

## **Integrated Experimentation and Trait-based Model Exploration of Algal-Bacterial Interaction Mechanisms.**

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**Project Goals:** The LLNL Bioenergy SFA seeks to support sustainable and predictable bioenergy crop production through a community systems biology understanding of microbial consortia that are closely associated with bioenergy-relevant crops. We focus on host-microbial interactions in algal ponds and perennial grasses, with the goal of understanding and predicting the system-scale consequences of these interactions for biomass productivity and robustness, the balance of resources, and the functionality of surrounding microbial communities. Our approach integrates ‘omics measurements with quantitative isotope tracing, characterization of metabolites and biophysical factors, genome-enabled metabolic modeling, and trait-based representations of complex multi-trophic biological communities, to characterize the microscale impacts of single cells on system scale processes.

Microalgal interactions with heterotrophic bacteria have substantial implications for carbon cycling in natural and engineered aquatic ecosystems, including beneficial effects on net primary productivity, but the relative importance of different mutualistic mechanisms is rarely explored from a functional trait perspective. In this work, *Phaeodactylum tricornutum*, a biofuel relevant microalgal species, was cultured with and without a heterotrophic bacterium of the *Marinobacter* genus, under cyclical light conditions and two nutrient regimes. *P. tricornutum* grown with *Marinobacter* exhibited a 10% increase in growth rate over the axenic culture. Carbon exchange and productivity increases were supported by a 19% enhancement in carbon (C) fixation by individual *P. tricornutum* cells as shown by nanoscale stable isotope probing (nanoSIP). The proposed beneficial roles of the heterotrophic bacteria include: (1) relief of algal CO<sub>2</sub> limitation through localized bacterial respiration of algal exudates (a function of biomass and growth yield); (2) oxidative stress alleviation via usage of O<sub>2</sub> as a terminal electron acceptor in bacterial respiration. These processes are coupled in reality, and to explore the relative contributions of these two mechanisms to enhanced algal growth, we have developed a dynamic energy budget (DEB) model of interactions between these phototrophic and heterotrophic microorganisms. Explicit representation of the competitive dynamics between DIC and O<sub>2</sub> in RuBisCO permitted direct comparison of the relative impact of the two mechanisms. Benchmark simulation results

show that the two mechanisms can account for most but not all of the enhanced carbon fixation, suggesting the possible importance of other mechanisms. The simulations specifically evaluated algal C exudation rates, bacterial growth yield (via maintenance costs), in addition to the affinities of algal RuBisCo for CO<sub>2</sub> versus O<sub>2</sub>. In doing so, the model revealed that algae with higher carbon exudation rates and bacteria with higher maintenance costs (and therefore lower carbon use efficiency) combine to result in higher algal growth yield. Therefore, factors that regulate algal retention and excretion of carbon, and microbial metabolic and biophysical traits that regulate carbon-use efficiency, are likely to influence the dynamics of algal-bacterial associations and the fitness of both parties. This provides a series of hypotheses that are being explored through integrated computational trait inference, experimentation and dynamic energy budget modeling.

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