Los Alamos
NATIONAL LABORATORY

DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

Medium Optimization with Recycled Elements (MORE) for Better Biomass

April 3, 2023
Advanced Algal Systems

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LANL

Project Overview

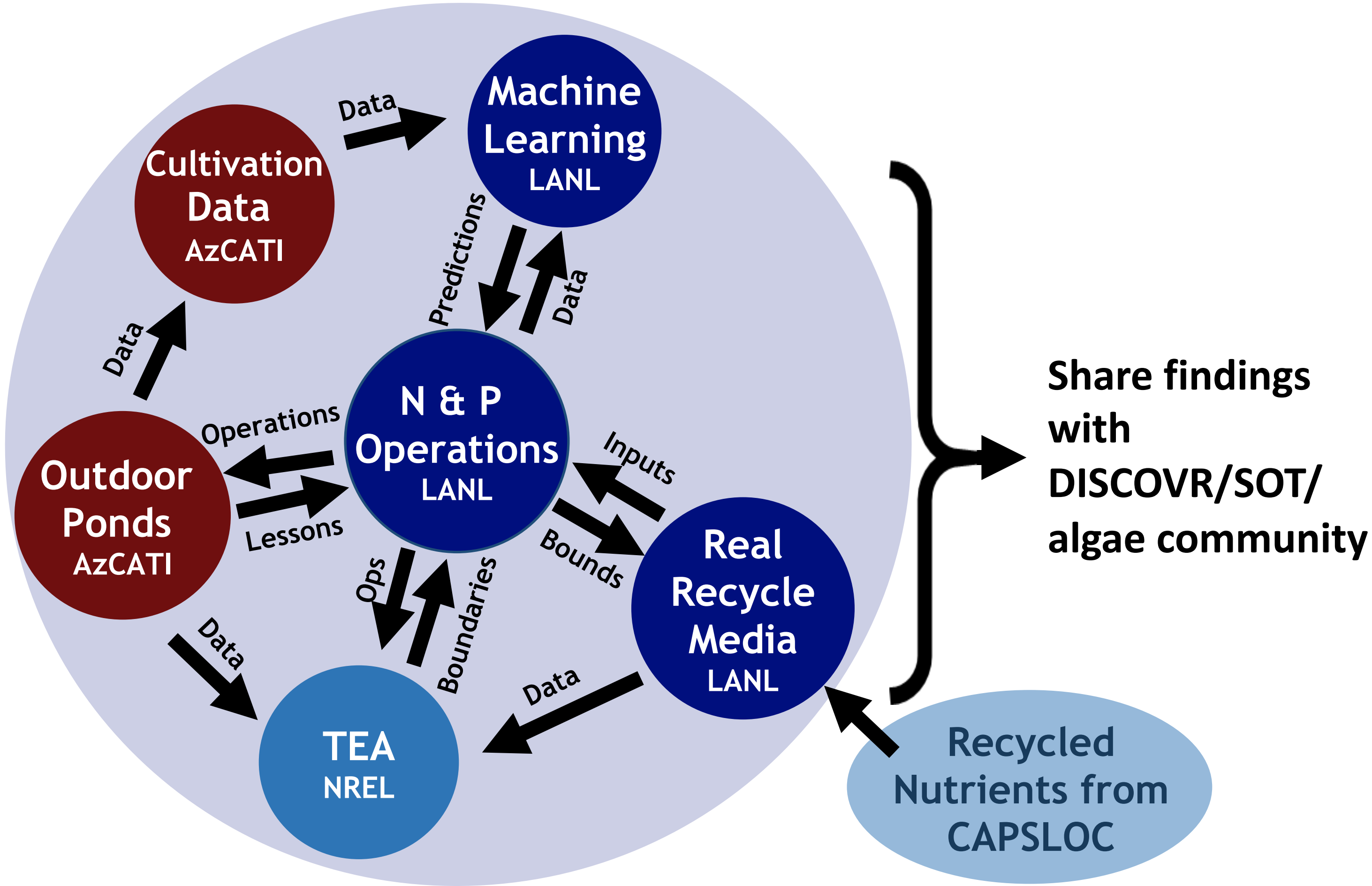
- **Challenge:** The cost of biomass is an impediment to the feasibility of large-scale implementation of microalgae as a petroleum replacement.
- **Project goals:** Create an updated approach to nutrient management. Use a combination of machine learning based on real outdoor data, indoor and outdoor growth experiments, application of recycled nutrients derived from extracted biomass, and technoeconomic analysis to understand the impacts of different nutrient inputs.
- **Importance:** Implementation of machine learning combined with technoeconomic analysis and experimentation is a new iterative approach to cultivation improvement.
- **Impact:** Support BETO's decarbonization goals by reducing the cost of producing algae biomass for conversion to sustainable aviation fuels and renewable chemicals.

1 – Approach
Project tasks

- **Task 1: Use SOT datasets, machine learning, and TEA to generate and bound new testable nutrient management hypotheses.**
  Couple existing outdoor data (AzCATI) with machine learning tools (LANL) to interrogate Algae SOT datasets for trends in nutrient management that can be harnessed for cultivation improvements. Use TEA (NREL) to bound machine learning predictions and experimental conditions.
- **Task 2: Test and verify nutrient management strategies in the laboratory and outdoors.**
  Evaluate how N and P uptake in fast-growing algae strains affects strain productivity, in order to develop nutrient management strategies that aim to maximize productivity (LANL). Iterate with AzCATI to verify strategy outcomes (AzCATI, LANL). TEA to bound strategies (NREL).
- **Task 3: Test recycled nutrient streams from the Combined Algal Processing pathway**.
  Apply learnings from Task 2 to SOT media that uses actual recycled nutrient (N, P) streams (LANL, NREL).

1 – Approach

Interplay between sites and tasks



1 – Approach
Technical objectives

- AzCATI outdoor pond data feeds into Machine Learning (ML)
- Technoeconomic analysis (TEA) bounds ML
- ML generates predictions for nutrient operations to maximize productivity
- Experiments utilizing various nutrient inputs conducted in environmental photobioreactors (ePBRs)
- Selected nutrient inputs tested outdoors at AzCATI
- Experimental data fed back into:
  - ML algorithms to enhance and confirm predictions
  - TEA to analyze the value impact of operational changes
- Recycled nutrients tested indoors and data included in TEA
- Dissemination of findings to algae community

1 – Approach
Challenges and risks

Challenge	Approach
A large amount of outdoor growth data has been collected for multiple species over several years, processing of that data is intense	Develop a machine learning model to process the data and provide meaningful outputs of parameters affecting pond productivity.
Outdoor experiments are limited by infrastructure and operational expenses	Employ environmental photobioreactors (ePBRs) to simulate outdoor conditions and test larger numbers of variables, utilizing machine learning to guide parameters

Risk	Mitigation
TEA design does not account for inputs identified in this project	Update the TEA Aspen Plus model to accept additional conditions and variables
Machine learning models trained on past available data exhibit high uncertainties in certain regions or prediction ranges are limited	Continue integrating data generated throughout the project and systematically augment the training data with targeted experiments and further refine models before making predictions
Biomass productivity in the ePBRs is more robust to changes in nutrients than expected	Shift focus from solely increases in biomass productivity to incorporate reductions in MBSP as outcomes.

1 – Approach

Go/no-go – 3/30/2023

Name	Description	Criteria
New approaches for the management of N and P in outdoor algae cultures reduces biomass production costs.	A TEA operational baseline is generated for this project using traditional nutrient management strategies. Results of ML and ePBR simulation experiments are fed into the TEA model to realize reduction in MBSP.	Changes in nutrient management strategies, informed my machine learning and experimentation, result in a reduction in nutrient contribution to MBSP by at least 15%, as compared to project TEA operation baseline for a top performing BETO priority strain.

- Experimental and Machine Learning data have demonstrated that reducing nutrient inputs from standard SOT f/2 media by greater than half do not reduce productivity.
- In combination with TEA we have shown that reduced nutrient inputs can reduce MBSP by $26 with 100% nutrient recycling.

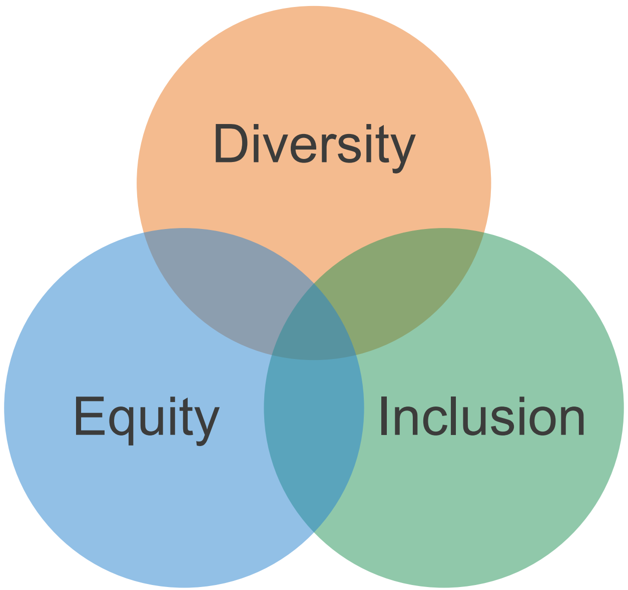
1 – Approach
Team core competencies

LANL	NREL	AzCATI
Claire Sanders – PI Project management Indoor cultivation	Bruno Klein Technoeconomic analysis	John McGowen Outdoor cultivation
Nilusha Sudasinghe Compositional analysis Indoor cultivation	Tao Dong CAPSLOC – nutrient recycling	
Erika Quezada Indoor cultivation Compositional analysis		
Shounak Banerjee Machine learning		

Management
- Monthly project meetings
- Regular communication between PI and each team member
- Monthly meetings with Program Manager

1 – Approach

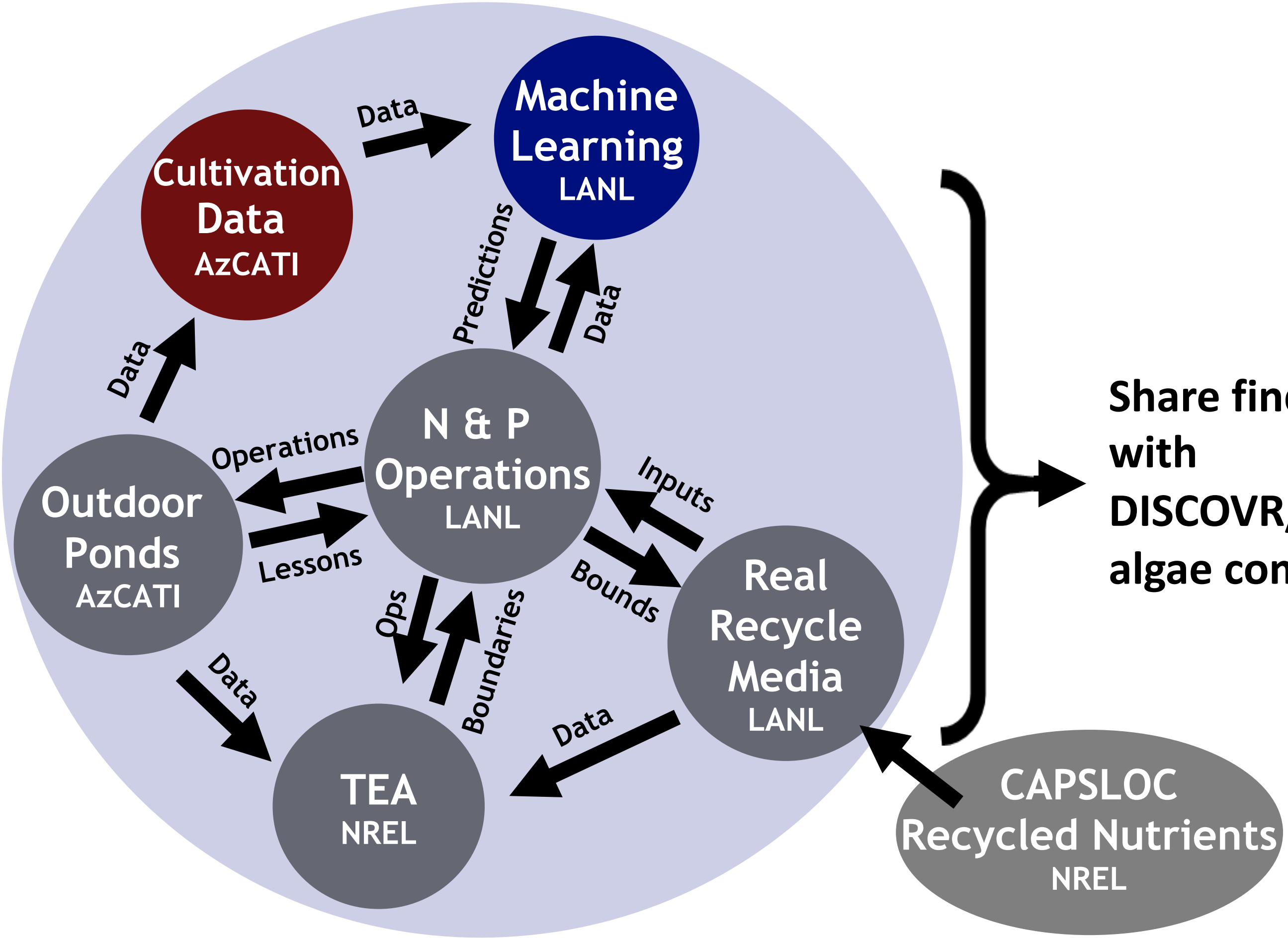
Diversity, equity, and inclusion



- Northern New Mexico College in Espanola, NM
  - Hispanic-serving institution
  - Located in an underserved rural community
  - Interacting with the Biology Department
    - Departmental seminars (LANL & NREL)
    - Laboratory tours (LANL)
- Arizona State University (AzCATI)
  - Hispanic-serving institution employing gender diverse and minority students and staff that contribute the data outdoor execution and data collection
- Project personnel are gender diverse and of varying backgrounds, including first generation and underrepresented groups.

2 – Progress and Outcomes

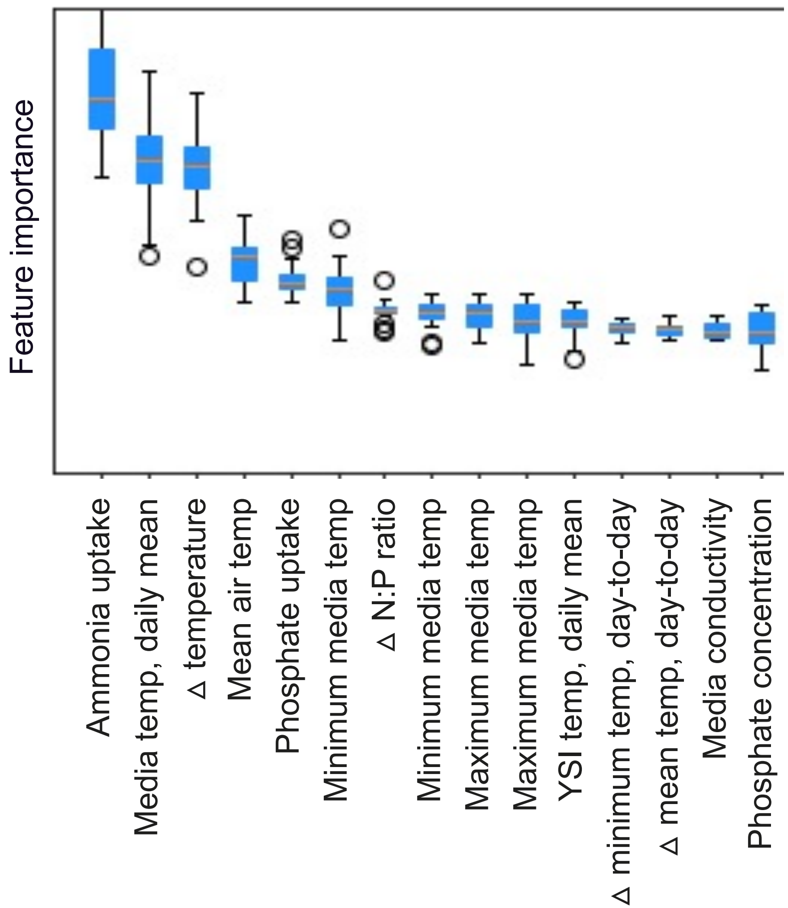
Task 1 – Machine learning using outdoor cultivation data



Share findings with DISCOVR/SOT/ algae community

2 – Progress and Outcomes

ML to connect specific parameters to outdoor productivity



Goal: Identify factors that are primary contributors to *P. celeri* productivity in outdoor ponds.

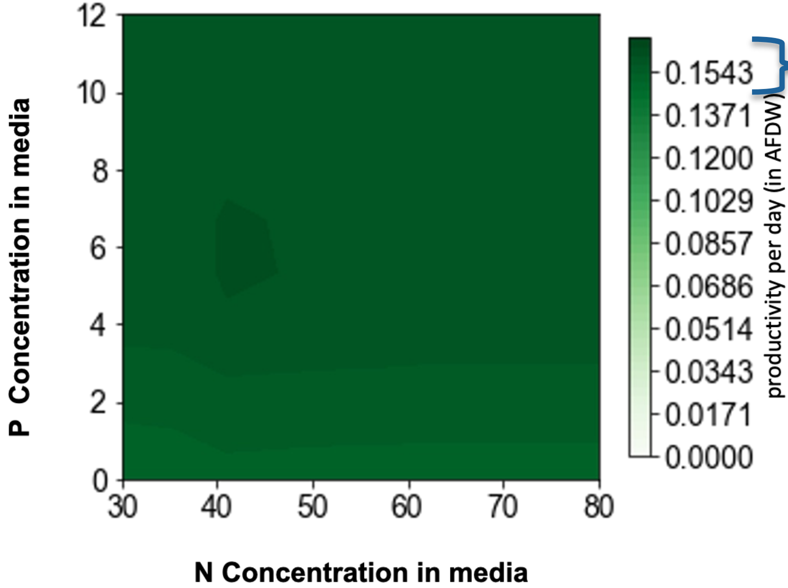
Method: Use multi-year pond data from 4.2 m^2 outdoor AzCATI ponds as inputs to a python based ML algorithm.

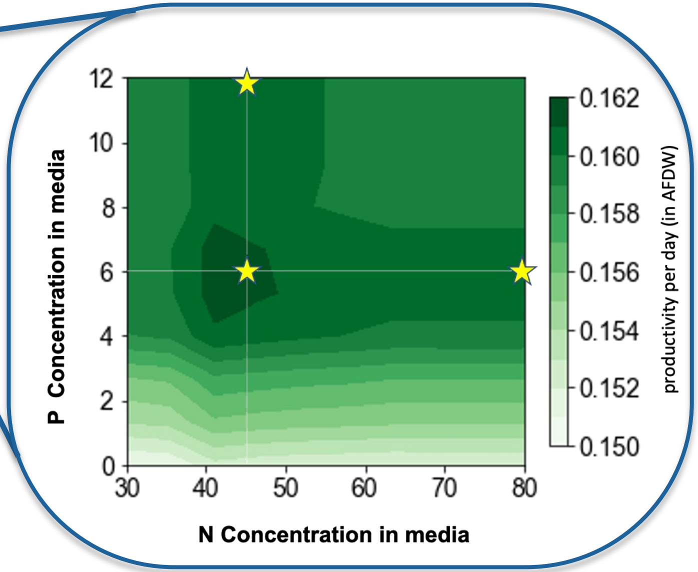
Outcome: Temperature and nutrient uptake from media are most predictive of pond productivity.

Impact: Identification of factors important to biomass productivity can direct operations towards increasing productivity.

2 – Progress and Outcomes

ML productivity predictions for P. celeri





Goal: Use ML to predict productivity maxima for *P. celeri* based on [N] and [P] in media.

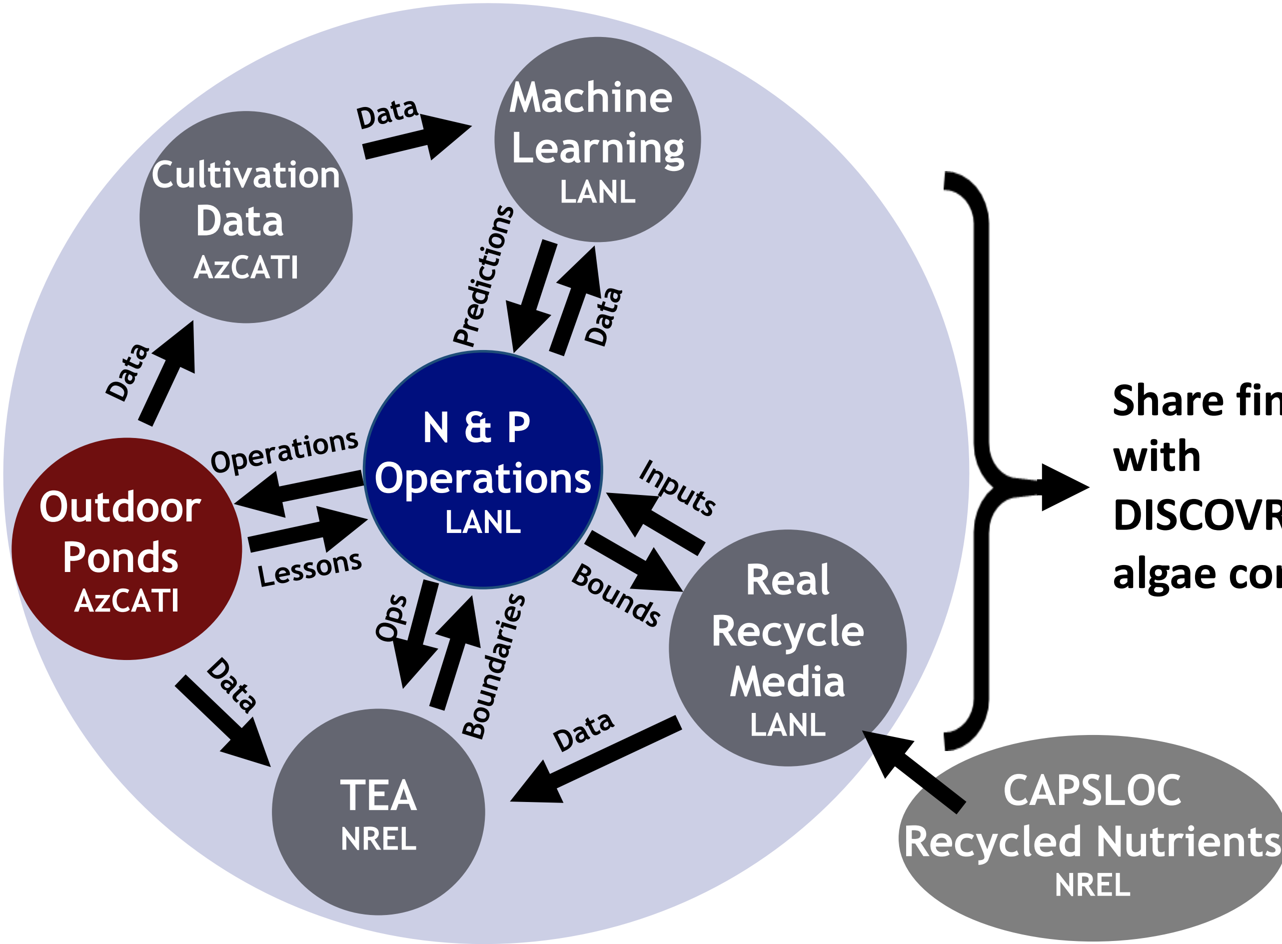
Method: All conditions except [N] and [P] are kept consistent and set to August 7, 2020.

Outcome: *P. celeri* shows robust productivity within the predictive ranges. Maximal growth at N & P concentrations of 45 ppm N / 6 ppm P (SOT f/2 70 ppm N / 9 ppm P).

Ongoing: Continue refining model and apply to *T. striata* pond data.

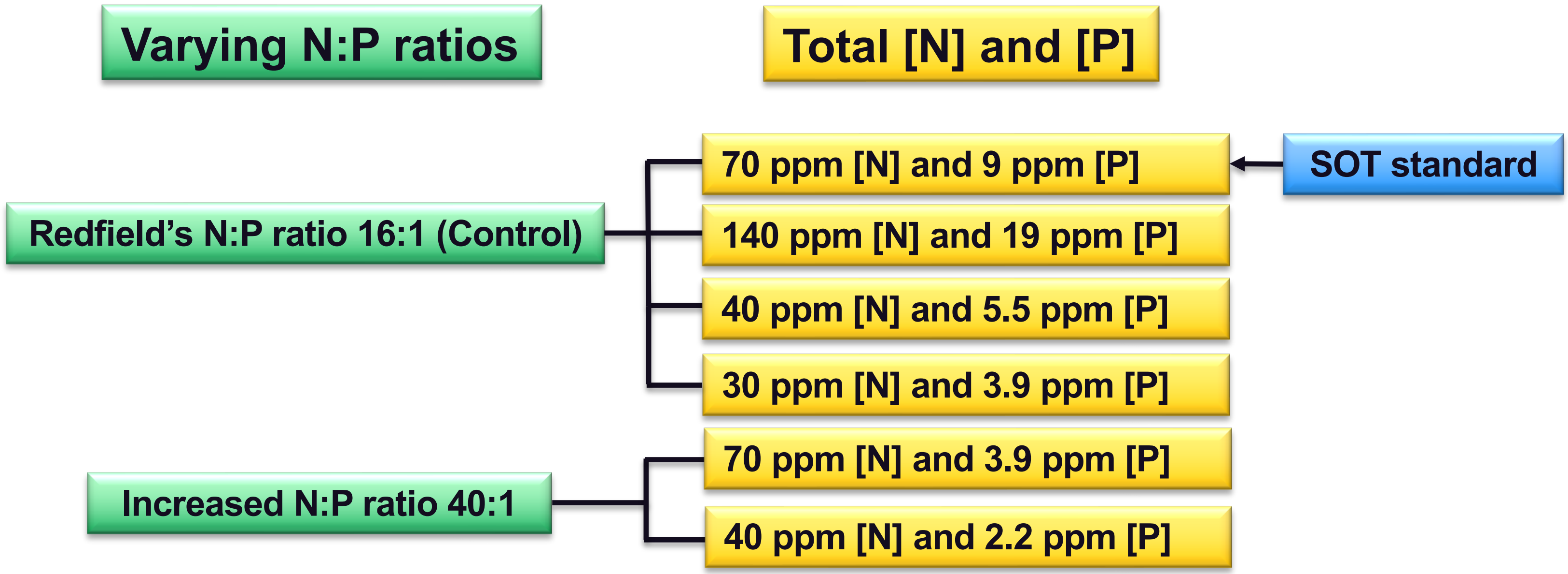
2 – Progress and outcomes

Task 2 – Cultivation and nutrient operations



Share findings with DISCOVR/SOT/ algae community

2 – Progress and Outcomes
ePBR P. celeri nutrient experiments

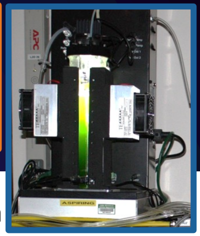


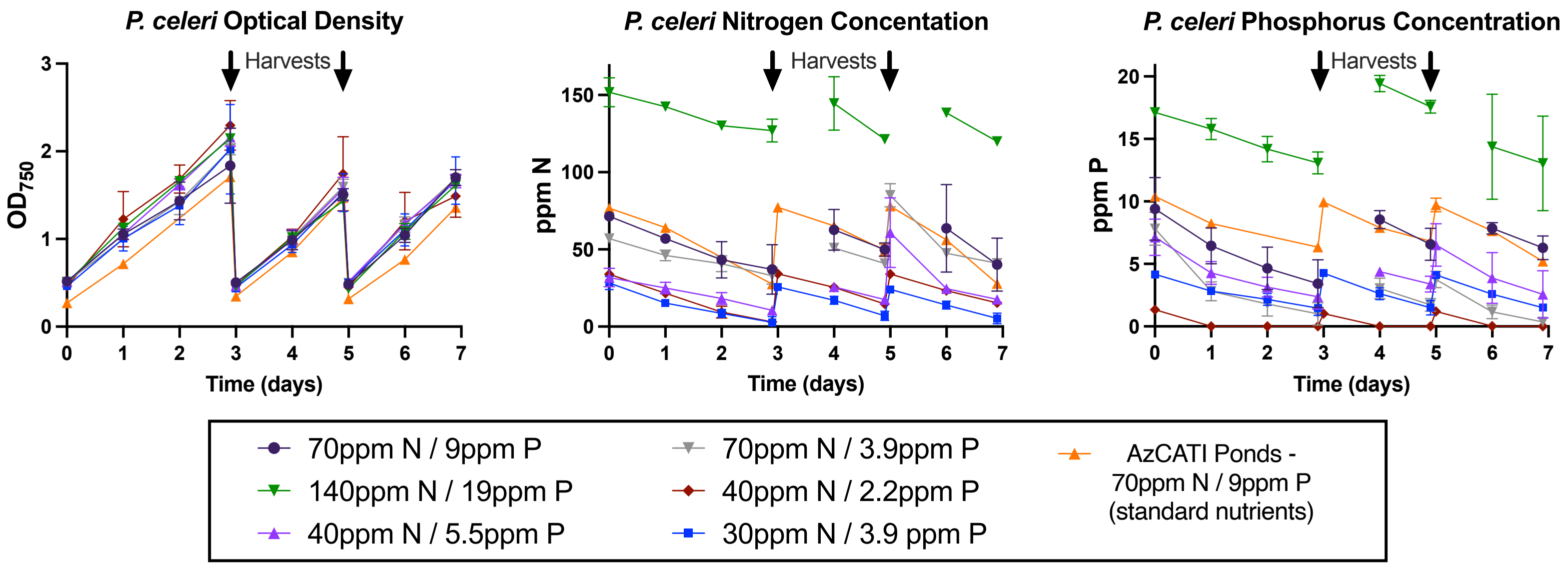
Goal: Measure the effects of varying nutrient concentrations on the growth of *P. celeri* and *T. striata*.

Method: Grow cultures in ePBRs with set nutrient inputs at the beginning of each grow out. Complete 3 grow-outs per condition in quadruplicate. Completed in parallel with ML.

2 – Progress and Outcomes

P. celeri ePBR productivity and nutrient uptake





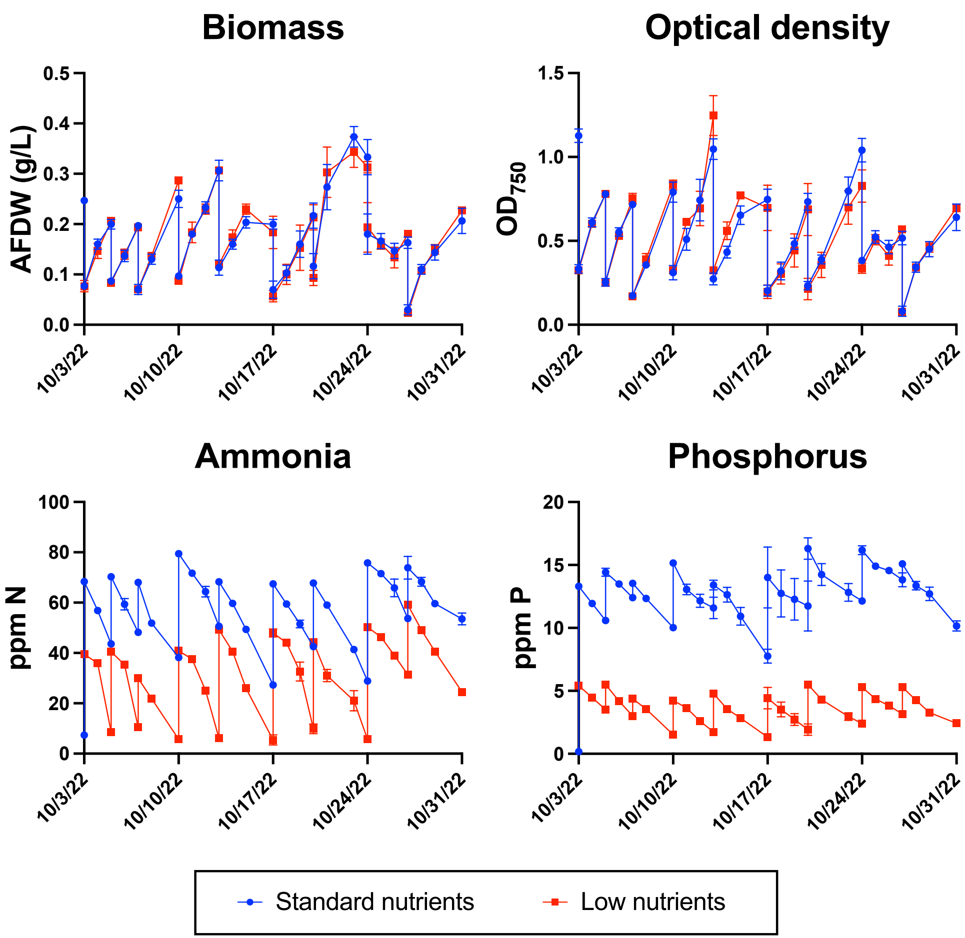
Goal: Test growth and nutrient uptake of *P. celeri* with different nutrient inputs.

Method: Cultures were grown in ePBRs on a script simulating light and temperature in Mesa, Az starting August 7, 2020. Nutrients added back at dilution were varied and measured daily, along with optical density.

Outcome: Growth by OD and nutrient uptake across conditions are consistent in ePBRs.

Impact: Nutrient inputs can be reduced to reach the same biomass productivity.

2 – Progress and Outcomes
P. celeri growth outdoors with reduced nutrients



Goal: Test growth of *P. celeri* outdoors in SOT f/2 with reduced N & P nutrient inputs.

Method: Grow ponds under two different nutrient regimes:

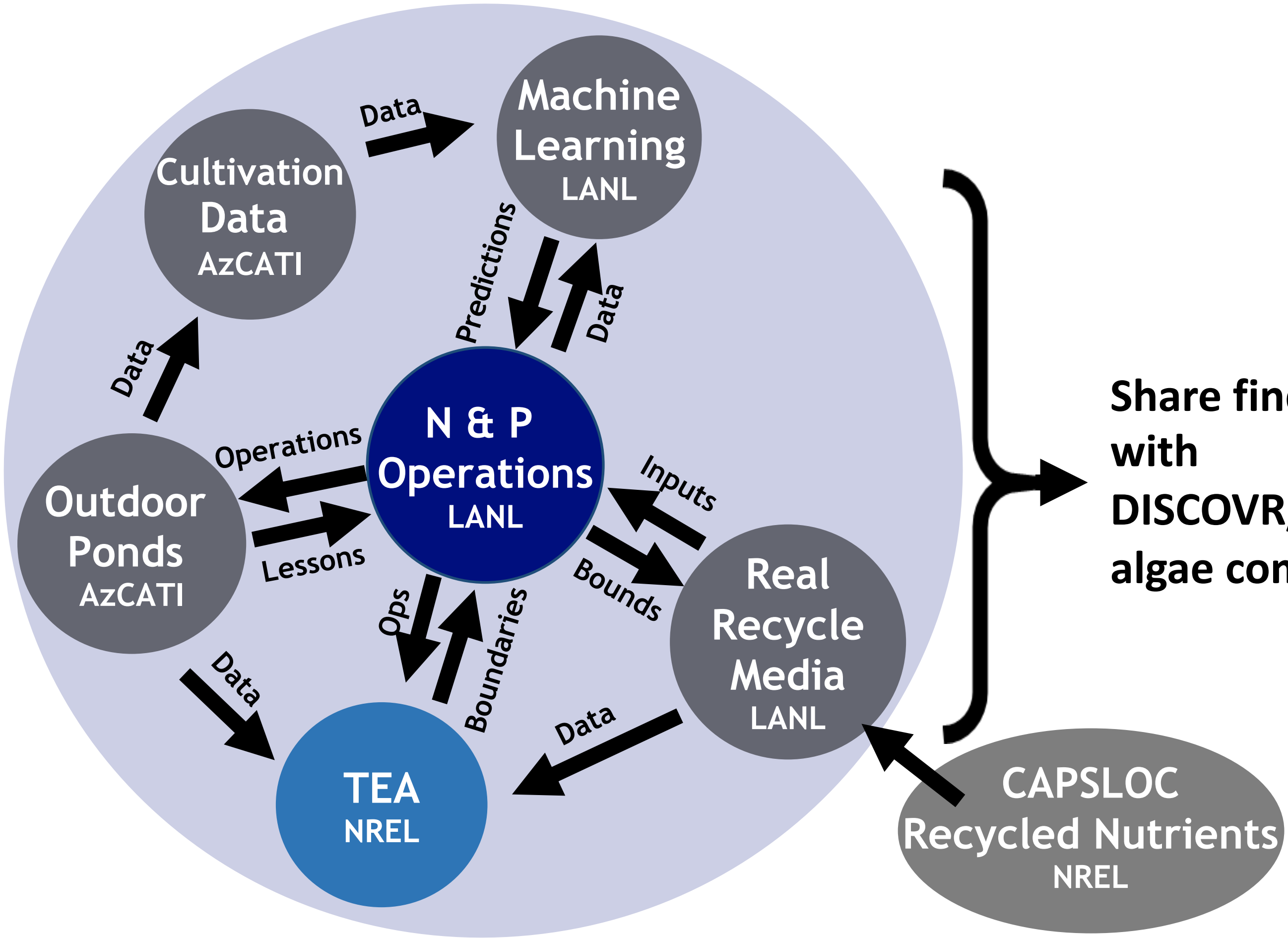
Condition	[N]	[P]
Standard nutrients	70-80 ppm	14-16 ppm
Low nutrients	40-50 ppm	5-6 ppm

Outcome: *P. celeri* culture grew the same with standard and reduced nutrients.

Impact: Outdoor experimental outcomes backup indoor results

2 – Progress and Outcomes

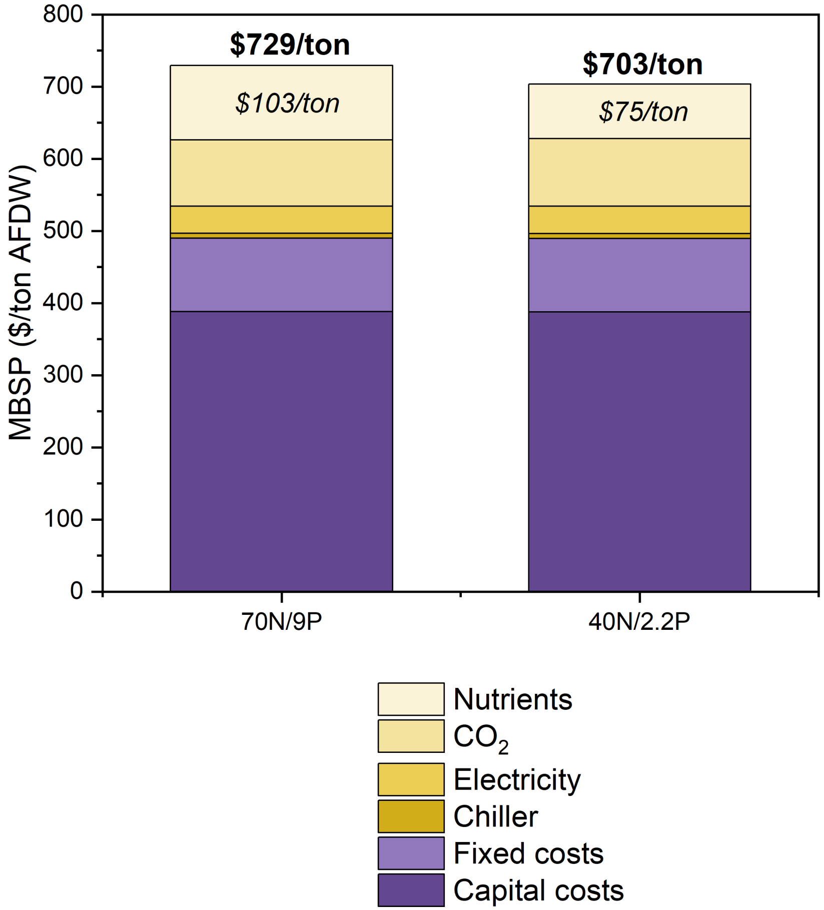
Task 1 – Technoeconomic analysis



Share findings with DISCOVR/SOT/ algae community

2 – Progress and Outcomes

Task 1 – *TEA analysis of differential nutrient inputs*



Goal: Determine effects of changing nutrient inputs on MBSP.

Method: Use nutrient uptake data from ePBRs to inform TEA using the Aspen Plus model. Consistent productivity and full water recycling is assumed.

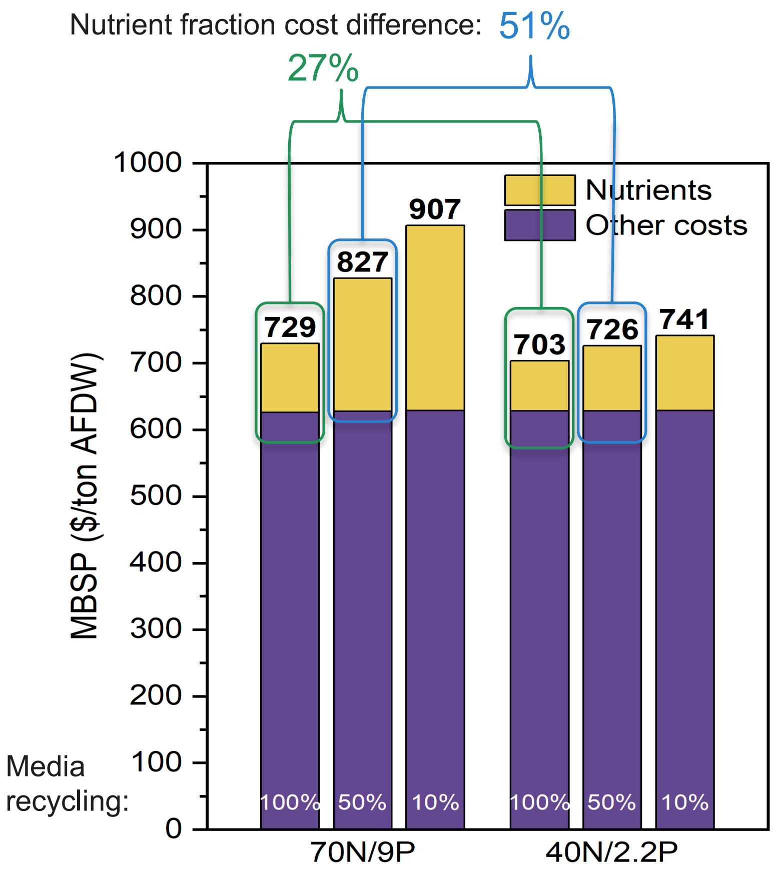
Outcome: Reducing nutrient inputs to levels that continue to maintain productivity saves $26/ton AFDW over the current SOT standard.

Impact: Cost savings are realized using more optimized nutrient levels.

Ongoing: Include inputs of actual biochemical composition in downstream processes.

2 – Progress and Outcomes

Task 1 – *TEA nutrient inputs with media recycling*



Goal: Determine the combined effects of changing nutrient inputs and media recycling on MBSP.

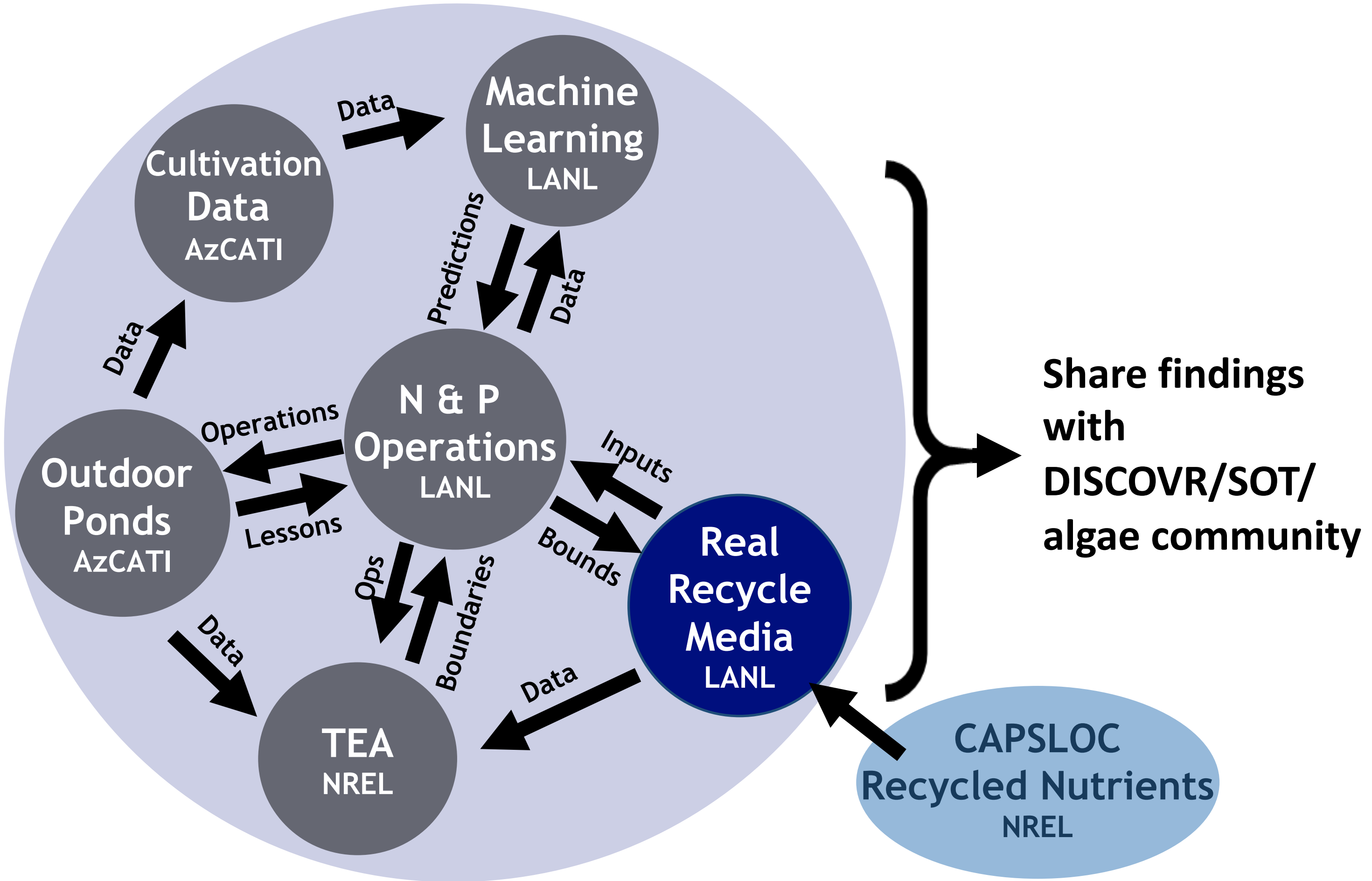
Method: Use nutrient uptake data from ePBRs to inform TEA using the Aspen Plus model. Consistent productivity is assumed but media recycling is varied.

Outcome: Non-perfect media recycling increases cost, particularly when excess nutrients are used.

Impact: TEA shows that optimizing media nutrients to reduce excess will contribute to considerable saving in cultivation.

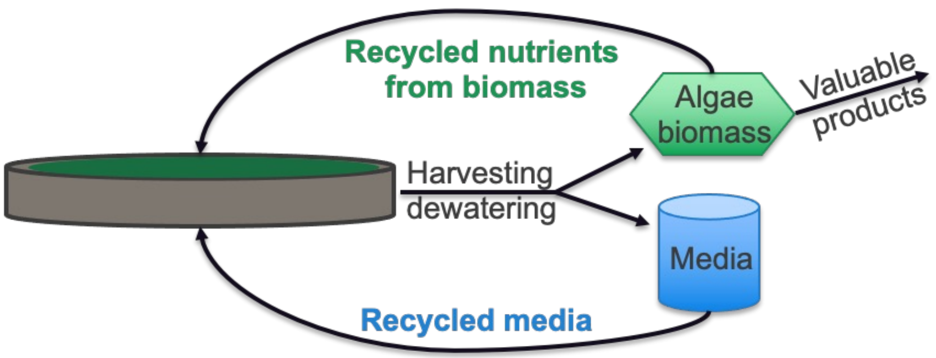
2 – Progress and Outcomes

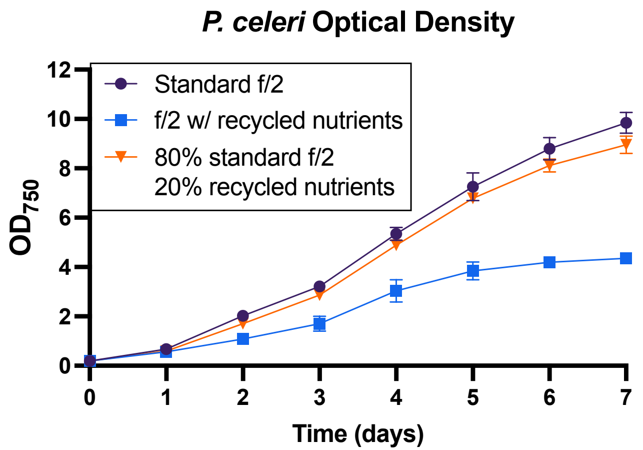
Task 3 – Recycled nutrients



2 – Progress and Outcomes

MOT liquor composition & P. celeri growth with MOT nutrients





Goal: Use biomass MOT extracted nutrients from the CAPSLOC project as an N & P source for f/2 media and test the growth of *P. celeri*.

Method: Track growth by OD of *P. celeri* in shake flasks in a 1% CO_2 incubator.

Outcome: Cells were able to utilize MOT nutrients for growth, however other liquor components may be reducing health and productivity.

Impact: Unused biomass nutrients can be extracted and used to feed cultures.

Ongoing: Conduct growth experiment utilizing varying amounts of MOT recycled nutrients in both *P. celeri* and *T. striata*.

3 – Impact

Machine learning and farm scale productivity modeling

- Development of Machine Learning tools
  - Designed around real multi-year outdoor data from AzCATI
  - Demonstrated implementation for two species (*P. celeri* & *T. striata*)
  - Includes growth, nutrient, and weather data and has been collated into a format that is ML readable, this can be expanded to additional species and test beds as it becomes important in further research

- Broadened TEA capabilities
  - Farm models in Aspen Plus updated to reflect more accurate cultivation approaches
  - Different nutrient levels and measured nutrient consumption from actual experiments as inputs, this can be expanded to different species
  - Variable inputs for non-perfect recycling of media
  - This is a widely used model in the BETO portfolio and these expanded capabilities can be implemented across projects.

3 – Impact

Optimization of nutrient inputs, including recycled nutrients

- Increased nutrient input efficiency
  - Reducing the nitrogen and phosphorus inputs into a growth system will decrease the costs associated with algae biomass production.
  - Understanding the nutrient uptake in different seasons and multiple species will change operations to optimize growth while reducing costs in large scale systems.

- Understanding the real generation, use, and impact of recycled nutrients from biomass will reduce waste and the cost of biomass production.

3 – Impact

Dissemination of results and community interaction

- Results have been and will be presented at industry conferences, including the *Algal Biomass Summit* and the *International Conference on Algal Biomass, Biofuels & Bioproducts.*

- We plan to submit papers focused on machine learning, nutrient uptake and growth effects combined with TEA, and growth with recycled nutrients.

- Interaction with Northern New Mexico College will increase the students understanding of the research occurring at LANL (local) and collaborative institutions. Our goal is to increase the confidence of students in their abilities to pursue STEM fields.

Summary

- **Approach:** MORE for Better Biomass takes an iterative approach to ML for predicting productivity, indoor simulations, outdoor ponds, growth with recycled nutrients, and technoeconomic analysis to improve biomass productivity and reduce nutrient costs in cultivation. We combine the expertise of researchers at LANL, NREL, and AzCATI to accomplish this.
- **Progress:** We have incorporated multiple years of data from two SOT species of algae, *P. celeri* and *T. striata*, into ML algorithms and confirmed predictions in ePBRs and outdoor ponds, showing robust growth through a range of nutrient conditions. TEA analysis has demonstrated a decreased MBSP using reduced nutrient inputs as compared to standard SOT f/2 media. Recycled nutrients from biomass have been generated and experiments incorporating that have begun, which is expected to further lower MBSP.
- **Impact:** Optimizing nutrient inputs while maintaining or increasing biomass productivity will move the industry towards the goal of cost effective biomass production.

Quad Chart Overview

Timeline

- *Project start date - 10/1/2021*
- *Project end date - 9/30/2024*

	FY22 Costed	Total Award
DOE Funding	$387,735 (LANL) $8,000 (NREL)	$1.5M ($1.38M to LANL, $120k to NREL)
Project Cost Share *	N/A	N/A

TRL at Project Start: 2
TRL at Project End: 4

Project Goal

Create an updated approach to nutrient management by using a combination of machine learning based on real outdoor data, indoor and outdoor growth experiments, application of recycled nutrients derived from extracted biomass, and technoeconomic analysis to understand the impacts of different nutrient inputs.

End of Project Milestone

At least one nutrient management strategy that increases biomass productivity by 15% in SOT media using recycled N and P streams from an algal biomass processing pathway.

Funding Mechanism

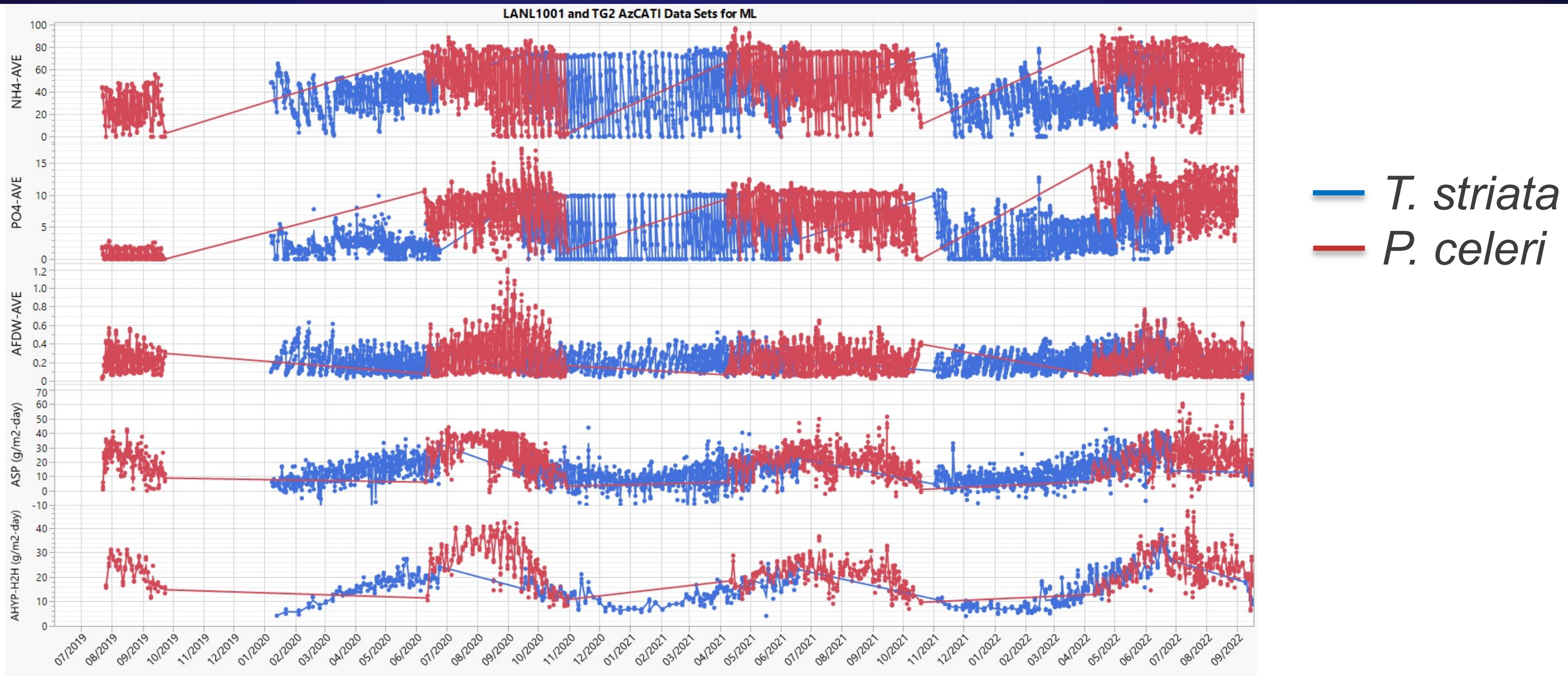
Lab Call FY22
Advanced Algal Systems
AOI 1c

Project Partners

- NREL – Bruno Klein, Tao Dong
- AzCATI – John McGowen

Additional Slides

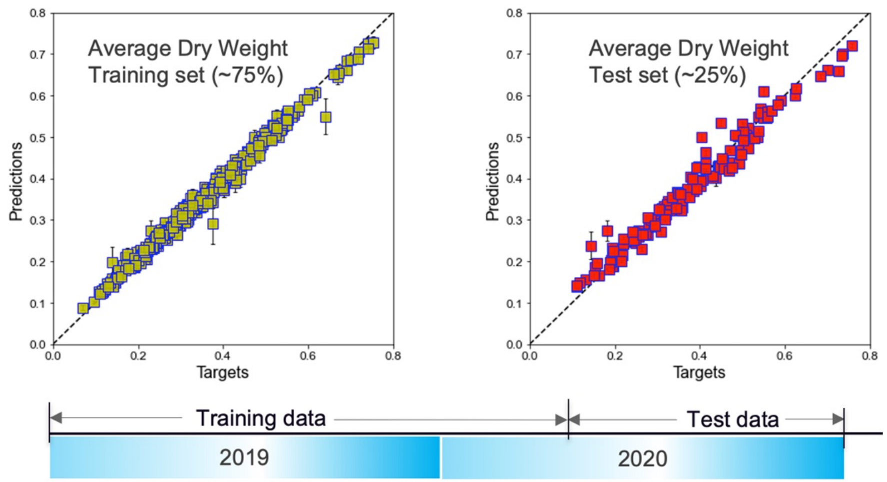
AzCATI pond productivity data used as inputs for ML



Goal: Provide multiple years of outdoor pond data for ML input.

Method: Collate over 6500 daily samples including measurements of biomass, [N], [P], continuous data for pH, water temp, dissolved oxygen, and weather at 5-15 min resolution.

Validation of ML model for assessment of pond productivity



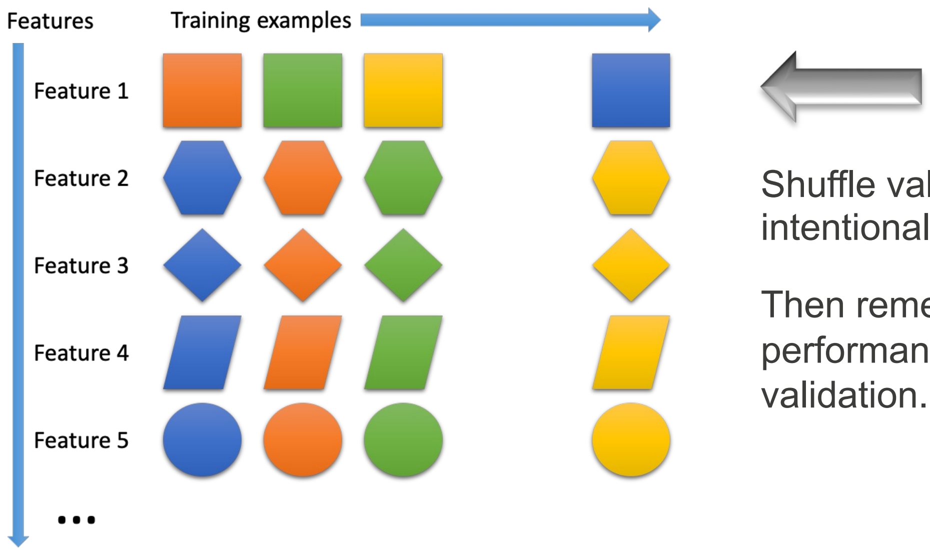
Goal: Train and validate the ML model.

Method: Use a subset of the data for model training and test predictions on additional data.

Outcome: ML model performs well using various training and test data, for both *P. celeri* and *T. striata*.

Impact: Model can be applied across large datasets and expanded to include new data for pond performance prediction and analysis.

ML approach for determination of feature importance



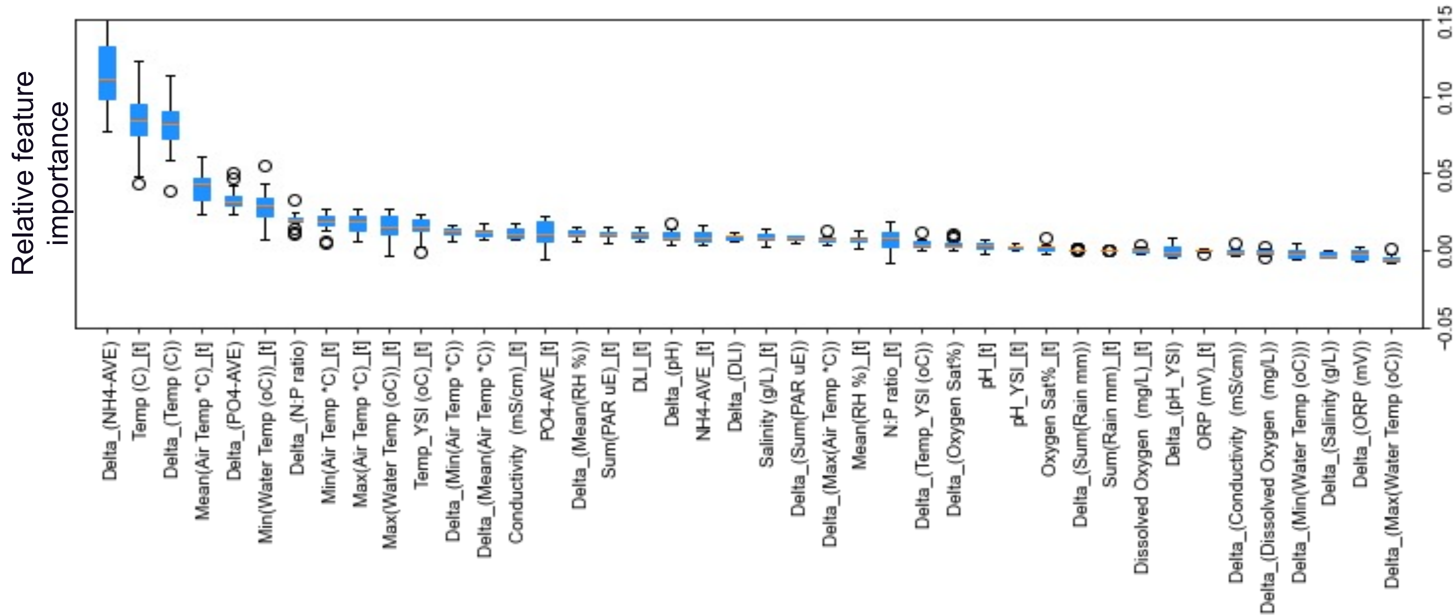
Shuffle values to intentionally corrupt data.

Then remeasure model performance using cross validation.

Goal: Develop an approach to determine relative feature importance.

Method: Use Random Forest Regressor architecture (# trees=100, maximum tree depth = 15 nodes, minimum # samples at leaves = 1) with value permutation for feature analysis and 20-fold cross-validation.

ML correlation between inputs and outdoor productivity

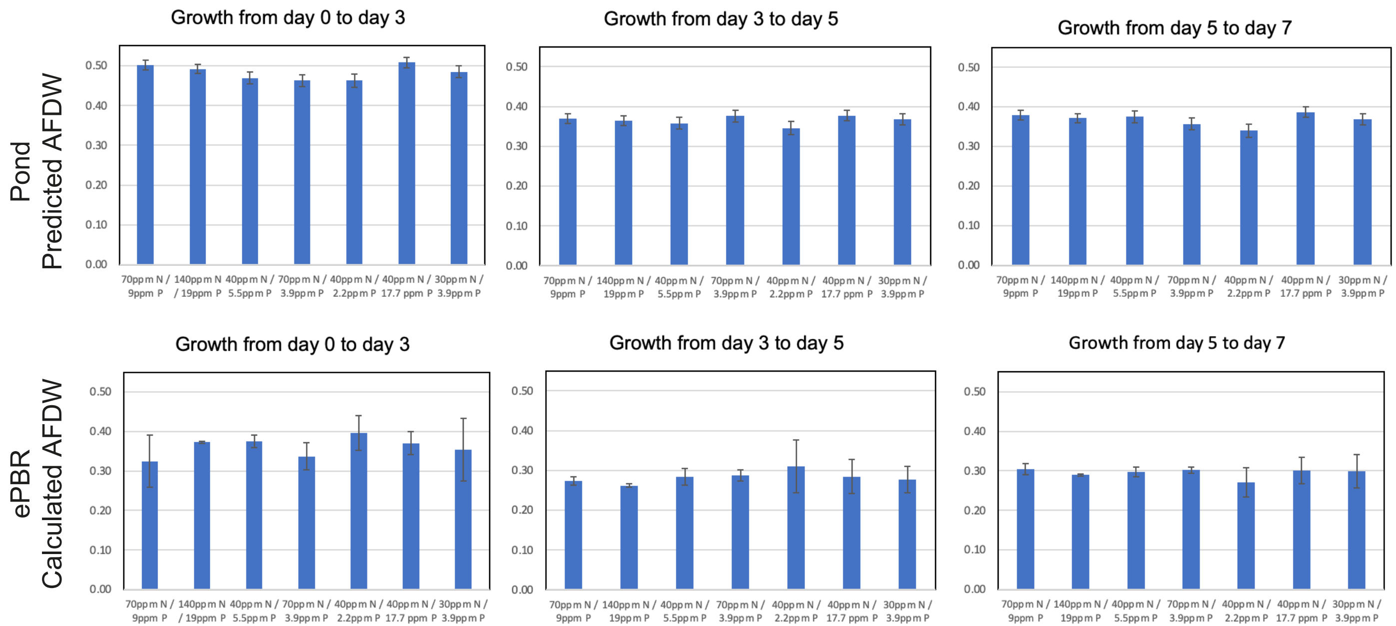


Goal: Identify features that are primary contributors to *P. celeri* productivity in ponds.

Method: Use multi-year pond data from 4.2m^2 outdoor AzCATI ponds as inputs to a python based Random Forest Regressor ML algorithm.

Outcome: Temperature and nutrient uptake from media are most predictive of pond productivity.

ML learning predictions for pond productivity and calculated ePBR productivity

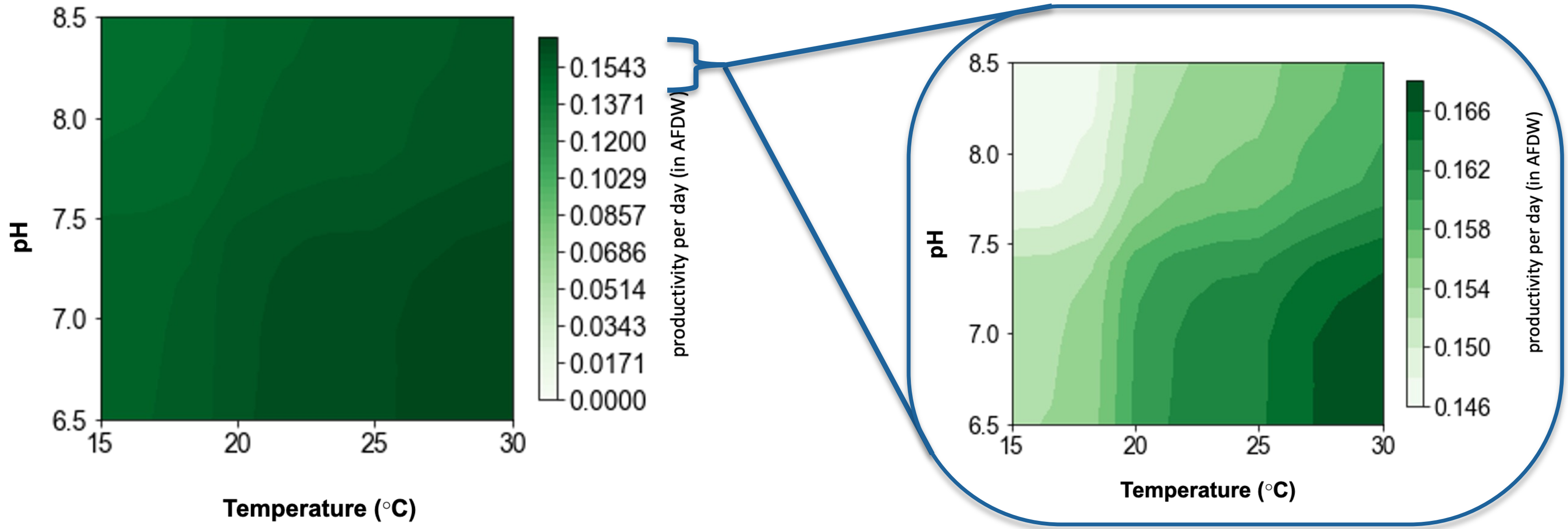


Goal: Use ML from pond data to predict productivity across nutrient conditions.

Method: ML algorithms are used to predict productivity and that is compared to calculated AFDW (using a standard curve) from ePBRs.

Outcome: Consistent productivity between conditions in both Predicted Pond and ePBRs. Highest productivity is seen in the first 3-day grow out compared to the subsequent 2-day.

ML productivity predictions for *P. celeri*

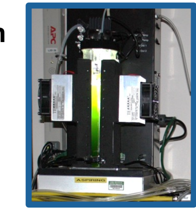


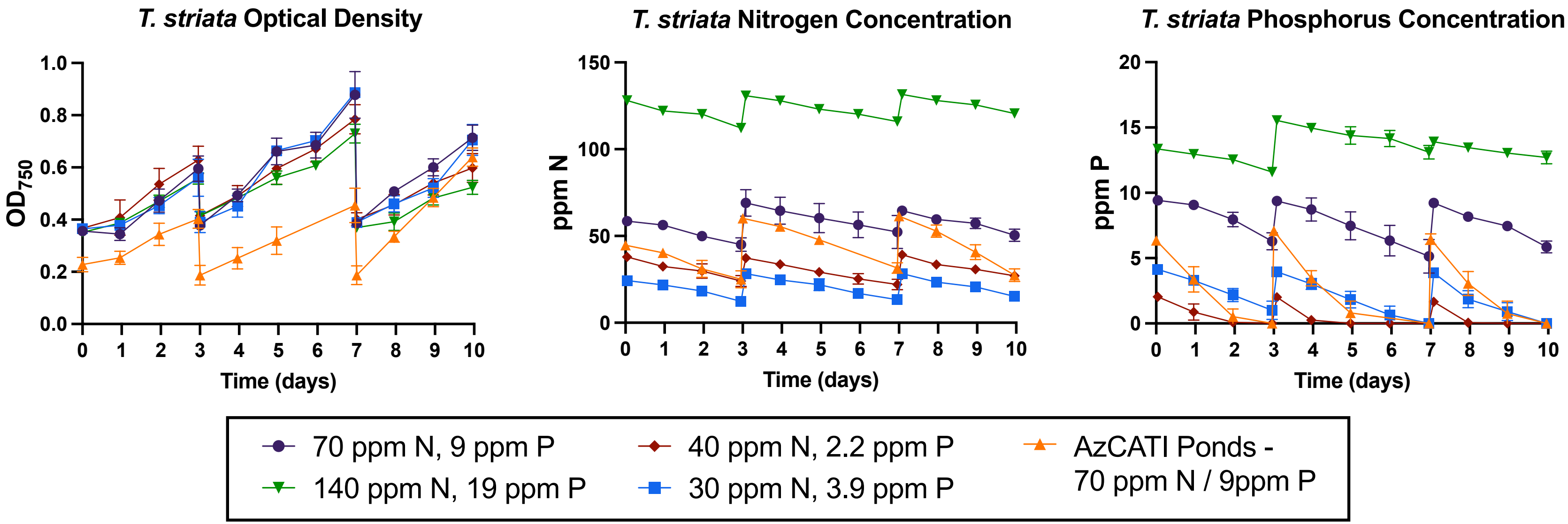
Goal: Use ML to predict productivity maxima for *P. celeri* based on culture pH and temperature.

Method: All conditions except pH and temperature are kept consistent and set to August 7, 2020.

Outcome: The ML model shows that decreasing pH and increasing temperature is predicted to increase *P. celeri* productivity.

T. striata ePBR productivity and nutrient uptake





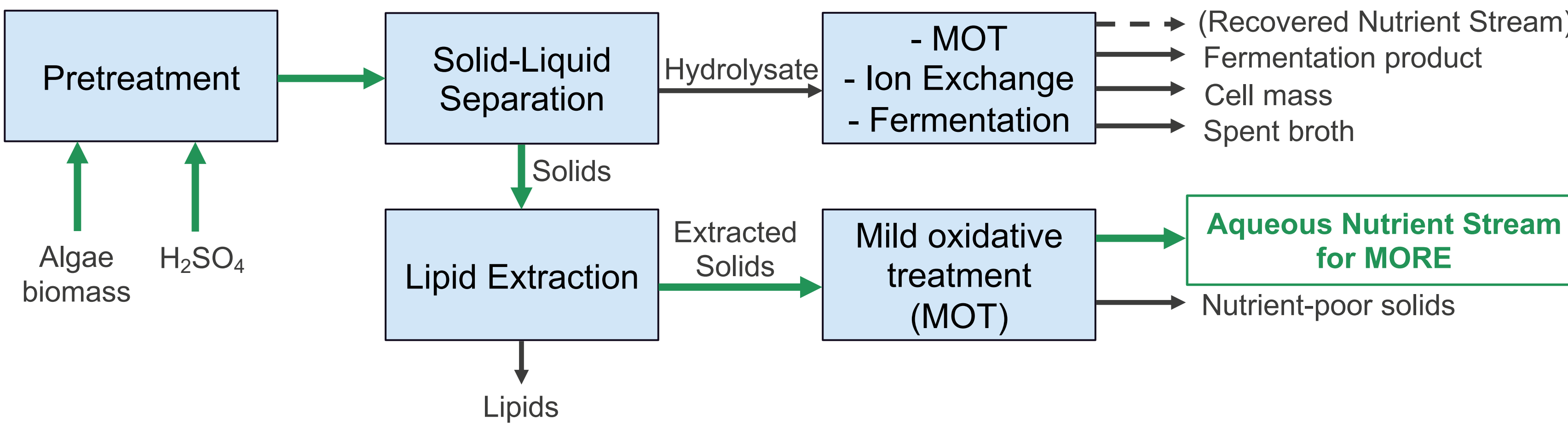
Goal: Test growth and nutrient uptake of *T. striata* with different nutrient inputs.

Method: Cultures were grown in ePBRs on a script simulating light and temperature in Mesa, Az starting January 24, 2022. Nutrients added back at dilution were varied and measured daily, along with optical density.

Outcome: Growth by OD and nutrient uptake across conditions are consistent in ePBRs.

Impact: Nutrient inputs can be reduced to reach the same biomass productivity.

CAPSLOC – generation of recycled nutrients



Goal: Use *P. celeri* and *T. striata* biomass from the AzCATI testbed to produce an aqueous nutrient stream containing N & P.

Method: Dried extracted solids were loaded into a parr reactor with water, pH adjusted to 2 with sulfuric acid, and mixed. The reactor purged with N_2, leak checked, and de-pressurized. The contents of the reactor were heated to 200°C then 20 bar zero air was introduced to the system. The reaction proceeded for 40 minutes, and then quenched in an ice-bath.

MOT Liquor composition

From P. celeri or T. striata biomass

Picochlorum celeri MOT Liquor			
Compound	**Concentration [g/L]**		
Ammonia	**5.9**		
Phosphate	**1.44**		
Formic acid	0.92	±	0.16
Acetic acid	0.78	±	0.13
Propanoic acid	0.46	±	0.19
Pyrrolidine	0.24	±	0.03
Fumaric acid	0.25	±	0.02
Succinic acid	1.42	±	0.15
Methyl succinic acid	0.27	±	-
Indole	0.29	±	-
Alanine	0.61	±	0.04

Tetraselmis sp. MOT Liquor			
Compound	**Concentration [g/L]**		
Ammonia	**7.5**		
Phosphate	**1.26**		
Formic acid	0.43	±	0.09
Acetic acid	0.56	±	0.05
Propanoic acid	0.43	±	0.01
Pyrrolidine	0.51	±	0.06
Fumaric acid	0.24	±	-
Succinic acid	0.56	±	-
Methyl succinic acid	0.32	±	0.11
Indole	ND		
Alanine	0.68	±	0.07

Goal: Characterize the components of recovered N and P from lipid extracted biomass.

Method: ICP for phosphorus, CHN for nitrogen, Propylchloroformate derivatization and GC-MS for other compounds.

Outcome: Primary extraction components are N and P in a concentration that is useful for adding to cultures.

Publications, Patents, Presentations, Awards, and Commercialization

Posters

- Nilusha Sudasinghe, Claire Sanders, Ghanshyam Pilania, Raul Gonzalez, John McGowen, Evan Taylor, Bruno Klein, Jacob Kruger, Taraka Dale. Media Optimization for Improved Biomass Productivity and Biomass Quality. Algal Biomass Summit; October 3-28, 2022; Virtual.
- Nilusha Sudasinghe, Claire Sanders, Erika Quezada, Bruno Klein, John McGowen, Taraka Dale. Media Optimization for Improved Biomass Productivity. International Conference on Algal Biomass, Biofuels and Bioproducts; June 12-14, 2023. Waikoloa Beach, Hawaii. Abstract accepted.