

Spatially-Resolved Carbon and Energy Transformations in a Vertically Laminated, Phototrophic Mat

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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.

The localization of carbon (C) fixation in vertically laminated phototrophic mat systems is largely controlled by photon accessibility and generally relegated to only an upper portion of the overall community. Light attenuation through the mat can produce ‘dark’ regions (negligible photosynthetically active radiation) below the photic zone, thereby precluding photoautotrophy from these laminae. Lack of *in situ* photoautotrophy below the photic zone means that the resident heterotrophs are reliant on the photosynthetic activity of upper mat layers and C transport processes to deliver fixed C deeper into the mat. **This work describes our team’s progress toward understanding the spatial controls on C and energy transformations in complex microbial mat communities.** Our studies are guided by two central hypotheses:

1. *Diel dynamics drive the localization and dispersal of fixed C within a photosynthetically driven system*
2. *Species richness in spatially laminated, photoautotrophically supported communities is decoupled from light energy input*

To address these hypotheses, we used stable isotope labeled (^{13}C) bicarbonate to track C fixation and accumulation into biomass within a phototrophic mat system under two sets of conditions: 1) field-based, stationary incubations of intact mat, using *in situ* medium and light (diel) conditions, and 2) laboratory flow-through incubations containing artificial medium and constant light intensity.

In the field-based incubations, we assessed C accumulation and migration over a complete natural diel cycle. Spatially resolved analysis (Figure 1) via laser ablation isotope ratio mass spectrometry (LA-IRMS) provided localized quantification of C uptake (day) and partial loss (night). We confirmed our hypothesis that net photoautotrophy (as recorded by accumulation of ^{13}C) was highly localized near the upper portions of the mat, above the maximum photic depth. C penetration below the photic zone progressed consistently during daylight, indicating that C slowly migrated while C was being actively fixed, versus a rapid spatial migration of C after a diel shift (i.e. light-to-dark). Proteomic analysis of isotopically labeled peptides was used to assess the degree to which photosynthetic products were transferred to associated heterotrophic community members. In an expanded set of field-based incubations, we used isotopically labeled (^{13}C) glucose and acetate as substrates to differentiate heterotrophic from autotrophic consumption. Proteins associated with autotrophs (i.e. cyanobacteria) dominated the labeled fraction for all substrates (bicarbonate, glucose, and acetate) but the bicarbonate-

derived ^{13}C labeled peptides from heterotrophs were used to identify trophic C exchange within the community. By clustering the labeled peptides based on their changing abundance over the diel cycle, we identified a disproportionately large increase in proteins incorporating acetate-derived C at night and bicarbonate and glucose-derived C during the day.

Similar C accumulation profiles were observed when the mat was incubated in a flow-through, laboratory-based system with (^{13}C) bicarbonate; namely a distinctive focusing of bicarbonate-derived C near the mat's surface. Shallower penetration of freshly fixed C into the mat profile was consistent with the reduced incident irradiance that was used in the laboratory versus the field. When the mat samples were exposed to constant light conditions, there was persistent migration of fixed C to deeper layers of the mat over the course of the experiment. In addition to measuring autotrophy rates, we used oxygen microsensors to determine *in situ* photosynthetic rates (both net and gross) and used 16S rRNA gene sequencing to determine the microbial diversity in cryosectioned mat laminae. We found an inverse relationship between community diversity and photosynthetic rates (Figure 2) with regions of highest O_2 production (near the mat surface) also having the lowest diversity based on number of observed OTUs.

Our studies of the spatial relationships between C and energy acquisition within photosynthetically driven microbial communities is helping to reveal basic principles about complex photoautotroph:heterotroph interactions. Together these data reveal new candidate principles to be validated with further experimentation:

1. Diel light cycling drives the timing of photosynthetically fixed C migration through the community and its exchange between phototrophs and heterotrophs.
2. Light energy input in spatially laminated, photosynthetically driven systems can constrain species richness.

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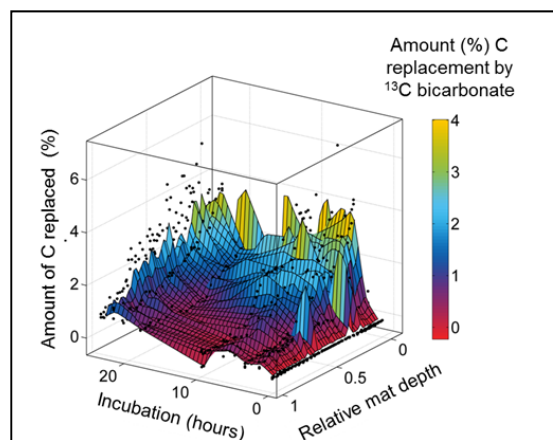


Figure 1: Spatially resolved isotope analysis to identify regions of label accumulation. We used LA-IRMS to pinpoint regions showing the highest accumulation of substrate-derived C (percent of native biomass replaced by the added substrate). Bicarbonate-derived C is predominately focused near the mat's surface (relative depth = 0) and displays a diel influx and efflux of photosynthetically derived C. Penetration of C below the photic depth is most prominent during the day suggesting a constant, depth-ward migration of C versus a stepwise transition linked to shifts in diel light regimes.

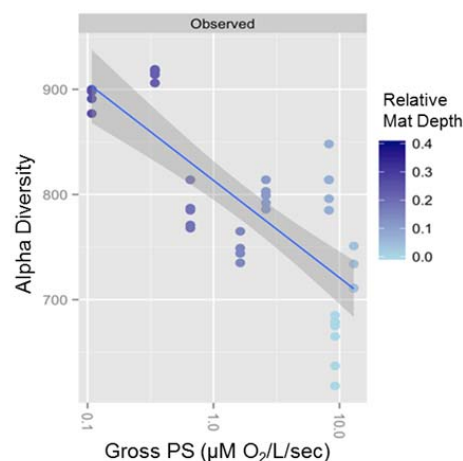


Figure 2: Depth-resolved microbial diversity (based on number of distinct OTUs) showed an inverse relationship with photosynthetic rates (both gross and net rates with gross shown above).