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College of
Agricultural Sciences

Current approaches and future directions for rhizosphere microbiome sampling methodologies

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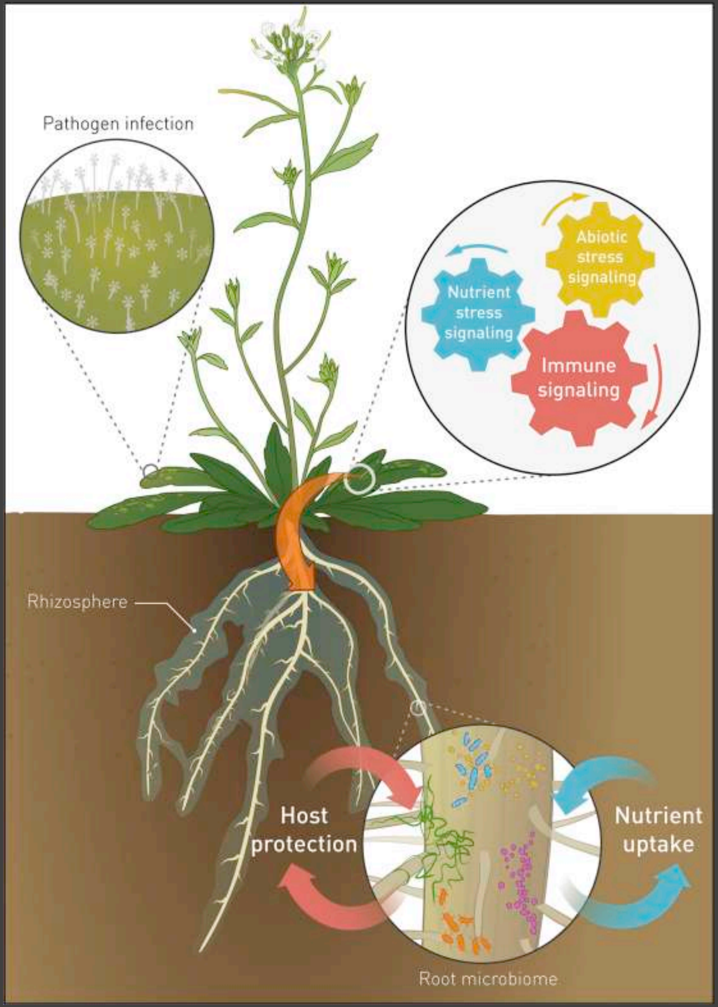
Many plant functions are microbially-mediated



Bakker et al. 2018, Trends in Plant Science

Fleishman et al. 2022, *In Review*

What exactly is the rhizosphere?

Narrow soil zone directly surrounding the root system and influenced by the root

(Reinhold-Hurek et al., Annu. Rev. Phytopathol. 2015)

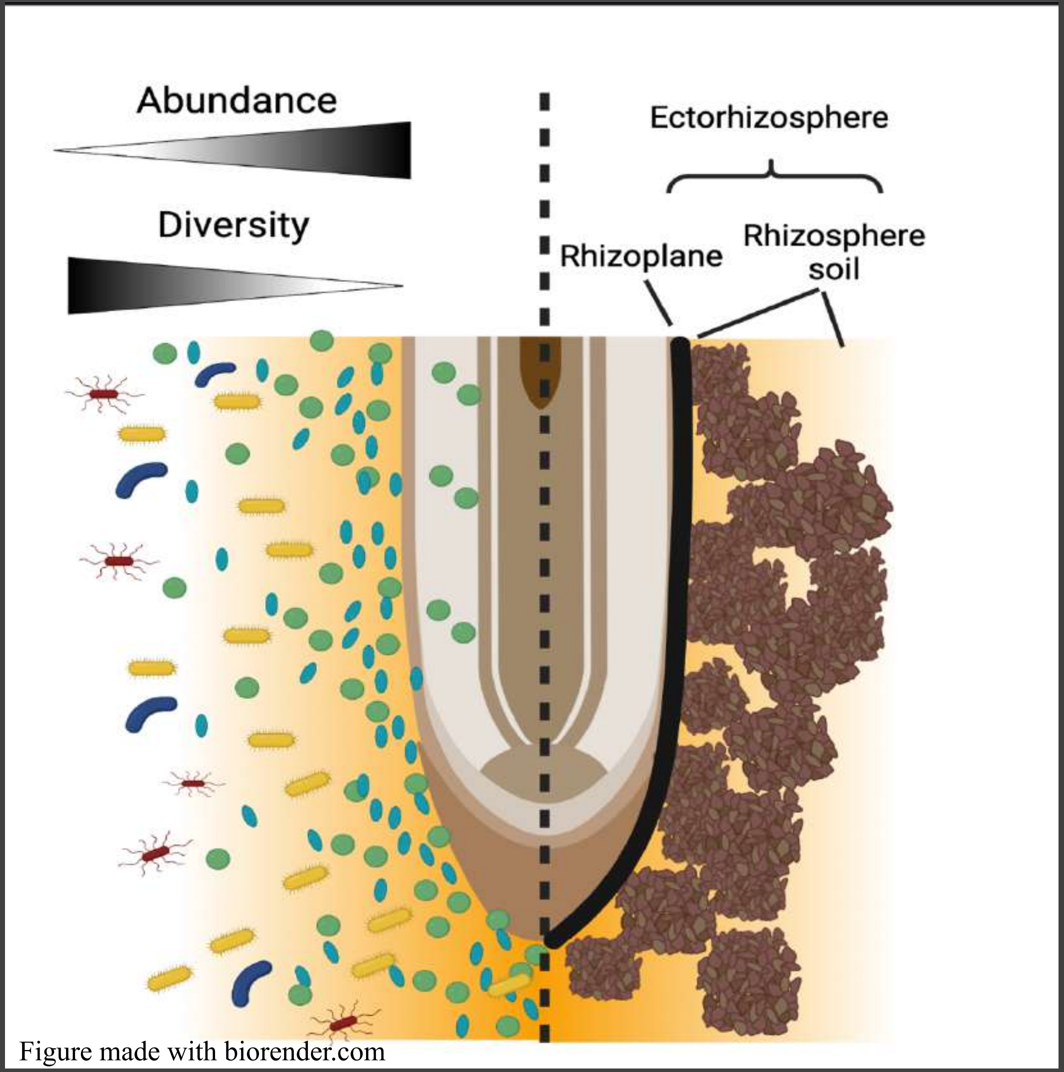


Figure made with biorender.com

Fleishman et al. 2022, *In Review*

No standard practices for root sampling and rhizosphere removal

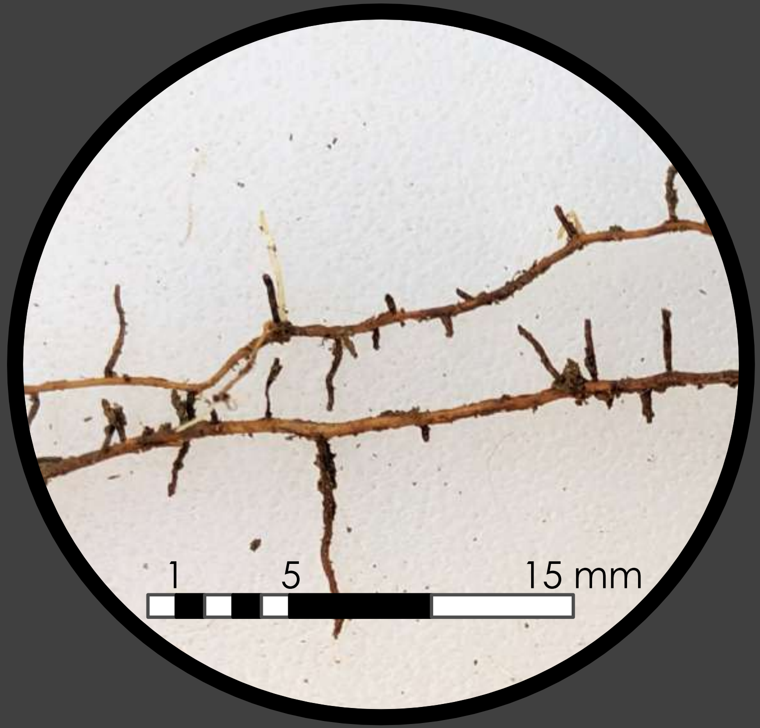
- **36% as potted studies**
- **In field studies, 30% sampled entire root system and 34% from the shallow depths**
- **88% on bacteria (16s)**
- **33% on fungi (ITS)**

Fleishman et al. 2022, *In Review*

How does the underlying functional and spatial heterogeneity of roots impact microbial composition?

Absorptive
vs.
Transportive

Developmental
stage



Spatial
location

Root systems are heterogeneous environments

Absorptive vs. transportive roots

PSU extension

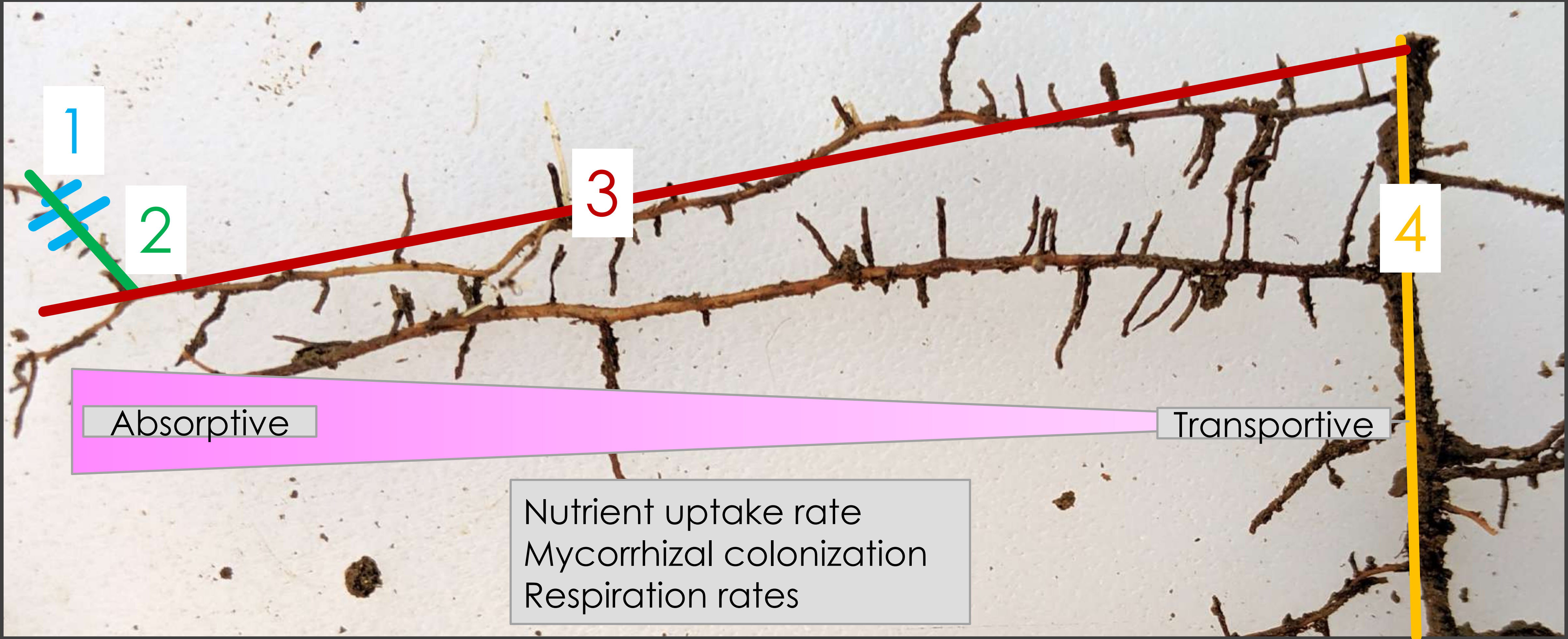
Absorptive vs. transportive roots

Root function can be classified by branching order

Root function can be classified by branching order

Root function can be classified by branching order

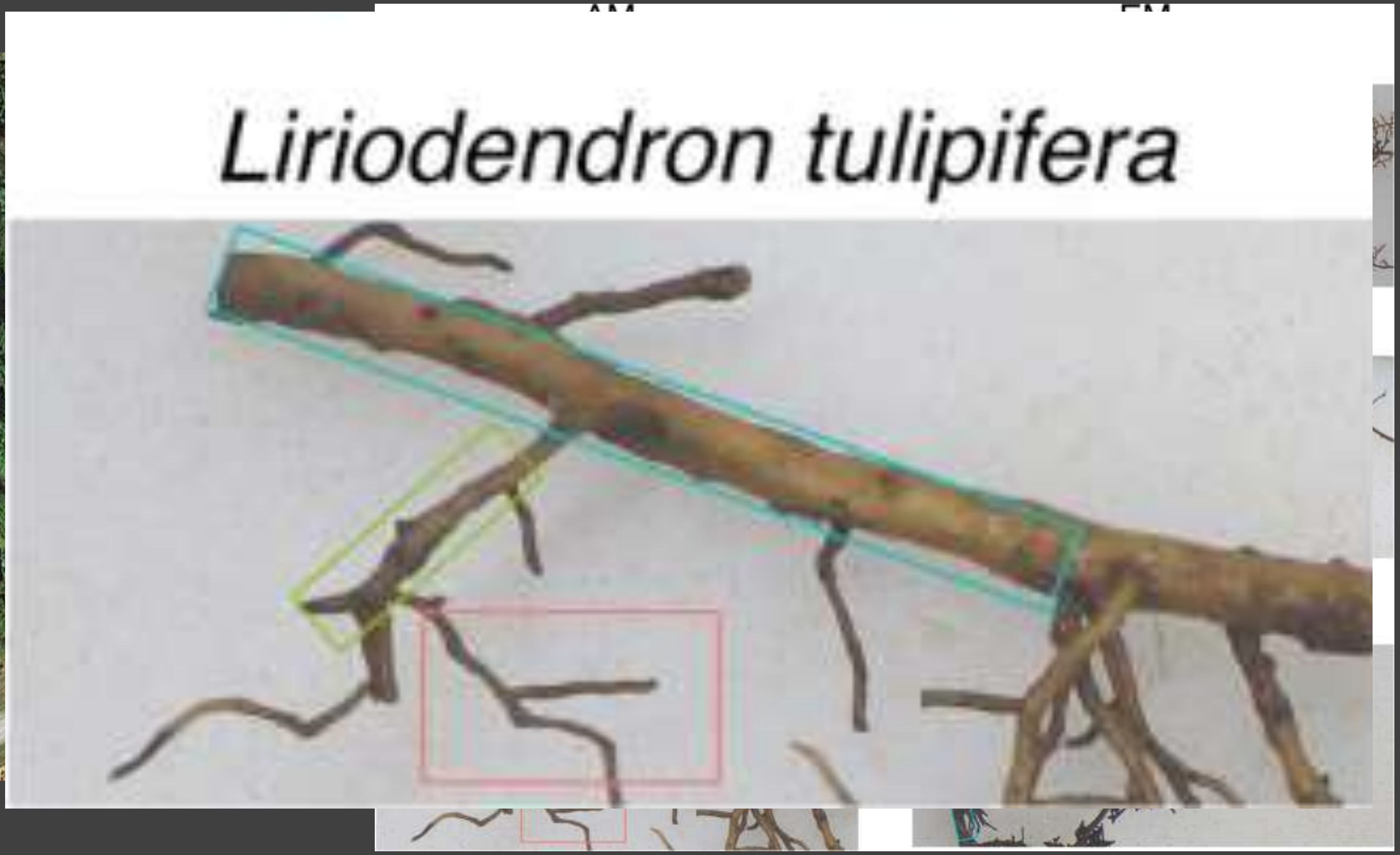
Root function can be classified by branching order



Nutrient uptake rate
Mycorrhizal colonization
Respiration rates

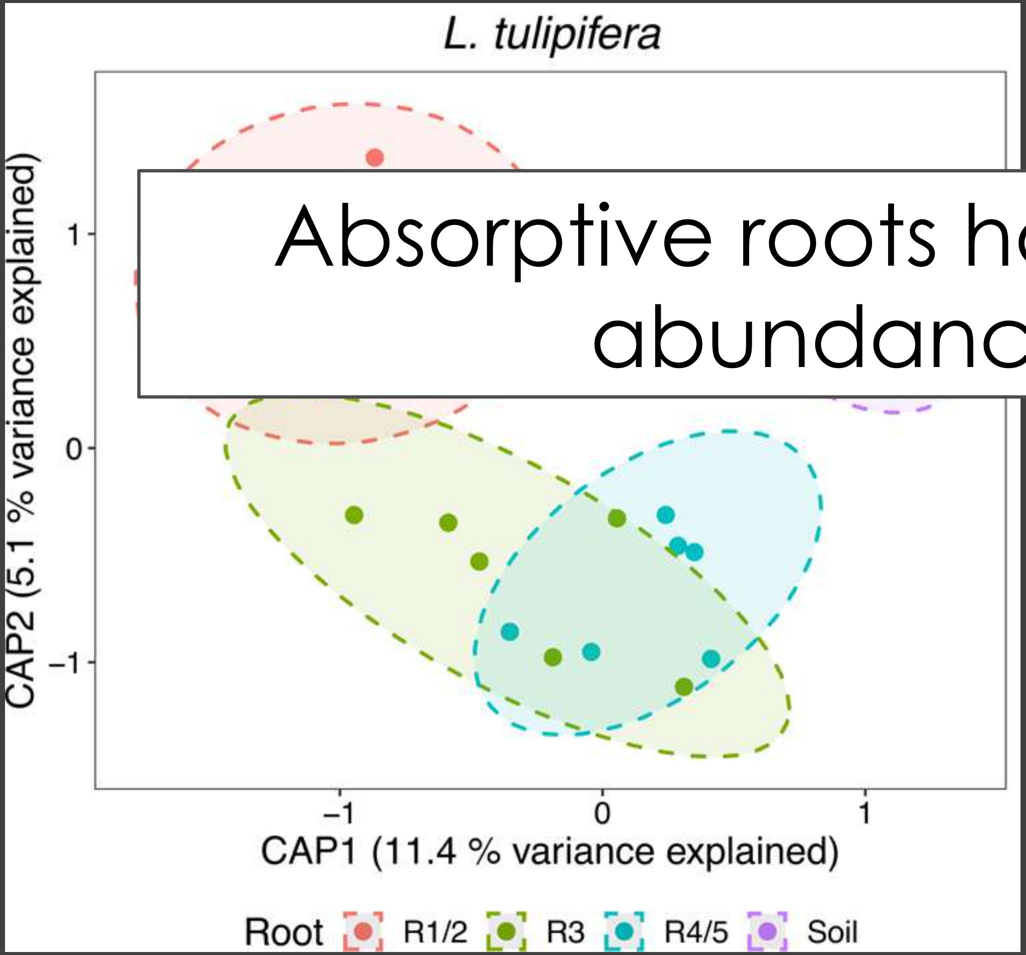
Do rhizosphere microbiomes vary with root branching order?

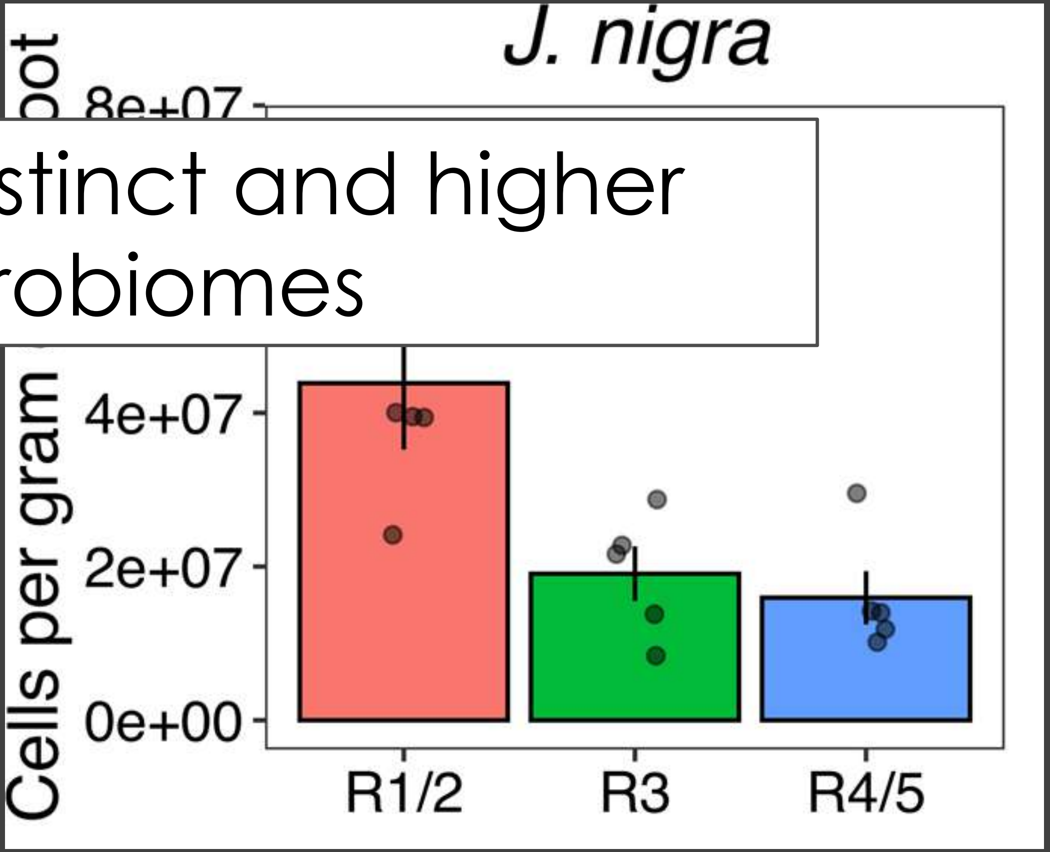
Common garden



King et al. 2021, *Communications Biology*

Root branching order determines composition





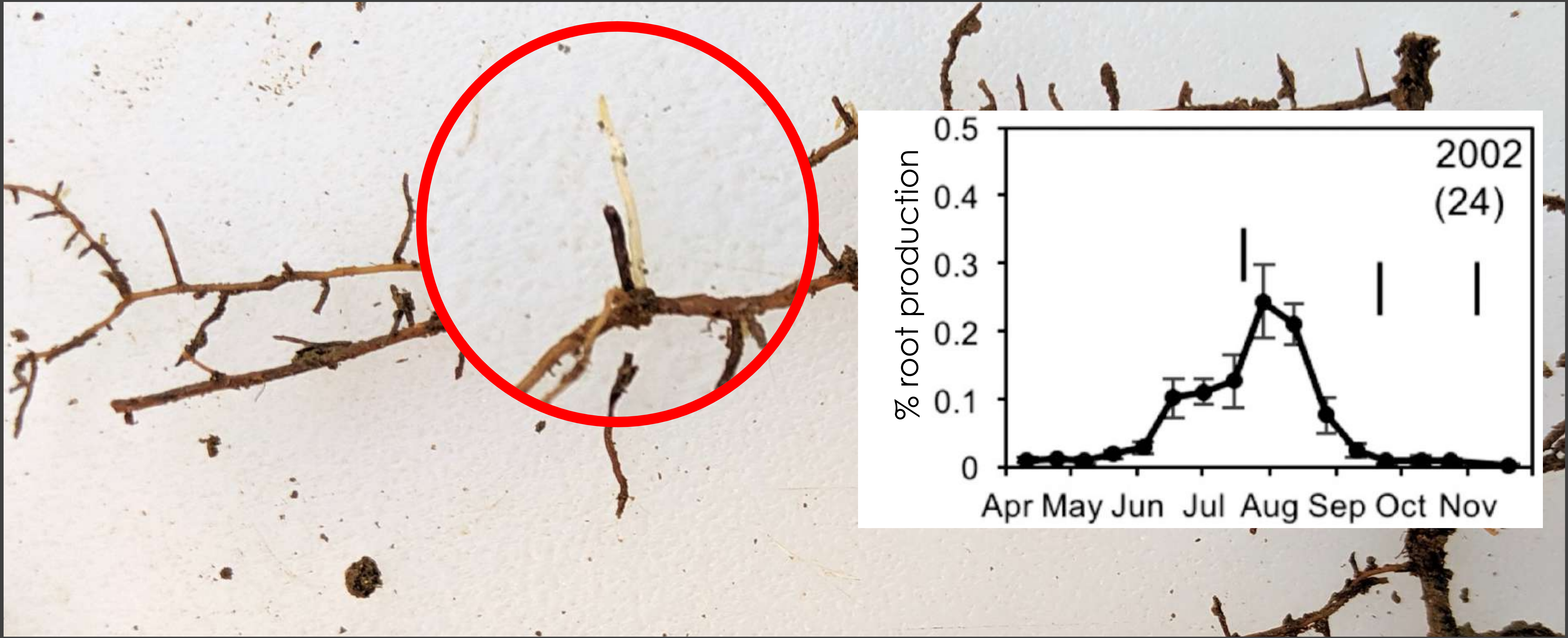
Absorptive roots have distinct and higher abundance microbiomes

* Pattern existed across all 6 tree species

King et al. 2021, *Communications Biology*

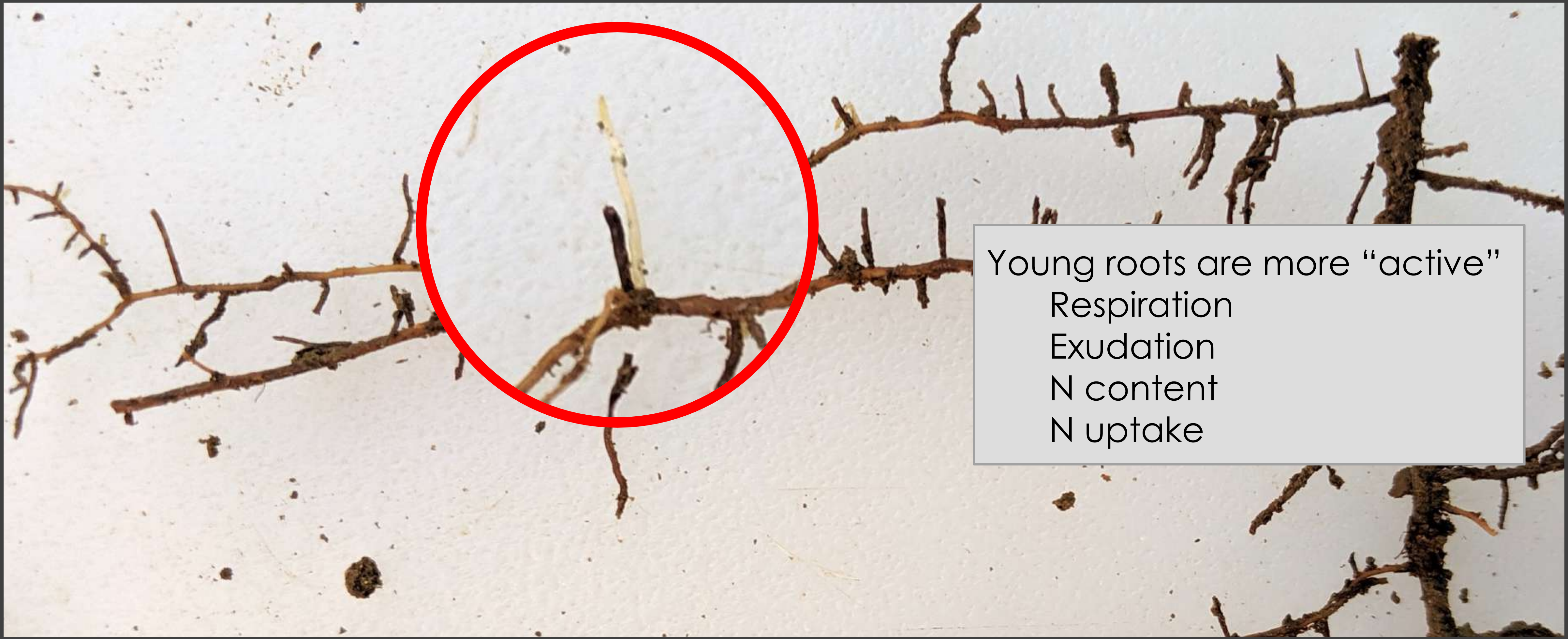
Absorptive roots differ in developmental stage

Absorptive roots differ in developmental stage



Radville et al. 2016, *J. Exp. Botany*

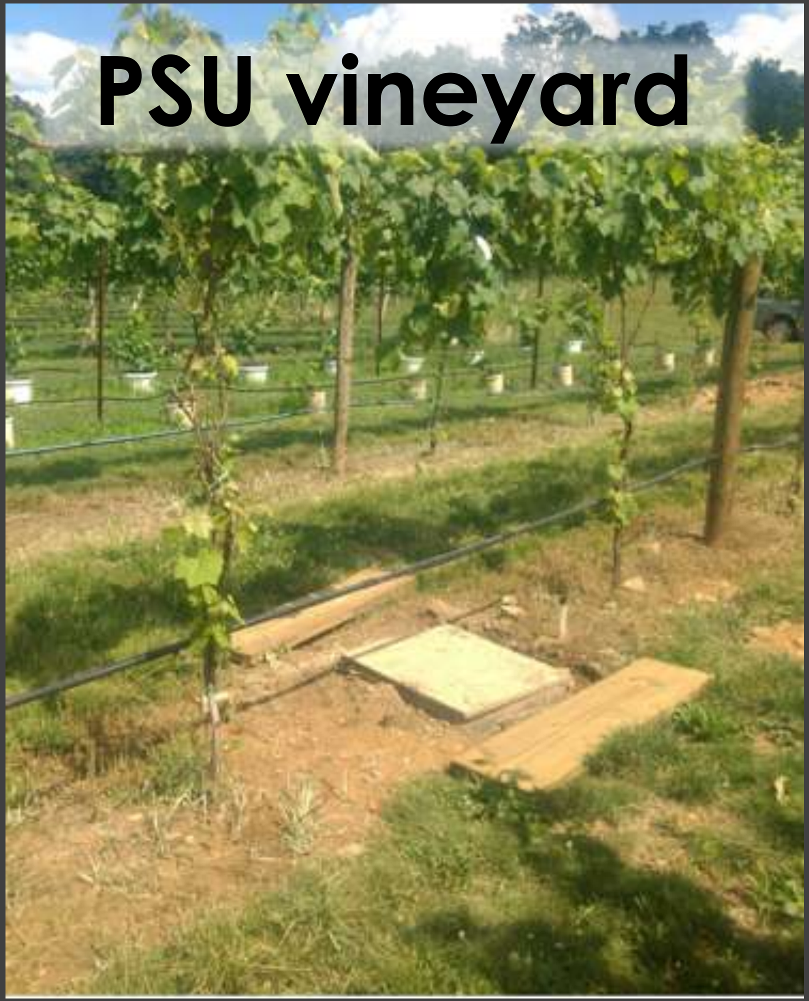
Absorptive roots differ in developmental stage



Young roots are more “active”

- Respiration
- Exudation
- N content
- N uptake

Monitoring root age in the field



Monitoring root age in the field

7 vines
98 individual roots
Age: 0.5 – 40 days

Microbial composition minimally differs between young (<11 days) and old (11-44 days) roots

Incredibly high root-to-root variation in rhizosphere bacterial and fungal composition

Persico et al. 2022, *In Prep*

Does root location explain microbial variation?

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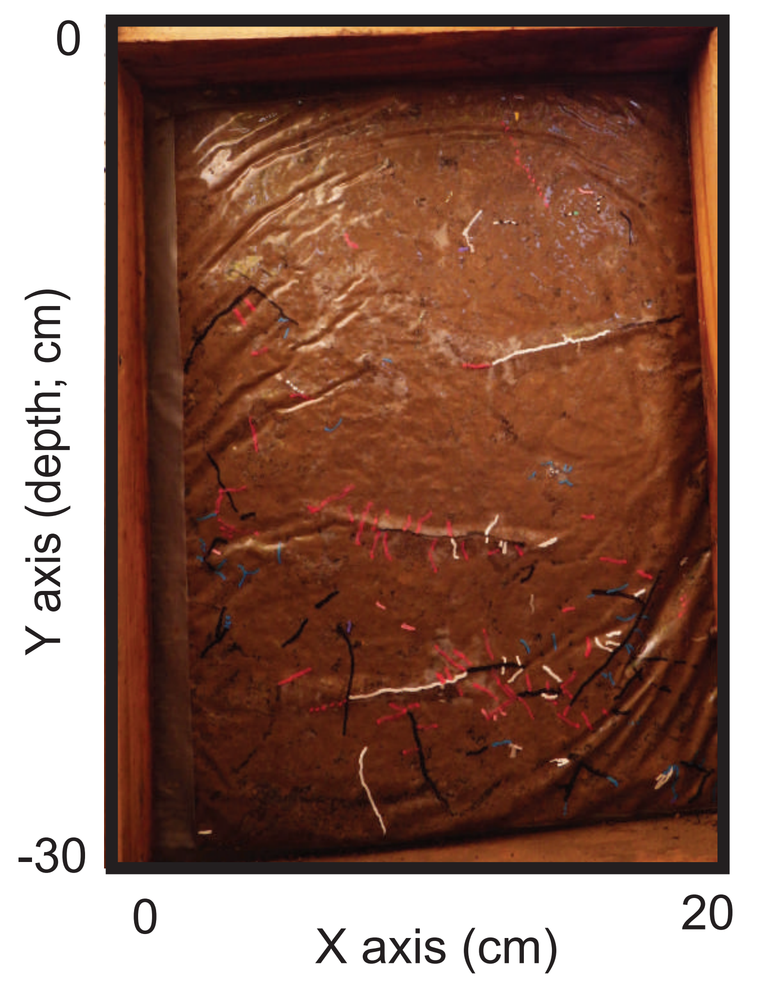
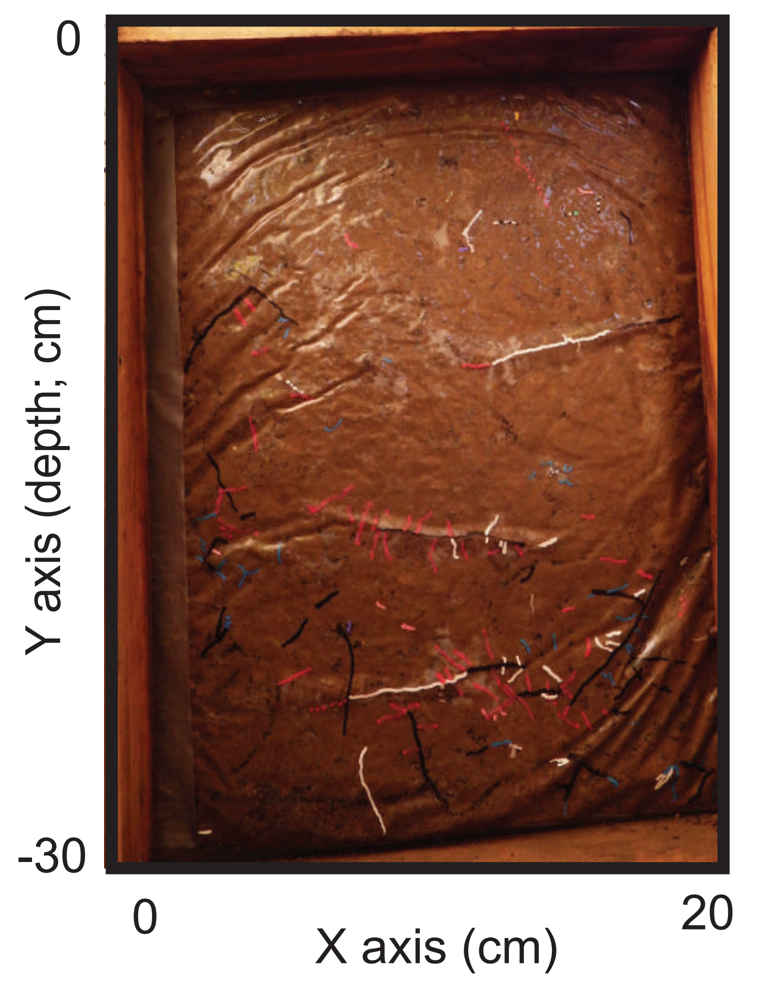
Mapping a root system



Variation Partitioning Analysis (VPA)

- Based on constrained ordinations (RDA) to model microbiome response
- Allows for R^2 generation for multivariate explanatory categories
- Allows for comparison of the relative importance of each category

Mapping a root system



Variation Partitioning
Analysis (VPA)

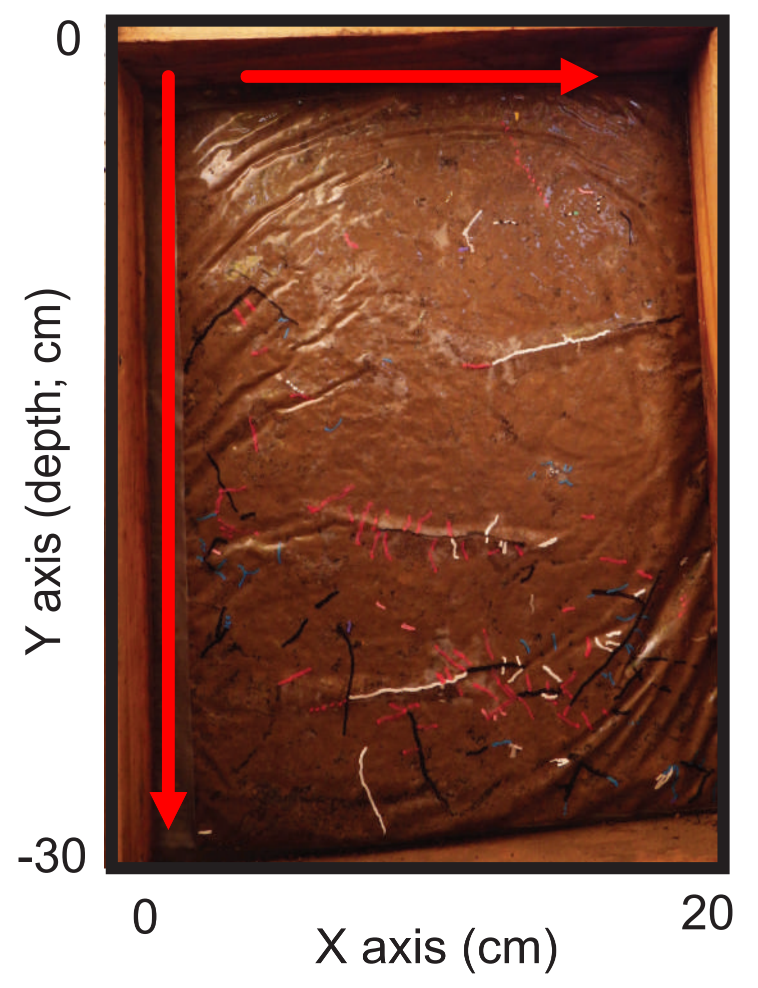
1. Root traits
   (branching order, age, color)

Mapping a root system

Variation Partitioning
Analysis (VPA)

1. Root traits
   (branching order, age, color)
2. Root cluster

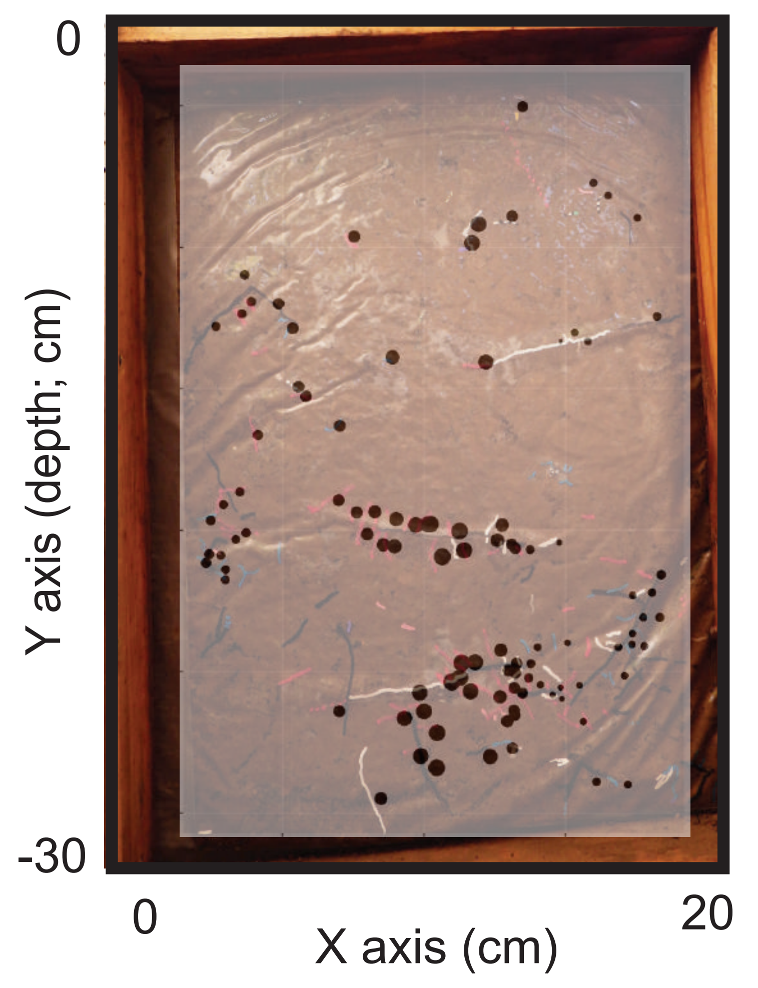
Mapping a root system



Variation Partitioning
Analysis (VPA)

1. Root traits
   (branching order, age, color)
2. Root cluster
3. Linear spatial trend (X,Y)

Mapping a root system



Variation Partitioning
Analysis (VPA)

1. Root traits
   (branching order, age, color)
2. Root cluster
3. Linear spatial trend (X,Y)
4. Local groupings (MEMs)

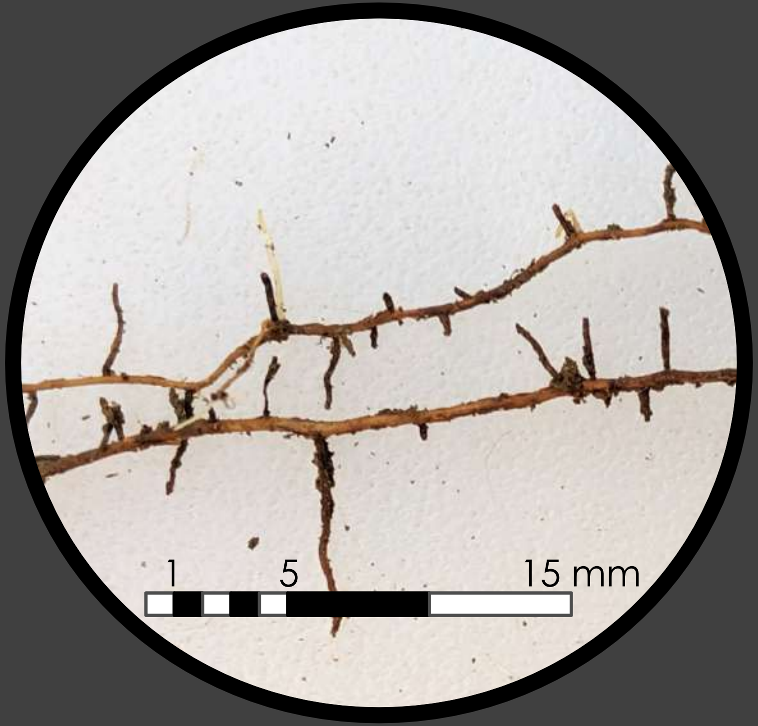
Root traits explain less variation than spatially structured factors

Fleishman et al. 2022, *In Review*

The underlying functional and spatial heterogeneity of roots impacts microbial composition

Branching order

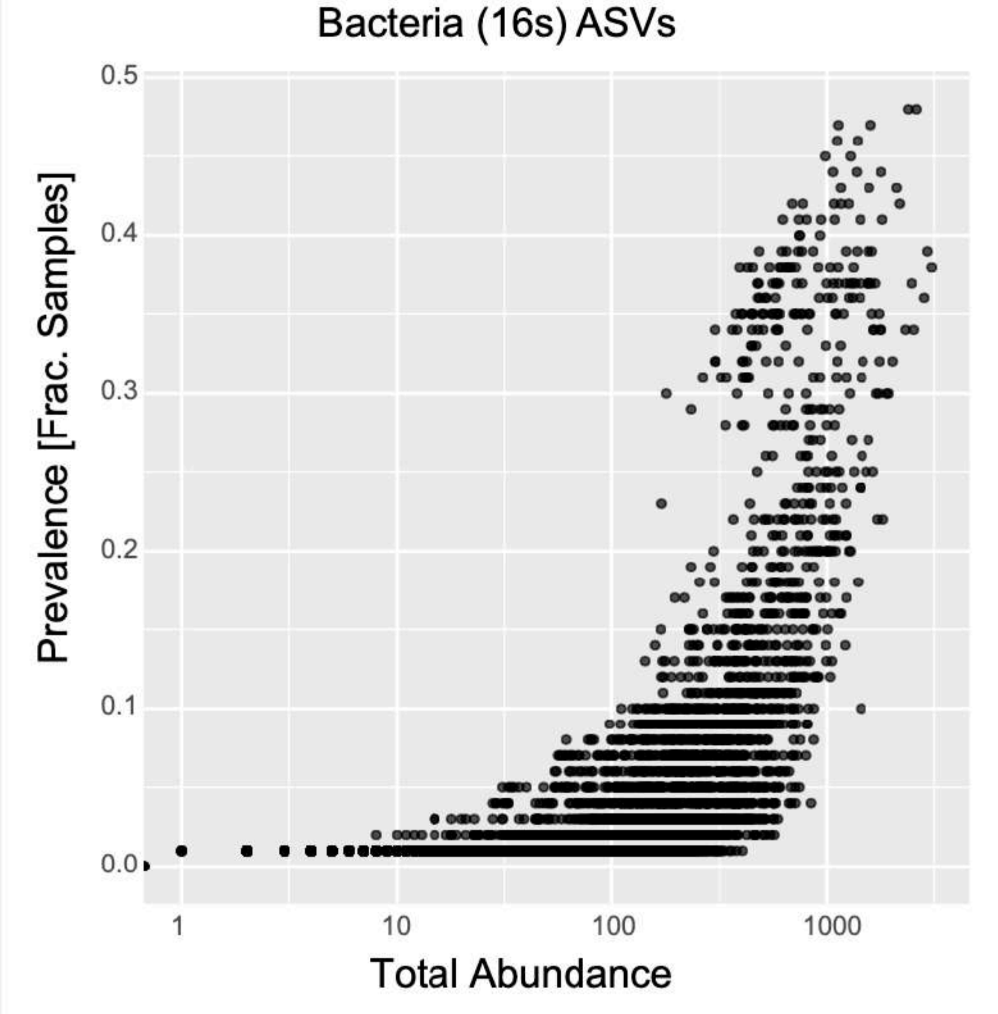
Developmental stage



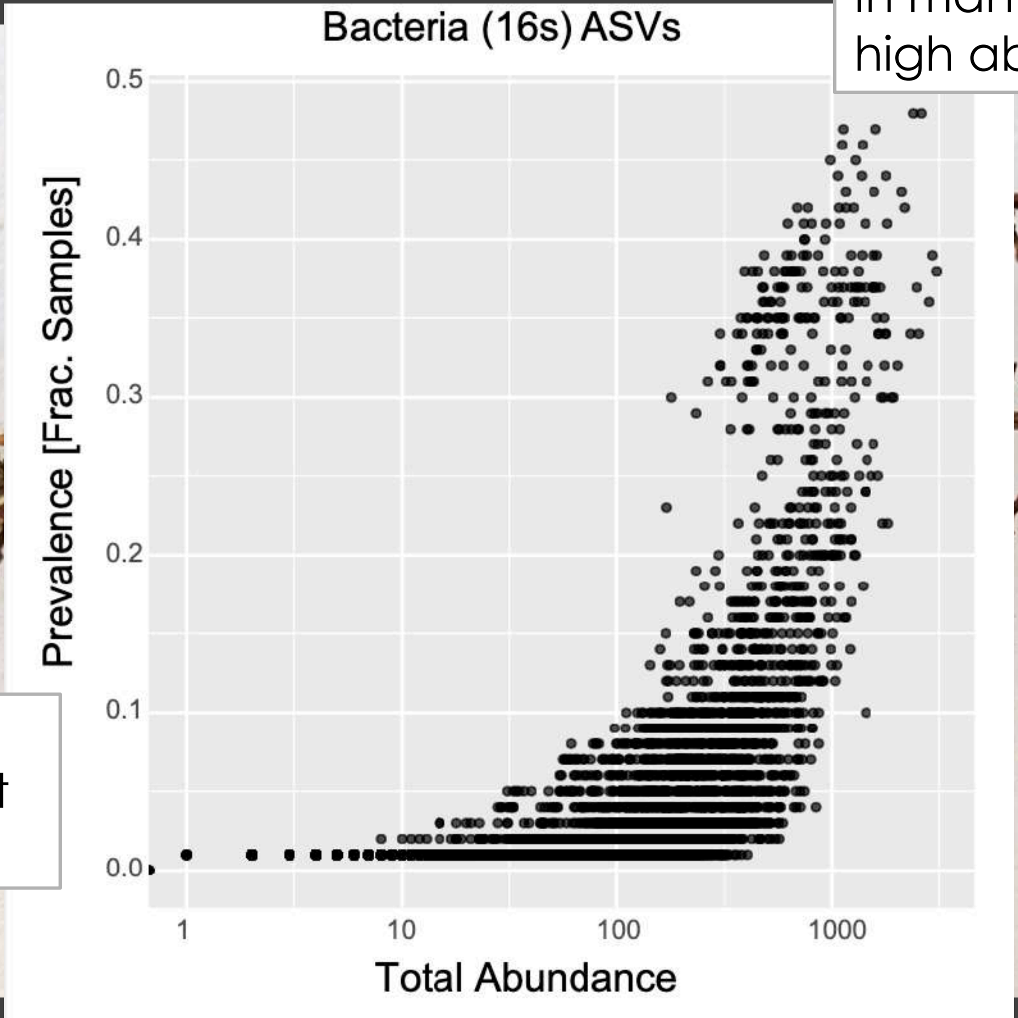
Spatial location

The consequences of pooling samples

The consequences of pooling samples



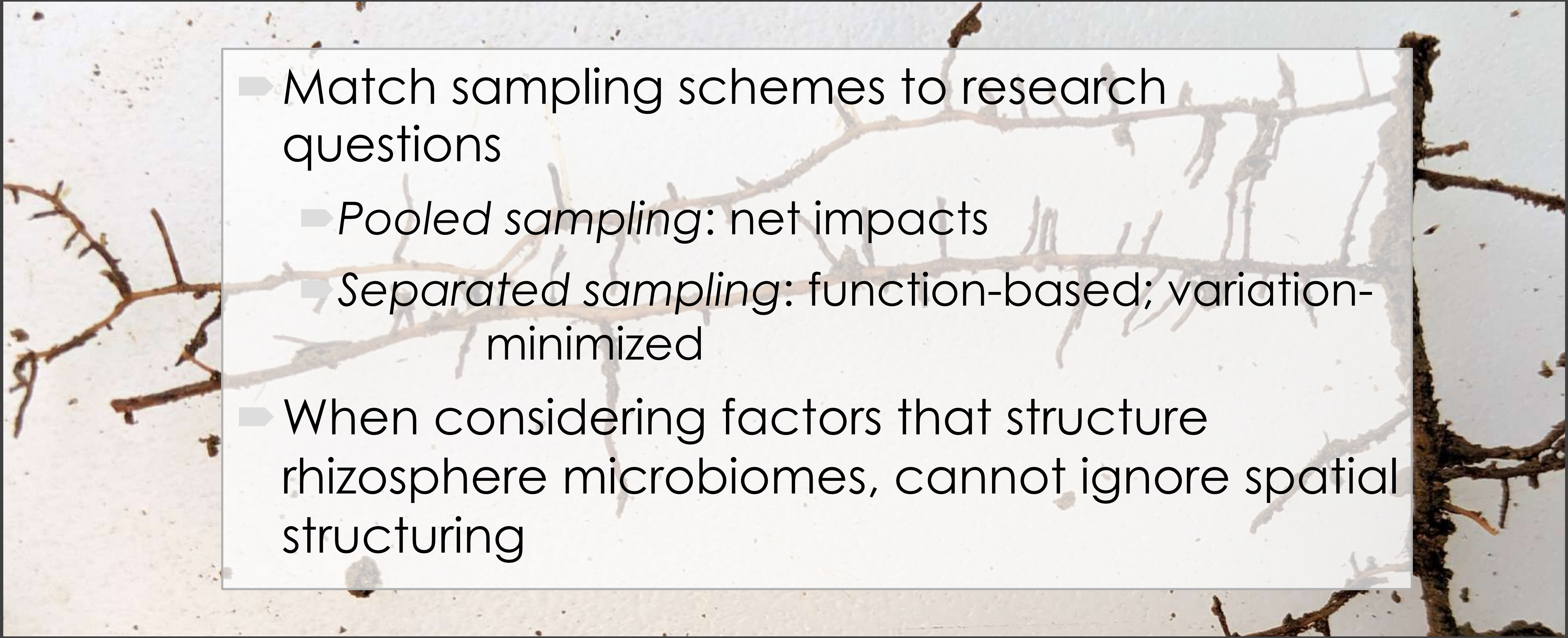
The consequences of pooling samples



"Core"
In many samples at
high abundance

"Rare"
In few samples at
low abundance

How can functionally explicit sampling advance rhizosphere microbial research?



- Match sampling schemes to research questions
  - *Pooled sampling*: net impacts
  - *Separated sampling*: function-based; variation-minimized
- When considering factors that structure rhizosphere microbiomes, cannot ignore spatial structuring

Applying these lessons in vineyards

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Bishopsvineyard.org

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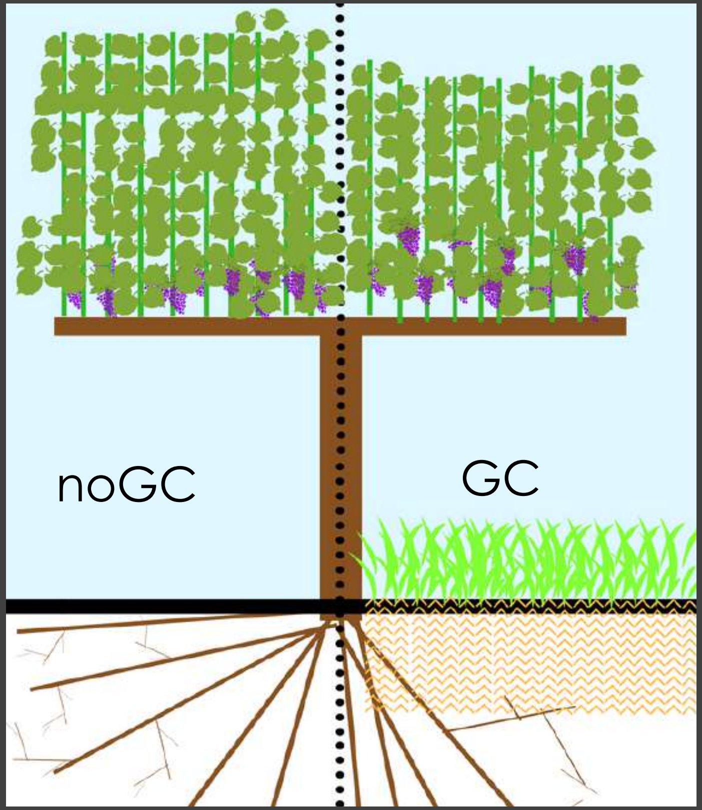
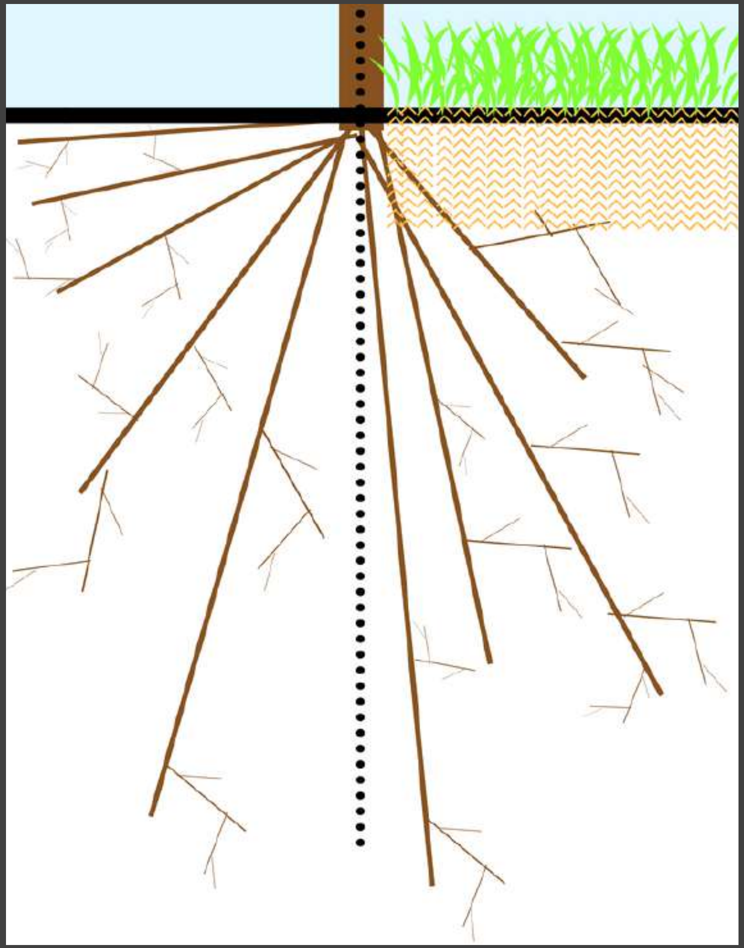
Bishopsvineyard.org

Grapevines shift roots deeper in response to groundcovers





Spatial heterogeneity across depth

- Soil moisture ↑
- Nutrient availability ↓
- Carbon content ↓
- Microbial biomass & diversity ↓

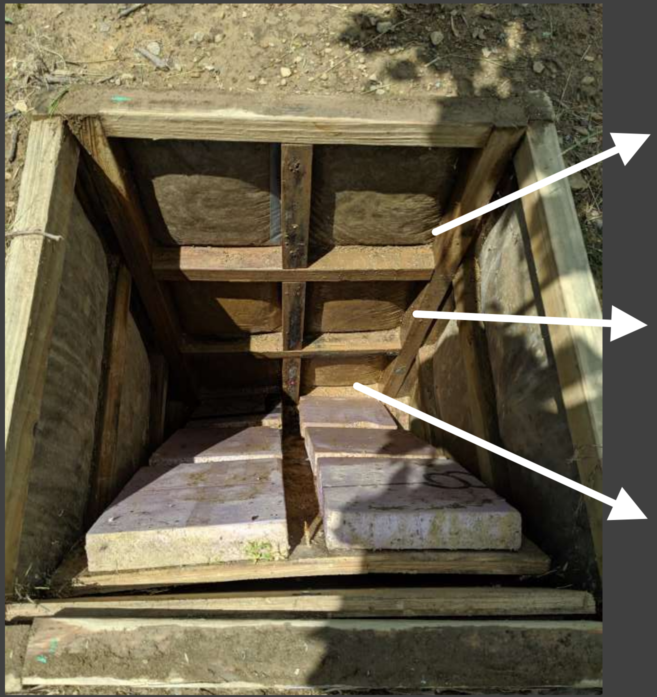
Study Site (2020)

- PSU vineyard at Rock Springs
- Noiret grapevines (*Vitis* hybrid)

- Groundcover treatment(fall 2016)
  - **noGC** (herbicide)
  - **GC** (Red Fescue; *Festuca rubra*)

- 32, 1-m deep root boxes

Competition varies with depth

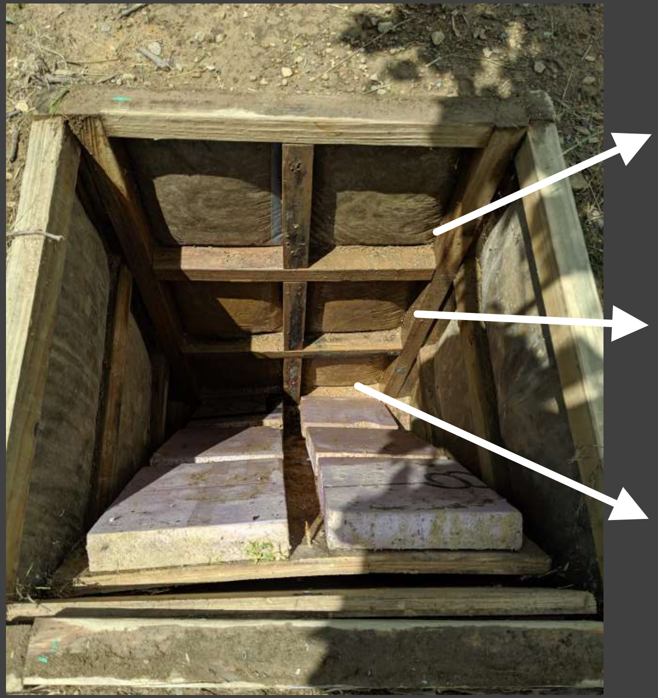


High competition
0-30 cm

Moderate competition
31-60 cm

Indirect competition
61-100 cm

Root distributions and sampling



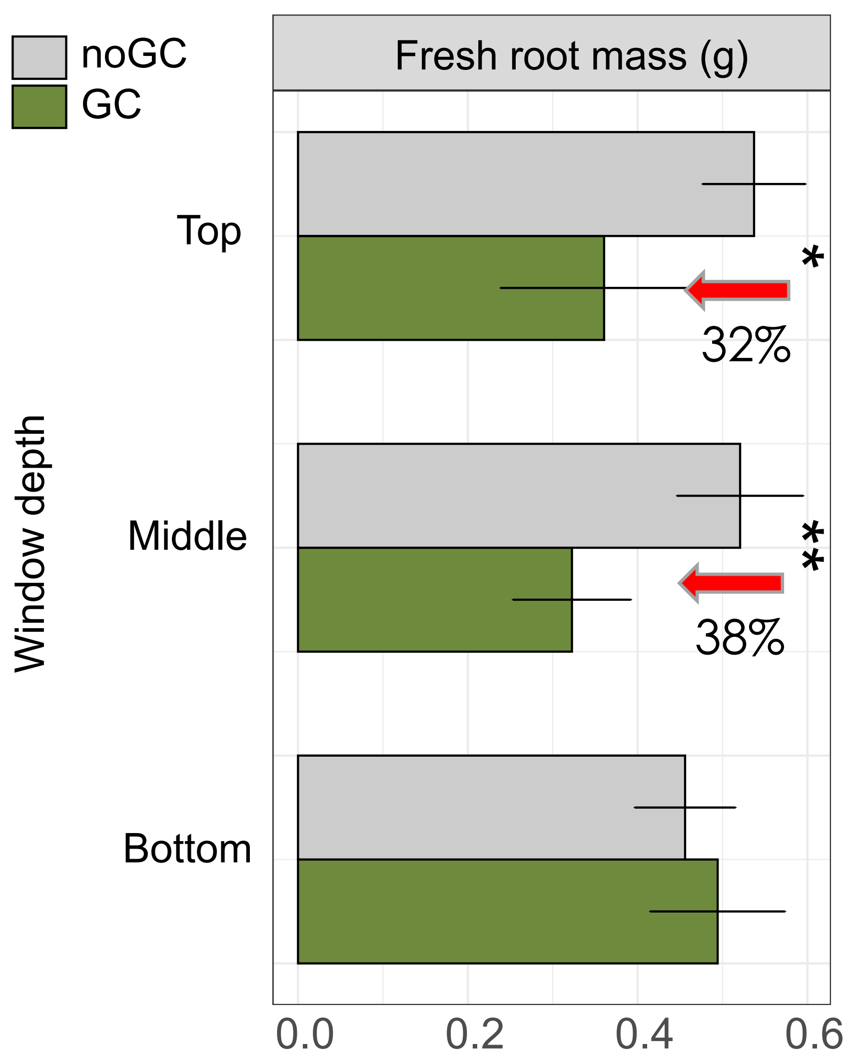
High competition
0-30 cm

Moderate competition
31-60 cm

Indirect competition
61-100 cm

- Absorptive root sampling during peak root growth (July 13-17, 2020)
- Fresh root weight (g)
- Counts of new white roots at each depth
- Rhizosphere removed by vortexing in PBS buffer in the field
- Concurrent study on root transcriptomics and metabolomics

Root systems shift deeper under GC



*$p<0.05$, **$p<0.01$, ***$p<0.001$

Fleishman et al. 2022, *In Prep*

Shifts beyond the groundcover rooting zone are closely related to the proportion of new roots at depth

Common practices of shallow soil sampling and pooled root sampling may have obscured findings

Fleishman et al. 2022, *In Prep*

Does the method of removing the rhizosphere matter?

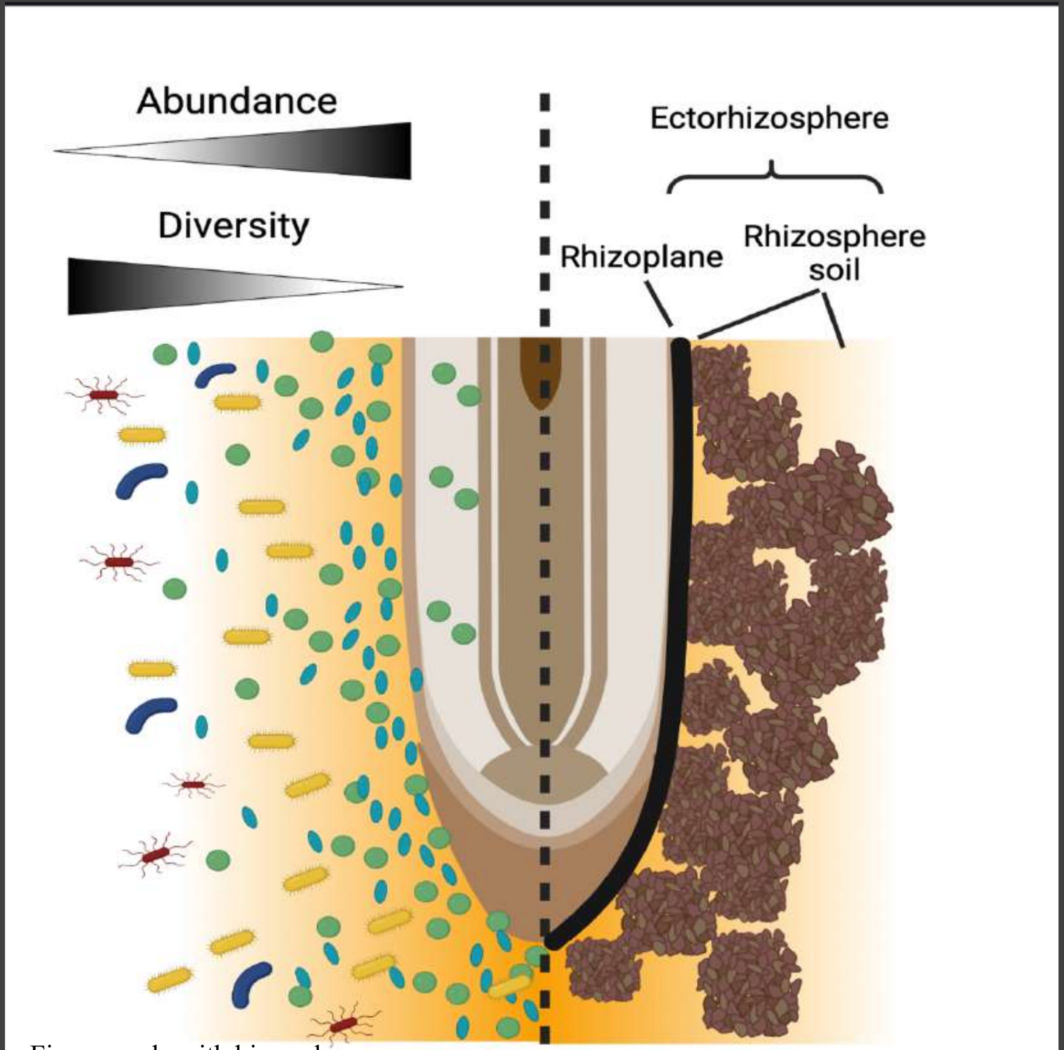
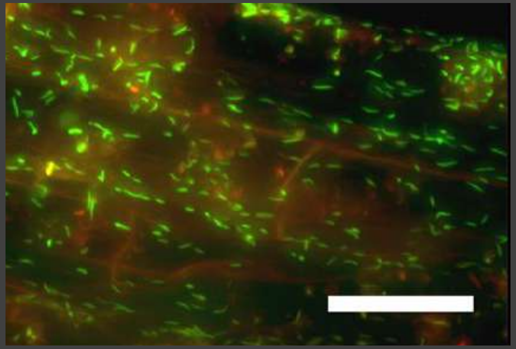


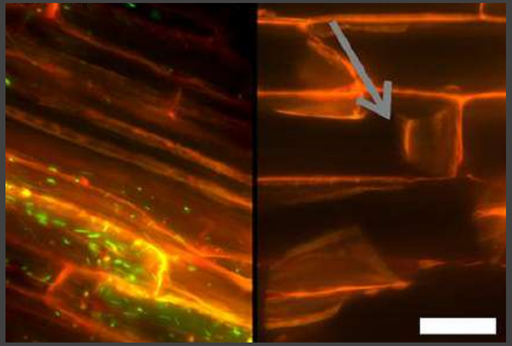
Figure made with biorender.com

Fleishman et al. 2022, *In Review*

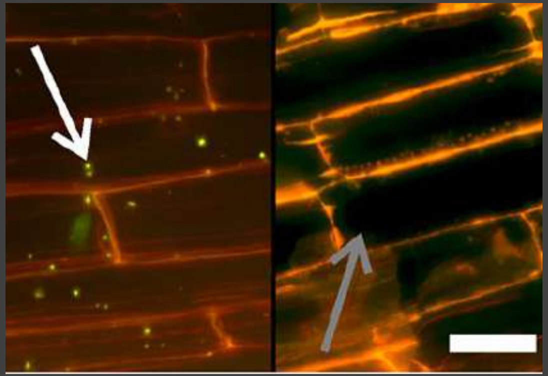
Untreated



Low intensity sonication

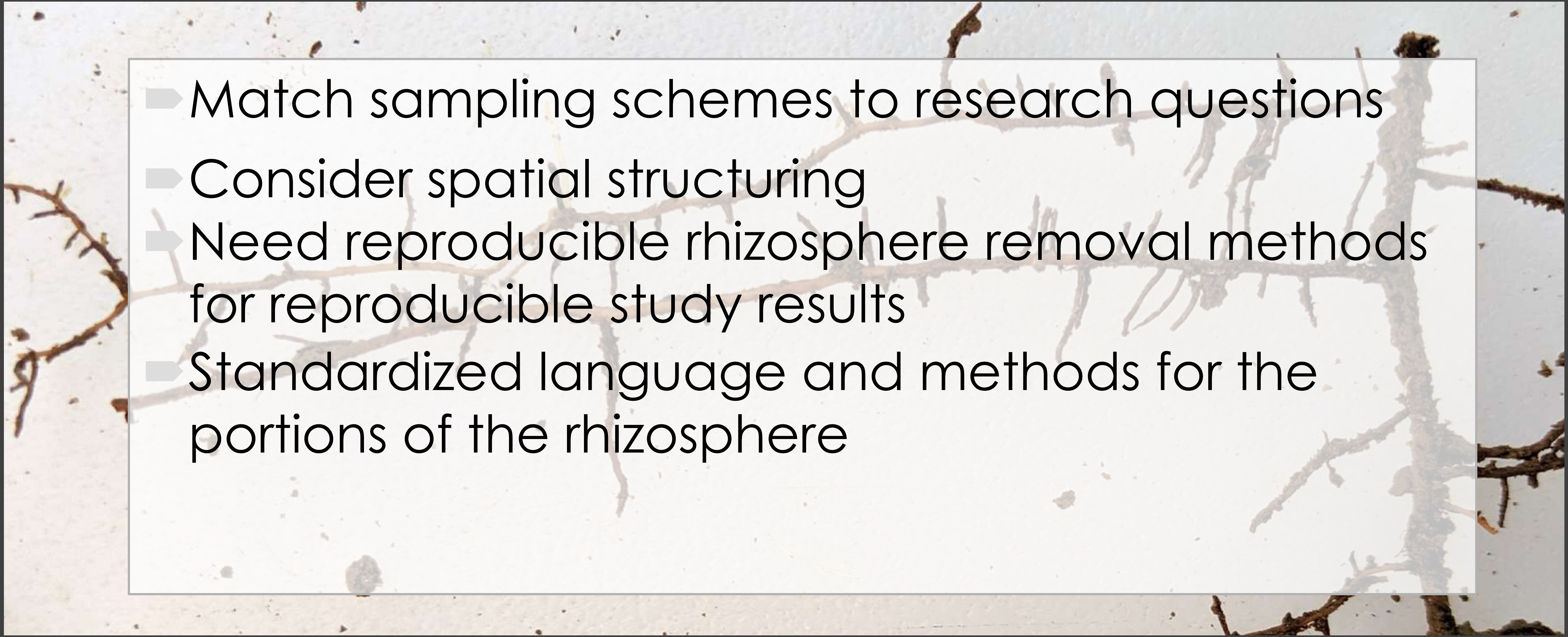


High intensity sonication



Richter-Heitmann et al., Front. Microbiol. 2016

How can we improve rhizosphere methodologies?



- Match sampling schemes to research questions
- Consider spatial structuring
- Need reproducible rhizosphere removal methods for reproducible study results
- Standardized language and methods for the portions of the rhizosphere

Acknowledgements

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King, W.L., et al., 2021. The hierarchy of root branching order determines bacterial composition, microbial carrying capacity and microbial filtering. Commun Biol 4, 483. https://doi.org/10.1038/s42003-021-01988-4

Penn State Microbiome Center

- Established in 2016
- 80 Research Groups from 10 different colleges and institutes (arts and anthropology to zygotes and zoology; human, animal, plant, environmental health)
- 40 weekly seminars and 2-3 workshops per semester
- Kick Start Program for beginners
- Data Analysis Working Group (DAWG) led by students and postdocs
- Industrial programming and partnerships (contact CaroleeBull@psu.edu for information)
- Formal Microbiome Sciences Dual Title being proposed
- NEW SENIOR LEVEL DIRECTOR being recruited

- Visit our website: microbiome.psu.edu
- Follow us on Twitter @PSUmBiome