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SHORT COMMUNICATION

Testing the effect of pitfall-trap installation on ant sampling

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Abstract Digging-in effect is related to higher epigaeic invertebrate catches immediately after pitfall-trap installation, as first reported for the Australian fauna. However, an installation effect has not been tested for arboreal pitfalls. We tested whether samples taken with pitfalls dug (epigaeic stratum) or tied in a tree (arboreal stratum) at the same time, but were opened for sampling after different time periods, showed some pattern of ant activity density and richness in Brazilian closed-forest habitat. We did not observe any effect for epigaeic activity density and species richness catches caused by pitfall installation. The lack of evidence of a digging-in effect is probably due to differences in habitat complexity and the natural history of the Australian and South American ant faunas. We also observed an increase for arboreal-ant activity density and species richness with increased time after the arboreal pitfall installation. Probably, arboreal ants avoid strange objects (pitfalls) in the tree in the early phase, and then, over time, they might become familiarized with the pitfall and start to fall into the trap. We term this process the familiarization effect, referring to the time that the ants require to become familiar with the trap, which was about 4–7 days after the installation. These results suggest that in closed-forest habitats, precautions to avoid a digging-in effect may be unnecessary for epigaeic samples, but that it is best to wait at least 5 days after an arboreal pitfall is installed to begin sampling ants.

Keywords Digging-in effect · Ant community · Sampling bias · Species richness · Sampling methods

Introduction

Pitfall trapping is a widespread method frequently used to sample arthropods, especially ants, in different areas of the natural sciences (Brown and Mathews 2016). Pitfall traps have proved to be a simple and efficient sampling technique for ant biodiversity surveys, bioindication, and conservation studies (Parr et al. 2001; Lopes and Vasconcelos 2008; Sabu et al. 2009; Silva et al. 2013). Nevertheless, technical features of pitfalls should also be taken into account for maximizing capture efficiency, such as the diameter and depth of the collecting container, the liquid content (Cheli and Coreley 2010; Brown and Mathews 2016), use of a cover (Buchholz and Hannig 2009; Brown and Mathews 2016) and bait (Wang et al. 2001), and trap design (Cheli

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and Corley 2010; Brown and Mathews 2016). Ant captures may be further biased depending on the ants' locomotion, activity, and avoidance of traps (Andersen 1983; Bestelmeyer et al. 2000; Ivanov and Keiper 2009). A bias in pitfall-trap catches due to their installation has also been reported (Bestelmeyer et al. 2000; Woodcock 2005), known as the digging-in effect (Joosse 1965; Greenslade 1973).

The digging-in effect consists of a temporary high capture frequency in the early period of epigaeic pitfall operation, in response to the physical disturbance of the soil during the installation (Joosse 1965; Greenslade 1973). High captures were reported for ant activity density (related to the number of ant workers and mentioned as ant abundance) immediately after installation, followed by a decline during the pitfall operating period in Australia (Greenslade 1973). According to Greenslade (1973), the initial high captures could be a result of (1) pitfall penetration in nest galleries, (2) environment exploration by ants learning different parts of the territory, and/or (3) traps installed on the ants' trail; and the subsequent decrease during the pitfall operating time could result from (4) depletion of populations. In addition, the output of CO_2 from dug soil may attract foraging invertebrates in the early stages of pitfall operation (Joosse and Kapteijn 1968; Schirmel et al. 2010).

In studies that aim to assess ant activity density (number of ant workers) and frequency, the digging-in effect could bias the results, since it would overestimate these aspects. Based on this, Greenslade (1973) suggested to invert the position of pitfall traps (initially installing the pitfall upside down) for a week to minimize the digging-in effect, and then start to sample. To overcome digging-in effects on both ant activity density and richness, many researchers have taken precautions in keeping pitfall traps closed or inverted after installation, for periods of 24 h to 7 weeks (Ballinger et al. 2007; Underwood and Cristian 2009; Pietersen et al. 2016); or using other techniques to minimize this effect, such as a soil corer to minimize the disturbance caused by pitfall installation (Morrison and Porter 2003; Williams et al. 2012).

Considering that soil disturbance can attract ants (Vasconcelos et al. 2014), the digging-in effect would be an advantage in biodiversity studies, by collecting more species of ants, but it could be a statistical bias. Williams et al. (2012) mentioned that the digging-in effect can attract some ant species. On the other hand, some researchers have considered the digging-in effect negligible, and although they mentioned this possible problem in their reports, they opened the pitfall traps immediately after installation (e.g., Botes et al. 2006; Munyai and Foord 2012). Indeed, most researchers have never taken precautions to overcome digging-in effect or even mentioned this effect in their studies (e.g., the most-used sampling protocol: ALL Protocol by Agosti and Alonso 2000). This lack of studies assessing the influence of the digging-in effect on ant activity density captures or ant diversity sampling has resulted in a lack of census among myrmecologists on the appropriate sampling protocol and the possible effects of pitfall-trap installation.

The few studies of the digging-in effect on ants (Ward et al. 2001; Schirmel et al. 2010), including the first study by Greenslade (1973), were conducted in grassland or grassy woodland, open forest, and sand-dune habitats, using only epigaeic pitfall traps, in Australia (e.g., Greenslade 1973; Ward et al. 2001) and Europe (e.g., Schirmel et al. 2010). In view of the known influence of habitat structure on pitfall captures (Woodcock 2005), it is also necessary to evaluate the digging-in effect in other habitat types, such as closed forests.

No studies have tested for analogous effects or bias with pitfall traps installed in other forest stratum, such as along tree trunks. The arboreal pitfall trap (Ribas et al. 2003) is widely used in studies of ant diversity (e.g., Andersen et al. 2006; Campos et al. 2006; Frizzo et al. 2012; Schmidt et al. 2013; Rabello et al. 2015). Differently from the epigaeic pitfall trap, the collecting container of the arboreal pitfall is tied on the tree trunk or stem, and therefore, the digging-in effect does not affect the catches of arboreal ants. However, installing an object on the tree may draw the ants' attention (C. R. Ribas personal observation), since it is a new element in the ants' foraging territory (Quinet et al. 1997), which could also introduce a bias for sampling in this stratum. Quinet et al. (1997) reported a rapid recruitment of ants when they discover new food resources in the trees, and conceivably, the same process could occur with a new object in the tree.

Simplifying the sampling method without compromising efficiency is one of the aims of any capture method. This study aimed to detect the possible bias caused by epigaeic and arboreal pitfall installation on estimates of ant activity density and richness, through captures in forest habitats, and if a bias was detected, we also aimed to detect the time range of the bias effect at different intervals after the pitfalls were opened. We hypothesized that samples representing epigaeic and arboreal-ant activity density and species richness would be higher in the early period (i.e., the first day after pitfall installation) of different pitfall opening-time intervals, according to the digging-in premise for the epigaeic stratum and our personal observations for the arboreal stratum.

Materials and methods

Study area

We conducted the study in three remnants of submontane semidecidous Atlantic Forest (IBGE 2012) in southern

Minas Gerais state, Brazil. The forest remnants were situated between the municipalities of Luminárias (21°30′22″S, 044°54′57″W) and Lavras (21°14′43″S, 044°59′59″W). The annual precipitation is about 1450–1650 mm, and the annual mean temperature is 19.4 °C. The three forest remnants lie within a region of transition between the Atlantic Forest and the Cerrado (Brazilian savanna) biomes. We obtained climate data for the Lavras region from the Estação Climatológica Principal de Lavras weather station for the days of our experiment (Online Resource 1).

Sampling ants

We sampled ants in September during the early rainy season (spring) in three forest remnants. In each forest remnant, we established two 6×6 m grids, 50 m distant from each other, and 150 m from the forest edge, totaling six grids. In each grid, we defined nine sampling points separated by 3 m, and installed one epigaeic (using a spade) and one arboreal unbaited pitfall trap at each sampling point (Bestelmeyer et al. 2000; Ribas et al. 2003; Fig. 1). The arboreal pitfalls were tied onto the tree trunk with a string, at about 1.5 m above the ground. The pitfall traps were 8 cm in diameter and 12 cm in depth, 1/3 full (200 ml) of water, salt (0.4%), and liquid soap (0.6%) (Canedo-Júnior et al. 2016), and each trap had a cover to protect against rain and sunlight.

We installed all pitfall traps on the same day in all our six grids. The pitfalls were kept closed by lids. We had nine pairs (epigaeic and arboreal stratum) of traps in all grids which were opened at different time intervals: 0 (opened at the installation), 1, 2, 3, 4, 5, 6, 7, and 15 days after installation. In this way, we had one replication for each time interval in each of our six grids. Therefore, we had 108 traps (54 for arboreal and 54 for epigaeic stratum) in a replication of six traps per time for both strata. We randomly defined, for each grid, which pitfall would be opened each day. Pitfalls of the same time interval were opened at the same day in all grids and remained open for 48 h as proposed in the ALL Protocol by Agosti and Alonso (2000); we performed this protocol in all grids. In every replication of the same interval, pitfalls were opened at the same day and some replications operated at the same time, respecting the interval of 48 h of sampling. By opening the pitfalls at different times, we were able to identify the digging-in effect bias or some possible effect of placing a new object that was strange to the ants on the trees. We could also identify the time range of these effects, to determine how long that precautions would be needed, if necessary at all, after the pitfall installation.

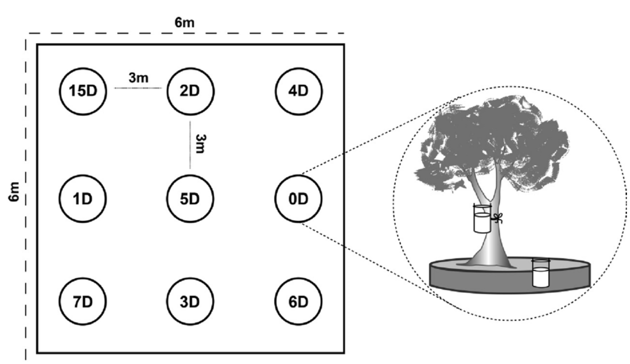

Fig. 1 Sampling design, showing one grid with nine sampling points. Each sampling point contained one epigaeic and one arboreal pitfall trap, which were opened at different time intervals (*0D* opened at the moment of installation, *1D* opened 1 day after installation, *2D* opened 2 days after installation, *3D* opened 3 days after installation, *4D* opened 4 days after installation, *5D* opened 5 days after installation, *6D* opened 6 days after installation, *7D* opened 1 week after installation, *15D* opened 2 weeks after installation). The traps were randomly placed in each grid

We sorted the ant specimens to morphospecies, and dry-mounted, pinned, labeled and deposited them in the collection of the *Laboratório de Ecologia de Formigas* at the *Universidade Federal de Lavras (UFLA)*. We counted the number of ant workers from each sample, which we considered as a measure of ant activity density. Ants were identified to genus following Baccaro et al. (2015) and to species level, whenever possible, through the literature and by matching the specimens with previously identified material at UFLA.

Statistical analyses

To determine whether there was an effect of pitfall installation (digging-in effect) on the epigaeic-ant activity density (number of ant workers) and species richness, and an analogous effect in the arboreal stratum, we constructed a generalized linear model with mixed effects (GLMM), using the Poisson distribution. We used the GLMM because of the dependence of each pitfall in the grids, and we thereby decreased the possible correlations among the subsamples by randomizing such samples, and then avoiding pseudoreplication effects (Pinheiro and Bates 2000). In this sense, differences of species richness between grids will be softened in the analyses. The response variables were ant activity density and species richness for both the epigaeic and arboreal strata, the explanatory variable was the opening-time intervals, and the grid was the random effect. As there was a longer interval between the 7th and 15th days of our experiment, we also performed all analyses without the data from the 15th day, to determine some outlier effect.

To evaluate the effect of climate influence, we verified if the opening-time intervals were collinear with temperature. We also performed a GLMM using the 3 days of each operating pitfall time mean temperature as explanatory variable.

These analyses were carried out with the software R 3.0.1 (R Development Core Team 2013) using the lme4 package (Bates et al. 2013).

Results

In total, we collected 1635 individuals, of which 1000 came from the epigaeic stratum and 635 from the arboreal stratum. We recorded a total of 50 ant species from 22 genera and seven subfamilies, with 38 species in the epigaeic stratum and 25 in the arboreal stratum. Those two stratum shared 13 species, the epigaeic stratum had 25 exclusive species, and the arboreal stratum had 12 exclusive species. The richest subfamily was Myrmicinae (25 species), followed by Formicinae (14 species), Dolichoderinae (4 species), Ponerinae (3 species), Ectatomminae (2 species), and Dorylinae and Pseudomyrmecinae (1 species each). The ant species frequency and activity density of all pitfalls are listed in Online Resource 2.

We detected a positive relationship between ant activity density and different opening-time intervals for the epigaeic stratum ($Z=5.09$; $p<0.001$) (Fig. 2a), where we observed a mean of ant workers for 0 days 6.5; 7 days 10.83; and 15 days 15.66. We observed three possible statistical outliers and so we repeated the analyses without them and found the same pattern ($Z=5.81$; $p<0.001$). The epigaeic-ant species richness showed no relationship

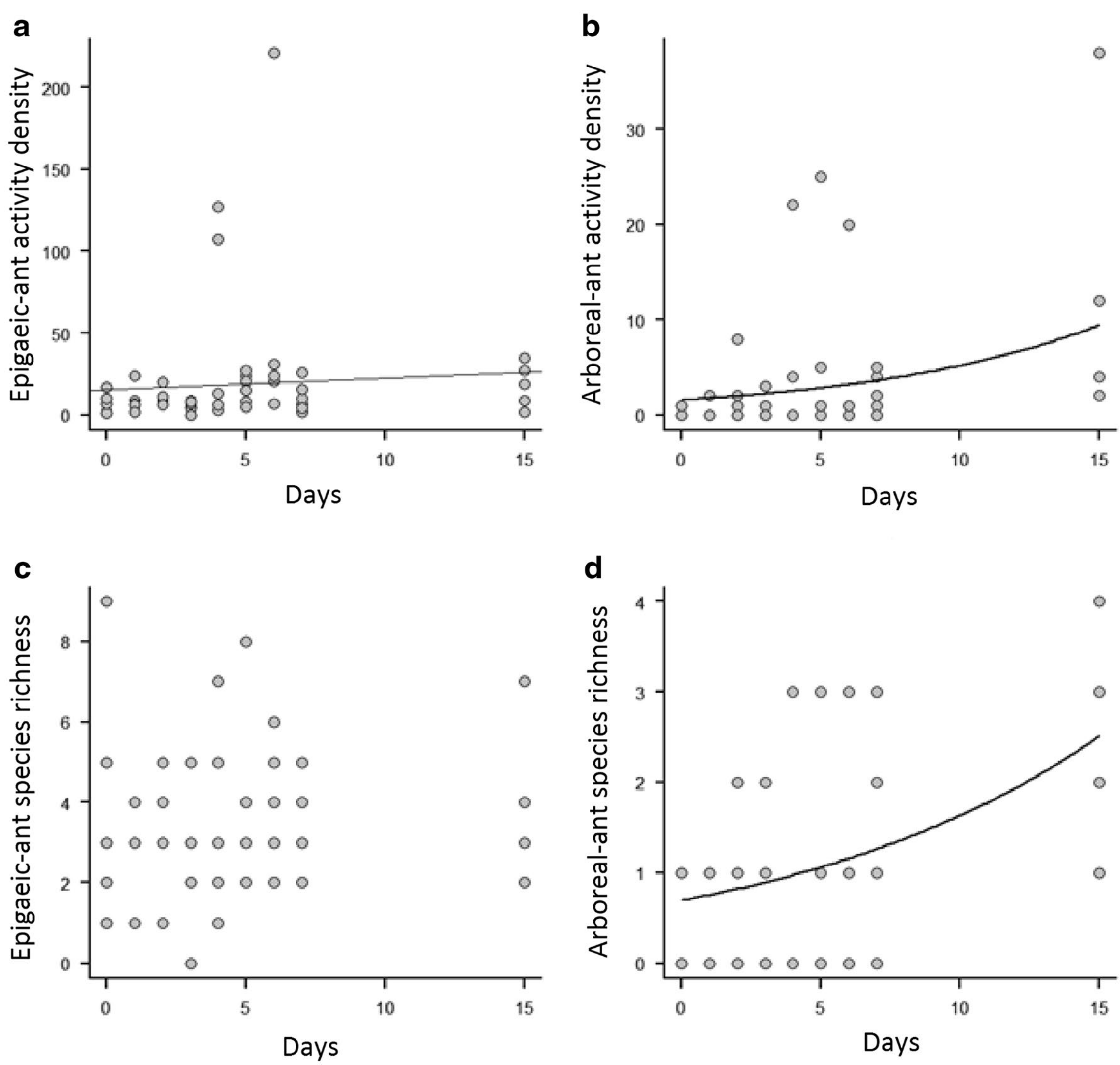

Fig. 2 Correlation between ant activity density (number of ant individuals per site) and richness (number of species per site) and days (opening-time intervals). **a** Correlation between epigaeic-ant activity density and days ($2.586071+0.03452x$; $Z=5.09$; $p<0.001$; $n=9$). **b** Correlation between arboreal-ant activity density and days ($0.19678+0.13028x$; $Z=9.63$; $p<0.001$; $n=9$). **c** Correlation between epigaeic-ant richness and days ($1.173680+0.007396x$; $Z=0.43$; $p=0.670$; $n=9$). **d** Correlation between arboreal-ant richness and days ($-0.36642+0.08585x$; $Z=3.43$; $p<0.001$; $n=9$)

to the opening-time intervals of the pitfall traps ($Z=0.43$; $p=0.670$) (Fig. 2c).

For the arboreal stratum, we found no relationship between ant activity density and different opening-time intervals ($Z = -1.92$; $p=0.055$). However, we had one super activity density datum in one of our arboreal pitfalls, which collected 454 individuals of *Solenopsis* sp. 1 (Online Resource 2, Arboreal Grid 5), perhaps because of the proximity of the trap to the colony entrance. Since this result was marginally significant and the activity density of other arboreal species did not exceed 35 individuals, we considered this datum for *Solenopsis* sp. 1 as a biological outlier and repeated the analyses without this species. We then detected a positive relationship between ant activity density and different time intervals for the arboreal stratum ($Z=9.63$; $p<0.001$) (Fig. 2b), where we observed a mean of arboreal-ant workers for 0 days 0.5; 7 days 2.33; and 15 days 10. The ant activity density increased according to opening-time intervals. We also observed a positive relationship between arboreal-ant richness and the intervals after the opening time of the pitfall traps ($Z=3.43$; $p<0.001$) (Fig. 2d), where we observed a mean of arboreal species richness for 0 day 0.5; 7 days 1.67; and 15 days 2.16. All analyses resulted in the same pattern, with and without the data of the 15th day of our experiment.

Opening-time intervals and mean temperature were not collinear (0.04). However, mean temperature of pitfall operating-time intervals also positively influenced epigaeic-ant activity density ($Z=12.71$; $p<0.001$) but did not influenced epigaeic-ant species richness ($Z=0.15$; $p=0.8800$), arboreal-ant activity density ($Z=0.14$; $p=0.8800$), and arboreal-ant species richness ($Z=1.15$; $p=0.2500$).

Discussion

We found no evidence of digging-in effect on epigaeic-ant activity density and species richness in these closed-forest habitats. Besides the positive relationship of ant activity density catches with opening-time intervals, we found that catches were not higher in the early days of pitfall operation. Moreover, the ant activity density did not decrease, as proposed by the digging-in premise, but rather continued to increase (even subtly) during the experiment. Regarding species richness, the lack of a relationship between epigaeic-ant species richness and pitfall opening-time intervals leads us to refute the idea that installing pitfall traps may attract more ant species in forest habitats. For arboreal ants, we also found that ant activity density and richness increased according to the opening-time intervals. This increase in arboreal activity density and species richness indicates a bias in the arboreal-ant catches that is probably caused by some installation effect.

Since we observed a positive relationship between epigaeic-ant activity density and opening-time intervals, in contrast to the digging-in effect premise, we could not verify the existence of this effect in a closed-forest habitat. Our results contrast with those of Ward et al. (2001), who also reported the digging-in effect, although in a grassy-woodland habitat. Importantly, we carried out our study in a different and more-complex vegetation type than the previous studies. Since the structural complexity can affect catches of epigaeic invertebrates (Woodcock 2005), the difference between our results and the others, which were obtained in a more simply structured environment, could be due to habitat complexity, since epigaeic ants may take longer to find traps in more-complex habitats (Greenslade 1973). However, our study is in accordance with Schirmel et al. (2010), who found no evidence of a digging-in effect for epigaeic ants in a grassland habitat. Even Schirmel et al.'s (2010) study was conducted in a simpler habitat than the habitat in our study, we believe that lower species richness and activity of European ant fauna could lead to this found.

Greenslade (1973) termed a "delayed digging-in effect" the peak of catches some time later in the pitfall operating period, with a subsequent decline. We found a different result, with epigaeic-ant activity density continued to increase (subtly) until the end of the experiment. Our results lead us to infer that high epigaeic captures (when it occurs) due to ants' trial, and ants exploiting areas and depletion of populations are not linked to opening-time intervals (or early stages) (see Online Resource 2). Another explanation for the contrasting results found by Greenslade (1973) and Ward et al. (2001) may be the immense ecological dominance of dolichoderines in the Australian ant fauna. This position of dominance is occupied by Myrmicinae species of the South American fauna, which results in several ecological divergences such as lower rates of activity and investment in worker production for South American Myrmicinae colonies (Campos et al. 2011). Thus, the Australian ant fauna might be more susceptible to depletion of populations over time than the South American fauna.

Ant catches are influenced by the climate, particularly the temperature and humidity (Bestelmeyer et al. 2000). In our experiment, this may be occurring due to temperature. However, we believe that warm temperatures did not cause the subtle increase in our experiment, once collinearity between temperatures and opening-time intervals was 0.04. Possibly, our later high catches from the epigaeic stratum were due to the ants becoming familiarized with the trap in the ground. This means that in the first moments, perhaps, the ants avoid the pitfall traps until they become familiarized with this strange object dug in the ground.

Regarding epigaeic-ant species richness, even soil disturbance has been reported to be an attractant for ants (Williams et al. 2012; Vasconcelos et al. 2014), our results did not show evidence that disturbance affected the epigaeic-ant species richness. Such a finding suggests that the digging action has no influence on ant species richness.

The higher catches in pitfalls that were opened later also occurred for arboreal-ant activity density. We believe that the same cause is linked to both stratum, but is more pronounced in the arboreal stratum. A tree trunk is considered to be a middle stratum that allows habitat-generalist ants to forage both in the canopy and on the ground (Hashimoto et al. 2006, 2010; Martinez 2015). Even considering that ants move around in different ways and at different speeds (Andersen 1991), it is possible that both faster and slower ants flee and avoid being caught, while they are trying to avoid a strange object on the tree. Furthermore, arboreal pitfall traps were reported to be an inefficient capture method when used without bait (Frizzo et al. 2012), probably because of pitfall avoidance. However, the ants could familiarize themselves with pitfall traps over time, as the pitfalls remained on the tree and the ants started to fall into them in larger numbers after they became familiarized with the strange object.

In relation to the high arboreal-ant species richness in the later period of the experiment, this seems to be more important result than arboreal-ant activity density, since pitfall data are best treated with ants incidence (species richness) than ants activity density or abundance (Gotelli et al. 2011). We suggest that some species may avoid a strange object (pitfall) at first moment, as described before. Bestelmeyer et al. (2000) reported that some ant species avoid epigaeic pitfall traps, but it appears that in the arboreal stratum, this effect was more pronounced. Over time, the arboreal pitfall could become familiar to the forager ants, and consequently, by chance, more species started to fall into the pitfalls. Here, we term this process the "familiarization effect". Based on this, the period of familiarization with the trap on the tree in forest habitats lasts about 4–5 days after the trap is installed.

There is no evidence of digging-in effects for epigaeic pitfalls traps in closed-forest habitats, and then, there is no need to keep pitfalls closed (or inverted) after installation in these habitats. We also detected a new effect, the familiarization effect, never described before when sampling the arboreal stratum using pitfalls. The main finding of this study is the increase in arboreal-ant catches (activity density and richness) after the pitfall installation. Most studies that use pitfall traps for sampling arboreal ants follow the same protocol of a 48-h sampling time that is used for epigaeic pitfall traps. This study demonstrated, for the first time, that starting to sample on the installation day results in only 1/3 or 1/2 of arboreal-ant activity density and richness captures in closed-forest habitats. Thus, studies that aim to improve sampling of arboreal ants in forest habitats should consider sampling with arboreal pitfall traps for at least 5 days when it is economic and logistically feasible. Future studies must include different vegetation habitats and sampling in different seasons, for a more comprehensive understanding of sources of bias when installing arboreal and epigaeic pitfalls.

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