

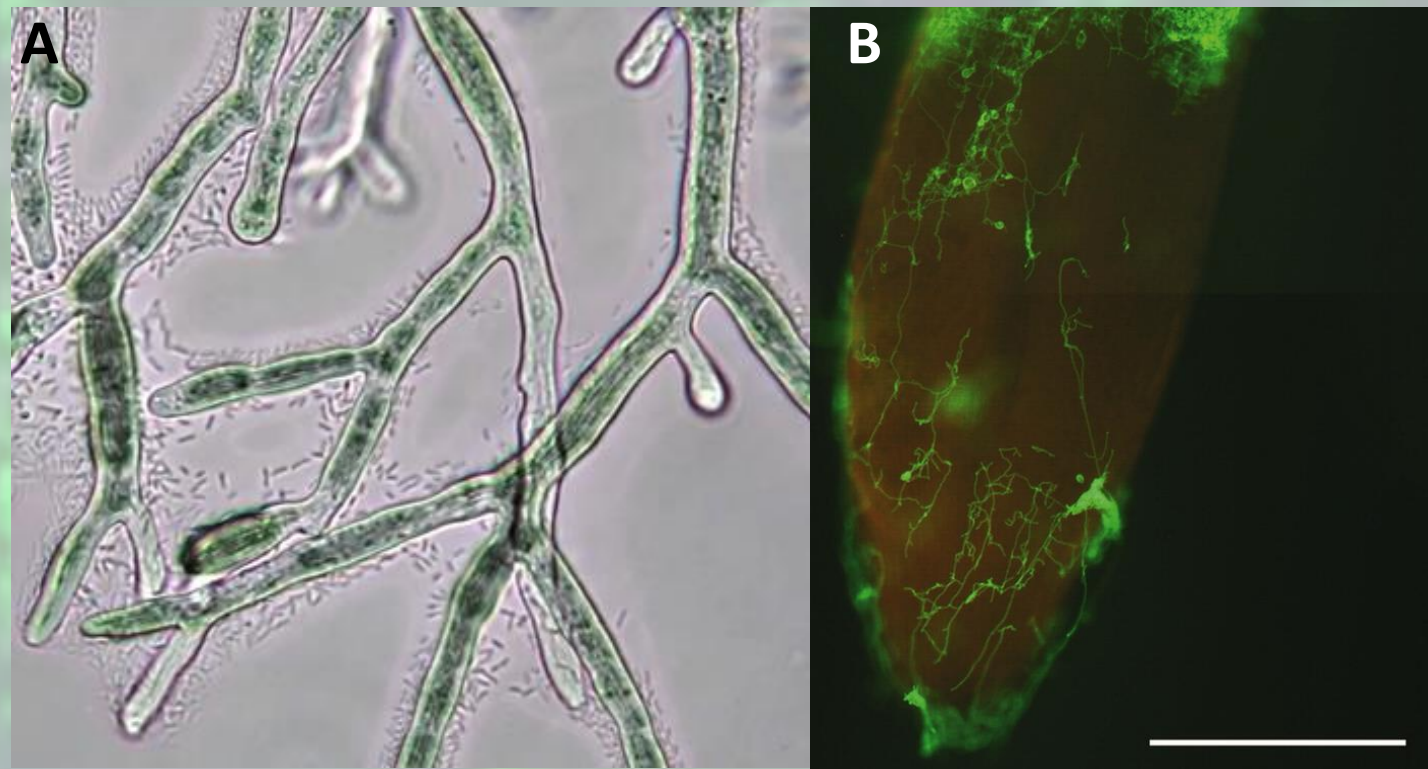
Do fungal endophytes facilitate colonization of bacterial endophytes in *Brachypodium distachyon*?

Julian Liber¹ and Gregory Bonito¹; @LiberJulian, liberjul@msu.edu

¹Department of Plant, Soil, and Microbial Sciences, Michigan State University

Introduction

- Bacteria move along hyphae on water films through solid media (cheese, soil)
- Fungal endophytes penetrate roots – provide channels
- Bacterial and fungal endophytes provide benefits to plants



A) *Serratia* cells swimming on *Mucor* hyphae. Zhang et al., 2018, *Nat. Comm.* **B)** *Serendipita indica* hyphae on barley. Deshmukh et al., 2006, *PNAS*

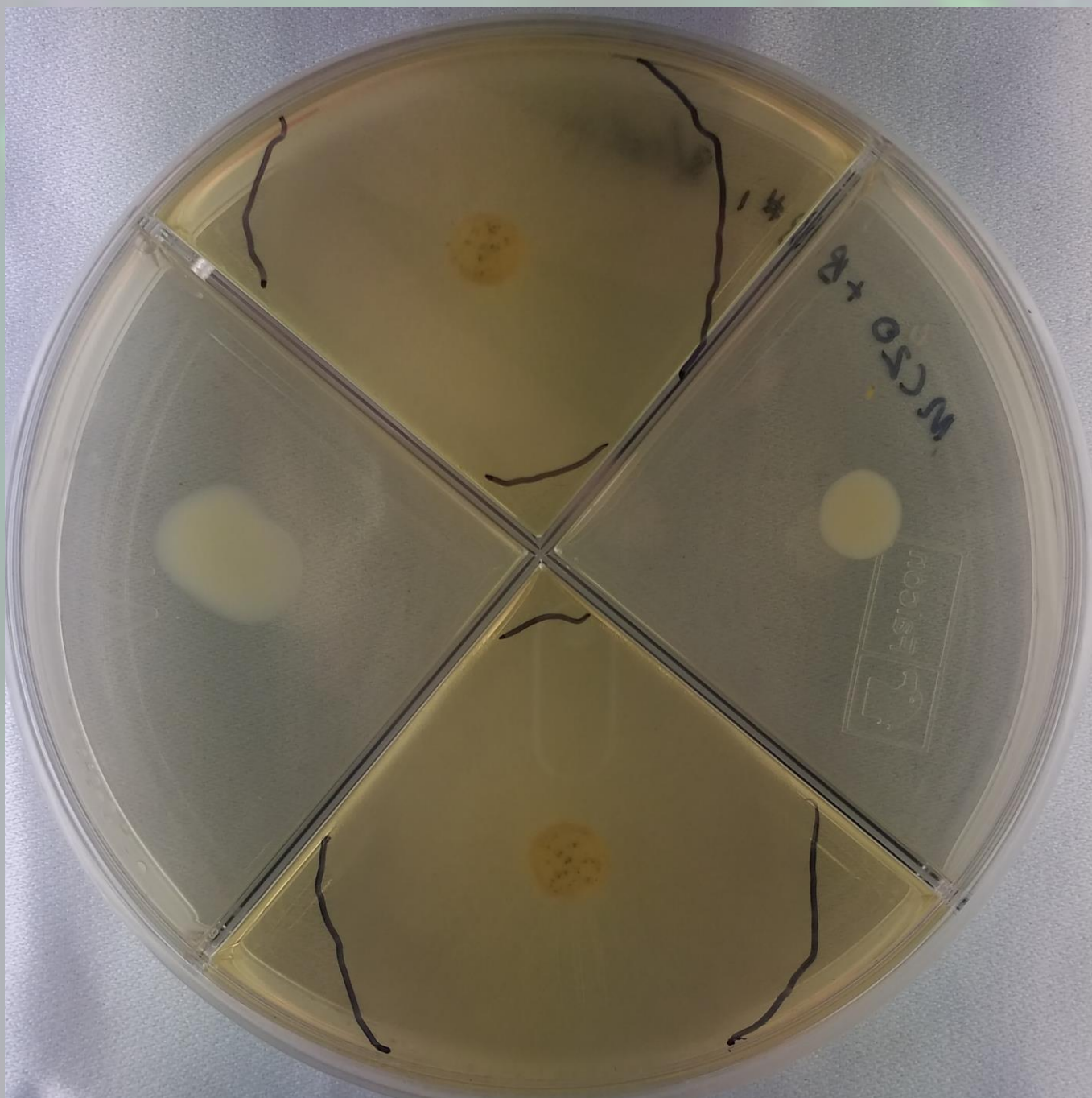
Questions

- Interaction – positive or negative between bacteria and fungi?
- Movement – bacteria on or near hyphae in roots?
- Abundance – amount of bacteria dependent on fungi?

Hypothesis

Presence of fungi facilitates bacterial movement into root apoplast, resulting in higher bacterial abundance within hyphae.

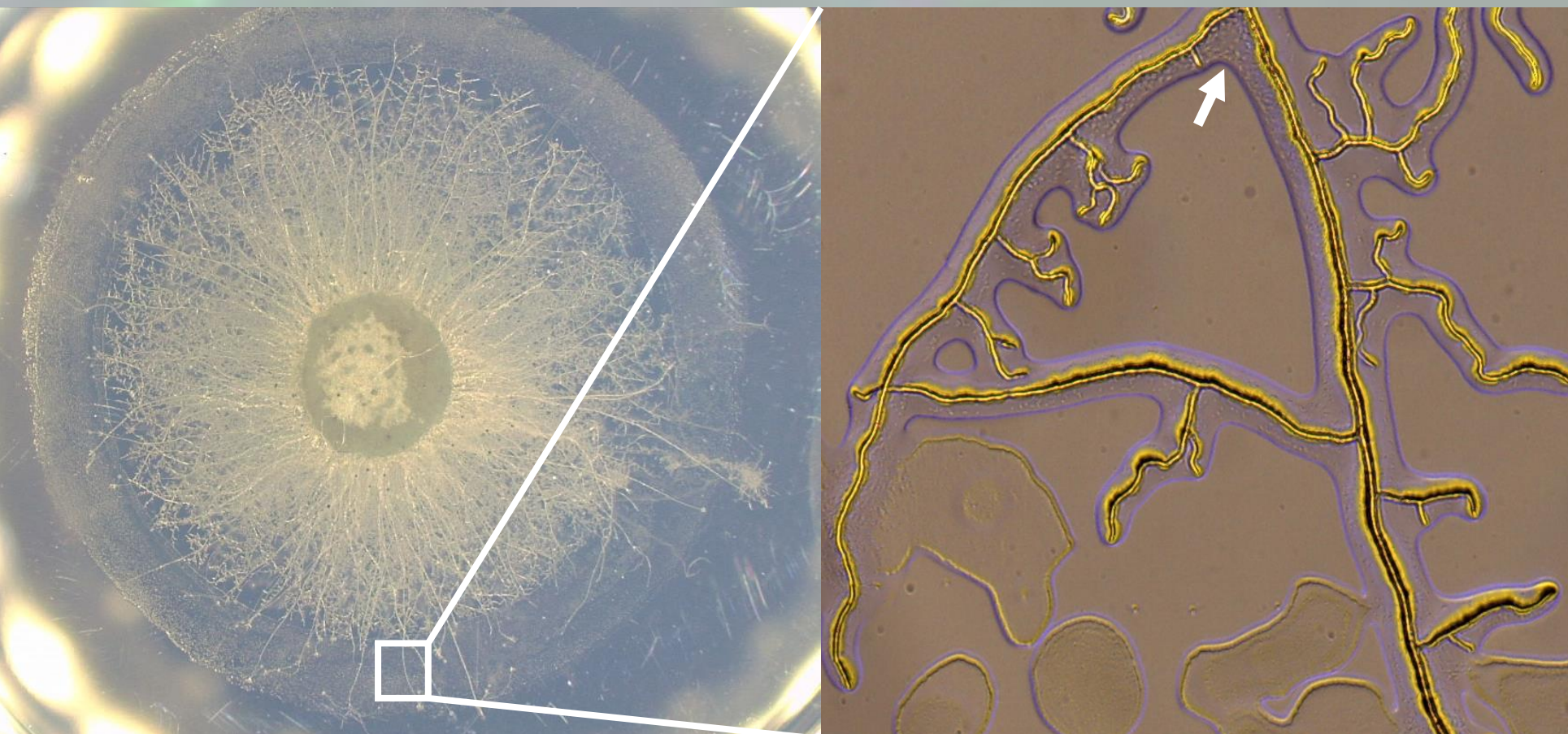
Methods



Volatile assay – Bacteria and fungi on quartered plate



Brachypodium + bacteria + fungus on plant media (- sucrose). Plant were used for dry weight, confocal microscopy, and qPCR.



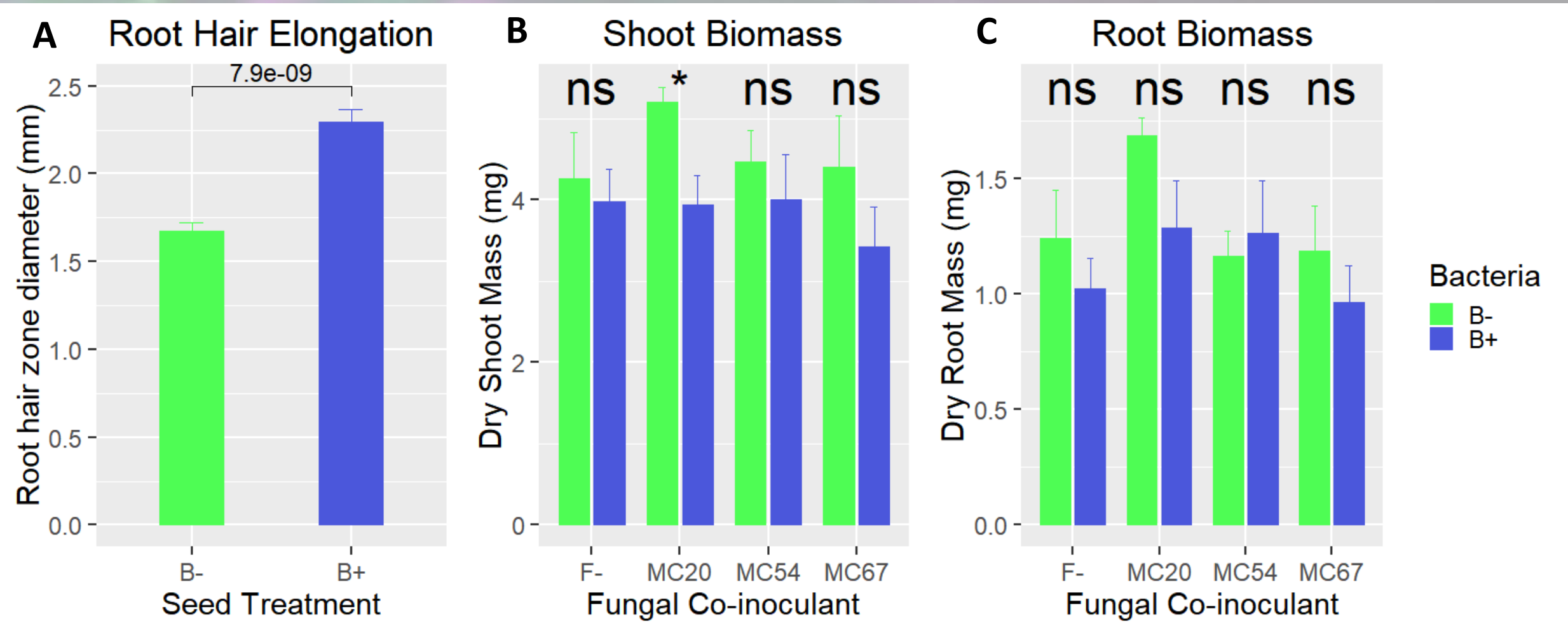
Motility assay – Bacteria and fungi on water agar

- Organisms used:
 - Brachypodium distachyon* Bd21
 - Enterobacter ludwigii* FCP2-01
 - Fungi isolated as endophytes
 - MC20 *Rhizopus oryzae*
 - MC50 *Alternaria alternate*
 - MC54 *Mucor circinelloides*
 - MC64 *Penicillium pinophilum*
 - MC67 *Fusarium solani* SC
 - MC68 *Fusarium oxysporum* SC
- CLSM used to look for co-localization of bacterial and fungi
- qPCR used to relatively quantify bacterial infection of roots, normalized by plant DNA amount

Bacterial and fungal endophytes compete for limited niche space in root apoplast

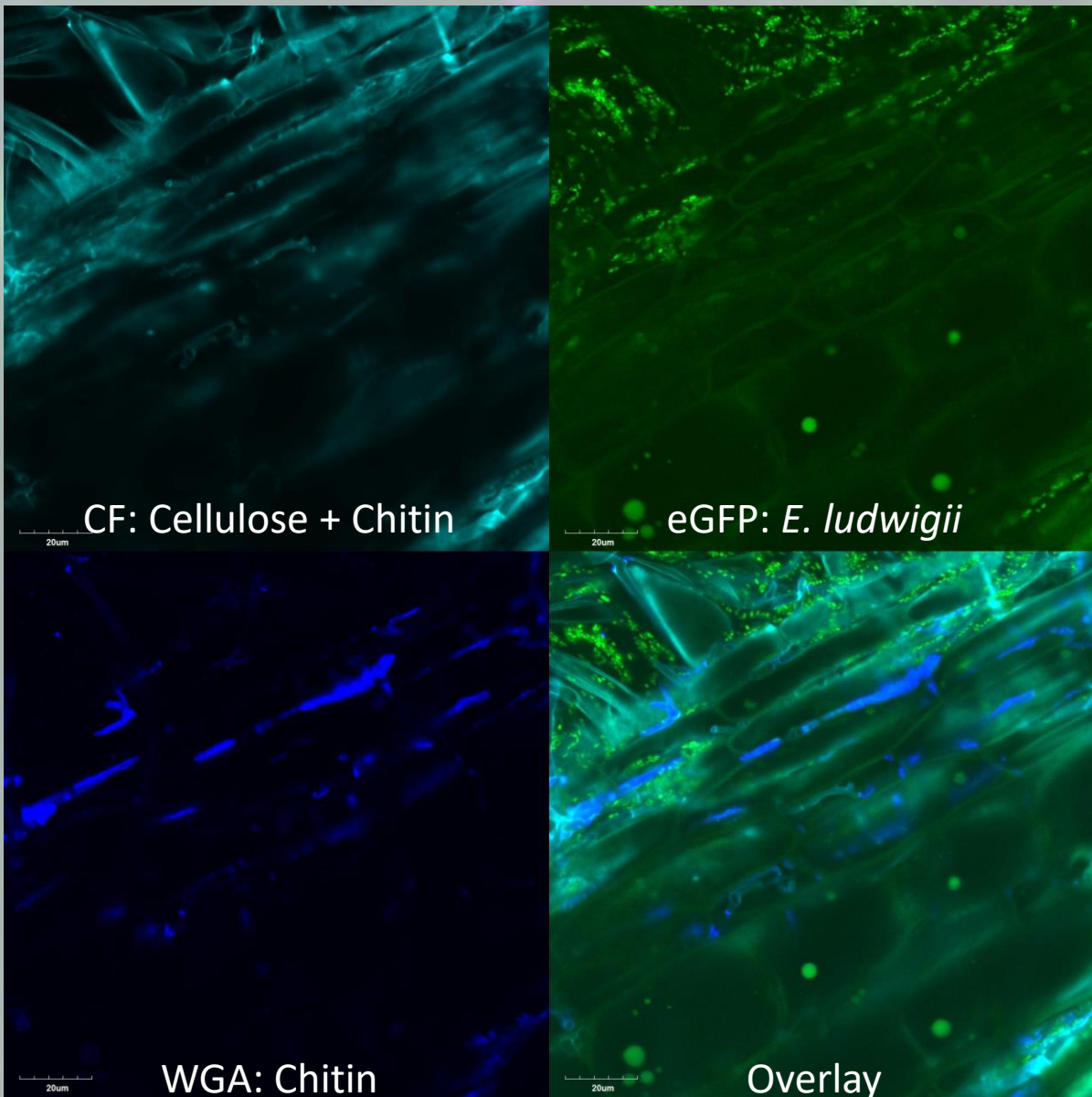
Results

Effects on plant traits

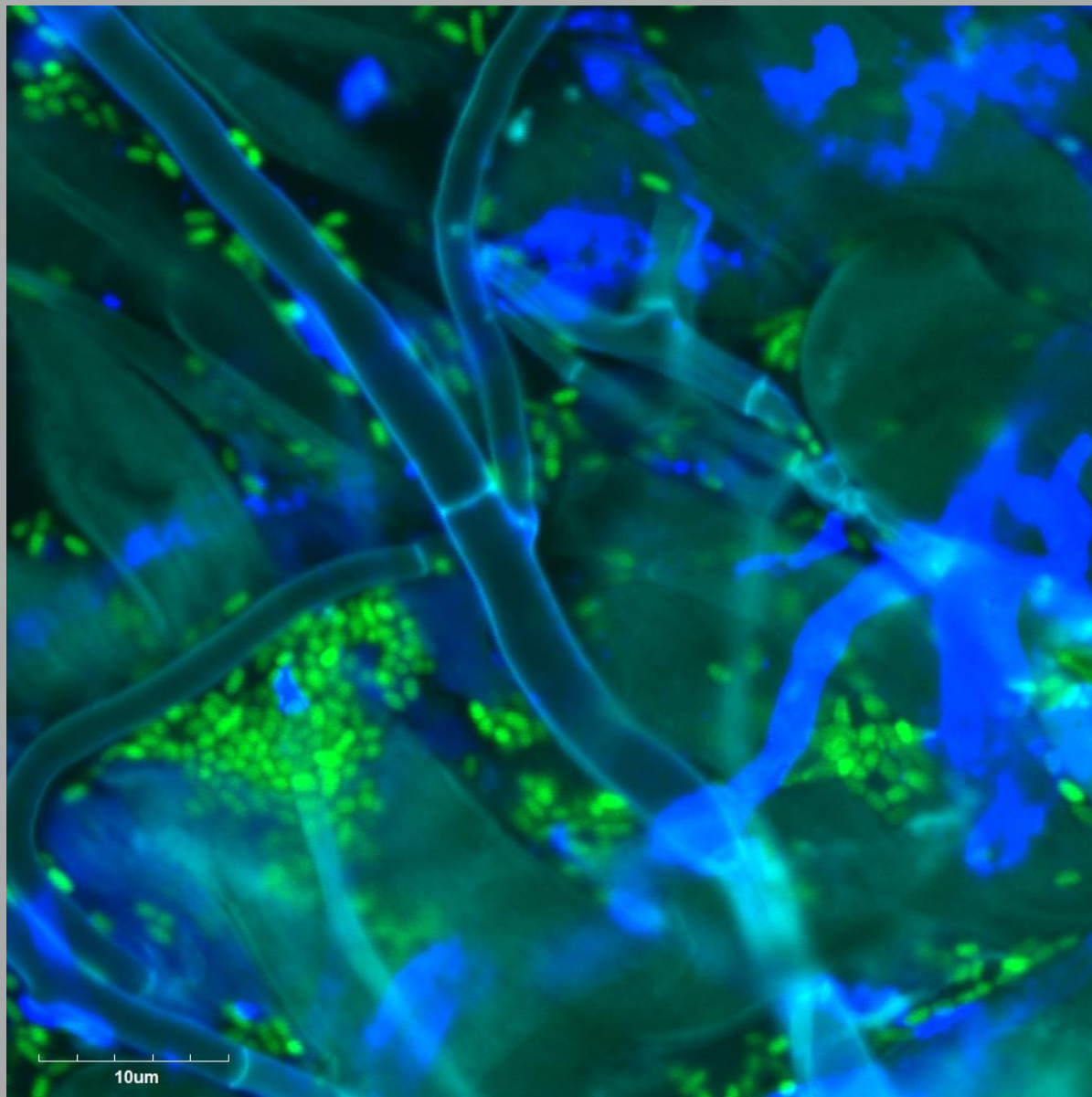


A) Endophytic bacteria demonstrated a positive effect on root hair zone diameter. **B)** Bacteria showed negative effect on plant shoot mass (ANOVA, $p = 0.028$), and MC20 showed difference between B- and B+ conditions. **C)** No significant effects of bacteria or fungus observed on root mass.

Confocal microscopy of roots

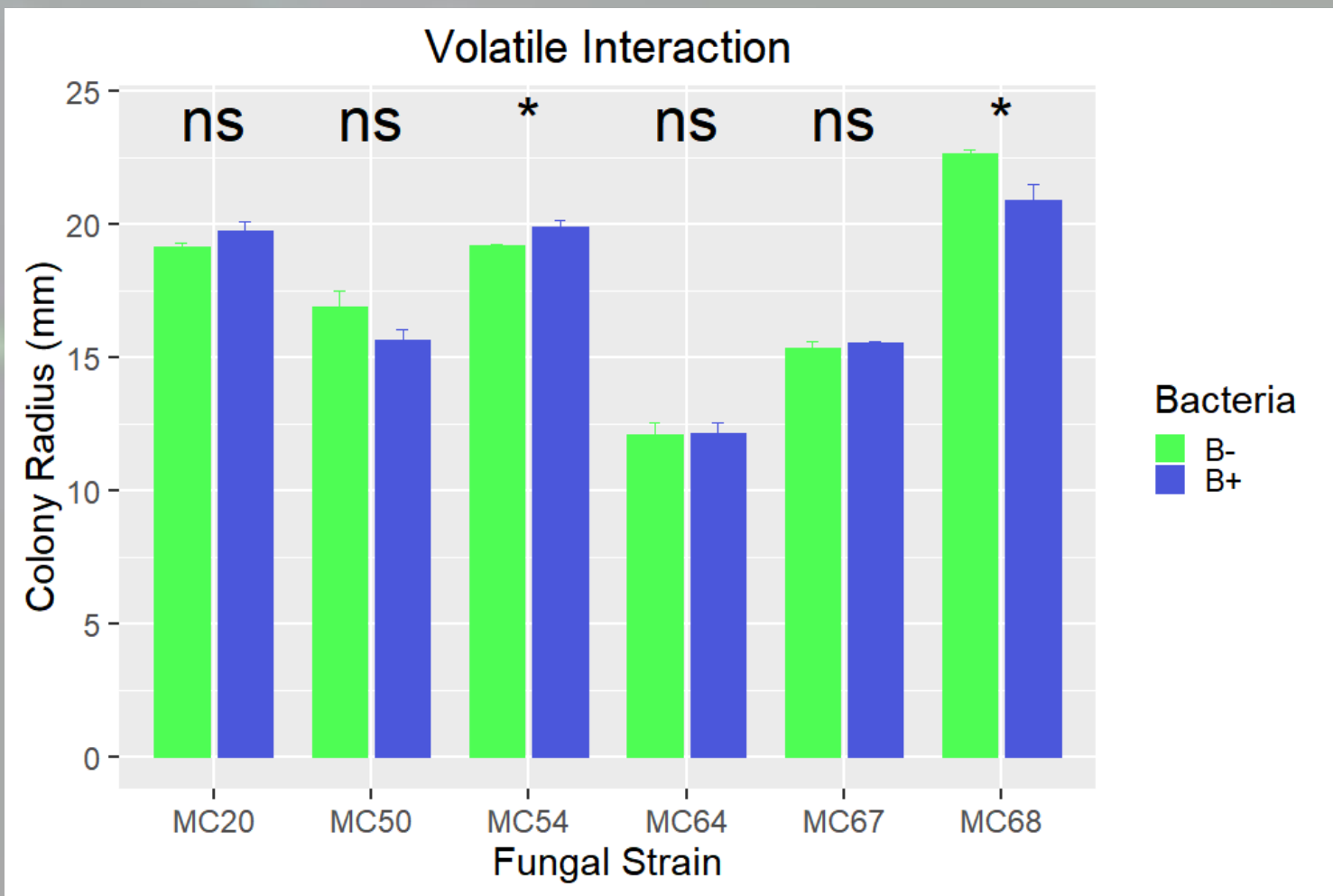


CLSM of root spaces, showing exclusion of bacteria by fungal hyphae (MC20). Staining with calcofluor white (CF) and wheat germ agglutinin (WGA). Scale bar = 20 μ m.



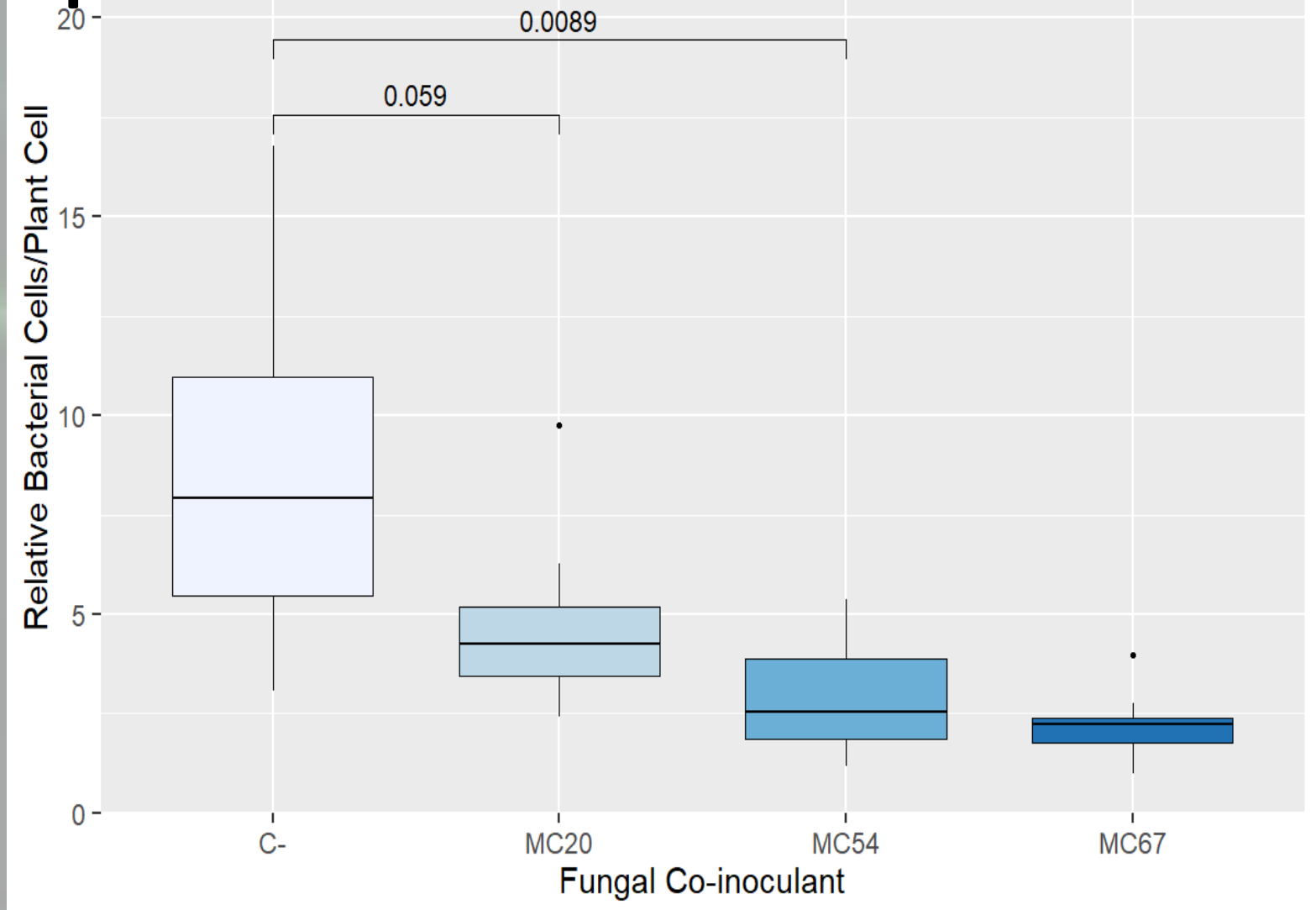
E. ludwigii and *R. oryzae* on the surface of a *Brachypodium* root. Scale bar = 10 μ m.

Bacterial effect on fungi



Comparison of fungi grown with expose to bacteria headspace to those grown without. Time of growth varies between strains, but not between bacterial +/- conditions.

qPCR-based abundance



Relative bacterial cell per plant cell measured with single copy genes. Fungal co-inoculant had a significant effect on bacterial infection (ANOVA, $F = 8.977$, $p < 0.0005$).

Discussion

- E. ludwigii* showed measurable effect on plant traits
- Some fungi had increased or decreased growth in response to bacteria volatiles
- All fungi facilitated movement of bacteria on agar plates
- Bacterial and fungi occupied different spaces in the roots
- Fungal inoculation decreased bacterial endophyte infection
- Next steps: microfluidic interaction and mobility assays, effect of plant viral disease state

Acknowledgments

- Morgan Chretien for strains, Jud Van Wyk for image measurements, and Pedro da Costa for experimental design help
- 2018 MSU iGEM team for developing bacterial endophyte system
- Research in the Bonito lab is supported though NSF - DEB 1737898 and MSU - AgBioResearch NIFA project MICL02416

