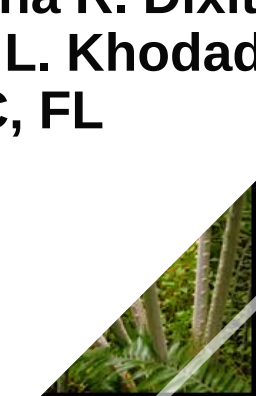


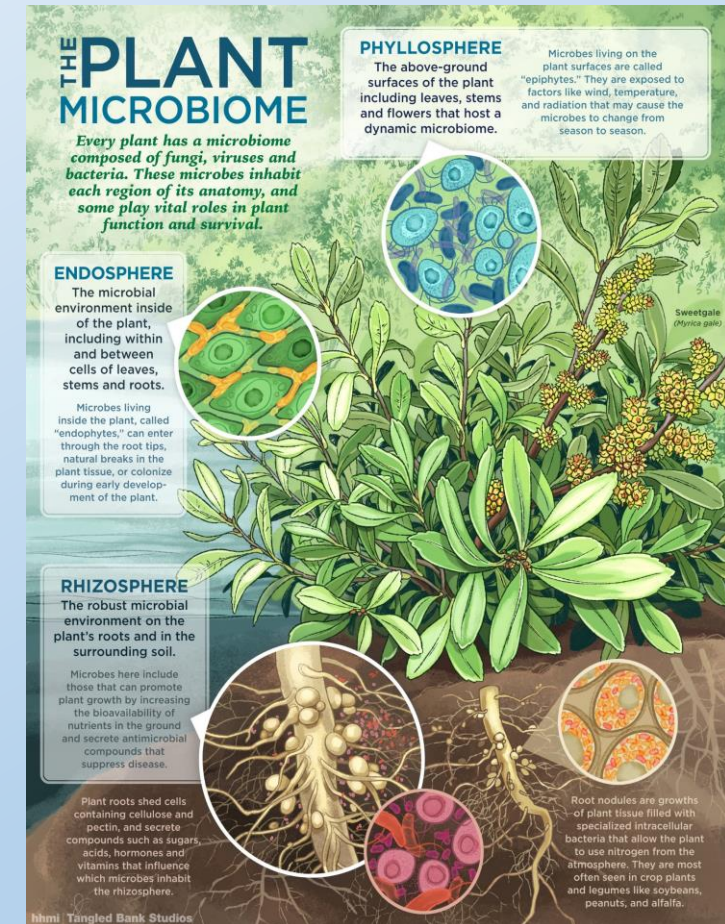
Plant Microbiome 101

Dr. Anirudha R. Dixit
Dr. Christina L. Khodadad
KSC, FL

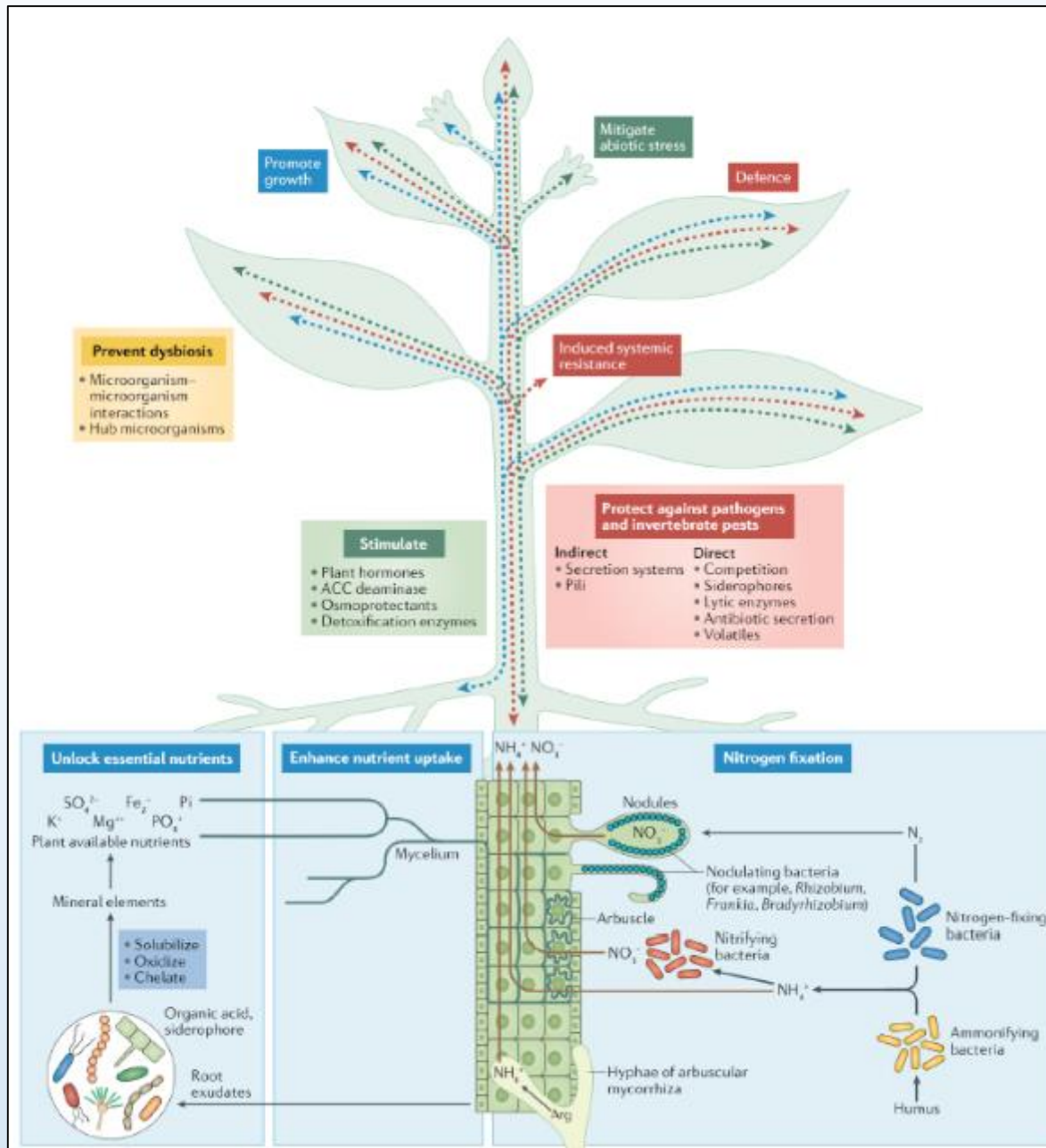


The Plant Microbiome

- The plant microbiome are distinct microbial communities associated with all plant host tissues and areas surrounding the plant tissue
 - ❖ key determinant of plant health and productivity
 - ❖ key players in global biogeochemical cycles
 - ❖ an extension of the plant genome
- ❖ Manipulation of the plant microbiome has the potential to
 - ❖ reduce the incidence of plant disease
 - ❖ increase agricultural production
 - ❖ reduce chemical inputs and reduce emissions of greenhouse gases
 - ❖ result in more sustainable agricultural practices



<https://medium.com/hhmi-science-media/visualizing-the-plant-microbiome>



Functions of plant-associated microbiomes

Promote Growth, Nutrient acquisition

- Nitrogen fixation
- Ammonification
- Phosphate solubilization
- Make other essential nutrients available to plants

Protection, Defense

- Protect against pathogens, competition
- Protect against pests
- Induce systemic resistance, antibiotic secretion
- Siderophores
- Volatiles

Stimulants

- Hormones
- Detoxification
- ACC deaminase
- Mitigate abiotic stress

What effects the microbiomes-biotic & abiotic

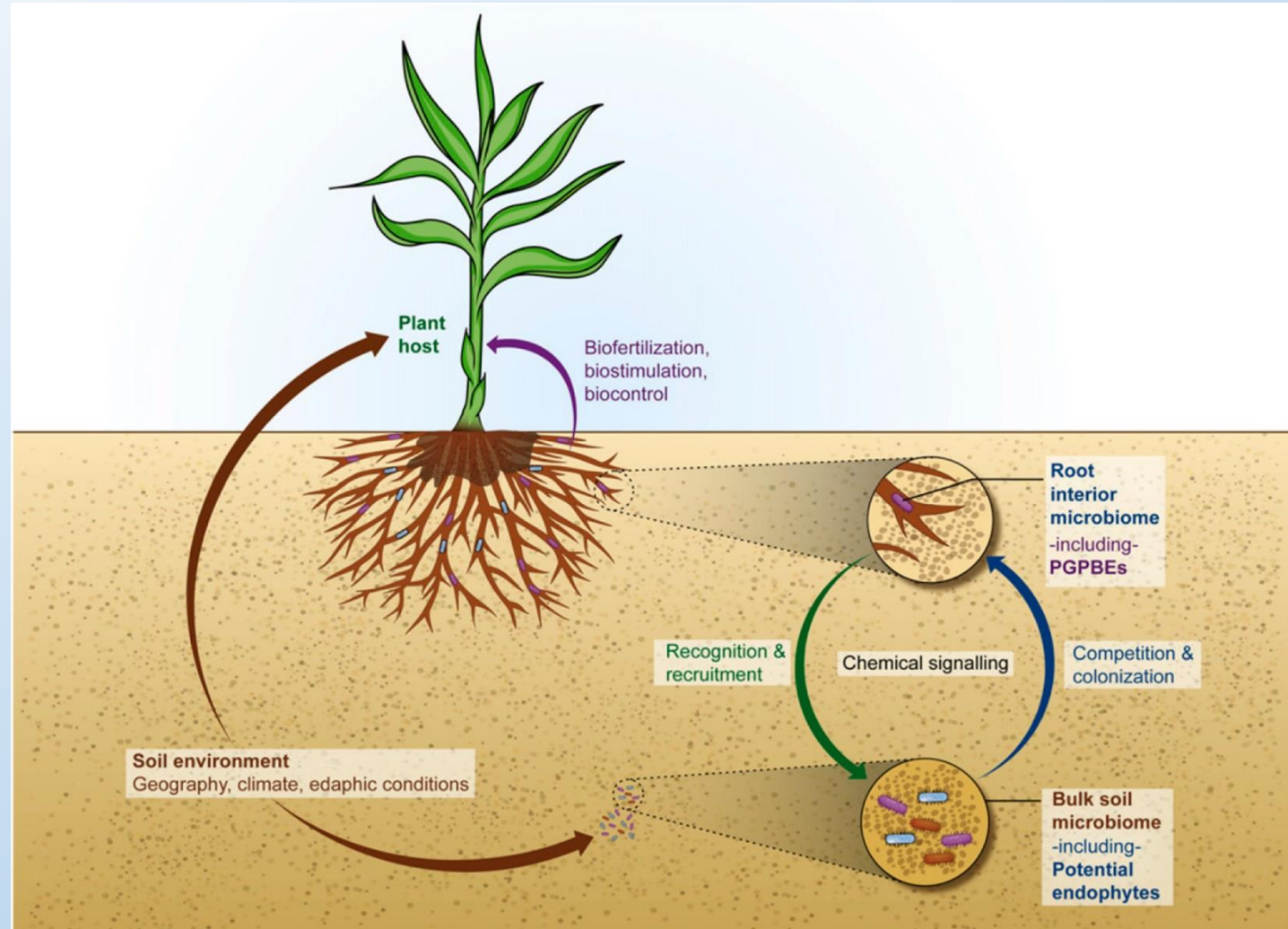
Phyllosphere & leaf endophytes

- Nutrient poor
- Low diversity
- Subject to extremes of temperature
- Radiation
- Moisture
- Plant genome

Rhizosphere & root endophytes

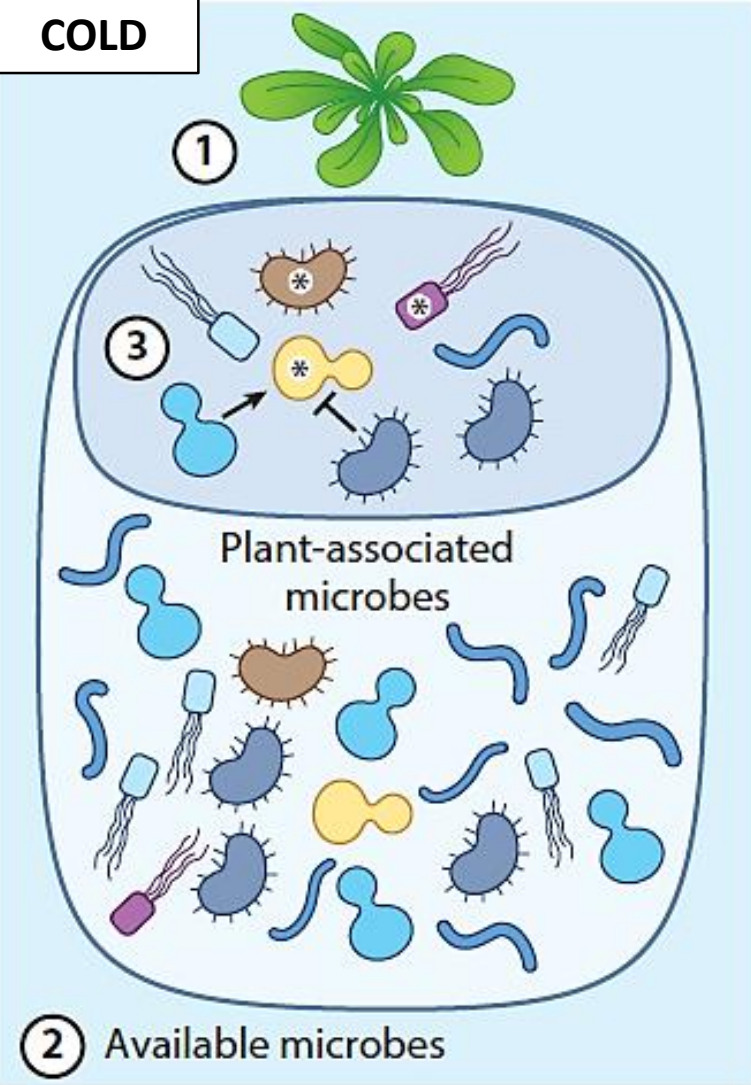
- Root exudates which vary between species & cultivars
- Soil content & conditions
- Root structure
- Plant selection, plant-microbe interactions
- Microbe-microbe interactions

Cross-talk

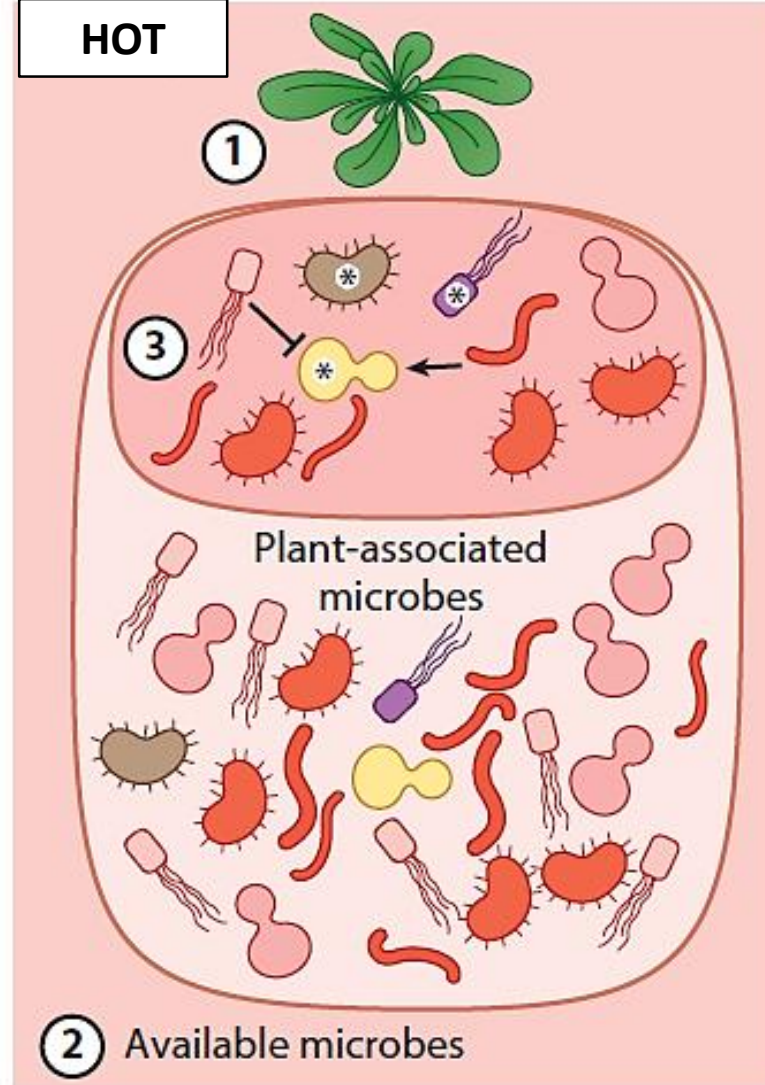


Gaiero, et al, 2013. Inside the root microbiome: Bacterial root endophytes and plant growth promotion. American Journal of Botany, 100:9, 1738-1750.

COLD



HOT



* Core plant microbiome members

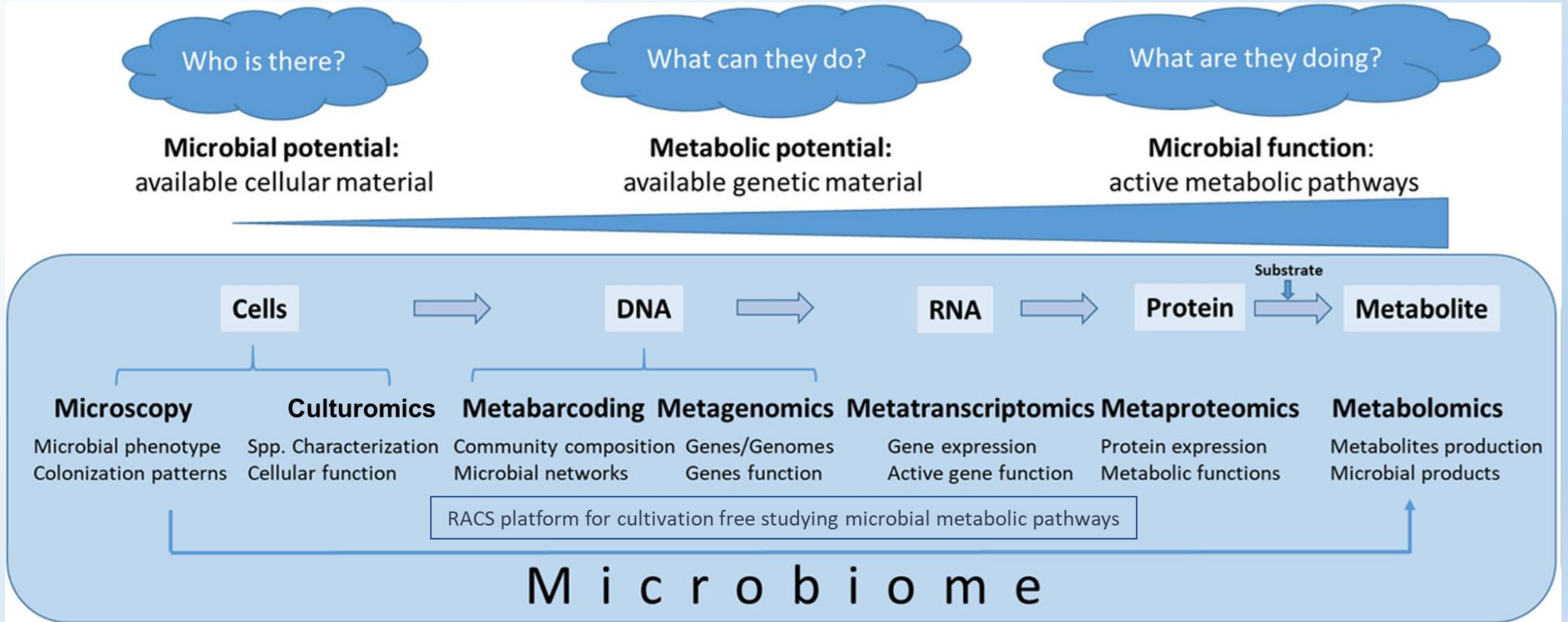
Factors responsible for shaping diversity and selection of plant microbiota

1 Host specific microbiota, genetically distinct populations

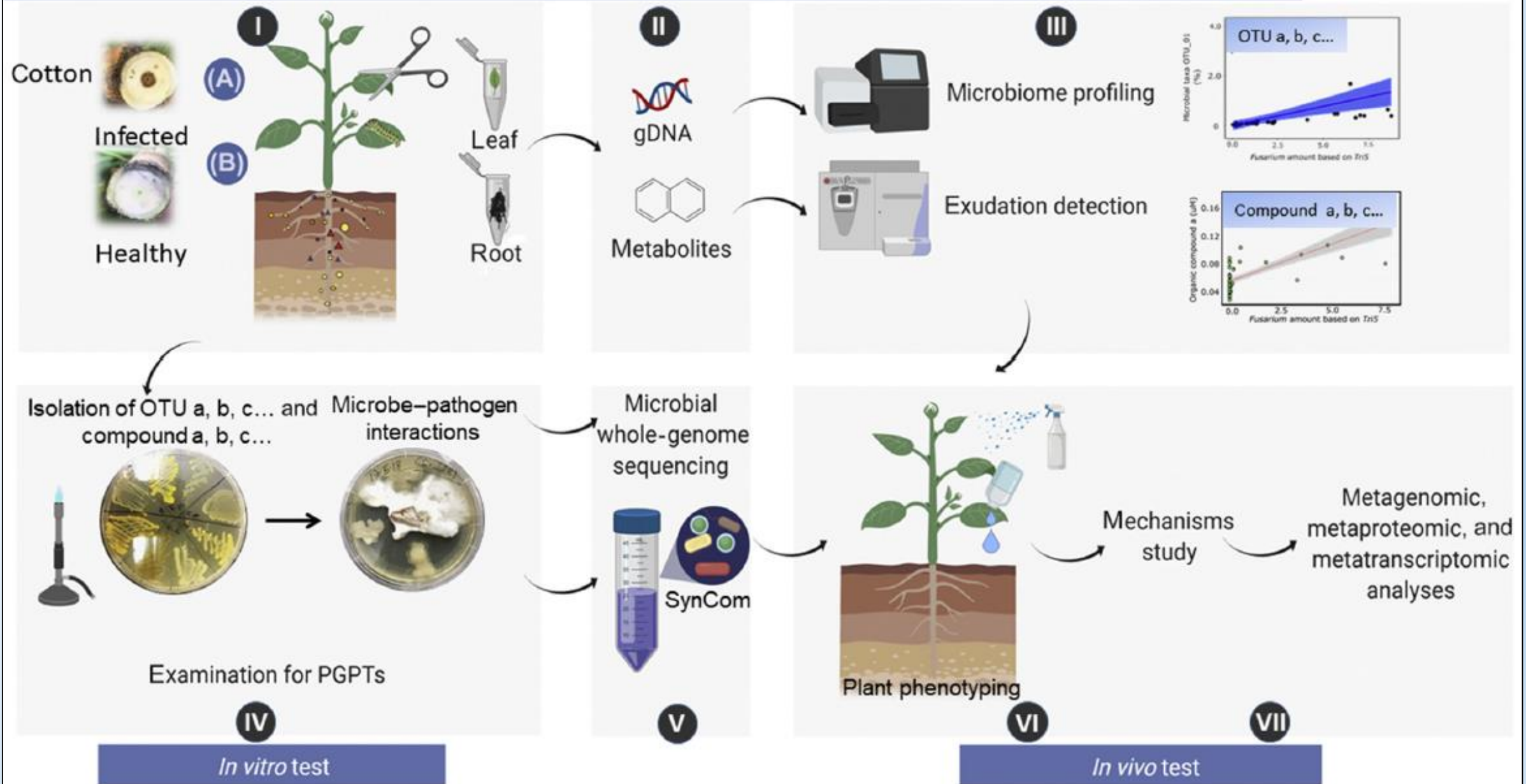
2 Geographic location and corresponding environmental features

3 Microbe-microbe interactions

How do we study the Microbiomes?



A combined metagenomics, metabolomics, and synthesized microbiome approach



Crop plants grown on ISS and ground

What do we know about these communities in space flight growth facilities

Two plant growth facilities on ISS

- Enclosed growth spaces
- Exposed to Station environmental parameters/conditions
- Investigated the communities (ISS vs Ground) under ISS conditions with exception of microgravity

General Knowledge

- In terrestrial & aquatic systems both require microbes
- Plants will actively recruit microbes that will adjust nutrient availability
- Recruit microbes that naturally fight off pathogens & reduce stress responses
- Hydroponic systems are more favorable to bacteria than fungi

Veggie

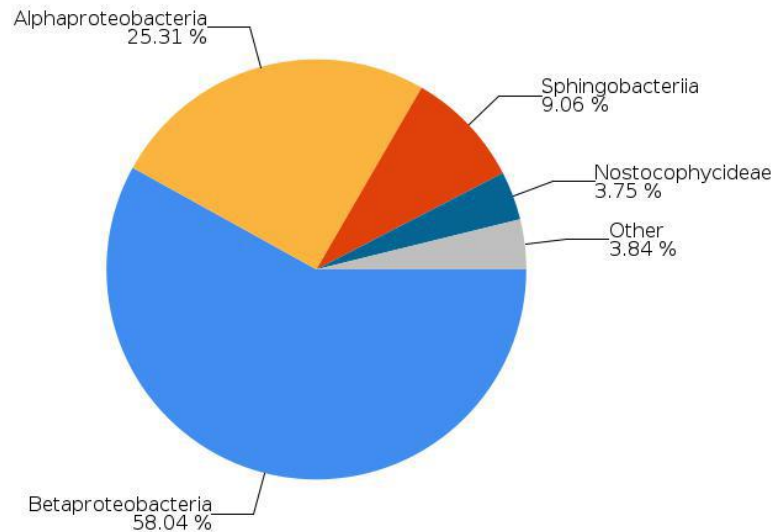


APH

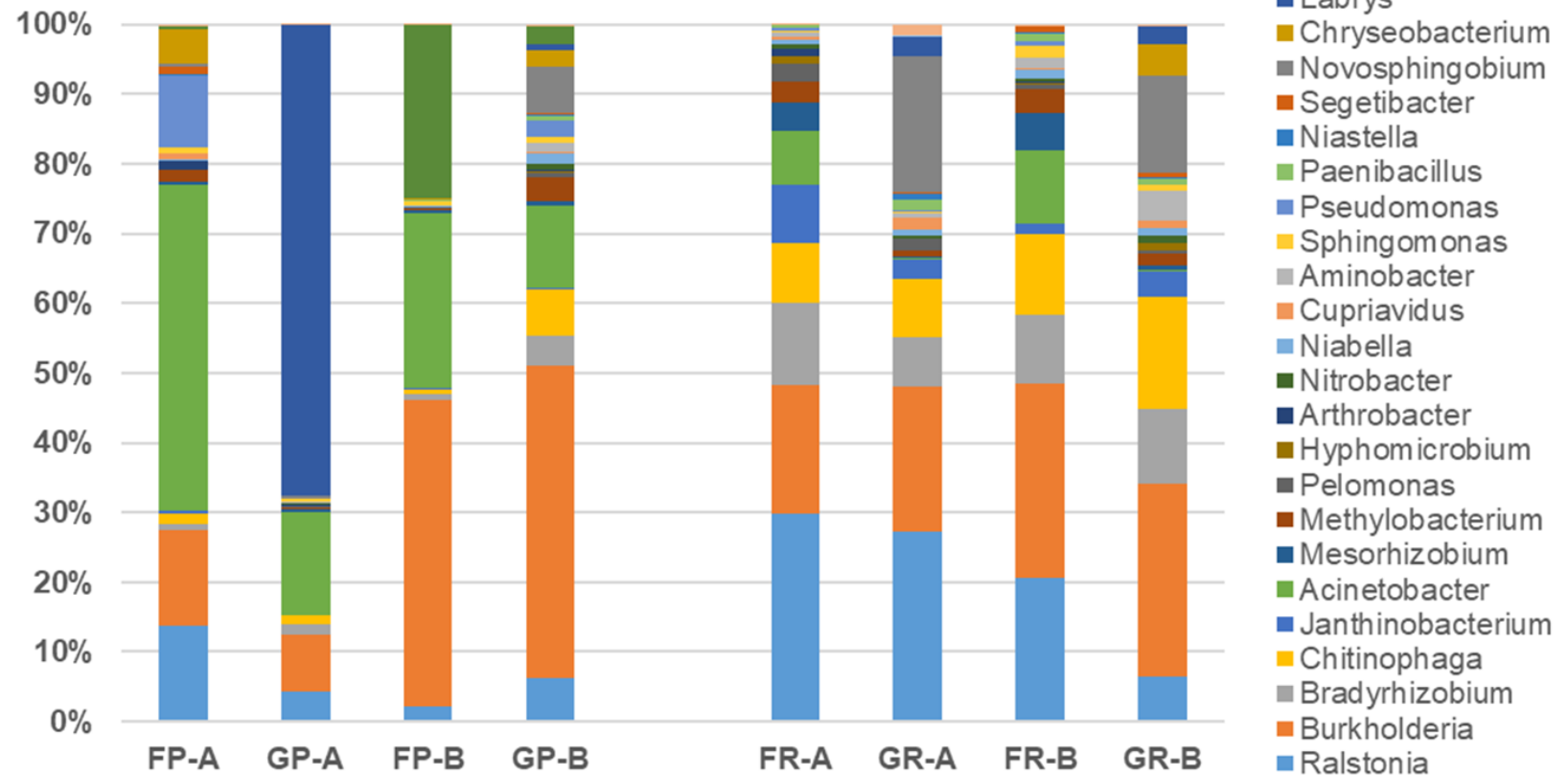


Veggie 'Outredgeous' lettuce flight-ground comparison

Top Class Classification Results

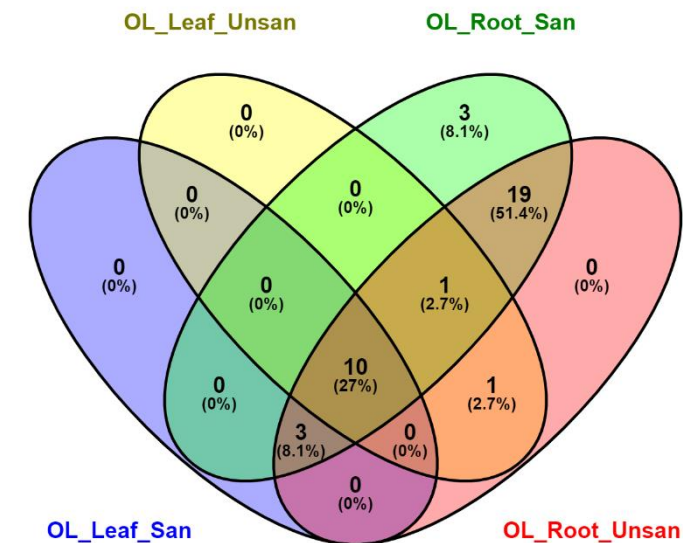


Veg 01A & B Plant & Root Bacterial Community
Flight vs Ground

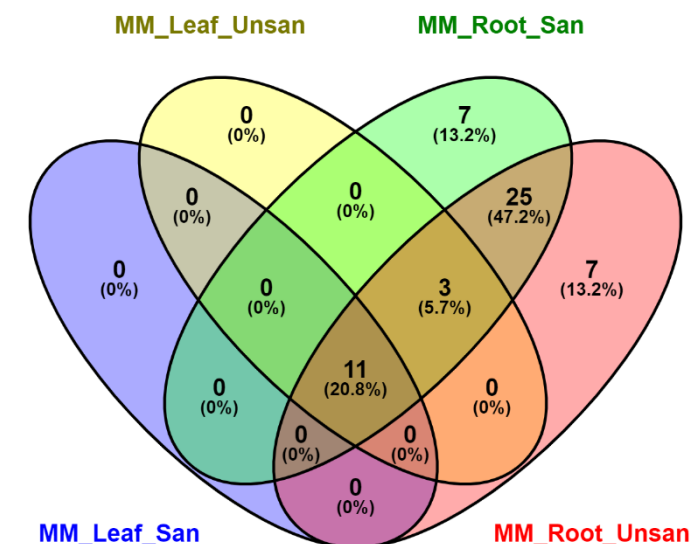


Microbiome Study

Core Microbiomes	
Red Romaine Lettuce	Mizuna Mustard
<i>Acinetobacter</i>	<i>Acinetobacter</i>
	<i>Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium</i>
	<i>Azospirillum</i>
	<i>Bacillus</i>
<i>Bradyrhizobium</i>	
<i>Burkholderia-Caballeronia-Paraburkholderia</i>	
<i>Escherichia-Shigella</i>	<i>Escherichia-Shigella</i>
<i>Massilia</i>	<i>Massilia</i>
	<i>Methylobacterium</i>
<i>Methylophilus</i>	<i>Methylophilus</i>
<i>Pseudomonas</i>	
<i>Ralstonia</i>	<i>Ralstonia</i>
<i>Sphingobium</i>	<i>Sphingobium</i>
<i>Sphingomonas</i>	<i>Sphingomonas</i>



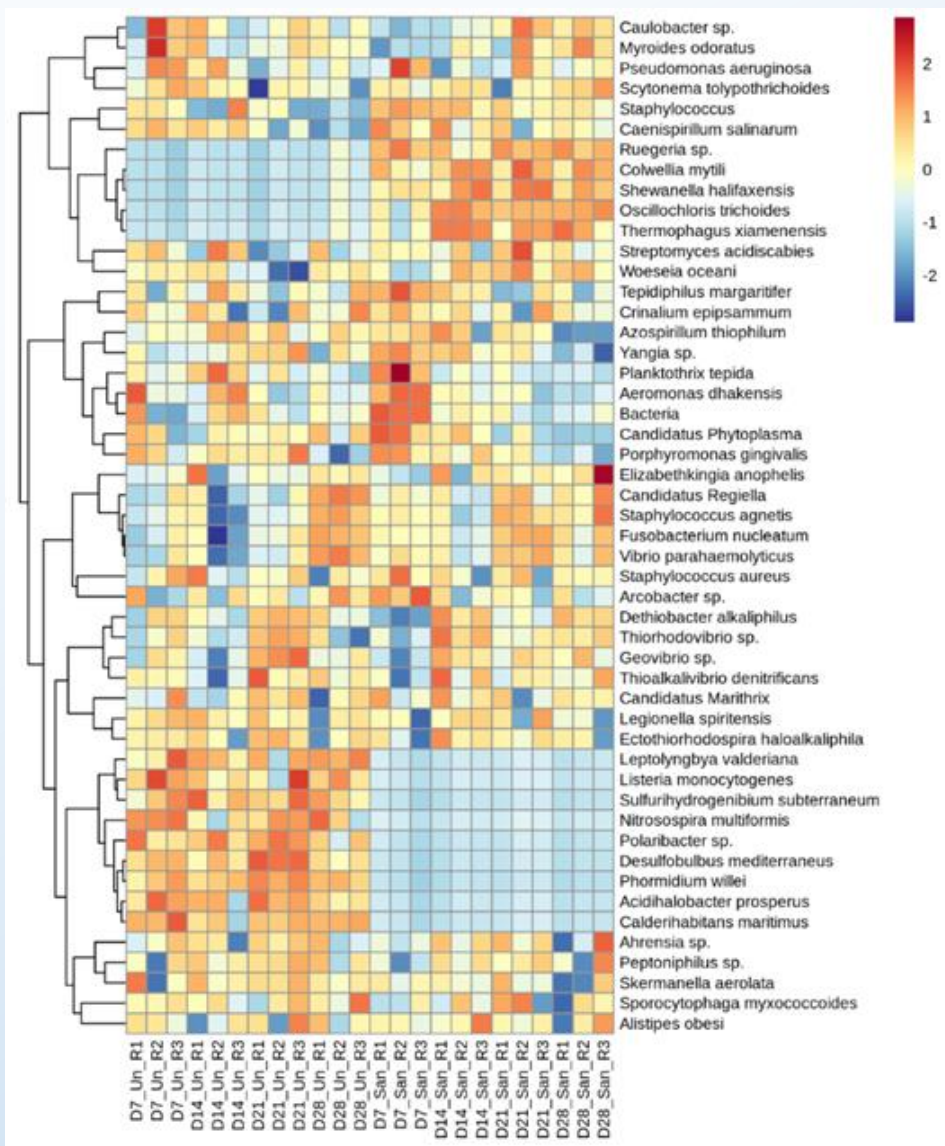
‘Outredgeous’ Lettuce



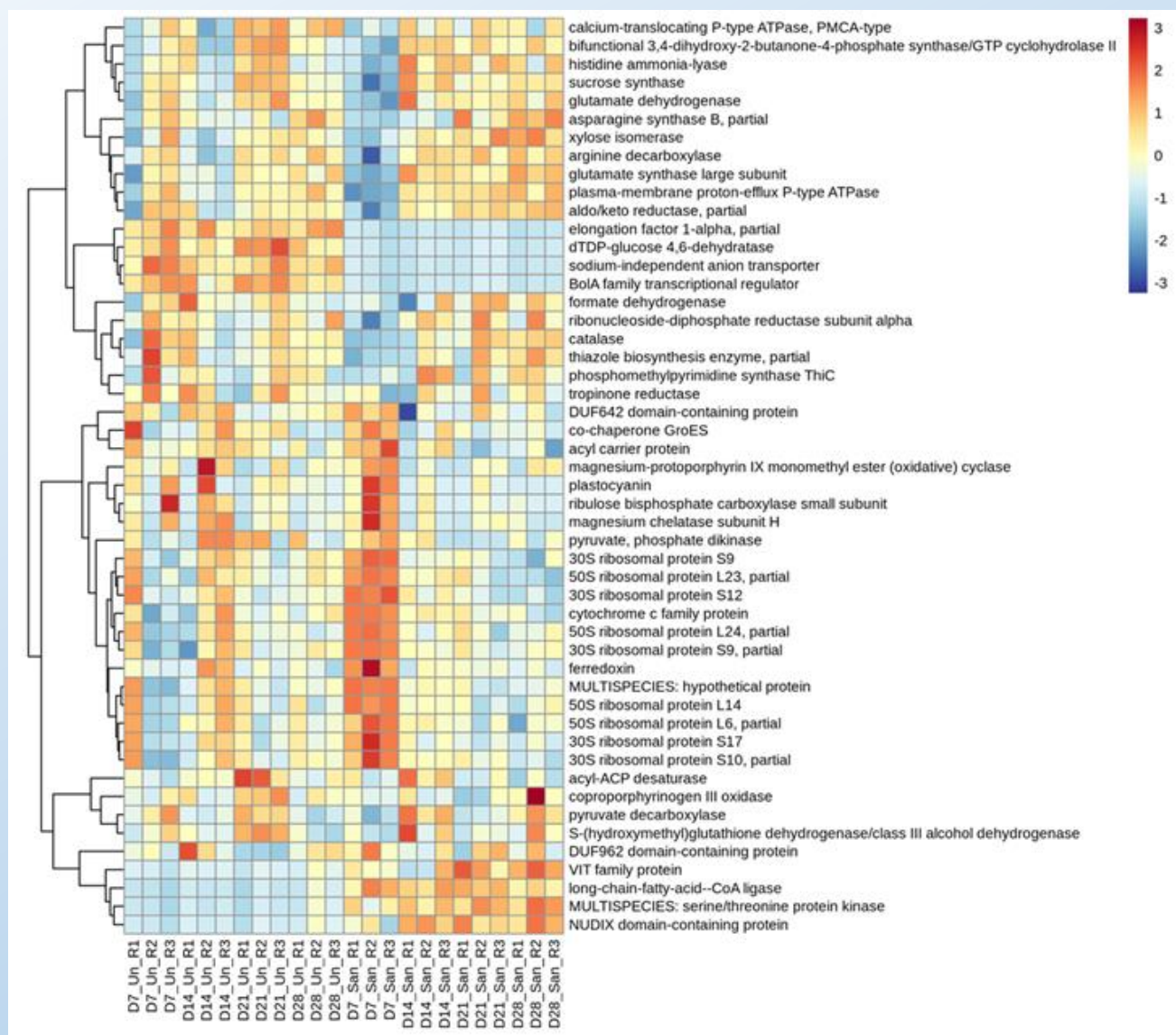
Mizuna mustard

Metatranscriptome – ‘Outredgeous’ Lettuce

Ref-Seq Organism

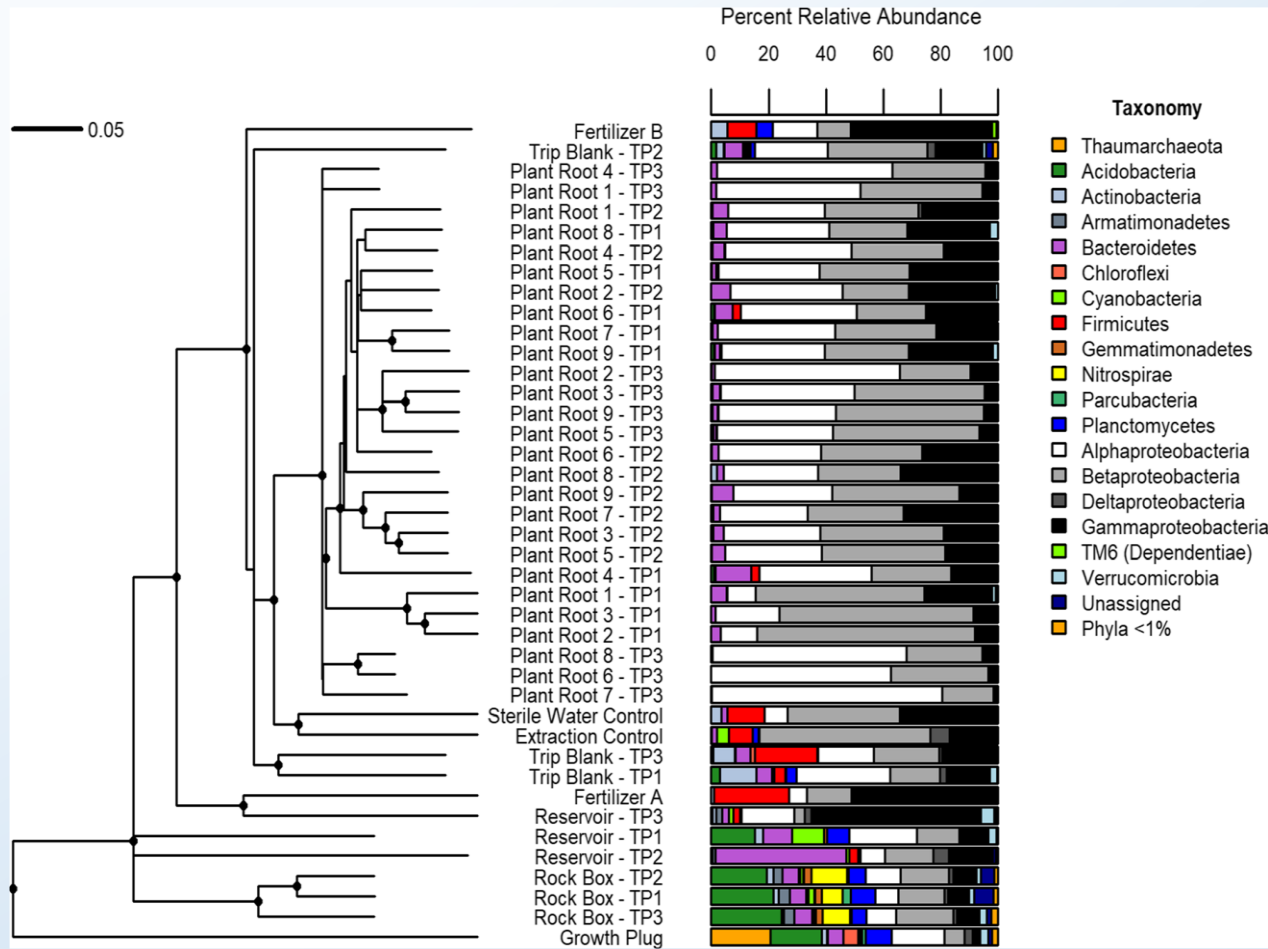


Ref-Seq Functional Groups



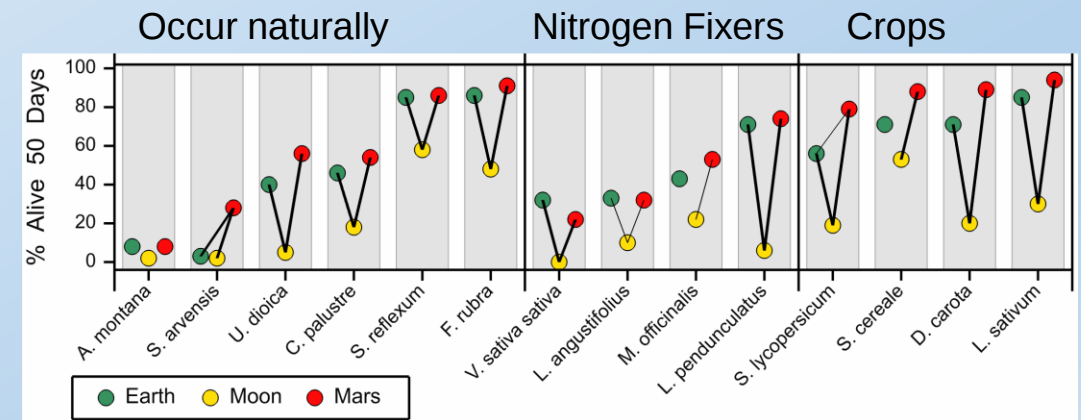
Other systems - Earth - Moon - Mars

AEROPONICS



Edmonds, et al., Antonie van Leeuwenhoek 2020

REGOLITH STUDY

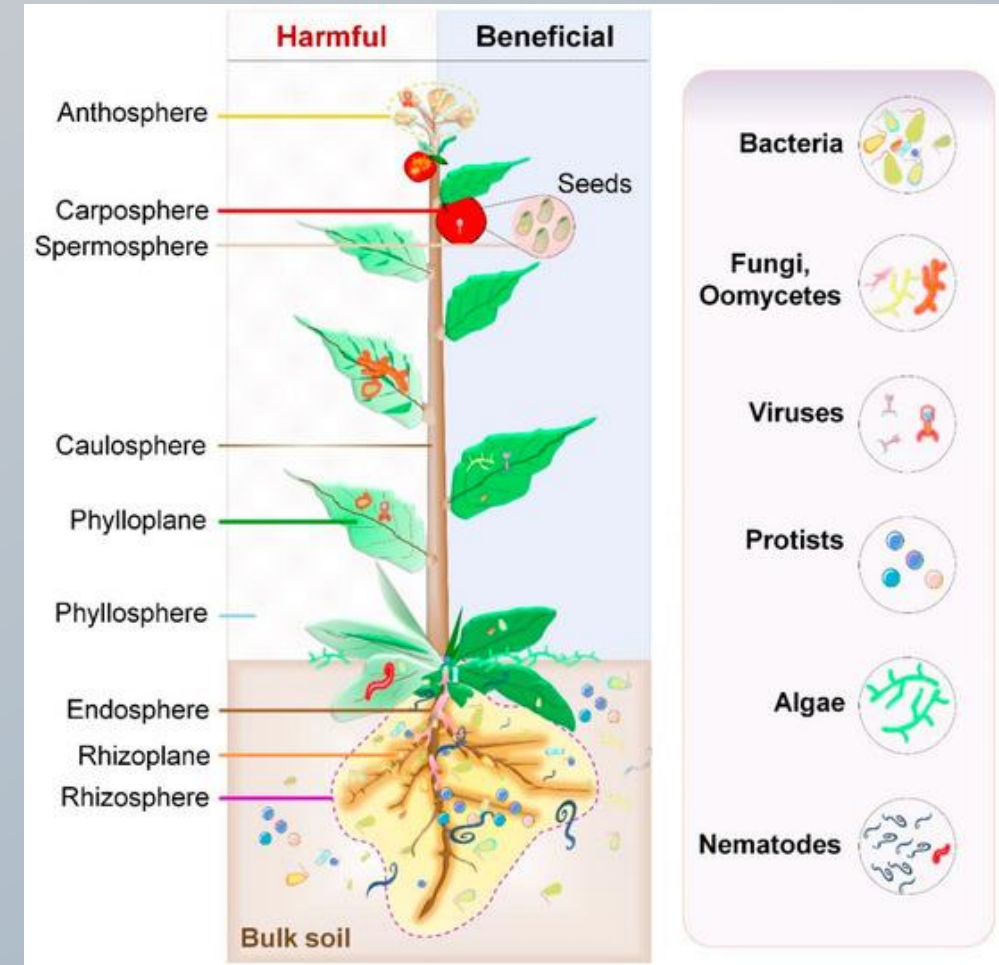


From Kamelink, et al, PLOS|One, 2014.

Good – Bad – Maybe?

Assay screening of isolates for benefits is needed to identify PGPB.

- IAA Production
- Phosphate Solubilization
- Siderophore Production
- Anti fungal potential
- Growth promotion studies

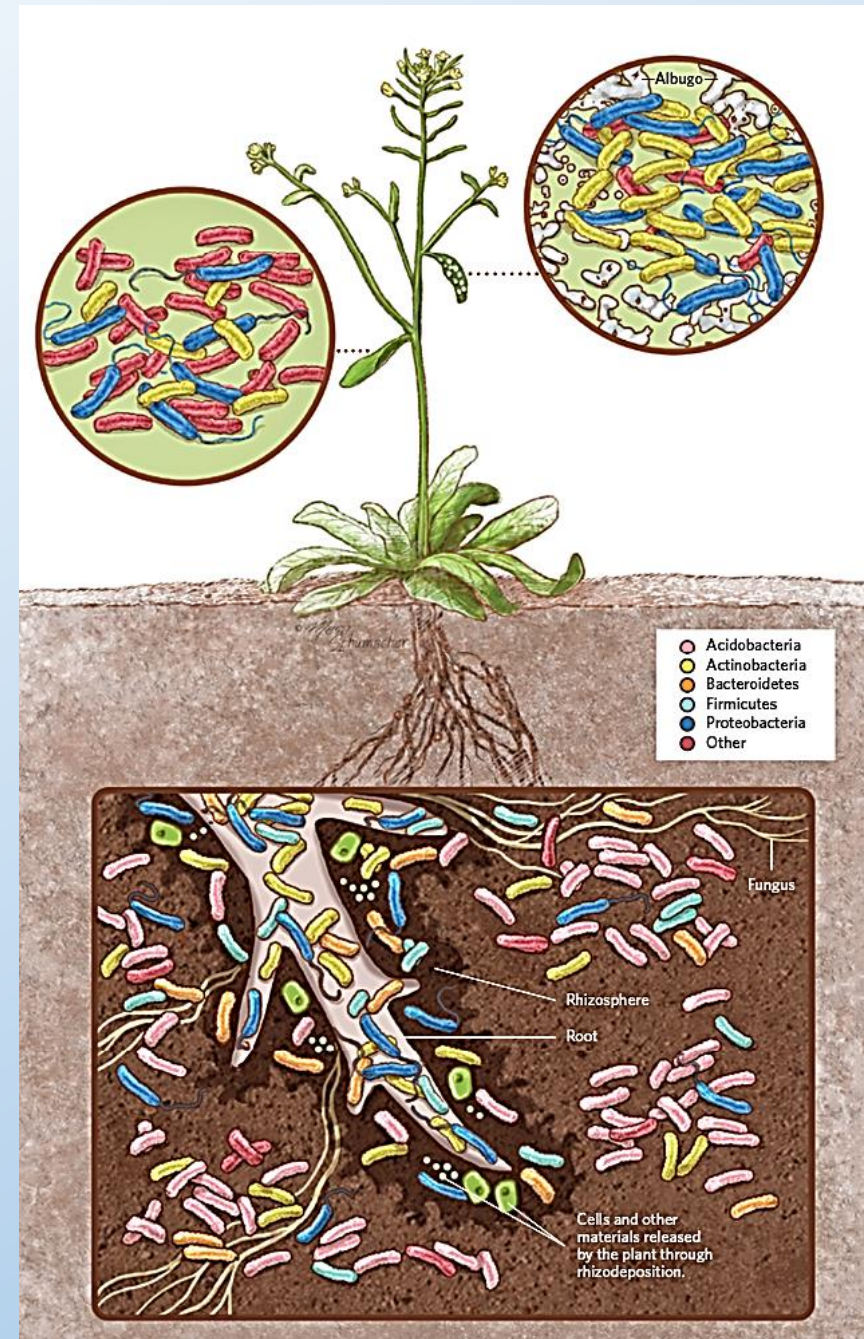


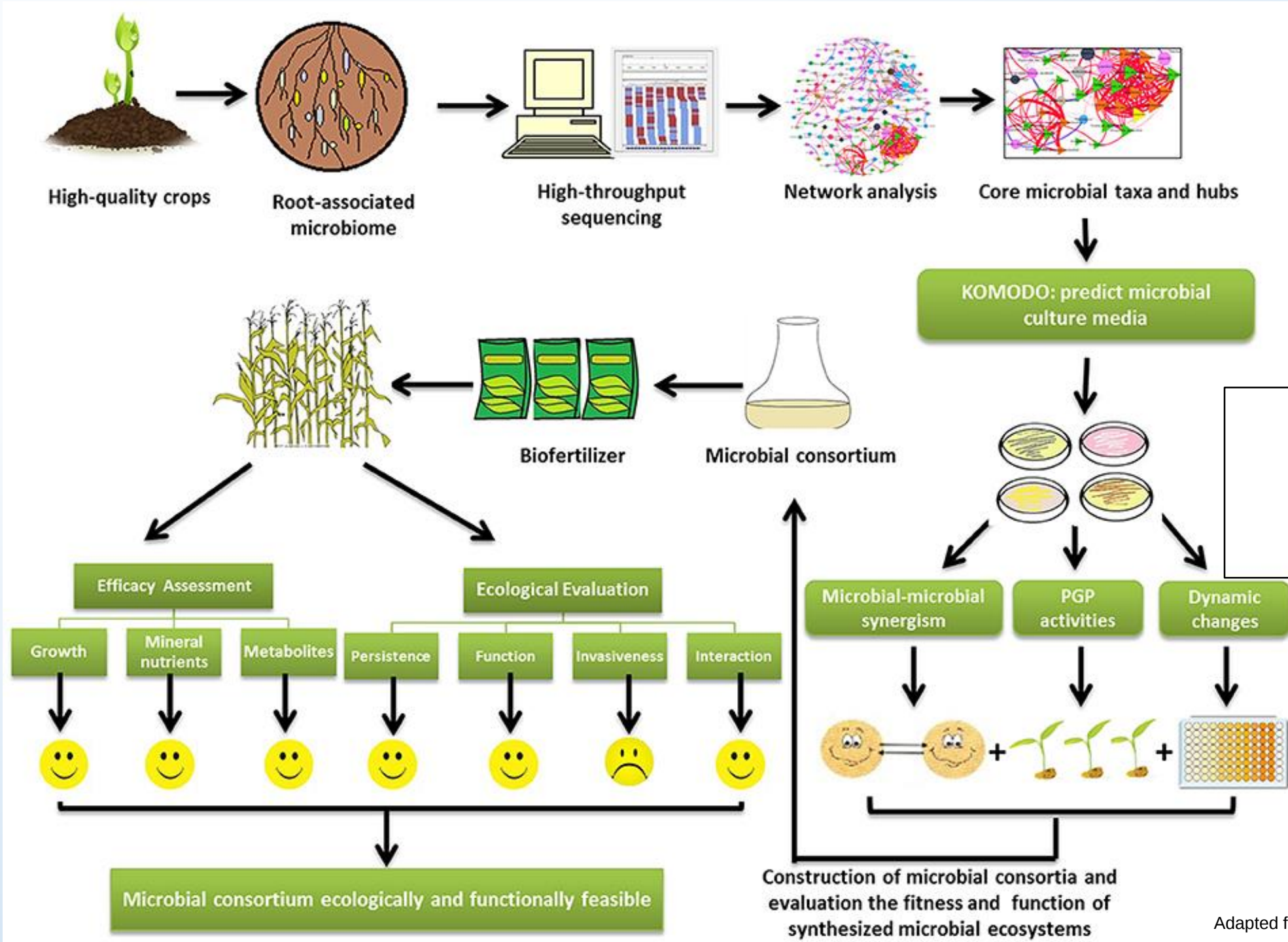
Understanding and exploiting plant beneficial microbes

Omri M Finkel^{1,2,*}, Gabriel Castrillo^{1,2,*}, Sur Herrera Paredes^{1,2,3},
Isai Salas González^{1,2,3} and Jeffery L Dangl^{1,2,3,4,5,6}



- **Screening of large number of isolates**
 - Despite of vast-diversity of soil microbes, plants are highly selective microbial niche
- **Ecological considerations of plant beneficial function of microbes**
 - Ability to invade and persist in the context of indigenous microbial populations
 - Environmental/abiotic conditions
 - Plant-microbe interactions in soil
- **Diversity of the inoculum**
 - Single vs. complex inoculum
- **Genetic control of beneficial plant-microbe interactions**
 - Host genetics contributes to plant microbiome assembly





Synthetic Microbial Consortium

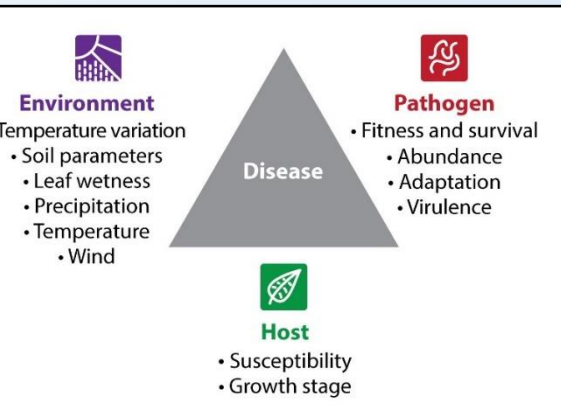
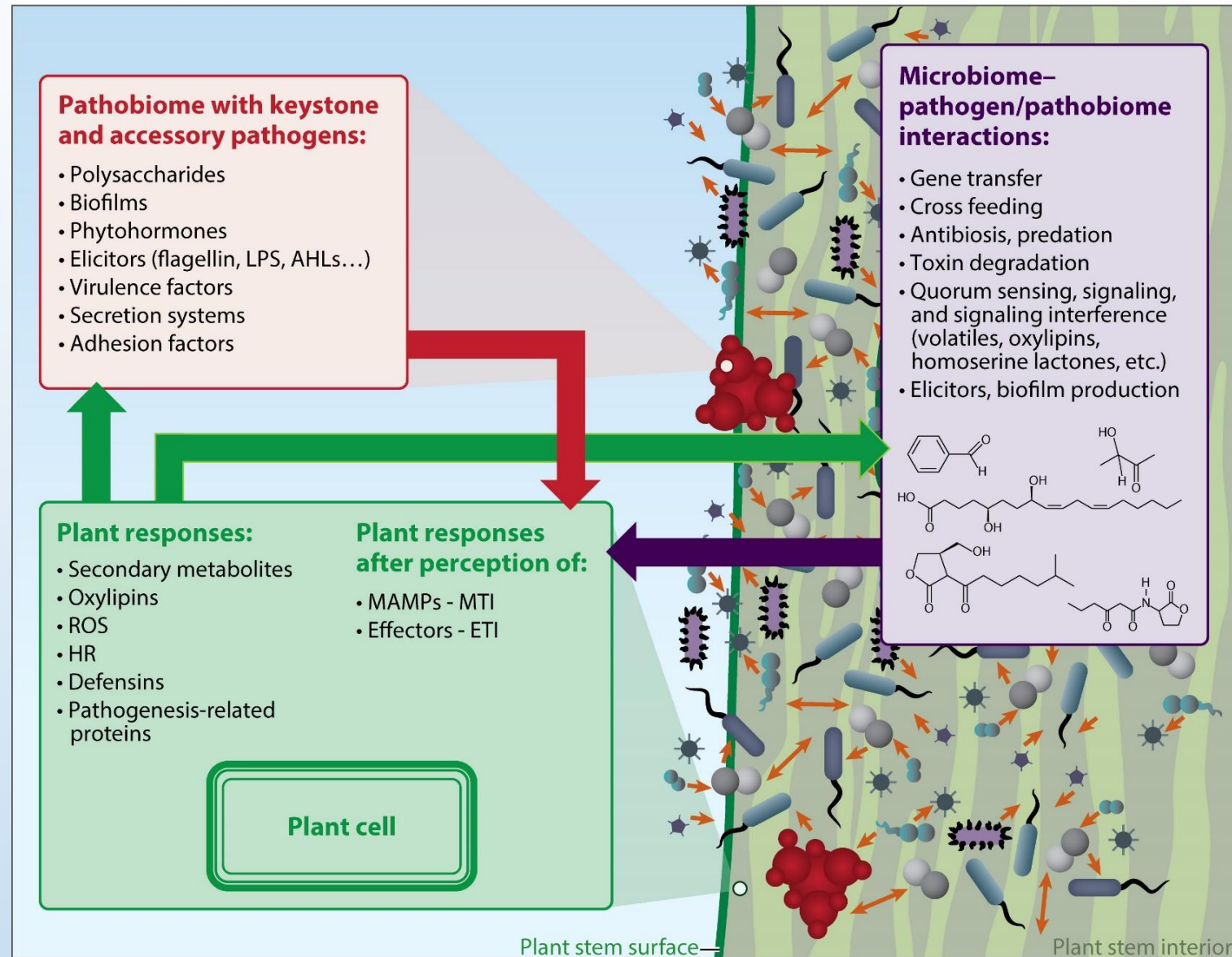


Pseudomonas syringae pv. *tomato*



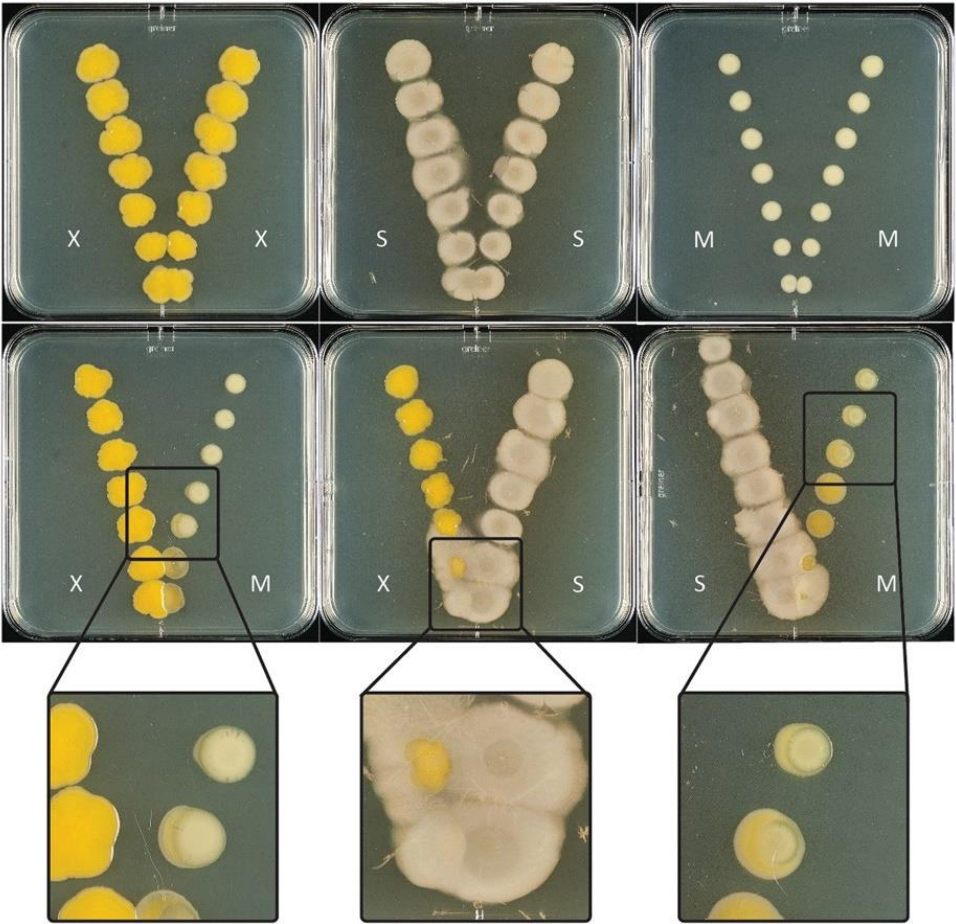
Sweet orange scab (SOS) by fungus *Elsinoë australis*,

Plant Microbiome and Pathogens



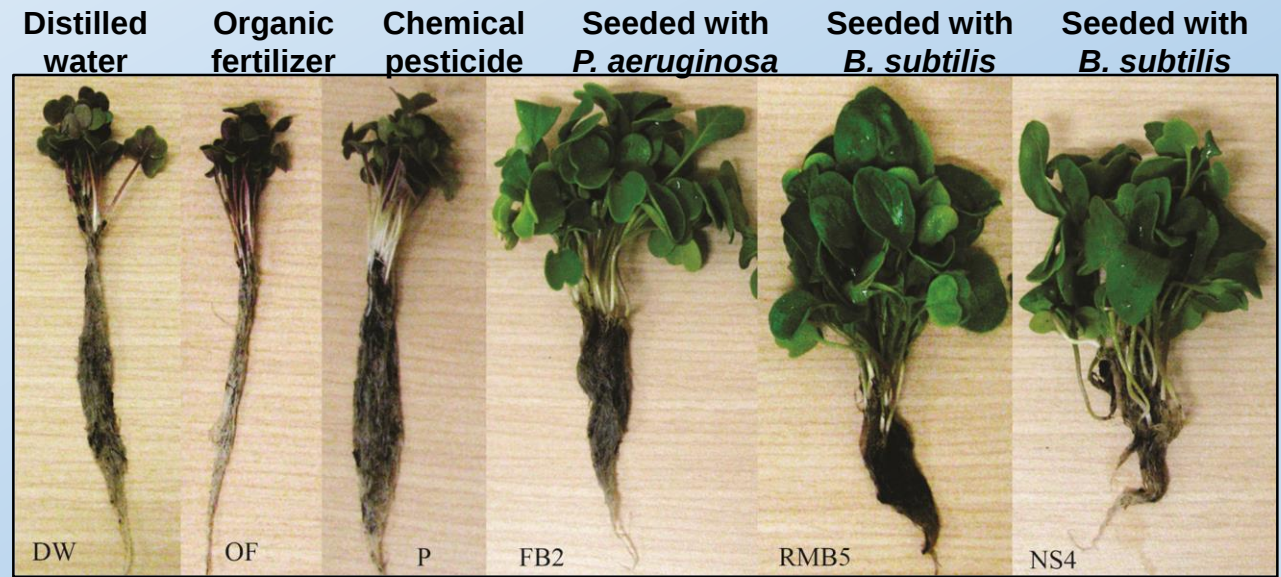
Plant Disease Triangle

Xanthomonas Stenotrophomonas Microbacterium



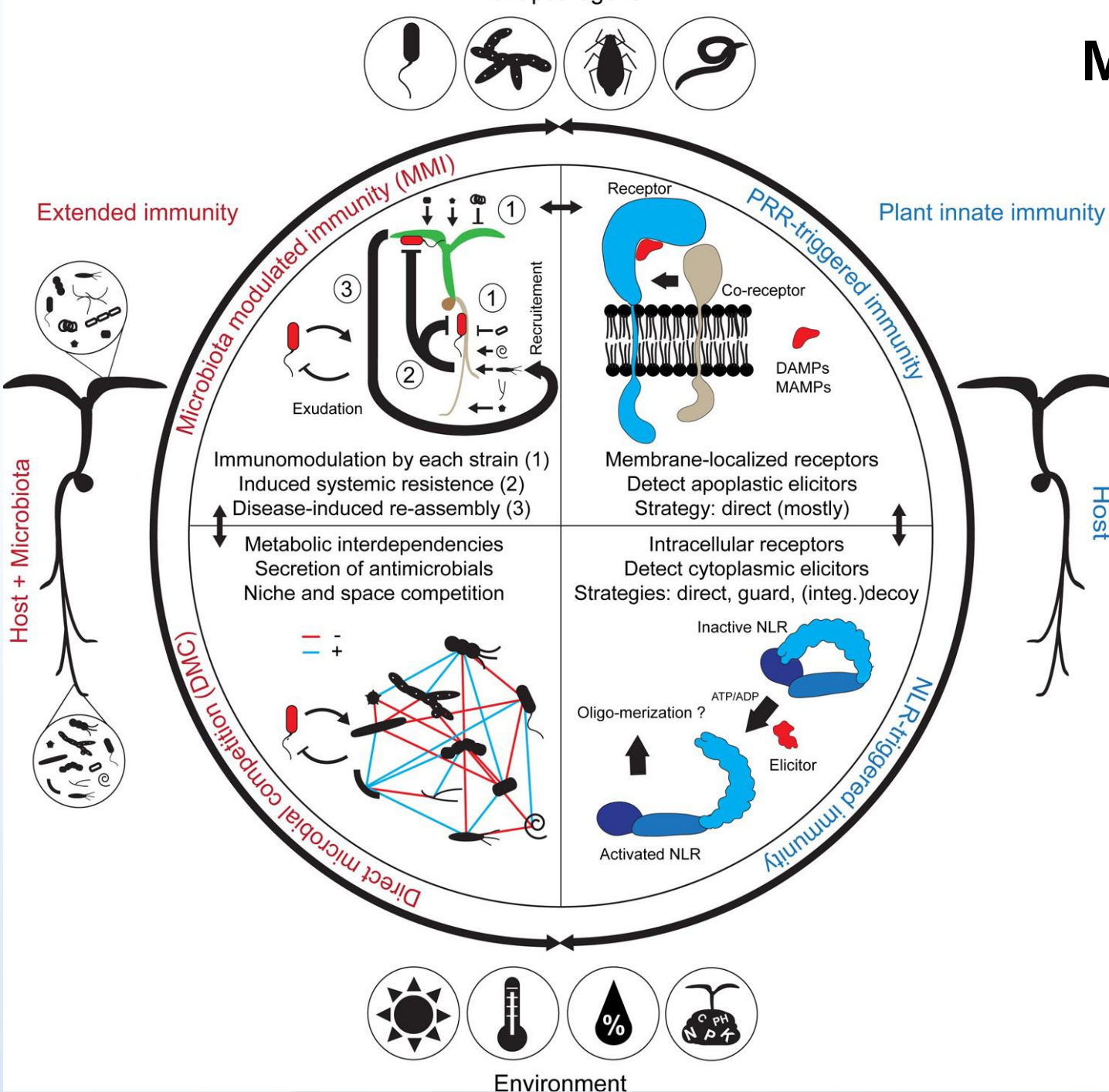
Synergistic interactions between *Xanthomonas*, *Stenotrophomonas*, and *Microbacterium* spp. upon foliar defense activation by the downy mildew pathogen *Hyaloperonospora arabidopsidis* in *Arabidopsis thaliana*. Berendsen et al., (2018) *The ISME Journal*

Pathogen Trigger	Effect	Host
<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	Enrichment of Proteobacteria, Actinobacteria, and Firmicutes (<i>Bacillus</i>)	Tomato
<i>Hyaloperonospora arabidopsidis</i> / <i>Pseudomonas syringae</i> pv. <i>tomato</i>	Assemblage of beneficial rhizosphere microbiome	Arabidopsis/Tomato
<i>Fusarium oxysporum</i> f. sp. <i>lini</i>	Increase of taxa associated to Fusarium wilt suppressiveness	Flax
<i>Botrytis cinerea</i>	Attraction of <i>Trichoderma harzianum</i> and inhibition of <i>Fusarium oxysporum</i>	Tomato; Cucumber



Plant growth and root health of Arugula plants grown in *Pythium* infested soil with various treatments. Ali et al., (2019) *Microbiological Research*.

Microbiota-mediated extension of the plant immune system



Plant Innate Immunity

- Pathogen Recognition Receptors (PRRs) triggered immunity
- Inside the plant cell, Nucleotide-binding domain and leucine-rich repeat-containing receptors (NLRs)

Extended Immunity

- Microbiota Modulated Immunity (MMI)
- Direct Microbial Competition (DMC)

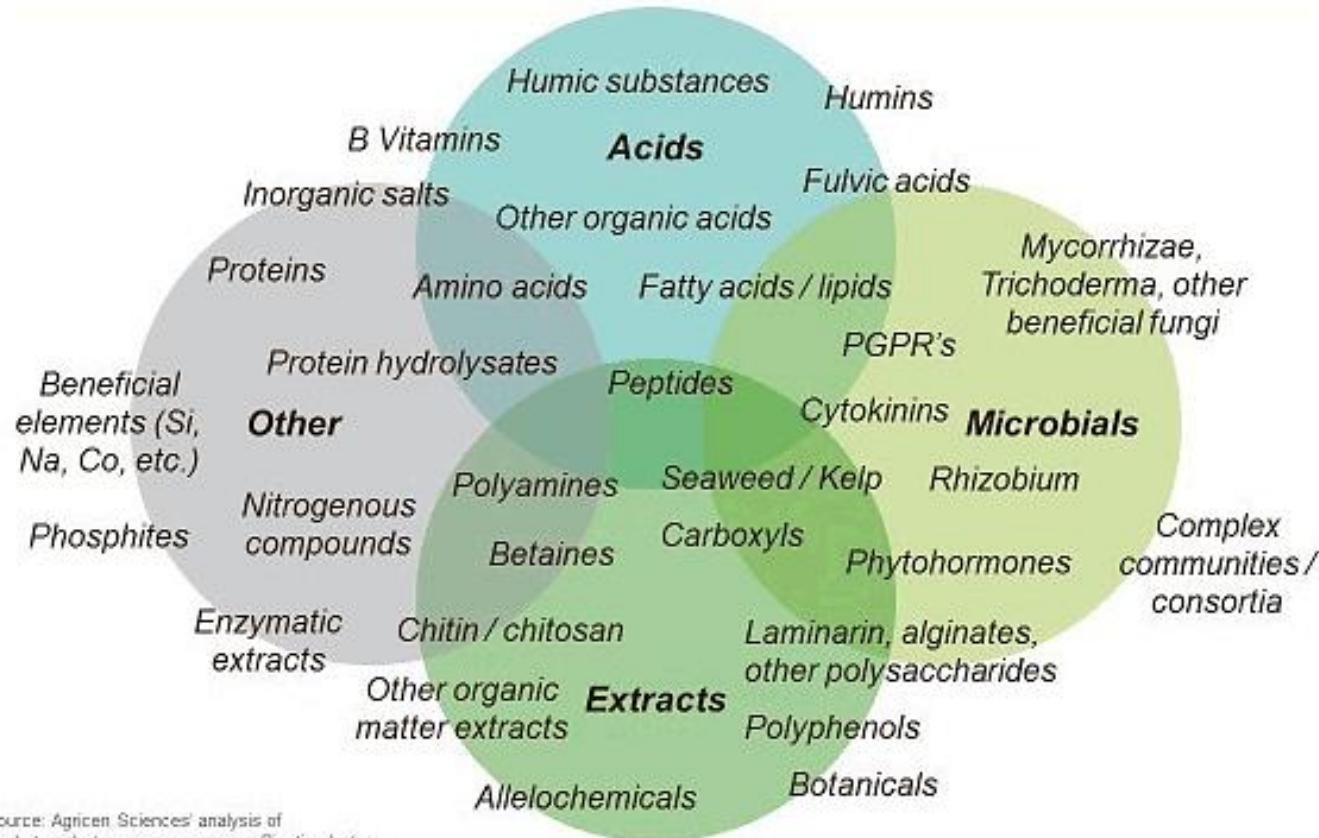
Key to synthetic community design

- Able to predict beneficial outcome
- Increased robustness of beneficial microbial trait expression is increased by **redundancy and dominance**

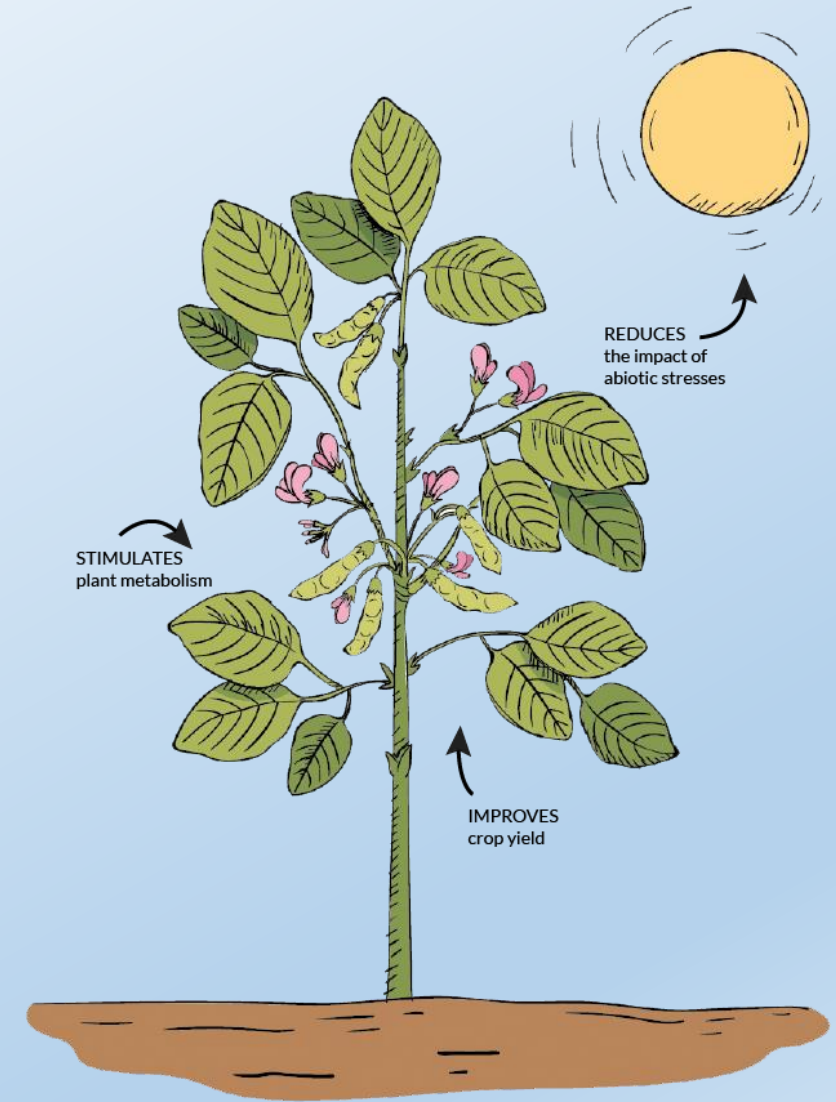
Plant Biostimulants: An approach to synthetic microbiome

“...a substance or micro-organism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield.”

Agriculture and Nutrition Act of 2018, Section 9201(c)

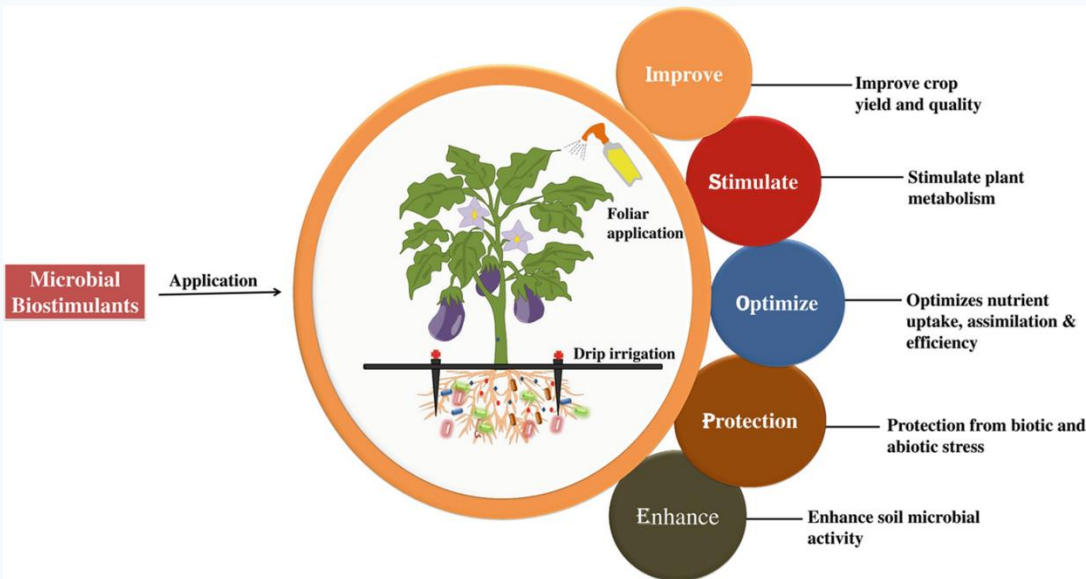


Source: Agricen Sciences' analysis of market analysts, survey papers on Biostimulants

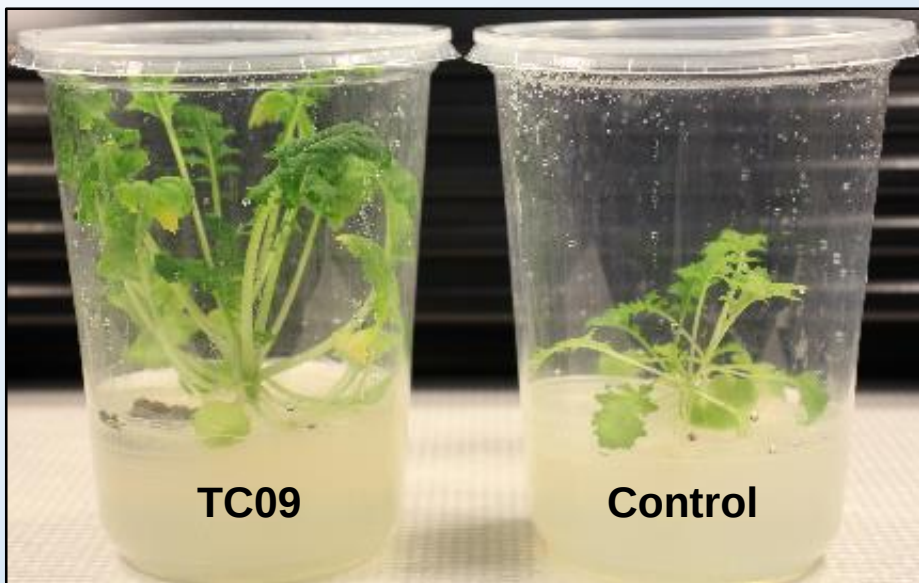


HOW DO BIOSTIMULANTS WORK?

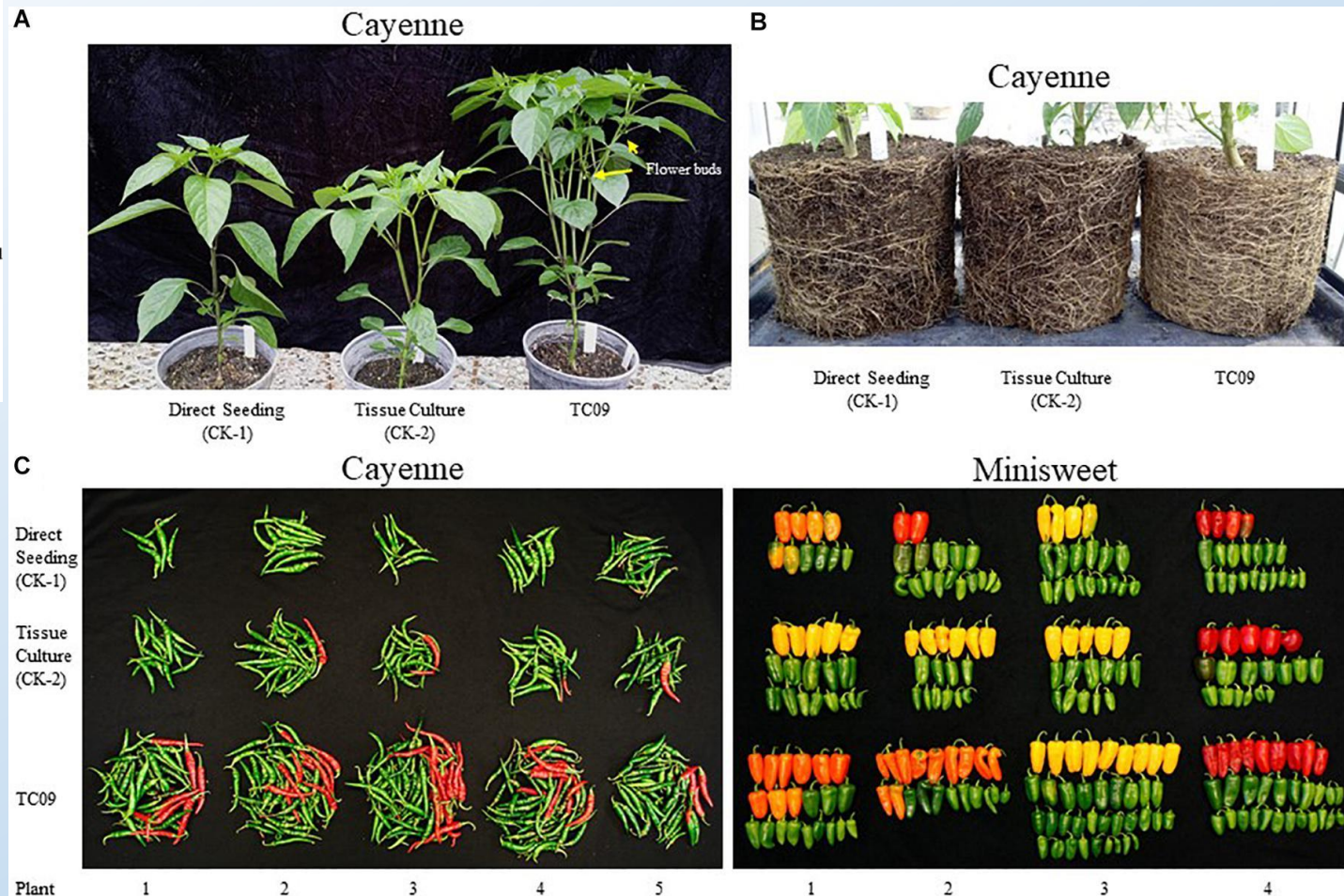
<https://biostimulants-agriculture.com/wp-content/uploads/2020/04/comment-marche-biosti-en-8.png>



https://encrypted-tbn0.gstatic.com/images?q=tbn%3AAND9GcQmugcK9G-rRD_lyF0N39PYGrn6Vn-AKR0cQ&usqp=CAU



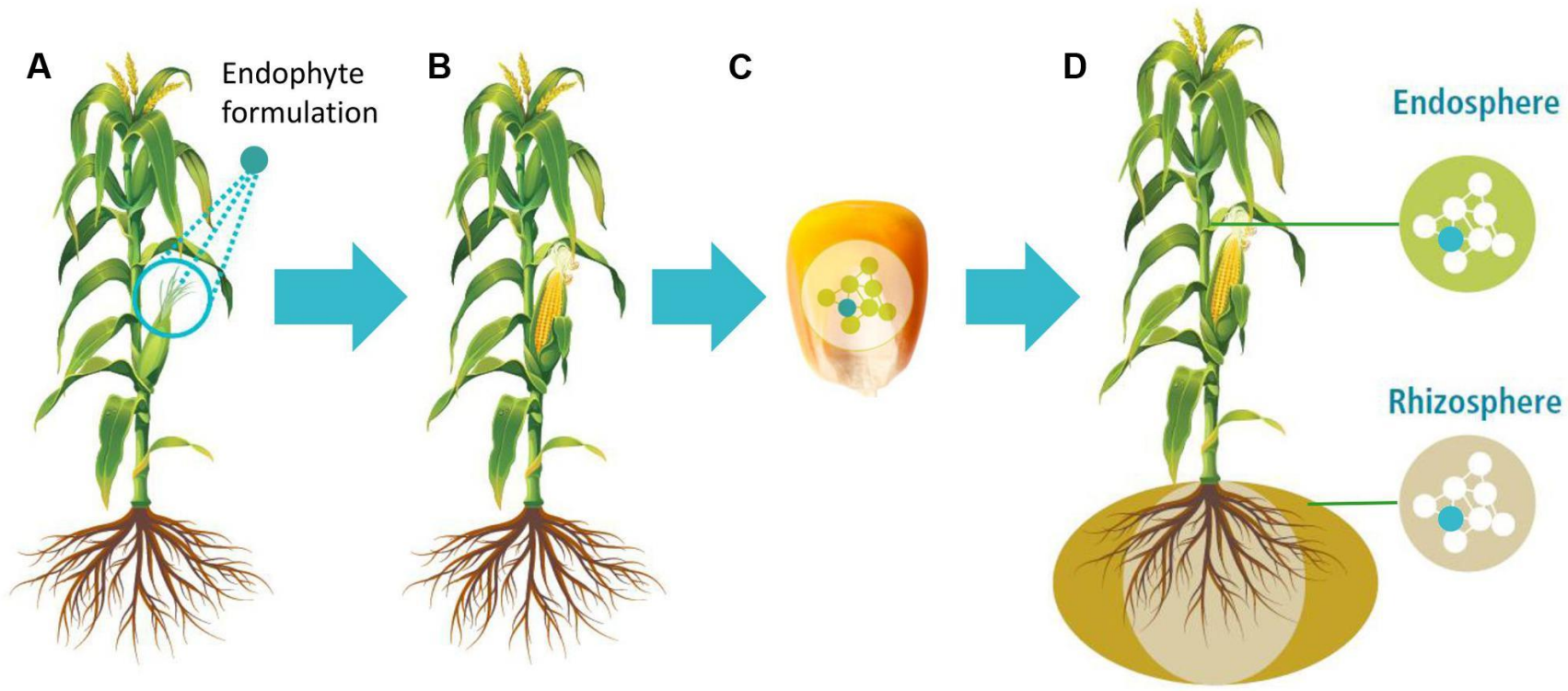
Growth promotion of Mizuna mustard by exposure to TC09



Plant growth and fruit production of pepper (*Capsicum annuum*) varieties cayenne 'Long Thin' and sweet bell 'Minisweet Pepper Mixed' promoted by exposure *in vitro* to *Cladosporium sphaerospermum* strain TC09. Adapted from Li et al., (2019) Frontiers in Plant Science.

Summary

- Plants and their associated microbiome interactions are immensely intricate and exclusive to the host
- Stability and succession of the rhizosphere microbiota depends upon plant type, soil, or artificial media composition
- Knowing who is there is helpful, but knowing how the community behaves is critical
- Carefully designed synthetic microbial communities or biostimulants can act as growth promoters as well as help plants alleviate biotic/abiotic stressors



Modify Plant Microbiomes and Traits by Introducing Beneficial Bacteria at Flowering into Progeny Seeds

