Friends with Benefits: Mycorrhizal Resource Exchange between *Serendipita bescii* and *Panicum hallii*

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ABSTRACT

Climate change has progressively increased severe drought conditions throughout the hemispheres, causing large reductions in crop yields, particularly biofuel crops. Utilizing the benefits of the symbiotic relationship between fungi and plants can possibly enhance drought tolerance of agricultural biofuel crops and increase production. In this paper, I focus on the fungi, *Serendipita bescii*, which has demonstrated plant-growth promoting characteristics in previous studies. My research seeks to investigate whether *Serendipita bescii* enriched soils provide plant-growth promoting characteristics for *Panicum hallii* in drought and typical-watered versus uninoculated typical-watered and drought treatments. This study explores the differences in aboveground and belowground biomass between treatments. I then examine whether *Serendipita bescii* produce higher rates of colonization in drought-treated conditions versus typical-water treated conditions to evaluate the differences in morphological traits of *Panicum hallii*. This study also investigates the differences in morphology of *Serendipita bescii* under drought versus typical-watered treated conditions of *Panicum hallii*. To assess whether *Serendipita bescii* provided plant-growth promoting characteristics for *Panicum hallii*, I analyzed aboveground and belowground Biomass weight. I used PCR and microscopy techniques to confirm the presence of *Serendipita bescii* in the system and determine the percent colonization rates in the treatments. My results indicate that *Serendipita bescii* inoculated-watered replicates had the highest aboveground biomass while control-drought replicates had the highest belowground biomass. This finding supports the hypothesis that *Serendipita bescii* provides plant-growth promoting characteristics for *Panicum hallii*. This research promotes developing a more comprehensive understanding of mycorrhizal fungi and its role in future biofuel crop production.

KEYWORDS

Biomass, Inoculate, Colonization, Mycorrhizae, Symbiotic Relationships

INTRODUCTION

Biofuels, a clean source of energy produced from plant biomass, have become an essential alternative to fossil fuels by effectively reducing greenhouse gas emissions and enhancing energy security. Thus, the production of biofuels has largely expanded in countries like the U.S. (Zhang et al. 2010), which continues to encourage the expansion of the biofuel industry. However, with drought conditions exacerbating globally as a result of climate change, biofuel crops are threatened by notable reductions in crop yields (Li 2009; Maes 2009). In addition, drought can also affect the biomass quality for biofuel production (Weijde et al. 2016). To preserve the feasibility of biofuels as an alternative to fossil fuels, drought-tolerance with increased biomass quality have become important traits that researchers and commercial farmers strive to breed in their biofuel crops (Weijde et al. 2016). The utilization of naturally occurring symbiotic microbes may enhance drought tolerance of agricultural biofuel crops and increase production (Ghimire and Craven 2011). This beneficial outcome relies on the symbiosis between plants and mycorrhizal fungi.

Various types of microorganisms form symbiotic relationships with plants, ranging from parasitic to mutualistic. The most widespread among these relationships is mycorrhizal symbiosis between fungi and the root system of vascular plants in the rhizosphere (Harley 1984; Harrison 2005). Typically, the mycorrhizal fungi cannot survive without the host plant, and the host plant cannot not thrive successfully without mycorrhizal fungi. For example, Arbuscular mycorrhizal fungi (AMF) is a model species of mycorrhizal fungi that has widely been studied. AMF is an endosymbiont, meaning it resides in the root cortical cells of its host. AMF delivers nutrients from the soil to the host plant, while the host plant supplies the AMF with photosynthetically-derived carbon and physical shelter (Harrison 2005). An essential characteristic of the symbiosis between AMF and its host plant is this bidirectional exchange of nutrients (Nuccio 2013). AMF can alleviate the effects of stresses such as drought on plant growth and forms extensive hyphal networks and produces other biochemicals that result in enhanced water and nutrient uptake, ultimately increasing the growth and production of its host plant (Miransari 2010). Other taxa of mycorrhiza fungi such as *Serendipita* endosymbiosis that offers plant-growth promoting characteristics for biofuel crops in the family *Panicum*; however very little is known about its specific roles and morphology.

Though limited knowledge of *Serendipita* exists, it is identified as a root-associated fungus that can proliferate and grow through the soil matrix without a host plant (Ray et al. 2018). Colonization of *Serendipita vermifera* was identified in different plants through migration and co-mingling, highlighting the first documentation of non-targeted colonization of mycorrhizal fungi (Ray et al. 2018). This discovery opposes the concept that mycorrhizae fungi need a host plant to grow, such as with AMF, which can be beneficial in using *Serendipita* inoculation for biofuel crops as a potential yield production enhancer. In addition, *Serendipita* has indicated plant-growth promoting abilities with little or no target-host specificity, indicating that they may be able to form symbiotic relationships with any host (Chi et al. 2018). Mycorrhizal fungi may also possess different morphological traits amongst various host plants and conditions, however the morphology of this fungus in different environments have yet to be established (Ray et al. 2018).

With *Serendipita* possessing plant-growth promoting characteristics and contributing several benefits to a variety of non-target-host plants, further research can provide insight on the benefits of *Serendipita* on nutrient poor and drought enhanced soil conditions. In a study focused on the inoculation of *Serendipita vermifera* in *Panicum virgatum*, a biofuel crop commonly known as switchgrass, the inoculated plants produced a higher biomass than the uninoculated control plants, under both watered and drought treatments (Ghimire and Craven 2011). Switchgrass is among some of the most promising perennial C4 grasses used for biofuel production with its broad soil adaptation characteristics and rapid growth rate (Joint Genome Institute 1997-2017). However, as a result of the large and complex genomes of switchgrass, it is difficult to investigate the basic biology of switchgrass under abiotic stress conditions and potential crop improvement techniques that can be applied to the crop (Sun et al. 2012). Thus, researchers have developed a simpler genomic model of perennial grasses to study in *Panicum hallii*, a cousin of switchgrass. Through studying a close relative model, crop improvement techniques for different stress conditions can be applied to Switchgrass and other potential biofuel perennial grasses (Joint Genome Institute 2018).

For this study I worked under Dr. Rachel Hestrin, a Postdoctoral Researcher at the University of California, Berkeley. This project lies within a larger scope of understanding the complex chemical, biological, and physical relationships between *Serendipita bescii* and *Panicum hallii*. I am focusing specifically on the colonization and morphology of these endosymbiotic interactions.

My research seeks to investigate whether *Serendipita bescii*, a subspecies of *Serendipita vermifera*, enriched soils provide plant-growth promoting characteristics for *Panicum hallii,* a species of *Panicum* similar to Switchgrass, in drought and watered treatments versus control-watered and control-drought treatments not inoculated with *Serendipita* bescii**.** The interactions between the two organisms have yet to be studied and understood, leaving a large gap of knowledge in how beneficial *Serendipita* is to biofuel crops grown in marginal (nutrient and water limited) soils. I then examine whether *Serendipita bescii* produce higher rates of colonization in drought-treated conditions versus typical water-treated conditions to evaluate the differences in morphological traits of *Panicum hallii.* Lastly, because the morphology of this fungus under different environments have yet to be established, this study seeks to investigate the notable differences in the morphology of *S. bescii* under drought versus water-treated conditions of *Panicum hallii*. Although largely unexplored, *Serendipita* shows great promise in enhancing the growth and drought-resistance of the plant, *Panicum hallii,* for the production of biofuels in drought-driven conditions. Through this research, I hope to develop a more comprehensive understanding of mycorrhizal fungi and its role in future biofuel crop production.

METHODS

I assisted in the setup, maintenance, and harvesting of replicates for the larger scope of the experiment. The project, headed by Hestrin, was designed to create a controlled environment to study the interactions between *Serendipita bescii* and *Panicum hallii.* To investigate the morphology of the endosymbiotic relationship between *Serendipita bescii* and *Panicum hallii,* I utilized techniques in biomass quantification, as well as fluorescence and light microscopy for a randomly selected subset of treated root samples.

Source material: Fungi, plants & soil

We obtained *Serendipita bescii* strains on agar petri plates from our collaborators at the Noble Research Institute (NRI) in Oklahoma. With these plates, we created a mycelium (concentrated hyphae) solution by inoculating the cultures into a flask of Hanahan's broth growth medium and cultured for 2 weeks.

We acquired the plant seeds from the Hawkes Lab at North Carolina State University, collaborators, who collected these seeds from a wild natural population field of *Panicum hallii* in Texas. We planted the seeds in unsterilized cones and grew them in the greenhouse for 4 weeks. All plants were watered to saturation weekly during this 4-week period, to ensure uniform growth prior to inoculation. After this plants' initial growth period, we added the liquid mycelium solution to a subset of randomly selected plant pots to inoculate with *Serendipita bescii*.

After this inoculation step, we transferred the plants from the unsterile starter cones to our experimental pots. The soil in the experimental pots consisted of a 50/50 mixture of sand and soil. Prior to mixing, the sand was autoclaved three times to sterilize it and kill any potential microorganisms that were in it, limiting experimental errors from contamination. During this transfer, we also added 5 grams of a clay particle inoculum of *Serednipita bescii* hyphae to each inoculated replicate pot. This inoculum was supplied by our NRI collaborators, similar to the liquid medium culture propagation process but with the addition of clay particles to ensure growth over all the particles.

Experimental setup: Replicate & chamber design

To establish a controlled environment for studying the interactions between *Serendipita bescii* and *Panicum hallii*, Hestrin created an experimental design consisting of two fungal treatments: *Serendipta bescii* inoculated plants and uninoculated control plants (Figure 1). For each inoculated and uninoculated treatment, we had two water level treatments. The first was a water-saturated "watered" treatment, and the second was a water-limited "drought" treatment. This design was used to explore the impacts of each environmental condition on plant growth, specifically focusing on whether *Serendipita bescii* positively influenced plant growth in comparison to the control. We had drought versus watered conditions to look at the different plant-growth responses for inoculated and uninoculated control plants, as well as the difference in *Serendipita bescii* colonization rates among the inoculated replicates.

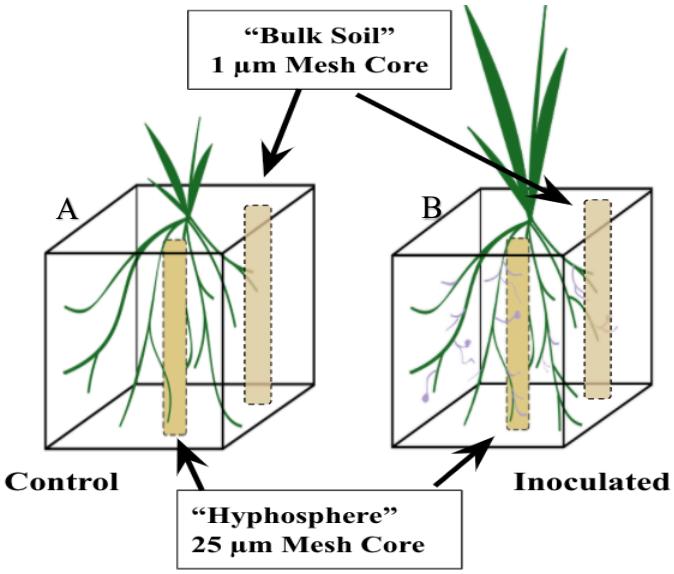

Figure 1: Illustration of pot replicate assembly. Tube like cores are shown in both **(A)** and **(B)**, representing the hyphosphere core and bulk soil core. **(A)** represents the control pots that is not inoculated with *Serendipita bescii.* **(B)** represents the pots that are inoculated with *Serendipita bescii* indicated by the purple lines coming out of the plant roots.

Each pot contained two manually assembled tube-like cores, consisting of the hyphosphere core and the bulk soil core (Figure 1 and 2b). The hyphosphere is the volume of soil influenced by the *S. bescii* fungal hyphae. The hyphosphere core, which contained 25 micrometer mesh barriers, were created to isolate the *S. bescii* strains from the rest of the soil in the pot, which most likely had other strains of fungi and microbes growing in it, due to the 50% soil portion that could not be sterilized. Only *S. bescii* hyphae should be able to pierce through the twenty-five-micrometer mesh barriers. The other cores of bulk soil were used as controls, to analyze what was naturally in the soil in contrast to what we inoculated the plants with. They contained one-micrometer mesh barriers which no fungi would be small enough to grow through.

These experimental pots were kept in the greenhouse inside the Environmental Plant Isotope Chambers (Figure 2). We had four 12CO2 labeled chambers and four 13CO2 labeled chambers for the larger scope of the research project. We allotted about 12 replicates per chamber, having a total of 152 replicates, however I focused on a subset of 100 replicates. These chambers provided a closed system environment for the plants to grow in. The CO2 levels were constantly measured and regulated. Moisture probes were used to monitor and maintain soil moisture continuously throughout the pots. Unfortunately, there was limited control over temperature and

light due to chambers being located in different areas of the greenhouse, therefore leaving more room for uncertainty in equal light and temperature among all pots.

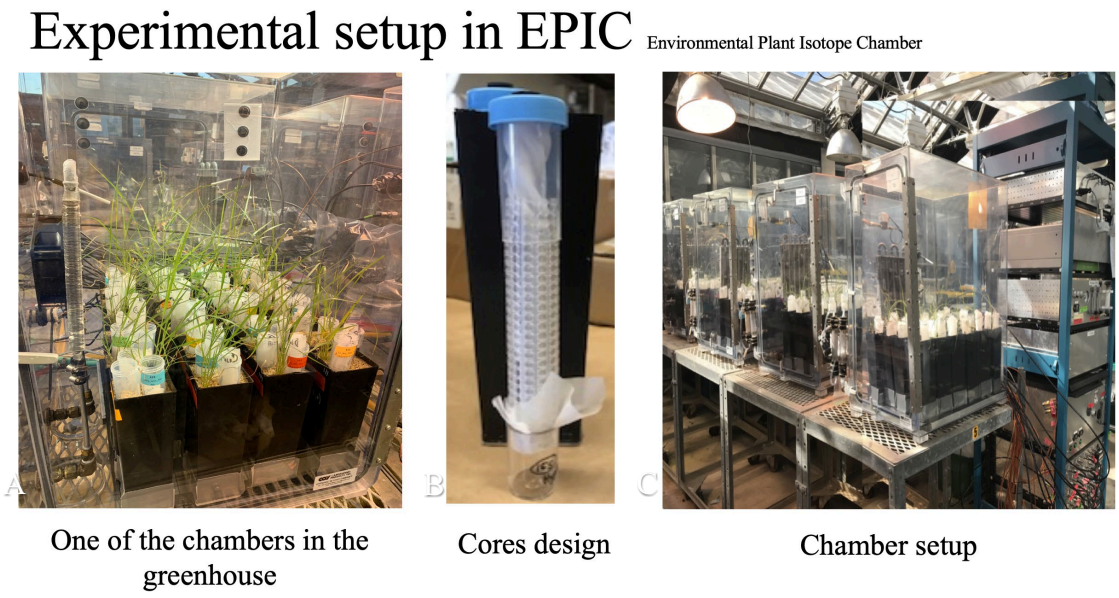

Figure 2: Images of the experimental setup. (A) A close-up view of an Environmental Plant Isotope Chamber containing 12 replicates inside it. **(C)** Design setup of the Environmental Plant Isotope Chambers, a closed system for the experimental replicates to grow in after inoculation or non-inoculation. **(B)** Design of the hyphosphere and Bulk soil core, which is pushed into the soil. The cores contain a mesh barrier (looks like white paper in the image). Hyposphere core contains a 25-micrometer mesh barrier and the bulk soil core contains a 1 micrometer mesh barrier.

As mentioned before, all replicates were initially watered to saturation in the starter cones. After transferring the plants to the experimental pots, we randomly chose half of the inoculated and half of the control replicates to be subjected to drought while the remaining halves were watered saturation. Each replicate was checked for soil volumetric water content (VWC) with a Field Scout TDR 100 soil moisture probe every 2-3 days. The plants subject to drought treatments were maintained at a minimum VWC of 1%, while the watered plants were maintained at a constant VWC of 10-20%.

Harvesting

For harvests, we retrieved a subset of pots to collect soil samples and the plant biomass. We had three harvesting time points to determine if treatments affected plants differently over

time. The replicates were chosen randomly during each harvest. The first harvest was at six weeks with thirty-two replicates chosen, the second harvest was at nine weeks with thirty-three replicates chosen, and the last harvest was at thirteen weeks with thirty-five replicates chosen. We obtained the plant biomass from each replicate by cutting off the entire plant above soil separately from the below-ground roots and placing both in 6x9 and 9x12 catalog envelopes. This separation was done to determine the effects of each treatment on the growth of the plants and conclude whether *Serendipita* enriched soils provide plant-growth promoting characteristics in drought and watered treatments versus control-watered and control-drought treatments. The aboveground biomass was separated from the belowground biomass, because I wanted to see the impacts that mycorrhizal fungi had on the root systems versus shoots, as well as the impacts that different water conditions had on the root systems versus shoots.

For each replicate, we also retrieved a 15 mL tube sample from the hyphosphere core, a 15 mL tube sample from the bulk soil core, and a 50 mL tube sample from the rhizosphere, which is considered to be any soil within 2 millimeters of the root system. These three soil sample tubes were collected to observe the presence and colonization rates of *Serendipita bescii* among each treatment and determine if it produces higher rates of colonization in drought-treated conditions versus typical water-treated conditions. After harvesting the root samples, we washed them in distilled water (DI water) to remove sand/soil and set them aside for Microscopy, and eventual DNA extraction/PCR use.

Examination of *Panicum hallii* morphological traits

Biomass data collection

To analyze the *Panicum hallii* biomass of each replicate, I dried the biomass roots and shoots in an oven for a week, then weighed them separately on a three-decimal scale. The shoot biomass accounted for the total plant biomass above the soil, and the root biomass accounted for the root system below the soil. I recorded the weight data onto a weigh sheet and transferred it to electronic form for data analysis.

Biomass data analysis

I calculated the averages of each individual timepoint for both belowground roots biomass and aboveground shoots biomass. From these averages I created bar graphs to highlight the trends between control-drought treatment, control-watered treatment, inoculated-drought treatment, and inoculated-watered treatment in each timepoint. I then combined all the averages of each treatment across all time points and graphed it on a scatter plot, to see the overall trendlines of growth over all three timepoints for each treatment. I analyzed the data using Microsoft Excel (Microsoft Excel 2020).

Identification of *Serendipita bescii*

Unfortunately, due to the recent COVID-19 Shelter in Place, I was unable to perform Microscopy and PCR on all my samples and was unable to collect a sufficient amount of data.

PCR

DNA extractions and Polymerase Chain Reaction (PCR) were planned for the roots to confirm that *Serendipita bescii* is present in the samples. I planned to perform DNA extractions with the DNEasy Plant Pro kit, which would be done to isolate high-quality total DNA from the roots through cell lysis (Qiagen 2013).

Using the DNA that was extracted, I would have conducted PCR, which is a technique used to amplify a specific fragment of DNA of interest from a complex pool (Garibyan and Avashia 2013). The PCR assay would have consisted of a template DNA of *Serendipita bescii*, primers, nucleotides (that include adenine, thymine, cytosine, and guanine), and DNA polymerase that links the nucleotides together to form the PCR product. The primers are used to specify the exact DNA product being amplified, the nucleotides are the building blocks, and the polymerase is the builder.

Microscopy

I planned to perform microscopy on all 100 replicates to observe the percent colonization of each replicate and the morphology of the *Serendipita bescii* among different conditions. I had two root-staining methods in order to view the roots under the microscope: ink/vinegar staining and Invitrogen Wheat Germ Agglutinin, Alexa Fluor 488 Conjugate (WGA-AlexaFluor488) staining. For the ink and vinegar staining, I placed the roots in 50% ethanol and left them overnight. After removing them from the ethanol, I rinsed them in DI water. I then left them in 20% KOH for 7 days at room temperature to clear the cytoplasmic contents of the cell for a higher quality image under the microscope. After 7 days, I rinsed them with DI water again 5 times and left them in water overnight. The next day, I submerged the roots in an ink and vinegar mixture of 1:50 v/v (volume per volume) and left it in a 4-degree Celsius fridge for 2 days, to stain all fungal structures. Lastly, I rinsed and destained the roots in DI water overnight in the 4-degree Celsius fridge and was ready to quantify the fungal colonization using a light microscope and the gridline intersection method (Giovannetti and Mosse 1980). This method consists of randomly dispersing cleared and stained roots in a dish with grid lines and assessing the mycorrhizal colonization under a dissecting microscope with fine forceps and a dissecting blade.

Staining the roots with WGA-AlexaFluor488 was for fluorescence microscopy, because the Alexa Fluor 488 conjugate of WGA exhibits the bright, green fluorescence of the Alexa Fluor 488 dye. What is happening here is the dye stains the fungal hyphae and the plant cell wall in a contrasting shade, causing the fungi to fluoresce (Redkar et al. 2018). All steps for this protocol are the same as the ink and vinegar protocol until removal of the 20% KOH and DI water rinse. After this step, I soaked the roots in 0.1 M hydrochloric acid (HCl) for 1-2 hours. I then rinsed them in 1x Phosphate-Buffered Saline (PBS) that was previously prepared and left them in it until the pH was at 7. Once the pH reached this, I removed PBS and added a PBS/ WGA stain working stock solution (2.5 ul WGA stock solution per 1 ml PBS for *S. bescii*), wrapped them in foil for light sensitivity, and stored them in the 4-degree Celsius fridge for 2 days. After this, they were ready to be used under confocal microscopy.

RESULTS

The results of my study are most abundant in the biomass data, having recorded the differences among the control-drought treatment, control-watered treatment, inoculated-drought

treatment, and inoculated-watered treatment in each timepoint, and across all timepoints. I will also discuss preliminary microscopy data.

Biomass at timepoint 1: 6 weeks after inoculation

Timepoint one occurred at six weeks after experimental start where thirty-two replicates were harvested. Both shoot biomass (aboveground plant growth) and root biomass (belowground plant growth) were collected and weighed on a three-decimal scale in grams. The aboveground shoot biomass and belowground root biomass of all thirty-two replicates were calculated to find the averages for each treatment (Figure 3 and 4).

The aboveground shoot biomass averages indicate that the inoculated-drought treatment had the highest average biomass while the control-drought treatment had the least amount of average biomass among all treatments (Figure 3). The control-watered treatment and the inoculated-watered treatment conveyed similar averages, with the control-watered treatment being slightly higher.

A

T1 Aboveground Shoot Biomass Average

Fungal-Water Treatment	Avg. Mass (grams)	Standard Deviation
Control wet	0.2524	0.0656
Control dry	0.1720	0.0692
Serendipita wet	0.2616	0.0880
Serendipita dry	0.2111	0.0860

B

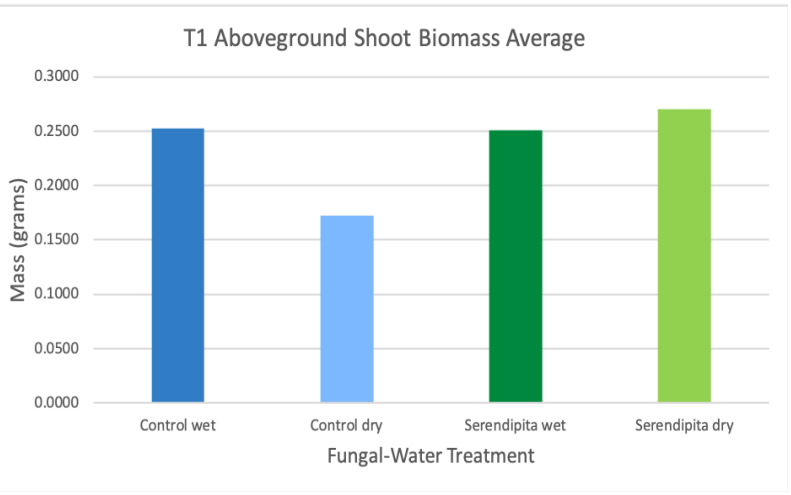

Figure 3: T1 Aboveground shoot biomass averages. (A) Averages collected from 32 replicates among four fungal-water treatments after six weeks. Control wet is control-watered treatment, control dry is control-drought treatment, Serendipita wet is inoculated-watered treatment, and Serendipita dry is inoculated-drought treatment. **(B)** Bars illustrate the averages of all four treatments. Control dry treatment was significantly lower in shoot biomass, while Serendipita dry treatment was the highest at timepoint 1.

The belowground root biomass averages indicate that the inoculated-drought treatment also had a significantly higher root biomass average than the other treatments (Figure 4). The inoculated-watered treatment had the second highest root biomass average but was similar to the control-drought and control-watered averages.

A

T1 Belowground Root Biomass Average

Fungal-Water Treatment	Avg. Mass (grams)	Standard Deviation
Control wet	0.0931	0.0582
Control dry	0.0933	0.0519
Serendipita wet	0.1163	0.0552
Serendipita dry	0.1298	0.0619

B

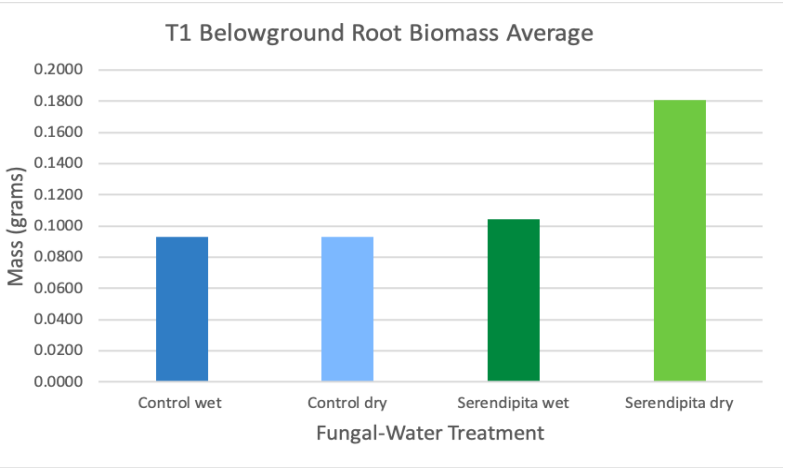

Figure 4: T1 Belowground root biomass averages. (A) Averages collected from 32 replicates among four fungal-water treatments after six weeks. Control wet is control-watered treatment, control dry is control-drought treatment, *Serendipita* wet is inoculated-watered treatment, and *Serendipita* dry is inoculated-drought treatment. **(B)** Bars illustrate the averages of all four treatments. *Serendipita* dry treatment was significantly higher in root biomass than all other treatments.

Biomass at timepoint 2: 9 weeks after inoculation

Timepoint two was harvested at nine weeks after the start of the experimental period. Thirty-three replicates were harvested. Both shoot biomass (aboveground plant growth) and root biomass (belowground plant growth) were collected and weighed on a three-decimal scale in grams. The aboveground shoot biomass and root biomass of all thirty-three replicates were calculated to find the averages for each treatment (Figure 5 and 6).

The aboveground shoot biomass averages indicate that the inoculated-drought treatment had the highest average biomass in timepoint two (Figure 5). The control-watered and control-drought treatment were also relatively high in average biomass. In contrast to timepoint one, the inoculated-drought treatment in timepoint two had the lowest average biomass among all treatments.

A

T2 Aboveground Shoot Biomass Average

Fungal-Water Treatment	Avg. Mass (grams)	Standard Deviation
Control wet	0.4696	0.2017
Control dry	0.4522	0.1794
Serendipita wet	0.5001	0.1278
Serendipita dry	0.3276	0.1254

B

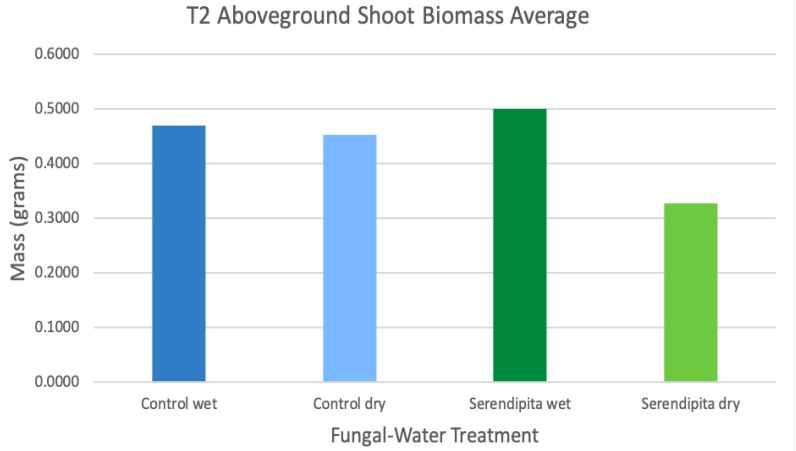

Figure 5: Aboveground shoot biomass averages. (A) Averages collected from 33 replicates among four fungal-water treatments after nine weeks. Control wet is control-watered treatment, control dry is control-drought treatment, *Serendipita* wet is inoculated-watered treatment, and *Serendipita* dry is inoculated-drought treatment. **(B)** Bars illustrate the averages of all four treatments. The *Serendipita* dry treatment is displayed to have a much lower average root biomass than the other treatments, while *Serendipita* wet treatment has the highest.

The belowground root biomass averages indicate that the control-drought treatment average biomass was immensely higher than the other three treatments (Figure 6). The control-watered treatment, inoculated-watered treatment, and inoculated-drought treatment were all similar in averages, with the inoculated-watered treatment being slightly higher. This data showed the most drastic differences across all three timepoints. Figure 6A clearly illustrates significant these differences in biomass between the control-drought treatment, and all the other treatments.

A

T2 Belowground Root Biomass Average		
Fungal-Water Treatment	**Avg. Mass (grams)**	**Standard Deviation**
Control wet	0.0993	0.0498
Control dry	0.2766	0.5373
Serendipita wet	0.1185	0.0931
Serendipita dry	0.0968	0.0653

B

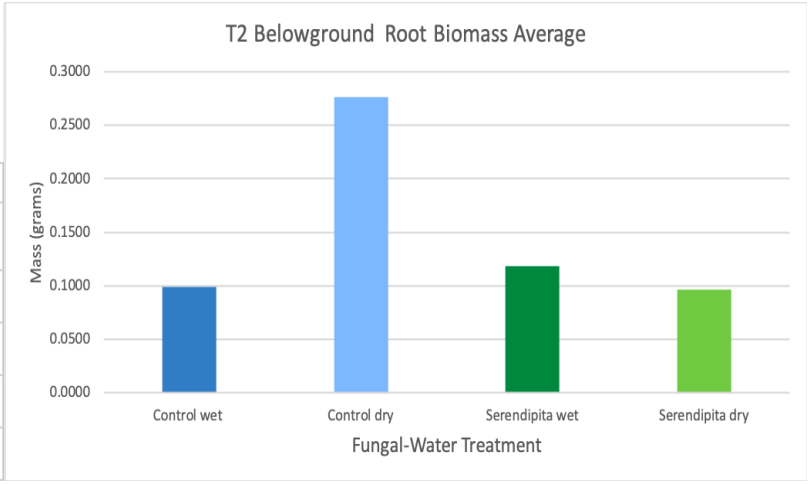

Figure 6: Belowground root biomass averages (A) Averages collected from 32 replicates among four fungal-water treatments after nine weeks. Control wet is control-watered treatment, control dry is control-drought treatment, *Serendipita* wet is inoculated-watered treatment, and *Serendipita* dry is inoculated-drought treatment. **(B)** Bars illustrate the averages of all four treatments. Control dry treatment displays the highest bar indicating the highest average root biomass.

Biomass at timepoint 3: 13 weeks after inoculation

Timepoint three was harvested at thirteen weeks after the start of the experimental period. Thirty-five replicates were harvested. Both shoot biomass (aboveground plant growth) and root biomass (belowground plant growth) were collected and weighed on a three-decimal scale in grams. The aboveground shoot biomass and root biomass of all thirty-five replicates were calculated to find the averages for each treatment (Figure 7 and 8). I focused most on this data, because reviled how the plants were impacted by their conditions after a considerable amount of time.

The aboveground shoot biomass averages indicate that the inoculated-watered treatment had the highest average biomass in timepoint three, similar to timepoint two (Figure 7). However here, the control-drought treatment has the lowest average biomass, which we also saw in timepoint one. The average aboveground biomass for control-watered and inoculated-drought is very similar, with control-watered being slightly higher.

A

T3 Aboveground Shoot Biomass Average

Fungal-Water Treatment	Avg. Mass (grams)	Standard Deviation
Control wet	0.6756	0.1486
Control dry	0.5761	0.0997
Serendipita wet	0.8130	0.1814
Serendipita dry	0.6685	0.1159

B

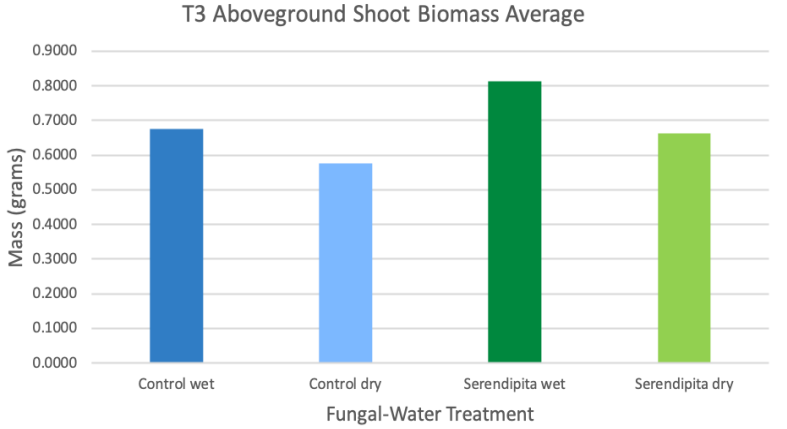

Figure 7: Aboveground shoot biomass averages. (A) Averages collected from 35 replicates among four fungal-water treatments after thirteen weeks. Control wet is control-watered treatment, control dry is control-drought treatment, *Serendipita* wet is inoculated-watered treatment, and *Serendipita* dry is inoculated-drought treatment. **(B)** Bars illustrate the averages of all four treatments. The *Serendipita* wet treatment bar displays how much higher it is in biomass than the other treatments, while the control dry treatment displays how significantly low it is.

The belowground root biomass averages indicate that the control-drought treatment average biomass was also significantly higher than the other three treatments (Figure 8). Although a lot lower, the inoculated-drought treatment shows the second highest root biomass average. The inoculated-watered treatment has the lowest average in timepoint 3.

A

T3 Belowground Root Biomass Average

Fungal-Water Treatment	Avg. Mass (grams)	Standard Deviation
Control wet	0.1493	0.0801
Control dry	0.2960	0.1519
Serendipita wet	0.1373	0.0614
Serendipita dry	0.1850	0.0816

B

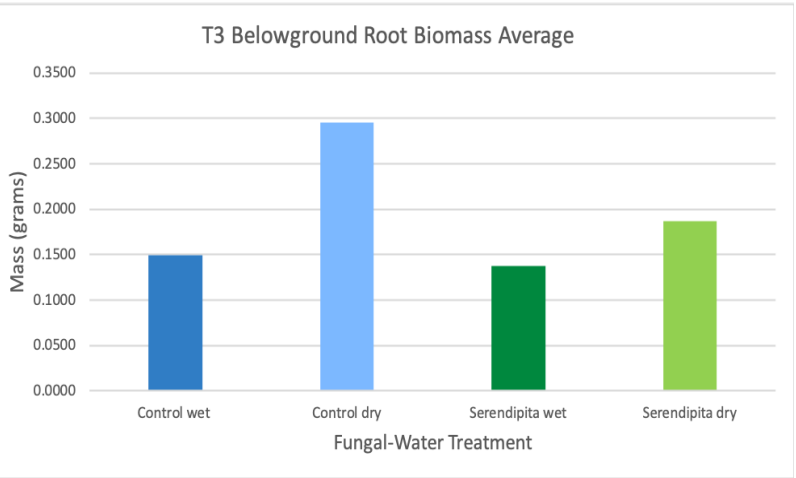

Figure 8: Belowground root biomass averages (A) Averages collected from 32 replicates among four fungal-water treatments after thirteen weeks. Control wet is control-watered treatment, control dry is control-drought treatment, *Serendipita* wet is inoculated-watered treatment, and *Serendipita* dry is inoculated-drought treatment. **(B)** Bars illustrate the averages of all four treatments. Control dry treatment displays the highest bar indicating the highest average root biomass similar to Figure 6B.

Aboveground shoot biomass change over time

After calculating the shoot averages for the four treatments in all three timepoints, I created a scatter plot that illustrated the linear trendlines of each treatment over time of the shoot biomass averages (Figure 9). The inoculated-watered treatment displays a trendline that progressively increases throughout all timepoints but has a slightly sharp increase from timepoint two to three. The inoculated-drought treatment displays a very gradual increase until timepoint two, where an abrupt sharp increase occurs all the way to timepoint three. The control-drought treatment displays the exact opposite pattern, where a sharp increase occurs from timepoint one to timepoint two, but then it becomes slow and steady from timepoint two to timepoint three. The control-watered treatment shows the most steady and gradual increase among all trendlines.

A

Aboveground Shoot Biomass Averages Over Time			
	Timepoint 1	Timepoint 2	Timepoint 3
Control wet	0.2524 g	0.4696 g	0.6756 g
Control dry	0.1720 g	0.4522 g	0.5761 g
Serendipita wet	0.2508 g	0.5001 g	0.8130 g
Serendipita dry	0.2698 g	0.3276 g	0.6635 g

B

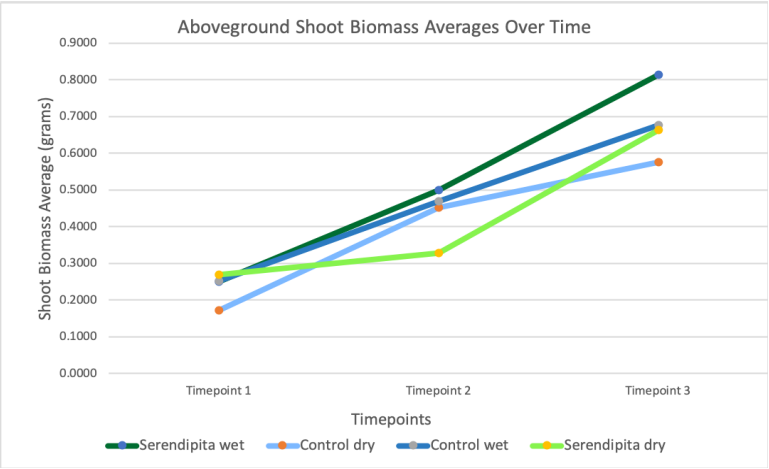

Figure 9: All three aboveground biomass averages across time for each treatment. (A) Aboveground shoot biomass averages of all four treatments across timepoints one, two, and three. **(B)** Linear trendlines illustrate the patterns of average shoot biomass over time, from six weeks, to nine weeks, to thirteen weeks. Shows the overall growth patterns of the replicates under the four different treatments.

Belowground root biomass change over time

After calculating the root averages for the four treatments in all three timepoints, I created a scatter plot that illustrated the linear trendlines of each treatment over time of the root biomass averages as well (Figure 10). The inoculated-watered treatment trendline displayed only a small increase overtime, and it was very gradual across all timepoints. The inoculated-drought treatment

shows a very abrupt and sharp decrease until timepoint two, where a sharp increase suddenly occurs and goes back to its initial root biomass average at timepoint three. The control-drought treatment shows the sharpest increase until timepoint two, where it becomes steady and gradual. Lastly, the control-watered treatment displays a very small increase from timepoint one to two, and then a slightly sharper increase to timepoint three.

A

Aboveground Root Biomass Averages Over Time

	Timepoint 1	Timepoint 2	Timepoint 3
Control wet	0.0931 g	0.0993 g	0.1493 g
Control dry	0.0933 g	0.2766 g	0.2960 g
Serendipita wet	0.1045 g	0.1185 g	0.1373 g
Serendipita dry	0.1808 g	0.0968 g	0.1870 g

B

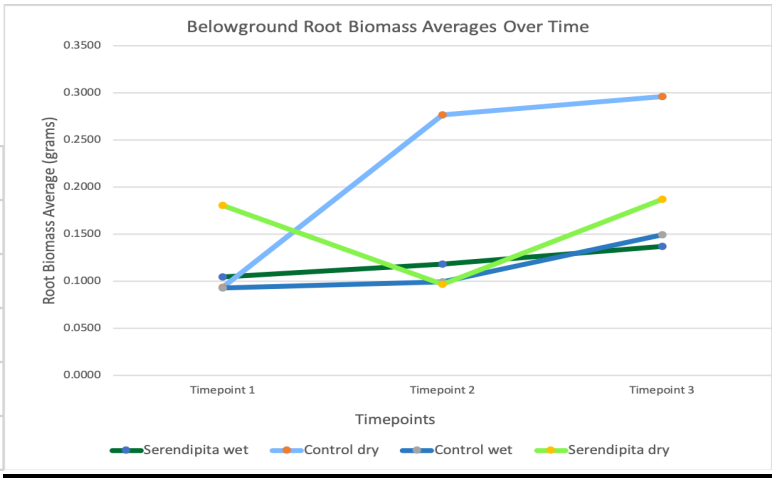

Figure 10: All three aboveground biomass averages across time for each treatment. (A) Aboveground shoot biomass averages of all four treatments across timepoints one, two, and three. **(B)** Linear trendlines illustrate the patterns of average shoot biomass over time, from six weeks, to nine weeks, to thirteen weeks. Shows the overall growth patterns of the replicates under the four different treatments.

Microscopy

Preliminary images of 3 replicates, all from the watered treatment inoculated with *Serendipita bescii,* are depicted under fluorescence microscopy and light microscopy (Figure 11). Mycorrhizal colonization is indicated by the circular bundles in both images. WGA-AlexaFluoro488 stains the *Serendipita bescii* hyphae, causing the hyphae to fluoresce (Figure 11A). *Serendipita bescii* hyphae is stained by ink and vinegar, causing a darker blue shade than the rest of the root cell wall (Figure 11B).

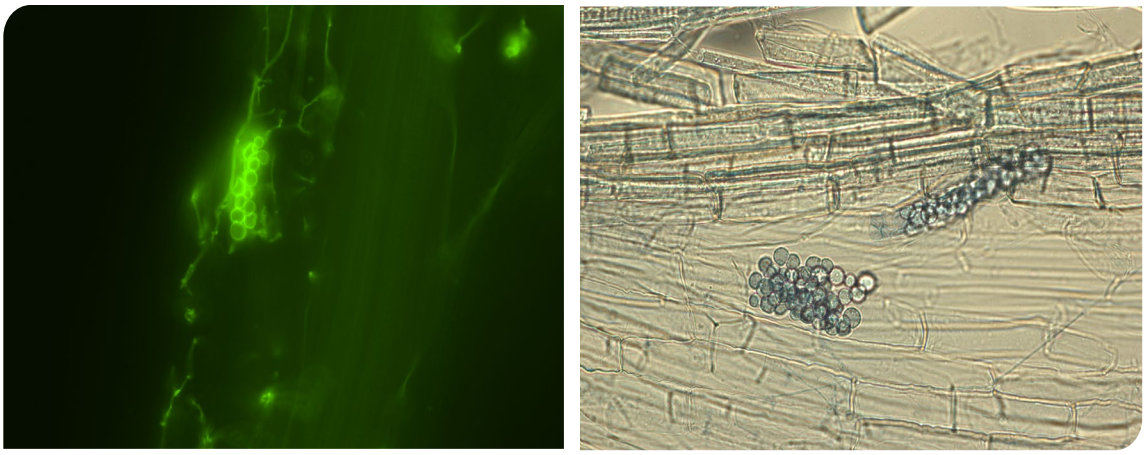

Figure 11: Visualization of *S. bescii* colonized root cells of Panicum hallii by **(A)** confocal microscopy and **(B)** light microscopy. Fungal colonization shown **(A)** Fungal hyphae stained with WGA-AlexaFluor488 staning. **(B)** Fungal hyphae stained with ink and vinegar staining.

DISCUSSION

In this project, I focused on understanding the symbiotic relationship between *Serendipita bescii*, a mycorrhizal fungus, and *Panicum hallii*, a promising biofuel crop. I collected data on the biomass of the roots and shoots of each replicate in each treatment to understand how the conditions of the treatments affected the biomass of the replicate's roots and shoots. From this, I was able to conclude whether *Serendipita bescii* enriched soils provide plant-growth promoting characteristics in drought and watered conditions versus soils that do not contain *Serendipita bescii* in both drought and watered conditions. Although data was collected from all three timepoints, I focused on timepoint three and the overall trendlines from all three timepoints to draw my conclusions. The data collected from timepoints one and two were more to track the growth and changes of the *Panicum hallii* plants and colonization rate of *Serendpita bescii.*

Aboveground shoot biomass implications

From the aboveground shoot biomass (Figure 8), I concluded that *Serendipita bescii* enriched soils in regularly watered conditions provide the most aboveground biomass growth-promoting characteristics. I hypothesized that *Serendipita bescii* enriched soils receiving the drought treatment would contain more growth promoting characteristics, which aligns with the

findings of Ray et al. (2018) that *Serendipita* is beneficial to enhancing biomass yield and drought tolerance, however this did not occur. I theorize that *Serendipita bescii* colonization rates were not as successful in the inoculated-drought treatments as they were in the inoculated-watered treatments, therefore not providing as many external nutrients to alleviate drought-stressed conditions.

Although my hypothesis was not supported, the inoculated-drought treatment replicates did just as well as the control-watered replicates by timepoint three, which supports my hypothesis and previous studies that *Serendipita bescii* provide growth promoting characteristics, even under stressed conditions. However, in timepoint two at nine weeks, the inoculated drought-treatment had the lowest shoot biomass average among all treatments including the control-drought treatment, which was unexpected. Because this harvest occurred only at six weeks, *Serendipita bescii* may not have fully colonized the roots, and therefore did not provide as many benefits to the plant. Unfortunately, I was not able to analyze the percent colonization of each replicate during each timepoint, which would have helped to better understand the alternating data among each timepoint. As a result, I focused on results from timepoint three, because thirteen weeks was enough time for the *Serendipita* to colonize, the plants to grow, and the benefits of their symbiotic relationship to occur.

Belowground root biomass implications

After receiving data, I realized how important it was to assess the root biomass data separately from the shoot biomass data, because the root biomass data was quite different from the shoot biomass data within the same replicate. Mycorrhizal fungi colonization can result in significant alterations to the root system morphology (Hooker et al. 1992). The root system morphology is often essential in determining the water and nutrient absorption and distribution, but its morphology can be influenced by other environmental factors, such as microbes (Yano et al. 1996).

The control-drought treatment replicates had significantly higher root biomass averages than any of the other treatments (Figure 9), despite having the lowest shoot biomass averages by Timepoint 3 (Figure 6). Two studies were reported on the effects of Arbuscular Mycorrhiza Fungi (AMF), a type of mycorrhiza fungi, on its host's root system. Both Price et al. (1989) and Berta et

al. (1995) reported that AMF colonization decreased the specific root length of the host plants. Patterns of AMF-influenced alteration of the root system morphology can vary between host plant species, meaning some plants will exhibit differences in their morphology while others do not (Yano et al. 1996). From these supporting studies, I concluded that the presence of *Serendipita bescii* in the inoculated watered and drought treatments altered the morphology of the *Panicum hallii* root systems, by decreasing the specific root length in both watered and drought conditions.

Because the control-drought replicates were under stressed conditions with little water availability, all energy and resources were possibly focused on developing the root system, which is the part of the plant that is essential in water and nutrient absorption/allocation, in order to sustain the plant. Typically, if a plant has limited resources, it will work harder to obtain those resources, such as water and nutrients, leading to an alteration of the root system. This process of working hard to attain those resources is known as the external-supply and internal-demand regulation system for the allocation of endogenous plant resources (Dunbabin 2002).

I did not observe this external-supply and internal-demand regulation take place in the *Serendipita bescii* inoculated-drought replicates, however. These replicates had relatively similar root biomass averages as the watered treatments most likely because the plant had the benefits of the mycorrhizal symbiotic relationship to rely on, providing an external nutrient supply. This external nutrient supply helped the *Panicum hallii* plants adjust its conditions, causing the plant to not rely as heavily on the root system under drought-stressed conditions like how the control-drought replicates exhibited.

Microscopy

The few images I took through microscopy displayed the presence of *Serendipita bescii* in the water-inoculated replicates. However, with such little microscopy data available at the moment, I am unable to draw a conclusion on the colonization rates and colonization patterns of *Serendipita bescii* in drought versus watered conditions.

Limitations and future directions

PCR and microscopy completion

Though my research asks larger questions of the percent colonization rates of *Serendipita bescii* among drought treatments versus water treatments as well as the differences in morphology of the fungi in these treatments, I acquired limited data due to mandated shelter-in-place as a result of COVID-19. In the future, I aim to complete DNA extractions and PCR analysis. PCR will allow me to analyze whether the DNA extracted from our root and soil samples match the DNA of *Serendipita bescii*. This analysis will confirm that the fungi were successful in colonizing our inoculated plants. PCR will also help determine whether *Serendipita bescii* is naturally present in the marginal soils, and if so, what the natural colonization rates are.

In addition, I hope to complete microscopy on each replicate to look at the presence of colonization as well as the colonization rate and percent colonization at each timepoint. This will better help me understand how much of an impact the fungi had on the plant's growth and success, as well as help understand the drastic alternations in biomass averages at each timepoint.

Broader implications

Ultimately, we expect that the symbiotic relationships between mycorrhizal fungi and crops can improve future biofuel production under these severe drought conditions resulting from climate change. If the fungi are providing host plants an external nutrient supply for plants to thrive even under drought stress conditions, we can use this benficial relationship to increase biofuel production among crops like switchgrass and *Panicum hallii* and create more environmental-friendly alternatives to fuel.

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