

Differences in leaf litter usage as food sources, refuge, and foraging substrates by invertebrates in forest and grasslands in the eastern Amazon

Diferenças no uso da serapilheira como fonte de alimento, refúgio e substrato de forrageamento por invertebrados em floresta e pastagem na Amazônia oriental

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Abstract: Forest conversion into grassland changes the environment, microhabitat, and food availability. Leaf litter microhabitats change from complex leaf variety piles to nutrient-poor grass heaps. In this context, this work aimed to investigate how the invertebrate compositions in the forest and grassland leaf litters differ in litter usage. To achieve this goal, litterbag traps were placed in four secondary forest samples and four grassland samples from the eastern Amazon. After litter exposure, the species were morphotyped and classified according to leaf litter usage as food, refuge, or foraging substrates. Disturbance-sensible groups characterized the secondary forest, while disturbance-tolerant species characterized the grasslands. The proportion of individuals using litter as food in grasslands is almost twice that in the secondary forest, while the percentage of individuals using leaf litter as refuge is eighteen times higher in the forest than in the grasslands. It seems that predators forage in the leaf litter of the forest just as much as in grasslands. The greater proportion of invertebrates using litter as a refuge in the forest relates to the limiting space in the habitat. In turn, food availability is scarce in grasslands, so there is urgency in using litter as a food source in this case.

Keywords: Detritivores. Land use. Litterbags. Microhabitat. Predators.

Resumo: A conversão de florestas em pastagens modifica o ambiente e a disponibilidade de microhabitats e de recursos. A serapilheira muda de amontoados de folhas de espécies florestais para amontoados de gramíneas com baixo valor nutricional. Nesse contexto, o objetivo deste estudo foi investigar como os invertebrados de serapilheira em floresta e pastagem se diferem com relação ao uso dessa serapilheira. Para isso, foram distribuídos quatro pacotes de serapilheira em amostras de floresta e quatro em pastagem na Amazônia oriental. Após o tempo de exposição, os invertebrados foram classificados em relação ao uso da serapilheira em espécies que a adotaram como comida, refúgio ou substrato de forrageamento. Os resultados da floresta indicaram comunidades de invertebrados sensíveis às modificações ambientais, enquanto pastagem, por invertebrados tolerantes. A proporção de indivíduos se alimentando da serapilheira foi duas vezes maior em pastagem. Porém, a proporção de indivíduos se refugiando na serapilheira foi 18 vezes maior na floresta. A mesma proporção de indivíduos utilizou a serapilheira como substrato de forrageamento nos dois ambientes. Maiores proporções de invertebrados refugiados em amostras de floresta devem-se à limitação de espaço no ambiente, enquanto, na pastagem, a disponibilidade de comida é menor, havendo, portanto, urgência em utilizar o recurso disponibilizado como fonte de alimento.

Palavras-chave: Detritívoros. Uso de terra. Pacotes de serapilheira. Microhabitat. Predadores.

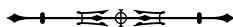
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INTRODUCTION

Forest conversion into land-use activities such as monoculture and pasture systems is one of the main threats to the Amazon (Malhi et al., 2008). In 2019, the region that accounts for at least a quarter of the world's terrestrial species showed a 30% increase in deforestation compared to 2018 (Malhi et al., 2008; PRODES, 2019). After the deforestation, the Amazon forest changes from a complex environment with a significant number of microhabitats (i.e., places for species to live or use as a refuge) and resources (i.e., organic material from where species may extract energy to live) to a simpler environment with harsher conditions and a smaller number of microhabitats and resources available (Lindsay & Cunningham, 2009; Ehlers Smith et al., 2017). With this change in vegetation, environmental conditions, microhabitats, and resource availability, the biological composition also changes (Andersen & Majer, 2004).

Specialist and disturbance-sensitive species that used to live in the forest environment can no longer support the new environmental conditions of the modified ambient and are replaced by generalist and disturbance-tolerant species, the ones that can support the new environmental conditions (Memmott et al., 2000; Andersen & Majer, 2004). Invertebrates account for at least 90% of the world's species. They are one of the biological groups that often present this composition change pattern, beyond being a significant part of Amazon's biological community (Andersen & Majer, 2004).

One of the significant sources of microhabitats and resources for invertebrates is the leaf litter that stands above the forest ground (Olson, 1994). Some species find the leaf litter attractive for its potential as a refuge for living in or a foraging substrate, such as ants that build their colonies in the leaf litter substrate and spiders looking for prey (Dobson, 1994; Queiroz et al., 2013). Other species are interested in the potential of leaf litter as a food source, like detritivore species such as mites and springtails, which degrade the leaves to extract energy for living (Richardson, 1992; Brückner et al., 2018).

This work aimed to investigate the changes in invertebrate composition that may occur after the conversion of forests to grasslands. The hypothesis was that the forest invertebrate composition would be different from that of the grasslands, with forests being characterized by disturbance-sensitive and specialist invertebrates concerning their environmental requirements and grasslands being characterized by disturbance-tolerant species that can live in harsh conditions. Moreover, the leaf litter usage by the invertebrates was determined based on morphospecies ecology. In forests, invertebrates would colonize by searching primarily for refuge since high-quality food is abundant in forest litter, so there should be no urgency for invertebrates to feed on the leaf litter provided in the habitat during the study (Richardson, 1992). However, space may be limited in the forest, which may increase the urgency to use litter as a refuge to hide from predators or build colonies (Richardson, 1992; Walls, 1995). Predators looking for forage substrates would also be more abundant in the forest litter since they are described as more diverse in forests (Moço et al., 2005). On the other hand, the leaf litter in grasslands would be colonized mainly by invertebrates looking forward to feeding on it since food sources are scarce in grasslands (Richardson, 1992; Xavier et al., 2011).

MATERIAL AND METHODS

STUDY AREA

This study was carried out in the School Farm of the Federal Rural University of the Amazon in Castanhal, Pará, Brazil, in the eastern Amazon (Figure 1). The farm area is a mosaic of land uses (from grasslands to agroforests), with secondary forest fragments preserved within the farm area for forty years. We sampled the invertebrate assemblage in four areas of secondary forest fragments and four areas of natural grassland, with the latter not having cattle and having been implemented since the inauguration of the farm forty years before, near lakes where pisciculture is developed.

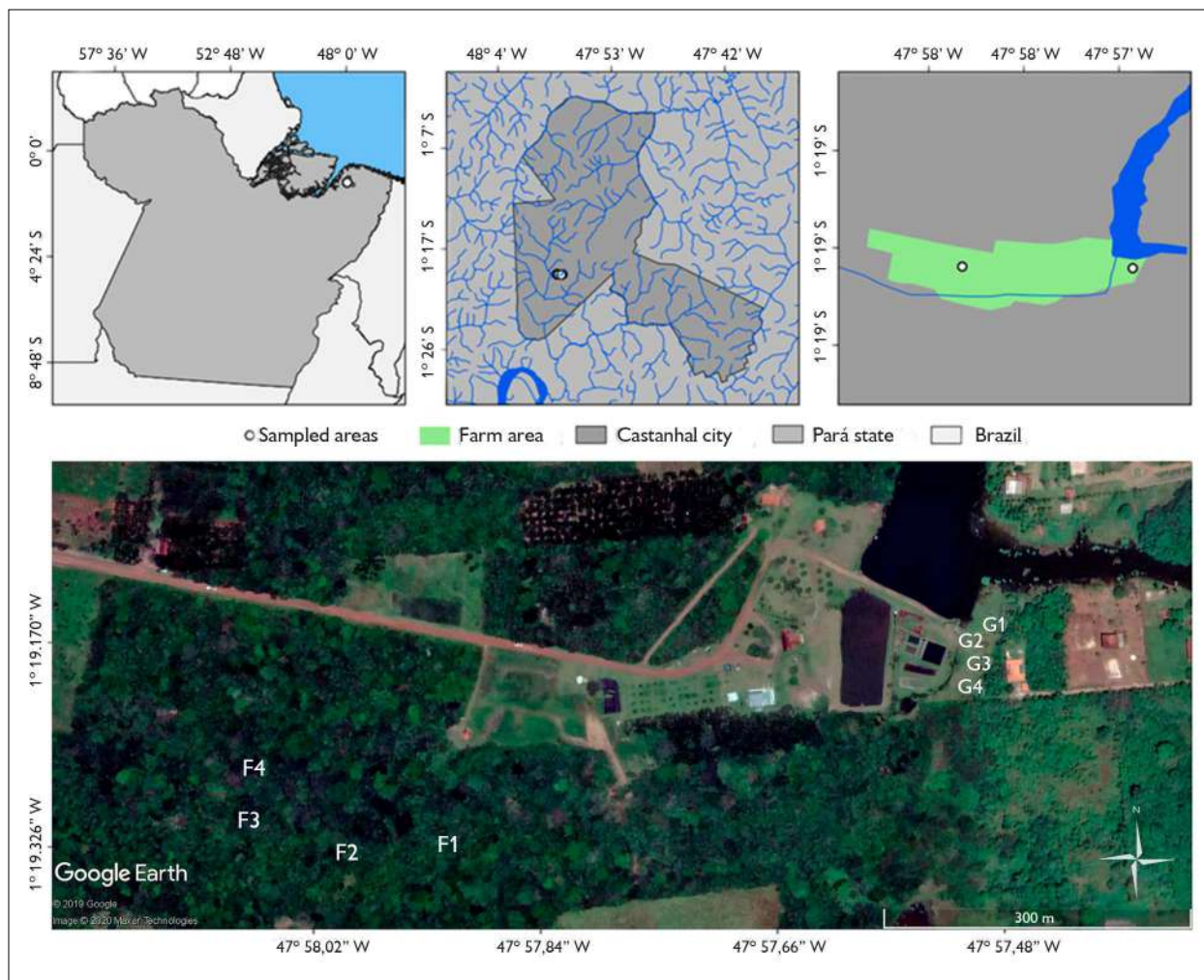


Figure 1. Location of the areas sampled in this study at the School Farm of the Federal Rural University of the Amazon in the city of Castanhal, Pará, Brazil, in the eastern Amazon. The samples from the secondary forest are identified with an F (e.g., F1, F2, F3, and F4), while grassland samples are identified with a G (e.g., G1, G2, G3, and G4). Map by Rafael Chagas, modified by Lucas Colares (2019).

Based on published literature, the climate of the region is humid tropical with two contrasting seasons: a dry one from June to November and a rainy season from December to May (Moraes et al., 2005). The mean annual rainfall reaches 2,432 mm, with a minimum of 63 mm in November and a maximum of 411 mm in March (Climate.Data, 2019). The mean annual temperature is 26.5 °C, with a minimum of 25.8 °C in March and a maximum of 27 °C in October (Climate.Data, 2019). This study was carried out during the rainy period, in December 2019.

SAMPLING METHOD

Leaf litter was collected from the secondary forest and then sifted so that most of the invertebrates could fall through the sieve openings. The leaves were also visually treated, aiming to remove any invertebrates that persisted after sieving. Then, the filtered leaf litter with no invertebrates was kept isolated in closed plastic bags to prevent new invertebrates from entering and colonizing it before the exposure time in grasslands and forest areas. All of the leaf litter was collected from the

secondary forest so the substrate of the leaf litter traps could be standardized with high-quality litter.

The filtered leaf litter was deposited in same-sized plastic bags with a large mesh (3 cm) so that they could allocate similar amounts of litter; these were the litterbags (Figure 2A) (Pereira et al., 2013). The large mesh allows any potential invertebrates from the micro, meso, or macrofauna to enter the bag and colonize it. Litterbags were then randomly distributed at four points of natural grasslands and four points of secondary forest, apart from each other for at least 30 m. Each litterbag was considered a different sample.

Therefore, four litterbags were exposed to grassland (Figure 2B) and four litterbags to the secondary forest (Figure 2C) environment for six days, and, although the colonization time is of a few days, the highest litter decomposition rates are documented to occur in the first days of litter exposure (Stripari & Henry, 2002). After the exposure time, the litterbags were collected in plastic bags (involving the bags from the top of the litterbag and closing at the bottom to prevent the escape of many invertebrates) with pieces of cotton engorged with acetate so invertebrates could be killed by asphyxia once the bag was closed. The leaves from each litterbag were cleaned with a brush, and the material that fell from the cleaning was analyzed through magnifiers in search of invertebrates. The analyzed content was deposited in the Zoological Didactic Collection of the Federal University of Pará.

The species were grouped into morphotypes and taxonomically identified to the taxonomic level of order or family (only adult individuals were accounted for) with the help of specialists and the taxonomy literature (Cohen & Weiner, 2004; Leite & Sá, 2010; Trautwein et al., 2012; Miyazawa et al., 2014; Insect Identification, 2020). The abundance was counted categorically due to the considerable presence of some taxa, and five abundance categories were defined: 0 if no individuals of the species were found; 1 if only one individual of the species was found; 10 if two to ten individuals of the species were



Figure 2. Photographs of (A) litterbags after removing all invertebrates and ready to be exposed to the environment of (B) grassland and (C) secondary forest in the School Farm of the Federal Rural University of the Amazon in Castanhal, Pará, Brazil, in the eastern Amazon. Photos: Lucas Colares (2019).

found; 25 if more than ten individuals of the species were found; and 100 if an uncountable number of individuals of the species was found.

To identify the factors influencing leaf litter colonization in the secondary forest and grasslands, the morphospecies were classified into three litter usage categories: 'using litter as food', 'using litter as a refuge', and 'using litter to forage'. Species were classified into these leaf litter usage categories according to autecology information from their taxonomic groups (Barnes et al., 2005). Taxa with detritivore species were always classified as 'using litter as food', predators were classified as 'using litter to forage', and other taxa were classified as 'using litter as a refuge'.

STATISTICAL ANALYSIS

To identify differences in invertebrate species composition between natural grasslands and secondary forest areas, a permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations was used (Legendre & Legendre, 2012). We employed the PERMANOVA to test for a dissimilarity matrix returned

by the Jaccard distance, which ranges from 0 to 1: the closer the value is to 0, the more similar two samples are concerning their species composition, and the closer the distance is to 1, the more dissimilar the two samples are concerning their species composition (Legendre & Legendre, 2012).

The proportion of individuals in each litter usage category relative to all individuals found in each sample (thus using abundance data to calculate the proportion) was calculated to assess the factors influencing leaf litter usage in the habitats. This proportion ranges from 0 to 100: the closer it is to 0, the fewer individuals from the leaf litter usage category are in the sample, whereas the closer it is to 100, the more individuals from the leaf litter usage category are in the sample. Differences in the proportion values were tested using individual T-tests with unequal variance for each type of leaf litter usage. This non-parametric test was chosen since the proportion data does not fit the assumptions for parametric analysis.

The categorical predictor for the T-test was the habitat (secondary forest or grassland), and the quantitative dependent variables were the proportions of each of the three litter usage categories.

The PERMANOVA was carried out using software R and the 'vegan' package (Oksanen et al., 2016), and the T-Tests we conducted in software Statistica (Hill & Lewicki, 2006). We considered a significance level of 0.05 in both analyses.

RESULTS

Forty morphospecies belonging to sixteen different invertebrate taxonomic orders were identified in the study (Table 1). Thirty-one of these species occurred in the secondary forest, with twenty-seven being exclusive of forests; in turn, twelve occurred in the grassland, with eight being exclusive to the land use (Table 1). Thus, the total number of species was higher in the secondary forest than in the grassland (Figure 3).

Table 1. Taxonomic composition, abundance, and litter usage category of invertebrates from leaf litter in secondary forest and grassland litterbags from the School Farm of the Federal Rural University of the Amazon in Castanhal, Pará, Brazil, in the eastern Amazon. The classification refers to whether a species uses leaf litter as food (FOOD), refuge (REFUGE), or forage substrate (FORAGE). (Continue)

Taxon	Abundance		Classification
	Secondary Forest	Grassland	
ARTHROPODA			
CHELICERATA			
Arachnida			
Acari			
Acari sp.	0	1	FOOD
Ixodida			
Ixodidae			
Ixodidae sp.	0	1	REFUGE
Mesostigmata			
Mesostigmata sp.	10	0	REFUGE
Sarcoptiformes			
Oribatida			
Oribatida sp.	21	0	FOOD
Poronota			



Table 1.

(Continue)

Taxon	Abundance		Classification
	Secondary Forest	Grassland	
Poronota sp.	11	400	FOOD
Trombidiformes			
Eupodidae			
Eupodidae sp.	1	0	FOOD
Araneae			
Araneae sp.1	0	1	FORAGE
Araneae sp.2	1	0	FORAGE
Araneae sp.3	1	0	FORAGE
Araneae sp.4	1	0	FORAGE
Araneae sp.5	0	1	FORAGE
Salticidae			
Salticidae sp.1	0	1	FORAGE
Salticidae sp.2	0	1	FORAGE
Salticidae sp.3	1	0	FORAGE
Pseudoscorpiones			
Pseudoscorpiones sp.1	2	0	FORAGE
Pseudoscorpiones sp.2	1	0	FORAGE
CRUSTACEA			
Malacostraca			
Isopoda			
Isopoda sp.	1	21	FOOD
HEXAPODA			
Entognatha			
Collembola			
Entomobryomorpha			
Entomobryidae			
Entomobryidae sp.1	2	0	FOOD
Entomobryidae sp.2	1	0	FOOD
Isotomidae			
Isotomidae sp.1	11	0	FOOD
Isotomidae sp.2	11	0	FOOD
Insecta			
Blattodea			
Blattaria			
Blattaria sp.1	2	0	FOOD
Blattaria sp.2	1	0	FOOD

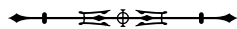


Table 1.

(Conclusion)

Taxon	Abundance		Classification
	Secondary Forest	Grassland	
Coleoptera			
Coleoptera sp.1	1	0	FOOD
Coleoptera sp.2	2	0	FOOD
Diptera			
Diptera sp.	1	0	REFUGE
Hymenoptera			
Formicidae			
Formicidae sp.1	10	0	REFUGE
Formicidae sp.2	110	0	REFUGE
Formicidae sp.3	100	1	REFUGE
Formicidae sp.4	10	1	REFUGE
Formicidae sp.5	0	11	REFUGE
Formicidae sp.6	1	0	REFUGE
Orthoptera			
Orthoptera sp.1	1	0	FOOD
Orthoptera sp.2	1	0	FOOD
Thysanoptera			
Thysanoptera sp.1	3	0	REFUGE
Thysanoptera sp.2	1	0	REFUGE
MYRIAPODA			
Diplopoda			
Diplopoda sp.1	1	0	FOOD
Diplopoda sp.2	0	36	FOOD
Diplopoda sp.3	1	0	FOOD
MOLLUSCA			
GASTROPODA			
Gastropoda sp.	0	1	FOOD
Total	324	603	

Collembola, Pseudoscorpiones, Blattodea, Coleoptera, Diptera, Orthoptera, and Thysanoptera were found exclusively in the secondary forest, while Gastropoda (a single individual) was found exclusively in grassland samples (Figure 3). The most abundant groups in the secondary forest and the grassland samples were ants

(Formicidae sp.2 and sp.3; Table 1; Figure 3A) and mites from the Poronota suborder (Table 1; Figure 3B), respectively. The ants developed a complete colony in one of the forest samples after the exposure time, evinced by the presence of all castes of the species, including eggs and a queen (Figure 3A).

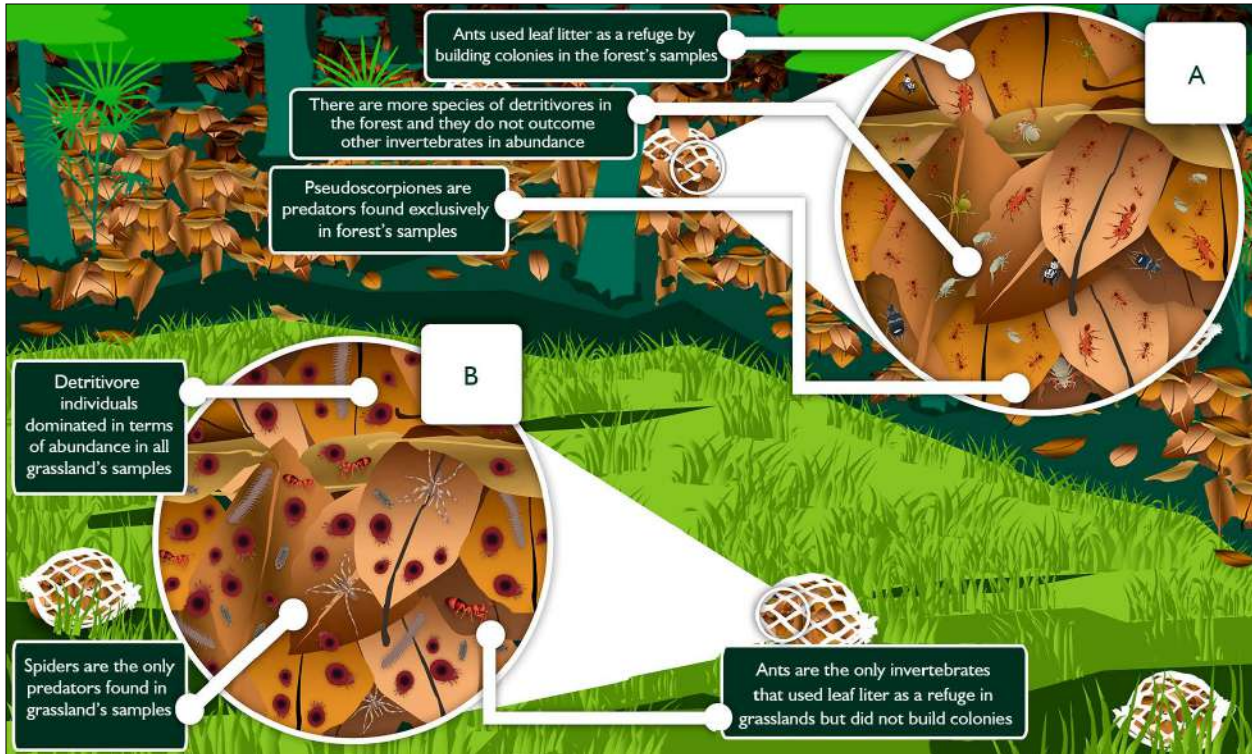


Figure 3. Digital illustration of the invertebrate compositions found in the litterbags of (A) the secondary forest and (B) the grassland. *Poronota* sp., *Araneae* sp.1, *Diplopoda* sp.2, *Isopoda* sp., and *Formicidae* sp.5 are illustrated in the grassland circle (B). *Formicidae* sp.2 and sp.3, *Araneae* sp.2, *Salticidae* sp.3, *Blattaria* sp.1, *Entomobryidae* sp.1, and *Pseudoscorpiones* sp.1 are illustrated in the secondary forest circle (A). The proportions and colors in this illustration are for visual purposes only and might not reflect reality.

The invertebrate species compositions were different between the secondary forest and grassland samples (PERMANOVA: Pseudo-F = 2.352; $R^2 = 0.282$; $p = 0.026$) (Figure 4).

The proportion of individuals using leaf litter as a food source was, on average, 47.07% in the secondary forest samples against 96.87% in the grassland samples. There were twice more individuals using leaf litter as a food source in grasslands than in the forest (T-test for unequal variances: $T = 2.63$; $DF = 6$; $p = 0.039$; Figure 5A).

Regarding the proportion of individuals using leaf litter as a refuge, on average, 47.75% of the individuals were doing so in the secondary forest samples against 2.57% in the grassland samples. This corresponds to eighteen times more individuals using litter as a refuge in secondary forest samples compared to the grassland samples

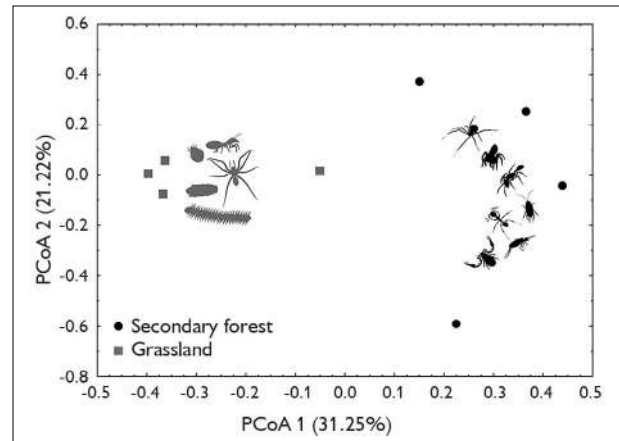


Figure 4. Principal coordinates analysis ordering of the invertebrate community in four secondary forest samples (closed circles) and four grassland samples (closed squares). The two areas are different in species composition, as shown by the PERMANOVA. The invertebrate silhouettes on the left are invertebrates found in grasslands, while silhouettes on the right are invertebrates found in the secondary forest. Silhouettes as shown in Figure 3.

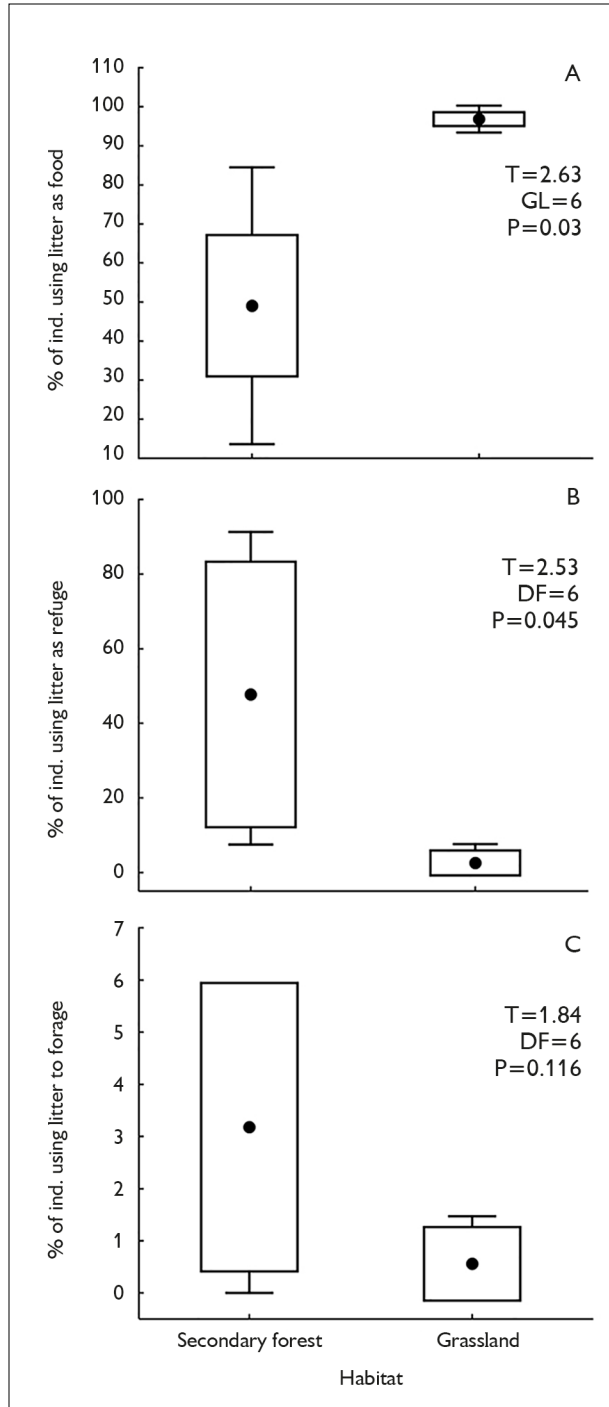


Figure 5. Boxplots representing the proportion (%) of individuals using the leaf litter as (A) food, (B) refuge, and (C) forage substrate in each environment. The middle points of the boxplots are the mean values, boxes are the standard deviations, and whiskers are the minimum and maximum values.

(T-test for unequal variances: $T = 2.53$; $DF = 6$; $p = 0.045$; Figure 5B).

Finally, the proportion of individuals using leaf litter as a foraging substrate to search for prey was, on average, 3.18% in the secondary forest samples against 0.56% in the grassland samples, with the difference in proportions not being statistically significant (T-test for unequal variances: $T = 1.84$; $DF = 6$; $p = 0.116$; Figure 5C).

DISCUSSION

Overall, this study evinced that the invertebrate compositions in the leaf litter of the secondary forest and grasslands are different. Disturbance-sensitive groups such as Collembola and Pseudoscorpiones occurred exclusively in the secondary forest, while disturbance-tolerant groups such as Formicidae and Acari characterized grasslands. Yet, different usages of leaf litter were found in the secondary forest and grassland samples. The proportion of individuals using leaf litter as a refuge in the secondary forest samples is eighteen times higher than in the grassland samples. On the other hand, twice as many individuals colonized leaf litter in grassland samples interested in using it as a food source compared to the secondary forest samples. Beyond this contrast between usage as refuge or food, there seems to be no difference in the proportion of individuals using the leaf litter as a foraging substrate between secondary forest and grassland samples.

The invertebrate composition changes when forest areas are converted into land use management, as made evident for grasslands. Forest areas provide a wet and warm environment due to the constant rain regime in the Amazon and the shade provided by the vegetation (Malhi et al., 2008). The microclimate in forest-ground leaf litter is even wetter and warmer due to the huddle of leaves always close to each other, which can retain rainwater and preserve mild temperatures close to 26 °C (Olson, 1994; Moço et al., 2005). These environmental conditions are ideal for the persistence of disturbance-sensitive species, as has been found for Pseudoscorpiones and springtails

(Collembola) occurring only in secondary forest areas (Figure 3A). The two groups are known to depend on mild temperatures and high soil moisture due to their considerable water loss through respiration (Harte et al., 1996; Lighton & Joos, 2002).

In turn, when forest areas are converted into grasslands, the environment tends to present harsher humidity and microclimate conditions (Breshears, 2006). Due to the predominance of grass and the absence of a canopy cover, there is a high incidence of solar rays on the soil, which raises the temperature near the ground close to 30 °C and accelerates the evaporation of water in the environment (Martens et al., 2000; Breshears, 2006). Thus, the microclimate in the leaf litter of grasslands tends to be dry and warmer than that in forests (Martens et al., 2000). The species found in the grassland environment must tolerate the hard conditions provided by the disturbed area, as shown here by the predominance of mites (Acari) from the Poronota suborder in the grassland areas (Figure 3B). Species from this suborder are known for being tolerant to a wide range of environmental conditions and often appear in high densities in agricultural systems (Maraun & Scheu, 2000; Murvanidze et al., 2011).

The leaf litter colonization by invertebrates looking for leaf litter as a food source was different between grasslands and secondary forests. Species that eat leaf litter are detritivores, those who break down the organic matter of the leaf litter, participating in the nutrient cycle of the ecosystem (Pereira et al., 2013). The more considerable presence of detritivores in the grassland litterbags may be due to resource limitations in the land-use since the leaf litter quality is lower than that of the forest (Xavier et al., 2011; Cofiteaux et al., 2016). Such quality depends on leaf nitrogen (Smith & Bradford, 2003) because, after deforestation, the soil nitrogen is depleted, and the amount of nitrogen that grass species have available to uptake is lower (Li et al., 2012). The quality of grassland leaf litter is lower than that of forest litter since the leaf nitrogen in grassland litter is not high (Cho et al., 2013). The input

of a forest-quality leaf litter in our study characterized a tremendous energy source to be used by detritivore species, which appeared in high abundance in the grassland samples. This proportion of detritivores in grasslands was represented mainly by mites from the Poronota suborder (Table 1), with over 100 individuals occurring in all grassland samples (Figure 3B).

In the forest samples, there was a lower proportion of detritivore species compared to the grassland samples. High-quality leaf litter is a resource always available since the complex vegetation of the forest continues to the leftover leaves that accumulate aboveground with higher amounts of nitrogen than in grasslands (Li et al., 2012). Therefore, detritivore species of forests still have plenty of high-quality food (Cho et al., 2013). Thus, a lower proportion of detritivores in the secondary forest sample may be due to the non-urgency in using leaf litter as a food source since there is plenty of leaf litter in the forest. Despite the lower proportion of detritivores in terms of abundance, forest areas surpass grasslands in terms of the number of detritivore species, having an even number of individuals per species, represented by springtails, cockroaches (Blattaria), and mites different from the ones found in the grassland samples (Table 1; Figure 3A).

There were eighteen times more invertebrate individuals using leaf litter as a refuge in the secondary forest samples than in the grassland samples. These species were using leaf litter as shelter in search of the microclimate conditions presented by the leaf litter microhabitat (Richardson, 1992). Some species that live in wet and warm environmental conditions like the ones provided by leaf litter may find a place to live among the accumulated leaves, just as we found a complete ant colony (Formicidae) developed in one of the forest litterbags (Figure 3A). The proportion of individuals using leaf litter as refuge is higher in the secondary forest samples than in the grassland litter, mainly due to space limitation. Since the Amazon forest has enormous biodiversity of invertebrates, the space to live in the forest is limited, so any microhabitat is swiftly colonized

as species try to find a place to live (Richardson, 1992). In turn, species present in grassland areas are already tolerant to the harsh environmental conditions, so they do not need refuge in leaf litter heaps (Richardson, 1992).

Due to the high biodiversity in leaf litter looking for either food or refuge, some predators started appearing to use the leaf litter as a place to hunt since there was enough prey available in the microhabitat (Moço et al., 2005). Spiders (Araneae) corroborated this inference by appearing in the grassland samples just as much as in the secondary forest samples (Table 1; Figure 3). The proportion of species using leaf litter as a foraging substrate did not differ between the secondary forest and grassland samples. This non-difference between the two areas evinces that predators are present in the trophic web of secondary forests and grasslands but represented by different species.

CONCLUSIONS

Forest leaf litter composition is characterized by specialist species that are sensitive to disturbance. Therefore, once forest areas are converted into grasslands, this composition changes, and these sensitive species are replaced by tolerant ones, which can support the new environmental conditions. Moreover, individuals using leaf litter as food (detritivores) in grasslands surpass the number found in the forest leaf litter. This higher proportion of detritivores in grasslands may be due to resource limitations in the environment. Conversely, the proportion of invertebrates using leaf litter as a refuge is eighteen times higher in the secondary forest than in grasslands, probably due to a limitation of space in the forest considering the high biodiversity of the Amazon. Finally, due to the high biodiversity of invertebrates looking for either food or refuge, leaf litter seems an ideal place for predators to forage in the secondary forest just as much as in grasslands.

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