Microbial communities associated with alfalfa in soils with stand establishment failures.

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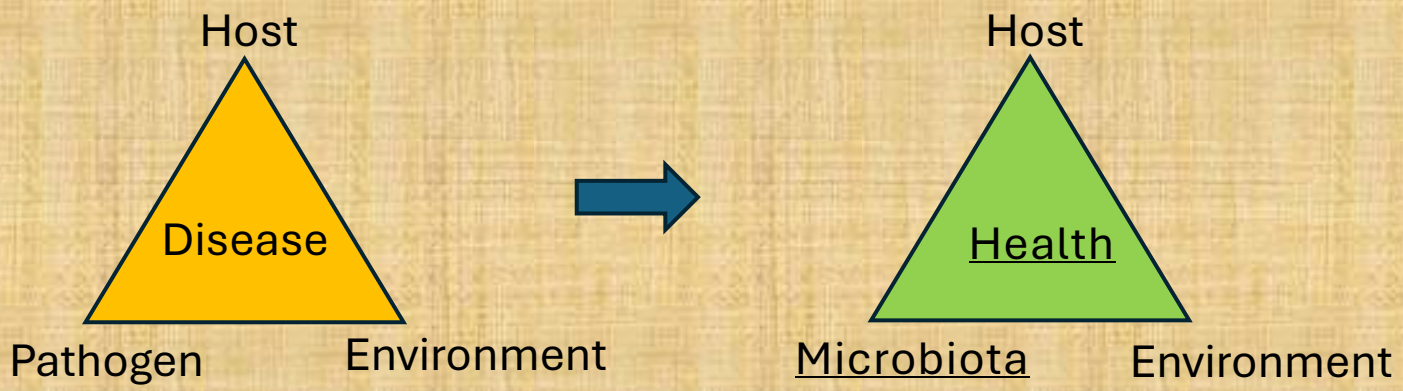
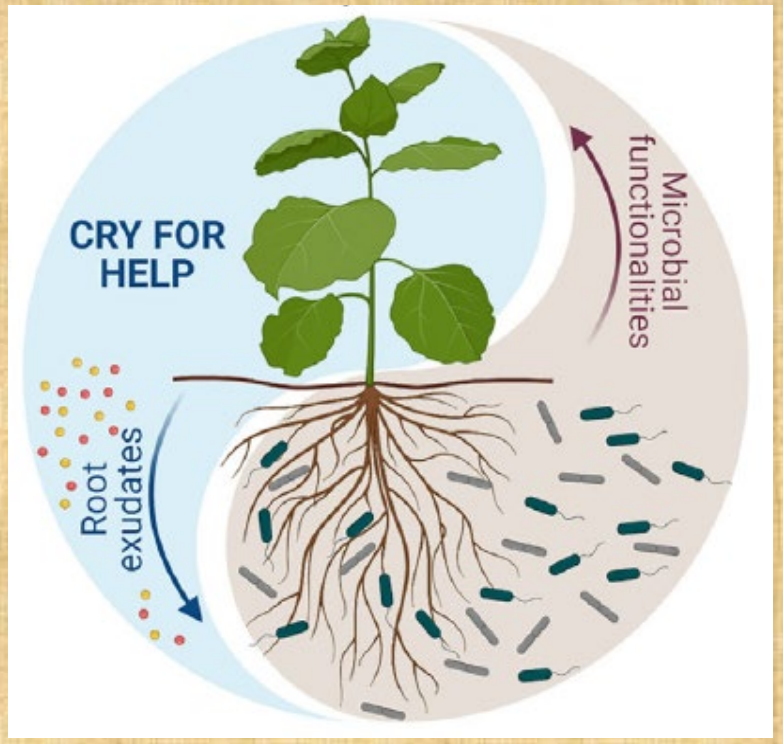
Background

- Root pathogens – an important cause of alfalfa stand establishment problems.
- Numerous oomycete and fungal pathogens cause damping off and seedling diseases.
- Diagnosing causal organisms of establishment failures can be challenging and laborious.

Plant microbiomes and changing paradigms

- Diverse plant-associated microbes play many roles in plant health.
- Plants can ‘recruit’ species- and genotype- specific microbial partners.
- ‘Cry for help’ model – stressed plants select for beneficial microbial taxa.





Goals

- What drives the composition and diversity of alfalfa microbiomes?

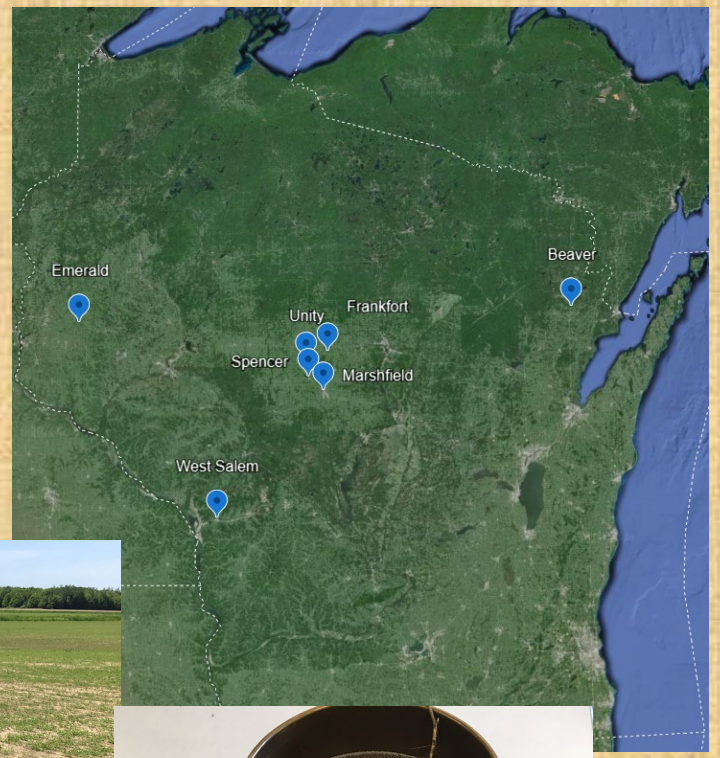
- What pathogens and potential pathogens are associated with soils with establishment problems?

- Can we identify microbial taxa associated with alfalfa pathogens?

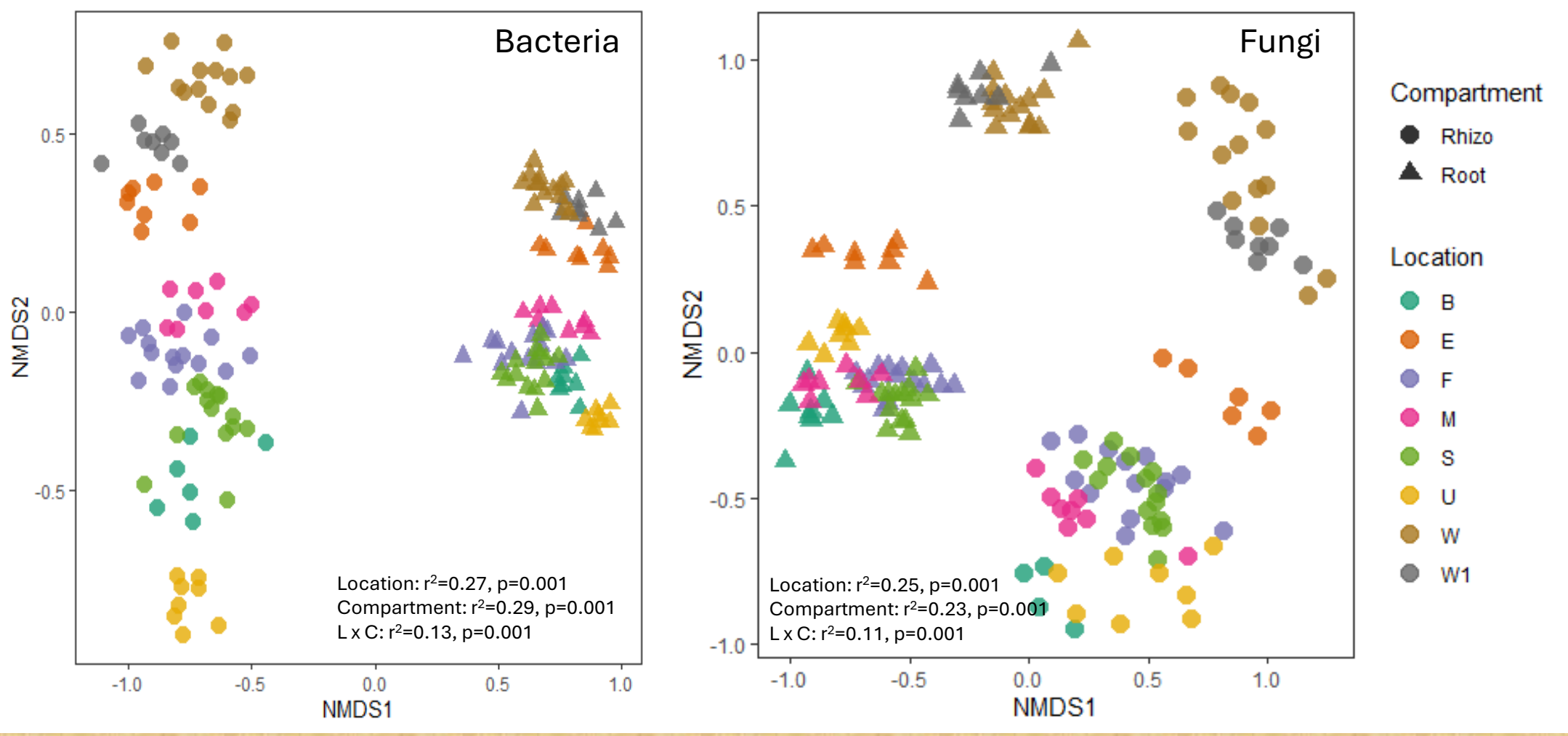
- Is there a consistent ‘core’ alfalfa microbiome?

Methods

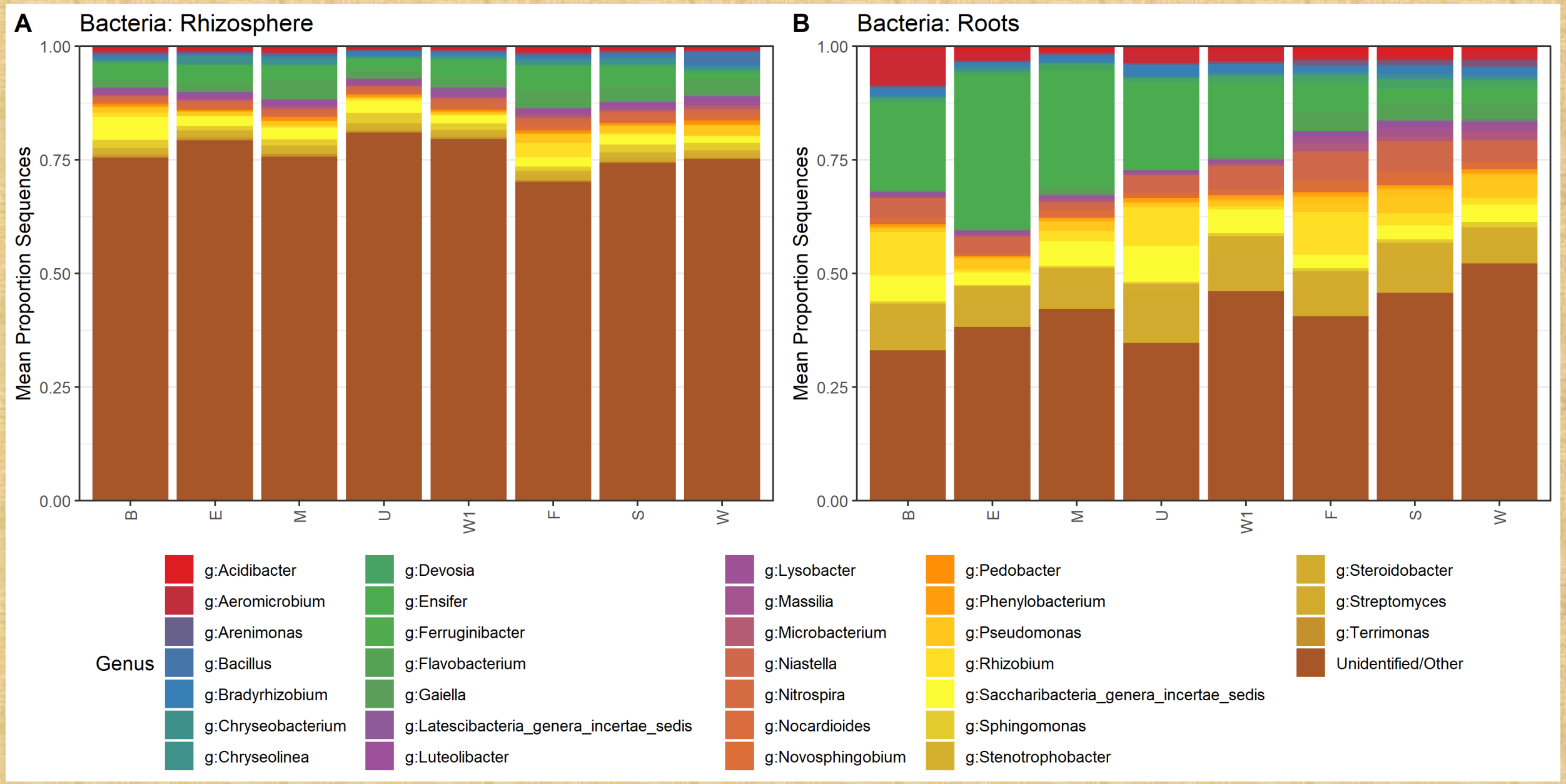
- 2 alfalfa varieties (Aphanomyces race 1 or race 1 and 2 resistance)
- 5 locations in 2020, 3 locations in 2021
- 2 plant compartments – rhizosphere and root.
- High-throughput sequencing of fungal and bacterial marker genes.
- qPCR quantification of soilborne pathogens
    - Aphanomyces euteiches
    - Phytophthora medicaginis
    - Pythium ultimum
    - P. irregulare
    - P. sylvaticum

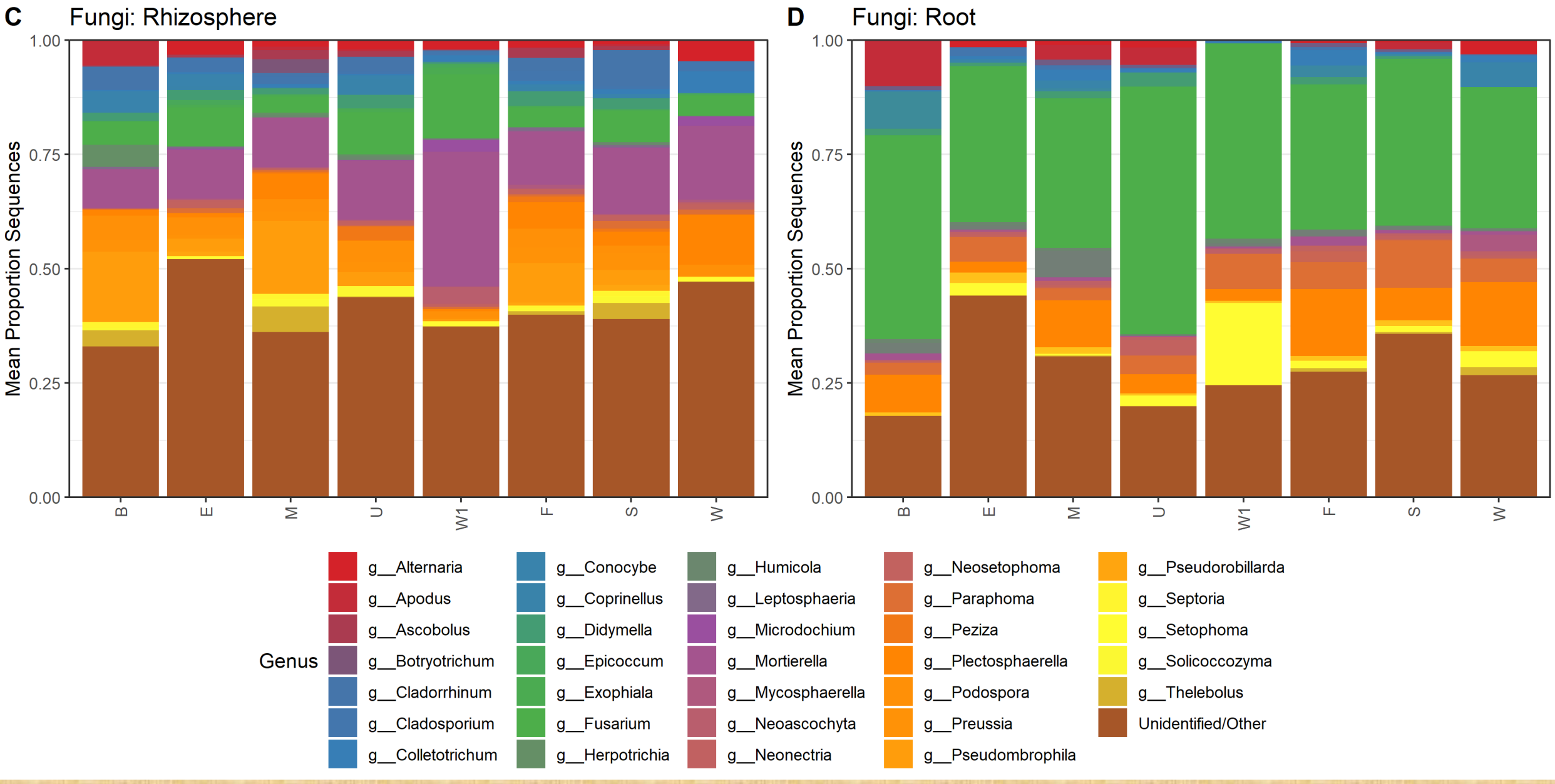


What drives the composition and diversity of alfalfa microbiomes?



Location and root compartment were strong drivers of alfalfa microbiome composition.


A
Bacteria: Rhizosphere
B
Bacteria: Roots
Mean Proportion Sequences
1.00
0.75
0.50
0.25
0.00
B
E
M
U
W1
F
S
W
Genus
g:Acidibacter
g:Aeromicrobium
g:Arenimonas
g:Bacillus
g:Bradyrhizobium
g:Chryseobacterium
g:Chryseolinea
g:Devosia
g:Ensifer
g:Ferruginibacter
g:Flavobacterium
g:Gaiella
g:Latescibacteria_genera_incertae_sedis
g:Luteolibacter
g:Lysobacter
g:Massilia
g:Microbacterium
g:Niastella
g:Nitrospira
g:Nocardioides
g:Novosphingobium
g:Pedobacter
g:Phenylobacterium
g:Pseudomonas
g:Rhizobium
g:Saccharibacteria_genera_incertae_sedis
g:Sphingomonas
g:Stenotrophobacter
g:Steroidobacter
g:Streptomyces
g:Terrimonas
Unidentified/Other


C
Fungi: Rhizosphere
D
Fungi: Root
Mean Proportion Sequences
1.00
0.75
0.50
0.25
0.00
B
E
M
U
W1
F
S
W
Genus
g__Alternaria
g__Apodus
g__Ascobolus
g__Botryotrichum
g__Cladorrhinum
g__Cladosporium
g__Colletotrichum
g__Conocybe
g__Coprinellus
g__Didymella
g__Epicoccum
g__Exophiala
g__Fusarium
g__Herpotrichia
g__Humicola
g__Leptosphaeria
g__Microdochium
g__Mortierella
g__Mycosphaerella
g__Neoascochyta
g__Neonectria
g__Neosetophoma
g__Paraphoma
g__Peziza
g__Plectosphaerella
g__Podospora
g__Preussia
g__Pseudombrophila
g__Pseudorobillarda
g__Septoria
g__Setophoma
g__Solicoccozyma
g__Thelebolus
Unidentified/Other

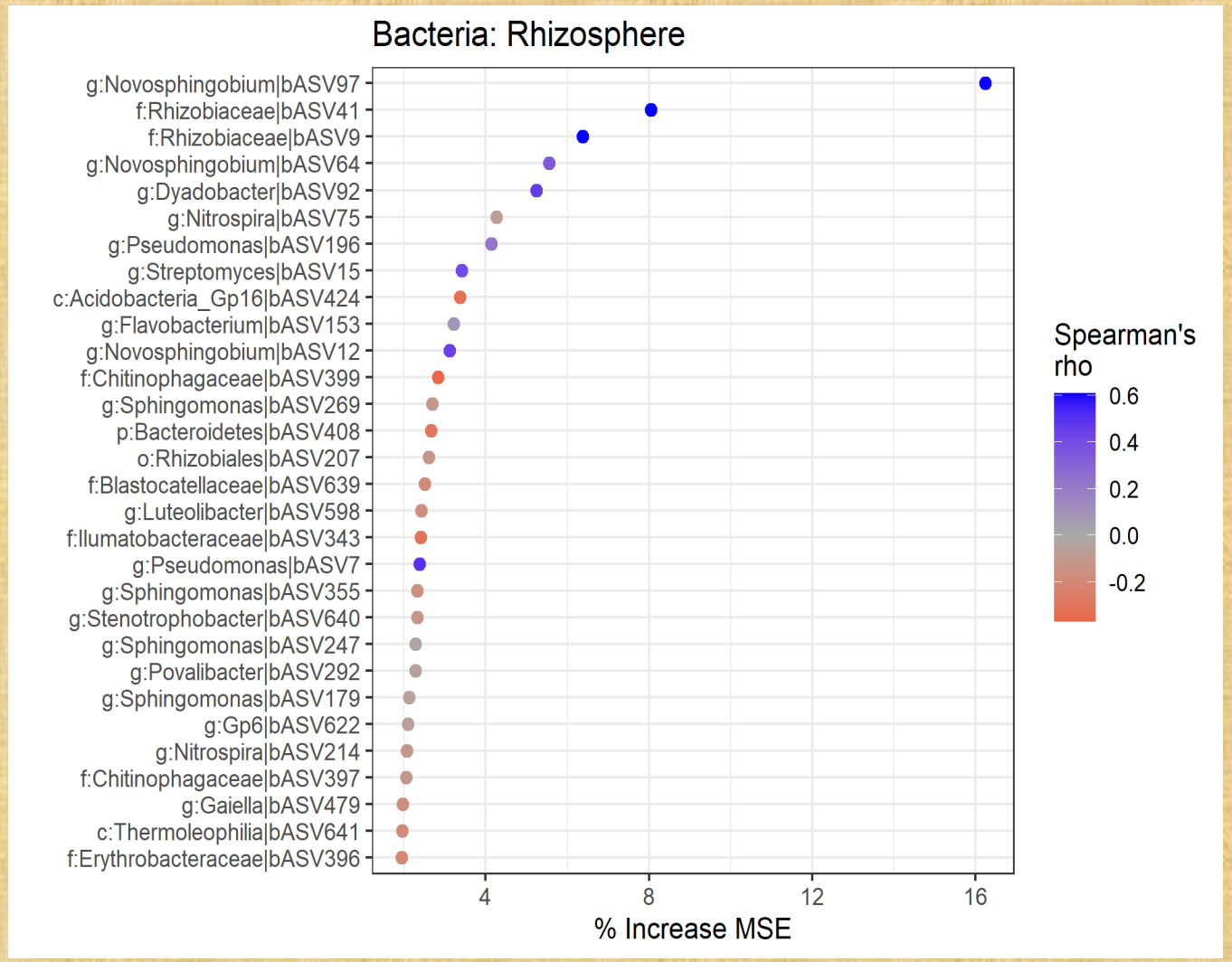
Pathogenic Fungi	F	H	WS21	B	E	MR	U	WS
Botrytis spp (1 ASV)	0 ±0 (0/14)	0 ±0 (0/14)	0 ±0 (0/14)	0 ±0 (0/4)	0 ±0 (0/6)	0 ±0 (1/8)	0 ±0 (0/8)	0 ±0 (0/8)
Colletotrichum spp (12 ASVs)	0.03 ±0.07 (14/14)	0 ±0.01 (14/14)	0.01 ±0.02 (13/14)	0 ±0 (4/4)	0.01 ±0.01 (6/6)	0.02 ±0.03 (8/8)	0.01 ±0.02 (6/8)	0 ±0 (7/8)
Coprinus spp (2 ASVs)	0 ±0 (0/14)	0 ±0 (0/14)	0 ±0 (0/14)	0 ±0 (0/4)	0 ±0 (0/6)	0 ±0 (0/8)	0 ±0 (0/8)	0 ±0 (2/8)
Cylindrocarpon spp (4 ASVs)	0 ±0 (0/14)	0 ±0 (1/14)	0 ±0 (11/14)	0 ±0 (0/4)	0 ±0 (5/6)	0 ±0 (0/8)	0 ±0 (0/8)	0 ±0 (8/8)
Phoma/Didymella spp (3 ASVs)	0.02 ±0.02 (14/14)	0.01 ±0 (14/14)	0 ±0 (6/14)	0.01 ±0.01 (4/4)	0.01 ±0.01 (6/6)	0.02 ±0.03 (8/8)	0.03 ±0.04 (8/8)	0 ±0 (8/8)
Fusarium oxysporum complex (2 ASVs)	0.07 ±0.07 (14/14)	0.1 ±0.04 (14/14)	0.06 ±0.04 (14/14)	0.07 ±0.03 (4/4)	0.05 ±0.04 (6/6)	0.08 ±0.07 (8/8)	0.15 ±0.05 (8/8)	0.13 ±0.05 (8/8)
Fusarium solani complex (8 ASVs)	0.24 ±0.14 (14/14)	0.23 ±0.11 (14/14)	0.24 ±0.17 (14/14)	0.37 ±0.08 (4/4)	0.29 ±0.2 (6/6)	0.23 ±0.11 (8/8)	0.39 ±0.16 (8/8)	0.3 ±0.08 (8/8)
Leptosphaeria spp (5 ASVs)	0 ±0 (10/14)	0 ±0 (9/14)	0 ±0 (2/14)	0 ±0 (0/4)	0 ±0 (0/6)	0 ±0 (1/8)	0 ±0 (2/8)	0 ±0 (1/8)
Microdochium spp (6 ASVs)	0 ±0 (11/14)	0 ±0 (10/14)	0.01 ±0.01 (14/14)	0 ±0 (3/4)	0 ±0 (5/6)	0 ±0 (4/8)	0 ±0 (8/8)	0.01 ±0 (8/8)
Mycoleptodiscus spp (1 ASV)	0 ±0 (0/14)	0 ±0 (1/14)	0.04 ±0.05 (13/14)	0 ±0 (0/4)	0 ±0 (6/6)	0 ±0 (0/8)	0 ±0 (0/8)	0 ±0 (8/8)
Paraphoma spp (12 ASVs)	0.06 ±0.04 (14/14)	0.1 ±0.06 (14/14)	0.05 ±0.07 (14/14)	0.03 ±0.04 (4/4)	0.05 ±0.04 (6/6)	0.03 ±0.03 (8/8)	0.04 ±0.04 (8/8)	0.08 ±0.04 (8/8)
Plectosphaerella spp (5 ASVs)	0.15 ±0.07 (14/14)	0.07 ±0.05 (14/14)	0.14 ±0.13 (14/14)	0.08 ±0.04 (4/4)	0.02 ±0.03 (6/6)	0.1 ±0.05 (8/8)	0.04 ±0.04 (8/8)	0.03 ±0.02 (8/8)
Pseudopeziza medicaginis (1 ASV)	0 ±0 (0/14)	0 ±0 (0/14)	0 ±0 (0/14)	0 ±0 (1/4)	0 ±0 (0/6)	0 ±0 (2/8)	0 ±0 (1/8)	0 ±0 (0/8)
Stagonospora spp (3 ASVs)	0 ±0 (2/14)	0 ±0 (0/14)	0 ±0 (0/14)	0 ±0 (0/4)	0 ±0 (0/6)	0 ±0 (1/8)	0 ±0 (1/8)	0 ±0 (0/8)
Stemphylium spp (3 ASVs)	0 ±0 (5/14)	0 ±0 (4/14)	0 ±0 (7/14)	0 ±0 (0/4)	0 ±0 (2/6)	0 ±0 (4/8)	0 ±0 (2/8)	0 ±0 (0/8)
Rhizoctonia/Thanatephorus (14 ASVs)	0.01 ±0.03 (4/14)	0.01 ±0.01 (12/14)	0.03 ±0.06 (11/14)	0 ±0 (0/4)	0.02 ±0.03 (6/6)	0.01 ±0.01 (8/8)	0 ±0 (3/8)	0 ±0.01 (3/8)

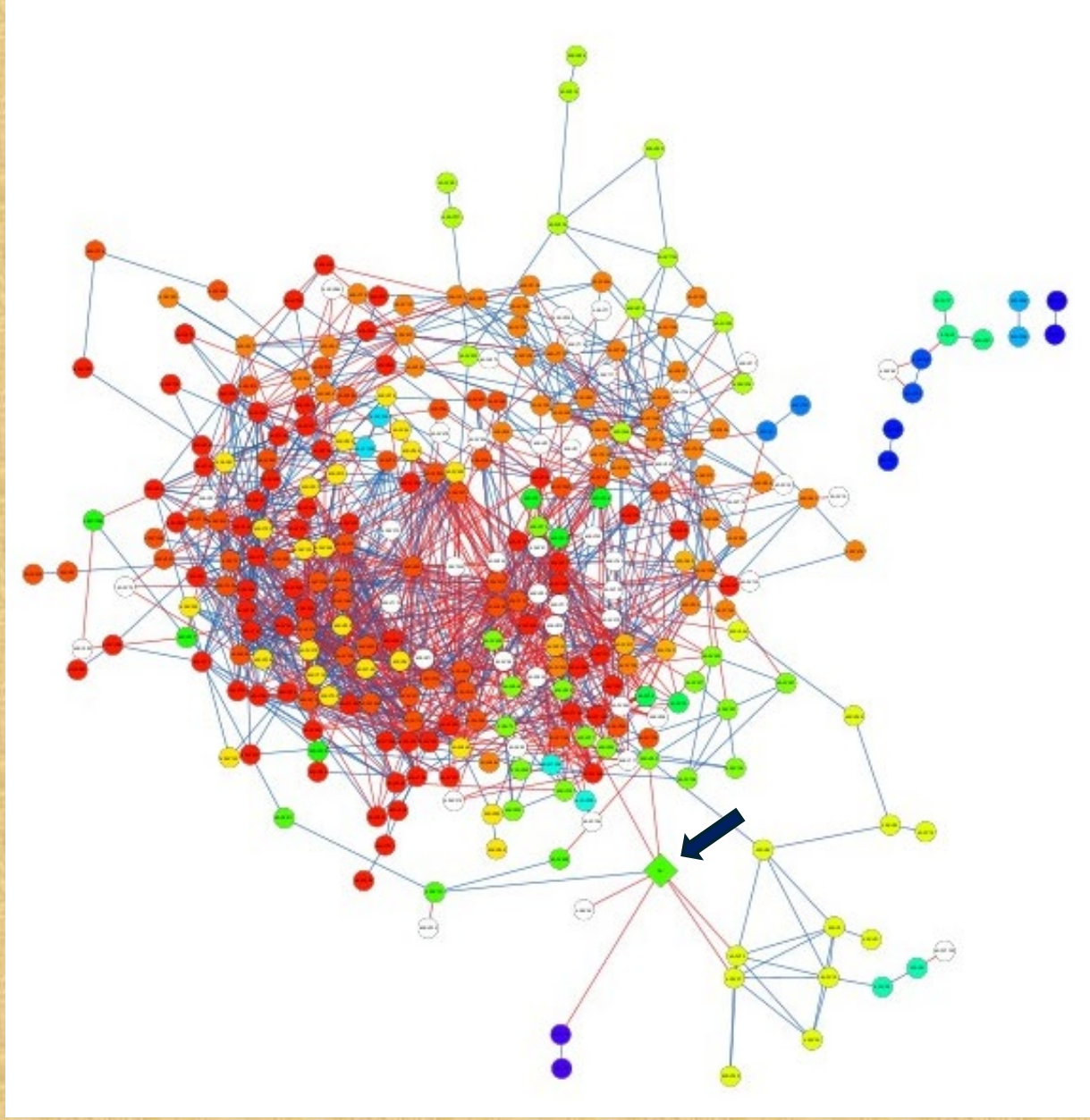
Can we identify microbial taxa associated with alfalfa pathogens?

- Robust associations in microbiome data can be challenging to establish.
- Random forest regression and co-occurrence networks are attractive approaches for identifying relationships.

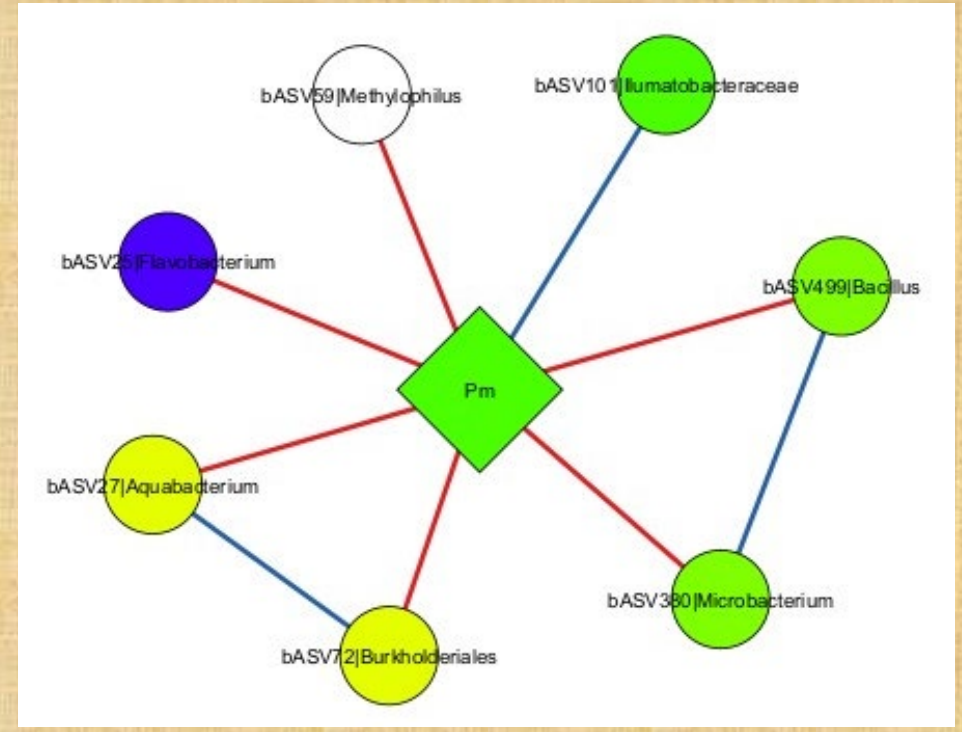
Random Forest Regression Coefficients (r2) from CLR-transformed abundances of top 200 taxa to predict pathogen abundances (ng DNA/g soil)

	Rhizosphere		Root	
Pathogen	Bacteria	Fungi	Bacteria	Fungi
A. euteiches	0.507	0.087	0.272	0.572
P. medicaginis	0.678	0.866	0.634	0.749
Py. Irregulare	0.215	0.3	0.563	0.408
Py. Ultimum	0.936	0.839	0.718	0.602
Py. Sylvaticum	0.49	0.195	0.103	0.195

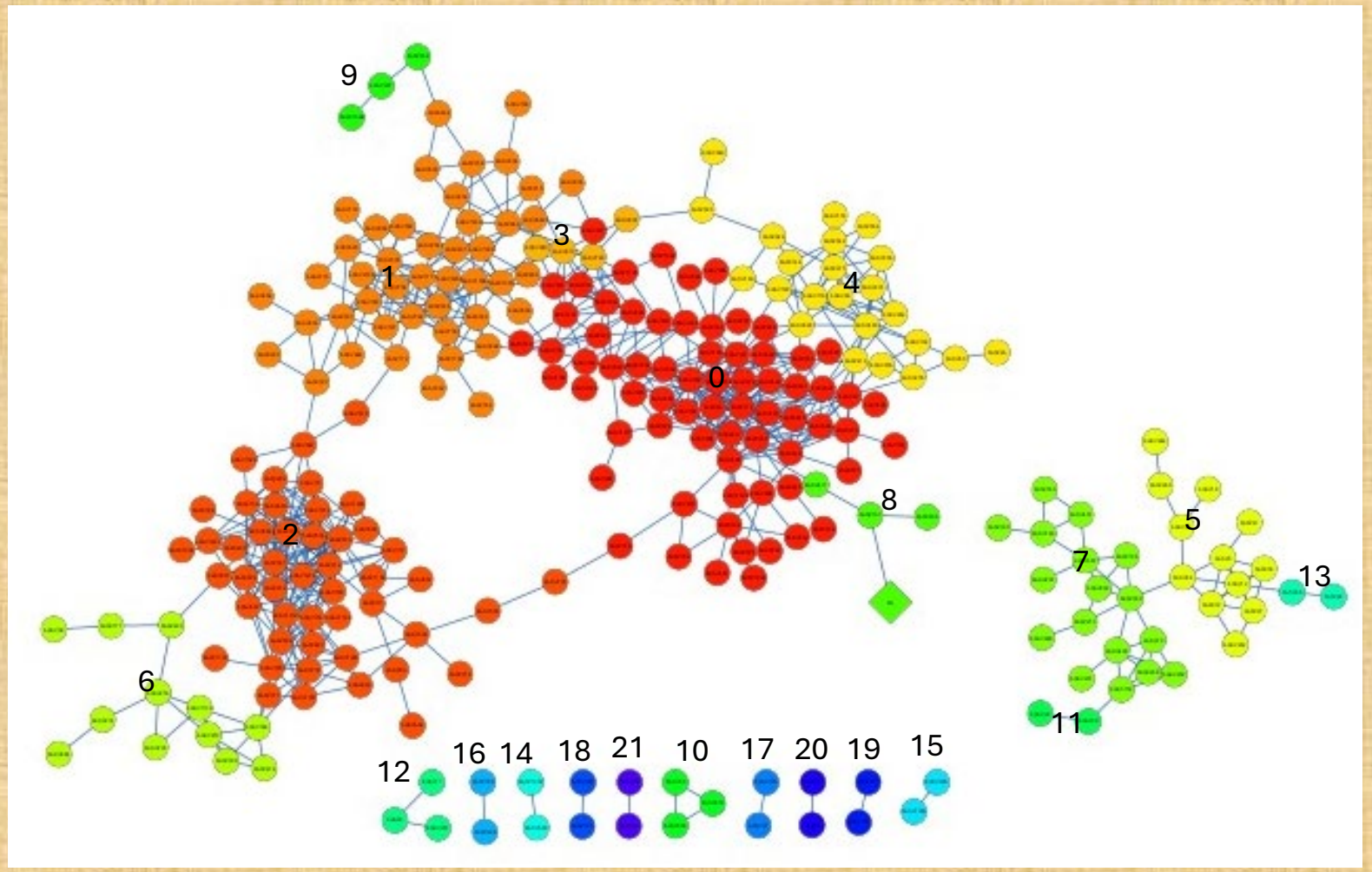


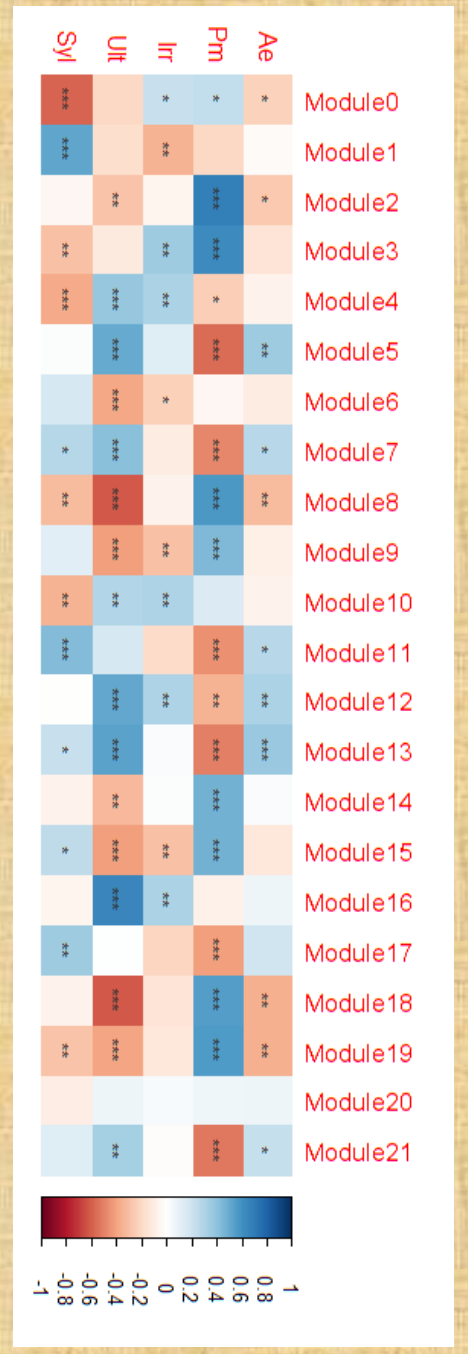


Microbial co-occurrence networks can offer insights into potential microbial interactions among taxa and with co-variates (e.g. pathogen abundances).



P. medicaginis subnetwork.





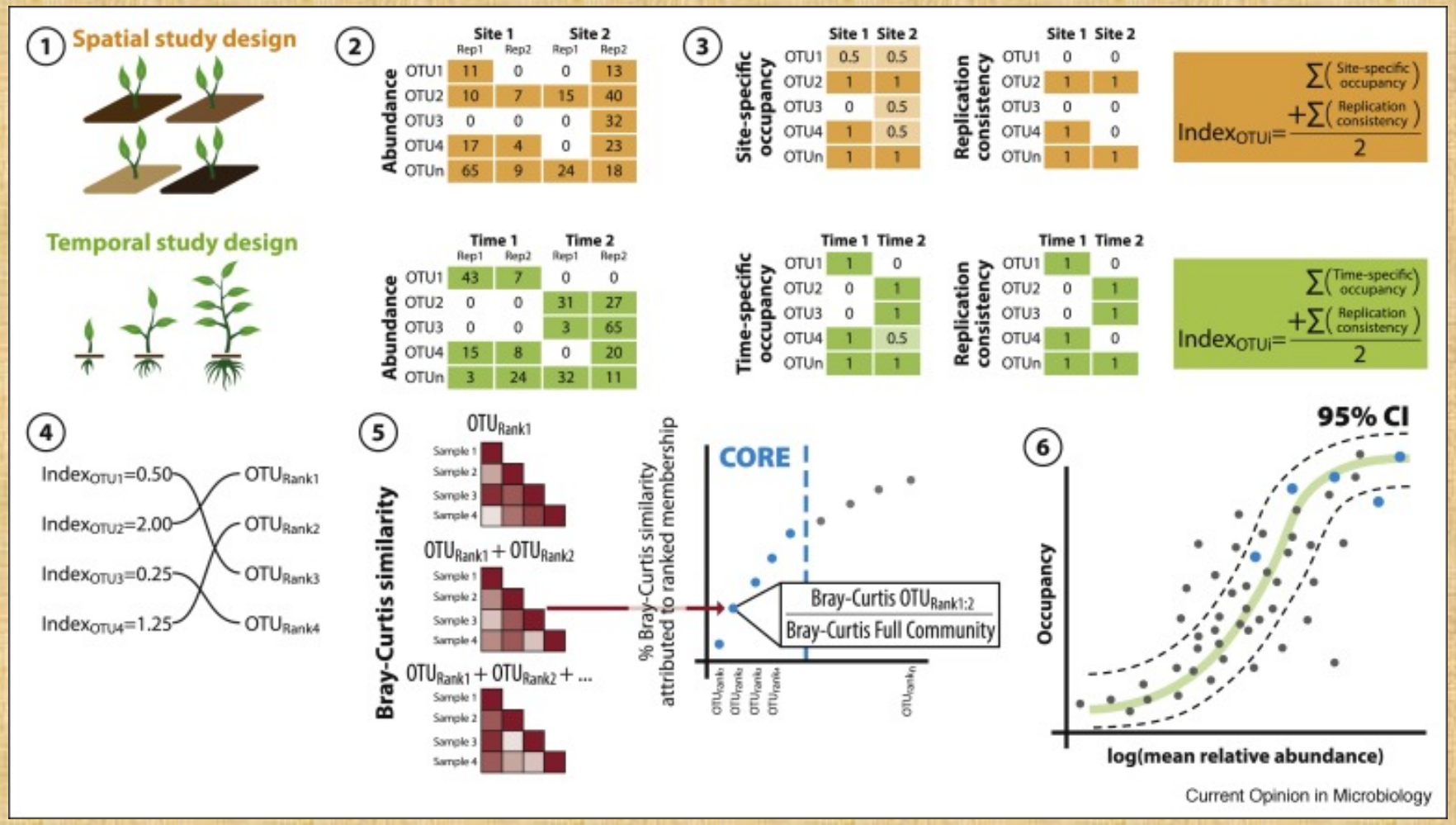
Networks can be decomposed into modules of co-occurring taxa, and sets of taxa can have distinct associations with pathogen abundances.

Prioritizing core members of alfalfa microbiomes

- Rank taxa based on their occupancy and contribution to Bray-Curtis dissimilarity.
- Model occupancy-abundance relationships.

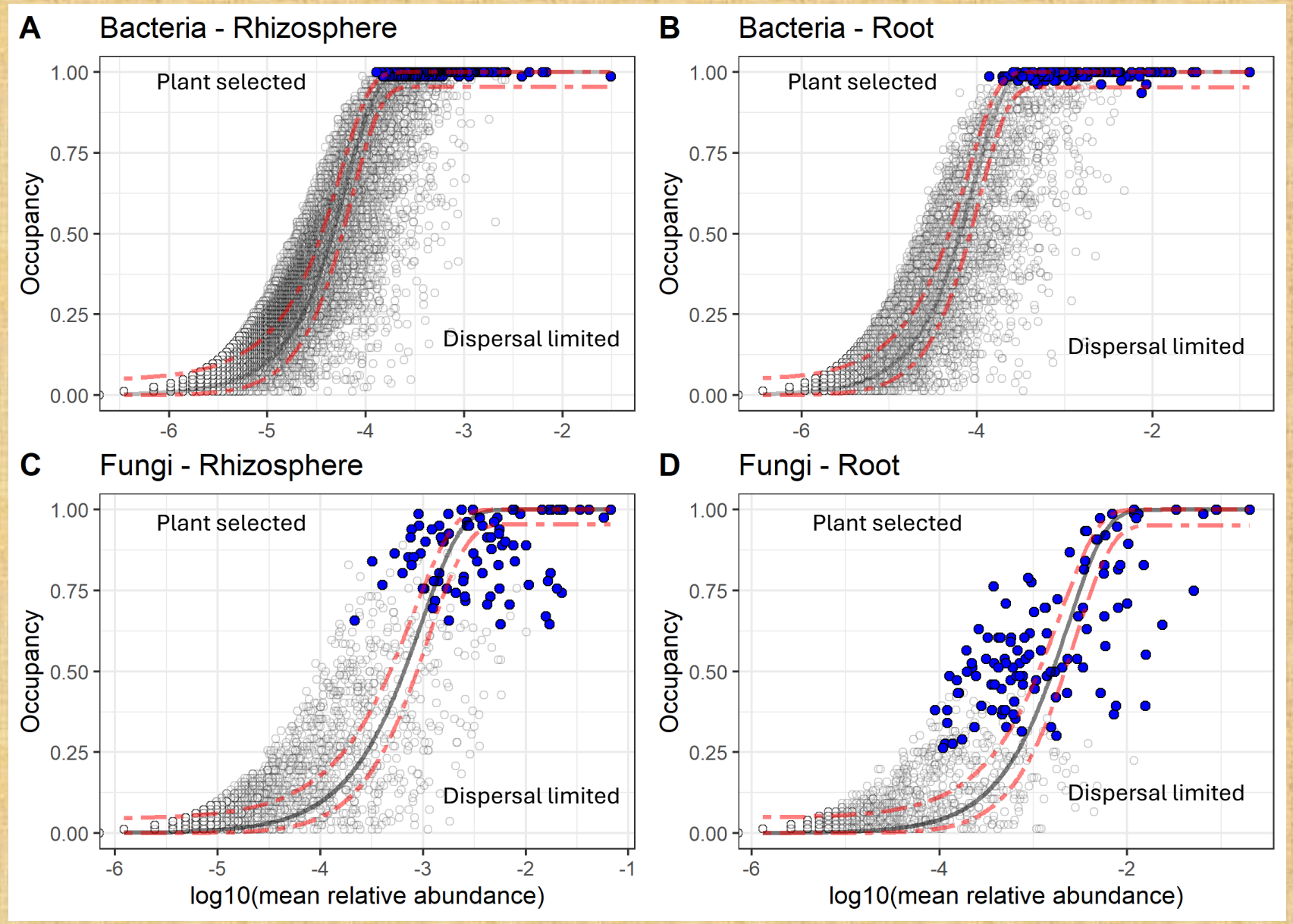
Neutral model assumes unlimited dispersal and no fitness differences among taxa.

Can infer neutral vs. deterministic assembly.

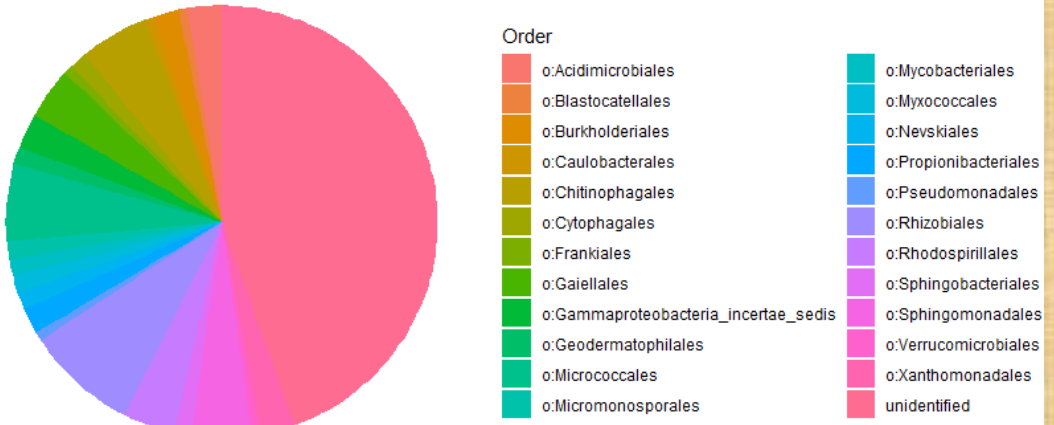


Shade and Stopnisek, 2019

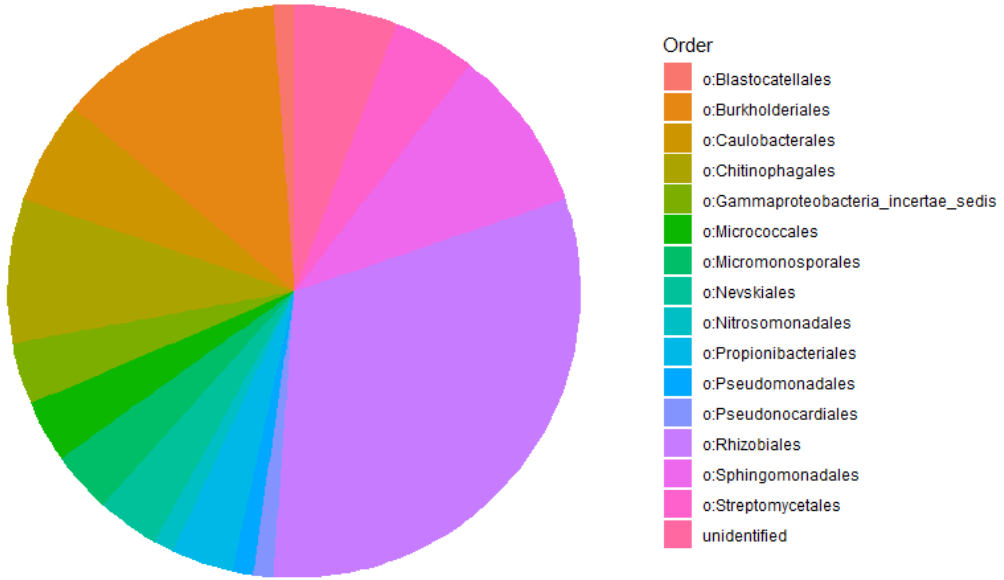
- Core bacterial taxa are generally ubiquitous in rhizosphere and root communities.
- Core fungal taxa have more variable distributions.

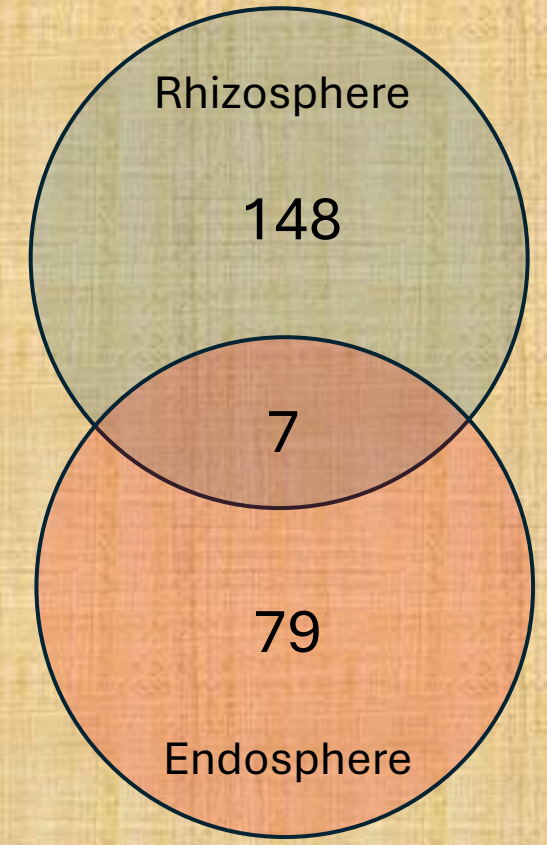


Rhizosphere-selected bacteria

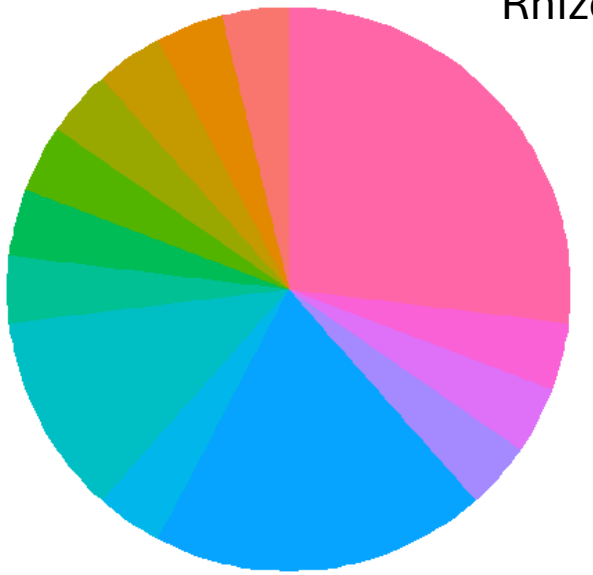


Root-selected bacteria





Generally little overlap between rhizosphere and root-selected bacteria.

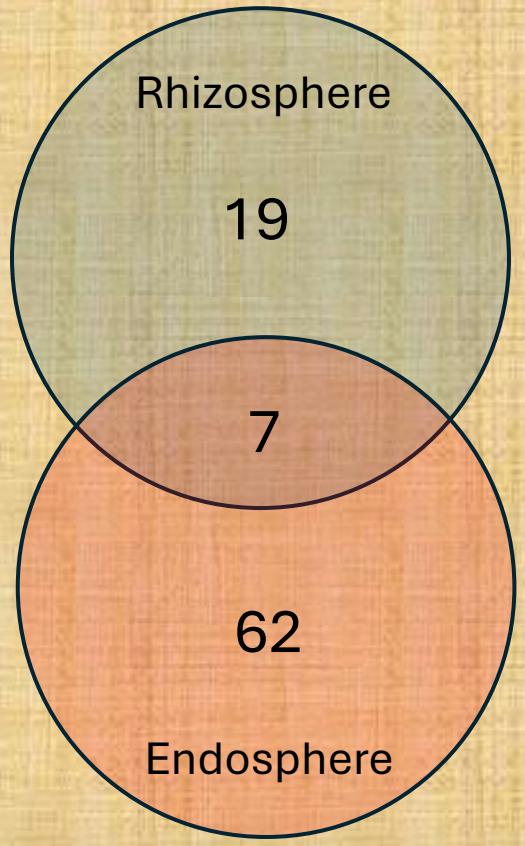


Rhizosphere-selected fungi

Family
- f__Bionectriaceae
- f__Chaetomiaceae
- f__Clavicipitaceae
- f__Ganodermataceae
- f__Herpotrichiellaceae
- f__Hypocreales_fam_Incertae_sedis
- f__Lasiosphaeriaceae
- f__Mortierellaceae
- f__Mrakiaceae
- f__Nectriaceae
- f__Orbiliaceae
- f__Phaeosphaeriaceae
- f__Piptocephalidaceae
- unidentified

Root-selected fungi

Family
- f__Bionectriaceae
- f__Boliniales_fam_Incertae_sedis
- f__Bulleribasidiaceae
- f__Chrysozymaceae
- f__Cladosporiaceae
- f__Clavicipitaceae
- f__Didymellaceae
- f__Filobasidiaceae
- f__Glomeraceae
- f__Glomerellaceae
- f__Helotiales_fam_Incertae_sedis
- f__Herpotrichiellaceae
- f__Lasiosphaeriaceae
- f__Microdochiaceae
- f__Mortierellaceae
- f__Mycosphaerellaceae
- f__Nectriaceae
- f__Orbiliales_fam_Incertae_sedis
- f__Phaeosphaeriaceae
- f__Piskurozymaceae
- f__Plectosphaerellaceae
- f__Pleosporaceae
- f__Sporidiobolaceae
- f__Sporormiaceae
- f__Xylariales_fam_Incertae_sedis
- unidentified



In contrast to bacteria, more core fungi are root-selected, including Nectriaceae (Fusarium) and Glomeraceae (AMF).

Take homes and next steps

- Rhizosphere and root microbiomes are a critical aspect of plant health.
- Sequencing approaches offer a broad picture of fungal pathogens present.
- Machine learning, networking, and ecologically motivated analyses can suggest important microbial taxa and consortia.
- Functional metagenomics and microbial cultures are needed to further develop and test hypotheses on microbial functions.

Thanks!

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