

Biotic and abiotic drivers of soil microbial communities in response to manure amendment



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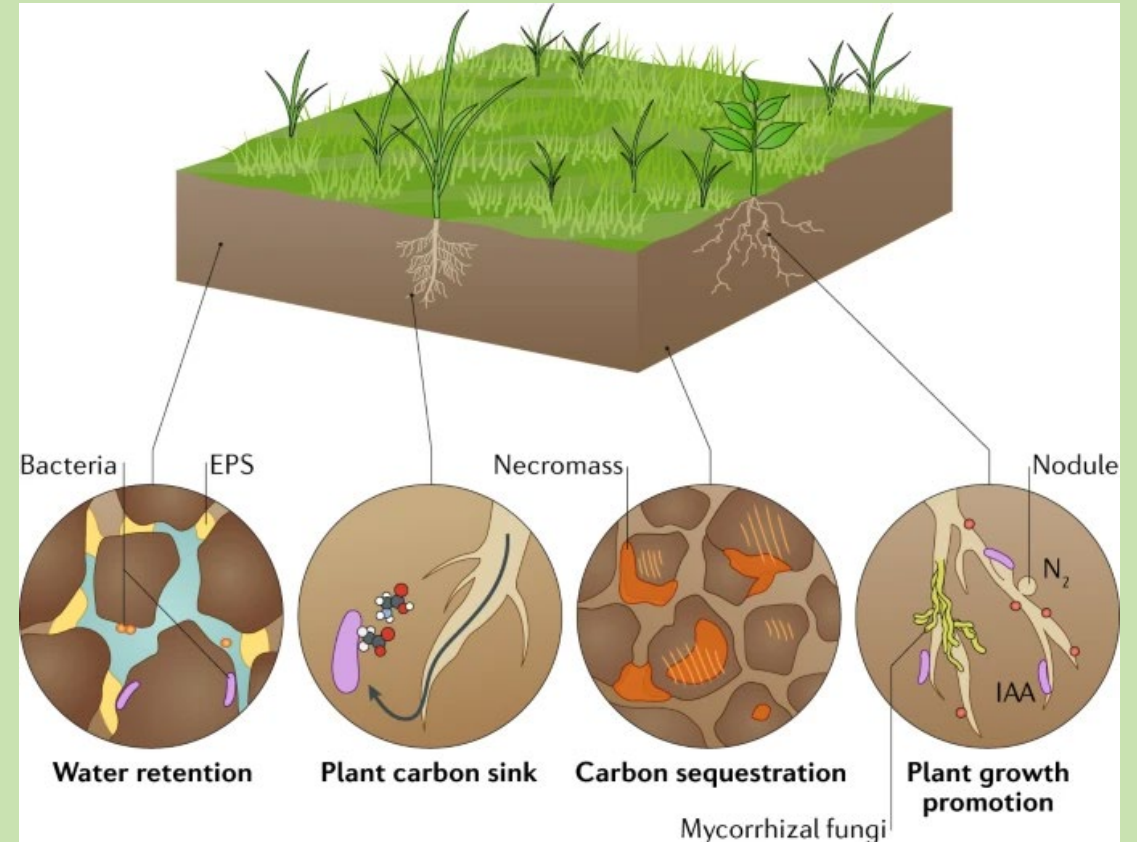
- Microbial communities play critical roles in soil processes

- Nutrient cycling
- Carbon fluxes / decomposition
- Water relations
- Plant productivity / stress tolerance

- Incredible biodiversity

- Composition, diversity, and function is highly dynamic

- Space
- Time
- Management practices

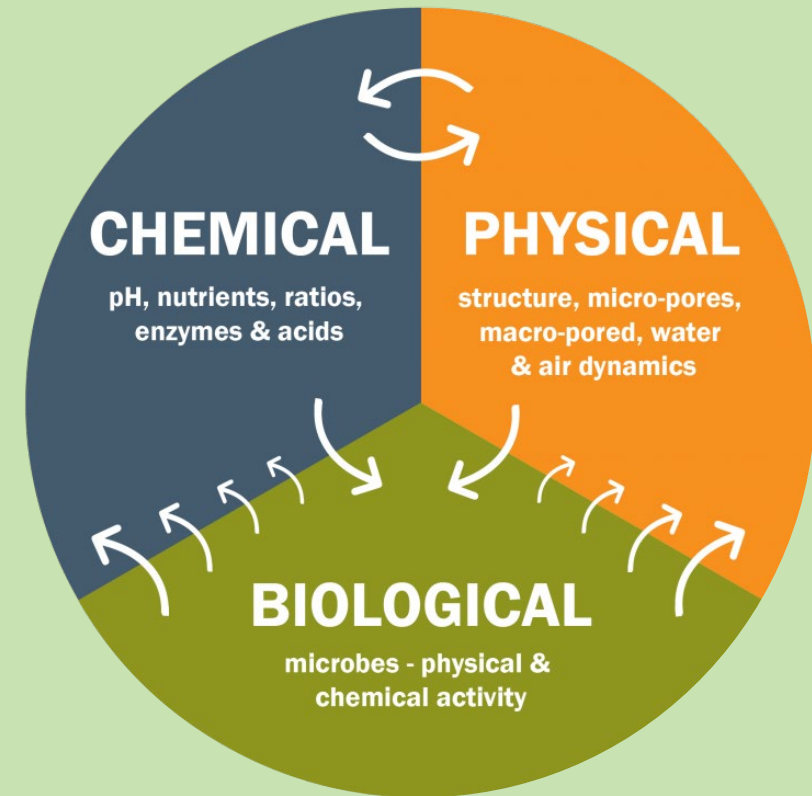


Janssen and Hofmockel, 2019

Challenging to deliberately manage microbial communities in agricultural systems

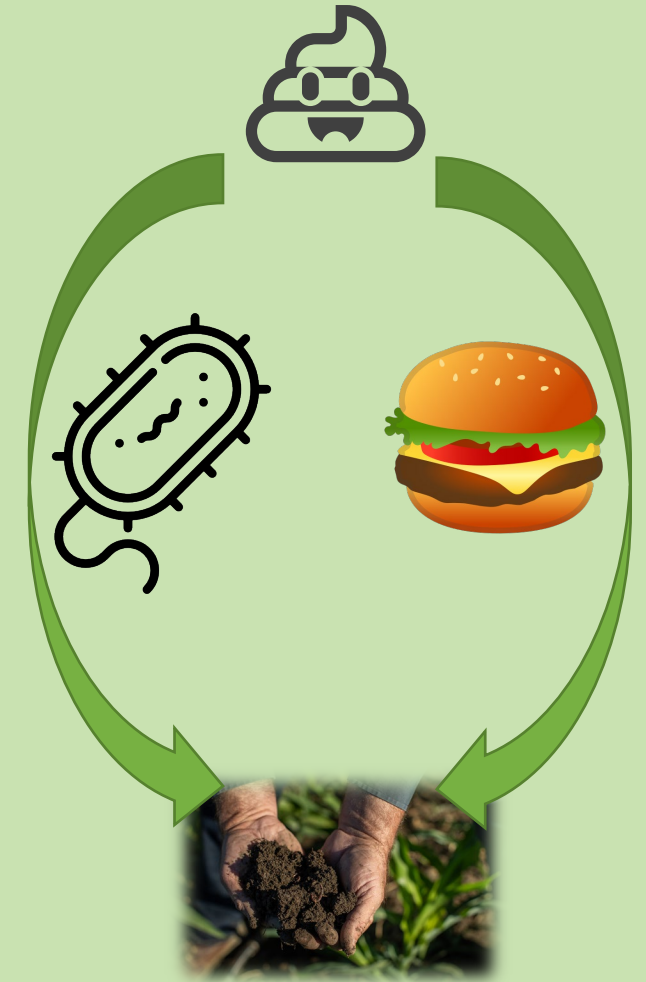
- Dairy manure is ubiquitous in dairy production systems.
- Valuable resource as soil amendment to promote soil health.
- Chemical: Source of N, P, K, other nutrients, and organic C.
- Physical: Promotes soil aggregation, water holding capacity.
- Biological: Increase microbial biomass, activity, diversity, nutrient cycling, pathogen suppression.

Microbial communities are key to understanding impacts of manure on soil ecosystems.



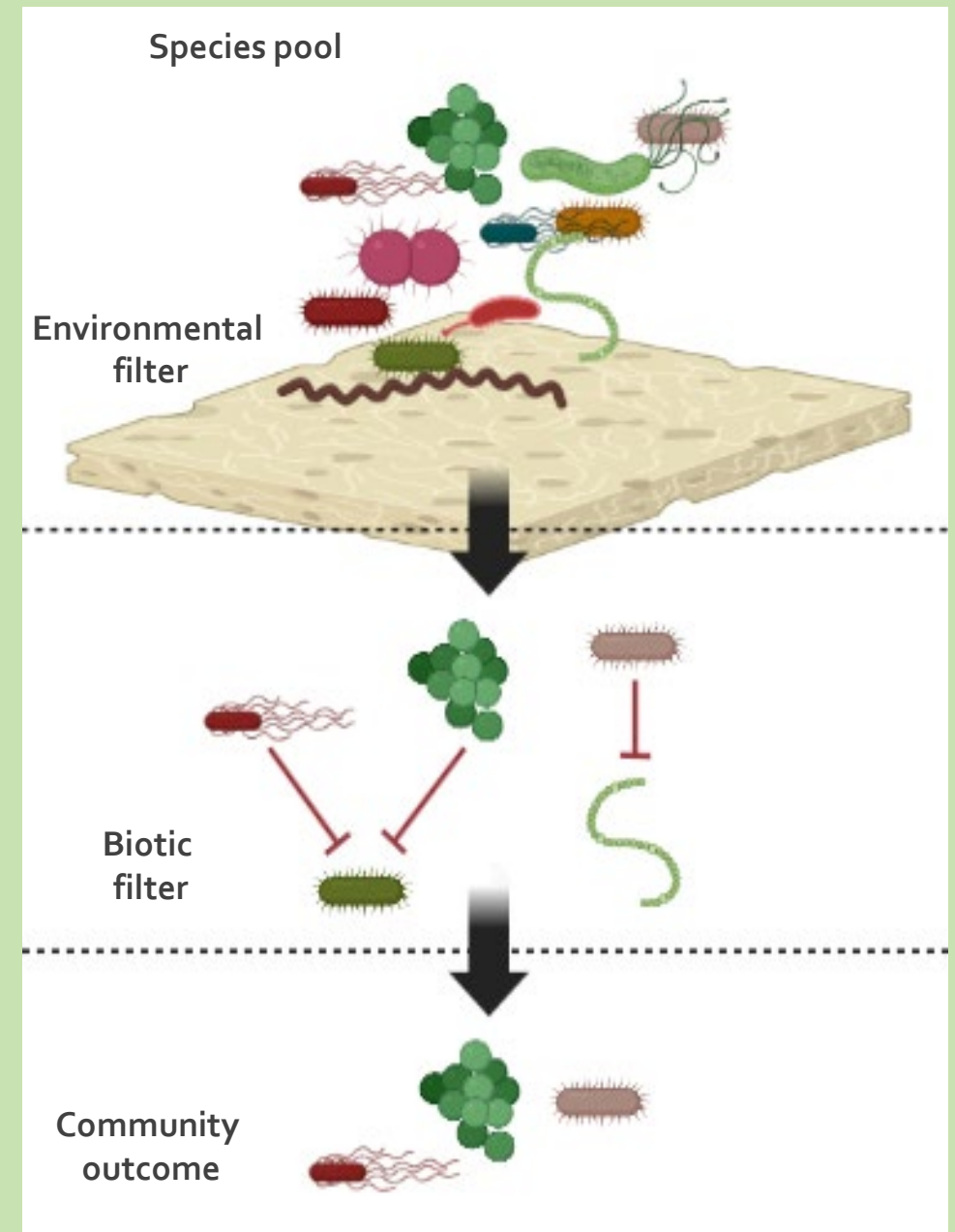
Manure and Microbes: Potential Mechanisms

- Direct introduction/survival of manure-borne microbes
 - Manure harbors an abundance of diverse microbes
 - Includes potential animal pathogens (*E. coli*, *Salmonella*)
 - Antibiotic resistance genes (ARGs)
- 'Activation' of indigenous soil microbes
 - Most microbes in soil are dormant.
 - Manure is a readily available substrate.
 - Potential consequences for plant symbionts (pathogens, pathogen antagonists)
- Changes in physical or chemical environment
 - pH, organic matter, N, P, K, bulk density, etc.



What roles do soil physical / chemical vs microbial communities play in the impacts of manure on soil microbes?

- Environmental filters (e.g. selection) are key to community assembly
- Abiotic – Soil physical and chemical environment limits the growth/persistence of introduced taxa
- Biotic – Indigenous microbes occupy available niche space, differences in intrinsic growth rates and competitive species interactions
- Both may contribute to resistance and resilience of soil communities.



Objectives

1. Determine how manure amendments modify soil community composition and diversity across a diverse range of soils.
2. Characterize the dynamics of manure-borne and indigenous microbes after manure amendment.
3. Investigate the role of abiotic and biotic factors in the assembly of soil communities after manure amendment.

Microcosm Incubation

Soil origin

- Minnesota (Waseca, Lamberton, Grand Rapids)
- Idaho (Kimberly)
- Pennsylvania (State College)

Soil – Manure mixtures

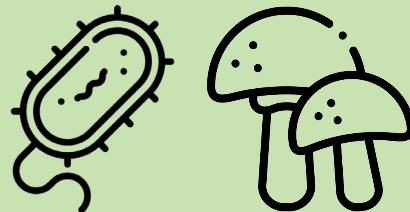
- Live soil – Live manure (LSLM)
- Live soil – Sterile manure (LSSM)
- Sterile soil – Live manure (SSLM)
- Live soil – No manure (Control)

Incubation time

- Sampled at 0, 30, and 60 days after incorporation

Composition and diversity of microbial communities

- Bacterial 16S rRNA sequencing (V4)
- Fungal ITS sequencing (ITS2)



LSLM



LSSM

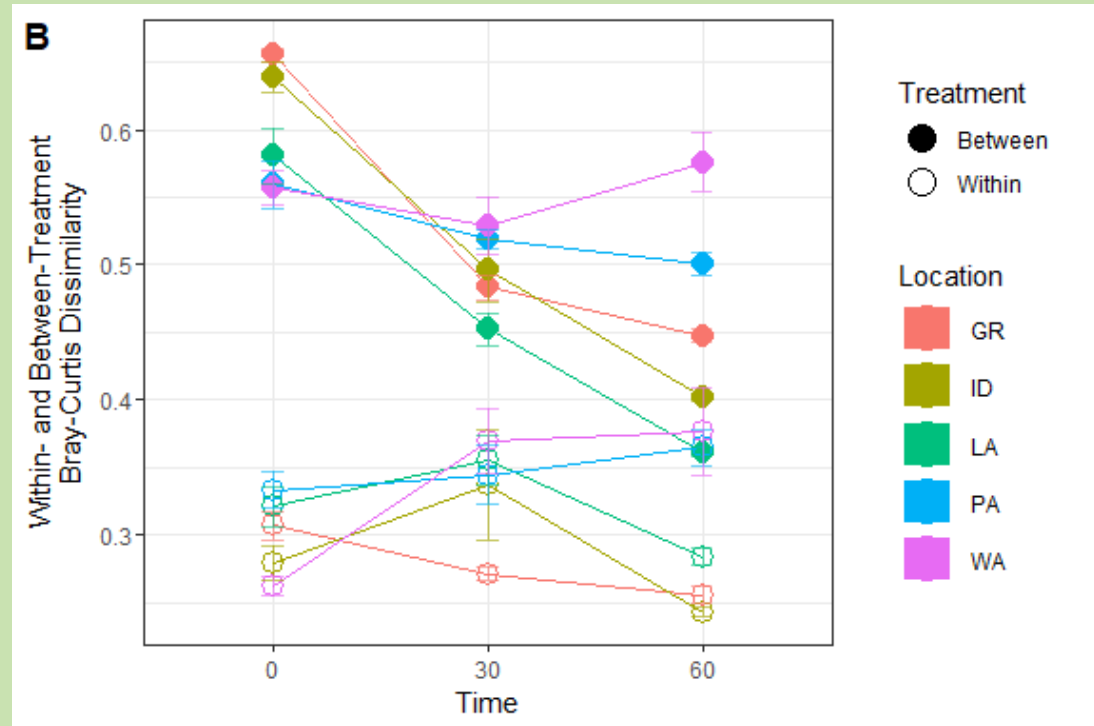
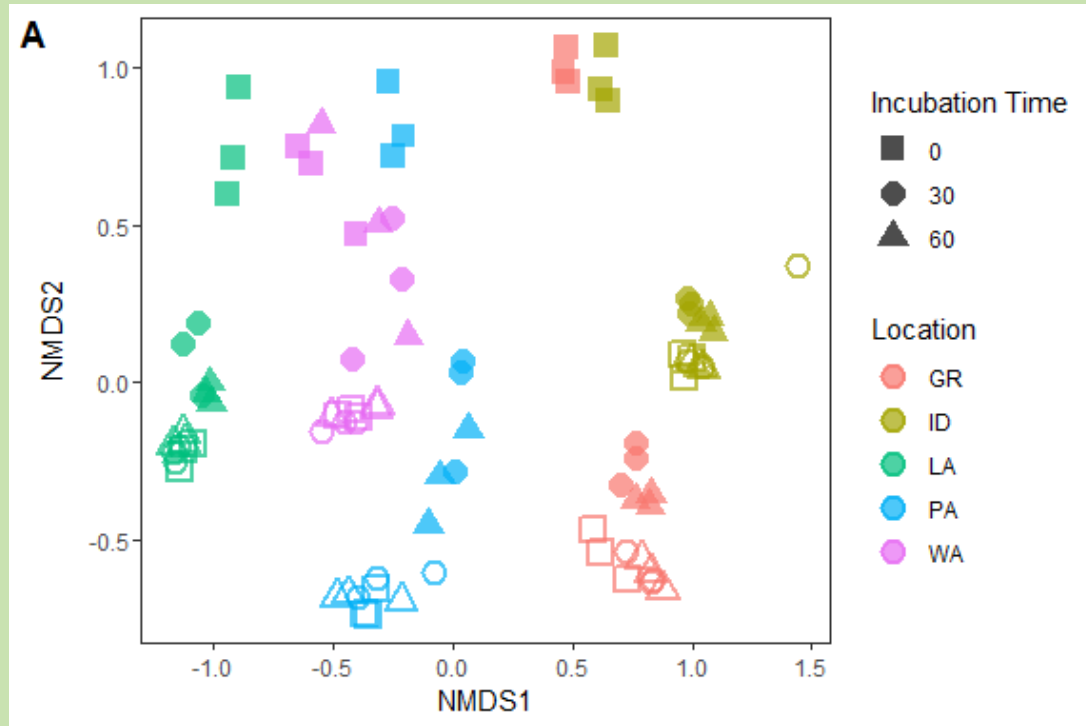


SSLM



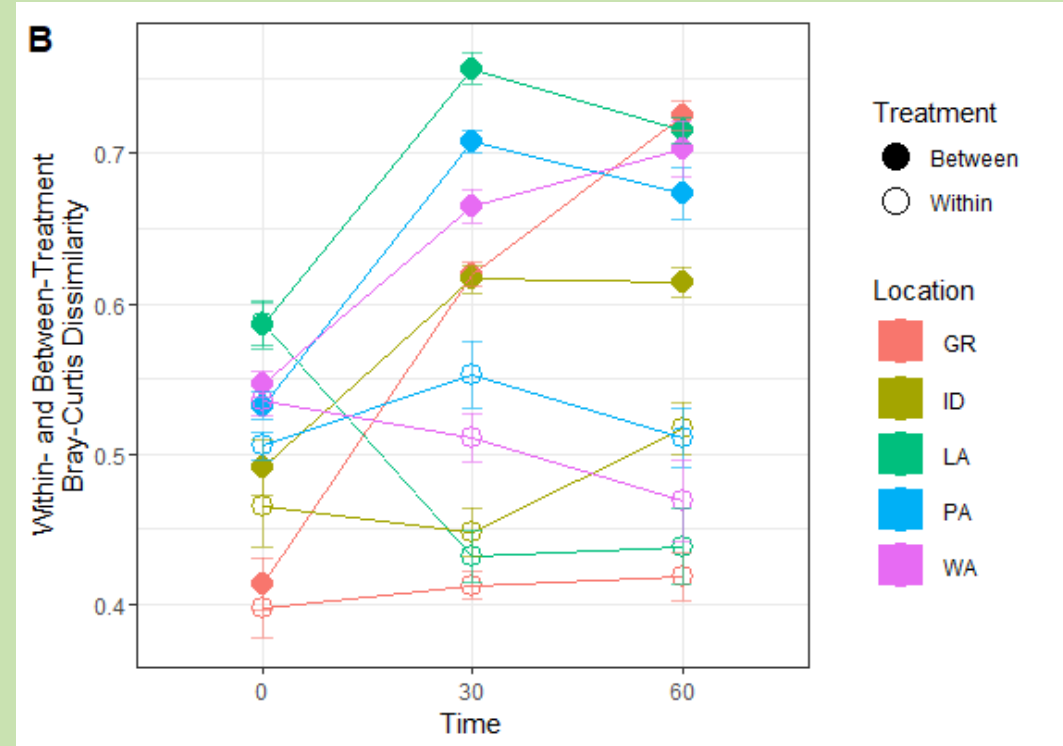
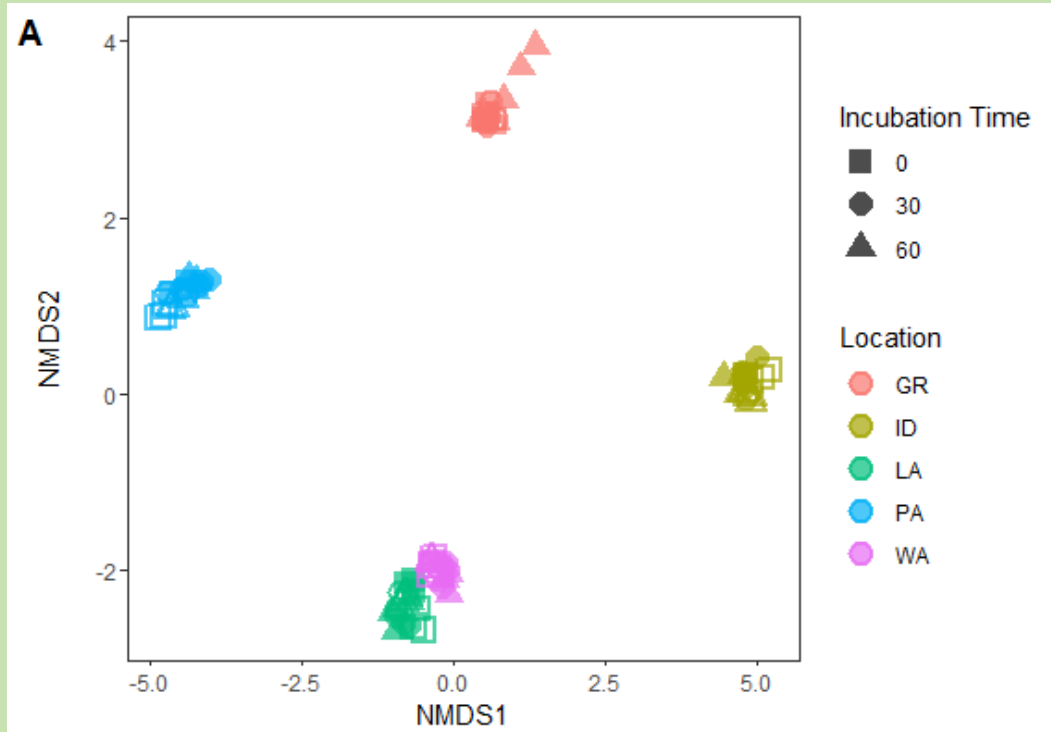
Control

LSLM vs Control



- Soil origin is a strong driver of bacterial community composition.
- Manure amendment drives strong shifts in bacterial community composition.
- Over time, most bacterial communities become more similar to unamended controls.

LSLM vs Control

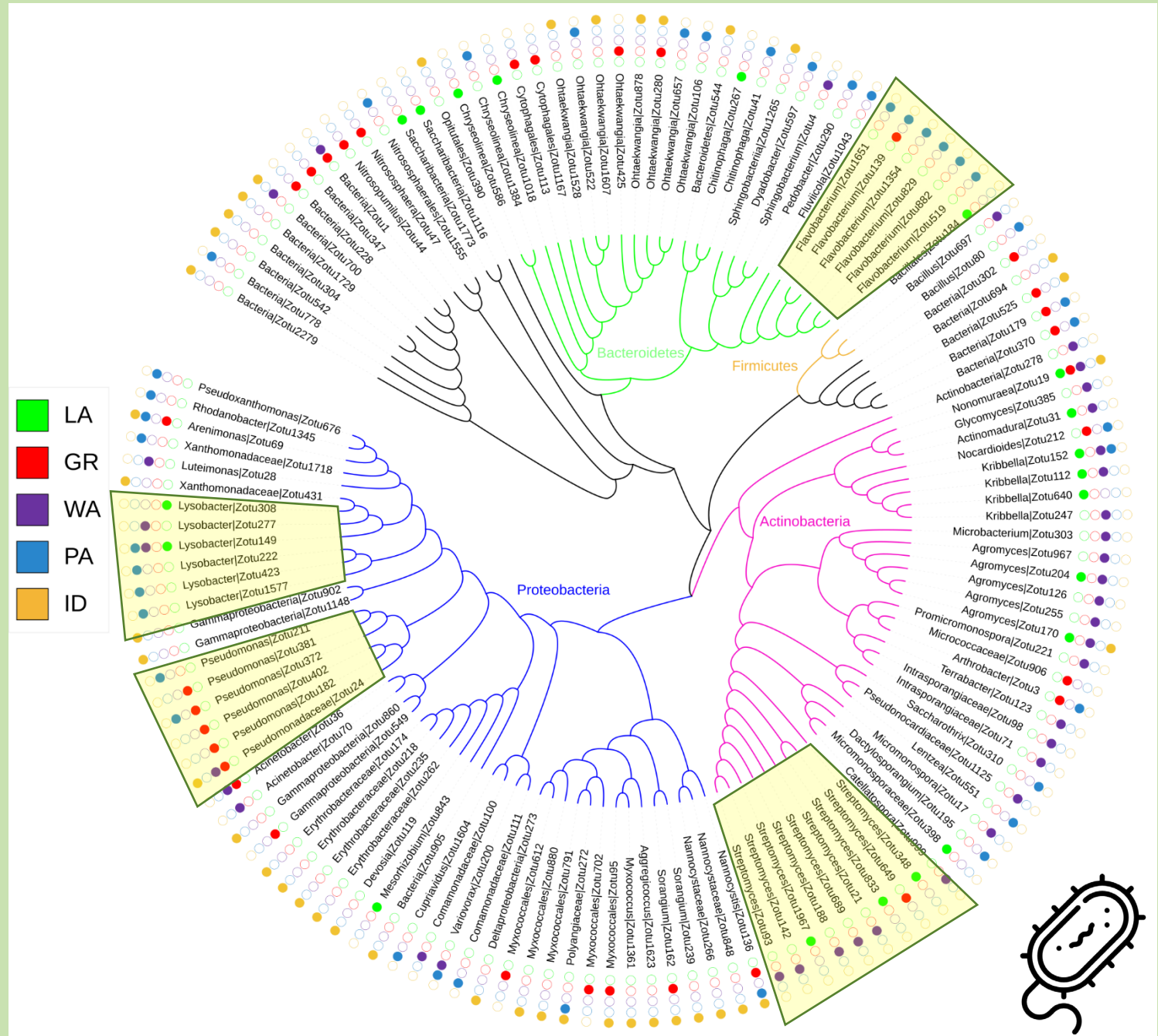


- Soil origin is a strong driver of fungal community composition.
- Manure amendment has little initial impact on fungal community composition.
- Over time, fungal communities become more differentiated from unamended controls.

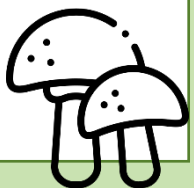
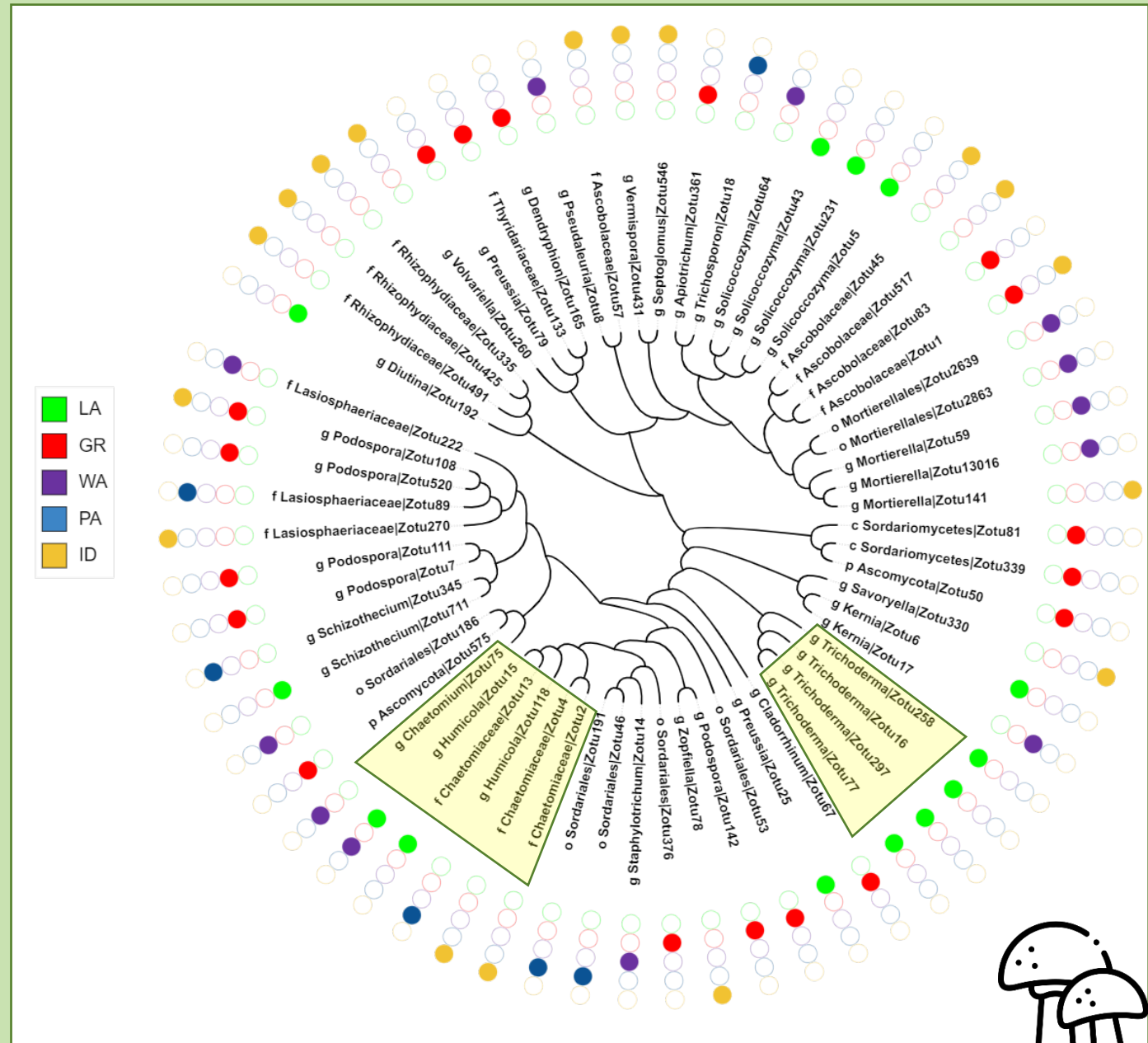
- Bacterial that increased in abundance in response to manure were typically soil specific.

- Primarily belonged to the phyla Proteobacteria, Actinobacteria, and Bacteroidetes.

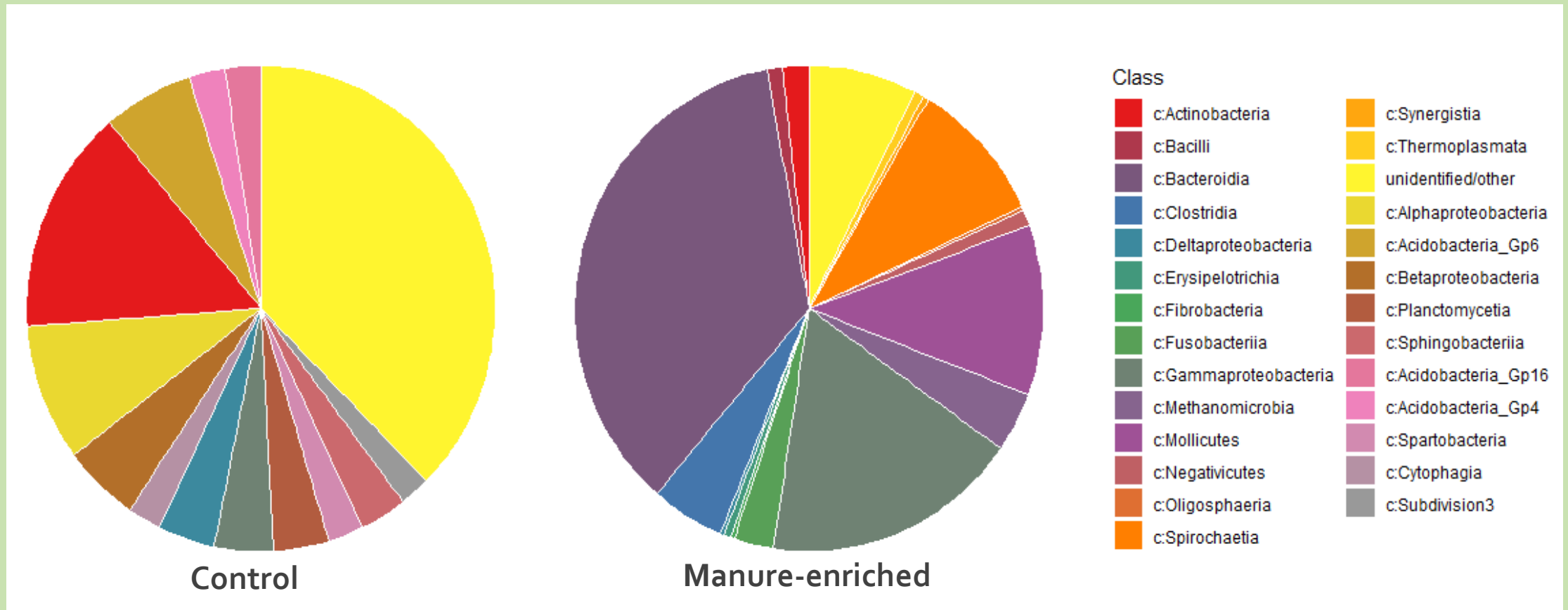
- Many manure responsive bacteria are considered copiotrophic, and potential plant symbionts.



- Fungal taxa responding to manure were also soil specific.
- Manure amendments enriched many Ascomycetes (esp. Sordariomycetes)
- Some clades are likely to play important roles in decomposition or plant health.



What is the fate of manure-born microbes?



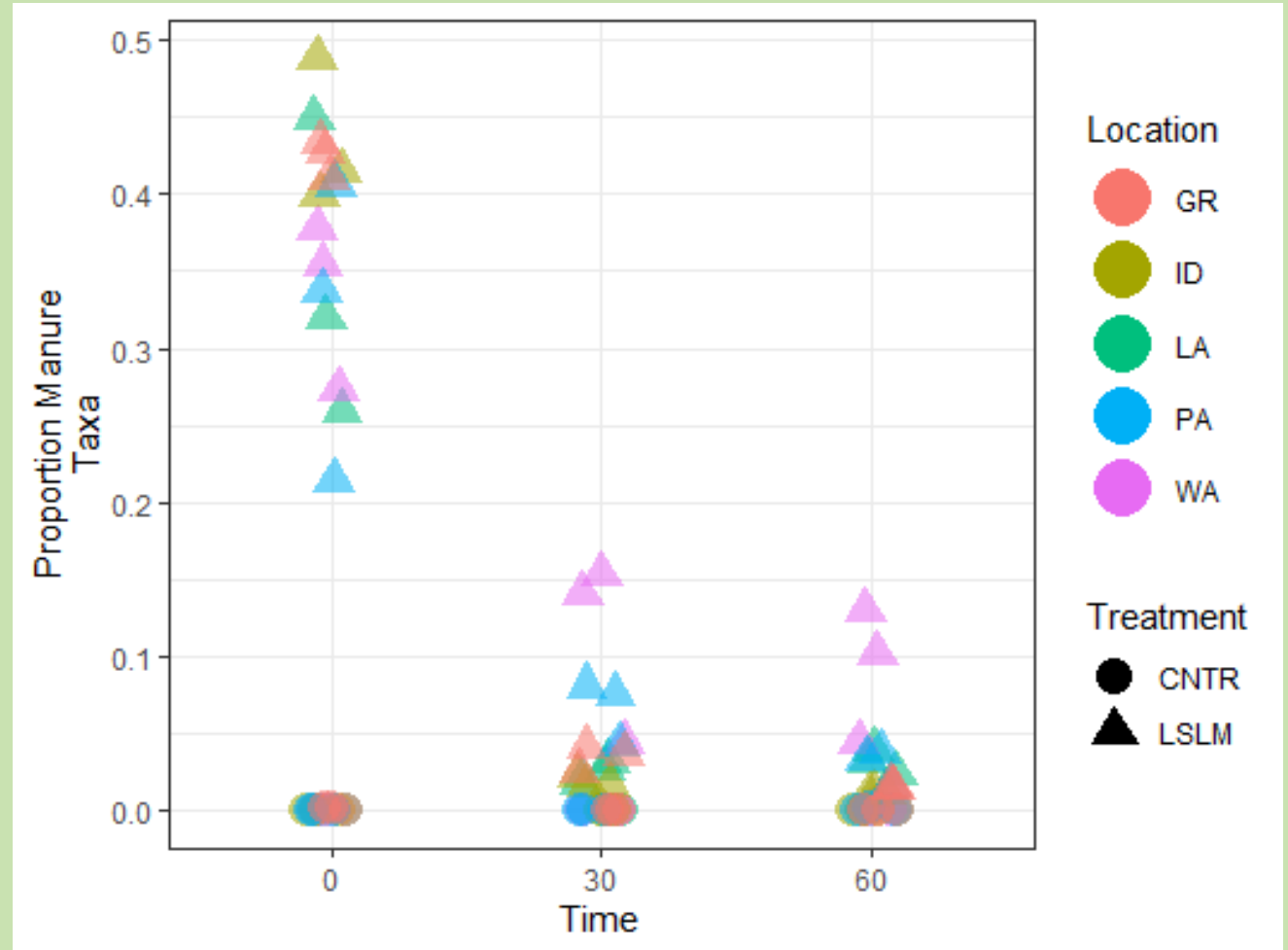
- Differential abundance analysis of manure-amended vs. control soils at time 0.
- Bacteria initially enriched by manure belonged to classes not typical of soil.
- Few fungi initially enriched by manure (e.g. *Neocallistigomycota*).



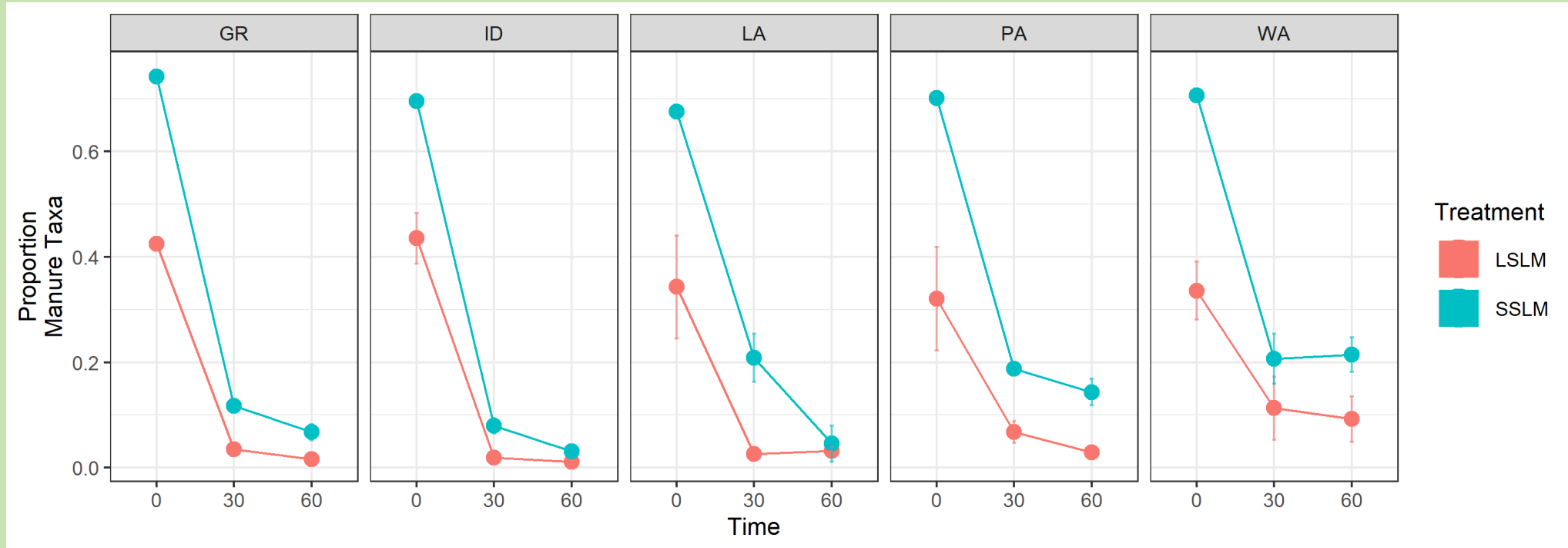
What is the fate of manure-born microbes in soil?



- Manure-associated bacteria declined substantially after 30 days incubation.
- WA and PA soils retained greater proportions of manure-associated bacteria.
- Suggests initial large shifts in manure-amended bacterial communities are driven by manure-borne taxa.

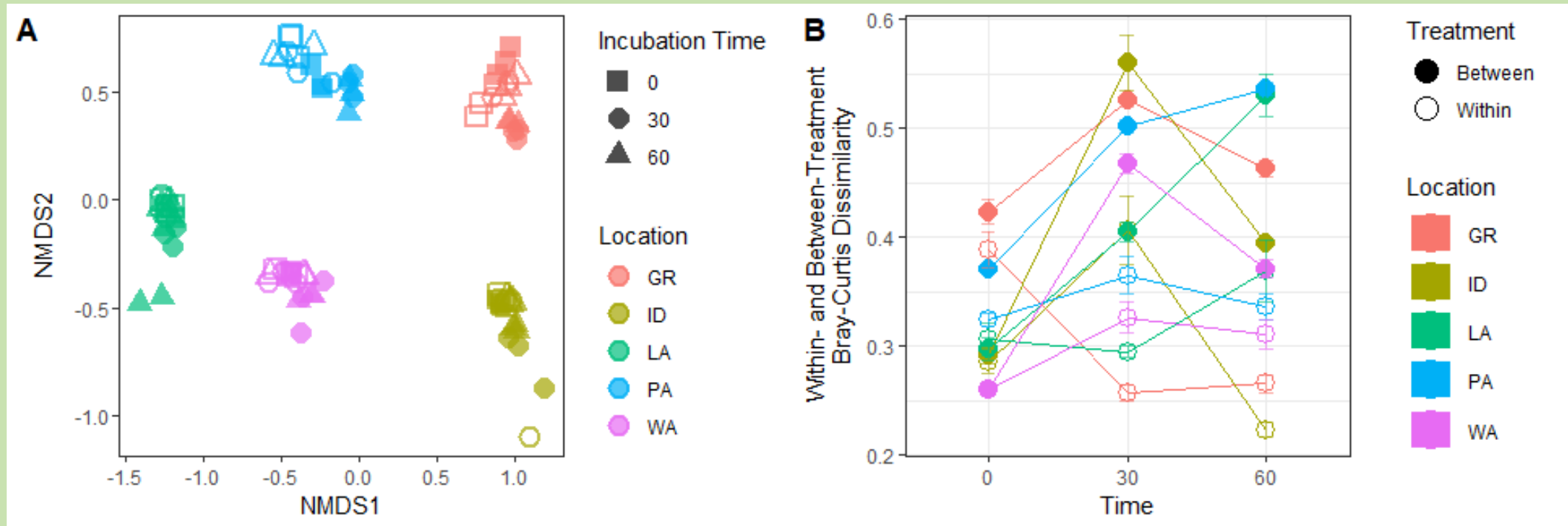


What forces drive the assembly of soil communities in response to manure amendments?



- Rapid die-off of manure-borne bacteria even without an indigenous community, though not as rapidly or to the same extent.
- Suggests soil abiotic environment is strong barrier to invasion, and that indigenous microbial communities provide a weaker, secondary barrier.

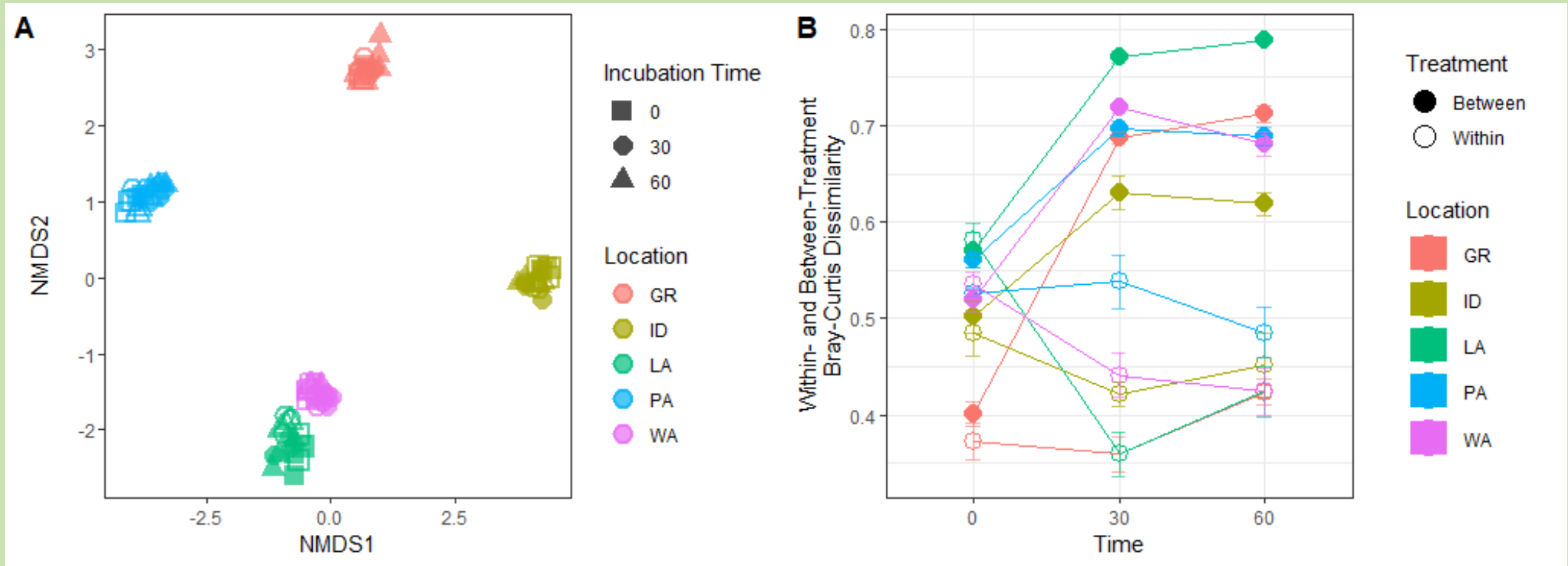
What forces drive the assembly of soil communities in response to manure amendments?



- Sterile manure amended to live soil did not have immediate impact on soil bacteria.
- Elicited similar response by indigenous taxa as live manure over time.
- Supports the idea that indigenous microbes are 'activated' over 30-60 day period.



What forces drive the assembly of soil communities in response to manure amendments?



- Impacts of sterile manure on soil fungi were similar to those of live manure.

Conclusions

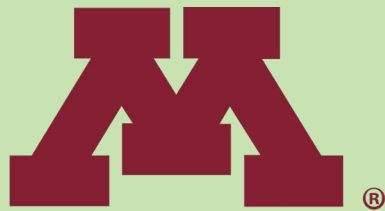
- Responses of soil communities to manure depended on soil origin.
- Manure promoted potential plant-beneficial taxa.
- Abiotic factors associated with manure drove changes in indigenous populations.
- Manure-borne bacteria drove substantial shifts in soil communities, but died off rapidly.
- Abiotic soil environment provides a strong filter against manure-borne microbes.

Future Directions

- Do shifts in bulk soil communities carry over to impact plant-associated microbiomes?
- Does manure 'prime' microbial activity in field settings?
- How does cattle diet, manure processing, or application method alter the impacts of manure on soil populations?

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Driven to DiscoverSM

Thanks!



**Agricultural
Research
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MN Manure Priming

Cropping system

Alfalfa-corn rotation (AAACC), three phases present

MN LTAR Sites

- Grand Rapids (A3, C1, C2)
- Lamberton (A1, C1, C2)
- Waseca (A1, C1, C2)

Fertility treatments (2019):

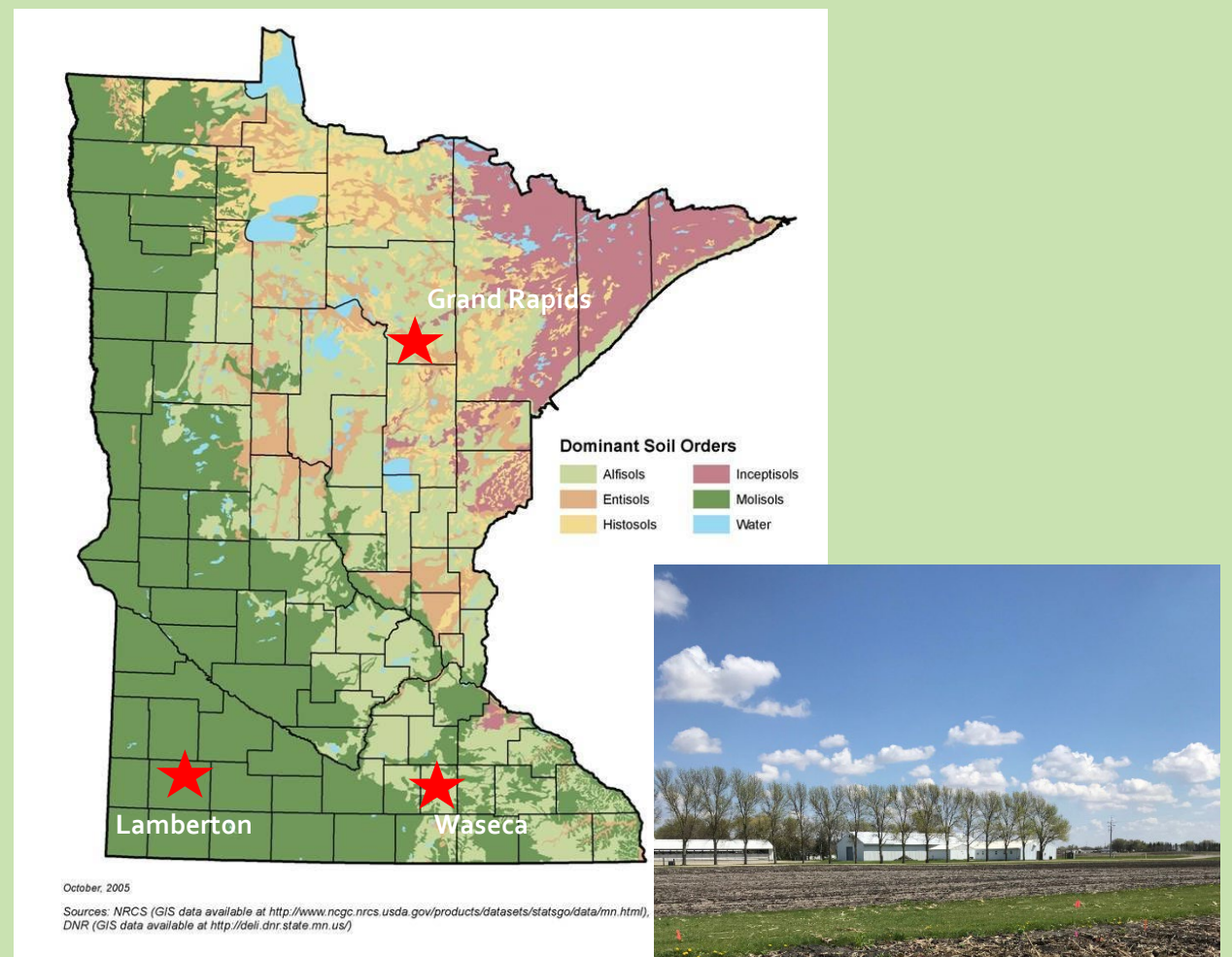
1. High manure (5000 gal/ac)
2. Low manure (3200 gal/ac)
3. Mineral fertilizer only
4. No amendment

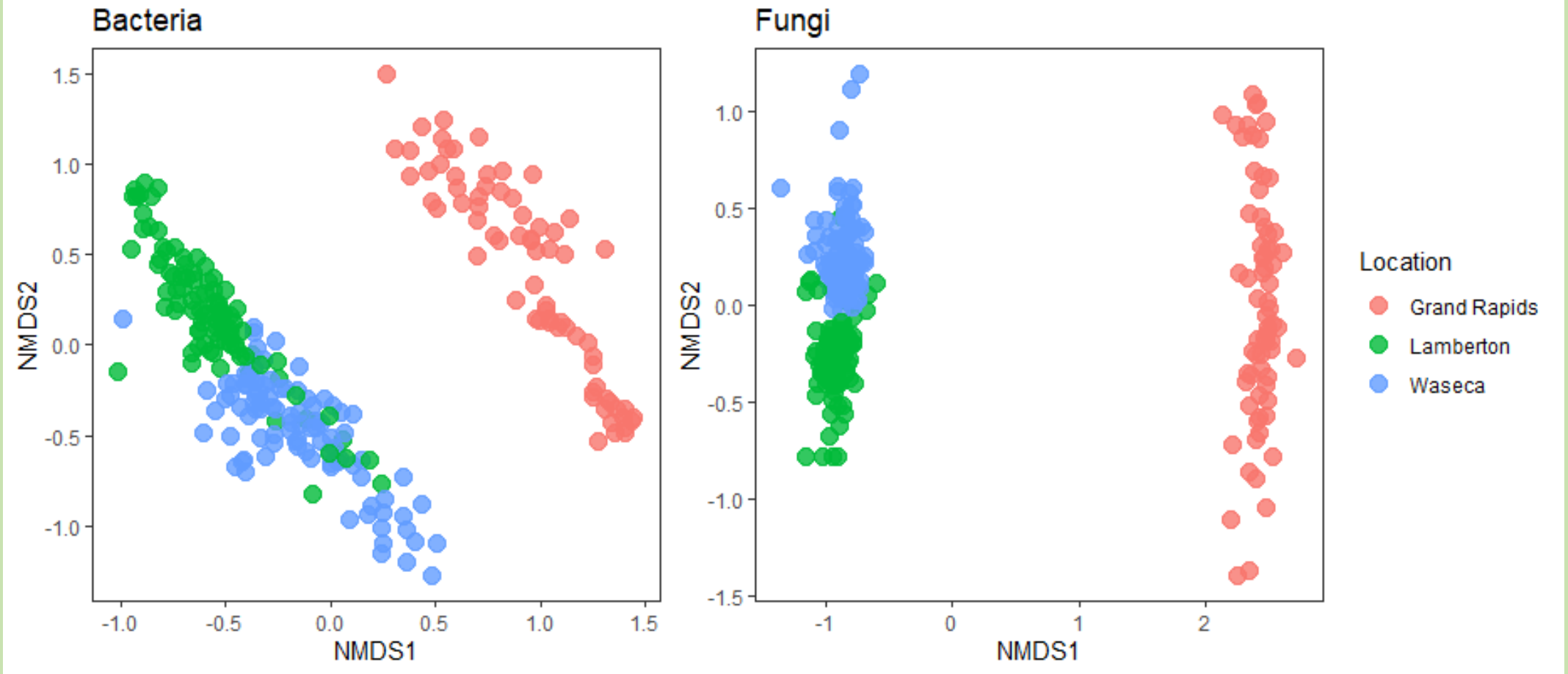
Sampling time

- Spring (pre-treatment)
- Fall (post-treatment)

Microbiome Data

- Bacterial 16S (V4)
- Fungal ITS (ITS2)





Location is the overwhelming driver of microbial community composition.

PERMANOVA: Bacteria $r^2=0.26$, $p=0.001$; Fungi $r^2=0.22$, $p=0.001$

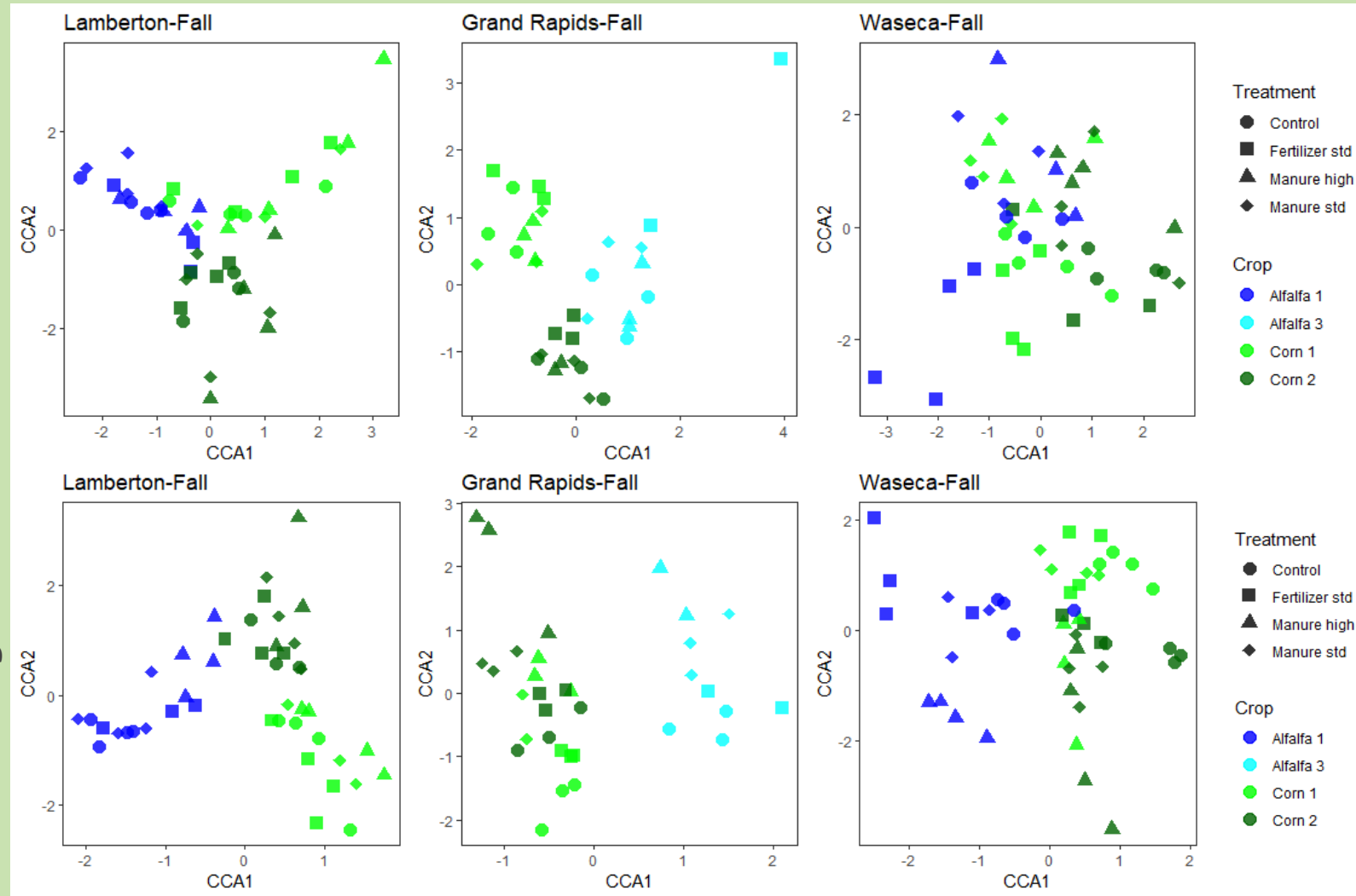
In each location, cropping phase is a significant factor structuring soil communities.

No response of overall microbial community structure to fertility treatment.

A small number of taxa (e.g. coprophilous fungi) appear to be enriched in manure treatments.

Bacteria

Fungi



CCA permutation test (F-value [p-value])				
		LA	GR	WA
Bacteria	Treatment	0.89 (0.82)	0.89 (0.93)	1.12 (0.13)
	Crop	2.30 (0.001)	1.50 (0.001)	1.45 (0.006)
Fungi	Treatment	1.01 (0.42)	1.09 (0.06)	1.08 (0.08)
	Crop	1.89 (0.001)	1.40 (0.001)	1.40 (0.001)

Microbiome Analyses



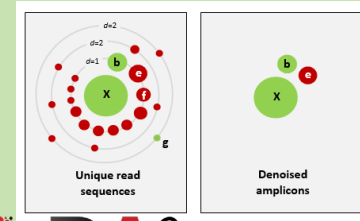
Sample collection + DNA extraction



PCR of marker gene (16S RNA, ITS)

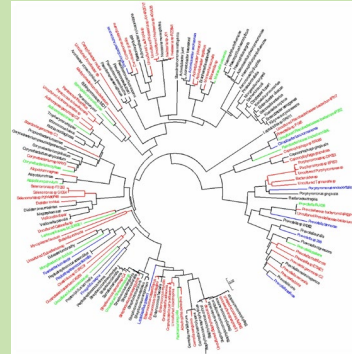


Barcoding and high-throughput sequencing



DADA2
QIIME2

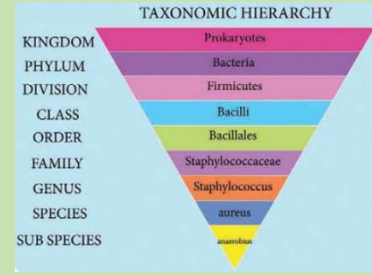
ASV/OTU clustering



Phylogeny

Sample ID	A	B	C	D	E
Otu2	571	322	273	229	381
Otu30	3	5	87	7	56
Otu36	19	0	0	1	16
Otu84	2	2	1	0	2
Otu85	76	4	25	7	5
Otu48	52	39	10	22	58
Otu8	24	13	32	55	360

OTU table



Taxonomy

Analyses of composition, diversity, differential abundance, etc.