



A comparative study between sampling methods for soil litter arthropods in conserved tree plots and banana crop plantations in Rwanda

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Abstract

The aim of this study was to compare trapping efficiency between Berlese-Tullgren funnels, pitfall traps and hand sorting sampling methods for soil litter arthropods. The study was carried out at the Arboretum of Ruhande and Rubona agricultural research station, in southern Rwanda. Biological indices indicated that pitfall traps collect a wide range of soil litter arthropod diversity, and chi-square test indicated the dependence between Berlese-Tullgren funnels and pitfall traps, and between pitfall traps and hand sorting. Z-test and univariate comparison indicated differences in means between tested sampling methods. The analysis of variance revealed that pitfall traps are less time consuming and the principal component analysis indicated that Formicidae is likely to be collected by pitfall traps and Berlese-Tullgren funnels, while Julidae, Porcellionidae and Geophilidae are likely to be collected by hand sorting. Research concluded that pitfall traps are more efficient than other studied sampling methods, but further studies should be conducted in other ecological zones, and different land uses in order to generate general information of these findings.

Keywords: Efficiency; Efficient; Meantime; Diversity; Evenness

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1. Introduction

The phylum Arthropoda is the largest in the animal kingdom and it includes more than one million species distributed in almost all habitats (Duelli et al., 1999). Soil litter arthropods control the stability and functioning of soil ecosystems (Bagyaraj et al., 2016), and participate in soil nutrient cycling through litter feeding and mineralization of nutrients, and contribute to the formation of soil structures through soil mixing, development of soil pores and formation of soil aggregates (Culliney, 2013). Soil litter arthropods are also ecosystem engineers that physically regulate the availability of resources for bacteria and fungi (Jones et al., 1994), thus minerals and nutrients of dead organisms become readily available in the soil for plant uptake (FAO, 2013).

Soil litter arthropods are frequently studied to understand their distribution for pest control, conservation purposes, understanding of the population dynamics, and to make predictions of future changes in abundance and diversity (Woodcock, 2005). In agricultural and forest systems, arthropods are studied in order to understand their economic benefits through pollination, seed dispersal (Isaac et al., 2009), predation (Wilson, 2005), and in the assessment of soil quality, soil health and environmental changes (Pankhurst et al., 1997). Recently, there is an increasing interest in studies by using arthropods, particularly insects in forensic and medical sciences (Bonebrake et al., 2010).

The species of soil litter arthropods captured during sampling is dependent on the sampling methods that were used (Ferrer-Paris et al., 2013), which are classified as either passive or active (Gullan and Cranston, 2005). The difference between active and passive methods is based on the intervention of the collector and the implication of the trap used (Yi et al., 2012). Simply, passive sampling methods are neutral and depend entirely on chance, while active sampling methods depend on the behavior of the targeted taxa and take advantages of the behavior and attractions either by chemicals, baits or colors (Yi et al., 2012). Berlese-Tullgren funnels and pitfall traps are passive sampling methods, while hand sorting is an active sampling method (Tuf and Tvardik, 2003).

Each of these sampling methods has constraints to trapping efficiency. Hand sorting is appreciated to be suitable for sampling large and abundant soil litter arthropods, but it is more laborious and time-consuming (Tuf, 2015). Pitfall traps are time efficient in sampling ground-dwelling arthropods (Smith et al., 2008), while Berlese-Tullgren funnels are suitable for sampling soil and leaf litter microarthropods (Southwood and Henderson, 1997). Compared to pitfall traps and hand sorting, researchers indicated that Berlese-Tullgren funnels are easy to use and less time consuming (Basset et al., 1997), but soil samples have to be processed quickly to avoid mortality of specimens (Yi et al., 2012).

Few studies have been done to critically evaluate criticisms, and compare the trapping efficiency between Berlese-Tullgren funnels, pitfall traps and hand sorting sampling methods (Krell et al., 2005). The best approach to collect a wide range of soil litter arthropods remains a topic of interest (Yi et al., 2012), and the capture effectiveness of these sampling methods need to be studied (Sabu and Shiju, 2010) in order to solve other questions such as the knowledge of the taxa that are most likely collected by each sampling method and the taxa that are best collected by specific sampling methods between Berlese-Tullgren funnels, pitfall traps or hand sorting, as well as the mean time required for each sampling method (Sabu et al., 2012).

The goal of this study is to compare the trapping efficiency between hand sorting, pitfall traps and Berlese-Tullgren funnels sampling methods for collecting a wide range and diversity of soil litter arthropods. The research presented here aims at determining the species diversity of soil litter arthropods from each sampling method, testing the trapping differences with which particular soil litter arthropods taxa were collected, assess the trap-wise differences in the capture efficiency of individual taxa per each sampling method, and determine the mean time required for trap fixation, trap collection, and extraction of specimens in traps for each sampling method.

2. Material and sampling methods

This research was conducted in the Arboretum of Ruhande and Rubona agricultural research station, in southern Rwanda. The arboretum of Ruhande is located at 2°36' South and 29°44' East with a maximum elevation of 1737 meters (Nsabimana et al., 2009). The surface area is approximately 200 hectares, divided into 504 plots of 50m x 50m each, and with 207 native and exotic trees species (Nsabimana et al., 2008). Rubona agricultural research station is located between 2°35' South and 29°43' East, at 1734 meters elevation (Nabahungu et al., 2011). The station covers a surface area of around 675 hectares, dominated by tree plantations, a woodland zone dominated by *Hyparrhenia* and *Acacia* species, and agricultural research zone dominated by leguminous species, cereals, tubers, banana, coffee, and fruit plantations (ISARA, 1989).

2.1. Data collection

Data on soil litter arthropods were collected three times separated by two weeks in-between, in March and April 2017, using Berlese-Tullgren funnels, pitfall traps, and hand sorting. At the Arboretum of Ruhande, data were collected in plots of *Eucalyptus maideni*, *Polyscias fulva*, *Cedrella serata* and *Grevillea robusta*, while at Rubona agricultural research station soil litter arthropod samples were collected in plots of four banana plantation varieties including *Mporogoma*, *Injagi*, *FHIA17*, and *FHIA25*. Three sampling points were selected randomly in each plot, and separated by at least six meters from another, by living five meters from the edge (Nsabimana, 2013). Each of the sampling points during the second and third sampling exercises was located at two meters ahead of the first sampling point to avoid the over sampling in the same sampling point (Sabu and Shiju, 2010). The time used for the trap fixation, trap collection, and extraction of specimens in the trap was recorded for each sampling method with a stopwatch.

2.1.1. Data collection by pitfall traps

Three pitfall traps placed randomly in each sampling site were used to collect soil litter arthropods. Each pitfall trap consisted of a transparent plastic bottle (6cm diameter, 10cm depth), buried in 20x20cm soil up to its rim and partly filled with 20ml of 75% ethanol after the removal of the leaf litter layer. Each trap was covered with cardboard fixed on nails in order to prevent the entry of rainwater, falling leaves, and debris which may facilitate trapped fauna to escape (Sabu and Shiju, 2010). Each trap was maintained for 24 hours

in order to avoid biases in captures which could arise from diurnal activities of fauna (Mommertz et al., 1996). The content of each trap was emptied into sterile plastic bottles filled with 20ml of 75% ethanol, and analyzed separately from others (Wang et al., 2014).

2.1.2. Data collection by hand sorting

Three sampling points selected randomly in each sampling site were sampled during this study by the use of the hand sorting sampling method. Soil litter arthropods were collected by using a meter square pick-up point sampling method (Mc Gavin, 2007), in five centimeter soil depth after the removal of the leaf litter layer (Sayad et al., 2012). Targeted soil litter arthropods were pulled out the soil with 11cm sharp-pointed forceps and fingers (Martin, 1997). Each collected individual arthropod was conserved in a sterile plastic bottle filled with 20ml of 75% ethanol. Each bottle was stored in laboratory, and analyzed separately from others (Wang et al., 2014).

2.1.3. Data collection by Berlese-Tullgren funnels

Three core soil samples (10cm x 10cm, 0 - 5cm depth) were taken randomly in each sampling site and bulked to give one representative sample, after the removal of the leaf litter layer and taken to the laboratory for the extraction of soil litter arthropods (Sakchoowong et al., 2008). Each representative soil sample was heated in Berlese-Tullgren funnels by a 60-watt bulb placed 10cm above the funnel for a period of 24 hours. The bottom of the apparatus was filled with 20ml 75% ethanol and catches biota as they drop from the funnel (Moço et al., 2010). Collected arthropods were conserved in a sterile container, and analyzed separately from others (Wang et al., 2014).

2.2. Data analysis

Samples of soil litter arthropods collected by each sampling method were taken to the laboratory for identification and classification to the family level using dichotomous keys in the literature (Mignon et al., 2016; Delvare and Aberlenc, 1989). Percentages, diversity and evenness indices were calculated to determine the abundance, diversity, and evenness of collected soil litter arthropods captured with each sampling method and to determine similarities or differences in Berlese-Tullgren funnels, pitfall traps and hand sorting sampling methods. Shannon diversity index (H') was used to evaluate the diversity (Shannon and Wiener, 1946), Pielou's evenness index (P') was used to calculate the evenness (Pielou, 1996), and the percentage of similarity (PS) was used to calculate the level of similarity between studied sampling methods (Henk, 1981).

The chi-square test was used to test for differences in the frequency with which particular soil litter arthropod taxa were collected by the three sampling methods (Sabu et al., 2012). The effect of sampling method on the proportion of arthropods captured was evaluated based on the significance of the chi-square test (Parasifka et al., 2007). Z-tests were used to assess the trap-wise differences in the capture efficiency of individual taxa among three sampling methods, while the univariate comparison was used to evaluate the

significance level of differences among medians. When significant differences were found, the honestly significant test was used to determine which pairs of sampling methods differed significantly (Weiss, 2007).

A principal component analysis (PCA) was used to analyze the level of variability between studied sampling methods and the abundance of collected soil litter arthropods, and to determine soil litter arthropods taxa that are likely to be collected by each studied sampling method (Quin and Keough, 2003). The mean time required for sampling soil litter arthropods using Berlese-Tullgren funnels, pitfall traps and hand sorting sampling methods was calculated focusing on the time needed for trap fixation, trap collection, and extraction of specimens in the trap for each sampling method. All statistical analyses were done by the use of SPSS and XL STAT software.

3. Results

A total of 1768 species of soil litter arthropods distributed in five classes, eleven orders and fifteen families were collected. Classes with the highest number of individual species were Insecta (49.3%), Diplopoda (32.9%), Chilopoda (9.8%), and Crustacea (7.9%). The class Arachnida had the lowest number of individual species (1.9%). The order Hymenoptera was abundant (38.9%) followed by Julida (25.3%), Coleoptera (9.5%), Isopoda (7.3%), Geophilida (6.6%), Isoptera (4.6%), Orthoptera (2.6%), Araneae (1.9%), Scolopendrida (1.1%), and Blattodea (1.7%). The families Formicidae (39.0%), Julidae (25.3%), and Porcellionidae (7.4%) were abundant compared to other identified families (Table 1).

Variations in abundance of collected soil litter arthropods were observed within each land use (Table 2), where banana crop plantations had higher abundance (58.9%) of collected soil litter arthropods than conserved tree plantations (41.2%). Higher abundance was found in *Mporogoma* (17.2%) and *FHIA17* (16.8%) banana varieties, while higher abundance in conserved tree plantations was found in *Grevillea robusta* (12.9%) and *Cedrella serata* (12.6%). Lower abundance was found in *Eucalyptus maideni* (5.7%) conserved trees and in *FHIA25* (11.9%) banana plantation.

Variations were also observed for each sampling method. The class Insecta was the most common class collected in pitfall traps (30.0%) and Berlese-Tullgren funnels (8.9%). The order Hymenoptera (Formicidae) comprised dominant species collected by pitfall traps (22.4%) and also by Berlese-Tullgren funnels (6.3%). The most abundant class collected by hand sorting was Diplopoda (24.5%) and Julida (18.2%). Collembola, (0.8%) were collected by pitfall traps and not found in Berlese-Tullgren funnels and hand sorting (Table 1 and Table 2).

Results of diversity and abundance of collected soil litter arthropods indicated less diversity and evenness for Berlese-Tullgren funnels ($H' = 0.48$, $P' = 0.15$), and hand sorting ($H' = 1.22$, $P' = 0.163$) sampling methods. A higher diversity was found for pitfall traps ($H' = 1.37$, $P' = 0.18$). Higher percentage of similarity were found between hand sorting and pitfall traps (PS=21.2%), while less percentage of similarity was found between Berlese-Tullgren funnels and pitfall traps (PS=12.6%), and between Berlese-Tullgren funnels and hand sorting (PS=12.2%).

Table 1. Abundance (%) of arthropod families obtained by studied sampling methods

Family	Berlese-Tullgren funnels		Pitfall traps		Hand Sorting		Total	%
	Number of Species	%	Number of Species	%	S Number of Species	%		
Araneidae	1	0.1	16	9.6	17	1.0	34	1.9
Blatteridae	0	-	13	0.7	17	1.0	30	1.7
Chrysomelidae	0	-	32	1.8	0	-	32	1.8
Staphylinidae	0	-	22	1.2	17	1.0	39	2.2
Tenebrionidae	9	0.5	28	1.6	61	3.5	98	5.5
Formicidae	112	6.3	396	22.4	181	10.2	689	39.0
Rhinotermitidae	10	0.6	0	-	0	-	10	0.6
Termitidae	25	1.4	39	2.2	8	0.5	72	4.1
Acrididae	0	-	2	0.1	2	0.1	4	0.2
Grillidae	2	0.1	24	1.4	16	0.9	42	2.4
Geophilidae	5	0.3	23	1.3	78	4.4	106	6.0
Porcellionidae	10	0.6	9	0.5	111	6.3	130	7.4
Julidae	65	3.7	60	3.4	323	18.3	448	25.3
Sclopendridae	2	0.1	0	-	18	1.0	20	1.1
Isotomidae	0	-	14	0.8	0	-	14	0.8
Total	241	13.6	678	38.3	849	48.0	1,768	100

Statistical analysis indicated the independence between Berlese-Tullgren funnels and hand sorting (chi-square=92.8, df=72, P=0.046, $\alpha=0.05$), while the dependence was found between Berlese-Tullgren funnels and pitfall traps (chi-square=110.8, df=88, P=0.104, $\alpha=0.05$), and between pitfall traps and hand sorting (chi-square=123.2, df=99, P=0.175, $\alpha=0.05$) sampling methods. The assessment of the trap-wise differences in capture efficiencies of individual species through Z-test indicated differences between means for Berlese-Tullgren funnels and hand sorting (P=0.046, $\alpha=0.05$), Berlese-Tullgren funnels and pitfall traps (P=0.038, $\alpha=0.05$), and between pitfall traps and hand sorting (P=0.010, $\alpha=0.05$).

Table 2. Abundance of soil litter arthropods by land use and by sampling method

Land use	Berlese-Tullgren funnels		Pitfall traps		Hand Sorting		Total	%
	Number of Species	%	Number of Species	%	Number of Species	%		
	<i>Polyscias fulva</i>	19	1.1	45	2.5	113		
Mporogoma	35	2.0	117	6.6	152	8.6	304	17.2
Injagi	38	2.1	107	6.1	84	4.8	229	13.0
<i>Grevillea robusta</i>	23	1.3	58	3.3	147	8.3	228	12.9
FHIA25	27	1.5	91	5.1	93	5.3	211	11.9
FHIA17	58	3.3	99	5.6	140	7.9	297	16.8
<i>Eucalyptus maideni</i>	7	0.4	53	0.3	40	2.3	100	5.7
<i>Cedrella serata</i>	34	1.9	108	6.1	80	4.5	222	12.6
Total	241	13.6	678	38.3	849	48.0	1768	100

Honestly significant tests to verify if pairs of sampling methods differ significantly indicated that there is no difference between the pairs of pitfall traps and hand sorting ($P=0.87$, $\alpha=0.05$), and between Berlese-Tullgren funnels and pitfall traps ($P=0.06$, $\alpha=0.05$). Significance differences were observed between Berlese-Tullgren funnels and hand sorting ($P=0.01$, $\alpha=0.05$). The test for similarity between tested sampling methods indicated positive Pearson's correlation between hand sorting and Berlese-Tullgren funnels ($\rho=0.76$), pitfall traps and hand sorting ($\rho=0.40$), and between Berlese-Tullgren funnels and pitfall traps ($\rho=0.81$).

Variations in time needed for each sampling method were observed for each step of the sampling process, where trap fixation required less time (2.65 ± 2.3) than trap collection (3.83 ± 3.2), and extraction of specimens in traps (7.58 ± 11.2). Berlese-Tullgren funnels required less time for the fixation of traps (3.13 ± 1.1) than pitfall traps (4.81 ± 1.6), but they required more time for a collection of traps (6.44 ± 2.9) than pitfall traps (4.63 ± 1.2). These differences qualify pitfall traps to be less time consuming (9.44 ± 2.8) than Berlese-Tullgren funnels (9.57 ± 3.9).

Hand sorting does not require time for trap fixation and trap collection, however, it requires more time for the collection of specimens from the soil (22.75 ± 5.2). This sampling method was more time consuming than Berlese-Tullgren funnels and pitfall traps. The analysis of variance indicated trap-wise differences in the length of time needed for trap fixation ($F_{2,45}=73.7$, $p=0.00$, $\alpha=0.05$), trap collection ($F_{2,45}=35.2$, $p=0.00$, $\alpha=0.05$), and for extraction of specimens ($F_{2,45}=309.7$, $p=0.00$, $\alpha=0.05$). The increase in the time for extraction of specimens in traps is due to the time spent for hand sorting.

The Principal Component Analysis (PCA) with which soil litter arthropod taxa were most likely collected by each sampling method indicated a high percentage of variability (99.7%). Eigen values equal 47.0 for PCA 1 (75.1%) and 15.4 for PCA 2 (24.6%). A biplot PCA (Figure 1) indicates that the families of Julidae, Porcellionidae, and Geophilidae loaded positively in PCA 2, and are most likely collected by hand collection, while the family of Formicidae loaded positively in PCA 1 and is most likely collected by pitfall traps and Berlese-Tullgren funnels (Figure 2).

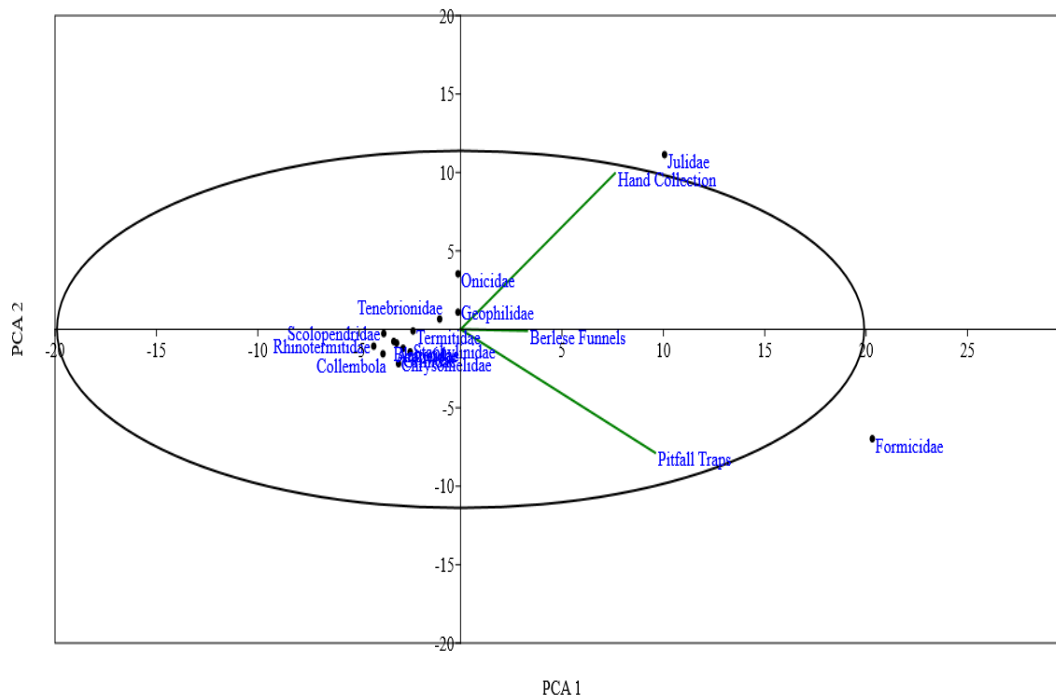


Figure 1. Principal Component Analysis between hand sorting, Berlese - Tullgren funnels and pitfall traps sampling methods based on the abundance of collected soil litter arthropods

4. Discussion

Results indicated that Berlese-Tullgren funnels collected less number as well as less diversity of soil litter arthropods compared to pitfall traps and hand sorting. Lower occurrence and less diversity of soil litter arthropods collected by Berlese-Tullgren funnels has been observed in other studies and could be caused by the heat from the apparatus, especially when specimens are collected from the moist area (Bestelmeyer et al., 2000). Because soil litter arthropods have been collected during the rain period, some of the soil litter arthropods, especially those of small size were likely to die by desiccation before dropping into the collecting jar (Sabu et al., 2012).

Despite high differences in means between independent and paired samples, positive correlations between hand sorting, pitfall traps, and Berlese-Tullgren funnels may suggest that when different sampling methods are paired, they can yield good results and collect a wide range of soil litter arthropods. A combination of different sampling methods has been highly recommended in other studies, especially when focusing on specific taxa (Yi et al., 2012). Pairing hand sorting with litter sifting has been shown to yield good results for sampling centipedes (Sabu and Shiju, 2010), and pairing pitfall traps with leaf litter collection yielded good results for sampling ground-dwelling carabid beetles with small size (Olson, 1994), while pitfall and stocking traps were effective in sampling Elateridae commonly known as Wireworms (Morales-Rodriguez et al., 2017).

The highest number and lower diversity of soil litter arthropod species collected by hand sorting might be due to the biases of this sampling method where observed and targeted arthropod species are collected, especially when they are big in size and abundant in the area of study (Woodcock, 2005). Similar findings were observed in savannah habitats (Druce et al., 2014), and in native forests, where hand sorting collected a large number of species in a high abundant species (Gaspar et al., 2014). As found in this study, hand sorting has been criticized for being time-consuming (Tuf, 2015). Another disadvantage of hand sorting is that, variations in individual skills and experience in sampling create differences in sampling efficiencies that affect the results (Berthold et al., 1999).

Even though hand sorting sampling method has several disadvantages, other studies have identified benefits of this sampling method, including targeted extraction of soil litter arthropods, minimum disturbance to the habitat and shorter sampling periods for targeted taxa, as well as reduction of unnecessary mortality of unwanted invertebrates (Smith et al., 2008). In addition, field workers may gain a better understanding of the environmental factors influencing soil assemblages through direct observations of correlations between changes in soil texture or moisture and invertebrate abundances; so that such observations may inform future data collection or help develop new hypotheses (Smith et al., 2008).

Suitability of Berlese-Tullgren funnels and pitfall traps for collecting individual species of the family of Formicidae has been documented in other studies where these sampling methods were efficient for sampling the majority of litter and soil dwelling arthropods (Paoletti et al., 1991). However, differences have been observed in other studies, where pitfall traps yielded good results for sampling soil litter ants (Peck et al., 1999), while Berlese-Tullgren funnels collect a large number of the larvae of dipterans due to accelerated hatching of eggs laid by flies due to the lamps' light, allowing the larvae to emerge during extraction period (Smith et al., 2008). This was not the case for this study because funnels were covered during extraction to prevent such contamination.

Pitfall traps collected a higher diversity of soil litter arthropods dominated by the class Insecta (Hymenoptera: Formicidae). Efficacy of this sampling method for Formicidae has been documented in other research (Osbrink et al., 2017). High diversity and large numbers of soil arthropod groups including Scorpionida, Isopoda, Diplopoda, Chilopoda, Symphyla, Araneae, Acari, Collembola, Coleoptera, and Formicidae have been collected by this sampling method in other studies (Frank et al., 2012; Skavarla et al., 2014), and this method is recognized for its trapping efficiency (Spence and Niemelä, 1994). Other studies indicated that pitfall traps can have different designs in terms of materials used and in size (Jud and Schmidt-Entling, 2008), so that they are suitable for studying the occurrence and relative abundance of litter and soil dwelling arthropods of different sizes (Phillips and Cob, 2005; Buchholz et al., 2010), and can contribute to the collection of nocturnal soil litter arthropod species, and hence reduce biases (Work et al., 2002).

5. Conclusion and recommendations

Results obtained from this study illustrate that pitfall traps and Berlese-Tullgren funnels are suitable sampling methods for soil litter arthropods dominated by Formicidae. Hand sorting sampling method was

suitable for sampling soil litter arthropods with a large size dominated by Julidae, Porcellionidae and Geophilidae. Pitfall traps showed greater efficiency in terms of collecting a higher diversity of soil litter arthropods, and meantime used for trap fixation, trap collection, and extraction of soil litter arthropod specimens, as well as higher percentage of similarity with hand sorting. Further studies comparing the trapping efficiency between Berlese-Tullgren funnels, pitfall traps and hand sorting sampling methods for soil litter arthropods in other ecological zones, different land uses and different seasons have to be conducted to better understanding differences among these sampling methods.

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