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RESEARCH ARTICLE

Mycorrhizal fungi affect growth of an endemic bunchgrass in pine savannas

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Abstract

1. Planting or sowing native perennial bunchgrasses is a common restoration practice in grasslands disturbed by agricultural or forestry operations. Bunchgrasses provide fine fuel loads for reinstating fire regimes that promote native plant community development. Wiregrass (*Aristida beyrichiana*) is a dominant bunchgrass used in the restoration of south-eastern US pine savannas. Amending disturbed soils with native soils or inoculating them with mycorrhizal fungi are restoration practices used to enhance plant growth or establishment. It is unknown, however, whether these practices increase wiregrass biomass, which would promote fire spread and reinstatement of frequent fire regimes
2. We grew wiregrass from seed in four treatments: (1) undisturbed soil from an undisturbed pine savanna (reference treatment), (2) soil disturbed by plantation forestry, (3) disturbed soil mixed with a small amount of undisturbed soil and (4) disturbed soil inoculated with commercial arbuscular mycorrhizal inoculum. After 6 months, we harvested plants and weighed above- and below-ground biomass. We compared total biomass, soil spore counts and root colonization between treatments.
3. Although the undisturbed and disturbed soils used in the experiment did not initially differ in nutrients or mycorrhizal inoculum potential, spore counts were significantly higher in the disturbed soils we collected. At the end of the experiment, total plant biomass (above- and below-ground) was significantly lower in the undisturbed treatment than in all other treatments, which were not significantly different from each other. There were no significant differences in percent root colonization at the end of the experiment, but soil spore counts were significantly higher in the disturbed and commercially inoculated soils than in the undisturbed and mixed soils.
4. Our results suggest that the mycorrhizal relationship might be enhanced in disturbed soils as arbuscular mycorrhizal fungi mobilize nutrients needed by

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wiregrass; alternatively, disturbed and commercially inoculated soils might harbour a different fungal community than native soils. Although plants had lower biomass in the undisturbed soils, this difference may disappear over time.

5. *Practical implication*. If the goal of savanna restoration is to re-establish fire regimes using wiregrass, inoculation is likely unnecessary. In contrast, inoculating the soil may support other objectives, such as increasing biodiversity or accelerating succession.

KEYWORDS
fungi, inoculum, pine savanna, restoration

1 | INTRODUCTION

The grass-dominated herbaceous layer is a key focus in efforts to restore grasslands and savannas. Understorey grasses are fundamental for reintroducing fire as a key process in these open-canopy ecosystems (Fill et al., 2016; Simpson et al., 2022; Wragg et al., 2018). Perennial bunchgrasses provide continuous fine fuels that promote fire spread, often resulting in abundant seed production and the establishment of numerous other herbaceous species (Ellsworth & Kauffman, 2010; Fill et al., 2012; Wragg et al., 2018). Understorey grasses also influence plant succession (e.g. plant–soil feedbacks) and structure plant communities (Tipton et al., 2022; Wragg et al., 2018). Thus, restoring bunchgrasses via planting seeds or plugs is a common initial practice for restoring ecosystem structure and function.

In grassland ecosystems, arbuscular mycorrhizal fungi (AMF) are a dominant fungal group that promote native grass establishment (Stover et al., 2018). AMF form symbiotic relationships with more than 70% of land plants, in which plants provide photosynthetically derived carbon compounds to the fungi in exchange for water and mobilized nutrients (Brundrett & Tedersoo, 2018; Jennings, 1995; Schnitzer et al., 2011; Smith & Read, 2008). Fire is frequent in many temperate and tropical grasslands; fungal communities typically comprise fire-tolerant or fire-adapted species that are not negatively affected by fire over the long term (Fox et al., 2022; Hansen et al., 2019; Hopkins et al., 2021). Although AMF abundance immediately post-fire might shift with changes in soil nutrients (Fox et al., 2022; Hopkins et al., 2021), and overall fungal community richness might decrease immediately post-fire, communities are resilient on long time-scales (Dove & Hart, 2017). Mechanical disturbances, such as tillage, however, can persistently alter soil microbial community structure (Brito et al., 2012; Rosner et al., 2018; Stevenson et al., 2014); mechanically disturbed soils may have homogenized AMF communities containing low AMF biodiversity (Liu et al., 2023; Lumini et al., 2010; Van der Heyde et al., 2017), resulting in lower plant productivity and diversity (van der Heijden et al., 1998). Experimental studies suggest that amending soils that have been mechanically disturbed encourages the re-establishment of the native microbial community, enhancing native plant biodiversity (Crawford et al., 2020; Duell et al., 2022; Koziol et al., 2022; Neuenkamp et al., 2019; Stevenson et al., 2014; Wubs et al., 2016). For example, commercial AMF inoculants have been used to increase the diversity of AMF communities in disturbed soils (Basiru et al., 2020; Basiru & Hijri, 2022).

Grassland restoration approaches that re-establish plant–fungal relationships are therefore becoming increasingly common (Koziol & Bever, 2017; Lyons et al., 2023). Soil amendments could include the addition of local soil fungal communities or commercial inoculum (Emam, 2016; Paluch et al., 2013). The use of either approach depends on restoration goals and context. For example, Emam (2016) found that adding local soil inoculum to existing AMF communities in a greenhouse experiment resulted in increased mycorrhizal colonization and shoot mass of *Stipa pulchra*, a perennial bunchgrass. In contrast, the addition of a commercial inoculum increased AMF colonization but had no effect on plant biomass (Emam, 2016). Thus, different approaches for restoring mycorrhizal symbioses might be differentially effective for plant vital rates, such as growth, survival and reproduction.

We investigated the relationship between the soil fungal community and the growth of a dominant perennial bunchgrass in southeastern US pine savannas. Wiregrass (*Aristida beyrichiana*) is an endemic, flammable bunchgrass used for reinstating the fire regime in southeastern pine savannas undergoing restoration (Clewell, 1989; Fill et al., 2016; Love et al., 2024; Wenk et al., 2011). Increasing grass biomass is a restoration target for supporting fire spread through the understorey (Fill et al., 2016; Wenk et al., 2011). Although wiregrass is known to establish relationships with mycorrhizal fungi (Fahey & Flory, 2022; Gordon & Rice, 1998), previous work has shown that wiregrass growth is affected by the soil microbial community but has not isolated the effect of fungi within the soil biota (Baruzzi et al., 2022). Moreover, to our knowledge, the efficacy of amending mechanically disturbed soils with native soil or commercial AMF inoculum for increasing wiregrass growth has not been previously investigated. We tested the response of wiregrass biomass to the soil fungal environment by growing wiregrass from seed in four treatments: (1) undisturbed soils from an undisturbed pine savanna (reference treatment), (2) soils disturbed by plantation forestry, (3) disturbed soils inoculated with a small amount of undisturbed soil and (4) disturbed soils inoculated with commercial arbuscular mycorrhizal inoculum. We hypothesized that wiregrass growth

would be affected by different levels of colonization in the different soils. We predicted that wiregrass growth would be greatest in undisturbed soils where mycorrhizal relationships would not have been disrupted. Identifying methods of increasing biomass during early establishment should, in turn, increase the likelihood of plant survival and better facilitate the spread of fire.

2 | MATERIALS AND METHODS

2.1 | Study design

We conducted this study as a greenhouse experiment at the University of Florida in Gainesville, FL, USA. We planted wiregrass seeds in four soil treatments: (1) disturbed soils typical of early restoration (*disturbed*), (2) undisturbed soils (*undisturbed*), (3) disturbed soils mixed with a small amount of undisturbed soil (*mixed*) and (4) disturbed soils inoculated with commercial AMF inoculum (*commercial*). The undisturbed treatment represented a state with naturally occurring soil biota, whereas the mixed, commercial and disturbed treatments represented different soil states in restoration projects. The disturbed soil in this study was affected by mechanical disturbance for forestry operations.

2.2 | Seed and soil collection and testing

We collected seeds from wiregrass populations in the xeric pine savannas of Ordway-Swisher Biological Station, located in Putnam County, Florida, USA (29°41′ N, 82°00′ W) in November 2022 from a unit that had burned in July 2022. We collected soil from Austin Cary Forest, Alachua County, FL (29°44′ N, 82°13′ W) in January 2023. All soils were xeric Ultisols. No special permits were required for the collection of materials. The undisturbed soil was from a management unit that had no recent record of soil disturbance (>30 years), abundant wiregrass and a history of fires every 1–2 years since 2010 (last burned in July of 2021). We collected disturbed soil from a management unit that was a sand pine plantation prior to 2013, when it was logged. The site was burned in 2016, and longleaf pines were planted in 2017, although most of the trees failed to establish. Most recently, the site was partially ploughed and used as a research unit to test the seasonal effects of fire on ruderal species and pollinators (see Adedoja et al., 2022). The disturbed site, last burned in February 2022, was dominated by yankeeweed (*Eupatorium compositifolium*). In the undisturbed site, we collected soil from at least 2 cm below the soil surface and directly beneath and adjacent to existing wiregrass plants. In a previous study, we determined that soils near and far from wiregrass have no effect on wiregrass growth within ecotypes (see Baruzzi et al., 2022). In the disturbed site where wiregrass was sparse, we collected soil at least 10 cm away from any existing plants (of all species) and at least 2 cm below the soil surface.

We also collected 10 soil samples each from disturbed and undisturbed soils. Half of the samples were submitted to the University of Florida (UF) Soil Testing Laboratory for nutrient analyses using standard methods (https://soilslab.ifas.ufl.edu/analytical-research-testing/). The other half were tested for soil spore counts and mycorrhizal inoculum potential (MIP) at the UF/IFAS Soil and Water Sciences Testing Laboratory (https://soils.ifas.ufl.edu/research/laboratory-services/soil-and-water-sciences-diagnostic-laboratories/). For the latter samples, we extracted mycorrhizal fungal spores from these soil samples using the wet sieving and decanting method described in Pacioni (1992). We then counted the extracted spores using a compound microscope. A subsample of approximately 3 grams, based on the dry weight, was taken from each sample to mix with soil growth media to grow model plant corn in three replicates in the greenhouse for 28 days. All plants received modified Hoagland's solution of low phosphorus concentration (less than 40 ppm) once a week and were watered as needed. After 28 days, the roots were cleaned from the soil, cleared with 10% potassium hydroxide, acidified with 1% hydrochloric acid and stained with 0.05% Trypan blue (The University of Kansas, 2024). Representative root samples were loaded on a slide to be examined under a light compound microscope. The MIP test is reported as percentages of root colonization in the corn, which reflects the spore viability to initiate colonization (Salomon et al., 2022).

2.3 | Experimental set-up

We planted three wiregrass seeds in each 2″ × 8″ plant band (Stuewe & Sons, Inc.), with 35 containers per treatment (undisturbed, disturbed, mixed and commercial). There was a total of 140 experimental units (35 reps × 4 treatments). The commercial inoculum used was MycoApply Endo, a granular mix of four species of endomycorrhizal fungi (*Glomus* spp.), obtained from Mycorrhizal Applications (https://mycorrhizae.com/mycoapply-endo/). For the commercial treatment, we added twice the amount of recommended inoculum, scaled to the amount of soil added to each container (following the methods of Koziol et al., 2020). For the mixed treatment, we used a ratio of 25:75 undisturbed:disturbed soil in each container, with the undisturbed soil added to the top of the disturbed soil but not mixed into it. Polyester stuffing was placed at the bottom of each plant band to prevent soil from falling out and to absorb moisture. To remove roots and debris, the soil was sieved (sieve mesh 1.0 cm). The seeds were cleaned using a 10% bleach solution and then rinsed several times with deionized water prior to planting to minimize bleach residue.

On 24 February 2023, we placed four trays (one tray for all the units of each treatment) into growth chambers set at 25°C and a 12L:12D light regime. We used distilled water to keep the soil moist, regularly adding water to ensure the soil surface did not dry out. Trays were rotated 180 degrees every week. The first seedlings that germinated in each pot were allowed to grow; the others were removed as they germinated. After 7 weeks, we transported trays to Ordway-Swisher Biological Station's outdoor set-up for growing wiregrass plants. After 7 weeks, the plants were placed on outdoor benches at the University of Florida's Ordway-Swisher Biological

Station (Melrose, FL, USA), where they were misted for 15 minutes twice a day, at 7 AM and 5 PM. This watering scheme is standard for establishing wiregrass plugs used for restoration (A. Rappe, pers. comm.). Trays were rotated once per week, both in the growth chambers and in the outdoor set-up.

2.4 | Post-experiment harvesting

We harvested surviving plants 6 months after germination. Final sample sizes were between 21 and 25 plants per treatment (see Table S4 for sample sizes). From these, we set aside eight haphazardly selected plants in each treatment to assess soils for spore counts (see methods above) and roots for colonization. The remaining plants were harvested for above- and below-ground biomass and dried in an oven at 60°C for 4 days before weighing them.

To evaluate mycorrhizal root colonization, we first washed wiregrass roots with distilled water to remove any soil particles. Cleaned root samples were cut into 1 cm segments and immersed in a 10% KOH solution at 90°C for 1 h. We then washed the root samples with distilled water and placed them in a 10% hydrogen peroxide solution for 15 min. After this step, root samples were washed again with distilled water, acidified in 1% hydrochloric acid and stained using a 0.05% Trypan blue solution with equal parts of lactic acid, glycerol and distilled water. We observed stained root samples under a light compound microscope and quantified root colonization using the gridline-intersect method described in Giovannetti and Mosse (1980).

2.5 | Statistical analysis

We ran pre-experiment analyses to determine whether the disturbed and undisturbed field soils used for the treatments initially differed in soil spore counts, MIP and soil nutrients. We used negative binomial regression with *glm.nb* to model spore counts and a beta regression with the *betareg* function (Cribari-Neto & Zeileis, 2010) to model MIP, transformed to a proportion (divided by 100). Spore counts and MIP were not significantly correlated (Kendall's correlation test $p > 0.05$). We ran individual linear models with *lm* to test whether each soil element (except percent organic matter, for which we used the beta distribution) differed between disturbed and undisturbed soil. Nitrogen (NH_4N and NO_3N) values were lower than the method detection limit in analysis and thus were considered to be minimal and excluded.

To determine the effect of our experimental treatments on wiregrass growth, we analysed differences in total biomass between all four treatments. Shoot biomass was positively correlated with root biomass (linear model; $p < 0.001$), and thus, total biomass, the sum of root and shoot biomass, was used for analyses (Figure S1). We used a linear model with *lm* in R to determine whether total biomass differed between treatments. We compared estimated marginal means using contrasts in the R package 'emmeans' (Lenth et al., 2018).

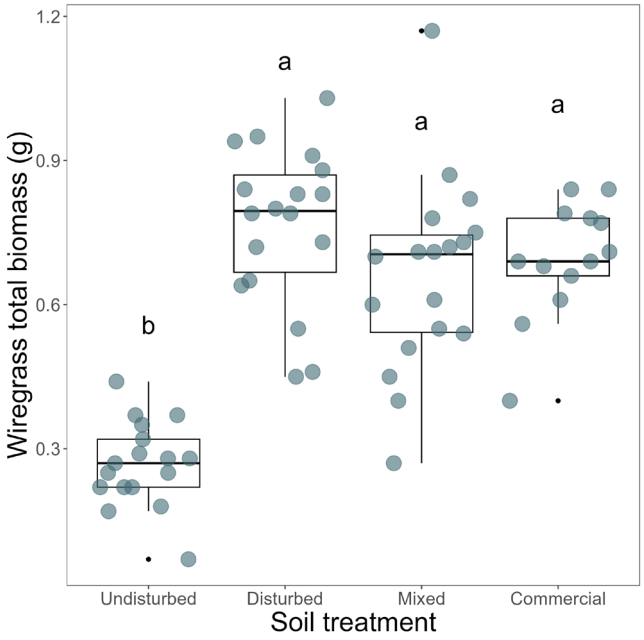


FIGURE 1 Total biomass (sum of root and shoot biomass) for wiregrass (*Aristida beyrichiana*) plants grown from seed in four soil treatments. Plant total biomass was significantly lower in the undisturbed treatment than in all other treatments, which were not significantly different from one another.

We also compared post-harvest soil spore counts and the percent root colonization between soils of the four treatments. We used negative binomial regression with *glm.nb* to model spore counts, and *betareg* to model the percent root colonization, transformed to a proportion (divided by 100). Spore counts and root colonization were not significantly correlated (linear model $p > 0.05$). We compared means using contrasts in the package 'emmeans' (Lenth et al., 2018). We did not test the correlation between biomass and soil spore counts or percent root colonization because these variables were measured on different samples than those harvested for biomass. All analyses were performed in R (R Core Team, 2023).

3 | RESULTS

Preliminary analyses indicate some similarity between the undisturbed and disturbed soils. There were no significant differences in nutrient concentrations between undisturbed and disturbed soils (Tables S1 and S2). Although MIP was not significantly different between soils, pre-experiment spore counts were significantly higher in disturbed than in undisturbed soils (Tables S1 and S2).

At the end of the experiment, plant total biomass was significantly lower in the undisturbed treatment than in all other treatments, which were not significantly different from each other (Figure 1; Table 1; Tables S3 and S4). There were no significant differences in percent root colonization (Figure 2; Table 1). Soil spore counts, however, were significantly higher in the soils of the

TABLE 1 Comparison of means of treatments for samples harvested post-experiment.

	Estimate	SE	*p*
Total biomass (g)			
Undisturbed–disturbed	−0.50	0.05	<0.001
Undisturbed–commercial	−0.43	0.06	<0.001
Undisturbed–mixed	−0.39	0.05	<0.001
Disturbed–commercial	0.07	0.06	0.566
Disturbed–mixed	0.11	0.05	0.173
Commercial–mixed	0.03	0.06	0.932
Soil spore counts			
Undisturbed–disturbed	−0.76	0.15	<0.001
Undisturbed–commercial	−0.51	0.15	0.006
Undisturbed–mixed	0.08	0.16	0.954
Disturbed–commercial	0.26	0.15	0.305
Disturbed–mixed	0.85	0.15	<0.001
Commercial–mixed	0.59	0.16	<0.001
Root colonization (%)			
Undisturbed–disturbed	−0.06	0.06	0.787
Undisturbed–commercial	−0.07	0.06	0.662
Undisturbed–mixed	−0.04	0.06	0.897
Disturbed–commercial	−0.01	0.07	0.997
Disturbed–mixed	0.02	0.07	0.996
Commercial–mixed	0.03	0.07	0.971

disturbed and commercial treatments than in the undisturbed and mixed treatments (Figure 3; Table 1; Tables S3 and S4).

4 | DISCUSSION

Contrary to expectations, we found that wiregrass growing in disturbed soils, amended or not, had greater above- and below-ground biomass than plants growing in undisturbed reference soils. Although the disturbed and commercially inoculated soils had higher spore counts, likely from weedy fungal species that produce abundant spores (Ishida et al., 2008), MIP did not differ significantly among our treatments, so all plants had equivalent colonization potential. Increased biomass might have resulted from structural changes in the soil or from chemical changes other than those we measured, which promoted the symbiotic aspects of the mycorrhizal association that increase plant growth. Although release from pathogen accumulation could also explain increased growth in disturbed soils, our previous research showed no evidence of negative plant–soil feedbacks (Baruzzi et al., 2022). Alternatively, or in addition, disturbed soils may have harboured AMF taxa different from those in native soil that promoted wiregrass growth or taxa that did not inhibit wiregrass growth. Previous studies have shown that AMF communities differ depending on site use history and the dominant plant species (House & Bever, 2018; Tipton et al., 2018, 2022).

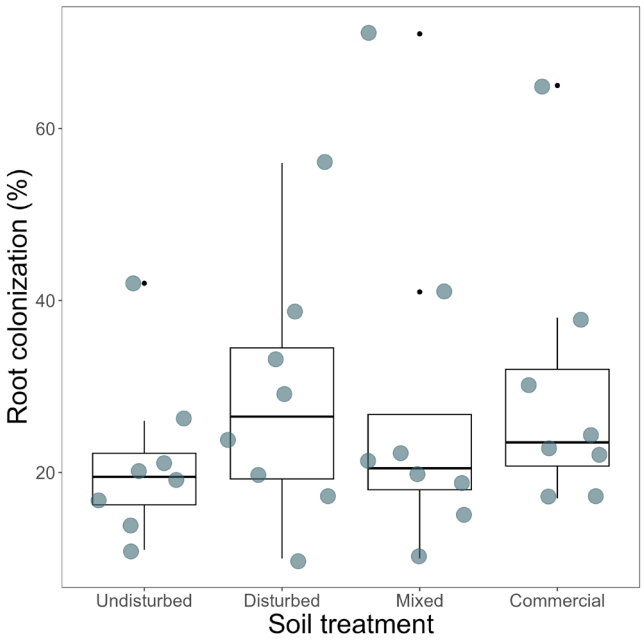


FIGURE 2 Percent root colonization for wiregrass (*Aristida beyrichiana*) plants grown from seed in four soil treatments. Percent root colonization for wiregrass plants did not differ significantly between the four soil treatments.

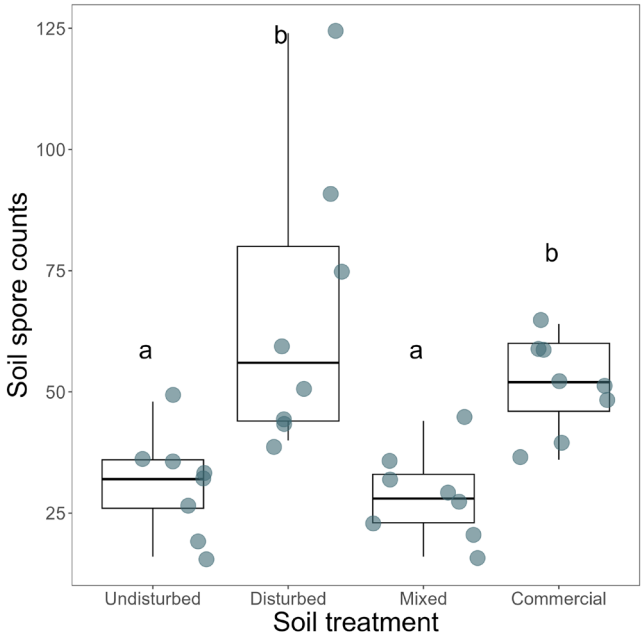


FIGURE 3 Spore counts in the soil of four treatments in which wiregrass (*Aristida beyrichiana*) plants were grown from seed. Soil spore counts were significantly higher in the disturbed and commercially inoculated soils than in the mixed and undisturbed soils post-harvest.

These results suggest that the symbiotic relationship might be enhanced in disturbed soils. Although plants had lower biomass in the undisturbed soils, this difference may disappear over time. Our experiment lasted for 6 months; over time, in natural stands, plants

may converge to similar sizes as the growth of mature plants is slow (Laucevicius Jr et al., 2021).

Inoculation of mechanically disturbed soils might not be necessary for increasing wiregrass growth in early restoration. Although research has shown that microbial communities in mechanically disturbed soils can become homogeneous and limit or reduce the successful establishment of local plant communities (Islam et al., 2021), we found no negative effects of soil disturbance on wiregrass growth. Wiregrass, in particular, has been shown to establish well in disturbed soils, including a reclaimed phosphorus mining site (Bissett, 1996). Inoculation could, however, still provide some benefit by filling niches in the soil where indigenous taxa may be lacking. A meta-analysis of 380 restoration field studies regarding mycorrhizal inoculation concluded that the addition of local or commercial inoculum generally increased plant biomass and height (Neuenkamp et al., 2019). A commercial AMF inoculant was found to establish with crop plants and promote plant growth in a three-year prairie study in Canada, in some instances coexisting but not necessarily overlapping with the existing AMF communities (Islam et al., 2021). Local inoculum, however, should be prioritized, as local adaptation is a major driver of plant–fungal relationships (Johnson et al., 2010; Karlsen-Ayala et al., 2022; Maltz & Treseder, 2015; Rúa et al., 2016).

The soil microbial environment is an important consideration for grassland restoration. If the goal of savanna restoration is to re-establish the fire regime using wiregrass (Baruzzi et al., 2023), inoculation is likely not necessary. In contrast, inoculating the soil may help reach other objectives, such as increasing biodiversity or accelerating succession. For instance, Koziol et al. (2022) showed that mycorrhizal fungi can advance plant succession and that the reintroduction of both whole soil and laboratory-cultivated native mycorrhizal fungi can be used as tools to improve native plant restoration following anthropogenic disturbance. Given the local soil and commercial inoculum have similar effects in our study, we recommend future research first examine the effects of local soil amendments on plant community succession. Freshly collected local soil is preferred in most cases, as inoculum preparation and handling have been shown to influence viability and efficacy (Gundale et al., 2017; Peacher & Meiners, 2020) and would be less likely to benefit invasive species (Xia et al., 2020). This study is a first step to better understanding the role of soil microbial communities in the restoration of southeastern US pine savannas.

AUTHOR CONTRIBUTIONS

Jennifer Fill, Raelene Crandall and Elena Karlsen-Ayala conceived the ideas. Adele Kimball, Debriana Love, Victoria Lopez-Scarim, April Zee, Abid Al-Agely and Raelene Crandall collected the data. Jennifer Fill analysed the data and led the writing of the manuscript. All authors contributed to the methodology, contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/2688-8319.70026.

DATA AVAILABILITY STATEMENT

The data used in this study are available on Zenodo (Kimball et al., 2025): https://doi.org/10.5281/zenodo.14961855.

RELEVANT GREY LITERATURE

You can find related grey literature on the topics below on Applied Ecology Resources: Fungi, Restoration.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1: Above- and belowground biomass for wiregrass (*Aristida beyrichiana*) plants grown in four soil treatments were significantly correlated.

Table S1: Model estimates, standard errors (SE), and significance levels for preliminary soil analyses.

Table S2: Descriptive statistics for soil variables tested in preliminary soil analyses.

Table S3: Parameters of models testing for differences in wiregrass total biomass, shoot biomass, soil spore counts, and root colonization.

Table S4: Descriptive statistics for variables analyzed between treatments.

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