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Research Article

Characterization of Ant Communities (Hymenoptera: Formicidae) in Twigs in the Leaf Litter of the Atlantic Rainforest and Eucalyptus Trees in the Southeast Region of Brazil

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Fragments of Atlantic Rainforest and extensive eucalyptus plantations are part of the landscape in the southeast region of Brazil. Many studies have been conducted on litter ant diversity in these forests, but there are few reports on the nesting sites. In the present study, we characterized the ant communities that nest in twigs in the leaf litter of dense ombrophilous forests and eucalyptus trees. The colony demographics associated with the physical structure of the nest were recorded. In the eucalyptus forests, the study examined both managed and unmanaged plantations. During five months, all undecomposed twigs between 10 and 30 cm in length containing ants found within a 16-m^2 area on the surface of the leaf litter were collected. A total of 307 nests and 44 species were recorded. *Pheidole*, *Solenopsis,* and *Camponotus* were the most represented genera. *Pheidole* sp.13, *Pheidole* sp.43 and *Linepithema neotropicum* were the most populous species. The dense ombrophilous forest and a eucalyptus plantation unmanaged contained the highest number of colonized twigs; these communities were the most similar and the most species rich. Our results indicate that the twigs are important resources as they help to maintain the litter diversity of dense rain forest and abandoned eucalypt crops.

1. Introduction

The Brazilian Atlantic Forest, which once covered about one million and two hundred thousand square miles, is reduced to 12% of its original area [1] and considered one of the most endangered biodiversity hot spots in the planet [2, 3]. Currently, part of its original area is occupied by *Eucalyptus* [4], which is a genus originating from Australia. In general, eucalyptus plantations can be found in approximately 50% of all tropical forests [5], but the leaf litter has a low nutritional quality [6, 7], which adversely affect the plant communities and various animals [8], including ants [9–11].

In tropical forests, approximately 50% of the ant fauna may be associated with the leaf litter [12], participating actively in the soil structure [13, 14]. However, the factors that structure their communities are poorly understood [15, 16], especially communities that live in the leaf litter of the Brazilian Atlantic Rainforest. One fundamental goal of ecology is to understand the structure and maintenance of diverse tropical assemblages [17]; the availability of nests is one of the factors structuring litter ant communities [18].

Ants primarily find food and nesting sites in the leaf litter of tropical forests [19, 20], and diverse nesting sites are a prerequisite for maintaining ant species richness in this forest stratum [21]. Their nests are found in interstices, in twigs, inside fruit, and between decomposing leaves or trunks [22]. Among the resources provided by the leaf litter, twigs/trunks are essential for ant nests to occur [23], and this microhabitat is part of the life cycle of several species [24, 25].

In Brazil, the leaf-litter ant fauna has been extensively studied in areas reforested with eucalyptus [9–11, 26], but few studies have addressed the communities found on fallen

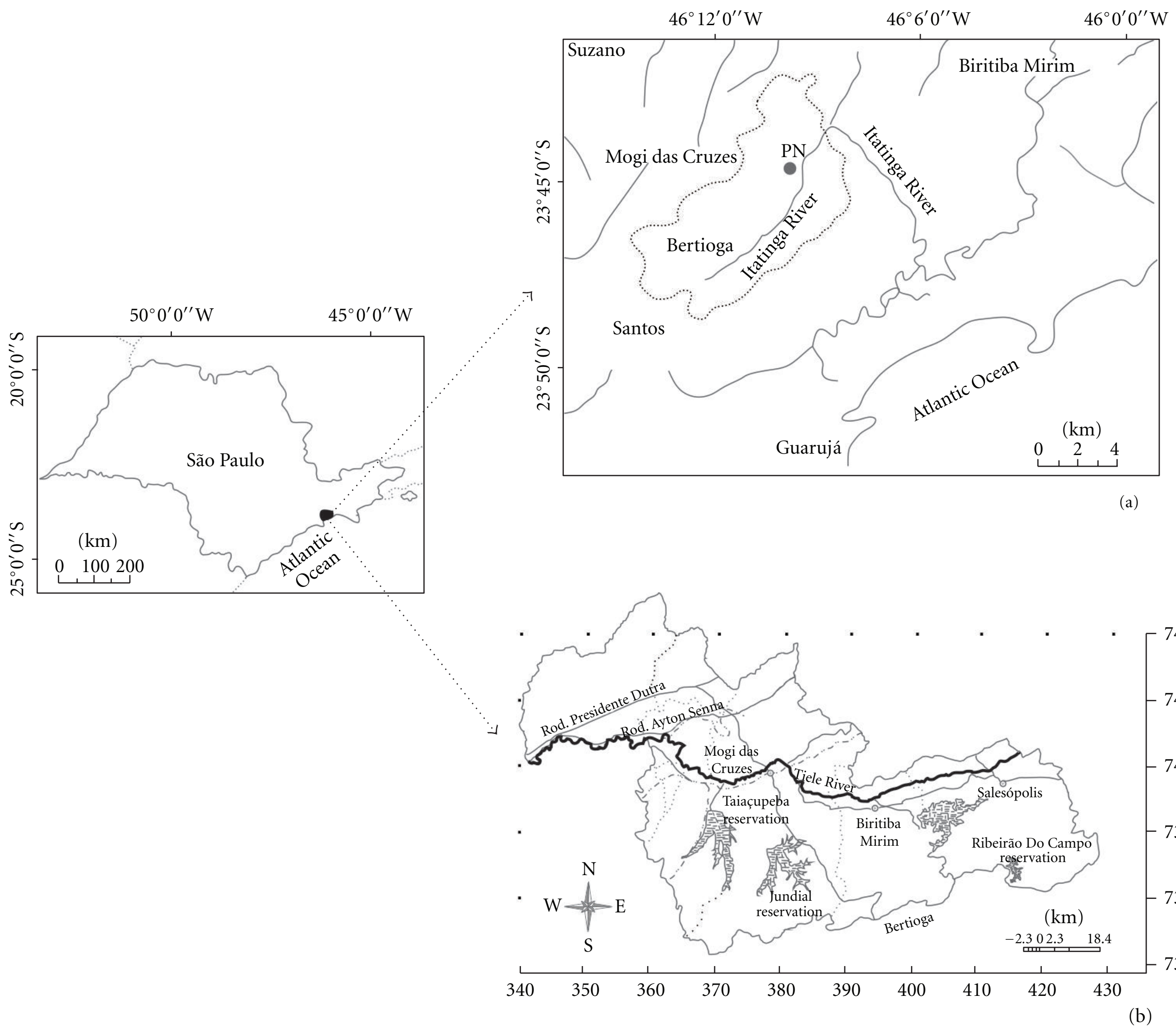

Figure 1: Location of the Itatinga (a) and Upper Tietê (b) river watersheds where the samples were collected.

twigs in this stratum. Thus, in the present study, we examined the ant communities in the twigs of the leaf litter from dense ombrophilous forests and eucalyptus plantations and compared the nest and population characteristics. Due to the specific abiotic and biotic conditions of eucalyptus forests, we expected to find smaller populations, a reduced number of species, and a decreased number of twigs containing colonies compared to in the native forests.

2. Methods

2.1. Study Area and Sampling. The colonies were collected in 20 areas located in the Itatinga and the Upper Tietê river watersheds (Figure 1) in the southeast region of Brazil. The types of forest analyzed can be described as follows.

(i) Dense ombrophilous forests (DOF, $n = 5$), characterized by a developed understory, the presence of herbaceous plants, and shrubs and trees between 2 and >20 m in height.

(ii) *Eucalyptus grandis* forests: plantations in areas of deforested DOF.

(1) Commercial plantation managed for 7 years (E1, $n = 5$) characterized by the absence of an understory, soil covered by grasses, and 25 m tall eucalyptus trees;

(2) commercial plantation managed for 28 years (E2, $n = 5$), characterized by a poorly developed understory, soil covered by grasses, and 25 m tall eucalyptus trees;

(3) plantation left unmanaged for 28 years (E3, $n = 5$), characterized by a well-developed understory with herbaceous plants, shrubs, and trees up to 20 m in height and 30 m tall eucalyptus trees.

The samples were collected weekly between December 2010 and April 2011, spanning the region's rainy season [27]. Each sampling expedition was conducted one day after rain in locations without trails and without flooded areas.

In each area, six 16 m^2 plots were delimited in each area along a linear transect. The plots were separated by 50 m to ensure the samples independence [28, 29]. The sampling effort for each plot was constant (collection time = 30 minutes and number of collectors = 3 per plot). All of the undecomposed twigs with lengths from 10 to 30 cm and containing ants were manually collected and individually placed in plastic bags for later identification. This twig size was selected because smaller twigs were generally decomposing in the sampling areas of dense ombrophilous forest, which would make it impossible to compare with fallen twigs in the litter of eucalyptus forests. Furthermore, the selected size was the approximate size of most ant-colonized twigs [23]. Only the twigs that were found on the surface were collected because they are the most recent resources in the leaf litter.

The twigs with colonies were counted (i.e., density of nests), and the ants were identified according to their genus and named according to Bolton et al. [30], except for the group *Prenolepis*, which followed the LaPolla et al. classification [31]. The species were identified by comparing them with specimens deposited in the reference collection at the Museum of Zoology, University of São Paulo, Brazil (Museu de Zoologia da Universidade de São Paulo—MZUSP). The classification proposed by Bolton [32] was used to classify the subfamilies. Voucher specimens were deposited in the Myrmecology Laboratory collection at the University of Mogi das Cruzes and at MZUSP.

2.2. Characterization of Nests, Demographic Data, and Litter Depth. Using a digital caliper, the total diameter of the twigs that contained colonies was measured; five measurements were conducted on each twig. The immature stages (eggs, larvae, and pupae) and workers were counted using a manual counter. The litter depth was measured using calipers at the corners and in the center of each 1 m^2 plot. These small plots were randomly marked within each 16 m^2 plots. The average of five values was used to define the stratum depth.

2.3. Data Analysis. Comparisons between forest types were performed using the number of occurrences of a species (presence and absence data). The species accumulation curves and estimated richness curves (Chao 2) were calculated based on the number of samples using the Estimates software [33]. The Jaccard similarity coefficient was calculated between the different forest types.

The species richness, nest abundance, abundance of immature stages, and the diameter of the twigs were compared between the different forest types using the Kruskal-Wallis test. The relationships between species richness and leaf-litter depth were identified using scatter plots and Pearson correlations. The same analyses were performed for nest abundance. All analyses were preceded by the Lilliefors test to check for data normality. The BioEstat software [34] was used for both of the tests with a 5% significance level.

3. Results

Out of the 1,920 m^2 of leaf litter, 307 nests were counted, including seven subfamilies, 18 genera, and 44 species. Myrmicinae was the richest subfamily, in both the number of nests (83) and species (19). *Pheidole, Camponotus,* and *Solenopsis* were the richest genera, with five species each (Table 1). Only one species was recorded from each twig.

The sampling effort was sufficient to record 93% of the species that live in fallen twigs in the leaf litter of the dense ombrophilous forest, 36% in commercial plantations managed for 7 years, 60% in commercial plantations managed for 28 years, and 73% in the plantations left unmanaged for 28 years (Figure 2). The Jaccard similarity coefficient ranged from 0.16 (E1 $\times$ E2) to 0.44 (DOF $\times$ E3) among different forest types.

The dense ombrophilous forest and the eucalyptus plantation left unmanaged for 28 years exhibited the most richness, including 28 and 26 species (Table 1) and an average richness of 11 ($\pm$3.36) and 11 ($\pm$2.61), respectively. The species density was 0.06 species/m^2 in the dense ombrophilous forest, 0.05 species/m^2 in crops left unmanaged for 28 years, and 0.02 species/m^2 in commercial crops managed for 7 years and commercial crops managed for 28 years.

The richness was significantly different between forest types (Kruskal-Wallis = 14.1064; df = 3; $P < 0.05$) (Figure 3(A)). The abundance of nests also differed between forest types (Kruskal-Wallis = 16.7154; df = 3; $P < 0.05$) (Figure 3(B)). The depth litter did not differ between forest types ($P > 0.05$) (Figure 3(C)). The number of workers (Kruskal-Wallis = 14.8629; df = 3; $P < 0.05$) and immature stages (Kruskal-Wallis = 16.3486; df = 3; $P < 0.05$) also differed between forest types (Figures 4(A) and 4(B)). The diameter of the twigs occupied by ant colonies did not differ between forest types (Kruskal-Wallis = 2.5200; df = 3; $P > 0.05$) (Figure 4(C)). The leaf-litter depth was related to the nest abundance ($r = -0.6299$; $P < 0.05$) (Figure 5), but not to species richness in twigs ($r = -0.3826$; $P > 0.05$).

The DOF fragments had the highest density of nests colonized by ants (0.34 nests/m^2), followed by E3 with 0.2 nests/m^2, E2 with 0.06 nests/m^2, and E1 with 0.03 nests/m^2. The colonies of *Heteroponera* sp.3, *Pheidole* sp.13, *Hypoponera* sp.4, *Linepithema neotropicum* (Alex Wild, described the species in 2007), and *Hypoponera* sp.4 occurred in the twigs with larger diameters (Table 1). The colonies of *Pheidole* sp.13 and *Pheidole* sp.43 were registered with the highest number of workers; the colonies of *L. neotropicum* have more immature individuals (Table 1).

The presence of alates and a queen was recorded for a higher number of species in the FOD fragments. The presence of more than one queen in the same colony was only recorded for *Solenopsis* sp.2 (Table 2).

4. Discussion

Many nests recorded in the litter of the different forest types can be characterized as ephemeral because only workers and immature stages were found [23]. These places of temporary

Table 1: Colony and nest structure of species recorded in twigs from the leaf litter of different forest types.

Subfamilies/ morphospecies/ species	Dense ombrophilous forest				Plantation managed for 7 years				Plantation managed for 28 years				Plantation unmanaged for 28 years			
	Nests*	Total Workers	Total Immature stages	Ø twig $\overline{X} \pm SD$	Nests*	Total Workers	Total Immature stages	Ø twig $\overline{X} \pm SD$	Nests*	Total Workers	Total Immature stages	Ø twig $\overline{X} \pm SD$	Nests*	Total Workers	Total Immature stages	Ø twig $\overline{X} \pm SD$
Dolichoderinae																
Linepithema neotropicum	12	468	2077	19.81 ± 7.84	2	50	150	34.29 ± 24.33					9	962	1231	14.94 ± 5.51
Linepithema iniquum													6	782	370	13.87 ± 4.44
Ectatomminae																
Gnamptogenys striatula	10	204	156	16.39 ± 5.72					1	72	8	22.1	4	58	84	18.02 ± 5.28
Formicinae																
Brachymyrmex incisus	2	18	14	9.54 ± 3.64	1	11	3	10.43	2	35	—	10.29 ± 7.52	9	827	448	16.94 ± 8.54
Camponotus (*Myrmaphaenus*)	2	81	41	17.91 ± 4.93												
Camponotus (*Tanaemyrmex*)									4	136	41	23.63 ± 14.63				
Camponotus sp.8	2	38	41	16.93 ± 3.94						93	38	16.91 ± 10.30	2	65	43	16.46 ± 3.19
Camponotus sp.9	2	43	42	10.75 ± 3.65												
Camponotus sp.10	12	402	622	13.65 ± 3.20												
Myrmelachista catharinae	10	710	472	17.57 ± 9.00									1	152	9	16.02
Myrmelachista ruszkii	12	621	829	12.48 ± 4.92					1	229	—	18.35	2	67	23	7.93 ± 1.15
Myrmelachista nodigera					1	22	7	9.06								
Nylanderia fulva													3	68	41	17.43 ± 5.9

Table 1: Continued.

Subfamilies/ morphospecies/ species	Dense ombrophilous forest				Plantation managed for 7 years				Plantation managed for 28 years				Plantation unmanaged for 28 years			
		Total		Ø twig		Total		Ø twig		Total		Ø twig		Total		Ø twig
	Nests*	Workers	Immature stages	$\overline{X} \pm SD$	Nests*	Workers	Immature stages	$\overline{X} \pm SD$	Nests*	Workers	Immature stages	$\overline{X} \pm SD$	Nests*	Workers	Immature stages	$\overline{X} \pm SD$
Heteroponerinae																
Heteroponera dentinodis	2	22	47	18.56 ± 6.20												
Heteroponera mayri	2	34	25	10.18 ± 5.27									2	23	7	9.38 ± 2.6
Heteroponera sp.3	2	300	41	26.54 ± 12.24												
Myrmicinae																
Acanthognathus ocellatus	1	5	1	9.2												
Acanthognathus rudis													1	14	18	27.82
Crematogaster gr. *Arthocrema*	9	234	366	15.82 ± 8.68	1	20	—	8.32	2	187	—	16.42 ± 4.2	2	170	160	19.62 ± 2.34
Crematogaster sp.7									1	118	54	9.55	2	177	12	12.96 ± 1.95
Hylomyrma sp.1													1	12	5	20.33
Mycetosoritis sp.1													1	14	7	28.9
Megalomyrmex iheringi					1	82	9	9.68					1	256	—	14.77
Pheidole sp.7	11	369	237	13.7 ± 7.62	3	205	35	10.75 ± 1.49					14	653	312	13.21 ± 4.70
Pheidole sp.13	36	1718	977	23.16 ± 17.77									11	689	417	12.60 ± 6.86
Pheidole sp.14	1	4	1	15.02												
Pheidole sp.19					1	4	16	19.61								
Pheidole sp.43	18	1378	1176	16.02 ± 5.41									5	192	97	15.21 ± 3.48
Procryptocerus sp.1	2	79	88	16.31 ± 0.56												
Procryptocerus sp.2	2	9	3	13.84 ± 1.83					8	163	142	13.99 ± 4.80	3	73	66	18.56 ± 2.59

Table 1: Continued.

Subfamilies/ morphospecies/ species	Dense ombrophilous forest				Plantation managed for 7 years				Plantation managed for 28 years				Plantation unmanaged for 28 years			
	Total			Ø twig	Total			Ø twig	Total			Ø twig	Total			Ø twig
	Nests*	Workers	Immature stages	$\overline{X} \pm$ SD	Nests*	Workers	Immature stages	$\overline{X} \pm$ SD	Nests*	Workers	Immature stages	$\overline{X} \pm$ SD	Nests*	Workers	Immature stages	$\overline{X} \pm$ SD
Solenopsis gr. *Diploroptrum*	1	39	22	11.68	1	230	14	11.97								
Solenopsis wasmannii	1	26	134	9.19												
Solenopsis sp.2					3	402	213	11.62 ± 4.96					1	31	56	11.74
Solenopsis sp.4													1	25	8	9.57
Solenopsis sp.5	1	124	17	11.26												
Ponerinae																
Hypoponera sp.4	1	13		19.88												
Hypoponera sp.7	6	100	119	13.21 ± 4.92	1	15	—	22.71					2	12	5	17.45 ± 4.86
Hypoponera sp.8	1	46	10	12.71												
Hypoponera sp.10													1	200	36	33.1
Pachycondyla crenata													3	28	5	15.6 ± 1.93
Pseudomyrmecinae																
Pseudomyrmex phyllophilus	1	9	29	11.33					11	160	239	9.76 ± 4.04	7	85	261	11.05 ± 3.55
Pseudomyrmex pallidus									1	29	78	9.26				
Pseudomyrmex sp.3	2	120	158	17.02 ± 7.24									2	7	23	8.38 ± 1.66
Pseudomyrmex sp.4									1	23	54	19.32				
Total number of species		28				10				10				26		
Total number of nests		164				15				32				96		

*Nest: ≥10 workers or ≤10 workers + immature stages.

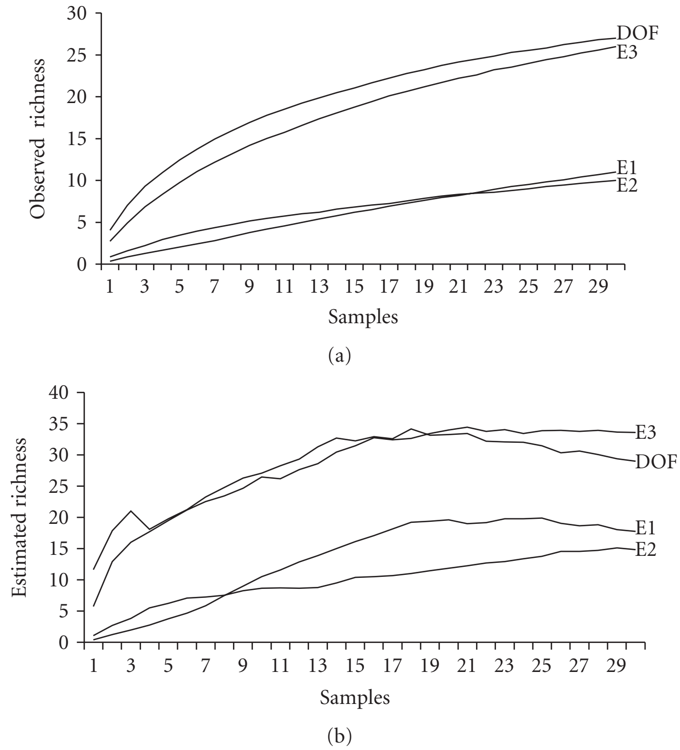

Figure 2: Curves for the observed (a) and estimated (b) richness of ants recorded in twigs from the leaf litter of the different forest types. DOF: dense ombrophilous forest, E1: commercial plantation managed for 7 years, E2: commercial plantation managed for 28 years, and E3: plantation left unmanaged for 28 years.

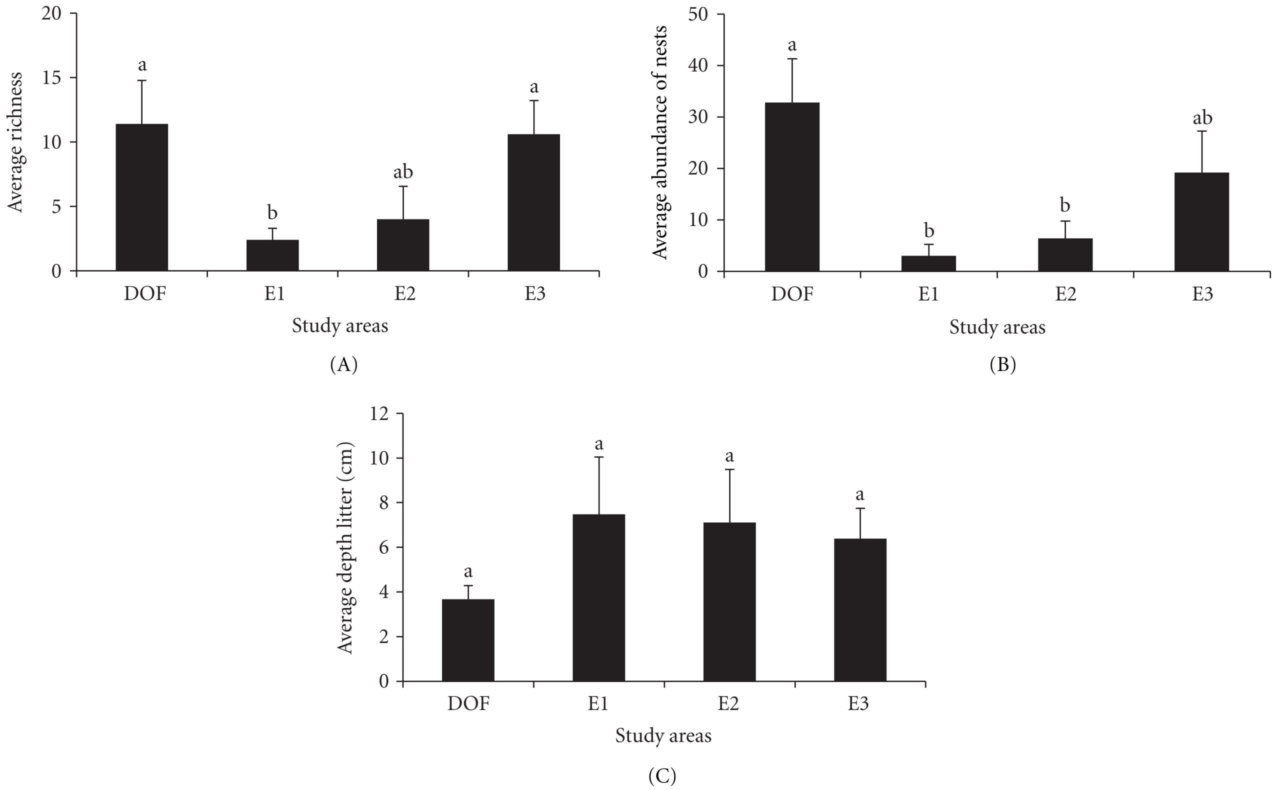

Figure 3: Species richness values (A), mean nest abundance (B), and average depth litter (cm) (C) differed between forest types. DOF: dense ombrophilous forest, E1: commercial plantation managed for 7 years, E2: commercial plantation managed for 28 years, and E3: eucalyptus plantation left unmanaged for 28 years. Vertical bar: standard deviation. Different letters: $P < 0.05$.

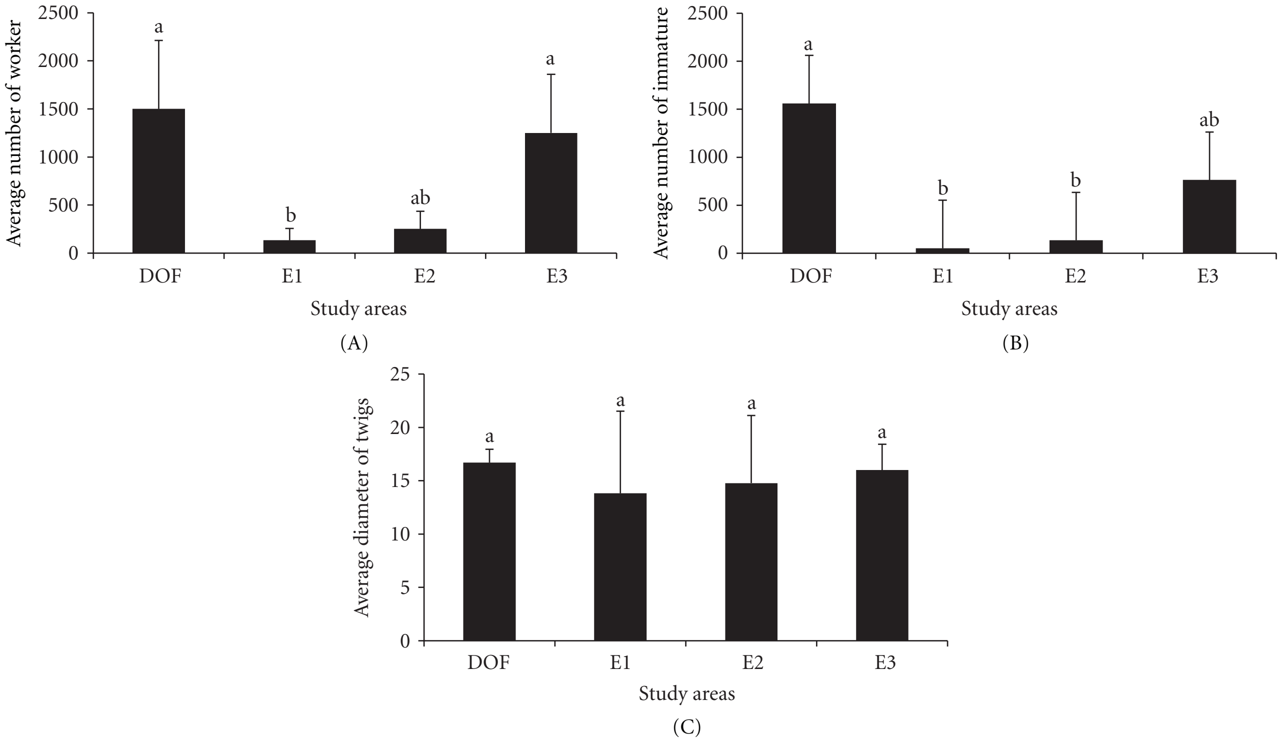

Figure 4: Average number of worker (A), immature stages (B), and the mean diameter of the twigs (C) containing ant colonies in each forest type. DOF: dense ombrophilous forest, E1: commercial plantation managed for 7 years, E2: commercial plantation managed for 28 years, and E3: eucalyptus plantation left unmanaged for 28 years. Vertical bar: standard deviation. Different letters: $P < 0.05$.

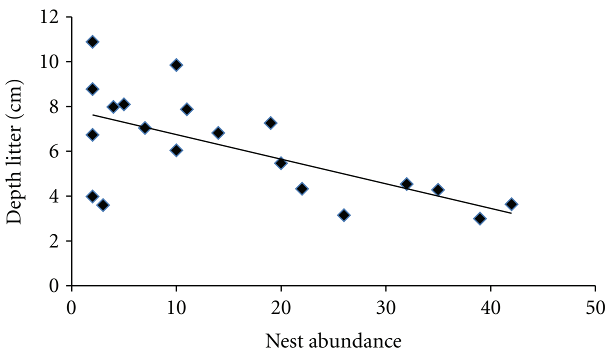

Figure 5: Relationship between depth litter (cm) and nest abundance in twigs of the leaf litter differed between the forest types.

shelter may represent satellite nests [23, 35] or polidomic nests, without the presence of queens [36]. Thus, the nest structure increases the chances of territory defense [37], survival of the colony itself, which is most at risk for predation when it is concentrated in a single place [38], increase foraging area [39] and the occupation of new areas [38, 40].

The most of the species recorded in the twigs are common in leaf litter of dense ombrophilus forest [41, 42] or eucalyptus crops [9–11]. Arboreal genera, such as *Crematogaster, Myrmelachista*, *Procryptocerus,* and *Pseudomyrmex* [23, 43–45], have also been reported in twigs, which can cause the occasional detection of arboreal ants in the leaf litter, as discussed by Delabie et al. [46]. Specifically *Myrmelachista*, which is an exclusively arboreal genus [44], may have external nests, with immature workers, queens and winged in fallen twigs in the Brazilian Atlantic forest litter [24, 25] was also recorded in eucalypt forests. In this case, the nests were found primarily in areas with understory. Probably, in the forests of eucalyptus, *Myrmelachista* species use more of understory vegetation for foraging and nesting; twigs/trunks of eucalyptus trees should be little occupied. In forests of *Araucaria angustifolia* (Bertol.) Kuntze [47] reported the presence of *Myrmelachista* nests in tree and fallen twigs near it.

Species diversity in ombrophilus forest was much higher than in eucalyptus forest, and this richness may be due to greater number of twigs in older forests [28, 48, 49], with attractive features (soft, hollow, wet, and twigs with holes) [23]; moreover, ant species having different body sizes and colony sizes may use a wider variety of nest sizes [50]. Regarding twigs originating from eucalyptus forests, they are hard, dry, and slightly moist, which makes difficult the colonization [49]. The moisture is an important factor for ants in colonizing new habitats [51, 52], but species of *Brachymyrmex, Camponotus, Crematogaster, Linepithema, Myrmelachista, Pachycondyla, Pheidole, and Pseudomyrmex* have also been recorded in bamboo [35, 53], which is a microhabitat with low humidity.

Along with other resources, twigs are important components of leaf litter [23] despite only supporting the colony

Table 2: Presence of a queen and alates according to the recorded species in twigs and forest type.

Species/morphospecies	Queen				Alates			
	Nests with a queen		Queens/nest		Nests with alates		Total number or range	
	FOD	E3	FOD	E3	FOD	E3	FOD	E3
Brachymyrmex incisus	2		1		2		2–20	
Camponotus (*Myrmaphaenus*)	1		1					
Camponotus sp.9	5		1		1		5	
Camponotus sp.10	1		1		1		1	
Crematogaster gr. *Arthocrema*	3		1		1		4	
Hylomyrma sp.1		1		1				
Hypoponera sp.7					1		1	
Linepithema iniquum						6		1–42
Linepithema neotropicum		1		1				
Mycetosoritis sp.1						1		13
Myrmelachista catharinae					9		1–15	
Myrmelachista ruszkii					12		1–18	
Pachycondyla crenata						3		1–16
Pheidole sp.7	3		1		12	14	1–18	1–38
Pheidole sp.13	6		1		26		1–12	
Pheidole sp.43	9		1					
Procryptocerus sp.2						3		1–10
Pseudomyrmex phyllophilus	1		1			1		9
Solenopsis gr. *Diploroptrum*					1		3	
Solenopsis sp.2		1		5		1		1
Total number of species	9	3			11	7		

for a short time because of the scarcity of food or the decomposition of the wood [28]. Twigs at different stages of decomposition can accommodate 0.88 nests/m^2 in Lowland rainforest (Equador) [16], 0.22 nests/m^2 in the central Amazonian rainforest (Brazil) [23], and 7.43 nests/m^2 in tropical wet forest (Costa Rica) [28]. The number of nests recorded in the present study for the dense ombrophilous forest was lower than previously reported but fell within the range of 0 to 23 nests/m^2 in twigs and between leaves in the leaf litter [28, 54, 55]. In *Eucalyptus citriodora* Hook forests with understory were recorded 0.2 nests/m^2 [49], corroborating the results of this work for the eucalyptus forest without management.

Variation in the density of nesting colonies and species richness between the different forest types may be related to the complexity of the vegetative structure. The structure and composition of ant communities are affected primarily by vegetation [56, 57], which provides the necessary resources for food and nesting [58, 59]. Changes in the floristic composition influence the arboreal ant communities [60], the soil [61], the leaf litter [60, 62], and, possibly, those who seek to use twigs for nesting.

Since twigs heterogeneity directly affects the ants diversity [63], species richness and abundance increase with natural twig density [64], the eucalyptus forests being more homogeneous, resulting in a less varied supply of resources, reduced richness and decrease in the organisms diversity [10, 65]. The release of different allopathic substances [66] that compromise nutrient cycling [67] and the size of macroinvertebrates [68], a canopy cover that exposes the leaf litter to intense sunlight, rain, and wind, thus modifying the microclimate, the decomposition of the leaf litter, and the composition of the local fauna [7] are also factors that influence the diversity of ants in the leaf litter of eucalyptus forests. These factors may also affect the ant species that seek the twigs as resources. Thus, even the litter of eucalyptus forests is similar to native forest, their resources are scarce and similar and, as consequence, the number of twigs colonized by ants is lower when compared to the dense ombrophilous forest.

The undecomposed twigs, despite harboring a smaller number of species compared to twigs in different stages of decomposition [23] and in leaf litter from dense ombrophilus forest [69] and eucalyptus forests [9–11], represent another source of resources for nesting. Consequently, undecomposed twigs compose part of the set of structural elements that maintain the diversity of ants that forage in leaf litter, especially in dense ombrophilus forest and abandoned eucalyptus plantations. The present study also shows that, with time, the ant communities that seek undecomposed twigs as resources in abandoned eucalyptus plantation are similar to those in native forests. These results are important for understanding the recovery process of ant diversity in the leaf litter of areas of dense ombrophilus forest following an anthropogenic action, in addition to providing information on the biology of some ant species of tropical forests.

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