Trent Northen, Berkeley Lab, JBEI, and JGI

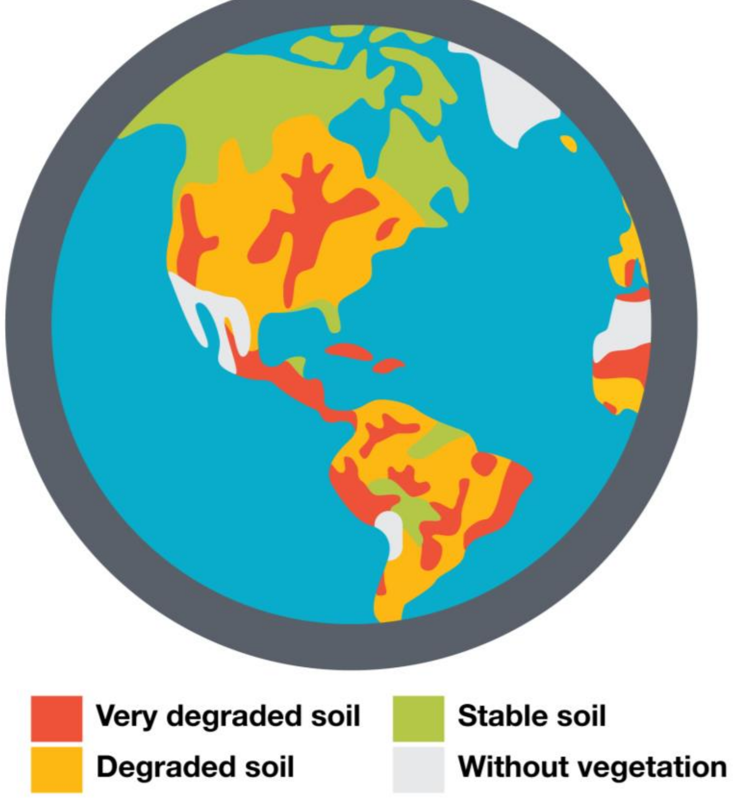
ICME

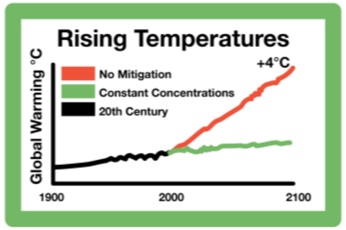
December 18th 2023

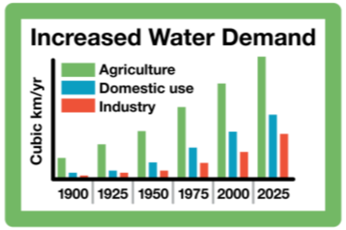
Engineering Microbiomes for Sustainable Agriculture and Soil Carbon Storage

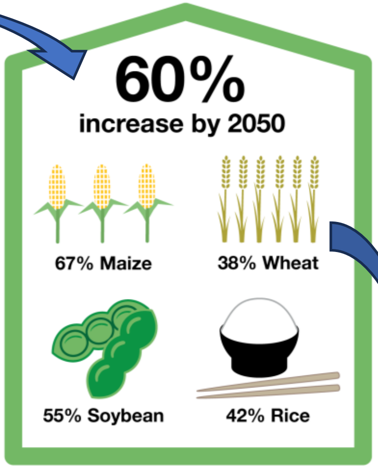
Climate change and degraded soils will make it very difficult to support a growing population

Improved soil fertility



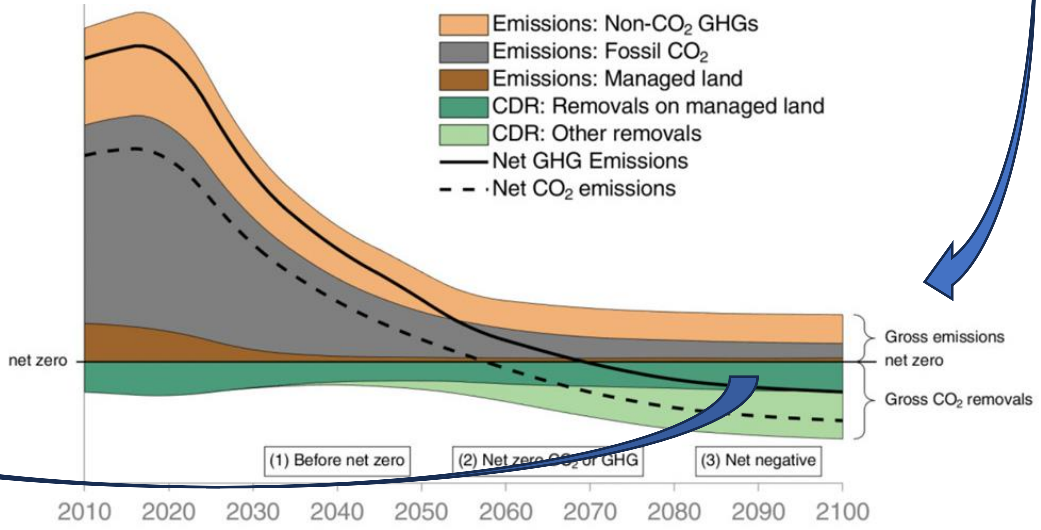


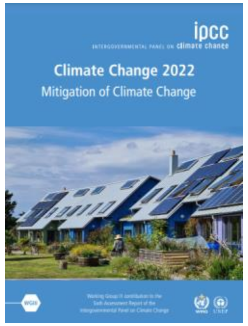




Harness agriculture to store carbon (esp. bioenergy)

Must sequester atmosphere carbon to stay below 1.5 C warming





Increase soil organics

Critical opportunity to harness soil microbes for sustainable agriculture and restoring soil carbon

MICROBIOLOGY SOCIETY

BRIEFING

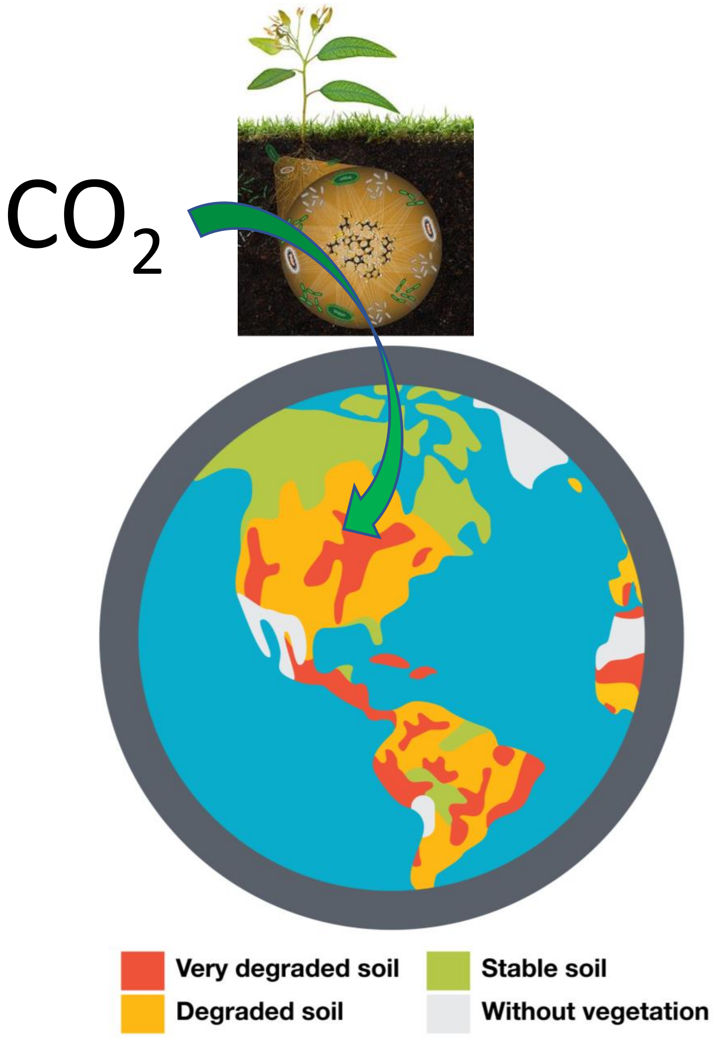
Food Security from the Soil Microbiome

- Soil micro-organisms are vital for soil health and food security.
- Intensive agricultural production often impacts the soil microbiome at a cost to productivity, sustainability and the environment.
- Microbiologists are investigating how the soil microbiome can be harnessed as a tool for sustainable agricultural intensification.

HOW MICROBES CAN HELP FEED THE WORLD

A REPORT FROM THE AMERICAN ACADEMY OF MICROBIOLOGY

AMERICAN SOCIETY FOR MICROBIOLOGY

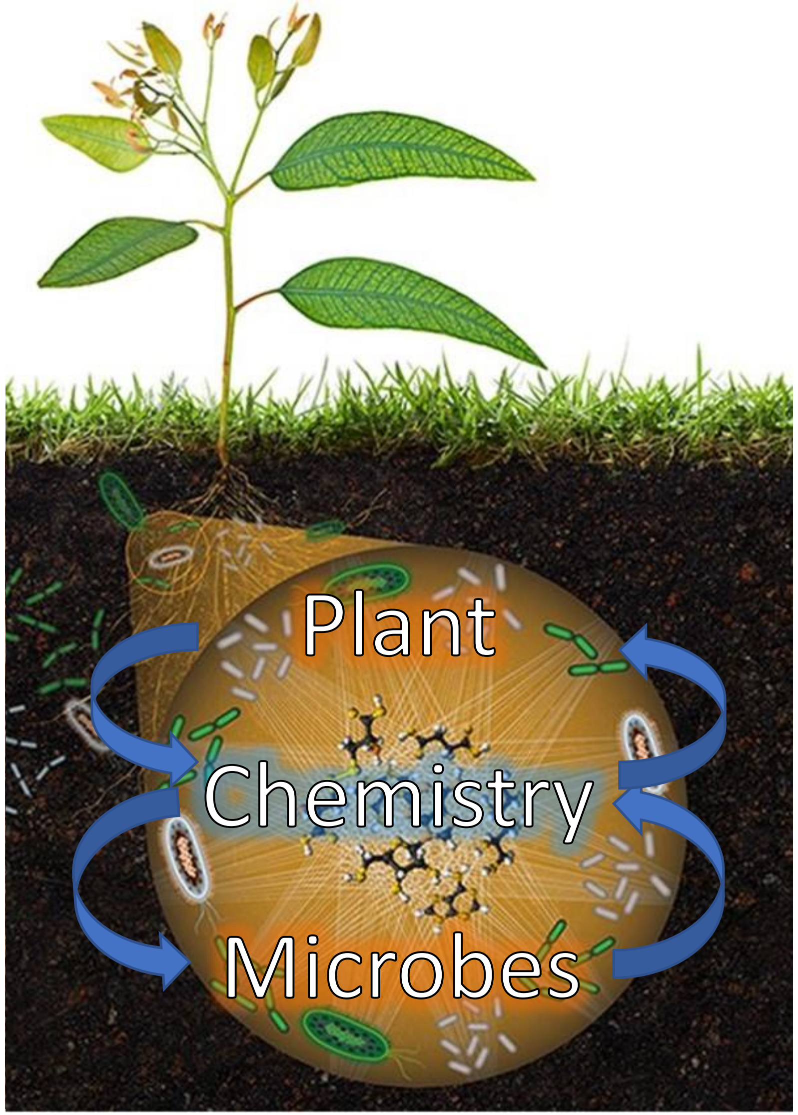


PIVOT BIO

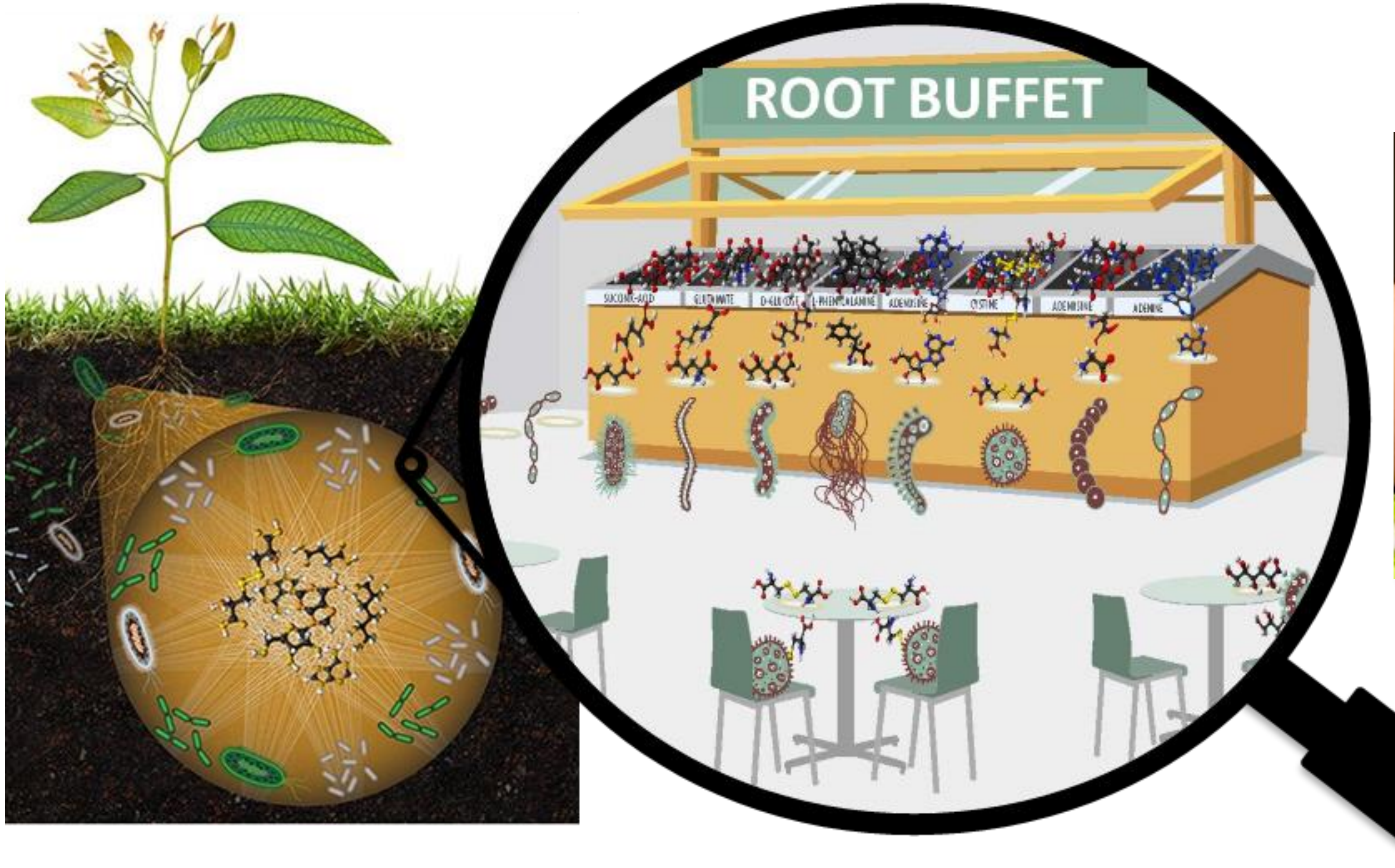
indigo

Challenge: Effectively matching beneficial microbes with their plant hosts

- Plants deposit up to 30% of their photosynthate into soil as rhizodeposits
- ~95% of plant inputs into soil are rapidly used by soil microbes and converted back into CO_2
- Drives changes in soil microbial community structure (rhizosphere effect)
- Challenging to get the beneficial microbes to engraft in the rhizosphere
- These processes are critical to sustainable agriculture and soil carbon restoration



Exudates are composed of diverse metabolites that act as substrates and signals for rhizosphere communities



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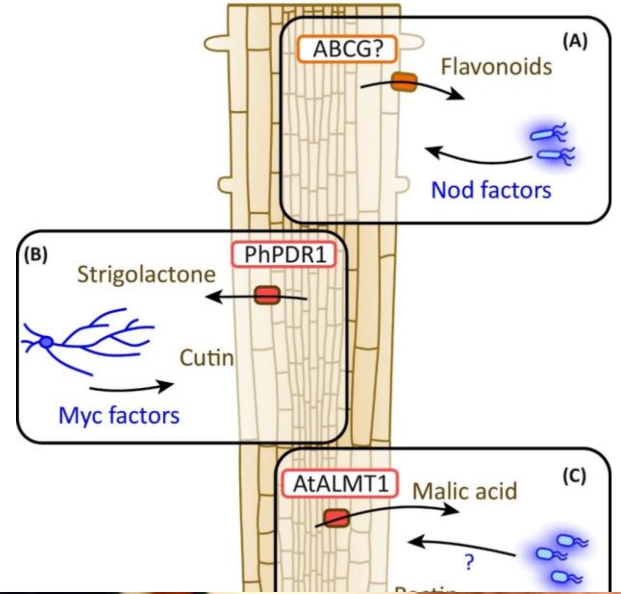
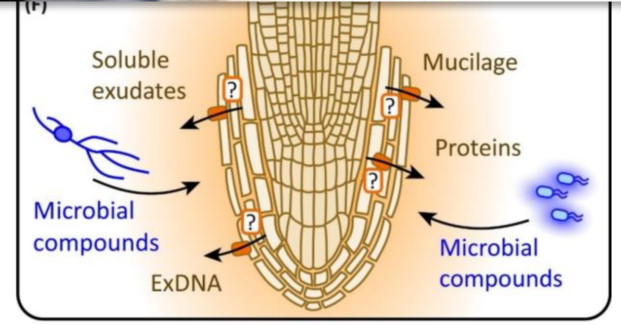


Soil bacteria are often selective 'eaters'

Baran et al Nature Communications 2015



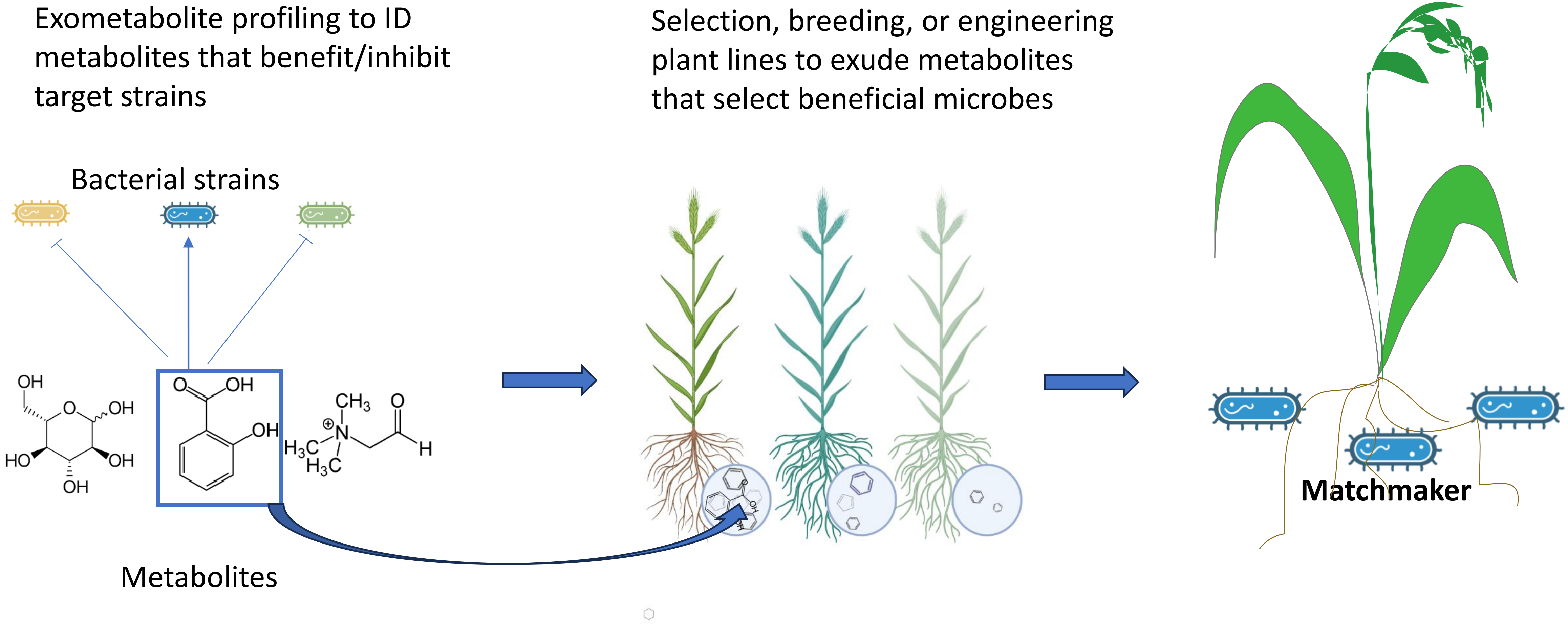
Sasse et al 2018

Trends in Plant Science

Exometabolomics: A simple experimental approach to measure metabolite inputs and outputs

Use LC/MS to measure the changes in metabolites

Rhizosphere microbiome engineering

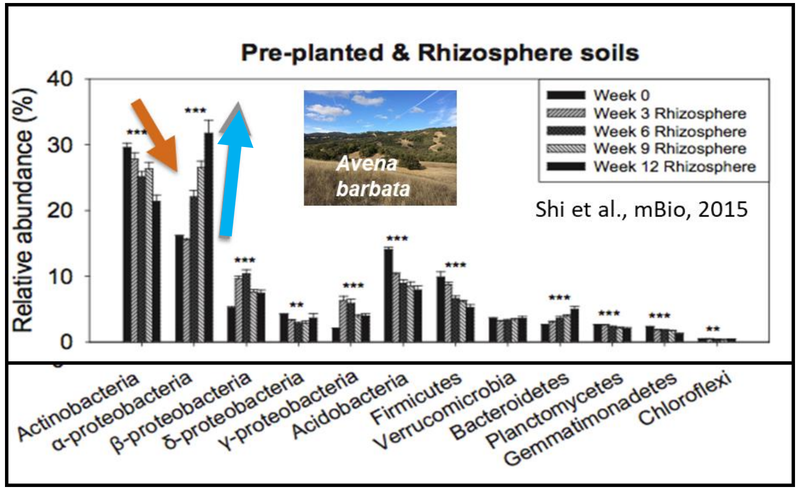


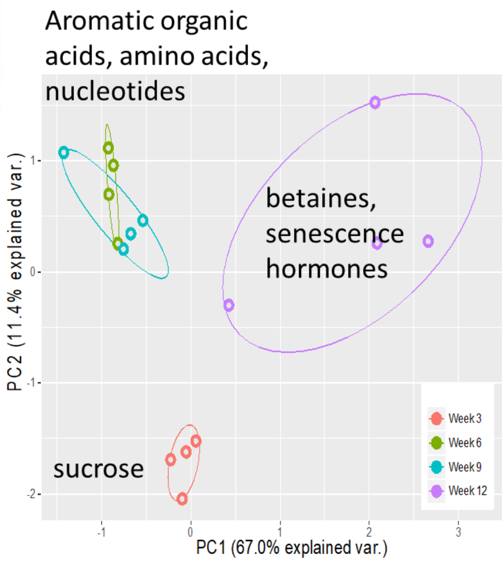
Exometabolite profiling to ID metabolites that benefit/inhibit target strains

Selection, breeding, or engineering plant lines to exude metabolites that select beneficial microbes

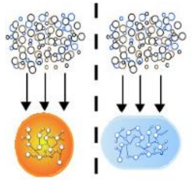
Exometabolite profiling to ID metabolites that benefit/inhibit target strains

Kate Zhalnina (LBNL)

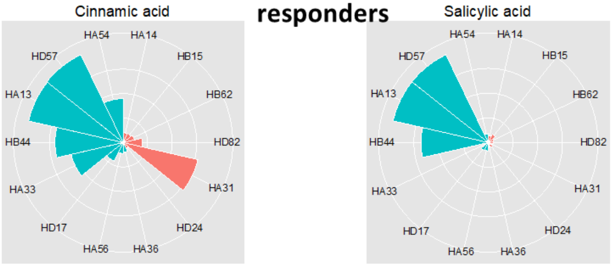




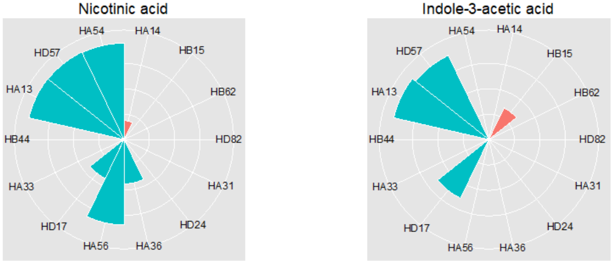
Fed exudates to rhizobacteria

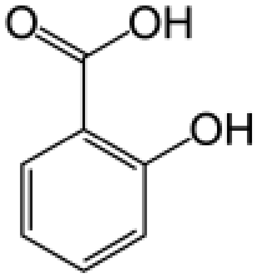


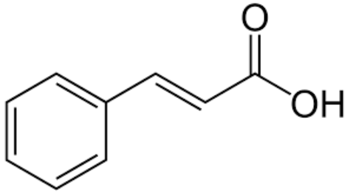
Phenolic acids are preferentially used by rhizosphere responders



Other aromatic acids





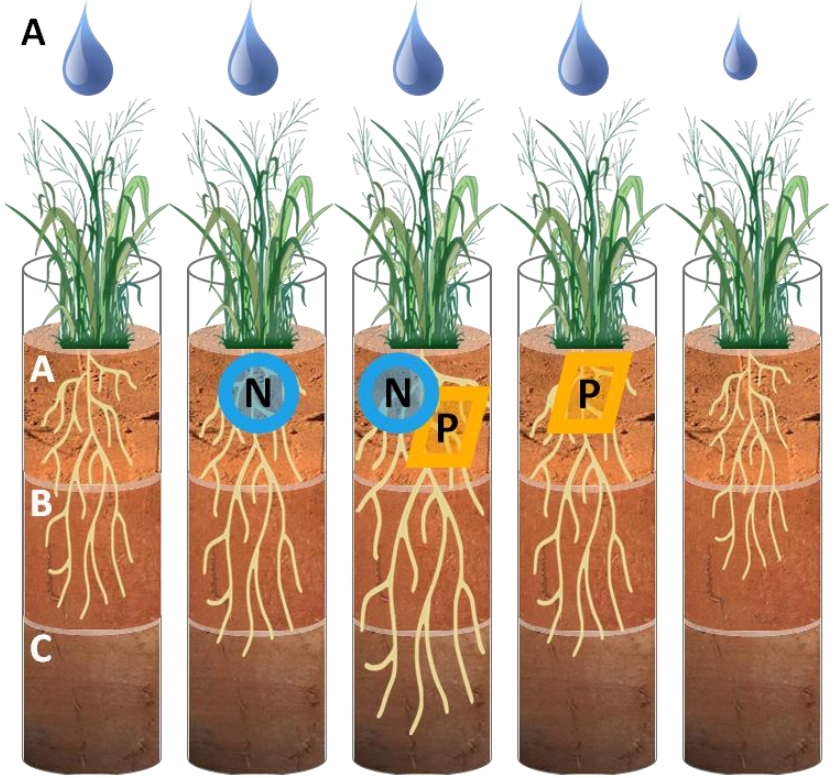


Zhalnina et al *Nature Microbiology* 2018

Mesocosm experiments to link metabolites to bacterial strains recruited during plant stress

Anadarko, Oklahoma

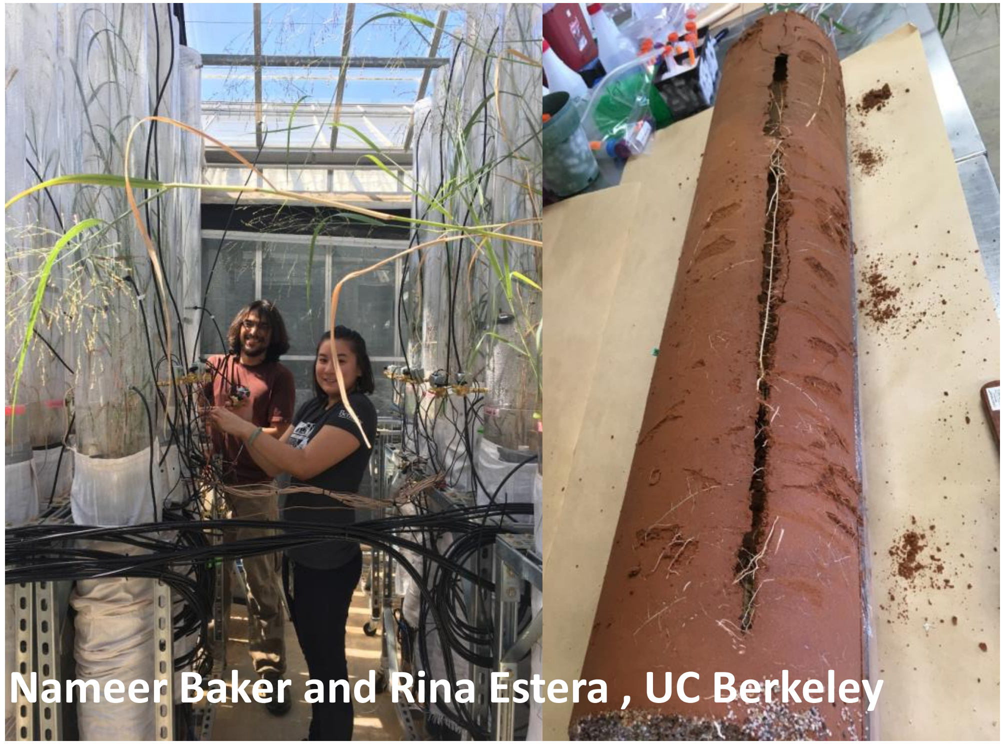
Berkeley, California



16S + Metabolomics

Kate Zhalnina (LBNL)

Mary Firestone
UC Berkeley

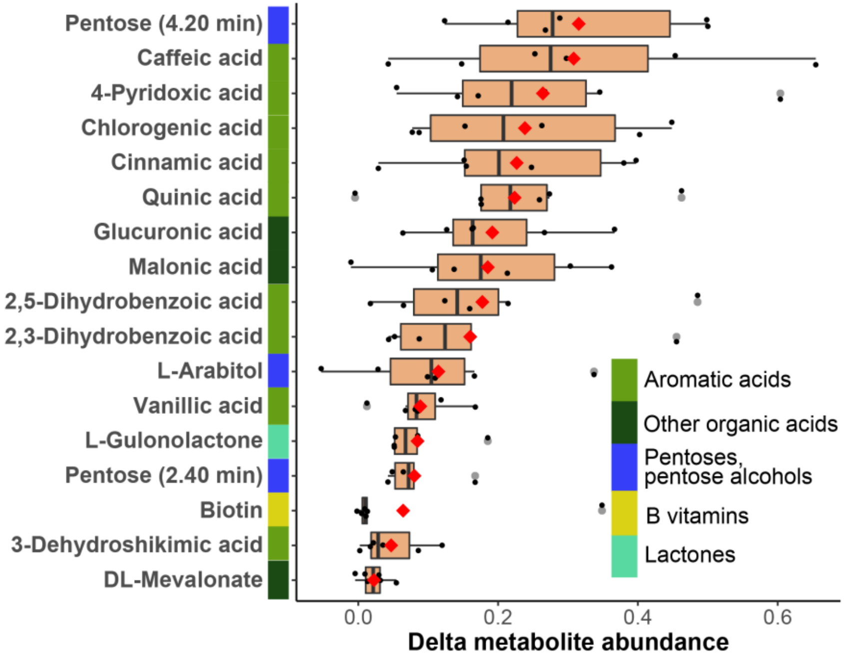


Nameer Baker and Rina Estera , UC Berkeley

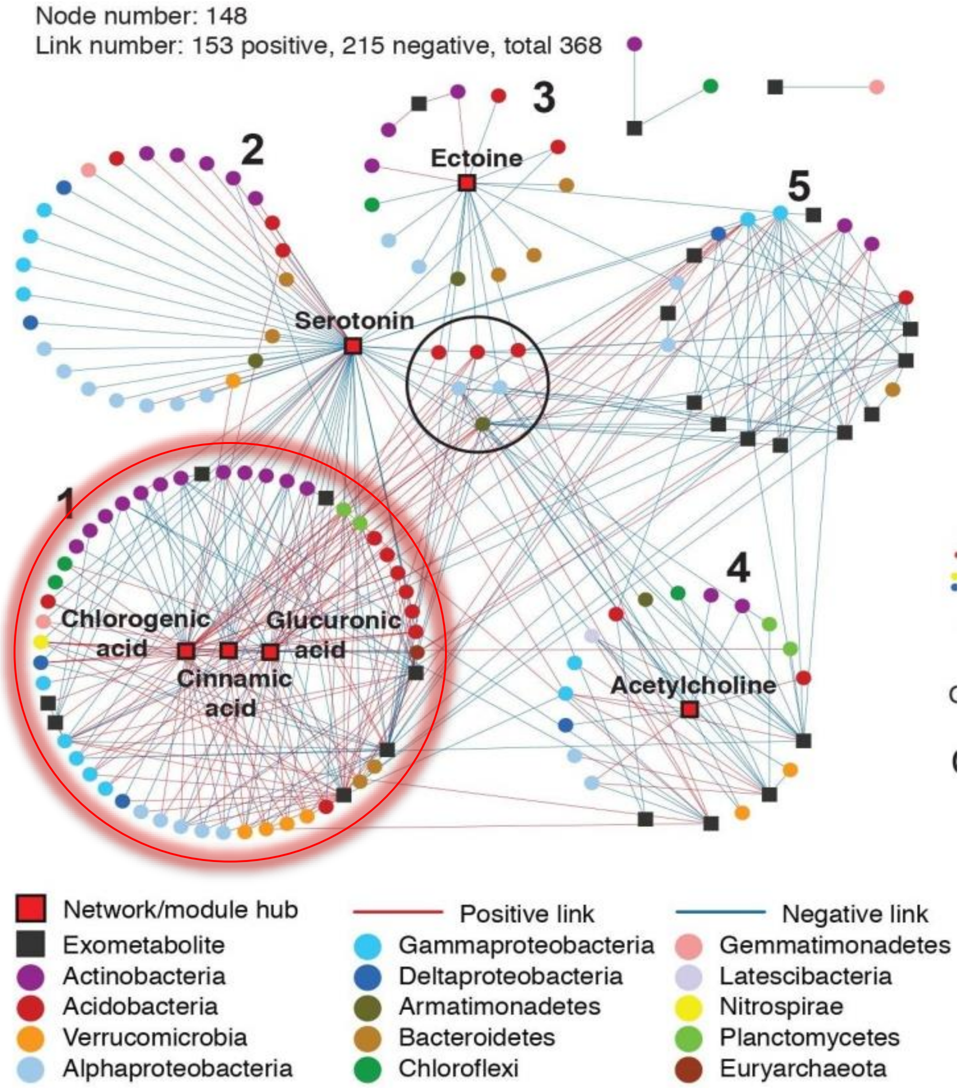
Baker and Zhalnina et al (doi: https://doi.org/10.1101/2022.06.20.496911)

Phenolic acids are selectively used by many beneficial metabolites and elevated during plant stress

Phenolic acids are elevated under N stress



Aromatic acids are correlated with diverse bacteria



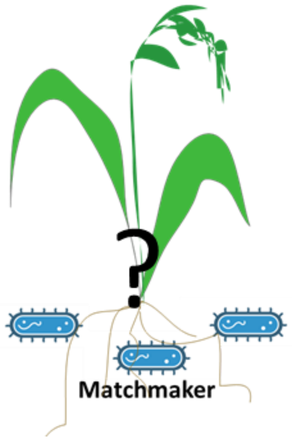
Kate Zhalnina (LBNL)

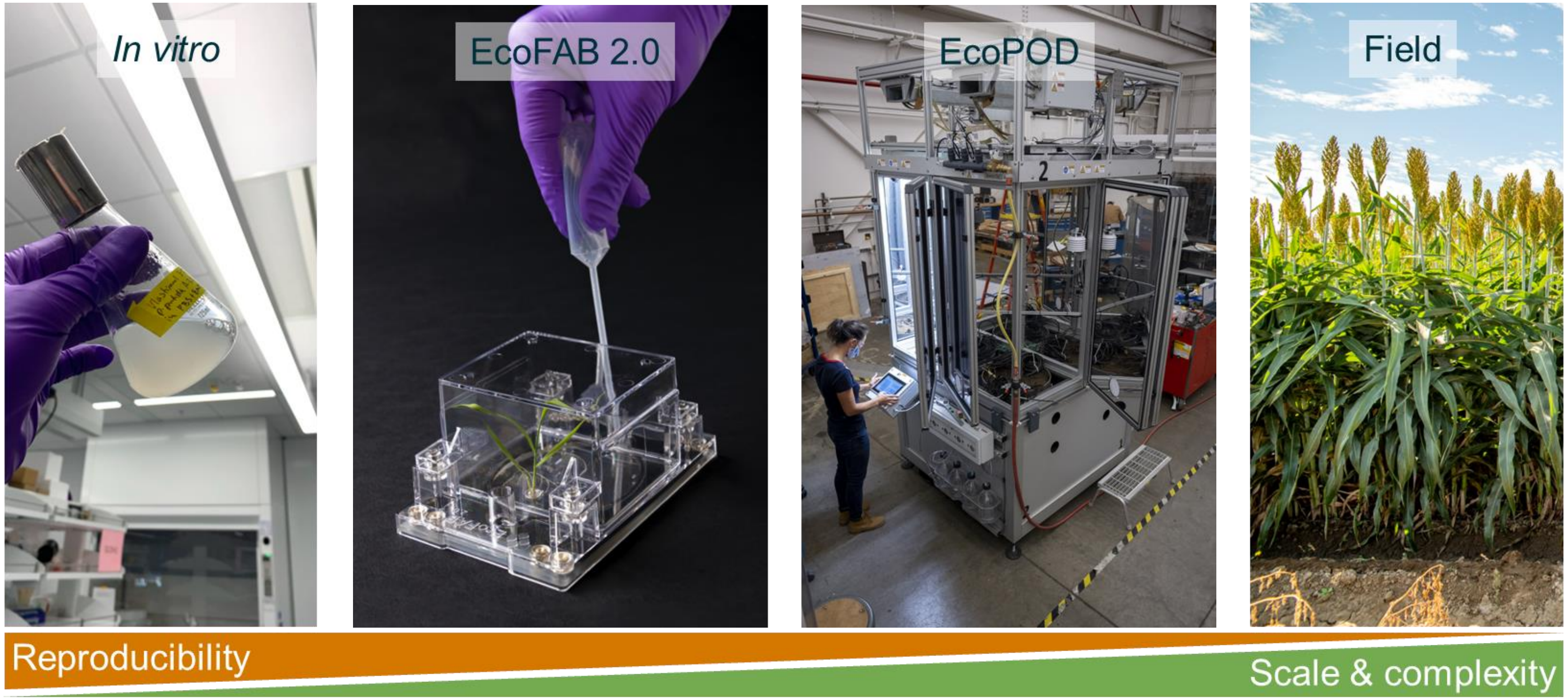
Nameer Baker (UC Berkeley)

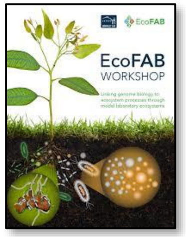
Mary Firestone UC Berkeley

Baker and Zhalnina et al bioRxiv doi: https://doi.org/10.1101/2022.06.20.496911

Need for standardized rhizosphere ecosystems to test predictions and deconstruct plant microbe interactions

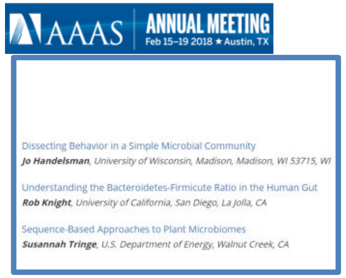






2016
EcoFAB
workshop

2017
EcoFAB
summit



AAAS ANNUAL MEETING Feb 15–19 2018 ★ Austin, TX

Dissecting Behavior in a Simple Microbial Community
***Jo Handelsman**, University of Wisconsin, Madison, Madison, WI 53715, WI*

Understanding the Bacteroidetes-Firmicute Ratio in the Human Gut
***Rob Knight**, University of California, San Diego, La Jolla, CA*

Sequence-Based Approaches to Plant Microbiomes
***Susannah Tringe**, U.S. Department of Energy, Walnut Creek, CA*

2018 AAAS
Session

nature methods

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Comment | Published: 21 June 2019

EcoFABs: advancing microbiome science through standardized fabricated ecosystems

2019 Perspective
paper

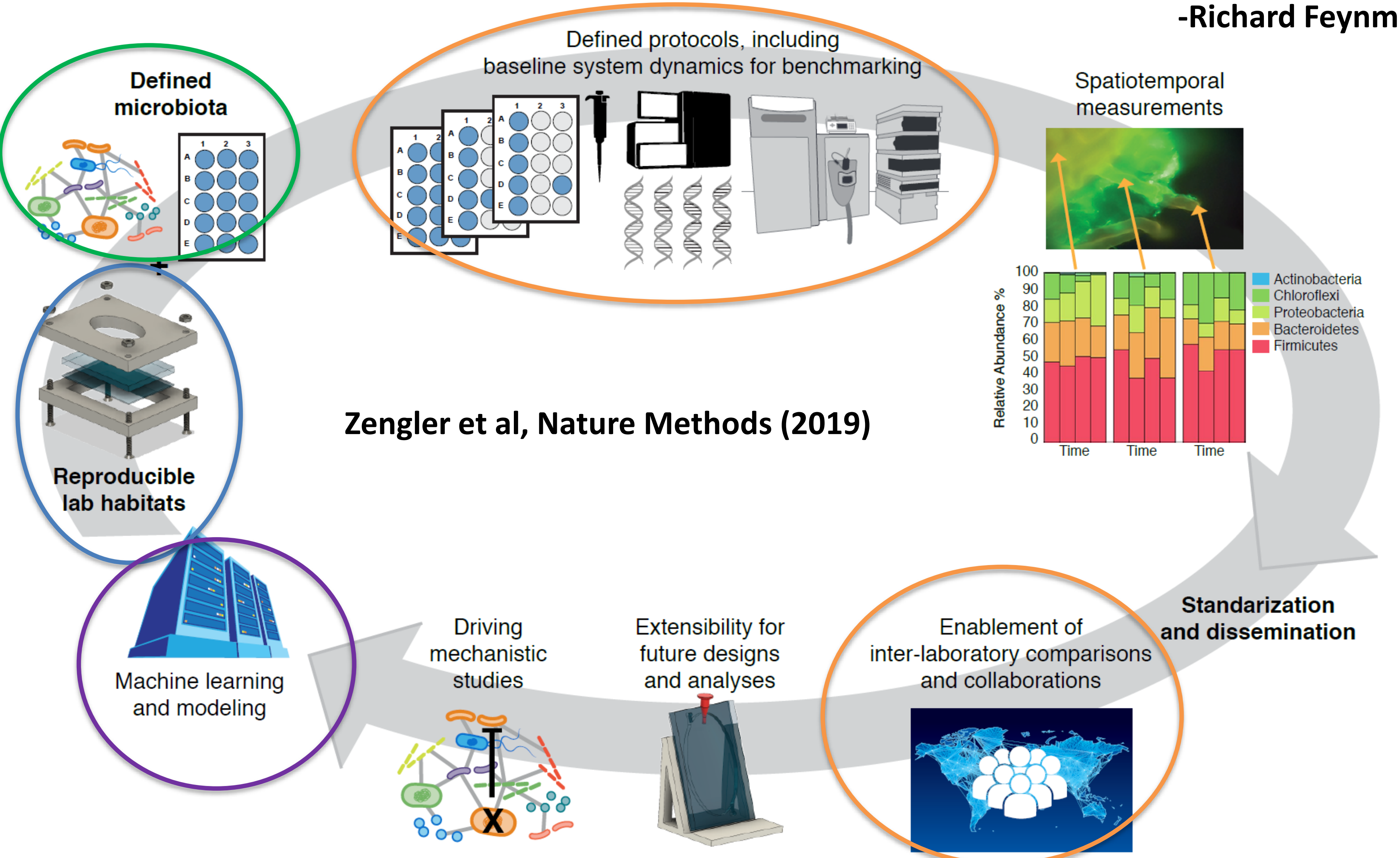
2020 AAAS Session

2021 EcoFAB mini-
conference

https://www.nature.com/articles/s41592-019-0465-0

Roadmap for using standardized fabricated ecosystems to accelerate microbiome science

“What I cannot create, I do not understand”
-Richard Feynman



Defined microbiota

Defined protocols, including baseline system dynamics for benchmarking

Spatiotemporal measurements

Standarization and dissemination

Enablement of inter-laboratory comparisons and collaborations

Extensibility for future designs and analyses

Driving mechanistic studies

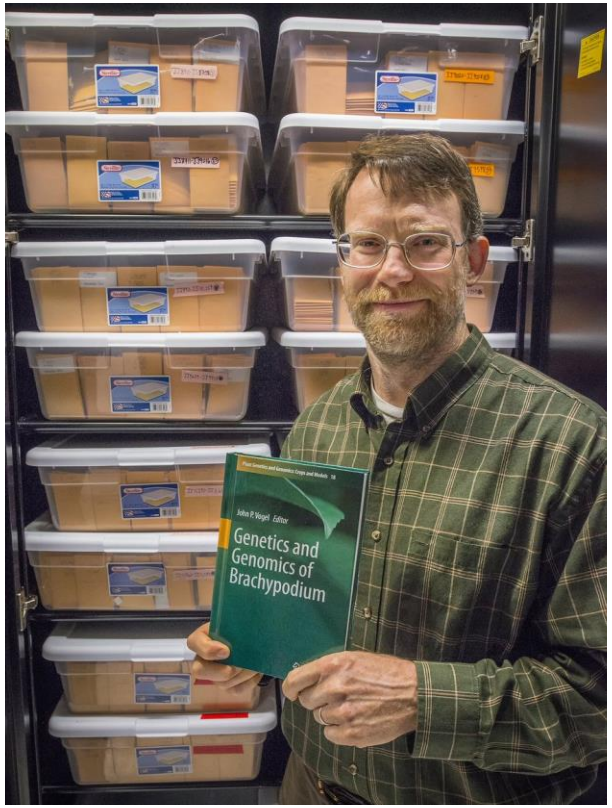
Machine learning and modeling

Reproducible lab habitats

Zengler et al, Nature Methods (2019)

EcoFAB summit

Focused on the model plant *B. distachyon*

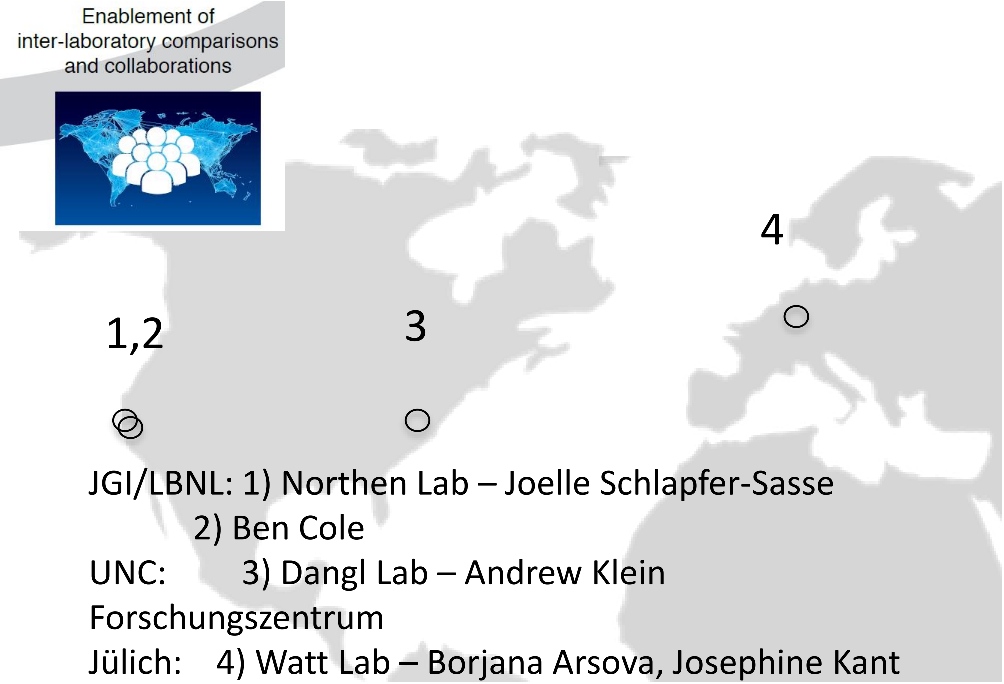


John Vogel (JGI)

Brachypodium distachyon

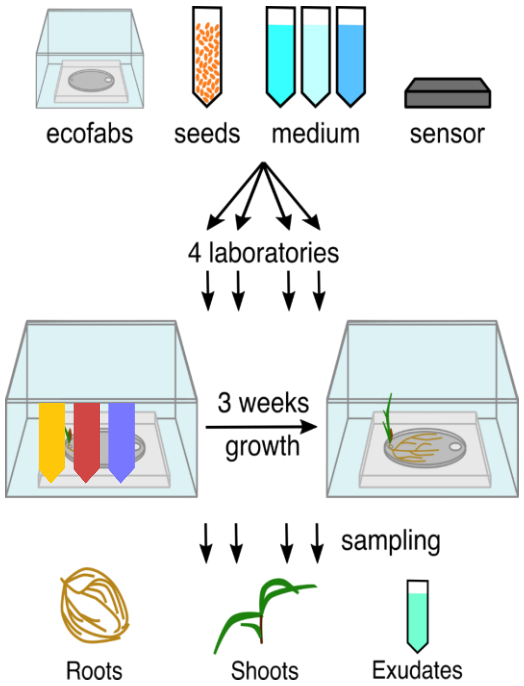
- JGI flagship Model for biomass grasses
- Mutant resources (>300,000 sequenced mutations)
- Experimentally tractable
  - Small, self fertile
  - Small genome (272Mb)
  - Short generation time, 8 weeks
  - Easily transformed
  - 'Finished' genome

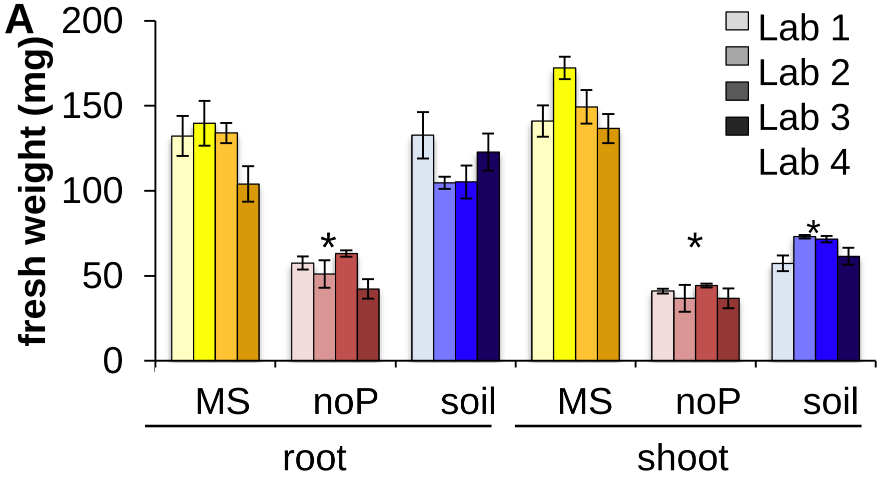
Multi-lab study demonstrated EcoFAB reproducibility

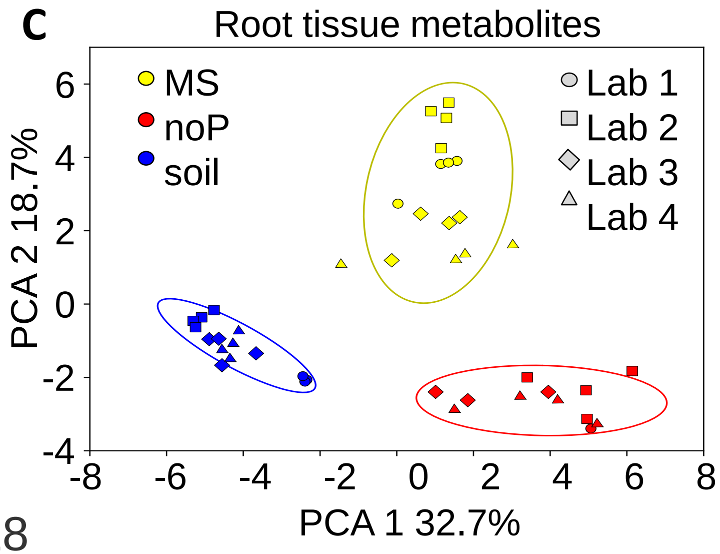


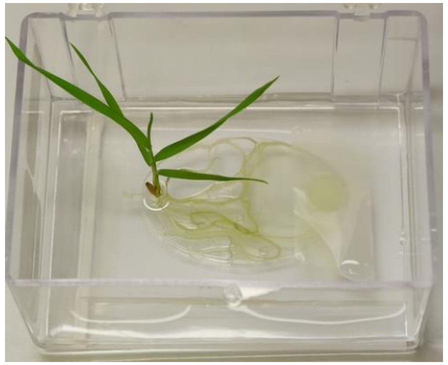
JGI/LBNL: 1) Northen Lab – Joelle Schlapfer-Sasse
2) Ben Cole
UNC: 3) Dangl Lab – Andrew Klein
Forschungszentrum
Jülich: 4) Watt Lab – Borjana Arsova, Josephine Kant

Joelle Schlapfer-Sasse









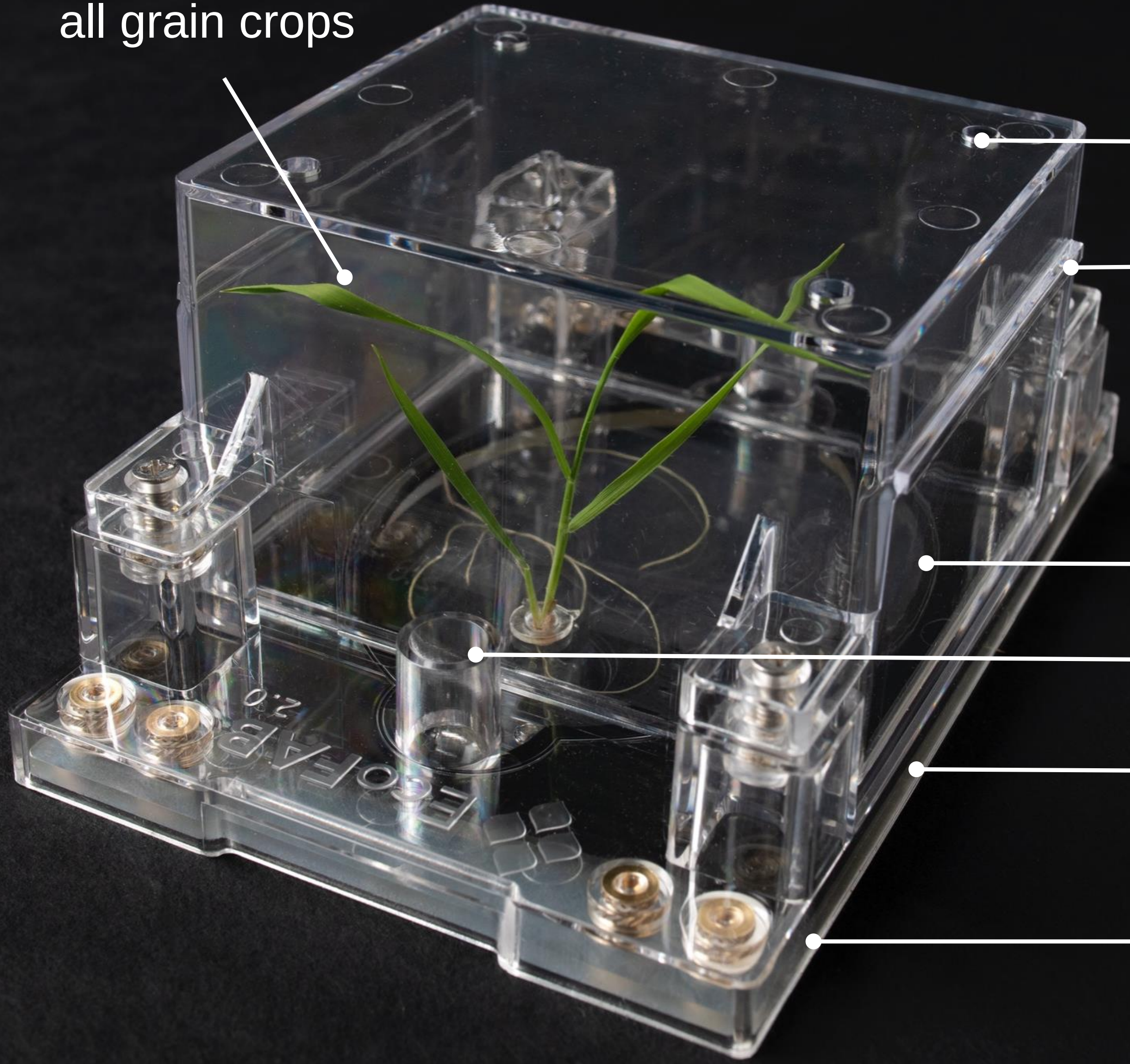
EcoFAB 1.0

Sasse et al 2018 https://doi.org/10.1101/435818

EcoFAB 2.0

Brachypodium distachyon

- Model grass relevant to all grain crops



Luer-compatible vents (gas analysis)

Gas-tight top chamber

Root chamber *V* = 10 ml

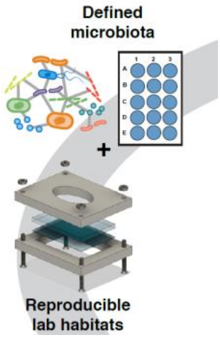
Sampling ports (sterile when sealed)

Transparent bottom (inverted microscopy)

Microplate-sized bottom plate
(equipment integration)

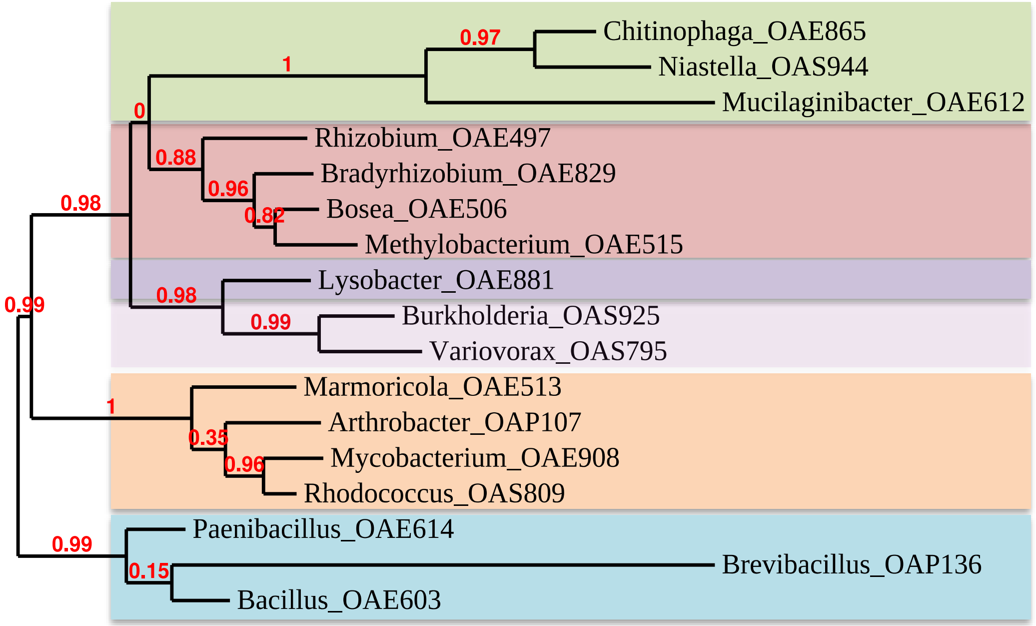
Novak & Andeer et al., bioRxiv (2023): 2023-01, Patent:16/876,415

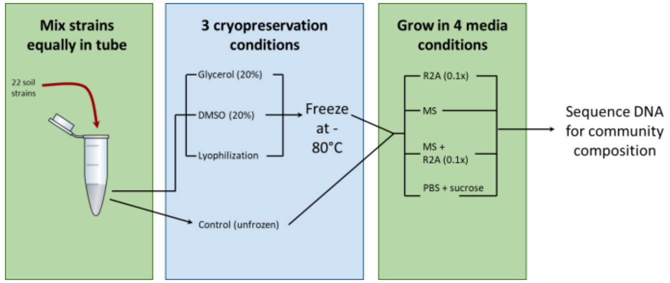
Defined microbiota: Developed a standardized 17-member SynCom



>1,000 isolates

Spans diversity and abundance in the field + relevance to other grasses + has many desired traits





Mix strains equally in tube

22 soil strains

3 cryopreservation conditions

Glycerol (20%)

DMSO (20%)

Lyophilization

Freeze at -80°C

Control (unfrozen)

Grow in 4 media conditions

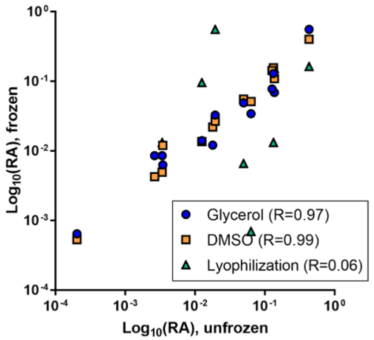
R2A (0.1x)

MS

MS + R2A (0.1x)

PBS + sucrose

Sequence DNA for community composition



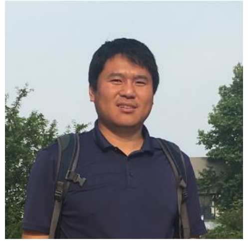
Kate Zhalnina (LBNL)

Karsten Zengler (UCSD)

Joanna Coker (UCSD)

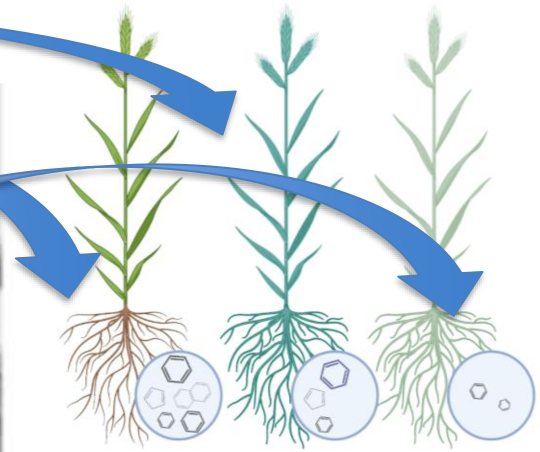
Coker et al mSystems 2022 https://doi.org/10.1128/msystems.00951-22

Exometabolite profiling of diverse *B. distachyon* lines to identify lines with elevated aromatic acid composition



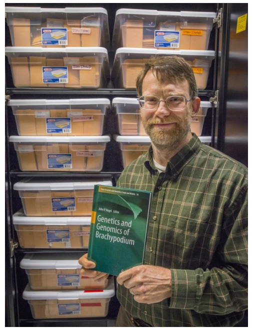
Yezhang Ding

Kate Zhalnina

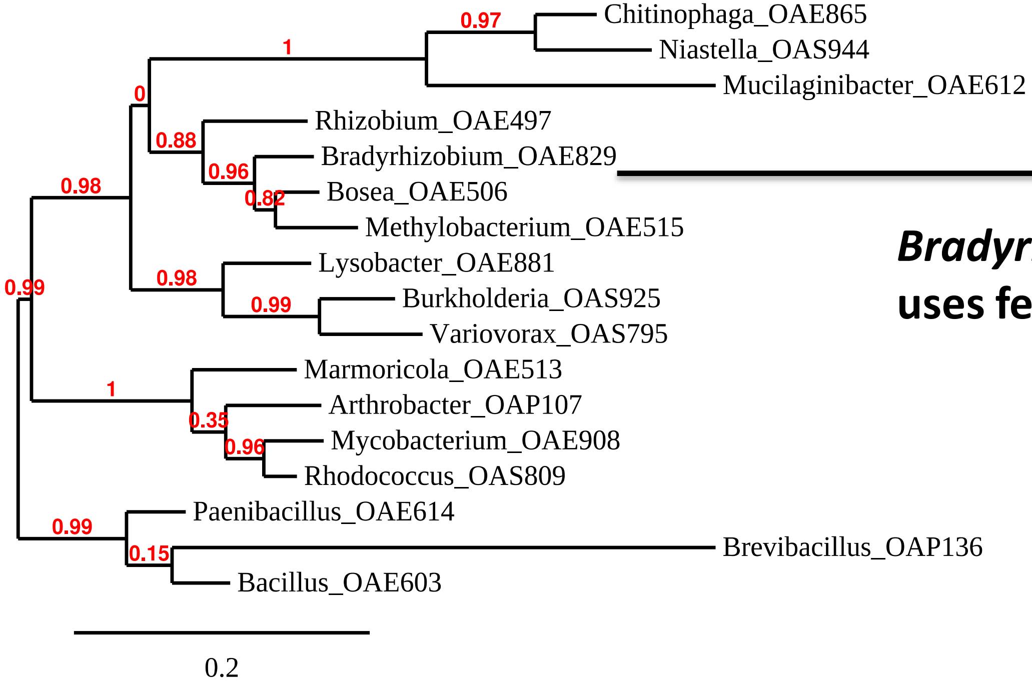


Gordon et al, Nature Communications 2017

John Vogel (JGI)



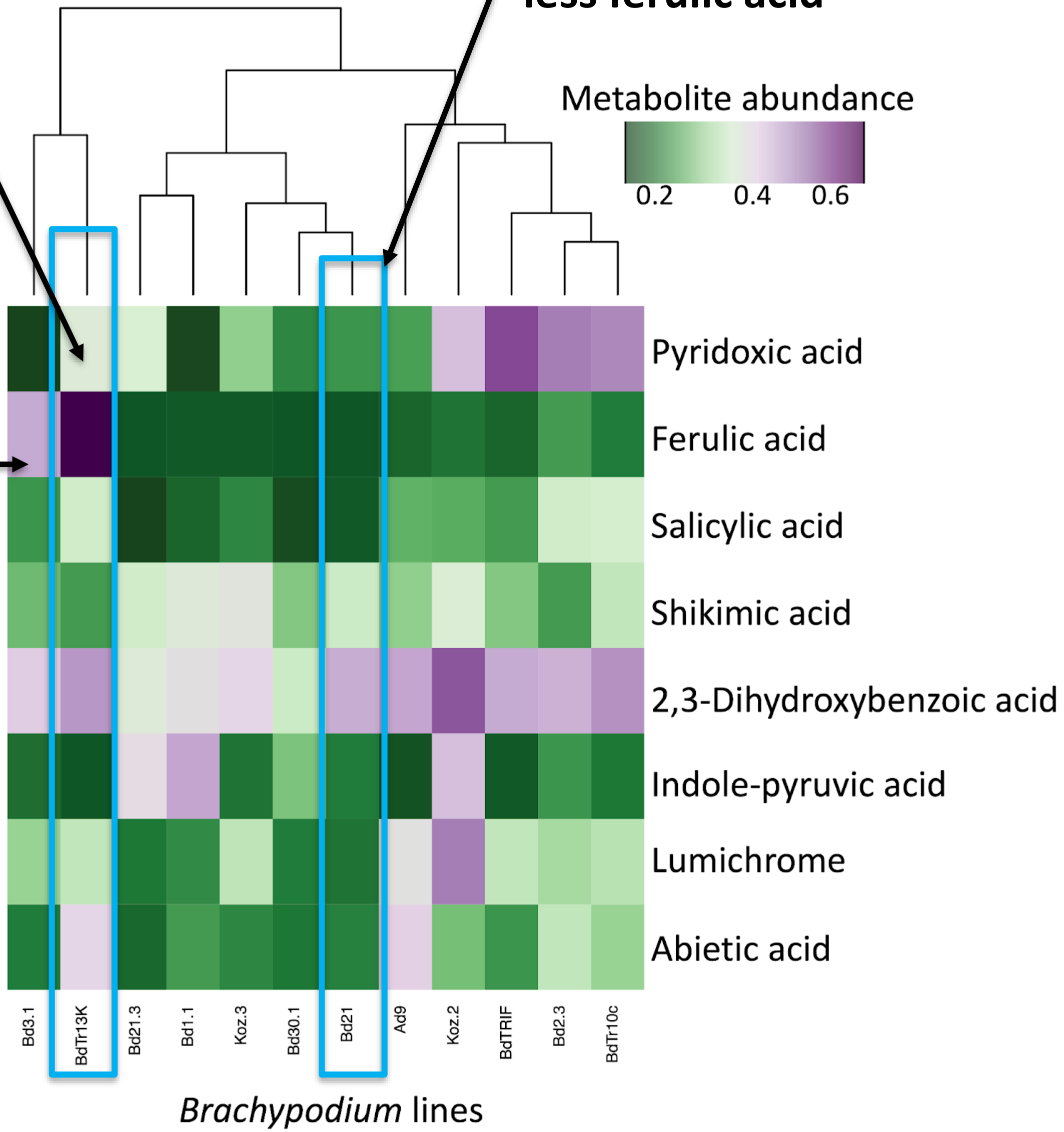
BdTR13 exudates have the highest levels of ferulic acid



Bradyrhizobium **uses ferulic acid**

The BdTR13K line has the highest levels of ferulic acid

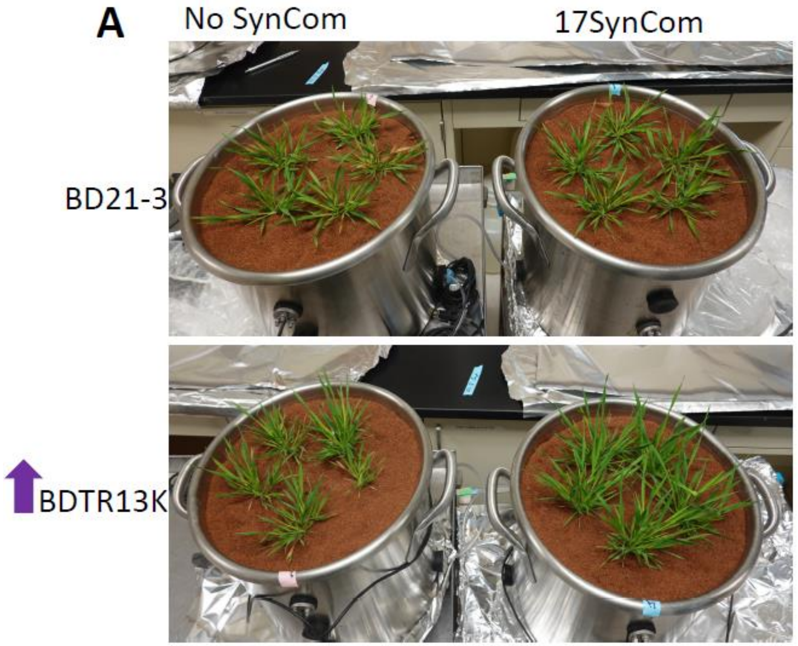
Bd21 exudes much less ferulic acid

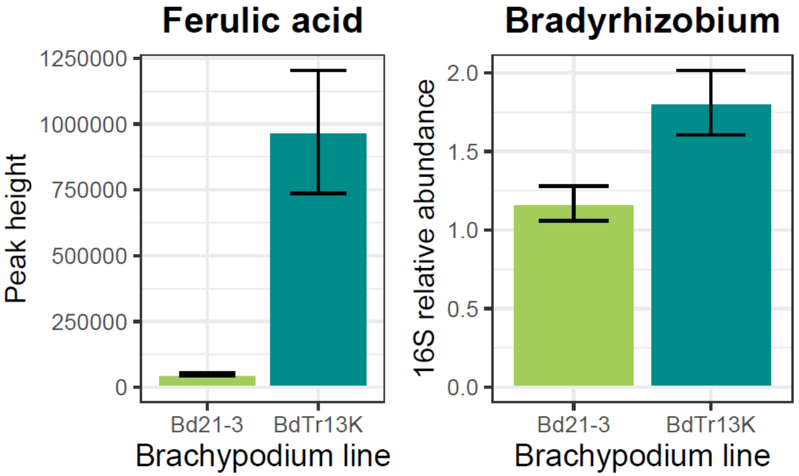


Using the EcoPOD to test if SynCom members that prefer aromatic acids are enriched in the high producing *B. distachyon* line

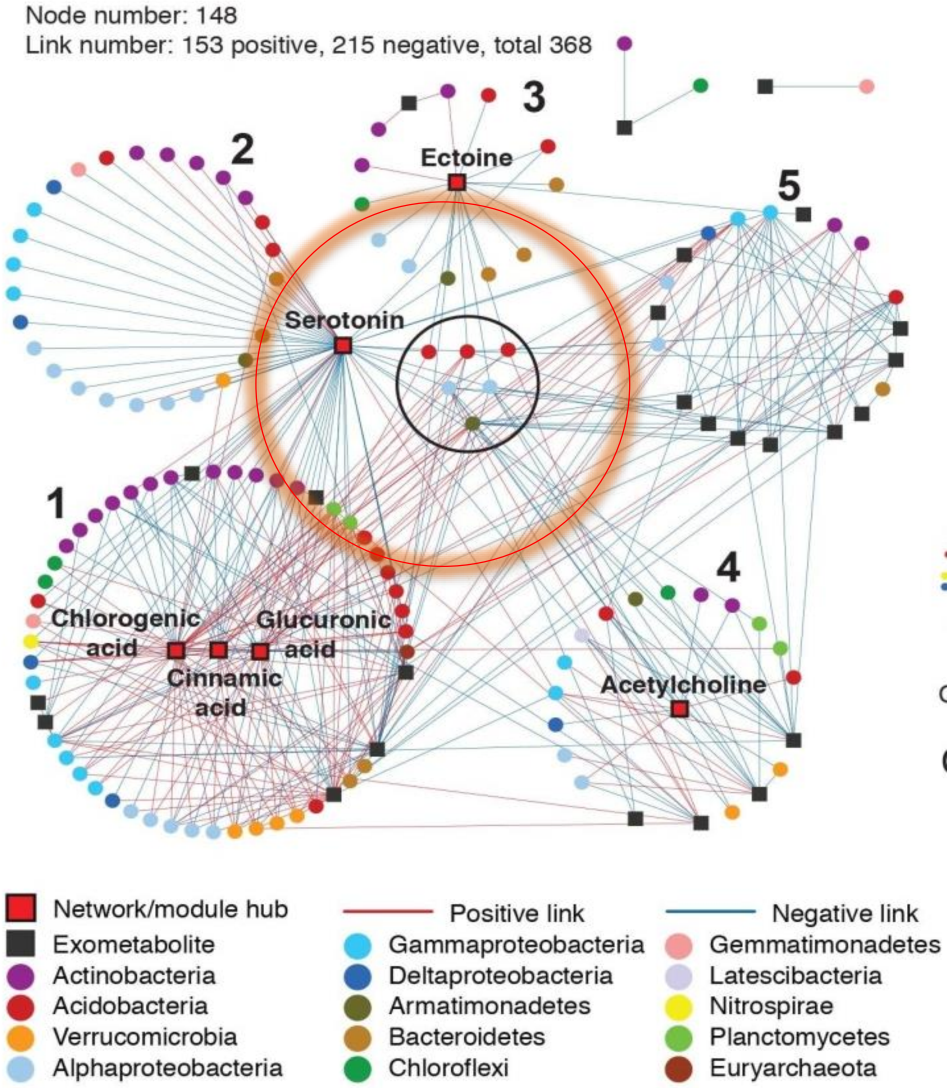
Kate Zhalnina

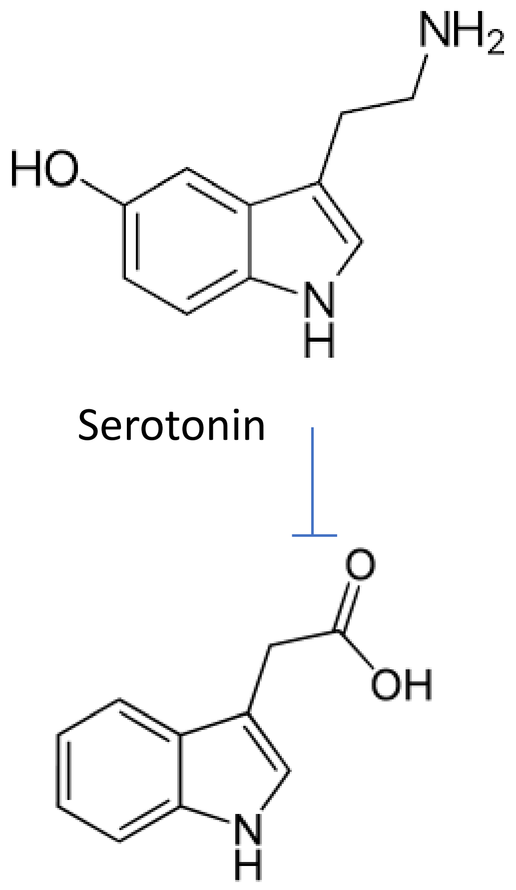
Albina Khasanova





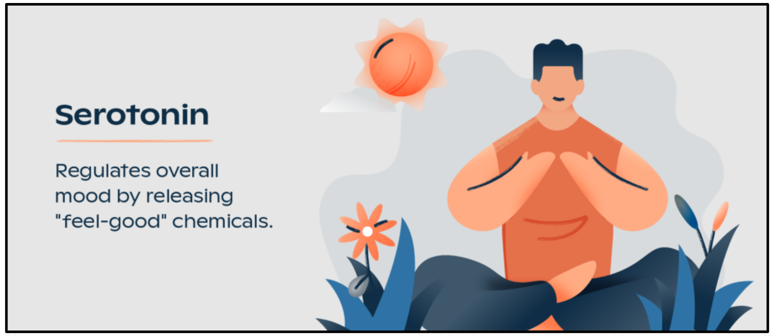
Serotonin is another 'hub' metabolite in our rhizosphere study





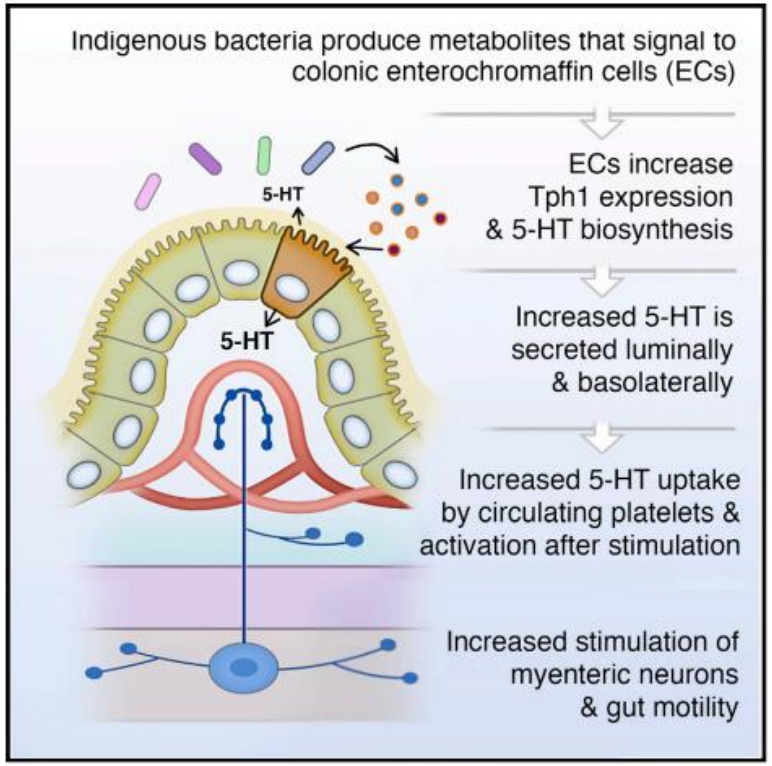
Serotonin

Indol-3-acetic acid



Serotonin

Regulates overall mood by releasing "feel-good" chemicals.



Indigenous bacteria produce metabolites that signal to colonic enterochromaffin cells (ECs)

ECs increase Tph1 expression & 5-HT biosynthesis

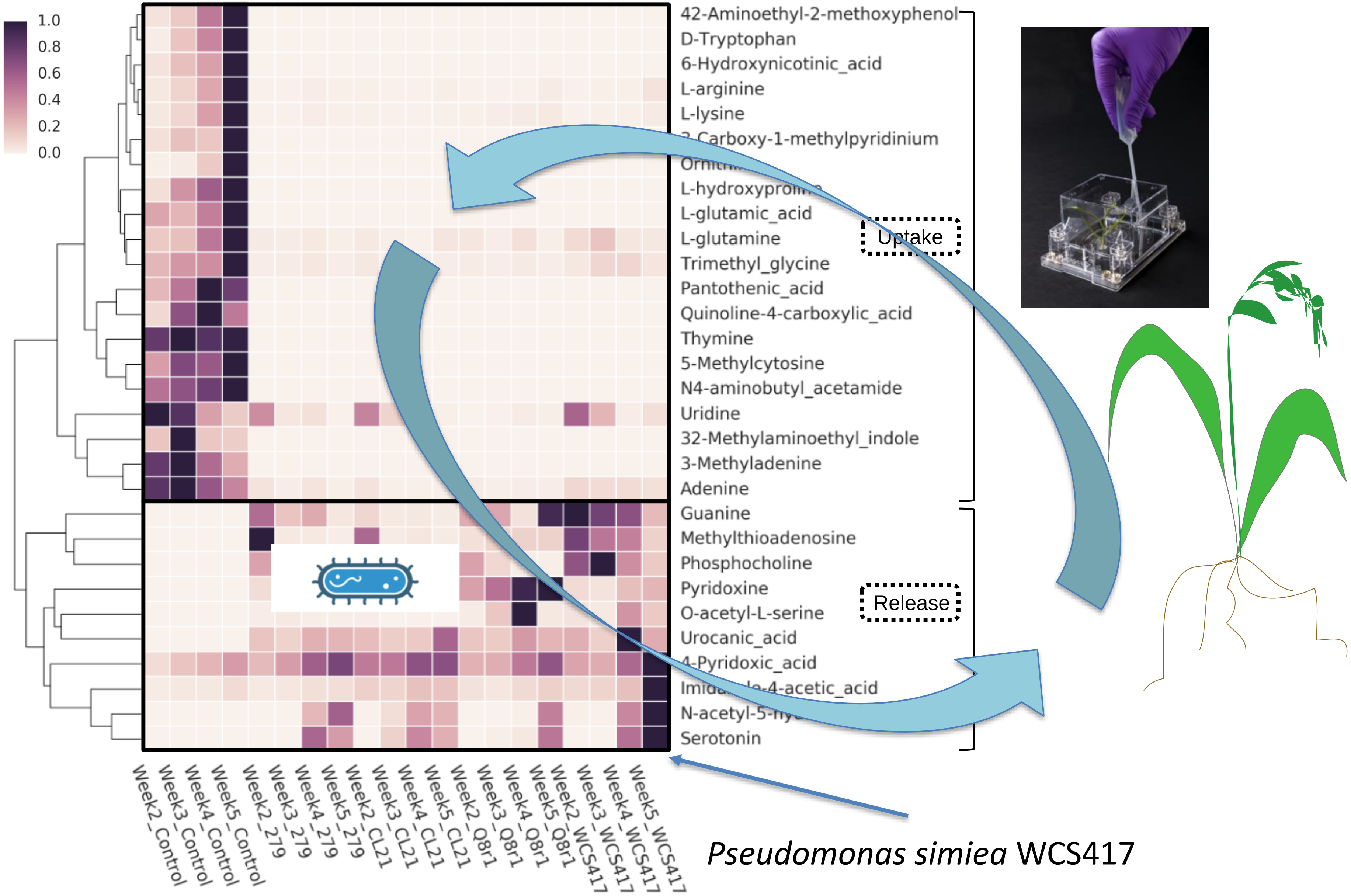
Increased 5-HT is secreted luminally & basolaterally

Increased 5-HT uptake by circulating platelets & activation after stimulation

Increased stimulation of myenteric neurons & gut motility

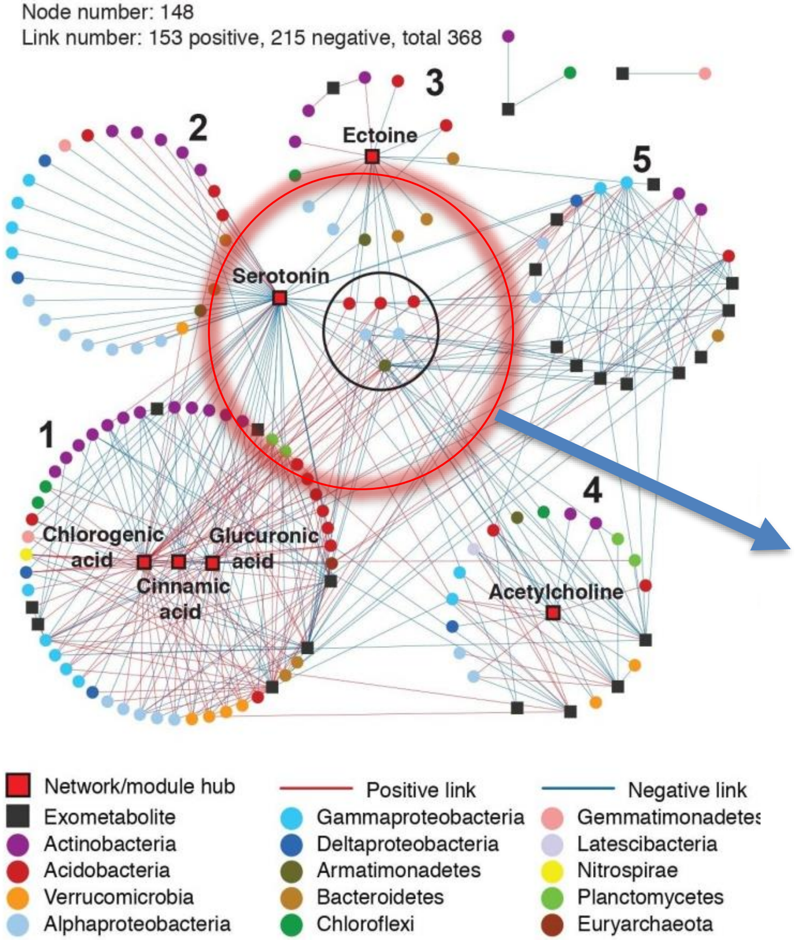
Yano et al *Cell* 2015
(Serotonin = 5-Hydroxytryptamine)

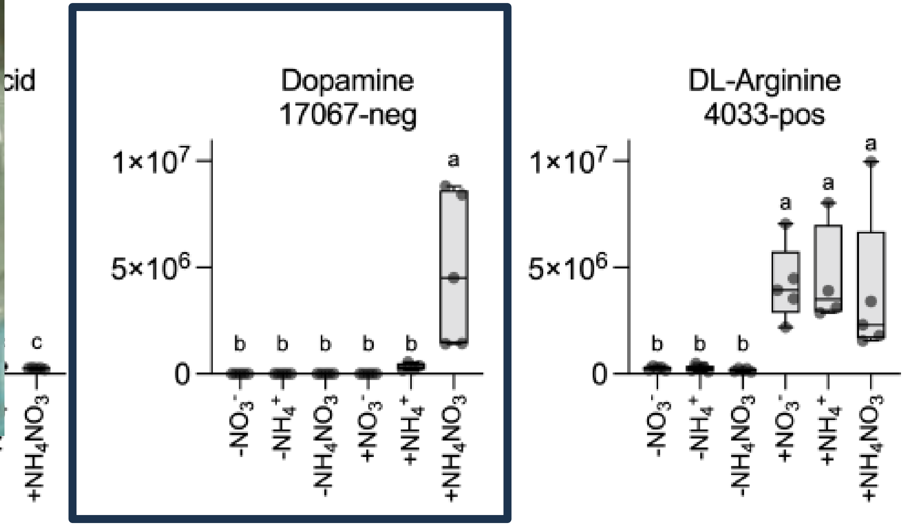
We have observed elevated serotonin when switchgrass is colonized by specific bacteria



Pseudomonas simiea WCS417

Now observed that dopamine is elevated in exudates from plants grown on ammonium nitrate





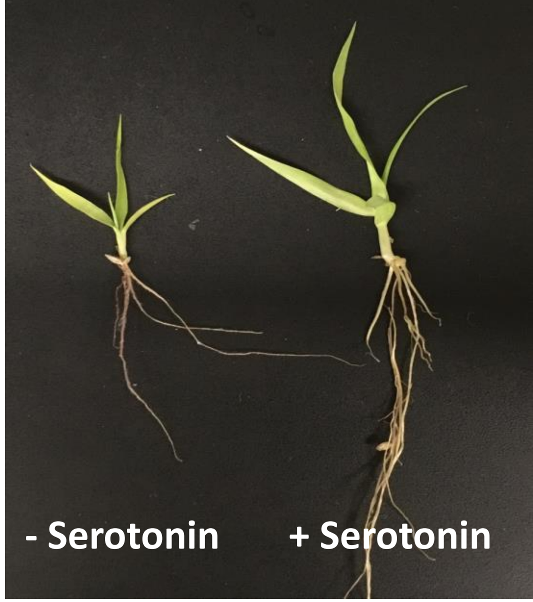
Vlastimil Novak

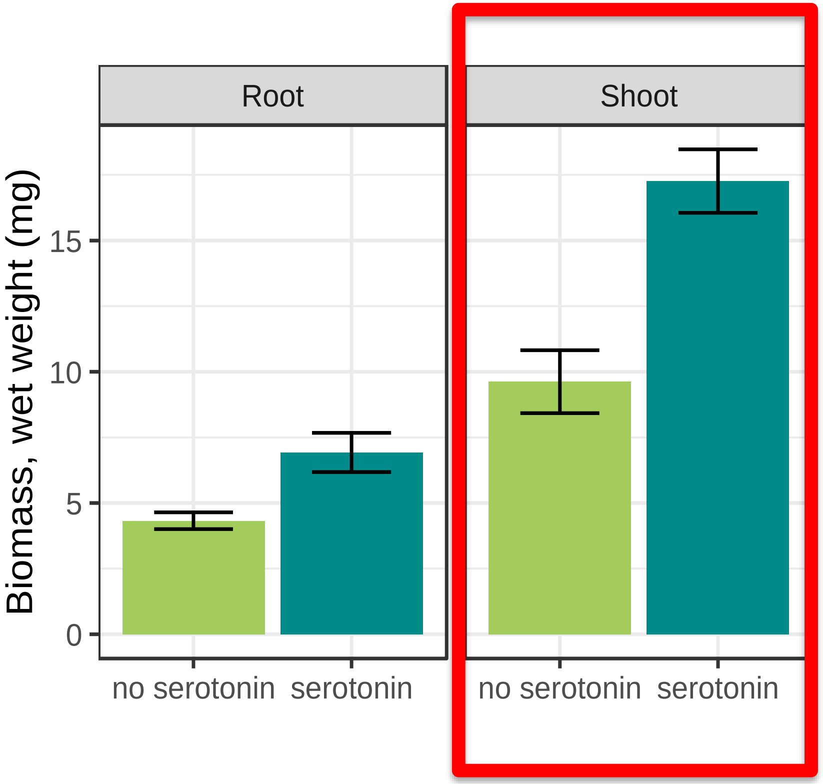
Novak et al, Science Advances (in press)

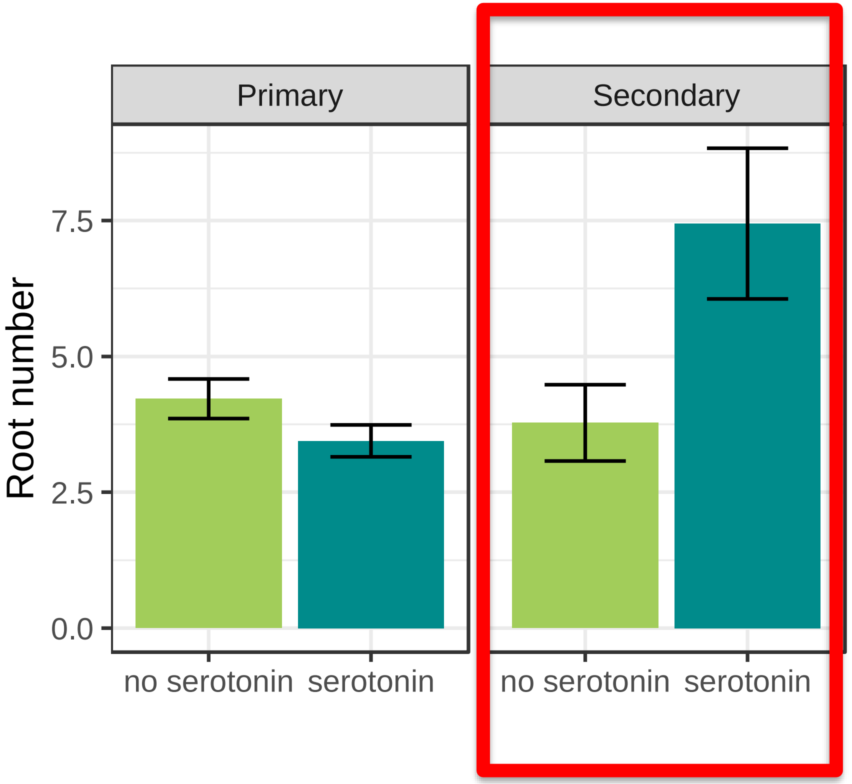
Serotonin promoted root and shoot growth and total length and number of secondary roots

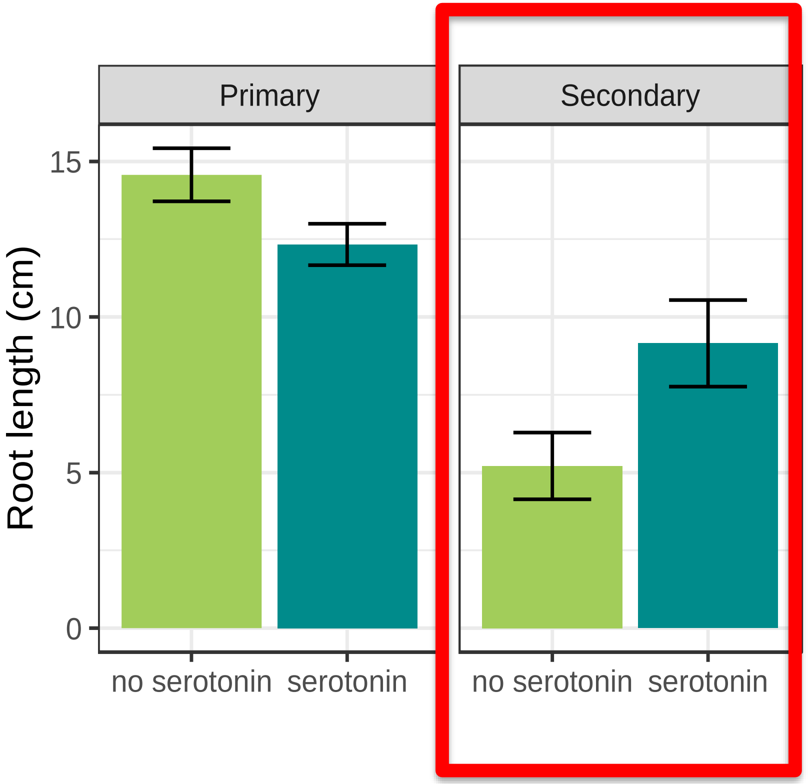
Added 100 uM serotonin to 7 day old switchgrass and incubated for 18 days.

Kate Zhalnina

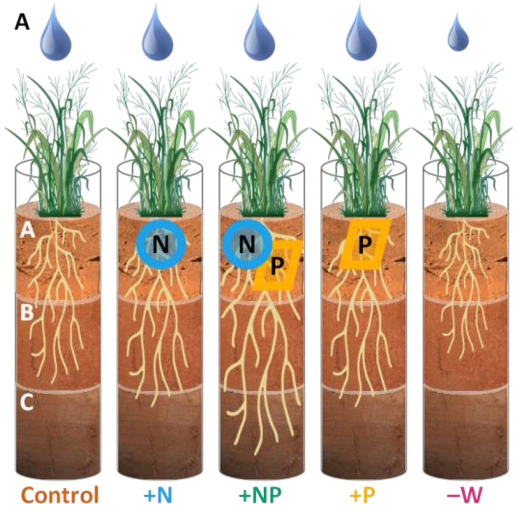


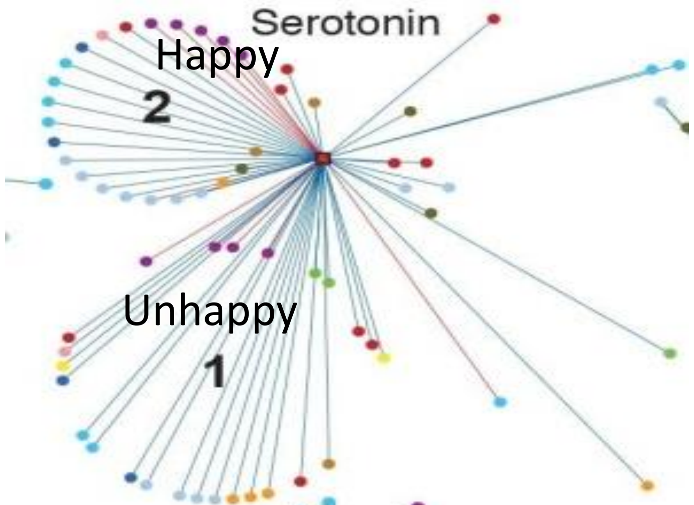


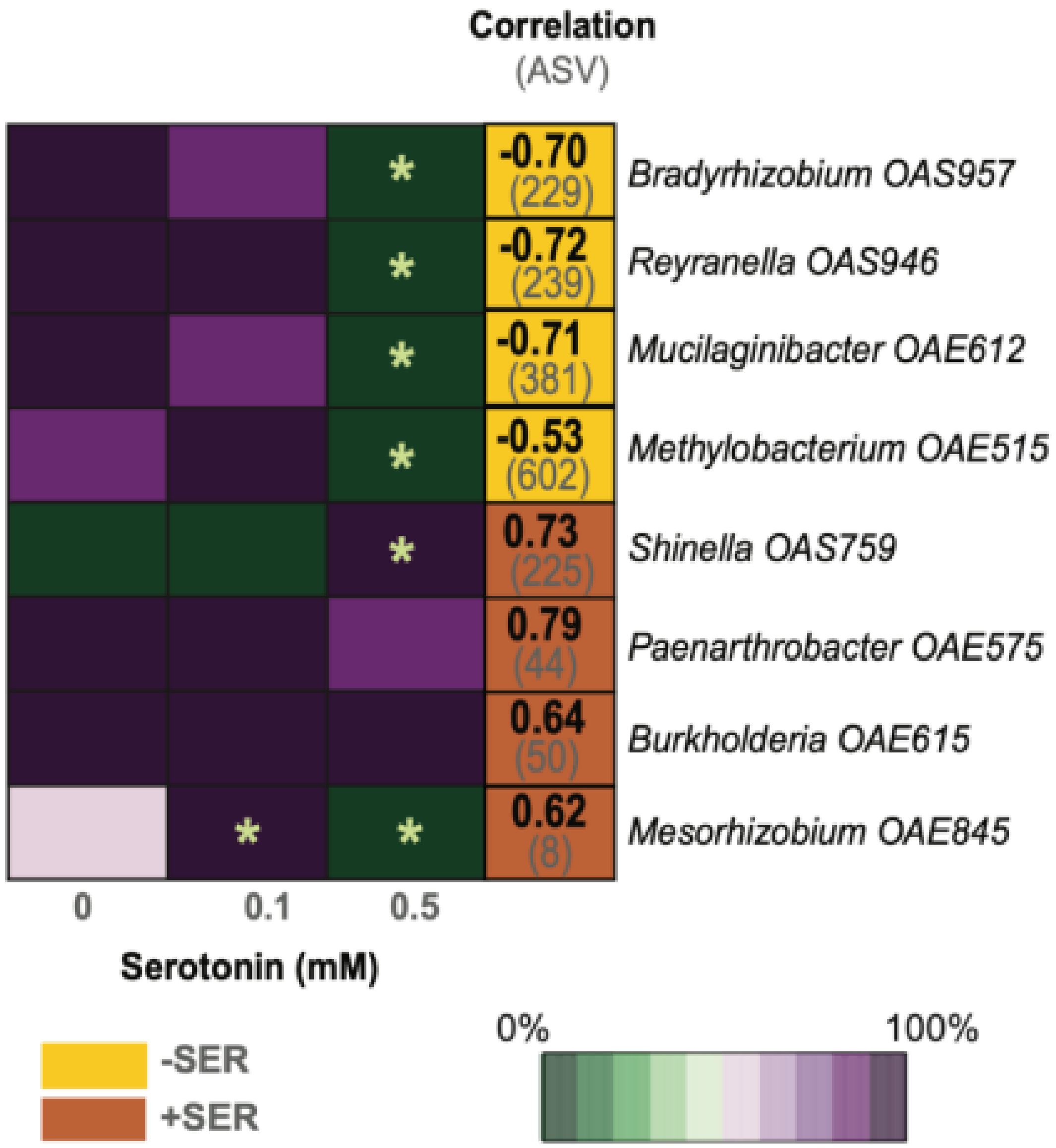




Observe differential bacterial responses to serotonin





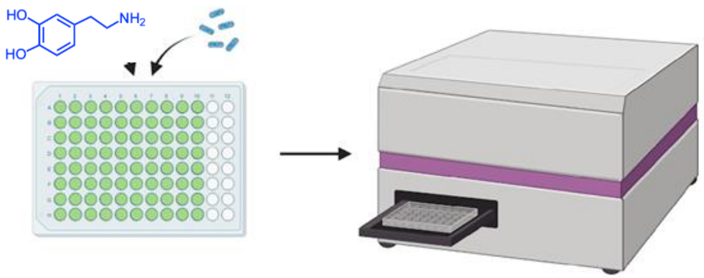


Anticorrelated with serotonin abundance

Correlated with serotonin abundance

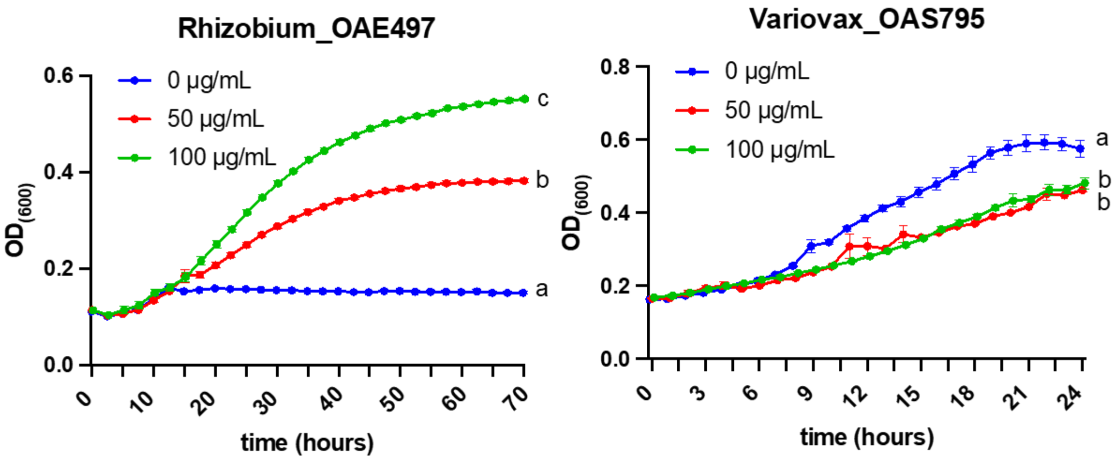
Baker and Zhalnina et al bioRxiv doi: https://doi.org/10.1101/2022.06.20.496911

Observe differential responses of individual SynCom members to catecholamines

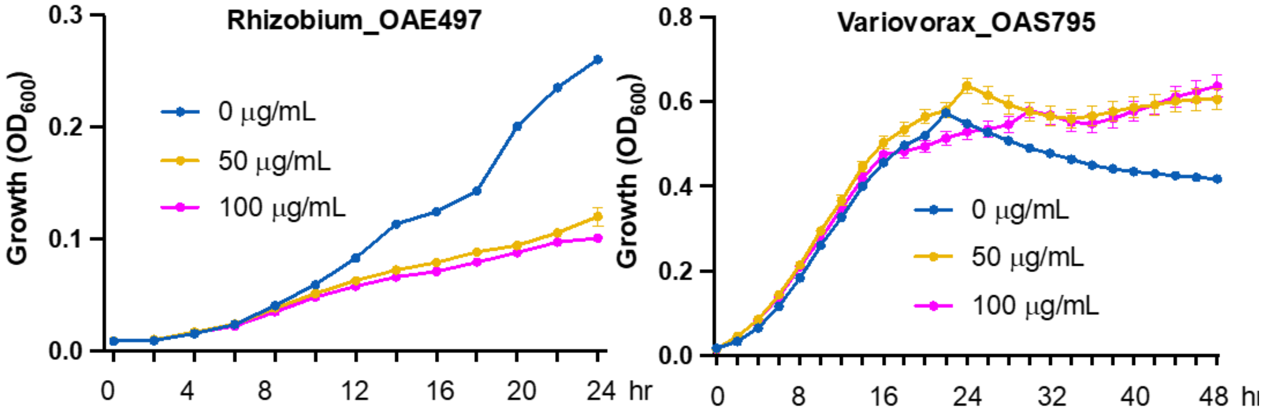


Hunter Vogel

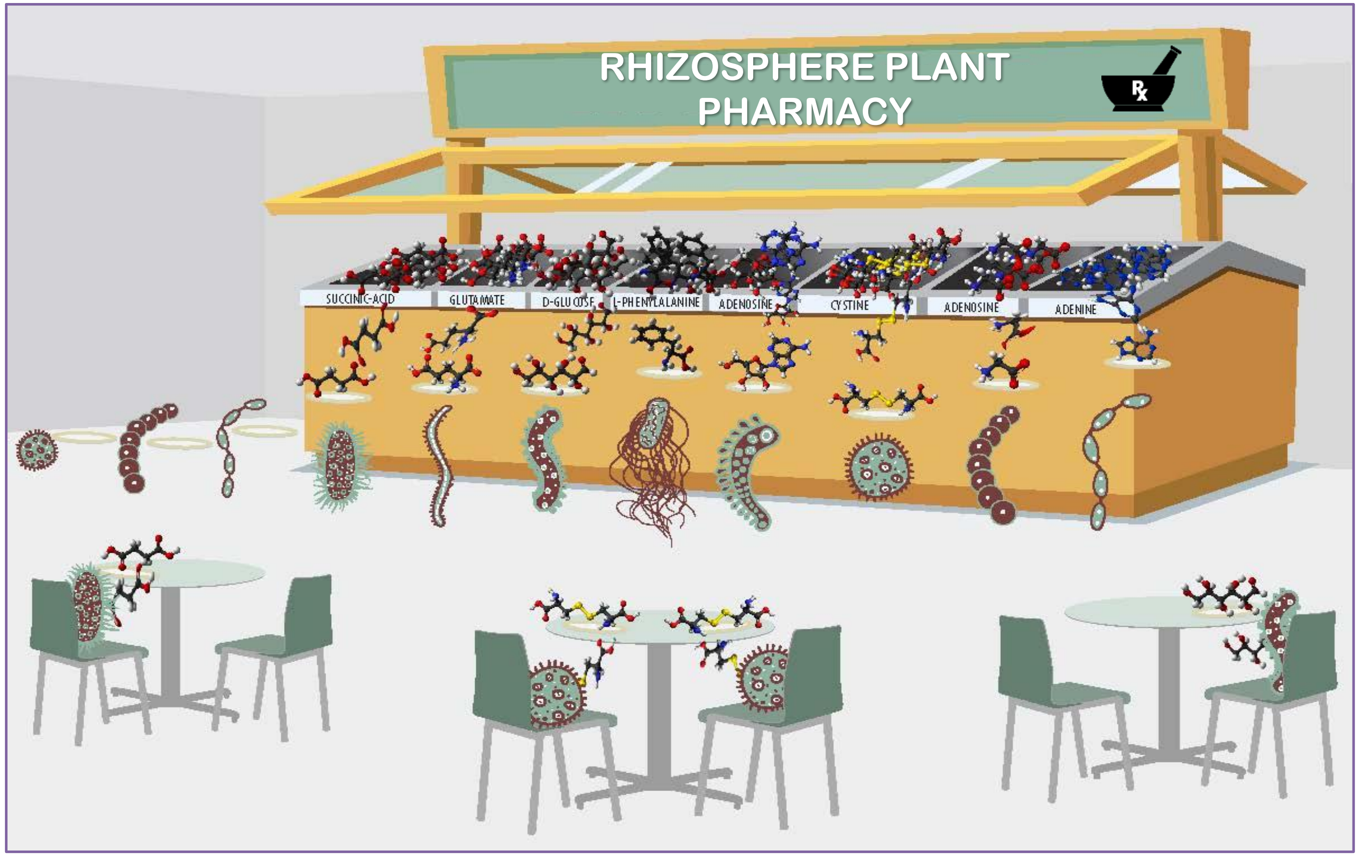
Dopamine

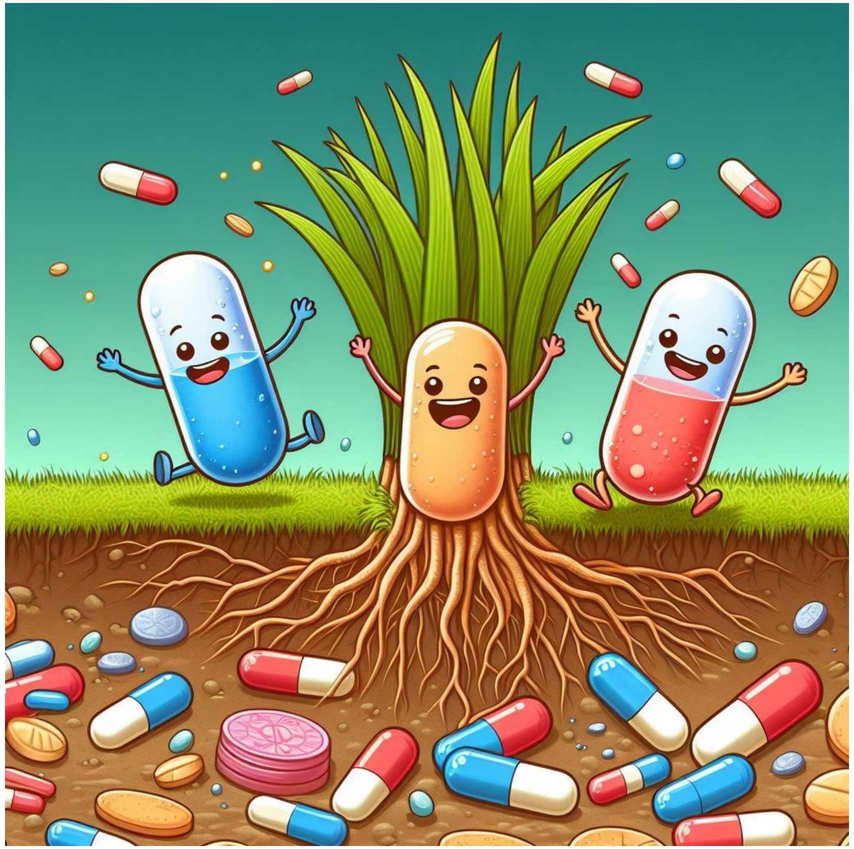


Serotonin



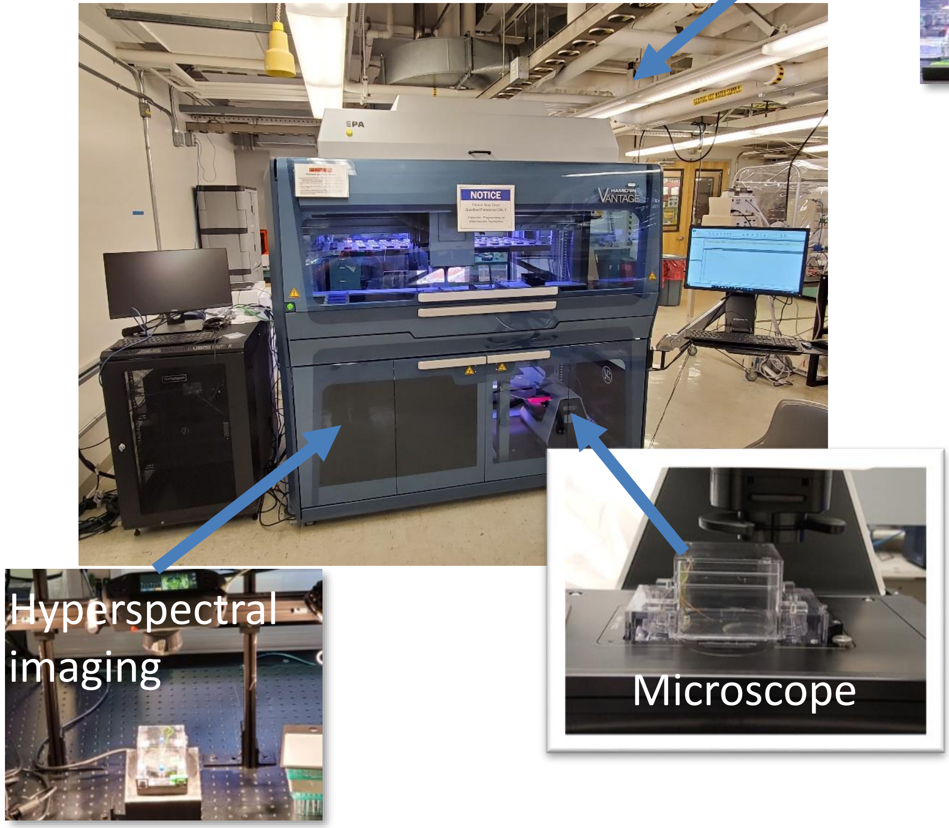
Need to consider the host-microbe feedbacks



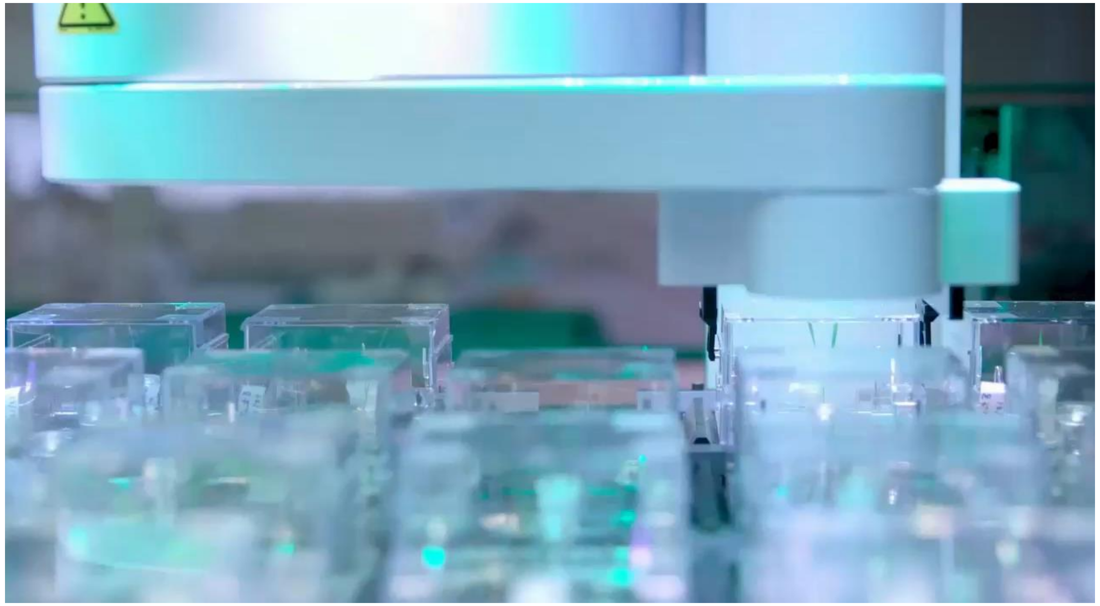


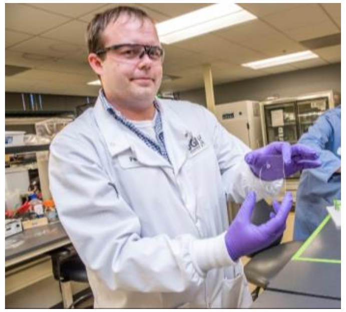
Automating EcoFAB experiments using the EcoBOT

Environmental chamber to reproduce light intensity and quality at a field site



Automation





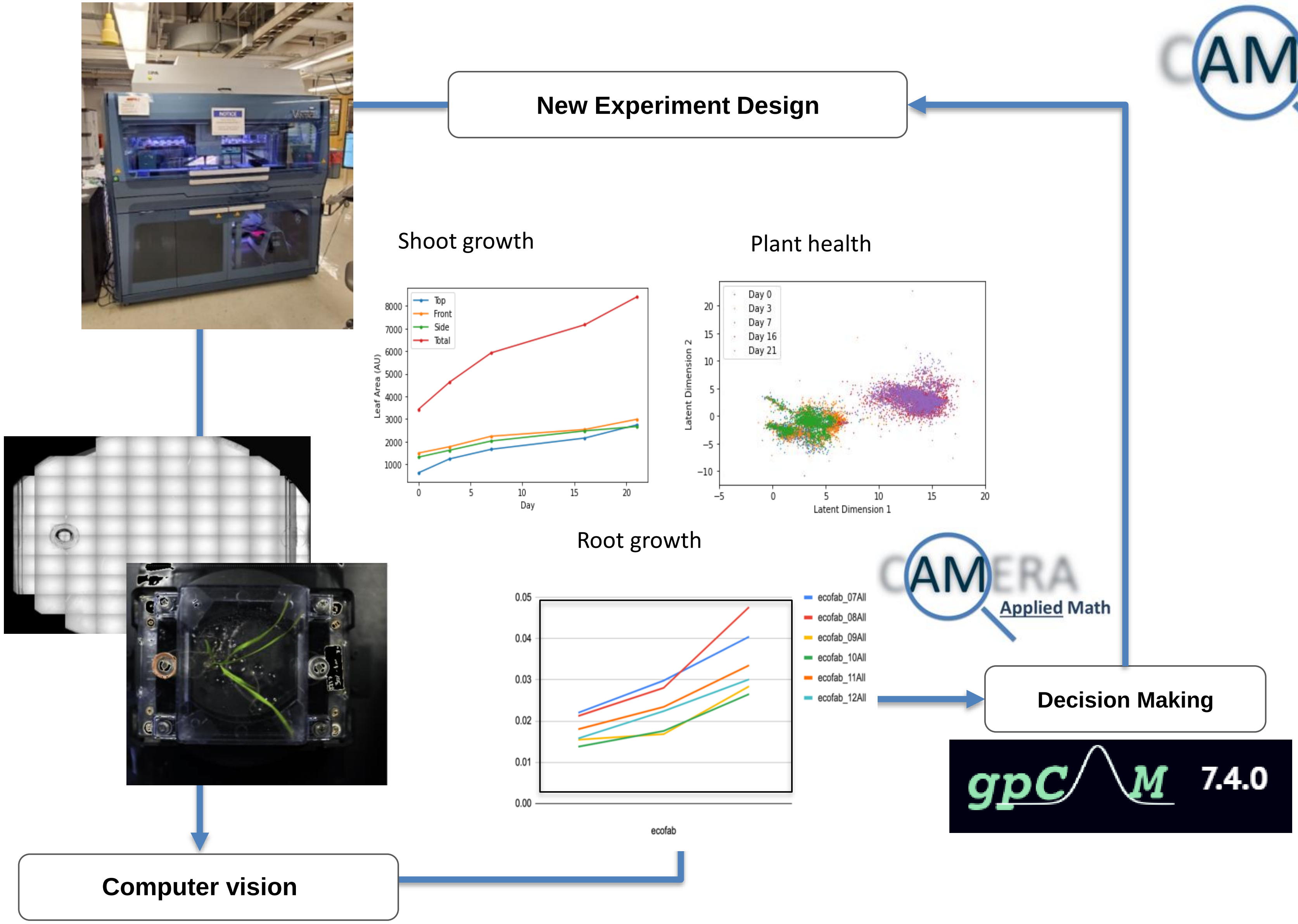
Peter Andeer

LT Cornmesser

Tom Vess

Stephen Tan

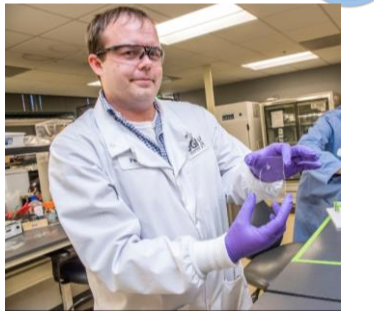
In progress: using machine learning and gpCAM to enable the EcoBOT to perform autonomous experiments



New Experiment Design

Decision Making

Computer vision



Peter Andeer

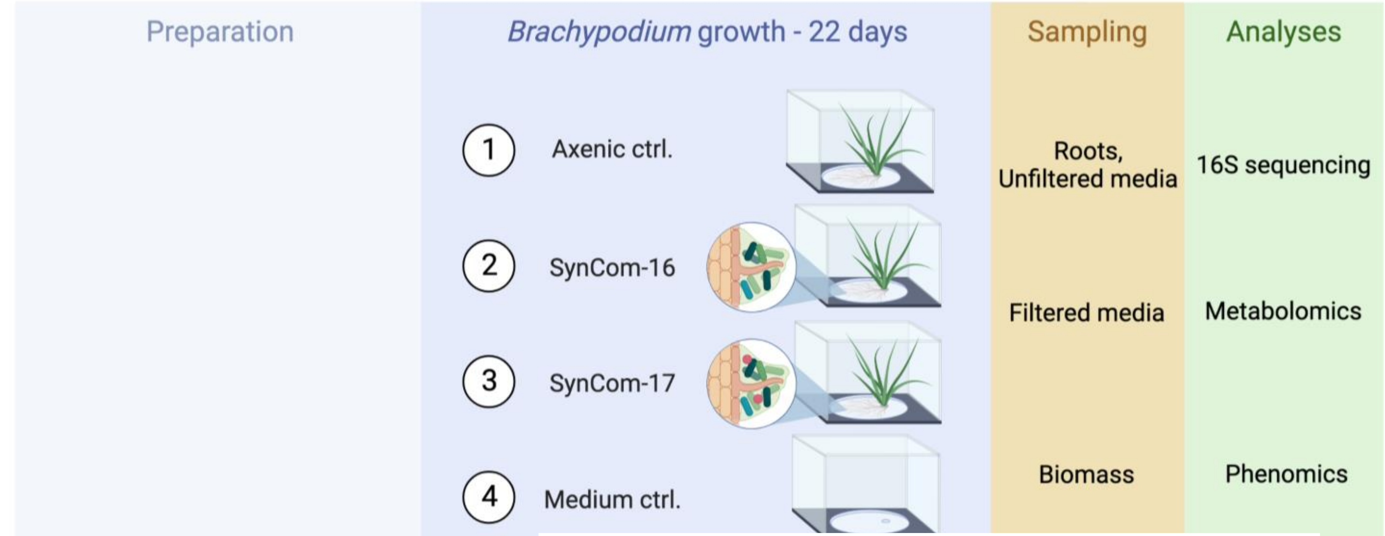
Daniela Ushizima

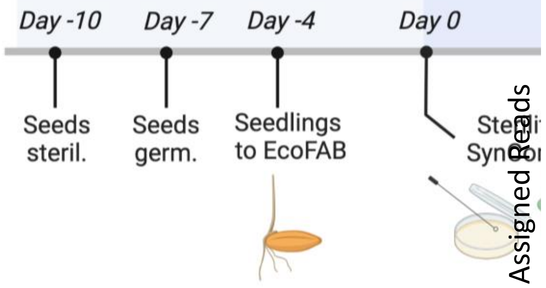
Peter Zwart

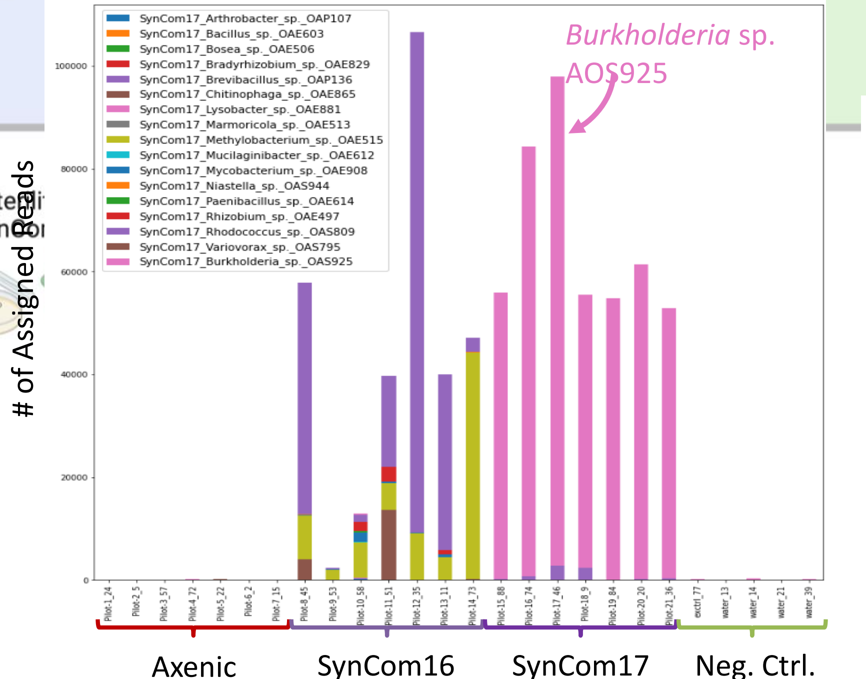
Marcus Noack

James Sethian

Standard methods and inter-lab comparisons: The first plant-microbiome ring-trial is now underway







J. Vogel
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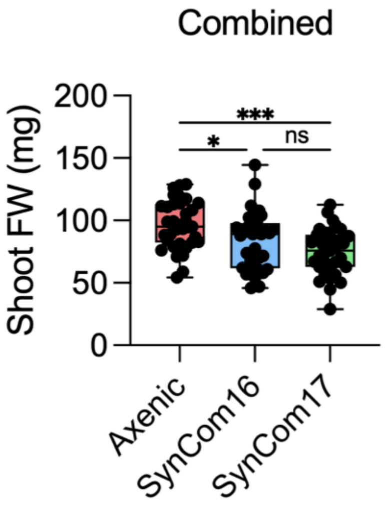
-Lefert

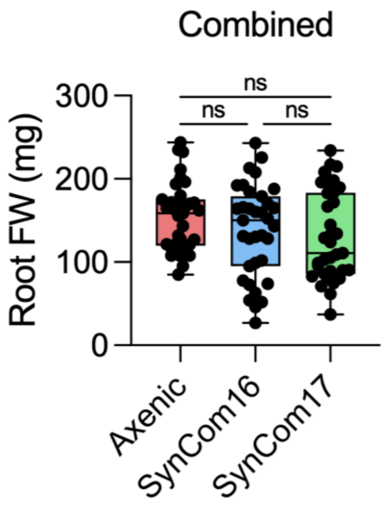
B. Arsova

M. Watt

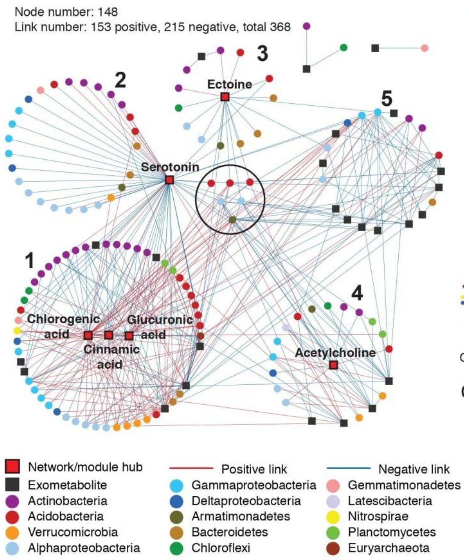
John Vogel

Vlastimil Novak





Summary



- Aromatic acids are preferentially consumed by bacteria that colonize the rhizosphere and elevated under nitrogen stress

- Serotonin for example, promotes the growth of the plant host and some bacteria, and its production can be elicited by bacteria
- Chemical between host and microbes greatly complicate rhizosphere microbiome engineering

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Roessner lab

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- **TEAMS project**
- **Twin ecosystem project**
- **Joint Genome Institute**
- **Switchgrass Program**
- **m-CAFEs SFA project**
- **Plant sustainability program (UCSD and MSU)**

teams Trial Ecosystems for the Advancement of Microbiome Science

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