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Research

Plant-Insect Interactions

Foraging Ecology of the Leaf-Cutter Ant, *Acromyrmex subterraneus* (Hymenoptera: Formicidae), in a Neotropical Cerrado Savanna

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Abstract

Fungus-farming ants cultivate a fungal symbiont inside the nest that serves as a food source. Leaf-cutter ants are distinctive among fungus-farmers because they forage for fresh plant material to nurture the fungus. Here we investigate the foraging ecology of *Acromyrmex subterraneus* (Forel) in the Brazilian cerrado savanna. We examined the species activity pattern, forage material collected, and the relationship between load mass and forager size. Ant activity peaked at night and was negatively related to temperature but positively related to relative air humidity. The majority of the items collected by ants was plant material: dry and fresh leaves, flowers, and fruits. Trunk trails ranged from 0.7 to 13 m and colony home ranged from 2 to 28 m^2, indicating that ants collect material nearby the nest. Total load mass was positively associated with forager size, especially in the case of leaves. The negative relationship between ant size and burden suggests that ants might optimize their delivery rate by collecting lighter substrates more frequently. Given their pest status, most studies on leaf-cutters are undertaken in human-altered environments. Information on *A. subterraneus* in native cerrado is imperative given the threatened status of this vegetation. Leaf-cutters thrive in disturbed cerrado and severe seedling herbivory may hinder vegetation recovery. Our fieldwork may provide insights for management techniques of *Acromyrmex* colonies in agroecosystems, as well as for restoration programs of degraded cerrado areas.

Key words: activity rhythm, foraging trail, fungus-farming ant, load carriage

Ants are dominant social insects that inhabit nearly all terrestrial biomes and participate in multiple types of interactions with organisms of diverse taxa (Hölldobler and Wilson 1990, Rico-Gray and Oliveira 2007). Due to their predominance and diverse ecological relationships, ants can alter the availability of resources for other organisms through biotic and abiotic changes of the environment, acting as important ecosystem engineers, with great impacts on soil properties and biological communities (Blüthgen and Feldhaar 2010, Philpott et al. 2010).

Fungus-farming ants (Formicidae: Myrmicinae: Attini: Attina) comprise nearly 250 species exclusive to the New World and provide a classic example of mutualism. The ants have an obligate symbiosis with cultivated fungi on which they feed and in return the ants provide the fungi with nourishment, dispersal to new locations, as well as a parasite and competition-free environment (De Fine Licht and Boomsma 2010, Mehdiabadi and Schultz 2010). Within fungus-farming ants, most genera do not cut leaves, with the exception of *Atta* and *Acromyrmex* that are known as leaf-cutting ants (Hölldobler and Wilson 2011). The genera *Trachymyrmex* and *Sericomyrmex* are considered transitional between the leaf-cutter and the nonleaf-cutter fungus-farming ants (e.g., *Mycocepurus*, *Mycetarotes*; Ješovnik et al. 2018) and their culturing substrates include fresh fallen plant material in addition to arthropod frass and carcasses (Leal and Oliveira 2000, Seal and Tschinkel 2008, De Fine Licht and Boomsma 2010, Ronque et al. 2019).

The leaf-cutter ant genera, *Atta* and *Acromyrmex*, have the largest and most complex societies within the fungus-farming ants, with thousands to millions of workers and marked polymorphism (Hölldobler and Wilson 2011). Due to their populous colonies and massive trunk trails, leaf-cutter ants can collect over 130 kg of fresh plant material per hectare per year (Wirth et al. 2003, Herz et al. 2007), making them major tropical pests of important crops such as citrus and cacao (Lewis 1975, Della Lucia et al. 2014). Leaf-cutter ants, however, can also act as important ecosystem engineers (Farji-Brener et al. 2017) and seed dispersers in tropical environments (Christianini and Oliveira 2009).

Although *Acromyrmex* has nearly twice the number of species compared with *Atta* (Bolton 2019), most studies on natural history and foraging of leaf-cutting ants have been performed with *Atta* species (Hölldobler and Wilson 2011), with some recent exceptions (Nickele et al. 2015, 2016; Caldato et al. 2016a; Verza et al. 2017; De Almeida et al. 2018; Jofre et al. 2018; Forti et al. 2019; Moreira et al. 2019). Ecological and natural history data about *Acromyrmex* species are needed to fully understand the ecological success of leaf-cutter ants and their role in Neotropical habitats. Studies on the behavior of *Acromyrmex* ants could also provide valuable data for the development of new sustainable methods of control in agroecosystems, as opposed to pesticides (Della Lucia et al. 2014). Here, we provide a natural history and ecological account of *Acromyrmex subterraneus* (Forel) in a reserve of cerrado savanna in Southeast Brazil. Specifically, we present qualitative and quantitative field data on daily activity pattern, types of substrate collected for fungiculture, relationship between load mass and forager size, foraging trails, and colony home range.

Materials and Methods

Study Site

Fieldwork was undertaken in a 470-ha reserve of cerrado savanna near Mogi-Guaçu, São Paulo state, Southeast Brazil (22°18′S, 47°11′W). Observations were carried out in the forest-like 'cerradão', whose physiognomy is formed of 50–90% of trees up to 10–12 m in height (Oliveira-Filho and Ratter 2002). The climate of the region consists of a dry/cold season (winter) from April to September and a rainy/hot season (summer) from October to March. The annual temperature ranges from 20.5 to 22.5°C and total rainfall varies from 250 to 300 mm in the winter to 1,100–1,200 mm in the summer (data provided by the climatological station at the cerrado reserve). Field observations were carried from 18 February to 31 March 2017, during the rainy/hot season, when ant activity is more pronounced in the cerrado (Leal and Oliveira 2000, Christianini et al. 2012, Ronque et al. 2018).

Activity Pattern

Ant colonies were located in the field by actively searching the characteristic nest mounds and foraging trails of this species. We determined the activity pattern of *A. subterraneus* (four colonies) through simultaneous censuses carried out over 24 h per colony during the rainy season. We recorded the number of workers exiting and entering the nest at intervals of 2 h in sessions of 20 min. Nests had only one entrance and were at least 20 m apart from one another. Air temperature and relative humidity were also noted before each sampling session. We used a generalized linear model (GLM) with Poisson distribution for ant activity pattern in relation to air temperature and air relative humidity. A pseudo-R^2 was calculated using the deviances of the final model as compared with the null model. This analysis was performed in R version 3.5 (R Core Team 2018).

Samplings of Substrate Collected by Ants

We sampled forage material from the ants and delimited foraging trails for six active colonies of *A. subterraneus* (three of which previously sampled for the activity pattern). For each colony, we sampled foragers and their respective loads in 1-h sessions, as follows: 5 min collecting foragers and substrates, followed by 10 min of trail delimitation. The sampling process was repeated until the session terminated. Samplings were intermittent from February to March 2017 and colonies were each monitored during 1-h sessions per night (totaling 10 nights). Accumulated duration of samplings for each focal colony ranged from 1 to 7 h (depending on the level of colony activity) totaling 27 h for the six monitored colonies altogether.

Collection of substrates was performed at foraging trails, 0.50–2 m from the nest entrance. In each sampling, the ant forager, the load item, and the hitchhiker(s) (when present; Fig. 1a) were collected and preserved in 70% alcohol. Hitchhikers refer to minor workers that ride on substrate carried by large nestmates (Feener and Moss 1990). We classified the substrates following previously defined categories (Rockwood 1975; Leal and Oliveira 1998, 2000). Our categories were fresh and dry leaves, flowers, fruits, mushrooms, and sap (Fig. 1a–c). Ant voucher specimens are deposited at the 'Museu de Zoologia da Universidade Estadual de Campinas', São Paulo (ZUEC, Campinas, Brazil; registration number 6261).

Delimitation of Foraging Trails and Colony Home Ranges

Foraging trails were delimited by following workers and marking their paths with flags (Fig. 1d), up to the most distant point they reached before returning to the nest. Each flag received a numbering code per colony and per trail branch and had its position mapped by using a measuring tape that provided flag-to-flag distance. A compass determined the cardinal direction of the flags in relation to each other. Each flag was then registered on a squared paper using a 1:10 scale (10 cm corresponding to 1 m), on which the direction and distance of each flag to the nest entrance was determined. The home range of each colony was estimated using R version 3.5 (R Core Team 2018, package 'adehabitatHR').

Worker Size and Load Carriage

Laden workers monitored in the field were preserved in 70% alcohol and brought to the laboratory to examine the relationship between worker size, load, and hitchhikers. Worker size was assessed by measuring the eye-to-eye head width to the nearest 0.01 mm (from the outer surface of an eye to the other), as previously used for other leaf-cutters (Wilson 1980a,b). Placed in frontal view through a Leica magnifier (model M205 C, Leica Microsystems, Germany), the worker was measured using the Leica Application Suite software (version 4.0).

Ants, together with their respective loads and hitchhikers, were oven-dried at 60°C for 48 h. Individual organisms and substrates were weighed separately to the closest 0.01 mg using an Ohaus Corporation analytical balance (model DV215CD, Class I, with a 0.01-mg detection sensitivity; Ohaus, Parsippany, NJ). Because laden workers sometimes had more than one associated hitchhiker, we considered the total weight of hitchhikers in the sample. The load weight relative to the ant weight, known as *burden*, was calculated using the formula $B = \frac{Lm}{Am}$, where B is the burden of the ant forager, Lm is the total load dry mass (substrate + hitchhikers), and Am is the ant dry mass (Wetterer et al. 2001). Dry weights were used because it was not possible to obtain substrate mass in the field (fresh weight).

Given that hitchhikers were frequently seen associated with laden workers (thus accounting for part of the load), we also investigated the relationship between forager head width and the number of hitchhikers in the samples. To do so, we performed a GLM with Poisson distribution and calculated a pseudo-R^2 as previously explained.

To investigate whether ant foragers carry substrates according to their body size, we performed linear mixed-effects models (LME; 'nlme' package, 'lme' function) to examine the relationship between the log-transformed values of forager head width and the total load

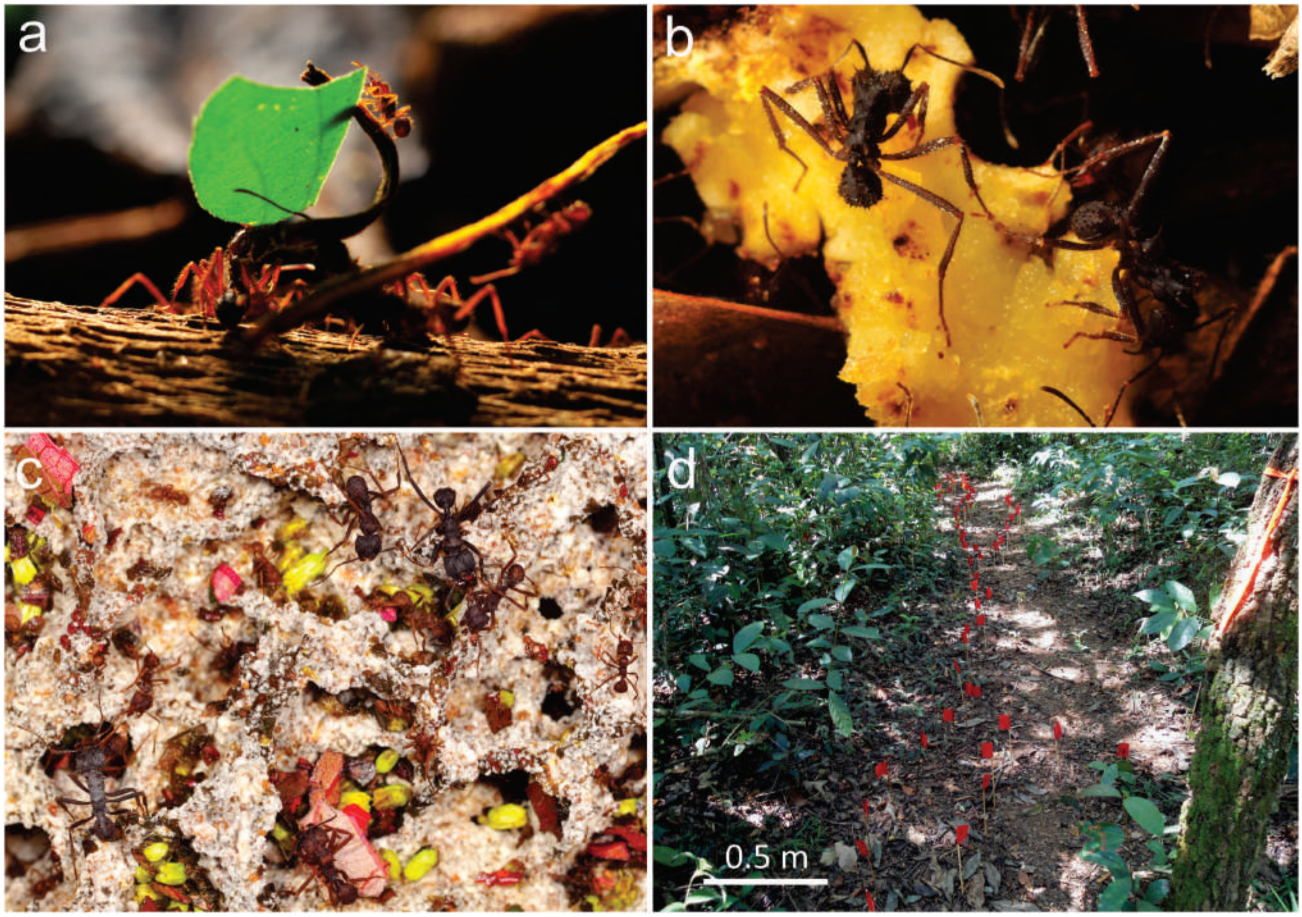

Fig. 1. Collection of substrates by *Acromyrmex subterraneus* in a cerrado reserve in Mogi-Guaçu, Southeast Brazil. (a) Worker carrying a leaf fragment with riding hitchhiker; (b) removal of pulp material from fallen fruit. (c) A view of the fungus garden showing workers using leaves of *Acalypha* (copperleaf) and flowers of *Solidago* (goldenrod) as culturing substrate. (d) Flags delimiting the trail system of a colony (nest N4 in Table 1).

dry mass. We included forager head width as the main explanatory variable, and colony as the random factor. To investigate whether burden varies in accordance with ant body size, we examined the relationship between the log-transformed values of forager head width and burden, including head width as the main explanatory variable and colony as the random factor. All analyses were performed in R version 3.5 (R Core Team 2018).

Results

Activity Pattern

Ant activity was predominantly nocturnal during the rainy/hot season, peaking between 08:00 p.m. and 02:00 a.m. (Fig. 2). Daily activity was positively affected by air relative humidity (z = 32.94, df = 47, P < 0.001, pseudo-R^2 = 0.18) and negatively affected by temperature (z = –20.59, df = 47, P < 0.001, pseudo-R^2 = 0.06).

Collected Material, Foraging Trails, and Colony Home Ranges

Nearly all items collected by *A. subterraneus* were plant material. The most collected items were fresh or dry leaves (71%), followed by flowers (18%) and fruits (10%; Figs. 1 and 3). Collection of Basidiomycete mushrooms and plant sap were each observed twice.

Most foraged material (including fresh leaves) was collected near the nests, including the cases in which foragers climbed onto nearby trees directly up the trunk or along attached vines. Except for fresh leaves, flowers, and plant sap, all other items were collected on the ground. Collection of plant sap was recorded when returning ants entered the nest carrying a droplet within their mandibles.

Trail length ranged from 0.7 to 13 m, and estimated colony home ranges varied from 1.8 to 27.6 m^2 (Table 1), indicating that most items are collected near the nests.

Worker Size and Load Carriage

Forager head widths ranged from 0.98 to 2.47 mm (mean ± SE = 1.87 ± 0.01 mm). Forager dry mass ranged from 0.33 to 4.51 mg (mean ± SE = 2.4 ± 0.04 mg). The pooled values of substrate and hitchhiker mass ranged from 0.03 to 22.78 mg (mean ± SE = 3.7 ± 0.13 mg). Hitchhikers (Fig. 1a) were present in 50% of all samples and represented 1–89% (mean ± SE = 28 ± 1%) of the total load carried by the foragers. The GLM indicated that the presence of hitchhikers was positively related to forager size (z = 3.6, P < 0.001, pseudo-R^2 = 0.01).

Total load was positively related to forager head width (t = 6.82, df = 590, P < 0.001; Fig. 4a), and bigger ants carried heavier substrates irrespective of the type of substrate (leaf: t = 4.76, df = 435, P < 0.001; nonleaf: t = 5.20, df = 147, P < 0.001; Fig. 4b). Inversely, burden was negatively related to forager head width (t = –2.50, df = 590, P = 0.013; Fig. 5a), indicating that bigger ants carried loads that were less heavy in relation to their own weight. This tendency,

Table 1. Estimated home ranges of *Acromyrmex subterraneus* in a cerrado reserve at Mogi-Guaçu, Southeast Brazil, during the rainy season (from Feb. to Mar. 2017)

Nest code	Number of trails	Range of trail length (m)	Estimated home range (m^2)
N1	3	0.7–4.4	4.5
N2	5	1.9–5.9	10.0
N3	1	6.2	4.2
N4	5	1.9–13.0	13.8
N5	9	3.0–10.8	27.6
N6	2	3.2–4.4	1.8

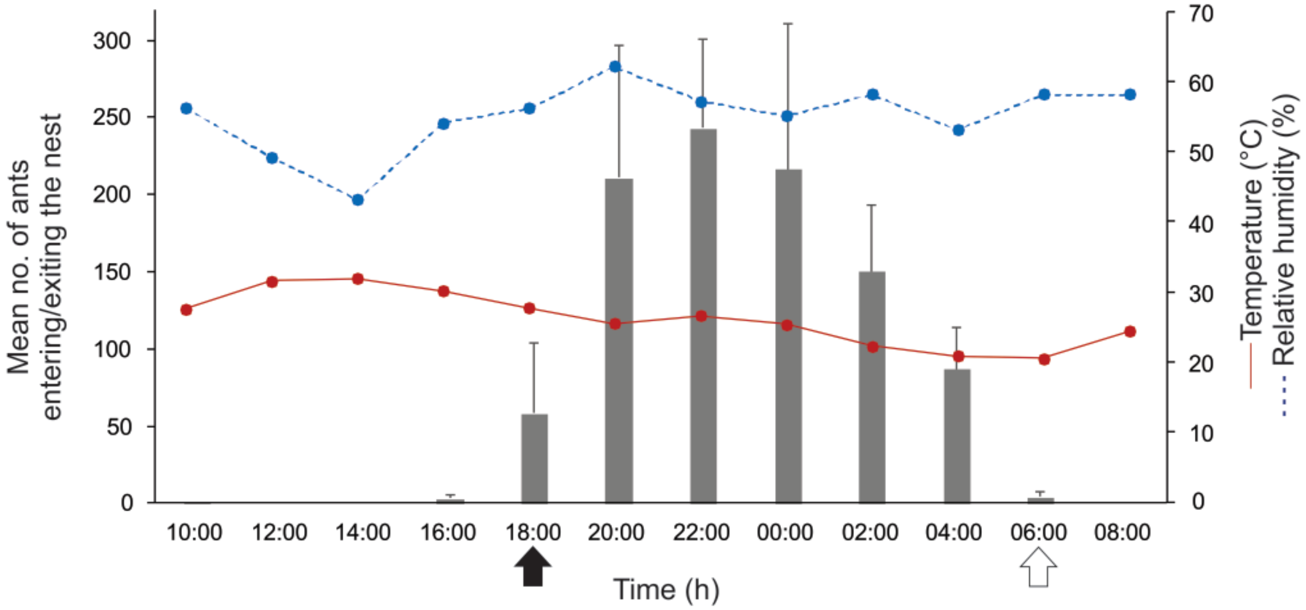

Fig. 2. Daily variation in the foraging activity of *Acromyrmex subterraneus* in a cerrado reserve in Mogi-Guaçu, Southeast Brazil. Foraging activity is expressed as the sum of inbound and outbound workers (mean ± SE, $n = 4$ nests). Air temperature and relative humidity were recorded simultaneously during each sampling of ant activity. The blue dashed line represents the relative humidity (%) and the red solid line represents the temperature (°C). The arrows indicate sunrise (white) and sunset (black). Data collected from February to March 2017.

however, depends on the type of substrate collected: it is significant for leaves but nonsignificant for nonleaf items (leaf: $t = -5.19$, df = 435, $P < 0.001$; nonleaf: $t = 0.49$, df = 147, $P = 0.624$; Fig. 5b).

Discussion

Overall, our study shows that *A. subterraneus* displays nocturnal activity during the rainy/hot season in the cerrado, with foragers collecting a wide variety of items. Trunk trails are short, indicating that ants collect material nearby the nest. Large foragers tend to carry heavier loads (leaf or nonleaf items) compared with smaller ants and sustain lower burdens when carrying leaves.

Diel activity of several ant species is known to be a consequence of their physiology and is affected by changes in abiotic factors, most notably temperature (Heinrich 1993). In tropical environments, ants tend to adopt nocturnal habits more frequently due to high temperatures and low humidity during the day (Orr and Charles 2007, Raimundo et al. 2009, Camargo and Oliveira 2012). The activity rhythm of *A. subterraneus* in our cerrado site matches the pattern of several other ant species, including leaf-cutters, which adjust their daily movements in accordance with optimal temperature and humidity levels (Hölldobler and Wilson 1990, Wirth et al. 2003, Caldato et al. 2016b; Ronque et al. 2018). Although leaf-cutting ants can exhibit both nocturnal and diurnal activity patterns (Wetterer 1990a, Nickele at al. 2016), nighttime foraging is frequently associated with avoidance of high temperatures. In such cases, foragers may exhibit nocturnal habits during the summer and shift to diurnal foraging during colder months (Fowler and Robinson 1979, Giesel et al. 2013, Nickele et al. 2016). Indeed, Maciel et al. (1995) and Nickele et al. (2016) recorded that nocturnal activity by *A. subterraneus* in forested areas was more intense during the summer compared to colder months. Air humidity is also known to influence activity rhythms of ant colonies (Levings 1983), which may intensify foraging at high relative humidity and temperature (Hölldobler and Möglich 1980). We showed that nocturnal foraging by *A. subterraneus* in cerrado was significantly influenced by humidity during summer, as also recorded for this ant species in forest plantations in South Brazil (Nickele et al. 2016). In addition, nocturnal foraging by leaf-cutter ants has been suggested as a strategy to avoid diurnal phorid parasitism (Feener and Moss 1990).

Acromyrmex species collect a wide range of culturing substrates, including seeds and fruits, insect frass, and arthropod carcasses (Oliveira et al. 1995, Wetterer et al. 1998, Leal and Oliveira 2000, Mehdiabadi and Schultz 2010). We showed that *A. subterraneus* collects mostly leaves (fresh and dry), including less frequently other types of plant-derived items such as flowers, fruits, and sap. The only nonplant matter collected by the ants was basidiomycete mushrooms, also recorded for *A. lundii* (Guérin-Méneville)

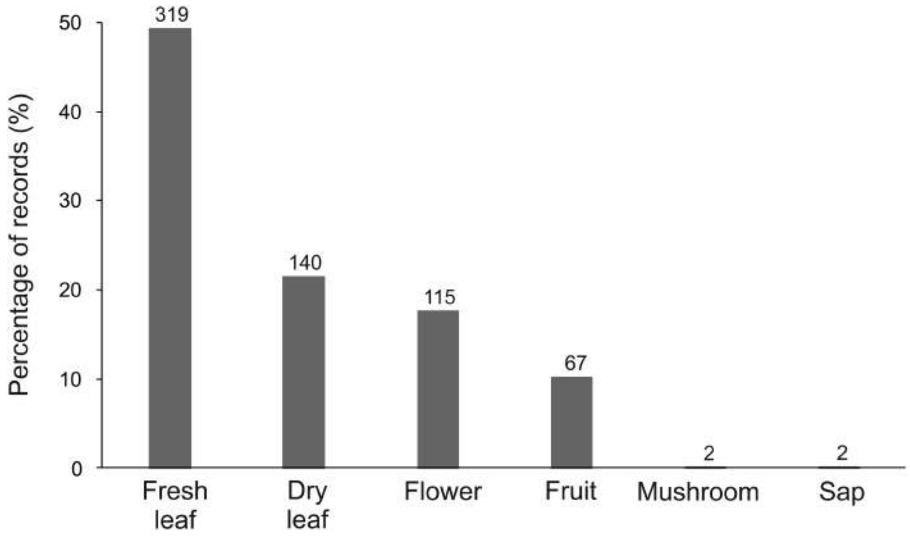

Fig. 3. Material collected by *Acromyrmex subterraneus* in the cerrado reserve of Mogi-Guaçu, Southeast Brazil. Numbers above the bars refer to number of records. Data are based on 27 h of observation (intermittent sessions) during foraging activity of six colonies. Data collected from February to March 2017.

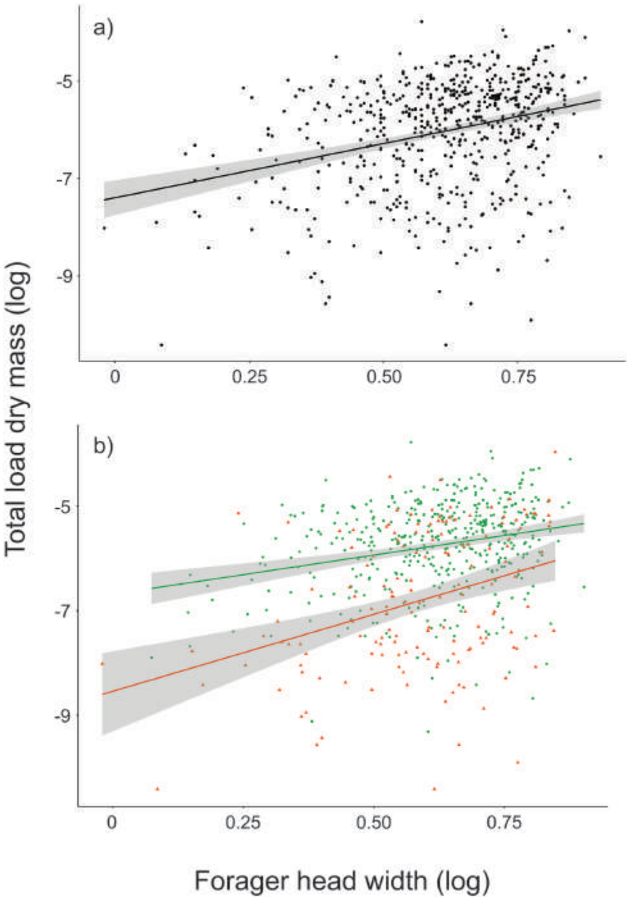

Fig. 4. Relationship between the log-transformed values of total load mass (g) as a function of forager head width (mm) in *Acromyrmex subterraneus*. Total load mass was (a) positively related to forager head width ($P < 0.001$), (b) regardless of the type of collected substrate (leaf: $P < 0.001$; nonleaf: $P < 0.001$). (b) Green dots represent leaves and orange triangles represent nonleaf substrates.

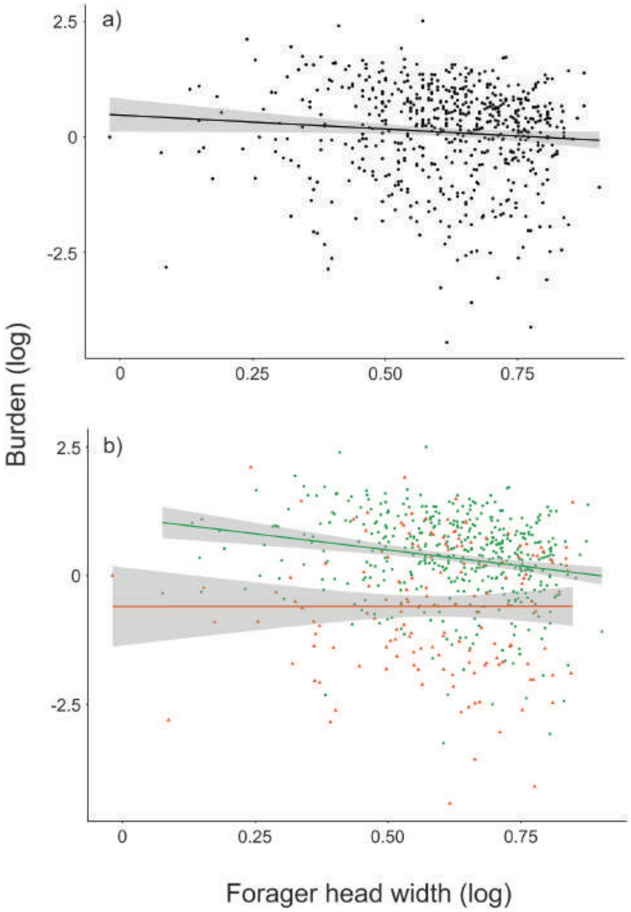

Fig. 5. Relationship between the log-transformed values of burden [load mass (g)/ant mass (g)] as a function of forager head width (mm) in *Acromyrmex subterraneus*. Burden was (a) negatively related to forager head width ($P = 0.013$), but (b) this relationship is dependent on the collected substrate (leaf: $P < 0.001$; nonleaf: $P = 0.624$). (b) Green dots represent leaves and orange triangles represent nonleaf substrates.

and *A. lobicornis* (Emery) in Argentina (Lechner and Josens 2012, Masiulionis et al. 2013). As seen in *A. subterraneus*, collection of liquid substrates has been recorded in nonleaf-cutter species in the *Cyphomyrmex* group (Murakami and Higashi 1997, Mehdiabadi and Schultz 2010). Foragers of some leaf-cutting species can feed directly on sap from leaves to acquire carbohydrates (Littledyke and Cherrett 1976, Bass and Cherrett 1995). Further investigation on the collection of liquids by leaf-cutting ants could elucidate whether this resource is solely for feeding or is also used as a culturing substrate.

Leaves represent nearly 70% of the total litterfall in the cerrado (Valenti et al. 2008), which matches the leaf fraction in our samples (≈71%) used by *A. subterraneus* for fungiculture. In our study area, annual plants abscise leaves from May to August (Mantovani and Martins 1988). Because nitrogen resorption is not efficient in the cerrado, the leaflitter presents high N concentration (Kozovits et al. 2007). Preference for N-rich culturing substrate by leaf-cutting ants has already been shown by Berish (1986) for *Atta cephalotes* (L.) in the Costa Rican wet forest and by Mundim et al. (2009) for *At. laevigata* (Smith) in the cerrado.

In cerrado areas of Southeast Brazil, including our study site, most plants produce flowers and fruits during the rainy season (Mantovani and Martins 1988). This may partially explain the high frequency of flowers and fruits collected by *A. subterraneus*, especially if these items are available in high quantities nearby (Fowler and Robinson 1979, Vasconcelos 1990, Leal and Oliveira 1998). Flowers are known to present lesser toughness and weight compared with leaves and tend to lack defensive secondary compounds (Nichols-Orians and Schultz 1989, Saverschek et al. 2010). These traits may allow foragers to cut and transport flowers more easily and faster compared with other substrates (Saverschek et al. 2010, Röschard and Roces 2011). Previous studies have shown a decrease in the collection of flowers by fungus-farming ants in cold/dry months in the cerrado (Leal and Oliveira 1998, 2000). Although we have not sampled during the dry season, we believe that *A. subterraneus* would collect more dry leaves (and less flowers and fruits) in this period compared with the rainy season.

Most *A. subterraneus* foragers collected items nearby their nests, using trails ranging from ≈2 to 13 m. *Atta* species can have trunk trails reaching several hundred meters (Wirth et al. 2003, Hölldobler and Wilson 2011, and references therein), whereas other fungus-farming ants, especially the nonleaf-cutters, usually forage within a small area (Leal and Oliveira 2000, Mehdiabadi and Schultz 2010, Ronque et al. 2019). Some nonleaf-cutter genera such as *Trachymyrmex* and *Sericomyrmex* tend to forage on fallen plant matter nearby their nests (Seal and Tschinkel 2008, Mehdiabadi and Schultz 2010, Ronque et al. 2019). Therefore, the foraging habits of *A. subterraneus* reported here can be considered intermediate between *Atta* species and other fungus-farming ants. Leaf-cutter foraging distance, however, can change with seasonal availability of culturing substrates. For instance, in the semiarid caatinga of Northeast Brazil, the foraging area of *At. opaciceps* Borgmeier colonies during the dry season was nearly fivefold that of the rainy season (Siqueira et al. 2018). Because our study was performed during the rainy season, it is possible that the observed short trails result from a high availability of potential culturing material in this period, which remains to be investigated.

We detected a positive relationship between the forager's head width and load mass in *A. subterraneus*, regardless of substrate type. Similar relationships were previously found in *Atta* (Rudolph and Loudon 1986, Wetterer 1990b, Van Breda and Stradling 1994, Segre and Taylor 2019) and *Acromyrmex* species (Wetterer 1990b, 1991, 1995, Roces and Núñez 1993, Norton et al. 2014) and have been interpreted as a direct consequence of the leaf-cutting process and/or food item quality. Because no transport chains or leaf caches were observed, we assume all carriers were also cutters, as previously reported for *A. subterraneus* (Nickele et al. 2015). Because cutting activity is energetically costly (Lighton et al. 1987), the mass of substrate load may be limited by ant size. Gomides et al. (1997) found no significant association between forager size and area of the collected material. Previous work, however, suggest that leaf-cutters are more influenced by substrate mass than by substrate size (Wetterer 1990b, Röschard and Roces 2002).

Burden was negatively related to forager head width in *A. subterraneus*, regardless of the type of substrate collected. The same relationship was recently reported for *At. sexdens* (L.), in which larger foragers carried substrates representing a lower proportion of their body mass, compared with smaller nestmates (Segre and Taylor 2019). Indeed, large leaf-cutter ants have proportionally shorter legs than small ants (Feener et al. 1988), which affects the forager's balance and load selection (Burd 2000). However, if only lighter nonleaf items are considered, burden is not significantly related to worker size in *A. subterraneus*. Indeed, Roces and Núñez (1993) suggested that leaf-cutting ants carry lighter pieces of substrate to improve information transfer because foragers can return faster to the nest, increasing recruitment to substrate sources. In addition, Segre and Taylor (2019) suggested that large ants may avoid using their maximal loading capacity to conserve energy to overcome challenges, such as winds and rain.

Our study provided novel data on the natural history and foraging ecology of *A. subterraneus* in cerrado vegetation. Our field account emphasizes the importance of collecting qualitative and quantitative data on the natural history, behavior, and ecology of a species of particular interest. Given their pest status in the Neotropics, most studies on leaf-cutting ants are carried out in human-altered environments (agriculture, cultivated forests, and pastures) with the main goal to design control methods for their management, so as to reduce economic loss (Della Lucia et al. 2014). Our study is a rare field account of *A. subterraneus* in a native ecosystem. Although ants are relatively well-studied insects due to their abundance and dominance in terrestrial ecosystems, lack of ecological data is especially evident for the tropical ant fauna (Wilson 2017). Information about leaf-cutting ants in the cerrado savanna is imperative given the threatened status of this vegetation, which had most of its natural landscape converted to agriculture and pasture (Mustin et al. 2017). Recent studies have shown that leaf-cutter ants thrive in fragmented areas of cerrado and act as ecological filters on plant recruitment by removing seeds and cutting seedlings, which hinders vegetation recovery (Costa et al. 2017). Our fieldwork may provide insights for management techniques of *Acromyrmex* colonies in tropical agroecosystems, as well as for restoration programs of degraded cerrado areas.

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