Maize Genetic Diversity and Microbial Interactions

Corey Schultz – Wallace Lab

PAG Exploring Phytobiomes – January 12th, 2022

UNIVERSITY OF GEORGIA

New challenges in agriculture:

Population Growth

Climate Change

Chemical Inputs

Soil Degradation

Global Demand

2020 - $3 Billion
2024 -> 10% Growth

BAYER Crop Science

indigo™

syngenta

Project Goals

Explore how the plant host affects the interaction with microbes.

Project 1:

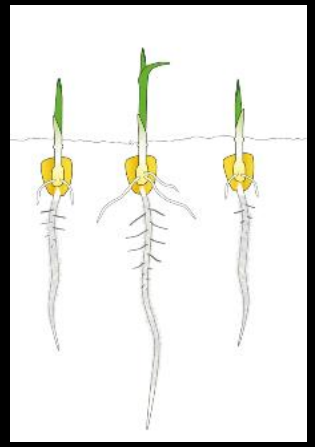
- Maize genotypes interact with endophytes

Project 2:

- Examine the community of inbred/hybrid maize

Project 1

The Role of Genetic Variation in Maize Response to Beneficial Endophytes



The Problem

Maize A (+)

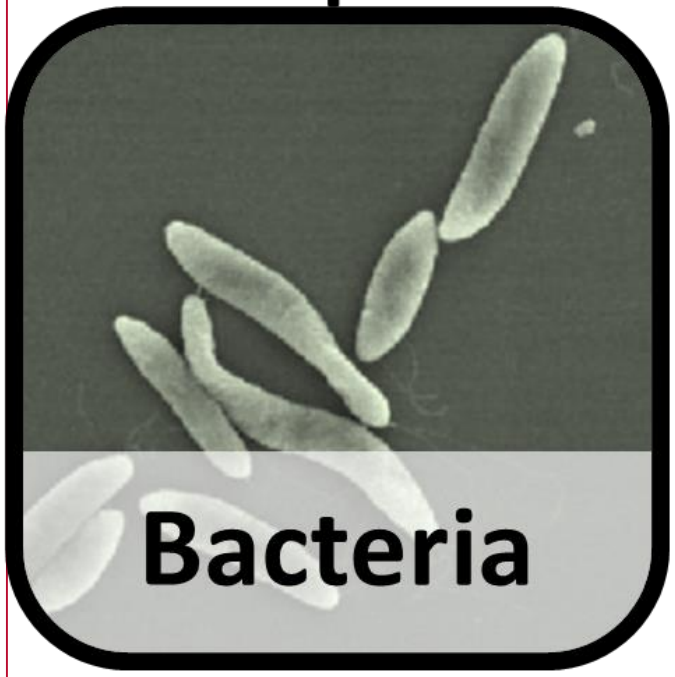
Maize B (-)

Maize C

http://clipart-library.com/clipart/1485838.htm

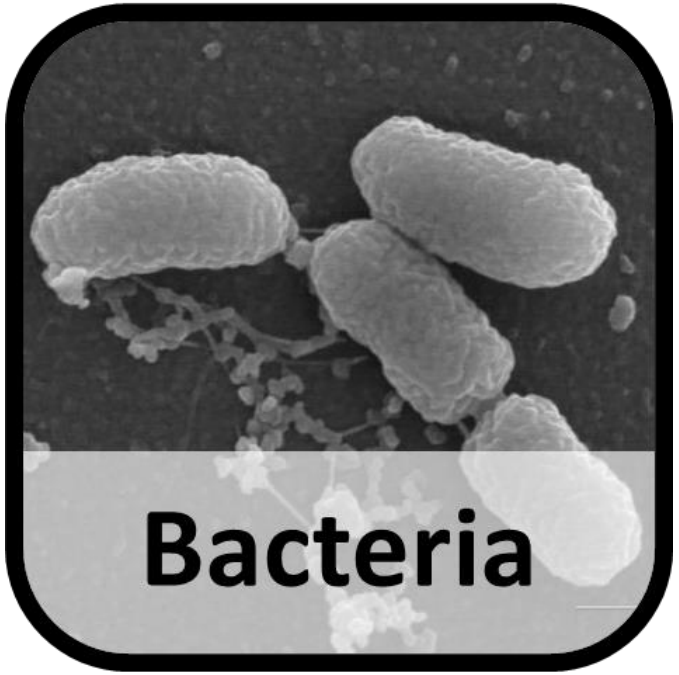
The Endophytes

Herbaspirillum



Rothballer et al 2006,Int J Sys Evol Microbiol 56:1341

Burkholderia

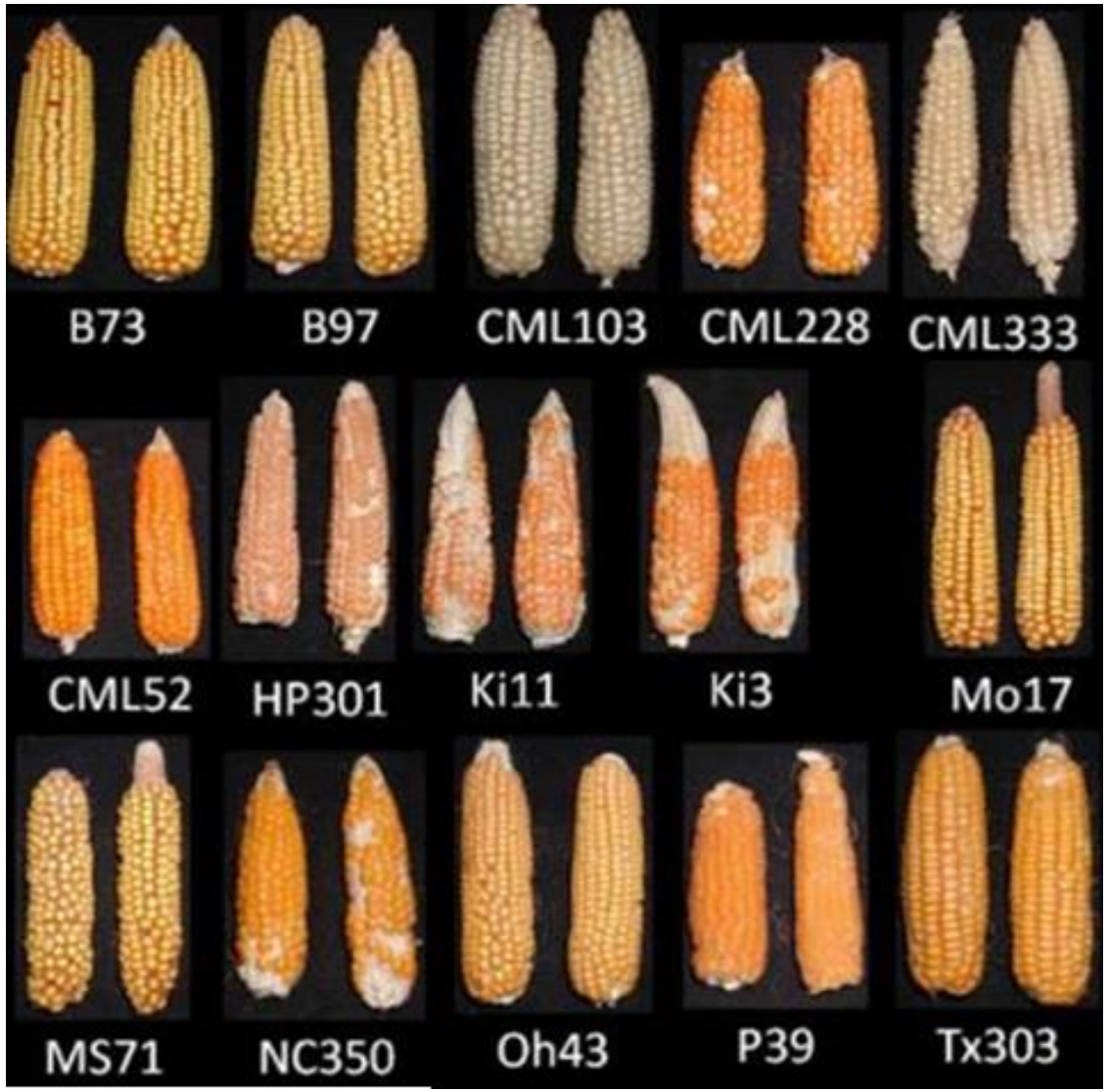


JGI

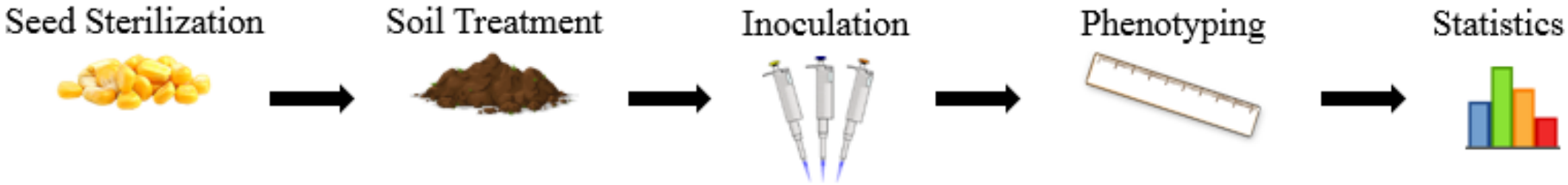
Serendipita

Ray & Craven 2016, World J Micrbiol Biotech 32(1)

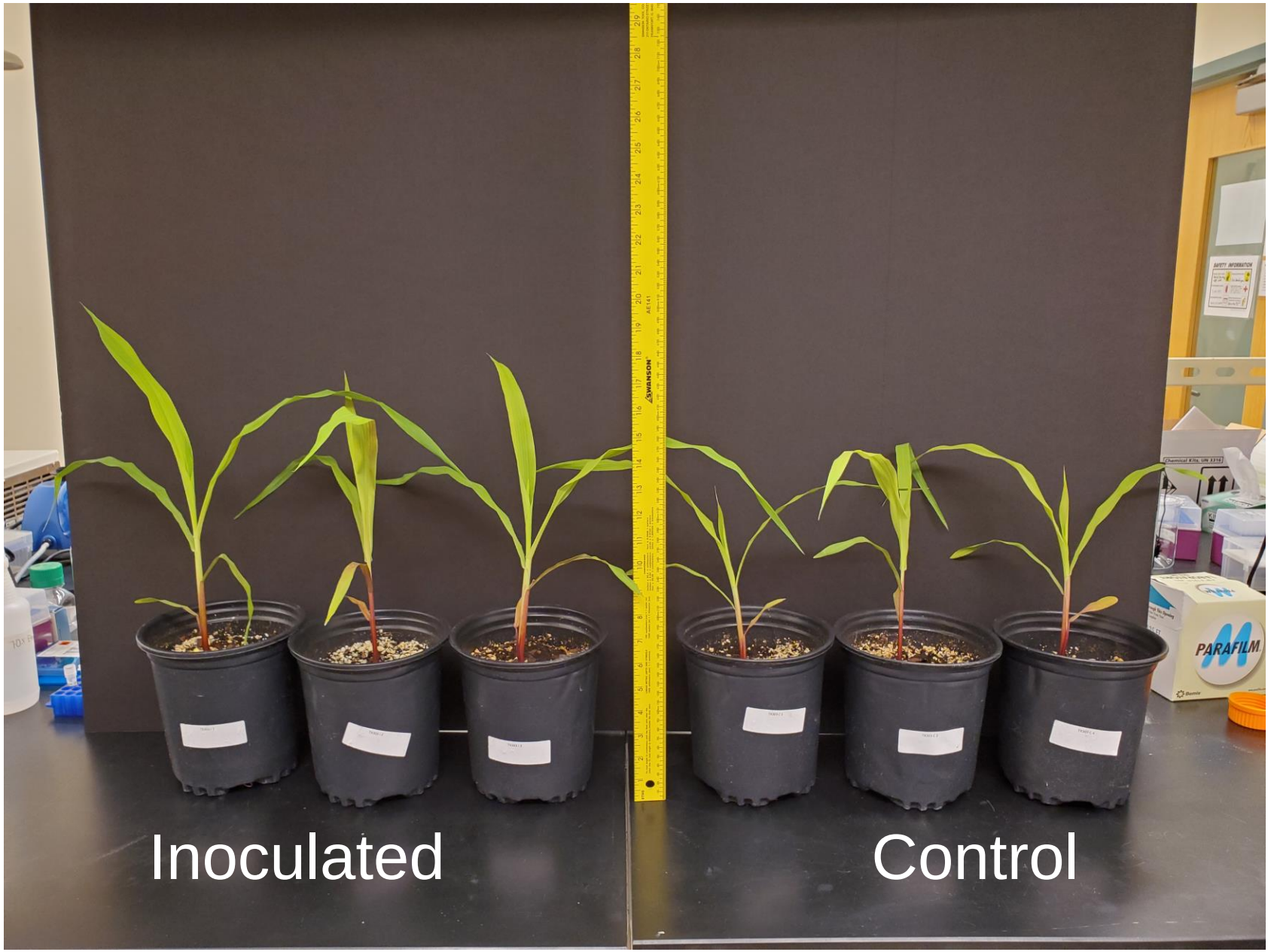
Maize Genotypes



Project Outline



- These are <u>three separate experiments</u> so there are variations for each endophyte.


Inoculated
Control
PARAFILM

Do these Endophytes Promote Growth?

Trait	P < 0.1	P < 0.05
Experiment 1 – ***H. Seropedicaea***		
Chlorophyll	CML103(+)	B73(+)
Plant Height	-	Mo17(+)
Leaf Area	-	-
Root Length	CML52(-), A635(+)	-
Root Volume	TX303(+)	CML228(+)
Experiment 2 – ***Burkholderia*** WP9		
Plant Height	-	-
Leaf Area	CML228(-)	-
Root Length	-	-
Root Volume	-	-
Experiment 3 – ***S. bescii***		
Plant Height	-	TX303(+), CML52(+)
Root Length	B73(-), CML103(-), A635(+)	-
Root Mass	KI11(+)	P39(+), CML52(+)
Shoot Mass	-	P39(+), NC350(+), TX303(+)

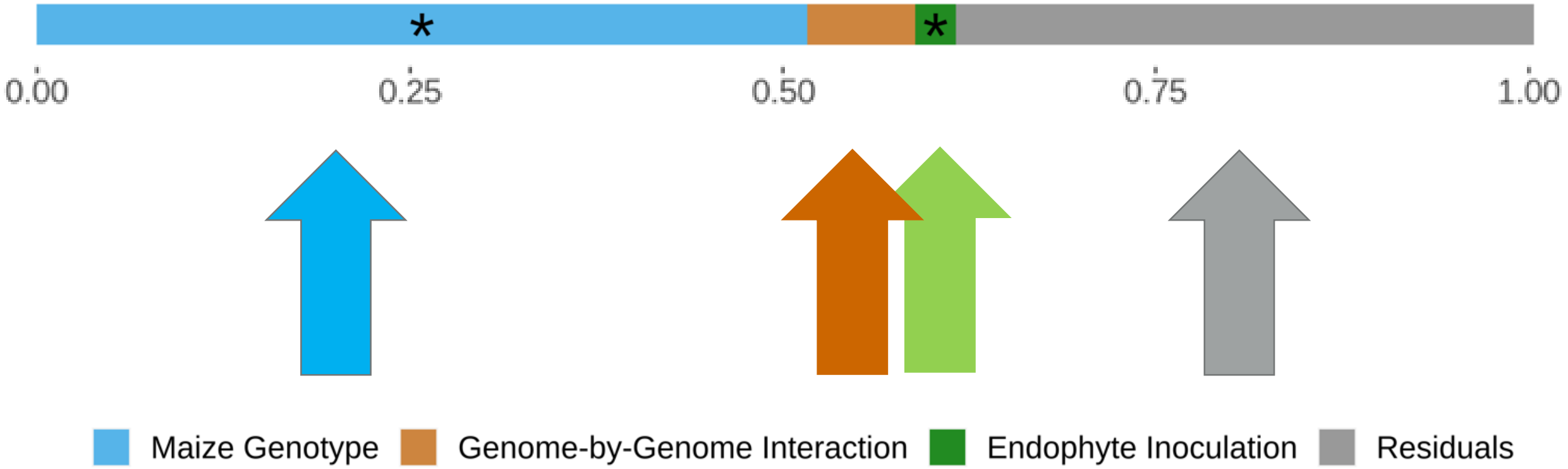
Do these Endophytes Promote Growth?

Trait	P < 0.1	P < 0.05
Experiment 1 – *H. Seropedicaea*		
Chlorophyll	CML103(+)	B73(+)
Plant Height	-	Mo17(+)
Leaf Area	-	-
Root Length	CML52(-), A635(+)	-
Root Volume	TX303(+)	CML228(+)
Experiment 2 – *Burkholderia* WP9		
Plant Height	-	-
Leaf Area	CML228(-)	-
Root Length	-	-
Root Volume	-	-
Experiment 3 – *S. bescii*		
Plant Height	-	TX303(+), CML52(+)
Root Length	B73(-), CML103(-), A635(+)	-
Root Mass	KI11(+)	P39(+), CML52(+)
Shoot Mass	-	P39(+), NC350(+), TX303(+)

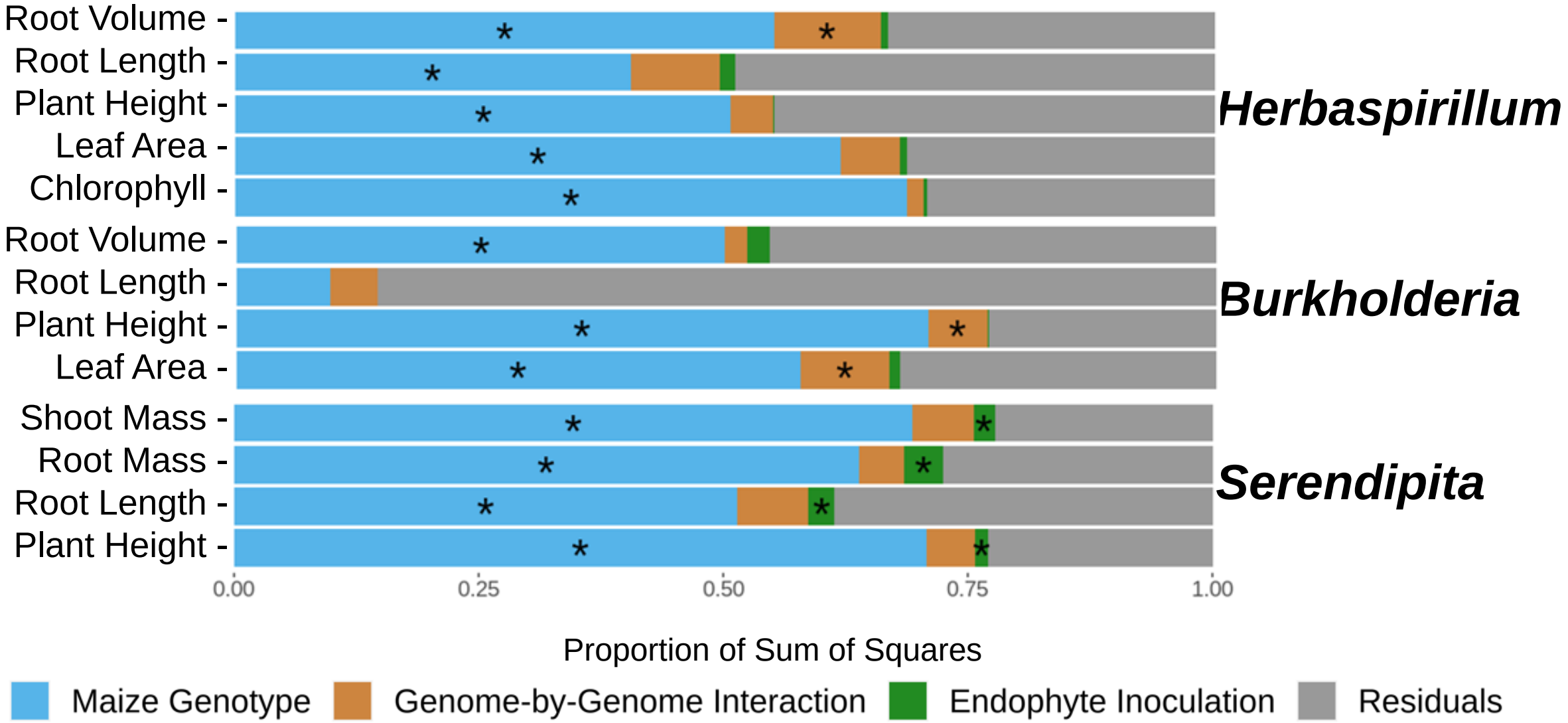
Do these Endophytes Promote Growth?

Trait	P < 0.1	P < 0.05
Experiment 1 – *H. Seropedicaea*		
Chlorophyll	CML103(+)	B73(+)
Plant Height	-	Mo17(+)
Leaf Area	-	-
Root Length	CML52(-), A635(+)	-
Root Volume	TX303(+)	CML228(+)
Experiment 2 – *Burkholderia* WP9		
Plant Height	-	-
Leaf Area	CML228(-)	-
Root Length	-	-
Root Volume	-	-
Experiment 3 – *S. bescii*		
Plant Height	-	TX303(+), CML52(+)
Root Length	B73(-), CML103(-), A635(+)	-
Root Mass	KI11(+)	P39(+), CML52(+)
Shoot Mass	-	P39(+), NC350(+), TX303(+)

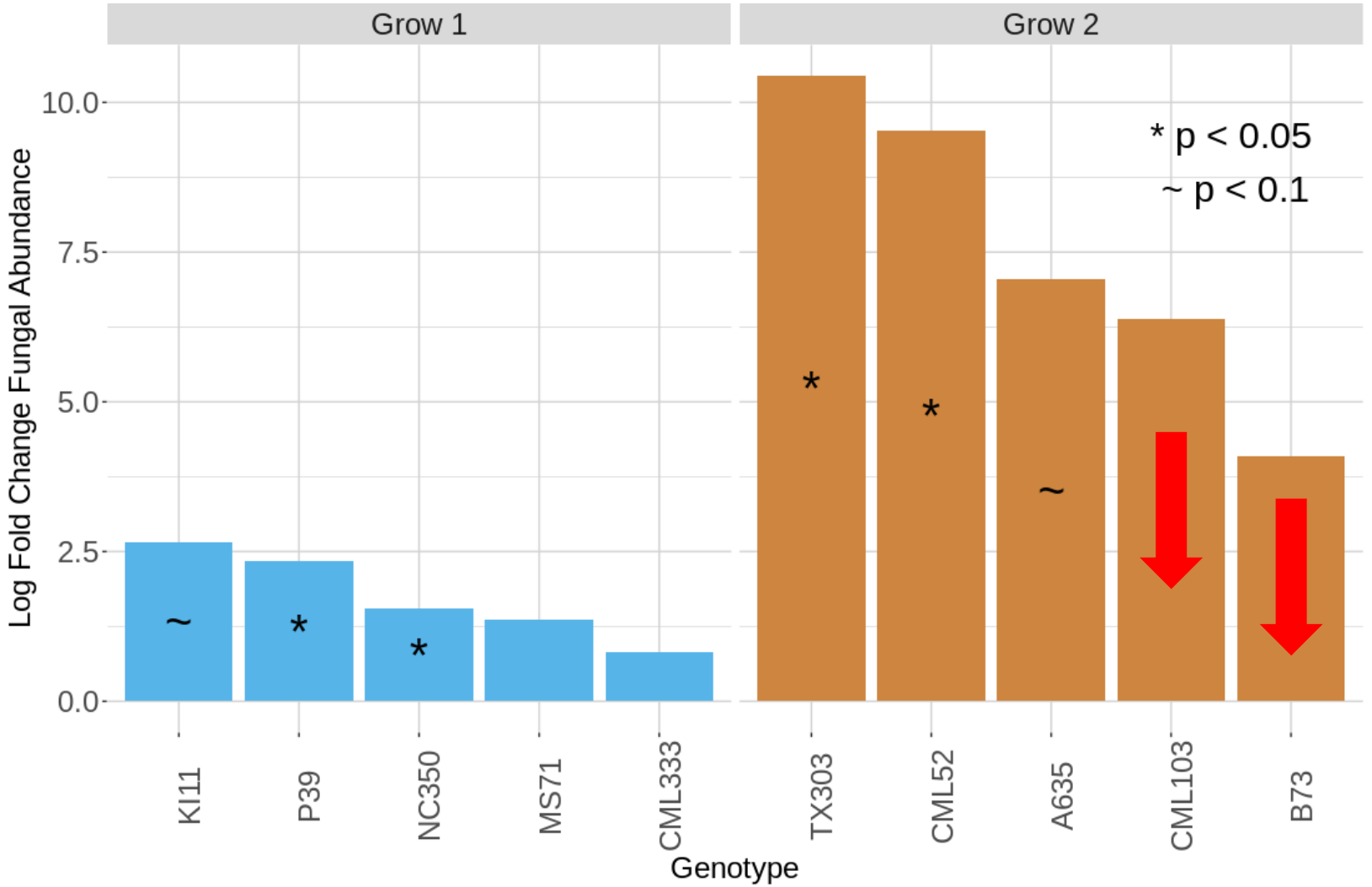
Sum of Squares Breakdown



Phenotypic Variance Primarily Comes from Genotype



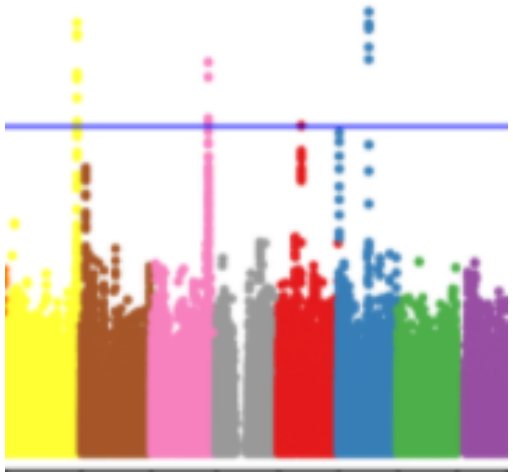
Growth Promotion may Correlate with Endophyte Amount



Take Aways

- Endophytes **differentially promoted growth** in diverse maize.

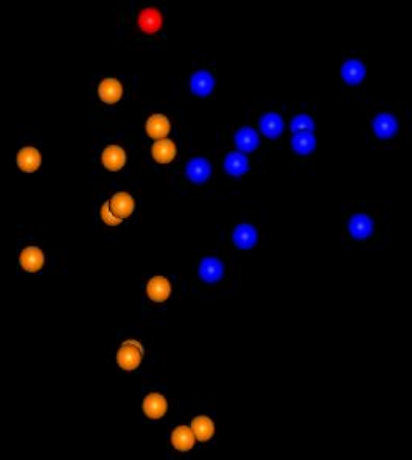
- Identifying genetic loci that play a role in this interaction may be **challenging.**



https://www.istockphoto.com/vector/corn-stalks-isolated-on-white-background-green-corn-plants-on-the-field-vector-gm1097345796-294670769

Project 2

Effects of Genetic Background on Microbial Community Diversity



Rhizosphere

Around the roots

Maize stem cross section by Josef Reischig, CC-SA-BY

Endosphere

Interior tissue

Rhizosphere

Around the roots

Maize stem cross section by Josef Reischig, CC-SA-BY

Experiments

2018 and 2019

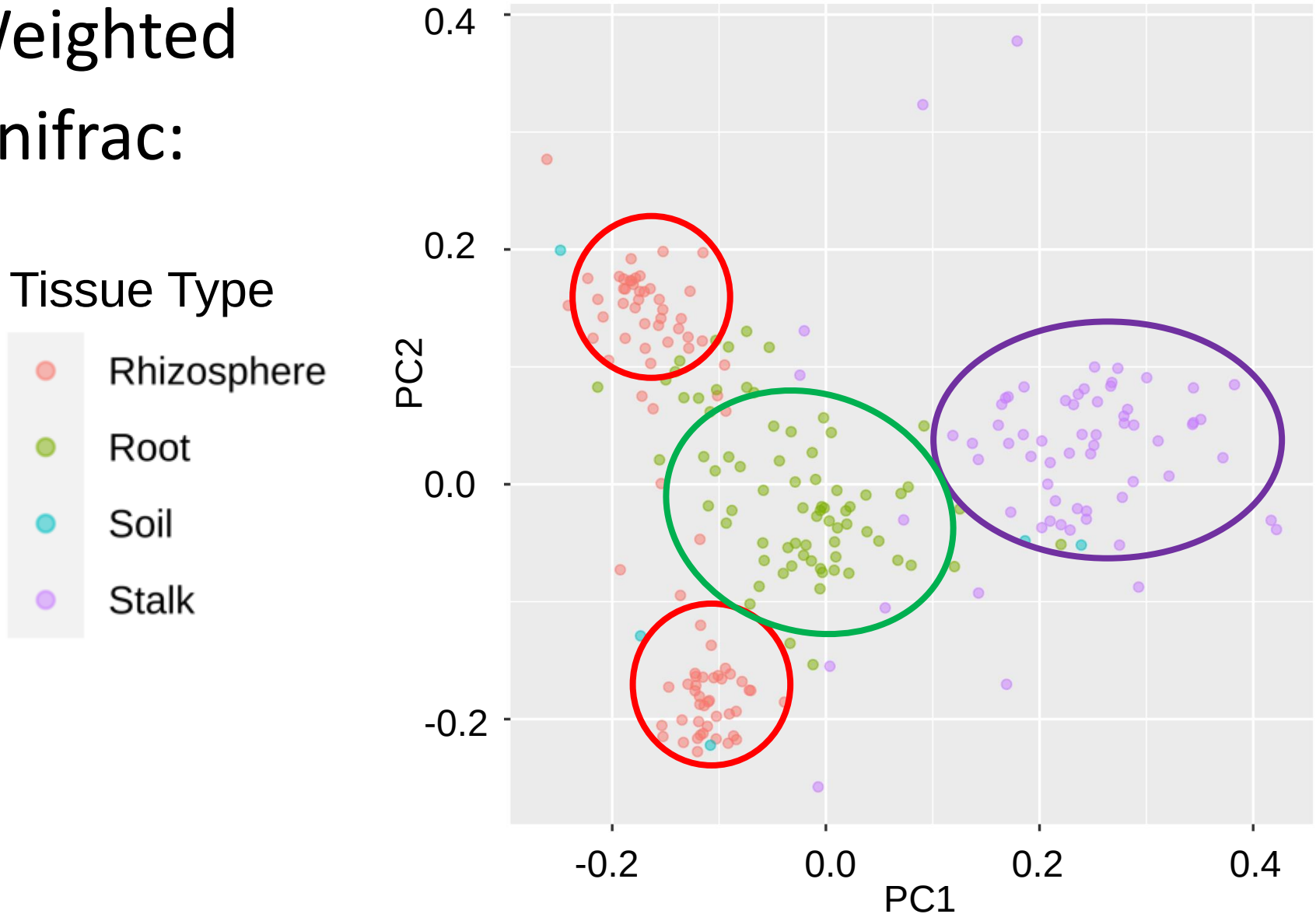
2019

Inbred Maize

Hybrid Maize

Open Pollinated

Weighted Unifrac:



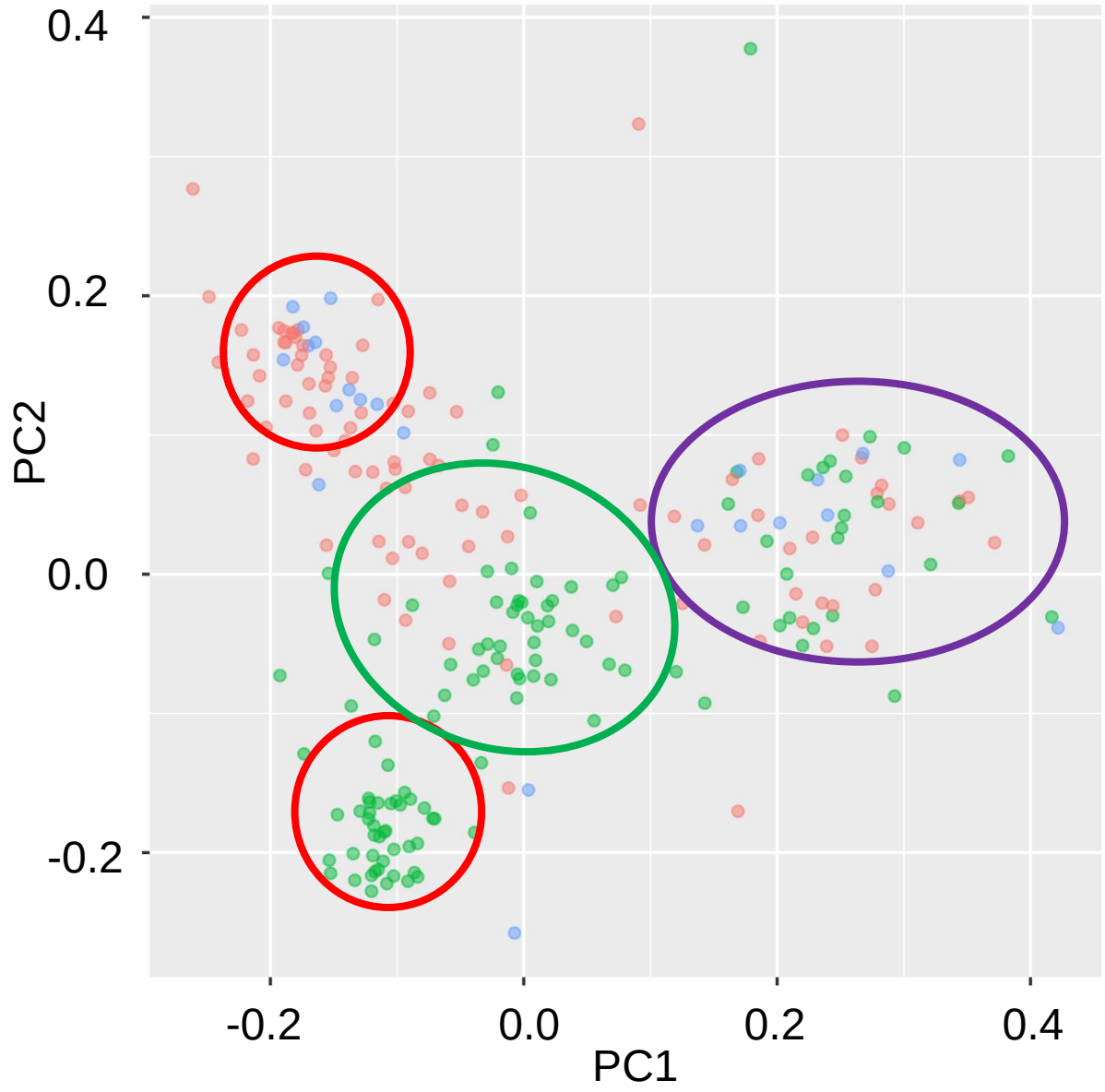
Weighted Unifrac:

Tissue Type

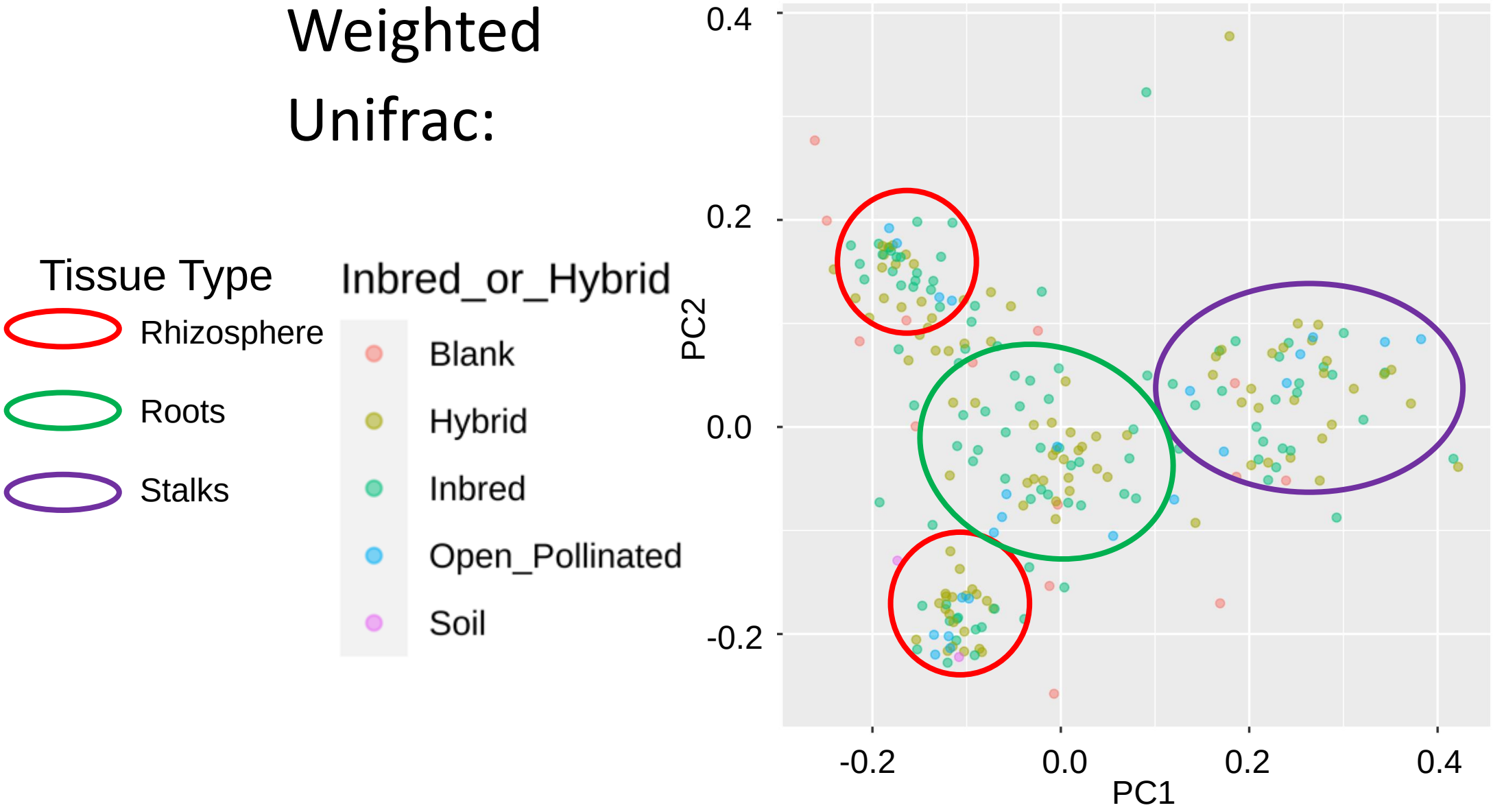
- Rhizosphere
- Roots
- Stalks

Experiment

- Field_1
- Greenhouse
- Field_2



Weighted Unifrac:

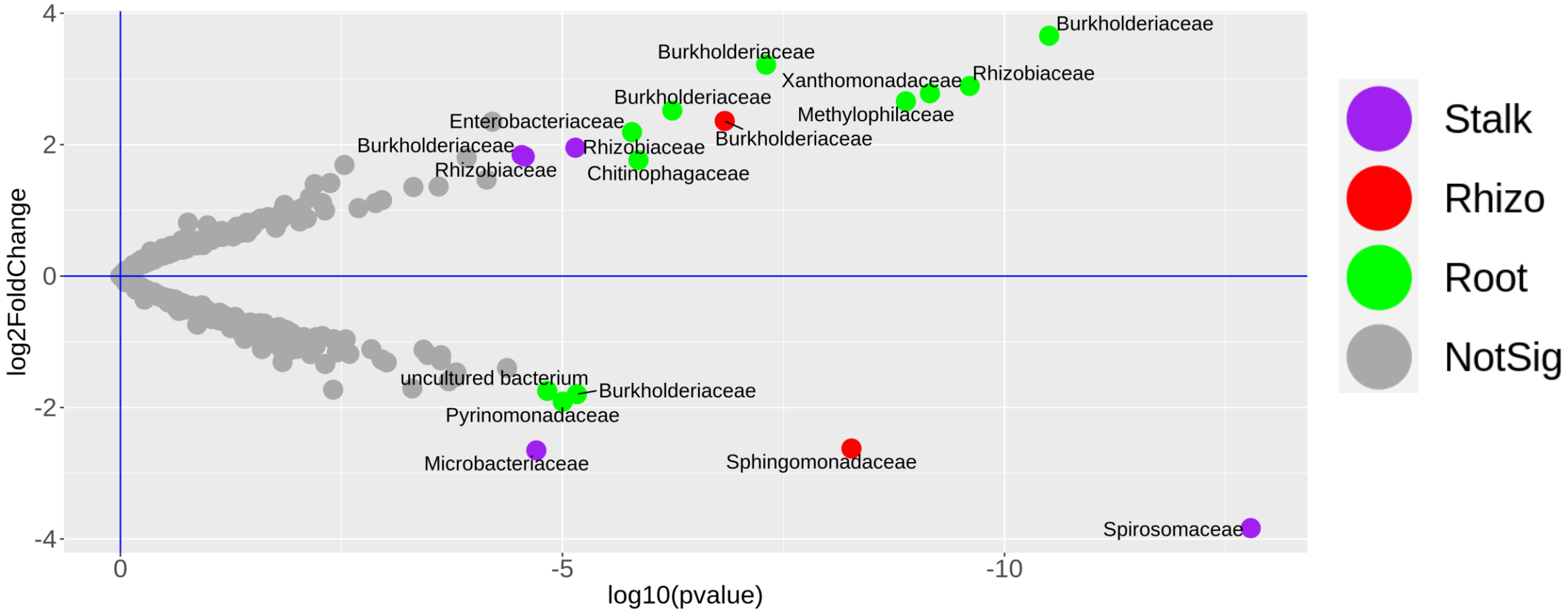


Bray Curtis Distance PERMANOVA

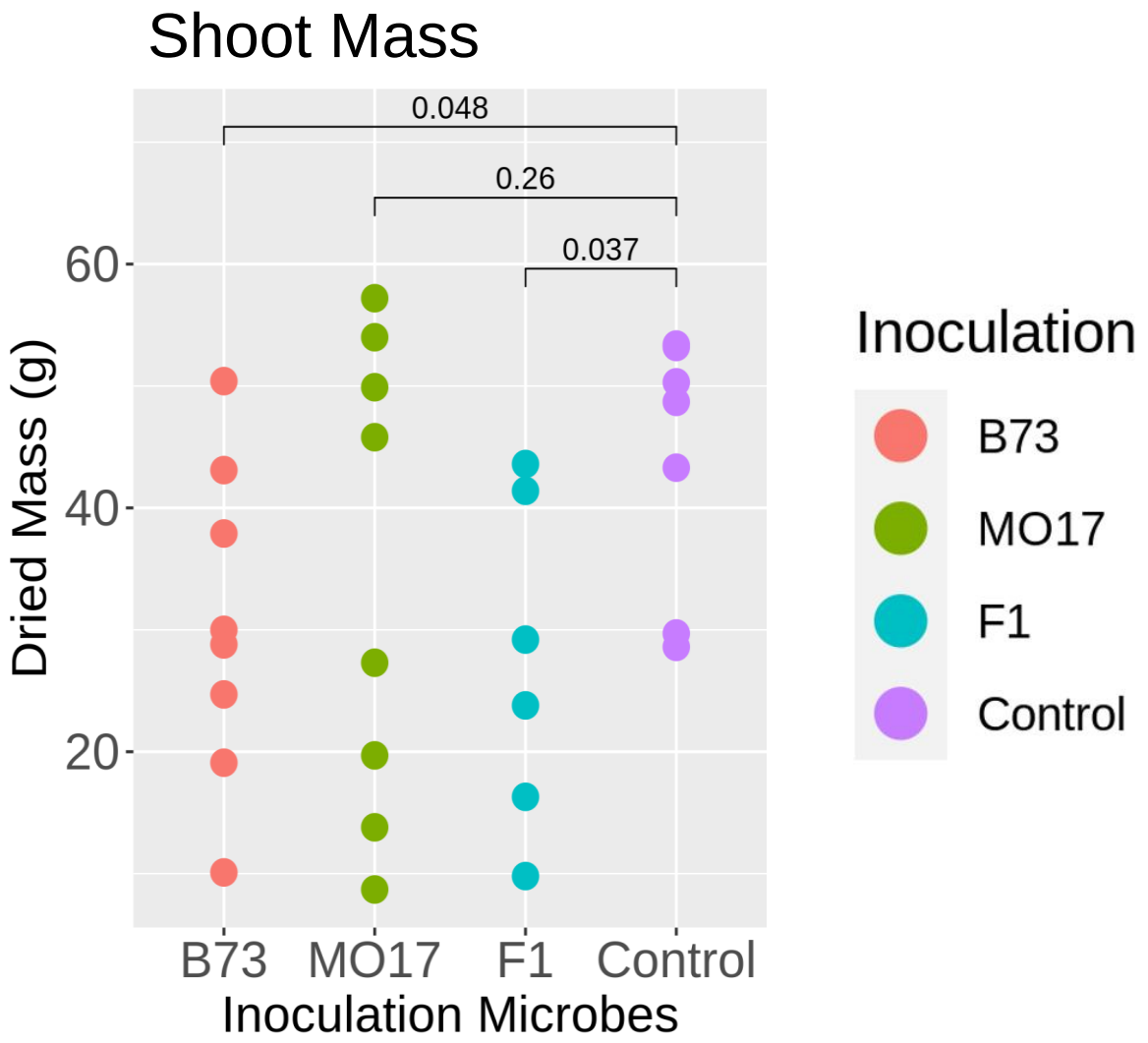
```
Number of permutations: 999

Terms added sequentially (first to last)

                          Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
$Experiment                2    15.372  7.6858 27.9891 0.15196  0.001 ***
$Tissue                    3    17.286  5.7620 20.9831 0.17088  0.001 ***
$Inbred_or_Hybrid          4     1.907  0.4768  1.7363 0.01885  0.004 **
$Genotype                 19     5.039  0.2652  0.9658 0.04981  0.651
$Location                  1     0.593  0.5929  2.1591 0.00586  0.011 *
Residuals                222    60.961  0.2746         0.60264
Total                    251   101.158                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

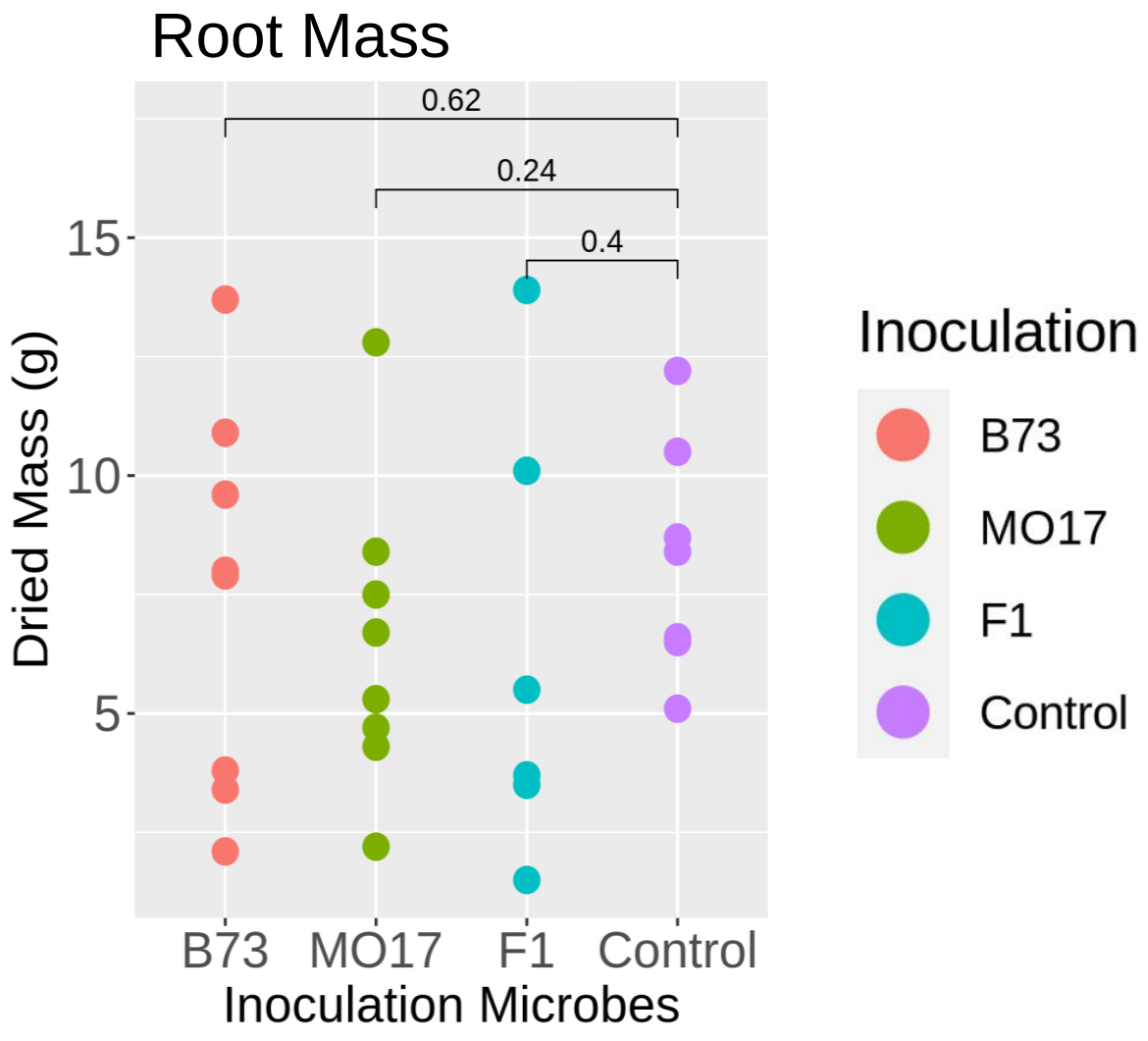
Experiment
Tissue
Inbred_or_Hybrid
Genotype
Location

Differential Abundance: Inbred vs Hybrids



Inoculated Fast-Flowering Mini Maize with Microbiomes



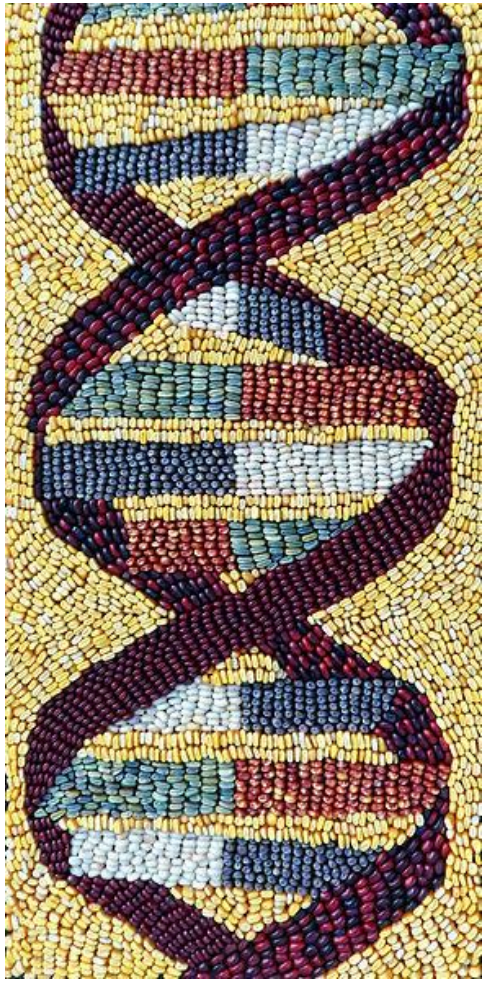


Take Aways – Project 2

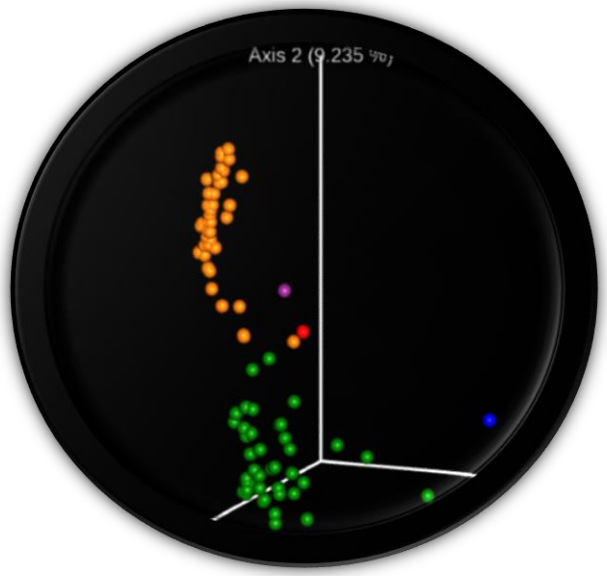
- Microbiome differed due to location and genetic background.

- Microbiome inoculation hurt above ground biomass.

Wrap up



Small but significant impact



Thanks!

- Committee Members:
  - Dr. Jason Wallace
  - Dr. Anny Chung
  - Dr. Elizabeth Ottesen
  - Dr. CJ Tsai

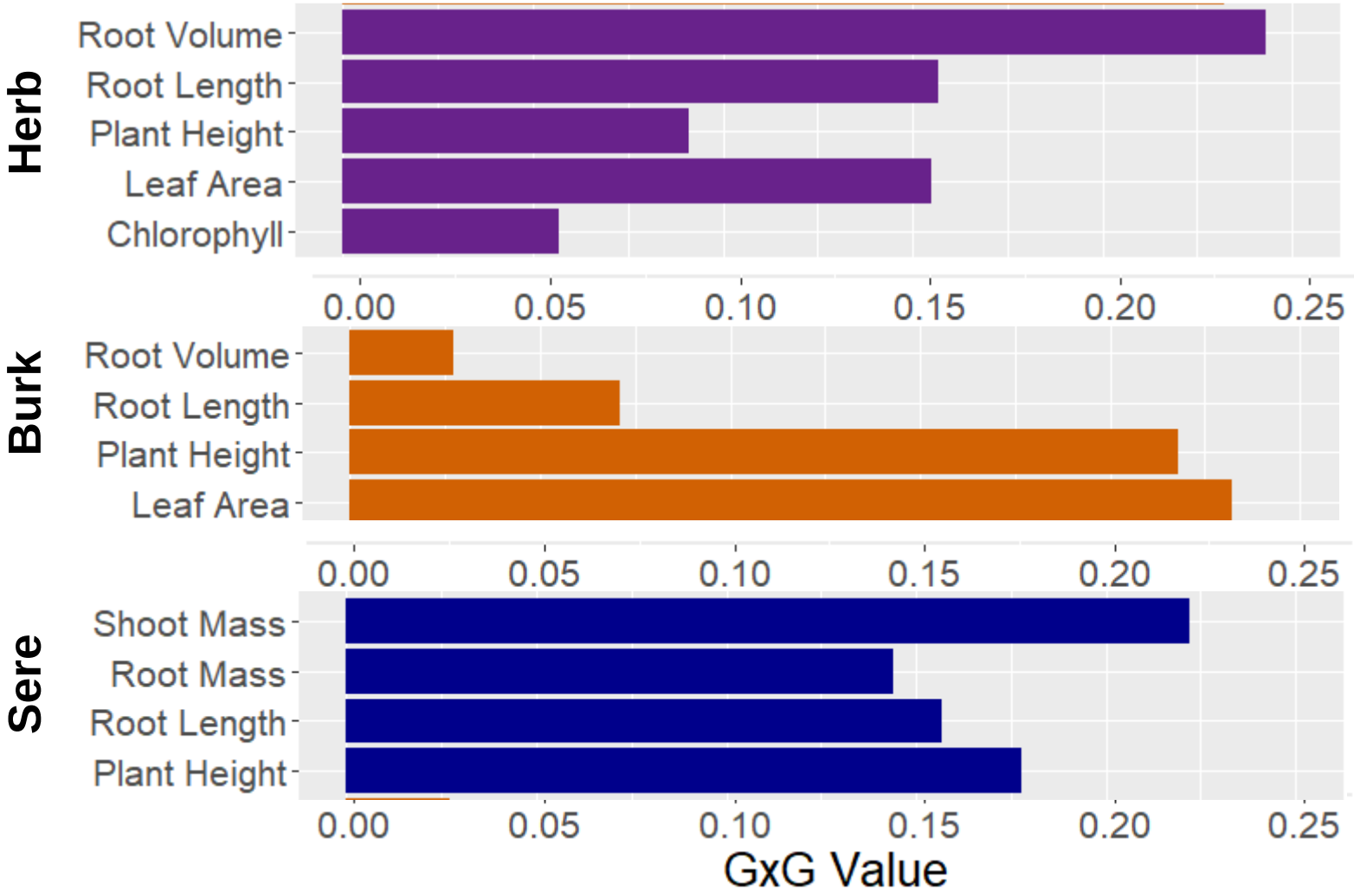
- Noble Research Institute
  - Dr. Ray Prasun
  - Dr. Kelly Craven
- University of Washington
  - Dr. Sharon Doty

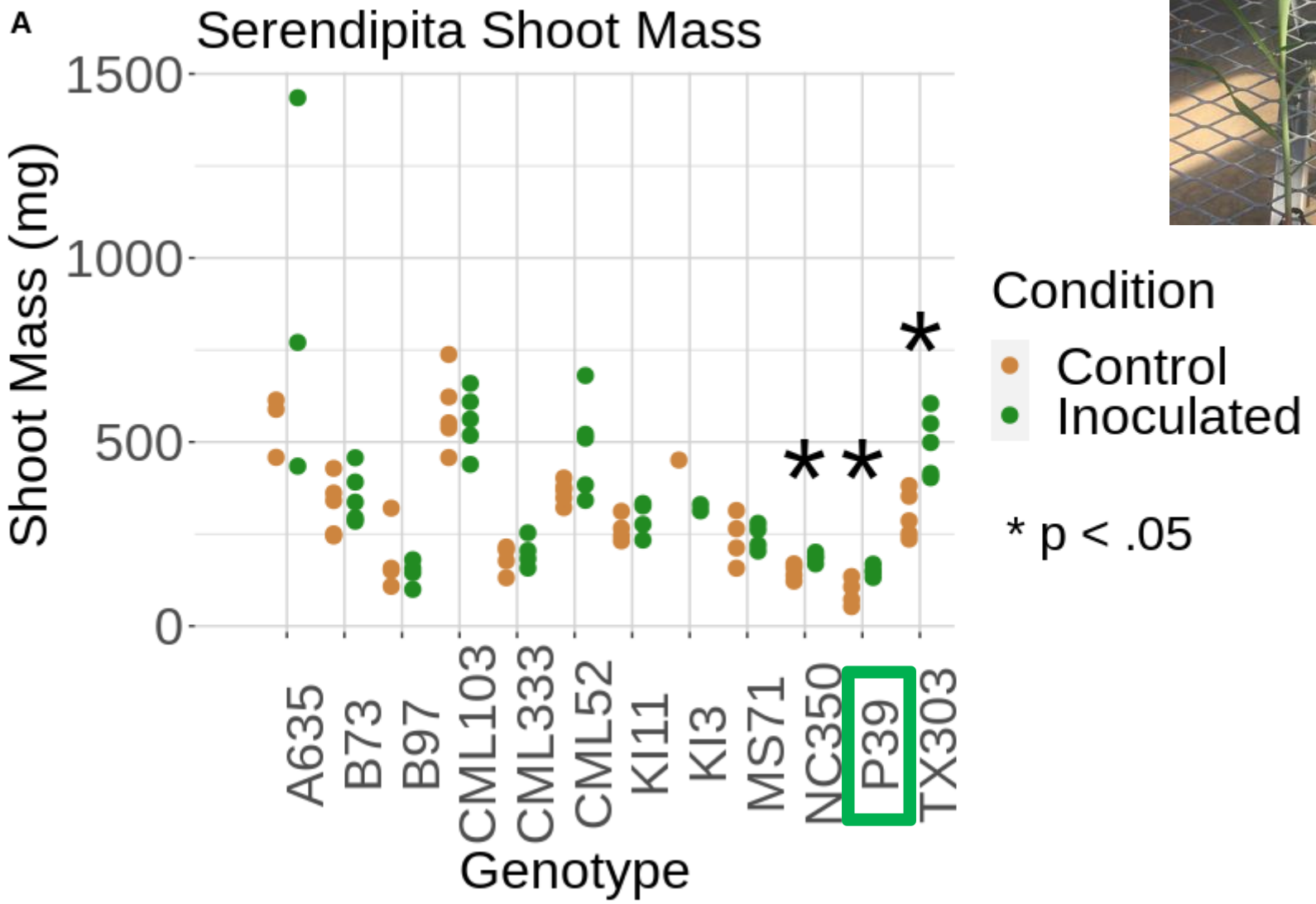
The Wallace Lab

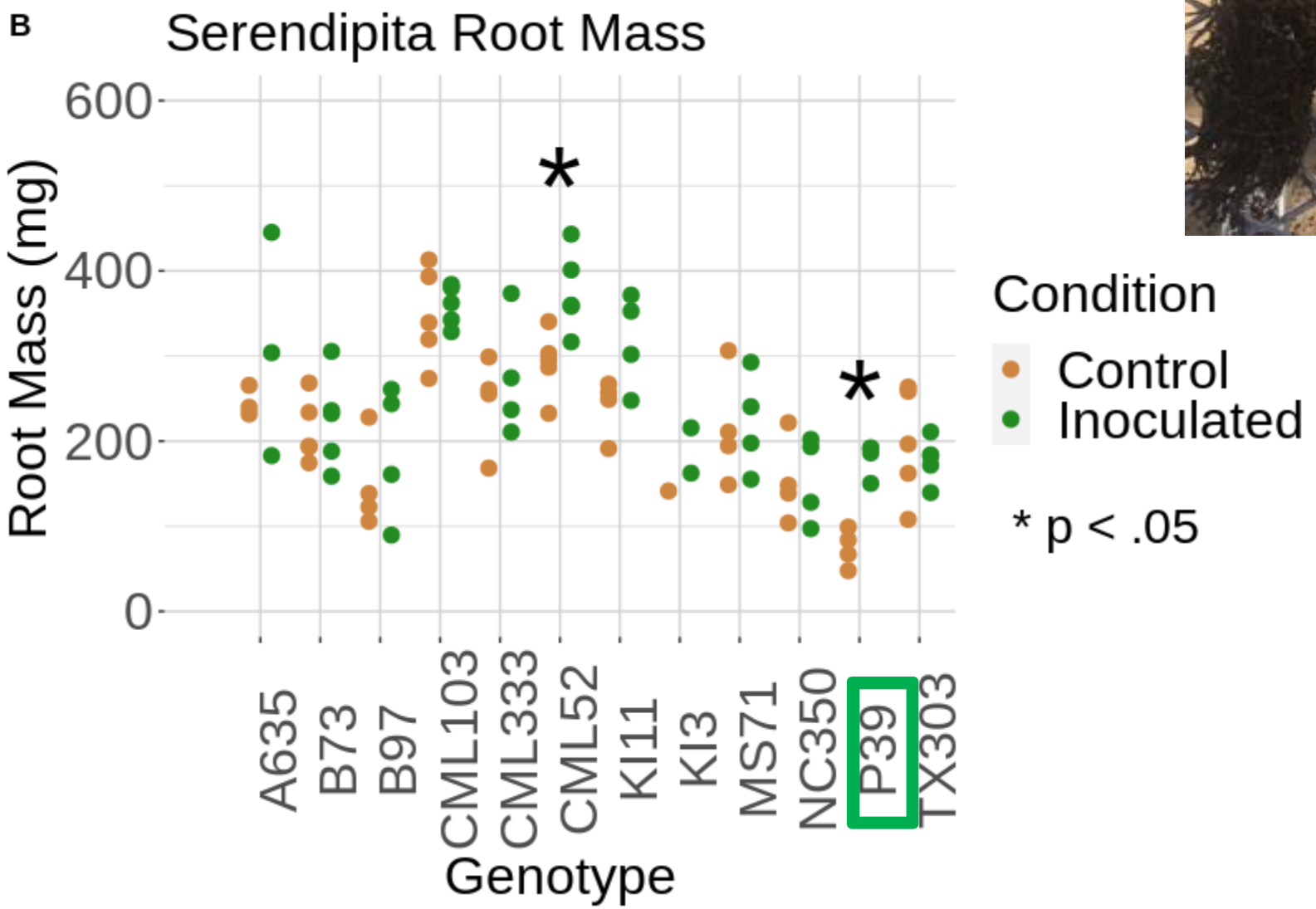
- <u>Funding</u>
  - Foundation for Food and Agriculture Research
  - National Corn Growers Association
  - University of Georgia Graduate School

Contact me:
Corey.Schultz@uga.edu
www.linkedin.com/in/corey-schultz

Questions?


Herb
Root Volume
Root Length
Plant Height
Leaf Area
Chlorophyll
0.00
0.05
0.10
0.15
0.20
0.25
Burk
Root Volume
Root Length
Plant Height
Leaf Area
0.00
0.05
0.10
0.15
0.20
0.25
Sere
Shoot Mass
Root Mass
Root Length
Plant Height
0.00
0.05
0.10
0.15
0.20
0.25
GxG Value


A
Serendipita Shoot Mass
Shoot Mass (mg)
1500
1000
500
0
A635
B73
B97
CML103
CML333
CML52
KI11
KI3
MS71
NC350
P39
TX303
Genotype
Condition
Control
Inoculated
* p < .05


B
Serendipita Root Mass
Root Mass (mg)
600
400
200
0
*
*
A635
B73
B97
CML103
CML333
CML52
KI11
KI3
MS71
NC350
P39
TX303
Genotype
Condition
Control
Inoculated
* p < .05

- *Herbaspirillum seropedicae* – nitrogen fixation in grasses.

- *Burkholderia* WP9 – increases uptake of phosphate and nitrogen.

- *Serendipita bescii* – promotes growth in switchgrass

Endophyte	Trait	GxG Interaction (0-1)
	Experiment 1	
Herbaspirillum	Chlorophyll	.057
Herbaspirillum	Plant Height	0.091
Herbaspirillum	Leaf Area	0.155
Herbaspirillum	Root Length	0.157
Herbaspirillum	Root Volume	0.243
	Experiment 2	
Burkholderia	Plant Height	0.218
Burkholderia	Leaf Area	0.232
Burkholderia	Root Length	0.071
Burkholderia	Root Volume	0.027
	Experiment 3	
Serendipita	Plant Height	0.178
Serendipita	Root Length	0.157
Serendipita	Root Mass	0.144
Serendipita	Shoot Mass	0.222

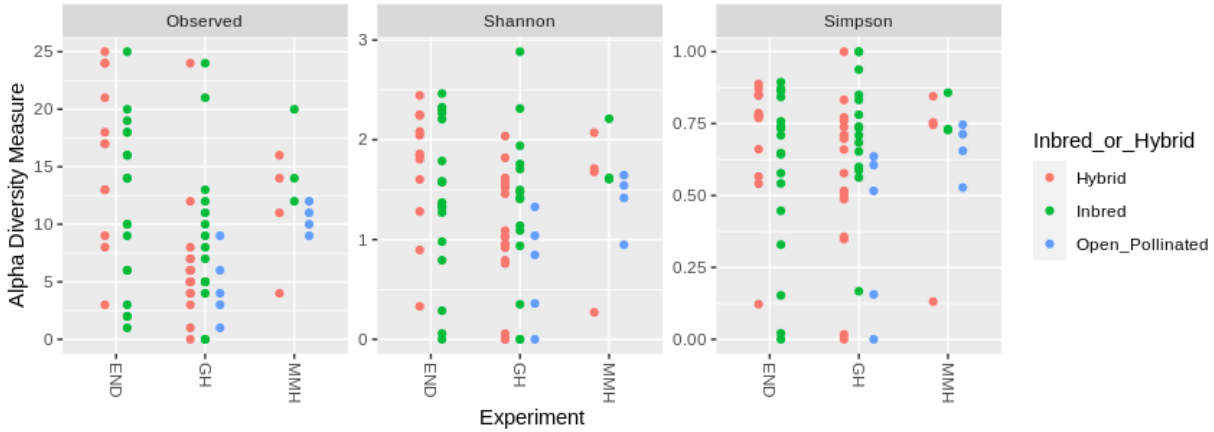
- *Herbaspirillum seropidicae* and *Serendipita bescii* **differentially promoted growth** in diverse maize.

- Our lower heritability calculations suggest that identifying genetic loci that play a role in this genotype-endophyte interaction may be **challenging.**

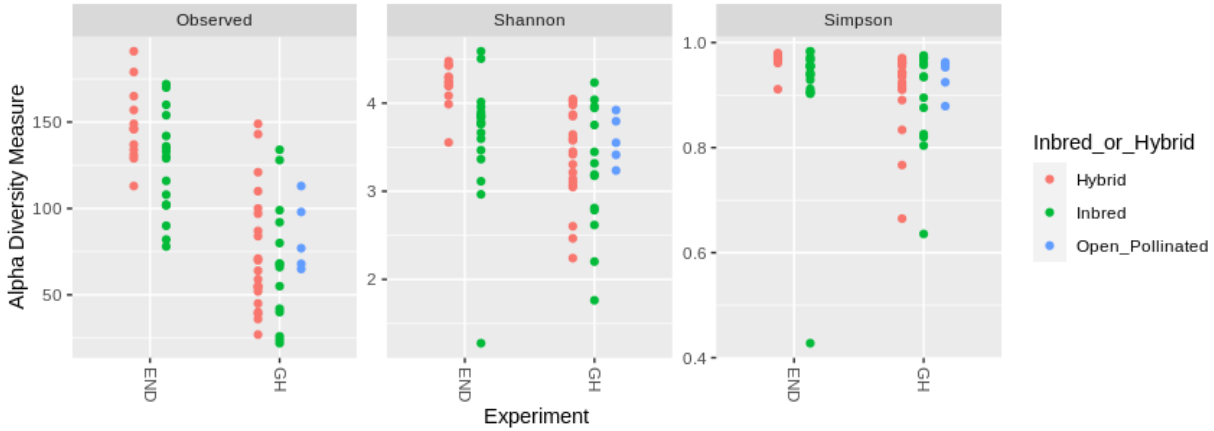
- Researchers and Companies need to test new bioinoculates on several lines
  - Waste Time
  - Waste Money
  - Worst Case Scenario – decrease growth

- Co-select crop lines with microbial consortia to maximize this interactions and growth.

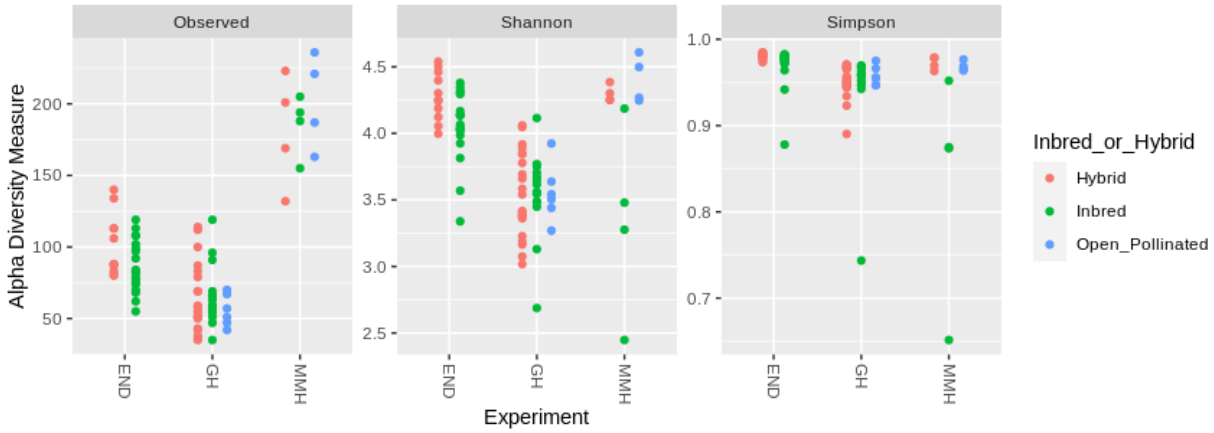
A Alpha Diversity: Combined Experiments - Stalks



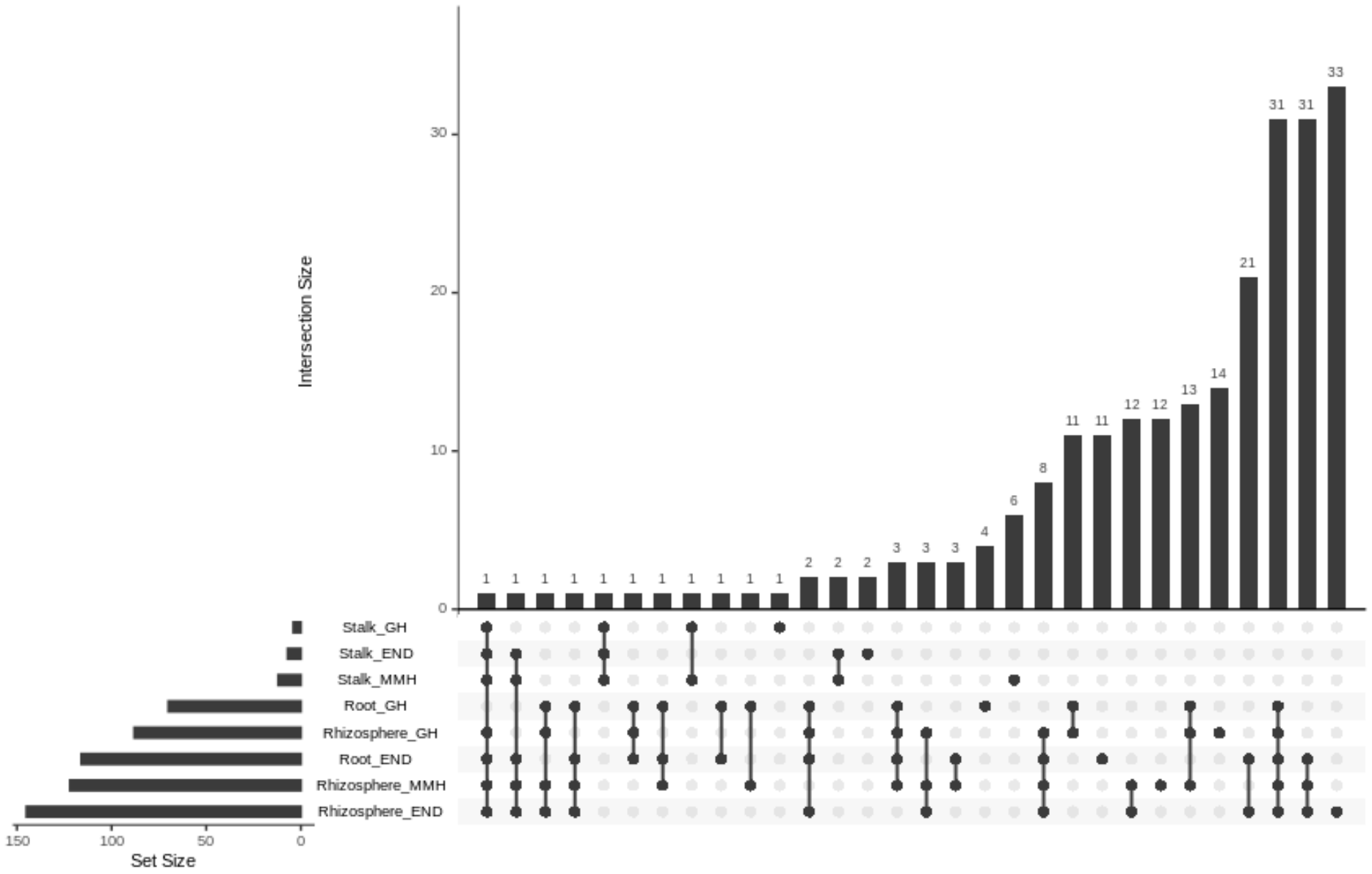
B Alpha Diversity: Combined Experiments - Roots



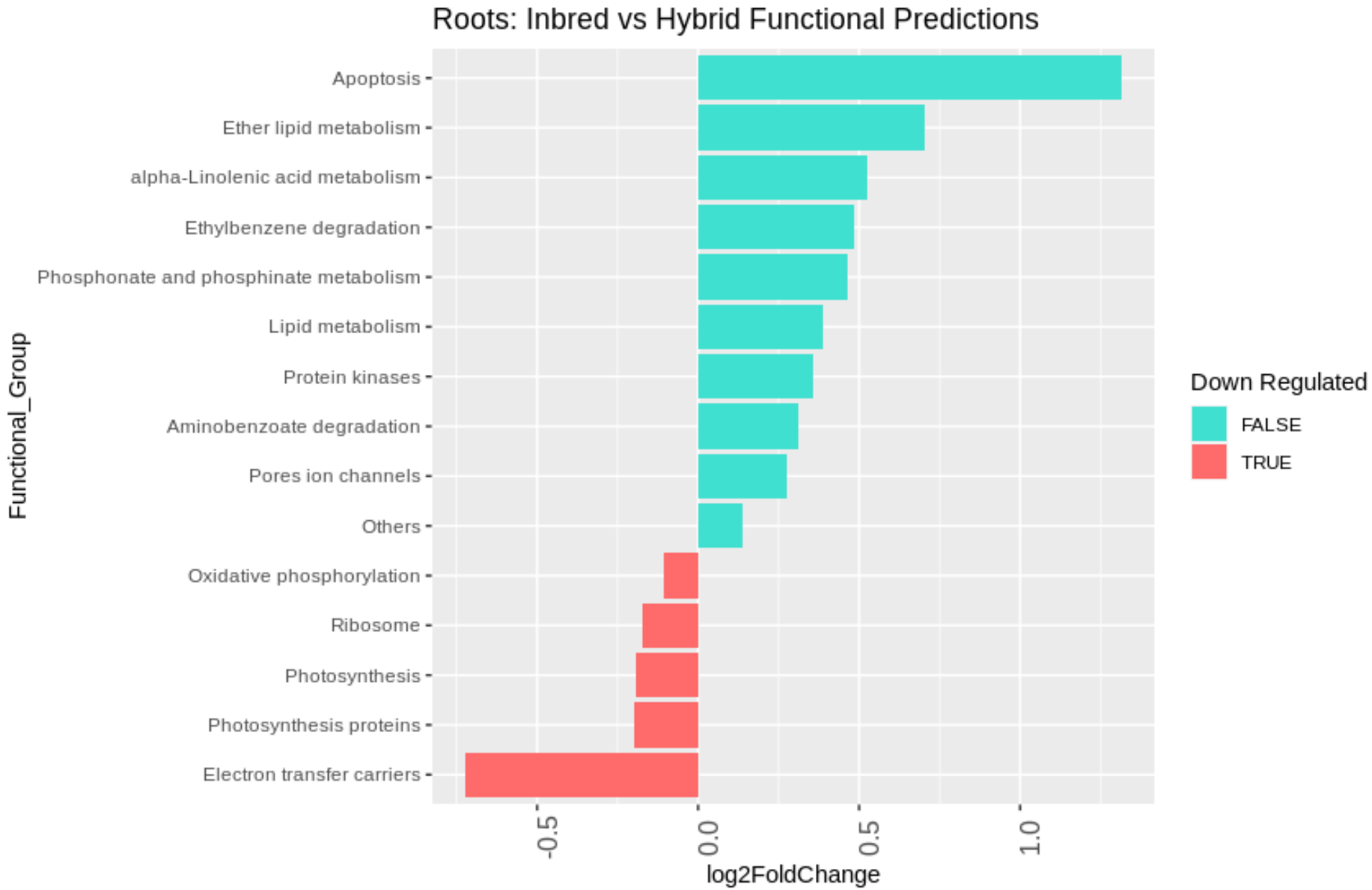
C Alpha Diversity: Combined Experiments - Rhizos



```
Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                                      Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
sample_data(phyCmbFilt)$Experiment     2    15.372  7.6858 27.9891 0.15196  0.001 ***
sample_data(phyCmbFilt)$Sample_Type    3    17.286  5.7620 20.9831 0.17088  0.001 ***
sample_data(phyCmbFilt)$Inbred_or_Hybrid   4     1.907  0.4768  1.7363 0.01885  0.004 **
sample_data(phyCmbFilt)$Genotype      19     5.039  0.2652  0.9658 0.04981  0.651
sample_data(phyCmbFilt)$Location       1     0.593  0.5929  2.1591 0.00586  0.011 *
Residuals                            222    60.961  0.2746         0.60264
Total                                251   101.158                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```


Intersection Size
0
10
20
30
1 1 1 1 1 1 1 1 1 1 1 2 2 2 3 3 3 4 6 8 11 11 12 12 13 14 21 31 31 33
Stalk_GH
Stalk_END
Stalk_MMH
Root_GH
Rhizosphere_GH
Root_END
Rhizosphere_MMH
Rhizosphere_END
150
100
50
0
Set Size

Compartment	Experiment	Comparison	Class Level	Genus Level	ASV Level	KO Groups
All	All	Inbred vs Hybrid	0	20	55	0
Stalks	All	Inbred vs Hybrid	2	2	5	0
Rhizos	All	Inbred vs Hybrid	0	0	2	0
Roots	All	Inbred vs Hybrid	0	8	11	15
All	All	IH Farm vs GH	13	151	475	38
Stalks	All	IH Farm vs GH	0	14	24	37
Rhizos	All	IH Farm vs GH	6	42	177	55
Roots	All	IH Farm vs GH	10	51	181	66


Roots: Inbred vs Hybrid Functional Predictions
Functional_Group
Apoptosis
Ether lipid metabolism
alpha-Linolenic acid metabolism
Ethylbenzene degradation
Phosphonate and phosphinate metabolism
Lipid metabolism
Protein kinases
Aminobenzoate degradation
Pores ion channels
Others
Oxidative phosphorylation
Ribosome
Photosynthesis
Photosynthesis proteins
Electron transfer carriers
-0.5
0.0
0.5
1.0
log2FoldChange
Down Regulated
FALSE
TRUE