Thakur et al. | J Pure Appl Microbiol | 15(3):1156-1172 | September 2021
Article 6824 | https://doi.org/10.22207/JPAM.15.3.05

Print ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Metagenomic Exploration of Bacterial Community Structure of Earthworms' Gut

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Abstract

Living organisms are naturally bestowed with unique and imitable qualities for maintaining ecological balance and earthworms are no exceptions. These so-called keystone species of terrestrial ecosystems are equipped with wonderful machinery, allowing them to nurture soil beautifully. Earthworm gut represents a potential microbial reservoir, having a complex interdependence with the host. The study aimed to profile bacterial community structure of three earthworm species belonging to two different life forms; *Perionyx excavatus* and *Eudrilus eugeniae* (epigeic), *Polypheretima elongata* (endogeic) respectively. Diversity analysis using 16S amplicon sequencing revealed that the dominant phyla were Proteobacteria (34.17-77.88) followed by Actinobacteria (13.43-35.54%), Firmicutes (1.69-15.45%) and Bacteroidetes (0.51-8.12%). The alpha diversity indices explicit similar gut microbiota of *Perionyx excavatus* and *Eudrilus eugeniae* and while higher alpha diversity was recorded in comparison to *Polypheretima elongata* gut. The taxonomic to the phenotypic annotation of 16S rRNA metagenomes revealed that dominance of Gram-negative bacterial community in all earthworm species while, *Polypheretima elongata* comprises higher percentage (78%) of Gram-negative bacterial community to *Perionyx excavatus* (32.3%) and *Eudrilus eugeniae* (38.3%). The oxygen requirement phenotypic analysis showed that all earthworm species were abundant with aerobic followed by anaerobic bacterial groups. Furthermore, functional metabolism phenotypic analysis revealed that a high abundance of ammonia oxidizers (29.3-80.2%), the gut microbiomes showed the relative abundance of sulphate reducer (22.6-78.7%), nitrite reducer (19.8-73.2%), dehalogenators (12.6-25.1%), illustrating in the role of these microbial communities in various degradation and bioremediation processes. The present study signifies the intrinsic gut microbiota of earthworm species for intensified biodegradation.

Keywords: Metagenomic study, earthworm gut microbiota, ammonia oxidiser, bioremediation, biodegradation

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(Received: December 18, 2020; accepted: June 08, 2021)

Citation: Thakur SS, Lone AR, Tiwari N, Jain SK, Yadav S. Metagenomic Exploration of Bacterial Community Structure of Earthworms' Gut. *J Pure Appl Microbiol.* 2021;15(3):1156-1172. doi: 10.22207/JPAM.15.3.05

INTRODUCTION

Earthworms are the most dominant members of terrestrial ecosystems, play vital role in biogeochemical and nutritional rhythm of soil[1,2]. These unsung hero of the soil ecosystems alter the soil texture, regulate water content, maintain the availability of nutrients for the plants[3] and regulate diverse biological functions by mixing the organic matter and other minerals within their gut[4,5]. In addition to shaping the soil structure, biogeochemical cycling and soil organic matter dynamics, earthworms have impacts on microbial communities in their gut, casts and drilosphere[6]. While the differences in the assimilation and digestion processes of earthworm indicate the possible occurrence of ecological group-specific gut microbial communities[7]. Thus earthworms also have an impact on the stability and the microbial diversity and properties of soil ecosystems[8]. Earthworm gut acts as a bioreactor and furnish favourable abode to microbes in comparison to the adjoining environment[9]. Earlier assumptions suggest that the earthworm gut microbiome depends on the bacterial diversity present in the surrounding environment[1,10] and therefore the environment have a significant role in shaping the gut microbiomes[1,11,12]. In addition earthworms have been identified as epigeic, anecic and endogeic forms, depending upon their burrowing and food habits within various soil horizons[13] which consequently regulating the core as well other bacterial communities within these three forms of earthworms. Moreover, the earthworm gut has the tendency to differentiate between the harmful and beneficial microbiota[10,14]. The earthworm harbours diverse microbial communities involved in metabolism, thereby maintaining the availability of essential nutrients and providing protection against pathogens[1,10,14]. The study evaluated taxonomic and functional profiling of gut microbiome of earthworms of diverse life forms and habitat.

MATERIALS AND METHODS

Collection of samples

The earthworms used in the present study were collected from Nauradehi wildlife sanctuary (23°32'55.3"N 79°12'03.5"E; 600 m sea level) Sagar, Madhya Pradesh, India. Fifteen adult earthworms' samples (five replicates for each experimental species) were collected following protocol described by Julka[15]. After considering morpho-anatomical parameters, worms were transferred into sterile plastic bags and delivered to Earthworm Biology Lab (23°50'03.7"N 78°47'01.2"E), Dr. Harisingh Gour Vishwavidyalaya (23°50'03.7"N 78°47'01.2"E), Sagar, Madhya Pradesh, India for further analysis.

Molecular identification of earthworms' species

One adult earthworm was randomly chosen from each sterile polythene bag and characterized using mitochondrial molecular marker *COI* following standard protocol[16]. Table 1 depicts the accession number of studied samples. All the *COI* sequences of the present study are accessible on BOLD web portal under the research project 'Diversity studies in earthworms of India' (IEW). In addition, 22 *COI* sequences including outgroup were retrieved from NCBI and BOLD database for the molecular analysis.

Sequence alignment and data analysis for earthworm species identification

25 *COI* sequences were analysed on MEGA software using the Kimura two-parameter[17]. *COI* dataset was searched on NCBI, the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov) to blast three query *COI* sequences and were aligned using Multiple sequence alignment program MUSCLE v3.8.31 (Multiple Sequence Comparison by Log-Expectation)[18]. Their phylogenetic estimation were inferred using neighbour-joining tree method following 1000 bootstraps using Molecular Evolutionary Genetics Analysis (MEGA X)[19].

DNA extraction of gut microbiome

The same earthworm from each group was selected for gut metagenomic study, that tissue used for molecular identification of earthworms species. The worms were washed three time with distilled water and placed on separate sterile petri dishes (one per dish), after the couple of minutes worms were dissected to take out their gut. The metagenomic DNA was extracted using Qiagen Blood and Tissue Kit (Qiagen, USA) following provided protocol. Their quality was assessed on the 0.85% agarose gel and quantified on NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA).

16S rRNA gene amplification

The V3-V4 (469 bp) hypervariable region

of 16S rRNA gene was targeted for amplification using primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3')[20]. In order to differentiate all samples, the reverse primer was labelled with a specific barcode for each sample. For PCR reaction, total of 20 µl reaction mixture was prepared comprising 17 µl reaction buffer, AccuPrime Supermix, 1 µl of DNA template and 1 µl of each forward and reverse primer. The PCR Thermocycler was set at 95 °C for 5 min as initial denaturation step, followed by 35 cycles of 94 °C for 30s, 52 °C for 40s and 72 °C for 60 s, with a final extension step at 72 °C for 5 min. The same parameters were used for negative control of PCR product. Their integrity were analysed after mixing equal volume of 1X loading buffer ran on agarose gel electrophoresis. Subsequently, the intact and sharp bands between 400–500 bp were used for construction of library.

Construction of library and sequencing

The Qiagen Gel Extraction Kit was used to purify the PCR products and the library prepared using TruSeq DNA PCR-Free Sample Preparation Kit following protocol provided by manufacturer. The quality of library products were evaluated on Qubit 2.0 Fluorometer and Agilent Bioanalyzer 2100 system. The sequencing of constructed library was performed on an Illumina HiSeq 2500 platform using standard protocol[21] at Nucleome Informatics Pvt. Ltd.

Data processing and *In silico* analysis

Paired-end raw sequences were filtered using FastQC[22] on CosmosID's bioinformatics pipeline (https://app.cosmosid.com; Rockville,

Table 1. Lists of samples, earthworms and outgroup taxa with accession numbers of *COI* gene sequence

Sl.No.	Species	Gene	Accession number (NCBI/BOLD)
1	*Drawida remiensis*	*COI*	BOLD:ADH0513
2	*Drawida remiensis*	*COI*	BOLD:ADH0513
3	*Drawida remiensis*	*COI*	BOLD:ADH0513
4	*Drawida remiensis*	*COI*	BOLD:ADH0513
5	*Eudrilus eugeniae*	*COI*	MN125034.1
6	*Eudrilus eugeniae*	*COI*	MT410736.1
7	*Eudrilus eugeniae*	*COI*	KC122194.1
8	*Eudrilus eugeniae*	*COI*	KX832072.1
9	S2	*COI*	BOLD:ACQ6907
10	*Eutyphoeus Kempi*	*COI*	BOLD:ADH6760
11	*Eutyphoeus Kempi*	*COI*	BOLD:ADH6760
12	*Eutyphoeus Kempi*	*COI*	BOLD:AAF0619
13	*Eutyphoeus Kempi*	*COI*	BOLD:AAF0619
14	*Moniligaster aiyeri*	*COI*	BOLD:ADH1655
15	*Moniligaster aiyeri*	*COI*	BOLD:ADH1655
16	*Perionyx excavatus*	*COI*	BOLD:ADC0803
17	*Perionyx excavatus*	*COI*	BOLD:ADC0803
18	*Perionyx excavatus*	*COI*	BOLD:ADC0803
19	S1	*COI*	BOLD:ADC0803
20	*Polypheretima elongata*	*COI*	BOLD:AAF0305
21	*Polypheretima elongata*	*COI*	BOLD:AAF0305
22	*Polypheretima elongata*	*COI*	BOLD:AAF0305
23	*Polypheretima elongata*	*COI*	BOLD:AAF0305
24	S3	*COI*	BOLD:AAF0305
25	*Calomera littoralis* (Outgroup)	*COI*	KX832072.1

MD, USA). Where, raw sequence files were uploaded to the CosmosID cloud application without set parameters or modified parameters. As reported earlier the application uses high-performance k-mer based algorithms and curated taxonomy databases (GenBook®) enable via the cloud interface[23-28]. Using CosmosID bioinformatics pipeline software the taxonomic community profiling, alpha diversity analysis (Chao1, Simpson and Shannon), Hierarchical clustering heatmap analysis at phylum and genus level was evaluated and plotted, to reveal microbial community composition in earthworms' gut.

Taxonomic to phenotypic analysis

The taxonomic abundance table generated by CosmosID (https://app.cosmosid.com) was uploaded on METAGENassist (http://www.metagenassist.ca/)[29] for taxonomic to phenotypic profiling. The generated data were normalised following Paul et al[30]. Further analysis of phenotypic subsets, Gram stating oxygen requirement and metabolism having various phenotypic characteristics were corelated with given taxa, pie charts and bar graphs were plotted to depict the fraction of percent of taxa characteristic. The supervised pie chart and bar graph were employed for each metabolic phenotype analysis[29].

RESULTS

Molecular identification of the species using the *COI* gene

Based on the molecular identification methods, these specimens were identified with the help of cytochrome oxidase subunit 1 (*COI*) gene partial sequence. The phylogenetic position of three query *COI* sequences was based on the BLASTN homology against the nucleotide sequence collection of the NCBI GenBank and BOLD sequence database and were identified as *Perionyx excavatus* (S1), *Eudrilus eugeniae* (S2), and *Polypheretima elongata* (S3). Obtained sequences showed 99% similarity with the available sequence in NCBI GenBank and BOLD databases were distantly related to the outgroup *Calomera littoralis* (Fig. 1).

Illumina Hiseq amplicon sequencing

Sequencing of the V3-V4 hypervariable region of 16S rRNA gene produced 2,54,807 high-quality reads of samples. The obtained reads were clustered into 1298 Operational Taxonomic Units (OTUs). The maximum, minimum and average

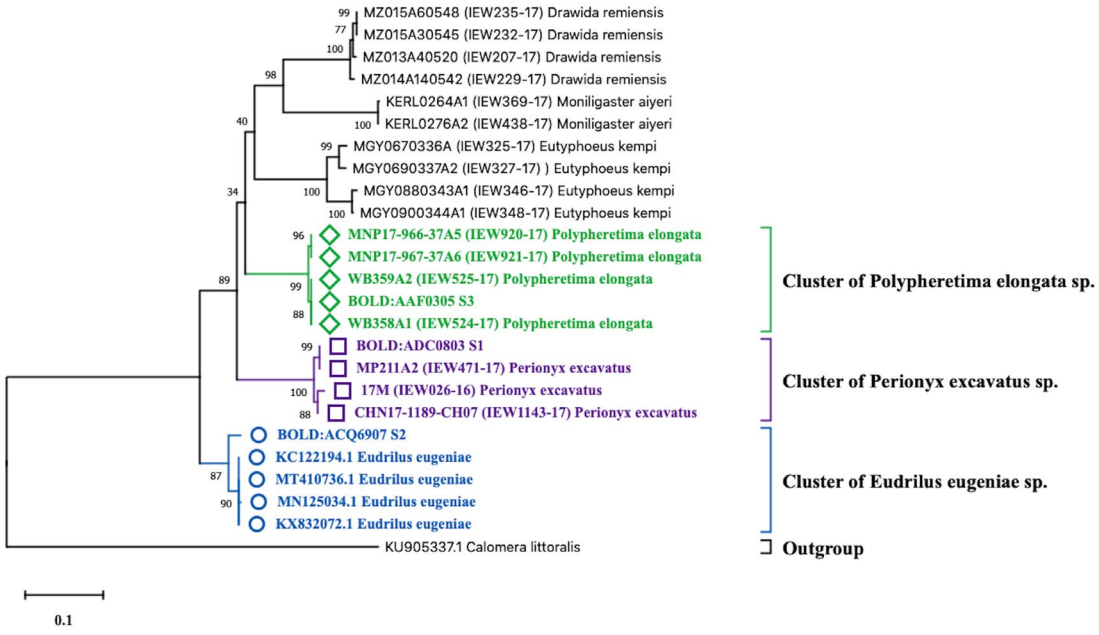


Fig. 1. Neighbour-joining tree with 1000 replication for selected strain of earthworms showing the relationship of S1, S2, and S3 with twenty four earthworm sequences and one outgroups (*Calomera littoralis*) using partial nucleotide sequence of *COI* gene.

number of reads per sample were 91938, 77455 and 84935 respectively.

Bacterial composition in earthworms' gut

99.98% reads hit with k-mer markers were accounted for bacterial sequences. The relative abundances of microbial communities across the earthworms species gut were analysed at the phylum and genus levels. Ten bacterial phyla were detected in all samples. As shown in Figure 2 the majority of reads belonged to Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes and Chloroflexi, which accounted for 90-95% of total classified sequences (Fig. 2) which represents "core microbiota" of earthworms gut. While, multiple specific fluctuations were recorded in the proportions to relative abundance of various taxa from phylum level to genus level. Bacterial phyla Actinobacteria (35.54%), Proteobacteria (34.17%), Firmicutes (8.98%), Bacteroidetes (8.12%) and Chloroflexi (3.05%) were the most abundant in *Eudrilus eugeniae*, comprising 90% of the total microbiota. Corresponding the above, Proteobacteria (36.10%), Actinobacteria (31.64%), Firmicutes (15.45%), Bacteroidetes (4.01%) and Chloroflexi (4.86%) were abundant in *Perionyx excavatus* sp. gut occupied 93% of the total microbiome. While, *Polypheretima elongata* sp. gut inhabited Proteobacteria (77.88%), Actinobacteria (13.43%), Firmicutes (1.69%), Chloroflexi (0.65%) and Bacteroidetes (0.51%) carrying 94% of total microbiota (Fig. 2). The sunburst chart depicted the relative abundance of dominant genus in *Polypheretima elongata* gut, were *Aeromonas* (47.72%) followed by *Enterobacter* (7.44%), and *Citrobacter* (2.43%) (Fig 3A). While, *Demequina* (5.37%) followed by *Mesorhizobium* (4.27%), *Cellulomonas* (2.62%) and *Rhodoplanes* (2.47%) were dominant in *Perionyx excavatus* gut (Fig 3B). And, *Demequina* (7.86%) followed by *Flavobacterium* (2.81%), *Salinibacterium* (2.20%), *Mizorhizobium* (1.74%) and *Cellulomonas* (1.50%) were more abundant in *Eudrilus eugeniae* gut (Fig. 3C).

Alpha diversity analysis of earthworms' gut microbiota

A total 1298 OTUs were obtained during the evaluation of bacterial diversity on CosmosID bioinformatics pipeline (https://app.cosmosid.com). A good coverage sequencing depth (99.8%) was found which represents capturing of majority of the bacterial diversity in all samples. The species richness (Chao1) was highest in *Perionyx excavatus* (947) followed by *Eudrilus eugeniae* (751) and lowest in *Polypheretima elongata* (678). Similar trends were found in Shannon index and Simpson index, the Shannon index for *Perionyx excavatus* (7.6139) was highest followed by *Eudrilus eugeniae* (7.2667) and lowest in *Polypheretima elongata* (5.1776). The Simpson index value for *Perionyx excavatus* (0.98757) was highest followed by *Eudrilus eugeniae* (0.98394) and lowest in

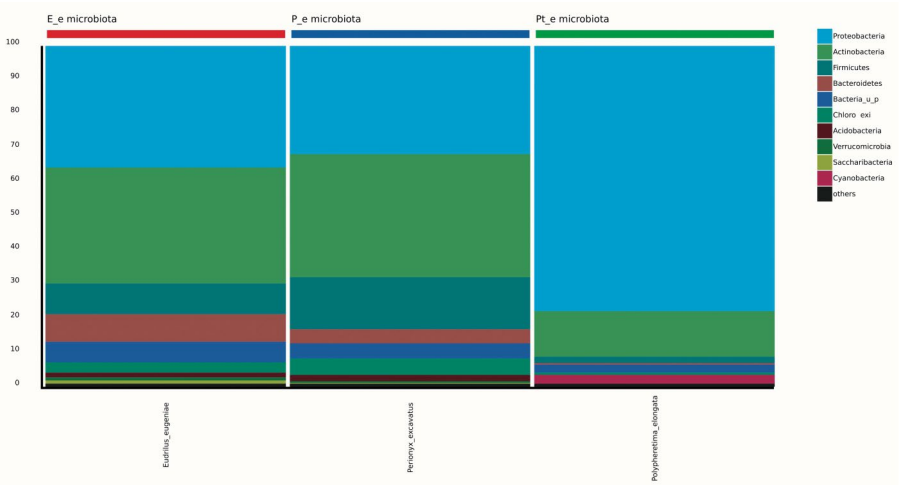
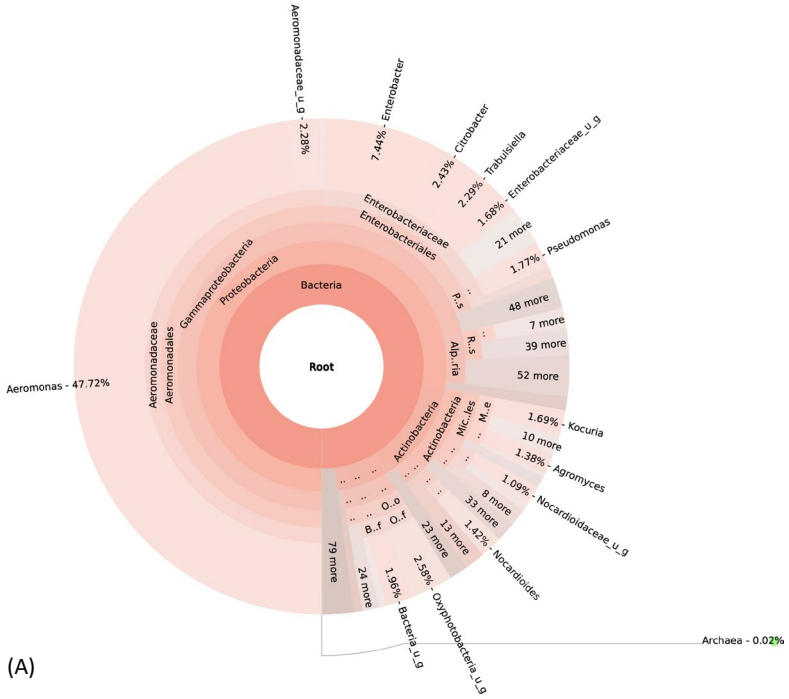


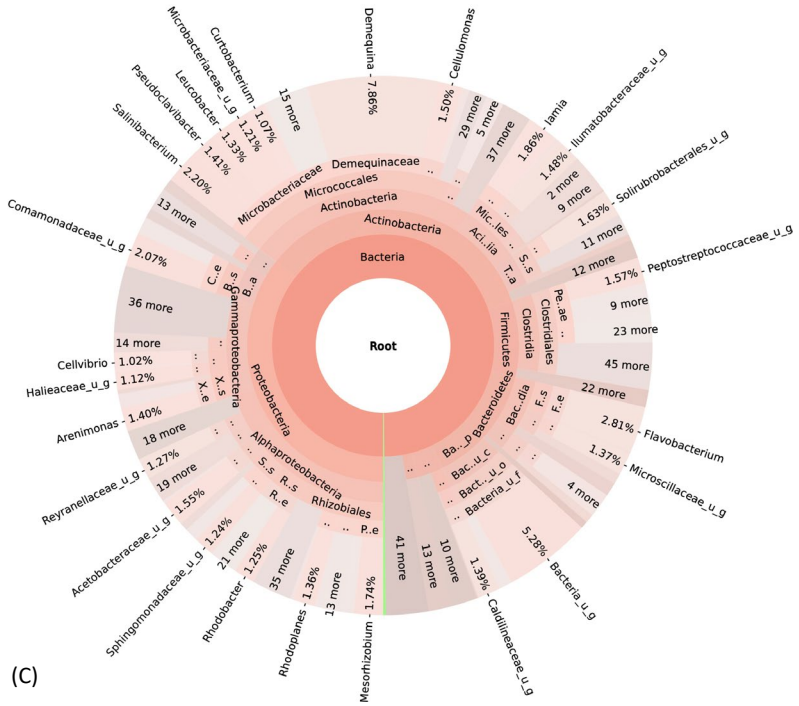
Fig. 2. Phylum level relative abundance of bacterial communities in different species of earthworms viz *Polypheretima elongata*; *Perionyx excavatus*; and *Eudrilus eugeniae*.

Polypheretima elongata (0.89856) (Table 2 & Fig. 4A-C). The alpha diversity indices analysis reflected high diversity in *Perionyx excavatus* followed by *Eudrilus eugeniae* and lowest in *Polypheretima elongata* (Fig. 4A-C).

Heatmap clustering analysis

The abundance of clusters and similarities were observed by plotting the heatmap, a graphical display of values in colour gradients of data matrix. Where, vertical clustering represents similarity in





(C)

Fig. 3. Sunburst chart analysis depicted the genus level relative abundance across the earthworms' species: (A) *Polypheretima elongata*; (B) *Perionyx excavatus*; (C) *Eudrilus eugeniae*.

abundance of different species in the samples. The lesser distances between two samples represents shorter branch lengths that expressed relatively similar abundance between samples. The colour gradient from blue to red symbolizes low to high relative abundance[31].

At phylum level

The relative abundance of microbiota in *Perionyx excavatus* was recorded the highest, followed by *Eudrilus eugeniae* and the lowest in *Polypheretima elongate* (Fig. 5A). The upper clustering tree indicates that the bacterial community in *Perionyx excavatus* and *Eudrilus eugeniae*, were more similar compare to the *Polypheretima elongata*.

At genus level

The heatmap analysis of top fifty genera is shown in Fig. 5B. For the top 50 genera, the abundant bacteria in *Polypheretima elongata, Perionyx excavatus* and *Eudrilus eugeniae* were barely overlapped (Fig. 5B). Those abundant bacteria in the *Polypheretima elongata* sample, such as *Aeromonas, Enterobacter, Citrobacter, Pseudomonas, Agromyces* and *Kacuria* were relatively low in *Perionyx excavatus* and *Eudrilus eugeniae* gut. Some genera including,

Table 2. Summary of the bacterial richness and diversity in different earthworm species

Parameters	*P. excavatus*	*E. eugeniae*	*P. elongata*
Total reads	91938	85414	77455
Good's coverage %	99.8	99.8	99.8
Chao1	947	751	678
Simpson	0.98757	0.98394	0.89856
Shannon	7.6139	7.2667	5.1776

Mezorhizobium, Rhodoplanes, cellulomonas, Salinibacterium, Pseudoclavibacter, lamia and *leucobacter* were abundant in *Perionyx excavatus* and *Eudrilus eugeniae* gut. Moreover, *Flavobacterium, Arenimonas* and *Rhodobacter* were abundant only in the *Eudrilus eugeniae* gut. *Bradyrhizobium, Hypomicrobium* and *Rummeliibacillus* were abundant only in *Perionyx excavatus*.

Taxonomic to phenotypic profiling

A web-based server METAGENassist was used to map taxonomic to the phenotypic profiling[29]. Differences were observed in the phenotype classes viz., Gram staining, metabolism

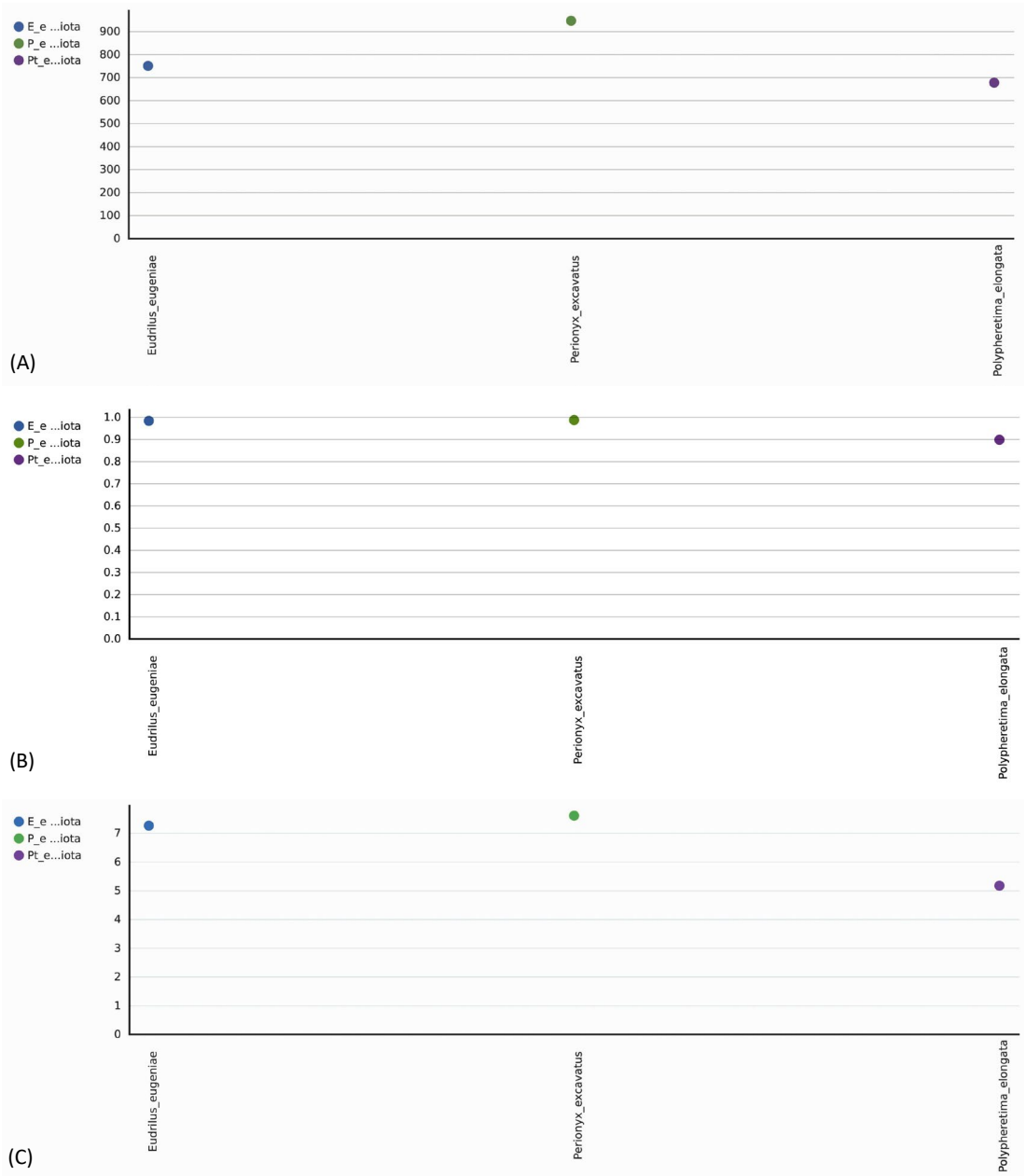


Fig. 4. Alpha diversity analysis of three different earthworms species gut microbiota. (A) Chao1; (B) Simpson index; (C) Shannon index.

and oxygen requirement. On considering Gram staining phenotypic analysis a significant increase in Gram-negative bacteria in *Polypheretima elongata* gut, was found as compare to *Perionyx excavatus* and *Eudrilus eugeniae* gut (Fig. 6A-C). While in case of another phenotypic category oxygen requirement, aerobic followed by anaerobic group were most dominant in all earthworms gut microbiota. It was worthy to note a very high range of unknown sequence reads were found 77.3%, 56.2%, 59.4.4% in *Polypheretima elongata, Perionyx excavatus* and *Eudrilus eugeniae* gut microbiota respectively (Fig. 7A-C). The metabolism phenotypic *In Silico* analysis demonstrated that the gut of earthworm can be looked at a microecological niche, where a number of different biogeochemical cycles, xenobiotic degradation, and lignocellulosic deconstructions are being performed. The taxonomic to the phenotypic characterization of the earthworm gut microbiome from three species showed that bulk of the bacterial communities were ammonia oxidizers in *Polypheretima elongata* (80.2%), whereas in *Perionyx excavatus* the relative

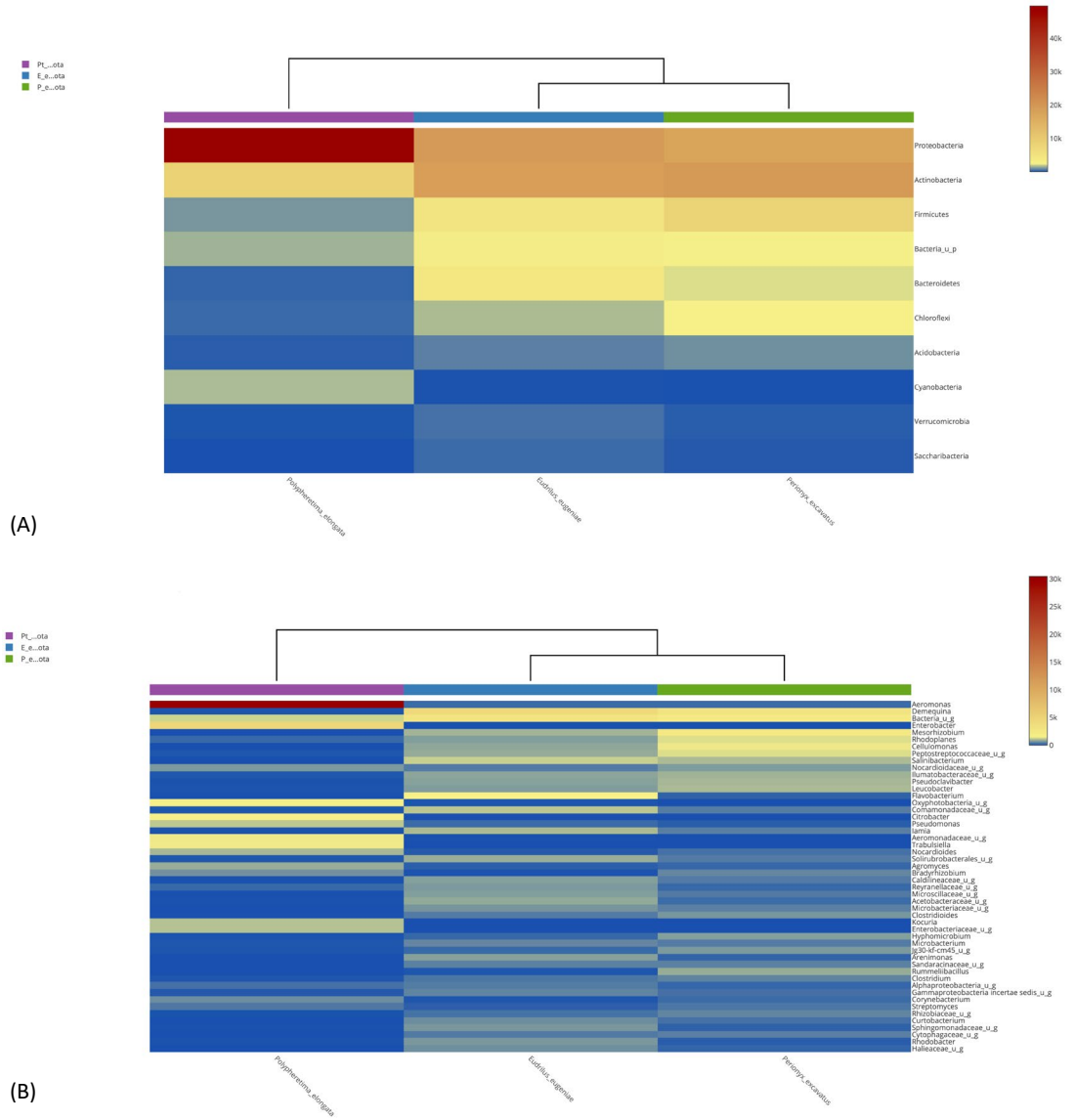
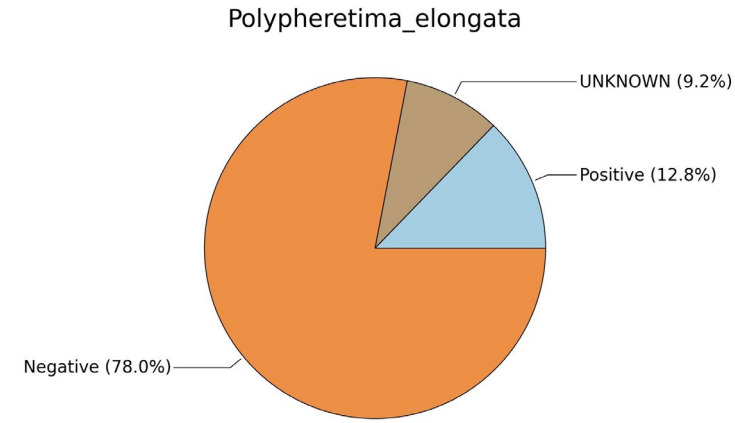
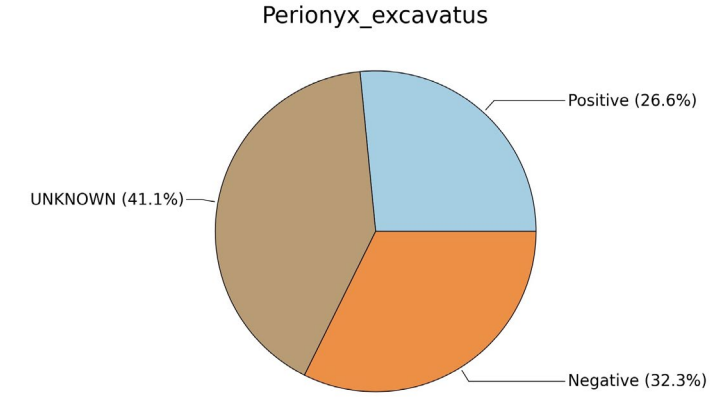


Fig. 5. Heat map of three different earthworms species gut microbiota. (A) at phylum level; (B) at genus level.



(A)



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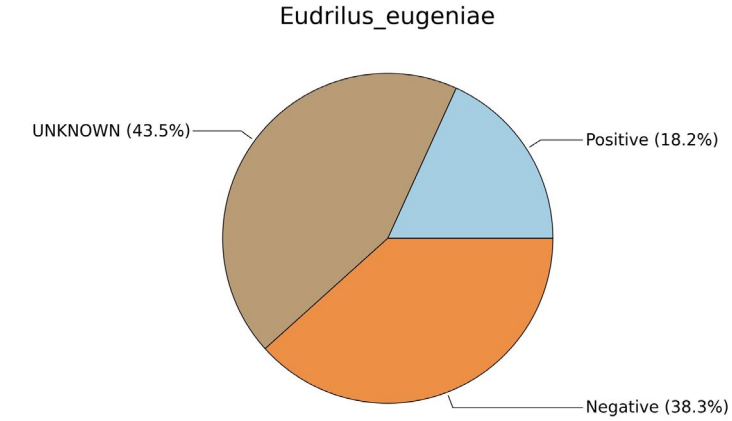
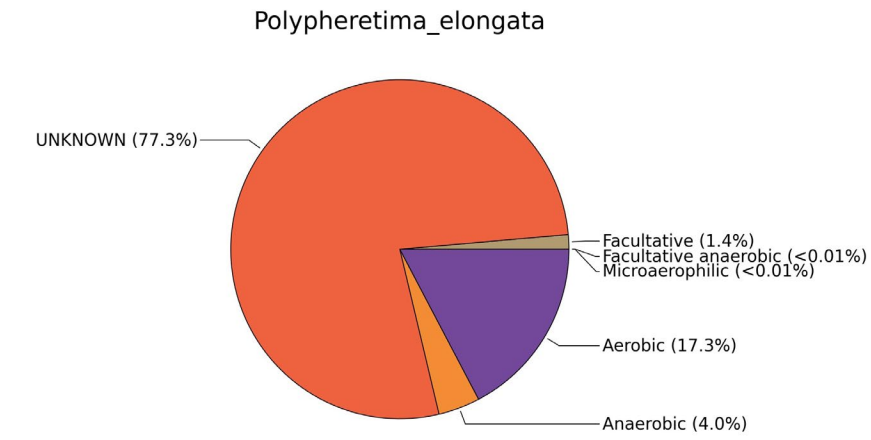


(C)

Fig. 6. Phenotypic analysis of three different earthworm sp. gut metagenomes on the basis of Gram staining using METAGENassist, (A) *Polypheretima elongata*; (B) *Perionyx excavatus*; (C) *Eudrilus eugeniae*.



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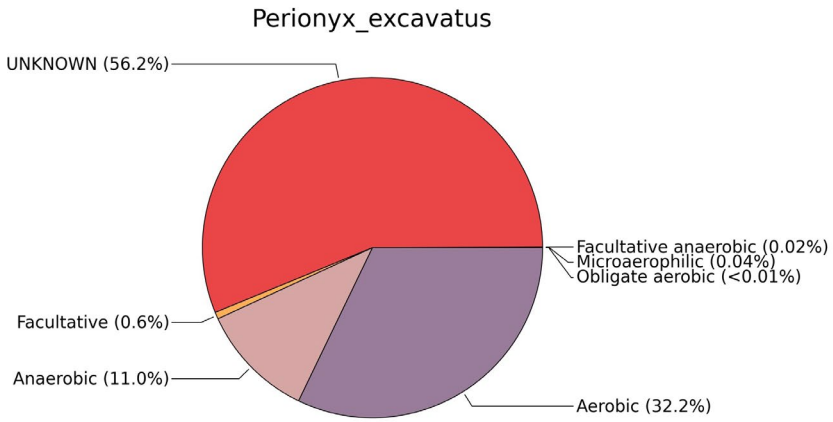

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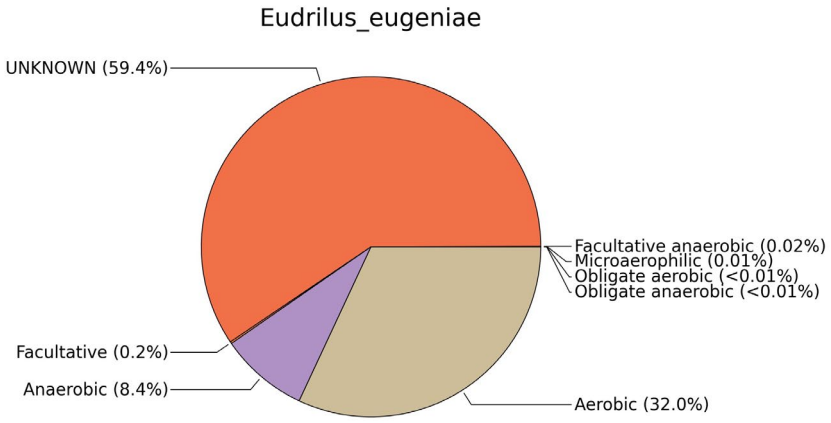


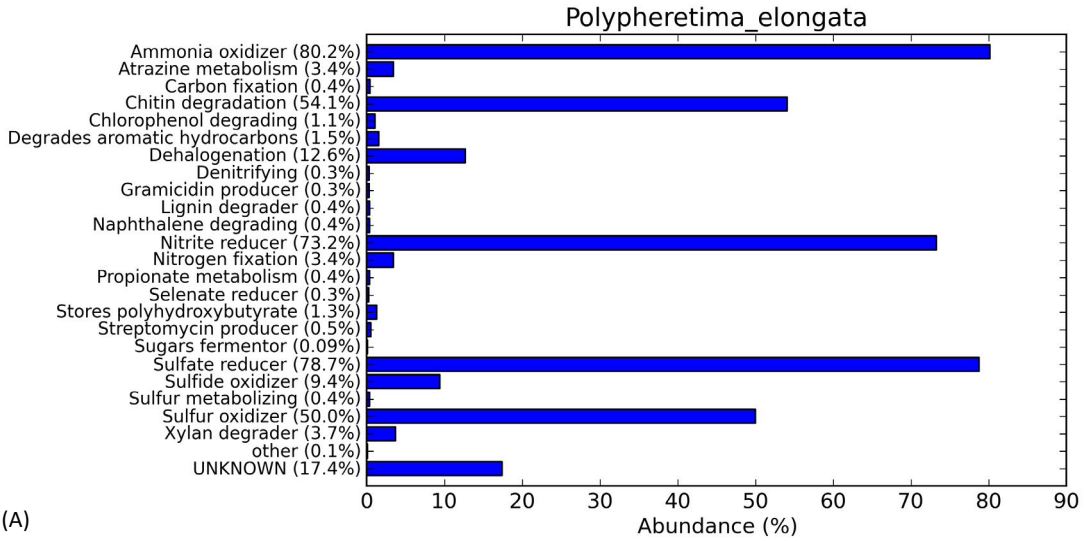
(C)

Fig. 7. Phenotypic analysis of three different earthworm sp. gut metagenomes on the basis of oxygen requirement using METAGENassist, (A) *Polypheretima elongata*; (B) *Perionyx excavatus*; (C) *Eudrilus eugeniae*.

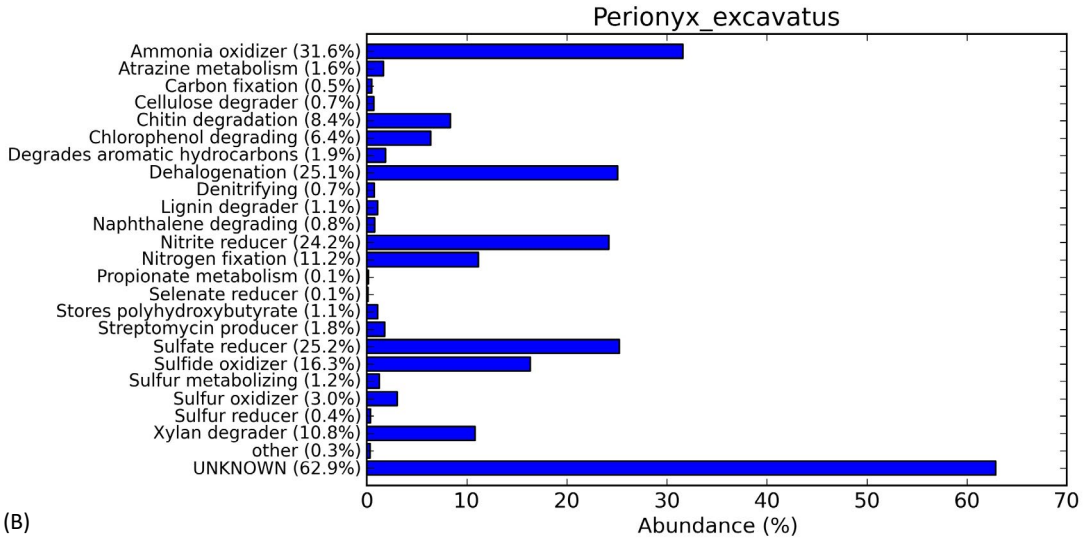
abundance of ammonia oxidizers was 31.6% and 29.3% in *Eudrilus eugeniae*. The metagenome of *Polypheretima elongata* has a relative abundance of sulfate reducer (78.7%), nitrite reducers (73.2%) and chitin degrader (54.1.7%) (Fig. 8A), whereas *Perionyx excavatus* has a relative abundance of sulfate reducer (25.2%), dehalogenators (25.1%), nitrite reducers (24.2%) and sulphide oxidizer (16.3%) (Fig. 8B). Similarly, *Eudrilus eugeniae* showed a relative abundance of sulfate reducer (22.6%), dehalogenators (22.0%), nitrite reducers (19.8%) and sulphide oxidizer (13.2%). In the metagenome of *Polypheretima elongata, Perionyx excavatus* and *Eudrilus eugeniae,* the relative abundance of nitrogen fixers were 3.4%, 11.2% and 10.2% respectively (Fig. 8A-C).

DISCUSSION

Earthworms play a vital role in the overall health and maintenance of soil ecosystems by altering soil texture, regulate water content, maintain the availability of nutrients for the plant; this diverse functionality of earthworms is mainly attributed to their gut microbiome[32]. Recently, scientists are gaining interest on functionality of earthworm gut[7,11,33-39] while, the studies on gut



(A)



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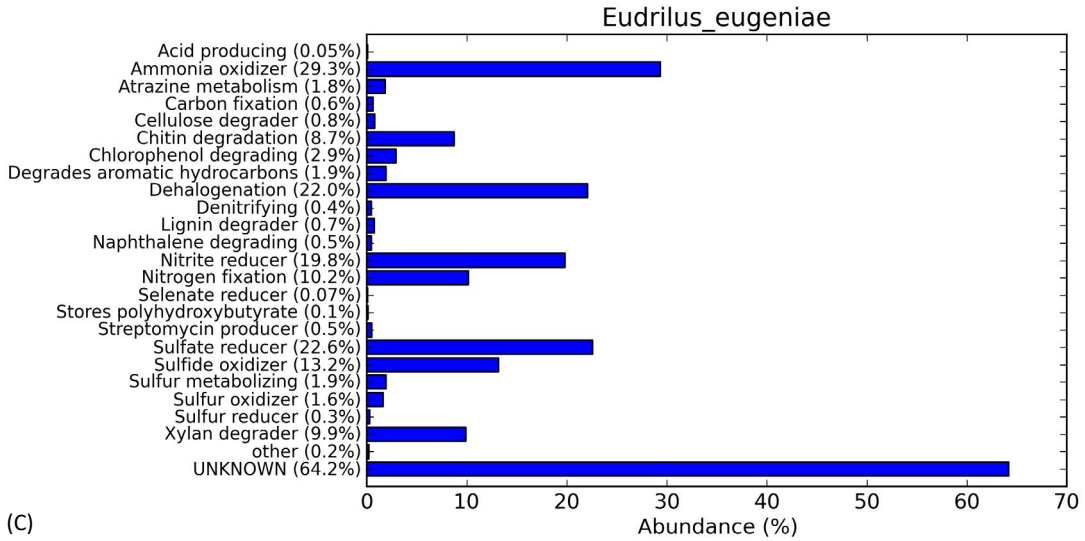

Fig. 8. Phenotypic analysis of three different earthworm sp. gut metagenomes on the basis of functional metabolism using METAGENassist, (A) *Polypheretima elongata*; (B) *Perionyx excavatus*; (C) *Eudrilus eugeniae*.

associated microbiome are still fragmentary. The present investigation revealed the community structures of gut bacteria of *Polypheretima elongata* by amplicon sequencing of 16S rRNA gene for the first time, although limited number of studies are available on other earthworm species especially, *Eudrilus eugeniae*[39-42] and *Perionyx excavatus* with metagenomic pyrosequencing[43,44]. We recorded a slight divergence in the diversity across the species, e.g the collective reads derived from the *Perionyx* sp. has the greatest value of Chao1 (947), followed by *Eudrilus* sp. (751), and *Polypheretima* sp. (678). Since there was no significant difference found in Good's coverage value among the samples (> 99%), reflects that sufficient amount of the bacterial diversity were captured in all the samples[45-47]. The Shannon index ranged from 5.177 to 7.613 and Simpson index ranged from 0.8985- 0.9875 across the samples, indicates *Polypheretima elongata* had the lowest bacterial diversity, while *Perionyx excavatus* has the highest diversity, with high species richness. The alpha diversity analysis (which is comprehensive indicator of species richness in community ecology) showed *Perionyx excavatus* gut had the highest diversity followed by *Eudrilus eugeniae* while *Polypheretima elongata* gut was the lowest according to Chao1, Shannon and Simpson values (Table 2 & Fig. 4A-C). The next generation sequences of soil microbiome with respect to soil depth suggested that the upper layer of topsoil have higher microbial diversity than the lower layer of topsoil, with increase in soil depth, microbiome abundance decreases[48,49]. The topsoil is made up of decomposed material of plants and leaves, which provides a favourable conditions for growth of soil microbes[50] and play a crucial role in formation of humus, nutrients and organic matter[51]. Since epigeic earthworms live and feed in upper layer of topsoil, get high exposure of soil microbiome, humus, nutrient and organic matter. Effect of surrounding environment (available substrate and feeding habit) may not be ignored on counting earthworms' gut microbiome[12,52]. Our observation on alpha diversity in gut microbiome of *Perionyx excavates, Eudrilus eugeniae* and *Polypheretima elongata* corresponds to above facts. The worms live in the lower layer of topsoil carry relatively low microbial abundance and less availability of nutrients. In addition, alteration in the relative abundance of bacterial communities in earthworms' gut may be interlinked with variation in their feeding behaviour pattern because of dependence on microbial colonization of host's feeding behaviour pattern[52]. The epigeic species *Perionyx excavatus* and, *Eudrilus eugeniae* feeding behaviour ranges at upper layer of topsoil, making them access to feed on soil minerals, humus

as well as remains of plant materials, while endogeic species *Polypheretima elongata* feeds on decomposing litter[53]. The study reported 5 major phyla of bacteria (Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes and Chloroflexi) in all three earthworm species, irrespective of their different feedings behaviours, which corresponded to the "core microbiota" of earthworms gut, although relative difference were observed at various taxonomical levels when compared for gut microbiota respectively. This could be explained due to co-evolution of certain core taxa via secretions of gut fluids that regulate microbial communities in earthworm gut[54], that remains largely unchanged with earthworms[12,55]. The progression of a particular microbial community depends on the food source and life forms. Moreover, various microbial communities are selected or favoured over other microbes in the tube-like gut, which is stable in the moisture and nutrient conditions, although it acts as unique anoxic micro-environment filtering agent for ingested microbial communities of microorganism pools[56].

The gut of *Polypheretima elongata, Perionyx excavatus* and *Eudrilus eugeniae* may be viewed as a bioreactor, in which diverse functions (biodegradation, bioremediation and biogeochemical cycling) goes simultaneously. On analysing homology datasets from the various online databases, Proteobacteria was found the predominant phylum, followed by Actinobacteria, Firmicutes and Bacteroidetes. The dominance of Proteobacteria may be due their fast-growing nature and its ability to employ available organic carbon sources and amino acids in the earthworm gut[32]. The predicted phenotypic analysis showed that gram negative bacteria's abundance depicts strong relationship between proteobacteria and ammonia oxidizers, sulfate reducers, nitrite reducers and chitin degraders. The nutrient poor environment carries high abundance of protobacteria[12,57] which play a vital role in the nitrogen cycle[58,59] and cellulose degradation[60]. It is noteworthy that similar trends have been recorded with predicted phenotype metabolism analysis in earthworms[42] and mammals gut microbiome[61,62]. In addition, few low abundance of certain phyla were also present such as Chloroflexi, Acidobacteria, Saccharibacteria and Verrucomicrobia. The earthworm's digestive system is involved in various processes such as oxidation and reduction, emission of N_2O and N_2, remediation, nitrogen fixation, denitrification and degradation processes. Deciphering the earthworm gut microbiome may enable researchers in understanding a much better perspective of their metabolic capabilities. Taxonomic to the phenotypic mapping on the basis of metabolism of the three species suggested that the earthworm gut microbiome played vital role in remediation, denitrification, nitrogen fixation, degradation of cellulose, reduction and sulfur oxidation processes. Isolation of such functionally active bacterial communities from earthworm gut may prove as a pearl of essential enzymes to degrade xenobiotic, lignocellulose for production of biofuels, environmental remediation and biogeochemical cycling.

CONCLUSION

The present study revealed Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes were the dominant phyla in earthworm sp. Functional characterization revealed that the majority of the bacterial groups were ammonia oxidizers followed by sulfate reducer and nitrite reducer. The study highlights that next-generation high throughput sequencing provides a much detailed and accurate insight into the gut microbiome than other conventional techniques. It can be hypothesized that the majority of the functional attributions of earthworms in the soil ecosystem may be related to their diverse gut microbiome instead the activity of soil microbiomes.

ACKNOWLEDGMENTS

Author SY is thankful to Department of Biotechnology, Ministry of Science and Technology, Govt. of India, New Delhi and SST for Environment Planning and Coordination Organisation (EPCO), Ministry of Environment, Govt. of Madhya Pradesh for the financial support to carry out the study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SST performed the experiment, recorded and analyzed data with help of available

bioinformatics tools and, wrote the manuscript with the support of SKJ and SY. ARL helped in performing experiment and computation of phylogenomic. NT helped in the draft preparation. All authors provided critical feedback and helped to shape the research, analysis, and manuscript.

FUNDING

Author (SY) received a financial grant under the project entitled Toxicity of NPs against earthworm and investigating its potential as bio-transforming agent with Grant ID: BT/PR1856/NNT/28/532/2011 from the Department of Biotechnology, Ministry of Science and Technology, Govt. of India.

DATA AVAILABILITY

The 16S rRNA amplicon sequences of earthworms gut were submitted in NCBI sequences read archive under the bio project SRA accession number PRJNA670329.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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