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Collecting arboreal arthropods: a technique for sampling plant-inhabiting arthropod communities in a tropical forest understory

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Abstract In this study, we describe a new low-cost technique to collect plant-inhabiting arthropods at a height of up to 10 m without the need for specialized and complicated methods such as rope climbing or fogging. We present a model to build and apply the technique in a tropical forest understory. This technique is important because it is not only efficient for arthropod collection, it also enables a variety of ecological studies such as stratification, habitat comparison, and species distribution.

Introduction

The loss of natural ecosystems has increased, mainly due to advancing agricultural frontiers, mining, deforestation, and pollution (Sánchez-Bayo & Wyckhuys, 2019). These factors negatively affect the diversity, abundance, distribution, and biomass of arthropods, including many insects, leading to the decline or even extinction of these organisms (Hallmann et al., 2017; Leather, 2018; Cardoso & Leather, 2019; Cardoso et al., 2019). Arthropods are the most abundant and diverse animal group in the world; however, information about the ecology and response to environmental changes is still scarce for this group (Cardoso et al., 2011; Basset et al., 2012), particularly in tropical ecosystems such as the Amazon tropical rainforest (Oliveira et al., 2016). The highest arthropod diversity of tropical rainforests is found in the aboveground vertical strata, notably in the forest canopy (Nakamura et al., 2017); however, sampling arthropods is difficult and more expensive in these higher locations (Basset et al., 2015).

In tropical rainforests, vertical stratification creates difficulty of access and there is no consensus in the literature regarding how it is structured. However, it is possible to recognize five strata (sensu Richards, 1971; Basset et al., 2015): the soil and litter layer, the shrub stratum in the understory, as well as the lower, mid-, and upper canopy in the canopy layer or tree crown (Aikens & Buddle, 2012). Thus, the understory is varied and can be defined as the 'vegetation layer between the tree canopy and the ground cover in a forest' (Lawrence, 2008). The understory consists largely of young trees, so there is no discrete limit between this layer and the lowest tree layers (Richards, 1971). The understory varies by location and can reach a height of 10 m or more (Richards, 1971). Therefore, to collect at this or greater heights requires the use of sampling techniques such as ladders, ropes, or cranes (Perry, 1978; Ozanne, 2005).

Some collection methods for arthropods inhabiting the understory can be separated into arthropods associated with understory environments (e.g., shade, constancy of microclimate, low wind speed) and arthropods associated with understory plants (Ozanne, 2005). In understory environments, the methods used to quantify plant-

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arthropod interactions become more complex and costly as you climb to strata higher than 2–3 m. Currently, at this height, the tree sampling techniques mostly used are beating trays (Basset & Novotny, 1999; Ozanne, 2005; McCaig et al., 2020) or manual collection after accessing the tree crowns with a simple rope-climbing technique (Perry, 1978). Recently, Lopes et al. (2019) created a new method for collecting plant-inhabiting arthropod assemblages in the tropical rainforest understory, the Amazonas-trap; however, this method is restricted to plants with a height of 3 m or less. Leponce et al. (2019) described arboreal bait line protocol to collect tree-dwelling ants, but this method was designed to collect only numerically dominant canopy ants. Although techniques for the study of the understory continue to be described today (Lopes et al., 2019), we still do not have low-cost techniques, easy to use, that collect a wide variety of organisms in strata higher than 3 m, and that can also be replicated in different environments (Quijano Cuervo et al., 2019).

In this study, we present a new technique for sampling arthropods inhabiting understory and trees from 1 to 10 m high, discussing its advantages and shortcomings. To test the efficacy of this technique, we conducted the study in seven transects in a forest area and seven transects in an area located in a previous mining area undergoing natural restoration. This way, we could insert an ecological factor to evaluate how anthropogenic drivers affect arthropod diversity associated with plants. We anticipated that ecological parameters of arthropod richness and abundance would be greater in reference sites (forests) and that composition would be modified between natural restoration sites and forest sites, even if we look at superior taxa. This research presents a new technique for collecting arboreal arthropods in an applied ecological experiment design to demonstrate its potential for replication and standardization in future sampling work.

Materials and methods

Concept of the arboreal arthropod collector

The collector is made using the basic principles of beating trays combined with the use of an aluminum telescopic pole spanning 4–8 m. The collector is composed of two parts: (1) a collector tray funneled into a collecting bag, and (2) a shaker stick with a hook at its upper end to pull on and shake the branches. The collector tray is made of a rectangular iron structure (70 × 50 cm), in which a primary fabric collector (white synthetic denim) with dimensions of 70 × 50 × 20 cm (depth) is sewn to the rectangular iron structure to collect the arthropods immediately after shaking the tree branches. The collector tray has at its center, in the lower part, an opening (a piece of PVC pipe of 10 cm in diameter and 10 cm high) with a plastic bag attached with a rubber band at the bottom, so that after shaking, it is

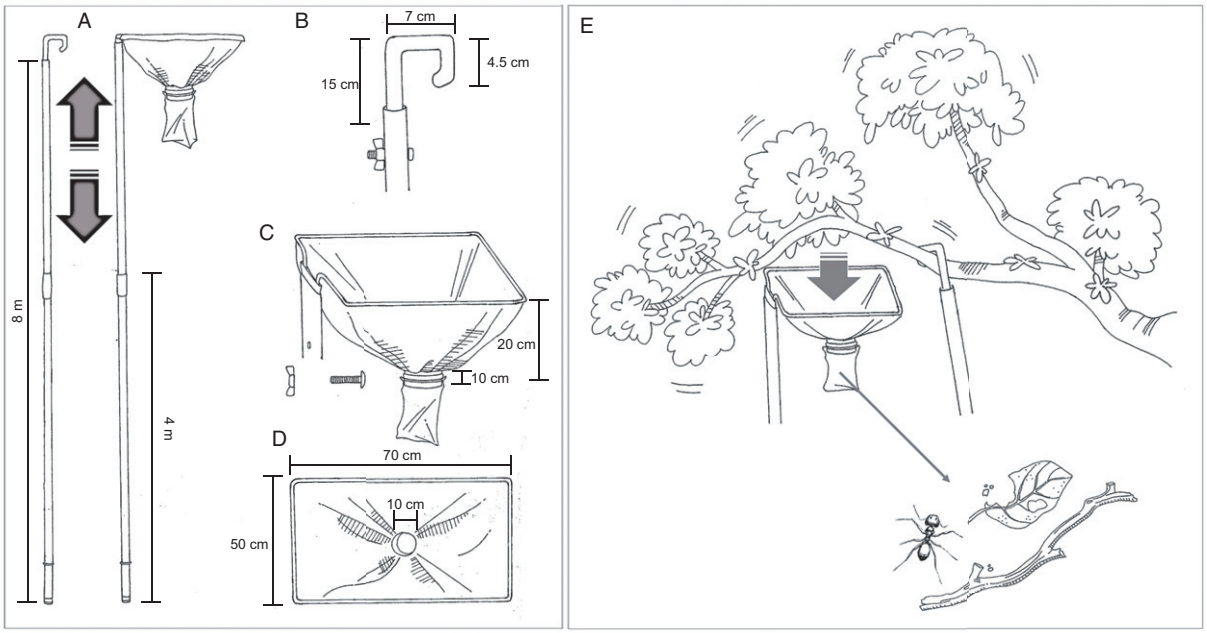

Figure 1 General design of the collector tray and beater stick for agitating foliage: (A) detail of the arboreal-arthropod collector with the telescopic pole expanded, (B) detail of the hook at the end of the beater stick, (C, D) side view and upper view of collector tray, and (E) operation of the arthropod collector on the branch of a tree. Illustration by Cleverton da Silva.

possible to pack all the arthropods in the plastic bag. The shaker stick is comprised of an aluminum hook attached to the end of a telescopic pole (Figure 1).

Study sites and sampling procedure

Sampling was conducted in a bauxite mining area (03°15′02″S, 47°44′03″W), in the municipality of Paragominas, Pará State, Brazil. This area is located in the northeastern region of the Brazilian Amazon with original vegetation covered by ombrophila dense forest. However, part of this area was previously converted to farmland and pasture and subsequently abandoned (El-Husny et al., 2003). Mining activities cleared much of the surrounding forest and secondary vegetation. After mining activities

Table 1 Location, number of trees sampled, and richness of trees in the 250-m-long transects in former mining area undergoing natural restoration and forest areas in the municipality of Paragominas, state of Pará, Brazil. Coordinates points 0 and 250 indicate the geographical positions of the start and endpoints of the linear transects where samples were taken, respectively

Treatment	Transect	Coordinates point 0	Coordinates point 250	Plant abundance	Plant richness
Natural restoration	I01	03°15'25"S, 47°42'90"W	03°15'32"S, 47°42'50"W	14	5
	I02	03°14'57"S, 47°42'20"W	03°15'40"S, 47°42'24"W	13	6
	I03	03°15'40"S, 47°41'39"W	03°15'10"S, 47°41'47"W	26	7
	I04	03°14'25"S, 47°42'25"W	03°14'31"S, 47°42'20"W	29	4
	I05	03°13'34"S, 47°42'90"W	03°13'41"S, 47°42'12"W	23	4
	I06	03°13'27"S, 47°43'90"W	03°13'36"S, 47°43'90"W	41	7
	I07	03°13'40"S, 47°42'19"W	03°13'00"S, 47°42'25"W	40	14
Forest	I08	03°15'10"S, 47°49'57"W	03°15'30"S, 47°49'54"W	23	12
	I09	03°15'24"S, 47°48'22"W	03°15'25"S, 47°48'14"W	31	16
	I10	03°14'40"S, 47°46'40"W	03°14'40"S, 47°46'47"W	17	15
	I11	03°12'34"S, 47°46'41"W	03°12'33"S, 47°46'33"W	20	17
	I12	03°13'27"S, 47°45'12"W	03°13'19"S, 47°45'16"W	25	17
	I13	03°14'16"S, 47°43'59"W	03°14'10"S, 47°43'59"W	29	14
	I14	03°12'56"S, 47°43'00"W	03°12'56"S, 47°43'90"W	37	24

Figure 2 Foliage agitation technique in operation in an Amazon tropical rainforest: (A) natural regeneration area, (B) forest area, (C) detail of the collector tray, and (D) detail of collecting bag after shaking the foliage. Note that two persons are involved, one operating the beater stick with the hook, the other holding the collector tray. Photo by Cesar Favacho. [Colour figure can be viewed at wileyonlinelibrary.com]

were concluded, natural restoration was one of the methods used by the company to restore the forest. For purposes of this study, we selected sites in primary forest remnants and natural restoration areas 5 years after mining activities were ceased. The landscape in this region is composed of forest fragments with different disturbance grades, agricultural areas, and secondary forests in different successional states (El-Husny et al., 2003). We selected 14 transects of 250 × 4 m (1 000 m^2) in two kinds of habitats: seven natural restoration areas of 5 years old and seven in forested areas (Table 1). All trees and shrubs with a circumference at breast height of >10 cm and leaf area height up to 10 m were sampled with a total of approximately 50–80 shakings of branches in various parts in each plant (Figure 2). We avoided branches with high densities of lianas and epiphytes to ensure that the collected arthropods were associated with the individual plant sampled. All arthropods collected were sorted into higher taxonomic levels (order and classes), quantifying their abundance (number of specimens), except for social insects of the orders Hymenoptera (Formicidae) and Blattodea (Isoptera). For both orders, we use its occurrence in the trees as indicative of its abundance – a colony is considered one ecological unit. The sampling was performed bimonthly from January to November, 2019.

Statistical analysis

To characterize the general structure of each habitat assemblage, species abundance distribution (SAD) was graphed with a Whittaker plot (McGill et al., 2007). Additionally, rarefaction and extrapolation curves were elaborated both for the number of arthropods collected (individual-based rarefaction) and for the overall number of trees sampled (sample-based rarefaction) in each habitat. There are extrapolated values 2× the size of each curve, acknowledging that those extrapolated beyond these values can be unreliable (Colwell et al., 2012; Chao et al., 2014). Extrapolations were done considering presence/absence data and abundance data, thus reducing a potential bias caused by rarely sampled species (Chao et al., 2014) with 1 000 randomizations to compare diversity between sampling habitats. In addition, we used non-parametric richness estimators Jackknife 1 and Chao (Colwell & Coddington, 1994), considered the best tools to estimate richness parameters of size-based samples (Palmer, 1990; Walther & Moore, 2005).

To assess whether the order richness, individual abundance, and composition of the communities collected differed between habitats, we ran generalized linear models (GLM; Nelder & Wedderburn, 1972) and permutational multivariate ANOVA (PERMANOVA; Anderson, 2001). The GLM of richness was fitted on quasi-Poisson error distribution, and the abundance model for the most representative orders was fitted on negative binomial error distribution when overdispersion was verified (Bolker et al., 2009). The PERMANOVA was running both a presence/absence matrix and an abundance matrix, using Jaccard index distance and Bray–Curtis index distance for each matrix, respectively. The PERMANOVA results were represented in a 'principal coordinates analysis' ordination (PCoA).

We performed all analyses in the platform R v.4.0.1 (R Core Team, 2019), using the packages iNEXT (Hsieh et al., 2016) for computing individual-– and sample-based extrapolation curves, and vegan (Oksanen et al., 2019) for rank abundance, non-parametric species richness estimation, ordination, and PERMANOVA.

Results

In total, we gathered 8 888 arthropod specimens – 1 157 in January, 2 159 in March, 1 525 in May, 1 587 in July, 1 682 in September, and 778 in November (Table 2). This total was classified into five Arthropoda classes: Insecta (5 813 individuals, 65.4%), Arachnida (2 986, 33.6%), Collembola (66, 0.74%), Malacostraca (19, 0.21%), and Chilopoda (1, 0.01%). The Insecta represented 16 orders in total, with Coleoptera (2 859 individuals), Hymenoptera (1 218), Hemiptera (950), Orthoptera (272), Blattodea (222), Diptera (102), and Lepidoptera (69) the most representative (Figure 3). The Whittaker plot showed a similar pattern of arthropod assemblages between treatments, with a slight progressive change in frequency of the most unusual and unique orders of forest area (Figure 3). From the 25 orders collected, six were exclusively found in the forested area and only one was sampled exclusively in the natural restoration area.

The individual- and sample-based extrapolation curves showed that the patterns for the two habitats were different, with the natural restoration area curve coming to an asymptote (Figure 4). This pattern was confirmed observing the non-parametric richness estimators for forest area (Sobs = 24, Jackknife 1 = 27.97, Chao = 31.95) and natural restoration area (Sobs = 19, Jackknife 1 = 21.98, Chao = 21.98; Figure 4). Notably, with the amount of trees, transects, and temporal replicas, we managed to capture approximately 90% of arthropod orders (except for Chao estimator in forest area, 75.1%) in the two habitats studied. As both estimators for the restoration area estimated the same value, we can say that this area is better represented in our collections in terms of the diversity of arthropod orders than the forest area.

The order richness was greater in the forest area (Figure 5), and only the Hemiptera, Orthoptera, and Blattodea presented significant differences in abundance

Table 2 Total numbers and percentages of arthropods sampled in the foliage shaking technique in January, March, May, July, September, and November 2019 in Paragominas city, Pará, Brazil. The reference columns indicate captures of each order in previous studies. ′+′ indicates capture, ′−′ indicates no capture, and ′?′ indicates uncertainty of the order captured. ′*′ indicates the most abundant orders. ′#′ indicates that the Isoptera order was inserted in the Blattodea order

Class	Orders	Total individuals		Lopes et al. (2019)[1]	Marques et al. (2006)[2]	Basset (2001)[3]	Kitching et al. (1993)[2]	Erwin (1989)[2]
		No.	%					
Arachnida				?				
	Araneae*	2755	31.0		+	+	+	+
	Acari	97	1.09		+	+	+	+
	Ixodida	2	0.023		−	−	−	−
	Opiliones	131	1.47		-	+	+	-
	Pseudoscorpiones	3	0.034		+	+	+	+
	Scorpiones	1	0.011		−	−	+	+
Collembola		66	0.74	+	+	+	+	+
Insecta								
	Archaeognatha	4	0.045	+	−	−	−	+
	Blattodea#	218	2.45	+	+	+	+	−
	Coleoptera*	2859	32.17	+	+	+	+	+
	Diptera	102	1.15	+	+	−	+	+
	Embioptera	5	0.056	−	−	−	−	+
	Hemiptera*	950	10.69	+	+	+	+	+
	Hymenoptera*	1218	13.70	+	+	+	+	+
	Lepidoptera	69	0.78	+	+	+	+	+
	Mantodea	11	0.12	+	−	−	+	−
	Neuroptera	10	0.11	−	+	+	+	+
	Orthoptera*	272	3.06	+	+	+	+	+
	Phasmatodea	8	0.090	−	−	−	+	−
	Plecoptera	1	0.011	−	+	−	−	−
	Psocoptera	59	0.66	+	+	+	−	+
	Thysanoptera	21	0.24	+	+	+	+	+
	Isoptera#	4	0.045	−	+	+	−	+
	Odonata	0	0	−	+	−	−	+
	Trichoptera	0	0	−	+	−	+	+
	Dermaptera	2	0.023	−	+	+	+	+
	Megaloptera	0	0	−	−	−	+	−
	Strepsiptera	0	0	−	−	−	+	−
	Mecoptera	0	0	−	−	−	+	−
	Thysanura	0	0	−	−	−	−	−
Chilopoda						+	?	?
	Scutigeromorpha	1	0.011	−	−			
Malacostraca				?				
	Isopoda	19	0.21		−	+	+	−
	Amphipoda	0	0		−	+	+	−
Diplopoda		0	0	+	−	+	+	+
Gastropoda		0	0	+	−	+	−	−
Total		8 888	100	1 423	15 744		9 967	82 391

Trap method:
[1]Amazonas-trap;
[2]Fogging;
[3]Literature review.

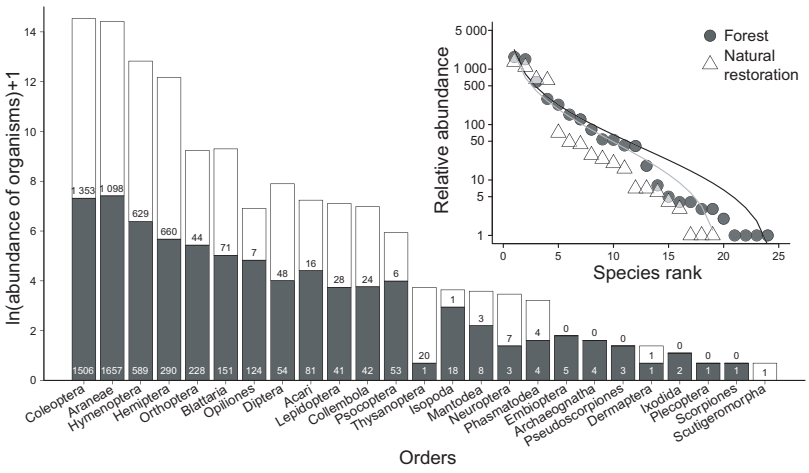

Figure 3 Distribution of the abundance [ln(x)+1] of 25 orders, and in the insert, lognormal rank abundance distribution, for the treatments 'forest area' and 'mined restoration area' (Whittaker plot).

between the forest area and the mined area undergoing natural restoration (Table 3, Figure 6). The sampled order composition differed among the habitat for presence/absence data matrix (PERMANOVA: $F_{1,12} = 5.99$, $P<0.001$; $R^2 = 0.33$) but not for abundance data matrix ($F_{1,12} = 2.08$, $P = 0.13$; $R^2 = 0.14$) (Figure 7).

Discussion

The collecting technique presented in this work can be standardized for arboreal arthropods. This technique was previously used in other studies for collecting entomofauna (Diodato & Fuster, 2016) and spiders (Quijano Cuervo et al., 2019); however, for the first time, here it is being described in detail, standardized, and compared for purposes of capture efficiency. The main advantage of this technique is in collecting arthropods at heights greater than 3 m without using specialized and laborious techniques such as rope climbing (Perry, 1978), fogging (Paarmann & Stork, 1987), or access with cranes (Basset et al., 2003). For the number of orders of arthropods collected (23) compared to other studies conducted in the Amazon forest [14 (Lopes et al., 2019) and 18 (Adis et al., 1998)], we show that this is an effective technique. The capture efficiency is possibly due to changes in the original use of the technique (Quijano Cuervo et al., 2019), such as using a collection bag at the end of the collection tray, which prevents organisms from escaping when the net is lowered.

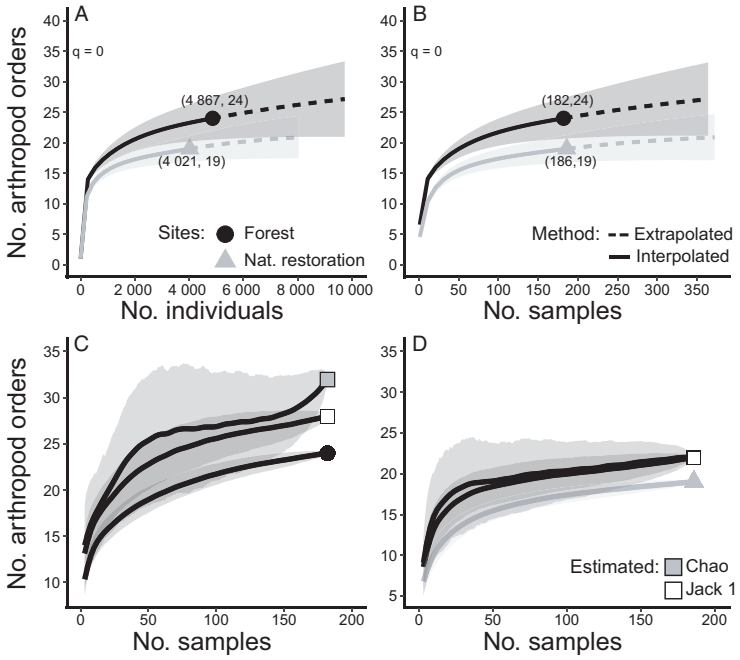

Figure 4 Rarefaction curves for arthropod orders in each habitat: interpolation and extrapolation curves based on (A) individuals and (B) samples, and rarefaction curves to (C) forests and (D) natural restoration area. The symbols indicate the non-parametric richness estimators Jackknife 1 (Jack1, white square) and Chao (gray square).

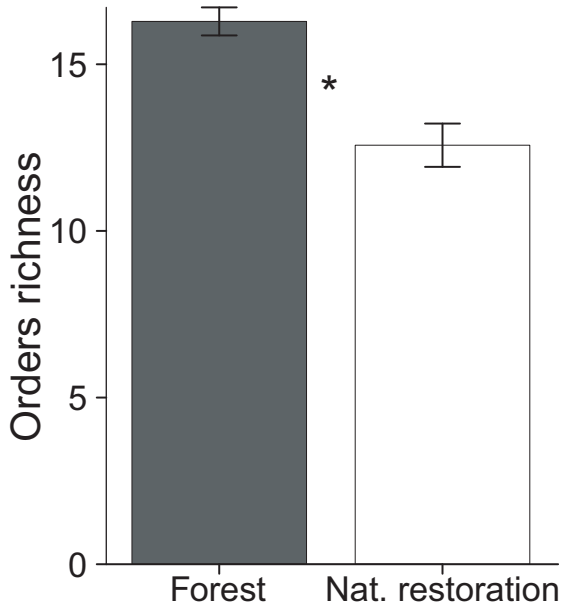

Figure 5 Mean (± SE; n = 14) richness of arthropod orders in forest area and natural restoration area in Paragominas, Pará, Brazil. The asterisk indicates a significant difference between treatments (GLM: $F_{1,12} = 21.59$, $P<0.001$).

Additionally, the use of a collection bag at the end of the collection tray freed us from the need of using other equipment, such as an entomological aspirator, making sampling faster. Another advantage is the use of shaking instead of beating, which could damage some plants, disrupting the habitat being sampled (McCravy, 2018). Therefore, this is an active collection technique that requires two people to execute it, one for the collection tray and one for the shaker stick.

We demonstrate that this technique is robust for making comparisons between habitats, even if we regard superior taxa. Forest sites sustain more richness and different composition than natural restoration areas, and these results are robust once we sample 3× more than recommended for the criteria of restoration success, by Ruiz-Jaen & Aide (2005). These authors suggest that multiple measures to evaluate restoration success, such as diversity, vegetation structure, and ecological processes, should be encouraged in restoration projects. Besides that, the data in this study will be used to assess the ecosystem services provided by interactions between organisms, once the sampled plants are identified. However, we are convinced that the vegetation structure of the natural restoration areas is very recent and at this stage (5 years) the ecological processes associated with restoration are not evident (Crouzeilles et al., 2016; Casimiro et al., 2019). Another important point is that the invertebrates represent the organisms most affected by regeneration processes when compared to mammals, birds, and amphibians (Crouzeilles et al., 2016).

The field tests showed that the arboreal arthropod collector for sampling plant-inhabiting arthropod communities in the tropical forest understory was effective in capturing these organisms. This efficiency was demonstrated as our results resemble those of other work in collecting arthropods that used different methods to assess canopy fauna. We suggest the use of this technique as a substitute for the classical beating tray, for both understory and canopy sampling, as its sampling can be performed at a higher range (from 0.5 to ca. 10 m) more efficiently and quickly than the traditional method. Our results highlight that plants harbor a diverse invertebrate-rich assemblage, whereby the versatility of this technique demonstrates that strata above 3 m can be sampled easily. This increases the sampling range in tropical forests. As such, we suggest that this method can be used with more detailed taxonomic resolution and replicated for the study of arthropod-plant interactions, as well as monitoring insect pests or the long-term monitoring of insect species distribution in response to climate change.

Table 3 Mean (± SE) abundance of the orders of arboreal arthropods in forest areas and mined area undergoing natural restoration in the municipality of Paragominas, Pará, Brazil

			Abundance (no. specimens)		
Order	Deviance	Residual deviance	Forest	Restoration	$Pr(>\chi^2)$
Araneae	1.87	14.74	236.71 ± 33.5	156.86 ± 44.50	0.17
Coleoptera	0.17	14.51	215.14 ± 30.8	193.29 ± 45.69	0.68
Hymenoptera	0.11	14.25	84.14 ± 11.9	89.86 ± 13.80	0.74
Hemiptera	5.56	14.69	41.43 ± 5.4	94.29 ± 32.35	0.018
Orthoptera	25.07	15.75	32.57 ± 4.6	6.29 ± 2.61	<0.0001
Blattodea	9.82	14.20	21.57 ± 4.0	10.14 ± 1.72	0.0017
Diptera	0.10	14.21	7.71 ± 2.2	6.86 ± 2.32	0.75
Lepidoptera	1.03	16.50	5.86 ± 0.8	4.00 ± 1.57	0.31

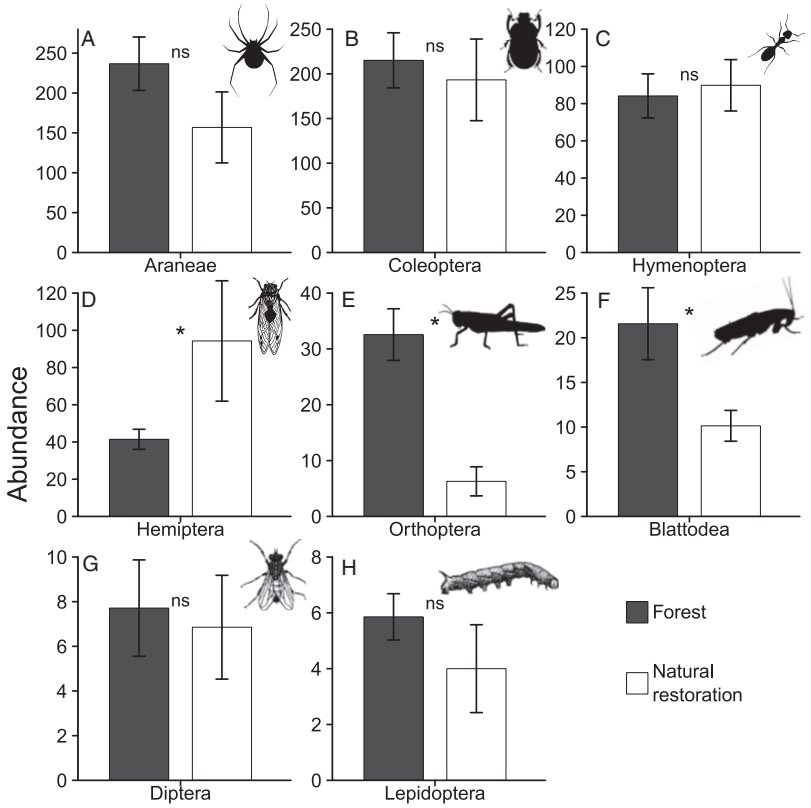

Figure 6 Mean (± SE) abundance (no. specimens) of the eight most representative arthropod orders in forest area and mined area undergoing natural restoration in Paragominas, Pará, Brazil. An asterisk indicates a significant difference between the two treatments (generalized linear models: $P<0.05$; ns, $P>0.05$).

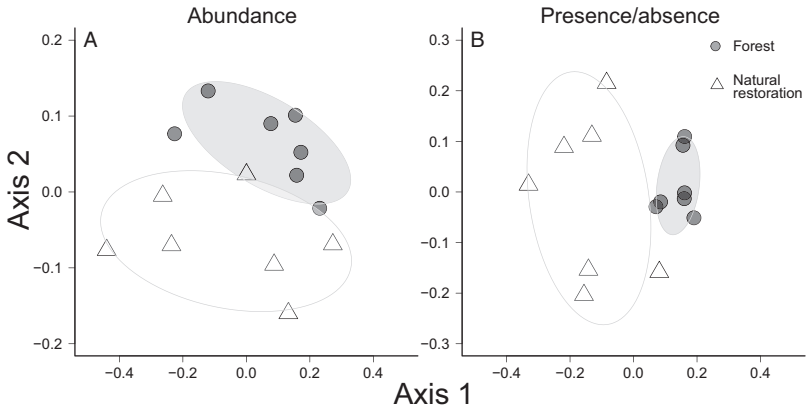

Figure 7 Principal coordinates analysis (PCoA) ordination diagrams of arthropod orders in forest area and mined area undergoing natural restoration: (A) abundance data matrix (Bray-Curtis index distance; $P>0.05$), and (B) presence/absence data matrix (Jaccard index distance; $P<0.05$).

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