19. Identifying environmental state variables governing the assembly of microbial communities

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Project Goals: To elucidate the underlying principles governing microbial community assembly by identifying the biotic and abiotic drivers determining recruitment and maintenance of phylogenetic and functional diversity in dynamic communities. Further, to examine how the interspecies interactions operating within a community are influenced by key environmental state variables, and how these effects feedback to community structure and function. To reveal how changes in community structure and function lead to alteration in higher-order community properties (e.g., resilience after perturbation) and predict environmental “tipping points” in community structural and functional state.

Microbial mats are compact ecosystems composed of highly interactive organisms in which complete energy and element cycles occur. These communities experience significant variation in key state variables (e.g., light quality and quantity, temperature, salinity, oxygen, pH) over both short (e.g., diel) and long (e.g., seasonal) time scales. To investigate overarching principles governing microbial community assembly, we examined temporal variation in disparate mat communities at multiple time points over seasonal cycles. We hypothesize that higher-order community properties (e.g., resistance to environmental change) emerge in microbial communities in predictable and generalizable ways based upon the structural and functional states of a community and that arise from the interspecies interactions operating between members. Two tractable and highly-structured microbial mat systems were chosen for this work (one phototrophic, one chemotrophic) to investigate system-independent principles of microbial interaction.

Hot Lake is a hypersaline lake that seasonally harbors a benthic, phototrophic mat that assembles each spring and disassembles each fall and is subject to greater than tenfold variation in salinity (primarily Mg2+ and SO42-) and irradiation over the annual cycle. Similarly, mat communities exposed to overlying flow, such as those occupying low-pH geothermal outflow channels in Yellowstone National Park, exhibit strong spatial and structural control by pronounced geochemical gradients. The temporal self-organization of these structured microbial mats provides opportunities for
elucidating key state variables that drive interspecies interactions within dynamic communities and govern the interplay between community structure and function.

Relative abundances of microbial phylotypes were obtained using amplicon sequences of the 16S rRNA gene (V4 region), which were cross-referenced to near-full-length 16S rRNA sequences for increased phylogenetic resolution. Despite extreme variation in environmental conditions over the season, the composition of the Hot Lake phototrophic mat community exhibited year-round stability in its key autotrophic (Leptolyngbya and Phormidium spp.) and heterotrophic (primarily, Rhodobacteraceae) membership. In contrast, by mid-fall the mat began to show signs of disassembly, with mat community richness and evenness declining precipitously under conditions of increasing salinity and decreasing irradiance and temperature. Elucidation of the principles governing interspecies interactions that drive microbial community assembly is challenging because of the diversity, complexity and intractability of natural system to in situ manipulation. Therefore we isolated two filamentous cyanobacteria and their co-isolated heterotrophic consorts from the Hot Lake mat to study primary succession in a mat-relevant model phototrophic community. The relative simplicity and tractability of these unicyanobacterial consortia allowed the characterization of the spatial and community structures of their assembling biofilms under controlled environmental conditions. Both consortia retained essentially the same suite of heterotrophic species, representing ten of the most abundant OTUs within the Hot Lake mat community. The abundance and growth rate of autotrophs dominated early in assembly, yielding to increasing rates of heterotroph growth late in succession. The two consortia exhibited similar patterns of succession at low taxonomic resolution, but substantial differences in the abundances of specific heterotrophic members depending upon which cyanobacterium served as primary producer. Taken together, these data suggest that, although similar niches are created by the cyanobacterial metabolisms, the resultant network of autotroph- heterotroph and heterotroph-heterotroph interactions are specific to each primary producer.

Likewise, community assembly in thermal chemotrophic communities displays similar patterns of autotroph-heterotroph succession. We studied community structure as a function of time and key state variables by temporal colonization experiments and by sampling along geochemical gradients. The distribution of autotrophic and heterotrophic community members varies depending upon pH and concentration of key electron donors and acceptors (S2-, Fe2+, O2). Temporal studies in Fe3+-oxide systems have also shown that autotrophic populations (especially Hydrogenobaculum sp.) are important colonizers in early mat development (2-30 days), and that heterotrophic populations generally establish in deeper mats (> 1 mm) that develop on time frames > 100 d. Metagenomic analyses revealed that many of these key heterotrophs are likely auxotrophs for synthesis of required cofactors, amino acids, and nucleotides, a result also observed in the Hot Lake unicyanobacterial consortia.

These data suggest that similar governing principles shape the succession of microbial communities inhabiting disparate environmental systems. Early colonization of prototrophic organisms leads to generation of niches for auxotrophic heterotrophs, which likely increase the functional capacity and efficiency of the community. Future work will focus on identifying specific state variables governing the interspecies interactions that shape community succession dynamics and the emergence of higher-order community properties. These genome-enabled studies will involve an iterative field-lab approach in which observations of the mat in the field are paired with laboratory-based perturbation studies of consortia, both assayed using integrated global molecular techniques (e.g., transcriptomics, proteomics, and metabolomics).

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