

Asian Journal of Research in Zoology

Volume 6, Issue 4, Page 107-118, 2023; Article no.AJRIZ.108952 ISSN: 2582-466X

# Abundance and Diversity of Leaf Litter and Subsoil Arthropods in Four Different Sites of Three Agroecological Zones of Northwest Part of Bangladesh

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#### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

#### Article Information

DOI: 10.9734/AJRIZ/2023/v6i4128

#### **Open Peer Review History:**

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/108952

**Original Research Article** 

Received: 06/09/2023 Accepted: 10/11/2023 Published: 15/11/2023

# ABSTRACT

Leaf litter and subsoil arthropods are biotic factors of an ecosystem that play a significant role in nutrient cycling between soil and vegetation. The present study aimed to explore the abundance and diversity of leaf litter and subsoil arthropods in four different sites of three agroecological zones of the Northwest part of Bangladesh. A total of 1920 individuals belonging to 17 orders, 28 families and 34 species were found in the present study where 1099 individuals (14 orders, 23 families, 28 species) from Rajshahi University Campus (Site A), 259 individuals (6 orders, 7 families, 8 species) from Rajshahi Fruit Research Center (Site B), 354 individuals (7 orders, 13 families, 13 species) from Kakon Hat Municipality (Site C) and 208 individuals (5 orders, 10 families, 10 species) from

Asian J. Res. Zool., vol. 6, no. 4, pp. 107-118, 2023

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Chalan Beel (Site D) were collected during the period from November 2021 to October 2022. In this study, within the three agroecological zones, the highest number of individuals was recorded in Site A (n=1099, 57.24%) and the lowest number in Site D (n=208, 10.83%). Among 17 orders Coleoptera (624, 32.50%) was the first dominant order, Hymenoptera (414, 21.56%) was the second largest order, followed by Araneae (202, 10.52%) and the least dominant order was Metastigmata (8, 0.42%). Formicidae was the most dominant family among collected 28 families, sharing 376 individuals of the total captured individuals and Staphylinidae, sharing 198 individuals, was the second largest family, whereas Tenebrionidae (n=8), Psychodidae (n=8), and Laelapidae (n=8) were the minor dominant families in this study. Among the total of 34 species, Lasioderma sp. was the most dominant species, sharing 189 individuals with 9.84% of the total collected individuals whereas Alphitobius sp., Psychoda sp., and Hypoaspis sp. were least dominant species, and each species of these three comprised of only 8 members. In this study, 15 species were very common, 5 were common, 7 were fairly common, and 7 were rare. The values of Shannon diversity index (H'), Simpson's diversity index (I-D), Margalef diversity index (D<sub>Ma</sub>), Menhinick Richness index  $(D_{Mp})$ , Berger Parker dominance index (d) and Pielou's evenness index (J') were 3.22, 0.95, 4.37. 0.78, 0.10, respectively. The study was the first attempt to examine the abundance and diversity of leaf litter and subsoil arthropods in four different sites of three agroecological zones in the northwest part of Bangladesh. Further study may be necessary for molecular identification of these species.

Keywords: Species abundance; species diversity; leaf litter arthropods; agroecological zone; diversity indices.

# 1. INTRODUCTION

Leaf litter or plant litter or litterfall is dead plant material, such as leaves, bark, twigs, etc., that have fallen down to the ground. Different parts of plants fall periodically and act as connectors between the vegetation and soil of the land [1]. More than 90% of the net primary production of an ecosystem goes back to the soil through litter decomposition [2]. Two major roles are played by leaf litter in the ecosystem. Firstly, litter fall is an inherent part of nutrient and carbon cycling, and secondly, litter forms a protective layer on the soil surface [3]. Due to the presence of litter in the forest, the seedling diversity increased [4]. The process of breaking down of leaf litter from organic to inorganic form is known as the nutrient cycling process [5]. Three main factors, such as climate, leaf litter quality and the abundance of decomposing organisms, manipulate the decomposition of leaf litter [6].

Among the organisms related to decomposition, arthropods play a vital role in decomposition [7]. Arthropods are considered as good bioindicators and show high sensitivity to temperature and [8]. Arthropods moisture changes assist microbes in litter decomposition by breaking down the litter into small pieces during feeding [9] and feeding activity of soil macrofaunal species is one of the most important initial processes in the decomposition of organic matter [10]. The litterfall ingested by microarthropods returns to the soil as feces which are chemically and physically different from the original vegetal material [11]. The faeces are more suitable for microbes and provide food for other soil organisms [12,13]. The consumption of leaf litter allows the return of nutrients to the soil from which surrounding plants take nutrients and minerals [14]. A different group of arthropods, such as millipedes (*Diplopoda*), Woodlice (Isopoda) and earwigs (Dermaptera), are litter transformers [15]. In contrast, ecosystem engineers such as termites (*Isoptera*) and ants (*Formicidae*) can affect patterns of soil formation [16].

Among the soil arthropods, acari and collembola are the most remarkable groups and the foremost investigated taxa [17-19]. A combination of mites and collembola weighs less than 2  $q/m^2$  [20] can be directly responsible for 15 to 28 percent of annual litter breakdown and release 1 percent of the potassium and 12 percent of the calcium in the forest soil [21]. The terrestrial isopods (Woodlice) indirectly contribute to litter decomposition by stimulating microbial activities, and it was quantified by Hassall et al. [22] in the laboratory. Ants (Hymenoptera) play a vital role in terrestrial ecosystems. They affect both closely and distantly related taxa directly or indirectly [23]. Most of the ant species build nests in the soil [24,25] and play different types of functions like scavenging, predation, granivory and omnivory [26-28].

The abundance and diversity of arthropods vary from one agroecological zone to another

because different physiographic units possess different types of soil. An agroecological zone indicates an area characterized by homogeneous agricultural and ecological characteristics. In the high Barind tract, deep red-brown terrace soil is found. In the Ganges floodplain, the soil is silt loam to silt clay loam, whereas the lower Atrai Basin is covered by non-calcareous dark grey soils, but the soil of wetlands varies from silty clay to clay [29]. Different type of soil possesses different type of vegetation. The life of almost all organisms affected by higher plants [30]. Soil macrofauna populations are affected by litter quality and quantity [31].

Recently, a paper on the abundance and diversity of soil-dwelling arthropods in Rajshahi University Campus has been published [32]. There are also several papers on arthropods in Bangladesh [33,34,32,35,36]. These were reported on specific arthropod species, but nothing has been reported on the abundance and diversity of leaf litter and subsoil arthropods in Bangladesh yet. Though soil microarthropods play a significant role in leaf litter decomposition, they are not noticeable due to a lack of study.

This study guided to explore the leaf litter and subsoil arthropods in four sites- Rajshahi University Campus (Active Ganges Floodplain), Rajshahi Fruit Research Centre (Active Ganges Floodplain), Kakon Hat Municipality (High Barind Tracts), Rajshahi and Chalan Beel (Lower Atrai Basin), Natore and make a comparison among the arthropods in these areas.

# 2. MATERIALS AND METHODS

#### 2.1 Study Area

Four different sites of three agroecological zones were selected for the study. Rajshahi University Campus (Site A) and Rajshahi Fruit Research Center (Site B) were included in the Active Ganges floodplain. Kakon Hat Municipality (Site C) was included in the High Barind Tract, and Chalan Beel (Site D) was under the Lower Atrai Basin.

# 2.2 Sample Collection

Sample collection, extraction and slide preparation were inspired by Hossain [37]. The samples were collected three times during the period from November 2021 to October 2022. To collect the sample,  $50 \text{ cm}^2$  quadrate was placed on the selected spots and 1.5 to 2.0 kg samples

were taken in gallon-size clear plastic bags on which collection date and location were written. After that, the samples were carried into the crop protection and toxicology laboratory for extracting small arthropods. Larger arthropods were picked up through hand or forceps and placed into plastic jars for further analysis.

#### 2.3 Sample Extraction

A homemade Berlese-Tullgren funnel was used to extract small leaf litter and subsoil arthropods from the collected litter and soil. In this method, samples were taken in a funnel on a sieving net and an electric bulb was placed over the sample. A beaker with 70% alcohol was placed under the funnel. Due to excess heat, small arthropods were moving or crawling downward and eventually fell into the beaker that was placed under the funnel. Larger arthropods were picked up by forceps. Then, the collected arthropods were preserved in 70% ethyl-alcohol for further analysis.

#### 2.4 Slide Preparation

Slide preparation was taken into consideration for smaller arthropods like ants and different body parts of larger arthropods. For slide preparation, first, the specimen was put on a slide, then 80%, 90% and 100% ethyl-alcohol were used for dehydration, and the slide was left for a few minutes to dry. After drying it up, Canada balsam and cover slip were used to mount every slide. Finally, prepared slides were taken under a light microscope and identified from the taxonomic level up to the genus level with the help of literature, articles, websites and consultation with specialists for particular taxa.

# 2.5 Status of the Species

According to the classification given by Mahdi et al. [36]; Chowdhury et al. [38] the collected leaf litter and subsoil arthropods were classified into five categories based on the abundance of specimens. The categories were VC, Very Common (> 50 sightings); C, Common (31-50 sightings); FC, Fairly Common (16-30 sightings); R, Rare (6-15 sightings); and VR, Very Rare (1-5 sightings).

#### 2.6 Data Analysis

All of the data was analyzed using Microsoft Office Excel 19 and PAST 0.43 [39]. Relative abundance was calculated using the following formula [40].

Relative Abundance (RA) = Number of individual for a Particular Species / Total Captured Individuals \*100

The following diversity indices were used to estimate the biodiversity of leaf litter and subsoil arthropods in the study areas.

#### 2.6.1 Shannon diversity index

This index is commonly used to characterize species diversity in a community based on the mathematical theory of communication proposed by Shannon [41].

$$H' = -\sum_{i=l}^{S} p_i \ln p_i$$

Here,

H' = Shannon diversity index  $P_i =$  The proportion (n/N) of individuals of one particular species Ln = The natural logarithm S = Total number of Species

#### 2.6.2 Simpson's diversity index

It is a diversity index given by Simpson [42].

 $1 - D = \sum_{i=1}^{S} p_i^2$ 

Here,

D= Simpson index

 $P_i$  = Proportion of individuals Numbers of a Particular species found divided by the total number of individuals found.

S = Total number of Species

#### 2.6.3 Margalef diversity index

It is used as a simple measure of species richness, introduced by Margalef [43].

 $D_{Mg} = \frac{(S-1)}{\ln N}$ 

Here,

 $D_{Mg}$  = Margalef Diversity index S = Total number of Species N = Total Number of Individuals Ln = The natural logarithm

#### 2.6.4 Menhinic richness index

The index was introduced by Menhinick, and it is used to estimate species richness, but it is independent of the sample size [44].

$$D_{Mn} = \frac{S}{\sqrt{N}}$$

Here,

 $DM_n$  = Menhinick richness index S = Total number of Species N = Total number of individuals

#### 2.6.5 Berger-parker dominance index

This index was proposed by Berger and Parker in 1970 [45]. It is an intuitively simple dominance measure and reveals the proportional abundance of the most abundant species.

 $d = \frac{Nmax}{N}$ 

Here,

 $\label{eq:max} \begin{array}{l} d = Berger-Parker \ dominance \ index \\ N_{max} = The \ number \ of \ individuals \ of \ the \ most \\ abundant \ species \\ N = Total \ number \ of \ individuals \end{array}$ 

#### 2.6.6 Pielou's evenness index

It was attained from the Shannon index by Pielou [46]. The proportion of the observed value of the Shannon index to the maximum value shows the Pielou evenness index result. The values are between 0 and 1. When the value is closer to 1, it indicates that the individuals are distributed equally or evenly.

$$\mathsf{J}' = \frac{\mathsf{H}'}{\mathsf{H}_{\max}}$$

Here,

J' = Pielou's evenness index H' = The observed value of Shannon-Weiner index  $H_{max} = lnS$  S = Total number of species Ln = The natural logarithm

#### 3. RESULTS AND DISCUSSION

The present study was conducted from November 2021 to October 2022 to collect leaf litter and subsoil arthropods from four different sites of three agroecological zones in the northwest part of Bangladesh. In this study, a total of 1920 individuals belonging to 34 species, 28 families and 17 orders were found where 1099 individuals were collected from Rajshahi University Campus (Site A), 259 individuals were found in Rajshahi Fruit Research Center (Site B), 354 individuals were found in Kakon Hat Municipality (Site C), and 208 individuals were found in Chalan Beel (Site D), Natore. Among the collected species *Lasioderma sp.*(n=189) was the most dominant with 9.84% of the total number of leaf litter and subsoil arthropods. About 7.34% were *Ponera sp.* (n=141), followed by *Philonthus sp.* (n=134, 6.98%). The lowest populations were found in *Alphitobius sp., Psychoda sp.* and *Hypoaspis sp.,* where the abundance and relative abundance were 8 and 0.42%, respectively (Table 1).

In Site A, Odontotermes sp. (n=115) was the most dominant species, and Alphitobius sp. (n=8) and Hypoaspis sp. (n=8) were recorded as the least dominant species. Philonthus sp. (n=61) was the most dominant species in Site B, whereas Philoscia sp. was recorded as the least dominant species. In Site C, Ponera sp. (n=64) was the most abundant species, and Psychoda sp. (n=6) was the least abundant species. Tetramorium sp. (n=55) was highly abundant in Site D and Psychoda sp. (n=2) was also least abundant in this site (Table 1).

The collected leaf litter and subsoil arthropods were categorized as follows: 15 were very common (VC), 5 were common (C), 7 were fairly common (FC), and 7 were rare (R). Four categories of individuals were found in the study (Table 1). In this study, the highest number of individuals were recorded in agroecological zone, Site A (n=1099, 57.24%), followed by Site C (n=354, 18.44%) and the lowest number in Site D (n=208, 10.83%) (Fig. 1).

In this study, within the three agroecological zones, the highest number of individuals was recorded in the family Formicidae (n=376), and the second largest family was Staphylinidae (n=198), followed by Ptinidae (n=189). The most minor families were Tenebrionidae (n=8), Psychodidae (n=8) and Laelapidae (n=8) (Fig. 2).

Among the 17 orders, Coleoptera was the most dominant order (n=624, 32.5%), followed by Hymenoptera (n=414, 21.56%) and the minimum number of individuals was found in the order Metastigmata (n=8, 0.42%) (Fig. 3).

Different diversity indices were used to calculate species diversity, richness, dominance and evenness of leaf litter and subsoil arthropods of the four respective areas. Based on the Shannon

diversity index value (3.09), the Site A was the most diverse community, and Site D was the least diverse (1.96) community. The values of the Simpson diversity index also indicated that Site A was the most diverse (0.95) community, whereas Site D was the least diverse (0.83) community. The values of the Margalef diversity index were highest (3.86) in Site A and lowest (1.26) in Site B. That means species richness was highest in Site A and lowest in Site B. The highest value of the Menhinick richness index was 0.84 in Site A, and the lowest value (0.50) in Site B. These values indicated that most species were found in Site A and the most minor species were found in Site B. The value of the Berger-Parker dominance index was greatest (0.26) in Site D and lowest (0.10) in Site A. These values indicated that a particular taxon was dominant in Site D and no distinct taxa was dominant in Site A, which means Site A was the most diverse community and Site D was the least diverse community. The value of Pielou's evenness index was most excellent (0.95) in Site B and lowest (0.85) in Site D. That means the species were most evenly distributed in Site B and least evenly distributed in site D (Table 2).

This is the first report on leaf litter and subsoil arthropods in the northwest part of Bangladesh. As a result of this study, a total of 1920 individuals under 34 species were recorded. In this study, Coleoptera was the most dominant taxa, comprising of 32.50% of total individuals, and Hymenoptera was the second largest taxa, sharing with 21.56% of total individuals. Previous investigation on the population abundance and diversity of soil-dwelling arthropods in Site A showed that Coleoptera was the most dominant sharing 37.67% of total captured taxon. arthropods, and Hymenoptera was detected as the second largest taxon, sharing 28.77% of total collected arthropods [32]. In a study on soil arthropods in Rajegwesimeru Betiri National Park, Hymenoptera was the most dominant taxon, followed by Coleoptera, with 283 out of 702 individuals identified as Hymenoptera and 145 as Coleoptera [47]. Mahdi et al. [32] studied soil-dwelling arthropods in Rajshahi University Campus and discovered that Formicidae was the most dominant family. In this study, Formicidae was investigated as the most dominant taxa also. In Site A, most of the taxa were found because of its high plant diversity and abundance. In Site B, the abundance of the plant was high, but plant diversity was lesser than that of Site A. In Site C, the number of high plants was less, but there was a different type of grasses on the floor of the

site. In Site D, tall plants were very few actually. it was a seasonal wetland. So, in the Site D, the abundance of species was low. This finding was supported by Koricheva & Hayes [48], who observed that species richness and dominance of arthropods were higher in a community with diverse plant groups. Whitlock illustrated that plant genetic diversity has a positive influence on plant productivity, serving as an efficient food source for more herbivore individuals [49]. According to the Shannon diversity index, Site A (3.09) was the most diverse community, and Site D (1.96) was the least diverse community. Site B and Site C were medium-diverse communities. Simpson's diversity index value also showed a similar result for all four sites. According to the Margalef diversity index value, most species were found in Site A (3.86) and the least number of species were found in Site B (1.26). Menhinick richness index values showed a similar result that Site A (0.84) was the richest area and Site B (0.50) was the least rich area, whereas Site C (0.69) and Site D (0.69) were medium-rich areas. According to Berger-Parker dominance index value, a particular taxon was dominant in site D (0.26), and no specific taxa was dominant in Site A (0.10). Pielou's evenness index value was most significant in Site B (0.95) and lowest in Site D (0.85). That means in Site B, the species were most evenly distributed though the number of species was least and in Site D, the species were most unevenly distributed, whereas the values of Pielou's evenness index were the same in Site A and Site C and that was 0.93.

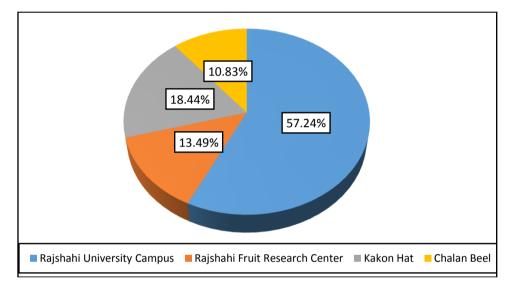


Fig. 1. Site-wise abundance of leaf litter and subsoil arthropods

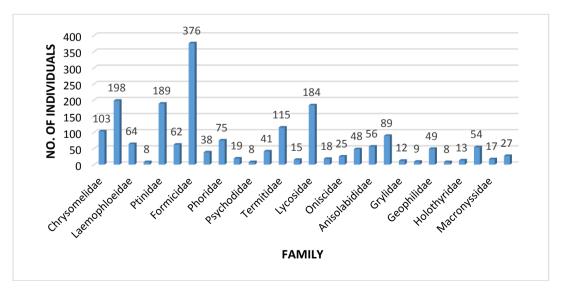


Fig. 2. Family-wise abundance of leaf litter and subsoil arthropods

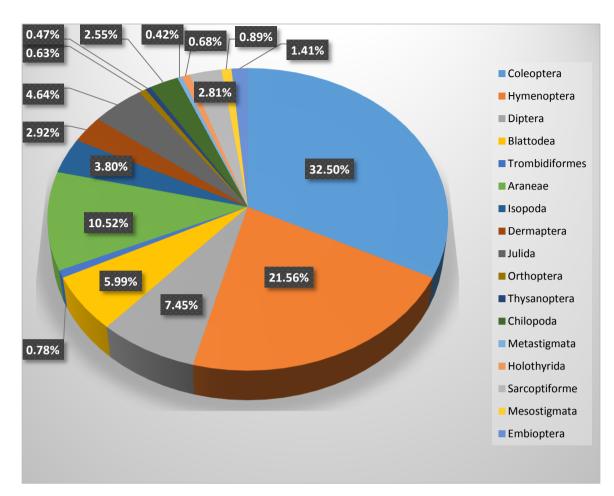
| Order          | Family          | Species               | Site A | Site B | Site C | Site D | Total | RA   | Status |
|----------------|-----------------|-----------------------|--------|--------|--------|--------|-------|------|--------|
|                | Chrysomelidae   | Chrysolina            | 29     | -      | -      | -      | 29    | 1.51 | FC     |
|                |                 | Callosobruchus sp.    | 16     | -      | 38     | 20     | 74    | 3.85 | VC     |
|                | Staphylinidae   | <i>Oxypoda</i> sp.    | 44     | -      | 20     | -      | 64    | 3.33 | VC     |
|                |                 | Philonthus sp.        | 73     | 61     | -      | -      | 134   | 6.98 | VC     |
| Coleoptera     | Laemophloeidae  | Cryptolestes sp.      | 27     | -      | 37     | -      | 64    | 3.33 | VC     |
|                | Tenebrionidae   | Alphitobius sp.       | 8      | -      | -      | -      | 8     | 0.42 | R      |
|                | Ptinidae        | Lasioderma sp.        | 94     | 39     | 16     | 40     | 189   | 9.84 | VC     |
|                | Curculionidae   | Sitophilus sp.        |        | -      | -      | 39     | 39    | 2.03 | С      |
|                |                 | Scolytus sp.          | 23     | -      | -      | -      | 23    | 1.20 | FC     |
| 11             | Formicidae      | Ponera sp.            | 77     | -      | 64     | -      | 141   | 7.34 | VC     |
|                |                 | Tetramorium sp.       | 52     | -      | -      | 55     | 107   | 5.57 | VC     |
| Hymenoptera    |                 | Acropyga sp.          | 83     | 45     | -      | -      | 128   | 6.67 | VC     |
|                | Eurytomidae     | Sycophila sp.         | 31     | -      | -      | 7      | 38    | 1.98 | С      |
|                | Phoridae        | Apocephalus sp.       | 29     | -      | 31     | 15     | 75    | 3.91 | VC     |
| Distance       | Simulidae       | Simulium sp.          | 12     | -      | 7      | -      | 19    | 0.99 | FC     |
| Diptera        | Psychodidae     | Psychoda sp.          |        | -      | 6      | 2      | 8     | 0.42 | R      |
|                | Ceratopogonidae | Leptoconops sp.       |        | -      | 22     | 19     | 41    | 2.14 | С      |
| Blattodea      | Termitidae      | Odontotermes sp.      | 115    | -      | -      | -      | 115   | 5.99 | VC     |
| Trombidiformes | Tetranychidae   | Tetranychus sp.       | 15     | -      | -      | -      | 15    | 0.78 | R      |
|                | Lycosidae       | Arctosa sp.           | 52     | 25     | 15     | 4      | 96    | 5.00 | VC     |
| Araneae        |                 | Hogna sp.             | 50     | 38     | -      | -      | 88    | 4.58 | VC     |
|                | Theridiidae     | Crustulina sp.        | 18     | -      | -      | -      | 18    | 0.94 | FC     |
| Isopoda        | Oniscidae       | Oniscus sp.           | 25     | -      | -      | -      | 25    | 1.30 | FC     |
|                | Philosciidae    | Philoscia sp.         | 35     | 13     | -      | -      | 48    | 2.50 | С      |
| Dermaptera     | Anisolabididae  | <i>Euborellia</i> sp. | 49     | -      | -      | 7      | 56    | 2.92 | VC     |
| Julida         | Julidae         | Julus sp.             | 65     | 24     | -      | -      | 89    | 4.64 | VC     |
| Orthoptera     | Gryllidae       | Gryllus sp.           | 12     | -      | -      | -      | 12    | 0.63 | R      |
| Thysanoptera   | Thripidae       | Thrips sp.            | 9      | -      | -      | -      | 9     | 0.47 | R      |
| Chilopoda      | Geophilidae     | Geophilus sp.         | 35     | 14     | -      | -      | 49    | 2.55 | С      |
| Metastigmata   | Laelapidae      | Hypoaspis sp.         | 8      | -      | -      | -      | 8     | 0.42 | R      |
|                | Holothyridae    | Holothyrus sp.        | 13     |        |        |        | 13    | 0.68 | R      |

# Table 1. Abundance and relative abundance of collected leaf litter and subsoil arthropods from Sites A to D

#### Mahdi et al.; Asian J. Res. Zool., vol. 6, no. 4, pp. 107-118, 2023; Article no.AJRIZ.108952

| Order         | Family        | Species               | Site A | Site B | Site C | Site D | Total | RA   | Status |
|---------------|---------------|-----------------------|--------|--------|--------|--------|-------|------|--------|
| Sarcoptiforme | Acaridae      | <i>Tyrophagus</i> sp. | -      | -      | 54     | -      | 54    | 2.81 | VC     |
| Mesostigmata  | Macronyssidae | Ornithonyssus sp.     | -      | -      | 17     | -      | 17    | 0.89 | FC     |
| Embioptera    | Oligotomidae  | Oligotoma sp.         | -      | -      | 27     | -      | 27    | 1.41 | FC     |
| 17            | 28            | 34                    | 1099   | 259    | 354    | 208    | 1920  |      |        |

Status: VC, very common (>50 sightings); C common 31-50 sightings); FC, fairly common (16-30 sightings); R, rare (6-15 sightings); VR, very rare (1-5 sightings). Site A, Rajshahi University Campus (Rajshahi); Site B, Fruit Research Center (Rajshahi); Site C, Kakon Hat Municipality (Rajshahi); Site D, Chalan Beel (Natore); RA, Relative Abundance



Mahdi et al.; Asian J. Res. Zool., vol. 6, no. 4, pp. 107-118, 2023; Article no.AJRIZ.108952

Fig. 3. Order-wise abundance of leaf litter and subsoil arthropods

| Diversity indices                           | Site A | Site B | Site C | Site D | Total |
|---|--------|--------|--------|--------|-------|
| Taxa (S)                                    | 28     | 8      | 13     | 10     | 34    |
| Individuals (N)                             | 1099   | 259    | 354    | 208    | 1920  |
| Shannon diversity index (H')                | 3.09   | 1.97   | 2.38   | 1.96   | 3.22  |
| Simpson diversity index (1-D)               | 0.95   | 0.85   | 0.89   | 0.83   | 0.95  |
| Margalef diversity index (D <sub>Mg</sub> ) | 3.86   | 1.26   | 2.04   | 1.69   | 4.37  |
| Menhinick richness index (D <sub>Mn</sub> ) | 0.84   | 0.50   | 0.69   | 0.69   | 0.78  |
| Berger-Parker dominance index (d)           | 0.10   | 0.24   | 0.18   | 0.26   | 0.10  |
| Pielou's evenness index (J')                | 0.93   | 0.95   | 0.93   | 0.85   | 0.91  |

#### 4. CONCLUSION

In conclusion, the abundance and diversity of leaf litter and subsoil arthropods play a crucial role in ecosystem dynamics and function. The findings of studies on the abundance and diversity of these arthropods shed light on the intricate web of life beneath our feet and the importance of preserving and conserving their habitats. The present study was the very first effort to make a checklist of leaf litter and sub soil arthropods in four sites of three respective agroecological zones of northwest part of Bangladesh and evaluate the population abundance, species diversity, species richness, species dominance and evenness of leaf litter and subsoil arthropods. A total of 1920 individuals were documented of 34 species, 28 families and 17 orders from all the four respective sites together. In this study many individuals were found but all were not listed due to lack of proper identification, only 34 species were listed. Further research may be necessary to detect most of the leaf litter and subsoil arthropods living in these areas.

### ACKHOWLEDGEMENTS

The authors acknowledge the support of the Department of Zoology, University of Rajshahi, Rajshahi, while conducting the study. They are also thankful to all teachers and students of the Laboratory of Crop Protection and Toxicology of the same university for their support.

# **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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Mahdi et al.; Asian J. Res. Zool., vol. 6, no. 4, pp. 107-118, 2023; Article no.AJRIZ.108952

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Peer-review history: The peer review history for this paper can be accessed here: https://www.sdiarticle5.com/review-history/108952