

# **MANAGING SOIL AND PLANT MICROBIOMES FOR BETTER CROP AND HUMAN HEALTH**

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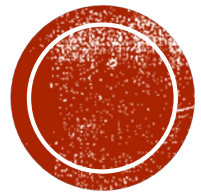
Purdue University



# OUTLINE

- ❖ What is a microbiome and why should we care?
- ❖ What factors are most critical in shaping microbiomes and the services they provide?
- ❖ Examples of what we've learned in my lab about how to manage root microbiomes and the implications of these microbiomes on crop health, quality and safety.
- ❖ Questions





# MICROBIOMES



# WHAT IS A MICROBIOME?

- **Definition:** the community of microorganisms that can usually be found living together in any given habitat



## Functions include:

- Aiding digestion
- Supporting our immune systems
- Producing essential vitamins
- Protection against pathogens
- Positively impacts mental health



# SOIL MICROBIOMES

## Functions include:

- Nutrient cycling & uptake
- Soil aggregation
- Water retention & uptake
- Pathogen suppression
- Pollutant sequestration and detoxification
- *Support our mental health*



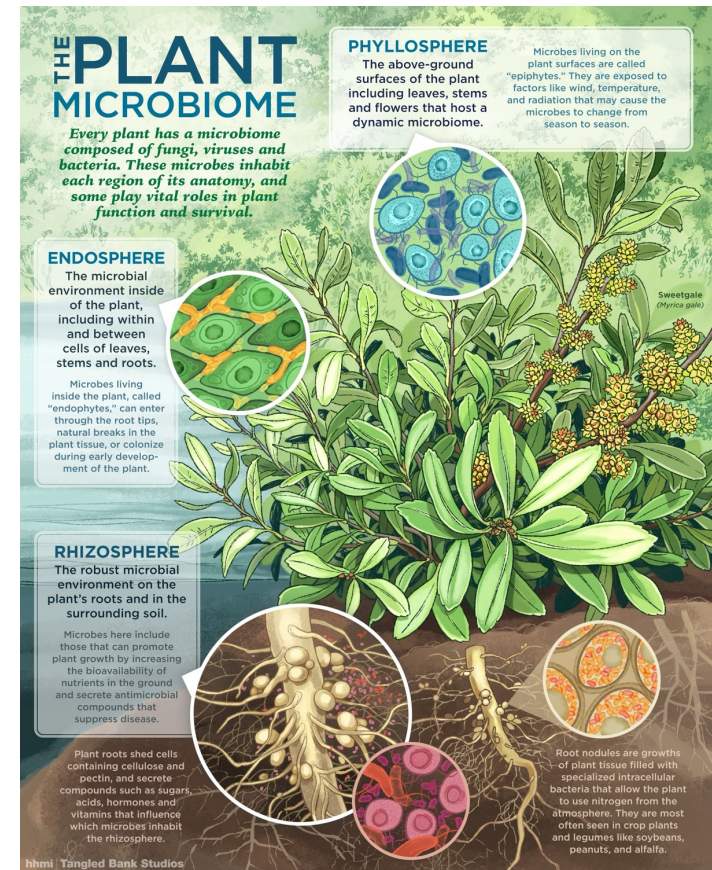
<https://mother-nature.ca/the-mental-health-benefits-of-gardening/>

***The most abundant and diverse microbial habitat on the planet***



# PLANT MICROBIOMES

- ❖ Plants are literally covered, inside and out with microorganisms
  - > *phyllosphere* (leaf/stem/fruit surfaces)
  - > *rhizosphere* (on the surface of roots)
  - > *endosphere* (inside the plant)
- ❖ More microbes than plants (total # of microbial cells actually outnumber those of the plant)
- ❖ **What are these microbes doing?**



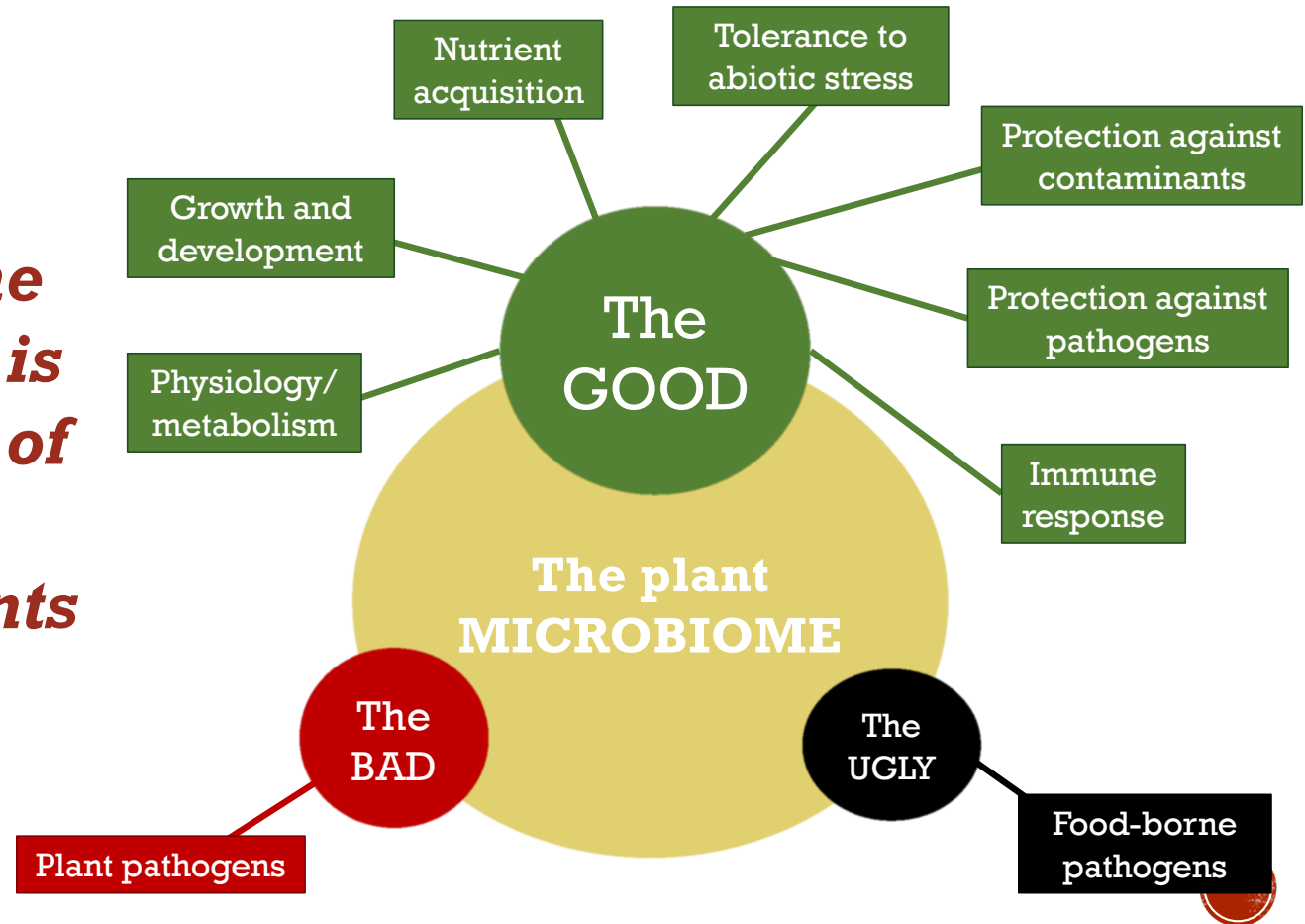
<https://imgur.com/gallery/dGmS3qy>

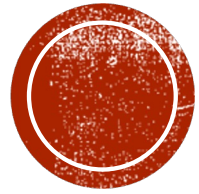


# The Good, The Bad, and The Ugly

(modified from Mendes et al., 2013)

*Composition of the plant microbiome is a key determinant of the health and productivity of plants*





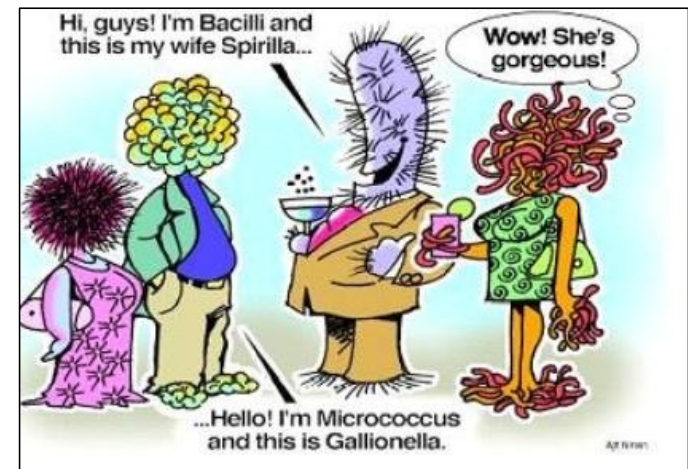
**CRITICAL FACTORS LIKELY TO  
PLAY A KEY ROLE IN  
SUPPORTING BENEFICIAL PLANT  
MICROBIOMES**





# MICROBIAL PRODUCTS

- Beneficial microbes isolated from soil and plant tissues with strong functional traits
- Microbes that were developed in the lab to produce strong functional traits (ie. *Trichoderma harzianum* T22)
- Efficacy in promoting plant growth and other functions such as pathogen suppression can be very dramatic under controlled conditions
- **HOWEVER, efficacy in the field is often highly variable and inconsistent**



***Are there other ways to support healthy crop microbiomes and perhaps enhance the efficacy of these types of products?***



# MOST PLANT MICROBIOMES ARE RECRUITED FROM BULK SOIL



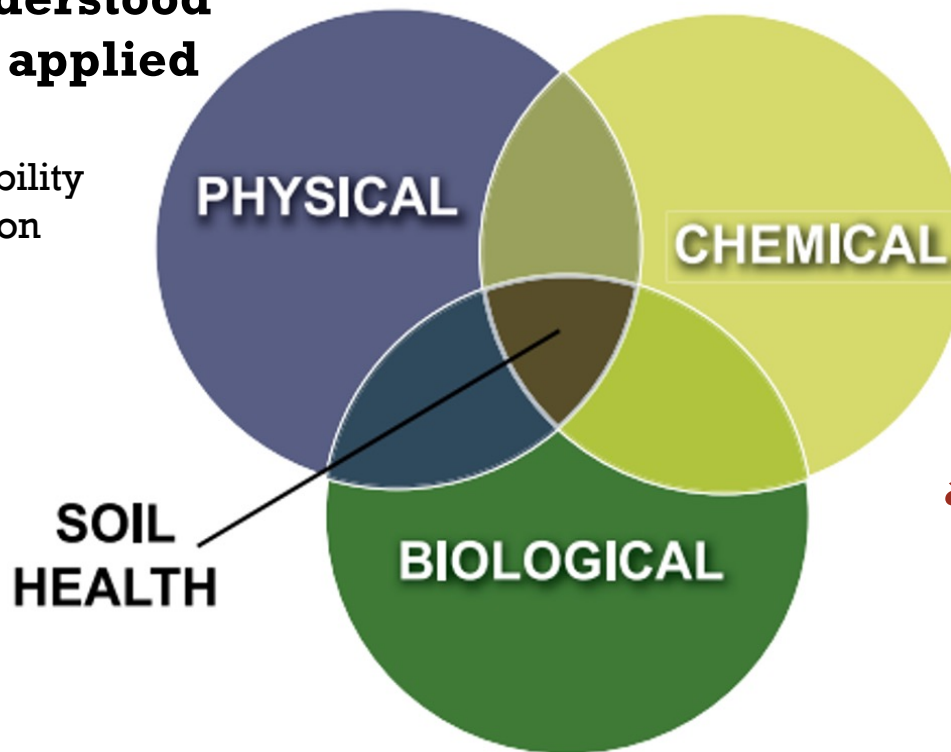
*Soil health provides the foundation for sustainable agricultural production systems*



# QUANTIFYING SOIL HEALTH

**Fairly well understood  
but less often applied**

- Bulk density
- Aggregate stability
- Water infiltration



**Well understood and  
most often applied**

- Soil pH
- Cation exchange capacity (CEC)
- Available mineral nutrients
- Electrical conductivity (EC)

***Least understood and  
applied due to difficulty in  
studying and quantifying,  
yet highly important!***

- Microbial activity
- Microbial community composition





# ROOT MICROBIOMES

*Opportunity to better understand connections between soil and plant health*

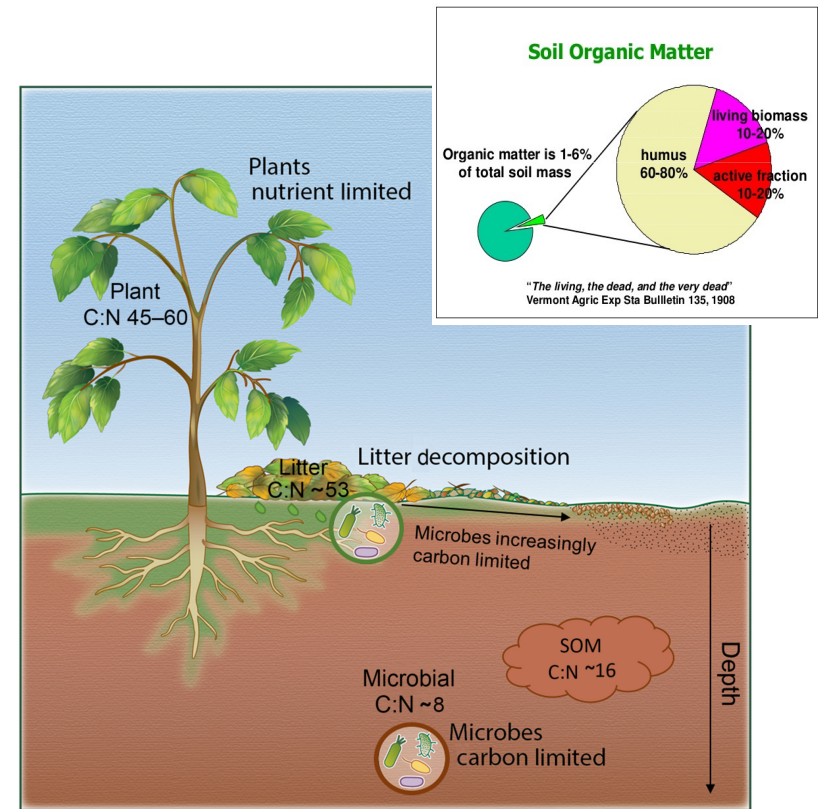
- Dynamic community of microorganisms living on the surface or inside plant roots
- Can positively or negatively affect plant health via a range of processes
- Are generally much more specialized than microbes residing in bulk soil
- While most root microbiomes are recruited from bulk soil, plants can also alter the composition of soil microbes via root exudates and other processes (*cries for help* and *plant-soil feedbacks*)



# WHAT CAN WE DO TO SUPPORT BENEFICIAL SOIL AND ROOT MICROBIOMES?

## Provide labile or active sources of carbon

- Unlike plants, soil microbes are carbon-limited
- Microbes can obtain carbon from plant roots (exudates) and organic matter, but not soil organic matter is available to all microbes
- Any time you start cultivating soil, you will deplete active soil carbon
- Soil carbon must be replenished to improve and retain soil health



EESA19-066

Soong et al., 2019

- ***How can labile pools of soil carbon be restored?***



# COVER CROPS

- Non-cash crops grown for the protection & enrichment of soil
- Most cost-effective way to restore soil organic matter and improve soil health
- Root exudates and crop residuals provide labile organic carbon compounds required to support soil microbiomes
- Also create pores for aeration and water storage that can support beneficial soil organisms



(E. Brennan)





## **EXAMPLE #1**

*Leguminous cover  
crop supply nutrients,  
improve soil health,  
alter root  
microbiomes and  
reduce susceptibility  
to soilborne  
pathogens*

(Rudisill et al., 2015 *HortScience*)  
(Rudisill et al., 2016 *Applied Soil Ecology*)





# HIGH TUNNELS (OR POLYHOUSES)

## ■ Potential benefits:

- season extension
- protection from some pests
- increase yield

## ■ Potential drawbacks:

- ***soil quality degradation***
  - intensively managed (*loss of SOM*)
  - high evapotranspiration/limited leaching (*issues with salinity*)
- ***build up of soil-borne pathogens***
  - limited crop rotation

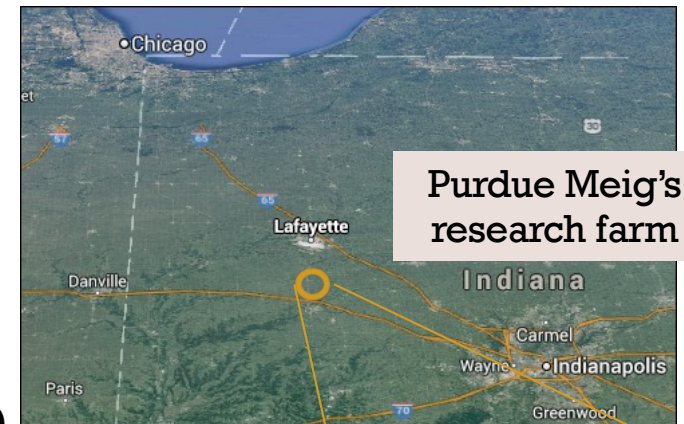


# PHASE I

***Can a green manure crop help supplement fertility needs in high tunnels while improving rather than depleting soil health?***

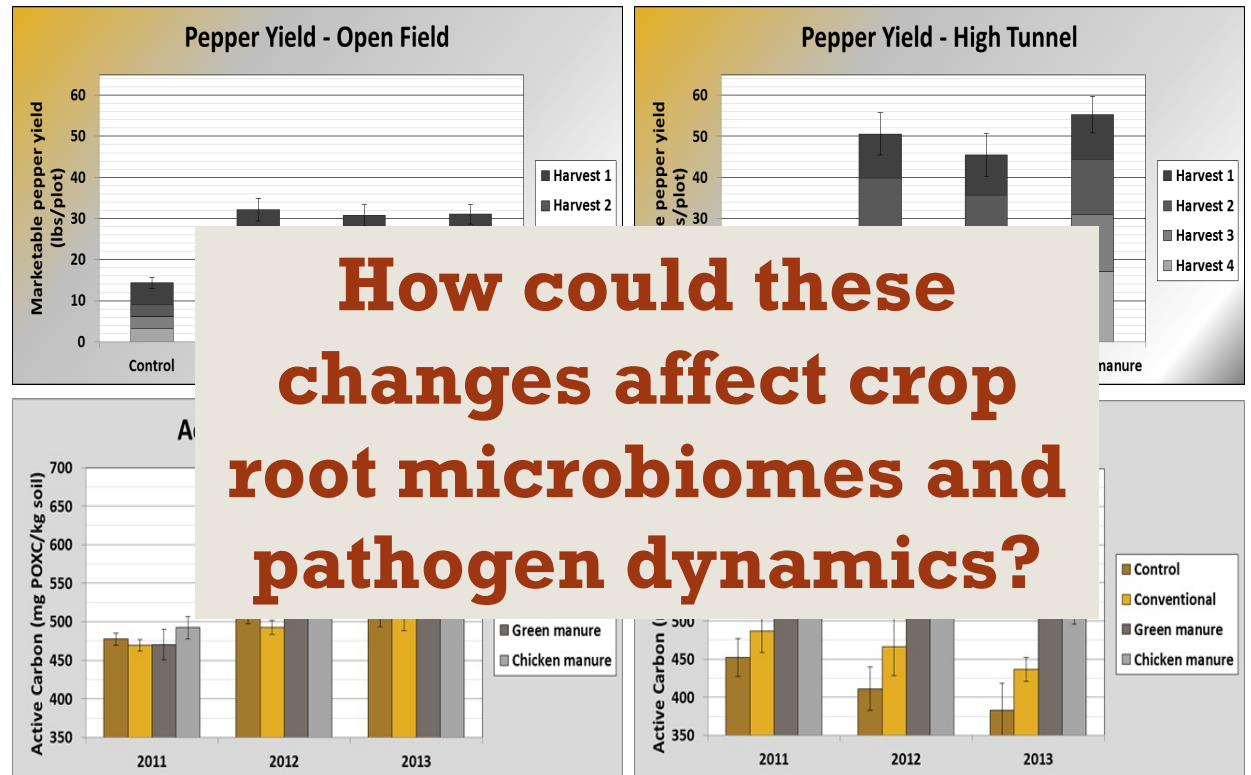
## ❖ Research design (2011-2013)

- Treatments (repeated annually):
  - 1) unamended control
  - 2) inorganic (urea)
  - 3) chicken litter compost (industry standard)
  - 4) green manure (*hairy vetch* + *alfalfa pellets*)
- RCBD w/ 4 replicates
- High tunnel and open-field
- Pepper (*rather than tomato*) planted annually



# IMPACTS ON SOIL AND CROP HEALTH

- ❖ Pepper yield greater in high tunnels
- ❖ All fertility treatments met plant nutrient needs
- ❖ Soil health declined in high tunnel conventional fertility treatment
- ❖ Soil health improved by green and chicken manure & microbes were more efficient at cycling carbon and nitrogen



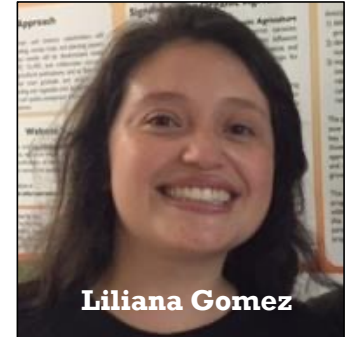
Rudisill et al., 2015 *HortScience*

Rudisill et al., 2016 *Applied Soil Ecology*



# PHASE II

**Objective:** determine how changes in soil health could affect susceptibility to soilborne pathogens and investigate potential mechanisms



## ■ Design:

- soil collected from field trials  
pasteurized or left untreated, amended  
with *Rhizoctonia solani* and planted  
with susceptible snap bean variety
- RCBD with six replicates

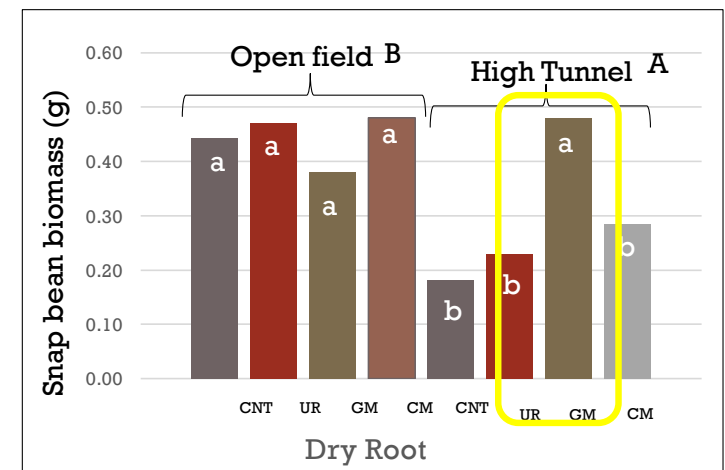


# IMPACTS ON SUSCEPTIBILITY TO *R. SOLANI*

- Soils collected from the high tunnel were more susceptible to *R. solani* than from the open field
- The HT green manure treatment was less susceptible to *R. solani* than the other treatments
- No difference between treatments and systems when soil was pasteurized (*the effects were microbially-mediated*)



*Rhizosphere soil collected for shotgun metagenomics and metranscriptomic assays*

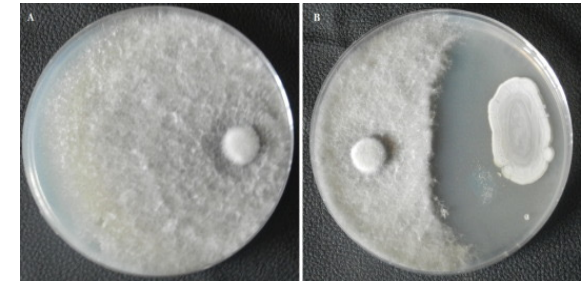


(Hoagland et al. in final prep, *Soil Biology and Biochemistry*)

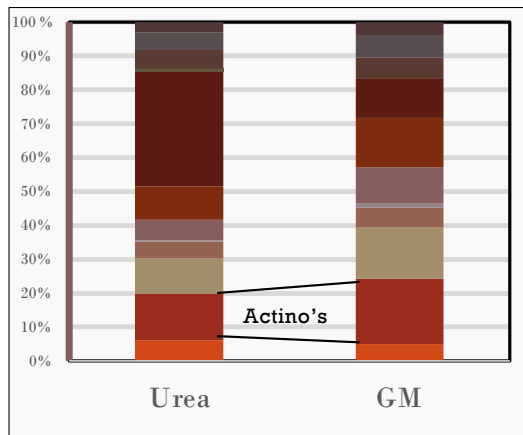


# IMPACTS ON ACTIVE MICROBIAL COMMUNITY STRUCTURE

- Several microbial taxa often implicated in pathogen suppressive activity **greater in GM**
  - *Actinomycetales*, *Bacillales* and *Pseudomanadales*



Antagonistic activity of *Actinomycetes* (right) against a fungal pathogen (left)



Relative abundance of dominant microbial in the rhizosphere by Order

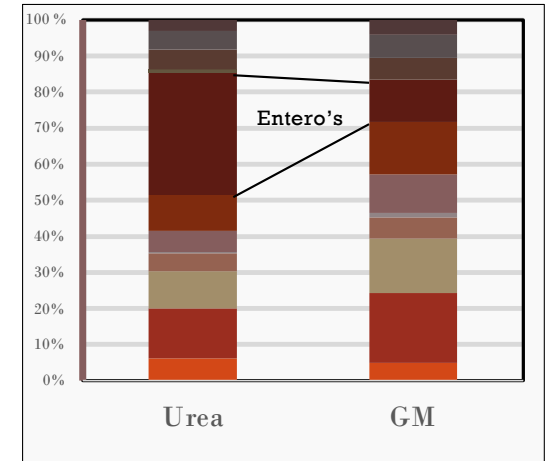
Phylum	Class	Order	Family	Genus	Urea (OTU's)	Green manure (OTU's)
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	15761	91297
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Arthrobacter	0	74621
Actinobacteria	Actinobacteria	Actinomycetales	Propionibacterineae	Nocardioide	0	69552
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	25217	86162
Proteobacteria	Gammproteobacteria	Pseudomanadales	Pseudomonadaceae	Pseudomonas	0	35078

(Hoagland et al. in final prep, *Soil Biology and Biochemistry*)



# IMPACTS ON ACTIVE SOIL MICROBIAL COMMUNITY STRUCTURE

- Enterobacteriaceae, including several genera of potential enteric bacteria, ***much greater in the urea treatment***

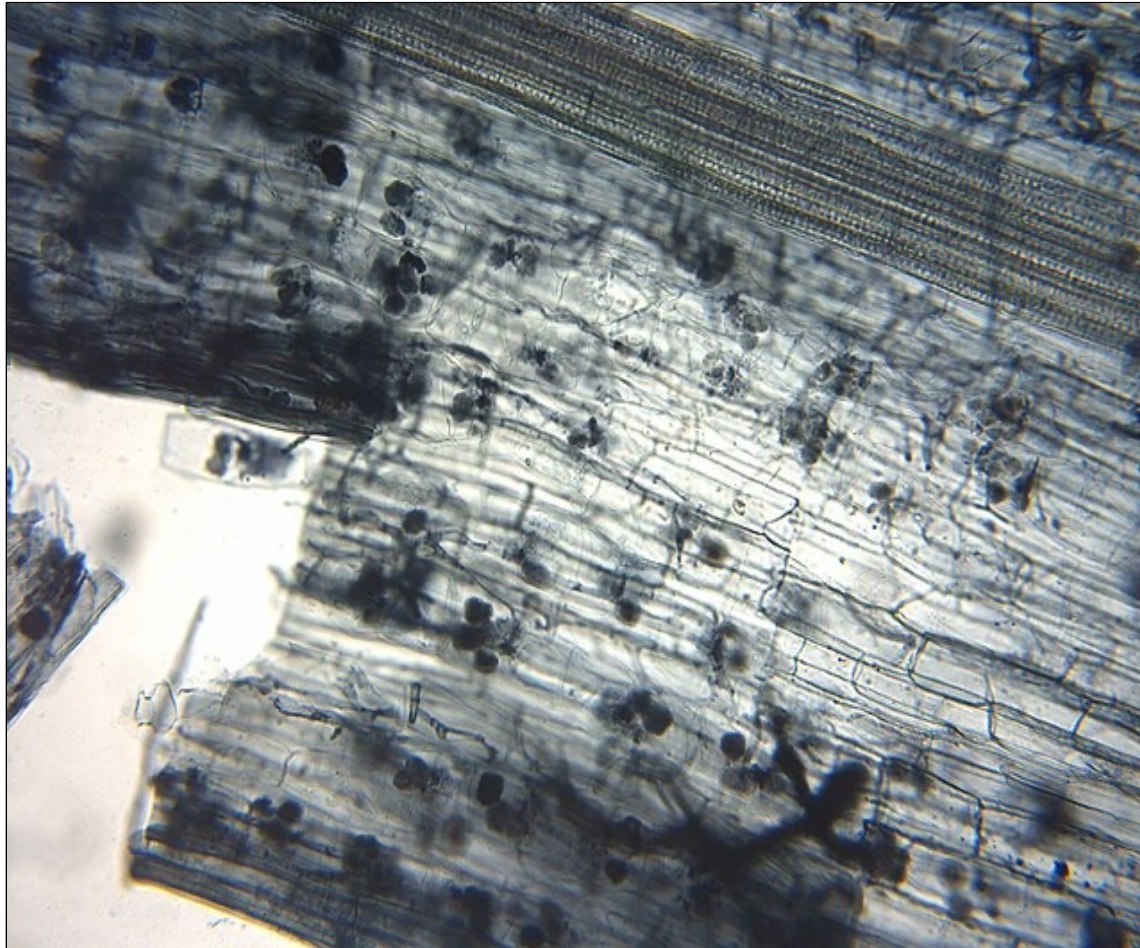


Relative abundance of dominant microbial in the rhizosphere by Order

Phylum	Class	Order	Family	Genus	Urea (OTU's)	Green manure (OTU's)
Proteobacteria	Gammproteobacteria	Enterobacteriales	Enterbacteriaceae	Escherichia/Shigella	391504	182033
Proteobacteria	Gammproteobacteria	Enterobacteriales	Enterbacteriaceae	Salmonella	166046	80493
Proteobacteria	Gammproteobacteria	Enterobacteriales	Enterbacteriaceae	Enterobacter	112383	30891
Proteobacteria	Gammproteobacteria	Enterobacteriales	Enterbacteriaceae	Cronobacter	54589	0
Proteobacteria	Gammproteobacteria	Enterobacteriales	Enterbacteriaceae	Yersinia	46807	0

(Hoagland et al. in final prep, *Soil Biology and Biochemistry*)





## **EXAMPLE #2**

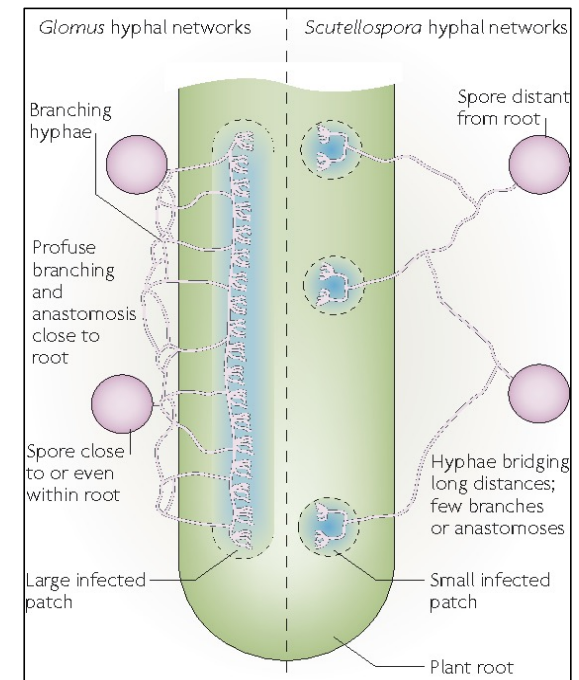
*Cover crops,  
arbuscular  
mycorrhizal fungi  
(AMF), and  
implications for the  
health of crops and  
people*





# ARBUSCULAR MYCORRHIZAL FUNGI (AMF)

- Beneficial symbiosis between plant roots and fungi within the Glomeromycota
- Most well-studied beneficial root symbiosis
- Ancient relationships (400 mya); highly conserved across most (80%) plant species
- Numerous benefits for plants and fungi
- Actually quite diverse (244-1600 species) and benefits likely depend on species
- AMF species likely respond differently to soil/crop management practices, but impact on their benefits is less clear



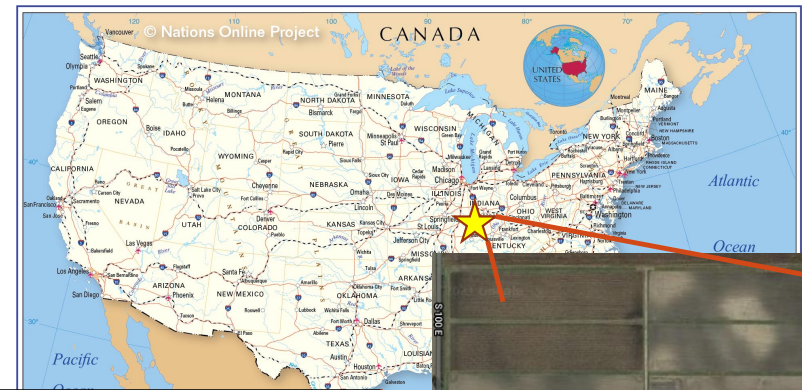
**Different hyphal growth and branching strategies in AMF (Parniske, 2008)**

**How can cover crops alter the composition and functional capacity of AMF?**



# CROP SYSTEMS TRIAL AT MEIG'S FARM

- Established on adjacent plots of land using organic and conventional management since 2000
- Four crop systems evaluated (2011-16)
- 4-year crop rotation in all systems  
popcorn -> **edible soybean** -> tomato -> **carrot**



**Conventional management system**

- Inorganic fertilizers
- Chemical pesticides

Nutrient concentrations similar between two systems

**Soil health declined**

**Soil-building organic management system**

- Animal manure based fertilizer
- Limited use of organic pesticides
- Winter cover crop mixtures

**Soil health improved**



- few differences in crop yield, but did observe lower pest incidence & higher nutrient density in crops from the organic soil-building system

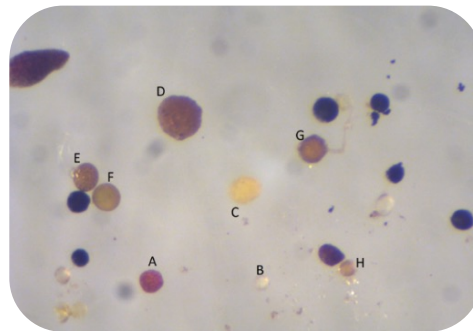


# IMPLICATIONS FOR ROOT MICROBIOMES AND RESISTANCE TO BIOTIC AND ABIOTIC STRESS

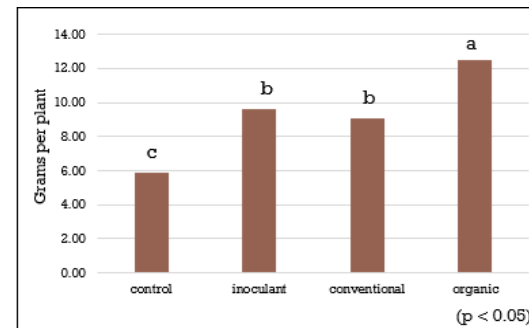
- Carrots grown in the organic soil-building system hosted endophytic microbes with greater capacity to suppress carrot diseases (Abdelrazek et al., 2020 *PLOS One*; Abdelrazek et al., 2020 *Scientific Reports*)
- Soybeans grown using **AMF** inoculum from the soil-building organic system helped soybean plants better resist drought and increased yield under stress (Zubieta, 2018)



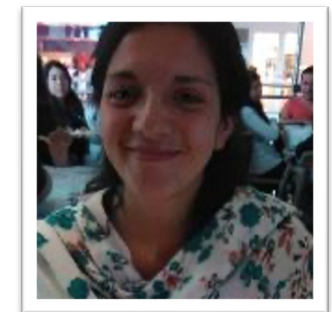
Dr. Sahar Abdelrazek



AMF spores types identified in soil from the organic soil-building system



Pod dry weight of soybean plants subject to water stress



Lisseth Zubieta



# HOW MIGHT DIFFERENCES IN AMF ALTER THE NUTRITIONAL QUALITY AND SAFETY OF CARROT CROPS?



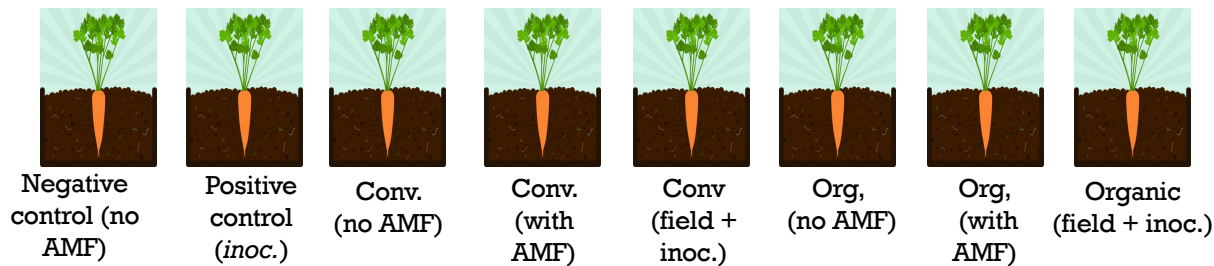
Hannah Komanapelli

- AMF are well-known for their capacity to help plant scavenge nutrients (*especially phosphorous, iron and zinc*), but the impact of AMF species on these dynamics is less clear
- AMF may also have potential to positively impact the uptake of toxic heavy metals and metalloids like cadmium, lead and arsenic
- Carrots can take up these toxic heavy metals putting consumers, especially babies at risk
  - *FDA's Closer to zero initiative*
- Can commercial inoculants displace native AMF species in soil and alter their benefits?



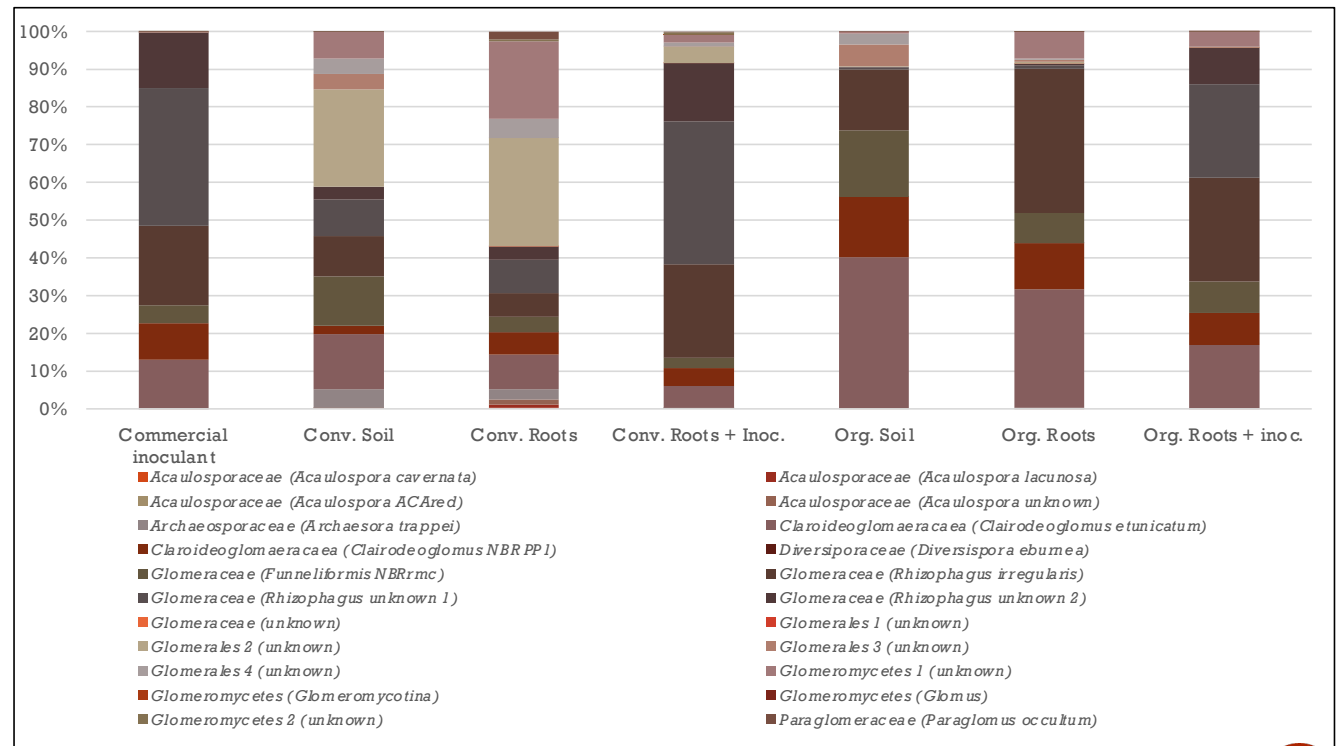
# EXPERIMENTAL DESIGN

- Soil/root fragments collected from field trial after cover crop in spring prior to carrot planting
- Soils from field trials (with and without AMF) and a commercial inoculant (*Rhizophagus irregularis*) used as inoculum in sterile sand:soil mix
- Carrot (cv. Napoli) planted in each pot
- Quantified AMF communities in fine roots, as well as taproot yield and elemental concentrations



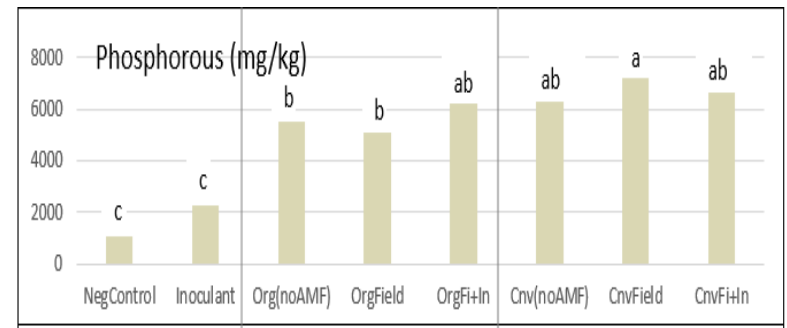
# RESULTS: IMPACTS OF SOIL MANAGEMENT/HEALTH ON THE COMPOSITION OF AMF AND THEIR CAPACITY TO HELP CARROT PLANTS OBTAIN NUTRIENTS AND PREVENT CD UPTAKE

- Commercial AMF inoculants were not pure, but dominated by *Rhizophagus* species
- AMF in conventional systems were more diverse and dominated by *Glomerales* & *Rhizophagus*
- AMF in organic systems had greater concentrations of *Clariodeglomus*
- AMF in carrot roots reflected inoculum in soil and were impacted by the addition of the commercial inoculant

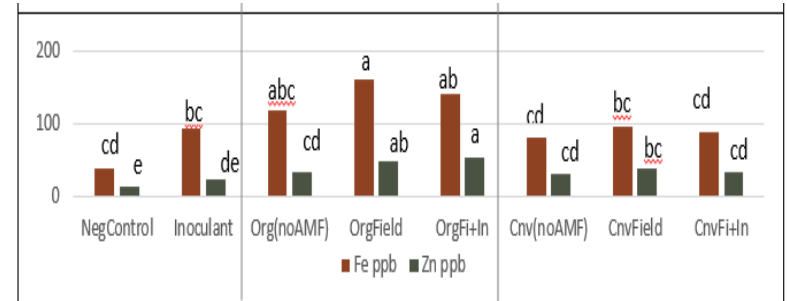


# RESULTS: IMPACTS OF SOIL MANAGEMENT AND HEALTH ON THE COMPOSITION OF AMF AND THEIR CAPACITY TO HELP CARROT PLANTS OBTAIN NUTRIENTS AND PREVENT CD UPTAKE

- Phosphorous was higher with AMF communities from both field trials than with the commercial inoculant
- Iron and zinc were higher in taproots grown with AMF from organic field
- Small reduction in Cd with whole microbiomes from organic system (*AMF not acting alone – follow up studies underway*)
- Commercial inoculant did increase Cd uptake relative to control, but did not disrupt benefits of AMF from either field



Impact of AMF communities on phosphorous in taproots



Impact of AMF communities on iron and zinc in taproots





## **EXAMPLE #3**

*Legume  
intercropping on  
the composition and  
functional capacity  
of coffee root  
microbiomes*





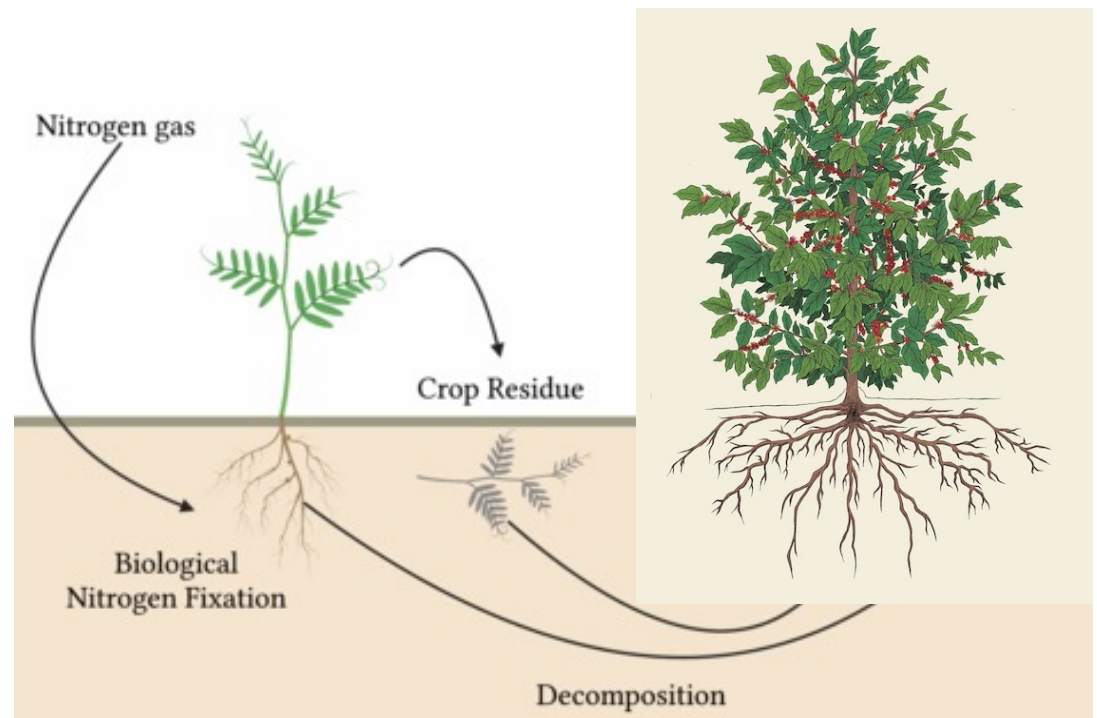


- Coffee: Main export crop in Colombia - **3rd** largest producer in the world.
- Nitrogen (N): The most critical nutrient in coffee production – influences growth, yield and caffeine content.
- Increasing price of fertilizers/dependence on imports: **Concern for coffee growers.**
- **Affordable and sustainable N sources are needed to decrease production costs and keep you caffeinated**



# LEGUME INTERCROPPING SYSTEMS HAVE POTENTIAL TO HELP MEET N NEEDS

- Legumes fix atmospheric N through microbial symbiosis with nitrogen-fixing bacteria.
- Legume intercropping systems also have potential to help mitigate soil erosion on steep hillsides.
- ***Integrating more diversity in an this critical agroecosystem through intercropping could also bring additional benefits for coffee plants by promoting beneficial root microbiomes.***



(Salinas et al., in prep)

Modified from University of Minnesota Extension, 2022



# METHODS – INTERCROPPING FIELD EXPERIMENT



Locations:  
3  
experimental  
farms

1. Caldas:
2. Quindio
3. Cauca



Legume treatments:  
*Tephrosia sp.* = Tephrosia  
*Cajanus cajan* = Guandul  
No legume = None

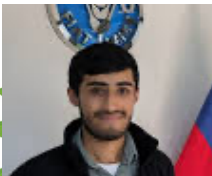
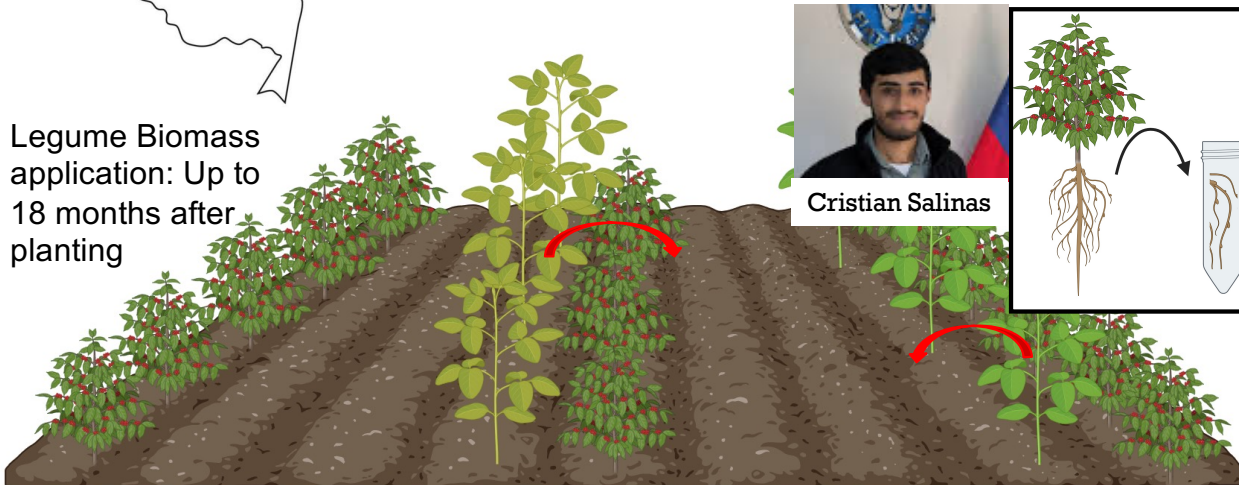
None: Caldas, Quindio, Cauca  
Tephrosia: Caldas, Quindio, Cauca  
Guandul: Caldas, Quindio



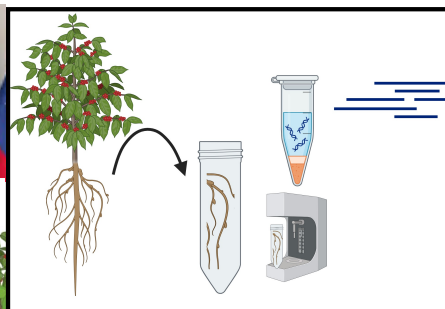
Nitrogen supply treatments:  
0 = 0% of N required by plant  
50 = 50% of N required by plant

None = 0 and 50 %  
Tephrosia = 50 %  
Guandul = 50 %

Legume Biomass  
application: Up to  
18 months after  
planting



Cristian Salinas



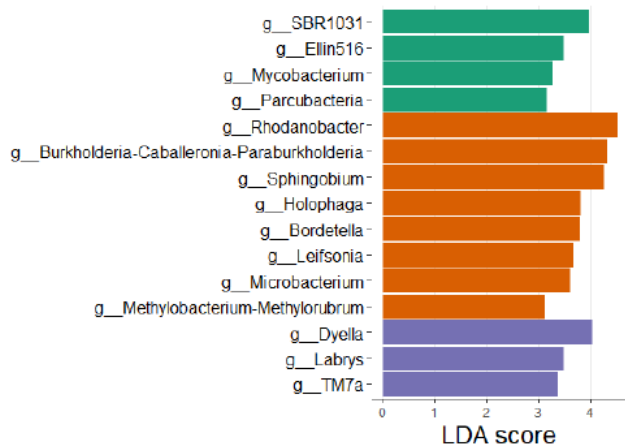
Dr. Hernan Gonzalez  
National Coffee Research Center - Cenicafe



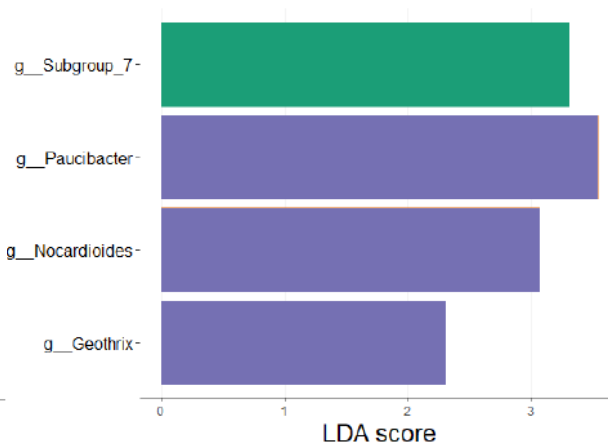
Dr. Lucio Navarro  
National Coffee Research Center - Cenicafe

# RESULTS: BACTERIA LEFSE ANALYSIS

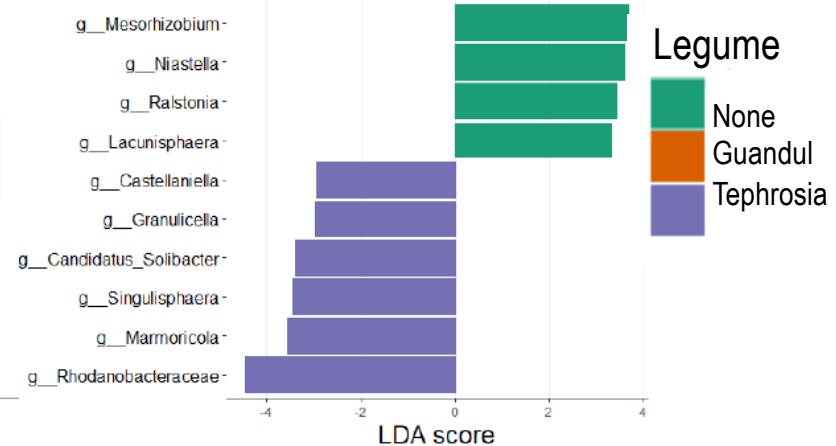
Location Caldas



Location Quindio



Location Cauca



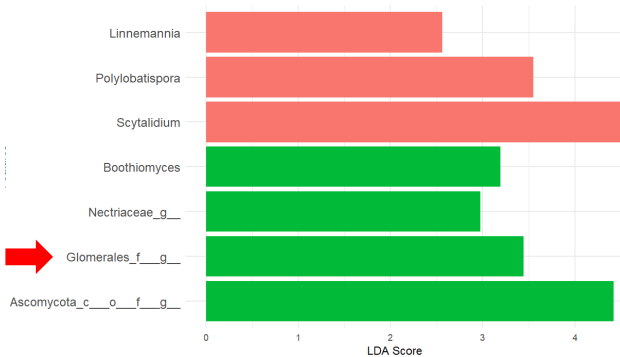
## Takeaways

- The most abundant bacterial genera in coffee roots varied across locations and legume treatment.
- *Sphingobium sp.* and *Leifsonia sp.* were enriched in coffee grown with the Guandul intercrop treatment.
  - *Leifsonia* could help coffee plants solubilize P and improve coffee growth
  - *Sphingobium* could produce plant growth hormones (gibberellins and ACC deaminase) that aid in root growth and nutrient uptake
- *Dyella*, *Labrys* and *Nocardioides* were enriched in coffee grown with the Tephrosia intercrop treatment
  - These organisms could support root growth, enhance N cycling, and suppress disease-causing pathogens.

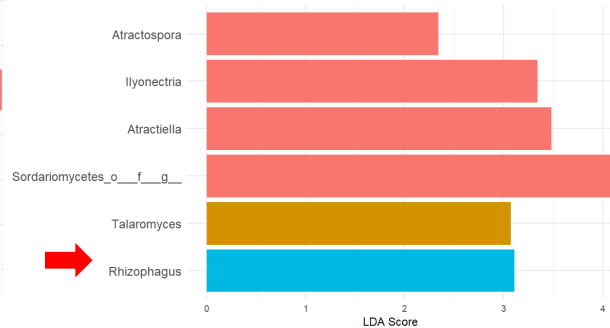


# RESULTS: FUNGI LEFSE ANALYSIS

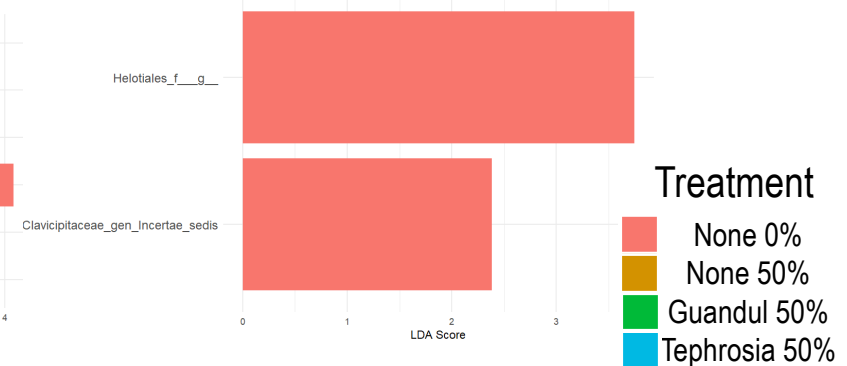
Location Caldas



Location Quindio



Location Cauca



## Takeaways

- The most abundant fungal genera in coffee roots also varied across locations and legume treatment.
- Arbuscular mycorrhizal fungi (AMF) species in coffee were differentially impacted by the legume intercrops
  - *Rhizophagus* had a higher relative abundance in coffee roots grown with the Tephrosia intercrop treatment
  - *Glomerales* sp. had a higher relative abundance in coffee roots grown with the Guandul intercrop treatment
  - Both sp. have potential to provide multiple benefits to coffee plants





## **EXAMPLE #4** ***(LAST ONE)***

*Leaf mold compost,  
soil health,  
microbial inoculants  
and pathogen  
dynamics*



# COMPOST

- Quickest way to build organic matter and improve soil tilth on degraded soils (*improve 'soil health'*)
- Can provide supplemental nutrients to meet crop fertility needs
- ***Some composts have also been demonstrated to make soils more disease suppressive***
- Compost quality (*and benefits*) depends on feedstocks and processing conditions



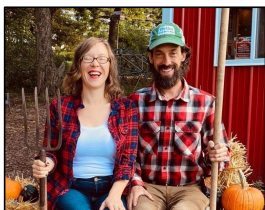
**Decomposed organic materials** (e.g. plant & animal wastes)



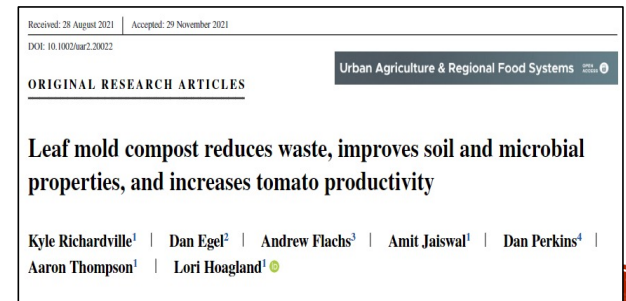
# FIELD TRIAL WITH 'LEAF MOLD' COMPOST



- Many benefits to farming in the city, but soils are often highly degraded, and in some cases contaminated
- Urban growers often apply ample amounts of composts to improve soils and meet plant fertility needs (but *can also add too much compost – soils often way over enriched in P*)
- ***Are there urban waste products that can improve soils and crops without enriching soil? Can they also better support microbial inoculants and support a more circular economy in cities?***
- Midwest cities have lots of deciduous tree leaves (*resource for fungi*)



Dan & Julie Perkins  
Good Earth Farm



**'Leaf mold'** – passive composting process (*thought to encourage fungi*); not contaminated with metals



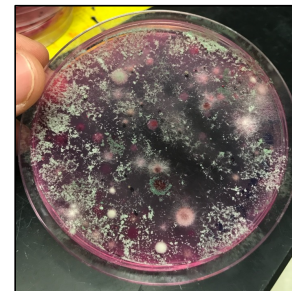
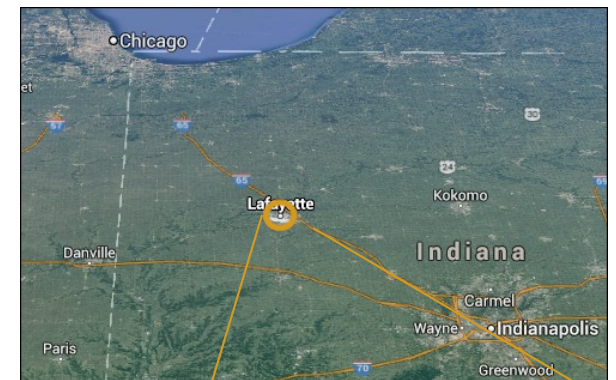
# EXPERIMENTAL DESIGN

- Field trial at Purdue's urban Student Farm on Cherry road (2019-2020)

## Research questions:

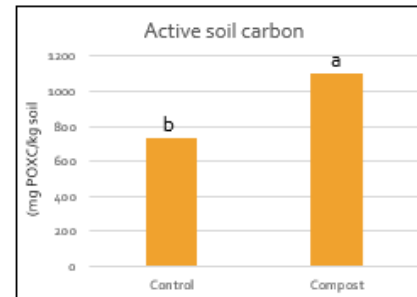
- 1) Can leaf mold compost improve soil and plant health and suppress disease in tomato?
- 2) Can leaf mold compost increase survival of a commercially available biocontrol inoculant (*Trichoderma harzianum* T22) in the field?

- RCBD w/ 2 tomato genotypes and 4 replicates; T22 applied at seeding in trays and again to soil in field; repeated in new location each year to verify results
- Tracked range of 'soil health' parameters (e.g. active organic matter, microbial activity, etc.) and crop responses (disease, yield, etc.)
- Tracked T22 inoculant using plate counts/qPCR

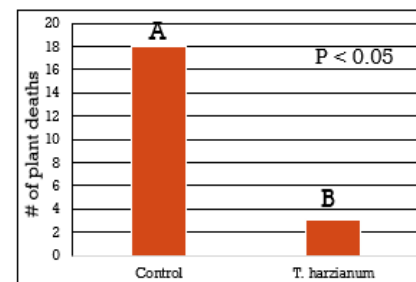


# RESULTS – DRAMATIC INCREASES IN SOIL HEALTH AND CROP PRODUCTIVITY

- **60% increase in marketable fruit yield!!!**
- Large increases in key ‘soil health’ properties due to compost -> (e.g. active soil carbon, microbial activity, & abundance of potentially ‘beneficial’ soil microbes)
- Reduction in tomato plant deaths after transplant when inoculated with T22
- Small reduction in foliar disease due to compost amendment
- Greater survival of T22 with compost & greater reduction in disease due to compost + T22



Impact of leaf mold compost on active soil carbon



Effect of *T. harzianum* on W55 transplant stress in 2020



# GREENHOUSE TRIALS WITH OTHER TYPES OF COMPOST

- Some, but not all composts reduced the spread of pathogen lesions on tomato leaves
- Black soldier fly compost (Dr. Laura Ingwell) and derivatives of the process more suppressive than other composts
- Suppressive activity of composts in both trials correlated with presence of unique microbiomes



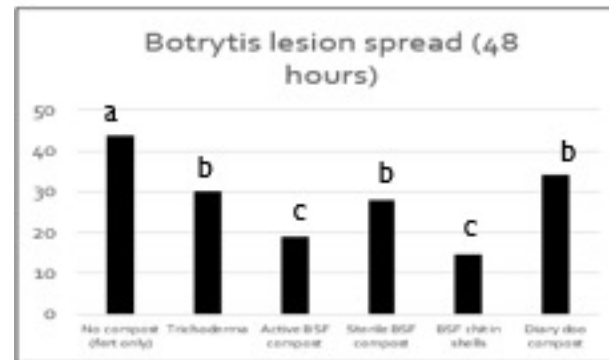
Dr. Laura Ingwell, Purdue



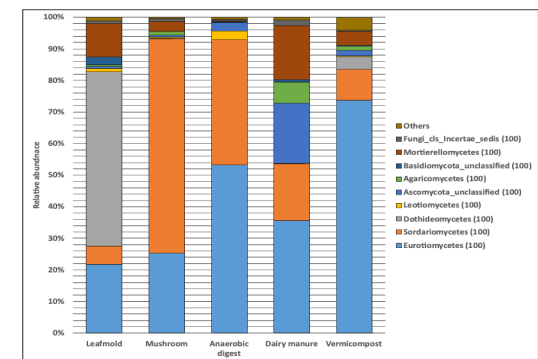
- Additional trials underway including options for 'seeding' beneficial microbiomes in transplants w/ Dr. Liz Maynard**



Dr. Liz Maynard  
Purdue



*Botrytis cinerea* lesion size on tomato leaves 48 hours after inoculation. Columns with different letters significantly different at  $p < 0.01$ . (unpubl.)



Relative abundance of fungal microbiomes in composts by class (unpublished)



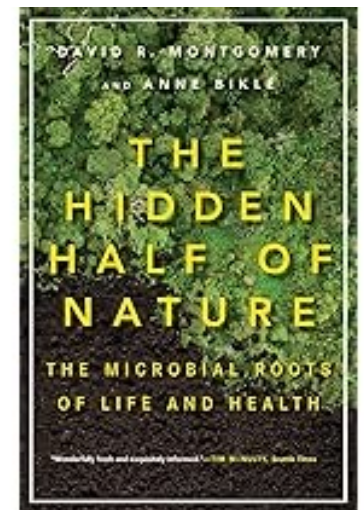


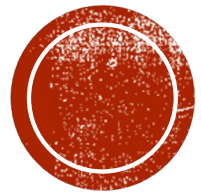
# **SUMMARY AND CONCLUSIONS**



# SUMMARY AND CONCLUSIONS

- Root microbiomes can influence the health and productivity of crop plants and this could also have important implications for human health
- It is possible to support beneficial root microbiomes in horticultural and field crops by integrating cover crops (*in both time and space*) and amending soils with high quality compost, *but be careful not to add too much compost*
- **The health of soils, plants and people are connected**





**QUESTIONS?**

