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Research Article

Metagenomics Shows That Termite Activities Influence the Diversity and Composition of Soil Invertebrates in Termite Mound Soils

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Background. Soil invertebrates are a significant part of the functioning and biodiversity of engineered soil. Nevertheless, it remains unclear how termite bioturbation that promotes soil nutrients affects the diversity and composition of invertebrates that dwell in soils from termite mounds. Therefore, we tested the premise that the rich nutrients accrued in soils from termite mounds encourage a complex variety of soil invertebrates. *Methods*. Whole DNA was extracted from soils from termite mounds and adjacent soils that were 10 m away from the mound. The soil samples were then sequenced using metagenomics. *Results*. Disparity in the composition of the soil invertebrate communities between the termite mound and their adjacent soils was clear from the results. Also, principal coordinate analysis showed that the structure of the soil invertebrate communities in termite mound soils was distinctive from that of the adjacent soils. The canonical correspondence analysis showed that phosphorus, soil pH, and soil organic carbon were the environmental factors that significantly explained the variation in the composition and diversity of the soil invertebrate communities between the two habitats. *Conclusion*. Metagenomics and chemical analysis jointly offered a route to examine the compositional and diversity variations in soil invertebrate communities in relation to termite bioturbation.

1. Introduction

Termites contribute significantly to regulating soil activities such as water preservation [1], nutrient decomposition [2], and the development and maintenance of soil structure [3, 4]. Data show that mound-constructing termites transform soil physicochemical properties by accumulating organic-mineral materials from diverse depths and dropping them in termite mound soil [5–7]. Therefore, termite mound soil is richer in nutrients than the adjacent soil [8]. Due to termite bioturbation and over a long period of time, termite mounds are weather-beaten, and their materials that are made up of rich soil nutrients are redistributed to the soil surface, possibly forming a soil environment more advantageous to soil health, plant development, and soil invertebrates [9].

Alterations in the fauna of disturbed sites might be assessed by examining the communities of biological indicators such as soil invertebrates. Soil invertebrates consist of many organisms that significantly contribute to ecological processes [10]. They form a series of trophic chains and serve as food for other living things, and they contribute to organic matter breakdown and nutrient recycling. They act as seed dispersers, predators, pollinators, and herbivores [11]. The role performed by these soil organisms is greatly linked to ecological conditions relating to chemical, physical, and structural changes in the environment [12]. Considering the significance of soil invertebrates for ecological functions, the goal of this study was to examine the change in the composition and diversity of soil invertebrate communities both in termite mound soils where termite activities are present and in adjacent soils without tangible termite activities.

Profiling the soil invertebrate community structure and diversity is vital, especially when formulating effective schemes for restoration and monitoring programs. Therefore, we tested the assumption that the rich soil nutrients accumulated in termite mounds influence the diversity of soil invertebrates from termite mound compared with soil invertebrates present in adjacent soils. This hypothesis was founded on the notion that two main fundamental soil properties (organic carbon and pH) known to drive the community structure of soil organisms are normally dissimilar between soils from termite mounds compared to nearby soils [8, 13].

Soil invertebrates, which are highly diverse, prevalent, and sensitive to ecological disturbances, form an expedient means of assessing the health of ecosystems [13]. However, the customary, manual taxonomic method based on morphological identification is very time- and resource-intensive, expensive, and laborious for routine biomonitoring [14]. Metagenomics is anticipated to overcome these drawbacks, and likewise, the process saves time, and it is more precise [15]. In past decades, metagenomics has mainly been used for various medical purposes and for microbial investigation. Only a limited number of studies have targeted the diversity and composition of metazoans, phytoplankton, macroinvertebrate larvae, and multiple taxa using metagenomics [15, 16]. Here, we present the first metagenomic examination of termite mound soil invertebrate communities based on Illumina MiSeq shotgun sequencing. This case study assesses the prospect of metagenomics as an instrument to monitor soil biodiversity as well as reveal previously unidentified species.

2. Materials and Methods

2.1. Study Locations and Soil Collection. Braklaagte and Zeerust (both South Africa) with the respective coordinates 25°26′13.5″S 26°05′50.4″E and 25°27′11.2″S 26°07′33.8″E serve as the study sites. They have a climate that is semidry, with winter (17° to 31°C) and summer (3° to 21°C). These study sites are located in the North-West Province with erratic and highly variable rainfall with a mean annual rainfall of 360 mm. We used a soil auger (diameter = 5 cm) to obtain four soil samples (50 g each) from termite mound from Braklaagte (T1a–d) and Zeerust (T2a–d), respectively. The samples were collected at a depth of 15 cm into the bottom of the mound where most of *Coptotermes* activities had an impact by constructing a carton nest that is rich in organic materials like cellulose and chewed wood, bound together with feces and saliva [17]. For morphological identification of *Coptotermes*, we targeted soldier termites using the procedure of Arif et al. [18] and Takematsu and Vongkaluang [19]. We further obtained four (50 g) soil samples from 0–15 cm depth [20, 21] from adjacent soils where termite activities were not felt from Braklaagte (S1a–d) and Zeerust (S2a–d). The termite mounds were 10 m apart from the adjacent soils [8]. The collected soil samples were well-preserved transitory in ice pack while still in the sites and then moved that same day to the Microbial Biotechnology Laboratory at North-West University, Mafikeng Campus, where they were sieved and each equally split into two parts, one for soil analysis (stored at 4°C) and the other for DNA extraction (stored at −20°C), within two weeks.

2.2. Analysis of Soil Physicochemical Properties. With the aid of a sieve, we removed dirt and debris from the soil samples prior to physical and chemical analysis of the samples. The parameters analyzed include the particle size analyses (clay, silt, and sand), potassium (K), magnesium (Mg), calcium (Ca), pH, organic carbon (OC), phosphorus (P), and total nitrogen following [22].

2.3. DNA Extraction, Illumina MiSeq, and Data Analysis. Of the soil samples collected, only 0.25 g from each was used for DNA extractions. We used the DNA Isolation kit (Power Soil) designed by MoBio Laboratories Incorporated for the extraction by following the manufacturer's protocol. Thereafter, the DNA concentrations were estimated with the Quant-iT PicoGreen dsDNA kit and subsequently assessed on a DQ 300 fluorometer. The dataset was then generated using a shotgun sequencing approach. For library preparations and for the determination of the library insert size, we used Nextera DNA Sample Preparation Kit (Illumina) and the Experion Automated Electrophoresis Station (Bio-Rad), respectively. 50 ng of DNA from each sample was used for the library preparations, while the library insert size was 500 bp on average. The respective library was put into a 600 cycles' V3 reagent cartridge and then sequenced with a 2×250 bp sequencing run on the Illumina MiSeq 2500 platform.

The MG-RAST pipeline [23] was used for the downstream analysis of our raw sequences. First, quality control (dereplication, ambiguous base (>5 bp, Phred score cutoff = 15), and host-specific species sequence filtering) was done in the pipeline. We also removed artefacts as determined by the pipeline. Thereafter, the remaining high-quality sequences were annotated with the BLAT algorithm and the M5NR database (a database that offers nonredundant integration of many databases) [24]. SEED Subsystem databanks were used for the taxon categorization under the following settings: a minimum identity of 60%, an *e*-value of $1e-5$, and 15 bp alignment lengths. We also turned on the normalized data selection of the pipeline to reduce the impact of the experimental noise. Since our focus was on soil invertebrates, we removed sequences that could not be properly annotated, as well as sequences stemming from other eukaryotes than soil invertebrates, prokaryotes, viruses, and fungi. The resultant taxonomic table was grouped properly to their respective taxon, and we retained the uncategorized reads for the purpose of statistics. Finally, the taxon abundances were expressed in percentages.

To evaluate the changes in the physicochemical properties between the comparable sites (i.e., T1 versus S1 and T2 versus S2), we employed ANOVA statistics (also used to check for any possible significant differences). We did Tukey's pairwise comparison test (p-value <0.05). To evaluate the alpha diversities of the soil invertebrates among the termite mound and the adjacent soils, we used the Shannon

and evenness indices and tested for significance using the Kruskal–Wallis test. These analyses were done using the Paleontological statistics software package (PAST) version 3.20 [25]. To depict the beta diversity between the soil samples, principal coordinate analysis (PCoA) was used, while the analysis of similarities (ANOSIM) was used to calculate (through p- and R-values) the strength of the significance [26]. The canonical correspondence analysis (CCA) was used to evaluate the environmental factors that best elucidate the differences in the soil invertebrate compositions. To test the significance of the CCA plot, we used the Monte Carlo permutation test with 999 random permutations. We used the Shinyheatmap (to plot the Heatmap) and the Circos software (https://circos.ca/) to show the relative abundance of soil invertebrate taxonomic groups [27].

3. Results

3.1. Analysis of Soil Properties from Both Soil Samples. Assessment of the soil physicochemical properties (Table 1) revealed higher values of K, Ca (except for T1a), and clay (except in T1b) in soils from termite mounds in relation to the adjacent soils. Conversely, the values of N and pH in adjacent soil samples were higher than those in the termite mound soils.

3.2. Analyses of the Sequencing Data. Prior to sequence quality control, 6,120,406–7,604,095 (T1) and 5,583,309–7,775,745 (T2) sequences were obtained from the termite mound soils while 5,377,078–8,524,325 (S1) and 4,723,364–9,271,857 (S2) were obtained the adjacent soils. After quality control was done in MG-RAST, 5,199,839–8,337,673 (S1) and 4,618,194–9,096,156 (S2) sequences were retained from the adjacent soils, while 5,932,964–7,376,055 (T1) and 5,228,212–7,047,788 (T2) were retained from the termite mound soils.

3.3. Community Structure of Soil Invertebrates across the Soil Samples. The abundance of soil invertebrate taxonomic groups was visualized using the Shinyheatmap (Figure 1) and Circos software (Figures 2). Among soil invertebrates at the phylum level, Arthropoda was the most common phylum across all soil samples. Nematoda, Mollusca, Platyhelminthes, and Annelida were also observed at the phylum level (Figure 1). At the class level (Figure 2), the relative abundance of class Insecta (except in Sb1) was predominant in termite mound soils, while the relative abundance of subclasses Ellipura (except in T1a) and Chromadorea (expect in Tb2) was predominate in adjacent soils. The relative abundance of the classes Gastropoda, Arachnida, Enoplea, Polychaeta, and Ascidiacea—as well as the clade Trematoda—did not follow a definite pattern as some were higher in termite mound soils and lower in adjacent soils, and vice versa. We also observed unclassified eukaryotes in both soil samples (Figure 1).

3.4. Alpha and Beta Diversity Assessment. The results showed no significant difference in the alpha diversity. All Shannon index values recorded were below the theoretical values of 2.81 [28], while the evenness index values were low (Table 2). For the beta diversity as depicted with principal coordinate analysis (Figure 3), a separate clustering by the related soil samples (i.e., T1 versus S1 and T2 versus S2) was observed. The analysis of similarity (ANOSIM) supported the strength of the separation with R-value and p value 0.50 and of 0.01, respectively.

3.5. Impact of the Environmental Parameters on the Soil Invertebrate Communities. The CCA revealed the relationship between the measured soil physical and chemical properties and the soil invertebrate communities. The CCA results (Figure 4) revealed that invertebrate community composition is possibly coupled to soil physicochemical properties (CCA permutation test = 0.03). From the CCA plot (Figure 4), we observed a positive correlation between the Gastropoda, Arachnida, Enoplea, Polychaeta, Ascidiacea, Trematoda, Insecta, and Chromadorea with Mg, clay, Ca, K, silt, N, OC, and P. Furthermore, Ellipura had a positive correlation with sand and pH and had a negative correlation with Ca, Mg, K, clay, silt, N, OC, and P. The Monte Carlo permutation test (via forward selection of all soil parameters in Table 1) revealed that P (p value = 0.008), pH (p value = 0.020), and OC (p value = 0.049) significantly contributed 30.9%, 18.2%, and 12.9%, respectively, of the variation (Table 3).

4. Discussion

The aptness of metagenomics for evaluating soil invertebrate diversity has been shown by earlier studies by several research studies [15, 16]. Thus, the metagenomic method of unraveling undescribed soil invertebrates seems to be reasonably promising. This study made an extensive effort to examine the diversity of soil invertebrate communities associated with soils from termite mounds and to understand if termite bioturbation influences their variance from their adjacent soils via metagenomics. Within the groups (at class levels), there was no significant difference (p-value > 0.05) (Table 2) in alpha diversity from our study. This shows that the diversity of the soil invertebrate characterized by the metagenomes in both soils did not come close to 2.82 (a value theoretically seen as richness standard) (Table 1) [28], suggesting that we did not observe higher numbers of different taxa in the samples. Annelida was not seen in T1a, T1b, T1c, T2c, S1a, and S1d. Though Annelida exists in large quantities, they are easily damaged or broken during the process of collection, making them hard to classify morphologically [29]. With low evenness values of approximately 0.1863–0.6391 (Table 2) recorded in our study, only some few soil invertebrates (such as Arthropoda) were predominant in each habitat. This could be as a result of the fact that, in the animal kingdom, the phylum Arthropoda is recognized as the principal and the most diverse group [30].

Table 1: Soil properties assessment from both soil samples.

Site	T1a	T1b	T1c	T1d	T2a	T2b	T2c	T2d	S1a	S1b	S1c	S1d	S2a	S2b	S2c	S2d
Ca	1500	1278.00	2215.00	2525	1790.00	2237.00	2417.00	2507.00	2010	997.00	1046.00	1921.00	873.00	1211	1140.00	1210.00
N	0.13	0.07	0.07	0.1	0.14	0.09	0.10	0.07	1.00	0.10	0.26	1.00	0.29	0.22	0.22	0.27
P	1.00	0.00	0.00	0.00	1.00	0.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
OC	0.10	0.10	0.10	0.94	0.10	0.10	0.10	0.10	0.10	0.10	0.12	0.11	0.10	0.11	0.10	0.11
pH	4.89	4.79.00	5.52	5.21	4.21	4.52	5.11	4.06	5.64	5.40	5.97	6.14	5.84	5.57	5.09	5.03
Mg	253.00	503.00	672.00	872.00	632.00	535.00	647.00	675.00	343.00	560.00	324.00	172.00	414.00	427.00	128.00	352.00
K	507.00	304.00	276.00	487.00	403.00	459.00	489.00	359.00	186.00	285.00	217.00	179.00	208.00	209.00	160.00	161.00
Silt	8.00	9.00	6.00	13.00	12.00	29.00	30.00	8.00	6.00	31.00	6.00	4.00	9.00	10.00	11.00	11.00
Sand	71.00	70.00	66.00	53.00	65.00	25.00	30.00	71.00	80.00	46.00	80.00	82.00	75.00	79.00	73.00	79.00
Clay	21.00	21.00	28.00	34.00	23.00	49.00	40.00	21.00	14.00	23.00	14.00	14.00	16.00	11.00	16.00	10.00

T1a–d and T2a–d mean termite mounds from Braklaagte and Zeerust, respectively, while S1a–d and S2a–d mean adjacent soils from Braklaagte and Zeerust, respectively.

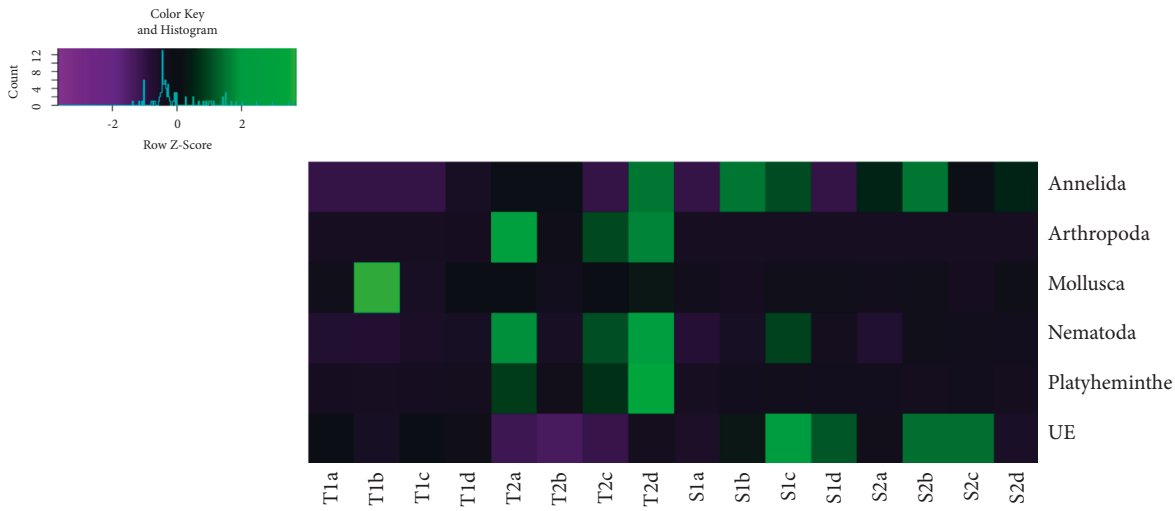

Figure 1: Soil invertebrate at the phylum level (relative abundance). T1a–d and T2a–d mean termite mounds from Braklaagte and Zeerust, respectively, while S1a–d and S2a–d mean adjacent soils from Braklaagte and Zeerust, respectively. UE = unclassified eukaryote. The relative abundance of the soil invertebrates was transformed with the z-score as expressed in colour saturation gradient seen in the scale bar.

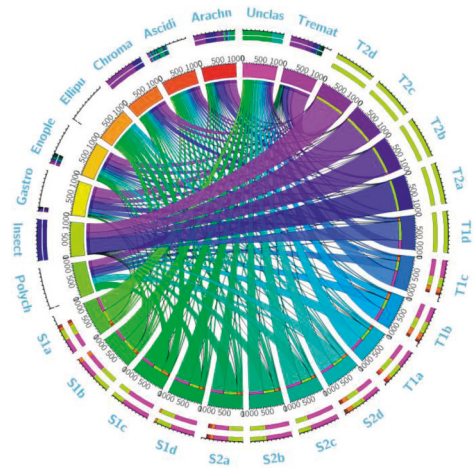

Figure 2: Soil invertebrate at class level (relative abundance). T1a–d and T2a–d mean termite mounds from Braklaagte and Zeerust, respectively, while S1a–d and S2a–d mean adjacent soils from Braklaagte and Zeerust, respectively. The labels Gastropoda, Arachnida, Enoplea, Polychaeta, Ascidiacea, Trematoda, Insecta, Ellipura, and Chromadorea were shortened to Gastro, Arachn, Enople, Polych, Ascidi, Tremat, Insect, Ellipu, and Chroma.

The dominance of Arthropoda in each habitat may connote their unique functional role in that habitat [31].

The PCoA analysis demonstrated that the structures of the soil invertebrate communities from termite mounds are distinctive to those of the adjacent soils. This was further buttressed by the analysis of similarity that showed strong distinction of samples (R- and p-values = 0.50 and 0.01, resp.). The variation in the soil physical and chemical properties (Table 1) serves to explain the significant separation observed between the two habitats sampled (Figure 4). The difference in the soil physical and chemical properties observed suggests that the influence of the *Coptotermes* activities is significant on the soil nutrients. The materials (such as chewed wood with a lot of cellulose glued with their undigested food particles and saliva) used by *Coptotermes* when building their nests help improve soil nutrients [8, 19].

The CCA results indicated that phosphorus (p-value = 0.008), pH (p-value = 0.020), and organic carbon (p-value = 0.049) significantly influenced the variation (Table 3; Figure 4). CCA also revealed that P significantly contributed 30.90% of the variation, pH significantly contributed 18.20%, and OC significantly contributed 12.90% (Table 3). The vector arms of K, clay, and Mg in the CCA plot suggest that, apart from pH, P, and OC, other soil properties can also drive the distribution of the soil invertebrate communities. Soil invertebrates tend to do well in environments of optimal pH and rich in organic matter [32, 33]. Since termite mound soils are rich in soil organic matter and slightly acidic [22, 34], they tend to support a nontrivial diversity of soil invertebrates. Some soil parameters from this study were associated with compositional shifts in the invertebrate community. For example, Gastropoda,

Table 2: Assessment of soil invertebrate diversity and evenness of the soil samples.

Indices	T1a	T1b	T1c	T1d	T2a	T2b	T2c	T2d	S1a	S1b	S1c	S1d	S2a	S2b	S2c	S2d	p-value
Shannon	0.9461	1.162	0.9518	0.9585	0.1113	0.4235	0.1961	0.229	0.9203	0.9995	1.07	0.9539	1.001	0.9725	0.9498	1.088	$P > 0.05$
Evenness	0.5151	0.6391	0.5181	0.4346	0.1863	0.2545	0.2433	0.2096	0.502	0.4528	0.4859	0.5192	0.4533	0.4408	0.4309	0.4948	$P > 0.05$

T1a–d and T2a–d mean termite mounds from Braklaagte and Zeerust, respectively, while S1a–d and S2a–d mean adjacent soils from Braklaagte and Zeerust, respectively.

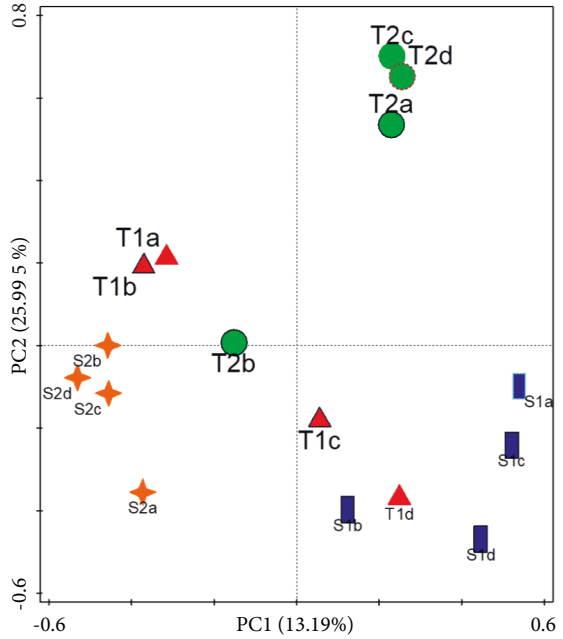

Figure 3: Evaluation of the beta diversity as depicted with principal coordinate analysis. T1a–d and T2a–d mean termite mounds from Braklaagte and Zeerust, respectively, while S1a–d and S2a–d mean adjacent soils from Braklaagte and Zeerust, respectively.

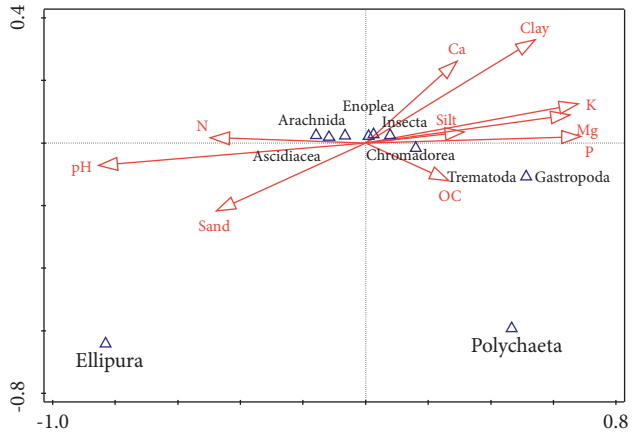

Figure 4: CCA of termite mound and adjacent soil invertebrate communities and soil physicochemical parameters.

Table 3: Forward selection of environmental variables, which best explain variation in invertebrate composition among samples.

Environmental parameters	Explanation (%)	Contribution (%)	Pseudo-F	p value
P	24.10	30.90	4.50	0.008
OC	10.10	12.90	2.00	0.049
pH	14.20	18.20	3.30	0.02
Clay	4.30	5.50	1.00	0.432
K	6.10	7.80	1.50	0.239
Silt	5.20	6.60	1.30	0.271
Sand	7.90	10.10	2.20	0.124
N	4.20	5.30	1.20	0.302
Mg	1.70	2.10	0.40	0.688
Ca	0.50	0.60	0.10	0.952

Arachnida, Enoplea, Polychaeta, Ascidiacea, Trematoda, Insecta, and Chromadorea were found to be correlated with Mg, silt, N, Ca, OC, clay, K, and P in the positive direction and correlated with pH and sand in the negative direction. Overall, the differences in the soil invertebrate diversity and composition in both habitats could impact the ecological

functions contributed by these organisms and show that termite bioturbation can have a significant impact on soil inveterate diversity and composition.

5. Conclusion

Our metagenomic examination of termite mound soil invertebrates revealed two overarching things. First, our study revealed that soil invertebrate community composition and diversity are possibly influenced by bioturbators as well as environmental properties. Furthermore, it shows that metagenomics can be employed as a powerful tool for profiling soil invertebrate communities as well as observing soil biodiversity. This can further lead to updating extant reference databases that will consequently help the assessment of the correct quantity of species by accruing sequencing data via additional molecular taxonomic assessment.

Data Availability

PRJNA526912 (https://www.ncbi.nlm.nih.gov/bioproject/526912) and PRJNA525146 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA525146) are the respective bioproject numbers for the high-quality sequences retained from termite mound and adjacent soil samples in the NCBI SRA database.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Ben Jesuorsemwen Enagbonma searched the literature, did the benchwork, executed the analyses and result interpretation, and wrote the paper. Olubukola Oluranti Babalola, the principal investigator, supervised the PhD, made funding acquisition, made critical revision, and proofread the drafts. Both authors approved the paper for publication.

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