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The Effects of Prescribed Fire on Ant-Mediated Seed Dispersal in Missouri

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B.Sc. Biology, College of William & Mary, 2016

A Dissertation Submitted to The Graduate School at the University of Missouri-St. Louis
in partial fulfillment of the requirements for the degree
Doctor of Philosophy in Biology with an emphasis in Ecology, Evolution, & Systematics

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Abstract

Many aspects of animal-mediated seed dispersal are vulnerable to disturbance, including partner identity and dispersal quantity and quality. This dissertation explores ant-mediated seed dispersal of the herb *Sanguinaria canadensis* in Missouri Ozark oak forests, where prescribed fire is a common land management tool. In Chapter 1, I test the definition of a keystone seed disperser using absolute and relative contributions of different ant species to the quantity and distance of seed dispersal, based on field observations of *S. canadensis* seed dispersal. I demonstrate that the ant *Aphaenogaster rudis* is better described as a numerically dominant rather than keystone seed disperser, while other species in the genera *Formica* and *Camponotus* provide higher quality dispersal in the form of longer dispersal distances. In Chapter 2, I explore the effects of prescribed burning and physical litter removal on the number of seeds dispersed, dispersal distances, and disperser identity, using field observations of *S. canadensis* seed dispersal in burned, litter removal, and control plots. The overall seed-dispersing ant community and different species therein responded differently to burning and litter removal between two field seasons. Burning increased dispersal distance in 2020, but reduced dispersal distance in 2021. Finally, in Chapter 3, I consider the effects of burning and litter removal on ground-foraging and litter-dwelling ant communities, using three rounds of pitfall traps in 2020 (April, July, and September), and one round of pitfall and Berlese litter extraction in 2021. In 2020, overall ant abundance was higher in burn compared to control plots in September, but diversity peaked in July and was negatively affected by burning when weighting species by their relative abundances. Although both burning and litter removal reduced leaf litter cover in 2021, litter removal increased ground-foraging ant abundance but decreased litter-dwelling ant abundance on an area basis, and only affected ant diversity when considering ant abundance in 2020 pitfall traps and 2021 Berlese samples. The results of this dissertation suggest Missouri Ozark ant communities and their seed dispersal services are either positively affected by or relatively resilient to prescribed burning.

Keywords: *myrmecochory; seed dispersal effectiveness; Formicidae;* Aphaenogaster*; keystone species; prescribed fire; ant diversity; plant-ant mutualism*

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Introduction

Myrmecochory, or the dispersal of seeds by ants, is found in at least 11,000 species of plants worldwide, often in environments where fire is a frequent disturbance (Lengyel et al., 2010; Pausas & Lamont, 2022). In Australia in particular, studies of myrmecochory in disturbed environments suggest fire might enhance the frequency and benefits of this dispersal mutualism (Parr et al., 2007; Beaumont et al., 2013, 2018), and a few studies elsewhere have also suggested similar outcomes (Bond et al., 1990). However, despite the resurgence in the use of fire as a management tool in eastern North American woodlands (Brose et al., 2001; Nowacki & Abrams, 2008; Ryan et al., 2013) and the prevalence of ant-dispersed herbs in the same area (Beattie & Culver, 1981; Vander Wall et al., 2017; Warren et al., 2021), there are no published studies to date on the interaction of fire and myrmecochory in this biome. This dissertation thus aims to answer questions of how fire and myrmecochory interact in eastern North America, with a focus on one common ant-dispersed plant, *Sanguinaria canadensis.*

The first chapter sets the stage for investigating myrmecochory in the Ozarks by describing the variation in dispersal services provided by ants. Initially, I planned to focus on one species pair: the ant *Aphaenogaster rudis* and the plant *Sanguinaria canadensis,* based on previous descriptions of *A. rudis* as the "keystone disperser" of myrmecochorous seeds in North America (Ness et al., 2009; Canner et al., 2012). However, my pilot studies revealed that other ant species were also providing significant dispersal services, leading me to question whether *A. rudis* could really be called a keystone disperser. More broadly, I also began to wonder whether a limited focus on a few "keystone dispersers" in myrmecochory (*e.g.,* Warren et al., 2014) was premature.

Thus, Chapter 1 investigates previous definitions of “keystone species”, proposes a new set of criteria to use when evaluating whether species are keystone seed dispersers, and applying those criteria to the case of seed-dispersing ants in Missouri. These criteria, when generalized, could also help provide a more specific definition of keystone species more broadly. Ultimately, my conclusions from this first chapter led me to consider the entire suite of seed-dispersing ants in my subsequent two chapters.

Chapter 2 focuses on the motivating question for this research: how does prescribed burning affect myrmecochory in Missouri? If my findings were to show increases in dispersal rates and distances after fire as other regions, it would add to existing arguments for using prescribed fire as a restoration and land management tool. Opposite results, however, would indicate a need for extra preventative or remedial action to ensure continued survival of the mutualism or myrmecochore populations after burning. Furthermore, the results from this chapter add to our overall understanding of how dispersal mutualisms respond to disturbance, and specifically, how myrmecochory varies in its response to disturbance across its range. Specifically, plant adaptation for ant-mediated seed dispersal has evolved over 101 times across the angiosperm phylogeny and occurs in every continent except Antarctica (Lengyel et al., 2010). Disturbance, including fire, has been hypothesized as a driver for this relationship (Andersen, 1988; Hughes & Westoby, 1992; Pausas & Lamont, 2022), but rarely tested in North America.

Finally, Chapter 3 focuses on the effects of prescribed burning on the entire ground-foraging ant community, as found in pitfall traps, as well as litter-dwelling ants found in Berlese-extracted leaf litter samples. Ant community responses to disturbance can also serve as gauges of larger-scale ecosystem recovery in other parts of the world

(King et al., 1998; Hoffman & Andersen, 2003), so this chapter also considered whether ants could serve as indicators of fire's effects in the Missouri Ozarks.

Finally, a stylistic note: each chapter is formatted and written in preparation for submission to a different peer-reviewed journal. Chapter 1 is written in the style of *The American Naturalist,* Ch. 2 is written in the style of *Ecology* articles, and Ch. 3 is intended for *Ecological Applications.*

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Chapter 1: The Winnow Ant (*Aphaenogaster rudis*): Keystone disperser or merely abundant?

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Abstract

Non-quantitative use of the term "keystone species" conflates abundance and importance, even though understanding the relationship between abundance and impact is crucial for conservation and management. Compared to other species in the disperser community, we propose a keystone disperser must 1) disperse overall more seeds, 2) provide higher quality dispersal, and 3) contribute to both the quantity and quality of seed dispersal more than expected based on abundance. We apply these criteria to *Aphaenogaster rudis,* often called the keystone disperser of North American myrmecochores, and co-occurring seed-dispersing ants, using observations of the amount and distance ants took *Sanguinaria canadensis* diaspores during one field season in a Missouri woodland. *Aphaenogaster rudis* accounted for 53% (137/247) of observed *Sanguinaria canadensis* seed transport events. However, four other ant species dispersed seeds 3.82–7.94 times (1.22–2.54 m) farther on average than *A. rudis*. *Camponotus chromaiodes* and *Formica pallidefulva* were 90.6% and 71.7% less common in pitfall traps but had higher relative importance-to-relative abundance ratios compared to *A. rudis.* We suggest *A. rudis* is better described as a numerically dominant seed disperser, and other seed-dispersing ant species may be more important than previously considered.

Introduction

In its original use, *keystone species* referred to species whose activities impacted the rest of the ecosystem's appearance, composition, and stability through time (Paine 1969). Use of the term then grew without a consistent definition, bringing its utility into question (Mills et al. 1993; Hurlbert 1997). One revised definition requires keystone species to have a disproportionately large ecological impact compared to their abundance or biomass (Paine 1995; Power et al. 1996), although other definitions continue to be used without consensus (Cottee-Jones and Whittaker 2012). A defining, albeit debated (Hurlbert 1997; Davic 2003; Cottee-Jones and Whittaker 2012) component of Power et al.'s (1996) definition classified species with large impacts but also proportionally large abundances as numerically dominant, not keystone, species (Supp. Fig. 1). This definition does not *de facto* exclude abundant species from being keystone species, but rather requires a consideration of relative abundance in addition to impact. For example, one of Paine's original (1969) examples, the abundant starfish *Pisaster ochraeus,* was still considered a keystone species by Power et al. (1996).

Despite the importance Power et al. (1996) placed on quantifying community impact while controlling for proportional abundance or biomass, directly quantifying the relationship between impact and relative abundance or biomass is rare in the literature. Perhaps this rarity is due to a lack of quantitative thresholds, logistic difficulties of manipulating species abundances, and alternative definitions. However, a few key exceptions have used Power et al.'s (1996) framework to some extent, including Kotliar (et al. 1999, 2000) and Catano and Stout (2015). Kotliar et al. (1999) compared the ecological impacts of prairie dogs (*Cynomys* spp.) to other native prairie herbivores, using per capita primary consumption estimates as well as a general assessment of prairie dog effects on other vertebrate species. Kotliar (2000) demonstrated prairie dog impact varied with prairie dog abundance, but in a non-linear manner unlike Power et al. (1996)'s linear assumption. Building on the idea that a species' impacts could vary with abundance, Catano and Stout (2015) quantified the relationship between gopher tortoise (*Gopherus polyphemus*) burrow abundance and vertebrate diversity. Additionally, Catano and Stout (2015) built upon Power et al. (1996)'s definition of a keystone species by considering the dynamic relationship between species abundance and impact, allowing keystoneness to vary at different rates with abundance.

Although use of the term keystone species is widespread, consensus on its use is lacking. Some argue the term's utility lies in the metaphor, but referring to any species of 'demonstrable importance for ecosystem function' as a keystone species (Cottee-Jones and Whittaker 2012) ignores the fact that a keystone species' importance arises from an exceptional contribution to ecosystem function beyond what its abundance would imply, whereas a numerically dominant species' exceptional contribution to ecosystem function directly stems from its abundance. Furthermore, understanding the relationship between abundance and impact can provide greater insight into the conditions under which keystoneness arises. For example, Kotliar (2000) viewed keystoneness as a dynamic property, and advocated studying the relationship between a species' impact and abundance to reveal not only if, but also under what conditions a species has greater impact than predicted by its abundance. Indeed, there exist thresholds of abundance below which a species ceases to contribute an ecological function, such as flying foxes whose seed dispersal importance depends on having high enough population densities to induce intraspecific aggression and associated fruit-dropping behavior (McConkey and Drake, 2006).

We examined the use of the term "keystone" in one specific context, that of seed dispersal by ants. In eastern North American forests, the winnow ant, *Aphaenogaster rudis* Enzmann, J., (1947; Formicidae: Myrmicinae; Fig. 1A), has been called a keystone mutualist due to its frequency as a seed disperser (Zelikova et al. 2008; Ness et al. 2009;

Canner et al. 2012, Lubertazzi 2012). Justification for calling *A. rudis* a keystone disperser usually stems from Ness et al. (2009), who performed a meta-analysis demonstrating that *Aphaenogaster* (mostly *A. rudis*) (1) accounts for the majority of myrmecochorous seed dispersal in North American forests, (2) is more likely than other ant genera to disperse seeds when encountered, and (3) is positively correlated with myrmecochore density and species richness in North American temperate deciduous forests. However, Ness et al. (2009) do not consider the Power et al. (1996) criterion for classification as a keystone species, in which a species' impact must be disproportionately large compared to its relative abundance. As *A. rudis* is common and abundant in eastern North American forests (King et al. 2013), its importance as a seed disperser could plausibly be a function solely of its abundance and thus be at odds with the Power et al. keystone definition, requiring a comparative study considering both relative impact and relative abundance. To remain consistent with Power et al., we will use the term "dominant" to describe numerically abundant species, different from the term's behavioral connotations in ant ecology. Although *Aphaenogaster rudis* may be numerically dominant, it is behaviorally subordinate and often aggressively displaced by more behaviorally dominant ants at baits (Lynch et al., 1980).

Seed dispersal effectiveness hinges on both the quantity and quality of dispersal (Schupp 1993, Schupp et al. 2010). Thus, even if other ant species do not disperse as many seeds as *A. rudis,* they could still be more effective dispersers if they provide higher quality of dispersal. Dispersal quality depends on the effects of dispersal on seed mortality, germination, establishment success, and subsequent production of adult plants (Schupp 1993). These outcomes can further be affected by seed treatment, microsite deposition, and distance to conspecifics and heterospecifics that determine the competitive, mutualistic, predator, and pathogenic environment (Schupp 1993). Although not as likely to disperse seeds when encountered as *Aphaenogaster rudis,* ants from the genera *Camponotus, Formica, Lasius, Myrmica, Stenamma,* and *Tapinoma,* and *Temnothorax* (as *Leptothorax*) have also been observed dispersing seeds in North American forests (Ness et al. 2009), and thus warrant consideration under a seed dispersal effectiveness framework as well.

We focus on dispersal distance as a proxy for dispersal quality here because of its impact on seedling establishment and population spread, as directly measuring seedling establishment is logistically more challenging than measuring dispersal distance. Although plant species vary in the presence and strength of density and distance-dependent mortality (Janzen-Connell effects, Song et al. 2021), dispersal distance has been positively linked with seedling establishment for ant-dispersed plants (Handel 1976; Andersen 1988; Fernandes et al. 2020). The cited average myrmecochorous dispersal distance of eastern deciduous forests is 35–45 cm (Warren et al. 2021), but longer-distance events could benefit population spread. Furthermore, in German deciduous forests, instances of medium and longer-distance seed dispersal are necessary to explain the observed population distribution and spread of the myrmecochorous herb *Melanpyrum pratense* (Winkler and Heinken 2007; Heinken and Winkler 2009). Population spread of myrmecochores could thus benefit disproportionately from rare long-distance dispersal events, such as dispersal by less common species with larger foraging ranges. Focusing on *Aphaenogaster rudis,* a common species with a relatively small foraging range, to the exclusion of less common species that travel farther distances, could produce an incomplete picture of local myrmecochory and the contribution of the entire ant community to the maintenance of plant species.

We investigated variation in impact among the ant species that transport seeds of bloodroot, *Sanguinaria canadensis* L. (Papaveraceae), in Missouri. We revisit the classification of *Aphaenogaster rudis* as a keystone seed disperser in forests of eastern North America by considering the relationship between impact and abundance, considering both quantity and quality of dispersal services (*sensu* Schupp et al., 2010) as a measure of impact. By considering the interactions of *A. rudis* and co-occurring ant species with myrmecochorous seeds and natural variation in ant abundance, this study aims to determine whether *A. rudis* is a keystone species under Power et al. (1996)'s definition (i.e., disproportionately important) or merely an abundant seed disperser. Following Power et al.'s definition of keystone species, we propose *A. rudis* or any other species must meet three criteria to be called a keystone seed disperser. Compared to other species in the disperser community, keystone dispersers should: 1) disperse a greater overall quantity of seeds compared to any other species 2) contribute a higher quality of dispersal (measured as distance in this study) compared to any other species, and 3) contribute to both the quantity and quality of seed dispersal more than expected based on absolute and relative abundance. We use data on the seed-dispersing ant community of *S. canadensis* at Shaw Nature Reserve, Gray Summit, Missouri, to test the hypothesis that *A. rudis* fits these three keystone disperser criteria.

Methods

Study System & Location: This research was conducted in forested portions of Shaw Nature Reserve (SNR) in Franklin County, Missouri (38°28'09.8"N, 90°49'15.8"W) a 988-ha property owned and managed by Missouri Botanical Garden. In two forest sites within SNR, we established 18 and 19 ant sampling plots sized 1 m × 3 m (Supp. Fig. 2). Canopy trees in both sites are dominated by *Quercus* spp. and *Carya* spp. (E.M. Colberg, unpublished data.).

Voucher specimens of *Aphaenogaster rudis* from SNR have been confirmed under current descriptions (B. DeMarco), although the species is polyphyletic (DeMarco and Cognato 2016) and part of the difficult-to-distinguish "*fulva–rudis–texana*" species complex (Umphrey 1996). Workers of *Aphaenogaster rudis* (*s.l.*) are typically 5.5 mm in length (Enzmann 1947), and behaviorally subordinate to more aggressive ants (Lynch et al. 1980, Fellers 1989). The species nests in soil, leaf litter, dead wood, tree cavities and under rocks (Talbot 1951; E.M. Colberg, unpublished data).

Although many North American forest herbaceous perennials are ant-dispersed (Handel et al. 1981; Warren et al. 2021), we chose to focus on the dispersal of one myrmecochorous species, *Sanguinaria canadensis,* due to its local abundance and seed availability. *Sanguinaria canadensis* is considered "at risk" in the U.S. due to harvest pressure for medicinal and artistic purposes (Furgurson et al. 2012; United Plant Savers 2021). In Missouri, it is locally abundant and associated with cooler, moister, north and north-east slopes of hardwood forests, including tulip poplar, hickory, red and white oak, as well as poplar stands (Furgurson et al. 2012; Albrecht and McCarthy 2009). It occurs patchily throughout SNR woodlands and forests (pers. obs.). Leaves emerge in April and fruits ripen in late May in Missouri, each fruit yielding around 25-34 elaiosome-bearing seeds (Schemske 1978). The oblong seeds average approximately 4 mm × 2 mm in length and width (EC, pers. obs.), and with an average dry weight of 7.05 mg and an average elaiosome dry weight of 4.9 mg (Schemske et al. 1978). In late May, 2020, we collected *S. canadensis* seeds from an SNR-origin garden population near Eureka, MO. Seeds were stored in sealed plastic bags in a -21°C freezer until field use.

Seed dispersal quantity & quality. From June 18 to July 18, 2020, we conducted seed-dispersal trials at SNR. At the center of each plot within two sites at SNR (Dana Brown Woods, 19 plots, and Trail House Woods, 18 plots), we placed three 10.16 cm × 10.16 cm index cards ("seed depots") in a straight line with 1 m spacing, the line perpendicular to the direction of the slope. We placed 20 seeds of *Sanguinaria canadensis* on each card*,* and for two hours per plot or until all seeds were taken, whichever came first, one observer recorded transport events, dispersal distances, dispersal locations, and collected ant voucher specimens. Specifically, to measure the quantity of seed dispersal by species, we recorded every time an ant took a seed >5 cm (in a straight linear distance parallel to the ground surface, to distinguish true transport from gravity-aided falls) from a depot. We then followed the seed-bearing ants, marking the seed's furthest recorded location, dispersal distance, and whether it reached a nest, was dropped, or lost to the observer. Additionally, we collected voucher specimens from located nests, to ensure accurate species identification. When too many ants transported seeds simultaneously than what a single observer could keep track of, we prioritized ants from apparently new nests, as differentiated by morphospecies and direction of movement.

Ant community sampling: On July 26, 2020, we used pitfall traps to sample the ground-foraging ant community at seed dispersal plots after all seed dispersal trials had concluded. At each corner of a 1 × 1 m square around the center of each plot, we dug a hole for a 60 mL plastic deli cup, which we filled with approximately 40 mL of a 1:10 unscented Schnucks brand detergent: water mix. Traps were collected after 24 hours, and the catch was rinsed and transferred to vials of 70% ethanol for storage and identification. Ant samples were then pinned and identified using the keys in J. Trager (unpublished manuscript), Fisher and Cover (2007), MacGown (2014), DeMarco (2015), and Antwiki (2020).

Statistical analysis. We considered a species at a plot as the replicate, and thus for dispersal quantity and quality, we combined data from all three cards per plot by ant species, and for abundance data, we combined all four pitfalls per plot by ant species. Unless otherwise noted, only plots with at least one ant observed were included in analyses for Criterion 1 and 2 (23 out of 37 plots), and only plots with at least one ant of the same ant species present in dispersal observations and pitfall traps were used to test Criterion 3. All analyses were conducted with R software (version 4.2.0, R Core Team 2022).

Criterion 1: Does A. rudis *transport more diaspores compared to other ant species?* To test whether *Aphaenogaster rudis* disperses more seeds than other species in the disperser community at the plot level, we used the number of seeds dispersed by each species at each plot as our outcome variable. This variable was a count with overdispersion, so we selected a negative binomial error distribution with a log-link function. Because our hypothesis that *A. rudis* contributes more to dispersal quantity than other species requires comparing *A. rudis* with the other ant species in the community, we treated species as a fixed effect and used planned treatment contrasts (*contr.treatment* with *A. rudis* as the base level) to compare *A. rudis* with every other species. We ran generalized linear mixed models (GLMMs) with site and plot nested within site as random effects (*glmer.nb* in “lme4” package). We used AICc values to compare the models with random effects to a generalized linear model (GLM, using *glm.nb* in “MASS” package), and found that GLM (that is, excluding site and plot) provided the best fit compared to more complex models (Supp. Table 3). Additionally, we tested whether *A. rudis* dispersed more seeds across all plots than any other species by comparing the total number of seeds transported by each ant species with a null distribution of equal probabilities using a chi-square goodness-of-fit test, followed with post-hoc Bonferroni-corrected chi-square comparisons between expected and actual number of seeds transported for each species.

Criterion 2: Does A. rudis *provide higher quality dispersal than other ant species?* To test whether *A. rudis* transports seeds farther than other ant species, we compared mean dispersal distances among ant species at the plot level. We log-transformed the mean dispersal distance recorded for a species at each plot to approximate a normal distribution, and used a linear model to compare *A. rudis* to every other species using planned treatment contrasts (*contr.treatment* with *A. rudis* as the base level). We used AICc values to decide whether to include site and plot nested within site as random effects (*lmer* in “lme4”), and found the best fitting model to be that without random effects (Supp. Table. 4). We also ran a linear regression of each species’ average dispersal distance against the fixed effect of ant body size (Weber’s length, mm), based on AntWeb (2022) specimens for each species (details and measures in Supp. Table. 1).

Criterion 3: Is A. rudis *a more effective seed disperser than expected based on its abundance in the ant community, in both (A) absolute and (B) relative terms?* Authors differ in whether to use absolute (Catano and Stout 2015) or relative (Power et al. 1996, Kotliar 2000) values to quantify the relationship between abundance and impact. We consider both approaches, testing the third criterion in terms of both absolute and relative values of dispersal quantity (number of seeds transported), quality (dispersal distance), and abundance. We hypothesized that *A. rudis* is a keystone disperser, and thus we expected a higher quantity and quality of dispersal from *A. rudis* compared to other species when controlling for abundance, whether absolute or relative. For both sets of analyses, we reduced our dataset to use only seed transport observations from plots at which a species was also collected in pitfall traps, to avoid assigning zeroes for species that had been present during dispersal trials but were not collected in pitfall traps.

Criterion 3A: Is A. rudis *a more effective seed disperser than expected based on* absolute *abundance and impact?* We tested whether *A. rudis* abundance had a greater impact on *S. canadensis* dispersal quantity compared to other ant species by running a negative binomial GLM with the number of seeds dispersed by a species at a plot as the dependent variable and worker abundance, ant species, and their interaction as fixed effects. To measure how absolute dispersal distance related to worker abundance, we ran an LMM with the log-transformed mean dispersal distance as our outcome variable, worker abundance, ant species, and the interaction of abundance and species as fixed effects, and site and plot nested within site as random effects, and performed planned contrasts between *A. rudis* and all other species. In both models, we included the interaction of abundance and species to determine whether, when compared to *A. rudis,* different species demonstrated different relationships to the outcome variable with increasing abundance (as would be indicated by a significant interaction term).

Criterion 3B: Is A. rudis *a more effective seed disperser than expected based on* relative *abundance and impact?* We compared the relative dispersal quantity and quality of each seed-dispersing ant species to its proportional abundance based on pitfall trap data. We calculated proportional abundance as the number of workers of a species found in traps at that plot divided by the total number of ants found in traps at a plot. We first considered the relationship between proportional abundance and proportional dispersal quantity, the number of seeds transported by a species relative to all seeds transported at that plot, to ensure we were considering relative impact. Because our outcome variable was an overdispersed proportion, the proportion of seeds transported by a species at a plot, we ran GLM and GLMMs with beta error distribution and a logit-link function (*glmmTMB* in package "glmmTMB"), using site and plot nested within site as random effects in the mixed models. Based on AICc values, adding random effects did not improve model fit, so we used the most parsimonious model of relative dispersal quantity as a function of relative abundance and species (Supp. Table 7).

We calculated relative dispersal distance as the average dispersal distance for each species at each plot divided by the maximum dispersal distance by any species at a plot. To model how relative dispersal distance related to relative abundance, we used a quasibinomial error distribution to run a GLM with relative dispersal distance as our outcome variable, proportional abundance and ant species as fixed effects, and planned contrasts between *A. rudis* and all other species to test the hypothesis that *A. rudis* as a keystone species, would contribute more to dispersal quality than other species after controlling for the effect of proportional abundance.

Finally, because dispersal quantity and dispersal quality are both aspects of overall seed dispersal effectiveness (SDE, *sensu* Schupp et al. 2010), we multiplied relative dispersal quantity by relative dispersal quality to give a measure of SDE. As this gave us SDE values between 0 and 1, we ran a beta-distributed GLM with a log-link error function with relative abundance and ant species as fixed effects (*glmmTMB,* family = "beta"). Additionally, since all three models using relative abundance as a fixed effect could only provide information of how different species compared to *A. rudis,* we also determined whether species' proportional contributions to dispersal quantity, quality, or SDE exceeded what would be expected if relative impact and relative abundance followed a null hypothesis of a 1:1 relationship. To do so, we made three graphs, plotting relative abundance against relative dispersal quantity, relative dispersal quality, and SDE by ant species, to demonstrate whether any species cleared the 1:1 line in terms of mean, confidence intervals, or both.

Results

Based on the number of seeds left at each depot at the end of two hours compared to the number of seeds we were able to record being transported, we observed 38% (247/654) of all transport events (Supp. Table 2). Seeds were moved by ants of three subfamilies: Myrmicinae (*Aphaenogaster, Myrmica*), Formicinae (*Camponotus, Formica, Lasius*), and Dolichoderinae (*Tapinoma*), comprising six genera and eleven species. The most common seed-dispersing species was *Aphaenogaster rudis,* which accounted for 53% of all observed transport events.

Criterion 1: Does A. rudis *transport more diaspores compared to other ant species? Aphaenogaster rudis* transported an average of 8.8 (± 8.0) seeds per plot when present, compared to an average of 4.3 (± 5.2) seeds for all other species combined. A GLM revealed significant differences among ant species in their contribution to the number of seeds moved (Table 1, Fig. 1B). Namely, *A. rudis* took significantly more seeds than *Camponotus castaneus* ($\beta = -1.769$, $z = -2.269$, $p = 0.023$) and *Formica subsericea* ($\beta = -1.287$, $z = -3.167$, $p = 0.002$), and marginally significantly more seeds than *C. chromaiodes* ($\beta = -1.076$, $z = -1.041$, $p = 0.052$), *C. pennsylvanicus* ($\beta = -1.259$, $z = -1.826$, $p = 0.068$), *C. subbarbatus* ($\beta = -2.175$, $z = -1.769$, $p = 0.077$), and *Myrmica latifrons* ($\beta = -2.175$, $z = -1.769$, $p = 0.077$).

When considering the number of seeds transported by each species across all plots, there were significant differences between our data and the null hypothesis that each species transported the same number of seeds ($\chi^2 = 632.8$, df = 9, $p < 0.001$). When comparing the proportion of seeds taken by *Aphaenogaster rudis* to every other species, *A. rudis* took significantly more seeds, even with Bonferroni correction (Bonferroni-adjusted $p < 0.001$ for every comparison).

Criterion 2: Does A. rudis *provide higher quality dispersal than other ant species?* A linear model revealed four ant species had significantly greater mean dispersal distances compared to *A. rudis,* one species had a significantly shorter mean dispersal distance, one species had marginally significantly greater distance, and one species had a marginally significantly shorter distance (Table 2, Fig. 1C). Specifically, *Camponotus castaneus* ($p = 0.086$), *C. chromaiodes* ($p = 0.004$), *C. pennsylvanicus* ($p = 0.008$), *Formica pallidefulva* ($p = 0.001$), and *F. subsericea* ($p < 0.001$) all transported seeds farther than *A. rudis,* whereas *Lasius americanus* ($p = 0.024$) and *Tapinoma sessile* ($p = 0.078$) transported seeds shorter distances than *A. rudis* (Table 2, Fig. 1C). Body size was significantly related to dispersal distance (adj. $R^2 = 0.637$, $F_{(1,8)} = 16.77$, $p = 0.003$, Supp. Fig. 3).

Criterion 3A: Is A. rudis *a more effective seed disperser than expected based on its* absolute *impact and abundance in the ant community*? *Aphaenogaster rudis* worker abundance was marginally significantly correlated with the number of seeds transported, with a 46% increase in dispersal quantity for every additional *A. rudis* worker found in pitfall traps ($p = 0.058$; Supp. Table 5, Fig. 2A). This relationship that was significantly steeper than that of two other species, based on significant interactions between worker abundance and species identity (abundance * *L. americanus*: IRR = 0.68, $p = 0.056$; abundance * *F. pallidefulva*: IRR = 0.61, $p = 0.098$), suggesting *A. rudis* does fit the keystone criterion when considering dispersal quantity and absolute abundance. However, this did not hold when considering the relationship between dispersal distance and absolute abundance (Supp. Table 6, Fig. 2B). *Camponotus chromaiodes* transported seeds significantly farther than *A. rudis* ($p = 0.049$), a relationship steeper than that of *A. rudis* when considering the interaction term of worker abundance ($p = 0.005$). Similarly, *F. subsericea* transported seeds farther than *A. rudis* when considering worker abundance, based on a marginally significant interaction between abundance and *F. subsericea* ($p = 0.063$). However, *C. pennsylvanicus* transported seeds significantly shorter distances than *A. rudis* ($p = 0.031$). Overall, when considering absolute impact and abundance, *A. rudis* somewhat, but not entirely, met the criterion for dispersal quantity, but did not meet the criterion for dispersal quality when using absolute distance as a measure.

Criterion 3B: Is A. rudis *a more effective seed disperser than expected based on its relative impact and abundance in the ant community?* We did not find support for our hypothesis that *A. rudis* would meet the keystone criterion of having a greater relative impact compared to relative abundance in the ant community (proportional abundance in pitfall traps compared to other ants present at a plot). Only *C. pennsylvanicus* accounted for a marginally significant difference from *A. rudis*, contributing a greater proportion of *S. canadensis* seed dispersal when accounting for proportional abundance (Estimate = 5.22, p = 0.080, Table 3A). Furthermore, only two species (*C. chromaiodes* and *F. pallidefulva*) had mean proportional dispersal quantities above a 1:1 line when considering proportional abundance (Fig. 3A). When considering relative dispersal distance while accounting for relative abundance, only *F. pallidefulva* was responsible for significantly greater proportion of dispersal distance compared to *A. rudis* (p = 0.037; Table 3). Although *A. rudis* fully cleared the 1:1 line when plotting proportional abundance against proportional dispersal distance, so did *C. chromaiodes, C. pennsylvanicus, F. pallidefulva,* and *M. latifrons* (Fig. 3B). However, when considering dispersal quantity and quality together (SDE) and controlling for relative abundance, *C. pennsylvanicus* provided significantly more SDE than *A. rudis* (p = 0.001) while *L. americanus* had significantly lower SDE than *A. rudis* (p = 0.025; Supp. Table 8). *Camponotus chromaiodes* and *F. pallidefulva* fell above the 1:1 line when plotting SDE against proportional abundance (Fig. 3C), while *L. americanus* and *A. rudis* fell below the 1:1 line. Overall, *A. rudis* did not meet our final criteria for keystoneness when considering the impact of relative abundance, not significantly exceeding (and in fact, being surpassed by) the relative dispersal quantity, quality, or combined SDE of other ant species.

Discussion

Our results show *Aphaenogaster rudis* is better described as a numerically dominant disperser than a keystone disperser; while its high abundance means it has a large contribution to dispersal quantity, less abundant species surpassed it in terms of distance that seeds were dispersed. Specifically, when considering the number of seeds transported at the plot level, *A. rudis* took more seeds than six out of nine other species, and took significantly more seeds than all other species when pooling all dispersal events together. Thus, *A. rudis* meets the first criterion of a keystone disperser by transporting more seeds than other ants in its community at two different spatial scales. Other studies have found similar results, with *Aphaenogaster* spp. (usually *A. rudis*) often recorded as the most important seed dispersing species in terms of quantity (Culver and Beattie 1978; Zelikova et al. 2008) and relative likelihood of transporting seeds when encountered (Ness et al. 2009).

Aphaenogaster rudis dispersed seeds an average of 0.32 (± 0.16) m (Supp. Table 2). When using dispersal distance as a proxy for dispersal quality, our results show *A. rudis* does not meet the second criterion of contributing more to quality than other species, as four formicine ants dispersed seeds significantly farther than *A. rudis,* with average dispersal distances 1.22–2.54 meters greater than that of *A. rudis*. This is supported by studies demonstrating that other species, particularly formicine ants such as *Formica subsericea,* transport seeds significantly farther than *A. rudis* (Pudlo et al. 1980). Indeed, Myrmicinae in general are considered short-distance dispersers, compared to

Formicinae which disperse seeds greater distances on average (Gómez and Espadaler 1998).

Our observed pattern of formicine ants transporting seeds farther than myrmicines is also consistent with global patterns of smaller ants accounting for shorter dispersal distances, whereas larger ants account for the tail of the dispersal curve (Gómez and Espadaler 2013). *Aphaenogaster rudis* is smaller than all species for which we measured longer dispersal distances (*C. castaneus, C. chromaiodes, C. pennsylvanicus, C. subbarbatus, F. pallidefulva,* and *F. subsericea*), except for *M. latifrons,* when using Weber's length (Geraghty et al. 2007; King and Trager 2007; Mackay 2019). Additionally, across all species, dispersal distance and worker abundance were not significantly correlated (Supp. Table 6). Of course, although we used dispersal distance as our measure of dispersal quality, other factors not considered here can also impact seed and seedling survival and fitness (Schupp 1993). In the future, other variables affecting seed fate and seedling establishment, such as the nutrient content of the substrate of deposition sites should also be considered (Farji-Brener and Werenkraut 2017), as well as distance from maternal plants rather than artificial seed depots.

Finally, when considering our final criterion and controlling for abundance, *A. rudis* does not unequivocally meet the requirements of a keystone disperser. If considering dispersal quantity and worker abundance, *A. rudis* only disperses marginally significantly more seeds than two other ant species. In turn, *A. rudis* did not contribute to relative dispersal quantity or quality more than other ant species when controlling for relative abundance. When accounting for absolute abundance, the number of seeds transported by *A. rudis* at a plot did not significantly differ from most other species

(Supp. Table 5). We encountered *A. rudis* more often in pitfall traps than any other ant (Supp. Table 2), although *L. americanus* had higher abundance when it occurred. However, our measure of dispersal quality, dispersal distance, was not well predicted by ant abundance in general. When considering dispersal quality and worker abundance, several less common formicine ants dispersed seeds farther than *A. rudis*. Using proportional dispersal distance and abundance instead of absolute values still resulted in a formicine ant contributing more than *A. rudis.* In part, this could be explained if ants with larger foraging distances have lower nest densities, as trap success depends in part on nest density (Schlick-Steiner et al. 2006). Finally, when considering proportional dispersal distance and dispersal quantity together as SDE and plotted against proportional abundance, only *C. chromaiodes* and *F. pallidefulva* fell above the 1:1 line, and did not fully clear the line with their confidence intervals. This suggests that *A. rudis* does not contribute more to seed dispersal than expected based on its relative abundance when considering dispersal quantity and dispersal quality, but other species do, at least in some circumstances.

Taken together, the high contribution of *A. rudis* to dispersal quantity alongside high abundance could better classify it as a dominant disperser rather than a keystone disperser. This trend is likely to hold true in other parts of *A. rudis'* range. Although the relative abundance of *A. rudis* varies throughout its range (King et al., 2013), its general frequency at baits is associated with the fact that it has high numbers of foragers per nest (Warren et al., 2020). However, *A. rudis* did not contribute as greatly to dispersal distance as rarer species did (*C. pennsylvanicus* when considering dispersal distance, or *F. pallidefulva* when considering relative dispersal distance), suggesting that when dispersal quality is incorporated in evaluating keystone dispersers, other species emerge as more important.

Our observations consider only one myrmecochorous plant, *Sanguinaria canadensis.* Other local plant species are also ant-dispersed, and both plant and ant species can affect outcomes of myrmecochory for plant and ant partners (Beattie et al. 1979; Turner anmd Frederickson 2013). Additionally, this study was conducted in daylight, but a study including nighttime observations noted *Camponotus castaneus* moved more seeds than *A. rudis* at night (Stuble et al. 2014). Thus, there could be variation in which ants emerge as dominant and important for myrmecochory depending on ant species, plant species, and time within a 24-hour cycle. However, we do not believe this potential variation is enough to affect our conclusions that *A. rudis'*s importance stems from its abundance as a disperser rather than keystone impacts, and that more rare species could have outsized impacts on dispersal quality. Nonetheless, other factors affecting species importance during myrmecochory, particularly other aspects of dispersal quality such as dispersal site and seed handling by ants, warrant future study.

The requirement that a keystone species have greater impact than expected by relative abundance, as stipulated by Power et al. (1996), has often been overlooked or modified in practice. Even Kotliar et al. (1999) and Catano and Stout (2015) consider absolute abundance rather than relative abundance. We agree with the underlying idea that abundance should be considered when evaluating candidate keystone species (Power et al. 1996, Kotliar et al. 1999, Catano and Stout 2015), but we go a step further to consider both absolute and relative abundance and impact. The differences are not enough to affect our main conclusions that *A. rudis* is a dominant seed disperser but not a keystone species, and that other, less abundant species have greater impacts on dispersal in comparison. That said, absolute abundance may be more readily understandable than proportional abundance. Ultimately, we recommend considering abundance and contributions of other species in the community when evaluating a species impacts, whether using the keystone concept or not. The North American seed-dispersing ant community is a prime example of how an abundant species can garner attention for numerically large impacts, while potentially equally important but less common species may have been overlooked and understudied in comparison.

Power et al.'s (1996) method of measuring whether a species meets keystone requirements did not account for the possibility of functional redundancy, wherein a drop in one species' abundance might not result in any change in ecosystem functioning if other species are able to compensate for that loss. Furthermore, species removal experiments such as those proposed by Power et al., (1996) would still fail to detect non-linear relationships between abundance and impact, such as cases of cryptic function loss where behavioral shifts lead to losses in species functionality despite continued presence (McConkey and O'Farrill, 2016). More recent approaches allowing for shifts in the relationship between abundance and importance (Kotliar 2000; Catano et al. 2015) create a fuller picture, but direct comparisons of abundance–impact relationships among species are rare (but see Kotliar et al. 1999). We add to this by revisiting the question of how to quantify keystoneness with data from multiple species, using natural variation in species abundance rather than a removal experiment. We find variation among species along different axes of importance (dispersal quantity versus quality). However, not all functions may correlate with abundance; for example, quantifying which species has a larger impact compared to its relative abundance in terms of dispersal quality can indicate when a species contributes more to dispersal than expected for its relative abundance, but aspects of dispersal quality may not be expected to directly relate to relative abundance until they are put into terms of seed survival and germination.

We do not intend to negate the importance of *A. rudis* for *S. canadensis,* but rather suggest the abundance of *A. rudis* is key to its impact, while also advocating for increased attention to less common but no less important seed-dispersing ants. As Kotliar (2000) suggested, we show that considering the relationship between impact and abundance can be more informative than blanket terms such as “keystone disperser”—namely, the impact of *A. rudis* is largely a factor of its worker abundance and contribution to dispersal quantity, but other species have outsized effects on dispersal distance. Particularly as communities face new forms of disturbance, considering the entire seed-dispersing community, not just the most important or abundant dispersers, can help predict how dispersal services will respond to a changing world. Several of the seed-dispersing ant species we consider here are known to respond differently to temperature increases (Stuble et al. 2013), which could lead to shifts in dispersal effectiveness as well as abundance. Warming temperatures can also shift the relative phenology of myrmecochores and their dispersers, leading to mismatches between when seeds are ready to be dispersed and when *A. rudis* is active (Warren et al. 2011). Phenological mismatch may not be as imminent a problem for *Sanguinaria canadensis* in Missouri, but could be of real concern for plants at the edges of its range as well as for earlier seeding myrmecochores (Warren et al. 2017). Additionally, some invasive ants may outcompete *A. rudis* and provide differing levels of dispersal quantity and quality (Prior et al. 2014;

Warren et al. 2015; Meadley-Dunphy et al. 2020), but the functional responses of other native species could be affected or become even more important in comparison. Indeed, how well other seed-dispersing ants compare to or even exceed the seed dispersal services provided by *A. rudis* could be important for understanding functional redundancy and variation within this ant-plant mutualism (Zamora 2000; Stuble et al. 2014). Thus, we urge future studies to consider the entire suite of seed-dispersing ants, rather than just *A. rudis.*

Conclusions

Drawing upon previous definitions of keystone species and seed dispersal effectiveness, we established three criteria for a species to be a keystone disperser, and tested their application using natural levels of seed-dispersing ant abundance and dispersal in a Missouri woodland ecosystem. We found that our hypothesized keystone disperser, *Aphaenogaster rudis,* meets some but not all criteria for being a keystone disperser of *Sanguinaria canadensis*. Although *A. rudis* abundance positively affects the number of seeds transported by *A. rudis*, this relationship is not significantly different from that of most other ant species' abundance and dispersal quantity. Furthermore, less abundant species such as *Camponotus chromaiodes* and *Formica pallidefulva* may provide higher quality dispersal by transporting seeds farther than *A. rudis,* with potentially large implications for plant spread. We suggest more attention should be focused on non-dominant ants and other aspects of dispersal quality beyond dispersal distance in this study system. More broadly, by using seed dispersal effectiveness, which depends upon both quantity and quality of dispersal, we highlight the importance of considering abundance when evaluating keystone species. Not doing so can distort the keystone species concept to focus on numerically dominant species that provide high quantity but not necessarily high-quality services, while species that might better fit the definition based on high quality services despite lower abundances could be overlooked. Additionally, quantifying impact in relationship to abundance also allows for variation in keystoneness, which could help conservationists and land managers determine and maintain abundance thresholds for species to provide keystone services.

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Tables & Figures

Table 1: Results of a negative binomial GLM of the number of seeds taken per plot, modeling species as a fixed effect, with planned comparisons between *Aphaenogaster rudis* and every other ant species.

	Seeds transported per plot		
Predictors	*Incidence Rate Ratios*	*CI (lower, upper)*	*p*
(Intercept)	8.80	5.96, 13.46	**<0.001**
Camponotus castaneus	0.17	0.03, 0.84	**0.027**
C. chromaiodes	0.34	0.11, 1.11	0.061
C. pennsylvanicus	0.28	0.07, 1.25	0.077
C. subbarbatus	0.11	0.01, 1.29	0.0783
Formica pallidefulva	0.87	0.44, 1.77	0.686
F. subsericea	0.28	0.12, 0.64	**0.002**
Lasius americanus	0.34	0.09, 1.45	0.118
Myrmica latifrons	0.11	0.01, 1.29	0.083
Tapinoma sessile	1.36	0.34, 9.64	0.701
Observations	42		

R^2 Nagelkerke	0.541

Note: We present incidence rate ratios (IRR) rather than raw estimates, for easier interpretation despite the use of a log-link function. The IRR depicts the change in number of seeds transported at a plot when the species is changed from the intercept (in this case, *A. rudis,* which transports an average of 8.8 seeds per plot) to the predictor species when holding all else equal. IRR <1 indicates a species disperses fewer seeds on average than *A. rudis,* and an IRR>1 indicates a species disperses more seeds than *A. rudis*. The R^2 value was obtained using trigamma estimation. Df = 41. Bold p-values indicate $p \leq 0.05$.

Table 2: Results of a linear model of average dispersal distance (in m, log-transformed).

	Log(distance)			
Predictors	*Estimates*	*CI (lower, upper)*	*p*	*df*
(Intercept)	-1.12	-1.54, -0.70	**<0.001**	32
Camponotus castaneus	1.07	-0.16, 2.30	0.086	32
C. chromaiodes	1.56	0.53, 2.59	**0.004**	32
C. pennsylvanicus	1.70	0.47, 2.93	**0.008**	32
C. subbarbatus	1.33	-0.35,3.01	0.118	32
Formica pallidefulva	1.35	0.64, 2.07	**0.001**	32
F. subsericea	1.64	0.89, 2.39	**<0.001**	32
Lasius americanus	-1.43	-2.66, -0.20	**0.024**	32
Myrmica latifrons	0.78	-0.90, 2.47	0.353	32
Tapinoma sessile	-1.51	-3.19, -0.18	0.078	32
Observations	42			
Marginal R^2 / Conditional R^2	0.635 / 0.533			

Note: Ant species values are in comparison to *Aphaenogaster rudis,* the reference category. Bold p-values indicate $p \leq 0.05$.

Table 3: Results for a beta GLM of the proportion of seeds transported, and a quasibinomial GLM of the proportion of plot maximum dispersal distance. Est. = estimate, CI = Confidence Interval (lower, upper).

	Proportion of seeds transported			**Proportion of plot maximum dispersal distance**		
Predictors	*Est.*	*CI*	*p*	*Odds Ratio*	*CI*	*p*
(Intercept)	0.31	0.15, 0.66	**0.002**	0.54	0.16, 1.73	0.307
Proportional abundance	4.52	0.43, 47.52	0.209	6.88	0.14, 427.59	0.336
Camponotus chromaiodes	0.62	0.14, 2.70	0.523	6.36	0.64, 183.25	0.156
C. pennsylvanicus	5.22	0.82, 33.20	0.080	NA	NA	NA
Formica pallidefulva	0.76	0.31, 1.87	0.555	5.49	1.24, 33.19	**0.037**
F. subsericea	0.42	0.12, 1.53	0.189	2.07	0.32, 14.68	0.443
Lasius americanus	0.31	0.07, 1.36	0.121	NA	NA	NA
Myrmica latifrons	0.28	0.03, 2.30	0.237	2.02	0.08, 58.26	0.636
Observations			29		27	
Marginal R^2			0.349		NA	

Note: Both models display the results of the fixed effect of proportional abundance, and ant species represent planned comparisons between *Aphaenogaster rudis* and every other ant species. NA indicates species for which the model could not estimate parameters due to insufficient samples and extreme values; thus, these were dropped from the final model. Bold p-values indicate $p \leq 0.05$.

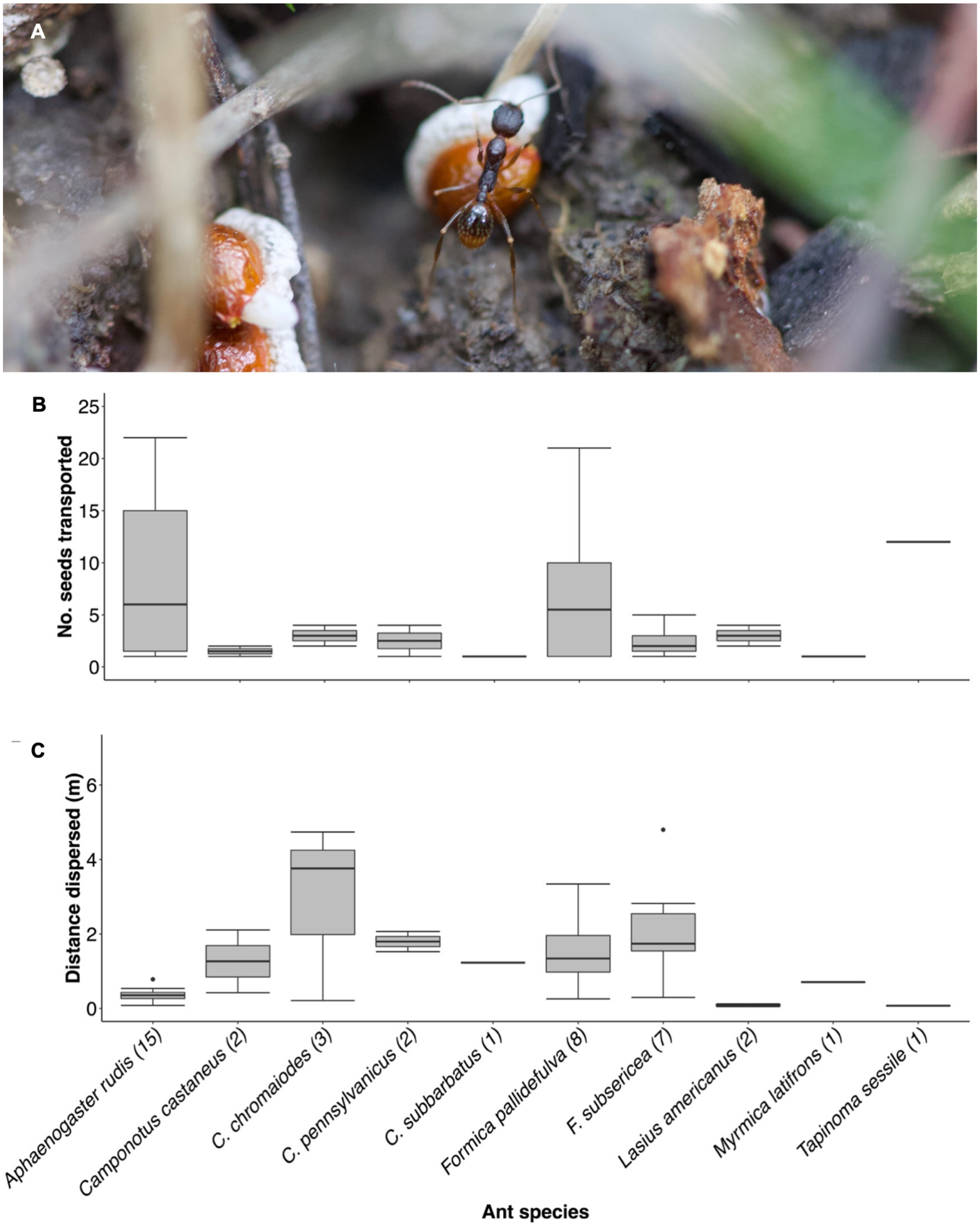


Fig. 1: *Aphaenogaster rudis* handling a diaspore of *Sanguinaria canadensis* (A), number of *S. canadensis* diaspores transported by each ant species (B), and mean distance ants transported *S. canadensis* diaspores (C), using plot as the replicate. Numbers by species

names denote the number of plots in which species were observed transporting seeds. Boxes represent first and third quartiles, black horizontal lines represent medians, whiskers represent 1.5 times the inter-quartile range, and dots represent values beyond 1.5 times the inter-quartile range.

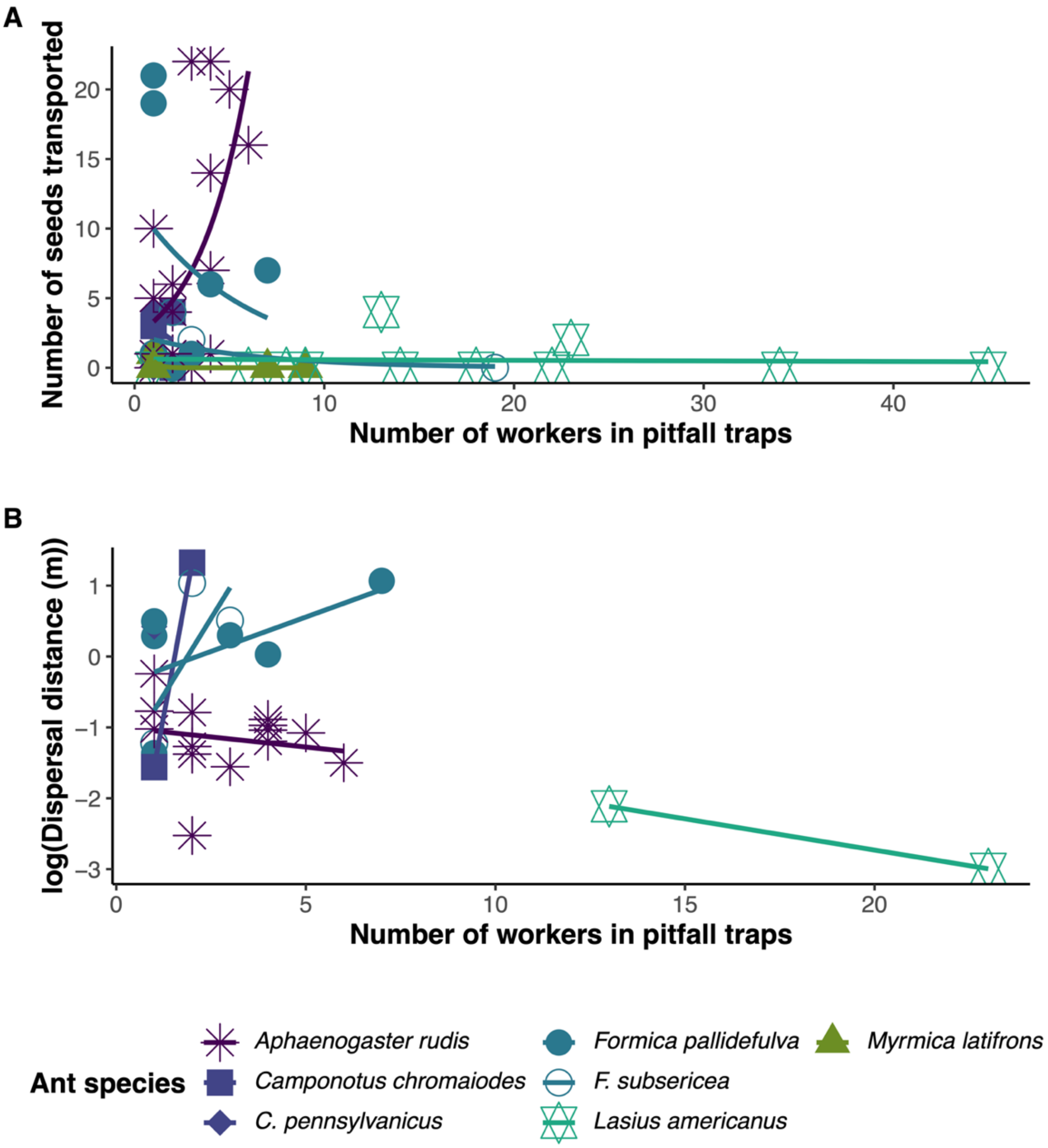


Fig. 2: Relationship between the abundance of ant workers in a plot's pitfall traps and the number of seeds species were recorded transporting at that plot (A), and between

abundance and mean dispersal distance (B). Curves in (A) are fitted with a Poisson error distribution, and normal curves are used to model log-transformed dispersal distance in (B). In both graphs, we would expect a keystone species to have a curve steeper than non-keystone species; *A. rudis* meets this expectation in (A) but not (B).

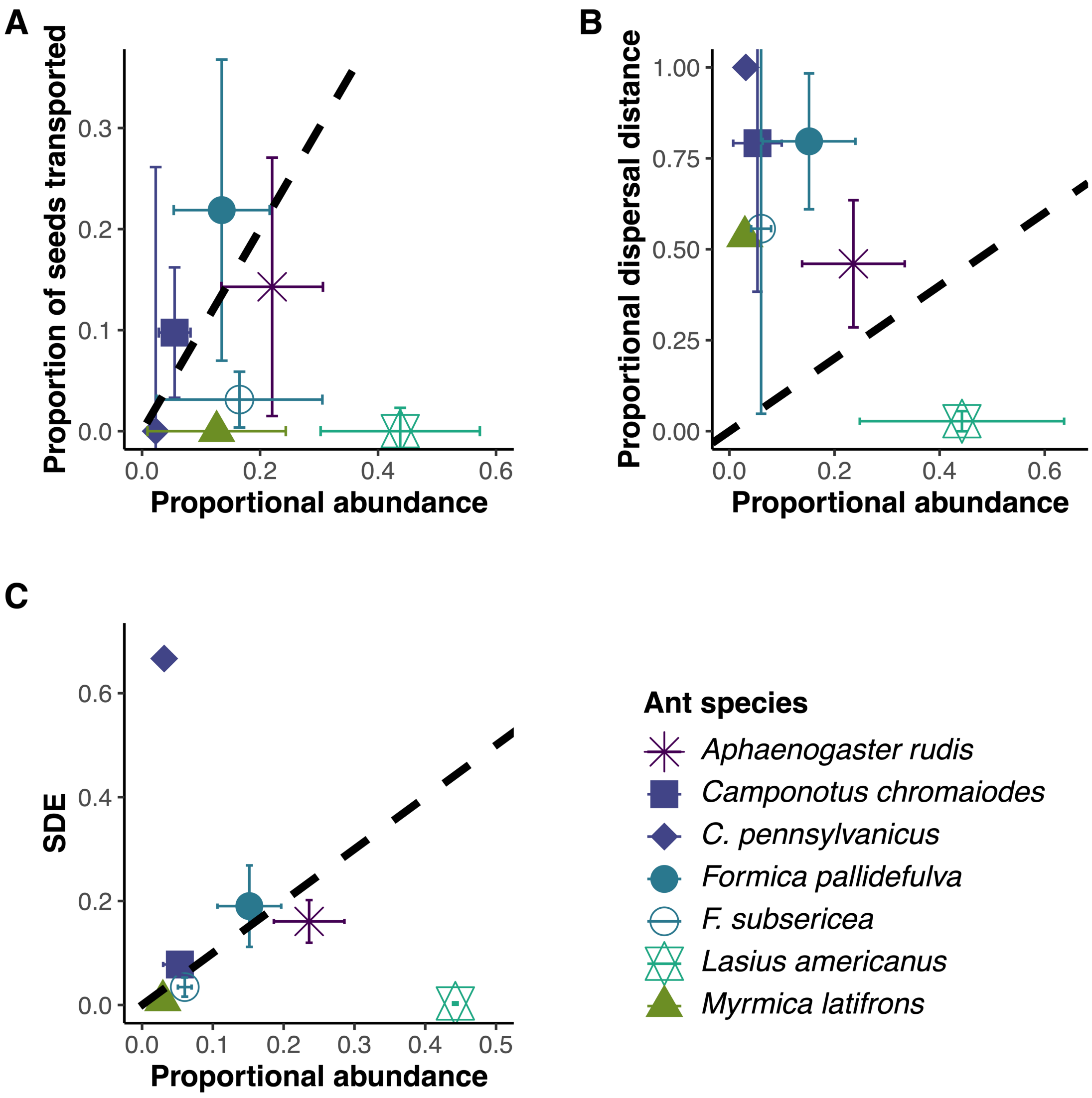


Fig. 3: Ant proportional abundance in pitfall traps compared to dispersal importance, in terms of (A) proportion of seeds dispersed, measured by the number of seeds transported by a species at a plot divided by the total number of seeds transported at that plot, (B) contribution to dispersal distance, represented by mean dispersal distance of a species at a plot divided by the plot maximum dispersal distance, and (C) seed dispersal effectiveness (SDE), defined here as the multiplied proportions of dispersal quantity and quality. Note different axis scales among graphs. Dashed black lines denote the null relationship, y =

x—when data points fall below this line, the species is not contributing to dispersal as much as expected based on its proportional abundance, and when data points fall above the line, a species is contributing more than expected based on its proportional abundance. Points represent mean values and whiskers represent 95% CI.

Supplementary Materials

Supp. Table 1: Weber's length used and number of AntWeb photos measured from.

Ant species	**Weber's length (mm)**	**Number photos measured**
Aphaenogaster rudis	1.73	6
Camponotus castaneus	3.45	5
C. chromaiodes	3.68	1
C. pennsylvanicus	2.89	3
C. subbarbatus	2.39	4
Formica pallidefulva	2.40	6
F. subsericea	2.50	4
Lasius americanus	1.25	5
Myrmica latifrons	1.50	4
Tapinoma sessile	1.03	7

Note: To obtain WL measures, we used all profile-view pinned worker specimen photos available for each species via AntWeb, measuring on-screen WL (distance from anterior point of pronotum abutting cervical shield to posterior end of propodeal lobe; *a*) and on-screen 1 mm scale bar length (*b*), dividing *a* by *b*, and taking the average from all available photos. We discarded photos in which the anterior of pronotum and posterior propodeal lobe were not visible (due either to importune leg placement or broken specimens). For species of *Camponotus,* we only measured minor workers.

Supp. Table 2: Number of observed seed transport events and number of plots species were found at during observation and pitfall sampling. SD = Standard deviation.

Ant species	**Transport events**	**Observation plots**	**Pitfall plots**	**Mean dispersal distance (m)**	**SD dispersal distance (m)**
Aphaenogaster rudis	132	15	23	0.32	0.16
Camponotus castaneus	3	2	6	0.99	0.97
C. chromaiodes	9	3	4	2.79	2.20
C. pennsylvanicus	5	2	6	1.63	0.24
C. subbarbatus	1	1	1	1.23	NA
Formica pallidefulva	61	8	8	1.25	0.89
F. subsericea	17	7	7	2.05	1.19
Lasius americanus	6	2	16	0.10	0.05
Myrmica latifrons	1	1	4	0.71	NA
Tapinoma sessile	12	1	12	0.07	0

In total, 247 seed transports were observed with species identification, out of 654 total transport events based on the number of seeds remaining at depots after 2 hours. Standard deviations were not attainable (NA) for species with only one observed transport event.

Supp. Table 3: ANOVA comparison table of GLM and two GLMMs of the number of seeds transported per plot, all with a negative binomial error distribution.

Model	**AICc**	**Log likelihood**	**Deviance**	χ^2	**df**	**p**
Number of seeds transported ~ Ant species	251.156	-110.18	220.36	--	--	
Number of seeds transported ~ Ant species + (1|Site)	259.356	-110.18	220.36	0	1	1
Number of seeds transported ~ Ant species + (1|Site) + (1|Plot:Site)	255.115	-110.18	220.36	0	1	1

Supp. Table 4: Results of ANOVA comparison of LM and LMM models of log-transformed average dispersal distances.

Model	**AICc**	**Log likelihood**	**Deviance**	χ^2	**df**	**p**
Dispersal distance ~ Ant species	119.921	-44.560	89.121	--	--	
Dispersal distance ~ Ant species + (1|Site)	122.930	-44.085	88.171	0.950	1	0.330
Dispersal distance ~ Ant species + (1|Site) + (1|Plot:Site)	127.171	-44.085	88.171	0.000	1	1.000

Supp. Table 5: Results of a negative binomial GLM of number of seeds taken, with worker abundance, ant species, and the interaction of species and abundance as fixed effects.

	Number of seeds taken		
Predictors	*Incidence Rate Ratios*	*CI*	*p*
(Intercept)	2.23	0.62, 7.98	0.218
Worker abundance	1.46	0.99, 2.16	0.058
C. chromaiodes	2.02	0.01, 487.81	0.802
C. pennsylvanicus	0.25	0.05, 1.33	0.103
Formica pallidefulva	4.67	0.67, 32.62	0.120
F. subsericea	1.27	0.13, 12.64	0.836
Lasius americanus	0.30	0.03, 2.73	0.287
Myrmica latifrons	22.39	0.00, Inf	1.000
Abundance * *C. chromaiodes*	0.46	0.02, 10.54	0.624
Abundance * *F. pallidefulva*	0.61	0.34, 1.10	0.098
Abundance * *F. subsericea*	0.54	0.26, 1.13	0.100
Abundance * *L. americanus*	0.68	0.45, 1.01	0.056

Abundance * *M. latifrons*	0.01	0.00, Inf	1.000
Observations	52		
R^2 Nagelkerke	0.677		

Note: Ant species values are in comparison to *Aphaenogaster rudis,* the reference category. Additionally, *M. latifrons* posed a problem for model estimation, likely due to the fact that it only took one seed at one plot, but showed up in pitfall traps in other plots.

Supp. Table 6: Results of LM of log-transformed dispersal distance as predicted by worker abundance, ant species, and their interaction.

	Log(dispersal distance)		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	-0.99	-1.77, -0.22	**0.015**
Abundance	-0.06	-0.29, 0.18	0.614
C. chromaiodes	-3.45	-6.56, -0.35	**0.031**
C. pennsylvanicus	1.47	0.01, 2.93	**0.049**
F. pallidefulva	0.58	-0.60, 1.76	0.316
F. subsericea	-0.63	-2.83, 1.56	0.551
Lasius americanus	0.02	-3.61, 3.66	0.989
Myrmica latifrons	0.71	-0.76, 2.17	0.322
Abundance**C. chromaiodes*	2.94	1.03, 4.86	**0.005**
Abundance * *F. pallidefulva*	0.25	-0.09, 0.59	0.141
Abundance * *F. subsericea*	0.92	-0.06, 1.90	0.063
Abundance * *L. americanus*	-0.03	-0.33, 0.27	0.830
Observations	29		

R^2 / R^2 adjusted	0.786 / 0.647

Note: Ant species values are in comparison to *Aphaenogaster rudis,* the reference category. Bold p-values indicate $p \leq 0.05$.

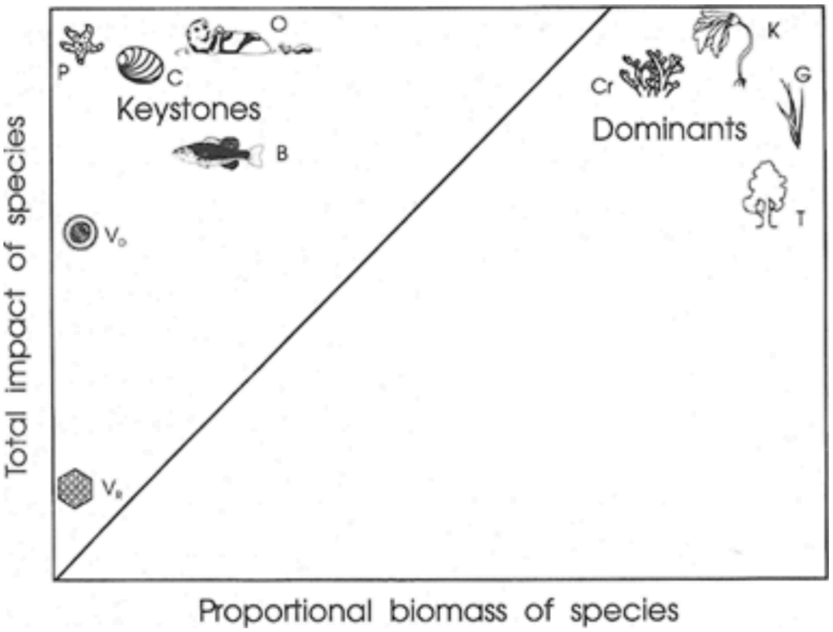
Supp. Table 7: Results of ANOVA comparison of beta-distributed GLM and GLMM models of relative dispersal quantity predicted by relative abundance and species identity.

Model	**AICc**	**Log likelihood**	**Deviance**	χ^2	**df**	**p**
Proportion seeds transported ~ Relative abundance + Ant species	-5.895	16.684	-33.368	--	--	--
Proportion seeds transported ~ Relative abundance + Ant species + (1|Site)	-1.146	16.684	-33.368	0	1	1
Proportion seeds transported ~ Relative abundance + Ant species + (1|Site) + (1|Plot:Site)	4.161	16.684	-33.368	0	1	1

Supp. Table 8: Results of a beta-distributed GLM of seed dispersal effectiveness (SDE) by ant species.

	SDE		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	-2.188	-2.968, -1.408	**<0.001**
Proportional abundance	1.537	-0.675, 3.749	0.173
Camponotus chromaiodes	0.210	-1.193, 1.612	0.769
C. pennsylvanicus	2.725	1.072, 4.378	**0.001**
Formica pallidefulva	0.432	-0.432, 1.296	0.327
F. subsericea	-0.471	1.744, 0.801	0.468
Lasius americanus	-1.749	-3.282, -0.216	**0.025**
Myrmica latifrons	-0.713	-2.765, 1.340	0.496
Observations	29		
R^2 marginal	0.299		

Note: Ant species values are in comparison to *Aphaenogaster rudis,* the reference category. Bold p-values indicate p ≤ 0.05.



Supp. Fig. 1: Power et al.'s (1996) proposed that keystone species were those whose total impact (measured as the absolute value of community importance) exceeded their proportional biomass or abundance (species above the solid line). Additionally, dominant species were proportionally abundant, but with impacts lower or equal to their proportional biomass (top right corner, but below solid line). In theory, species not abundant nor impactful would be neither keystone nor dominant. Original figure from Power et al. 1996, reproduced from Oxford University Press under license number 5390860763379.

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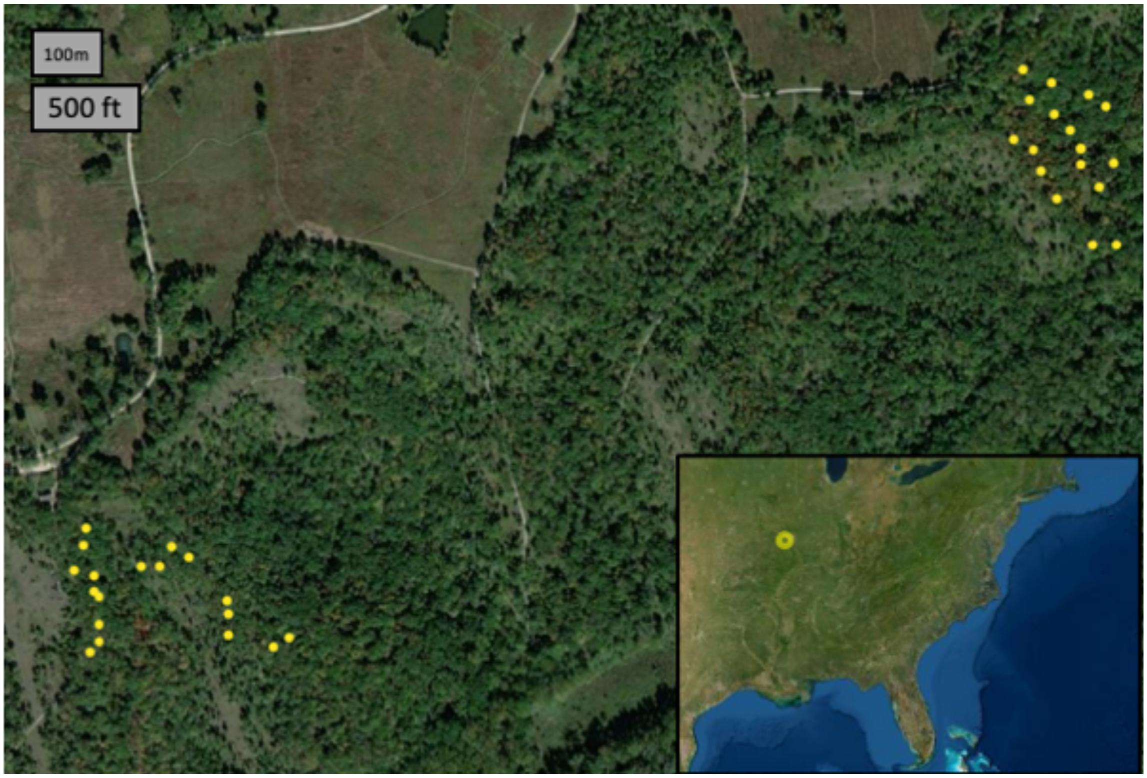
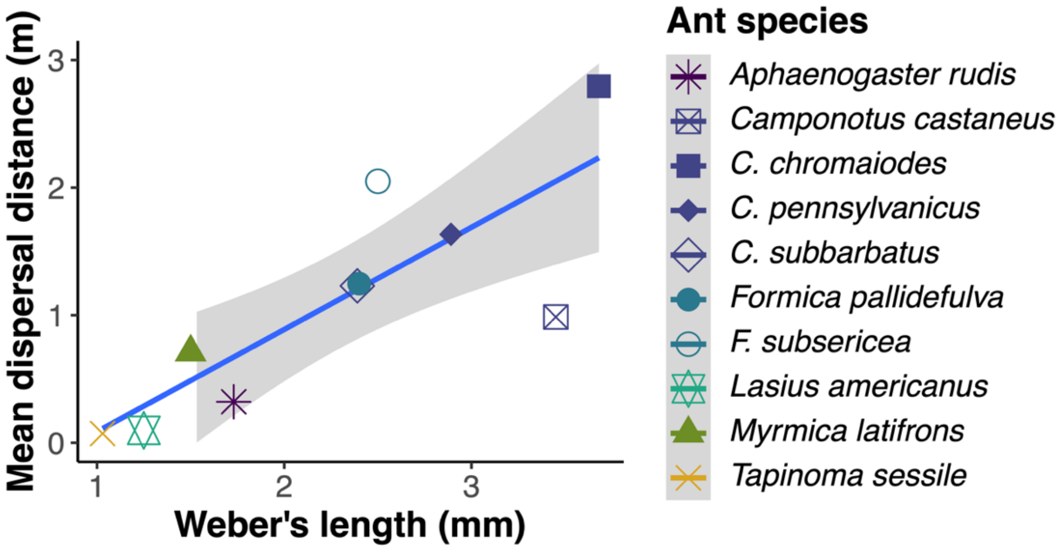


Supp. Fig. 2: Yellow circles represent plot locations at Shaw Nature Reserve (SNR). Yellow circle on inset shows the location of SNR.



Supp. Fig. 3: Relationship between ant size and average dispersal distance. Line depicts linear regression of the equation *Distance =-0.714 + 0.801*Weber length*. Residual standard error = 0.529 on 8 degrees of freedom, multiple $R^2 = 0.677$, adj. $R^2 = 0.637$, $F_{(1,8)} = 16.77$, $p = 0.003$.

Chapter 2: Resilience of ant-mediated seed dispersal to prescribed burning and litter removal in Missouri

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Abstract

Ant-mediated seed dispersal is frequently cited as a relationship benefitting from fire, in part due to increased abundance of seed-dispersing ants and dispersal distances in burned areas, but concrete tests of this hypothesis are both few and limited in location. Fire is a common land management tool in temperate deciduous North American woodlands. Increased understanding of whether and how burning affects ant-provisioned seed dispersal could improve plant and ant conservation and management practices. In two seasons and locations of field experiments in the Missouri Ozarks, we compared the dispersal rates, distances, and ant species transporting *Sanguinaria canadensis* seeds in burned, litter removal, and control plots. Based on short-term decreases in ant abundance after fire in the Ozarks, we hypothesized that seed dispersing ant abundance and dispersal rates would decrease in burned and litter removal plots. Based on myrmecochory studies from Australia, we hypothesized that reduction of leaf litter by fire, as well as manual litter removal, would increase ant-mediated seed dispersal distance compared to control areas. We found variation between study years and locations as well as among ant species—in the first field season, ants dispersed over twice as many seeds and carried seeds 68 % (0.35 m) farther in burned plots compared to unburned controls. In our second field season, average dispersal distances were 57% (1.06 m) shorter in burn compared to control plots, but not significantly different between litter removal and control or between burn and litter removal treatments. The ant *Aphaenogaster rudis* was responsible for the majority of observed dispersal events in both seasons, and *Formica* and *Camponotus* spp. transported seeds farther than *A. rudis* but were variably affected by burning and litter removal. Overall, we found the effects of fire and litter removal to be inconsistent across sites and years, and found much of our variation was explained by ant size, species identity, and slope aspect (only measured in 2021), suggesting this seed dispersal mutualism is more affected by factors other than fire and litter removal.

Keywords: *myrmecochory; disturbance; fire management; ant foraging; ant-plant interactions*

Introduction

Seed dispersal facilitates the spread of plant populations and can benefit plant populations by reducing conspecific density (Comita et al., 2014). While many types of anthropogenic disturbance may threaten seed dispersal (Neuschulz et al., 2016), fire is a disturbance that can have both negative and positive impacts on seed dispersal, with varying implications for conservation and management (Whelan, 1986; Pyke et al., 2010; Paolucci et al., 2016). Myrmecochory, or ant-mediated seed dispersal, has independently evolved over 100 times throughout the angiosperm phylogeny and occurs worldwide (Lengyel et al., 2010), and is a common dispersal syndrome in fire-prone areas (Pausas & Lamont, 2022). However, most studies to date have focused on the importance of fire for breaking myrmecochorous seed dormancy rather than the effects of fire on ant-seed interactions (Pausas & Lamont, 2022). Exceptions include studies in Australia, which have shown increased rates of myrmecochory (Beaumont et al., 2011), dispersal distance (Parr et al., 2007; Beaumont et al., 2011, 2013), and seed-dispersing ant abundance in burned areas (Beaumont et al., 2011, 2012). In contrast, fire in the Amazon rainforest decreased seed dispersal rates for myrmecochorous seeds (Paolucci et al., 2016). Here, we provide the first study of the effects of fire on myrmecochory in a temperate deciduous forest in North America.

The effects of fire on myrmecochory are mediated by the effects of fire on ant abundance and behavior, which vary based on habitat and ant physiological preferences (Dafni et al., 2012; Paolucci et al., 2016; Beaumont et al., 2018). In Australian woodlands and savannas, South African fynbos, and western North American chaparral, ants enhance seed survival by depositing seeds in safe sites prior to burning (Auld 1986, Bond et al., 1990; Boyd, 2001; Arnan et al., 2010; Beaumont et al., 2018). Australian studies have further demonstrated increases in frequency and distance of myrmecochory after burning (Parr et al., 2007; Beaumont et al., 2013). These increases are attributed to positive effects of fire on the abundance (Beaumont et al., 2013) and foraging ranges (Parr et al., 2007) of seed-dispersing ants. In part, these effects could be mediated by the reduction of leaf litter in burned areas (Parr et al., 2007; Beaumont et al., 2011), thereby reducing energetic costs of foraging (Nielsen, 2001). However, myrmecochory does not respond to fire in the same way everywhere. For example, in the transition zone between Cerrado savanna and Brazilian Amazon rainforest, fire increased the time needed by ants to find seeds and reduced the number of seeds they transported (Paolucci et al., 2016). In part, these differences could be due to habitat preferences; ants adapted to shadier and more humid conditions of a rainforest transition zone could be more sensitive to fire and post-fire conditions than the myrmecofauna of more open and fire-adapted woodlands, savannas, and chaparral.

Eastern North American oak-hickory forests, including those in the Ozark Mountains, are disturbance-adapted ecosystems wherein regular fire prevents succession of more mesic plants and allows oaks to persist and regenerate (Abrams, 1992; Dey & Guyette, 2000; Nowacki & Abrams, 2008). As lightning-caused fires are rare in these forests, humans are considered the most likely historical ignition source since their settlement in the area at least 14,000 years ago (Dey & Guyette, 2000; Ryan et al., 2013). Native Americans maintained the landscape with low intensity burns every decade or so prior to displacement by Europeans (Abrams, 1992; Guyette & Cutter, 1997; Guyette et al., 2002; McEwan et al., 2007). In recent decades, land managers have begun to use low-intensity fire as a tool to restore these disturbance-adapted communities (Brose et al., 2001; Van Lear, 2004; Arthur et al., 2012; Brose, 2014).

Myrmecochory is a common dispersal strategy of herbaceous understory plants in the Ozarks and eastward through North America (Handel et al., 1981; Yatskievych & Steyermark, 1999; Warren et al., 2016b). We focused on one myrmecochorous species, *Sanguinaria canadensis* (Papaveraceae), a perennial rhizomatous herb valued culturally and economically (Furgurson et al., 2012). This species grows in well-drained soils, and although it can grow in shade, responds to higher light conditions with increased vegetative growth (Marino et al., 1997). However, *S. canadensis*'s response to fire remains unclear; a 16-year study in the Missouri Ozarks demonstrated a positive relationship between the number of prescribed fires and *S. canadensis* herbaceous cover, but a negative relationship between *S. canadensis* cover and the time since the study's start (Johnson, 2017).

Dispersal of *Sanguinaria canadensis* seeds depends upon seed-dispersing ants (Pudlo et al., 1980). The ant *Aphaenogaster rudis* is widely referred to as the "keystone disperser" of *S. canadensis* and other North American woodland myrmecochores (Ness et al., 2009), but other co-occurring ants within the genera *Aphaenogaster, Camponotus, Crematogaster, Formica, Lasius, Myrmica,* and *Solenopsis* also contribute to dispersal of *S. canadensis* seeds (Ness et al., 2009), sometimes at much greater distances (Colberg et al., in prep.; Ness et al., 2009). Here, we consider all the species we observed dispersing seeds of *S. canadensis* to comprise the "seed-dispersing ant community," and consider community as well as individual ant species responses to our treatments.

Although this study is the first to address the effects of fire on myrmecochory in North American forests, other authors have demonstrated the short-term effects of fire on the overall ant community in North American hardwood forests. Fire and fire intensity in Ozark forests have demonstrated immediate negative effects on arthropod communities, including ants, as measured a few hours post-burn (Verble-Pearson & Yanoviak, 2014). Burning in the Ozarks during February and March also reduced ant abundance and species richness 30–59 days after burning, but the magnitude of both did not differ from controls after 60 days (Verble & Yanoviak, 2013). In upland oak forests in Iowa, ants were generally more abundant in unburned plots compared to plots with a history of yearly burning even when sampled in the fall, a year after the most recent burn (Vincent et al., 2009).

The short-term responses of ant and arthropod communities to fire could be due to both direct and indirect effects of burning. Direct effects of fire on ants could include temperatures—in oak-hickory forests, temperatures 10 cm above the ground can average 222°C during burning, soil temperatures can increase by around 10°C during burning, and soil temperatures may remain elevated for several hours (Iverson & Hutchinson, 2002). However, for taxa whose nests provide protection from direct burning and heat due to the insulating properties of soil, bark, and damp wood, fire could still affect behavior indirectly by altering the foraging environment and habitat characteristics. Prescribed burns in the Missouri Ozarks can consume virtually all leaf litter on the forest floor (Kolaks et al., 2004), requiring two years to regain roughly half the equilibrium amount of leaf litter (Stambaugh et al., 2006). With reduced surface complexity and increased insolation at the soil surface, conditions of burned areas could affect ant community composition by altering habitat and microclimatic conditions to fall outside individual physiological preferences and tolerances. For example, after burns in the Ozarks, more mesic species such as *Aphaenogaster carolinensis* and *Stenamma* spp. were only recorded in unburned areas (Verble & Yanoviak, 2013). Additionally, fewer dead leaves obstructing movement at the ground surface could affect foraging behavior (Beaumont et al., 2011).

Seed dispersing ants can vary by species in terms of the quantity and quality of their dispersal services (Colberg et al., in prep.). Different ant species tend to travel shorter or longer distances, and have different secondary dispersal behaviors (Beaumont et al., 2013; Meadley-Dunphy et al., 2020). If different species of ants vary in terms of the dispersal services they provide, the composition of disperser species in a community should have a direct effect on seed dispersal quantity and quality (Rey & Manzaneda, 2007). For example, although *Aphaenogaster rudis* is responsible for the greatest amount of seed dispersal in eastern North America (Ness et al., 2009), other ants, such as *Camponotus* and *Formica* spp. in the subfamily Formicinae*,* are responsible for longer distances (Gómez and Espadaler, 1998; Colberg et al., in prep.). Differences in dispersal distance are partly explained by body size, with larger ants taking seeds farther (Gómez and Espadaler, 2013). Furthermore, seed dispersal can also vary with colony needs and activity levels, so ant presence and abundance may not completely indicate dispersal quantity and quality (Gordon et al., 2019). Thus, if prescribed fire alters ant abundance, community composition, or behavior, there could be repercussions for the extent and quality of seed dispersal services.

To determine the effects of prescribed fire on ant-mediated seed dispersal in temperate North American forests, we studied the dispersal of *Sanguinaria canadensis* by ants during two field seasons, each at a different fire-managed reserve in the Missouri Ozarks. We compared dispersal quantity and distance, and dispersing ant species composition in burned and control plots in 2020. In 2021, we added a litter removal treatment to test whether differences between burned and control treatments were due to the reduction of leaf litter or other aspects of burning. Based on previously demonstrated short-term negative effects of fire on overall ant abundance in similar habitats (Vincent et al., 2009; Verble & Yanoviak 2013, 2014), we hypothesized that seed-dispersing ant abundance would be lower in burned compared to control plots and would lead to ants transporting seeds less frequently in burned (2020, 2021) and litter removal (2021) treatments compared to unburned areas (H1). Independent of ant abundance, however, we expected ants to transport seeds farther in burn (2020, 2021) and litter removal treatments (2021) due to less obstruction to foraging from reduced leaf litter in burned and litter removal treatments. In all cases, we consider the treatment response of the entire seed-dispersing ant community, as well as species-specific and size-based responses.

Methods

Study sites and focal species:

In 2020, field experiments were conducted at Shaw Nature Reserve (SNR; (38°28'09.8"N, 90°49'15.8"W) in Franklin County, Missouri, a 988-ha private reserve consisting of prairie, stream, glade, and woodland habitats managed for public use and ecological restoration. As part of SNR's management strategy, various sections of the reserve are subjected to controlled burns every few years to control invasive species and maintain habitat, and thus our replication of each treatment was limited by the type, amount, and spatial separation of forest units burned within the year prior to 2020 seed dispersal experiments. In 2021, to increase burn replicates and provide more control over differences in burn and control locations, we used a location 24 km east of SNR: the Tyson Research Center (TRC; 38°31’35.8” N, 90°33’41.8” W) in St. Louis County, Missouri, an 800-ha forested landscape owned by Washington University in St. Louis. In 2016, TRC established 16, 2.5-acre experimental forest plots, consisting of 8 burn treatment plots paired with 8 control plots. Aside from fires administered in experimental burn plots since 2017, TRC had not been subject to burning (prescribed or otherwise) or logging since 1942, but was selectively logged and grazed in the early 1900s (Zimmermann & Wagner 1979; J. Myers pers. comm.).

The woodland herb *Sanguinaria canadensis* grows patchily throughout wooded areas in both SNR and TRC. In addition to its ant-dependent seed dispersal strategy, it is a clonal species with rhizomatous growth contributing to a clumped distribution. Leaves emerge in early spring, with mature plants flowering in April and fruits maturing in late May (Colberg, unpublished). The average number of seeds per fruit is 25-34 (Schemske, 1978), and each seed bears an elaiosome containing oleic acids that attract ants (Kusmenoglu et al., 1989; Sheridan et al., 1996). In late May of each year, we collected *S. canadensis* seeds from an SNR-origin garden population in Eureka, MO, and stored seeds in plastic bags in the freezer (-21°C) to preserve elaiosome freshness until use in the field.

Experimental setup:

In 2020, ant-sampling plots (each 1 × 3 m) were divided between four SNR sites, two of which were burned that year as part of SNR's fire management program ("burn" treatment). One burn site (Morton West, MW: 52 ha) was burned January 7, 2020, with relative humidity levels ranging from 39–51% and windspeeds ranging from 22–24 mph, and the other site (Morton East, ME: 78 ha) was burned March 6, 2020, with relative humidity of 29% and a surface windspeed of 13 mph (C. Maginel, pers. comm.). The two SNR sites not burned within the past year ("control" treatment) were previously burned in November 2017 (Trail House, TH: 82 ha, 18 plots) and March 2018 (Dana Brown, DB: 117 ha, 19 plots). At each site, ant-sampling plots (18 at MW, ME, and TH; 19 at DB) were established at least 20 m apart from each other (Supp. Fig. 1a). Including the 2020 burns, one burn site (MW) was burned twice in the preceding decade, and the other burn site (ME) and both control sites (TH and DB) were burned four times in the preceding decade. Slopes faced predominantly one or two directions within a site in 2020; mostly east–northeast in ME, southwest in MW, southeast–south in DB, and a wider range of southwest–southeast at TH.

Ant sampling plots in 2021 were placed to take advantage of TRC's Species-Pool, Fire & Diversity (SPFD) experiment, in which eight 2.5 ha (160 × 160 m) sites were burned April 1–15, 2021, with humidity during burns ranging from 25–52% and windspeeds ranging from 5–14 mph (J. Myers, pers. comm.). These burn sites were previously burned in 2017 (and not prior at least since 1942). Each TRC burn site was paired with eight 2.5 ha (160 × 160 m) unburned sites (control treatment), and each site spanned a drainage valley. Within each burn and control site, two 10 × 10 m ant sampling plots were established, one on each side of the site's drainage valley (Supp. Fig. 1b). Two litter removal plots were placed next to each burn-control site pair, 20–200 m away from SPFD plots and facing opposite slopes. From April 15–May 5, 2021, all leaves down to the duff were removed within the 10 × 10 m area of each litter removal plot using a leaf blower and rake, taking care to scatter leaves at the edges to prevent build-up that could alter microhabitat conditions. In sum, we had 16 plots of each treatment (burn, control, and litter removal), with two plots per treatment at each of eight sites for a total of 48 ant sampling plots (Supp. Fig. 1b).

We quantified seed dispersal in a similar manner in both years. We placed one 4 × 4" index card with 20 seeds of *S. canadensis* ("seed depot") at the center of each ant sampling plot, and one more seed depot 1 m to the left and right of the center (when facing uphill; Supp. Fig. 2). We noted the start time and recorded all ant-seed transport events within the first two hours of seed placement, noting every time an ant took a seed from a depot and then following those ants back to their nests until the end of two hours or until all seeds had been taken from all index cards. When multiple ants transported seeds at the same time, we prioritized following and tracking ants from nests not previously recorded, as determined by morphospecies and direction of transport. We logged a total of 129 observation hours across 76 plots in 2020 (June 18–July 18) and 94 observation hours across 48 plots in 2021 (May 31–June 29). Nests were flagged to return to at the end of two hours, to collect voucher specimens and measure the distance dispersed from the appropriate depots. In 2020, we followed all ants without consideration to the 1 × 3 m plot boundaries. In 2021, we noted when ants crossed the 10 × 10 m plot border, and only continued pursuit outside of plot borders if no other ants were active at that time. In both years, we visually estimated the percent leaf litter cover within a 1 × 1 m quadrat centered at the middle seed depot, ensuring it added up to 100% with all other cover types (rock, soil, stem, wood). Finally, as plots in 2021 were paired across opposite-facing slopes, we used a compass to take the slope aspect facing downhill at the center of each plot to include as a random effect in all models for the TRC dataset.

Following seed dispersal trials each year, we set pitfall traps to determine general abundance of ant workers in our plots. At each plot, we placed one pitfall trap at each corner of a 1 × 1 m square, centered around the middle seed depot (Supp. Fig. 2). Each trap consisted of a 60 mL plastic deli cup dug into the ground until the rim sat flush with the soil surface. After allowing buried traps to settle for at least 3 days with the lids still on, we filled traps with approximately 40 mL of a 1:10 unscented Schnucks brand detergent: water mix. Traps were collected after 24 hours in 2020 (July 25-July 26, 2020), and after 48 hours in 2021 (July 6–8 for sites 1–4 and 13–16, and July 13–15 for sites 5–12), and the catch was rinsed and transferred to vials of 70% ethanol for storage and identification.

All ants collected from seed-dispersal trials and pitfall traps were identified to species level using the keys in Trager (in prep.), Fisher & Cover (2007), MacGown (2014), and AntWiki (2020), with confirmation by J. Trager. As this study only focused on seed dispersal and not ant community responses to fire, we reduced our pitfall trap dataset to only include ants from species which we recorded transporting seeds during each year ("seed-dispersing ants"). To obtain an estimate of body size for each recorded species, we measured Weber's length (WL) on AntWeb specimen photos (Supp. Fig. 3). Using all available profile-view pinned worker specimen photos for a species, we measured WL as the length from the anterior point of pronotum abutting the cervical shield to posterior end of propodeal lobe, and then scaled down to life size by dividing the photo WL by the length of 1 mm on the photo's scale bar. We discarded photos where the anterior of pronotum and posterior propodeal lobe were not visible (due either to importune leg placement or broken specimens), as well as four photos labelled as *A. fulva* that appeared to be misidentified *A. rudis* workers. For *Camponotus,* we only measured minor workers. We took the average from all available photos to obtain one WL value per species (Supp. Table 1).

Statistical Analysis:

Due to differing experimental designs and locations between years, we analyzed data from each year separately. All analyses were conducted using R version 4.2.0 (R Core Team, 2022).

H1: Due to negative effects of fire and litter removal on seed-dispersing ant abundance, ants will transport seeds less frequently in burned (2020, 2021) and litter removal (2021) treatments compared to unburned areas. To test this hypothesis, we first considered whether burning and litter removal treatments reduced seed-dispersing ant abundance. Our outcome variable of seed-dispersing ant abundance, measured as the number of workers of seed dispersing ants found in pitfall traps within a plot, was a count with overdispersion (*check_overdispersion* function in the "performance" package), so we used a negative binomial distribution with a log link function. We ran a separate negative binomial GLMM for each year (*glmmTMB* function with *family = "nbinom2"* in "glmmTMB" package), with treatment (control and burn in 2020; control, burn, and litter removal in 2021) as a fixed effect. For our 2020 model, we used site nested within treatment in 2020 (two sites per treatment), plot nested within site (18–19 plots per site), and ant species as random effects. For 2021, we used site (eight total), plot nested in site (6 plots per site, 2 of each treatment), slope aspect, and ant species as random effects. However, due to singularity (and redundance between plot and slope aspect), we dropped the nested effect of plot in site for the final model. For ease of interpretation of negative binomial GLMM results, we present Incidence Rate Ratios (IRRs), or the ratio of the outcome variable at a given level of the predictor compared to the control, rather than direct estimates. Multiplying the IRR by 100 gives the percent increase in the outcome variable, with an IRR < 1 indicating a negative effect.

We then tested whether the number of seeds transported over two hours per depot differed among treatments, and whether this number was affected by seed-dispersing ant abundance. The number of seeds transported was also a nonnormal count with overdispersion, so again we used a negative binomial error distribution and log link function. For 2020 data, we ran a GLMM with the number of seeds dispersed per depot after two hours as the outcome; treatment (control or burn), total number of seed-dispersing ant workers found in pitfall traps at that plot (ant abundance), and the interaction of treatment and abundance as fixed effects; and site nested within treatment and plot nested within site as random effects. The random effect of site nested within treatment had a singular fit, so to balance Type I error with power and retain only the most maximal model structure supported by the data (Matuschek et al., 2017), we removed the nested variable of site within treatment. For all further models with singular fits, we used the same principle to reduce models to eliminate singularity. For 2021 data, we ran a GLMM with number of seeds dispersed per depot after two hours as the outcome; treatment (control, burn, or litter removal), seed-dispersing ant abundance from pitfall traps, and the interaction of treatment and ant abundance as fixed effects; and site, plot nested within site, and slope aspect as random effects. However, the random effects of site and plot nested within site yielded a singular fit, so we refit the model with only slope as a random effect. Finally, we estimated marginal means for each treatment, based on the model (*emmeans* in package "emmeans").

Finally, to determine whether species-specific responses were driving the seed-dispersing ant community response, we tested whether the number of seeds taken in each treatment was independent of ant species identity. As 13 out of 22 expected values in 2020 and 5 out of 24 expected values in 2021 were <5, in both years we used Fisher's exact test instead of a chi-square test (Kim, 2017) to compare species-specific dispersal amounts among treatments (*fisher.test* in base R with simulated p-value based on 2000 replicates) and post-hoc Fisher comparisons with a Benjamini-Hochberg correction for multiple comparisons (*fisher.multcomp* in the package "RVAideMemoire").

H2: Ants will transport seeds farther in burn (2020, 2021) and litter removal treatments (2021) due to reduced leaf litter. We began by evaluating the effect of treatment on leaf litter cover at the plot level. To test whether the response variable of percent litter cover varied with treatment each year, we used beta-distributed GLMMs with a logit link function (*glmmTMB* with *family = beta_family(link = "logit")*). For 2020, we used treatment (control or burn) as the fixed effect and site (nested within treatment) as a random effect. For 2021, we used treatment (control, burn, or litter removal) as the fixed effect and site and slope aspect as random effects. However, site and slope aspect resulted in singularity, so we removed the random effects of site and slope to fit a beta-distributed GLM instead (*betareg(link = "logit")* in "betareg"). We lost

% litter cover estimates for three plots in 2020 and eight plots in 2021, so we omitted those plots for all analyses requiring % litter cover.

To then test whether leaf litter cover affected dispersal distance, we ran linear mixed models using natural log-transformed dispersal distance (m) as the outcome variable. For 2020 data, we used treatment (control or burn) and percent litter cover (as an integer) as fixed effects, and ant species, site nested within treatment, and plot nested within site as random effects. For 2021 data, we used treatment (control, burn, or litter removal) and percent litter cover (as an integer) as fixed effects, and ant species, site, plot nested within site, and slope aspect as random effects. However, including plot nested in site yielded a singular fit, so we removed the nested variable of plot within site. We back-transformed resulting estimates and 95% confidence intervals to obtain more interpretable response ratios rather than attempting to interpret natural log-transformed responses. In addition to looking at model terms, we performed pairwise comparisons of estimated marginal means for each treatment in both years, with a multivariate *t* distribution correction (*pairs(adjust = 'mvt')* in "emmeans").

To determine species-specific dispersal distance responses to treatment, we ran linear mixed models (function *lmer* in "lmer4") using log-transformed dispersal distance as our response variable, and treatment (control or burn in 2020; control, burn, or litter removal in 2021), ant species with deviation contrasts (to compare each species to the mean of all species' means, the grand mean), and the interaction of treatment and ant species (to detect within-species treatment differences). We expected any interaction between species and treatment to indicate a species-specific effect of treatment on dispersal distance. Our random effects for 2020 were initially site nested within treatment and plot nested within site, but due to singularity, we removed first plot nested within site and then site nested within treatment, such that our final model used only site as a random effect. Our random effects for 2021 were site, plot nested within site, and slope aspect.

Finally, to determine whether ant size affected species-specific responses to treatment, we ran another set of models using natural log-transformed dispersal distance as our outcome variable, with Weber's length, treatment, and their interaction as fixed effects. If Weber's length affected ant response to treatment, we expected to see a significant interaction term indicating that the effect of size on dispersal distance varied with treatment. For our 2020 dataset we used ant species, site nested in treatment, and plot nested in site as random effects, and for our 2021 dataset we used ant species, site, and plot nested within site as random effects.

Results

In 2020, out of a total of 2610 seeds taken at least 5 cm from notecards in 2 hours in 70 plots, we recorded 891 dispersal events (34%). Due to lost data, we dropped three plots from further analysis (all from the burn site ME). The remaining 1719 dispersal events were seeds that disappeared when we were watching other depots, most likely also transported by ants. We followed 725 of those 891 (81.4%) seeds to an ant nest (i.e., without the ant dropping the seed, or without the researcher losing sight of the ant), from which we sampled and identified 1–3 workers per nest to species. In 2021, we observed 915 of 1152 transports across 48 plots, or 79.4%, of which we were able to follow 902 to a nest (as in 2020, not all ants brought the seed to nests nor were we able to track all the rest back to nests). Of the seed-dispersing ants that we followed all the way to the nest in

2021, we were able to collect and identify 884 to species. At SNR in 2020, we found 12 ant species dispersing *S. canadensis* seeds, from 6 genera within three subfamilies (Dolichoderinae: 1 species, Formicinae: 7 species, and Myrmicinae: 4 species; Supp. Table 2). In 2021 at TRC, we found 8 species dispersing *S. canadensis* seeds, from 3 genera within 2 subfamilies (Formicinae: 6 species and Myrmicinae: 2 species; Supp. Table 2).

H1: Due to negative effects of fire and litter removal on seed-dispersing ant abundance, ants will transport seeds less frequently in burned (2020, 2021) and litter removal (2021) treatments compared to unburned areas. Contrary to our expectations, the burn treatment had a marginally significant positive impact on ant abundance in 2020 (IRR = 1.60, p = 0.080, Table 1, Fig. 1a). Additionally, increasing ant abundance was positively correlated with dispersal quantity, with every additional ant increasing the number of seeds dispersed by 3% (IRR = 1.03, p = 0.008; Table 2). The relationship between ant abundance and dispersal quantity was not affected by any interaction of abundance with burn treatment (IRR = 0.99, p = 0.603; Table 2), but burning on its own positively impacted dispersal. Ants took 2.2 times as many seeds in burn plots in two hours compared to control plots, a significant difference (IRR = 2.18 p = 0.006, Table 2, Fig. 2a). Based on estimated marginal means, ants transported 6.9 (SE = 0.903) seeds in control plots versus 13.4 (SE = 1.609) seeds in burned plots.

Unlike our 2020 results, treatment (control, burn, or litter removal) was not a significant predictor of seed dispersing ant abundance in 2021 (Table 1, Fig. 1b), and neither burning nor litter removal had any significant effect on the number of seeds taken after two hours (Table 2, Fig. 2b). Using estimated marginal means, ants transported 8.2

(SE = 1.57) seeds in control plots, 7.8 (SE = 1.38) in burn plots, and 7.7 (SE = 1.41) in litter removal plots. Likewise, neither seed-dispersing ant abundance nor the interaction of seed-dispersing ant abundance and treatment were significant predictors of dispersal quantity (Table 2).

Although we saw an overall increase in the number of seeds dispersed in burn compared to control plots in 2020, only a few ant species accounted for this trend, and a Fisher's exact test revealed how species varied in the number of seeds species taken in burn and control plots (simulated $p < 0.001$; Fig. 3a). *Aphaenogaster rudis* was responsible for the majority (54.5%) of observed dispersal events in 2020, followed by *Formica pallidefulva* (30.3%) and *Myrmica latifrons* (6.3%), all of which dispersed more seeds in burn compared to control plots (Fig. 3a, Supp. Table 3). However, six species showed the opposite trend, specifically *Camponotus castaneus*, *C. chromaiodes, C. pennsylvanicus, F. subsericea*, *Lasius americanus,* and *Tapinoma sessile* (Fig. 3a, Supp. Table 3).

We also found significant differences in the numbers of seeds transported among treatments and among species in 2021 (Fisher's exact simulated $p < 0.001$; Fig. 3b). In 2021, *Aphaenogaster rudis* accounted for the majority (81.8%) of dispersal events, across all treatments. *Camponotus pennsylvanicus, F. pallidefulva,* and *F. subsericea* all took more in burn plots than in control plots, and *Camponotus subbarbatus* transported more seeds in control compared to burn plots (Supp. Table 4, Fig. 3b). Additionally, *A. rudis* took more seeds in litter removal compared to burn plots, but at a lower ratio than *C. americanus,* and an opposite pattern from *C. pennsylvanicus* and *F. pallidefulva*, which both took more in burn than litter removal plots.

H2: Ants will transport seeds farther in burn (2020, 2021) and litter removal treatments (2021) due to reduced leaf litter. Burning and litter removal reduced leaf litter in both years. In 2020, burn treatment significantly decreased percent litter cover by 85 ± 10.8 % (IRR = 0.15, $p = 0.011$; Supp. Table 5). In 2021, the burn treatment significantly decreased percent litter cover by about 87 ± 5.8% (IRR = 0.13, $p < 0.001$), and litter removal significantly decreased percent litter cover by about 96 ± 1.9 % (IRR = 0.04, $p < 0.001$; Supp. Table 5).

In 2020 at SNR, ants took seeds an average of 0.858 ± 0.352 m in burn plots, and an average of 0.509 ± 0.229 m in control plots (Supp. Table 6). Overall, burning had a marginally significant positive effect on dispersal distance (IRR = 1.68, $p = 0.080$; Table 3; Fig. 4a), but percent litter cover did not significantly affect dispersal distance. (Table 3, Supp. Fig. 4a).

In 2021, ants took seeds an average of 0.80 ± 0.289 m in burn plots, 1.86 ± 0.823 m in control plots, and 1.23 ± 0.449 m in litter removal plots (Supp. Table 6). Dispersal distances were only significantly different between burn and control plots (odds ratio = 0.433 ± 0.098, $p = 0.001$; Table 3, Supp. Table 6, Fig. 4b). Percent litter cover did not significantly affect dispersal distance (Table 3, Supp. Fig. 4b).

Dispersal distance varied with ant species in both years. In control plots in 2020, several ant species (*Camponotus chromaiodes:* IRR = 2.28, $p = 0.013$; *C. pennsylvanicus*: IRR = 2.00, $p = 0.069$; and *Formica subsericea*: IRR = 2.86, $p < 0.001$) transported seeds farther than the grand mean (the mean of each species' mean dispersal distance). Also in control plots, *Aphaenogaster rudis* (IRR = 0.51, $p = 0.008$) and *Lasius americanus* (IRR = 0.11, $p = 0.003$) transported seeds shorter distances compared to the grand mean (Table

4). *Camponotus chromaiodes* and *C. pennsylvanicus* dispersal distances were greater than grand mean in burn compared to control plots (burn × *C. chromaiodes* estimate = 4.24, p = 0.051; burn × *C. pennsylvanicus* estimate = 6.62, p = 0.027; Table 4; Supp. Fig. 5a).

In 2021, different ant species took seeds different distances among treatments (Table 4; Supp. Fig. 5b). *Camponotus chromaiodes* (IRR = 9.56, p = 0.001), *C. pennsylvanicus* (IRR = 12.01, p < 0.001), and *Formica pallidefulva* (IRR = 4.87, p < 0.001) transported seeds farther than the grand mean in control plots (Table 4). Significant interaction terms indicated species differed from the grand mean to different extents between control and treatment. For three species, dispersal distance was shorter than the grand mean in the burn treatment compared to control (*Aphaenogaster. rudis* IRR = 0.24, p = 0.001; *C. chromaiodes* IRR = 0.17, p = 0.008; *C. pennsylvanicus* IRR = 0.04, p < 0.001), and three were shorter in the litter removal treatment compared to control (*A. fulva:* IRR = 0.09, p < 0.001; *A. rudis:* IRR = 0.18, p = 0.001; and *C. chromaiodes* IRR = 0.10, p = 0.003; Table 4, Supp. Fig. 5b).

Ant size, as measured by Weber's length, positively impacted dispersal distance in both years, but treatment affected the magnitude of this slope differently in 2020 and 2021. Although every 1 mm increase in Weber's length more than tripled the dispersal distance with all else held equal (2020 IRR = 3.26, p < 0.001; 2021 IRR = 3.25, p = 0.014; Table 5, Fig. 5), this effect was further increased by 33% in burn plots in 2020 (burn × Weber's length IRR = 1.33, p = 0.047; Table 5, Fig. 5a) but decreased by 48% in burn plots in 2021 (burn × Weber's length IRR = 0.52, p = 0.034; Table 5, Fig.5b).

Discussion

In 2020, we found a marginally significant positive effect of burning on seed-dispersing ant abundance and a significant positive effect of burning on the number of seeds dispersed at SNR. Our 2021 results from TRC did not show any effect of burning or litter removal on seed-dispersing ant abundance or the number of seeds dispersed. Thus, our results did not support our first hypothesis that burning and litter removal would reduce seed-dispersing ant abundance and number of seeds dispersed. Furthermore, our 2020 findings run counter to other results from the Ozarks showing lower ant abundances in burned plots compared to controls less than 60 days after burning, after which abundances did not significantly differ between treatments (Verble & Yanoviak, 2013). In contrast, we found increased ant abundances in burned plots when surveying ant communities 104–192 days after burns in 2020 at SNR. Our 2021 results were more similar to those in Verble & Yanoviak (2013), although our sampling at TRC began 46 days after burning that year, still within the timeframe when Verble & Yanoviak found differences in ant communities between treatments. Nonetheless, after sampling from 46–89 days after burning, we found no effect of treatment on ant abundance or number of seeds dispersed in 2021.

Although different from our second field season and other Ozark data (Verble & Yanoviak, 2013), our 2020 dispersal quantity results resemble findings from other parts of the world. Experiments in Australia have shown increased ant-mediated seed dispersal rates in burned areas accompanied by increases in the abundance of seed-dispersing ant species (Beaumont et al., 2011, 2013). Overall, ant abundance also increases in burned areas in Australian temperate forests, particularly driven by the functional groups that include the region's seed-dispersing species (Beaumont et al., 2012). In contrast, our observed lack of burn effect on mymecochory amounts in 2021 is more similar to results from the Brazilian Cerrado, where ant-mediated seed dispersal was resilient to fire-induced changes in vegetation cover and structure metrics (Alcolea et al., 2022).

The greater quantity of seeds transported in burn compared to control plots in 2020 was mainly due to the responses of two common species, *A. rudis* and *F. pallidefulva*, which both carried more seeds in burn plots compared to control plots, unlike most other seed dispersing ants in that dataset. *Aphaenogaster rudis* also paralleled the community trend in 2021, mirroring the overall lack of a treatment effect by transporting similar amounts of seeds between control and burn plots in 2021. This bears resemblance to a common seed-dispersing ant in South Australia, *Rhytidoponera metallica,* which can drive overall community seed dispersal trends through its response (or lack thereof) to burning (Beaumont et al., 2011). *Aphaenogaster rudis* and a complex of morphologically similar species are found throughout mesic deciduous forests in eastern North America (Lubertazzi, 2012; DeMarco & Cognato, 2016), with *Aphaenogaster rudis* being slightly more tolerant of warm temperatures (Warren et al., 2016a) and occupying slightly drier microhabitats within the mesic forest ecosystem (Warren et al., 2012). Thus, the conditions of recently burned forest are likely well within the thermal tolerances of *A. rudis,* explaining its positive or lack of response to prescribed burning and litter removal.

Our second hypothesis, wherein we expected to find greater dispersal distances in burn and litter removal compared to control plots, was also not fully supported. Although burning and litter removal had the hypothesized effect of reducing litter cover in both years, dispersal distance did not respond to reduced litter cover or treatment the way we predicted. In fact, percent litter cover did not significantly affect dispersal distance in either year. Burning increased dispersal distance by an average of 0.35 m compared to control plots in 2020, but reduced dispersal distance by more than one meter (1.06 m) compared to control plots in 2021. We added a manual litter removal treatment in 2021 to better test whether litter cover was driving the differences we saw in 2020 dispersal distances, and expected litter removal and burn plots alike to have longer dispersal distances than control plots. However, dispersal distance in litter removal plots was not significantly different from control or burn treatments.

Dispersal distance has been shown to increase in Australian savanna, where mean dispersal distance was twofold higher in burned plots three weeks after burning compared to dispersal in those areas three weeks prior to burning (Parr et al., 2007). The authors hypothesized this was due to ants traveling farther over ground where leaf litter had been removed by fire (Parr et al., 2007). Indeed, habitat complexity can affect ant foraging behavior, reducing the time to discover bait, but varies with region (Gibb & Parr, 2010). However, we did not see any significant effect of litter cover on dispersal distance. In part, this could be due to abiotic landscape heterogeneity—perhaps non-leaf materials functioned similarly to leaf litter in obstructing ant foraging behavior. Furthermore, the effect of burning on dispersal distance was not consistent between our sampling years, suggesting factors beyond burning might be driving dispersal distances.

Both ant size and species identity helped explain some of the variation in dispersal distance. Unsurprisingly, larger ants carried seeds farther distances, a pattern found in seed-dispersing ants elsewhere in North America (Ness et al., 2004) and worldwide (Gómez & Espadaler, 2013). However, the general positive relationship between Weber's length and dispersal distance was positively impacted by burning in 2020, but negatively impacted by burning in 2021. However, within species, there were also treatment-specific responses. In other studies, increased dispersal distances in burned treatments have been driven by species-specific responses; for example, *Iridomyrmex* sp. 21 in Australia showed a marked increase in dispersal distance from pre- to post-burn sampling (Parr et al., 2007). In our dataset, *Camponotus pennsylvanicus* and *C. chromaiodes,* two of the largest species we observed, both carried seeds farther in burn plots compared to control plots in 2020, but in 2021 both species actually carried seeds farther in control plots than burn plots. We found a similar pattern for *Aphaenogaster rudis*, although the difference in 2020 was not significant for this species. We suspect our sampling method was biased against *Camponotus* and *Formica* spp., which although easier to follow due to their size, were also faster and thus easy to miss and lose track of. Improving dispersal records for these genera could provide more information for the tail of the dispersal curve and add more certainty to our results on the impacts of burning and litter removal on dispersal distance, as both contain larger species capable of traveling long distances.

Our different results between years and locations could also be due to site-specific factors. Land-use legacy can have lasting impacts even as restoration progresses (Brudvig et al., 2021)—although SNR and TRC share some compositional characteristics and regional history (e.g., clearcut logging within the past century and a half; followed by a period of fire suppression), each site within the two properties has its own specific land use history. If site differences are the underlying cause of the increased seed dispersal and ant abundance in burn compared to control plots in 2020 at SNR, however, this could also indicate that general site characteristics are more important to shaping ant communities than the incidence of fire.

Whether our observed increase in dispersal quantity in burn plots is the result of burn effects or site differences, burning did not decrease the number of seeds dispersed in 2020. This suggests a high degree of resilience of seed-dispersing ant communities and dispersal quantity to burning. That is, the community takes very little time (less than a full year) to return to its initial state, with the same main species participating in the same interactions (Holling, 1973; Pimm, 1984). The case for resilience is further bolstered by the lack of detectable burn or litter removal effect on the number of seeds dispersed in 2021 at TRC. Other ant communities and ant-seed dispersal relationships have shown similar degrees of resilience to fire and disturbance, albeit with different responses. For example, in Australian savanna, the overall ant community showed no reduction in abundance or species richness after fire (Parr & Andersen, 2008); and fire in the same system increased seed dispersal amount and distance (Parr et al., 2007). However, our distance results show contrasting patterns in 2020 and 2021, perhaps explained in part by the relatively lower contribution of larger ants with longer foraging distances in 2021 compared to 2020.

Understanding how abiotic and biotic processes respond to burning, including interspecific interactions and seed dispersal, is an important aspect of using fire management for biodiversity conservation (Driscoll et al., 2010). Our findings suggest that ant-mediated seed dispersal quantity is resilient to prescribed burning, but also indicate areas for future study. We addressed the short-term effects of burning on myrmecochory, but the impacts of fire can vary with time scale, and resilience can be affected by changes in the disturbance regime (Johnstone et al., 2016). Separate from short-term effects, burning could also affect myrmecochory in the long term, as decades of fire management can result in increased abundance and diversity of ants in the Missouri Ozarks (Wright, 2013). Additionally, there could be differing effects of burning and interactions of burning and myrmecochory on the plant life stages before and after seed dispersal, which we do not address here (Beaumont et al., 2012; Beaumont et al., 2018). Finally, *Sanguinaria canadensis* is just one of a diverse range of myrmecochores in eastern North America. Plant species identity affects dispersal likelihood as well as post-fire myrmecochory in other ecosystems (Beaumont et al., 2011), and the same is likely to be true in the temperate forests of eastern North America. Thus, myrmecochory of other plant species might respond to fire in a different manner, warranting future study.

Conclusions

We saw different responses of myrmecochory after burning between two locations and field seasons, but no net loss of seed dispersal quantity, suggesting that ant-mediated seed dispersal is resilient to prescribed burning in temperate North America. We also added a litter removal treatment in our second field season, and found no effect of litter removal on dispersal quantity or dispersal distance. We saw opposite impacts of burning on dispersal distance between years, however, and found dispersal distance was largely driven by species-specific variation. The variability in our results between sites and years also indicates that other aspects of habitat could have a larger impact on myrmecochory than prescribed fire, but the long-term effects of repeated burning on myrmecochory in this region remain unknown.

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Tables and Figures

Table 1: Results of negative binomial (log link) GLMMs on seed-dispersing ant abundance in 2020 at Shaw Nature Reserve and 2021 at Tyson Research Center.

	Ant abundance, 2020 $\theta = 2.55$			**Ant abundance, 2021** $\theta = 3.34$		
Predictors	*IRR*	*CI*	*p*	*IRR*	*CI*	*p*
(Intercept)	2.06	1.14, 3.74	**0.017**	3.23	1.76, 5.93	**<0.001**
Treatment [Burn]	1.60	0.95, 2.71	0.080	1.06	0.72, 1.54	0.775
Treatment [Litter removal]	--	--	--	1.18	0.82, 1.71	0.372
Random Effects						
σ^2	0.57			0.46		
τ_{00}	0.57 (Ant species)			0.41 (Ant species)		
	0.05 (Site, within Treatment)			0.09 (Site)		
	0.02 (Plot, within Site)			0.09 (Slope aspect)		
ICC	0.53			0.56		
N	12 (Ant species)			8 (Ant species)		
	2 (Treatment)			8 (Site)		
	4 (Site)			42 (Slope aspect)		
	63 (Plot)					
Observations	223			148		
Marginal R^2 / Conditional R^2	0.042 / 0.548			0.005 / 0.564		

Note: Incidence Rate Ratios (IRR) represent ratio of response variable in a level of treatment compared to control, such that an IRR of 1.51 indicates a 51% increase in ant abundance in burn plots compared to controls, whereas an IRR below 1 indicates a decrease. θ denotes the negative binomial dispersion parameter. Bold indicates $p \leq 0.05$.

Table 2: Effects of negative binomial (log link) GLMMs testing effects of treatment (relative to control) and seed-dispersing ant abundance on number of seeds taken from seed depots after 2 hours in 2020 at Shaw Nature Reserve and 2021 at Tyson Research Center.

	No. seeds dispersed, 2020 $\theta = 5.16$			**No. seeds dispersed, 2021** $\theta = 1.40$		
Predictors	*IRR*	*CI*	*p*	*IRR*	*CI*	*p*
(Intercept)	4.49	3.11, 6.47	**<0.001**	5.55	3.07, 10.03	**<0.001**
Ant abundance	1.03	1.01, 1.04	**0.008**	1.02	0.99, 1.04	0.158
Treatment [Burn]	2.18	1.26, 3.79	**0.006**	0.81	0.35, 1.87	0.616
Treatment [Litter removal]	--	--	--	1.23	0.51, 3.00	0.642
Burn × Ant abundance	0.99	0.97, 1.02	0.603	1.01	0.98, 1.04	0.667
Litter removal × Ant abundance	--	--	--	0.99	0.96, 1.02	0.470
Random Effects						
σ^2	0.26			0.61		
τ_{00}	0.36 (Plot, within Site)			0.18 (Slope aspect)		
ICC	0.57			0.23		
N	2 (Treatment)			3 (Treatment)		
	4 (Site)			8 (Site)		
	63 (Plot)			42 (Slope aspect)		
Observations	189			138		
Marginal R^2 / Conditional R^2	0.289 / 0.697			0.085 / 2.93		

Note: θ denotes the negative binomial dispersion parameter. Bold indicates $p \leq 0.05$.

Table 3: Results of LMMs using dispersal distance (m) as an outcome variable in 2020 at Shaw Nature Reserve and 2021 at Tyson Research Center. Estimates and confidence intervals are exponentiated from the natural log.

	Dispersal distance (m), 2020			**Dispersal distance (m), 2021**		
Predictors	*IRR*	*CI*	*p*	*IRR*	*CI*	*p*
(Intercept)	0.47	0.16, 1.38	0.169	2.25	0.73, 6.93	0.156
Treatment [Burn]	1.68	1.68, 3.02	**0.080**	0.43	0.28, 0.66	**<0.001**
Treatment [Litter removal]	--	--	--	0.66	0.27, 1.61	0.363
% Litter cover	1.00	0.99, 1.01	0.728	0.99	0.98, 1.00	0.305
Random Effects						
σ^2	0.30			0.24		
τ_{00}	1.55 (Ant species)			0.54 (Slope aspect)		
	0.23 (Plot, within Site)			0.49 (Ant species)		
	0.04 (Site, within Treatment)			0.13 (Site)		
ICC	0.86			0.83		
N	12 (Ant species)			31 (Slope aspect)		
	51 (Plot)			8 (Ant species)		
	4 (Site			8 (Site)		
Observations	725			721		
Marginal R^2 / Conditional R^2	0.021 / 0.861			0.055 / 0.838		

Note: Bold indicates $p \leq 0.05$.

Table 4: Results of LMMs using dispersal distance (m) as an outcome variable, and treatment (compared to control), ant species (compared to the grand mean), and the interaction of treatment and species as fixed effects for 2020 at Shaw Nature Reserve and 2021 at Tyson Research Center. Estimates and confidence intervals are exponentiated from the natural log.

	Dispersal distance (m), 2020			**Dispersal distance (m), 2021**		
Predictors	*IRR*	*CI*	*p*	*IRR*	*CI*	*p*
(Intercept)	0.54	0.28, 1.07	0.078	0.41	0.16, 1.04	0.061
Treatment [Burn]	0.98	0.23, 4.16	0.982	2.26	0.94, 5.45	0.070
Treatment [Litter removal]	--	--	--	5.57	1.85, 16.81	**0.002**
Aphaenogaster fulva	1.11	0.39, 3.18	0.840	2.95	0.95, 9.15	0.062
A. lamellidens	2.08	0.63, 6.90	0.230	--	--	--
A. rudis	0.51	0.31, 0.84	**0.008**	1.25	0.50, 3.08	0.635
Camponotus americanus	--	--	--	0.43	0.28, 0.65	**<0.001**
C. castaneus	2.76	0.80, 9.55	0.108	--	--	--
C. chromaiodes	2.28	1.19, 4.36	**0.013**	9.56	2.62, 34.94	**0.001**
C. pennsylvanicus	2.00	0.95, 4.20	0.069	12.01	3.82, 37.75	**<0.001**
C. subbarbatus	1.61	0.47, 5.57	0.451	1.83	0.65, 5.18	0.254
Formica pallidefulva	1.45	0.88, 2.38	0.147	4.87	2.74, 8.67	**<0.001**
F. subsericea	2.86	1.61, 5.06	**<0.001**	--	--	--
Lasius americanus	0.11	0.03, 0.49	**0.003**	--	--	--
Myrmica latifrons	0.93	0.39, 2.21	0.868	--	--	--
Treatment [Burn] × *A. fulva*	--	--	--	0.37	0.11, 1.26	0.113
Treatment [Litter removal] × *A. fulva*	--	--	--	0.09	0.02, 0.33	**<0.001**
Treatment [Burn] × *A. rudis*	1.85	0.51, 6.66	0.349	0.24	0.10, 0.57	**0.001**

Treatment [Litter removal] × *A. rudis*	--	--	--	0.18	0.06, 0.53	**0.002**
Treatment [Burn] × *C. americanus*	--	--	--	2.08	0.68, 6.38	0.200
Treatment [Burn] × *C. castaneus*	0.65	0.07, 5.81	0.703	--	--	--
Treatment [Burn] × *C. chromaiodes*	4.25	0.99, 18.23	0.051	0.17	0.05, 0.63	**0.008**
Treatment [Litter removal] × *C. chromaiodes*	--	--	--	0.10	0.02, 0.45	**0.003**
Treatment [Burn] × *C. pennsylvanicus*	6.62	1.24, 35.38	**0.027**	0.04	0.01, 0.13	**<0.001**
Treatment [Litter removal] × *C. pennsylvanicus*	--	--	--	1.17	0.25, 5.46	0.844
Treatment [Burn] × *C. subbarbatus*	--	--	--	0.12	0.00, 17.28	0.401
Treatment [Burn] × *F. pallidefulva*	2.37	0.65, 8.59	0.189	--	--	--
Treatment [Burn] × *F. subsericea*	0.53	0.13, 2.11	0.368	--	--	--
Random Effects						
σ^2	0.41			0.24		
τ_{00}	0.12 (Site)			0.30 (Slope aspect)		
				0.08 (Site)		
ICC	0.22			0.62		
N	4 (Site)			8 (Site)		
				38 (Slope aspect)		
Observations	725			884		
Marginal R^2 / Conditional R^2	0.527 / 0.633			0.452 / 0.790		

Note: Bold indicates p ≤ 0.05.

Table 5: Results of LMMs using dispersal distance (m) as an outcome variable, and treatment (compared to control), ant size (Weber's length, mm), and the interaction of treatment and size as fixed effects for 2020 at Shaw Nature Reserve and 2021 at Tyson Research Center. Estimates and confidence intervals are exponentiated from the natural log.

	Dispersal distance (m), 2020			**Dispersal distance (m), 2021**		
Predictors	*IRR*	*CI*	*p*	*IRR*	*CI*	*p*
(Intercept)	3.79	1.07, 13.36	**0.038**	10.17	1.07, 96.63	**0.043**
Treatment [Burn]	0.88	0.41, 1.90	0.742	1.65	0.55, 4.99	0.373
Treatment [Litter removal]	--	--	--	2.53	0.77, 8.39	0.128
Weber's length (mm)	3.26	1.95, 5.44	**<0.001**	3.25	1.27, 8.30	**0.014**
Treatment [Burn] × Weber's length (mm)	1.33	1.00, 1.77	**0.047**	0.52	0.28, 0.95	**0.034**
Treatment [Litter removal] × Weber's length (mm)	--	--	--	0.59	0.31, 1.11	0.100
Random Effects						
σ^2	0.30			0.24		
τ_{00}	0.23 (Plot, within Site)			0.57 (Slope aspect)		
	0.40 (Ant species)			0.13 (Site)		
	0.04 (Site, within Treatment)			0.39 (Ant species)		
ICC	0.69			0.82		
N	12 (Ant species)			8 (Ant species)		
	2 (Treatment)			8 (Site)		
	4 (Site)			31 (Slope aspect)		
	51 (Plot)					
Observations	725			721		
Marginal R^2 / Conditional R^2	0.284 / 0.778			0.100 / 0.838		

Note: Bold indicates $p \leq 0.05$.

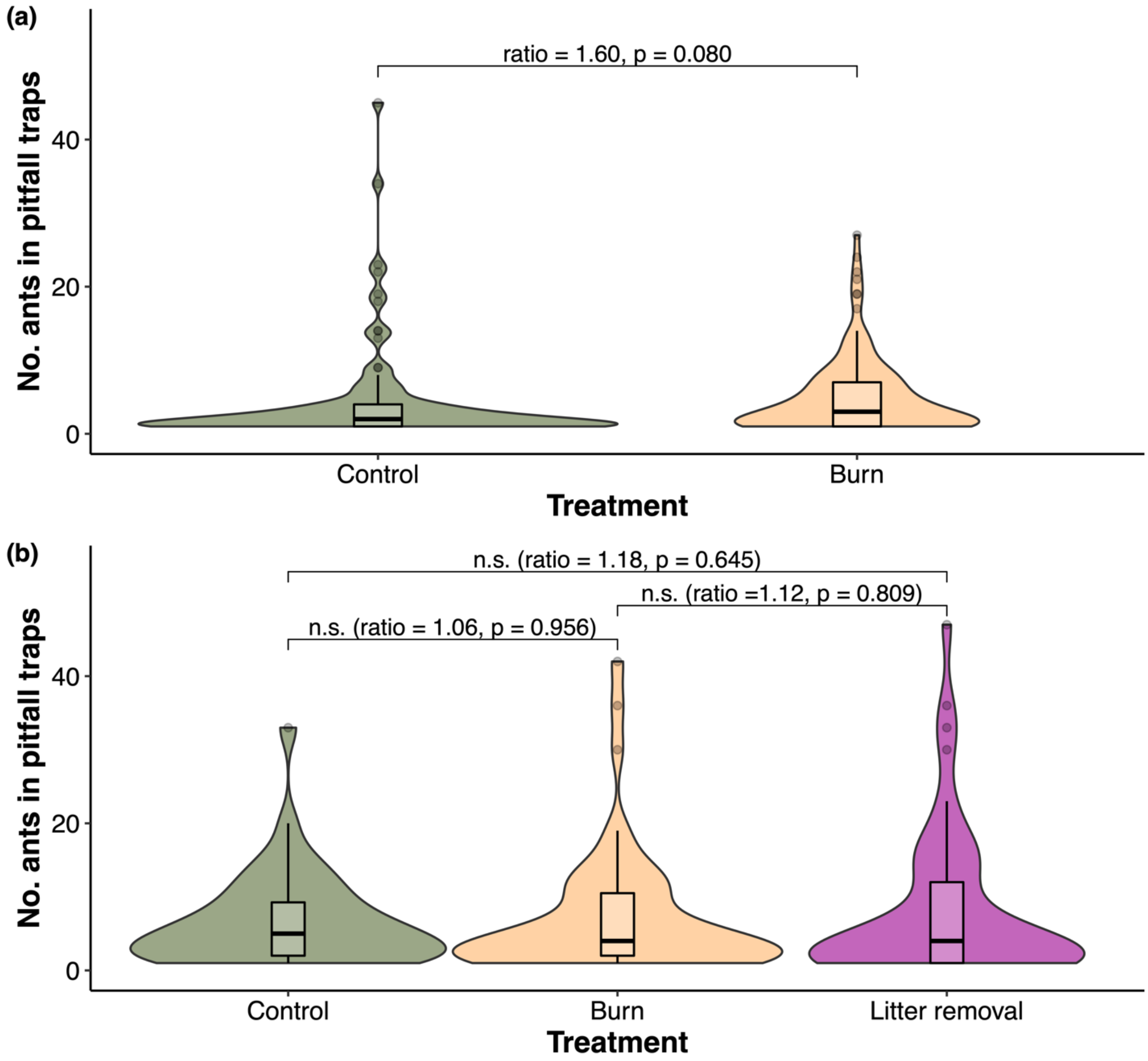


Figure 1: Seed-dispersing ant worker abundance in pitfall traps per plot in (a) 2020 at Shaw Nature Reserve and (b) 2021 at Tyson Research Center. Violin plots reflect the density distribution of values, and boxplots represent medians (black lines), first and third quartiles (hinges), 1.5 × the interquartile range (whiskers), and outliers (dots). Estimates and p-values are from post-hoc pairwise Tukey tests. Ratios indicated are exponentiated from log estimates, and should be interpreted as the right side of the bracket compared to the left.

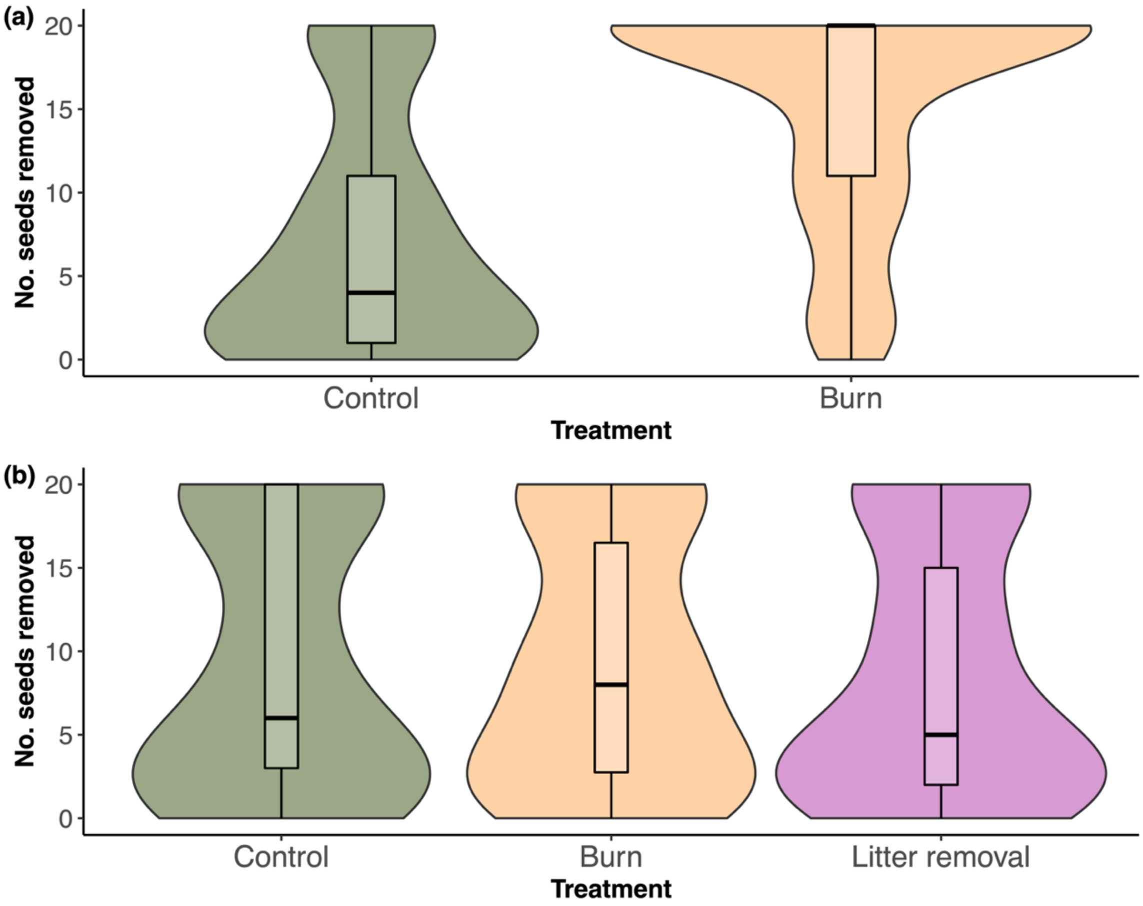


Figure 2: Kernel density plots of the number of *S. canadensis* seeds taken (out of 20 per depot) by ants over the span of 2 hours, by treatment (a) at Shaw Nature Reserve in 2020 and (b) at Tyson Research Center in 2021. Boxplots inside violin plots represent median (thick horizontal line), first and third quartiles (thinner horizontal lines), 1.5 × interquartile ranges (vertical lines), and outliers (dots). More than twice (2.18) as many seeds were taken from depots at burn plots compared to control plots in 2020, a significant difference ($p = 0.006$) based on our negative binomial GLMM. There was no significant difference among treatments for the number of seeds transported per depot in 2021.

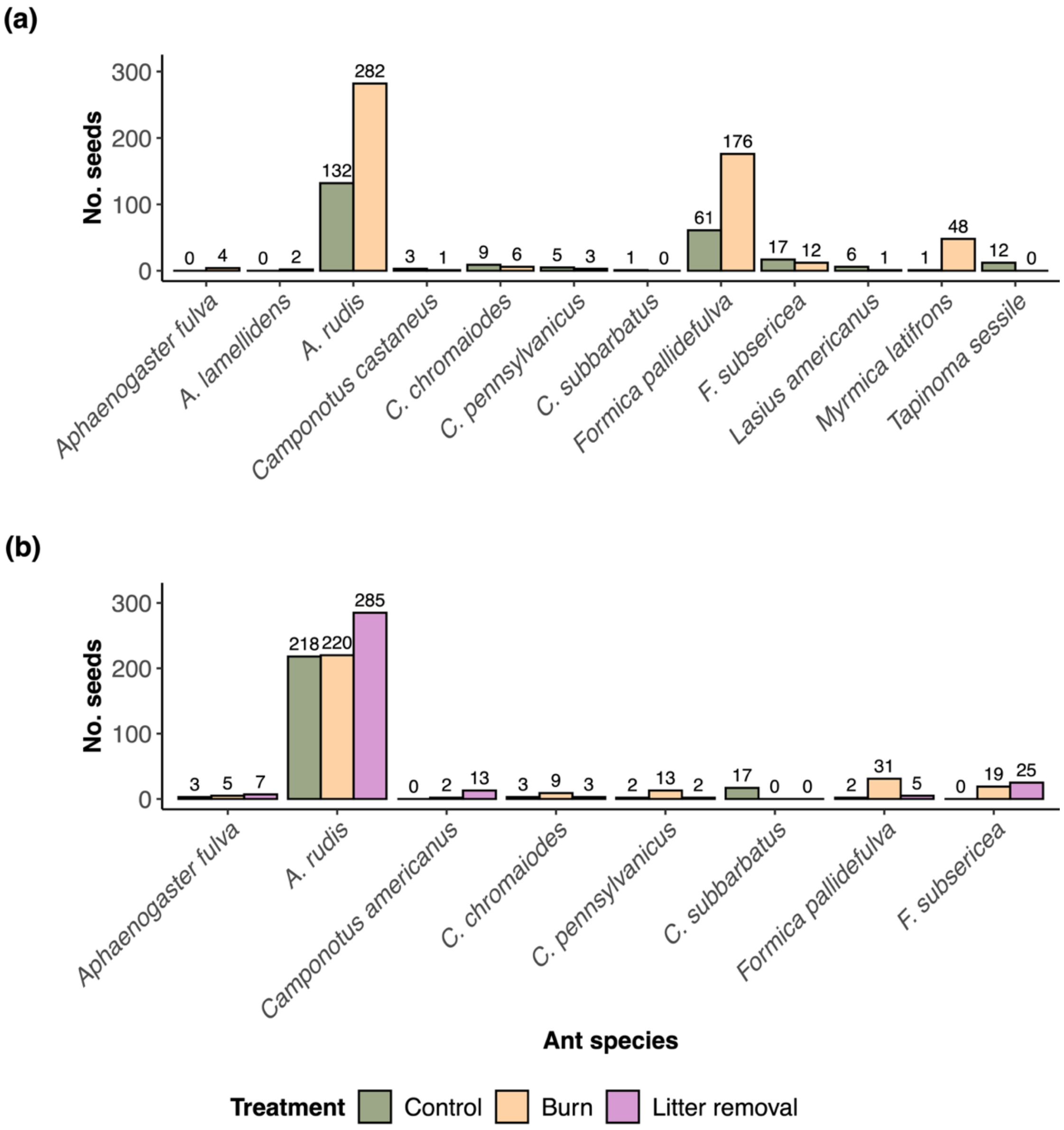


Figure 3: Total number of seeds dispersed across all plots, by ant species and treatment, in (a) 2020 at Shaw Nature Reserve and (b) in 2021 at Tyson Research Center. Numbers atop bars represent number of seeds transported by a species in a given treatment.

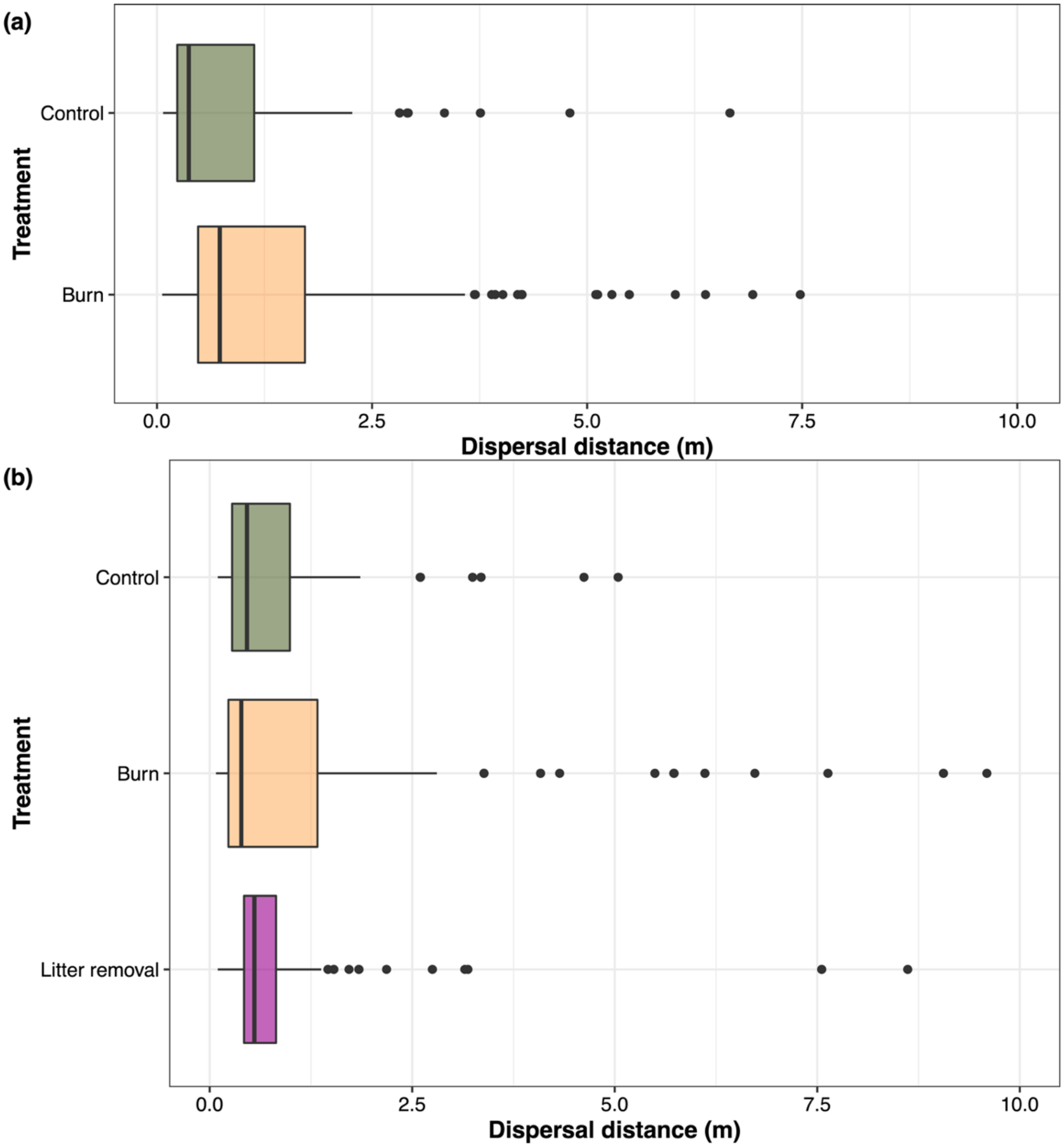


Figure 4: Seed dispersal distance by treatment from (a) 2020 at Shaw Nature Reserve and (b) 2021 at Tyson Research Center. Boxplots show medians (black lines), first and third quartiles (hinges), 1.5 × the interquartile range (whiskers), and outliers (dots). Note the truncated x-axis omits in (a) one outlier at 17.3 m in the burn treatment, and in (b) two outliers at 24.5 and 25.2 m in the litter removal treatment.

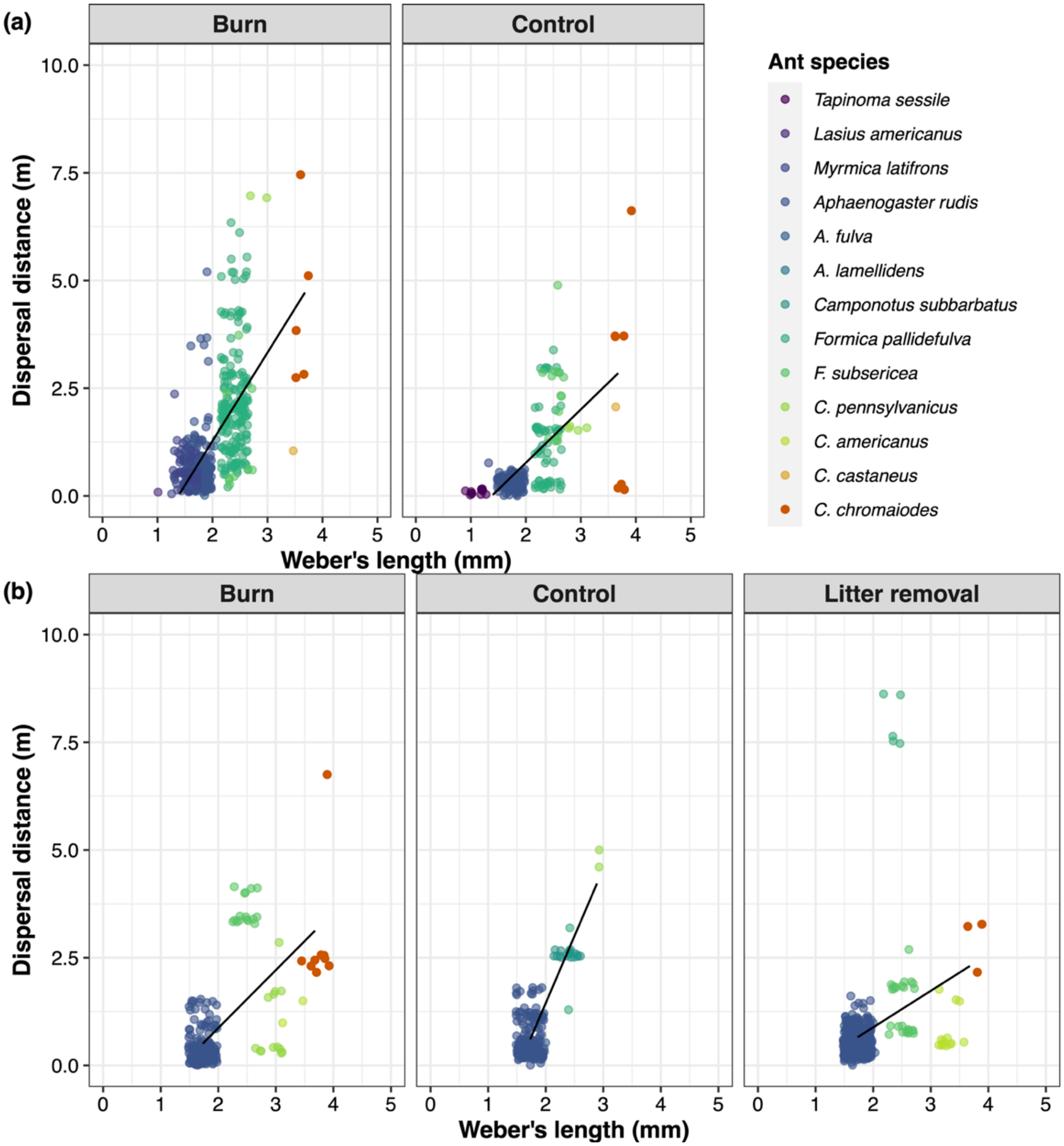


Figure 5: Seed dispersal distance by ant species and treatment, with x axis scaled by Weber's length, for (a) 2020 at Shaw Nature Reserve and (b) 2021 at Tyson Research Center. Each point is a dispersal event, and points are horizontally jittered.

Supplementary Materials

Supp. Table 1: Average Weber's length and number of AntWeb specimens measured.

Ant species	Weber's length (mm)	Number photos measured
Aphaenogaster fulva	1.85	6
A. lamellidens	1.98	7
A. rudis	1.73	5
Camponotus americanus	3.35	5
C. castaneus	3.45	5
C. chromaiodes	3.68	1
C. pennsylvanicus	2.89	3
C. subbarbatus	2.39	4
Formica pallidefulva	2.40	6
F. subsericea	2.50	4
Lasius americanus	1.25	5
Myrmica latifrons	1.50	4
Tapinoma sessile	1.03	8

Note: To obtain WL measures, we used all profile-view pinned worker specimen photos available for each species via AntWeb, measuring on-screen WL (distance from anterior point of pronotum abutting cervical shield to posterior end of propodeal lobe; *a*) an on-screen 1 mm scale bar length (*b*), dividing *a* by *b*, and taking the average from all available photos. We discarded photos where the anterior of pronotum and posterior propodeal lobe were not visible (due either to importune leg placement or broken specimens), as well as four photos labelled *A. fulva* that appeared to be misidentified *A. rudis* workers. For *Camponotus,* we only measured minor workers.

Supp. Table 2: Ants observed dispersing *Sanguinaria canadensis* diaspores during seed-dispersal trials in 2020 at Shaw Nature Reserve and 2021 at Tyson Research Center.

Ant species	**Subfamily**	**No. seeds dispersed in 2020**	**No. seeds dispersed in 2021**
Aphaenogaster fulva Roger, 1863	Myrmicinae	4	15
A. lamellidens Mayr, 1886	Myrmicinae	2	0
A. rudis Enzmann, J., 1947	Myrmicinae	414	723
Camponotus americanus Mayr, 1862	Formicinae	0	15
C. castaneus (Latreille, 1802)	Formicinae	4	0
C. chromaiodes Bolton, 1995	Formicinae	15	15
C. pennsylvanicus (De Geer, 1773)	Formicinae	8	17
C. subbarbatus Emery, 1893	Formicinae	1	17
Formica pallidefulva Latreille, 1802	Formicinae	237	38
F. subsericea Say, 1836	Formicinae	29	44
Lasius americanus Emery, 1893	Formicinae	7	0
Myrmica latifrons Stärcke, 1927	Myrmicinae	49	0
Tapinoma sessile (Say, 1836)	Dolichoderinae	12	0

Supp. Table 3: Benjamini-Hochberg-corrected p-values from multiple comparison Fisher's exact tests between species from dispersal quantities in burn and control plots from 2020 at Shaw Nature Reserve.

Burn: Control	*A. ful*	*A. lam*	*A. rud*	*C. cas*	*C. chr*	*C. pen*	*C. sub*	*F. pal*	*F. sub*	*L. ame*	*M. lat*	*T. ses*
A. ful												
A. lam	1.000											
A. rud	0.529	1.000										
C. cas	0.286	0.587	0.223									
C. chr	0.197	0.378	0.125	1.000								
C. pen	0.196	0.638	0.529	1.000	1.000							
C. sub	0.377	0.537	0.231	1.000	1.000	1.000						
F. pal	0.790	1.000	**0.030**	0.148	**0.030**	0.109	0.453					
F. sub	0.125	0.377	**0.028**	1.000	1.000	1.000	1.000	**0.004**				
L. ame	*0.053*	0.196	**<0.001**	1.000	0.550	0.790	1.000	**0.010**	0.573			
M.lat	1.000	1.000	**<0.001**	**0.004**	**<0.001**	**0.001**	0.120	**<0.001**	**<0.001**	**<0.001**		
T. ses	**0.004**	**0.040**	**<0.001**	0.446	0.066	0.130	1.000	**<0.001**	0.032	0.566	**<0.001**	

Note: Only the first three letters of the specific epithet are used for space; see Supp. Table 2 for full names. Bold indicates $p \leq 0.05$.

Supp. Table 4: Benjamini-Hochberg-corrected p-values from multiple comparison Fisher's exact tests between species from dispersal quantities from 2021, subdivided by treatment comparison.

Burn:Control	
Species pair	*p*
Aphaenogaster fulva:A. rudis	9.656e-01
A. fulva:Camponotus americanus	1.000e+00
A. fulva:C. chromaiodes	8.846e-01
A. fulva:C. pennsylvanicus	5.089e-01
A. fulva:C. subbarbatus	**4.427e-03**
A. fulva:Formica pallidefulva	*9.674e-02*
A. fulva:F. subsericea	*5.361e-02*
A. rudis:C. americanus	8.217e-01
A. rudis:C. chromaiodes	2.639e-01
A. rudis:C. pennsylvanicus	**2.202e-02**
A. rudis:C. subbarbatus	**9.103e-05**
A. rudis:F. pallidefulva	**3.851e-06**
A. rudis:F. subsericea	**2.786e-05**
C. americanus:C. chromaiodes	1.000e+00
C. americanus:C. pennsylvanicus	1.000e+00
C. americanus:C. subbarbatus	**1.965e-02**
C. americanus:F. pallidefulva	1.000e+00
C. americanus:F. subsericea	1.000e+00
C. chromaiodes:C. pennsylvanicus	8.792e-01
C. chromaiodes:C. subbarbatus	**1.419e-04**
C. chromaiodes:F. pallidefulva	2.110e-01
C. chromaiodes:F. subsericea	1.054e-01
C. pennsylvanicus:C. subbarbatus	**4.232e-06**
C. pennsylvanicus:F. pallidefulva	8.706e-01
C. pennsylvanicus:F. subsericea	3.345e-01
C. subbarbatus:F. pallidefulva	**7.293e-10**
C. subbarbatus:F. subsericea	**3.257e-09**
F. pallidefulva:F. subsericea	8.355e-01
Burn:Litter removal	
A. fulva:A. rudis	1.000e+00
A. fulva:C. americanus	3.345e-01
A. fulva:C. chromaiodes	3.741e-01
A. fulva:C. pennsylvanicus	*9.121e-02*
A. fulva:C. subbarbatus	1.000e+00
A. fulva:F. pallidefulva	**1.727e-02**
A. fulva:F. subsericea	1.000e+00
A. rudis:C. americanus	*7.787e-02*
A. rudis:C. chromaiodes	*9.382e-02*
A. rudis:C. pennsylvanicus	**4.427e-03**
A. rudis:C. subbarbatus	1.000e+00
A. rudis:F. pallidefulva	**5.244e-06**
A. rudis:F. subsericea	1.000e+00

C. americanus:C. chromaiodes	**7.813e-03**
C. americanus:C. pennsylvanicus	**7.616e-04**
C. americanus:C. subbarbatus	1.000e+00
C. americanus:F. pallidefulva	**1.354e-05**
C. americanus:F. subsericea	1.249e-01
C. chromaiodes:C. pennsylvanicus	8.792e-01
C. chromaiodes:C. subbarbatus	1.000e+00
C. chromaiodes:F. pallidefulva	6.617e-01
C. chromaiodes:F. subsericea	2.021e-01
C. pennsylvanicus:C. subbarbatus	1.000e+00
C. pennsylvanicus:F. pallidefulva	1.000e+00
C. pennsylvanicus:F. subsericea	**1.965e-02**
C. subbarbatus:F. pallidefulva	1.000e+00
C. subbarbatus:F. subsericea	1.000e+00
F. pallidefulva:F. subsericea	**4.701e-04**
Control:Litter removal	
A. fulva:A. rudis	8.355e-01
A. fulva:C. americanus	1.388e-01
A. fulva:C. chromaiodes	8.786e-01
A. fulva:C. pennsylvanicus	8.706e-01
A. fulva:C. subbarbatus	**7.567e-04**
A. fulva:F. pallidefulva	1.000e+00
A. fulva:F. subsericea	*5.311e-02*
A. rudis:C. americanus	**4.334e-03**
A. rudis:C. chromaiodes	1.000e+00
A. rudis:C. pennsylvanicus	1.000e+00
A. rudis:C. subbarbatus	**1.034e-05**
A. rudis:F. pallidefulva	9.541e-01
A. rudis:F. subsericea	**1.800e-05**
C. americanus:C. chromaiodes	*5.593e-02*
C. americanus:C. pennsylvanicus	*9.752e-02*
C. americanus:C. subbarbatus	**1.754e-07**
C. americanus:F. pallidefulva	2.110e-01
C. americanus:F. subsericea	1.000e+00
C. chromaiodes:C. pennsylvanicus	1.000e+00
C. chromaiodes:C. subbarbatus	**3.513e-02**
C. chromaiodes:F. pallidefulva	8.725e-01
C. chromaiodes:F. subsericea	**1.699e-02**
C. pennsylvanicus:C. subbarbatus	*7.500e-02*
C. pennsylvanicus:F. pallidefulva	8.706e-01
C. pennsylvanicus:F. subsericea	**4.433e-02**
C. subbarbatus:F. pallidefulva	**2.441e-03**
C. subbarbatus:F. subsericea	**3.298e-10**
F. pallidefulva:F. subsericea	*9.674e-02*

Note: Bold indicates $p \leq 0.05$.

Supp. Table 5: Beta-distributed models (with a logit link function) showing the effects of treatment on percent litter cover in 2020 at Shaw Nature Reserve (GLMM) and in 2021 at Tyson Research Center (GLM).

	% Litter cover, 2020 $\theta = 9.42$			**% Litter cover, 2021** $\theta = 2.85$		
Predictors	*IRR*	*CI*	*p*	*IRR*	*CI*	*p*
(Intercept)	4.93	1.77, 13.75	**0.002**	5.22	2.76, 9.90	**<0.001**
Treatment [Burn]	0.15	0.03, 0.62	**0.009**	0.13	0.05, 0.31	**<0.001**
Treatment [Litter removal]	--	--	--	0.04	0.02, 0.10	**<0.001**
Random Effects						
σ^2	-0.05					
τ_{00}	0.51 (Site, within Treatment)					
ICC	1.10					
N	2 (Treatment) 4 (Site)					
Observations	70			40		
Marginal R^2 / Conditional R^2	0.667/ 1.033			0.698		

Note: Bold indicates $p \leq 0.05$.

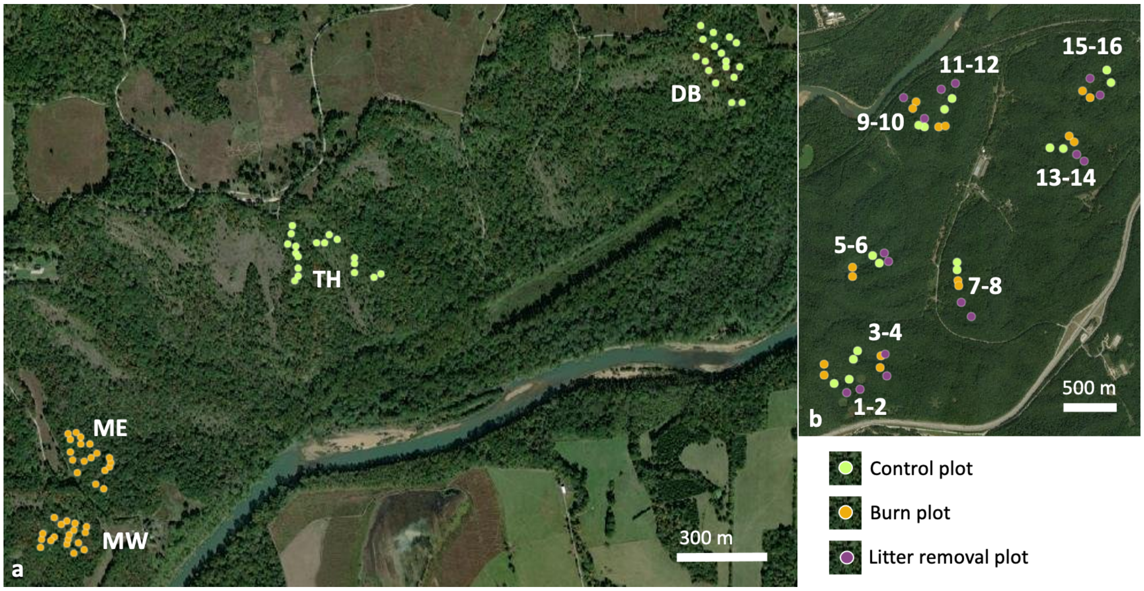
Supp. Table 6: Summary table of descriptive statistics, estimated marginal means (Est. Marg. Mean), and comparisons of estimated marginal means from LMMs using dispersal distance (m) as an outcome variable at Shaw Nature Reserve in 2020 and Tyson Research Center in 2021. Full model results can be found in Table 3. Estimates and SEs are exponentiated from the natural log.

	Dispersal distance (m), 2020					**Dispersal distance (m), 2021**				
Treatment	*Est. Marg. Mean*	*SE*	*df*	*Median*	*Max.*	*Est. Mar. Mean*	*SE*	*df*	*Median*	*Max.*
Burn	0.858	0.352	11.3	0.730	17.3	0.80	0.289	17.9	0.390	6.73
Control	0.509	0.229	12.9	0.370	6.66	1.86	0.823	26.2	0.499	5.04
Litter removal	--	--	--	--	--	1.23	0.449	19.1	0.552	25.2
Comparison	*Odds ratio*	*SE*	*df*	*t-ratio*	*p*	*Odds ratio*	*SE*	*df*	*t-ratio*	*p*
Burn v. control	1.68	0.569	2.13	1.543	0.255	0.433	0.098	98.5	-3.692	**0.001**
Litter removal v. control	--	--	--	--	--	0.663	0.315	35.6	-0.866	0.665
Burn v. litter removal	--	--	--	--	--	0.652	0.218	36.2	-1.276	0.419

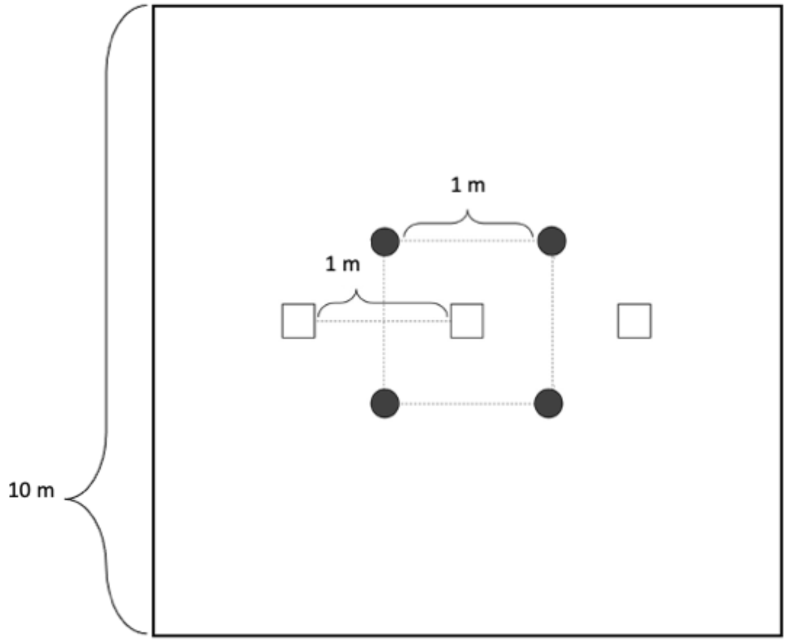
Supp. Table 7: Results of negative binomial GLMMs on seed-dispersing ant abundance in 2020 at Shaw Nature Reserve and 2021 at Tyson Research Center.

	Ant abundance, 2020 $\theta = 2.66$			**Ant abundance, 2021** $\theta = 3.52$		
Predictors	*IRR*	*CI*	*p*	*IRR*	*CI*	*p*
(Intercept)	7.61	2.33, 24.90	**0.001**	4.32	0.60, 31.33	0.147
Treatment [Burn]	1.56	0.59, 4.16	0.374	2.36	0.86, 6.48	0.095
Weber length (mm)	0.56	0.34, 0.92	**0.022**	0.90	0.44, 1.84	0.778
Treatment [Burn] * Weber length (mm)	1.03	0.69, 1.52	0.901	0.73	0.50, 1.06	0.102
Treatment [Litter removal]	--	--	--	3.39	1.24, 9.28	**0.018**
Treatment [Litter removal] * Weber's length (mm)	--	--	--	0.66	0.46, 0.97	**0.032**
Random Effects						
σ^2	0.57			0.45		
τ_{00}	0.33 (Ant species)			0.33 (Ant species)		
	0.05 (Site, within Treatment)			0.07 (Site)		
	0.02 (Plot, within Site)			0.09 (Plot, within Site)		
ICC	0.41			0.52		
N	12 (Ant species)			8 (Ant species)		
	2 (Treatment)			8 (Site)		
	4 (Site)			46 (Plot)		
	63 (Plot)					
Observations	223			148		
Marginal R^2 / Conditional R^2	0.176 / 0.516			0.092 / 0.567		

Note: Bold indicates $p \leq 0.05$.

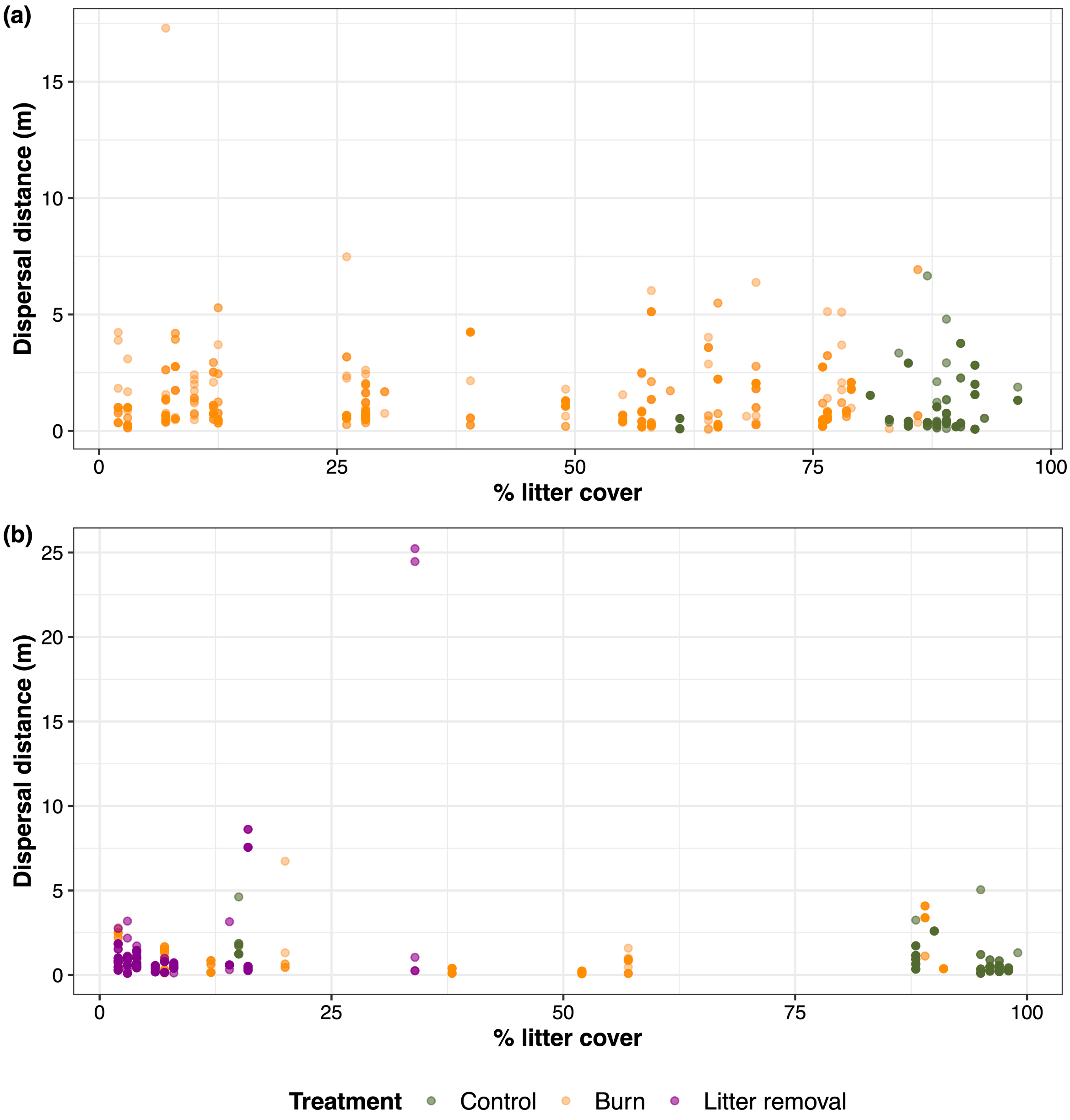


Supp. Fig. 1: At (a) Shaw Nature Reserve in 2020, we had two burn sites (ME and MW) with 18 plots each, and two control sites (TH and DB) with 18 and 19 plots each, and at (b) Tyson Research Center in 2021, we had eight sites (denoted by numbers nearest a cluster of six plots) with two burn, two control, and two litter removal plots each.

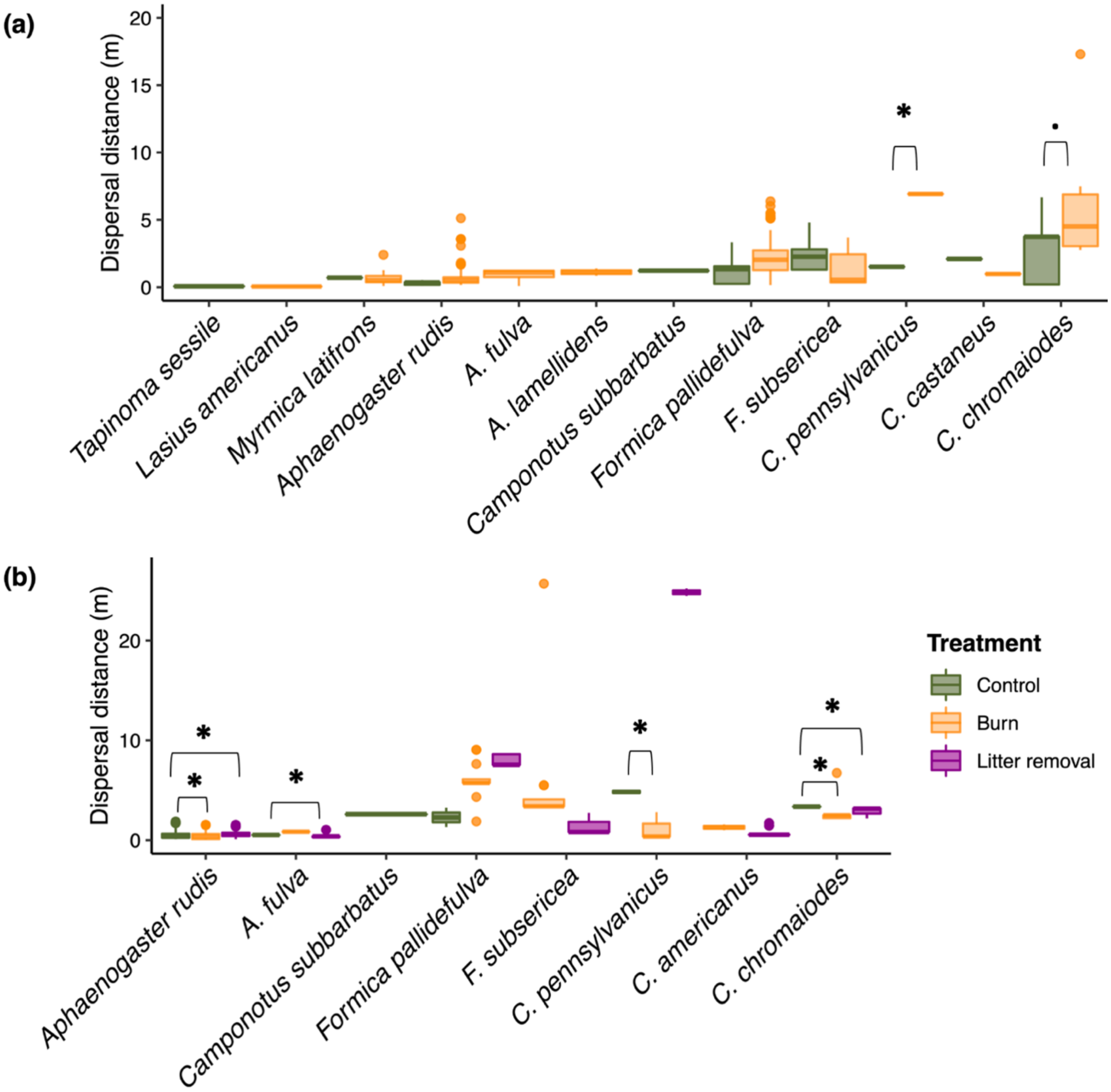


Supp. Fig. 2: Conceptual diagram of seed dispersal plot setup. At the center of a 10 x 10 m square, one seed depot (smallest square in diagram) was placed, and one meter to each side of the central depot, a second and third depot were placed. The line formed by these depots ran parallel to the contour of the slope, such that an observer could watch all three depots by standing 1 m downslope of the center depot. After dispersal trials (see text), we returned to the dispersal plot center points and set up four pitfall traps (black circles) in a 1 x 1 m square, with two traps upslope of the line where seed depots had been, and two traps downslope. Plot borders were 10 x 10 m, but we followed ants outside the borders both years.

Supp. Fig. 3: Modified photo of *Aphaenogaster fulva* demonstrating how we used AntWeb photos to measure Weber's lengths (from the anterior edge of the propodeum to the posterior edge of mesonotum; white line on photo) and compare to the provided scale. Original image from April Noble (CASENT0103585 from www.antweb.org).



Supp. Fig. 4: Dispersal distance by treatment and percent litter cover (a) in 2020 at Shaw Nature Reserve and (b) 2021 at Tyson Research Center. Note slightly different y-axes between (a) 2020 and (b) 2021 due to different maximum dispersal distances (17.3 m in 2020, 25.7 in 2021).



Supp. Fig. 5: Species-specific dispersal distance responses to treatment in (a) 2020 at Shaw Nature Reserve and (b) 2021 at Tyson Research Center. Ant species are ordered by size, with labels for significant interaction terms (for details, see Table 4); * indicates $p < 0.05$, • indicates $p < 0.10$.

Chapter 3: Ground-foraging and litter ant resilience to prescribed burning and litter removal in the Missouri Ozarks

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Abstract

Understanding how fire affects biodiversity is crucial for land management in eastern North America. To better understand the effects of prescribed burning on an ecologically important insect group, we sampled ant communities in the Missouri Ozarks at one location in 2020 using three rounds of pitfall traps (April, July, and September), and at another location in 2021 using one round (July) of pitfall and Berlese samples. Each year, we used sites burned that year and control sites not burned that year. We hypothesized ant abundance, species richness, and diversity would be negatively impacted by fire but would recover with time since burning in 2020. We further hypothesized that the negative effects of burning on ant abundance and alpha diversity would be due to reduced leaf litter. To test this latter hypothesis, we added a physical litter removal treatment in 2021. Finally, we expected ant community composition to shift toward more open-habitat species in burn and litter removal treatments compared to controls. Across both years, we found 44 species from 20 genera. In 2020 pitfall traps, ant abundance peaked in July, but was higher in burn compared to control treatments in September. In 2021 pitfall traps, we found more ants in litter removal compared to control treatments, but found the opposite pattern in 2021 Berlese samples. Ant species richness was not different among treatments in either year, or among sampling rounds (April, July, or September) in 2020. However, in 2020, July control plots had the highest Shannon and inverse Simpson values. Litter removal had higher Shannon and inverse Simpson diversity than burn and control in 2021 Berlese samples, likely a product of reduced litter volume. Ant composition varied among treatments in pitfall samples from 2020 and Berlese samples from 2021, but not among treatments in 2021 pitfall samples. Pitfall traps and Berlese litter samples were compositionally different from each other. No species was associated with the same treatment in both years. Overall, our results suggest that in the short term (< 1 year), ant communities are resilient to or positively impacted by prescribed burning in eastern North America.

Key words: *Ant community; indicator species; prescribed fire; litter removal; Berlese sampling; biodiversity; temperate forests; Formicidae*

Introduction

Ants (Formicidae) have been used in different parts of the globe as indicators of ecosystem recovery and resilience after disturbance and restoration (Underwood & Fisher, 2006; Casimiro et al., 2019). Ant community diversity and composition are relatively easy to measure, and often show similar responses to disturbance as other invertebrates, soil microbial biomass, and birds (Andersen & Majer, 2004; Majer et al., 2007a; Peters et al., 2016). Ants are routinely used as indicators of ecosystem recovery after mining and other disturbances in Australia (Majer et al., 2007b), and have also shown promise as indicators of disturbance in North American grasslands (Peters et al., 2016) and of ecosystem response to restoration in both tropical and temperate regions (Casimiro et al., 2019). In addition to providing information on specific ecosystem responses to a disturbance, a good indicator group or species should respond to the same disturbance predictably across sites (Andersen & Majer, 2004). However, ants do not always meet these requirements, and have proven to be unreliable indicators in some contexts and habitats (Calcaterra et al., 2014), and thus require an initial assessment before they can be used as indicators in different habitats. In particular, few studies have considered whether ants could reliably serve as indicators of fire impact in eastern North American forests and woodlands (but see Verble & Yanoviak, 2013), where fire is a common tool for land management and ecological restoration.

Eastern North American oak-hickory forests are adapted to a frequent, low-intensity fire regime (Abrams 1992; Nowacki & Abrams, 2008), which was maintained in part by Indigenous use of fire until European settlement (Abrams 1992, Guyette et al., 2002; Stambaugh et al., 2015). Subsequent genocide and displacement of Indigenous peoples alongside changes in human populations and land use disrupted fire regimes, and the introduction of national fire suppression policies in the early 1900s led to the incursion of more mesic forest plants (Nowacki & Abrams, 2008; Vose & Elliott, 2016). To reverse this “mesophication” and promote open understory conditions and regeneration of economically valuable oak trees, public and private land managers throughout the eastern United States are returning to the use of fire as a management and restoration tool (Brose et al., 2001; Ryan et al., 2013; Brose, 2014). As such, understanding the impacts of prescribed burning on biodiversity and ecologically important groups is a crucial aspect of land management, monitoring, and conservation.

The response of ant communities to fire depends on habitat and timeframe, and can include both direct (mortality from combustion and heat) and indirect (food resource fluxes, habitat alteration) effects (Certini et al., 2021). A global meta-analysis of the effects of fire on ant communities found that fire tended to decrease ant diversity in forests but not in deserts, grasslands, or savannas (Vasconcelos et al., 2017). However, such responses are not always apparent in more fire-adapted ecosystems such as oak-pine forests (Coleman & Rieske, 2006). Andersen (2019) posited that ant community responses to fire are largely mediated by resilience and adaptation for changes in habitat and microclimate, particularly openness. Citing ant community responses to fire in multiple ecosystems, Andersen (2019) hypothesized that fire and other disturbances would have less negative impacts in open and fire-adapted habitats such as grasslands and woodlands, compared to more closed-canopy habitats with less frequent fire regimes. For example, in South African savannas, pitfall-sampled ant communities showed no significant difference in species richness and abundance between burned and unburned plots (Parr et al., 2004). In Australian savannas and open woodlands, ant species richness increased with fire frequency (Vanderwoude et al., 1997), although not all functional groups responded positively to fire. However, a study in the Siskiyou Mountains of Oregon and California demonstrated that although ants in open fens had higher species richness in burned than unburned fens, burned forests had lower species richness than unburned forests (Sanders et al., 2007). Collectively, these studies suggest resilience or even positive responses of ant communities to fire in open habitats, whereas fire in closed forest habitats can have more negative effects.

Nonetheless, the impact of habitat openness on ant response to disturbance may depend upon how openness is defined. A more recent study from Greece suggested openness at the ground level may more relevant than canopy openness for predicting ground-foraging ant community responses (Bishop et al., 2021). From the point of view of a ground-foraging ant, perhaps the most immediate type of openness is the litter layer, which can be drastically reduced by fire. In the Missouri Ozarks, prescribed burns can consume the entire litter layer (Kolaks et al., 2004), a loss that can take two years to reaccumulate just half of pre-burn amounts (Stambaugh et al., 2006). Removing the temperature buffer of leaf litter, whether by burning or physical litter removal, exposes soils to a wider range of temperatures (Pearse, 1943), and could potentially reduce options for shelter and nests. These effects on leaf litter amount could operate in conjunction with or in addition to the other physical, chemical, and heat-related effects of burning (Bond & Keeley, 2005; Kral et al., 2017; McLauchlan et al., 2020).

In temperate North American ecosystems, ant communities show variable responses to burning. In North American grasslands, burn responses are often driven by dominant ants (Moranz et al., 2013; Bonoan & McCarthy, 2022) and specific functional groups (Izhaki et al., 2003; Bonoan & McCarthy, 2022). However, less is known about ant responses in oak forests. In Arkansas Ozark forests, ant community abundance declined immediately after burning (Verble-Pearson & Yanoviak, 2014), and burned communities continued to show lower ant abundance and species richness through multiple sampling methods (Berlese funnels, pitfall sampling, and baiting) 30–60 days after burning (Verble & Yanoviak, 2013). However, Verble & Yanoviak (2013) stopped data collection in July, leaving unknown whether fire effects could resurge later, when the heat of summer might exacerbate any exposure from litter removal. The timing of sampling relative to burning is important both in the short term and in the long term. A longer-term study in the Missouri section of the Ozarks found that annual burning over six decades increased ant abundance in pitfall traps, and less frequent (every four years) burning over the same sixty-year period increased Shannon diversity (i.e., diversity weighted by relative abundance; Wright, 2013).

Fire can also change ant community composition, which generally takes longer to return to pre-disturbance levels than species richness (Dunn 2004). In South African savanna woodlands, community composition was significantly different between burn treatments, although species-specific responses varied with location (Parr et al., 2004). In Australian savanna, burning every 1–2 years increased the prevalence of two functional groups associated with open habitats, Dominant Dolichoderinae and Hot-Climate Specialists (Vanderwoude et al., 1997). In North American sandplain forests burned 8–10 years prior to pitfall sampling, the widespread forest species *Aphaenogaster rudis* and *Myrmica punctiventris* were more abundant in burn compared to control (not burned in "recent times") areas, and species of *Camponotus* (particularly *C. pennsylvanicus*) and *Formica* were only found at burn sites (Banschbach & Ogilvy, 2014). Another *Aphaenogaster* species, *A. carolinensis,* along with other mesic species such as *Strumigenys clypeata* and *Stenamma* spp., were present in control plots but absent from burned areas in the Ozarks when sampled in the growing season following burning (Verble & Yanoviak, 2013). Although these suggest a trend toward more open and generalist species in burned areas compared with more mesic and forest species in control areas, such patterns are not always the case in North American forests. For example, a study from Georgia forests found that fire had little effect on ant community composition (Graham et al., 2008). However, the authors suggested the lack of compositional response could be due to their use of pitfall traps instead of litter sampling (Graham et al., 2008). Pitfall and litter sampling methods each target a different subset of the ant community (ground-foraging versus litter-dwelling ants), and so using only pitfall traps could leave out the response of cryptic species (small species with little activity outside the soil or litter layers and thus little interaction with other ant species, *sensu* Andersen, 1995) that are more easily found in litter samples (Martelli et al., 2004).

With this study, we aimed to understand patterns in the responses of the ant community and individual ant species to prescribed fire and leaf litter removal in Missouri oak woodlands. We also wished to determine whether the ant community or individual ant species show consistent responses to fire, and could thus be used as indicators of the short-term (< 1 year) impacts of fire in the region. We studied ground-foraging ant communities in two locations in eastern Missouri where prescribed burns are used for land management and experimental purposes. We hypothesized that ant abundance, species richness, and diversity would be negatively impacted by fire but would recover with time since burning within the year (H1). To test H1, we compared ant abundance, species richness, and diversity across three rounds (April, July, and September) of pitfall trapping in burned and control plots at Shaw Nature Reserve in 2020. The following year, we used a burn experiment at Tyson Research Center to further test how burning affected ant communities, and added a physical litter removal treatment to test whether the reduction of leaf litter was driving the effect of fire. If fire's effects were primarily mediated by the reduction of the litter layer, we expected burning and litter removal treatments to show similar impacts on ants. Specifically, we expected ant abundance, species richness, and diversity to be negatively impacted by burning and litter removal compared to control plots, due to reduced leaf litter (H2). We also expected ant community composition to shift toward more open-habitat species in burned and litter removal compared to control plots (H3).

Methods

Study locations and land use history: In the northern extent of the Ozark Mountains in Missouri, we used forests in two different study locations: in 2020, Shaw Nature Reserve (SNR; (38°28'09.8" N, 90°49'15.8" W) in Franklin County, Missouri, and in 2021, Tyson Research Center (TRC; 38°31'35.8" N, 90°33'41.8" W) in St. Louis County, Missouri. Shaw Nature Reserve is a 988-ha private reserve owned by Missouri Botanical Garden, and contains prairie, stream, glade, and woodland habitats managed for public use and ecological restoration. At SNR, prescribed burning is used as a restoration tool, with various sections of the reserve subjected to burning every few years to control invasive species and maintain habitat. Twenty-four kilometers east of SNR, TRC is an 800-ha forested landscape owned by Washington University in St. Louis. At TRC, 16 2.5 ha (160 × 160 m) experimental forest plots were set up in 2016 as part of the Species-Pool, Fire & Diversity (SPFD) project, eight of which were burned in 2017 and 2021, and eight of which serve as controls. The 2021 burns took place April 1–15 (relative humidity 25–52%, windspeed 5–14 mph, J. Myers, pers. comm.; Sup. Fig. 1a), and together with the 2017 burns were the first fires at TRC in recent history after the land was selectively logged and grazed in the early 1900s (Zimmermann & Wagner 1979; J. Myers pers. comm.).

In 2020 at SNR, we established 73 1 × 1 m pitfall trap plots across four SNR sites, including two sites burned that year as part of SNR's fire management program ("burn" treatment) and two sites that were not burned that year ("control" treatment). Burn treatments were administered on January 7 (Morton West, MW: 52 ha, relative humidity 39–51%, windspeed 22–24 mph) and March 6 (Morton East, ME: 78 ha, relative humidity 29%, surface windspeed 13 mph) in 2020 (C. Maginel, pers. comm.). The two control sites were not burned within the two years prior to sampling, with their most recent burns taking place March 2018 (Dana Brown, DB: 117 ha) and November 2017 (Trail House, TH: 82 ha). Including the 2020 burns, MW was burned twice in the preceding decade, and ME, TH, and DB were burned four times in the preceding decade. At each site, 18 (MW, ME, and TH) and 19 (DB) 1 × 1 m pitfall trapping plots were placed with at least 20 m spacing (Fig. 1a).

In 2021 at TRC, we placed 48 10 × 10 m ant sampling plots of three treatments across 8 sites (Fig. 1b). Each "site" consisted of two ant-sampling plots placed within an SPFD burn plot, two ant-sampling plots placed within an SPFD control plot, and two 10

× 10 m litter removal plots within 200 m of the site's SPFD plots. As with burn and control plots, litter removal plots were placed to approximate opposing slope aspects within a site (Fig. 1b). From April 15–May 5, 2021, we used a leaf blower and rake to remove all leaves down to the duff. We ensured leaves were scattered at the edges, rather than mounded, to prevent build-up that could alter microhabitat conditions. We placed two 10 × 10 m ant-sampling plots in each SPFD plot, one on each side of the plot's drainage valley but avoiding ridge tops and valley bottoms, as well as one in each litter removal plot, yielding 16 ant-sampling plots per treatment (burn, control, and litter removal), a total of 48 ant sampling plots (Fig. 1b). As each plot within a treatment at a site was paired with a plot on an opposite-facing slope, we measured slope aspect as a covariate by noting the compass direction when facing directly downhill from the center of each plot.

Pitfall traps are simple to operate, catch ants active during both the day and night, and sample fast-moving species that are harder to capture in litter-based sampling methods (Andersen, 1991; Lopes & Vasconcelos, 2008; Vargas et al., 2009). In contrast, litter sampling methods that include post-sample processing such as Berlese funnels can be biased toward mymicine and ponerine species active during the day, but can catch smaller, more cryptic species that do not wander into pitfall traps (Martelli et al., 2004). Due to logistical and COVID-19 pandemic-related constraints, we used only pitfall traps our first year of sampling (2020 at SNR), but used both pitfall and Berlese litter sampling methods in 2021 (TRC) to gain a more thorough sample of ant diversity.

Pitfall traps: At the center of each ant-sampling plot, we placed one pitfall trap at each corner of a 1 × 1 m square (Supp. Fig. 2). We used 60-mL plastic deli cups as our pitfall traps, which we dug into the ground until the rim sat flush with the soil surface. We allowed buried traps to settle for at least 3 days with the lids still on, and then removed the lids and filled traps with approximately 40 mL of a 1:10 unscented Schnucks brand detergent: water mix. At SNR in 2020, we conducted three rounds of pitfall sampling, leaving the traps open for 24 hours before collection: Round 1, April 15–16 (traps set 39 and 98 days since burning); Round 2, July 25–26 (140 and 199 days since burning); and Round 3, September 22–23 (199 and 258 days since burning). Between Round 1 and Round 2, we conducted seed dispersal trials at the same plots (see Ch. 2 for details). In 2021 at TRC, we left traps open for 48 hours for logistical reasons, and split our eight sites into two bouts of pitfall setting and trapping, sampling four sites July 6–8, and the other four July 13–15. Additionally, we used rain covers at TRC, created by cutting white plastic cups into three- or four-legged covers and secured by poking sewing pins into the ground (Supp. Fig. 1b). Similarly to SNR, we conducted seed dispersal trials at all TRC plots in the month prior to pitfall sampling (Ch. 2). In both years, after collecting pitfall traps, we rinsed the contents of each trap over fine fabric gauze, removed debris by hand, and transferred ants and bycatch to vials of 70% ethanol for storage and identification.

Berlese funnels: We collected leaf litter samples for Berlese extraction from each plot at TRC from July 2–9, 2021 (78–99 days after burning and 58–85 days after litter removal), sampling two sites at a time due to logistical constraints. At each corner of a 10 x 10 m ant sampling plot, we collected all leaf litter (including woody debris) down to the duff within a 0.5 × 0.5 m frame of PVC piping and elbow joints. We combined these four 0.5 × 0.5 samples from a single plot for further processing; Berlese samples were thus standardized by area, with the volume of litter from a total area of 1 × 1 m collected per plot. We used cloth canvas sifting bags (Supp. Fig. 1c) to agitate litter samples and dislodge arthropods on-site, and then poured the siftate (fine material that made it past the mesh) into drawstring cotton bags with cardstock labels and retained the remaining leaf matter (coarse litter) to dry and weigh in brown paper grocery bags. We combined all siftate from a plot into one litter sample per plot, which we then poured into Berlese funnels. Berlese funnels consisted of a metal funnel with a 1 cm mesh wire plate preventing litter from falling through, and a cup of ethanol (70%) below the funnel opening. Funnel and collection cup were centered in a 2-gallon bucket, and covered with an incandescent lightbulb and domed metal lid (Supp. Fig. 1d). In some control samples where litter cover was particularly thick, we divided the siftate between two Berlese funnels to avoid siftate touching the light bulb. We transferred collection cup contents into Falcon tubes for transport after 48 hours, and poured all remaining siftate into paper bags for drying and weighing. We poured the contents of each Falcon tube into a weigh boat, and used forceps to transfer ants to smaller vials of 70% EtOH for storage and identification.

All ants collected from pitfall traps and Berlese-processed litter samples were identified to species level using the keys in Trager (in prep.), Fisher & Cover (2007), MacGown (2014), and AntWiki (2020), with confirmation by J. Trager. We also measured litter cover on August 19, 2020 at SNR, and May 31–June 29, 2021 at TRC, at each plot within each treatment with the exception of missing data from eight plots in 2021 (four burn and four control plots). To measure litter cover, we visually assessed the percent litter cover within the 1 × 1 m quadrat framed by pitfall traps. In 2021, we also used sifted coarse litter as an additional measure of treatment effect on litter. Specifically, we placed paper bags with coarse litter into a 55–60°C drying oven until litter was dry to touch (at least two days). We then sorted the coarse litter into leafy and woody matter, considering petioles as leaf matter but twigs and acorns as woody, and took the mass of dried, sorted leaf matter.

Statistical analyses: All statistical analyses and data visualizations were performed using R version 4.2.0 (R Core Team, 2022). We analyzed data from each year (2020 and 2021) separately due to differences in study design and location, and further separated 2021 data by sampling method (pitfall traps and Berlese-processed litter samples), as pitfall traps and sifted litter samples are known to differ in ant species composition (Martelli et al., 2004).

H1: Ant abundance, species richness, and diversity will be negatively impacted by fire but will recover with time since burning.

Although the exact time since burning to each sampling round depended on site, we treated sampling round as an ordered categorical variable (in temporal order of April, July, and September) to allow for seasonal shifts in ant activity and community composition. To measure the effect of burning and sampling round on ant abundance, we used total ant worker abundance per trap as our outcome variable. We used a generalized linear mixed model (GLMM) with a negative binomial distribution and a log link function (*glmmTMB* with family = nbinom2 in package "glmmTMB") to allow for overdispersion of our outcome variable, ant abundance (a count variable). We used treatment (burn compared to control), sampling round (Round 1, 2, or 3), and the interaction of treatment and sampling round as fixed effects, and site nested within treatment as a random effect on intercept, and plot nested in site as a random effect on intercept and slope, to allow the effect of time on ant abundance to vary among plots (based on explanations of random effects in Zuur et al., 2009). We expected a significant interaction term (between treatment and round) to indicate that the effect of treatment varied with sampling round. We calculated differences between treatments and among rounds by comparing estimated marginal means (*emmeans* and *pairs* in package “emmeans”).

When considering species diversity, we first considered sample coverage (*sensu* Chao et al., 2020), an estimate of sample completeness based on the proportion of observed individuals compared to the number of observed and undetected individuals. To test for differences in species richness and diversity, we compared the interpolated and extrapolated Hill numbers within each treatment and sampling round (Chao et al., 2014). Hill numbers are estimates of the effective number of species as designated by the parameter q, an order designating how much weight to give rare species when calculating diversity (Hill, 1973, Chao et al., 2014). When $q = 0$, rare species are given the same weight as common ones and the Hill number is equal to species richness. When $q = 1$, species are weighted by their relative abundance and is equivalent to exponentiated Shannon entropy, and when $q = 2$, the most weight is given to the numerically dominant species (by taking the arithmetic mean of relative abundances) and is equivalent to inverse Simpson diversity (Hill, 1973; Jost, 2006). To assess how species richness and diversity varied with treatment and round, we calculated individual-based interpolation and extrapolation curves for burn and control treatments within each sampling round, based on each order of q (package “iNEXT”; Hsieh et al., 2020). We considered the curves to be significantly different when 95% confidence intervals did not overlap.

Finally, as a potential mechanism of overall treatment effects, we ran a beta-binomial (logit link) GLMM with percent litter cover from 2020 as the outcome variable and treatment (control or burn) as a fixed effect, with site nested in treatment as a random effect.

H2: Ant abundance, species richness, and diversity will be negatively impacted by burning and litter removal compared to control plots, due to reduced leaf litter.

To test this hypothesis, we used two datasets collected from TRC in 2021: pitfall trap samples and Berlese-processed litter samples. We kept pitfall and Berlese data separate due to differences in sampling method and replication. For pitfall trap data, we considered each trap as a replicate nested within a plot, but Berlese litter samples had one sample per plot.

We first evaluated the effect of treatment on leaf litter amount by running a beta-binomial (logit link) GLMM with percent litter cover from 2021 as the outcome variable and treatment (control, burn, or litter removal) as a fixed effect. We used site as a random effect, but this gave a singular fit, so we dropped the random effect of site from the final model and used a GLM with a beta-binomial (logit link) distribution and treatment as a fixed effect. We also ran a model using coarse litter mass (g) as the outcome variable and treatment as a fixed effect. As litter mass was highly right-skewed, we used a Gamma (link = log) error distribution. We used site as a random effect, this yielded a singular fit so we dropped it from the final model, reducing to a Gamma GLM.

To measure the effects of burning and litter removal on ant abundance in 2021, we ran negative binomial (log link) GLMMs with total ant worker abundance per replicate as the outcome variable. We used treatment (burn and litter removal compared to control) as a fixed effect in each model. As random effects, we used site and plot nested in site for the 2021 pitfall sample model, and only site for the 2021 Berlese litter samples. However, including site as a random effect in the model for 2021 Berlese litter samples yielded a singular fit, so we dropped the random effect of site to obtain a non-singular fit. This reduced our model of ant abundance in 2021 Berlese litter samples to a GLM, with only one fixed effect.

Just as we had for H1, we compared species richness and diversity between treatments within each 2021 dataset (pitfall traps and Berlese litter samples) using interpolated and extrapolated Hill numbers: diversity weighted by rare species (species richness, order q = 0), common species (inverse Simpson diversity, q = 2) and a mix of both (exponentiated Shannon entropy, q = 1; Hill 1973, Jost 2006). Separately for pitfall trap samples and Berlese litter samples, we calculated individual-based interpolation and extrapolation curves for burn, control, and litter removal treatments (package “iNEXT”; Hsieh et al., 2020). If 95% confidence intervals did not overlap, we did considered curves to be significantly different.

H3: Ant community composition will shift toward more open-habitat species in burned and litter removal compared to control plots.

To fully determine whether the entire ant community or specific species could serve as indicators of fire impact and recovery, we looked for differences in overall both ant community composition and species-specific responses. To determine compositional differences in ordination space between treatments but within each dataset (2020 pitfall samples, 2021 pitfall samples, and 2021 Berlese samples), we used separate non-metric multidimensional scaling (NMDS; function *metaMDS* in package “vegan”) based on Bray-Curtis distances with Wisconsin double-standardization by species and sites (Anderson et al., 2006) for each dataset (each pitfall round in 2020, pitfall samples from 2021, and Berlese samples in 2021). Wisconsin double-standardization treats abundance data prior to calculating Bray-Curtis distances by standardizing values by the sum of abundance in a plot and within a species (Anderson et al., 2006). For 2021 Berlese samples, we also square-root transformed our abundance matrix due to high species abundances (Supp. Table 1), and resolved near-zero stress in 2021 pitfall trap ordination by adding a dummy species with an abundance of one in each plot (Clarke et al., 2006). For each dataset, we chose dimensions by using scree plots to evaluate the change in stress using 1–7 dimensions (*dimcheckMDS* in “goeveg” package) and choosing the number of dimensions beyond which our reduction in stress would be small. When scree plot results were ambiguous, we cross-referenced our selected dimension choice with a stress plot showing observed dissimilarity in relation to ordination distance (*stressplot* in “vegan”), and evaluated whether changing dimensions reduced scatter from the trend. Final dimensions and stress levels can be found in Supp. Table 1.

Community composition can differ in both location of centroids and dispersion away from centroids, but using a combination of permutational dispersion tests (PERMDISP) and permutational multivariate analysis of variance (PERMANOVA) procedures can differentiate between the two (Anderson, 2006; Anderson & Walsh, 2013). We used PERMANOVA (*adonis2* in “vegan”) to determine whether ant community centroids differed among treatments, using Bray-Curtis distances and 999 permutations. We then assessed whether our ant communities had homogenous variances (similar levels of dispersion from centroids) among treatments using PERMDISP (*betadisper* and *permutest* with 999 permutations, “vegan” package).

To examine species-specific responses to treatment and determine which, if any, species were associated with control, burn, or litter removal treatments, we used Indicator Species Analysis (ISA), which calculates the square root of the Indicator Value index (IndVal), which uses a species’ abundance and frequency of occurrence to determine group associations (Dufrêne & Legendre, 1997). Specifically, the IndVal is a compound index that calculates a species’ association with designated groups (in this case, treatments) by multiplying A_{ij}, the mean abundance of a species *i* in plots of a certain treatment *j* divided by its abundance in all plots, and B_{ij}, the number of plots of *j* at which species *i* occurs divided by the total possible number of plots of treatment *j* (Dufrêne & Legendre, 1997; Borcard et al., 2011). Indicator Species Analysis involves running permutational tests for statistical significance of relationships between species and treatments, requiring separate comparisons for each species (De Cáceres & Legendre, 2009); as such, we used a Benjamini-Hochberg correction to reduce the false discovery rate (Benjamini & Hochberg, 1995). Although ISA can help determine which species are more associated with a certain treatment and whether certain treatments are more likely to contain certain species, an ecologically useful indicator would be consistently associated with the same treatment across different sites (Bakker, 2008). Thus, we ran ISA (function *multipatt* in package “indicspecies”) separately for each dataset (2020 pitfall traps, 2021 pitfall traps, and 2021 Berlese litter samples), but compared results of burn and control associations between 2020 and 2021 datasets to see if any species were consistently associated with a certain treatment type.

Finally, to evaluate whether compositional shifts were due to habitat preferences, we searched the literature for habitat records of our observed species. We ran separate Google Scholar searches for each species' name and synonyms with the word "habitat", sorted through results for papers that presented information on collection or observation of species in different habitat types, and then checked the AntWiki page for each species to search for additional habitat references to do the same with. From the references we found, we noted whether each species was recorded in xeric, dry, mesic, or hydric habitat as well as open, forest, or some combination. We then used these habitat records to evaluate whether indicator species associated with different treatments had habitat preferences for dry and open or mesic and forested habitat. References for habitat preferences can be found in Supp. Table 2.

Results

Across all three sampling rounds in 2020 at SNR, we found 34 species of ants comprising 18 genera and 4 subfamilies (Amblyoponinae, Dolichoderinae, Formicinae, Myrmicinae; Supp. Table 3; Supp. Fig. 3), and reached 97–100% sample coverage (the proportion of individuals of detected species compared to all individuals in the community, *sensu* Chao et al., 2020). However, there was differentiation among sampling rounds and treatments, with only 14 species found in all three rounds and 26 found in both control and burn treatments (Supp. Table 3, Supp. Table 4, Supp. Fig. 3). Of the four species found only in one treatment type, none occurred in more than two plots (Supp. Table 3), but together provided a mix of habitat preferences within each treatment (Supp. Table 3). Also of note, the widespread forest ant *Aphaenogaster rudis* occurred in the most plots in each treatment overall, and ranked within the five most abundant ants for each treatment and sampling round in 2020 pitfall traps.

At TRC in 2021, there was differentiation in the species found in each sampling method. In pitfall traps, we found a total of 26 ant species comprising 14 genera and 4 subfamilies (Dolichoderinae, Formicinae, Myrmicinae, Ponerinae; Supp. Table 3), and in Berlese litter samples, we found 21 ant species comprising 14 genera and 4 subfamilies (Formicinae, Myrmicinae, Ponerinae, Proceratiinae; Supp. Table 3). Twelve species were found in both pitfall and Berlese litter samples, 9 were unique to Berlese litter samples, and 14 were unique to pitfall samples (Supp. Table 3, Supp. Fig. 4). Genera found only in pitfall traps included *Camponotus* and *Formica,* both of which contain large, fast-moving species able to easily escape litter samples, whereas ants found exclusively in Berlese litter samples included the genus *Ponera* and some species of *Stenamma, Strumigenys,* and *Temnothorax*. Our 2021 pitfall samples reached 98–99% coverage, and our Berlese litter samples reached 99–100% coverage. As in 2020, *Aphaenogaster rudis* occurred in the most plots within each treatment in both pitfall and Berlese litter samples in 2021, but the other common species varied with treatment and dataset (Supp. Table 3). When considering all the species we found in 2020 and 2021 combined, nine were reported in the literature to occupy mainly open or xeric/dry sites (Supp. Table 3). However, six of those nine were found from only one plot across all sampling methods and years, and the remaining three open/xeric/dry species occurred in both control and at least one treatment plot (Supp. Table 3).

H1: Ant abundance, species richness, and diversity will be negatively impacted by fire but will recover with time since burning.

We found an overall effect of sampling round on ant abundance when pooling burning and control treatments for 2020 pitfall traps, with estimated marginal means showing a 62.9 (±23) % increase (p = 0.002) from Round 1 to Round 2 (Fig. 2a; Supp. Table 5). Round 3 also had 60.6 (±23) % more ants than in Round 1 (p = 0.003), but Rounds 2 and 3 were not significantly different from each other (Fig. 2a; Supp. Table 5). Ant abundance increased from Round 1 to 2 in control treatments (IRR = 1.65, p = 0.025) and from Round 1 to 3 in burn plots (IRR = 1.99, p = 0.014; Table 1, Supp. Table 5). Within rounds, however, ant abundance was only significantly different between burn and control treatments in Round 3, with 2.38 times more ants in burn plots compared to control plots (p = 0.017; Fig. 2a, Supp. Table 5).

Ant species richness was not significantly different between treatments in any round in 2020, nor between sampling rounds (Fig. 3a). However, there were some differences between rounds and treatments when weighting species by their relative abundances: exponentiated Shannon entropy (q = 1) and inverse Simpson diversity (q = 2) peaked in Round 2 compared to other rounds. Within Round 2, burning decreased both exponentiated Shannon and inverse Simpson metrics (Fig. 3a).

H2: Ant abundance, species richness, and diversity will be negatively impacted by burning and litter removal compared to control plots, due to reduced leaf litter.

We found a negative effect of treatment on percent litter cover in both years, and of treatment on litter mass in 2021: in 2020, percent litter cover was reduced by 85% in burn compared to control plots (IRR = 0.15, p = 0.008; Supp. Table 6), and in 2021 percent litter cover was reduced by 87% in burn plots (IRR = 0.13, p < 0.001) and 96% in litter removal plots (IRR = 0.04, p < 0.001; Supp. Table 6). In 2021, leaf litter mass was also significantly negatively affected by both treatments, with 93% less litter in burn compared to control plots (IRR = 0.07, $p < 0.001$) and 94% less litter in litter removal compared to control plots (IRR = 0.06, $p < 0.001$; Supp. Table 6).

Despite burn treatments having less leaf litter than controls, we did not find a consistent effect of burning on ant abundance, species richness, or diversity across all rounds in 2020. Additionally, although both burning and litter removal significantly reduced leaf litter in 2021, we found significant but opposite effects of only litter removal on ant abundance in 2021 pitfall traps and 2021 Berlese samples (Table 2, Fig. 2b,c). Compared to control plots, litter removal significantly increased ant abundance in pitfall traps (IRR = 1.50, $p = 0.046$; Table 2), but reduced ant abundance by 79% in Berlese samples (IRR = 0.21, $p < 0.001$; Table 2). Ant abundance in Berlese samples was also significantly lower in litter removal plots compared to burn plots (odds ratio = 0.346 ± 0.129, $p = 0.020$).

Pitfall traps from 2021 showed no treatment differences in interpolated or extrapolated effective number of species when weighting all species equally (species richness), weighting by relative abundance (exponentiated Shannon entropy), or weighting dominant species (inverse Simpson values; Fig. 3b). However, Berlese samples from 2021 showed significantly higher exponentiated Shannon entropy and inverse Simpson values in litter removal compared to both burn and control plots (Fig. 3).

H3: Ant community composition will shift toward more heat-tolerant and open-habitat species in burned and litter removal compared to control plots.

In 2020 pitfall traps, ant community composition differed with round and treatment. Specifically, PERMANOVA showed that community centroids were significantly different between burn and control treatments (sum of squares = 6.448, F =

20.185, p = 0.001), as well as among rounds (sum of squares = 2.857, F = 8.945, p = 0.001), and between treatments among rounds (sum of squares = 0.916, F = 2.900, p = 0.002). However, PERMDISP showed that compositional dispersion was heterogenous between treatments (sum of squares = 0.271, F = 18.961, p = 0.001), but homogenous among rounds (sum of squares = 2.345, F = 1.958, p = 0.125). NMDS dimension 1 provided the most differentiation among rounds (particularly Round 2 compared to Rounds 1 and 3; Fig. 4a,c,e), and NMDS dimension 2 provided the most separation among burn treatment and control plots (Fig. 4a,b,d). However, there was still some overlap between treatments and rounds in all NMDS dimensions. Within-round PERMANOVA revealed significant differences between burn and control communities in all three rounds (Supp. Table 7), and within-round PERMDISP revealed differences in dispersion between treatments (Supp. Table 7).

Pitfall traps in 2021 showed no significant differences among treatments for community composition using PERMANOVA (p = 0.48; Supp. Table 7) or community variance using PERMDISP (p = 0.647; Supp. Table 7), and treatments did not separate using NMDS (Fig. 5a–c). However, Berlese samples from 2021 revealed significant differences in centroid locations among treatments with PERMANOVA (p = 0.024; Supp. Table 7), although treatments did not clearly separate in NMDS (Fig. 5d–f). Nonetheless, these differences were due to centroid differences, as 2021 Berlese sample data did not have different dispersions among treatments (p = 0.195; Supp. Table 7).

In terms of species-specific responses, thirteen species had significant indicator values (IndVal) for treatment, sampling round, or some combination thereof in 2020 pitfall traps (Fig. 6, Supp. Table 8). One species, *Tapinoma sessile,* had a significant

IndVal for control treatments across all three rounds (IndVal = 0.496, Benjamini-Hochberg p = 0.013; Fig. 6, Supp. Table 8), and two species had significant IndVals for a sampling round across both treatments (*Camponotus pennsylvanicus* for Round 2: IndVal = 0.466, Benjamini-Hochberg p = 0.003; and *Prenolepis imparis* for Round 1 and 3: IndVal = 0.670, Benjamini-Hochberg p = 0.003; Fig. 6, Supp. Table 8). In 2021 pitfall data, no species had a significant IndVal for any treatment after Benjamini-Hochberg p-value adjustments. Finally, in 2021 Berlese samples, only *Aphaenogaster rudis* was significantly associated with control treatments (IndVal = 0.580, Benjamini-Hochberg p = 0.004; Supp. Fig. 6).

Discussion

We did not find consistent support for any of our three hypotheses about ant community responses to burning and litter removal; in some cases, this was due to a lack of detectable response, and in others, due to variable responses between treatments across years and/or sampling methods. For example, at Shaw Nature Reserve, we found ant abundance to only be significantly different between treatments in September during our third sampling round, 199 or 248 days after burning, depending on the location, with burn plots having more ants than control plots. This contrasts with our hypothesis that the largest difference between burn and control treatments would be in April (Round 1), our sampling round closest to the time of burning. This result also contrasts with our hypothesized direction of effect, showing a positive response where we had expected a negative effect of burning on ant abundance. Overall, we found ant abundance was lowest in our first round of sampling in April, a result we expected based on seasonal patterns of ant activity (Lynch, 1981). When considering treatments separately, abundance in control plots increased sooner (from Round 1 to Round 2) compared to burn plots (from Round 1 to Round 3), and ant abundance only differed between burn and control plots in Round 3, when we found more ants in burn plots (Fig. 2A). Finding such a difference only in the third sampling round is somewhat unexpected given other studies show ant communities in burned areas tend to return to pre-burn or control conditions between two and six months post-burning (Izhaki et al., 2003; Verble & Yanoviak, 2013; Vasconcelos et al., 2017). Perhaps our September results are due to an increased effect of late-summer heat in exposed, burned areas, as leaf litter in the Ozarks can take multiple years to recover to pre-fire levels (Stambaugh et al., 2006). Such a late-summer effect could have been missed by previous work in the Ozarks that only sampled post-fire ant communities through July (Verble & Yanoviak, 2013). Further replication and measurements of temperature and moisture co-variates could help test this hypothesis.

We did not detect any difference in interpolated or extrapolated ant species richness between treatments or sampling rounds in 2020. However, when weighting species by relative abundance or emphasizing the most abundant species, we found higher exponentiated Shannon and inverse Simpson diversity values in our July samples (Round 2) compared to April (Round 1) or September (Round 3), and more so in control than in burn plots. Thus, the number of common and dominant species peaked in July, and overall ant abundance was highest in July and September compared to April. These results somewhat align with patterns of June-July peaks in ant abundance and exponentiated Shannon diversity found in monthly litter samples from mature forest in Maryland (Lynch, 1981).

We found partial support for our second hypothesis that ant abundance, species richness, and diversity would be negatively impacted by burning and litter removal due to reduced litter cover. Burning reduced the amount of leaf litter compared to control plots in both years, as did physical litter removal in 2021. However, these treatment-driven reductions in litter cover and quantity were not accompanied by consistent reductions within a treatment between years (burn effects in 2020 vs. 2021) or between treatments within a year (burn vs. litter removal effects in 2021) in ant abundance, species richness, or diversity.

When considering the effect of burning, ant abundance in 2020 pitfall traps was actually higher in burn compared to control plots in September, and we did not find a significant effect of burn compared to control on ant abundance in 2021 pitfall traps or Berlese litter samples (both of which were collected in July 2021). Although burning did not affect species richness in either year or sampling method, we found some differences in ant diversity between burn and control plots. In 2020, these differences varied with sampling round; in July, control plots had more common and highly abundant species (higher exponentiated Shannon entropy and inverse Simpson diversity values) than burn plots, but in April, burn plots had more common and highly abundant species than control plots. The July pattern was only slightly repeated in 2021, where inverse Simpson diversity was significantly higher in control compared to burn Berlese litter samples, but the difference in the number of effective species was less than 1.

In 2021, our litter removal treatment significantly affected ant abundance, but the direction of the impact depended on the sampling method. In 2021 pitfall traps, litter removal increased ant abundance, but the opposite was true for 2021 Berlese litter samples, likely because we collected Berlese litter samples on a per-area rather than per-volume basis but the number of ants that a Berlese litter funnel can extract depends upon the starting amount of litter. Furthermore, we did not find a significant effect of litter removal on any Hill number in 2021 pitfall traps, but we found higher exponentiated Shannon and inverse Simpson values in 2021 Berlese samples. This could also be a byproduct of collecting litter samples by area rather than volume, as having less leaf litter could reduce the encounter rate for rare species and thereby boost more common species' relative abundances.

These differences in treatment effect by sampling method could also be due in part to the biases of pitfall and litter samples toward different ant taxa, with Berlese litter samples catching more litter-dwelling species that might also be more sensitive to drastically reduced levels of leaf litter. However, burning also reduced leaf litter, and the fact that ant diversity did not respond to burning and litter removal in a similar manner suggests the influence of another mechanism; for example, perhaps insects were translocated along with leaf litter in litter removal treatments, compared to burn treatments where insects might have sought refuge but then continued on with life as normal after the burns.

Support for our final hypothesis, that of compositional shifts toward more open-habitat species in burned and litter removal plots, required evidence of compositional differences between treatments, which we found in some but not all cases. In 2020 at Shaw Nature Reserve, ant communities differed in dispersion with treatment and compositionally with sampling round, but did not fully separate in our NMDS results. In pitfall data from 2021, we did not find differences in composition among treatments. However, we found compositional differences among treatments in 2021 Berlese litter samples, although these differences were not easily discernible in our NMDS results.

Finding compositional differences between treatments was necessary but insufficient for our third hypothesis, and ultimately these differences did not support the hypothesis that community composition would shift toward more open-habitat ants in burned and litter removal treatments. Instead, we found a mix of habitat preferences in each treatment, and did not find any species with consistent responses between years. Within 2020 pitfall samples, we found few species that were consistently associated with only control or burn treatments across sampling rounds. Of the ants that did associate with a single treatment type, we found various habitat preferences inconsistent with our hypothesis of a shift toward open-habitat species in burn plots. For example, of the species we found associated with only burn treatments in 2020, *Formica pallidefulva* is considered a closed canopy forest species but can also be found in more open habitats (Trager et al., 2007), *Stenamma impar* is a general forest associate (Carter, 1962)*,* and *S. schmitti* is known from forest and bog habitats (Ellison et al., 2012). We did not find any species significantly associated with burn or litter removal treatments in 2021, because no species had significant IndVals in 2021 pitfall traps, and the one species with a significant IndVal in 2021 Berlese litter samples was associated with control in that dataset (*A. rudis*).

In 2020 pitfall traps, we found five species to be associated with only control plots: *Lasius americanus, Monomorium minimum, Nylanderia faisonensis, Solenopsis molesta,* and *Tapinoma sessile*. In contrast to what we expected for species associated with our control plots, *M, minimum, S. molesta,* and *T. sessile* are known open habitat associates (Lynch, 1981; Supp. Table 3). *Monomorium minimum* is also known to generally inhabit drier areas, including ridges and grassy habitat (Wheeler et al., 1994; Wang et al., 2001). *Tapinoma sessile* could be considered a control indicator for our plots at SNR, at least in 2020, as this species was associated with our control plots in each sampling round despite overall seasonal shifts in ant abundance and community composition. However, we cannot say whether this species would be a reliable indicator of unburned areas, as we found it in only three plots at TRC in 2021, two of which were burn and one of which was a litter removal plot.

Only one species, *Aphaenogaster rudis,* was significantly associated with control treatments in 2021 Berlese litter samples. However, this association of *A. rudis* with control treatments in 2021 litter samples is likely an artifact of sampling method, as our Berlese litter samples were collected on a per-area rather than per-volume basis, and thus there was a greater volume of leaf litter to find ants in with control plots compared to burn or litter removal plots. Furthermore, *A. rudis* was associated with burn plots and in all three rounds as well as control plots in Round 2 and 3 in 2020, and did not show any significant associations for one treatment over the others in 2021 pitfall traps. In fact, *A. rudis* was the most widespread ant species in all sample datasets, occurring in more plots than any other ant species in each treatment in each year and sampling type.

Our finding of more open-habitat species in our control plots and more mesic-habitat species in our burn plots in 2020 could also indicate site differences prior to our sampling. Indeed, our control site TH was more exposed and had more plots on south-facing plots than our other control and two burn sites. If such is the case, then burning still did not overcome these site differences and shift community composition toward more open-habitat species. When considered with our 2021 results indicating no difference in ground-foraging ant community composition among treatments, and the influence of sampling method, our findings suggest that the composition of the ant community is generally resilient (*sensu* Holling, 1973; Pimm, 1984) to prescribed burning and litter removal within the months following fire. This is similar to studies in other fire-adapted ecosystems, such as South Africa (Pryke & Samways, 2012) and Australia (Parr & Andersen, 2008), where ant communities showed resilience to fire in the form of unchanged species richness and unchanged or even increased abundance one year post-fire.

Ultimately, we found only a few species that were significant indicators of only burn or control treatments when using ISA for 2020 pitfall traps, and we did not find any species that consistently were associated with the same treatment across years and sampling sites. When coupled with the lack of distinct or consistent patterns in abundance, species richness, diversity, or community composition across years, individual species and the overall ant community did not respond consistently to fire and thus would not be useful indicators for measuring the impacts of prescribed fire in Missouri. This is in contrast with ants in other habitats, such as more open savanna and woodlands in Australia (Andersen & Majer, 2004). Our results also do not support the hypothesis (Andersen, 2019) that fire impacts ant communities more in closed compared to open habitats.

A singular low-intensity burn is likely to alter habitat structure, but repeated burns can lead to more open canopy and understory conditions and thus have larger and more lasting effects on ant communities (Andersen et al., 2014; Knapp et al., 2015). Our different locations had different recent fire histories, which could partly explain why we saw different responses of the ant community between 2020 and 2021, as long-term repeated fire can have a larger and more consistent effect on ant communities than one singular fire event. For example, in upland oak forests in Iowa, plots with a yearly burn history showed lower ant abundance compared to control plots (Vincent et al., 2009). Yet, ant communities in Vermont sandplain forests showed increased species diversity and evenness in areas burned 7–10 years prior to sampling when compared to controls (Banschback & Ogilvy, 2014). In the Missouri Ozarks, annual burning over six decades increased abundance of soil- and litter-dwelling ants, and less frequent (every four years) burning over the same sixty-year period increased Shannon diversity and litter-dwelling ants (Wright, 2013). At Shaw Nature Reserve, each of our sites had a different burn history, but all experienced more burning prior to this experiment compared to Tyson Research Center, where only one burn had occurred prior to our experimental burn. Thus, we cannot discount that some of differences in ant community responses could have be influenced by these differences in burn histories, rather than the occurrence of the most recent burn. However, if that is the case, it would also support the hypothesis that our ant communities are resilient to a single, recent prescribed burn, and are instead more affected by long term burn history and other habitat variables.

Conclusion

We found Missouri Ozark ant species richness and community composition were resilient to or benefitted from burning and litter removal depending on year and location. The lack of a consistent response does not support the use of ants as indicators in this context. Our results show some differences in ant abundance based on sampling date, location, and method of sampling, generally following seasonal trends but having higher abundances in burn compared to control plots in September 2020, and showing lower abundances in litter removal treatments in litter sampling. We also found more common species in pitfall traps in July compared to April and September samples in 2020, and in Berlese samples from litter removal compared to burn and control treatments in 2021. Overall, this indicates that ant communities are resilient to prescribed burning in the winter and spring in the Missouri Ozarks, and adds to general evidence of ant community resistance to burning in areas where fire is a common occurrence.

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Tables and Figures

Table 1: Results of a negative binomial GLMM on ant abundance in pitfall traps from 2020 at Shaw Nature Reserve.

	Ant abundance, 2020 pitfall traps $\theta = 2.33$		
Predictors	*IRR*	*CI*	*p*
(Intercept)	2.94	1.69, 5.13	**<0.001**
Treatment [Burn]	1.20	0.57, 2.49	0.632
Sampling round [2]	1.65	1.07, 2.55	**0.025**
Sampling round [3]	1.14	0.73, 1.77	0.566
Treatment [Burn] × Sampling round [2]	0.98	0.57, 1.68	0.934
Treatment [Burn] × Sampling round [3]	1.99	1.15, 3.44	**0.014**
Random Effects			
σ^2	0.50		
τ_{00} (Plot, within Site)	0.37		
τ_{00} (Site, within Treatment)	0.08		
τ_{11} (Plot, Sampling Round 2, within Site)	0.44		
τ_{11} (Plot, Sampling Round 3, within Site)	0.36		
ρ_{01} (Plot, Sampling Round 2, within Site)	-0.82		
ρ_{01} (Plot, Sampling Round 3, within Site)	-0.07		
ICC	0.48		
N (Site)	4		
N (Plot)	73		
N (Treatment)	2		
Observations	567		
Marginal R^2 / Conditional R^2	0.098 / 0.534		

Note: Incidence Rate Ratios (IRR) represent ratio of ant abundance in a treatment compared to control, such that an IRR of 1.51 indicates a 51% increase in ant abundance in burn plots compared to controls, whereas an IRR below 1 indicates a decrease.

Table 2: Results of negative binomial (log link) GLMMs on worker ant abundance in 2021 datasets from Tyson Research Center pitfall trap samples and Berlese funnel-processed litter samples.

	Ant abundance, **2021 Pitfall traps** $\theta = 6.33$			**Ant abundance,** **2021 Berlese samples** $\theta = 1.38$		
Predictors	*IRR*	*CI*	*p*	*IRR*	*CI*	*p*
(Intercept)	5.61	3.96, 7.94	**<0.001**	133.36	87.84,216.24	**<0.001**
Treatment [Burn]	1.22	0.85, 1.77	0.285	0.66	0.35, 1.20	0.177
Treatment [Litter removal]	1.50	1.01, 2.22	**0.046**	0.21	0.11, 0.40	**<0.001**
Random Effects						
σ^2	0.27					
τ_{00}	0.10 (Site)					
	0.17 (Plot, within Site)					
ICC	0.50					
N	8 (Site)					
	43 (Plot)					
Observations	154			42		
Marginal R^2 / Conditional R^2	0.045 / 0.521			0.465		

Note: Incidence Rate Ratios (IRR) represent ratio of ant abundance in a treatment compared to control, such that an IRR of 1.51 indicates a 51% increase in ant abundance in burn plots compared to controls, whereas an IRR below 1 indicates a decrease.

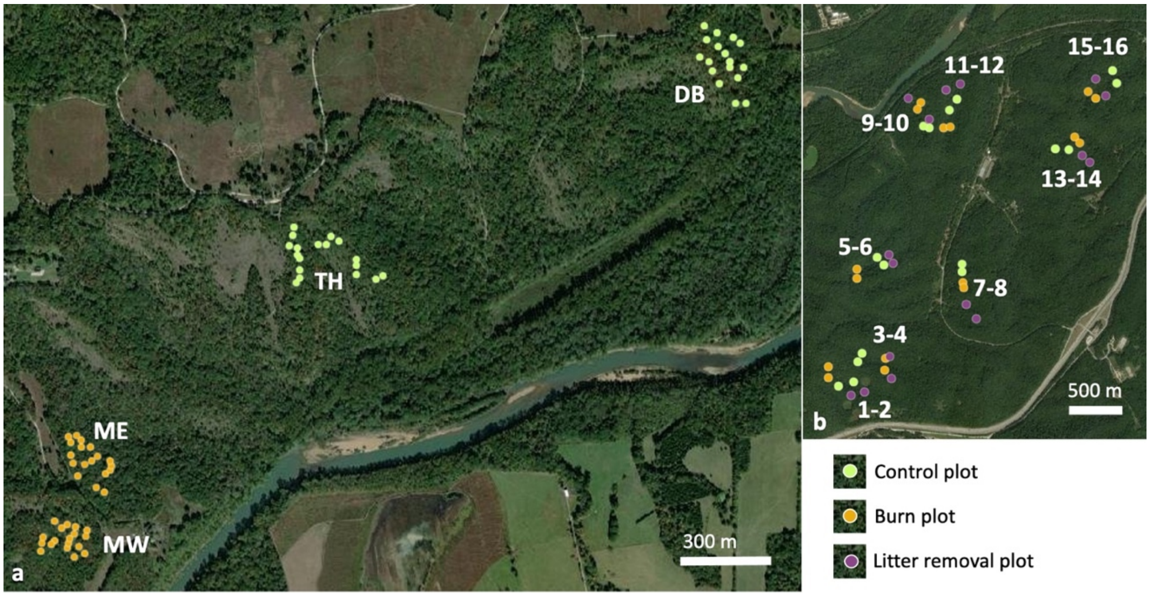

Figure 1: Map of plot locations for experiments from (a) 2020 at Shaw Nature Reserve and (b) 2021 at Tyson Research Center. Sites are labeled with site abbreviations or number codes.

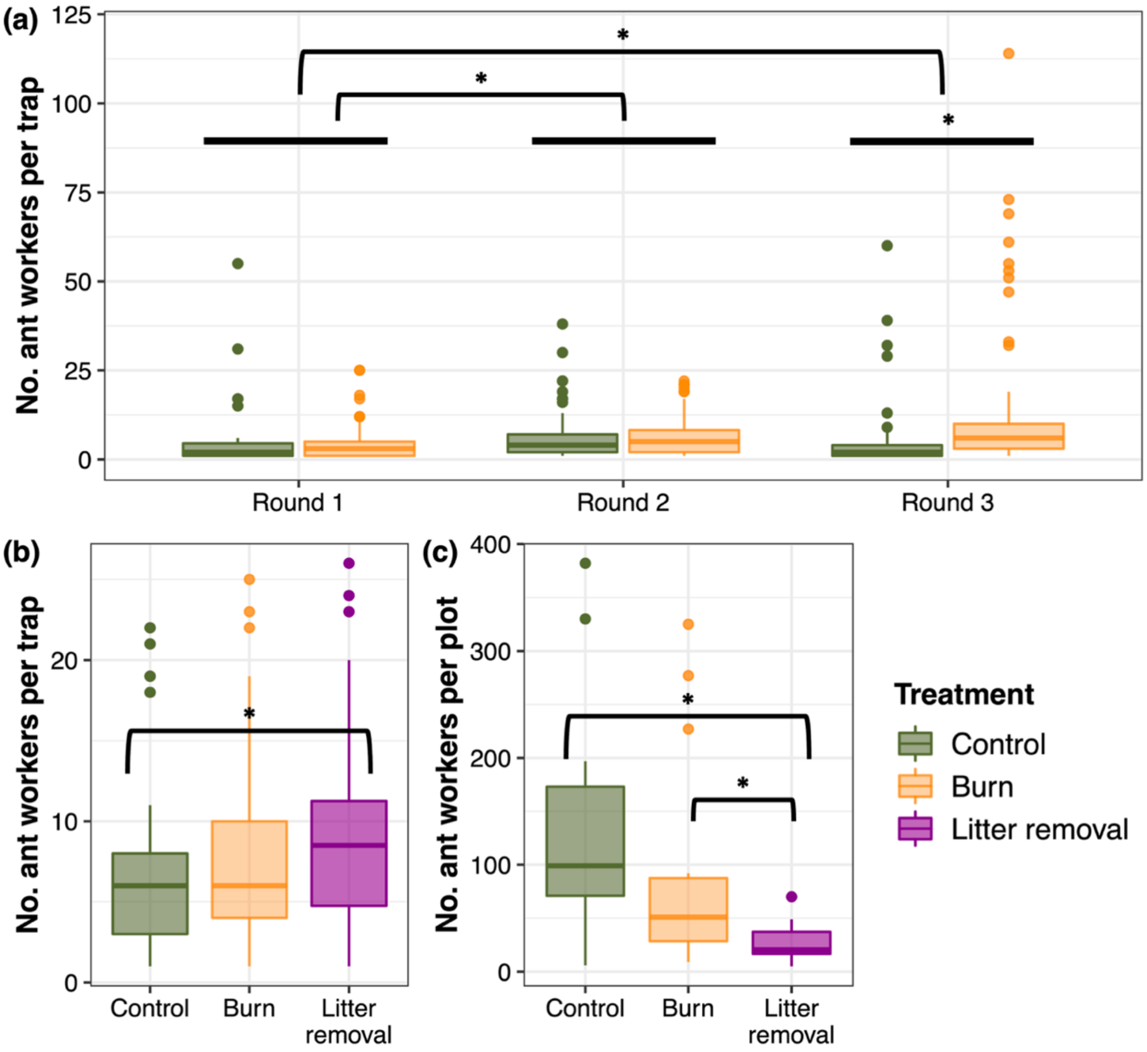

Figure 2: Ant worker abundance from (a) Shaw Nature Reserve pitfall traps from three sampling rounds in 2020: Round 1 (April), Round 2 (July), and Round 3 (September); (b) Tyson Research Center pitfall traps in 2021 (July), and (c) Tyson Research Center Berlese samples in 2021. Asterisks indicate significant differences ($p \leq 0.05$) between groups based on results from Tables 2 and 3.

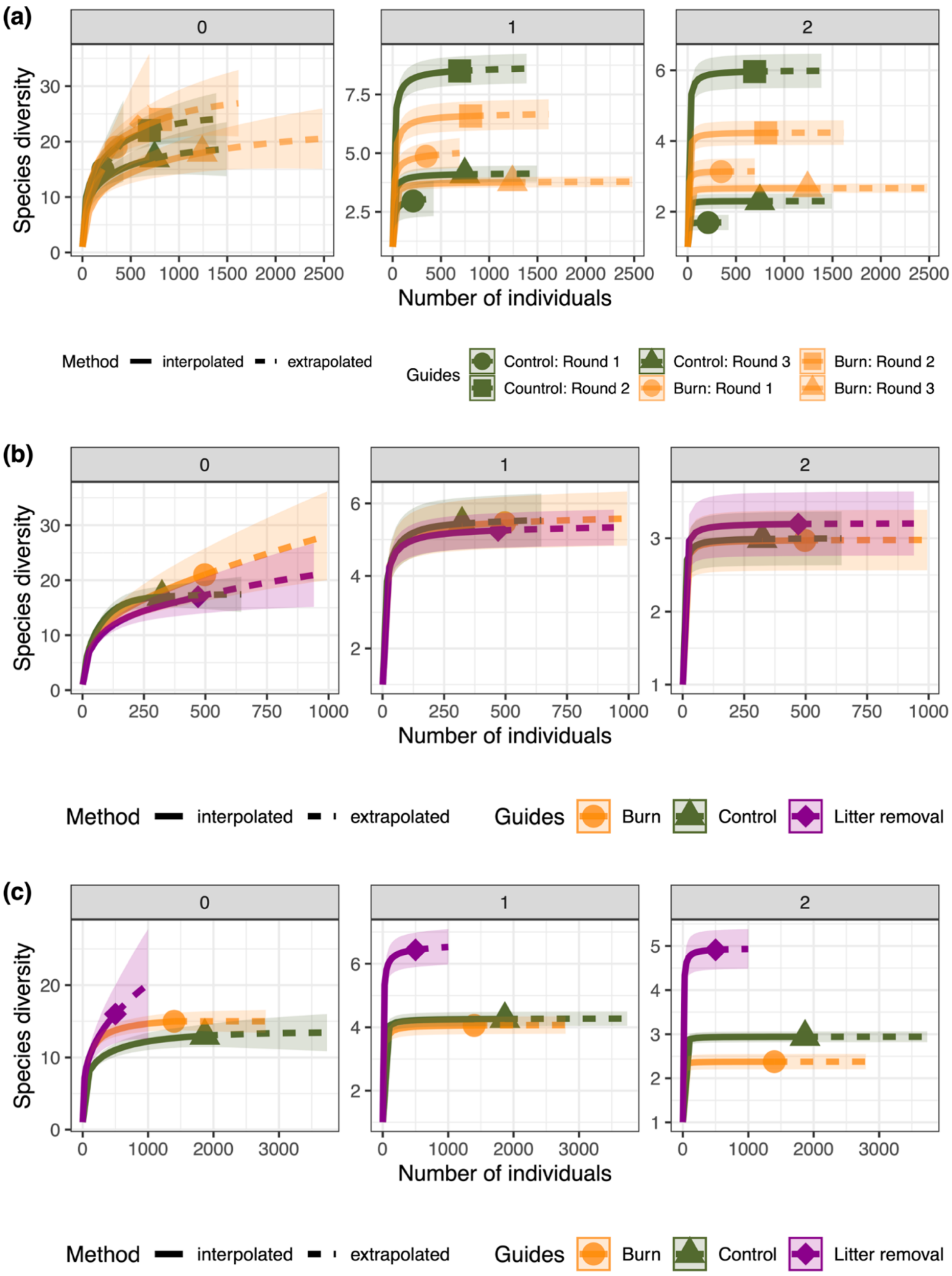

Figure 3: Interpolated (solid lines) and extrapolated (dashed lines) ant diversity with

95% confidence intervals from (a) Shaw Nature Reserve pitfall traps in 2020 by round and treatment, (b) Tyson Research Center pitfall traps in 2021 by treatment, and (c) Tyson Research Center Berlese samples in 2021 by treatment. Hill numbers presented are q = 0 (species richness), 1 (exponentiated Shannon entropy), and 2 (inverse Simpson diversity).

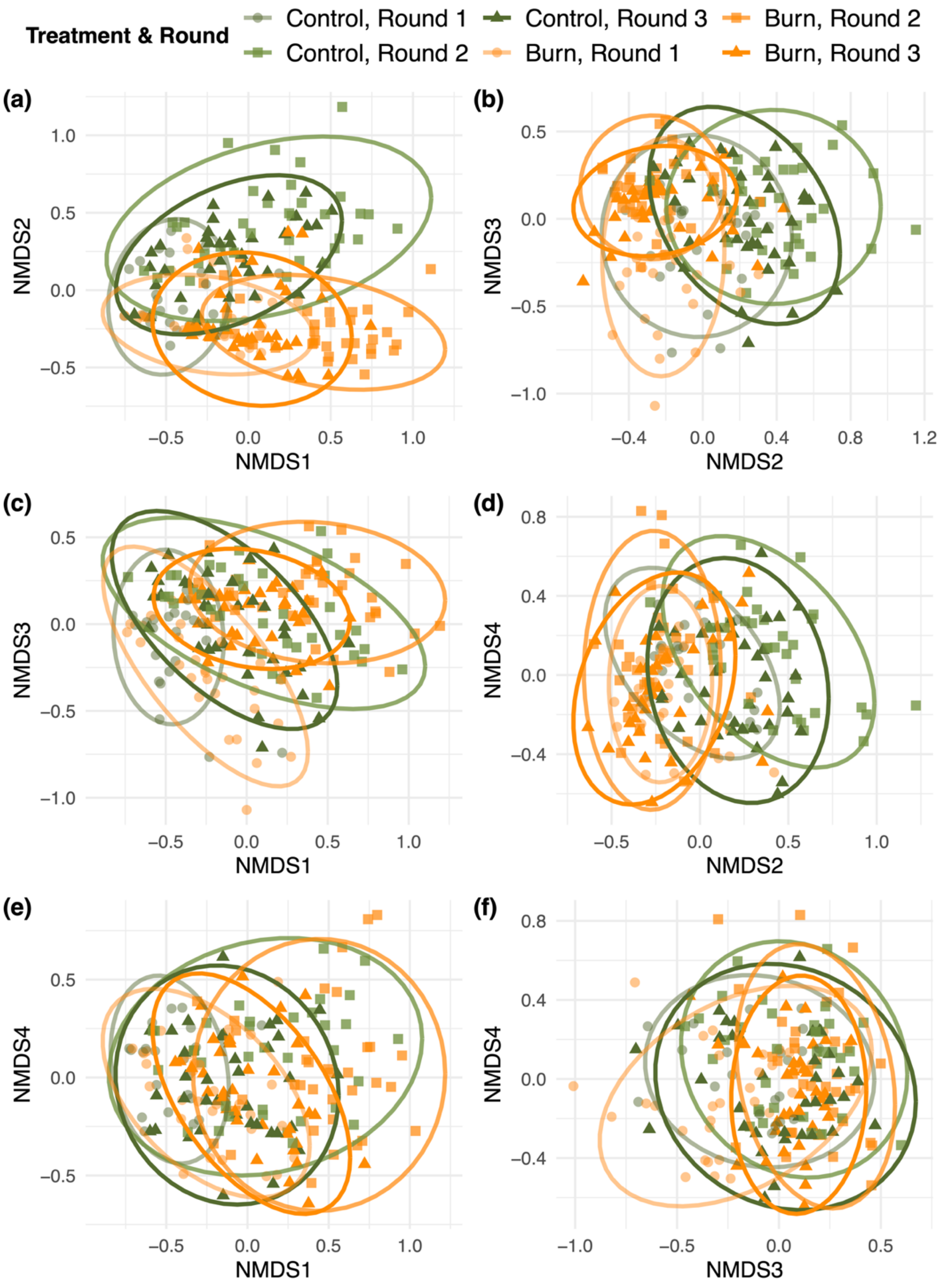

Figure 4: Results of NMDS from pitfall traps collected at Shaw Nature Reserve in 2020, using four dimensions. Ellipses represent 95% confidence intervals.

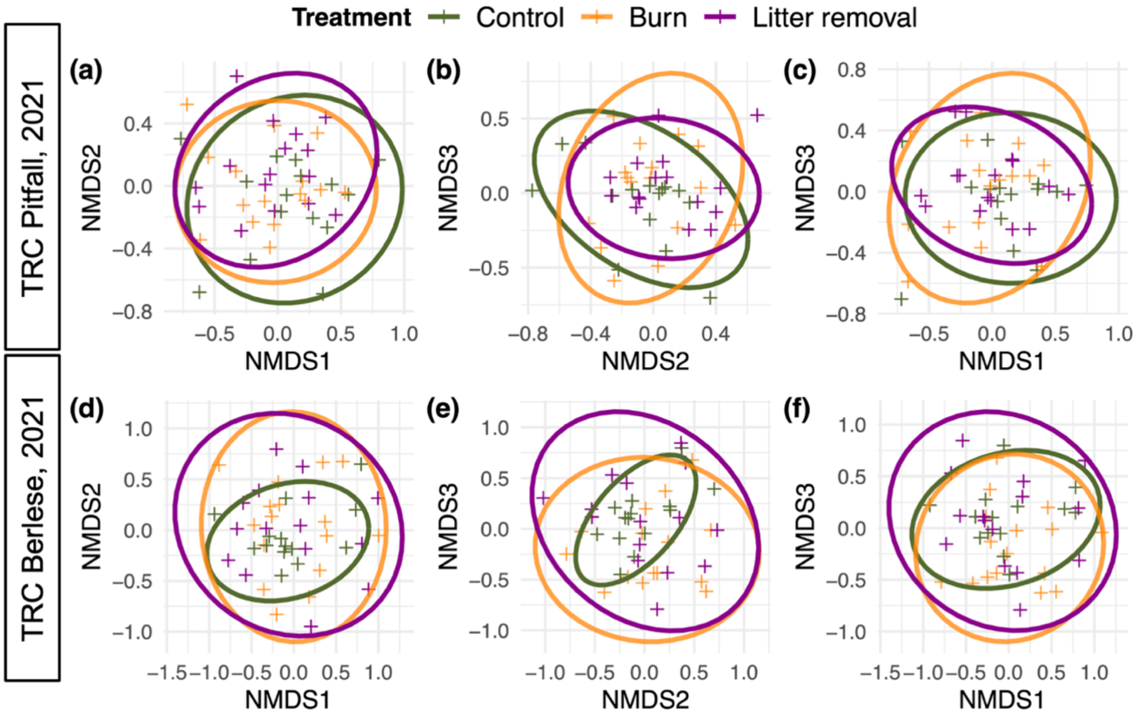

Figure 5: Results of NMDS based on Bray-Curtis dissimilarities from Tyson Research Center in 2021: (a–c) pitfall trap samples, and (d–f) Berlese funnel litter samples. Ellipses represent 95% CIs.

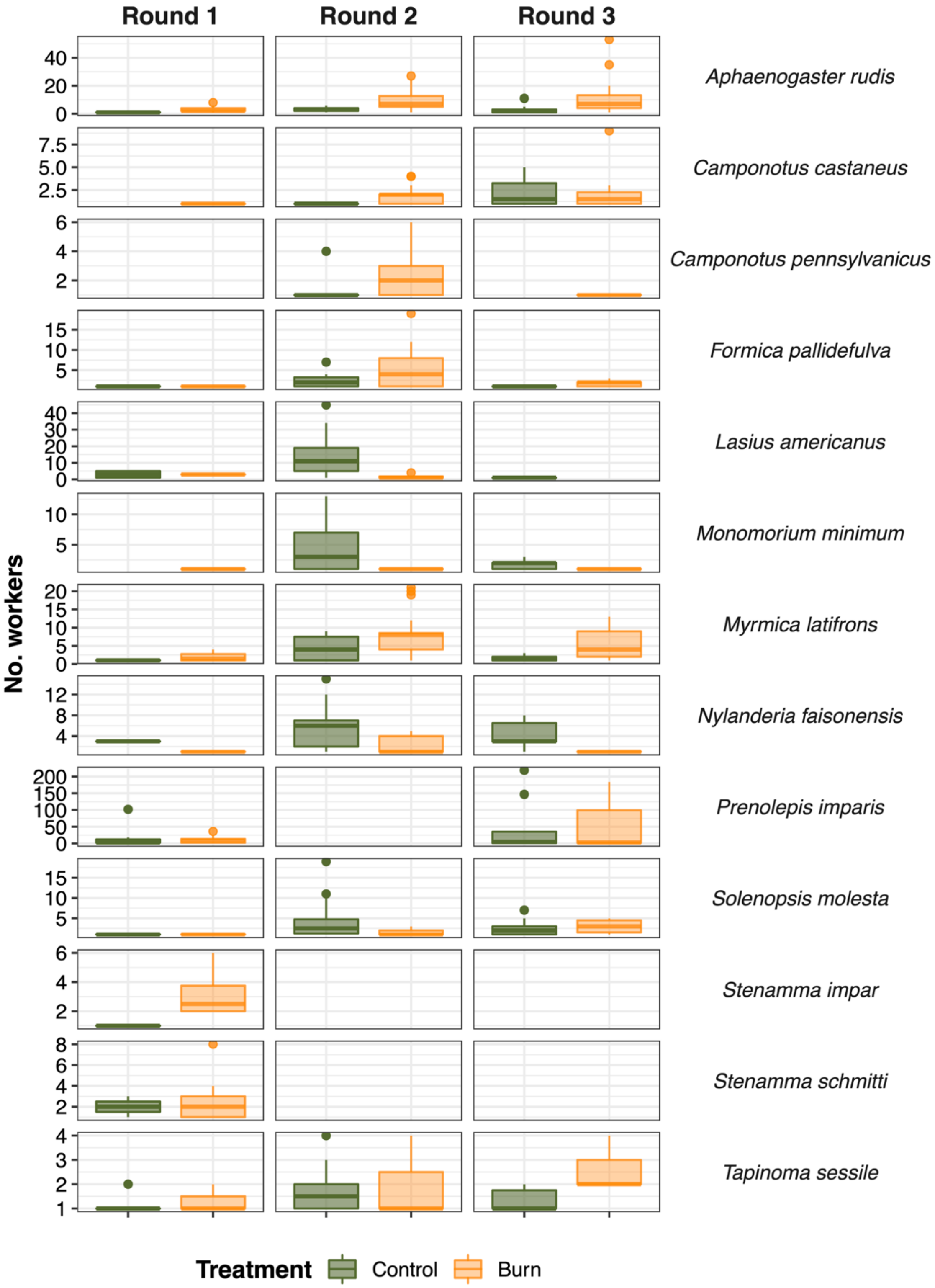

Figure 6: Ant abundance per plot from 2020 pitfall traps at Shaw Nature Reserve, by

treatment, sampling round, and indicator species.

Supplementary Materials

Supplementary Table 1: Specifications from NMDS tests using data from 2020 at Shaw Nature Reserve and 2021 at Tyson Research Center.

Sample year	*Dataset*	*Transformation*	*Dimensions*	*Tries*	*Solutions*	*Stress*
(a) 2020, SNR	Round 1 pitfall traps	Wisconsin	3	20	2	0.139
	Round 2 pitfall traps	Wisconsin	5	20	2	0.114
	Round 3 pitfall traps	Wisconsin	4	20	2	0.136
	Pitfall traps (all rounds)	Wisconsin (square root, dummy species)	4	20	2	0.136
(b) 2021, TRC	Pitfall traps	Wisconsin (dummy species)	3	27	2	0.163
	Berlese samples	Wisconsin (square root)	3	20	2	0.173

Supplementary Table 2: List of ant species found in this study, functional group classification based on Andersen (1997), and habitat types based on listed references.

Ant species	*Functional group*	*Habitat type*	*Reference*
Aphaenogaster fulva	Opp.	Forest	1, 2
A. lamellidens	Opp.	Forest	1, 2
A. rudis	Opp.	Forest	3
Brachymyrmex depilis	Cry.	Open–forest	1
Camponotus americanus	Sub. Camp.	Forest	1
C. castaneus	Sub. Camp.	Open–forest	1
C. chromaiodes	Sub. Camp.	Forest	2
C. nearcticus	Sub. Camp.	Forest	1
C. pennsylvanicus	Sub. Camp.	Open–forest	1, 2
C. subbarbatus	Sub. Camp.	Mesic, forest	1
Crematogaster lineolata	Gen. Myr.	Mesic, open–forest	1, 2
Formica biophilica	Opp.	Mesic–hydric, open	4
F. dolosa	Opp.	Dry, open	4
F. pallidefulva	Opp.	Dry–mesic, open–forest	4
F. subsericea	Opp.	Forest	2
Lasius americanus	Opp.	Forest	5
Monomorium minimum	Gen. Myr.	Open	1, 6
Myrmecina americana	Cold	Forest	1
Myrmica latifrons	Opp.	Open–forest	7
M. pinetorum	Opp.	Forest	1
M. punctiventris	Opp.	Forest	1
Nylanderia faisonensis	Opp.	Mesic, forest	8
N. terricola	Opp.	Open	8
Ponera exotica	Cry.	Xeric–mesic, forest	1
P. pennsylvanica	Cry.	Mesic, forest	1
Prenolepis imparis	Cold	Forest	2
Proceratium silaceum	Cry.	Forest	1
Solenopsis molesta	Cry.	Open	1, 6
Stenamma diecki Emery, 1985	Cry.	Mesic, forest	9
S. impar	Cry.	Forest	1
S. schmitti	Cry.	Mesic–hydric, forest	5
Stigmatomma pallipes (Haldeman, 1844)	Cry.	Mesic, forest	9
Strumigenys abdita Wesson, L.G. & Wesson, R.G., 1939	Cry.	Open–shaded	10, 11
S. ornata Mayer 1887	Cry.	Mesic, forest	10
S. pilinasis Forel 1901	Cry.	Forest	11
S. rostrata Emery 1895	Cry.	Dry, forest	10
Tapinoma sessile (Say, 1836)	Opp.	Open	6
Temnothorax americanus (Emery, 1895)	Opp.	Dry, open–forest	*
T. curvispinosus (Mayr, 1866)	Opp.	Dry, open–forest	2, 5
T. floridanus	Opp.	Forest	2
T. longispinosus (Roger, 1863)	Opp.	Forest	7
T. texanus (Wheeler, W.M., 1903)	Opp.	Open	12
Tetramorium immigrans Santschi, 1927	Opp.	Open, disturbed	13
T. tsushimae Emery, 1925	Opp.	Xeric, open	14

Notes: Opp. = Opportunist, Cry. = Cryptic species, Sub. Camp. = Subordinate Camponotini, Gen. Myr. = Generalized Myrmicinae, Cold = Cold-climate specialist.

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*Based on habitat preferences of hosts in 2, 5; hosts listed in: Beibl, J., Stuart, R.J., Heinze, J. and Foitzik, S., 2005. Six origins of slavery in formicoxenine ants. *Insectes Sociaux, 52*(3), pp.291–297.

Supplementary Table 3: Number of plots each species occurred in, by treatment, in 2020 pitfall traps from three rounds of sampling at Shaw Nature Reserve (SNR) combined, 2021 pitfall traps at Tyson Research Center (TRC), and 2021 Berlese litter samples at TRC. Abbreviations in table note. References for habitat type can be found in Supp. Table 2.

			Pitfall, 2020 (SNR)		**Pitfall, 2021 (TRC)**			**Berlese, 2021 (TRC)**		
Ant species	*Subf.*	*Hab.*	*B*	*C*	*B*	*C*	*LR*	*B*	*C*	*LR*
Aphaenogaster fulva	Myr.	F	1	2	2	0	2	2	0	0
A. lamellidens	Myr.	F	2	2	1	1	0	0	0	0
A. rudis	Myr.	F	92	55	16	14	16	14	13	11
Brachymyrmex depilis	For.	O,F	1	0	0	1	0	2	0	1
Camponotus americanus	For.	F	4	2	10	7	10	0	0	0
C. castaneus	For.	O,F	25	14	0	0	0	0	0	0
C. chromaiodes	For.	F	13	7	9	8	10	0	0	0
C. nearcticus	For.	F	0	0	1	0	1	0	0	0
C. pennsylvanicus	For.	O,F	14	6	10	8	4	0	0	0
C. subbarbatus	For.	M,F	12	4	1	0	2	0	0	0
Crematogaster lineolata	Myr.	M,O,F	5	3	3	4	2	3	3	3
Formica biophilica	For.	M,H,O	1	0	0	0	0	0	0	0
F. dolosa	For.	D,O	0	0	0	1	0	0	0	0
F. pallidefulva	For.	D,M,O,F	39	10	5	2	6	0	0	0
F. subsericea	For.	F	6	12	3	1	3	0	0	0
Lasius americanus	For.	F	6	22	0	0	0	0	0	0
Monomorium minimum	Myr.	O	3	27	0	0	0	0	0	0
Myrmecina americana	Myr.	F	4	1	1	2	2	6	6	3
Myrmica latifrons	Myr.	O,F	50	11	0	1	0	0	0	0
M. pinetorum	Myr.	F	11	1	0	0	0	0	0	0
M. punctiventris	Myr.	F	1	1	2	1	2	1	0	0
Nylanderia faisonensis	For.	M,F	9	31	6	5	9	12	11	9
N. terricola	For.	O	0	1	0	0	0	0	0	0
Ponera exotica	Pon.	X,M,F	0	0	0	0	0	0	2	1
P. pennsylvanica	Pon.	M,F	3	3	0	0	0	10	11	10
Prenolepis imparis	For.	F	34	23	0	0	1	0	0	1
Proceratium silaceum	Pro.	F	0	0	0	0	0	0	0	1
Solenopsis molesta	Myr.	O	12	51	2	5	3	8	9	6
Stenamma diecki	Myr.	M,F	1	1	0	0	0	0	0	0
S. impar	Myr.	F	10	3	0	0	0	0	1	0
S. schmitti	Myr.	M,H,F	17	2	0	0	0	0	0	0
Stigmatomma pallipes	Amb.	M,F	0	0	0	0	1	0	0	0
Strumigenys abdita	Myr.	O,S	0	0	0	0	0	0	1	0
S. ornata	Myr.	M,F	0	1	0	0	0	2	4	3
S. pilinasis	Myr.	F	0	1	0	0	0	1	0	1
S. rostrata	Myr.	D,F	0	0	3	0	0	7	11	8
Tapinoma sessile	Dol.	O	9	32	2	0	1	0	0	0
Temnothorax americanus	Myr.	D,O,F	1	0	0	0	1	0	0	0
T. curvispinosus	Myr.	D,O,F	6	6	4	4	0	13	12	10

T. floridanus	Myr.	F	2	0	1	1	0	5	5	4
T. longispinosus	Myr.	F	0	0	1	0	0	0	0	0
T. texanus	Myr.	O	0	0	0	0	0	0	0	1
Tetramorium immigrans	Myr.	O, Di	0	0	0	0	0	1	0	0
T. tsushimae	Myr.	X,O	0	1	0	0	0	0	0	0

Abbreviations: Subf. = Subfamily, B = Burn, C = Control, LR = Litter removal, Myr = Myrmicinae For. = Formicinae, Pon. = Ponerinae, Pro. = Proceratiinae, Amb. = Amblyoponinae, Dol. = Dolichoderinae; F = Forest, O = Open, M = Mesic, H = Hydric, D = Dry, X = Xeric, Di = Disturbed.

Supplementary Table 4: Number of plots ant species were observed at, from 2020 pitfall sampling at Shaw Nature Reserve.

	Round 1 (April)		*Round 2 (July)*		*Round 3 (September)*	
Ant species	*Burn*	*Control*	*Burn*	*Control*	*Burn*	*Control*
Aphaenogaster fulva	0	0	1	2	0	0
A. lamellidens	0	0	2	2	0	0
A. rudis	22	7	34	23	36	25
Brachymyrmex depilis	0	0	1	0	0	0
Camponotus americanus	0	0	3	0	1	2
C. castaneus	1	0	16	6	8	8
C. chromaiodes	0	1	10	4	3	2
C. pennsylvanicus	0	0	11	6	3	0
C. subbarbatus	0	0	7	1	5	3
Crematogaster lineolata	1	0	4	3	0	0
Formica biophilica	0	0	0	0	1	0
F. pallidefulva	2	1	29	8	8	1
F. subsericea	0	3	4	7	2	2
Lasius americanus	1	4	4	16	1	2
Monomorium minimum	1	0	1	16	1	11
Myrmecina americana	1	0	1	1	2	0
Myrmica latifrons	6	1	23	4	21	6
M. pinetorum	3	0	6	1	2	0
M. punctiventris	0	0	1	1	0	0
Nylanderia faisonensis	1	1	5	16	3	14
Nylanderia terricola	0	0	0	0	0	1
Ponera pennsylvanica	1	1	1	2	1	0
Prenolepis imparis	18	11	0	0	16	12
Solenopsis molesta	2	2	3	30	7	19
Stenamma diecki	1	1	0	0	0	0
S. impar	10	3	0	0	0	0
S. schmitti	17	2	0	0	0	0
Strumigenys ornata	0	0	0	1	0	0
S. pilinasis	0	0	0	0	0	1
Tapinoma sessile	3	6	3	12	3	14
Temnothorax americanus	1	0	0	0	0	0
T. curvispinosus	1	0	5	2	0	4
T. floridanus	0	0	2	0	0	0
Tetramorium tsushimae	0	1	0	0	0	0

Supplementary Table 5: Estimated marginal mean comparisons from the models in Table 2. Pairwise comparisons use Tukey method for p-adjustment.

Comparison	*Odds ratio*	*SE*	*df*	*t-ratio*	*p*
Round 2 vs Round 1	1.629	0.230	553	3.453	**0.002**
Round 3 vs Round 1	1.606	0.230	553	3.303	**0.003**
Round 3 vs Round 2	0.986	0.127	553	-0.111	0.993
Round 1: Burn vs Control	1.20	0.447	553	0.479	0.632
Round 2: Burn vs Control	1.17	0.367	553	0.499	0.618
Round 3: Burn vs Control	2.38	0.867	553	2.385	**0.017**
Control: Round 2 vs Round 1	1.648	0.366	553	2.246	0.065
Control: Round 3 vs Round 1	1.138	0.256	553	0.574	0.834
Control: Round 3 vs Round 2	0.691	0.127	553	-2.012	0.110
Burn: Round 2 vs Round 1	1.610	0.273	553	2.811	**0.014**
Burn: Round 3 vs Round 1	2.266	0.389	553	4.764	**<0.001**
Burn: Round 3 vs Round 2	1.407	0.248	553	1.937	0.129

Supplementary Table 6: Results of beta-binomial (logit link) GLM(M)s showing effect of treatment on % litter cover at Shaw Nature Reserve in 2020 and Tyson Research Center in 2021, and (a Gamma (log link) GLM demonstrating effect of treatment on leaf mass (g) in 2021 at Tyson Research Center.

	% Litter cover, 2020 **SNR** (θ = 9.23)			**% Litter cover, 2021** **TRC** (θ = 2.85)			**Leaf mass (g), 2021** **TRC**		
Predictors	*IRR*	*CI*	*p*	*IRR*	*CI*	*p*	*IRR*	*CI*	*p*
(Intercept)	4.94	1.83, 13.35	**0.002**	5.22	2.76, 9.90	**<0.001**	455.35	335.86, 638.95	**<0.001**
Treatment [Burn]	0.15	0.04, 0.61	**0.008**	0.13	0.05, 0.31	**<0.001**	0.07	0.04, 0.10	**<0.001**
Treatment [Litter removal]	--	--	--	0.04	0.02, 0.10	**<0.001**	0.06	0.04, 0.10	**<0.001**
Random Effects									
σ^2	-0.05								
τ_{00}	0.48 (Site, within Treatment)								
ICC	1.11								
N	4 (Site)								
Observations	73			40			44		
Marginal R^2 / Conditional R^2	0.677/ 1.035			0.698			0.918		

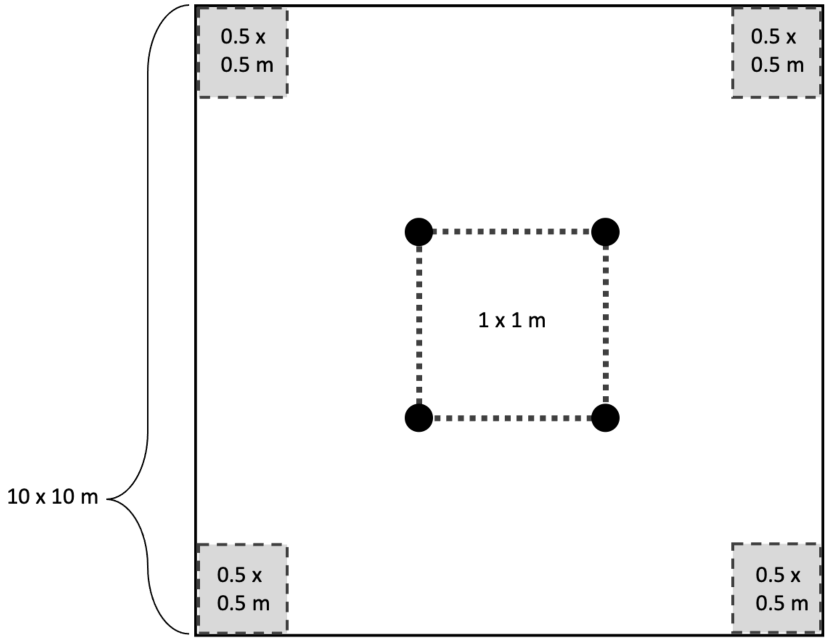
Supplementary Table 7: Full results of PERMANOVA and PERMDISP tests using species abundance data.

			PERMANOVA				**PERMDISP**			
	Dataset	*Group*	*df*	*Sum of Squares*	*F*	*p*	*df*	*Sum of Squares*	*F*	*p*
2020 Pitfall traps	Round 1	Treatment	1	1.196	3.444	0.002	1	0.069	5.58	0.022
	Round 2	Treatment	1	4.873	19.542	0.001	1	0.236	19.138	0.001
	Round 3	Treatment	1	2.883	9.615	0.001	1	0.198	13.383	0.001
	All	Treatment	1	6.448	19.992	0.001	1	0.272	18.961	<0.001
	All	Round	1	2.857	8.860	0.001	2	0.047	1.958	0.144
2021 Pitfall traps		Treatment	2	0.363	0.978	0.448	2	0.026	0.501	0.622
2021 Berlese samples		Treatment	2	1.119	1.831	0.019	2	0.063	1.897	0.161

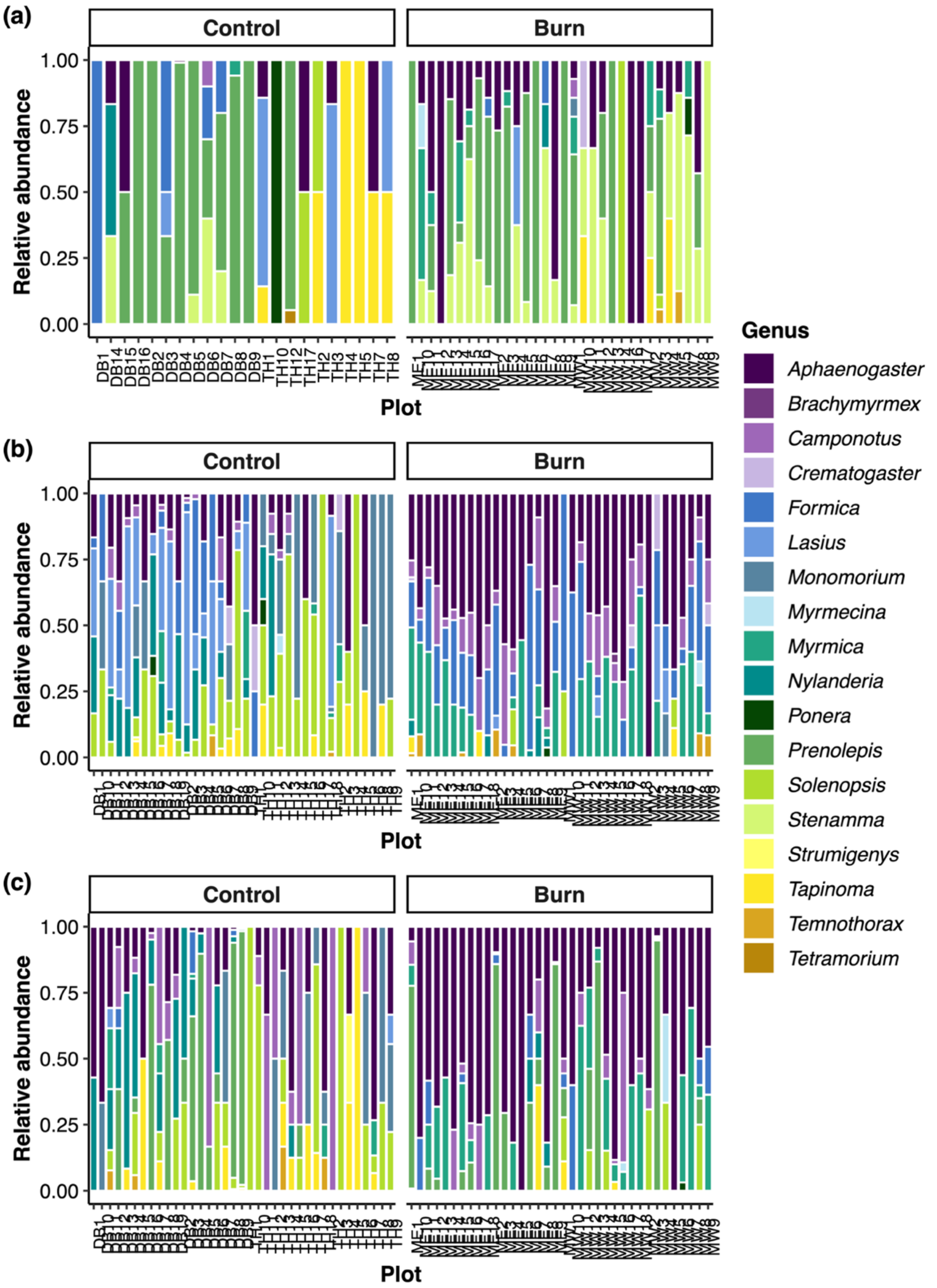
Supplementary Table 8: Indicator species analysis results for 2020 pitfall traps for each combination of round and treatment. Shaded association cells indicate a species is not associated with that treatment in that round. P-values are Benjamini-Hochberg adjusted.

	Association:							
	Burn			*Control*				
Species	*Rd. 1*	*Rd. 2*	*Rd. 3*	*Rd. 1*	*Rd. 2*	*Rd. 3*	*IndVal*	*p (adj.)*
Aphaenogaster rudis	1	1	1	0	1	1	0.884	**0.003**
Camponotus castaneus	0	1	1	0	0	1	0.515	**0.003**
C. pennsylvanicus	0	1	0	0	1	0	0.466	**0.003**
Formica pallidefulva	0	1	0	0	0	0	0.799	**0.003**
Lasius americanus	0	0	0	0	1	0	0.622	**0.003**
Monomorium minimum	0	0	0	0	1	1	0.597	**0.003**
Myrmica latifrons	0	1	1	0	0	0	0.721	**0.003**
Nylanderia faisonensis	0	0	0	0	1	1	0.600	**0.003**
Prenolepis imparis	1	0	1	1	0	1	0.670	**0.003**
Solenopsis molesta	0	0	0	0	1	1	0.751	**0.003**
Stenamma impar	1	0	0	0	0	0	0.522	**0.003**
S. schmitti	1	0	0	0	0	0	0.680	**0.003**
Tapinoma sessile	0	0	0	1	1	1	0.496	**0.013**

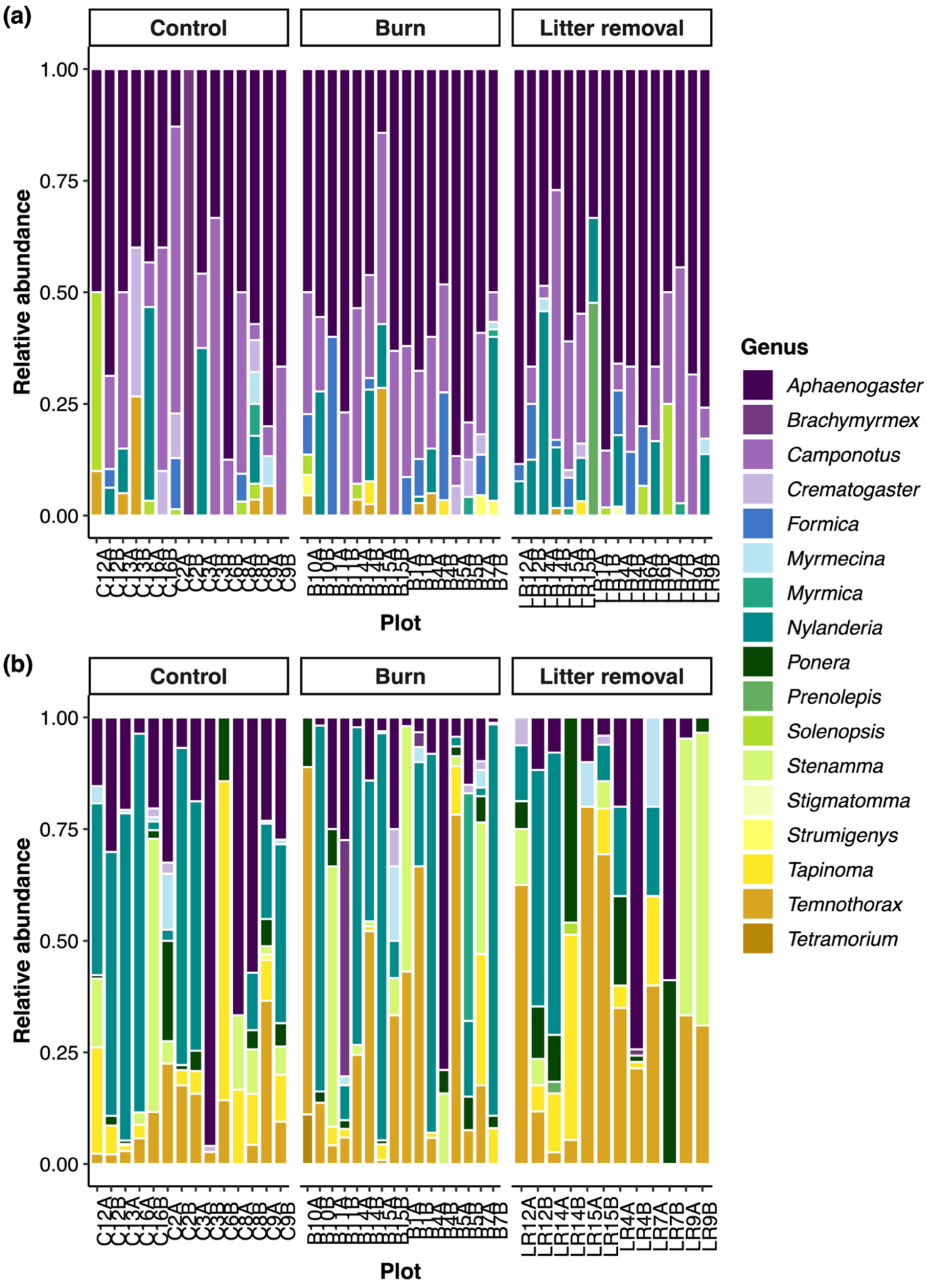
Supplementary Figure 1: (a) Edge of a burn site at Tyson Research Center in April, 2021, (b) pitfall trap with rain cover, (c) litter sample in cloth sifter, and (d) Berlese funnels running.



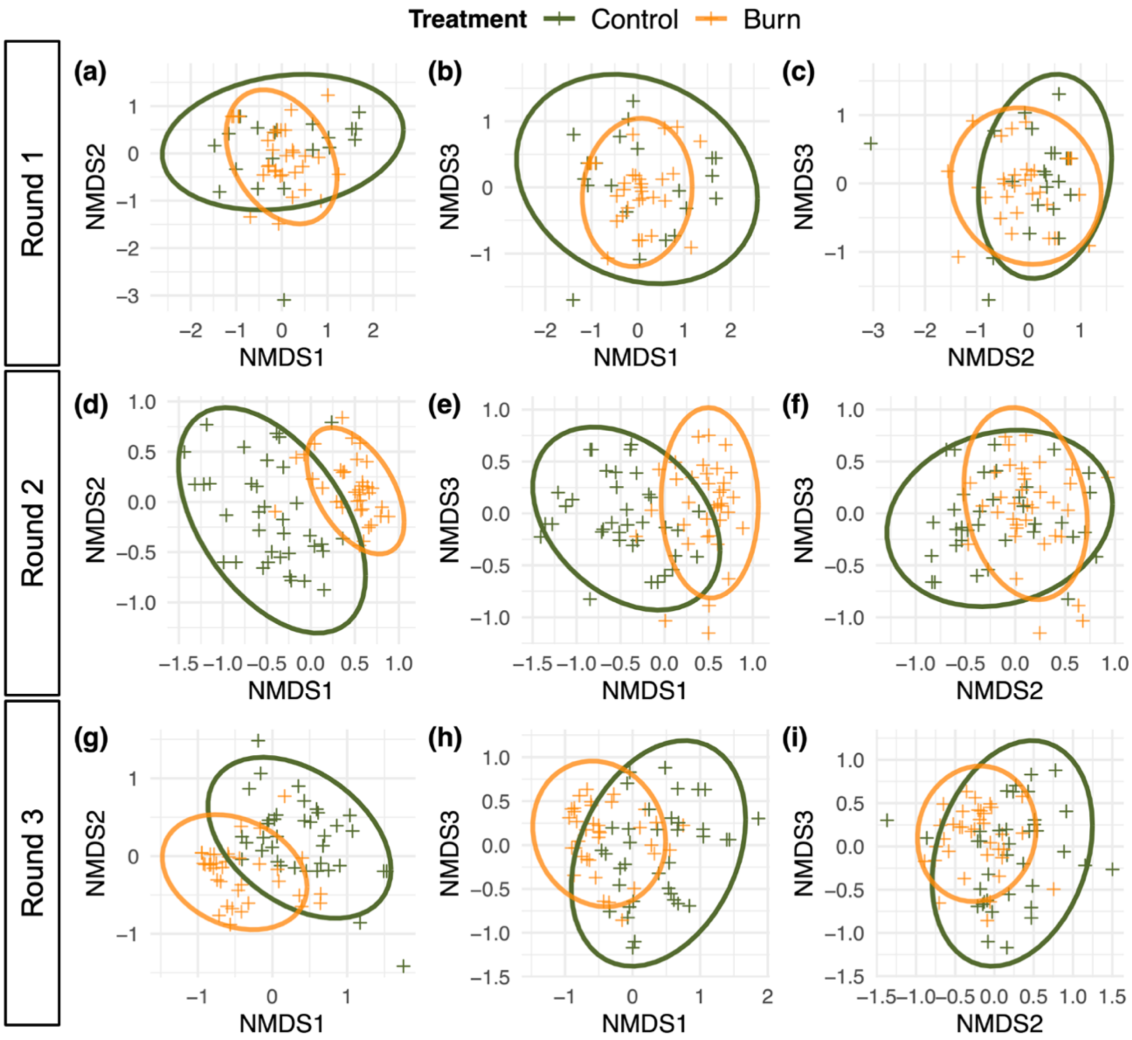
Supplementary Figure 2: Sampling plot design. At both Shaw Nature Reserve in 2020 and Tyson Research Center in 2021, four pitfall traps (black circles) were placed at each corner of a 1 x 1 m square at plot center (dotted lines) within which percent litter cover was estimated. In 2021 at Tyson Research Center, one 0.5 x 0.5 m litter sample (gray shaded squares) was collected from each corner of a 10 x 10 m plot surrounding the pitfall plot before being sifted and aggregated for Berlese funnel sampling.



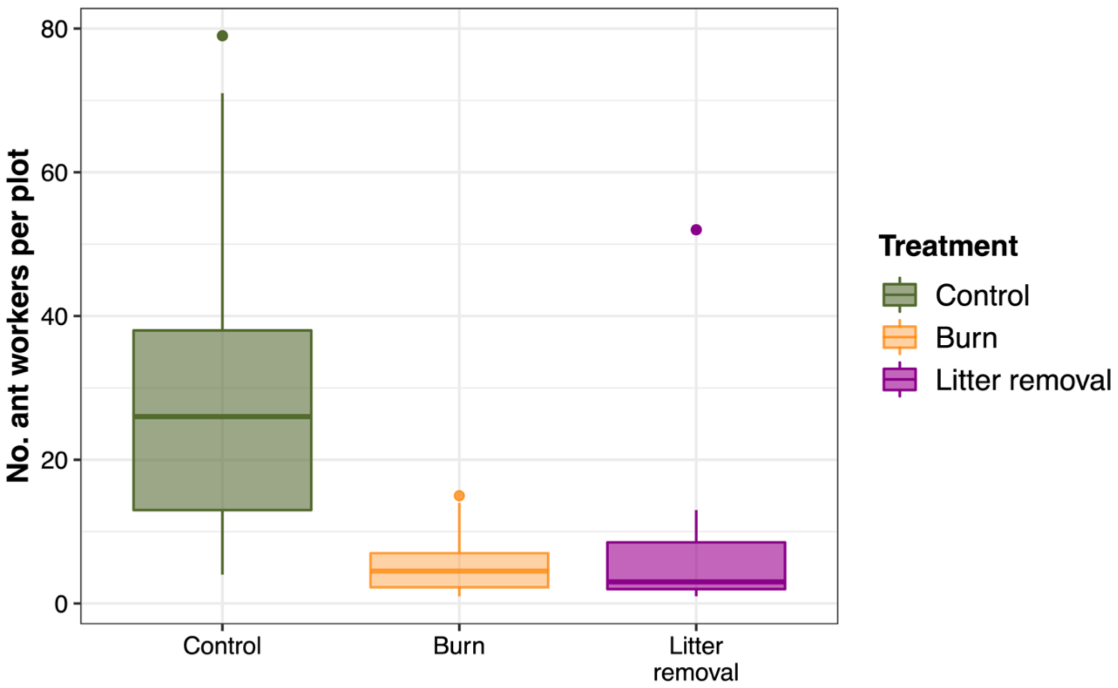
Supplementary Figure 3: Ant worker relative abundance per plot by genus and treatment, from Shaw Nature Reserve in 2020: (a) Round 1 in April, (b) Round 2 in July, and (c) Round 3 in September.



Supplementary Figure 4: Ant worker relative abundance per plot by genus and treatment, from Tyson Research Center in 2021: (a) pitfall samples and (b) Berlese samples.



Supplementary Figure 5: Results of NMDS based on Bray-Curtis distances from 2020 pitfall traps (a–c) Round 1 in April, (d–f) Round 2 in July, and (g–i) Round 3 in September. Ellipses represent 95% confidence intervals for each dimension.



Supplementary Figure 6: *Aphaenogaster rudis* abundance per plot in Berlese litter samples from Tyson Research Center in 2021.