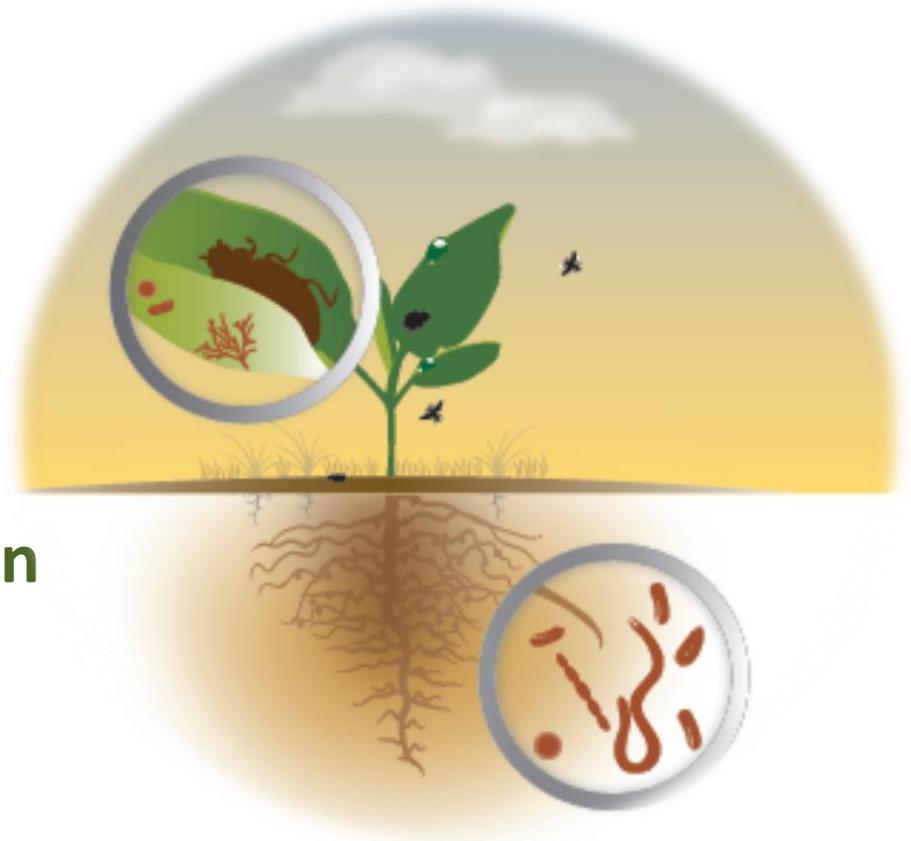


UNDERSTANDING PHYTOBIOMES FOR PHYTOSEQUESTRATION

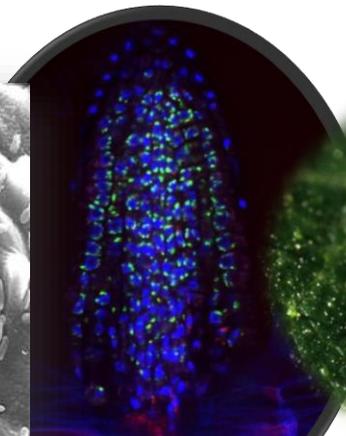
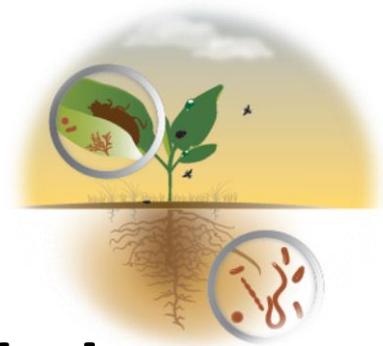
**Jan E. Leach
Colorado State University**

**ARPA-E Phytosequestration
Workshop, Chicago
July, 2015**

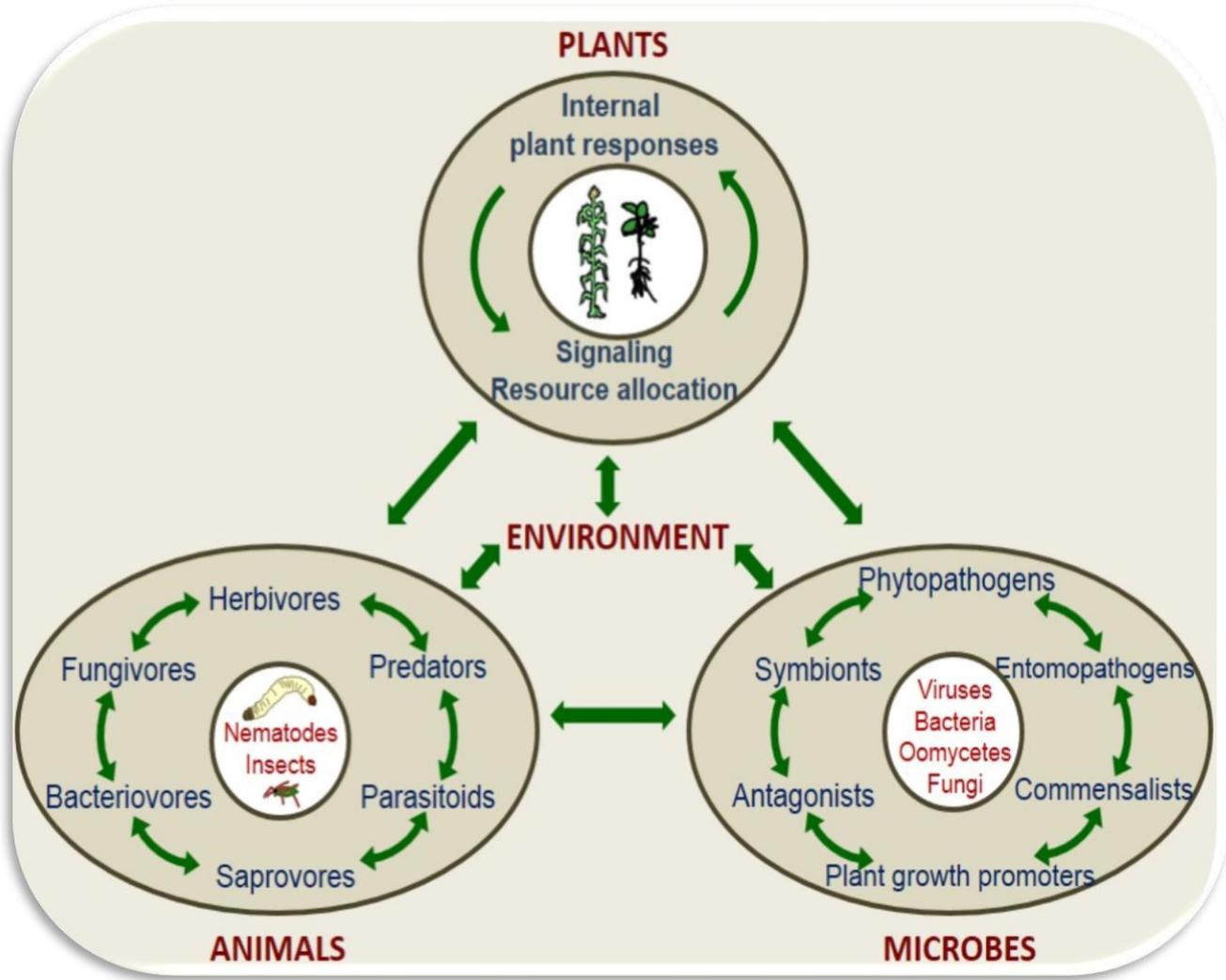


What is the *Phytobiome*:

- All organisms living in, on and around plants
 - microbes (the plant & soil microbiomes)
 - animals (insects, nematodes, etc)
 - other plants
- the Environment

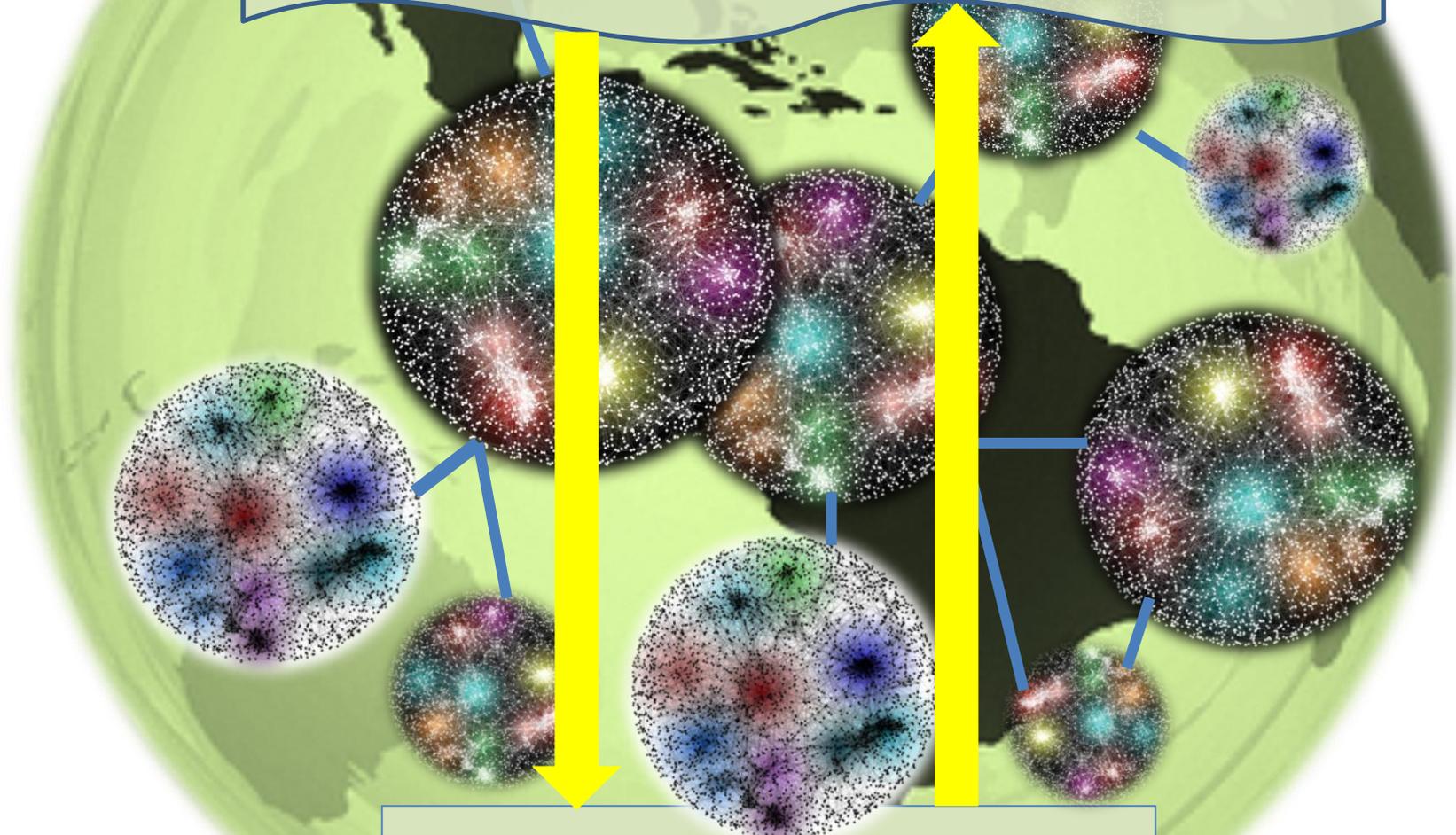


The plant microbiome is one component of the phytobiome system:



Other components of the system likely contribute to sequestration ...not just the microbiome

**(GENOME-GENOME
INTERACTIONS)ⁿ**



ENVIRONMENT

How are plant microbiomes studied?



Sample collection

Community
DNA extraction



Community RNA, protein,
metabolite extraction



PCR amplify
(16S-18S rRNA, ITS,
cpn60)

Amplicon
sequencing

Metagenome
sequencing

Meta-
transcriptome
sequencing

Metaproteome
analysis

Metabolome
analysis

Species (taxa)
number, abundance,
composition

Community function

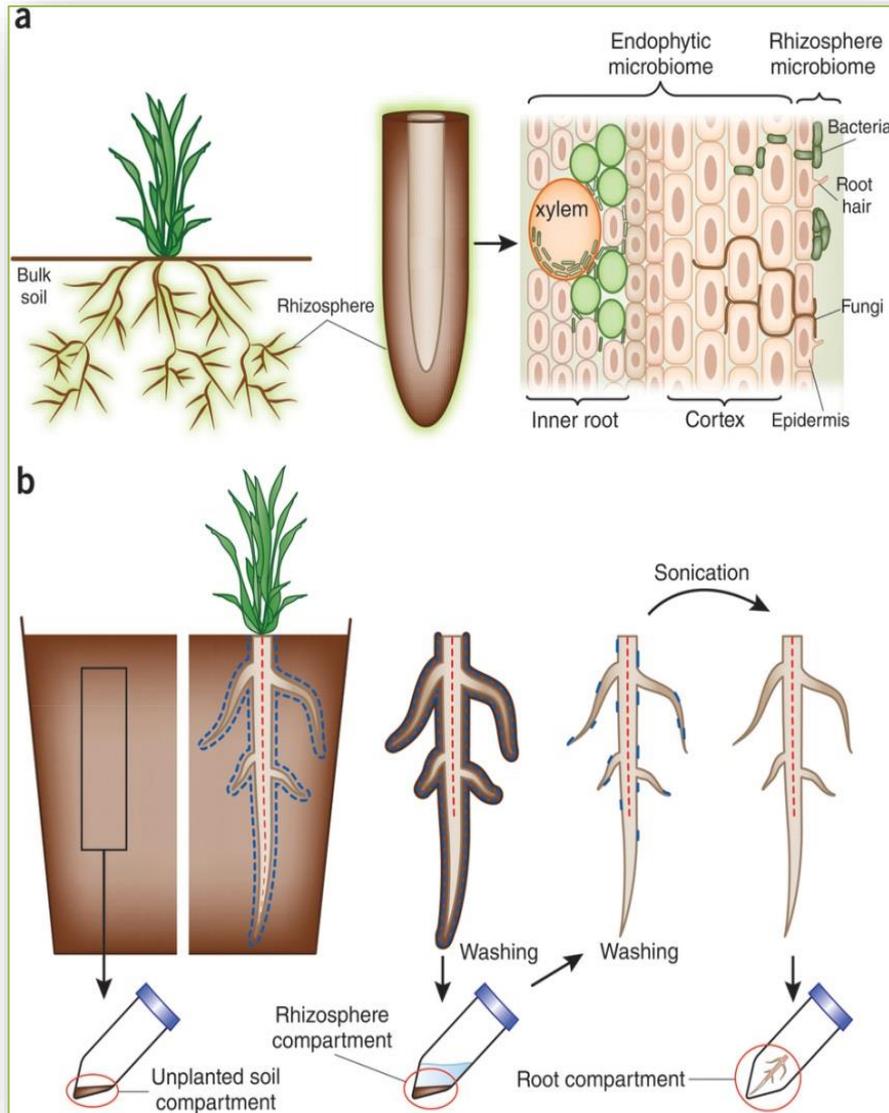
Community activity

“Who is there?”

“What can they do?”

“What are they doing?”

Is there a core rhizosphere microbiome?



- DNA from microbes in the soil, rhizosphere, and endophytic compartments
- amplicon sequencing

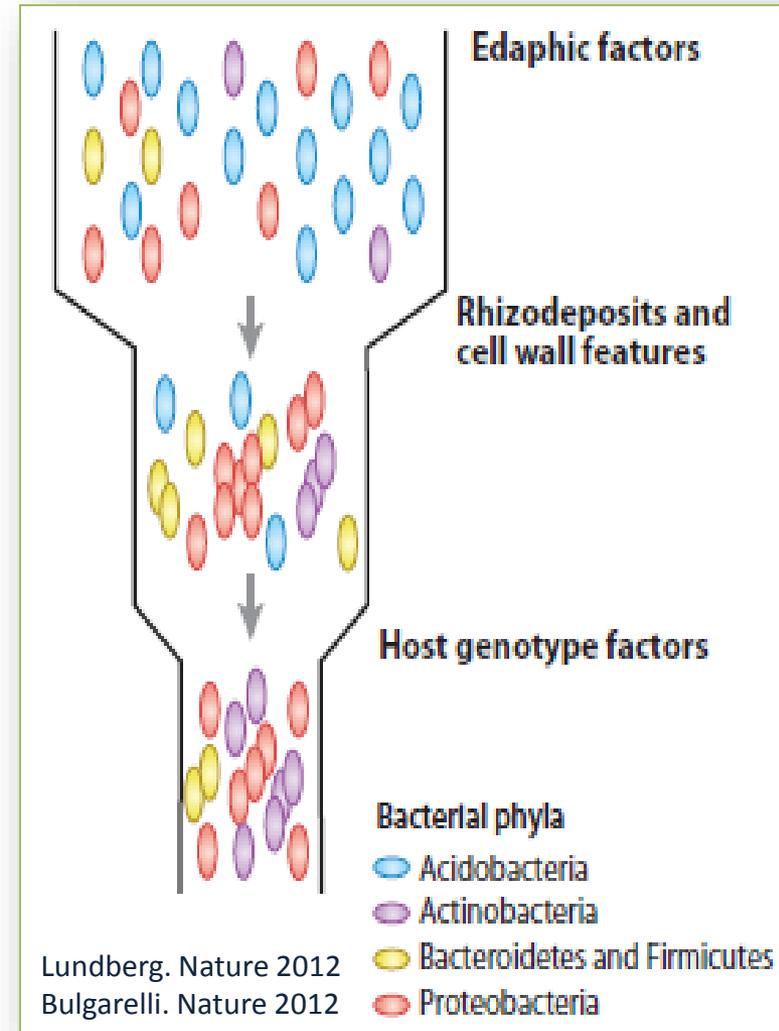
Lundberg et al, Nature 2012

Bulgarelli et al, Nature 2012

Hirsch et al, Nature Biotech 2012

What did they find?

- Many microbes abundant in the soil were **NOT** found in the **endophytic** communities
- Endophytic communities in roots from different parts of the world were surprisingly **similar**
- **Host genotype** –dependent selection within the root corpus fine-tunes community profiles
- **Bottom line, microbiome communities:**
 - are not a product of random assembly,
 - may be predicted based on knowledge of the processes that drive their development
 - can be ‘improved’ through plant breeding



Are endophytic microbial populations only selected from the rhizosphere?

No.....



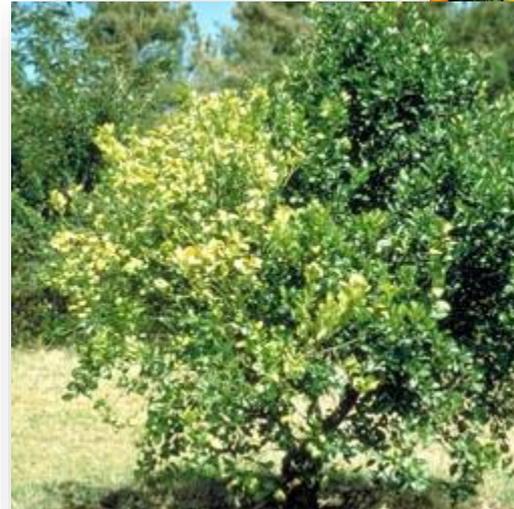
Johnston-Montje et al, 2014. BMC Plant Biol

- Plants regulate entry and/or establishment of select soil bacteria
- BUT, plants also transmit bacterial endophytes from generation to generation through seed

Influence of plant physiological state (disease) on the rhizosphere microbiome?

- Extracted DNA from bacteria in the rhizosphere for:
 - Amplicon sequencing (Who is there?)
 - Analysis of functional genes via hybridization (What can they do?)

Healthy orange tree

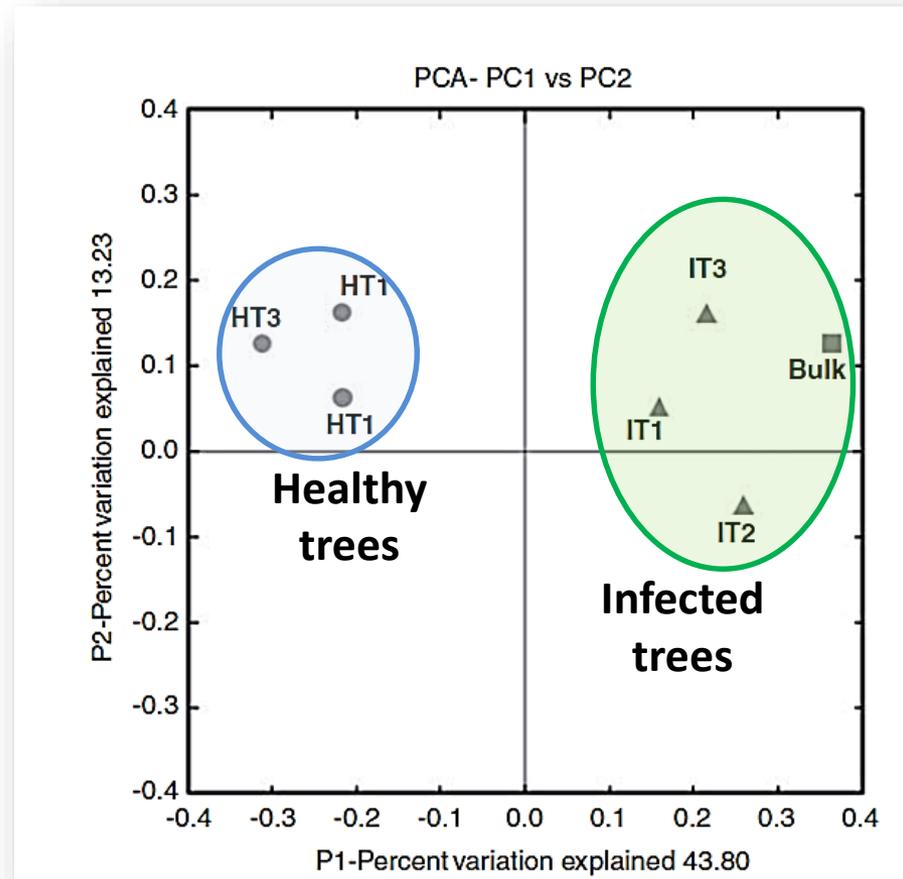


Tree with citrus greening (Huanglongbing)

Does the genome of the pathogen affect the genome response of the plant, and alter the genome content/function of the microbiome???

What did they find?

- Rhizosphere communities on infected trees were different from those on uninfected trees
→ Disease is associated with shifts in the microbiome

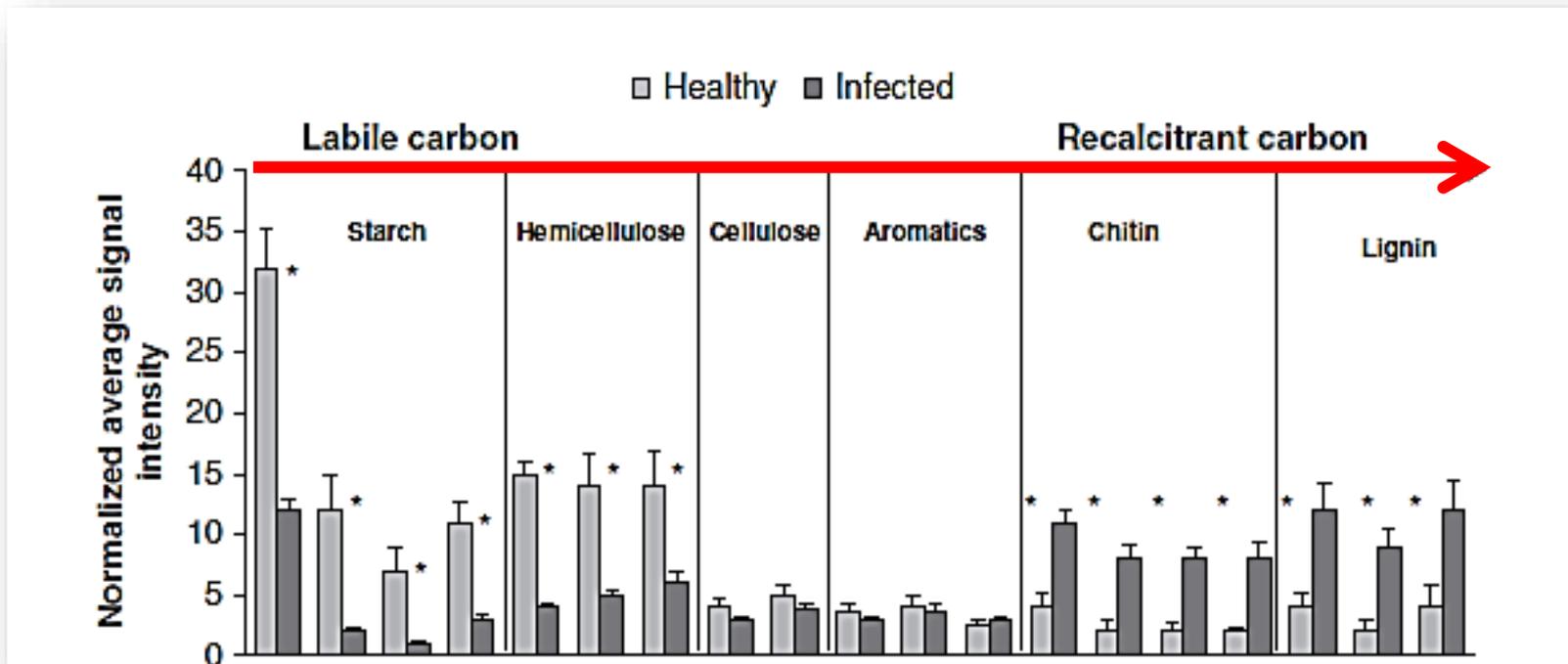


Function?

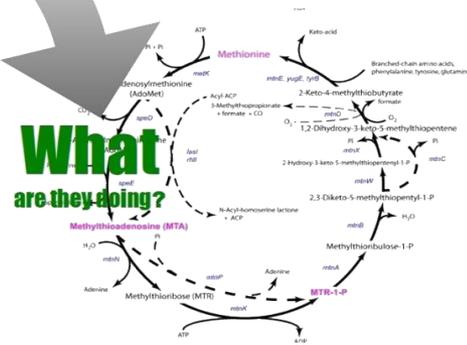
Citrus greening associated with:

- a shift away from use of easily degraded carbon sources to more recalcitrant forms
 - *consistent with carbohydrate repartitioning during disease*
(↓ *photosynthate to roots...*)

Trivedi et al 2012. ISME J



How do plant roots influence the rhizosphere microbiome composition?



Seedling



Vegetative



Bolting

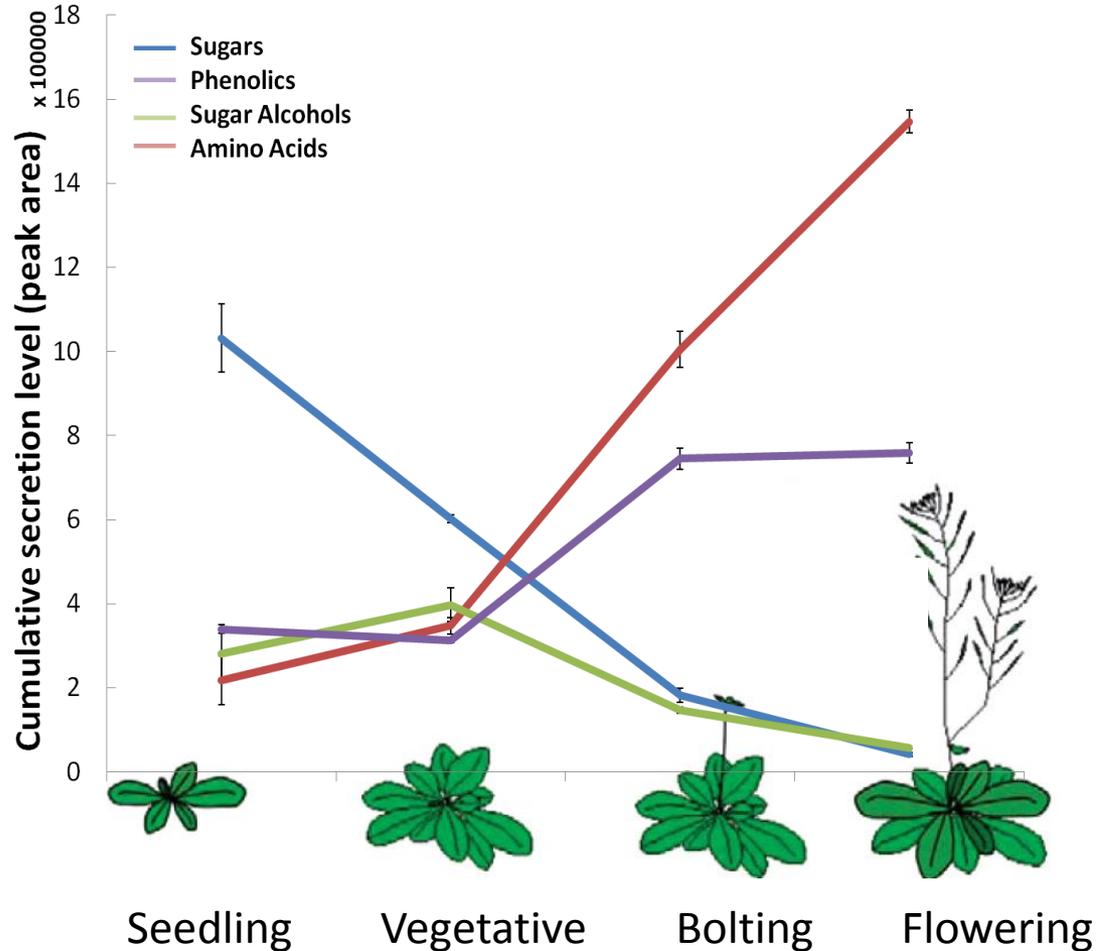


Flowering

At different plant developmental stages, measured:

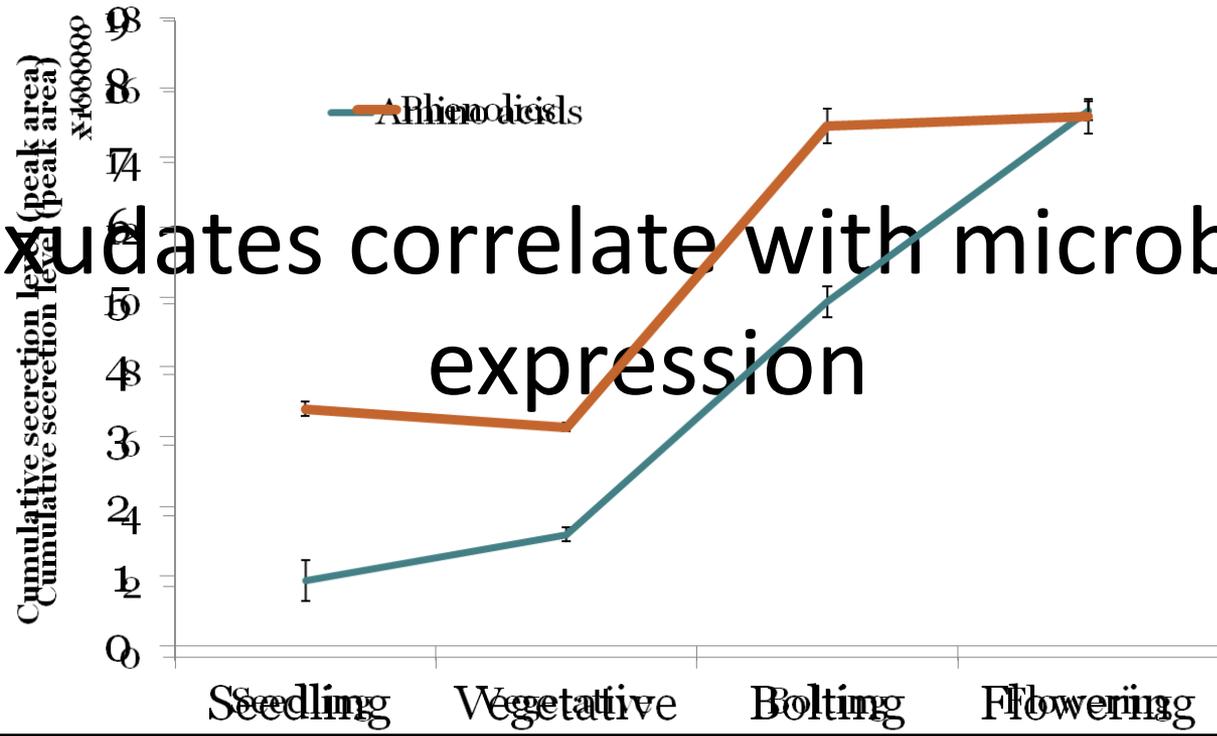
- root exudate composition
- rhizosphere microbiome composition
- microbiome function via transcriptome analysis

Root exudate composition changes during plant development



- Plant development influences the classes of chemicals in exudate
 - *Increased phenolics and amino acid content with age*

Root exudates correlate with microbial gene expression



Corresponding functional genes in each sample Abundance of corresponding functional genes in each sample

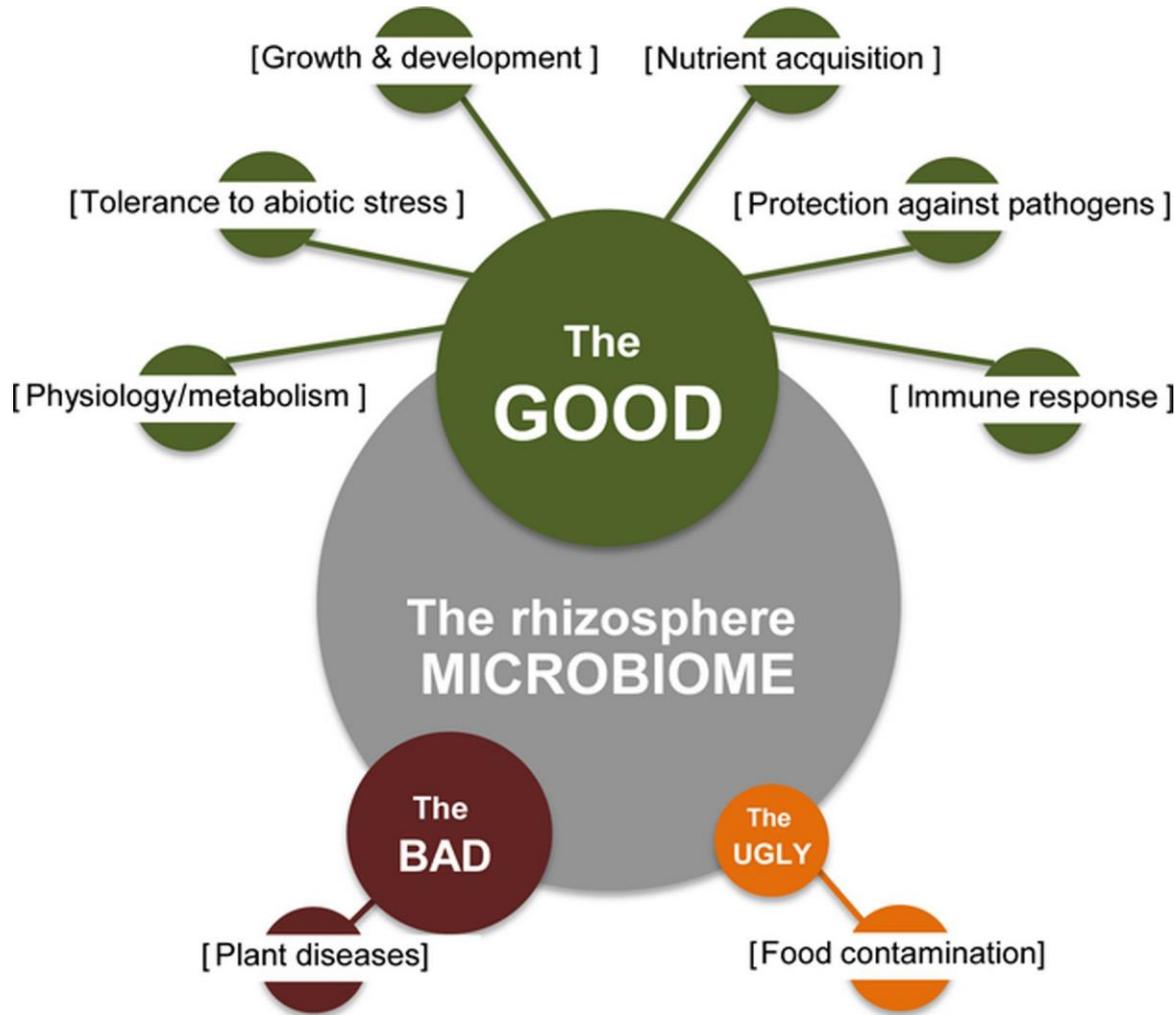
	Pearson	Spearman	Pearson	Spearman
Amino Acids	0.86	1.00	0.63	0.80
Phenolics	0.95	0.80	0.98	0.80



Bottom line

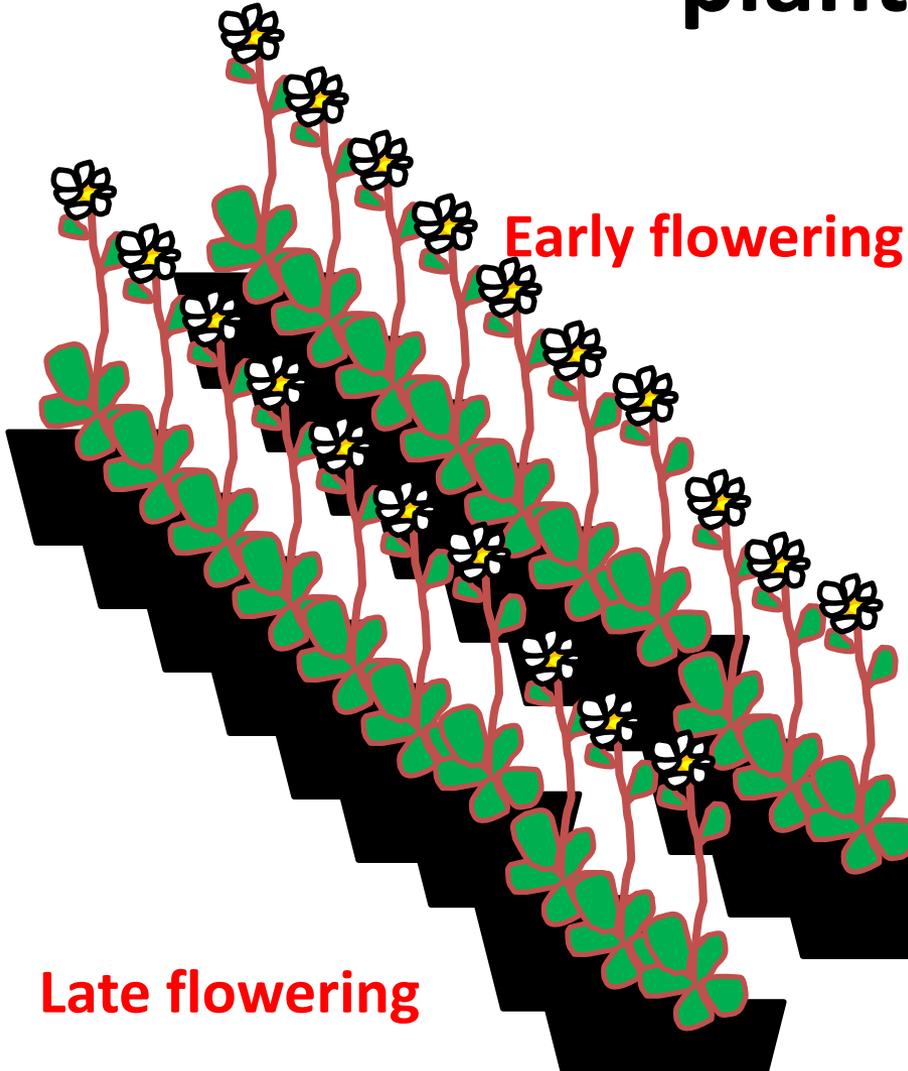
- Plant developmental stage and physiological state impact microbiome
- One way: root exudates modulate the rhizosphere microbiome

Also, microbiomes influence plant traits!



- **Disease resistance** (Mendes et al., 2011; etc.)
- **Abiotic stress tolerance** (Lau & Lennon, 2012; Bainard et al., 2013; etc.)
- **etc**

Can enriched microbiomes be generated that reliably influence plant traits?



The diagram illustrates a population of plants with varying flowering times. The plants are arranged in a diagonal line from the top-left to the bottom-right. The plants on the left are taller and have flowers that are higher up, labeled 'Early flowering' in red text. The plants on the right are shorter and have flowers that are lower down, labeled 'Late flowering' in red text. The plants have green leaves and brown stems. The ground is represented by a black, stepped surface.

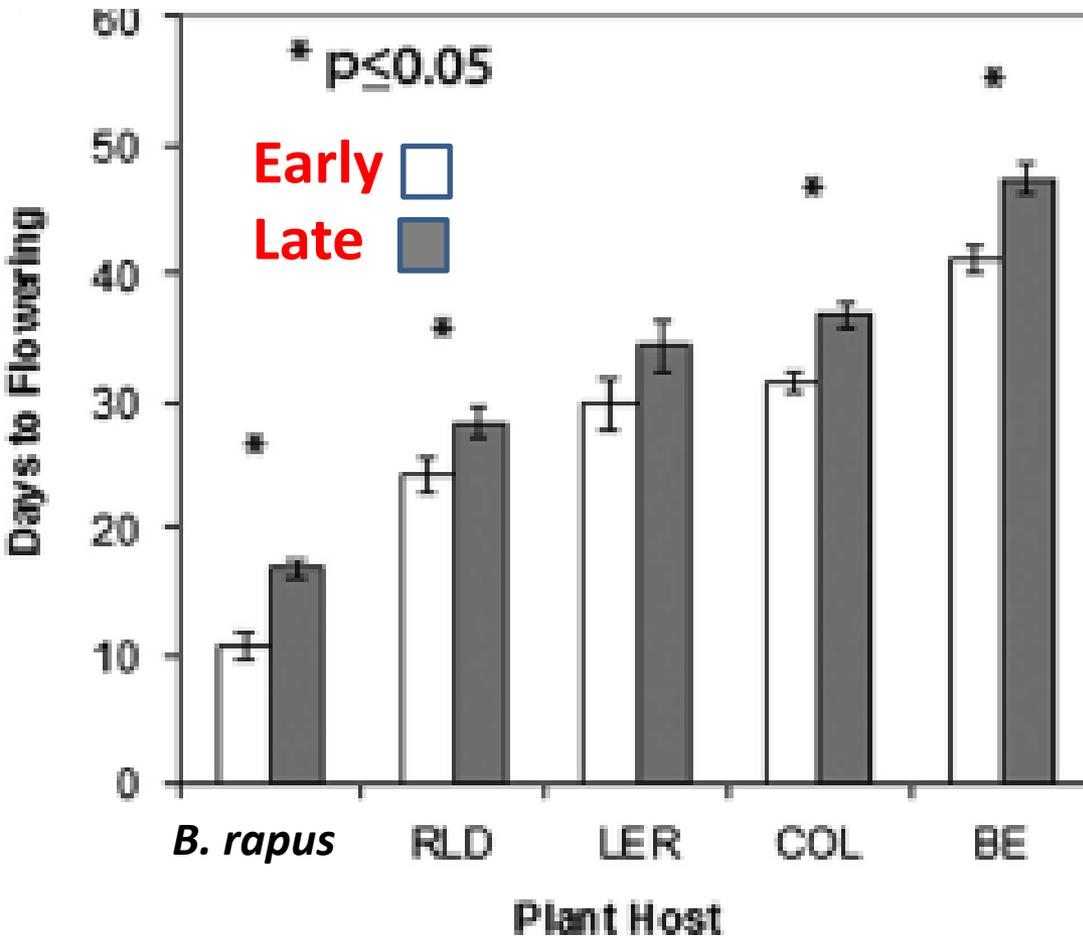
Early flowering

Late flowering

Multi generation selection of microbiome inoculants

- Plants selected at each generation for early flowering vs late flowering
- Soil from each 'phenotype' used as inoculant for next generation; repeated for 10 generations
- Plant genotype held constant: same seed pool used for all plantings

Soil microbial inoculants conferred Early or Late flowering traits!



- Inoculation of soil with ‘adapted’ microbiome predictably delayed or accelerated flowering in original genotype, other ecotypes, and other genera!



Phytobiomes
Initiative

Research

New
Phytologist



Simultaneous profiling of seed-associated bacteria and fungi reveals antagonistic interactions between microorganisms within a shared epiphytic microbiome on *Triticum* and *Brassica* seeds

Rodri
Johan
Peter
Matthew G. Lin
Tim J. Dumon

frontiers in
MICROBIOLOGY

PERSPECTIVE ARTICLE

published: 03 December 2013

doi: 10.3389/fmicb.2013.00355



Reshape microbiome therapy to manage plant diseases

Natural rice rhizospheric microbes suppress rice blast infections

Carla Spence^{1,2†}, Emily Alff^{2,3†}, Cameron Johnson⁴, Cassandra Ramos⁴, Nicole Donofrio³, Venkatesan Sundaresan⁴ and Harsh Bais^{2,3*}

Root exuda
Tizian) as a

Phylogeny and Functions of Bacterial Communities Associated with Field-Grown Rice Shoots

TAKASHI OKUBO^{1†}, SEISHI IKEDA^{1,2}, KAZUHIRO SASAKI¹, KENSHIRO OHSHIMA³, MASAHIRA HATTORI³, TADASHI SATO¹, and KIWAMU MINAMISAWA^{1*}

1:17

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biota

holt^{1*}

Rice root-associated bacteria: insights into community structures across 10 cultivars

erview
plants

Pablo Rodrigo Hardoim^{1,5}, Fernando Dini Andreote², Barbara Reinhold-Hurek³, Angela Sessitsch⁴, ...

Many questions remain:

- How do phytobiomes affect plant performance?
- How does the phytobiome influence plant tolerance to biotic or abiotic stresses?
- What useful organisms, genes and products can be mined from phytobiomes?
- Can phytobiomes be 'managed' to maintain soil health, rebuild depleted soils, or remediate contaminated soils?
- Can phytobiomes be manipulated to promote 'healthy' animal and human microbiomes?



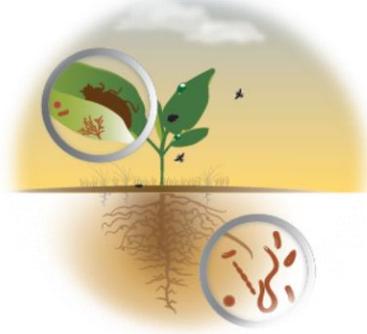
Constraints:

Phytobiome data are diverse & often ill-defined

- Plant health/disease status
- Plant physiological state
- Plant developmental stage
- Soil composition/ health/ 3-D structure
- Community metabolome, meta-transcriptome, metaproteome
- Environment (°C, humidity, etc)
- Etc.

Needs:

- **Standardized protocols**
- Capability to scale
 - **Single cell vs organ vs plant vs population?**
- High throughput
 - Continuous or multiple times
 - Across environments
- Ability to integrate & store diverse data
 - Novel & improved algorithms
 - Increased personnel trained in analysis of microbiome data
 - Access to sufficient computing power
- Reduced costs



Developing a Roadmap for Phytobiomes Research and Translation

- Establish current and future priorities in phytobiomes research
 - Knowledge gaps?
 - Technological gaps?
 - Infrastructural gaps?
 - Training gaps?



@phytobiomes

www.phytobiomes.org