

ARTICLE

Spore traits mediate disturbance effects on arbuscular mycorrhizal fungal community composition and mutualisms

Jacob R. Hopkins  | Alison E. Bennett 

Evolution, Ecology & Organismal Biology,
The Ohio State University, 318 W 12th
Avenue Aronoff Laboratory, Columbus,
Ohio 43210, USA

Correspondence

Jacob R. Hopkins
Email: hopkins.982@osu.edu

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Abstract

Trait-based approaches in ecology are powerful tools for understanding how organisms interact with their environment. These approaches show particular promise in disturbance and community ecology contexts for understanding how disturbances like prescribed fire and bison grazing influence interactions between mutualists like arbuscular mycorrhizal (AM) fungi and their plant hosts. In this work we examined how disturbance effects on AM fungal spore community composition and mutualisms were mediated by selection for specific functional spore traits at both the species and community level. We tested these questions by analyzing AM fungal spore communities and traits from a frequently burned and grazed (bison) tallgrass prairie system and using these spores to inoculate a plant growth response experiment. Selection for darker, pigmented AM fungal spores, changes in the abundance and volume of individual AM fungal taxa, and altered sporulation, were indicators of fire and grazing effects on AM fungal community composition. Disturbance associated changes in AM fungal community composition were then correlated with altered growth responses of *Schizachyrium scoparium* grass. Our work shows that utilization of trait-based approaches in ecology can clarify the mechanisms that underly belowground responses to disturbance, and provide a useful framework for understanding interactions between organisms and their environment.

KEYWORDS

AM fungal mutualisms, disturbance, fire, grasslands, grazing, mycorrhizae, plant–soil (belowground) interactions, trait-based approach, traits

INTRODUCTION

Trait based approaches in ecology are invaluable for understanding interactions between organisms and their environment. This approach has proven particularly

useful in community ecology for understanding how processes like disturbance, succession, and trait interactions impact community assembly and ecological function (Day et al., 2020; Laughlin, 2014; Zakharova et al., 2019). Recent plant studies have identified traits which vary

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across disturbance regimes and climates, and influence plant community composition and ecosystem level processes like fire regimes and productivity (Cui et al., 2020; Kandlikar et al., 2022; Li et al., 2018). Since soil microbial communities also vary across disturbance regimes and climates, trait-based approaches may also be useful for understanding microbial community and functional responses to disturbance and aboveground processes (Chaudhary et al., 2022; Deveautour et al., 2020; Treseder & Lennon, 2015; Zanne et al., 2020). For example, traits related to microbial stress tolerance (e.g., melanin content and life history strategy) and function (e.g., nutrient foraging and plant–fungal interactions) vary between clades, suggesting that selection on stress tolerance traits could drive changes in microbial functions and belowground processes. Further, spore morphology traits have already been linked to arbuscular mycorrhizal (AM) fungal community assembly (Chaudhary et al., 2020; Deveautour et al., 2020), which can influence plant community diversity and productivity. Therefore, identifying ecologically relevant microbial traits would not only help our understanding of microbial community assembly (Clemmensen et al., 2015; Hart et al., 2016), but may also be useful for understanding belowground responses to aboveground changes.

Trait-based approaches may be particularly useful for understanding how interactions between above- and belowground communities respond to disturbance. Grasslands provide key examples, since many grassland plants possess traits associated with surviving recurrent fire and grazing by bison (Archibald et al., 2018; Daubenmire, 1968; Ford, 2010). Since microbial symbionts like AM fungi are closely associated with grassland plant communities and productivity (Hartnett & Wilson, 1999; van der Heijden et al., 2008), understanding disturbance relevant traits that mediate AM fungal community assembly could inform disturbance effects on plant–fungal interactions. Traits that help AM fungi survive high fire temperatures, carbon limitation following grazing, or altered soil conditions following disturbance could not only influence AM fungal community composition, but also AM fungal mutualisms. Since AM fungi can influence plant productivity, and are affected by fire (van der Heyde et al., 2017) and grazing (Heyde et al., 2017), it is imperative that we identify the functional traits that mediate AM fungal responses to disturbance (Chaudhary et al., 2022).

AM fungal spore traits vary greatly between taxa in terms of color, size, ornamentation, and cell walls. This variation can be used to identify AM fungal species (INVAM, 2022; Powell et al., 2009), and it is increasingly evident that spore traits are related to life history strategies (Chagnon et al., 2013; Chaudhary et al., 2022;

Deveautour et al., 2020; Powell & Rillig, 2018; van der Heyde et al., 2017). Since AM fungal sporulation is also a meaningful fitness metric (Bever, 2002; Bever et al., 1996), trait responses to forces like disturbance are not only informative of AM fungal community dynamics, but also AM fungal mutualisms. In common disturbance contexts (i.e., prescribed fire and grazing), spore traits like color, size, and abundance may be related to the ability to resist environmental disturbance. For example, small, spored taxa with high sporulation rates may be beneficial in frequently grazed environments where plant hosts allocate fewer resources to root symbionts (Allsup et al., 2021; van der Heyde et al., 2019). Darker colored spores created by high melanin and/or pigment contents could help spores survive arid conditions (Deveautour et al., 2020; Henson et al., 1999), temperature fluctuations during fire (Cordero & Casadevall, 2017), or post-fire UV exposure (observed in Hopkins et al., 2021). If fire and grazing have predictable effects on AM fungal community composition through specific spore traits, then this could have disturbance dependent implications for AM fungal mutualisms and plant communities.

Healthy grassland plant and microbial communities (e.g., AM fungi) display long-term adaptation to fire and grazing disturbance (Daubenmire, 1968; Ford, 2010); but, understanding the mechanisms that underly these disturbance regime effects on ecosystems has been difficult. However, trait-based approaches may be helpful for understanding these mechanisms, since AM fungal taxa vary greatly in terms of spore traits (Chaudhary et al., 2022; INVAM, 2022; Powell et al., 2009), responses to disturbance (Allsup et al., 2021; Eom et al., 1999; Klopatek et al., 1988; van der Heyde et al., 2019), and effects on plant hosts (Hoeksema et al., 2010; Sikes et al., 2009). Therefore, understanding how disturbance selects for specific spore traits may clarify post-disturbance AM fungal community assembly processes and symbiotic outcomes (Beals et al., 2020). For example, fire and grazing (or their combined effects) could select for different spore traits that drive variation in post-disturbance AM fungal community composition. If disturbance selected spore traits are also associated with changes in the abundance of AM fungal taxa with higher or lower mutualistic capabilities, disturbance driven variation in AM fungal community composition could also influence plant community productivity. Therefore, understanding how traits mediate disturbance effects on AM fungi communities and mutualisms is key for understanding the above- and belowground mechanisms that underly grassland ecosystems.

We tested how prescribed fire and bison grazing effects on AM fungal spore community composition and mutualisms were mediated by selection for spore traits. We sampled AM fungal spore communities from tallgrass

prairie sites with different fire and grazing regimes at Nachusa Grasslands during the Summer and Fall of 2021. Spore communities were quantified and either used for trait analyses (Fall spores) or inocula for a green house plant growth assay (Summer spores). We conducted a plant growth assay using the AM fungal spores from burned and grazed plots to test how disturbance effects on AM fungal spore communities altered interactions with plant growth. This allowed us to test three questions: (1) how do fire and grazing independently and interactively influence AM fungal community composition; (2) does management disturbance select for specific AM fungal spore traits at the species and/or community levels, and is this correlated with shifts in community composition; and (3) do changes in traits and community composition alter AM fungal mutualisms? Note that this work tests both the evolutionary (selection on traits within species) and the community ecology (filtering of species by environmental factor based on trait combinations; “community level”) definitions of selection. We hypothesized that prescribed fire and grazing would favor specific spore traits related to disturbance, and that this selection would drive differences in AM fungal spore community composition and mutualisms. We predicted that fire would select for darker coloration and increased pigmentation, which could provide resistance to high temperatures (Cordero & Casadevall, 2017) and protection from oxidation in post-fire environments (Tereshina, 2005). We also predicted that grazing would favor smaller volumed spores which may have smaller carbon requirements beneficial in carbon limited, grazed environments where plant hosts may allocate less C to mutualists (van der Heyde et al., 2019). Finally, we predicted that selection for spore traits would be linked to disturbance related changes in community composition, and changes in benefit provided to plant hosts. Our findings suggest that disturbance influences AM fungal mutualisms through selection for spore traits and changes in community composition.

MATERIALS AND METHODS

Study system

We conducted the field component of this work at The Nature Conservancy's Nachusa Grasslands property in Ogle County, IL, USA (41°53'28.0674' N, 89°20'35.2314' W; Figure 1). This region of Illinois has a 5–6 month long growing season between April and September, with an annual average of 940 mm of precipitation coming primarily in the Summer and Fall. Native vegetation is characterized by *Schizachyrium scoparium*, *Andropogon gerardii*,

Baptisia australis, and *Silphium laciniatum*. Nachusa Grasslands is divided into two primary units (North and South) that are managed with prescribed fire and grazing by bison. Fires generally take place in late Winter and early Spring, with fire return intervals of ~2 years. Fires in 2021 took place between February and April. Bison graze throughout both units outside enclosure plots.

Field sampling

Soil samples (July 600 mL; September: 100 mL) were collected from 13 bison enclosures in July of 2021 and 16 enclosures (3 unburned enclosure sites were added to sampling effort) in September of 2021 (Figure 1). A larger volume of soil was collected in July to inoculate the greenhouse experiment. Sampling effort was expanded in September since smaller soil volumes were collected and determined to be less disruptive of the soil. Of the 16 enclosures, 6 were located in burned (B+), and 10 in unburned (B) portions of Nachusa Grasslands. Each enclosure was comprised of a fenced off plot (no grazing; G) and an adjacent open plot (grazing; G+; Appendix S1: Figure S1 inset). For July spore communities this produced: six B+G+ plots, six B+G plots, seven B G + plots, and seven B G plots. For September spore communities this produced: six B+G+ plots, six B+G plots, 10 B G+ plots, and 10 B G plots. From each plot, one soil sample (July: 600 mL; September: 100 mL) was collected from the base of a single *S. scoparium* plant using a hand trowel that was sterilized with ethanol between plots.

Soil samples were stored at 4°C for 2 weeks to promote AM fungal sporulation. Then spores were extracted from soil samples using 2 mm and 38 μ m sieves, followed by centrifugation with 60 sucrose solution. July spores were extracted from 600 mL of soil using three separate 200 mL extractions ($n = 78$, three replicates per plot), while September spores were extracted from 100 mL of soil using one extraction ($n = 32$). Different extraction volumes were necessary because a larger quantity of July spores were required to inoculate the greenhouse experiment.

Spore community and trait analyses

AM fungal spore communities were quantified using a Nikon SMZ800N dissection scope (Nikon, Tokyo, Japan) at 30 \times under the same light conditions. Spores were sorted into morphotypes based on size, color, internal contents, hyaline appearance, hyphal connection, and shape, then putatively identified (Appendix S1: Section S1). Species were counted based on morphology, and checked for viability by assessing cell wall integrity and internal lipid

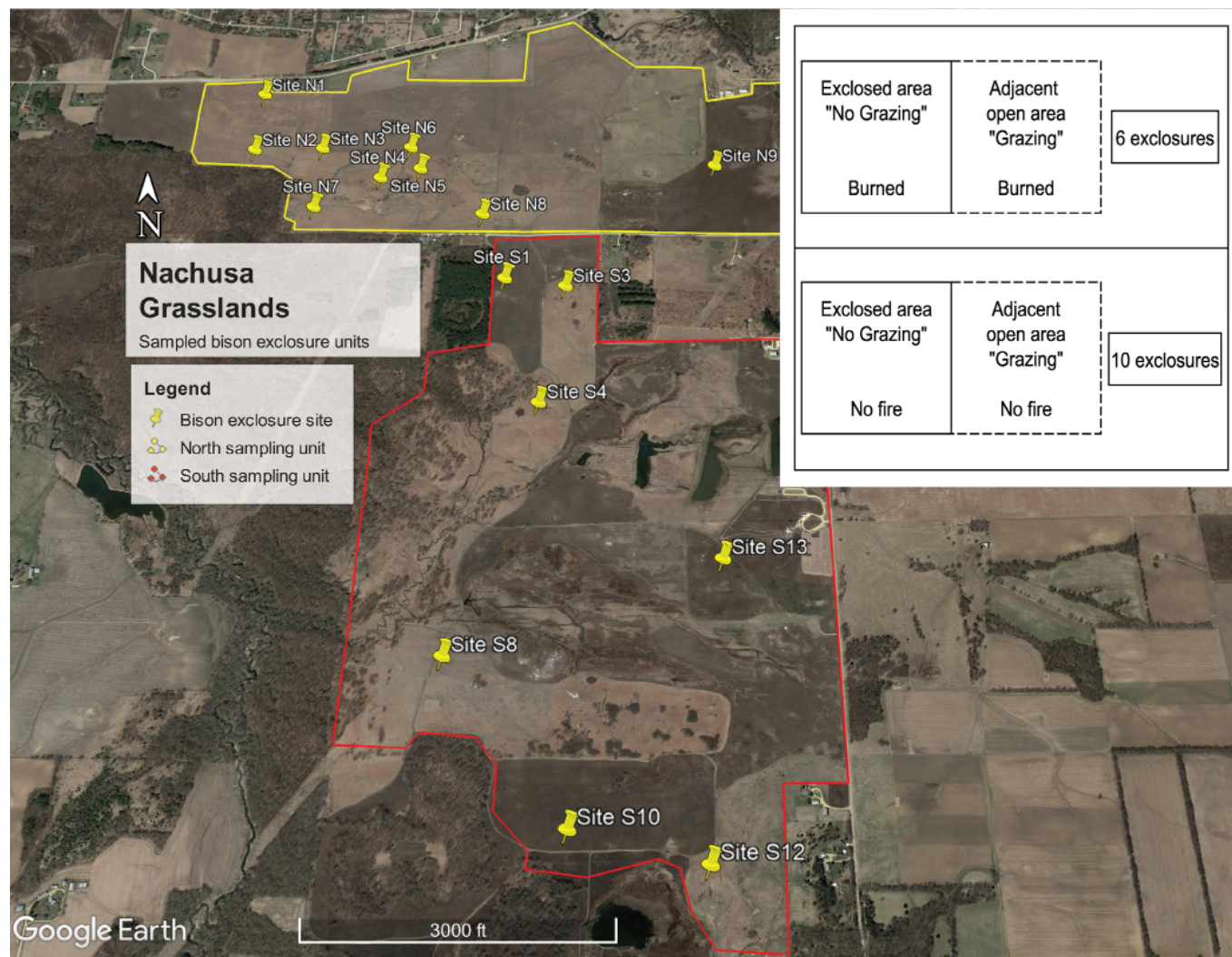


FIGURE 1 Map of bison exclosures at Nachusa Grasslands and exclosure layout details. The bison units at Nachusa are divided into northern (yellow outline) and southern (red outline) sections each containing bison exclosures (marked with pins). The exclosure plots (inset) are comprised of a fenced off section (no grazing) and an adjacent open section (grazing). Exclosures were either located in burned or unburned portions the bison units.

contents by crushing (if necessary). For trait analyses, pictures of each AM fungal spore were taken using a Nikon DS-Fi3 microscope mounted camera at 30× under the same light conditions. The NIS-Elements AR 5.02.01 software was used to measure the radius, volume, luminance, saturation, hue, mean red, mean green, and mean blue, for each spore. When spore shape was not a perfect sphere (e.g., spheroid, ellipse), the radius and volume were calculated by overlaying a circle shape of similar size to the spore. Pictures were calibrated in micrometers, and colorimetric variables (red-green-blue; RGB model) were obtained from pixels in manually overlaid regions of interest on each spore. Equations for trait variables are included below.

$$\text{spore volume (m}^3\text{)} = \frac{4}{3}\pi r^3; \quad r = \text{radius,}$$

$$\text{saturation} = \max(R, G, B) - \min(R, G, B),$$

$$\text{luminance} = (0.216 \times R) + (0.7152 \times G) + (0.0722 \times B)$$

Hue (H) was calculated using the following two equations:

$$H = \arccos \left[\frac{R - \frac{1}{2}G - \frac{1}{2}B}{(R^2 - G^2 - B^2 - RG - RB - BG)^{0.5}} \right],$$

$$H = \begin{cases} 360^\circ - H & \text{if } B > G \\ H & \text{if } B < G \end{cases}$$

Saturation is the purity of the spore's color, with 0 being gray and 255 being pure color. Luminance describes how dark a spore's color is (0 = black, 255 = white). Hue refers

to spore color on a 0° to 360° color wheel. Traits were recorded for individual spores and averaged across communities to test for disturbance effects within and across taxa. Total spore volumes for small (0–35 μm radius), medium (36–99 μm), and large (100+ μm) spores were also calculated at the community level.

Green house experimental set up

To test *S. scoparium* responses to disturbance effects on AM fungal spore communities we set up a plant growth assay experiment with the July AM fungi spores. Background soil was collected from Nachusa grasslands and mixed 1:1 with sand and autoclaved twice (2 h at 120°C). *S. scoparium* seeds were purchased from Prairie Moon Nursery (Winona, MN), started in sterilized potting soil (autoclave; 2 h at 120°C), then grown for 3 weeks.

Spores extracted from July samples were kept separate between sites and used as AM fungal inocula solution. To isolate AM fungal effects on plants from the effects of other soil biota, we also created microbial washes from the inocula solutions collected during spore extractions. Following extractions, liquid from each inocula treatment was vacuum filtered through a Buchner funnel with a Whatman filter (90 mm diameter, 11 μm pore size). Half of each microbial wash and AM fungal inocula solution were then autoclaved to create sterile controls for the microbial washes and AM fungal inocula solutions (30 min, 120°C). Using the sterilized soil, inocula, and microbial washes, we set up a greenhouse experiment using 175 mL Cone-tainers (Stuewe & Sons, Tangent, OR). To each pot, 170 mL of sterile background soil was added, then an *S. scoparium* plant was planted, and 1 mL of AM fungal inocula or microbial wash was added to each pot as appropriate. Briefly, AM fungal inocula and autoclaved microbial wash was added to AM fungal + pots, but not AM fungal – pots which instead received microbial wash and autoclaved AM fungal inocula. This allowed us to test the effect of AM fungi relative to the background microbial communities. Each grazing \times AM fungal inocula \times plot treatment included five replicates for a total of 260 pots. Plants were grown in a greenhouse for 3 months with supplemental daytime lighting. Each pot received 2 mL of 200 ppm 15–0–15 (NPK) fertilizer on a bi-weekly basis. Plants were harvested after 3 months. After harvesting, plants were dried for 3 days at 60°C, and biomass was recorded.

Statistics

Analyses were conducted in R version 4.1.3 (R Core Team, 2022). We tested how prescribed fire and grazing

influenced Summer and Fall AM fungal spore community composition using principal coordinates analysis (PCoA) and permutational multivariate analysis of variance (PERMANOVA) with the Vegan package (Oksanen et al., 2013). Bray–Curtis dissimilarity matrices and PCoAs for AM fungal spore communities were produced using the `vegdist()` and `princomp()` functions. Following ordination, PERMANOVAs were used to test for disturbance effects on AM fungal spore community composition. PERMANOVA models included terms for fire, grazing, site age (time since restoration; remnants \approx 100 years), management unit, exclosure plot nested within management unit, and fire \times grazing interactions. Generalized linear mixed effect models (GLMERs) with poisson link functions tested for changes in abundance of each AM fungal taxa found in whole field sampling and spore densities with the `glmer()` and `joint_tests()` functions in the `lme4` (Bates et al., 2015) and `emmeans` (Lenth, 2018) packages. Each GLMER included similar terms as the PERMANOVA models. Estimated marginal means were extracted with the `emmeans()` function and tested with contrasts using the `contrast()` function.

Disturbance effects on Fall AM fungal spore traits at the species (saturation, luminance, volume) and community (saturation, luminance, small spore volume, medium spore volume, and large spore volume) level were assessed using multivariate analysis of variance (MANOVA). The MANOVA model included identical terms as the PERMANOVA with the addition of spore hue as a covariate. Note that the species level MANOVA also included a species term to test for grazing and fire effects on spore traits within species. Spore hue was included as a covariate in models since spore hue can determine other colorimetric traits. MANOVA model error terms were visually assessed for normality following analyses, and met model assumptions. For species level traits model, contrasts similar to those for the GLMER models were applied. For the community level model, linear mixed effect models (LMERs) with the `lmer()` function were used following the MANOVA to analyze treatment effects on individual spore traits. Each LMER included prescribed fire treatment, grazing treatment, site age, spore hue as well as a fire and grazing interaction term as fixed effects and covariates. Since the bison enclosures are divided into Northern and Southern units, LMER models included management unit as a random effect term. We then used structural equation modeling (SEM) to evaluate how disturbance influenced AM fungal spore communities through spore traits with the `lavaan` package's `sem()` function (Rosseel, 2012). Appendix S1: Tables S1 and S2 describe variables and justifications for model pathways. All trait variables were scaled prior to analysis with the `scale()` function, and upon convergence,

fit measures and parsimony were used to assess model fit with the `fitmeasures()` function. Model fitting started with a highly saturated model, removed poorly supported pathways iteratively (e.g., $p > 0.75$, $p > 0.5$, $p > 0.25$) until fit statistics were maximized. See Appendix S1: Section S4 for details of model fitting process and model fit statistics.

Disturbance effects on AM fungal mutualisms with *S. scoparium* were tested using LMERs with total plant biomass and root:shoot ratios as response variables. LMERs contained all independent and interactions terms for fire, grazing, and AM fungal inocula treatments, as well as an independent site age covariate, and terms for greenhouse block, management unit, and inocula source plot as random effects.

RESULTS

Disturbance alters AM fungal spore communities

Disturbance altered AM fungal spore community composition, spore densities, and the abundance of individual AM fungal taxa. In total, nine AM fungal species were identified, and all nine were found in burned and mowed treatments. Prescribed fires were associated with differences in July ($F_{1,77} = 24.2$, $p = 0.001$, Table 1, Figure 2a) and

September ($F_{1,31} = 2.28$, $p = 0.035$, Figure 2b) spore communities between burned and no burn sites, while differences between grazed and no graze sites were marginal at both time points (July: $F_{1,77} = 2.05$, $p = 0.09$; September: $F_{1,31} = 1.77$, $p = 0.1$). Differences in AM fungal community composition were associated with changes in spore density (Appendix S1: Table S3). During both the Summer ($F_{1,Inf} = 16.6$, $p < 0.0001$; Figure 2c) and Fall ($F_{1,Inf} = 8.01$, $p = 0.0047$; Figure 1d), fire reduced spore density, while grazing promoted spore density in the Fall ($F_{1,Inf} = 29.7$, $p < 0.0001$). Additionally, spore densities were reduced in plots that were both burned and grazed ($F_{1,Inf} = 5.43$, $p = 0.0198$).

Disturbance effects on AM fungal spore community composition varied across taxa and sampling times (Appendix S1: Tables S4–S12, Figure S1). During July, *Glomus species 1* ($F_{1,Inf} = 6.53$, $p = 0.011$), *Cetranspora pellucida* ($F_{1,Inf} = 10.3$, $p = 0.001$), and *Funneliformis* sp. ($F_{1,Inf} = 5.88$, $p = 0.015$) spore counts were lower in burned versus no burn sites. Further, an interaction between fire and grazing altered the abundance of *Gigaspora* sp. ($F_{1,Inf} = 7.08$, $p = 0.008$) and *Funneliformis* sp. ($F_{1,Inf} = 7.8$, $p = 0.005$), with *Gigaspora* sp. counts increasing in burned grazed sites, and *Funneliformis* sp. counts reduced in all sites relative to sites that were not burned or grazed. *Rhizophagus* sp., *Acaulospora* sp. 2, and *Acaulospora* sp. 1 abundances did not vary across treatments.

TABLE 1 Disturbance effects on July and September arbuscular mycorrhizal (AM) fungal community composition.

Collection time	Model term	df	Sum squares	R^2		p Value
July spores	prescribed fire	1	1.3475	0.15506	24.2316	0.001***
	bison grazing	1	0.1141	0.01313	2.0521	0.09*
	site age	1	0.2263	0.02605	4.0703	0.003**
	management unit	1	0.3619	0.04164	6.5076	0.001***
	fire × grazing	1	0.1106	0.01272	1.9885	0.097*
	exclosure site (within unit)	9	3.0262	0.34824	6.0465	0.001***
	residual	63	3.5034	0.40315		
	total	77	8.69	1		
September spores	prescribed fire	1	0.3344	0.0411	2.2786	0.035*
	bison grazing	1	0.2592	0.03186	1.7663	0.1*
	site age	1	0.7217	0.0887	4.9169	0.001***
	management unit	1	0.5614	0.069	3.8248	0.004**
	fire × grazing	1	0.1386	0.01704	0.9445	0.463
	exclosure site (within unit)	12	4.0662	0.49976	2.3087	0.001***
	residual	14	2.0548	0.25255		
	total	31	8.1364	1		

* $p < 0.1$; ** $p < 0.05$; *** $p < 0.001$.

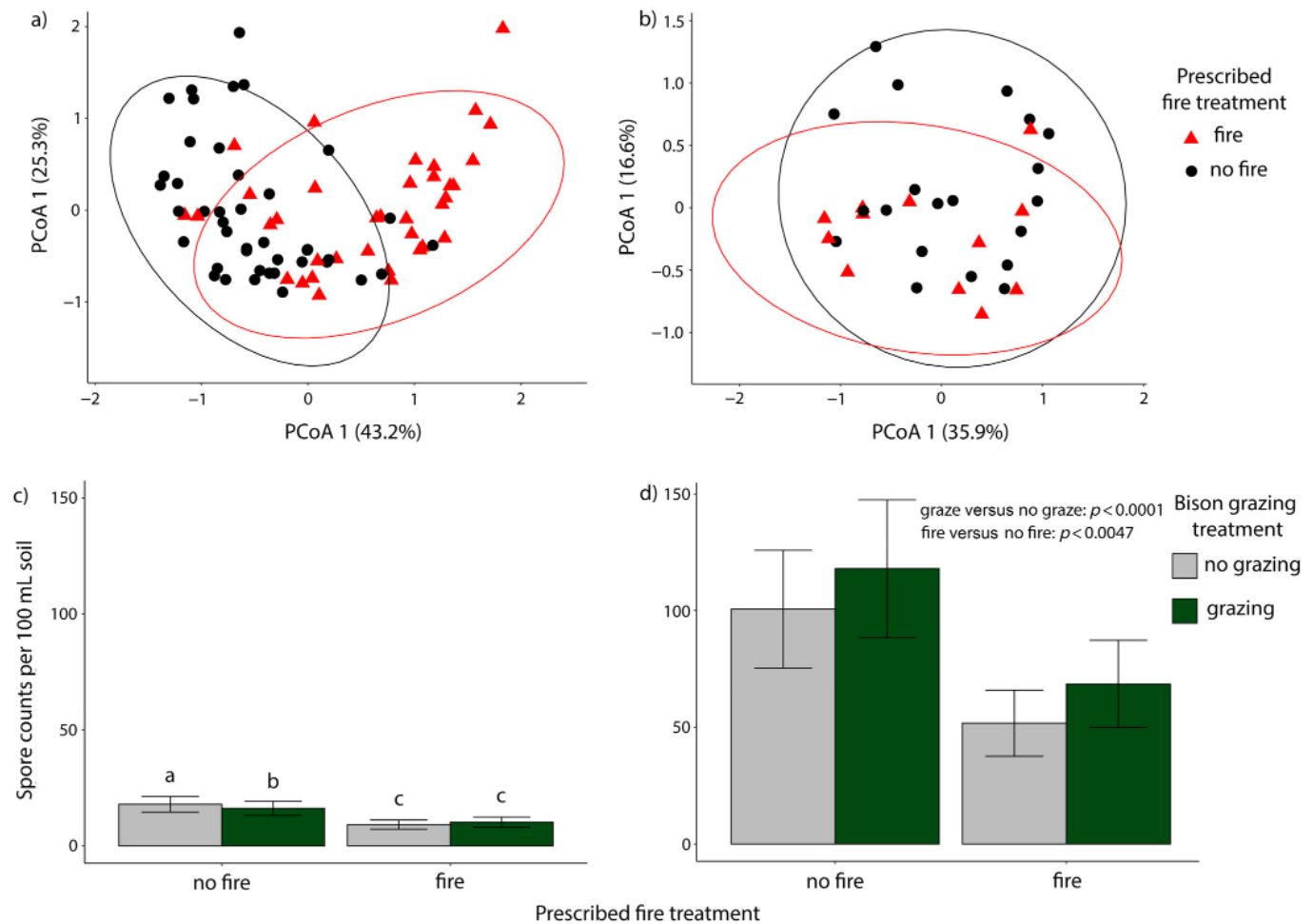


FIGURE 2 Disturbance effects on arbuscular mycorrhizal (AM) fungal spore community composition and spore densities. Ellipses in panels (a) and (b) represent 95% CI. Lower case letters represent differences between Disturbance treatments ($p < 0.05$). Error bars represent the mean \pm 1 SE. Prescribed fire altered AM fungal spore community composition in both (a) July and (b) September of 2021. Prescribed fire also reduced AM fungal spore density in both (c) July and (d) September, while (d) grazing promoted AM fungal sporulation in September. PCoA, principal coordinates analysis.

During September, grazing and prescribed fire influenced the abundance of AM fungal taxa (Appendix S1: Tables S13–S24, Figure S2). *Glomus* species 1 ($F_{1,Inf}$ 40.6, $p < 0.0001$) and *C. pellucida* ($F_{1,Inf}$ 5.87, p 0.015) spore counts were higher in grazed sites, however, in the absence of grazing, fire reduced their abundance. *Rhizophagus* sp. counts were higher in sites that received any kind of disturbance ($F_{1,Inf}$ 7.94, p 0.005), while *Glomus* sp. 2 counts were lowest in sites that were grazed and burned ($F_{1,Inf}$ 7.63, p 0.006). *Funneliformis* sp. spore counts increased in both grazed and burned sites; however, spore counts were lowest in sites that were both burned and grazed ($F_{1,Inf}$ 19, $p < 0.0001$). *Gigaspora gigantea* and *Gigaspora* sp. did not vary across fire or grazing treatments. In summary, disturbance shifted AM fungal spore community composition through changes to spore density and the abundances of individual AM fungal taxa.

Disturbance selected for specific AM fungal spore traits within species

Disturbance treatments were associated with variation in AM fungal spore traits at the species level ($F_{12,1689}$ 41.6, $p < 0.0001$; Figure 3; Appendix S1: Tables S25–S32, Figure S3a–g). With the exception of *Glomus* sp. 1, spore color saturation for all AM fungal taxa observed in Fall 2021 had higher color saturation values (purer color) in burned sites. Fire's effect on spore color saturation was often modified by grazing however, with *Funneliformis* sp., *G. gigantea*, *Glomus* sp. 2, *R. irregularis*, and *Glomus* sp. 1 spores displaying lower saturation values in sites that were both burned and grazed.

Fire was also associated with lower spore luminance values (less bright); however, as with saturation, this effect was modified by grazing (Appendix S1: Figure S4a–g).

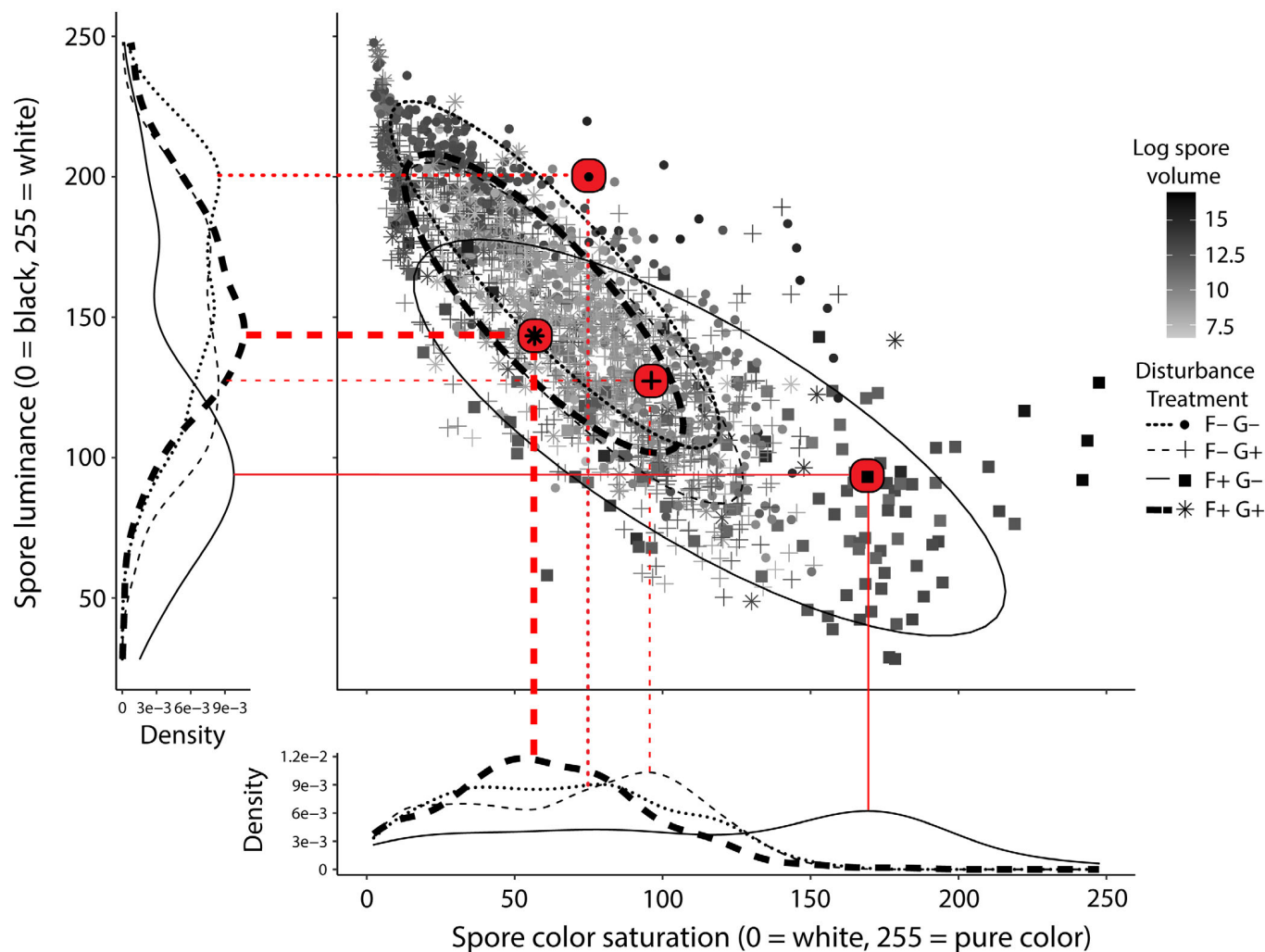


FIGURE 3 Trait profile plot of September 2021 arbuscular mycorrhizal (AM) fungal spores. Each point represents a single AM fungal spore, with shading corresponding to spore volume and shape representing the disturbance treatment the spore was found in. F+/ and G+/ denote the occurrence of prescribed fire and grazing in the plot a spore was found in. Note that line pattern also represents disturbance treatment. The density plots illustrate the most common spore luminance and color saturation values in each disturbance treatment. Red lines and circles denote the most common trait profile of AM fungal spores in each treatment.

C. pellucida and *G. gigantea* spore luminance values were lower in burned sites, while *Glomus* sp. 2, *R. irregularis*, *Glomus* sp. 1, and *Funneliformis* sp. luminance values were lowest in burned sites without grazing. *Gigaspora* sp. luminance values were reduced following any disturbance activity.

Disturbance was also associated with spore volume; however, this effect was not uniform across AM fungal taxa (Appendix S1: Figure S5a–c). *Gigaspora* sp. 1 and *G. gigantea* spore volumes were lower in grazed relative to no graze sites, and along with *C. pellucida*, highest in burned only sites. The other observed AM fungal taxa did not display disturbance associated variation in spore volume. In summary, disturbance was associated with intra-species variation in spore color saturation, luminance, and volume, but these effects varied between taxa.

Disturbance selected for specific AM fungal spore traits at the community level

Disturbance treatments were associated with variation in AM fungal spore colorimetric traits at the community level. While there were not strong overall effects of prescribed fire ($F_{1,25} = 1.79$, $p = 0.19$; Appendix S1: Table S33) or grazing ($F_{1,25} = 0.5$, $p = 0.49$) on AM fungal spore traits, fire did have varying effects on individual spore traits ($F_{1,25} = 4.97$, $p = 0.004$; Appendix S1: Tables S34–S38, Figure S6a–e). Specifically, spore color luminance values were lower (darker spores; $F_{1,24,29} = 4.43$, $p = 0.05$) and spore saturation values were higher (purer color relative to white; $F_{1,24,29} = 6.53$, $p = 0.06$) in burned relative to no burn sites. Disturbance did not affect total small (0–35 m), medium (36–99 m), or large (100+ m) spore volumes, however.

While disturbance did not select for suites of AM fungal spore traits, darker, non-white spores did increase in abundance after fire.

Disturbance influenced spore communities through traits

Disturbance treatments influenced AM fungal spore community composition through spore traits (Figures 4 and 5, Appendix S1: Tables S39 and S40). In the following paragraphs numbers in parentheses represent standardized regression coefficients which allow for the direct comparison of SEM pathways. A lower case “d” or “i” in parentheses refers to direct pathways (arrow linking two variables) and indirect pathways (variables linked through mediators)

in the model. Spore hue was negatively correlated with site age (*d*: 0.419) meaning that in older restorations and remnants, spores tended to be more yellow and orange. Prescribed fire was associated with higher spore color saturation (*d*: 0.292) and lower color luminance (*d*: 0.338) values. Additionally, spore communities in older sites tended to have higher color saturation (*d*: 0.382), lower luminance (*d*: 0.36), and have higher amounts of medium sized spores (*d*: 0.136).

Variation in AM fungal spore communities was influenced by spore traits, site characteristics, and disturbance (Figure 6). AM fungal spore traits were the strongest determinants of community composition, with total small spore volume (1.26), total medium spore volume (0.949), color saturation (0.693), and hue (0.398) describing the bulk of variation. Site age was also an important

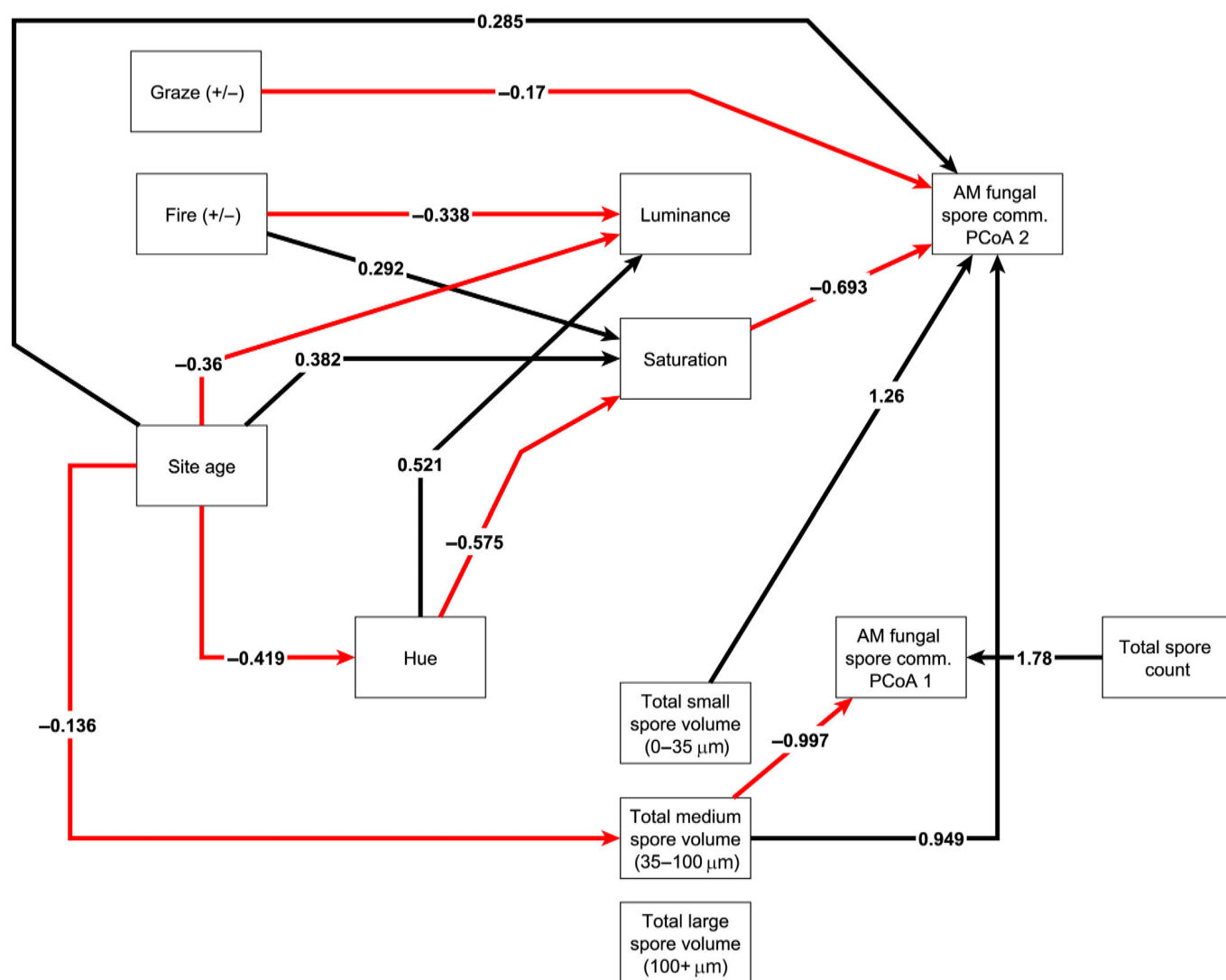


FIGURE 4 Final SEM diagram relating disturbance effects on arbuscular mycorrhizal (AM) fungal community composition through spore traits. Red and black arrows denote negative and positive interactions, respectively. Path coefficients are standardized regression coefficients, which allows for the comparison of path strengths within the model. Prescribed fire disturbance was associated with higher spore color saturation, and indirectly influenced AM fungal spore community composition. PCoA, principal coordinates analysis.

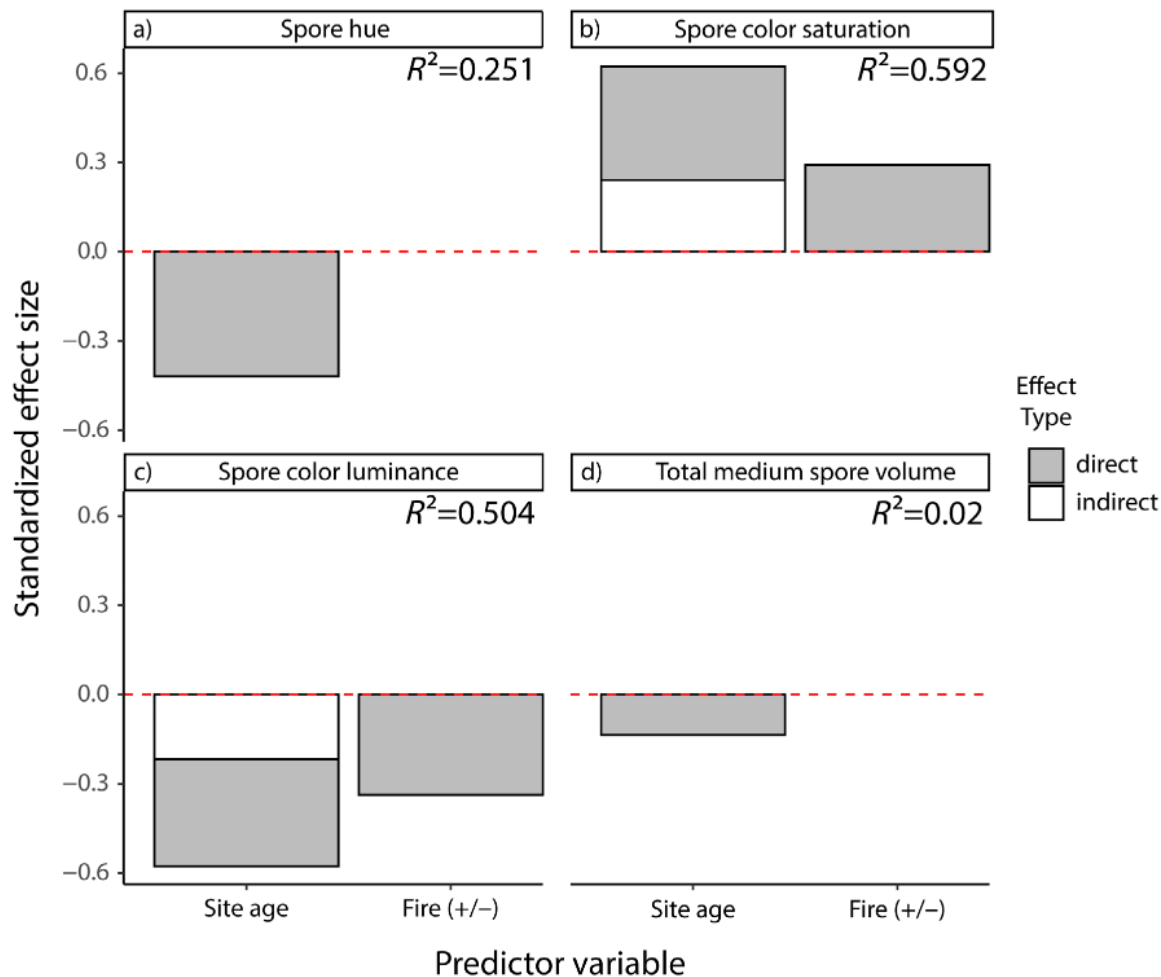


FIGURE 5 Structural equation modeling (SEM) results—Disturbance and site characteristics effects on arbuscular mycorrhizal (AM) fungal spore traits. Each panel represents the standardized effect of predictor variables on AM fungal spore traits. The use of standardized effect coefficients allows for direct comparability of each predictor's impact on the response variable. Gray bars represent “direct” pathways between predictor and trait variables, while white bars represent “indirect” pathways between variables that were mediated by another variable. (a) As site age increased, Spore hues (i.e., “color”) became more yellow and orange. (b) Spore color saturation increased (non-white, purer color) while (c) spore luminance decreased (darker color, less bright) in older and burned sites. (d) The total volume of medium sized spores (35–99 μm) decreased slightly in older sites.

determinant of AM fungal community composition both directly (0.285) and indirectly through effects on spore traits (0.153). Finally, disturbance also influenced AM fungal community composition with bison grazing altering community composition through direct, unmeasured pathways (0.17), and fire indirectly through effects on spore traits (0.2). To summarize, disturbance treatment and site age drove differences in AM fungal spore community composition through spore trait linked pathways.

Disturbance effects on AM fungal mutualisms

Disturbance effects on AM fungal spore communities influenced *S. scoparium* growth. While total *S. scoparium* biomass was higher when pots were inoculated with AM

fungi ($F_{1,251.2} = 5.61, p = 0.019$; Appendix S1: Table S41), prescribed fire and grazing effects on AM fungal communities did not influence total plant biomass. However, prescribed fire did alter root:shoot ratios ($F_{1,245.3} = 3.1, p = 0.08$; Appendix S1: Tables S42 and S43, Figure S7). Root:shoot ratios were lower in pots receiving AM fungi from burned sites versus microbial washes from burned sites ($p = 0.068$). Overall, disturbance effects on AM fungal inocula did not drive strong differences in *S. scoparium* biomass, but did influence biomass allocation.

DISCUSSION

Disturbance selected for specific AM fungal spore traits at the species and community level, which altered both AM fungal spore community composition and plant

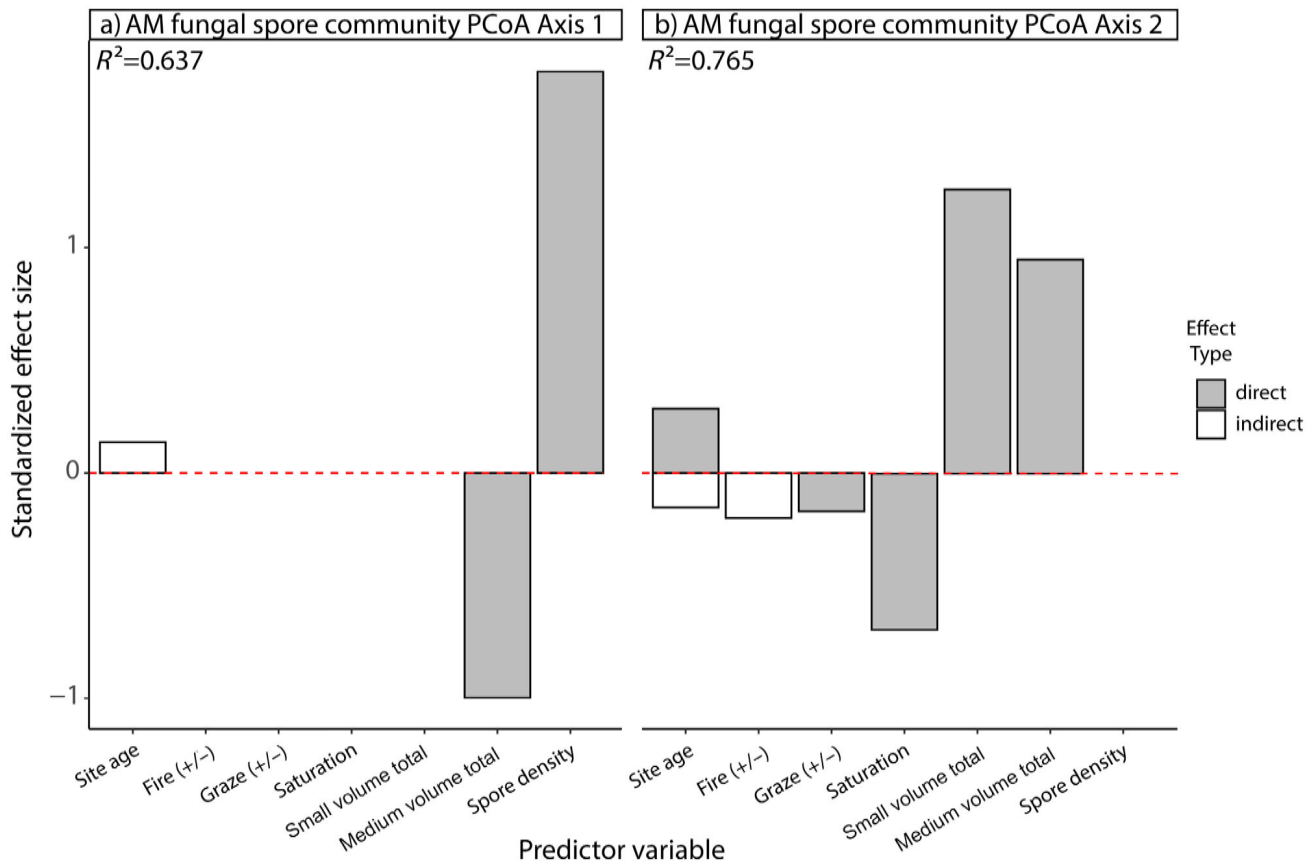


FIGURE 6 Structural equation modeling (SEM) results—Disturbance, site characteristic, and spore trait effects on arbuscular mycorrhizal (AM) fungal spore community composition. Each panel represents the standardized effect of predictor variables on AM fungal spore traits. The use of standardized effect coefficients allows for direct comparability of each predictor’s impact on the response variable. Gray bars represent “direct” pathways between predictor and trait variables, while white bars represent “indirect” pathways between variables that were mediated by another variable. AM fungal spore traits were the strongest predictors of spore community composition, with spore volume size classes, spore color saturation, and sporulation influencing composition the most along principal coordinates analysis (PCoA) axes 1 (panel a) and 2 (panel b). Disturbance also influenced AM fungal spore community composition (panel b), both indirectly through spore traits (prescribed fire) and directly through unmeasured pathways (grazing).

resource allocation. AM fungal spore community compositional shifts due to fire and grazing were linked to increased spore color saturation (purer colored spores), decreased spore luminance (darker spores), changes in the abundance of AM fungal taxa, and variation in sporulation. Important to note is that all nine identified AM fungal species were found in both burned and grazed plots, suggesting that changes in community composition are due to changes in abundance rather than species turnover. Disturbance driven changes in AM fungal communities (particularly prescribed fire) in turn were correlated with altered *S. scoparium* growth strategies. By linking disturbance effects on AM fungal community composition and function to spore traits, we have identified community assembly processes that can help explain fire and grazing effects on AM fungi (Dove & Hart, 2017; Hamman et al., 2007; Hart et al., 2016; Heyde et al., 2017; van der Heyde et al., 2019). Since disturbance associated

selection of AM fungal spores can alter AM fungal communities and mutualisms, understanding how disturbance influences both AM fungi and plant communities may be particularly important for understanding the above- and belowground responses of ecosystems to fire and grazing.

AM fungi and their associated plant communities are important components of terrestrial ecosystems. Therefore, understanding how fire and grazing influence AM fungi can also be informative of plant community responses (Beals et al., 2020). In this work, both fire and grazing effected AM fungal taxa and sporulation differently, and fire in particular selected for darker, purer colored spores with larger volumes; while grazing selected for lighter colored spores with less pure color and smaller volumes. Since fire associated effects on AM fungi were linked to altered *S. scoparium* growth responses (root:shoot ratios), it is possible that disturbance could

influence plant productivity through belowground pathways. This means that other well-known effects of fire and grazing on AM fungal traits like altered sporulation (Eom et al., 1999; Pattinson et al., 1999), changes in colonization (Dove & Hart, 2017; Eom et al., 2001), and taxa specific responses (Allsup et al., 2021; Carson et al., 2019; van der Heyde et al., 2017) should be considered in above- and belowground responses to disturbance. For example, when fire is too severe, AM fungal spore densities (Pattinson et al., 1999) and colonization (Klopatek et al., 1988) can be reduced, and potentially decrease the plant host benefit. In this work, fire reduced Summer and Fall spore densities, while grazing promoted sporulation in the Fall. This suggests that even low severity prescribed burns can influence AM fungal sporulation, but this effect may be reduced by grazing. The different effects of fire and grazing on AM fungal sporulation and taxa in this study show that aboveground disturbances have complex, temporal effects and indicates that the effects of other factors like disturbance history, intensity, and frequency on AM fungal communities and mutualisms should also be considered (Ford, 2010; Hulbert, 1986). Linking AM fungal community assembly and function to spore traits not only provides a framework for understanding disturbance effects on AM fungal community assembly, but also shows the utility of trait-based approaches for elucidating how AM fungi interact with their environment.

AM fungal responses to fire and grazing varied based on specific spore traits and taxa. Specifically, spore traits associated with color were particularly important in determining prescribed fire effects on AM fungal spore communities. As hypothesized, darker (low luminance) and purer colored spores (high saturation) were associated with burned sites, while grazing was associated with brighter, less pigmented spores (Figure 3). While the exact mechanism is unclear, darker coloration, especially if associated with melanin content, could help spores survive high temperatures (Cordero & Casadevall, 2017), post-fire UV exposure (Gessler et al., 2014; Hopkins et al., 2021), or arid conditions common after fire (Deveautour et al., 2020). Lighter coloration and lower pigmentation in grazed relative to burned sites may reflect an unconsidered grazing associated stressor where whiter spore coloration is beneficial, or white pigmentation may be a conserved trait in AM fungal taxa that respond to grazing (van der Heyde et al., 2017). We also found that spore size was related disturbance, however, this was only true of medium (*C. pellucida*) and larger volume (*Gigaspora* sp. 1 and *G. gigantea*) taxa. Grazing was associated with smaller spore volumes, which may reflect reduced carbon allocation from plant hosts following grazing activity (Allsup et al., 2021; van der Heyde et al., 2019). Fire on

the other hand was associated with higher spore volumes in the absence of grazing. This result is surprising given that increased surface area is often associated with higher flammability (Murray et al., 2013), however, *C. pellucida* and the two *Gigaspora* species all possess large amounts of internal lipids which could act as effective insulators against high temperatures or provide reserves for post-fire recovery. The importance of spore size (and other traits) may also vary with factors like disturbance intensity and frequency, when long-term effects of disturbance on community assembly processes have time to take effect. It is also important to note that AM fungal responses to disturbance varied between sampling times, for example *Funnelformis* sp. sporulation was lower in burned sites during July, but then higher relative to no burn sites in September. This variation between sampling times may be explained by fire's waning effect with time, or that fire disturbance can drive changes in AM fungi phenology and sporulation time. In addition to the traits considered in this work, AM fungal taxa display an array of other traits that could be relevant in other management, disturbance, and ecological contexts (Chagnon et al., 2013; Chaudhary et al., 2022; Treseder & Lennon, 2015; Zanne et al., 2020). AM fungal spore traits have already been used to explore the influence of aridity on AM fungal community assembly (Deveautour et al., 2020), but other traits like competitive ability (Bennett & Bever, 2009; Chagnon et al., 2013), hyphal foraging strategy (Declerck et al., 2004; Hart & Reader, 2002), or sporulation (Koch et al., 2017) could be informative of AM fungal responses to stressors and disturbances like fertilization, soil acidification, flooding, and tillage. While this study focused on AM fungi, trait-based approaches have already been applied in other contexts (e.g., plant communities; Day et al., 2020; Laughlin, 2014; Zakharova et al., 2019), and are powerful tools for understanding above- and belowground interactions.

Conservation of both above- and belowground ecosystem components is critical to maintaining healthy systems. Since soil biota consist not only of AM fungi, but also bacteria, fungi, and archaea, trait-based approaches can inform disturbance effects across both ecosystems and domains of life. Trait-based approaches could also be used to explore how rhizobial nodule characteristics are influenced by tillage in old-field and restored ecosystems (Torabian et al., 2019), the connection between spore morphology and factors like dispersal and niche (Norros et al., 2015; Pringle et al., 2015), how life history characteristics of plant-pathogens respond to fire (Katan, 2000), and how grazing intensity is associated with the spore reserves and carbon requirements of plant symbionts (van der Heyde et al., 2019). Changes in non-AM fungal groups may also explain the root:shoot ratio changes

observed in this work. We found that inoculation with AM fungi from burned sites reduced root:shoot ratios relative to microbial washes from burned sites. If fire harms beneficial root associated microbes (Beals et al., 2020), then this could explain the increased root growth we observed. In addition to testing plant–microbial interaction type questions, trait-based approaches could also inform disturbance effects on other ecological processes like decomposition and nutrient cycling (Butler et al., 2019; Hopkins et al., 2020). Utilizing trait-based approaches in disturbance contexts provides new insight into the mechanisms that shape community assembly and the function, and are invaluable for understanding how organisms interact with their environment.

In conclusion, disturbance driven selection on AM fungal spore traits was associated with changes in community composition and AM fungal mutualisms. This work is the first to test fire and grazing disturbance effects on AM fungal spore traits at the species and community levels, and builds on previous work linking traits to life history (Chagnon et al., 2013; Chaudhary et al., 2022; Deveautour et al., 2020; Powell & Rillig, 2018; van der Heyde et al., 2017). Using a trait-based framework, we confirmed that disturbance influences AM fungal mutualisms, and showed the trait related mechanisms that underly these pathways. This both demonstrates the power of trait-based approaches in soil ecology for understanding interactions between microbes and their environment, and reveals how understanding the ecological significance of traits can improve our knowledge of AM fungal community assembly. Future work should consider AM fungal trait responses to other types of disturbance, as well as how intra-species trait variation is related to aboveground processes. The applicability of trait-based approaches in ecology promise to not only provide new insights into the processes that underly ecosystems (de Souza et al., 2020; McGill et al., 2006; Zakharova et al., 2019; Zanne et al., 2020), but also help predict how changes in ecosystems will impact the organisms that live there.

AUTHOR CONTRIBUTIONS

Jacob R. Hopkins and Alison E. Bennett developed and designed all experiments. Jacob R. Hopkins performed the field, traits, and greenhouse portions of the research. Jacob R. Hopkins collected and analyzed all data. Jacob R. Hopkins and Alison E. Bennett wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT


The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Hopkins & Bennett, 2023) are available in Dryad at <https://doi.org/10.5061/dryad.63xsj3v5f>.

ORCID

Jacob R. Hopkins  <https://orcid.org/0000-0003-2409-3099>

Alison E. Bennett  <https://orcid.org/0000-0002-1037-0713>

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SUPPORTING INFORMATION

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

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