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Ground-dwelling Ant Diversity in Maliau Basin, Borneo: Evaluation of Hand-sorting Methods to Estimate Ant Diversity

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ABSTRACT The ground-dwelling ant fauna in tropical forests remains the "final frontier" in the study of Formicidae biodiversity. This is mainly due to a lack of convenient sampling methods for the inventory of ants in soil. In the present study, employing the hand-sorting of small soil cores, we carried out the first survey of ground-dwelling ants in Maliau Basin Conservation Area, in Sabah, Borneo, to compare disturbed and undisturbed forest ant communities and to evaluate the sampling method's performance in assessing species richness and characterizing community structure of the ant fauna. For this purpose we set a 5 m long and 1 m wide transect, consisting of five 1-m^2 sections, in disturbed and undisturbed forest plots in the area, and compared the sampling results of hand-sorting between the two plots. A total of 50 ant species, in 28 genera and eight subfamilies, were collected in the two plots, of which 32 species in 20 genera were collected from the undisturbed plot and 24 species in 18 genera from the disturbed plot. According to estimates of expected species richness, the hand-sorting of soil cores allowed for the collection of 66.7 to 80.0% of the ant species expected in the undisturbed plot, and 83.3 to 88.9% in the disturbed plot. Besides lower species richness, the ant community in the disturbed plot had lower species evenness and a different species composition in comparison to undisturbed plot, which may be related with environmental changes in the plot, due to open canopy. These results indicate that hand-sorting of small soil cores is a method that could provide reliable information, not only to estimate species richness at a site, but also to evaluate differences in community structure between sites.

Key words: Community structure, disturbed and undisturbed plots, formicidae, ground fauna, sampling method

INTRODUCTION

Ants are a major component of the arthropod fauna in tropical forests, in terms of species richness and biomass (Fittkau and Klinge 1973, Hölldobler and Wilson 1990). They use a wide variety of food resources in the forests and contribute to species richness by their manifold interactions with other tropical organisms (Agosti et al. 2000). Therefore, ants are considered useful bioindicators for biodiversity measuring and environmental monitoring in tropical forests (Underwood and Fisher 2006). Ground-dwelling ants are thought to be particularly appropriate for monitoring of environmental impact. Most ground-dwelling ant species have small body-size, small stationary nests and a fairly restricted foraging range (Hölldobler and Wilson 1990). These attributes guarantee a tight habitat connection for the ants and make them sensitive to environmental changes (Agosti et al. 2000). Additionally, they are recognized as main predators of other underground arthropods (Carroll and Janzen 1973), and ecosystem engineers enhancing the mineralisation of nutrients (Gunadi and Verhoef 1993) and microbial activity in the soil (Dauber and Wolters 2000).

Despite their potential importance for biodiversity and environmental monitoring, the ground-dwelling ant fauna in tropical forests remains the "final frontier" in the study of Formicidae biodiversity (Longino and Colwell 1997, Wilkie et al. 2007). This is mainly due to a lack of appropriate sampling methods for the inventory of ants in the soil layer. For sampling ants in the soil, subterranean baiting and soil extracting, e.g. with the Berlese-Tullgren funnel and Winkler extractor, have usually been used (Longino and Colwell 1997, Agosti et al. 2000, Wilkie et al. 2007, Andersen and Brault 2010). However, for biodiversity inventory, baiting methods are not appropriate, because their efficiency depends on the behavioral responses of each ant species to the bait, which leads to biased estimates of biodiversity. Therefore, methods of soil extraction are preferable in biodiversity inventories, but Berlese-Tullgren funnels and Winkler extractors are logistically highly demanding methods, with high costs of equipment and time (Smith et al. 2008). The devices use a heat producing light source or natural drying to drive out the ants from soil samples to a collection pot, and thus they require a lot of space under cover and/or an electricity supply for drying soil in the field. Furthermore, the samples from these methods include all animals extracted from the soil, with a lot of extracted soil particles and organic debris, therefore requiring a long sorting time to pick up ants from the samples. Owing to these problems in sampling, biodiversity inventory for the hidden ant fauna is often considered "too difficult" and consequently ignored or incompletely attempted in previous biodiversity studies of tropical ants (Berghoff et al. 2003).

Hand-sorting of soil cores represents another extraction method that is inexpensive and easy to use (Smith et al. 2008). The method is less demanding logistically in comparison to the Berlese-Tullgren funnel and Winkler extractor, and so can be carried out in the field. Furthermore, the method can target extraction of ants alone, and so can avoid a long sorting time. Therefore, hand-sorting of soil cores may be the most convenient method for biodiversity inventory of ground-dwelling ants, but this method is seldom used for ant inventory. This is mainly because the extraction efficiency of ants by the method depends on visual detectability, which may lead to the underestimation of biodiversity, especially for soil samples of large volume. However, this may be avoided by taking many small soil cores, instead of a few large soil cores. Hence, in the present study, employing hand-sorting of small soil cores, we sampled ants from the soil layer ranging from the ground surface to 12cm depth in Maliau Basin Conservation Area in Sabah, Borneo. Our first aim was to conduct the first survey of ground-dwelling ants in Maliau Basin, to demonstrate the biodiversity value of this unique area, which is fully encircled and guarded by cliffs and remained virtually unknown until 1970. Our second aim was to test the adequacy of hand-sorting small soil cores as sampling methods for biodiversity inventory of ground dwelling ants. To do this, we chose two plots of forests, one with no evidence of disturbance and the other in disturbed areas where were lightly logged in 1996 for timber to build the base camp, in Maliau Basin, and compared sampling results between the two plots. Based on past studies (Brühl et al. 2003, Bickel and Watanasit 2005), we expected there to be differences in biodiversity structure, such as species diversity and abundance structure, between sites. Therefore, this study evaluated the hand-sorting for method of assessing ground-dwelling ant diversity by checking adequacy to characterize the ant assemblages inhabiting different forest types. Our third aim was thus to characterize differences in the soil ant fauna between these two conditions.

MATERIALS AND METHODS

Study site

This study was conducted in forest near Agathis Camp (04 °41′N, 116°54′E), at an altitude of c. 1100 m, in Maliau Basin Conservation Area in Sabah, Borneo, for four days in May 2001. The study site is within lower montane forest, dominated by *Shorea platyclados*, *Agathis borneensis* and *Casuarina sumatrana*. Ant sampling was undertaken in undisturbed and disturbed plots in the study sites. The undisturbed plot (hereafter referred to as UP) contained many large trees and a closed canopy at a height of around 20 to 25 m, with emergents up to 35 m. In the disturbed plot (hereafter referred to as DP), because large trees were logged in 1996 (i.e. five years before the sampling period) to build facilities of the Agathis Camp, most trees were thin with an open canopy. This plot was roughly 200 m south of the UP.

Sampling protocol

Within each plot, one transect was set up, 5 m long and 1 m wide, and divided into 5 contiguous sections (each 1 m x 1 m in area), which was located at least 50 m inside the forest from trails. Surface leaf litter was removed and 20 soil cores, using a 7 cm diameter tube sampler, were collected to a depth of 12 cm, from each section. Ants were extracted from the soil cores by hand-sorting in the field on a white tray, and then transferred and preserved in vials with 80% alcohol. All ants collected were identified to genus, using the keys of Bolton (1994) and Hashimoto (2003), and then to species or morphospecies level, using the reference collection of Asian ants established by the International Network for the Study of Asian Ants (ANeT). Voucher specimens were deposited in ITBC of Universiti Malaysia Sabah, and the Museum of Nature and Human Activities, Japan.

Data analysis

Our analyses are divided into two parts: (1) evaluation of sampling efficiency and (2) comparison of biodiversity structure of the ground-dwelling ant community in DP and UP. To evaluate the efficiency, species richness in each section was estimated with five non-parametric estimators of species richness, ICE, Chao2, Jacknife1, Jacknife2 and Bootstrap, using EstimateS software (version 8.0; Colwell 2005), as so far no best performing estimator exists (Soberón and Llorente 1993), and the proportion of observed species number to the estimated species number was calculated. For an easier assessment of sampling efficiency between sections, the mean of these five estimators was also calculated and compared with observed species number in each section. Furthermore, we used sample-based accumulation curves as a tool to evaluate sampling efficiency, based on visual inspection of the shape. To compare the biodiversity structure of the ant community between UP and DP, we analyzed species turnover, and species diversity and evenness, using PRIMER 6 (Clarke and Warwick 2005). The turnover (dissimilarity) of the species between UP and DP was measured calculating a Bray-Curtis dissimilarities matrix of incidence data (species presence and absence data) between the sections. Based on these distance measurements, Non-Metric Dimensional Scaling (NMDS) was performed to graphically analyze community dissimilarities between the two plots, and one-way Analysis of Similarity (ANOSIM; Clarke and Ainsworth 1993) was also carried out to statistically detect dissimilarity between the communities in the two plots. If ANOSIM showed significant differences between the plots, similarity percentage analysis (SIMPER; Clarke and Ainsworth 1993) was used to determine which ant species contributed most to this dissimilarity. For analyzing species diversity and evenness, we used number of occurrences (the total number of times a species is captured independently in the samples, ignoring the number of individuals of a species in any one sample) as an indication of abundance for each species, because ants are social organisms and the presence of many individuals may be simply due to collecting a nest or a column of foragers. Based on the abundance data pooled in each plot, species diversity indexes of the communities in UP and DP were calculated using the Shannon index (H). Furthermore, for comparing diversity between the two plots, Rényi diversity ordering was used. This diversity ordering method can perform well for all size communities and display graphically the differences

Table 1. Ant species collected in the undisturbed plot (UP) and disturbed plot (DP). Table entries are number of sections in each plot in which each species occurred.

Subfamily Amblyoponinae	Species	UP	DP
Aenictinae	*Aenictus* sp.1	1	0
Amblyoponinae	*Amblyopone* sp.1	1	0
	Mystrium camillae	1	0
	Prionopelta kraepelini	0	2
Cerapachyinae	*Cerapachys* sp.1	1	0
	Cerapachys sp.2	0	1
Dolichoderinae	*Technomyrmex* sp.1	0	1
	Tecnhomyrmex karaepelini	1	0
Formicinae	*Acropyga inezae*	2	0
	Paratrechina opaca	1	0
	Paratrechina sp.1	0	1
	Pseudolasius sp.1	0	1
	Pseudolasius sp.2	1	0
	Pseudolasius sp.3	1	0
	Pseudolasius sp.4	1	0
	Pseudolasius sp.5	1	0
	Pseudolasius sp.6	0	2
Leptanillinae	*Leptanilla* sp.1	0	1
Myrmicinae	*Acanthomyrmex ferox*	0	1
	Crematogaster sp.1	4	0
	Meranoplus malaysianus	0	1
	Monomorium sp.1	1	0
	Myrmecina sp. 1	0	1
	Oligomyrmex sp.1	2	0
	Pheidole aristotelis	2	0
	Pheidole inornata	1	0
	Pheidole schoedli	2	4
	Pheidole tawauensis	0	1
	Pheidole parvicorpus	3	1
	Pheidole poringensis	1	0
	Pyramica mitis	0	1
	Solenopsis sp.1	0	3
	Strumigenys sp.1	1	0
	Tetramorium parvum	2	0
	Tetramorium sp.1	1	1
Ponerinae	*Anochetus graeffei*	1	0
	Centromyrmex feae	0	1
	Cryptopone sp.1	1	0
	Hypoponera sp.1	1	0
	Hypoponera sp.2	2	1
	Hypoponera sp.3	0	1
	Hypoponera sp.4	1	1
	Hypoponera sp.5	0	2
	Hypoponera sp.6	1	0
	Leptogenys sp.1	0	1
	Pachycondyla sp.1	2	0
	Pachycondyla sp.2	4	3
	Ponera sp.1	3	0
	Ponera sp.2	0	1
	Ponera sp.3	2	0
	Total species number	32	24

Table 2. Numbers (rounded) of Species observed (Sobs) and that estimated by five different methods (ICE, Chao2, Jack1, Jack2 and Bootstrap) for the ant communities in the sections in the two plots. Sampling efficiency (percentage value of observed species divided by the number of estimators) is given in brackets.

Plot	Section	Sobs	ICE	Chao2	Jack1	Jack2	Bootsrap	Mean
UP	U1	12	16 (75.0)	13 (92.3)	17 (70.6)	16 (75.0)	15 (80.0)	15 (80.0)
	U2	8	12 (66.7)	9 (88.9)	12 (66.7)	13 (61.5)	10 (80.0)	11 (72.7)
	U3	10	13 (76.9)	11 (90.9)	14 (71.4)	14 (71.4)	12 (83.3)	13 (76.9)
	U4	9	14 (64.3)	12 (75.0)	14 (64.3)	16 (56.3)	11 (81.8)	13 (69.2)
	U5	10	17 (58.8)	14 (71.4)	16 (62.5)	18 (55.6)	13 (76.9)	15 (66.7)
DP	D1	6	7 (85.7)	7 (85.7)	8 (75.0)	8 (75.0)	7 (85.7)	7 (85.7)
	D2	8	9 (88.9)	9 (88.9)	10 (80.0)	9 (88.9)	9 (88.9)	9 (88.9)
	D3	6	7 (85.7)	7 (85.7)	8 (75.0)	8 (75.0)	7 (85.7)	7 (85.7)
	D4	5	6 (83.3)	7 (71.4)	6 (83.3)	6 (83.3)	6 (83.3)	6 (83.3)
	D5	7	8 (87.5)	8 (87.5)	9 (77.8)	8 (87.5)	8 (87.5)	8 (87.5)

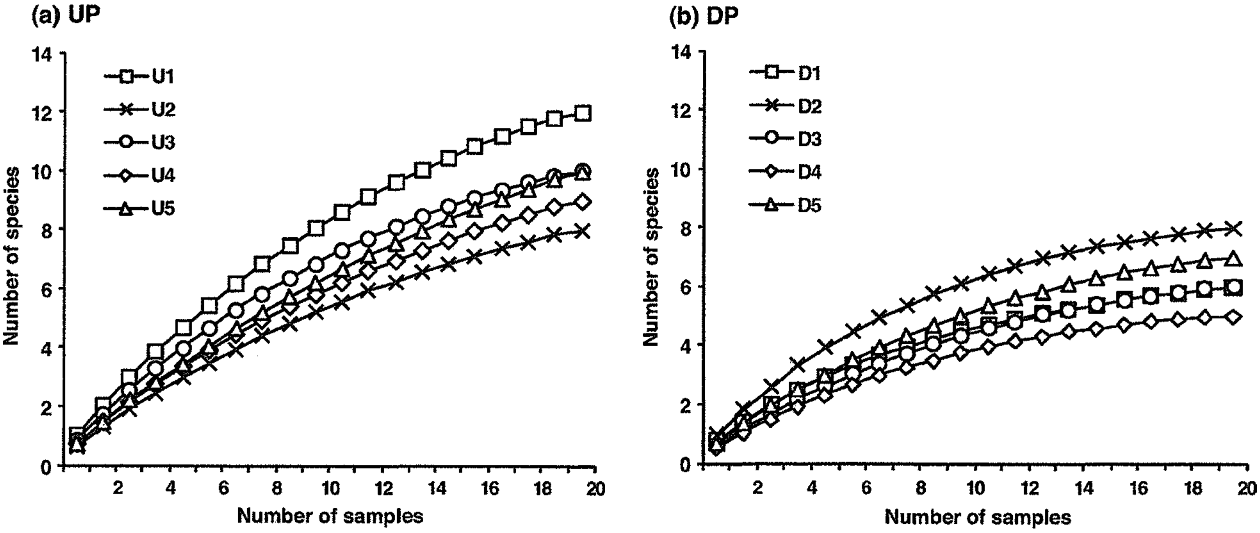

Fig. 1. Randomized species accumulation curves of the ant communities of the sections in the undisturbed plot (a) and disturbed plot (b).

of community diversity structure (Tóthmérész 1995). With the Rényi index $H(\alpha)$ calculated over the scale parameter α, $H(\alpha)$ diversity was plotted against the scale parameter and the resulting curves were compared. If $H(\alpha)$ values are higher over the full range of α and curves do not cross, a community is ranked as more diverse. To analyze species evenness of the ant communities in UP and DP, an abundance-rank curve was plotted for the community in each plot, and the shape of curves was compared between the two plots.

RESULTS

Species number and Sampling efficiency

A total of 50 ant species, in 28 genera and eight subfamilies, were collected in the two plots (Table 1). UP

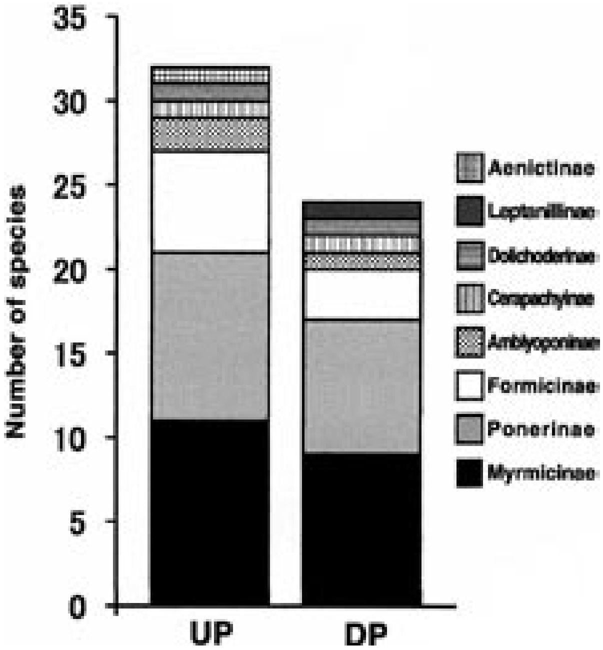

Fig. 2. Number of species from subfamilies at the undisturbed plot (UP) and disturbed plot (DP).

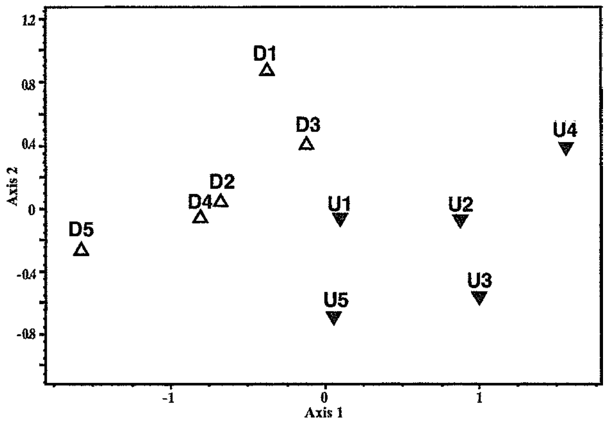

Fig. 3. Non-metric multidimensional scaling ordination of the ant communities of the sections in the undisturbed plot (U1-5) and disturbed plot (D1-5) (stress=0.06).

had 32 species in 20 genera, while DP had 24 species in 18 genera. The highest species number collected per section was recorded in UP (12 species at U1) and the lowest species number was found in DP (five species at D4) (Table 2). The mean number of species per section was significantly higher in UP (9.81 ± 1.48 SD) than in DP (6.4 ± 1.14 SD) ($P<0.05$; Two-sided Mann–Whitney *U*-test). The mean number of species per core sample was also significantly higher in UP (2.01 ± 1.06 SD) than in DP (1.55 ± 0.80 SD) ($P=0.003$; Two-sided Mann–Whitney *U*-test).

Sample efficiency was mostly between 70 to 90% for all estimators. The highest estimated efficiency for any single estimator was found in UP (Chao2 estimator) with 92.3%, the lowest in UP (Jack2 estimator) with 55.6%. The mean sampling efficiency (observed species number/ mean number of the five estimators) in each section ranged from 83.3 to 88.9% in DP, and 66.7 to 80.0% in UP (Table 2). Species accumulation curves for each site were similar in shape for both plots, but the curves in DP were rising less steeply toward the right-hand end than those in UP (Fig. 1). None of the curves reached an asymptote, but they showed little increase of new species in the last ten samples in both sections (2-3 new species in UP, about 1 in DP).

Comparison of community structure in DP and UP

The taxonomic structure of the ant community sampled was similar for both plots, in which Myrmicinae was the most speciose subfamily, followed by Ponerinae and Formicinae (Fig. 2). However, the proportion of Formicinae was recognizably lower in DP, compared with UP. This was due to lower number of species of *Pseudolasius* and *Acropyga* in DP.

Overlap of species in the two habitats was very low: of 50 species, only 6 were found in both plots (12%). NMDS analysis also showed clear separation between UP and DP graphically (stress value: 0.06) (Fig. 3), and ANOSIM comparisons showed this difference to be significant ($P=0.008$, $R=0.724$). Results of SIMPER analysis showed that *Pheidole schoedli* (percentage contribution: 36.7%), *Pachycondyla* sp. 2 (18.3%) and *Hypoponera* sp. 5 (17.5%) were the three species contributing most to the similarity within DP (Average similarity: 27.39), and *Crematogaster* sp.1 (23.8%),

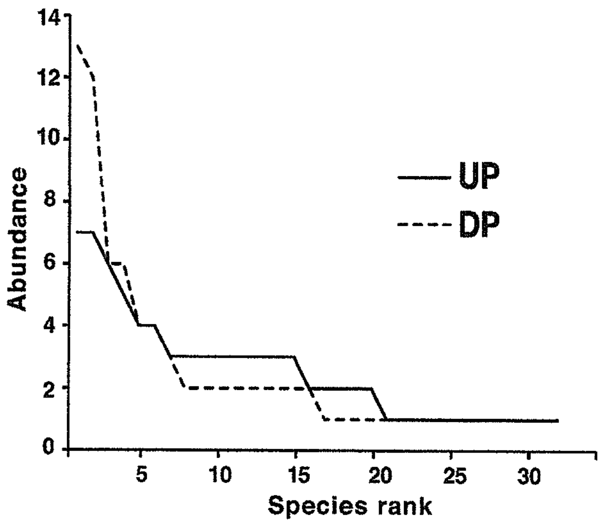

Fig. 4. Rank abundance distributions of the ant communities in the undisturbed plot (UP) and disturbed plot (DP).

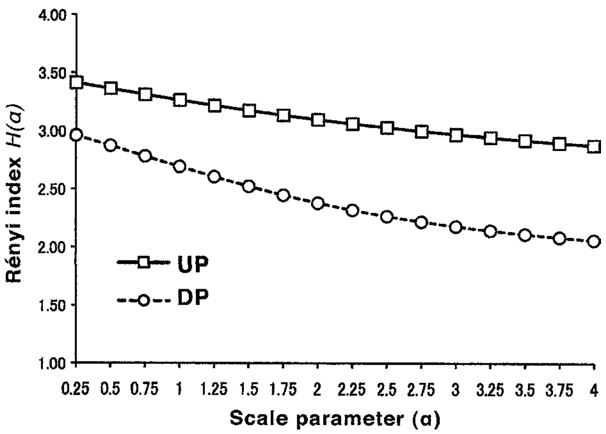

Fig. 5. Rényi index *H(α)* along the scale parameter in the ant communities of the undisturbed plot (UP) and disturbed plot (DP).

Pachycondyla sp.2 (23.8%) and *Pheidole parvicorpus* (13.1%) contributed most to similarity within UP (25.41). For the dissimilarity between the two plots (Average dissimilarity: 89.62), the top three of the most contributing species were *Solenopsis* sp.1 (8.8%), *Pheidole schoedli* (8.1%), *Crematogaster* sp.1 (5.5%).

The rank abundance curve for UP showed a long tail, indicating a high number of species with low occurrences (Fig. 4). On the other hand, the curve for DP showed a more compressed shape, resulting from the higher dominance of the first and second ranked species, *Pheidole schoedli* and *Solenopsis* sp. 1, and a smaller number of rare species. Shannon's diversity index was 3.26 in UP and 2.69 in DP and Diversity Ordering (the Rényi index) in UP demonstrated higher values over all scale parameters than in DP (Fig. 5).

DISCUSSION

The present study is the first published record to survey the ground-dwelling ant fauna in Maliau Basin. We collected a total of 50 ant species from ten 1-m^2 of soil samples in the area. Although there are no comparable studies of inventory for the ground-dwelling ants in Maliau Basin, the number of species sampled by hand-sorting of soil cores was relatively high compared with the results of Winkler extraction in Mount Kinabalu in Sabah, Borneo, at the same altitude (25 species from ten 1-m^2 soil samples) (Brühl et al. 1999). According to estimates of expected species richness, the hand-sorting of soil cores performed well for inventory of ground-dwelling ants. The method allowed for the collection of over 80% of the ant species expected in each section in DP, and generally over 65% in UP. The relatively low values in UP indicated the probability that total species richness in the plot would increase with additional sampling effort. However, since species accumulation curves for each section in UP showed relatively minor increase in species over the last five samples, there would be no dramatic increase in species number with further sampling effort. Consequently, the generally high sampling efficiency together with visual inspection of species accumulation curves suggest that the method of hand-sorting performed well in the survey of species richness of ants living in the soil layer.

Comparing ground-dwelling ant diversity in DP and UP, the results indicated that species richness decreased with disturbance. Furthermore, contrary to UP, the structure of the DP community was characterized by high relative abundance of dominant species and fewer rare species, leading to low diversity of the ant community. Lower species richness and evenness are typical of ant communities in disturbed forests, as shown by previous studies (Brühl et al. 2003, Bickel and Watanasit 2005, Fayle et al. 2010). By intercepting solar radiation, trees reduce the amount of light reaching the ground layer (Belsky and Amundson 1992). Consequently, in open canopy habitats, such as logged forests and plantations, soil temperatures and potential evapotranspiration tend to be higher than in closed-canopy forests, making them a more stressful environment for ground-dwelling ants, leading to decline of species richness and changes in the community structure (Turner and Foster 2006). These fundamental environmental disparities may have occurred in the two plots surveyed in present study.

This is also suggested by differences in the community composition between the two plots (although we should be cautious of some differences between the plots may reflect spatial community turnover rather than impact of disturbance). Levings and Windsor (1984) demonstrated that patterns of species composition of ground-dwelling ants in relation to disturbance were determined by their adaptability to resist desiccation stress in soil layer. Our analysis of SIMPER showed that *Pheidole schoedli* was the species most responsible for similarity of community composition within UP and dissimilarity between DP and UP. The species is known to be a true subterranean nester and forager, and is considered to have resistance to desiccation stress in soil because it nests not only in undisturbed forests but also in disturbed forests (Brühl 2001, Eguchi et al. 2006). The dominance of the species in DP might indicate that the microclimate in the plot became hotter and drier than in

UP, due to the open canopy. Furthermore, our results showed that *Pseudolasius* and *Acropyga* much fewer in species number in DP. These ants have trophobiotic relationships with subterranean mealybugs feeding on plant roots. Bunzli (1937) and Malsch et al. (2001) pointed out that changing soil-layer conditions to dry and hot was responsible for decreasing their food supply, due to the increasing drying out of the fine root system or in directly affecting the mealybugs themselves. Thus, the decrease in species richness of *Pseudolasius* and *Acropyga* in DP may also suggest desiccation of the soil layer caused by direct insolation.

Ant community structure differed greatly between UP and DP, as shown by previous studies, and this difference could be detected well from the results of hand-sorting. Furthermore, the sampling intensity used was found to catch a relatively high proportion of the ant species expected in the plots. A goal of biodiversity inventory is to record as many of the species present at a site as possible. However, if the inventory is to be of use in environmental monitoring or describing patterns of diversity between sites, it should contribute more than just a species list. In the ideal inventory the sampling method employed should make the goal of creating species lists compatible with the goals of comparing species richness or community structure between sites. The method of hand-sorting employed in the present study provides sufficient information, not only to estimate species richness at a site, but also to evaluate differences in community structure between sites. Furthermore, this method is easy to use, cheap, fast and not incredibly time-consuming. Such features seem especially suited to a sampling method for the inventory of ground-dwelling ants in tropical areas, because they are among the most diverse and abundant members of the soil fauna, making inventory a time-consuming and labor-intensive task. The advantages of the hand-sorting method are also appropriate to inventory work in tropical developing countries where the remaining tropical forests are often inaccessible and scientific infrastructure in the field is often still rudimentary. Therefore, the method of hand-sorting small soil cores could be used in measuring species richness, characterizing community structure and monitoring environmental changes, thus having wide-ranging applications for ground-dwelling ant inventories in tropical areas.

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