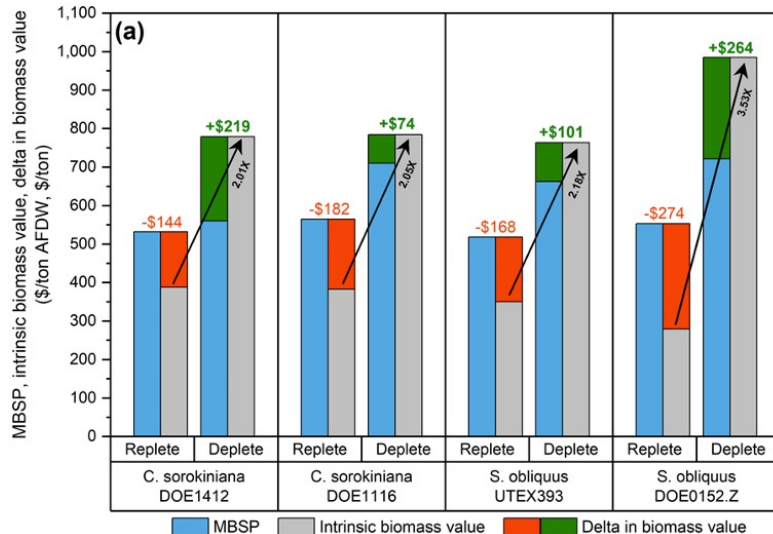DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review:

WBS 1.3.2.005
Metabolic Carbon Fluxomics during Compositional Shifts

April 3, 2023
Advanced Algal Systems
Lieve M. Laurens
National Renewable Energy Laboratory

Identify critical factors for economic development of the algae bioeconomy

- Build quantitative framework of biomass composition, productivity for important algae
- Integrate algae compositional dynamics to identify a co-product portfolio that increases intrinsic value of biomass above production cost

	Replete → Deplete	
Productivity (g/m²/day)	27.2	14.4
Ferm Carbs (%DW)	14.1	37.4
Lipids (as FAME) (%DW)	9.4	19.7
MBSP ($/ton)	**$558**	**$767**

Analysis
- Ash
- Carbohydrate
- FAME
- other
- Protein

High production cost penalty

Impact: Contribution custom-designed feedstock for BETO-SAF targets

Klein, B., Davis, R., Laurens, LM., 2023 (under review)

Aim: Increase the value and opportunity for algal biomass
Today: Challenging to target pathways towards consistent high biomass value and composition manipulation
Importance: Biomass high in carbohydrate and lipids can increase low-carbon feedstock to fill SAF supply gap
Risk: Species- and environmentally-specific biochemical response leads to unpredictable value projections

Project Overview (FY22 New Start)

Project Objectives: Identify metabolic predictors of composition shift in outdoor production relevant algal strains to:

1. Identify nutrient conditions to enable rapid composition shift without significant loss in biomass productivity

2. Propose metabolic engineering targets/pathways implicated in composition shift

Challenge: Lack of comparable, high fidelity targeted and untargeted metabolomics data under variety of environmental and physiological conditions to determine "true" metabolic predictors for top algae species

Outcome: Mature metabolomics workflow and toolbox, with a dissemination strategy, in addition to readily implementable strategies for tailoring biomass composition for more carbon-efficient SAF production
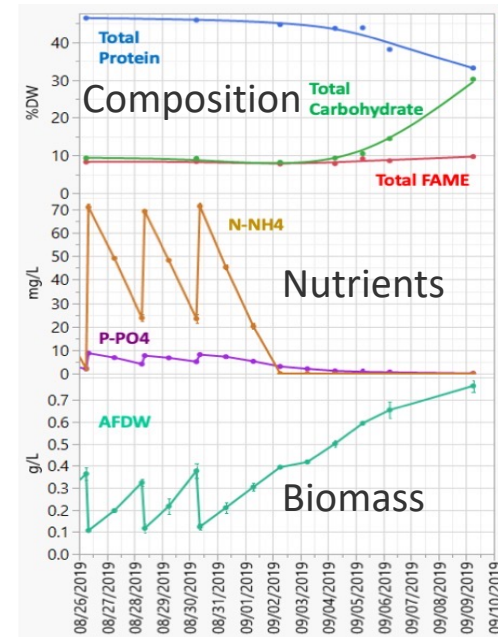

Outdoor productivity (22 - 32 g.m^{-2}.day^{-1} and composition for UTEX393 in relation to nutrient availability at replicate testbed ponds at AzCATI

| 26BAM	Ash	FAME	Protein	Carbs
Day 1	6.36 (n=1)	9.83±0.18	44.79±1.02	11.79 (n=1)
Day 4	3.87±0.41	14.68±1.63	21.38±1.56	38.48±1.47
Day 5	3.06±0.06	20.18±0.89	15.17±0.39	40.81±0.91
Day 6	2.80±0.05	24.10±0.69	13.26±0.09	39.89±0.85

Composition shift of Monoraphidium biomass under controlled conditions

Approach (Innovations)

- Build extensive metabolomics dataset that includes different strains under varying nutrient conditions resulting in different carbon storage composition‡ to unravel metabolic predictors to control fate of carbon allocation

  - ➤ Optimize and consolidate outdoor-relevant physiological environment mimic to collect metabolites

  - ➤ Design computational metabolite selection workflow amenable to machine learning

- **Contribution to BETO goals**: Selection of implementable physiological strategies to improve feedstock and **most compelling economics for biofuel/bioenergy precursors**

‡ https://www.nrel.gov/bioenergy/microalgae-analysis.html
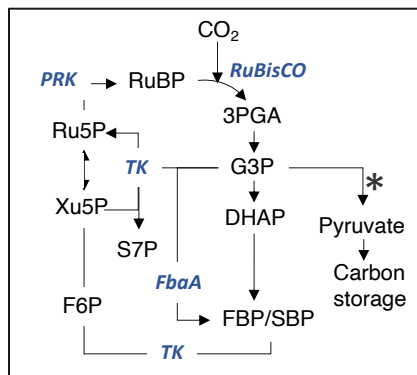
Simplified CBB cycle diagram showing control points* for carbon storage allocation
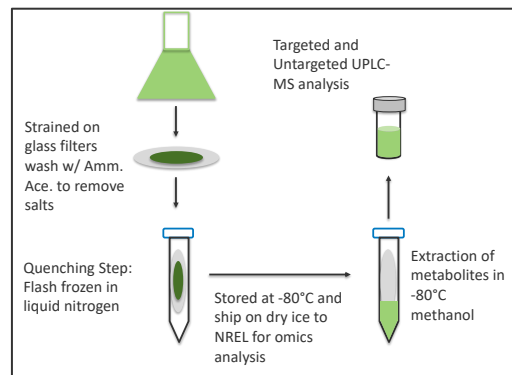
Typical metabolomics rapid quenching workflow for most representative central carbon metabolite profile
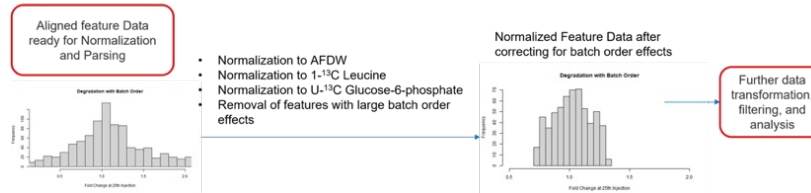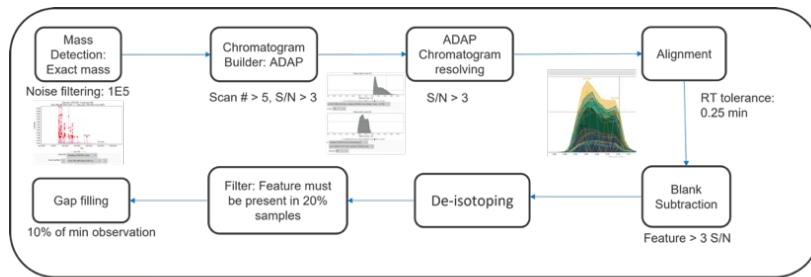
Optimization of computational m/z feature selection workflow and normalization strategy

Approach (Management)

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┌─────────────────────────────┐
│     PI: Lieve Laurens       │
└─────────────────────────────┘
              │
┌─────────────────────────────┐
│  Instrumentation and        │
│  Method Development         │
│  [Steven Rowland]           │
└─────────────────────────────┘
              │
┌─────────────────────────────┐
│  Metabolomics and           │
│  Experimental Fluxomics     │
│  [Arnav Deshpande]          │
└─────────────────────────────┘
              │
┌─────────────────────────────┐
│  Compositional Analysis     │
│  and Carbon Utilization     │
│  [Mauro Lua, Alicia Sowell] │
└─────────────────────────────┘
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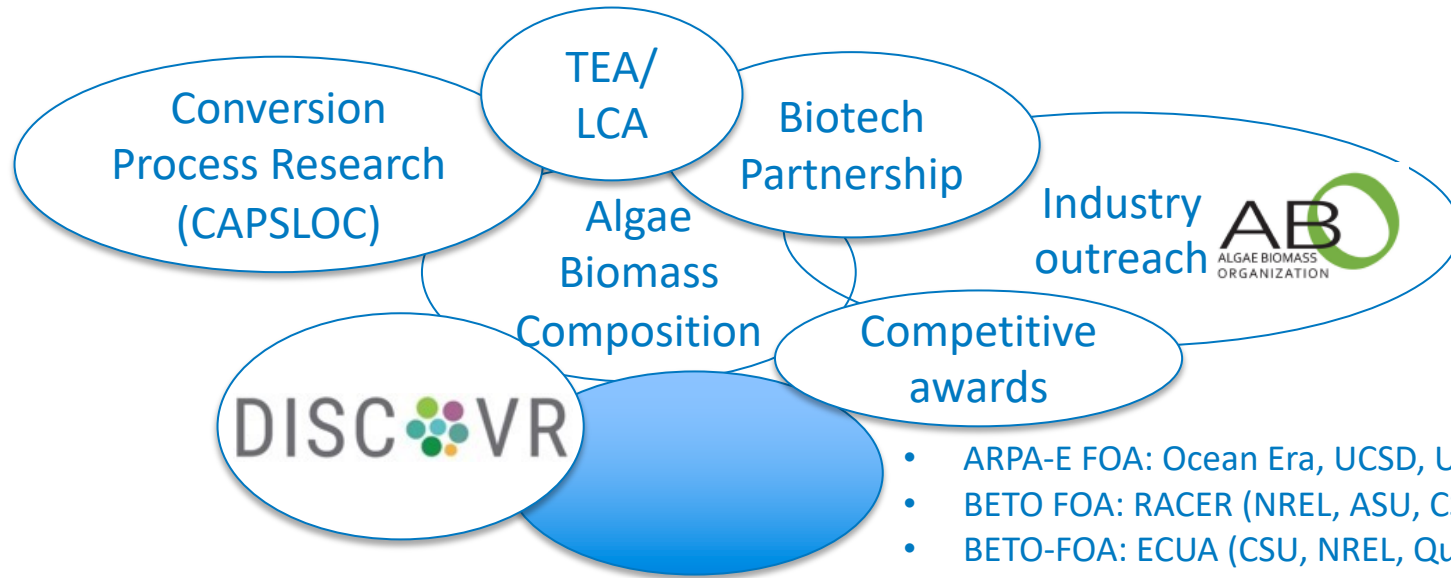
Complementary projects:
- **DISCOVR** (PNNL *et al.*)
- **MORE** (LANL)
- **Translational Genomics** (LANL)
- **CAPSLOC** (NREL)

Communications Plan:

- Monthly meetings with BETO, bi-weekly meetings with project team, 1-on-1 meetings with team members as needed

- Milestones structured to support quantitative and predictive correlations , which quantifies project progress via improvement in TEA and LCA outcomes

- Disseminate results via publications, presentations, patent applications, technical reports

- Leverage expertise of collaborating partners and projects to develop new concepts

Conversion Process Research (CAPSLOC)

TEA/ LCA

Biotech Partnership

Industry outreach

ALGAE BIOMASS ORGANIZATION

Algae Biomass Composition

Competitive awards

DISC VR

- ARPA-E FOA: Ocean Era, UCSD, UH, LBNL, NREL
- BETO FOA: RACER (NREL, ASU, CSU)
- BETO-FOA: ECUA (CSU, NREL, Qualitas)
- BETO-FOA: CIPA (GAI, NREL)
- BETO-FOA: ACCESS CARBON (Lumen, NREL)
- CRADA: ExxonMobil
- BETO-FOA: APEX (ASU, NREL, Carbon Collect)

Approach
(Diversity, Equity and Inclusion)

- Building a diverse, supportive, safe and welcoming science-focused project environment:
  - Build collaborative training with MSIs integrated in Algae Technology Education Consortium (ATEC)
  - National Consortium for Graduate Degrees for Minorities in Engineering and Science (GEM) candidate in FY23, with faculty continued collaboration

- Algae carbon farming can enable new workforce with jobs in underserved communities on waste resources and impact equity
  - Enable broader and more diverse community through sustainable jobs

- Freely-available reports democratize science access and enable broad community access
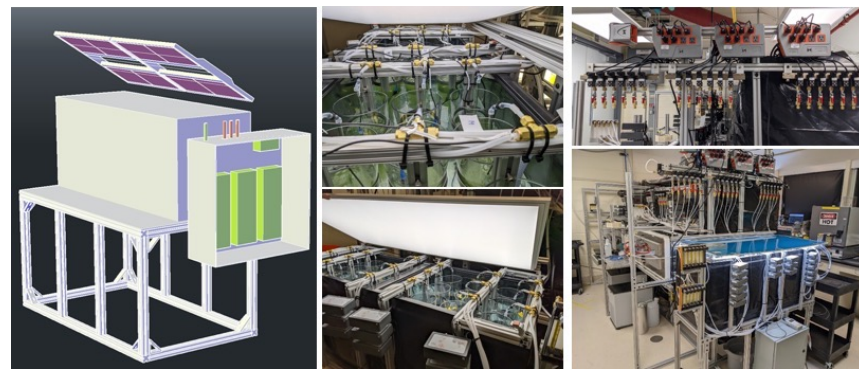

THE NATIONAL GEM CONSORTIUM
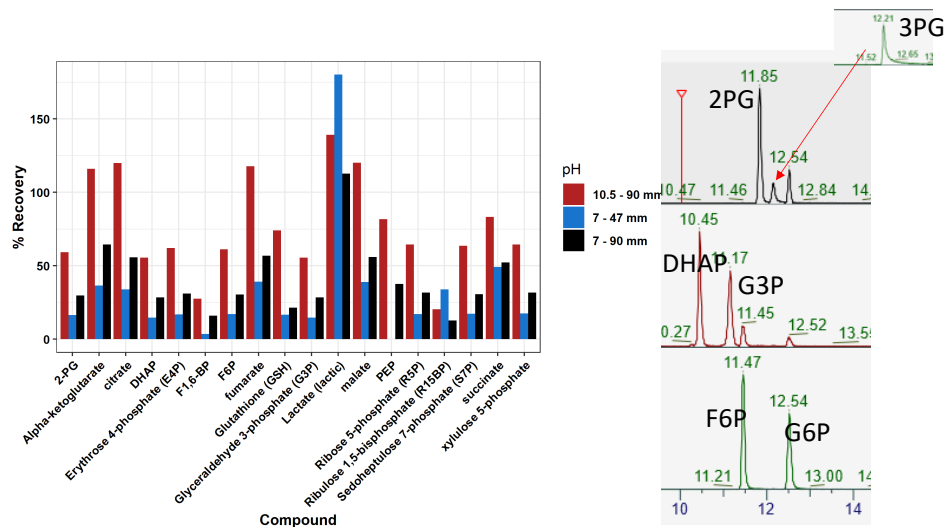https://www.gemfellowship.org/gem-fellowship-program/)

Approach (Risk Mitigation)

Risk	Mitigation Strategy
Feedstock composition and rates of changes are highly species-dependent	Cast a broad enough physiological environment experimental strategy to deconvolute the metabolite predictors of composition
Controlled photobioreactor cultivation does not capture all the outdoor pond biotic complexities	Aim to coordinate metabolomics with outdoor cultivation composition shift experimentation aligned to identify metabolite conservation
May not capture all metabolites that contribute to prediction of carbon allocation dynamics	Extensive analytical development to ensure proper recovery of difficult-to-analyze metabolites
Changing algae composition and species-specific compositional shift rates can challenge quantification of project progress	Dialogue with downstream projects (including TEA/LCA) produces traceable metrics around impact of growth and biomass composition on cost and carbon intensity metrics

Progress and Outcomes (Workflow Development)

New, fully controlled, 18-position photobioreactor allows for rapid and repeatable comparison of physiological conditions

- Design simulated physiological testing environment for high-throughput cultivation testing with CO_2 capture and storage:
  - ➢ New, 18-position environmentally-simulating bioreactor
  - ➢ Align with spring season productivity, leverage DISCOVR experimental validation
- Optimize extraction and HPLC MS workflow → Elucidate intermediates between inorganic carbon and organic carbon storage, metabolomics
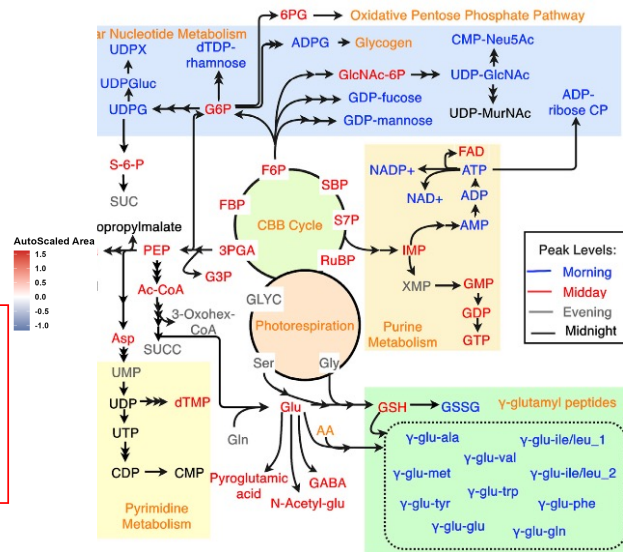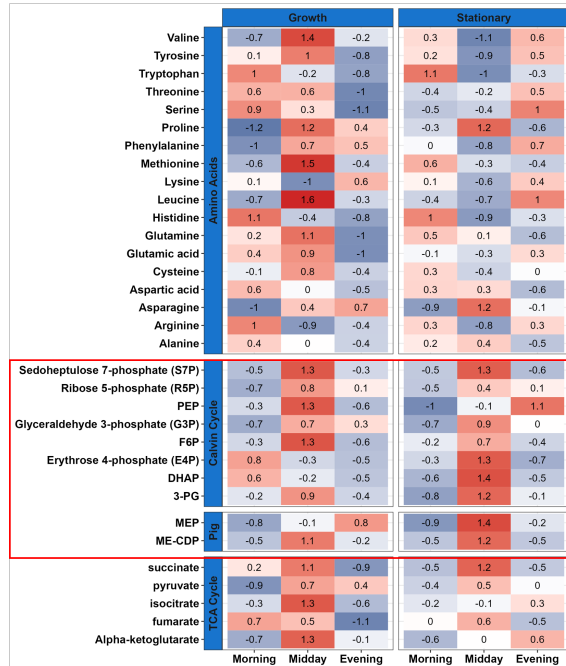

(l) optimized metabolite extraction protocol and (r) HPLC-MS baseline resolution of subset of 43 metabolites on Q-Exactive instrument
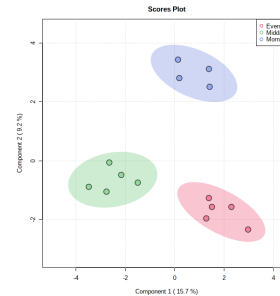
Progress and Outcomes (Dataset Development)

2 strains│28 different conditions (N, N:P, Diel vs Continuous, phase of growth, time of day) → Different composition shifts
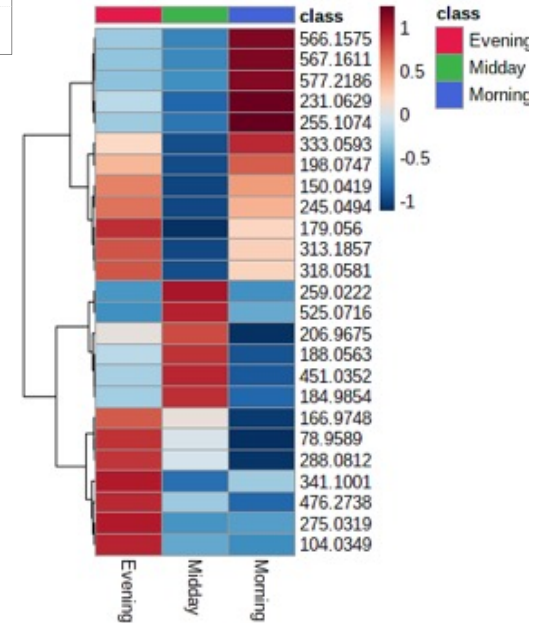
Untargeted Metabolomics for unique signature identification

	Growth			Stationary		
	Morning	Midday	Evening	Morning	Midday	Evening
Amino Acids						
Valine	-0.7	1.4	-0.2	0.3	-1.1	0.6
Tyrosine	0.1	1	-0.8	0.2	-0.9	0.5
Tryptophan	1	-0.2	-0.8	1.1	-1	-0.3
Threonine	0.6	0.6	-1	-0.4	-0.2	0.5
Serine	0.9	0.3	-1.1	-0.5	-0.4	1
Proline	-1.2	1.2	0.4	-0.3	1.2	-0.6
Phenylalanine	-1	0.7	0.5	0	-0.8	0.7
Methionine	-0.6	1.5	-0.4	0.6	-0.3	-0.4
Lysine	0.1	-1	0.6	0.1	-0.6	0.4
Leucine	-0.7	1.6	-0.3	-0.4	-0.7	1
Histidine	1.1	-0.4	-0.8	1	-0.9	-0.3
Glutamine	0.2	1.1	-1	0.5	0.1	-0.6
Glutamic acid	0.4	0.9	-1	-0.1	-0.3	0
Cysteine	-0.1	0.8	-0.4	0.3	-0.4	0
Aspartic acid	0.6	0	-0.5	0.3	0.3	-0.6
Asparagine	-1	0.4	0.7	-0.9	1.2	-0.1
Arginine	1	-0.9	-0.4	0.3	-0.8	0.3
Alanine	0.4	0	-0.4	0.2	0.4	-0.5
Calvin Cycle						
Sedoheptulose 7-phosphate (S7P)	-0.5	1.3	-0.3	-0.5	1.3	-0.6
Ribose 5-phosphate (R5P)	-0.7	0.8	0.1	-0.5	0.4	0.1
PEP	-0.3	1.3	-0.6	-1	-0.1	1.1
Glyceraldehyde 3-phosphate (G3P)	-0.7	0.7	0.3	-0.7	0.9	0
F6P	-0.3	1.3	-0.6	-0.2	0.7	-0.4
Erythrose 4-phosphate (E4P)	0.8	-0.3	-0.5	-0.3	1.3	-0.7
DHAP	0.6	-0.2	-0.5	-0.6	1.4	-0.5
3-PG	-0.2	0.9	-0.4	-0.8	1.2	-0.1
Plg						
MEP	-0.8	-0.1	0.8	-0.9	1.4	-0.2
ME-CDP	-0.5	1.1	-0.2	-0.5	1.2	-0.5
TCA Cycle						
succinate	0.2	1.1	-0.9	-0.5	1.2	-0.5
pyruvate	-0.9	0.7	0.4	-0.4	0.5	0
isocitrate	-0.3	1.3	-0.6	-0.2	-0.1	0.3
fumarate	0.7	0.5	-1.1	0	0.6	-0.5
Alpha-ketoglutarate	-0.7	1.3	-0.1	-0.6	0	0.6

AutoScaled Area: 1.5, 1.0, 0.5, 0.0, -0.5, -1.0

Heatmap of metabolite upregulation at 3 time points, illustrating accelerated CBB cycle at mid-day, with downstream protein synthesis in the evening

Peak Levels:
- Morning
- Midday
- Evening
- Midnight

Data are consistent with prior published work (Jaiswal, et al., 2021)

→ Central carbon metabolism is dynamic during the day, with longer time scales for composition shift

Untargeted metabolite profile clustered by sampling and compositional fingerprint

class: Evening, Midday, Morning

566.1575, 567.1611, 577.2186, 231.0629, 255.1074, 333.0593, 198.0747, 150.0419, 245.0494, 179.056, 313.1857, 318.0581, 259.0222, 525.0716, 206.9675, 188.0563, 451.0352, 184.9854, 166.9748, 78.9589, 288.0812, 341.1001, 476.2738, 275.0319, 104.0349

FY23 Go/No-Go Milestone (March 2023): "Demonstrate quantitative relationship between carbon metabolomics and storage sink carbon allocation in at least two contrasting algal species, projected over average biomass productivity"

➤ Excess N environmental conditions can be utilized to deconvolute strain dependent response from nutrient concentration-induced composition shift response

➤ Unsupervised learning on untargeted metabolomics data sets can lead to novel metabolic predictors

Low carbs
High carbs shift
No shift

25 ppm / 50 ppm — Day 2, Day 3, Day 4

PC2 (16.2%)
PC1 (48.9%)
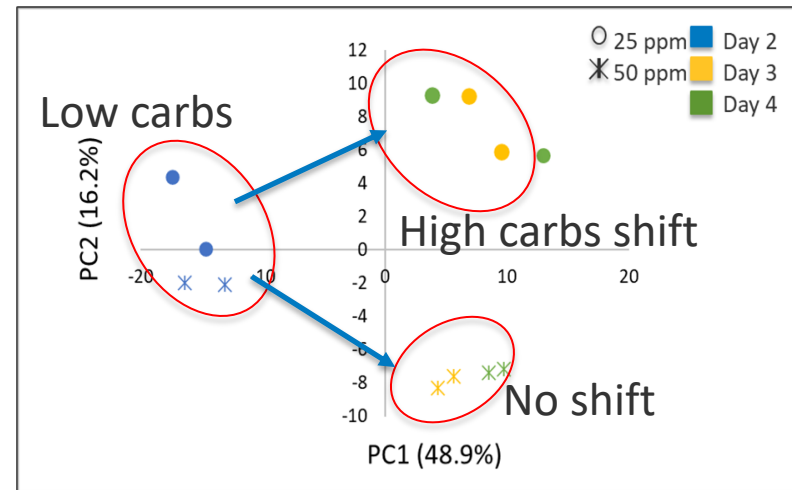
Excess Nitrogen Two Way ANOVA Analysis

Strain 93
Nitrogen 0
Interaction 0
97
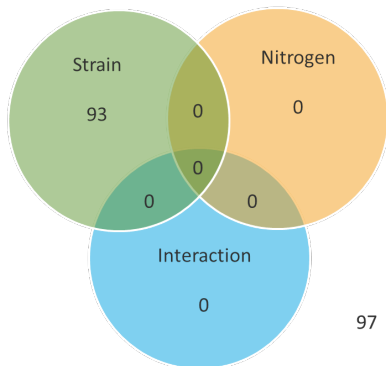
Two-way ANOVA under excess **N conditions – elucidates 93 strain-specific metabolites**

PCA Loadings

PC2
PC1

Top putative predictors
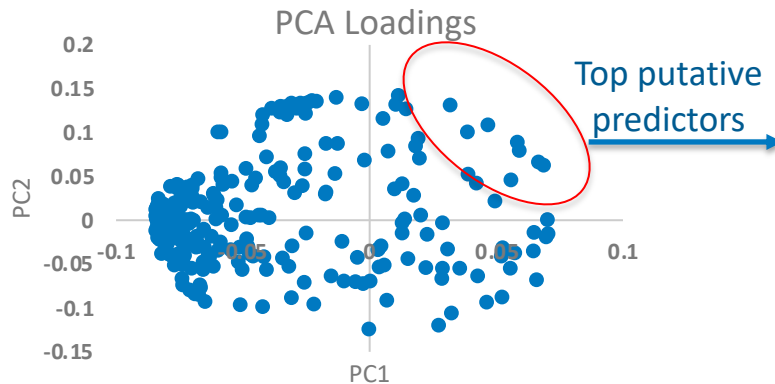
Triose sugar
Methionine
Tryptophan
Mevalonolactone
Dicarboxylic acid
Glutamate derivative
Sorbitol/Mannitol
Sucrose/Trehalose/Sugar

PCA and loading plot **of untargeted metabolomic** response under excess (50 ppm N) and limiting (25 ppm N) conditions
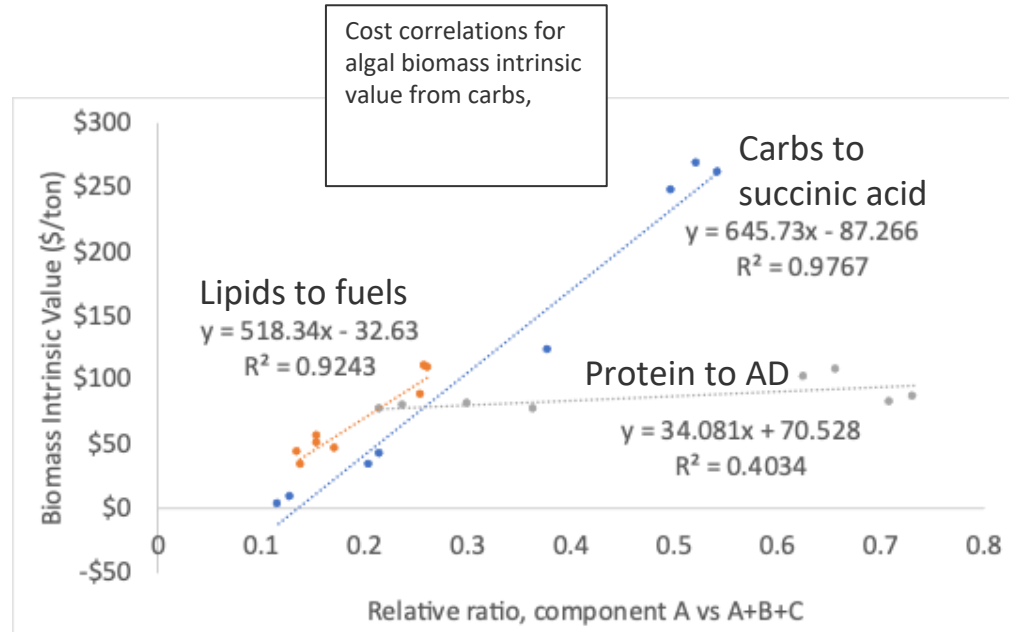
Impact (BETO Goals)

- Determination of carbon allocation from a mechanistic perspective provides foundation for <u>engineering of more productive and fast-shifting organisms</u>
- The tool-set built allows for predicting carbon allocation into SAF-relevant biomolecules (carbohydrates and lipids) and alleviate barriers in top species

- Outdoor deployment scale-up work is sufficiently de-risked to support future commercial SAF production targeting BETO's 2040 goals of 17MM gallons of commercial production and physiological conditions identified here can increase the SAF-precursor output from a farm
- Inclusion of a machine learning application towards improving the rapid carbon allocation shift will allow for an objective identification of targets for compositional optimization of algal biomass
- Identification of biomarkers may enable more rapid screening approaches that may be deployed at scale

Impact
(Biomass Quality and Value)

- Supports concept of "value" of algal biomass based rapidly achieving higher value compositional profile (lipids & carbohydrates) based on valorization in a conversion process

- Applied to DISCOVR strains run under environmental (nutrient) replete and deplete harvesting

- Correlations indicate <u>strong relation to high carbohydrate and lipid content in biomass as value drivers</u>

- Commercial adoption of physiological strategies for production-relevant species

Cost correlations for algal biomass intrinsic value from carbs,

Carbs to succinic acid
$y = 645.73x - 87.266$
$R^2 = 0.9767$

Lipids to fuels
$y = 518.34x - 32.63$
$R^2 = 0.9243$

Protein to AD
$y = 34.081x + 70.528$
$R^2 = 0.4034$

Biomass Intrinsic Value ($/ton)

Relative ratio, component A vs A+B+C

Summary

Management

- Progress tracked by quantitative metrics around metabolite relation to carbon storage rates
- Publish and disseminate findings as open access tools, datasets and algorithms

Approach

- Apply mature advanced analytical methodology workflow to designed physiological experiments in top algae species
- Propose metabolic engineering targets that are predictive of rapid compositional shifts
- Design and build computational metabolite selection approach to enable machine learning
- Disseminate metabolomics datasets for multiple species for strain-agnostic response tracking

Impact

- Alleviate barriers for primary carbon allocation into SAF-relevant biomolecules (carbohydrates and lipids)
- Optimize value of algae in response to cultivation and physiological stressor management, leading to improved nutrient uptake efficiency
- Commercialization of control of biomass composition

Progress & Outcomes

- Developed and implemented workflow for strain-agnostic metabolomics fingerprinting
- Built statistically-robust data set with at least 28 different physiological conditions with metabolites and compositional fingerprints correlated
- Unsupervised clustering already demonstrates unique relationships between metabolites/pathways and compositional profiles

Team:

Arnav Deshpande   Alicia Sowell
Steven Rowland   Mauro Lua
Stefanie Van Wychen   Seth Steichen
Bonnie Panczak

DOE BETO project managers:

Liz Burrows   Frank Fields

U.S. DEPARTMENT OF ENERGY

BioEnergy Technologies Office

Thank You

www.nrel.gov

www.nrel.gov/bioenergy/microalgae-analysis.html

Lieve.Laurens@nrel.gov ✉

NREL
Transforming **ENERGY**

Quad Chart Overview

Timeline
- October 1, 2021 (FY22)
- September 30, 2024 (FY24)

FY22 Budget	Total Award (FY22-FY24)
DOE Funding	

Collaborations/ Connecting projects	**LANL: Shawn Starkenburg, Taraka Dale (MORE)** **PNNL (DISCOVR): Scott Edmundson, Michael Huesemann**
DOE TM Lead	Liz Burrows

TRL at Project Start: 3
TRL at Project End: 4

Project Objective
Reduce the carbon intensity of algae-based sustainable aviation fuels, by identifying the biomass compositional metabolic markers that correlate with increased biochemical carbon storage (carbon sink strength) in algae for multiple, DISCOVR-relevant species, through utilization of quantitative metabolomics, coupled with machine learning

End of Project Goal:
Selection of implementable physiological strategies to shift biomass compositional quality and value in outdoor-relevant conditions towards more complete carbon storage in biomass, with a minimal impact on biomass productivity

Diversity Equity and Inclusion
Workforce development collaboration with ATEC

Funding Mechanism
Lab Call FY22 – New Start

Additional Slides

Responses to Previous Reviewers' Comments

- N/A

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Publications, Patents, Presentations, Awards, and Commercialization

- Planned presentations at national and international conferences (iABBB)

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