Selectively Changing the Microbiome of the Rhizosphere

Karsten Zengler

www.zenglerlab.com

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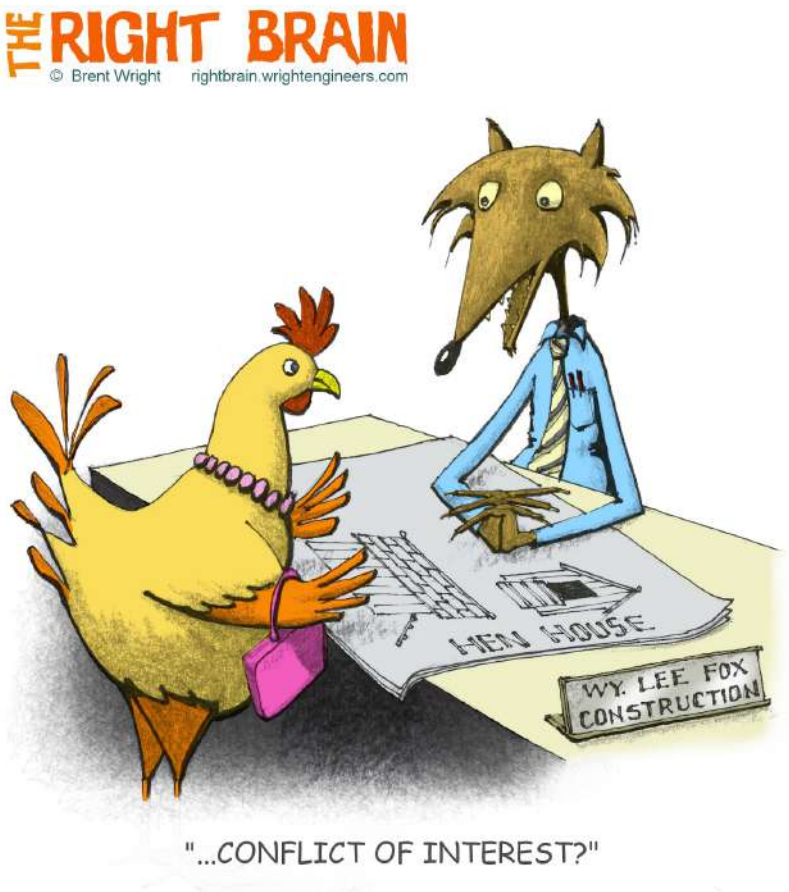
Disclosures



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Disclosures

Co-founder **Isolation Bio** (San Carlos), developing high-throughput platform for **microbiome research**.

Co-founder **Native Microbials** (San Diego), developing microbial solutions for your **animals**.

Co-founder and SAB member **Allive Biosciences** (San Diego), improving health by **reducing inflammation**.

SAB member **DiscitisDX** (La Jolla), developing diagnostics for intervertebral **disc surgery**.

SAB member **Triton Algae Innovations** (San Diego), introducing new ingredients for **future foods**.

Consultant **Procter&Gamble** (St. Louis), consumer skin care.

Former SAB member

Joyn Bio (Boston)

ProdermIQ (San Diego)

Syngip (Vaals, The Netherlands)

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Microbiome Sciences

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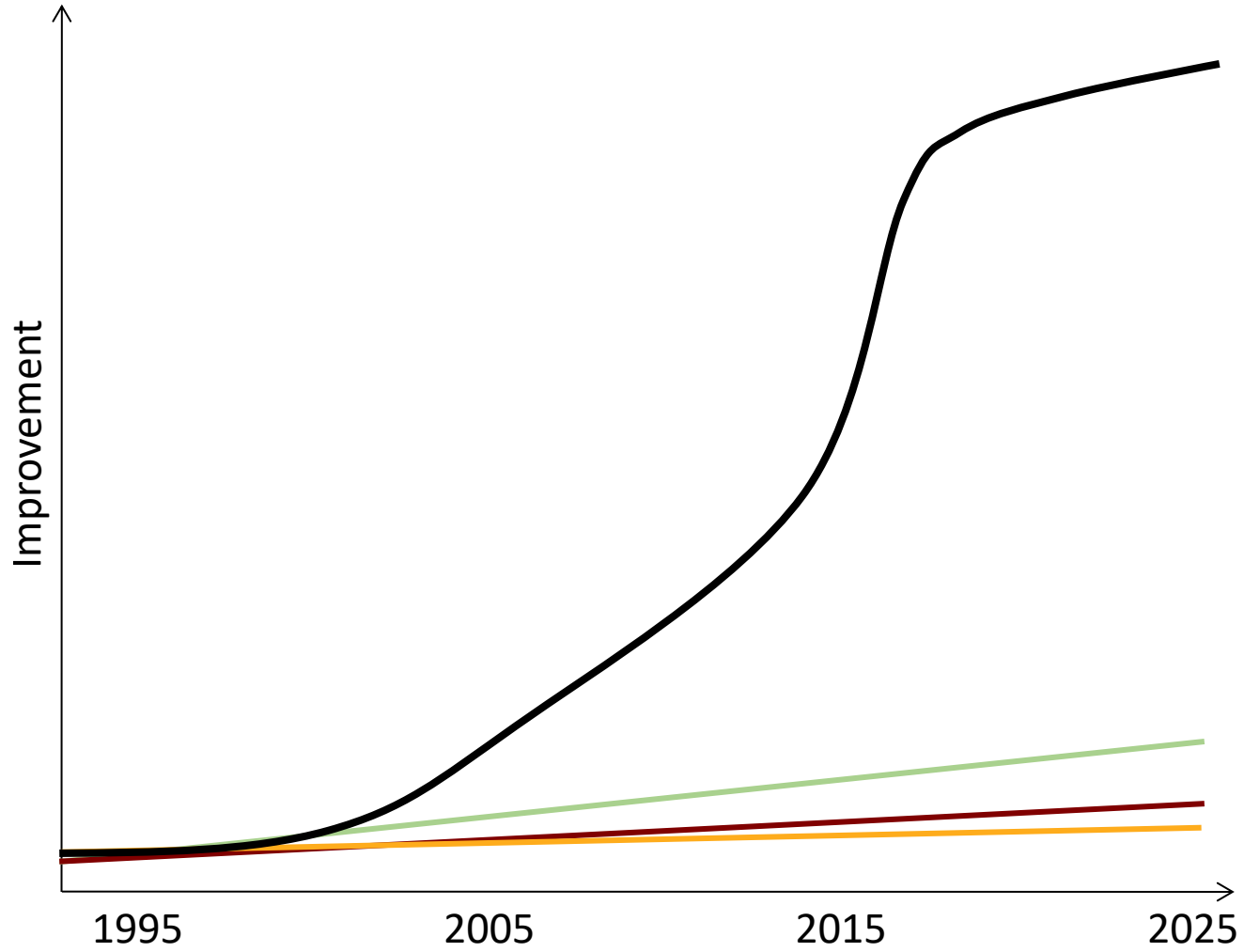
Progress in Microbiome Research

(my personal view)



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Progress in Microbiome Research

Why?
…are they doing it?

How?
…are they doing it?

What?
…are they doing?

Who?
…is there?

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Progress in Microbiome Research

Why?
…are they doing it?

How?
…are they doing it?

Who else lives there?

Transplant organisms?

What?
…are they doing?

Build/create?

Who?
…is there?

Predict?

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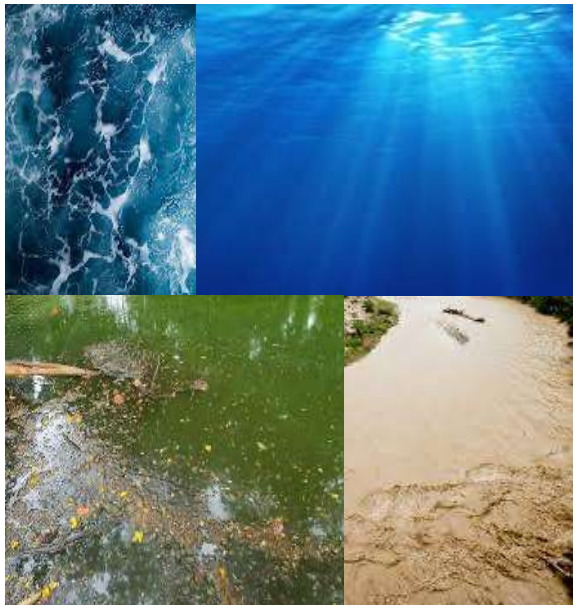
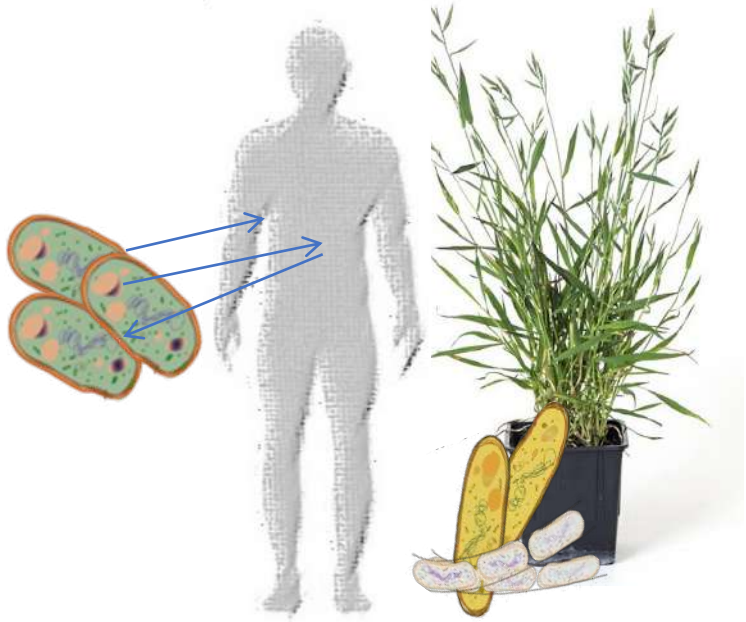
Open Questions in Microbiome Research

Terrestrial

Aquatic



Host-Associated



…how do communities response to perturbations?

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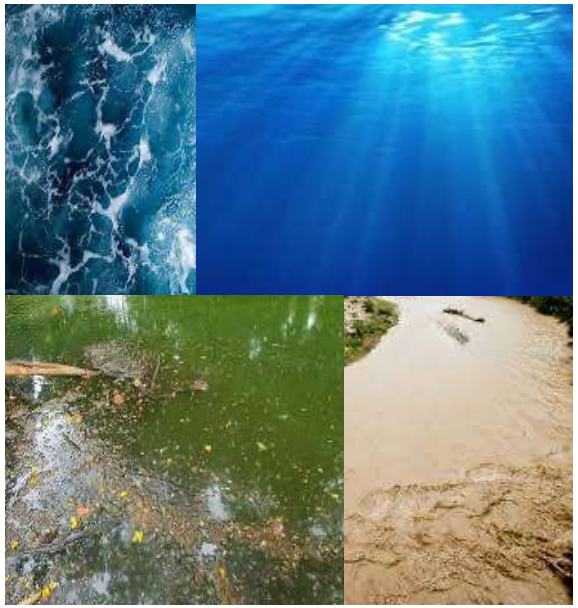
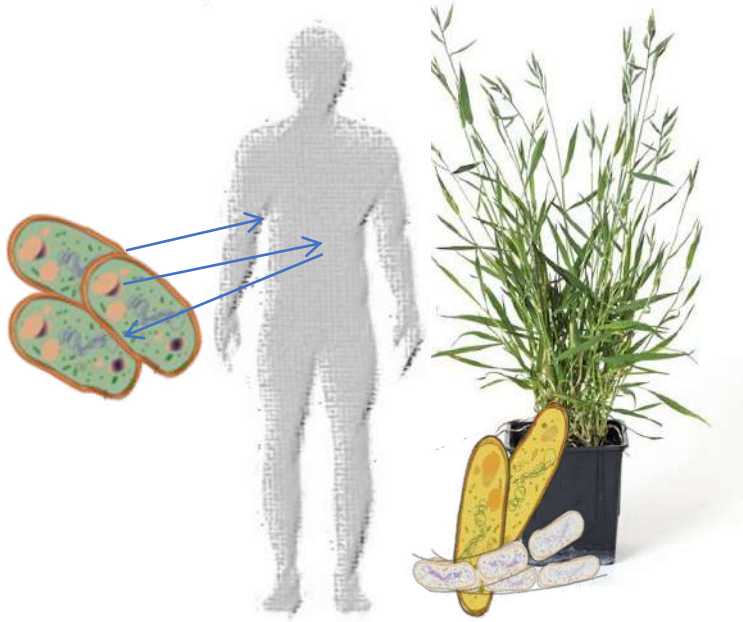
Open Questions in Microbiome Research

Terrestrial

Aquatic



Host-Associated



…how do communities response to perturbations?

…can we predict outcomes?

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Open Questions in Microbiome Research

Terrestrial

Aquatic

Host-Associated

Control – Change – Rational Design

…how do communities response to perturbations?

…can we predict outcomes?

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Processes in the Rhizosphere

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How do we study microbial communities?

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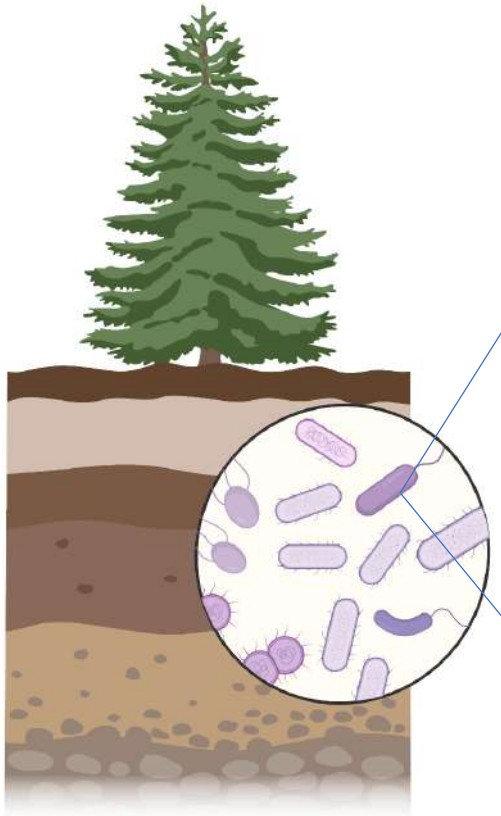
Microbiome Science Tools

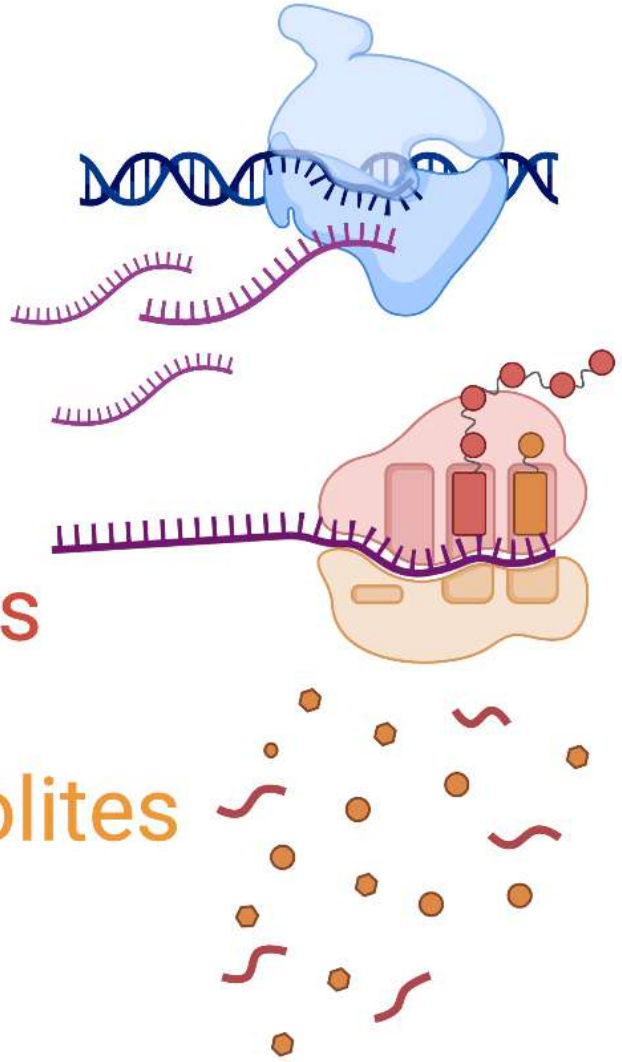


DNA

mRNA

Proteins

Metabolites



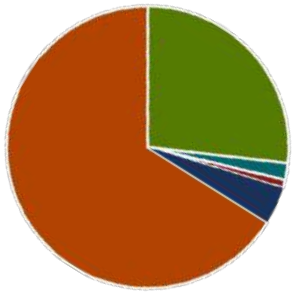
16S rRNA
Who is there

Genomics
What is possible

Transcriptomics
What appears to be happening

Proteomics
What makes it happen

Metabolomics
What is happening



% microbiome studies
(last 10 years, >68,000 articles)

- 16S rRNA (66%)
- Metagenomics (27%)
- Metatranscriptomics (2%)
- Metaproteomics (1%)
- Metabolomics (4%)

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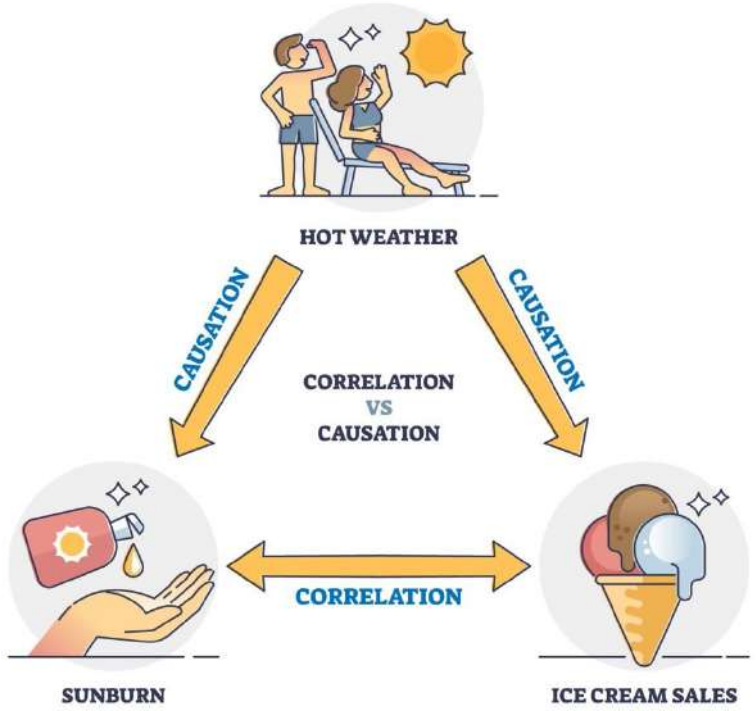
Microbiome science is mostly descriptive & correlation-based



www.simplypsychology.org/correlation.html

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Microbiome science is mostly descriptive & correlation-based

…often NOT predictive

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Microbiome science is mostly descriptive & correlation-based

…establish causation and make it predictive!

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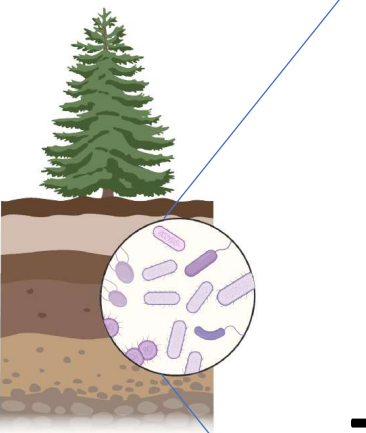
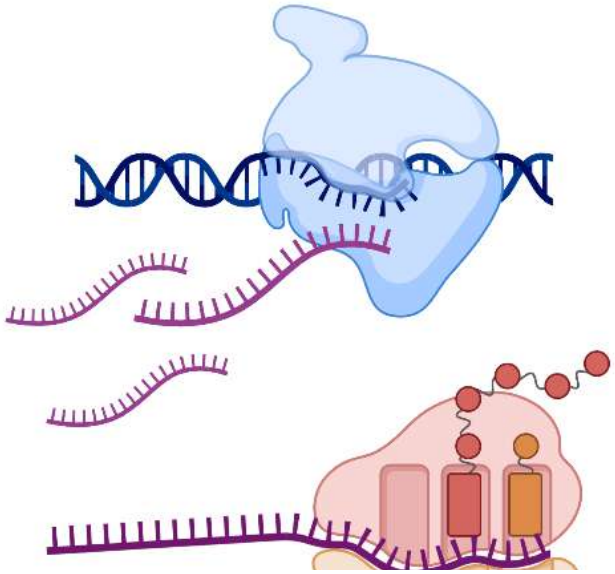
What defines the phenotype?



DNA

mRNA

$$$



16S rRNA

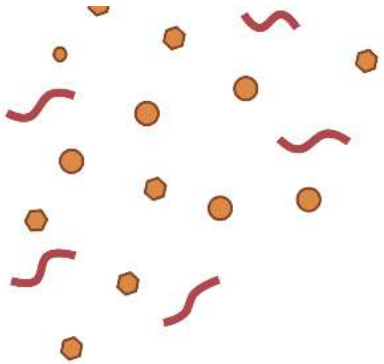
Genomics

Transcriptomics

TRANSLATION???

Translation is the most expensive process in the cell

Metabolites



Metabolomics

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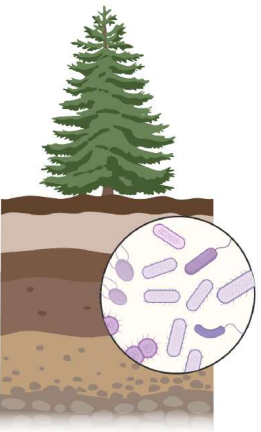
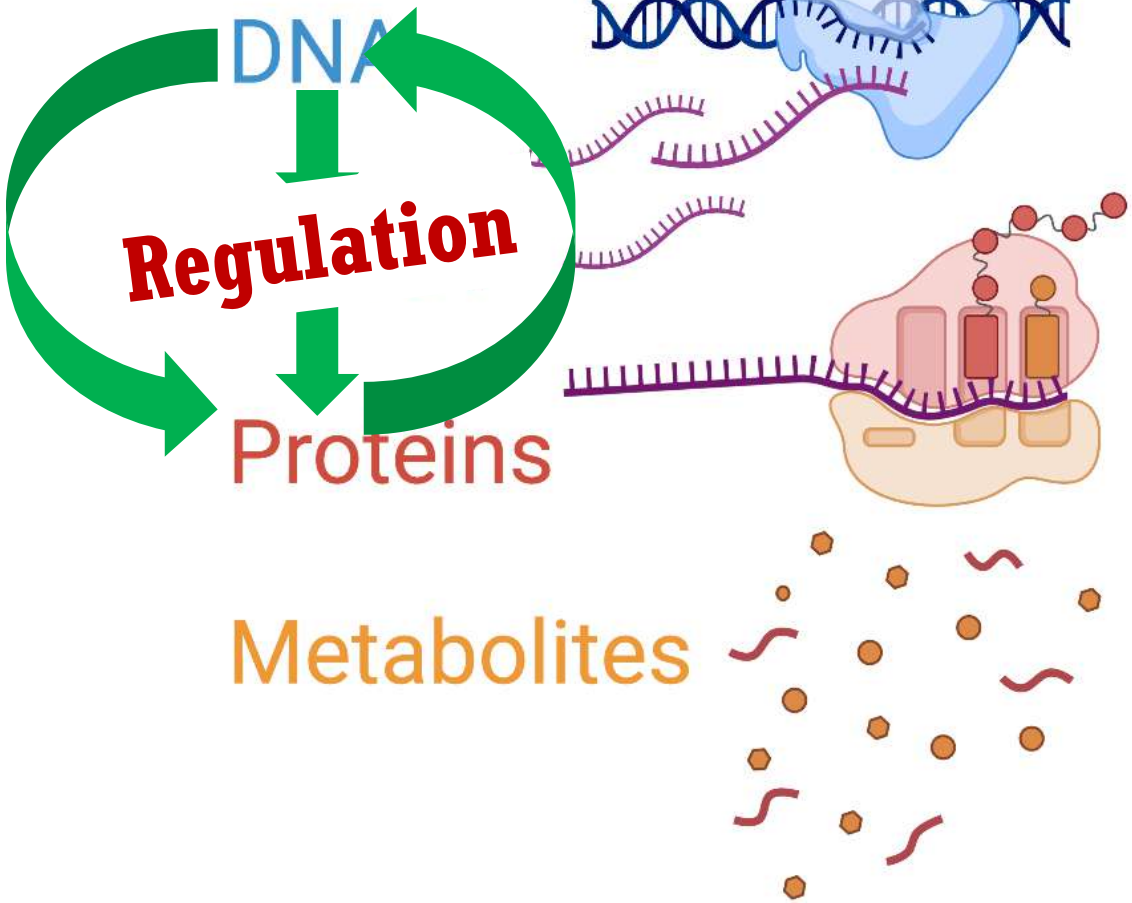
What defines the phenotype?





16S rRNA

Genomics

Transcriptomics

TRANSLATION

Proteomics

Metabolomics

Al-Bassam et al. *Nature Communication* 2018

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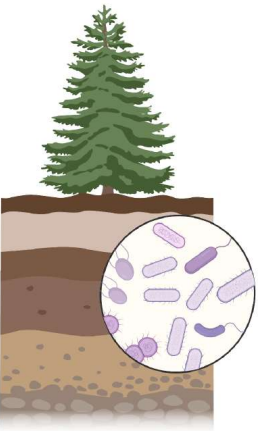
What defines the phenotype?

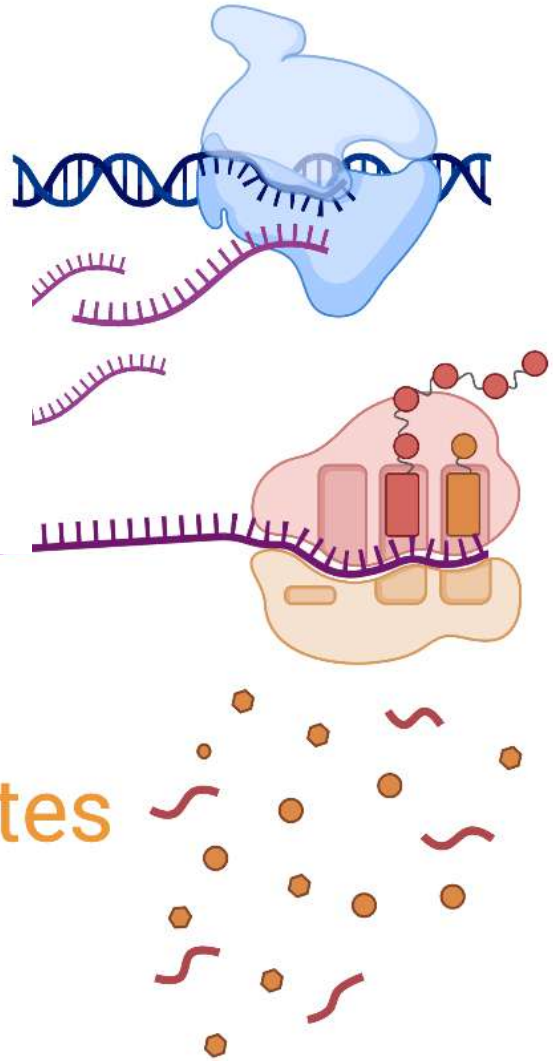


DNA

Translational Efficiency

Proteins

Metabolites



16S rRNA

Genomics

Transcriptomics

TRANSLATION

Proteomics

Metabolomics

Al-Bassam et al. *Nature Communication* 2018

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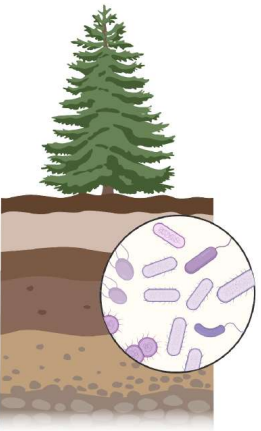
What defines the phenotype?

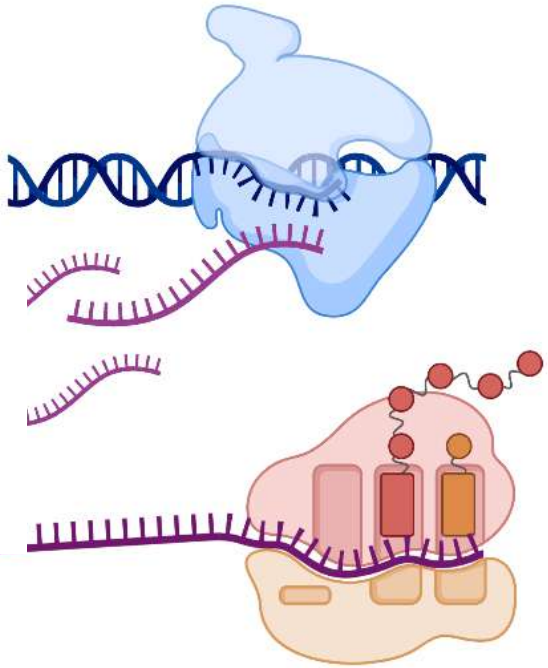


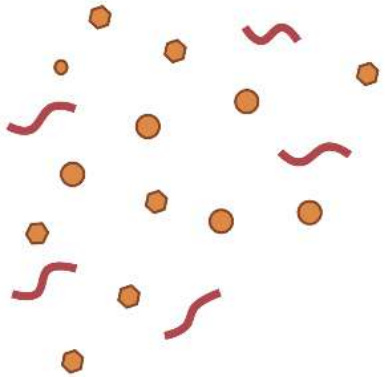
DNA

Translational Efficiency

Proteins

Metabolites





16S rRNA

Genomics

Transcriptomics

Translatomics
(Ribo-Seq)

Proteomics

Metabolomics

Al-Bassam et al. *Nature Communication* 2018

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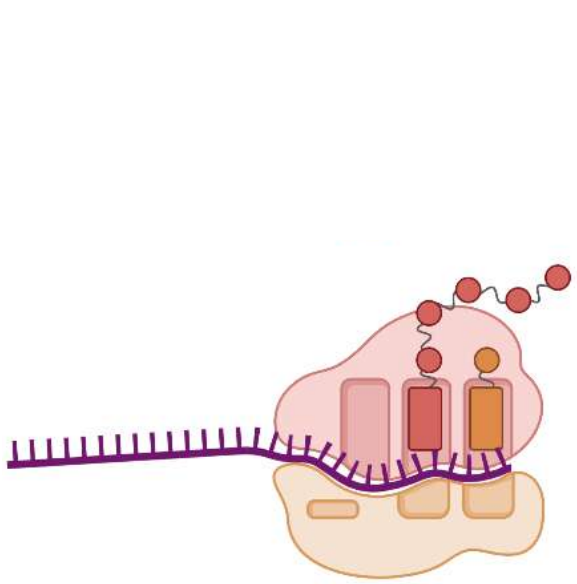
Translatomics – Ribo-Seq



Stop Translation ⇨ Digest mRNA ⇨ Sequence

Al-Bassam et al. *Nature Communication* 2018

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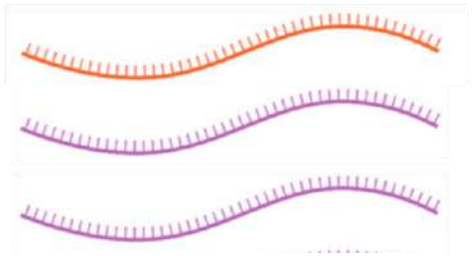
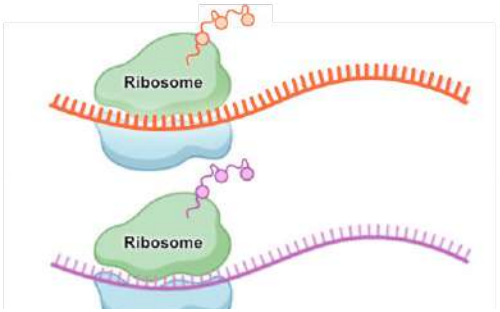
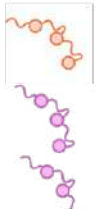
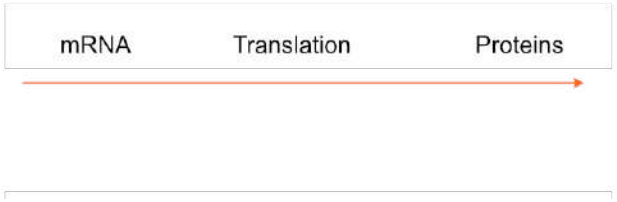
Translational Efficiency (TE)





TE: how many mRNAs are being translated





The cell controls its phenotype through translational efficiency (resource allocation)

Al-Bassam et al. *Nature Communication* 2018

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Resource Allocation

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Resource Allocation

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Resource Allocation

Resource allocation defines a person's preferences

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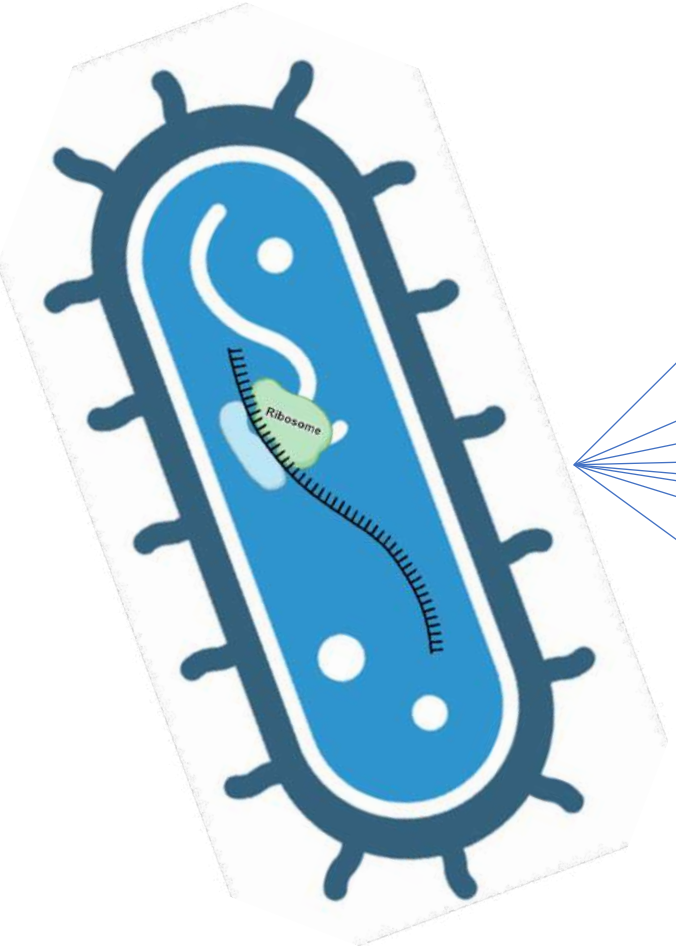
Resource Allocation



Amino Acid Synthesis

Division

Sugars

DNA

N-fix

Fatty Acid Metabolism

RNA

Motility

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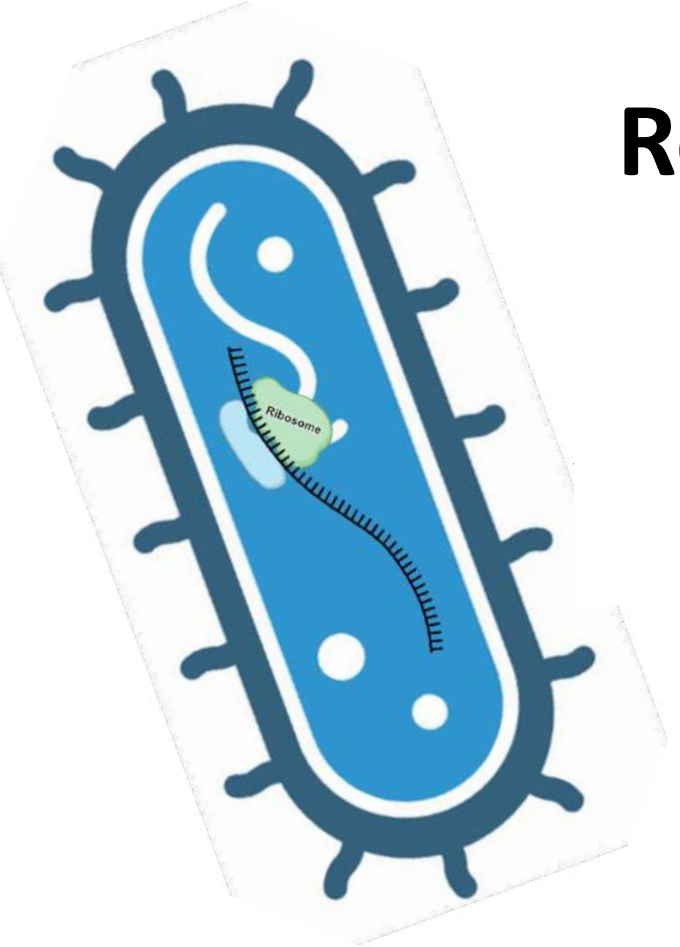
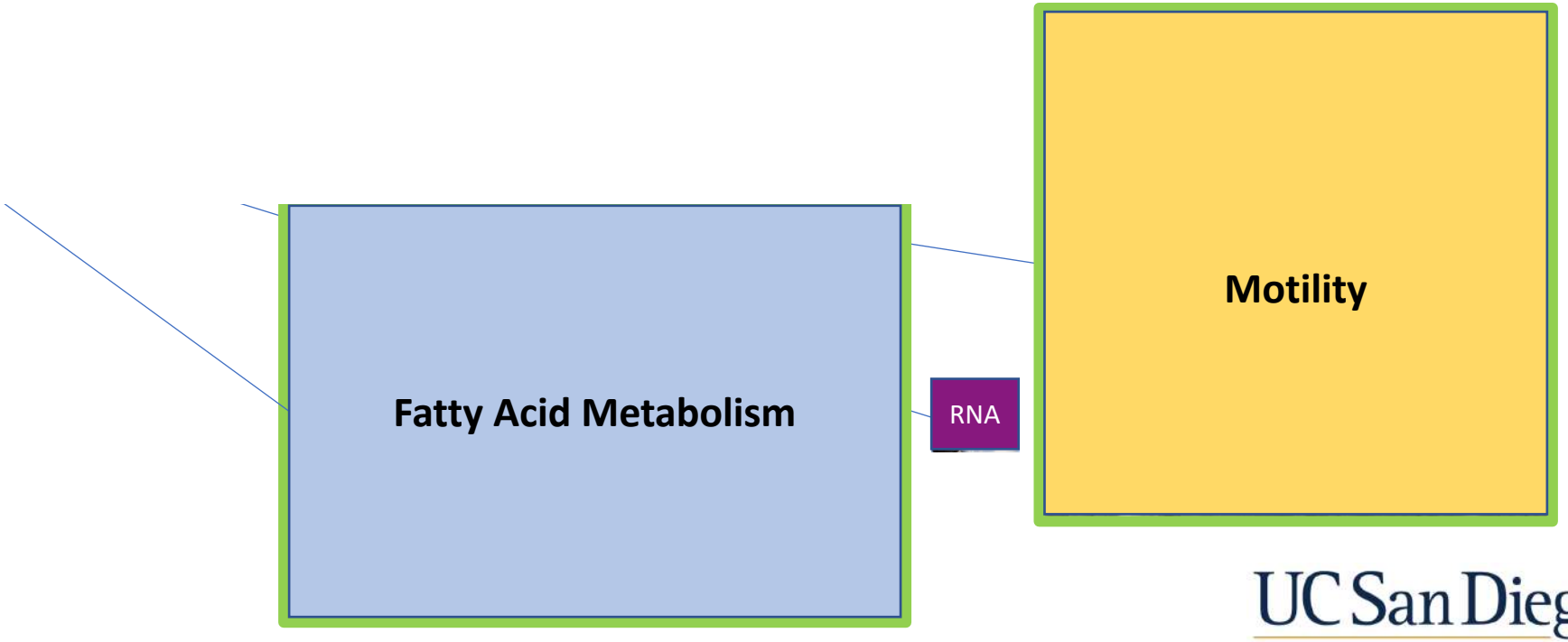
Resource Allocation



Resource allocation defines a cell's preferences



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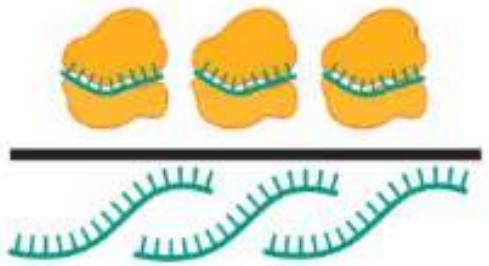
TE in Communities

Translational Efficiency

$$TE = \frac{\text{MetaRibo-Seq}}{\text{MetaRNA-Seq}}$$



Microbes Preferences

Functional microbiome classification **(Guilds)**

Control – Change – Rational Design

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Method Validation

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Synthetic Community (SynCom)

16 strains

Isolated from switchgrass rhizosphere

Taxon	Strain
Lysobacter	OAE881
Burkholderia	OAS925
Variovorax	OAS795
Chitinophaga	OAE865
Chitinophagaceae	OAS944
Mucilaginibacter	OAE612
Rhizobium	OAE497
Bradyrhizobium	OAE829

Taxon	Strain
Bosea	OAE506
Methylobacterium	OAE515
Arthrobacter	OAP107
Mycobacterium	OAE908
Rhodococcus	OAS809
Marmoricola	OAE513
Brevibacillus	OAP136
Paenibacillus	OAE614

Coker et al. *mSystems* 2022

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Experimental Design

16 strains SynCom

0.1X R2A medium
7 days, 30 °C
4 biological replicates

Multi-omics

Metagenomics

Metatranscriptomics

Metatranslatomics

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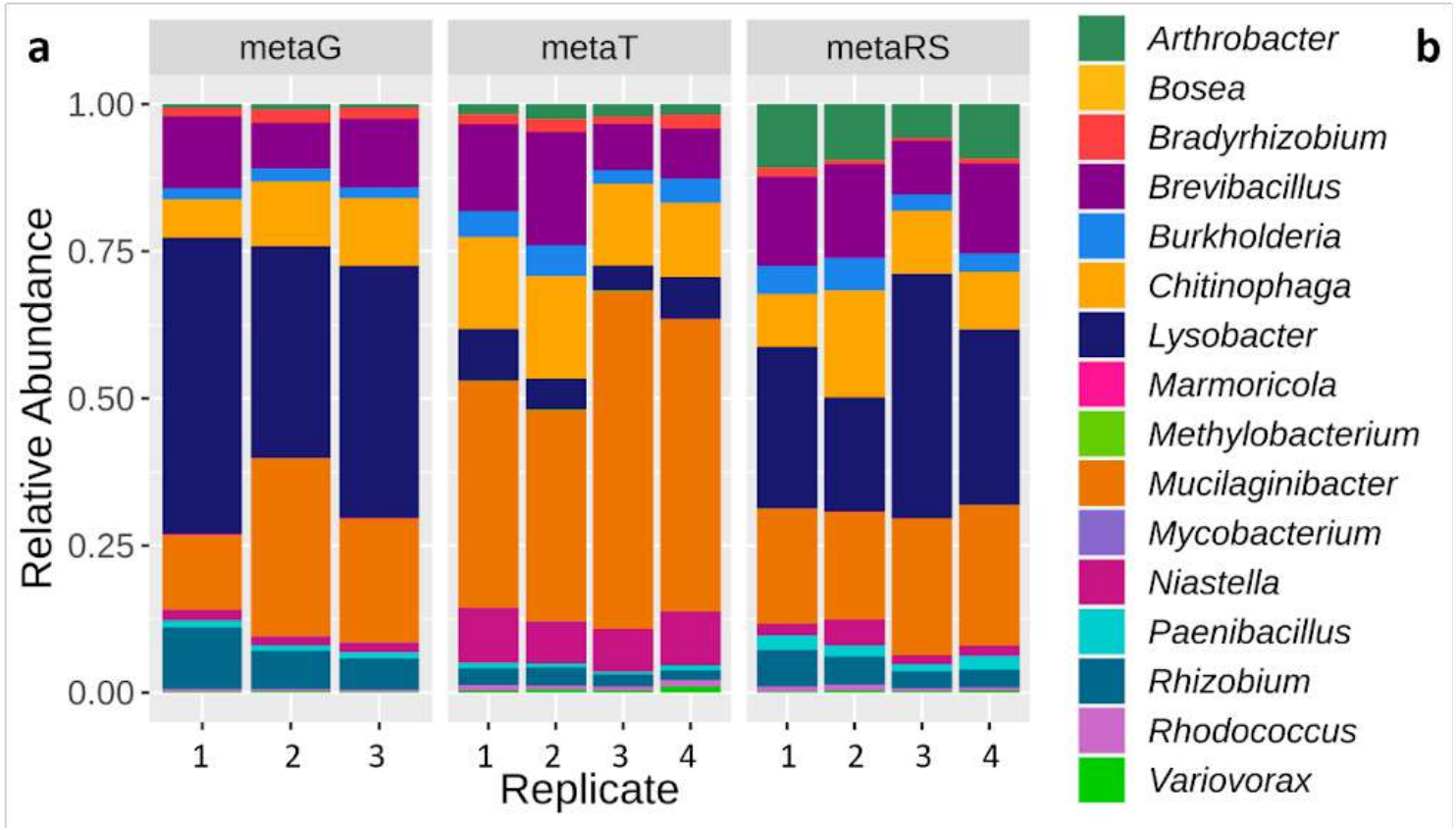
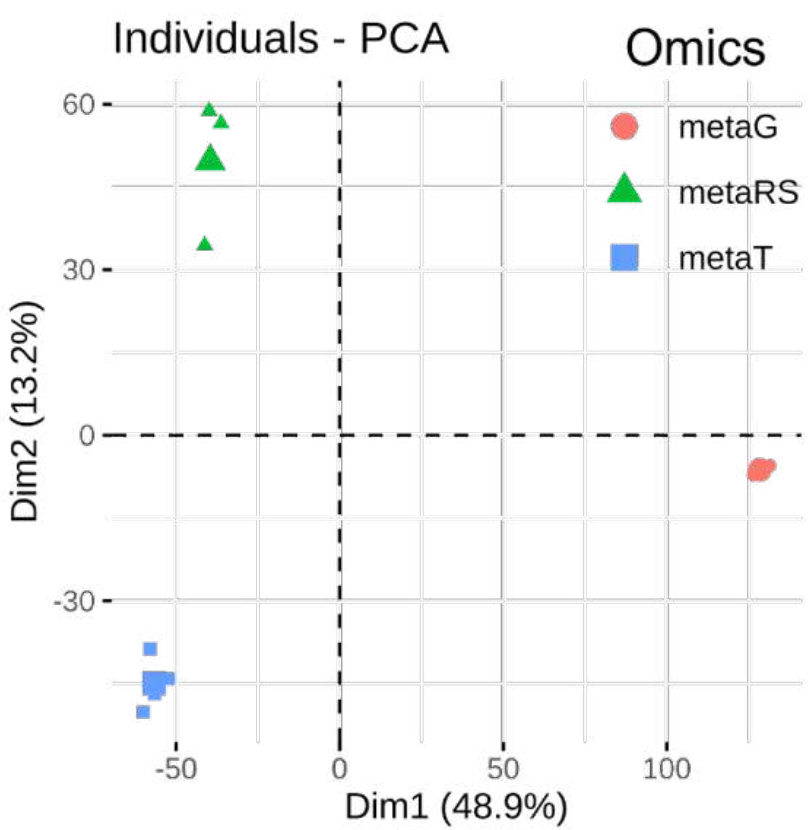
Comparison of -omics





- **Excellent reproducibility within –omics**

- **Distinct profiles between -omics**

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Guilds and Microbial Niche Determination (MiND)

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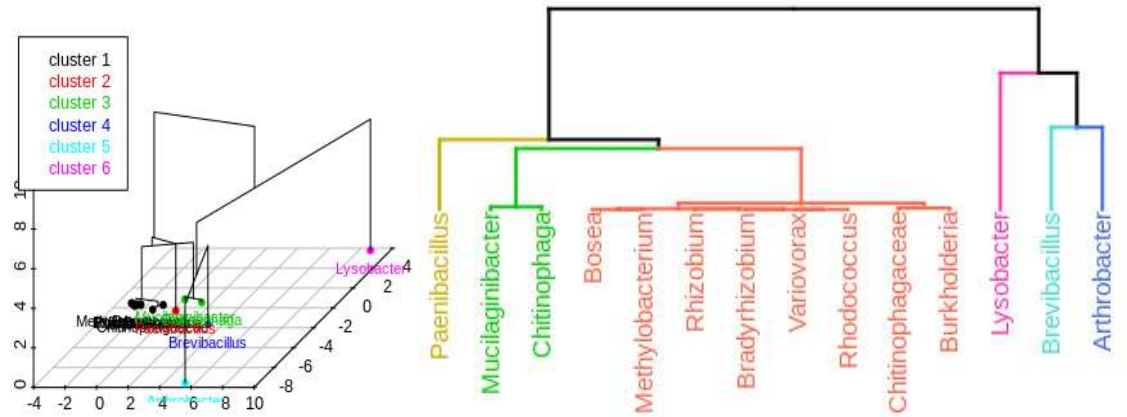
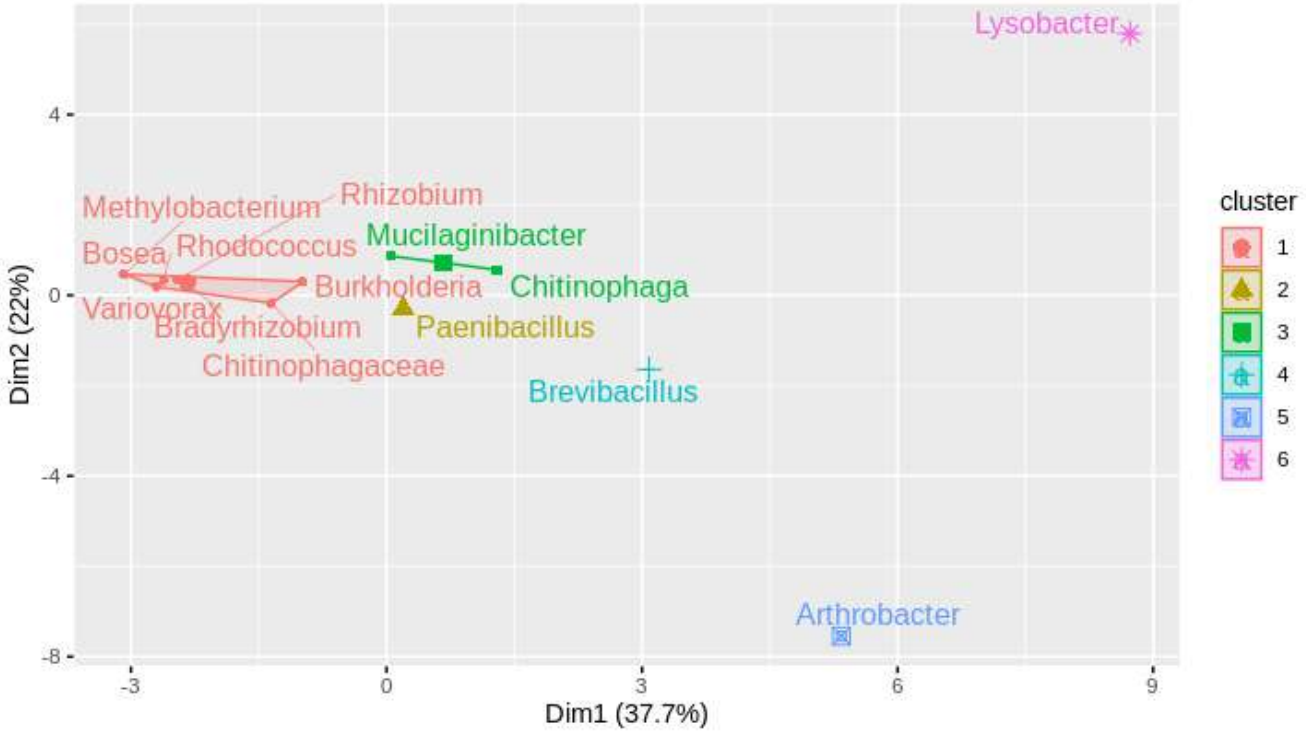
Guilds





$$TE = \frac{\text{MetaRibo-Seq}}{\text{MetaRNA-Seq}}$$

16 strains, 275 metabolic pathways (KEGG)

Average 4 replicates

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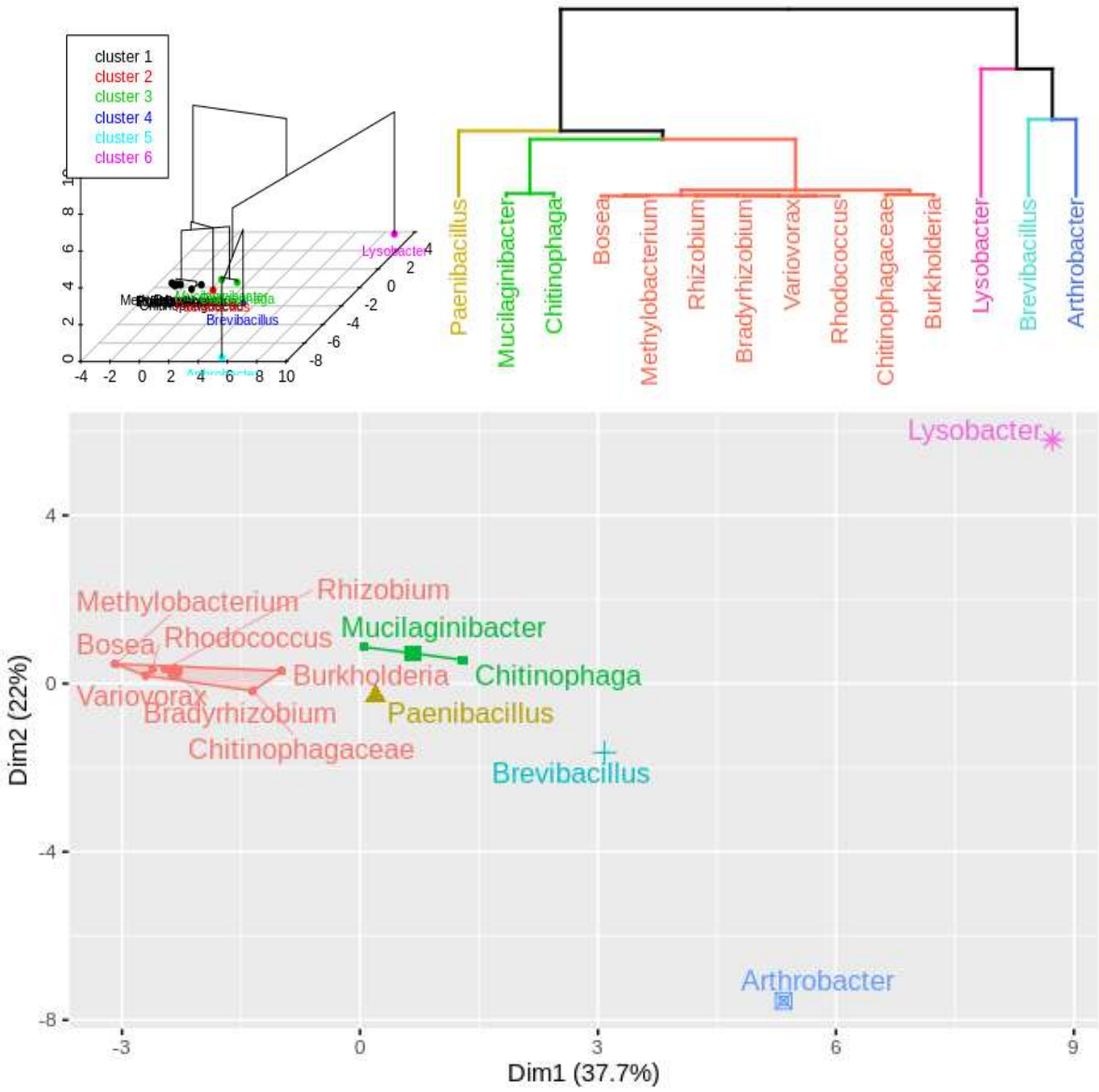
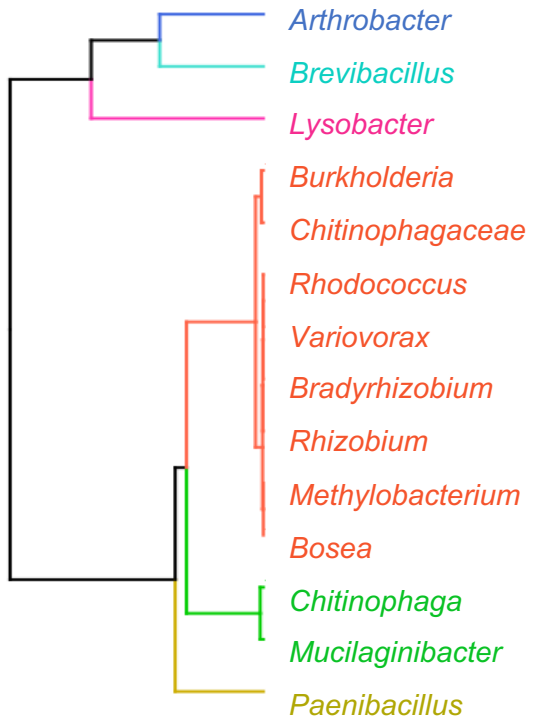
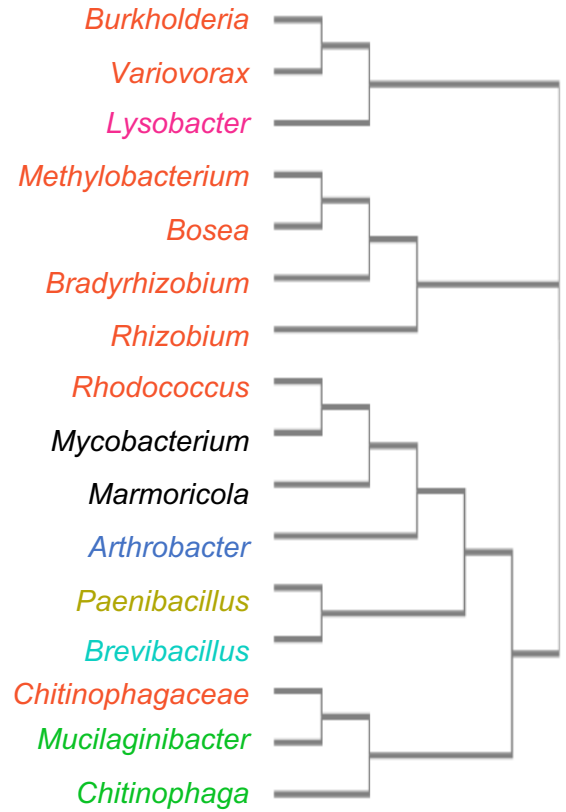
Guilds vs Phylogeny



Guilds based on TE



Phylogenetic tree based on 16S rRNA



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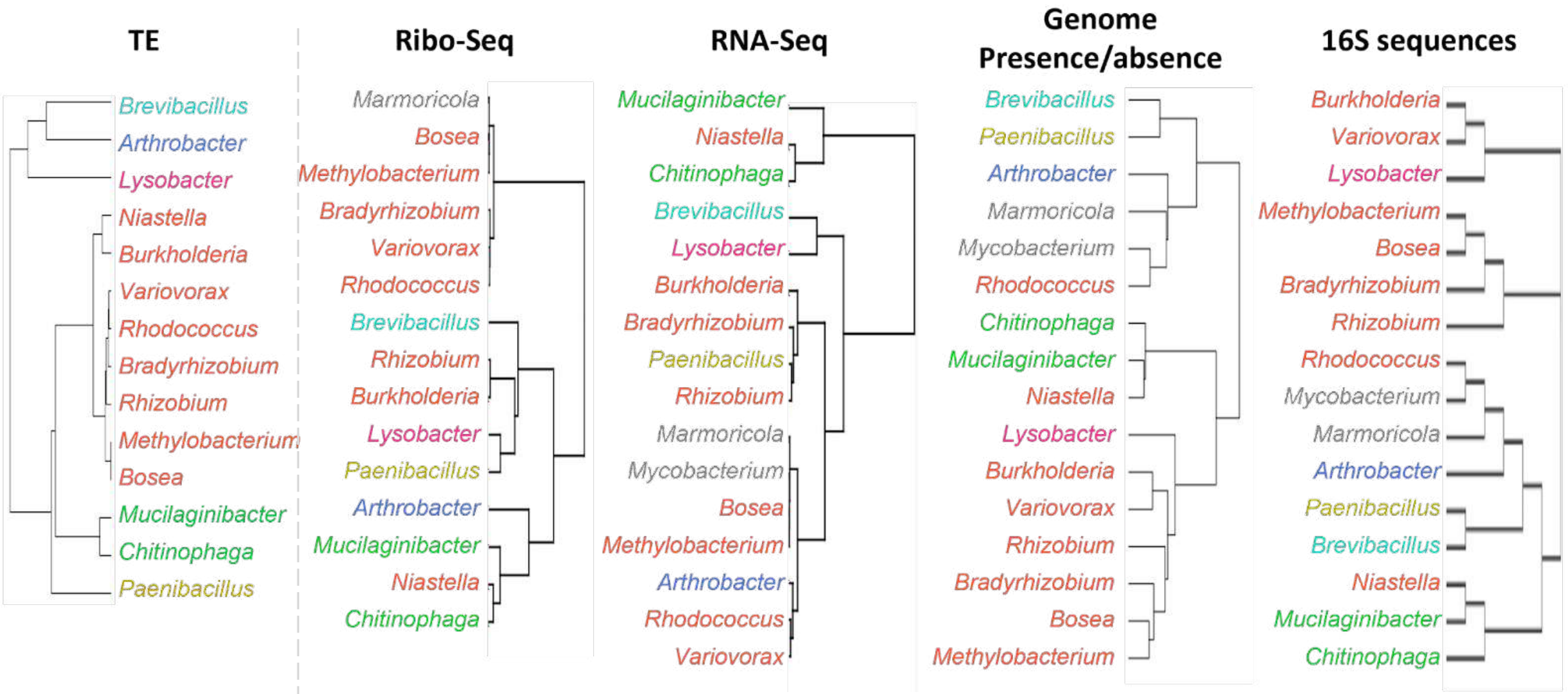
Guilds vs Phylogeny



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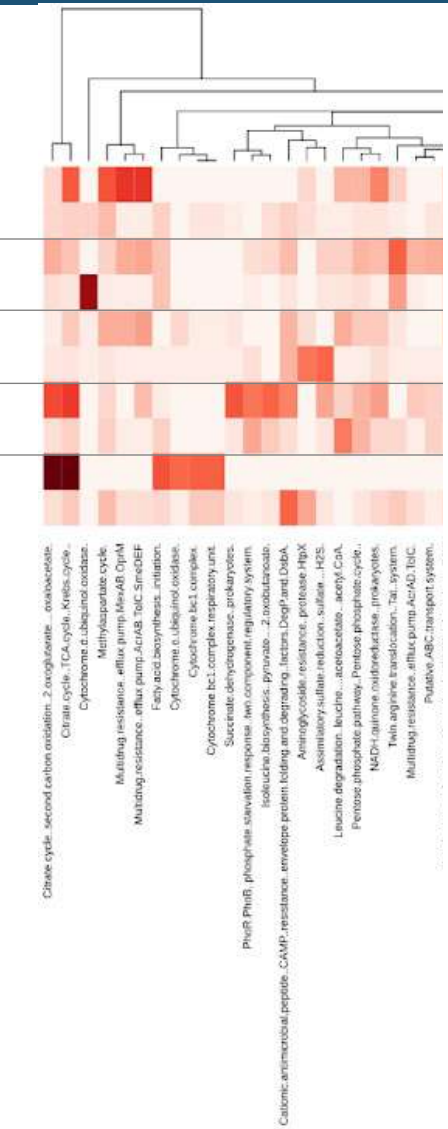
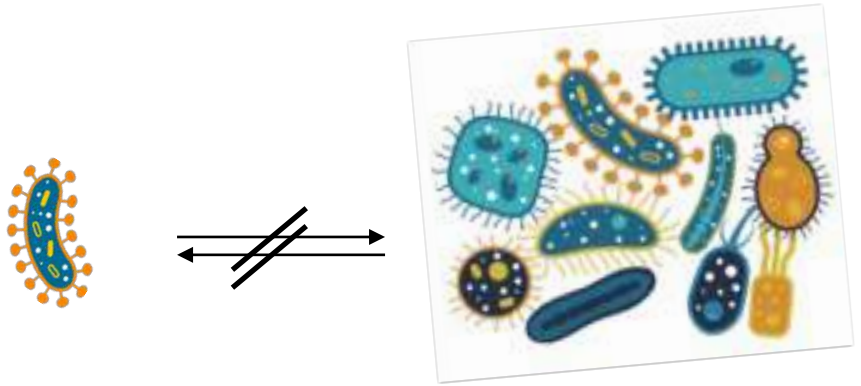
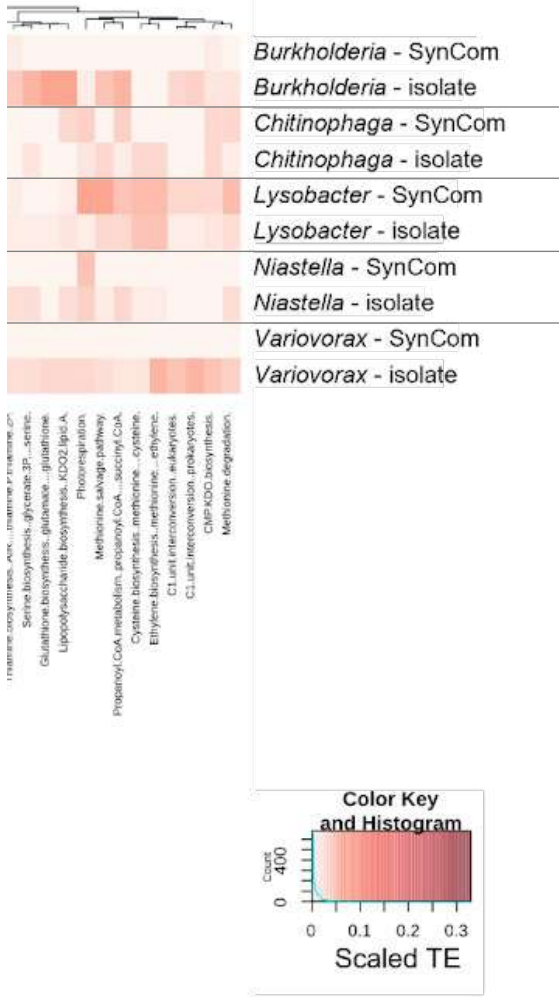
Pathway Prioritization: SynCom vs. Axenic Culture





Organisms prioritize different pathways when in a community!



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Can guilds predict intervention outcomes?

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❑ Modifying community composition

REMOVAL

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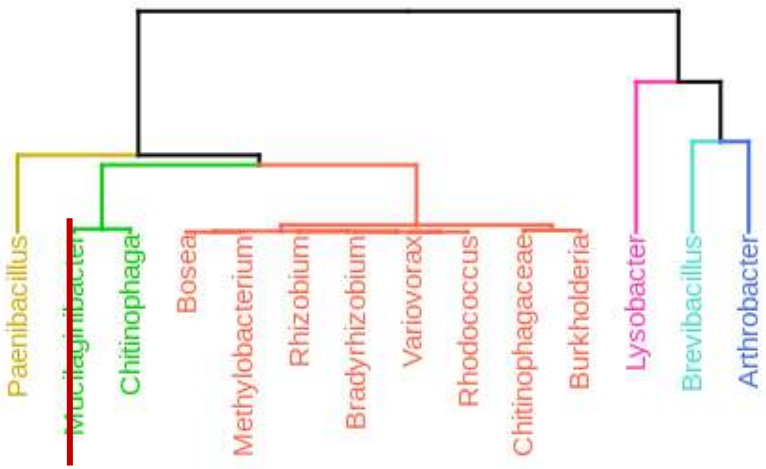
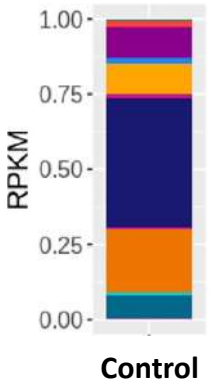
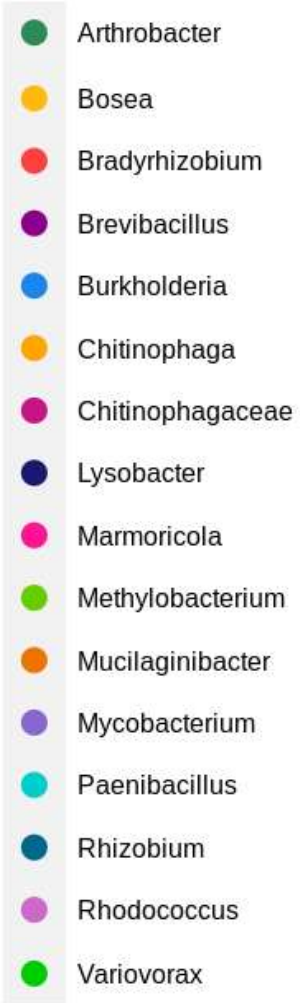
Response to the Removal of Microbes

Metabolic Guilds based on TE





- *Mucilaginibacter*



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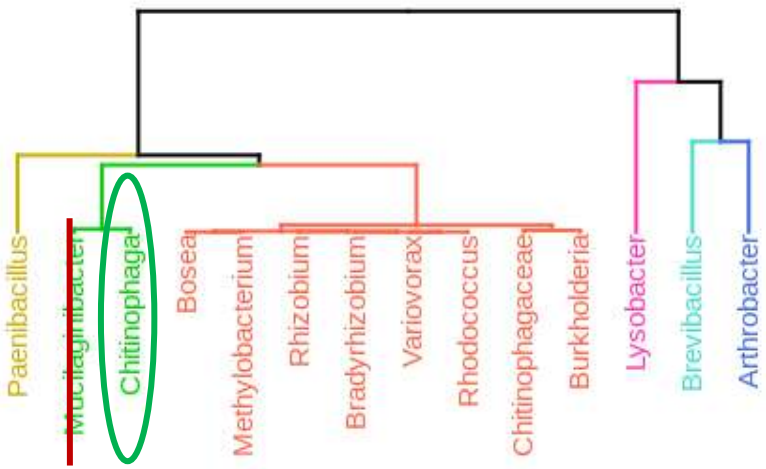
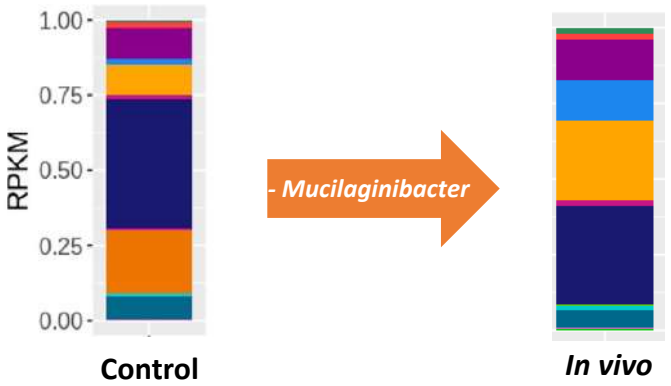
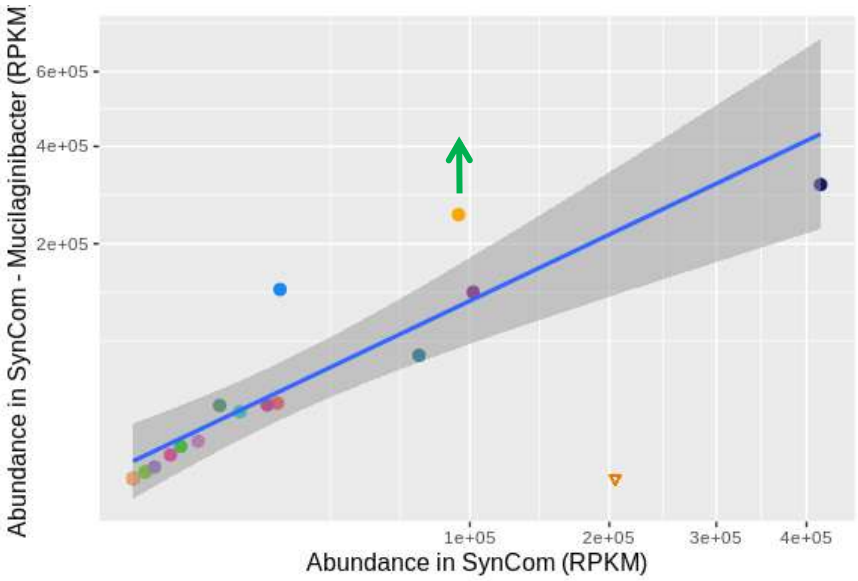
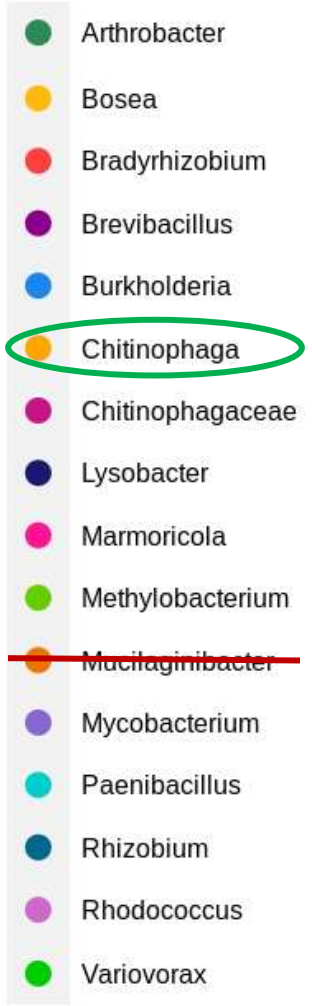
Response to the Removal of Microbes

Metabolic Guilds based on TE









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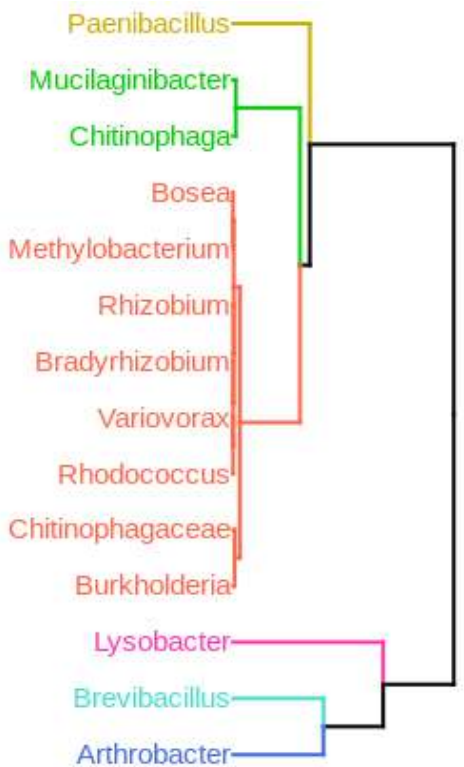
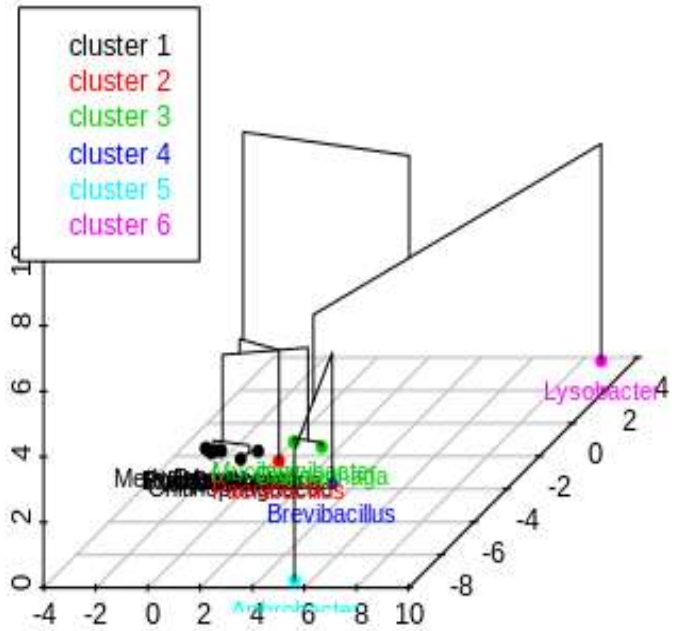
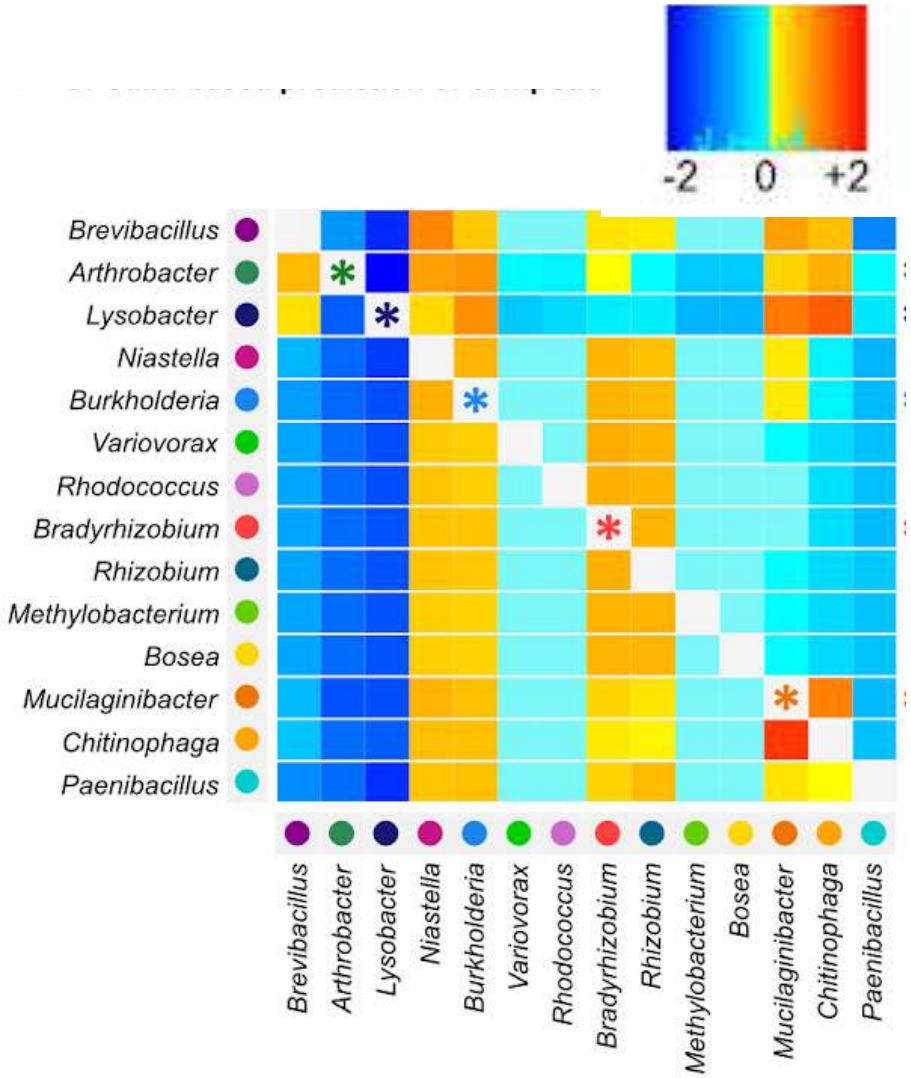
Calculating Competition Score







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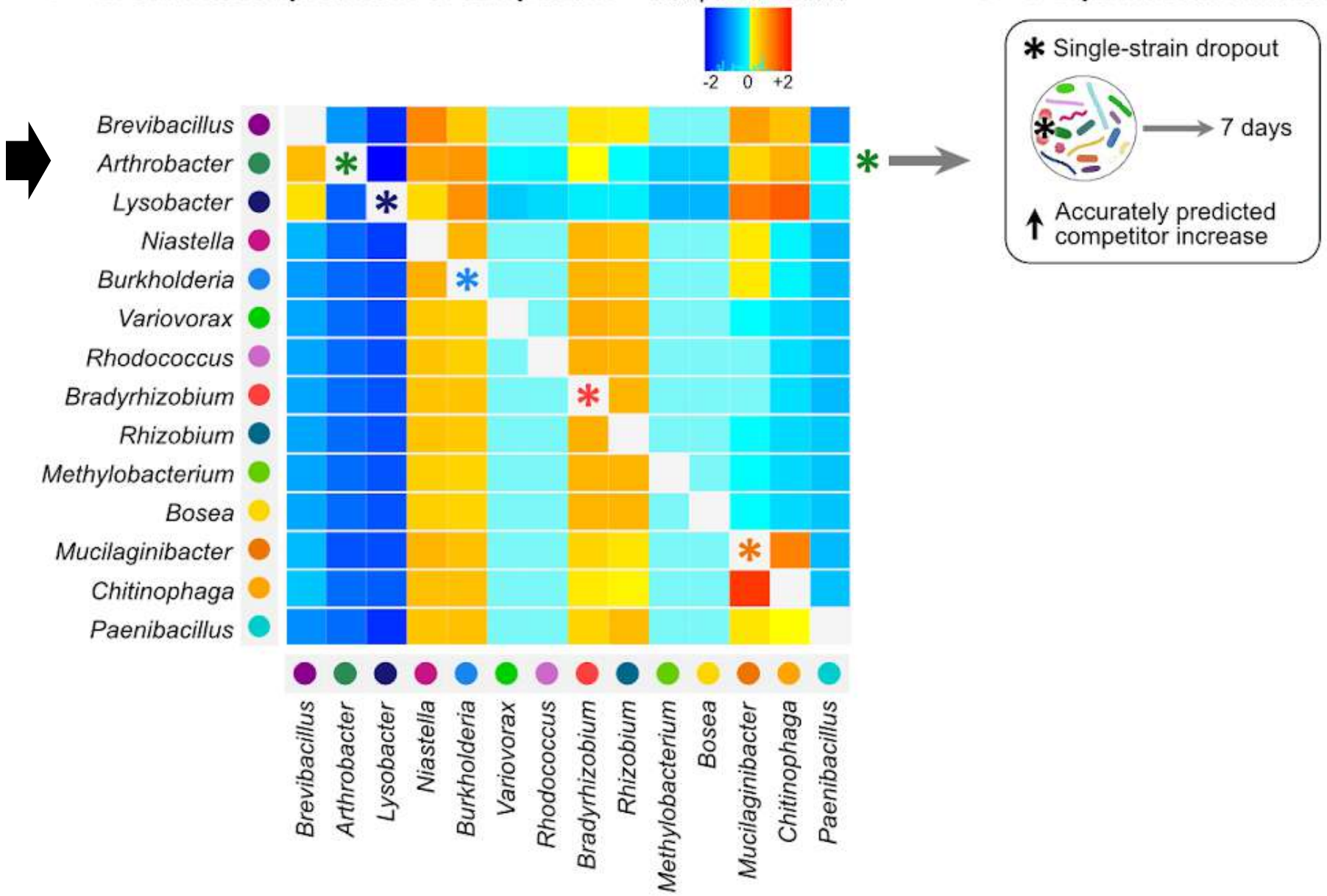
Response to the Removal of Microbes



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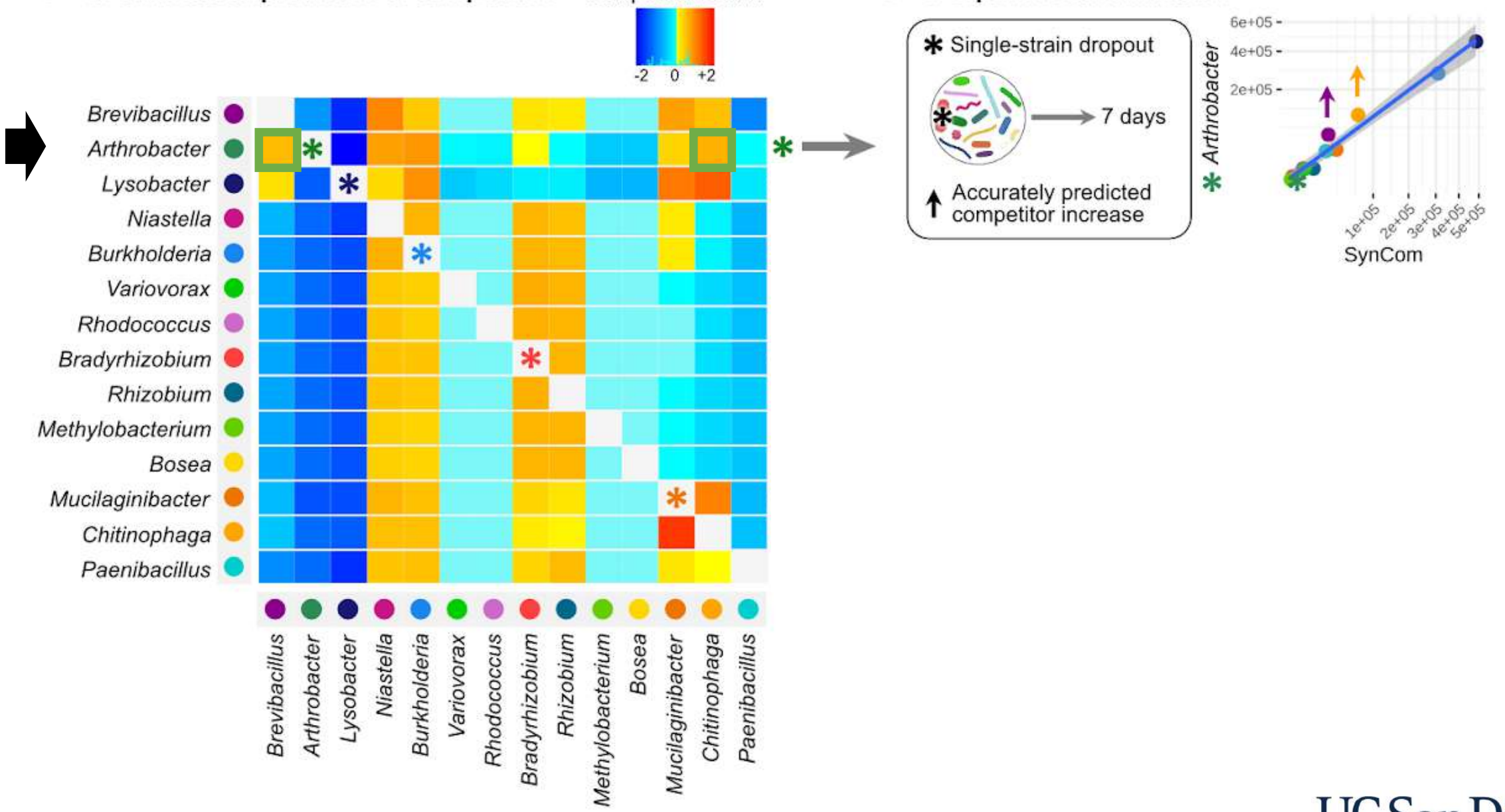
Response to the Removal of Microbes



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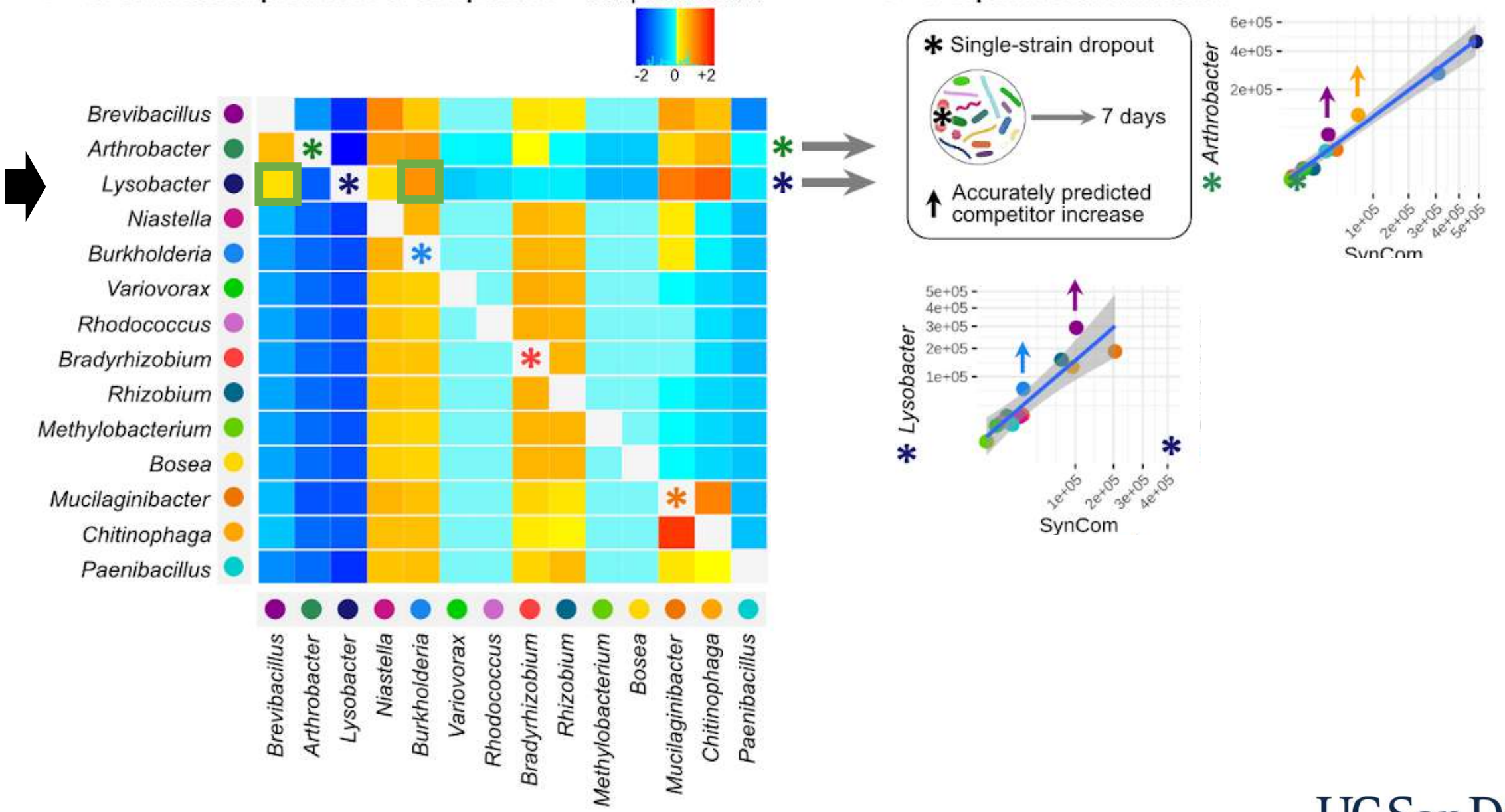
Response to the Removal of Microbes



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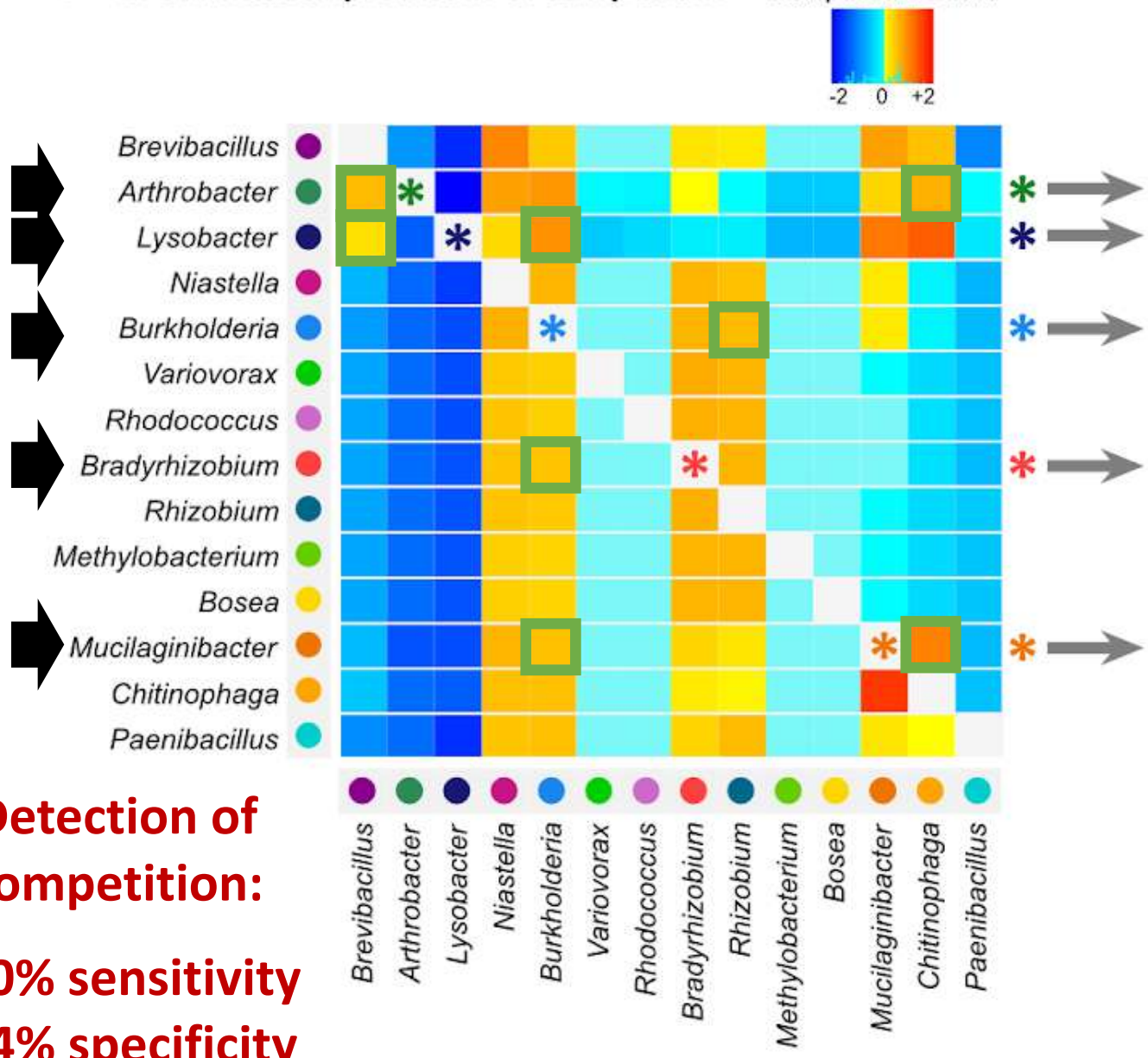
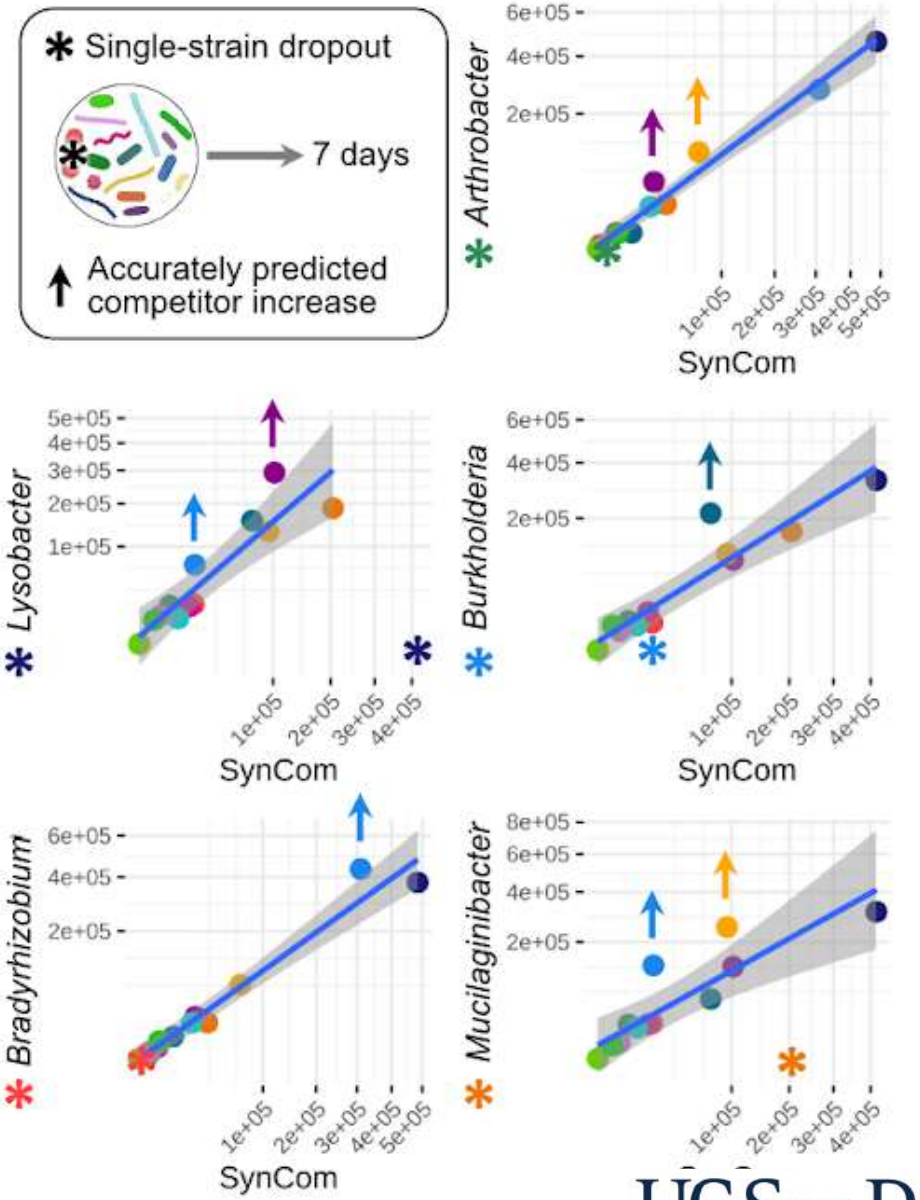
Response to the Removal of Microbes





Detection of competition:

100% sensitivity

74% specificity

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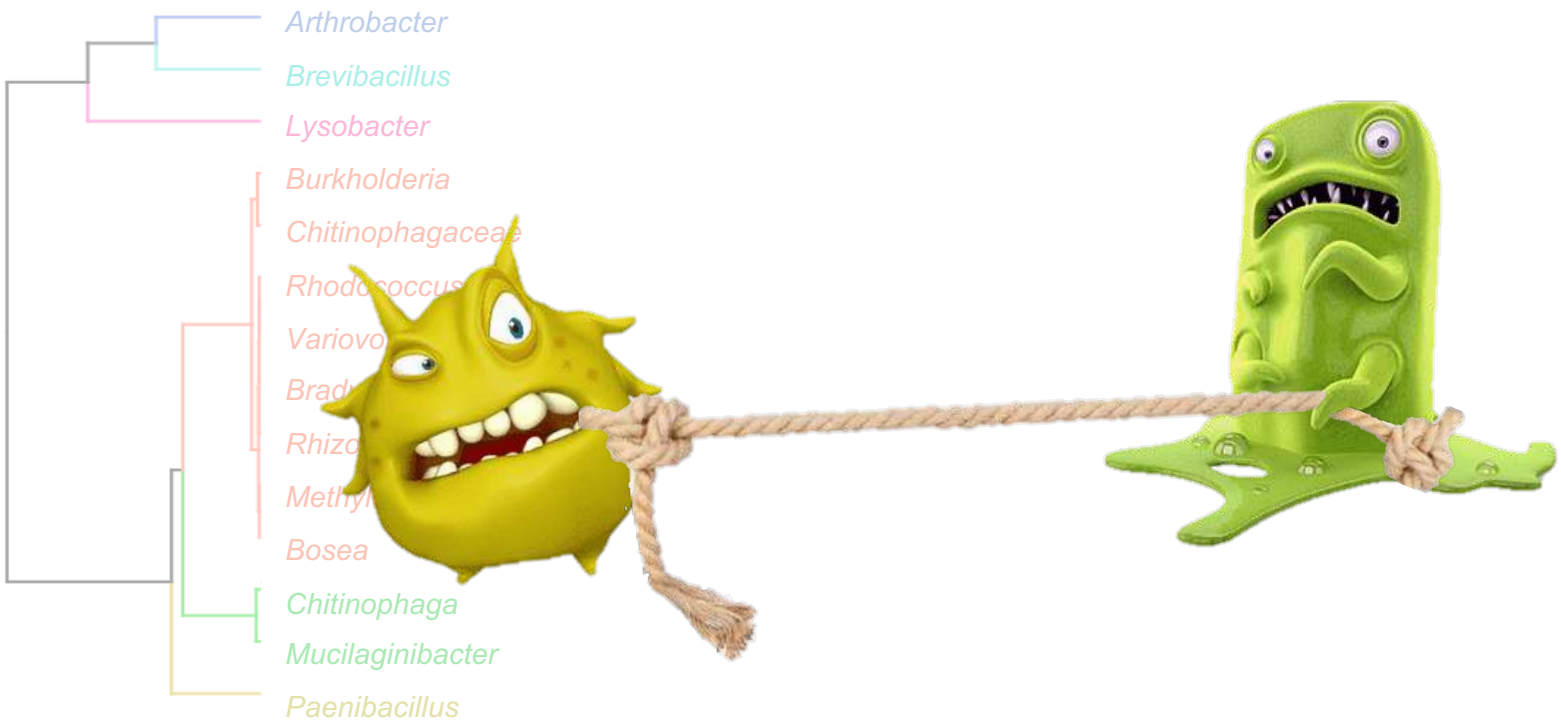
Hypothesis



Bacteria in the Same Guild are Competitors

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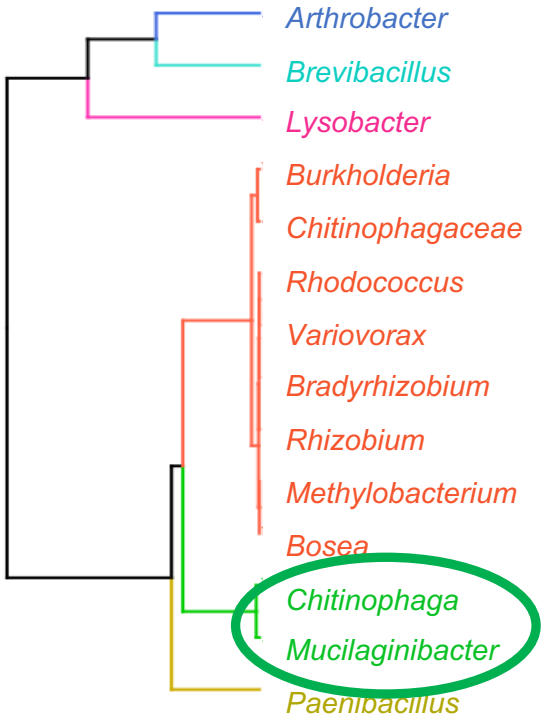
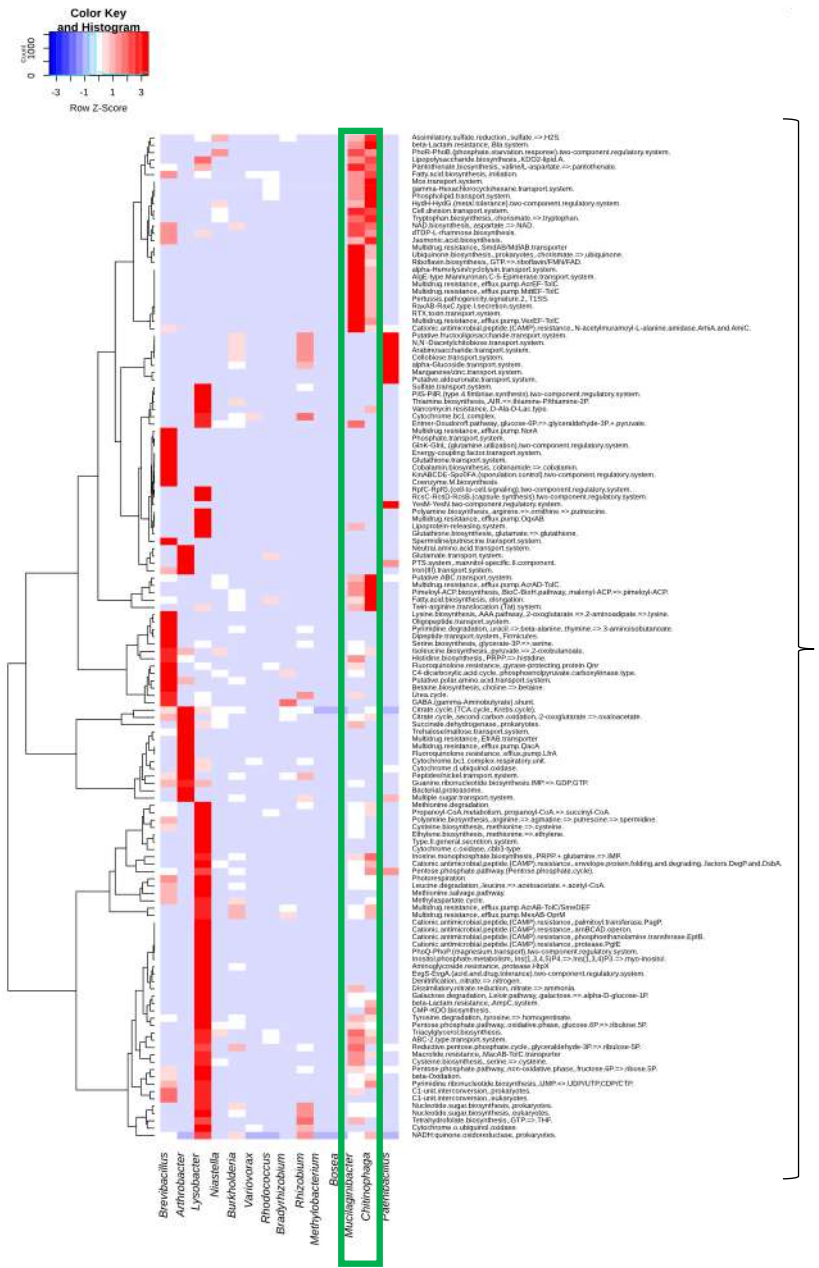
Antimicrobials





- Beta-Lactam resistance
- Multidrug resistance transporter
- Multidrug resistance efflux pump
- Rax Type 1 secretion system
- RTX toxin transport system
- Antimicrobial peptide resistance

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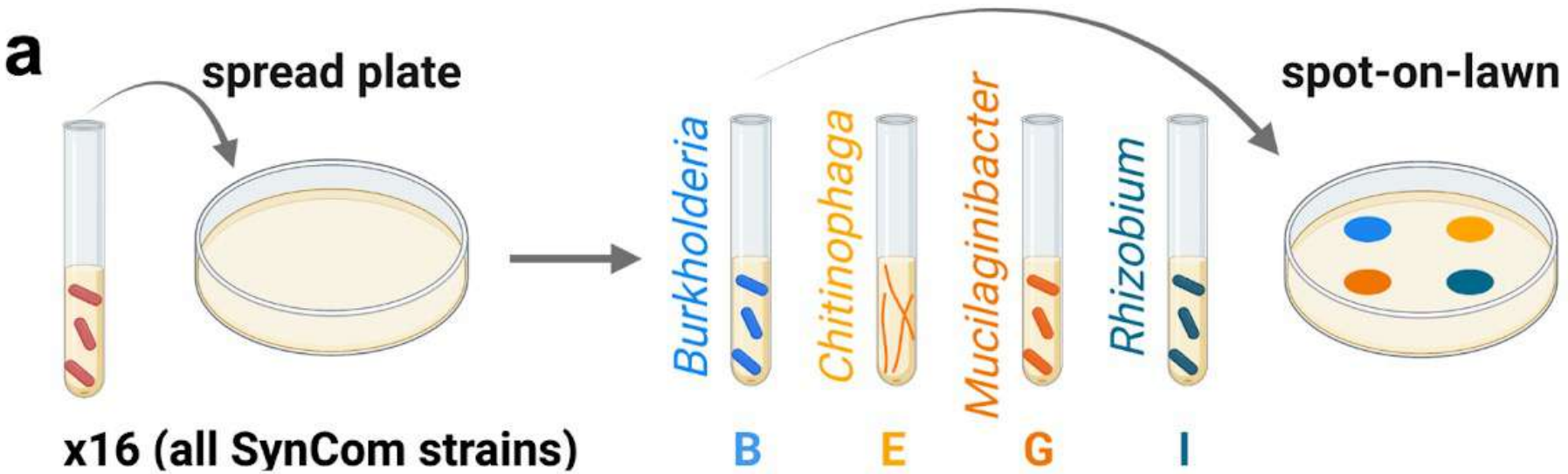
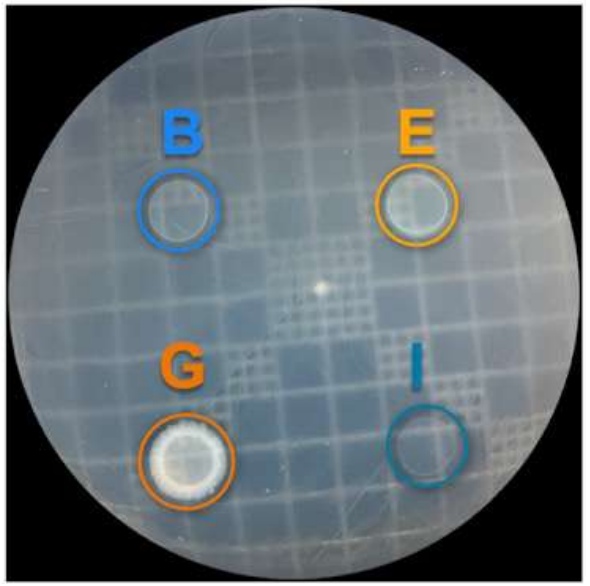
Antimicrobials





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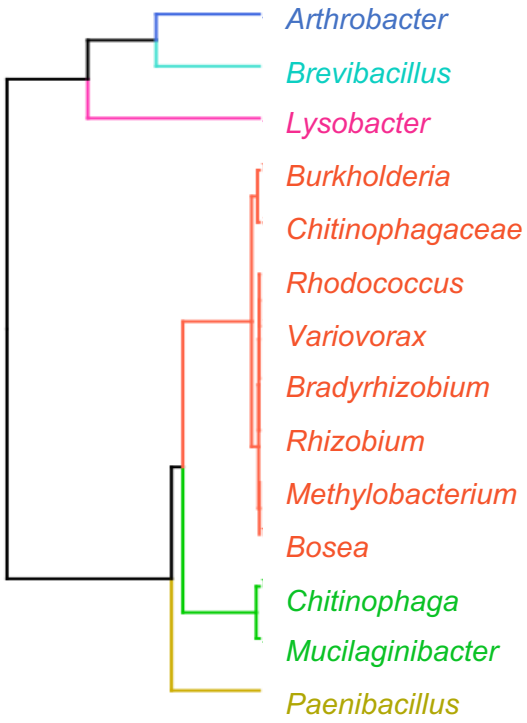
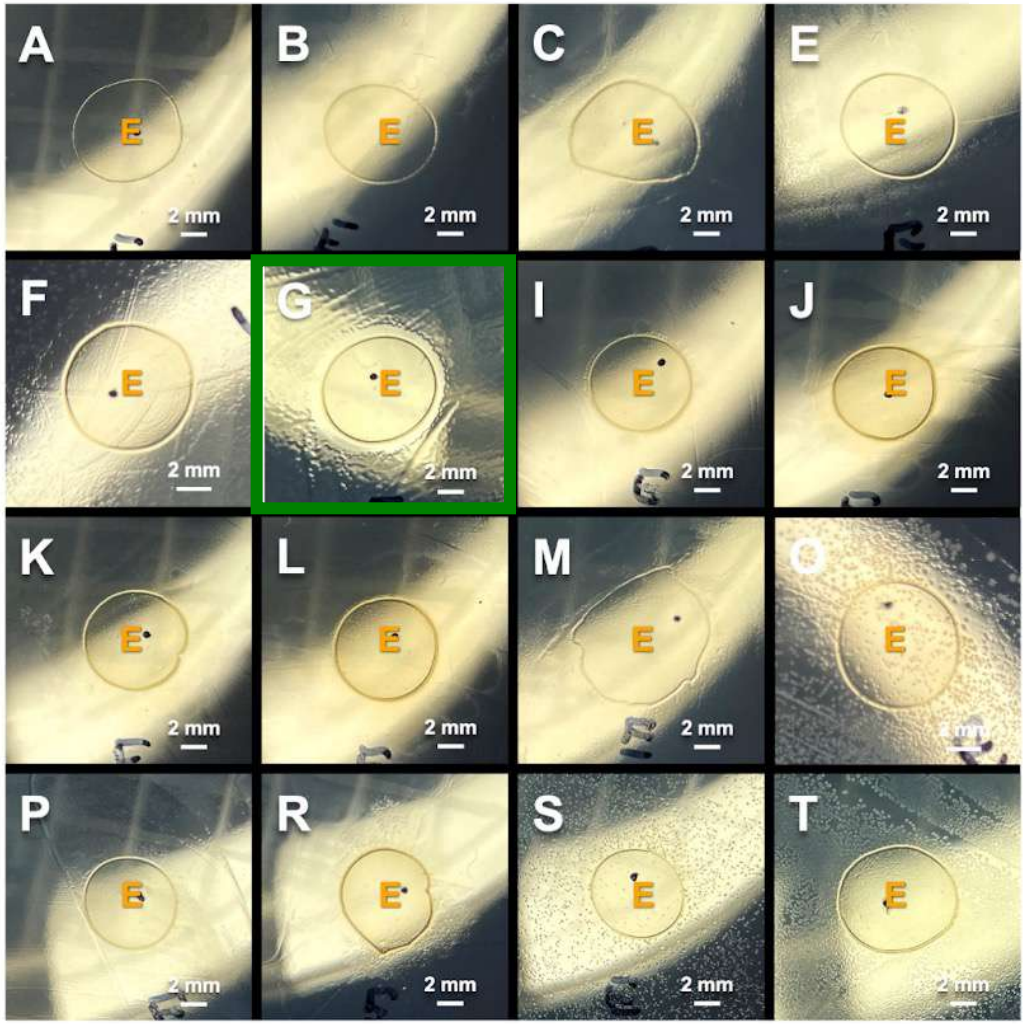
Antimicrobials



E=
Chitinophaga



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❑ Modifying community composition

ADDITION

PROBIOTIC INTERVENTION

ADDITION

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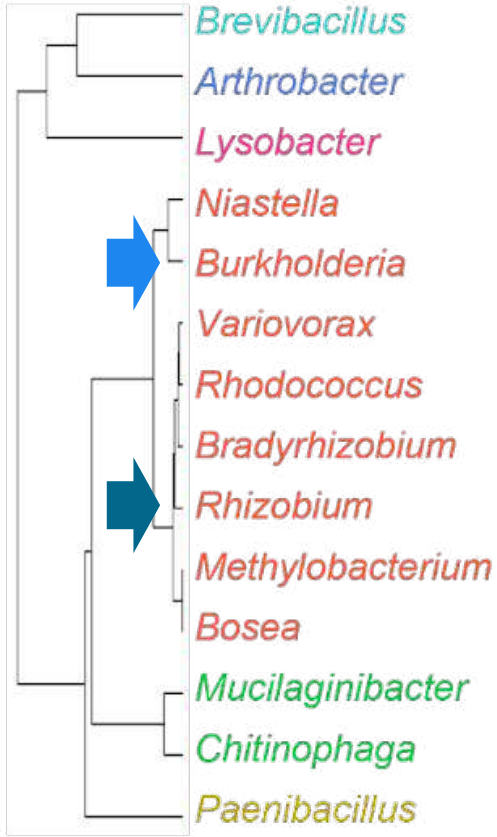
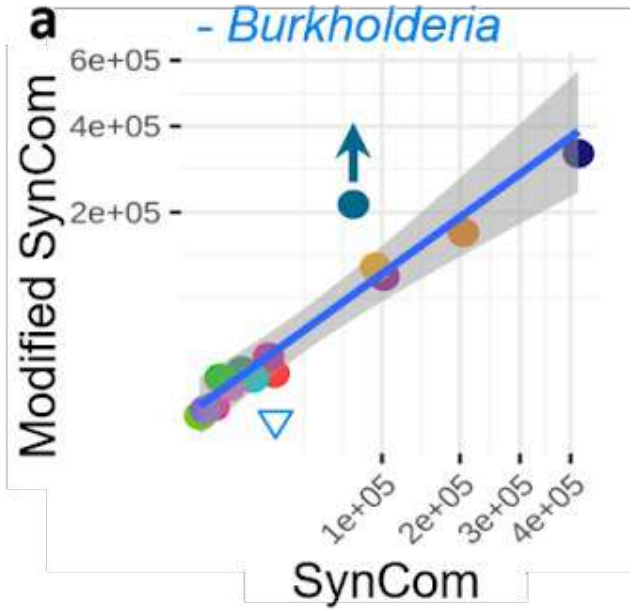
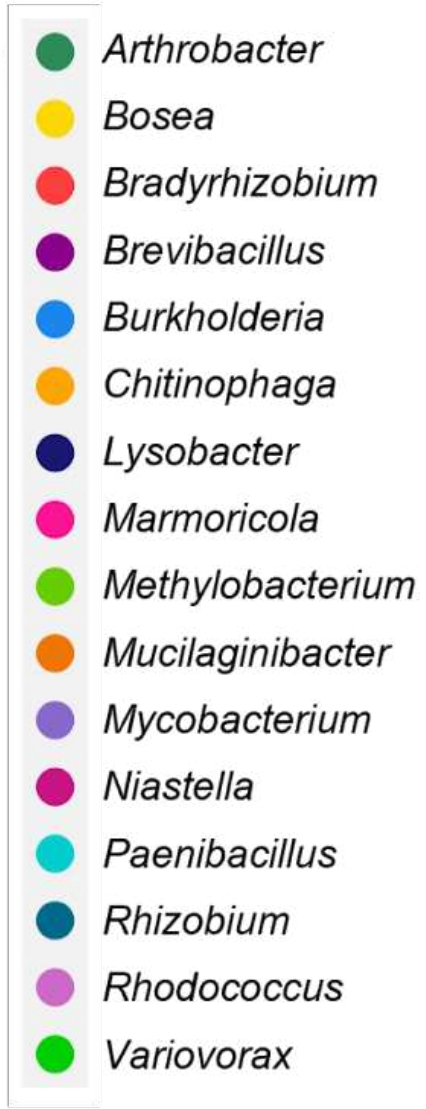
Response to the Addition of Microbes

Burkholderia/
Rhizobium







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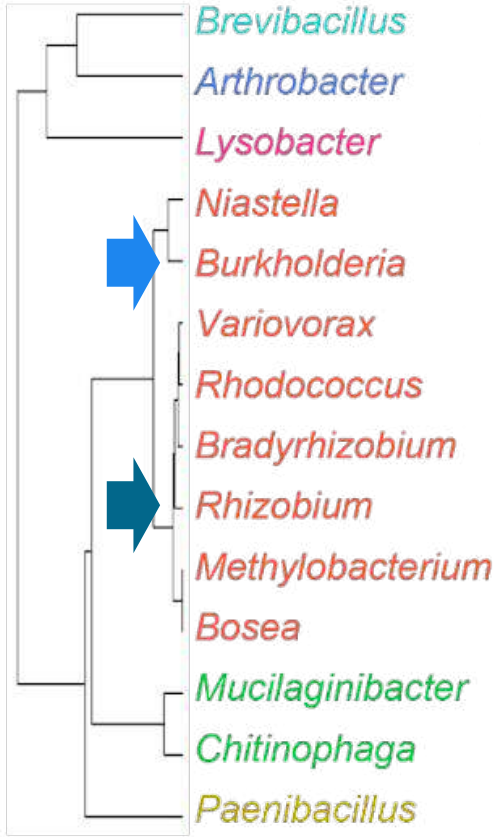
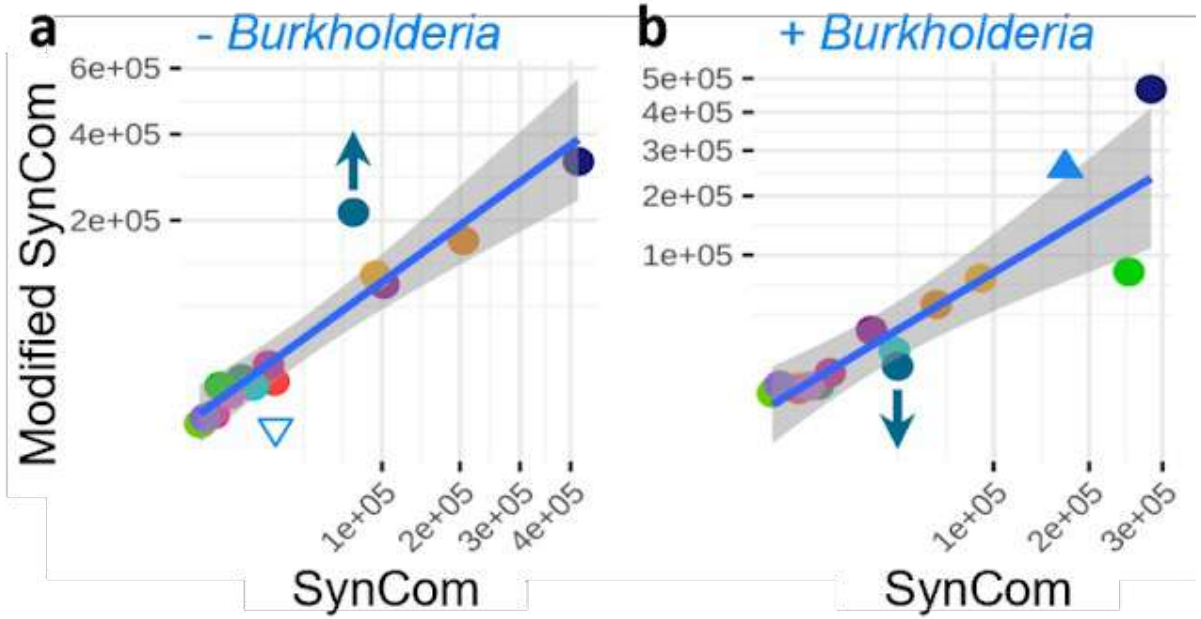
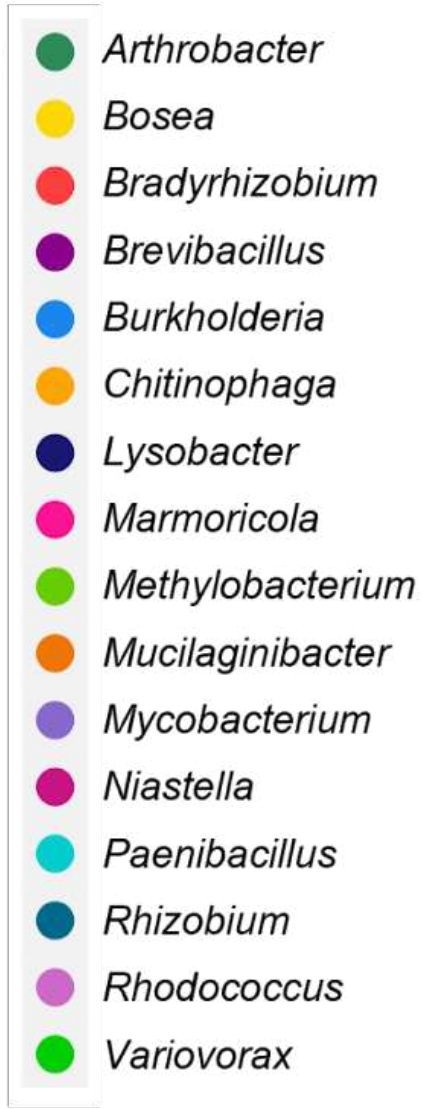
Response to the Addition of Microbes

Burkholderia/
Rhizobium







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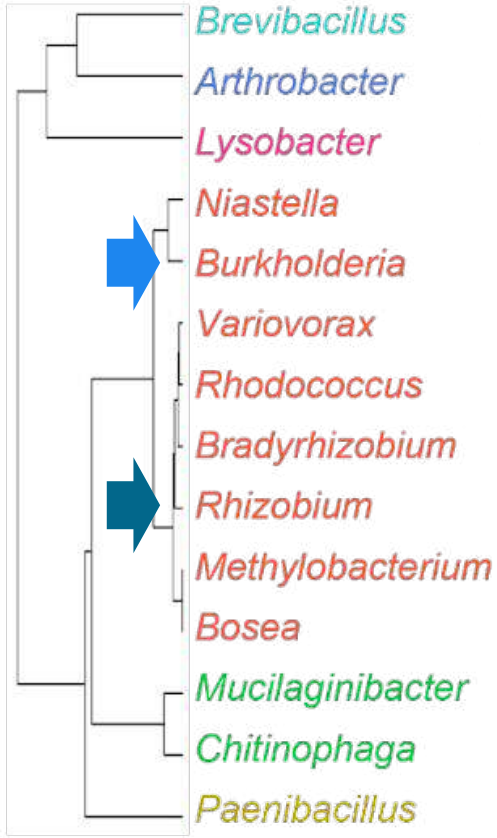
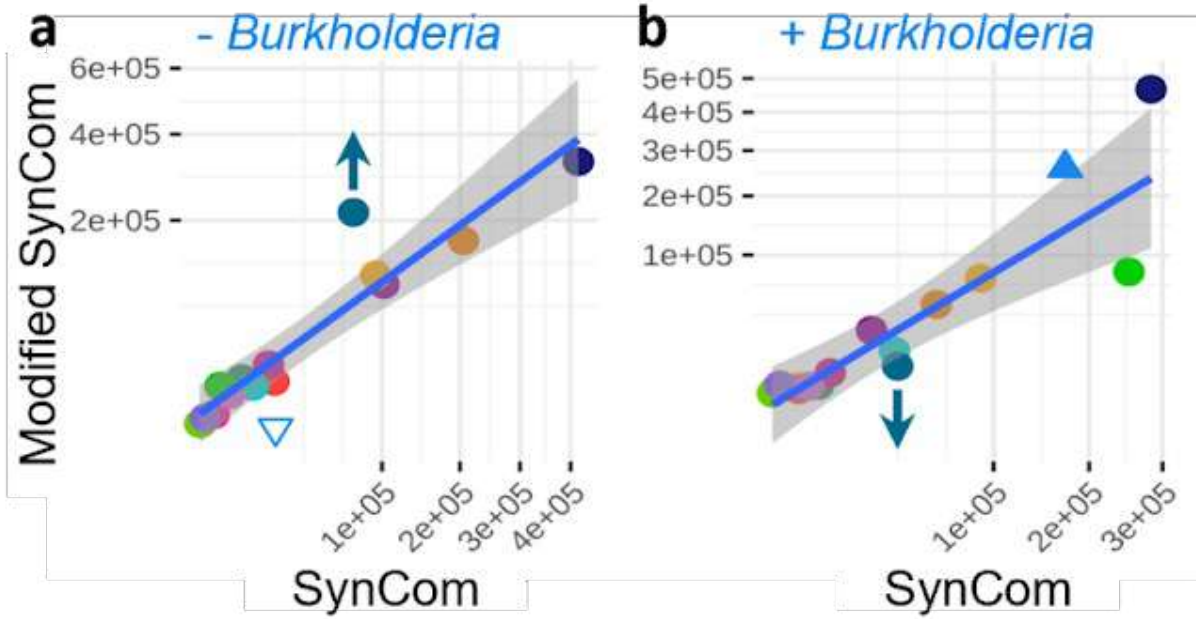
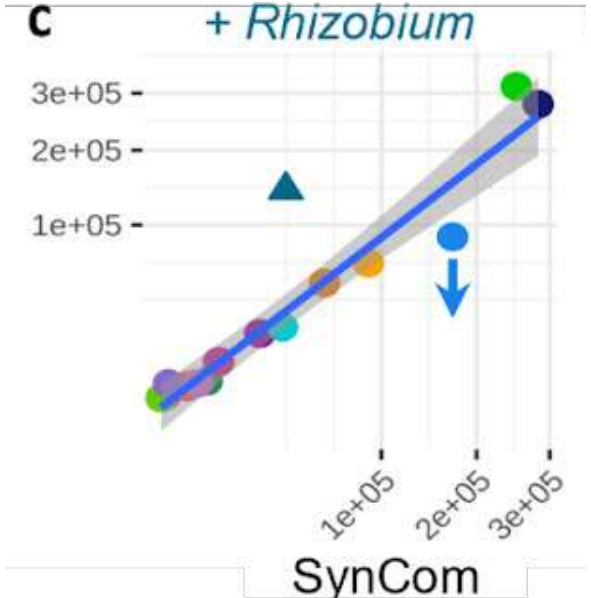
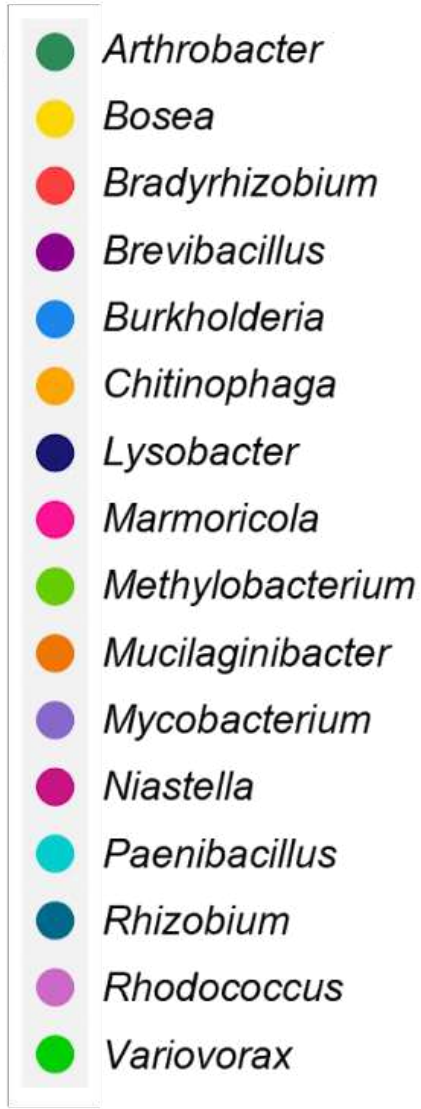
Response to the Addition of Microbes

Burkholderia/
Rhizobium









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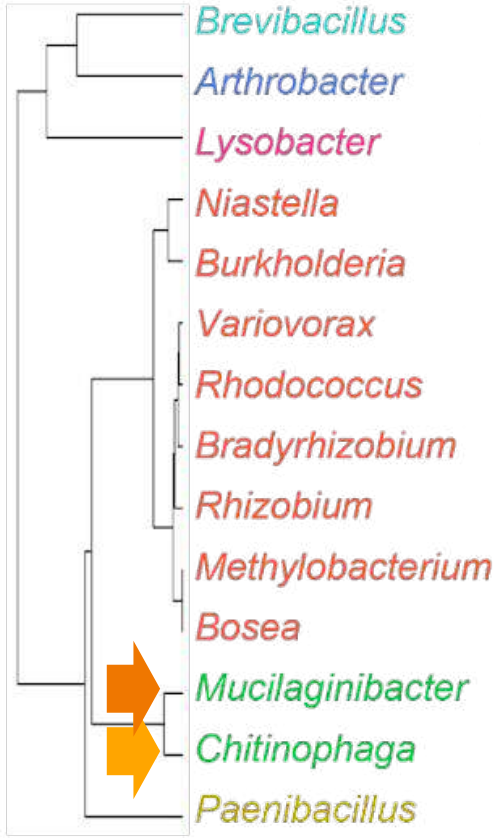
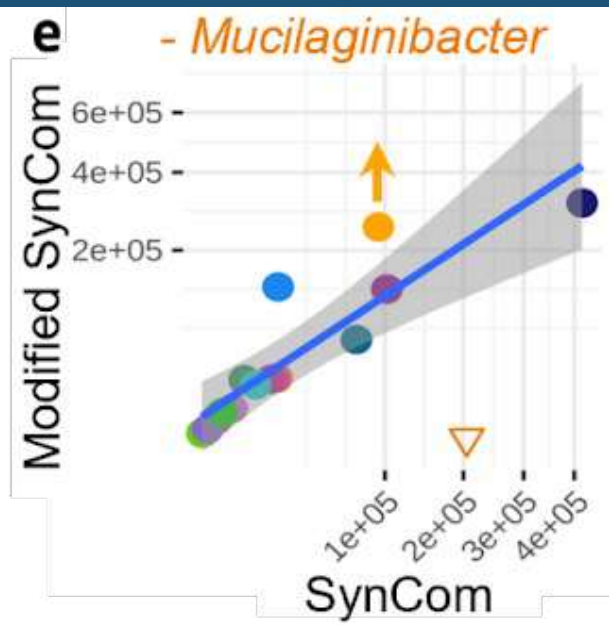
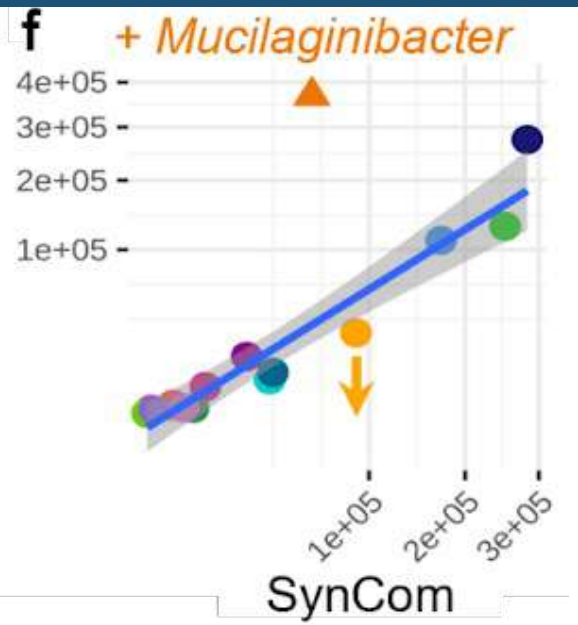
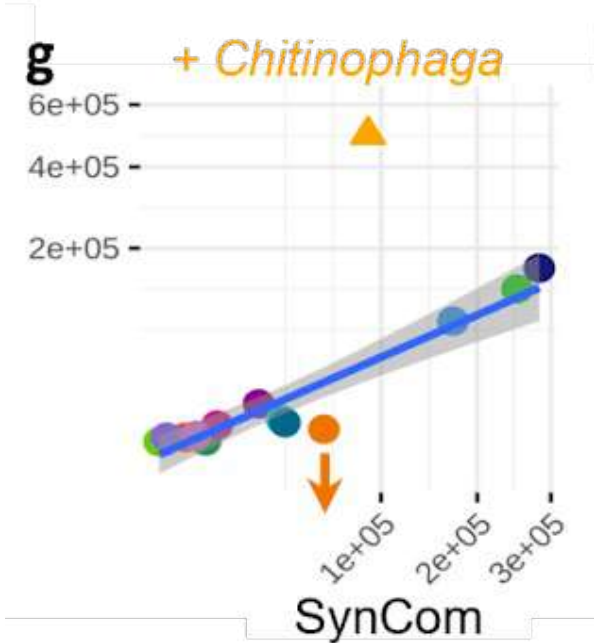
Response to the Addition of Microbes

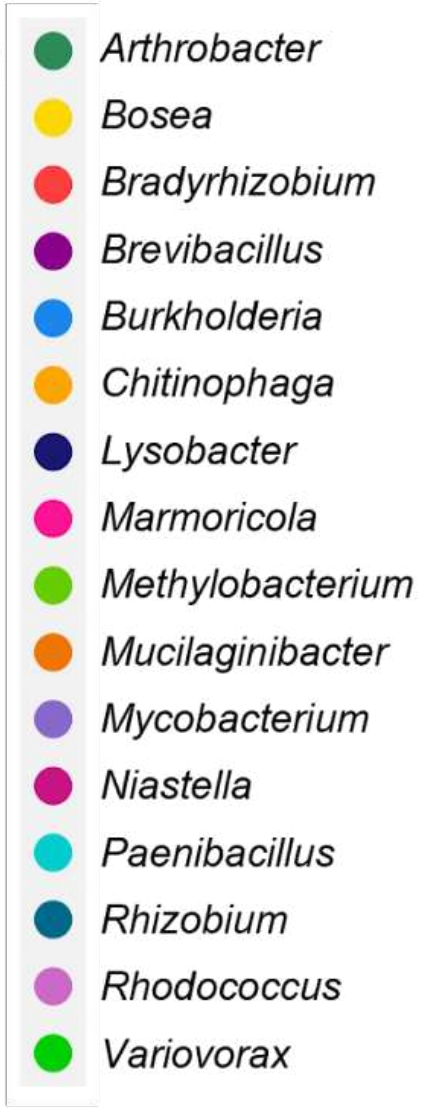
Mucilaginibacter/
Chitinophaga











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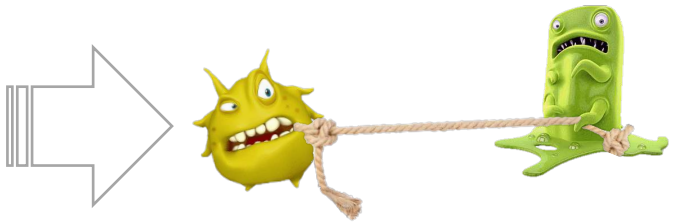
PROBIOTIC INTERVENTION

✓ Modifying community composition



✓ Modifying community composition

Adding metabolites

PREBIOTIC INTERVENTION

Adding metabolites

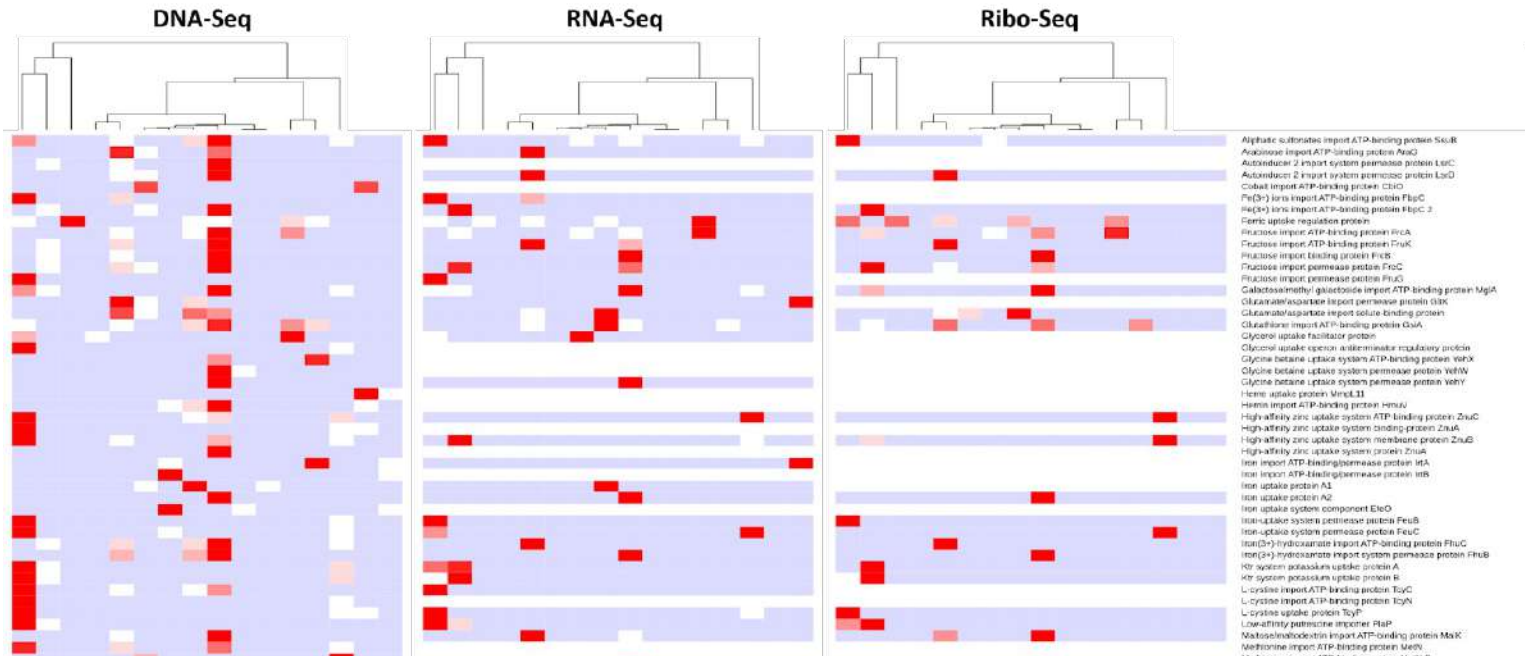
PREBIOTIC INTERVENTION

Adding metabolites

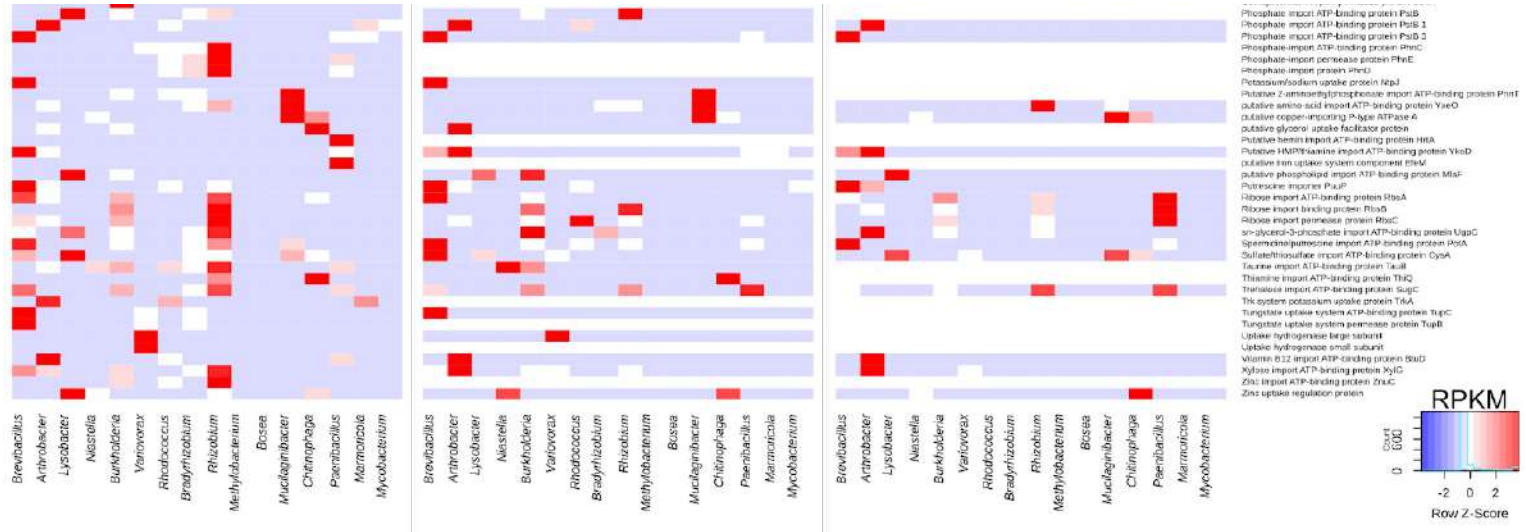
PREBIOTIC INTERVENTION

Adding metabolites

Importer Proteins



88 in metaG data



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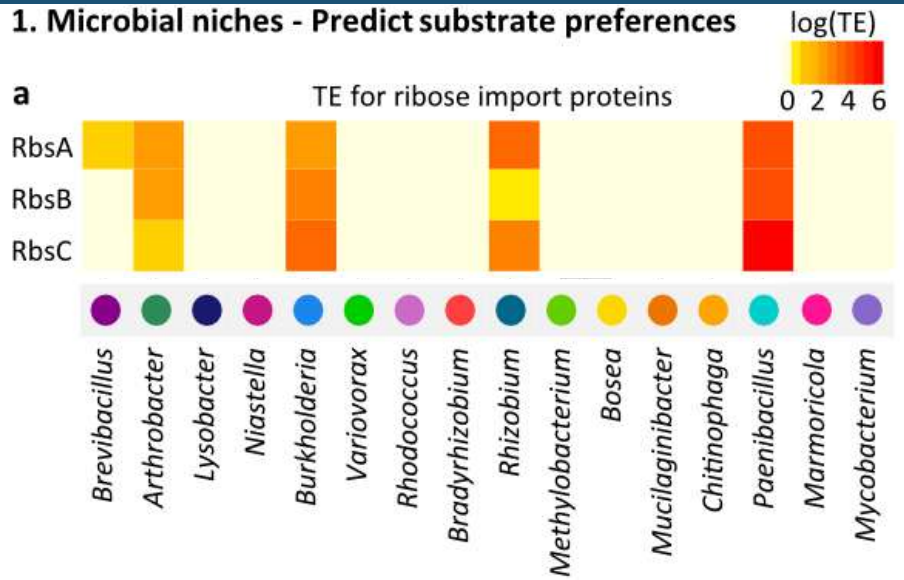
Microbial Niche Determination (MiND)

1. Microbial niches - Predict substrate preferences



3. Experimental validation: Prebiotic interventions

+ Ribose 5 g/L

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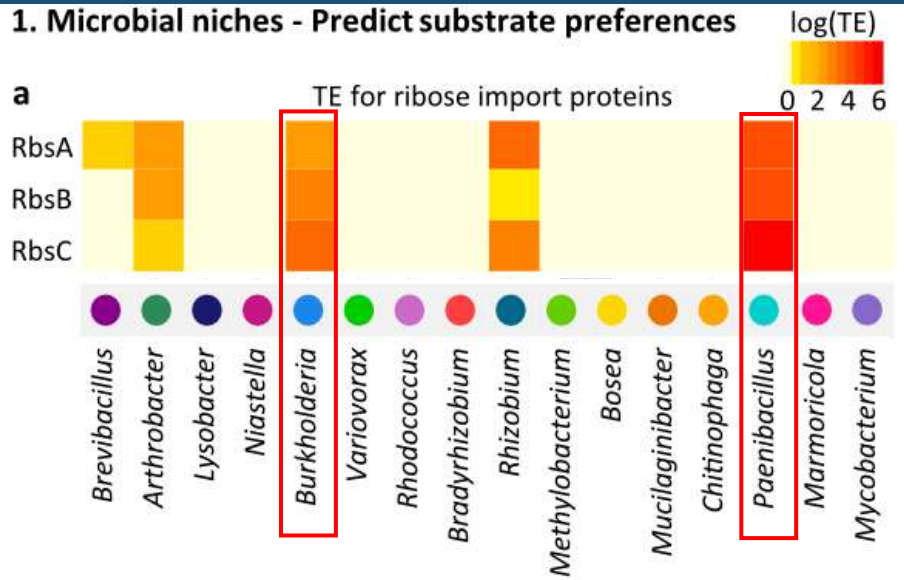
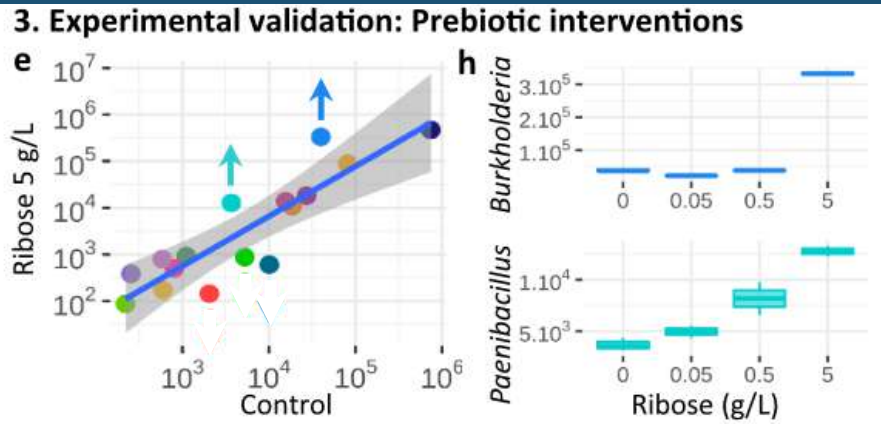
Microbial Niche Determination (MiND)





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Microbial Niche Determination (MiND)

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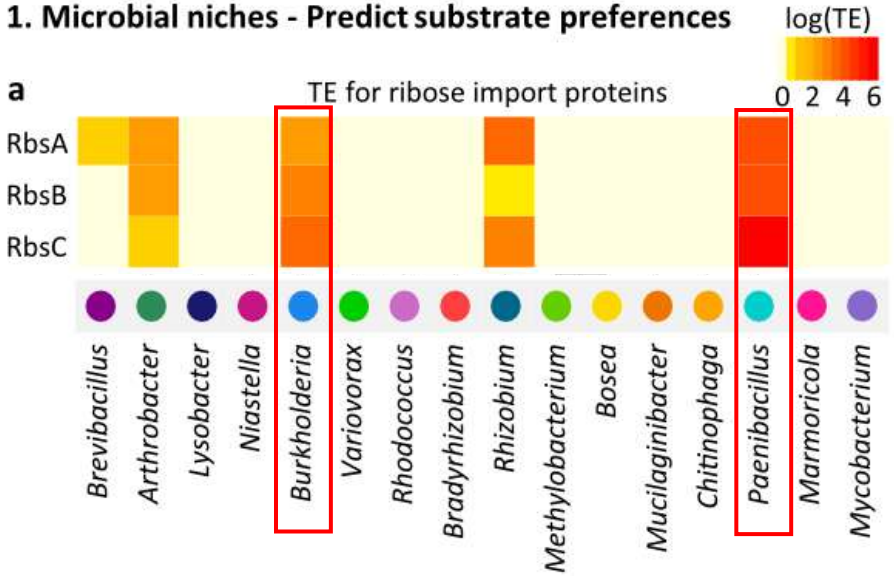
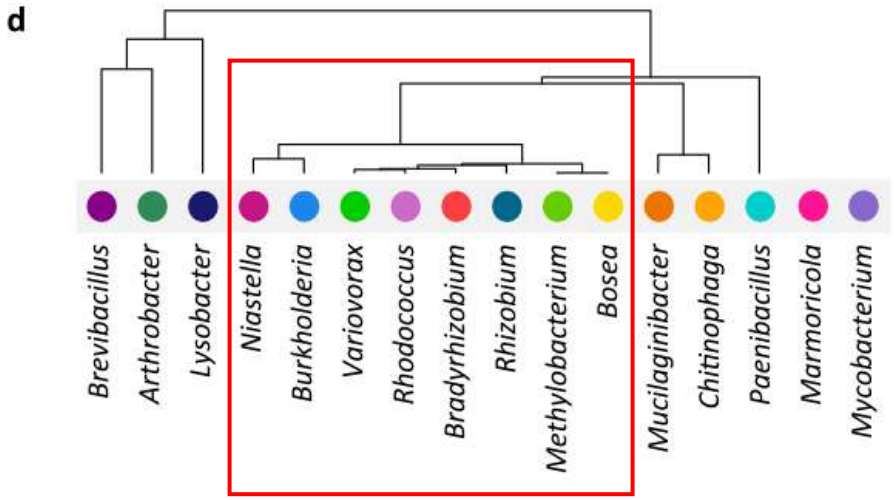
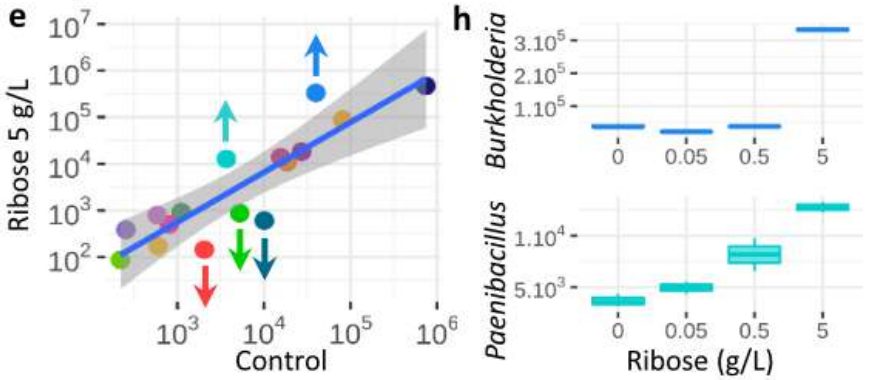


2. Microbial guilds - Predict competition



3. Experimental validation: Prebiotic interventions



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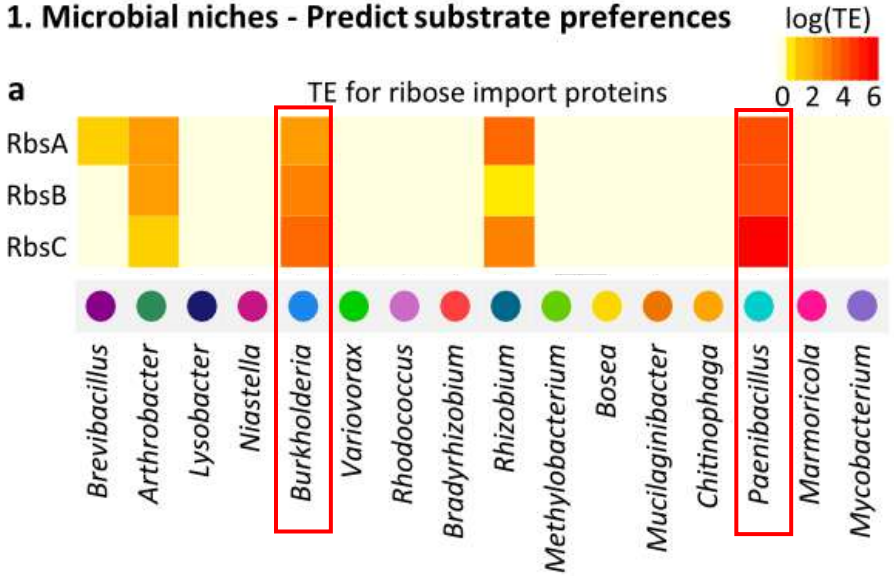
Microbial Niche Determination (MiND)

1. Microbial niches - Predict substrate preferences



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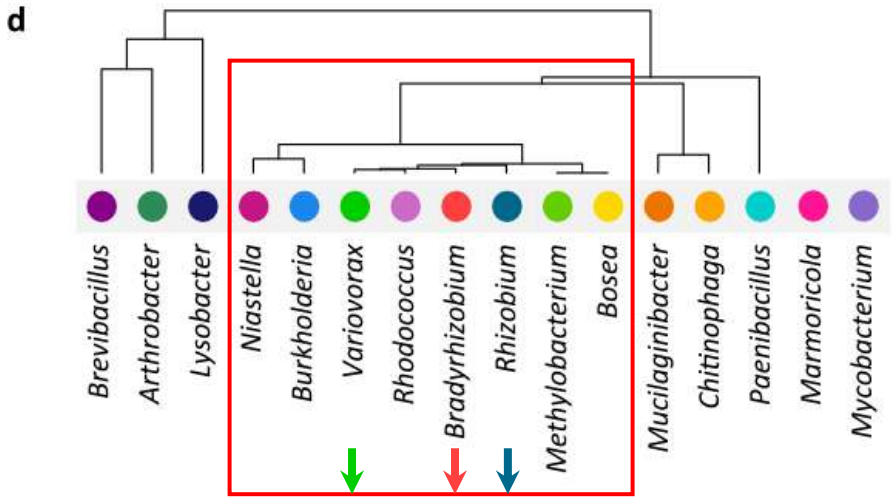
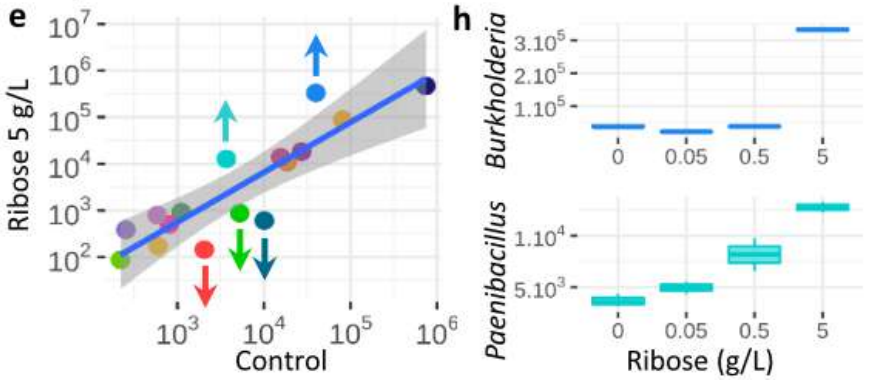


3. Experimental validation: Prebiotic interventions



Competition for Resources

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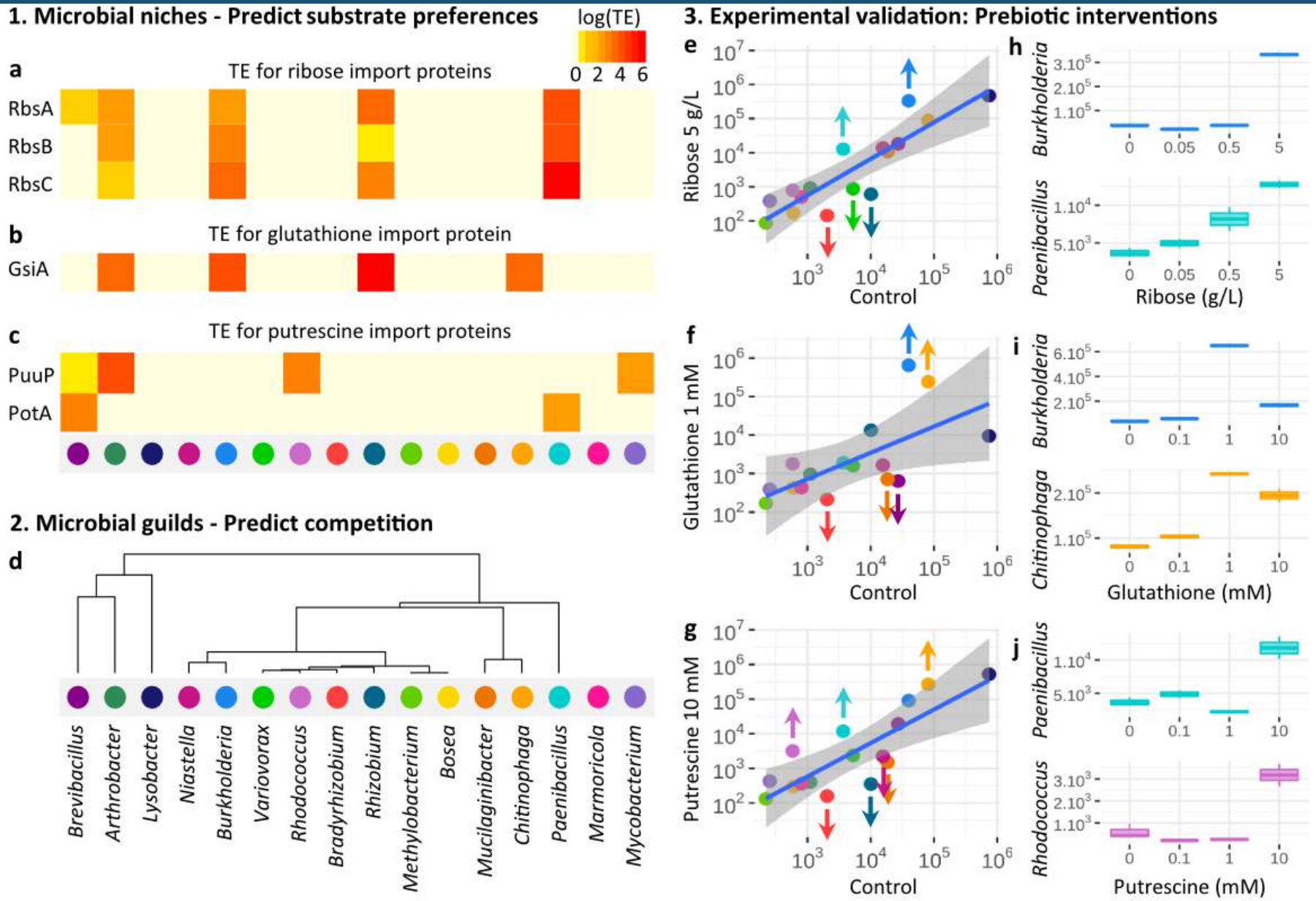
Microbial Niche Determination (MiND)



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Predicting Response to 11 Metabolites*

	Sensitivity	Specificity	Accuracy
Primary Target: ↑ (increase)	54%	83%	79%
Secondary Target ↓ (decrease)	93%	65%	70%

*Fructose, Galactose, Ribose, Trehalose, Xylose, Maltodextrin, Glutamate, Glutathione, Putrescine, Spermidine, Sulfate+Thiosulfate

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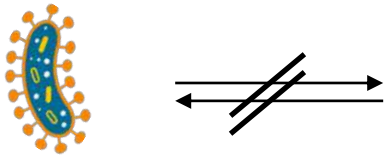
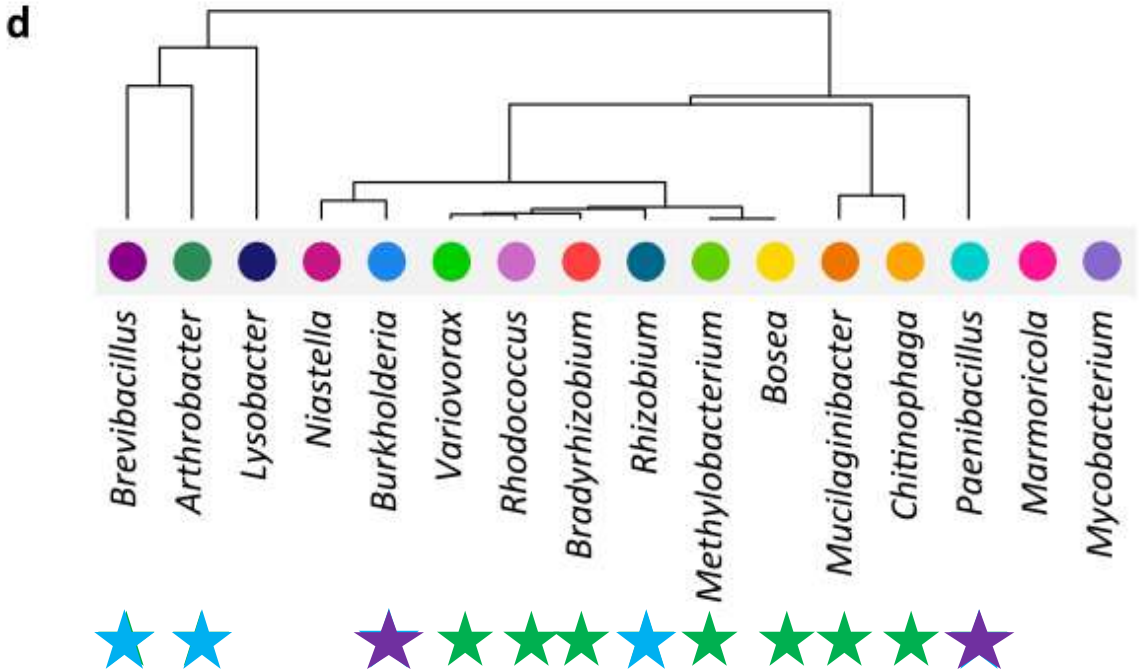
Axenic vs. Community Growth

very hard to predict!





12 isolates metabolize ribose axenically

5 try to grow with ribose in the community

2 isolates succeed

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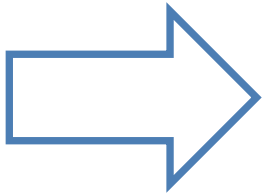
- ✓ Modifying composition (Probiotics)
- ✓ Adding metabolites (Prebiotics)

Complex



Complicated

SynCom

Complex

Soil

Complicated

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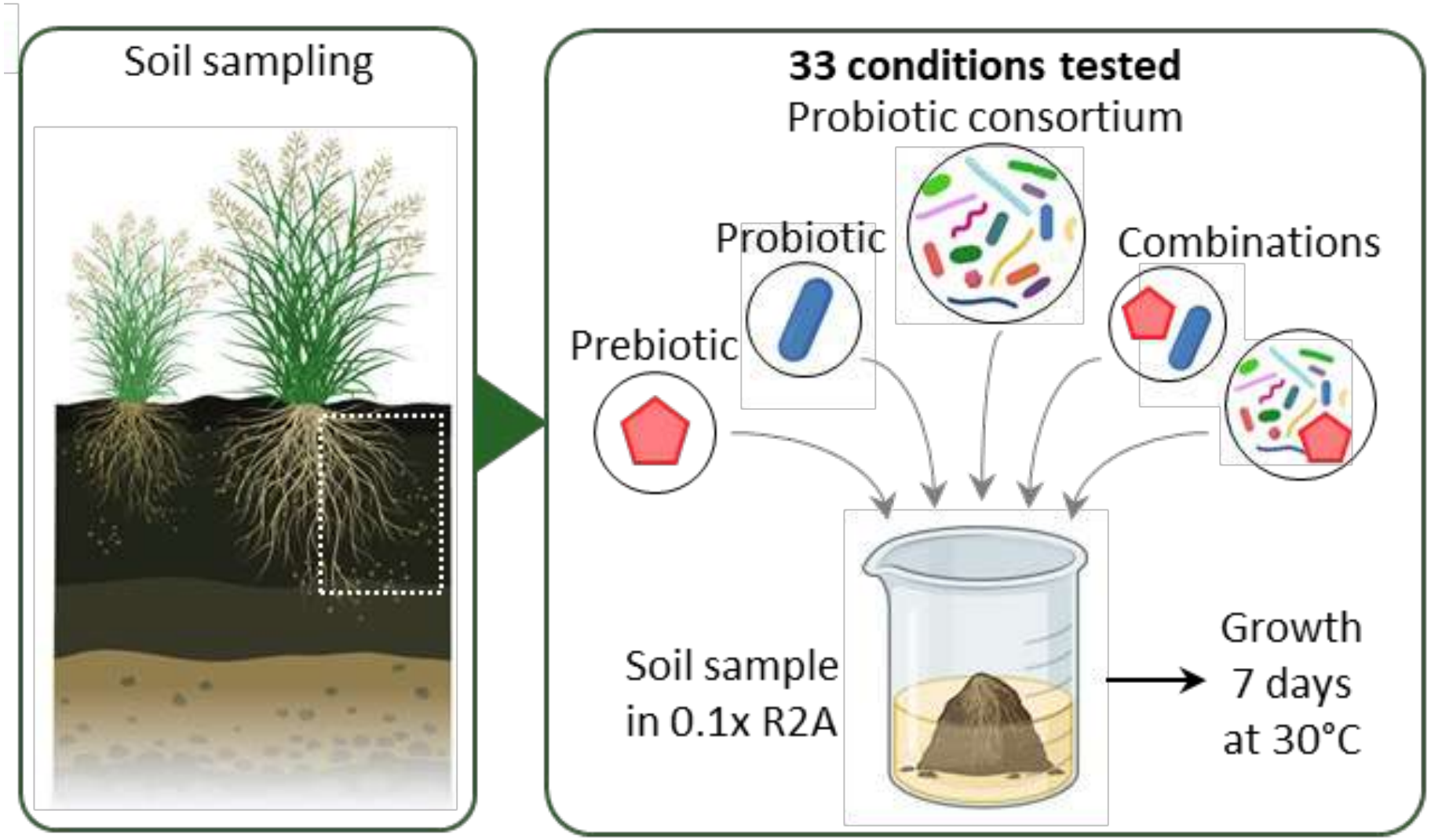
Experimental Setup



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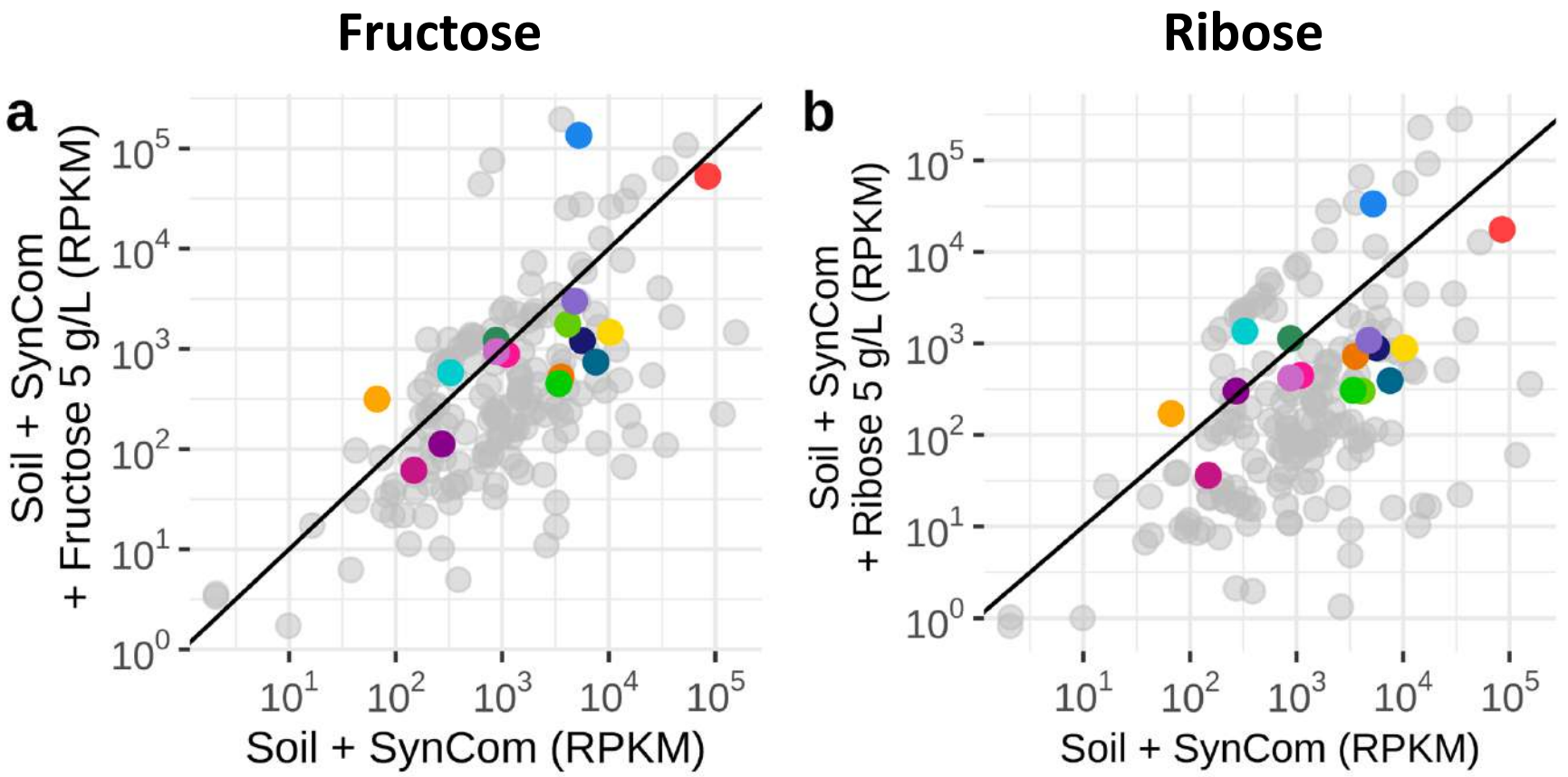
Soil



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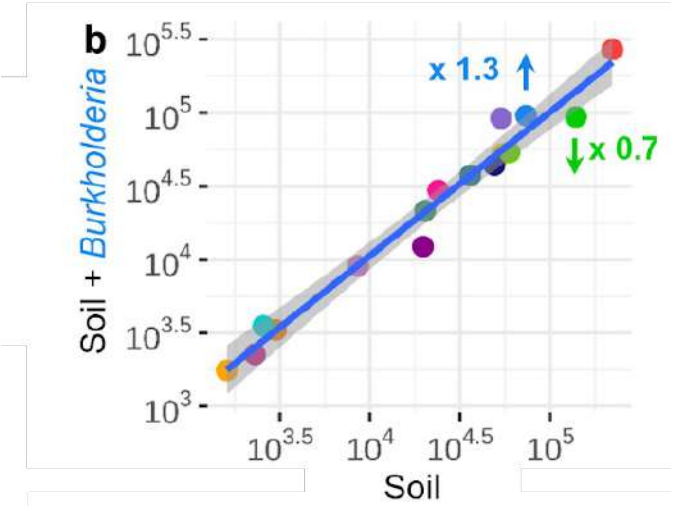
Soil (only SynCom Members Shown)



Glutathione

Glutathione

SynCom strains

- *Arthrobacter*
- *Bosea*
- *Bradyrhizobium*
- *Brevibacillus*
- *Burkholderia*
- *Chitinophaga*
- *Lysobacter*
- *Marmoricola*
- *Methylobacterium*
- *Mucilaginibacter*
- *Mycobacterium*
- *Niastella*
- *Paenibacillus*
- *Rhizobium*
- *Rhodococcus*
- *Variovorax*

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Targeted Interventions in Soil

Intervention in soil 	Total number of tested conditions	Number of conditions in which primary targets ↑ increased	Number of conditions in which secondary targets ↓ decreased
Probiotic Single Strain	7	4/7 (57%)	4/4 (100%)
Prebiotic	7	6/7 (86%)	6/6 (100%)
Prebiotic + Probiotic Single Strain	10	8/10 (80%)	6/8 (75%)
Prebiotic + Probiotic Consortium	7	7/7 (100%)	7/7 (100%)

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synCom in Soil

Soil

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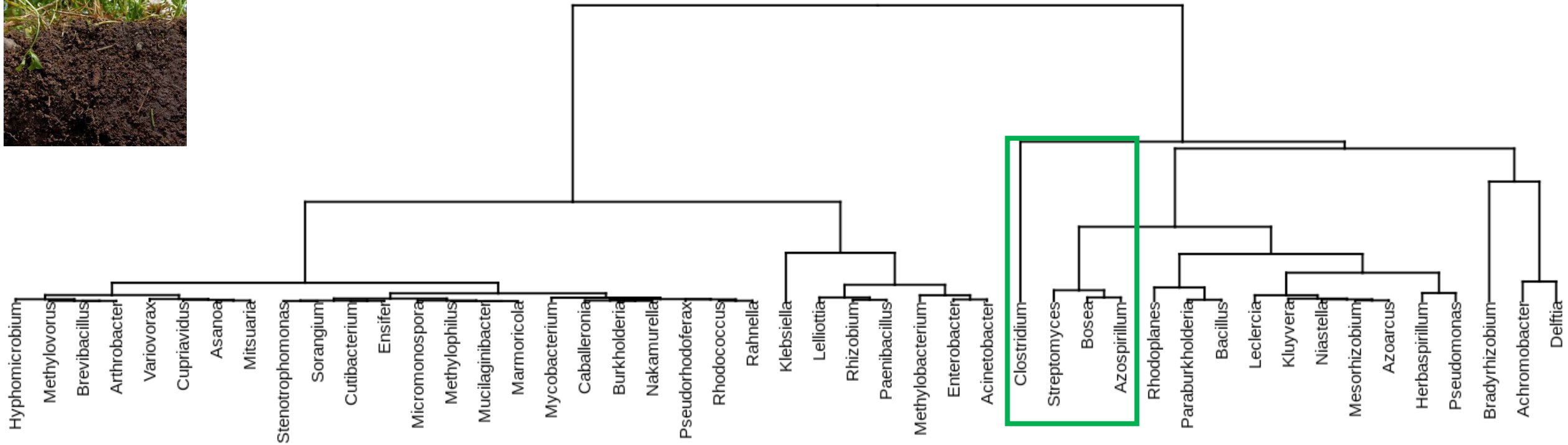
Guilds based on TE in soil



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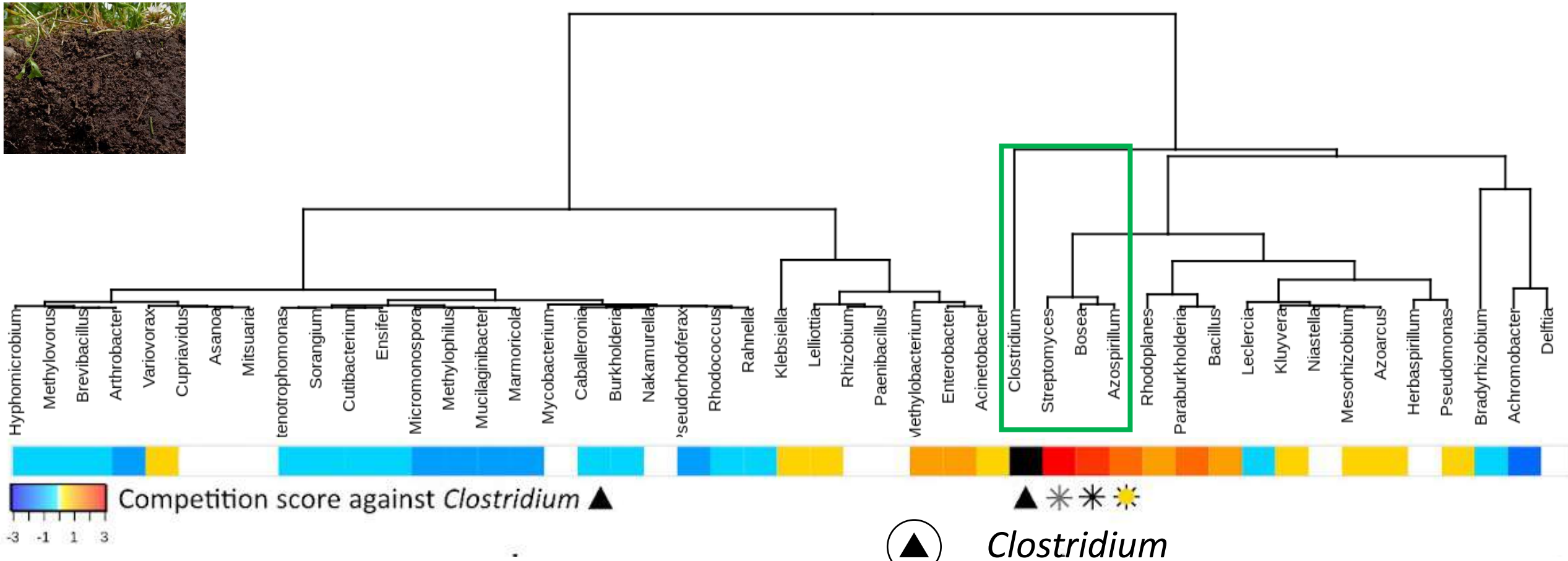
Guilds based on TE in soil



Clostridium

Bosea

Azospirillum

Streptomyces

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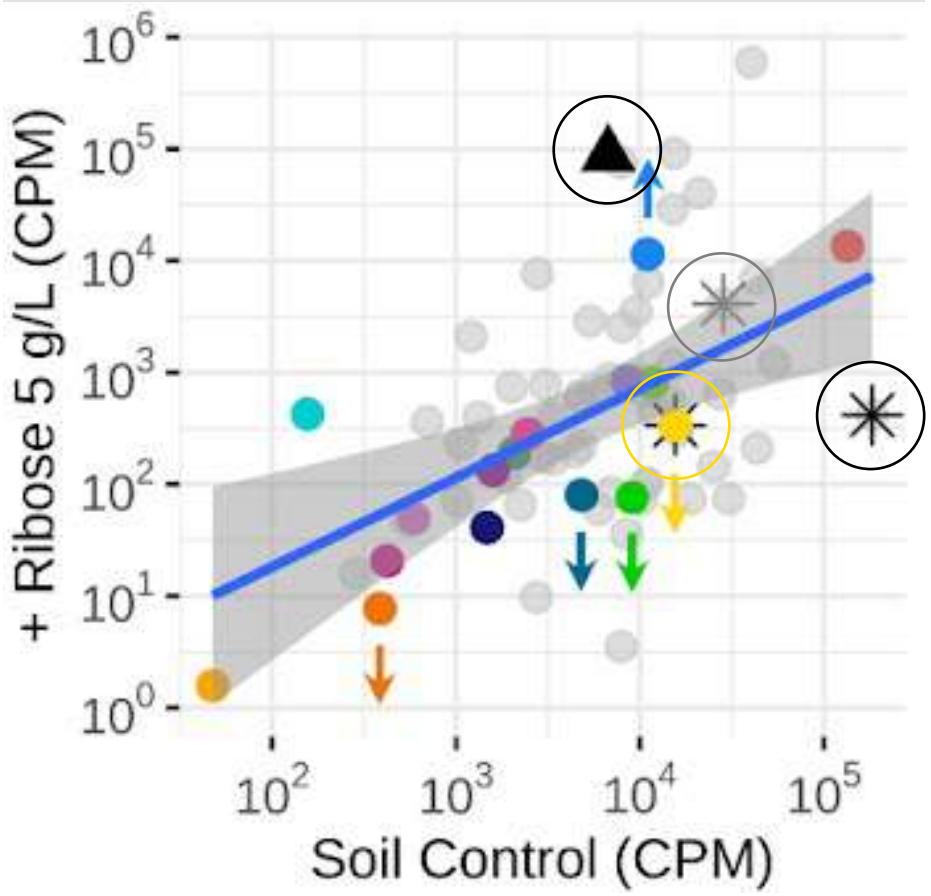
Soil – Substrate Addition

+ Ribose



Clostridium

Bosea

Azospirillum

Streptomyces

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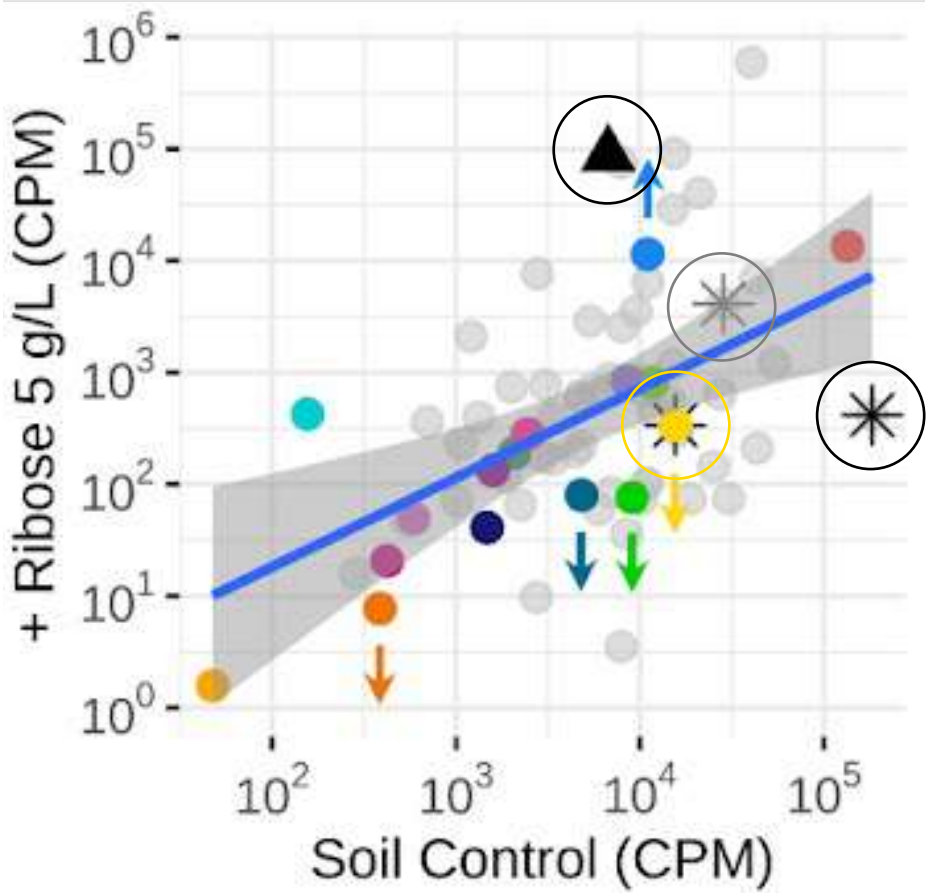
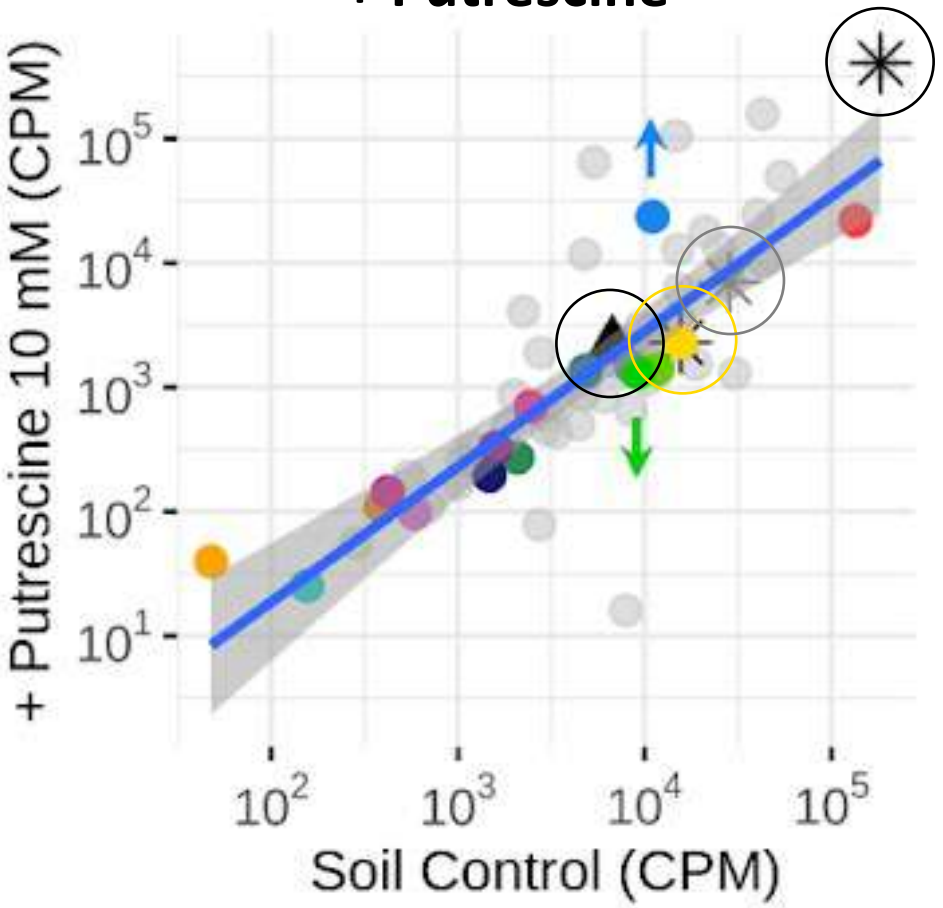
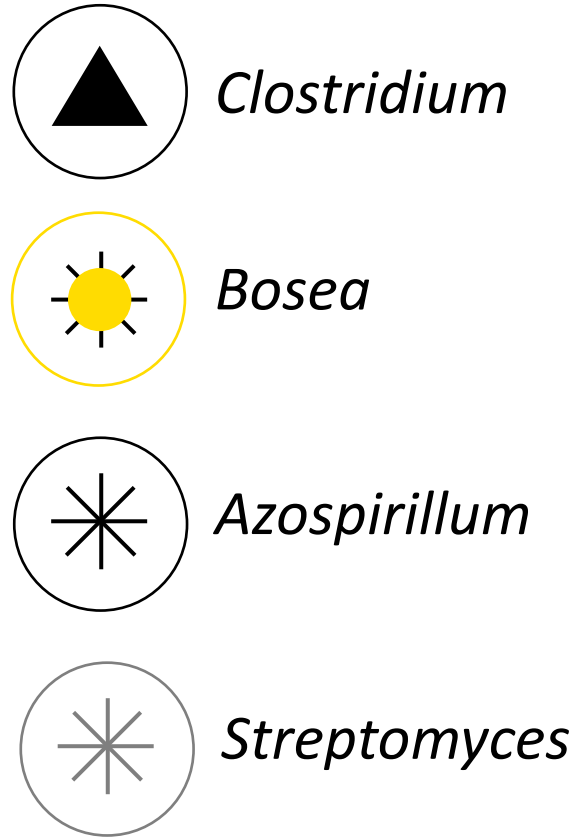
Soil – Substrate Addition

+ Ribose



+ Putrescine





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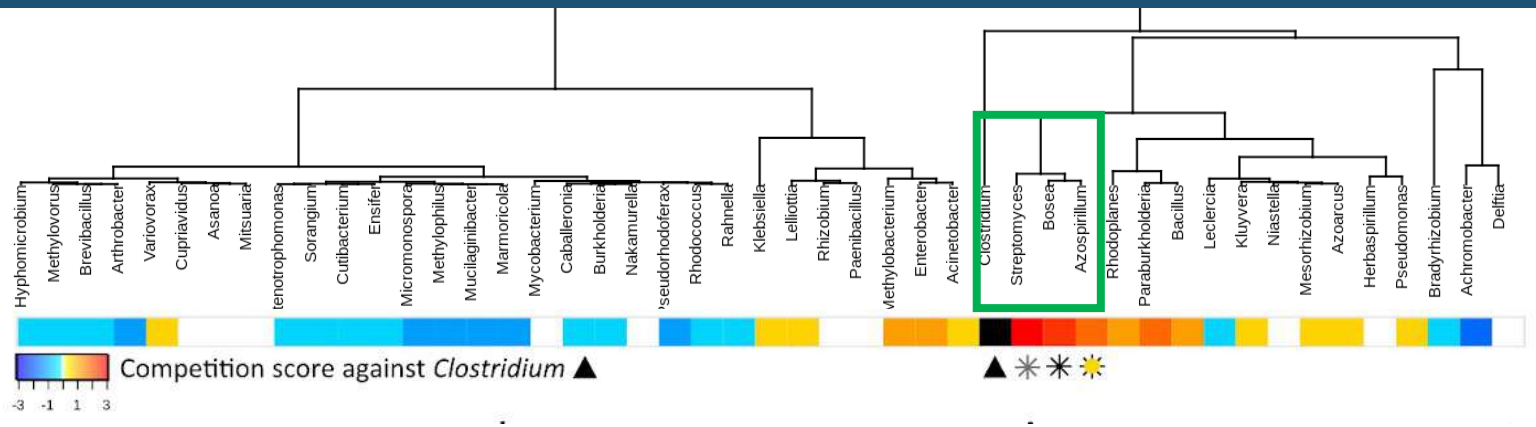
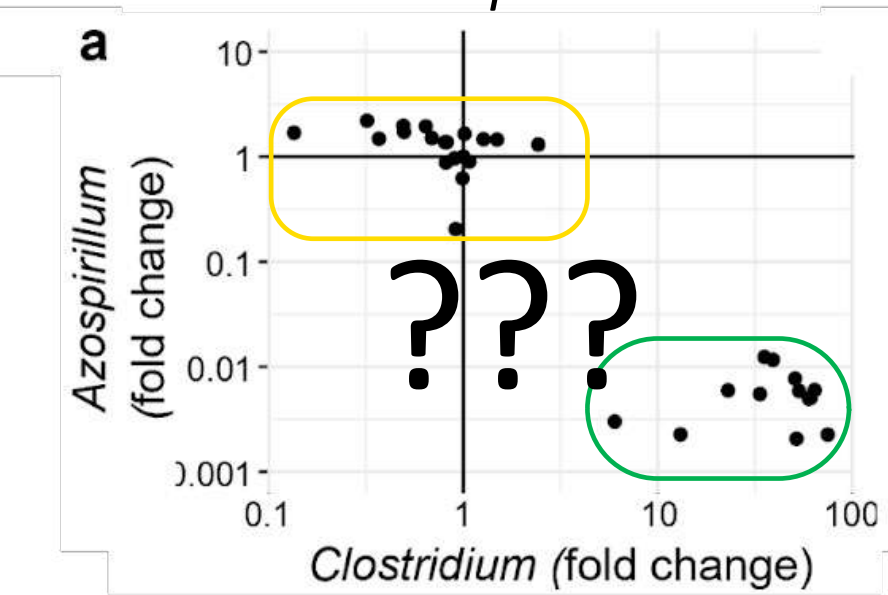
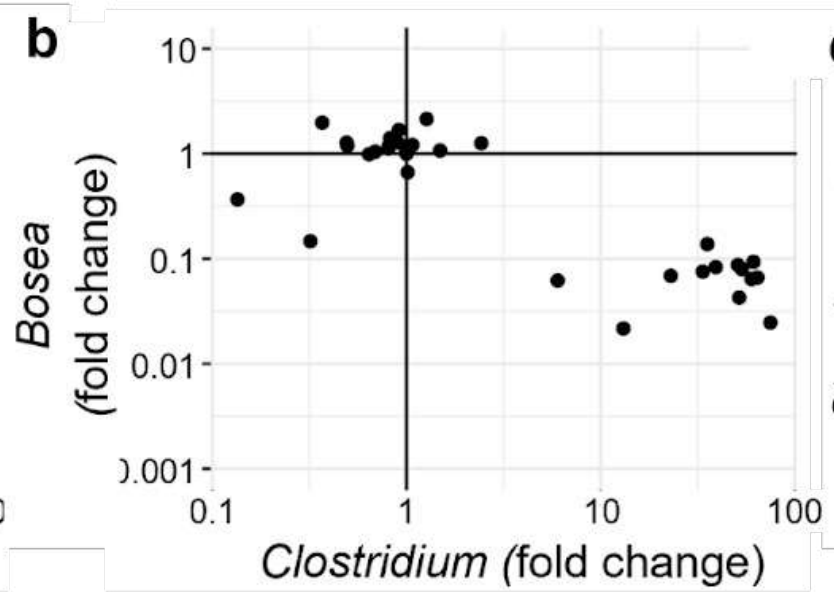
Log-Fold Change Under Many Conditions



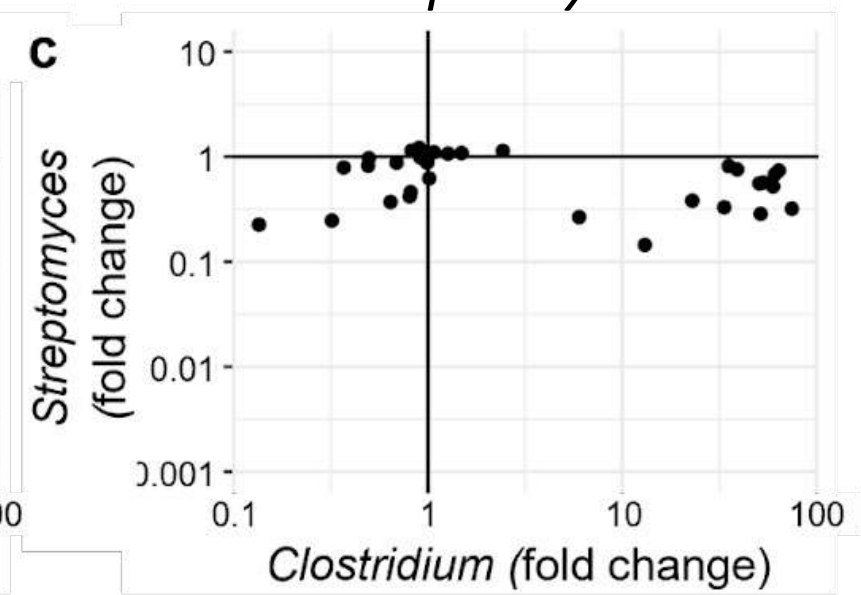
Azospirillum



Bosea



Streptomyces



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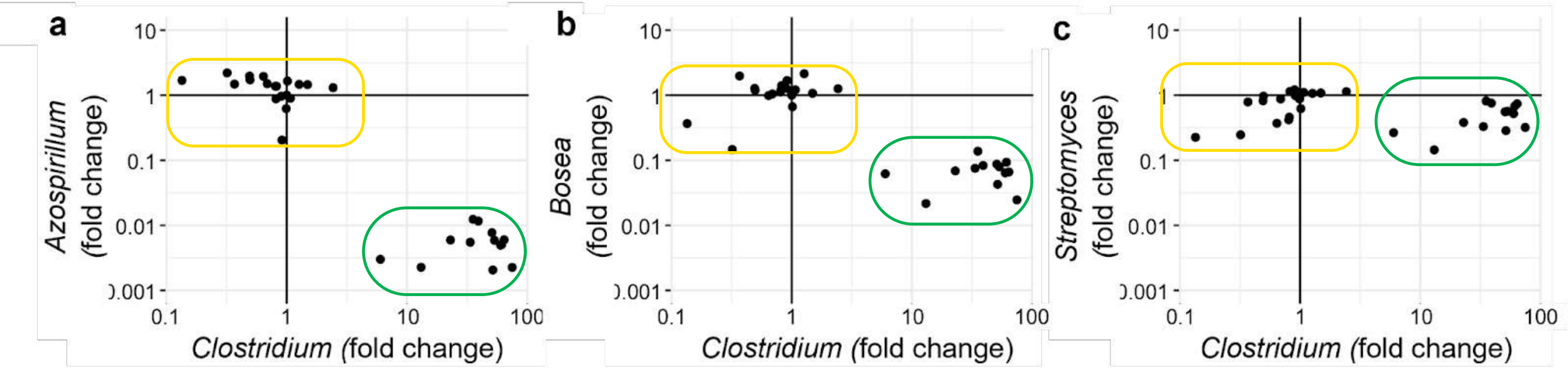
Log-Fold Change Under Many Conditions

Non-Sugars

(Glutathione, putrescine, sulfate/thiosulfate)

Sugars

(Fructose, ribose, maltose, trehalose)



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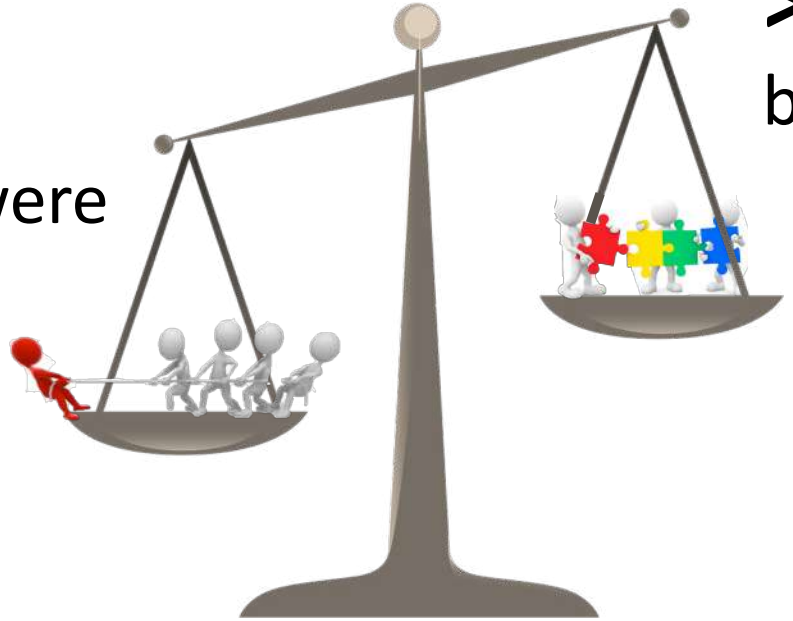
Competition versus Collaboration

81% of all interactions were explained by competition



>19% of all interactions are based on collaboration

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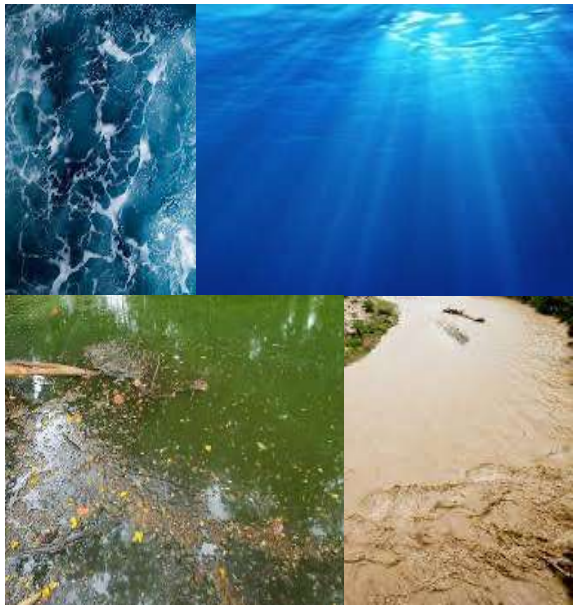
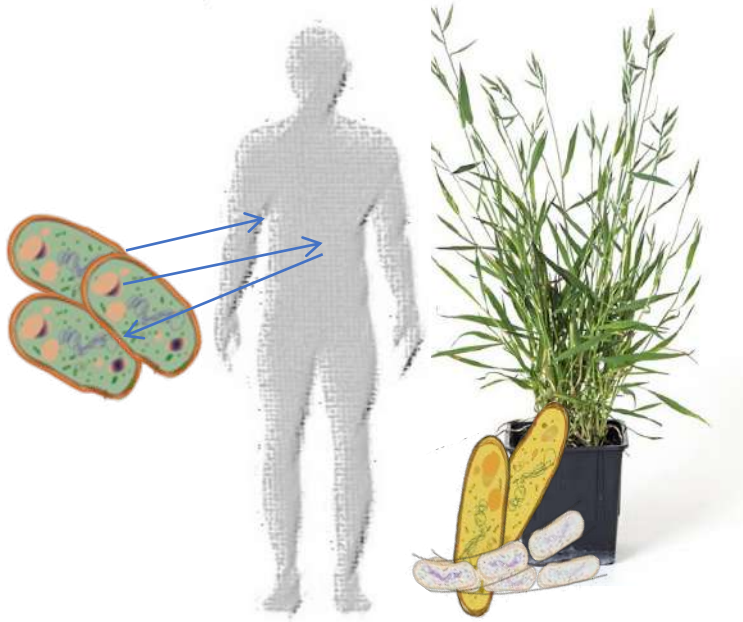
Open Questions in Microbiome Research

Terrestrial

Aquatic



Host-Associated



…how do communities response to perturbations? ✓

…can we predict outcomes? ✓

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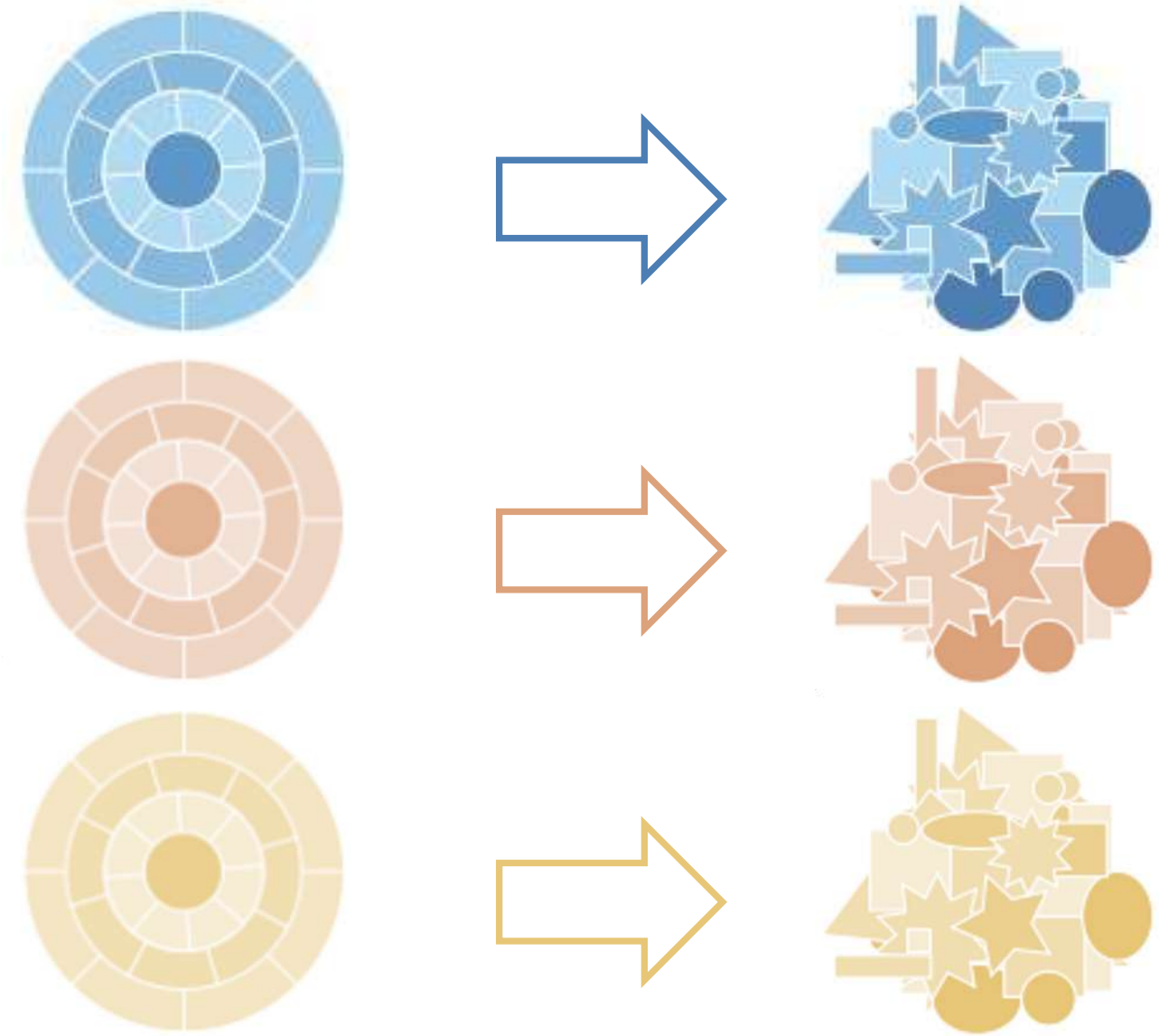
Broadly Applicable Method



Complex

Complicated

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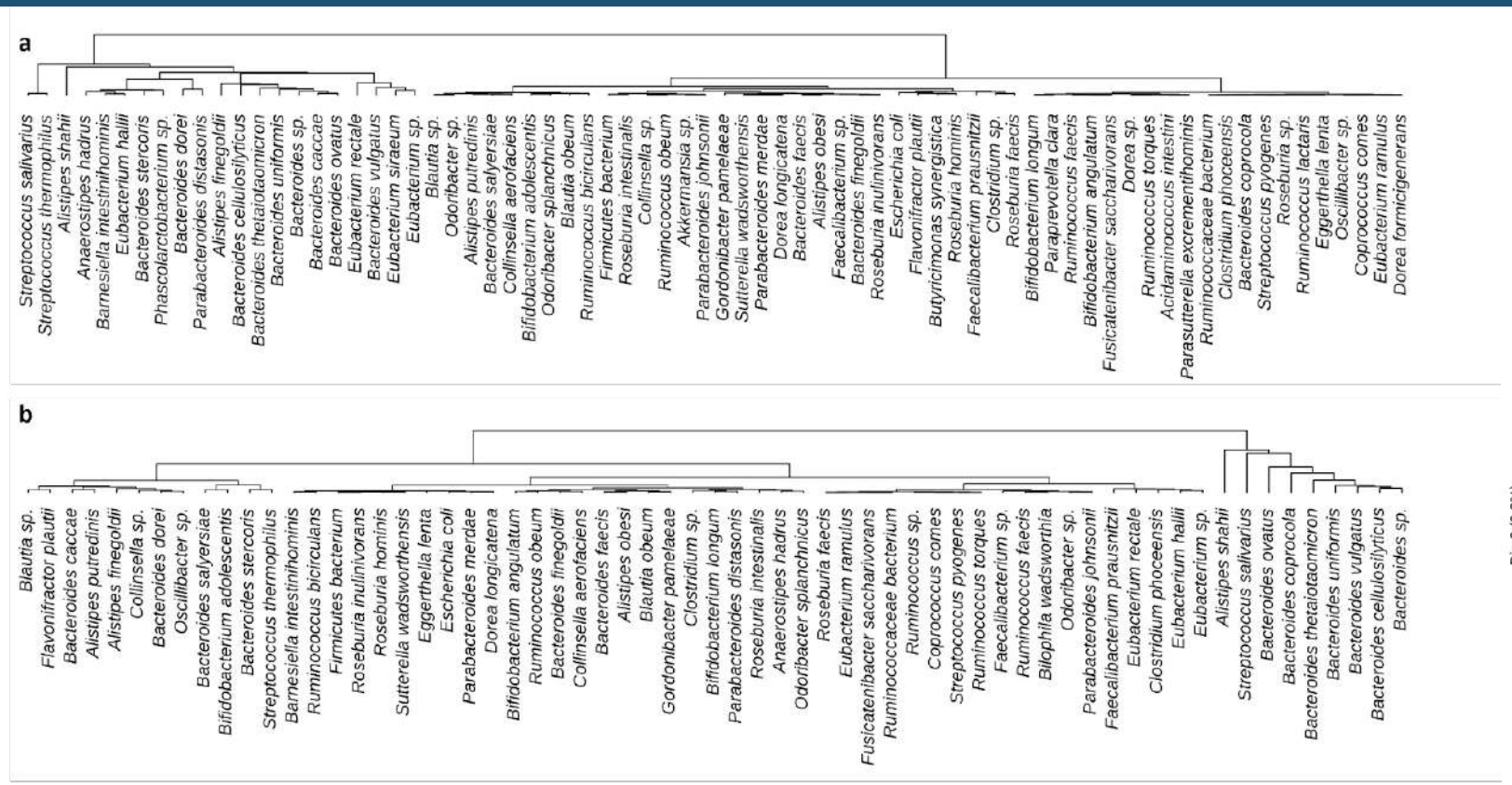
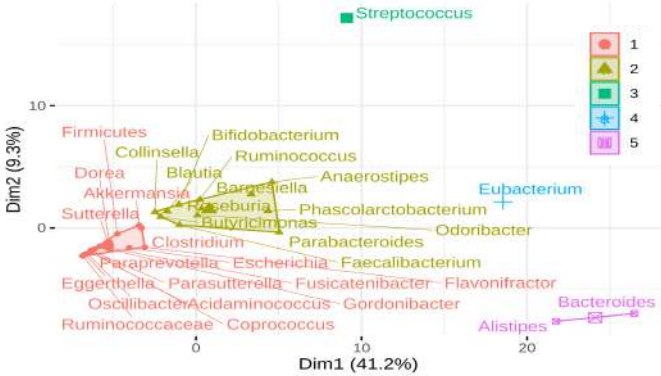
MiND and Guilds in Human Stool Samples





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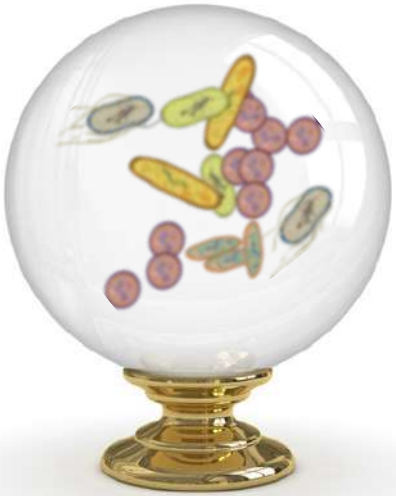
Summary

Predicting Community Function

- ✓ Translational Efficiency
- ✓ Predicting Metabolic Niches and Guilds
- ✓ Identifing Interactions (Competition)
- ✓ Designing Interventions



Changing/Engineering Microbiomes

- ✓ Organism-Level, i.e. *Probiotics*
- ✓ Metabolite-Level, i.e. *Prebiotics*
- ✓ Scalable Technology, i.e. *Soil, Stool*

- ✓ Patent filed

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Microbial Niche Determination (MiND) can predict outcomes in complex communities

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Microbial Niche Determination (MiND) can predict outcomes in complex communities

MiND and guild association identifies intervention strategies to selectively alter the microbiome

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Questions?

www.zenglerlab.com

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