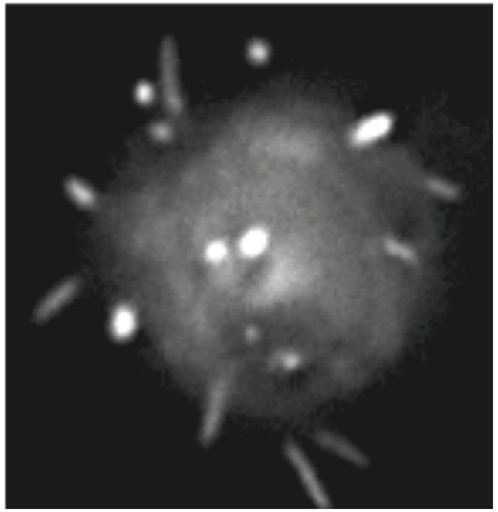
DOE BIOENERGY TECHNOLOGIES OFFICE (BETO)
2021 PROJECT PEER REVIEW

MICROBIOME ENGINEERING OF *DESMODESMUS* TO ALLEVIATE CARBON LIMITATION

WBS 1.3.2.652, CID NL0033320



March 22, 2021
Advanced Algal Systems

Xavier Mayali
Lawrence Livermore National Laboratory

Project Overview

THE NATIONAL MICROBIOME INITIATIVE

- 2017 BETO Productivity and Enhanced Toolkit (PEAK) identified *Desmodesmus intermedius* C046 as State of Technology for summer season

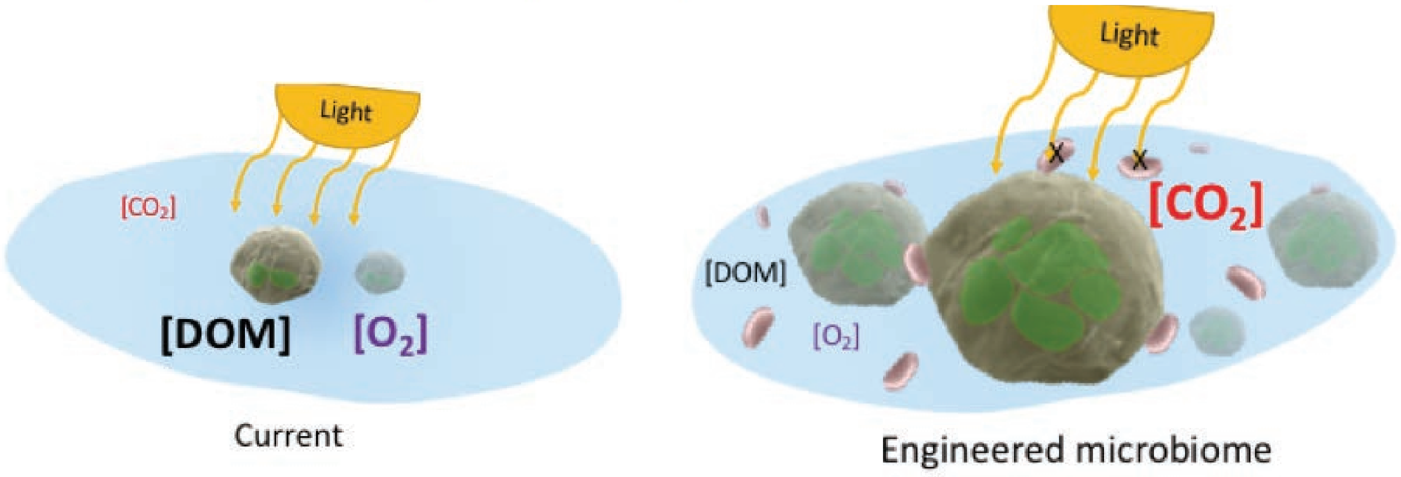
- **Project Goal:** R&D to identify strategies for altering the algal microbiome to increase biomass production under summer conditions (high light and high temperature)

- The microbiome impacts <u>all</u> ecosystems (aquatic, soil, animal)

  Hypothesis: altering the algal microbiome can increase biomass production, decrease cost

Season	2016 BETO SOT ($g/m^2/day$), ash free basis	2020 PEAK Performance Target ($g/m^2/day$)
Spring	11.1 *(Nanno)*	22
Summer	13.3 (*Desmo*)	26
Fall	7.0 (*Desmo*)	14
Winter	5 *(Nanno)*	10
Annual average	9.1	18

**Nanno* refers to *Nannochloropsis maritima KA32* (saline media) and *Desmo* refers to *Desmodesmus sp. C046* (saline media)



1 – Management (DOE lab-led competitive project)

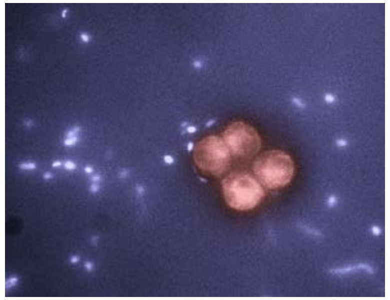
microbiome enrichments

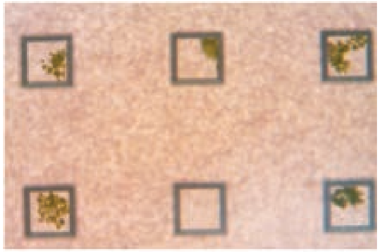
GENERAL AUTOMATION LAB TECHNOLOGIES

microwell cultivation

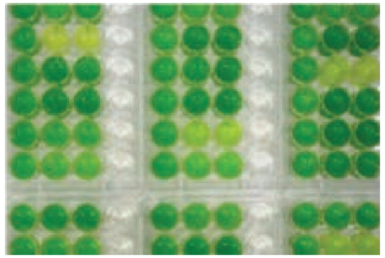
milliliters

Liters

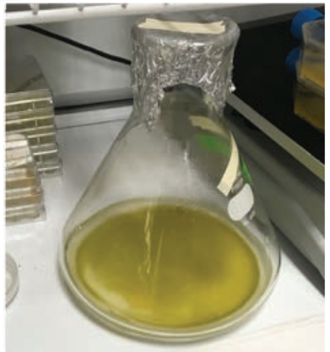


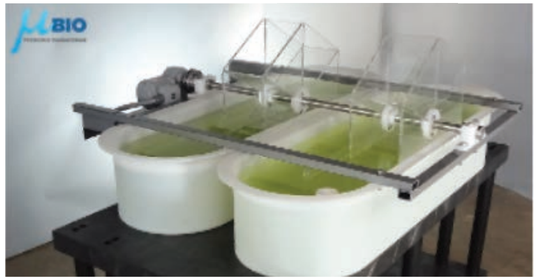


nanoliters



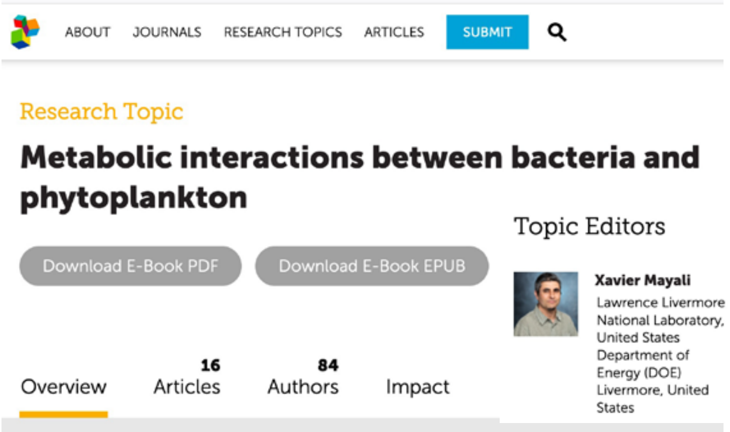
microliters





- **Expertise**
  - LLNL: microbial ecology, microscale algae-bacteria interactions, algal cultivation
  - GALT: high-throughput microbiome cultivation start-up

- **Related projects & collaborations**
  - LLNL AOP, anti-grazing probiotic bacteria (R. Stuart)
  - LLNL SEED AOP, ecology of chytrid infections (T. Samo)
  - DOE Office of Science (BER) SFA on microbial interactions: R. Stuart (LLNL), X. Mayali (LLNL), T. Lane (Sandia), S. Merchant (UC Berkeley), C. Buie (MIT)
  - Strong link between basic (BER) and applied (BETO) algal microbiome science



1 – Management

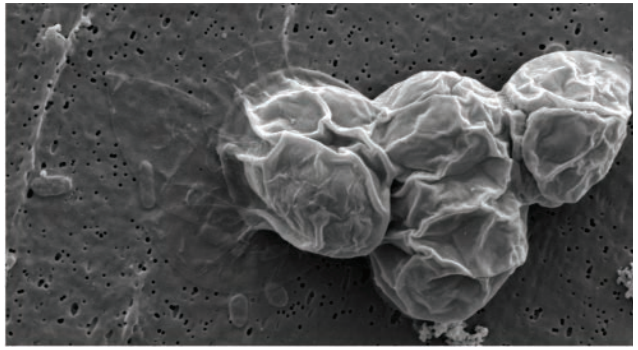
Engagement with stakeholders

- Strong & frequent interaction with BETO (project updates every 6 weeks, quarterly reports, monthly lab updates via LLNL Laboratory Relationship Manager)
- Active and vocal participation at algae workshops (2019 Bio-restore, 2020 Rules of Life Algae, 2021 Crop Protection)
- Plans for increased future collaboration (Bowling Green State, U. Toledo, Montana State, NREL)

Risks and *mitigation strategies*

- GALT microwell system not previously tested with algae: *significant R&D during Years 1 &2, dilution to extinction experiments tested as alternative*
- Results do not scale up from nanoliter to multi liters: *all lab experiments at all scales under simulated outdoor conditions (sinusoidal light/temperature)*
- Mechanisms of mutualism are uncharacterized: *investigate fundamental science with BER funding; iterate between fundamental and applied research*

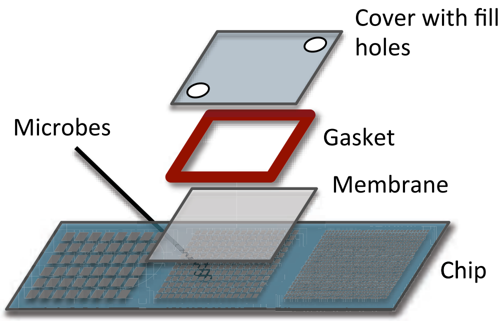
2 – Approach



Background

- Excessive light and high temperature are known to make algae less photosynthetically efficient (wasted resources excreted as organic carbon)
- Hypothesis: interactions with bacteria can alleviate temperature/light stress

Technical methods

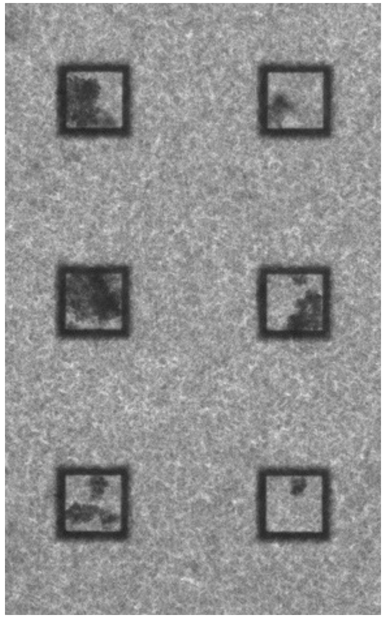


- GALT microwell system for high-throughput cultivation, low volume, allows testing of thousands of combinations
- Microbial community analysis (PCR amplification of 16S rRNA gene)
- Isotope tracing and NanoSIMS to quantify C fluxes between bacteria and microalgae

Potential challenges

- Lab-optimized microbiomes will be lost once moved outdoors: *focus on algal-attached bacteria to maintain mutualism*
- Bacterial mutualistic impacts are modest: *examine combination of microbiome and added nutrients*

2 – Approach (cont)

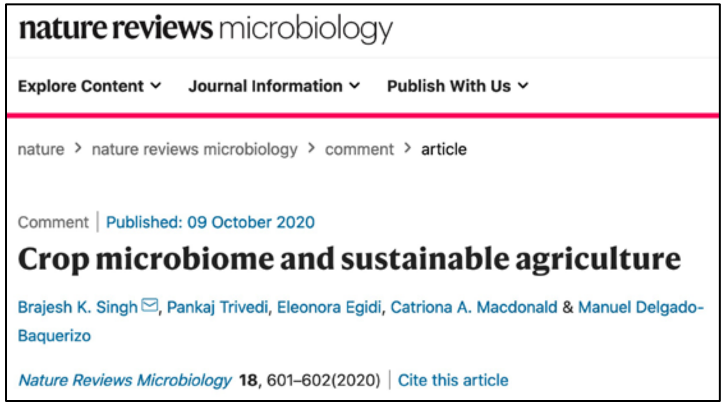


- **Go/No-Go decision points**
  - Verification (BP1): GALT system compatible with microalgae, *Desmodesmus* can grow without bacteria
  - Mid-project verification (Dec. 2019): Milestone of 100% productivity improvement not achieved (we achieved 40% greater AFDW than bacteria-free culture)
  - Rescoped BP3: simulation of outdoor conditions in the lab (aerosol input of microbes, iron dust, guano); outdoor trial at end of project

- **Technical metrics**
  - Experimental demonstration that the *Desmodesmus* microbiome can confer increased biomass production under stress conditions
  - Test that microbiomes do not alter biomass composition (lipid, protein)
  - Investigate interaction between microbiome and nutrient status (organic N, iron)

3 – Impact

- The microbiome plays a role in animal/plant health, but most algal growth efforts ignore bacteria until pond failures
- All options are on the table to decrease costs (e.g. PEAK goal of 80 GGE fuel ton^{-1} of biomass), including farming the microbiome for increased efficiency
- Terrestrial agriculture is using microbiome science to improve sustainability
- Presentations (2020): Algal Biomass Summit, NSF-BETO Rules of Life, Florida A&M DOE Minority Workforce Colloquium; one drafted manuscript, one in prep
- Two summer students trained (winner LLNL best poster 2019)


nature reviews microbiology

Explore Content ⌄ Journal Information ⌄ Publish With Us ⌄

nature > nature reviews microbiology > comment > article

Comment | Published: 09 October 2020

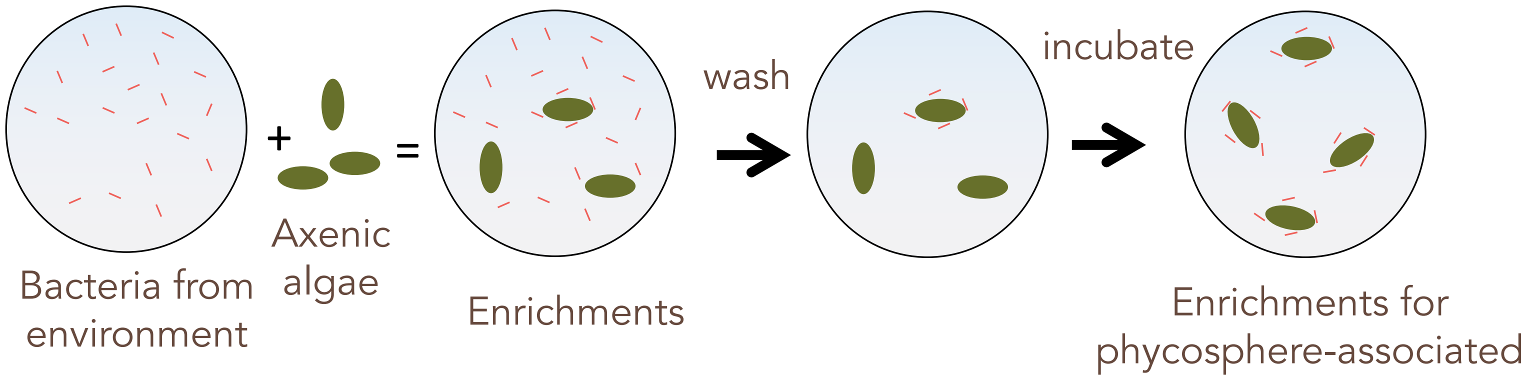
Crop microbiome and sustainable agriculture

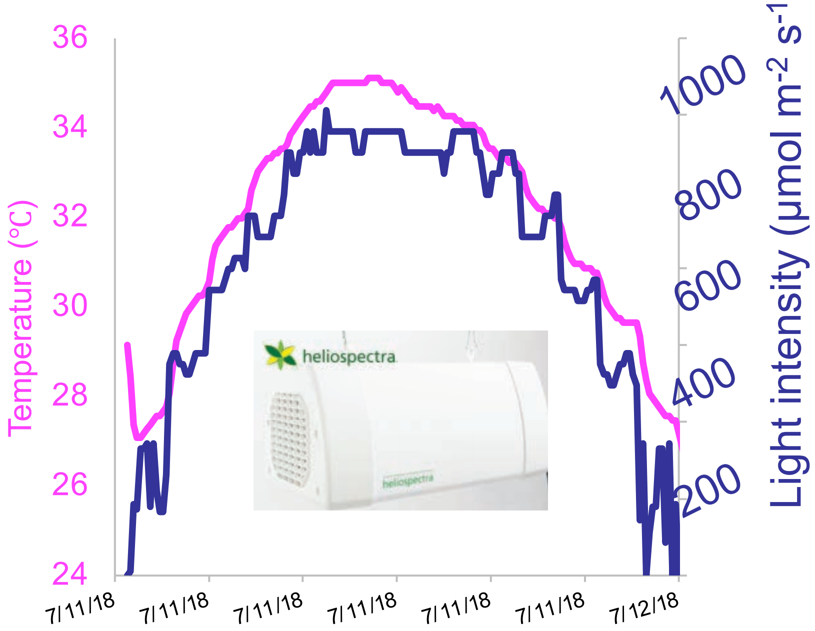
Brajesh K. Singh, Pankaj Trivedi, Eleonora Egidi, Catriona A. Macdonald & Manuel Delgado-Baquerizo

Nature Reviews Microbiology **18**, 601–602(2020) | Cite this article

4 – Progress and Outcomes: Task 1

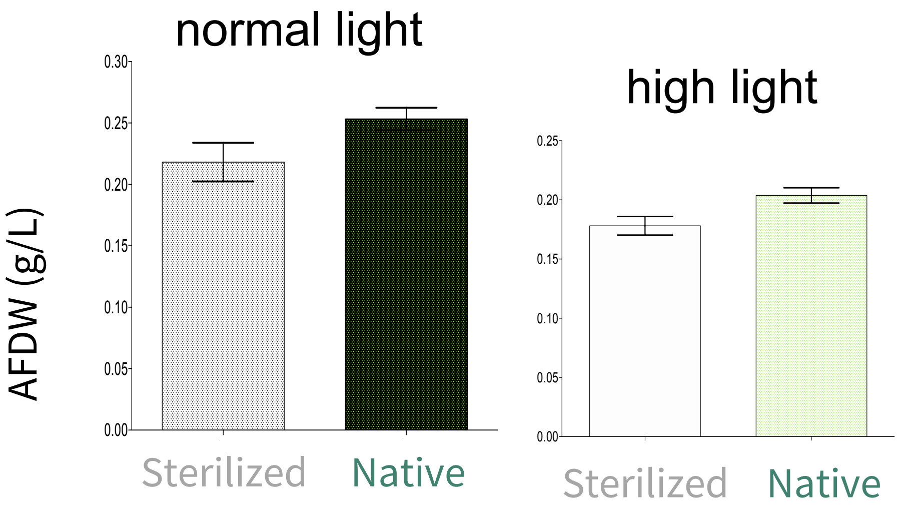
- **Goal**: Verification: bulk microbiome enrichments, GALT microwell system
- **Accomplishment**:
  - Removed native *Desmodesmus* microbiome, perform microbiome transplants
  - Optimized GALT microwell cultivation for microalgae under summer conditions
- **Impact**: GALT bacterial cultivation system applied to algal microbiome

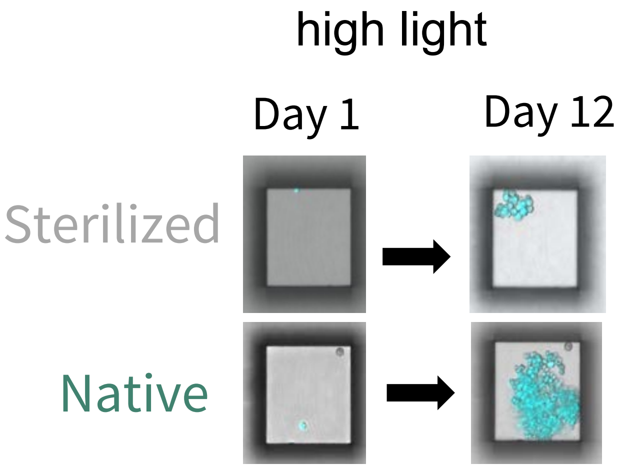


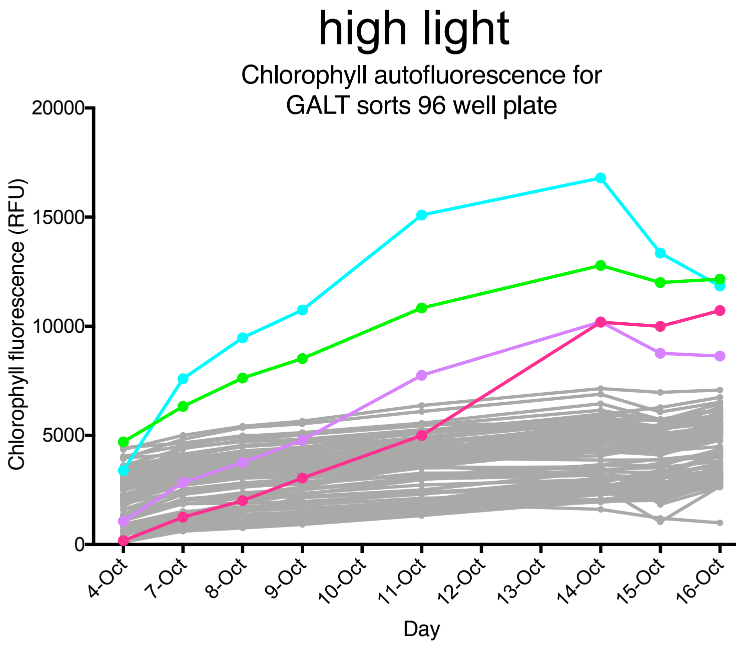


4 – Progress and Outcomes: Task 2

- **Goal**: obtain simplified microbiomes that increase *Desmodesmus* growth under high heat/light
- **Accomplishments**:
  - Bulk and microscale analyses of algal & bacterial biomass
  - Develop and test toolkit #1 (GALT screening & sampling for algal growth)
- **Impact**: Identified that microbiome affects algal biomass accumulation under simulated summer conditions

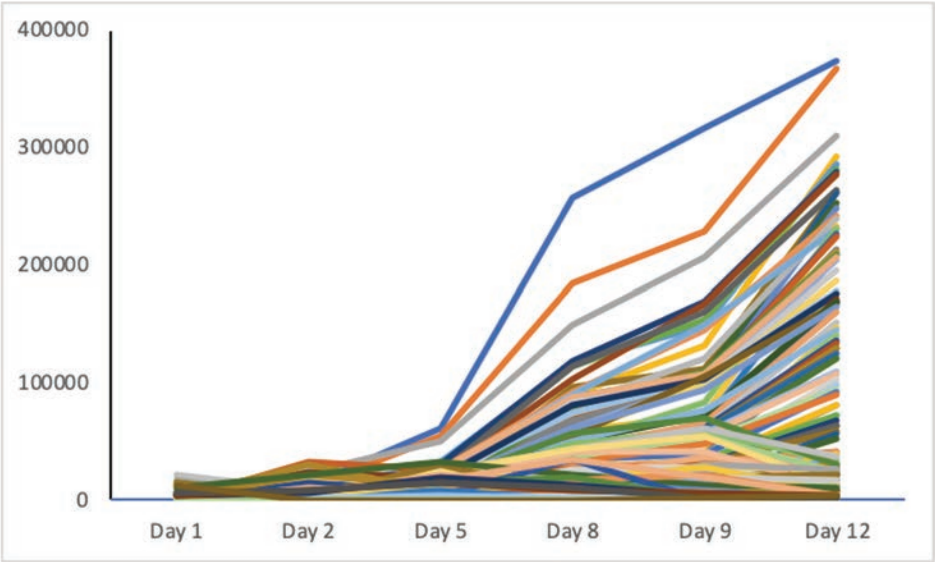


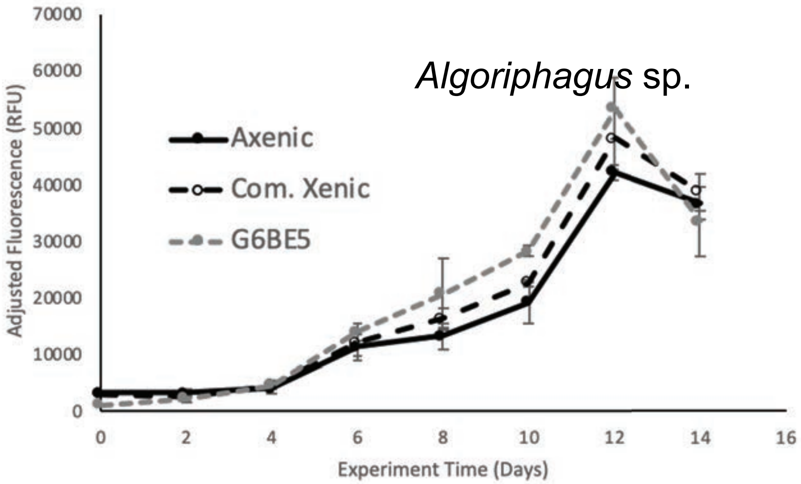




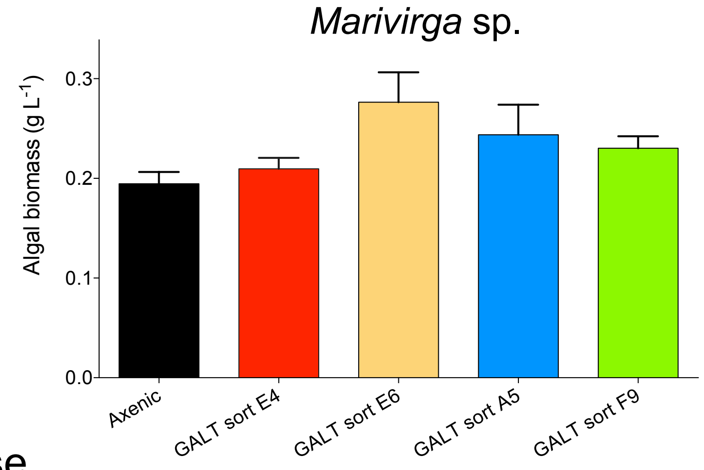
4 – Progress and Outcomes: Task 2 (cont)

- **Goal**: obtain simplified microbiomes that increase *Desmodesmus* growth under high heat/light
- **Accomplishments**:
  - Scale-up testing of simplified microbiomes (10,000 cultures screened 10 nL scale)
  - Tested/optimized DOM well-plate assay
  - Identified microbial communities associated with high growth
- **Impact**: Identified that microbiome affects algal biomass accumulation under simulated summer conditions





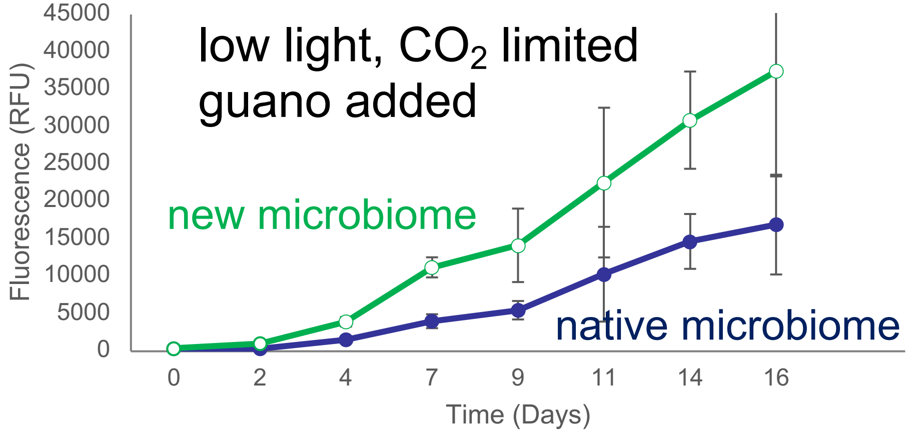
top microbiome = 19% chlorophyll increase compared to wild type (xenic) at day 10

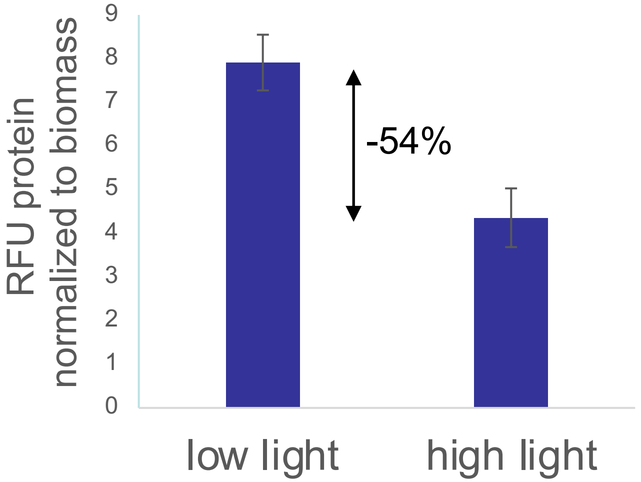


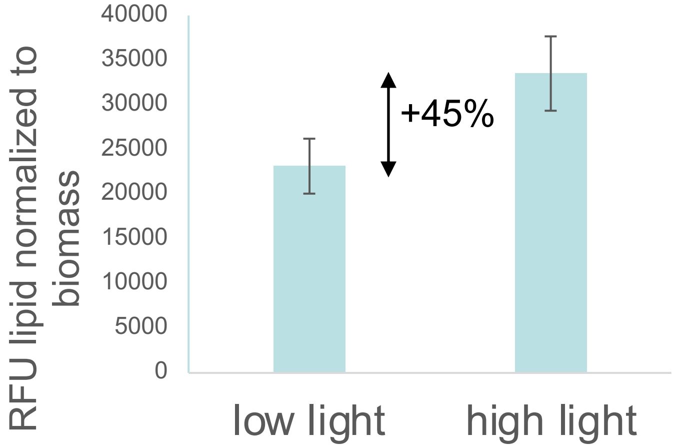
40% increased AFDW vs. sterilized (axenic)

4 – Progress and Outcomes: Task 3

- **Goal**: quantify microbiome impacts with increased complexity from lab to outdoors
- **Accomplishments**:
  - Simulate airborne invasions in the laboratory (minimal impact on biomass, some impact on microbial community structure)
  - Test influence of added bird guano and iron dust under normal and high light: identity of microbiome impacts mutualism
- **Impact**:
  - Cryptic organic N and inorganic Fe nutrient limitation
  - High light/temperature stress: higher lipid/biomass, lower protein/biomass

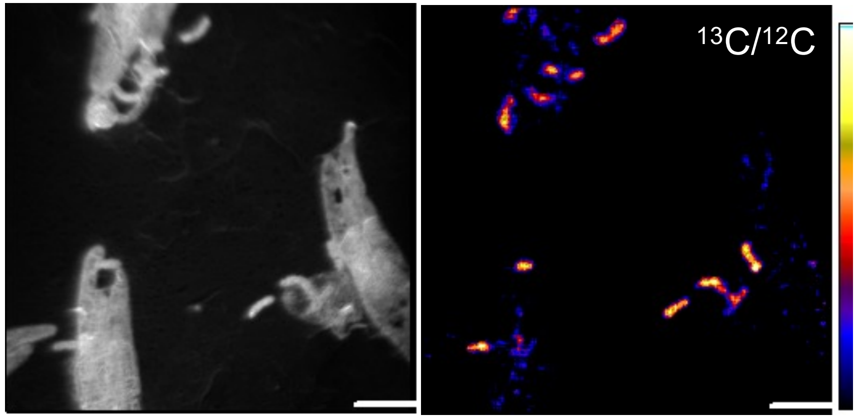


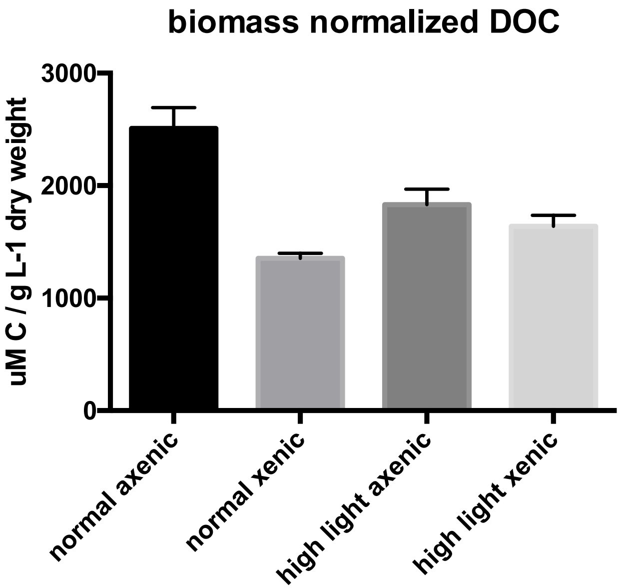


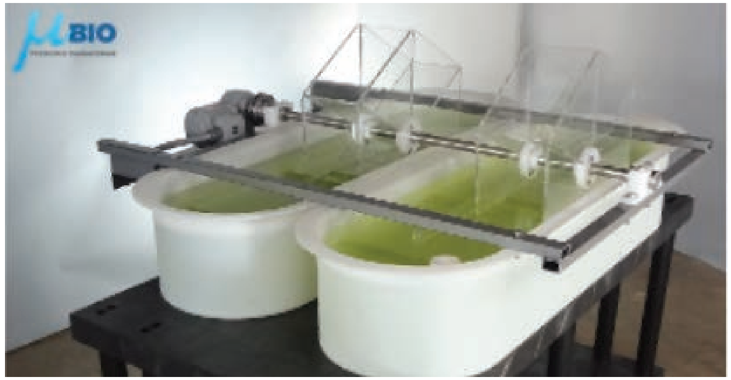


4 – Future work: Task 3

- **Goal**: Link microbiome impact and DOM disappearance
- **Experiments**:
  - Microscale quantification of carbon remineralization via NanoSIMS stable isotope tracing to explain low DOC production/utilization under high light
  - Use toolkit #2 to quantify DOM in different cultures
  - Test outdoors (100 L mini-raceways)
- **Impact**: Develop a microbiome screening toolkit to apply to other strains & conditions







SUMMARY

- **Overview**: optimize a microbiome engineering approach to screen for bacterial mutualism
- **Approach**: High-throughput, microscale screening of single-cells, scale up in volume and environmental complexity, quantify activity of attached bacteria
- **Results**:
  - The algal microbiome impacts biomass under temperature and light stress
  - Optimized microbiomes offer modest increases compared to original xenic culture (but up to 50% higher than axenic and dozens of unoptimized microbiomes)
  - Additions of organic nitrogen and particulate iron rescue adverse temperature/light effects
- **Relevance**: Sustainable algal cultivation will <u>require</u> microbiome understanding and control
- **Future work**: link microbiome to C recycling at bulk and single cell level, carry out 100L scale outdoor experiment

Acknowledgements

LLNL:

Ty Samo

Kristina Rolison

Rhona Stuart

Courtney Swink

Dan Flowers

Adrienne Ly

Adrian Tolentino

GALT:

Suzan Yilmaz

AJ Hallock

Jude Dunne

Peter Christey

Maria Villancio-Wolter

BETO:

Devinn Lambert

Jamie Meadows

Philip Lee

Evan Mueller

Amanda Barry

Quad Chart Overview

Timeline
- Project start 1/1/2018
- Project end 9/30/2021

	FY20 Costed	Total Award
DOE Funding	$341K	$1,539K
Project Cost Share		$173K

Project Partners
- Lawrence Livermore National Laboratory
- General Automation Lab Technologies

Project Goal
Using a high-throughput microfluidic screening system, identify mutualistic bacteria with high respiration metabolism and protective pigmentation that lead to increased Desmodesmus growth under summer conditions, test at different lab and outdoor scales

Barriers Addressed: AFT-C. BIOMASS GENETICS & DEVELOPMENT: The productivity and robustness of algae strains against such factors as temperature, seasonality, predation, and competition needs to be improved

End of Project Milestone
Two toolkits (a high-throughput microchip-based approach to isolate microbiomes with algal growth promoting properties, and a medium-throughout assay to quickly identify low DOC cultures);

Demonstration of increased productivity at pilot scale

One submitted manuscript, one in prep manuscript

Funding Mechanism
PEAK FOA, 2017

Additional Slides

Response to reviewer comments

- **2019 Peer Review**
  - "Outdoor demonstration of the technology might be challenging given the additional ecological complexity": *Partially due to COVID shutdown, the SOPO was rescoped in spring 2020 to replace mid-project outdoor trial with indoor experiments with simulated outdoor effect such as airborne microbial contamination, bird guano, dust input.*
  - "The biomass composition will need to be evaluated to ensure there is no negative impact of the presence of the microbial community": *we have tested this empirically and not found any differences in algal lipid and protein content with the addition of difference microbiomes*
  - "The main weakness noted was in providing a better explanation/justification on the use of isotope tracing": *the NanoSIMS isotope tracing has been moved towards the end of the project (ongoing as of March 2021) and will provide direct evidence for bacterial metabolism and remineralization of algal-derived organic matter through cell-specific net flux quantification.*
- **Go/No-go Reviews**
  - "Algal cultivation with optimized microbiomes should be compared to original "xenic" culture, not axenic culture, since BETO SOT calculation used xenic culture": *this was addressed in all subsequent experiments*
  - "Verification team recommends cell counts for future growth productivity improvement experiments" *addressed for certain 100 mL scale experiments, but chlorophyll is more robust due to algal cell size differences*
  - "The team has not shown a baseline AFDW or GGE estimation": *we calculated a baseline of 8.97 g/m2/day for the xenic culture under extreme light and temperature stress in the laboratory*

Publications, Presentations, Awards

Presentations

- Mayali X, Samo TJ, Rolison K, Christie P, Hallock AJ (2020) Scaling up Algal-bacterial Co-cultures to Alleviate Temperature and Light Stress, Algal Biomass Summit Virtual Conference, oral presentation
- Mayali X, Samo TJ, Rolison K, Christie P, Hallock AJ (2020) Bacteria-Algae Interactions under the Hot Sun, Rules of Life: Complexity in Algal Systems Virtual Summer Symposium, oral presentation
- Mayali X (2020) Aquatic microbes breathe and eat more than we do: using DNA and the periodic table to uncover their secrets, Florida A&M DOE Minority Workforce & Pipeline Colloquium

Awards

- Global Security Directorate best summer student poster, Ly A, Mayali X, Samo TJ (2019) De-stressing biofuel microalgae: Leveraging beneficial microbiomes to alleviate harmful conditions that reduce algal growth

Publications

- Swink C, Samo TJ, Rolison K, Yilmaz S, Christey P, Mayali X (in preparation) Influence of heterotrophic bacteria on microalgal productivity under light and temperature stress
- Mayali X (in press) Microscale Carbon Cycling Between Bacteria and Algae under the sun; in Microbes: the foundation stone of the biosphere, Advances in Environmental Microbiology, C. Hurst, ed., Springer Nature