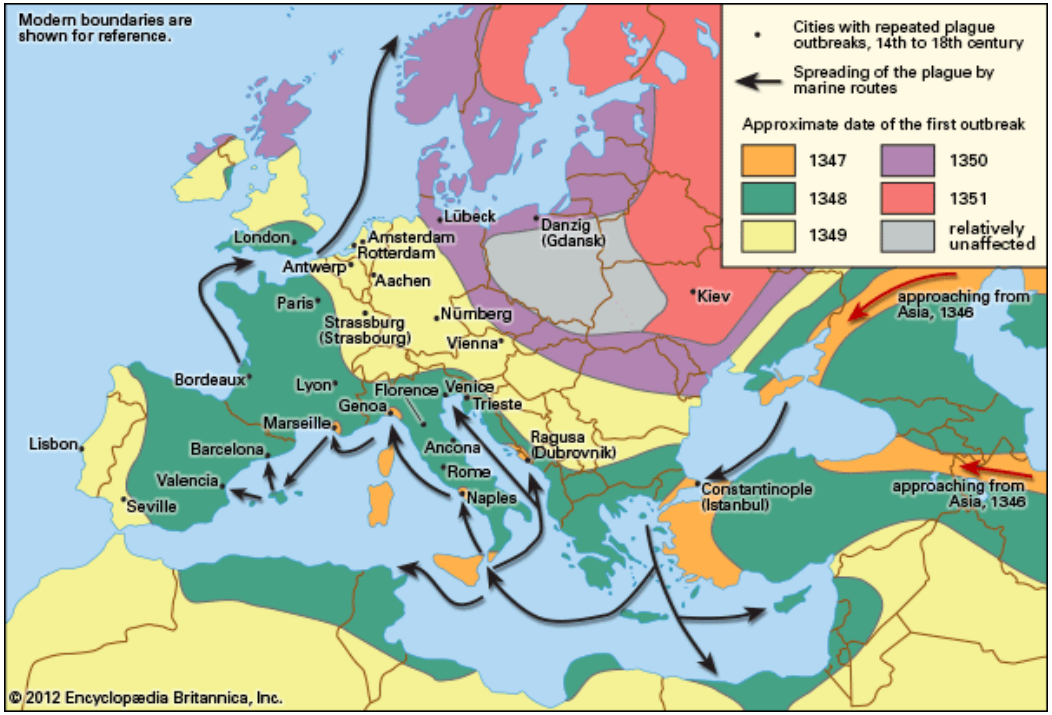
The plant microbiome in plant health and disease

Cara Haney
Department of Microbiology and Immunology/
Michael Smith Labs
(Associate member of Botany)

The University of British Columbia
Vancouver, Canada

Microbes make us sick, and their control is essential to medicine

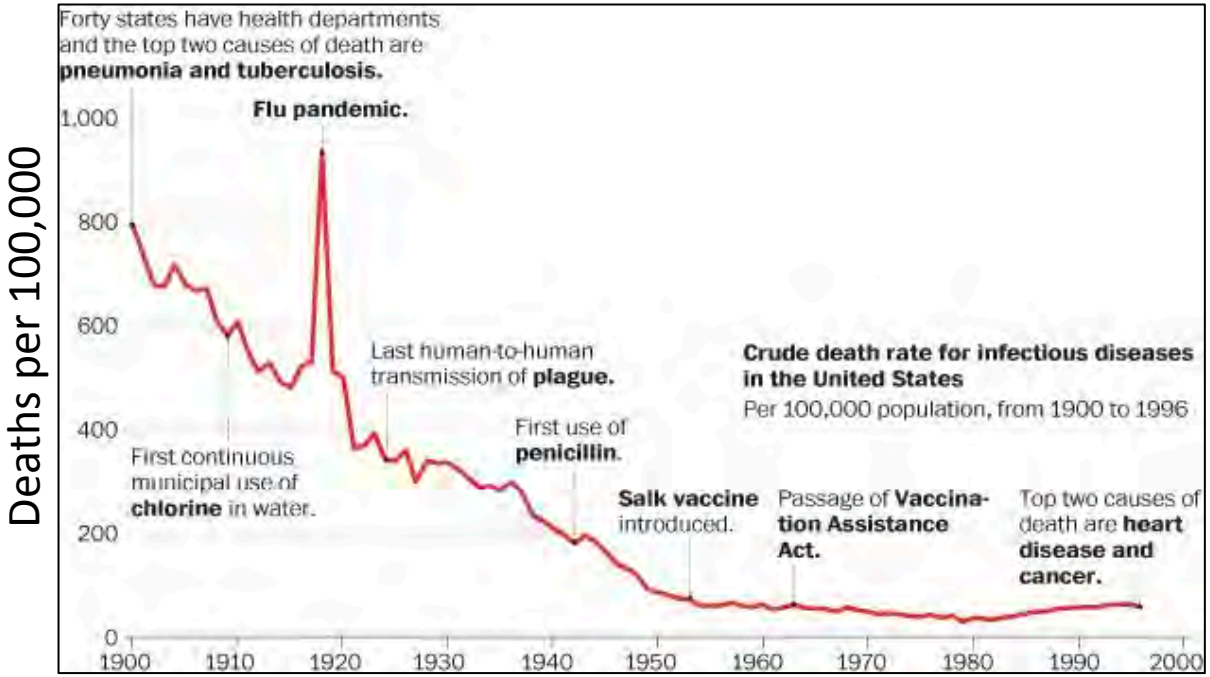
- The black plague killed nearly a third of all people on earth (up to 200 million people) from 1346-1353



- *Yersenia pestis* is the **bacterial pathogen** responsible for the black plague

Microbes make us sick, and their control is essential to medicine

- Sanitation, antibiotics and vaccines have dramatically reduced deaths from infectious diseases



The vast majority of microbes are not harmful

- Those which are harmful often get more attention
- Without microbes, earth's entire ecosystem would change drastically

Algal bloom from space

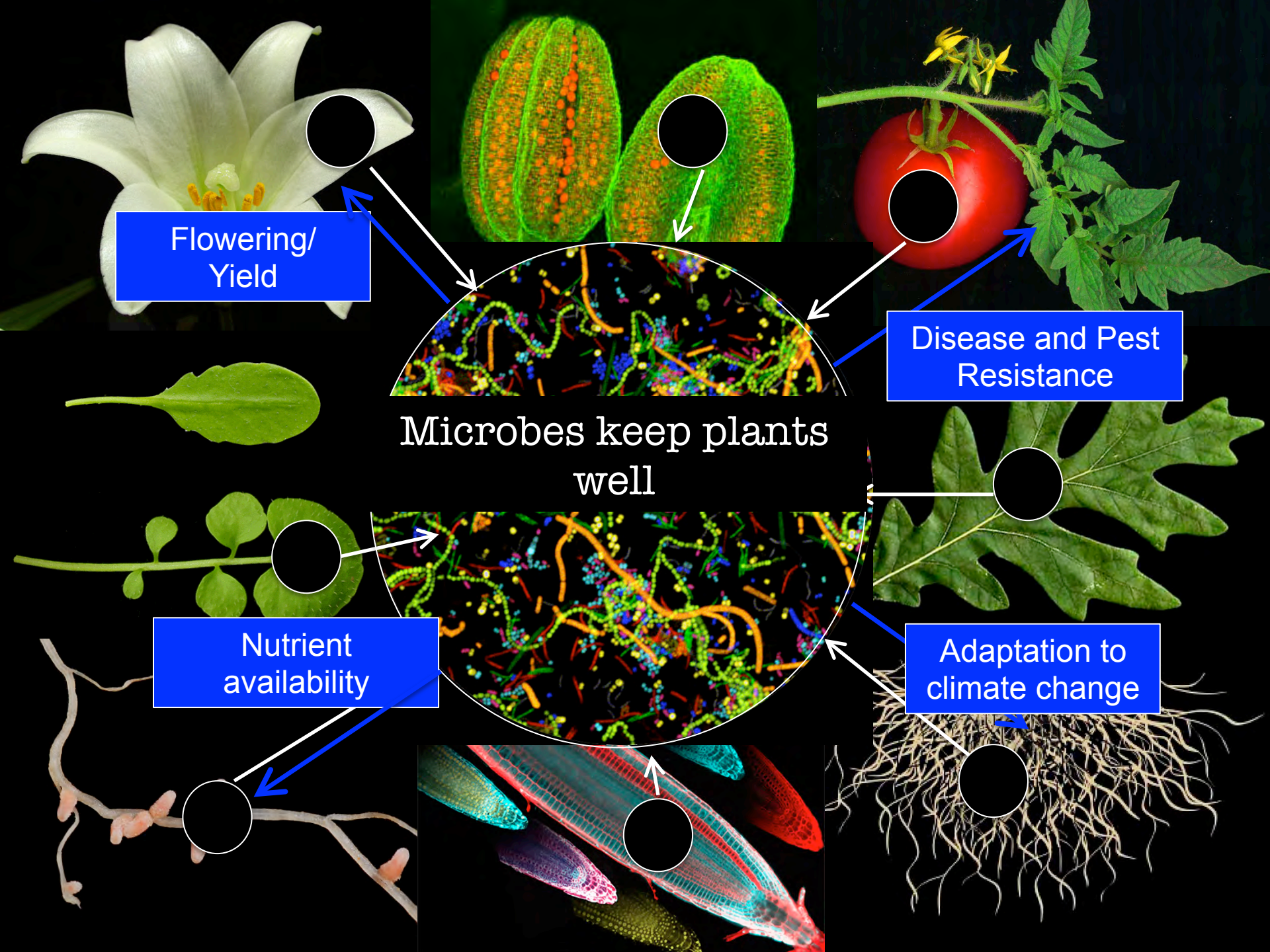
Microbes (our "microbiota") keep us well

- Each of us has our own ecosystem of microbes (our "microbiome")
- Microbes colonizing our gut are essential for normal nutrient absorption
- We have 10X more microbial cells than human cells
  - 100 trillion of our own cells and 1 quadrillion microbial cells
- **Competitive exclusion** by **normal flora** discourages the growth of **pathogens**

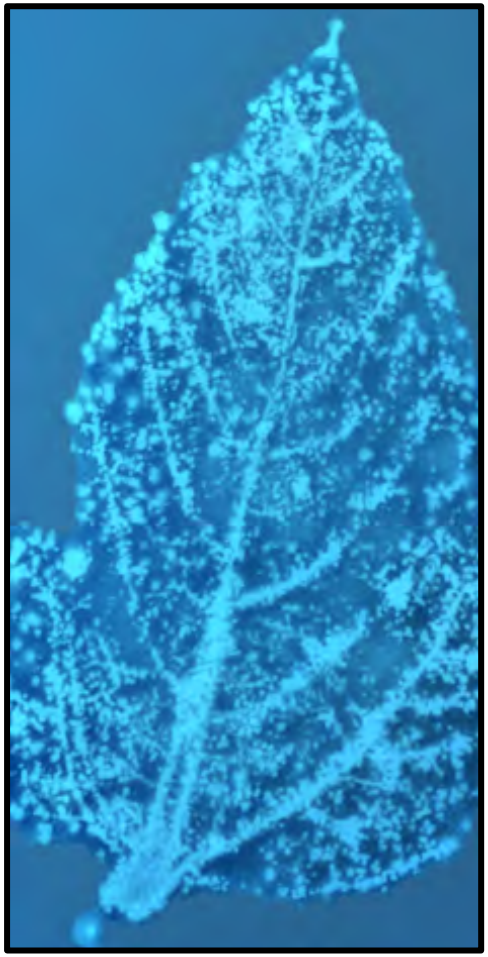
Microbes make plants sick, and their control is essential to human survival

Late blight of potatoes

- Late blight caused the Irish potato famine of 1845-1852
- Famine resulted in 1 million deaths (~12% of Irish population)
- 1 million more fled


Flowering/
Yield
Disease and Pest
Resistance
Microbes keep plants
well
Nutrient
availability
Adaptation to
climate change

If microbes keep plants and animals well, what happens when we alter these microbial communities ("disbiosis")



What happens when we alter our normal flora?

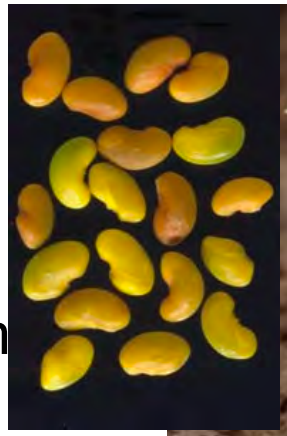
- Babies normally acquire microbes as they pass through the birth canal
- Babies born by C-section are more likely to have autoimmune issues

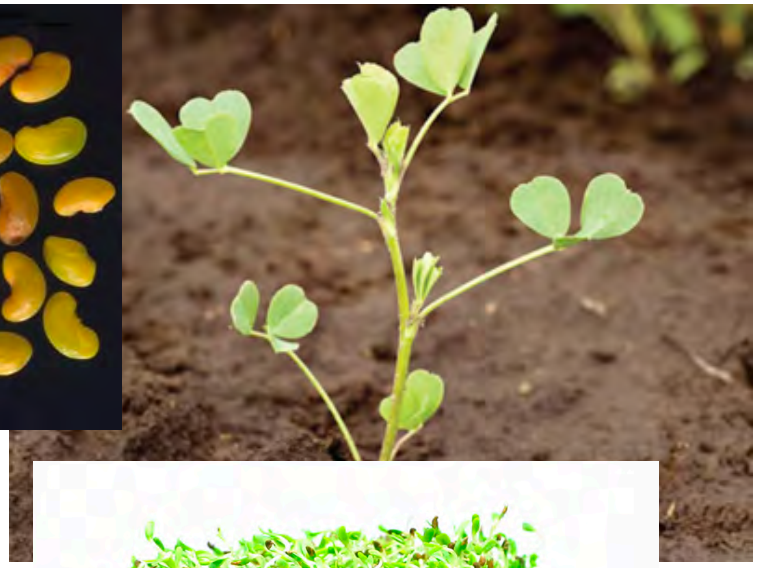
- Long-term or heavy doses of antibiotics alter our gut microbiome
- This can cause infections such as *C. diff (Clostridium difficile*).

What happens when we alter the normal flora of plants?

- Plants normally acquire a small subset of their microbiome from their parent
- Most of the plant microbiome is acquired from soil

- For sprouts, removal of microbes from seeds and absence of soil likely leaves plants vulnerable to outside infection and colonization by non-specialized microbes





How do we restore normal microbes?: Microbial transplants and *C. diff*.

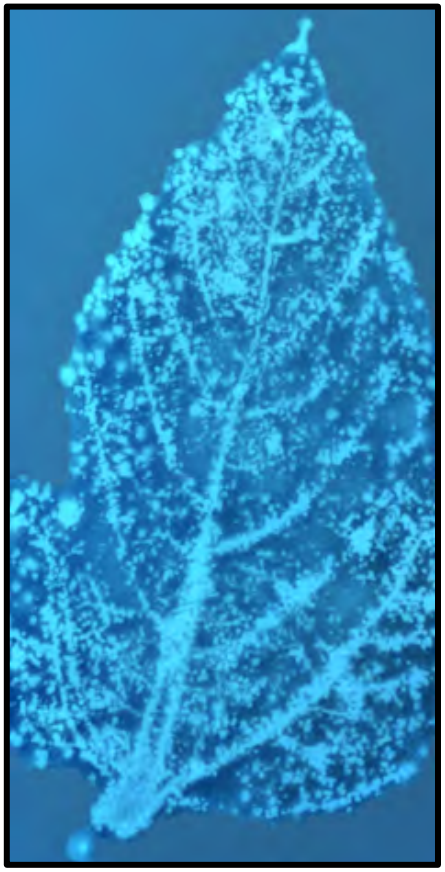
- *C. diff* is an an infection caused by overgrowth of normal gut microbes ("disbiosis")
- *Clostridium difficile* is normally kept in check by the gut microbiome.
- After antibiotic treatment, *C. diff* can get out of control
- The treatment?
  - More antibiotics? NO!
  - Competitive exclusion by good microbes? YES!

Microbial transplants for sprouts?

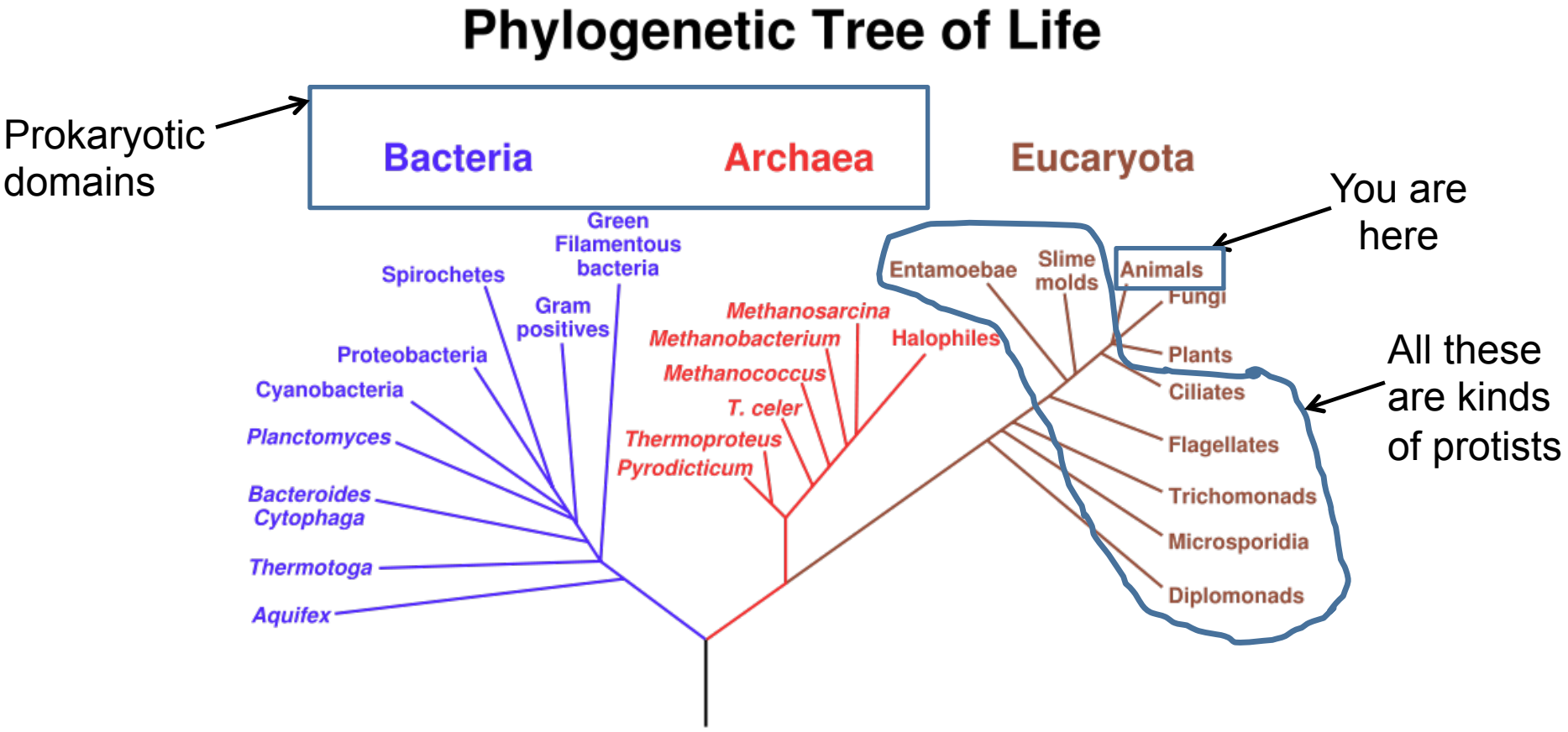
- The problem: human pathogens present on sprouts
- Is this a problem because of sprout disbiosis?
  - Sprouts never see soil and so may lack a large component of their normal microbiota
  - Some regulations agencies require sterilization of seeds
- By using microbes from healthy plants, can we prevent overgrowth of human pathogens?
  - *Salmonella* and *E. coli* are poorly adapted to plants
  - But, the goal is to eliminate these pathogens, rather than reduce their ability to cause disease on the host

The plant microbiome in plant health and disease

- What is a microbe?
- Brief history of use of microbes in agriculture
- What we've learned about the plant microbiome that might be relevant for sprouts
  - What effect does plant species or cultivar have on the microbiome?
  - How do microbes protect plants from plant pathogens?

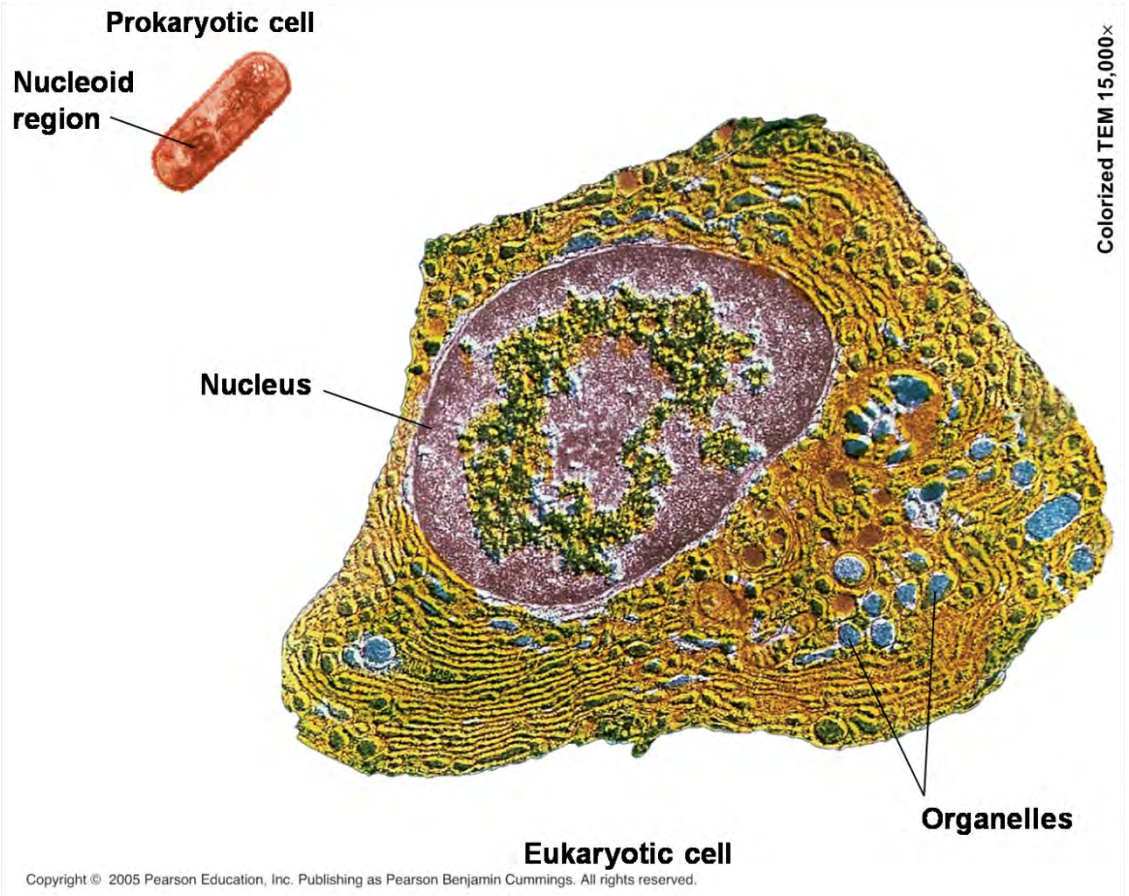


What's alive on plant roots that you can't see: the microscopic domains of life.



How are bacteria and archeal cells different from plants and animals?

- **Prokaryotic**- smaller and simpler (bacteria and archaea)
- **Eukaryotic**- larger and more complex (eukarya only)
- Eukaryotes- “you”



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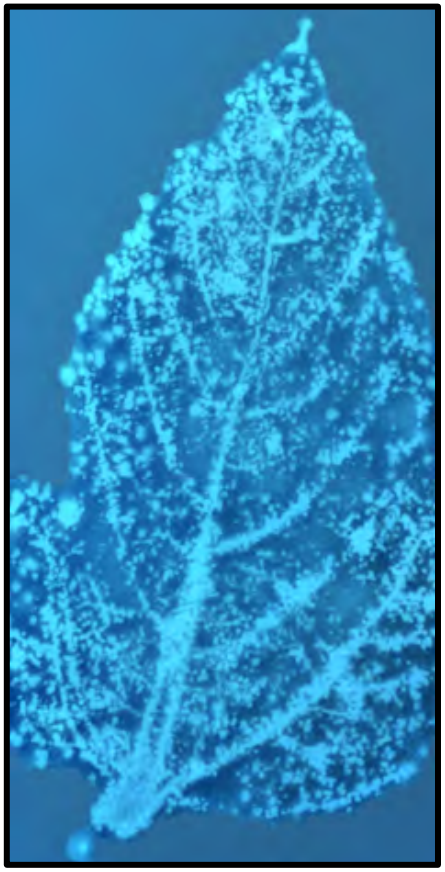
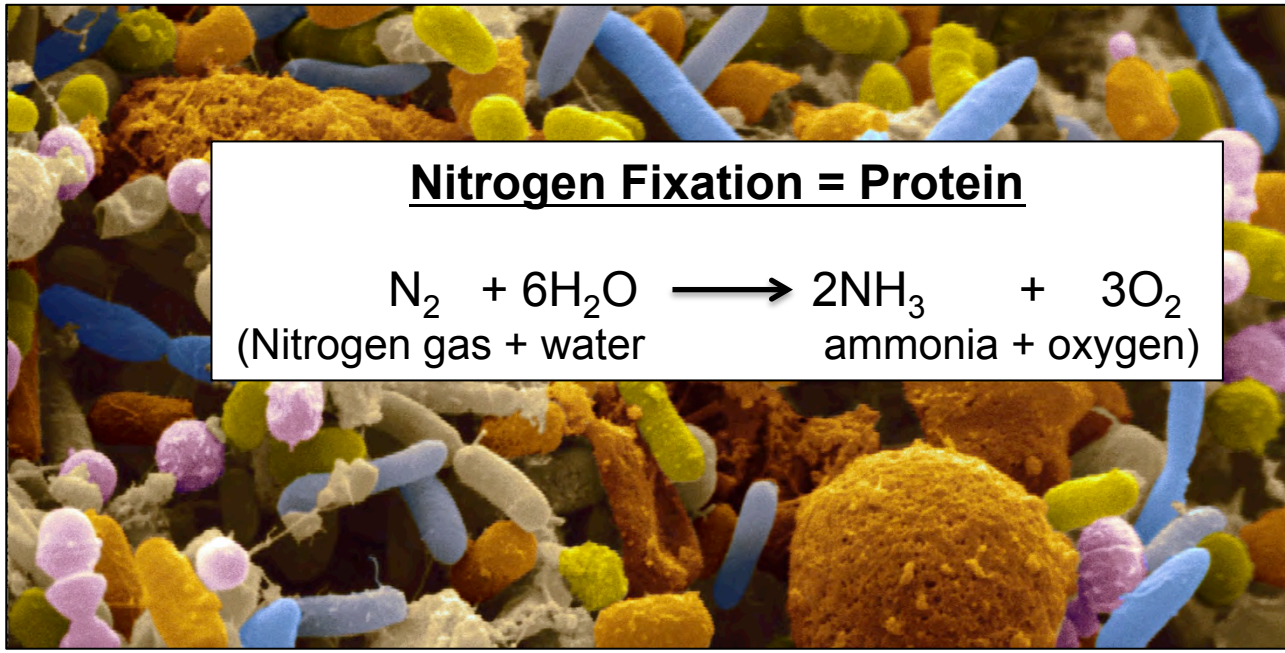


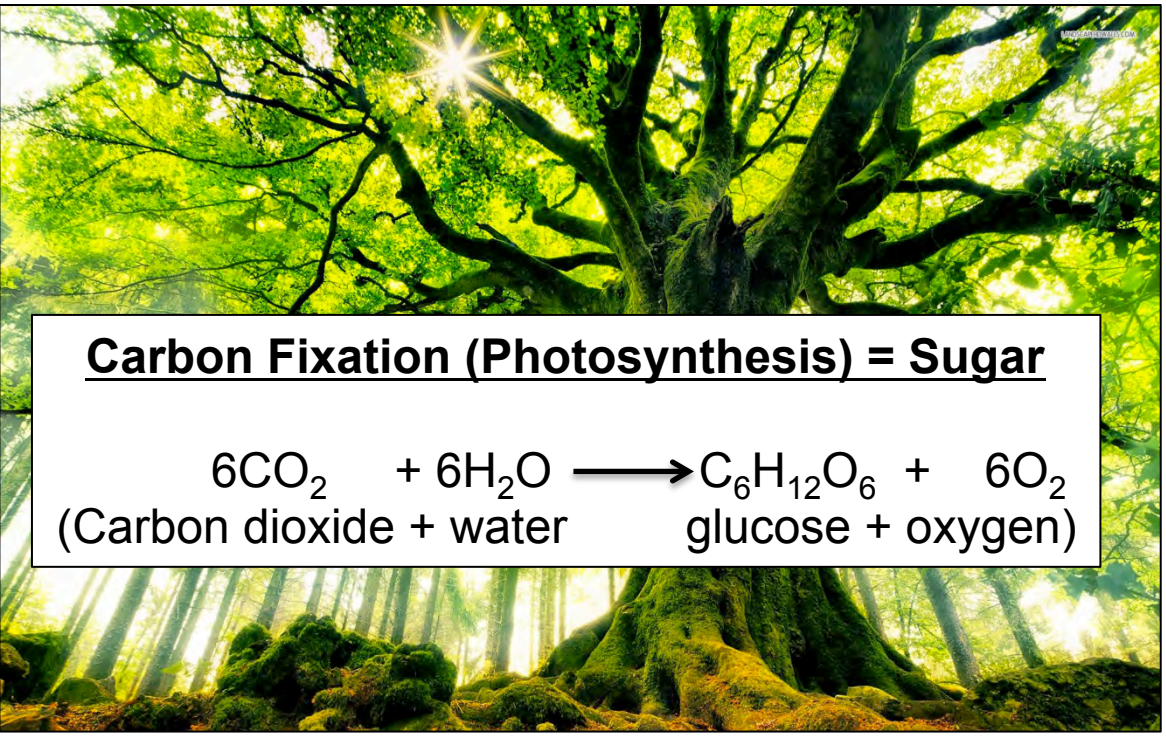
Chemical reactions



Nitrogen Fixation = Protein

$N_2 + 6H_2O \longrightarrow 2NH_3 + 3O_2$

(Nitrogen gas + water → ammonia + oxygen)



Carbon Fixation (Photosynthesis) = Sugar

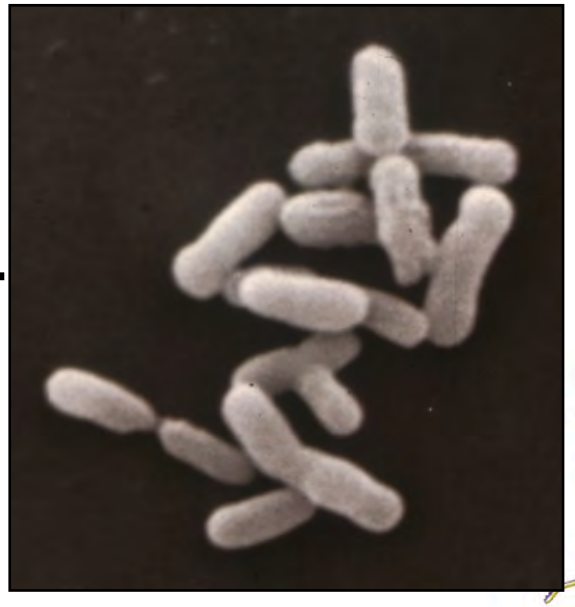
$6CO_2 + 6H_2O \longrightarrow C_6H_{12}O_6 + 6O_2$

(Carbon dioxide + water → glucose + oxygen)

that are essential to grow food

Plants and microbes have joined forces to fuel food production

+



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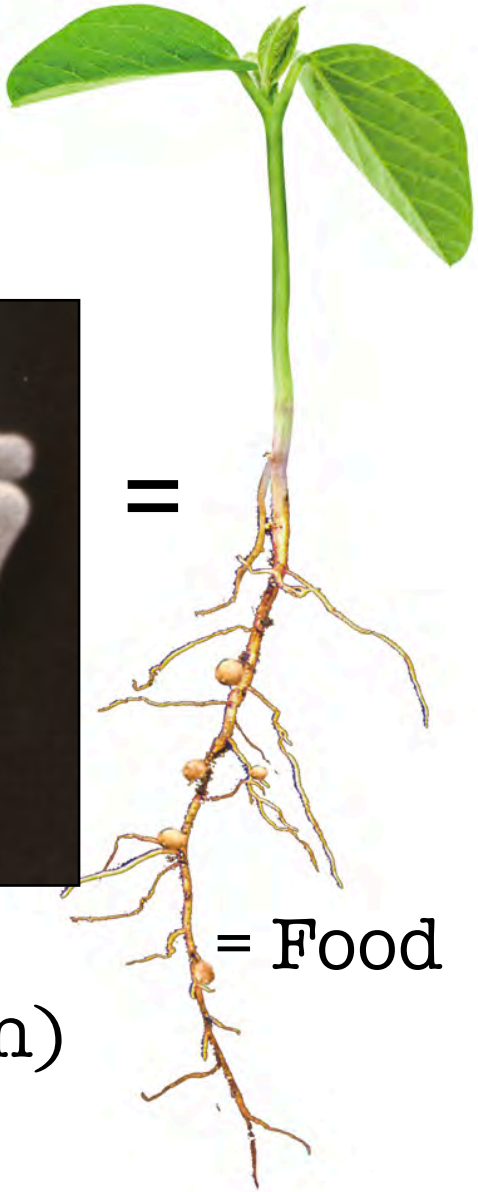


Sugar
(Fixed Carbon)

+

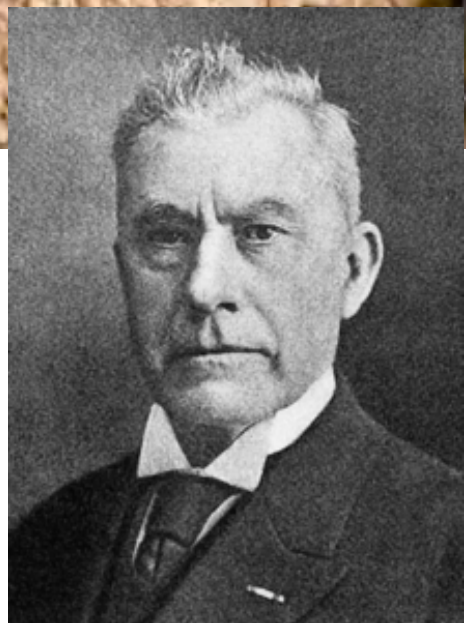
Protein
(Fixed Nitrogen)

= Food

Use of microbes to enhance agricultural production has occurred for thousands of years

- Romans as early as the 5th century BC realized that alternating legumes (bean, peas, etc) with other crops could enhance productivity.

- In 1901, Martinus Beijerinick Discovered that leguminous nitrogen fixation was driven by microbes, which he named “Rhizobium”



Martinus Beijerinick

Nitrogen fixation is limited to legumes

How can we harness this chemistry for other plants?

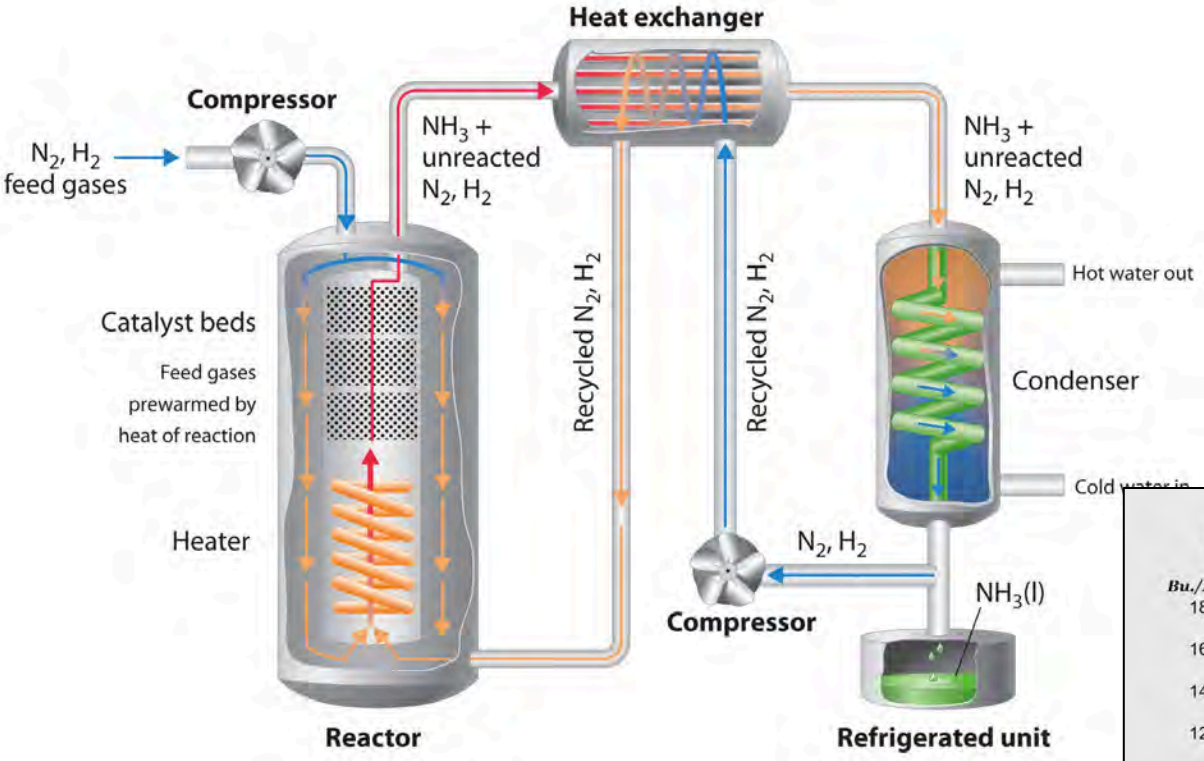
The green revolution was fueled by artificial nitrogen fixation

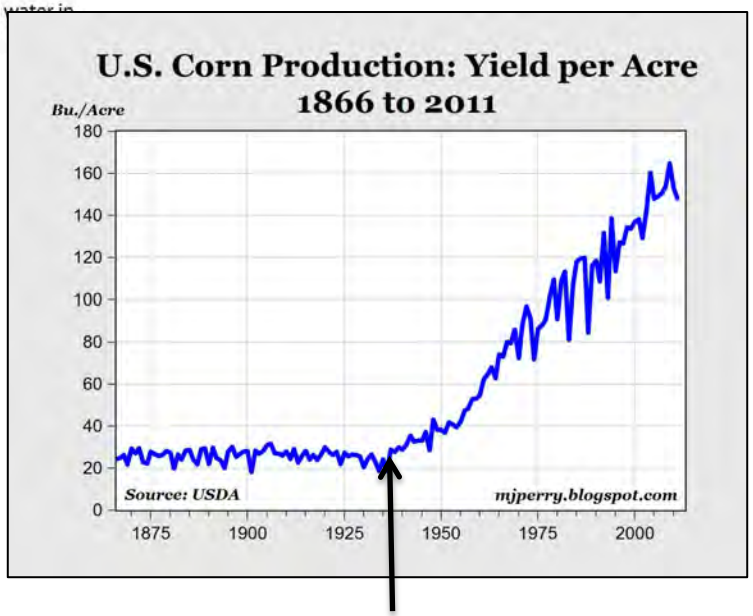
- In the early 1900s, Fritz Haber and Carl Bosch discovered an artificial method of generating ammonium from atmospheric nitrogen

- While developed for agriculture, the "Haber-Bosch process" provided the German army with ammunition during World War I

Fritz Haber

30-80% of nitrogen atoms in the human body originated from Haber-Bosch

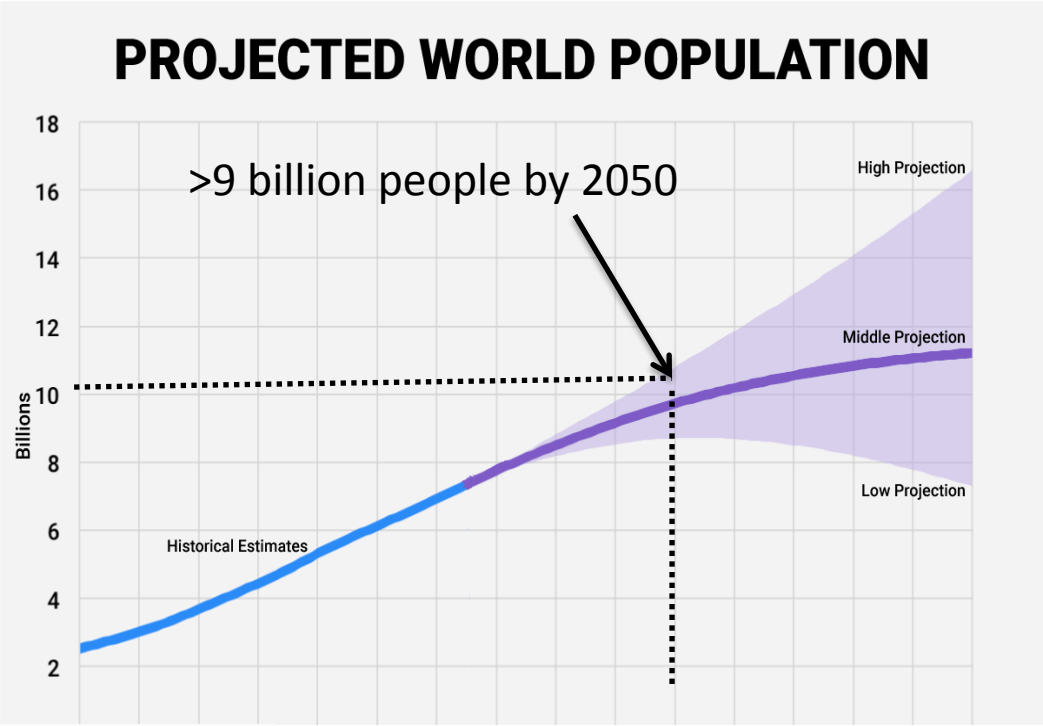


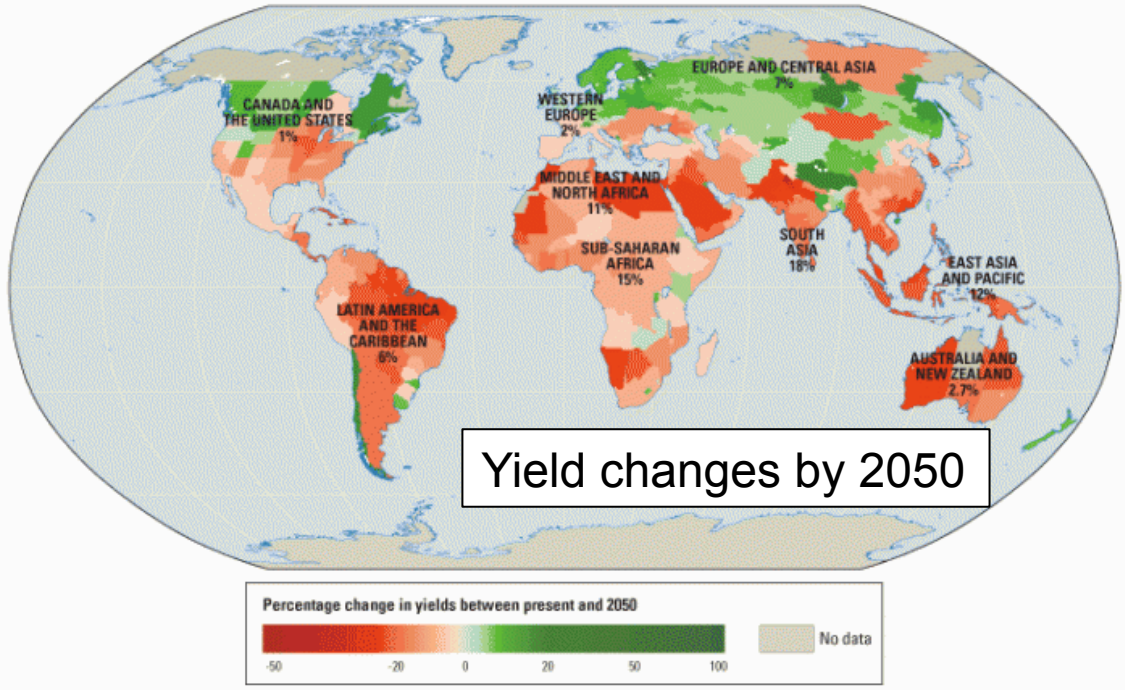


Fertilizers and pesticides are economically and ecologically expensive

- 5.2 million tons of N fertilizer applied in Canada each year costing $4.2 billion
- 1000 tons of pesticides applied in Canada each year costing $2.1 billion
- Less than half of N fertilizer is incorporated into plant tissue; the rest remains in the environment

Current agricultural practices

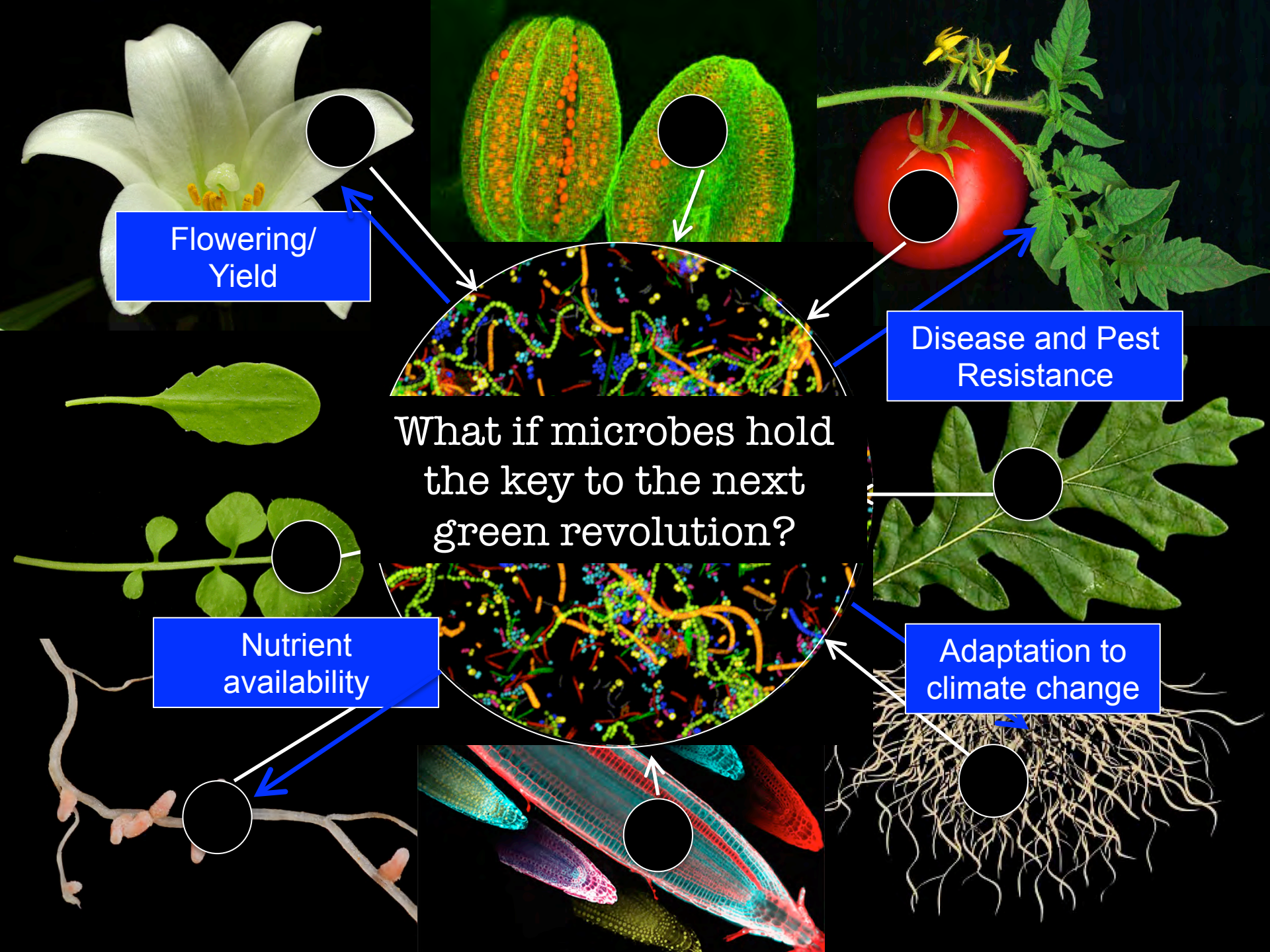




are failing to meet global food needs

We need a second green revolution that

- meets global food demands
- addresses climate change
- is sustainable
- is affordable


Flowering/
Yield
Disease and Pest
Resistance
What if microbes hold
the key to the next
green revolution?
Nutrient
availability
Adaptation to
climate change

A few microbial products in agriculture are effective

Serinade: a fungicide

Bt toxin: an insecticide

Most microbial products fail in the field

Why?

The soil has tens of thousands of microbes

Plant roots enrich for ~1000 species

An introduced microbe must:

- Compete in the soil
- Compete in the plant rhizosphere
- Be compatible with the plant species/cultivar
- Adapt to local climate

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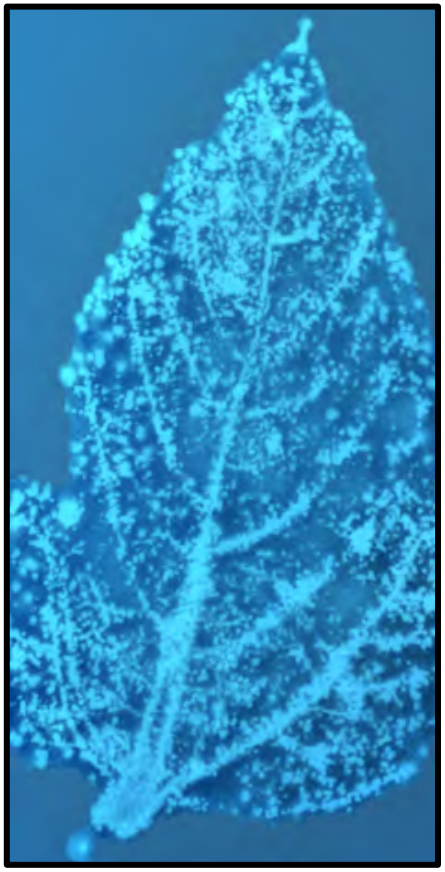
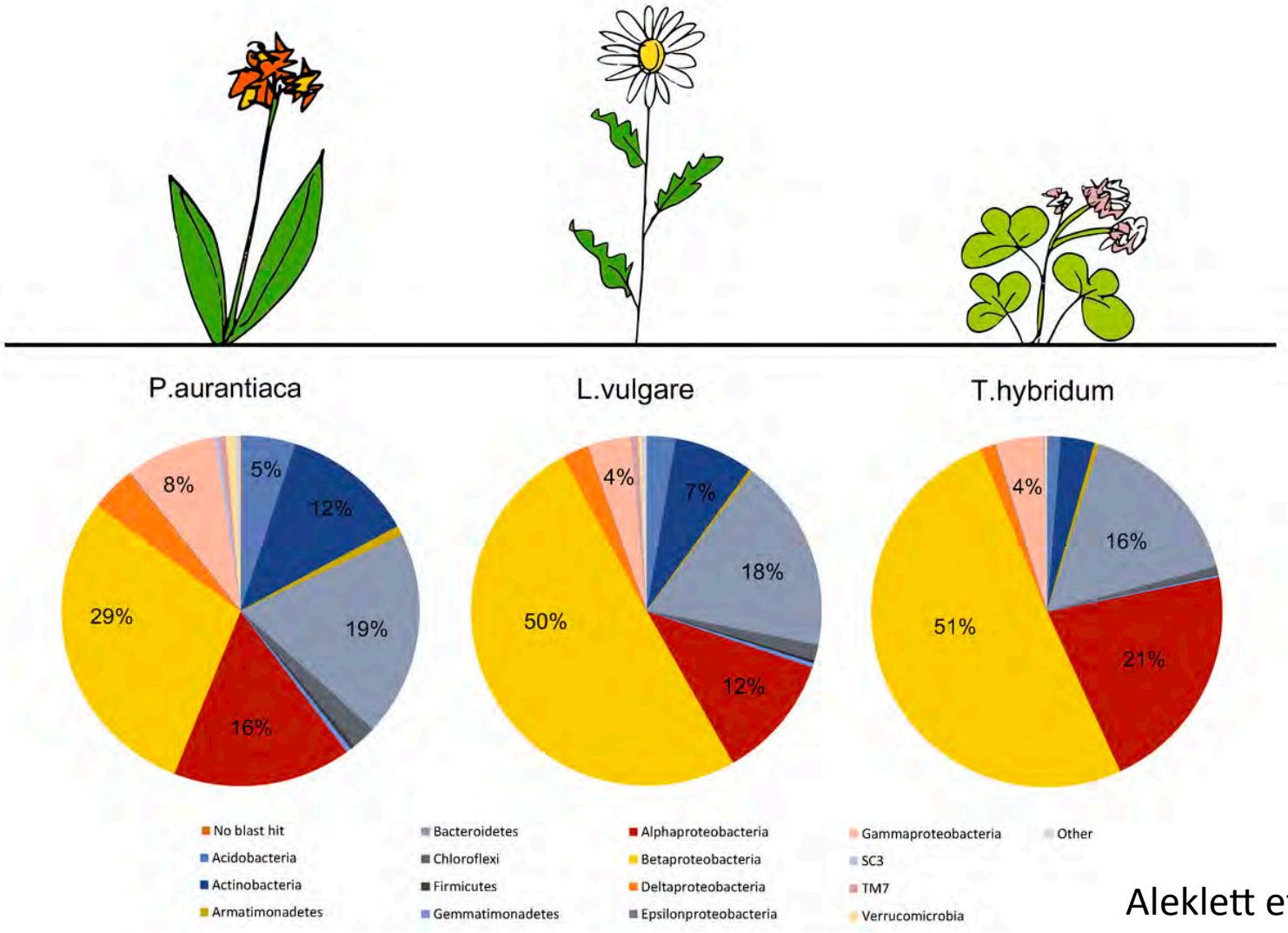
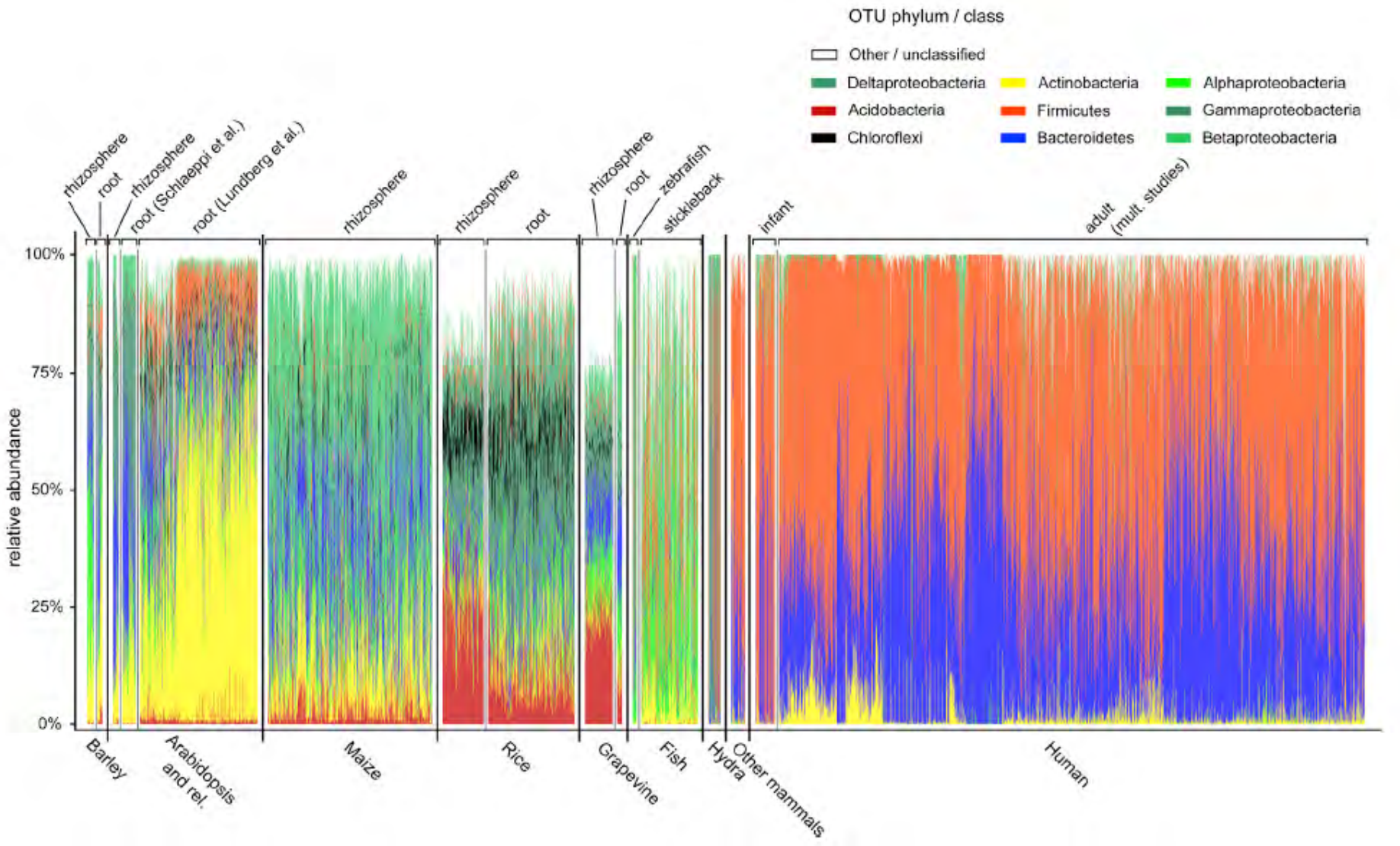


Modern DNA sequencing methods allow us to ID all microbes in a given environment



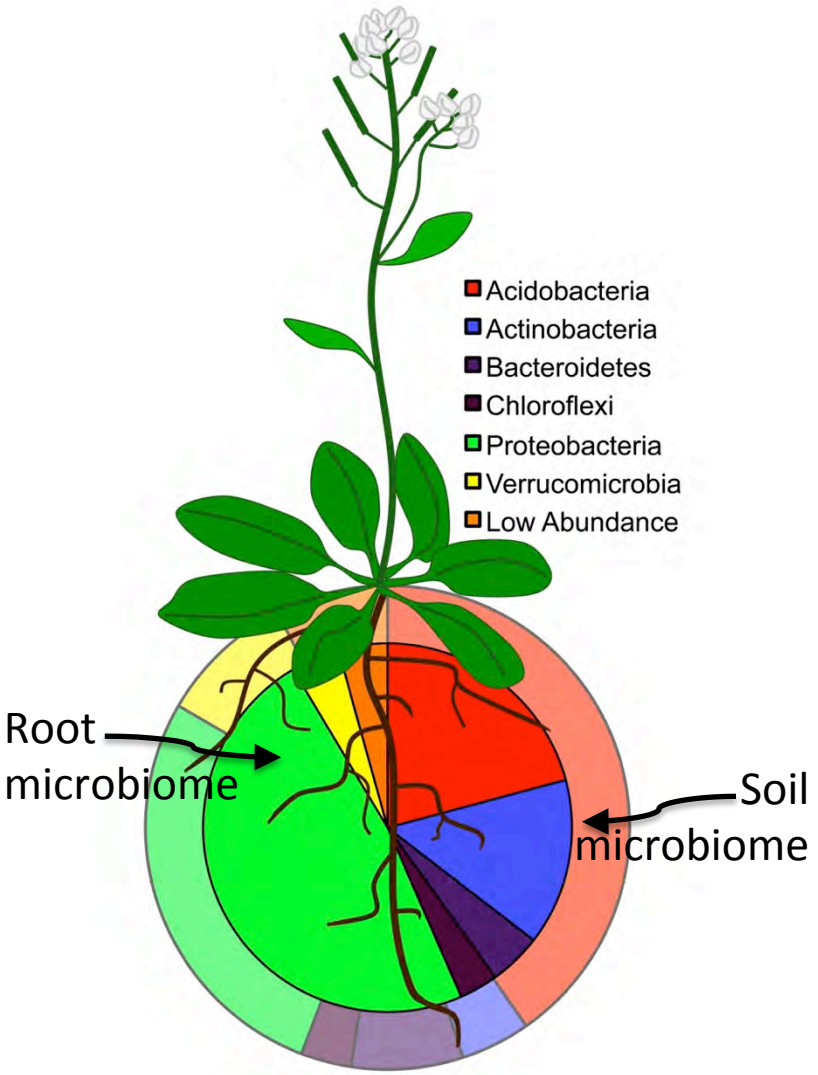
Aleklett et al. 2015

Plant root and animal gut microbiotia look very different



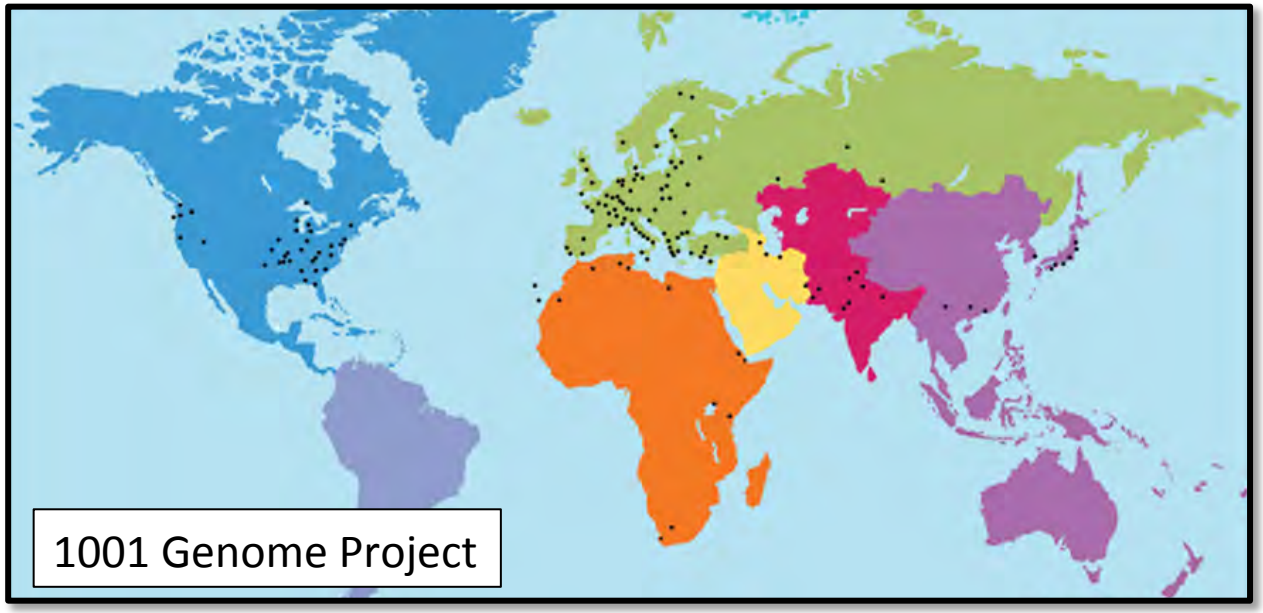
Understanding the complexity of the plant microbiome

- Rhizosphere community is consistent; 10^3 bacterial species/ plant
- Largest effect is the environment; small but significant genetic effect
- Naturally colonized by culturable bacteria (*Pseudomonas, Bacillus*, *Streptomyces*)
- Single strains are sufficient to confer benefits

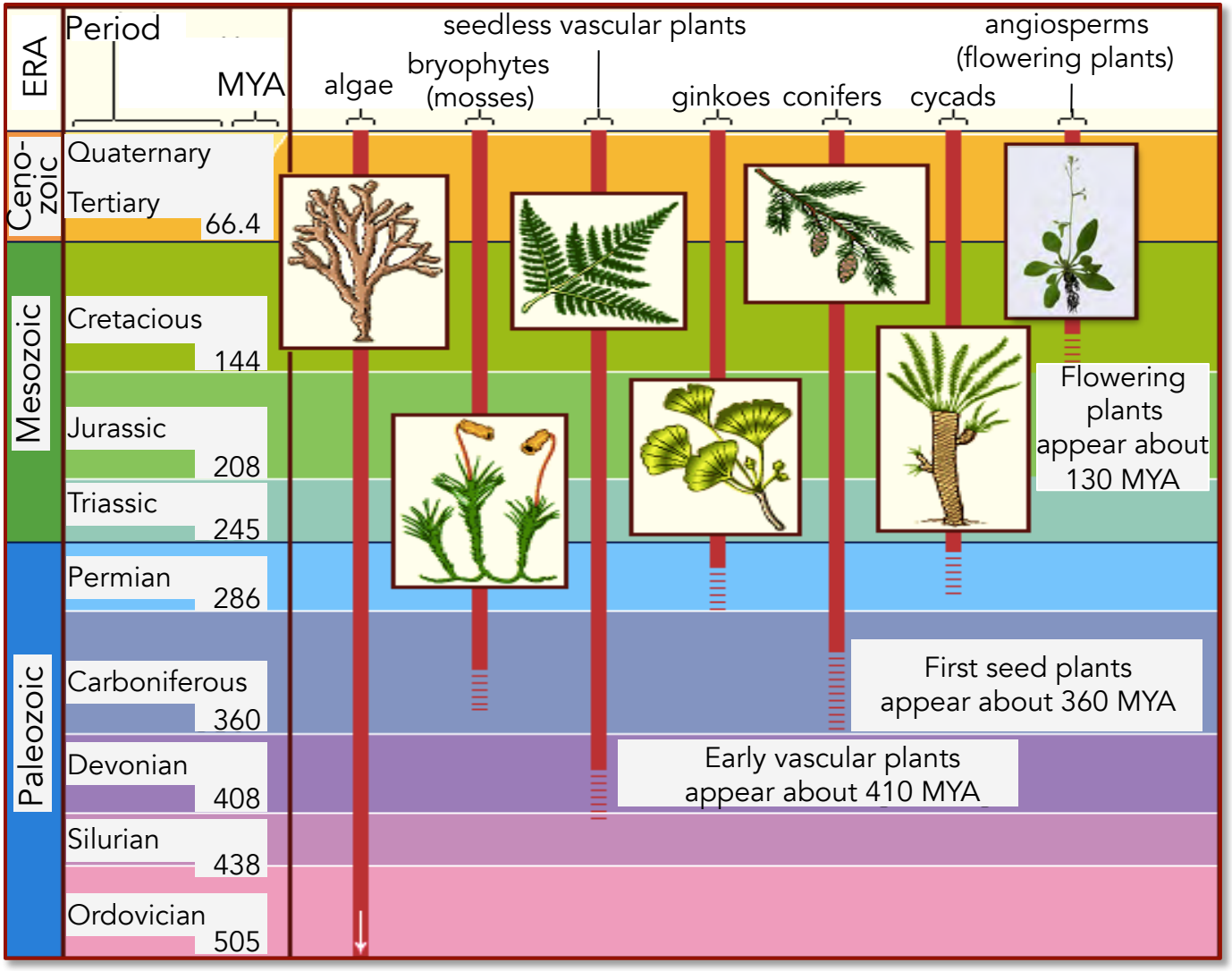


We use a wild brassica, *Arabidopsis thaliana*, for microbiome studies

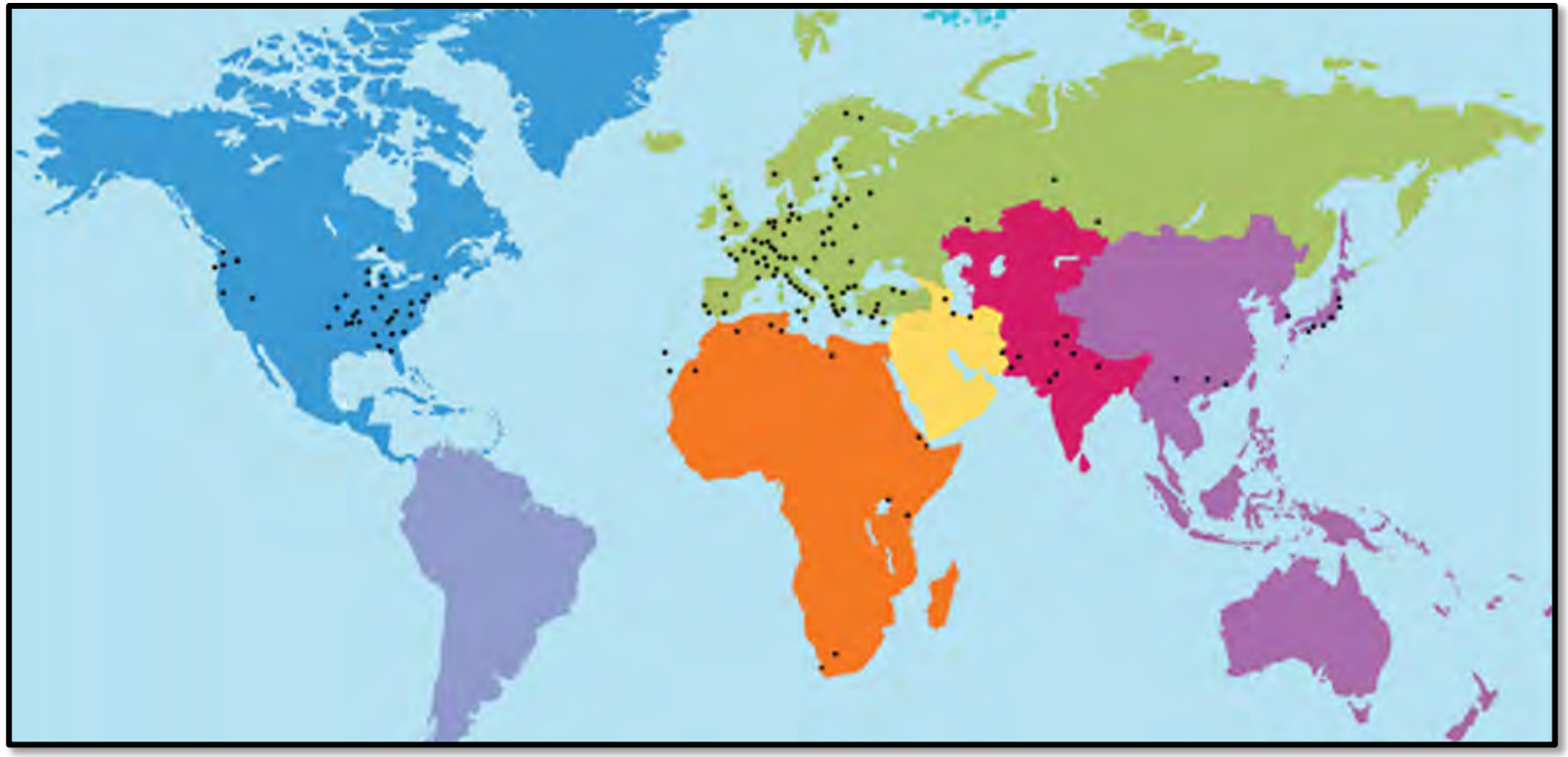
- Excellent system for genetics (the “fruit fly” of the plant world)
- Similar to other angiosperms – validated “reference” plant
- Lots of natural variation (wild “accessions”)
- Wild species



Angiosperms Appeared about 130 MYA and Radiated Rapidly

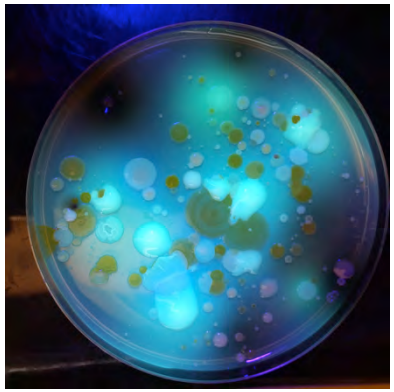


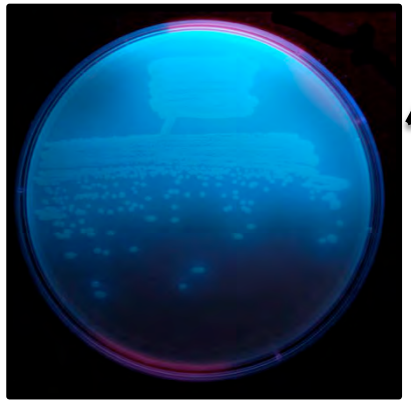
Do different varieties ("ecotypes") of Arabidopsis have different associations with microbes?

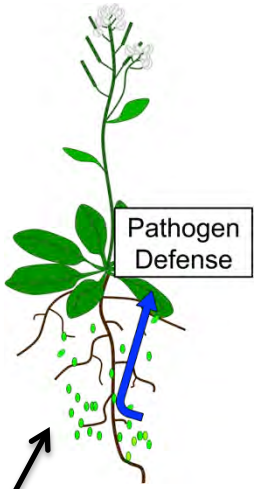


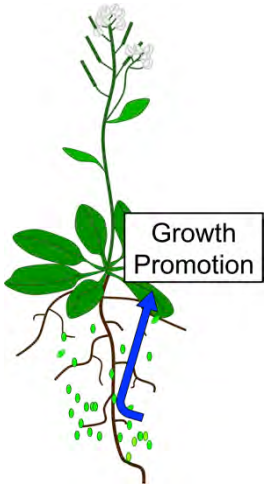
Could differences in crop varieties explain why microbial treatments fail in the field?

We perform microbial transplants from wild plants to those in the lab

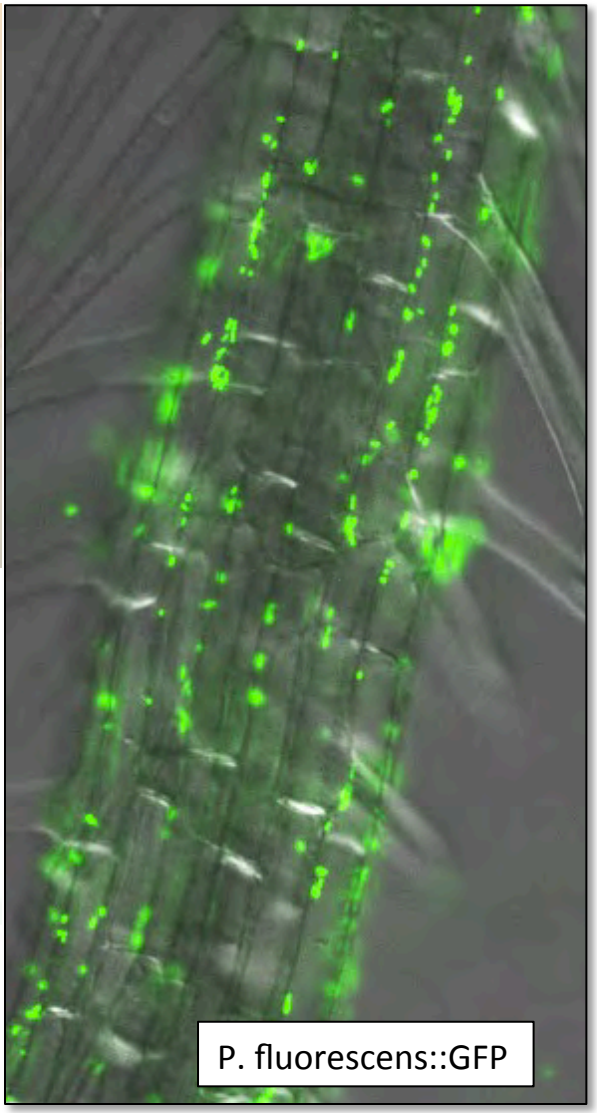


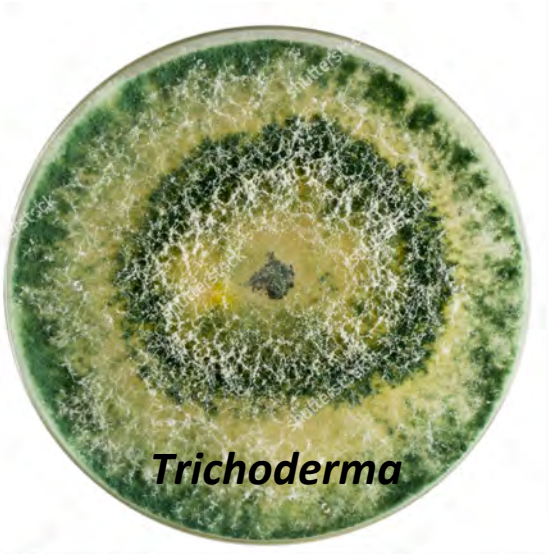






~50% of plant-associated microbes are readibly culturable in the lab

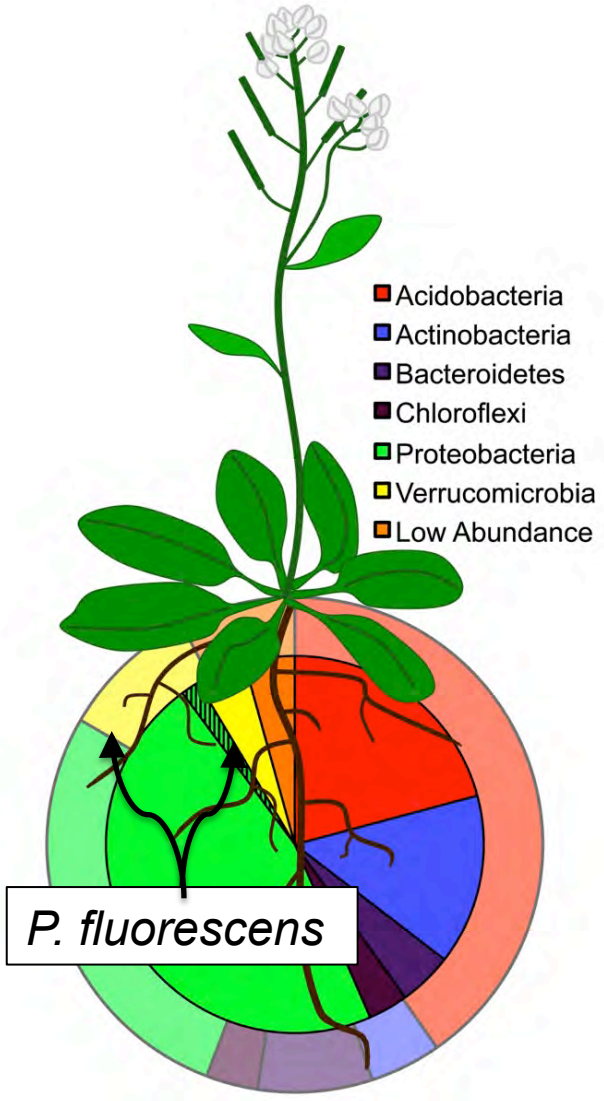




Arabidopsis-*Pseudomonas fluorescens* as a model for microbiome studies

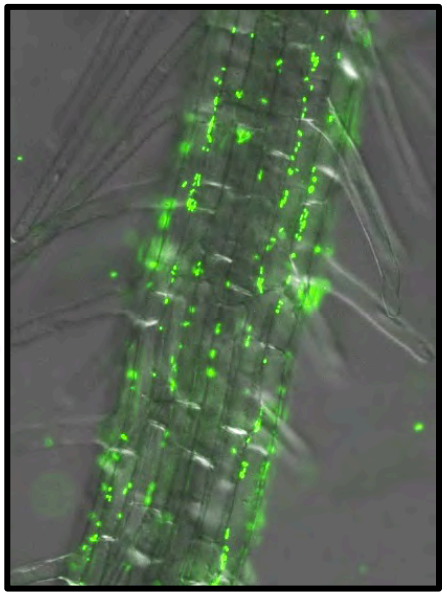
P. fluorescens:

- is well adapted to the plant rhizosphere
- is beneficial to plants
  - protects from pathogens
  - promotes growth



Automated quantification of *P. fluorescens* growth in the rhizosphere

Arabidopsis plants are grown in 48-well plates

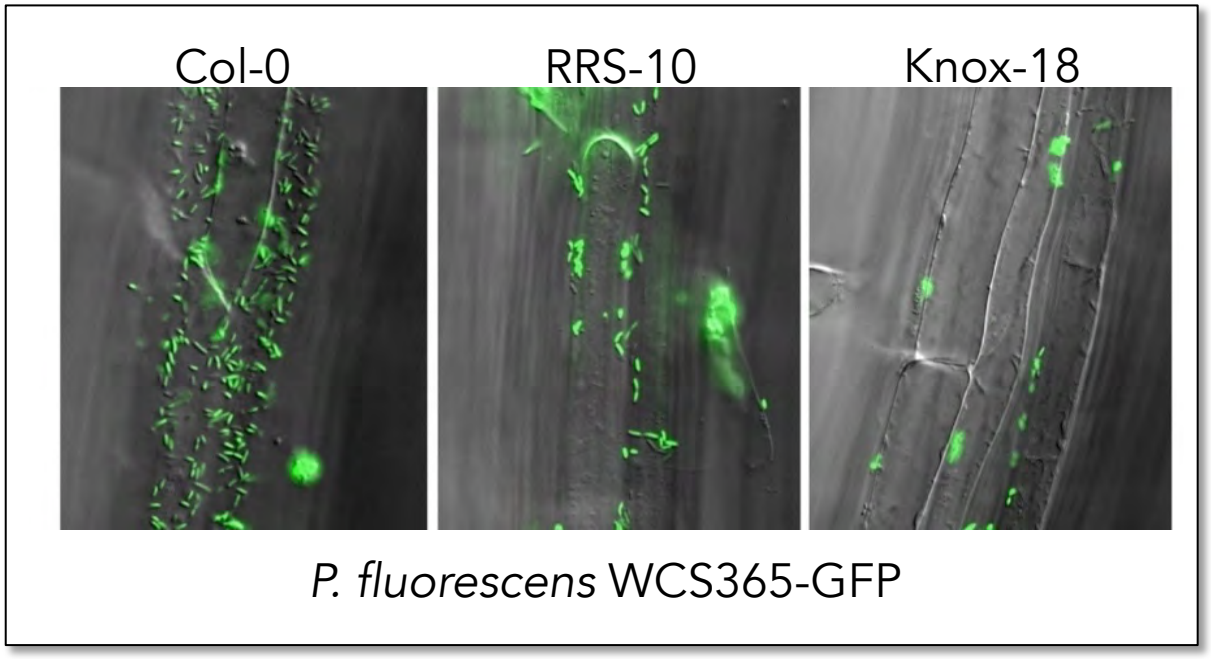


Seedlings are inoculated with *P. fluorescens*-GFP

Plates are read from the bottom with a fluorescent plate reader

Haney et al. (2015) *Nature Plants*

Some varieties of Arabidopsis can't support growth of this beneficial bacterium



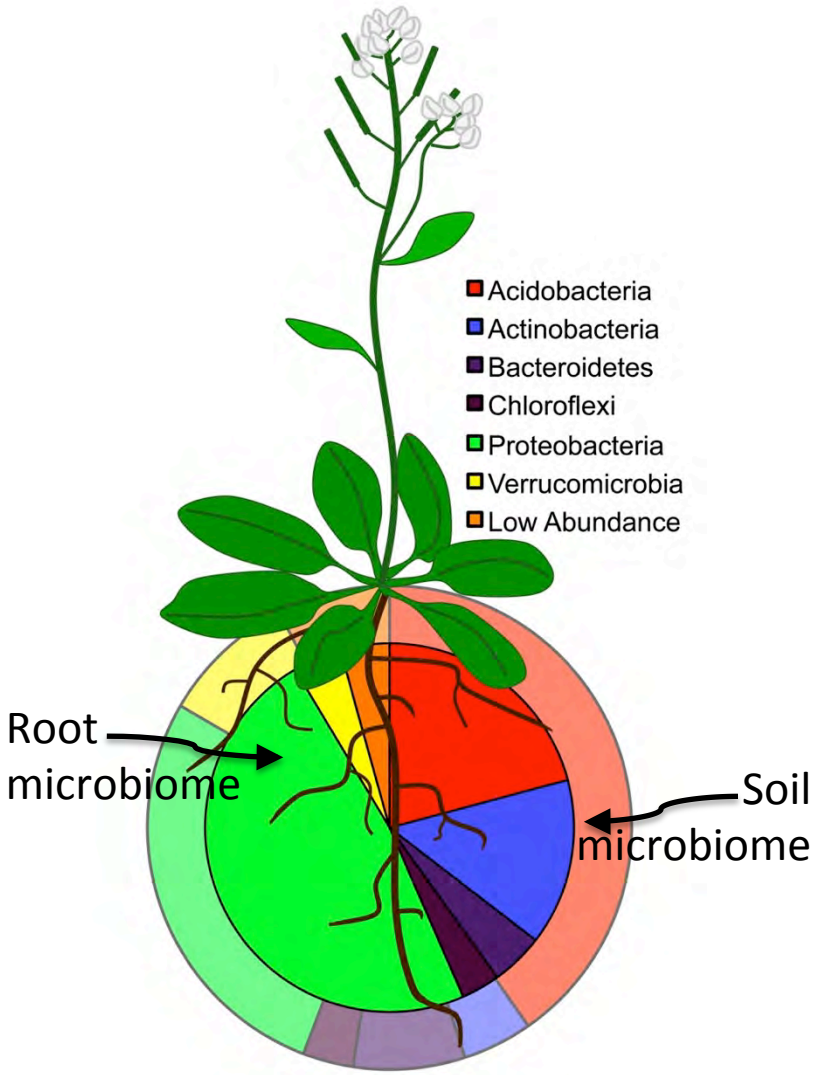
Cara Haney, *Nature Plants*, 2015

The presence of this beneficial bacterium can protect from a plant pathogen

Many bacterial strains can protect from pathogens

The effect of plant species and the microbiome

- Not all beneficial microbes can colonize even closely related plants
  - The same microbes may not work on alfalfa and radish
- Colonization is essential to get protection from plant pathogens



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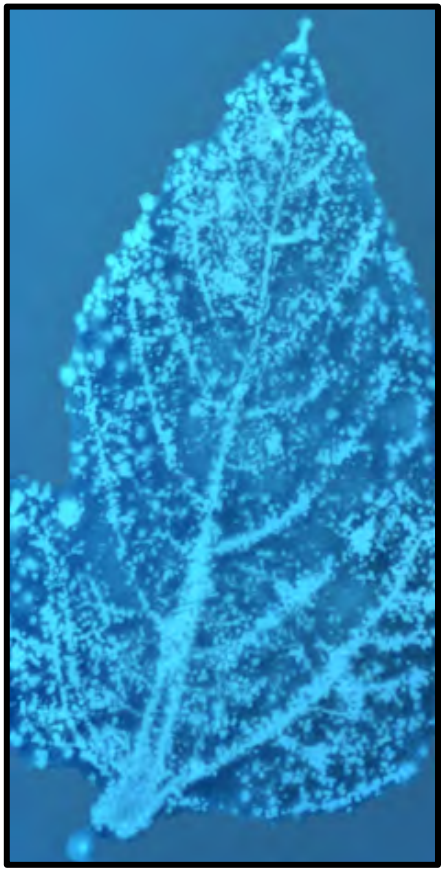
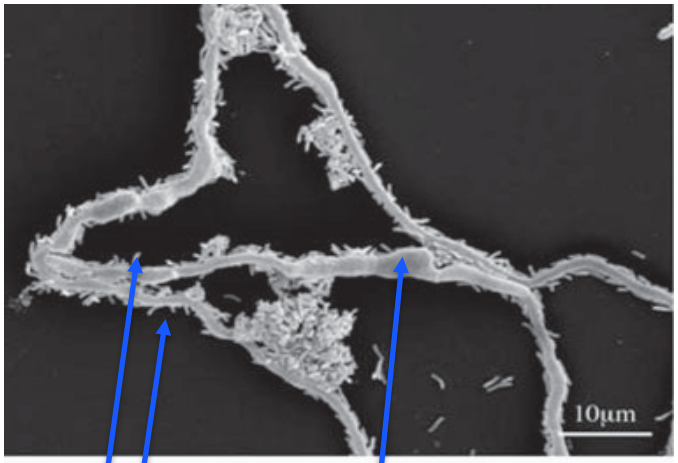
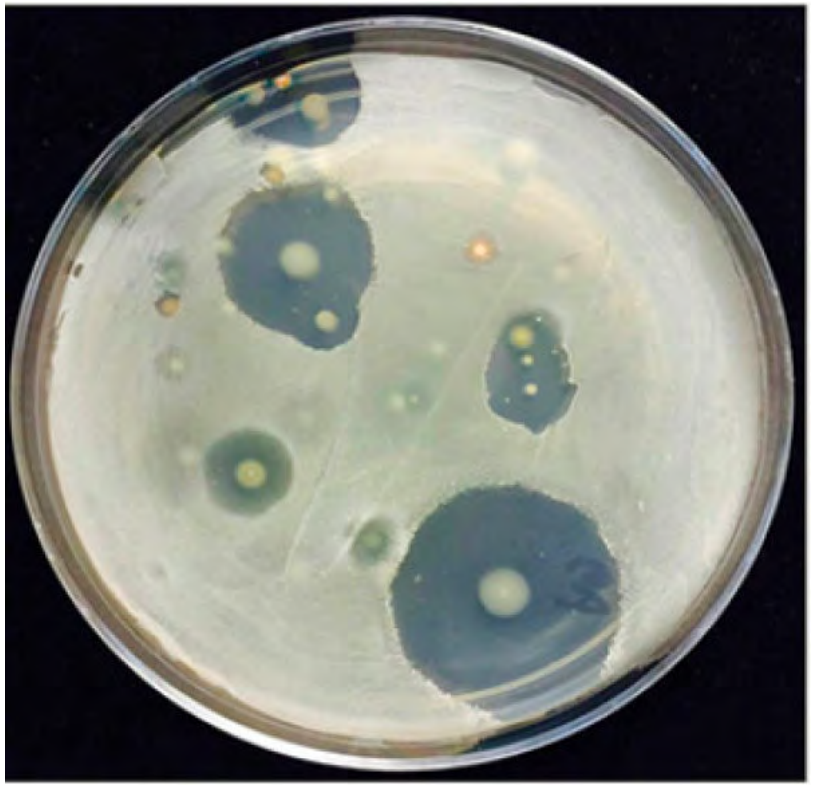


How do microbes protect plants from plant pathogens?

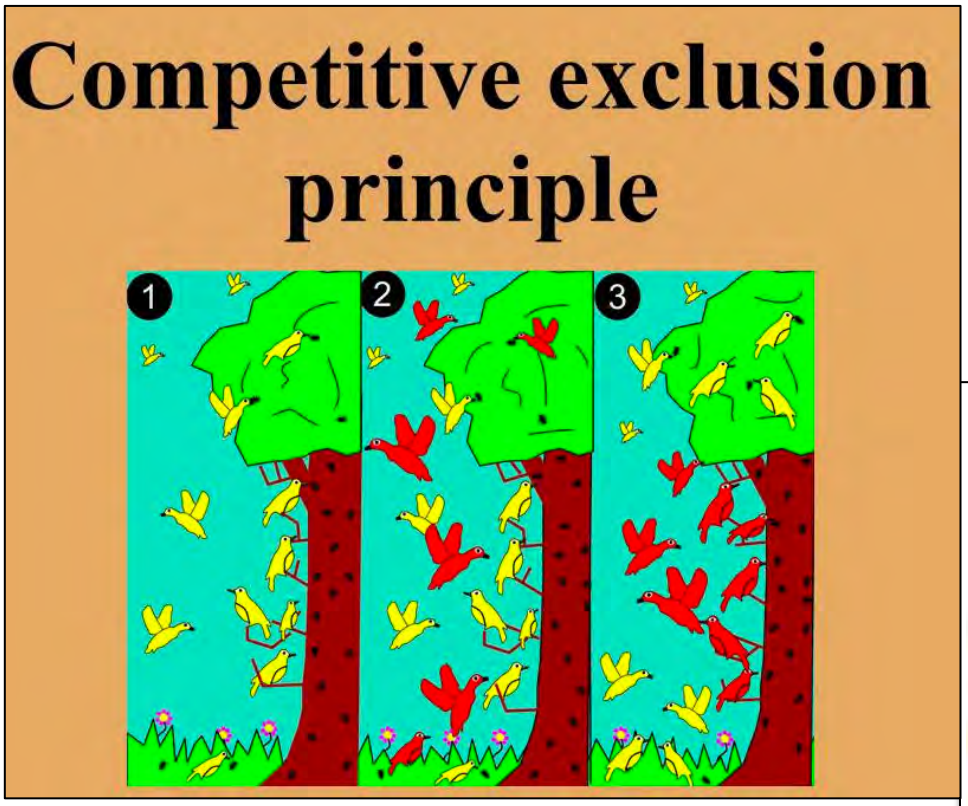
- Production of antimicrobials
- Competitive exclusion
- Modulation of the plant immune system

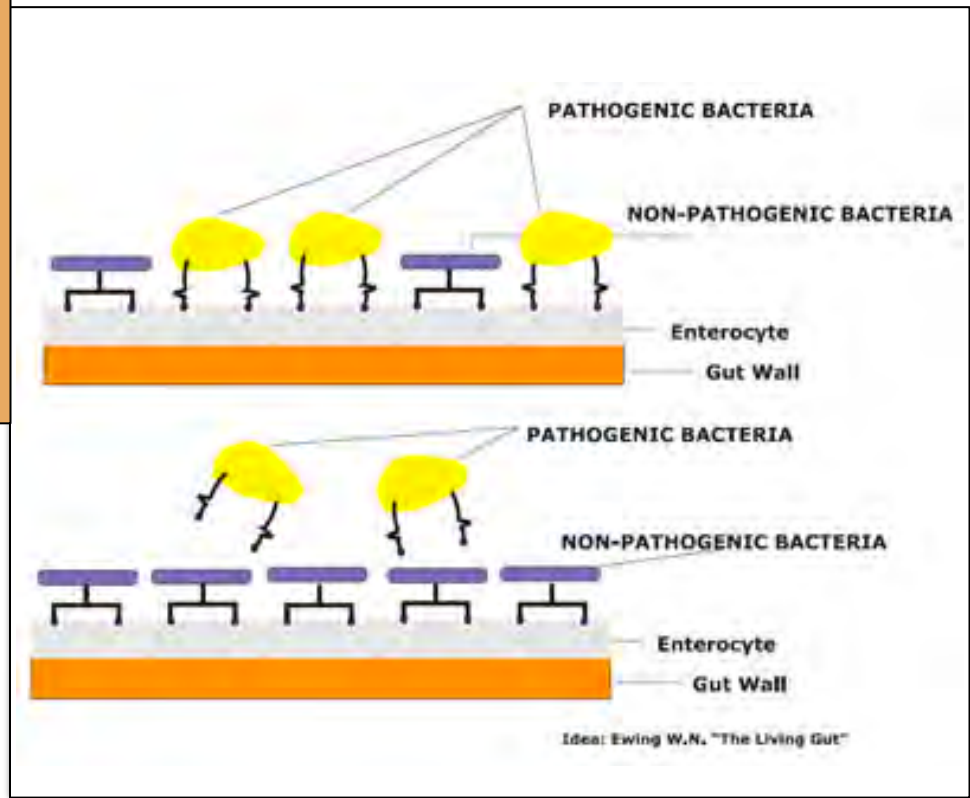
Production of antimicrobials/direct killing





Competitive exclusion





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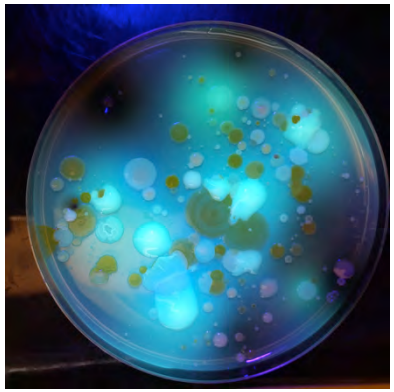
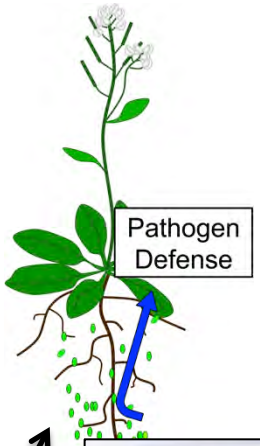
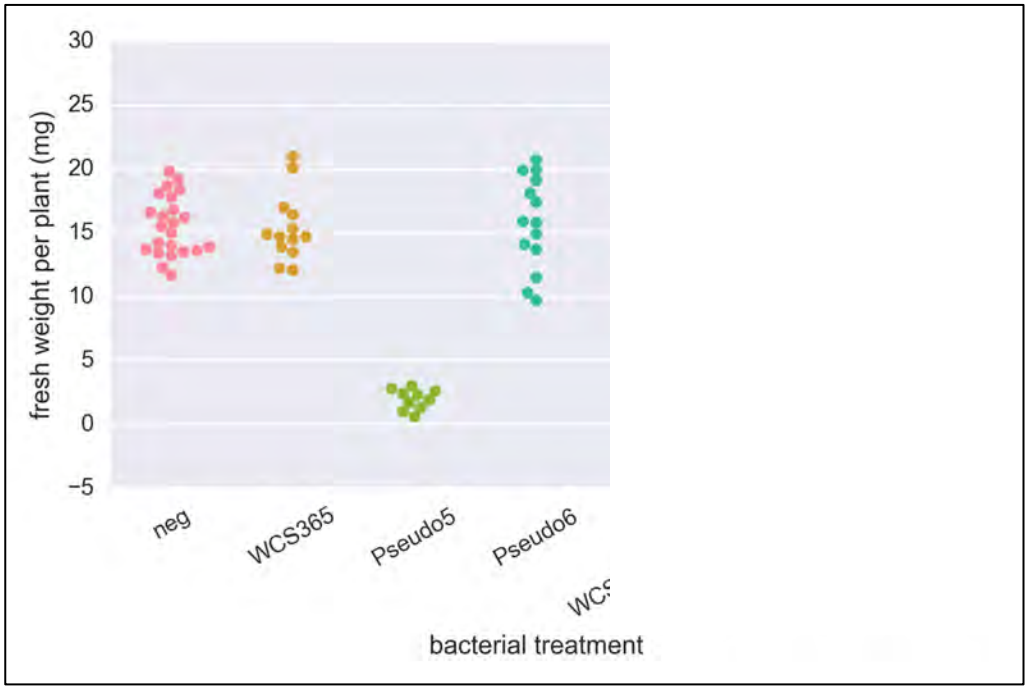




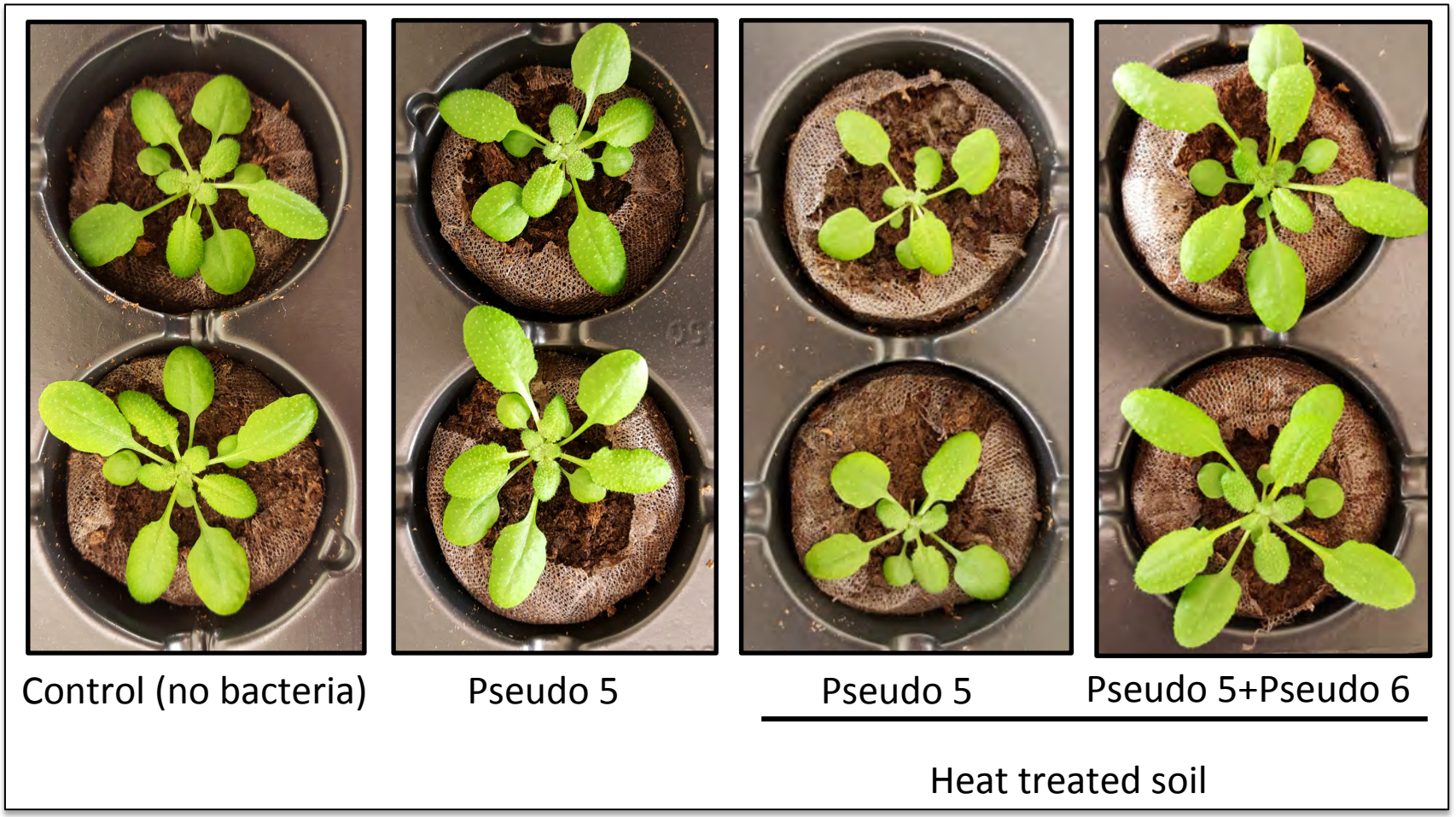
The plant microbiome in the lab: the good and the bad

- In the absence of a protective microbiome, “harmless” microbes can be pathogens
- A single good microbe can protect from bad ones



Ryan Melnyk

Microbes in soil offer some protection from potential pathogens



Ryan Melnyk

The soil has tens of thousands of microbes

Plant roots enrich for ~1000 species

An introduced microbe must:

- Compete in the soil
- Compete in the plant rhizosphere
- Be compatible with the plant species/cultivar
- Adapt to local climate

Microbial transplants/probiotics for sprouts?

- Compared to soil-grown plants, sprouts are relatively controlled
  - No soil
  - Environment is controlled

- What are the native microbes in sprout seeds?
- Do these offer protection from introduced human pathogens?
- Can we develop a microbial probiotic cocktail to give sprouts some protection?

Thanks!

Haney Lab

Ryan Melnyk
Joël Richard
Christina Weismann
Frank Liu
Polina Beskrovnaya
Sarzana Houssain
Gloria Han

Collaborators
Fred Ausubel
Buck Samuel
Jenifer Bush

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