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ORIGINAL ARTICLE

Sampling techniques and environmental variables influence the distribution of pseudoscorpions in urban forest fragments in the central Amazon

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ABSTRACT

The growth of urban landscapes has genarally reduced biodiversity worldwide. Invertebrates have explored different environments, and it usually takes different sampling techniques to get a representative sample of the species assemblage in a given location. Some studies have sought to determine the minimum necessary number of sampling techniques, including ecological relationships or costs to guide the sampling protocol. In the Amazon, the effect of soil characteristics on invertebrate distribution is well known. We evaluated if sampling techniques have a complementary effect on the detection of pseudoscorpion assemblages and tested whether environmental variables affect the distribution of pseudoscorpion species. The study sites were two urban forest fragments in the city of Manaus, in the central Amazon. In each fragment, we sampled 20 palm trees using the beating technique, and installed transects with 12 sampling points for collection of soil and litter samples for extraction of arthropods in a Berlese funnel and a Winkler extractor, respectively. We collected 267 individuals of 11 species of pseudoscorpions. Most records were obtained through the Winkler extraction in both fragments. The assemblage from the palm trees was different from that in the edaphic samples. Pseudoscorpion species composition also differed significantly between soil and litter, and was influenced by potassium concentration. The number of species in the fragments and the environmental effect on the distribution of pseudoscorpions was similar to that recorded in environmental protection areas, evidencing that urban forest fragments can serve as an efficient repository of Amazonian pseudoscorpion biodiversity.

KEYWORDS: Berlese funnel, biodiversity surveys, entomological umbrella, tropical rain forest, invertebrates, Winkler extractor

Métodos de amostragem e variáveis ambientais influenciam a distribuição de pseudoescorpiões em fragmentos florestais urbanos na Amazônia central

RESUMO

O crescimento das paisagens urbanas geralmente reduziu a biodiversidade em todo o mundo. Os invertebrados exploram diferentes ambientes, e geralmente são necessárias diferentes técnicas de amostragem para obter uma amostra representativa da assembleia de espécies em um determinado local. Alguns estudos têm buscado determinar o número mínimo necessário de técnicas de amostragem, incluindo relações ecológicas ou custos para orientar o protocolo de amostragem. Na Amazônia, o efeito das características do solo na distribuição dos invertebrados é bem conhecido. Nós avaliamos se as técnicas de amostragem têm um efeito complementar na detecção de assembleias de pseudoescorpiões e testamos se as variáveis ambientais afetam a distribuição das espécies de pseudoescorpiões. Os locais de estudo foram dois fragmentos florestais urbanos na cidade de Manaus, Amazônia central. Em cada fragmento, amostramos 20 palmeiras com guarda-chuva entomológico e instalamos transectos com 12 pontos amostrais para coleta de solo e serrapilheira para extração de artrópodes em funil de Berlese e extrator de Winkler, respectivamente. Coletamos 267 indivíduos de 11 espécies de pseudoescorpiões. A maioria dos registros foi obtida com Winkler em ambos fragmentos. A assembleia em palmeiras foi diferente das amostras edáficas. A composição de espécies de pseudoescorpiões também diferiu significativamente entre solo e serapilheira, e foi influenciada pela concentração de potássio. O número de espécies nos fragmentos e o efeito ambiental na distribuição de pseudoescorpiões foram semelhantes aos registrados em áreas de proteção ambiental, evidenciando que fragmentos florestais urbanos podem servir como um eficiente repositório da biodiversidade de pseudoescorpiões amazônicos.

PALAVRAS-CHAVE: Funil de Berlese, levantamentos de biodiversidade, guarda-chuva entomológico, floresta tropical, invertebrados, extrator de Winkler

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INTRODUCTION

Population growth in recent decades and the consequent expansion of cities has converted natural into artificial landscapes at an accelerated rate (McKinney 2002; Laurance and Engert 2022). The growth of cities often also suppresses adjacent landscapes and their associated biodiversity, creating environments disconnected from the original forest matrix (Fischer and Lindenmayer 2007; McKinney 2008; Laurance and Engert 2022), with environmental impacts comparable to those of activities such as agriculture and forestry (Simkin *et al*. 2022). These fragmented environments are characterized by the isolation of native vegetation patches that are often spatially and temporally dynamic (McIntyre *et al*. 2001; McKinney 2002) and associated with low biodiversity (Vitousek *et al*. 1997; McIntyre *et al*. 2001). Urban landscapes are associated with high heterogeneity and can occasionally harbor great species diversity, providing relevant information to foster discussions on biodiversity management (Savard *et al.* 2000).

In tropical forests worldwide, terrestrial invertebrates constitute approximately 94% of the animal biomass (Fittkau and Klinge 1973; Ellwood and Foster 2004; Basset *et al.* 2012), and invertebrates also have been widely used for biomonitoring in aquatic systems (Hawkins *et al*. 2000). However, despite providing valuable indications of changes in biological integrity and ecosystem functioning in terrestrial environments, invertebrates have been used less frequently by environmental agencies in terrestrial biomonitoring (Andersen and Majer 2004).

No sampling technique manages to capture all invertebrates present in a given area. Studies on minimum effort to achieve sampling sufficiency usually evaluate which technique or combination of techniques is most efficient for an inventory with an emphasis on records of diversity and abundance of taxa (e.g., Garden *et al.* 2007; Roy *et al.* 2007). Some studies have shown that there may be some redundancy in the concomitant use of some sampling techniques (Souza *et al.* 2012; Porto *et al.* 2016; Tourinho *et al.* 2014; 2018).

Soil characteristics and vegetation structure are the most frequently tested predictors in distribution models of edaphic organisms (Costa *et al.* 2015; Dambros *et al.* 2020). Environmental factors such as soil texture and chemistry generate microhabitat variability that can affect the spatial distribution pattern of invertebrates at local scales (Mezger and Pfeiffer 2011), as has been determined in the Amazon for oribatid mites (Moraes *et al.* 2011), cockroaches (Tarli *et al.* 2014), termites (Dambros *et al.* 2017), and ants (Souza and Araújo 2020; Torres *et al.* 2020).

Pseudoscorpions (Arthropoda: Arachnida: Pseudoscorpiones) are tiny (0.5 to 10 mm), mostly solitary arachnids with a low ability to disperse and colonize (Weygoldt 1969; Harvey 1986; 2002; Adis 2002; Bedoya-Roqueme and Tizo-Pedroso 2021), found in several terrestrial environments, except the poles (Harvey 2013a). Although pseudoscorpions are considered relevant predators and regulators of the density of small arthropods in soil and litter (Weygoldt 1969; Adis 2002), few studies have tested their role as bioindicators (Yamamoto *et al.* 2001). Although the knowledge on the taxonomy and ecology of pseudoscorpions is incipient (Mahnert and Adis 2002; Adis *et al.* 2009), some studies have detected that physicochemical soil characteristics, temperature, vegetation cover and structure affect the distribution and predation patterns of pseudoscorpions (Mahnert and Adis 1985; Aguiar *et al.* 2006; Adis *et al.* 2009; Moura *et al.* 2018). Most studies on invertebrate fauna in the central Amazon were carried out in Ducke Reserve, a 10,000-ha environmental protection area on the outskirts of the city of Manaus (Adis 2002; Magnusson *et al.* 2014). Some more recent studies exist for other parts of the Brazilian Amazon (e.g., Tourinho *et al.* 2019; Demetrio *et al.* 2021; Souza and Fernandes 2021). However, to our knowledge, there is no published study on pseudoscorpion species diversity and distribution in urban forest fragments in the Amazon.

Our objective was to evaluate the diversity of pseudoscorpions in two urban forest fragments in the central Brazilian Amazon. Specifically, we aimed to (a) evaluate if different sampling techniques have a complementary effect on estimates of pseudoscorpion diversity and assemblage composition and (b) test whether environmental variables are associated with the diversity and distribution of pseudoscorpions. For the characterization of richness, abundance, and composition of the pseudoscorpions in the two fragments, we used different sampling techniques to evaluate the diversity in different habitat compartments (soil, litter, and understorey vegetation).

MATERIAL AND METHODS

The study was conducted in two highly vulnerable forest fragments subjected to constant human action in the city of Manaus, the capital of Amazonas state, Brazil. One is located in the environs of an oil refinery (Isaac Sabbá Refinery – Reman) (03°08'19.5"S, 59°57'27.05"W) and has an area of 0.86 km^2. The other is located on the banks of a stream (Igarapé Cururu) in the area of the Marine Operations Battalion of the Brazilian Navy (03°07'47.72"S, 59°56'34.20"W), with an area of 0.72 km^2. Hereafter the fragments are referred as Reman and Cururu (Figure 1). Both fragments have a total area of 1.58 km^2. The minimum distance between the sampling points and the fragment's edge was 150 m in Cururu and 200 m in Reman. In each fragment, twelve sampling points approximately 15 m apart were demarcated. Two sampling sessions were carried out in each area, in September-October 2006 and February-April 2007. We used three sampling

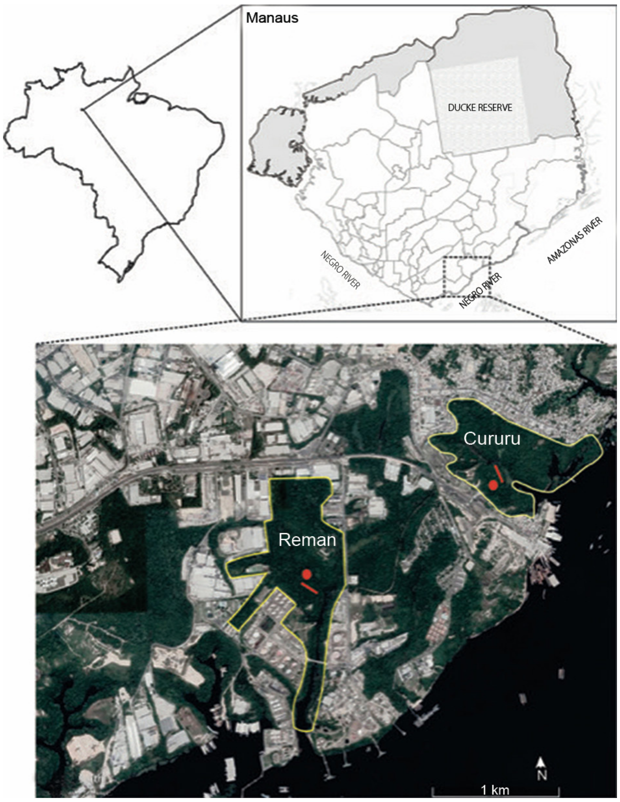

Figure 1. Location of the two urban forest fragments sampled for pseudoscorpions in the city of Manaus, Amazonas, Brazil. This figure is in color in the electronic version.

methods: beating (entomological umbrella), Berlese-Tullgren funnel, and Winkler extractor.

The beating technique was used on 20 understorey palm trees in each fragment. The palms belonged to *Astrocaryum ginacanthus* Mart. (14 individuals), *Bactris simplicifrons* Mart. (22) and *Geonoma* Willd (4). These palms accumulate organic material between the leaves suspended 0.5 m to 2 m above the ground. The beating was carried out for 45 min during daytime in each fragment by beating each palm tree 20 times with a stick. The collections took place once from September- to October 2006 and once from February- to April 2007. We used a 1-m^2 white fabric attached to a frame placed under the bushes to collect the invertebrates that fell from the palms. The invertebrates were placed in plastic bottles with 80% alcohol and taken to the laboratory for later identification.

For the Berlese method, 12 soil samples were collected in each fragment using a 5x5x5cm probe. The samples (without litter) were placed in a Berlese-Tullgren funnel with 16 lamps (25W; 120V) installed on top as heat sources, one lamp for each funnel. Styrofoam plates were drilled to support the sieves containing the samples and also to isolate the temperature of the upper compartment. In the lower portion, collector cups containing 1% formalin and detergent with 4% glycerin were placed. After the extraction period (7 days), the material was filtered, washed, and deposited in bottles with 80% alcohol.

For the Winkler method, 12 litter samples were collected at the same points of soil sampling in each fragment from a bounded area of 1m^2. The litter was collected manually and placed in a 1-cm^2 mesh sieve. In the laboratory, the samples were placed in a Winkler extractor for 48 hours for extraction. After extraction, the material was deposited in bottles with 80% alcohol.

Pseudoscorpion specimens in the samples were identified using the keys to families and genera in Harvey (1992) and Mahnert and Adis (2002). The identifications were supervised and confirmed by Dr. Nair O. Aguiar. The voucher specimens were deposited in the Paulo Bührnheim Zoological Collection located at the Federal University of Amazonas - UFAM.

We also collected environmental variables related to soil (clay content, pH, organic matter, sodium, phosphorus, and potassium concentration) due to their influence on invertebrates in previous studies in the Amazon region (Aguiar *et al.* 2006; Tarli *et al.* 2014; Dambros *et al.* 2017; Souza and Araújo 2020; Torres *et al.* 2020). In each fragment, 12 soil samples were collected along the aforementioned transects (same points as for Berlese and Winkler samples) using an auger. The soil samples were collected up to 10 cm deep after removal of the leaf litter and large roots, and were sent to the laboratory for analysis of texture and chemical characteristics, following the protocols of EMBRAPA (Silva 2009; Teixeira *et al.* 2017).

We calculated the number of species concerning the number of collected individuals using Hill numbers (Chao *et al.* 2014; Hsieh *et al.* 2016). We used observed samples to calculate diversity estimates for rarefied and extrapolated samples and the 95% confidence intervals. The generated curves plot the diversity estimates to the sample size (Chao *et al.* 2012).

We evaluated the species composition associated with each sampling technique in each fragment using cluster analysis (Everitt 1980; 1993), based on the Jaccard coefficient of similarity. The clustering dendrograms were obtained from the similarity matrix, using the unweighted pair-group method using arithmetic averages – UPGMA (Sneath and Sokal 1973).

We evaluated the effect of soil variables (clay content, pH, organic matter, sodium, phosphorus and potassium concentration) on species composition using permutational multivariate analysis of variance - PERMANOVA (Anderson 2001). In the analysis, we also tested whether the two fragments and the edaphic sampling techniques (Winkler and Berlese) affected species composition. To control for a possible pseudo-replication effect of sampling, we include the sampling points as a covariate in the model. Environmental variables were previously analyzed for their collinearity using the Pearson correlation and the correlated variables were not included in the model. We tested the effect of the soil variables on pseudoscorpion species composition collected with the edaphic sampling techniques. We used a stratified permutation procedure to keep the nested structure of the data (sampling techniques nested in fragments) in the PERMANOVA to control for possible spatial autocorrelation of the data. The PERMANOVA probability values were based on 999 permutations. All analyses were performed in the R environment version 4.2.0 (R Core Team, 2022) using the vegan 2.6-2 (Oksanen *et al.* 2022) and iNEXT 2.0.20 (Hsieh *et al.* 2020) packages.

RESULTS

We collected 267 pseudoscorpions belonging to eight families and 11 species. In Reman, 10 species of 8 families were present, two being exclusive to this fragment. In Cururu, we recorded nine species of six families, with one species exclusive to this fragment. The rarefaction and interpolation curves indicated that the number of species would remain the same in Cururu, and Reman would have an addition of one species with approximately 200 individuals (Figure 2). In both fragments, the largest number of species was collected with the Winkler extractor (8), followed by beating and Berlese-Tullgren (6 each). Beating and Berlese-Tullgren had a unique species each. The highest abundance in both areas was also recorded with the Winkler extractor, followed by beating and Berlese-Tullgren. The results indicate that the edaphic techniques, especially the Winkler extractor, sample more species and individuals than beating (Table 1).

In the cluster analysis, beating samples formed a distinct group from the other assemblages, thus separating them from the edaphic fauna (Figure 3). The next grouping separated the Berlese from the Winkler samples. The techniques were more efficient in sampling pseudoscorpion assemblages from distinct environments than assemblages of different fragments (Figure 3).

The sampling techniques influenced species composition regardless of the sampled fragment. The species composition sampled with Winkler differed significantly from that of Berlese (PERMANOVA: $F_{7, 54} = 8.320$; $R^2 = 0.12$; $p < 0.001$;

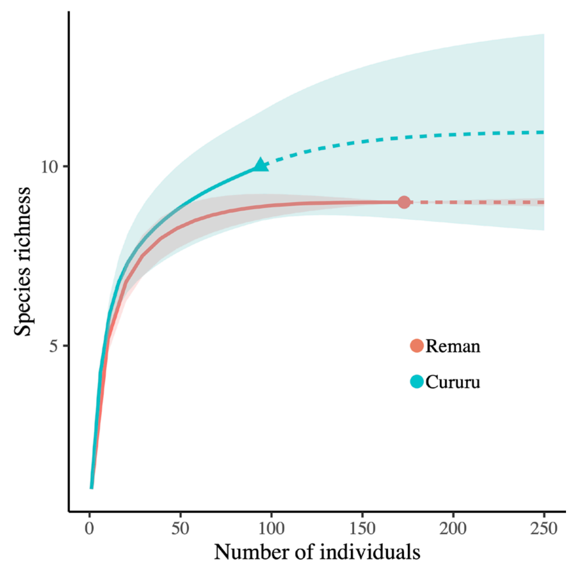

Figure 2. Diversity curves (rarefied and extrapolated values) and their 95% confidence intervals for pseudoscorpion species sampled in two urban forest fragments (Reman and Cururu) in Manaus, Amazonas, Brazil. The solid line indicates calculated values and, the dashed line indicates extrapolated values. This figure is in color in the electronic version.

Table 1. Abundance and richness of pseudoscorpion species by sampling technique in two urban forest fragments (Reman and Cururu) in Manaus, Amazonas, Brazil. Values are the number of individuals of each species captured with each of three sampling methods (beating of understory palm trees, Winkler extractor and Berlese-Tullgren funnel) and overall.

Family	Taxon	Reman				Cururu				Overall
		Beating	Winkler	Berlese	Total	Beating	Winkler	Berlese	Total	
Chthoniidae	*Pseudochthonius homodentatus* Chamberlin, 1929	12	3	2	17		2	1	3	20
	Tyranochthonius minor Mahnert, 1979		2	1	3		5		5	8
Ideoroncidae	*Albiorix* aff. *arboricola*	8	1	3	12		5	1	6	18
Syarinidae	*Ideoblothrus brasiliensis* (Mahnert, 1979)		2	14	16		5	7	12	28
Geogarypidae	*Geogarypus amazonicus* Mahnert, 1979	8			8	45	8		53	61
Olpiidae	*Apolpium* aff. *vastum*	1	2		3	7	11		18	21
	Pachyolpium irmgardae Mahnert, 1979	3	15		18	9	9		18	36
Cheiridiidae	*Apocheiridium* sp.			1	1					1
Atemnidae	*Brazilatemnus browni* Muchmore, 1975		14	1	15		34	7	41	56
	Paratemnoides nidificator (Balzan, 1888)					17			17	17
Chernetidae	*Parachernes* sp.	1			1					1
Total abundance		33	39	22	94	78	79	16	173	267
Richness		6	7	6	10	4	8	4	9	11

Table 2; Figure 4). Potassium concentration also affected species composition, but with a more moderate effect (PERMANOVA: $F_{7, 54} = 2.918$; $R^2 = 0.04$; $p < 0.05$; Table 2; Figure 4). Among the species collected with the Berlese funnel, *Ideoblothrus brasiliensis* (Mahnert, 1979) tended to have greater abundance at low potassium concentrations, while *Brazilatemnus browni* Muchmore, 1975 tended to be more abundant at points with higher potassium concentrations (Figure 4). The other species were uniformly distributed among points or were collected at a single point (Figure 4a). Among the species sampled with the Winkler extractor, *Pachyolpium irmgardae* Mahnert, 1979 tended to be more abundant in soil with lower potassium concentrations, while *Apolpium* aff. *vastum*, *Geogarypus amazonicus* Mahnert, 1979, and *Brazilatemnus browni* tended to be more abundant with higher potassium concentrations. The other species had an approximately uniform distribution or were sampled at a single point (Figure 4b). pH, organic matter content, sampling site and sampling points within sampling sites did not affect the distribution of the pseudoscorpions (Table 2).

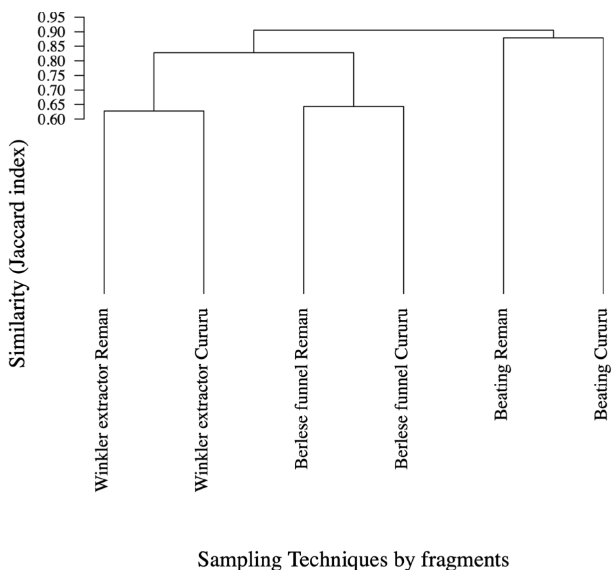

Figure 3. Dendrogram of species composition of pseudoscorpions sampled with three sampling techniques in two urban forestfragments in Manaus, Amazonas, Brazil. Obtained from the matrix of the Jaccard coefficient of similarity and the grouping method UPGMA.

Table 2. Results of PERMANOVA for the effects of soil varibles (clay content, pH, organic matter content, potassium concentration), sampling sites, sampling techniques, and sampling points within sites on species composition of the pseudoscorpions sampled with Winkler extractor and Berlese-Tullgren in two urban forest fragments (Reman and Cururu), in Manaus, Amazonas, Brazil. Significant values are in bold.

Variable	F model	R^2	P partial
Clay content	0.4913	0.00695	0.714
pH	– 0.1931	– 0.00273	0.989
Organic matter content	2.4537	0.03469	0.056
Potassium concentration	2.9184	0.04126	**0.044**
Sampling site	0.6970	0.00985	0.567
Sampling technique	8.3195	0.11762	**0.001**
Sampling point	2.0464	0.02893	0.103

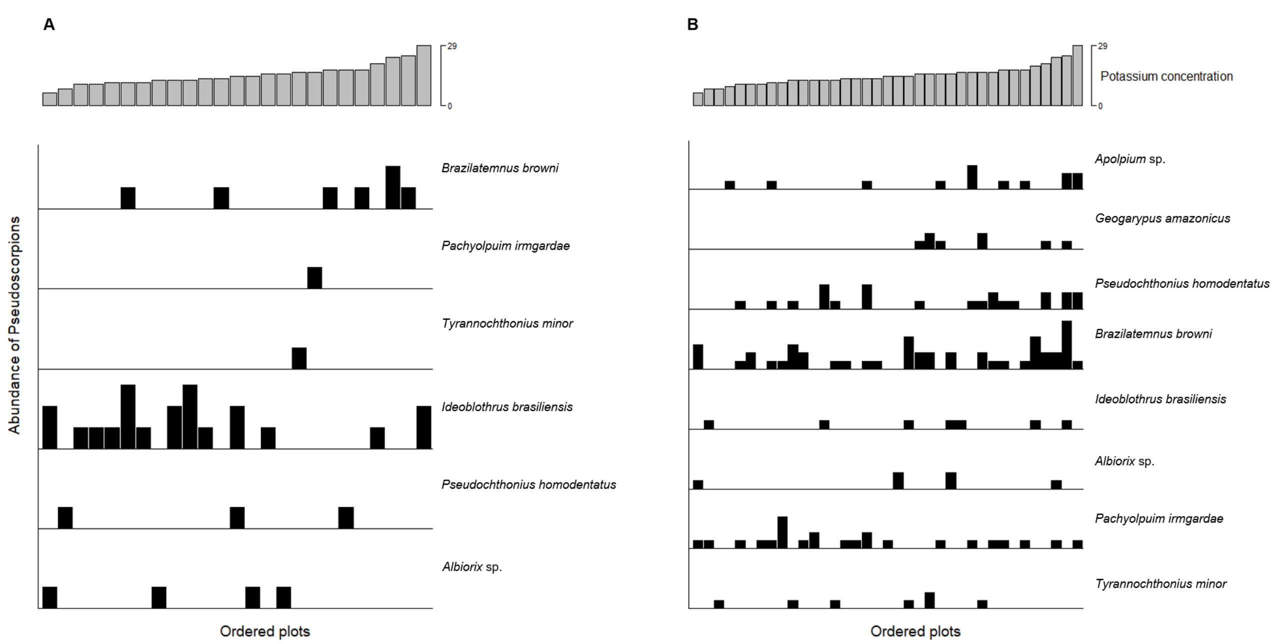

Figure 4. Distribution of pseudoscorpion species relative to soil variables in 12 sampling points in each of two urban forest fragments in Manaus (Amazonas, Brazil) sampled with Berlese funnel (A) and Winkler extractor (B).

DISCUSSION

Pseudoscorpions are considered a meso-diverse group with approximately 3850 species formally described worldwide (Harvey 2013a; Benavides *et al*. 2019). In Brazil, 66 genera and 174 species are currently known (Harvey 2013b). In the Brazilian Amazon, 65 species of 11 families are known, and 52 of them occur in the municipality of Manaus, (Mahnert and Adis 2002), 18 of which in the Ducke Reserve, a 10,000-ha primary forest area near Manaus (Aguiar *et al.* 2006; Adis *et al.* 2009). In a dense tropical rainforest in Coari (central Amazon), 15 pseudoscorpion species were recorded in understorey palm trees (Aguiar and Bührnheim 2003). Ten of the 11 species recorded in our fragments also occur in Ducke Reserve. The exception is *Apocheiridium* sp., which was found only in soil samples in Reman. Although urbanization and its effects are usually classified as deleterious to biodiversity (Vitousek *et al*. 1997; McIntyre *et al*. 2001; Laurance and Engert 2022), we recorded 11 species of pseudoscorpion in urban forest fragments. This corresponds to 60-70% of the species richness recorded in tropical rainforest areas without anthropic influence and with a much larger sampling effort (Aguiar and Bührnheim 2003; Aguiar *et al.* 2006). A similar trend was observed in the same urban forest fragments for ground-dwelling ants, with a high richness of genera and species compared to surveys carried out in protected forests (Souza and Araújo 2020).

Regardless of the fragment, the Winkler extractor sampled the largest number of species (8), while beating recorded 7, and Berlese 6. In Ducke Reserve, a combination of manual collection and Berlese funnel recorded 10 species of 7 genera and 4 families (7 species and 81.5% abundance with manual collection, and 8 species with Berlese) (Aguiar *et al.* 2006).

The clustering dendrograms grouped the species assemblage obtained through beating apart from those obtained through the edaphic techniques, suggesting that the pseudoscorpion fauna living on vegetation is differentiated from that living on the ground. The assemblages sampled with edaphic techniques formed more similar yet differentiated groupings, equally suggesting some differentiation between pseudoscorpion soil and litter fauna. These results highlight the need to use more than one sampling technique to obtain representative samples of pseudoscorpion fauna. The complementary effect of sampling methods has also been observed in other sites with a wider variety of phytophysiognomies in the Amazon for pseudoscorpions (Aguiar *et al.* 2006), spiders and harvestmen (Tourinho *et al.* 2014; 2018) and ants (Souza *et al.* 2007; 2012). However, this is the first time that the effect of complementarity between sampling techniques has been recorded for pseudoscorpions in urban forest fragments. The vertical stratification of the invertebrate fauna is quite pronounced in some groups (Amorim *et al.* 2022), so that techniques that sample at different heights should probably be useful in most cases.

Regardless of the sampled fragments, the species composition in our edaphic samples was influenced by the potassium concentration in the soil, and varied significantly with the sampling techniques, corroborating the results of the cluster dendrograms. Environmental variables (slope and altitude of terrain) explained 7 to 8% of the variance in the distribution of pseudoscorpions in Ducke Reserve (Aguiar *et al.* 2006). Studies with other invertebrates in the Amazon have also detected the effect of environmental variables on species richness and composition (Oliveira *et al.* 2009; Souza *et al.* 2009; Gomes *et al*. 2018; Torres *et al*. 2020), often with subtle effects (Moraes *et al*. 2011; Franklin *et al*. 2013; Souza and Araújo 2020) as those observed in our study. The magnitude of environmental effects may be related to the spatial scale of the sampling effort, as the variability of certain environmental factors can only be detected across larger scales. For instance, while our survey comprised 24 sampling points in an area of1.58 km^2, the survey by Aguiar *et al*. (2006) was carried out in 72 plots in an area of 72 km^2. Also, environmental complexity increases with the area, making ecological interpretation more difficult (May 1994). Ecological responses associated with frequently used diversity metrics (richness, abundance, and composition) form the basis of most biodiversity sampling protocols, therefore it is crucial to extend this information to understudied groups such as pseudoscorpions.

Urban forest fragments are usually considered environments of high vulnerability and low diversity under constant anthropogenic impact, and the consequences of urbanization are generally harmful to wildlife (Vitousek *et al.* 1997; McIntyre *et al.* 2001; Fischer and Lindenmayer 2007; McKinney 2002; 2008; Laurance and Engert 2022; Simkin *et al.* 2022). While the impact of urbanization is comparable to high-impact human activities such as agriculture and deforestation (Simkin *et al.* 2022), there also are positive effects such as the emergence of new environments that do not occur elsewhere (Niemelä 1999; Marshall and Shortle 2005), favoring species with colonizing abilities (McIntyre 2000; Egerer *et al.* 2017). In this sense, the small forest fragments studied, although located in an inhospitable matrix and distant from larger forest areas, still harbor a relevant complement of the known pseudoscorpion species in the region.

CONCLUSIONS

Anthropically impacted urban forest fragments still maintained a high diversity of the pseudoscorpion fauna known for the central Brazilian Amazon. The Winkler extractor produced the most diverse and abundant sample, although complementarity with other sampling techniques provided a better overview of pseudoscorpion diversity in the fragments, especially the method the sampled higher strata. The arboreal species assemblage was differentiated from the edaphic assemblage (soil and litter). The edaphic assemblage was subtly affected by the potassium concentration in the soil, suggesting that complex processes act on assemblage structure, even in environmentally disturbed sites, highlighting the importance of forest fragments for maintaining biodiversity in urban areas.

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