214. Assessing In Situ Metabolic Carbon Dynamics in a Phototrophic Microbial Mat

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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.

We tracked C from stable isotope (13C) labeled substrates into a phototrophic microbial mat system to assess metabolic interactions responsible for directing C flow over a complete diel cycle. We used substrates directed towards photoautotrophic (13C bicarbonate addition) and heterotrophic (13C-glucose and 13C-acetate additions) members of the community to specifically target C exchange between these functional groups. The different fates of the 13C-substrates were assessed in terms of chemical transformation (catabolic remineralization versus anabolic uptake to different biomass constituents), spatial localization (along a depth profile), functional utilization (protein and storage polymer synthesis), and phylogenetic activity (specific protein synthesis).

Hot Lake is a hypersaline, meromictic lake located in an endorheic basin in north-central Washington which, despite extreme salinity and seasonal water temperatures (> 55 ˚C), hosts dense, phototrophic benthic microbial mats. We extracted mature mat from this system (figure 1) and performed lake-side, batch incubations using ambient light, native lake water, and while maintaining natural temperature. A single pulse of labeled substrate (13C bicarbonate, glucose, or acetate) was added to different incubations and we performed timeseries subsampling around a complete diel cycle (noon to noon).

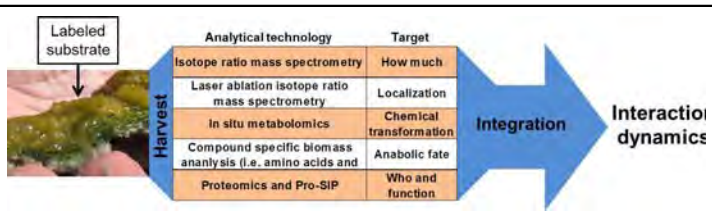

Figure 1: Experimental approach. Mat is harvested then incubated in the presence of ^{13}C-labeled substrates and subsampled over a diel timeseries. A suite of analytical techniques are applied to track overall carbon uptake into the mat and its spatial, chemical, and functional fate.

Bulk stable isotope mass spectrometry revealed distinct patterns between the different substrates in terms of uptake into mat biomass. Bicarbonate and acetate uptake tracked the diel cycle (presumably governed by light and oxygen availability respectively) while glucose showed a nearly uniform uptake rate despite large-scale diel shifts in redox conditions within the mat. Sample washing with dilute HCl removed a significant fraction of 13C associated with bicarbonate and glucose uptake, suggesting conversion of 13C

from these substrates into to acid-labile or water soluble components. In comparison, little acetate-derived 13C was removed by the HCl washing. We speculate that a portion of the removed C can be attributed to acid labile and water soluble EPS components (i.e. polysaccharides), suggesting preferential conversion of bicarbonate and glucose-derived C to EPS versus acetate C. 13C-metabololite analysis demonstrated anabolic uptake of applied substrates into various monomers (i.e., glucose conversion in a distinct C6 unit directly into trehalose) that serve in osmotic regulation or as metabolic storage compounds. Similar processes (using minimal chemical bond transformations for synthesis) may enable high rates of conversion of extracellular glucose C to EPS components. Interestingly, however, is the lack of water-soluble, acid labile C derived from acetate 13C. We are exploring whether acetate may be preferentially shunted towards other compound classes (e.g. lipids).

In incubations with either of the organic substrates (glucose and acetate) we observed a large 13C flow towards remineralization to dissolved inorganic carbon (DIC) at multiple points in the diel cycle. We additionally observed conversion of C from all substrates to solid-phase, inorganic carbon (e.g., carbonate) although this represented a relatively small C flux and the mechanism for this conversion is unclear, but likely includes intermediate remineralization to DIC.

Proteomic analysis was enabled by metagenome sequences of unicyanobacterial consortia (UCC) developed from this system (See Nelson poster for details). For all three substrates, there was a higher C flux into phototroph associated proteins versus those of heterotrophic organisms. Both bicarbonate and glucose derived 13C showed a larger flux into heterotrophic proteins than acetate 13C. The relative high abundance of bicarbonate derived 13C in the heterotroph proteins highlights the close C coupling between autotrophs and heterotrophs; autotroph fixed C being converted to heterotroph proteins as quickly as or more quickly than exogenously applied glucose or acetate respectively. We also observed diel dependence in conversion of 13C substrates to protein with the bulk of conversion not occurring until after the diel dark period. We hypothesize the mat undergoes day-time nitrogen limitation and that freshly fixed N2 during the night, when the mat goes anoxic, may be needed for protein synthesis.

Taken together, our data highlights the different C conversions and interactions revealed in this mat by the combined stable isotope, metabolomics, and proteomic approach we applied. Specifically, the three applied substrates showed distinct patterns of uptake into microbial biomass; bicarbonate 13C preferentially converted to protein, glucose 13C showing the highest conversion to water/HCl soluble compounds, and acetate 13C to water/HCl insoluble compounds. Further, the higher conversion of bicarbonate 13C to heterotroph associated proteins highlights the tight metabolic interactions between these groups as autotrophically fixed C was preferred for protein synthesis versus exogenous C.

This research was supported by the U.S. Department of Energy (DOE), Office of Biological and Environmental Research (BER), as part of BER's Genome Sciences Program (GSP). This contribution originates from the GSP Foundational Scientific Focus Area (FSFA) at the Pacific Northwest National Laboratory (PNNL).