MUTUALIST MATTERS: EFFECTS OF INOCULA PROVENANCE ON TEXAS RED OAK SEEDLING PERFORMANCE UNDER DROUGHT

by

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DEDICATION

To the chthonic network and unspeakable mystery.

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ABSTRACT

Mycorrhizae are ubiquitous symbioses serving a critical role in plant resilience to stress. Inoculant can improve restoration outcomes; however, success varies depending on inocula provenance. Source of inocula can influence the availability of suitable mutualists, and consequences of variation in mutualist quality have more pronounced effects under drought conditions. In this study we tested the importance of inocula source under water scarcity for Texas red oak (*Quercus buckleyi*) seedlings. Inocula came from either their natal old-growth juniper-oak forest beneath elder conspecific trees (Home-Near), from the same forest beneath elder juniper trees (Home-Far), from a nearby disturbed site beneath live oak-juniper mottes (Away-Near), or from clearings at the same site (Away-Far). Inocula treatments were replicated within 13 blocks and blocks were split into well-watered and droughted halves. The drought treatment was imposed for 2 months during which time photosynthetic rates were measured and seedlings were monitored for leaf desiccation. Additionally, post-harvest biomass, percent mycorrhization and mycorrhizal diversity were analyzed. Drought reduced photosynthetic rate, biomass, and mycorrhizal frequency and diversity. Seedlings were highly ectomycorrhizal with evidently similar community compositions across inoculation treatments, indicating that mycorrhizae were not constrained by inocula source.

Nonetheless, inocula did result in significant physiological differences, most notably higher photosynthetic rates in the mid-late drought and longer time to total leaf desiccation for Home-Far seedlings, especially as compared with Away-Far. Thus, proximity to juniper trees at the home site seemed to increase drought tolerance and slow the rate of leaf desiccation. Although we cannot attribute performance differences to specific mycorrhizal associations (they were not resolved by morphotyping), perhaps synergies involving other members of the soil

microbial community influenced seedling physiology. These results may be of interest to land stewards as juniper trees are disproportionately targeted for removal.

I. INTRODUCTION

Root-fungal symbionts

Chthonic fungal symbioses serve an integral function in the lives of most plants. Of the various types of plant-fungal symbioses, mycorrhizae, the intimate associations between certain taxa of fungi and plant root systems, have been most researched, especially in agricultural science, but increasingly in ecology, where their important role in plant community restoration is acknowledged. Other root-fungal symbionts such as root endophytes (who inhabit roots without forming specialized interface structures) and the recently described feremycorrhizae (Kariman et al., 2014), (which occur in the rhizosphere but do not penetrate roots) are only beginning to garner attention and recognition as mutualists (Kariman et al, 2018). For example, dark septate endophytes, long thought to be pathogenic or otherwise harmful to plants, have been confirmed as mutualists (Newsham, 2011) especially in higher stress environments (Malicka, Magurno, and Piotrowska-Seget, 2022).

Spanning the parasitic to mutualist spectrum, depending on partners and context (Johnson, Graham, and Smith, 1997), mycorrhizae generally exhibit reciprocity: plants provide photosynthate including sugars and fatty acids (Keymer et al., 2017) to fungi and fungi relay soil nutrients (Smith and Read, 2008) and confer other benefits to plants, including pathogen protection (Jung et al., 2012), increase in photosynthetic efficiency (Augé, Toler, and Saxton, 2016), and amelioration of abiotic stress including drought stress (Ruiz-Sánchez et al., 2010). Early versions of these partnerships likely enabled plants' establishment on dry land devoid of organic nutrients ~460-475 million years ago (Brundrett and Tedersoo, 2018), when relationships were forged between proto-roots and fungi; the vast majority of modern plants

maintain coevolving mycorrhizal partnerships (Hoeksema et al., 2010).

Gymnosperms are seemingly all mycorrhizal, and mostly obligately so; an estimated 80% of angiosperm species and 94% of angiosperm families are mycorrhizal (Wang et al., 2006). Although the few general types of mycorrhizal association tend to be phylogenetically conserved within plant families or genera (Orchidaceae and Ericaceae have their own familiar mycorrhizal types), some taxa of plants are capable of associating with more than one type (Teste et al. 2019), including members of globally widespread families and genera such as *Populus* and *Quercus*. These plants are termed "dual" mycorrhizal, and this status can be contemporaneous or successional (ibid).

Generalizations about mycorrhizal status are problematic, generalizations about mycorrhizal community composition even more so, even within a well-studied plant taxon, because of the highly contextual nature of these symbioses. Mycorrhizal community composition can shift with abiotic variables such as aspect (Wei, Song, and Jia, 2021), and soil moisture and chemistry (Erlandson et al, 2016) but also with plant stand age (Odriozola et al., 2020) and genotype (Gehring et al., 2018). Additionally, despite their ancient origins, ubiquity, and critical role, very few ecoregions and plant species have been studied with respect their mycorrhizal community and its influence on plant health.

Arbuscular mycorrhizae (AM) are considered the ancestral form. Obligate symbionts from Mucoromycota (subphylum Glomeromycotina), they develop specialized structures within the root cortical cells of more than 80% of all terrestrial plant species (Brundrett and Tedersoo 2018). By contrast, ectomycorrhizae (EM), a nonmonophyletic group consisting of ~20,000 taxa of fungi from Mucoromycotina, Basidiomycota, and Ascomycota (Tedersoo, May, and Smith, 2010) evolved independently at least 80 times in association with forest trees (Raven and

Andrews 2010; Tedersoo et al., 2013). EM remain external to root cells and form hyphal mantels around lateral root tips in about 2% (~8,500 species) of vascular plants (Brundrett and Tedersoo, 2018). The prominent morphological and physiological distinctions between EM and AM provide different benefits to the plant. For instance, EM mine nutrients directly from soil organic matter (Read and Pérez-Moreno 2003) and may offer more protection from pathogens by physically enveloping root tips (Marx 1972; Tedersoo, Bahram and Zobel, 2020), whereas AM require less carbon to maintain (Leake et al 2004) - they do not produce fruiting bodies - and scavenge inorganic nutrients (DeForest and Snell 2020). AM are also more resilient to higher temperatures and extremes in soil moisture (Teste et al, 2019).

Inoculation for restoration success

Altered soil microbiology and physicochemical properties at degraded sites makes plant establishment a challenge; compatible fungal mutualists are limited. However, even small increases in mycorrhization can reduce plant mortality at restoration sites (Markovchick et al., 2023). Ecological restoration of plant communities can also be enhanced by the proactive introduction of mycorrhizal inocula. Appropriate inoculant application augments plant species richness and facilitates approximation of restored communities to reference sites (Neuenkamp et al., 2019). However, inoculants vary in quantity and quality of fungal mutualist composition, with concomitant variability in restoration success (Maltz and Treseder, 2015). Native mycorrhizal inocula procured from intact plant communities can improve abundance, richness, and diversity at restored sites (Koziol, Crews, and Bever, 2020), whereas commercial inoculant may not even contain viable mycorrhizal fungi (Karlsen-Ayala et al., 2022; Salomon et al., 2021) and its dispersal also presents concerns about introduction of competitive non-native fungi. Mycorrhizal fungi viable for commercialization are generally early successional, disturbance

adapted taxa, which do not provide the same benefits as native co-adapted fungi (Koziol et al., 2018), whether that native inocula is provisioned by whole soil or lab-cultured from soil of old-growth sites (Koziol et al, 2022). Many types of mycorrhizal fungi, which may include those most beneficial to restoration, are difficult if not impossible to culture (Hawkins, Jones, and Kranabetter, 2015)

Acquiring inocula directly from the field could be a more feasible and reliable practice for restoration. Nevertheless, practical questions around provenance of mycorrhizal inocula remain: For example, is it necessary to acquire inocula from more ecologically pristine sites to capture the most beneficial fungal mutualists or might even a disturbed site, with related but different species, provide adequate inocula? Does collection beneath a conspecific, congeneric, or otherwise compatible host optimize mycorrhization? To unpack these questions, what follows is a summary of plant root-fungal symbioses, focusing on mycorrhizae and highlighting the challenges around determining which inocula sources might be optimal for specific restoration target plants, in the case, Texas red oak trees.

Mycorrhization and Drought

A crucial contribution of mycorrhization to plants' health is via mitigation of drought stress. This occurs through a variety of mechanisms including direct uptake and transfer of soil water (Püschel et al. 2020; Marulanda et al., 2003; Egerton-Warburton et al., 2003), regulation of phytohormones (such as ABA) (Tedersoo et al., 2013; Ouledali et al., 2019) and aquaporin expression (Bárzana et al., 2014; He et al., 2016), protection from oxidative damage (Lehto and Zwaizek 2011; Santander et al., 2017), enhanced nutrition (Augé 2001; Li et al., 2021) and photosynthetic efficiency (Porcel and Ruiz-Lozano 2004; Augé et al., 2016), and improved soil physical properties (Bitterlich et al., 2018; Augé 2004).

With increasing water scarcity, mycorrhizal community composition may shift towards AM predominance (Gehring et al 2006). Reduced soil moisture likely decreases EM abundance and diversity (Kilpeläinen 2017) and alters oak EM community composition (de Jalón et al., 2020), which frequently shifts towards higher abundance of long-exploration type hyphae with hydrophobic mantels (rhizomorphs) (Shahin et al., 2013; di Pietro et al., 2007). However, there is evidence for strong tree genetic influence on EM community composition (Gehring et al., 2018; Patterson et al., 2018). EM community composition can differ at the phylum level between drought-tolerant and drought-intolerant pinyon genotypes (Patterson et al., 2018); drought-tolerant tree genotypes maintain distinct ectomycorrhizal communities, even when inoculated with soil from trees of a different genotype (Gehring et al., 2018).

In dual mycorrhizal plants, AM may become more prevalent during drought with a greater diversity of oak AM taxa (Jafarian et al., 2024). For example, *Q. agrifolia* became associated almost exclusively with AM as EM disappeared from the upper rhizosphere during severe drought (Querejeta et al 2009). AM can increase stomatal conductance; this effect increases with drought severity (Ruíz-Sánchez et al, 2011). The severity of the drought may determine whether and how much mycorrhizal richness and abundance change, with some evidence that EM will be higher under intermediate drought stress (Swaty et al., 2004).

Oak dual status

Quercus is a speciose and wide-ranging genus of ecological, economic, and sociocultural import, and globally in decline (Kowasari and Karimi, 2023). Oak restoration efforts are typically focused on reducing plant neighbor competition rather than augmenting fungal mutualist facilitation (Johnson, Shifley, and Rogers, 2005). However oak mycorrhizal partners are known to enhance nutrient acquisition (Dickie et al., 2005; Dickie, Koide, and Steiner 2002),

growth (Dickie et al 2006), drought tolerance (Sebastiana et al., 2018; Li et al, 2021), and survivorship (Tonn and Ibáñez, 2017; Burkett et al., 2005), making it plausible that inoculation with beneficial mycorrhizae could improve restoration outcomes. Although presence of AM and benefits derived from oak AM association are less commonly demonstrated (Ibáñez et al., 2015; Dickie et al., 2002; Tonn and Ibáñez, 2017; Reyes-Jaramillo, Montaño, and Silva-González, 2020), both AM and EM can support some oak species (Ksentini, Meddad-Hamza, and Beddiar, 2020; Egerton-Warburton and Allen, 2001; Olivera-Morales et al., 2011).

A few species (*Q. robur, Q. rubra, Q. suber, Q. agrifolia, Q. ilex*) from a few ecoregions dominate the oak mycorrhizae literature. Studies have indicated red and white oak share the same taxa of ectomycorrhizae (Walker, 2001), and that proximity to congeneric oak can help seedlings (Dicke, Koide, and Steiner 2002), however in a study of two pairs of oak sister species with habitat differentiation, host-specific soil microbes facilitated their plant partners and inhibited the closely related oaks (Wu, Brown, and Ricklefs, 2022). Currently for most oak species, partner specificity and dual status is unknown.

Mycorrhizal Availability

Proximity, identity, and age of compatible plant hosts can strongly influence mycorrhizal community composition (Janowski et al 2022; Grünfeld et al, 2019; Martínez-García et al., 2015). Mutualist potentials are constrained by host plant availability for both AM (Whitcomb and Stutz 2007; Grünfeld et al., 2022) and EM (Pickles et al., 2012; Peay et al 2010). In closed forest, *Q. suber* and *Q. canarienses* seedlings developed more EM and the extent of EM roots was associated with survival in the first year (Ibáñez et al., 2015). Further from forest edge, EM diversity and abundance declines precipitously (Peay, Garbelotto, and Bruns (2010). A 20 meter (Dickie and Reich, 2005) or even 5 m distance (Frank et al., 2009) from mature oak was

sufficient to prevent mycorrhization in experimental oak seedlings. These studies indicate that mutualist limitation in sites removed from forest edge can impinge on growth and curtail survivorship.

Community composition may be more important than proportion of mycorrhizal roots (Baxter and Dighton, 2001) or species richness of mycorrhizal partners (Anthony, 2025; Livene-Luzon et al., 2017); this composition is greatly influenced by edaphic factors and plant neighborhood. Within forests of AM-EM co-dominant neighbors, some predominantly EM trees' mycorrhizal communities are altered by a heterospecific AM neighbor (Otsing et al 2021; Haskins and Gehring 2005; Hubert and Gehring 2008). In juniper-oak and juniper-pine studies, for example, presence of AM *Juniperus* sp. neighbor can increase prevalence of AM (Williams et al., 2013) and reduce EM (Haskins and Gehring, 2005). Sometimes mycorrhizal community composition changes without reducing species richness (Bermúdez-Contreras; Hubert and Gehring, 2008); dual mycorrhizal oaks can become more AM in these circumstances, which may (Olivera-Morales et al., 2011) or may not (Dickie, Koide, and Steiner, 2002; Ibáñez et al., 2015) benefit oak seedlings.

Site type and soil legacy can influence mycorrhizal community composition more than specific plant host and neighbor species in some instances (Dean et al., 2015). Mature forests contain greater phylogenetic diversity of mycorrhizal fungi (Yoo et al., 2022); even AM fungi – long thought to be generalists - have shown strong niche differentiation by site successional age and host specificity (Martínez-García et al. 2014; Veresoglou and Rillig, 2014). In degraded sites, legacy effects of disturbance persist for decades and have deleterious impacts on native soil microbial communities (Meinhardt & Gehring 2012; Koziol & Bever 2017). Numerous studies on oak forests and woodlands reveal shifts in mycorrhizal community composition and richness

of degraded sites (Bermúdez-Contreras, 2022), with reductions in overall fungal abundance (Xiong et al., 2021) and diversity (Tonn and Ibáñez, 2017), but sometimes increased prevalence of AM (Ksentini, Meddad-Hamza, and Beddiar, 2022; Xiong et al., 2021).

Common Mycorrhizal Network and Inoculation

Seedlings obtain mycorrhizal partners largely by anastomosis (filamentous fungal cell fusion) with the existing Common Mycorrhizal Network (van der Heijden and Horton 2009). Nutrients can be transferred and signals transmitted (van der Heijden et al., 2015; Gorzelak et al., 2015) by way of this extensive belowground network, which connects plants of disparate phylogenies through their shared mycorrhizal partners (He et al., 2019); however, given the exceeding complexity of the network, effects can be challenging to demonstrate unequivocally (Figueiredo, Boy and Guggenberger, 2021). Nonetheless, tree seedlings tend to benefit from proximity to mature trees with compatible partners (Selosse et al., 2006) and fungal richness is highest near the base of these trees (Teste, et al., 2009). Some researchers consider these elder trees to be "hubs" in the network, with more extensive and diverse connections; these hub trees may be more important to network topology at xeric sites (Beiler, Simard, and Durrall, 2015). Dual plants can serve as nodes connecting AM and EM Common Mycorrhizal Networks.

Greenhouse grown seedlings isolated in pots lack access to Common Mycorrhizal Networks, though they can still benefit from mycorrhizal inoculation. However, most greenhouse studies are conducted using commercial and non-native inoculant despite evidence that more complex (Hoeksema et al. 2010) and sympatric (Rúa et al, 2016; Remke et al., 2020[a,b], Remke et al., 2022) inoculant provide greater benefits. Inocula provenance influences abundance, richness, and composition of mycorrhizal fungi. If inocula is procured from a site removed from compatible plant hosts and/or the accustomed plant community type and neighborhood, this

could result in mutualist limitation and consequently, negative health outcomes for seedlings, especially under drought stress. There is some evidence that inoculum provisioned from "home" can facilitate oak seedlings' connections with the fungal mutualists most likely to promote growth and survival (Olivera-Morales et al., 2011; Tatsumi et al., 2021), but what constitutes home?

Study goals

Compatible fungal mutualists can help germinants survive the vulnerable early stages of life and get established in intermittently dry conditions (Sebastiana et al., 2013; Pereira et al., 2021; Atala et al., 2012). However, inocula of potential symbionts are patchily distributed and seedlings may be constrained by their availability. The distribution of fungi in soil is influenced by the interrelated factors of proximity to particular plant hosts (Frank et al., 2009; Selosse et al., 2006; Dickie, Koide, and Steiner 2002), plant neighborhood (Otsing et al 2021; Haskins and Gehring 2005; Hubert and Gehring 2008; van der Heijden and Horton, 2009), site type and soil legacies (Swaty et al., 1998; Dean et al., 2015; Ibáñez et al., 2015). To address uncertainty about the constraints that inocula provenance may exert on seedling mycorrhizal formation and their effects on function under drought, I conducted a greenhouse experiment using inocula from two distinct sites and different distances from mature oak, under two water regimes.

This study contributes knowledge of oak-fungal symbioses by investigating influences of inocula provenance on mycorrhizae and how these associations impact seedling growth and drought response in an ecoregion increasingly prone to hot drought. The focal species, Texas red oak (*Quercus buckleyi*), is endemic to juniper (*Juniperus ashei*)-oak forests and woodlands on limestone hills from southcentral Texas into northcentral Oklahoma with the highest remaining concentrations along the rapidly urbanizing Balcones Escarpment. Texas red oak regeneration is

impinged upon by overabundant white-tailed deer (*Odocoileus virginianus*), oak wilt fungus (*Bretziella fagacearum*), and extreme drought, among other threats. The seedling stage is particularly vulnerable both to herbivory (Russell and Fowler 2004) and water scarcity (Cavender-Bares and Bazzaz 2000). In this ecoregion, juniper cover protects oak seedlings from browsing by deer (Russell and Fowler 2004), and canopy cover of mature oak and juniper trees enhances survival in co-occurring *Quercus sinuata* var. *breviloba* seedlings (O'Donnell et al., 2020). Boost to survivorship was attributed to the mitigation of abiotic stress via shading with speculation that mycorrhizae played a role, but the contribution of soil symbiont access mediated by the proximity of adult trees has not been addressed directly.

Most studies examining mycorrhizal inoculant influences on plant performance contrast very different inocula sources – for example, whole soil inocula from sites with distinct climatic and edaphic characteristics (Remke et al., 2020). In this study we seek to isolate inoculant effects under the controlled setting of a greenhouse and examine more subtle contrasts: natal Home soil from a quintessential habitat for the focal plant and a site in the same local preserve system but with a different land-use history and plant community (Away). In this way we are more able to discern influence of site legacy and dominant plant community on mycorrhizae. From each site type, inocula were provisioned from beneath oak (Near) or removed from oak (Far). Home-Near inocula came from mature conspecifics; Home-Far came from mature co-dominants. Away-Near inocula came from mottes of congeneric and codominant trees; Away-Far came from clearings removed from trees.

The expectation is that Texas red oak is somewhat specific in their mycorrhizal associations and that forest inocula will provide the most beneficial mutualists. With access to a greater EM functional diversity under mature conspecifics (Home-Near), seedlings may achieve

higher rates of photosynthesis and greater biomass under well-watered conditions, however effects of drought stress may be ameliorated by the AM mutualists provisioned by old-growth juniper (Home-Far). These more drought resilient mutualists may also enable seedlings to endure increasingly severe drought before total leaf desiccation.

When inocula is provisioned from the disturbed "Away" site, the expectation is that seedlings will lack access to the most beneficial mutualists. The mottes of juniper-live oak (Away-Near) will likely provide some helpful symbionts; however, the land-use history and absence of conspecifics will alter the mycorrhizal community composition such that seedlings will obtain fewer and less diverse partners. Inocula from the clearings (Away-Far) will be devoid of compatible partners; this mutualist limitation will impinge on photosynthetic rates and growth. These seedlings' leaves will desiccate earliest.

In this study, we test the following predictions:

1. Experimental oak seedlings will form AM and EM.
2. Rate of ectomycorrhization and EM diversity will be greatest with Home-Near inocula. Away-Far inoculated seedlings will have the lowest ectomycorrhization and EM diversity.
3. Ectomycorrhization and EM diversity will be drastically reduced by drought.
4. Droughted seedlings will have higher proportions of AM, especially with Home-Far inoculum.
5. Plants with Home inocula will have higher rates of photosynthesis (higher assimilation and stomatal conductance); Home-Far inoculant will slow the decline in photosynthetic function under drought. Away-Far seedlings will have the lowest photosynthetic rates, especially under drought.

6. Under well-watered conditions, biomass will be highest with Home-Near inoculant; Away-Far seedlings will be smallest.

7. Among droughted groups, Away-Far seedlings' leaves will reach total desiccation earliest; Home-Far will retain viable leaves longest; Home-Near and Away-Near will be intermediate.

II. METHODS

Experimental Design

The experiment was conducted in a greenhouse under well-watered or droughted conditions using four inoculants designated as Home-Near, Home-Far, Away-Near and Away-Far. Home inocula were provisioned from an old-growth forest site and Away from a disjunct tract of the same preserve system (Balcones Canyonlands Preserve) in central Texas. Near inocula were collected in the immediate vicinity of *Quercus* sp., and Far at a minimal distance of either 5 m (Home-Far) or 10 m (Away-Far) from the nearest oak taller than 0.5 m. Treatments were arranged in blocks split by drought treatment. Each half-block contained 16 plants, composed of 4 replications of each inoculant treatment in a random position. The experiment consisted of a total of 13 blocks (N = 416).

Seed collection and seedling preparation

Acorns were collected beneath several parent trees under closed-canopy forest from mid-October into early November 2022 at Reicher Ranch in Austin, Texas, USA (30.330521 N, -97.936728 W), a City of Austin Wildland Conservation Division-managed tract of the Balcones Canyonlands Preserve (BCP). After discarding seeds that floated in water, each acorn was weighed, assigned a unique identification number and sown into 1.5” wide x 8.25” deep conical containers within a week of collection. Conetainers were filled with an autoclaved seedling potting mix comprised of a blend of granite and cow manure-based compost (Geogrowers Thunderdirt, 2110 US Hwy 290, Dripping Springs, TX), shredded coconut coir pith, and super coarse vermiculite in a ratio of 2:1:1 (v/v/v), respectively. Conetainers were watered weekly, and emergence was documented from December 2022 until March 1st, 2023. In the last week of March, seedlings of similar height without evidence of pest damage were randomly assigned to

treatment groups and blocks for transplant.

Inocula collection

Home and Away inocula were collected from two tracts of the Balcones Canyonlands Preserve near Austin, Texas, USA: for the Home inocula, the old growth Ashe juniper-oak forest at Reicher Ranch (source of acorns for the study), and for the Away inocula, Cortaña (30.372514 N, -97.873492 W), a disjunct parcel 8.5 km to the northwest, which was cleared of most woody plants in the winter of 2003-2004 and now hosts primarily herbaceous vegetation with mottes of juniper and escarpment live oak (*Quercus fusiformis* Small, hereafter live oak). (Fig. 1)



Figure 1a. Map of Home inocula collection locations. Red triangles indicate collection sites of Home-Far inocula; blue pentagons indicate collection sites of Home-Near inocula.

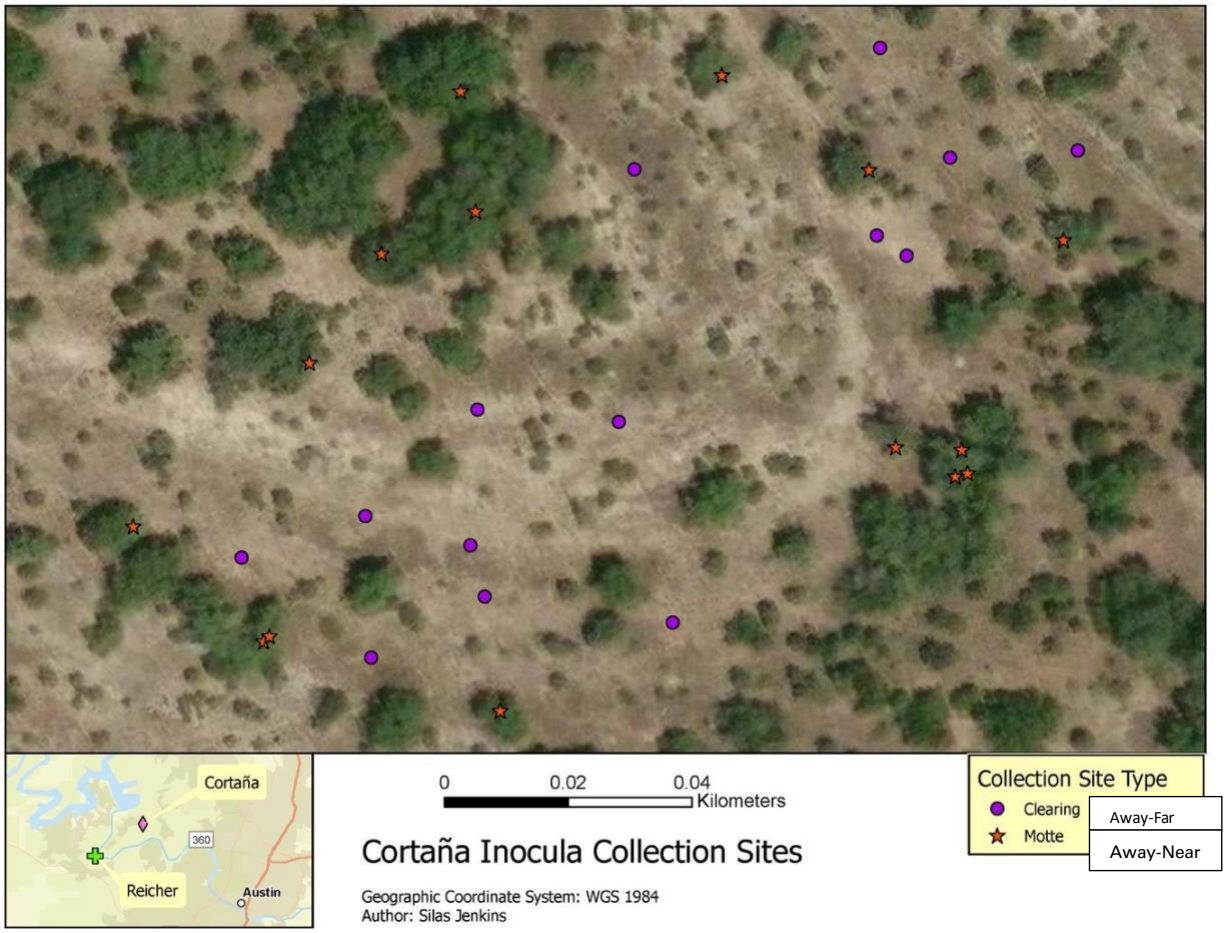


Figure 1b. Map of Away inocula collection locations. Purple circles indicate collection site of Away-Far inocula (2 missing from map b/c flags not found); red stars indicate approximate collection site of Away-Near inocula (one missing).

The Home-Near inoculant soil was collected at the bases of elder red oak trees under juniper-oak canopy (Fig 2a). Home-Far inoculant soil was collected under juniper canopy beneath elder juniper trees located no closer than 15 m from the nearest red oak tree over 0.5 m in height, and at least 5 m from any congeneric taller than ~0.5 meters (Fig 2b). Elder trees of both species had circumferences at ground level ≥ 100 cm.

Away-Near soil was collected under the canopy of live oak-juniper mottes, within 0.5 m of both adult juniper and live oak (Fig 2c). Away-Far soil was collected from clearings in the root zone of herbaceous plants at least 5 m from any woody vegetation and at least 10 m from any oak tree (Fig 2d). Further collection site characteristics are shown in Table 1.

Inoculant material was gathered from 16-20 locations per treatment and from at least two holes on opposite sides of trees (when present) at each location. Approximately 500 mL of soil including fine roots was collected at each sampling point and aggregated and mixed by inoculant type just prior to transplanting.



Figure 2a. Home-Near site photo



Figure 2b. Home-Far site photo



Figure 2c. Away-Near site



Figure 2d. Away-Far site

Table 1. Characteristics of inoculation collection locations. Includes neighbor species present at 3 or more locations.

INOCULANT TREATMENT	SITE-PROX	DISTANCE TO WOODY PLANT	DISTANCE TO CONGENERIC (>0.5 M TALL)	DISTANCE TO CONSPECIFIC (> 0.5 M TALL)
Clearing	Away-Far	≥ 5 m	≥ 10 m	absent
Neighbors: *Thamnosma texana, Evax prolifera, Wedelia acapulcensis* var. *hispida, Croton monanthogynus, Senna roemeriana, Cooperia pedunculata, Allium* sp., *Sida* sp., *Schizachyrium scoparium, Muhlenbergia reverchonii, Polygala lindheimeri*				
Motte	Away-Near	~ 0.25 m	≤ 0.5 m	absent
Neighbors: *Yucca rupicola, Rhus virens, Berberis trifoliolata, Carex planostachys, Diospyros texana, Prunus serotina, Cooperia pedunculata, Sideroxylon lanuginosum, Smilax* sp., *Vitis* sp.				
Juniper	Home-Far	~ 0 m	≥ 5 m	≥ 15 m
Neighbors: *Cercis canadensis, Lespedeza texana, Garrya ovata, Cocculus carolinus, Forestiera pubescens, Prunus serotina, Sideroxylon lanuginosum, Smilax* sp., *Parietaria pensylvanica, Quercus sinuata, Quercus fusiformis, Quercus buckleyi, Styphnolobium affine, Juniperus ashei, Matelea reticultata, Yucca rupicola, Toxicodendron radicans, Acourtia runicinata, Ageratina havanensis, Berberis trifoliolata, Opuntia* sp., *Celtis* sp., *Carex planostachys, Diospyros texana, Tragia* sp., *Dermatophyllum secundiflorum*				
Oak	Home-Near	~ 0 m	n/a	~ 0 m
Neighbors: *Rhus virens, Berberis trifoliolata, Celtis* sp., *Juniperus ashei, Diospyros texana, Dermatophyllum secundiflorum, Nolina lindheimeriana, Frangula caroliniana, Prunus serotina, Sideroxylon lanuginosum, Smilax* sp., *Vitis* sp				

Transplantation and Blocking

The transplant potting mixture consisted of sieved topsoil from an upland construction site approximately 2.4 km from the homesite, sand, and rice hulls in a 2:2:1 (v/v/v) ratio. The potting mixture was autoclaved in 12 L batches for 45 minutes at 121 degrees and pressure 15 psi in plastic bags with soil no deeper than 10 cm to ensure complete sterilization. Transplantation occurred within 24 hours of inocula collection, on April 1st and April 3rd, 2023. Inoculant soils remained at ambient conditions in buckets out of direct sunlight in the interim. Tree pots (10 cm x 10 cm x 24 cm) were lined at the bottom with mats of coconut coir to prevent soil loss. Transplant potting mixture was added by hand around the root of the transplant, while 112 ml of the inoculant was gradually poured around the center taproot. Soil was gently tapped down and covered with rice hulls as mulch to deter fungus gnats.

Pots were arranged in trays accommodating 16 pots each; four replicates of inoculation

treatments were randomly distributed within each tray. Trays opposite one another on the greenhouse bench constituted a block, split at random into watered and droughted treatment groups. The experiment consisted of 13 such blocks, totaling 416 plants and 52 replicates of each treatment.

All transplants were watered to saturation one day a week for seven weeks to allow them to recover from transplant stress and for mycorrhizal connections to establish (Erland and Finlay, 1992). The well-watered treatment group continued to be watered once a week. The droughted treatment group plants were not watered after May 24th.

Monitoring

Seedlings were monitored weekly for leaf bud break, change in leaf number, wilting, damage from disease or other causes. Changes in color and texture indicating drought-induced leaf death were documented; day of total leaf desiccation, when all leaves reached this state, was recorded.

Leaf gas exchange measurements using CIRAS-3 DC (PP Systems, 110 Haverhill Rd, Suite 301, Amesbury, MA 01913 USA) were conducted at least once each week from June 8th through July 5th. Measurements were taken on plants from 4 or 5 blocks each data collection session. Blocks were selected at random without replacement until all blocks had been sampled once; then the random selection resumed until all blocks were sampled a second time. Eleven of thirteen blocks were sampled from three times over the course of the experiment. Measurements were taken on one replicate per block, randomly selected without replacement. Leaves were large enough to fill the 25 mm x 18 mm leaf chamber. The CIRAS was set to an incident light level of 500 mE m-2, representing the maximal light intensity in the greenhouse, and ambient temperature, which ranged from 27.4 to 41.9 ºC; relative humidity was set to approximately 50%.

RH in the greenhouse ranged from 40-60.7%.

Harvest

In the drought treatment, plants were harvested within a week of displaying total leaf desiccation, meaning all leaves were pallid and thoroughly crispy instead of supple and verdant. We did not want complete plant death since we aimed to harvest live roots to examine mycorrhizae. Harvest began in the first week of July, 6 weeks after the last water application. This rolling harvest continued for three weeks, as droughted seedlings reached total leaf desiccation status, culminating on July 24 when very few seedlings retained any viable leaves. On July 25th, a random subsample of 39 plants per inoculation treatment (3 replicates per block) in the well-watered group were harvested.

Root systems were carefully separated from the soil during harvest, placed in airtight plastic bags, and immediately stored in a freezer. Leaves, stems, and root systems not subsampled for microscopy were dried at 70º C for at least three days and weighed separately.

Mycorrhizal microscopy

Frozen root samples were briefly soaked in water and carefully rinsed to remove remaining soil particles. Fragments at least 2.5 cm long with adherent fine roots were subsampled from different parts of each plant's root system. For each seedling, about ten subsamples were collected for AM analysis and about 15 for EM. Microscopy analysis of mycorrhizal morphotypes' relative abundance and overall percent colonization was conducted in the Gehring Lab at Northern Arizona University. To examine for AM, roots were cleared with 7.5% KOH and stained with trypan blue. EM root tip colonization counts were determined using the grid intersect method (Brundrett et al., 1996). Morphotypes were counted for a minimum of 100 root tips per sample. The initial survey identified 21 recognizable morphotypes as determined by shape, color and texture of mycorrhizal

root tips. For the ten most common morphotypes, at least three replicates from different plants and treatments were sequenced. Briefly, PCR products were selected for Sanger sequencing using the ITS4 primer; sequences were compared with NCBI GenBank database (http://www.ncbi.nlm.nih.gov) using the BLASTn algorithm (see Appendix C). Further molecular work is underway to clarify ectomycorrhizal taxonomy.

Statistical analysis

Leaf gas exchange

All statistical tests were conducted in R studio. Assumptions or normality and heteroscedasticity were checked visually using histograms, boxplots with means, q-q plots, and by plotting residuals against fitted values. Treatment effects on seedling physiological status (stomatal conductance, assimilation, and water use efficiency) were tested using a lmer model with the main factors water regime, site, and proximity crossed and block as random effect, and with the same model but using inoculation treatment as a single factor with four levels instead of divided into site and proximity with 2 levels each. When significant to marginally significant (p-value ≤ 0.10) effects were revealed, post-hoc tests using the emmeans package (Lenth, 2024) determined which treatments differed and were used to contrast percent differences in effect size. For leaf gas exchange, three readings logged around peak assimilation were averaged. Data collected on consecutive days were combined to increase sample size. Data were analyzed by each collection session separately and cumulatively, and different transformations were utilized to avoid violating parametric test assumptions of normality and homoscedasticity. Seed mass was included as a covariate in the initial by session analysis, but only had a marginally significant effect on stomatal conductance on one occasion ($p=0.055$), and no significant effect on assimilation. Therefore, seed mass was removed from these models; however seed mass was used in the full model for

cumulative photosynthesis data, which included day of session as a covariate. The full model was compared with reduced models using corrected Akaike's Information Criterion with bbmle package (Bolker, 2023) to identify best fit models for assimilation and stomatal conductance overall.

Final biomass and size

Seed mass and root to shoot ratios were normally distributed, but all other continuous size variables were log-transformed for analyses. Biomass variables were analyzed using the lmer model with main effects of water regime and inoculation treatment (4 levels), random block effects, and seed mass as covariate.

Difference leaf count increase was analyzed using glm (family = poisson) with post-hoc comparisons using emmeans.

Time to desiccation

A log-rank time to event analysis was conducted using the "survival" (Therneau and Grambsch, 2000), "survminer" (Kassambara, Kosinski, and Biecek, 2024) and "dplyr" (Wickham et al., 2023) packages to test differences among inoculation treatments in time to complete leaf desiccation.

Mycorrhization and diversity

For mycorrhization, lmer (with main effects of water regime and inoculation treatment and block as random effect) tested differences in arcsin square-transformed proportions of living EM and total EM per seedling; EM rate was also analyzed separately in the well-watered group to compare pre-drought mycorrhization potential. Linear regressions (lm code) tested relationships between mycorrhization and seed mass; ANOVAs tested relationships between EM rate and above-ground biomass variables. Shannon Diversity Indices using morphotype data (of 14

morphotypes accounting for >99.5% of EM root tips) were analyzed using lmer with interacting main effects of water regime, site, and proximity. GLM (family=Poisson) evaluated relationship between mycorrhization rate and days until total leaf desiccation.

III. RESULTS

Leaf gas exchange

Within 4 weeks of drought treatment application, assimilation rates and stomatal conductance for well-watered and droughted groups fully diverged (Fig. 3). Prior to that split, an inoculation main effect (and site*proximity interaction) occurred on day 16, when assimilation in Away-Far was elevated 22% over Away-Near ($p=0.041$). By day 22, droughted Home-Near seedlings' photosynthetic rates fell 41-45% below other droughted groups. They were significantly reduced compared with both droughted Away-Near and Away-Far ($p=0.029$ and 0.048), and marginally reduced as compared with droughted Home-Far ($p=0.084$). A week later, all droughted inoculation treatment groups had similarly reduced photosynthetic function.

Intrinsic water use efficiency (assimilation divided by stomatal conductance) 34 days into drought treatment was higher in Far than Near ($p=0.001$); among droughted groups the contrast was greatest between Home-Far and Home-Near ($p = 0.002$). WUE in Home-Far was elevated over Away-Near ($p=0.028$) and Home-Near ($p=0.078$); WUE in Away-Far was higher than Away-Near ($p=0.063$) (Fig 5). Proximity continued to be significant thirty-seven days into the drought (Far > Near) for both assimilation ($p = 0.012$) and stomatal conductance ($p=0.024$). At this time, stomatal conductance was also higher for Home than Away ($p=0.016$) (Fig 4). Home-Far had the highest assimilation rates and stomatal conductance, with average carbon assimilation 22-23% higher than Home-Near ($p=0.008$) and Away-Near ($p=0.034$), and 17% higher than Away-Far ($p=0.074$); average stomatal conductance significantly exceeded Away-Near ($p=0.012$) (by 21%). By the final data collection session on day 42, Away-Far had the lowest assimilation rates. Contrasts from the significant site*proximity interaction ($p=0.038$) revealed a marginally significant differences between Away-Far and both Away-Near and Home-Far ($p=0.067$; 0.060).

Results from photosynthesis analyses by session can be seen in Table 2.

The full models combining all photosynthesis measurements throughout the data collection period (Table 3) were pared down to determine best-fit models (Table 4) for both assimilation and stomatal conductance. The model which best described assimilation includes interactions of water regime with data collection day as covariate, and site with day, plus block as a random effect. Both interactions, both main effects, and block are significant in this model; the AICc score is 1164.5 as compared with 1196.3 for the full model. A model with inoculation as a single factor of four levels reveals significant inoculation treatment main effects on assimilation ($p=0.041$); the strongest contrast is between Home-Far and Home-Near ($p=0.116$). For stomatal conductance, the model providing the best fit includes the 3-way interaction between water regime, site, and proximity, plus block as random effect and seed mass as a covariate. In this model, only water regime and block are significant; the AICc score was 2967.7 as compared with 3019.6 for the full model. In model with inoculation as a single factor, inoculation is not significant ($p=0.283$).

Prediction 5, that (a) Home-inoculated seedlings will have higher water use efficiency and photosynthetic rates, (b) Home-Far inocula will slow the decline in photosynthetic function under drought, and (c) Away-Far seedlings will have the lowest photosynthetic function, is partly supported. The Home versus Away contrast was only significant once - 36 days into the drought treatment application for stomatal conductance, and this was attributable to higher conductance in Home-Far; generally home inocula did not outperform Away inocula. Under drought, Home-Near seedlings' photosynthetic rates plummeted first and subsequently their WUE was reduced, especially relative to their Home-Far counterparts. In the latter half of the drought, Home-Far was the only inoculation treatment with any significantly elevated photosynthetic function, with the highest WUE on day 34, and highest assimilation and stomatal conductance on day 37. By the end

of the experiment, assimilation was significantly reduced in Away-Far. Overall, full and even simplified best-fit model contrasts could not be readily parsed, however, with inoculation as a single factor, assimilation in Home-Far exceeded Home-Near.

Table 2. LMER results for gas exchange data by session. Block effects (not included in table) were significant for gs in session 5, A in session 6, and WUE in session 1. Response variables were transformed with log (for WUE 1 and 2), square root (for gs 5), √(max(x+1)-x) (for A 4 and 6), and ^2 for WUE 4. All others were not transformed; Session 3 was eliminated due to abnormality as was Session 6 WUE. Highlighted values indicate emmeans post-hocs were conducted. If they are not included in the post-hoc tables (in appendix), contrasts' p-values were > 0.1.

Session (Day)	Factor	A		gs		WUE	
1 (16)		F	p	F	p	F	p
	WATER	0.485	0.493	2.185	0.154	3.149	0.090
	SITE	0.497	0.488	0.495	0.489	0.001	0.970
	PROX	3.069	0.093	2.060	0.166	0.375	0.547
	WATER*SITE	2.116	0.159	**4.883**	**0.038**	**7.224**	**0.014**
	WATER*PROX	**4.338**	**0.048**	2.995	0.098	0.017	0.896
	SITE*PROX	**5.398**	**0.029**	1.176	0.290	0.798	0.382
	WATER*SITE*PROX	0.046	0.832	0.076	0.785	0.012	0.913
2 (22)		F	p	F	p	F	p
	WATER	0.164	0.690	0.555	0.464	2.568	0.124
	SITE	**4.270**	**0.051**	2.288	0.145	0.249	0.623
	PROX	1.806	0.193	1.528	0.230	0.155	0.698
	WATER*SITE	3.297	0.084	1.261	0.274	0.117	0.736
	WATER*PROX	2.315	0.143	1.419	0.247	0.017	0.897
	SITE*PROX	1.141	0.298	0.757	0.394	0.006	0.938
	WATER*SITE*PROX	**5.119**	**0.034**	1.412	0.248	1.059	0.317
4 (34)		F	p	F	p	F	p
	WATER	**9.248**	**0.006**	**35.560**	**<0.001**	**5.903**	**0.024**
	SITE	1.034	0.320	2.805	0.110	0.436	0.516
	PROX	1.900	0.182	0.567	0.461	**.902**	**0.001**
	WATER*SITE	0.926	0.346	0.348	0.562	0.265	0.612
	WATER*PROX	2.001	0.171	0.018	0.894	**6.517**	**0.018**
	SITE*PROX	0.563	0.461	0.025	0.875	0.004	0.947
	WATER*SITE*PROX	0.576	0.456	0.042	0.840	0.136	0.715
5 (37)		F	p	F	p	F	p
	WATER	**276.485**	**<0.001**	**324.941**	**<0.001**	0.643	0.426
	SITE	1.939	0.169	**6.078**	**0.016**	0.156	0.695
	PROX	**6.700**	**0.012**	**5.340**	**0.024**	1.530	0.221
	WATER*SITE	0.022	0.882	0.818	0.369	0.067	0.797
	WATER*PROX	0.001	0.981	1.700	0.197	0.643	0.426
	SITE*PROX	**4.344**	**0.041**	0.308	0.581	3.080	0.084
	WATER*SITE*PROX	0.960	0.331	0.005	0.945	1.884	0.175
		F	p	F	p		
	WATER	**289.982**	**<0.001**	**177.388**	**<0.001**		
	SITE	2.368	0.129	2.153	0.147		
	PROX	1.960	0.167	0.068	0.795		

6 (42)	WATER*SITE	0.000	0.994	0.080	0.779		
	WATER*PROX	0.940	0.336	2.172	0.146		
	SITE*PROX	**4.500**	**0.038**	1.290	0.260		
	WATER*SITE*PROX	2.778	0.101	**5.918**	**0.018**		

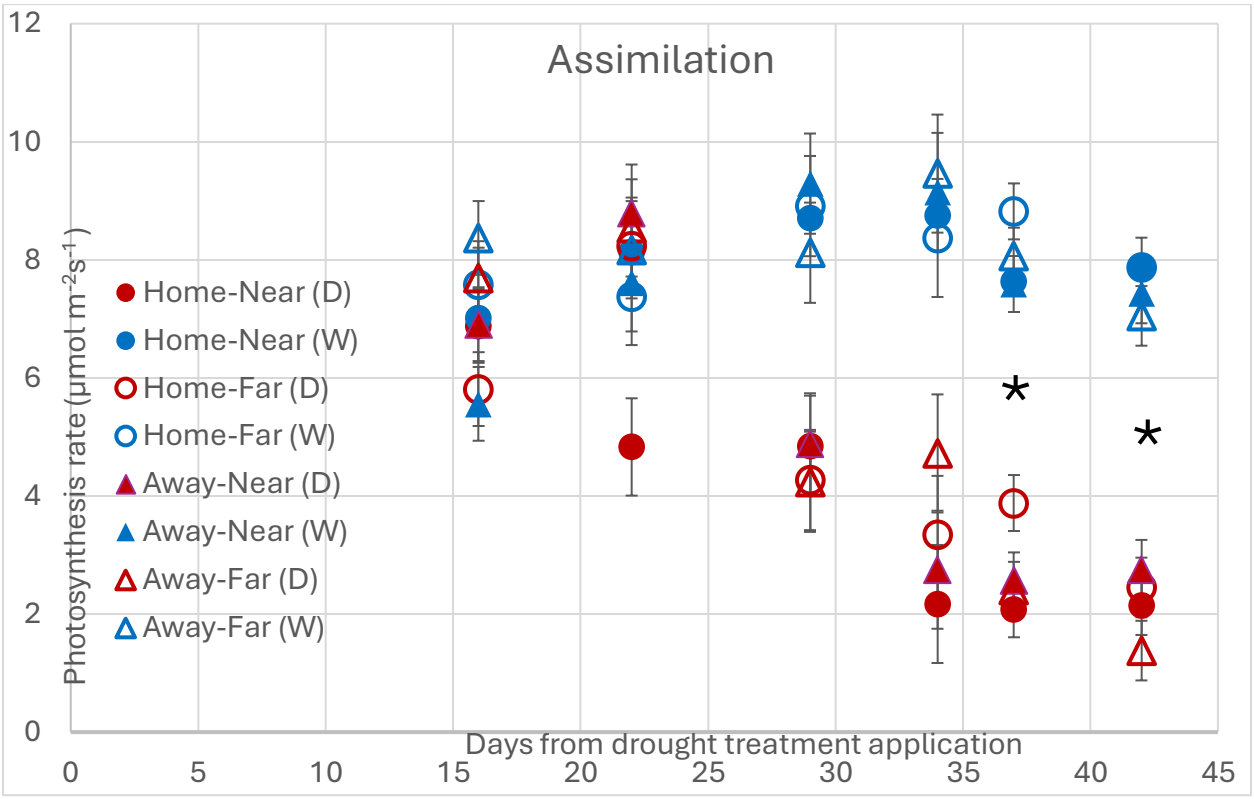


Figure 3. Asssimilation rates emmeans for each treatment and session ± emmeans SE. Asterisks indicate statistically significant site-proximity interaction and inoculation main effect.

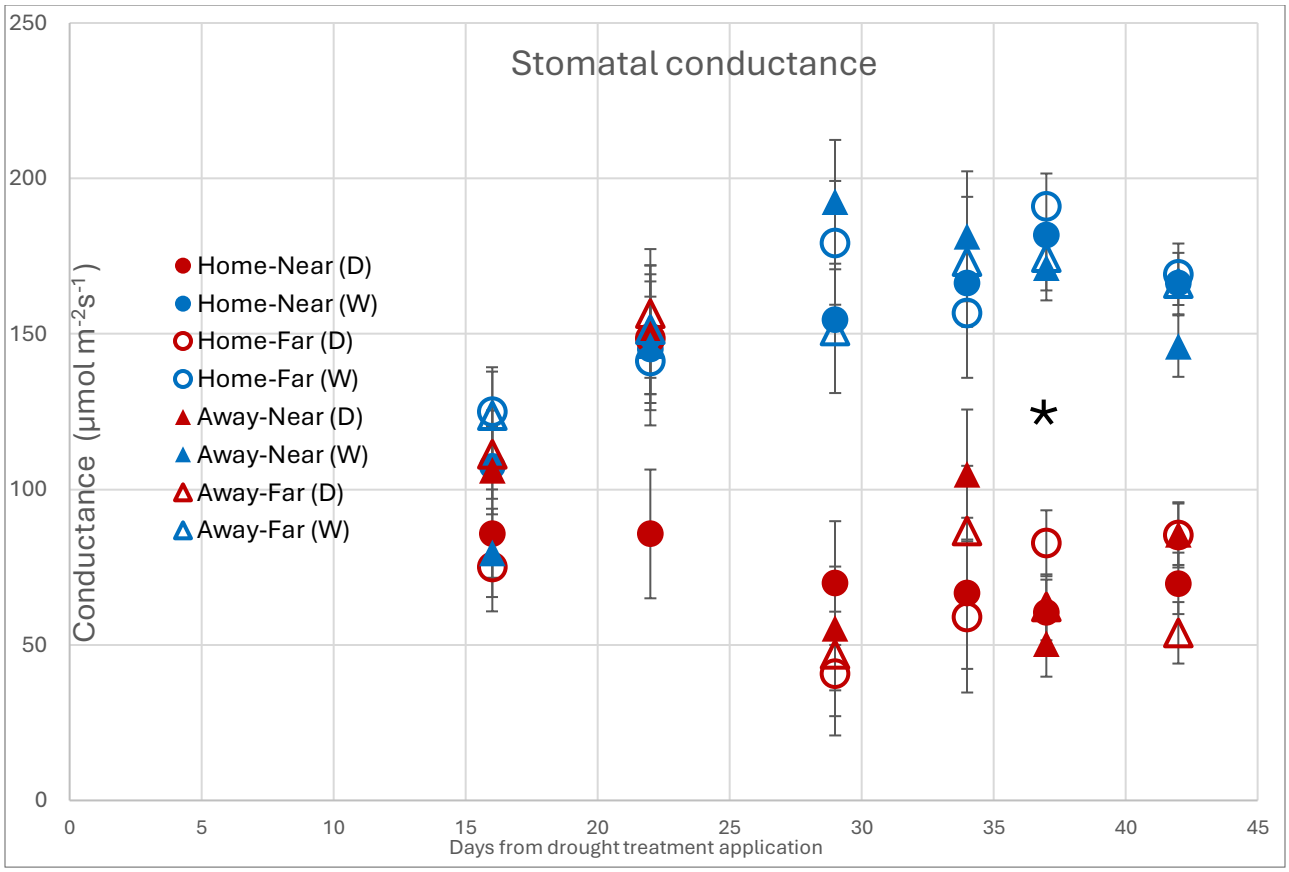


Figure 4. Stomatal conductance emmeans for each treatment and session ± emmeans SE. Asterisk indicates statistically significant inoculation main effect.

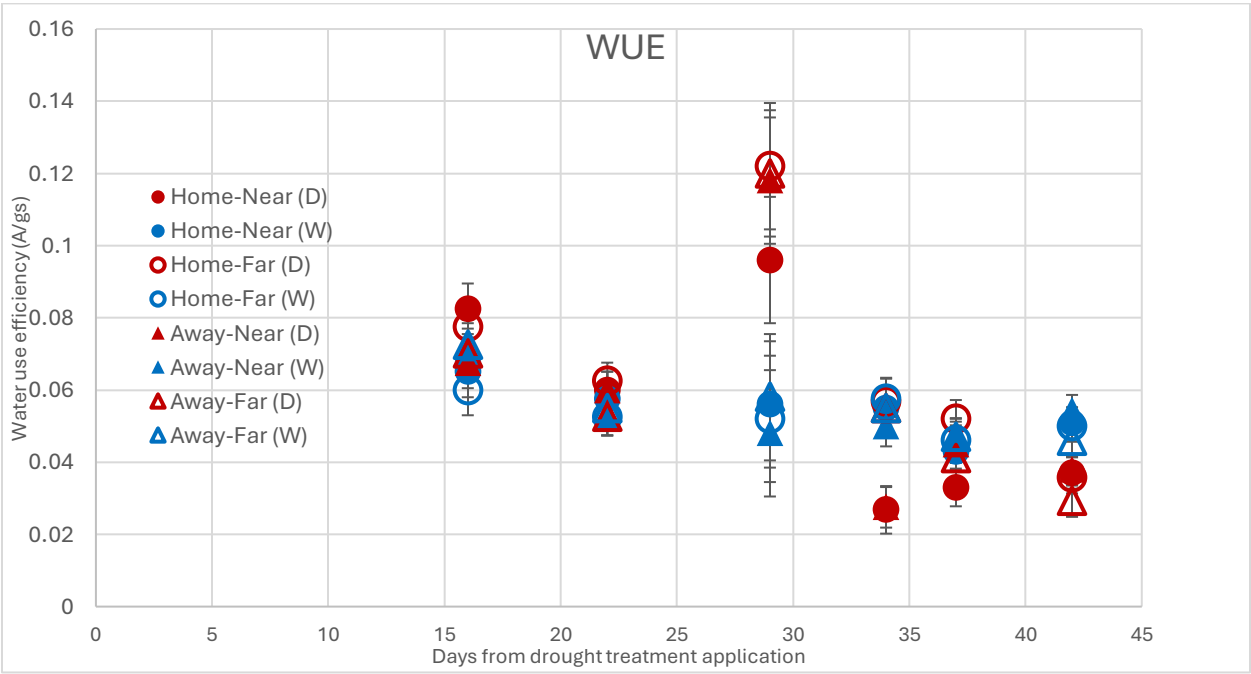


Figure 5. WUE (A/gs) for each treatment and session ± emmeans SE. Asterisk indicates statistically significant site*proximity interaction and inoculation main effect.

Table 3. Results of full linear mixed effects model for cumulative assimilation and stomatal conductance. (X~WATER*SITE*PROXIMITY*DAY + SEEDMASS + (1|BLOCK)

FULL MODEL	A		gs	
	F	p	F	p
WATER	**16.946**	**<0.001**	2.467	0.117
SITE	**4.024**	**0.046**	3.241	0.073
PROX	2.225	0.136	0.554	0.458
DAY	**90.440**	**<0.001**	0.291	0.590
WATER*SITE	2.216	0.138	2.267	0.133
WATER*PROX	0.069	0.793	0.011	0.916
SITE*PROX	2.130	0.146	0.027	0.870
WATER*DAY	**97.041**	**<0.001**	**40.907**	**<0.001**
SITE*DAY	**4.364**	**0.038**	3.543	0.061
PROX*DAY	1.175	0.279	0.290	0.591
WATER*SITE*PROX	0.274	0.601	0.174	0.677
WATER*SITE*DAY	1.458	0.228	1.639	0.202
WATER*PROX*DAY	0.094	0.760	0.000	0.984
SITE*PROX*DAY	3.504	0.062	0.224	0.637
WATER*SITE*PROX*DAY	0.031	0.861	0.461	0.498
SEEDMASS	0.617	0.433	0.755	0.386
BLOCK		**<0.001**		**0.014**

Table 4. Results of the best-fit model for assimilation and stomatal conductance across all data collection sessions. Assimilation model includes the interactions of water* day and site*day, plus block as a random effect; the stomatal conductance model includes a three-way interaction among water, site, and proximity, seed mass as a covariate, and block as a random effect.

BEST MODEL	A ~WATER*DAY + SITE*DAY + (1|BLOCK)		gs ~WATER*SITE*PROX + SEED MASS + (1|BLOCK)	
	F	p	F	p
WATER	**17.552**	**<0.001**	**297.590**	**<0.001**
SITE	**3.903**	**0.049**	0.007	0.934
PROX	-	-	0.976	0.324
DAY	**88.966**	**<0.001**	-	-
WATER*SITE	-	-	1.173	0.280
WATER*PROX	-	-	0.086	0.769
SITE*PROX	-	-	1.016	0.314
WATER*DAY	**98.263**	**<0.001**	-	-
SITE*DAY	**4.150**	**0.043**	-	-
PROX*DAY	-	-	-	-
SEEDMASS	-	-	0.027	0.870
BLOCK		**<0.001**		**0.038**

Biomass

Growth and development

Seed mass had significant positive effects on all biomass variables (Table 5).

The water treatment significantly impacted growth, with greater dry mass at harvest in well-watered seedlings across all dry mass fractions (Table 5, Fig. 6). Root mass was most reduced by drought; leaf mass was least impacted. Droughted seedlings' root mass was 37% lower on average than well-watered seedlings'; stem mass was reduced by 23.5%, and leaf mass by 17%.

Inoculation treatment main effect was significant for some biomass variables. Stem mass was largest in Away-Far, significantly exceeding Away-Near (by 16%) ($p=0.007$). Above ground biomass in Away-Far was also greater than other inoculation treatment groups, with a marginally significant difference (13% increase) over Home-Near ($p=0.082$). Home-Far seedlings' root to shoot ratio was 14.5% larger than Away-Far ($p=0.015$) (Fig 7.). There were no significant differences in size among droughted seedlings.

Leaf dry mass, root dry mass, and total dry mass did not differ significantly among inoculation treatments.

Home-Near seedlings put on fewer new leaves over the course of the experiment. They produced significantly fewer new leaves than Away-Near ($p=0.007$) (1.9 versus 2.8) across water treatments. Among well-watered groups, average leaf increase in Home-Near was 2.5, significantly lower than both Away-Near ($p=0.006$) and Home-Far ($p=0.033$) (with 3.9 and 3.6 new leaves on average).

None of prediction 6 - that (a) watered Home-Near will be largest; (b) among droughted seedlings Home-Far might be largest; (c) Away-Far will be smallest - is supported. Home-Near seedlings were not larger and put on the fewest new leaves; droughted seedlings did not differ in

size. Away-Far seedlings had the largest stem and aboveground biomass.

Table 5. LMER results for biomass. Seed mass used as covariate and all analyses conducted using random block effects. All biomass variables were log-transformed for analysis except seed mass and root to shoot ratio.

	LEAF		STEM		ABOVE		ROOT		ROOT TO SHOOT		TOTAL	
	F	p	F	p	F	p	F	p	F	p	F	p
WATER	**16.837**	**<0.001**	**67.392**	**<0.001**	**32.084**	**<0.001**	**269.705**	**<0.001**	**46.897**	**<0.001**	**185.610**	**<0.001**
INOC	0.993	0.396	**4.101**	**0.007**	2.053	0.107	1.988	0.117	**3.443**	**0.018**	0.292	0.831
WATER*INOC	1.330	0.265	1.517	0.210	1.190	0.313	0.511	0.675	1.578	0.196	0.649	0.584
SEED MASS	**16.800**	**<0.001**	**30.808**	**<0.001**	**21.603**	**<0.001**	**71.467**	**<0.001**	1.734	0.190	**69.046**	**<0.001**
BLOCK		**<0.001**		**<0.001**		**<0.001**		0.294		**0.013**		0.072

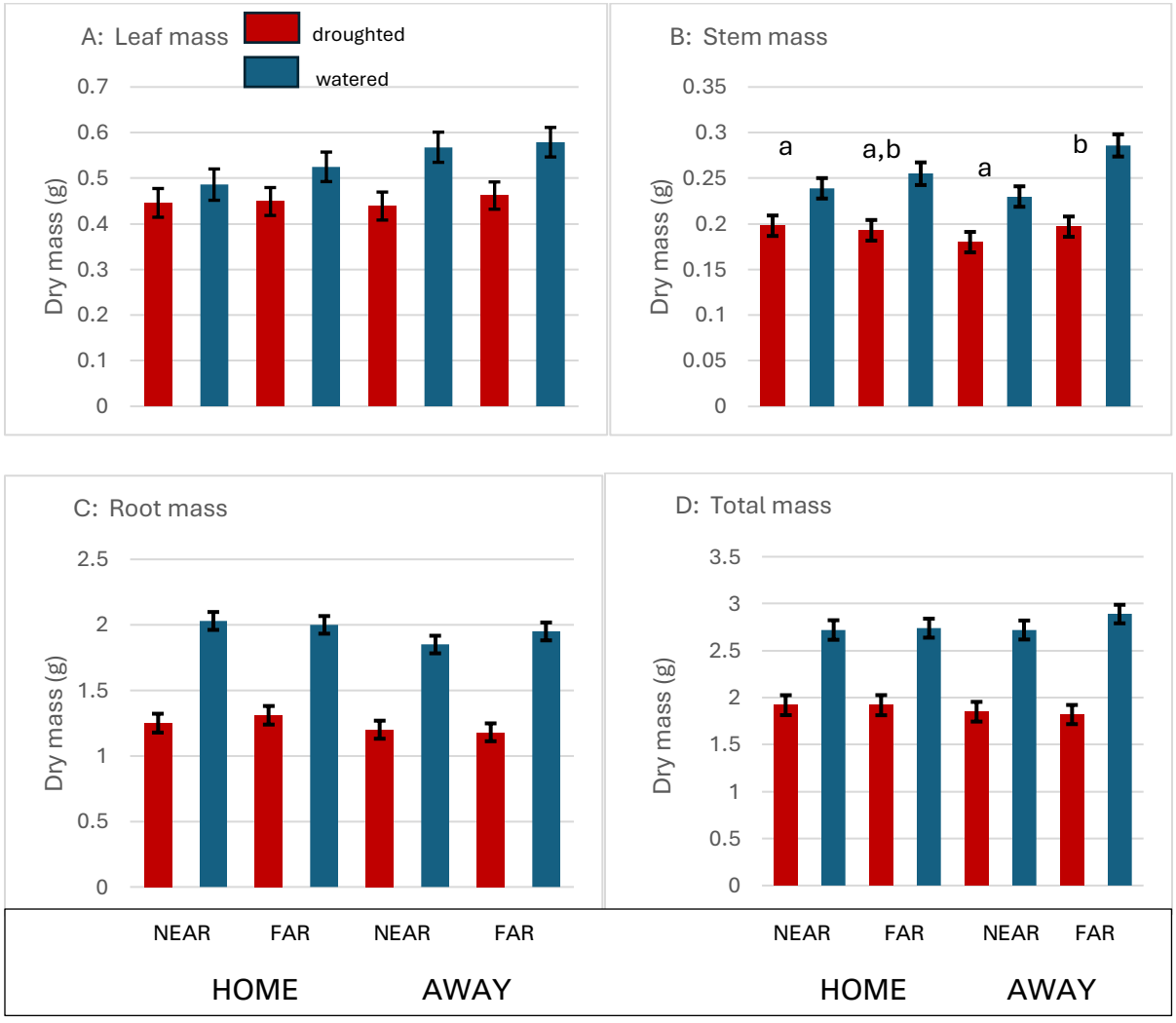


Figure 6. Dry biomass emmeans for each treatment ± emmeans SE for leaf (a), stem (b), root (c), and total (d). Letters indicate significant differences in inoculation treatment main effects.

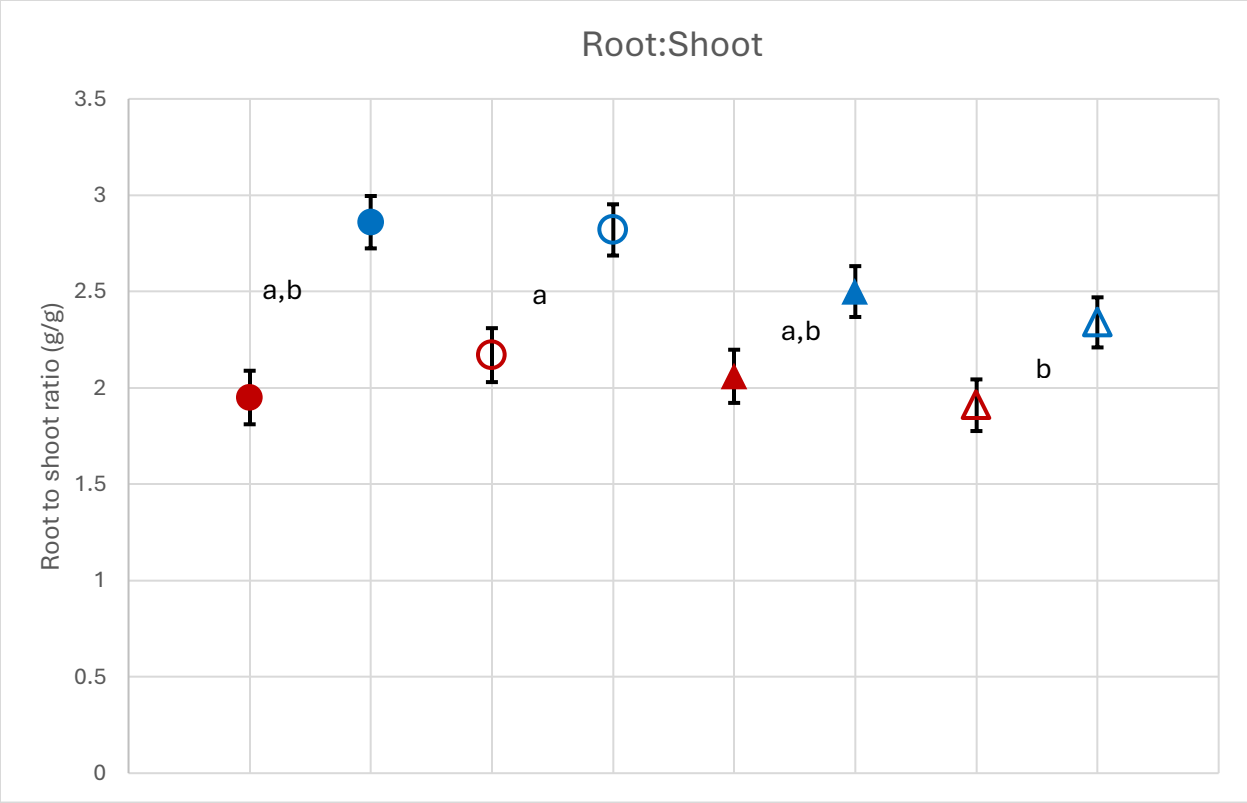


Figure 7. Emmeans root to shoot ratios for each treatment ± emmeans SE. Letters indicate significant differences in inoculation treatment main effects.

Days to desiccation

Log-rank time to event analysis in the drought treatment group revealed significant inoculation effects on time to total leaf desiccation (Chisq = 11.7 on 3 degrees of freedom, p = 0.009), with Home-Far seedlings retaining viable leaves furthest into drought and Away-Far seedlings desiccating soonest (Fig. 8). Pairwise comparisons show significant difference between Home-Far and Away-Far (p=0.002), and marginally significant difference between Away-Near and Away-Far (p=0.078). Contrast between Home-Far and Home-Near was not statistically significant (p=0.178). These results align with prediction 7.

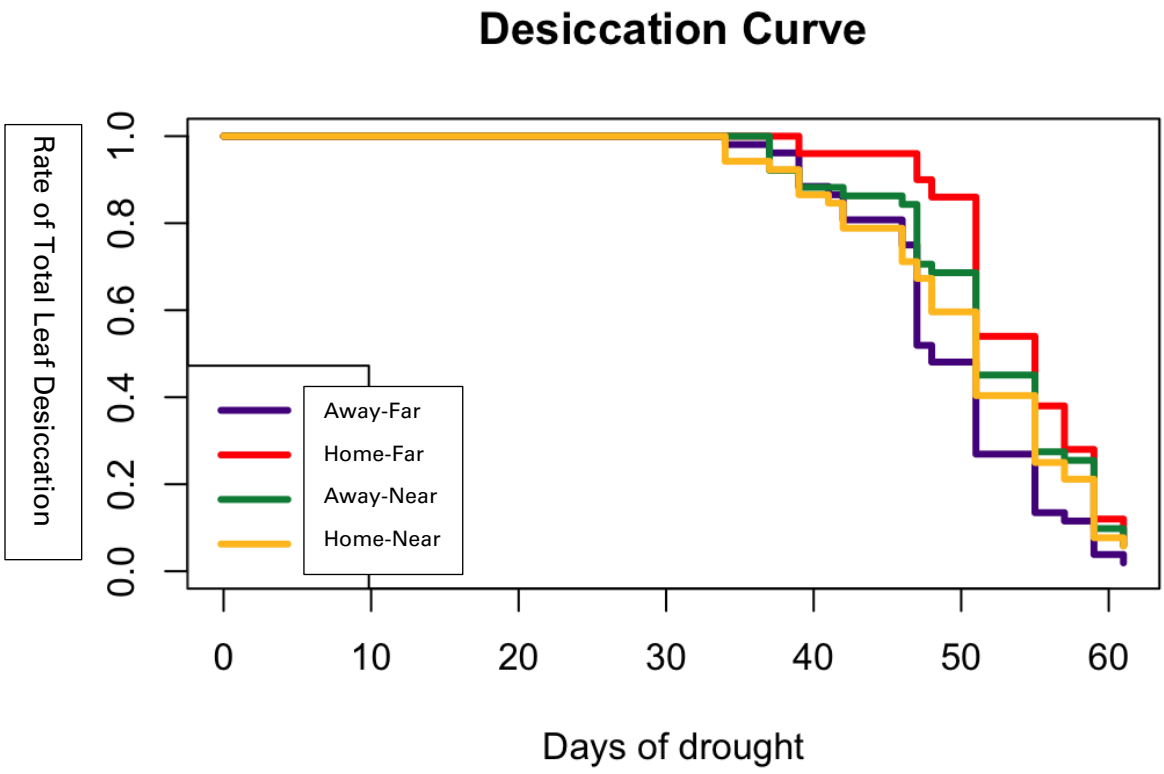


Figure 8. Rate of total leaf desiccation for droughted plants by inoculation treatment

Mycorrhization

All seedlings' root samples examined were ectomycorrhizal. Across inoculation treatment groups in well-watered seedlings ~70-90% of root tips were live EM (Fig. 9). Drought reduced levels of live ectomycorrhization to ~30-40% ($p < 0.001$); the drought treatment also lowered rates of overall mycorrhization (proportion of living and dead EM root tips) ($p=0.020$) (Fig 10.) Mycorrhization potential, as assessed by overall mycorrhization in the well-watered group only, did not differ by inoculation treatment ($p=0.760$), however live EM proportions were higher in Home-Far, especially as compared with Away-Near, though this contrast was not statistically significant ($p=0.114$).

Seed mass did not correlate with overall mycorrhization ($p=0.476$), or living mycorrhization ($p=0.953$). Mycorrhization (living or overall) did not influence any aboveground biomass variable in a statistically significant way, with only a marginally significant difference in leaf biomass in relation to overall mycorrhization ($p=0.092$). Neither did mycorrhization affect days to total leaf desiccation (living $p=0.384$; overall $p = 0.408$). Living mycorrhizal root tips were more frequent in the Home-Far group, especially as compared with Home-Near (Fig 11), though inoculation was not a statistically significant predictor of mycorrhization rate ($p= 0.216$).

Community diversity, as evaluated by Shannon Diversity Indices of morphotype categories, was reduced by drought ($p<0.001$), but not influenced by inoculation treatment ($p=0.432$) (Fig 12). In the 3-way analysis with site and proximity as separate factors, Away seedlings had higher diversity than Home, but this did not reach the threshold of statistical significance ($p=0.110$). Fourteen morphotypes accounted for 99.5% of all root tip observations across inoculation treatments (Fig 13). The five morphotypes with frequencies over 5% accounted for ~85% of all EM root tips (Table 6). So far molecular work has revealed that the community is

dominated by Ascomycetes. Most successful sequences have been from the Order Pezizales in the families Pezizaceae, Pyronemataceae, or Tuberaceae. The most common morphotype (M4) has yet to be successfully sequenced.

Arbuscular mycorrhizae, if present at all, are uncommon. Of the small subset of each treatment group examined, only 3 samples contained hyphae which may be AM, but no vesicles are arbuscules. Dark septate endophytes were prevalent across inoculation treatment groups, especially in well-watered treatments.

Predictions 1 (dual status) and 4 (increase in AM with drought) are not supported, as there was no evidence of AM. Prediction 2, that (a) rate and diversity of EM would be highest with Home inocula (b) especially with Home-Near inocula, and that (c) Away-Far seedlings would have the lowest rates and diversity, is not supported. Prediction 3, that ectomycorrhization and EM diversity will be drastically reduced by drought, is supported.

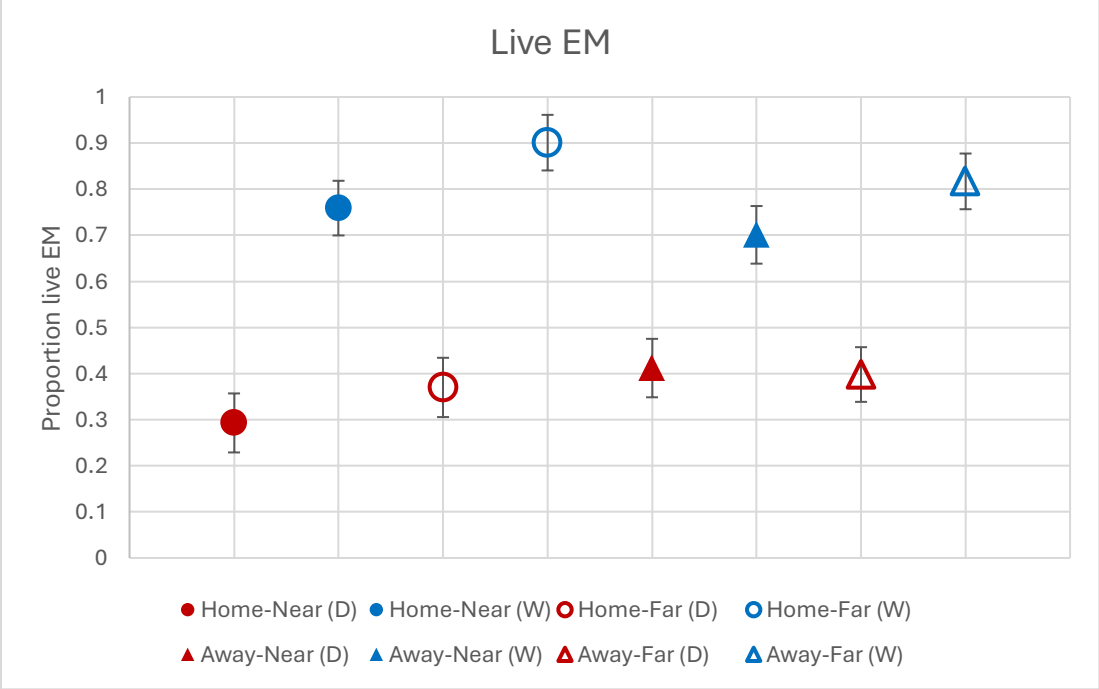


Figure 9. Proportion of living ectomycorrhizal root tips emmeans by treatment ± emmeans SE.

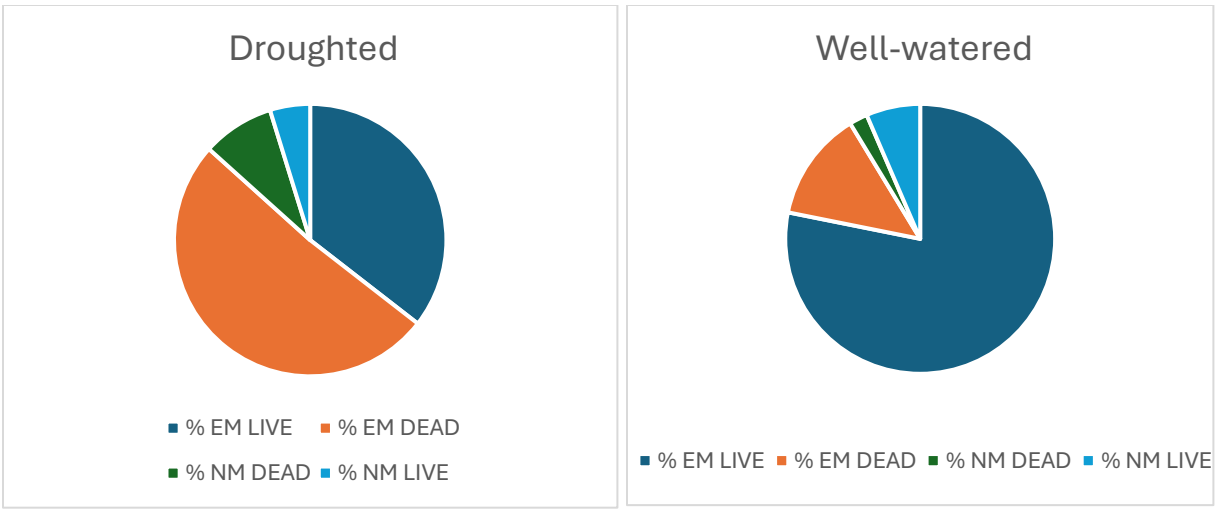


Figure 10. Percent root tips with living ectomycorrhizae (EM LIVE), dead ectomycorrhize (EM DEAD), dead nonmycorrhizal (NM), or live nonmycorrhizal (NM LIVE), averaged across inoculation treatments.

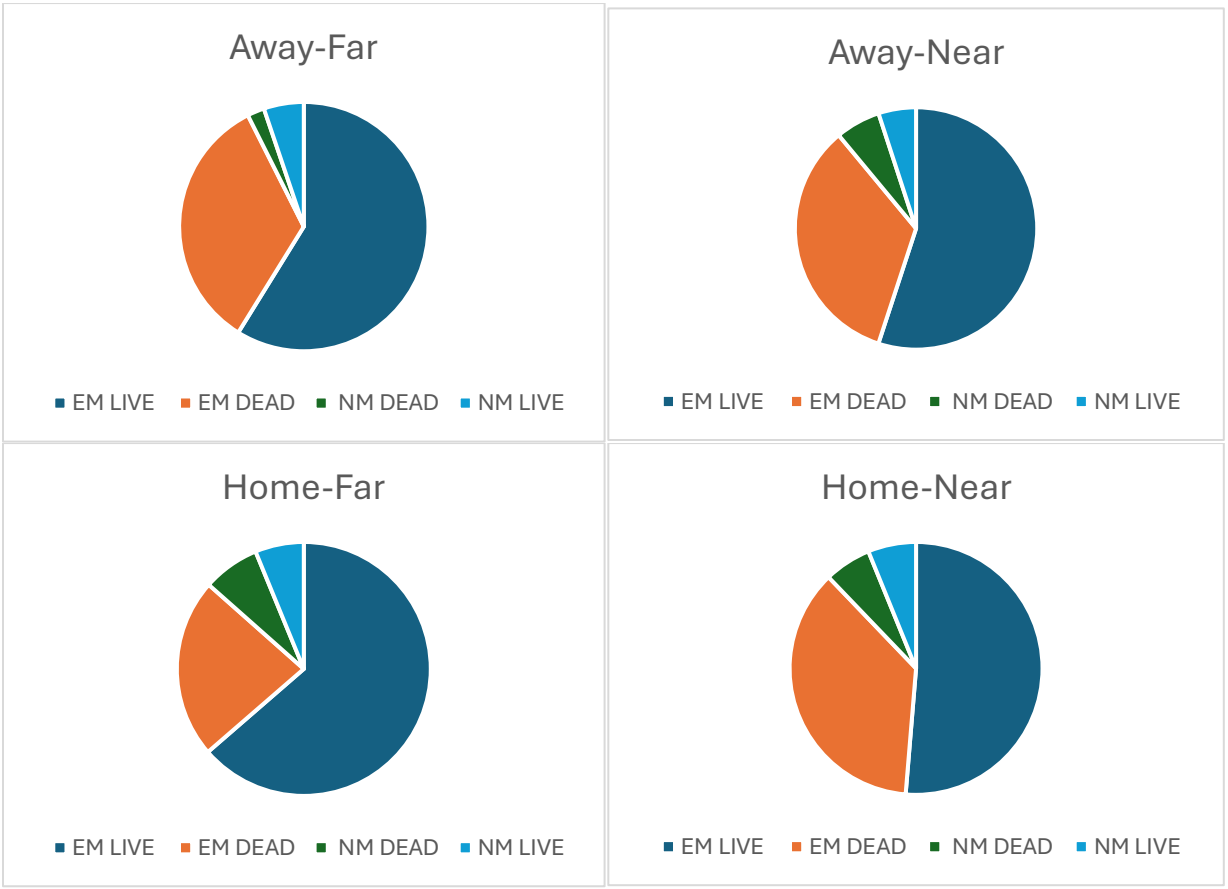


Figure 11. Percent root tips with living ectomycorrhizae (EM LIVE), dead ectomycorrhize (EM DEAD), dead nonmycorrhizal (NM), or live nonmycorrhizal (NM LIVE), averaged across water treatments.

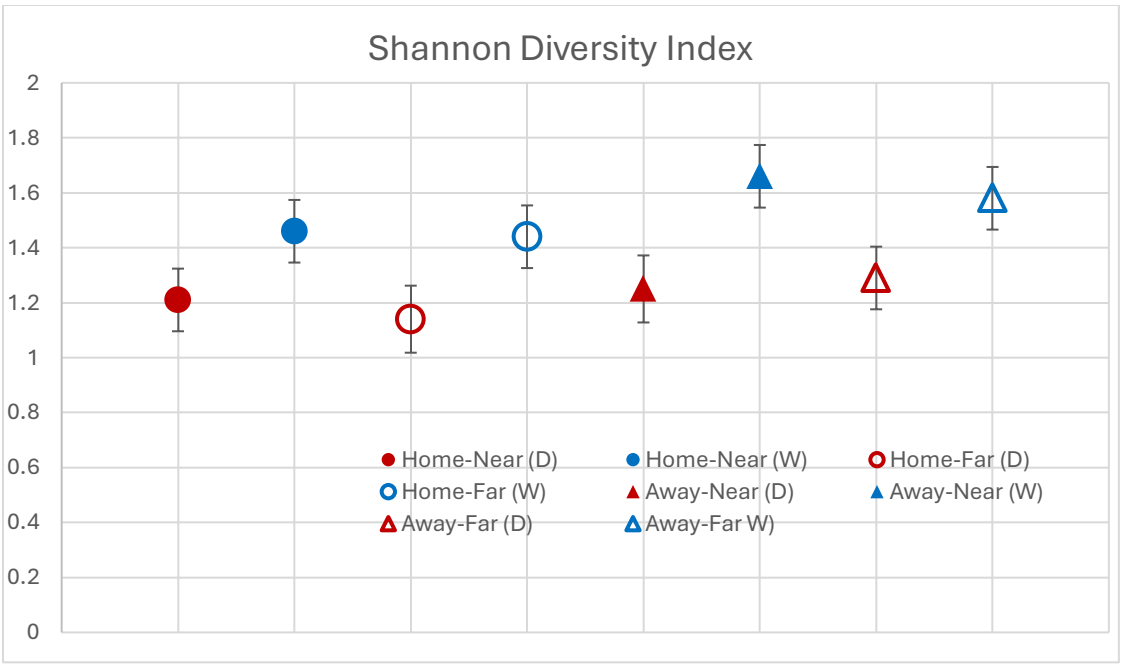
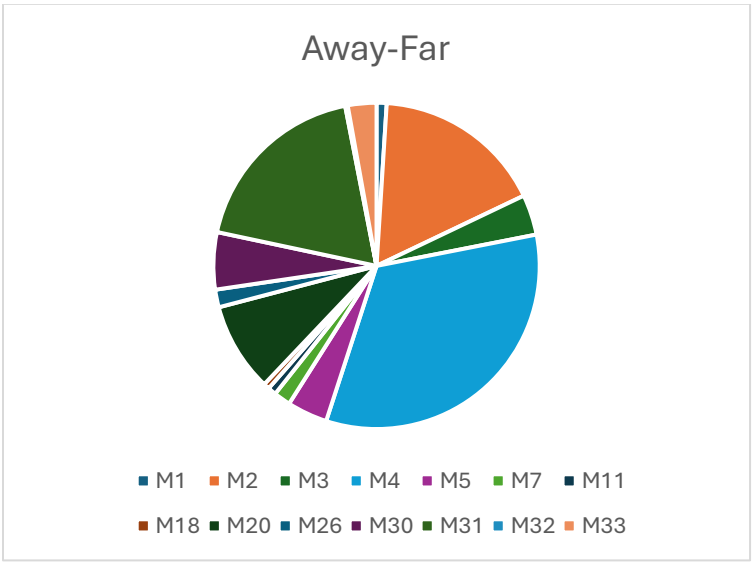


Figure 12. Shannon Diversity Indices emmeans by treatment ± emmeans SE.



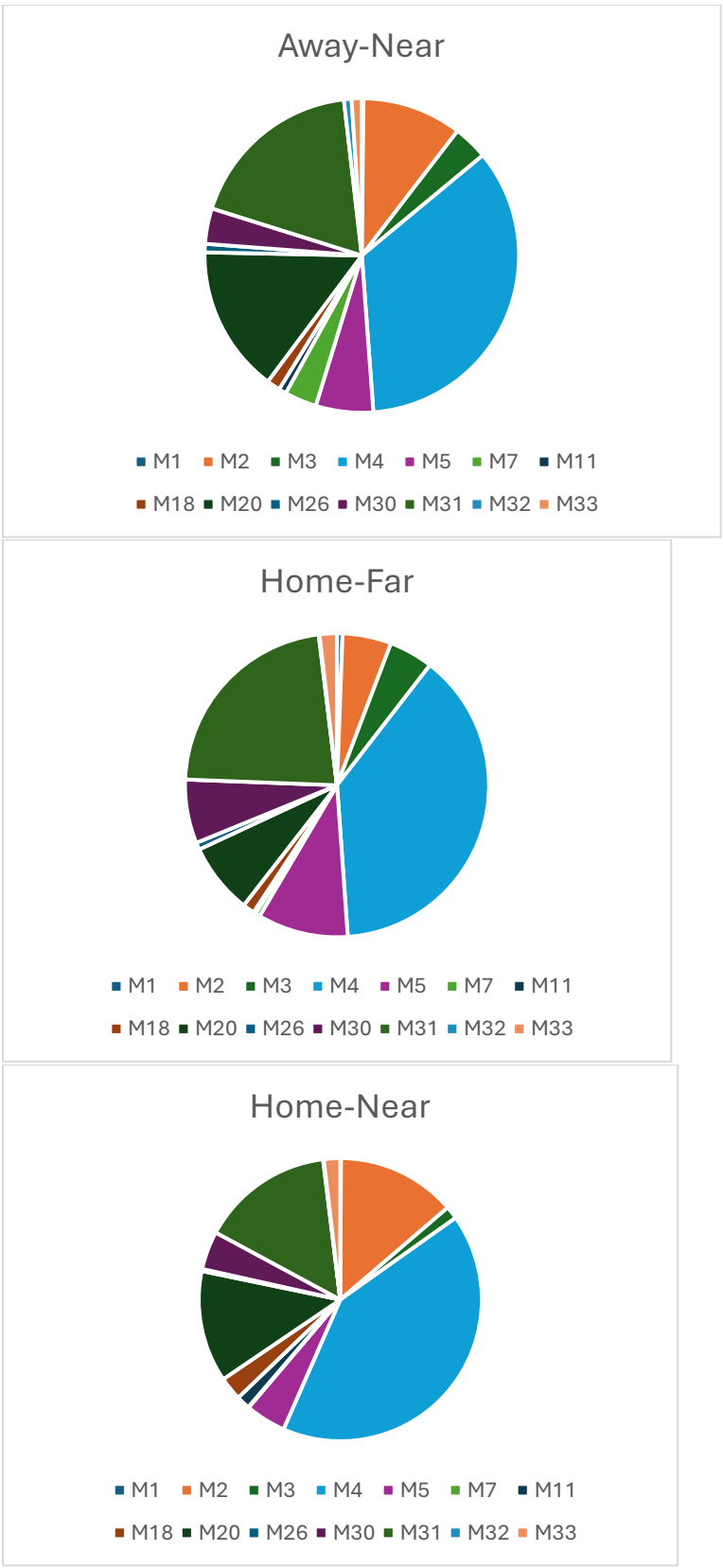
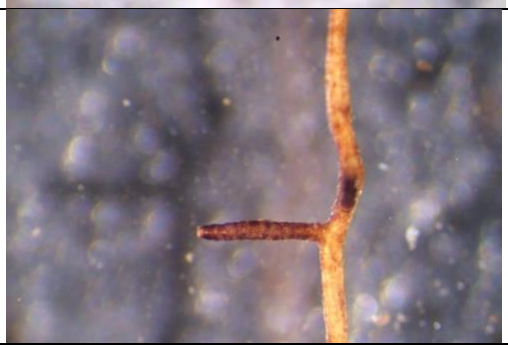


Figure 13. Pie charts depicting proportion of the 14 morphotypes accounting for >99.5% of all live EM root tips across inoculation treament groups.

Table 6. Descriptions and photos of the 5 most common morphotypes. Cumulatively accounted for >85% of morphotype diversity.

MORPH	NAME	DESCRIPTION	TAXA	PHOTO
M2	CATTAIL	bulbous part a beige ("cinnamon sugar") cattail flower before going to seed; occasionally some white stringy hyphae, more likely attached to maroon part or slight fuzz on the cattail. Cattail velvety-felty, straight-sided or sometimes bent; some forked but if branching cluster -> M18. "Stalk" maroon-red/orange usually velvety-felty, sometimes grainy, sometimes white fuzzy/felty hyphae or occasionally a few stringy hyphae. Occasionally just cattail seed head and no stalk.	Tuber sp.	
M4	Maroon White-Tip	Color diagnostic – if orangey at all probably M5, color should be deeper red- dark maroon If branched -> M15 Texture smooth to velvety-felty. Tapering to bright white tip.	unsuccessful	
M5	Orange Light Tip	Same texture and shape as M4 but a yellow/orange-orange/red color.	inconclusive	

M20	Dark Maroon	M4 but no white tip. Could be older version of M4. Many senescing roots look like this. Often white felty hyphae.	inconclusive	
M31	Mottled Dark Morph	Similar to M20 but with more mottled/grainy appearance.	inconclusive	

IV. DISCUSSION

Synthesis of results

Soil provenance did not constrain mycorrhizal inocula potential as all seedlings were highly mycorrhized by a seemingly similar ectomycorrhizal community; however, inocula did significantly influence seedling physiology, indicating potential mutualist limitation and/or enemy introduction by inoculation treatment. The trends in performance indicate that Home-Far inoculum most mitigated drought stress, as evidenced by their elevated photosynthetic rates in the mid-late drought and longer time to total leaf desiccation; Away-Far inocula was least beneficial to seedlings, as they succumbed most readily to drought pressure. These results concur with my predictions. In contrast to expectations, Home-Near inocula did not promote increases in growth or photosynthetic rate. Although this study cannot parse the microbial contributors to these results, it seems likely that the soil community influenced outcomes for seedlings.

Treatment effects on seedling performance

Seedlings inoculated with rhizosphere soil from beneath junipers at the home site performed better under drought than all other treatments - including those inoculated with rhizosphere soil from conspecific oaks at the same site - contrary to most logic suggested by the scientific literature, which tends to assert that seedlings under EM canopy benefit from positive plant-soil feedbacks (Liang et al., 2020). Contrary to expectation, Home-Near inocula did not improve seedling performance and in some cases even decreased it: For example, their photosynthetic rates plummeted earliest into drought, and they produced the fewest new leaves. Home-Far seedlings, however, had higher WUE and photosynthetic rates at certain points; their rate of photosynthesis over the course of the entire experiment was also highest. Additionally, they

retained viable leaves longer and had the greatest root to shoot biomass ratio. Away-Far inoculated seedlings had the lowest root to shoot ratio, lowest photosynthetic rates by the end of the drought, and their leaves desiccated most rapidly. While total leaf desiccation is not necessarily fatal, drought-induced leaf drop is not a feature of this oak's typical phenology (pers. obs), and whether they would have the energy required for a new flush of leaves would depend on the severity and duration of drought. Overall, results suggest that neither site nor proximity were influential factors on their own, but that inoculation treatment did impact seedlings: homesite juniper-inoculated seedlings (Home-Far) had higher photosynthetic function and demonstrated the most drought resilience.

Acorn biomass is one of the most consistent predictors of early size in oaks (Quero et al, 2007), and it strongly influenced seedling biomass in this experiment. Until seedlings deplete their seed reserves, mycorrhizae may be less important for growth (Tateishi et al., 2001). In our study, biomass at harvest was only significantly affected by the drought treatment, except for stem biomass, which differed between the well-watered Away-Near and Away-Far seedlings, but not between the Home and Away treatments as predicted. Without good explanation for this result and affecting only 10% of the total seedling biomass, we consider this potentially spurious and conclude that seedling size at harvest was chiefly limited by seed provisioning and by drought. It is also worth mentioning that for slow-growing long-lived species, putting on weight early in life is not necessarily the goal; nutrient content would be a more important indicator of health status than size.

Duality

An assumption of duality undergirded most hypotheses, including and especially that seedlings would benefit most from compatible AM mutualists provided by old-growth juniper

inocula under drought conditions. Although there was an occasional plant with some possible AM hyphae, this was uncommon, and exchange structures were not observed. This is consistent with data collected from roots of adult Texas red oak from the same preserve system (Gehring, personal communication, October 2025). The mycorrhizae of most western United States oaks have not been studied; neither have Texas red oak's closest kin, *Quercus shumardii* and the endangered *Quercus acerifolia*. Their next closest relative, according to at least one phylogeny (Hipp et al., 2019), is *Quercus rubra*, who have been shown to form AM but not to benefit from them (Dickie et al., 2002). Given that AM propagules are widespread, it seems safe to conclude that Texas red oak does not usually associate with AM.

Inocula Potential and Mycorrhization

Predictably, the fraction of live EM was more than halved by drought; however, contrary to expectations mycorrhization was high across inoculation treatments, with no more EM in Home-Near seedlings than other groups. Diversity was also reduced by drought as expected, however even the water regime did not dramatically influence community composition. A few of the rarest morphotypes (accounting for less than 1% of EM root tips) dropped off with drought, but the most prevalent partners remained without major shifts in abundance. However, root senescence in the drought treatment made determining EM status and morphotype difficult; we cannot know for certain which EM were present.

Relative abundance of mycorrhizal morphotypes was not influenced by inoculation treatment as predicted. The same suite of morphotypes occurred in all treatments; five of them accounted for most EM roots. The slight shifts in community composition with inoculation showed the opposite trend as expected, with higher diversity in Away than Home, although this did not rise to the level of significance. Except for reduction of a *Tuber* sp. in Home-Far treatments, there are

no evident significant differences in relative abundance of any given morphotype by inoculation treatment.

With morphotyping as a coarse proxy for fungal mycorrhizal identity and few molecular results, it appears that inoculation yielded similar compositions of mycorrhizal partners for oak seedlings. Further molecular work may reveal that communities are distinct, however at this juncture we cannot attribute differences in seedling performance to their mycorrhizae. Morphotyping followed by sequencing of a small subset of root tips is unlikely to pick up on the rarest fungal taxa (Grove et al., 2019), however to the extent that morphotype categories approximate OTUs, the bulk of the root tips were mycorrhized by the same symbionts regardless of treatment. This method provides a picture of the predominant members of the mycorrhizal community.

This study indicates that inocula potentials were not constrained by either site or proximity. Outside of the rooting zone of a compatible host, a seedling relies on the spore bank to establish (Ashkanejahad and Horton, 2006). Limitations on sporocarp production in semi-arid places and spore dispersal distances which generally do not exceed a few meters (Li, 2005), mean that proximity to mature trees or persistence of durable propagules are the methods by which an oak seedling would access mycorrhizal partners. In the case of our Away-Far sites, the spore bank would likely consist of a mixture of recalcitrant propagules who withstood the 20 years without forest, and those who dispersed from sporocarps near mottes onsite. At this site, live oak is still common and may be a host to many of these mycorrhizal fungi; spores from live oak mottes may disperse into clearings. Most spores do not travel far; basidiospores in particular disperse within only about a meter of the sporocarp (Galante, Horton, and Swaney, 2011).

There are few studies on spore viability over long time periods. The absence of living host

trees – at least for nine years - does not necessarily reduce EM abundance, but does change the community composition (Mueller et al., 2009). However, twenty years should have been sufficient to also significantly reduce inocula potential. In a study on sites extirpated of pinyon, 16 years was enough to alter community composition and reduce percent mycorrhization (Montes and Taylor, 2020); diversity was more severely impacted at sites of extirpation by drought rather than human intervention.

There is evidence that some spores, such as the mammal-dispersed Rhizopogon, can remain viable for at least 4 years and actually increase inoculum potential over time (Bruns et al., 2009), whereas other species loose viability (Ashkannejhad and Horton, 2006). Basidiospores do not seem to persist or accumulate in spore banks as much as ascospores (Nara, 2008), and Ascomycota are more prevalent under drought conditions (Gehring et al., 2014). Most of the taxa we were able to identify are Ascomycetes.

Tuber species were somewhat more frequent in Away-Far treatment seedlings and are dispersed via fecal pellets of mycophagous rodents (Frank et al., 2009). Rodent-dispersal of hypogeous spores were credited for second-year *Quercus garryana* forming mycorrhizal partnerships up to 15 m from mature oak (ibid). Additionally, evidence suggests *Tuber* may be able to associate as endophytes with herbaceous plants not regarded as ectomycorrhizal hosts (Gryndler et al., 2014; Schneider-Maunoury et al., 2018), so they may be able to proliferate more readily outside forest habitats – possible explanations for their higher relative abundance in the Away-Far seedlings.

Likewise, at the homesite, whether or not inocula was collected proximate to adult EM hosts, EM fungal partnerships formed. Although attempts were made to collect Home-Far inocula outside of an oak network, oak seedlings were present in the immediate vicinity, and we cannot

truly claim that Home-Far inocula was outside of oak CMN. Oak seedlings have been observed to have roots entangled with adult oaks more than 13 m away in this preserve system (O'Donnell et al., 2020). It is unlikely that future studies will be able to parse the effects of old-growth Ashe juniper inocula from an oak network, both because oak invariably co-occur with this juniper, and because old-growth juniper has practically vanished from the Edwards Plateau. Nearly 30 years ago, only a few documented old-growth juniper forests remained; one of which is this homesite (Diamond, 1997).

Greenhouse Mycorrhizae

Inocula potential was not reduced by treatment; however, the fact that this study was conducted in the greenhouse imposes limits on the ability to test mycorrhization effects. Mycorrhizal community composition can differ starkly between greenhouse and field-grown seedlings, even when greenhouse seedlings are provisioned with field soil as inoculant (Sýkorová et al, 2007). In one study on *Quercus garryana*, greenhouse seedlings partnered with *Laccaria* sp. and *Tuber* sp.; field-grown seedlings' assemblage consisted of 13 species, none of which appeared in the greenhouse (Southworth et al., 2009). In another study, chestnut (*Castanea dentata*) seedlings inoculated with *Q. rubra* soil from their former range, partnered with 14 EM species that barely overlapped with the 38 species observed in field-grown seedlings (Dulmer et al., 2014). The researchers suggest that the inocula provided the seedlings with resistant propagules that became active in the greenhouse.

Tree seedlings generally obtain their mycorrhizal community from mature trees (Nara and Hogetsu, 2004; Tedersoo et al., 2008); hyphal connections to the Common Mycorrhizal Network may offer resource supplementation (Booth and Hoeksema, 2010; Figueiredo, Boy, and Guggenberger, 2021). By contrast, for greenhouse seedlings growing in isolation, any benefit they

obtain from mycorrhization must come from an increase in function that exceeds the cost of symbiosis. Greenhouse conditions, with frequently lower light levels, may therefore not be able to express higher function, even if the beneficial fungal species are present. In addition, in this experiment, the greenhouse conditions were harsh with temperatures frequently exceeding 40°C. This would have further limited available carbon for fungi, although the overall rate of mycorrhization was high. Under carbon-limited conditions, rare taxa can be lost (Pena et al., 2010), and this may have reduced diversity in our experimental seedlings.

The stress of separation from the network in tandem with the hot conditions of this greenhouse may have enabled only the most resilient taxa to endure and promoted formation of more connections via spore than from mycelia. Most EM fungal mycelia cannot persist for long without host plants (Drigo et al., 2012); although we aimed to introduce inocula once the root systems were robust enough to forge numerous connections, the temperature of the greenhouse may have reduced viability of some taxa. More durable spore types, which may more likely be melanized spores from early successional species (Nguyen, Hynsons, and Bruns, 2012), belong to taxa which are also more likely to be able to withstand extreme conditions and the absence of mature neighbors. Given that Texas red oak is not an early successional species, it may be that they did not form partnerships with their typical (or most beneficial) mycorrhizal partners.

Additionally, the severity of the experimental drought compounded by the hot greenhouse conditions produced fatally high stress conditions for seedlings. The intermediate disturbance hypothesis suggests that seedlings would derive the most benefit from their mycorrhizal partners under conditions less stressful than the ones imposed by this study. It may be the case that our study does not capture the greatest inoculation contrasts because the circumstances were so severe.

Other symbionts

Experimental seedlings were not just inoculated with mycorrhizal fungi. Myriad biota was conveyed through inoculation; perhaps these other symbionts contributed to health outcomes. It is possible that the Home-Near seedlings did not acquire the most beneficial oak-specific mutualists, but did obtain specific diseases from these mature trees. We do not know to what extent pathogenic or endophytic biota differed.

AM and EM plants can share non-mycorrhizal fungal symbionts (Toju and Sato, 2018), so maybe the combination of old growth juniper-associated endophytes and absence of oak enemies promoted the most beneficial seedling microbiome, one that mitigated drought stress. It is beyond the scope of this study to investigate the holistic composition of these seedlings' microbiome; however, we do know dark septate endophytes were present, and that DSE have been shown to benefit oak, even when mycorrhizae did not (Reyes-Jaramillo et al, 2020); DSE may be more abundant in warmer, drier ecosystems (Newsham, 2011).

Especially for ecotypes from warm and dry homesites and under stressful conditions, sympatric plant-soil pairings can result in higher frequency of fungal symbionts and better health outcomes (Remke[2] et al., 2020). This has been explained by a confluence of the microbial mitigation-exacerbation hypothesis (David et al., 2018), which predicts that the microbiome will exert a mitigating influence under stressful circumstances - but an exacerbating influence under benign circumstances - and the sympatric advantage hypothesis (Hoeksema, 2010), which predicts that coevolved, sympatric mutualisms will be most beneficial. Allopatric plant-soil pairings can even reduce plant performance as compared with a sterile control (Remke[1] et al., 2020).

The soil from each inoculation treatment was likely biochemically distinct, as evidenced by different soil color and texture, however we did not perform any soil analyses for this study. Home-Far inocula was practically sympatric, but also from a drier area of the homesite than the

Home-Near inocula (Jenkins, unpublished, 2022). Given the evidence that sympatric pairings and familiar moisture regimes produce the best outcomes for plants (Remke[1] et al., 2020), perhaps Home-Far inocula provided more drought-tolerant – and yet familiar – mutualists.

Implications for restoration and conclusion

The home range of Texas red oak faces increasing intensity of hot droughts. Although the greenhouse experiment sacrificed realism with pot isolation, ambient conditions may have approximated the higher stress environments becoming more frequent in the ecoregion. While greenhouse conditions and severance from the mycorrhizal network restrict which mycorrhizae develop, seedling community in the field may be determined by priority effect (Kennedy, Peay, and Bruns, 2009) – whoever is most immediately available determines which partnerships develop; even when inocula is introduced, it may not recreate the community from which it was procured. In a study inoculating with either forest or *Cystis*-invaded soil near forest edge or in clearings, inoculation did not increase mycorrhizal abundance, richness, or change community composition – it was proximity to forest itself that influenced community - and increased mycorrhization and survival (Grove et al., 2019).

Land management practices across the southwest disproportionately target *Juniperus* sp. for removal. In northern New Mexico, the Forest Service commonly prescribes juniper eradication or disproportionate thinning, despite any evidence that this promotes forest resilience or benefits codominant pinyon in any way (De'Scoville, 2025). Myths around juniper are pervasive and have informed land management prescriptions for decades (McGreevy, 2021). This study contributes further evidence that juniper neighbors can benefit co-dominant trees.

Experimental seedlings seemed to benefit from old growth juniper-conditioned soil, although perhaps not for the reasons presupposed. This research presents strong evidence that AM

fungi are not partners of Texas red oak; despite this, it was the inocula provisioned from Homesite juniper which enhanced seedling drought resilience, suggesting the microbial community assisted oak seedlings. The forces contributing to differences in performance and drought tolerance remain unclear. Although it opened more queries than it resolved, this investigation lends support to the growing body of literature on oak and their multitudinous collaborators.

Soil is frequently treated as a "black box"; as such, most controlled experiments reduce complexity in attempts to tease apart causal relations among plants and the myriad soil components. Plant-fungal networks challenge reductionist and binary thinking foundational to Western science. Increasingly, ecologists are recognizing the need for "two-eyed seeing", integrating traditional indigenous ways of knowing with the conventions of scientific practice (Ortiz et al., 2025). This study was premised on the notion that mycorrhizal communities would differ by provenance of whole soil inocula; while we did not find evidence that this was the case, clearly the inoculation treatment influenced outcomes for seedlings. We can only speculate about how and why.

APPENDIX SECTION

APPENDIX A

PHOTO POST-HOCS BY SESSION

	CONTRAST	SESS 1					
		A		gs		WUE	
		t.ratio	p	t.ratio	p	t.ratio	p
WATER	D-W	-	-	-	-	1.774	0.090
PROX	FAR > NEAR	1.752	0.094	-	-	-	-
WD*SITE	D Home - W Home	-	-	-2.608	0.072	**3.155**	**0.028**
WD*PROX	W Far > W Near	2.711	0.058	-	-		
SITE*PROX	Away Far > Away Near	**2.882**	**0.041**	-	-	-	-

	CONTRAST	SESS 2	
		A	
		t.ratio	p
SITE	Away > Home	**2.066**	**0.051**
WD*SITE	D Away > D Home	**2.745**	**0.054**
WD*SITE*PROX	D Home Far > D Home Near	3.098	0.084
WD*SITE*PROX	W Away Far > D Home Near	3.039	0.094
WD*SITE*PROX	D Away Far > D Home Near	**3.374**	**0.048**
WD*SITE*PROX	D Away Near > D Home Near	**3.606**	**0.029**
WD*SITE*PROX	D Home Near < W Home Near	-3.002	0.101

	CONTRAST	SESS 4					
		A		gs		WUE	
		t.ratio	p	t.ratio	p	t.ratio	p
WD	D < W	**3.002**	**0.007**	**-6.157**	**<0.001**	**-2.399**	**0.026**
SITE	AWAY > HOME	-	-	-	-	-	-
PROX	FAR > NEAR	-	-	-	-	**3.728**	**0.001**
WD*PROX	D FAR > D NEAR	-	-	-	-	**4.280**	**0.002**
WD*PROX	W FAR > D NEAR	-	-	-	-	**4.326**	**0.002**
WD*PROX	D NEAR < W NEAR	-	-	-	-	**-3.500**	**0.001**

	CONTRAST	SESS 5			
		A		gs	
		t.ratio	p	t.ratio	p
WD	D < W	**-16.624**	**<0.001**	**-18.024**	**<0.001**
SITE	AWAY < HOME	-	-	**-2.465**	**0.016**
PROX	FAR > NEAR	**2.588**	**0.012**	**2.311**	**0.024**
SITE*PROX	HOME FAR > AWAY NEAR	**2.793**	**0.034**	-	-
SITE*PROX	AWAY FAR < HOME FAR	-2.476	0.074	-	-
SITE*PROX	HOME FAR > HOME NEAR	**3.328**	**0.008**	-	-

	CONTRAST	Session 6	
		A	gs

		t.ratio	p	t.ratio	p
WD	D < W	**-17.023**	**<0.001**	**-13.309**	**<0.001**
SITE*PROX	AWAY FAR < AWAY NEAR	-2.513	0.067		
SITE*PROX	AWAY FAR < HOME FAR	-2.563	0.060		

APPENDIX B

POST-HOCS BIOMASS using emmeans

STEM MASS

CONTRAST	estimate	t.ratio	p-value
C > J	0.029	1.543	0.413
C > M	0.064	**3.464**	**0.003**
C > O	0.039	2.097	0.156
J > M	0.036	1.896	0.232
J > O	0.010	0.545	0.948
M < O	-0.025	-1.360	0.525

ABOVE

CONTRAST	estimate	t.ratio	p-value
C > J	0.030	1.535	0.418
C > M	0.033	1.694	0.329
C > O	0.048	2.386	0.082
J > M	0.003	0.176	0.998
J > O	0.018	0.886	0.812
M > O	0.014	0.712	0.892

Root to Shoot

CONTRAST	estimate	t.ratio	p-value
C < J	-0.370	**-3.022**	**0.015**
C < M	-0.154	-1.248	0.597
C < O	-0.279	-2.254	0.113
J > M	0.217	1.725	0.314
J > O	0.091	0.725	0.887
M < O	-0.126	-1.002	0.749

APPENDIX C

DNA sequencing methods (Kovacs):

For molecular identification, 3-5 representative tips per ectomorphotype were excised under a stereoscopic microscope and kept in 1.5 mL Eppendorf tubes at -20 °C. DNA was extracted following a modified version of the Park et al. (2022) protocol, as described by Floriani et al. (2024). Briefly, a small portion (~2 mm) of each ectomorphotype was cleaned in sterile distilled water, placed in 1.5 mL Eppendorf tube containing 40 µL of TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0) and 5 µL of 200 mM KOH, and then mechanically crushed using micropestles. Tubes were incubated 65 °C for 10 min, followed by the addition of 5 µL of 100 mM $(NH_4)_2SO_4$ (Floriani et al. 2024). PCR amplification was performed using the fungal-specific primer ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′; Gardes and Bruns 1993) and the universal primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′; White et al. 1990). PCR reactions were performed in 15 µL using the Dream Taq Hot Start Green PCR Master Mix (Thermo Scientific), with the following final concentrations: 0.06 U/ µL polymerase, 1× reaction buffer, 2 mM MgCl2, 0.2 mM dNTPs, 0.25 µM of each primer. One µL of DNA extraction without prior purification was used as template. PCR was performed in a Model Thermal Cycler (brand) under the following conditions: an initial denaturation step at 94 °C for 4 min, 35 cycles of 30 s at 94 °C, 45 s at 50 °C, and 70 s at 72 °C, and a final extension of 10 min at 72 °C. PCR products were analyzed by electrophoresis in 1.5% (w/v) agarose gels, stained with xxxx (xxxx), and visualized under a UV transilluminator (brand and model). Single-band PCR products were selected for Sanger sequencing using an ABI3730 with the primer ITS4. Chromatograms were visually inspected and manually edited using Chromas software. Sequences were compared against the NCBI GenBank database (http://www.ncbi.nlm.nih.gov) using the BLASTn algorithm and assigned to species based on a ≥97% ITS sequence similarity threshold. Nomenclature and taxonomic classification followed the Index Fungorum database (https://www.indexfungorum.org/names/names.asp).

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