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A High-Throughput System to Measure Beneficial Plant-Microbial Interactions

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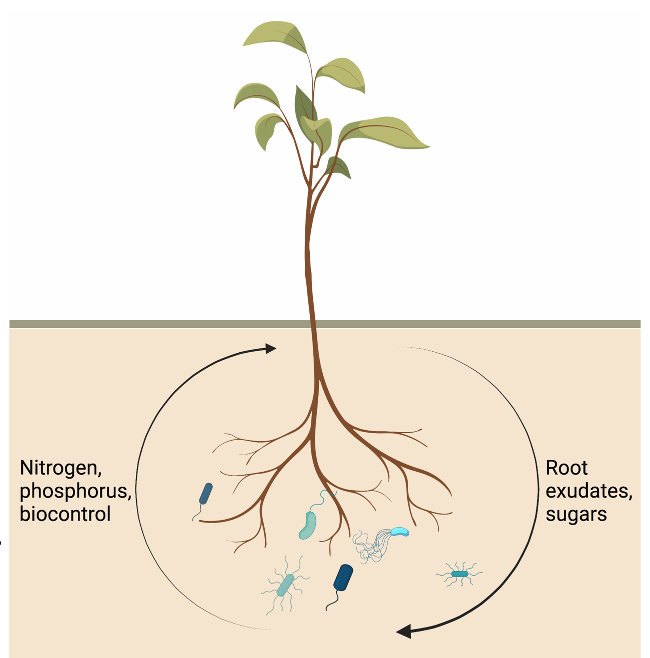
Abstract

Plant-microbial interactions have a significant impact on plant health and growth, and better understanding these systems could lead to new ways to improve the yield and resilience of agricultural crops. However, they are difficult to study because of the inherent complexity of both soil structure and soil microbial communities; thus, there is a demand for a standardized system to study complex soil microbiomes in a reproducible fashion. Here, we developed a system for growing *Brachypodium distachyon* in a model soil system in a container dubbed the minirhizotron. To determine the organic matter to add to model soil, we grew *B. distachyon* in minirhizotrons with five different types of ground organic matter: *Sorghum bicolor, B. distachyon*, and leaf litter from red maple, red oak, and black birch trees. Plants were destructively harvested after three weeks of growth and measured for wet and dry biomass, as well as total leaf area, to determine plant health. Carbon dioxide production from model soils post-harvest was also measured as an indicator of microbial activity. Unexpectedly, red maple leaf litter caused inhibition of plant growth, as indicated by reduced total dry weight, leaf area, and specific leaf area. Microbial respiration was significantly higher in model soil containing ground sorghum, red oak, and red maple. From these results, we conclude that either ground sorghum or red oak leaf litter should be added to model soil systems to optimize both plant and microbial growth.

Introduction

Plants in a natural environment are exposed to thousands of pathogenic, commensal, and beneficial bacteria on a daily basis. By modifying the chemical composition of root exudates, plants are able to selectively promote or inhibit specific species of bacteria, cultivating a rhizosphere microbiome of symbiotic microorganisms[1]. Plants provide bacteria with carbon-rich exudates, and in return soil microbes provide a variety of services, including nitrogen fixation and solubilizing phosphorus and other inorganic nutrients. Thus, soil microbes are important in a variety of plant functions and could impact growth, health, and resilience of agricultural crops.



Created with BioRender.com

Researchers have been increasingly interested in studying plant-microbe interactions in recent years in response to growing demand for a way to increase agricultural productivity without the use of chemical fertilizers. There is currently no standardized system to study these interactions. Researchers will sometimes inoculate with a single strain or simple coculture of bacteria, which generally do not reflect the diversity of natural microbial communities[2]. Conversely, inoculating with environmentally obtained microbes lacks reproducibility. Therefore, there is significant demand for a replicable, standardized system for studying plant-microbial interactions.

Methods

Model soil Preparation

Base formula

90% acid washed sand + 6.67% kaolinite clay + 3.33% bentonite clay

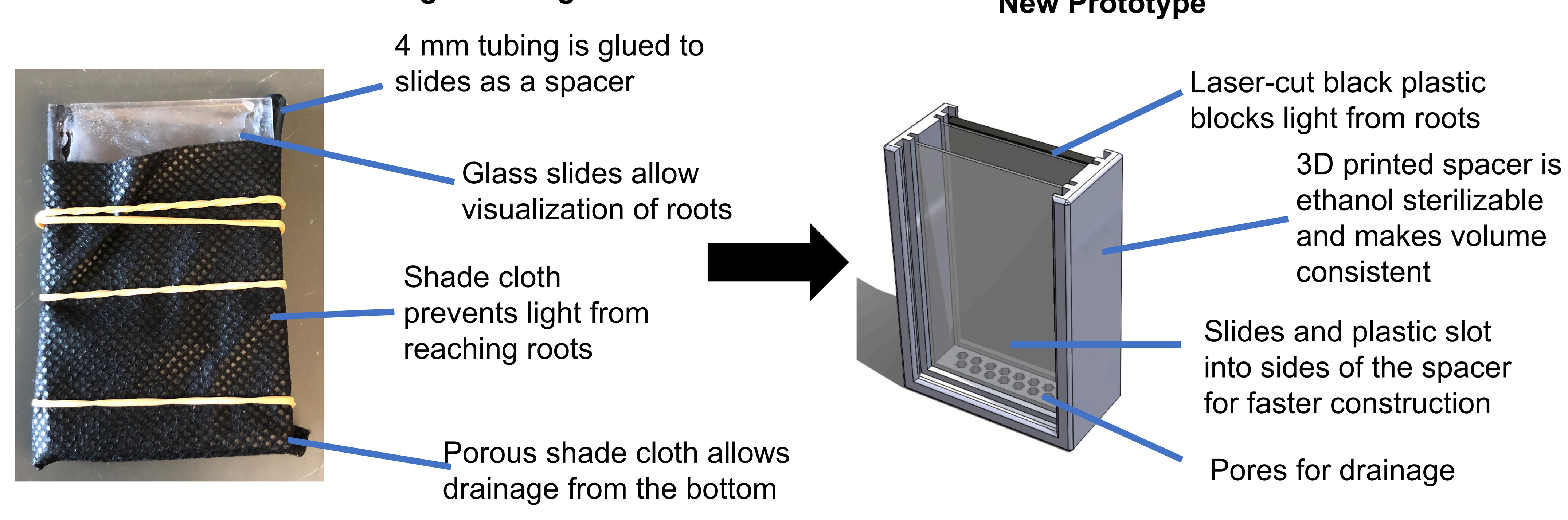
\+

One type of ground organic matter (3% by weight)

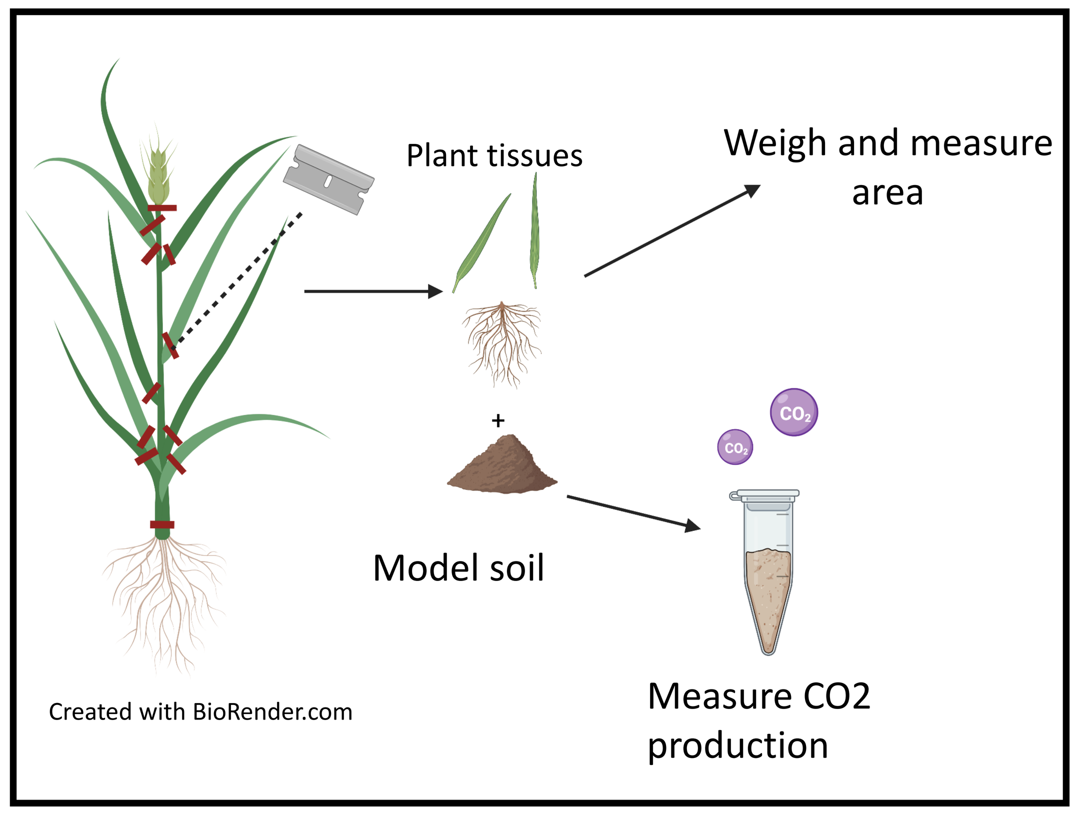
Nothing (control)	Red oak leaf litter
Sorghum	Black birch leaf litter
Brachypodium	Red maple leaf litter

Methods

Minirhizotrons as a growth container



Destructive harvests



Results

Analysis of Growth Components

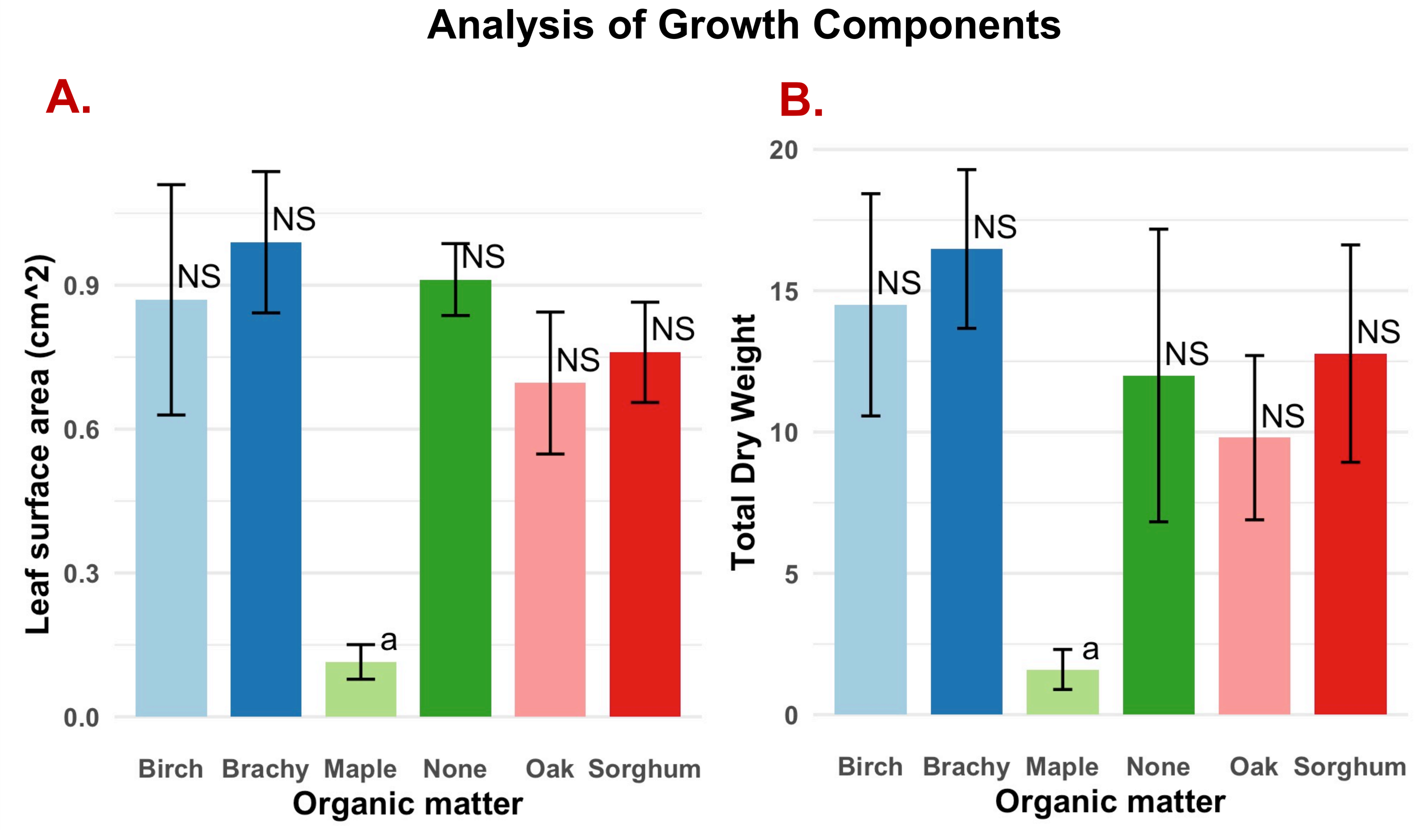


Figure 1: Letters indicate statistical significance; error bars represent standard deviation. A) Total leaf area, a: p=0.00016 B) Total dry weight, a: p= 0.0488313

Figure 2: Representative pictures from each treatment three weeks after planting. From left to right: No organics, ground *B. distachyon*, ground *S. bicolor*, black birch, red maple, red oak.

Biomass by Organic Matter Type

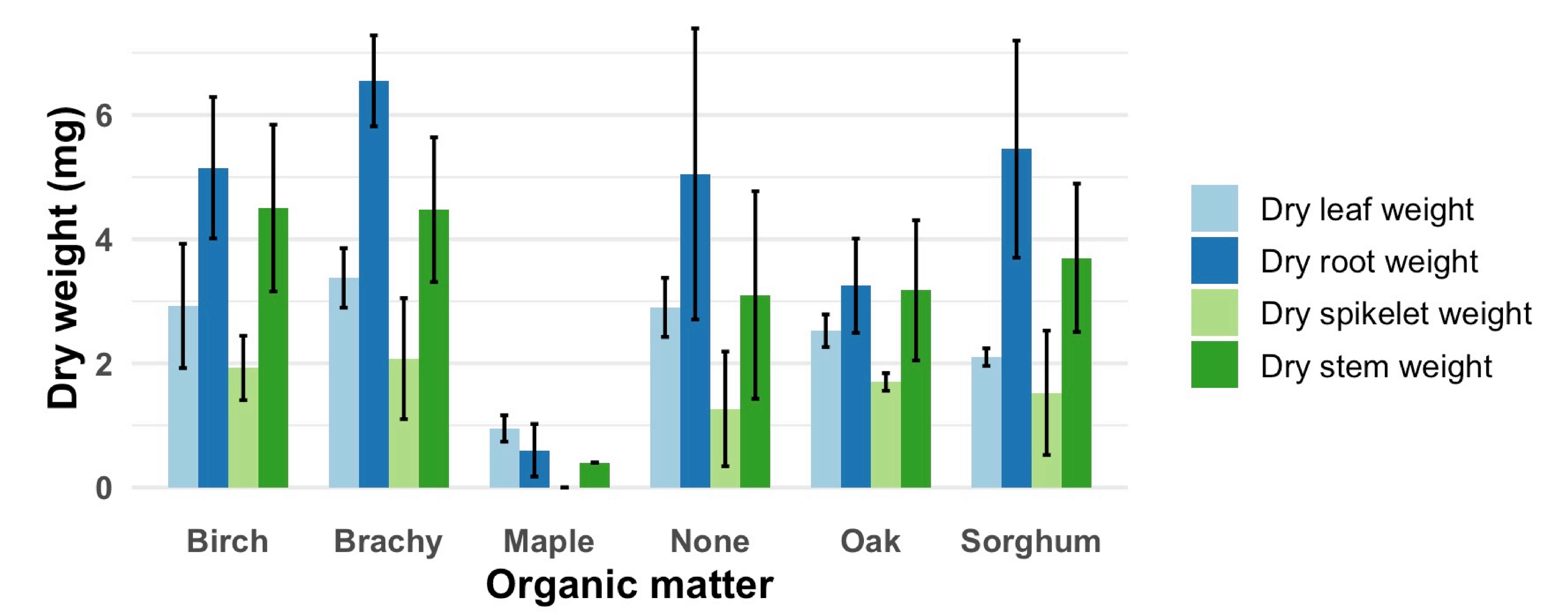


Figure 3: Mean dry mass of leaves, roots, and stems, for each species three weeks post planting, in each organic matter type. Error bars represent standard deviation.

Microbial Respiration

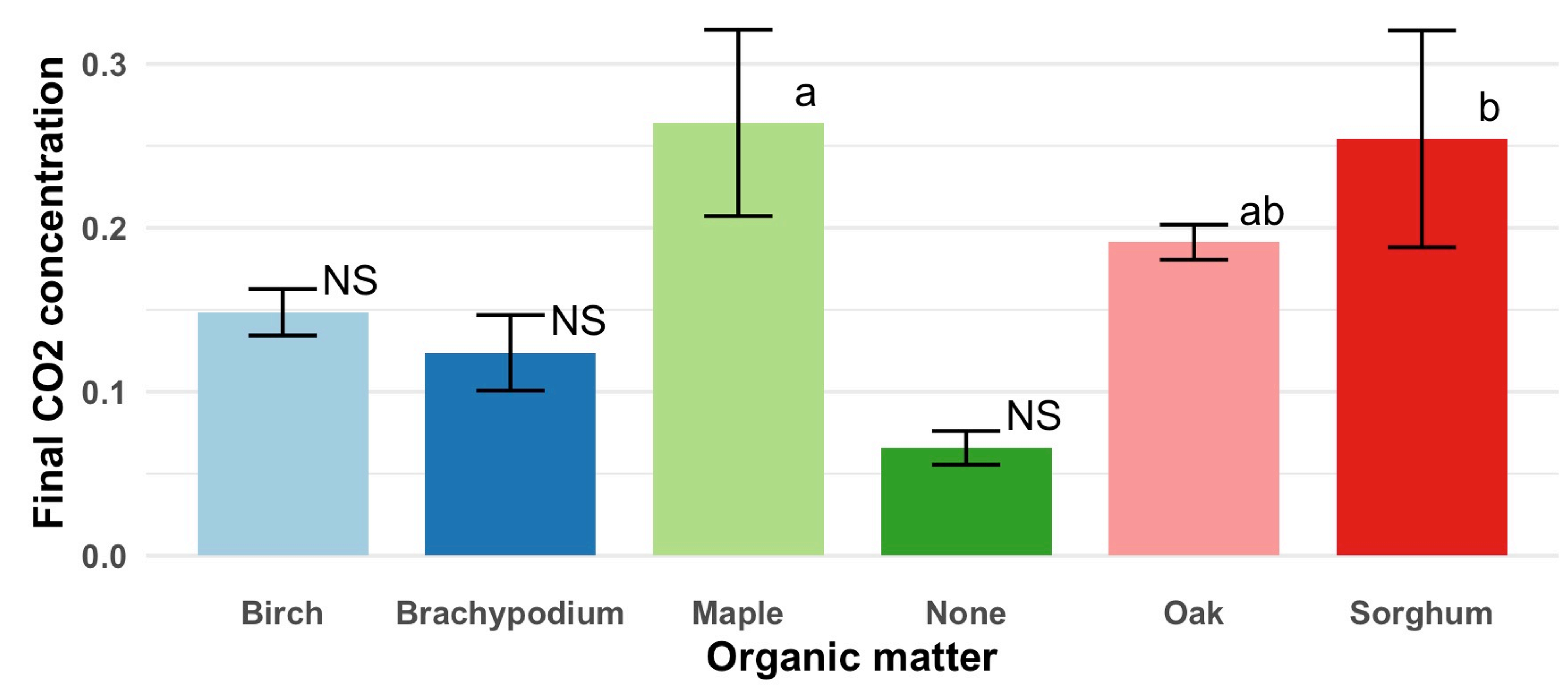


Figure 4: Microbial respiration in each treatment, measured by CO_2 production following destructive harvest. Letters represent statistical significance from ANOVA + Post Hoc Tukey Tests. a: p= 0.0006565, b: p= 0.000018, , ab: p= 0.0020966. Error bars represent standard deviation.

References

[1]Hartmann, A., Schmid, M., Tuinen, D.v., Berg, G. Plant-driven selection of microbes. *Plant Soil* **321**, 235–257 (2009). https://doi.org/10.1007/s11104-008-9814-y

[2]Wolfe, B. E. *Msystems* **3**, 00161-17 (2018)

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