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REVIEW ARTICLE

Bacterial community present in the earthworm's gut and its role in soil biology and health

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Abstract

Earthworms are known as ecological engineers due to their significant role in enhancing soil health and productivity. Various factors such as temperature, moisture, acidity, pH, sunlight and the availability of organic matter influence their presence in soil. Earthworms exhibit diverse feeding and burrowing behaviors, which lead to crucial ecological processes within terrestrial ecosystems. Their interactions with soil result in the colonization of their gut and surrounding soil by diverse bacterial communities, including key species such as *Escherichia coli*, *Streptomyces*, *Bacillus* and *Pseudomonas*. These bacteria aid in the digestion of organic and inorganic matter, thereby altering soil physio-chemical properties and enhancing nutrient mineralization, which promotes plant growth. Additionally, earthworms influence nutrient cycling by modifying microbial soil populations and the bacterial communities in their gut and adjacent soil contribute to phytoremediation. This review delves into the types of bacterial populations found in the earthworm's gut and surrounding soil, elucidating their specific roles and contributions to the terrestrial ecosystem. By understanding these complex interactions, we can better appreciate the vital role earthworms and their associated bacterial communities play in soil biology. This knowledge is essential for developing sustainable agricultural practices and improving soil management strategies, ultimately contributing to healthier and more productive ecosystems.

Keywords

earthworm; bacterial population; plant; soil; ecology

Introduction

The earthworm belongs to the class Oligochaeta, it consists of 800 genera and 8000 species and is found in both terrestrial and aquatic ecosystems (1). Earthworms are a few mm in length and are regarded as major macrofauna in many soils. Pastures and meadows have plenty of species in ecosystems inhabited by earthworms, accompanied by agricultural lands as well as forests. The earthworm's presence in soil is stimulated by factors like temperature, moisture, acidity, pH and sunlight, along with the accessibility of the organic matter. Earthworm species exhibit different feeding and burrowing behavior and are categorized into 3 different ecotypes named as epigeic, endogeic and anecic (2). Epigeic species such as *Lumbricus rubellus* and *Eisenia foetida* subsist over the surface of mineral soil, burrow and become a substitute for litter. Endogeic species, including *Aporrectodea caliginosa* and *Octolasion lacteum,* dwell in the upper layers of the soil, consuming large volumes of the soil, forming horizontal burrows and nourishing the rhizosphere. Anecic species like *Lumbricus terrestris* and *Aporrectodea longa* reside in the deeper layers of the soil. They are known to ingest soil in neutral quantity and drag litter into their burrows, and feed upon it. Generally, the soil is the habitat for the microbial community on the earth. Microbiomes associated with earthworms' gut and soil have been catching attention due to their potential role in ecosystem services (3). The microbial community helps in the enrichment of the soil. Bacteria accelerate the organic matter decomposition while the earthworms feed upon the denitrifying bacteria, which is capable of surviving in anaerobic conditions present in the gut (4). Soil macrofaunae like beetles, termites and earthworms act as the basic moderators for soil production as well as operation. The principal macroinvertebrates play a magnificent role in the fertility of the soil. They influence the process of nutrient cycling and growth of plants by the microbes present in the earthworm's gut and its surroundings in a terrestrial habitat (5). Earthworms also influence microbial and soil interactions (6). The actions of earthworms are known to intervene in the establishment of both micros as well as macroaggregates (7).

When soil travels from the earthworm's gut, it encounters various physical and chemical microbial modifications. The different life forms of earthworms are known to have different impacts on the diversity of soil microbes, which explains the changes occurring in the earthworm cast and gut processes (8). The bacterial number in the casts is significantly larger if compared with the adjoining soil, although the organization of bacterial colonies inside the earthworm as well as the soil seems to be much more similar (9).

Microbes present in the earthworms' gut are known to perform a dynamic function in the genesis of soil (10), while the microbial actions increase the nutrient cycling of the drilosphere (11). The drilosphere is the soil zone directly influenced by earthworms' burrowing and digestive activities, enhancing soil structure and microbial activity. Gut microbes contribute to the weathering of the soil. Phosphorus and potassium solubilizing bacteria have also been separated from the gut of earthworms (12).

Over the last decade, researchers have understood the significant role of the worm's gut microflora, which contributes to nutrient alteration (13). It is acquired chiefly as extreme progress in the selection of bacteria as well as the separation process, containing unculturable bacterial species having standard isolation and next-generation classification methods (14).

The amount of anaerobic as well as anaerobic bacteria present in the earthworms' gut is about 12-20 folds, which is about 10-4000 folds more than that present in the soil. Most of the bacteria present in the earthworm's intestine are derived from the adjoining soil surrounding the worm (15). The anaerobic nature exhibited by the earthworm's gut also provides a micro-environment for the facultative anaerobic bacterial species, which accounts for the large difference between the bacterial concentration of the earthworm's gut and the surrounding soil. The number of elements such as N, P, C and S present in the earthworm's gut has been reported to be greater than that present in the surrounding soil (10). These nutrients are beneficial for the remediation of pollutant alteration due to the presence of heavy metals and microplastics (16).

Microbes are essential in the environment due to their potential applications. They play a significant role in the fields of agriculture and pollutant remediation. Presently, the improvement is going on in the context of microbe potential for various applications in environmental management (17). The microbes present in the earthworm's guts are much more significant if compared to the microbes present in the soil. The dominant bacterial communities become selectively active inside the worm's gut, stimulating various enzymatic actions (18).

The study with context to the bacterial community present in the earthworm's gut and soil is essential as it can be beneficial in environmental-related applications like pollutant remediation and plant growth. The bacterial community is also an important parameter for studying the quality and efficiency of manure and fertilizers, which supports the agriculture sector in influencing the economy. The microbial communities also act as biological indicators of the terrestrial ecosystem. The presence of a bacterial community inside the earthworm's gut is related to the surrounding soil and it also plays a dynamic role in the nutrient cycle which is an important aspect of maintaining balance in the ecosystem. Thus, the main objective of this paper is to focus on the bacterial richness of the soil ecosystem and its potential in the structuring and functioning of natural environmental processes.

Earthworms' gut and bacterial community present in it

The gut of an earthworm is a straight tube-like structure that holds a constant temperature even during the regulatory procedures, which helps in speeding up various biological processes. The gut acts like a bioreactor and helps in enzyme inhibition, which occurs due to the huge temperatures. Gizzards in earthworms crushes particles into very small size approximately less than 2 mm. This small size of the particles thereby increases the surface area, which is more suitable and favourable for microbial growth.

The gut is essential to record the complementary influence of harmful constituents like microplastics, heavy metals, and other pollutants upon bacterial action as well as upon the diversity of other microbes present in earthworm species (19). The gut in *Metaphire guillelmi* extends from mouth to anus, having an average length of 90-250 mm and a width of 5-10 mm (19). Because of oxygen reduction, the gut favors anaerobic conditions, which inhabit bacterial species like Actinobacteria, Proteobacteria, Firmicutes and Bacteroidetes (10). The gut surroundings of the earthworm can play the role of a definite strainer and fermenter for bacteria and fungi species associated with the bacterial cells that exist along with the gut passage and can show multiplication while entering into the hindgut.

The earthworm's gut is regarded as a perfect environment for microbes like bacteria, as many surveys show the presence of huge microbial populations in the guts exposed to the soil. It was also reported after an analysis that qualitatively, the microbial community present in the earthworm's gut is somewhat like the microbial population present in the vicinity of the surrounding soil.

The *in-situ* environment of the earthworm's gut possesses anoxic conditions along with a higher concentration of organic substrate. They stimulate bacteria present in the soil. It shows anaerobiosis (20). The presence of increased amounts of anoxia, organic acids, mucus, plant saccharides, nitrous oxide and hydrogen shows that the gut of an earthworm works like a bioreactor. It offers a huge diversity of biochemical as well as metabolic actions with context to the bacterial community.

It was found that the earthworm's gut contains different types of symbiont microbes. The population of microbes is higher in the foregut and it progressively decreases in the midgut as well as in the hindgut. It is also found in trace amounts in the casts. Earthworm's guts provide a supreme environment for microbial growth, mainly for bacterial and fungal communities. Casts laid by earthworms extensively have enriched bacterial counts if compared to the soil present in the surroundings.

Microbes as well as enzymes derived from the earthworm's gut can influence the microbial growth occurring over the particles of soil, and the gut can act as a source of nourishment for them. Earthworms keeps wandering in the soil and thus, soil particles easily flow into their gut. The constitution of the microbial community in the earthworms gut was transformed as bacterial formation increased (21).

The bacterial community plays a significant and secondary role in the diet of worms and serves as a chief source of nutrients. Earthworms present in the disinfected environment can survive with certain customs of bacteria and fungi, whereas they can be best produced with combinations of different microbes. This symbiotic association between microbes and earthworms leads to the decomposition and disintegration of organic matter.

Seven bacterial species, namely *B. thurigiensis*, *B. sphaericus*, *B. pabuli*, *B. pasteurii*, *B. megaterium*, *B. insolitus* and *B. breris,* were recognized from the Bacillus genus inside the intestine of the species *Onychochaeta borincana* (22). All these species are standard bacteria present in the soil. A reduction (from front to back portion) in the weight of microbes inhabiting the intestinal area was reported and for further analysis, different procedures were followed and they came out in the support of the context recognizing *Aeromonas, Azotobactor*, *Bacillus, Enterobactor, Klebsiella, Pseudomonas* and *Serratia* species (23).

Some common bacterial species present in the earthworms gut and its surrounding soil are *Clostridium* and *Bacillus*. The worm gut contains a high content of nitrogen and carbon, whereas low oxygen levels make it much more beneficial for inhabiting anaerobic microbes (24). Although some investigations have also reported that the gut of earthworms favors the settlement of bacterial species like *Aeromonas* and *Staphylococcus* which are anoxic in nature (25). There is a variation in the arrangement of bacterial colonies among various species of earthworms depending upon their habitat and environmental conditions. Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria show the maximum contribution to the earthworms' gut.

By using Single Strand Conformation Polymorphism (SSCP), approximately 8 bacterial strains were taken from the guts of *Lumbricus terrestris* and *Aporrectodea caliginosa* species of earthworms and adjoining soil. A specific primer named 16S rRNA gene targets bacteria like Bacteroides, *Verrucomicrobia*, *Alphaproteo*, *Betaproteo*, *Gammaproteo* and *Deltaproteo*. Firmicutes as well as Planctomycetes act as traditional primers for SSCP, along with DNA and RNA templates. The use of fluorescence in the case of *in-situ* hybridization makes the bacterial population more prevalent in the soil as well as in the casts. Bacterial Taxonomic Unit help in maintain microbial diversity and contributes to enhancing their population during the passage through the intestinal tract of earthworm species (26).

Earthworms gut contains nitrogen-producing bacteria

Earthworms act as the main source to produce nitrogen gas. Nitrous oxide is a greenhouse gas that is known to be released by earthworms from both forests and garden soils. They may produce about 30% of total nitrous oxide from the soil where they reside and about 85-99 % from their gut contents (15). The microbes associated with nitrogenous gas production inhabit the earthworms guts and are known to have been derived from the soil ingested by them.

Species like Acidobacteria are present in large quantities in the soil, whereas in very fewer amounts in the earthworms gut. The reason for low counts of Acidobacteria might be the presence of large levels of nitrogen and carbon contents in earthworms gut as Acidobacteria is mostly known to be present in conditions that have a limited supply of nutrients (29). Thus, concentrations of nutrients, as well as oxygen levels, act as essential and critical parameters for distinguishing the bacterial arrangement inside the earthworms gut and in the adjoining soils.

Genera *Flavobacterium* and *Paenibacillus* generally constitute nitrate reducers derived from the soil. These are present inside earthworms gut microflora and show their contribution to emitting nitrous oxide which helps in the decomposition of matter by involving the role of hydrolytic enzymes (30). *Pseudomonas* bacterial species help in nitrogen fixation and promote plant growth. These bacteria which are capable of solubilizing phosphate are also found in the earthworm's alimentary canal. Phosphate-solubilizing bacteria present in the earthworms' gut affect the growth of microbes and influence the enzymatic action, thereby increasing the availability of phosphate (31).

Agrobacterium, *Chthoniobacter* and *Rathayibacter* are some other bacterial species found in the soil whereas some of them are also present in the earthworm's gut (8). The soil having higher contamination shows the presence of *Pseudomonas* strains resisting heavy metals (32). *Rhodobacter* bacterial species also offer high resistance to various heavy metals (33). Fig. 1 represents the different abilities of bacteria that play an essential role in soil health and biology.

Benefits of bacterial populations present inside earthworms' gut and adjoining soil

Earthworms are known to play a significant role in the fertilizer industry as they have the capability of converting organic waste into organic fertilizer, as shown in Fig. 2 (24). The individuality of the substrate used in composting influences the arrangement of a bacterial colony in vermicompost and earthworms gut.

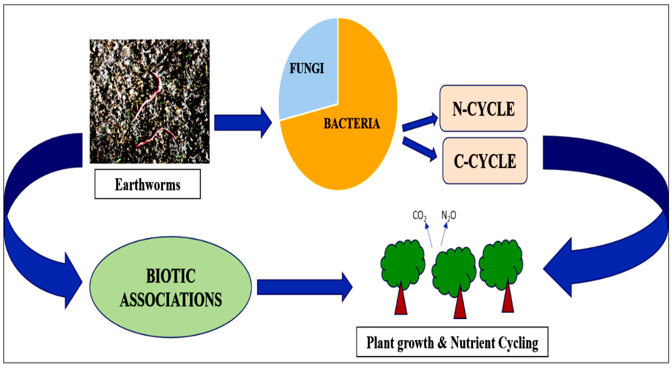

Fig. 2. Bacteria present in earthworm gut have the potential to enhance the fertilizer industry.

Different types of manures, like those of cows and horses, promote the growth of Proteobacteria whereas that of pigs promote the colonization of Firmicutes in the vermicompost (8).

Scientists have found that the procedure for vermicomposting increases the abundance of anoxic bacterial species as a result of digestion processes occurring inside the gut of earthworms. The organization of the bacterial species in the intestine of earthworms varies according to the diversity of worm species. The composting of *Eisenia andrei* changes the quantity of Firmicutes from 18.8 % to 62.7 % in the composting substrate and up to 35.7 % in the earthworms' gut (34). Likewise, composting of *Ei-*

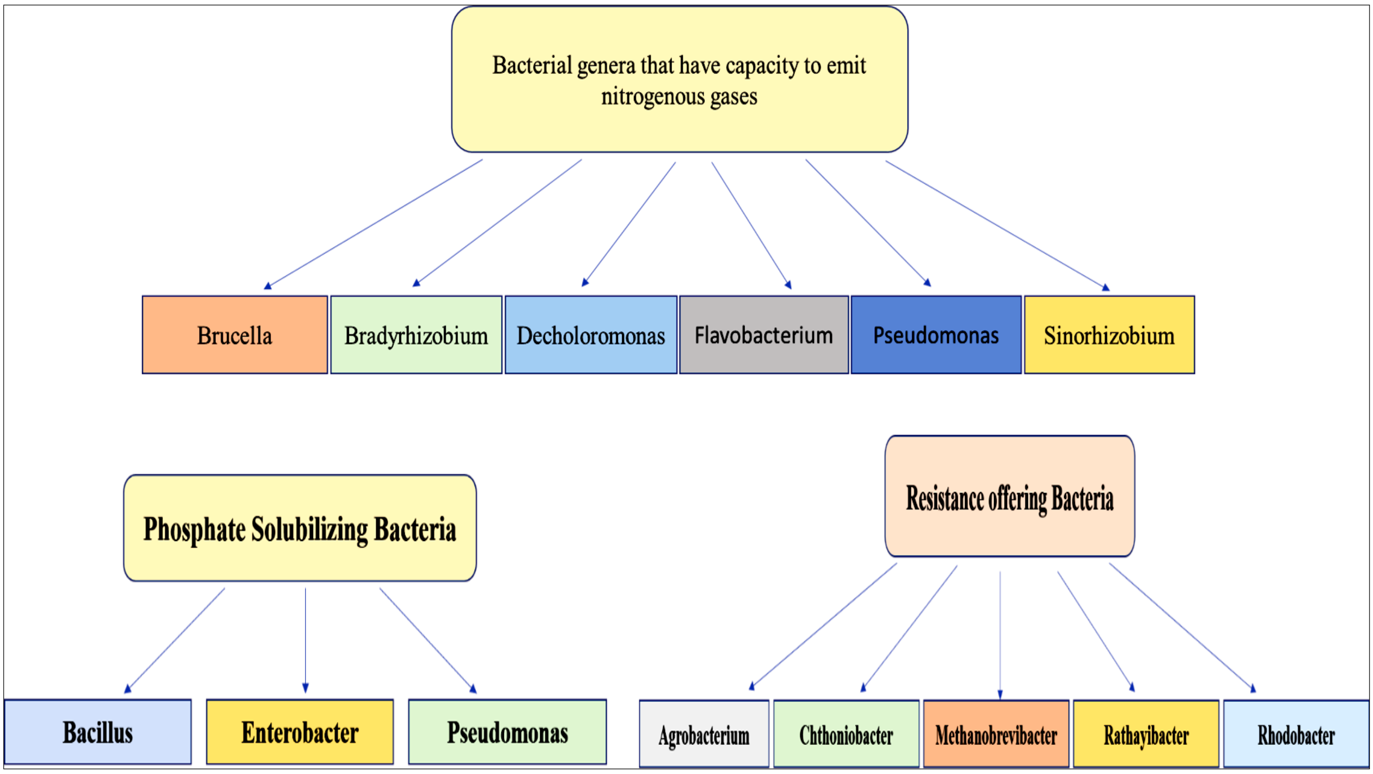

Fig. 1. Abilities of bacteria that play an essential role in the soil health and biology.

senia foetida enhances the % of Firmicutes in vermicompost and demarcates the quantity of Gram-negative as well as Gram-positive bacteria present in the vermicompost and earthworms gut (35).

After being passed from the earthworms gut, vermicompost contains an elevated bacterial diversity along with a great abundance of gram-positive and anoxic bacterial species with context to the earthworms gut and adjoining soil. Technology and industry have been fast moving towards advancement, which has also led to an increase in the harmful pollutants being discharged into the soil, containing heavy metals, antibiotics, microplastics and chemicals. This has established a major risk to the production of agricultural products as well as public health (36).

The bacterial community present in the earthworm's gut serves to detoxify pollutants through various steps such as alteration, assimilation and bioaccumulation occurring in the biodegradation and bioremediation processes as shown in Fig. 3 (37). Thus, the earthworm's gut is gaining the interest of many scientists because of its significant action in contributing to the constancy of the terrestrial ecosystem.

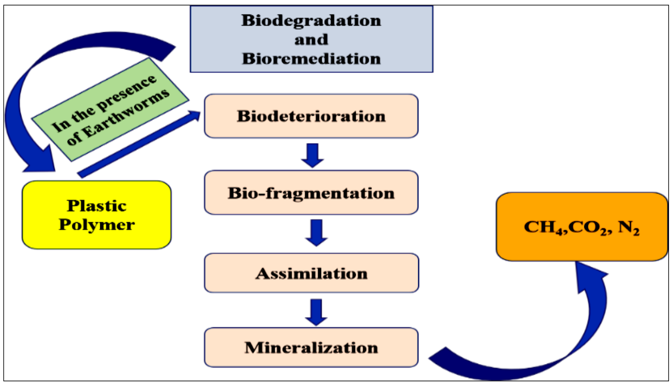

Fig. 3. Presence of bacteria leads to biodegradation and bioremediation process.

The bacterial species present in the earthworm's intestine also act as biomarkers or indicators of soil pollution, as shown in Fig. 4. They show different reactions when exposed to distinct concentrations and types of pollutant dosages. Pollutants with low dosage concentrations are known to initiate the modification in the intestine of the worm by increasing the quantity of the bacterial population but do not show any alteration in its arrangement. It results in influencing the physio-chemical changes in the earthworms (38).

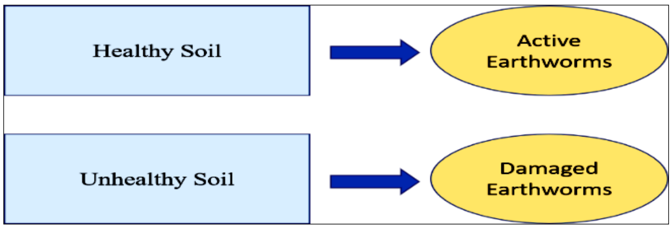

Fig. 4. Biomarkers of pollution.

On exposure to 10 mg of tetracycline for about 14 days, the bacterial population of Planctomycetacia showed a significant decline from about 33.05 to 3.28 %; whereas the Actinomycete population showed an increase from about 2.47 to 23.65 %. It remarks on the short-term stress produced as a result of tetracycline, showing a transitory variation in the intestinal bacterial population (39). The gut of microbes is a complex zone constituting various microbes. The population of bacteria present in the intestine of a worm acts as a basic constituent for performing a vital action in the preservation of the sustainability of the gut microflora. The bacterial population of earthworms' intestines is useful in depicting the status of pollution and estimating the effects on earthworms that occur as a result of harmful pollutants (39).

Exposure of worm intestinal bacteria to low-dose pollutants for longer durations may lead to slight and slow variations that may become permanent later. Although long-term exposures to such pollutants result in the development of resistance and detoxification abilities which leads to the creation of a new population (40).

Microbes have an essential role in the weathering of soil. Microorganisms, including bacteria, fungi, cyanobacteria and lichens, are said to be well-known 'soil engineers' actively participating in the pedogenesis through commencing the process of biological weathering of rocks, decomposition of organic matters and nutrient cycling. Microorganisms produce carbonic acid and organic acids that chemically break down minerals in the soil. They can also release chelating agents that bind to metal ions, enhancing mineral dissolution. They expel out the organic substances, which are known to initiate various physical as well as chemical processes (41).

In the 18th century, microbes played a crucial yet largely unrecognized role in the mining industry through a process called bioleaching. This biological method uses bacteria to extract valuable metals like copper, gold and uranium from ore deposits. Microorganisms such as *Acidithiobacillus ferrooxidans* thrive in the acidic environments of mining sites, breaking down sulfide minerals and releasing metal ions into the solution. Bioleaching proved particularly useful for extracting metals from low-grade ores, which were not economically feasible with traditional smelting techniques. Although the intentional application of microbial mining developed later, the principles observed in the 18th century laid the foundation for modern biohydrometallurgy, underscoring the significant yet initially overlooked contributions of microbes to metallurgy (42).

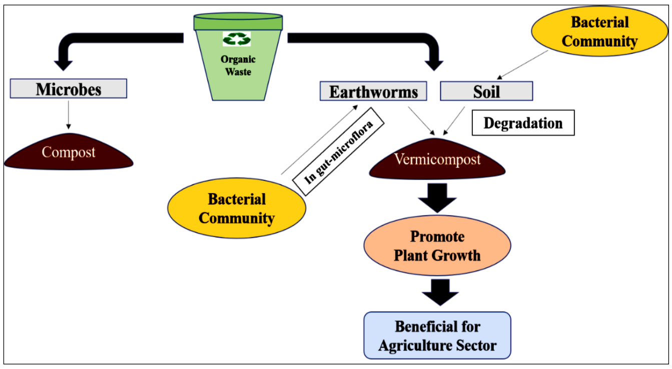

Fig. 5. Transformation of garbage into black gold.

Earthworms have the capability of transforming garbage into gold as illustrated in Fig. 5. Earthworms are known as 'unheralded soldiers of mankind' as stated by Charles Darwin, whereas they were also regarded as the 'intestine of the earth' by Aristotle because of their ability to ingest organic substances. The selection of food serves as a source of energy by enhancing organic matter digestion and thereby the release of intestinal and cutaneous mucus.

Earthworms participate in the formation of soil, turnover of carbon, degradation of cellulose, and in the collection of hummuses. These actions also influence the physio-chemical and biological characteristics of the soil. They feed on organic waste and consume just a little part of the waste to carry out their metabolic processes.

A wide range of microbes are inhabited inside the earthworm's gut. Enzymes as well as hormones helping in the rapid disintegration of incompletely digested substances transform them into vermicompost in a very short time which is about 4-8 weeks respectively. It takes about 20 weeks to manufacture compost. When organic matter passes through the earthworm's gizzard, it is crushed into very fine powder-like particles. Enzymes and microbes, along with other fermenting substances, contribute to the decomposition processes occurring inside the worm's gut. Finally, the cast is excreted out and they are then accomplished by the microbes associated with the gut which further leads to the formation of vermicompost (43).

Earthworms as ecosystem engineers tend to affect the soil processes

Earthworms are also known as ecosystem engineers, as they perform a significant task in the formation of soil and in nutrient cycling. Disintegration and mineralization with context to litter supports the burrowing and feeding habits that enhance the characteristic features of the soil. It performs an essential role in the soil specification of soil ecology. Earthworms are known to manipulate the rhizosphere. They are also called allogenic engineers which contribute to the accumulation of organic matter in the soil along with a pathway for securing soil organic carbon through the establishment of nutrient concentration in the soil.

There is an intimate relationship between earthworms and soil offering various advantages as described in Fig. 6. Earthworms are responsible for organic matters decomposition. They are known to accelerate the organic matter breakdown by causing an increase in the surface area of organic matter which occurs because of comminution. By burrowing, feeding and digestion actions, earthworms are known to improve and enhance organic matter assimilation into the soil along with macroaggregate production (44).

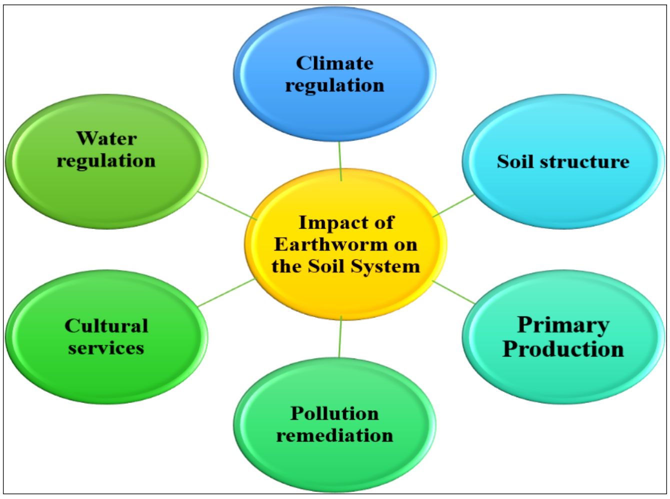

Fig. 6. Impact of earthworm on the soil system affecting other factors.

Earthworm action has the potential to increase Nitrous oxide production by transforming the breakdown of residue in the presence of an aerobic process, leading to higher denitrification along with N_2O production (45).

The major biomass of a terrestrial ecosystem is made up of earthworms, and it is known that plants have evolved along with earthworms by adapting to the various factors occurring in the soil. Earthworms show a positive impact with respect to the development of plants.

Based on the earthworm's diversity and weather conditions, a 7 % loss was seen in the water-storing capacity of the soil and as a result, a rise in the bulk density of endogeic species was seen. It can be dangerous for the development of plants in dry and harsh conditions (46). They have an impact on the microbial communities, cycling of nutrients and growth of plants. Earthworms are known to increase the yield of plants in sandy soil with a slightly acidic pH (47).

Earthworms offer various cultural services. Charles Darwin has written in his book 'Origin of Species that earthworms play a vital role in guarding and preserving everything that falls on the soil surface. They bury it under their castings for longer periods of time. An earthworm can be employed as an efficient tool for waste remediation. It has been recycling organic waste for 300 million years. Some variables are known to influence the earthworm's impact on the texture and nutrient content of the soil (48).

There is a connection between earthworms and soil, as soil provides shelter for earthworms, whereas earthworms are known to improve nutrient levels along with the permeability and structure of the soil (10). A study also reports that some bacterial species belonging to the phylum Firmicutes are present in the earthworm's gut but are absent in the surrounding soil. Further study also shows that bacterial arrangement is the same in the gut as well as in the adjoining grass soil in the case of *L. terrestris* and the contribution of the phylum Actinobacteria in the gut was accounted to be 30 % which is comparatively more as only 8 % is recorded from the soil. Some bacterial species, along with their role in the soil have been mentioned in the following Table 1.

Feeding habits of earthworms

Some soil organisms prevent eating fresh leaf litter as it can be poisonous because they do not have the capability to digest cellulose and are thus dependent upon the microbes for energy in the form of some necessary amino acids (59). Soil invertebrates like earthworms are known to digest large amounts of soil as well as organic matter including a wide diversity of microbes. Bacteria present along with the passage of the intestinal tract in earthworm

Table 1. Bacteria and its role in the soil.

Sl. No.	Name of bacteria	Role in soil	References
1.	*Pseudomonas fluorescence*	Growth and curcumin content effected	(49)
2.	*Pseudomonas putida*	Decrease plant growth	(50)
3.	*Azospirillum brasilense*	Accounts for the shoot and root weight	(51)
4.	*Pseudomonas corrugate*	Grain yield	(52)
5.	*Rhizobium japonicum*	Enhanced root nodule formation	(53)
6.	*Actinomycetes, Streptomyces* species	Bioremediation of contaminated soil	(54)
7.	*Azospirillum brasilense*	Causes increase in the height of plant, Causes increase in the diameter and length of roots	(55)
8.	*Bacillus subtilis*	Phosphate solubilization	(56)
9.	*Azotobacter, Azosprillum*	Nitrogen fixation in soil	(57)
10.	*Rhizobium trifolii*	Promotes the growth of leguminous plants	(58)

species can influence the constitution of the bacterial community of the soil. Composition of bacteria inside some earthworm species is shown in Table 2. Moreover, it is important to take earthworm's gut passage into more consideration while examining the danger of discharging exotic bacterial species (like genetically engineered species) into the soil ecosystem. Earthworms play an essential role in maintaining and shaping the dynamics and structure of the soil. Apart from this, they influence the microbes present in their guts and casts as shown in Fig. 7. Thus, they are also known as autogenic engineers of the soil ecosystem (60).

Table 2. Composition of bacteria inside some earthworm species.

Sl. No.	Earthworm species	Composition of bacterial community inside the intestine of the worm		Reference
1.	*Lumbricus terrestris*	Actinobacteria	3 %	(27)
		Bacteroidetes	6 %	
		Chloroflexi	9 %	
		Firmucutes	30 %	
		Planctomycetes	7 %	
		Proteobacteria	50 %	
		Tenericutes	13 %	
		Verrucomicrobia	3-4 %	
2.	*Eisenia foetida*	Actinobacteria	9 %	(28)
		Bacteroidetes	7 %	
		Chloroflexi	-	
		Proteobacteria	55 %	
		Tenericutes	33 %	
		Verrucomicrbia	-	

Earthworms are among the most significant faunal groups present in the soil, both in terms of population and biomass. They have a crucial impact on the microbial communities of the soil. Earthworms possess a high consumption rate of the soil. Geophagous worms can consume 200 to 6700 mg of soil per day and epigeic earthworms are known to consume about 3 to 50 mg of dung and litter per day (69).

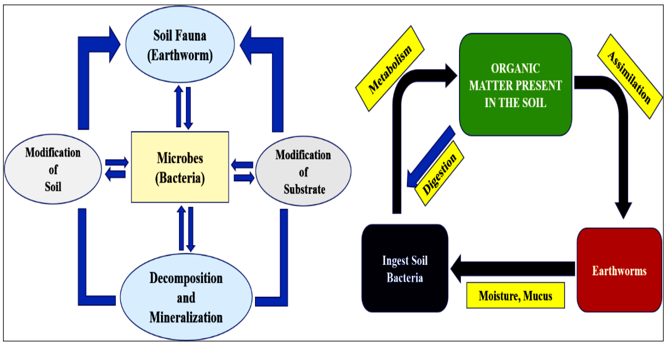

Fig. 7. Interaction of earthworm and bacteria in the soil.

Digestive enzymes like protease, amylase, chitinase, urease, lipase and cellulase are known to be present inside the alimentary canal of the earthworm. These microbes present in the earthworm's gut is mainly for carrying out various biochemical activities. Earthworms are also known for causing an enlargement in the surface area which is useful for microbial deprivation including the most active and efficient phase occurring in the vermicomposting process.

When the organic substrate enters the gut, it gets mixed with microbes along with several digestive enzymes. Then, at last, it comes out of the gut in the form of casts. Later, the microbes cause a disintegration process, which contributes to the maturation phase. Earthworms are known to selectively feed upon substances enriched with organic matter like some polymers and their residues which are produced from plants or microbes after their breakdown process (48). The diet along with phylogeny and anatomy of the host, gives an idea about the arrangement of microbial colonies inside the animal gut as shown in Table 3. Although, information about microbial population as well as its composition about a bridge between gut

Table 3. Food preference of earthworm.

Sl. No.	Type of food ingested by worms	Action occurring inside the gut	Reference
1.	Protozoans	Leads to the maturation of earthworm species	(62)
2.	Fungal hyphae and spores	Their length decreases when they pass from the gut	(48)
3.	Bacteria	Their digestion also occurs inside the gut Some culture-reliant bacteria show the tendency of multiplication	(48),(63)

diversity and ecological groups is absent. The relationship between the feeding habits and the gut microbial population of earthworms is not so clear, but the microbial outline present in the gut can be assumed to be an efficient indicator of the metabolism of earthworms (61).

Worms are known to consume trash as well as soil as a food source. The intricate amount of soil microbial biomass present inside the earthworms' gut microflora can be predicted by the nutrition they take. The gut of an earthworm harbours endemic microbes too, but still, the microbial biome inhabited in its gut appears like the ingested matter (64).

The ingested bacteria that are capable of anaerobic development are generally derived due to the anoxia condition of the earthworm's guts which lacks detectable oxygen levels. The stimulation of ingested microbes is also influenced by some other factors, like increased levels of carbonaceous substrates which are produced by the hydrolysis of carbohydrate-enriched mucus released by the pharyngeal glands of earthworms (48).

The worm's gut also has the capability to catalyse a high fermentation process that occurs during anoxic conditions and this serves as an illustration of the stimulation of anaerobic actions of the gut microflora (10).

The effective handling of earthworm bioresources has the capability of producing major environmental as well as economic advantages. By swallowing, modifying and combining mineral soil with organic substances, earthworms show an influence on the operation and ecosystem structure, which also shows an alteration in the biology and chemistry of the soil (68). The connection between the bacterial populations is closely related to the earthworm's gut and its ecology. By using ARISA and IGS area in between 16S and 23S rRNA genes the bacteria were differentiated.

Earthworms influence Biogeochemical cycles and nutrients modification of soil microbial populations

The most prevalent organisms in terrestrial environments, earthworms are crucial to the soil's biogeochemical and nutritional cycles. These underappreciated heroes of the soil ecosystems modify the soil's texture, control its water content and keep nutrients available to plants. They control a variety of biological processes by blending organic materials and other minerals in their gut (65).

For the modification of bio-geochemical cycles along with the dynamics of soil structure and organic matter, earthworms are known to exhibit an impact on the microbial populations present in their gut and cast (66). While the variations in earthworm digestion and absorption processes suggest that ecological group-specific gut microbial populations may exist (5). Thus, earthworms also affect the stability, microbial diversity and other characteristics of terrestrial ecosystems. Compared to the surrounding environment, the earthworm's gut serves as a bioreactor and provides favorable living conditions for bacteria (67). According to earlier theories, the bacterial variety in the environment influences the earthworms gut microflora. (24) and as a result, the environment plays a big part in determining the gut microbiomes (18).

Earthworms are organic matter eaters and they tend to release nutrients through digestion and excretion processes, which has an impact on plant growth. Information about the microbial populations associated with earthworms are needed to figure out the influence of earthworms in various processes, like the cycling of nutrients and waste remediation processes. The ecological relationships between microbial functions were altered by the presence of earthworms. Certain microbial species are stimulated by earthworms, which elevates the significance of keystone roles. According to a recent study, about 70 microbial functions of the rhizosphere are related to biosynthesis as well as the symbiosis of plants and microbes, which are known to be altered by *P. corethrurus* species of earthworm (70).

Earthworm species can exhibit both positive as well as negative effects on the richness and diversity of microbial populations. Although no negative consequences have been reported about the earthworm and bacterial population present in the adjoining soil (71). It was reported that the bacterial Operational Taxonomical Units and the richness estimation of soil were not affected by the endogeic species like *Aporrectodea* trapezoids. The beneficial results with respect to the diversity of bacterial community were seen (72). Epigeic earthworm species such as *E. fetida* and *Eudrilus* are known to increase the bacterial population during the early stages of the vermicomposting process (73).

It is also known that earthworms, especially endogeic and geophagous earthworms, encourage soil C and N mineralization (73), mostly by altering the decomposition rate of organic matter present in the soil through a priming effect (74). Epigeic species like *E. fetida* as well as *P. excavates*, are known to enhance the decomposition rates of the organic matter present in the soil. The nutrient recycling of nitrogen and phosphorus present in organic matter is encouraged by the priming effect. An increase of 2 to 3-fold has been seen in the mineralized carbon with regard to the casts produced by *A. caliginosa* and its adjoining soil. This occurs as a result of the priming effect of the ingestion and digestion processes occurring in the case of earthworms (75).

Earthworms are known to enhance the physiochemical properties of the soil, resulting in its fertility. An easy-to-understand conceptual model of how earthworms affect soil characteristics to improve soil nitrogen cycling Earthworms have an impact on plant development by increasing the plants' access to nutrients. Organic substances act as the primary source of food for earthworms in the soil. Consequently, they are crucial for the cycling of biological materials. The organic material inside the earthworms' gut is mixed with the soil during digestion and the resulting mixture is added to the soil profile. In the earthworm-incorporated system, nitrogen and nitrate losses will be reduced by 6-8 times (76).

The enzymes associated with the earthworm gut are amylase, cellulase, xylanase, cellobiase, endoglucanase, acid phosphatase, alkaline phosphatase and nitrite reductase. These enzymes are secreted when the microbial population increases inside the earthworm gut (77). It has been shown in Fig. 8. The presence of these enzymes makes earthworms versatile bioreactors, beneficial for sustainable agriculture and waste management. Nitrate, nitrite as well as denitrifying bacteria present in the earthworm gut result in the release of N_2O (78).

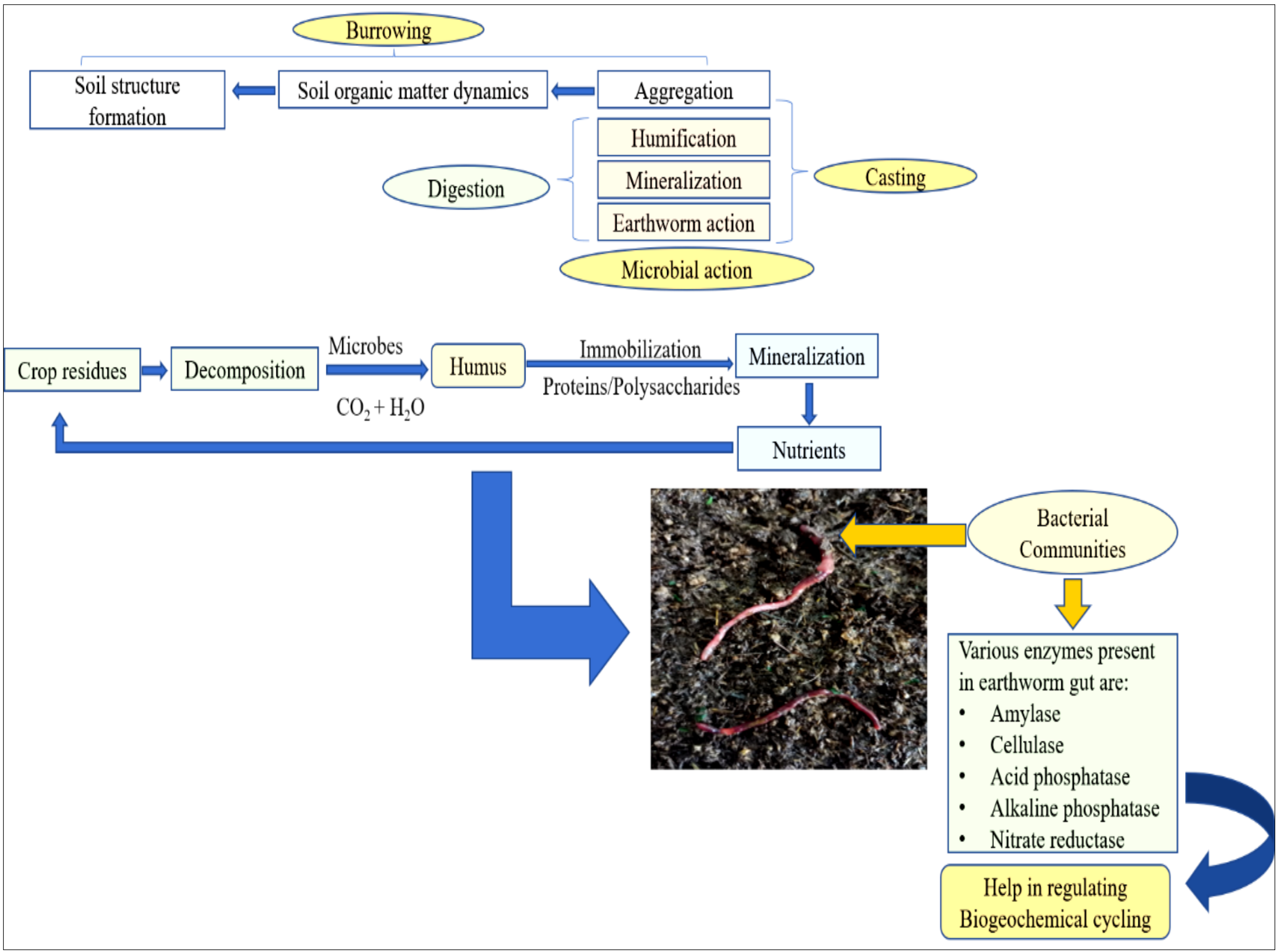

Fig. 8. Earthworm gut inhabit various enzymes that contribute into biogeochemical cycling.

Earthworms have the capability to alter the physical (79), chemical (80) and biological (81) characteristics of the soil. The presence of earthworms causes an increase in micronutrient uptake, ultimately contributing to the functioning of biogeochemical cycles. Moreover, the introduction of exotic earthworm species into the soil can increase the natural biogeochemical cycling process by accelerating the nutrient availability of the soil promoting plant growth.

Proteobacteria present inside the earthworm gut are essential for biogeochemical cycles existing on the earth (82). Proteobacteria are associated with host energy metabolism, as the gut helps in fermenting food, which results in the digestion as well as absorption of nutrients. In addition to Proteobacteria, other bacteria phylum such as Actinobacteria, Bacteroidetes and Firmicutes are present in abundance in the gut of earthworm also play role in the biogeochemical cycling (8, 39).

Nitrogen producing bacterial species found in the earthworm gut result in the formation of Nitrite (NO_2^-) and Nitrogen dioxide (N_2O) which is essential for the regulation of nitrogen cycle whereas the phosphate solubilizing bacteria like *Pseudomonas* and *Bacillus* present inside the earthworm gut are essential to carry out the cycling of phosphorus in nature as they contributes to the immobilization as well as mineralization of the organic phosphorus. Likewise, other biogeochemical cycles like carbon cycle and oxygen cycle, all are directly or indirectly being influenced by the earthworm species whose gut constitute of various useful bacterial communities. These microbial communities may seem too smaller in size but their influence plays an essential role in the vital processes such as biogeochemical cycling occurring on the earth's surface.

During complete denitrification, nitrate is converted to N_2 and aerobes that can accelerate the denitrification process work best in anoxic surroundings. Soil homogenates also have the ability to denitrify but homogenates of earthworms' gut show more potential for denitrification by employing accelerated rates. Intermediates like nitrite and N_2O are present as transient products in the worm's gut microflora but cannot be detected in the soil biome (83).

The potential of the denitrification process is quite different in the case of the worm's gut and soil; however, it is a fact that worms could ingest denitrifying bacteria when they consume soil. This may be because the worm's gut contains a huge number of carbonaceous substrates that are present in the form of sugars or fatty acids. They are essential for carrying out the process of denitrification, as the presence of denitrifying microbes in the gut is not limited (48).

In laboratory conditions, intermediates like nitrate and nitrate, along with an anoxic atmosphere cause the induction of N_2O production by the gastrointestinal constituents of earthworms. Earthworms also result in *in-vivo* emission of nitrogen oxide, which occurs due to the denitrification process, where the presence of cultured denitrifiers can be seen abundantly in the stomach of earthworms. By ingesting a significant volume of soil, earthworms can directly control the microbial population. As a result, some bacteria are driven out of the earthworm's digestive tract while others grow (84).

Microbes are an essential food source for soil invertebrates like earthworms. Numerous events point towards the significance of fungi and bacteria as key food suppliers, as they lack the innate ability to digest cellulose. Thus, they depend on the microbes for the intake of critical amino acids. Earthworms avoid fresh leaf litter because it can be hazardous for them. It was postulated that gut-specific microbiota in earthworms provide them with more energy and nutrients than bacteria found in the adjoining soil they absorb (85).

It is anticipated that the priming effect increases nutrient recycling, mainly organic N and P. In some studies, it has been seen for *P. corethrurus* species (86). Earthworms are known to enhance the nutrient mineralization process.

Earthworm and soil interaction promote plant growth

The portion of the soil known as the drilosphere is affected by earthworm activities. It is a portion of soil that is rich in microbial load and contains earthworm burrows and castings. Major roles were performed by ecosystem engineers, such as earthworms working with microflora, in the creation of the biogenic structures. Earthworms make up most of the organisms involved in the digestion of complex organic compounds. It suggests that earthworm activity in the drilosphere improves the microflora and other biological elements' microbial activity. They act as dominant living things in the drilosphere and are responsible for converting complicated organic molecules into a variety of biogenic forms.

Anecic species create permanent burrow systems in soil that are beneficial as a pathway for root growth and microbial dissemination activity, whereas endogeic species show more impact on microbial populations.

Earthworms make it easier for roots to penetrate the soil, absorb nutrients and exchange gases. It is recognized that the beneficial impacts of earthworms upon the growth of plants and yield are somewhat related to enhancing the physio-chemical variables of the soil. Recently, the soil microbiota's beneficial effects have also been linked to the activation of bacteria that produce signal molecules. Despite all the evidence linking earthworms to beneficial impacts on plant development and modifications to the N cycle.

The rhizospheric region in plant roots is composed of a thin layer of soil that is enriched with microbes and tends to adhere to the surface of the root, showing enhanced action of the earthworms. They have an essential role in soil structure creation. Earthworms also aid in the improvement of soil penetration and reduction in soil compaction. They are also known as natural tillers because they promote aeration, porosity, hydraulic conductivity and infiltration capacity of the soil, especially in the case of soils that are water resistant with lower bulk density (87). The impact of worms on the growth of plants cannot be completely explained by an increase in the mineralization of nutrients (47). The concurrent ways like signal molecule emission in the earthworm presence show an impact on the growth of the plants. The variety of bacterial communities present in different earthworm species varies along with their role in the soil as shown in Table 4.

Table 4. Bacterial communities present inside different earthworm species.

Sl. No.	Name of bacteria	Species	Role in soil	References
1.	*Nitrobacter* and *Nitrosomonas* (Free-living nitrogen fixers) Solubilizer of phosphate ammonification bacteria	*Eudrilus* sp.	Promotion of plant growth by nitrification, phosphate solubilization and suppression of plant disease	(73)
2.	*Actinobacteria* *Bacteroidetes* *Firmicutes* *Proteobacteria* *Verrucomicrobia*	*Eisenia fetida*	Shows antifungal activity against, *Colletotrichum coccodes, Fusarium moniliforme, P. capsica, P. ultimum* and *R. solani*	(88)
3.	*Brodyrhizobium japonicum*	*L. terrestris*	Shows improvement in the nodule distribution over the soyabean roots	(89)
4.	*Rhizobium japonicum*	*Lumbricus rubellus*	Promotion of plant growth	(90)
5.	*Brodyrhizobium japonicum*	*L. terrestris*	Shows improvement in the nodule distribution over the soyabean roots	(89)
6.	*Pseudomonas oxalaticus*	*Pheretima* sp.	Degradation of oxalate	(91)
7.	Filamentous actinomycetes	*Lumbricus terrestris*	Suppress *Fusarium oxysporum* and *Fusarium proliferatum* in *Asparagus*	(92)

Various methods to examine bacterial variety present in earthworms gut

The majority of microbes cannot be cultivated in conventional media as functional gene key analysis plays a magnificent role in the soil's vital processes such as nitrification, denitrification, oxidation of methane as well as in nitrogen fixation. The detection of changes occurring in the gut bacterial community of earthworm acts as an indicator of environmental contamination. Such changes occurring in the structural as well as functional parameters, can be used for toxicity evaluation (93). In Oligochaetes, the fluctuations occurring in the bacterial populations can be examined by using fluorescent microscopy techniques. It has also shown the ignorance of taxonomy, classification and diversity through the involvement of molecular technology. Culture methods are used to study the bacterial communities present inside the earthworms' gut (94). However, independent culture methods like sequencing of 16S ribosomal RNA gene are also used nowadays (95). These microbial studies especially focus on the bacteria, ignoring other microbes present in earthworm diets. The fundamental microbiology-associated cultural techniques that are essential for the identification of the bacterial presence inside the earthworms gut is shown in Table 5.

Table 5. Molecular techniques used in the identification of bacterial diversity within the digestive tract of earthworm.

Earthworm	Technique	Bacteria identified	References
Onychochaeta borincana	Electron microscopy and PCR	Identified the Genus *Bacillus* with 7 different species and b-bacteria hemolitica	(96)
Eisenia feotida	PCR-16s DNA	Identified 22 bacteria	(97)
Onychochaeta borincana	Electron microscopy	Identified 7 bacteria of the Genus *Bacillus* sp.	(96)
Lumbricus rubellus	Fluorescent *in situ* hybridisation techniques and 16s RNA	Identified *Acidobacteria,* *Paenibacillus, Pseudomonas* sp., *Actinobacteria*	(98)
Lumbricus rubellus	*In situ* hybridization technique	*Bacillus megaterium* within the digestive tract	(66)

Further Perspectives

Earthworm's gut cum soil bacteria can come out as a potential tool for the removal of heavy metals and their microbial as well as metabolic actions such as biotransformation, biocatalysis and biodegradation, which can be more explored for framing up the remediation strategies. Some bacterial strains can also be utilized for promoting plant growth. Exploration and understanding of the earthworm as well as the soil bacterial community, will help in widening the knowledge. Ultimately, it will open new doors for unexplored research and discoveries with regard to ecosystem functioning. The current review also supports reliable information that can be utilized for dealing with new challenges like waste management and pollutant degradation.

Additionally, recent advancements in metagenomics and biotechnology have significantly enhanced our understanding of the earthworm gut microbiota. Utilizing high-throughput sequencing technologies, researchers can now conduct comprehensive profiling of these microbial communities, uncovering complex bacterial interactions and functions. Metagenomic studies have shed light on the genetic potential of these microbes, revealing novel genes critical for nutrient cycling and soil health. Moreover, biotechnological innovations, including gene editing and synthetic biology, provide new methods to exploit these bacteria for boosting soil fertility and promoting sustainable agriculture. These breakthroughs pave the way for more targeted and efficient use of earthworm-associated bacteria in environmental management.

Conclusion

Earthworms are known to be the 'keystone species of terrestrial food webs. They act as degraders and stimulators of the soil vicinity. Soil provides a habitat for earthworms, whereas earthworms help in the improvement of soil structure. Earthworm and soil interactions promote the flow of energy and the cycling of nutrients. Earthworms and soil are home to several bacterial species that are beneficial for carrying out various physio-chemical processes in the terrestrial ecosystem. Earthworms guts have anaerobic conditions due to fewer oxygen levels, thus marking the presence of bacterial species like Actinobacteria, Proteobacteria, Firmicutes and Bacteroidetes. Although bacterial arrangements vary depending upon the type of species. The bacterial colonies present inside the earthworm's gut make it capable of carrying out a degradation process, which is helpful for waste remediation, and more research about this aspect can open doors for waste management strategies in the future in need of an hour. Earthworms and soil show a mutual relationship with one another, and the bacterial populations residing in them also contribute to the fertilizer industry and other biological processes like nutrient cycling as well as plant growth promotion.

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Authors' contributions

All the authors have played an important role in publishing this manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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None

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