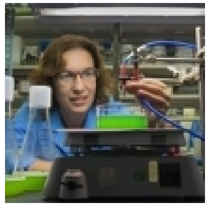
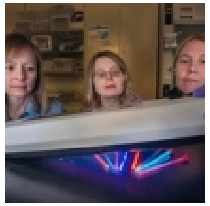
SAND2021-12153C

Sandia National Laboratories

Tracking mixotrophic carbon utilization from plant substrate to algal biomass and high-value metabolites






October 6, 2021

Chuck Smallwood

Sandia National Laboratories

Sandia LEAF Team: Morgan Mackenzie, Jenna Schambach, Wittney Mays, Bryce Ricken and Amanda Barry (PI)

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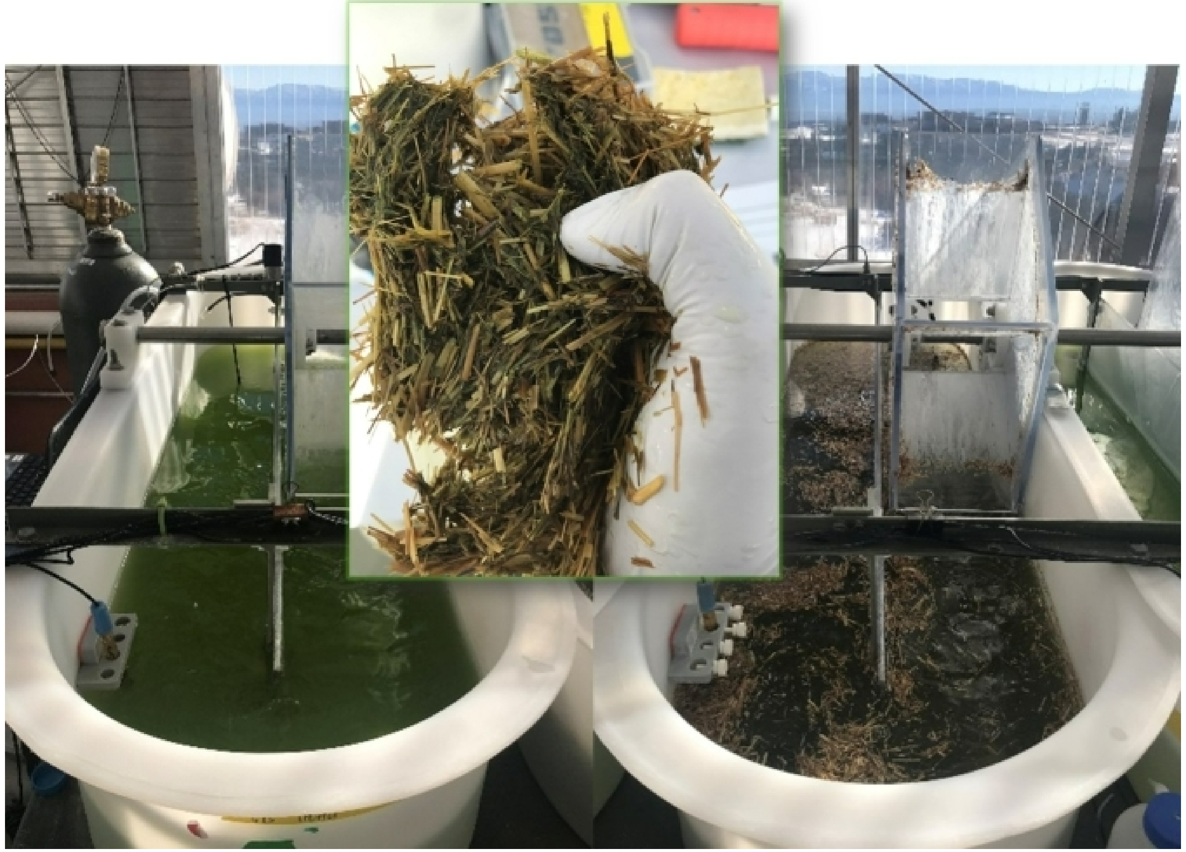
LEAF: Leveraging Algal Traits for Fuel

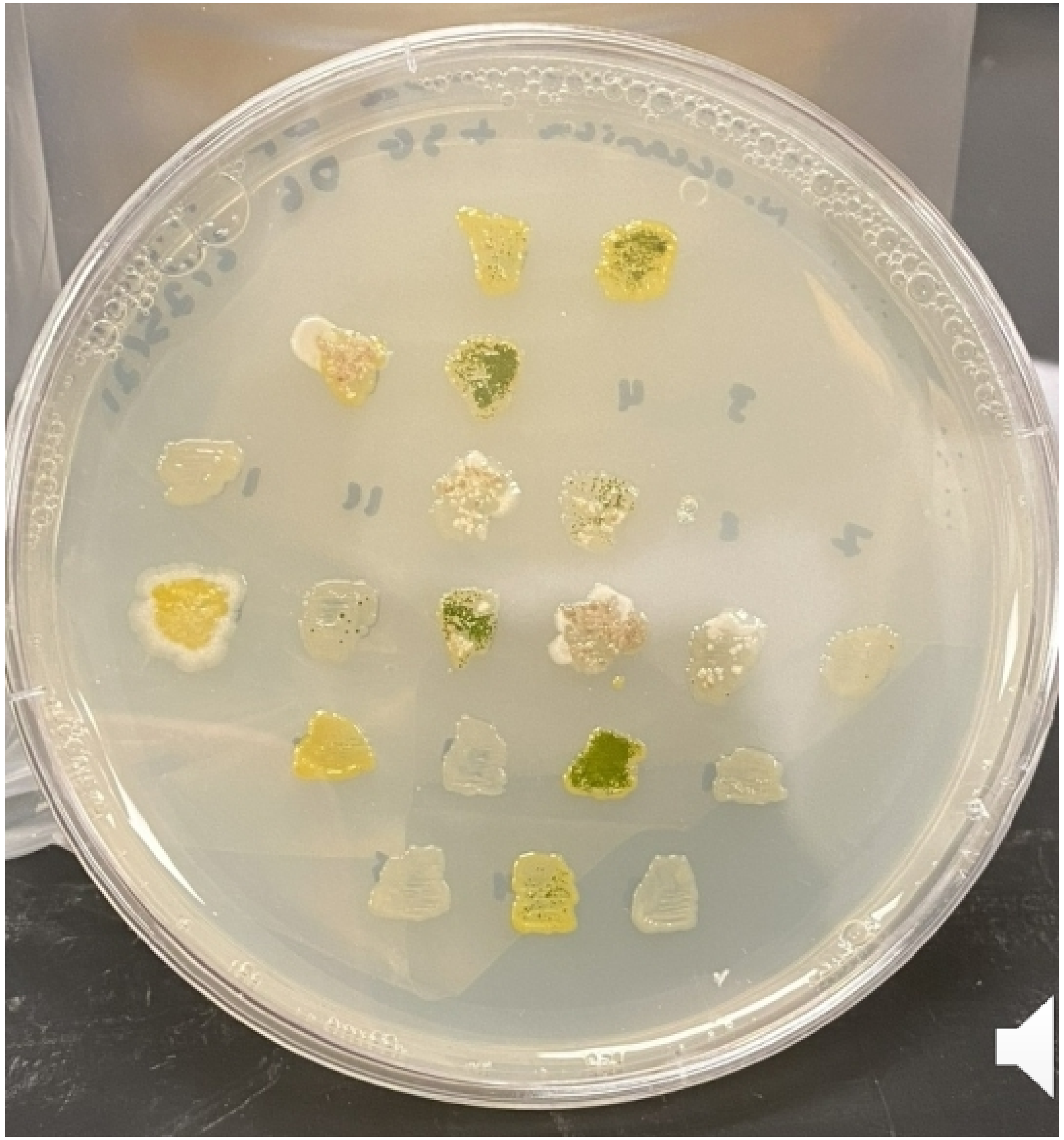


Schambach, J., Finck, A., Kitin, P., Hunt, C., Hanschen, E., Starkenburg, S., Vogler, B.W., and Barry, A.N. 2020. Growth, total lipid, and omega-3 fatty acid production by *Nannochloropsis sp.* cultivated with raw plant substrate. *Algal Research*

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

BIOENERGY TECHNOLOGIES OFFICE

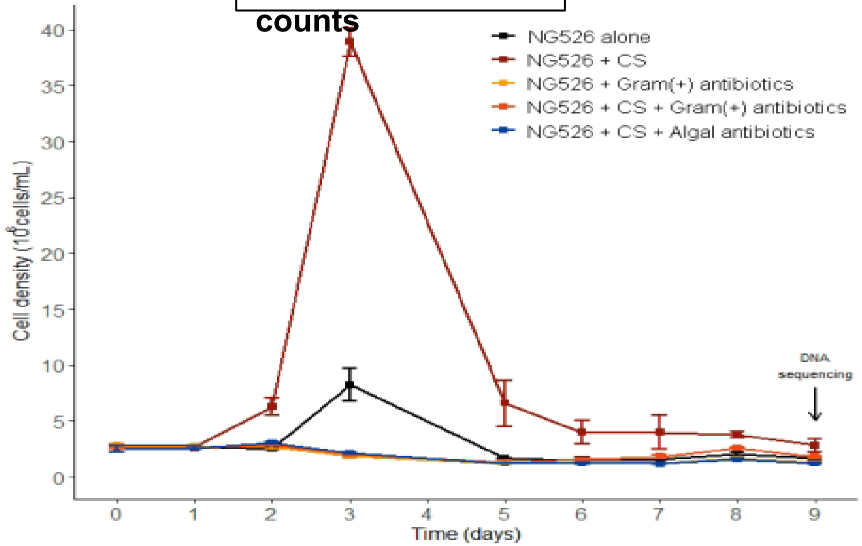
Are bacteria flora friend or foe or frenemy?!



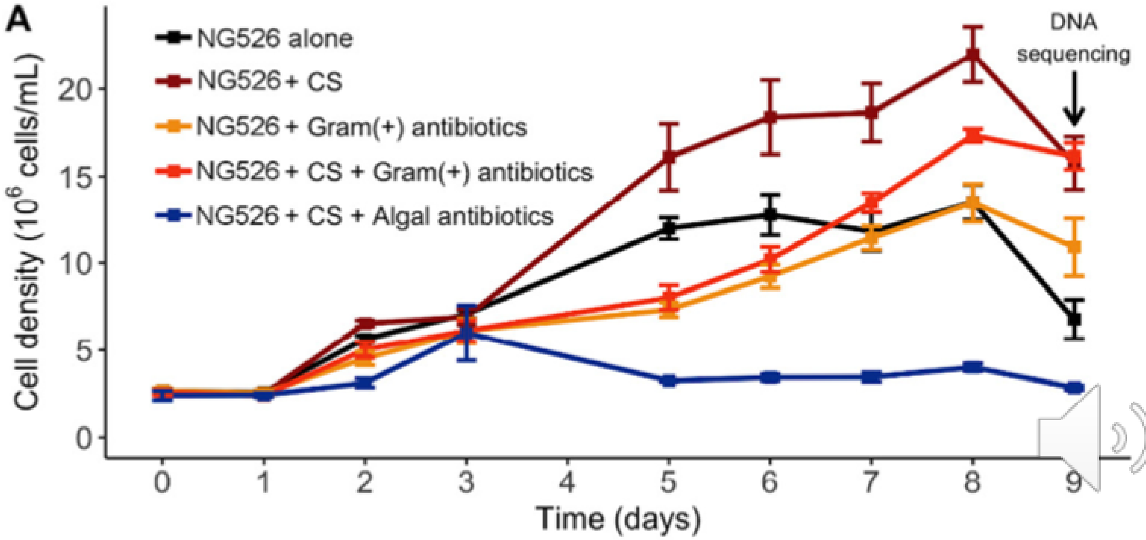
Tuning Algal Microbiome Members May Improve Biomass

- Algae–bacterial symbiosis exists that may contribute to observed growth patterns on switchgrass.
- Symbiosis between *N. gaditana* and bacterial “hitch-hiker” species improves growth under mixotrophic conditions.
- Interplay between algae and bacterial community at different points during growth offers potential to improve algal growth.

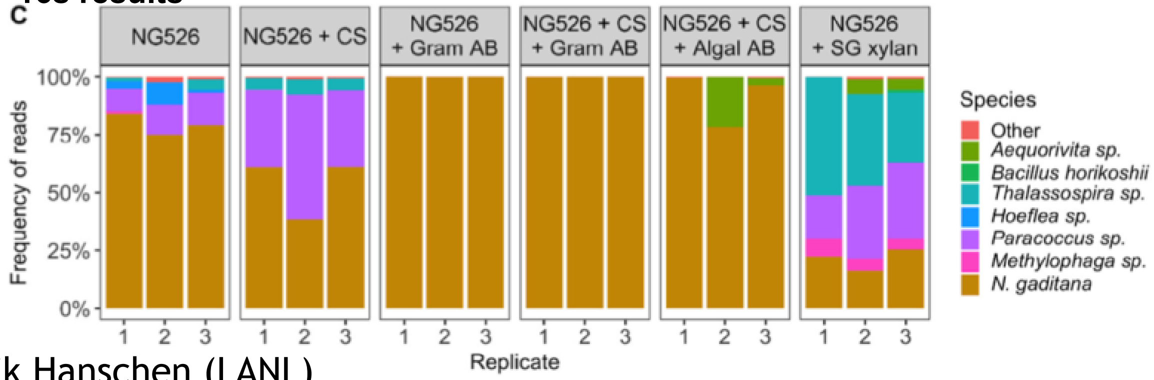
Bacterial cell counts



N. Gaditana **CCMP526 cell counts on biomass with and without antibiotics**



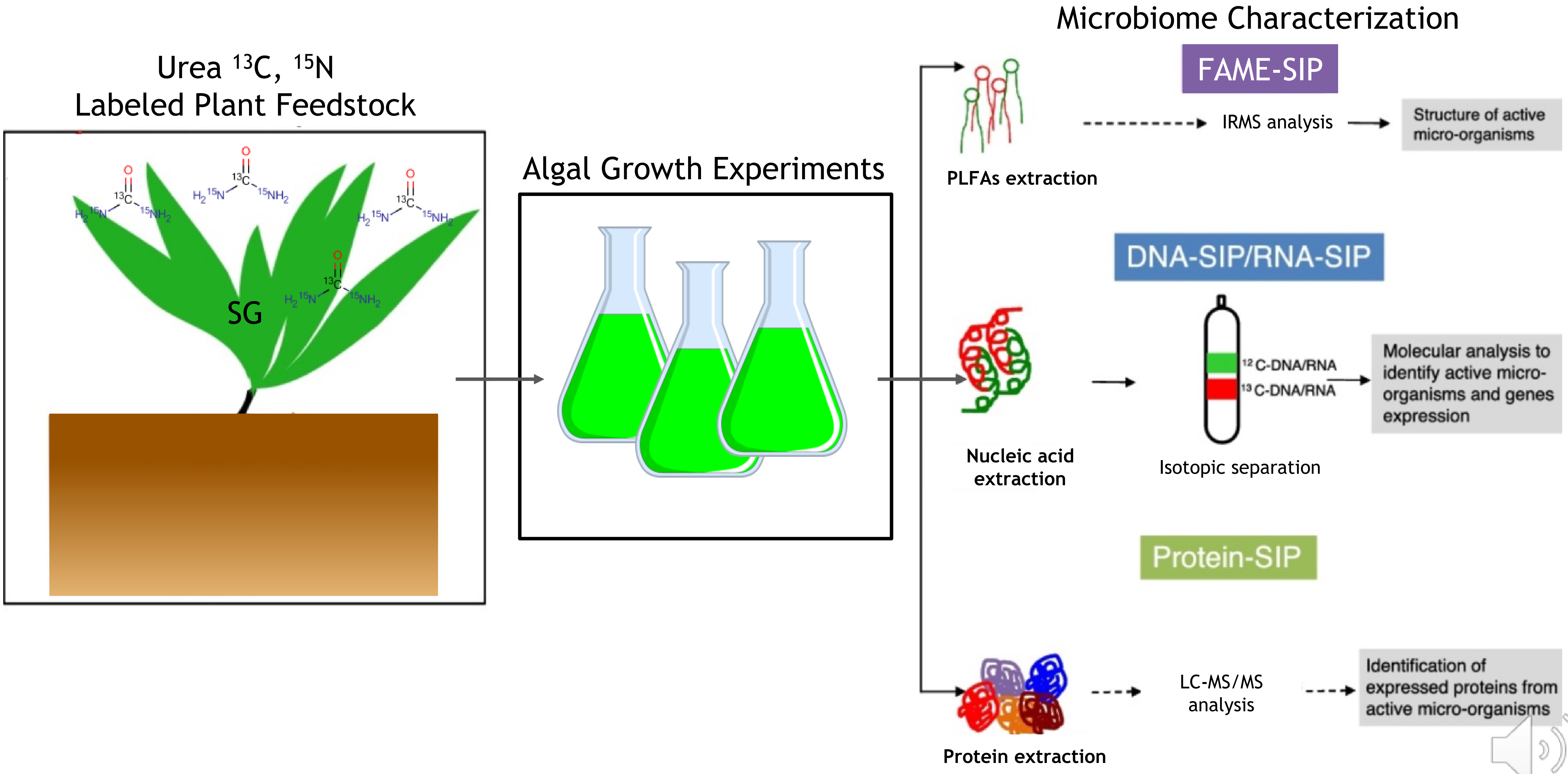
Final relative abundance of N. Gaditana chloroplast and bacterial 16s results



Erik Hanschen (LANL)

Schambach et al. Growth, total lipid, and omega-3 fatty acid production by Nannochloropsis T spp. cultivated with raw plant substrate . Algal Research 51, (2020) 102041

Metabolic Tracking With Isotopically-labeled Plant Substrate



Preparing Plant Material for IRMS Isotopic Analysis

Harvest Shoots

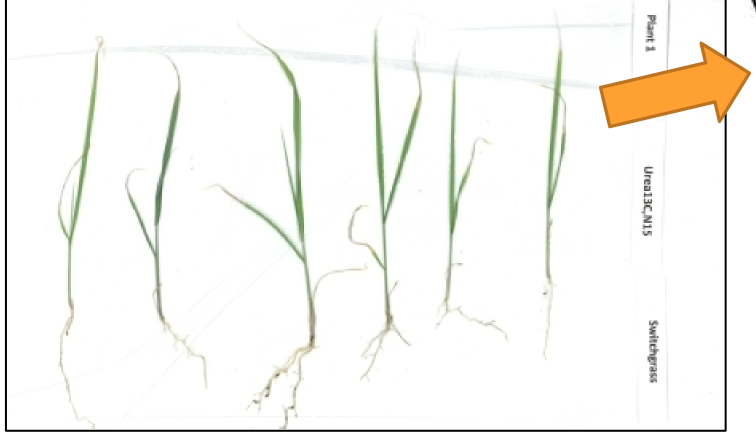
Drying Biomass at 65°C

Cultivation Experiments

- PLFAs extraction
- Nucleic acid extraction
- Protein extraction

Bulk Biomass

SIP Incorporation into Root/Shoot



Sample Prep for IRMS

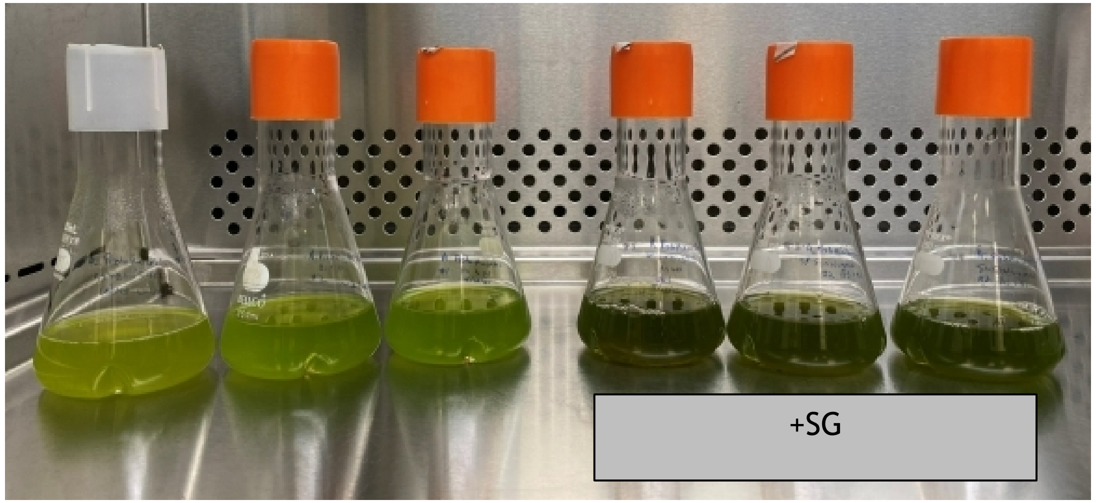
Bulk Isotopic Analysis

Low 13-carbon and high 15-nitrogen plant tissue enrichment

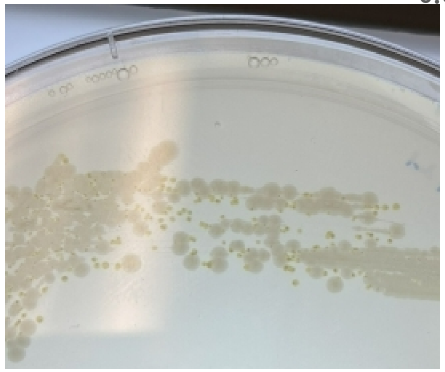
Flask Scale Growth with ^{13}C,^{15}N Labeled Switchgrass

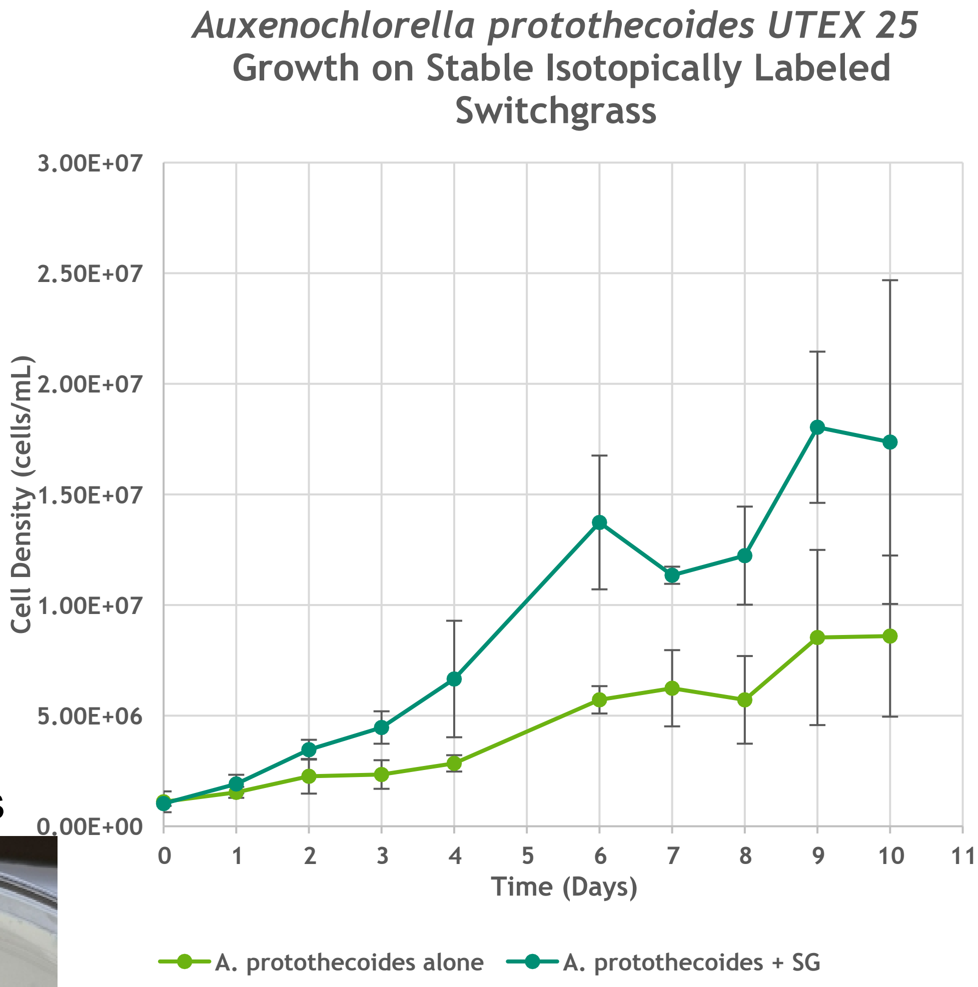
- Grown in temperature controlled incubator
- 12:12 Diurnal 217µE warm white light
- Shaking 150rpm in baffled flasks
- Collect samples (400µL samples) on Day 9 and 15
  - Freeze samples with 10% Glycerol in -80 for future DNA/RNA extractions
- Bacterial isolates collected each day and streaked on Difco Marine 2216 media plates for isolation and preservation.

Day 10:
A. protothecoides

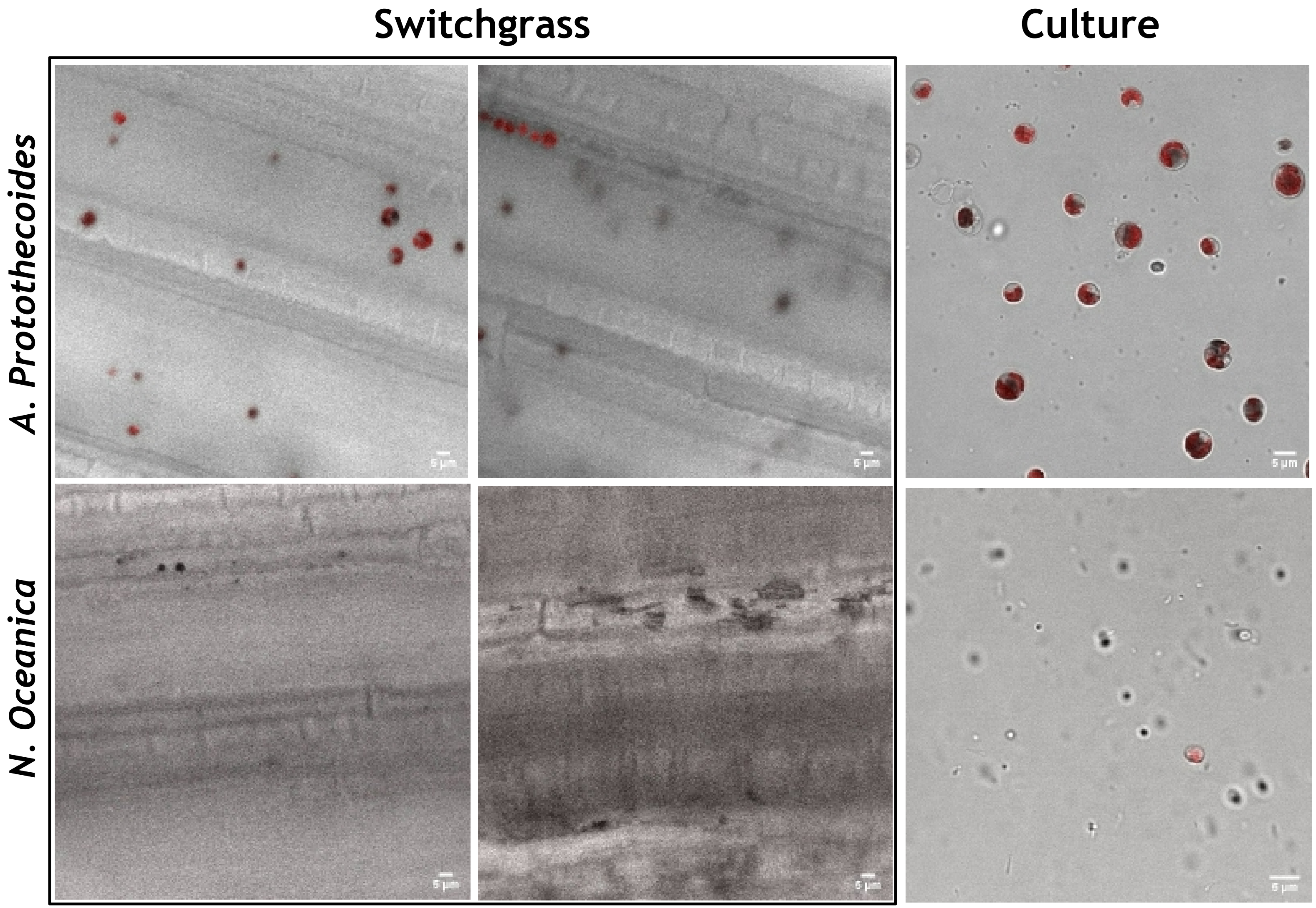


Bacterial isolates



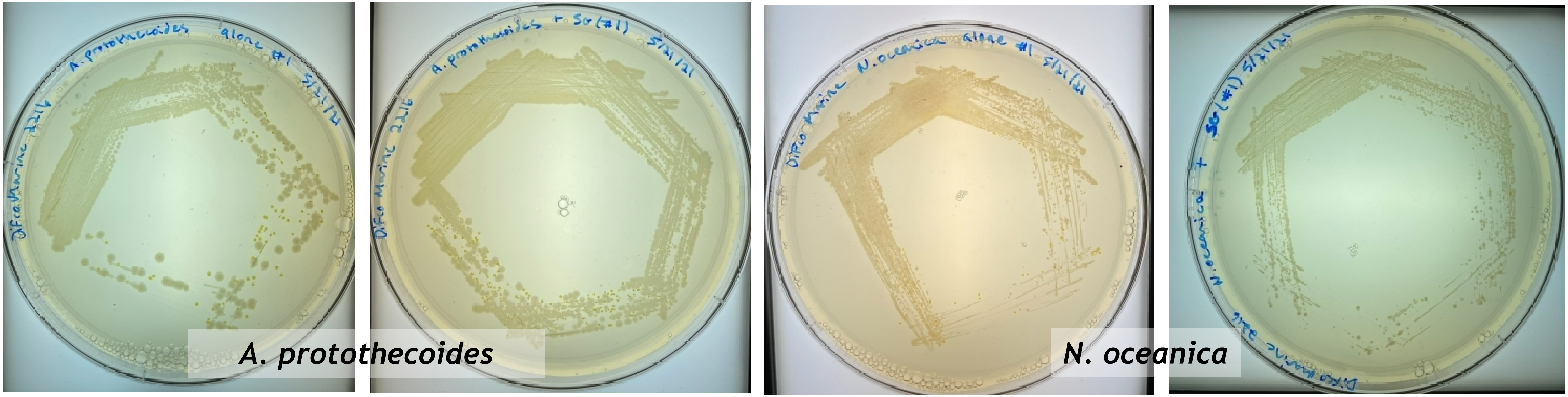


Confocal Microscopy of Algae attachment on Switchgrass



- Algae+SG cultures were screened by microscopy at the conclusion of each experiment
- *A. protothecoides* was adhered along the entire length of switchgrass shoots
- Little to no *N. oceanica* was observed on switchgrass tissue
- Notable difference in apparent bacteria between *A. protothecoides* and *N. oceanica*

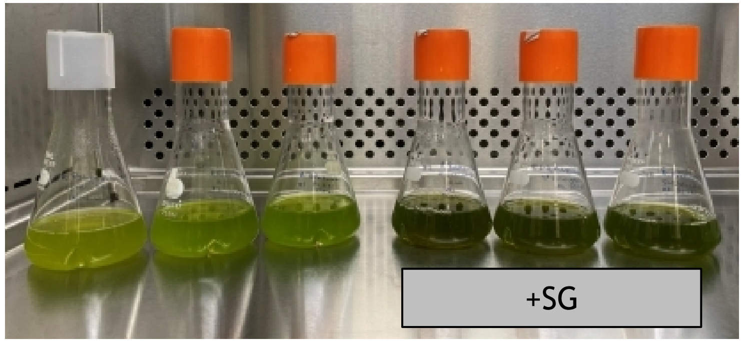
Algae Only Grow On DM Plates With Bacteria



- Bacterial isolates cultured on Difco Marine 2216 (DM)
- Clear difference of bacterial concentration between algae
- Algae only grow on DM in presence of microbes

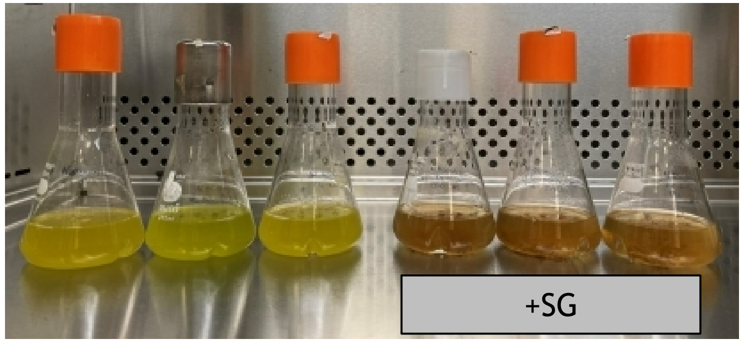
Day 10

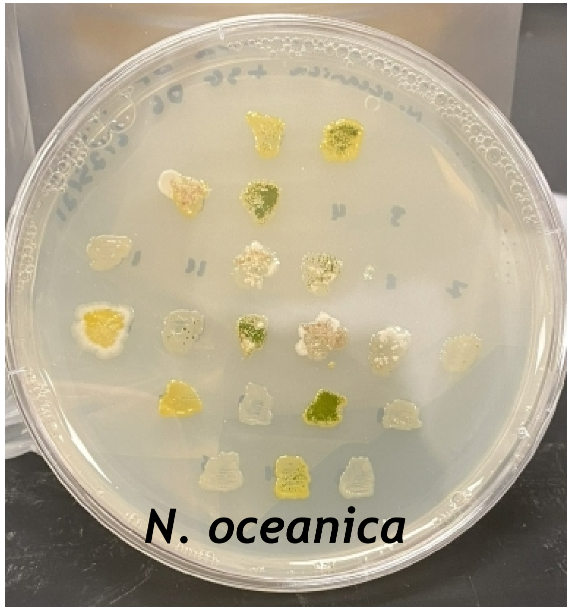
A. protothecoides

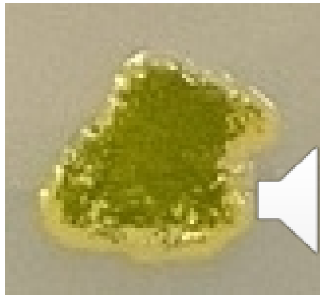


Day 10

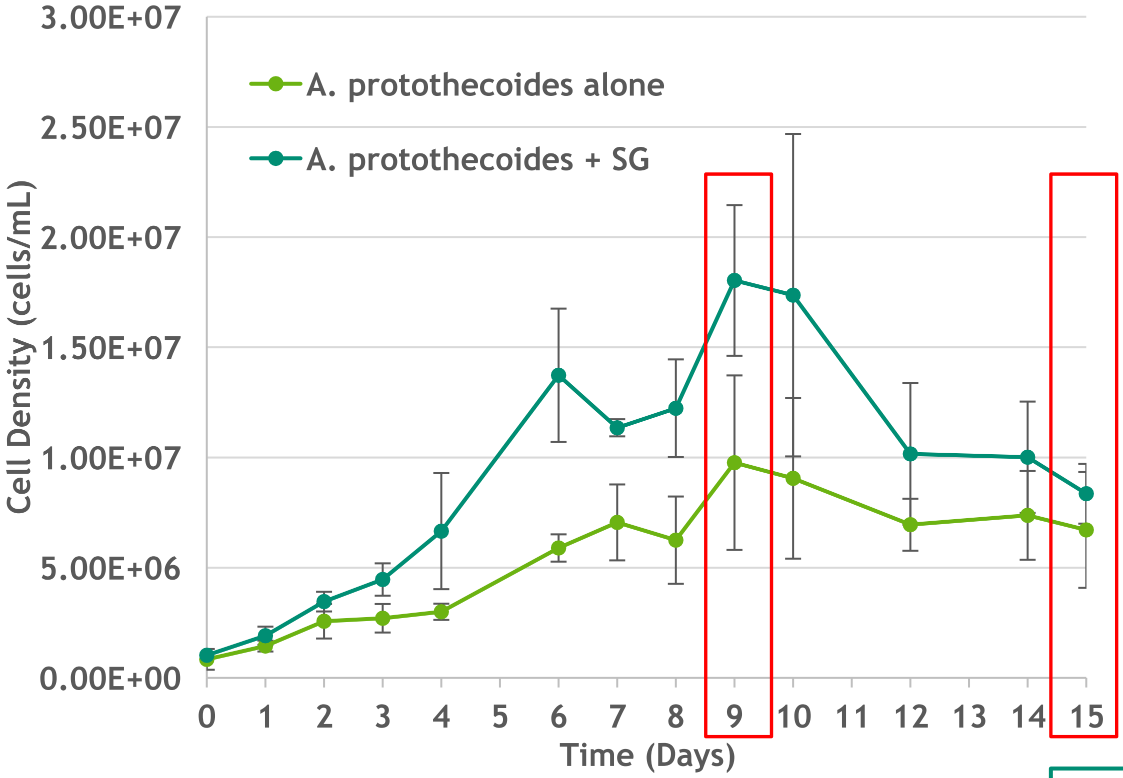
N. oceanica



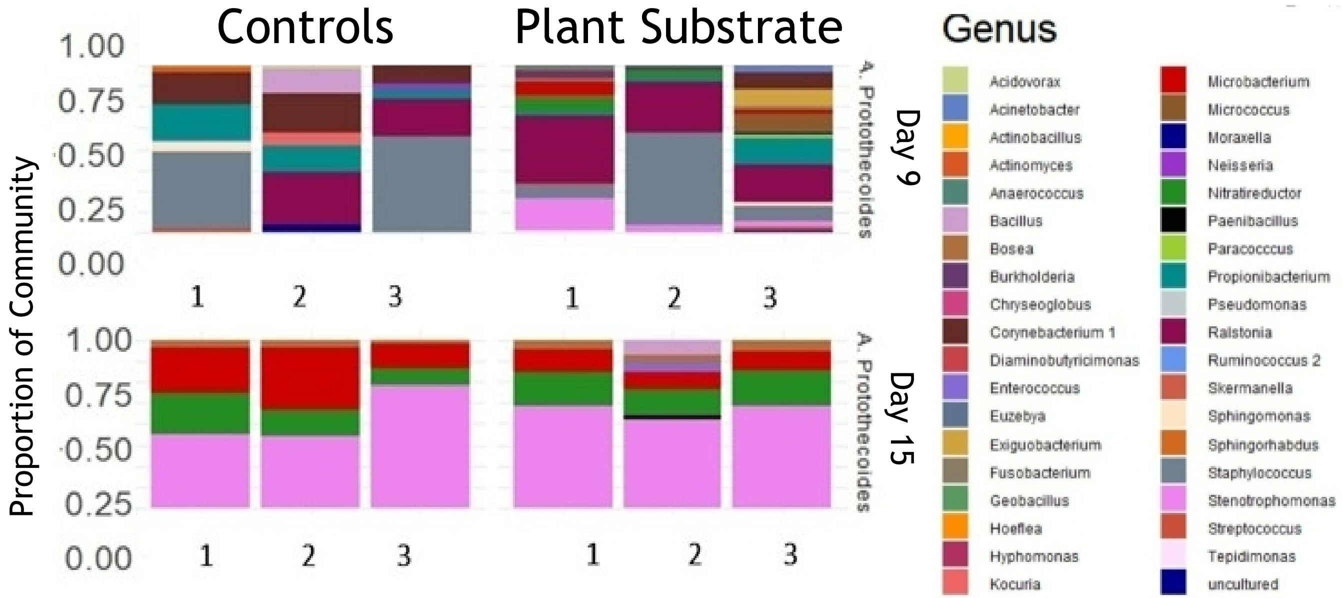


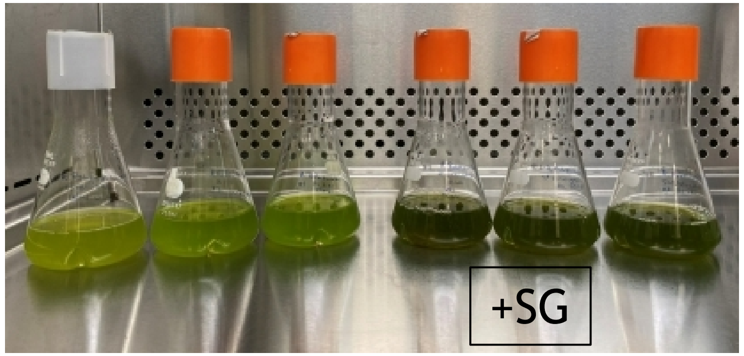


A.protothecoides 16s Diversity Increases During Plant Substrate Utilization



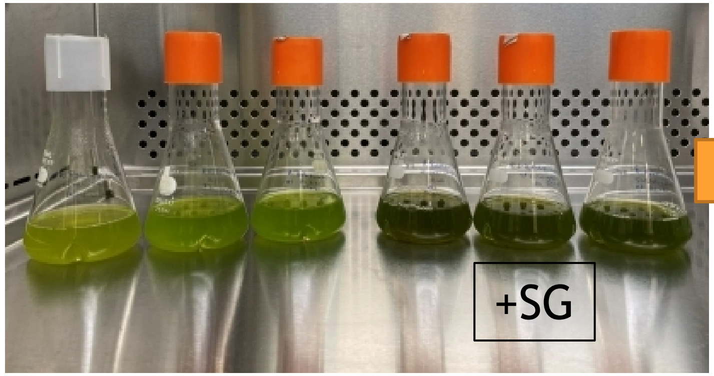
Taxonomic composition of bacterial communities based on 16S rRNA gene analysis of non-fractionated DNA



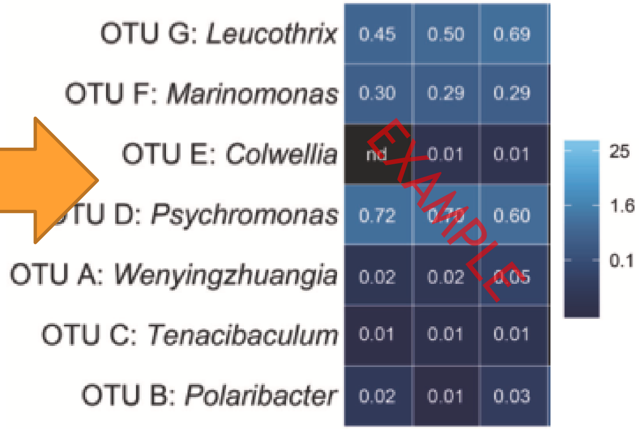


- Overall bacterial diversity was greater for plant substrate samples for both time points.
- Genus diversity is greater for both the control and plant substrate day 9 cultures with prominent species from genus of *Staphylcoccus*, *Corynebacterium*, *Ralstonia* and *Propionibacterium*.
- Day 15 cultures are much more homogenous with bacteria from genus *Stenotrophomonas*, *Nitratireductor*, and *Microbacterium* dominating cultures.

Tracking Plant 13-Carbon & 15-Nitrogen in Algal Microbiomes



^{13}C-,^{15}N-incorporating OTUs



SNL greenhouse facility with environmental controls for GMO algae cultivation.

Role of the microbiome:

- Analyze 16s rRNA sequencing analyses at multiple timepoints
- DNA-SIP fractionation for 16s sequencing to resolve metabolic distribution of ^{13}C, ^{15}N in OTUs
- Sequencing plate isolated bacteria from various time points
- Scale experiments to mini raceway ponds

Biochemical pathways involved in utilization:

- ^{13}C, ^{15}N tracking with 16s metagenomic metabolic analysis
- Transcriptomic sampling at multiple timepoints

LEAF Team Members:
SNL - Morgan Mackenzie, Wittney Mays, Jenna Schambach, Amanda Barry
LANL - Shawn Starkenburg, Erik Hanschen
USDA - Chris Hunt, Peter Kitin
CSU – Peter Chen
Heliae - Steven Pflucker

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