



ISSN: 2447-3359

REVISTA DE GEOCIÊNCIAS DO NORDESTE

*Northeast Geosciences Journal*

v. 7, nº 2 (2021)

<https://doi.org/10.21680/2447-3359.2021v7n2ID19861>



## A SURVEY OF SOIL MESOFAUNA IN ENVIRONMENTAL PROTECTION AREAS IN MARANHÃO STATE, BRAZIL

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### Abstract

Knowledge of the composition of soil mesofaunal communities is an important tool to understand the influence of soils on living organisms and may help elucidate how soil fauna is affected by anthropization. This study aimed to survey soil mesofaunal orders and investigate their dynamics in the Municipal Environmental Protection Areas of Buriti do Meio and Inhamum, Maranhão State. Samples were collected twice in the dry season (2018) and twice in the rainy season (2019), totaling six collections. Mesofauna individuals were sampled using Provid traps and Berlese–Tullgren funnel batteries. At the laboratory, individuals were screened and counted. Data were analyzed using Excel and ANAFAU software. A total of 12,486 mesofauna individuals were recorded. Individuals from the order Collembola were predominant at both sites. By comparing the abundance of mesofauna individuals between collection methods, it was found that Provid traps were more efficient than Berlese–Tullgren funnels. The (Shannon–Wiener) diversity of soil mesofaunal orders was compared between the two environments, revealing that the Buriti do Meio Environmental Protection Area had the highest diversity index, a finding confirmed by Pielou's evenness index.

**Keywords:** Berlese–Tullgren; Diversity; Provid.

### LEVANTAMENTO DA MESOFAUNA EDÁFICA EM ÁREAS DE PROTEÇÃO DO MARANHÃO

#### Resumo

O conhecimento da composição da comunidade da mesofauna edáfica é uma ferramenta importante para compreender a

influência dos solos nos seres vivos e também para elucidar como a fauna do solo é afetada pela antropização. Neste sentido, a pesquisa tem como objetivo avaliar o levantamento das ordens da mesofauna edáfica e sua dinâmica na Área de Proteção Ambiental Municipal do Buriti do Meio e a Área de Proteção Ambiental do Inhamum. As coletas foram realizadas duas vezes durante o período seco (2018), e duas vezes no período chuvoso (2019), totalizando seis coletas. Para a amostragem da mesofauna, foi utilizado armadilhas do tipo Provid e bateria de extratores Berlese-Tullgre. No laboratório foram feitas a triagem e quantificados os indivíduos e os dados foram analisados no Excel e ANAFAU. Foram contabilizados 12.486 indivíduos da mesofauna. Os indivíduos da ordem Collembola foram predominantes nas duas áreas. Ao comparar a abundância de indivíduos coletados nas duas armadilhas. Notou-se que a armadilha Provid se mostrou mais eficiente em relação a armadilha de Berlese Tullgren. Foi analisada a diversidade das ordens entre os dois ambientes estudados (Shannon-Wiener), com isso, a Área de Proteção Ambiental Municipal do Buriti do Meio obteve o maior índice de diversidade, confirmado pelo Índice de Pielou (J).

**Palavras-chave:** Berlese–Tullgren; Diversidade; Provid.

## ENCUESTA DE MESOFAUNA EDAFICADA EN ÁREAS PROTEGIDAS DE MARANHÃO

### Resumen

El conocimiento de la composición de la comunidad mesofauna edáfica es una herramienta importante para entender la influencia de los suelos en los seres vivos y también para dilucidar cómo la fauna del suelo se ve afectada por la antropización. En este sentido, la investigación tiene como objetivo evaluar el estudio de las órdenes de la mesofauna edáfica y su dinámica en el área de Protección Ambiental Municipal de Buriti do Meio y el Área de Protección Ambiental de Inhamum. Las muestras fueron recogidas dos veces durante la estación seca (2018), y dos veces en la temporada de lluvias (2019), totalizando seis colecciones. Para el muestreo de la mesofauna, se utilizaron trampas Provid y la batería extractora de Berlese–Tullgren. En el laboratorio, los individuos fueron evaluados y cuantificados y los datos fueron analizados en Excel y ANAFAU. Se contaron un total de 12.486 individuos de la mesofauna. Los individuos de la orden Collembola fueron predominantes en ambas áreas. Al comparar la abundancia de individuos recogidos en las dos trampas. Se notó que la trampa de Provid demostró ser más eficiente en relación con la trampa berlese tollgren. La diversidad de pedidos entre los dos ambientes estudiados (Shannon-Wiener) se analizó, con esto, el Área Municipal de Protección Ambiental de Buriti do Meio obtuvo el índice de diversidad más alto, confirmado por el índice Pielou (J).

**Palabras-clave:** Berlese–Tullgren; Diversidad; Provid.

## 1. INTRODUCTION

Soil fauna have been widely used as soil indicators, particularly in environments subjected to anthropogenic activities (BARETTA *et al.*, 2006; PEREIRA-JÚNIOR *et al.*, 2010; VICENTE *et al.*, 2010; CAPRONI *et al.*, 2011). However, soil faunal composition is influenced by several biological factors that interact with each other and are sensitive to edaphoclimatic conditions (KORASAKI *et al.*, 2013), such as rainfall and soil temperature, which are known to affect the distribution and diversity of many taxa (ALMEIDA *et al.*, 2015).

It is important to emphasize that there are several approaches to classify soil fauna. Body diameter or length is usually the main criterion, although mobility characteristics, eating habits, and soil function may also be considered (MORAIS *et al.*, 2013). The soil mesofauna encompasses all invertebrate organisms that live or spend part of their life cycle in the soil and measure between 0.2 and 2.0 mm in length. This group includes Acari (mites and ticks), Collembola (springtails), Diplura (two-pronged bristletails), and Protura (coneheads), among others (Almeida *et al.*, 2013). Collembola and Acari dominate in terms of abundance and diversity; the latter, for instance, is represented by more than 1,000 species in Brazil (MELO *et al.*, 2009). These organisms perform important soil functions, such as organic matter decomposition, nutrient cycling, and soil structure formation, serving as bioindicators of environmental quality (BERUDE *et al.*, 2015).

Knowledge of soil mesofaunal community structure is an important tool to understand faunal effects on soil processes and elucidate how soil fauna is affected by anthropization and changes in soil use (ZAGATTO, 2014). Soil use changes were observed in the Municipal Environmental Protection Areas (EPAs) of Buriti do Meio and Inhamum, which have been exposed to increasing levels of anthropic disturbances. This study surveyed soil mesofaunal orders in the Buriti do Meio and Inhamum EPAs in an attempt to provide a scientific contribution, minimize environmental impacts, guide the development of adequate management practices, and identify a set of actions for a more sustainable use of local biodiversity.

## 2. METHODS

The research was carried out in the Buriti do Meio EPA (site I) and Inhamum EPA (site II) in Caxias, eastern Maranhão State, Brazil (Figure 1). The Buriti do Meio EPA, created by Law No. 1540/2004, covers an area of 58,347.30 ha in the settlement project of Buriti do Meio and Santa Rosa, second district, 35 km away from the urban perimeter of Caxias (IBGE, 2010). The Inhamum EPA is located on the left side of the BR-316 federal highway, close to the urban perimeter of Caxias, and transversely crossed by the MA-127 state highway, which connects Caxias and São João do Sóter (ALBUQUERQUE, 2012).

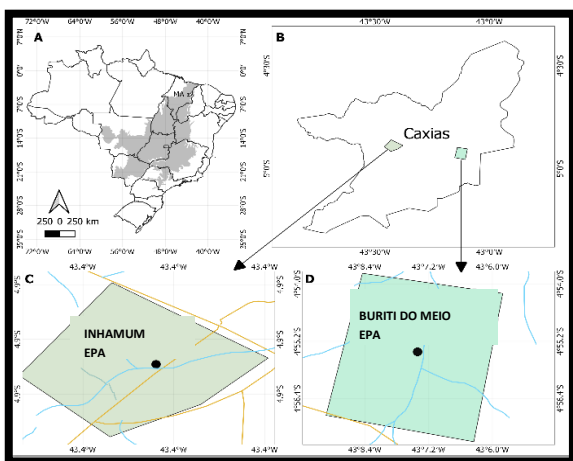


Figure 1 – (A, B) Location map of the Municipal Environmental Protection Areas (EPA) of (C) Inhamum and (D) Buriti do Meio in Caxias, Maranhão State, Brazil. Source: the authors (2021).

The vegetational structure of Buriti do Meio EPA is composed of different physiognomies of natural and anthropized Cerrado vegetation on sandy, acidic, nutrient-poor, and fragile soils (IBGE, 2010). By contrast, Inhamum EPA is characterized by grasslands in flat relief areas as well as Cerradões, Chapadas, Cerrado, and small fragments of closed-canopy forest that provide the necessary conditions for the survival of several animals, contributing to biodiversity (ALBUQUERQUE, 2012). In addition to Cerrado vegetation, the area possesses seasonal semi-deciduous forests composed predominantly of babassu palms and patches of Cerrado vegetation and gallery forests (CONCEIÇÃO *et al.*, 2012).

Sampling was performed by the Provid trap and Berlese–Tullgren funnel methods. Four parallel transects about 10 m apart were marked with five equidistant sampling points (10 × 10 m), totaling 20 points per experimental site (Figure 2).

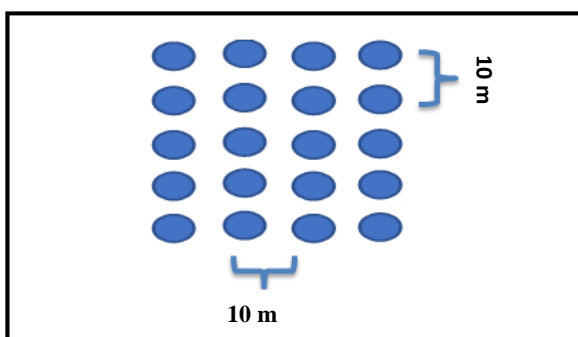


Figure 2 – Schematic representation of the distribution and spacing of sampling points. Source: the authors (2021).

Sample collections at sites I and II using Provid traps were performed in September 2018, October 2018, February 2019, and March 2019. Each Provid trap consisted of a 2 L PET bottle with

four 2 × 2 cm holes at a height of 20 cm from the base and containing 200 mL of a 5% detergent solution and 5 drops of formaldehyde p.a. (GIRACCA *et al.*, 2003; FORNAZIER *et al.*, 2007). Traps were buried with the holes at the soil level and were kept in the same place in all collections (ALMEIDA *et al.*, 2007). During each collection, traps remained in the field for 96 h (DRESCHER *et al.*, 2007).

After the 96 h period, traps were collected, identified with the sampling point and collection date, and transported to the Laboratory of Soil Fauna (LAFS, CESC, UEMA). Samples were washed over a 0.25 mm sieve and stored in plastic pots containing 70% ethyl alcohol. Mesofauna individuals were identified at the order level, counted, and separated (BORROR; DELONG, 1969; COSTA *et al.*, 2006) using entomological forceps and a stereomicroscope (Carl Zeiss Stemi DV4).

Collections for estimation of soil macrofaunal communities by the Berlese–Tullgren method were performed at sites I and II in February and March 2019. Samples of soil and plant litter were collected using metal rings measuring 4.8 cm in diameter and 3 cm in height. Sampling rings were pushed into the ground by successive hammer strokes until completely covered with soil. Then, rings and surrounding soil were moistened with water to prevent samples from detaching, and a spatula was introduced externally to collect the rings. Excess soil was removed, and rings were wrapped between two disc-shaped pieces of white fabric, one made of tulle and the other of non-woven fabric. Samples were carefully stored in plastic trays inside plastic bags to minimize water loss.

Shortly after collection, samples were processed through a battery of Berlese–Tullgren funnels for extraction of soil organisms, as storage periods longer than 24 h would probably cause the death of sensitive individuals (MELO, 2002). The method is based on the downward migration of insects from the soil sample in response to a temperature increase caused by light bulbs placed near the soil surface. Insects fall into the funnel and subsequently into a 240 mL glass vessel containing 30 mL of 70% ethanol.

The Berlese–Tullgren funnel battery was equipped with 20 lamps (25 W) and divided into two compartments. The upper compartment contained rings with soil samples and lamps, and the lower compartment comprised funnels and glass bottles with ethanol solution for collection. Samples were kept in the extractor for 96 h under light and heat, with a maximum temperature (at the top of the ring) of 42 °C. The radiation emitted by lamps during the 96 h period dried the soil in a descending manner, forcing the organisms to migrate to deeper layers and eventually fall into the collection vessel. The apparatus was sealed with nylon screen to prevent collection of nocturnal insects attracted by lamp lights. After sampling, the collected individuals were transferred to Petri dishes, counted, and identified at the order level using a stereomicroscope.

Soil microfaunal data were tabulated using Microsoft Excel software. Faunal analyses were carried out using ANAFAU software for determination of dominance, abundance, frequency, constancy, Shannon–Weaver diversity ( $H'$ ), Pielou evenness ( $J'$ ), and Margalef richness ( $I$ ) indices (MORAES *et al.*, 2003).

### 3. RESULTS AND DISCUSSION

#### 3.1. Abundance, frequency, constancy, and dominance of taxa

A total of 12,486 mesofauna individuals were sampled, 2,834 at site I (distributed in four orders) and 9,652 at site II (distributed in five orders). Site II had the highest order richness (Table 1). Abundance was higher at site II than at site I, attributed to the fact that the former was less affected by anthropogenic activities. Site

I has a land history of deforestation and burning for crop planting, resulting in an imbalance in soil fauna. According to Alvarez *et al.* (2001), soil fauna and microorganisms are influenced at different degrees of intensity by agricultural practices. Thus, the lower abundance of individuals at site I is likely related to agricultural activities. Another factor that might have contributed to the higher abundance of site II was the greater amount of decomposing organic matter, which serves as a food source to many organisms.

Table 1 – Number of individuals, abundance, dominance, frequency, and constancy of taxa sampled using Provid traps in the Buriti do Meio Environmental Protection Area (EPA) (site I) and the Inhamum EPA (site II), Caxias, Maranhão State, Brazil. Source: the authors (2021).

Taxa	Site I							Site II						
	NI	%	NC	A	D	F	C	NI	%	NC	D	A	F	C
<b>Acari</b>	700	24.0	64	va	d	f	Y	3412	35.4	54	d	va	f	Y
<b>Collembola</b>	2083	73.5	69	va	d	vf	Y	6135	63.6	68	d	va	vf	W
<b>Diptera</b>	19	0.8	10	va	d	f	Y	40	0.4	12	d	va	f	Y
<b>Diplura</b>	32	1.7	8	va	d	f	Y	41	0.4	7	d	va	f	Y
<b>Hemiptera</b>	-	-	-	-	-	-	-	24	0.3	9	d	va	f	Y
<b>TOTAL</b>	<b>2834</b>	<b>100</b>						<b>9652</b>	<b>100</b>					

Legend: NI, number of individuals; NC, number of collections; A, abundance; va, very abundant; D, dominance; d, dominant; F, frequency; f, frequent; vf, very frequent; C, constancy; W, constant; Y, accessory.

At site I, the orders with the highest abundance were Acari, with 700 individuals (24%), and Collembola, with 2,083 individuals (73.50%). Both orders are known to comprise some of the largest populations of soil mesofauna (COLEMAN; CROSSLEY, 1995). Acari, Collembola, Diptera, and Diplura were found to be dominant. Given that the frequency of taxa does not depend on diversity, the results showed that, at site I, Collembola was very frequent. Acari, Collembola, Diptera, and Diplura were classified as accessory with regard to constancy. Hemiptera was not identified at site I.

At site II, the orders with the greatest abundance of individuals were Acari, with 3,412 individuals (35.35%), and Collembola, with 6,135 individuals (63.56%). Acari, Collembola, Diptera, Diplura, and Hemiptera were categorized as dominant. Collembola was classified as very frequent and constant.

Overall, individuals of the order Collembola were predominant at both study sites. As discussed by Atonioli *et al.* (2013), the abundance of Collembola individuals is associated with their ability to multiply and grow rapidly. Of note, Collembola was more abundant at site II, probably associated with the greater environmental stability and preservation of this site compared with site I. Rieff *et al.* (2010) investigated the diversity of edaphic families belonging to Acari and Collembola in *Eucalyptus* plantations and native vegetation. The authors found that Collembola individuals develop better in native vegetation areas, where plant species variety and soil composition contribute to a greater soil organism diversity.

The exclusivity of Hemiptera to site II is likely associated with low anthropogenic disturbance, in contrast to the conditions of site I, which is constantly subjected to burning and soil tillage. According to Mortimer *et al.* (1998), Hemiptera individuals are good indicators of disruptions, as they respond immediately to changes in plant diversity. These organisms are directly related to vegetation quality and structure (KOROSI *et al.*, 2012). Furthermore, the reduced quantity and quality of organic waste at site I is a limiting factor to the establishment of certain taxa (BAINI *et al.*, 2012; ASHFORD *et al.*, 2013). Thus, the exclusivity of Hemiptera to site II might be related to the site's greater conservation.

Acari and Collembola were the most dominant taxa, with high dominance in both rainy and dry periods (Table 2). The order Acari is known for its adaptation to long periods of drought, explaining its constant occurrence, and Collembola adapts well to milder soil temperatures (FORMIGA, 2014). Acari had the lowest abundance in September 2018, with 57 individuals at site I and 26 at site II. This finding may be related to the dry period, when resource availability is low. By contrast, in the rainy season (March 2019), 399 individuals were sampled at site I and 2,860 at site II. The lowest abundance of Collembola was observed in September 2018 at site I (234 individuals) and in February 2019 at site II (341 individuals), whereas the highest abundance occurred in March 2019 at site I (895 individuals) and in October 2018 at site II (4,469 individuals).

Table 2 - Abundance of individuals sampled using Provid traps as a function of period and rainfall in the Buriti do Meio Environmental Protection Area (EPA) (site I) and the Inhamum EPA (site II), Caxias, Maranhão State, Brazil. Source: the authors (2021).

Site	Period	Rainfall* (mm)	Taxa				
			Acari	Collembola	Diptera	Diplura	Hemiptera
I	September 2018	13.5	57	234	1	32	0
	October 2018	42.5	131	423	14	0	0
	February 2019	182.2	399	494	2	0	0
	March 2019	373.9	113	895	2	0	0
II	September 2018	13.5	26	785	34	0	16
	October 2018	42.5	47	4469	0	41	2
	February 2019	182.2	2860	341	0	0	4
	March 2019	373.9	435	540	6	0	2
<b>TOTAL</b>			<b>4111</b>	<b>8181</b>	<b>59</b>	<b>73</b>	<b>24</b>

Legend: \* Rainfall levels were the same at both sites.

Overall, the number of sampled individuals varied according to collection period. Formiga *et al.* (2014) argued that an increase or decrease in the number of individuals can be attributed to the opportunistic characteristics of certain organisms, which may have a seasonal variation.

### 3.2. Efficiency of sampling methods for evaluating mesofauna composition and abundance

Mesofauna composition did not differ between the Provid trap and Berlese–Tullgren funnel at either site (Table 3), as both traps captured only four orders in each collection period. Regarding abundance, the Provid trap was more efficient than the Berlese–Tullgren funnel. Provid traps remained in the field for a longer period than sampling rings used in the Berlese–Tullgren method, thereby favoring the capture of mesofauna.

Table 3 - Efficiency of Provid trap and Berlese–Tullgren methods in sampling soil mesofauna in the Buriti do Meio Environmental Protection Area (EPA) (site I) and the Inhamum EPA (site II), Caxias, Maranhão State, Brazil. Source: the authors (2020).

Taxa	Provid trap		Berlese –Tullgren funnel	
	Site I	Site II	Site I	Site II
Acari	512	3295	156	141
Collembola	1426	881	10	30
Diptera	4	6	11	23
Hemiptera	-	7	-	1

According to Sandler *et al.* (2010), some of the limitations of Berlese–Tullgren funnels include the possibility of escape, sampling point location, and nondetection of immature or low-mobility organisms, impairing assessment. The inefficiency of the method can also be attributed to the escape of organisms during sampling. Another possibility is that some individuals might have died in the soil without migrating through the funnel (ANTONIOLLI *et al.*, 2006).

### 3.3. Shannon–Weiner diversity, Pielou evenness, and Margalef richness indices

The Shannon–Weiner diversity and Pielou evenness indices of sites I and II per collection period are presented in Table 4. At site I, the highest diversity index was observed in September 2018 ( $H' = 0.787$ ,  $J' = 0.316$ ) and the lowest in October 2018 ( $H' = 0.363$ ,  $J' = 0.183$ ). At site II, the highest ( $H' = 0.734$ ,  $J' = 0.362$ ) and lowest ( $H' = 0.152$ ,  $J' = 0.041$ ) diversity indices were

recorded in March 2019 and October 2018, respectively. The fact that the diversity index was lowest in October 2018 at site II might be associated with the greater abundance of individuals in this period. During the dry season, there is a greater deposit of leaf litter on the soil surface, resulting from intense defoliation. As highlighted by Mercante *et al.* (2004), the quality and quantity of organic matter and leaf litter, as well as climate conditions, greatly influence abundance and diversity and, consequently, soil faunal composition. This is in agreement with the observation that seasonality directly influences the occurrence of individuals, as well as their metabolic functions (MANHÃES, 2011). Manhães (2011) characterized soil fauna in different vegetation types and found that, in dry periods, individuals showed greater density. The authors attributed this finding to greater litterfall production and nutrient availability. In the rainy season, however, the soil fauna was more diverse. Thus, low diversity and evenness indices are associated with the higher abundance of individuals promoted by seasonal variations and nutrient availability.

Table 4 - Shannon diversity index ( $H'$ ) and Pielou evenness index ( $J'$ ) of soil mesofauna in the Buriti do Meio Environmental Protection Area (EPA) (site I) and the Inhamum EPA (site II), Caxias, Maranhão State, Brazil. Source: the authors (2021).

Site	Period	$H'$	$J'$
I	September 2018	0.787	0.316
	October 2018	0.649	0.356
	February 2019	0.702	0.452
	March 2019	0.363	0.183
II	September 2018	0.392	0.120
	October 2018	0.152	0.041
	February 2019	0.348	0.175
	March 2019	0.734	0.362

The overall diversity, evenness, and richness indices of sites I and II are described in Table 5. In general, diversity was higher at site II ( $H' = 0.7165$ ) than at site I ( $H' = 0.6529$ ), as was evenness (site I,  $J' = 0.4731$ ; site II,  $J' = 0.4452$ ). Diversity indices were similar between both sites. The Margalef richness

index was higher at site II ( $I = 0.4360$ ) than at site I ( $I = 0.3774$ ) (Table 5). As previously mentioned, these results can be attributed to differences in site conservation, collection period, and rainfall.

Table 5 - Shannon diversity, Pielou evenness, and Margalef richness indices of soil mesofauna in the Buriti do Meio Environmental Protection Area (EPA) (site I) and the Inhamum EPA (site II), Caxias, Maranhão State, Brazil. Source: the authors (2021).

Index	Site I	Site II
Shannon diversity	0.6529	0.7165
Pielou evenness	0.4731	0.4452
Margalef richness	0.3774	0.4360

Araújo *et al.* (2009) investigated the influence of rainfall on mesofauna at a Caatinga site in the semi-arid region of Paraíba. The authors concluded that collection period influenced faunal density, species richness, Shannon diversity index, and Pielou evenness index and that rainfall favored taxa abundance and richness. Our results indicate that the higher diversity and richness indices of site II might be related to conservation status, collection period, and rainfall.

#### 4. FINAL CONSIDERATIONS

The most abundant mesofaunal orders at both sites were Collembola and Acari.

The most frequent, constant, and dominant order at both sites was Collembola.

The Provid trap method was more efficient than the Berlese-Tullgren method in estimating abundance.

Site II had the highest taxonomic richness and diversity.

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## 6. ACKNOWLEDGMENTS

The authors thank the Soil Fauna Laboratory (LAFS/CESC) for their assistance with data analysis and the State University of Maranhão (UEMA).

Received in: 13/02/2020

Accepted for publication in: 09/06/2021



