#### **Research Article**

Species Evenness and Diversity of Soil Invertebrates at Different Agricultural Lands and a Forest Reserve at Kota Belud, Sabah

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Received 26 September 2023 | Accepted 10 May 2024 | Published 31 August 2024 DOI: <u>https://doi.org/10.51200/jtbc.v21i.5389</u>

#### ABSTRACT

Increasing agricultural activities can lead to changes in soil ecosystems, potentially impacting soil invertebrate communities as they are highly responsive to soil disturbances. The composition of soil invertebrates within each agricultural habitat provides insights into how these communities respond to their environmental conditions. This study aimed to investigate the evenness and diversity of soil invertebrates in agricultural areas of Kota Belud, Sabah. Soil samples were collected from three different agricultural sites, a rubber plantation, an oil palm plantation, and a paddy field, as well as a forest reserve as a control site. Pitfall traps and Berlese-Tullgren funnels were used to collect soil invertebrates. A total of 180 soil samples and 474 individuals of soil invertebrates were collected and analyzed. Physicochemical analyses, including moisture content, soil texture, pH, soil nutrient content, and organic matter, were conducted on all soil samples to assess their influence on the composition of soil invertebrates. Results indicated that Julida, Coleoptera, Gastropoda and Araneae were frequently associated with high organic matter, pH, moisture content and phosphorus. On the other hand, Haplotaxida appeared to be more sensitive to potassium levels. Notably, the Blattodea, Orthoptera, Hymenoptera, Isopoda, Polydesmida and Diptera, were found to be abundant in areas with lower organic matter and pH in the control site, i.e., forest reserve area. These findings underscore the significance of soil layer activities in influencing the presence and survival of soil invertebrates. Given their vital role in sustaining life, prioritizing the enhancement of soil invertebrate populations is crucial, particularly in Sabah, a prominent food crop-producing state in Malaysia.

Keywords: Soil invertebrates; agriculture physicochemical; Sabah; evenness; diversity.

#### INTRODUCTION

Soil invertebrates are the communities that are responsible for a variety of functions in soil ecosystems both above and below ground; earthworms, termites, and ants are examples of "ecosystem engineers" that change the soil's physical composition and movement of nutrients, meanwhile the microarthropods, known as "litter transformers", break up decaying litter to increase the number of bacteria that can access it (Coleman et al., 1999). Despite the growing spotlight on these soil fauna, other factors, such as the acceleration of agricultural productivity, are given far greater consideration (Maqtan et al., 2021). The abundance of soil fauna gives positive results on the soil structure and fertility, e.g., by improving aeration, drainage,

biological pest management, soil mixing, specific cropping, transportation of microorganisms and enhancement of food and space conditions for microorganisms and higher plants (Hill, 1985).

To assess soil health, it is crucial to take into consideration both biological and physicalchemical soil characteristics and to see the influence of these indicators on the abundance and diversity of the soil fauna groups (Cardoso et al., 2013).

Therefore, the focus of the current research was to study the diversity of soil invertebrates by showing the relationship of their diversity with the physicochemical properties of the agricultural soil located in Kota Belud, Sabah. By taking a forest reserve as the control site, we can compare the difference in the effect of the environmental variables (soil organic matter, soil moisture content, pH, soil nutrients, soil texture) on the diversity of soil invertebrates among different agricultural activities.

#### MATERIAL AND METHODS

#### **Study location**

Extensive soil sampling was carried out on a wide range of agricultural lands and a forest reserve at Kota Belud, Sabah. Three study areas located at Kampung Sarang (6°34'14.9"N 116°33'03.6"E), Kampung Timbang (6° 29' 31.9986"N, 116.5495937E) and Kampung Dudar (6° 35' 22.3074"N 116° 33' 22.824"E) represent agricultural areas and each agricultural area has a different type of plantation, such as rubber, paddy, and oil palm respectively (Figure 1). Meanwhile, the forest reserve is located at Kampung Ulu Kukut and is gazetted under the Forests (Amendment) Enactment 2014, as a Class 1 forest reserve. We observed that, in terms of their soil type, the forest reserve soil predominantly, consisted of a mixture of sandy clay loam meanwhile rubber plantation was in sandy loam soil, oil palm plantation was clay loam soil and paddy was cultivated in sandy clay loam soil.



Figure 1: Locations of soil sampling at different plantation types and a forest reserve in Kota Belud, Sabah. Base map modified from Google Maps.

## Sampling method

Two sampling methods were applied to collect samples of the soil invertebrates, namely pitfall trap for mobile invertebrates and modified Berlese Tullgren funnels for soil micro invertebrates where soil macroinvertebrates were heat-extracted from the soil samples (Tuf, 2015). In each sampling plot, a quadrant of  $30m \times 30m$  was set up and applied in this study. Each quadrant was divided into 5 line transects and each line transect consisted of 3 pitfall traps with measurements of 10 cm depth and 10 cm diameter. The distance for each trap was 10 meters (Figure 2). A total of 15 pitfall traps were installed at each plot using a soil auger. Extraction of invertebrates inside the 10 cm soil cores was directly made using the hand-collection method. Extracted invertebrates were kept inside a specimen bottle with 70% alcohol solution for preservation. After the hand-collecting method was performed, the soil samples were then put into Berlese Tullgren funnels for 3 days to extract the remaining macroinvertebrates. The same 10 cm soil core sample that was taken from each plot was transferred to the laboratory for soil physicochemical analysis.

Three replicates of soil sampling were performed at each site, as per the methodology outlined by Arshad and Martin (2002). Specifically, from each study site, 15 pitfall traps were excavated, and only 9 soil cores were collected from these traps making a total of 27 soil samples (N=27) for each study site. This procedure yielded a sufficient total of 108 soil samples, which were subsequently transferred to the laboratory for further physicochemical analysis.



Figure 2:  $30m \times 30m$  quadrant used in this study.

# Soil invertebrate identification

Soil invertebrates were identified in the laboratory through meticulous examination employing a laboratory stereo microscope, following an established identification key (Saxena & Rao, 2015). Each soil invertebrate specimen was systematically identified to the Order level. Subsequently, the abundance of each Order was recorded by aggregating the total number of individuals within each Order.

# Soil physicochemical parameters

## Soil moisture content

In this study, 10g of air-dried soil samples were taken and sieved before being placed in an oven at 105°C for 24 hours. Soil samples were cooled in a desiccator before being weighed. The percentage of moisture content (MC) in the soil was recorded using the formula below (Shukla et al., 2014):

MC % = (<u>Weight of air - dried soil + porcelain basin</u>) - (<u>Weight of oven-dried soil + porcelain basin</u>) × 100 (Weight of oven - dried soil + porcelain basin) - Weight of porcelain basin

#### Soil organic matter

In the analysis of organic matter in soil samples, the Loss of Ignition (LOI) method was conducted (Heire et al., 2001). Soil samples from the moisture content determination above were then put in the furnace at 500°C for 24 hours. After drying overnight, the samples were cooled in the desiccator before the weighing process. The percentage of organic matter in the soil was calculated by using the formula as stated according to Heire et al. (2001):

 $\begin{array}{l} \text{Organic matter } \% = (\underbrace{\text{Weight of oven} - \text{dried soil} + \text{porcelain basin}) - (\underbrace{\text{Weight of mass of ignited soil} + \text{porcelain dish})}_{(\text{Weight of oven} - \text{dried soil} + \text{porcelain basin}) - \text{weight of porcelain dish})} \times 100 \\ \end{array}$ 

## Soil pH

In this study, the pH of soil samples was taken by first weighing 10g of air-dried soil, putting it into a beaker, then adding 25 ml of distilled water to the soil sample. The mixture was then stirred and left for 30 minutes. After 30 minutes, a pH meter was used to measure the pH. pH was measured 3 times for each sample, with the electrode washed with distilled water between each reading.

#### Percentage composition

Calculations were made to determine the percentage composition as well as the relative abundance of the observed individuals at each study site. Relative abundance is the percentage composition of each order recorded at each study site relative to the number of organisms in the area (Hamzah et al., 2020). Percentage composition was calculated by dividing the number of individuals for each order by the total number of abundance from each site (Bufebo et al., 2021).

Percentage Composition =  $\underline{\text{Number of individuals in each order}} \times 100$ Number of abundances of all orders from each site

#### **Biological indices**

The diversity of soil invertebrates was calculated using PAST 4.03 software (Hammer et al., 2001). Four diversity indices were computed namely Shannon-Wiener, Evenness, Simpson and Menhinick's indices to compare the diversity of soil invertebrates in the forest reserve and the three different agricultural areas.

#### Shannon-Wiener Index (Shannon and Wiener, 1949)

Species diversity was calculated using a formula by Shannon and Wiener (1949) as below:

 $H' = -\Sigma Pi(lnPi)$ 

Where;

H' = Shannon-Wiener Diversity Index

Pi = Proportion of grand total of abundance recorded by each taxon

# Evenness

E=H/Hmax, Hmax = ln (N)

Where; E= evenness H= Shannon-Wiener Diversity Index Hmax = Natural Logarithm of the number of specific categories (Number of samples in the study area)

## **Species Richness**

In this study, species richness was calculated based on the taxa collected from each study area. Species richness is simply defined as the total number of species in an assemblage of a sample (Gotelli & Chao, 2013). Other than that, species richness was measured by computing Menhinick's index (Shah & Pandit, 2013) based on the data collected from all study sites.

## Statistical analysis

The differences in the relative abundance of recorded soil invertebrates from each study site were statistically analysed using IBM SPSS Statistics software version 28. A normality test was conducted on the data and the value for Shapiro-Wilk showed that the data collected from each study site was normal (P > 0.05). Thus, a comparison of the differences in the relative abundance for each taxon between different study sites was calculated using one-way analyses of variance (ANOVA) followed by Tukey's significant difference (HSD) posthoc tests. Finally, a canonical-correlation analysis (CCA) was conducted by using PAST software (4.03) to see the relationship between the soil physicochemical parameters and the abundance of soil invertebrates at each study site.

# **RESULTS AND DISCUSSION**

## Relative abundance and percentage composition of soil invertebrates

A total of 496 individuals were collected composed of 17 Orders throughout the sampling period (Table 1). The highest abundance of soil invertebrates was recorded from the forest reserve (234 individuals, 16 Orders), followed by rubber plantation (110 individuals, 12 Orders), oil palm plantation (86 individuals, 6 Orders) and paddy field (65 individuals, 8 Orders) (Table 2). Altogether, the forest reserve was the most order-rich site, while the least number of Orders were found at oil palm plantations (44 Hymenoptera, 17 Haplotaxida, 5 Coleoptera, 12 Blattodea, 2 Orthoptera). Haplotaxida, Coleoptera and Araneae were found in both agricultural sites and the forest reserve. Hymenoptera recorded the highest abundance in the forest reserve with 96 individuals (40.85%) and 44 individuals (51.16%) in the oil palm plantation. Followed by Haplotaxida which showed the highest abundance in the rubber plantation with 66 individuals (60%), and Coleoptera showing the highest abundance in the highest number of abundances of Hymenoptera, Haplotaxida and Isopoda lead to the highest number of abundances among the study sites showing their superiority over other organisms (Table 1). However, there was no significant difference in the relative abundances among the study sites (P > 0.05).

Our findings indicate that the oil palm plantation typically lacks species that are more common in the forest reserve (Table 1). In the oil palm plantation as opposed to the forest reserve, largebodied species and those from higher trophic levels were significantly rarer, similar to finding of Senior et al. (2013). In addition, less disturbance (Class 1) and better soil cover are the fundamental characteristics of a forest reserve, which improve the circumstances for the existence of soil organisms.

Taxon	Forest Reserve	%	Rubber Plantation	%	Oil Palm Plantation	%	Paddy Field	%	Total
Hymenoptera	96	40.85	11	10.00	44	51.16	0	0.00	
Haplotaxida	32	13.62	66	60.00	17	19.77	13	20.00	
Isopoda	28	0.12	9	8.18	0	0.00	5	7.69	
Coleoptera	21	8.94	4	3.64	6	6.98	32	49.23	
Araneae	15	6.38	7	6.36	5	5.81	8	12.31	
Blattodea	11	4.68	1	0.91	12	13.95	0	0.00	
Othoptera	7	2.98	1	0.91	2	2.33	0	0.00	
Gastropoda	4	1.70	2	1.82	0	0.00	2	3.08	
Julida	2	0.85	1	0.91	0	0.00	1	1.54	
Geophilomorpha	2	0.85	1	0.91	0	0.00	3	4.62	
Polydesmida	2	0.85	0	0.00	0	0.00	0	0.00	
Protura	2	0.85	0	0.00	0	0.00	0	0.00	
Diptera	6	2.55	0	0.00	0	0.00	0	0.00	
Glomerida	1	0.43	0	0.00	0	0.00	0	0.00	
Hemiptera	5	2.13	6	5.45	0	0.00	1	1.54	
Archaeognatha	1	0.43	0	0.00	0	0.00	0	0.00	
Collembola	0	0.00	1	0.91	0	0.00	0	0.00	
Total nr. Ab.	235		110		86		65		496

**Table 1:** Abundance and percentage composition of soil invertebrates collected from different study sites in Kota

 Belud, Sabah.

# **Diversity of soil invertebrates**

The community of soil invertebrates in agricultural areas was less diverse and showed lower species richness (Order level) than the forest reserve (Table2.) Shannon-wiener diversity differs among sites (Table 2) where the highest diversity was recorded in the forest reserve (H'= 1.984). The values of Simpson, Evenness and Menhinick indices were again highest in the forest reserve (0.7843, 0.4546 and 1.042 respectively) while they were lower in the rubber plantation (0.5927, 0.5457 and 0.6928 respectively), oil palm plantation (0.5927, 0.5457 and 0.6928, respectively) and paddy field (0.6649, 0.5679 and 0.889, respectively) (Table 2.). Species richness was highest in the forest reserve (16 Orders) and lowest at the oil palm plantation (6 Orders) (Table 2).

The forest reserve had more species diversity (Order level), explaining the fact that the conversion of forests into agricultural areas negatively affects soil invertebrate species diversity (Gibson et al., 2011). Diversity has been shown to gradually decline as land use in the system became more intensive (Flynn et al., 2009). The abundance and diversity of soil invertebrates are believed to fluctuate depending on residue inputs and soil management practices. Other studies have shown that monoculture agriculture has lower diversity compared to agroforestry (Sisay & Ketema, 2015).

		Forest Reserve	Rubber Plantation	Oil Palm Plantation	Paddy Field	Р
Mean (SD)		14.69 (23.81)	9.17 (18.24)	14.33 (15.50)	8.86 (11.10)	0.844
Diversity Parameters	Richness	16	12	6	7	
	Shannon	1.984	1.483	1.186	1.38	
	Simpson	0.7843	0.6142	0.5927	0.6649	
	Evenness	0.4546	0.367	0.5457	0.5679	
	Menhinick	1.042	1.144	0.6928	0.889	

**Table 2:** Mean (SD) of relative abundance of soil invertebrates and values of diversity parameters from each study site in Kota Belud, Sabah.

The mean relative abundance of soil invertebrates across the studied sites was highest at a value of 14.69 (23.81) in the forest reserve area (Table 2) However, these differences were not statistically significant (P = 0.844), suggesting relatively consistent abundance levels across the different land-use types. Nevertheless, the analysis of diversity parameters revealed variations in species richness and diversity indices among the study sites. The groups of soil invertebrates living in the forest reserve may have benefited from better quality of litter such as decomposed leaves from trees that may have encouraged the presence of ants (Hymenoptera). The availability of surface on the soil may influence the occurrence of litter-invertebrates (Burghouts et al., 1992).

## Physicochemical characteristics of soil

The soil physicochemical parameters were measured and showed significant differences among study sites (Table 3). Soil physicochemical characteristics influenced the abundance and diversity of soil invertebrate communities including soil moisture content, pH, and organic matter. The forest reserve had higher soil moisture content compared to two agricultural areas yet there was no significant difference in soil moisture content of the forest reserve and the paddy field which were 21.41% and 20.00% respectively. This was due to the type of soil in the paddy field which was sandy loam and its watery characteristics; greyish colour and subangular blocky structure reflected flooding and high organic matter (Dou et al., 2016). In Table 3, the paddy field recorded the highest organic matter which was 7.00 %. Organic matter content may also be impacted by crop species and soil types (Zhou et al., 2014). All study sites had acidic soil where the forest reserve had a pH of 4.90, the rubber plantation a pH of 4.44, the oil palm plantation a pH of 4.48 and the paddy field a pH of 5.54.

The level of organic matter produced affects the soil water and pH with significant effects on the community of soil invertebrates. The forest reserve and the paddy field showed normal moisture content level, while the rubber plantation and the oil palm plantation recorded lower soil moisture content level that showed the soil was drier compared to the forest reserve and paddy field. Other studies showed that soil moisture content in an oil palm plantation is lower than in a secondary forest (Ngau et al., 2022). The conversion of forests into monoculture plantations affects the soil quality including soil moisture content and soil organic matter (Nguyen et al., 2020). The natural habitat in which the soil invertebrates dwell may be impacted by an ecological imbalance caused by any significant changes to the environmental variables (Bufebo et al., 2021).

**Table 3:** Physicochemical properties of soil in different agricultural activities and a forest reserve, in Kota Belud, Sabah. All data are represented in Mean  $\pm$  SD (N=27) followed by different letters that showed significant differences at P < 0.05.

	<b>Forest Reserve</b>	<b>Rubber Plantation</b>	<b>Oil Palm Plantation</b>	Paddy Field
Moisture content %	$21.41\pm7.95^{\rm a}$	$8.03\pm4.57^{\rm b}$	$6.26\pm2.98^{\text{b}}$	$20.00\pm10.37^{\rm a}$
pН	$4.90\pm0.86^{\rm b}$	$4.44\pm0.48^{\rm b}$	$4.48\pm0.49^{\rm b}$	$5.54\pm0.59^{\rm a}$
Organic matter %	$5.08 \pm 1.43^{\mathrm{b}}$	$4.22\pm1.63^{\rm b}$	$4.25\pm1.23^{\mathrm{b}}$	$7.00\pm2.63^{\rm a}$

#### Relationship of soil physicochemical characteristics and abundance of soil invertebrates

CCA is a multivariate analysis, an efficient way to relate the distribution of soil invertebrates with soil physicochemical parameters (soil organic matter, soil moisture content and pH). The CCA plot (Figure 3) revealed that the soil physicochemical influenced the abundance and distribution of soil invertebrates and the relationship between the presence of soil invertebrates with soil organic matter, moisture content and pH. Based on the CCA, Coleoptera and Geophilomorpha were abundant in the paddy field, where most of the Coleoptera were composed of ground beetles (Family: Carabidae). In high-yielding rice types, these insect pests and their natural opponents were more prevalent (Hussain Khan, 2013). In Figure 3, both Coleoptera and Geophilomorpha were associated with high soil moisture content and pH, but lower organic matter. Meanwhile, Glomerida, Archaeognatha and Polydesmida seem to be more sensitive to higher soil organic matter as well as both Hymenoptera and Orthoptera. Isopoda is located between higher organic matter and moisture content and more likely a lower pH. Next, Hemiptera seems to be more sensitive towards these three variables (organic matter, moisture content and pH). Lastly, Collembola located far from the point of three variables revealed this taxon seems to be not affected by these 3 environmental variables.



Figure 3: Canonical Correspondence Analysis (CCA) plot for the abundance of soil invertebrates in the different study sites in Kota Belud, Sabah.

## CONCLUSION

The abundance of soil invertebrates depends on land use practice. Observing the results of this study, it is important to consider the activity in the soil layer that affects the existence and survival of soil invertebrates. Given their importance in maintaining life, proper attention should be paid to enhance the presence of soil invertebrates especially in Sabah, which is one of the food crop-producing states in Malaysia. This study is significant in that it adds baseline information to researchers and parties involved.

## ACKNOWLEDGEMENTS

We would like to thank Universiti Malaysia Sabah for the research grant (SDN0074) and the Institute for Tropical Biology and Conservation for the technical support. We would also like to thank Sabah Biodiversity Council (SaBC) for the access license to collect soil samples and soil invertebrate specimens at Ulu Kukut, Forest Reserve, Kampung Sarang, Kampung Dudar and Kampung Timbang with Licence Ref. No. JKM/MBS.1000-2/2 JLD.13 (115). Our appreciation goes to all the officers and rangers at the forest reserve for providing facilities and support.

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