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Complementarity among sampling methods for harvestman assemblages

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ABSTRACT

Tropical arthropod surveys generally use a combination of complementary sampling methods to increase the detection of species and individuals, and to decrease the number of singletons. However, given the high arthropod abundance and the taxonomic challenges of arthropod surveys, the combination of different sampling methods may be inefficient and may increase survey costs. Harvestmen were sampled using beating tray, nocturnal search, leaf-litter manual sorting and Winkler apparatus in 70 plots distributed in two areas in Central Amazonia. Every sampled method documented different assemblages, and only the nocturnal search method proved to be efficient in representing both harvestmen richness and composition. Given the data collected from leaf-litter manual sorting, Winkler apparatus and beating tray can be used in inventories to increase the number of species collected, but may be less useful for applied or monitoring studies. Although pooling data from three methods was effective to obtain an overview of species richness, it may not be the more efficient strategy for studies of assemblage associations with environmental variables. As each method may sample distinct assemblages that have different responses to the surrounding environment, pooling data from these different methods may obfuscate patterns of assemblage composition related to environmental factors instead of clarifying them.

Introduction

Biological surveys play an essential role in studies of systematics, taxonomy, ecology and conservation biology. A robust survey is necessary to provide information of species occurrence that can be used for comparisons between different areas if both, sampling methods and effort are standardized (Longino et al. 2002). Arthropod surveys are usually designed to estimate species diversity and facilitate taxonomic investigations using a combination of complementary sampling methods that aim to increase detection of individuals and decrease the number of singletons; (=species represented by only one specimen) (Coddington et al. 1991, 2009; Scharff et al. 2003). However, given the high abundance and the taxonomic challenges of species identifications for most arthropod groups (Lawton et al. 1998), researchers often face a trade-off between sampling intensity and laboratory work to meet project deadlines (Gardner et al. 2008; Souza et al. 2012).

Arachnids have strong relationships with microhabitats (Mestre and Pinto-da-Rocha 2004; Curtis and Machado 2007; Proud et al. 2012). Therefore, comprehensive arachnid surveys often use several sampling methods to access different types of microhabitats. For at least two decades attempts have been made to find the most useful and efficient protocol to be applied in arachnid surveys (Coddington et al. 1991, 2009; Pinto-da-Rocha and Bonaldo 2006; Carvalho et al. 2011). Six methods (hand-searching, beating trays, sweep nets, pitfall traps, litter sifting/extraction and bark/log fragmentation) are normally chosen as the basic methods to sample arachnids in the tropics (Coddington et al. 1991), but detailed analysis of their performance have only been undertaken for spider assemblages (but see Pinto-da-Rocha and Bonaldo 2006).

Most tropical arachnid surveys have sampled spiders and harvestmen together, using the combination of several sampling methods (Bragagnolo and Pinto-da-Rocha 2003; Pinto-da-Rocha and Bonaldo 2006; Bragagnolo et al. 2007; Bonaldo et al. 2009). Using several sampling techniques at the same time may be useful to maximize estimates of species richness. However, a combination of several sampling methods also increases the types of microhabitats explored, the field effort and time spent, consequently increasing field work complexity and possibly reducing survey efficiency.

As is true for many taxonomic groups, harvestmen are considered good models for biogeographic and conservation studies because they have specific biological requirements that limit their species distributions (Giribet and Kury 2007; Curtis and Machado

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2007). Harvestmen species are susceptible to dehydration, being highly dependent on humidity and temperature, and normally have low dispersal ability (Pinto-da-Rocha et al. 2007). In addition, the local diversity of harvestmen assemblages in the Neotropics is relatively low compared to other invertebrate groups (ranging from 12 to 52 species), which makes species sorting faster (Kury 2011). Historically, the combination of nocturnal search, beating tray, leaf-litter manual sorting and Winkler apparatus have been employed to represent harvestman and spiders alpha diversity (number of species per sampling area) and assemblage composition in the Amazon forests (Pinto-da-Rocha and Bonaldo 2006; Venticinque et al. 2008; Bonaldo et al. 2009; Rego et al. 2009). Although several studies compared the utility of different sampling methods for spiders in different regions of the world (Cardoso et al. 2008; Coddington et al. 2009), sampling method efficiency and harvestmen assemblage response to small-scale differences in habitat structure is still poorly studied (e.g. Pinto-da-Rocha and Bonaldo 2006; Proud et al. 2012).

Sampling designs and methods depend on the research questions being posed, and therefore, sampling the largest number of species may not always be crucial. This paper discusses the results and major implications of pooling the data collected by four sampling methods for harvestman assemblages in two locations. Specifically, (i) we compared the number of species and composition of harvestmen sampled with nocturnal search, beating tray, leaf-litter manual sorting and Winkler extraction with all combined data, and (ii) we also evaluated whether the assemblage composition obtained using one technique responded similarly to combined sampling techniques to several environmental descriptors.

Materials and methods

Study sites

The study was conducted at the Experimental Farm of the Federal University of Amazonas – FEX-UFAM, located near the BR 174 highway, 38 km north of Manaus (02°39′ S, 60°07′ W), and at Porto Urucu, a petroleum/natural gas production facility belonging to Petrobras S.A. located in Coari municipality, on the north side of the Urucu River, Solimões River basin (4°30′ S, 64°30′ W), 650 km west of Manaus (Fig. 1). At FEX-UFAM, the mean temperature ranges from 24.1 °C to 27.1 °C and the relative humidity may vary between 75% in August, the driest month of the year, and 95% during the peak of rainy season, in April (Oliveira et al. 2011). Total annual rainfall is about 2200 mm, and the monthly rainfall may be less than 100 mm between July and September (Luizão et al. 2004). The Experimental Farm is covered by an old-growth upland forest, with altitude ranging between 40 and 130 m a.s.l. The study site includes areas of plateau, slope, floodplains and *campinaranas*, which reflect the topography, soil type and vegetation composition (Ribeiro et al. 1999). *Campinaranas* are sandy plains that may have large accumulations of litter, both near streams and in areas of higher altitude. The plateaus have clay soils with good drainage and harbor the tallest trees, reaching up to 60 m. The lowland areas close to the streams have sandy soils, which sometimes can be flooded during the rainy season. Slopes show intermediate values of soil physical characteristics between the plateaus and areas around streams (Hopkins 2005).

At Porto Urucu the annual mean temperature is 25.9 °C, but it ranges from 26.4 °C to 25.5 °C, and the relative humidity is 91%. The area is covered by undisturbed dense forests, with uniform canopy, presenting low diversity of lianas and epiphytes (Lima Filho et al. 2001). The phytophysionomic diversity in Urucu is characterized by uniformity, with a few flooded areas ("*igapó*" forests) occurring on the banks of the Urucu River or its main tributaries. Notable changes in the vegetation structure occur only in areas with poor soil drainage or in artificial forest gaps, opened in the dry land for natural gas and oil exploitation. Sampling was undertaken in artificial forest gaps. Artificial gaps were formed by removal of soil material for the construction or maintenance of the Porto Urucu road network.

Sampling design

At FEX-UFAM data were collected in 40 permanent plots along the Brazilian Research Program for Biodiversity (PPBio) trail grid. The trail grid covers 24 km^2 and the spatial sampling design followed the RAPELD system (Magnusson et al. 2005; Costa and Magnusson 2010). The plots are 250 m long and follow the topographic contour lines to minimize variation in soil type and topography (Pezzini et al. 2012). Twenty plots were sampled in riparian and 20 in non-riparian areas (Fig. 1). At Porto Urucu, 33 artificial forest gaps were sampled during three months (between June and November, 2006). Each forest gap contained one sampling plot (Fig. 2).

Sampling, techniques and identification

We used manual sorting of leaf-litter, beating trays and nocturnal search to sample harvestmen in FEX-UFAM. Sampling was undertaken in four 30 m × 5 m sub-plots, separated by 5–10 m, along the main axis of the 250-m plots. In each plot, 2 m^2 of litter was harvested, placed in plastic bags and all harvestman were manually collected. A beating tray consists of a cloth that is usually stretched out using a frame. The frame is held under a shrub and the vegetation foliage is shaken, the arthropods fall from the shrubs on the cloth and are subsequently collected manually by the collector. Beating trays were placed under 20 shrubs up to 3 m tall during the day in each sub-plot. The vegetation above each tray was struck 20 times with a wooden stick. Night visual search was undertaken between 19:00 and 02:00. One collector searched for arachnids in each sub-plot during 1 h. We made six 2–14 day expeditions between April and October 2010. Overall, 80 m^2 of litter was manually sorted and 800 trees distributed in 24,000 m^2 were sampled using beating trays. Total sampling effort employed during nocturnal search lasted 40 h and covered 24,000 m^2 at FEX-UFAM.

At Porto Urucu, the harvestmen were sampled using Winkler apparatus, beating tray and nocturnal search. At each sampling location, 10 m^2 of litter were sifted and placed in Winkler extractors for two days (48 h). Beating was standardized by time: 1 h beating in each plot. Nocturnal search was standardized by time (1 h) and area (300 m^2) per collector. Sampling was carried out between June and August 2006. Overall, 330 m^2 of litter and 203 h were spent during Winkler and beating tray sampling, respectively. Total sampling effort employed during nocturnal search lasted 170 h and covered 51,000 m^2 at Porto Urucu.

Species identifications were provided by specialists in harvestmen taxonomy (Ana Lúcia Tourinho and Ricardo Pinto-da-Rocha). Whenever possible, identifications to species level were provided; otherwise morphospecies were defined. Only adult individuals were used because most juveniles cannot be adequately identified. The *taxa* in poor taxonomic state of knowledge were not included in any specific genera (e.g. Zalmoxidae sp1, Zalmoxidae sp2, Cosmetidae sp1 and Cosmetidae sp.2), and were not included in genera counting, for this set of species it is only possible to confirm whether the species belong to the same genus or not after a taxonomic revision. All the new genera and species, very common in any inventory undertaken in the Tropics, were referred to as genus or species followed by their number (e.g. Gagrellinae, genus 1 sp 1).

Sampling and transport of biological material was authorized by the Brazilian Institute of Environment and Renewable Natural

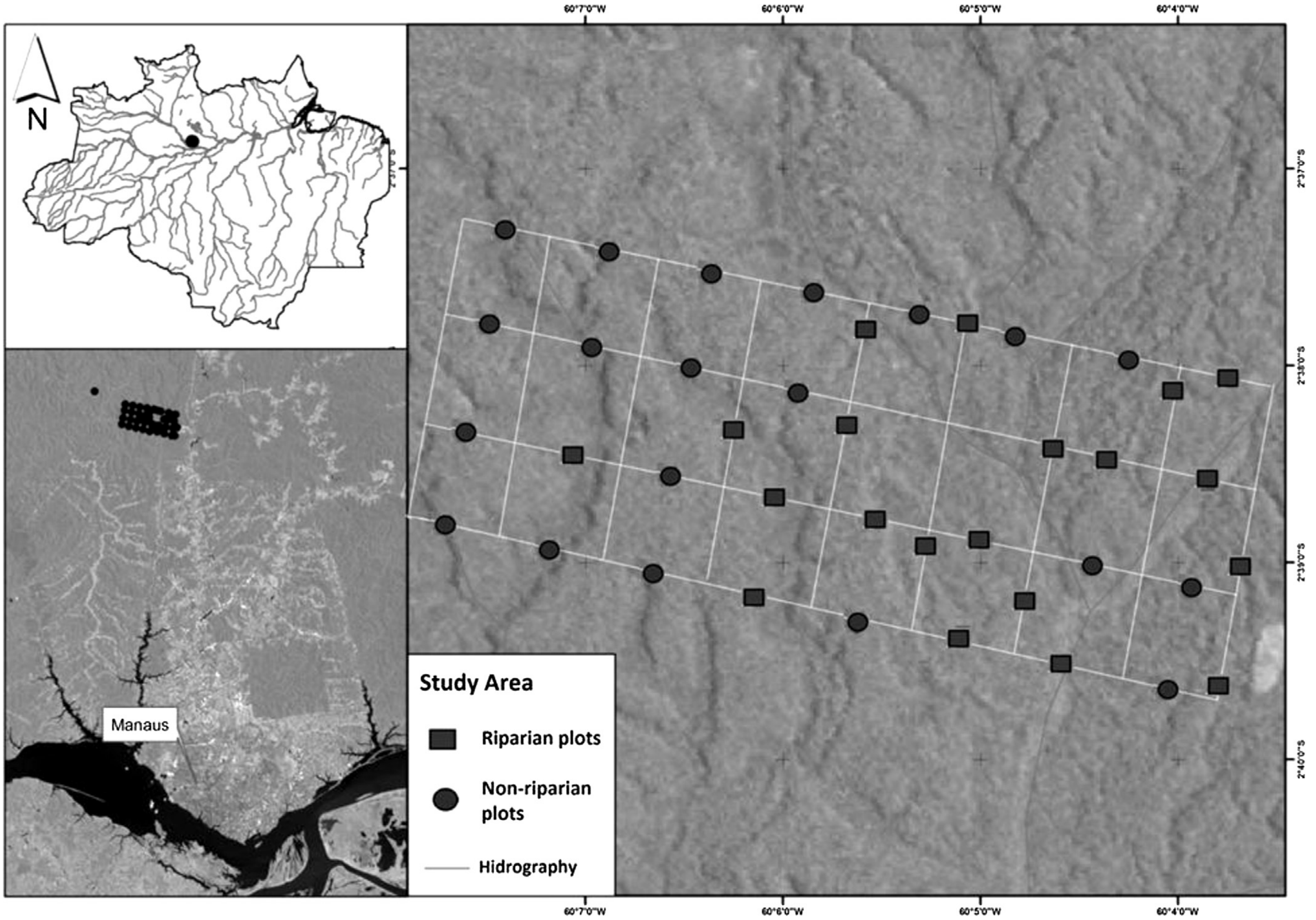

Fig. 1. Map of the FEX-UFAM. The squares and circles represent the 250-m plots sampled in riparian and non-riparian areas (Orgs. Marcelo dos Santos Junior and Larissa Lança).

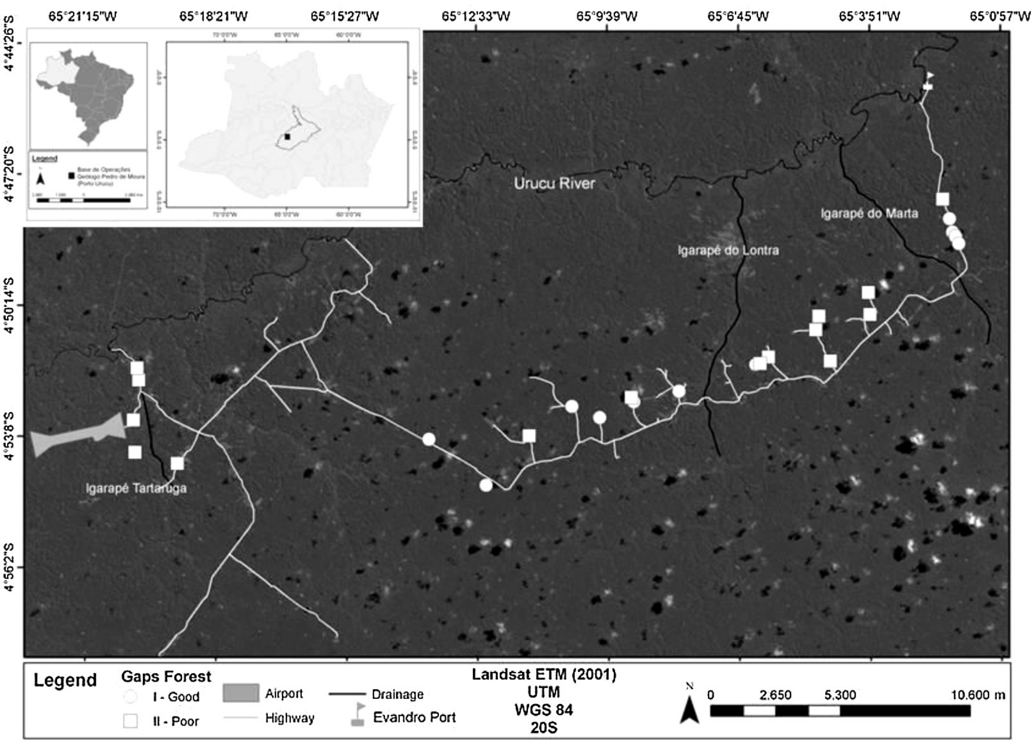

Fig. 2. Map of the Porto Urucu. The squares represent the 250-m plots sampled (Orgs. Sidclay Dias and Larissa Lança).

Resources (IBAMA permit number a. 21825-1). All the specimens collected at FEX-UFAM were deposited in the arachnology collection of Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil. Voucher specimens from Porto Urucu were deposited in the arachnological collection of the Museu Paraense Emílio Goeldi – MPEG, Belém, Pará, Brazil.

Environmental variables

We used several environmental descriptors to correlate with harvestman diversity and distribution at the FEX-UFAM sampling grid. The number of understory palms was used as a surrogate for vegetation density. Palm trees taller than 30 cm were counted in each sub-plot. Litter depth was measured at 10 points along the midline of each sub-plot with a measuring tape. The resulting mean was used as an estimate of depth and the variance as a measure of litter heterogeneity. The volume of coarse litter (fallen woody stems with diameter >1 cm) was obtained in each plot from measurements in 25 ten square meter transects, each separated by 10 m. All woody items with a diameter >1 cm at the intersection point of each transect were measured with a metric tape and their volume was estimated by the formula,

$$V = \frac{(w^2/d)\sum dt^2}{\sum xj}$$

where V is volume per unit area (m^3/ha), di is the diameter of item i at the intersection point (m), and sj is the length of segment j (m) (modified from Barbosa et al. 2009). Altitude data were obtained based on Shuttle Radar Topography Mission (SRTM) images (Miranda 2005). The distance between each plot and the closest stream was measured using a measuring tape (Rojas-Ahumada and Menin 2010). Percentage clay and the amount of soil organic matter per plot were determined from six equidistant 5 cm depth samples taken every 50 m in each plot (Rojas-Ahumada and Menin 2010).

Data analysis

The effectiveness of any sampling method depends on sampling intensity. One m^2 of litter, a beating tray, and 1 h of night visual search do not necessarily provide samples of comparable sampling intensity. Therefore, comparative analyses of sampling-method performance can be biased by variation in sampling intensity between techniques. We used the average species accumulation curves from 999 random permutations of actual individuals to assess how many individuals would be needed to record a comparable number of species for each method per site. In this analysis the possible effect related to sampling intensity was minimized. We used two types of standardization between sampling methods, at the FEX-UFAM site beating trays were standardized by area, and in Porto Urucu beating trays were standardized by time. Leaf-litter searches were standardized by area in both locations and nocturnal searches were standardized by both time and area in both study sites. Species accumulation curves were computed in EstimateS 9.0 (Colwell 2013) using the Mao Tau method that provides non-closed" confidence intervals at the right-hand tail of the curve.

The dimensionality of the assemblages sampled from each sampling method per site was reduced using Nonmetric Multidimensional Scaling (NMDS, Minchin 1987) based on the Sørensen similarity index. The data were standardized for presence/absence to minimize differences in the abundance of individuals between sampling techniques. Non-parametric MANOVA (Anderson 2001) was used to test for differences in harvestman assemblage composition among sampling methods. We also used the average distance to the group centroid (i.e. multivariate dispersion) as a measure of overall species turnover, or beta-diversity for each sampling method (Anderson et al. 2006). In our case, this analysis evaluated whether species turnover among plots differed between sampling methods. Tukey's test was used for pairwise comparisons between methods. The significance of non-parametric MANOVA and multivariate dispersion was calculated based on 999 random sample permutations.

At the FEX-UFAM site, mean litter depth, distance to the closest stream, altitude and number of palms per plot were used as predictor variables in analyses. The remaining variables were discarded because they were auto-correlated (Pearson's product-moment correlation >0.4) with one or more variables selected (Table 1). Redundancy analysis (RDA) was used to estimate how much of the variance in the harvestman composition matrix can be explained by the environmental variables and to determine if the ecological patterns recovered using all sampling methods combined were also recovered using only one sampling method. RDA is a direct extension of multiple regression analysis to model multivariate response data (Borcard et al. 2011). The statistical significance of RDA models was tested using 9999 permutations per test. The ordinations and RDA analyses were done in R 2.14 (R Development Core Team, 2011) with the package "vegan" (Oksanen et al. 2011).

Results

Harvestmen diversity

Combining all sampling methods from both sites resulted in 3208 harvestmen adults collected. In FEX-UFAM, 1067 harvestmen, 26 species, 12 genera, 12 families and three suborders were sampled (Table 2). The highest number of species per plot was 12 and the lowest 3. The families with the largest numbers of species registered were Stygnidae (5), Manaosbiidae (4); Sclerosomatidae (4), and Cosmetidae (3). Sclerosomatidae and Cosmetidae were the most abundant families (35% and 32% of individuals respectively). We registered only one singleton and three doubletons at FEX-UFAM. In Porto Urucu 2141 harvestmen, distributed in 27 species, 14 genera and 10 families, were collected. The families with the largest numbers of species registered were Cosmetidae (11) and Stygnidae (4). Cosmetidae was the most abundant family (46% of individuals). We registered only one doubleton at this site.

Sampling methods performance

In FEX-UFAM the beating-tray method sampled 37 individuals, 4 families, 3 genera and 4 species (Table 2). Most species collected using this method belong to the Zalmoxoidea (1 Fissiphallidae and 1 Zalmoxidae), Samooidea (1 Samoidae) and Sclerosomatidae (1 Gagrellinae). Only one species of Gagrellinae was collected by the three methods. The beating-tray sampled fewer species and individuals, but one species of both, Samoidae and Fissiphalliidae, were exclusively sampled using this method. Using manual leaf-litter sorting, we collected 65 harvestmen belonging to 7 families, 8 genera and 10 species (Table 2). The suborder Cyphophtalmi, with *Metagovea oviformis* Martens, 1969, Kimulidae Pérez Gonzalez, Kury and Alonso-Zarazaga, 2007 and genus *Auranus* Mello Leitão, 1941 of the Stygnidae were exclusively sampled using this method. The other four species were more effectively sampled by nocturnal search, except for Zalmoxidae sp.1, which was consistently sampled using leaf-litter sorting and only one individual was recorded using the beating tray method.

Table 1
Average of environmental variables collected in 20 riparian and 20 non-riparian 250 m-long plots. Minimum and maximum values are given in parentheses.

	Riparian	Non-riparian
Amount of soil organic matter (g/kg)	28.64 (15.32–53.58)[a]	29.60 (9.92–52.26)
Clay content (%)	13.94 (3.00–48.80)[a]	56.89 (2.80–85.20)
Distance to nearest stream (m)	37.54 (1.70–133.36)	437.23 (106.00–1093.40)
Elevation (m a.s.l.)	61.90 (42.00–92.00)	94.85 (67–121)
Litter depth – mean (cm)	3.03 (1.91–4.08)	2.26 (0.86–4.25)
Litter depth – variance	3.56 (1.13–6.35)	1.88 (0.63–5.39)
Number of palms	167 (110–330)	141 (74–289)
Volume of course litter (m^3/ha)	21.07 (9.80–35.67)[a]	22.97 (12.44–35.89)

[a] Values based in 10 plots.

In Porto Urucu, the beating-tray method sampled 1238 harvestmen, 7 families, 11 genera and 18 species (Table 3); however, only one species of Cosmetidae was exclusively collected using this method. With the manual Winkler apparatus, we collected 236 harvestmen, 9 families, 11 genera and 15 species (Table 3) while only family, Samoidae, was exclusively sampled using the Winkler apparatus.

The overall number of species was similar between sites, with 26 species sampled at Fex-UFAM and 27 species sampled in Porto Urucu. The species accumulation curve for the nocturnal search increased more rapidly than for the other techniques, indicating that the nocturnal search was the most effective method used for sampling harvestman in both sites (Fig. 3). In fact, in Porto Urucu, the species accumulation for nocturnal searches was similar to the accumulation curve using data from all methods combined (Fig. 3).

In both areas, nocturnal searches sampled the largest number of species (FEX-UFAM = 20, Urucu = 24), and the largest number of exclusive species (FEX-UFAM = 13, Urucu = 3) (Tables 2 and 3). The results of other methods were less congruent among sites. At Fex-UFAM, litter sorting sampled more species than the beating-tray method, but those sampling methods sampled similar numbers of species in Porto Urucu (Fig. 3). Litter sampling using the Winkler apparatus at Urucu was the only sampling method which did not reach the asymptote.

The species composition of harvestmen (Fig. 4) differed among the sampling methods in both sites (non-parametric MANOVA: $F_{2,75} = 84.891$; $P = 0.001$ and $F_{2,94} = 118.39$; $P = 0.001$, for Fex-UFAM and Porto Urucu, respectively). Despite the fact that nocturnal searches showed more overlap with the other techniques, the assemblage composition sampled by the beating tray method and

Table 2
List of the harvestmen species collected and number of individuals using three different methodologies at Experimental Farm of the Federal University of Amazonas, Brazilian Amazon.

Species	Nocturnal	Litter sorting	Beating	Total
CYPHOPHTALMI – NEOGOVEIDAE				
Metagovea oviformis Martens, 1969	0	2	0	2
EUPNOI – SCLEROSOMATIDAE				
Prionostemma sp.1	92	0	0	92
Genus 1 sp.1	41	0	0	41
Genus 1 sp.2	158	3	15	176
Genus 2 sp.1	67	1	0	68
LANIATORES – AGORISTENIDAE				
Genus 1. sp.1	6	0	0	6
LANIATORES – BIANTIDAE				
Genus 1 sp.1	0	20	0	20
LANIATORES – COSMETIDAE				
Genus 3 sp.1	95	0	0	95
Eucynortella duapunctata Goodnight and Goodnight, 1943	244	6	0	250
LANIATORES – CRANAIDAE				
Phareicranaus manauara Pinto-da-Rocha, 1994	57	0	0	57
LANIATORES – FISSIPHALLIIDAE				
Fissiphallius martensi Pinto-da-Rocha, 2004	2	0	0	2
Fissiphallius sp.1	0	0	12	12
LANIATORES – KIMULIDAE				
Genus 1 sp.1	0	2	0	2
LANIATORES – MANAOSBIIDAE				
Saramacia lucasae Jim and Soares, 1991	37	0	0	37
Manaosbia sp. 1	22	0	0	22
Mecritta sp. 1	27	0	0	27
Genus 1 sp.1	19	0	0	19
LANIATORES – SAMOIDAE				
Genus 1 sp.1	0	0	4	4
LANIATORES – STYGNIDAE				
Stygnus simplex Roewer, 1943	37	0	0	37
Stygnus pectinipes Roewer, 1943	12	3	0	15
Protimesius longipalpis Roewer, 1943	18	0	0	18
Auranus sp.1	1	0	0	1
Auranus sp.2	0	10	0	10
LANIATORES – ZALMOXIDAE				
Zalmoxidae sp.1	1	14	0	15
Zalmoxidae sp.2	4	0	6	10
Zalmoxidae sp.3	17	4	0	21
Total	965	65	37	1067

Table 3
List of the harvestmen species collected and number of individuals using three different methodologies at Porto Urucu, Brazilian Amazon.

Species	Nocturnal	Litter (Winkler)	Beating	Total
EUPNOI – SCLEROSOMATIDAE				
Prionostemma sp.1	96	0	13	109
Prionostemma aureomaculatum H. Soares, 1970	139	1	55	195
LANIATORES – AGORISTENIDAE				
Avima sp.1	3	0	4	7
Avima sp.2	1	0	0	1
LANIATORES – BIANTIDAE				
Stenostygnus pusio Simon, 1879	1	1	9	11
LANIATORES – COSMETIDAE				
Cosmetidae sp.1	28	1	3	32
Cosmetidae sp.2	1	0	1	2
Cosmetidae sp.3	5	0	2	7
Cocholla simoni Roewer, 1927	7	1	0	8
Cynorta sp.1	58	10	377	445
Cynorta juruensis (Mello-Leitão, 1923)	54	4	0	58
Paecilaema graphicum Roewer, 1947	0	0	2	2
Paecilaema lobipictum Roewer, 1947	10	0	87	97
Paecilaema manifestum Roewer, 1927	98	10	192	300
Paecilaema marajoara Soares, 1970	6	0	0	6
Protus insolens Simon, 1879	12	0	25	37
LANIATORES – ESCADABIIDAE				
Escadabiidae sp.1	5	3	0	8
LANIATORES – GONYLEPTIDAE				
Ampycinae gen.sp.n	8	2	0	10
Discocyrtus sp.1	2	1	0	3
LANIATORES – MANAOSBIIDAE				
Paramicrocranaus cf. *difficilis*	14	0	32	46
Saramacia lucasae (Jim and Soares, 1991)	37	2	2	41
LANIATORES – SAMOIDAE				
Samoidae sp.1	0	179	0	179
LANIATORES – STYGNIDAE				
Imeri sp.1	9	8	39	56
Protimesius longipalpis (Roewer, 1943)	6	0	0	6
Stygnidius sp.1	21	7	180	208
Stygnus pectinipes (Roewer, 1943)	41	1	7	49
LANIATORES – ZALMOXIDAE				
Zalmoxidae sp.1	5	5	208	218
Total	667	236	1238	2141

manual litter sorting were remarkably different in both sites, highlighting the role of microenvironment characteristics for harvestman species distribution.

Species turnover within sampling methods differed only at FEX-UFAM (PERMDISP, $F_{2,75} = 3.962$, $P = 0.023$), where the nocturnal search was the technique with the lowest species turnover (lower average distance to centroid). However, the beating tray method and litter sorting did not differ significantly in species turnover (Tukey's post hoc test; $P = 0.28$), indicating that the assemblage heterogeneity sampled by each technique was similar. The species turnover within sampling methods were similar at Porto Urucu (PERMDISP, $F_{2,94} = 2.664$, $P = 0.074$). The three sampling methods showed a similar variation in assemblage composition (distance to centroid).

Relationships with environmental gradients

RDA analysis showed that the harvestman assemblage composition for all data of FEX-UFAM was significantly correlated with mean litter depth, distance to the closest stream, altitude and number of palms. This pattern was not detected with leaf-litter sorting or beating tray methods (Table 3). When the nocturnal search method was used to determine harvestman assemblages, it was the only method that responded similarly to the combined data set (Tables 3 and 4).

Discussion

We tested the efficiency of four sampling methods frequently used to describe the assemblage composition of harvestman. Each method focuses on a different microhabitat, because harvestmen species vary among microhabitats (Proud et al. 2012). The beating tray method focuses on the herb and shrub layer, leaf-litter manual sorting and the Winkler apparatus capture ground

Table 4
Proportion of variance in the harvestman assemblage composition explained by the environmental variables in Redundancy Analysis (RDA) sampled at Experimental Farm of the Federal University of Amazonas, in the Brazilian Amazon. Predictor variables used in the RDA analyses were the mean litter depth, distance to the closest stream, altitude and number of palms.

Sampling methods	R^2_{adj}	F	P
All	0.154	1.601	0.035*
Mean litter depth		1.456	0.161
Distance to the closest stream		1.919	0.065
Altitude		0.669	0.687
Number of palms		2.266	0.038*
Beating	−0.113	0.618	0.860
Mean litter depth		0.396	0.829
Distance to the closest stream		0.678	0.549
Altitude		1.261	0.235
Number of palms		0.288	0.865
Litter sorting	0.053	1.297	0.210
Mean litter depth		1.767	0.118
Distance to the closest stream		0.942	0.430
Altitude		1.818	0.124
Number of palms		0.434	0.832
Nocturnal search	0.155	1.605	0.038*
Mean litter depth		1.403	0.197
Distance to the closest stream		1.962	0.059
Altitude		0.598	0.765
Number of palms		2.355	0.034*

* $P < 0.05$

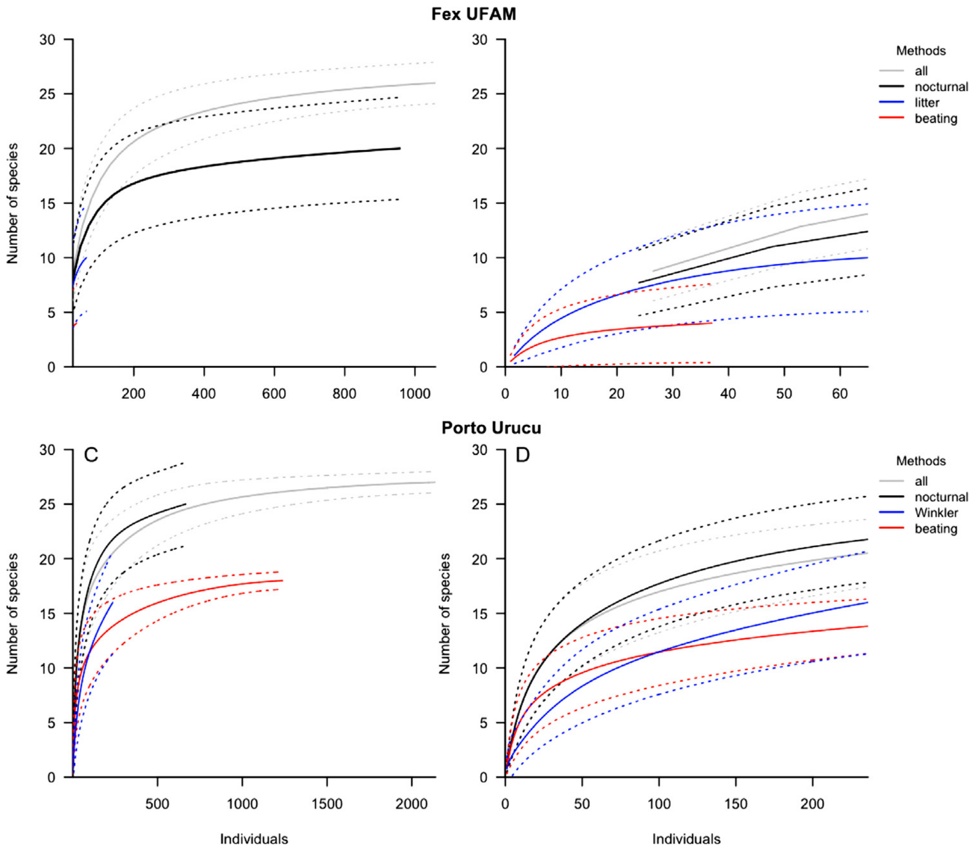

Fig. 3. Harvestman species/individual curve comparisons of sampling methods. (A) Differences in sampling methods lead to large differences in the number of individuals collected at FEX-UFAM. (B) The same curves from FEX-UFAM truncated at 70 individuals. (C) Curves for the three sampling methods at Porto Urucu. (C) The same curves from Porto Urucu truncated to the total number of individuals collected with Winkler method. All curves are sample-based curves re-scaled to individuals. Dashed lines show the 95% CI for each curve.

leaf-litter species, and nocturnal searches access at least four different types of microhabitats also sampled using the first two methods in addition to capturing harvestmen with nocturnal activity. The four methods were complementary, sampling different harvestman assemblages. Therefore, when combined, these four methods should give a better picture of harvestman diversity.

Although beating normally samples fewer species, it also consistently samples specific families and species in the Amazon forests, such as small harvestmen of Fissiphalliidae. These species are mostly arboreal and live in crevices and on leaf surfaces (Tourinho and Pérez-González 2006). The beating tray method also samples some nocturnal ground species that climb the vegetation during their active period, and use suspended leaf litter and vegetation as a refuge during the day (species of Cosmetidae, Gagrellinae, Manaosbiidae and sometimes Stygnidae in the Amazon). The high abundance of palms with suspended leaf litter in the plots may

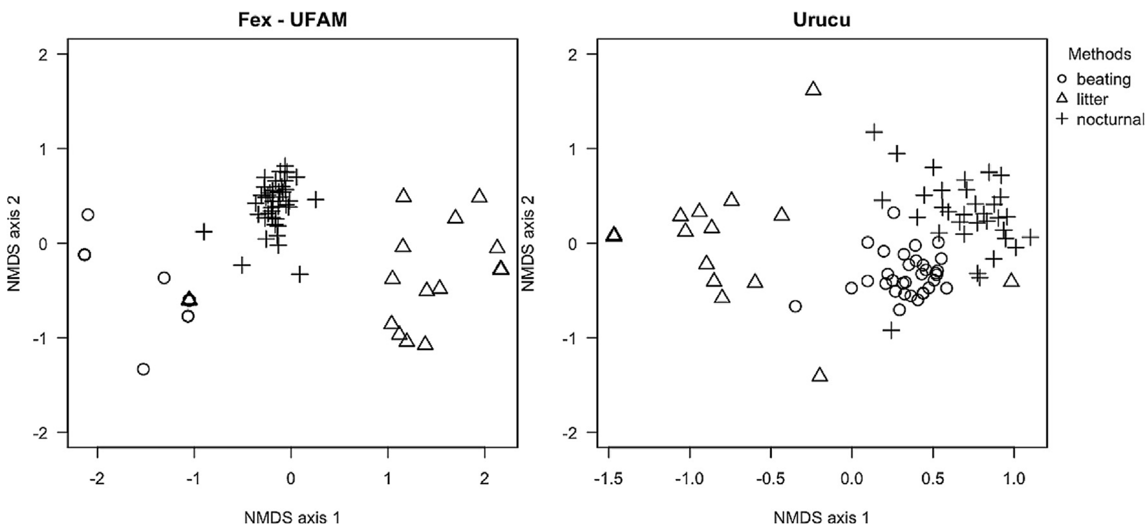

Fig. 4. NMDS ordination plot indicating the complementarity in harvestman species composition among three sampling techniques collected in 40 plots of the Experimental Farm of UFAM and 33 plots at Porto Urucu, in the Brazilian Amazon. The triangles represent litter manually searched at Fex-UFAM and Winkler extractions at Porto Urucu.

explain the uncommon and extremely large and rich harvestman assemblages collected using beating in Porto Urucu. In these samples, Cosmetidae, Gagrellinae, Manaosbiidae and Stygnidae represented the majority of the species sampled. Beating is frequently used to sample spiders, but not usually for harvestmen likely because in most Neotropical sites the number of harvestmen species captured using the beating tray method is very low or zero (e.g. Atlantic forest sites). Recently, the beating tray method was used more often in harvestmen inventories in the Amazon basin, but still less than other methods. As this technique requires a lot of effort and field time, it should be used only if a more detailed inventory of harvestmen assemblage is required.

The number of species sampled in our surveys was comparable with previous studies (Pinto-da-Rocha and Bonaldo 2006; Bonaldo et al. 2009). However, our surveys had the lowest number of singletons for Amazon forests, especially if the sampling effort is taking into account. This result was reflected in the accumulation curves, where the number of species sampled tended to stabilize. Most part of the accumulation curves suggests that our sampling effort was a reasonable estimate of harvestmen diversity at the site and microhabitat scale. The only exception was for the Winkler apparatus at Urucu site, which did not reach an asymptote. The higher number of stemless palms may also explain the steep accumulation curve of Winkler samples at Porto Urucu. Stemless palms, such as *Attalea attaleoides* or *Astrocaryum acaule*, both very common at Porto Urucu, usually trap fallen litter, increasing the habitat complexity and litter volume available (Vasconcelos 1990). Therefore, more complex environments may offer a variety of microhabitats allowing the co-occurrence of more harvestman species at the plot scale (Proud et al. 2012).

The efficiency of the Winkler method is closely related to extraction time, which can be 3–7 days for a good spider survey (Krell et al. 2005). However, the high humidity requirements of most harvestman species and the similar number of species sampled in leaf litter at both sites, suggests that the 48 h Winkler extraction was sufficient in sampling the majority of individuals and species at Porto Urucu. Also, since the manual litter survey at FEX-UFAM was extensive and identified very small harvestman in the leaf litter, we are confident that we have not overlooked individuals during manual searching. However, to what extent the differences between number of harvestmen sampled by the Winkler apparatus and manual sorting is related to site characteristics or sampling method efficiency needs further investigation.

In both sites, the species composition differed among sampling methods, but the harvestmen turnover within methods (assemblage composition dispersion) was different only in Fex-UFAM. This difference was related to the small turnover in species composition among nocturnal samples. At Fex-UFAM, more species were sampled during the nocturnal search, but the similarity of species composition among plots was also very high, appearing clustered in the NMDS ordination. In general, a random plot sampled using a nocturnal search at Fex-UFAM seems to be enough to collect most species. This pattern did not hold for Porto Urucu, where the species composition was less similar among plots, even when sampled using nocturnal searches.

Microhabitat features may account for the differences in assemblage composition between sampling methods. For instance, the majority of harvestmen species seem to require microhabitat specific requirements to complete their life cycle. Microhabitat characteristics have been recognized as an important factor controlling the distribution of harvestman species in other regions (Proud et al. 2012). In fact, despite the use of pitfall traps as the preferred method for community studies (Curtis 2007), often more harvestmen species and individuals are collected by nocturnal searches (Pinto-da-Rocha and Bonaldo 2006; Tourinho and Pérez-González 2006; Bonaldo et al. 2009). The success of this method may be related to the nocturnal activity of most harvestmen species and because the nocturnal search method covers ground/litter microhabitats as well as the vegetation layer.

Pooling the data collected by three methods is an appropriate approach to sample more species. However, the combination of different methods was not necessary to determine general relationships between harvestman assemblage composition and the environment. As different methods sampled different assemblages, is not surprising that each assemblage responded differently to the same predictor variables. While we are not arguing that harvestman assemblage composition sampled by the beating tray method should be related with litter depth, or that the assemblage composition sampled by litter manual search should be related with palm number, all sampling methods were modeled with the same environmental predictors, and possible relationships between variables and species composition could be determined in this way. At Fex-UFAM, the assemblage composition sampled with three methods combined was correlated with the number of palms. Our results are in line with previous studies suggesting that harvestman composition may respond to variation in habitat structure (Bragagnolo et al. 2007; Curtis and Machado 2007; Proud et al. 2012). Acaulescent palms may increase the habitat complexity by trapping fallen litter (Vasconcelos 1990) while arborescent palms are closely related with soil properties–proportionally less palm basal area is related to areas with well-structured soils (Emilio et al. 2013). Therefore, number of palms may be reflecting the natural variation in microhabitat structure for harvestman species. But only the nocturnal search was able to retrieve the main ecological pattern detected with all methods combined. The correlative result between palm number and all sampling methods combined was achieved only with nocturnal search data. Nocturnal search data also showed an accumulation curve similar to the overall assemblage accumulation curve, and collected more exclusive species than all the other four methods combined. Additionally, we found little effect of collector experience on data as has been reported in previous studies using nocturnal search. In fact, inexperienced collectors became good collectors in a very short period of time and possible discrepancies due to wide variety of taxonomic interests among personnel were statistically indistinguishable (Coddington et al. 1991). Therefore, this method may be a good proxy for both, the relationships between environment and harvestmen assemblage composition, and an estimate of harvestman species number in an area.

In our study, reducing the number of sampling methods decreased the number of species sampled probably by decreasing the number of habitats accessed, but it also reduced sampling redundancy saving time and money, and avoiding confounding environmental relationships of species assemblages. Therefore, where time and economic costs limit the number of techniques applied, such as in most Amazonian biodiversity-monitoring programs, the use of nocturnal search alone is a efficient alternative for adequately sampling harvestman assemblages.

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