INTERACTIVE EFFECTS OF FUNGAL COMMUNITY STRUCTURE AND SOIL MOISTURE ON WYOMING BIG SAGEBRUSH PERFORMANCE

by

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The following individuals read and discussed the thesis submitted by student Arden Marlene Engel, and they evaluated the student's presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

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DEDICATION

I dedicate this work to my mother, Katherine Engel, for her selfless support and guidance during the execution of this work, and to Megan Pease, Ph.D., whose mentorship in my early academic career inspired me to pursue studies in the wild, wonderful world of ecology.

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ABSTRACT

Large swathes of the North American sagebrush steppe have been converted to grazing and agricultural use. Remaining fragmented areas are threatened by invasive annual grasses and associated perpetuation of increased fire frequency, with efforts to restore sagebrush after fire largely unsuccessful. Lack of success may be due, in part, to poor interactions between sagebrush seed sourced from distant locations and unfamiliar soil fungi.

A greenhouse experiment was designed to evaluate effects of soil fungal community composition on sagebrush reestablishment by answering: (1) How does inoculation with 'coevolved' versus 'foreign' fungal inoculum impact sagebrush fitness? (2) How does moisture availability modify the advantage different fungal communities provide?

Wyoming big sagebrush seed was collected from a single population at the wet end of its range and grown in sterilized soil inoculated with either its coevolved wet-site fungi or foreign fungi from a drier sagebrush-dominated site, with sterilized-inoculum controls. Seedlings received either a wet-precipitation treatment simulating that of the seeds' and wet-site inoculum's source site or a dry-precipitation treatment simulating that of the drier foreign inoculum's source site, producing eight treatment combinations. Quantification of $\delta^{13}C$ in sagebrush tissue (a drought stress proxy) established that plants given dry-site precipitation experienced more drought stress and photosynthesized less than those given wet-site precipitation. Sagebrush biomass, root morphology, and visible

fungal root colonization were assessed after 6-7 months of growth. Generalized linear models were fitted to evaluate plant response variables as functions of inoculum source site, viability, and precipitation regime.

ITS fungal DNA sequence data demonstrated that the in-situ fungal rhizosphere communities of mature sagebrush at the sites from which inoculum was derived differed markedly in fungal taxonomic composition. Seedling rhizosphere community composition at harvest also differed by treatment group, confirming that different inoculation treatments were effectively applied. Arbuscular mycorrhizal fungal taxa, which this experiment initially aimed to assess specifically, however, were entirely absent from all but one seedling's rhizosphere by the end of the growth period, instead requiring assessment of the fungal community broadly. Rhizospheres of seedlings inoculated with live inoculum from either site were enriched in fungi of *Phlyctochytrium sp.* (and family Chytridiaceae) as well as fungi belonging to unknown families and genera, compared to sterilized inoculum, and dry-site inoculum treatments also had significantly greater abundances of *Paraphoma sp.* (and family Phaeosphaeriaceae). The majority of root-colonizing fungi visible under light microscopy were dark septate endophytes, a functional group with variable documented effects on plant growth and morphology – though, root colonization by DSE was not correlated with growth responses, consistent with some prior studies.

Live fungal inoculum from both sites enhanced sagebrush seedling biomass when the precipitation regime simulated that of the inoculum's source site, when compared to sterilized counterparts. Live dry-site inoculum also produced longer, finer roots under dry-site conditions. Both findings suggest adaptation of the soil fungal community to

local environmental conditions and reflect that the positive impacts of inoculation on sagebrush growth and morphology are contingent on a match between the inoculum and soil moisture. Applying local-environmentally-adapted fungal inoculum, derived from the intended restoration site, could potentially assist sagebrush in optimizing water uptake and maintaining productivity under drought conditions, thereby encouraging sagebrush establishment. Further research into the role of the differentially abundant taxa and DSE on sagebrush responses, and classification of as-yet unidentified fungal taxa may elucidate mechanistic explanations of the observed effects of inoculation.

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LIST OF ABBREVIATIONS

AMF	arbuscular mycorrhizal fungi
DSE	dark septate endophytic fungi
HS	hyaline septate fungi
TNS	thin non-septate fungi
SRL	specific root length
GLM	generalized linear model
GLMM	generalized linear mixed model

INTRODUCTION

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Background

The sagebrush ecosystem is declining in spatial extent and growing increasingly fragmented, now occupying only 56% of its historic range in North America (Davies et al., 2011). This alarming loss has led to the local or regional extirpation of numerous sagebrush obligate and facultative species (Davies et al., 2011). In low elevation sites occupied by Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*), invasion by exotic annual grasses generate fine fuels that increase fire frequency, perpetuating further invasion (Eiswerth et al., 2009). As big sagebrush does not resprout after burning and can only disperse seed over short distances (~16 m on average) (Applestein et al., 2022), it is slow to recover after fire, resulting in habitat conversion of sagebrush steppe to annual grassland (Eiswerth et al., 2009; Knutson et al., 2014; Requena-Mullor et al., 2019; Wijayratne et al., 2012). Despite land managers investing millions of dollars annually to restore sagebrush landscapes via transplanting or seeding after wildfire, rates of successful restoration are low (Arkle et al., 2014; Davies et al., 2018; Requena-Mullor et al., 2019; Shriver et al., 2018). Mortality of sagebrush seedlings is high, but adult shrub mortality is low (Cawker, 1980). Thus, factors that influence early seedling establishment, such as water availability, have substantial effects on a key bottleneck in recruitment and successful restoration, making understanding the role of the soil fungal community on germination, early survival, and initial growth of sagebrush critical.

Increasing recognition that feedbacks between plants and the soil microbial community are important drivers of plant fitness (van der Putten et al., 2013) has prompted interest in the potential for soil fungi to impact restoration success, particularly in dryland ecosystems that experience extreme seasonal drought such as the sagebrush-steppe in which symbiotic fungi interact with sagebrush to enhance uptake of resources that are limited in abundance and mobility.

Most studies of interactions between plants and soil fungi have focused on arbuscular mycorrhizal fungi (AMF), symbiotic biotrophic fungi which penetrate the cortical root cells of vascular plants, receiving organic carbon from their hosts in exchange for enhancing the plants' water and nutrient uptake (Berruti et al., 2016). Approximately 80% of vascular plants are colonized by AMF to some degree in natural settings (Berruti et al., 2016).

Though understudied compared to AMF, growing evidence illustrates non-mycorrhizal root-associated fungi can influence the physiological traits of host plants as well, especially when mycorrhizal fungi are absent or present in low abundance in the soil (Andrade-Linares & Franken, 2013; Hughes et al., 2020; Porras-Alfaro & Bayman, 2011). Dark septate endophytic (DSE) fungi, for instance, comprise a poorly understood polyphyletic functional group of fungi that colonize healthy plant roots and form melanized inter- and intracellular hyphae and microsclerotia (Santos et al., 2021). DSE can alter plant shoot and root growth, enhance seed germination, prompt adventitious root formation, affect the accumulation of phytochemicals and phytohormones, induce systemic pathogen resistance mechanisms, increase mineral nutrient solubility and/or uptake, and both enhance and reduce resistance to abiotic stresses (Andrade-Linares &

Franken, 2013; Hou et al., 2021). Their effects on plant growth, morphology, and hormones vary with environmental conditions, genotype, developmental stage, and nutritional status of the plant and the fungus during their association (Andrade-Linares & Franken, 2013; Hou et al., 2021; Hughes et al., 2020).

Symbiosis with arbuscular mycorrhizae improves Wyoming big sagebrush seedlings' ability to obtain moisture from soil compared to non-mycorrhizal seedlings (Stahl et al., 1998), likely also ameliorating limitations on nutrient uptake incurred by soil drying (Davidson et al., 2016). These mechanisms contribute to AMF's improvement of plant drought tolerance (Berruti et al., 2016) and seedling survival across a range of low soil water potentials (Stahl et al., 1998). However, in semiarid and arid regions such as the sagebrush steppe, low AMF spore densities in the soil of disturbed landscapes may inhibit root colonization, reducing sagebrush seedling survival and establishment as well as water use efficiency (Davidson et al., 2016). Consequently, restoration practitioners have considered inoculating big sagebrush seedlings with AMF to increase propagule density (Davidson et al., 2016). In prior field and mesocosm experiments, *A. tridentata* ssp. *wyomingensis* inoculated with AMF native to the planting site exhibited increased root colonization by AMF and improved seedling survival (Davidson et al., 2016). These experiments established that application of known symbiotic classes of fungi confers growth and survival advantages to big sagebrush, However, they used a single inoculum type and did not examine 1) how inoculum composition, including non-mycorrhizal fungi such as DSE, influences success, or 2) whether the impacts of fungal inocula depend on patterns of local adaptation between both the inoculum and the sagebrush population as well as between the inoculum and the environment.

Artemisia tridentata ssp. *wyomingensis* seed is often translocated over large geographic distances for restoration efforts (Davidson & Germino, 2020; Innes, 2019), outside of the environmental conditions, both abiotic and biotic, to which its source population is locally adapted. Soil fungal community structure varies across soil moisture gradients, with soil moisture exerting a stronger impact on mycorrhizal community structure than host plant species (Collins et al., 2018; Drenovsky et al., 2009; Jacobson, 1997; Trent et al., 1994). Climatic and edaphic factors can shape distinct fungal assemblages across both local and global scales (Peay et al., 2016; Tedersoo et al., 2014). Thus, the composition of the soil fungal community is likely to be highly variable and radically different between seed source and intended restoration sites, depending on geographical and climatic disparities between them.

Often, substantial variation is found within a species or subspecies across its range that reflects local adaptation to contrasting environments, in which local genotypes outperform foreign transplants (Kawecki & Ebert, 2004; Wadgymar et al., 2017). While existing literature acknowledges that the unfamiliar abiotic conditions in a new environment can reduce plant fitness after transplantation (Miglia et al., 2007; Bennington et al., 2012), and local adaptation to either the abiotic or the biotic environment has been quantified in multiple experiments and meta-analyses (Rice & Knapp, 2008; Walsh et al., 2016; Wilhelmi et al., 2017), few studies have assessed the context dependence of local adaptation due to interactions between abiotic and biotic factors (Briscoe Runquist et al., 2020). Most studies have evaluated adaptation to abiotic conditions at the extremes of abiotic gradients or have assessed adaptation to biotic conditions based on the categorical presence or absence of biotic variables, rather than

experimentally replicating the extent of natural variation of both abiotic and biotic factors to examine how organisms may be locally adapted to particular combinations of realistic biotic and abiotic conditions (Briscoe Runquist et al., 2020).

To improve restoration efforts, we must understand the relative impacts of both local adaptation of sagebrush and the soil fungal community to abiotic conditions and of local adaptation of both partners to one another on sagebrush growth. The nature of the plant-fungal interaction may be contingent on soil moisture and change along a gradient of abiotic conditions. Application of fungal inoculum native to a target restoration site, and therefore adapted to its abiotic conditions, could be expected to improve big sagebrush survival and growth compared to the fungal community from the sagebrush seed source location, especially in arid environments in which soil symbionts are more critical for nutrient uptake due to lack of soil moisture. Alternatively, it is possible that nutrient and water transfer between sagebrush and its "familiar" native symbiotic fungal community could be more efficient than "foreign" communities with which it is inoculated, due to coevolution and selective pressures imposed by their shared abiotic conditions (water availability, soil type, temperature, etc.), which vary across sagebrush's range (Hoeksema, 2010).

We expect that environmental conditions will shape the potential for positive species interactions (Maestre et al., 2009). Potentially, due to local adaptation of the rhizosphere fungal community to its abiotic environment, the relative advantage conferred by familiar and foreign fungal communities may depend on the abiotic conditions at the target restoration site, with inocula only conferring benefits to their host when exposed to environmental conditions similar to their origin site. Familiar fungi may confer some

"home-field" advantage to the sagebrush with which it coevolved when adequate moisture (i.e., levels of moisture comparable to the source location) is available. However, under drier conditions, this advantage may erode as the adaptation of the foreign fungi to dry soil makes it more beneficial to the sagebrush. Given the expected increase in drought frequency in many parts of the world, including the North American West (Pachauri et al., 2015), understanding the manner in which environmental conditions affect the interaction between sagebrush and rhizosphere fungi based on fungal community composition is critical.

This research project sought to assess how the species composition of the local soil fungal community affects sagebrush growth and its allocation of resources to shoot and root growth under different moisture-availability conditions. Specifically, it asked: (1) How does inoculation with coevolved familiar versus foreign soil fungal communities impact sagebrush growth, morphology, and resource allocation? (2) How does soil moisture modify the relative growth benefit provided by different fungal communities to sagebrush?

To answer these questions, a greenhouse experiment was conducted in which seeds from a single Wyoming big sagebrush population were inoculated with sagebrush-rhizosphere fungal consortia from sites with contrasting climatic conditions and grown under contrasting simulated precipitation regimes. Plants were grown for 6-7 months before harvest and subsampling. Stable isotope ^{13}C in seedling root and shoot tissue was measured to evaluate relative water stress experienced by plants in each precipitation regime.

This research has tangible applications in sagebrush steppe restoration, as understanding how interactions with particular fungal assemblages contribute to the success or failure of seed translocations has the potential to bolster restoration success and maximize the efficacy of resources.

Hypotheses

I) When provided moisture characteristic of its source site, the familiar wet-site fungal inocula will confer a stronger growth advantage to the sagebrush ecotype with which it coevolved than the foreign dry-site fungal inocula adapted to dry-site soil conditions.

II) Under drought stress, the dry-adapted foreign fungal inocula will confer greater growth benefit to the sagebrush than the familiar fungal community that coevolved with the sagebrush seed under wet-site soil conditions (Fig. 1).

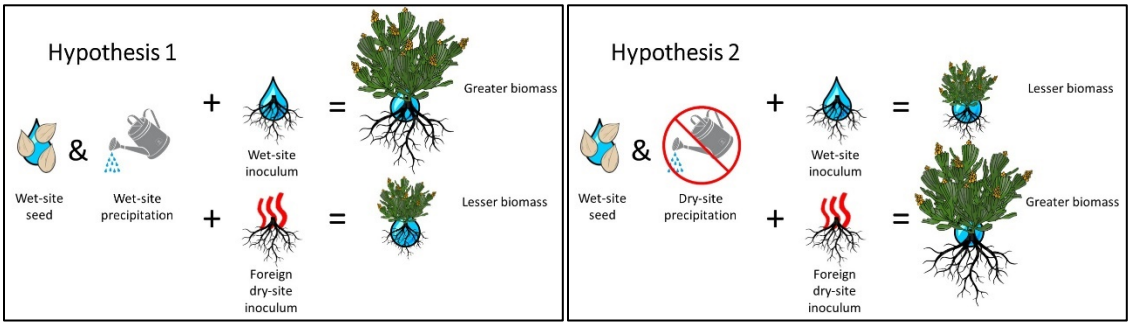

Figure 1. Hypothesized effects of live fungal inoculum on sagebrush growth under differing moisture regimes.

MATERIALS & METHODS

Experimental Design

To evaluate how symbiotic fungi presence, fungi source and soil moisture interact to affect sagebrush seedling growth and resource allocation, I planted sagebrush seeds in a greenhouse experiment with a full factorial design involving eight combinations of microbial and watering treatments. Plants received either a) wet-site inocula; b) dry-site inocula; or c) sterilized versions of these two inoculum types, and then received either a wet-site precipitation or dry-site precipitation treatment. (Table 1).

Table 1. Eight treatment combinations were produced from manipulation of (1) inoculum source (wet-site or dry-site), (2) whether the inoculum added was live or sterilized by autoclaving to serve as a baseline, and (3) the amount of moisture supplied to the pots (N=80 cone-tainers).

	Fungal Inoculum Source	Fungal Inoculum Viability	Precipitation Treatment
 Wet site population seed	 Wet site "Familiar"	 Live	Wet site precip. 
			Dry site precip. 
		 Sterile (control)	Wet site precip. 
			Dry site precip. 
	 Dry site "Foreign"	 Live	Wet site precip. 
			Dry site precip. 
		 Sterile (control)	Wet site precip. 
			Dry site precip. 

Sample Collection

Two sagebrush steppe sites dominated by Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) were selected based on their mean annual precipitation (MAP) and mean annual temperature (MAT). Nancy Gulch, within the Reynold's Creek Experimental Watershed (43.16867 N, -116.713 W), was chosen to be the "wet site", as its MAP of 446.17 mm/year (NACSE, 2021) is at the wetter end of the climatic spectrum of Wyoming big sagebrush's range; MAT at NG is 8.28 °C. The Snake River Birds of Prey National Conservation Area (43.23641 N, -116.344 W), with a MAP of 227.66 mm/year and MAT of 10.57 °C (NACSE, 2021) was located at the drier end of the range, and was selected as the "dry site". All Wyoming big sagebrush seed used in this experiment was collected from the wet site in November 2020.

Soil cores were collected with a hammer corer (5 cm diameter; ~15 cm depth) from the two sites on June 03, 2021. Samples were collected directly beneath the canopies of 10 mature sagebrush located at least 10 m apart from one another at each of the two sites to serve as the source of fungal inoculum (5 cores per shrub). The stainless-steel core collection chamber was sterilized with 70% ethanol and dried between shrubs. Each of the total 100 cores was deposited in its own sterile sample bag. Additionally, to create a common soil for use in the greenhouse experiment, bulk soil was collected by trowel (~10 cm depth) from both sites in 5-gallon buckets. Roughly equal volumes of this surface bulk soil were gathered from under the canopies of randomly chosen sagebrush within the general vicinity of those from which cores were taken and from interspaces between sagebrush; the area from which bulk soil was taken therefore generally encompassed the core sampling zone, with a few trowel-volumes of soil being taken from canopy and interspace microsites located between the sampled shrubs. The common soil was produced by mixing the bulk soil from the wet site (Nancy Gulch), bulk soil from the dry site (Snake River), and silica pool sand (to reduce soil compaction) in equal parts, all of which were passed through a 2 mm sieve. This common soil was homogenized and sterilized via autoclaving to kill fungi and bacteria in the soil This sterilized common soil served as the primary substrate in which sagebrush were grown.

Manipulation of Fungal Inoculum

The fungal inocula applied to the common soil in which the plants were grown, were derived from a mixture of a subset of the soil samples from either the wet or dry site. In sterile plastic tubs, the 5 soil cores from each shrub were combined and

homogenized; half of this total soil core volume from each shrub was combined with half of that of a second shrub from the same site to produce 10 "soil core combinations" from each site (Appendix A, Table A.1.). Fungal spores were derived from these combinations to inoculate the sterilized common soil in cone-tainers in which the sagebrush seeds were planted. This combination of two shrubs' rhizosphere communities was done with the goal of producing inocula representative of the site without artificially inflating fungal species diversity (as might be the case if all 10 cores from a site were combined).

Spores were derived from cores by first wet sieving the soil through a 500 μm sieve and 38 μm sieve, and then adding the material collected atop the 38 μm sieve to a sucrose gradient in 50 mL centrifuge tubes and centrifuging the tubes. Spores from the sucrose layers were collected on a 25 μm mesh and rinsed for at least 5 minutes to remove the sucrose; spores were rinsed from the mesh into 125 mL foil-wrapped Erlenmeyer flasks, producing suspensions of spores in deionized water, each roughly 15 mL in volume (Dierks et al., 2019; Klironomos, 2002). The spore concentration (measured as number of spores per milliliter) was determined for a subsample of inoculum suspensions via visualization under a dissecting scope (Fig. 2). Spore density was similar for both soil types (roughly 1200 spores/mL suspension). To account for changes in soil chemistry due to inoculation, half of each spore suspension (~8 mL) was transferred to a separate flask and sterilized via autoclaving to serve as a control against which its live counterpart could be compared to isolate the effects of microbial activity alone.

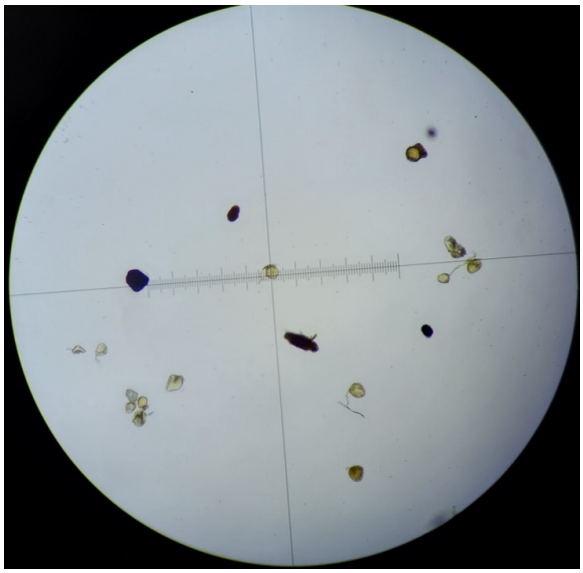

Figure 2. Fungal inoculum derived via sucrose gradient centrifugation from sagebrush rhizosphere soil, viewed under dissecting microscope.

Planting

Plants were grown in SC10 cone-tainers for this experiment. Prior to planting, the cone-tainers were sterilized by soaking for 10 minutes in 10% bleach, then rinsed with deionized water. To avoid soil loss, the bottom of each cone-tainer was lined with a piece of Vigoro WeedBlock Weed Control Film ~11 cm x 10 cm (cleaned via soaking in 10% bleach for 10 minutes). To each cone-tainer, ~175 g of autoclaved common soil was added. Cone-tainers were prepared two days prior to planting by adding 50 mL of sterile deionized water (5 mL at a time by micropipette) to all 80 cone-tainers and allowing them to freely drain to reach field capacity.

Planting occurred on June 19, 2021 using a total of 80 pots, including 10 replicates of each of 8 treatment combinations of soil moisture and fungal inoculum type (Table 1). Immediately prior to planting seeds, 2.5 mL of each inoculum suspension was added to the appropriate cone-tainers by sterile micropipette. A metal spatula was used to

spread out scraped surface soil over top of the inoculum (to keep spores from drying out) before 20 wet site sagebrush seeds were lightly pressed into the soil surface of each cone-tainer, which can increase sagebrush germination rates.

All seeds were gently pressed onto the soil surface at the time of planting such that they were all visible when daily counts of germinated seeds were conducted. A seed was considered to have "germinated" when any sort of green tissue/cotyledon was observed emerging from the seed. All but one per cone-tainer were culled once one seed had promisingly established in each. Germination rates and seedling survival to time of culling (6.5 weeks after planting) were assessed for all treatment combinations.

Precipitation Regime Manipulation

Precipitation treatments (wet-site vs. dry-site precipitation regimes) commenced on August 30, 2021, 10 weeks after planting to ensure seedling establishment prior to incurring water stress. Precipitation treatments were designed to simulate the monthly precipitation of each site in May, the point in the growing season when the difference in precipitation between the wet and dry sites is maximized (Fig. 3). Cone-tainers in the wet-site precipitation treatment received 69.7 mL /month (11.6 mL / 5 days), corresponding to the 55.45 mm 30-year mean May precipitation received at the wet site, while those in the drought treatment received 39.5 mL /month (6.6mL /5 days) to emulate the (31.43 mm average May precipitation at the dry site (NACSE, 2021).

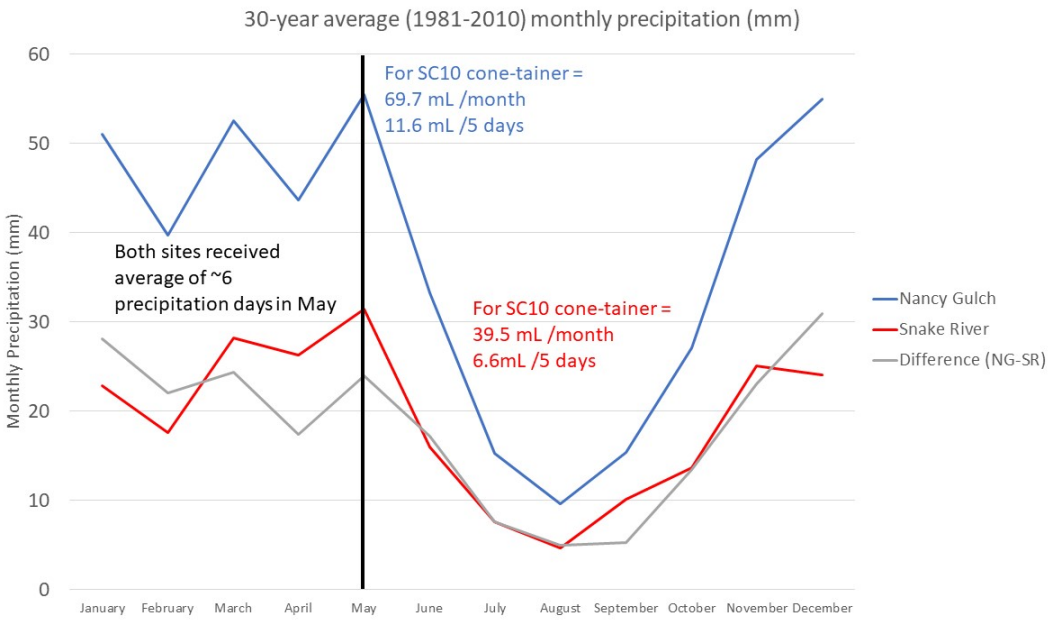

Figure 3. 30-year average monthly precipitation data at 800 m resolution for the wet site and dry site. Retrieved from https://prism.oregonstate.edu/explorer/ on 13 May 2021.

Biomass Harvest

Of the 80 cone-tainers seeded, 72 seedlings established and survived to time of harvest (See Appendix D for details). 32 seedlings were harvested on December 14, 2021, while the remaining 40 seedlings were grown for a month longer and harvested on January 20, 2022. No additional seedling mortality occurred during this interval. Aboveground biomass of all plants was cut at the soil surface. Collected roots were cleaned of soil aggregates using deionized water. Shoots and roots were oven-dried in envelopes at 65°C for 48+ hours and weighed to determine dry aboveground biomass and dry root biomass, respectively. For the January-harvested seedlings, dead aboveground biomass ("litter" biomass) was placed in separate envelopes from live/photosynthesizing biomass, as the inclusion of biomass incapable of absorbing the $^{13}C - CO_2$ would skew the calculation of total ^{13}C in sagebrush aboveground biomass.

From each plant, a root subsample (9- 20 mg) was stored in a sterile Nalgene microcentrifuge tube at -80°C for later extraction of root DNA.

Subsequently, the remaining contents of each cone-tainer were root-picked over a 2mm sieve to recover the root system of each seedling. Since root systems of the seedlings were small and were visually assessed to be differ minimally in size, the root-picking duration was standardized to 5 minutes to ensure equal sampling effort of all replicates. Roots were transferred to a 250 μm sieve and cleaned of aggregates and organic matter in deionized water. Cleaned roots were arranged in an acrylic scanner tray with 1 cm water depth to allow roots to float and scanned. Scans were processed by the image analysis system WinRHIZO (version 2009) (Fig. 4) to compile root morphology metrics on each, namely specific root length, calculated as sample total root length divided by sample dry weight (cm/g), average root diameter, and the length of roots in the smallest diameter size class (<0.5 mm). Root morphology and architecture metrics were evaluated to further characterize belowground responses to drought and fungal association, as these measures have been observed to change in coordination with root biomass and dry mass fractions (Garbowski et al., 2020). As some root morphology variables tend to be correlated with overall root system size, all morphology metrics were standardized by dry root biomass.

A subsample of the moist root (exact mass recorded) was refrigerated, to be stained and used to quantify levels of root colonization by fungi.

The remainder of the root was placed in a pre-weighed glassine envelope, weighed to calculate the moist mass of remaining root, oven-dried at 65°C for 48+ hours,

and re-weighed to calculate wet:dry root biomass ratio, then homogenized via ball-milling.

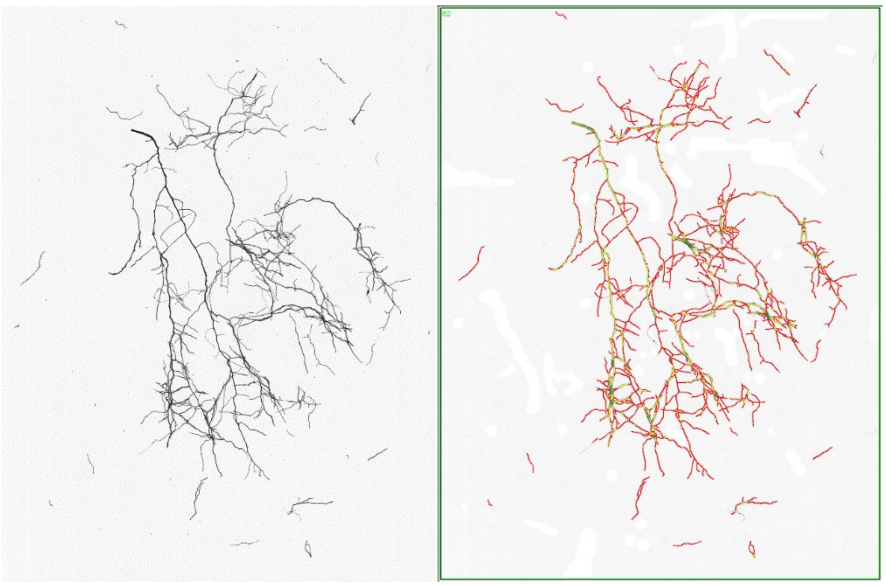

Figure 4. (left) scan of sagebrush seedling roots, (right) root scan color-coded by diameter size classes in WinRHIZO.

Endpoint Soil Sampling

At the time of enriched-seedling harvest, a ~3 g subsample of soil (hereafter "endpoint soil") from the zone of root influence was taken in a sterile DNA-free plastic vial and stored at -80°C for later extraction of soil DNA to characterize the composition of the soil fungal community. Another ~5 g of soil from the seedling rhizosphere was subsampled and stored at -80°C to be freeze-dried and undergo phospholipid fatty acid and neutral lipid fatty acid analysis. A 5-7 g subsample of soil from the rhizosphere was taken and oven-dried at dried at 65°C for 48+ hours, to be root-picked, homogenized via ball-milling, and assessed for ^{13}C content.

Assessment of Fungal Community Structure

DNA from three sample types was used to characterize the species composition of the soil fungal community, totaling 111 DNA extracts. DNA was extracted from a ~0.25 g subsample of each of the 20 soil core combinations used to derive fungal spores for inoculation (20 "core combo" samples + 2 negative controls), as well as a ~0.25 g subsample of the soil from cone-tainers in the zone of root influence of each of the 40 sagebrush seedlings that underwent labeling with $^{13}C - CO_2$ after harvest (40 "endpoint soil" samples + 2 uninoculated and unseeded soil blanks + 3 negative controls). DNA was also isolated from subsamples of root tissue from all 40 January-harvested seedlings at the time of harvest (40 root samples + 4 negative controls). DNA was extracted using the QIAGEN DNeasy PowerSoil Pro kit and the QIAGEN DNeasy Plant Pro kit. DNA was eluted in 75 µL 10 mM Tris-HCl, pH 8.5 (Solution C6 in the PowerSoil Pro kit, or Buffer EB in the Plant Pro kit), and stored at -80°C.

PCR was carried out with fungal primers 5.8S-Fun and ITS4-Fun to amplify the ITS2 region of the rDNA (spacer DNA between the small-subunit ribosomal RNA (rRNA) and large-subunit rRNA genes) (Taylor et al., 2016). Primers included an overhang adapter to enable downstream addition of barcodes for sample differentiation. Samples were amplified in a total volume of 20 µL with the following reagent concentrations and volumes per sample: 2 µL of 5 µM forward primer, 2 µL of 5 µM reverse primer, 5 µL PCR-grade water, 10 µL Q5® High-Fidelity 2X DNA Polymerase Master Mix (New England Biolabs), and 1 µL DNA extract. The thermal cycler protocol included denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 seconds, primer annealing at 58°C for 20 seconds, and extension at 72°C for 30

seconds, with a final extension period at 72°C for 2 min. In addition to the DNA extraction negative controls, every plate of samples subjected to PCR included a PCR-negative control consisting of PCR-grade water in lieu of DNA extract. Success of PCR was assessed by visualizing PCR products via gel electrophoresis at 80V for 60 min on a 1.5% agarose gel, using 3 µL PCR product + 1 µL 6X GelRed/ cresol red, followed by UV imaging. PCR product was cleaned using magnetic beads (Rohland & Reich, 2012) with a bead solution:PCR product ratio of 0.9.

Arbuscular mycorrhizal primer set FLR3/FLR4 was also used to amplify the D2 region of the LSU rDNA (Gollotte et al. 2004; Leckberg et al. 2013).

A second amplification was performed to index each sample with a unique pair of 8-nucleotide barcodes ligated to the forward and reverse strands (Hamady et al., 2008). A 2.5 µL aliquot of each bead-cleaned PCR product was barcoded in this second PCR step with a total volume of 20 µL, consisting of 10 µL Q5® 2X DNA polymerase, 2.5 µL of 2 µM of a barcode forward primer, and 2.5 µL of 2 µM of a barcode reverse primer. The thermocycler program used was identical to that used in the first amplification, except only 8 amplification cycles were performed, and the annealing temperature was set to 55°C. Barcoded PCR products were visualized via gel electrophoresis and cleaned with magnetic beads, identically to the first amplification PCR products. The DNA concentrations of the cleaned, dual-indexed ITS2 amplicons were quantified fluorometrically using a Qubit dsDNA HS (High Sensitivity) Assay Kit (Qiagen), with an excitation wavelength of 495 nm and emission measured at 525 nm on a BioTek Synergy multimode microplate reader using Gen5 software. These concentrations were used to determine the volume of each barcoded PCR product required to add 25 ng of DNA from

each sample to a pooled sample tube; this ITS2 amplicon pool was bidirectionally sequenced on an Illumina Mi-Seq platform at the University of Texas at Arlington Life Sciences Core Facility, rendering 2 x 300 bp paired-end read lengths.

The program Cutadapt was used to find and remove priming sites and poor-quality bases at the 5' and 3' ends of these reads to prevent these regions from interfering with downstream analysis (Martin, 2011). Resultant sequences were processed in R using the "DADA2" package (Callahan et al., 2016) as follows: Using The *filterAndTrim()* function, reads were truncated at the first instance of a quality score less than or equal to 2, and reads that contained any unassigned bases (Ns) or had an overall expected error score higher than 2 were discarded. The DADA2 algorithm, which utilizes quality scores and the number of times each sequence was observed to learn the run-specific error rate for forward and reverse reads and infer biologically real/true sequences and likely sequencing errors, thus enabling assignment of reads and cluster analysis at the species level. Forward and reverse reads were then merged. De novo chimeras were identified and removed using the *removeBimeraDenovo()* function, after which 0.9739% of total reads and 75.64% of SVs were retained. Taxonomy was assigned using the UNITE database and two *Artemisia* sequences from GenBank, with the *assignTaxonomy()* function.

As the ITS amplicon varies in length from 396-514 base pairs, read lengths were inspected to evaluate and remove short sequences less than 300 base pairs long, resulting in 2602 SVs within the reasonable length range. The *isContaminant()* function in the "decontam" package was implemented to identify run contaminants based on their presence in the blanks, and these contaminants were removed from the data (Appendix B,

Table B.3.). Soil core ITS samples were rarefied to 36,735 ITS reads, while endpoint rhizosphere soil samples were rarefied to 7,863 reads based on rarefaction curves.

Root Colonization

Prepared microscope slides of linear root segments from the 40 January-harvested seedlings were assessed for colonization by fungi as described in McGonigle et al. (1990). In short, root subsamples were cut into 1-2 cm linear segments and decolorized/bleached by autoclaving in 5-10% KOH solution for 3 minutes, rinsing in deionized water 3 times for 2 minutes, soaking in phosphate-buffered saline (PBS) for 20 minutes, and incubating in refrigerated lectin solution for 16-24 hours. Fungi were then stained by rinsing in PBS 3 times for 5 minutes, and incubating in Vector® VIP peroxidase substrate solution (Vector Laboratories) for 3-5 minutes under a dissecting microscope until a color change of the root from colorless to pinkish-purple was observed, after which the roots were rinsed for 5 minutes in deionized water. Stained roots were mounted on microscope slides in a 50% glycerol solution in linear rows.

Colonization on each root subsample was assessed via visualization under 20-40X magnification. The field of view was moved along each row in increments of 5 mm, with the presence/absence of fungal structures (hyphae, spores, and/or sclerotia) that intersected with the vertical crosshair in the field of view noted at each location. When marking a certain fungal structure as present/absent intersecting with the vertical crosshair at a given position, the entire width of that root segment cross section that intersected was assessed (i.e. the field of view was shifted up/down so as to evaluate the whole cross section of the root if the entire cross section was not fully visible in the field

of view at high magnification); consequently, the magnification under which each slide was viewed did not affect the area of the root that was assessed for fungal colonization. If the field of view at a particular 5 mm increment position did not contain a root cross section, this location was skipped and the slide was shifted 5 mm to the next location.

Visualized fungi were classified into three categories: thin non-septate fungi (TNS; fungi lacking both septa and arbuscules or vesicles characteristic of Glomeromycota, and with hyphae are < 5 μm in diameter), dark septate endophytic fungi (DSE; darkly pigmented fungi with septate hyphae and possible sclerotia present), and hyaline septate fungi (HS; unpigmented fungi with septate fungi, which may include DSE, as DSE become pigmented only at later stages of development) (Fig. 5).

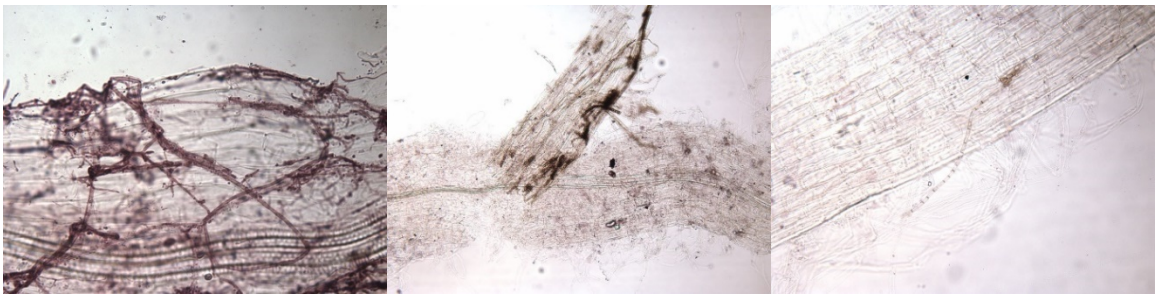

Figure 5. Fungal root colonization visualized under 40X magnification by thin non-septate fungi (left), dark septate endophytic fungi (center), and hyaline septate fungi (right)

^{13}C Analysis

$\delta^{13}C$ in subsamples of sagebrush shoot and root biomass and rhizosphere soil was assessed via elemental analyzer isotope radio mass spectrometry (EA-IRMS). Each of the dried shoots, roots, and soil samples from all 40 January-harvested individuals; each of the shoots and roots of the 32 December-harvested seedlings; and soil samples from 5 randomly selected December-harvested seedlings was homogenized via ball milling.

Background levels (a.k.a. the natural of abundance) of ^{13}C in the seedlings were used as a proxy measure of the degree of drought stress experienced by seedlings in all treatments, as plants incorporate more ^{13}C under greater water limitation (Fry, 2008). While site-specific conditions such as soil fertility, nitrogen content, and relative humidity can contribute to significant variation in $\delta^{13}C$ (Scheidegger et al., 2000), the growth of seedlings in a common greenhouse on a sterilized common soil substrate allowed us to isolate the effect of precipitation on $\delta^{13}C$ content.

Once powdered, ~0.5 mg of each shoot or root sample, ~20 mg of each unenriched soil sample, or ~2 mg of each enriched soil sample was folded into a tin capsule and subjected to $\delta^{13}C$ isotopic analysis using a Thermo Delta V Plus isotope ratio mass spectrometer (IRMS) coupled with a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies Inc.). Data were normalized relative to VPDB with USGS 40 and USGS 41a (glutamic acid), with NIST 1547 peach, Montana soil II, and a keratin powder run as controls as appropriate.

The value of $\delta^{13}C$ was calculated according to Formula 1 below.

$$\delta = [(R_{SAMPLE}/R_{STANDARD} - 1)] * 1000,$$

where

$$R = {}^{H}F/{}^{L}F \quad \text{and}$$

F = fractional abundance of the heavy $({}^{H}F)$ or light $({}^{L}F)$ isotope.

(Fry, 2008)

Statistical Analyses & Modeling

Analyses of all response variables were performed using RStudio (version 2022.12.0). All scripts will be posted to GitHub with submission of the manuscript associated with this thesis.

Germination rate was modeled with a generalized linear mixed model (glmm) with a binomial distribution, including a varying intercept for conetainer, to account for the nesting of seed-level observations within pots (n=20-30 per conetainer). Seedling survival probability to 6.5 weeks (i.e., to time of culling) was also modeled using a binomial glmm with a varying intercept for conetainer. Each model included variables for inoculum origin, inoculum viability (live or sterilized), and an interaction between these two terms. Precipitation regime was not included in models of germination or survival, as no treatment was applied until after final germinant culling occurred on August 05, 2021.

Differences in biomass metrics (total seedling biomass, aboveground biomass, root biomass, root:shoot biomass ratio) and root morphology metrics (specific root length, average root diameter, and total root length in the smallest diameter size class less than 0.5 mm diameter) were evaluated with generalized linear models (glms), with a Gamma distribution and logit link function. All models for biomass and root morphology

metrics included variables for inoculum origin, inoculum viability, precipitation treatment, and all two-way and three-way interactions between this set of variables.

To evaluate differences in the composition of the fungal communities present in the soil at the two inoculum source sites, and differences in composition at the end of the experiment, following experimental inoculation, non-metric multidimensional scaling (NMDS) analyses were performed on weighted on Bray–Curtis dissimilarities for rarefied ITS region data from the original soil core combinations and from the endpoint rhizosphere soil and root samples, respectively. Significant differences in mean centroids between inoculum source sites were tested for via permutational multivariate analysis of variance (PERMANOVA). The PERMANOVA assumption of homogeneity in dispersion was verified using the *betadisper()* function in the "vegan" package , which demonstrated no significant differences in dispersion among core samples ($p=0.8077$) or among endpoint soil samples ($p=0.2494$).

Fungi were assigned to functional groups at the genus level using the FungalTraits database, which contains 10,210 fungal genera annotated with 17 lifestyle related traits, including primary guild, which is expected to be the most characteristic of each genus, and capacities for biotrophic functions such plant pathogen, endophyte, and saprotroph (Põlme et al., 2020).

Differential abundance analysis was used to identify fungal genera and fungal functional groups whose abundance varied between treatment combinations. Differential abundance of an amplicon sequence variant (ASV) is analogous to differential expression of a gene from RNA-Seq data, with an considered differentially abundant if its mean proportion is significantly different between two or more predefined treatment classes

(McMurdie & Holmes, 2014). Differential abundance analyses were performed using the "ANCOM-BC" package in R (Lin & Peddada, 2020).

Differential abundance methodologies require read counts to be normalized to account for differences in sampling fractions (the ratio of expected absolute abundance of a taxon in a sample to its corresponding absolute abundance in the ecosystem, estimated by the ratio of library size to the microbial load) across samples (Lin & Peddada, 2020). Rarefaction to even depth, as has been commonly used to normalize microbial count data, often discards samples that can be accurately clustered by alternative methods and inflates the variances in all samples to the largest value among them, reducing the statistical power of subsequent analyses (McMurdie & Holmes, 2014). ANCOM-BC is designed to analyze unrarefied read count data, and accounts for sampling fraction by introducing a sample-specific offset term in a linear regression framework, that is estimated from the observed data. The offset term serves as the bias correction, and the linear regression framework in natural log scale is analogous to log-ratio transformation to deal with the compositionality of microbiome data (Lin & Peddada, 2020). Differential abundance analysis with the *ancombc2()* function was performed with FDR p-value adjustments for multiple comparisons and a prv_cut value of 0.05 to exclude taxa that were present in less than 5% of endpoint soil samples from the analysis.

Since the type of precipitation regime simulated in the greenhouse had no impact on endpoint soil fungal community composition within the same live inoculum treatment group (PERMANOVA, p=0.308 for all endpoint soil samples, p=0.350 for live-inoculum soil samples only), differential abundance analysis with ANCOM-BC was performed with endpoint soils grouped into three categories: sterile inoculum (all four combinations

of inoculum source site and precipitation regime), live wet-site inoculum (both precipitation regimes) and live dry-site inoculum (both precipitation regimes).

To investigate the relationship between seedling response metrics and fungal abundance, glms were fitted with each plant response metric (aboveground biomass, root biomass, specific root length, and total root length <0.5mm diameter) as the response variable and the regularized log transformed counts of differentially abundant fungal families as additive predictors, with a Gamma distribution and logit link function. An offset of 0.5 was added to the rarefied ITS read count totals of each differentially abundant fungal family, and the result was $\log_2$-transformed.

Shoot and root natural abundance of $\delta^{13}C$ between precipitation regimes in the unenriched seedlings was evaluated as a proxy measure of drought stress. The absolute values were taken of the inherently negative $\delta^{13}C$ values, and modeled as using a glm with a Gamma distribution. Small sample sizes limited the statistical power of this model, and required that the full model including all interactions between inoculum viability, inoculum source site, and precipitation regime be reduced to modeling $\delta^{13}C$ as a function of precipitation regime alone.

Root colonization (total fungal colonization and colonization by each of the aforementioned fungal classes) was modeled with glmms using the *glmer()* function in the lme4 package. Models were fitted to a binomial distribution as the total number of intercepts observed per slide varied between slides, with a fungal structure of a particular type either "present" or "absent" at each, a binomial response; consequently the probability that a given intercept has a fungal structure present is constrained between 0 and 1. Slide identity was included as a varying intercept to account for both the variation

in the total number of intercepts observed per slide, and grouping of observations by slide due to the tendency of roots on the same slide to have more similar numbers and types of fungal structures than roots on different slides.

Model fit was assessed via comparison of the mean absolute error (MAE) to sample mean, and computation of R^2 values (marginal and conditional R^2 values for Gamma and binomial glmms and Nagelkerke's R^2 for Gamma glms).

RESULTS

Seedling Germination & Survival to 6.5 Weeks

We examined whether our inoculum and precipitation treatments influenced seedling germination or survival to time of culling at 6.5 weeks. However, model fit associated with fixed effects was low, so results are not discussed or interpreted further here. Parameter estimates and model summaries are available in Appendix D.

Seedling Biomass

Parameter estimates from seedling biomass glms and estimates of model fit are summarized in Table 2. Total dry biomass of sagebrush seedlings averaged 0.1062 g across all treatments, ranging from 0.0281 g to 0.1867 g. Overall, precipitation substantially increased seedling biomass ($p<0.001$), with average predicted total biomass of seedlings across all inoculum types 0.07 g (95% CI: 0.06 - 0.08) in dry-site precipitation treatments and 0.13 g (95% CI: 0.11 - 0.16) in wet-site precipitation treatments.

Under dry conditions, addition of live dry-site inoculum increased predicted total biomass by 0.02439 g ($p=0.04401$; MAE=0.0232; Nagelkerke's $R^2=0.388$) and increased predicted aboveground biomass by 0.01527 g ($p=0.01816$; MAE=0.0126; Nagelkerke's $R^2=0.358$) compared to sterile dry-site controls (Fig. 6C; Fig. 6A). Live inoculum of

either type (wet-site or dry-site) had no significant impact on root biomass or root:shoot biomass under dry conditions ($p>0.05$) (Fig. 6D).

Under wet conditions, addition of live wet-site inoculum increased predicted total biomass by 0.04400 g ($p=0.008375$) (Fig. 6C) compared to sterile wet-site inoculum, but did not significantly increase aboveground biomass ($p=0.1212$) (Fig. 6A). Addition of live wet-site inoculum increased predicted root biomass by 0.02986 g ($p=0.003392$; MAE= 0.0141; Nagelkerke's $R^2=0.340$) (Fig. 6B) and increased predicted root:shoot biomass by 0.2544 g ($p=0.04087$; MAE=0.2150; Nagelkerke's $R^2=0.144$) (Fig. 6D) under wet conditions.

No effect of dry-site inoculum under wet-site conditions or of wet-site inoculum under dry conditions was observed on total biomass or aboveground biomass (Fig. 6C; Fig. 6A).

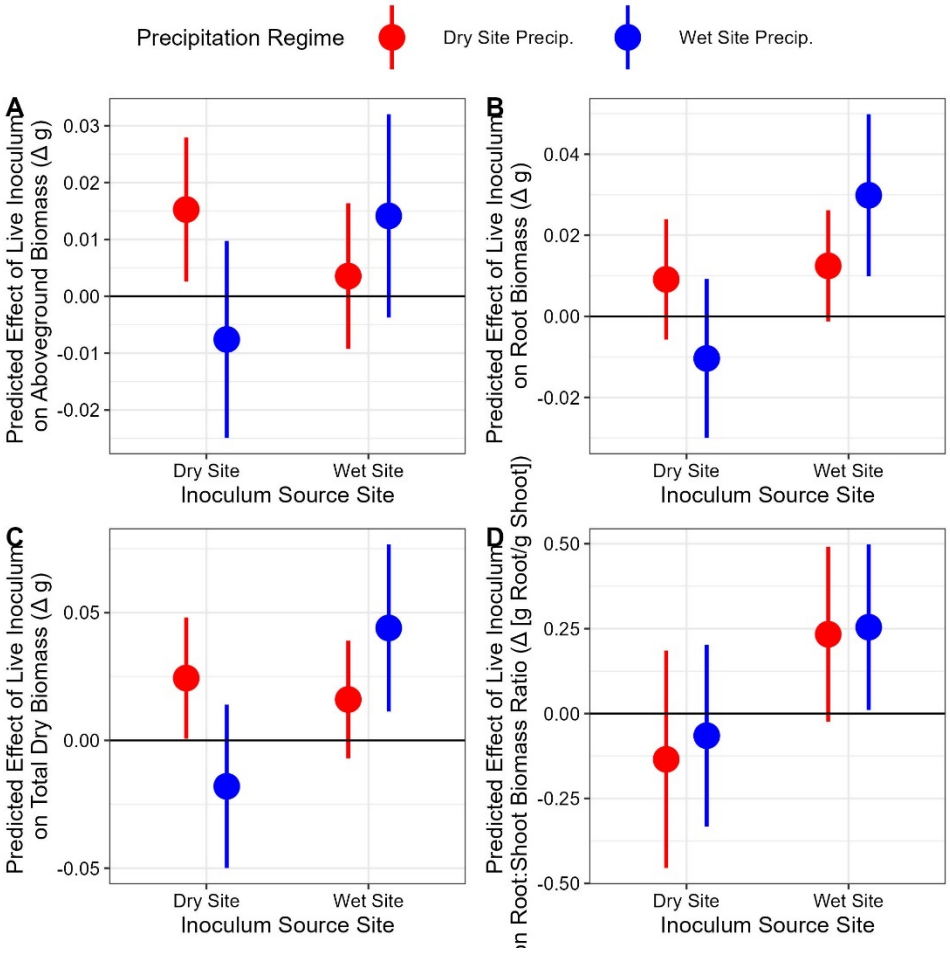

Figure 6. Predicted mean contrasts (and 95% CI) between sterile control and live inoculum treatments derived from dry and wet sites for seedling (A) aboveground dry biomass, (B) root dry biomass, (C) total dry biomass, and (D) root:shoot dry biomass ratio. Plants were grown under simulated dry-site and wet-site precipitation conditions in the greenhouse.

Table 2. Summary of parameter estimates from generalized linear models of plant biomass metrics.

	Total Biomass		**Shoot Biomass**		**Root Biomass**		**Root:Shoot Biomass**	
Predictors	*Estimate*	*p-value*	*Estimate*	*p-value*	*Estimate*	*p-value*	*Estimate*	*p-value*
(Intercept)	-2.6843 ± 0.1098	**<0.001**	-3.3338 ± 0.1085	**<0.001**	-3.42300 ± 0.14880	**<0.001**	-0.03714 ± 0.13210	0.7795
Inoculum source (Wet-site)	0.1262 ± 0.1463	0.3917	0.2650 ± 0.1447	0.0717	-0.05148 ± 0.19840	0.79610	-0.35915 ± 0.17613	**0.0456**
Inoculum viability (Live)	0.3055 ± 0.1503	**0.0462**	0.3564 ± 0.1486	**0.0194**	0.24665 ± 0.20376	0.23054	-0.15065 ± 0.18089	0.4080
Precip. (Wet-site precip.)	0.6767 ± 0.1431	**<0.001**	0.7102 ± 0.1415	**<0.001**	0.63867 ± 0.19402	**0.00162**	-0.06324 ± 0.17224	0.7147
Inoculum source X viability	-0.1176 ± 0.2033	0.5651	-0.2827 ± 0.2010	0.1644	0.09131 ± 0.27561	0.74151	0.44866 ± 0.24467	0.0713
Inoculum source X precip.	-0.3986 ± 0.1980	**0.0484**	-0.4204 ± 0.1958	**0.0356**	-0.37835 ± 0.26849	0.16362	0.01891 ± 0.23835	0.9370
Inoculum viability X precip.	-0.4490 ± 0.1986	**0.0272**	-0.4669 ± 0.1964	**0.0204**	-0.43043 ± 0.26929	0.11488	0.07594 ± 0.23906	0.7518
(Inoculum source X viability) X precip.	0.6189 0.2757	**0.0282**	0.5983 ± 0.2726	**0.0318**	0.64809 ± 0.37374	0.08772	-0.04089 ± 0.33179	0.9023
Nagelkerke's R^2	0.388		0.358		0.340		0.144	

Root Morphology

Adding live dry-site inoculum to a pot subjected to a dry climate (Fig. 7A) increased predicted specific root length (SRL, in m of root length per g root (DW)) by 21.08 m/g, producing longer, finer roots ($p=0.005966$, MAE=9.7965; Nagelkerke's $R^2=0.438$), (Fig. 7B) compared to sterile dry-site inoculum). It also increased predicted root length in the smallest diameter size class (<0.5 mm) by 74.22 cm ($p=0.01144$, MAE=37.2236; Nagelkerke's $R^2=0.795$), increased predicted number of root forks by 299.82 ($p=0.001461$; MAE=121.263; Nagelkerke's $R^2= 0.838$), and increased predicted root surface area by 12.82 cm^2 ($p=0.0004415$, MAE=4.9612; Nagelkerke's $R^2=0.898$). Root length <0.05 mm diameter, root forks, and root surface area were all highly correlated with one another, all reflecting higher surface area as a result of more fine roots (which have a higher surface area to volume ratio) with more branches. No significant effects of live dry-site inoculum under wet-site climate or live wet-site inoculum under dry-site or wet-site climate conditions was detected (Table 3).

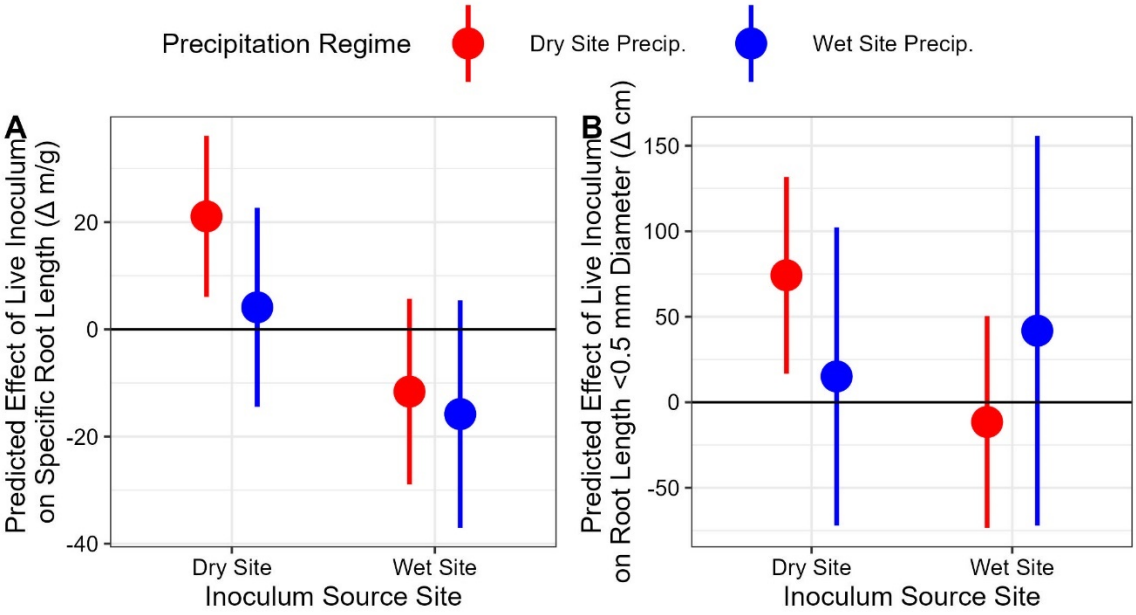

Figure 7. Contrasts and surrounding 95% CI between live inoculum treatments and corresponding sterile controls for root morphology metrics, predicted by generalized linear models. Standardized by dry root biomass, live dry-site inoculum + dry-site precipitation (A) increased predicted specific root length (SRL) and (B) increased predicted root length in the smallest diameter size class (<0.5 mm), compared to sterile dry-site inoculum.

Table 3. Summary of parameter estimates from generalized linear models of root morphology metrics.

	Specific Root Length		**Root Length <0.5 mm Diameter**		**Root Surface Area**		**Number of Root Forks**	
Predictors	*Estimate*	*p-value*	*Estimate*	*p-value*	*Estimate*	*p-value*	*Estimate*	*p-value*
(Intercept)	3.5749 ± 0.1143	**<0.001**	4.4454 ± 0.1584	**<0.001**	2.1872 ± 0.1347	**<0.001**	4.4367 ± 0.2166	**<0.001**
Inoculum source (Wet-site)	0.4807 ± 0.1617	0.00565	0.2909 ± 0.1626	0.08364	0.3805 ± 0.1382	**0.009926**	0.4573 ± 0.2223	**0.048467**
Inoculum viability (Live)	0.4641 ± 0.1617	**0.00732**	0.4256 ± 0.1609	**0.01285**	0.5119 ± 0.1368	**<0.001**	0.8060 ± 0.2200	**<0.001**
Precip. (Wet-site precip.)	0.4592 ± 0.1617	**0.00788**	0.6517 ± 0.1829	**0.00125**	0.7647 ± 0.1555	**<0.001**	0.8564 ± 0.2501	**0.001804**
Inoculum source X viability	-0.6889 ± 0.2356	**0.00642**	-0.4892 ± 0.2381	**0.04873**	-0.6093 ± 0.2024	**0.005253**	-0.8140 ± 0.3256	**0.018112**
Inoculum source X precip.	-0.2044 ± 0.2286	0.37825	-0.3088 ± 0.2343	0.19754	-0.3582 ± 0.1992	0.082202	-0.3178 ± 0.3204	0.329266
Inoculum viability X precip.	-0.3938 ± 0.2286	0.09493	-0.3709 ± 0.2274	0.11331	-0.4811 ± 0.1933	**0.018595**	-0.8284 ± 0.3109	**0.012288**
(Inoculum source X viability) X precip.	0.3800 ± 0.3283	0.25595	0.5817 ± 0.3389	0.09645	0.5513 ± 0.2881	0.065313	0.6981 ± 0.4635	0.142469

Total dry root mass	-	-	9.5340 ± 2.7941	**0.00186**	14.7536± 2.3753	**<0.001**	20.2421± 3.8207	**<0.001**
Nagelkerke's R^2	0.438		0.795		0.898		0.838	

Background ^{13}C Assessment of Drought Stress

Averaged across inoculum types, seedlings subjected to dry-site precipitation averaged a background shoot $\delta^{13}C$ level of -31.8385‰, while those subjected to wet-site precipitation averaged slightly more negative -32.5421‰. For seedlings pulse-labeled after 6 months and thus enriched in $\delta^{13}C$, mean shoot $\delta^{13}C$ values across all inoculum types were -1.9871‰ for dry-site precipitation, and 331.6459‰ for wet-site precipitation.

Predicted average background shoot $\delta^{13}C$ was -32.54‰ in wet-site precipitation plants and -32.84‰ in dry-site precipitation plants (0.70 ‰ more negative in wet-site precip.; estimate=0.021860; p= 0.00687; MAE=0.5292; Nagelkerke's R^2=0.218). Predicted average background root $\delta^{13}C$ was -30.82‰ in wet-site precipitation plants and -30.15‰ in dry-site precipitation plants (0.67‰ more negative; estimate= 0.021970; p= 0.0173; MAE=0.5853; Nagelkerke's R^2=0.174) in the unenriched wet-site precipitation regime plants than in the unenriched dry-site precipitation regime plants (Fig. 8).

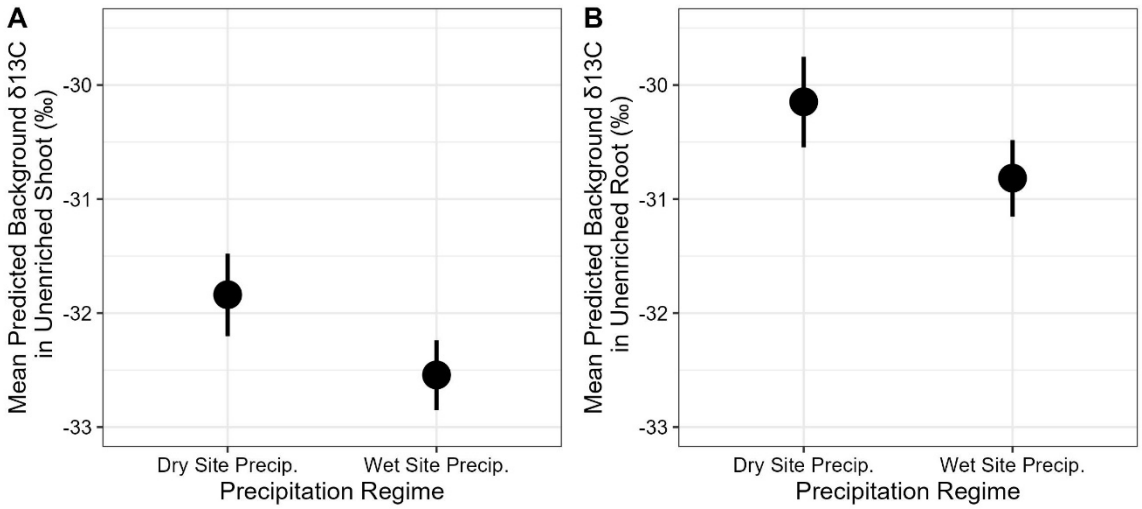

Figure 8. Means and 95% CIs for natural abundance of ^{13}C in photosynthesizing shoot tissue (A) and root tissue (B), expressed as $\delta^{13}C$ ‰ values. Points represent the $\delta^{13}C$ value by precipitation treatment averaged across all four inoculum types.

Fungal Community Structure

Taxonomic composition of the in-situ soil fungal community differed between the wet site and the dry site as evaluated from the original soil core samples (PERMANOVA, p=0.001) (Fig. 9). By the end of the greenhouse experiment, the fungal community in live inoculum-treated pots was compositionally different than sterile inoculum soils (PERMANOVA, p=0.001), and the pots treated with dry and wet-site inoculum differed from one another (PERMANOVA on subset of soil samples from live inoculum-treated cone-tainers, p=0.048) (Fig. 10). Precipitation regime did not significantly impact composition within treatments with the same live inoculum (PERMANOVA, p=0.308 for all endpoint soil samples, p=0.350 for subset of soil samples that received live inoculum).

Members of the phylum Glomeromycota, which encompasses all fungi that form arbuscular mycorrhizas, were detected in 18 of the 19 original soil cores from which fungal inocula were derived, comprising 1.02% (11,165 of 1,092,883 total) of the

unrarefied ITS reads detected in the soil cores. However, Glomeromycota were entirely absent from all root samples and all but a single endpoint soil sample (soil 46).

Observed fungal taxon richness in the rarefied ITS data from endpoint soil cores was not significantly different between any of the treatment combinations (ANOVA; $p=0.241$). ASV richness was low in all treatment combinations, ranging from a mean richness of 4.2 ASVs in sterile dry-site inoculum + wet-site precip. endpoint soil to a mean richness to 9.8 ASVs in live dry-site inoculum + wet-site precip. endpoint soil, prior to rarefaction. Thus, while the live and sterile versions of each inoculum (wet-site and dry-site) were found to differ significantly in fungal community composition, the sterile versions were equally as species-rich. See Appendix B, Fig. B.2 for details on ASV richness by treatment combination in endpoint soils.

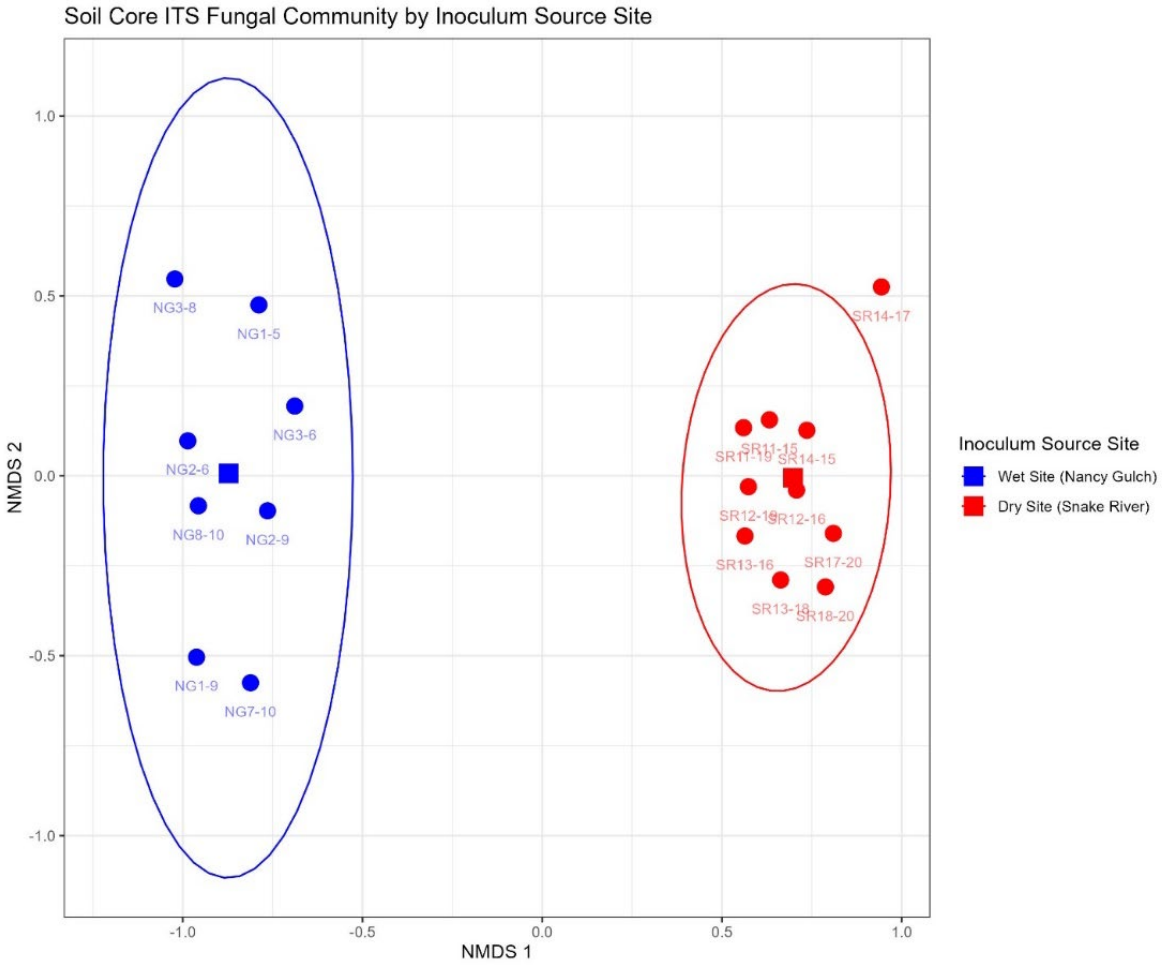

Figure 9. NMDS of weighted on Bray–Curtis dissimilarities and 95% CI normal ellipses for rarefied ITS region data of soil cores from which inoculum was derived (stress = 0.0899).

ANCOM-BC identified 36 genera and 24 fungal families as differentially abundant between the original soil cores, after FDR p-value adjustment to account for multiple comparisons ($q<0.05$) (Appendix B, Table B.1. & Table B.2.).

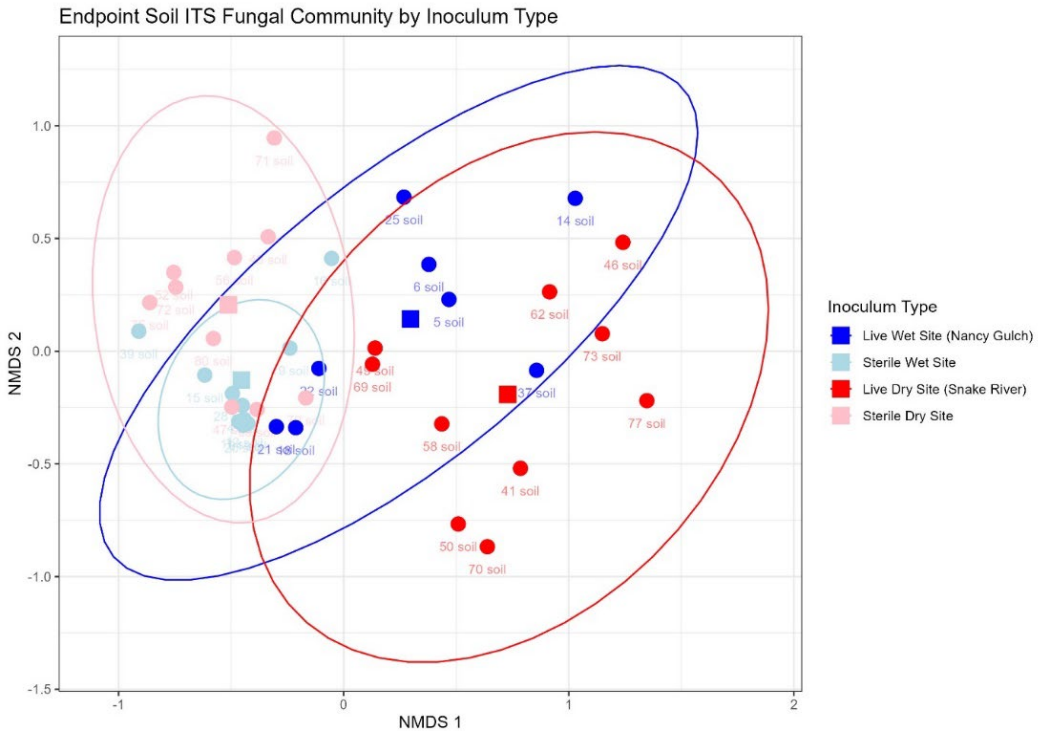

Figure 10. NMDS of weighted on Bray–Curtis dissimilarities and 95% CI normal ellipses for rarefied ITS region data of endpoint soil samples taken from the rhizosphere of seedlings at the time of harvest (stress = 0.1578).

17 fungal genera and 21 fungal families were present in at least 5% of endpoint soil samples, and thus included in all ANCOM-BC differential abundance analyses.

ANCOM-BC identified 2 families (Table 4), 3 genera (Table 5), and 3 sequence variants (Table 6) as differentially abundant in live wet-inoculated and live dry-inoculated endpoint soil samples compared to sterile endpoint soils. All fungal families and genera found to be differentially abundant in the endpoint rhizosphere soils and roots were detected at some level in the original soil cores, but not all were differentially abundant between the wet site cores and dry site cores.

Table 4. Fungal families identified by ANCOM-BC to be differentially abundant in endpoint soil between inoculum treatments. Natural log-fold-change (LFC) values and FDR-adjusted p-values (q-values) for ITS read abundances in live wet-site treatments and live dry-site inoculum treatments compared to aggregated sterile-inoculum treatments, with green indicating greater abundance and red indicating lower abundance compared to sterile treatments.

Family	Live Wet Site vs. Sterile Endpoint Soil	Live Dry Site vs. Sterile Endpoint Soil	Number of Fungal Genera in Family by Primary and Secondary Lifestyles (FungalTraits)							
			Saprotroph	Decomposer	Plant Pathogen	Non-plant Parasite (lichen,	Symbiont	Foliar Endophyte	Unknown	Total
Phaeosphaeriaceae	LFC = 2.1312 q = 0.5085	LFC = 4.9403 q = 0.0115	67	0	19	4	0	0	0	90
			42	4	4	0	0	2	-	52
Chytridiaceae	LFC = 5.4582 q = 0.0077	LFC = 5.9076 q = 0.0034	4	0	0	3	0	0	0	7
			4	1	0	0	1	0	-	6
Unknown (All taxa for which family is unknown)	LFC = 2.7096 q = 0.3567	LFC = 5.5791 q = 0.0034	NA							

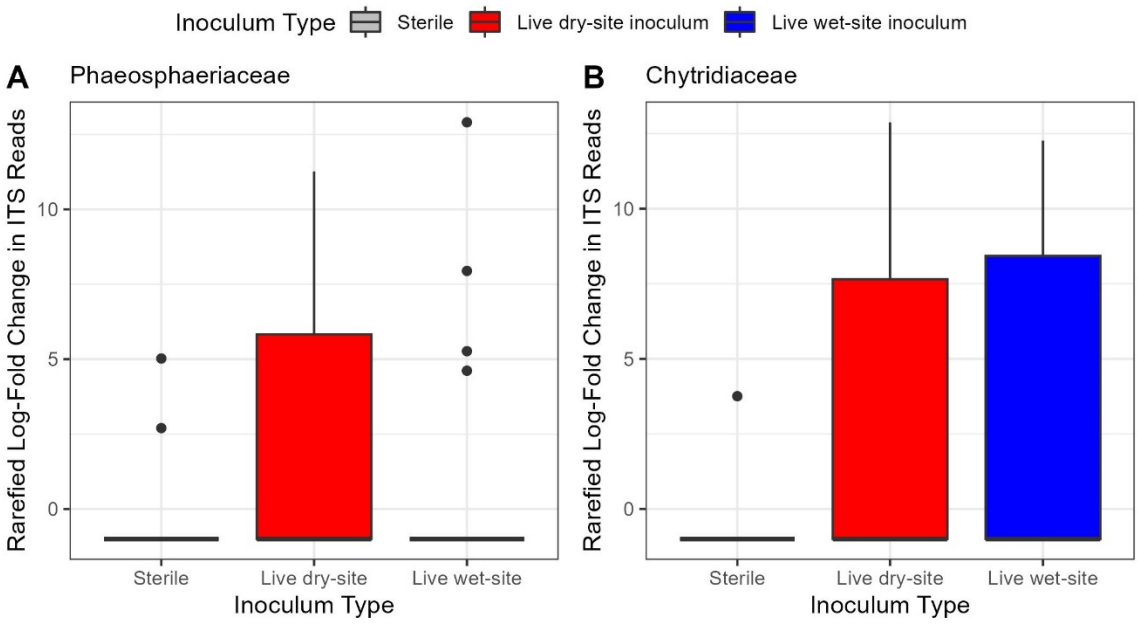

Figure 11. Regularized abundances of differentially abundant fungal families, (A) Phaeosphaeriaceae and (B) Chytridiaceae by inoculum type, sterile vs. live dry-site vs. live wet-site.

The rhizosphere of seedlings that received live wet-site inoculum and those that received live dry-site inoculum had significantly greater abundances of family Chytridiaceae (234.67 times more reads in live wet-site treatments; 367.82 times more in live dry-site treatments) and genus *Phlyctochytrium* (220.15 times more in live wet-site treatments; 217.37 times more in live dry-site treatments) in particular, compared to sterilized inoculum. In addition, live dry-site inoculum treated seedlings were additionally enriched in the family Phaeosphaeriaceae (139.81 times more) and the genus *Paraphoma* in particular (124.86 times more) (Fig. 11; Fig. 12; Fig. 13).

Table 5. Fungal genera identified by ANCOM-BC to be differentially abundant in endpoint soil between inoculum treatments. Natural log-fold-change (LFC) values and FDR-adjusted p-values (q-values) for ITS read abundances in live wet-site treatments and live dry-site inoculum treatments compared to aggregated sterile-inoculum treatments, with green indicating greater abundance and red indicating lower abundance compared to sterile treatments.

Genus	Live Wet-Site vs. Sterile Endpoint Soil	Live Dry-Site vs. Sterile Endpoint Soil	Primary Lifestyle (FungalTraits)
Paraphoma	LFC = 2.4914 q = 0.2700	LFC = 4.8272 q = 0.0033	plant pathogen / DSE
Aspergillus	LFC = -4.2551 q = 0.0911	LFC = -6.6165 q = 0.0016	unspecified saprotroph
Phlyctochytrium	LFC = 5.3943 q = 0.0071	LFC = 5.3816 q = 0.0033	algal parasite
Unknown (All taxa for which genus is unknown)	LFC = 2.7979 q = 0.2597	LFC = 5.5891 q = 0.0018	NA

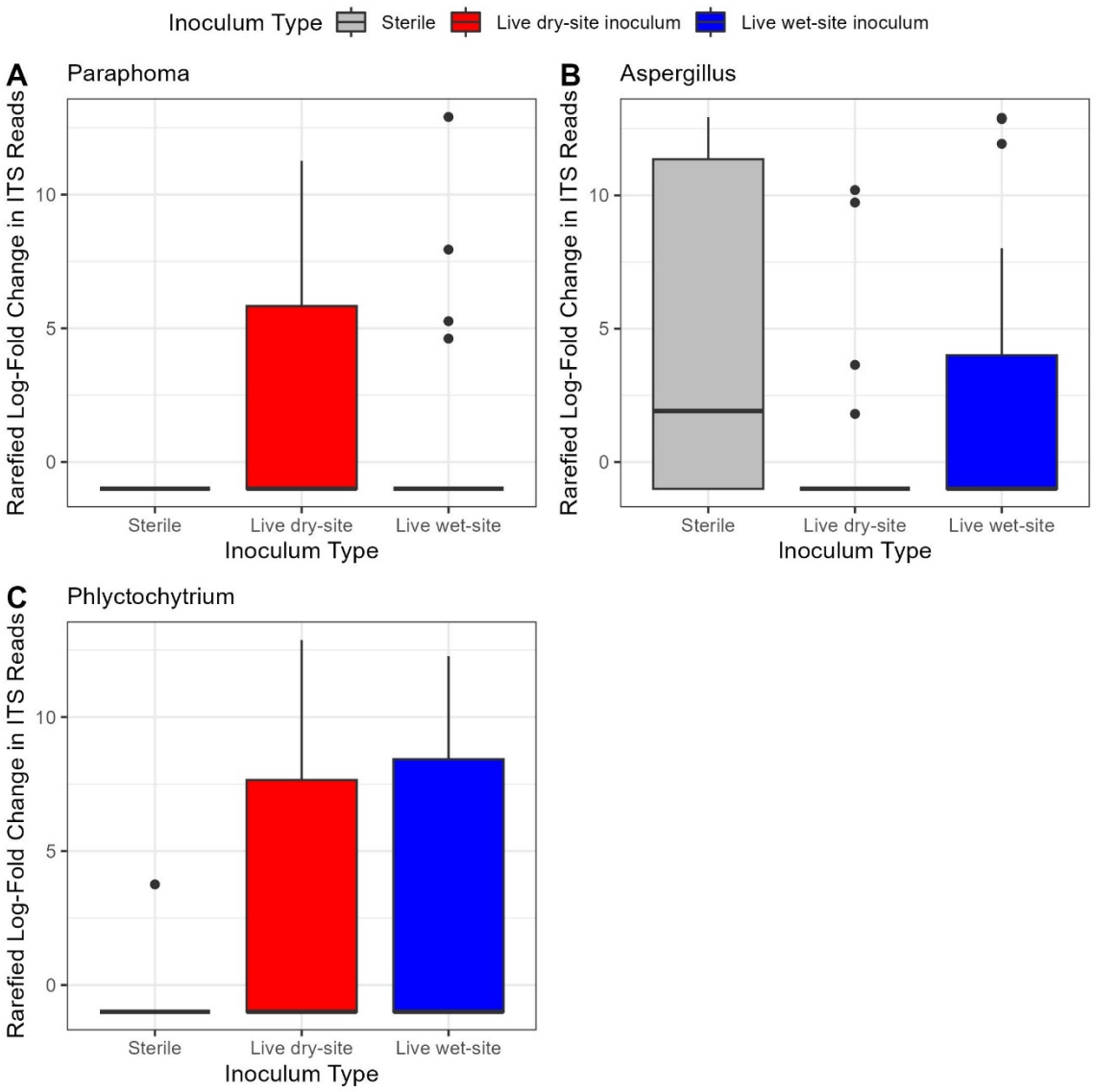

Figure 12. Regularized abundances of differentially abundant fungal genera, (A) *Paraphoma sp.*, (B) *Aspergillus sp.*, and (C) *Phlyctochytrium sp.* among inoculum treatments: live inocula from the dry site, live inocula from the wet site, and sterilized inocula.

ANCOM-BC analysis at the sequence variant level: demonstrated that two sequence variants, i0001 (*Aspergillus hiratsukae* in both UNITE and BLAST) and i0031 *(Pleurotheciella sp*. in UNITE, *Pleurotheciella saprophytica* in BLAST) were significantly less abundant in live dry-site inoculum treatments compared to sterile

treatments, potentially suggesting that the live dry-site community more effectively out-competed these potential greenhouse colonizers.

One ASV, i0033 (*Phlyctochytrium africanum* in UNITE, but *Spizellomyces acuminatus* in BLAST) was significantly more abundant in live wet-site inoculum treatments compared to sterile treatments (Table 6). *Spizellomyces acuminatus* is the currently accepted name for basionym *Phlyctochytrium acuminatum* (Barr, 1984).

Table 6. Fungal sequence variants identified by ANCOM-BC to be differentially abundant in endpoint soil between inoculum treatments. Natural log-fold-change (LFC) values and FDR-adjusted p-values (q-values) for ITS read abundances in live wet-site treatments and live dry-site inoculum treatments compared to aggregated sterile-inoculum treatments, with green indicating greater abundance and red indicating lower abundance compared to sterile treatments.

ASV	Live Wet-Site vs. Sterile Endpoint Soil	Live Dry-Site vs. Sterile Endpoint Soil
i0031 *Pleurotheciella sp*. (UNITE) *Pleurotheciella saprophytica* (BLAST)	LFC = -2.7961 q = 0.2311	LFC = -4.4488 q = 0.0224
i0001 *Aspergillus hiratsukae*	LFC = -3.9645 q = 0.1637	LFC = -8.1296 q = 0.0001
i0033 *Phlyctochytrium africanum* (UNITE) *Spizellomyces acuminatus* (BLAST)	LFC = 3.9352 q = 0.0196	LFC = -1.6109 q = 0.4039

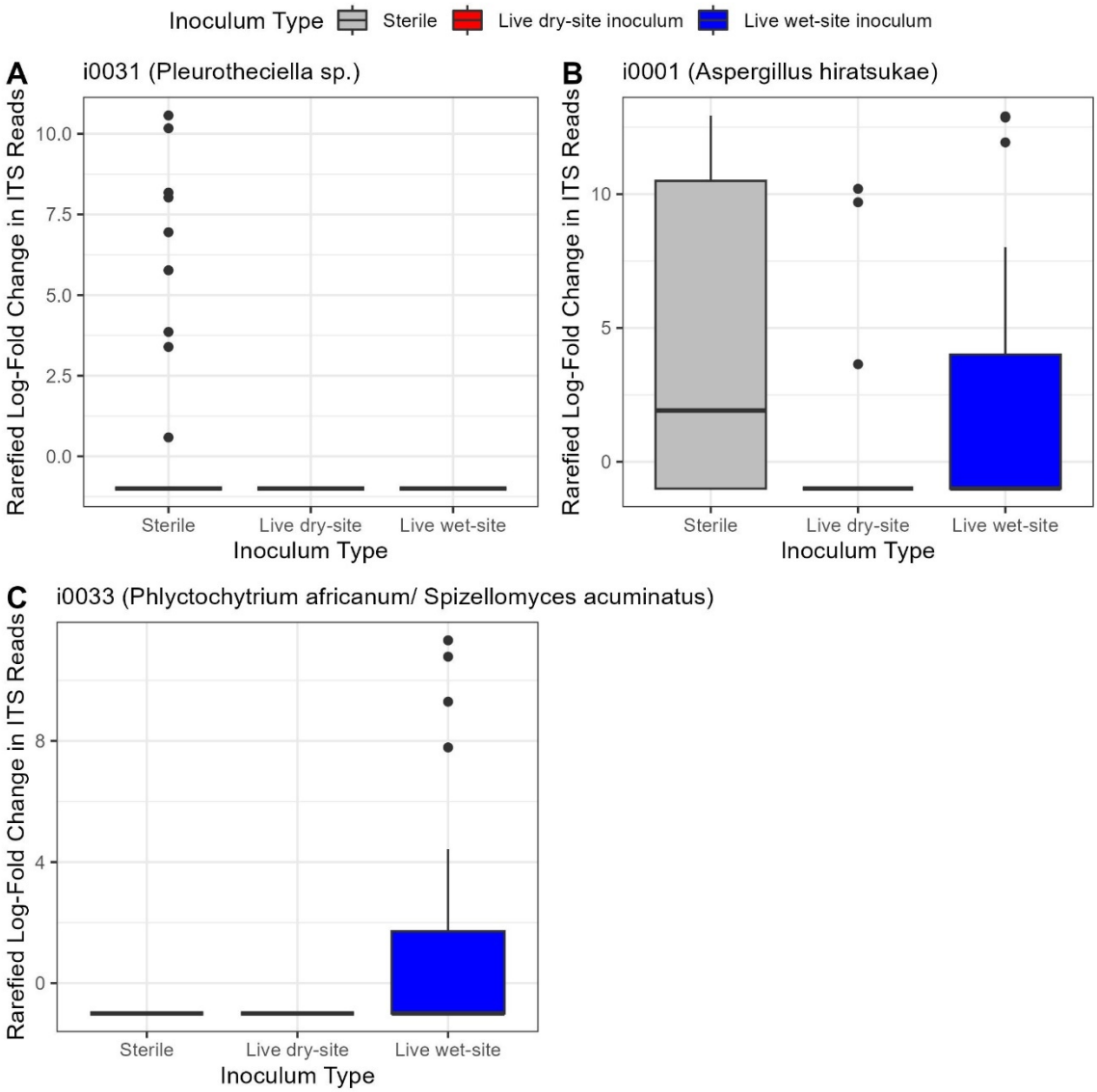

Figure 13. Regularized abundances of differentially abundant fungal sequence variants, (A) i0031 *Pleurotheciella sp.*, (B) i0001 *Aspergillus hiratsukae*, and (C) i0033 *Phlyctochytrium africanum/ Spizellomyces acuminatus* by inoculum type, sterile vs. live dry-site vs. live wet-site.

In glmms of plant responses containing the two fungal families identified as differentially abundant (Phaeosphaeriaceae and Chytridiaceae), the only significant effect of any fungal family's regularized abundance on any plant response was a slight positive effect of Chytridiaceae abundance on root biomass (estimate=0.03099; p= 0.0391), but

the low explanatory power of this model (Nagelkerke's R^2=0.089) suggests that other latent factors are present and that root biomass changes cannot be ascribed to the abundance of this family alone (Table 7).

Table 7. Parameter estimates for plant response glms as functions of regularized abundances [log2(rarefied ITS counts + 0.5)] of the two fungal families identified by ANCOM-BC as differentially abundant in endpoint soil samples.

	Aboveground Biomass			Root Biomass			SRL			Root Length <0.5mm Diam.		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	-2.85	-2.94 – -2.76	**<0.001**	-3.08	-3.19 – -2.97	**<0.001**	4.02	3.91 – 4.13	**<0.001**	5.44	5.28 – 5.61	**<0.001**
Phaeosphaeriaceae + 0 5 [log2]	0.01	-0.02 – 0.04	0.655	-0.00	-0.04 – 0.03	0.904	0.01	-0.02 – 0.03	0.486	0.01	-0.03 – 0.05	0.645
Chytridiaceae + 0 5 [log2]	0.01	-0.01 – 0.03	0.370	0.03	0.00 – 0.06	**0.035**	-0.01	-0.03 – 0.01	0.545	0.02	-0.01 – 0.05	0.287
Observations	71			71			38			38		
R^2 Nagelkerke	0.027			0.089			0.015			0.060		

The genus *Aspergillus* was determined to likely have colonized pots over the course of growth in the greenhouse, rather than being intentionally added via inoculation, as it was significantly more abundant in sterile inoculum treatments. Models of plant responses as functions of the regularized abundances of *Paraphoma*, *Phlyctochytrium*, and *Aspergillus* generally did not reveal any significant effects of the abundances of these genera (Table 8). While there was a statistically detectable positive effect of *Phlyctochytrium* abundance on dry root biomass, explanatory power was poor for this model (Nagelkerke's R^2=0.091) so we hesitate to ascribe biological significance to the apparent response of root biomass the presence of this taxon individually.

Table 8. Parameter estimates for plant response glms as functions of regularized abundances [log2(rarefied ITS counts + 0.5)] of fungal genera identified by ANCOM-BC as differentially abundant in endpoint soil samples.

	Aboveground Biomass			Root Biomass			SRL			Root Length <0.5mm Diam.		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	-2.85	-2.95 – -2.75	**<0.001**	-3.09	-3.22 – -2.96	**<0.001**	3.96	3.76 – 4.17	**<0.001**	5.56	5.27 – 5.87	**<0.001**
Paraphoma + 0 5 [log2]	0.01	-0.02 – 0.04	0.677	-0.00	-0.04 – 0.03	0.817	0.01	-0.01 – 0.04	0.408	0.00	-0.04 – 0.04	0.988
Phlyctochytrium + 0 5 [log2]	0.01	-0.01 – 0.03	0.388	0.03	0.00 – 0.06	**0.037**	-0.00	-0.02 – 0.02	0.730	0.01	-0.02 – 0.05	0.400
Aspergillus + 0 5 [log2]	0.00	-0.01 – 0.02	0.982	0.00	-0.02 – 0.02	0.795	0.01	-0.01 – 0.03	0.469	-0.01	-0.04 – 0.02	0.393
Observations	71			71			38			38		
R^2 Nagelkerke	0.027			0.091			0.027			0.078		

Root Colonization

Parameter estimates and p-values for generalized linear models of root colonization are summarized in Table 9.

DNA analysis demonstrated that the fungal community in the soil and roots of seedlings at the end of the greenhouse experiment significantly differed in composition between the two live-inoculum treatments and between live and sterile treatments. However, observable root colonization did not reflect this difference, consistent with the absence of AMF DNA in endpoint rhizosphere soil and root samples (Table 9).

The precipitation regime simulated in the greenhouse significantly affected total root colonization by all fungal classes combined (thin non-septate fungi, dark septate endophytic fungal hyphae and sclerotia, and hyaline septate fungi, with colonization under dry-site conditions higher than under wet-site conditions (estimate=-2.7840; p=0.019; MAE= 0.0946; marginal R^2=0.308; conditional R^2=0.539). Unexpectedly, whether seedlings received live or sterilized versions of the fungal inoculum had no significant effect on total root colonization (p=0.078; Table 9). For plants inoculated with wet-site inoculum, total colonization was higher in live treatments (though not

significantly) in both dry-site precipitation and wet-site precipitation treatments. However, for plants given dry-site inoculum plants, total colonization was similar for live and sterile treatments in wet-site conditions, and higher in sterile than live treatments in dry-site conditions (Fig. 14).

Since DSE can produce non-pigmented hyaline septate hyphae as well as melanized hyphae and sclerotia, (Mandyam & Jumpponen, 2005), it is not possible to distinctly divide DSE and hyaline-septate colonization, justifying the analysis of colonization by all septate fungi combined (dark septate hyphae + dark septate sclerotia + hyaline septate fungi). When these were combined, inoculum viability had a significant effect on septate colonization, with live-inoculum plants unexpectedly colonized less by septate fungi than sterile-inoculum plants (estimate= -2.7919; p=0.0444; marginal R^2=0.392; conditional R^2=0.652). As with total colonization, septate colonization was also decreased by wet-site precipitation treatment compared to dry-site precipitation (estimate=-3.6280; p=0.0175).

No factors (inoculum source site, inoculum viability, or precipitation regime) were found to have significant effects on colonization by thin non-septate fungi.

Table 9. Parameter estimates from root colonization generalized linear mixed models.

	Total Colonization			Septate Colonization		
Predictors	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*
(Intercept)	-3.07	-4.39 – -1.74	**<0.001**	-3.20	-4.76 – -1.64	**<0.001**
amf source site [1]	1.13	-0.64 – 2.91	0.211	1.16	-0.96 – 3.27	0.283
amf viability [1]	-1.91	-4.04 – 0.22	0.078	-2.79	-5.51 – -0.07	**0.044**
precip [1]	-2.78	-5.10 – -0.46	**0.019**	-3.63	-6.62 – -0.64	**0.017**
amf source site [1] × amf viability [1]	2.13	-0.58 – 4.84	0.123	2.86	-0.53 – 6.24	0.098
amf source site [1] × precip [1]	0.65	-2.28 – 3.58	0.665	0.11	-3.69 – 3.91	0.955
amf viability [1] × precip [1]	2.16	-1.18 – 5.50	0.206	2.59	-1.93 – 7.11	0.262
(amf source site [1] × amf viability [1]) × precip [1]	-1.57	-5.76 – 2.62	0.462	-0.77	-6.30 – 4.76	0.785
Random Effects						
σ^2	3.29			3.29		
τ_{00}	1.65_{ID}			2.46_{ID}		
ICC	0.33			0.43		
N	40_{ID}			40_{ID}		
Observations	2895			2895		
Marginal R^2 / Conditional R^2	0.308 / 0.539			0.392 / 0.652		

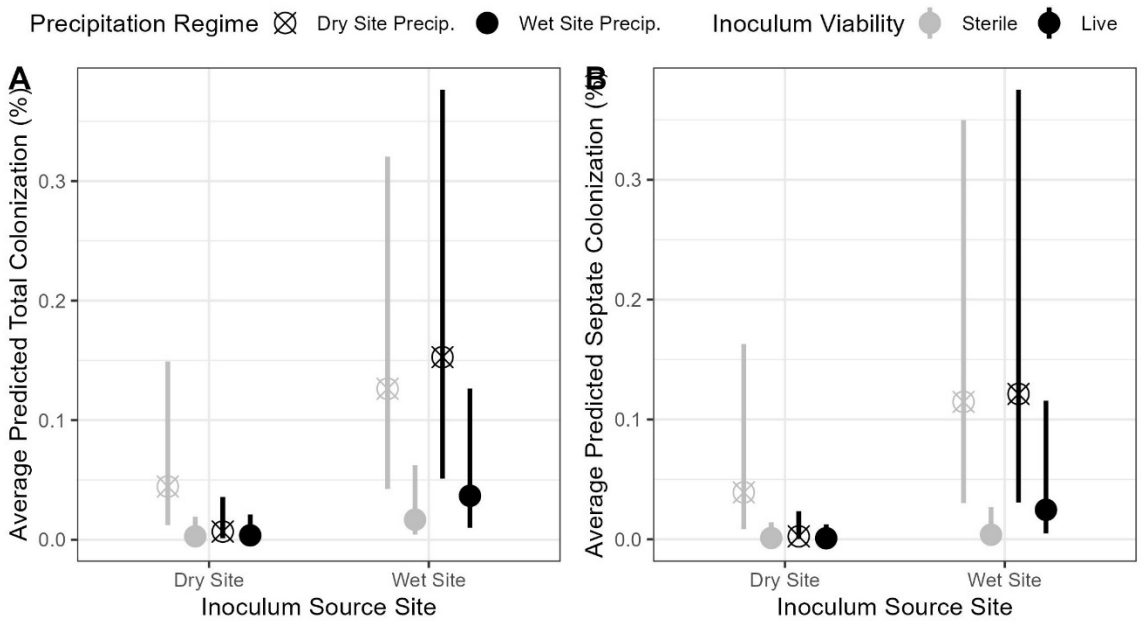

Figure 14. Probability of (A) root colonization by all fungal classes and (B) root colonization by septate fungi, predicted by glmms with variable intercept for slide identity.

DISCUSSION

This study initially aimed to assess the influence of AMF on sagebrush. However, absence of AMF in the rhizosphere at the termination of the experiment prohibited assessment of the impact of this group specifically. Fungal taxonomic richness was lower and fewer taxa were differentially abundant between treatments in endpoint soils than in the soil cores taken from the in-situ rhizosphere of the mature Wyoming big sagebrush at the two sites. Nonetheless, communities differed throughout the experiment. This allowed me to test the question of how the fungal community composition between sites impacted sagebrush growth under different precipitation regimes. Differences in fungal assemblages in soil of live-inoculum treated cone-tainers at the end of the greenhouse experiment were driven by the site from which the inoculum was derived, with the type of precipitation regime simulated in the greenhouse having no significant impact on composition within the same live inoculum treatment group. In endpoint soils, fungi of *Phlyctochytrium sp.* (and family Chytridiaceae) were more abundant in live inoculum treatments from both sites compared to sterile treatments, with the dark septate endophytic genus *Paraphoma sp*. (and family Phaeosphaeriaceae) also enriched in live dry-site inoculum treatments. I confirmed that biologically meaningful different precipitation treatments were applied in my greenhouse study, as dry-site precipitation significantly altered seedling physiology, evident in significant differences in $\delta^{13}C$ in

shoot and root tissue between precipitation treatment groups that indicated lower photosynthetic rate in dry-site precipitation plants.

Data showed that 1) association with live fungal inoculum enhanced sagebrush seedling biomass when the precipitation regime reflected the home site of the inoculum; 2) live dry-site fungal inoculum produced longer, finer roots under dry-site conditions despite sagebrush seed being sourced from the wet-site. In both cases, live inoculum had an impact on growth and morphology, but was only helpful to the sagebrush if the soil moisture conditions matched the inoculum's home site conditions in the greenhouse study. Both findings reflect that the positive impacts of fungal inoculum on sagebrush growth and morphology are contingent on a match between the inoculum and the soil moisture conditions under which it evolved. Thus, results suggest that seedlings grown with microbial communities adapted to the local climate may be better able to optimize water uptake and maintain productivity under drought conditions.

Plant responses to fungal inocula were contingent on precipitation regime

$\delta^{13}C$ analysis confirmed that the two precipitation regimes simulated did effectively impose biologically meaningful differences in the level of water-stress experienced by the seedlings in this experiment. Documented changes in $\delta^{13}C$ signatures as a result of isotopic fractionation in plant tissue samples can be very small, with leaf $\delta^{13}C$ in big sagebrush following 20 years of growth under different seasonal irrigation regimes differing by approximately 1 - 2.5 ‰ (Germino & Reinhardt, 2014). Thus, the small estimates for the effect size of precipitation on $\delta^{13}C$ content in the plants grown for

only 6 months still represent a biologically significant impact on plant physiology between precipitation regimes.

Both biomass and root morphology metrics were assessed to characterize slightly different aspects of seedling responses. Biomass was evaluated as a proxy of resource allocation to root and shoots, while root morphology variables were considered as plant responses to stress and/or microbial chemical signals. Sagebrush seedling shoot and root biomass were increased with live inoculation compared to sterile controls, but only under the precipitation conditions that reflected the inoculum's source site. This result is consistent with microbial community adaptation to local soil moisture conditions that maximizes benefits to its host plant under those conditions (Rúa et al., 2016). While, under wet-site conditions, total biomass was increased by live wet-site inoculum compared to its sterile counterpart, there was a relatively greater allocation of biomass to roots vs. shoots in this treatment, evident in a higher root:shoot ratio. This suggests wet-adapted inoculum prompted seedlings to allocate relatively more resources to root development when moisture was relatively abundant. The investment in greater root biomass relative to shoot biomass by plants that received live wet-site inoculum under wet-site conditions is consistent with the life history strategy of Wyoming big sagebrush, a slow growing dryland subspecies which invests resources preferentially in rapid long root growth to withstand long dry periods in xeric sites where basin and mountain big sagebrush cannot survive (Welch & Jacobson, 1988). It is reasonable to expect that sagebrush plants with greater available resources would thus be larger overall, but still invest more resources in root growth compared to shoot growth. The lack of a similar positive effect of live dry-site inoculum on total, shoot, or root biomass under the wet-site

simulated precipitation compared to sterile inoculum could suggest that live wet-site soil symbionts are more effective in conveying water and nutrients to their plant hosts when moisture is relatively abundant than dry-adapted soil microbiota are.

Root morphology and architecture govern plant resource acquisition and mediate interactions between plants and the rhizosphere microbiome (Herms et al., 2022). Therefore, evaluating the responses of sagebrush root structure (specific root length and diameter-stratified root length) to inoculum treatments under differing precipitation regimes is critically important to understand the likelihood of successful seedling establishment. Under greater water stress, live dry-site inoculum produced seedlings with higher root surface area and more fine roots, which have a higher surface area to volume ratio with more branches. It may be the case that the consortium of fungal and unevaluated bacterial taxa in the dry-site inoculum induce root morphological changes under the dry conditions to which they are adapted. Soil microorganisms interact with plants through the production and exchange of chemical signals in the rhizosphere, either in the form of volatiles or solutes in the soil solution (Splivallo et al., 2009). While most literature regarding fungal-induced changes on root architecture focus on AMF's ability to induce more profuse root branching (Berta et al., 1993; Cosme & Wurst, 2013; Gamalero et al., 2002; Hooker et al., 1992), other fungal groups have been found to affect root morphogenesis as well (Splivallo et al., 2009).

High SRL is considered a drought avoidance trait thought to optimize water uptake (Garbowski et al., 2020), in contrast with drought tolerance strategies such as investment in greater root biomass generally (Zhou et al., 2018). Meta-analysis has shown that drought is associated with significantly decreased SRL and overall root length

(Zhou et al., 2018). Such results could suggest that the sagebrush seedlings in my experiment subjected to the dry-site precipitation regime experienced relatively less drought stress when provided microbial associates that persist/occur naturally under those environmental conditions, as they had significantly higher SRL. This would be consistent with local adaptation of the microbial community to local environmental conditions, in which soil symbionts are better able to convey water and nutrients to their plant hosts under the soil moisture availability with which the microbes evolved. However, the direction of causation cannot be assigned. Mycorrhizal fungi often preferentially inhabit lower order roots, so sagebrush seedlings grown with microbial communities adapted to the local climate may have improved opportunities to form beneficial symbioses with these fungi.

Seedling rhizosphere communities differed in abundance of dark septate endophytes and chytrids, and were unaffected by simulated precipitation regime

While our two climatically disparate Wyoming big sagebrush-dominated sites hosted significantly different rhizosphere fungal communities (Fig. 10), the endpoint rhizosphere soil samples were less taxonomically rich than the original soil cores (Appendix B: Fig. B.1 & Fig. B.2). This was intentional to some degree because the goal was to isolate AMF spores for inoculation, but contrary to treatment expectations, arbuscular mycorrhizal fungal DNA was absent from all seedling root samples and all but one endpoint soil sampled at time of harvest. Low levels of AMF DNA (i.e., phylum Glomeromycota) were detected in the original soil cores from which inocula were derived, suggesting that either the AMF spores were lost during the process of spore

isolation from the soil, or that applied spores failed to germinate and colonize roots, possibly due to spore mortality during an extreme summer heat event (as ambient outdoor temperatures reached approximately 42 °C for several days approximately 2 weeks after seeding, and incubation experiments have documented cessation of fungal growth above 45 °C (Bárcenas-Moreno et al., 2009)).

The absence of AMF at the end of this experiment may also have been caused by antagonistic interactions with other soil microorganisms. Prior studies have demonstrated that *Alternaria alternata*, a generally saprophytic but opportunistically pathogenic fungus that was found to be present in endpoint rhizosphere soil in this experiment, produced soluble and volatile substances that inhibited the germination of spores of the arbuscular mycorrhizal fungus *Glomus mosseae* (McAllister et al., 1996). This saprophyte decreased AMF root colonization and metabolic activity both in plants inoculated with spores (McAllister et al., 1996) and in soil in the absence of a plant, suggesting a direct interaction between the two fungi independent of the plant host (McAllister et al., 1997). Additionally, fungi in the genus *Phlyctochytrium*, also present in endpoint soil, hyperparasitize AMF spores (Daniels & Menge, 1980). Though a speculative suggestion, such interactions between fungi could be a contributing factor to the absence of AMF from the rhizosphere soil at the end of the greenhouse experiment. Poor colonization by arbuscular mycorrhizal fungi may pose a challenge to improving restoration efforts via inoculation with AMF spores alone, as the germination of spores has been shown to be slowed or inhibited by soil salinity and exudates and volatile compounds produced by some plants and soil saprophytic fungi (Cantor et al., 2011; Delvian & Rambey, 2019; McAllister et al., 1996).

Though specific fungal taxa were present in significantly different abundances between live dry-site inoculum, live wet-site inoculum, and sterile-inoculum treated endpoint soils, all treatments' endpoint rhizospheres were equally species-rich. This finding means that cone-tainers given sterile inocula were likely colonized by ambient fungi in the greenhouse environment. No differential diversity treatment was applied so plant responses observed as a result of live vs. sterile inoculum from the same source site were likely driven by the particular consortium of fungal species in the live inoculum.

The differential abundance analysis conducted here could detect differences only down to the ASV level, as this was the finest taxonomic resolution at which fungi could be identified with the 5.8S-Fun/ ITS4-Fun primer set and the UNITE database. Potentially, local selection pressures could result in sub-ASV level genetic variation between populations of the same fungal taxon from different inoculum source sites (Yung et al., 2015). Though such variation could manifest in different physiological impacts on sagebrush, it would not be identified by ANCOM-BC differential abundance analysis. Additionally, ITS sequence data characterizes all fungal DNA present in the soil, regardless of whether the taxa it belongs to are viable and metabolically active. Consequently, DNA that was not destroyed by the autoclaving process in the sterile inoculum treated endpoint soils could have been sequenced, overrepresenting the presence of viable taxa in the sterile treatments and obscuring their enrichment in live inoculum treatments.

Live inocula were distinguished from sterile inocula by dark septate endophytes and chytrids. As *Phlyctochytrium* belongs to the Chytridiaceae family and the effect sizes and p-values for the effects of this genus and family on root biomass were nearly

identical, the genus *Phlyctochytrium* is the likely driver of the differential abundance of Chytridiaceae and the effect of this family on root biomass. The genus *Paraphoma* belongs to the Phaeosphaeriaceae family, so this genus is likely the primary driver of the differential abundance of this family.

Functional types of differentially abundant fungal genera were appraised to identify potentially mechanistic explanations for observed plant responses. *Spizellomyces*/*Phlyctochytrium sp.,* present in significantly greater abundance in endpoint soils given live inoculum from either source site, are classified as parasites of algae by FungalTraits, and are documented parasites of nematodes and oospores of other fungi, in addition to their saprotrophic role (Barelli et al., 2020; Daniels & Menge, 1980). We are not aware of any studies documenting direct effects of *Spizellomyces*/*Phlyctochytrium sp.* on plant responses, but their role as antagonists of potential plant pathogens (Barelli et al., 2020) may have resulted in the suppression of harmful soil microorganisms in live inoculum treatments, to the benefit of the host sagebrush seedlings.

The genus *Paraphoma*, present in significantly greater abundance in live dry-site inoculum endpoint soil samples, has been classified as containing dark septate endophytic (DSE) fungi (Andrade-Linares & Franken, 2013; Santos et al., 2021). The FungalTraits database classifies *Paraphoma sp.* as primarily plant pathogens and secondarily saprotrophs, but the functional role of the polyphyletic group of DSE taxa is poorly understood (Almario et al., 2017; Santos et al., 2021). Calls for more research on root-associated DSE have been made (Newsham, 2011) because they can impact physiological traits of host plants, particularly in mycorrhizae-poor soils (Andrade-Linares & Franken, 2013; Hughes et al., 2020; Porras-Alfaro & Bayman, 2011).

Altered patterns of plant shoot and root growth, enhanced seed germination, adventitious root formation, both improved and hindered resistance to abiotic stresses, and various modifications to plant biochemistry and soil chemistry have been documented in response to DSE presence in the soil or DSE root colonization (Andrade-Linares & Franken, 2013; Hou et al., 2021). For example, a study of the effect of dark septate endophytes on plant performance of *Artemisia ordosica* under varying salt stress found that inoculation with *Alternaria chlamydosporigena* or with *Paraphoma chrysanthemicola* promoted root growth (root length, root volume and root surface area) under unsalted conditions and mild salt stress, and *Paraphoma* continued to have this effect under severe salt stress (Hou et al., 2021). Inoculation with either DSE species also increased root biomass and root:shoot biomass ratio under unsalted conditions, with *Paraphoma* again continuing to have this effect under mild and severe salt stress (Hou et al., 2021). While drought stress cannot be equated with salt stress, this study demonstrated that the DSE genus enriched in live dry-site inoculated endpoint soils in this greenhouse experiment has substantial impacts on the physiological root characteristics of *Artemisia sp.* As DSE exhibit low host specificity and have been isolated from both mycorrhizal and non-mycorrhizal plant roots across a wide range of ecosystem types (Mandyam & Jumpponen, 2005; Santos et al., 2021), it is probable that *Paraphoma sp.* would also interact with North American species and subspecies of *Artemisia*. Though not statistically significant, enrichment of this DSE genus in live dry-site inoculated treatments may have contributed to the altered root morphology observed in seedlings that received live dry-site inoculum and dry-site precipitation.

The impacts of the abundance of any one fungal genus on plant responses may be obfuscated by potential contingency on the presence of one or more others due to interactions between microbes, as has been found in some prior studies. For instance, one field study demonstrated that administration of a core consortium of five species of rhizosphere bacteria, but not individual member species, significantly reduced seedling mortality from a pathogenic fungus, *Alternaria sp.* (Santhanam et al., 2015). Thus, the relationship between a particular fungal genus and a host sagebrush seedling may span a spectrum from pathogenic to commensalistic to mutualistic, based not only on environmental conditions, but also on the presence or absence of other soil microbes.

The majority of studies on synergistic effects of plant growth promoting microorganisms on plant responses have focused on pairs or consortia of rhizosphere-colonizing bacteria and root-colonizing fungi (largely AMF and fungi in the genus *Trichoderma*) (Santoyo et al., 2021). A meta-analysis of such studies identified that the synergistic effect on plant growth depends on the consortia microorganisms present, the genotype of the host plant, and soil nutrient composition (Santoyo et al., 2021). The context-dependency of microbial interactions requires that further research be conducted to identify whether pairs/consortia of fungi have growth-promoting effects on sagebrush specifically.

While DNA analysis demonstrated that the fungal community in the soil and roots of seedlings at the end of the greenhouse experiment significantly differed in composition, observable root colonization by stainable hyphae did not reflect this difference. DSE made up the majority of fungal structures observed on sagebrush roots under light microscopy, but DSE colonization may be even more abundant than reported

here, as numerous studies (Haselwandter & Read,1982; Newsham, 1999; Yu et al., 2001) have documented the formation of non-melanized, hyaline hyphae that were continuous with melanized hyphae and were thus clearly produced by the same DSE fungus (Mandyam & Jumpponen, 2005). While I assessed colonization by all septate fungi collectively, melanized or not, to try to address this uncertainty, some of these prior studies have found hyaline hyphae at high prevalence in roots that were not visible with ordinary light microscopy or staining, likely due to "poor chitinization or poor development of the fungal cell wall during host colonization" (Barrow & Aaltonen, 2001) and consequently low affinity for chitin targeting stains (Mandyam & Jumpponen, 2005). The production of poor-staining, non-melanized hyphae by DSE likely results in an underestimation of actual abundance of DSE when assessing root colonization visually based on observation of melanized hyphae and sclerotia. Difficulty in quantifying DSE colonization visually may be partially responsible for the apparent lack of a correlation between root colonization by DSE/septate fungi and plant growth responses in this experiment as well as in prior studies (Berthelot et al., 2017; Hughes et al., 2020). Alternatively, it has been proposed that DSE indirectly influence plant growth by degrading organic matter and accelerating the mineralization of organic compounds, increasing the solubility of minerals in the rhizosphere (Newsham, 2011), or through the production of secondary metabolites such as antimicrobial compounds or plant hormones that affect plant biomass and morphology (Andrade-Linares & Franken, 2013; Hughes et al., 2020).

The absence of a statistically significant effect of visible root colonization or of the regularized abundance of any individual differentially abundant fungal taxon (see

Tables 7 & 8), on shoot biomass, root:shoot ratio, SRL, or fine root length, may be the result of limited replication and, therefore, statistical power. Further, at both the genus level and family level, fungi of unknown taxonomy were differentially abundant in endpoint soil samples, demonstrating that our knowledge of the soil fungal community composition is incomplete; unidentified individual fungi or consortia may have induced observed plant responses. Reciprocal transplant experiments in the field or in larger pots could potentially yield different results, as larger soil volumes containing larger pools of water and mineral nutrients would increase the resources that root-associated fungi could transfer to host plants.

CONCLUSIONS

Fungal community composition in the rhizosphere of Wyoming big sagebrush differed markedly between the two inoculum source sites. Application of live fungal inoculum appeared to only impact Wyoming big sagebrush biomass and root morphology when grown under a precipitation regime that simulated that of the inoculum source site, suggesting local adaptation of the soil microbial community to environmental conditions. Thus, while soil chemistry may be a latent variable affecting fungal community assemblage, differences in mean annual precipitation and temperature at the two sites are likely drivers of soil fungal community structure in the sagebrush rhizosphere.

The soil microbiomes of seedlings at the end of the greenhouse experiment, while still significantly different in fungal composition, featured fewer genera that differed in abundance than did the soil cores between the two sites, and rarefaction curves of ITS read data demonstrated that the cores were far more taxonomically rich than the endpoint soil samples. Both suggest the loss of fungal richness in the preparation of inoculum or during the greenhouse experiment and some degree of convergence in the fungal communities in this study.

Small fine root diameters and high specific root length are both root traits associated with maintaining plant productivity under drought by optimizing water uptake. The effect of environmental conditions on the phenotypic responses of this sagebrush population was dependent on the composition of the soil microbial community.

Enhancement of sagebrush biomass and advantageous root characteristics by association with precipitation regime-adapted fungi suggest that applying local-environmentally-adapted microbial inoculum from the site of intended restoration could potentially help improve sagebrush establishment.

As seeds from the dry site were not also grown in this study to evaluate whether dry-site seed germination would benefit from live dry-site inoculum and suffer with wet-site inoculum, we cannot estimate whether sagebrush seeds are adapted to the local soil fungal community based on these results. However, future studies could assess this possibility with a full-factorial experiment. While the small number of rhizosphere soil samples in which each fungal family or genus was detected prohibited identification of plant morphological responses to the presence or abundance of particular taxa, there was a clear effect of inoculation with distinctly different live microbial consortia from the two source sites on shoot and root growth and morphology. Further research is needed to clarify the taxonomy and mechanism(s) of the wide range of relatively distantly related non-mycorrhizal fungi that have been documented to impact plant physiology. Such research could also reveal particular fungal taxa or coalitions of fungi and/or bacteria that play key roles in affecting sagebrush growth and morphology.

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APPENDIX A

Experimental Design Supplementary Material

Table A.1. Soil core sample pairing regime to produce 20 unique combinations of two core samples from which microbial inocula were derived. NG = Nancy Gulch (wet site); SR = Snake River (dry site)

1.	½ volume of NG shrub #2 cores & ½ volume of NG shrub #6 cores (NG2&6)	11.	½ volume of SR shrub #12 cores & ½ volume of SR shrub #16 cores (SR12&16)
2.	½ volume of NG shrub #1 cores & ½ volume of NG shrub #9 cores (NG1&9)	12.	½ volume of SR shrub #11 cores & ½ volume of SR shrub #19 cores (SR11&19)
3.	½ volume of NG shrub #4 cores & ½ volume of NG shrub #5 cores (NG4&5)	13.	½ volume of SR shrub #14 cores & ½ volume of SR shrub #15 cores (SR14&15)
4.	½ volume of NG shrub #7 cores & ½ volume of NG shrub #10 cores (NG7&10)	14.	½ volume of SR shrub #17 cores & ½ volume of SR shrub #20 cores (SR17&20)
5.	½ volume of NG shrub #3 cores & ½ volume of NG shrub #8 cores (NG3&8)	15.	½ volume of SR shrub #13 cores & ½ volume of SR shrub #18 cores (SR13&18)
6.	½ volume of NG shrub #4 cores & ½ volume of NG shrub #7 cores (NG4&7)	16.	½ volume of SR shrub #14 cores & ½ volume of SR shrub #17 cores (SR14&17)
7.	½ volume of NG shrub #2 cores & ½ volume of NG shrub #9 cores (NG2&9)	17.	½ volume of SR shrub #12 cores & ½ volume of SR shrub #19 cores (SR12&19)
8.	½ volume of NG shrub #8 cores & ½ volume of NG shrub #10 cores (NG8&10)	18.	½ volume of SR shrub #18 cores & ½ volume of SR shrub #20 cores (SR18&20)
9.	½ volume of NG shrub #1 cores & ½ volume of NG shrub #5 cores (NG1&5)	19.	½ volume of SR shrub #11 cores & ½ volume of SR shrub #15 cores (SR11&15)
10.	½ volume of NG shrub #3 cores & ½ volume of NG shrub #6 cores (NG3&6)	20.	½ volume of SR shrub #13 cores & ½ volume of SR shrub #16 cores (SR13&16)

APPENDIX B

Fungal Community Supplementary Figures & Tables

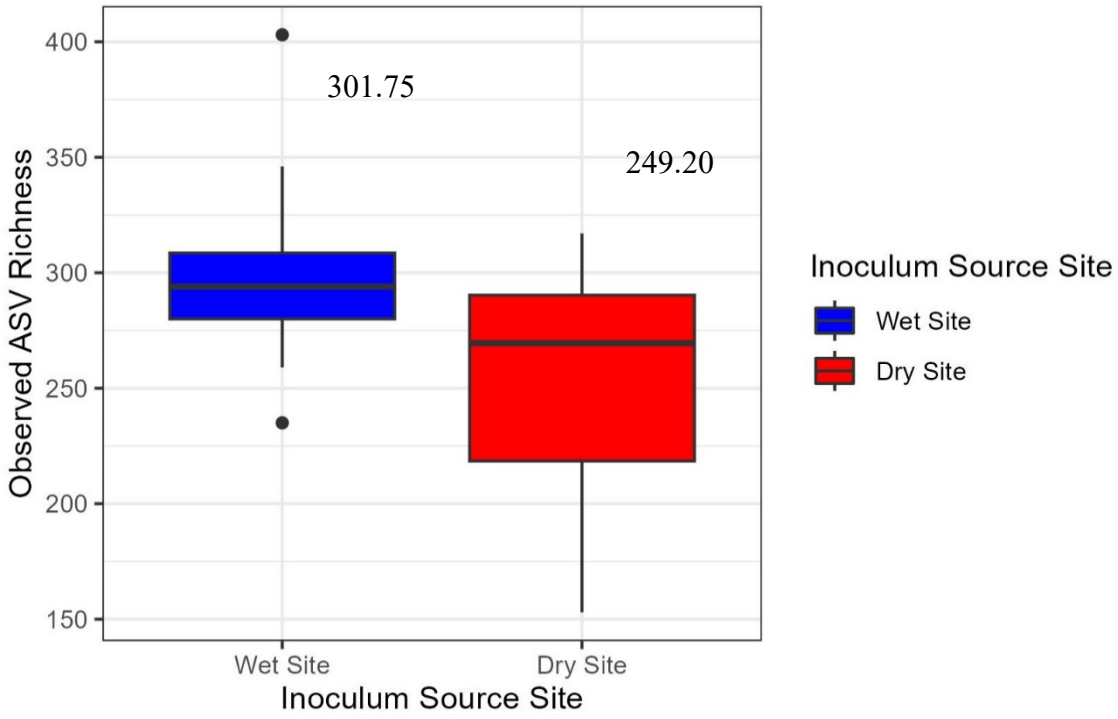

Figure B.1. Observed fungal ITS sequence variant richness in unrarefied soil core samples by source site. Mean ASV richness: 301.75 ASVs in wet-site soil core samples, 249.20 ASVs in dry-site soil core samples.

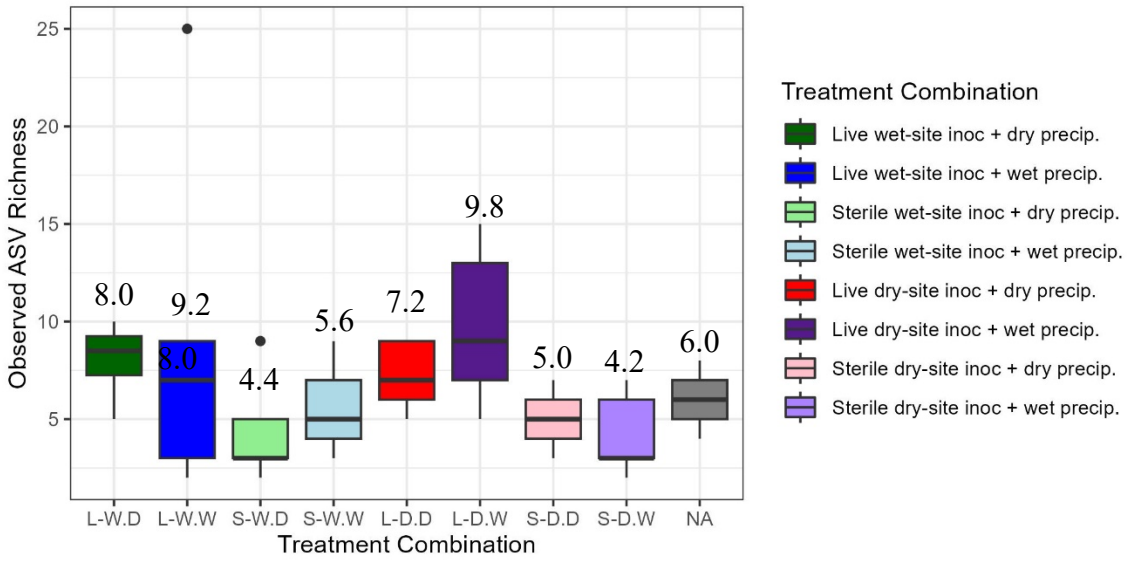

Figure B.2. Observed fungal ITS sequence variant richness in unrarefied endpoint soil samples by treatment combination. Mean ASV richness: L-W.D=8.0, L-W.W=9.2, S-W.D=4.4, S-W.W=5.6, L-D.D=7.2, L-D.W=9.8, S-D.D.=5.0, S-D.W=4.2, NA=6.0

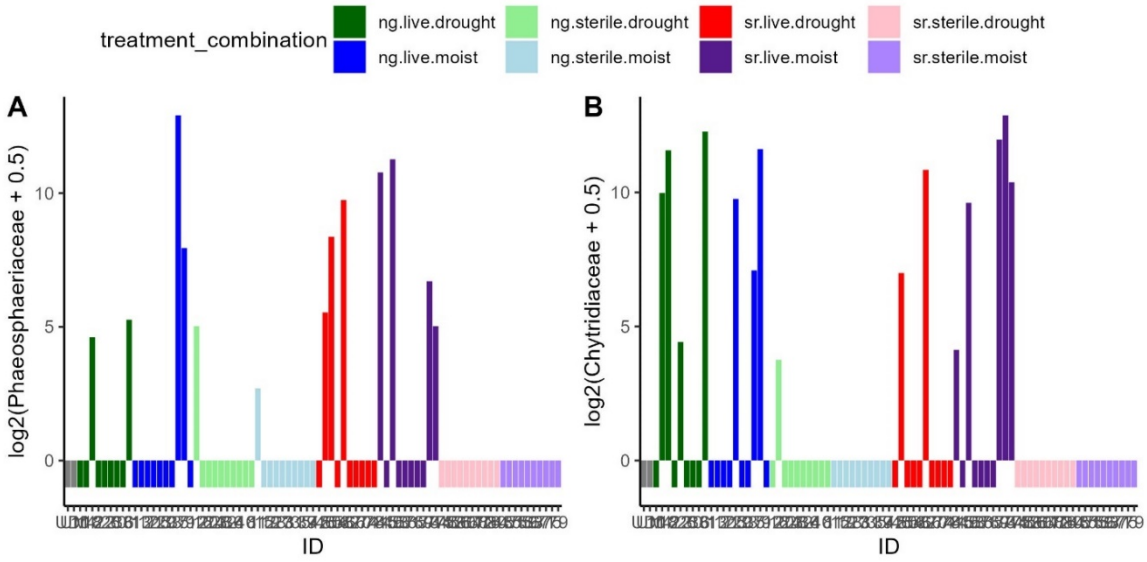

Figure B.3. Regularized abundances of differentially abundant fungal families in endpoint soils by sample ID.

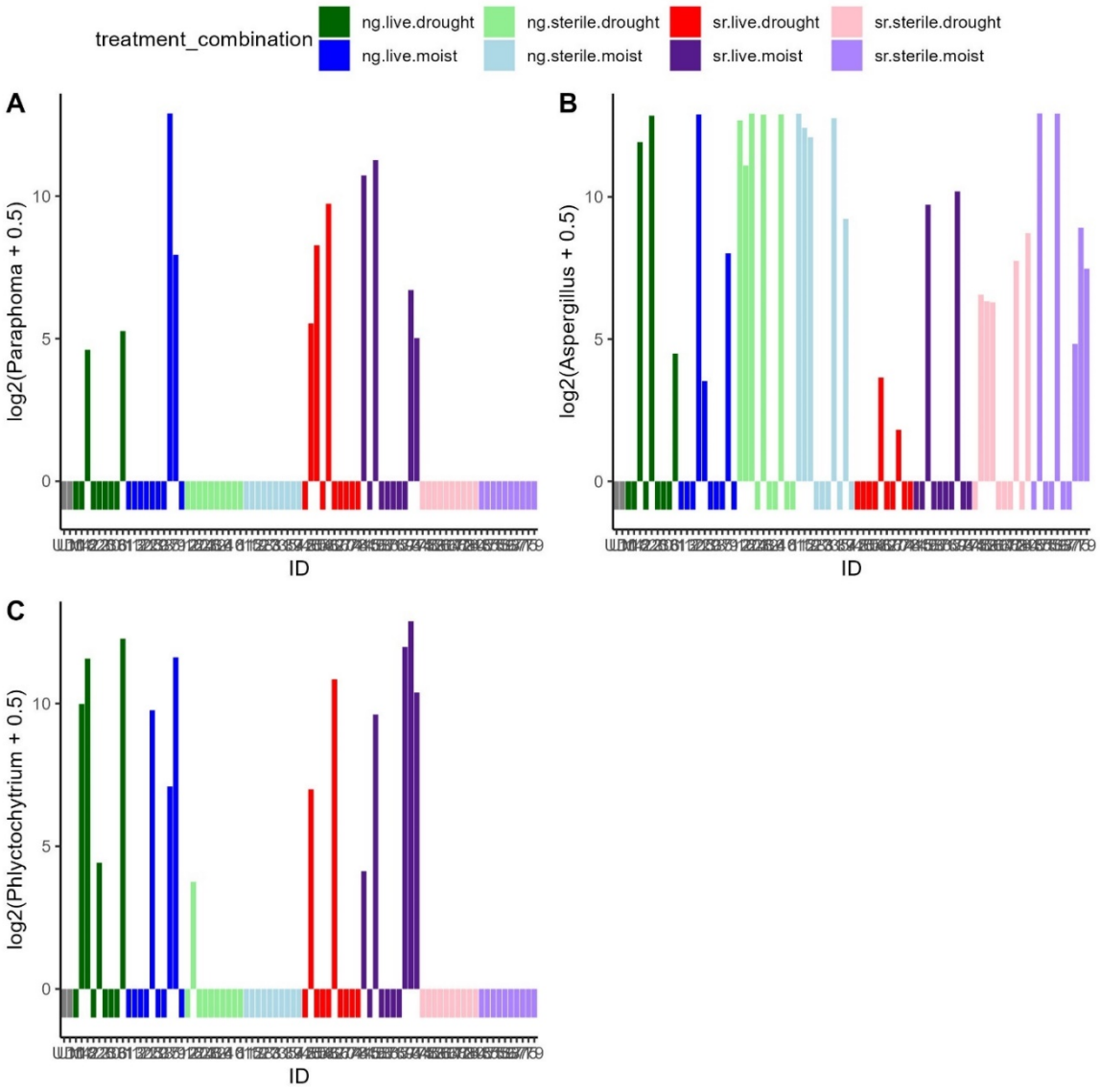

Figure B.4. Regularized abundances of differentially abundant fungal genera in endpoint soils by sample ID.

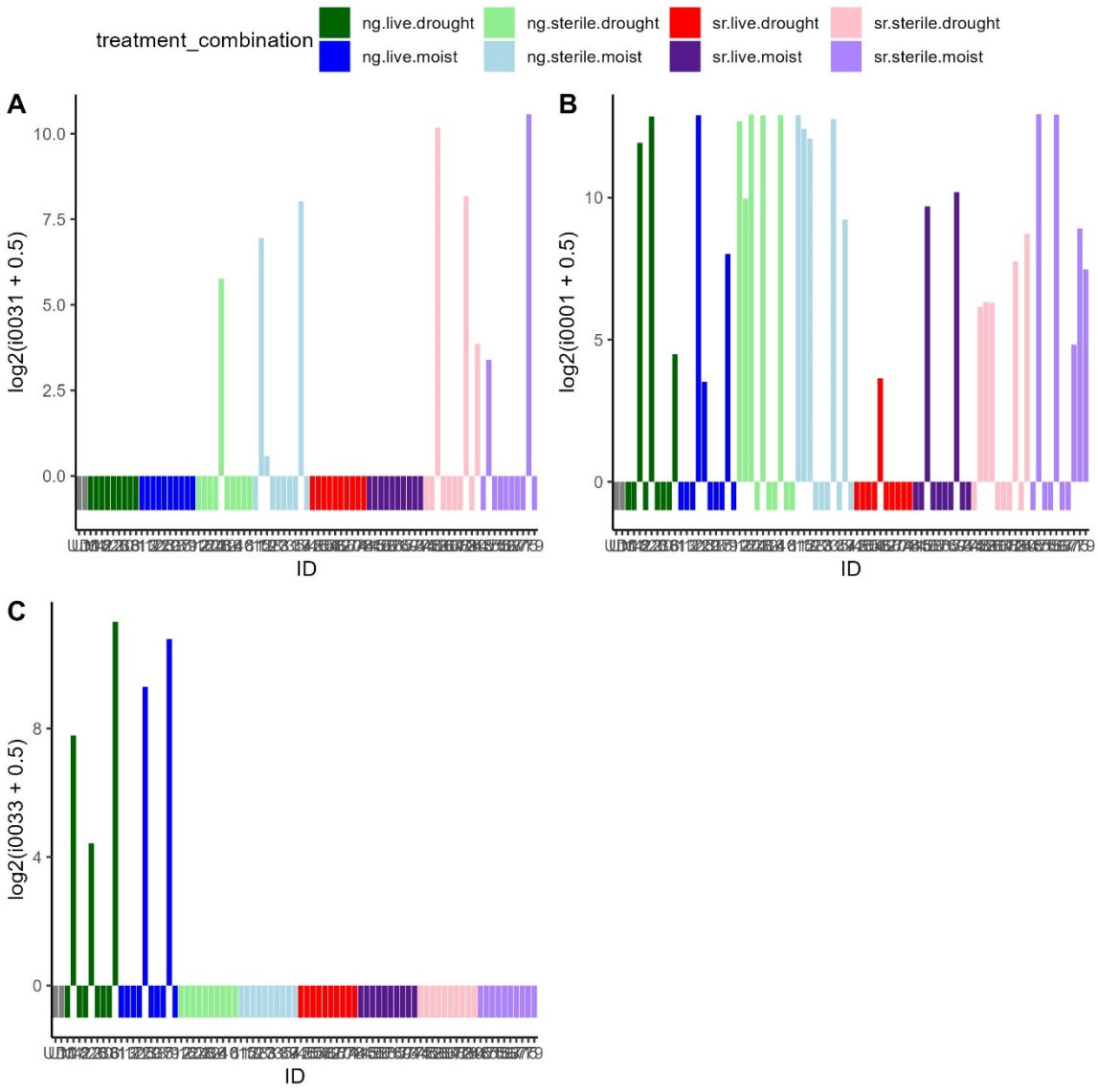

Figure B.5. Regularized abundances of differentially abundant fungal sequence variants in endpoint soils by sample ID.

Table B.1. Fungal families identified as differentially abundant between soil cores samples from the wet site and dry site. Natural log-fold-change (LFC) values and FDR-adjusted p-values (q-values) of ITS read counts in dry site cores relative to wet site cores

Family	Natural LFC (Dry Site vs. Wet Site)	q-value
Rozellomycota_fam_Incertae_sedis	2.919232	0.009216
Pleosporaceae	3.386179	0.003525
Sordariomycetes_fam_Incertae_sedis	-2.44988	0.005987
Capnodiaceae	-3.70835	3.50E-05
Cucurbitariaceae	-3.26121	0.003684
Annulatascaceae	-2.68408	0.003684
Dothideaceae	2.884399	0.018922
Trichomeriaceae	2.557541	0.018922
Cyphellophoraceae	-3.62026	0.00082
Clavicipitaceae	2.342676	0.038784
Xylariales_fam_Incertae_sedis	-2.68722	0.003732
Ajellomycetaceae	-4.70074	7.29E-06
Pezizaceae	2.934029	0.001499
Magnaporthaceae	-3.16759	0.000146
Patellariaceae	2.870375	0.018922
Glomeraceae	-2.29833	0.020439
Lipomycetaceae	-2.77824	0.005843
Psathyrellaceae	-2.8015	0.011916
Bolbitiaceae	-4.71175	7.38E-08
Pleurotaceae	4.069416	0.002698
Crepidotaceae	-4.16402	6.89E-08
Claroideoglomeraceae	-3.72384	1.30E-09
Piskurozymaceae	-3.85538	0.000599
Chytridiaceae	-3.65023	0.003732

Table B.2. Fungal genera identified as differentially abundant between soil cores samples from the wet site and dry site. Natural log-fold-change (LFC) values and FDR-adjusted p-values (q-values) of ITS read counts in dry site cores relative to wet site cores

Genus	Natural LFC (Dry Site vs. Wet Site)	q-value
genus:Alternaria	2.669631	0.019225
genus:Phlyctochytrium	-3.65128	0.003815
genus:Pleurophragmium	-2.45092	0.005375
genus:Ilyonectria	-6.54576	3.46E-64
genus:Peziza	4.656435	1.08E-05
genus:Knufia	2.553064	0.010424
genus:Trichocladium	-5.00267	3.69E-09
genus:Metapochonia	2.601227	0.03079
genus:Oidiodendron	4.59016	7.65E-07
genus:Solicoccozyma	-3.85235	0.000375
genus:Paraboeremia	-4.77505	2.85E-10
genus:Ajellomyces	-3.85066	0.002337
genus:Podospora	-4.92531	4.33E-11
genus:Conocybe	-4.65106	1.35E-08
genus:Neocamarosporium	4.787092	4.51E-27
genus:Rhizodiscina	2.869332	0.021384
genus:Pyrenochaeta	-3.26226	0.003401
genus:Cyphellophora	-3.8745	2.51E-05
genus:Herpotrichia	2.865956	0.028711
genus:Simocybe	-4.16506	1.21E-08
genus:Ophiosphaerella	-3.75884	4.42E-06
genus:Lipomyces	-2.77928	0.005372
genus:Collembolispora	-2.39644	0.042677
genus:Leuconeurospora	-2.93981	0.003401
genus:Plenodomus	2.635539	0.014716
genus:Basidioascus	-4.36787	1.82E-10
genus:Dominikia	-2.8385	0.002371
genus:Hansfordia	-2.68826	0.003401
genus:Slopeiomyces	-2.66501	0.00409
genus:Mucor	2.373689	0.009335
genus:Hydeomyces	2.73427	0.001769
genus:Paramicrosporidium	2.918189	0.009335
genus:Kalmusia	2.948526	0.000304
genus:Coniosporium	-2.37155	0.005133
genus:Rhizophagus	-2.49522	0.03114
genus:Claroideoglomus	-3.35692	1.56E-06

Table B.3. Contaminants that identified in negative controls and removed by the *isContaminant()* function in the "decontam" package and removed from the ITS Illumina sequencing data. These contaminants accounted for 7.2% of the reads after removal of too-short sequences.

SV ID	Phylum	Class	Order	Family	Genus	Species	Length
i0005	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	Chaetomium	angustispirale	322
i0013	Ascomycota	Eurotiomycetes	Onygenales	Onygenales fam_Incertae_sedis	Chrysosporium	pseudomerdarium	315
i0017	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	Schizothecium	<NA>	321
i0036	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Paraphoma	chlamydocopiosa	310
i0085	Ascomycota	Leotiomycetes	Helotiales	Helotiales fam_Incertae_sedis	Leohumicola	<NA>	316
i0848	Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae	Thanatephorus	cucumeris	398

APPENDIX C

^{13}C Labeling

Plants were grown for 6 months, before half were pulse labeled with ^{13}C-CO_2 with the intention of tracing ^{13}C into shoots, roots and the fungal community to measure carbon allocation via lipid extraction and analysis of phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) markers (Dierks et al., 2019; Olsson & Johansen, 2000). However, measured PLFA/NLFA levels in soil samples were too low to meaningfully analyze. The natural abundance of ^{13}C in unlabeled plants was measured to evaluate relative water stress experienced by plants in each precipitation regime.

40 randomly-selected sagebrush seedlings (5 per treatment combination) were pulse-labeled with the stable isotope ^{13}C with the intention to evaluate relative allocation of C to shoot growth, root growth, and the soil microbial community via comparison of ^{13}C in PLFA/NLFAs in samples of each type, relative to the background levels in each treatment combination, as well as assess drought stress levels between precipitation regimes at a single timepoint. These seedlings were placed in a sealed Plexiglas labeling chamber and pulse-labeled with the stable isotope ^{13}C – CO_2 on January 20, 2022. Seedlings were watered according to their assigned precipitation treatments prior to placement in a sealed Plexiglass labeling chamber to ensure that their stomata remained open and they continued to actively photosynthesize, thereby taking in and incorporating the ^{13}C into their tissues.

Carbon dioxide concentrations within the chamber were measured using an infrared gas analyzer (IRGA) (model 820-LC, LI-COR, Lincoln, NE, USA). To draw down the concentration of ^{12}C to maximize the levels of ^{13}C that the seedlings could absorb, CO_2 was scrubbed from the air in the chamber with soda lime granules overnight

prior to labeling. A gas tank filled with 99.9 % $^{13}C - CO_2$ was attached and CO_2 was continuously added with pulse button until it reached a concentration of 1000 ppm. Seedlings remained in the labeling chamber at this concentration for 13 hours, before being removed and allowed to sit at ambient $^{13}C - CO_2$ in the greenhouse overnight to permit time for the transport of ^{13}C throughout plant tissues.

Prior to labeling with ^{13}C, on December 14, 2021, after approximately 6 months of growth, a randomly-selected set of 32 of the surviving 72 seedlings were harvested to establish background ^{13}C signatures, against which ^{13}C levels in the shoots, roots, and the rhizosphere of ^{13}C -labeled seedlings (hereafter, the "enriched" seedlings) were intended to be compared to evaluate allocation of C resources under different treatments. These 32 plants harvested prior to labeling (hereafter, the "unenriched" seedlings) were included in evaluation of germination, seedling survival to 6.5 weeks, and biomass differences between treatments, as well as background levels of ^{13}C in sagebrush shoots, roots, and the rhizosphere. All other response variables were measured only for the remaining 40 seedlings.

While natural abundance analysis represents the long-term accrual of $\delta^{13}C$ incorporation between planting on June 19, 2021 and harvest of the 32 unenriched/unlabeled plants on December 14, 2021, separate statistical analysis of $\delta^{13}C$ levels in enriched shoot and root tissue of the 40 labeled plants also demonstrated significant differences between precipitation regimes at a single timepoint on January 20, 2022, and indicated that the more water-restricted plants photosynthesized significantly less (i.e. had less negative/higher $\delta^{13}C$ values) (shoot $p= 2.06e-05$; root $p= 0.000121$).

APPENDIX D

Germination & Survival to 6.5 Weeks

Seedlings were harvested from the 72 out of 80 seeded cone-tainers. Of the 8 cone-tainers in which no seedling persisted until the end of the greenhouse growth period, all seeds failed to germinate in 3 cone-tainers (NG2&6SM, SR12&19LD, and SR11&15LD), one or more seeds germinated but all died in the cotyledon stage within the first 3 weeks prior to culling in 4 cone-tainers (NG1&9SD, NG8&10LD, SR12&16SD, SR13&18SD), and in only 1 cone-tainer, seeds germinated and survived to time of culling at 6.5 weeks, but the remaining seedling died thereafter (SR14&17SD).

Germination probabilities for seeds treated with sterile wet-site and sterile dry-site inocula were approximately equivalent (42.72% +/- 17.07% and 38.75% +/- 17.31%, respectively), and both were lower than live wet-site and higher than live dry-site (Fig. 6; Table D.1.).

Live dry-site inoculum decreased predicted germination probability, compared to sterile dry-site inoculum, from 38.75% (+/- 17.31%) to 28.47% (+/- 17.54%). In contrast, live wet-site inoculum increased predicted germination probability compared to sterile wet-site inoculum from 42.72% (+/- 17.07%) to 52.90% (+/- 17.05%). However, the proportion of variance captured by these fixed effects was low (marginal R^2= 0.037; conditional R^2 = 0.134), precluding biologically meaningful interpretation of these results.

Neither inoculum source site nor viability significantly impacted probability of seedling survival from germination to 6.5 weeks of age (time of culling), though model

fit of survival probability as a function of these predictors was similarly low (marginal R^2= 0.013; conditional R^2 = 0.013).

Table D.1. Summary of generalized linear model parameters of seed germination and survival to 6.5 weeks. Inoculum type variables have been coded as binary operators, with inoculum source site: 0=dry site & 1=wet site; inoculum viability: 0=sterile & 1=live.

	Germination			Survival		
Predictors	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*
(Intercept)	-0.46	-0.80 – -0.12	**0.008**	0.82	0.16 – 1.48	**0.015**
amf source site [1]	0.16	-0.31 – 0.64	0.498	0.52	-0.41 – 1.45	0.277
amf viability [1]	-0.46	-0.95 – 0.02	0.060	-0.13	-1.08 – 0.81	0.783
amf source site [1] × amf viability [1]	0.87	0.20 – 1.55	**0.011**	-0.38	-1.68 – 0.93	0.569
Random Effects						
σ^2	3.29			3.29		
τ_{00}	0.37_{ID}			1.37_{ID}		
ICC	0.10			0.29		
N	80_{ID}			79_{ID}		
Observations	1670			677		
Marginal R^2 / Conditional R^2	0.037 / 0.134			0.013 / 0.303		

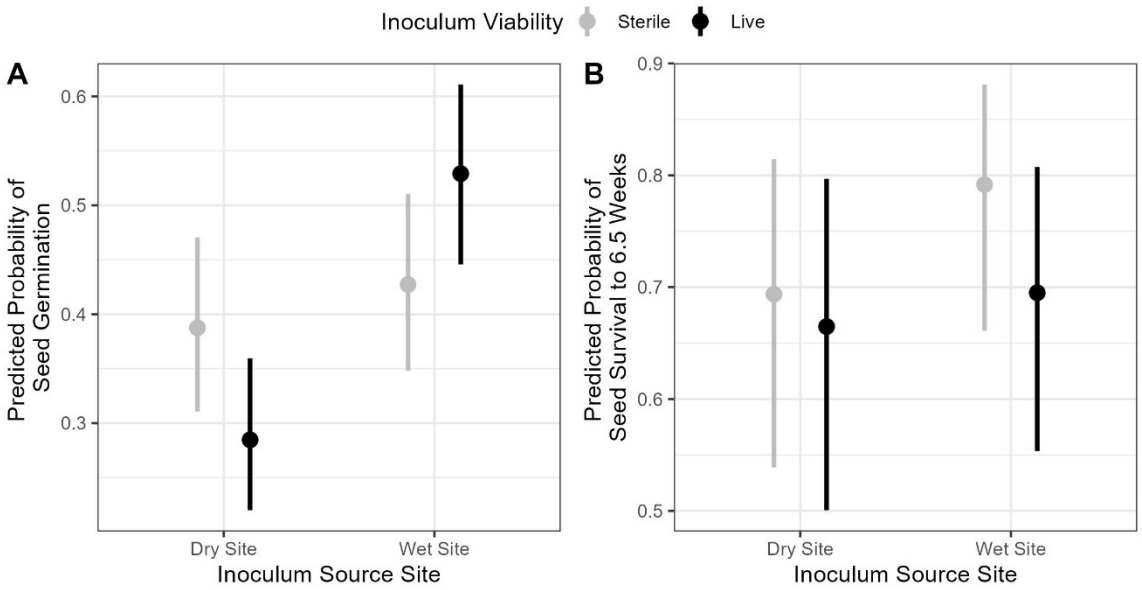

Figure D.1. Predicted mean and surrounding 95% CI for the average cone-tainer's A) germination rate, and B) survival rate to age 6.5 weeks, in each of the 4 combinations of source site and viability.

Administration of live inoculum substantially modified germination rate compared to the sterile inoculum controls, though the direction of this effect differed between inoculum source sites. In the presence of abundant water, as was applied to all treatments at the beginning of the experiment, live foreign dry-site inoculum appeared to hinder germination of wet-site-ecotype seeds, while live wet-site inoculum enhanced germination of seeds from the same site. A mechanism by which fungi could affect germination rate would likely also incur differences in early-stage seedling mortality, which was not observed. Poor model fit and this lack of an identifiable mechanism may suggest that a correlated latent variable, such as the presence of growth promoting bacteria or pathogenic bacteria, that differed between inocula could be responsible for differences in germination rate.

Bacteria present on spores or within empty spore coats of the live inoculum could be responsible for the differences in germination rate between live wet-site and live dry-site treatments; discerning whether this was the case would require further analysis of the 16S rRNA gene to identify bacterial species. Many species of bacteria synthesize ethylene, which despite being best recognized as a ripening hormone, also stimulates germination, and breaks dormancy of seeds (Esashi, 1991). Further, so-called plant growth promoting rhizobacteria are known to synthesize and export other plant growth regulating hormones capable of impacting seed germination (Hayat et al., 2010). For example, seeds of Indian mustard (*Brassica campestris*) coated with bacterial strain *Mesorhizobium loti* MP6 exhibited enhanced seed germination, in addition to improved early vegetative growth and grain yield as compared to uninoculated controls (Chandra et al., 2007).