

MICROBIOMA E MICROBIOTA

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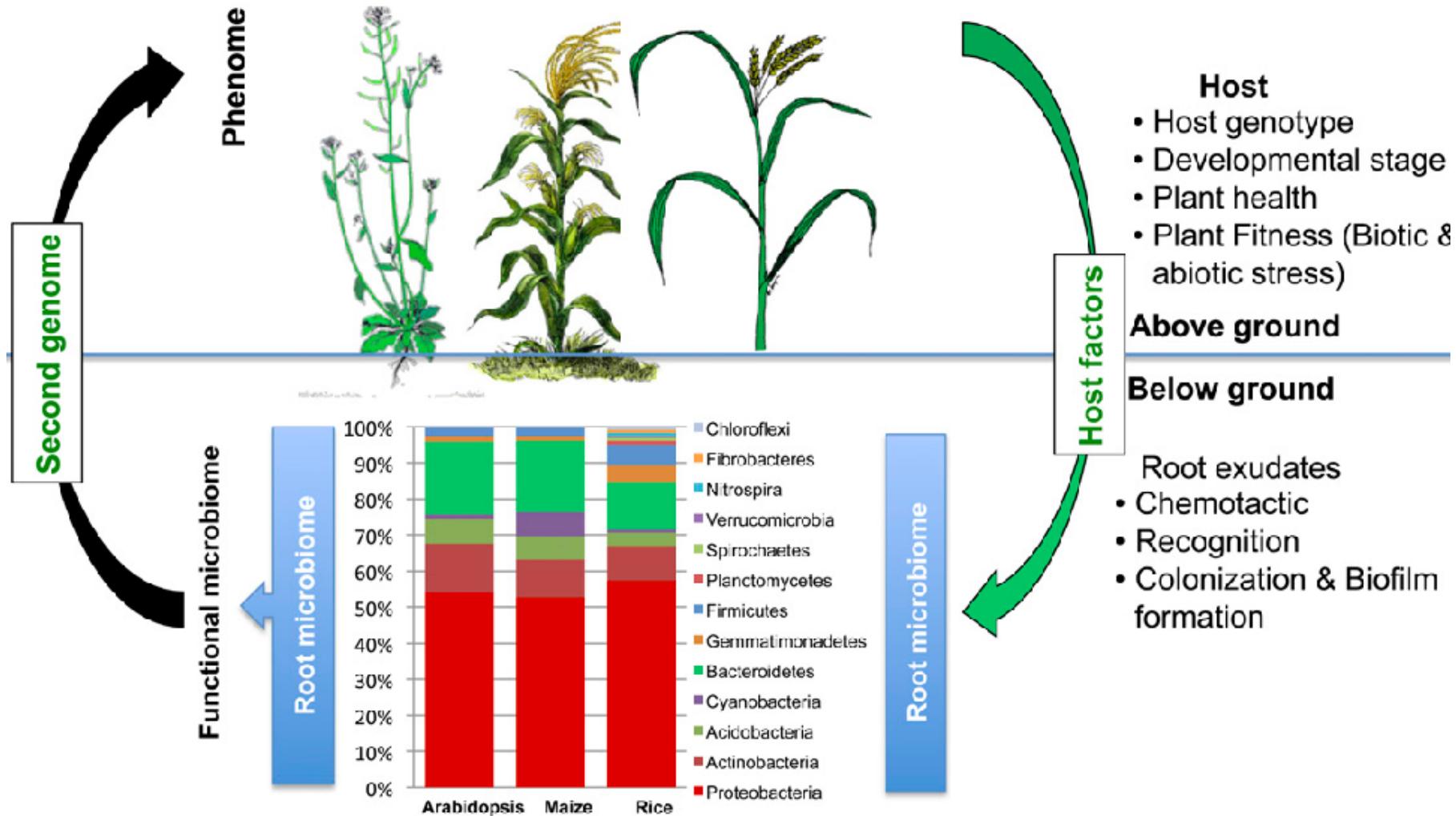
Recap



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Can the plant modify the composition of the associated
microbiota?

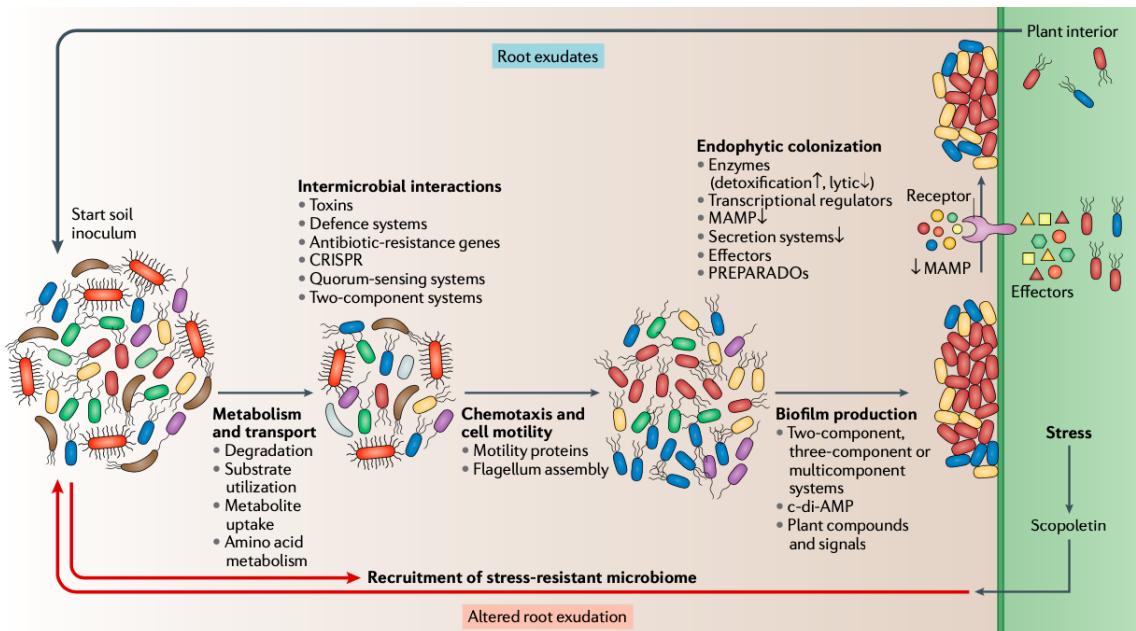
Plants modify the composition of microbiota



Plants modify the composition of microbiota

Plant sensing and the initiation of colonization

1. Plants release exudates
 - o **Plant exudates:** complex mixtures of soluble organic substances that are secreted by living plants
2. Plant-associated microorganisms use **chemotaxis** to sense and respond to plant-derived signals
3. Once a signal is perceived, **microorganisms move towards the plant** with the use of flagella
4. Microorganisms attach to the root surface and form a **biofilm**
5. Genes that encode proteins involved in bacterial chemotaxis, flagella assembly, bacterial motility and biofilm formation are **highly abundant in the rhizosphere compared with the bulk soil**

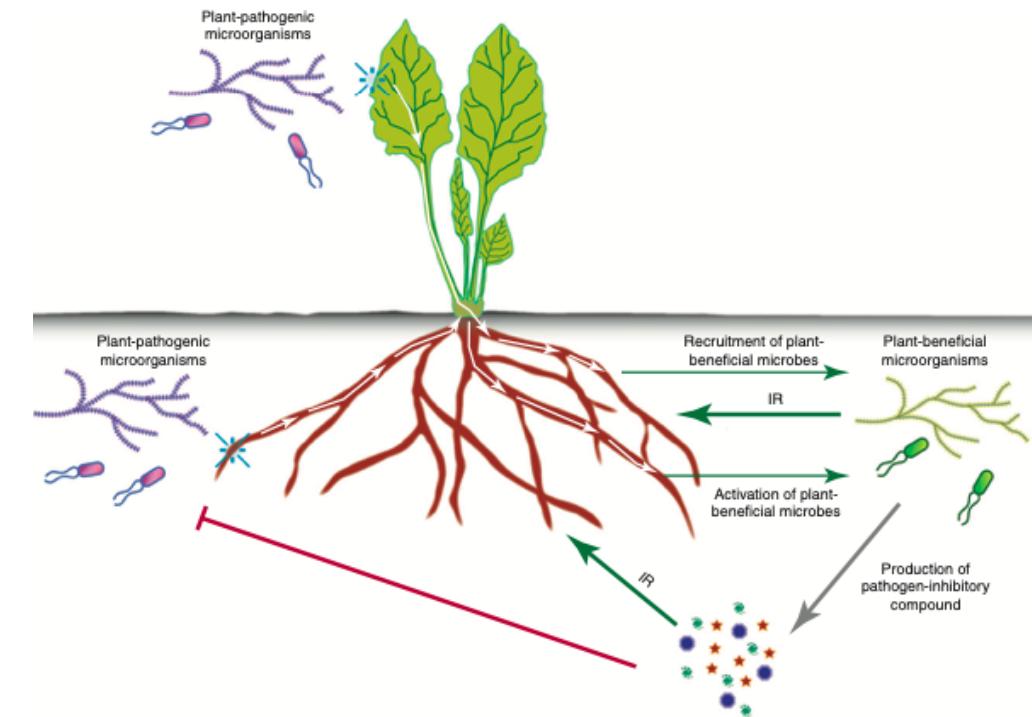


(Trivedi, Leach, Tringe, Sa, and Singh, 2020)

Plants modify the composition of microbiota

How plant can recruit beneficial microbes

- 💡 The plant-associated **microbiota is shaped** by complex interactions among the plant, microorganisms and the environment
- ❗ Metabolic diversification of root exudates within the plant kingdom may provide a basis for communication and recognition that directs the **assembly and maintenance** of a distinct microbiota tailored to the needs of the host
- Plant in response to local environments can **recruit distinct plant-associated microbial communities**

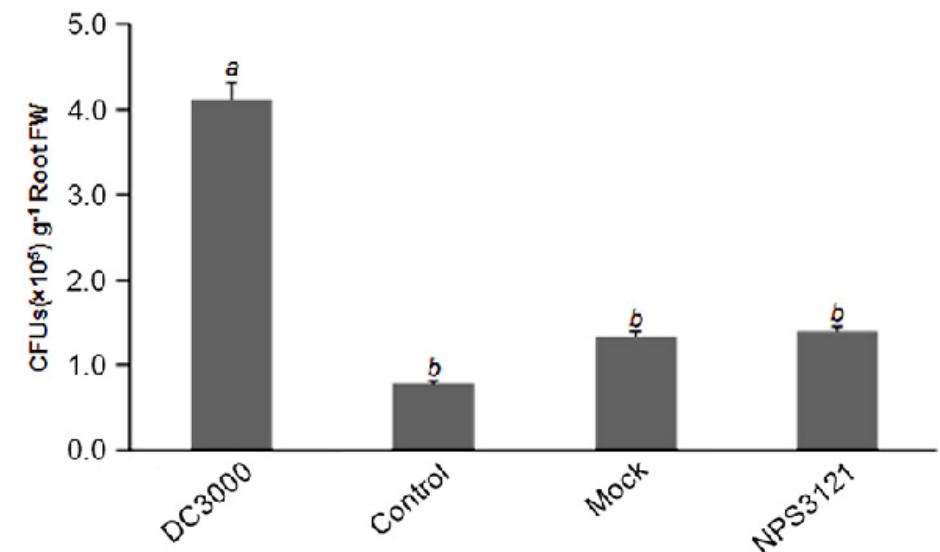
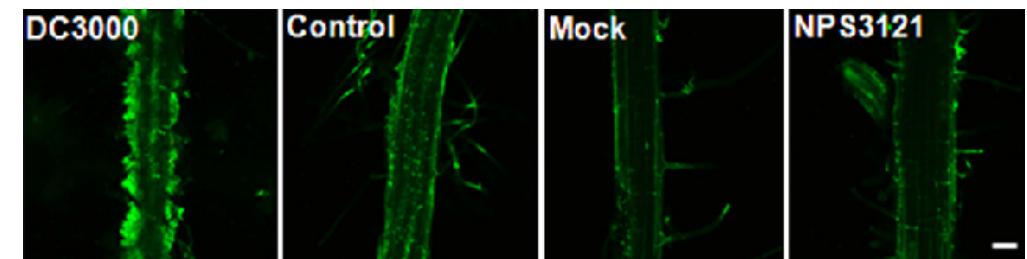


(Berendsen, Pieterse, and Bakker, 2012)

Plants modify the composition of microbiota

How plant can recruit beneficial microbes

- Demonstration that roots selectively secrete Malic acid and effectively signal beneficial rhizobacteria establishes a regulatory role of root metabolites in recruitment of beneficial microbes
- Aerial infection with *Pseudomonas syringae* strains Pst DC3000 **correlate with changes in root symbiont colonization** (*Bacillus subtilis* strain FB17) implicated root exudate involvement in the beneficial microbe recruitment



(Rudrappa, Czymbek, Paré, and Bais, 2008)

Plants modify the composition of microbiota

How plant can recruit beneficial microbes

- ⓘ Can leaf infection trigger changes in the composition of root metabolites?

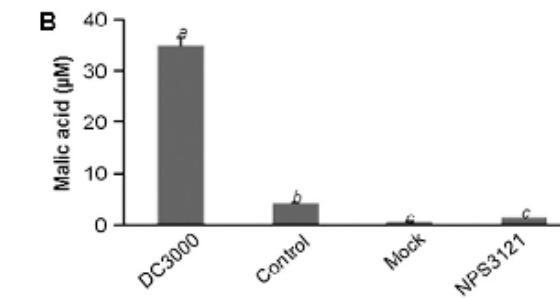
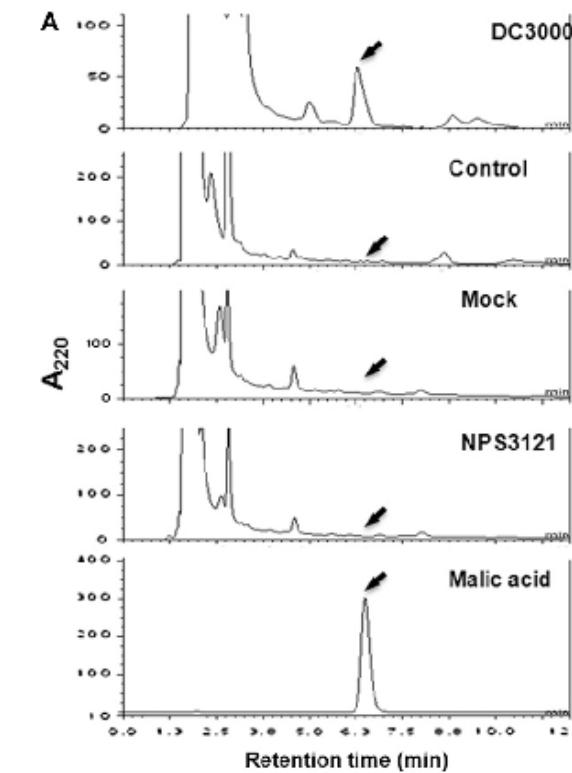


Spurious correlation

Plants modify the composition of microbiota

How plant can recruit beneficial microbes

- Can leaf infection trigger changes in the composition of root metabolites?
- Root secretions from plants subjected to different aerial bacterial infection treatments were collected and chemically analyzed by HPLC
- The peak was characterized by liquid chromatography-mass spectrometry and determined to be **malic acid**



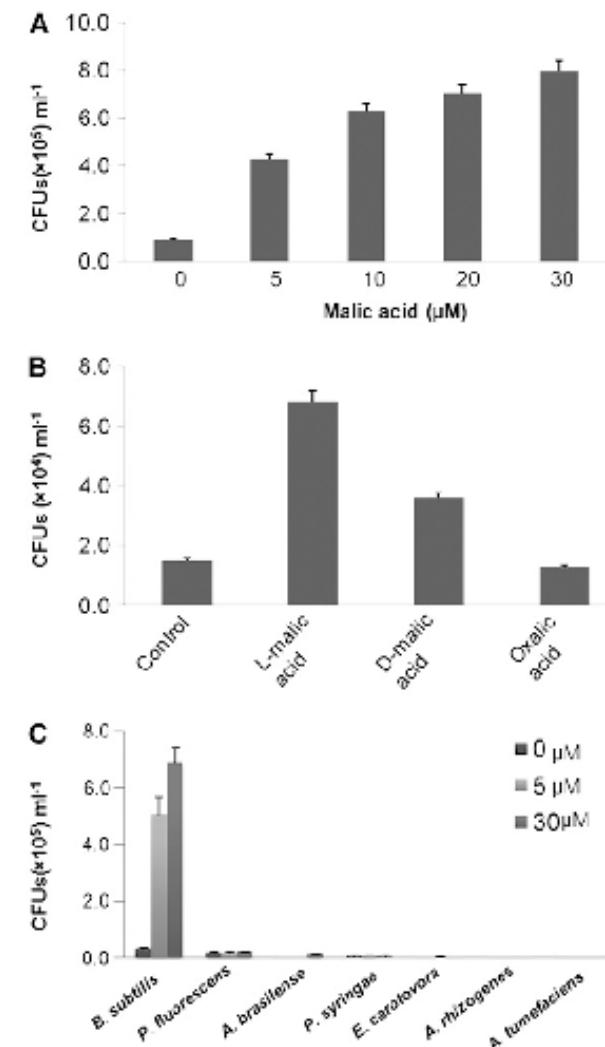
(Rudrappa Czymbek, et al., 2008)

Plants modify the composition of microbiota

How plant can recruit beneficial microbes

- **?** Is the malic acid able to selectively recruit the symbiont colonization (*Bacillus subtilis* strain FB17)?
- *B. subtilis* exhibits positive chemotaxis towards Malic Acid
- L-MA specifically chemoattracts FB17
- None of the tested bacteria shows any significant motility toward L-MA compared to *B. subtilis*

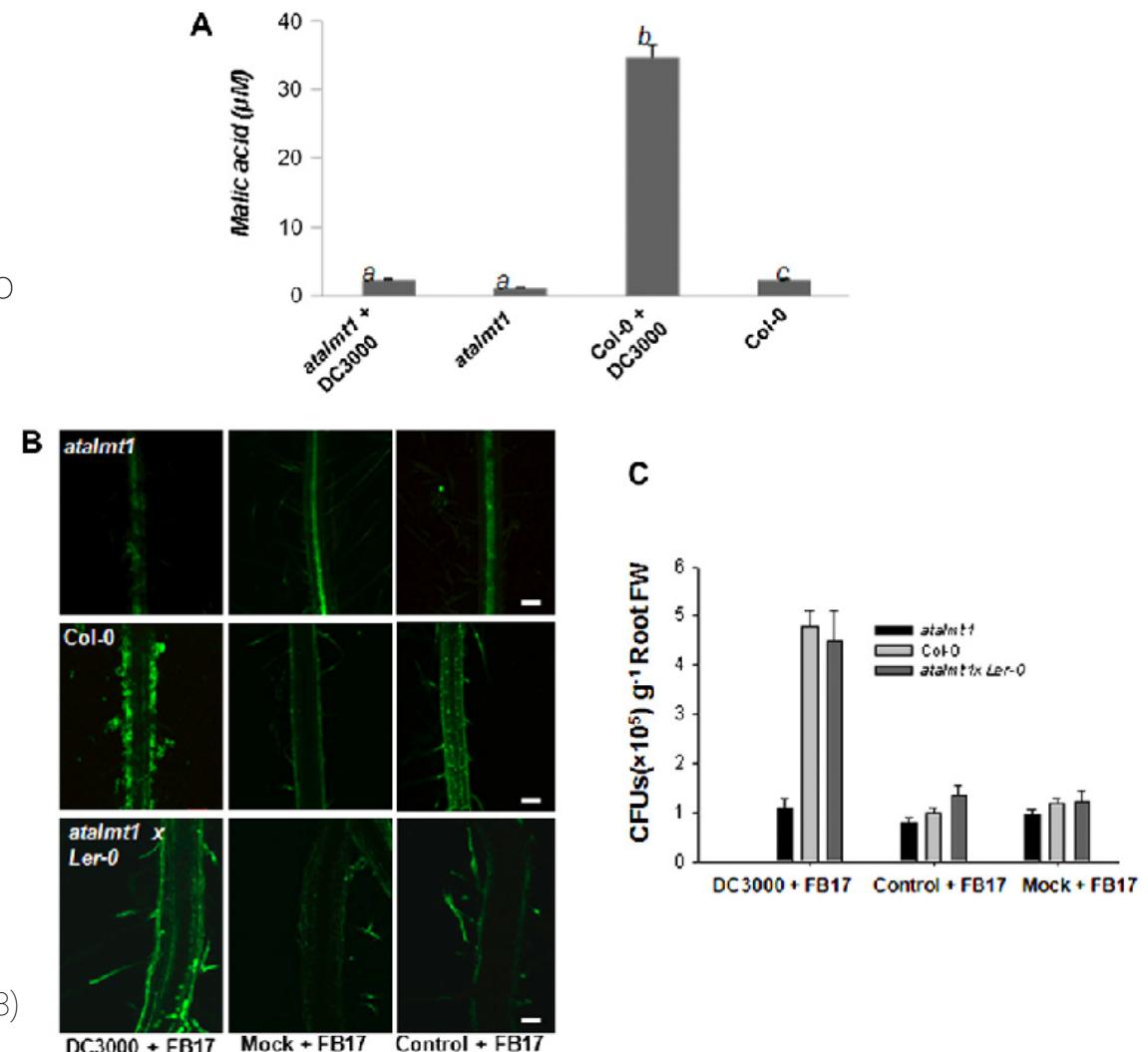
(Rudrappa Czymbmek, et al., 2008)



Plants modify the composition of microbiota

How plant can recruit beneficial microbes

- Does MA transporter mutant fails to recruit *B. subtilis* onto the root surface?
- Arabidopsis knockout mutant *Atalmt1* **deficient in root MA secretion** was assayed to confirm the role of MA secretions in recruiting FB17
- *Atalmt1* x *Ler-0* line **restored wild-type Col-0 phenotype** under Pst DC3000 infection

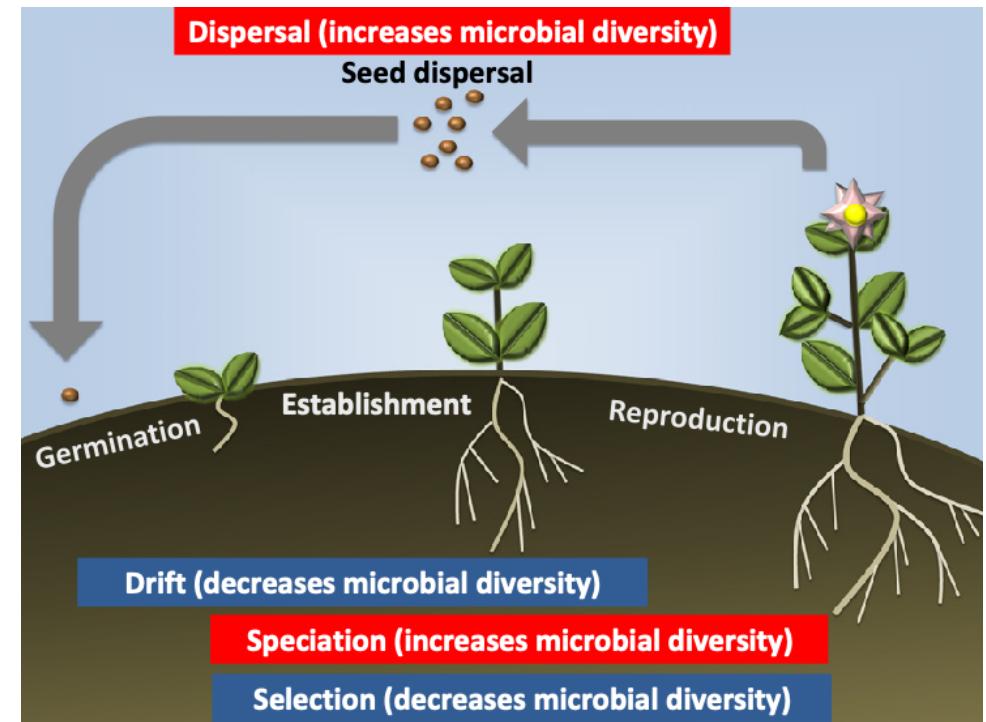


(Rudrappa Czymbek, et al., 2008)

Plants modify the composition of microbiota

Dynamics of the plant-associated microbiome

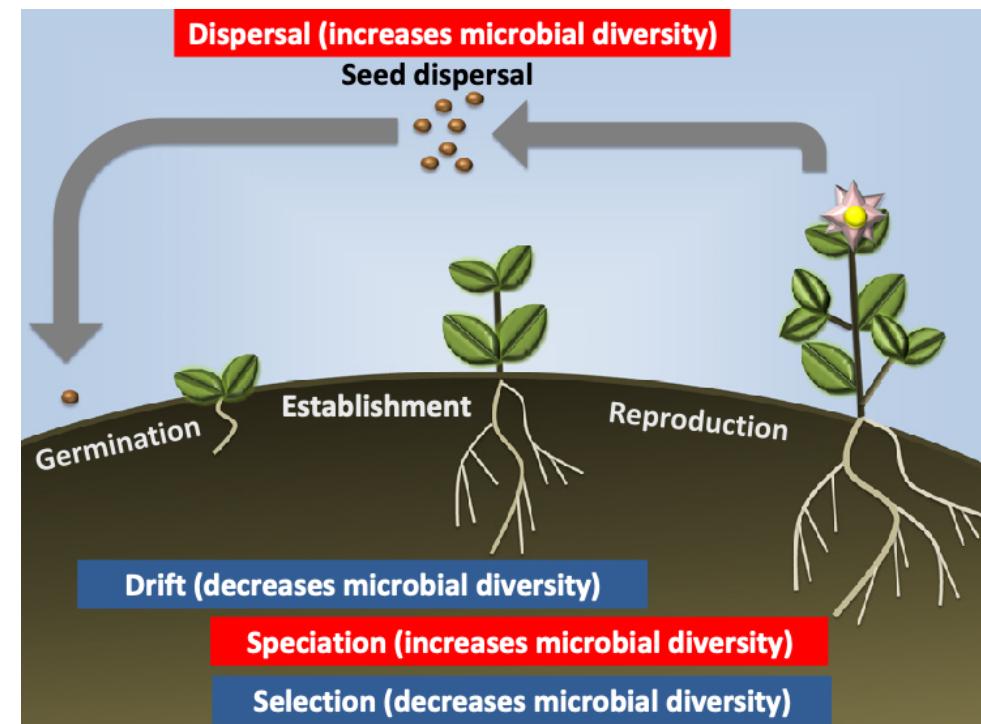
- ⓘ The assembly of a plant-associated microbiome is a **successional, multistep process** that is determined by dispersal, species interactions, the environment and the host
- **Early colonizers** could be **transmitted vertically** ↑, via the parents, through seed transmission pathways
- Once **seeds germinate**, microbiome assembly is likely to be driven by **horizontal transfer** ↔:
 - seed-borne microorganisms preferentially become associated with aboveground plant tissues
 - soil-derived microorganisms are mainly associated with the rhizosphere and roots



Plants modify the composition of microbiota

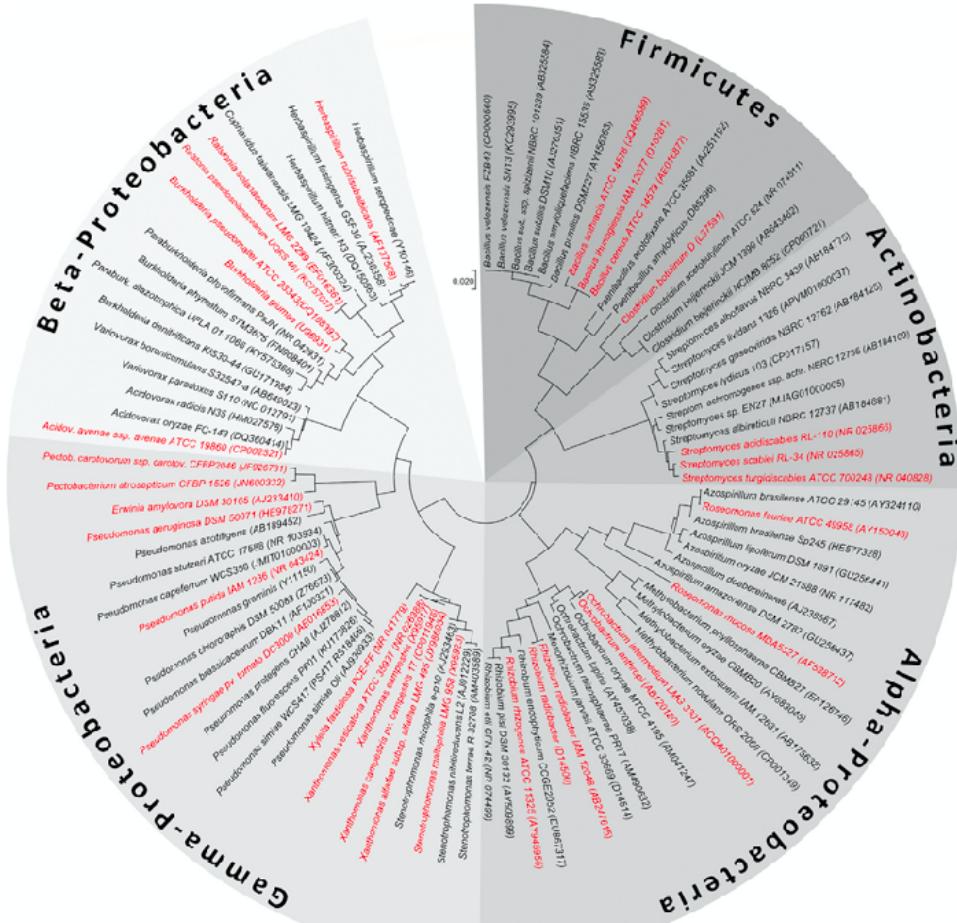
Dynamics of the plant-associated microbiome

- Microbiomes is **highly dynamic** in the early vegetative phase 🌱, begins to **converge** throughout vegetative growth 🌿 and **stabilizes** during the reproductive phase ↗
- A small fraction of microbial taxa belonging to the **core microbiota** are consistently maintained throughout plant development
- Specific traits (drought tolerance, disease resistance) that are conferred by associations with a particular microbial group can be **passed from mother plants to offspring** ↕



Plants modify the composition of microbiota

Evolutionary Relationship of PGPB and Pathogenic Bacteria



(Berendsen Pieterse, et al., 2012)

Can we exploit the microbiota?

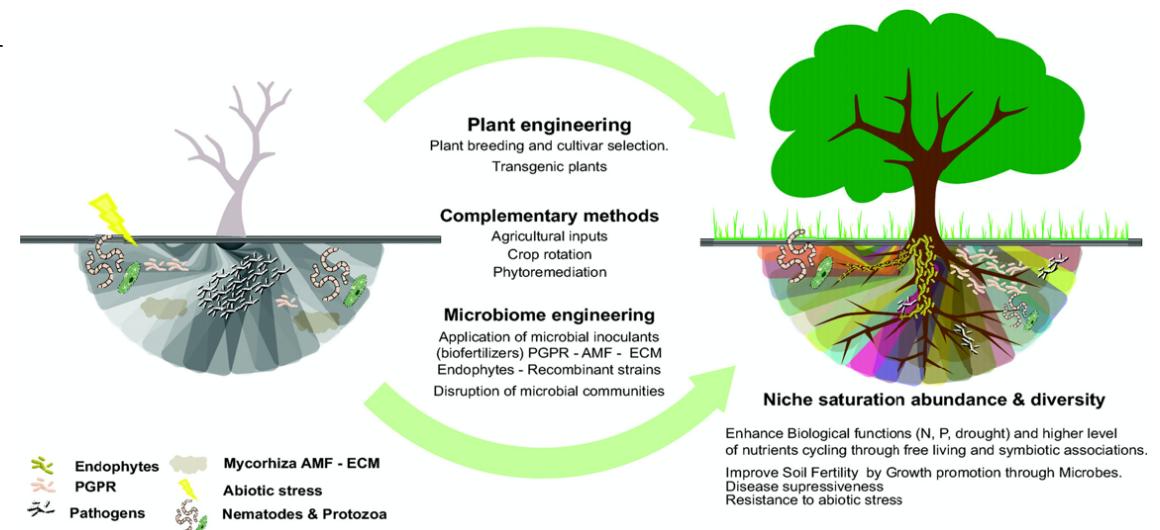
Can we exploit the microbiota?

Inoculation of microbial consortium

- Microorganisms have long been applied as inoculants for biocontrol or biostimulation

BUT

- their field efficacy varies with the climate, soil type and other environmental factors

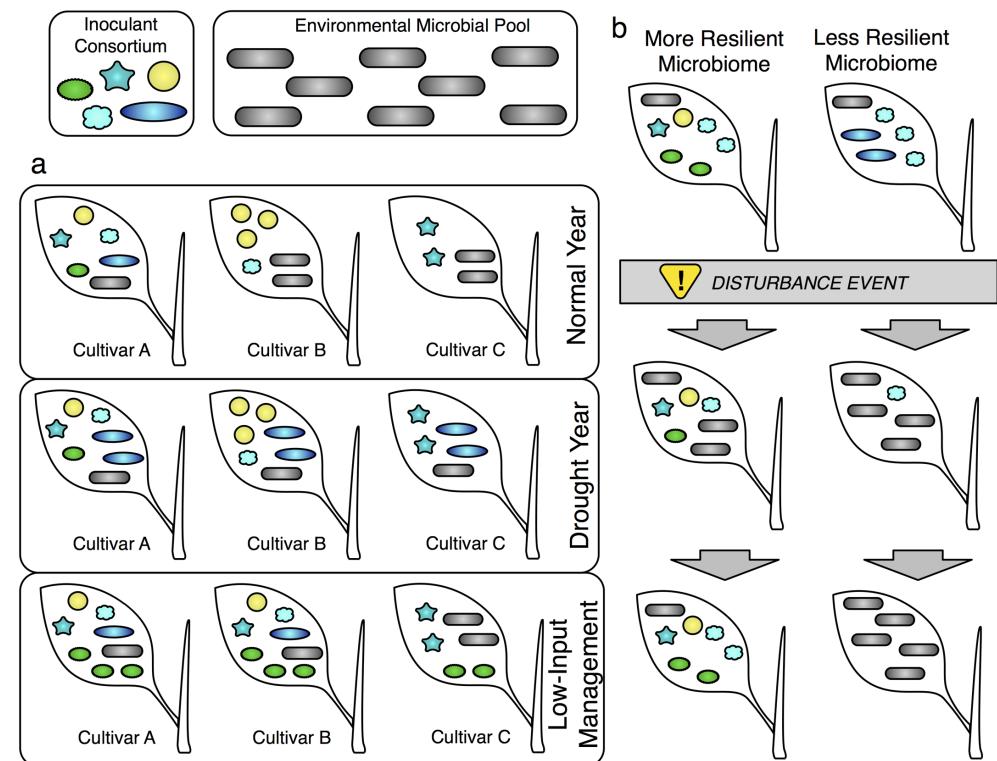


(Quiza, St-Arnaud, and Yergeau, 2015)

Can we exploit the microbiota?

Outcomes of inoculation with a microbial consortium

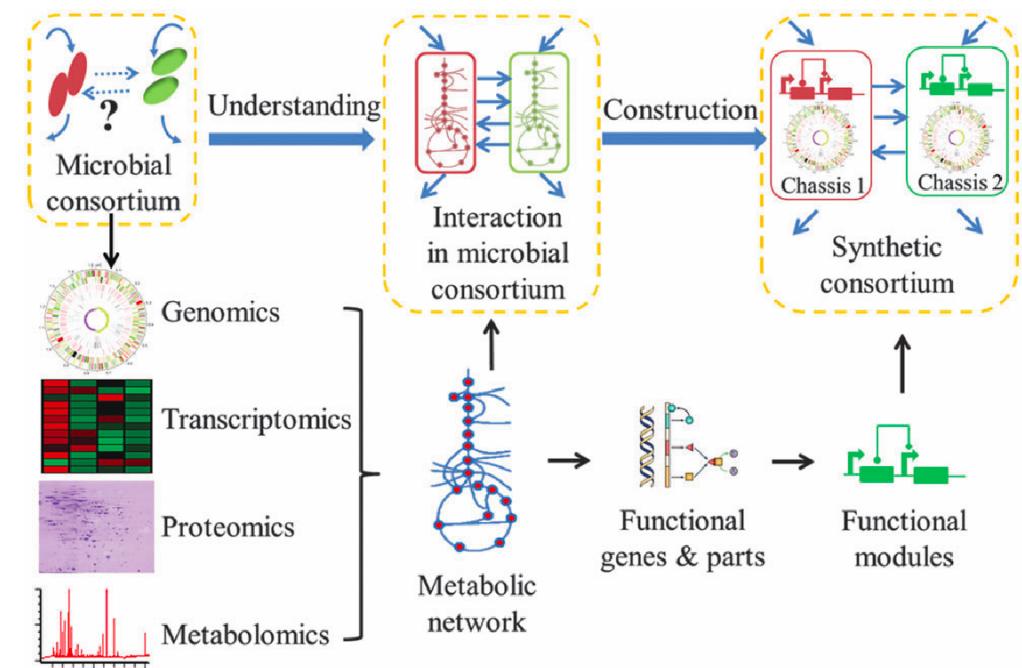
- **Outcomes of inoculating** with a five-member consortium in the presence of a diverse environmental microbial pool
- Three cultivars are depicted growing in **three different regimes**: a normal year, a drought year, and low-input management
- **Genotype effects**:
 - In a normal year, Cultivar A is colonized by all five members of the inoculant consortium
 - Cultivar B is colonized only by yellow spheres
 - Cultivar C is colonized only by teal stars
- **Environment/ management effects**:
 - The drought year shows higher colonization by blue ovals
 - Under low-input management all cultivars show increased colonization by the green spiky ovals
- **Temporal dynamics** of two communities after a disturbance event:
 - The more resilient community recovers to its initial state after perturbation
 - The less resilient community does not recover to its initial state after perturbation



Can we exploit the microbiota?

Microbiome engineering using synthetic microbial communities

- ! There is NOT a "one size fits all" microorganism community
- 💡 Synthetic microbial communities (synComs) of varied complexity have been constructed using bottom-up combinations
- To build a synCom selection of core functions are more important than taxonomical selection
- synComs with different complexities can be designed through predictive modelling that evaluates trait redundancy, dominance, modularity, interactions and assembly



(Song, Ding, Jia, Ma, and Yuan, 2014)

Companies Examples

Can we exploit the microbiota?



BioConsortia Inc. is devoted to developing solutions through **enhanced crop productivity** and is aware that **microbial products have a major role** to play in future mainstream agricultural crops

<https://bioconsortia.com/>

Can we exploit the microbiota?

Founded in New Zealand in 1994

The company specializes in the discovery and development of natural microbial products

Breakthrough in Advanced Microbial Selection (AMS) process in 2009

Focused on perfecting the innovative R&D platform for AMS

In 2014, the decision was made to globalize

BioConsortia raised \$15 million from Khosla Ventures and Otter Capital, and establishing the headquarters and labs in Davis, CA

Strong R&D Expertise

30 highly experienced experts from industry and academia, including 12 PhDs

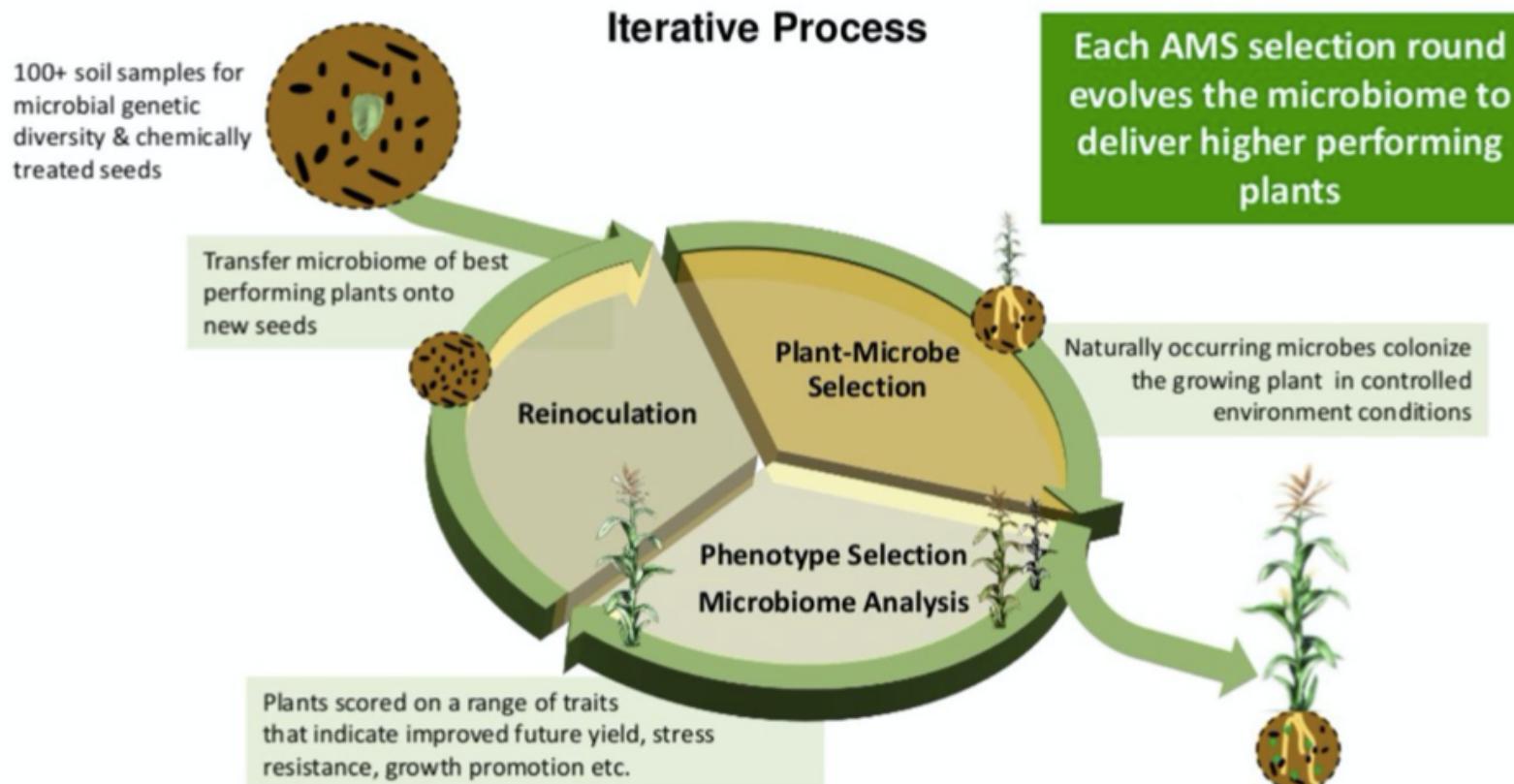
Highest level of competency in ultra-high-throughput biological screening

They now hold one of the world's largest collections of pre-screened and characterized microorganisms, comprising over 45,000 microbes, including 9,000 endophytes

Can we exploit the microbiota?

Evolving the Microbiome

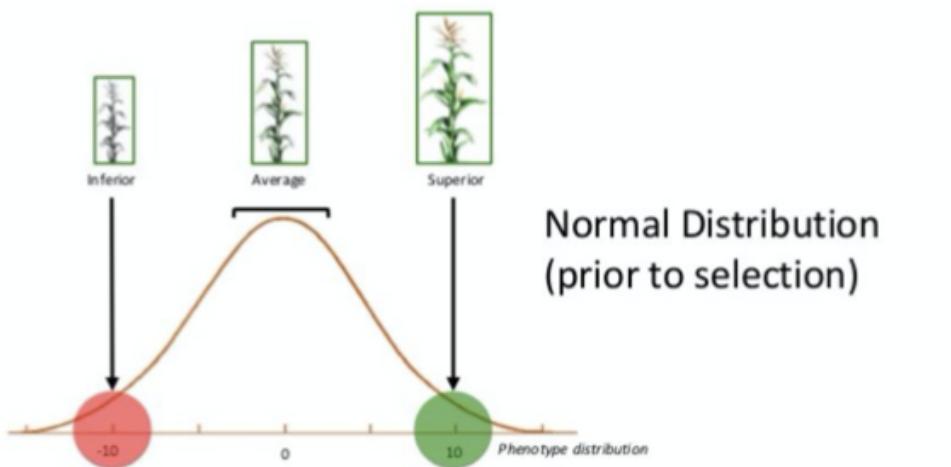
Advanced Microbial Selection (AMS)



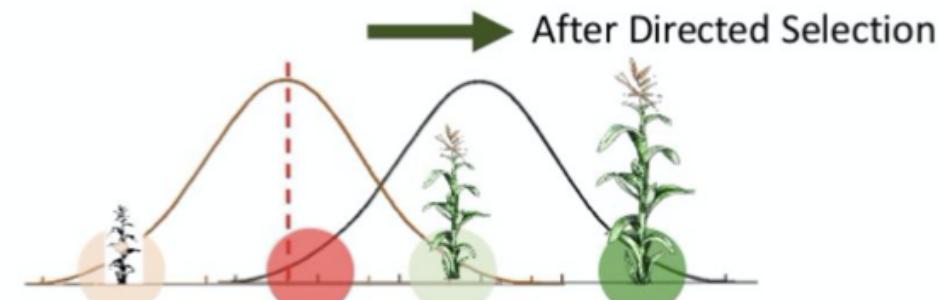
Proprietary, BioConsortia patented process

Can we exploit the microbiota?

Directed Selection



Normal Distribution
(prior to selection)



After Directed Selection

Proprietary, BioConsortia patented process

The AMS Process

Selecting the superior phenotype & associated microbiome
Selection in both ideal & stressed environments.

Changing the microbial community
Advancement of the most beneficial microbes through iterative selection rounds.

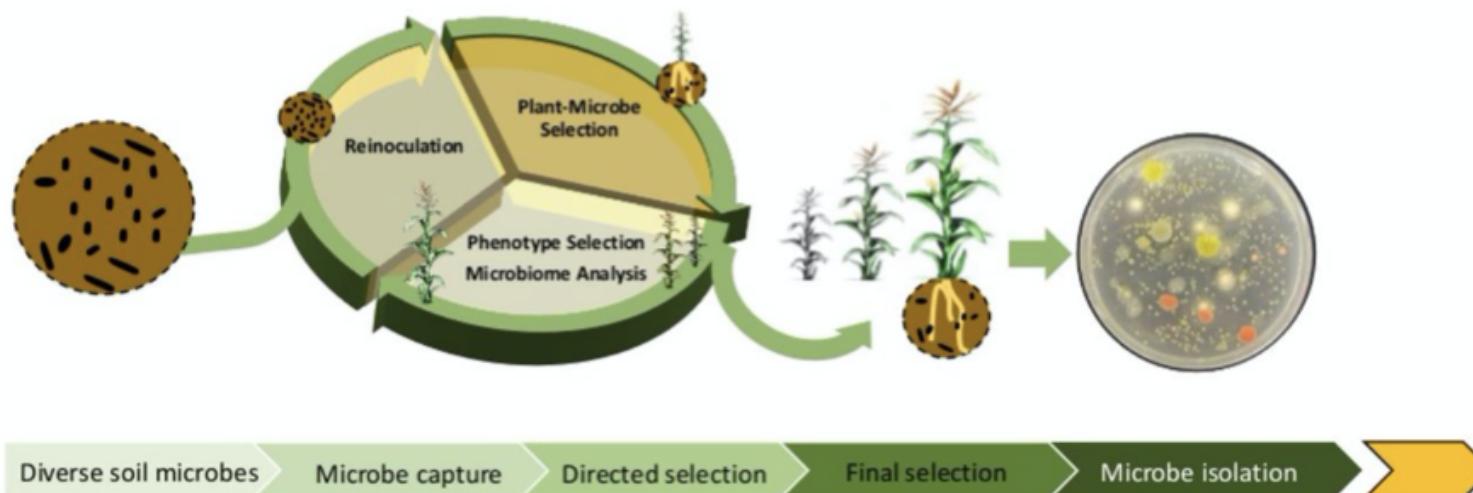
Driving an improvement in trait performance
Results in an accumulation of microbes responsible for enhanced targeted traits.

$$G \times E \times M = P$$

Can we exploit the microbiota?

Advanced Microbial Selection (AMS)

We select the crop trait -- the plant selects the microbes



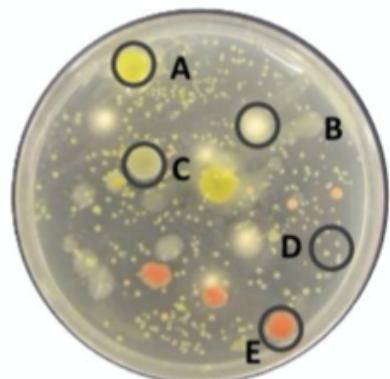
Process and selection informed by DNA and microbiome analysis

Proprietary, BioConsortia patented process

Can we exploit the microbiota?

Identify Effective Consortia

Informed by high-throughput DNA sequencing of plant microbiome



Eliminate:
• Pathogenic (plants, humans, etc.)
• Hard to produce or deliver

Microbes

AB, AC, AD, AE, BC,
BD, BE, DE, ABC, ABD,
ABE, ACD, ACE, ADE,
BCD, BCE, CDE, ABCD,
ABCE, A B C D E

To replicate or
improve past
performance



Microbe Evaluations including a range of:

- Soils
- Hybrids/varieties
- Base seed-applied chemistries

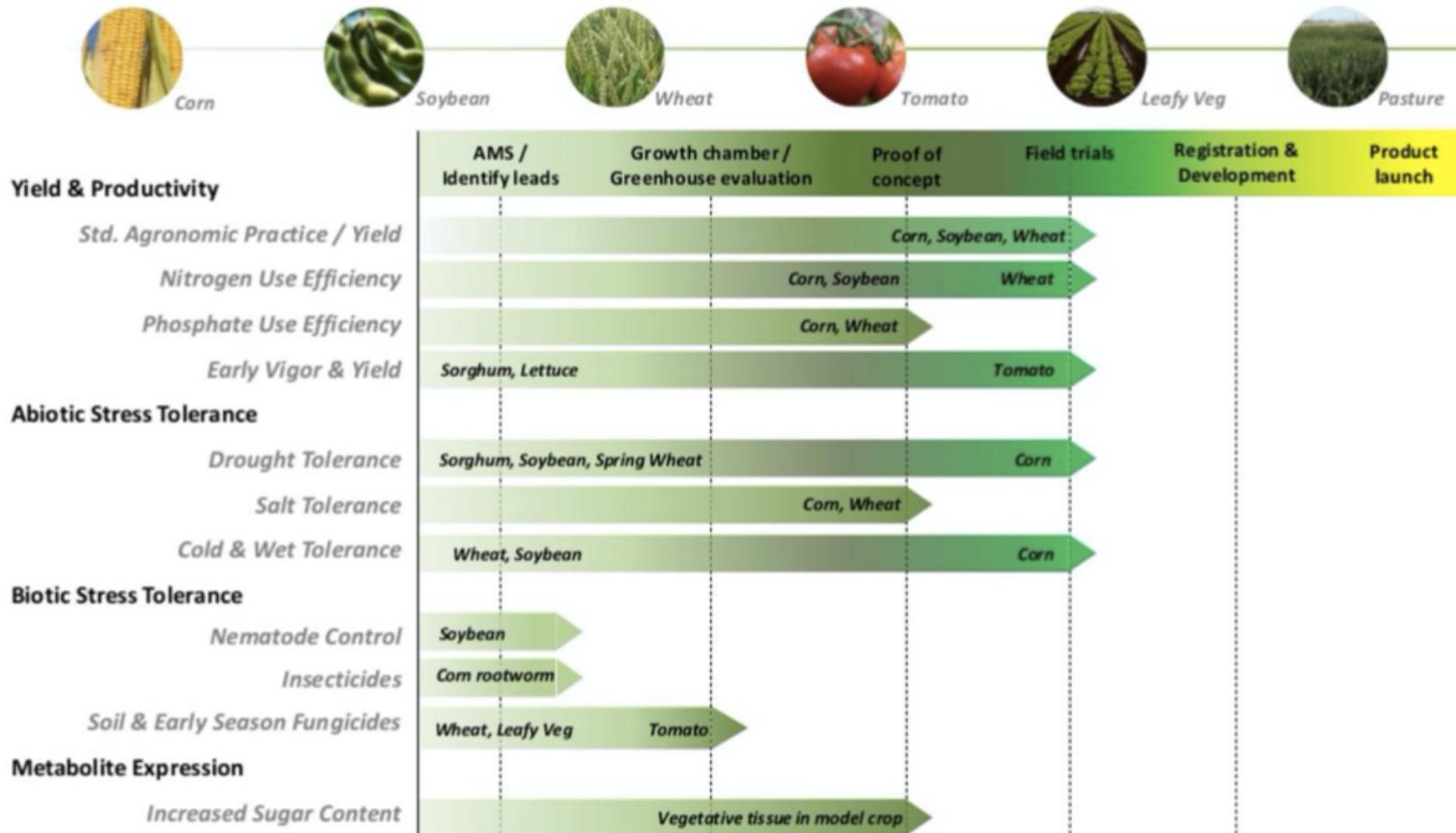
**Isolate & identify
microbes**

Prepare combinations
(key microbial consortia)

Final screens
(determine best consortia)

Can we exploit the microbiota?

R&D Pipeline: seed treatment, in-furrow, drench



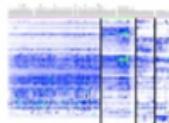
Plus R&D collaborations with multinational companies on additional crops and combination products

Can we exploit the microbiota?

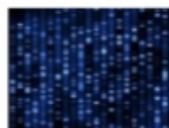
Underpinning Technology Platform



- Plant Growth & Phenotyping



- Microbiome Analysis



- DNA sequencing



- Microbial Phenotyping



- Plant Gene Expression

Can we exploit the microbiota?



At Mycorrhizal Applications, we harness the power and wisdom of natural systems to promote living soils and increase quality, productivity, and health in all industries involving soils, plants, and people

<https://mycorrhizae.com/>

Can we exploit the microbiota?

Founded in USA

Mycorrhizal Applications was established in the Dr. Amaranthuses' garage, with a focus on producing ectomycorrhizal slurries for use in forest restoration

Large-scale production in 2002

The company moved to its first large-scale production and office building

Entered into the agriculture industry in 2004

Its MycoApply® Micronized Endo product being utilized as a seed coat, kick starting the next growth phase for the company

Products for international markets in 2016

New highly-concentrated MycoApply® products for the Agriculture markets and non-Agriculture sectors like Horticulture, Nurseries and Turf Management

Development and launch of new formulations in 2020

New advanced carrier technology for agriculture and for horticulture and turf

Can we exploit the microbiota?



MycoApply 4 Species Consortium & Benefits



Beneficial Factors Attributed to 4-Species MycoApply Endomycorrhizal Fungi and Their Relationships with Plants

	Endomycorrhizal Fungi			
	<i>Glomus mosseae</i>	<i>Glomus aggregatum</i>	<i>Glomus intraradices</i>	<i>Glomus etunicatum</i>
Plant Nutrition Attributes				
Increased Nitrogen (N) uptake	X	X	X	X
Increased Phosphorus (P) uptake	X	X	X	X
Can access organic forms of N and P			X	
Increases mineral uptake			X	X
Effective root colonization with time-release fertilizers	X	X		
Tolerant of high fertility levels		X		
High levels of enzyme activity benefiting nutrient and micronutrient acquisition	X		X	X
Plant Growth and Establishment				
Improved performance of woody perennials	X		X	X
Increases fruiting and flowering	X		X	X
Improves plant performance in sandy soils		X		
Improves performance of palms and fruit trees		X	X	
Increases crop yields	X		X	X
Improves growth and performance of turf grasses, agricultural crops and nursery stock	X		X	
Very effective in agricultural soils	X		X	X
Improved plant establishment	X		X	X
Well adapted to a wide variety of plants and soil conditions	X		X	
Improved growth of grain crops	X		X	
Increases production of vegetable crops	X		X	
Improved growth of tropical and sub-tropical fruits		X	X	



Endomycorrhizal Fungi Species & Benefits



Beneficial Factors Attributed to Endomycorrhizal Fungi and Their Relationships with Plants

	Endomycorrhizal Fungi								
	<i>Glomus mosseae</i>	<i>Glomus aggregatum</i>	<i>Glomus intraradices</i>	<i>Glomus etunicatum</i>	<i>Glomus deserticola</i>	<i>Glomus clarum</i>	<i>Glomus monosporum</i>	<i>Gigaspora margarita</i>	<i>Paraglomus brasiliense</i>
Plant Nutrition Attributes									
Increased Nitrogen (N) uptake	X	X	X	X	X	X		X	
Increased Phosphorus (P) uptake	X	X	X	X	X	X	X	X	X
Can access organic forms of N and P			X						
Increases mineral uptake			X	X	X				
Effective root colonization with time-release fertilizers	X	X							
Tolerant of high fertility levels		X							
Increases N fixation activity					X	X			
High levels of enzyme activity benefiting nutrient and micronutrient acquisition	X		X	X			X		X
Plant Growth and Establishment									
Improved performance of woody perennials	X		X	X					
Increases fruiting and flowering	X		X	X	X	X	X	X	
Improves plant performance in sandy soils		X			X		X		
Improves performance of palms and fruit trees		X	X						
Increases crop yields	X		X	X	X	X	X		X
Improves growth and performance of turf grasses, agricultural crops and nursery stock	X		X						
Very effective in agricultural soils	X		X	X	X	X	X		
Improved plant establishment	X		X	X					
Well adapted to a wide variety of plants and soil conditions	X		X				X		
Improved growth of grain crops	X		X		X	X	X		
Increases production of vegetable crops	X		X		X	X	X		X
Improved growth of tropical and sub-tropical fruits		X	X			X	X	X	X

Can we exploit the microbiota?

Biome Makers

leaders in soil functionality analytics



Global scope

Our goal is to recover the health of our soils and create a more sustainable future.



Advanced Technology

Proprietary technology combining DNA Sequencing, Bioinformatics, and Intelligent computing to enable functional interpretation of microbiome communities.



Rock star team

Highly skilled team in genetics, software engineering, microbiology, agronomy, and data science. Meet them!

<https://biomemakers.com>

Can we exploit the microbiota?

2015

Biome Markers is born in San Francisco, taking action to recover soil health through technology

2016

Tails and market validation for advance soil analysis in vineyard

2017

Launched the operational portal with all the features to be an easy and friendly platform

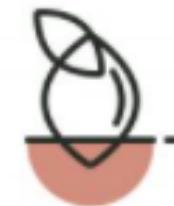
Community recognition

Many awards for providing the best technology for any crop and soil analysis

2018

Launch BeCrop and Ggeom platforms (even on mobile)

Can we exploit the microbiota?



BECROP
Smart Agriculture

Farmers & Agronomists

BeCrop is the most advanced technology for functional soil analysis, combining DNA Sequencing and intelligent computing.

Crop specific data-driven insights on what the soil really needs to be healthy for **any crop and any soil**.

Can we exploit the microbiota?

FOUR STEPS,

a complete crop status evaluation



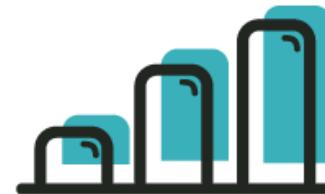
- 1 Order BECROP

You will receive your
Soil Collection Kit



- 2 Collect the samples

Just three
spoonfuls of soil



- 3 Get the results

PDF report

Quick
turnaround



- 4 Optimize your farming

Get the most out of
your field!

Can we exploit the microbiota?

TRANSLATING DNA

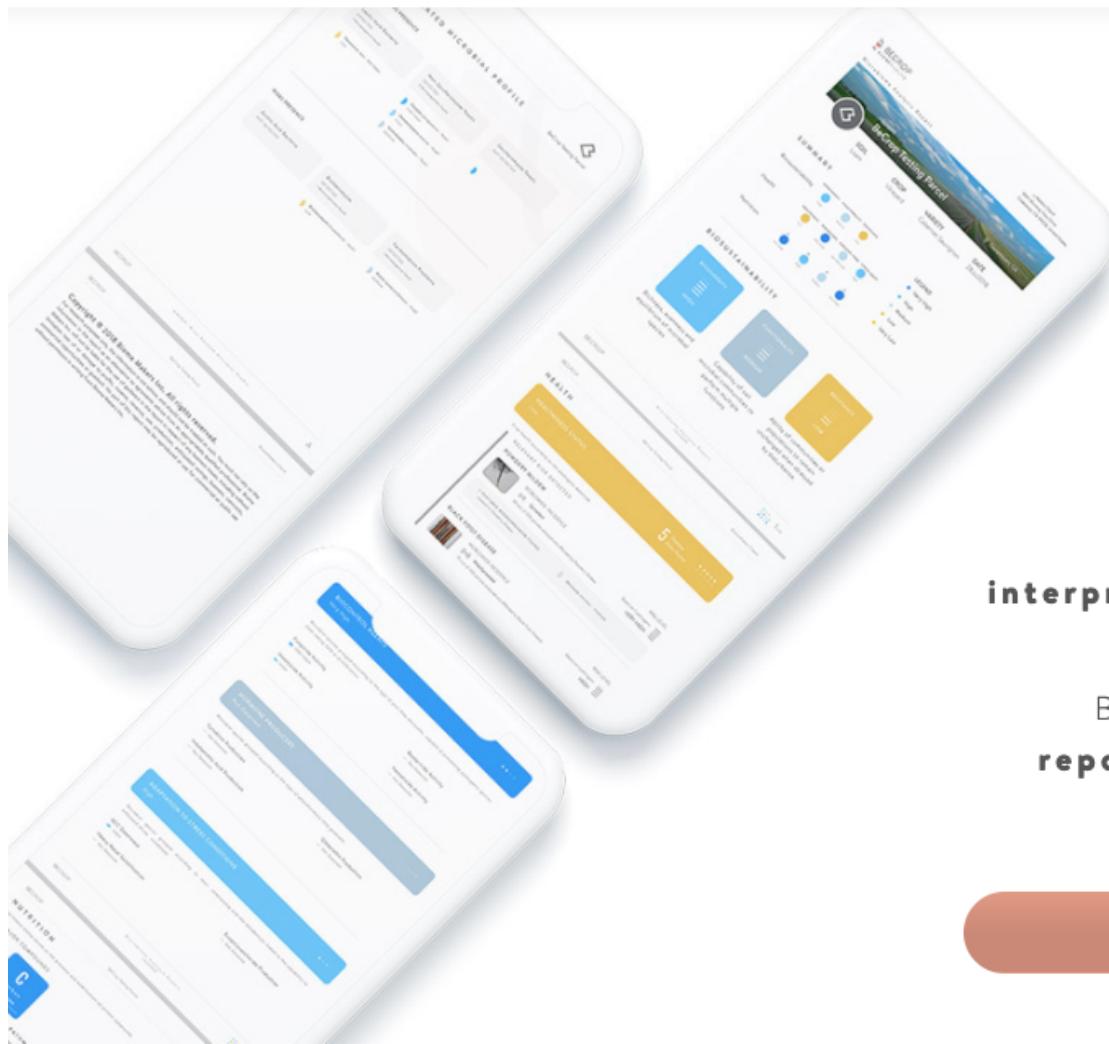
into actionable data

BECROP is a proprietary technology developed in-house by **BIOME MAKERS**

integrating genomics, AI and AgData to identify the microbial biomarkers

providing a meaningful explanation of soil function in agriculture.

Can we exploit the microbiota?



TAKE
CONTROL
of your crop

Get the complete microbial analysis and **customized interpretation of your microbiome** based on its biodiversity, vulnerability and nutrition pathways.

BeCrop turns your samples into an **easy-to-understand report** with all the information you need to make the best farming decisions.

 LAUNCH BECROP

Can we exploit the microbiota?

SPECIFIC SOLUTIONS

for particular needs

BeCrop has been design to be adapted to **any** **crop** and to process data specifically per crop.



IMPROVING THE YIELD OF SUGAR CANE

By identifying the needs of the sugar cane parcels using BeCrop technology, one of the largest sugar producers has achieved an increase in yield of their farm.



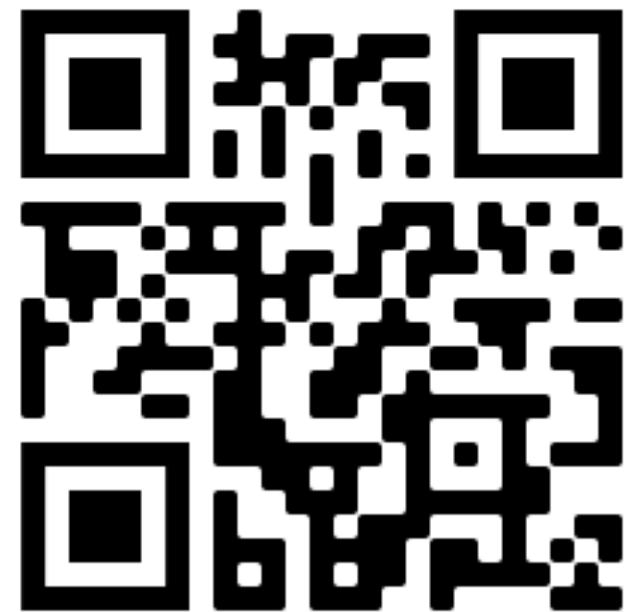
IMPROVING ALMOND DISEASE DIAGNOSIS

When a Californian almond farmer used soil microbiome to identify the fungal problem killing his trees, he was surprised that BeCrop showed a dagger nematode problem instead.

Questions about the lesson



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<https://bit.ly/2ZAYzky>