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On the Effectiveness of Hand Collection to Complement Baits When Studying Ant Vertical Stratification in Tropical Rainforests

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Abstract

A key factor to study ants is the choice of an appropriate sampling method since distinct sampling methods can capture distinct ant fauna and, therefore, leading to bias in the interpretation and conclusion of the patterns observed. Despite it is well known that the ant fauna is vertically stratified, some of the sampling methods cannot be used throughout the whole vertical stratum (e.g., fogging and Winkler extractor). Here, we compared and evaluated the complementarity of distinct sampling methods in ant surveys on canopy and forest floor in a tropical rainforest of Mexico. Sampling in 10 trees in canopy and on the forest floor around the trees using two baits (tuna and honey) and a standardized hand collecting, we found a total of 44 ant species, belonging to 17 genera and five subfamilies. Ant species composition was different among sampling methods at both vertical strata. Besides, hand collecting yielded higher ant species richness and more exclusive species than either bait at both vertical strata, but both tuna and honey baits also led to the detection of some, though fewer, exclusive ant species. The combination of hand collecting, tuna, and honey baits should thus be considered complementary tools for ant inventories, since using the three methods together yielded more complete inventories at both vertical strata. All methods tested here can be used on distinct vertical strata, ensuring that data in different vertical strata are comparable, allowing more reliable comparisons among these different habitats, i.e., vertical stratification.

Introduction

Myrmecologists have developed many ingenious sampling methods to collect ants (García-Martínez et al., 2018; Lutinski et al., 2013; Oliveira-Santos et al., 2009; Pacheco & Vasconcelos, 2012; Smith & Tschinkel, 2009), and most of the collecting efforts have the objective of testing ecological questions (Gotelli et al., 2011). However, because each sampling method can only record a portion of the community, even though they are used in the same space and time, the sampling method itself acts as a filter (Lee et al., 2019). Indeed, concerns about the choice of sampling methods deserve special attention in the literature, and these works provide valuable information to be known before starting sampling (Gotelli et al., 2011; Lee et al., 2019; Souza et al., 2016). Specifically, comparisons between distinct sampling methods should help researchers to evaluate the trade-offs between sampling completeness and the costs and time required (Souza et al., 2012). Also, the choice of sampling method should be adapted to the scientific question under consideration to avoid bias in the interpretation of results and conclusions (Lee et al., 2019). Therefore, understanding the shortcomings and advantages of each sampling method is important for myrmecological investigations (Gotelli et al., 2011).

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Hand collecting is one of the sampling methods that has been recognized as efficient to sample ants, often used to capture arboreal (Adams et al., 2017; Yanoviak & Kaspari, 2000) and ground-dwelling ants (Bestelmeyer et al., 2000; Longino et al., 2002). This is one of the primeval sampling method to capture insects, requiring little equipment, as forceps or aspirators (Packard, 1873). Hand collecting can be more efficient for recording ants than most other sampling methods, including pitfall traps or baits (Gotelli et al., 2011) and is often at least, if not more efficient than other sampling methods (Ellison et al., 2007; Pisarski et al., 1982; Romero & Jaffe, 1989). To obtain comparable samples, it is recommended that the ant survey be standardized in space and time (Gotelli et al., 2011; Kaspari, 1996). While comparisons between hand collecting and other sampling methods have received attention in the literature (Gotelli et al., 2011), these comparisons are limited to a few habitats and circumstances; for example, among vertical strata.

Distinct vertical strata in tropical forests present differences in their abiotic conditions and, hence, in biotic communities that occupy these habitats (Brühl et al., 1998; Shaw, 2004). For example, ant assemblages from different vertical strata differ in their nutrient restrictions; ground assemblages have less access to carbohydrates, while canopy assemblages are more limited in protein (Brandão et al., 2012; Yanoviak & Kaspari, 2000). Accordingly, the use of honey baits (carbohydrate source), tuna baits (protein source) or a mix of both is widely used to sample ants (Bestelmeyer et al., 2000; Yanoviak & Kaspari, 2000). Also, the use of tuna and honey baits separately can affect the composition and abundance of sampled ants even in the same strata. For instance, *Myrmica* species preferred honey whereas Formicine genera preferred tuna bait in temperate environments (Véle et al., 2009). Moreover, it is known that baits are often dominated by few, resource-dominant species, that restrict the presence of subordinate or submissive species, both on the ground and in the canopy (Bestelmeyer et al., 2000; Camarota et al., 2018; Carval et al., 2016). Baits are widely used by myrmecologists, especially because they are less demanding in time and cost than other methods, such as pitfall traps or Winkler (Souza et al., 2012). Even though baits may record fewer species than other sampling methods, they can be useful for detecting changes in ant assemblages among habitats (Lopes & Vasconcelos, 2008). Therefore, it has been suggested that baits and hand collecting combined are a highly efficient way to sample ants, for instance, in the canopy (Adams et al., 2017; Yanoviak & Kaspari, 2000). However, no study has tested the efficiency of such combination across vertical strata.

Many studies have performed intensive efforts to sample ants across vertical strata, from the forest floor to the treetops, i.e., vertical stratification of ants (Brühl et al., 1998; Wilkie et al., 2010), however, most of these studies use different sampling methods at each vertical stratum. If the ecological question does not involve comparison among vertical strata, but rather detect the total ant diversity, it is better to use as many sampling methods as possible (Gotelli et al., 2011). On the other hand, when different sampling methods are used at different vertical strata, it is difficult or impossible to determine whether apparent differences in richness and composition among habitats are due to true differences or to differences in the ant fauna that each sampling method record (Gotelli et al., 2011). When it is not possible to use several sampling methods in all habitats to be compared, it is recommended that fewer sampling methods, even if only one, be used in a standardized way across all sampling sites (Steiner et al., 2005). Accordingly, to compare habitats with distinct conditions, hand collecting is recommended instead, for instance, litter sampling or pitfall traps, as it can be used in sites that have no leaf litter or are rocky, steep or too disturbed by human and domestic animal traffic (Gotelli et al., 2011), or on distinct vertical strata. Specifically, it is impossible to use, for instance, fogging on the forest floor, due to the inherent limitations of these sampling methods. In contrast, hand collecting and baits are widely used to record ants at all vertical strata (Adams et al., 2017; Leponce et al., 2019). Baits and hand collecting combined are a widely used sampling method to sample ants, especially in the canopy strata (e.g., Adams et al., 2017; Yanoviak & Kaspari, 2000; Yanoviak et al., 2008). Therefore, it is necessary to use standardized sampling methods to compare ant fauna over space and time (King & Porter, 2005; Longino et al., 2002; Lopes & Vasconcelos, 2008; Romero & Jaffe, 1989; Tista & Fiedler, 2011), including across vertical strata.

Most comparisons between sampling methods are concentrated on the forest floor, without considering other vertical strata. Here, we aimed to evaluate the effectiveness of the combination of hand collecting, honey bait, and tuna bait in recording ants on the forest floor and in the canopy of a Mexican tropical rain forest. We expected that hand collecting would collect more ant species than tuna or honey baits because baits are known to collect few ant species per bait, e.g. due to competition (Baccaro et al., 2010; Bestelmeyer et al., 2000). Unlike bait samples, hand collecting should allow sampling cryptic, submissive, and less abundant ants. We also expected that all sampling methods combined would improve the ant survey, leading to a better description of the ant community (Longino & Colwell, 1997) spread over distinct vertical strata. Our results will provide a framework that emphasizes the reliability of the combination of hand collecting and baits to sample ants on the ground and in the canopy.

Method

Study area

The study was conducted in a 130 ha fragment of tropical rainforest in the municipality of Ixhuatán in the state of Veracruz, Mexico (18°2’22.99” N, 94°21’27.61” W, 20-60m a.s.l.). The fragment is inside a privately protected area (*Área de Protección y Desarrollo de Ceratozamia*) established in 2015 by the Braskem Idesa company, as a management unit for wildlife conservation (Retes López et al., 2010). The climate of the study area is warm and humid with a mean annual temperature of 27 °C and an annual rainfall of 1,800 mm. Half of the vegetation of the fragment is composed of grasslands with isolated trees. The other half is composed of a remnant of secondary lowland tropical rainforest with characteristic species *Bursera simaruba* (L.) Sarg. (Burseraceae), *Cecropia obtusifolia* Bertol. (Urticaceae), *Coccoloba hondurensis* Lundell (Polygonaceae), *Cupania dentata* Moc. & Sessé ex DC. (Sapindaceae), *Guazuma ulmifolia* Lam. (Malvaceae), *Miconia argentea* (Sw.) DC. (Melastomataceae) (Ortiz-Lozada et al., 2017).

Ant sampling

We established four transects, covering most of the forest fragment, that were at least 100 m apart from each other. Then, we selected 10 trees, at least 50 m apart, among the tallest and safest to climb. Tree crowns (hereafter called "canopy strata") were accessed using the 'single rope climbing technique' (Perry, 1978). Ants were also sampled on the forest floor around each climbed tree. The sampling in both strata was performed between 09:00 and 16:00 h in May 2016, at a rate of one tree per day (total sampling period= 10 days). We used three widely used sampling methods to collect ants, namely tuna bait, honey bait, and hand collection, to sample ants on the canopy and forest floor (e.g., Adams et al., 2017; Yanoviak & Kaspari, 2000). The tuna and honey baits consisted of approximately one teaspoon of tuna and one teaspoon of honey. Tuna and honey were added separately on plastic plates, for the ground samples, and in plastic pots, for the canopy samples. One tuna and one honey bait per tree were placed near the main fork, as high in the canopy as possible (10-15 m above the ground, depending on tree height) and on the forest floor, at the base of each tree. The baits were left for 60-90 min, then collected in individual plastic bags (Bestelmeyer et al., 2000). Ants were also collected by hand using forceps for 10 minutes at each stratum. In the canopy, ants were collected on the trunk and branches and leaves, and the forest floor, around each tree. All samples were stored in 70% ethanol and transported to the laboratory for sorting, mounting, and identification to the lowest taxonomic level, morphospecies or species when possible. We used a reference collection determined by the *Colección entomológica IEXA* (*Instituto de Ecología A.C.*), "AntWeb" (Fisher, 2002), and "Ants of Costa Rica" (Longino, 2007).

Data analyses

For all analyses, we only focused on comparisons of sampling methods within each vertical stratum, without comparing the canopy and forest floor, following our hypothesis. We performed ant species accumulation curves in relation to our sampling units (i.e., trees) for each method and stratum separately and for all sampling methods combined for each vertical stratum. To do this, we used rarefaction and extrapolation methods (doubling the sample size as suggested by Chao et al. (2014)). We implemented these methods in R (R Core Team, 2017) using the package iNEXT (Hsieh et al., 2016).

We used Generalized Linear Mixed Models, (GLMM) (Bolker et al., 2009) because our observations were not independent (Hurlbert, 1984), to test whether the ant species richness differed among sampling methods, following the data exploration proposed by Zuur et al. (2010). We built the structure of the fixed-effect model using ant species richness as a function of the sampling method and we used as the random effect the tree (sampling points, n = 10). We considered each tree (canopy and forest floor) as a random effect because each sampling point would present particular abiotic conditions, and biotic communities, due to the high diversity of organisms in tropical forests, even though in small scales (Benson, 1985; Dejean et al., 2008). All models followed the Poisson distribution and logarithmic link function, frequently used for count data (O'hara & Kotze, 2010; Zuur & Ieno, 2016). We examined the distribution of the model residuals to confirm the appropriateness of the Poisson error distribution.

To test the differences in species composition among the sampling methods, we performed a generalized linear multivariate model (GLM_{mv}) (Wang et al., 2012) using a presence-absence data matrix and a binomial error distribution. We performed these analyses using the "*mvabund*" package (Wang et al., 2012), R software (R Core Team, 2017).

Results

With all sampling methods combined, we recorded 44 ant species belonging to 17 genera and five subfamilies (Table 1); 23 species were recorded from the canopy and 37 on the forest floor. The subfamily Myrmicinae represented the highest species richness (41%, 18 ant species), followed by Pseudomyrmecinae (18%, eight ant species), Dolichoderinae (16%, seven ant species), Formicinae (14%, six ant species), and Ponerinae (11%, five ant species) (Table 1).

We found that ant species composition differed between the sampling methods (hand collecting, tuna bait, honey bait) both in the canopy (GLM_{mv}: deviance = 63.39, $p < 0.05$) and on the forest floor (GLM_{mv}: deviance = 157.52, $p < 0.001$). Using tuna bait, we recorded two and six exclusive species for canopy and forest floor, while using honey bait we found three and one exclusive species. Using hand collecting, we recorded 17 and 21 exclusive species for canopy and forest floor (Fig 1a-b). In the canopy, the total sampling coverage was 86.6%, hand collecting was 91.4%, tuna bait was 47.8%, and honey bait was 55 % (Fig 1c). On the forest floor, the sampling coverage total was 86.6%, hand collecting was 88.2%, tuna bait was 32.6% and honey bait was 69.4% (Fig 1d).

Table 1. Occurrence of ant species sampled with different sampling methods at the forest floor and canopy level of a fragment of lowland tropical forest, Gulf of Mexico.

Subfamily	Species	Canopy			Forest floor			Canopy	Forest floor
		Hand collecting	Tuna bait	Honey bait	Hand collecting	Tuna bait	Honey bait	Total	Total
Dolichoderinae									
	Azteca alfari Emery, 1893	3	-	-	-	-	-	3	-
	Azteca forelii Emery, 1893	5	1	-	1	-	-	6	1
	Azteca instabilis (Smith, 1862)	-	-	-	-	-	-	-	-
	Azteca nigra Forel, 1912	-	-	-	2	-	-	-	2
	Dolichoderus bispinosus (Olivier, 1792)	2	-	-	9	1	-	2	10
	Dolichoderus lutosus (Olivier, 1792)	1	-	-	-	-	-	1	-
	Linepithema sp1	-	-	-	2	-	-	-	2
Formicinae									
	Camponotus brettesi Forel, 1899	5	1	-	1	-	-	6	1
	Camponotus linnaei Forel, 1886	1	-	-	1	-	-	1	1
	Camponotus mucronatus Emery, 1890	1	-	-	2	-	-	1	2
	Camponotus novogranadensis Mayr, 1870	1	1	1	4	-	-	3	4
	Camponotus planatus Roger, 1863	-	-	-	3	-	-	-	3
	Nylanderia sp1	-	-	-	-	1	2	-	3
Myrmicinae									
	Carebara sp1	-	-	-	-	1	-	-	1
	Cephalotes basalis (Smith, 1876)	1	-	-	-	-	-	1	-
	Cephalotes minutus (Fabricius, 1804)	-	-	-	2	-	-	-	2
	Cephalotes scutulatus (Smith, 1867)	2	-	-	2	-	-	2	2
	Cephalotes umbraculatus (Fabricius, 1804)	2	-	-	1	-	-	2	1
	Crematogaster curvispinosa Mayr, 1862	1	-	-	1	-	-	1	1
	Crematogaster torosa Mayr, 1870	1	-	-	-	-	-	1	-
	Nesomyrmex pleuriticus (Kempf, 1959)	-	-	-	1	-	-	-	1
	Pheidole absurda Forel, 1886	-	1	-	1	-	-	1	1
	Pheidole flavens Roger, 1863	-	-	-	2	1	6	-	9
	Pheidole punctatissima Mayr, 1870	-	-	-	-	1	-	-	1
	Pheidole simonsi Wilson, 2003	-	-	-	-	-	1	-	1
	Pheidole susannae Forel, 1886	-	-	-	2	1	1	-	4
	Pheidole sp1	-	-	-	-	1	-	-	1
	Solenopsis geminata (Fabricius, 1804)	-	-	-	3	3	2	-	8
	Solenopsis sp1	-	-	-	-	1	-	-	1
	Trachymyrmex intermedius (Forel, 1909)	-	-	-	1	-	1	-	2
	Wasmannia rochai Forel, 1912	3	4	2	1	2	1	9	4
Ponerinae									
	Neoponera carinulata (Roger, 1861)	1	-	-	1	-	-	1	1
	Neoponera unidentata (Mayr, 1862)	-	-	-	2	-	-	-	2
	Neoponera villosa (Fabricius, 1804)	1	-	-	-	-	-	1	-
	Odontomachus ruginodis Smith, 1937	-	-	-	-	1	-	-	1
	Pachycondyla harpax (Fabricius, 1804)	-	-	-	2	-	-	-	2
Pseudomyrmecinae									
	Pseudomyrmex boopis (Roger, 1863)	-	-	-	6	-	1	-	7
	Pseudomyrmex cubaensis (Forel, 1901)	1	-	-	-	-	-	1	-
	Pseudomyrmex elongatulus (Dalla Torre, 1892)	1	1	-	5	-	-	2	5
	Pseudomyrmex elongatus (Mayr, 1870)	3	-	-	3	-	-	3	3
	Pseudomyrmex oculatus (Smith, 1855)	1	-	-	-	1	-	1	1
	Pseudomyrmex gracilis (Fabricius, 1804)	2	-	-	3	-	-	2	3
	Pseudomyrmex salvini (Forel, 1899)	-	-	-	2	1	-	-	3
	Pseudomyrmex subater (Wheeler & Mann, 1914)	2	-	-	2	-	-	2	2

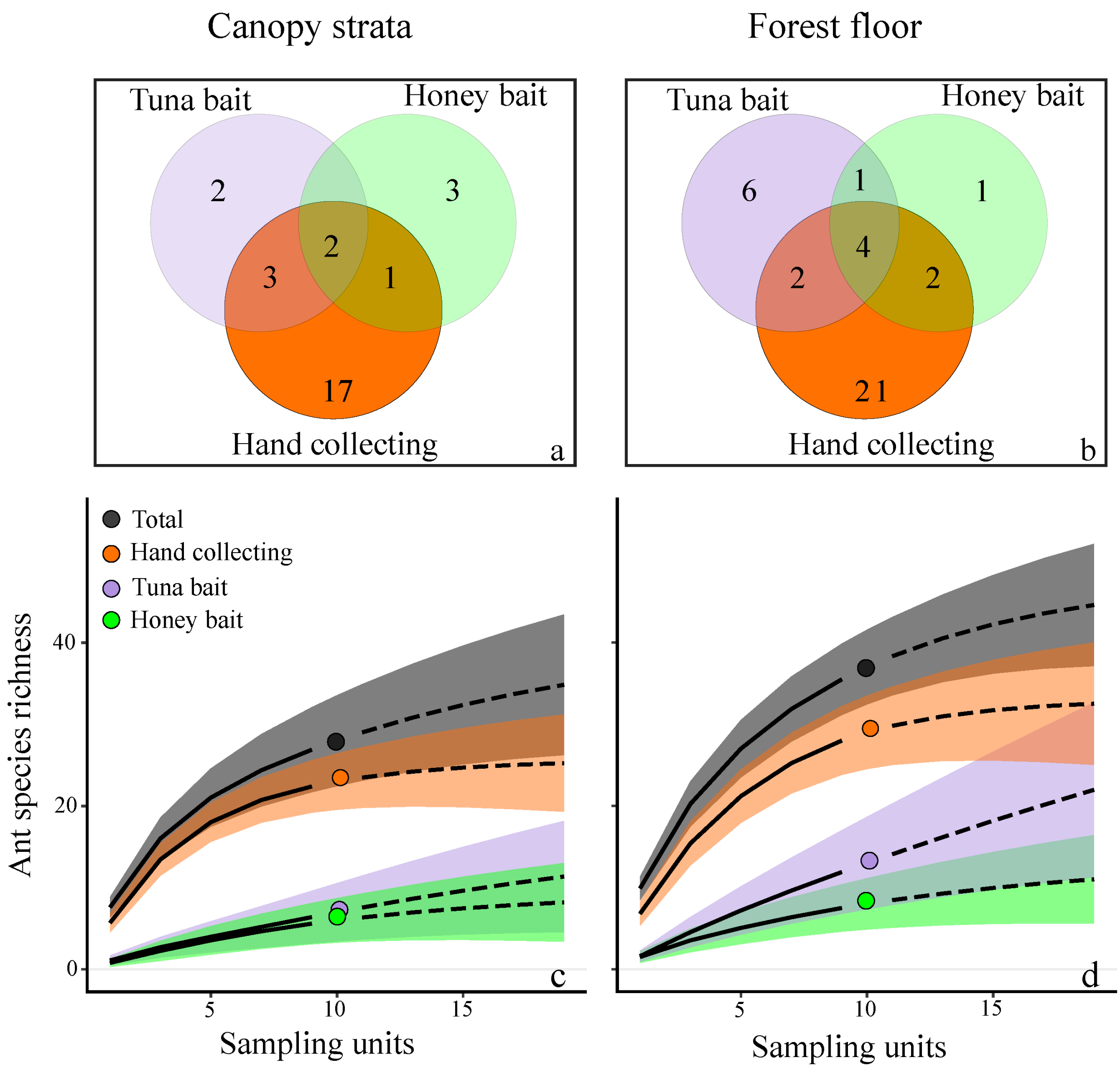

Fig 1. Venn diagrams of the comparisons of ant sampling methods in the canopy (a) and on the forest floor (b). Sample-size-based rarefaction (solid lines) and extrapolation curves (dashed lines, up to double the sample size) of ant species diversity using different sample methods for the canopy (c) and the forest floor (d) of a tropical rainforest fragment, Gulf of Mexico. Shaded regions represent the 95% confidence intervals. The total sampled ant species are denoted by a black circle sign, while the red circles represent the hand collecting method, the purple circles represent tuna and the green circle honey bait methods.

Ant species richness was higher using hand collecting (canopy, mean ± SD: 4 ± 2.36, forest floor: 5.2 ± 1.62) than tuna (canopy: 0.9 ± 0.57, forest floor: 1.6 ± 0.70) and honey baits (canopy: 0.3 ± 0.48, forest floor: 1.5 ± 0.85) at both vertical strata (canopy: $\chi^2 = 31.6$; $df = 2, 26$; $P < 0.001$; forest floor: $\chi^2 = 46.55$; $df = 2, 26$; $p < 0.001$) (Fig 2).

Discussion

We found that hand collecting recorded about four times more ant species in the canopy and on the forest floor than tuna or honey bait. Each sampling method recorded different ant species composition, and although tuna and honey baits also recorded some exclusive ant species in both vertical strata, hand collecting was remarkably the sampling method with which most exclusive ant species were recorded. Our findings allow us to infer that hand collecting is a suitable sampling method across vertical strata and using it together with baits yields a comprehensive inventory.

Important ecological questions have been addressed over the past few years using hand collecting in association with tuna and honey baits to sample ants, especially in the canopy strata (e.g., Adams et al., 2017; Yanoviak & Kaspari, 2000; Yanoviak et al., 2008). However, it had not been evaluated whether the use of these sampling methods combined increases the efficiency in sampling ants on distinct vertical strata. Since we collected during the day on only 10 trees using hand collecting for 10 min on distinct vertical strata in addition to tuna and honey baits, we recorded a satisfactory number of ant species for canopy (23) and forest floor (37) in

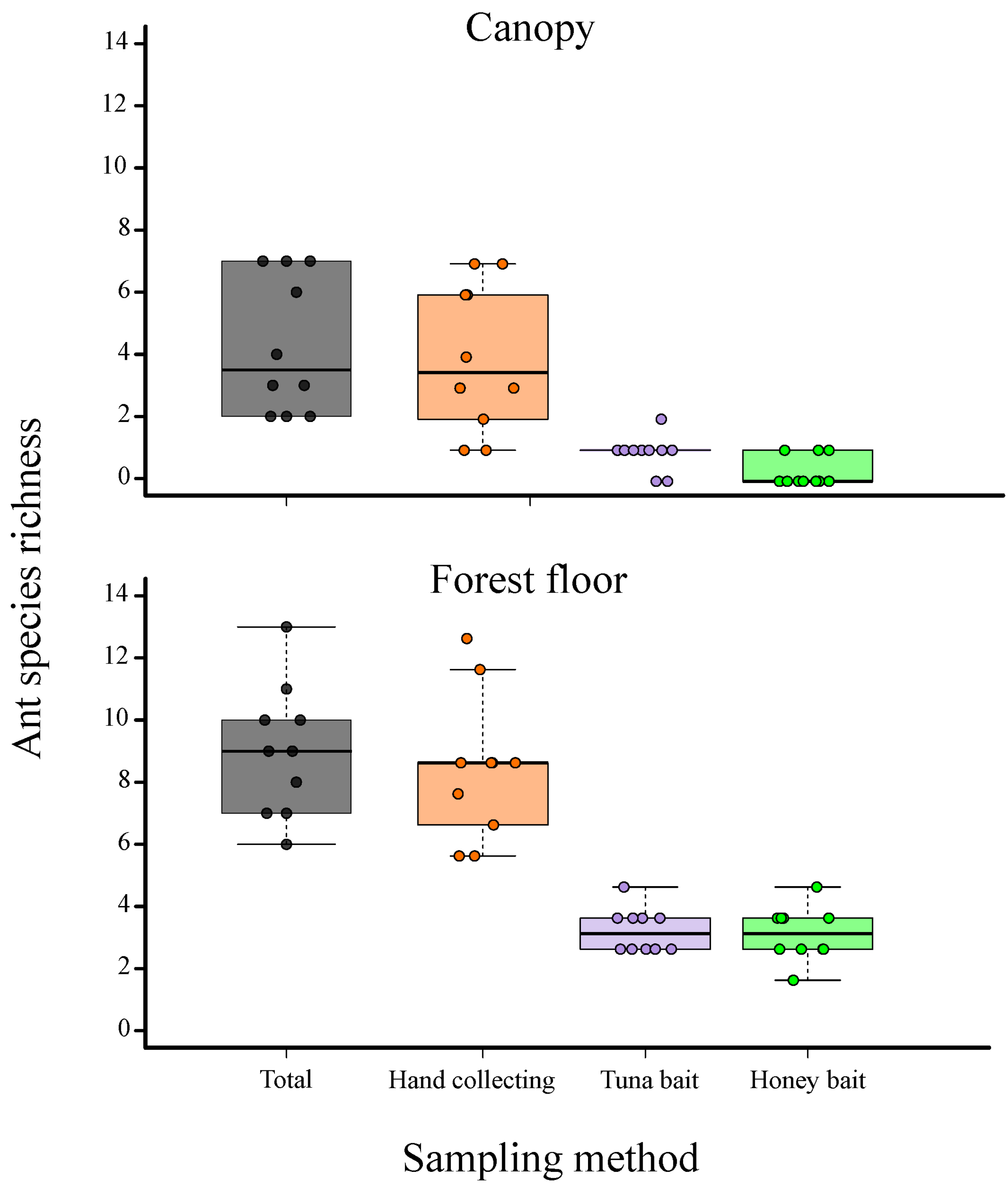

Fig 2. Box plots of the ant species richness total and per sampling method for canopy (top) and forest floor (bottom).

the tropical forest studied. Certainly, other sampling methods, e.g. fogging in the canopy (Longino et al., 2002) and Winkler extractor on the forest floor (Gotelli et al., 2011), may be more effective for capturing ant fauna in that habitat. However, fogging is time-consuming both in the field and the laboratory (Adis et al., 1984), and it is difficult to determine from which vertical stratum the sampled ant came from, besides that it is not equally efficient in sampling all canopy microhabitats (e.g. ant fauna living in epiphytes; Yanoviak et al., 2003). Similarly, the use of the Winkler extractor to sample ground-dwelling ants represents an increase in cost and time compared to other sampling methods (Souza et al., 2012). Therefore, we have validated the combination of hand collecting, tuna, and honey baits to capture ants and to test ecological questions, such as vertical stratification of ants.

Sampling by hand collecting in the canopy is not always an easy task since it involved climbing (Lowman et al., 2012), but the single rope technique, the use of canopy cranes and other methods can circumvent this limitation (Basset et al., 2003). Here, we found that hand collecting allows us to record most ant species richness in the canopy and on the forest floor, with a high rate of exclusive ant species, in comparison with tuna or honey baits. Also, hand collecting can be easily standardized in terms of time and survey area when carried out by the same collector(s) in all treatments or habitats (Gotelli et al., 2011). "Collector effect" results from differences among collectors in their sampling efficiency in sampling ants (Longino et al., 2002) and can be a serious confounding variable even if the collections were carried out over the same space and time. The collector effect can be accounted for using statistical tools, such as including the identity of the collector in the statistical model as a random effect. Thus, it is possible to obtain the effect of the variables of interest in the statistical model independent of the undesirable effect of differences among collectors (Crawley, 2013). This is useful because the sampling efficiency of hand collecting increases with more collectors and also more time devoted (Morrison, 1996), allowing better comparisons between distinct sampling points (Bestelmeyer et al., 2000). Thus, the use of hand collecting to collect ants shows many advantages for the ant fauna survey in both vertical strata, mainly when compared to tuna or honey baits.

Despite we have found more species richness and more exclusive ant species with hand collecting, we also found exclusive ant species on baits. Also, species composition was different among sampling methods. Thus, the use of baits can improve the ant inventory on the canopy and forest floor. Baits often attract only trophic generalists, a significant proportion of ant fauna worldwide (Bestelmeyer et al., 2000), allowing to be used in most habitats to sample ants. Besides, some factors can lead to sample fewer ant species on baits, such as competition by exclusion among resource dominant ant species (Baccaro et al., 2010; Ribeiro et al., 2013). In this context, many ant species can first find and occupy the baits, subsequently being removed by the dominant species, due to their most aggressiveness and recruitment rates (Davidson, 1998). Also, ant species exhibit preferences for carbohydrates or proteins, leading to preferences for distinct bait types, as honey or tuna (Yanoviak & Kaspari, 2000), being better mixing tuna and honey in one bait type, as widely used by myrmecologists (Bestelmeyer et al., 2000; Leponce et al., 2019; Yanoviak et al., 2007). It is important to highlight that a potential limitation of our study related to the comparison of sampling methods is that the covered area where we searched ants using hand collecting could not be the same area under the influence of bait attractiveness. However, we emphasized here the comparison of such sampling methods in the tree crown and forest floor around these trees, despite the inherent characteristics of each sampling methods, and hence the difficulty in comparing them, even if in the same site, at the same time (Lee et al., 2019).

Although the use of distinct sampling methods can be redundant in the ant fauna obtained from comprehensive surveys, it is known that each of them records certain ant fauna, e.g. Winkler captures small ants vs. pitfall traps capture large ants, being complementary (Parr & Chown, 2001). Specifically related to our findings, ant fauna recorded by tuna and honey bait was a subset of hand collecting species. However, these baits also recorded some exclusive ant species, corroborating the potential complementarity of these sampling methods to capture ants in the vertical strata studied. Here, we emphasized that hand collecting, tuna, and honey baits combined can be used both in the canopy and on the forest floor, allowing comparisons between distinct vertical strata. Besides, baits can be used to record ants in different microhabitats and periods and can provide information related to habitat use and activity patterns by ants on very fine scales (Luque & Reyes-López, 2007). Also, since the bait technique is widely used in several ant surveys (Bestelmeyer et al., 2000), the use of this sampling method allows comparisons among previous studies. Ground-based techniques using baits to sample ants on the canopy have been improved in recent years, such as the recently described "arboreal bait line technique" (Leponce et al., 2019). This sampling method consists of hoisting the tuna and honey baits using a line strung by slingshot from the ground. Using this sampling method it is possible to determine with precision height and vertical strata and, because it uses the same method along the whole vertical strata, data are comparable, though the number of species recorded is limited (for more detail, please see Leponce et al., 2019). Our results evidenced that baits are reliable to compare the distinct vertical strata, consistent with a previous study that compared ant fauna from distinct habitats using only baits (Lopes & Vasconcelos, 2008).

In short, our results highlight the importance of comprehensive sampling methods, including hand collecting, tuna bait, and honey bait to record ants, on distinct vertical strata. While hand collecting was by far the more efficient sampling method to record ants in both vertical strata, tuna and honey baits also provided some exclusive ant species. Finally, it is important to consider appropriate methodologies for answering ecological questions, e.g. using the same sampling methods when the interest is to compare different vertical strata or between any distinct habitats.

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Authors Contribution

RA and WD conceived and designed the study, JPM and LOL collected the data. RA conducted the data analysis. RA wrote the manuscript with support from WD, ML, DA.

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