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The Ecology of Central Florida's Thief Ants (Hymenoptera: Formicidae: Solenopsis)

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THE ECOLOGY OF CENTRAL FLORIDA'S THIEF ANTS (HYMENOPTERA: FORMICIDAE: *SOLENOPSIS*)

by

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B.S. Boise State University, 2016

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
in the Department of Biology
in the College of Sciences
at the University of Central Florida
Orlando, Florida

Summer Term
2019

Major Professor: Joshua R. King

ABSTRACT

Thief ants of the genus *Solenopsis* are a diverse group of ants that are found in ant communities throughout the world. They have long been purported to practice lestobiosis, an interaction between small and larger-bodied ants, where small ants cryptically tunnel into larger-bodied ant nests within the subterranean environment and steal brood or eggs for consumption. Thief ants are extremely small, measuring 1-2 mm in length and many of the species within this group practice a subterranean life history, where they live the entirety of their lives exclusively belowground. Due to these key characteristics, the ecology and natural history of this group of ants has remained largely unknown despite their noted high abundance within the southeastern United States, especially in upland ecosystems. The purpose of this thesis is to improve our understanding of the ecology of this enigmatic group, providing a solid foundation for future work on their behavior, biology, and natural history. Therefore, this project first attempts to identify key abiotic environmental variables that potentially drive the diversity and distribution of this group in upland ecosystems. Next a field manipulation experiment was conducted in areas of high thief ant density to determine biotic effects between thief ants and the aboveground ant community. This was done by removing thief ants using belowground toxic baits and monitoring co-occurring ant worker abundances throughout a period of approximately 1 year. We found evidence that thief ants dominate belowground and diversity. Our field experiment also yielded evidence indicating that thief ants exert potential top-down regulation on entire ant communities.

ACKNOWLEDGEMENTS

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INTRODUCTION

Ants are an abundant and diverse group of ecosystem engineers and bio indicators (Hölldobler & Wilson 1990; Andersen et al. 2004). They serve essential roles in environmental processes such as soil ecosystem functions (Folgarait 1998; Nkem et al. 2000; Wagner & Jones 2004; Tschinkel & Seal 2016). However, an often under-studied area lies in the activities and distribution of subterranean ants, ants that exclusively nest and forage below the soil surface (Wong & Guénard 2017). Available literature on subterranean ant communities predominantly focuses on the New World Tropics (Pacheco & Vasconcelos 2012) where a foundational study from 2007 indicated a largely unexplored subterranean ant fauna (Wilkie et al. 2007, 2010). Even in well-studied ant communities, such as those in Florida (Lubertazzi & Tschinkel 2003; Deyrup 2016), subterranean ant literature is mostly comprised of anecdotal accounts (Hölldobler 1973, Hölldobler & Wilson 1990 ,Wheeler 1901).

At the global scale, subterranean communities include many genera, but Florida's subterranean community is believed to mainly consist of species from the thief ant group of the genus *Solenopsis*. The moniker of "thief ant" refers to their assumed behavior of stealing brood and larvae from other species of ants. However, the existence of such behavior has not been adequately assessed and stands mostly as conjecture (Hölldobler & Wilson 1990). Despite the morphological taxonomic certainty of Florida's complex of *Solenopsis* (Thompson 1980, 1989), thief ant ecology has been rarely investigated since Thompson's dissertation (1980). A more apt description would be that the majority of knowledge on this group of ants is conjecture. Reasons behind the paucity in research can be attributed to the difficulty of investigating subterranean ecology and behavior. However, central Florida serves as an ideal location to study subterranean

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ants. Its soils are composed of mostly pure sand which, given that they spend the entirety of their life belowground, makes their study logistically feasible. Furthermore, Florida's subterranean ant community is mostly comprised of a single genus that has also been taxonomically well-described and therefore provides an informed study system. This project aimed to take advantage of the unique conditions available in the area to assess thief ant distributions and diversity as well as their potential ecological impacts on co-occurring ant species.

The genus *Solenopsis* is known mostly because of the fire ants, a widespread, common and abundant genus of Myrmicine ants (Tschinkel 2006). However, this genus includes two taxonomically distinct groups. The most familiar group includes fire ants, with larger sizes and polymorphic workers. The other group, the *Diplorhoptrum* or thief ants, are much smaller (including some of the smallest ant species), and workers are mostly monomorphic (a few species are weakly polymorphic) (Creighton 1930; Moreno Gonzalez 2001). *Diplorhoptrum* was originally proposed in 1855 and then synonymized with *Solenopsis* in 1862 by Mayr (Mayr 1855, 1862). However, this subgenus has gone through numerous revisions including being elevated to the status of genus but then being reverted (Kempf 1969; Pacheco & MacKay 2013). Today, the classification of thief ants as a subgenus is considered outdated and instead they are classified as a group within *Solenopsis* as they are similarly related to fire ants in terms of morphology but differ ecologically as most thief ants are strictly subterranean (Pacheco & MacKay 2013). This thief ant group is abundant on a global scale in communities ranging from the warm temperate to the tropical zones with ~ 86 described species occurring across the globe as a common and conspicuous group in most ant communities (Pacheco & MacKay 2013). They are characterized by their small size and cryptic morphology which also makes this group very difficult to work with and as a result, many more potential species likely remain undescribed

throughout the globe. Thankfully, all of Florida's thief ant taxonomy has been extensively

investigated and species descriptions have remained intact and supported for decades (Thompson

1980, 1989; Moreno Gonzalez 2001; Deyrup 2016)

CHAPTER 1: DIVERSITY AND DISTRIBUTION BELOWGROUND

Abstract

Subterranean ant communities are vastly understudied relative to aboveground ant communities. The thief ants of the genus *Solenopsis* are a globally abundant and widespread group that is a conspicuous and important part of the belowground ant community. Thief ant ecology, including their distribution and diversity at local scales, has also rarely been documented. In this study we sampled the subterranean ant community of central Florida, a region with conspicuously high subterranean thief ant abundance. We used a stratified-random sampling protocol and collected soil environmental variables at each sampling plot to model subterranean ant diversity in relation to abiotic conditions in the soil environment. Furthermore, we utilized non-parametric ordination methods and permutation-based analyses of variance (PERMANOVAs) to visualize and quantify associations of species based on habitats and soil strata. Our study yielded 15 species from six genera of which 5 were thief ant species. These 5 *Solenopsis* species represented 64% of all ants found. We also identified distinct differences in species composition between 2 habitat types and significant effects of soil abiotic conditions on the diversity of the subterranean community. This study finds that thief ants dominate belowground and respond predictably to soil habitat conditions. Biotic effects among ant species may be important given their purported lestobiotic behaviors.

Subterranean ants nest and forage almost entirely belowground. They are a group that may represent the final unexplored frontier for global ant biodiversity (WILKIE & al. 2007). In general, these ants are usually small-bodied and cryptic in their morphology, most likely a result of a hypogaeic life history (WONG & GUÉNARD 2017). Despite recent evidence of the diversity represented in subterranean communities as well as their potential impacts on soil ecosystems, little information exists on their basic biology and ecology (WILKIE & al. 2007, SCHMIDT & DIEHL 2008, ANDERSEN & BRAULT 2010, WILKIE & al. 2010, RIBAS & al. 2012, WONG & GUÉNARD 2017). This also extends to what little is known about the ecology and belowground activities of most epigaeic ants. Subterranean sampling has not been integral to ant diversity assessments and its practice has only recently become more widespread (SCHMIDT & SOLAR 2010). Most sampling of subterranean ant communities has only been done in the Neotropics (WONG & GUÉNARD 2017). Thus, subterranean ant distributions and interactions with other soil invertebrates are scarcely known. This dearth of information is because of the difficulties associated with sampling belowground where traps and direct soil sampling are usually the only logistically feasible approaches.

Given this sparse background, an important question is: what are the potential drivers of subterranean ant species distributions at local scales? At broader scales, soils (type, compression, temperature) and elevation have been shown to affect subterranean ant diversity (LYNCH & al. 1988, WILKIE & al. 2010, BERMAN & ANDERSEN 2012, CANEDO-JÚNIOR 2015). However, substantial variation exists among local sample sites in the above studies. For example, subterranean ant abundances in Ecuador are not predicted by some soil conditions, such as soil pH or mineral content (JACQUEMIN & al. 2012). However, another study in the Brazilian

savannah showed significant effects of soil temperature and compression in association with changing subterranean ant species compositions (CANEDO-JÚNIOR 2015). Collectively, these few studies represent most of what is known about environmental factors affecting subterranean ant diversity and distributions (WONG & GUÉNARD 2017). These studies suggest that the relationship between the diversity and distributions of subterranean ants and soil conditions may jointly depend on broad-scale geography and the local composition of the local subterranean ant community.

Subterranean ant communities, especially in the tropics, contain a variety of genera. However, the genus *Solenopsis* is found globally in belowground communities and is among the most abundant group of species in these communities and thus warrants special attention (WILKIE & al. 2007, BERMAN & ANDERSEN 2012, PACHECO & MACKAY 2013). Thief ants in the genus *Solenopsis* are a group of relatively small-bodied, largely subterranean or litter-dwelling species (although there are even some arboreal species) that are abundant in communities from the warm temperate to the tropical zones (PACHECO & al. 2007, ANDERSEN & BRAULT 2010, HERNÁNDEZ 2010, PACHECO & MACKAY 2013). About 86 described thief ant species occur across the globe as a common and conspicuous group in most ant communities (MACKAY & MACKAY 2002, PACHECO & MACKAY 2013). In Florida, the thief ant species considered to be completely subterranean are *Solenopsis tonsa* (THOMPSON, 1989), *Solenopsis pergandei* (FOREL, 1901), and quite possibly *Solenopsis tennesseensis* (SMITH, M.R., 1951). Some (or perhaps most) thief ant species are purported to be "lestobiotic", nesting near the nests of host ant colonies, tunneling belowground into their nests, and stealing their brood (HÖLLDOBLER 1973, HÖLLDOBLER & WILSON 1990, TSCHINKEL 2006, DEYRUP 2016). Thief ants are assumed to practice lestobiosis upon a wide range of ant species that are often much larger in size, as this

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interaction often emphasizes the interaction between small and large-bodied ants (HÖLLDOBLER & WILSON 1990). Although thief ants may also be dietary generalists and even predators of other ants when not stealing brood as they have also been observed actively preying on founding queens (WHEELER 1901, BLUM & al. 1980, THOMPSON 1980, BUREN 1983, LAMMERS 1987, NICHOLS & SITES 1991, VINSON & RAO 2004, DEYRUP 2016). The small body size of thief ants (which includes some of the smallest workers among all ants) may also allow them to move through soil and escape via pathways not accessible to their larger-bodied prey (KASPARI & WEISER 1999). This potential behavior coupled with their high abundance and broad, global distribution suggests that lestobiosis by thief ants, and preying directly on brood and, especially, founding queens (LAMMERS 1987, NICHOLS & SITES 1991, VINSON & RAO 2004), may be an important regulator of both subterranean and aboveground ant communities.

What is actually known about subterranean ant interactions with other ants is largely based on a few descriptions (WHEELER 1901, SCHNEIRLA & al. 1944, DEYRUP 2016). This gap in knowledge is all the more important in regions such as the southeastern US, and especially upland habitats in Florida, where thief ants dominate subterranean ant diversity and abundance (LUBERTAZZI & TSCHINKEL 2003, KING & PORTER 2007, DEYRUP 2016). Furthermore, the subterranean thief ant complex from these localities have been taxonomically well described for many years, meaning that community diversity analyses may be confidently conducted (THOMPSON 1980, THOMPSON 1989, MORENO GONZALEZ 2001).

In the most comprehensive treatment of thief ant ecology to date, THOMPSON (1980) found that thief ant species composition differed between shrubby and grassy habitat types. Otherwise, only unpublished observations inform the ecology of thief ant distributions. Depth to water table or soil moisture content may be the main environmental drivers of thief ant

distribution and diversity as long-term soil moisture dynamics may limit the foraging capabilities

of these ants (LAMMERS 1987). It is also known that thief ants are sensitive to low humidity

when being raised in a laboratory setting but in the wild are incapable of building mounds like

the fire ant (*Solenopsis invicta* (BUREN,1972)) to escape inundation (THOMPSON 1980,

TSCHINKEL 2006). Therefore, well-drained soils in otherwise mesic regions likely maintain

conditions ideal for thief ant populations. In Florida, upland habitats such as drier pine flatwoods

and especially high pine sandhills (MYERS & EWEL 1990) appear to support robust populations

of a number of thief ant species (THOMPSON 1980). Nearby habitats (e.g., more mesic flatwoods

and dry prairies) are more prone to flooding (MYERS & EWEL 1990) and appear to have reduced

subterranean ant diversity and abundances (DEYRUP 2016). We therefore conducted this study in

upland sandhill and flatwood habitats to determine if there are differences in thief ant

communities associated with these common habitat types in this region.

   To better understand the factors affecting ant distribution and activity belowground, we

sampled belowground foraging ants in the two habitat types (sandhill and flatwoods) using baits

and collected associated soil environmental variables to identify relationships between the

subterranean ant community and local habitat conditions. Considering that many subterranean

ants are known for their small-bodied form and cryptic morphology we specifically targeted

small-bodied ants in our sampling. We understand that not all subterranean ants are small-bodied

as seen in WONG ET AL. (2017) but based on previous surveys and studies in central Florida and

in other parts of the state we have evidence that subterranean ants in our locality were small-

bodied (THOMPSON 1980, PRUSAK 1997, LUBERTAZZI & TSCHINKEL 2003, KING & PORTER

2007, KING 2010). Furthermore, our primary focus, the *Solenopsis* thief ants, are all small-

bodied (THOMPSON 1989, DEYRUP 2016). However, not all the ants that were baited truly

practice a subterranean life history, that is, nesting and foraging entirely belowground but they were still classified as part of the subterranean ant community for the purpose of this study. Therefore, we defined the "subterranean ant community" to be composed of ants with a hypogaeic life history as well as the ants that were found to co-occur with them in our subterranean sampling. These co-occurring species may forage or nest aboveground but may be opportunistically foraging belowground as well. We later differentiate subterranean versus other ant species, based on what is known of their natural history. Nevertheless, even small-bodied aboveground foraging or nesting ants that forage opportunistically belowground likely play a role in the subterranean ant community.

We asked: (1) Do subterranean ant communities (with an emphasis on thief ants) differ in composition and abundance between flatwood and sandhill habitats? (2) Do soil environmental gradients predict the species diversity of this subterranean ant community? (3) Do these gradients also predict the occurrence of thief ant species?

We also compared those data to the only 2 other subterranean sampling studies conducted in Florida (THOMPSON 1980, LUBERTAZZI & TSCHINKEL 2003). LUBERTAZZI & TSCHINKEL (2003) carried out their subterranean assessment in the longleaf pine forest of the Apalachicola National Forest outside of Tallahassee, Florida. THOMPSON (1980) conducted a sampling survey comprised of 2 total plots, 1 in turkey oak woods and the other in an open field outside of Gainesville, Florida. Comparisons to aboveground ant diversity and relative abundance in our study site were also made possible using aboveground pitfall sampling data (from 2012) collected from the same areas as our subterranean sampling.

Study Site

Sampling was conducted during the months of July and August, 2017, at Wekiva Springs State Park (2,750 Hectares) situated in Orange County, Florida at 28.7118° N, 81.4628° W. Average annual rainfall in the area is approximately 1350 mm. The general seasonality of the site involves a cycle of wet and dry seasons with the wet season beginning around May and ending in November and the dry season occurring December-April. We distinguished 2 main habitat types within this park to conduct our survey, high pine sandhills and mesic pine flatwoods.

High pine sandhill is a pyrogenic habitat characterized by well-drained sandy soils, an overstory of longleaf pine (*Pinus palustris*), and a groundcover dominated by wiregrass (*Astrida beyrichiana*) (MYERS & EWEL 1990). The sandhill sites selected for this study were in areas maintained by low intensity fires. High pine sandhill habitats gradually transition downhill to pine flatwoods, which are distinct in vegetation as a result of more poorly-drained soils due to a higher water table and subsequent proneness to flooding (ABRAHAMSON & HARTNETT 1990). Sandhill soils are generally categorized into droughty course sands, sandy clays, or loamy sands; our sites were mostly composed of coarse sand classified as Entisols that are generally low in nutrients (ABRAHAMSON & HARTNETT 1990). Flatwood soils are usually acidic and hold insignificant amounts of extractable nutrients (GHOLZ & FISHER 1982, MYERS & EWEL 1990). Soil moisture of the flatwoods is usually influenced by soil organic matter content as well as a mulching effect from the litter layer (MYERS & EWEL 1990).

A stratified-random sampling design was used in both habitat types, where habitat type

boundaries were first identified in the field (based on vegetation) using a handheld GPS. These

coordinates were used to generate polygons representative of the 2 habitat types in ArcMap (ESRI

2017). Coordinates for our sample plots (16 per habitat type) were then randomly generated in

ArcMap within the habitat type polygons. A minimum distance of 36 meters between sample

plots avoided site overlap. Sample plots were randomly assigned a sampling depth of 10 cm or

20 cm. As a result, 8 plots in each of two habitat types were sampled at each of two depths (32

total sample plots) (Fig. 1).
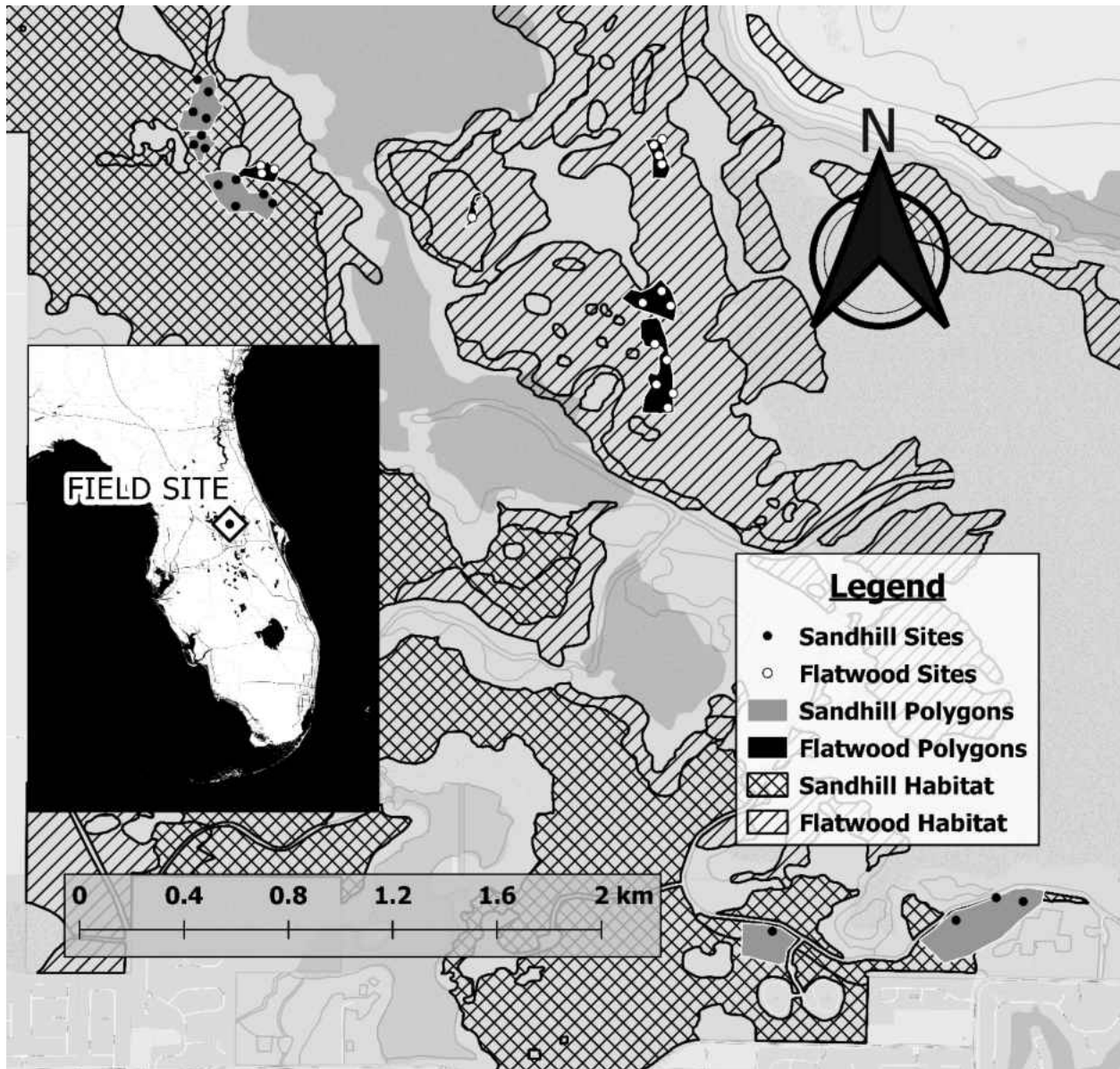
Figure 1: Map of study site (Wekiva Springs State Park) with sampling sites.

Baits

Baits were made using plastic capped vials 70 mm tall and 30 mm in diameter. A ~5 mm

diameter hole was made near the bottom edge of the vial and covered with 1 mm screening to

exclude larger animals (e.g., fire ants) but permit entry by subterranean ants. This was done to

specifically target small-bodied subterranean ants as well as other non-subterranean ants that

may forage opportunistically within the subterranean environment. Each bait was loaded with ~3-4 cm^3 of sugar cookie (Pecan Sandies). To deploy the baits a battery-powered 24v drill and a 24-inch auger-bit was used to drill into the soil to a specified depth. The baits were then placed in the holes and covered up with the previously extracted soil. Baits were deployed in the morning and retrieved using a hand trowel ~72 hours later. Specimens were kept in sandwich bags and stored in a freezer.

Habitat Variables

Soil temperature and soil moisture were recorded at each bait site. Soil temperature was recorded for the entirety of the 72-hour baiting period using data loggers (iButton, Maxim). Each plot had 2 data loggers installed on both east and west sides at 10 cm below the soil surface to record temperatures every hour during the baiting period. Using those data, we extrapolated average minimum, maximum, and temporal changes in soil temperatures per site. Soil moisture was collected by using a soil moisture sensor at 10 cm depth (Procheck, Decagon Devices). 10 readings were taken from each plot at the time of retrieval and averaged to represent the soil moisture level of the plot.

<div align="center">Sorting</div>

All ants collected from the baits were sorted to species utilizing identification pointers from DEYRUP (2016). Additional reference specimens from J. R. King's personal collection were used to confirm identifications.

Aboveground Sampling

Aboveground ant communities were sampled previously in the same area and habitat types of the park as the belowground sampling. In August 2012, three 100 m linear transects were established in each habitat type (a total of 6 transects), separated by at least 100 meters from one another or

forest roads. In each transect, sampling was performed using pitfall traps placed at 5-meter

intervals for a total of 20 traps per site and 120 traps for the 2 habitat types. Pitfall traps were 85

mm long plastic vials with 30 mm internal diameter partially filled with ~ 15 ml of non-toxic,

propylene-glycol antifreeze. Traps were buried with the opened end flush with the surface of the

ground and operated for 7 days. Traps were installed using a hand-held, battery-powered drill

using an auger bit.

Analyses

Each occurrence of a species in a baited vial was considered an occurrence of 1 colony of that

species based on the spatial distances between baits (KING & PORTER 2007, KING 2010).

Potential differences in community composition between habitats and depths were evaluated

with nonmetric multi-dimensional scaling (NMDS), which is a nonparametric ordination method.

Subsequent permutation-based analyses of variance (PERMANOVAs) were used to test for

significant differences between detected clusters. The NMDS utilized beta diversity distances

based on the Bray-Curtis index, a measure of dissimilarity that allowed for the separation of sites

based on differences in species composition (while also accounting for species abundance as

measured by frequency of occurrences). Bray-Curtis distances are also robust to sampling errors

and preferred to other beta diversity measures (SCHROEDER & JENKINS 2018). Potential effects of

environmental gradients on ant diversity were modeled using both linear mixed-effect models

and linear regressions. Species estimators were also calculated using Chao1 estimators (all

values listed in Appendix S1, as digital supplementary material to this article, at the journal's

web pages) to provide further evidence of the robustness of sampling methods. The response

variable for all models was the Jost Diversity index ($D = e^{H'}$; JOST 2006) per site calculated using

number of species occurrences per site. Independent variables included depth of the baited vial,

soil temperature (averages of maximum, minimum, and daily range), and average soil moisture

(Table 1). Model assumptions were evaluated based on residual diagnostic plots (Appendix S2,

as digital supplementary material to this article, at the journal's web pages). Finally, the

occurrence of all species in the baited vials was modeled using logistic regressions, where the

occurrence of each ant species was predicted by soil parameters. All regressions were compared

and ranked using corrected Akaike Information Criterion weights (AICc w_i) from the R package

'bbmle' (BOLKER & TEAM RDC 2017) as they allowed an appropriate comparison for model

parsimony compared to evaluating individual R^2-values (Table 1). Logistic regressions were also

evaluated with pseudo-R^2 values calculated by subtracting the null deviance of the model from

the residual deviance and dividing the total by the residual deviance (Table 2). All soil

environmental variables were standardized during analyses and all statistical analyses were

conducted using R 3.4.1 statistical software (R DEVELOPMENT CORE TEAM 2017). Mixed-effect

models were computed using the R package 'lme4' (BATES & al. 2015) and the 'vegan' package

(OKSANEN & al. 2017) was used to compute NMDS ordinations and PERMANOVAs. All

graphics for regressions and ordinations were done using the R package 'ggplot2' (WICKHAM

2009).

<div align="center">Results</div>

<div align="center">*Ant Diversity and Abundance*</div>

A total of 15 species encompassing 6 genera were captured and identified from all our

belowground baits (full species list in Appendix S3, as digital supplementary material to this

article, at the journal's web pages). 98% of the 1152 baited vials deployed were recovered; 23

baited vials were lost during sampling. Species-sampling estimates indicate that all existing

species were observed in most samples (Appendix S1, as digital supplementary material to this article, at the journal's web pages). We assessed relative abundances as the occurrence of a species at each baited vial. The most common genus was *Solenopsis* (in 70% of baits), followed by *Pheidole* (21.5%) and *Brachymyrmex* (8.3%). The last three genera, *Forelius*, *Hypoponera*, and *Nylanderia* occurred in 1 baited vial, each. *Solenopsis* was the most species-rich genus with 6 species (all thief ants except for the introduced fire ant, *S. invicta*). The 8 most common species were *Solenopsis pergandei* (occurring in 209 baited vials, 27.6% of total), *Solenopsis carolinensis* (FOREL, 1901) (98, 12.9%), *Solenopsis nickersoni* (THOMPSON, 1982) (93, 12.3%), *Pheidole floridana* (EMERY, 1895) (69, 9%), *Brachymyrmex depilis* (EMERY, 1893) (63, 8.3%), *Solenopsis tenneessensis* (50, 6.6%), *Solenopsis invicta* (40, 5.3%), and *Pheidole morrisii* (FOREL, 1886) (39, 5.2%).

<center>*Soil stratum comparison*</center>

Most ant taxa other than *Solenopsis, Nylanderia wojciki* (TRAGER, 1984)*,* and *Pheidole dentata* (MAYR, 1886) were less frequently sampled at the greater depth (20 cm). *Nylanderia wojciki* and *Pheidole dentata* were relatively rare and were only detected at 20 cm (Appendix S3, as digital supplementary material to this article, at the journal's web pages). Among the *Solenopsis* species, *S. carolinensis* occurrence decreased 42% from 10 cm to 20 cm soil depth and *S. nickersoni* occurrence decreased (25%), but *S. pergandei* occurrence increased (78%), *S. tennesseensis* occurrence had no change, and *S. tonsa* occurrence increased (145%). The most frequently captured species at both depths was *S. pergandei*. Based on an NMDS analysis and a subsequent PERMANOVA, depth did not significantly affect species compositions (PERMANOVA, $P > 0.05$).

Brachymyrmex (1 occurrence in high pine sandhills, 62 occurrences in pine flatwoods), was more prevalent in the flatwoods than in sandhill habitats. *Forelius* (1,0), *Hypoponera* (1,0), and *Nylanderia* (1,0) were present in flatwoods but absent in the sandhills. *Pheidole* (125, 38) and *Solenopsis* (359, 169) were more common in the sandhills. Within *Solenopsis, S. nickersoni* was found more commonly in flatwoods than in sandhill habitats. However all other thief ant species (*S. carolinenesis*, *S. pergandei*, *S.tennesseensis*, *S. tonsa*) were more prevalent in the sandhills.

The NMDS analysis (Fig. 2, Fig. 3) showed a distinct separation between communities of the 2 habitat types along with the separation of species that was congruent with our raw data. A subsequent PERMANOVA verified significant separation of centroids in this analysis (P<0.05). All thief ant and *Pheidole* species, except for *S. nickersoni*, *P. dentata*, and *P. morrisii*, were clustered tightly within the sandhill cluster. Positions for *S. tonsa* and *Pheidole adrianoi* (NAVES, 1985) in the NMDS were furthest away from the flatwood cluster. The species within and around the flatwood cluster had a higher degree of spread, most likely due to several species (*Forelius. pruinosus* (ROGER, 1863), *Hypoponera. opacior* (FOREL, 1893), *N. wojciki*, and *P. dentata*) having been collected only once. *Brachymyrmex depilis*'s position in the NMDS mirrors *S. tonsa* and is one of the few frequently collected species in the flatwoods. Finally, the fire ant, *S. invicta,* is positioned more along the upper edge of the flatwood cluster and towards the center between both habitat clusters. To further validate these results, we removed singletons from the species by site matrix (3 total species/columns removed) and ran the NMDS at the same dimensions (k =2) with the same number of starting iterations (1000) and found no differences in patterns. The stress value remained the same at ~0.127.=
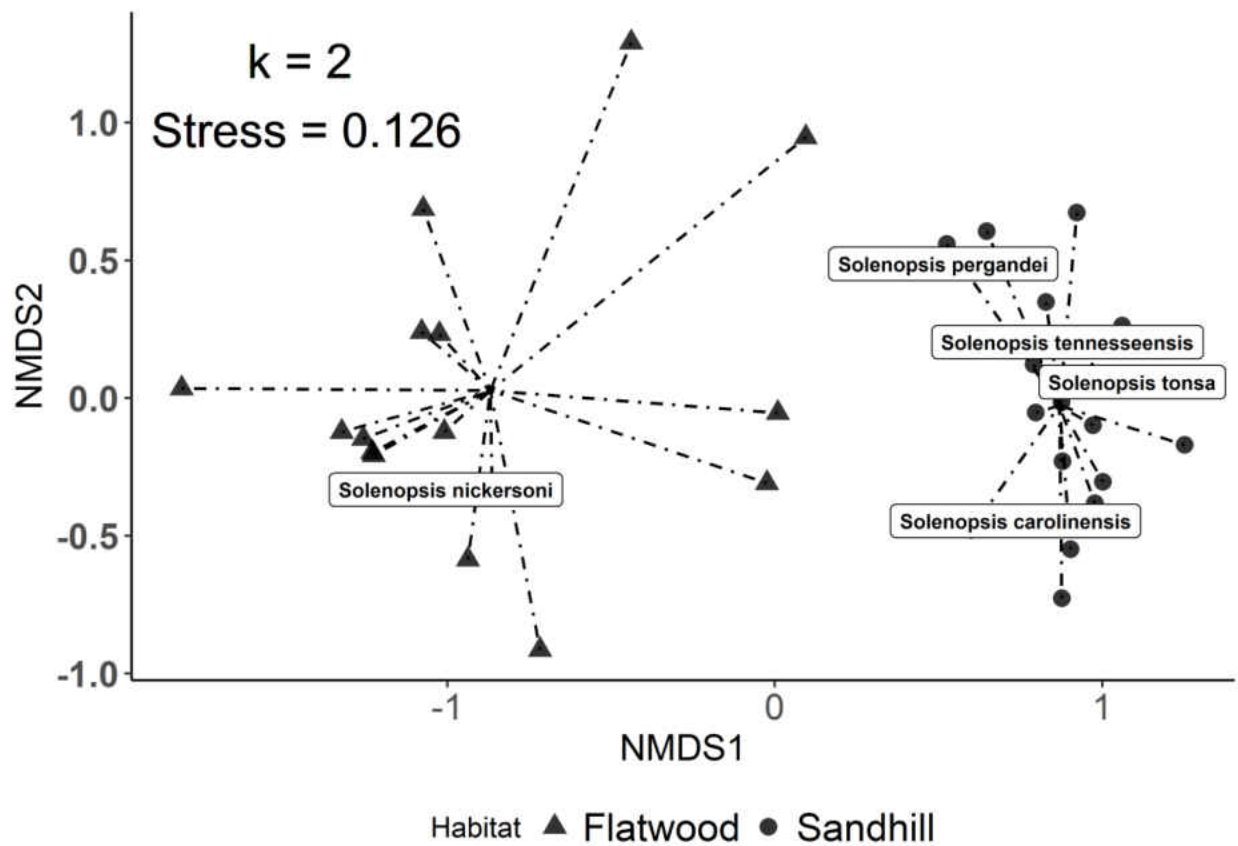
Figure 2: Nonmetric multi-dimensional analysis of the species by site matrix from the subterranean sampling. Triangles represent pine flatwood sites and circles represent high pine sandhill sites. Lines connect the sites to each habitat's respective centroid in multivariate space. Labels for thief ant species represent the position of species within this space. The analysis had acceptable stress values of 0.126 at 2 dimensions (k = 2)
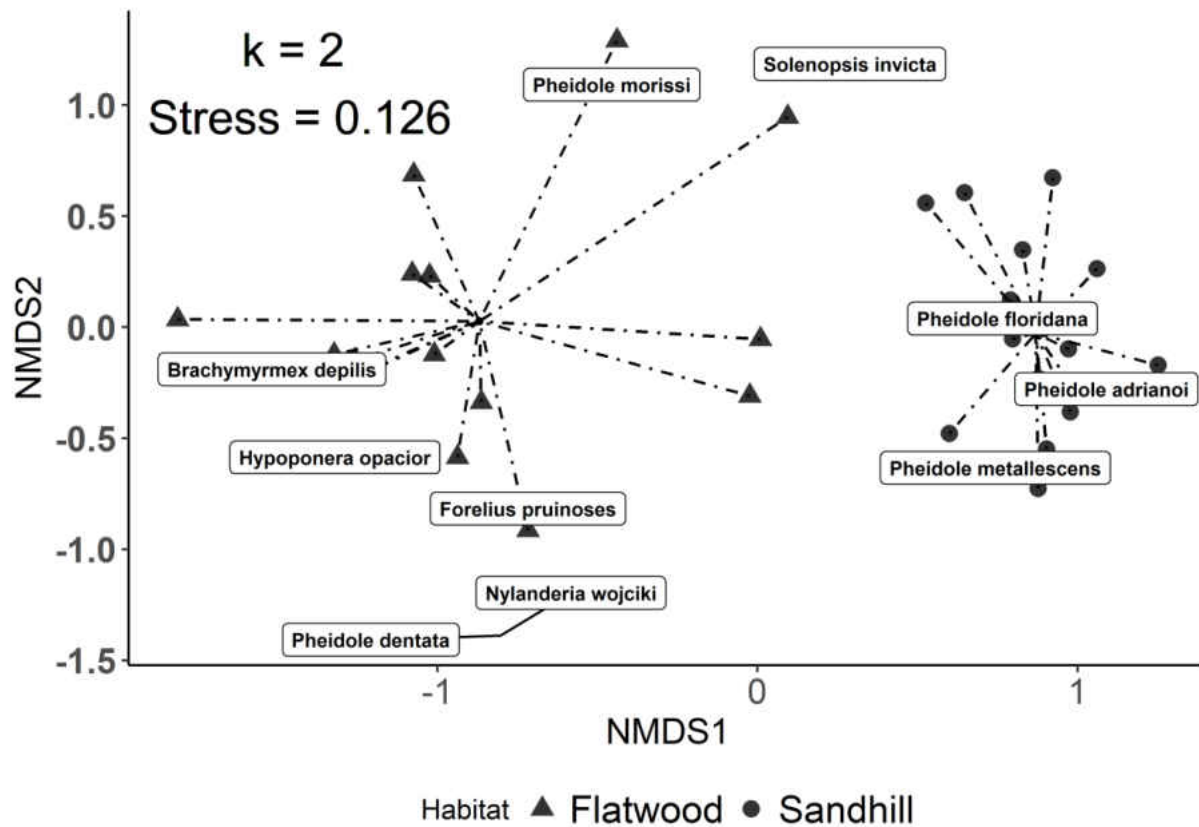
Figure 3: A replicate nonmetric multi-dimensional analysis visual of Figure 2. Labels differ here to show the position of non-thief ant species. Flatwood species labels have a higher degree of spread due to extreme low occurrences of some species (e.g. Pheidole dentata, Nylanderia wojciki)

Modeling Species Diversity

Although not all species caught at our baits are truly subterranean ants, for the purposes of this study, we included species captured in belowground samples as part of the subterranean community as these species were clearly actively foraging belowground. Subterranean ant diversity was most effectively explained in regression models as an interaction between habitat types and average daily soil temperature range (*AICc* w_i = 0.34, Table 1). This model represented a majority of variance in ant diversity (*P* = 0.02, R^2 = 0.60). Residuals met assumptions of the model. The simple linear regression model outperformed the random-intercept model, and conditional pseudo *R*-squared values indicated that random intercepts explained very little

variation and both models indicated approximately the same effect sizes. A second linear model also included an interaction between habitat type and average soil maximum temperature ($AICc$ $w_i = 0.14$). However, the model using average daily temperature ranges accounted for more variation and was more plausible. In all our initial models we added soil depth as a covariate but the differences between the top-ranked models with and without the covariate was negligible as effect sizes and adjusted- R^2 values barely differed.

Table 1: A table showing the top 5 performing simple linear regression models under AICc (Akaike Information Criterion with correction for small sample sizes) rankings. Predictor variables for each model are shown along with each model's AICc score, the change in AICc for every lower ranked model, AICc weights, and the adjusted R^2.

Model	AICc	ΔAICc	Weight (w_i)	Adjusted-R^2
D ~ Habitat * Avg. Change in Daily Soil Temp.	94.4	0	0.34	0.60
D ~ Habitat * Avg. Soil Maximum Temp.	96.2	1.8	0.14	0.57
D ~ Habitat + Avg. Soil Moisture * Avg. Soil Temp.	97.2	2.9	0.08	0.58
D ~ Habitat + Avg. Soil Minimum Temp.	97.4	3.0	0.08	0.53
D ~ Habitat + Avg. Soil Moisture	97.6	3.3	0.07	0.53
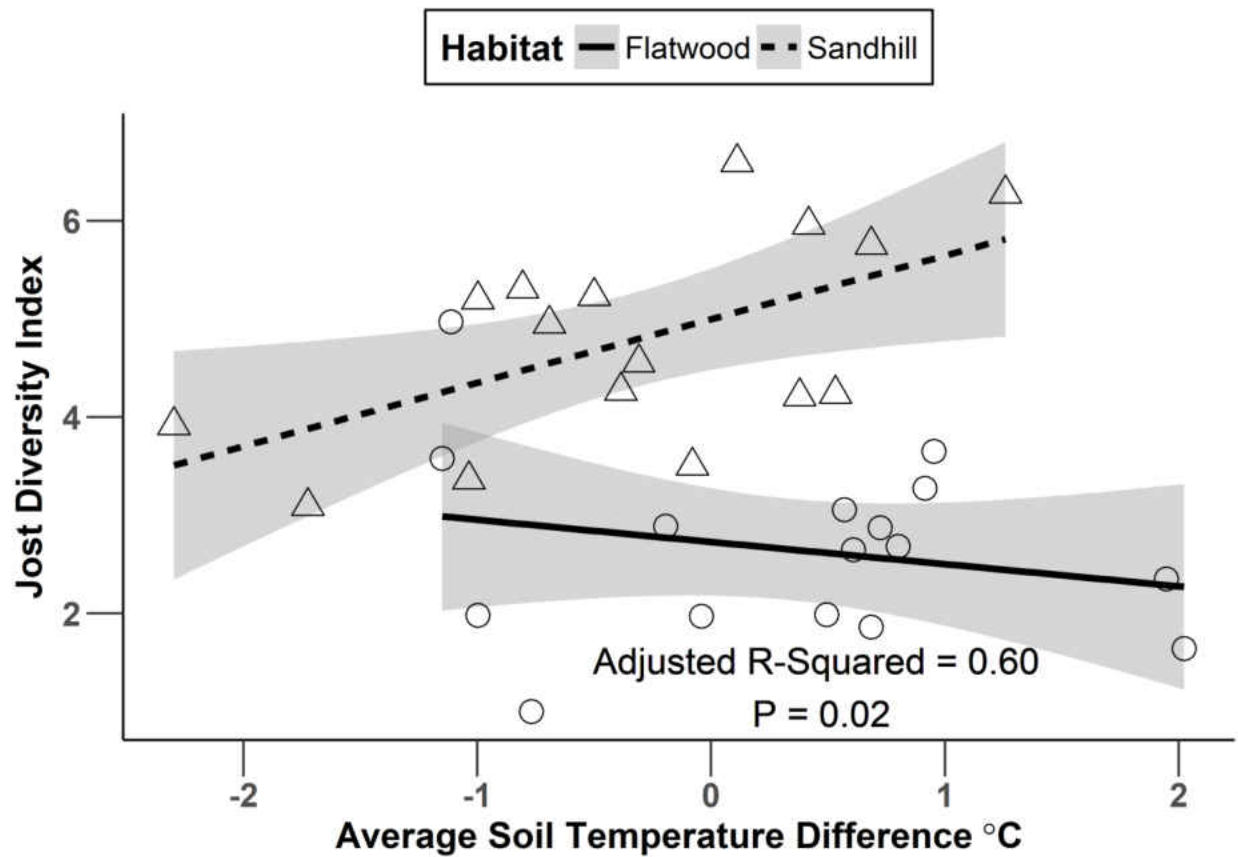
Figure 4: Simple linear model coefficients and their 95% confidence intervals for the top 5 most plausible models in predicting diversity based on AICc (Akaike Information Criterion corrected for small sample sizes) rankings. Coefficients represent changes in the Jost diversity index relative to different soil abiotic variables. Bolded coefficients were significant at P<0.05. All coefficients are based on the flatwood habitat as being the reference level in the model and all quantitative predictor variables were standardized.

Table 2: *Simple linear model coefficients and their 95% confidence intervals for the top 5 most plausible models in predicting diversity based on AICc (Akaike Information Criterion corrected for small sample sizes) rankings. Coefficients represent changes in the Jost diversity index relative to different soil abiotic variables. Bolded coefficients were significant at P<0.05. All coefficients are based on the flatwood habitat as being the reference level in the model and all quantitative predictor variables were standardized.*

Independent Variables	D ~ Habitat + Avg. Soil Moisture * Avg. Soil Temp.	D ~ Habitat * Avg. Soil Maximum Temp.	D ~ Habitat * Avg. Change in Daily Soil Temp.	D ~ Habitat + Avg. Soil Moisture	D ~ Habitat + Avg. Soil Minimum Temp.
Intercept	**2.25±0.66**	**2.82±0.53**	**2.73±0.49**	**2.59±0.51**	**2.69±0.50**
Sandhill	**2.55±1.03**	**2.15±0.73**	**2.27±0.69**	**2.25±0.74**	**2.05±0.71**
Avg. Change in Daily Soil Temp.	-	-	-0.23±0.48	-	-
Avg. Soil Maximum Temp.	-	-0.39±0.59	-	-	-
Soil Minimum Temp.	-	-	-	-	-0.20±0.36
Avg. Soil Moisture	0.31±0.48	-	-	0.19±0.37	-
Avg. Soil Temp.	-0.08±0.53	-	-	-	-
Sandhill:Avg. Change in Daily Soil Temp.	-	-	**0.87±0.70**	-	-
Sandhill:Avg. Soil Maximum Temp.	-	**0.85±0.76**	-	-	-
Avg. Soil Moisture: Avg. Soil Temp.	**-0.53±0.43**	-	-	-	-

Logistic regression models of thief ant species occurrence per site using soil environment variables significantly predicted 4 of 5 thief ant species and helped in further understanding the NMDS result (full models listed in Appendix S4, as digital supplementary material to this article, at the journal's web pages); only *S. nickersoni* occurrence was not predicted. *Solenopsis pergandei'*s most plausible model was a function of the interaction between average soil moisture and average minimum soil temperature ($P = 0.02$, Pseudo- $R^2 = 0.59$, Table 2). *Solenopsis tonsa*'s most plausible model was a function of the additive effects of average soil moisture and temperature ($P=0.02$, 0.01 respectively, Pseudo- $R^2 = 0.33$). *Solenopsis carolinensis*'s most plausible model was also a function of the same predictors ($P = 0.04$, $P = 0.04$, Pseudo- $R^2 = 0.16$). Finally, *S. tennesseensis*'s most plausible model was a function of average soil temperature ($P = 0.01$, Pseudo- $R^2 = 0.23$).

Other co-occurring ant species found in our samples were also modeled by logistic regression, though not all species had sufficient occurrences to model (Table 5, models listed in Appendix S5, as digital supplementary material to this article, at the journal's web pages). *AICc* model selection on the logistic regressions for *P. adrianoi* and *S. invicta* showed the null model being ranked the best indicating the lack of any statistical signal in their species-respective models. *Brachymyrmex depili*s' most plausible model was a function of the interaction between average soil moisture and average minimum soil temperature ($P = 0.04$, Pseudo- $R^2 = 0.74$). *Pheidole floridana*'s most plausible model was a function of average maximum soil temperature ($P = 0.05$, Pseudo- $R^2 = 0.11$). *Pheidole metallescen*s' (EMERY, 1895) most plausible model was a function of the additive effects of average soil moisture and average minimum soil temperature ($P = 0.07$, 0.09, Pseudo- $R^2 = 0.12$). It's important to note that the next plausible model for *P.*

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metallescens was the null model, and the two models were only different by a *ΔAICc* of 0.2 with similar *AICc* weights (Appendix S5, as digital supplementary material to this article, at the journal's web pages). Therefore, we did not evaluate *P. metallescens* occurrences. *Pheidole. morrisii*'s most plausible model was a function of average minimum soil temperature ($P = 0.02$, Pseudo-$R^2 = 0.21$).

Table 3: Pseudo R^2 values for most plausible logistic regression model of successfully modeled species collected in the subterranean sampling (8 of 15 possible species). Model predictor variables are also displayed.

Species	Pseudo- R^2 of most plausible model	Model
Solenopsis carolinensis	0.16	Occurrence ~ Avg. Soil Moisture + Avg. Soil Temp.
Solenopsis pergandei	0.59	Occurrence ~ Avg. Soil Moisture * Avg. Minimum Soil Temp.
Solenopsis tennesseensis	0.23	Occurrence ~ Avg. Soil Temp.
Solenopsis tonsa	0.33	Occurrence ~ Avg. Soil Moisture + Avg. Soil Temp.
Brachymyrmex depilis	0.74	Occurrence ~ Avg. Minimum Soil Temp. * Avg. Soil Moisture
Pheidole floridana	0.11	Occurrence ~ Avg. Maximum Soil Temp.
Pheidole metallescens	0.12	Occurrence ~ Avg. Minimum Soil Temp + Avg. Soil Moisture
Pheidole morrisii	0.21	Occurrence ~ Maximum Soil Temp.

Table 4: *Logistic regression model coefficients and their 95% confidence intervals for the most plausible model for every successfully modeled thief ant species. Coefficients represent the log odds of the occurrence of the ant species relative to different soil abiotic conditions. Bolded coefficients were significant at P<0.05. All predictor variables were standardized for the models.*

Independent variables	*Solenopsis carolinensis* coefficients	*Solenopsis pergandei* coefficients	*Solenopsis tennesseensis* coefficients	*Solenopsis tonsa* coefficients
Intercept	0.02±0.79	**2.76±2.49**	0.37±0.84	-0.57±0.94
Avg. Minimum Soil Temp.	-	**-2.40±2.20**	-	-
Avg. Soil Moisture	**-1.04±0.99**	**-3.61±3.06**	-	**-1.85±1.37**
Avg. Soil Temp	**-1.04±1.01**	-	**-1.42±1.07**	**-1.71±1.50**
Avg. Soil Moisture: Avg. Minimum Soil Temp.	-	**2.36±2.02**	-	-

Table 5: Logistic regression model coefficients and their 95% confidence intervals for the most plausible model for every successfully modeled non-thief ant species. Coefficients represent the log odds of the occurrence of the ant species relative to different soil abiotic conditions. Bolded coefficients were significant at P<0.05. All predictor variables were standardized for the models.

Independent variables	*Brachymyrmex Depilis* coefficients	*Pheidole floridana* coefficients	*Pheidole Metallescens* coefficients	*Pheidole Morrisi* coefficients
Intercept	-3.26±0.91	-0.27±0.75	-0.77±0.82	-0.75±0.89
Avg. Maximum Soil Temp.	-	**-0.86±0.85**	-	**-1.48±1.26**
Avg. Minimum Soil Temp.	**4.49 ±1.06**	-	-0.83±0.97	-
Avg. Soil Moisture	5.56±1.08	-	-1.01±1.07	-
Avg. Soil Temp	-	-	-	-
Avg. Soil Moisture: Avg. Minimum Soil Temp.	**-3.70±1.02**	-	-	-
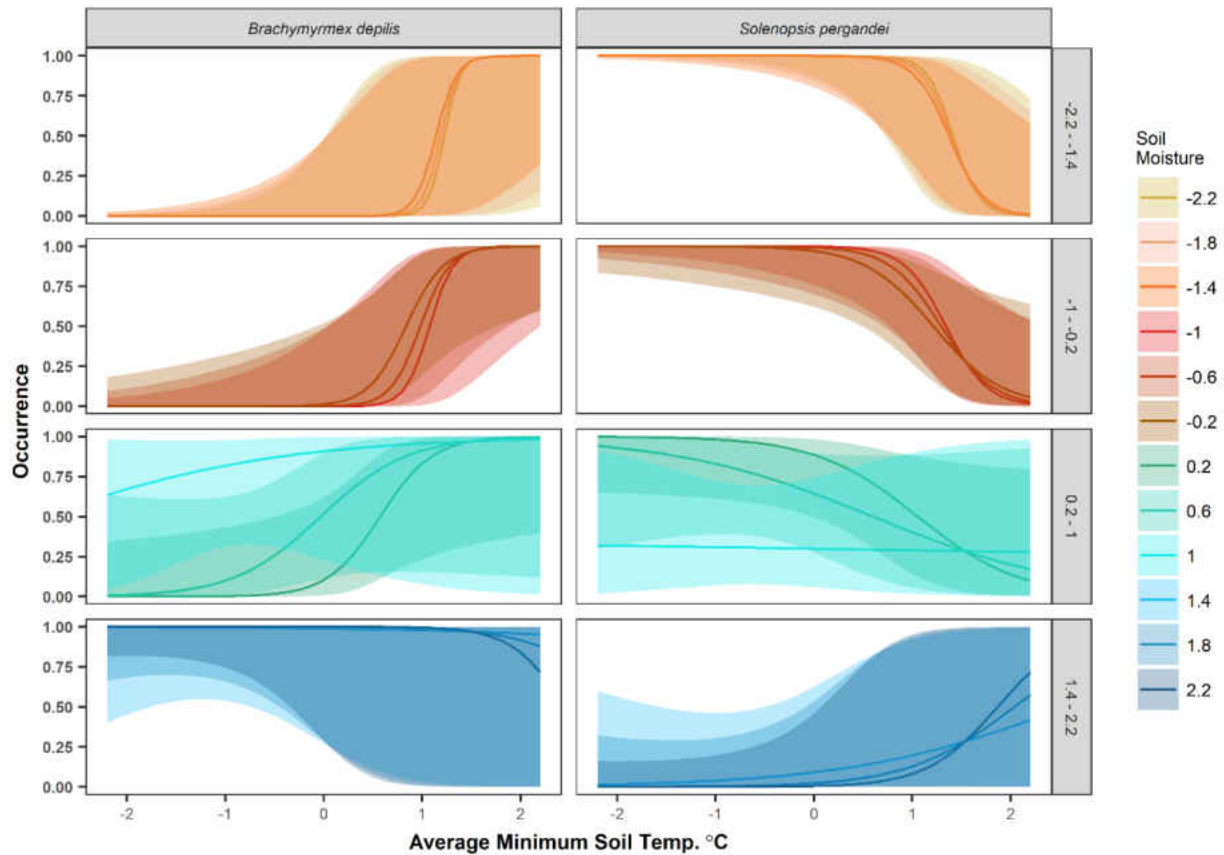
Figure 5: Logistic regression models of Brachymyrmex depilis (left) and Solenopsis pergandei (right). Y-axis represents occurrence; X-axis represents average minimum soil temperature (standardized). The interaction between average minimum soil temperature and average soil moisture (standardized) is represented through 4 facets (labels on right). Each facet shows the model at 3 different average soil moisture levels and average soil moisture increases from the top to the bottom facet. Colors differ for each average soil moisture level and colored shading represents the 95% confidence intervals of the model at various levels of moisture. Pseudo R values for B. depilis and S. pergandei moderls were 0.74 and 0.59 and P-values for each model's interaction were 0.04 and 0.02 respectively.

Overall, subterranean ant diversity was dominated by *Solenopsis* species and different in composition between high pine sandhills and pine flatwoods. Those patterns appeared to be related to soil temperature and moisture, which consistently predicted belowground ant diversity and species' occurrences in the two different habitat types.

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Differences among habitats

Distinct multivariate differences between sandhill and flatwood sites are consistent with the expectation that ant communities differ between habitat types at local scales (BERMAN & ANDERSEN 2012, CROSS & al. 2016) (Fig. 2 & 3). Distinct species compositions existed between habitats, but sandhill sites were more similar to one another than flatwood sites, indicating the greater homogeneity in soil habitat conditions in the sandhills. This suggests that heterogeneous soil habitat conditions affecting thief ants in flatwoods may result in more variation in the species present in any given area. This clustering also indicates the presence of a potential ecological driver (soil temperature and moisture conditions by regressions) for dissimilar species rosters found in both habitats. Such drivers may be environmental filters resulting in different survivorship or competitive abilities among species, ultimately resulting in different species found in pine flatwoods and high pine sandhills. Results here describe patterns in species composition; elucidating actual drivers of these patterns will require experiments and careful observation of species' natural histories.

The known natural history of most of these species agrees with their positions within the NMDS. Of the sandhill thief ant species, only *S. tonsa,* one of the few truly subterranean species, is expected to occur strictly in sandhill (DEYRUP 2016). *Solenopsis pergandei*, another true subterranean species can be found in other soils but tends to be most common in open sandy areas such as sandhills. *Solenopsis tennesseensis*, a suspected subterranean but also litter-dwelling thief ant, is a supposed habitat generalist but in this case, was closely associated with the sandhill sites. Other species that were tightly clustered to the sandhills were *P. metallescens,*

P. adrianoi, and *P. floridana*. *Pheidole metallescens* is considered a predominantly upland species that is usually found in high pine sandhills and usually co-occurs with *P. adrianoi*. *Pheidole floridana* is associated with drier habitats, like the sandhills, and is less likely to be found in moist forested areas (DEYRUP 2016). Flatwood species other than *S. nickersoni* included *B. depilis* and *P. morrisi*. *Brachymyrmex depilis*, predominantly sampled in the flatwoods, is considered a generally subterranean species like subterranean thief ants except they are usually found in a wider variety of habitat types across North America (DEYRUP 2016). However, its general absence in the sandhills may be indicative of it preferring mesic conditions or being out-competed by the thief ants or species of small-bodied *Pheidole*. The fire ant, *S. invicta,* is a known invasive and weedy species, capable of surviving in inundation-prone habitats (TSCHINKEL 2006). Its position in the NMDS analysis indicate its prevalence in both habitats (Fig. 2 & 3) which would be logical considering its ability to establish in a variety of conditions, especially if there are forest roads or other disturbances nearby.

Environmental gradients with Diversity and Species Occurrence

Local scale ant diversity is often weakly correlated with abiotic conditions and is usually more strongly associated with local vegetation (CROSS & al. 2016). However for subterranean communities, gradients of abiotic conditions such as soil moisture and temperature may heavily influence their distribution at local scales (THOMPSON 1980, LUBERTAZZI & TSCHINKEL 2003, WILKIE & al. 2010). Teasing apart how local scale abiotic conditions affect diversity can be useful in discerning drivers of diversity. Here we found that diversity was predicted by an interaction between habitat types and average daily soil temperature range, where subterranean ant diversity increased with average daily temperature range in sandhill habitats but decreased slightly in the flatwoods (Fig. 4, Table 3). Flatwood sites also experienced higher variation in

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average daily soil temperature range than sandhill sites. This result may indicate a more dynamic environment in the flatwoods, where soil temperature can be influenced by flooding events due to poorly drained soils. Flooding events in these areas as well as shallow water tables may strongly constrain habitat space for these ants (LAMMERS 1987, LUBERTAZZI & TSCHINKEL 2003, TSCHINKEL & al. 2012). Another possible explanation is that some ant species may not be able to tolerate the wide temperature differences and therefore prefer the lower soil temperature variation. It was surprising to find no significant effects on diversity from soil moisture as it could be a better proxy for indicating periodic flooding. However, the relatively brief study did not collect moisture data throughout a wet-dry season cycle, so the full variation of soil moisture that may affect colony distributions was not fully evaluated.

Depths to water tables and inundation dynamics may not drive species composition and diversity differences between the two habitat types. Logistic regressions showed that environmental soil gradients serve a significant role in the occurrence of thief ants and co-occurring ants found in our sampling. For example, in low soil moisture, cooler minimum soil temperature increases the chance of *S. pergandei* occurrence but in high moisture soils, lower minimum soil temperature decreases the chances of occurrence. This suggests that *S. pergandei* might be sensitive to the synergistic effects of both soil moisture and temperature.

The logistic regression for *S. carolinensis* showed significant negative effects on the chances of its occurrence as soil temperature and moisture increased. The same significant effect on the same parameters were also observed for *S. tonsa*. Finally, *S. tennesseensis* occurrence was negatively affected by increasing soil temperature. Across these four thief ant species there is thus a trend of decreasing occurrence as soil moisture or temperature increases (Table 4). These four species were also all positioned tightly within the same sandhill cluster from the NMDS

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analysis suggesting, again, soil abiotic conditions as a potential driver for that thief ant clustering. This is congruent with previous assumptions found from THOMPSON (1980) that highly moist and inundation-prone areas may not be suitable for the persistence of these species as well as a study from Texas (LAMMERS 1987) where it was suggested that subterranean foraging by thief ants may be limited by soil moisture.

When considering the occurrence of other non-thief ant species in flatwoods within the context of the NMDS analysis, only *B. depilis* occurrence was modelled successfully in the flatwoods. A sandhill species, *S. pergandei*, was modeled with the same predictors but responded in opposite directions (Fig. 5, Table 4, Table 5). These contrasting patterns suggest environmental filtering as potential mechanism explaining their occurrence in disparate habitats. *Brachymyrmex depilis* could be more sensitive to xeric conditions as indicated by lower occurrences at lower levels of soil moisture while *S. pergandei* tends to show the opposite trend. These results supports previous suggestions that Florida's subterranean thief ants may occur more frequently in well-drained soils (e.g., high pine sandhill ecosystems) (THOMPSON 1980, LAMMERS 1987). A wider range of environmental conditions in other habitats and locations should also be considered to verify the patterns observed here, in sandy soils.

Although our models show evidence indicative of environmental filtering in certain subterranean species, patterns of occurrence of thief ants may also be affected by the occurrence and distributions of potential prey in the context of the purported lestobiotic interactions that thief ants have with other ants, especially larger-bodied ant species. To further understand the role that species interactions may play in shaping subterranean ant distributions, there is a need for detailed information on, for example, the local distribution of thief ant colonies in relation to

other colonies. Unfortunately, no such data exists but we can cautiously infer patterns of co-occurrence from aboveground pitfall data.

A Comparison of Studies

This study showed the dominance of thief ants among small-bodied ants in the subterranean environment of central Florida's sandy soils. Furthermore, our community analyses indicate significantly distinct subterranean ant communities between flatwood and sandhill habitat types. Moreover, the diversity of these communities can be predicted using soil abiotic conditions. Subterranean thief ant diversity patterns remain largely enigmatic in most regions of the world, so the results of this study are the first quantitative assessments of the diversity and distribution of an abundant group of subterranean ants and the abiotic predictors of that diversity.

This study complements two other subterranean sampling studies in Florida (Tallahassee and Gainesville) and is one of few studies globally to assess abiotic predictors of subterranean ant diversity patterns (THOMPSON 1980, LUBERTAZZI & TSCHINKEL 2003). Ants in the *Solenopsis* genus dominate the subterranean thief ant communities in both north and central Florida. Fifteen total species were found in belowground samples here while 20 species were captured in north Florida (LUBERTAZZI & TSCHINKEL 2003). *Solenopsis pergandei*, was the most dominant species in our study, but not in north Florida. THOMPSON (1980) described *S. pergandei* as an "occasional dominant" species in north-central Florida (Gainesville). The dominant thief ant in both the Tallahassee and Gainesville studies was *S. carolinensis*. This indicates a transition between *S. pergandei* and *S. carolinensis* as dominant thief ants between central and north Florida. Other species occurrences, including *P. dentata*, *P. floridana, P. metallescens*, and *B. depilis* were found in studies of THOMPSON (1980), LUBERTAZZI & TSCHINKEL (2013), and results here. Our study provides further evidence of the widespread,

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high abundances of thief ants in this region. It is also clear that the subterranean ant communities of semi-tropical and temperate Florida are not as diverse as subterranean communities in the Neotropics (THOMPSON 1980, LUBERTAZZI & TSCHINKEL 2003, WILKIE & al. 2007) where as many as 47 species were recorded at local scales.

Sampling methods differed between 2012 aboveground sampling (pitfall traps) and belowground baits in this study; comparisons are made with caution. Aboveground samples collected more species (37 species in 18 genera), and abundances were more evenly distributed among general than in our belowground sampling. Aboveground, the genus *Pheidole* is most abundant followed closely by *Solenopsis* and *Campanotus*. *Solenopsis pergandei* and *S. tonsa*, two truly subterranean species, were not recorded in any of the aboveground traps. However, belowground, *Solenopsis* remains dominant by quite a large margin (Fig. 6). Aboveground species richness remains relatively the same with 32 species in the flatwoods and 35 in the sandhill. The aboveground ant community seems to have a higher abundance of individuals across the genera present in sandhill habitat when compared to flatwoods habitat. However, several genera show the opposite trend, including *Formica* and *Nylanderia*. Considering the temporal difference in the pitfall data and the subterranean data we suggest that it is possible that sandhill habitats may serve as areas of higher abundance of larger-bodied ants that can serve as potential prey for thief ants.

Figure 6: Abundance of aboveground and belowground sampling. Y-axis represents ant taxa at the genus level. X-axis represents the proportion of total abundance per sampling type. Dark sections of the bars represent abundance found in pine flatwood areas and lighter sections represent abundance found in high pine sandhill areas. Note: *Solenopsis invicta* has been removed from the datasets represented in the figure.

Figure 6: Abundance of aboveground and belowground sampling. Y-axis represents ant taxa at the genus level. X-axis represents the proportion of total abundance per sampling type. Dark sections of the bars represent abundance found in pine flatwood areas and lighter sections represent abundance found in high pine sandhill areas. Note: Solenopsis invicta has been removed from the datasets represented in the figure

Lestobiosis and subterranean ant communities

This study affirms the general dominance of thief ants in Florida upland soils (THOMPSON 1980, LUBERTAZZI & TSCHINKEL 2003). If thief ants are truly lestobiotic, then their widespread abundance, now shown by three studies in Florida (including this one), suggests potential for substantial effects on co-occurring ants, including direct and indirect effects via brood raiding and generalist predation (THOMPSON 1980, BUREN 1983, LAMMERS 1987, NICHOLS & SITES 1991, YAMAGUCHI & HASEGAWA 1996, VINSON & RAO 2004). Further sampling is needed to evaluate subterranean ant communities among various ecosystems, and the environmental

conditions that may potentially predict the diversity and distributions of these lesser-known ant communities.

CHAPTER 2: EXPERIMENTAL EVIDENCE THAT SUBTERRANEAN SOLENOPSIS THIEF ANTS (HYMENOPTERA: FORMICIDAE) EXERT TOP-DOWN EFFECTS ON THE ABOVEGROUND ANT COMMUNITY

Abstract

Interspecific ant interactions in natural subterranean habitats have rarely been studied. Instead, research has primarily focused on aboveground interspecific competition. This study sheds new light on predation by an abundant and globally cosmopolitan group of ants, the *Solenopsis* thief ants. Thief ants are hypothesized to prey on brood or larvae of larger ant species in belowground nests after stealing them from the host ant. To assess their potential belowground impacts on the surrounding ant community, subterranean thief ant populations were experimentally reduced in field plots over the course of ~1 year. Aboveground ant sampling quantified potential effects of thief ant reductions on aboveground ants. The reduction methodology, a novel contribution, proved to be successful with significant reductions of both thief ant abundances and occurrences in plots targeted for reduction compared to control plots. Among aboveground ants, only *Dorymyrmex bureni* clearly increased given reduced thief ants; other aboveground ant species were not clearly affected. Based on our results, thief ants selectively affect aboveground ant communities, suggesting predator-prey coevolution.

Introduction

Biotic interactions between organisms serve as an important component in niche-based processes and influence community-level structure. These interactions, such as competition and predation, can mold an ecological community's structure by playing a pivotal role in regulating organisms

at the local population level (Lynch et al. 1979). Although views that counter this idea exist (Ricklefs 2008), previous studies have shown the significant effects that these interactions can have at the local scale and across trophic levels (Thorp & Cothran 1984). Perhaps one of the most direct and significant interactions in regulating organisms at the population level is predation. Assessing the effects of predation on prey in the natural environment is often difficult with some methods involving the assessment of species co-occurrence patterns at various scales (Bell et al. 2010, Jenkins 2006) or the estimation of predicted biomass consumption directly after a predation event while accounting for predation rates (Kaspari et al. 2011).

Ants serve as an excellent group in quantifying and understanding biotic interactions at the community level as they represent a well-studied and abundant group of organisms with established methods available for assessing their abiotic and biotic interactions. Ants have ecosystem-level effects through a variety of interactions such as seed dispersal, soil turnover, and mutualisms with other arthropods (Nkem et al. 2000, Levey & Byrne 1993, Styrsky & Eubanks 2006). These interactions justify the need to study interspecific interactions that may affect the total abundance or species composition of ant communities. Interspecific interactions likely play a strong role in the dynamism and structure of an ant community as evidence from exotic ants show the capacity of these interactions, often times antagonistic, in generating community-level changes (Sanders et al. 2003).

Ant communities are often well-studied in the context of interspecific competition (Sanders & Gordan 2000, Morrison 2000). These competition-based interactions are shown to drive a structured hierarchy of behavioral dominance among some species in a variety of ant communities (Greenslade 1976, Andersen 1997). The subsequent effects of a structured hierarchy based on behavioral dominance plays a role in our perception of the overall structure

of ant communities. However, there is a good deal that we do not know about how other interspecific interactions, such as ant-ant predation, affect community structure. Ant predation on other ants has rarely been studied except for predation by some army ant species (Kaspari et al. 2011). Army ants have been shown to exert a form of top-down regulation on the co-occurring arthropod community, especially ants (Breton et al. 2007, LaPolla et al. 2002, Swartz 1998).

It is probable that predation plays a significant role in the structure and assembly of ant communities at local scales (LaPolla et al. 2002, Powell & Clark 2004). The importance of predation driving top-down regulation of animal communities has been repeatedly shown in vertebrate and invertebrate communities, alike (Krebs 2009). In the specific case of ants, understanding how predation shapes community structure has traditionally been very difficult because most ants spend a significant portion of their life belowground. Interspecific predation occurring belowground is likely very important but also very hard to document and verify. For example, predation of founding ant queens by other ants likely has a direct effect on population-level dynamics in ant communities (Lammers 1987, Nichols & Sites 1991, Vinson & Rao 2004) but has rarely been documented.

This study investigates the interactions between aboveground-foraging ants and subterranean ants that occur in the belowground environment. Subterranean ants spend almost the entirety of their lives exclusively belowground (Wong & Guénard 2017). Studies like this are non-existent in the literature likely due to the difficulty in finding and studying ants belowground under natural conditions. Current hypotheses of the origin and evolution of ants suggest that basal lineages stem from species that were subterranean (Lucky et al. 2013, Wilson & Hölldobler 2005) and recent evidence supports these hypotheses (Rabeling et al. 2008). Thus, studying the ecology of subterranean ants may provide important insights into understanding the evolutionary

biology of ants, generally, and will provide specific information about subterranean interactions between ant species that are likely ancient selective forces. Subterranean ants are often cryptic in their morphology and species delimitation has proven challenging for a number of genera (Pacheco & MacKay 2013, Wong & Guenard 2017). Therefore, not only do many taxa remain undescribed but the conventional morphological approaches to describing ants may not be as useful for describing many subterranean species. However, there are certain groups of subterranean ants that have been morphologically well-described, such as thief ants (Pacheco MacKay 2013). Collectively, there are a number of challenges to studying subterranean ant communities but improving our understanding of their ecology is justified.

Thief ants are a globally conspicuous member of most ant communities throughout the world, ranging from tropics to temperate zones. In Florida, they are a dominant group in belowground ant communities, often comprising over half the total abundance of ants (Lubertazzi & Tschinkel 2003, Ohyama et al. 2018) and have been taxonomically described without any significant changes for decades (Moreno-Gonzalez 2001, Pacheco & MacKay 2013, Deyrup 2016, Thompson 1980, Thompson 1989). Furthermore, Florida's thief ant natural history is better understood relative to their congeners around the world. However, our understanding of their natural history has resulted primarily from a very limited number of studies and a handful of anecdotal accounts of their behavior and ecology (Blum et al. 1980, Deyrup 2016, Hölldobler 1973, Thompson 1980, Wheeler 1901). Existing evidence suggests that there is a high probability that most thief ant colonies are lestobiotic, preying on other ground-nesting species of ants that forage aboveground and are regularly found near thief ant colonies.

Lestobiosis is a subterranean predatory interaction typically involving a small-bodied "thief" ant species that nest and forage belowground near the nests of larger-bodied host ant

colonies, tunnel into their hosts' nests, and steal their brood (Deyrup 2016, Hölldobler 1973, Hölldobler and Wilson 1990, Tschinkel 2006). Thief ants are assumed to be lestobiotic upon a wide range of ant species that are often much larger in size, although thief ants may also be dietary generalists and even predators of other ants when not stealing brood as they have also been observed actively preying on founding queens (Blum et al. 1980, Deyrup 2016, Thompson 1980, Buren 1983, Lammers 1987, Nichols and Sites 1991, Vinson and Rao 2004, Wheeler 1901).

Whether thief ants specialize solely on the brood and larvae of other ants or are generalist omnivores like their sister group, the fire ants, remains unknown. And the effects of lestobiosis or general predation by thief ants on co-occurring ant populations is unknown. Thief ants are often very abundant locally in a wide variety of subtropical and tropical ecosystems. If highly abundant thief ants are actively lestobiotic and regulate co-occurring aboveground-foraging ant populations, then release from lestobiosis through multiple generations of brood and workers should quantifiably increase aboveground-foraging ant worker abundances. This study's objective is to test that hypothesis.

To test this hypothesis, a field experiment was conducted where thief ant populations were significantly reduced in treatment plots compared to control plots. Co-occurring ant abundances were then quantified and compared between treatment types.

Predation is the assumed regulatory mechanism of co-occurring aboveground-foraging ants as thief ants practice a completely different life history strategy compared to the majority of the co-occurring ant community. They forage belowground in a different stratum than most ants (Ohyama et al. 2018) and are smaller than almost all other ants found in the same community (Deyrup 2016, King & Porter 2010, Pacheco & MacKay 2013). These distinct differences allow

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the probabilistic assumption that competition plays a negligible role in the relationship between thief ants and co-occurring aboveground-foraging ants.

Field studies involving the removal or significant reduction of ants from large plots are not common. They are logistically difficult to execute and often require long time periods to determine the effectiveness of the removal or reduction and whether impacts on co-occurring species has occurred. Removing or reducing specific ant species from a community adds another layer of difficulty as removal or reduction methods must be specific, only affecting the species or group of interest. Despite such difficulties, removal or reduction experiments provide a robust and elegant approach to determining the impact of a species in a community.

In the past, ant removal or reduction field-based studies have often relied on techniques that center on the prevention of ants from gaining access to areas in a form of exclusion (e.g. caging, fencing) (Andersen & Patel 1994, Gibb & Hochuli 2004, Zelikova et al. 2011). Other methods of reduction or removal are short temporal reductions of a species from localized areas (LeBrun et al. 2007). There are even fewer studies that focus on large-scale and long-term reduction of ants from plots of natural habitat, most likely because they prove to be logistically challenging. One of the few studies that has been able to successfully reduce certain groups of ants from large areas in the field over multiple years comes from King & Tschinkel (2006), where colonies of the red-imported fire ant, *Solenopsis invicta,* were successfully reduced over the course of three years. The same study involved the monitoring of co-occurring ants in these plots to assess the competitive effects of the invasive species.

For this study, subterranean thief ants were reduced in the field over the course of almost one year. Similar to King & Tschinkel (2006), co-occurring ant populations were assessed throughout the duration of the experiment in treated and control plots.

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Methods

Study site and design

20 plots were randomly placed in sandhill habitat of Wekiva Springs State Park (2,750 hectares)

situated in Orange and Seminole County Florida. Sandhill was used for the location of this

experiment given results from a study by Ohyama et al. (2018). The authors established that this

habitat holds a high diversity and very high abundance of subterranean thief ants. High pine

sandhill is a pyrogenic habitat characterized by well-drained sandy soils, an overstory of longleaf

pine (*Pinus palustris*), and a groundcover dominated by wiregrass (*Astrida beyrichiana*) (Myers

& Ewell 1990) . The placement of sandhill sites were randomly generated in ArcMap and placed

in areas maintained by low intensity prescribed fires. 10 of these were randomly chosen to

receive a thief ant reduction treatment while the other 10 plots were left as control plots,

receiving no treatments. Plots were 18 m × 18 m. 16 flags were positioned in these plots in a grid

spaced out by 6 meters, these flags were point flags that represented the borders and gridlines

within each plot. Another 16 flags were set up within a 1-meter radius of the point flags. These

flags represented points where thief ant reduction and sampling were done. These flags were

randomly moved within a 1-meter radius of the point flags for every sampling and thief ant

reduction event to avoid the resampling of the same position.

Thief ant reduction and belowground sampling

The thief ant reduction treatment consisted of loading plastic capped vials 70 mm tall and 30 mm

in diameter with AMDRO™. This approach is an adaptation of sampling methods used by

Ohyama et al. (2018). AMDRO is an ant-specific pesticide with a main chemical compound of

hydramethylnon (Meer et al. 1982). It's effects on other arthropods are minimal, especially when

the target species collect the majority of the toxic bait (Apperson et al. 1984). A ~5 mm diameter hole was made at the bottom of the plastic vial and covered with 0.75 mm screening to exclude larger insects and arthropods (e.g., fire ants) but permit entry by thief ants. This approach reduces the likelihood of non-target effects as the vast majority of ants with access to the pesticide baits were thief ants. We note that a few species of small *Pheidole* (*P. metallescens*, *P. floridana*) still had the capacity to enter these vials and did so in relatively small numbers in some of the plots (see Results).

The screening used for insecticide treatment vials in this study had smaller openings compared to those used by Ohyama et al. (2018). As a result, the traps permitted fewer species and in lower numbers of non-thief ant species to enter the vial. Specifically, Ohyama et al. 2018 showed that screening with 1 mm aperture still allowed species such as *Forelius pruinosus*, *Pheidole morrisii*, and *Nylanderia wojciki* to pass through. Thus, we adopted the 0.75 mm aperture screening for this study.

Pesticide-loaded vials were delivered belowground to a depth of ~10 cm below the soil surface using a 24-inch auger bit with a 24-volt battery-operated drill. Vials remained underground for 2 weeks then removed and replaced with a vial with fresh bait. The treatment was carried out during the last two weeks of every month starting from February 2018 to November 2018. After every pesticide treatment a subterranean sampling of all plots was done to evaluate the effects of the pesticide on thief ant relative abundances and occurrences. Sampling was not done for the month of October because of difficult field conditions. These sampling events utilized a vial with the same design as the one used to deploy AMDRO belowground but were filled with Pecan Sandies™ cookies. Vials were planted 10 cm belowground and left for 3 days. An initial sampling of the plots prior to the first pesticide treatment was conducted in the

month of February to establish baseline abundances of both above and belowground ants for all plots.

Aboveground pitfall sampling

4 pitfalls were randomly set up in each plot and operated for 3 days in tandem with the subterranean sampling. Pitfall traps were 85 mm long plastic vials with 30 mm internal diameter partially filled with ~ 15 ml of non-toxic, propylene-glycol antifreeze. Traps were buried with the opened end flush with the surface of the ground. Traps were installed using a hand-held, battery-powered drill using an auger bit. All ants were pooled into one sample to represent the abundance and occurrence per plot rather than per trap.

Seasonality

It's important to note that the study site experienced abnormal weather patterns during the wet season (months of April to August) in 2018. These abnormalities included heavy pulses of rain and intermittent weeks of droughty conditions that likely reduced ant foraging (personal observation) relative to 'normal' years where ant worker activity peaks in the summer months. As a result, pitfall trap abundances were unusually low from the months of June through September. Comparisons among treatments and controls still remain valid as all plots were subject to the same conditions. All ants from all pitfalls were identified to species and counted by the authors. Voucher specimens were deposited in the University of Central Florida Collection of Arthropods.

Analysis

To validate the reduction of thief ants in the plots both graphing and statistical modeling were used. Occurrences and abundances were modeled as a function of treatment type and a covariate

of the initial abundances or occurrences in the plots. The covariate was included as a model

parameter to account for the natural variation of ant abundance or occurrences for each plot and

comes from the first month of sampling (February 2018) prior to the first treatment of pesticide.

This was necessary as some plots had been randomly generated in areas with high concentrations

of thief ants. Therefore, in order to assess the effectiveness of the thief ant reduction treatment it

was necessary to account for this initial and unequal variance. A mixed-effects model with a

negative binomial error distribution and a random intercept for months that accounted for

repeated sampling was used with the initial data as a covariate (scaled to z-scores). Reduction of

thief ants was also assessed using heat maps at the plot level for the months of August to

November. This was done because a single belowground bait could at times hold up to ~600

thief ant workers. In some cases, this one bait could skew and misrepresent the abundance of

thief ants throughout the entire plot.

A similar modeling approach was used with pitfall data to assess the potential effects of

thief ant reduction on aboveground ants. Any species found in the belowground baits were

removed from the aboveground pitfall analyses as these species would have had access to the

pesticide (10 species total, average of 2 species per plot, per month). A mixed-effects model,

with a negative binomial error distribution, and a random intercept to account for repeat

sampling was used. The negative binomial distribution was selected for all models due to

overdispersion in the count data.

Initial sampling data from February was not included in the response variable for the

models as the data was collected prior to the first pesticide treatment. All models were run using

R statistical programming and the package lme4 (R Development Core Team 2018, Bates et al.

2015). All plots were done using the package 'ggplot2' (Wickham 2009). Pseudo-R^2 values for marginal and conditional effects were generated using the R package 'MuMIn' (Barton 2018).

<div align="center">Results</div>

<div align="center">*Thief ant reduction*</div>

Thief ant abundances and occurrences were reduced throughout the duration of 8 sampled months in plots treated with AMDRO. Significant reduction was not observed until the months of May and June for both abundances and occurrences, respectively. Subsequent months after the initial signs of reductions showed rapidly increasing differences between pesticide-treated and control plots (Figure 7 & 8). Non-thief ant species caught in our belowground bait traps included small-bodied species of *Pheidole* (*P. adrianoi*, *P.floridana*, *P. metallescens*, and *P. moerens*), *Nylanderia wojciki* and *Wasmannia auropunctata*. The latter two species only occurred once and twice respectively across all samples. There were a total of 3 *N. wojciki* and 84 *Wasmannia* individuals recorded from the sampling while the total belowground ant count was 197,400 ants. Finally, heat maps of average thief ant abundance generated for the months of August, September, and November (Figure 9) indicate that the average thief ant worker abundance at the individual plot scale were lower in thief ant reduced plots versus control plots across the majority of the 16 sampling points per plot.
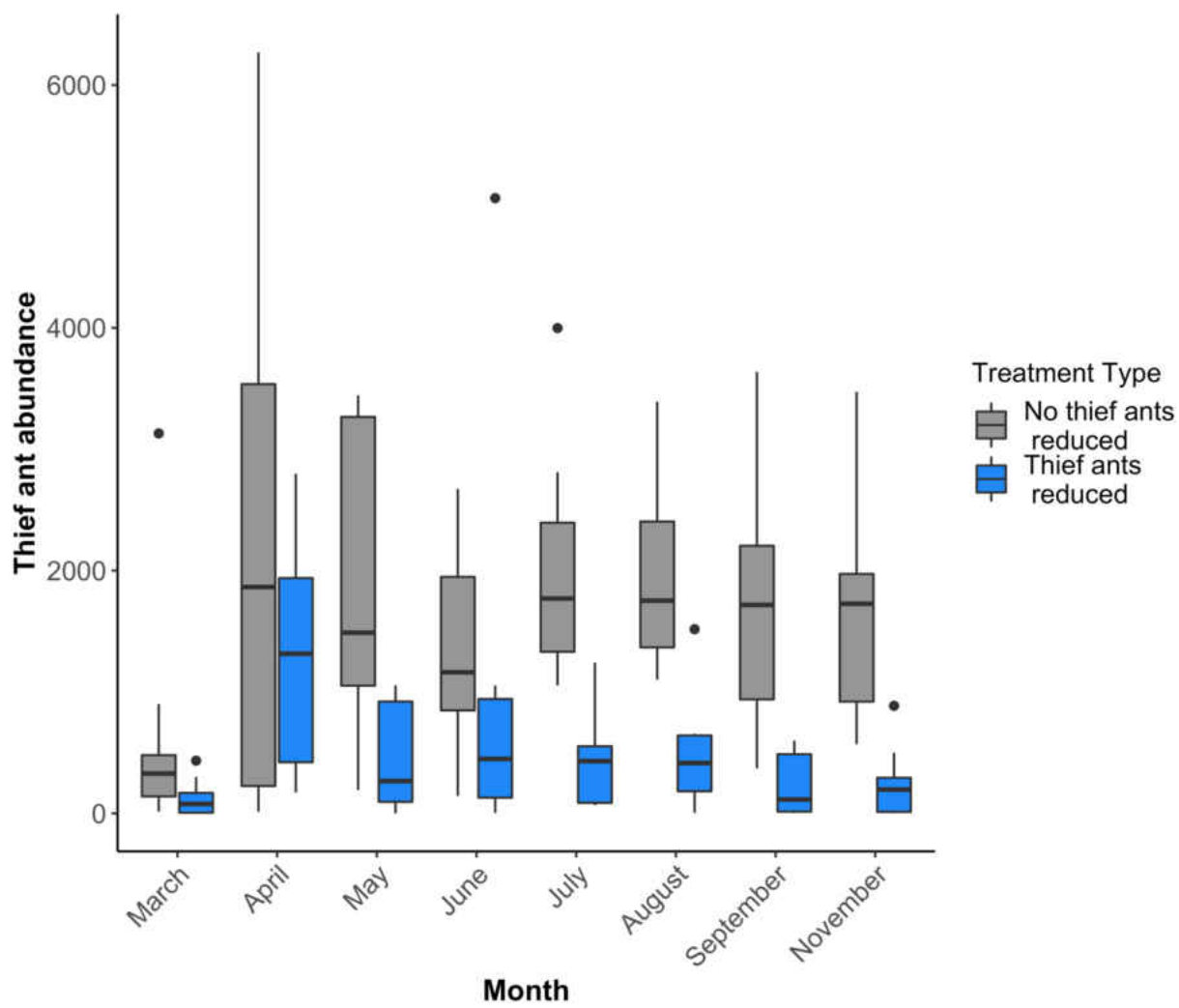
Figure 7: Boxplots showing the distribution of thief ant worker abundance (Y – axis) in control and treatment plots for every month of sampling (X – axis).
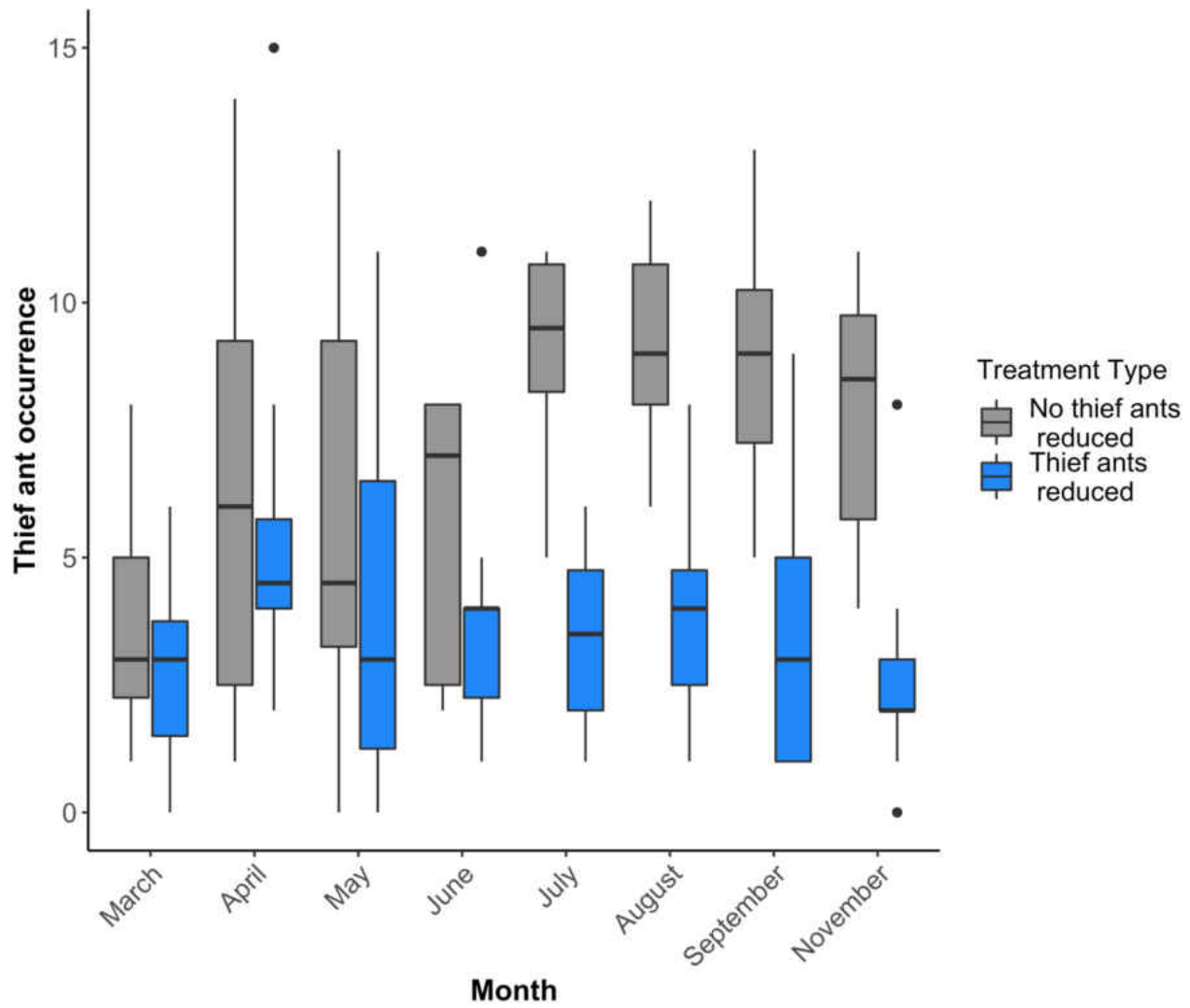
Figure 8: Boxplots showing the distribution of thief ant occurrence (Y – axis) in control and treatment plots for every month of sampling (X – axis).
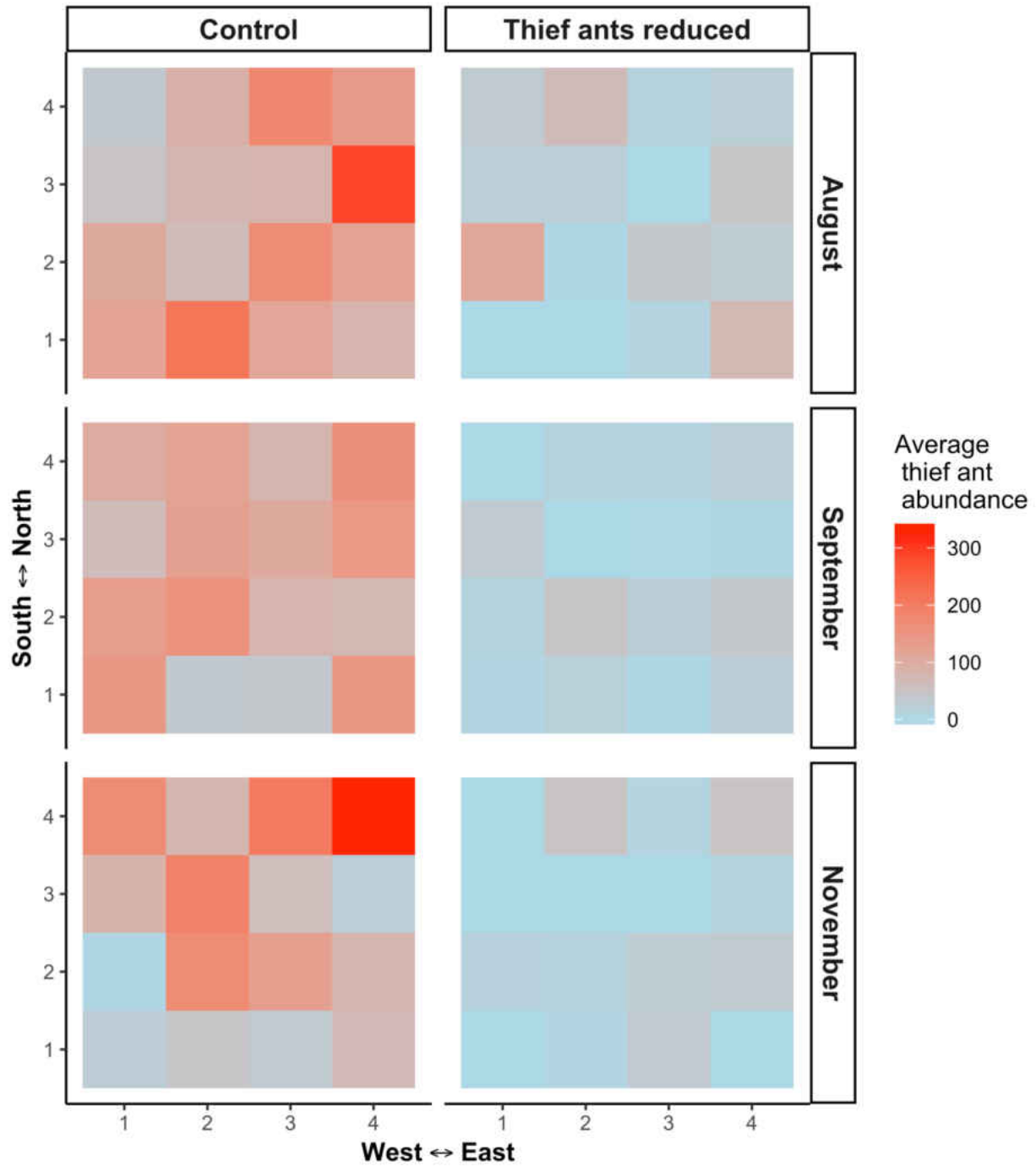
Figure 9: Heat maps of average thief ant abundance at the plot level for 16 sampling points across plots based on treatment type and the month of sampling.

The total abundance and occurrence of the belowground sampling also show proportionally large total differences between control and thief ant reduced plots (Fig 11 & Fig 12). Across all plots, thief ants made up 88.67% of the total ant abundance and 66.62% of all ant occurrences in belowground samples.

There was a lag effect in thief ant reduction during the course of the year as thief ants did not instantly decrease in number as treatments were applied (Figure 7 & 8). However, the months of May and June saw a significant reduction of abundance and occurrence. The two most likely, not mutually exclusive explanations for this lag effect are: 1) it took ~4-5 months to reduce the number or size of colonies in the treated plots to a detectable level, and 2) thief ants may increase foraging activities in the months of May, therefore increasing encounters with pesticide during the subsequent months (Figure 10). By August, the effects of the treatment were apparent at the plot level (Figure 9) with average thief ant abundances at almost every sampling point within treated plots being lower than abundances in the control plots.
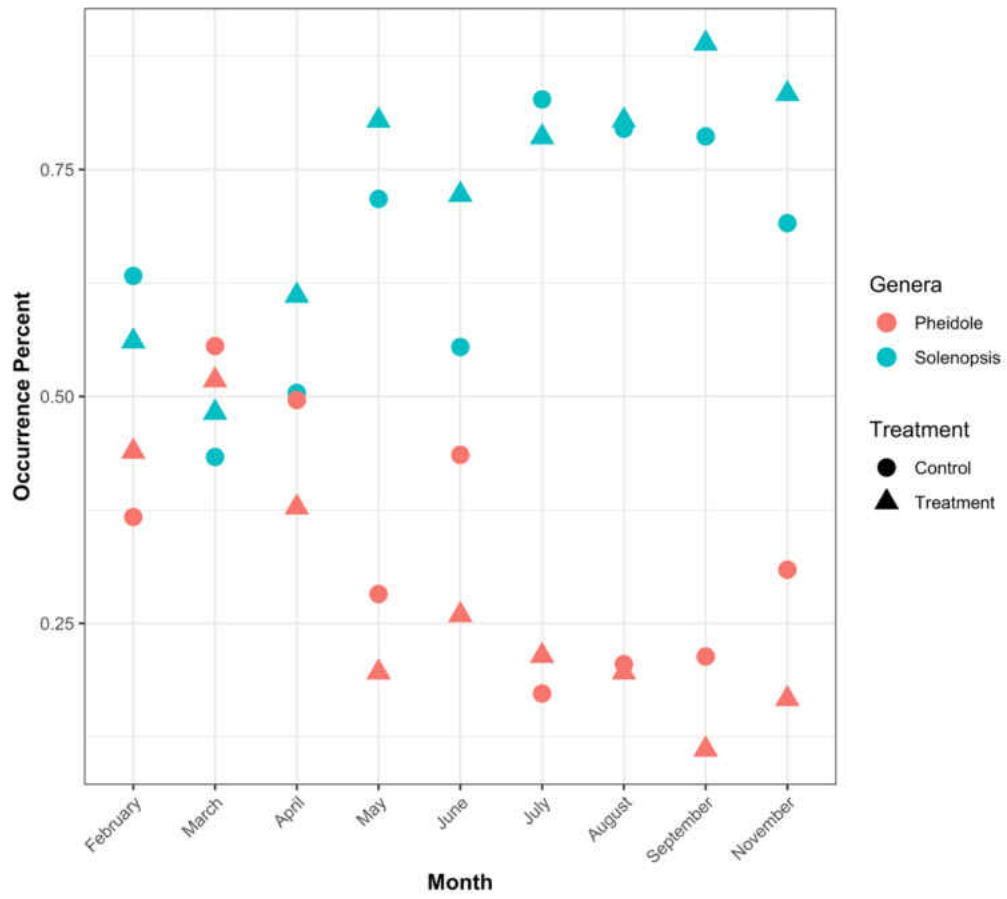
Figure 10: Plot showing the occurrence percentage of the genera Pheidole and Solenopsis (Y – axis) across sampled months (X – axis) from belowground sampling. Shapes represent treatment types and colors represent taxa.
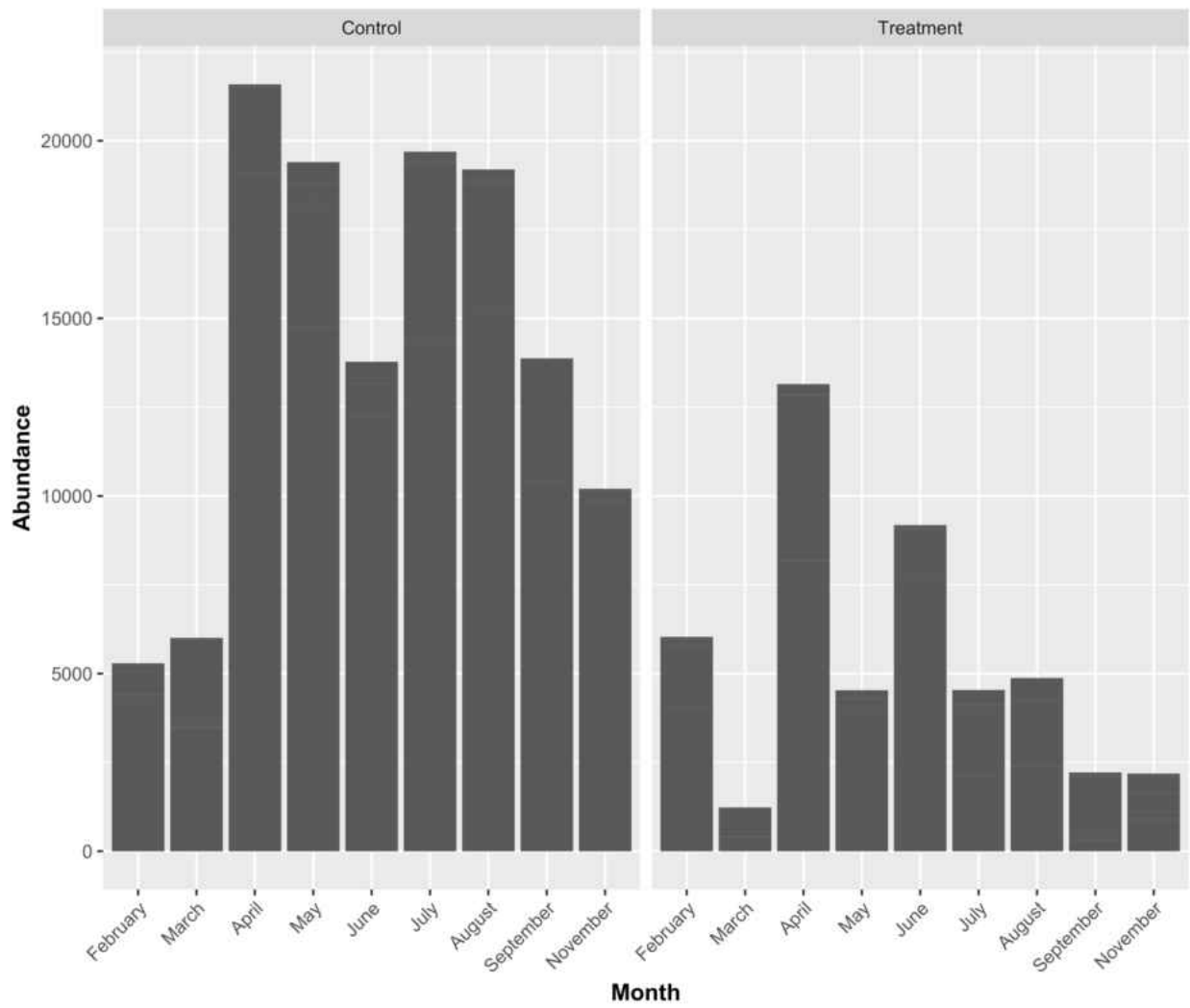
Figure 11: Column graph showing the total summed abundance of thief ant workers for each month of sampling
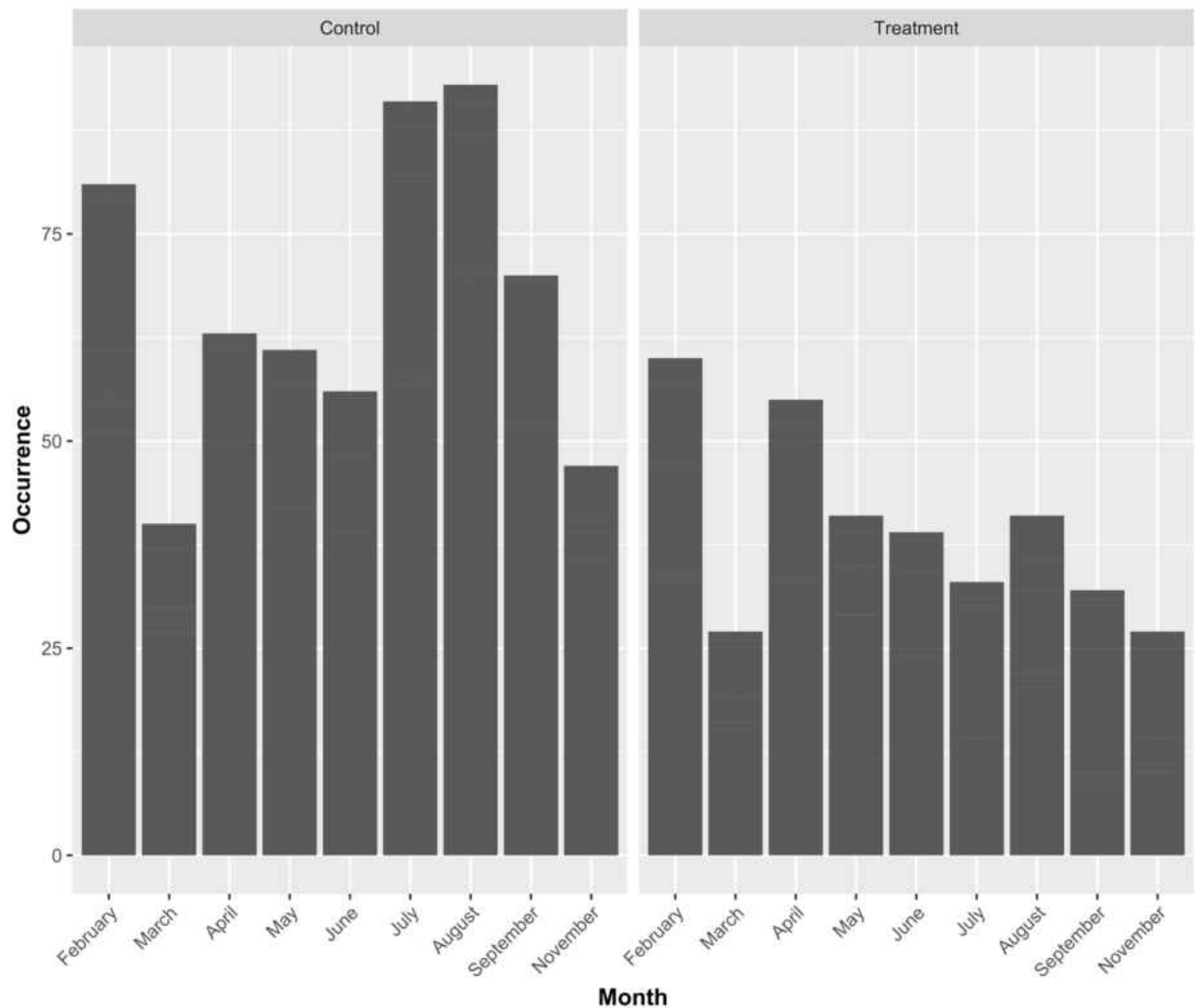
Figure 12: Column graph showing the total summed occurrence of thief ant workers for each month of sampling

Fixed effects of the model showed significant reduction of thief ant occurrence in thief ant reduced plots relative to control plots (Table 6, Figure 13). Marginal and conditional Pseudo-R^2 values were 0.23 and 0.27 where marginal values represent the variation explained by the fixed effects and conditional values represent the variation explained by the both fixed and random effects. These values were calculated using the tri-gamma function, a method that is best suited for distributions using logarithmic links (Barton 2018). It's important to note that pseudo-

R^2 values for mixed-effect models are not fully reliable and may misrepresent the model's ability to fully explain variation in the data. Therefore, although helpful, these values should be interpreted with some caution.

Table 6: Model output from a negative binomial mixed-effects model assessing thief ant occurrence as a function of treatment type and an initial occurrence covariate. The variation from the random effects of months as well as the fixed effects estimates and their respective standard errors are shown.

Model: Thief ant occurrence ~ Initial occurrence + Treatment type + (1|Month)

Random effects	Variance	Standard deviation		
Months	0.2167	0.1472		
Fixed effects	Estimate	Standard error	z – value	Pr(>|z|)
Intercept (Control)	1.86715	0.08390	22.253	<2e-16
Initial occurrence	0.1397	0.05196	2.573	0.0101
Treated	-0.50178	0.10282	-4.880	1.06e-06

Fixed effects from the mixed-effects model assessing thief ant abundance showed significant reduction of abundance in thief ant reduced plots relative to control plots (Table 7, Figure 14). Pseudo-R^2 values based on the tri-gamma function show that the marginal R^2 was 0.13 and the conditional R^2 was 0.18.

Model: Thief ant abundance ~ Initial abundance + Treatment type + (1|Month)

Random effects	Variance	Standard deviation		
Months	0.1785	0.4225		
Fixed effects	Estimate	Standard error	z – value	Pr(>|z|)
Intercept (Control)	7.3685	0.2018	36.506	<2e-16
Initial abundance	0.08428	0.1017	0.829	0.407
Treated	-1.26095	0.1942	-6.493	8.39e-11
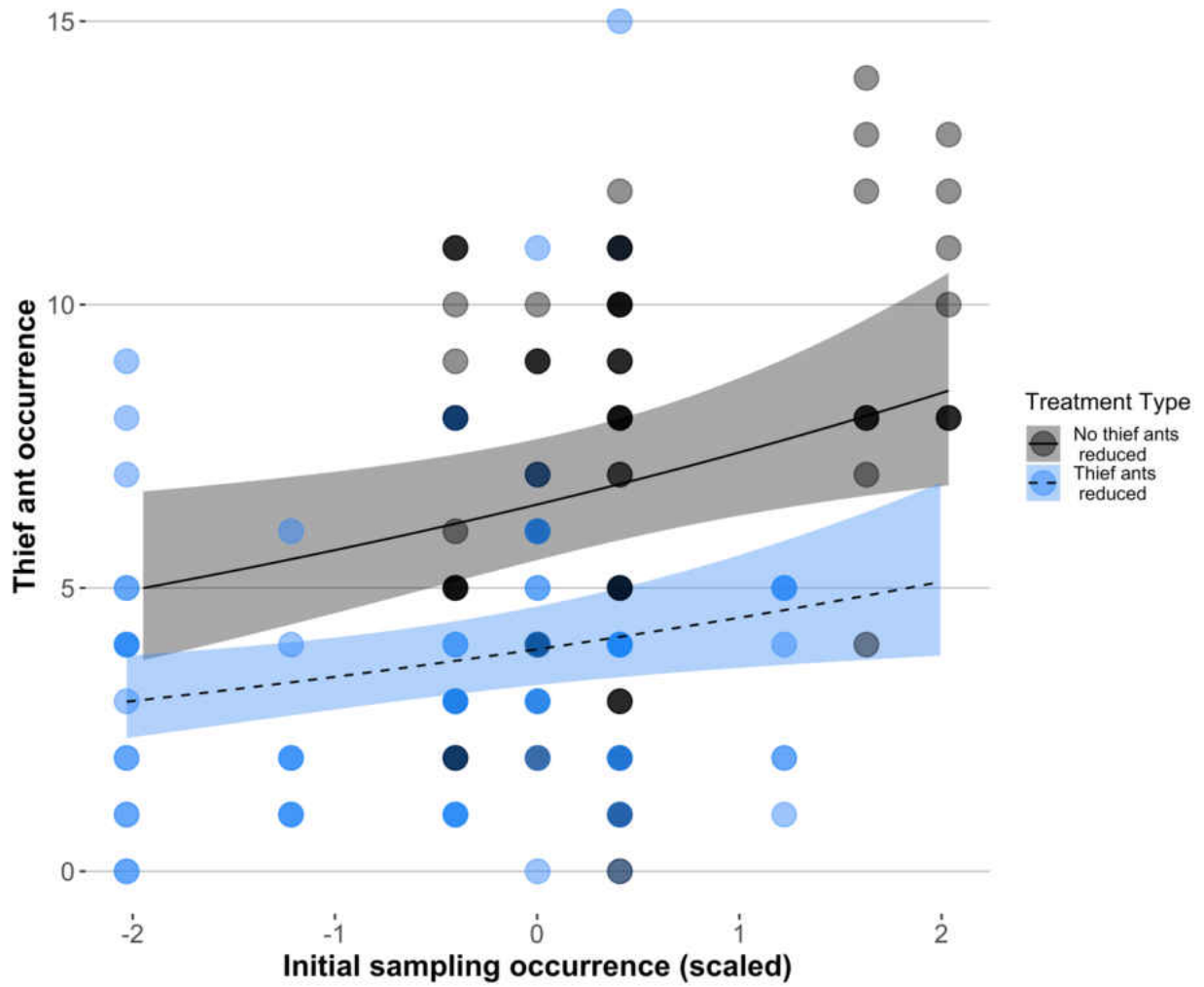
Figure 13: Fixed effects of a mixed-effect negative binomial model fitted regression values of thief ant occurrence by treatment type. X − axis represents initial sampling of occurrence scaled by z-scores. Y − axis represents the back-transformed predicted values from the model. Shaded regions represent 95% confidence intervals that account for the uncertainty of the fixed effects. Raw data represented by colored points (blue, black). The uncertainty of the random effects is not accounted for in the confidence intervals therefore caution is necessary when interpreting them.
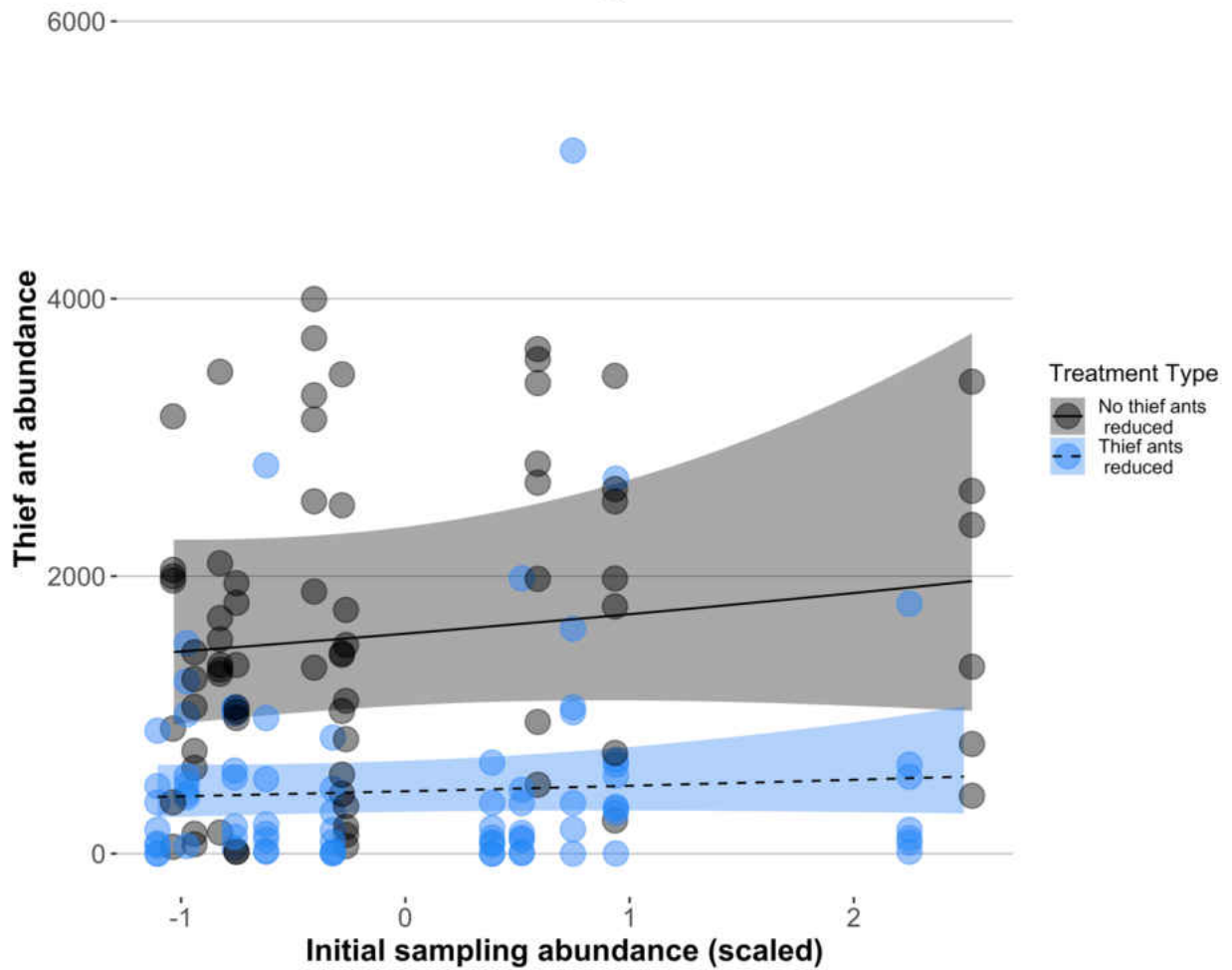
Figure 14: Fixed effects of a mixed-effect negative binomial model fitted regression values of thief ant abundance by treatment type. X – axis represents initial sampling of abundance scaled by z-scores. Y – axis represents the back-transformed predicted values from the model. Shaded regions represent 95% confidence intervals that account for the uncertainty of the fixed effects. Raw data represented by colored points (blue, black). The uncertainty of the random effects is not accounted for in the confidence intervals therefore caution is necessary when interpreting them.

Aboveground ant effects

Prior to analyzing the aboveground ant data obtained from pitfall sampling, species that were

found in belowground baits were removed from the dataset to avoid false inferences for species

that could have been affected by the pesticide. The species removed were *Nylanderia wojciki*,

Pheidole adrianoi, *P. floridana*, *P. metallescens*, *P. moerens*, *Solenopsis carolinensis*, *S. invicta*,

S. nickersoni, *S. tennesseensis*, and *Wasmannia auropunctata*. These 10 species accounted for

40% of the ant abundance and 34% of the occurrences in the aboveground pitfall dataset. Of the

remaining species, 38 species were pooled as aboveground ant abundance because they appeared

in very low numbers or sporadically in some months. One species, *Dorymyrmex bureni,* was

well-represented in data every month and provided a robust response for the repeated measures

statistical model.

Thief ant reduced plots consistently held higher abundances of aboveground ants over

time than in control plots. The differences in abundances were detectable as the mixed-effects

model of aboveground ant abundance showed a positive estimate in thief ant reduced plots

compared to control plots (Table 8, Figure 15). The random intercept of months helped parse out

the signal from this data and this is shown by the variance and standard deviation of the random

intercept output from the model (Table 3) as well as the tri-gamma pseudo-R^2 value where the

marginal (0.08) and conditional (0.24) pseudo-R^2 values indicate ~ 66% of the total explained

variance contributed by the random effect.

Table 8: Model output from a negative binomial mixed-effects model assessing aboveground ant abundance as a function of treatment type and an initial abundance covariate. The variation from the random effects of months as well as the fixed effects estimates and their respective standard errors are shown.

Model: Aboveground abundance ~ Initial abundance + Treatment type + (1|Month)

Random effects	Variance	Standard deviation		
Months	0.3059	0.5531		
Fixed effects	Estimate	Standard error	z – value	Pr(>|z|)
Intercept (Control)	3.4491	0.2255	15.296	<2e-16
Initial abundance	0.3638	0.0879	4.137	3.52e-05
Treated	0.4746	0.1620	0.293	0.003
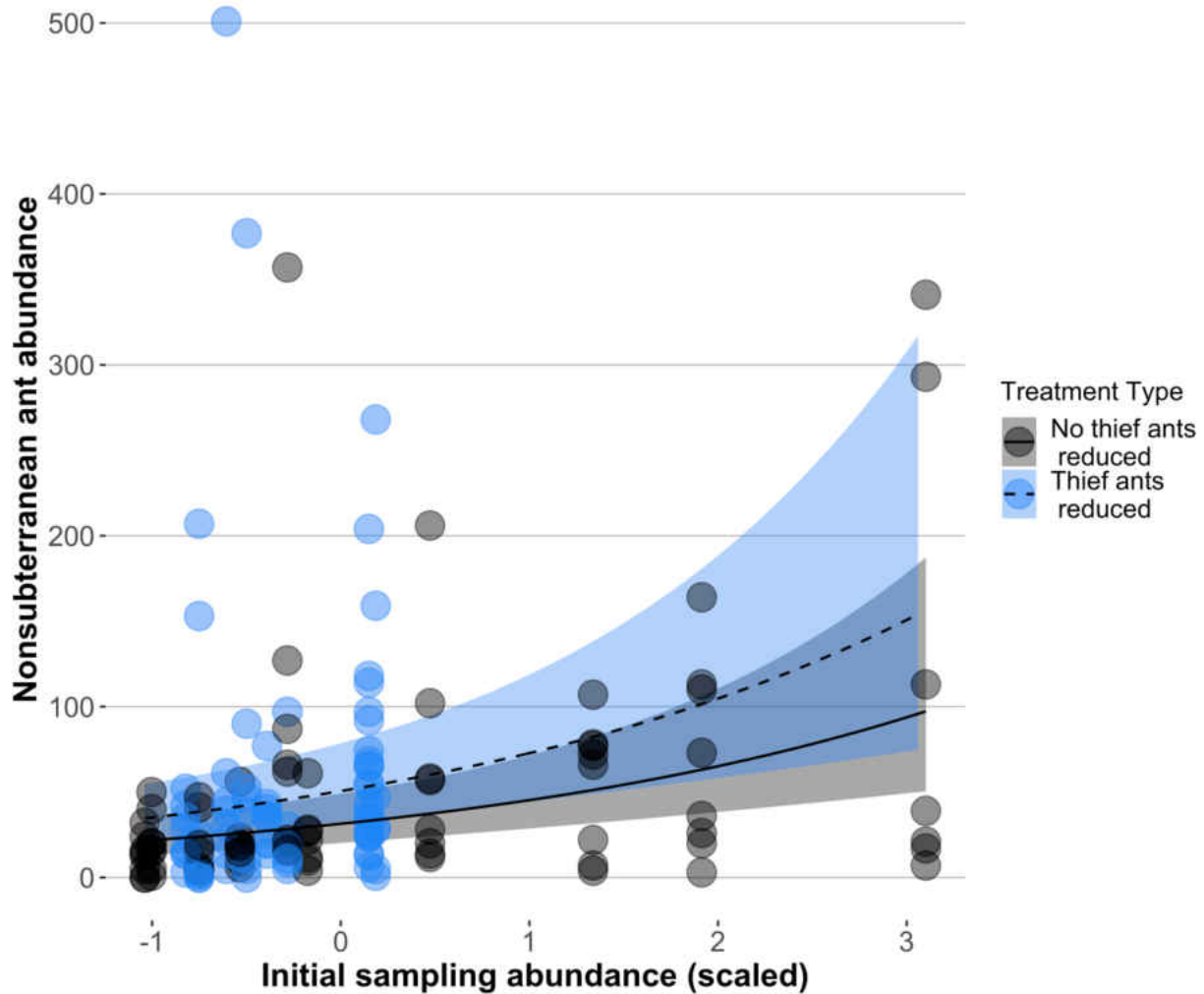
Figure 15: Fixed-effects of a negative binomial model fitted regression values of aboveground ant abundance by treatment type. X – axis represents initial sampling of abundance scaled by z-scores. Y – axis represents the back-transformed predicted values from the model. Shaded regions represent 95% confidence intervals that account for the uncertainty of the fixed effects. Raw data represented by colored points (blue, yellow). The uncertainty of the random effects is not accounted for in the confidence intervals therefore caution is necessary when interpreting them.

The final mixed-effects model was the abundance of *Dorymyrmex bureni* worker abundance as a function of an initial sampling covariate and treatment type (Figure 16). The model showed a significant positive estimate for *D. bureni* abundance in thief ant reduced plots

versus control plots (Table 9). The marginal and conditional pseudo-R^2 values were 0.11 and

0.14 respectively.

Table 9: Model output from a negative binomial mixed-effects model assessing Dorymyrmex bureni ant abundance as a function of treatment type and an initial abundance covariate. The variation from the random effects of months as well as the fixed effects estimates and their respective standard errors are shown.

Model: *Dorymyrmex bureni* abundance ~ Initial abundance + Treatment type + (1|Month)

Random effects	Variance	Standard deviation		
Months	0.1107	0.3328		
Fixed effects	Estimate	Standard error	z – value	Pr(>|z|)
Intercept (Control)	1.7954	0.1925	9.328	<2e-16
Initial abundance	0.5270	0.1050	5.019	5.19e-07
Treated	0.7908	0.2101	3.764	0.000167
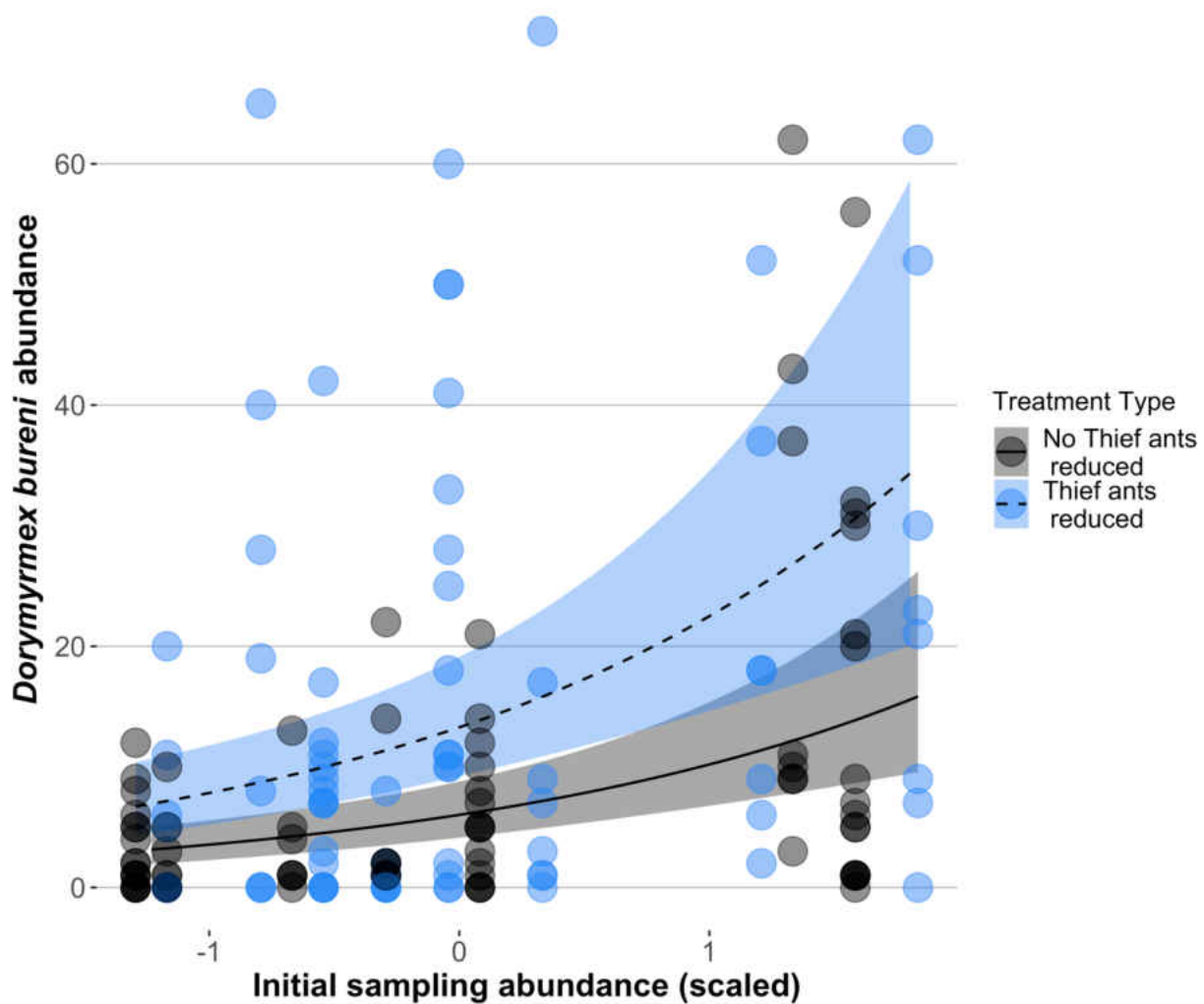
Figure 16: Fixed-effects of a negative binomial model fitted regression values of Dorymyrmex bureni abundance by treatment type. X – axis represents initial sampling of abundance scaled by z-scores. Y – axis represents the back-transformed predicted values from the model. Shaded regions represent 95% confidence intervals that account for the uncertainty of the fixed effects. Raw data represented by colored points (blue, black). The uncertainty of the random effects is not accounted for in the confidence intervals therefore caution is necessary when interpreting them.

<u>Discussion</u>

Thief ant reduction

The methods utilized to reduce thief ant diversity were effective as the models showed the

effectiveness of AMDRO applied in screened, belowground traps in reducing thief ant

abundance and occurrence belowground. Although a small number of ant species other than thief ants, such as small *Pheidole* species, were potentially impacted by treatments of pesticide, the reduction of these other species was minimal relative to the impact of the treatments on thief ants. *Pheidole* species baited belowground decrease in abundance and occurrence around the onset of the wet season suggesting a shifting of foraging strategies to the aboveground surface (Figure 10). At the same time, thief ants start increasing in their activities belowground. Furthermore, a study done in the same park in 2017 established that thief ants make up 70% of the belowground ant fauna in sandhill habitat (Ohyama et al. 2018). Therefore, pesticide treatments had the greatest impact on the most abundant ants in the belowground environment, thief ants. Nevertheless, we excluded non-thief ant species found in both below and aboveground sampling from our aboveground pitfall trap analyses to account for any potential of non-target effects due to pesticide exposure.

Results of this novel approach to experimentally manipulate subterranean ants show the value of implementing consistent field applications, in the manner of a press experiment, every month instead of a single pulse event. This is important as seasonal fluctuations in abundance or other factors affecting abundance over short time spans could make interpretation of data more challenging. Furthermore, as ants are territorial, new colonies may quickly move into areas that have been reduced. In order to sustain a reduction of the species of interest, a consistent application of control measures was necessary.

Effects on aboveground communities

Our results show increases in aboveground ant, and especially *Dorymyrmex bureni,* abundance corresponding with the experimental reduction of thief ants belowground. The effects of thief ant reduction took some time to become apparent in the aboveground ant community. Such lag

effects can be a result of seasonal fluctuations in aboveground-foraging ant worker abundance, for example. The majority of aboveground foraging ants in these communities begin the production of their reproductive sexual castes in the spring season (Deyrup 2016). During this time, sexual-producing colonies produce fewer workers and more sexuals. Among species here, the fire ant, *S. invicta*, is the best studied example of this annual cycle, where the tradeoff between producing sexuals or worker castes results in a diminished number of foragers during the spring (Tschinkel 2011). Forager numbers do not increase until the end of the mating season and the beginning of Fall. Therefore, under normal conditions, the initial signs of community effects on aboveground ants would most likely begin to show in the later months of September. It's likely that another year of treatments and sampling would result in clearer patterns showing effects of the removals on the aboveground ant community.

Based on evidence that aboveground ant abundance was higher than in thief ant reduced plots, it is probable that the aboveground ant community is going through a form of release from predation on brood by thief ants. This makes this study the first of its kind to show experimental evidence that lestobiosis may play a significant top-down regulatory role in ant communities. While it is possible that thief ants may only impact some species more than others, our data show potential impacts affecting multiple species.

One species, *Dorymyrmex bureni,* showed strong effects from the reduction of thief ants, in part because it was more consistently abundant. *Dorymyrmex bureni* is a likely species to be the first to show any substantial changes in worker abundance given that they are one the most abundant members of Florida's ant communities, have shorter generation cycles, and are also often viewed as opportunists (Deyrup 2016, King & Porter 2007). Our model shows that in plots with naturally high abundances of *D. bureni*, the effects from reducing thief ants was greatest

compared to plots with naturally lower levels of abundances (Figure 16). Although weaker, this pattern is also observed from the model assessing general aboveground ant abundance (Figure 15). Therefore, as prey density increases the effects of predation also increase suggesting that the magnitude of brood predation by thief ants is likely density-dependent and occurs across the spectrum of prey density. This further suggests that thief ant predation could be a form of apex predation as it is a constant pressure on the ant community.

The implications of this study for ant community ecology are important. Here we show the smallest-bodied ants in a community, which is a group of subterranean thief ants, likely play a top-down regulatory role on the aboveground ant community through brood predation in the belowground environment. Our experimental evidence suggests that belowground ecological interactions between ants in the form of predation influences ant community structure. Considering that most ants spend a significant portion of their in-nest lives belowground these interactions are most likely equally or more important than the effects of ant-ant predation that occur aboveground (e.g. predatory effect of spiders, vertebrates, other ants on foragers). Further, these results suggest that many of the ecosystem services provided by ant communities are potentially indirectly influenced by the presence and abundance of thief ants.

Predation occurring in the subterranean environment is an understudied aspect of ant ecology and our study creates a new perspective on how ant communities are regulated. Furthermore, the type of predation that is most likely occurring belowground involves the targeting of brood or larvae rather than fully-developed workers suggesting that these thief ants pose a survival hurdle to aboveground-foraging ants at very specific life stage in their development. Therefore, it's most likely that these belowground ecological interactions play a

significant role in what is observed in the ant community aboveground. The smallest members of the ant community thus appear to exert an oversized effect on the entire community.

<p style="text-align:center">Lestobiosis</p>

Lestobiosis by thief ants plays an important role in regulating abundance of multiple aboveground-foraging ants, and possibly for specific species. The mechanisms driving this study's results, particularly with *D. bureni*, may include direct predation on queens but more likely is predation on larvae and pupae.

Different effects among different prey ant species may be due to nest architecture or in-nest behaviors (e.g., frequency of brood visits, brood care, or protection). We hypothesize that brood predation acts as a selective pressure for nest architecture, where nests that are diffuse and connected by long tunnel networks are more vulnerable to brood predation by thief ants. Such architecture is seen in *Dorymyrmex bureni* nests which are comprised of small flat chambers spread out vertically through a single tunnel system (Tschinkel 2003, Tschinkel 2015). While diffuse distribution of chambers may be more vulnerable, it may also positively affect overall survivorship of colony resources and brood by spreading risk via multiple small targets compared to architectural designs that utilize one major chamber. The relationship between ant nest architecture, in-nest brood care behaviors, and the ability of potential subterranean predators, like thief ants, to access brood piles has not been considered as part of the selective forces shaping ant nest architecture (Tschinkel 2003) but should be.

Considering the high abundance and widespread distributions of thief ants across the warm temperate, subtropical, and tropical regions of the earth, it is reasonable to assume that the evolution of lestobiotic behavior has contributed to the success of this group of *Solenopsis*

species and the evolution of other ants. Unfortunately, the lack of natural history studies on this group impedes additional productive speculation. Nevertheless, this study has shown that the smallest of ants may be among the mightiest of predators within eusocial insect communities. This represents a new and exciting line of research that will help us better understand differences among ant species and how ant communities are structured.

APPENDIX: COPYRIGHT PERMISSION

Re: Copyright agreement

Schlick-Steiner & Steiner <birgit.florian@gmail.com>
Sun 1/6/2019 04:33
To: Leo Ohyama <leoohyama@Knights.ucf.edu>

Dear Leo,
Happy New Year.

This is to grant you permission to include the publication "Ohyama L, King JR, Jenkins DG (2018) Diversity and distribution of Solenopsis (Hymenoptera: Formicidae) thief ants belowground. Myrmecological News 27, 47-57." as a chapter in your PhD thesis.

Best wishes for completing your thesis!
Warmest,
Florian
Florian Steiner, Editor-in-Chief Myrmecological News

Am Fr., 4. Jan. 2019 um 21:56 Uhr schrieb Leo Ohyama <leoohyama@knights.ucf.edu>:
Hi Florian,

Happy new year.

I am in the process of writing my thesis and one of my chapters was already published in Myrmecological News (Diversity and distribution of Solenopsis thief ants belowground) last year. Part of the requirements of the written thesis is to receive permission from the copyright holder in order to incorporate the article as a chapter. I also need to include any copyright release/permission letter(s) in the appendix of the thesis. I was wondering if I could gain permission as well as receive any form of documentation (be it copyright release/permission letters) from the journal?

Thank you so much,
Leo Ohyama

Leo Ohyama
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