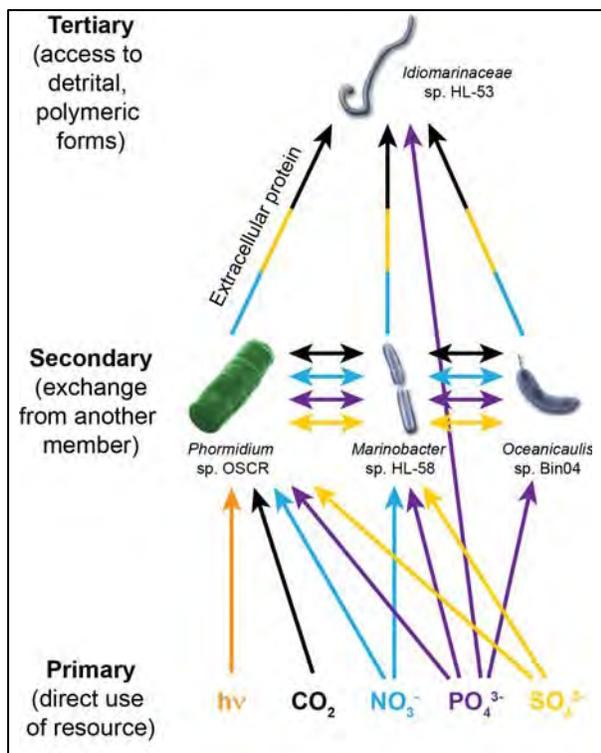
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Project Goals: The PNNL FSFA is focused on discovering fundamental principles that determine microbial community structure, function, and dynamics, and applying these principles to rational design of microbial communities for desirable outcomes. Our strategy integrates field studies of microbial mat communities and laboratory studies of field-derived consortia and isolates to gain a mechanistic understanding of the environmental and biotic drivers of community higher-order properties such as resistance and resilience. Genome sequence-enabled approaches, including chemical probes directed against specific functions, are being used to elucidate underlying interaction mechanisms. These data are in turn being used to inform community metabolic, spatial and regulatory network models. Our research plan supports DOE goals to achieve a predictive understanding of microbes and microbial communities and to provide foundational knowledge enabling rational design of microbial systems.

In contrast to macroorganisms, which tend to occupy clear positions in food webs based upon producer-consumer relationships, the majority of known microbes encode the capacity to assimilate at least some of



their nutrients directly in their mineral forms as well as through exchange of organic metabolites. However, the ability to directly assimilate mineral resources (e.g., CO₂, NO₃⁻, PO₄³⁻, SO₄²⁻) is not uniformly distributed among members of microbial communities, constraining some organisms to acquire lacking nutrients through interactions with other members. The standard categorizations of microbial metabolisms (photoautotroph, chemoautotroph, photoheterotroph, and chemoheterotroph) have focused solely upon carbon and energy acquisition. We suggest that trophic levels for microbes should be examined for each element required for life, as such trophic interactions govern the flow of these elements through communities. These flows determine which community members are able to grow, shaping community dynamics. We hypothesize that, under conditions where an organism has primary access to a nutrient, its relative abundance will increase over conditions where its access to the same nutrient is constrained by interspecies interactions. Figure 1. Conceptual diagram of trophic levels potentially occupied by four of the species in consortium UCC-

O under standard cultivation conditions. Idiomarinaceae sp. HL-53 can also occupy the secondary trophic levels for all nutrients; these interactions are not pictured for the sake of clarity. Using species-resolved

metagenome bins (see Nelson et al. poster) generated from uncyanobacterial consortia¹ derived from microbial mats populating epsomitic Hot Lake, WA², we evaluated each member species for the genome-encoded mechanisms by which it may acquire energy, carbon, nitrogen, phosphorus, and sulfur. These genome-based predictions were used to assign these organisms to one or more trophic levels they could potentially occupy under given cultivation conditions. Organisms with a predicted capability to assimilate a nutrient directly from an inorganic source were assigned to the primary trophic level, those that can acquire an element via a small molecule produced within the consortium to the secondary trophic level, and those that can depolymerize extracellular macromolecules and salvage monomers to the tertiary trophic level. These assignments permitted a reconstruction of the possible trophic networks within the community. We further investigated the impact of changes in trophic levels with respect to nitrogen upon the community's composition, spatial structure, and patterns of element flow by amending growth medium with ammonium. Because the UCCs are routinely cultivated with excess NO₃⁻ as the nitrogen source, the primary organisms by which nitrogen enters the consortia will be those capable of reducing NO₃⁻ to NH₄⁺ under standard conditions¹. Both cyanobacteria possess cytoplasmic nitrate and nitrite reductases, as do all of the consortial gammaproteobacteria except for *Idiomarinaceae* sp. HL-53, allowing them to serve as primary producers with respect to nitrogen acquisition. All members of the consortia contain Amt-like NH₄⁺ transporters and can assimilate NH₄⁺ via glutamine synthetase, meaning all of our members can occupy the first trophic level of nitrogen when NH₄⁺ is present. As expected, supplementation with ammonium essentially eliminated nitrate uptake. Ammonium amendment significantly altered the community composition and the biofilm structure of UCC-O in succession, unexpectedly suppressing the growth of species predicted to be unable to directly incorporate NO₃⁻ (e.g., *Idiomarinaceae* sp. HL-53). Furthermore, ammonium amendment increased the total biovolume and density of organisms in UCC-O, and eventually terminated in a crash in autotrophic biomass late in succession. In contrast, ammonium amendment of UCC-A resulted in only minor changes to successional dynamics. Because the consortia share the same suite of heterotrophic members¹, these results suggest that these members occupy significantly different niches in each consortium with respect to nitrogen flow that are difficult to predict from the presence or absence of nitrate assimilation pathways. These data suggested large-scale alteration in carbon and nitrogen flow between members, a conclusion further supported by changes in the spatial patterns of H¹³CO₃⁻ and ¹⁵NH₄⁺ or ¹⁵NO₃⁻ as visualized using nanoSIMS. In sum, these results suggest that lack of primary access to a nutrient may not significantly impede an organism's growth within a consortium, but that community compositional and spatial structure are likely determined by large-scale patterns of resource flow. This informs the design of consortia by suggesting that supplementation with resources predicted to favor expansion of a desired species are likely to have unpredictable and distributed impacts upon community structure and function.

References:

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2. Lindemann, S. R. et al. The epsomitic phototrophic microbial mat of Hot Lake, Washington: community structural responses to seasonal cycling. *Front. Microb. Physiol. Metab.* 4, 323 (2013).

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