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Diversity and Resilience of Seed-Removing Ant Species in Longleaf Sandhill to Frequent Fire

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Abstract: Prescribed fire is used globally as a habitat restoration tool and is widely accepted as supporting biotic diversity. However, in fire-prone ecosystems, research has sometimes documented post-fire reduction in ant diversity and accompanying changes in seed removal behavior. This is concerning because ants provide important ecosystem services that can aid in restoration efforts, including seed dispersal. In this study, we examined the immediate impacts of fire in the well-studied ant community of longleaf pine forests (LLP) in the SE USA. We surveyed seed-removing ant species in a LLP sandhill ecosystem to investigate the effects of prescribed fire and coarse woody debris (CWD), a nesting and foraging resource, on ant community composition and ant–seed interactions. Seed-removing ants comprised a significant portion of detected ant species (20 of 45); eight of these species are documented removing seeds for the first time. Following an experimentally applied low-intensity summer burn, decreases in seed remover detection were observed, along with reductions in the number of seeds removed, across both burned and unburned areas; neither prescribed fire nor proximity to CWD significantly influenced these factors. Together, these results show that seed-removing ant species constitute a substantial proportion of the LLP sandhill ant community and are relatively robust to habitat changes mediated by low-intensity prescribed burning during the growing season. Considering ant community resiliency to fire, we can infer that using prescribed fire aligns with the goals of restoring and maintaining biotic diversity in this fire-prone ecosystem.

Keywords: Formicidae; *Crotalaria rotundifolia*; *Pinus palustris*; leaf litter; National Ecological Observatory Network (NEON); *Pheidole*; restoration; seed dispersal; tuna–honey bait; *Aphaenogaster*

1. Introduction

Prescribed fire is used globally as a habitat restoration and conservation tool [1,2]. Successful long-term restoration and conservation of fire-prone habitats are contingent upon understanding the relationships between fire and the organisms each habitat supports. Research in various ecosystems has investigated the effects of prescribed fire on the diversity and composition of many floral and faunal groups, including ants.

Ants provide many important ecosystem services that can aid ecosystem restoration efforts [3,4], including soil turnover, aeration, decomposition, and seed dispersal [5]. Ant–seed interactions include both predation and mutualism—many ant species consume seeds, disperse seed, or both [6]. Both dispersing and predatory relationships can be characterized along a spectrum ranging from specific—occurring between select plants and ant species with specialized diets, e.g., phylogenetically constrained seed granivores are highly specialized seed predators; other species are keystone dispersers of plants with nutritious "elasiosome" seed appendages [7–9]—to diffuse, occurring between a diversity of plants and opportunistic, omnivorous generalist ants [10,11]. Proposed benefits of seed dispersers—ants that remove but do not consume seeds—include increasing plant fitness by carrying seeds farther than passive dispersal limits [12] and by depositing seeds in locations with nutrient-rich soil [13]. By moving seeds below ground or into wood, ants

may potentially benefit plants by reducing seed loss to rodent predation and fire [14]. In contrast, seed-predating ants can decrease plant fitness by directly consuming seeds and preventing germination by caching seeds far belowground [15]. Of course, seed predators occasionally act as seed dispersers—seeds cached near the soil surface may germinate before consumption, and discarded seed may germinate in refuse piles [16–18]. By these processes, seed predation and dispersal shape plant communities [8,19–21].

A large body of literature documents the general effects of fire on ant communities (reviewed by Andersen [22]). This research shows that the largest community composition differences are seen in habitats managed with highly contrasting fire regimes. Ants that are most susceptible to direct mortality from fire are those that nest in flammable substrates such as twigs and leaf litter [22]. Studies in fire-prone ecosystems have shown that reductions in ant diversity and abundance are common in temperate forests for as long as six months post-fire, with litter-dwelling ants more adversely affected than soil dwellers [23]. Other ant species are impacted indirectly by changes in habitat mediated by fire [22,24]. One way that fire effects on ant communities impact the broader ecosystem is through seed dispersal. Observed changes in seed dispersal behaviors post-fire include increased seed removal rates [25–27], changes in the composition of seed-removing ant species [25], and shifts in the relative activity of seed-removing species [26,27].

Fire management may affect ant–seed interactions by altering the availability of coarse woody debris (CWD), a resource that contributes to overall biodiversity in forest systems [28–30]. Many invertebrates, such as termites and saproxylic beetles depend on decaying wood, and other taxa use it for nesting or foraging [31,32]. For ants, CWD is known to provide nesting habitat to species in the genera *Camponotus*, *Formica*, *Pheidole* and *Aphaenogaster*, among others [33–35], but the importance of wood presence to other ant taxa is unclear. Ant species richness has been shown to increase in the immediate vicinity of logs as compared to 3 m away [36], and the presence of both fire and CWD has been shown to benefit specific ant species [36]. CWD may offer wood-nesting ants protection from heat- and fire-inflicted mortality, as it does for termites [37]. Additionally, decaying CWD retains moisture during and after burning, which maintains a more hospitable microclimate for ants [31]. For this reason, CWD may be favored by wood-nesting species such as *Aphaenogaster*, a genus generally associated with high-humidity logs [34] and other taxa attracted to cool, humid microclimates. Consequently, CWD may increase the survival of wood-associated ants both during and after fire and mitigate the disruption of seed dispersal after prescribed burns.

In the Southeastern U.S., prescribed fire is commonly used to manage and restore biodiverse longleaf pine forest (LLP) ecosystems [1,38], which are characterized by the presence of *Pinus palustris* Mill. (Pinales: Pinaceae). At present, about only 3% of LLP habitat that existed before European settlement remains intact, and many sites have experienced fire suppression for decades [39]. In areas undergoing restoration and conservation, frequent, periodic prescribed fire is consistently applied. In LLP ecosystems, frequent fire maintains a diverse herbaceous groundcover [1,39–42] and provides a habitat for open-canopy pine specialist animal species, including the gopher tortoise, red-cockaded woodpecker, upland snakes, and brown-headed nuthatch [43,44]. While multiple studies have addressed ant diversity in LLP ecosystems [45–50], few have identified which ant species interact with plant seeds [51–54], and little is known about the impact of restoration activities (including prescribed burning) on seed-removing ants.

Studies specific to LLP ecosystems suggest that prescribed burning has short-term negative impacts on the native ant community. Izhaki et al. [46] detected a decrease in ant abundance and richness following a winter burn, with a return to pre-burn richness and abundance 6 months post-fire. However, ant genera respond variably to fire [45,46], which may influence competition for resources post-burn. Higher disturbance regimes are correlated with the presence of non-native invasive species such as the red-imported fire ant [45,50], which competes with native ants for seeds [52,53]. While fire is generally

thought to reduce CWD volumes, studies have shown variable effects of fire frequency on the volume of CWD in LLP ecosystems.

Prescribed fire and CWD resource use interact with seasonal changes to influence ant community dynamics in LLP ecosystems. It is well established that ant activity varies seasonally [55]. For example, in the well-studied LLP-dwelling *Pogonomyrmex badius* (Latreille), foraging intensity is correlated with energy demands related to colony phenology, with foraging peaking when reproductive castes are being produced [56]. For *P. badius* and others (e.g., *Camponotus* and *Temnothorax* spp.,) peak foraging occurs during the wet, growing season [56,57], while others (e.g., *Prenolepsis imparis* Say [58]) are more active in the dry, dormant season. Previous research on ant community response to prescribed fire in LLP has been limited to dormant season burns [45] or growing season burns without informative controls [46,50]. Based on these studies, it is not possible to disentangle the effects of fire management from seasonal peaks of activity related to abiotic/biotic factors, including the presence of CWD. Growing season burns are preferred in restoration efforts as they induce flowering in the dominant groundcover wiregrass species (*Aristida stricta* Michx./*Aristida beyrichiana* Trinius and Ruprecht) (Poales: Poaceae) [59]. However, growing season burns likely coincide with periods of high energy demands for colonies of many under-studied ant species.

To explore these dynamics, we conducted a two-year study assessing ant community dynamics in relation to fire in a LLP sandhill ecosystem in north-central Florida. We investigated whether prescribed fire and CWD were associated with discernable changes in ant community composition and seed removal behaviors over time. This field study was designed to test the effects of conservation management on ground-dwelling ant species by (1) identifying the seed-removing ant species in LLP sandhill habitat and (2) determining the influence of prescribed fire and the presence of CWD on seed-removing ant species community composition and activity within the context of seasonal change. We expected seed-removing species to be resilient to prescribed fire, resulting in relatively short-term, minor declines in seed-removing activity post-fire, and even smaller declines in seed-removing activity in areas where CWD was present.

2. Materials and Methods

2.1. Study Site

This study was conducted at Ordway-Swisher Biological Station (OSBS), a >9500 acre biological research station owned and operated by the University of Florida in Putnam County, Florida (29.67° N, 82.03° W). The site was xeric sandhill LLP savanna with intact, biodiverse groundcover managed with growing season burns every 2–4 years, on average, for at least the past 20 years [60]. Sandhill, one of six recognized LLP ecosystem types, is found throughout the Southeastern United States Coastal Plain [39] and is characterized by widely dispersed *P. palustris*, a midstory of oaks and shrubs and a dense, grassy, and herbaceous groundcover, dominated by wiregrass [59]. The seeds of at least seven plant species in sandhills at OSBS are known to be dispersed by ants (Table S1).

2.2. Experimental Design

We implemented a Before-After-Control-Impact (BACI) study by conducting ant sampling before and after application of prescribed fire to half our study plots. We established 10 plots, each of which consisted of two 5 × 5 m paired subplots spaced 10 m apart, at least 15 m from other plots, and at least 25 m from management unit edges (Figure 1). Each plot was considered independent from others; plots were distanced farther apart than the average foraging distance of an individual ant colony in eastern North American forests, and thus far enough to attract ants from separate nests [61]. For each plot, subplots were selected with CWD naturally present in one and naturally absent in the other. CWD was defined as, minimally, one segment of a dead, decaying oak or pine tree trunk or branch at least 6 cm in diameter and 1 m in length. All downed wood meeting these criteria were

summed for a measure of total CWD volume (Table S2). The average volume of CWD per plot was 960.1 cm^3 ± 173.7 cm^3 SE.
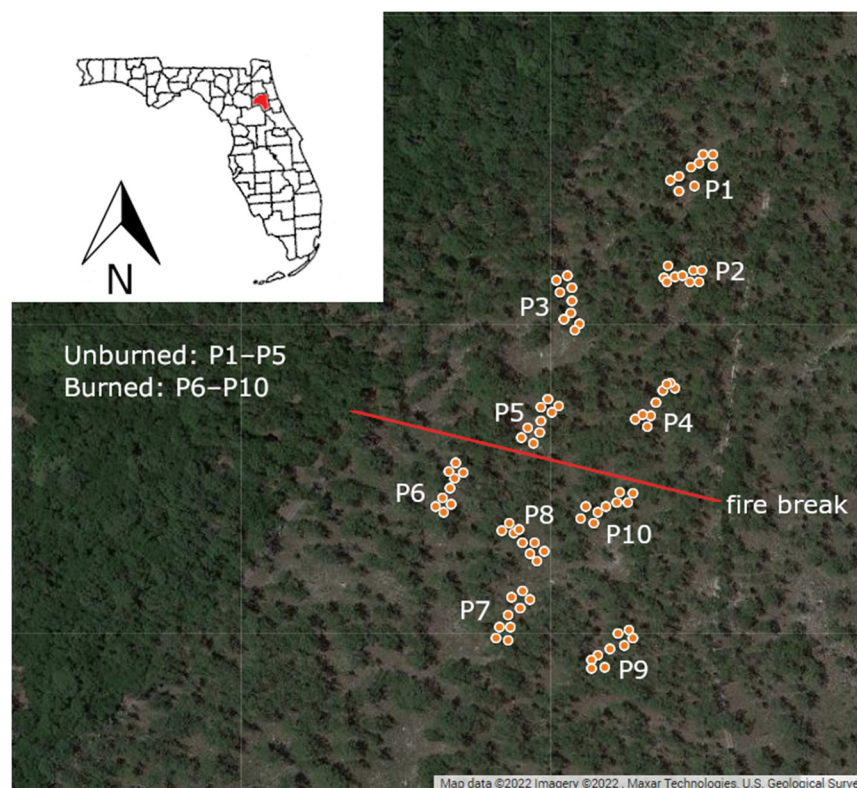

Figure 1. Diagram of the 10 treatment plots, each containing a pair of treatment subplots, at Ordway-Swisher Biological Station in Putnam Co. FL. Red line represents the division between burned and unburned sites. One experimental burn was conducted south of the red line; north of the line remained unburned. Each 25 m^2 subplot is represented by four orange circles, each represents the Global Positioning System referenced locations of a subplot corner.

Prescribed Fire

A low-intensity prescribed fire was lit during the growing season to align with the timing of historically prevalent lightning-ignited fires [62]. A fire break bisected the unburned (UB) and prescribed burn (PB) study plots (Figure 1). A backing fire was lit midmorning on 24 July 2017 along the fire break and progressed 15–30 m south, after which a series of strip fires were used to carry the fire [63] across half of the experimental plots. High relative humidity (>70%) in combination with a relatively low Keetch–Byram Drought Index (287) resulted in a low-severity fire. Ignition was completed after approximately 2 h [64]. A combination of ignition methods promoted fire behavior to vary across the management unit, creating independently altered microsites. The fire was heterogeneous in intensity, severity, and patchiness across the landscape as is typical in LLP ecosystems where subtle variation in elevation, soil moisture, and fuel loading interact to affect the fire regime [65–68].

2.3. Specimen Sampling

Over 15 months, we sampled a LLP sandhill ant community by conducting 12 rounds each of seed trials and tuna–honey bait trials as well as 400 leaf litter sample collections. Activity and composition of seed foragers and non-seed foragers were monitored before and after prescribed burning (sampling periods: 1–3 months pre-, 1–4 months post-, and 10–12 months post-burn (Table 1), hereafter referred to as pre-, post-, and 1-year post-burn), in areas with and without CWD. Ants were sampled using three complementary methods: (1) baiting with *Crotalaria rotundifolia* seed, (2) baiting with a tuna–honey mixture, and

(3) systematic leaf litter sampling. Seed baiting identified seed-removing species specifically, whereas other sampling methods captured the breadth of the larger ant community.

Table 1. Sampling period dates by sampling method. Prescribed burn was conducted on 24 July 2017.

	Sampling Period		
Sampling Method	**Pre-Burn 2017**	**Post-Burn 2017**	**1-Year Post-Burn 2018**
Seed trials	13 June to 9 July	2 Aug to 6 Nov	22 May to 28 June
Tuna–honey trials	4 May to 5 July	31 July to 15 Oct	5 June to 25 July
Leaf litter sampling	12 June to 9 July	1 Aug to 26 Sept	31 May to 30 July

2.3.1. Ant Identification and Characterization

Collected ants were identified to the species level using MacGown [69] as a reference and stored in 95% EtOH at −20 °C at the University of Florida (Gainesville, FL, USA). A representative collection of all identified species was deposited at the Florida State Collection of Arthropods (Gainesville, FL, USA). Ant species in LLP known to forage for seeds were identified by a literature review (Table 2). Species lacking literature records of ant–seed interactions were considered newly identified seed removers. We considered an ant to be removing a seed when the ant had lifted the seed off the substrate and moved a minimum distance of approximately 2.5 cm with the seed retained in its mandibles.

Table 2. Seed-removing ant species present in LLP ecosystems identified in this and other studies.

Species Name	Our Study	Stamp and Lucus [51]	Stuble et al. [52]	Cumberland and Kirkman [54]	Studies in Non-Longleaf Habitat
Aphaenogaster floridana M.R. Smith	✓				
Aphaenogaster treatae Forel	✓	✓			Disperser: Hilley and Thiet [70]
Brachymyrmex depilis Emery			✓	✓	
Crematogaster lineolate (Say)			✓		
Cyphomyrmex rimosus (Spinola)			✓		
Dorymyrmex bossutus (Trager)	✓				
Dorymyrmex bureni (Trager)	✓		✓	✓	
Forelius pruinosus (Roger)	✓	✓	✓		
Forelius sp A	✓				
Formica pallidefulva Latreille	✓				Disperser: Beattie and Culver, Bale et al., Giladi, Ness [71–74]
Nylanderia arenivaga (Wheeler)	✓		✓		
Nylanderia parvula (Mayr)	✓				Disperser: Beattie and Culver [71]
Nylanderia wojciki (Trager)	✓				
Odontomachus brunneus (Patton)	✓				
Pheidole bilimeki Mayr	top	✓			
Pheidole dentata Mayr	✓	✓	✓	✓	Disperser: Giladi [74]
Pheidole metallescens Emery	✓	top			
Pheidole morrisi Forel	✓	✓			
Pheidole navigans Wheeler	✓				

Table 2. *Cont.*

Species Name	Our Study	Stamp and Lucus [51]	Stuble et al. [52]	Cumberland and Kirkman [54]	Studies in Non-Longleaf Habitat
Pogonomyrmex badius (Latreille)	✓	✓			Predator: Harmon and Stamp, Smith, Tschinkel and Domínguez [75–77]
Solenopsis carolinensis Forel			✓		
Solenopsis geminata (Fabricius)	✓				Predator: Horvitz, Carroll, and Risch, Horvitz and Schemske, Kaspari, Cuautle et al., Chauhan et al., Motzke et al., Hernandez-Cumplido et al. [78–86]
Solenopsis invicta Buren			top	top	
Solenopsis nickersoni Thompson	✓				
Solenopsis truncorum Forel			✓		
Tapinoma sessile (Say)			✓		
Trachymyrmex septentrionalis (McCook)	✓				

Studies previously identifying species as dispersers or predators are listed as "Studies in non-longleaf pine habitat". The top seed remover for each study is noted. Stuble et al. and Cumberland and Kirkman [52,53] did not identify all ants to the species level. Some species were identified to the genus level (*Pheidole, Nylanderia, Monomorium*); therefore, those ants cannot be added to this table.

2.3.2. Seed Trials

Seed removal trials served to identify seed-removing ants and monitor their activity in subplots with and without CWD. Each plot was sampled four times during each of the experiment's three sampling periods. Seed trials took place between 8:00 and 13:00 UTC when ant activity was high. During each seed trial, we placed ten *Crotalaria rotundifolia* (Walter) Poiret (Fabales: Fabaceae) seeds on each of two 3 × 5 in index cards, spaced 1 m apart in the center of each subplot. Observers watched ant activity within each plot for 30 min, alternating between subplots at 5 min intervals. Ants observed removing seeds were collected using an aspirator.

Seeds of the common, native herbaceous perennial *C. rotundifolia* (commonly called rabbitbells) were used because fruits are continuous and abundant from March to December [87]. *Crotalaria* seeds do not possess a nutritious elaiosome appendage; their attractiveness to ants may be a result of chemical mimicry, but its mechanism remains unknown. Despite their lack of elaiosomes, *C. rotundifolia* seeds are known to attract a wide variety of genera [51,53]. Ants characteristically carry these seeds by using mandibles to grasp an indentation in the seed coat; this is where the seed attaches to the fruit wall before detaching upon ripening [51]. Seeds were collected from ripe fruit and stored in a refrigerator set to 4 °C for up to 4 months until use. While other ant-dispersed plants were present, seeds could not be collected in the quantity necessary for seed trials.

In addition to spacing plots at least 15 m apart to avoid overlapping recruitment to baits, we tested for spatial autocorrelation by performing Mantel tests on the plot level pre-, post-, and one-year post-burn composition of seed removers. Plot distances were calculated from the distances between the midpoints of each plot. In each test, we failed to reject the null hypothesis that plots were not spatially autocorrelated (pre: $p = 0.071$, post: $p = 0.59$, one-year post: $p = 0.054$), indicating that they can be considered independent replicates [88].

2.3.3. Tuna–Honey Bait Trials

Tuna–honey baits were used to monitor generalist ants attracted to fat, protein, water, and sugar. Singly or combined, tuna and honey are commonly used to estimate and monitor ground-foraging ant richness and composition [89,90]. Each subplot was sampled four times during each sampling period. Tuna–honey bait stations consisted of a 2 cm mound of a 5:2 tuna to honey mixture atop an index card. Because tuna–honey baits can become dominated by individual fast-recruiting species and we wanted to capture the breadth of generalist ants, we set up four bait stations in each subplot, each located 1 m toward the plot center from each outer corner. We did not quantify the amount of tuna–honey bait removal (as with seed trials) after the initial establishment of all bait stations; rather, each subplot was observed once approximately every 40 min over a 4-hour period for a total of 5 separate collections. Ants at each station were collected by aspirator and pooled by subplot.

2.3.4. Leaf Litter Sampling

Leaf litter sampling was conducted to assess overall community composition in the burned and unburned areas and provide a more complete characterization of ant community composition than baits alone [89]. Each 50 m transect sample consisted of 10 individual 1 m^2 leaf litter collections, sifted and bagged every 5 m along the transect. During each sampling period, litter samples were collected from 20 transects: 10 from the burned area and 10 from the unburned area. Each transect location was assigned using a stratified random protocol, which maintained a 25 m buffer at the perimeter of the management unit and did not allow intersection with subplots. Arthropods were extracted from each sample over 48 hours using the mini-Winkler method [91] in a greenhouse at the University of Florida (Gainesville, FL, USA) set to 26 °C.

2.3.5. Corroborative Samples

To validate the completeness of community capture using the three sampling techniques described above, we compared our resulting community composition with the ant species recovered from pitfall traps administered at OSBS by the National Ecological Observatory Network (NEON). Four NEON sampling sites were chosen for comparison; each had a fire management history similar to our study unit and sites were spaced across OSBS to isolate the effects of ecosystem type and limit similarity in species composition attributable to proximity effects (Figure S1). Pitfall traps (3/site; 11 surveys) were administered following NEON protocol [92] between May and September 2017, coincident with our pre- and post-burn sampling periods. All ants were identified to species-level as described above and are retained by NEON.

2.4. Statistical Analyses

Differences in ant species diversity and composition across treatments and plots over time were evaluated using R [93]. Species accumulation curves and sample coverage estimates for each sampling method were created to assess the completeness of sampling efforts using the iNEXT R package [94] (Figure 2). To visualize how species' abundances varied across sampling periods, heat maps depicting average frequency of species detection were produced for each sampling technique (Figures S2 and S3).

To contextualize the diversity of seed-removing species within the broader ant community, seed-removing ant composition was compared to leaf litter sampling composition. For every ant species detected by litter sampling we calculated individual ant species' contribution to beta diversity (SCBD) with the adespatial R package [95] using the method developed by Legendre and De Cáceres [96]. With these calculations, we identified the seed-removing species whose contribution to beta diversity was higher than the community mean as well as how much seed-removing species as a subset contributed to the beta diversity of the overall community.
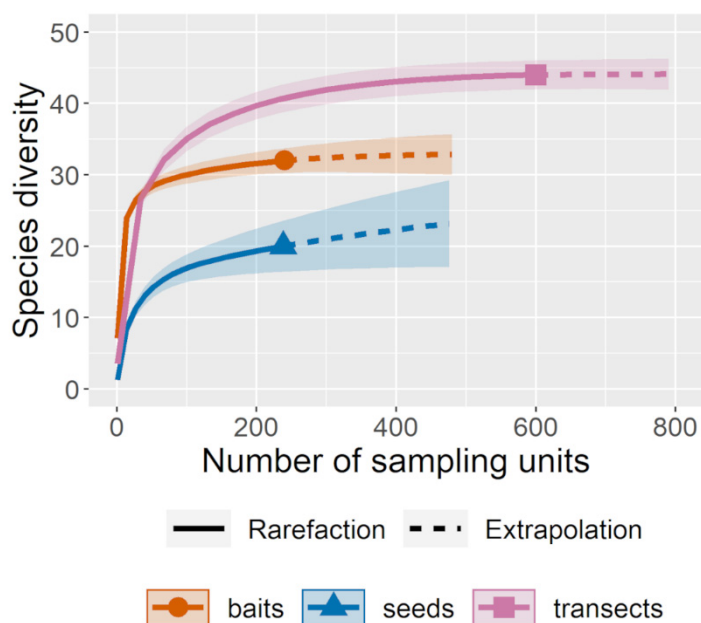
Figure 2. Ant species richness accumulation and extrapolation by sampling type: leaf litter quadrats, tuna–honey bait recruitment, and seed bait removals.

To investigate seed-removing species' resilience to prescribed fire we assessed the interactive effect of burn treatment and sampling period on the relative abundance and composition of these species compared to the ant community response as a whole. Using leaf litter sampling data, we defined "composition" as the relative detection rate of each species by transect, i.e., the proportion of samples the species was present in out of the total samples (10) per transect. Nonparametric multivariate analyses of variance (npMANOVA) were conducted on resultant matrices with 999 permutations using the adonis function in the vegan R package [97]. Results were visualized with non-metric multidimensional scaling (NMDS) using Bray–Curtis dissimilarity distance measures. Homogeneity of multivariate dispersions was tested using the betadisp function in the vegan R package [97]. Pairwise comparisons of sampling period were conducted with the RVAideMemoire R package [98] using the Bonferroni correction for multiple comparisons.

We evaluated the effects of prescribed fire, CWD, and sampling period on seed trial species richness using a generalized linear model (GLM) with Poisson distribution. Separately, we tested the effect of these variables on the proportion of seeds removed using a GLM with a quasibinomial distribution to account for overdispersion. For each GLM, model selection was determined by comparing AICc or quasi AICc (QAICc) values of all possible variable combinations with the MuMIn R package [99] and choosing the model where the change in AICc or QAICc was less than 2. Significant effects were determined with Wald chi-square tests (type II ANOVA) and then evaluated by pairwise Tukey comparisons with the emmeans R package [100]. To further address whether CWD affects seed removal, we determined if potential wood nesters removed more seed than non-wood nesters with a Wilcoxon rank sum test, and whether potential wood-nesting species were detected more frequently than non-wood nesters in CWD subplots (using tuna–honey bait trial data) with a Welch two sample *t*-test.

3. Results

Over the course of this study, we detected 45 total ant species, 20 of which removed plant seeds (Table S3, Figure 3). Species accumulation curves indicate that richness was well sampled across sampling methods (Figure 2). Sample coverage estimates were 99.95% for both leaf litter and tuna–honey bait sampling and 98.66% for seed removal sampling. Several species found in this study were not found in the pitfall samples collected by NEON.

Overall, the three sampling methods in this study captured nearly all species recovered from NEON pitfall samples with the exception of six (largely nocturnal) species (Table S3).
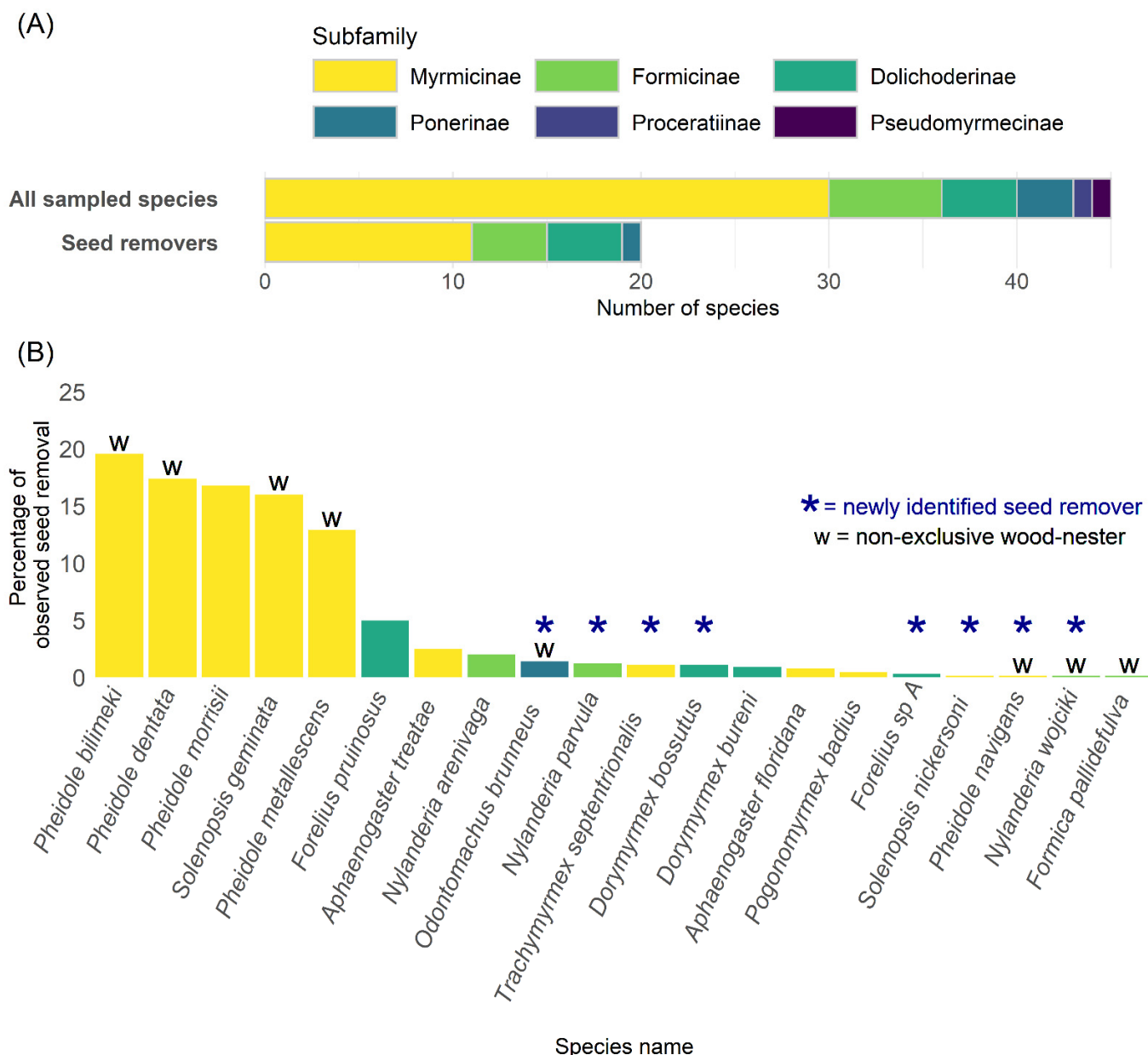

Figure 3. (**A**) Subfamily breakdown of all species sampled compared to the subset of species identified as seed removers. (**B**) Seed-removing ant species and the percent of seeds each species removed out of 644 total seeds removed across the entire study. Asterisks denote new observations of seed removal for a species. "w" identifies the species as a non-exclusive wood nester.

3.1. Seed-Removing Species

Seed removers were a subset of species collected at tuna–honey baits, which were themselves a subset of the more extensive community of ant species recovered from leaf litter samples (Figure 3, Table S3). Collectively, ants in the subfamily Myrmicinae removed the majority of seeds (Figure 3A): *Pheidole bilimeki* Mayr was responsible for 19.4% of the seeds removed over the course of the study, *Pheidole dentata* Mayr: 17.4%, *Pheidole morrisii* Forel: 16.7%, *Solenopsis geminata* (Fabricius): 16.1%, and *Pheidole metallescens* Emery: 12.9% (Figure 3B). Other species observed removing less than 5% of seeds included two Forelius spp. (dolichoderines), three *Nylanderia* spp. (dolichoderines), and two *Aphaenogaster*

spp. (myrmicines). Seed-removing behavior is reported for the first time for eight ant species (Table S3; Figure 3B). The top five seed removers—*Pheidole bilimeki*, *Pheidole dentata*, *Pheidole morrisii*, *Solenopsis geminata*, and *Pheidole metallescens*—were also the most frequently encountered seed-removing species, each present at 13–27% of seed trials. These five species were also among the most common at tuna–honey baits, with each species present at over 25% of tuna–honey baits; *P. bilimeki* and *P. dentata* were present at ≥70%. High prevalence at both seed and tuna–honey baits suggests these five species are among this habitat's most abundant generalists. Each top *Pheidole* species was also detected in 11–28% of the total transects, making the genus among the most commonly sampled species across methods. *Solenopsis geminata* was less common in leaf litter samples (present in 4% of transects) than in tuna–honey baits and seed trials. The remaining seed-removing species were detected at ≤6% of the seed baits, indicating they were less abundant competitors for seed resources in our sampling.

Many seed-removing species were large drivers of beta diversity. Beta diversity measures the variation in community composition among samples; species with high species' contribution to beta diversity (SCBD) vary the most in abundance among samples and are neither rare nor relatively widespread [101,102]. In leaf litter samples, 10 of the 16 species that contributed more than the mean SCBD were seed removers (Figure S4). This indicates that many seed-removing species were detected regularly by litter sampling, but detections fluctuated over time and/or space.

Eight seed-removing ant species nest non-exclusively in CWD (Figure 3B). Collectively, wood-nesting species were not detected in CWD subplots more often than species that do not nest in wood (t (10.5) = −1.4, *p* = 0.9). On average, potential wood nesters were approximately 3.5% more likely to be detected in CWD than no CWD plots, while non-wood nesters were approximately 2.6% more likely to be detected in plots without CWD than with CWD. Two seed-removing species were detected in subplots with CWD at least 15% more often, on average, than in subplots without CWD: *P. bilimeki* (21%) and *Odontomachus brunneus* (Patton) (17%), which are both potential wood nesters.

3.2. Community Resiliency to Fire

Prescribed fire was associated with differences in community composition for both the overall litter ant community and among seed-removing species (Figure 4). Multivariate permutation analysis indicated that burn treatment explained significant differences in the composition of the seed removers within the litter ant community (F = 4.6; df = 1, 53; *p* = 0.001) (Table 3, Figure 4A). Differences in composition between burned and unburned areas can be partly attributed to greater variability in species detection rates in burned habitat, i.e., variances between burn treatments were heterogeneous (F = 6.8; df = 1, 57; *p* = 0.012). Similarly, multivariate permutation analysis indicated that burn treatment explained significant differences in the composition of the overall litter ant community (F = 6.3; df = 1, 53; *p* = 0.001), with heterogeneous variances (F = 5.1; df = 1, 57; *p* = 0.028) also attributable to greater variation in composition among burned than unburned litter transects (Table 3, Figure 4B). The sampling period was an additional significant predictor of overall litter ant community composition (F = 2.3; df = 1, 53; *p* = 0.001). Variances between sampling periods were also heterogeneous (F = 3.7; df = 2, 56; *p* = 0.031), with the most composition variability among transects in the post-burn sampling period. The composition of the overall litter community was different between pre-burn and 1-year post-burn sampling (*p* = 0.033) and post-burn and 1-year post-burn sampling (*p* = 0.048) across burn treatments. Although burn treatment and sampling period (for the overall ant community) were found to be significant predictors of community composition, these predictor variables explained a small proportion of the variability in the observed data (burn treatment R^2 = 0.9 for the overall ant community and R^2 = 0.07 for seed removers; sampling period R^2 = 0.07 for the overall ant community). Importantly, we did not detect interactive burn treatment and sampling period effects for either the seed-removing subset or the

overall community, suggesting that prescribed growing season fire does not significantly alter the LLP ecosystem ant species assemblage.
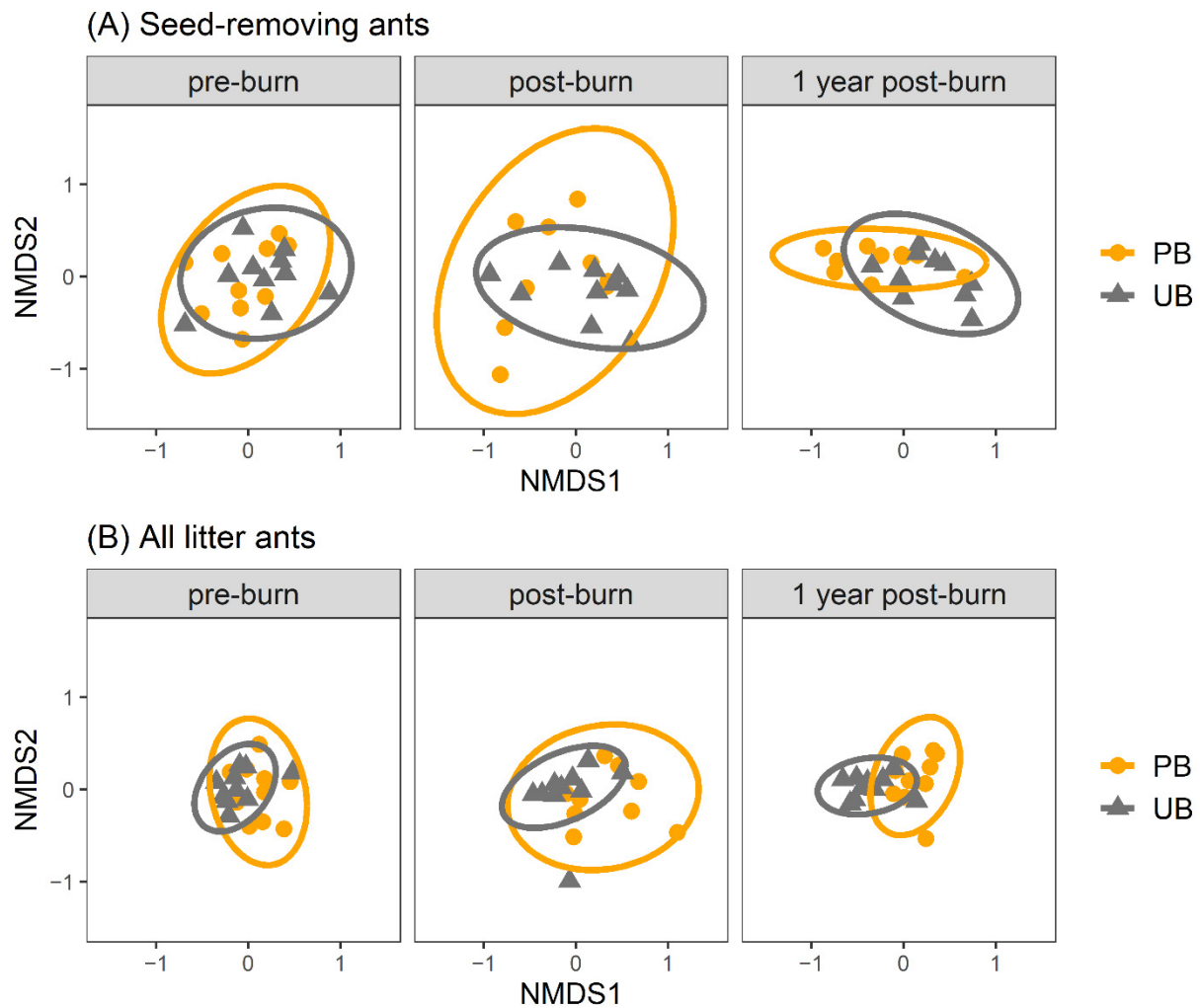
(A) Seed-removing ants

(B) All litter ants

Figure 4. NMDS for ant community composition. Burn treatment is labeled "PB" for prescribed burn and "UB" for unburned. (**A**) Seed-removing species: species frequency of occurrence in transects using Bray–Curtis distance metric. (**B**) Overall community: species frequency of occurrence in transects using Bray–Curtis distance metric.

Table 3. Non-parametric multivariate analyses of variance of leaf litter community composition.

		Seed Removing Species					Whole Ant Community				
	DF	Sum Sqs	Mean Sqs	F-Model	R^2	*p*-Value	Sum Sqs	Mean Sqs	F-Model	R^2	*p*-Value
Burn treatment	1	0.8	0.8	4.6	0.07	0.001 *	0.71	0.71	6.3	0.09	0.001 *
Sampling period	2	0.45	0.22	1.3	0.04	0.225	0.51	0.26	2.3	0.07	0.002 *
Burn x Sampling period	2	0.31	0.15	0.89	0.03	0.585	0.29	0.14	1.3	0.04	0.179
Residuals	53	9.3	0.17		0.86		6.0	0.11		0.8	
Total	58	10.8			1		7.5			1	

Asterisks (*) denote significant predictor variables.

3.3. Seed Removal Activity

3.3.1. Ant Richness

　　Seed-removing ant richness remained stable regardless of burn treatment or CWD proximity. The best-fitting GLM model had sampling period as the sole predictor variable, predicting ~32% of deviance (χ^2 = 12.4, *p* = 0.002). Comparisons across burn and CWD treatments determined that seed-removing species richness was 1.9 times greater pre-burn than post-burn (z = 3.49; *p* = 0.0014), with an average of approximately six species removing seeds per sampling round compared to approximately three species, on average, post-burn. Moreover, species richness of seed-removing ants was 1.6 times less post-burn than 1-year post-burn (z = −2.55; *p* = 0.03). Species richness of seed-removing ants pre-burn and 1-year post-burn did not differ (z = 0.99, *p* = 0.58) (Figure 5A).
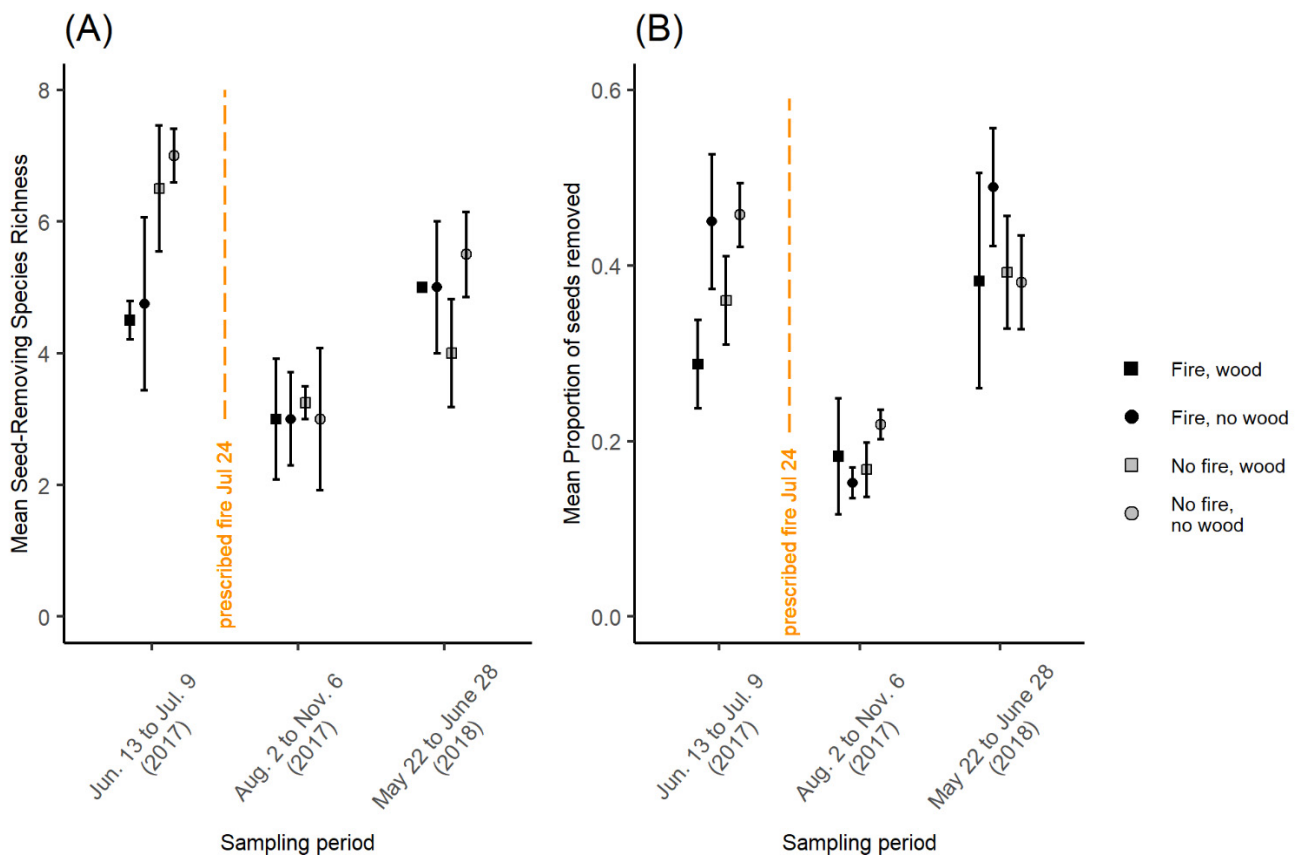

Figure 5. Sampling period effects in seed trials. Error bars are ±1 SE. (**A**) Effect on species richness. (**B**) Effect on number of seeds removed.

3.3.2. Proportion of Seeds Removed

　　The number of seeds removed was unaffected by prescribed fire or CWD alone or prescribed fire in combination with CWD. The best-fitting GLM model included sampling period and CWD as predictors, with sampling period as the sole significant explanatory variable explaining ~46% of the total deviance (χ^2 = 36.2, *p* < 0.0001) (Table 4). Pairwise Tukey comparisons across burn and CWD treatments indicated that the proportion of seed removed pre-burn and 1-year post-burn was greater than twice that of post-burn (z = 5.08; *p* < 0.0001, and z = −5.64; *p* < 0.0001, respectively). By contrast, the proportions of seeds removed pre-burn and 1-year post-burn were not significantly different (z = −0.64; *p* = 0.82) (Figure 5B). Although CWD was included in this model, it was a nonsignificant predictor, explaining approximately 4% of total deviance (χ^2 = 3.6, *p* = 0.06). Additionally, across subplots, wood-nesting species did not remove more seeds, on average, than non-wood nesters (W = 40.5, *p* = 0.73).

Table 4. GLM model analysis of deviance: Proportion of seeds removed by ants across time in subplots with and without coarse woody debris (CWD).

	DF	Deviance	Residual DF	Residual Deviance
Null model			47	547.83
Sampling period *	2	252.88	45	294.94
Coarse woody debris (CWD)	1	22.93	44	272.01
Sampling period x CWD	2	10.0	42	262.01

Asterisks (*) denote significant predictor variables.

4. Discussion

Pine forests of the Southeastern United States Coastal Plain have a rich assemblage of ant species. This study detected a total of 45 ant species within one LLP sandhill site (<4 hectares) in north-central Florida, with an additional six species in similarly managed sandhill habitats detected by NEON pitfall traps. These 51 species represent approximately 20% of the total ant diversity currently documented in Florida. Seed-removing ants were prevalent throughout the site, regardless of treatment, and accounted for nearly half (44.4%) of the total species detected at the LLP site sampled (Figure 3A).

4.1. Seed-Removing Species

This study indicates that seed removal is a relatively common behavior among ant species in longleaf pine sandhill habitat. We documented a high number of ant species (20) removing seeds; these species represent the four most common ant subfamilies we detected (Myrmicinae, Formicinae, Dolichoderinae, Ponerinae) and were found to be abundant using three detection methods: seed baits, tuna–honey baits, and leaf litter sampling. The eight newly documented seed removers include the poorly studied LLP sandhill endemic ant species, *Dorymyrmex bossutus* (Trager) and an unnamed *Forelius* species (designated sp A by Deyrup [35]), as well as the closely related and less habitat-restricted species, *Dorymyrmex bureni* (Trager) and *Forelius pruinosus* (Roger), respectively. The most unexpected seed remover was *O. brunneus*, a trap-jaw ant and arthropod predator, which uses its snapping jaws to stun prey [35]. We observed these ants exhibiting snapping behavior upon discovering seeds, which were thrown into the air, and then retrieved. Three tropical species in this genus are known to collect seeds [103], but this marks the first record of this behavior by *O. brunneus*. In contrast to the richness of seed removers detected here, the majority of seed dispersal in other ecosystems (e.g., North American deciduous forests and Australian chaparral) is performed by a primary keystone species, a single species, or species complex that is mutualistic with myrmecochorous plants [7,104].

The twelve ant species previously documented removing seeds in longleaf pine habitat include both seed specialists and ants with a broad generalist diet. *Solenopsis geminata* and *P. badius* are well-known granivores [78–86], but most are generalists not previously considered to be seed dispersers or predators (Table 2). In *Pheidole* (the genus containing four of the five top seed removers), major workers possess large mandibles that can grind seeds [35]. Despite their morphology, *P. dentata* has been previously identified as a seed disperser [74]. Other seed removers in our study that were previously identified as dispersers include *Formica pallidefulva* Latreille, *Nylanderia parvula* (Mayr), and *Aphaenogaster treatae* Forel (Table 2 and references therein). However, additional knowledge of species-specific seed behavior beyond the classification as predators or dispersers is required to assess seed fate.

Ant species differ in behaviors that influence plant fitness [105], including seed dispersal distance [51,72] and the spatial arrangement of discarded seeds (clumped versus diffuse). For example, researchers have observed *P. metallescens* frequently dropping seeds in depressions upon return to the nest and *P. badius* carrying seeds into nests and later discarding them, uneaten, on the soil outside [51]. In our study, *Pheidole* spp. and *S. geminata* removed the most seeds and were the most numerous at seed baits, but we did not determine seed fate. Less frequently detected ants may be more effective seed dispersers, exerting a greater

influence on plant community assembly than ants with higher seed recovery rates if ants which retrieve relatively greater amounts of seeds also exhibit behaviors that negatively or neutrally affect plant fitness. For example, ant body size has been positively correlated with seed dispersal and foraging distances [72], and while singly, each of the two *Aphaenogaster* spp., *O. brunneus*, *P. badius*, and *F. pallidefulva* accounted for less than 5% of the observed seed removal, they were the largest seed-removing ants. Lower incidences of seed removal by other species—e.g., *Forelius* sp A was only observed removing seed twice and four other species (Figure 3B, right of *Forelius* sp A) were observed once—do not suggest they have a large collective impact on plant fitness, but contribute to our observation that generalist ants frequently interact with seeds in this ecosystem.

Daily fluctuations in species' foraging behavior can also determine seed fate. We conducted our seed trials between 8:00 and 13:00 UTC to avoid the warmest part of the day when many ant species decrease foraging activity, but *F. pruinosus* and *P. badius* were observed at later, hotter times when other species became less active. The diversity of ant species attracted to seeds in this habitat suggests that phenology of seed release could determine whether a seed is picked up by *S. geminata* or *Pheidole* spp., or whether more heat-tolerant species have a higher likelihood of seed discovery and retrieval.

Ant recruitment to seed may also depend on plant-specific dispersal mechanisms. Some plants, such as *C. rotundifolia*, use ballistic dispersal for initial seed release with ants as secondary agents of dispersal. In this case, individual seeds will likely be spread out. Other species simply drop seeds so recruitment to seeds concentrated around these plants seems more likely. While our sampling methods were successful at detecting seed removers, natural recruitment was limited. All individuals observed removing seeds were collected before they could recruit nestmates, but some ants were able to retrieve seeds and return to the nest during the 5 min breaks between observation periods. This may have influenced our data on relative seed removal in one of two ways. First, our data on percent seed removal may be skewed towards ants with faster recruitment times, although we cannot determine whether non-collected ants recruited nestmates. For example, in our study, *Pheidole* spp. and *S. geminata* removed the most seed and were the most numerous at seed baits. They also have relatively large colonies and fast recruitment. Second, inhibiting recruitment by removing some workers may have given other species the opportunity to remove seed without being excluded from it by dominantly aggressive species. For instance, we frequently observed *Pheidole* spp. biting each leg of large-bodied *Aphaenogaster* spp. at tuna–honey baits, thereby immobilizing them and preventing food retrieval. While these concerns do not invalidate the data presented here, greater attention to these methodological limitations should be taken into account by future researchers.

4.2. Community Resilience to Fire

Although frequent fire temporarily removes much living plant material and raises the heat index of aboveground microhabitats, ants are resilient in two ways: (1) many species nest far enough belowground to avoid direct mortality from fire, and (2) more susceptible ant species such as litter dwellers can survive in and recolonize from unburned areas.

The soil-nesting habits of the seed removers in LLP sandhill likely contribute to their resilience in this community. We found that ants identified as seed removers were common surface foragers and active across sampling periods, both before and after fire management (Figures 4, S2 and S3), and no significant change in ant community composition caused by fire was detected. The LLP ant community appears to be robust to direct mortality and fire-inflicted habitat change. None of the seed-removing species detected nest exclusively in leaf litter or wood, where they would be most vulnerable to direct fire mortality. All seed-removing species nest in soil, three also nest in wood, and five also nest in wood and leaf litter. Belowground nesting is advantageous in a fire-prone habitat, as exposure to lethal soil temperatures during a fire is typical only at the surface to a couple centimeters deep [106,107]. Soil nest depth is not known for all seed removers but typically ranges between 15 and 25 cm for *D. bossutus* [108] at the most shallow to up to 3 m for *P. badius* [109].

Many herbaceous plants quickly re-sprout after low-intensity fires in LLP sandhill and other fire-prone habitats. As such, ants are not facing a barren habitat post-fire. Re-sprouting and unburnt or partially burnt surviving plants provide food in the form of honeydew resources from sap-sucking insects and by providing habitat for invertebrate ant prey.

Unburned plots or microsites provide reservoirs for aboveground nesters. For the overall community for which sampling period was a significant predictor variable, community composition 1-year post-burn differed significantly from both the pre-burn and post-burn sampling periods. Mean species richness was the highest during the 1-year post-burn sampling period (mean = 16.2 ± 1.16 SE for burned transects and mean = 17.5 ± 0.65 SE for unburned transects), and there was less species turnover among transects than in the post-burn sampling period, suggesting that heterogeneous fire regimes support higher ant diversity.

While no significant effect of fire or CWD was found, beta diversity patterns suggest that burning affects community composition through species turnover. The heterogeneous variances observed between burned and unburned areas for both seed-removing and overall community composition indicate greater species turnover and richness differences among burned than among unburned transects through time. Species turnover, i.e., variability in species detections among transects, is largely driven by species with a relatively high SCBD (Figure S4). Seed removers accounted for 42.7% of beta diversity in leaf litter samples and there was more variance in seed-removing ant community composition, evidenced by the larger spatial extent of dots in the NMDS plots (Figure 4A), than that of the more-inclusive overall ant community (Figure 4B). This likely corresponds to greater habitat heterogeneity in the burned compared to the unburned treatment, where uneven fire contributed to variable microhabitats, with areas left unburned with intact groundcover and burned areas with little to no leaf material in which ants could actively forage or nest. While seed-removing and overall ant composition between burn treatments largely overlapped (Figure 4), they were still determined to be significantly different (Table 3). Four unique species were found in each burn treatment, but all were relatively rare (found in 2% of transects or fewer), indicating that species turnover, rather than unique species distinctions, drove the significant differences between burn treatments across time.

4.3. Seed Removal Activity

Seed removal activity was resilient to fire and CWD influences. Sampling period was the most important predictor of seed-removing species richness and the number of seeds removed (Figure 5, Table 4). While our study defined sampling periods with respect to burn timing, differences observed between sampling periods are the same for both burned and unburned plots and are therefore attributable to seasonality of ant behavior. We observed the same phenological trend across CWD subplots in both burned and unburned plots of high seed-removing ant species richness in early summer, followed by a drop in richness in late summer into fall, and a higher richness during the following spring and summer (Figure 5A). This same pattern was observed for seed removal: the lowest seed removal activity was recorded across burn treatments in the post-burn sampling period (Figure 5B). These same patterns across treatments may indicate a seasonal dip in seed foraging—following a decreased abundance of seed into the cooler months. This trend is unlikely to be explained by a general decrease in ant activity in the late summer and fall, because species richness recovered at tuna–honey baits increased (from 11.5 to 13.25, on average) during the same period.

Coarse woody debris has been shown to be a resource to ants in past studies [31–35]. In particular, Ulyshen et al. [32] found an association between *P. dentata* and burned logs in pine forests. In our study, some seed removers such as *P. bilimeki* and *O. brunneus* were detected more often in plots with CWD, but species richness and the number of seeds removed did not vary with CWD presence. Unlike other studies where species nesting in wood was measured [32], or where specific measured distances from wood was a predictor variable for species richness [36], we did not check CWD for nests, and, while our seed trials

were conducted at the center of each subplot, CWD occupied varying space and position within subplot parameters. While this study was unable to establish relationships between seed removers and CWD, future species-specific studies may reveal significant associations that necessitate more direct study. Moreover, this site has been managed for biodiversity for more than 2 decades, making it a relatively undisturbed example of the sandhill ecosystem. Southeastern U.S. pine habitats historically managed for timber resources have densely planted pines that shade out groundcover and inhibit biodiversity until they are restored by thinning and other management practices, so CWD may be more beneficial at LLP sites during the restoration process.

4.4. Conclusions

Overall, these results demonstrate high resilience to fire among ants in LLP sandhill, an important ecosystem that is undergoing extensive biodiversity restoration in the Southeastern United States. Considering ant community resilience to fire, we can infer that using prescribed fire aligns with the goals of restoring and maintaining biotic diversity in this fire-prone ecosystem.

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