

# Quantifying Variation of Soil Arthropods Using Different Sampling Protocols: Is Diversity Affected?

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## 1. Introduction

In ecological studies, the use of different sampling methods for the same purpose influence data quality and thus the resulting conclusions (Coddington *et al.* 1996; Fisher 1999). For example, to collect arthropods from soil and litter samples a soil corer or a shovel may be used. Soil corers compact the soil (Meyer 1996) making difficult for organisms to leave the sample while shovels create a large disturbance (Longino *et al.* 2002) promoting mobile organisms to leave and reducing their apparent abundance in the sample. As a consequence the diversity of collected arthropods will vary between these two procedures, resulting in either an under- or overestimate of the diversity of the collected fauna (André *et al.* 2002). These different results will lead the researcher to infer different conclusions. Therefore it is essential to assess how different procedures affect the abundance, richness and species composition of the retrieved arthropods.

Arthropods are usually retrieved from soil/litter samples with Berlese-Tullgren funnels (Walter *et al.* 1987; Rohitha 1992; MacFadyen 1961; Bremner 1990; Lakly & Crossley 2000; MacFadyen 1953; Haarlov 1947). In these funnels, a source of heat (i.e. a light bulb) is placed above the sample, and a collecting vial filled with a killing solution (e.g. 70% ethanol) is placed below the sample. Light from the bulb has a double effect because light per se forces photophobic organisms to move away from the source, and light heats the sample. As the sample dries, a temperature and humidity gradient is created between the upper and lower surfaces of the sample (Haarlov 1947; Block 1966). As this gradient moves downwards, animals are forced down into the collecting liquid (Coleman *et al.* 2004). By increasing the temperature within the funnel, heat speeds drying (Coleman *et al.* 2004) but may also burn organisms before their collection and thus decreases estimates of their abundance (Walter *et al.* 1987). Alternatively, in remote field conditions, extractions without light are logistically more affordable and feasible, in which case the establishment of the gradient and the drying out of the sample depends on the room temperature in which the extractions are performed (Krell *et al.* 2005). Both, extractions with and without light, create different conditions within the sample, as a consequence, the use, or no use, of light during extractions, can result in

different groups of arthropods being extracted, and thus a different set of data (Agosti *et al.* 2000).

The duration of arthropod extraction can also affect diversity estimates. Extraction periods reported in the literature vary from 2 d (Burgess *et al.* 1999), 3 d (Hasegawa 1997), to 4 d (Oliver & Beattie 1996; Bestelmeyer *et al.* 2000) and up to 7 d (Chen & Wise 1999; Walter *et al.* 1987). Long extraction periods are generally assumed to result in more complete extractions and higher abundance of the extracted fauna (Oliver & Beattie 1996) as organisms with low mobility require more time to exit the sample, but longer extraction periods may expose the samples to potential contamination with foreign organisms. On the other hand, to establish an adequate period of extraction, the environment of origin and the developmental stage should be taken into account (André *et al.* 2002). For example, organisms adapted to extreme environments, such as areas devoid of vegetation cover that have large temperature fluctuations, may require longer extraction periods than organisms adapted to less extreme environments. Furthermore, organisms from the same habitat but occurring in the dry or wet seasons (Oliver & Beattie 1996) or different developmental stages (Søvik & Leinaas 2002) may differ in the extraction period required to retrieve them. As a consequence, in order to collect reliable data, it is necessary to assess how an adequate duration of the extraction varies among environments of origin and developmental stages of the focal organism.

The present study was carried out in the Caribbean island of Puerto Rico, specifically in tropical dry and wet forests with contrasting environmental conditions (Ewel & Whitmore 1973). The objective of this study was to assess how the diversity of extracted arthropods was affected by variations in the collection and extraction methodologies, and by variations in the duration of the extraction. We present abundance, richness and composition of the collected fauna. The information presented here will provide researchers with data to simplify the logistics of arthropod sampling and extraction, and to better choose a specific procedure for a given focal organism in a given habitat.

## 2. Materials and methods

### 2.1 Study site

This study was carried out in north-eastern Puerto Rico in two forests of contrasting conditions. Samples from the litter and soil horizon (0-5 cm) were obtained in March 2003 from a wet forest site at the El Verde Field Station (Luquillo Experimental Forest, 18.33080, -65.82320, WGS 84), and from a dry forest site in the former Roosevelt Roads Military Base (Ceiba, 18.24800, -65.63290, WGS 84).

The wet forest site is located in the Luquillo Experimental Forest, where mean monthly temperature ranges from 23.5°C in January to 27°C in September (<http://www.lternet.edu/sites/luq/fulldescription.php?site=LUQ>), and total annual precipitation is 3524 mm yr<sup>-1</sup> (García-Martínó *et al.* 1996) with a mild dry season from January to April (Schowalter & Ganio 1999). Soils are highly weathered; soil nutrients are 0.49% S, 0.35% N, 4.92% C, 0.30 P mg/g soil, and the C/N ratio is 14.2 (Gould *et al.* 2006); humus accumulation is low because there is rapid decomposition. Vegetation at the site is described as closed evergreen broad leaf forest that lays within the subtropical wet forest Holdridge life zone (Gould *et al.* 2006). The forest is dominated by *Dacryodes excelsa* and *Manilkara bidentata* (Schowalter & Ganio 1999; Gould *et al.* 2006).

The dry forest site is located within the former Roosevelt Roads Military Base, where mean monthly temperature is 27.5°C and annual precipitation is 1,262 mm yr<sup>-1</sup> (Gould *et al.* 2006). It has a pronounced dry season that runs from November to April, and a wet season that usually runs from May to October (<http://www.ceducapr.com/ceiba.htm>). The soils are sandy or clayey with a developed organic matter (<http://www.ceducapr.com/ceiba.htm>). Soil nutrients are 0.06% S, 0.61% N, 6.34% C, 0.48 P mg/g soil, and the C/N ratio is 10.4 (Gould *et al.* 2006). This is a closed, mixed-evergreen deciduous, broad leaf forest that lays within the subtropical dry forest Holdridge life zone (Gould *et al.* 2006). This forest is dominated by *Bucida buceras* and *Guapira fragrans* (Gould *et al.* 2006).

In summary, these forests present contrasting conditions because, the wet forest has lower temperature and higher precipitation than the dry forest. In addition, the dry forest has a pronounced dry season while in the wet forest; the dry season is measured as number of days with no effective rain. The organic horizon is thin in the wet forest, and thick in the dry forest. As a consequence, the wet forest is warm and humid with thin litter and almost no humus, while the dry forest is hot and dry with deep litter and humus.

## 2.2 Data collection

In each forest, a 50 m x 50 m area was located, and within this area 40 litter samples were collected. Each sample was 100-cm<sup>2</sup> (10 cm x 10 cm), and was collected down to mineral soil. Litter depth was measured three times inside each of the 100-cm<sup>2</sup> areas. Inside the same 100-cm<sup>2</sup> area and after collecting the litter samples, two soil samples were collected: one using a soil corer (4.3 cm diameter and 5 cm height) and another one using shovels. For the shovel sampling, soil was collected with a shovel and served into a corer to assure that the soil volume in the shovelled sample was similar to that obtained with the soil corer. This sampling design resulted in 40 litter samples, 40 soil shovelled samples and 40 soil cored samples from each forest, giving a total of 120 samples in each forest type.

In the laboratory, the litter and soil shovelled samples were each placed in small Berlese-Tullgren funnels (Bioquip 2845) (10 cm height and 11 cm diameter) (Fig. 1A & 1B). The soil corer samples were placed in hand made funnels. For this, a wooden skeleton was built with basal holes covered with a mesh (Fig. 1C). Over each hole, a corer was placed and covered with a metallic funnel (11 cm x 5 cm) dia. The metallic funnels were then covered with a wooden ceiling (Fig. 1D). All funnels had the ceiling with an opening.

Samples were randomly assigned to two treatments in which extraction was done with or without light. For this, the 40 litter samples were split into two groups: 20 samples were extracted with light and 20 samples were extracted without light. The 40 soil shovelled samples and the 40 soil cored samples were similarly randomly assigned to one of these extraction treatments. When extraction was with light, a 20V-bulb was hanging through the funnel's opening, and was kept at maximum intensity during all the extraction period to control for the effect of changing light intensity during extractions. When extraction was without light, no bulb was placed over the funnel. All samples were located simultaneously in the same room where temperature and humidity were controlled.

Vials containing the killing solution (ethanol 70%) and collected arthropods were retrieved at 24, 48, 72, 144 and 168 hours after placement in the funnels. Thus each of the 120 samples

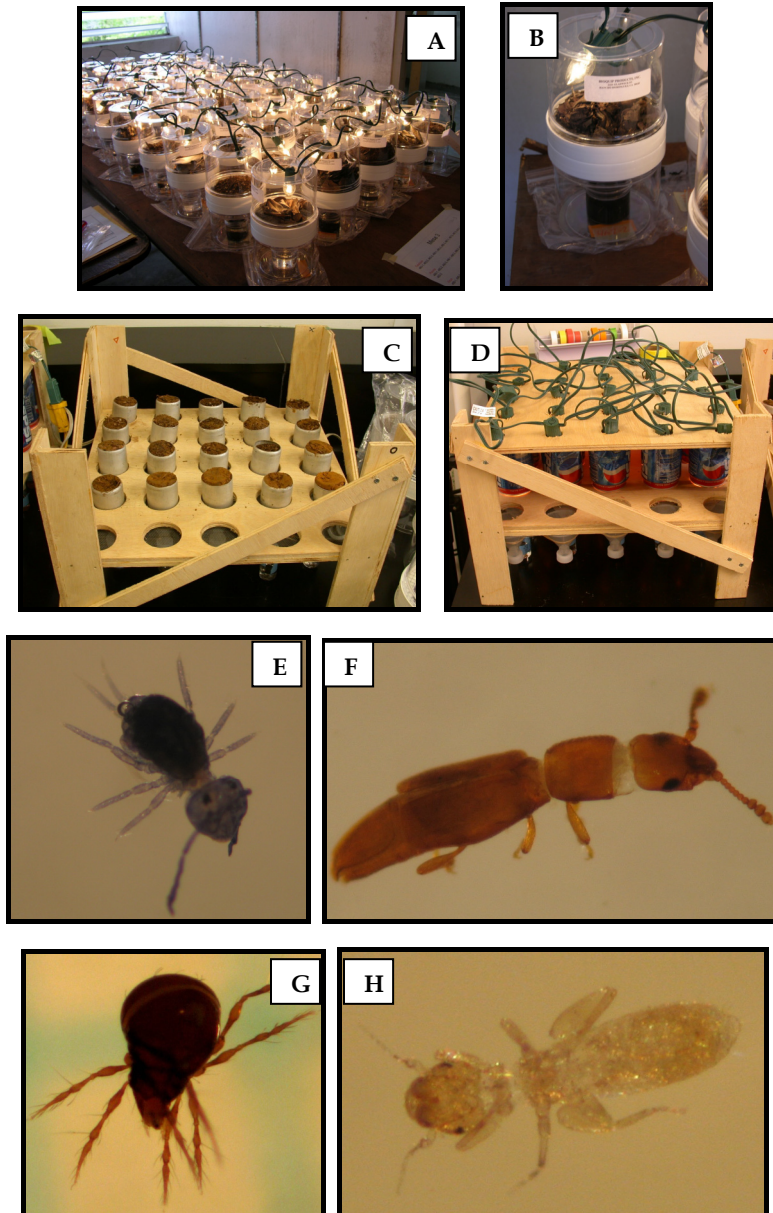


Fig. 1. A and B: Berlese-Tullgren funnels (Bioquip 2845) used for the litter and shovelled soil samples. (Photos A and B provided by M. F. Barberena-Arias, Universidad del Turabo). C and D: wooden skeleton used for the cored soil samples. E through H: examples of collected arthropods. E: *Collembola Sminthurida*, F: *Coleoptera Corylophidae*, G: *Acari Oribatida*, H: *Psocoptera*. (Photos C through H provided by G. González, Soil Ecology Program, IITF-US Forest Service).

per forest was retrieved at five sequential times giving a total of 600 samples per forest that were processed separately. For each sample, arthropod abundance was recorded, and arthropods were identified to the lowest category possible such as class, subclass, order or suborder, and classified as adult or immature (MacAlpine 1989; Triplehorn & Johnson 2004; Krantz 1978) (Fig. 1E through 1H). Collembola were not separated as adults or immature because it is difficult to differentiate among developmental stages.

All litter and soil samples were weighed before extraction, and after extraction all samples were oven dried at 65°C for a week and then weighed again. During the experiment, sample temperature and humidity were not measured but from reviewing literature, we assumed that the use of a light bulb during extractions increased sample temperature and dried the sample resulting in a gradient of temperature and humidity within the sample, while in the extractions without light, the establishment of the gradient would depend on the temperature and humidity of the room where extractions were performed (MacFadyen 1953; MacFadyen 1961; Haarlov 1947; Søvik & Leinaas 2002; Block 1966).

### 2.3 Data analyses

Data analyses were done using SigmaStat 3.0 (Systat Software Inc., [www.sigmaplot.com](http://www.sigmaplot.com)) and PCORD 4.0 (PC ORD - Multivariate Analysis of Ecological Data, [home.centurytel.net/~mjm/pcordwin.htm](http://home.centurytel.net/~mjm/pcordwin.htm)). Litter depth, and litter and soil dry weights were compared between forests using a Mann Whitney Rank Sum test (Sokal & Rohlf 1994). Arthropod abundance was standardized to individuals per square meter. Two way ANOVAs were used to establish the effect of light and duration of the extraction (time) on litter arthropod abundance and on the abundance of developmental stages. Three-way ANOVAs were used to establish the effect of corer vs shovel, light and duration of the extraction (time) on soil arthropod and developmental stage abundance. Non Metric Multidimensional Scaling (NMS) and a Multi Response Permutation Procedure (MRPP) were used to establish the identity and sequence in which arthropods were extracted. NMS was run with the Sorensen dissimilarity index based on presence/absence and a maximum of three axes were allowed. Non Metric Multidimensional Scaling places sampling units in space based on similarity such that close points have a similar composition, and MRPP establishes significant differences based on the dissimilarity matrix calculated with the Sorensen index (McCune & Grace 2002). The MRPP was used to establish statistical differences in composition due to collection, extraction and duration of the extraction (time), and the significant value was set at 0.05. Throughout the text, results are expressed as mean  $\pm$  standard error.

### 3. Results

There were significant effects of forest type (dry vs wet) on litter depth (Mann Whitney,  $n=120$ ,  $T= 9843.5$ ,  $p<0.001$ ), litter dry weight (Mann Whitney,  $n=40$ ,  $T= 2410.0$ ,  $p<0.001$ ) and soil bulk density (Mann Whitney,  $n=80$ ,  $T= 7206.0$ ,  $p=0.009$ ). Mean litter depth was higher in the dry forest, 3.7 cm ( $\pm 0.1$ ), than in the wet forest, 2.2 cm ( $\pm 0.1$ ), and mean litter dry weight was 5.7 kg m<sup>-2</sup> ( $\pm 458.8$ ) in the dry forest and 0.8 kg m<sup>-2</sup> ( $\pm 54.5$ ) in the wet forest. Mean soil bulk density was higher in the wet forest, 0.6 g cm<sup>-3</sup> ( $\pm 0.02$ ), than in the dry forest, 0.5 g cm<sup>-3</sup> ( $\pm 0.02$ ).

### 3.1 Litter arthropods

Acari and Collembola were dominant in both forests. Acari were significantly more abundant in the dry forest while, Collembola were more abundant in the wet forest (Table 1). In the dry forest, there were, on average, 655 Acari m<sup>-2</sup> and 583 Collembola m<sup>-2</sup>, while in the wet forest, there were 623 and 635, respectively.

	Dry forest		Wet forest	
	Mean(±s.e.)	%	Mean(±s.e.)	%
Acari	655 (± 106 )a	30	623 (± 101 )b	28
Collembola	583 (± 92 )a	26	635 (± 92 )b	29
Diptera	187 (± 33 )a	8	179 (± 33 )b	8
Hymenoptera	166 (± 65 )a	7	152 (± 65 )a	7
Araneae	155 (± 20 )b	7	144 (± 19 )a	7
Pseudoscorpiones	138 (± 27 )a	6	142 (± 28 )b	6
Homoptera	104 (± 31 )a	5	101 (± 32 )a	5
Isopoda	85 (± 17 )b	4	93 (± 18 )a	4
Coleoptera	70 (± 16 )a	3	60 (± 11 )b	3
Thysanoptera	58 (± 24 )a	3	48 (± 24 )a	2
Others	14 (± 4 )		16 (± 5 )	
Total	2214 (± 436 )		2192 (± 428 )	

Table 1. Mean abundance m<sup>-2</sup> (±standard error) and percent dominance of litter arthropods in dry and wet forests. Alphabets indicate significant difference between forests for a particular group (Mann-Whitney Rank Sum Test,  $\alpha=0.05$ ,  $n=40$  for each forest).

In both forests, there was a significant effect of light on the abundance of litter arthropods, being higher when extractions were done with light. In the dry forest, there were significant effects of light and time on the abundance of the extracted arthropods while in the wet forest only time had a highly significant effect (Table 2). In the dry forest, >20,000 ind m<sup>-2</sup> were extracted with light and in the wet forest <5,000 ind m<sup>-2</sup> were extracted with light (Fig. 2). Through time, in both the dry and the wet forests, a high abundance was obtained in the first 24 h, with a slight increase at 48 h (Fig. 3). In the dry forest, some individuals were still recovered after 168 h, while in the wet forest all individuals were collected in the first 48 h. In general, in the dry forest, >90% of total extracted individuals was obtained after 144 h (6 d) of extraction with light, while in the wet forest >90% of total individuals was obtained after 48 h (2 d).

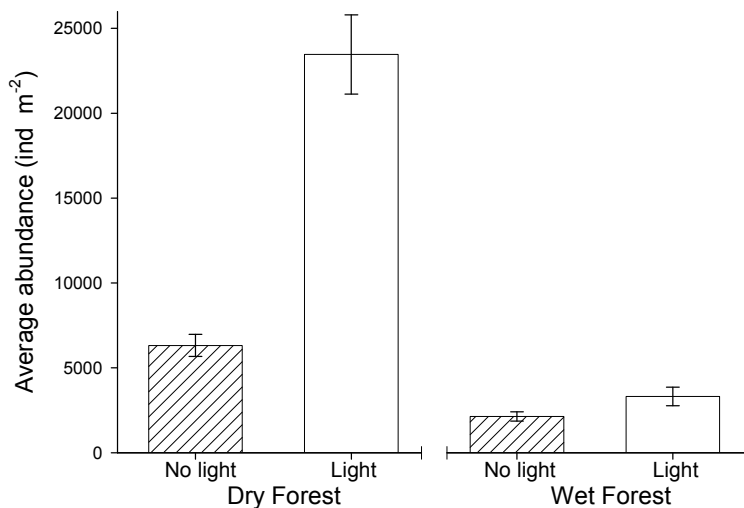


Fig. 2. Mean abundance of litter arthropods (ind m<sup>-2</sup>) extracted with and without light. Bars represent standard error.

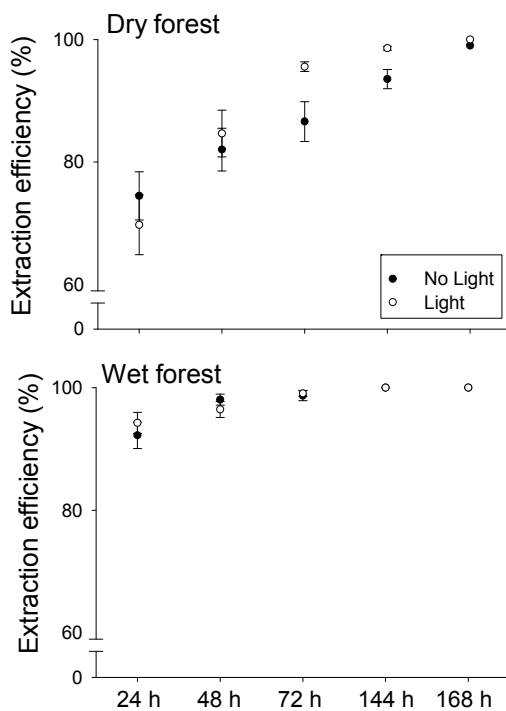


Fig. 3. Relative extraction efficiency (below) in dry and wet forests. Bars represent standard error.

In both forests, duration of the extraction (time) and light significantly influenced the identity and sequence in which arthropods were extracted (NMS, MRPP) (Fig. 4). In the dry forest and during the first 24 h, Blattodea and Protura were extracted mainly without light, while Acari (Fig. 1G), Collembola (Fig. 1E) and Pseudoscorpiones were extracted mainly with light. These groups represent organisms that are mostly considered detritivores, omnivores or predators, but microbivores (mainly Acari, Oribatida) were still recovered after 144 h of extraction. In the wet forest, all groups were extracted in the first 24 h, Chilopoda, Hymenoptera and Symphyla were extracted without light after the first 24 h, and Acari, Diplopoda and Diptera were extracted with light in the same extraction period.

Forest	Source	df	Abundance		Developmental stage			
			F	P	immatures		adults	
			F	P	F	P	F	P
Dry	Light	1	48.66	<0.001	41.72	<0.001	35.49	<0.001
	Time	4	57.42	<0.001	48.29	<0.001	42.40	<0.001
	Light x time	4	14.91	<0.001	13.88	<0.001	10.50	<0.001
	Residual	189						
	Total	198						
Wet	Light	1	3.98	0.05	6.48	0.01	2.80	0.10
	Time	4	73.26	<0.001	66.55	<0.001	60.06	<0.001
	Light x time	4	3.20	0.01	6.50	<0.001	2.09	0.08
	Residual	190						
	Total	199						

Table 2. Effect of use of light during extraction (with and without light) and duration of the extraction (time) (24 h, 48 h, 72 h, 144 h and 168 h) on litter arthropods in dry and wet forests (two 2-way AOV,  $\alpha = 0.005$ ,  $n=199$  for the dry forest and  $n=200$  for the wet forest). The effects were evaluated for total abundance (ind m<sup>-2</sup>) and abundance per developmental stage (ind m<sup>-2</sup>).

Developmental stages. There were significant effects of light and time on the abundance of both immature and adults in the litter (Table 2). In the dry forest, both immature and adults were more abundant when extraction was done with light, and in the wet forest immature were more abundant when extraction was done without light, but adults were abundant in both extraction treatments (Table 3). Through time, both immature and adults followed the same pattern as established before: in the dry forest, >90% was obtained after 144h (6 d), while in the wet forest, >90% was obtained after 48h (2 d).

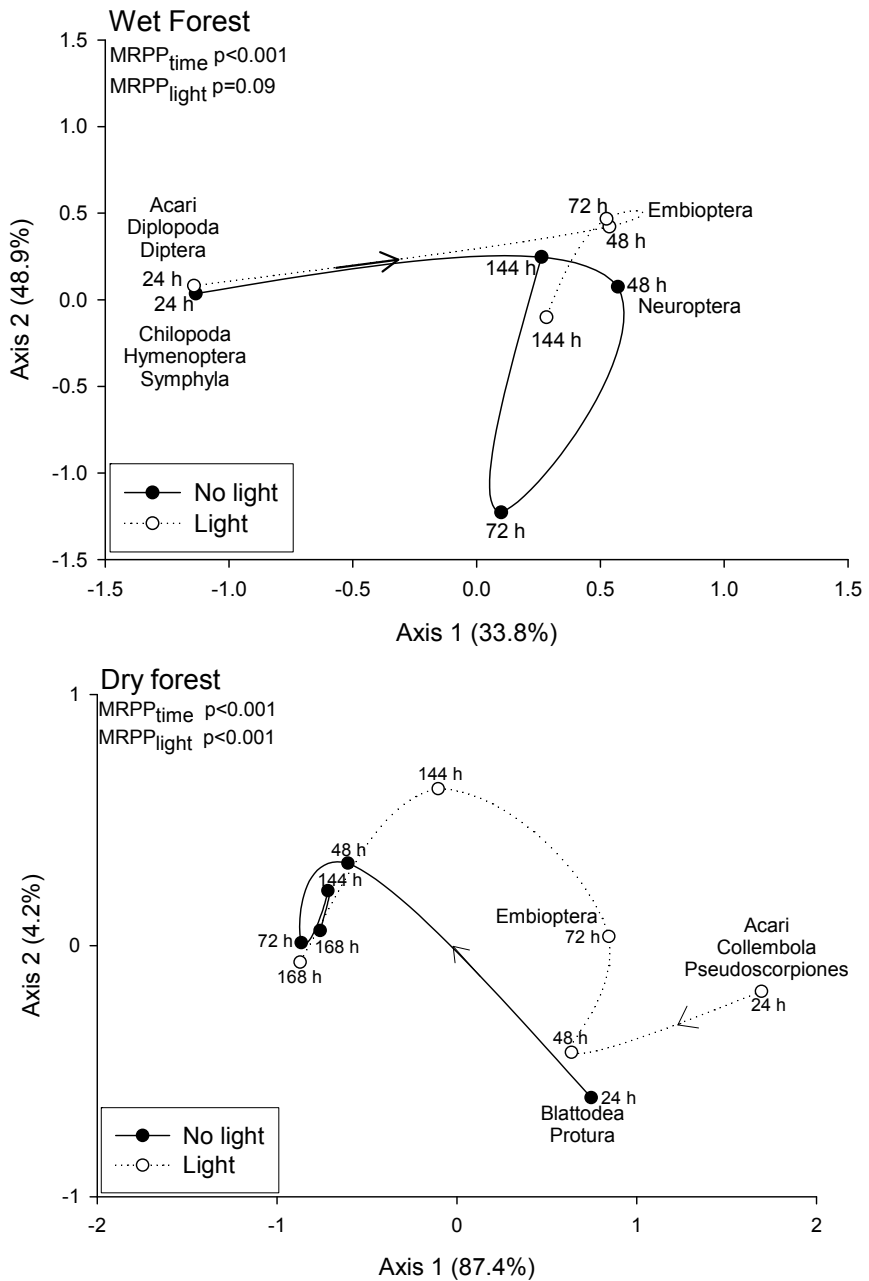


Fig. 4. Identity and sequence of extraction of litter arthropods extracted with and without light in dry and wet forests. The sequence of extraction within each type (with and without light) through time is connected by a line.

Sample	Stage	Collection	Dry forest		Wet forest	
			No light	Light	No light	Light
Litter	Immatures		2,115(±10113)b	8,120(±947)a	630(±90)a	345(±72)b
	Adults		4,205(±487)b	15,245(±1857)a	1,790(±239)a	2,690(±497)a
Soil	Immatures	Corer	11,877(±10113)a	10,924(13367)a	5,132(±5,091)a	1,466(±2,562)b
		Manual	19,062(±11,969)a	7,038(±7,724)b	2,346(±1,867)a	2,859(±3,027)a
	Adults	Corer	13,196(±17,395)a	5,792(±7,939)b	15,249(±8,968)a	4,179(±2,744)b
		Manual	11,070(±7,604)b	16,496(±8,015)a	9,310(±9,937)a	3,739(±2,912)b

Table 3. Mean abundance per square meter (+/- standard error) of litter and soil arthropods in dry and wet forests. Alphabets indicate significant differences between extraction methods (with and without light) for a particular developmental stage in a specific forest (Mann-Whitney Rank Sum Test,  $\alpha=0.05$ ,  $n=40$  for litter arthropods in each forest, and  $n=80$  for soil arthropods in each forest).

### 3.2 Soil arthropods

Overall, soil arthropod abundance was higher in the dry forest than in the wet forest (Table 4). Acari and Collembola were dominant in both forests, but both orders were significantly more abundant in the dry forest. There were, on average, 14,021 Acari  $m^{-2}$  and 3,904 Collembola  $m^{-2}$  in the dry forest, while in the wet forest, there were 2,511 and 2,786 ind  $m^{-2}$  respectively.

In both forests, there were significant effects of sampling technique, and of light and time, on the abundance of the extracted arthropods (Table 5). In both forests, extraction without light rendered higher abundance of soil microarthropods than extraction with light (Fig. 5). In the dry forest, collection with a corer and extraction without light rendered 5,015 ind  $m^{-2}$  while corer with light rendered 3,343, and shovel without light and shovel with light rendered 6,026 ind  $m^{-2}$  and 4,780, respectively. In the wet forest, corer without light had 4,076 ind  $m^{-2}$ , corer with light had 1,129, shovel without light had 2,331.4 and shovel with had 1,319. As established before, in the dry forest, arthropods continued to be collected after 144 h of extraction, and samples collected with shovel and extracted without light rendered the highest abundance. In the wet forest, arthropods were collected within the first 48 h except for the corer without light where arthropods were collected even after 144 h and this collection method rendered the highest abundance (Fig. 6).

In both forests, time significantly influenced the identity and sequence in which soil arthropods were extracted (NMS, MRPP) (Fig. 7). In the dry forest, Isoptera was extracted with corer with light in the first 24 h, Pseudoscorpiones with corer without light in the same time period 24 h, and Protura and Hemiptera with corer without light after 48 h. Also, Hymenoptera, Isopoda, Diplopoda and Chilopoda were extracted with shovel after 24 h (Fig. 7). In this forest, organisms representing several trophic groups (such as predators and omnivores) were extracted in the first 48 h, but those representing microbivores (mainly Oribatida) were still collected after 144 h. In the wet forest, Isoptera was extracted with corer with light after 24 h, Diplopoda and Chilopoda were extracted with shovel with light after 24 h, and Hymenoptera and Collembola were extracted with shovel without light after 24 h.

	Dry forest		Wet forest	
	Mean( $\pm$ s.e.)	%	Mean( $\pm$ s.e.)	%
Acari	14021 ( $\pm$ 1166 )a	60	2511 ( $\pm$ 380 )b	28
Collembola	3904 ( $\pm$ 538 )a	17	2786 ( $\pm$ 343 )b	29
Hymenoptera	1650 ( $\pm$ 813 )a	7	1723 ( $\pm$ 531 )a	8
Diplopoda	1100 ( $\pm$ 184 )a	5	18 ( $\pm$ 18 )b	7
Diptera	605 ( $\pm$ 390 )a	3	642 ( $\pm$ 145 )a	7
Coleoptera	440 ( $\pm$ 112 )a	2	220 ( $\pm$ 59 )b	6
Pseudoscorpiones	403 ( $\pm$ 111 )a	2	220 ( $\pm$ 64 )b	5
Protura	385 ( $\pm$ 135 )a	2	861 ( $\pm$ 170 )b	4
Araneae	183 ( $\pm$ 106 )a	1	587 ( $\pm$ 124 )b	3
Homoptera	183 ( $\pm$ 66 )a	1	422 ( $\pm$ 111 )b	2
Chilopoda	147 ( $\pm$ 96 )a	1	73 ( $\pm$ 36 )a	1
Hemiptera	128 ( $\pm$ 70 )a	1	37 ( $\pm$ 26 )b	0
Others	63 ( $\pm$ 9 )a	0	92 ( $\pm$ 11 )a	1
Total	23212 ( $\pm$ 1060 )a		10189 ( $\pm$ 264 )b	

Table 4. Mean abundance per square meter ( $\pm$ -standard error) and percent dominance of soil arthropods in dry and wet forests. Alphabets indicate significant difference between forests for a particular group (Mann-Whitney Rank Sum Test,  $\alpha=0.05$ ,  $n=80$  for each forest).

Developmental stages. For immature arthropod abundance, there were significant effects of light and duration of the extraction (time) in both forests (Table 5). For adult arthropod abundance, there were significant effects of collection and duration of extraction (time) in the dry forest and, of collection, light and duration of the extraction (time) in the wet forest (Table 5). In the dry forest, immature were significantly more abundant when the sample was collected with shovels and extracted without light (Table 3). The abundance of adults depended on the collection method: for samples collected with a corer, a higher abundance was obtained when extracted without light; but for samples collected with shovel a higher abundance was obtained when extracted with light. In the wet forest, all soil samples, both corer and shovel that were extracted without light rendered a higher abundance than their counterparts extracted with light (Table 3).

Forest	Source	df	Abundance		Developmental stage			
			F	P	Immature		Adults	
			F	P	F	P	F	P
Dry	Collection	1	4.05	0.045	0.64	0.424	5.30	0.022
	Light	1	5.76	0.017	9.91	0.002	0.21	0.646
	Time	4	79.02	<0.001	39.01	<0.001	54.47	<0.001
	Collection x light	1	0.12	0.727	7.21	0.008	11.66	<0.001
	Collection x time	4	6.90	<0.001	3.90	0.004	4.08	0.003
	Light x time	4	0.42	0.797	0.42	0.792	1.14	0.337
	Collection x light x time	4	0.43	0.787	6.33	<0.001	3.99	0.003
	Residual	380						
	Total	399						
Wet	Collection	1	5.59	0.019	1.10	0.295	4.65	0.032
	Light	1	36.29	<0.001	5.64	0.018	31.62	<0.001
	Time	4	43.75	<0.001	15.25	<0.001	31.55	<0.001
	Collection x light	1	8.67	0.003	9.90	0.002	3.45	0.064
	Collection x time	4	12.80	<0.001	6.87	<0.001	8.23	<0.001
	Light x time	4	2.62	0.350	4.09	0.003	1.72	0.146
	Collection x light x time	4	8.52	<0.001	2.72	0.030	8.13	<0.001
	Residual	380						
	Total	399						

Table 5. Effect of collection (corer and shovel), extraction (with and without light) and duration of the extraction (time) (24 h, 48 h, 72 h, 144 h and 168 h) on the abundance of soil arthropods (two 3-way AOV,  $\alpha = 0.005$ ,  $n=400$  for each forest ). The effects were evaluated for total abundance ( $\text{ind m}^{-2}$ ) and abundance per developmental stage ( $\text{ind m}^{-2}$ ).

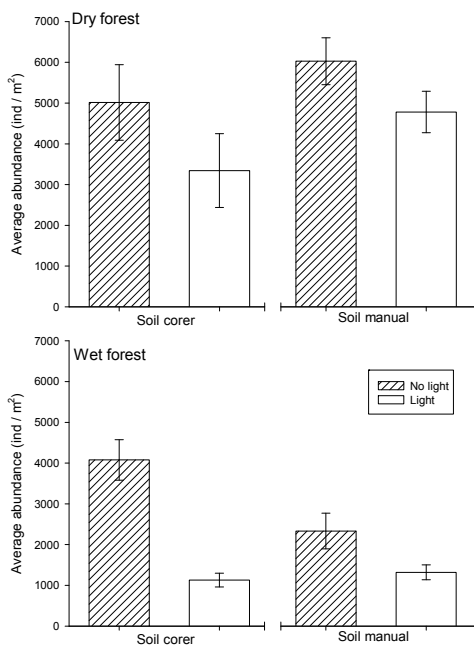


Fig. 5. Mean abundance of soil arthropods (ind m<sup>-2</sup>) collected by corer or shovel, and extracted with and without light. Bars represent standard error.

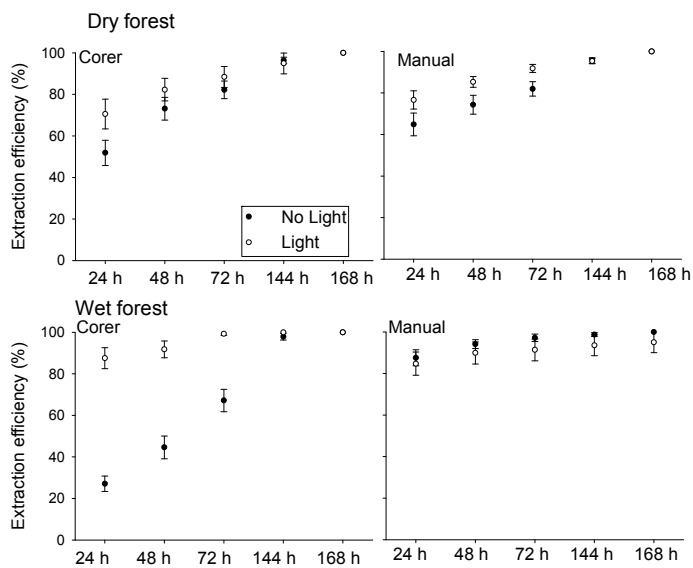


Fig. 6. Extraction efficiency in dry and wet forests. Bars represent standard error.

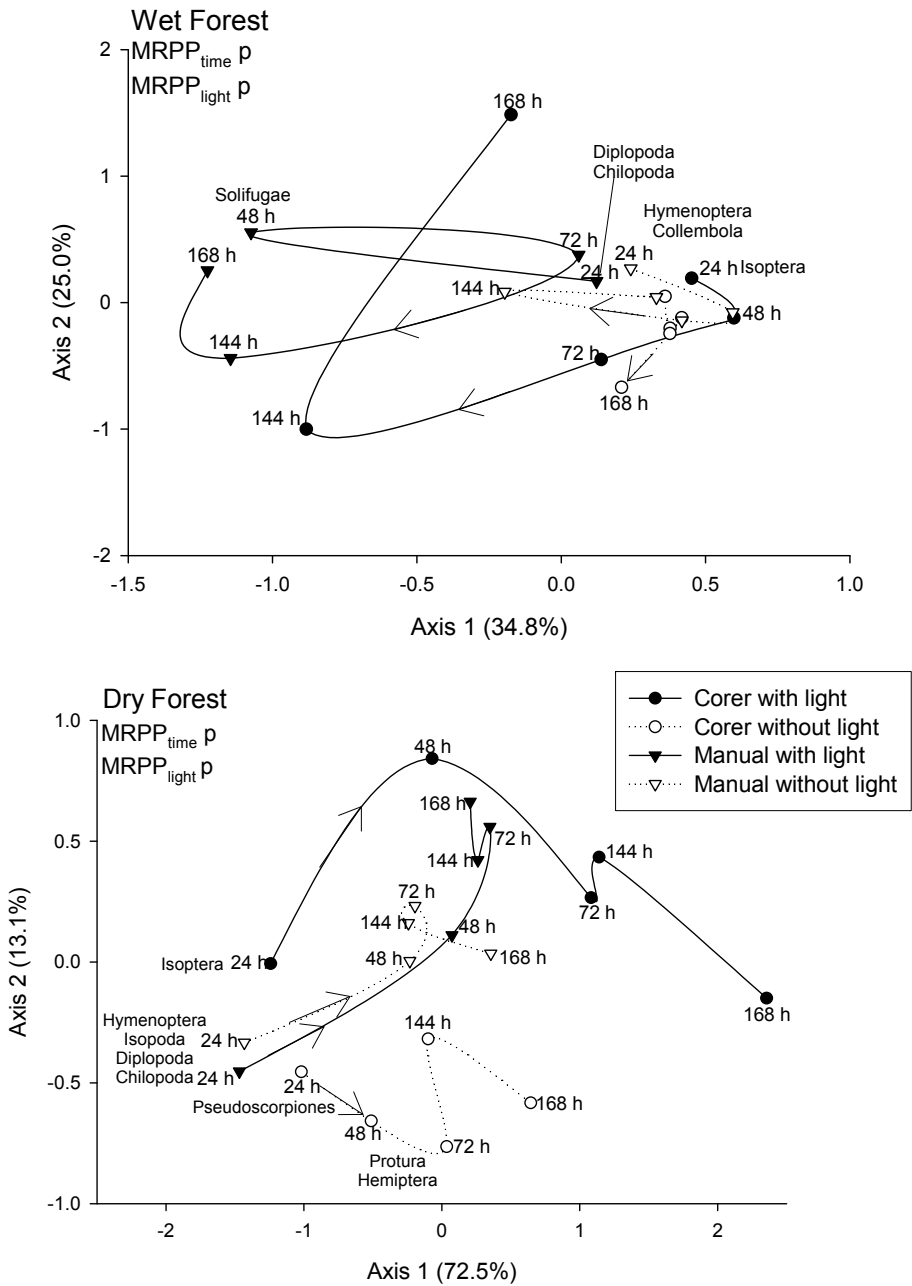


Fig. 7. Identity and sequence of soil arthropods collected by corer or shovel, and extracted with and without light in dry and wet forests. The sequence of extraction within each collecting technique with extraction type through time is connected by a line.

#### 4. Discussion

The objective of this study was to determine how the diversity of retrieved arthropods was affected by collection technique, use of light during extractions in Berlese-Tullgren funnels and duration of the extraction. For use of light during extractions, we found that litter arthropod abundance was highest when extraction was done with light but soil arthropods were highest when extraction was done without light. We found that forest type (tropical wet vs dry forest) influenced the sampling technique that was best suited because, in the wet forest, soil arthropod abundance was highest when collection was done with soil corers, while in the dry forest soil arthropod abundance was highest when collection was done with shovels. Finally, we found that forest type also influenced duration of the extraction because in the wet forest, 90% of arthropods were recovered within the first 24 h while, in the dry forest the same percent was obtained after 144 h of extraction.

Litter and soil arthropods responded differently to the use of light during extractions. One explanation is that the use of light may have speeded the desiccation of the sample forcing more litter animals to exit the sample than in the extractions without light. On the contrary, soil arthropods are more sensitive to increasing temperature or decreasing humidity than litter arthropods, in which case the use of light during extractions would made arthropods inactive before leaving the sample and thus their apparent abundance would decrease. Furthermore, litter arthropods inhabit a clear and warm habitat (litter) (Eviner & Chapin 2003) and thus may require an increase in temperature and in light incidence to exit the samples. But soil arthropods inhabit a comparatively cooler and darker habitat (soil) (Eviner & Chapin 2003) and thus may be sensitive to increasing temperature and light incidence to the point that the use of light during extraction may have resulted in an underestimation of soil arthropod abundance. In both forests, we retrieved more adults than immature, possibly because the soft cuticle of immature makes them more susceptible to the decreasing humidity within the extraction funnel, and because immature organisms, such as mites (majority of the immatures retrieved in this study), undergo inactivity when moulting and thus cannot leave the sample (Søvik & Leinaas 2002). As a consequence another extraction methodology, such as flotation, should be more suitable for immature forms (Hale 1964; Walter et al. 1987; Lakly & Crossley 2000; Søvik & Leinaas 2002).

In both forests, soil arthropods left the shovelled samples faster than the cored samples. Also, abundance was highest from shovelled samples in the dry forest, and in the wet forest abundance was highest from the cored samples although the pace of retrieval was slow. One explanation is that the loose structure of shovelled samples retained less humidity and dried out faster allowing the temperature/humidity gradient to be established sooner than in the compact cored samples where more humidity could be retained (MacFadyen 1953). This would have a dual effect, in wet forest samples, the gradient resulting from drying out the cored sample at room temperature moved slowly downwards forcing arthropods to leave the sample but not being large enough to kill them (as would occur in the shovelled samples) resulting in higher arthropod abundance in cored than in shovelled samples. In the dry forest samples, the gradient resulting from drying the shovelled sample at room temperature reached higher critical levels and became larger than in cored samples, forcing arthropods outwards, resulting in higher estimates of arthropod abundance in the shovelled samples.

Arthropods from the wet forest were recovered faster than arthropods from the dry forest. Macfadyen (1961) proposed that humidity-loving animals require high levels of humidity to be active, as a consequence they respond quicker to changes in humidity than humidity-resistant animals. On the contrary, humidity-resistant animals require high temperatures and low humidity to be forced to exit the sample. During extractions, temperature of the sample begins to increase immediately with a significant increase after 16 - 24 h, and humidity within the sample drops simultaneously with the significant increase in temperature (Haarlov 1947; Block 1966). Following MacFadyen (1953), we can explain why arthropods from the wet forest exited the sample faster than those from the dry forest. Arthropods from the wet forest left the sample within the first 24 h in response to the increase in temperature and the decrease in humidity that occurred in the sample when extraction begins. As the sample became hotter and drier, any animal remaining in the sample could have become inactive (or killed) by low humidity and high temperature. On the other hand, arthropods from the dry forest required longer extraction times because for these humidity-resistant animals, the critical levels of humidity required for them to leave the sample, took longer to be established.

The environmental characteristics of the two forests studied here were different and contribute another explanation to our results. Dry periods in the wet forest and the concomitant response of the biota to these periods are based on the number of dry days, because monthly rainfall is always above 100mm (Cuevas *et al.* 1991; Cuevas & Lugo 1998). On the contrary, dry days in the dry forest are the common condition, an average of 200 dry days per year, with pulses of heavy rainfall occurring during the wet season (<http://cirrus.dnr.state.sc.us/cgi-bin/sercc/cliMAIN.pl?pr8412>). As a consequence, arthropods from the dry forest come from a habitat with higher temperatures and longer periods of drier conditions than do arthropods from wet forest. The more extreme conditions in the dry forest may have make arthropods less responsive to higher temperature and drier air within the extraction funnel, resulting in longer extraction times.

We found that arthropod abundance was significantly higher in the dry forest than in the wet forest. Litter depth and dry weight were higher in the dry forest, litter was 40% deeper and 86% heavier in the dry forest than in the wet forest, suggesting that habitat and resource availability significantly influenced arthropod abundance (Mulder *et al.* 1999). Several researchers have found that Berlese-Tullgren extractions underestimate arthropod abundance. Nevertheless, in this study we found that total abundances fall within similar ranges to those reported in the literature, such as in Mexico and Perú, where abundances are reported to be 4,303 - 6,409 ind/m<sup>2</sup> (Lavelle *et al.* 1992) respectively. In addition, Berlese-Tullgren funnels are the predominant methodology to collect arthropods from litter/soil samples, but care should be taken because some groups are sensitive to light and are not effectively recovered with funnel extraction, such as collembolans in the family Onychiuridae (Coleman *et al.* 2004), or in our case, Proturans that were much more abundant in extractions without light and almost absent when light was used.

The structure of the retrieved community was affected by duration of extraction. Other authors have also found that during extractions, different taxonomic groups leave the sample at different times (Krell *et al.* 2005; Block 1966). In an extraction that lasted three days (Block 1966), Mesostigmata mites left the sample during the first day, while the majority of Collembola and Cryptostigmata mites left the sample mainly during the second day, and

Prostigmata began to leave the sample at the third day. Also, Krell *et al.* (2005) using an alternative extraction method, Winkler bags, also found that duration of the extraction affected the structure of the retrieved community, for example 70% of adult beetles and ants were retrieved within three days of extraction but Chilopoda required 3 to 4 wk. By using Berlese-Tullgren funnel, we found that both Collembola and Acari began to leave the sample during the first 24 h, also we retrieved few adult Coleoptera (the majority of Coleoptera were larvae), and the majority of ants and centipedes left the samples within the first 24 h of extraction.

## 5. Conclusion

Krell *et al.* (2005) proposed that if the aim of the study is to rapidly assess the litter/soil fauna, short extraction times should be enough. On the contrary, if the aim of the study is to exhaustively assess this fauna, then the methodology should be standardized, such as assessing optimum extraction times and biases due to collection methods. Our results also suggest that methodology standardization is necessary because (1) to reach similar percents (90%), extraction periods were longer for samples from dry forests than from wet forests, (2) the use of light promoted litter arthropods to leave the sample producing high abundances, but for immature and soil arthropods the use of light resulted in low abundances, and (3) cored samples rendered higher abundances in wet forests than in dry forests where shovel samples rendered higher abundances. In addition, our data suggest that samples from dry environments should be extracted for longer periods than those coming from wet environments. Also, if the focal organisms are soil arthropods, then extraction without light should result in high abundances. Finally, the collection method best suited depends on the environment to be sampled: in this study for wet habitats cored soil samples resulted in higher abundances than shovelled samples which resulted in highest abundances when sampling dry habitats.

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