(ISSN: 0973-7049(P))

The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 21(4): S.I (4),751-770, 2026 www.thebioscan.com

Comparative Assessment of Earthworm Biomass, Excreta Production, and COI Gene-Based Species Identification in Chilli and Paddy Agroecosystems of the Vidarbha Region in Sakoli Tehsil, Maharashtra, India

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DOI: https://doi.org/10.63001/tbs.2026.v21.i04.S.I(4).pp751-770

KEYWORDS

Earthworms; Chilli field; Paddy field; Soil health; Biomass; Excreta production; COI gene; Agroecosystem; Vidarbha.

Article Info

Received: 30/08 /2026
Accepted :18 /09/2026
Published: 28/10/2026

Abstract

Present study evaluated the influence of crop type and cultivation stage on earthworm communities in chilli and paddy fields of the Sakoli Tehsil in Vidarbha region, Maharashtra, India. Earthworms were sampled during the pre-sowing and harvesting stages, and their biomass, fresh body weight, excreta production, excreta production rate, and hourly excretion rate were determined. Adult earthworms were identified morphologically and further confirmed through cytochrome c oxidase subunit I (COI) gene sequencing and phylogenetic analysis. Statistical comparisons were performed using an unpaired t-test. Earthworm biomass remained relatively stable in chilli fields but declined significantly in paddy fields after harvest. Physiological parameters such as fresh body weight and excreta production exhibited crop-specific responses, indicating that soil texture, moisture, nutrient availability, and agricultural practices strongly influenced earthworm activity. COI-based molecular identification confirmed the occurrence of *Eisenia fetida* and *Travoscolides changannueres* in both cropping systems, whereas *Amynthas mayshanesis* was restricted to chilli fields. The findings demonstrate that earthworm physiological performance and species composition vary with agroecosystem characteristics and cultivation stage, highlighting their value as bioindicators of soil health and sustainable agricultural management.

Introduction

As essential pedofauna, earthworms significantly influence soil structure and fertility, yet their ecological role remains heavily impacted by intensive management practices in plantation systems (Abdi et al., 2025). While land-use conversion frequently triggers shifts in community composition, the transition from rainforest to monoculture often facilitates the dominance of invasive, peregrine species like *Pontoscolex corethrurus* at the expense of native richness (Garcia-Ulloa et al., 2018; Potapov et al., 2021). These shifts in community structure are fundamentally linked to fluctuations in biomass and density, which can increase significantly in mature monocultures despite a marked decrease in functional diversity (Potapov et al., 2021a, 2021b). Furthermore, the conversion of forest land into such plantations often results in an energetic reallocation where earthworms compensate for broader losses in soil arthropod communities, thereby stabilizing below-ground processes despite a simplified trophic network (Potapov et al., 2024). Beyond these shifts in population density, the resulting changes in species assemblages directly alter rates of excreta production and litter incorporation, which are critical for soil organic matter cycling

(Peerawat et al., 2018). Specifically, the homogenization of earthworm communities in intensified agricultural landscapes often leads to a decline in larger-bodied native species, shifting the overall size spectrum and potentially reducing the efficiency of nutrient mineralization and soil macropore formation (Dewi & Senge, 2015; Potapov et al., 2019). These functional shifts are further exacerbated by intensive management, as anecic and endogeic groups demonstrate heightened sensitivity to tillage and chemical inputs compared to more resilient epigeic species (Briones et al., 2019). Consequently, the ecological resilience of these plantation soil systems is frequently maintained by a narrow cohort of species that thrive in disturbed environments, albeit with distinct alterations in nutrient processing rates (Tondoh et al., 2019; Wong et al., 2016). Evaluating the efficacy of these systems requires an understanding of how specific agricultural regimes, such as the shift from conventional to semi-organic management, can modulate earthworm density and restore elements of taxonomic richness (ABDI et al., 2025). Such evaluations are increasingly urgent, as the depletion of diverse soil invertebrate communities often driven by the removal of native litter layers and vegetation compromises the long-term sustainability of ecosystem services provided by these habitats (Cifuentes-Croquevielle et al., 2020; Krashevska et al., 2019; Tůma et al., 2019). Beyond these structural changes, the inherent physical and chemical properties of plantation soils, such as moisture availability and nitrogen supplementation, act as critical mediators that can either mitigate or further suppress earthworm biomass and diversity (Naik et al., 2024). Moreover, the degradation of habitat quality often prevents recolonization by native taxa, leaving croplands dependent on a reduced functional pool that struggles to maintain necessary soil structure and drainage, (Briones & Schmidt, 2017). In the present study change in the distribution of earthworm functional groups, biomass, and excreta production rates within agricultural plantations is evaluated to determine how diverse management strategies influence these vital pedological indicators (Lago et al., 2019; Naik et al., 2023). By integrating data on cast deposition and species-specific turnover rates, this research elucidates how varying intensity levels modulate the mineralization of organic residues (Fonte & Six, 2010).

Methods

Study site

The study sites were located in the Vidarbha region in Sakoli Tehsil of Maharashtra, India, where chili and rice have been cultivated for over 50 years. The soil in the chili fields consisted of well-drained sandy loam, while the rice fields utilized clayey loam soil, favored for its excellent water-retention capacity.

Field methods

In this study, earthworm sampling was conducted in chilly and rice fields during the pre-sowing and harvesting stages in November 2024 to February 2025 and July 2025 to November 2025, respectively. At each replicate site, four randomly located pits were dug to a depth of 30 cm to collect soil. Upon collection, earthworms were separated from the soil using the hand-sorting method, placed in petri dishes lined with moist cotton towels, and counted in the laboratory. To record fresh biomass, the earthworms were placed in petri dishes; after the earthworms voided their gut contents, their excreta and fresh body weight were measured. Based on this data, we calculated the relative excreta production rate and the relative change in the hourly excretion rate.

The formula used as:

Excreta production rate = (Excreta released (mg)) / (Earthworm weight (g))

Hourly excretion rate = (Excreta (mg))/ (Weight (g) x 24)

Earthworms were categorized into three age classes as juvenile, adolescent, and mature based on the development of the clitellum, the reproductive organ involved in cocoon production that forms a distinct band around the midsection segments. Juveniles lacked a visible clitellum, whereas adolescents were identified by the lightening of segments where the clitellum was developing; mature earthworms were characterized by a fully developed clitellum.

COI gene based Earthworm identification

Mature earthworms were euthanized with ethanol and identified using keys provided by Schwert (1990). To confirm species-level identification, earthworms from both paddy and rice fields were targeted for COI gene analysis. Tissue DNA was isolated using an Expure tissue DNA isolation kit. In the PCR protocol, the following primers were used:

LCO 1490 5’ GGTCAACAAATCATAAAGATATTGG 3’

HCO2198 5’ TAAACTTCAGGGTGACCAAAAAATC A 3’

In a PCR reaction, 5µl of DNA added in 25µl of PCR reaction solution (1.5µl forward primer and reverse primer, 5µl deionised water, and 12µl of Taq master mix.) The PCR reaction carried out using following conditions-

Initial denaturation (95°C, 25min); Denaturation (95°C, 30sec); Annealing (40°C, 30sec); Extension (72°C, 2min); Final extension (72°C, 10min) and total 25 cycles of reaction and PCR kept on hold at 4°C. Further PCR product purified by using Montage PCR clean up kit and then sequenced by using the primers. Here sequencing reaction were performed by using a ABI PRISM, Big Dye TM Terminator Cycle sequencing kit with AmpliTaq DNA polymerase (FS enzyme)(Applied Biosystems). In a process, single pass sequencing was done. Further obtained sequences were then analyzed for homology using BLASTN program and identity of each earthworm has been confirmed (total six as per discrete morphology) upto species level. These earthworms then phylogenetically analyzed to confirm their evolutionary relationship and identity.

Statistical Analysis

Relative changes in earthworm parameters in chili and rice fields during the sowing and harvesting stages were analyzed using an unpaired t-test ($P < 0.05$) in GraphPad Prism software.

Result and Discussion

Earthworm Biomass

Chilli field

In the chilli fields, earthworm biomass recorded before sowing (83 ± 11 g/m^2) was comparable to levels observed at harvest (87 ± 17 g/m^2), with no statistically significant change ($P > 0.6141$) in change (Table 1). Conversely, rice fields exhibited a marked increase in earthworm biomass, rising from 62 ± 9 g/m^2 during the pre-sowing period to 105 ± 14 g/m^2 at harvest, a difference likely driven by the moisture-retentive properties of the clayey loam soil which supports higher abundance of soil-dwelling invertebrates (Khan et al., 2022; Sankar & Patnaik, 2018). This trend in biomass accumulation correlates with regional soil health, as earthworms represent nearly 80% of total soil invertebrate biomass, serving as vital bioindicators in agroecosystems (Vabeiryureilai et al., 2020).

Paddy field

In the paddy fields, earthworm biomass recorded before sowing (48 ± 7.8 g/m²) was significantly higher than the recorded post-harvest (26 ± 5.6 g/m²) ($P<0.0011$),

indicating a marked reduction in earthworm biomass associated with the harvesting stage (Table 2). This decline may be attributed to the physical disruption of soil structure during paddy harvest, which often subjects earthworms to increased desiccation and predation risks (Lalthanzara et al., 2020). These disturbances, compounded by the removal of crop residues that serve as critical surface mulch, significantly reduce available organic matter and microhabitat stability within the topsoil layer (Torppa et al., 2023). Such variations in population dynamics align with findings that non-cultivated habitats often maintain greater stability and species abundance compared to more intensive agricultural systems (Singh et al., 2020).

Excreta production rate and Hourly excretion rate

Evaluation of adult earthworms from paddy fields indicated that fresh body weight remained stable throughout the growing season. Specifically, the fresh weight recorded before sowing was 0.82 ± 0.65g, which was statistically comparable to the 0.72 ± 0.34 g observed at harvest, showing no significant difference (P>0.6933) (Table 3). In contrast, earthworms collected from the chilli fields displayed a more pronounced fluctuation in physiological output, suggesting that differences in soil texture and irrigation regimes influence metabolic activity and waste deposition across distinct agricultural habitats (Lal et al., 2025). These physiological variances likely reflect adaptive responses to varying organic matter availability, as earthworm biomass and individual development are highly sensitive to the nutritional quality and water-holding capacity of the soil matrix (Chassain et al., 2024; Hallam & Hodson, 2020). Furthermore, the presence of these essential invertebrates is further regulated by physicochemical parameters, as high concentrations of soil organic carbon and mineral nutrients are positively correlated with increased population density and sustained community stability (Kalu et al., 2015).

Similarly, excreta production by earthworms in paddy fields did not vary significantly between the sowing and harvesting stages, with values of 660 ± 520 mg and 590 ± 280 mg, respectively (P> 0.7562)(Table 4). This consistency in physiological output suggests that the paddy environment provides a relatively stable substrate for earthworm metabolism throughout the cropping cycle. Conversely, the observed fluctuations in the chili fields may be attributed to periodic variances in soil moisture and nutrient availability, which are key drivers

of earthworm activity and physiological turnover (Bertrand et al., 2015). Furthermore, the application of organic amendments, such as manure, may mitigate these environmental fluctuations by enhancing habitat quality and providing a consistent food resource for epigeic and endogeic populations (Duddigan et al., 2023; Liu et al., 2025).

Regarding the excreta production rate, no statistically significant change (P>0.1434) was observed in paddy fields between the sowing and harvesting stages, with values recorded as 790 ± 34 mg/g and 820 ± 34 mg/g body weight, respectively (Table 5). In the chili fields, however, the hourly excretion rates exhibited greater variability, potentially reflecting shifts in soil moisture and organic carbon availability that dictate the metabolic turnover of these invertebrates (Bhosale et al., 2025). These physiological shifts highlight the environmental sensitivity of earthworms, where habitat composition and pH directly influence both their mobility and the bioavailability of essential nutrients (Chatelain & Mathieu, 2017).

Finally for hourly excretion rate, earthworms of paddy recorded during sowing and harvesting as 19000±820 mg/g/h and 20000± 820 mg/g/h, respectively which remained statistically non-significant in change(P>0.1436) (Table 6). In contrast, the chilli fields showed significantly higher hourly excretion rates at harvest compared to pre-sowing periods, likely resulting from the accelerated mineralization of crop residues and increased availability of assimilable nutrients (Bhadauria & Saxena, 2009). These patterns reinforce the role of earthworms as critical drivers of nutrient cycling, where their metabolic processes enhance the availability of essential elements like phosphorus and nitrogen within the soil matrix (Suntoro et al., 2024).

In the chilli fields, the fresh body weight of earthworms was 0.74 ± 0.38 g during the sowing stage, which significantly declined to 0.44 ± 0.18 g at harvest (P< 0.0433). This substantial reduction suggests that harvesting practices and associated environmental changes negatively affect the physiological status of earthworms (Table 7). Such population declines underscore the vulnerability of soil-dwelling invertebrates to the physical disruption and habitat modification inherent in intensive agricultural land-use systems (Naik et al., 2024). Indeed, the intensification of agricultural practices frequently leads to a sharp decrease in earthworm biomass and diversity due to

the cumulative effects of soil degradation and chemical stress (Naik et al., 2023).

Similar trend recorded with excreta released by earthworms recorded during sowing and harvesting stages of chilly. Here values recorded as 600 ± 310 mg and 340 ± 150 mg with significant change (P>0.0382) respectively indicating dependencies on plant growth and resultant impact on excreta production and reduction in excreta production during harvesting (Table 8). These metabolic variations underscore how epigeic and endogeic species regulate biogeochemical cycling, as their burrowing and feeding activities directly modify soil physicochemical properties and nutrient availability (Guarderas et al., 2022). Moreover, these metabolic activities promote the mineralization of soil nutrients, effectively transforming inaccessible organic forms into plant-available fractions that foster long-term agricultural sustainability (Jing et al., 2023).

In case of chilly, earthworm's excreta production rate during sowing and harvesting stage, values recorded as 810±96 mg/g/h and 770±89 mg/g/h, respectively showing no significant difference (P>0.3681)(Table 9). Conversely, the hourly excretion rate of 21000 ± 950 mg/g/h observed at sowing in chili fields increased to 28000 ± 1100 mg/g/h at harvest, indicating that metabolic turnover may be stimulated by the increased availability of organic residues and microbial activity following crop maturation (Iordache, 2023; Wu et al., 2023). These metabolic shifts reflect the capacity of earthworms to act as ecosystem engineers, as they produce nutrient-rich casts that integrate organic matter and essential minerals into the soil profile (Ahmed & Al-Mutairi, 2022; Saharan et al., 2025).

Here hourly excretion rate also remained unaffected with respect to plant sowing and harvesting stages for sampled earthworms. Here the values recorded as 19000±2300 mg/g/h versus 18000±2100 mg/g/h, respectively with no significant difference (P> 0.3659) (Table 10). These findings align with broader observations that intensive agricultural disturbances, including frequent tillage and agrochemical exposure, significantly diminish the functional diversity and resilience of lumbricid communities. Given that cast production serves as a highly sensitive behavioral biomarker, these metabolic declines provide early evidence of ecological stress induced by chemical and mechanical disturbances (Jouni et al., 2018).

COI gene based identification of earthworm

In this study, adult earthworms prevalent in the soil environments of paddy and rice fields were initially identified using Schwert's keys, with select specimens further confirmed through COI gene sequencing and homology analysis.

In this study, *Eisenia fetida* and *Travoscolides changannueres* were commonly present during the sowing and harvesting stages in both paddy and chili fields. Furthermore, *Amynthas mayshanesis* was specific to the chili fields throughout both plantation stages. The phylogenetic homology for all COI genes targeted in both paddy and chili plantations is presented in the Figure 1. These genetic analyses confirm the presence of distinct taxonomic groups, further illustrating how varied land-use types maintain specific earthworm assemblages despite anthropogenic pressures (Bibi et al., 2026; Teng, 2012). This taxonomic differentiation highlights the niche-specific adaptations of lumbricid species, which dictate their distribution across contrasting soil environments (Gudeta et al., 2022). These distinct taxonomic assemblages further demonstrate that species-specific traits, such as burrowing depth and feeding preferences, are instrumental in determining how different ecological categories respond to environmental disturbance (Forey et al., 2023; Pathma & Sakthivel, 2012).

Conclusion

The study demonstrated that earthworm populations and physiological performance differed between chilli and paddy cultivation systems and across crop growth stages. Variations in biomass, body weight, and excreta production reflected the influence of soil type, moisture availability, crop management, and harvesting practices on earthworm ecology. Molecular identification using COI gene sequencing confirmed the presence of distinct earthworm species and validated morphological identification. These findings support the use of earthworms as reliable indicators of soil quality and ecological sustainability. Integrating biological indicators such as earthworm diversity and activity into agricultural monitoring can improve soil health assessment and guide sustainable farming practices.

Limitations of the Study

1. The study was limited to selected chilli and paddy fields within the Vidarbha region, limiting broader geographical applicability.

2. Sampling was conducted only during pre-sowing and harvesting stages, excluding seasonal fluctuations throughout the cropping cycle.
3. Environmental variables such as soil pH, organic carbon, nutrient status, and pesticide residues were not directly correlated with earthworm responses.
4. COI gene sequencing was performed only for representative mature specimens, which may not capture the complete genetic diversity of the earthworm community.
5. The relatively small sample size may reduce statistical power for detecting subtle ecological differences.
6. Long-term monitoring across multiple years would provide a better understanding of temporal changes in earthworm populations.

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Table 1. Comparison of Earthworm Biomass in Chilli Fields During Pre-Sowing and Post-Harvesting Stages		
	Chilly (Pre)	Chilly (Post)
Minimum	68	65
Median	87	85
Maximum	95	110
Mean± Std. Deviation	83±11	87±17
P value : 0.6141 Non significant		

Table 2. Comparison of Earthworm Biomass in Paddy Fields During Pre-Sowing and Post-Harvesting Stages		
	Paddy (Pre)	Paddy (Post)
Minimum	39	20
Median	45	25
Maximum	59	35
Mean	48	26
Std. Deviation	7.8	5.6
P value : 0.0011 Significant		

Table 3. Comparison of Fresh Body Weight of Adult Earthworms Collected from Paddy Fields During Sowing and Harvesting Stages		
	Paddy (Sowing) Fresh weight	Paddy (Harvesting) Fresh weight
Minimum	0.067	0.049
Median	0.69	0.89
Maximum	2.0	1.0
Mean± Std. Deviation	0.82±0.65	0.72±0.34
P value : 0.6933 non-significant		

Table 4. Comparison of Excreta Production by Adult Earthworms Collected from Paddy Fields During Sowing and Harvesting Stages		
	Paddy (Sowing) Excreta	Paddy (Harvesting) Excreta
Minimum	49	36
Median	540	730
Maximum	1500	840
Mean± Std. Deviation	660±520	590±280
P value 0.7562 non-significant		

Table 5. Comparison of Earthworm Excreta Production Rate (EPR) in Paddy Fields During Sowing and Harvesting Stages		
	Paddy (Sowing) EPR	Paddy (Harvesting) EPR
Minimum	730	730
Median	790	830
Maximum	860	850
Mean± Std. Deviation	790±34	820±34
P value : 0.1434 non-significant		

Table 6. Comparison of Hourly Excretion Rate (HER) of Adult Earthworms in Paddy Fields During Sowing and Harvesting Stages		
	Paddy(Sowing) HER	Paddy(Harvesting) HER
Minimum	18000	18000
Median	19000	20000
Maximum	21000	20000
Mean± Std. Deviation	19000±820	20000±820

Table 7. Comparison of Fresh Body Weight of Adult Earthworms Collected from Chilli Fields During Sowing and Harvesting Stages		
	Chilly (Sowing) Fresh weight	Chilly (Harvesting)Fresh weight
Minimum	0.15	0.23
Median	0.79	0.41
Maximum	1.3	0.81
Mean± Std. Deviation	0.74±0.38	0.44±0.18
P value 0.0433 Significant		

Table 8: Comparison of Excreta Production by Adult Earthworms Collected from Chilli Fields During Sowing and Harvesting Stages		
	Chilly (Sowing) Excreta	Chilly (Harvesting)Excreta
Minimum	100	180
Median	620	330
Maximum	1200	660
Mean± Std. Deviation	600±310	340±150
P value 0.0382: Significant		

Table 9: Comparison of Earthworm Excreta Production Rate (EPR) in Chilli Fields During Sowing and Harvesting Stages		
	Chilly (Sowing) EPR	Chilly (Harvesting)EPR
Minimum	670	540
Median	790	800
Maximum	1000	830
Mean± Std. Deviation	810±96	770±89
P value0.3681: Non significant		

Table 10. Comparison of Hourly Excretion Rate (HER) of Adult Earthworms in Chilli Fields During Sowing and Harvesting Stages

	Chilly (Sowing) HER	Chilly (Harvesting) HER
Minimum	16000	13000
Median	19000	19000
Maximum	24000	20000
Mean± Std. Deviation	19000±2300	18000±2100
P value 0.3659: Non significant		

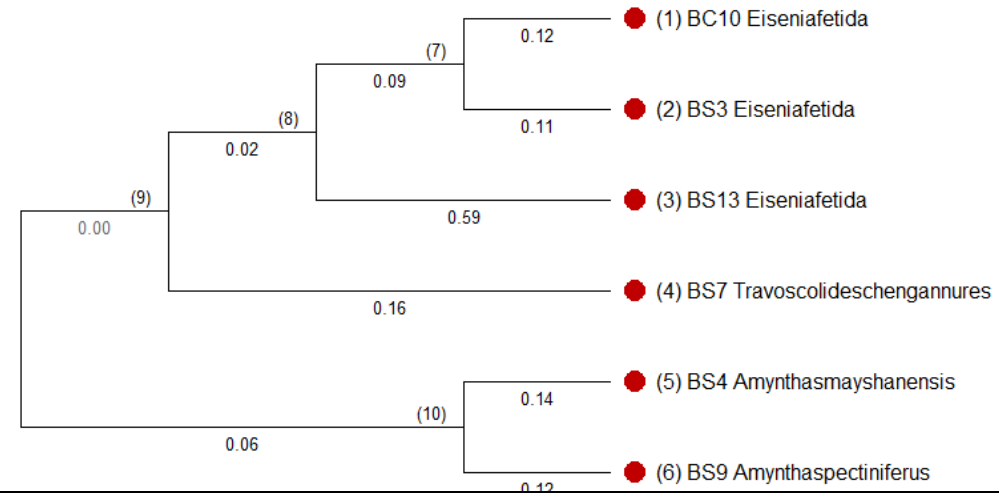


Figure 1. Phylogenetic Tree Based on COI Gene Sequences Showing the Evolutionary Relationships Among Earthworm Species Collected from Chilli and Paddy Fields