



## RESEARCH ARTICLE - ANTS

### Evaluation of Sampling Techniques and Influence of Environmental Variables on Ants in Forest Fragments in an Oil Extraction Area in the Amazon

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#### Abstract

Around the world, urban landscapes are environments modified by man, generally related to low diversity. The efficiency of a biodiversity inventory is linked to the choice of the sampling technique since the taxonomic and ecological responses of the taxons vary between methods. Here we investigate differences in the ant's composition sampled using three different techniques in two fragments of the urban forest in the Brazilian Amazon. We also assessed whether the different techniques maintained the same ecological responses. We sampled 12 collection points at each fragment, at vegetation, and manual collection and Winkler extractor on the ground. At the same points, soil samples were collected to determine their granulometry, pH, and concentrations of organic matter, sodium, phosphorus, and potassium. We collected 115 taxa and 4720 ants. The Cururu was the richest site with more species in general, as well as in the techniques of manual collection and Winkler. We detected a complementary effect on sampling techniques, which collected different ants' assemblages. The potassium concentration positively influenced the assemblage's composition, but its effect varies according to the sampling techniques used. The studied fragments revealed diversity very similar to those registered in continuous Amazonian forests. The use of sampling techniques together improves the representation of the diversity of ants in the studied fragments. Edaphic environmental variables seem to have a predominant effect on ants, affecting their distribution in the landscape even in urban fragments. This highlights the importance of urban forest fragments and their inherent ecological processes.

#### Introduction

The expansion of cities is a conspicuous cause of the conversion of land into landscapes modified by man (McKinney, 2002; Santos, 2016). These urban landscapes are usually spatially and temporally dynamic (McIntyre et al., 2001), and often associated with low biodiversity (Vitousek et al., 1997; McIntyre et al., 2001; Yamaguchi, 2004; 2005). All over the world, these urban landscapes are characterized by high heterogeneity, which can provide useful information on the management of biodiversity in other ecosystems (Savard et al., 2000).

Environments that ants inhabit are modified by natural disturbances, which vary in extent, magnitude, and frequency. Also, many terrestrial ecosystems have been altered due to various human activities, including urbanization (Philpott et al., 2010). Urbanization is a driving force behind habitat destruction and has powerful effects on ants' richness and composition. Ecological surveys of urban ants usually address changes in species richness and composition (Gibb & Hochuli 2003; Lessard & Buddle 2005; Pacheco & Vasconcelos 2007; Yamaguchi 2004), but little has been done regarding the choice or efficiency of sampling techniques.



There is no suggestion of an ant sampling protocol for urban environments (Munhae, 2017), which makes comparisons between works difficult. In urban green areas (i.e. parks or forest fragments) among the most used techniques, we have a manual collection, Winkler extractors (if there is litter), and beating, which is on a smaller scale (Munhae, 2017). The manual collection has a very low cost, which facilitates its use. However, the effectiveness of this technique depends on the researcher's experience and the sampling effort (Bestelmeyer et al., 2000). The Winkler has a high initial cost and depends on the existence of a litter, however, it has the advantage of sampling a large portion of ants that are not detected with other methods (Olson, 1991; Bestelmeyer et al., 2000). Beating on vegetation is often used to complement other techniques, as it underestimates species by not accessing the canopy (Majer & Delabie 1994; Munhae, 2017).

No sampling technique is effective for capturing all invertebrates present in a given area. Some studies with several sampling techniques to capture ants have revealed that the information obtained by these techniques may be complementary to obtain a more complete survey of the richness of genera and species in the area (Olson, 1991; Delabie et al., 2000; Antoniazzi et al. 2020). But may have some degree of redundancy of information (Parr & Chown, 2001; Lopes & Vasconcelos, 2008; Souza et al., 2012). Studies of a minimum number of sampling techniques usually seek to know which technique or combination of techniques is most efficient for an inventory with an emphasis on records of the diversity and abundance of taxa and are usually conducted in natural landscapes (e.g. Garden et al., 2007; Roy et al., 2007; Ribeiro-Junior et al., 2008). However, little is known about the choice of sampling techniques in urban environments (Munhae, 2017).

In aquatic systems, invertebrates have been widely used for biological monitoring (Hawkins et al., 2000), however, in terrestrial environments they have been used less frequently (Andersen & Majer, 2004). While providing valuable indications of changes in biological integrity and ecosystem functions, terrestrial invertebrates are usually not considered by management agencies (Andersen & Majer, 2004) due to the high costs, especially those resulting from the long time needed to identify this megadiverse group, together with the lack of taxonomists and parataxonomists. However, some change has occurred in recent times and long-term inventories on biodiversity have included ants in their focus groups (e.g. Fernandes & Souza, 2018).

Among the most commonly used environmental characteristics for modeling the distribution of organisms are soil, topography, and vegetation structure (Zuquim et al., 2007). For invertebrates, environmental factors, such as topography, texture, and soil chemistry, generate microhabitat variability that can affect the spatial patterns of invertebrate assemblages at local scales (Vasconcelos et al., 2003; Oliveira et al., 2009; Mezger & Pfeiffer, 2011). In the Amazon, some studies already find effects of these variables on oribatid-mites

(Moraes et al., 2011), pseudoscorpions (Aguilar et al., 2006), ants (Oliveira et al., 2009; Souza et al., 2009; Gomes et al., 2018; Torres et al., 2020), cockroaches (Tarli et al., 2014) and termites (Dambros et al., 2016). To be considered efficient, the sampling protocol must satisfy the taxonomic, ecological and financial aspects of the research and some studies have used the responses of taxa to the environment to calibrate sampling protocols in the Amazon (Souza et al., 2012; 2016).

Both the forest fragments areas around the Isaac Sabbá Refinery (Reman) and the surroundings of the Cururu stream are now fragmented forest fragments with high vulnerability and constant anthropic action. Although they are located within the city of Manaus, there is no study report on surveys to characterize the terrestrial invertebrate fauna of these forest fragments, neither now nor in the past. This is the first biodiversity survey of the insects in both sites. The majority of the studies on the fauna of Manaus invertebrates were carried out in the Ducke Reserve, a forest reserve belonging to the National Research Institute of Amazonia - INPA (Magnusson et al., 2014). There is only one study evaluating the impact of the oil spill that occurred in 1985, on the water and edaphic insects of the riverbank, in Cururu Igarapé (Couceiro et al., 2006).

Biodiversity surveys are one of the first steps to determine the conservation or sustainable use of a location or ecosystem (Scott et al., 1987; National Research Council, 1992). For the characterization of ant's assemblages in the two study areas, different sampling techniques were used, covering the available habitats, such as soil and vegetation of the understory. Information on environmental variables that possibly affect the distribution of ant's assemblages was collected in areas located around the Isaac Sabbá Refinery - Reman and the Marine Command Base at Igarapé Cururu. Our goals are: 1) Evaluate if sampling techniques have a complementary effect on ant's assemblages on urban forest fragments and 2) Test if environmental variables affect the distribution of the ant's assemblages in the forest fragments studied. For this, we tested two hypotheses. The first is that the collection techniques would have a null or reduced complementary effect on biodiversity survey, due to the constant anthropic effect on the fragments. Also, the ecological response of the ants collected with the techniques together will remain with the use of them separately.

## Material and Methods

### Study Area

The study was conducted in two urban forest fragments, the first around the Isaac Sabbá Refinery – Reman (03° 08'13.47 "S – 59° 57'24.7" W). The second area sampled was the forest on the slope of the Cururu Igarapé, owned by the Marine Operations Battalion of the Brazilian Navy (03° 07'47.72 "S – 59° 56'34.20" W), hereafter Reman and Cururu respectively (Fig 1). The minimum distance between the sampling points and the fragment's edge was 150m in Cururu and 200m in Reman. Two sampling collections were carried

out in two areas, the first from September 14 to October 27, 2006, and the second from February 14 to April 02, 2007. Each sampling technique was used on different days, in a single collection event per year.



**Fig 1.** Location of the two urban forest fragments near an oil and gas refinery in the city of Manaus, Amazonas, Brazil.

#### *Sampling Techniques and Identification*

To sampling ants, three different techniques were used: Beating (entomological umbrella), manual collecting, and Winkler extractor. In both areas, 12 sampling points approximately 15 m apart were allocated in plots, one in each fragment, installed on the ground, in the flattest location possible. Each sampling technique was performed on the same plot in each fragment but on different days. The length of the plots was 180m for the techniques of manual collection and Winkler extractor. For the beating, the length of the plots was variable to accommodate 10 palm trees, with a minimum distance of 10 m between sampled plants.

The Beating (entomological umbrella-hunting) technique was used to collect ants in the palms of the Genera *Astrocaryum*, *Bactris* and *Geonoma*, which are found in the understory. These palms accumulate organic plant material in decay between the leaves and suspended from the ground between 0.5 m to 2 m in height. Ten palm trees were selected for this study in each area. The palms were hit to dislodge ants from a tray underneath. This technique sampled the fauna associated with palm trees (Bestelmeyer et al., 2000).

The manual collection and Winkler extractor were used for sampling ground-dwelling ants. In the manual collection, the litter samples were removed from a bounded area with 0.50 x 0.50 m (0.25 m<sup>2</sup>), placed in a white tray for the sorting of ants (Bestelmeyer et al., 2000). The litter samples for Winkler extraction were taken from a bounded area of 1 m<sup>2</sup>. The whole litter was collected manually and placed in a 1 cm<sup>2</sup> mesh sieve. The sieve was stirred for about one minute and the sieved material was transported to the laboratory. In the laboratory, the samples were placed in the Winkler extractor for 48 hours for extraction (Bestelmeyer et al., 2000; Souza et al., 2012). The manual collection and Winkler extractor were collected 24 samples for each method in both areas.

Ants were identified at the Genus level using the keys provided in Baccaro et al. (2015). Later, this material was identified by morphospecies or species whenever possible, using available taxonomic keys, comparison with material previously identified by specialists and deposited in the National Institute of Amazon Research (INPA) entomological collections.

#### *Environmental predictors*

The environmental variables related to soil factors (clay content, pH, organic matter, sodium, phosphorus, and potassium concentration) were selected due to their influence in previous research in the Amazon basin (Vasconcelos et al., 2003; Oliveira et al., 2009; Gomes et al., 2018; Torres et al., 2020). Along each of the plots, 12 soil samples were collected using an auger, at the same sampling points as the ants. The soil samples were collected up to 10 cm deep after removal of the leaf litter and large roots. After collecting the soil, the material was sent for analysis of texture and chemical characteristics, following the protocols, established by EMBRAPA (Silva, 2009).

#### **Data Analysis**

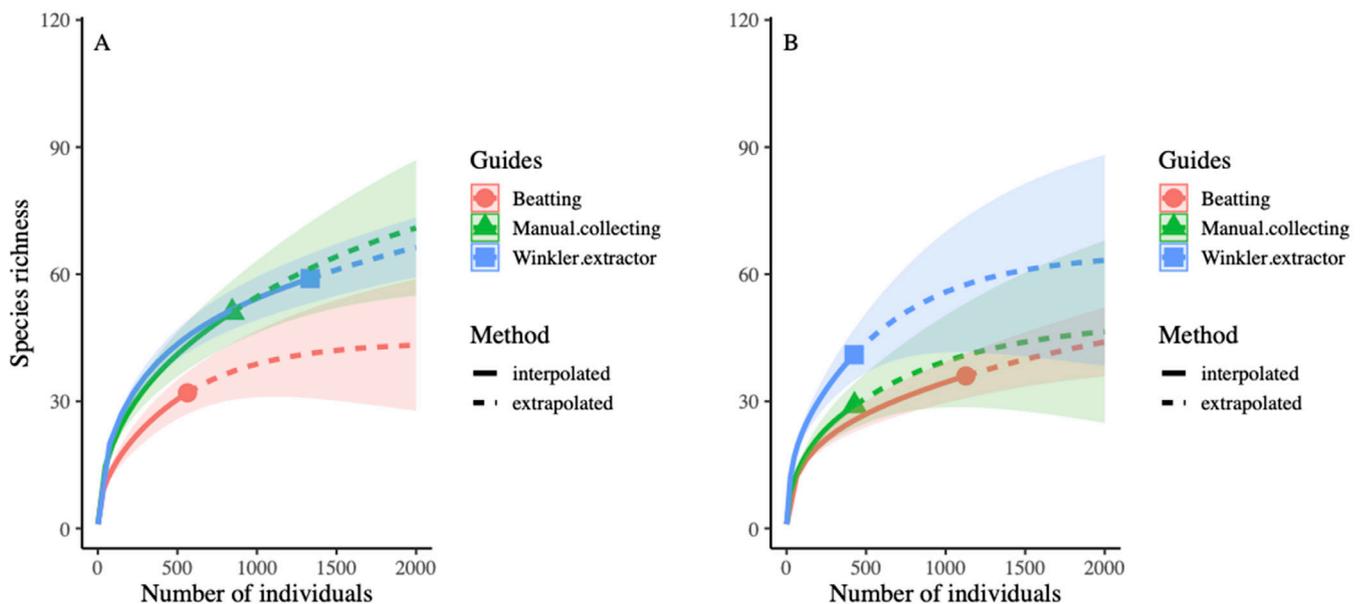
We used frequency data (presence/absence) to avoid the overestimation of species with larger nests (Hölldobler & Wilson, 1990; Gotelli et al., 2011). Some ant species have mass foraging strategies and their nests, or foraging/displacement trails (e.g. Dorylinae) may be close to the sampling point (Hölldobler & Wilson, 1990; Brühl et al., 2003). The frequency was calculated for each sampling point (trap). The data from the two sampling events (2006 and 2007) were grouped and analyzed jointly. Thus, we have 12 Winkler extractors, 12 manual collections and 10 palm samples for each fragment.

We calculated the number of species in relation to the number of individuals collected using Hill numbers (Chao et al., 2014; Hsieh et al., 2016). Observed samples were used to calculate diversity estimates for rarefied and extrapolated samples and the confidence intervals associated with 95%. The generated curves plot the diversity estimates in relation to the sample size (Chao & Jost, 2012; Colwell et al., 2012).

To evaluate the complementary between the sampling techniques, we used the Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson, 2001). We also evaluated the effect of the sites and their interaction with sampling techniques on the composition of the ant assemblage. We created a stratified permutation procedure to keep the nested structure of the data (plots nested in sites) in the PERMANOVA to control for possible spatial autocorrelation of the data. The PERMANOVA probability values were based on 999 permutations.

The ground-dwelling ants collected in the Winkler extractor and manual collection techniques were correlated with environmental variables related to soil factors (clay content, pH, organic matter and, potassium concentration).

We used redundancy analysis (RDA) to estimate how much of the variance in the response variable (ant's species composition matrix) can be explained by the environment. We carry out these analyzes with the sampling techniques together and separately. We also include as variables in the RDA, the collection technique and the sampling site. RDA is a direct extension of multiple regression analysis to model multivariate response data (Borcard et al., 2011). The statistical significance of RDA models was tested by 1000 permutations per test. All analyses were performed with the R environment version 4.0.2 (R Core Team, 2020) using the vegan 2.5-6 (Oksanen et al., 2019), car 3.0-3 (Fox & Weisberg, 2019), iNEXT 2.0.19 (Hsieh et al., 2019) packages.



**Fig 2.** Species accumulation curves based on 1,000 randomization (rarefied and extrapolated values) for ant species sampled with three techniques in the Cururu (A) and Reman (B) urban forest fragments, in the city of Manaus, Amazonas, Brazil. Confidence intervals were calculated to 95%.

## Results

We collected 4720 ants, belonging to 8 subfamilies and 115 species. At Cururu, we observed 98 species of ants and 74 species in Reman. Of these, 41 species occurred only in the Cururu area, 17 exclusively in Reman and 57 species were recorded in both areas. The rarefaction and interpolation curves indicate that the number of species tends to increase with the addition of individuals. Winkler extractor was the technique that sampled the highest ant's richness in both forest fragments. At the Cururu site, it registered 59 species of ants and at Reman 41. With the extrapolation to 2000 individuals, the estimate for Cururu was 88 species and 65 for Reman. In the Cururu fragment, manual collection registered 51 species (88 estimated) followed by the beating that registered 32 (44 estimated). In the Reman fragment, there was an inversion and the beating surpassed the manual collection in the number of species, collecting 36 (57 estimated) and 29 (49 estimated) species respectively (Fig 2, Table 1). In Cururu, the greatest abundance of ants was recorded with Winkler, followed

by manual collection and beating. At Reman, the greatest abundance of ants was detected at the beating; Winkler and the manual collection had similar abundances (Table 1).

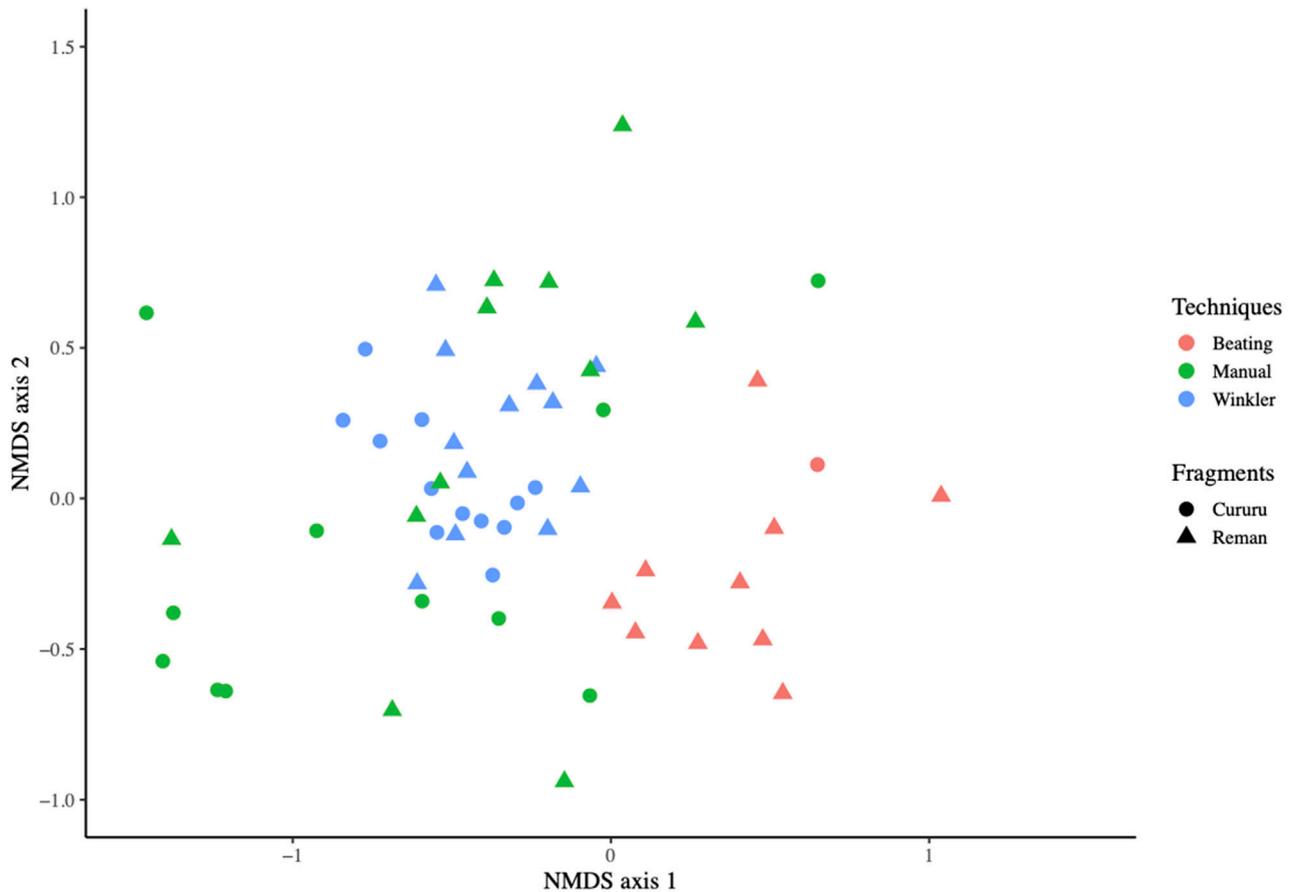
Sampling techniques have an effect on the ant species composition (PERMANOVA:  $F_{5,53} = 6.811$ ;  $R^2 = 0.19$ ;  $p < 0.001$ ). The assemblage sampled with the Winkler (blue symbols) is concentrated in the center of the figure. Around it, is the points of the manual collection samples (green symbols), and a more distinct group on the right (red symbols) are the points of the beating (Fig 3). The effect of forest fragments on the composition of ants is small (PERMANOVA:  $F_{5,53} = 2.490$ ;  $R^2 = 0.03$ ;  $p < 0.01$ ), so there is no evident separation between them (Fig 3). The interaction term between sites and sampling techniques was not significant (PERMANOVA:  $F_{5,53} = 1.332$ ;  $R^2 = 0.04$ ;  $p = 0.120$ ).

Considering the Winkler and manual collection techniques together, the ant assemblage composition was significantly correlated with tested variables (RDA = 0.3975;  $p > 0.001$ ). But, the environmental variables have a small effect on the ant's assemblages sampled in the two forest fragments.

The greatest detected effect on the assemblage is the sampling techniques ( $F = 12.4165$ ;  $p > 0.001$ ), followed by the effect of potassium concentration ( $F = 2.1492$ ;  $p > 0.05$ ; Fig 4-A). Analyzing Winkler separately, the analysis is not significant ( $RDA = 0.2726$ ;  $p = 0.093$ ), but the partial effect of potassium concentration is still detected ( $F = 2.1155$ ;  $p > 0.05$ ; Fig 4-B). Similarly, the manual collection also has a non-significant result ( $RDA = 0.2577$ ;  $p = 0.113$ ), this time, without partial effects of environmental variables.

## Discussion

Although urban environments are usually associated with low biodiversity, we detected high biodiversity of ants in the studied urban forest fragments. We also verified the complementary effect of the sampling techniques used. Besides, we detect that ants' responses to environmental variables are subtle, and their detection will depend on the sampling technique used.

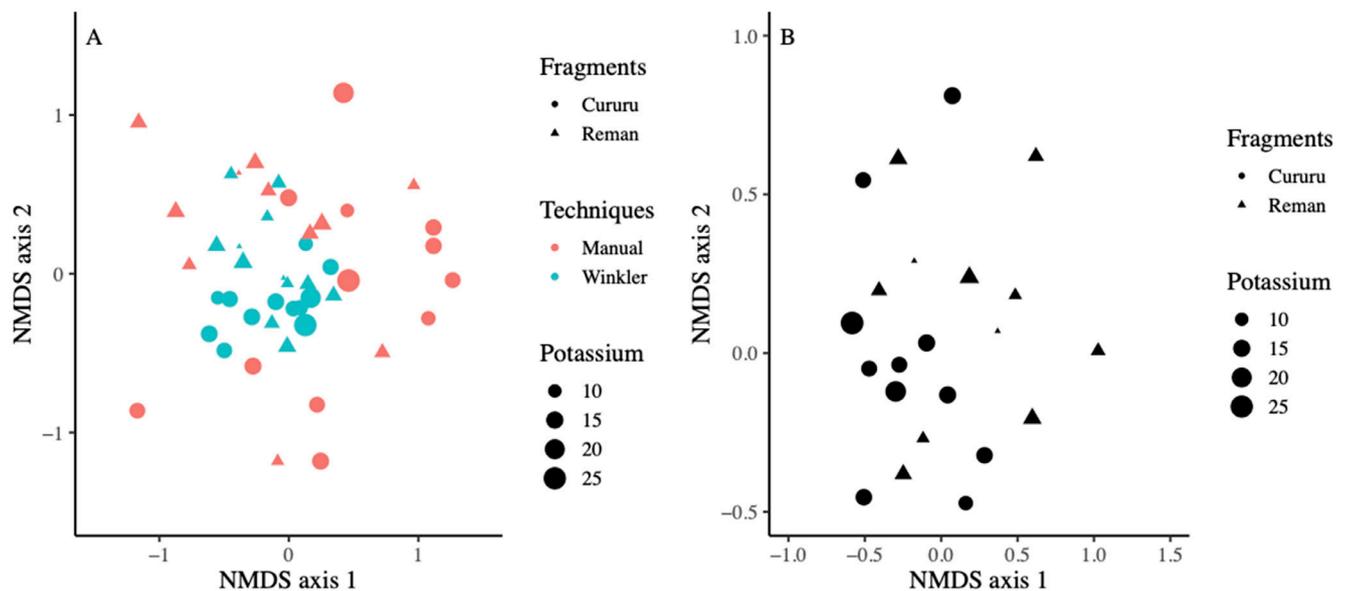


**Fig 3.** An NMDS ordination (Stress = 0.122) plot indicating the congruence in ant assemblages' associations among two urban forest fragments and the three sampling techniques in the city of Manaus, Amazonas, Brazil. Circular symbols indicate sampling points of the Cururu fragment and triangular ones from Reman. Different colored symbols indicate different sampling techniques.

Ants form a mega-diverse group with several species and high abundance. In the gradient along the Amazon River on the Amazon basin were recorded 42 genera and 166 species (Vasconcelos et al., 2010). A non-urban fragment of dense ombrophilous forest near the city of Manaus record 31 genera and 151 ant species (Vasconcelos et al., 2003). In sites of dense ombrophilous forests near Manaus, 51 genera and 219 species (Torres et al., 2020) and 54 genera and 237 species were recorded (Oliveira et al., 2009; Souza et al., 2012). Contrary to our hypothesis, the number of genera and ant species (42; 117 respectively) found in this work conducted in urban forest fragments, was very similar to the numbers found in studies carried out in ombrophilous forests in the Amazon basin with greater sampling effort. Ants are an example of successful arthropods in urban or fragmented landscapes (Menke et al., 2011).

The effects of urbanization are usually characterized as damaging to biodiversity (Vitousek et al., 1997; McIntyre et al., 2001; Yamaguchi, 2004; 2005), however, ecologically they can have positive effects, with the creation of new environments that do not occur anywhere else (Niemelä, 1999; Marshall & Shortle, 2005). This indicates that the ant's assemblages of the studied sites, although located in a forest fragment near to the oil refinery, and disconnected from larger areas of ombrophilous forest, harbor a relevant part of the genera and species already registered in the Amazon region.

The rarefaction curves of sampling techniques in both sites have similar trends and do not reach an asymptote. This is a recurrent fact in studies with ant's assemblages where most of them usually do not reach the asymptote regardless of the sampled environment or collection technique employed



**Fig 4.** A-An NMDS ordination (Stress = 0.167) plot indicating the congruence in ant assemblages' associations among two urban forest fragments and the two edaphic sampling techniques in the city of Manaus, Amazonas, Brazil. B- An NMDS ordination (Stress = 0.229) plot indicating the congruence in ant assemblages' associations among two urban forest fragments and the Winkler extractor technique in the city of Manaus, Amazonas, Brazil. Circular symbols indicate sampling points of the Cururu fragment and triangular ones from Reman. The size of the symbols indicates the magnitude of their value.

(e.g. King & Porter, 2005, Vasconcelos & Vilhena, 2006; Lopes & Vasconcelos, 2008; Souza et al., 2012). Although they have similar tendencies, the techniques have different efficiencies between and within each fragment. In Cururu, manual collection and Winkler have similar efficiencies, with Winkler collecting slightly more species and individuals. The beating was much less efficient both in species and in ant's abundance. At Reman, Winkler was distinctly the most efficient, collecting the most species. Manual collection and beating had similar efficiencies, sampling a resembling number of species, but differing in the total of individuals. The Winkler is an efficient method in forest environments, as it collects species neglected by other techniques (Bestelmeyer et al., 2000) and seems to maintain this efficiency in forest fragments. Manual collection can be as efficient as other techniques and is widely used in work in urban areas (Bestelmeyer et al., 2000; Munhae, 2017). However, it seems that this technique is more sensitive to environmental differences and that is why it had different efficacy between the fragments, which may prove to be a limiting factor in its use. The beating added a few species to the total, and this fact had already been reported in the literature (Majer & Delabie, 1994). Perhaps because of the three techniques used here, this is the least frequent in studies in the urban area (Munhae, 2017). Depending on the question to be answered and the financial and time cost of the survey, this increase in the sampling effort may render the survey unviable (Souza et al., 2012).

Some studies have already indicated the complementary effect of sampling methods on the study of ants (Olson, 1991; Delabie et al., 2000, Fisher, 1999) and this effect were also confirmed by some studies in the Amazon region (Souza et

al., 2007; Souza et al.; 2009). The successful and long-term conservation of a site depends on knowledge of its biological diversity, associated with efforts to protect and manage this biodiversity (National Research Council, 1992). The initial way of knowing the biodiversity of a given location or an ecosystem is to conduct a biodiversity survey (Scott et al., 1987; Gardner et al., 2008). In this study, the ant assemblages sampling through edaphic techniques (Winkler extractor and manual collecting) are more similar, forming a separate group from the group of species sampling by the beating. This shows that the sampling techniques are quite particular, and the use of combined techniques is indicated to better portray the diversity of the fragments area. However, it is possible to find some degree of redundancy using more than one sampling method (Parr & Chown, 2001; Lopes & Vasconcelos, 2008), and depending on the research objective, the use of a single sampling technique can be efficient and more economical (Souza et al., 2012).

Some studies have used ecological responses, based on environmental variables that determine the distribution of organisms, to evaluate the decision to reduce the number of sampling techniques (Souza et al., 2012) or the selection of higher-taxon (Souza et al., 2016). Contrary to our hypothesis, the ecological response has changed between the sampling techniques used. Only the Winkler was able to maintain an ecological response similar to that detected with the two sampling techniques together. This efficiency of Winkler in maintaining the ecological responses had already reported in other areas in the Amazon (Souza et al., 2012). Investigating ecological relations, in addition to the usual metrics of diversity (richness and composition), increase more information when

defining sampling protocols. Depending on the research objective, the choice of a sampling technique that not only collects more ant species, but that is also efficient in detecting environmental responses can prove to be useful, especially in the situation of environments where the creation of a protocol is still eminent.

Anthropically impacted environments, such as forest fragments near the oil refinery and Navy barracks, still maintain a high diversity of ants. The use of various sampling

techniques provided a better overview of the ant diversity in the study sites and indicated that the tree fauna is quite different from that associated with the litter-soil interface. The effect of environmental variables on the distribution of ants were small, but they suggest that there are some complex processes in the structuring of assemblages, even in places considered environmentally simpler. Its reinforces the importance of these forest fragments to maintain biodiversity in urban areas.

**Table 1.** Abundance and richness of ant species by each sampling technique in both studied sites in the city of Manaus, Amazonas, Brazil.

Subfamily / Taxon	Cururu			Total	Reman			Total	Grand Total
	Beating	Manual collecting	Winkler extractor		Beating	Manual collecting	Winkler extractor		
<b>Dolichoderinae</b>									
<i>Azteca</i> sp.01	1			1	2			2	3
<i>Azteca</i> sp.02	1			1	1			1	2
<i>Dolichoderus attelaboides</i> (Fabricius, 1775)					5			5	5
<i>Dolichoderus</i> sp.01		1		1			1	1	2
<i>Tapinoma</i> sp.01	2		4	6			10	10	16
<i>Tapinoma</i> sp.02			2	2					2
<b>Dorylinae</b>									
<i>Neocerapachys splendens</i> (Borgmeier, 1957)						3		3	3
<b>Ectatomminae</b>									
<i>Ectatomma brunneum</i> Smith, 1858	2	1	8	11			1	1	12
<i>Ectatomma edentatum</i> Roger, 1863		1	4	5					5
<i>Ectatomma lugens</i> Emery, 1894		1	1	2			1	1	3
<i>Ectatomma tuberculatum</i> (Olivier, 1792)			2	2					2
<i>Gnamptogenys tortuolosa</i> (Smith, F., 1858)			69	69		1		1	70
<i>Gnamptogenys</i> sp.02		8	16	24					24
<i>Gnamptogenys</i> sp.03		1		1					1
<b>Formicinae</b>									
<i>Brachymyrmex</i> sp.01	105	3	1	109	165		15	180	289
<i>Brachymyrmex</i> sp.03		1	7	8	3	3	1	7	15
<i>Camponotus atriceps</i> (Smith, 1858)	3			3	1			1	4
<i>Camponotus bidens</i> Mayr, 1870			4	4	1			1	5
<i>Camponotus latangulus</i> Roger, 1863	1			1	1			1	2
<i>Camponotus trapezoideus</i> Mayr, 1870	22			22	6			6	28
<i>Camponotus</i> sp.02					3			3	3
<i>Camponotus</i> sp.03		1		1					1
<i>Gigantiops destructor</i> (Fabricius, 1804)			3	3	1			1	4
<i>Nylanderia</i> sp.01			1	1			1	1	2
<i>Nylanderia</i> sp.02			1	1		3	5	8	9
<i>Nylanderia</i> sp.03		5	17	22					22
<i>Nylanderia</i> sp.04	31	7		38	167			167	205
<b>Myrmicinae</b>									
<i>Acanthognathus</i> sp.01			1	1					1
<i>Acromyrmex</i> sp 01			1	1					1
<i>Atta sexdens</i> (Linnaeus, 1758)	1		3	4					4
<i>Blepharidatta brasiliensis</i> Wheeler, 1915		196	193	389		1	19	20	409

**Table 1.** Abundance and richness of ant species by each sampling technique in both studied sites in the city of Manaus, Amazonas, Brazil. (Continuation)

Subfamily / Taxon	Cururu			Total	Reman			Total	Grand Total
	Beating	Manual collecting	Winkler extractor		Beating	Manual collecting	Winkler extractor		
<b>Myrmicinae</b>									
<i>Carebara</i> sp.01			4	4			1	1	5
<i>Cephalotes atratus</i> (Linnaeus, 1758)	2			2					2
<i>Cephalotes minutus</i> (Fabricius, 1804)					1	1	1	3	3
<i>Cephalotes opacus</i> Santschi, 1920		1		1	1		1	2	3
<i>Cephalotes pallens</i> (Klug, 1824)			1	1			1	1	2
<i>Cephalotes pinelii</i> (Guérin-Méneville, 1844)	1			1					1
<i>Crematogaster arcuata</i> Forel, 1899					6			6	6
<i>Crematogaster brasiliensis</i> Mayr, 1878	2			2	463		6	469	471
<i>Crematogaster curvispinosa</i> Mayr, 1862	1			1	16			16	17
<i>Crematogaster erecta</i> Mayr, 1866		2	5	7	25		1	26	33
<i>Crematogaster flavosensitiva</i> Longino, 2003						35		35	35
<i>Crematogaster foliocrypta</i> Longino, 2003							11	11	11
<i>Crematogaster limata</i> Smith, 1858	4	67	6	77	25		2	27	104
<i>Crematogaster sotobosque</i> Longino, 2003	6	4		10	11	84	69	164	174
<i>Crematogaster tenuicula</i> Forel, 1904	7	1		8					8
<i>Crematogaster</i> sp.01					1			1	1
<i>Cyphomyrmex</i> sp.01		67	13	80		2	15	17	97
<i>Megalomyrmex</i> sp.01		3		3		1		1	4
<i>Megalomyrmex</i> sp.02	2			2		1		1	3
<i>Mycocepurus smithii</i> (Forel, 1893)						1		1	1
<i>Myrmicocrypta</i> sp.01							2	2	2
<i>Ochetomyrmex semipolitus</i> Mayr, 1878	55	2	24	81	25		4	29	110
<i>Octostruma betschi</i> Perrault, 1988		3	3	6			2	2	8
<i>Octostruma iheringi</i> (Emery, 1888)		2		2					2
<i>Pheidole</i> sp.01		12		12			2	2	14
<i>Pheidole</i> sp.02		1		1					1
<i>Pheidole</i> sp.03		93	165	258	20	79	54	153	411
<i>Pheidole</i> sp.04		10	1	11					11
<i>Pheidole</i> sp.05					5			5	5
<i>Pheidole</i> sp.06					7			7	7
<i>Pheidole</i> sp.08		1	3	4		2	1	3	7
<i>Pheidole</i> sp.09		1	103	104	2	136		138	242
<i>Pheidole</i> sp.10					1			1	1
<i>Procryptocerus attenuatus</i> (Smith, 1876)	1			1					1
<i>Strumigenys appretiata</i> (Borgmeier, 1954)			6	6					6
<i>Strumigenys beebei</i> (Wheeler, 1915)			1	1					1
<i>Strumigenys cincinnata</i> (Kempf, 1975)		1		1					1
<i>Strumigenys denticulata</i> Mayr, 1887		7	117	124		1	30	31	155
<i>Strumigenys elongata</i> Roger, 1863		4	2	6					6
<i>Strumigenys infidelis</i> Santschi, 1919		1	2	3			2	2	5
<i>Strumigenys perparva</i> Brown, 1958			3	3					3
<i>Strumigenys stenotes</i> (Bolton, 2000)		1	3	4			3	3	7
<i>Strumigenys trinidadensis</i> Wheeler, 1922		1		1					1
<i>Strumigenys tridifera</i> Kempf & Brown, 1969			22	22			5	5	27

**Table 1.** Abundance and richness of ant species by each sampling technique in both studied sites in the city of Manaus, Amazonas, Brazil. (Continuation)

Subfamily / Taxon	Cururu			Total	Reman			Total	Grand Total
	Beating	Manual collecting	Winkler extractor		Beating	Manual collecting	Winkler extractor		
<b>Myrmicinae</b>									
<i>Rhopalothrix</i> sp.01			1	1					1
<i>Rogeria</i> sp.01	1		1	2		1		1	3
<i>Rogeria</i> sp.02		1		1					1
<i>Rogeria</i> sp.03	1			1	2			2	3
<i>Sericomyrmex</i> sp.01			1	1			1	1	2
<i>Solenopsis</i> sp.01		117	134	251	82	19	23	124	375
<i>Solenopsis</i> sp.02	13		52	65	9	2	12	23	88
<i>Solenopsis</i> sp.03		13	15	28		13	25	38	66
<i>Solenopsis</i> sp.04	2	2	9	13	1		1	2	15
<i>Solenopsis</i> sp.05			4	4					4
<i>Solenopsis</i> sp.06			5	5					5
<i>Solenopsis</i> sp.07	122	21		143		1		1	144
<i>Paratrachymyrmex</i> sp.01		1	2	3	1			1	4
<i>Paratrachymyrmex</i> sp.02			1	1					1
<i>Paratrachymyrmex</i> sp.03		1		1		3	1	4	5
<i>Paratrachymyrmex</i> sp.04			1	1					1
<i>Wasmannia auropunctata</i> (Roger, 1863)	162	53	163	378		11	74	85	463
<i>Wasmannia rochai</i> Forel, 1912		1		1					1
<b>Ponerinae</b>									
<i>Anochetus diegenis</i> Forel, 1912		2		2			1	1	3
<i>Anochetus emarginatus</i> (Fabricius, 1804)					63			63	63
<i>Anochetus horridus</i> Kempf, 1964		7	19	26		5	1	6	32
<i>Hypoponera</i> sp.01		71	80	151	1	5	16	22	173
<i>Hypoponera</i> sp.02		31		31		1		1	32
<i>Hypoponera</i> sp.03	1			1					1
<i>Leptogenys linearis</i> (Smith, 1858)						1		1	1
<i>Mayaponera constricta</i> (Mayr, 1884)		6	6	12					12
<i>Neoponera apicalis</i> (Latreille, 1802)					1			1	1
<i>Neoponera crenata</i> (Roger, 1861)	6			6					6
<i>Odontomachus haematodus</i> (Linnaeus, 1758)		3		3		3	1	4	7
<i>Odontomachus</i> sp.01			1	1					1
<i>Pachycondyla crassinoda</i> (Latreille, 1802)			1	1					1
<i>Pachycondyla harpax</i> (Fabricius, 1804)			1	1					1
<i>Pachycondyla</i> sp.01			12	12		8		8	20
<i>Thaumatomyrmex atrox</i> Weber, 1939		1		1					1
<b>Proceratiinae</b>									
<i>Discothyrea neotropica</i> Bruch, 1919		2	3	5			2	2	7
<b>Pseudomyrmicinae</b>									
<i>Pseudomyrmex flavidulus</i> (Smith, 1858)					2			2	2
<i>Pseudomyrmex</i> sp.02	1			1					1
<i>Pseudomyrmex</i> sp.03	1			1					1
<i>Pseudomyrmex</i> sp.04	2			2					2
<i>Pseudomyrmex</i> sp.05	1			1					1
Abundance	563	844	1334	2741	1127	427	425	1979	4720
Species richness	32	51	59		36	29	41		

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