PLANT NEIGHBOR HAS STRONGER INFLUENCE ON PINYON PINE ECTOMYCORRHIZAL FUNGI THAN SOIL HISTORY OF FIRE

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ABSTRACT

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Mycorrhizal fungi have the ability to improve plant responses to stressors such as heat and drought and may play a critical role in seedling establishment in semi-arid landscapes. Wildfire and the introduction of novel species have been shown to change mycorrhizal fungal communities, including in the long-term. As wildfire frequency and severity increases across the Southwest due to climate change, the recovery of pinyon pine (*Pinus edulis*) and juniper (*Juniperus osteosperma*) woodland ecosystems and their fungal communities following disturbance is becoming increasingly uncertain. We sought to understand the interactions between soil history of wildfire, introduced grasses, and pinyon pine mycorrhizae using a plant neighbor greenhouse experiment. Pinyon seedlings were grown in soils collected at Mesa Verde National Park from areas that burned in a stand-replacing fire in 2002 or in soils from adjacent intact woodlands. Each pinyon was grown in the same pot as an invasive grass (cheatgrass, *Bromus tectorum*), a native grass (western wheatgrass, *Pascopyrum smithii*) or another pinyon seedling. We measured mycorrhizal abundance and fungal community composition, as well as metrics of plant performance, including biomass and plant water stress. Our results show that even twenty years after fire, ectomycorrhizal community composition differs from that of intact pinyon-juniper woodlands, and that fire history and plant neighbor interact to affect fungal abundance. Importantly, the presence of either invasive or native grasses had a stronger negative effect on pinyon mycorrhizae than burning, resulting in an average 38% drop in colonization and an altered community. Seedling water stress, however, was controlled by soil fire history, despite controlling for other environmental conditions. This work has the potential to inform post-fire management strategies and suggests that interactions among plant species, long-term effects of fire on soil, and mycorrhizal fungi may help determine the trajectory of recovery in western ecosystems following disturbance.

Key words**:** Mycorrhizae, ectomycorrhizae, fire, invasive species, pinyon pine, cheatgrass, western wheatgrass, plant-soil interactions

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INTRODUCTION

In recent decades, wildfires and drought have intensified across the Western United States as part of a pattern of global climate change, including in forested ecosystems (Abatzoglou and Williams, 2016; Jolly et al., 2015; Westerling, 2016). As disturbance patterns shift, the resiliency of forests to degradation or state changes into new ecosystem types may be undermined (Johnstone et al 2016). Fire has the potential to alter forest soil microbiota through a myriad of mechanisms, including direct heating, alteration of nutrient availability, creation of water-repellent soils, and changes to post-fire vegetation communities (Hart et al 2005). Changes to soil characteristics and soil communities may be persistent, though long-term effects of fire on soil fungi are currently understudied (Cairney and Bastias 2007, Gill et al. 2022).

The vast majority of terrestrial plants form mutualistic mycorrhizal relationships in their roots with fungi (Brundrett and Tedersoo 2018). In these associations, the plants exchange the products of photosynthesis for water and nutrients procured by the fungi from the soil (Smith and Read 2008). Ectomycorrhizal (EM) relationships are common between many tree species and a wide diversity of soil fungi and play a critical role in tree seedling establishment worldwide (Tedersoo et al. 2010). Each plant may associate with multiple taxa of fungi within its root system, with some species providing greater benefit to the plant than others (Gehring et al., 2017). Fire has been shown to reduce EM species richness and *in-situ* colonization of host plant roots (Dove and Hart, 2017), and recovery of full soil diversity after severe fire can frequently require decades (Karst et al 2014, Taudière et al 2017). If important plant mutualists are among the taxa lost, the ability of trees to reestablish could be hampered by reduced soil microbial diversity.

Invasive plants can negatively affect soil fungi and mycorrhizal colonization of native plants, though interactions vary by taxa (Grove et al., 2017). Potential mechanisms of inhibition include chemical changes to the soil, direct competition from roots, and aboveground competition for light with native mycorrhizal host plants, which could subsequently reduce carbon resources available to mycorrhizal mutualists (Bennett and Klironomos, 2019; Grove et al 2017). These direct and indirect effects on soil microbial communities can lead to shifts in plant community dynamics (Bever et al 2010).

In the Western United States, increased cover of invasive grasses such as cheatgrass (*Bromus tectorum*) may also be inhibiting tree seedling regeneration after fire. Cheatgrass can out-compete native species following fire and contribute to shorter fire return intervals by creating a carpet of fine fuel, thus reinforcing its advantage over native species (D'Antonio and Vitousek, 1992). It can also alter soil characteristics and change the composition of bacterial and arbuscular mycorrhizal fungal communities (O'Connor et al., 2015; Reitstetter et al., 2022, Hawkes et al., 2006, Busby et al. 2013, Owen et al., 2013). However, effects of cheatgrass on EM fungi are poorly understood. Seeding of grasses after fire is a common management tactic to control erosion and potentially restore native vegetation, yet long-term consequences of this practice on tree regeneration and ectomycorrhizal communities is likewise unclear. While seeding efforts have the potential to introduce invasive species through contamination of seed mixes, the native grass species may themselves compete effectively against tree seedlings, reduce available ectomycorrhizal inoculum, or otherwise alter soil microbial communities (Peppin et al. 2010).

Along with other dryland forest ecosystems, pinyon-juniper (*Pinus edulis* and *Juniperus osteosperma*) woodlands are experiencing high-severity fire over increasingly large areas with unprecedented frequency (Singleton et al., 2019). However, the climate conditions needed for seedling regeneration and post-fire recovery of these forests are becoming less common, leaving pinyon-juniper woodlands less resilient in the face of fire and other disturbance (Stevens-Rumann et al., 2018). Changes in climate and fire regime can also render pinyon-juniper ecosystems more vulnerable to invasion by non-native fire-adapted species such as cheatgrass at the expense of native taxa (Kerns et al., 2020). Despite covering over 40 million hectares of western landscapes, these woodlands have been historically understudied from a conservation perspective, particularly over extended time periods (Hartsell et al., 2020, Romme et al., 2009).

Within Mesa Verde National Park and Ute Mountain Ute tribal lands, large swaths of mature pinyon-juniper woodlands have been lost in a series of stand-replacing fires, despite historically rarely burning (Floyd et al., 2004). Our research team surveyed soil and vegetation characteristics at three sites that burned in the early 2000s within these parks and found no tree seedlings in any of the areas that burned (Phillips et al., 2022). In contrast, tree seedlings of both species were abundant in nearby intact woodlands surveyed as controls. Hotter, drier conditions in burned areas relative to intact woodlands may be preventing seedling success (Floyd, et al., 2015), but changes in the soil microbiota and rapid establishment of novel grasses following burning may also be important. Cheatgrass presence has increased across burn areas in Mesa Verde National Park, and in 2019 constituted nearly a quarter of vegetative cover in burn scars on the mesa where our field collections occurred (Floyd and Hanna 2021, Rondeau et al. 2022). Western wheatgrass was not present in Mesa Verde's pinyon-juniper woodlands prior to fire but is now well-established in our study site burn area due to seeding, with 20% cover documented in 2019 (Rondeau et al 2022). More research is needed to understand how novel grasses, trees, and fungi interact, and whether long-term effects of fire on soil and plant communities changes these interactions.

To fill this research gap, we conducted a greenhouse experiment in which ectomycorrhizal fungal communities were measured on pinyon pine seedlings that were each grown in a shared pot with a cheatgrass neighbor, a western wheatgrass neighbor, or a pinyon neighbor. We also tested for long-term effects of fire on soils by growing each plant neighbor treatment group in live field soil taken from areas of Mesa Verde National Park that burned in a stand replacing wildfire and in soils from adjacent intact pinyon-juniper woodlands to create a full factorial design. We used water potential readings, a measure of tension in plant xylem that can indicate plant drought stress, as a metric of seedling stress response to the experimental conditions. We also collected plant biomass data to quantify effects on seedling growth. Although a body of ecological niche-partitioning theory suggests that plants may perform better in mixed culture than monoculture due to greater intraspecific competition for resources compared with interspecific competition, we suspected that in our experiment the positive effects of shared ectomycorrhizal communities for pinyon pines and the highly competitive nature of grasses would lead to better plant performance in pinyon conspecific pairings (Davis et al., 1998, Bever et al., 2010). Our hypotheses were:

H1. EM colonization and pinyon seedling size will be greatest and plant stress lowest in the pinyon-pinyon plant neighbor pairing, performance will be poorest in the pinyon-cheatgrass pairing, and wheatgrass will have intermediate effects,

H2. EM colonization and pinyon seedling size will be greatest and plant stress least in the intact soil treatment, and

H3. The negative effects of a cheatgrass neighbor will be greater for pinyons growing in burned soils than intact soil, while the other species pairings will not show an interaction effect with soil.

Through our analyses, we hope to better understand the effects of wildfire on plants, fungi, soils, and the relationships between them in pinyon-juniper woodlands in order to inform post-fire management and restoration. By investigating whether mycorrhizal interactions change in post-fire landscapes, we can better predict patterns of regrowth and determine appropriate restoration strategies.

METHODS

Soil Collection, Seed Collection, and Planting

We collected soil samples from the top 15 cm of topsoil in May of 2021 from four locations within an area of Mesa Verde National Park that had burned in the Long Mesa fire nineteen years prior, and had been mature pinyon-juniper woodland prior to the fire. Four intact woodland (control) soil samples were collected from an area of unburned pinyon-juniper woodland that was previously contiguous forest with the area that burned and had similar stand characteristics prior to the fire. Both the burned area and intact woodland control were located on Morefield loam soils with a 3-6% east-facing slope (Soil Survey Staff, 2023). All samples were taken a minimum of 50 m from another sample, and at least 5 m from a road or forest edge. Each soil replicate was amended with perlite (4:1 ratio of field soil to perlite by volume). Rocks larger than 2 cm in diameter and roots larger than 2 mm in diameter were removed. All seeds used in the experiment were collected by our research team or collaborating research groups from local sources on the Colorado Plateau, with the exception of the western wheatgrass seed, which was sourced from Southwest Seed Company, Cortez, Colorado. The pinyon seeds used were sourced from multiple mother trees and had a minimum seed weight of 0.2 g. Pinyon seeds were surface sterilized in 10% bleach for twenty minutes, then rinsed three times in reverse-osmosis water before being planted into the live field soils in 656 mL conical pots. Sixteen pots were planted per treatment, with four pots per soil sample replicate. Seed source and soil replicate were stratified within treatments. Cheatgrass and western wheatgrass seeds were surface sterilized in 10% bleach for five minutes, then rinsed three times in reverse-osmosis water, and planted into the appropriate pots one week after the pinyon seedlings to allow for all species to germinate at approximately the same time. Both grass species were thinned to 4-6 plants per pot, and pinyons thinned to two seedlings per pot after germination, with one pinyon randomly assigned as the “experimental” plant and the other as the “neighbor” plant. Seedlings were grown in the NAU Research Greenhouse for six months from the date each pinyon seedling germinated with supplemental light and under temperatures ranging between 17-26°C. The position of the plants was rotated regularly within the greenhouse to avoid position effects, and all plants were watered twice weekly until four days prior to harvest, at which point water was withheld to facilitate soil sampling and plant harvest. Due to failed germination, final sample sizes ranged between 13-16 plant replicates per treatment.

Data Collection

To assess treatment effects on plant stress, two water potential readings for each pinyon seedling were taken on the same day at pre-dawn (4 AM- 8:30 AM, with plants kept in a dark room until reading was taken) and midday (11 AM-2 PM) four to six days prior to harvest, and the difference between the two readings for each plant was calculated. Readings were taken on individual needles using a Model 1505D PMS Instrument Company Pressure Chamber. All readings were taken on well-watered plants, and needles used for water potential readings were saved for inclusion in biomass measurements. We assumed that the predawn measurement indicated the baseline of lowest water stress for the plants, as none were actively photosynthesizing, and that the midday reading indicated the point of greatest water stress (McDowell et al. 2008). Thus, we felt that the difference between the two readings was an appropriate metric of the level of water stress each seedling was under on the day of the measurements.

Three 1 cm diameter by 9 cm length soil cores were taken per pot immediately prior to harvest for future nutrient analysis. At the time of harvest, we separated roots from shoots, then measured fresh shoot mass for both target experimental plants and conditioning neighbor plants. We measured pinyon pine shoot length and number of plants for the grasses. Roots were gently rinsed to remove soil, then frozen for future processing.

On a randomly selected subset of the pinyon pines from each treatment group (n=5 per treatment) we visually sorted the EM root tips by morphological characteristics under a dissecting microscope and counted the total number of colonized and uncolonized root tips, as in Mueller et. al., (2019), while also counting the number of dead colonized and uncolonized root tips in each sample. We calculated percent EM colonization as a measure of total fungal abundance by dividing the total number of live colonized EM root tips by the total number of root tips for each seedling, including living and dead uncolonized root tips. Because we observed a large number of dead EM root tips in several of the treatments, we also calculated the proportion of living to total colonized EM root tips to document the unexpected levels of senesced mycorrhizal root tips. Root tips of each morphotype were saved in separate tubes and frozen for DNA analysis. Following assessment for mycorrhizal structures, both roots and shoots were dried at 60 °C for 48 hours, then weighed to determine dry biomass. On the subset of plants for which morphotyping had occurred and thus drying of roots could be accomplished, we calculated the total plant biomass and root to shoot ratio to better understand resource allocation within the seedlings.

Molecular Analysis

Fungal DNA from individual root tips representing all morphotypes and treatment groups was extracted using the High Molecular Weight Extraction Protocol described by

Mayjonade *et al* (2016). Multiple root tips from each morphotype were selected when possible. The internal transcribed spacer region was amplified using ITS1F and ITS4 fungal primers and Dreamtaq Green Hotstart II polymerase. The samples were then subjected to an additional bead clean-up step before being eluted in water and sequenced using the Sanger method on an ABI 3730 machine in the NAU Genetics Core Facility. Sequence quality was checked and sequences were trimmed using Whitehead Institute for Biomedical Research's Staden PreGap4 program (Bonfield et al., 1995). We then used the NCBI nucleotide BLAST tool to compare our sequences to published GenBank databases, and ascribed taxonomic designations to each sample at the genus level when percent identity was 95% or greater, and at the species level when percent identity was 98% or greater (Altschul et al., 1990; Kõljalg et al., 2013). Taxa are reported at the genus level or higher in this work. The relative abundance of each fungal taxon was calculated by dividing the number of live root tips of the corresponding morphotype by the total number of live colonized root tips on the seedling.

Statistical Analysis

We used two-way Analysis of Variance (ANOVA) to test for main effects (H1 and H2) and interactions (H3) between two fixed effects: soil type (burn or intact) and plant neighbor (cheatgrass, western wheatgrass, or pinyon). ANOVAs were also used to assess whether there were significant differences between soil replicates within each soil type. All data were checked for Normality using the Shapiro-Wilk test and by plotting the residuals against fitted values. Where data were not normally distributed, a log transformation (dry shoot biomass, total plant biomass, root to shoot ratio) or arc-sine square root transformation (percent live root tips) was performed to meet the assumptions of the ANOVA. Results were interpreted as significant when p-values were greater than α=.05, and marginally significant when p-values fell between 0.5-0.1. Where more than two groups were considered, Tukey's method of p-value adjustment was used. Differences in EM community composition were analyzed through Permutational Analysis of Variance (PERMANOVA) using the 'adonis' function of the 'vegan' package in R (Oksanen 2022, R Core Team 2021). Principal Component Analysis (PCA) was used to visualize the data and determine which fungal taxa had the greatest influence on differences among treatments. All figures were created using the 'ggplot2' package and all analyses were conducted in R Version 2022.07.2+576 (R Core Team 2021; Wickham 2016).

RESULTS

Mycorrhizal Colonization

Plant neighbor and soil type interacted to significantly influence ectomycorrhizal abundance on pinyon seedling roots, while the presence of a grass neighbor, regardless of soil history, reduced mycorrhizal abundance. Soil type did not have a significant main effect ($F_{1,23}$ = 2.2449, P = 0.1477), while the effect of plant neighbor was highly significant ($F_{2,23}$ = 35.3796, P < 0.0001). There was a significant interaction between soil type and plant neighbor ($F_{2,23}$ = 3.8739, P =.03548), with greater differences in colonization among neighbor treatments in the intact soil compared with the burn soil (Fig. 1). Pinyon seedlings with pinyon neighbors had the highest colonization in the intact soil, where grass neighbors also had the strongest negative effect. Importantly, the two species of grass had similarly negative effects on EM colonization of pinyon seedlings, on average reducing colonization by 38%. Pairwise contrasts between the neighbor species treatments showed that pinyon seedlings grown with a pinyon neighbor had significantly greater colonization than those grown with either a cheatgrass or wheatgrass neighbor ($P < .0001$ for each) but that percent colonization was only marginally different between the two grass neighbor treatments (t = -2.438, $P = 0.0574$).

Fungal Community Composition

Fungal community composition differed significantly among treatments (Fig. 2). Using a PERMANOVA with 999 permutations, the differences were driven by plant neighbor main effects ($F_{2,28} = 9.7844$, $P = 0.001$), while soil main effects were not significant (PERMANOVA soil $F_{1,28} = 1.7734$, $P = 0.123$) and there was no significant interaction between the two treatments ($F_{2,28} = 0.7650$, $P = 0.632$). The major genera returned by the BLAST sequence search were *Geopora* and *Rhizopogon*. including *Geopora pinyonensis* and unknown *Geopora* spp., as well as *Rhizopogon guzmanii, Rhizopogon armeniacus,* and an unknown *Rhizopogon* sp*.* Other taxa returned included *Sebacina* and *Peziza.* Several of the less common morphotypes observed were visually distinctive but could not be successfully sequenced, and thus are listed as unknowns in this thesis. *Geopora* was the most abundant genus across treatments, followed by *Rhizopogon*. Levels of *Geopora* were significantly higher in the pinyon-pinyon pairing than either of the grass treatments ($F_{2,23} = 7.391$, $P = 0.003$ for plant neighbor main effect) interactions with soil were not significant ($F_{2,23} = 1.0069$, $P = 0.381$), nor was the main effect of soil alone ($F_{1,23} = 0.385$, $P = 0.541$). *Rhizopogon* was 37% more abundant on pinyon seedling roots when the pinyon was grown in the presence of either grass species in the intact soil treatment, and 8-9% more abundant on pinyon roots in the presence of either grass species in the burned soil treatment. The PCA (Fig. 3) revealed that the fungal communities of each treatment were distinct, that the plant neighbor treatments could be predicted by the fungal community data, and that the majority of variation in the data (60%) was explained by *Geopora and Rhizopogon.*

Water Potential Measurements

The difference between predawn and midday leaf water potential readings, a measure of plant stress, was greater on pinyon seedlings grown in the burned soil than in the intact soil ($F_{1,87}$ = 4.7270, *P* = 0.03241) but did not differ by plant neighbor treatment ($F_{2,87}$ = 0.4489, *P* = 0.63979; Fig. 4). The interaction between the two factors was marginally significant ($F_{2,87}$ = 2.5699, *P* = 0.0823) and was driven by differences in the cheatgrass neighbor treatments. Specifically, the mean difference in water potential for pinyons with a cheatgrass neighbor in intact soils was significantly greater than the difference for pinyon seedlings with a cheatgrass neighbor in burned soils (*t* = 3.110, *P* = 0.0025). No other pairwise contrasts were significant.

Pinyon Seedling Growth

Pinyon seedlings showed differences in growth driven by plant neighbor treatment, as well as by the interaction between plant neighbor and soil type. The effect of soil type on dry shoot biomass when averaged across plant neighbor treatments was only marginally significant ($F_{1,83}$ = 3.0935, *P* =.0823), while the plant neighbor main effects were highly significant ($F_{1,83}$ = 22.8262, *P* < .0001; Fig. 5). The shoot biomass of pinyon seedlings grown with western wheatgrass did not differ from that of those grown with cheatgrass (*t* = -0.880, P = 0.6543), suggesting that both grasses had similar suppressive effects on pinyon growth. Differences in shoot dry biomass between pinyon seedlings grown with a pinyon neighbor and those grown with a grass neighbor were more pronounced in the intact soil than in the burn soil. Similar patterns were observed for dry root biomass and total plant dry biomass. The root:shoot ratio of the pinyon seedlings across treatments did not differ, except for seedlings with a cheatgrass neighbor having a higher root:shoot ratio in the intact soil treatment compared with the burned soil treatment (t = -2.077, P =.0487).

Relationship between Plant Biomass and EM Abundance

Pinyon shoot dry biomass was positively correlated with percent EM colonization (R^2 =0.2, $F_{1,27}$ = 7.9989, P = 0.0087), confirming that plant performance is linked with mycorrhizal abundance (Fig. 6). The correlation was driven entirely by the intact soil treatment. When considered alone, the size of the pinyon seedlings grown in the burned soil did not correlate with percent colonization (R^2=0.01, $F_{1,13}$ = 0.1403, P = 0.714) while seedlings grown in the intact woodland soil that grew larger also had higher mycorrhizal abundance (R^2=0.43, $F_{1,12}$ = 9.1538, P = 0.01055).

DISCUSSION

Our results show that both plant neighbor and soil history of fire affect mycorrhizal communities of pinyon pine roots, and that they interact to change plant performance and mycorrhizal colonization of seedlings. Our hypotheses were partially supported: grass neighbor species did have negative effects on pinyon EM colonization and seedling growth, but did not increase plant stress as measured with water potential differences (H1). Burned soil did increase plant stress, but did not have main effects on plant growth, nor EM abundance (H2). Interactions were observed between soil type and plant neighbor for seedling growth and EM abundance, but did not control community composition nor water potential, and where they were significant, worked to increase differences between neighbor treatments in intact soil rather than burn soil.

While fungal abundance appears to have recovered after fire to levels similar to undisturbed woodlands, the composition of the community remains different from nearby intact ecosystems after twenty years. This result matches trends from similar studies evaluating the long-term effects of burning on pine ectomycorrhizae (Karst et al 2014). As specific taxa of EM fungi provide varying levels of benefit to plants, this suggests that pinyon seedlings may not have an optimal community of fungal mutualist partners back even decades after fire. Of the taxa we observed, both *Rhizopogon* and *Geopora* are known to be frequent mycorrhizal partners of pines and have been previously associated with disturbance (Bruns et al. 2019; Fujimura et al., 2005). *Geopora*, however, is also common in undisturbed pinyon-juniper systems and is known to improve pinyon seedling response to drought (Gehring et al. 2017). The relative abundance of *Geopora* was reduced by the presence of either grass species; it is possible that this may correlate with reduced seedling resilience to stress under these conditions. We were surprised to find that despite a correlation between seedling growth and EM colonization for seedlings grown in intact woodland soil, there was essentially no correlation between seedling biomass and EM colonization in the burned soil treatment. This may reflect a mycorrhizal community in the burned soil that is less beneficial to the pinyon host plant.

The presence of a grass neighbor also affected both EM community composition and abundance, even though grasses do not form EM associations (Brundrett and Tedersoo 2018). However, there is evidence of cheatgrass negatively affecting other soil and plant-associated biota (Belnap et al 2005, Gehring et al., 2016; Owen et al., 2013; Reitstetter et al., 2022). Grasses in general are known to be highly competitive against tree seedlings for water and nutrients (D'Onofrio et al., 2015, Davis et al., 1998). There is also evidence other plant taxa influence EM fungal communities (Hubert & Gehring, 2008; McHugh and Gehring 2006). Mechanisms for the observed reduction in EM fungi could include belowground interactions between grass roots and soil conditions, including antifungal compounds released by the grasses, nutrient depletion, pH changes, or creation of conditions that favor other soil biota that compete with or antagonize EM fungi (Bennett & Klironomos, 2019). While we are unable to distinguish between these potential mechanisms with the current study, this is an area for potential future investigation. Reductions in photosynthesis from aboveground shading of pine seedlings by grasses could also potentially explain reduced mycorrhizal colonization due to reduced resource allocation of carbon to fungal partners (Bennett & Klironomos, 2019, Bever et al 2010), yet shading was not observed in this study and all plants were kept under supplemental light in the greenhouse. However, we did observe that in the grass neighbor treatments, pinyon seedlings appeared to have formed EM root tips, but that these root tips subsequently died. In contrast, nearly all EM root tips in the pinyon neighbor treatments were alive. This suggests that the grasses may be killing the EM root tips after they are formed, potentially through chemical means. It is possible that both belowground competition and suppressive effects on mycorrhizal fungi are both contributing to reductions in pinyon growth, as these two mechanisms are not mutually exclusive.

Surprisingly, both species of grass had strong negative effects on EM colonization levels, despite the fact that cheatgrass is an invasive, non-native species and western wheatgrass is native to the Colorado Plateau and had been intentionally seeded after fire. However, western wheatgrass is not a typical member of pinyon-juniper woodlands at the study site, which instead have understories characterized by bunchgrasses and forbs (Rondeau et al 2022). Western wheatgrass, in contrast, is a fast-growing and deep-rooted rhizomatous grass species. The efficacy of post-fire seeding of native grasses on controlling erosion and preventing invasive plant invasion are equivocal, and may bring about conversion to grassland in some circumstances (Peppin 2010, Andrus et al., 2022)

Plant stress, measured in this study as water potential differences between predawn and midday readings of pinyon needles, responded to soil type despite being grown in a controlled greenhouse environment with supplemental lighting and ample water. This is surprising, as many of the environmental conditions expected to increase seedling water stress in burn scars, such as lack of shade and drier soil conditions were held constant in this experiment. Thus, differences in seedling performance are almost certainly due to soil properties rather than differences in climate. These properties could be biotic (ie, mycorrhizae or other microbes) or abiotic (soil texture, nutrient availability, etc.). Soil samples from the study have been reserved for future nutrient analysis to follow up on this finding. While it is possible that soil temperatures were greater in burned soils due to their darker color, this is still controlled by an attribute of the soil rather than external environment. Plant neighbor overall did not significantly influence water potential measurements in our study, despite our expectation that greater belowground competition for water between pinyon and grass roots may lead to increased water stress for pinyon seedlings.

Seedling growth was influenced primarily by plant neighbor, as well as by the interaction between plant neighbor and soil type. Differences between neighbor treatments were most pronounced in intact soils, indicating that intact pinyon-juniper woodland seedling survival may be particularly vulnerable to negative effects of introduced grasses. Our results also suggest that in sites with a history of fire, high levels of seedling growth and EM colonization of seedlings are possible if grasses are excluded. Because the site where our burned soils were taken from has a history of cheatgrass and western wheatgrass presence, while our intact woodland soil source location did not, it is also possible that the results we observed are driven by soil microbial communities having already adjusted to grasses in the burned soil. If this is the case, we might expect a smaller response in plant performance and mycorrhizal colonization to grass introduction in the burned soils than in the intact soils where the effects of these grass species are more novel. In any case, our results suggest that interactions between soil history and plant community matter for pinyon seedling success, and that the presence of both invasive and seeded grasses has the potential to significantly reduce pinyon pine seedling growth and ability to form EM associations.

CONCLUSION

Management for forest resilience under increasingly disturbance-prone climate conditions is one of the major challenges facing land managers today (Millar & Stephenson, 2015). Wildfires and introduction of novel grass species have the potential to drive ecosystem shifts from woodland into grassland (Andrus et al., 2022). Our results suggest that complex interactions between EM fungi, tree seedlings, soil characteristics, and grasses may mediate these changes. Importantly, while fire can have long-lasting changes on soil communities, we observed that vegetation following fire has an even greater impact. This work has the potential to inform land manager decisions about whether or not to seed grasses following fire, as well as the utility of grass removal when trying to promote tree regeneration. It also suggests that pinyon replanting efforts may benefit from additions of soil inoculum from intact woodlands, as these EM communities appear to better promote seedling growth. Future work is needed to understand the complex relationships between fire, introduced species, plant-soil feedbacks, and land management strategies for protecting and restoring dryland forests.

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FIGURES

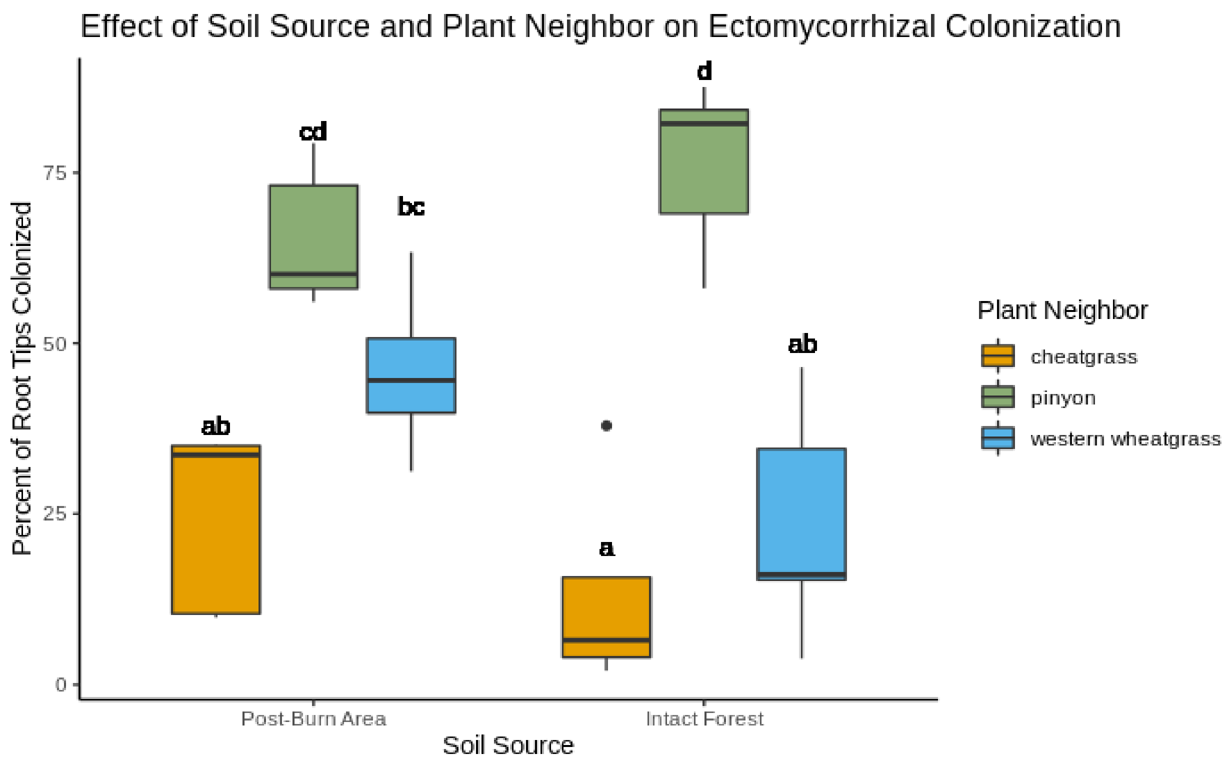

Figure 1: Percent ectomycorrhizal colonization of root tips in pinyon seedlings was greatest with a pinyon neighbor, and suppressed by the presence of both cheatgrass and western wheatgrass. Differences in plant neighbor treatments were greatest in the soil from intact woodlands, suggesting an interaction between the two treatments.

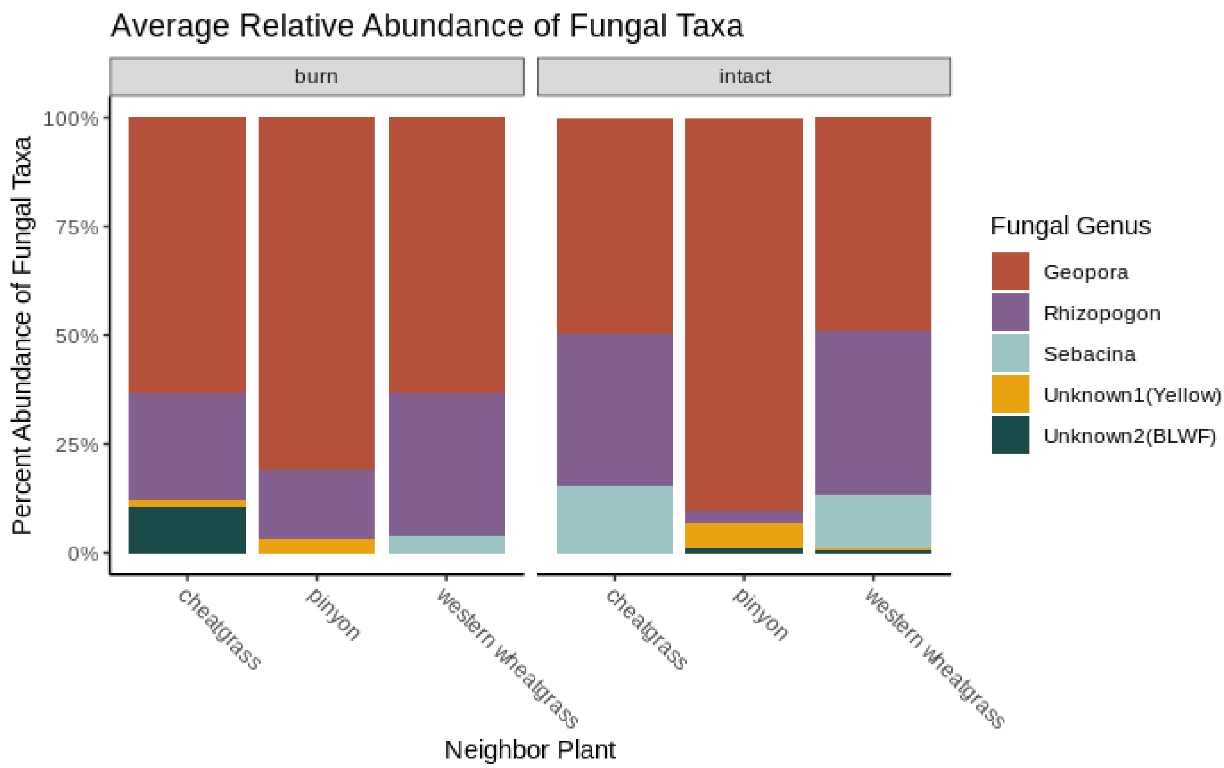

Figure 2: Relative abundance of fungal taxa in each treatment category. Fungi in the genus Geopora were the most abundant, followed by Rhizopogon.

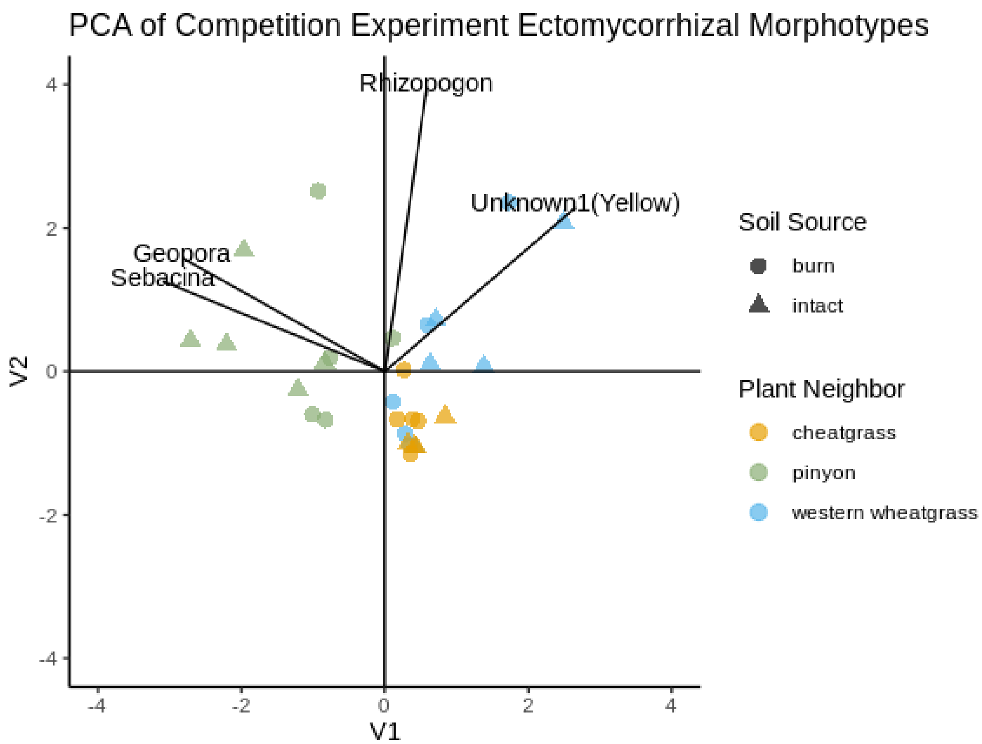

Figure 3: Principal Component Analysis (PCA) showing that EM fungal communities differ by plant neighbor treatment (indicated by point color) and by soil treatment (indicated by point shape). The genera Geopora and Rhizopogon drive the majority of the variation in the data.

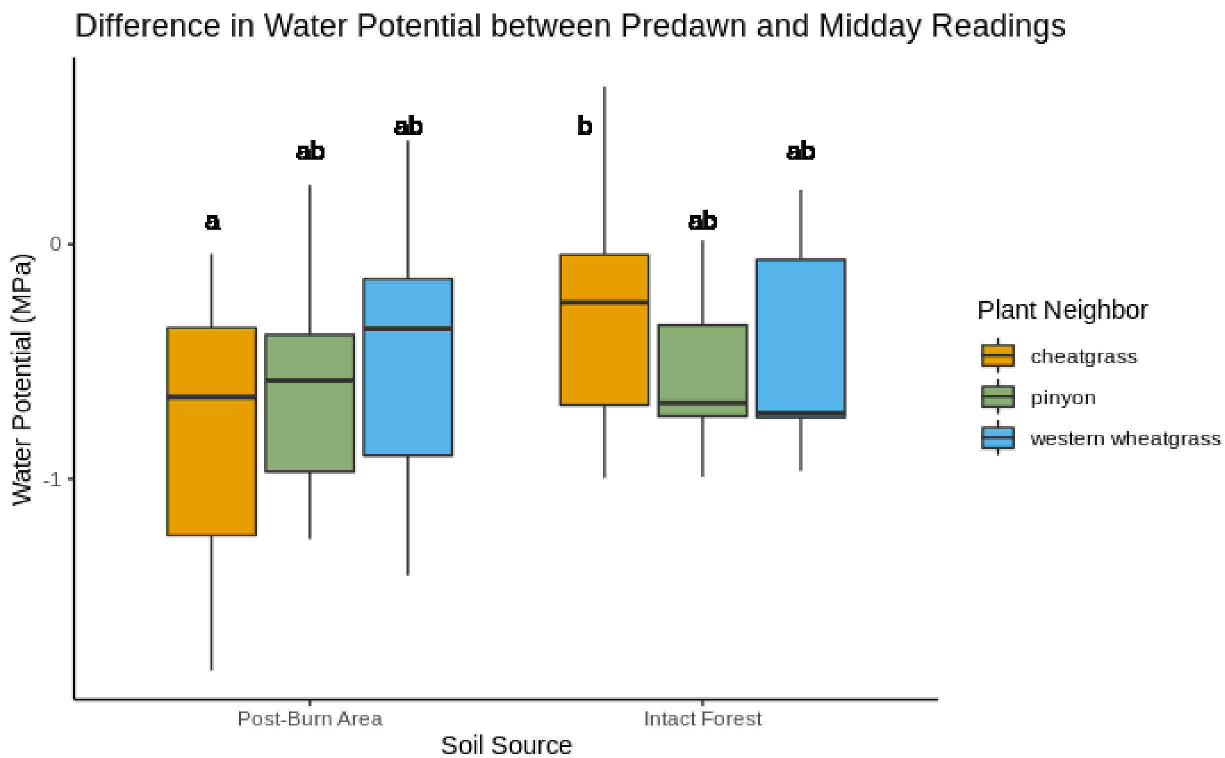

Figure 4: The difference between soil midday and predawn water potential readings, a measure of plant stress, was greater in soils from intact woodlands than in burned soils, despite both treatments receiving ample water in the greenhouse. No differences were observed between plant neighbor treatments within each soil type. Statistically significant differences (α=.05) are denoted with letters, and based on pairwise-contrasts.

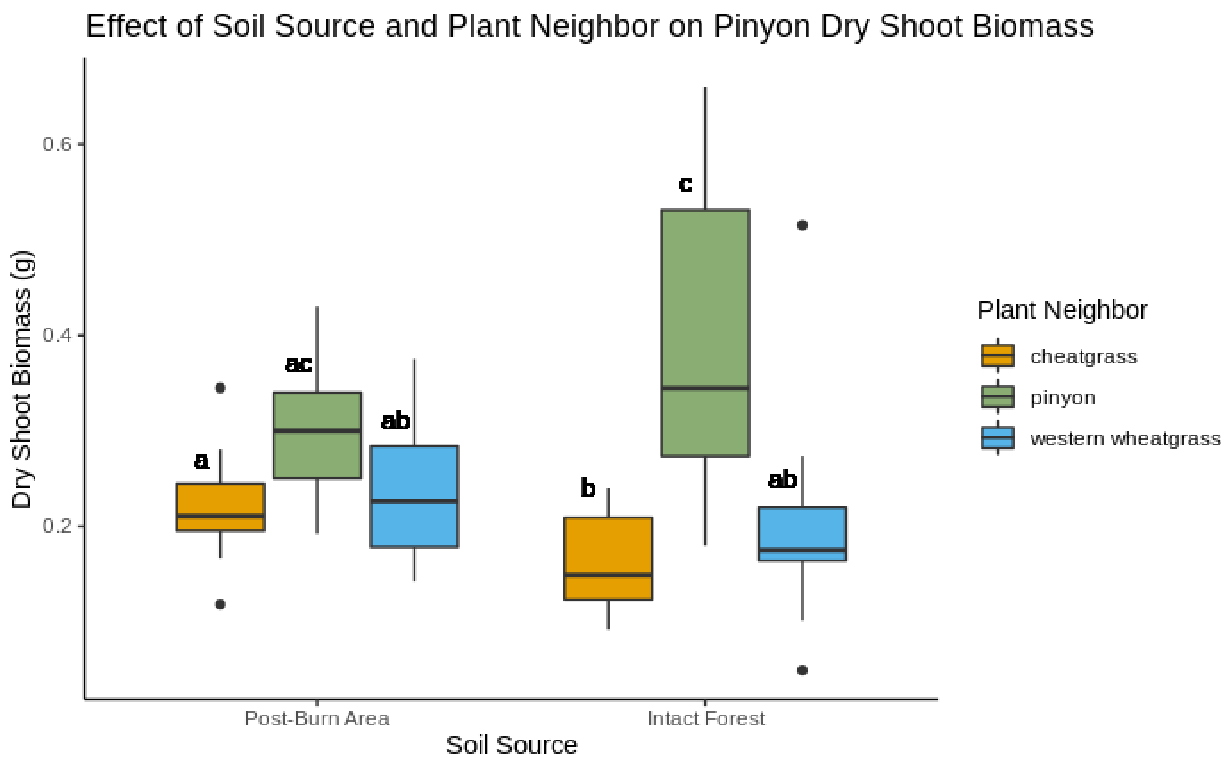

Figure 5: Differences between in seedling shoot biomass were influenced by the interaction between plant neighbor and soil type. Grass neighbor plants had the strongest negative effect on pinyon seedling growth in soils sourced from intact woodlands. Statistically significant differences (α=.05) are denoted with letters, and based on pairwise-contrasts.

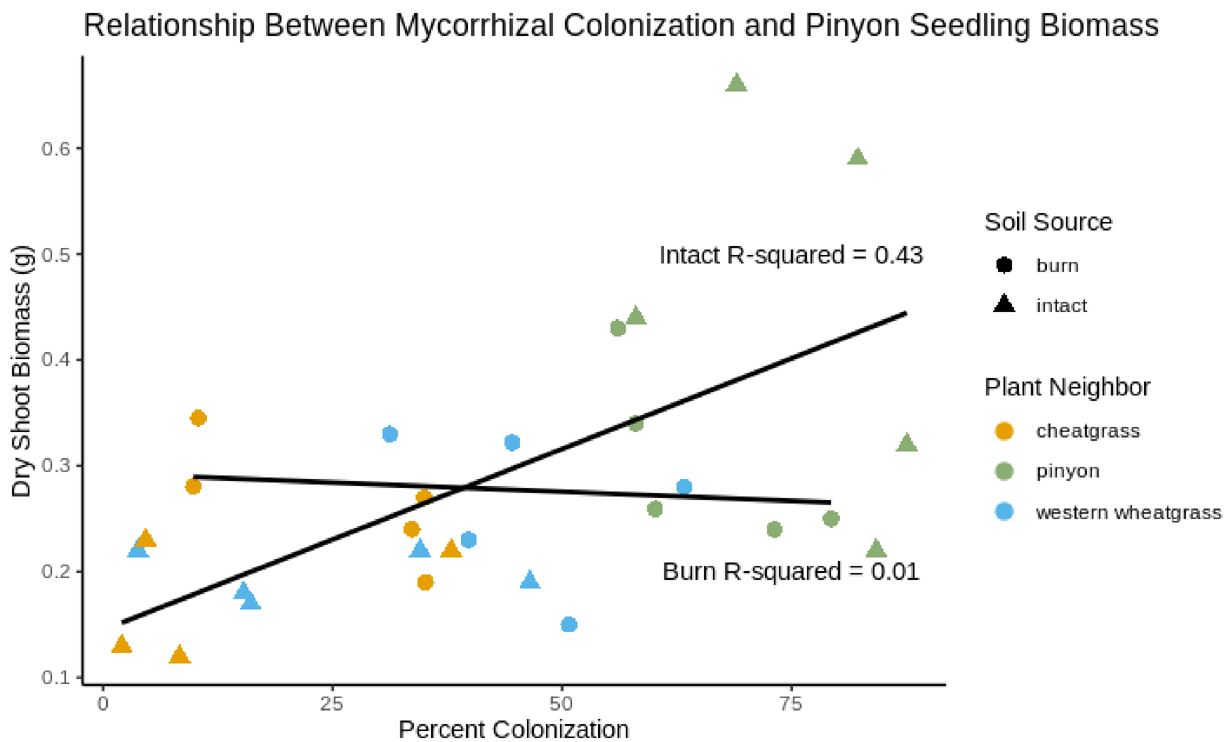

Figure 6: Correlation between pinyon seedling dry shoot biomass and mycorrhizal abundance, measured as percent colonization. While seedlings grown in the intact soil showed a positive relationship between size and EM abundance, those grown in the burned soil did not.