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The Amazonas-trap: a new method for sampling plant-inhabiting arthropod communities in tropical forest understory

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Abstract

Methods to quantify plant-insect interactions in tropical forests may miss many important arthropods and can be time consuming and uneven in capture efficiency. We describe the Amazonas-trap, a new method that rapidly envelops the target plant for sampling arthropods. We evaluated the efficiency of the Amazonas-trap by comparing it with two commonly used sampling methods to collect arthropods from plants: the beating tray and manual collection. Samples were collected in 10 permanent plots, in the Ducke forest reserve, Manaus (Amazonas, Brazil). In each plot we sampled 18 plant individuals of *Protium* sp. (Burseraceae): six by a beating tray, six by manual collection, and six using the Amazonas-trap. All insects were identified to the family level and those belonging to the order Hymenoptera were identified to the species and morphospecies level. The new method sampled more insect families and more Hymenoptera species than tree beating and manual collection. Of the 75 total families collected, 20 were sampled exclusively by the Amazonas-trap, seven were only collected with a beating tray, and seven were sampled exclusively with manual collecting. A similar pattern was found for abundance: Amazonas-trap sampled more individuals, followed by the beating tray and manual collection. Small and winged arthropods were more abundant in Amazonas-trap, explaining the highest richness of Hymenoptera and insect families sampled with this method. The new method sampled more spiders, wood-fungi feeders, sap suckers, omnivorous, parasitoids, and insect predators than the other methods, but was equally effective in sampling leaf-feeders and ants. Amazonas-trap was more time consuming in the field, but for all diversity parameters evaluated, the new method showed better performance for collecting invertebrates on plants.

Introduction

Plants represent a primary resource and the base of complex interactive food web networks in terrestrial ecosystems. Herbivorous insects are the most abundant and diverse group of organisms generally found on vegetation (Strong et al., 1984). Plants also harbor other important arthropod functional groups, such as predators that use plants as substrate to find their prey (Wise, 1993), decomposers that find shelter on plants (Santos et al., 2003), parasitoids of eggs and larvae (Fernandéz & Sharkey, 2006), and ants that nest in domatia or forage for food on extrafloral nectaries (Oliveira & Brandão, 1991). Therefore, every plant individual supports assemblages of arthropods from many trophic levels and represents an

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appropriate ecological unit to investigate the occurrence, diversity, and interactions among arthropod communities (Farrell et al., 1992). A group of plants, in the same way, can be used in ecological studies of coevolution or insect-plant interaction, provided that appropriate collection methods are applied.

The group of individuals that interact with a plant and its associated fauna was initially called the 'component community' (Root, 1973). However, this term is rarely used in the recent literature, perhaps because of the difficulty of exhaustively sampling all invertebrates on a plant at the same time. The primary challenge in sampling arthropods on plants is that flying insects represent most of the arthropod diversity associated with plants. Comprehensive methods for sampling flying insects in the forest understory (e.g., Malaise traps and windowpane traps; see Lamarre et al., 2012) are broadly used to relate the insect community to the plant community in a given forest habitat (Lamarre et al., 2016). These methods produce broad surveys of arthropod composition, but they cannot be performed at the level of an individual plant. Malaise-based studies can therefore only indirectly link the arthropod diversity with local habitats. In studies focusing on arthropod-plant interactions at the level of individual plants, a method of active sampling (beating tray or manual collection) is more often employed (Basset & Novotny, 1999).

The beating tray technique allows fast and practical sampling of invertebrates resting or feeding on plants and can be considered as a selective method, as the insects falling from the vegetation are mainly wingless or less mobile (Ozanne, 2005). Manual collection, although widely used, does not sample the entire arthropod community present on a plant. Small individuals or camouflaged/cryptic species may not be noticed by the collector, and more active species have a high probability of escape. Consequently, the sampled fauna depends partly on the ability and the experience of the collector, which can create significant bias that in turn can be challenging to standardize for comparison among sites and studies (Basset et al., 1997). Each method produces its own result and no method is efficient enough to exhaustively sample invertebrate communities on a plant at a single time. Choosing the method of collection depends on the purpose of the study and on the targeted groups of arthropods.

In this study we describe the Amazonas-trap, a new method for exhaustively collecting plant-inhabiting arthropod assemblages in the tropical rainforest understory. This method, developed and designed by GPA Lamarre, consists of a rapid and complete bagging of juvenile individuals, and differs from methods that involve removing or enveloping branches or other plant parts only (e.g., foliage bagging; Ozanne, 2005). To evaluate the performance of the new method, we compared the structure and composition of the most abundant insect communities found on tropical plants, either sampled with the Amazonas-trap, or with the most widely used sampling methods: the beating tray and manual collection. We compared the method's performance at both the plant and the plot scale, which are the most common scales used in insect-plant interaction studies. For a more comprehensive evaluation of the Amazonas-trap, we also compared the time spent during survey among the three sampling methods.

Materials and methods

Concept of the Amazonas-trap

The trap is made of white polyester (100%), a light and resistant fabric measuring 3 m long and 3 m wide (Figure 1; see also the photographs in Figure S1). The two lateral sides of the fabric have a velcro strip along the entire length of the trap. The bottom part of the fabric is folded and sewn to form a hem of 3 cm wide, through which a rope is inserted. The top part of the fabric also has a hem, of 5 cm wide, through which a weldable PVC pipe of 2 cm diameter and 1.5 m long is inserted. In one of the ends of the pipe a three-way T-shaped connector (2 cm diameter) is attached with bolt and nut. During installation, the other end is inserted and manually screwed into the connector, forming a circle with the collector around the plant, and an aluminum tube (2 cm diameter, 2.5 m long) is also attached to this connector. A 5-m rope with a loop knot at the tip is inserted into a hem sewn 15 cm below the top of the collecting cloth.

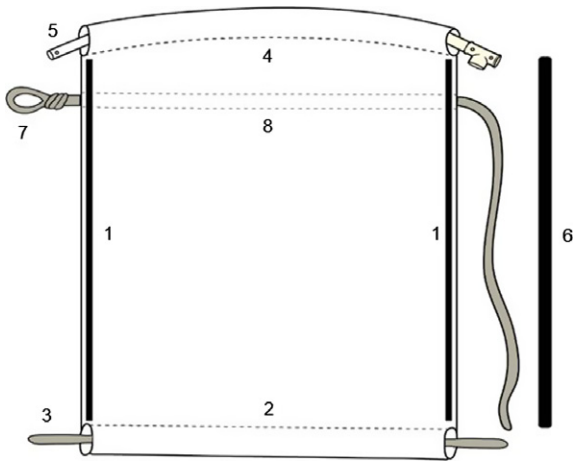

Figure 1 General structure of the Amazonas-trap: (1) velcro strips, (2) bottom hem, (3) rope, (4) top hem, (5) PVC pipe, (6) aluminum tube, (7) rope with loop knot, and (8) rope hem with loop knot.

Installation and sampling procedure

The trap is designed to sample free-standing plants of 1–3 m high that are at least 0.5 m from neighboring plants. The trap is placed on the ground around the target plant and the bottom part is tightly attached to its lowest branch (Figure 2A). The weldable PVC pipe is also placed around the plant with the two end-sections joined together forming a circle over the trap around the plant. The velcro is tightly closed throughout its entire length, from the top to the bottom, preventing escape by any arthropods. The end of the rope is passed through the loop knot. Finally, the aluminum tube is joined into the connector (Figure 2B). As the installation of the trap may create some disturbance, chasing away insects, in this test the target plant was left alone for 2 min before the trap was activated, allowing the invertebrate fauna to return to the target plant. Meanwhile, other traps may be installed on other target plants. Longer recovery time is probably more effective than the 2 min used here.

With the Amazonas-trap installed the activation is conducted as follows. First, the entire structure of the trap is energetically pulled up by lifting the device upwards with the help of the aluminum tube in order to completely envelope the target plant. Simultaneously the rope is pulled to close the top of the trap (Figure 2C). All invertebrates present on the plant are now trapped.

With all invertebrates trapped, the sampling procedure can start. A small opening of the velcro allows the collector to stick in her/his hand and vigorously shake the whole plant usually by holding the most solid part of the trunk to avoid physical damage (Figure S1D). All plant-inhabiting arthropods fall into in the lower part of the trap, or are resting in the fabric allowing very easy inspection by eye and collection using an aspirator in the trap (Figure S1E, F).

Testing Amazonas-trap performance

We compared the performance of the Amazonas trap with two classical sampling methods, the beating tray and manually collecting, based on intensive field sampling at the Ducke Forest Reserve (02′55′–03°01′S, 59°53′–59°59.5′W), located in Manaus (Amazonas, Brazil). Reserva Ducke is a 10 000-ha rainforest reserve covered by typical 'terra-firme' forest on moderately rugged terrain (elevation 50–120 m a.s.l.). The climate is tropical humid with a mean (± SD) annual temperature of 26 ± 3 °C and mean annual precipitation of 2.2 m, which varies seasonally (Marques-Filho et al., 1981).

The surveys took place in 10 previously installed permanent plots, at least 1 km apart (Magnusson et al., 2013). The plots were distributed over 10 km^2 and cover the natural environmental variation found at Ducke: from the clay poorly drained soils in the valleys to the clayed well-drained soils on the plateaus. These plots represent a gradient of local environmental conditions (Oliveira et al., 2008). In each plot, we selected, marked, and identified 18 *Protium* sp. tree saplings (Burseraceae) of 1.5–2.5 m high. *Protium* is a widespread and locally abundant tropical

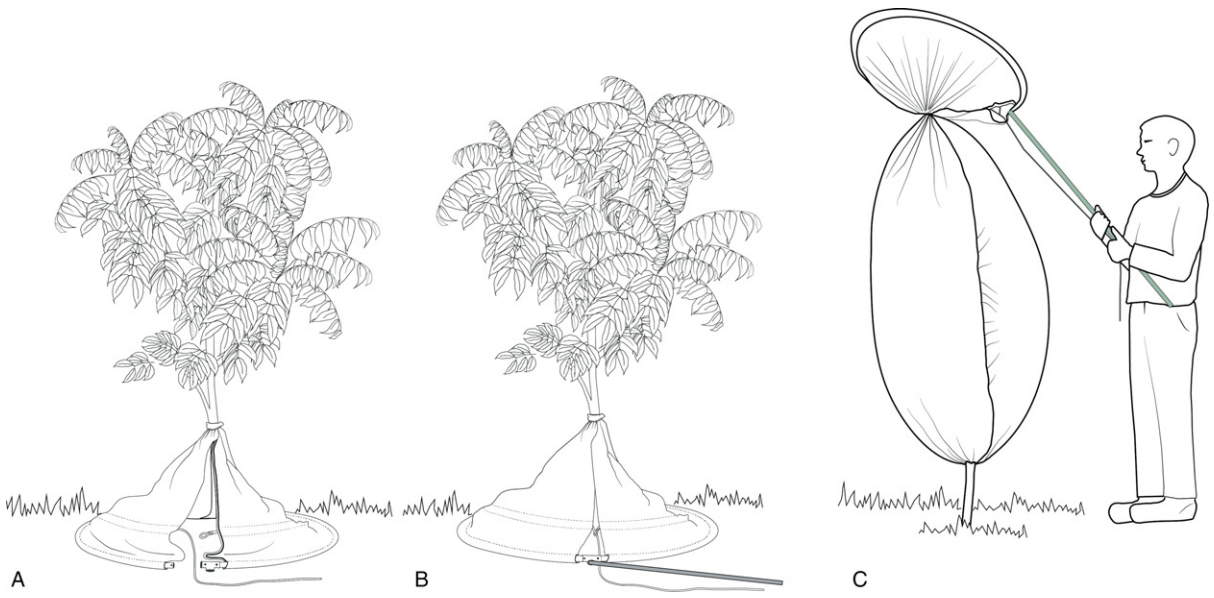

Figure 2 Installation of the Amazonas-trap: (A) tying on the plant stem, (B) closing up of velcro and attaching the aluminum tube, and (C) lifting and closing up by the collector.

genus that occurs across the environmental gradient. We focus on one plant lineage to minimize the influence of variation of plant secondary compounds on insect assemblages (Salazar et al., 2018). The field work was carried out in March and April 2016.

Sampling procedure

We sampled arthropod communities on focal plants using (1) the classical beating tray, (2) manual collection, and (3) the new Amazonas-trap. Each method was used on six *Protium* plant individuals per plot. For the beating tray, individual plants were agitated above a standard-size collection cloth (1 × 1 m) and the invertebrates were collected using an entomological aspirator. This process was repeated until no more invertebrates fell on the cloth. For the manual collection, the plants were carefully inspected until all observed individuals were collected using entomological aspirator and forceps. This method was always performed by the same collector. The Amazonas-trap collection was performed as previously described. The time taken to sample the individual plants was measured for each sampling method.

All insects collected were identified to family level. Insect family level provides a practical diversity resolution, sufficient for detecting taxonomic and functional patterns of assemblage composition in tropical forests (Lamarre et al., 2016). We also sorted Hymenoptera (the most abundant order sampled) further to species/morphospecies level (Fernandéz & Sharkey, 2006; Rafael et al., 2012; Baccaro et al., 2015). The specimens belonging to the classes Arachnida, Malacostraca, Chilopoda, and Diplopoda were classified at the order level. Finally, to examine ecological correlates of invertebrate assemblages, we grouped all invertebrates sampled into guilds based on the feeding habits and taxonomy of adults (Moran & Southwood, 1982; Basset & Arthington, 1992; Rafael et al., 2012; Lamarre et al., 2016). The following guilds were erected: ant, spider, insect predator, leaf-feeder, sap sucker, wood-fungi feeder, omnivorous, and parasitoid.

Statistical analysis

Rarefaction curves were constructed for the number of insect families or Hymenoptera species sampled by collection method per plant. Also, we constructed accumulation curves for Hymenoptera species and insect families over the total time of each sampling method. The 95% confidence interval was estimated by the Mao-Tau method that does not collapse around the mean at the highest values (Colwell et al., 2004). This approach permits the comparison of rarefaction curves with higher shared sampling effort.

Subsequently, the number of insect families, Hymenoptera species, and the total number of individuals sampled by each method per plot were compared by ANOVA. For both matrices, number of insect families or Hymenoptera species and the abundance of individuals per plot were the dependent variables, and collection method was the independent variable. We used the same analytical scheme to compare the guild abundance between methods. Comparisons among sampling methods were made with Tukey's post hoc test.

The composition of the communities captured with each collection method (families of all captured insects and Hymenoptera species) were compared by multivariate ANOVA by permutation (PERMANOVA), based on a dissimilarity matrix generated by the Bray–Curtis index (Anderson, 2001). In all analyses, the sampling unit was the plot, and the results were based on 999 permutations. Simple ordering plots were created to present the composition and identity of the taxa (family and hymenopteran species) sampled by each method. We also used a PERMANOVA to test possible bias toward plant species secondary compounds. In this analysis, we compare the composition of *Protium* species sampled by each method per plot, based on Bray–Curtis distance. All analyses and graphs were done in R v.3.4.4 (R Core Team, 2017). All data generated during this study are included in Table S1.

Results

Overall, 21 *Protium* species were sampled. Even with the high number of replicates (180), ca. 62% (13) of *Protium* spp. were sampled by at least two of the sampling methods. The remaining eight species were sampled by only a single method. However, possible differences in arthropod composition related with plant species secondary defenses were minimized given that the number of unique *Protium* species sampled were quite similar among sampling methods: beating tray (3), manual collection (2), and Amazonas-trap (3). In addition, the *Protium* assemblage composition per plot was similar between sampling methods (PERMANOVA: $F_{2,27} = 0.478$, $P = 0.96$).

We collected in total 1 423 arthropod specimens among the four main classes: Hexapoda (n = 1 039), Arachnida (365), Diplopoda (14), and Malacostraca (5). Hexapoda was the most abundant and species-rich group collected in the understory of Amazonian tropical forests, representing a total of 11 orders and 75 families. The most abundant order was Hymenoptera (390 individuals), mostly composed of Formicidae (349 individuals), followed by Collembola (272 individuals) and Coleoptera (175 individuals).

The Amazonas-trap sampled on average (± range) ca. 8 ± 4 families more than beating tray and manual collection per plot (ANOVA: $F_{2,27} = 28.2$, $P<0.001$). For

Hymenoptera, the Amazonas-trap sampled on average ca. 4 ± 2 more species compared with other methods per plot (ANOVA: $F_{2,27} = 10.47$, $P<0.001$), whereas the beating tray and manual collection sampled a similar number of Hymenoptera species per plot (Tukey's test: $P = 0.26$). At site and individual plant scales, the Amazonas-trap sampled more families and Hymenoptera species than the other two sampling methods (Figure 3).

This pattern was even stronger when considering insect abundance. The Amazonas-trap sampled more individuals per plot than the other two methods together (ANOVA: $F_{2,27} = 14.08$, $P<0.001$). The number of insects sampled (abundance) per plot was similar between the beating tray and manual collecting (Tukey's test: $P = 0.19$). The Amazonas-trap sampled 617 individuals, followed by the beating tray with 295 individuals, and manual collection with 127 individuals sampled.

The composition of the sampled families differed among the three collection methods (PERMANOVA: $F_{2,27} = 3.74$, $P = 0.001$). Of the total of 75 families collected, 20 were sampled exclusively when using the Amazonas-trap, seven were only collected with beating tray and seven with manually collecting (Figure 4). However, there was no difference in the composition of the sampled Hymenoptera species among the collection methods (PERMANOVA: $F_{2,27} = 1.01$, $P = 0.41$). Formicidae was the most representative family in all methods. Of the 27 species of Hymenoptera manually collected, 24 were Formicidae, whereas of the 32 species sampled with the beating tray, 26 were Formicidae. For the Amazonas-trap 60 species of Hymenoptera were collected, including 40 Formicidae species (Figure 5).

The Amazonas-trap sampled more spiders (ANOVA: $F_{2,27} = 30.11$), wood-fungi feeders ($F_{2,27} = 10.83$), sap suckers ($F_{2,27} = 13.62$), omnivorous ($F_{2,27} = 13.99$, all $P<0.001$), parasitoids ($F_{2,27} = 6.79$, $P = 0.004$), and insect predators ($F_{2,27} = 17.38$, $P<0.001$) than the other methods (Figure 6). However, the number of ants (ANOVA: $F_{2,27} = 2.08$, $P = 0.15$) and leaf feeders ($F_{2,27} = 2.19$, $P = 0.14$) were similar among the three sampling methods. The manual collection sampled fewer individuals for all guilds, except for leaf-feeders (Figure 6).

The Amazonas-trap was more time consuming in the field, taking ca. 3× longer to sample the same number of plants (Figure 7). On average, 11.4 arthropods were sampled per min using the beating tray, 8.3 per min using the Amazonas-trap, and 4.1 per min with manual collection. Although manual sampling collects hymenopteran species faster, the three methods accumulate practically the same number of insect families per unit of time (Figure 7).

Discussion

We described the Amazonas-trap, a new collection method able to comprehensively sample the arthropod community associated with plants. We also compared the

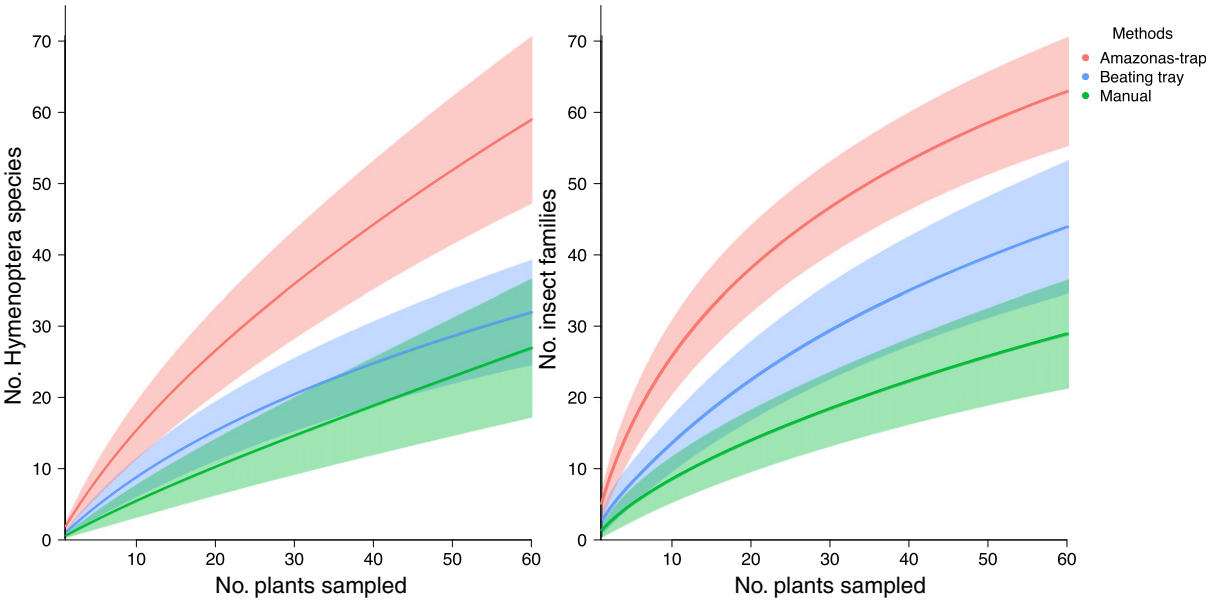

Figure 3 Rarefaction curves for Hymenoptera species and insect families sampled with Amazonas-trap, beating tray, and manual collection. The continuous lines represent accumulation and the polygon areas represent 95% confidence intervals. [Colour figure can be viewed at wileyonlinelibrary.com]

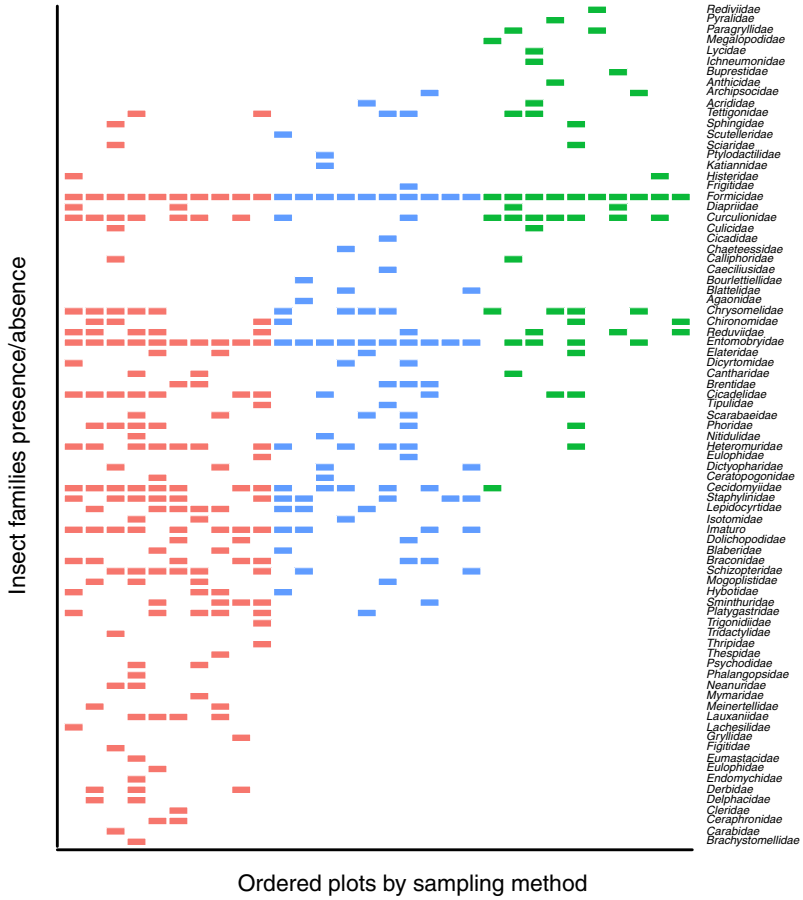

Figure 4 Distribution of insect families sampled with the three collection methods on *Protium* saplings. The columns represent the plots. The 10 columns on the left (red bars) represent the Amazonas trap, the 10 columns in the middle (blue bars) the beating tray, and the 10 columns on the right (green bars) the manual collection. [Colour figure can be viewed at wileyonlinelibrary.com]

Amazonas-trap performance with that of two other often used sampling methods and found that our new method proved to be better than the methods traditionally used for most parameters tested. The new Amazonas-trap collected more species of Hymenoptera, more families of insects, and more individuals from most guilds than the widely used tree beating and manual collection. Although somewhat more time consuming, the Amazonas-trap sampled similar numbers of individuals and species per unit of time compared with the other two methods. Overall, the Amazonas-trap provided a more accurate and exhaustive picture of the plant-inhabiting insect assemblages in this lowland Amazonian rainforest.

The entomological beating tray, a widely used method for insect sampling on plants (Ozanne, 2005), collected nearly half of the species of hymenopterans and ca. 70% of the insect families sampled by the Amazonas-trap. It is likely that during the physical disturbance of the plant by the beating tray, agile specimens escape, reducing efficiency. Winged insects and jumpers are particularly difficult to be sampled when using the beating tray. Entirely enclosing the plant allows the collection of winged and fast-moving insects, explaining the overall greater abundance and richness of the insect families and in particular of Hymenoptera species sampled by the Amazonas-trap.

Very small insects can also be sub-sampled by beating tray or manual sampling. For instance, manual collection yielded the smallest number of individuals, species, and families sampled, which is probably related to the difficulty of capturing agile, cryptic, or very small specimens directly on the plant. However, entirely enclosing the plant allows the collection of very small or cryptic individuals, even without seeing them in the field. That happens because all the fine plant material that accumulates inside the trap, together with the invertebrates, can be sampled with an insect aspirator and then sorted under a stereomicroscope. The families Mymaridae (Hymenoptera) and Thripidae (Thysanoptera) are examples of small insects that are relatively difficult to observe and that were exclusively sampled with Amazonas-trap.

The composition of Hymenoptera species in all three methods was dominated by ants, which is one of the most abundant groups in tropical forests and relatively easy to collect with each of the three methods. Winged

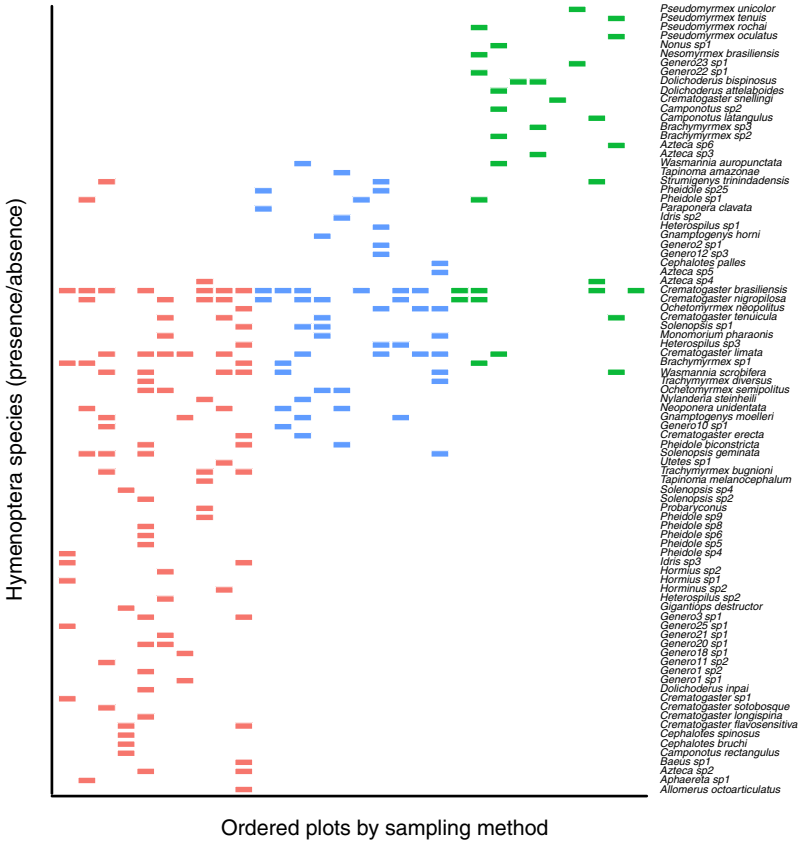

Figure 5 Distribution of Hymenoptera species sampled with the three collection methods on *Protium* saplings. The columns represent the plots. The 10 columns on the left (red bars) represent the Amazonas trap, the 10 columns in the middle (blue bars) the beating tray, and the 10 columns on the right (green bars) the manual collection. [Colour figure can be viewed at wileyonlinelibrary.com]

Hymenoptera, such as parasitoids, on the other hand, were more common in the Amazonas-trap than in the other methods because of the increased facility of collecting winged individuals. However, how many species were not sampled by beating and manual sampling is not a simple question to answer. There were only five winged hymenopteran species collected with the beating tray and three by visual inspection. For the Amazonas-trap this bias was minimized, as we collected at least 15 winged Hymenoptera species.

Overall, the number of unique families collected was also higher using the new trap than the other methods. For instance, orthopterans that are highly mobile individuals were only sampled using the Amazonas-trap. Other herbivorous winged, agile, and sometimes difficult to collect individuals, such as sap-suckers, were also more abundant in the Amazonas-trap. However, the abundance of overall leaf-feeders (mainly orthopterans and Coleopterans) did not differ among the sampling methods. Therefore, depending of the herbivorous taxa, entirely enveloping the plant may not necessarily be a better option.

Sorting plant debris under the stereomicroscope also substantially increased the efficiency of the new method for wood-fungi feeders. This guild is composed mainly by small cryptic individuals, such as Collembola and Psocoptera that live under the bark of the trees and between decomposing fine organic matter. Collembola are very difficult to observe and collect manually due to their small size and agility. For comparison, in the Amazonas-trap samples, 188 springtails were collected, compared to 79 individuals sampled by beating tray and eight individuals sampled by hand (Table S1).

Although not directly included because of the lack of knowledge of arthropod taxonomy in tropical forests, the collection of spiders and mites indicated very promising results using the new sampling method. The Amazonas-trap sampled more spiders than the other methods. In fact, the number of spiders sampled with the Amazonas-trap was similar to the number of ants, which is regularly cited as the most abundant invertebrate taxon on plants (Stork, 1988; Ellwood & Foster, 2004). Other predators were also better sampled, suggesting that the Amazonas-trap provides more robust and comprehensive pictures of insect-plant assemblages in hyper-diverse tropical forests.

Despite the higher efficiency, one limitation of the new insect trap proposed is the time of installation; it takes more time than the two other methods. However, the greater time spent installing and using the

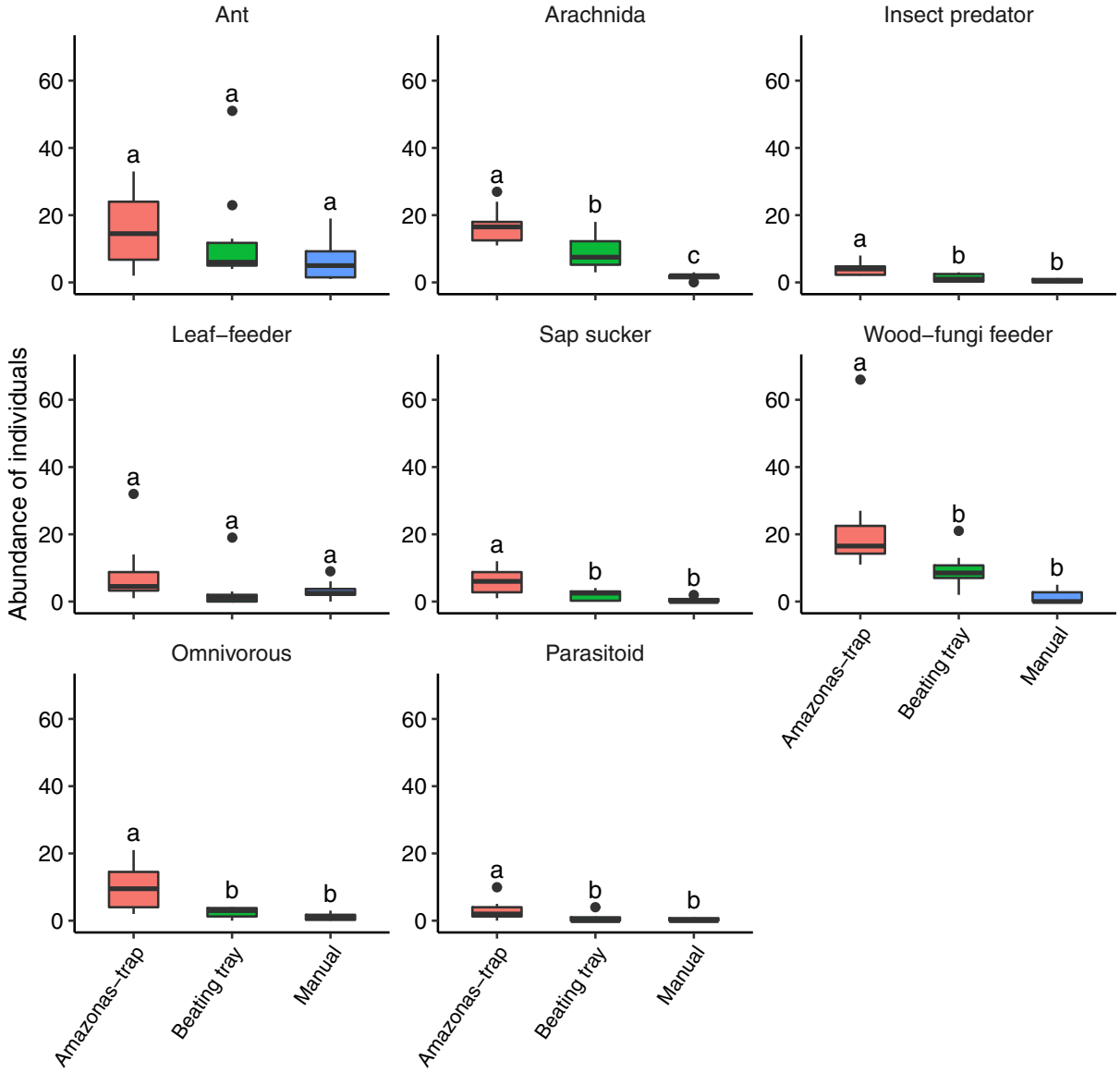

Figure 6 Abundance (no. individuals) of eight arthropod functional groups found on *Protium* saplings sampled with the three collection methods: Amazonas-trap, beating tray, or manually. Means within a panel capped with different letters are significantly different (Tukey's post hoc tests: $P<0.05$). The boxes and whiskers represent the 50th and 95th percentile around the median (bold line), respectively. The dots indicate outliers. [Colour figure can be viewed at wileyonlinelibrary.com]

Amazonas-trap is balanced by the collection of more individuals and species per plant. The results from the rarefaction curves per unit of time indicated that the three collection methods are equally time-efficient, accumulating the same number of insect families per unit of time. For Hymenoptera species composition, the efficiency of the three methods was also equivalent. This result is probably due to the ease of collecting Formicidae by the three methods, which was the richest Hymenoptera family in this study.

The new Amazonas-trap was the most effective sampling method tested for plant-associated invertebrates in this hyper-diverse tropical forest understory. Our results indicate that plants harbor a diverse invertebrate-rich assemblage, which may generally be undersampled when using the traditional sampling methods. The Amazonas-trap has great potential to be used for targeted collection for behavioral studies, through observation of live insects in the trap, and also for ecological studies (community or population), as well as for the study of relations between

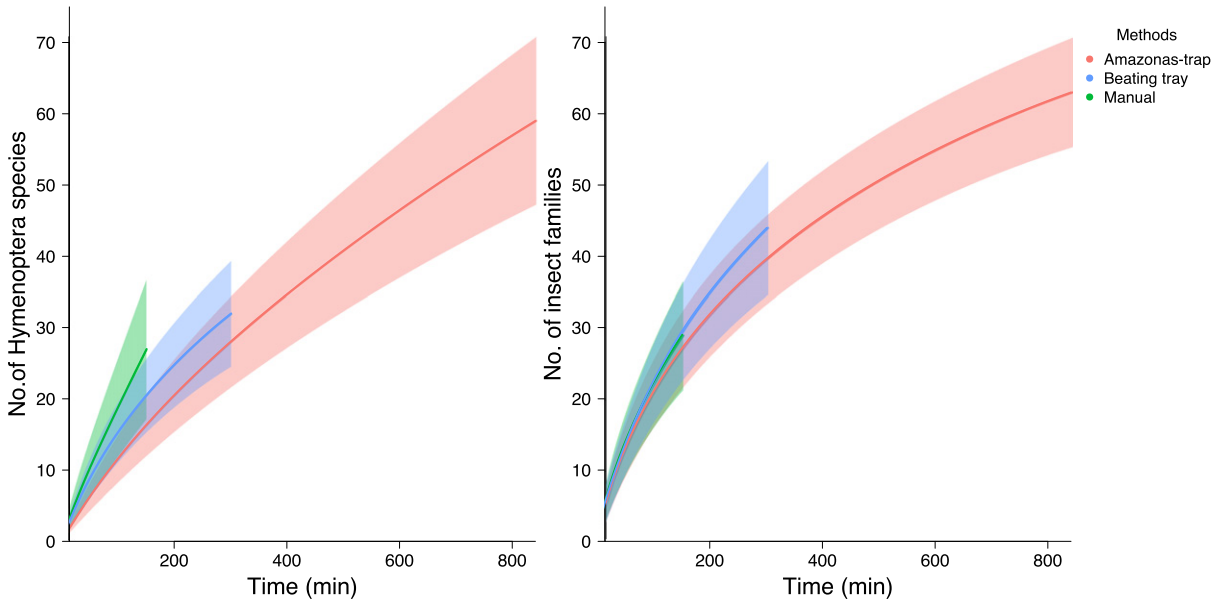

Figure 7 Rarefaction curves for Hymenoptera species and insect families sampled with Amazonas-trap, beating tray, and manual collection, per unit of time. The continuous lines represent accumulation and the polygon areas represent 95% confidence intervals. [Colour figure can be viewed at wileyonlinelibrary.com]

herbivorous insects and their host plant architecture, chemical profiles, and anti-herbivore functional traits. The new method may also be applied in the monitoring of insect pests, studies on interaction between herbivores and parasitoids (e.g., for biological pest control), and finally for long-term monitoring of insect species distribution in responses to climate changes (Basset et al., 2017).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Installation and procedure of the Amazonas-trap. (A) Tying on the plant stem. (B) Closing of velcro and installation of the aluminum tube. (C) Lifting and closing up of the collector. (D) Agitation of the plant. (E, F) Collection of specimens. This test tree is 3.5 m tall and 2 m in diameter.

Table S1. Abundance of arthropod classes, insect families, and hymenopteran species in 180 *Protium* plants distributed in 10 plots at Reserva Ducke, Manaus, Brazil.