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Watching Grass Grow: How Soil Moisture Affects Vesicular-Arbuscular Mycorrhizae and Growth in Little Bluestem

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ABSTRACT

Vesicular-arbuscular mycorrhizae (VAM) are an ancient mutualism in which soil-dwelling fungi enhance plant absorption of phosphorus and nitrogen in exchange for photosynthates. VAM are sensitive to changes in soil moisture and nutrient content, fluctuating between mutualism and parasitism depending on conditions of drought stress and nutrient deficiency. Understanding how VAM respond to precipitation changes is crucial for both conservation and agricultural purposes. To test how soil moisture changes the effects of VAM colonization and growth in little bluestem (*Schizachyrium scoparium*), a common prairie grass, I planted 300 seeds in a greenhouse in sterilized soil and soil inoculated with VAM fungi spores. I applied five watering treatments for 13 weeks and then harvested the seedlings. Increasing soil moisture had a negative effect on biomass and shoot height, but no significant effect on number of leaves or root length. Soil inoculation did not have a significant effect on plant growth, and the fungi did not colonize any of the 15 root samples examined. Results suggest that little bluestem grows taller in dry soil, and may benefit from mild drought conditions. The phosphorus content of the potting soil may have been too high to incentivize young seedlings to recruit VAM fungi. As a precaution, future greenhouse studies involving VAM fungi should incorporate nutrient-poor soil to encourage colonization.

ACKNOWLEDGEMENTS

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INTRODUCTION

Vesicular-arbuscular mycorrhizae (VAM) are an ancient mutualism consisting of interactions between the roots or rhizoids of most land plants and specialized soil-dwelling fungi (Allen 1991). VAM are the most common mycorrhizal symbiosis and belong to a monophyletic group, phylum Glomeromycota (Stürmer 2012). There is evidence of this mutualism in the fossil record dating back more than 400 million years, and they likely coevolved with the first land plants around 475 million years ago (Wang and Qiu 2005, Corradi and Bonfante 2012, Hoysted et al. 2018). VAM continue to form important mutualisms, and are capable of associating with more than 80 percent of land plant families (Wang & Qiu 2006). Most VAM fungi are generalists, with an estimated 250 species described to date colonizing more than 200,000 plant species (Bahadur et al. 2019).The coevolution of VAM with plants is so tightly knit that VAM fungi are obligately dependent on their hosts, and some mycorrhizal plants are unable to complete their life cycle in nutrient-poor soil without fungal symbionts (Anderson et al. 1994).

VAM function as root extensions, helping host plants acquire phosphorus and nitrogen in exchange for carbohydrates (Allen 1991). VAM fungi colonize root cortical cells with exchange organs called arbuscules, through which they deliver phosphorus and nitrogen to their host (Lee et al. 2013). VAM fungi extend beyond the rhizosphere via a net of filamentous hyphae that mine soil for phosphorus and nitrogen at a low energy cost (Field et al. 2015). VAM fungi access soil phosphorus and nitrogen more effectively because hyphae are longer and have a larger surface area to volume ratio than roots and root hairs, allowing them to probe smaller pore spaces (Allen 1991). As mycorrhizal hyphal length can range up

to 50 m per ml of soil, the capacity to exploit a given soil volume increases dramatically when a plant is mycorrhizal (Allen 1991).

Mycorrhizal fungi have a dramatic impact on ecosystems through their interactions with host plants. VAM modify soil aggregations and can stimulate or retard decomposition of soil organic matter through promoting degradative enzymes, modifying root production, and regulating microbial communities in the rhizosphere (Wei et al. 2019). In grasslands, competition between mycorrhizal and non-mycorrhizal plants predicts plant diversity and mediates succession (Collins & Foster 2009). VAM increase plant diversity when competitively subordinate plants benefit from the mutualism, giving them a relative advantage and increasing likelihood of coexistence. By contrast, VAM decreases plant diversity when VAM primarily benefit only dominant plants (Collins & Foster 2009). VAM can facilitate or inhibit proliferation of invasive species, depending on the degree of similarity between native VAM communities and those carried with invasive plants (Aslani et al. 2019). Similar VAM community composition between the two plants tends to maintain stands of native plants, whereas highly different VAM fungi tend to modify soil structure in favor of the invaders (Aslani et al. 2019).

VAM confer many advantages to mycorrhizal plants. One advantage they confer is protection against soil-borne pathogens via induction of systemic resistance (Cameron et al. 2013, Pozo 2007). A mycorrhizal plant is able to absorb a much larger volume of nitrates and phosphates than a non-mycorrhizal plant in nutrient-poor soil. In exchange for up to 20 percent of the host plant's carbohydrate supply, VAM may provide up to 80 percent of the host plant's nitrogen and 100 percent of the plant's phosphorus required for growth and

reproduction (Hoysted et al. 2018). VAM improve their host plant's drought tolerance by improving water status and influencing plant hormonal pathways, modifying photosynthetic rate, root hydraulic conductivity, and root architecture to adapt to drought conditions (Bahadur et al. 2019). Mycorrhizal plants release larger quantities of root exudates during times of stress (Graham et al. 1982). Estimates suggest that plants can exude up to 40 percent of their photosynthates from roots to recruit beneficial soil organisms such as VAM fungi (Cameron et al. 2013). Root exudates enhance VAM spore germination, and fungal hyphae grow 20 times faster in the presence of root exudates than not (Gadkar et al. 2001).

Mycorrhizae appear to be most beneficial to the host plant during times of acute stress and under poor growing conditions. In mutualistic association, VAM can confer stress tolerance to abiotic factors such as drought, high soil salinity, heat, cold, oxidative stress, and heavy metal toxicity to their host plants (Singh et al. 2011). On the other hand, during times of stress or abundance, plants may divert resources away from their VAM in order to conserve energy, thereby reducing mycorrhizal prevalence (Owens et al. 2011). When stress is low, an arbuscular mycorrhizae relationship can shift from mutualism to parasitism by the fungus on the plant, depending on environmental conditions (Anderson et al. 1994). Elevated soil phosphorus has been shown to induce the host plant to inhibit arbuscular mycorrhizal development in petunias (Nouri et al. 2013) and little bluestem (Frater 2012).

High-phosphorus environments in particular eliminate resource limitation so that mycorrhizal fungi can become a carbon sink, suppressing plant growth (Collins & Foster 2009). Soil temperature tends to have a positive impact on VAM colonization, but the effects vary by soil nutrient and light availability for the host plant. Soil temperature has a

positive effect on VAM formation in sudangrass (*Sorghum vulgare*), either through a direct effect of temperature on the fungus or an indirect effect springing from increases in root exudates (Graham and Leonard 1982).

VAM affect the hydration balance of drought-stressed plants in subtle ways (Augé 2001). The relationship between VAM and soil moisture is highly variable. Water shortage hampers VAM spore germination, colonization capacity, sporulation, and extra-radical hyphal elongation, yet mycorrhizal recruitment by host plants is greater during water shortages, benefiting the VAM (Bahadur et al. 2019). VAM colonization improves the establishment of hyphal networks and glomalin secretion that improves water and nutrient uptake and enhances soil structure, but drought still negatively affects VAM (Bahadur et al. 2019). VAM increase stomatal conductance, transpiration, and phosphorus acquisition in host plants over nonmycorrhizal plants during drought episodes, but there is no difference in stomatal conductance, transpiration, and phosphorus acquisition in mycorrhizal plants between drought and non-drought conditions (Augé 2001). Changes in VAM colonization can be related to direct mycorrhizal effects on root hydraulic conductivity or shifts in soil water retention and conductivity as a result of increase water consumption by the host plant facilitated by VAM (Bitterlich et al. 2018). Because research suggests that VAM responses to changes in moisture are multifaceted and nonadditive (Owens et al. 2012, Augé 2001), they may have a stronger impact than is currently believed.

VAM determine soil structure in both natural habitats and agricultural fields, so developing a better understanding of their behavior in response to changes in rainfall is crucial. In a study of *Leymus chinenis* and *Puccinellia tenuiflora*s growth under simulated

climate warming, VAM increased plant growth significantly by mobilizing soil phosphorus, helping to offset negative impacts of higher temperatures (Mei et al. 2019). In a study of rye and spelt under a range irrigation levels, planting of an overwinter mycorrhizal cereal cover crop reduced density of nonmycorrhizal weeds by growing the extraradical mycelium in the soil (Trinchera et al. 2019). This effect was stronger in dry soil than in wet soil, indicating that VAM's response to moisture is important to study in an agricultural context as well.

Given the near-ubiquity and ecological importance of VAM, even a modest response in mycorrhization due to precipitation changes could have an impact on terrestrial ecosystems. Shifts in freshwater availability and rainfall from climate change make understanding the response of VAM to precipitation changes even more important. In its fifth assessment report, the Intergovernmental Panel on Climate Change (2014) projected significant increases in average annual rainfall in eastern North America by the end of the 21st century. Understanding how VAM respond to climatic variability is an important part of understanding how mycorrhizal and non-mycorrhizal plants change in their distributions over time, and in how ecosystems and mycorrhizal crops are responding to climate change.

This study was an investigation of the relationship between mycorrhizae, growth, and precipitation in *Schizachyrium scoparium* (hereafter referred to as little bluestem), a prairie grass common across the continental United States and southern Canadian provinces. With its coarse root system, little bluestem has a high affinity for VAM, and Owens et al. (2012) and Anderson et al. (1994) found it accumulated greater biomass with VAM than without it in unmodified prairie soil. I planted little bluestem seedlings in sterilized soil and inoculated half of the soil with VAM spores. Then, I applied five moisture treatments to investigate the

relationship between VAM, precipitation, and host plant growth by answering the following questions:

(1) How does soil moisture impact little bluestem growth?

(2) How does soil moisture affect VAM colonization of little bluestem roots?

(3) How does soil moisture change VAM's effect on little bluestem growth?

I hypothesized that increased soil moisture would increase plant growth, decrease the colonization of root tissue by VAM, and decrease VAM's positive impact on plant growth. I predicted that grass growth would increase more with soil moisture in sterile soil than in inoculated soil. I predicted that I would find more fungal organs in roots grown in dryer soil than wetter soil. Lastly, I expected grass to grow the largest in dry inoculated soil and the smallest in dry, uninoculated soil.

METHODS

Ecology of Little Bluestem

Little bluestem is a perennial, warm-season (C_4), mycorrhizal bunchgrass native to all lower Canadian Provinces and continental U.S. states, with the exception of Nevada and Washington (Tober & Jensen 2013). It is a deep-rooted tallgrass and can grow from 1-3 feet fall. It typically grows in sandy or clay-loam soils on dry upland sites such as ridges, hilltops, and steep slopes. Moderately drought tolerant, little bluestem thrives in areas receiving 250 to 1,500 mm of mean annual precipitation (Tober & Jensen 2013). It sprouts from seed and begins growth in late spring, often forming dense mats from short rhizomes, especially in

wetter habitats. Little bluestem is one of the best grasses for wildlife nesting and roosting habitat due to its clustered growth pattern, and its seeds and leaves provide high-quality nutrition for many small mammals, birds, and insects. Understanding little bluestem's growth patterns with respect to environmental change is important for prairie conservation.

Design of Greenhouse Experiment

To test for the effect of soil moisture on arbuscular mycorrhizae and grass growth, I grew 300 seedlings of little bluestem from seed in a greenhouse for 13 weeks. I grew the seedlings in two batches: the first in the spring (March through June 2019), and the second in the summer (June through September 2019). The greenhouse fluctuated with ambient temperatures and was artificially lit to a consistent day length of 16 hours throughout the growing period. To test the effects of mycorrhizal fungi on growth, I grew half of the seedlings in sterile soil mixed with MycoBloom commercial VAM inoculum, and the other half in sterile soil (Fig.1). I then examined the inoculated roots for arbuscules, hyphae, and vesicles (storage organs). I applied five watering treatments based on (1) average monthly precipitation in the state of Connecticut in the month of April (when mycorrhizal fungi spores typically germinate), (2) the prediction for precipitation trends in eastern North American by the end of the 21st century, supplied by the Intergovernmental Panel on Climate Change Assessment Report 5 (IPCC 2014), and (3) the holding capacity of the soil I used. My five watering treatments were as follows: 12 ml as a simulation of drought, 21 ml, 27 ml as a control corresponding to Connecticut's average rainfall in April, 33 ml, and 39 ml as a simulation of consistent heavy rainfall (Table 1).

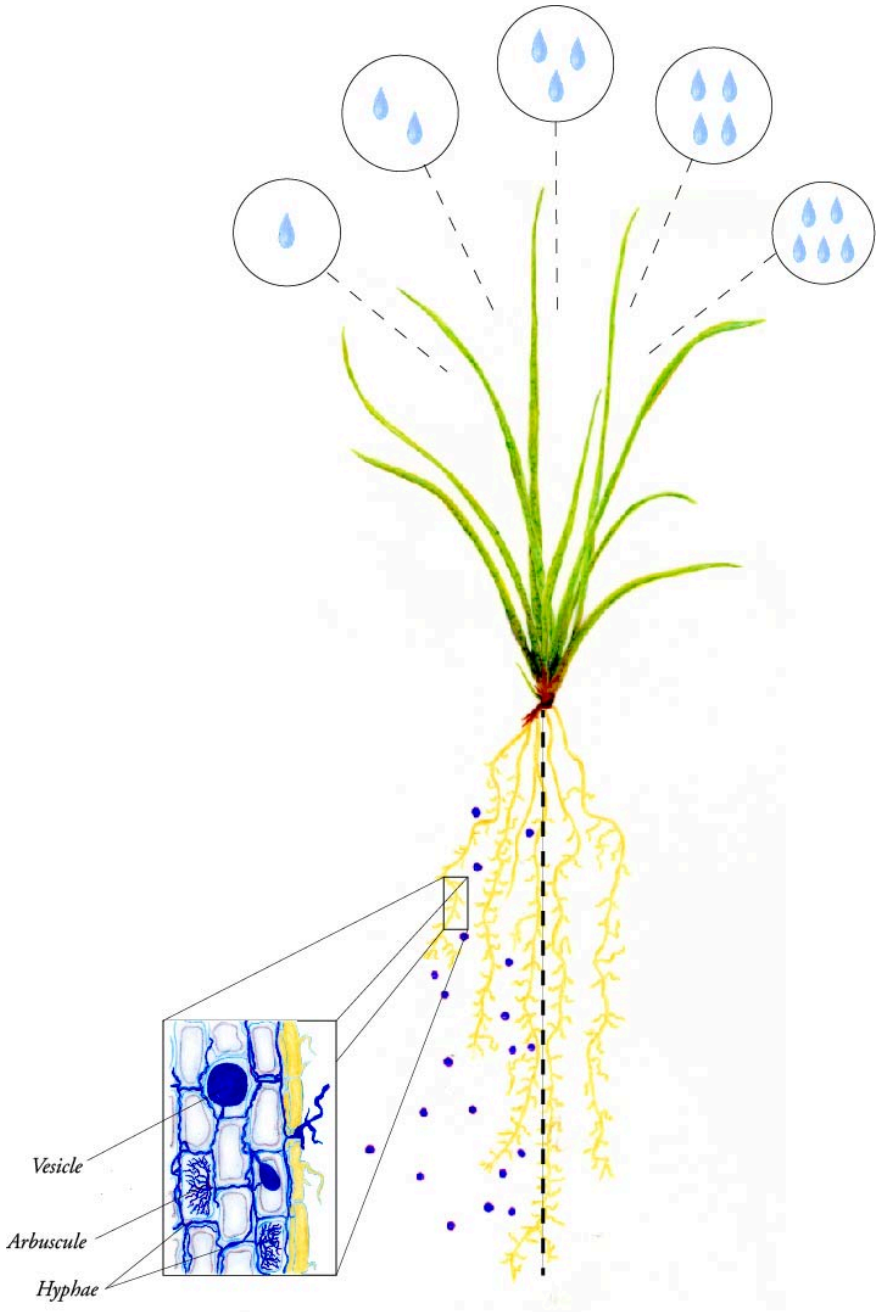

Figure 1. *Illustration of five watering treatments applied to 150 seeds planted in sterile soil, and 150 planted in soil inoculated with VAM. Anatomy of VAM within root tissue also shown, longitudinal section.*

Table 1. Treatments applied to 10 seedling cohorts out of a total of 300 individuals.		
Water received per week (mL)	**Sterilized Soil**	**Inoculated Soil**
12	30 seeds planted	30 seeds planted
21	30 seeds planted	30 seeds planted
27	30 seeds planted	30 seeds planted
33	30 seeds planted	30 seeds planted
39	30 seeds planted	30 seeds planted

Soil Selection, Sterilization, and Inoculation

MycoBloom EndoMycorrhizal Mix (MycoBloom LLC, Indiana University) contained a blend of spores from seven species of VAM (Glomeromycota) common in mid-western U.S. prairies: *Claroideoglomus claroideum*, *Funneliformus mosseae*, *Cetraspora pellucida*, *Claroideoglomus lamellosum*, *Acaulospora spinosa*, *Racocetra fulgida*, and *Entrophosphora infrequens*. I used Miracle-Gro organic loamy potting soil mix with perlite and grew my seedlings in long, narrow "cone-tainers" from Stuewe & Sons. Replacing traditional growing pots with cones gave the seedlings adequate space for root growth and soil drainage. I sterilized all of my soil by autoclaving it. I used commercial potting soil mix because field-collected soil molded quickly in storage. After sterilizing, I added 2.5 percent MycoBloom inoculum by volume to half of the soil. I also used a LaMotte agricultural soil test kit to measure soil pH and nitrogen, phosphorus, and potassium content to detect potential effects of soil nutrient levels on plant-VAM interactions.

Using a protocol adapted from gardening company Vegatronix, Inc., I calibrated the soil's saturation to ensure that my watering treatments were truly distinct, and not masked by excess drainage. I did this by measuring 100 g of soil on a balance scale, and adding tap

water 10 ml at a time while stirring until water began to pool in the container. I considered the volume added to this point the soil's holding capacity, and used it to constrain my watering treatments.

Seed Selection and Surface Sterilization

I purchased little bluestem seeds from Prairie Moon Nursery and planted several as a preliminary test for germination rate over the course of two weeks. Before planting, I sterilized the seed coats to eliminate other spores or microbes that might alter the plants' growth. I sterilized them by mixing them in 0.6 percent bleach and rinsing with autoclaved tap water three times. After drying the seeds, I planted five in each cone and transferred them immediately to the greenhouse. I randomly assigned positions to cones of each treatment in the two trays.

Harvesting Seedlings and Measuring Little Bluestem's Growth

I watered the plants three times weekly with autoclaved tap water. To track changes in growth rate over the course of the 13-week growing period, I measured the shoot height and number of leaves of each seedling once weekly between week three and week twelve. After growing the seedlings for 13 weeks, I harvested them and measured their growth in terms of wet biomass, shoot height, root length, and number of leaves. I collected root fragments weighing about 0.1 g from each inoculated seedling to examine for VAM colonization. I placed these samples in 70 percent ethanol and refrigerated them until I cleared and stained them. Next, I dried the harvested seedlings in paper bags at 40 °C for 24 hours. After drying, I measured their dry biomass.

Clearing Roots and Staining VAM to Assess Colonization

To prepare my inoculated root samples for scoring under a microscope, I cleared, acidified, and stained them using a protocol designed for quantifying arbuscules in roots (Giovanetti & Mosse 1980, McGonigle et al. 1990, Vierhlig et al. 2005). I cleared the roots in 10 percent potassium hydroxide solution for 48 hours. I then rinsed them with tap water and acidified them via soaking in 2 percent hydrochloric acid for 20 minutes. I transferred the roots directly into the stain solution, which consisted of 0.3 percent by mass Chlorazol Black E in a 1:1:1 solution by volume of lactoglycerin in water. Chlorazol Black E is an acidic stain that specifically binds to chitin in fungal cell walls, and is routinely used in the study of mycorrhizae (Vierhlig et al. 2005). After soaking the roots in the stain solution at room temperature for 48 hours, I transferred them to storage in 50 percent glycerin in water. I mounted a total of 30 root samples on slides and examined them to confirm mycorrhizal colonization under 100X and 400X magnification.

Analysis

I analyzed the relationships between water treatment, fungal inoculation, and plant wet biomass, dried biomass, leaf number, shoot height, and root length by fitting a linear regression. I performed Welch two sample t-tests to determine if the two seedling cohorts significantly differed from each other based on growing period. I used statistical computing software R (R Core Team 2018) to fit linear models and R package ggplot2 (Wickam 2016) to create figures.

RESULTS

Soil Moisture and Growth in Little Bluestem

Increased soil moisture had a significant negative impact on little bluestem biomass (Fig. 2) and shoot height (Fig. 3). Every 1 mL per week increase in soil moisture decreased biomass by 4.1 mg on average ($p = 0.00012$), and decreased shoot height by 2.1 mm on average ($p = 5.68 \times 10^{-5}$). Increased soil moisture did not have a significant effect on number of leaves ($p=0.34$, Fig. 4) or root length ($p=0.13$, Fig. 5).

Soil Moisture and VAM Colonization of Little Bluestem

I did not identify any arbuscules, vesicles, or hyphae in the 15 root samples I stained and examined under a microscope. The soil nutrient test showed that the potting soil was rich in both phosphorus and nitrogen (Table 2).

Interaction of Soil Moisture and VAM's Effect on Growth in Little Bluestem

There was no evidence of interaction between the effects of soil moisture and VAM on little bluestem's growth. There was not a significant difference in biomass accumulation ($p = 0.44$, Fig. 2) or shoot height ($p = 0.62$, Fig. 3) between sterile and inoculated soils. Soil inoculation did not significantly affect number of leaves ($p = 0.57$, Fig. 4) or root length ($p=0.15$, Fig. 5).

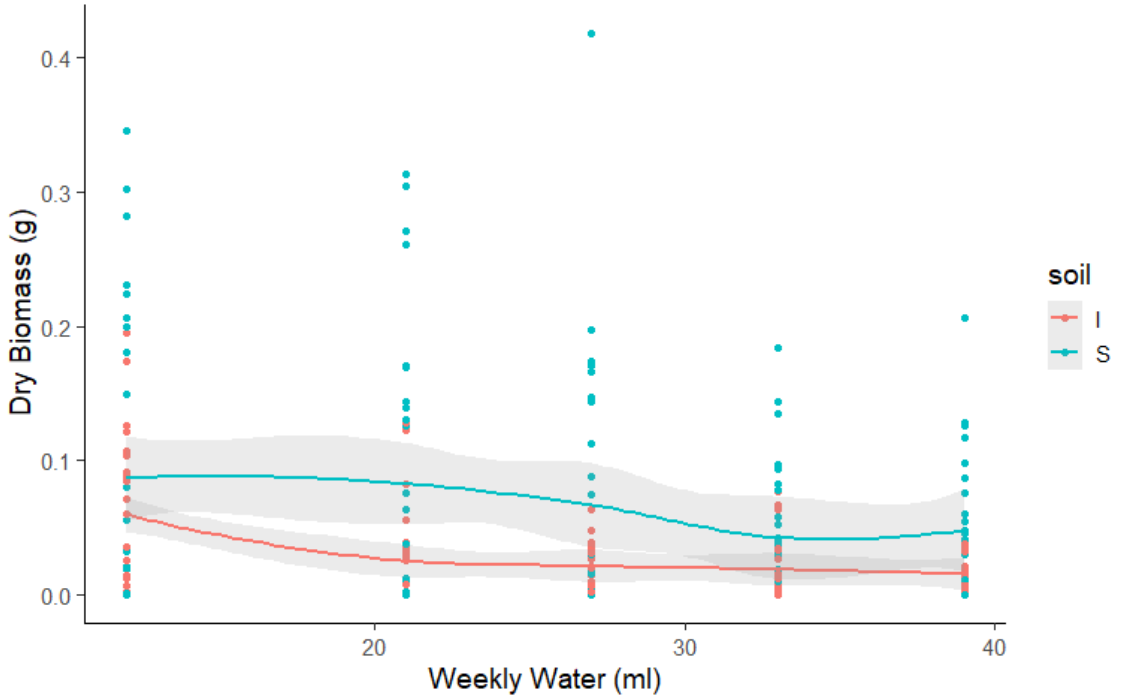

Figure 2. Total dry biomass of little bluestem at harvest as a function of five watering treatments in inoculated (I) and sterile (S) soils. Both seedling cohorts are combined.

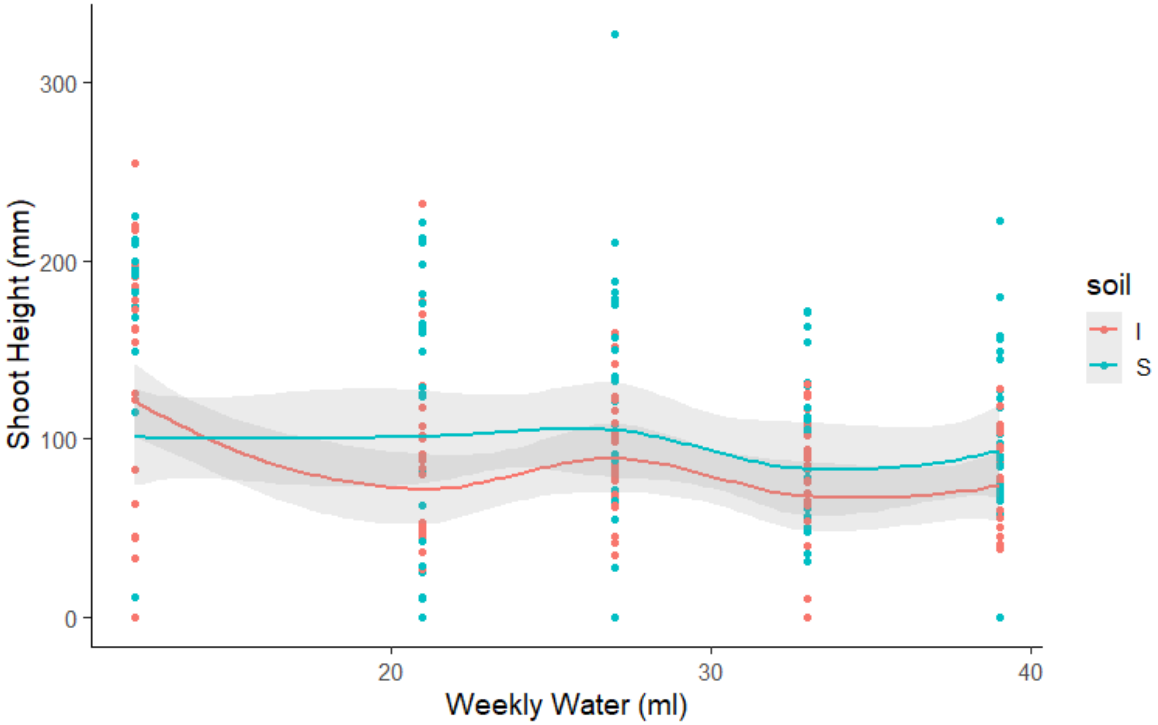

Figure 3. Variation in grass shoot height at harvest as a function of water received in inoculated (I) and sterile (S) soils. Both seedling cohorts are combined.

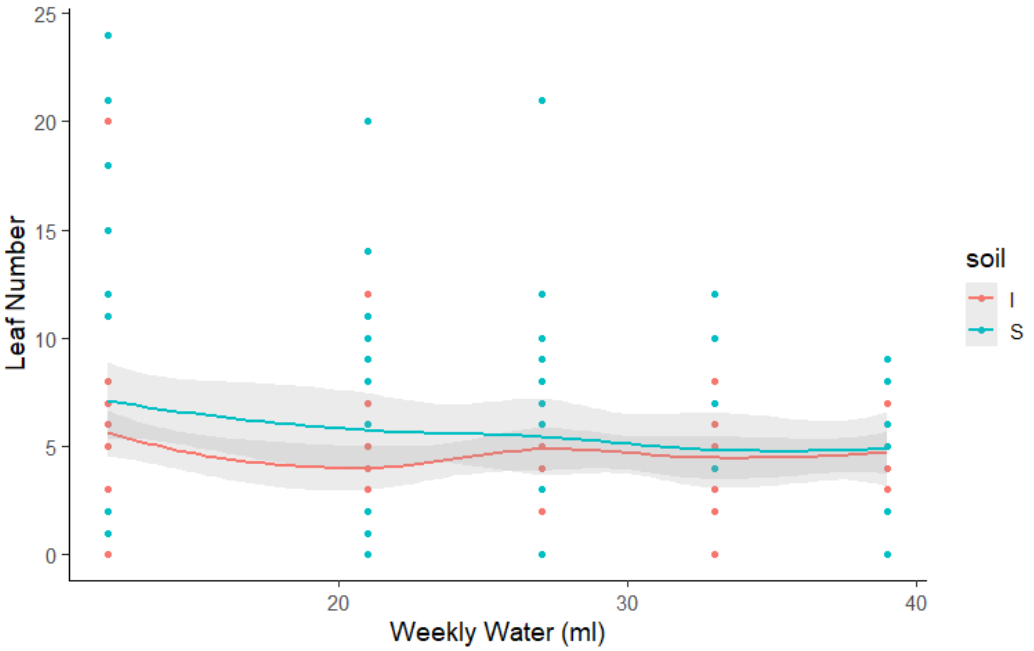

Figure 4. Variation in leaf number at harvest as a function of water received in inoculated (I) and sterile (S) soils. Seedling cohorts are combined.

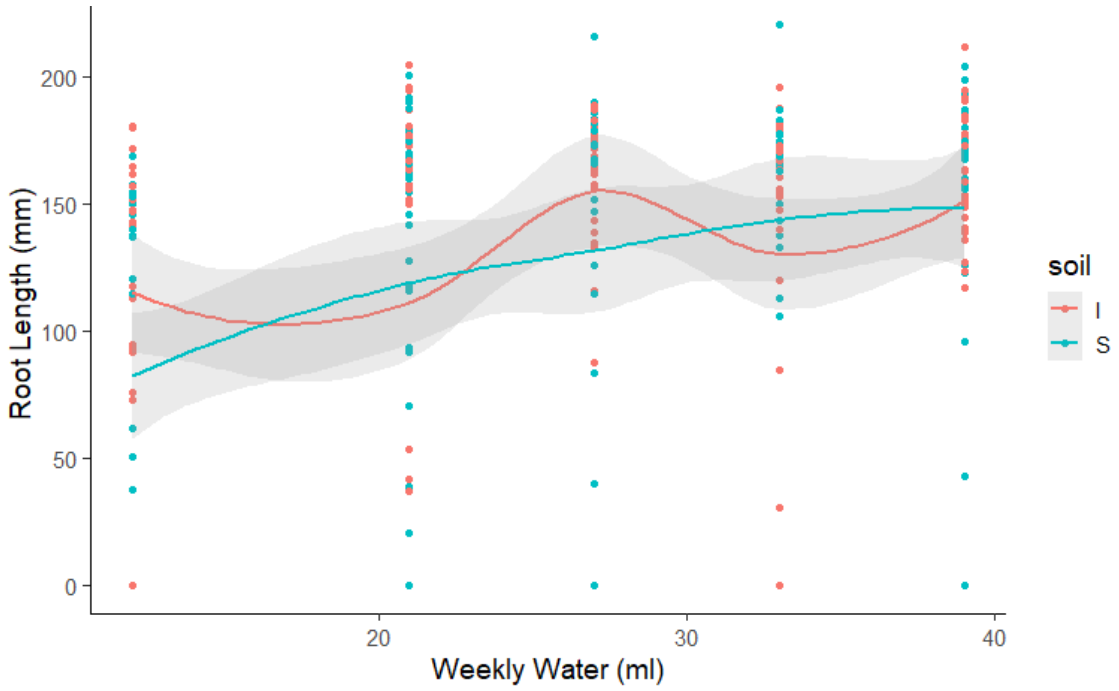

Figure 5. Variation in root length at harvest as a function of water received in inoculated (I) and sterile (S) soils. Seedling cohorts are combined.

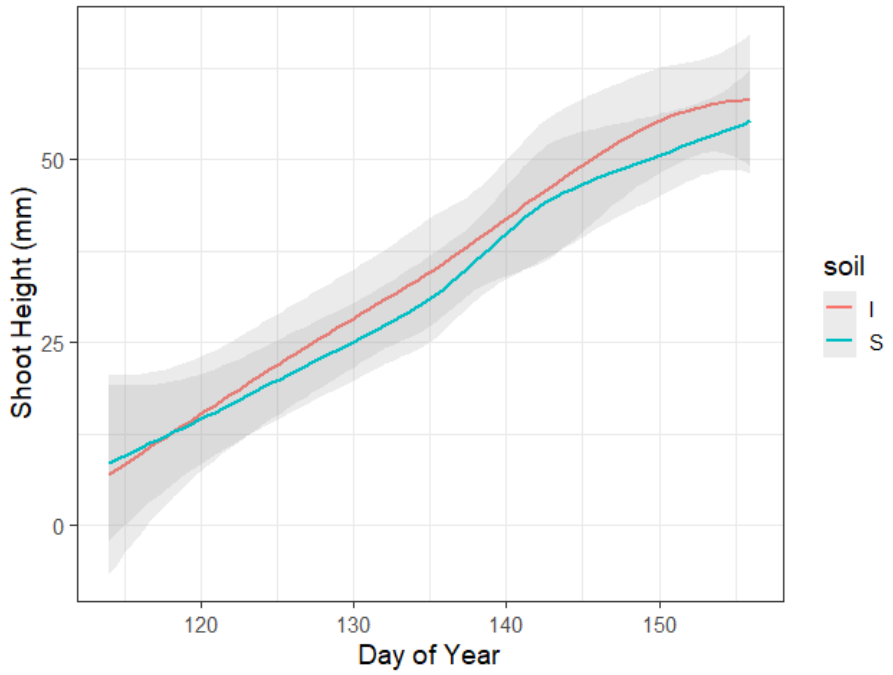

Figure 6. Growth of seedlings in inoculated (I) and sterile (S) soil in the first cohort as measured weekly from April through June 2019.

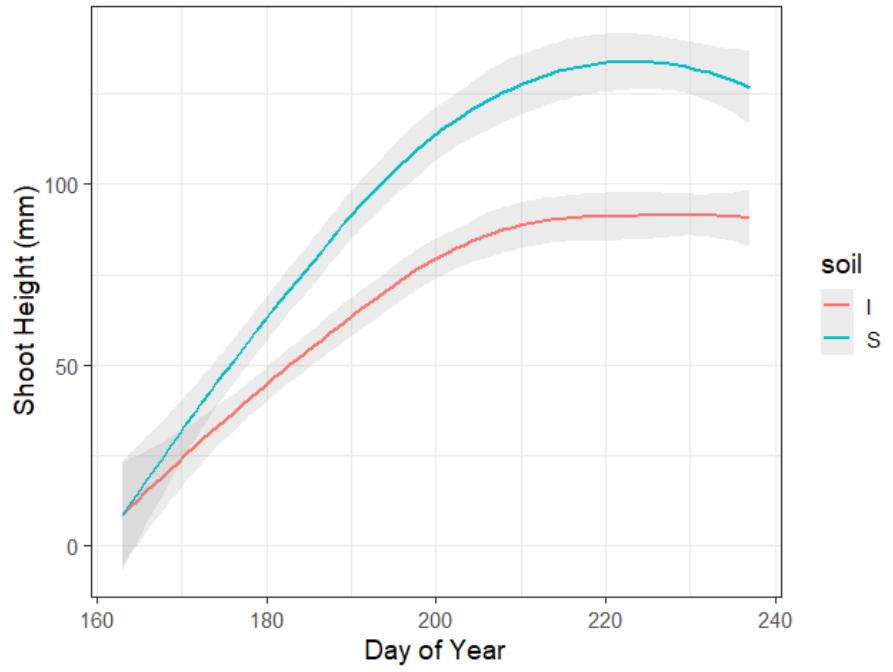

Figure 7. Growth of seedlings in inoculated (I) and sterile (S) soil in the second cohort as measured weekly from May through August 2019.

Table 2. Results of LaMotte Soil Nutrient Test	
Soil pH	6.5
Nitrogen concentration	high, > 67 kg/ha
Phosphorus concentration	very high, > 112 kg/ha
Potassium concentration	high, > 135 kg/ha

There was a significant difference between the average heights of seedlings grown in the spring cohort (38.0 mm) and the summer cohort (80.2 mm, $p < 2.2 \times 10^{-16}$, Welch two sample t-test). In the spring cohort, seedlings in sterile and inoculated soil had a similar growth pattern over time (Fig. 6). In the summer cohort, seedlings in sterile soil grew taller than seedlings in inoculated soil but this difference was not significant ($p = 0.10$) and had disappeared by harvest date (Fig. 7). Drought-stressed grass seedlings typically sprouted more leaves and grew laterally, whereas adequately watered seedlings primarily grew vertically.

DISCUSSION

Growth of little bluestem seedlings was not significantly impacted by soil inoculation with VAM spores in any of the treatments at any point in time. Dry biomass and shoot height were both negatively impacted by increasing soil moisture, whereas the effect of water on root length and leaf number weren't significant. These results refute my hypothesis that little bluestem's growth would be positively affected by increasing soil moisture, and positively affected by VAM in dry soil but not wet soil. No arbuscules could be found in root samples after harvest, so the VAM were probably unable to germinate and colonize the roots properly. While it is possible that the sample of roots examined (10 percent of seedlings in

inoculated treatments) was too small to detect low levels of colonization, such low colonization rates arguably would have a negligible effect on little bluestem populations.

There are four possible explanations as to why the fungi were not present in the root samples. The first possibility is that the spores were unable to germinate for some reason, or died before harvest. Spore germination may be affected by a number of factors, including root exudates or volatiles, soil pH, temperature, and day length (Maia and Yano-Melo 2001). VAM fungi that colonize warm-season grasses usually germinate in April or May, and I planted the seedlings in the first cohort on April 1st, so day length and temperature are unlikely to be the main culprits. Because little bluestem grew in a range of soil moisture conditions and none of these treatments had fungi, soil moisture is unlikely to be the limiting factor for germination. The seedlings were grown in full sunlight in sterilized soil with a pH of 6.5, so it also is unlikely for sunlight or pH to have prevented spore germination. Because growing conditions were kept constant throughout the growing periods for both cohorts, and because there was no significant difference in plant growth between sterile and inoculated soils at any point before or at harvest, it's unlikely that VAM colonized roots and then died before staining.

A second possible reason as to why I did not find mycorrhizae is that the fungal organs did not stain properly. I calibrated a widely used staining technique to my root samples, which were small, delicate, and easily stained. There is no reason to think that a faulty stain caused the fungi to be invisible. A third explanation is that the inoculum I used was not viable.

The fourth possibility is that the high concentrations of nitrogen and phosphorus in the potting soil inhibited the plant's recruitment of mycorrhizae. These two nutrients influence root colonization by VAM in such a way that the plant controls its symbionts depending on how limited its access is to nitrogen and phosphorus, inhibiting the symbiosis by restricting flow of photosynthates in high phosphorus, high nitrogen soil, and promoting it by exudate plenty of photosynthates for the fungi so long as they are limited by one of the two major nutrients (Nouri et al. 2014). Plant exudates from roots enhance VAM spore germination, but are not required for this process (Gadkar et al. 2001). Even after germination, if the fungi do not receive adequate nutrition from the plant, such as in high quality soil, the symbiosis will not survive. In this case, the only evidence that would support initial colonization and then die-off by VAM is the difference in shoot heights in the second cohort of seedlings during the growing period (Figure 10); however, this difference was never significant. Furthermore, seedlings typically respond to mycorrhizal fungal colonization by reducing root length and growing thicker roots (Anderson & Liberta 1992). Regardless of the cause, the fact that I didn't find any arbuscules, vesicles, or hyphae in the stained root samples indicates that soil inoculation did not significantly affect plant growth in this study. The variations I observed in plant growth are mostly likely responses purely to the different watering treatments.

The negative relationship between soil moisture and little bluestem dry biomass and shoot height might be explained by little bluestem's high drought tolerance. The watering treatments were assigned based on average precipitation for the month of April in Connecticut (when mycorrhizae typically germinate), on precipitation increases predicted by

the IPCC's fifth assessment report for the east coast of North America from 2080-2100 (IPCC 2014), and on the saturation point of the potting soil used. Although little bluestem is native to Connecticut, it is much more prolific in the Great Plains of the midwestern U.S. states and southern Canadian provinces. These prairies are maintained by precipitation patterns which are too dry and irregular to support forests, and so they are colonized by drought-tolerant herbs, forbs, grasses, and shrubs. Little bluestem is a grass well adapted to prairie conditions. Connecticut is almost completely forested and consistently receives high levels of rain through every season. Thus, I may have overestimated how much water my seedlings would need to grow, waterlogging the roots of plants in wetter treatments and making it harder for them to grow. Soil carbon dioxide is an essential carbon source for hyphal growth; excess water in the soil may have blocked the percolation downward of carbon dioxide from the air (Gadkar et al. 2001). Little bluestem's adaptation to drought is the most likely cause of its negative growth response to water.

The main implication of this study is that increasing soil moisture negatively impacts growth in little bluestem. Changes in amount, timing, and intensity of rainfall affect nutrient availability and soil microbial community composition, including VAM fungi (Cavagnaro 2016). Along the east coast of North America, where annual precipitation is expected to increase, little bluestem's fitness may be reduced. In the Great Plains, where regional droughts are likely to become more severe, little bluestem and other drought-tolerant C_4 grasses may become dominant over cool-season C_3 grasses.

The main limitation in this study is that VAM fungi did not colonize the roots. I recommend that future greenhouse experiments with VAM use soil low in phosphorus and

nitrogen to encourage plants to accept mycorrhizae. In the interest of saving time, VAM fungi colonization should be monitored before the end of the growing period. This can be done either by germinating spores in agar culture prior to planting, by snipping and staining root samples partway through the growing period. It may also be useful to extend this study to field experimentation in garden plots. Plants in a greenhouse are unable to compete or interact with each other, including through mutually shared VAM hyphal networks (Allen 1991). Field experiments incorporate a wider range of variables, especially soil chemistry and microbes, making them both more complex and more realistic. Mycorrhizae tend to be affected by many interacting factors simultaneously, so holistic study of grasses planted in different soil microenvironments in the field may make specific interactions clearer. Finally, mycorrhizae are also sensitive to interannual variability, so field studies are most useful when conducted over several field seasons (Owens et al. 2012).

CONCLUSION

VAM in grasses transform prairie community composition and restoration by modifying soil chemistry and conferring competitive advantages to mycorrhizal species over non-mycorrhizal species in phosphorus and nitrogen-deficient soils (Middleton & Bever 2012). The responses of VAM fungi to changes in soil moisture are variable and interactive with other facets of the soil environment, such as quantity of root exudates released by the host plant. Little bluestem is a common, mycorrhizae-dependent, and ecologically important grass native to prairies across North America. Climate change is projected to result in drier and

more variable climates for many regions of the world, including the heart of the range of little bluestem in the midwestern U.S. Extreme weather are already increasing abiotic and biotic stress to plants. It is important to understand how mycorrhizal fungi respond to changes in rainfall, both because they are nearly universal, and because of their capacity to transform ecosystems.

I predicted that in my study of the impact of variations in soil moisture on growth and VAM inoculation in little bluestem, water would positively affect plant growth and negatively affect VAM colonization. The reality was that water had a slightly negative effect on biomass and shoot height in little bluestem, indicating that it is highly drought tolerant with or without fungal symbionts. VAM from the commercial inoculum used did not successfully colonize roots in my system, likely indicating that (1) the spores in the inoculum were dead, or (2) that the grass seedlings did not permit the VAM to colonize their roots because the medium in which they were grown was already so rich in phosphorus. I recommend that in future replications of this study design, roots be sampled and tested frequently for colonization to ensure that mycorrhizae are forming. These results suggest that little bluestem will be of minimum conservation concern in the case of short-term regional drought. They also suggest the possibility that high soil nutrient content may inhibit VAM colonization or germination in the first place, an idea which warrants further study.

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