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The Ecology of Nest Cavity Use by Arboreal Ants in the Brazilian Cerrado: Resource Availability, Nest Modification, and Trophic Interactions

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**The Ecology of Nest Cavity Use by Arboreal Ants in the Brazilian Cerrado:
Resource Availability, Nest Modification, and Trophic Interactions**

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B.A. Biology, The College of Wooster, 2009

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

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Abstract

Arboreal ants in the Brazilian Cerrado rely on cavities in living trees as nest sites. These cavities are created by a community of, wood-boring beetles, which act as ecosystem engineers. Despite the importance of these cavities as a resource, little is known about their natural abundance and heterogeneity, how ants use and modify them as nest sites, and how this interaction between cavities and their ant occupants influences trophic interactions on cerrado trees. Here I use natural history observations and manipulative experiments to address these questions. In the first chapter I quantified the occurrence, heterogeneity, and use of beetle-created cavities by ants in six cerrado tree species. I found that cavity abundance differs significantly among tree species and within different branch sizes. Furthermore, patterns of cavity use suggest that competition for large cavities is far greater than that for abundant smaller cavities. Finally, a strong correlation between ant body size and cavity entrance size suggests an important axis of variation upon which arboreal ant species partition cavity resources, allowing for high ant diversity on individual trees. In the second chapter, I describe how ants modify the entrance size of cavities to better correspond to their body size. I found that entrance modification reduced entrance area of otherwise unsuitable cavities. In doing so the ants expand availability of a limiting resource without sacrificing nest defensibility. In the third chapter, I report a year-long experiment to test the effects of ant exclusion and increased cavity resources on levels of herbivory for two species of cerrado trees, *Caryocar brasiliense* and *Sclerolobium aureum*. I found that while excluding ants significantly increased the amount of leaf tissue consumed by herbivores, adding cavities had no measurable effect on herbivory. These results point to the important role of specific ant species that use large nest cavities in reducing herbivory on trees. Overall this work has further developed our understanding of relationships between host trees, cavities, and arboreal ants by demonstrating that cavity availability and use by arboreal ants has significant ramifications for the ecology and evolution of ants, trees, and arthropod herbivores in the Cerrado ecosystem.

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**Characterization of arboreal ant nest cavities in Brazilian Cerrado:
Abundance, heterogeneity, and usage**

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Abstract

Many arboreal ant species nest exclusively in cavities in trees created by the feeding activity of wood-boring beetles during their larval stage. In doing so, wood-boring beetles act as ecosystem engineers to create cavities that are an important resource for these ants. Previous work has shown that these cavities impact arboreal ant diversity and have been an important driver of evolution in the arboreal ant genus *Cephalotes*. Understanding the heterogeneity and distribution of available nest cavities and their use by arboreal ants is key to understanding arboreal ant community assembly and diversity. This is the first study to quantify the abundance and diversity of arboreal cavity-nest resources in nature, and how ants use these resources. In this study we dissected branches from six common tree species in the Brazilian Cerrado, taking measurements of various cavity characteristics and recording the identity of the occupants. We sampled over 1 km of branch length which contained 2,310 individual cavities containing 576 ant nests from 25 arboreal ant species. We found significant differences among tree species in the proportion of stem length bored by beetles as well as the number of cavities available to ants, suggesting that the large observed differences in availability of nesting sites among tree species may help account for differences in abundance and diversity of arboreal ants associated with different tree species. Although tree species had similar ranges of cavity entrances available, the most commonly used entrance sizes were sevenfold more abundant in some tree species than in others. Furthermore, we found that 36 percent of the most common entrance sizes (1.5-2 mm) were occupied by ants, while 79 percent of all cavities with entrances 4 mm and larger were occupied, suggesting that competition for cavities with large entrances may be higher than for those with the most common entrance sizes. A strong correlation between ant head width and cavity entrance diameter suggests that diversity of cavity entrance sizes is one axis on which cavity resources are partitioned among competing arboreal ant species, potentially allowing high observed levels of arboreal ant diversity on individual trees.

Keywords: arboreal ants, wood-boring beetles, resource use, cerrado, cavity, diversity, ecosystem engineering

Introduction

Fundamental questions in ecology concern how organisms are organized into ecological communities and what processes allow species to co-occur and co-exist despite using similar resources. One commonly overlooked interaction in ecological communities is the role of physical ecosystem engineers in the creation of novel resources. Physical ecosystem engineers are organisms that cause physical state changes in the environment in a way that creates a resource used by other organisms (Jones *et al.*, 1997). Novel resources created by ecosystem engineers can create new niches in a number of ways, including the creation of enemy free space and the moderation of abiotic conditions, thus potentially impacting species co-occurrence and local biodiversity.

Ecosystem engineering is a ubiquitous yet often overlooked process. In some systems ecosystem engineering is likely to play an important role in community structure, ecological processes (such as trophic interactions and competition), and evolutionary processes (Wright & Jones, 2003). Studies that have addressed these issues have found that ecosystem engineers are important for the maintenance of biodiversity (Wild *et al.*, 2011), ecosystem structure (Sousa *et al.*, 2009), ecosystem dynamics (Cardinale *et al.*, 2004), and ecosystem function (Folgarait, 1998). Ecosystem engineering has also been implicated in altering evolutionary trajectories in engineering species through niche construction, a process by which feedback from the engineered environment drastically alters the selective environment of the engineer species (Laland *et al.* 1999). Despite this, resource bases produced by ecosystem engineers are often poorly characterized and quantified (but see Marquis and Lill 2010), and as a result, the mechanisms by which ecosystem engineers influence community structure and other ecological and evolutionary processes remain poorly understood. Characterizing engineered resource bases is fundamental for our understanding of the mechanisms and scope of the impacts of ecosystem engineers on ecological and evolutionary processes.

Ecosystem engineers should have a greater impact on ecological communities when the resource created is durable and long-lasting (Wright & Jones, 2003). One such example, which exists virtually worldwide, is the creation of cavities in the wood of living and dead trees by the larvae of wood-boring beetles. These cavities provide a long-lasting and durable shelter that is used by a variety of organisms, notably ants. The interaction between wood-boring beetles and ants is likely ubiquitous in both temperate and especially in tropical ecosystems, where both wood-boring beetles and ants are abundant in tree canopies (Powell *et al.*, 2011; Berkov & Tavakilian, 1999). Many species of arboreal ants rely on beetle cavities in dead and living wood for nesting sites (Jiménez-Soto & Philpott, 2015; Klimes *et al.*, 2012; Powell *et al.*, 2011).

A number of studies to date have explored the relationship between arboreal ants and their cavity nest sites both through the use of artificial cavities and by sampling naturally-occurring nests. These studies have shown that availability of nest sites is limiting for colonizing arboreal ants and that this limitation may depend on factors such as management intensity (as in agroecosystems) (Philpott & Foster, 2005) and habitat differences, as shown in the variation in occupation levels of artificial cavities between studies (Powell *et al.*, 2011; Philpott & Foster, 2005; Novais *et al.*, 2017). Although availability of nests may be limiting in general, other factors such as suitability of entrance size, location in respect to existing ant nests, and dispersal ability (Powell, 2009; Powell *et al.*, 2011), are likely to play an important role in whether a given cavity is

occupied. Heterogeneity of cavity entrance sizes (Powell, 2011; Jiménez-Soto & Philpott, 2015) and the diversity of tree species from which artificial cavities are derived (Armbrrecht *et al.*, 2004) have each been shown to increase species richness of colonizing arboreal ant communities. Moreover, colonization of newly available cavities tends to be rapid and considerable, supporting an important role for cavity resources in species abundance and richness in arboreal ant communities (Powell, 2009/ Powell *et al.*, 2011; Jiménez-Soto & Philpott, 2015).

Cavity entrance size seems to be an important factor in nest selection for arboreal ants. At least one study found a strong positive correlation between ant body size and entrance size (Novais *et al.*, 2017). Furthermore, Powell (2009) demonstrated that colonies of *Cephalotes persimilis* nesting in cavities within a limited range of preferred entrance-hole sizes had higher survival than those that nested in cavities with larger entrance-holes. This interaction between members of the genus *Cephalotes* and their cavity resources has led to the evolution of specialized head discs on the ants, which are used to block entrance holes to protect the colony from threats (Powell, 2008; Powell, 2009). Both the correlation between ant body size and entrance size of arboreal ant species and the evolution of cavity blocking defenses in *Cephalotes* suggest that cavity entrance size is important for colony survival and is likely an important factor in partitioning cavity niche space among arboreal ant species. Studies on cavity-dwelling *Leptothorax* in acorns and stems on the forest floor in Eastern North-America have demonstrated that these ants show preferences for cavities with larger volume in addition to those with particular entrance diameters (Herbers & Banschbach, 1995; Pratt & Pierce, 2001). This suggests that cavity characteristics such as cavity length, diameter, and volume may also be important factors for nest site selection by arboreal ants, but preference for, and advantages conferred by these characteristics have yet to be explored for ants inhabiting cavities created by wood-boring beetles.

While a number of studies have made important contributions by using artificial cavities (e.g. Jiménez-Soto & Philpott, 2015; Powell *et al.*, 2011, and others), or have quantified the abundance of occupied ant cavity nests (e.g. Klimes *et al.* 2012), we still lack estimates of the dimensions, diversity, or distribution of naturally occurring cavities, as well as information on how their natural availability influences arboreal ant communities. Characterizing availability of naturally-occurring nest cavities will help us to better understand competition, resource limitation, and nest site selection, as well as patterns of diversity and community structure in arboreal ants. Furthermore, understanding how arboreal ants interact with their nesting cavity resource will allow us to better understand how patterns of ant occurrence scale up from individual trees to the landscape or ecosystem level.

Assessing the availability and heterogeneity of beetle cavities is prerequisite to understanding how cavity-dwelling arboreal ants interact and are able to co-exist at such high levels of species diversity. Key to this pursuit is understanding which beetle species create these cavities during their larval stages. Differences in the size of cavities produced by beetle larvae, as well as specialization of beetle species on particular host trees can create a heterogeneous resource for ant occupants. Tropical wood-boring beetles have specialized on one or a small group of related host tree species as a result of evolved resistance to, or tolerance of, various plant defenses (Berkov & Tavakilian, 1999). Furthermore, nutritional requirements corresponding to larval body size and high levels

of interspecific competition dictate preferences by beetles for specific branch diameters (Hespenheide, 1976; Stiling & Strong, 1984). This evidence suggests that beetle diversity and specialization may be an important driver of heterogeneity in ant nesting resources.

In this study we sought to describe and quantify the resource base represented by beetle-produced cavities at a site in the Brazilian savannah (Cerrado), and how it is used as a shelter resource for arboreal cavity-nesting ants. The aims of this study were to 1) quantify the damage caused by wood-boring beetles in stems of six common Cerrado tree species; 2) describe and quantify the nest cavity resource base; 3) determine patterns of cavity use by arboreal ants.

Methods

Study site:

This study was conducted at the Estação Ecológica do Panga administered by the Universidade Federal de Uberlândia (UFU). Panga is a 404 ha reserve, 30 km from Uberlândia, MG, Brazil. It consists of mixed densities of Cerrado vegetation. Cerrado is a savanna ecosystem characterized by a distinct wet season (approx. Nov.-April) and dry season (May-Oct). This study focused on six cerrado tree species which are common and characteristic of Cerrado vegetation in general, and are abundant in our study site: *Caryocar brasiliense* (Caryocaraceae); *Stryphnodendron polyphyllum* (Fabaceae); *Qualea grandiflora* (Vochysiaceae); *Kielmeyera coriacea* (Clusiaceae); *Machaerium opacum* (Fabaceae); and *Sclerolobium aureum* (Fabaceae).

Each of our six focal tree species is subject to herbivory by wood-boring beetles which feed on xylem tissue during their larval stage in both live and dead wood. Most species pupate in their feeding cavities and emerge as adults, abandoning the structure. These hollow branch cavities are used by a diverse arboreal ant community composed primarily of ant species which nest exclusively in cavities in wood. Previous work in our study site has found 117 ant species sampled in vegetation, of which 51 were never encountered in samples from the ground (Camacho & Vasconcelos, 2015), and up to 20 species of ants co-occurring on a single tree (Powell *et al.*, 2011).

Stem Sampling:

In June-August 2012 and June-August 2013, we sampled branches from 120 individual trees in mid to low-canopy density (Cerrado *sensu stricto*). We removed a single 10-cm branch with all attached biomass from 12 individuals of each tree species. Only large trees with more than two 10-cm diameter branches were sampled in order to minimize impact on trees. We also chose trees that were at least 15 m from conspecifics. Because of the growth form and availability of *Stryphnodendron polyphyllum*, branches sampled from this species ranged 7.5-10 cm at the base (samples from only 2 individuals were under 9.0 cm). From an additional six individuals of each tree species, we sampled between six and fifteen 3-cm branches depending tree size as measured by bole diameter (10 cm above soil). All measurements were taken using digital calipers, except for cavity and branch lengths greater than 10 cm which were taken using a measuring tape or a string which was then measured (when branching or curvature made other methods inaccurate).

The 10-cm diameter branches and all attached biomass greater than 3 cm diameter was cut using a chainsaw at 10-cm intervals and split lengthwise to expose any cavities.

Xylem diameter was measured at the basal end of each 10-cm length and total length of cavities was measured. When present, we measured entrance-hole diameter, taking multiple measurements for non-circular shapes and averaging for analysis. Cavity diameter was measured at 10-cm intervals along the length of the cavity and then averaged for analyses. Cavity inhabitants were identified in the field, taking voucher specimens to the lab for confirmation when necessary. Because of the scarcity of dead stems of larger diameter, all stems from this dataset were living wood.

Small stems (those 3 cm diameter and under) were measured for total length, basal xylem diameter, and then split lengthwise to expose cavities. Measurements were taken on all cavities encountered including occupant species, cavity length, minimum and maximum diameter, diameter of any entrance holes as well as whether the cavity was located in live or dead wood. Voucher specimens of ants were collected for identification.

Ant Identification:

Ant specimens from both datasets were identified to species or morphospecies using expertise and voucher collections at the Instituto de Biologia at the Universidade Federal de Uberlândia, Laboratório de Ecologia de Insetos Sociais. Vouchers were deposited in this same collection.

Analysis:

All analyses were conducted in R Studio and figures were produced using the ggplot2 package in R and Microsoft Excel. For analyses including individual trees as sampling units, stem datasets (large stems > 3 cm diameter and small stems < 3 cm diameter) were analyzed separately because stems were collected from different individuals and there were often tree species-times-dataset interactions. Prior to parametric analyses, assumptions were checked and any necessary data transformations were conducted. All figures and summaries of data were back-transformed prior to inclusion in the results. All proportional data for parametric tests were normalized using logit transformations in R Studio using the “car” package. All tests comparing means among tree species were conducted using individual trees as the sampling units by obtaining a tree average. Averages were used when multiple measurements were taken for cavity characteristic such as wood diameter, cavity length, cavity width, and entrance-hole diameter. Cavity volumes were calculated using the standard equation for volume of a cylinder where the cavity diameter represented an average for cavities with multiple measurements (multiple measurements were taken every 10 cm for cavities exceeding 10 cm length). Cavity data occasionally lacked measurements for entrance-hole diameter, either because none was present, because none was discovered, or because entrance holes were sometimes destroyed during sampling. All cases missing a relevant measurement were removed from the analysis, but may have been used in other analyses when relevant data were present.

Results

We dissected and sampled 1,108 meters of stems from our six focal tree species which contained 2,310 individual cavities. Of the cavities sampled, 576 were occupied by ants representing 25 species in 13 genera, and 97 contained wood boring beetle larvae, pupae, or adults.

Beetle activity on trees

The proportion of stem length bored by beetles varied both by stem diameter and tree species, and the interaction between the two. In general, large stems 3-10 cm diameter showed higher proportions of stem length bored (0.32) than did small stems < 3 cm diameter (0.14), but there was a strong tree species interaction between large and small stem ($F_{(1,5)}=7.06$, $p<0.001$ (data logit transformed)). Separate ANOVAs on each of the large and small stem datasets revealed that tree species differed significantly in proportion of total stem length bored by beetles (large stems: $F_{(5,66)}=15.26$, $p<0.0001$, small stems: $F_{(5,42)}=18.47$, $p<0.0001$, data logit transformed). Tukey's HSD test revealed that the proportion of stem length bored in the large stem data was significantly higher for *Caryocar*, *Machaerium*, and *Stryphnodendron* than for both *Kielmeyera* and *Qualea*, though *Kielmeyera* was also significantly higher than *Qualea* (Tukey's HSD) (Fig. 1a).

In the small stem data, *Caryocar* and *Sclerolobium* had significantly higher levels of damage than all other species except *Stryphnodendron*. *Stryphnodendron* and *Kielmeyera* were significantly higher than *Machaerium* and *Qualea* (Fig. 1b).

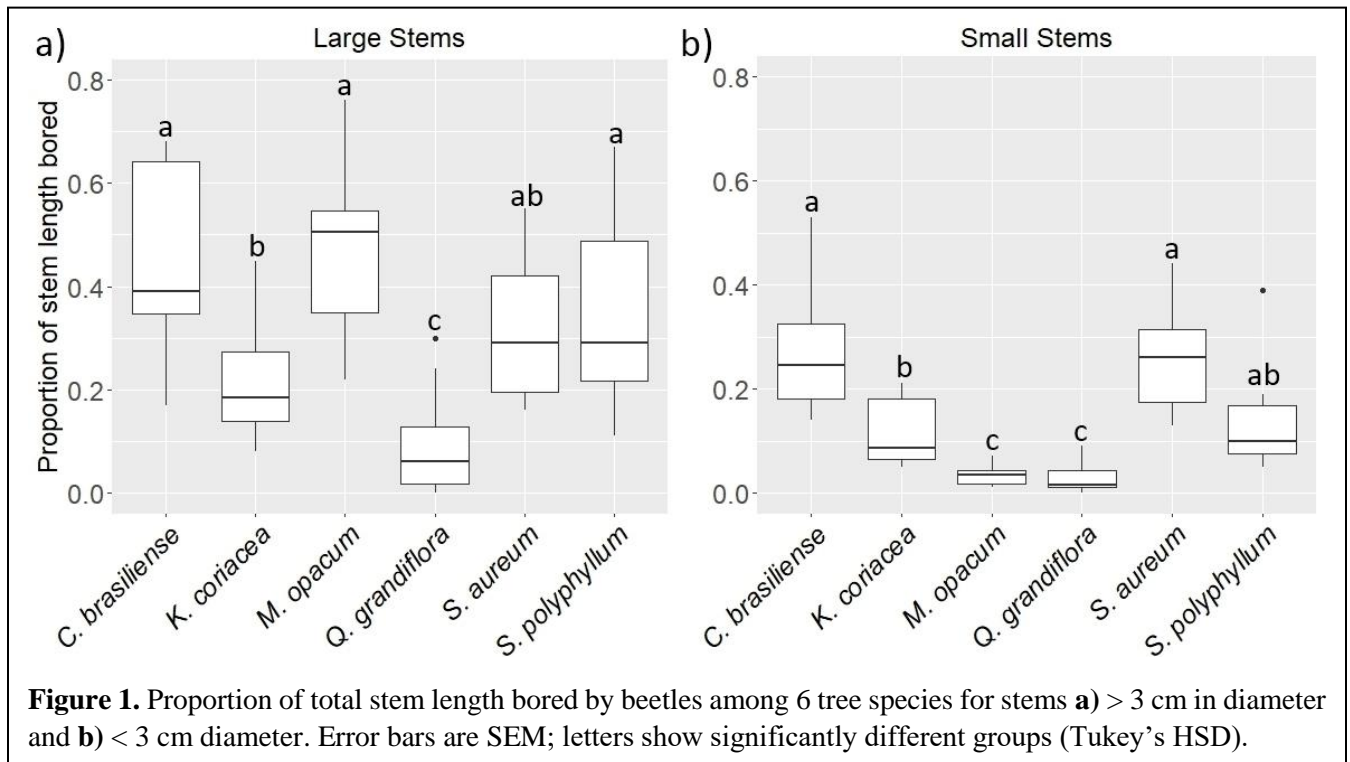


Figure 1. Proportion of total stem length bored by beetles among 6 tree species for stems a) > 3 cm in diameter and b) < 3 cm diameter. Error bars are SEM; letters show significantly different groups (Tukey's HSD).

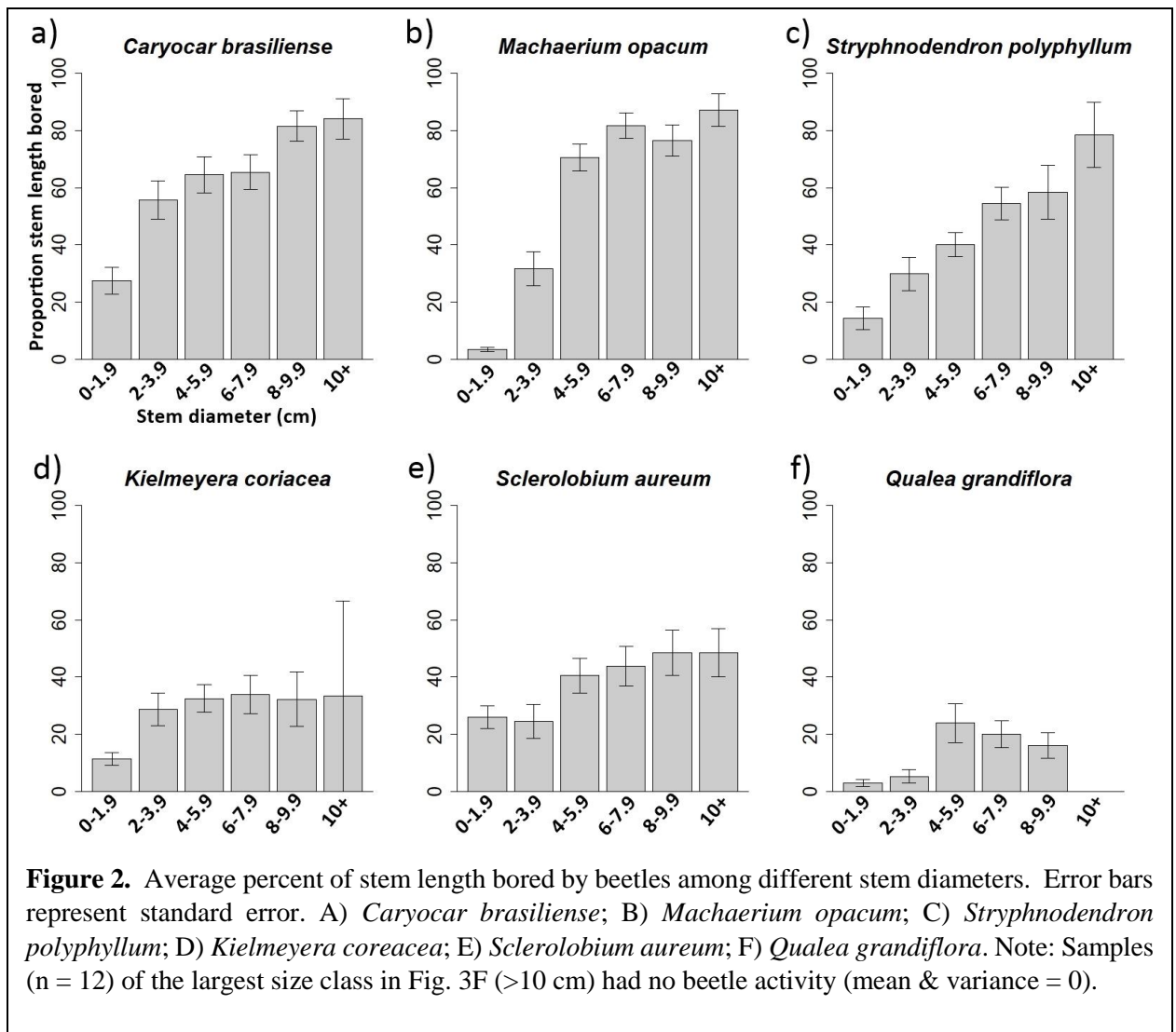
Overall, *Caryocar* and *Qualea* had the highest and lowest levels of beetle damage respectively in both large and small stems while *Machaerium* had high levels of beetle damage in its large stems but very little damage in the smaller ones. Large differences between tree species in the amount of beetle damage present, ranging from less than 10% of total length affected in *Qualea*, to more than 50% in larger stems of *Caryocar* and *Machaerium*, suggests that different tree species may contain vastly different levels of cavity nesting resources for ants as a result of different levels of beetle damage

Table 1. Total numbers of beetles (including larvae, pupae, and adults) sampled in each of the small and larger stem sampling.

Tree Species	Number beetles in stems < 3 cm diameter	Number beetles in stems 3-10 cm diameter	Total
<i>Caryocar brasiliense</i>	9	2	11
<i>Kielmeyera coriacea</i>	4	2	6
<i>Machaerium opacum</i>	28	25	53
<i>Qualea grandiflora</i>	6	2	8
<i>Sclerolobium aureum</i>	15	0	15
<i>Stryphnodendron polyphyllum</i>	1	3	4

The number of beetle pupae, larvae, and adults encountered in the branch sampling was not related to the amount of damage observed for that tree species. While *Machaerium* had among the lowest levels of beetle damage in stems less than 3 cm diameter, it had much higher numbers of beetle larvae in those stems than other species. While *Caryocar* and *Machaerium* had similarly high levels of beetle damage in stems greater than 3 cm in diameter, we found 2 and 25 beetles respectively in stems of these species (Table 1). This lack of correspondence between the number of beetles present and the amount of damage recorded for these tree species suggests that a single sampling in time of beetle occupants is not sufficient to gauge the dynamic patterns of beetle damage on trees which accumulates over multiple seasons, or in the case of larger branches, over many years of stem growth.

In addition to differences among tree species in the proportion of stem length bored, the amount of beetle boring also varied with the diameter of branches within individual tree species. We identified three general patterns of stem-boring in relation to stem diameters: high levels of stem-boring with dramatic increases in percent bored with increasing stem diameter as seen in *Caryocar*, *Machaerium*, and *Stryphnodendron* (Figs. 2a-2c); relatively low beetle consumption with no dramatic increase with stem diameter as seen in *Kielmeyera* and *Sclerolobium* (Figs. 2d & 2e); and very low levels of stem-boring with most damage in the middle-size branches, and no stem-boring in the largest class, as in *Qualea* (Fig. 2f).



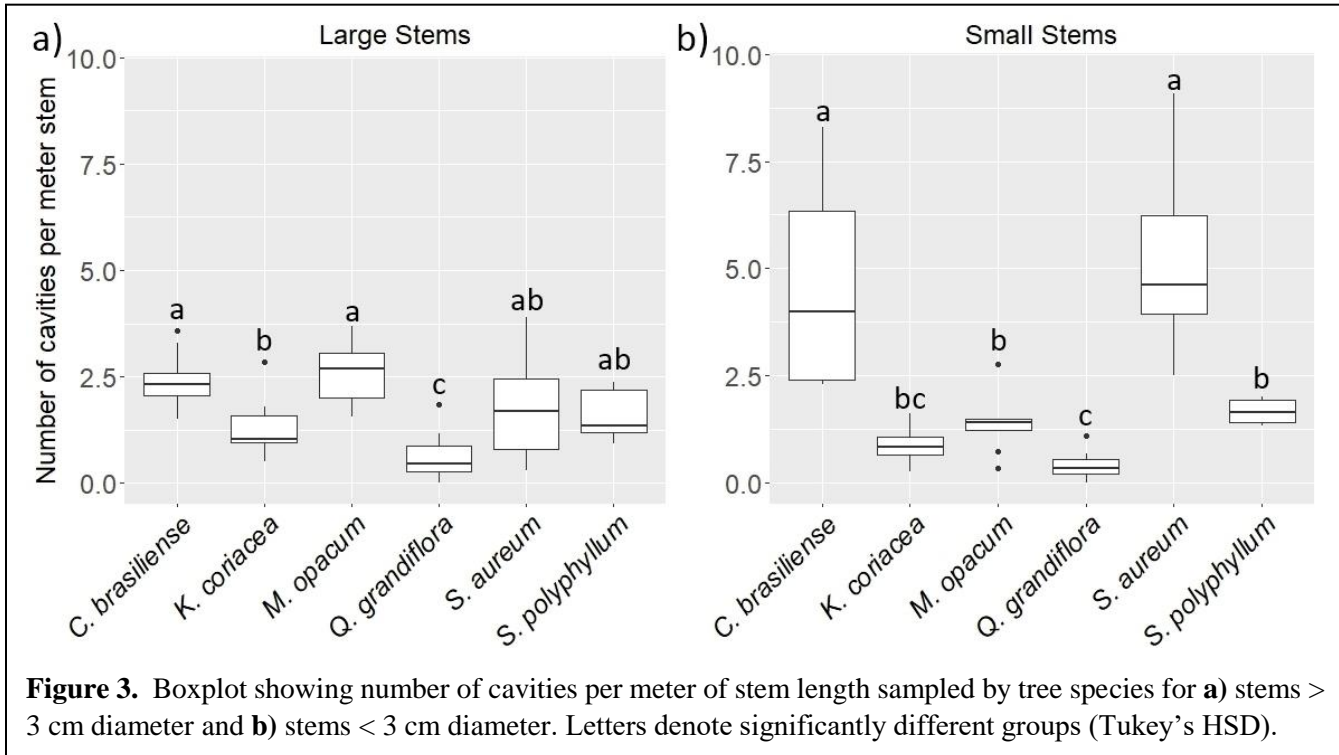
When looking at the relationship between the diameter of cavities and the branches in which they occur, we found that stem diameter explained 44% of the variance in cavity diameter (data \log_{10} -transformed, $R^2 = 0.44$, $p < 0.001$). This relationship is expected given that branch diameter puts an upper limit on the diameter of a cavity that is possible in any given branch size. Despite this, there were fewer small diameter cavities in large-diameter branches suggesting that, in general, larger diameter stems contain larger diameter cavities (Supplemental material Fig. 1).

Cavity Resource Base

Tree species differed significantly in the number of cavities present per cm of stem sampled, ANOVA $F_{(1,5)}=33.79$, $p<0.001$, but there was a significant tree species times dataset interaction, ANOVA $F_{(1,5)}=12.62$, $p<0.001$) (cavities per cm square-root transformed). When analyzed separately we found significant differences between tree

species in the number of beetle cavities per cm sampled for both large stems ($F_{(5,66)}=12.80, p<0.001$) (Fig. 3a), and small-stems ($F_{(5,42)}=26.09, p<0.001$) (Fig. 3b) (cavities per cm square root-transformed).

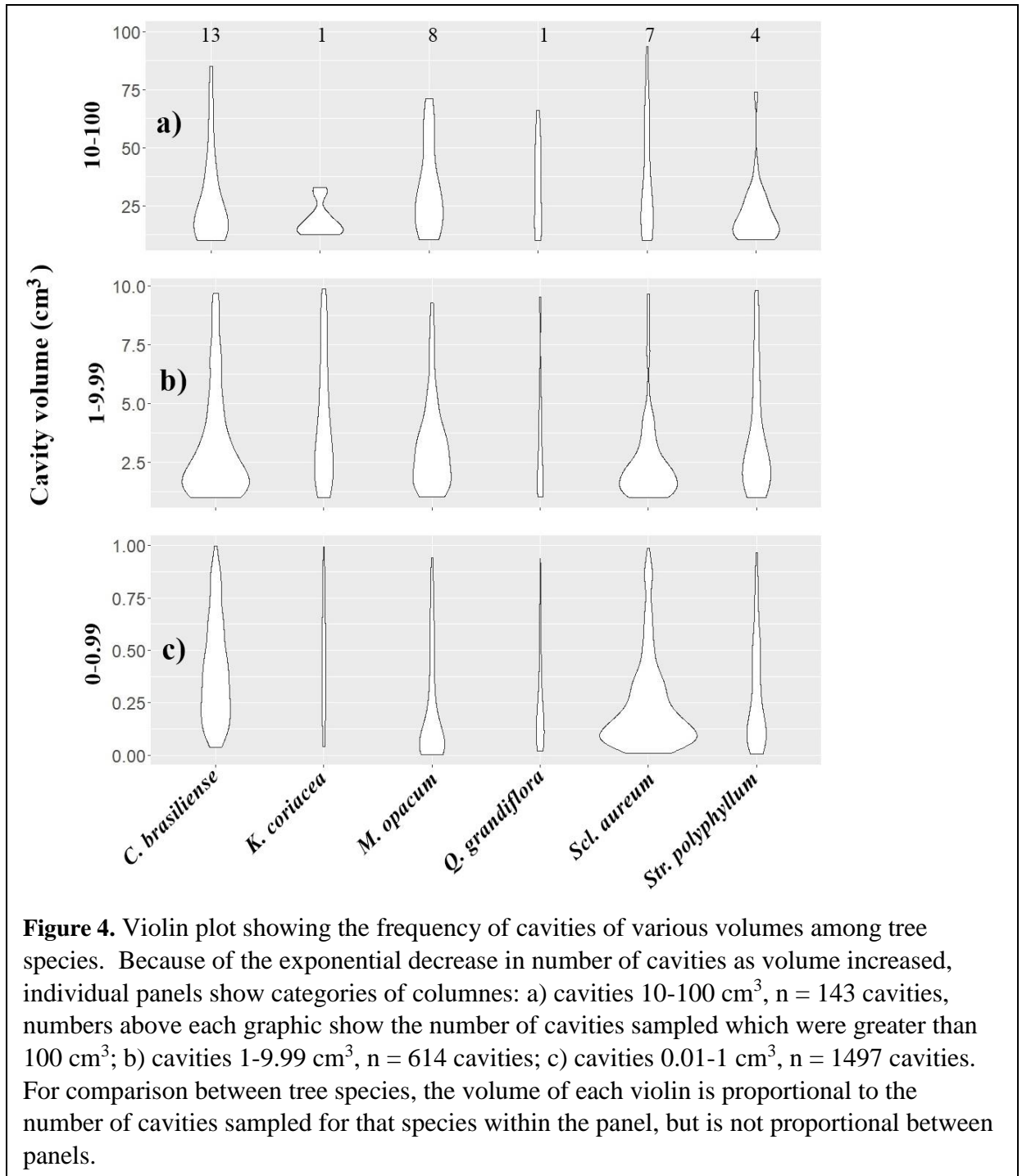
In the large stem dataset *Caryocar*, *Machaerium*, *Sclerolobium*, and *Stryphnodendron* had significantly higher numbers of cavities per stem length than both *Kielmeyera* and *Qualea*, followed by *Kielmeyera* which was significantly higher than *Qualea* (Tukey's HSD) (Fig. 3a). In the small stem dataset both *Caryocar* and *Sclerolobium* have significantly higher numbers of cavities per stem length than all other species, which had a similar number of cavities per centimeter of stem length (Tukey's



HSD) (Fig. 3b).

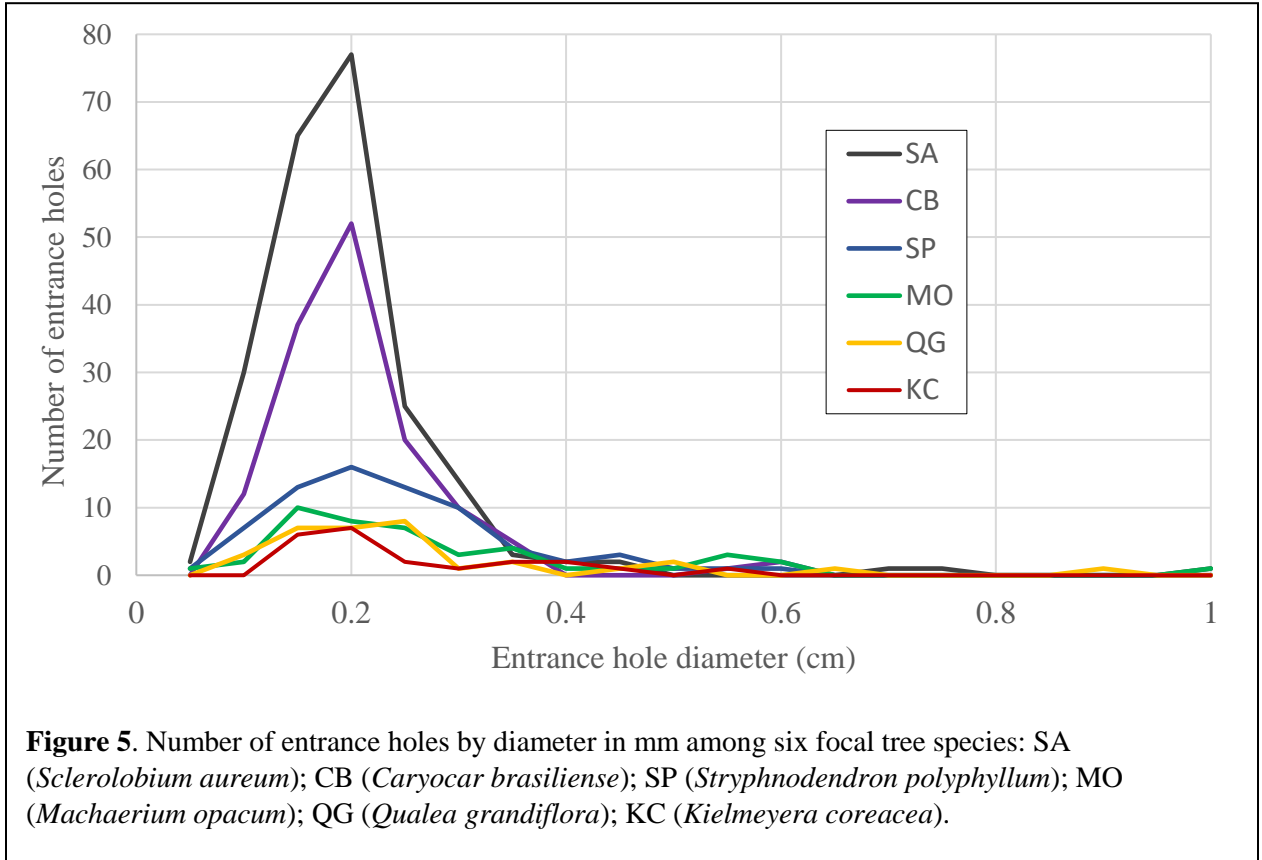
Tree species showed similar relative ranking in number of cavities when compared to proportion of stem bored (Fig. 2 vs. Fig. 3). However when comparing proportion of stem length bored to number of cavities, the number of cavities was similar between large and small datasets whereas proportion bored was much higher in large stems than small stems, indicating that large stem cavities were generally longer than those in small stems, this is expected given that there are positive correlations between all cavity dimensions and stem diameter (Table S1).

All tree species sampled had high levels of variability in cavity volumes, ranging from a fraction of a cubic centimeter to several cavities which were over 500 cubic centimeters. Cavities of small size (less than 1 cm³) were more than twice as abundant as medium cavities (1-1.99 cm³), and more than eightfold more abundant than cavities larger than 10 cm³ (see the legend for Fig.4 for numbers sampled). The frequency of cavities of different volumes differed among tree species. *Sclerobium* for example had



more cavities in the smallest size class (Fig.4c), while *Caryocar* and *Machaerium* had much higher numbers of cavities in the largest stem class than *Sclerolobium* (Fig.4a).

Cavity entrance sizes had similar ranges across all tree species but the availability of entrance holes differed greatly among tree species. Entrance holes between 1.5 and 2.5 mm were the most common among all tree species and showed particularly high abundance in *Sclerolobium* and *Caryocar*, mirroring differences in the number of small cavities per stem length in small diameter stems (Fig. 3). Entrance holes greater than 3.75 mm diameter were uncommon across all tree species (Fig. 5).



Patterns of cavity use by ants

We found 576 cavities occupied by 25 species of ants. *Azteca* sp.1 was the most common occupant of cavities in our study site, accounting for 45% of all cavities with ants. Of the 13 genera found during stem sampling, *Camponotus* was the most diverse with 6 species found nesting in the stem cavities (Table 2).

Table 2. Ant species sampled from stem nest cavities with total number of nests sampled per species. Mean head diameter represents measurements from soldier castes when present or workers for species without soldiers; measurements were taken from individuals collected from the same study site as the experiment.

Genus	Species	Nests sampled	Mean head diameter (mm)
<i>Azteca</i>	sp.1	261	1.1
<i>Camponotus</i>	<i>atriceps</i>	9	3.3
	<i>bonariensis</i>	5	1.6
	<i>melanoticus</i>	1	3.3
	<i>senex</i>	11	2.0
	<i>sericeiventris</i>	9	4.1
	sp.15	3	0.8
<i>Cephalotes</i>	<i>pusillus</i>	33	1.7
<i>Crematogaster</i>	<i>ampla</i>	29	1.0
<i>Dolichodorus</i>	<i>lutosus</i>	2	1.1
<i>Myrmelachista</i>	sp.1	4	-
<i>Neoponera</i>	<i>villosa</i>	5	2.5
<i>Nesomyrmex</i>	sp.1	3	1.0
<i>Pseudomyrmex</i>	<i>curacaensis</i>	6	0.8
	<i>elongatus</i>	6	-
	<i>gracillis</i>	7	1.8
	<i>urbanus</i>	41	0.7
<i>Solenopsis</i>	sp.1	6	0.5
	sp.2	2	-
	sp.3	4	-
	sp.4	1	-
<i>Strumingenys</i>	sp.1	1	-
<i>Tapinoma</i>	sp.1	17	0.5
	sp.3	1	-
<i>Wasmania</i>	sp.1	2	0.5

Tree species differed in the percent of available cavities occupied by ants in large stems (3-10 cm diameter) (ANOVA, $F_{(5,66)}=3.59$, $p=0.006$, data logit transformed), but not for small stems (< 3 cm diameter) (ANOVA, $F_{(5,42)}=1.14$, $p=0.35$, data logit transformed). For large stems, *Stryphnodendron* had a significantly higher percent of cavities occupied than both *Caryocar* and *Qualea*, but there were no significant differences among other species (TukeyHSD) (Table 3).

There were also significant differences in the number of occupied cavities per meter of stem for both large stems and small stems (large stems: ANOVA, $F_{(5,66)}=10.39$, $p < 0.001$; small stems: ANOVA, $F_{(5,42)}=3.84$, $p=0.006$; data log transformed). In the large stems, *Machaerium* had significantly more occupied cavities per meter of stem than *Kielmeyera*, and *Qualea* had significantly fewer than both *Stryphnodendron* and *Machaerium* (TukeyHSD). In the small stem data set both *Caryocar* and *Sclerolobium* had significantly more occupied cavities per meter than *Qualea* (TukeyHSD) (Table 3).

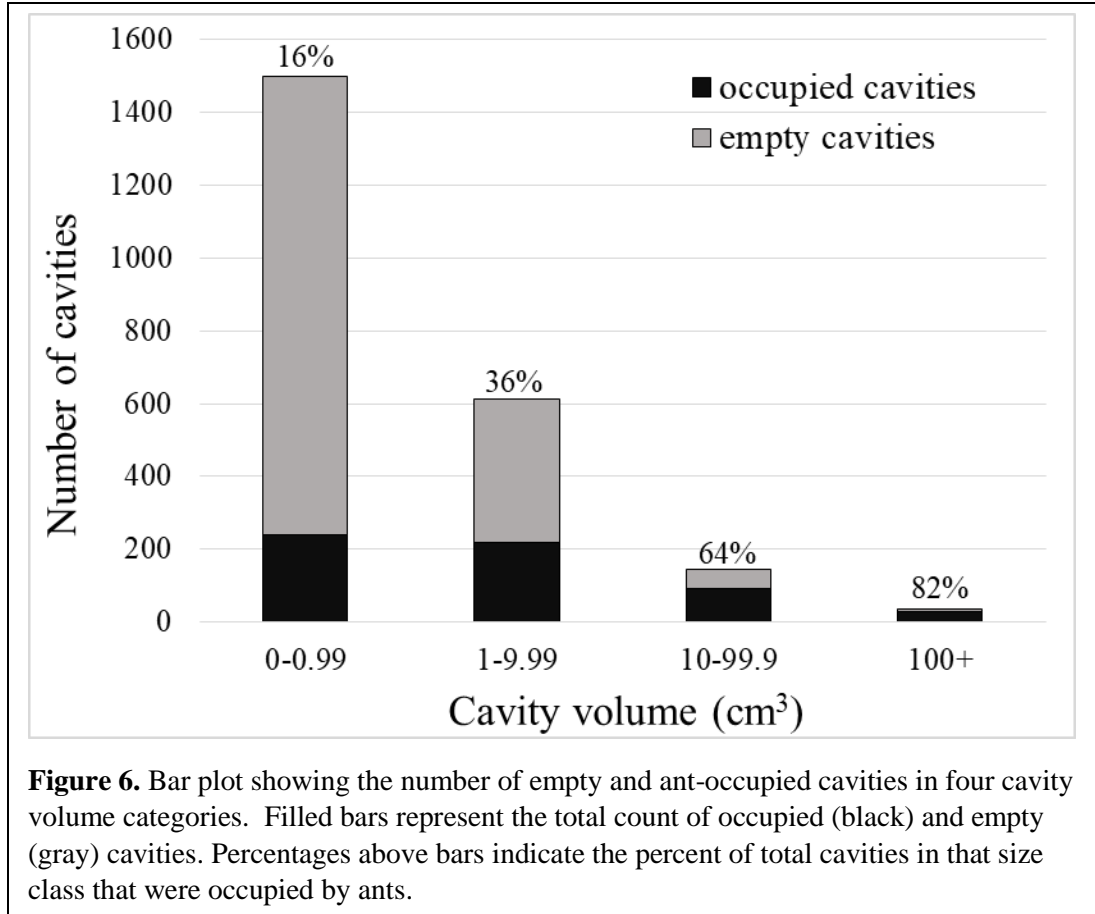
Table 3. Tree species averages for percent of total cavities occupied (large and small stem datasets) and for number of ant-occupied cavities per ten meters of branch length.

<u>Tree species</u>	<u>percent occupied (large)</u>	<u>percent occupied (small)</u>	<u>occupied cavities per 10 meters (large)</u>	<u>occupied cavities per 10 meters (small)</u>
<i>Caryocar brasiliense</i>	23.5	15.8	5.8	7.9
<i>Kielmeyera coriacea</i>	28.7	24.7	4.1	2.0
<i>Machaerium opacum</i>	44.8	9.5	11.3	1.4
<i>Qualea grandiflora</i>	26.2	22.4	1.9	1.2
<i>Sclerolobium aureum</i>	33.7	20.6	5.4	10.4
<i>Stryphnodendron polyphyllum</i>	61.5	32.7	9.2	5.5
species combined means	36.4	21.0	6.3	4.7

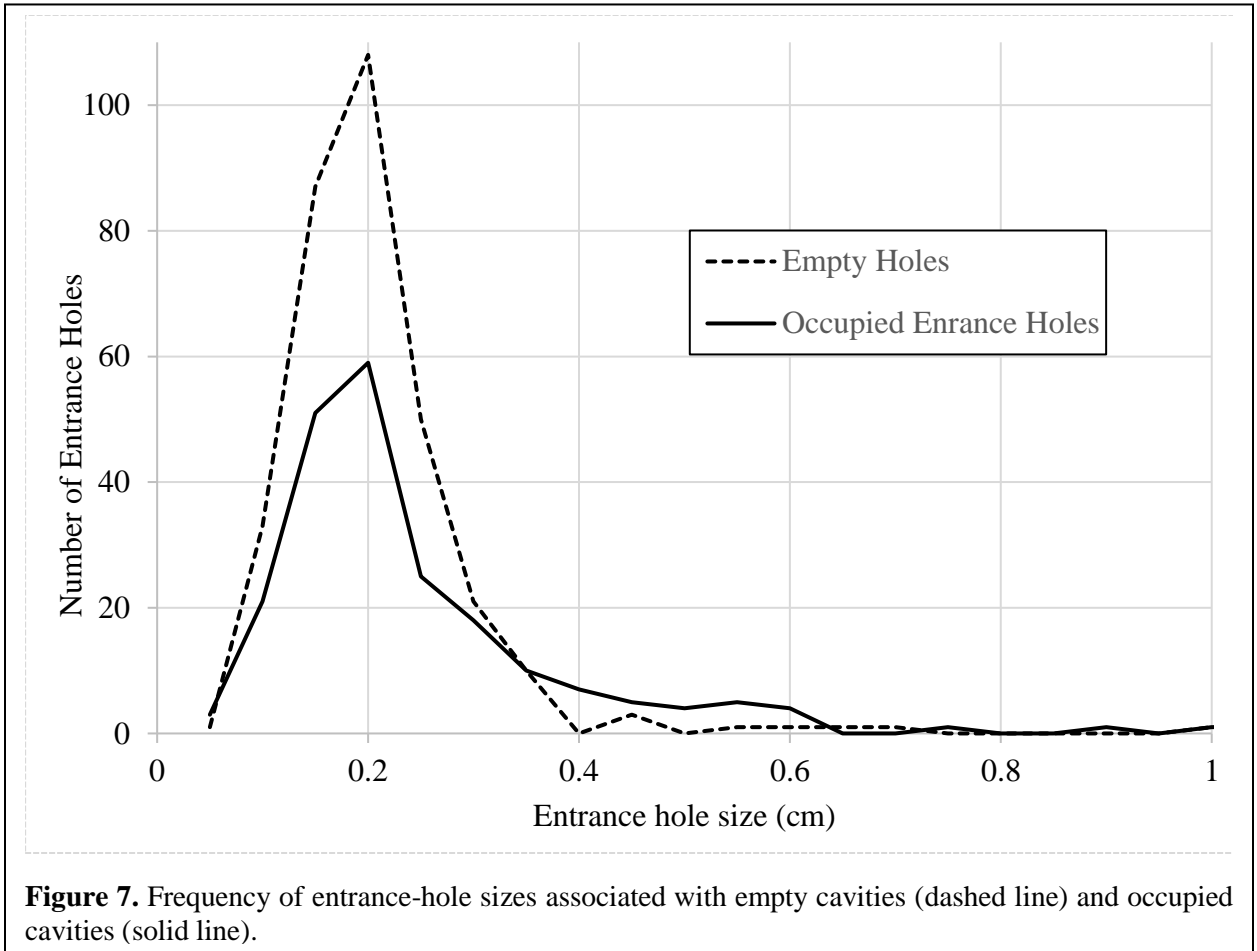
Seventeen percent of the small stems (stems < 3 cm diameter) were dead or partially dead and 19% of all cavities from this dataset were located in dead wood. In order to test whether ants were more likely to occupy cavities in live or dead wood, we first tested for tree species differences in the relative proportion of cavities in live and dead wood as well as the relative proportion of occupied cavities between species. In comparing living versus dead stems, we found no significant differences in the proportion of cavities in live versus dead wood by tree species (Fisher's exact test, $p = 0.07$, p-value simulated based on e^7 replicates), nor did we find differences in the number of occupied versus empty cavities among species (Fisher's exact test, $p = 0.19$, p-value simulated based on e^7 replicates). We therefore lumped all tree species to test whether the number of occupied versus unoccupied cavities in live and dead wood were different than expected. Given the relative abundance of cavities in live and dead wood and relative proportions of occupied cavities we found that significantly fewer cavities in dead wood were occupied than expected (Fisher's Exact test, $p < 0.001$, p-value simulated based on e^7 replicates). Among cavities in dead wood, 11.5% were occupied by ants whereas 21.9% of cavities in living wood were occupied, suggesting that either ants generally prefer living wood, or, that more cavities in dead wood were unsuitable for nesting ants.

Cavity use by ants differed significantly among cavity volume classes (Fisher's exact test, $p < 0.0001$, p-value simulated based on e^7 replicates). Pairwise Chi Squared tests with Bonferroni Correction for multiple tests revealed that occupation levels for size-classes 0-0.99 cm^3 , 1-9.99 cm^3 , and 10-99.9 cm^3 differed significantly from each other (all p-values < 0.0001). The largest two size classes (10-99.9 and 100+) did not differ significantly (Chi square, $p = 0.06$). Abundance of cavities declined rapidly with increasing cavity volume and the proportion of cavities occupied by ants increased with

increasing cavity size (Fig.6). This pattern suggests that there is likely less competition for small cavities and/or small cavities are less frequently suitable for occupation.



Entrance-hole size also affects the probability that a cavity is occupied. Only 36% of the cavities with the most common entrance hole sizes (0.15-0.2 cm) were being used, while the less common cavities with entrance holes (0.4+ cm) had 79% ant occupancy (Fig. 7). Similar to the results for cavity volume, this suggests that ants compete more for larger entrance-hole sizes, or fewer of the cavities with smaller entrance holes are suitable for use by ants.



Measurements of cavity characteristics (diameter, length, and entrance hole size) and ant body size measurements (head width, head length, femur length, tibia length, and pronotum width) were highly correlated for transformed data. Pearson's coefficients for pairwise comparisons in cavity size measurements ranged from 0.48 to 0.67 (data \log_{10} transformed) (Table S1) while coefficients in pairwise comparisons for ant size measurements ranged from 0.93 to 0.99 (data \log transformed) (Table S2). Because of high levels of correlation among both ant body measures and cavity measures, head width and cavity entrance diameter were selected as biologically relevant variables to represent the relationship between cavity size and ant body size. We found a significant linear relationship between head diameter and cavity entrance-hole diameter (adj. $R^2 = 0.7848$, $P < 0.001$) (Fig. 8).

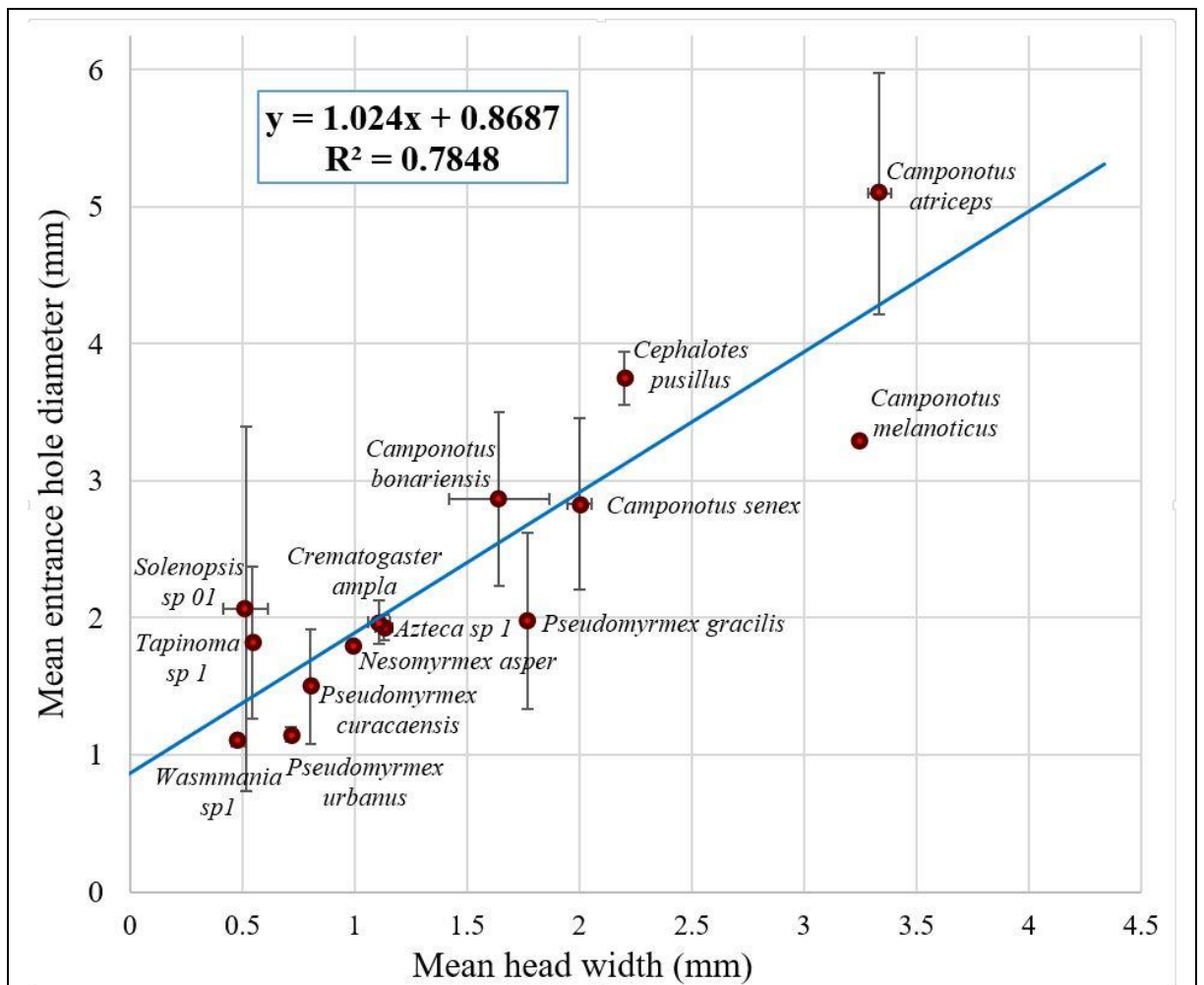


Figure 8. Relationship between head ant head diameter and entrance-hole size (ant species for which both measurements were available). Points represent species means; for species with multiple castes head width size of the soldier caste was used. Points for *Camponotus melanoticus*, *Nesomyrmex asper*, and *Wasmmmania sp.1* represent single observations. *Neoponera villosa* and *Camponotus sericeiventris* were not included in this figure because these large-bodied species use entrance holes many times larger than their body size, representing a different nest-defense strategy, and therefore do not adhere to this relationship between head size and entrance-hole size seen in smaller species, for plot including these species see Fig.S2.

Two large-bodied ant species (*Camponotus sericeiventris* and *Neoponera villosa*) were removed from this analysis because these species each inhabit cavities with entrance sizes many times their body size (for plot with these species included see supplementary material Fig.S2). These large and very aggressive species did not conform to the linear relationship found for other species relating body size to cavity entrance size. This is probably due to different defense strategies used by these ants, which likely contend more with large mammalian or avian predators as opposed to smaller ant species that face threats primarily from other ants.

Discussion

In this study we sampled stems from six common Cerrado tree species to characterize nest cavity resources and their use by arboreal ants. Cavities created by wood-boring beetles in tree stems represent an important shelter resource for arboreal cavity-dwelling ants and are an example of ecosystem engineering. Despite work on the relationship between arboreal ants and the cavities they inhabit, little is known about the resource base itself. Previous work on mechanisms that facilitate diversity and co-occurrence in arboreal ant communities has uncovered a number of important factors, including nest cavity diversity (Powell *et al.*, 2011; Jimenez & Philpott), canopy connectivity and tree size (Powell *et al.*, 2011), and ant species-specific traits (Camarota *et al.*, 2016). Furthermore, although differences in arboreal ant communities between tree species have been observed (Camarota *et al.*, 2016), no study has yet investigated whether this may reflect differences in the availability and diversity of nesting resources. Understanding patterns of abundance and distribution of naturally-occurring nest cavities is fundamental for understanding the influence that cavity nest resources have on community composition, competition, and evolution of arboreal cavity-nesting ants. In this study we were able to provide the first detailed description of diversity and availability of naturally-occurring cavity nesting resources and how these cavities are used by a number of species within an ant community across six species of Cerrado trees.

Significant differences in the proportion of total stem length bored by beetles among tree species demonstrates that cerrado trees are subjected to different amounts of consumption by wood-boring beetles. Most tropical wood-boring beetles exhibit some level of host specificity, whereby the majority of species use a small number of closely related trees as hosts (Berkov & Tavakilian, 1999). Furthermore, because beetle attack represents a cost to trees through removal of living tissue, deterioration of structural integrity, and potential introduction of pathogens, many tree species have defenses against wood-boring insects, such as sap and resins (Christainsen *et al.*, 1987), toxins and antinutritive compounds (Villari *et al.*, 2015), and thick bark with low nutritional quality or high moisture content (Hanks, 1999). Therefore the amount of damage on a given tree species is influenced by a combination of factors including the presence and identity of beetle species capable of using that tree as a host, host specificity of those beetle species, tree defenses, plant ontogeny (Boege & Marquis, 2005), and fluctuations in beetle populations. This relationship between beetles and their tree hosts is probably responsible for the stark differences in levels of beetle damage observed in our study, e.g., between *Caryocar* which had 45% of stems larger than 3 cm diameter bored, and *Qualea*, which had only 10% bored. While beetle damage can be measured on trees as the outcome of the relationship between trees and the beetles that use them as hosts, a-priori predictions about the amount of cavity resources a tree or tree species may contain will require detailed information about the identity of wood-boring beetles and their host use. While some studies have begun to investigate the impact of specific beetles on cavity resource creation (e.g., Novias *et al.*, 2017 and Satoh *et al.* 2016), or host use by a

community of beetles (Berkov & Tavakilian, 1999), documenting host use and cavity creation for a whole community of beetles will be a formidable undertaking.

Differences in levels of beetle damage were also discovered among different branch sizes of a single tree species. Overall, 32% of stem length was bored in stems greater than 3 cm diameter and 14% in stems smaller than 3 cm. In *Machaerium* only 3% of the total length of stems was bored in stems less than 3 cm diameter while, in contrast, 47% of the total length of larger stems of this species contained beetle damage. Differences in levels of damage between different stem diameters are likely driven by differences in the species of beetles that use different stem diameters. Previous work has shown that certain beetle species use a limited range of stem diameters for larval development (Reagel *et al.*, 2012). This stem diameter preference means that branches of different diameters are subjected to different levels of beetle damage. Satoh, *et al.* (2016) found that in a temperate forest, the preference for specific stem sizes by a few beetles translates to partitioning of branch sizes among ant species inhabiting the resulting cavities, suggesting this relationship may be consistent in many systems involving beetle cavity use by arboreal ants.

The proportion of stem length with damage increased dramatically with stem diameter for some species, while for others it was relatively constant. In *Qualea*, which had the lowest overall proportions of stem length consumed by beetles, the highest levels of beetle damage were in stems 4-6 cm in diameter; the largest size had no damage in any of the individuals we sampled while other species had upwards of 80% of stem lengths bored in these largest stems. Patterns of increases in beetle damage with increasing stem diameter in *Caryocar*, *Stryphnodendron*, and *Machaerium* are consistent with both accumulation of beetle damage as an individual branch grows and, potentially, preference by some beetle species for larger diameter stems. In *Kielmeyera*, a constant level of beetle damage across all branch sizes except the smallest is consistent with scenarios in which either small branches are consumed by beetles but not larger branches, or branches of all sizes are consumed by beetles but die and then fall from the tree. For *Qualea*, the initial increase in beetle damage across stem sizes from 0-6 cm diameter suggests an accumulation of damage with branch age, while the decrease in beetle damage across stems of 6+ cm resulting in no cavities in the largest size class, suggests that branches initially attacked by beetles tend not to survive to the largest size class. Additionally, low levels of beetle activity in this species, in general, may indicate that it is defended against beetle consumption, possibly an evolutionary response to the apparent lower longevity of branches with beetle damage. Lower levels of damage in large stems of *Qualea* may also indicate the absence of a beetle species which use larger branch sizes in these species, or that larger branches in these species are more highly defended against beetles.

Differences in the level of beetle damage among tree species and among different stem diameters is most likely the result of differences in host use by wood-boring beetles (including differences in beetle communities using each host species, preferences for a subset of stem diameters, and differences in tree defense), and differences among tree species in the survival and growth of branches or trees subjected to beetle damage. Interestingly, samples of beetle larvae, pupae, and adults from the stems revealed that the

abundance of beetles from our single sampling do not correspond to the levels of damage found in individual tree species. *Machaerium*, for example, contained the highest number of beetle larvae in its small stems, yet had one of the lowest levels of beetle damage for those same stems. The lack of correspondence between the presence of beetles and damage levels in our samples highlights the fact that the accumulation of beetle damage in stems happens on a long time scale, particularly for larger (and therefore older) stems. Beetle species that have short larval stages may only be present in stems during certain seasons, while species which have a multi-year larval stage may represent rare events for individual tree branches, but the legacy of their wood consumption, in the form of cavities, may last the lifetime of the tree and beyond.

Understanding the ecosystem engineers in this system (beetles) and their relationship with trees is crucial for understanding and predicting how resource heterogeneity is produced. When looking at cavity measurements, we found that entrance-hole diameter, cavity diameter, and cavity length were all correlated, and positively related to the diameter of the stem in which the cavity was located. This is in part due to the fact that stem diameter dictates an upper limit to the possible entrance hole and cavity diameter. However, previous work has shown that cerambycid larval survival and adult body size are positively related to stem diameter, while larval survival decreased with density of larvae due to food availability and interspecific competition (Hanks et al, 2005; Reagel *et al.*, 2012). Furthermore, exit holes of at least one species, *Rosalia alpina* (Cerambycidae), were found to be strongly correlated with adult body size (Ciach & Michelcewicz, 2013), suggesting that cavity diameter and length may also be correlated with adult beetle size. Together these studies suggest that the identity of members of the beetle community present in the system, together with their preferences for tree species and different stem sizes, will dictate the diversity and distribution of cavity resources. Future studies that combine information on beetle-host tree relationships with the resulting cavity resource could help us to both better understand observed patterns in ant communities such as the positive response of ant diversity to tree diversity (Ribas *et al.*, 2003) and higher ant diversity in secondary than primary forest (Klimes, *et al.* 2012). Such studies may also aid in scaling up predictions about arboreal ant diversity from community ecology to the landscape and ecosystem level.

The proportion of stem length damaged by beetles for individual tree species generally corresponded to the number of cavity nest sites, these discrete cavities being the biologically relevant unit for arboreal ants. In both large and small stems there were significant differences in the number of cavities per meter of stem length, indicating that different tree species provide vastly different quantities of potential nest sites for arboreal ants. Furthermore, differences in the number of potential nesting sites per meter between small and large branches (for example in *Machaerium* which had the highest levels of cavities in large stem and very low levels in small stems, and *Sclerolobium* which had the highest level of cavities in small stems and intermediate levels in large stems) indicate that some tree species offer more nesting resources in stems of certain diameters.

Cavity entrance-hole size has been shown to be an important factor in cavity selection by colonizing ant species. Previous studies have demonstrated a correlation

between cavity entrance size and ant body size (Jiménez-Soto *et al.*, 2015; Novais *et al.*, 2017) and that the relationship between body size and entrance size is important for nest defense (Powell, 2009). When looking at the distribution of cavity entrance sizes across different tree species, we found that ranges of available entrances were similar, but the abundance of those sizes differed dramatically between tree species. While entrance holes larger than 3.5 mm diameter were rare in all tree species, the most common entrance sizes, 1 mm to 3 mm were much more common in some species than others. For entrances 2 mm in diameter, for example, *Sclerolobium* had more than seven times higher abundance than *Machaerium*, *Qualea*, and *Kielmeyera*. Differences in the availability of entrance holes of specific diameters may influence ant community assembly on individual trees and may be partially responsible for differences in ant communities between different cerrado tree species (Camarota *et al.*, 2016). For example, 2 mm entrance size was the most frequent size used by *Azteca* ants in our study, and the very high relative abundance of these cavities on *Sclerolobium* might explain the significant association found between *Azteca* and *Sclerolobium* by Camarota *et al.* (2016).

Similar to a previous study by Jimenez-Soto & Philpott (2015), we found a strong relationship between the head diameter of the largest caste of each ant species and the average entrance hole used, suggesting one mechanism by which co-occurring arboreal ants may be partitioning nesting resources. This relationship, however, did not apply to the two largest species sampled (*Camponotus sericeiventris* and *Neoponera villosa*). Each of these large-bodied, aggressive species used nests with entrances many times their body size. It is possible that these species have a different strategy for nest defense than the other species in this study. A close relationship between ant body size and entrance size is likely an effective defense against neighboring ant colonies where blocking or constraining the entrance of enemy ants into the colony (particularly when the enemy species is too large to fit) is important for colony success. It is possible that these larger species contend more frequently with threats from large mammalian or avian predators rather than neighboring ants. In these cases, it may be beneficial to have larger nest entrances for movement of large numbers of individuals in and out of the nest.

Ant occupancy differed among entrance-hole sizes. We found that the most frequent entrance-hole sizes available were 1.5-2 mm, and accounted for 57% of all cavities and 50% of all occupied cavities. When looking at entrance hole use, cavities 2 mm and under were occupied in 36% of cases, while those greater than 2 mm were occupied in 60% of cases suggesting that although more ants use cavities with small entrance holes, there is higher levels of competition for cavities with larger entrance holes.

Cavity volume showed similar patterns of distribution and use to those of entrance holes. There was relatively low occupancy in the smallest cavities which were very abundant, but the number of cavities decreased exponentially as cavity volume increased. The percent of cavities that were occupied increased dramatically as volume (and rarity) increased, from 16% in the smallest cavities to 82% for cavities over 100 cm³. Like entrance-hole diameter, cavity volume is likely a biologically important factor in nest site selection by arboreal ants. This has been demonstrated for ants nesting in cavities of fallen branches and acorns (Herbers & Banschbach, 1995; Pratt & Price, 2001), and for ants occupying domatia in *Cecropia* (Cogni & Venticinque, 2003) and *Vachellia*

(Campbell et al., 2013). It is likely that cavity volume is equally important for ants occupying cavities created by wood-boring beetles in tree stems since these cavities also impose discrete spatial limitations for arboreal ants. Given the limited number of large-volume cavities, we predict that cavity volume will be particularly important for ant species with relatively large body size, large colony sizes, and those that are not polydomous. Furthermore, differences among tree species in the abundance of large cavities may help to predict occurrence of species which rely on very large cavities, such as *Camponotus sericeiventris*, which nested almost exclusively on large *Caryocar* trees, where high-volume single cavities were most common (pers. obs., present study).

Although the amount of beetle boring increases linearly with stem diameter, the branching structure of trees results in an exponential decrease in the abundance of stems as stem size increases, meaning that there are far fewer large cavities located in large stems than there are small cavities on any given tree. While some ant species in our site are limited to these rare large singular cavities (e.g., *Camponotus sericeiventris* and *Neoponera villosa*), this disparity between the abundance of large cavities and small cavities could be one reason for the high frequency of polydomous species in arboreal ant communities (Levings & Traniello, 1981) (for a full discussion of potential reasons for polydomy see Debout *et al.*, 2007). The concurrent use of multiple nesting sites by some ant species potentially enables ants with larger body and colony sizes to reduce limitations imposed by scarcity of single larger cavities.

This study provides detailed information about the abundance and distribution of potential cavity nest resources for arboreal ants across six species of cerrado trees. These data will allow us to formulate hypotheses and predictions about how differences in the cavity resource base translate to the dynamics of competition and community assembly of arboreal cavity-nesting ants. Future work combining detailed information about the identity of ecosystem engineers (wood-boring beetles) and their patterns of host use will further elucidate how differences in beetle communities using individual tree species translates to cavity resource heterogeneity and distribution. Furthermore, studies investigating ant species-specific requirements for nest cavities, and the factors (beyond the body-size, entrance size relationship) that make cavities suitable for habitation will aid in linking information on resource availability and ant community composition.

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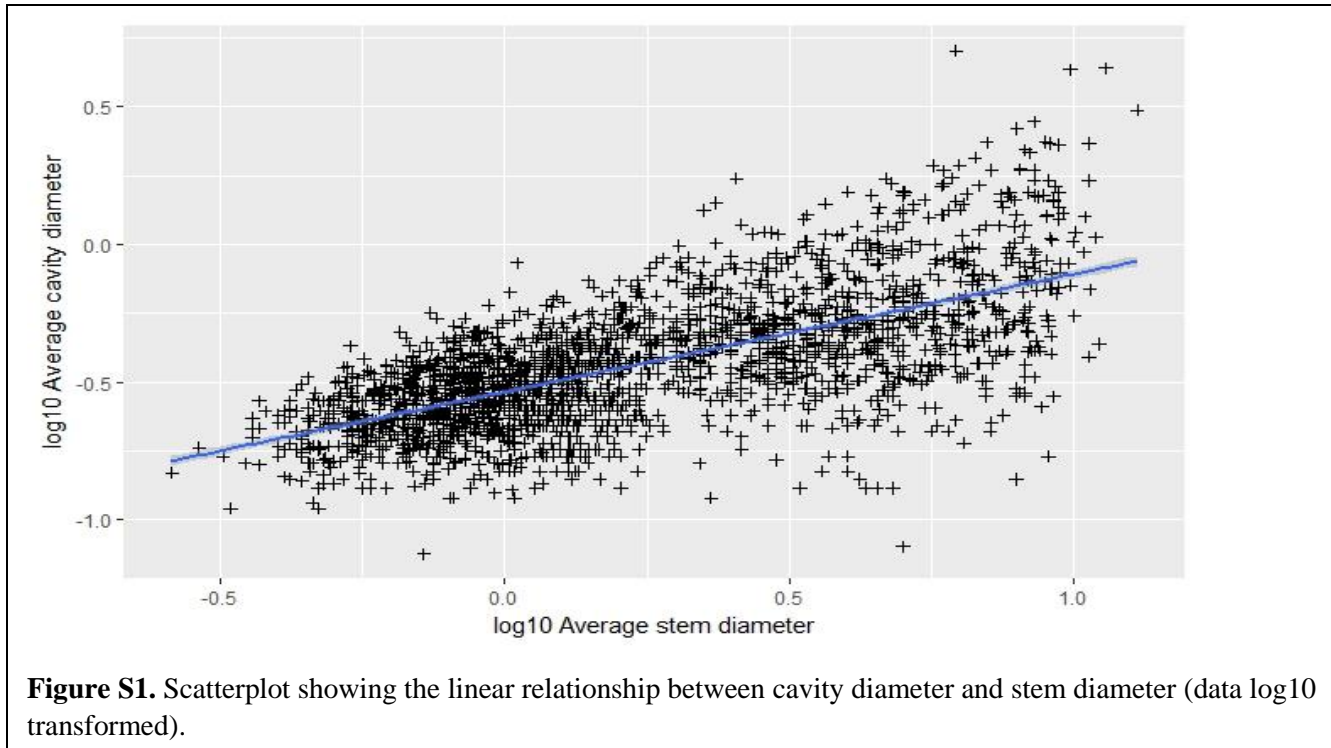
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Supplementary Material



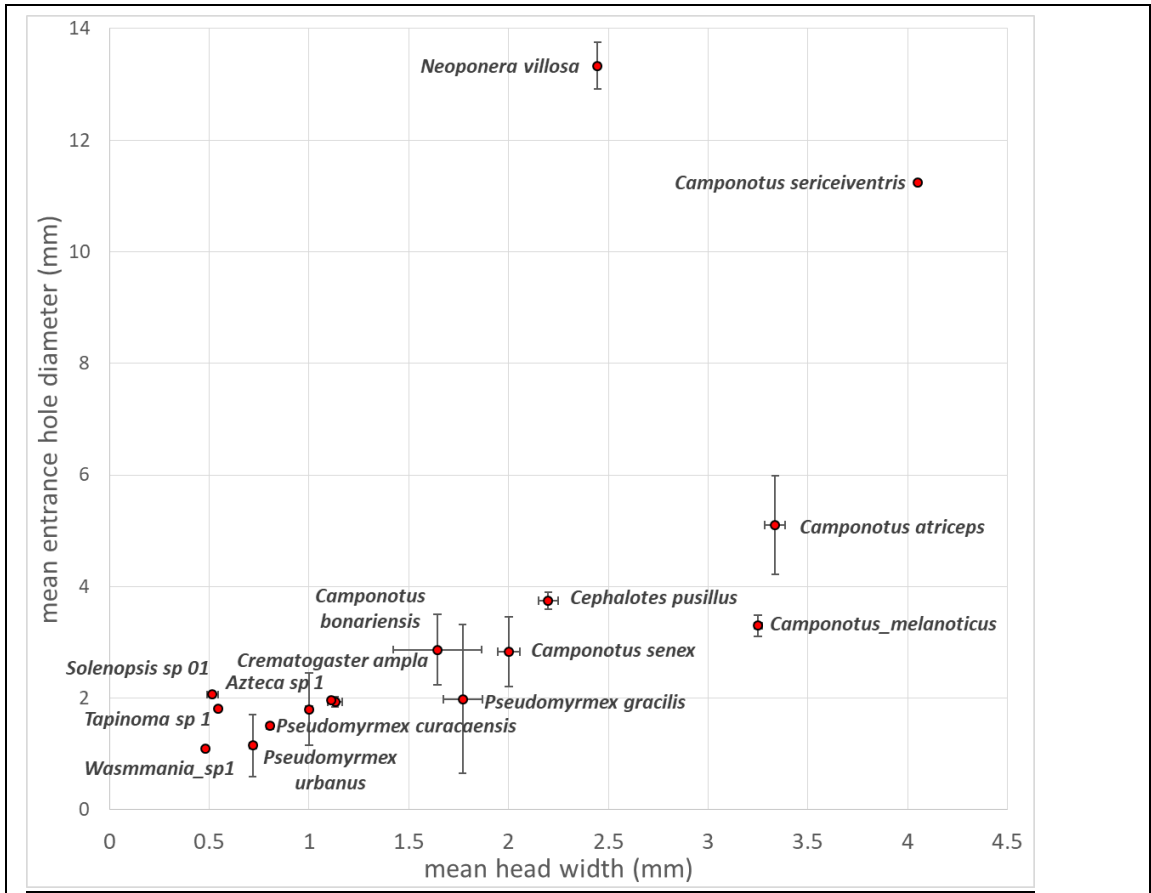


Figure S2. Relationship between head ant head diameter and entrance-hole size including *Neoponera villosa* and *Camponotus sericeiventris* which were excluded in Fig.6. Points represent species means; for species with multiple castes head width size of the soldier caste was used.

Table S1. Pearson's correlation results for pairwise comparisons of measured cavity variables (data \log_{10} transformed).

<u>Cavity Measurement Variables</u>	<u>t-value</u>	<u>df</u>	<u>p-value</u>	<u>Pearson's coefficient</u>
cavity diam. x wood diam.	41.1	2077	<0.0001	0.67
cavity diam. x entrance diam.	13.6	433	<0.0001	0.55
cavity diam. x cavity length	30.2	2077	<0.0001	0.55
wood diam. x entrance diam.	12.9	433	<0.0001	0.53
wood diam. x cavity length	25.0	2077	<0.0001	0.48
entrance diam. x cavity length	12.7	433	<0.0001	0.52

Table S2. Pearson's correlation results for pairwise comparisons of measured ant size variables (species means, data log transformed).

<u>Ant Size Variables</u>	<u>t-value</u>	<u>df</u>	<u>p-value</u>	<u>Pearson's coefficient</u>
head width x head length	23.5	16	<0.0001	0.99
head width x pronotum width	27.2	16	<0.0001	0.99
head width x femur length	13.8	16	<0.0001	0.96
head width x tibia length	12.3	16	<0.0001	0.95
head length x pronotum width	14.1	16	<0.0001	0.96
head length x femur length	19.8	16	<0.0001	0.98
head length x tibia length	17.5	16	<0.0001	0.97
pronotum width x femur length	10.9	16	<0.0001	0.94
pronotum width x tibia length	10.1	16	<0.0001	0.93
femur length x tibia length	30.5	16	<0.0001	0.99

Arboreal cavity-dwelling ants modify nest cavity entrances to increase suitability of a limiting resource

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Abstract

Nest entrance modification may be common among arboreal cavity-nesting ants and could have important ecological and evolutionary ramifications for their constructors. Despite this, no study to date has described this phenomenon in detail, nor investigated the occurrence and function of modifications to nest entrances in arboreal cavity-nesting ants. Here, we investigate nest entrance modification in a community of arboreal ants in the Brazilian Cerrado. In this study, we recorded the frequency of modification, differences in construction materials used, and the species of ants participating in this behavior. Given that nest sites are a limiting resource for arboreal ants, and entrance size is important for nest defense, we asked whether nest modification expands the availability of nest sites by allowing ants to alter suitability of otherwise potentially indefensible nests. To do this we placed 720 artificial nest cavities in 120 experimental trees of four different species for one year. At the end of this period, we measured entrance size and recorded inhabiting species and nest modification characteristics. Cavity modification was common, with 10 ant species occupying 39% of recovered cavities, and entrance modifications were present on 28% of cavities containing ants, including cavities of 7 of the 10 ant species. Modifications fell into eight classes based on the materials used. These materials were similar among host tree species, but were different between the two most common ant species. Cavity modification reduced entrance size of cavities on average by 35%. Distributions of cavity entrances before and after modification, when compared to unmodified cavities, suggested that ants use modifications to alter the entrance size of otherwise unusable cavities. By modifying entrances to nesting sites, some arboreal ants are able to increase the suitability of cavities with larger entrances, thereby expanding the availability of limited nesting resources without sacrificing nest defensibility. Interestingly, this behavior contrasts with that of other arboreal ant species in the community in the genus *Cephalotes*, that instead have specialized further on a subset of the resource base and developed head discs to block cavity entrances, resulting in even higher resource limitations for these species because of the need for cavity entrances to closely match head-disc size for successful defense.

Keywords: arboreal ants, cavity nest, resource modification, resource expansion, *Pseudomyrmex gracilis*, *Camponotus bonariensis*

Introduction

Arboreal ants are a diverse and ecologically important group in the tropics. They are dominant both numerically and with respect to biomass in tropical canopy fogging samples (Tobin, 1995; Davidson & Patrell-Kim, 1996; Davidson, 1997), and are important primary consumers and predators (Davidson *et al.*, 2003; Floren *et al.* 2002). Many species of arboreal ants rely exclusively on cavities in dead or living wood produced by wood-boring beetles as nesting sites (Powell *et al.* 2011; Philpott & Foster, 2005). Wood nest cavities provide a durable, long-lasting shelter which is defensible from predators and competitors while ameliorating abiotic conditions. Competition for these nest sites may be an important factor in determining abundance, diversity, and community structure of arboreal ants (Philpott & Foster, 2005; Powell *et al.* 2011; Powell 2008; Jiménez-Soto & Philpott, 2015). Predation and attack from neighboring ant colonies is a constant threat to cavity dwelling ants as species turnover in individual cavities can be high (Arbrecht *et al.* 2004), and some ants have evolved specialized morphologies for the defense of their nest cavities (Powell, 2009).

Variability in cavity resources is important in determining community structure and supporting co-occurrence of ant species on individual trees. Specifically, experiments using artificial cavities have shown that treatments with diverse nest entrance sizes result in higher species diversity of cavity occupants per tree than those of uniform entrance size (Jiménez-Soto & Philpott, 2015; Powell *et al.* 2011). Nest entrance sizes also are closely correlated with the body size of the ant occupant (Priest, *unpublished data*; Novais *et al.* 2017), and this correspondence between entrance size and body size seems to be important for nest defense (Powell, 2008; 2009). This evidence suggests that individual ant species should use a subset of available cavity entrances corresponding to their body size in order to maximize survival.

Competition for, and defense of, nest sites are likely key factors in colony fitness for arboreal cavity-dwelling ants. According to Oster & Wilson (1978), the evolution of polymorphism for specialized tasks is favored in scenarios with competition for a variable resource base, but behavioral flexibility may also be a successful strategy. Powell (2008) found that, contrary to this prediction, the evolution of specialized soldier castes (with both behavioral and morphological specialization) in *Cephalotes* seems to have been driven by ecological specialization on a subset of cavity entrance sizes, resulting in a narrowing of the resource base along a gradient of specialization in the genus. Here we present evidence that other arboreal ant species seem to have evolved a behavioral solution to the problem of variability in nest entrance size: they modify entrances by constructing structures that reduce the size of large (potentially indefensible) entrances to better suit their requirements.

Despite the high frequency of entrance modification in some abundant and widely distributed species of arboreal ants, no studies have reported the frequency or the potential function of this behavior. To our knowledge, only two studies have mentioned this behavior in cavity-dwelling ants, both from the genus *Leptothorax*, which inhabit cavities in wood and acorns on the forest floor. Herbers & Banschbach (1995) noted that

the cavity-dwelling ant *Leptothorax longispinosus* in North America sealed up entrance holes of birch dowels with mud and debris, yielding a consistent entrance-hole size of approximately 1 mm width. Pratt & Pierce (2001) noted a similar behavior in the acorn-dwelling *Leptothorax curvispinosus*, demonstrating that rims of soil and leaf litter were constructed by this species to reduce entrance size. Both studies suggested that this behavior likely serves to constrain entrance size for the purpose of nest defense. Modifications to nest entrances may serve important functions, and these modifications on cavity nest entrances could significantly influence the ecology and evolution of the species that do this.

In this study, we describe and quantify variation in nest entrance modification by arboreal ants to test whether nest modification characteristics are consistent with the hypothesis that modification is used to expand the available nest resource base, and suggest possible functions of nest entrance modification. To do this we addressed the following questions: 1) How frequently, and with what materials are nest entrances modified? 2) Do ant species differ in the modifications they employ? 3) What factors influence the presence and type of modification? 4) Does nest entrance modification expand the availability of cavities by allowing ants to alter suitability of otherwise unsuitable nest sites?

Methods

Study Site and Focal Species

Fieldwork was conducted at the Panga Ecological Station (Estação Ecológica do Panga), administered by the Universidade Federal de Uberlândia, 30 km from Uberlândia, Minas Gerais, Brazil. The site is a 400 hectare reserve consisting of mixed Cerrado vegetation, though the current study was focused in *cerrado sensu strictu* with approximately 30% canopy cover (Oliveira-Filho & Ratter, 2002). The four tree species selected for this study were those most commonly occurring in the study site; *Caryocar brasiliense* Camb. (Caryocaraceae), *Machaerium opacum* Vogel (Fabaceae), *Qualea grandiflora* Mart. (Vochysiaceae), and *Sclerolobium aureum* (Tul.) Baill (Fabaceae).

Experimental Design & Data Collection

Artificial cavities were constructed from locally-obtained wood dowels (3cm diameter) commonly used for tool handles, and were of a variety of hard-wood species. Dowels were cut into 10cm lengths and drilled lengthwise from one end to a depth of 9cm using a 1cm diameter bit. A single entrance hole was drilled perpendicular to the cavity bore approximately one-third the distance from the closed end. A flashlight was used to check that the entrance-hole and main cavity bore intersected (Supplement Figure 1). Four entrance sizes were used (approx. 4.4 mm², 6.6 mm², 12.4 mm², 31.7 mm²) and one entrance was created in each artificial cavity in a ratio of 1:2:2:1 respectively. The main bore of the cavity was closed by securely fitting a rubber stopper.

As part of a larger experiment, cavities were placed on 120 experimental trees (30 individuals of each of the four tree species). Cavities were placed on trees in July-August 2015 prior to the beginning of the rainy season when most ants reproduce and new

queens establish colonies. Cavities were attached to relatively small trees, 15-45 cm in circumference at the bole (measured 10cm above the soil) (Supplement, Table 1). Bole circumference was used because of the tendency for Cerrado trees to branch below breast height. Two pieces of bailing wire were used to secure each cavity to the tree, insuring maximum contact between the cavity and the tree branch. Cavities (six per tree) were spread evenly throughout the canopy on branches greater than 2cm diameter.

Cavities were collected after one year in July-August 2016. Once removed from the tree, clear packing tape was used to cover cavity entrance holes to prevent the escape of any inhabitants. In cases where hole modifications extended above the surface of the wooden cavity (Figure 1a), a metal ring made from bailing wire was placed around the entrance hole prior to taping in order to prevent damage to sometimes delicate modifications.

Entrance holes were photographed using an Olympus e-M5 camera equipped with an Olympus M. Zuiko 12-50mm F/3.5-6.3 lens in macro mode. Photos were taken looking directly down into the entrance while insuring sharp focus on the edge of the opening. A set of digital calipers was used, placing the jaws on the same focal plane as the entrance to minimize distortion and insure accurate scale. If necessary, a second photo was taken with cavity modifications removed to measure area of the unmodified entrance. All entrance areas were measured using ImageJ and the Fiji plugin (Schindelin *et al.*, 2012; Rueden *et al.*, 2017), using the brightness threshold tool to delineate entrance area. In some cases photos were adjusted using PhotoScape photo editing software to facilitate calculation of entrance area.

Prior to photographing, cavities were left in a household freezer for at least 24 hours to insure easy removal of inhabitants. Cavity occupants were removed from the cavities by removing the stopper and tapping the cavity over plain paper. All occupants including adult ants and nest parasites were counted and stored in 90% ethanol. Vouchers were pointed on pins for later identification. The presence of any queens, brood, or alates was noted. Queens and alates were included in the total count for adult ants, but brood were recorded as present or absent and not counted. Ants were identified using expertise and extensive voucher collections at the Universidade Federal de Uberlândia, Laboratório de Ecologia de Insetos Sociais (LEIS) under the direction of Dr. Heraldo Vasconcelos. Vouchers were deposited in the LEIS collections. All statistical testing and figures were done in R-Studio, and plots were created using the ggplot2 package.

Results

Modification frequency and materials

Of the 720 artificial cavities placed out, we recovered 653 from 116 experimental trees. Cavities that fell off the tree or were destroyed by termites were discarded. The sample of four tree species included 29 individuals of *Caryocar brasiliense* (162 cavities recovered), 29 *Machaerium opacum* (163 cavities recovered), 28 *Qualea grandiflora* (156 cavities recovered), and 30 *Sclerolobium aureum* (172 cavities recovered). Of the 653 cavities, 252 (38.6%) were inhabited by ants, 7 (1.1%) contained bee pupae or

remains, one (0.2%) contained Isoptera, one (0.2%) contained a spider, and 392 (60.0%) were empty.

Modifications to entrance holes were present on 117 or 17.9% of all recovered cavities and 71 or 28.2% of cavities that were inhabited by ants. Of the cavities with modified entrances, 101 (86.3%) of the entrances had modifications that reduced the area of the entrance hole, while 13 (11.1%) had modified structures that did not alter entrance hole area, and 3 cavities (2.6%) had entrances that were completely closed.

We categorized entrance-hole modifications into eight types based on the primary component used to create the modification. White fibers were the most common material used in constructing entrance modifications, accounting for 27.4% (32 cavities) of all modified entrances. These white fibers were fine and closely compacted, likely sourced from the same silk used by *Camponotus* species to cover their pupae. Brown organic matter was the second most common, accounting for 18.0% (21 cavities) of modified entrances. These modifications appeared to consist of organic material that had been finely chewed or digested such as frass, or plant material. Wood fibers accounted for 16.2% (19 cavities) of modified entrances and were often whitish in color, more coarse than silk, and distinctly straight. These fibers appeared similar to those on the outside of the artificial cavity itself but may have been sourced from other available wood. White-colored organic matter accounted for 15.4% of modified entrances (18 cavities). Similar to the brown organic matter, the white organic matter appeared to be paper-like, possibly sourced from lichen, fibers, and other organic matter. Resin, likely sourced from the host tree, accounted for 10.3% (12 cavities) of modified entrances. Soil, sand, and wood chips accounted for the remaining modifications (Fig. 1). Soil was characteristic of entrances modified by bees or wasps which used cavities for pupal development (Krombein, 1967; Mesquita & Augusto, 2011). In three cases these cavities were occupied by ants which probably occupied cavities after bees completed development. For bee-modified cavities, it is unclear whether ant occupants further altered entrance holes after it was used by bees.

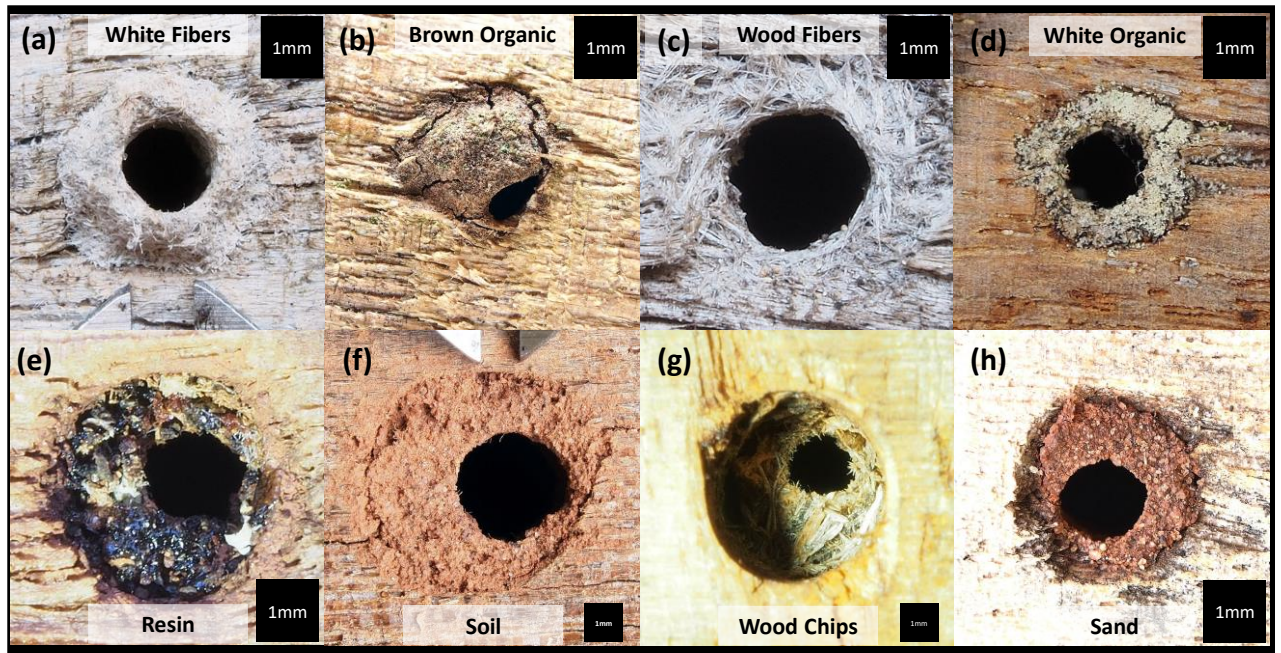


Figure 1. Photos of modification classification types. Black squares represent 1 mm² for scale. Photos show examples of the eight modification categories used: a) white fibers; b) brown organic; c) wood fibers; d) white organic; e) resin; f) soil (possibly constructed by bees or wasps); g) wood chips; h) sand.

Species differences in modification

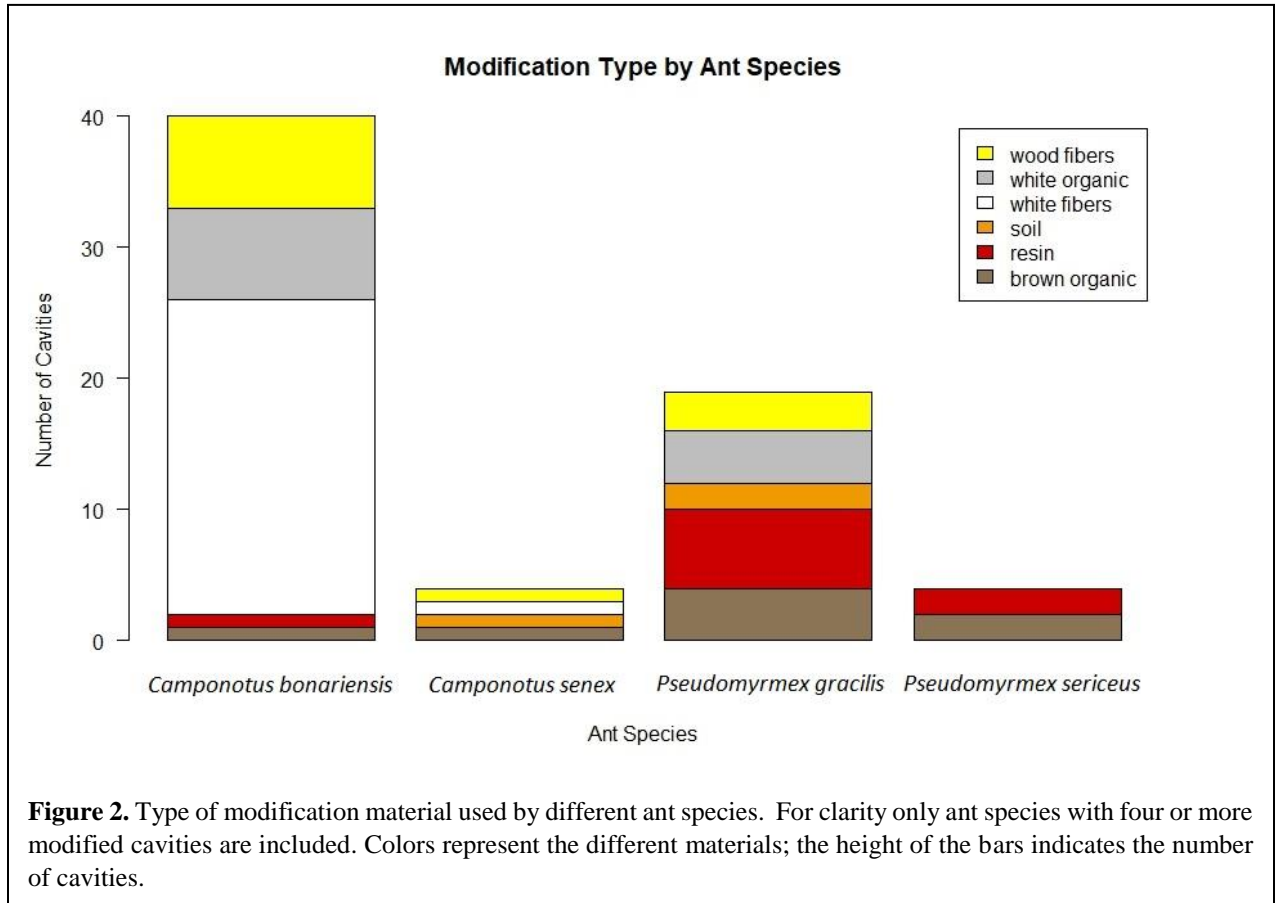
Most ant species that used the artificial cavities inhabited cavities with modified entrances at least some of the time. Of the three species with the most observations, 36% of cavity entrances used by *Pseudomyrmex gracilis* (Fabricius) were modified, 28% of *Camponotus bonariensis* (Mayr) entrances were modified, and 13% of *Camponotus senex* cavities were modified (Table 1).

Table 1. Total number of cavities sampled and percent of occupied cavities with modification by ant species.

Ant Species	Total Number of Cavities	% of Cavities Modified
<i>Camponotus atriceps</i>	4	0.00%
<i>Camponotus bonariensis</i>	143	27.97%
<i>Camponotus senex</i>	30	13.33%
<i>Cephalotes bormeyeri</i>	4	25.00%
<i>Cephalotes depressus</i>	1	0.00%
<i>Cephalotes minutus</i>	1	0.00%
<i>Cephalotes pusillus</i>	6	16.67%
<i>Pseudomyrmex gracilis</i>	56	35.71%
<i>Pseudomyrmex sericeus</i>	4	100.00%
<i>Solenopsis</i> sp.	1	100.00%

When comparing the materials used to construct entrance modifications we found that individual ant species used cavities modified with a variety of different materials. Differences between species in the materials they used were significant (Fisher's exact test, $p < 0.0001$, p -value simulated based on $1e+7$ replicates). The two species for which

we had larger sample sizes, *Camponotus bonariensis* and *Pseudomyrmex gracilis*, differed strikingly in the materials they used. While both species used wood fibers, white organic matter, and brown organic matter, *Camponotus bonariensis* used white fibers the majority of the time while *Pseudomyrmex gracilis* never used white fibers and instead used resin more than any other material (Fig. 2).



Factors influencing presence and type of modification

We found that different tree species had similar types and amounts of modifications (Fisher's exact test $p=0.12$ (simulated p -value with e^7 replicates)). Notable, however, were the absence of resin-based modifications on *Machaerium opacum* (which produces large amounts of red resin when damaged) and the absence of any white organic-based modifications on *Sclerobium aureum*. In each case, these types of modification were relatively common on other tree species (Fig. 3).

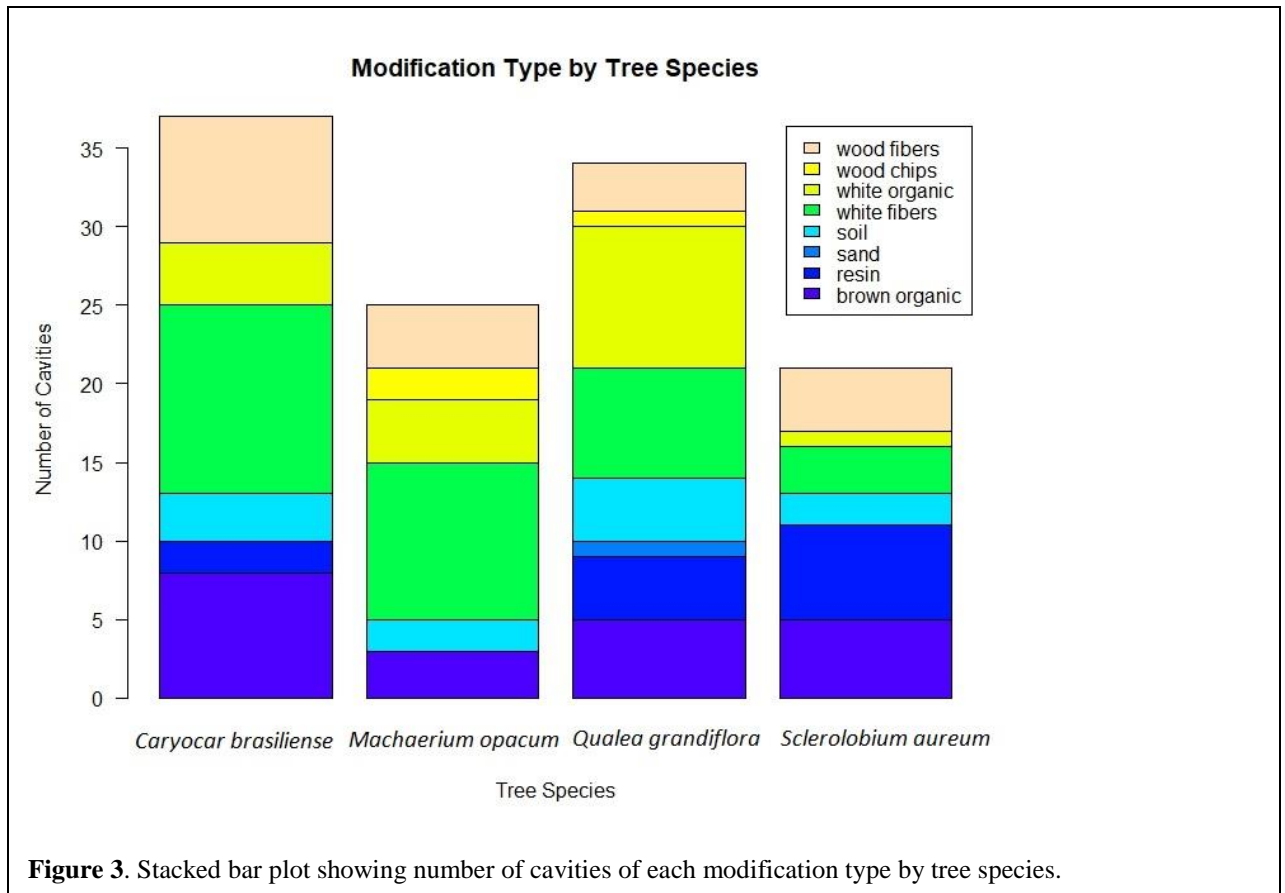
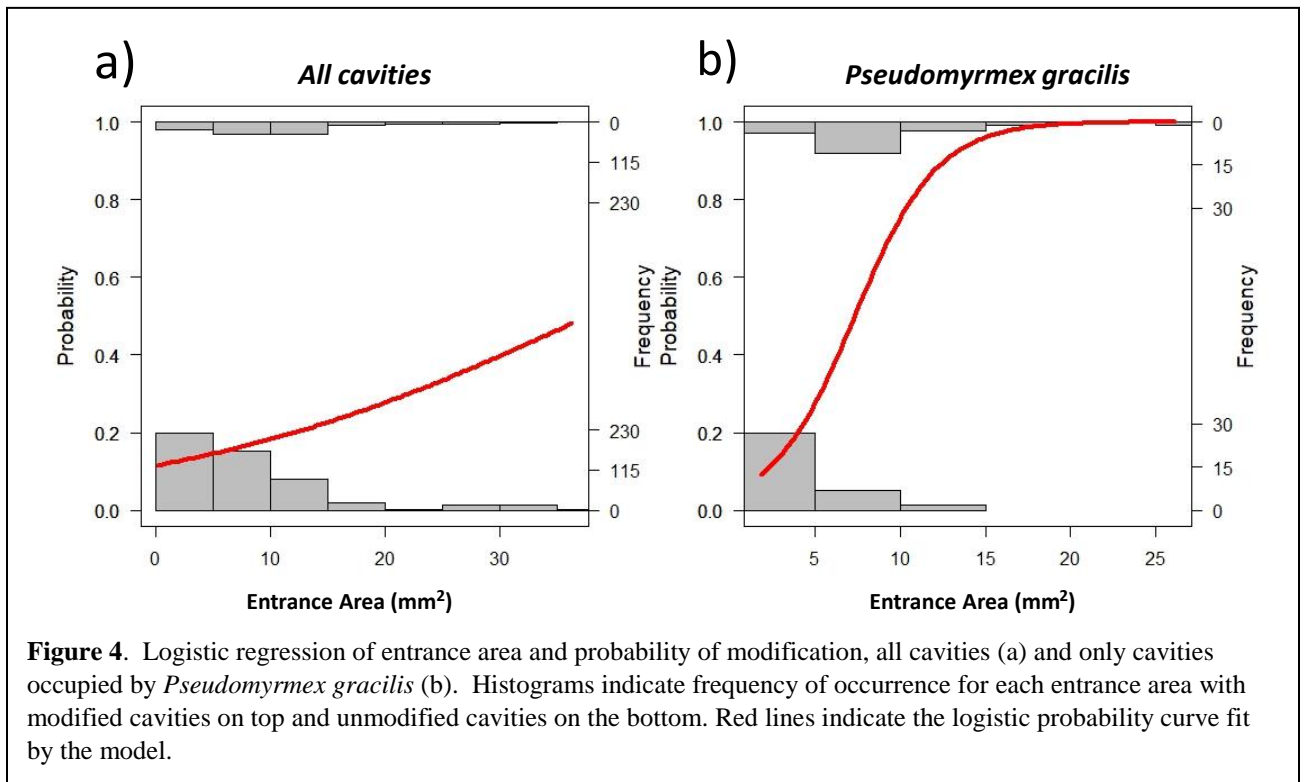


Figure 3. Stacked bar plot showing number of cavities of each modification type by tree species.

Logistic regression was used to model the relationship of the probability that a cavity entrance was modified versus the original area of the entrance. When all cavities were included in the model, entrance area was a significant predictor in the model ($p < 0.0001$). However the small difference between the null deviance (614.00, $df = 652$) and the residual deviance (596.43, $df = 651$) indicates that the inclusion of entrance area in the model only marginally increases the predictive power of the model as compared to when the response variable was predicted by the model using the intercept alone. Therefore although probability of modifications being present does increase with increasing entrance area, entrance area is not a good predictor of the presence of modification (Fig. 4a). Results were similar when running the model with only cavities occupied by ants and only cavities occupied by *Camponotus bonariensis*.

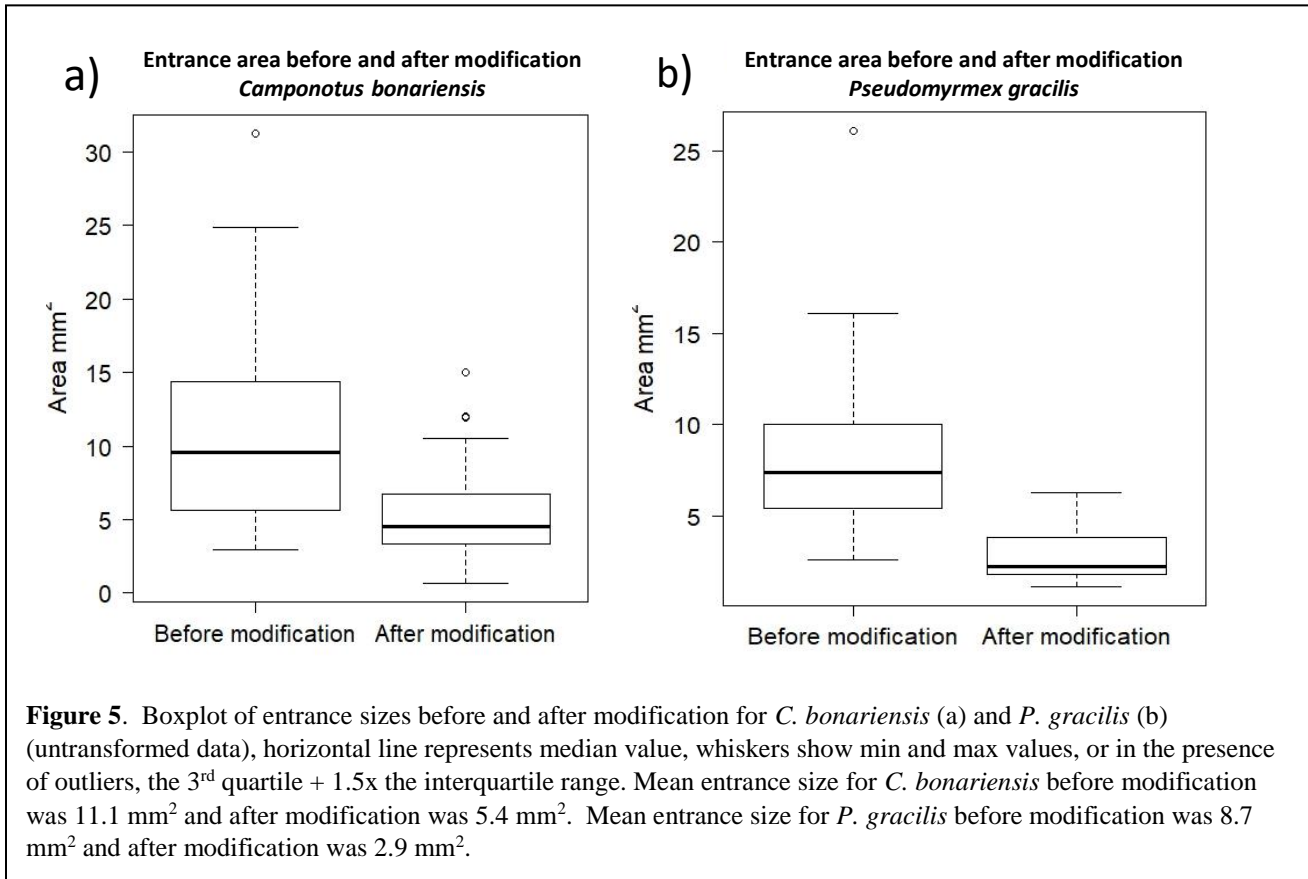
When running the logistic regression model for *Pseudomyrmex gracilis* alone, entrance area again was a significant predictor in the model ($p=0.0016$). The comparison of null deviance (73.0, $df=55$) and residual deviance (56.3, $df=54$) suggested that entrance area of the cavity had higher predictive power relative to other models in regards to the presence of modification. For cavities of *Pseudomyrmex gracilis*, entrances measuring less than 5 mm² were more likely to have no modification, whereas cavities over 5 mm² were more likely to be modified. According to the model, the turning point for probability of being modified or not was at an entrance area of approximately 7 mm² (Figure 4b).



Because nest entrance size may represent a balance between defensibility and worker traffic we tested for relationships between number of individuals (as a proxy for potential traffic) and entrance size. We found that for *Camponotus bonariensis* entrance size was unrelated to the number of adult individuals found in the nest (linear regression, $F=2.61$, $n=135$, $p=0.109$, $\text{adj R-squared}=0.012$, data square root transformed). The results were the same for *Pseudomyrmex gracilis* (linear regression, $F=0.10$, $n=54$, $p=0.753$, $\text{adj R-squared}=-0.0166$, data square root transformed). This suggests that entrance size is important for reasons other than the rate that individuals may pass into or out of the nest.

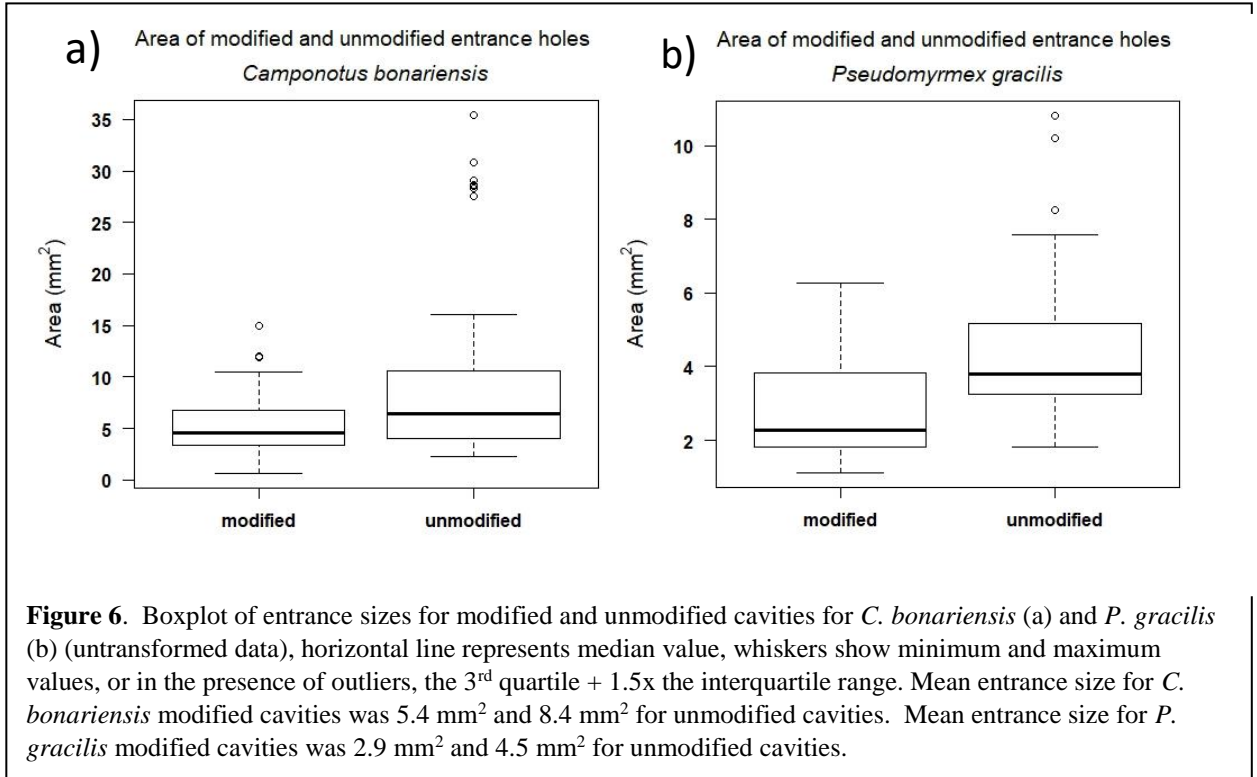
Changes to entrance size resulting from modification

Comparing cavity entrance sizes before and after modification, we found that modification significantly reduced entrance size (Paired T-test, $t=14.145$, $df=107$, $p<0.0001$, data square root transformed). The mean difference in entrance area before and after modification was 6.95 mm^2 or a 53.4% mean reduction in area compared to the original entrance pre-modification (range 0%-94.2%, $sd=28.2$). When considering cavities occupied by *C. bonariensis* (Figure 5a) and *P. gracilis* (Figure 5b) individually, again we found significant reductions in the entrance area as a result of modification (*C. bonariensis* $t=5.99$, $df=33$, $p<0.0001$; *P. gracilis* $t=7.36$, $df=19$, $p<0.001$, entrance areas natural log transformed).

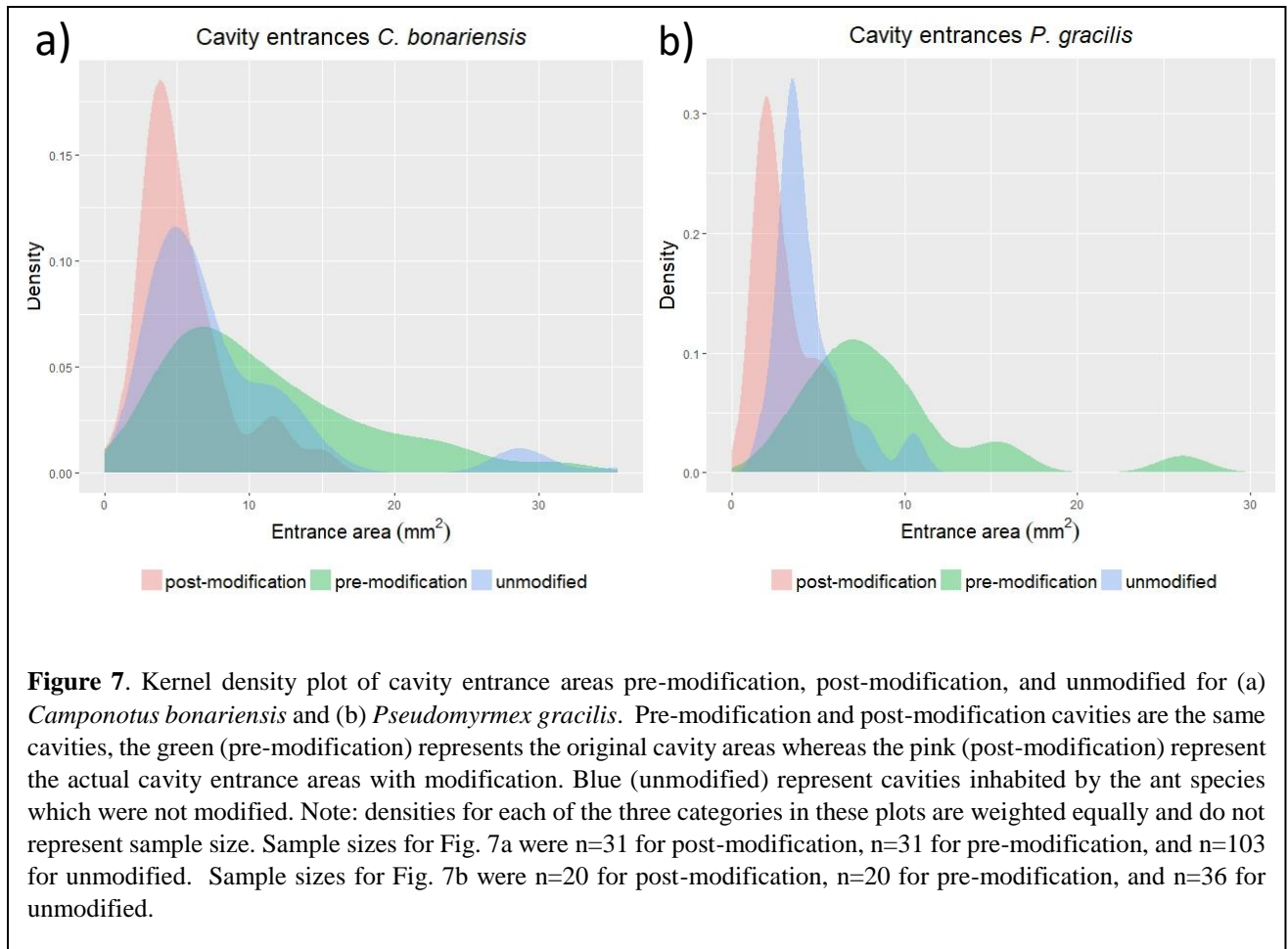


Modified cavities of both *C. bonariensis* and *P. gracilis* had significantly smaller entrance areas than unmodified cavities used by those species (Welch's two-sample T-test, *C. bonariensis*: $t = -3.26$, $p = 0.0018$; *P. gracilis*: $t = -3.532$, $p = 0.0013$, data natural log transformed) (Fig. 6).

For *C. bonariensis* and *P. gracilis*, when comparing density plots of entrance areas prior to modification to modified entrance areas (post-modification or unmodified),



we confirmed what we learned from the previous t-tests. Modification allows these species to use a number of cavities with entrance areas that are generally larger than those preferred by that species. Thus modification of entrance areas enables these species to alter otherwise marginal resources to increase the number of cavities that are available to them (Fig. 7).



Discussion

Although sample sizes for some species in this study are low, the frequency of modification to nest cavity entrances observed (28.2% of all cavities occupied by ants), suggests that entrance modification may be a common behavior for arboreal cavity-nesting ants. The number of species using cavities with at least some modified entrances (7 out of 10 species sampled, and all 4 genera), suggests that this behavior also has a wide taxonomic distribution. Nest cavity entrance modification likely has important implications for the evolution and ecology of cavity-nesting arboreal ants because it apparently impacts the suitability (and therefore availability) of a critically important limiting resource (Jiménez-Soto & Philpott, 2015; Philpot & Foster, 2005; Powell, 2009, Powell *et al.* 2011).

Ants are well known for their construction abilities, using a variety of materials and substrates to build and modify their nest sites (Von Frisch, 1974; Hansell, 1984). Thus, modification to cavity entrances is not novel within the repertoire of ant behaviors, but it is probably an adaptation of existing construction behavior to suit the constraints of arboreal and cavity-dwelling life. The range of materials used to construct modifications were similar across all four tree species, yet there were distinct differences in the materials used between *Camponotus bonariensis* and *Pseudomyrmex gracilis* (namely the use of white fibers and resin respectively), suggesting that materials used for entrance

modification are more characteristic of the ant species than of the materials available on the host tree. Unoccupied cavities with modifications from the present study, as well as high rates of turnover in cavity occupants in other studies (e.g., Armbrecht et al. 2004) suggest some amount of turnover in cavity occupancy. As a result, we suspect that the repertoire of modification types constructed by any one ant species is more limited than the types of modifications present on the cavities they inhabit. This is because a second ant species using an individual nest might reuse or reconfigure any existing modification left by the previous inhabitants. Inheritance of modifications from previous tenants may explain some of the variation in nest materials used by an individual species.

Some species sampled in our study rarely used cavities with entrance modifications. Only 4 out of 30 nests of *Camponotus senex* (13.3%), and only 2 out of the 12 nests from the genus *Cephalotes* (16.7%) had modified entrances. It is possible that, when modification occurs infrequently, particular ant species may not modify cavities at all, but rather inherit modified entrances from the previous occupant. For some ant species, modifying nest entrances may not be important to their nest for defense, moderating abiotic conditions, or nest recognition. Expanding observations of nest entrance modification across a greater number of species in conjunction with detailed analysis of the materials used in modification may shed light on whether all or only some species are capable of nest modification, and the extent to which modifications are inherited from previous tenants.

This study demonstrated that nest entrance modification significantly reduces the area of cavity entrances (53.4% average reduction in area). When comparing distributions of cavities prior to modification to those of cavities used by ants (post-modification or unmodified) for *C. bonariensis* and *P. gracilis*, it is evident that modifications are used to reduce the entrance size of cavities bringing them into the range of useable entrance sizes for that species. Previous work has shown that cavity entrance size is related to average ant body size (Priest, *unpublished data*; Novais et al. 2017) but cavity entrances used by a single species still vary considerably. This variability may be explained by intraspecific variation in body size, particularly when a species has more than one caste. *C. bonariensis*, for example, has both a worker caste and a much larger soldier caste, but not all cavities of *C. bonariensis* in this study contained soldiers. It is possible that either young colonies, which do not yet produce soldiers, or individual nests of mature colonies, which are not occupied by soldiers, have much smaller entrances to accommodate smaller castes contributing to higher levels of variability in entrance area. *P. gracilis* in contrast has lower levels of variability in entrance size and does not have multiple castes but high levels of intraspecific variation in individual size is also present. Future studies might clarify the relationship between entrance area and ant body size by comparing entrance sizes of cavities to the individuals that occupy them rather than species' averages. We found that the likelihood of modification increased with entrance size for *P. gracilis* but not overall or for *C. bonariensis*, and that this may be due to larger variability in entrance sizes used by *C. bonariensis* as a result of greater variation in body size when compared to *P. gracilis*.

A comparative study on nest entrances of stingless bees (Meliponini) showed that species-specific nest entrance size was the result of a tradeoff between defensibility favoring smaller entrances and accommodation of forager traffic favoring larger entrances (Couvillon et al. 2008). We used the number of individuals present in a nest

cavity as a proxy for potential traffic but found no relationship between entrance size and number of individuals in the nest. The lack of relationship between the number of individuals and the area of the entrance hole suggests that either number of individuals in the nest is not a good metric of traffic, or entrance area is not as important for the rate at which individuals can pass in and out of the nest. If this were the case we might expect that nests with more individuals have larger entrances to facilitate higher nest traffic. For the species examined in this study, either the rate of traffic into and out of the nest is not high enough that larger entrances would be beneficial, or the benefit of nest defensibility far outweighs any constraint on entrance traffic.

Defensibility of a nest entrance appears to be the main factor in selecting a nesting cavity, whereby colonies that inhabit nests with entrances not well-suited to their defensive strategy may suffer higher rates of predation by neighboring ants or other arthropods (Jiménez-Soto & Philpott, 2015; Novais *et al.*, 2017; Powell, 2008; Powell, 2009; Powell *et al.* 2011). While some species in the genus *Cephalotes* have evolved a highly specialized, and morphologically distinct, soldier class to remedy this problem, this adaptation limits these species to very specific entrance sizes (Powell, 2008; Powell, 2009). Once a cavity with a suitable entrance is encountered, these species have an effective strategy for defending it, but the number of available suitable cavities is probably severely limiting, possibly explaining the relatively low abundance of these species in the community. It seems, however, that some of the dominant ant species in this community have evolved an alternative solution to the problem of entrance size suitability. Rather than evolve morphologically specialized castes to optimize use of a certain entrance size, these species have evolved the ability to simply alter the entrance size of a cavity to suit their needs. Thus, while some species, of *Cephalotes* have evolved to increase their competitive ability by specializing on a subset of the available cavity resources, other species, such as *C. bonariensis* and *P. gracilis*, may have increased their competitive ability by evolving behaviors allowing them to modify otherwise unsuitable cavities to fit their needs, thereby expanding the resources available to them.

Evidence from this study suggests that cavity entrance modification is an adaptation for increasing nest defensibility of otherwise un-defendable cavities, however, hypotheses for alternative function were not explored. Although relatively rare, in 13 cavities of 117 that had entrance modifications, the area of the entrance was not altered, *i.e.*, a structure was constructed around the entrance hole but did not make the entrance smaller. This indicates that cavity modifications may serve functions in addition to nest defense. While cavity entrance modifications likely serve more than one purpose, here we suggest three hypotheses for the function of these modifications.

First, given that arboreal ants show preference for a subset of entrance sizes, together with the limited availability of suitable nest sites, cavity modification may serve to reduce the effective size of entrances which would otherwise be unsuitable for that species. Under pressures of nest site limitation and threat of predation or aggression from neighboring colonies, these modifications could serve to ameliorate competition for nests of the required size for that species by increasing the availability of potential nest sites without incurring loss of nest defensibility. Furthermore, because many arboreal ant species are polydomous, nest entrance modification may serve to increase the number of suitable nest cavities within the existing territory of the colony, allowing for larger colony size and better control of foraging territory.

Second, nest entrance modifications may serve to moderate abiotic conditions experienced inside the nest cavity. Restricting the area of the connection between the inside of the cavity and the outside conditions may serve to regulate humidity, particularly in dry seasons when water may be at a premium, or heat when solar radiation or outside air pass through the nest entrance. Some entrance modifications have a raised structure (a volcano-like shape, Figure 1a) while others are completely covered such that the entrance to the structure is horizontal to the cavity rather than perpendicular (like a hut, (Figure 1b, Supplement Figure 2)). These structures may be effective ways to keep water out of cavities during heavy rains, acting like levies around the cavity entrances, or completely inclosing them from flowing water.

Third, modifications may aid in nest recognition. Ants are known to recognize nest entrances both through imprinting of nest scent and learning of nest visual characteristics (Cammaerts, 2013). Modifications may aid in nest recognition in two ways. First, they offer a distinguishable and unique visual landmark upon which ants can identify cavities, and second, they may act as a location where pheromones are deposited by inhabiting ants while entering and exiting the nest.

Because entrance modifications are easily removed, the alternative functions for entrance modification proposed here could be tested. Effects of entrance modification on abiotic factors could be tested by measuring changes in humidity, temperature, and entrance of water, within the cavity before and after removal of the structure. Similarly, nest recognition behavior may be observed by testing the reaction of inhabitants returning to the nest under a scenario comparing disturbance resulting in modification removal and disturbance without removal. Responses to chemosensory cues and analysis of chemicals on the structure could provide insight into the function of modification in nest recognition, and whether they act as a visual or chemical cue for nest inhabitants.

This study provides an initial look into cavity entrance modification by arboreal ants. We found that modification of entrance cavities by ants is a common behavior among some arboreal ant species and probably has wide taxonomic distribution. The ability of some ants to reduce cavity entrance size of unsuitable cavities to suit their needs has strong implications for the relationship between ant body size and cavity entrance size, and likely plays an important role in expanding availability of nesting sites, a limiting resource for arboreal cavity-nesting ants. Further investigation of the scope and function of nest entrance modification by arboreal ants may yield better understanding of the dynamics of nest resource limitation and their effects of community assembly, insights into adaptive responses to arboreal life, and the ecological and evolutionary consequences of adaptations that reduce resource limitations, effectively expanding the available niche.

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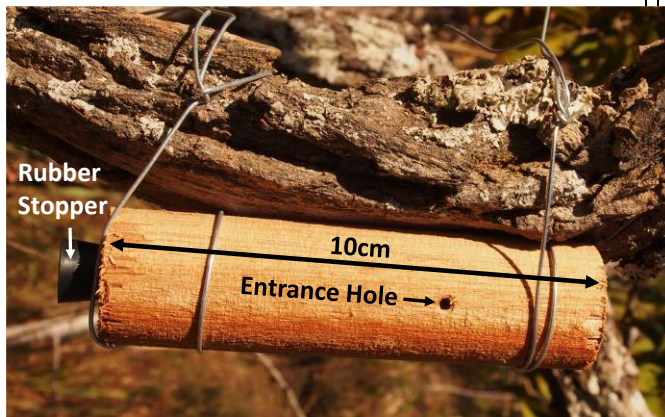
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Supplementary Material

Supplement Table 1. Table of mean and standard deviation of tree bole diameter by species.

<u>Tree Species</u>	<u>Mean Bole Circumference</u>	<u>Standard Deviation</u>
<i>Caryocar brasiliense</i>	34cm	6.48
<i>Machaerium opacum</i>	25cm	6.22
<i>Qualea grandiflora</i>	29cm	7.65
<i>Sclerobium aureum</i>	27cm	6.70



Supplement Figure 1. Artificial nest cavity attached to experimental tree.



Supplement Figure 2. 'Hut-shaped' entrance modification on a cavity of *Solenopsis* spp. Calipers show 1mm for scale. Original entrance hole is perpendicular to 'hut' entrance located under the structure.

Increased nesting resources for ants does not impact herbivory on two cerrado tree species, despite an overall positive effect of ants

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Abstract

Studies of ant-plant protection mutualisms on trees in the Brazilian Cerrado have demonstrated that the presence of ants frequently results in benefits to the plant in the form of reduced herbivory. Many of the arboreal ants involved in these interactions nest in cavities in tree stems created by wood-boring beetles. It has been proposed that this beetle-mediated association between arboreal ants and cerrado trees is one reason for the dominance of ants on cerrado vegetation in general. Beetle cavities have been shown to be an important limiting resource for arboreal ants, and they modulate ant abundance and diversity on trees. Despite this, no study has directly investigated the impact of resources for cavity nesting on ant-herbivore interactions occurring on host trees. To test whether cavities suitable for ants indirectly impact herbivory on host trees, we experimentally increased cavity nesting resources on individual branches of two common cerrado tree species, *Caryocar brasiliense* and *Sclerolobium aureum*, in a year-long experiment, and measured subsequent herbivory at the end of the experiment. While experimental cavities were colonized by ants, we found no effect of cavity addition treatments on number of ants, nor on herbivory. Ant exclusions, however, significantly increased herbivory on these same experimental trees. Ant exclusions resulted in an 86% reduction in mean number of individual ants per branch and a 70% reduction in the mean number of ant species when compared to control branches. These results, in conjunction with those from recent studies, suggest that the abundance of cavity resources alone do not impact herbivory at the branch level because cavities are not necessarily occupied by ant species that reduce herbivory on host trees. Rather, evidence from this, and other studies, suggests that the abundance of large cavities, which are more commonly used by aggressive and dominant ant species, may have a much stronger indirect impact on herbivores and herbivory on the trees in which they are located.

Keywords: Ecosystem engineering, cavity nesting ants, arboreal ants, herbivory, cerrado, facultative ant-plant mutualism, symbiosis, *Caryocar brasiliense*, *Sclerolobium aureum*

Introduction

Community ecologists seek to understand how interactions between groups of species and resources shape ecological outcomes. One such outcome of species-interactions is the consumption of plant tissues by herbivores. Studies of herbivory have focused extensively on how predators, and ants in particular, impact plants through their interference with primary consumers (Beattie, 1985; Huxley & Cutler, 1991; Koptur, 1992; Davidson & McKey, 1993; Bronstein, 1998; Heil & McKey, 2003). In the Brazilian Cerrado, arboreal ants are important predators of herbivorous insects on trees. Oliveira & Freitas (2004) suggested that the dominance of ants on cerrado foliage is the result of two main factors: the frequency of plants bearing extrafloral nectaries, and the abundance of ants nesting in hollow cavities within the vegetation. While a number of studies have investigated the impact of ants tending extrafloral nectaries on herbivory in the cerrado (Oliveira & Freitas, 2004, and references therein), no study has directly tested the impact on herbivory of cavity nesting resources used by ants.

Trees in the cerrado contend with challenging abiotic conditions such as nutrient-poor, often toxic soils, strong annual cycles of precipitation, which result in a long season with little or no rainfall, and fires. In addition to harsh abiotic conditions, cerrado plants are subjected to herbivores (primarily insects) that consume living tissues (Marquis *et al.*, 2002). Insect herbivores not only feed on leaves and reproductive tissues of plants, but also on woody stems. Wood-boring beetles feed on living and dead stems during their larval stages, creating hollow cavities in which they usually pupate before emerging as adults. Consumption of living stems by beetles represents a cost to trees through removal of living tissue, deterioration of structural integrity, and potential introduction of fungal pathogens. In one study on the mangrove tree *Rhizophora*, in Belize, indirect loss of leaf area as a result of stem-boring was equal to, or exceeded, that of direct consumption by folivores, suggesting that the cost of damage to live stems by wood-boring beetles can be considerable (Feller & Mathis, 1997). A previous study on six common cerrado tree species demonstrated that damage from wood-boring beetles, which is substantial, varies dramatically among tree species and stem diameters. The proportion of stem length bored by wood-boring beetles ranged from less than 10% in *Qualea grandiflora* to more than 50% in *Caryocar brasiliense* in stems greater than 3-cm diameter (Priest, *et al.* 2018).

By feeding on tree stems, wood-boring beetles create long lasting, durable shelters that act as nest sites for a wide range of arboreal ant species (Novais *et al.*, 2017; Klimes *et al.*, 2012; Powell *et al.*, 2011; Priest, *et al.*, 2018, Satoh *et al.*, 2016). In creating nesting sites for ants, wood-boring beetles act as physical ecosystem engineers that create resources for other organisms by causing physical state changes in their environment (Jones *et al.*, 1997). Previous studies on the use of cavities in stems (both artificial and natural) by ants have provided evidence that these beetle cavities are a limiting resource for arboreal ants (Armbrecht, *et al.*, 2006; Philpott & Foster, 2005), and are important for arboreal ant diversity (Powell *et al.* 2011, Armbrecht *et al.*, 2004), abundance (Klimes *et al.*, 2012), and niche partitioning (Jiménez-Soto & Philpott, 2015; Satoh *et al.*, 2016; Novais *et al.*, 2017; Priest *et al.*, 2018).

Ants that forage on cerrado trees for prey and extrafloral nectar have been shown to have a significant impact on herbivory. Ant exclusion experiments on these trees have demonstrated that the presence of ants reduces abundance of specialist herbivores (Oliveira, 1997), decreases leaf consumption by herbivorous insects (Del-Claro *et al.*, 1996; Koch *et al.*, 2016), and alters larval and adult oviposition behavior of herbivores (Oliveira & Freitas, 2004). Most ant defense mutualisms can be placed in one of two categories: myrmecophyte symbioses, involving plants that have evolved domatia to house ant colonies; and non-symbiotic or facultative mutualisms in which plants employ food rewards to attract ants not nesting on the plant (Heil & McKey, 2003). Non-symbiotic ant-plant mutualisms tend to have more variable outcomes with respect to reducing herbivory due to having more diverse and unpredictable ant partners and levels of association (Rosumek *et al.*, 2009). Cavity-containing cerrado trees fall somewhere between the two categories of plant defense mutualisms. While these trees have not evolved specialized domatia to house ants, consumption by wood-boring beetles nevertheless creates an analogous resource for ants. Furthermore, while the ants that use these cavities are more diverse than those found in most typical symbiotic ant-plant systems (Powell *et al.*, 2011; Priest *et al.*, 2018), the presence of ants in cavities on the tree creates a much more intimate and long-lasting association between ants and their host tree compared to facultative ant-plant interactions where only food rewards are present.

Despite numerous studies on both the effects of ants on herbivory, and the use by ants of nest cavities created by wood-boring beetles, no studies have measured the indirect effect that cavity nest resources have on herbivory via the ants that inhabit them. In this study, we sought to test experimentally the indirect impact of cavity nesting resources on herbivory. To do this we addressed the following questions: 1) Does the addition of cavity resources to two species of cerrado trees increase ant abundance and diversity? 2) Does the addition of cavity resources indirectly impact herbivory? 3) How does the impact on herbivory of additional cavity resources compare to the impact of ant exclusion?

Methods

Study Site and Focal Species

Fieldwork was conducted at the Panga Ecological Station (Estação Ecológica do Panga), administered by the Universidade Federal de Uberlândia, 30 km from Uberlândia, Minas Gerais, Brazil. The site is a 400-hectare reserve consisting of mixed Cerrado vegetation, though the current study was focused in *cerrado sensu strictu* with approximately 30% canopy cover (Oliveira-Filho & Ratter, 2002). The tree species selected for this study were among those most common in the study site; *Caryocar brasiliense* Camb. (Caryocaraceae), and *Sclerolobium aureum* (Tul.) Baill (Fabaceae).

Experiment set up

The experiment, which was conducted between the second week of May, 2015, and the second week of August, 2016, was a fully-blocked split-plot design with two factors: ant exclusion and cavity addition. Thirty individual trees of each species were

assigned to cavity-addition or control (no cavity addition) treatments (for a total of 60 trees per species), and each individual tree was considered a ‘plot’ with an ant exclusion branch and a control (no exclusion) branch.

The sixty experimental trees of each species were distributed throughout the reserve in areas of similar vegetation physiognomy (*cerrado sensu strictu*). Individuals were selected based on accessibility and availability of branches for experimental treatment insuring at least 5 meters between experimental trees. We selected relatively small trees for the experiment, 5-15 cm in diameter at the bole (measured 10cm above the soil). Bole circumference was used because of the tendency for some Cerrado trees to branch below breast height. Mean bole diameter of experimental trees was 10.8-cm (SD = 2.1) for *Caryocar* and 8.6-cm (SD = 2.1) for *Sclerolobium*.

In May-June 2015, during the dry season, when no leaves were present, two branches of equal diameter (approximately 3-6 cm) were selected on each tree and randomly assigned to control and ant-exclusion treatments. In the selection of branches, we considered similarity in branch height, orientation, sun exposure, and amount of new growth. Furthermore, none of the chosen branches were in contact with other branches or surrounding vegetation. On ant-exclusion branches we wrapped a 10-cm long section of the branch in a layer of cotton, making sure to fill any crevices in the bark where ants would be able to pass, and secured the cotton by wrapping it in several layers of packing tape. Over the packing tape, we put a thick layer (approximately 5-10 mm) of Tanglefoot® insect barrier (Scotts Company LLC), insuring complete coverage around the circumference of the branch (Fig. S1a). We did not do a ‘mock manipulation’ on control branches because we decided the risk of interfering with ant visitation on controls outweighed the risk of unintended effects of manipulating branches.

To insure no ants remained on the ant exclusion branch, we physically removed any visible ants by hand and placed insecticidal baits. Insecticidal baits were placed on trees for 3 days and consisted of a 80 ml capacity plastic cup, 5 cm in height with a 5 cm opening diameter, which was baited with 25 ml of a 1:1 mixture of urine and water, which contained 5 ml per liter of Decis® 25EC (Bayer LLC) (a 2.5 % general pyrethroid ester insecticide). Bait cups were wired to the ant exclusion branch and covered with a lid. Each lid had one 8-mm hole to allow entry of ants while excluding larger non-target arthropod species. A piece of cotton and a piece of twine were placed in each cup to allow ants to feed and return to their nest, delivering the delayed-action insecticide to the entire colony (Fig. S1b).

In July-August, 2015, prior to the beginning of the rainy season, when most ants reproduce and new queens establish colonies, we placed artificial cavities on half of the experimental trees, insuring a relatively even spacing of cavity-treatment and control trees throughout the site. Artificial cavities were constructed from locally-obtained wood dowels (3-cm diameter) commonly used for tool handles, and were of a variety of hardwood species. Dowels were cut into 10-cm lengths and drilled lengthwise from one end to a depth of 9 cm using a 1-cm diameter bit. A single entrance hole was drilled perpendicular to the cavity bore approximately one-third the distance from the closed end. A flashlight was used to check that the entrance-hole and main cavity bore

intersected (Supplement Figure 1). Four entrance sizes were used (approx. 4.4 mm², 6.6 mm², 12.4 mm², 31.7 mm²) in a ratio of 1:2:2:1 respectively. The main bore of the cavity was closed by securely fitting a rubber stopper. Two pieces of bailing wire were used to secure each cavity to the tree, insuring maximum contact between the cavity and the tree branch. Cavities (six per tree) were spread evenly throughout the canopy on branches greater than 2cm diameter (for a photo of artificial cavities, see Priest & Marquis, 2018).

Data collection

In May 2016, we used arboreal pitfall traps to sample ant diversity and abundance on each of our control and the ant-exclusion branches. Traps consisted of an 80-ml capacity plastic cup, 5 cm in height with a 5 cm opening diameter, which was baited with 25-ml of a 1:1 mixture of urine and water with a small amount of liquid detergent to increase capture and killing efficiency. Traps were wired directly onto the branch and manipulated to maximize the area of contact between the cup lip and the branch. One trap was placed directly distal to the Tanglefoot® treatment on the ant-exclusion branches, and one was placed on the control branch on a section of the stem with equal diameter to that of the treatment. Traps were set for 48 hours before collection. All samples were stored in alcohol for later identification. While most studies that use this method of ant exclusion (i.e., Tanglefoot) suggest constant maintenance of ant exclusion (e.g., Del-Claro *et al.*, 1996), a previous attempt to conduct this experiment, which was later destroyed by a fire, showed that when applied thickly, Tanglefoot® maintains its sticky texture and is effective for a full year. Because we placed our exclusion devices on branches separated from other vegetation, contact with other plants was not an issue. If the efficacy of Tanglefoot® diminished over time, our pitfall sampling at the conclusion of the experiment would be representative of the minimum efficacy of our ant exclusion treatments. Despite the long interval of exclusions without constant maintenance, our treatments were effective at excluding, or at least significantly reducing the number of, ants attending exclusion branches (see Results section).

After allowing the experiment to proceed for one year, artificial cavities and leaves were collected simultaneously from experimental trees in July-August 2016. Cavities were removed from the tree, and clear packing tape was used to cover cavity entrance holes to prevent the escape of any inhabitants. Cavities were left in a household freezer for a minimum of 24 hours to insure easy removal and processing of inhabitants. Cavity occupants were removed from the cavities by removing the stopper and tapping the cavity over plain paper. All occupants, including adult ants, were counted and stored in 90% ethanol.

Leaves were then collected from each the control and exclusion branches. We observed that leaves towards the apical end of a new stem were more heavily damaged by herbivores than more basal leaves, probably representing an increase in herbivores later in the plant's leaf-flushing period. For this reason we sampled all leaves on individual active meristems in order to assure proportional sampling of both early-flushing and late-flushing leaves. On each branch, all leaves were sampled or until the 1-gallon plastic bag used for sampling was full. Leaves were transported to the laboratory for analysis. In the laboratory leaves were placed on a white surface with a ruler scale and flattened under a

piece of clear acrylic. Photographs were taken directly from above the leaves using a tripod. The camera was leveled to insure no spatial distortion in the photo. Photos were taken in indirect natural light to reduce glare from the acrylic sheet and taken at a high F-stop with long exposure to insure clarity of focus across the entire photo. Herbivory (proportion of leaf area removed) was analyzed in ImageJ. Leaf margins were drawn in using photo editing software and images were converted into binary before processing. In some cases, photos had to undergo adjustments to exposure and contrast to insure correct transformation of the image into binary black and white for analysis. The ruler in each photo was used to calibrate the scale of images accurately, and allow calculations of measurements of total leaf area and the amount of leaf area removed square centimeters.

Ant identification

Vouchers were pointed on pins and dried for identification. Ants were identified using the extensive voucher collection from this site at the *Universidade Federal de Uberlândia, Laboratório de Ecologia de Insetos Sociais* (LEIS) under the direction of Dr. Heraldo Vasconcelos. Identifications were confirmed by members of the Vasconcelos lab and vouchers were deposited in the LEIS collections.

Data analysis

Data were analyzed using R Studio. Proportions and percentages were logit transformed, and other variables were transformed when required to attain near normal distributions. Proportion leaf area removed for individual branches (ant-exclusion and control for individual trees) represents the total area removed divided by the total leaf area sampled. Mean number of ants per tree and ant species per tree represent data collected from control branches only (those that were not affected by ant exclusions) unless otherwise noted. Data regarding artificial cavities such as percent of cavities occupied, and number of ants in cavities represent only trees that received the cavity addition treatment. Welch’s t-tests for unequal variance were used to test for differences among treatments. For comparisons of herbivory and ants between exclusion and control branches we used a paired design in order to account for differences between individual trees in their levels of primary defenses and local effects such as differences in nearby ant colonies and herbivore pressure.

Results

Artificial cavities were successfully colonized by arboreal ants. Of the cavities recovered, 39 percent were occupied by ants, representing nine species in four genera. Percent of cavities occupied by ants, mean number of ants per cavity, and mean number ant species in cavities were similar for *Caryocar* and *Sclerolobium* (Table 1).

Table 1. Percent of cavities occupied, mean ants per cavity, mean number of ants species in cavities per tree and total number of ant species nesting in cavities for each tree species.

Tree species	Percent of cavities occupied	Mean ants per cavity	Mean number of ant species per tree	Total number of ant species
<i>Caryocar brasiliense</i>	36%	9.6	1.4	7
<i>Sclerolobium aureum</i>	41%	10.1	1.2	7

Of the ant species nesting in artificial cavities in the experiment, *Camponotus bonariensis* was the most common, occupying 21 percent of all cavities and accounting for 53 percent of cavities occupied by ants. Six out of the nine species nesting in artificial cavities were found on both tree species, and all ant species that occurred in more than four cavities were found on both tree species. Mean number of ants per cavity ranged from 1 to 99 for different species with the highest number of individuals per cavity occurring in *Solenopsis* sp.1, the smallest of the ant species found nesting in our artificial cavities (Table 2).

Pitfall sampling revealed that 34 ant species from 14 genera were present on experimental trees. *Camponotus senex* was the most abundant species in pitfall samples in terms of number of individuals and it also occurred in the most number of samples, while *Camponotus bonariensis*, the most common ant nesting in the artificial cavities,

Table 2. Number and percent of artificial cavities occupied and mean number of ants per cavity by ant species.

Ant Species	Total number of cavities occupied	% of cavities occupied	Mean number of ants per cavity	Tree species
<i>Camponotus atriceps</i>	2	0.5%	16	both
<i>Camponotus bonariensis</i>	70	21.0%	24	both
<i>Camponotus senex</i>	15	4.5%	32	both
<i>Cephalotes borgmeyerii</i>	4	1.2%	54	<i>S. aureum</i>
<i>Cephalotes minutus</i>	1	0.3%	1	<i>S. aureum</i>
<i>Cephalotes pusillus</i>	3	0.9%	27	<i>C. brasiliense</i>
<i>Pseudomyrmex gracilis</i>	32	9.6%	15	both
<i>Pseudomyrmex sericeus</i>	3	0.9%	61	both
<i>Solenopsis</i> sp.1	1	0.3%	99	<i>C. brasiliense</i>
Empty	203	60.7%	NA	both

was the fourth most abundant with respect to individuals and it occurred in the second highest number of samples. *Camponotus* was the most abundant and diverse genus in our pitfall samples, with 12 different species. While some ant species were only sampled from one of the two tree species in the study, all species that occurred in more than four samples were found on both *Caryocar* and *Sclerolobium* (Table 3).

Table 3. Occurrence of ant species in total number of individuals and number of samples from pitfall sampling.

Ant Species	Number Individuals	Number Pitfalls	<u>Tree species</u>
<i>Atta</i> sp.1	70	2	both
<i>Azteca</i> sp.1	885	29	both
<i>Brachymyrmex</i> sp.1	2	1	<i>C. brasiliense</i>
<i>Camponotus atriceps</i>	146	6	both

<i>Camponotus blandus</i>	200	11	both
<i>Camponotus bonariensis</i>	348	70	both
<i>Camponotus cingulatus</i>	527	34	both
<i>Camponotus egregius</i>	3	1	<i>C. brasiliense</i>
<i>Camponotus fastigatus</i>	11	2	both
<i>Camponotus ledigii</i>	1	1	<i>S. aureum</i>
<i>Camponotus renggeri</i>	1	1	<i>C. brasiliense</i>
<i>Camponotus senex</i>	916	105	both
<i>Camponotus sp.1</i>	12	3	both
<i>Camponotus sp.15</i>	12	4	both
<i>Camponotus sp.2</i>	11	6	both
<i>Cephalotes attratus</i>	1	1	<i>C. brasiliense</i>
<i>Cephalotes depressus</i>	16	3	both
<i>Cephalotes persimilis</i>	2	1	<i>S. aureum</i>
<i>Cephalotes pusillus</i>	121	11	both
<i>Crematogaster ampla</i>	51	2	<i>S. aureum</i>
<i>Gnamptogenys suleata</i>	2	2	<i>S. aureum</i>
<i>Neivamyrmex</i>	1	1	<i>S. aureum</i>
<i>Neoponera villosa</i>	2	2	both
<i>Nesomyrmex asper</i>	4	4	both
<i>Pheidole sp.1</i>	4	2	<i>S. aureum</i>
<i>Pseudomyrmex curacaensis</i>	2	1	<i>S. aureum</i>
<i>Pseudomyrmex elongatus</i>	16	6	both
<i>Pseudomyrmex gracilis</i>	83	43	both
<i>Pseudomyrmex maculatus</i>	1	1	<i>C. brasiliense</i>
<i>Pseudomyrmex simplex</i>	3	3	<i>C. brasiliense</i>
<i>Pseudomyrmex unicolor</i>	1	1	<i>C. brasiliense</i>
<i>Pseudomyrmex urbanus</i>	43	17	both
<i>Solenopsis sp.1</i>	674	22	both
<i>Tapinoma sp.1</i>	126	3	<i>C. brasiliense</i>

Pitfall ant sampling revealed no significant differences among ant species in the mean number of individuals (Welch's t-test, $t_{(93)} = -0.59$, $p = 0.56$, data log transformed) nor number of species per tree (Welch's t-test, $t_{(93)} = 1.62$, $p = 0.12$) between *Caryocar* and *Sclerolobium*. Overall levels of herbivory on *Sclerolobium* were lower than on *Caryocar*, but the increase in herbivory between control and ant exclusion branches were similar (Table 4). The number of ants per tree collected in pitfalls did not differ between cavity addition trees and control trees for *Caryocar* ($t_{(42)} = 0.77$, $p = 0.44$) or *Sclerolobium* ($t_{(31)} = 1.45$, $p = 0.16$) (Welch's t-test, data log transformed). For *Caryocar*, the number of ant species per tree in pitfall samples did not differ significantly between cavity-addition trees and controls (Welch's t-test, $t_{(49)} = 0.14$, $p = 0.89$), but *Sclerolobium* had significantly higher numbers of species on cavity addition trees than on

controls (Welch's *t*-test, $t(40) = 3.26$, $p = 0.002$, data log transformed). Mean per tree species diversity for pitfall traps was 2.96 for cavity addition trees and 2.00 for controls.

Tree species	Mean herbivory ant control	Mean herbivory ant exclusion	Mean bole diameter	Mean # ants per branch per tree	Mean ant species per branch per tree
<i>C. brasiliense</i>	8.4%	12.1%	10.6 cm	41	2.9
<i>S. aureum</i>	5.0%	8.5%	8.7 cm	17	1.9

Because of inherent differences between tree species in their primary herbivore defenses (*i.e.*, secondary chemistry, and physical defenses) and associated communities of herbivores, *Caryocar* and *Sclerolobium* were each tested separately for effects of ant exclusion and cavity addition on herbivory.

Caryocar brasiliense

We used a hierarchical series of *t*-tests to determine whether the proportion of leaf area removed by herbivores or average leaf size differed between our cavity treatments and our ant exclusion treatments. We found no significant difference in the proportion of leaf area removed between cavity addition trees and control trees (Welch's two sample *t*-test, $t = 0.89$, $p = 0.38$, proportion leaf area removed logit transformed), nor did the average leaf size differ between cavity addition and control trees (Welch's two sample *t*-test, $t = -0.5$, $p = 0.60$). Mean percent herbivory was 11.0% for the cavity addition treatment and 9.6% for the control treatment, and mean leaf area was 101 cm² for cavity addition and 104 cm² for control trees.

To test whether the number of occupied cavities on a tree impacted herbivory, we fit a linear regression model for control branches on cavity addition trees. We found no association between the number of occupied cavities on a tree and the proportion of leaf area removed by herbivores (linear regression, $F_{(20)} = 0.55$, $SE_{resid.} = 0.61$, adjusted $R^2 = -0.02$, $p = 0.47$, data logit transformed), nor did we find a relationship between the total number of ants occupying cavities and proportion of leaf area removed (linear regression, $F_{(20)} = 0.02$, $SE_{resid.} = 0.61$, adjusted $R^2 = -0.06$, $p = 0.89$, data logit transformed).

Because sample means showed no difference for cavity treatment, we combined cavity treatment trees to test whether ant exclusion treatment impacted proportion of leaf area removed. Ant exclusion branches had significantly less damage than non-excluded branches (Welch's paired sample *t*-test, $t_{(50)} = -4.1$, $p = 0.0001$, data logit transformed). The mean pairwise difference in proportion area removed by herbivores between exclusion and control treatments was 3.7%. Overall, 12.1% of the leaf area was removed from ant exclusion branches, and 8.4% from control branches (Fig. 1a). Mean leaf area did not differ between ant exclusion and control treatments (Welch's paired sample *t*-test, $t_{(50)} = 0.71$, $p = 0.47$). Ant exclusion treatments were not equally successful on all trees, however.

Ant exclusion treatments successfully reduced the number of ants on exclusion branches, but did not exclude ants altogether. Significantly fewer ants were recovered

from pitfall traps on ant exclusion compared to control branches (Welch's paired-sample t -test, $t_{(50)} = 8.9$, $p < 0.0001$, data $\log x + 0.01$ transformed), mean ant abundance in pitfalls on control branches was 31, compared to 4 in pitfalls on exclusion branches (Fig. 2a). Ant exclusion treatments also significantly reduced the average number of ant species in pitfalls from 2.8 on control branches to 0.9 on exclusion branches (Welch's paired-sample t -test, $t_{(50)} = 8.8$, $p < 0.0001$).

To test whether the number of ants on trees influenced herbivory, we fit a linear regression model relating the number of pitfall ants to proportion of leaf area removed on branches without ant exclusions. We found no relationship (linear regression, $F_{(47)} = 0.47$, $SE_{resid.} = 0.52$, adjusted $R^2 = -0.01$, $p = 0.50$, data logit transformed). Nor were the number of ant species in pitfall traps related herbivory on control branches (linear regression, $F_{(49)} = 0.25$, $SE_{resid.} = 0.55$, adjusted $R^2 = -0.02$, $p = 0.62$, data logit transformed).

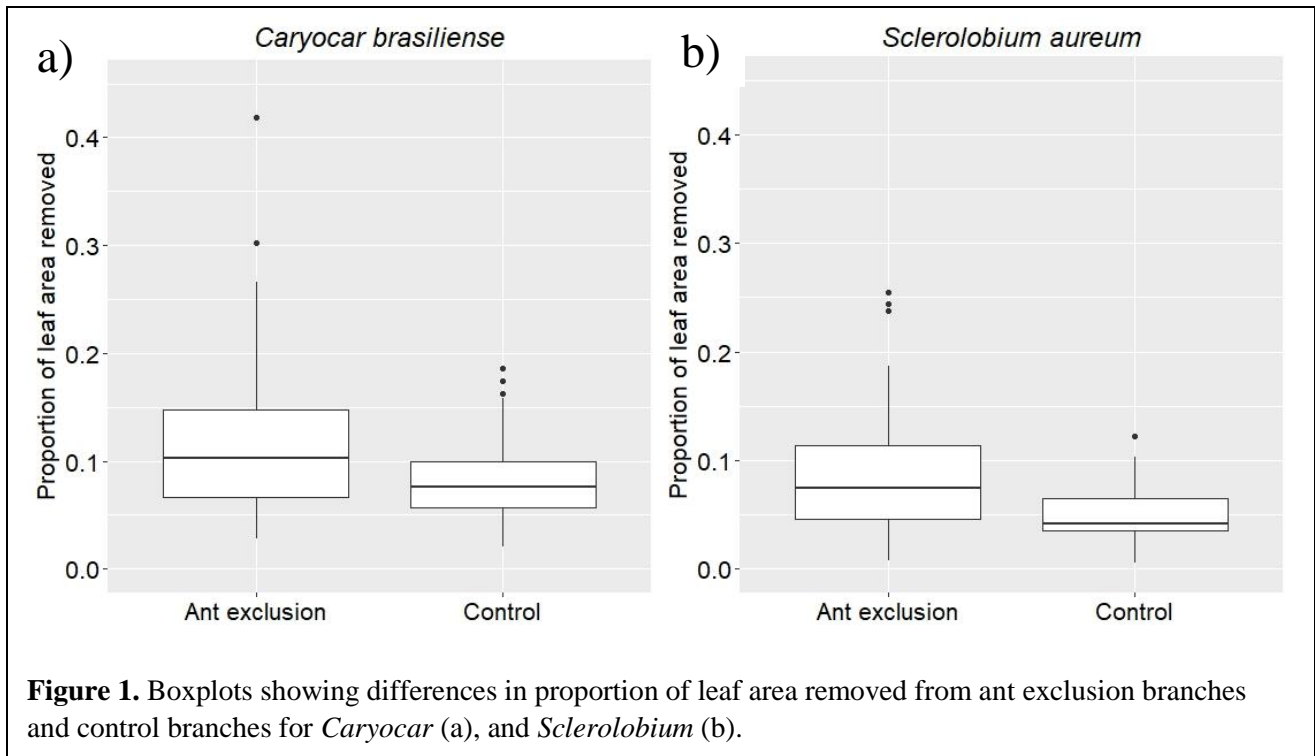
Sclerobium aureum

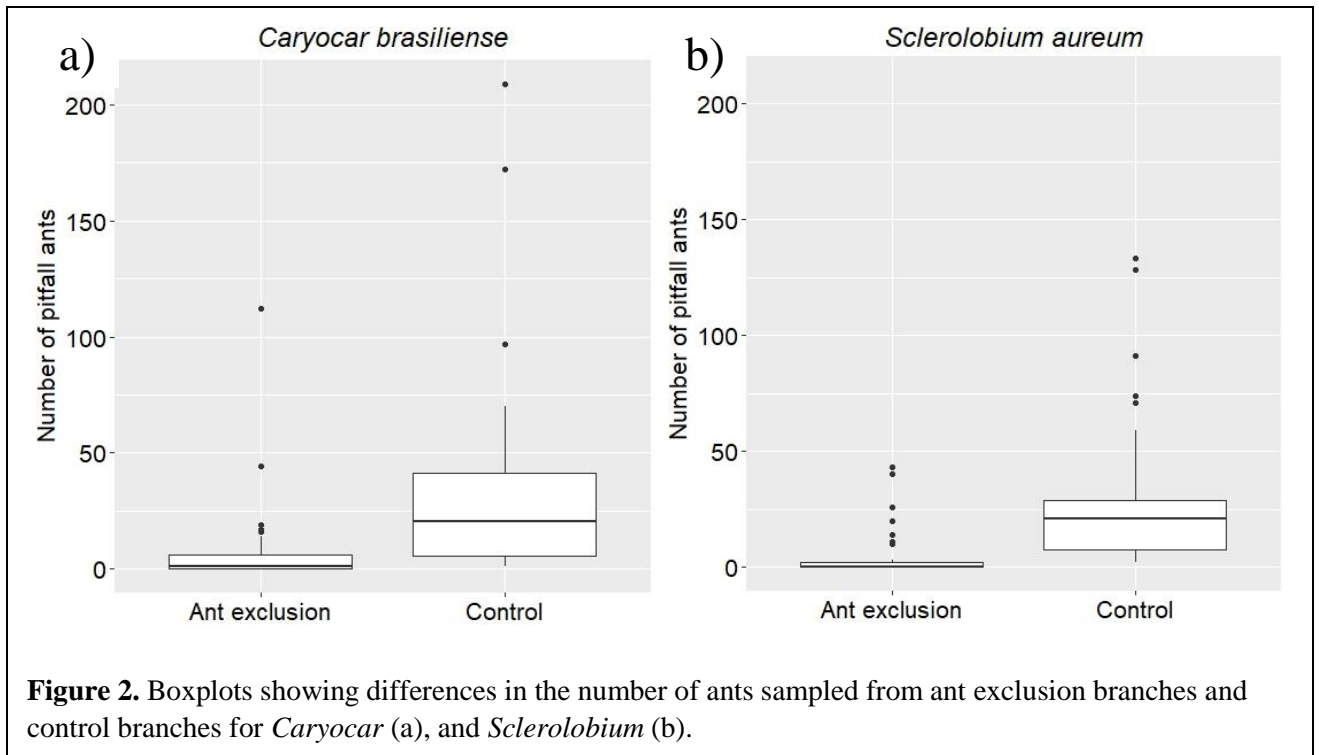
The proportion of leaf area removed did not differ between cavity addition trees and control trees (Welch's two sample t -test, $t = -0.52$, $p = 0.60$, proportion leaf area removed logit transformed): mean percent herbivory was 6.3% for the cavity addition treatment and 7.2% for the control treatment. To test whether the number of occupied cavities on a tree impacted herbivory, we fit a linear regression model for control branches on cavity addition trees. We found no association between the number of occupied cavities on the tree and the proportion of leaf area removed by herbivores (linear regression, $F_{(42)} = 0.37$, $SE_{resid.} = 0.60$, adjusted $R^2 = -0.01$, $p = 0.55$, data logit transformed), nor was there a relationship between the total number of ants found in cavities and proportion of leaf area removed (linear regression, $F_{(42)} = 0.08$, $SE_{resid.} = 0.62$, adjusted $R^2 = -0.03$, $p = 0.78$, data logit transformed).

Because sample means did not differ among cavity treatments, we again lumped cavity treatment trees to test whether ant exclusion treatment impacted proportion of leaf area removed. Ant exclusion branches had significantly less damage than non-excluded branches (Welch's paired sample t -test, $t_{(41)} = -4.2$, $p = 0.0001$, data logit transformed). The mean difference in proportion area removed by herbivores between exclusion and control treatments was 3.5% (mean proportion leaf area removed was 5.0% in control treatments and 8.5% in ant exclusion treatments) (Fig. 1b).

To test the effect of ant exclusion treatments we used paired samples t -tests to compare number of ants and number of ant species in pitfall traps between control and ant exclusion branches. Significantly fewer ants were caught in pitfall traps on ant exclusion branches compared to control branches (Welch's paired-sample t -test, $t_{(41)} = 9.8$, $p < 0.0001$, data $\log x + 1$ transformed); mean ant abundance in pitfalls on control branches was 34, compared to 5 in pitfalls on exclusion branches (Fig. 2b). Ant exclusion treatments also significantly reduced the average number of ant species in pitfalls from 2.5 on control branches to 0.7 on exclusion branches (Welch's paired-sample t -test, $t_{(41)} = 8.8$, $p < 0.0001$).

To test whether the number of ants on trees influenced herbivory, we fit a linear regression model using the number of pitfall ants and proportion of leaf area removed on branches without ant exclusions. We found no relationship between the number of pitfall ants and the proportion of leaf area removed (linear regression, $F_{(37)} = 0.07$, $SE_{resid.} = 0.53$, adjusted $R^2 = -0.03$, $p = 0.80$, data logit transformed). The number of ant species in pitfall traps and herbivory on control branches also were unrelated (linear regression, $F_{(40)} = 0.06$, $SE_{resid.} = 0.61$, adjusted $R^2 = -0.02$, $p = 0.81$, data logit transformed).





Discussion

Herbivory between ant exclusion branches and control branches differed significantly in both *Caryocar* and *Sclerolobium*, demonstrating that the presence of ants does impact herbivory on each of these tree species, and exclusion of ants from branches significantly increases the proportion of leaf area removed by herbivores. Observed levels of herbivory on *Caryocar* as well as the difference in herbivory between ant exclusion and controls were much higher in the present study than those recorded in a previous study in this site (12.1% and 8.4% for ant exclusion and control branches, respectively, in the current study versus approximately 3.4% and 2.8% in Koch et al., 2016). These differences likely reflect the durations of treatment (90 days versus approximately 1 year in the present study).

Given that herbivory levels were significantly higher on ant exclusion branches than on control branches for both tree species, we expected the number of ants and/or ant species in pitfalls (as a proxy for ant activity on the tree) and the amount of herbivory to be related. The lack of a relationship between number of individuals or species of ants visiting a tree and the level of herbivory contradicts experimental results showing that ant exclusion reduced herbivory. One possible explanation for these seemingly contradictory results is that a single sampling of the ant community using pitfall traps may not have been a good measure for ant activity on the tree. Ants were sampled over only a single time interval whereas herbivore activity lasted the entire growing period. Because arboreal ant colonies inhabit stationary cavities year-round and forage year-round, we

expected that ants sampled by pitfall trapping at the end of the experiment would reflect ant activity on a given tree. Repeated pitfall sampling (which kills the ants it captures) potentially alters the abundance of foragers and therefore impacts colony survival. While observing ant abundances at baits is a non-destructive alternative, this technique is challenging because strong diel shifts in ant foraging by different species requires constant monitoring through the 24-hour period, and therefore significant human resources particularly for repeated sampling of individual trees. Beating techniques for sampling ants also suffer as a result of diel shifts in ant species activity because only ants active during the time of sampling will be recorded.

Although pitfall sampling in this study might not have been sufficiently reflective of ant visitation to demonstrate a relationship with herbivory on our experimental trees, ants occupying artificial cavities represent permanent residents on that tree. Despite this, neither the number of occupied cavities nor the number of ants in those cavities impacted herbivory on the home tree. This strongly suggests that the particular ants that colonized artificial cavities in the experiment have little impact on herbivory in general. The majority of our artificial cavities were occupied by *Camponotus bonariensis*, a nocturnal species which is not known for aggressiveness, and is not classified among the dominant species in other studies (Camarota *et al.*, 2016; Koch *et al.* 2016).

Koch *et al.* (2016) showed that tree size in *Caryocar brasiliense* is a contributing factor in the degree of herbivory reduction as a result of ant exclusion. Smaller trees had significantly less reduction in herbivory as a result of ant exclusion than did larger trees. The trees used in this study fell primarily between the small and medium-size trees used in Koch *et al.* (2016) suggesting that tree size in this study may have influenced our ability to detect significant impacts of ant abundance and diversity on herbivory, or an effect of artificial cavity addition. Koch *et al.* (2016) proposed two potential causes of the lower effect of ants on herbivory in smaller trees. First, larger trees may invest less in primary defenses such as chemical defenses than smaller trees, and second, larger trees may contain more nesting resources for ants and therefore have more ants to defend leaves. They did not, however, find significant differences in the levels of tannins between large and small trees and suggest that differences in availability of cavity resources and the presence of certain aggressive ants in larger trees may account for this pattern.

In this study we found no impact of additional cavity resources on herbivory for these smaller trees, which initially suggests that the presence of dominant ant species, not the abundance of cavity resources, is responsible for the difference in the impact of ants on herbivory between large and small trees. Ant identity, however, is critically linked to what cavities are available for use as nest sites. Dominant and aggressive ant species in this study site require large cavities that only occur in large diameter stems (and therefore only in larger trees) (Priest *et al.* 2018). The artificial cavities used in the present study had a volume of approximately 7cm³, whereas dominant ant species in this community such as *Camponotus sericeiventris* and *Neoponera villosa* exclusively use cavities many times this volume (Priest, *et al.*, 2018; Priest, *unpublished data*). As a result, none of the dominant ant species that are predicted to have large impacts on herbivores colonized our

artificial cavities, which likely explains why we found no effect of cavity addition on herbivory, despite successful colonization and occupation of these cavities by ants.

In November, 2014 (approximately 6 months before the beginning of the experiment), a fire burned approximately 80% of the reserve, and affected all of the areas where the present study was conducted. Although the precise effects of the recent fire on this study are unknown, previous work has shown that fire likely impacted herbivory and ant abundance and diversity in this experiment. Lopes & Vasconcelos (2011) demonstrated that herbivory on *Caryocar* and other tree species in this study site was increased over twofold on plants that had been burned in the previous year over those that had not, and that this was largely due to an increase in damage by chewing insects. This study also showed that burned trees extended the period during which they produced new leaves, potentially influencing the impact of herbivores through phenological shifts. In another study, fire increased herbivory overall, but also reduced the impact of ants on herbivory in the Cerrado plant *Peixota tomentosa* (Malpighiaceae) (Del-Claro & Marquis, 2015). Thus, the interaction between plants, ants, and herbivores in the Cerrado is strongly impacted by the effects of recent fire, and therefore likely influenced the outcome of this study through changes in tree phenology, herbivore pressure, and ant interactions.

Overall we observed lower ant diversity both in pitfall sampling and in occupants of placed artificial cavities than previous studies in the same study site (Koch *et al.*, 2016; Powell *et al.*, 2011). Cerrado vegetation is adapted to frequent fire events and recovers rapidly. The area where the study was conducted, however, had experienced a long period of fire suppression prior to the burn that preceded the study (the most recent previous burn in our study site was in 2006) (Koch *et al.*, 2016), which likely amplified the effects of the fire. Previous work on experimental burn plots in cerrado showed a rapid recovery of ant communities after a burn event (Maravalhas & Vasconcelos, 2014). This study, however, was conducted in plots adjacent to unburned plots which may have contained source populations for rapid re-colonization of arboreal ants in burned areas. Furthermore, the higher frequency of burns in these plots may have reduced severity of burns and therefore lessened their impact on the ant fauna. In contrast, approximately 80% of our 400 ha study site burned in addition to large areas outside the reserve. This could have slowed recovery of the arboreal ant community because of a lack of nearby source populations for re-colonization, particularly because arboreal ants in this system appear to be dispersal-limited (Powell *et al.*, 2011). Potential impacts of this burn include dramatic shifts in the abundance and diversity of arboreal ants present during our study. For example, *Camponotus bonariensis* was the most frequent occupant of artificial cavities in this study but was much less frequent in studies from the same site prior to the fire. Similarly *Cephalotes pusillus* was extremely abundant and a common occupant of artificial cavities in this site prior to the fire, but was relatively rare afterward (Powell *et al.*, 2011). Fire may also be partially responsible for increased herbivory in our study compared to herbivory levels on *Caryocar* prior to the fire (Koch, *et al.*, 2016), however this is confounded by differences in the duration of the two experiments.

Future studies investigating the impact of ants, and specifically nest-cavity resources on herbivory in Cerrado vegetation will benefit from understanding which ant species impact herbivory on host trees and to what extent. While ant communities are fairly well characterized, herbivore communities and how they are influenced by the presence of certain ant species remain poorly characterized. Predictions about the impact of cavity-nesting ants on herbivory in trees will require more information on species-specific interactions occurring in the plant-ant-herbivore network. Coupled with information on the cavity resources required by various ant species, this information can be used to design experiments that successfully test the impact of dominant ant species on herbivory in cerrado trees. This study demonstrates that non-dominant ant species nesting in our artificial cavities did not significantly impact herbivory, but current information suggests that there should be a strong impact on herbivory of cavity resources used by dominant ant species (namely large cavities). Our results also suggest that these resources (and their occupants) may be responsible for differences in plant-ant-herbivore interactions among trees of different sizes and especially among different tree species.

This study has demonstrated that ant exclusion results in increased leaf removal by herbivores when compared to control branches with no ant exclusion for two Cerrado tree species. Despite the general effect of ants on herbivory found in this study, there was no relationship between the number or diversity of ants from pitfall sampling and the amount of leaf area removed. We also demonstrated that the addition of cavity nest resources to trees did not significantly impact the abundance of ants sampled in pitfall traps, nor did they impact the levels of herbivory when compared to trees where no cavities were added. Previous work has shown that the size of the trees used in this study may not support dominant and aggressive ant species that have a larger effect on herbivores. Furthermore, artificial cavities added to trees in our experimental were not colonized by these dominant species. This suggests that the absence of dominant species from small trees is not the result of fewer cavities in general, but may be due to the absence of large cavities, or to other factors such as the amount of foraging area and food resources available on an individual tree. It is widely acknowledged that the specific identity of ants involved in plant-ant-herbivore interactions is important for the resulting outcome in terms of herbivory (e.g. Rizali, *et al.*, 2018). Together, the results of this study suggest that herbivory on Cerrado trees may be more strongly influenced by the size of the cavities they contain, rather than the quantity, because large cavities are required for most of the dominant and aggressive ant species which have higher impacts on herbivory.

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Supplementary Material

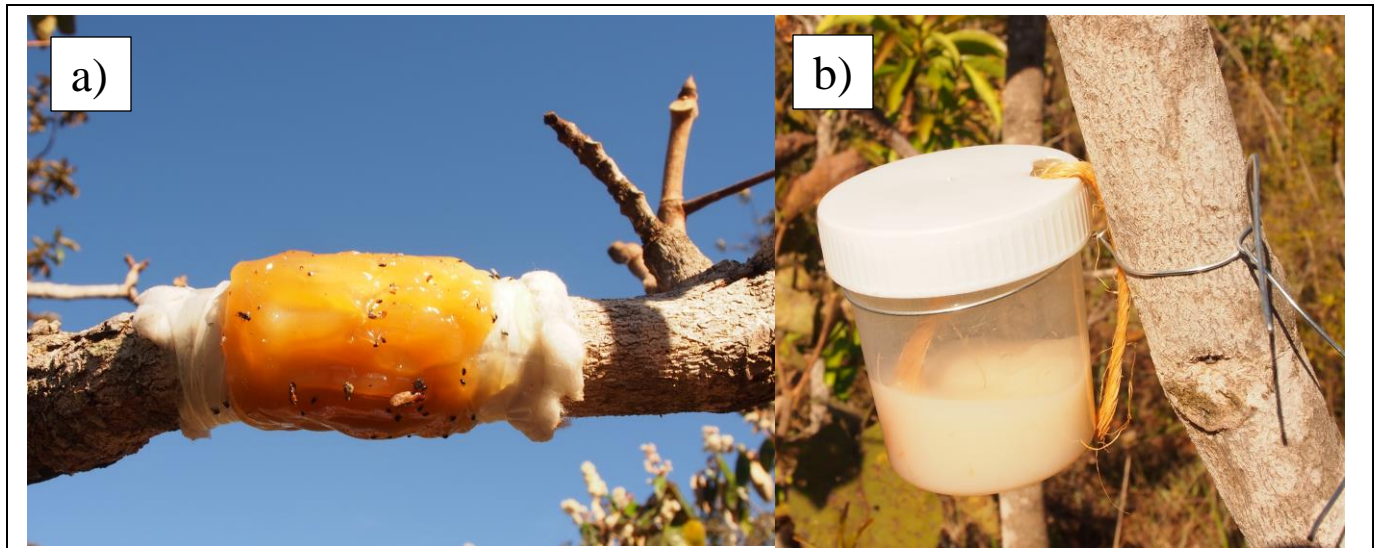


Figure S1. Photos showing a) ant exclusion using cotton, packing tape, and Tanglefoot and b) poison bait trap used for killing ants on ant exclusion branches.