



## RESEARCH ARTICLE - ANTS

### A comparison between time of exposure, number of pitfall traps and the sampling cost to capture ground-dwelling poneromorph ants (Hymenoptera: Formicidae)

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#### Abstract

Using effective survey protocols to address the effects of environmental change are key to saving time, resources and costs. Although exhaustive sampling in any location has been shown as impractical, biodiversity sampling projects must capture sufficient information to show how species assemblages change with the environmental variables. This study investigated time of exposure in the field and the number of pitfall traps that efficiently sampled poneromorph ants in 30 250 m long plots across an area of 25 km<sup>2</sup> of tropical rain forest in Brazil. The treatments used for the surveys included two days and 300 traps, 14 days and 300 traps, 14 days and 750 traps, and were considered the minimum, intermediate and maximum sampling efforts, respectively. We characterized each assemblage of ants in relation to a gradient of soil texture, terrain slope and leaf and branch litter volume, and then tested whether the ecological relationships observed with the maximum effort were comparable to data on intermediate and minimum sampling efforts. We also estimated the cost-effectiveness of using the protocols in survey programs. The assemblage of species sampled during 14 days was similar to the assemblage captured during two days, indicating that the number of days influenced the assemblage similarity more than the number of sampling traps. All ecological patterns detected with the maximum effort were also captured with lesser sampling efforts. Overall, both the intermediate and minimum sampling efforts represented savings around 26-40% of total project costs and 43-45% of time to process the samples. We recommend that two days of trapping time combined with 300 pitfall traps is a highly effective shortcut for monitoring assessment, which can be applied to large-scale biodiversity surveys in tropical forests.

#### Introduction

Assessing the effects of environmental changes on biodiversity is expected to increase with the increasing evidence of biodiversity loss. Additionally, information gathered through biodiversity assessment can play an important role in conservation plans. Nevertheless, conservation planning needs to be based fundamentally on biodiversity data, which requires taxonomic knowledge (Fisher, 2005). However, in order to transfer this important scientific knowledge to decision makers biodiversity assessment is required to be more than

just species lists, but surveys which highlight the relationships among the community and the changing environmental variables (Landeiro et al., 2010; Franklin et al., 2013). In addition, the serious lack of funding for ongoing taxonomical monitoring (Tahseen, 2014) dictates that the costs of the survey should not exceed the potential economic benefits resulting from the investigations (Evans & Viengkham, 2001). Thus, independent of their study aims, researchers often face a conflict of interest to decide between area sampled and sampling intensity in initial or sequential environmental assessment (Souza et al., 2012; Pos et al., 2014).



Ants are a mega-diverse invertebrate group with a wide geographical distribution and functional importance at various trophic levels, providing several advantages for indicating environmental changes (Folgarait, 1998; Andersen & Majer, 2004; Silva & Brandão, 1999; Osborn et al., 1999). They can be easily surveyed in large numbers in most ecosystems using several sampling methods and are relatively easily distinguished at morphospecies level (Bestelmeyer et al., 2000; Souza et al., 2012; 2016). Because ants are widely used as bio-indicators in land management and monitoring, shortcuts are necessary to improve the efficiency of the survey (Andersen & Majer, 2004; Souza et al., 2016). There are about 14,000 species of ants described worldwide with approximately 30% of these found in the Neotropics (Agosti & Johnson, 2005; Baccaro et al., 2015).

The Poneromorph ants have currently 71 genera and are defined as an informal (not monophyletic) group (Bolton, 2003), which remains primitive in some of their general characteristics (Schmidt & Shattuck, 2014). These ants are distributed throughout the world's zoogeographic regions and occupy a great diversity of ecological niches, from both small and cryptic, to large and remarkable (Ouellette et al., 2006). The poneromorph ants have a great ecological importance, occupying different trophic levels (Brandão et al., 2011), thus participating in mutualistic plant associations (Pereira et al., 2013), facilitating seed dispersal (Leal et al., 2014). This group of insect also plays a prominent role in bioindicators of anthropic impacts such as the use of pastures (Dias et al., 2008), fires (Endanger et al., 2008), and mining (Ribas et al., 2012).

Several studies have evaluated the efficiency of pitfall traps in catching ground-dwelling ants in comparison with other techniques (Wang et al., 2001; Ivanov & Keiper 2009; Souza et al., 2012). Traditionally, pitfall traps remain in the field for 48 hours in studies with ground-dwelling ants (Pik et al., 1999; Agosti & Alonso 2000; Bestelmeyer et al., 2000; Vasconcelos et al., 2003; Oliveira et al., 2009; Souza et al., 2009; Baccaro et al., 2012, 2013; Souza et al., 2012). Nevertheless, it is argued that the permanence of the traps for longer periods could capture more diversity (Bestelmeyer et al., 2000). Although the shortest period of 48 hours has been proven adequate to show the representative assemblage of ants (Borgelt & New, 2006), longer periods are recommended in order to capture rare species (Borgelt & New, 2006; Schirmel et al., 2010). The efficiency of sampling with pitfall traps is also affected by the number of samples collected (Borgelt & New, 2006; Schirmel et al., 2010) and trap spacing (Wang et al., 2001).

Inventories of invertebrates involve many hours of processing samples and sampling of soil from an extensive area, and this process is hampered due to limitations of time and money (Jiménez-Valverde & Lobo, 2006), which in turn limits work to a few years (Danielsen et al., 2003). The major constraint to the expansion of existing ant surveys in tropical forests is the labor-intensive laboratory effort in sorting and identifying these invertebrates (Lawton et al., 1998; Purvis &

Hector, 2000; Souza et al., 2009, 2012). The biggest challenge to investigate a more efficient use of resources is to ensure that sufficient ecological gradients are captured in relation to changing environmental variables (Souza et al., 2009).

Tropical Amazonian rainforests contain mosaic textures, which are correlated with forest architecture and linked to the altitudinal gradient (Guillaumet, 1987; Costa & Magnusson, 2010). These combinations of environmental variables account for the variation associated with ant's assemblage structure across tropical landscapes (Vasconcelos et al., 2003; Oliveira et al., 2009). Investigating these types of relationships between ants and environmental variables is of more practical importance than simply showing correlations between the numbers of taxonomic entities (Souza et al., 2016). In fact, some studies have shown how environmental variables can be used to evaluate decisions about sampling techniques (Souza et al., 2012) or the use of genus as a surrogate for species (Souza et al., 2016) to optimize ant surveys.

Using poneromorph ants, we compared two trapping times and the number of pitfall traps to test the efficiency of three sampling efforts. We first checked for variations in poneromorph ant composition in each sampling combination. Secondly, we tested whether the ecological patterns observed with the maximum effort could be retrieved from data on reduced efforts. We investigated the influence of biotic (leaf and branch litter volume) and abiotic factors (terrain slope and soil clay content) on the poneromorph ant species composition, and compared with the results obtained with different trapping times and number of pitfall traps. Lastly, we checked the consequent gain in terms of time and costs, and the loss of information of each effort. We hypothesize that fewer sampling days and a smaller number of traps would reduce the catch, while still maintaining enough information to capture the relationship with environmental variables, thus improving the efficacy of the survey.

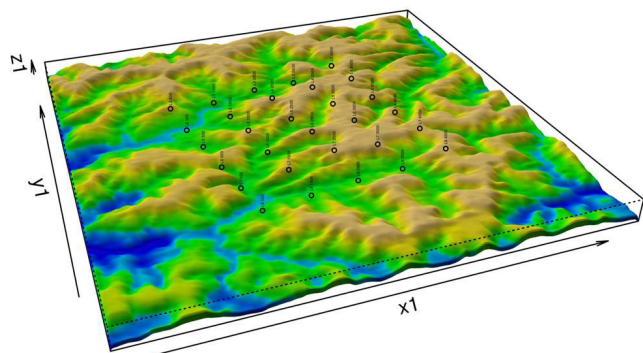
## Materials and Methods

### *Study site*

The Ducke Reserve is located 26 km on the Manaus-Itacoatiara Highway (3°00'S, 59°55'W), near to Manaus City, Amazonas State, Brazil. The reserve is covered by a terra-firma evergreen forest along a moderately uneven terrain (30–140 m a.s.l.) covering 10,000 hectares (Ribeiro et al., 1999). The nutrient-poor soils are classified as yellow clay latosols (xanthic hapludox) in the higher, well drained, flat plateaus, grading to clay-sand (typic epiaquods) on the slopes, and sandy soils (typic endoaquods) in the wet or temporarily floodplain (Chauvel et al., 1987; Santos et al., 2006). The climate is characterized by a rainy season from November to May, with a relatively dry season (less than 100 mm of monthly rainfall) occurring from July to September (Luizão et al., 2004). Mean daily air humidity and mean daily temperature between 2008 and 2011 were 77.7 percent and 25.7°C, respectively (Coordination of Environmental Dynamics, INPA).

### Ant sampling

The Ducke Reserve contains a grid of six regularly spaced north-south and six east-west trails. Each trail is 5 km long, forming a  $5 \times 5$  km grid (<https://ppbio.inpa.gov.br/sitios/ducke>; Fig 1). The east-west trails have five 250m long plots that follow terrain contours to minimize the variation in soil features (RAPELD method, Magnusson et al., 2005). The grid allows access to 30, 250m long plots, located 1 km apart from each other along the trails.



**Fig 1.** Location of the 25 km<sup>2</sup> sampling grid on Reserva Ducke in the Brazilian Amazon. Points indicate 1-km equidistant sample plots.

Between July and September 2006, poneromorph ants were sampled in the 30 plots using pitfall traps, which is the most widely used sampling technique for arthropods, such as ants (Schirmel et al., 2010). The traps consist of 500 ml plastic containers (6.5 cm diameter; 8 cm depth; 200 mL volume), buried with the rim at ground level and partially filled with the killing/preservative agent, and remaining open during 14 days (336 hours). The invertebrates that were active on the surface and fell into the trap were preserved in 70% alcohol. In total 750 samples were taken from 25 pitfall traps installed at 10 m intervals in each plot. We also used data from Oliveira et al. (2009), which used the same pitfall traps in the same period and area but with a different sampling effort, using a total 300 samples taken from 10 pitfall traps installed at 25 m intervals in each plot and left open for 48 hours. Thus, using data from both surveys, we are analyzing three sampling efforts in the 30 plots: two days and 300 traps, 14 days and 300 traps, 14 days and 750 traps were considered as the minimum, intermediate and maximum effort, respectively.

All ants were identified to genus using the taxonomic keys provide by Baccaro et al. (2015). After that, the ants were sorted to morphospecies, and whenever possible were identified to a species level (*sensu* Bolton et al., 2005), using the available taxonomic keys or by comparison with specimens in collections previously identified by experts. Vouchers are deposited in National Institute for Amazonian Research (INPA) Entomological Collection.

### Environmental variables

Data for independent variables that are recognized as important for ants were measured at the same 30 plots where

the ants were sampled. Environmental variable data included the soil clay content, leaf and branch litter volume and terrain slope (available in: <https://ppbio.inpa.gov.br/repositorio/dados>). To determine soil clay content and terrain slope, we sampled and combined six soil subsamples to a depth of 5 cm and at least 50 m distant from each other for each plot and analyzed them at the Soil Laboratory of the Agronomy Department at INPA. Litter volume was collected in the same 30 plots and period where the ground dwelling ants were sampled. About 1 m<sup>2</sup> of litter was put on a graduated plastic bucket to measure the volume (in liters). In each plot, 10 subsamples were collected and pooled to derive an average litter volume per plot. The environmental datasets and the descriptions of each sampling protocol (metadata) for each variable are available in the PPBio web site (<http://ppbio.inpa.gov.br/>). There were no significant interaction between soil clay content and terrain slope (Pearson's product-moment correlation  $t = -0.994$ ,  $df = 28$ ,  $p$ -value = 0.329,  $cor$ :-0.185), clay content and litter and branch volume (Pearson's product-moment correlation  $t = -0.174$ ,  $df = 28$ ,  $p$ -value = 0.863,  $cor$ :-0.033), and terrain slope and litter and branch volume (Pearson's product-moment correlation  $t = -0.453$ ,  $df = 28$ ,  $p$ -value = 0.654,  $cor$ :-0.085). The environmental variable were used merely to show whether an effect - significant or not - detected in one analysis can still be detected in a subsequent analysis based on reduced-effort sampling.

### Data analysis

The presence and absence data was used to reduce the influence of social behavior, foraging and mass distribution of the ant nests in leaf litter (Hölldobler & Wilson, 1990). The effectiveness of sampling technique depends on sampling intensity, and results of comparative analyses can be biased by variation in sampling intensity between efforts (Souza et al., 2012). To assess how many samples are needed to record a comparable number of species, the average species-accumulation curves from 1000 randomizations were used. The time and number of pitfall-traps could affect the composition of species in the area, so we used the Permutational Multivariate Analysis of Variance (np-MANOVA, Anderson, 2001) to test for differences in ant assemblages between time of exposure and the number of samples.

We tested hypothesis considering that the composition of the poneromorph ant assemblages in each sampling effort were different. The congruence between ant assemblages by each sampling effort was tested. The composition of the assemblages were reduced with the technique of Non-metric Multidimensional Scaling (NMDS, Minchin, 1987), applied to an association matrix using the Sørensen Index for qualitative data. The congruence between the NMDS ordinations for each sampling effort was quantified by Procrustean Superimposition with 1000 Monte Carlo permutations to test for statistical significance (Peres-Neto & Jackson, 2001).

The assemblages of ants and their relationship to environmental variables were compared for the maximum,

intermediate and minimum sampling efforts. Multiple regression test was used to verify that the environmental variables volume of litter, clay content and terrain slope affected the distribution of species (NMDS axes). Pearson's correlation was used to prevent errors of collinearity between variables to avoid putting correlated variables in the same regression model. All analyses were run in the R environment for statistical computing (R Core Team, 2017, version 4.3), using vegan package 2.4-6 (Oksanen et al., 2018).

Time and monetary costs for the all-sampling efforts were considered in relation to the maximum effort and the fractions of these costs were calculated for each reduced effort. The costs were based on the acquisition of material, maintenance, field sampling and laboratory activities (mainly salary and scholarships). The laboratory costs were those associated with species sorting, mounting, identifying, and chemicals for the conservation of voucher species. As recommended by Gardner et al. (2008), capital costs, which vary greatly among projects, such as non-perishable laboratory equipment (e.g. microscopes) and accommodation buildings for field staff, were not included. The time to carry out the work was the sum of the time spent to collect samples in the field and the time spent sorting and identifying ants in the laboratory.

## Results

Sixty-four species/morphospecies of poneromorph ants were recorded combining the results of the maximum, intermediate and minimum efforts. Using the maximum effort (14 days with 750 samples), a total of 944 poneromorph ants distributed in 58 species/morphospecies and 16 genera were recorded (Table 1). Using the minimum effort (2 days with 300 samples), 440 individuals distributed in 39 species and 14 genera were recorded. *Ectatomma* spp. and *Pachycondyla* spp. were the most abundant genera, independent of the applied effort. *Hypoponera* spp. was the most diverse with 12 species. Thirteen species were collected only once in 30 plots. Fourteen genera were also captured in both reduced efforts. Only two singletons genera (*Platythyrea* sp. and *Typhlomyrmex* sp.) were sampled with longer sampling period (14 days). The number of individuals caught decreased to more than a half (~53%) with a reduced number of pitfall traps, but the number of species/morphospecies still represented about 67% (minimum effort) and 74% (intermediate effort) of the maximum effort.

The regression line trends for the species accumulation curves were similar for all efforts (Fig 2). The slope for the intermediate effort increased more rapidly compared to the

**Table 1.** Abundance and frequency of poneromorph ant species (*sensu* Bolton et al., 2005), sampled with pitfall traps in 30 250-m long plots in an Amazonian rain forest. This data is deposited in the digital repository of the Brazilian Biodiversity Research Program (PPBio).

Taxon	Sampling effort					
	14 days				2 days	
	750 sub-samples		300 sub-samples		300 sub-Samples	
	Abundance	Frequency	Abundance	Frequency	Abundance	Frequency
<i>Acanthostichus bentoni</i> Mackay, 1996	13	6	1	1	4	2
<i>Anochetus diegenis</i> Forel, 1912	3	1	0	0	6	4
<i>Anochetus emarginatus</i> (Fabricius, 1804)	1	1	0	0	1	1
<i>Anochetus horridus</i> Kempf, 1964	12	6	8	4	4	3
<i>Centromyrmex gigas</i> Forel, 1911	2	2	0	0	1	1
<i>Ectatomma edentatum</i> Roger, 1863	145	23	94	19	70	17
<i>Ectatomma lugens</i> Emery, 1894	243	21	104	18	117	21
<i>Ectatomma tuberculatum</i> (Olivier, 1792)	7	4	2	2	0	0
<i>Gnamptogenys horni</i> (Santschi, 1929)	52	19	17	13	21	13
<i>Gnamptogenys lineolata</i> Brown, 1993	8	5	6	3	0	0
<i>Gnamptogenys moelleri</i> (Forel, 1912)	28	1	0	0	20	1
<i>Gnamptogenys relicta</i> (Mann, 1916)	9	4	2	1	0	0
<i>Gnamptogenys</i> sp. 02	5	3	1	1	3	2
<i>Gnamptogenys</i> sp. 04	0	0	0	0	1	1
<i>Gnamptogenys</i> sp. 07	1	1	0	0	0	0
<i>Gnamptogenys minuta</i> (Emery, 1896)	1	1	1	1	0	0
<i>Gnamptogenys striatula</i> Mayr, 1884	1	1	2	2	0	0
<i>Gnamptogenys tortuolosa</i> (Smith, 1858)	33	11	9	7	13	6
<i>Hypoponera</i> sp. 01	9	1	0	0	8	2
<i>Hypoponera</i> sp. 02	0	0	0	0	2	2
<i>Hypoponera</i> sp. 03	1	1	0	0	2	1
<i>Hypoponera</i> sp. 04	4	2	3	2	10	5



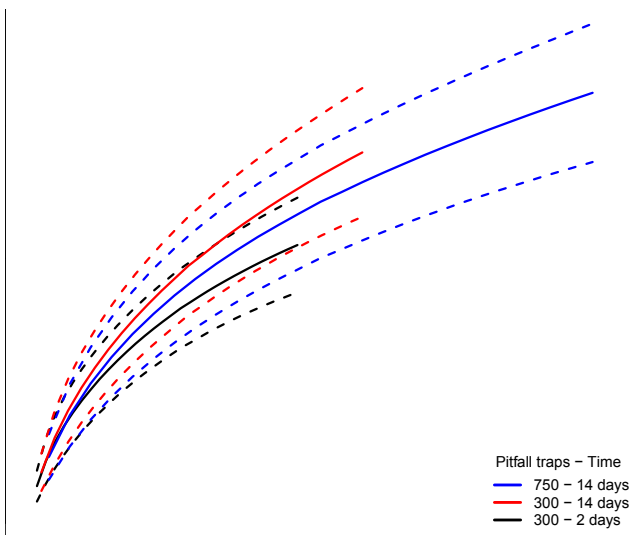
**Table 1.** Abundance and frequency of poneromorph ant species (*sensu* Bolton et al., 2005), sampled with pitfall traps in 30 250-m long plots in an Amazonian rain forest. This data is deposited in the digital repository of the Brazilian Biodiversity Research Program (PPBio). (Cont.)

Taxon	Sampling effort					
	14 days		2 days			
	750 sub-samples		300 sub-samples		300 sub-Samples	
	Abundance	Frequency	Abundance	Frequency	Abundance	Frequency
<i>Hypoponera</i> sp. 05	0	0	0	0	1	1
<i>Hypoponera</i> sp. 06	10	7	4	3	1	1
<i>Hypoponera</i> sp. 07	3	3	2	2	1	1
<i>Hypoponera</i> sp. 08	0	0	0	0	1	1
<i>Hypoponera</i> sp. 09	1	1	0	0	0	0
<i>Hypoponera</i> sp. 10	1	1	1	1	0	0
<i>Hypoponera</i> sp. 11	4	4	2	2	0	0
<i>Hypoponera</i> sp. 12	1	1	0	0	0	0
<i>Leptogenys gaigei</i> Wheeler, 1923	16	9	7	6	5	4
<i>Leptogenys pusilla</i> (Emery, 1890)	1	1	1	1	1	1
<i>Leptogenys wheeleri</i> Forel, 1901	1	1	0	0	3	2
<i>Leptogenys</i> sp. 03	2	2	2	2	0	0
<i>Leptogenys</i> sp. 04	3	3	2	2	0	0
<i>Leptogenys</i> sp. 05	1	1	1	1	0	0
<i>Mayaponera constricta</i> (Mayr, 1884)	109	20	51	16	29	14
<i>Neoponera apicalis</i> (Latreille, 1802)	15	7	10	5	2	2
<i>Neoponera commutata</i> (Roger, 1860)	10	4	5	2	1	1
<i>Neoponera laevigata</i> (Smith, 1858)	1	1	0	0	0	0
<i>Neoponera marginata</i> (Roger, 1861)	2	2	0	0	0	0
<i>Neoponera unidentata</i> (Mayr, 1862)	1	1	0	0	0	0
<i>Neoponera verenae</i> (Forel, 1922)	1	1	1	1	0	0
<i>Neoponera villosa</i> (Fabricius, 1804)	1	1	1	1	0	0
<i>Odontomachus bauri</i> Emery, 1892	2	2	1	1	0	0
<i>Odontomachus brunneus</i> (Patton, 1894)	0	0	0	0	1	1
<i>Odontomachus caelatus</i> Brown, 1976	16	7	12	5	10	3
<i>Odontomachus haematodus</i> (Linnaeus, 1758)	9	3	5	2	2	2
<i>Odontomachus laticeps</i> Roger, 1861	0	0	0	0	5	1
<i>Odontomachus meinerti</i> Forel, 1905	6	3	5	2	3	1
<i>Odontomachus opaciventris</i> Forel, 1899	2	1	2	1	9	4
<i>Odontomachus scalptus</i> Brown, 1978	12	5	2	1	2	1
<i>Odontomachus</i> sp. 01	6	5	1	1	0	0
<i>Pachycondyla crassinoda</i> (Latreille, 1802)	94	18	39	13	54	17
<i>Pachycondyla harpax</i> (Fabricius, 1804)	22	14	16	10	19	13
<i>Pachycondyla impressa</i> (Roger, 1861)	3	2	3	2	0	0
<i>Paraponera clavata</i> (Fabricius, 1775)	2	2	1	1	1	1
<i>Platythyrea pilosula</i> (Smith, 1858)	1	1	1	1	0	0
<i>Prionopelta punctulata</i> Mayr, 1866	10	6	4	2	3	3
<i>Pseudoponera stigma</i> (Fabricius, 1804)	1	1	0	0	2	2
<i>Rasopone arhuaca</i> (Forel, 1901)	14	5	6	3	1	1
<i>Rasopone lunaris</i> (Emery, 1896)	2	1	2	1	0	0
<i>Typhlomymex rogenhoferi</i> Mayr, 1862	1	1	0	0	0	0
Total number of individuals	944		441		440	
Total number of species	58		43		39	

other treatments, although there was no significant difference amongst treatments. In general, all efforts are equivalent, since their confidence intervals overlap.

The species composition of poneromorph ants (Fig 3) differed for period of time and number of samples (np-MANOVA:  $F_{2, 81} = 83.07$ ;  $r^2 = 0.664$ ;  $p \leq 0.001$ ). The congruence between assemblages was affected by the time the pitfall traps were operating in the field. The three efforts had moderate similarity ( $r > 0.40$ ). Assemblages sampled during longer periods (14 days) were more similar to each other ( $r \sim 0.66$ ) than when compared with the assemblage captured during two days ( $r \sim 0.43$ ), regardless of the number of sampling traps used (Table 2).

Multivariate regressions analysis indicated that the ecological pattern captured with the maximum effort was still captured in the two reduced effort treatments. In all tested efforts, soil clay content significantly affected the composition of the ground-dwelling ants. In addition, a consistent pattern of no significant relationship was captured for the terrain slope



**Fig 2.** Accumulation curves for poneromorph ant species assemblages captured in 30, 250 m long plots in the Amazonian rain forest. Dotted lines mark the 95% confidence intervals.

**Table 2.** Congruence between the poneromorph ant datasets sampled by the minimum (two days and 300 sampling traps), intermediate (14 days and 300 sampling traps), and maximum (14 days and 750 sampling traps) effort sampled along 30 250 m long plots in an Amazonian rain forest. Congruence was evaluated by symmetric Procrustean rotations using Sørensen dissimilarity index calculated for poneromorph ant species presence/absence data. P values were estimated by 999 Monte Carlo permutations. Significance levels: \* $p \leq 0.01$ ; \*\* $p \leq 0.001$ .

Sampling effort	Maximum effort treatment	Medium effort treatment	Minimum effort treatment
Maximum effort treatment	1		
Medium effort treatment	0.657**	1	
Minimum effort treatment	0.498**	0.427*	1

and volume of litter across all effort treatments (Table 3). Along the clay concentration gradient, some species that were mostly singletons and doubletons were restricted to the extremes of the gradient. Species with a wider distribution tended to occupy more uniformly the entire gradient (Fig 4).

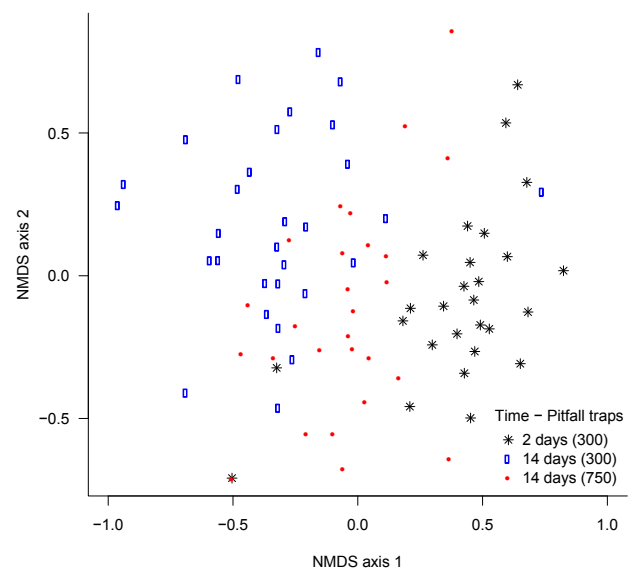
The relative monetary and time costs differed among efforts (Table 4). The maximum effort was the most expensive. Reduced efforts accounted to 60-74% of the total costs and 55-57% of the total time.

**Discussion**

Although trapping time influenced the similarity of the assemblages more than the number of sampling traps, the minimum effort (two trapping days using 300 pitfall traps) was still more effective at capturing ants compared with the

**Table 3.** Proportion of variance on the poneromorph ant species, assemblage composition explained by the environmental variables in multivariate regression models. Significance levels: \* $p \leq 0.01$ ; \*\* $p \leq 0.001$ .

Environmental variables	Sampling effort		
	14 days 750 sampling traps	300 sampling traps	2 days 300 sampling traps
Clay content (partial)	0.0093**	0.0252*	0.0093**
Litter volume (partial)	0.6145	0.4243	0.6145
Terrain slope (partial)	0.1634	0.1026	0.1634
R <sup>2</sup>	0.3188	0.3435	0.3188
P	0.0172*	0.0196*	0.0172*



**Fig 3.** An NMDS ordination (Stress = 0.148) plot indicating the congruence in poneromorph ant species associations among period of time and number of sampling traps in 30, 250-m long plots in an Amazonian rain forest.

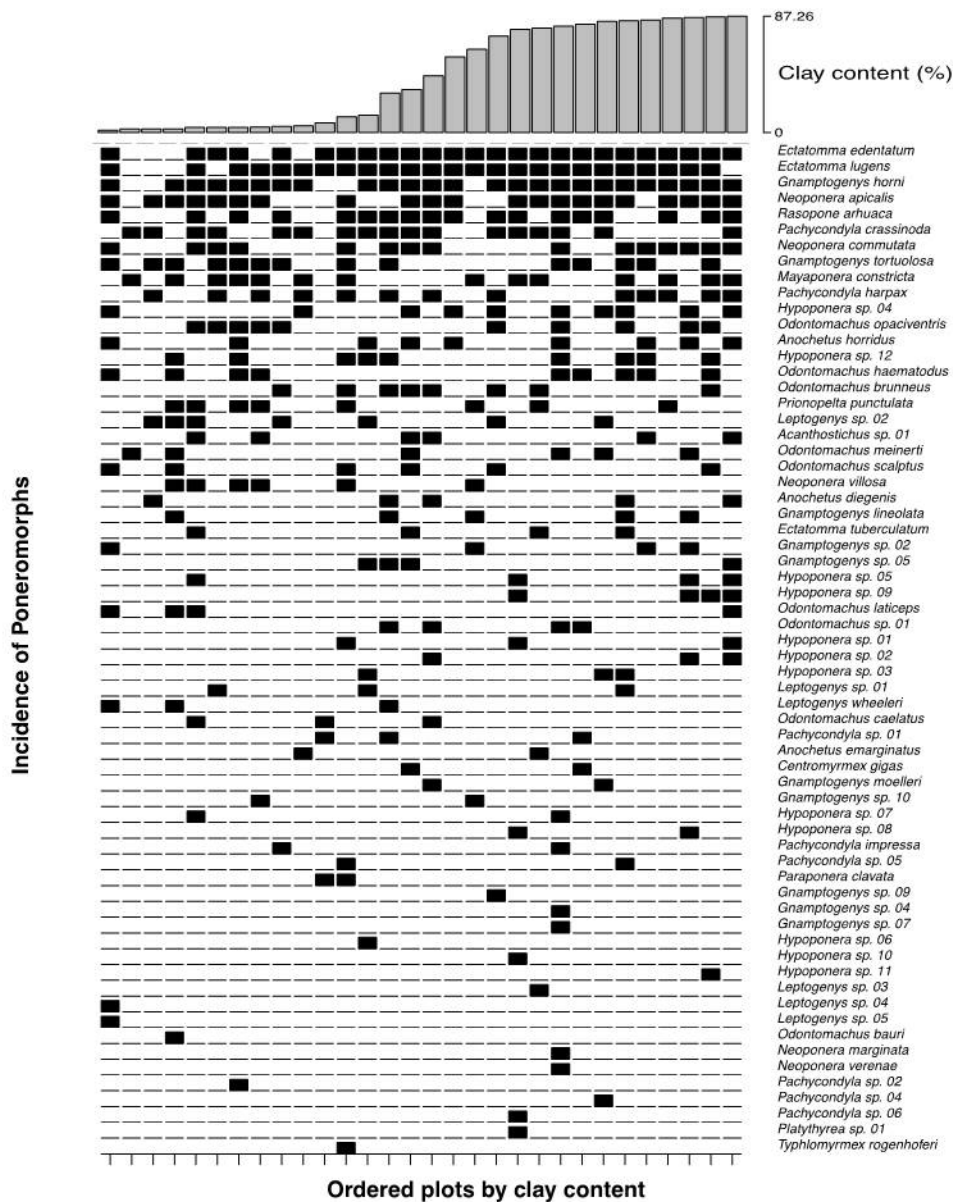


Fig 4. Distribution of poneromorph ant species along clay content gradient in 30, 250m long plots at the Ducke Reserve.

**Table 4.** Summary of the relative effort (cost and time) required to process the material for poneromorph antspecies assemblages captured in two and 14 days in 30, 250-m long plots in an Amazonian rain forest.

Sampling effort	Cost	Time
	%	
14 days/750 sampling traps	100	100
14 days/300 sampling traps	74	57
2 days/300 sampling traps	60	55

intermediate and maximum efforts. The sampling effort using 48 hours of pitfall-trap exposure has already been reported in previous studies (Olson, 1991; Bestelmeyr et al., 2000), as well as other studies that have evaluated the exposure time of the sampling traps (Delabie et al., 2000; Borgelt & New, 2006; Schirmel et al., 2010). The 48 hours sampling

time protocol in this study did reduce the abundance of ants in traps to more than a half but was robust enough in describing the diversity of ground-dwelling ants, revealing the presence of more than 60% of the poneromorph ant species present in the surveyed area. The ecological patterns of ground-dwelling ants detected with the maximum effort treatments were also captured with reduced effort treatments, indicating that this pattern can be detected faster and cheaper, both in the field and in the laboratory. Pitfall trapping time depends on the objective of work (Delabie et al., 2000) and from the survey and monitoring standpoint. Thus, reducing the number of pitfall traps from 750 to only 300 units and from 14 to only two days will greatly improve surveys effectiveness. This reduced time and trap sampling protocol will save about 40% of the cost and 45% of the resources used by the maximum effort treatment while maintaining the integrity of the ecological information collected.

Most studies evaluating ant-sampling techniques usually test the taxonomic aspect, i.e. they use metrics related to species richness or composition (Fisher, 1999; Delabie et al., 2000; Tista & Fildler, 2010). As a result, most of the investigations on ant sampling protocols were also based on these metrics (Olson, 1991; Bestelmeyer et al., 2000; Lopes & Vasconcelos, 2008). More recent sampling protocol approaches have included ecological responses and environmental variables to evaluate the efficiency of the collection techniques (Souza et al., 2009; 2012). This way, our analyses provide insights into which environmental variable is explaining the pattern of ant distribution. Although it was expected that the reduced effort treatment may have incurred a loss of ecological information, all assemblages of ground-dwelling ants captured within each one of the three treatments have their spatial distributions influenced by soil clay content. The ecological pattern of non-significant results for the effect of terrain and volume of litter also was maintained across all efforts. These results reveal that the use of reduced efforts is efficient in representing the ants sampling with maximum effort in both taxonomic and ecological approaches.

Changes in soil characteristics are related to the topography in the Amazon basin (Chauvel et al., 1987), the highest clay percentage is concentrated on the uplands, and the soils along stream valleys are particularly poorly drained (Ranzani, 1980). In tropical rainforest, soil clay content has also been shown to affect the distribution of palms (Costa et al., 2009), frogs (Menin et al., 2007), oribatid mites (Franklin et al., 2013) and ants (Oliveira et al., 2009; Vasconcelos et al., 2003; Souza et al., 2007; 2012; 2016). The effect of each environmental factor varies depending on the scale of analysis (total area surveyed and the number of sampling units). Due to the broad scale of this survey and the number of pitfall traps, the detected trends of significant and non-significant values of the environmental variables would be consistent even with efforts higher than those that we used here.

Especially in the Amazon, financial costs limit the extent of biodiversity studies (Costa and Magnusson, 2010). The cost (time and money) of environmental monitoring is a crucial factor for the viability of biodiversity studies (Margules & Pressey, 2000), and any reliable alternative should be used to increase the area covered or the duration of the time series. In 2010, approximately US \$ 0.01 per hectare was invested in monitoring actions in the Brazilian Amazon, and no increase is expected in the near future (Magnusson et al., 2013). Thus, we must consider that the financial resources saved both in the field and in the laboratory using lesser efforts can be used for biodiversity surveys in other areas.

We suggest that only two days of exposure in the field and smaller numbers of pitfall traps is sufficient to capture a representative amount of individuals and species, and is effective in describing basic ecological attributes of poneromorph ants. The rarest species in this study were located at the extremes of clay content gradient, which are

either valley areas with low soil clay content or high lands with a high concentration of clay. Therefore, these extremes are peculiar and important environments for the maintenance of rare species.

We believe that due to the ecological plasticity of ants, as well as their association with other organisms and their use as bioindicators, the results found here are likely to be used in general studies with ants, especially in situations where the time and cost of financing are limiting. Consequently, investigators can process a smaller volume of biological material in the laboratory, and give faster results. Effective monitoring is a vital tool for use in almost any program aiming at measure changing in the ecosystem. Survey managers may want to apply the results of this study to extrapolate to another sampling universe. The consequence of a substantial reduction in exposure time and the number of sampling traps in the field, can decrease the time required to process samples in the laboratory, but can be crucial in the appropriate sampling of taxa or groups of taxa for quick monitoring and inventory of biodiversity.

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