JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2026, 27(10), 1–12
https://doi.org/10.12911/22998993/220463
ISSN 2299–8993, License CC-BY 4.0

Received: 2026.05.23
Accepted: 2026.08.04
Published: 2026.09.01

Multivariate analysis of soil factors shaping earthworm communities

Aqsa Sarwar[1], Javaria Altaf[1*], Shabab Nasir[1], Azhar Rafique[1]

[1] Department of Zoology, Government College University Faisalabad, Faisalabad 38000, Pakistan
* Corresponding author's e-mail: javariaaltafuaar@yahoo.com; uqjaltaf@uq.edu.au

ABSTRACT

Earthworms are key regulators of soil ecosystem and influence soil structure, nutrient dynamics, and physicochemical interactions. This study investigated the relationships between soil abiotic properties and the distribution patterns of four earthworm species (Metaphire hawayana, Metaphire posthuma, Lumbricus trapezoides, and Lumbricus rubellus) across six diverse microhabitats in Faisalabad, Pakistan. A total of 1,800 soil samples were analyzed for texture, pH, electrical conductivity, total dissolved solids, major macro and micronutrients, and other physicochemical parameters. Hierarchical cluster analysis was applied to identify patterns of association among soil variables within species-specific habitats. Distinct clusters among soil physicochemical factors, indicate clear difference in ecological preferences of earthworm species. Electrical conductivity, soil pH, moisture content, and nutrient concentrations emerged as the most important variables for species distribution in different microhabitats. Microhabitat-based redundancy analysis (RDA) further demonstrated that vegetation composition and soil characteristics contribute significantly to spatial variability in earthworm communitiesOverall, the findings highlight the strong role of soil abiotic heterogeneity in shaping earthworm distribution and community organization in agro-ecosystems. This study provides useful insights for soil quality evaluationandbiodiversity conservation,for futuresustainable land-management strategies

Keywords: earthworms, cluster analysis, soil physicochemical properties, microhabitats, heterogeneity, Faisalabad

INTRODUCTION

Earthworms are the most common creature in the soil as they contribute 80% of the soil biomass (Singh et al., 2016) in the humid soils of tropical and temperate areas (Nouri-Aiin et al., 2021). Due to their substantial contribution to the production of biomass earthworms are known as soil engineers and are intestines of the earth (Goswami and Mondal, 2015). They contribute significantly to structure the soil and water infiltration (Lone et al., 2021; Fu et al., 2022; Manna et al., 2023). Being thin skinned organisms, these are exposed to variations in the chemical and physical characteristics of the soil (Rutgers et al., 2016). Their populations often occur in spatially distinct patches, largely due to variations in soil physicochemical properties across different sites. Understanding these properties can facilitate the prediction of earthworm species occurrence and distribution within specific habitats (Van Schaik et al., 2016). Quantifying the spatial distribution of earthworms across diverse agroecosystems is therefore important for evaluating the influence of abiotic soil processes and for linking earthworm abundance with the spatial distribution of soil. Information on species composition and abundance in different habitats can also provide valuable insights into ecosystem functionality, stability, and overall efficiency. In addition to soil physicochemical characteristics, the type of agroecosystem such as cultivated versus non-cultivated land plays a significant role in determining earthworm abundance and distribution patterns (Singh et al., 2020).

Soil constitutes one of the largest reservoirs of global biodiversity, with approximately 59 % of all species depending on soil at some stage of their life cycle (Anthony et al., 2023). Among soil biota, earthworms are comparatively well studied (Guerra et al., 2020), largely due to their relatively large body size, ease of sampling, and longstanding scientific interest dating back to the pioneering work of Charles Darwin (Darwin, 1881; 1838). Despite extensive research, important knowledge gaps remain regarding their ecology and functional roles in different environmental contexts (Blouin et al., 2013). Nevertheless, the critical contributions of earthworms to soil processes including the improvement of nutrient cycling, soil structure, and overall soil quality are now widely recognized (Lemtiri et al., 2014; Bartz et al., 2013; Frund et al., 2011; Blouin et al., 2013). Factors such as soil texture, temperature, pH, calcium, vegetation, management practices and organic matter have a significant effect on earthworm species activity and abundance (Rajkhowa et al., 2015; Edwards et al., 2022).

Earthworm sensitivity towards variations in these environmental factors shows that there is an association between their biodiversity and soil physico-chemical parameters. This makes it strong candidate as an indicator species (Jiang et al., 2023). Their impact on soil microbial activity, biogeochemical cycles and soil modification is important in terrestrial ecosystems (Lavelle and Spain, 2024). Anecic earthworms such as *Pheretima sp*. (Waqar et al., 2019) and *Lumbricus sp*. consume the surface leaf litter and takes it to the mineral layer of the soil which increases nutrient cycling (Addison, 2009) and has a strong impact on the distribution of microbial colonies, micro-fauna and epigeic invertebrates (Ferlian et al., 2018). The *Lumbricidae* family is an important part of the earthworm community of soil. In temperate agricultural soils, Lumbricids make up to 90% of the invertebrate biomass. This makes them the most abundant invertebrate (Bottinelli and Capowiez, 2021). *Lumbricidae* also known as "the ecosystem engineers" are important for the soil environment (Mazur-Paczka et al., 2019) as they improve the soil structure and quality (Schon et al., 2017).

Understanding the relationships between earthworm species and soil physicochemical parameters is crucial for evaluating soil health and ecosystem sustainability. Cluster analysis provides a powerful multivariate tool for the identification of associations among abiotic variables and species-specific habitat preferences.

MATERIALS AND METHODS

Study area and sampling

Faisalabad, the third-largest city of Pakistan, is located in central Punjab between 30°40′–31°47′ N latitude and 72°42′–73°40′ E longitude. The district covers an area of approximately 5,856 km² with an estimated population of 2.6 million (Ul-Allah et al., 2014). The region is predominantly agricultural and experiences a semi-arid subtropical climate with hot summers, mild winters, and an average annual rainfall of about 350 mm, mainly during the monsoon season (July–September).

The study was conducted in Tehsil Faisalabad, the most urbanized subdivision of the district, encompassing urban settlements, irrigated farmlands, green spaces, and managed forest areas. To represent habitat heterogeneity, six sampling sites were selected: University of Agriculture Faisalabad (UAF), Ayub Agricultural Research Institute Faisalabad (AARI), Government College University Faisalabad (GCUF), Jinnah Park, Gatwala Wildlife Park, and Punjab Forestry Research Institute (PFRI) (Figure 1).

Sampling and identification of earthworms

At each sampling site, 25 soil samples were collected monthly for a period of one year (May 2023 through April 2024), resulting in a total of 1,800 samples (6 sites × 25 samples × 12 months). A **s**tandardized soil corer (20 cm diameter, 0–25 cm depth) was used to extract soil from the active earthworm zone. Sampling points within each site were selected randomly along parallel transects to minimize spatial bias and ensure representative coverage of the microhabitats.

Collected soil blocks were carefully hand-sorted in the laboratory to extract earthworms. Each sample was properly labeled with the station name, microhabitat ID, date, and collector name. Samples were then transported to the Biochemistry Laboratory, Department of Zoology, Government College University Faisalabad, for subsequent analyses.

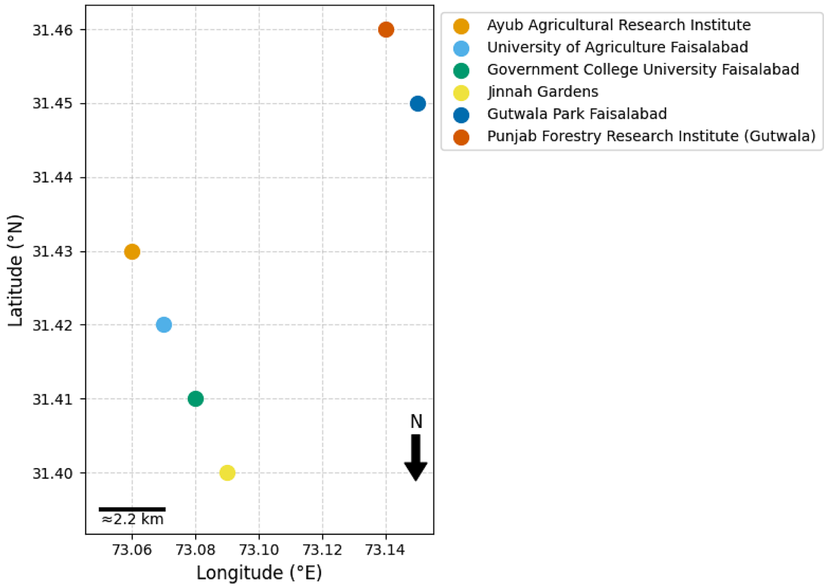

Figure 1. Sampling sites in district Faisalabad

Physico-chemical analysis

Soil samples collected from each study site were analyzed to determine their physico-chemical properties, which influence soil fertility and biological activity and serve as explanatory variables for multivariate cluster analysis. The parameters measured included soil texture, pH, electrical conductivity (EC), total dissolved solids (TDS), nitrogen (N), phosphorus (P), potassium (K), organic carbon (OC), ash, sodium (Na), calcium (Ca), lithium (Li), and heavy metals.

Soil texture was determined using the hydrometer method of Bouyoucos, (1962). Measurements of pH, EC, and TDS were obtained with a digital multi-parameter meter (Eutech Instruments, PCSTestr 35 series). Total Kjeldahl Nitrogen (TKN) was estimated following the method of (Bremner and Mulvaney, 1982), while organic carbon and ash content were determined according to (Nelson and Sommers, 1996). Phosphorus concentration was measured by the method of (John, 1970) using a Systronics UV/Visible Spectrophotometer (Model 117). The concentrations of sodium, potassium, calcium, and lithium were analyzed with a Systronics Flame Photometer (Model 128), calibrated with certified standard solutions prior to each run.

Statistical analysis

Hierarchical cluster analysis was performed using Minitab version 18 to explore relationships among soil physicochemical variables across earthworm species and microhabitats. All variables were standardized prior to analysis to remove scale differences and ensure fair comparison. Similarity matrices were calculated using Euclidean distance, which quantifies straight-line differences between samples across all variables, making it suitable for continuous soil data. Clusters were formed using Ward's linkage method, as this approach minimizes within-cluster variance and produces compact, ecologically meaningful groupings. Cluster stability and validity were assessed by examining consistent clustering patterns across multiple species and microhabitats, ensuring that the observed groupings reflect genuine ecological relationships rather than random variation. The resulting dendrograms illustrate patterns of association among soil variables and habitat types, providing a robust framework for interpreting species-specific soil preferences.

RESULTS

Soil physicochemical clustering in earthworm habitats

Hierarchical cluster analysis revealed distinct patterns of association among soil physicochemical variables across the habitats of the four earthworm species, reflecting species-specific ecological preferences and sensitivity to environmental gradients. All analyses were performed on standardized variables using Euclidean distance and Ward's linkage, ensuring robust and interpretable clustering.

Cluster analysis of abiotic factors of soil of *M. hawayana*

The clusters show that the variables of abiotic factors of soil were exhibited different relationship in the microhabitats where *M. hawayana* was found (Figure 2). The maximum similarity (nearly 87%), was found in cluster 3 (potassium) and 12 (pressure). More than >87% similarity was found in cluster 7 (electrical conductivity) and cluster 13 (pH). The similarity found in cluster 6 (total dissolved solids) and 14 (carbonates) was about (>85%). More than >82% similarity was found in cluster 9 (% moisture) and 10 (dissolved oxygen). More than (>82%) similarity was found in cluster 1 (sodium) and 8 (moisture content). About (>81%) similarity was found in cluster 6 (total dissolved solids, carbonates) and 15 (bicarbonates). Similarity (>72%) was found in cluster 3 (potassium, pressure) and 9 (% moisture, dissolved oxygen). the similarity (>70%) was found in cluster 4 (barium) and 5 (lithium). More than 57% similarity was found in cluster 2 (calcium) and 11 (temperature). Nearly 53% similarity was found in cluster 1 (sodium, moisture content) and 7 (electrical conductivity). 32% similarity was found in cluster 3 (potassium, pressure, % moisture, dissolved oxygen) and 4 (barium, lithium). The similarity (>30%) was found in cluster 1 (sodium, moisture content, electrical conductivity, pH and 2 (calcium, temperature). About similarity (>20%) was found in cluster 1 (sodium, moisture content, electrical conductivity, pH, calcium, temperature) and 6 (total dissolved solids, carbonates, bicarbonates).

Cluster 1 (sodium, moisture content, electrical conductivity, pH, calcium, temperature, total dissolved solids, carbonates, bicarbonates) and 3 (potassium, pressure, % moisture, dissolved oxygen, barium, lithium) exhibited very low similarity, nearly 4% (Table 1). Overall, the dendrogram highlights that *M. hawayana* habitats are strongly influenced by soil chemical and moisture-related variables, with distinct clusters reflecting species-specific preferences.

Cluster analysis of abiotic factors of soil of *M. posthuma*

The clusters show that the variables of abiotic factors of soil were shown different relationship in the microhabitats where *M. posthuma* was found (Figure 3). The maximum similarity (nearly 97%), was found in cluster 7 (electrical conductivity) and 13 (pH). More than >91% similarity was found in cluster 1 (electrical conductivity) and 2 (calcium). The similarity found in cluster 10 (dissolved oxygen) and 3 (potassium) was about (>89%). More than (>88%) similarity was found in cluster 5 (lithium) and 14 (carbonates). About (>85%) similarity was found in cluster 6 (total dissolved solids) and 12 (pressure). Greater

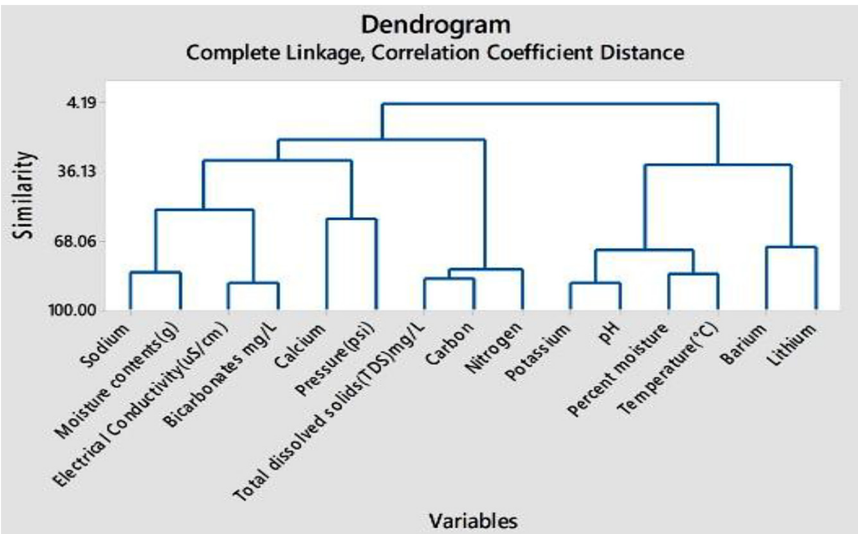

Figure 2. Dendrogram showing hierarchical cluster analysis of soil physicochemical variables associated with the habitat of *M. hawayana*

Table 1. Hierarchical cluster analysis of *M. hawayana*

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of obs. in new cluster
1	14	87.5334	0.24933	3	12	3	2
2	13	87.4142	0.25172	7	13	7	2
3	12	85.1020	0.29796	6	14	6	2
4	11	82.9945	0.34011	9	10	9	2
5	10	82.5241	0.34952	1	8	1	2
6	9	81.1198	0.37760	6	15	6	3
7	8	72.0293	0.55941	3	9	3	4
8	7	70.6706	0.58659	4	5	4	2
9	6	57.4061	0.85188	2	11	2	2
10	5	53.2343	0.93531	1	7	1	4
11	4	32.9098	1.34180	3	4	3	6
12	3	30.7441	1.38512	1	2	1	6
13	2	20.6663	1.58667	1	6	1	9
14	1	4.1913	1.91617	1	3	1	15

than 84% similarity was found in cluster 3 (dissolved oxygen, potassium) and 9 (% moisture). The similarity was found in cluster 7 (electrical conductivity, pH) and 8 (moisture content) nearly (>79%). More than 68% similarity was found in cluster 1 (sodium, calcium) and 7 (electrical conductivity, pH, moisture content).

About similarity (>66%) was found in cluster 4 (barium) and 15 (bicarbonates). The similarity >64% was found in cluster 5 (lithium, carbonates) and 11 (temperature). Moderate similarity 49% similarity was found between cluster 3 (dissolved oxygen, potassium, % moisture) and 4 (barium, bicarbonates) and about 37% similarity was found in cluster 1 (sodium, calcium, electrical conductivity, pH, moisture content) and 6 (total dissolved solids, pressure). Similarity greater than (>20%) was found in cluster 1 (sodium, calcium, electrical conductivity, pH, moisture content, total dissolved solids, pressure) and 5 (lithium, carbonates, temperature). Very low similarity (~0.45%) was observed between cluster 1 (sodium, calcium, electrical conductivity, pH, moisture content, total dissolved solids, pressure, lithium, carbonates, temperature) and 3 (dissolved oxygen, potassium, % moisture, barium, bicarbonates) (Table 2). The dendrogram illustrates that *M. posthuma* distribution is shaped by interacting chemical gradients,

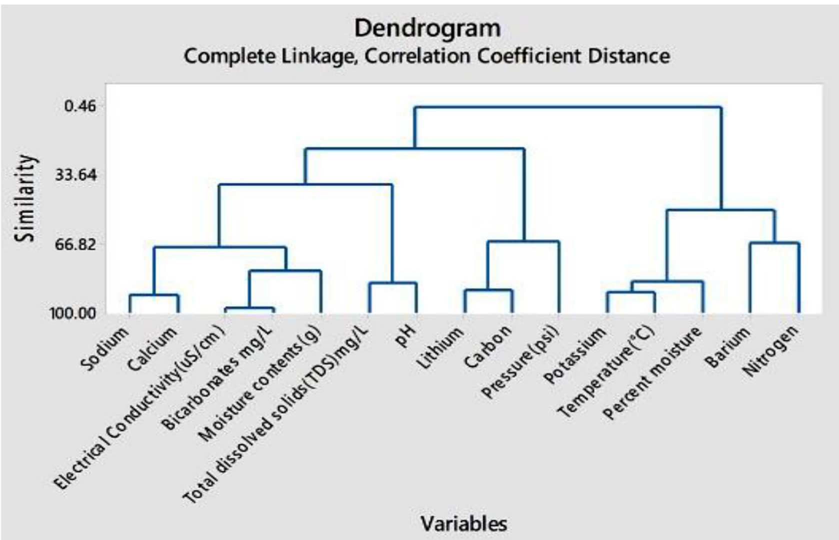

Figure 3. Dendrogram showing hierarchical cluster analysis of soil physicochemical variables associated with the habitat of *M. posthuma*

Table 2. Hierarchical cluster analysis of *M. posthuma*

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of obs. in newcluster
1	14	97.3035	0.05393	7	13	7	2
2	13	91.2539	0.17492	1	2	1	2
3	12	89.8358	0.20328	3	10	3	2
4	11	88.8830	0.22234	5	14	5	2
5	10	85.5306	0.28939	6	12	6	2
6	9	84.2593	0.31481	3	9	3	3
7	8	79.2180	0.41564	7	8	7	3
8	7	68.1829	0.63634	1	7	1	5
9	6	66.1825	0.67635	4	15	4	2
10	5	64.8913	0.70217	5	11	5	3
11	4	49.8559	1.00288	3	4	3	5
12	3	37.9891	1.24022	1	6	1	7
13	2	20.9849	1.58030	1	5	1	10
14	1	0.4586	1.99083	1	3	1	15

moisture, and aeration, as shown by closely associated clusters.

Cluster analysis of abiotic factors of soil of *L. trapezoides*

The clusters show that the variables of abiotic factors of soil were shown different relationship in the microhabitats where *L. trapezoides* was found (Figure 4). The maximum similarity (nearly 98%) was found in cluster 2 (calcium) and 5 (lithium). 97% similarity was found in cluster 1 (sodium) and 4 (barium). More than 96% similarity was found in cluster 1 (sodium, barium) and 2 (calcium, lithium). Greater than (> 91%) similarity was found in cluster 7 (electrical conductivity) and 13 (bicarbonate). The similarity (> 87%) was found in cluster 8 (moisture content) and 15 (nitrogen). More than 84% similarity was found in cluster 1 (sodium, barium, calcium, lithium) and 10 (temperature). More than similarity (> 79%) was found in cluster 1 (sodium, barium, calcium, lithium, temperature) and 12 (pH). Nearly 74% similarity was found in cluster 6 (total dissolved solids) and 7 (electrical conductivity, bicarbonate).

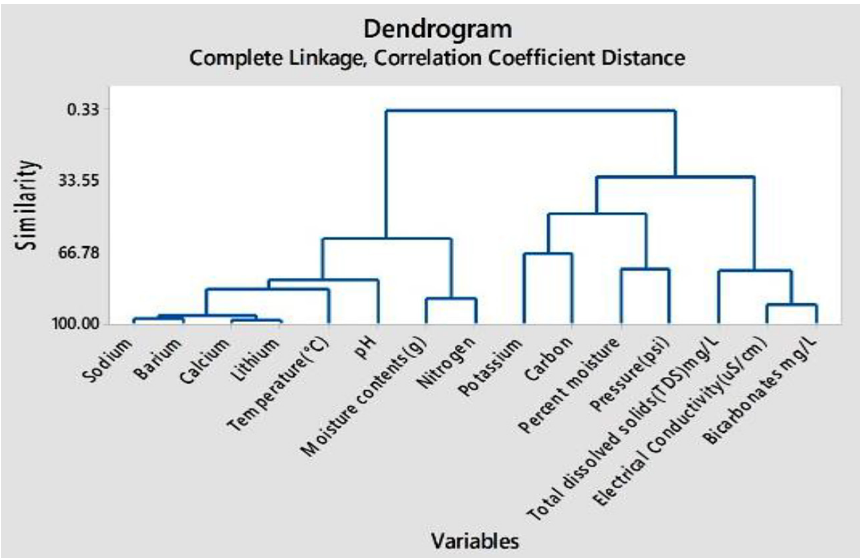

Figure 4. Dendrogram showing hierarchical cluster analysis of soil physicochemical variables associated with the habitat of *L. trapezoides*

More than similarity (> 74%) was found in cluster 9 (% moisture) and 11 (pressure). About (> 66%) similarity was found in cluster 3 (potassium) and 14 (carbon). Nearly 59% similarity was found in cluster 1 (sodium, barium, calcium, lithium, temperature, pH) and 8 (moisture content, nitrogen). Greater than 48% similarity was found in cluster 3 (potassium, carbon) and 9 (% moisture, pressure). The maximum similarity (nearly 31%) was found in cluster 3 (potassium, carbon, % moisture, pressure) and 6 (total dissolved solids, electrical conductivity, bicarbonate). Very low similarity (0.33%) was found between cluster 1 (sodium, barium, calcium, lithium, temperature, pH, moisture content, nitrogen) and cluster 3 (potassium, carbon, % moisture, pressure, total dissolved solids, electrical conductivity, bicarbonate) (Table 3). Dendrogram demonstrates that *L. trapezoides* habitats are primarily influenced by mineral composition, moisture, and temperature, forming clear, ecologically meaningful clusters.

Cluster analysis of abiotic factors of soil of *L. rubellus*

The clusters show that the variables of abiotic factors of soil were shown different relationship in the microhabitats where *L. rubellus* was found (Figure 5).The maximum similarity (nearly 98%) was found in cluster 1 (sodium) and 4 (barium) followed by similarity in cluster 1 (sodium, barium) and cluster 2 (calcium) i.e.> 94%. More than 93% similarity was found in cluster 6 (total dissolved solids) and 7 (electrical conductivity). The similarity found in cluster 1 (sodium, barium, calcium) and 12 (pH) was about (> 83%). More than 81% was found in cluster 5 (lithium) and 8 (moisture content). More than 79% similarity was found in cluster 6 (electrical conductivity, total dissolved solids) and 13 (bicarbonate). About 74% similarity was found in cluster 9 (percent moisture) and 11 (pressure). Nearly 71% similarity was found in cluster 3 (potassium) and 15 (nitrogen). 60% similarity was found in cluster 1 (sodium, barium, calcium, pH) and 14 (carbon). More than (> 59%) similarity was found in cluster 9 (% moisture, pressure) and 10 (temperature). (>48%) similarity was found in cluster 1 (sodium, barium, calcium, pH, carbon) and 5 (lithium, moisture content). Greater than 47% similarity was found in cluster 6 (electrical conductivity, total dissolved solids, bicarbonate) and 9 (% moisture, pressure, temperature). More than (> 33%) similarity was found in cluster 3 (potassium, nitrogen) and 6 (TDS, bicarbonate, % moisture, pressure, temperature). There was very less similarity (0.45%) in cluster 1 (sodium barium, calcium, pH, carbon, lithium, moisture content) and 3 (potassium, nitrogen, electrical conductivity, total dissolved solids, bicarbonate, % moisture, pressure, temperature) (Table 4). The dendrogram indicates that *L. rubellus* distribution responds to combined salinity, nutrient, and moisture factors, with clusters revealing integrated soil preferences.

Table 3. Hierarchical cluster analysis of *L. trapezoides*

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of obs. in new cluster
1	14	98.2016	0.03597	2	5	2	2
2	13	97.7249	0.04550	1	4	1	2
3	12	96.0407	0.07919	1	2	1	4
4	11	91.1696	0.17661	7	13	7	2
5	10	87.8638	0.24272	8	15	8	2
6	9	84.1163	0.31767	1	10	1	5
7	8	79.1617	0.41677	1	12	1	6
8	7	74.9838	0.50032	6	7	6	3
9	6	74.1446	0.51711	9	11	9	2
10	5	66.9514	0.66097	3	14	3	2
11	4	59.9189	0.80162	1	8	1	8
12	3	48.7113	1.02577	3	9	3	4
13	2	31.4777	1.37045	3	6	3	7
14	1	0.3303	1.99339	1	3	1	15

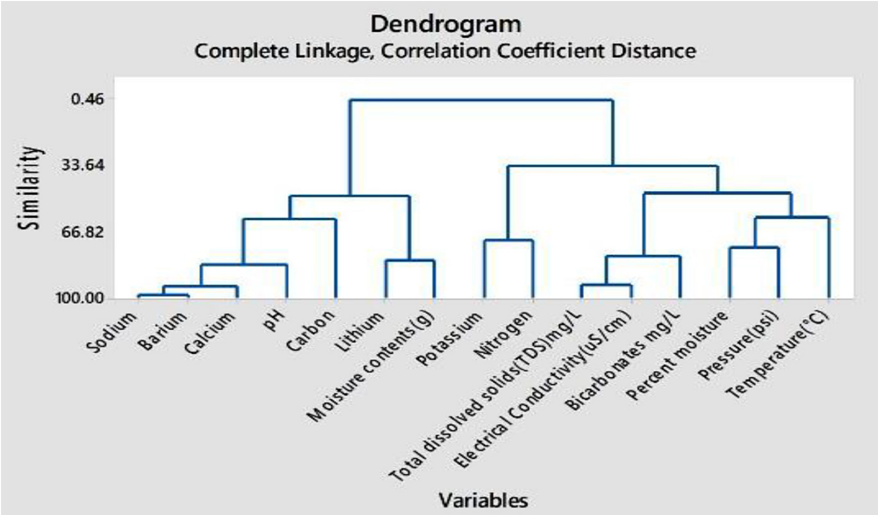

Figure 5. Dendrogram showing hierarchical cluster analysis of soil physicochemical variables associated with the habitat of *L. rubellus*

Table 4. Hierarchical cluster analysis of *L. rubellus*

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of obs. in new cluster
1	14	98.2408	0.03518	1	4	1	2
2	13	94.2038	0.11592	1	2	1	3
3	12	93.4953	0.13009	6	7	6	2
4	11	83.0417	0.33917	1	12	1	4
5	10	81.0893	0.37821	5	8	5	2
6	9	79.1357	0.41729	6	13	6	3
7	8	74.5827	0.50835	9	11	9	2
8	7	71.1066	0.57787	3	15	3	2
9	6	60.3148	0.79370	1	14	1	5
10	5	59.1784	0.81643	9	10	9	3
11	4	48.6127	1.02775	1	5	1	7
12	3	47.5397	1.04921	6	9	6	6
13	2	33.7352	1.32530	3	6	3	8
14	1	0.4558	1.99088	1	3	1	15

Cluster analysis of different microhabitat

Cluster analysis of microhabitats including *Mangifera indica*, open edge, water channel, *Citrus lemon, Morus nigra, Psidium guajava*, Nursery, *Syzygium cumini, Rosa rubiginosa, Dalbergia sisso, Phoneix dactylifera, Citrus siensis, Cactaceae, Azadirachta indica, Vachellia nilotica* and *Ficus carica* evealed patterns of similarity among habitats associated with the four earthworm species

The cluster analysis shows that microhabitat of the *Lumbricus* species have similarity among each other (Figure 6). The maximum similarity (nearly 66%) was found in cluster 9 (*Rosa rubiginosa*) and 15 (*Vachellia nilotica*). More than >66% similarity was found in cluster 4 (Citrus lemon) and 8 (*Syzygium cumini*). The similarity found in cluster 7 (Nursery) and 10 (*Dalbergia sisso*) was about >61%. More than >60% was found in cluster 1 (*Mangifera indica*) and 14 (*Azadirachta indica*). Greater than 59% similarity was found in cluster 13 (*Cactaceae*) and 16 (*Ficus carica*). About 58% similarity was found in cluster 3 (water channel) and 11 (*Phoenix dactylifera*). Nearly 56% similarity was found in cluster 1 (*Mangifera indica, Azadirachta indica*) and 5 (*Morus nigra*). More than (>56%) similarity was found in cluster 6 (*Psidium guajava*) and 7 (*Nursery, Dalbergia sisso*). Nearly 55%

similarity was found in cluster 3 (water channel, *Phoneix dactylifera*) and 9 (*Rosa rubiginosa, Vachellia nilotica*).

About similarity (>54%) was found in cluster 2 (open edge) and 13 (*Cactaceae, Ficus carica*). 51% similarity was found in cluster 3 (water channel, *Phoneix dactyla, Rosa rubiginosa, Vachellia nilotica*) and 4 (*Citrus lemon, Syzygium cumini*). The similarity (>47%) was found in cluster 1 (*Mangifera indica, Azadirachta indica, Morus nigra*) and 12 (*Citrus sinensis*). About 45% similarity was found in cluster 2 (open edge, *Cactaceae, Ficus carica*) and 6 (*Psidium guajava*, Nursery, *Dalbergia sisso*). More than 42% similarity was found in cluster 1 (*Mangifera indica, Azadiracuta indica, Morus nigra, Citrus sinensis*) and 2 (open edge, *Cactaceae, Ficus carica, Psidium guajava, Nursery, Dalbergia sisso*). There was very less similarity about (>33%) in cluster 1 (*Mangifera indica, Azadiracuta indica, Morus nigra, Citrus sinensis,* open edge, *Cactaceae, Ficus carica, Psidium guajava*, Nursery, *Dalbergia sisso*) and 3 (water channel, *Phoneix dactylifera, Rosa rubiginosa, Vachellia nilotica, Citrus lemon, Syzygium cumini*) (Table 5).

These patterns confirm that plant composition and associated soil conditions strongly influence earthworm distribution, and cluster

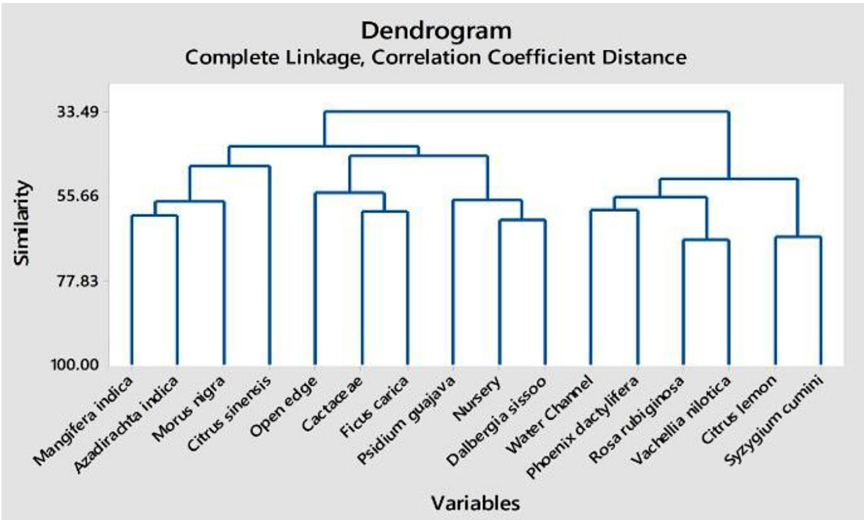

Figure 6. Dendrogram showing hierarchical cluster analysis of microhabitats based on earthworm species distribution, with highest occurrence of *Lumbricus trapezoides*.

Table 5. Hierarchical cluster analysis showing relationship among microhabitats and earthworm species

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of obs. in new cluster
1	15	66.9488	0.66102	9	15	9	2
2	14	66.2141	0.67572	4	8	4	2
3	13	61.9233	0.76153	7	10	7	2
4	12	60.5310	0.78938	1	14	1	2
5	11	59.6900	0.80620	13	16	13	2
6	10	58.9997	0.82001	3	11	3	2
7	9	56.7628	0.86474	1	5	1	3
8	8	56.5192	0.86962	6	7	6	3
9	7	55.5874	0.88825	3	9	3	4
10	6	54.8139	0.90372	2	13	2	3
11	5	51.0746	0.97851	3	4	3	6
12	4	47.4155	1.05169	1	12	1	4
13	3	45.0622	1.09876	2	6	2	6
14	2	42.5123	1.14975	1	2	1	10
15	1	33.4925	1.33015	1	3	1	16

analysis effectively highlights how microhabitat similarity correlates with species-specific ecological preferences. Microhabitats under *Rosa rubiginosa* and *Vachellia nilotica* were found to be the most suitable for the distribution of earthworm species.

DISCUSSION

The present study used hierarchical cluster analysis to explore relationships among soil physicochemical variables and the distribution of four earthworm species (*M. hawayana*, *M. posthuma*, *L. trapezoides*, and *L. rubellus*) across heterogeneous microhabitats in Faisalabad. The analysis revealed distinct clustering patterns among soil variables, reflecting species-specific habitat preferences and the complex environmental gradients that influence earthworm distributions.

Soil moisture, pH, electrical conductivity (EC), and nutrient content are widely recognized as key determinants of earthworm abundance and distribution (Edwards and Arancon, 2004; Singh et al., 2020). In agroecosystems, principal component and cluster analyses often identify EC, pH, total dissolved solids (TDS), and texture as major drivers of habitat suitability, affecting both nutrient availability and soil structure. In *M. hawayana*, potassium, pressure, EC, and pH formed strongly associated clusters, indicating that chemical soil conditions co-vary in its preferred microhabitats. Moisture content and dissolved oxygen also clustered together, suggesting this species favors soils with adequate hydration and aeration. These findings align with prior research showing that endogeic earthworms select habitats with optimal moisture and nutrient availability (Decaens et al., 2009; Chan and Barchia, 2010).

M. posthuma exhibited high similarity between EC, pH, and calcium, suggesting that electrical and chemical soil gradients significantly influence habitat selection. These results are consistent with studies highlighting the role of multiple interacting soil properties in shaping earthworm communities, rather than single limiting factors (Singh et al., 2020). Potassium and dissolved oxygen were also highly correlated, emphasizing the importance of both nutrient content and aeration. *L. trapezoides* showed clustering of sodium, barium, and calcium with pH and moisture content. Notably, moisture content and nitrogen exhibited strong associations, suggesting that nutrient-rich and adequately hydrated soils favor this species. This finding is consistent with previous reports indicating that anecic earthworms prefer nutrient-rich environments that support their burrowing and litter-processing behavior (Addison, 2009; Ferlian et al., 2018).

Similarly, *L. rubellus* habitats demonstrated strong associations among sodium, barium, and calcium, while TDS and EC also clustered together. The moderate clustering of potassium and nitrogen indicates that multiple nutrients collectively influence habitat suitability. These results corroborate studies showing that Lumbricidae distribution is shaped by integrated chemical and physical soil factors (Mazur-Paczka et al., 2019; Bottinelli and Capowiez, 2021).

Cluster analysis of microhabitats indicated that vegetation type influences earthworm distributions. Microhabitats dominated by woody vegetation (e.g., *Rosa rubiginosa* and *Vachellia nilotica*) exhibited higher similarity, suggest that plant litter and root structures modify soil properties and promote habitat suitability. This aligns with observations that vegetation indirectly affects earthworm assemblages via organic matter inputs and microhabitat conditions (Decaens et al., 2009; Chan and Barchia, 2010).

The use of Euclidean distance with Ward's linkage in hierarchical clustering, combined with prior standardization of all soil variables, ensured that differences in scale among variables did not bias the analysis. This approach maximizes within-cluster similarity while minimizing between-cluster variance, providing robust identification of ecologically meaningful patterns. Cluster validation was supported by consistent patterns across multiple species and microhabitats, increasing confidence in the multivariate interpretation.

This multivariate perspective reveals species-specific ecological requirements and the critical role of microhabitat heterogeneity in agro ecosystem soil biodiversity (Singh et al., 2020). From an applied standpoint, these insights are useful for soil quality assessment, biodiversity monitoring, and sustainable land-management practices, such as maintaining vegetative cover and optimizing soil nutrient balance to support earthworm-mediated ecosystem functions. Future studies on temporal dynamics, interspecific interactions, and will be helpful for the development of predictive models of earthworm distribution and soil ecosystem functioning.

CONCLUSIONS

This study demonstrates that cluster analysis is an effective tool for exploring the relationships among soil abiotic factors and earthworm distributions across heterogeneous microhabitats. Distinct clustering patterns of soil variables highlight species-specific ecological requirements and reflect how soil physicochemical environments govern earthworm occurrence. The strongest associations were identified among electrical conductivity, pH, moisture content, and nutrient elements, emphasizing their integrative role in shaping soil earthworm communities. These findings support the use of earthworms as bioindicators of soil health and underscore the importance of managing soil conditions to sustain functional biodiversity in agricultural and managed ecosystems.

Acknowledgement

The author sincerely thanks Mr. Zahid Aziz for his support in the lab work.

REFERENCES

1. Addison, P., 2009. Nutrient cycling and earthworm activity in agricultural soils. *Applied Soil Ecology* 42(2), 123–131. https://doi.org/10.1016/j.apsoil.2009.01.005
2. Anthony, M. A., Bender, S. F., & van der Heijden, M. G. A. (2023). Enumerating soil biodiversity. *Proceedings of the National Academy of Sciences, 120*, e2304663120. https://doi.org/10.1073/pnas.2304663120
3. Bartz, M. L. C., Pasini, A., & Brown, G. G. (2013). Earthworms as soil quality indicators in Brazilian no-tillage systems. In *Applied Soil Ecology, 69: Progress and Priorities in Latin American Oligochaete Research* (pp. 39–48). https://doi.org/10.1016/j.apsoil.2013.01.011
4. Blouin, M., Hodson, M. E., Delgado, E. A., Baker, G., Brussaard, L., Butt, K. R., Dai, J., Dendooven, L., Peres, G., Tondoh, J. E., Cluzeau, D., & Brun, J.-J. (2013). A review of earthworm impact on soil function and ecosystem services. *European Journal of Soil Science, 64*, 161–182. https://doi.org/10.1111/ejss.12025
5. Bottinelli, N., Capowiez, Y., 2021. Lumbricid earthworms as ecosystem engineers in temperate soils. *Soil Biology and Biochemistry* 157, 108238. https://doi.org/10.1016/j.soilbio.2021.108238
6. Bouyoucos, G.J., 1962. Hydrometer method improved for making particle size analyses of soils. *Agronomy Journal* 54, 464–465.
7. Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen total. In: Page, A.L. et al. (Eds.), *Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties*. Soil Science Society of America, Madison, WI, pp. 595–624.
8. Chan, K.Y., Barchia, I.M., 2010. Soil texture and moisture as determinants of earthworm distribution. *Applied Soil Ecology* 44(3), 209–215. https://doi.org/10.1016/j.apsoil.2010.01.004
9. Darwin, C. R. (1838). On the formation of mould [Read 1 November 1837]. *Proceedings of the Geological Society of London, 2*, 574–576.
10. Darwin, C. R. (1881). *The Formation of Vegetable Mould through the Action of Worms, with Observations on Their Habits.* John Murray, London.
11. Darwin, C., 1981. *The formation of vegetable mould through the action of worms*. John Murray, London.
12. Decaens, T., Jimenez, J.J., Rossi, J.P., 2009. Spatial patterns and community structure of earthworm assemblages in tropical soils. *Journal of Tropical Ecology* 25(2), 143–153. https://doi.org/10.1017/S0266467409005781
13. Edwards, C.A., Arancon, N.Q., 2004. The influence of soil properties on earthworm population dynamics. In: Edwards, C.A. (Ed.), *Earthworm Ecology*, 2nd ed. CRC Press, Boca Raton, pp. 89–113.
14. Edwards, C.A., Arancon, N.Q., 2022. Earthworm biomass responses to environmental conditions. In: Edwards, C.A. (Ed.), *Earthworm Ecology and Nutrient Cycles*. Springer, Cham, pp. 30–55. https://doi.org/10.1007/978-3-030-94701-2_3
15. Ferlian, O., Engl, A., Eisenhauer, N., et al., 2018. Earthworm-induced modification of microbial communities affects soil biodiversity. *Soil Biology and Biochemistry* 127, 92–102. https://doi.org/10.1016/j.soilbio.2018.09.012
16. Frund, I., Haenel, N. V., & Wichmann, F. A. (2011). Inference for psychometric functions in the presence of nonstationary behavior. *Journal of Vision, 11*(6), 16–16.
17. Fu, M., Liu, F., Abbas, G., Zhou, S., Ling, S., Zhang, W., Zhou, B., 2022. Cytotoxicity profiling of decabromodiphenyl ethane to earthworm (*Eisenia fetida*): Abnormity recovery dysregulation physiological pattern reflects the coping mechanism. *Science of the Total Environment* 813, 152607.
18. Goswami, R., Mondal, C.K., 2015. A study on earthworm population and diversity with special reference to physicochemical parameters in different habitats of South 24 Parganas district in West Bengal. *Records of the Zoological Survey of India* 115, 31–38.
19. Guerra, C. A., Heintz-Buschart, A., Sikorski, J., Chatzinotas, A., Guerrero-Ramirez, N., Cesarz,

S., Beaumelle, L., Rillig, M. C., Maestre, F. T., Delgado-Baquerizo, M., Buscot, F., Overmann, J., Patoine, G., Phillips, H. R. P., Winter, M., Wubet, T., Küsel, K., Bardgett, R. D., Cameron, E. K., … Eisenhauer, N. (2020). Blind spots in global soil biodiversity and ecosystem function research. *Nature Communications, 11*, 3870. https://doi.org/10.1038/s41467-020-17688-2

20. Jiang, X., Cao, J., Ye, Z., Klobucar, G., Li, M., 2023. Microplastics-back to reality: Impact of pristine and aged microplastics in soil on earthworm *Eisenia fetida* under environmentally relevant conditions. *Environmental Science and Technology* 57(44), 16788–16799.
21. John, M.K., 1970. Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. *Soil Science* 109, 214–220.
22. Lavelle, P., Spain, A.V., 2024. *Soil ecology: Fundamentals and applications*, 2nd ed. Springer, Cham.
23. Lemtiri, A., Colinet, G., Alabi, T., Cluzeau, D., Zirbes, L., Haubruge, E., & Francis, F. (2014). Impacts of earthworms on soil components and dynamics: A review. *Biotechnologie, Agronomie, Societe et Environnement, 18*, 1–15.
24. Lone, A.R., Thakur, S.S., Tiwari, N., Sokefun, O.B., Yadav, S., 2021. Disentangling earthworm taxonomic stumbling blocks using molecular markers. *Journal of Threatened Taxa* 13(11), 19566–19579.
25. Manna, S., Ray, A., Mukherjee, S., Ray, M., Ray, S., 2023. Nano zinc oxide induced lipid peroxidation, oxidative stress, genotoxicity, phagocytic alteration, and detoxification response in the coelomocytes of an anecic earthworm of India. *Applied Soil Ecology* 190, 105024.
26. Mazur-Pączka, A., Kania, I., Borsuk, P., 2019. Soil structural improvements by Lumbricidae in agricultural landscapes. *Ecological Engineering* 127, 12–19. https://doi.org/10.1016/j.ecoleng.2018.12.015
27. Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter. In: Sparks, D.L. et al. (Eds.), *Methods of Soil Analysis. Part 3: Chemical Methods*. Soil Science Society of America, Madison, WI, pp. 961–1010.
28. Nouri-Aiin, M., Schall, J.J., Keough, C.A., Wen, Y., Görres, J.H., 2021. Identifying the unidentifiable: A PCR multiplex protocol for the diagnosis of invasive pheretimoid earthworm species, verified by morphological and barcode identification. *Applied Soil Ecology* 161, 103822.
29. Rajkhowa, D.J., Bhattacharyya, P.N., Sarma, A.K., Mahanta, K., 2015. Diversity and distribution of earthworms in different soil habitats of Assam, north-east India, an Indo-Burma biodiversity hotspot. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* 85(2), 389–396.
30. Rutgers, M., Orgiazzi, A., Gardi, C., Römbke, J., Jänsch, S., Keith, A.M., De Zwart, D., 2016. Mapping earthworm communities in Europe. *Applied Soil Ecology* 97, 98–111.
31. Schon, N.L., Mackay, A.D., Gray, R.A., van Koten, C., Dodd, M.B., 2017. Influence of earthworm abundance and diversity on soil structure and the implications for soil services throughout the season. *Pedobiologia* 62, 41–47.
32. Singh, R., Sharma, A., Kumar, P., 2020. Multivariate analysis of earthworm distribution across agricultural habitats. *BMC Ecology* 20, 15. https://doi.org/10.1186/s12898-020-00296-5
33. Singh, S., Sharma, A., Khajuria, K., et al., 2020. Soil properties change earthworm diversity indices in different agro-ecosystems. *BMC Ecology* 20, 27. https://doi.org/10.1186/s12898-020-00296-5
34. Singh, S., Sharma, A., Khajuria, K., Singh, J., & Vig, A. P. (2020). Soil properties influence earthworm diversity indices in different agro-ecosystems. *BMC Ecology, 20*(1), 27. https://doi.org/10.1186/s12898-020-00323-0
35. Singh, S., Singh, J., Vig, A.P., 2016. Earthworm as ecological engineers to change the physico-chemical properties of soil: Soil vs vermicast. *Ecological Engineering* 90, 1–5.
36. Ul-Allah, S., Khan, A.A., Burkert, A., Wachendorf, M., 2014. Socio-economic aspects of fodder production in urban and peri-urban areas of Faisalabad. *Pakistan Journal of Agricultural Sciences* 51(2).
37. Van Schaik, L., Palm, J., Klaus, J., Zehe, E., & Schroder, B. (2016). Potential effects of tillage and field borders on within-field spatial distribution patterns of earthworms. *Agriculture, Ecosystems and Environment, 228*, 82–90. https://doi.org/10.1016/j.agee.2016.05.015
38. Waqar, A., Shah, G.M., Bakhat, H.F., Shahid, M., Aslam, M., Ashraf, M.R., Rashid, M.I., 2019. The earthworm species *Pheretima hawayana* influences organic wastes decomposition, nitrogen mineralization and maize N recovery. *European Journal of Soil Biology* 90, 1–8. https://doi.org/10.1016/j.ejsobi.2019.04.001